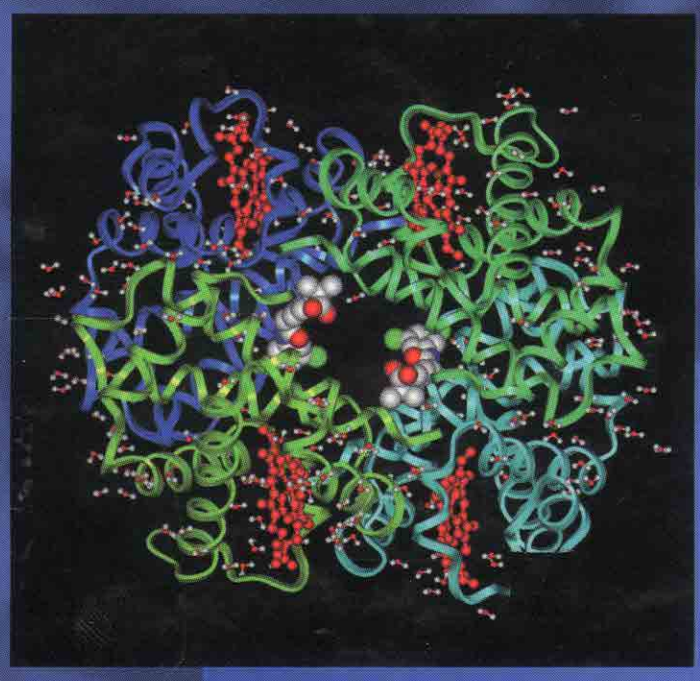
Sixth Edition

BURGER'S Medicinal & Chemistry Drug Discovery

Edited by

Donald J. Abraham



VOLUME 6

Nervous System Agents

BURGER'S MEDICINAL CHEMISTRY AND DRUG DISCOVERY

Sixth Edition

Volume 6: Nervous System Agents

Edited by

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BURGER'S
MEDICINAL CHEMISTRY
AND
DRUG DISCOVERY

CHAPTER ONE

Adrenergics and Adrenergic-Blocking Agents

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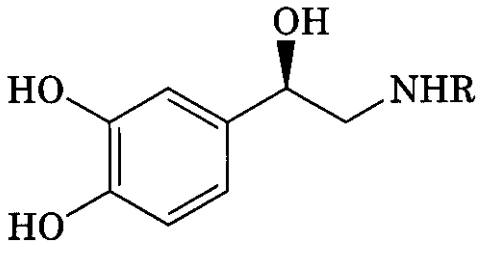
1 INTRODUCTION

In both their chemical structures and biological activities, adrenergics and adrenergic-blocking agents constitute an extremely varied group of drugs whose clinical utility includes prescription drugs to treat life-threatening conditions such as asthma and hypertension as well as nonprescription medications for minor ailments such as the common cold. This extensive group of drugs includes synthetic agents as well as chemicals derived from natural products that have been used in traditional medicines for centuries. Many adrenergic drugs are among the most commonly prescribed medications in the United States, including bronchodilators, such as albuterol (13) for use in treating asthma, and antihypertensives, such as atenolol (46) and doxazosin (42). Nonprescription adrenergic drugs include such widely used nasal decongestants as pseudoephedrine (5)and naphazoline (29). Most of these varied drugs exert their therapeutic effects through action on adrenoceptors, G-protein-coupled cell surface receptors for the neurotransmitter norepinephrine (noradrenaline, 1), and the adrenal hormone epinephrine (adrenaline, 2).



(1) norepinephrine, R = H
(2) epinephrine, R = CH_3

Adrenoceptors are broadly classified into α- and preceptors, with each group being further subdivided. Identification of subclasses of adrenoceptors has been greatly aided by the tools of molecular biology and, to date, six distinct α-adrenoceptors (α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, α_{2C}), and three distinct β-adrenoceptors (β_1, β_2, β_3) have been clearly identified (1), with conflicting evidence for a fourth type of β (β_4) (1–3). In general the most common clinical applications of a,-agonists are as vasoconstrictors employed as nasal decongestants and for raising blood pressure in shock; α_2-agonists are employed as antihypertensives; a,-antagonists (a-blockers) are vasodilators and smooth muscle relaxants employed as antihypertensives and for treating prostatic hyperplasia; β-antagonists (β-blockers) are employed as antihypertensives and for treating cardiac arrhythmias; and β-agonists are employed as bronchodilators. The most novel recent advances in adrenergic drug research have been directed toward development of selective β_3-agonists that have potential applications in treatment of diabetes and obesity (4–8).

2 CLINICAL APPLICATIONS

2.1 Current Drugs

U.S. Food and Drug Administration (FDA)-approved adrenergic and antiadrenergic drugs currently available in the United States are summarized in Table 1.1, which is organized in general according to pharmacological mechanisms of action and alphabetically within those mechanistic classes. Structures of the currently employed drugs are given in Tables 1.2–1.6 according to chemical class. Drugs in a given mechanistic class often have more than one therapeutic application, and may or may not all be structurally similar. Furthermore, drugs from several different mechanistic classes may be employed in a given therapeu-

Table *1.1* Adrenergic and Antiadrenergic Pharmaceuticals

Class and Generic Name	Trade Name[a]	Originator	Chemical Class	Dose[bc]
General agonists				
Amphetamine ***(3)***	Adderall, Dexedrine	SmithKline & French	Phenylethylamine	5–60 mg/day
Dipivefrin ***(4)***	Propine	Klinge	Phenylethylamine	1 drop 2 × daily 0.1% soln.
Ephedrine *erythro*-(**5**)	various		Phenylethylamine	50–150 mg/day for asthma 10–25 mg i.v. for hypotension
Epinephrine ***(2)***	Adrenaline	Parke-Davis	Phenylethylamine	0.3–1.5 mg s.c. 2–10 μg/min i.v. 160–250 μg inh.
Mephentermine ***(6)***	Wyamine	Wyeth	Phenylethylamine	30–45 mg, i.m.
Norepinephrine (**1**)	Levophed	Sterling	Phenylethylamine	0.5–30 μg/min i.v.
Pseudoephedrine *threo*-(**5**)	various		Phenylethylamine	60-240 mg/day
α_1-Agonists				
Levonordefrin ***(7)***	na	Winthrop	Phenylethylamine	1:20,000 in local anesthetics
Metaraminol (**8**)	Aramine	Sharpe & Dohme	Phenylethylamine	2–10 mg, i.m.
Methoxamine ***(9)***	Vasoxyl	Burroughs Wellcome	Phenylethylamine	10–20 mg, i.m.
Midodrine ***(10)***	ProAmatine	Oesterreichische Stickstoffwerke	Phenylethylamine	30 mg/day
Naphazoline ***(29)***	various	Ciba	Imidazoline	1–2 drops 0.05% nasal 0.03% ophthalmic
Oxymetazoline ***(30)***	various	Merck	Imidazoline	1–2 drops 0.05% nasal 0.025% ophthalmic
Phenylephrine ***(11)***	various	F. Stearns & Co.	Phenylethylamine	1–3 drops 0.25–0.5% soln. nasal 0.1–0.5 mg i.v. for shock
Tetrahydrozoline (**31**)	Various	Sahyun	Imidazoline	1–2 drops of 0.05% soln.
Xylometazoline (**32**)		Ciba	Imidazoline	2–3 drops of 0.1% soln.
α_2-Agonists				
Apraclonidine (**33**)	Iopidine	Alcon	Aminoimidazoline	3–6 drops 0.5–1% soln.
Brimonidine (**34**)	Alphagan	Pfizer	Aminoimidazoline	1 drop 0.2% soln., 3× daily
Clonidine (**35**)	Catapress	Boehringer	Aminoimidazoline	0.2–1.2 mg/day
Guanabenz (**36**)	Wytensin	Sandoz	Arylguanidine	8–32 mg/day
Guanfacine (**37**)	Tenex	Wander	Arylguanidine	1–3 mg/day
Methyldopa ***(12)***	Aldomet	Merck	Aromatic amino acid	500–2000 mg/day

w

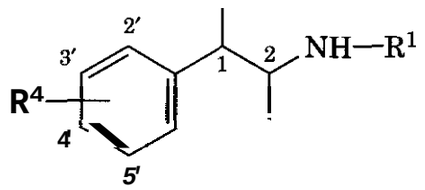
Table 1.1 (Continued)

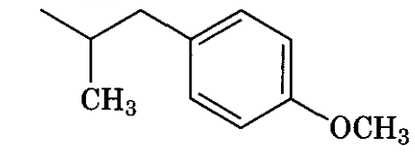
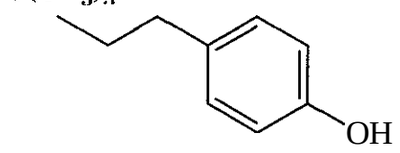
Class and Generic Name	Trade Name[a]	Originator	Chemical Class	Dose[bc]
β-Agonists				
Albuterol (13)	Proventil, Ventolin	Allen & Hanburys	Phenylethylamine	12-32 mg/day p.o. 2.5 mg 3–4× daily, neb.
Bitolterol (14)	Tornalate	Sterling	Phenylethylamine	0.74–2.22 inh.
Formoterol (15)	Foradil	Yamanouchi	Phenylethylamine	12 pg, 2× daily inh.
Isoetharine (16)	Bronkosol	I. G. Farben	Phenylethylamine	2 mL 0.25% soln. inh.
Isoproterenol (17)	Isuprel	Boehringer	Phenylethylamine	120-262 pg, 2–6× daily inh. 0.5–5.0 μg/min, i.v.
Levalbuterol (13)	Xopenex	Sepracor	Phenylethylamine	0.63–1.25 mg 3× daily neb.
Metaproterenol (18)	Alupent, Metaprel	Boehringer	Phenylethylamine	60–80 mg/day p.o. 1.3–1.95 mg, 6–8x daily, inh.
Pirbuterol (19)	Maxair	Pfizer	Pyridylethylamine	0.2–0.4 mg 4–6X daily, inh.
Ritodrine (20)	Yutopar	Philips	Phenylethylamine	150-350 μg/min, i.v. 120 mglday
Salmeterol (21)	Serevent	Glaxo	Phenylethylamine	42 μg, 2× daily, inh.
Terbutaline (22)	Brethine	Draco	Phenylethylamine	7.5–15 mg/day
Antiadrenergics				
Guanadrel (38)	Hylorel	Cutter	Guanidine	10-75 mglday
Guanethidine (39)	Ismelin	Ciba	Guanidine	10–50 mg/day
Reserpine (60)	reserpine	Ciba	Alkaloid	0.05–0.5 mg/day
Metyrosine (23)	Demser	Merck	Aromatic amino acid	14 g/day
a-Antagonists				
Dapiprazole (61)	Rev-Eyes	Angelini-Francesco	Piperidinlytriazole	2 drops 0.5% soln.
Phenoxybenzamine (62)	Dibenzylime	SmithKline & French	Haloalkylamine	20-120 mglday
Phentolamine (40)	Regitine	Ciba	Imidazoline	5–10 mg i.v.
Tolazoline (41)	Priscoline	Ciba	Imidazoline	40-200 mg/day
Selective a,-antagonists				
Doxazosin (42)	Cardura	Pfizer	Quinazoline	1–16 mg/day
Prazosin (43)	Minipress	Pfizer	Quinazoline	1–9 mglday for BPH 6–20 mg/day for hypertension
Tamsulosin (24)	Flomax	Yamanouchi	Phenylethylamine	0.4–0.8 mg/day
Terazosin (44)	Hytrin	Abbott	Quinazoline	5–20 mg/day

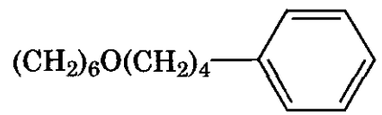
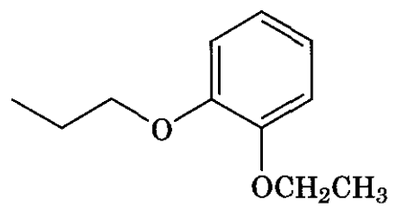
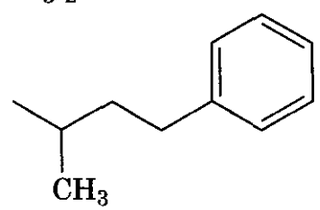
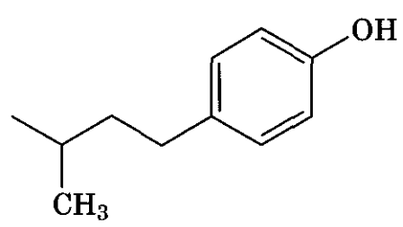
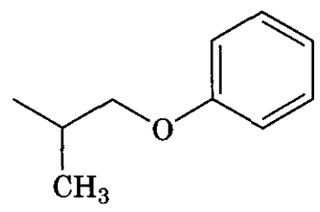
β-Antagonists				
Acebutolol (45)	**Sectral**	May & Baker	Aryloxypropanolamine	**200–1200** mg/day
Atenolol (46)	Tenormin	ICI	Aryloxypropanolamine	**25–150** mg/day
Betaxolol (47)	Betoptic, Kerlone	Synthelabo	Aryloxypropanolamine	Hypertension: **10–20** mg orally Glaucoma: **1–2** drops **0.5% soln.** 2× daily
Bisoprolol (48)	Zebeta	Merck	Aryloxypropanolamine	1.25–20 mg/day
Carteolol (49)	**Cartrol, Ocupress**	**Otsuka**	Aryloxypropanolamine	2.5–10 mg/day
Esmolol (50)	Brevibloc	American Hospital Supply	Aryloxypropanolamine	**50–100 μg/kg/min**
Levobetaxolol *S*-(−)-**(47)**	**Betaxon**	**Alcon**	Aryloxypropanolamine	**1** drop **0.5%** soln., 2× daily
Levobunolol (51)	**Betagan**	Warner-Lambert	Aryloxypropanolamine	**1–2** drops **0.5%** soln., 1–2× daily
Metipranolol (52)	**OptiPranolol**	Boehringer	Aryloxypropanolamine	**1** drop **0.3%** soln.., 2× daily
Metoprolol (53)	Lopressor, Toprol-XL Toprol-XL	AB **Hässle**	Aryloxypropanolamine	100–450 **mg/day** **XL 50–100** mg/day
Nadolol (54)	**Corgard**	Squibb	Aryloxypropanolamine	**40–320** mg/day
Penbutolol (55)	Levatol	Hoechst	Aryloxypropanolamine	**20–80** mg/day
Pindolol (56)	Visken	Sandoz	Aryloxypropanolamine	10–60 mg/day
Propranolol (57)	Inderal, Inderal LA	ICI	Aryloxypropanolamine	160–640 mg/day
Sotalol (25)	**Betapace**	Mead Johnson	Phenylethylamine	**160–320** mg/day
Timolol (58)	**Timoptic**	**Frosst**	Aryloxypropanolamine	Hypertension: **10–60** mg/day Glaucoma: **1** drop **0.25%** soln., 2× daily
alp-Antagonists				
Carvedilol (59)	Coreg	Boehringer	Aryloxypropanolamine	**13–50** mg/day
Labetalol (26)	Normodyne	Allen & Hanburys	Phenylethylamine	**200–2400 mg/day**
Agonist/Antagonists				
Dobutamine **(27)**	Dobutrex	**Lilly**	Phenylethylamine	**2–20** μg/kg/min, i.v.
Isoxsuprine (28)	**Vasodilan**	**Philips**	Arylpropanolamine	30–80 mg/day

"Not all trade names are listed, particularly for drugs no longer under patent.
[b]All dose information from Drug Facts and Comparisons 2002 **(14)**.
"Not all doses and dosage forms are listed. For further information consult reference (14).

Table 1.2 **Phenylethylamines (Structures** 1–28)



Compound	R1	R2	R3	R4	Receptor Activity"
(1)	H	H	OH	3′,4′-diOH	$\alpha + \beta$
(2)	CH_3	H	OH	3′,4′-diOH	$\beta \geq \alpha$
(3)	H	CH_3	H	H	$(\alpha + \beta)^b$
(4)	CH_3	H	OH	3′,4′-di-$O_2CC(CH_3)_3$	$(\beta \geq \alpha)^c$
(5)	CH_3	CH_3	OH	H	$(\alpha + \beta)^d$
(6)	CH_3	2,2-diCH_3	OH	H	$(\alpha + \beta)^b$
(7)	H	CH_3	OH	3′,4′-diOH	a
(8)	H	CH_3	OH	3′-OH	a!
(9)	H	CH_3	OH	2′,5′-diOCH_3	a!
(10)	$COCH_2NH_2$	H	OH	2′,5′-diOCH_3	a!
(11)	CH_3	H	OH	3′-OH	α
(12)	H	2-CH_3, 2-CO_2H	H	3′,4′-diOH	$\alpha_2{}^c$
(13)	$C(CH_3)_3$	H	OH	3′-CH_2OH, 4′-OH	β_2
(14)	$C(CH_3)_3$	H	OH	3′,4′-bis($O_2CC_4H_4$-*p*-CH_3)	$\beta_2{}^c$
(15)		H	OH	3′-NHCHO, 4′-OH	β_2
(16)	$CH(CH_3)_2$	CH_2CH_3	OH	3′,4′-diOH	β
(17)	$CH(CH_3)_2$	H	OH	3′,4′-diOH	P
(18)	$C(CH_3)_3$	H	OH	3′,5′-diOH	β_2
(19)	$C(CH_3)_3$	H	OH	2′-aza, 3′-CH_2OH, 4′-OH	β_2
(20)		CH_3	OH	4′-OH	β_2

(21)		H	OH	3′-CH_2OH, 4′-OH	β_2
(22)	$C(CH_3)_3$	H	OH	3′,5′-diOH	β_2
(23)	H	2-CH_3, 2-CO_2H	H	4′-OH	na[e]
(24)		CH_3	H	3′-SO_2NH_2, 4′-OCH_3	α_1-blocker
(25)	$CH(CH_3)_2$	H	OH	4′-$NHSO_2CH_3$	β-blocker
(26)		H	OH	3′-$CONH_2$, 4′-OH	α_1,β_1,β_2-blocker
(27)		H	H	3′,4′-diOH	β_1[f]
(28)		CH_3	OH	4′-OH	α_1-blocker, β-agonist

[a]Agonist activity unless indicated otherwise.
[b]Indirect activity through release of norepinephrine and reuptake inhibition.
[c]Prodrug.
[d]Mixed direct and indirect activity.
'Norepinephrine biosynthesis inhibitor.
[f]Net sum of effects of enantiomers.

Table 1.3 Imidazolines and Guanidines (Structures 29–41)

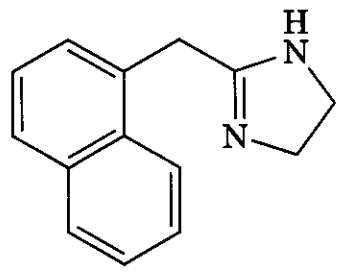
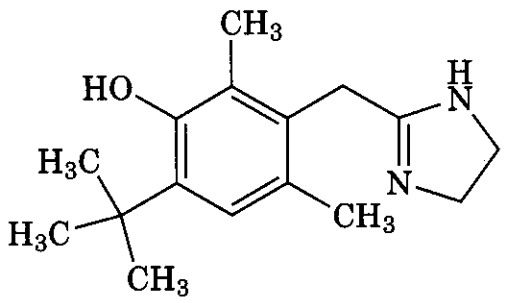
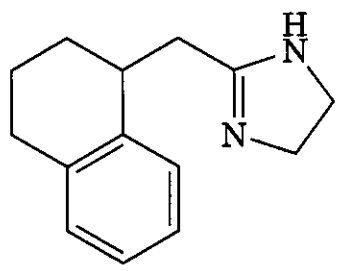
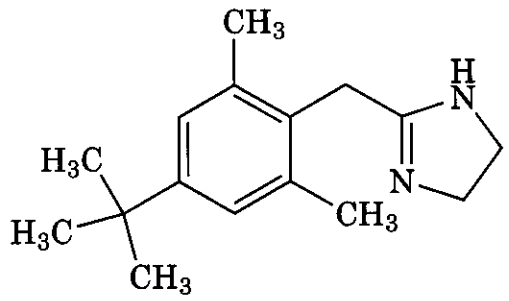
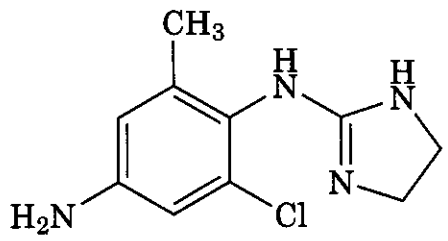
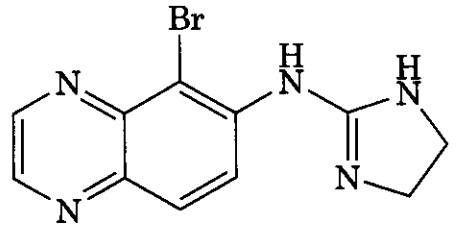
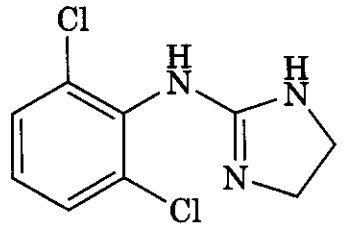
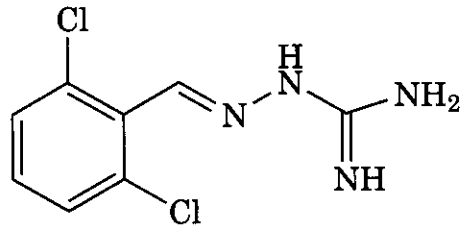
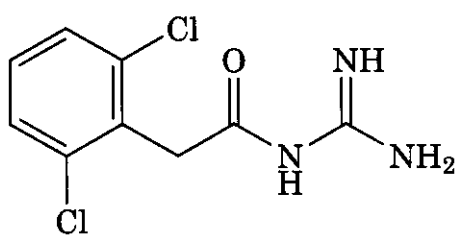
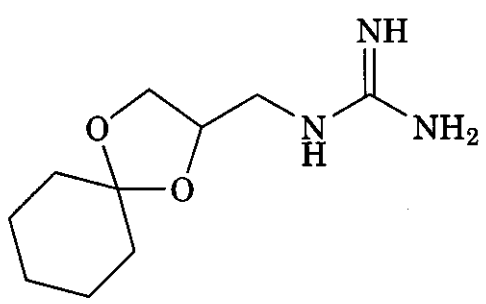
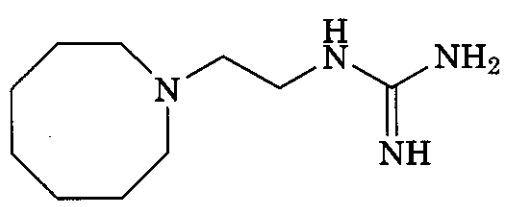
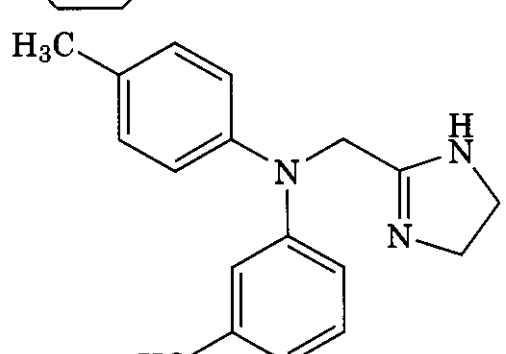
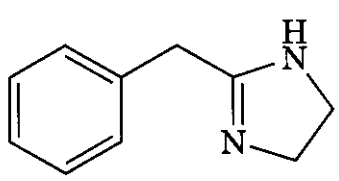
Compound	Structure	Receptor Activity
(29)		α_1-agonist
(30)		α_1-agonist
(31)		α_1-agonist
(32)		α_1-agonist
(33)		α_2-agonist
(34)		α_2-agonist
(35)		α_2-agonist
(36)		α_2-agonist

Table 1.3 (Continued)

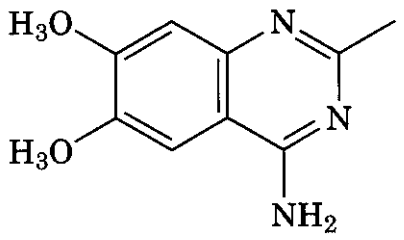
Compound	Structure	Receptor Activity
(**37**)		α_2-agonist
(**38**)		na[a]
(**39**)		na[a]
(**40**)		α_1-antagonist
(**41**)		α_1-antagonist

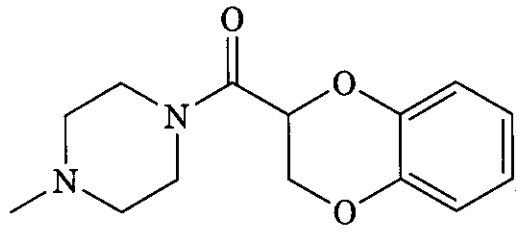
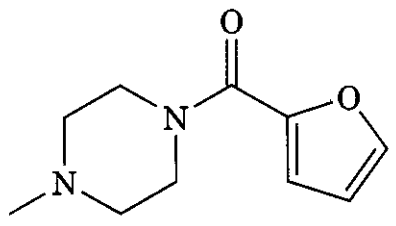
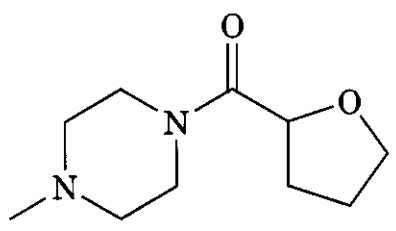
"Inhibit release of norepinephrine.

tic application; for example, β-blockers, α_1-blockers, and a,-agonists are all employed to treat hypertension.

2.1.1 Applications of General Adrenergic Agonists. The mixed a- and β-agonist norepinephrine (**1**)has limited clinical application because of the nonselective nature of its action in stimulating the entire adrenergic system. In addition to nonselective activity, it is orally inactive because of rapid first-pass metabolism of the catechol hydroxyls by catechol-*O*-methyl-transferase (COMT) and must be administered intravenously. Rapid metabolism limits its duration of action to only 1 or 2 min, even when given by infusion. Because its α-activity constricts blood vessels and thereby raises blood pressure, (**1**)is used to counteract various hypotensive crises and as an adjunct treatment in cardiac arrest where its β-activity stimulates the heart. Although it also lacks oral activity because it is a catechol, epinephrine (2)is far more widely used clinically than (**1**)Epinephrine, like norepinephrine, is used to treat hypotensive crises and, because of its greater β-activity, is used to stimulate the heart in cardiac arrest. When administered intravenously or by inhalation, epinephrine's β_2-activity makes it useful in relieving bronchoconstriction in asthma. Because it has significant a-activity, epinephrine is also used in topical nasal decongestants. Constriction of dilated blood vessels by a-agonists in mucous membranes shrinks the membranes and re-

Table 1.4 Quinazolines (Structures42–44)



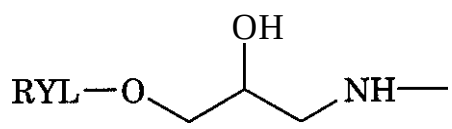
Compound	R	Receptor Activity
(**42**)		α_1-antagonist
(**43**)		α_1-antagonist
(**44**)		α_1-antagonist

duces nasal congestion. Dipivefrin (4) is a prodrug form of (**2**), in which the catechol hydroxyls are esterified with pivalic acid. Dipivefrin is used to treat open-angle glaucoma through topical application to the eye where the drug (4) is hydrolyzed to epinephrine (**2**), which stimulates both α- and β-receptors, resulting in both decreased production and increased outflow of aqueous humor, which in turn lowers intraocular pressure.

Amphetamine (3) is orally active and, through an indirect mechanism, causes a general activation of the adrenergic nervous system. Unlike (**1**)and (**2**), amphetamine readily crosses the blood-brain barrier to activate a number of adrenergic pathways in the central nervous system (CNS). Amphetamine's CNS activity is the basis of its clinical utility in treating attention-deficit disorder, narcolepsy, and use as an anorexiant. These therapeutic areas are treated elsewhere in this volume.

Ephedrine *erythro*-(**5**) and pseudoephedrine threo-(5) are diastereomers with ephedrine, a racemic mixture of the R,S and S,R stereoisomers, and pseudoephedrine, a racemic mixture of R,R and S,S stereoisomers. Ephedrine is a natural product isolated from several species of ephedra plants, which were used for centuries in folk medicines in a variety of cultures worldwide (9). Ephedrine has both direct activity on adrenoceptors and indirect activity, through causing release of norepinephrine from adrenergic nerve terminals. Ephedrine is widely used as a nonprescription bronchodilator. It has also been used as a vasopressor and cardiac stimulant. Lacking phenolic hydroxyls, ephedrine crosses the blood-brain barrier far better than does epinephrine. Because of its ability to penetrate the CNS, ephedrine has been used as a stimulant and exhibits side effects related to its action in the brain such as insomnia, irritability, and anxiety. It suppresses appetite and in high doses can cause euphoria or even hallucinations. In the United States the purified chemical ephedrine is considered a drug and regulated by the FDA. However, the dried plant material ma huang is considered by law to be a dietary supplement, and not subject to FDA regulation. As a result there are a large number of ma huang-containing herbal remedies and "nu-

Table 1.5 Aryloxypropanolamines (Structures 45–59)



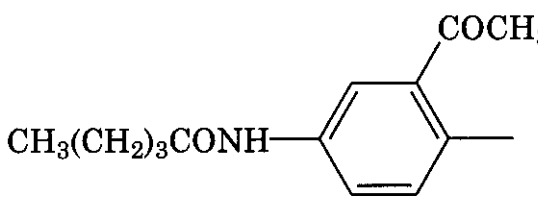
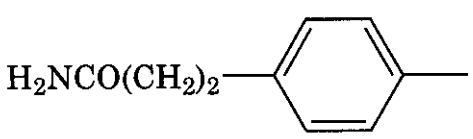
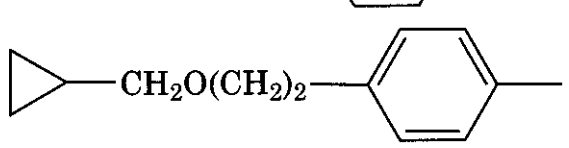
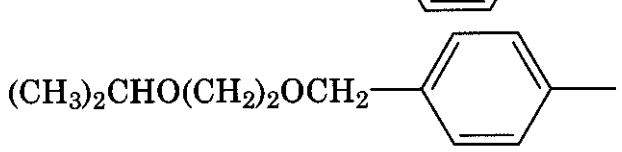
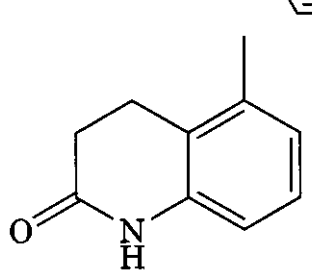
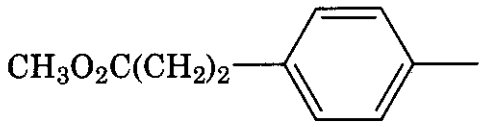
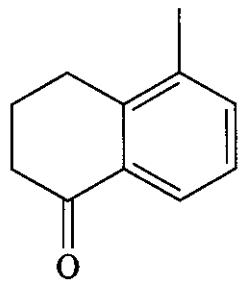
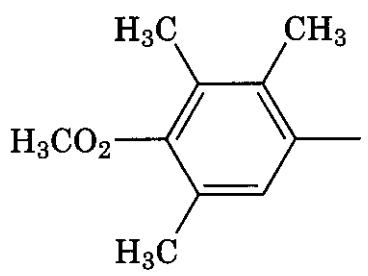
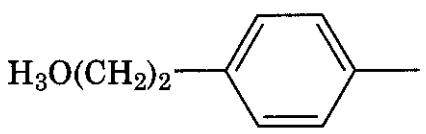
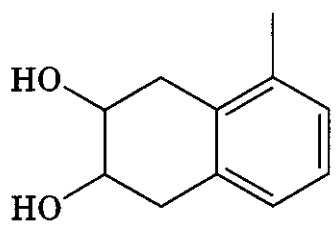
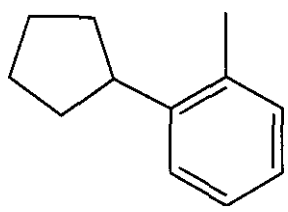
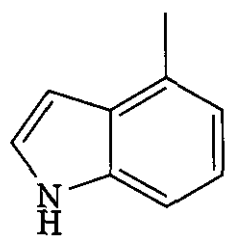
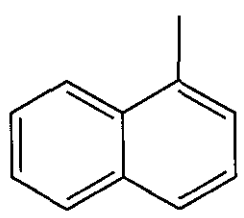
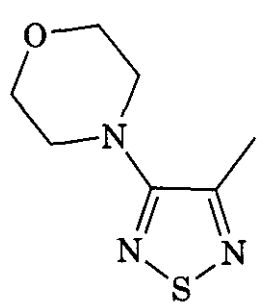
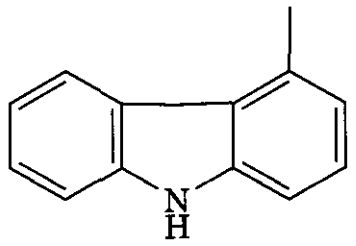
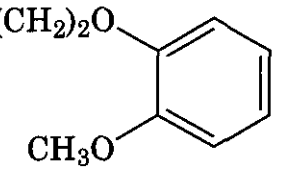
Compound	ARYL	R	Receptor Selectivity[a]
(**45**)		$CH(CH_3)_2$	β_1
(**46**)		$CH(CH_3)_2$	β_1
(**47**)		$CH(CH_3)_2$	β_1
(**48**)		$CH(CH_3)_2$	β_1
(**49**)		$C(CH_3)_3$	β_1, β_2
(**50**)		$CH(CH_3)_2$	β_1
(**51**)		$C(CH_3)_3$	β_1, β_2
(**52**)		$CH(CH_3)_2$	β_1, β_2
(**53**)		$CH(CH_3)_2$	β_1
(**54**)		$C(CH_3)_3$	β_1, β_2

Table *1.5 (Continued)*

Compound	ARYL	R	Receptor Selectivity[a]
(**55**)		$C(CH_3)_3$	β_1, β_2
(**56**)		$CH(CH_3)_2$	β_1, β_2
(**57**)		$CH(CH_3)_2$	β_1, β_2
(**58**)		$C(CH_3)_3$	β_1, β_2
(**59**)		—$(CH_2)_2O$— 	α_1, β_1, β_2

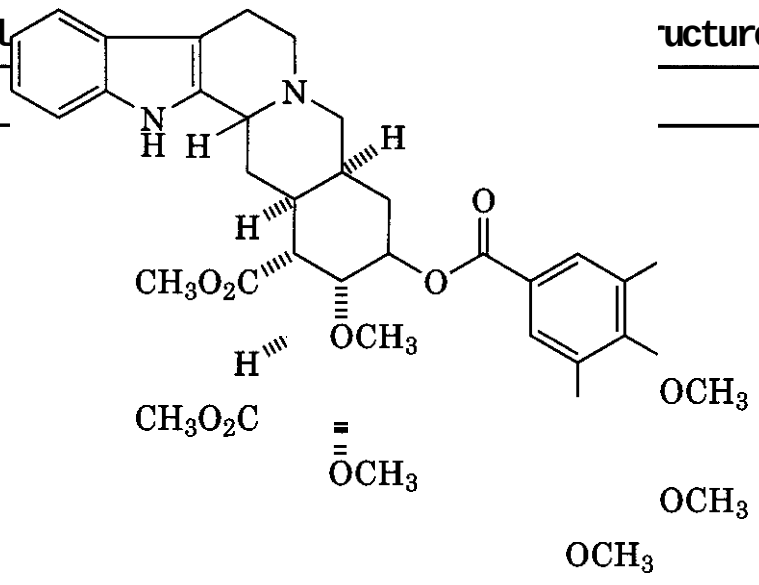
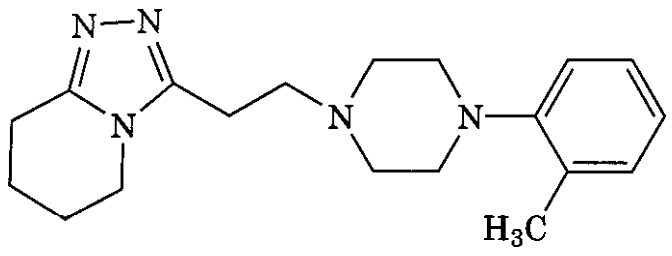
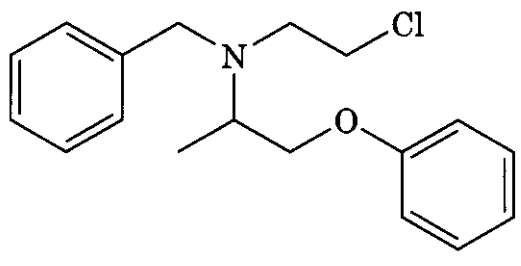
[a]Antagonists.

triceuticals" on the market whose active ingredient is the adrenergic agonist ephedrine. Pseudoephedrine, the ***threo*** diastereomer, has virtually no direct activity on adrenergic receptors but acts by causing the release of norepinephrine from nerve terminals, which in turn constricts blood vessels. Although it too crosses the blood-brain barrier, pseudoephedrine's lack of direct activity affords fewer CNS side effects than does ephedrine. Pseudoephedrine is widely used as a nasal decongestant and is an ingredient in many nonprescription cold remedies.

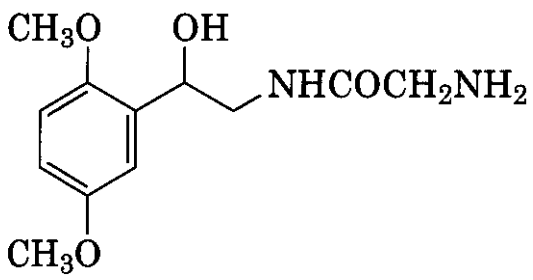
Mephentermine (**6**) is another general adrenergic agonist with both direct and indirect activity. Mephentermine's therapeutic utility is as a parenteral vasopressor used to treat hypotension induced by spinal anesthesia or other drugs.

2.1.2 Applications of α_1-Agonists. All selective a,-agonists are vasoconstrictors, which is the basis of their therapeutic activity. The sole use of levonordefrin (7)is in formulations with parenteral local anesthetics employed in dentistry. Vasoconstriction induced by the a-agonist activity of ***(7)*** helps retain the local anesthetic near the site of injection and prolongs the duration of anesthetic activity. Metaraminol (8)and methoxamine (9) are both parenteral vasopressors selective for α-receptors and so have few cardiac stimulatory properties. Because they are not substrates for COMT, their duration of action is significantly longer than that of norepinephrine, but their primary use is limited to treating hypotension during surgery or shock. Methoxamine is also used in treating supraventricular tachycardia. Midodrine (10) is an orally active glycine-

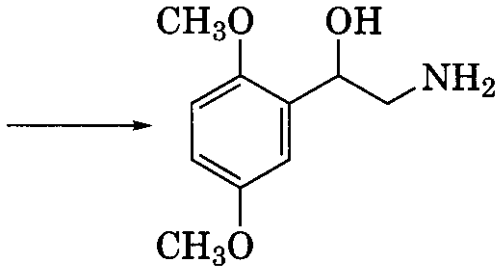
Table 16 Miscell **uctures 60-62)**

Compound	Pharmacological Activity
(**60**) 	Antiadrenergic
(**61**) 	α-Antagonist
(**62**) 	α-Antagonist

amide prodrug, hydrolyzed **in** *vivo* to (**63**), an analog of methoxamine, and a vasoconstrictor. Midodrine is used to treat orthostatic **hypo**tension.



(**10**)



(**63**)

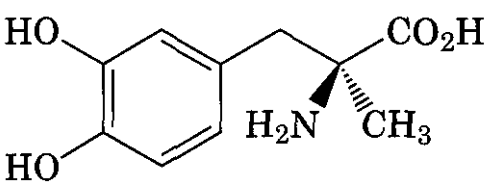
Phenylephrine (**11**), also a selective α-agonist, may be administered parenterally for severe hypotension or shock but is much more widely employed as a nonprescription nasal decongestant in both oral and topical preparations.

The imidazolines naphazoline (**29**), **oxy**metazoline (**30**), tetrahydozoline (**31**), and **xy**lometazoline (32) are all selective a,-agonists, widely employed as vasoconstrictors in topical nonprescription drugs for treating nasal congestion or bloodshot eyes. Naphazoline and oxymetazoline are employed in both nasal decongestants and ophthalmic preparations, whereas tetrahydrozoline is currently marketed only for ophthalmic use and **xylometa**zoline only as a nasal decongestant.

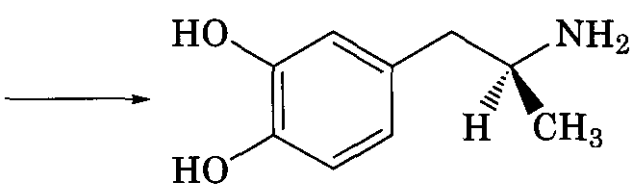
2.1.3 Applications of α_2-Agonists. Aminoimidazolines apraclonidine (33) and **bri**monidine (34) are selective a,-agonists employed topically in the treatment of glaucoma. Stimulation of a,-receptors in the eye reduces production of aqueous humor and enhances outflow of aqueous humor, thus reducing **in**traocular pressure. Brimonidine is substantially more selective for a,-receptors over α_1-

receptors than is apraclonidine. Although both are applied topically to the eye, measurable quantities of these drugs are detectable in plasma, so caution must be employed when the patient is also taking cardiovascular agents. Structurally related aminoimidazoline clonidine (35) is a selective α_2-agonist taken orally for treatment of hypertension. The antihypertensive actions of clonidine are mediated through stimulation of α_2-adrenoceptors within the CNS, resulting in an overall decrease in peripheral sympathetic tone. Guanabenz (**36**) and guanfacine (37) are ring-opened analogs of (**35**), acting by the same mechanism and employed as centrally acting antihypertensives.

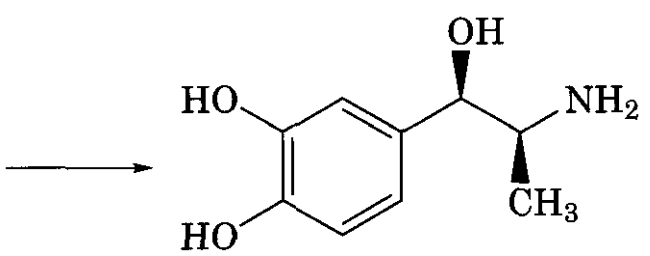
Methyldopa (12) is another antihypertensive agent acting as an a,-agonist in the CNS through its metabolite, α-methyl-norepinephrine (65). Methyldopa [the drug is the L-(*S*)-stereoisomer] is decarboxylated to α-methyldopamine (64) followed by stereospecific β-hydroxylation to the (1*R*,2*S*) stereoisomer of a-methylnorepinephrine (65). This stereoisomer is an α_2-agonist that, like clonidine, guanabenz, and guanfacine, causes a decrease in sympathetic output from the CNS.



(12)



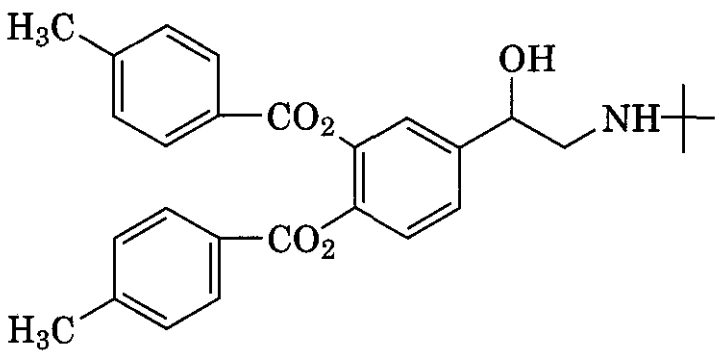
(64)



(65)

2.1.4 Applications of β-Agonists. Most of the β-selective adrenergic agonists, albuterol (13; salbutamol in Europe), bitolterol (**14**), formoterol (**15**), isoetharine (**16**), isoproterenol (**17**), levalbuterol [*R*-(−)-(**13**)], metaproterenol (**18**), pirbuterol (**19**), salmeterol (**21**), and terbutaline (22) are used primarily as bronchodilators in asthma and other constrictive pulmonary conditions. Isoproterenol (**17**) is a general β-agonist, and the cardiac stimulation caused by its β_1-activity and its lack of oral activity attributed to first-pass metabolism of the catechol ring have led to diminished use in favor of selective β_2-agonists. Noncatechol-selective β_2-agonists, such as albuterol (**13**), metaproterenol (**18**), and terbutaline (**22**), are available in oral dosage forms as well as in inhalers. All have similar activities and durations of action. Pirbuterol (19) is an analog of albuterol, in which the benzene ring has been replaced by a pyridine ring. Similar to albuterol, (19) is a selective β_2-agonist, currently available only for administration by inhalation. Bitolterol (**14**) is a prodrug, in which the catechol hydroxyl groups have been converted to 4-methylbenzoic acid esters, providing increased lipid solubility and prolonged duration of action. Bitolterol is administered by inhalation, and the ester groups are hydrolyzed by esterases to liberate the active catechol drug (**66**), which is subject to metabolism by COMT, although the duration of action of a single dose of the prodrug is up to 8 h, permitting less frequent administration and greater convenience to the patient. More recently developed selective β_2-agonist bronchodilators are formoterol (**15**) and salmeterol (**21**), which have durations of action of 12 h or more. Terbutaline (**22**), in addition to its use as a bronchodilator, has also been used for halting the contractions of premature labor. Ritodrine (20) is a selective β_2-agonist that is used exclusively for relaxing uterine muscle and inhibiting the contractions of premature labor.

2.1.5 Applications of Antiadrenergics. Guanadrel (38) and guanethidine (39) are orally active antihypertensives, which are taken up into adrenergic neurons, where they bind to the storage vesicles and prevent release of neurotransmitter in response to a neuronal impulse, which results in generalized decrease in sympathetic tone. These drugs are available but seldom used.



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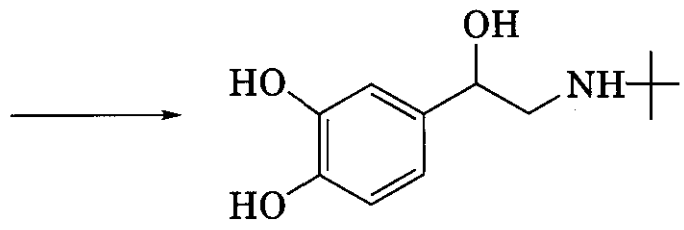


(66)

Reserpine (60)is an old and historically important drug that affects the storage and release of norepinephrine. Reserpine is one of several indole alkaloids isolated from the roots of Rauwolfia serpentina, a plant whose roots were used in India for centuries as a remedy for snakebites and as a sedative. Reserpine acts to deplete the adrenergic neurons of their stores of norepinephrine by inhibiting the active transport Mg-ATPase responsible for sequestering norepinephrine and dopamine within the storage vesicles. Monoamine oxidase (MAO) destroys the norepinephrine and dopamine that are not sequestered in vesicles. As a result the storage vesicles contain little neurotransmitter; adrenergic transmission is dramatically inhibited; and sympathetic tone is decreased, thus leading to vasodilation. Agents with fewer side effects have largely replaced reserpine in clinical use.

Metyrosine (23, a-methyl-L-tyrosine), a norepinephrine biosynthesis inhibitor, is in limited clinical use to help control hypertensive episodes and other symptoms of catecholamine overproduction in patients with the rare adrenal tumor pheochromocytoma (10). Metyrosine, a competitive inhibitor of tyrosine hydroxylase, inhibits the production of catecholamines by the tumor. Although metyrosine is useful in treating hypertension caused by excess catecholamine biosynthesis in pheochromocytoma tumors, it is not useful for treating essential hypertension.

2.1.6 Applications of Nonselective a-Antagonists. Because antagonism of a,-adrenoceptors in the peripheral vascular smooth muscle leads to vasodilation and a decrease in blood pressure attributed to a lowering of peripheral resistance, alpha-blockers have been employed as antihypertensives for decades. However, nonselective a-blockers such as phenoxybenzamine (**62**), phentolamine (**40**), and tolazoline can also increase sympathetic output through blockade of inhibitory presynaptic a,-adrenoceptors, resulting in an increase in circulating norepinephrine, which causes reflex tachycardia. Thus the use of these agents in treating most forms of hypertension has been discontinued and replaced by use of selective a,-antagonists discussed below. Current clinical use of the nonselective agents (**40**), (**41**), and (62) is primarily treatment of hypertension induced by pheochromocytoma, a tumor of the adrenal medulla, which secretes large amounts of epinephrine and norepinephrine into the circulation. Dapiprazole (61) is an ophthalmic nonselective α-antagonist applied topically to reverse mydriasis induced by other drugs and is not used to treat hypertension.

2.1.7 **Applications of Selective a,-Antagonists.** Quinazoline-selective a,-blockers doxazosin (**42**), prazosin (**43**), and terazosin (44) have replaced the nonselective a-antagonists in clinical use as antihypertensives. Their ability to dilate peripheral vasculature has also made these drugs useful in treating Raynaud's syndrome. The a,-selective agents have a favorable effect on lipid profiles and decrease low density lipoproteins (LDL) and triglycerides, and increase high density lipoproteins (HDL).

Contraction of the smooth muscle of the prostate gland, prostatic urethra, and bladder neck is also mediated by α_1-adrenoceptors, with a„ being predominant, and blockade of these receptors relaxes the tissue. For this reason the quinazoline a,-antagonists doxazosin (**42**), prazosin (**43**), and terazosin (44) have also found use in treatment of benign prostatic hyperplasia (BPH). However, prazosin,

doxazosin, and terazosin show no significant selectivity for any of the three known a,-adrenoceptor subtypes, a„, a„, and a„ (11). The structurally unrelated phenylethylamine α_1-antagonist tamsulosin (24) is many fold more selective for a„-receptors than for the other α_1-adrencoceptors. Tamsulosin is employed only for treatment of BPH, given that it has little effect on the α_{1B}- and α_{1D}-adrenoceptors, which predominate in the vascular bed (12) and have little effect on blood pressure (13).

2.1.8 Applications of β-Antagonists. p-Antagonists are among the most widely employed antihypertensives and are also considered the first-line treatment for glaucoma. There are 16 β-blockers listed in Table 1.1 and 15 of them are in the chemical class of aryloxypropanolamines. Only sotalol (**25**) is a phenylethylamine. Acebutolol (**45**), atenolol (**46**), bisoprolol (**48**), metoprolol (**53**), nadolol (**54**), penbutolol (**55**), pindolol (**56**), and propranolol (**57**) are used to treat hypertension but not glaucoma. Betaxolol (**47**), carteolol (**49**), and timolol (**58**) are used both systemically to treat hypertension and topically to treat glaucoma. Levobetaxolol [*S*-(−)-(**47**)], levobunolol (**51**), and metipranolol (52) are employed only in treating glaucoma. Betaxolol (racemic 47) is available in both oral and ophthalmic dosage forms for treating hypertension and glaucoma, respectively, but levobetaxolol, the enantiomerically pure *S*-(−)-stereoisomer is currently available only in an ophthalmic dosage form. Esmolol (50) is a very short acting β-blocker administered intravenously for acute control of hypertension or certain supraventricular arrhythmias during surgery. Sotalol (**25**) is a nonselective β-blocker used to treat ventricular and supraventricular arrhythmias not employed as an antihypertensive or antiglaucoma agent. β-Antagonists must be used with caution in patients with asthma and other reactive pulmonary diseases because blockade of β_2-adrenoceptors may exacerbate the lung condition. Even the agents listed as being β_1-selective have some level of β_2-blocking activity at higher therapeutic doses. Betaxolol is the most β_1-selective of the currently available agents.

2.1.9 Applications of α/β-Antagonists. Carvedilol (**59**), an aryloxypropanolamine, has both a- and β-antagonist properties and is used both as an antihypertensive and to treat cardiac failure. Both enantiomers have selective a,-antagonist properties but most of the β-antagonism is attributable to the *S*-(−) isomer. Labetalol (**26**) is also an antihypertensive with both selective a,-antagonist properties and nonselective β-antagonism. Labetalol is an older drug than carvedilol and is not as potent as carvedilol, particularly as a β-antagonist.

21.10 Applications of Agonists/Antagonists. Dobutamine (27) is a positive inotropic agent administered intravenously for congestive heart failure. The (+)-isomer has both a and β agonist effects, whereas the (−)-isomer is an α-antagonist but a β-agonist like the enantiomer. The β-stimulatory effects predominate as the a-effects cancel. As a catechol it has no oral activity and even given intravenously has a half-life of only 2 min. Isoxsuprine (28) is an agent with a-antagonist and β-agonist properties, which has been used for peripheral and cerebral vascular insufficiency and for inhibition of premature labor. Isoxsuprine is seldom used any more.

2.2 Absorption, Distribution, Metabolism, and Elimination

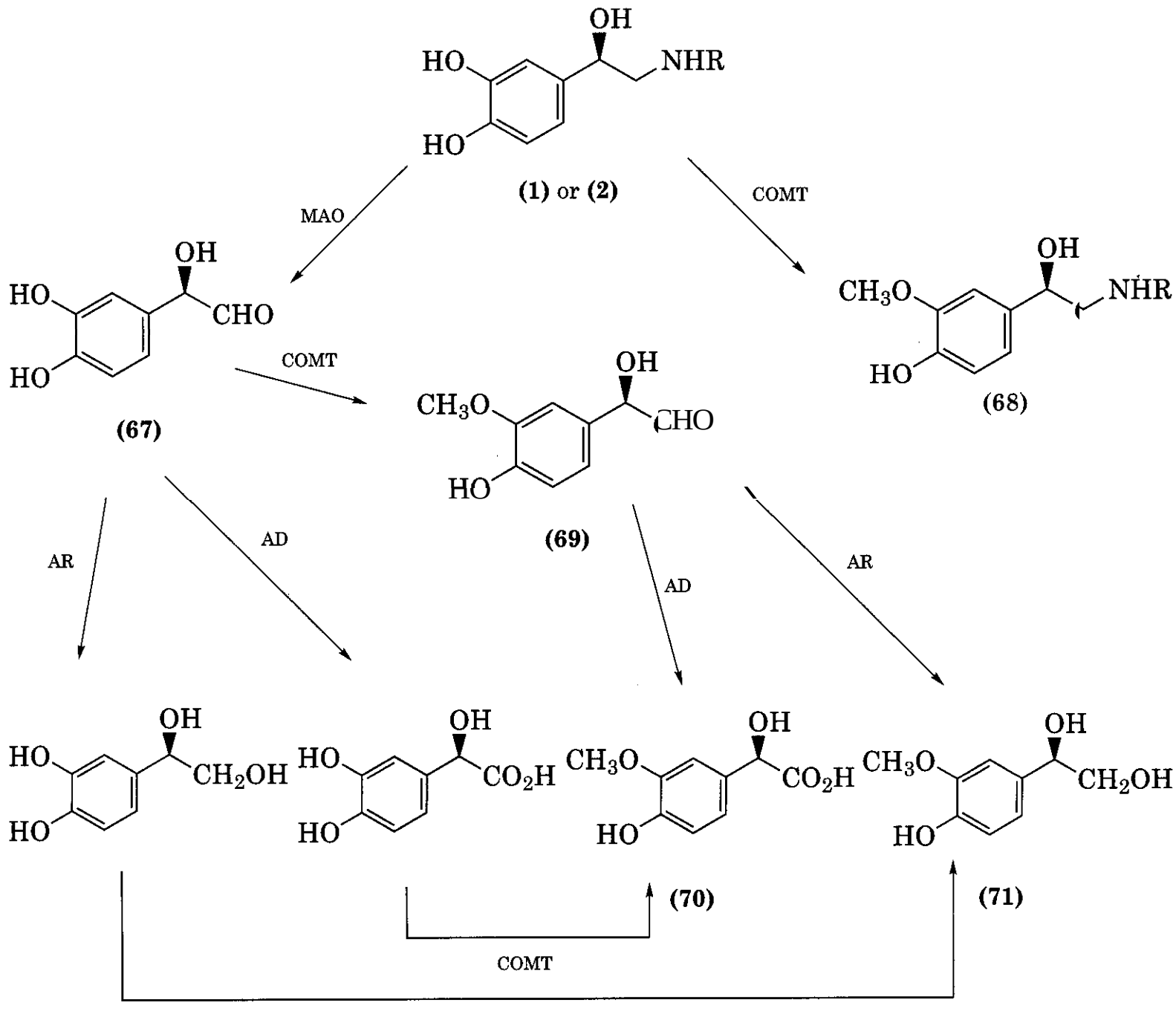
Because of the large numbers of chemicals acting as either adrenergics or adrenergic-blocking drugs, only representative examples will be given and limited to metabolites identified in humans. Because drugs with similar structures are often metabolized by similar routes, the examples chosen are representative of each structural class. Although it contains no structural details of metabolic pathways, ***Drug*** *Facts* and ***Comparisons*** (14) is an outstanding comprehensive compilation of pharmacokinetic parameters such as absorption, duration of action, and routes of elimination for drugs approved by the FDA for use in the United States.

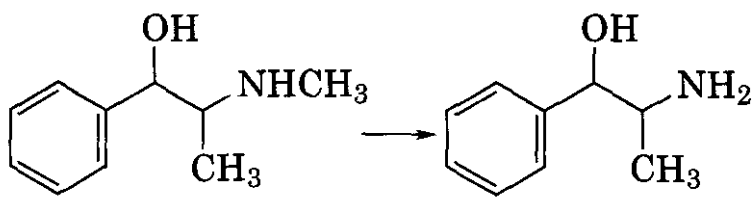
2.2.1 Metabolism of Representative Phenylethylamines. Norepinephrine **(1)** and epinephrine (2) are both substrates for MAO,

which oxidatively deaminates the side chain of either to form the same product DOPGAL (**67**), and for catechol-*O*-methyltransferase (COMT), which methylates the 3'-phenolic OH of each to form (68). Metabolite (68) is subsequently oxidized by MAO to form aldehyde (**69**), and aldehyde (68) may be methylated by COMT to also form (**69**). This aldehyde may then be either oxidized by aldehyde dehydrogenase (AD) to (70) or reduced by aldehyde reductase to alcohol (**71**). Alternate routes to (70) and (71) from (67) are also shown. Several of these metabolites are excreted in the urine as sulfate and glucuronide conjugates (15). As previously mentioned, neither (**1**) nor (2) is orally active because of extensive first-pass metabolism by COMT, and both have short durations of action because of rapid metabolic deactivation by the routes shown. Any catechol-containing drug will also likely be subject to metabolism by COMT.

Ephedrine (**5**), a close structural analog of (**2**), having no substituents on the phenyl ring, is well absorbed after an oral dose and over half the dose is eliminated unchanged in the urine. The remainder of the dose is largely desmethylephedrine (**72**), deamination product (**73**), and small amounts of benzoic acid and its conjugates (**16**). No aromatic ring-hydroxylation products were detected. This is in marked contrast to the case with amphetamine (**3**), in which ring-hydroxylated products are major metabolites.

Albuterol (**13**) is not subject to metabolism by COMT and is orally active but does have a 4'- OH group subject to conjugation. The major metabolite of albuterol (**13**) is the 4'-0-sulfate (74) (17). The sulfation reaction is ste-





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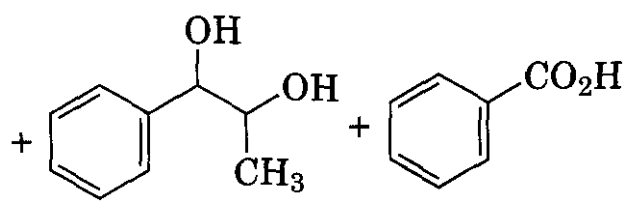

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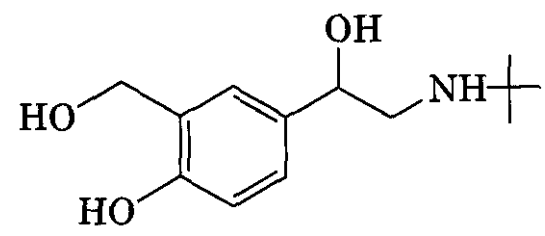

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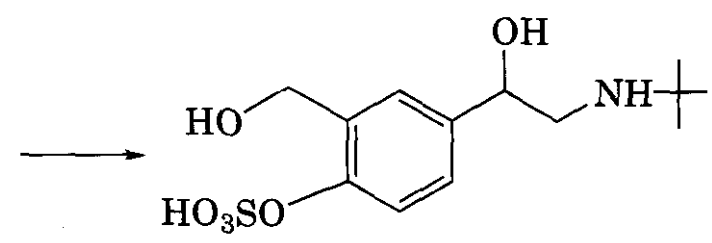


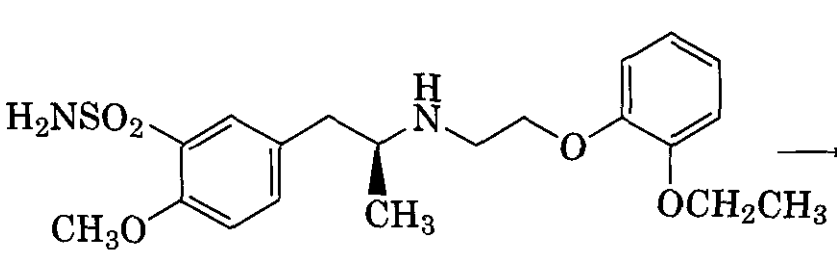
(74)

reoselective for the active R-(−)-isomer (18–20), resulting in higher plasma levels of the less active S-(+)-isomer after oral administration or swallowing of inhaled dosages.

Tamsulosin (24) is metabolized by CYP3A4 to both the phenolic oxidation product (75) and deaminated metabolite (76) and their conjugation products (21–23). The other products generated from the remainder of the drug molecule during formation of (76) were not explicitly identified. Tamsulosin is well absorbed orally and extensively metabolized. Less than 10% excreted unchanged in urine.

2.2.2 Metabolism of Representative Imidazolines and Cuanidines. In humans, clonidine (**35**) is excreted about 50% unchanged in the urine and the remainder oxidized by the liver on both the phenyl ring and imidazoline ring to (**77**), (**78**), and (**79**). Oxidation of the imidazoline ring presumably leads to the ring-opened derivatives (80) and (**81**). All metabolites are inactive but do not appear to be further conjugated.

In contrast, less than 2% of guanabenz (**36**), a ring-opened analog of (**35**), is excreted unchanged in the urine (**24**). The major metabolite (35%) is the 4-hydroxylated compound (82) and its conjugates, whereas guanabenz-N-glucuronide accounts for about 6%. Also identified were 2,6-dichlorobenzyl alcohol (**83**) (as conjugates) and the *Z*-isomer of



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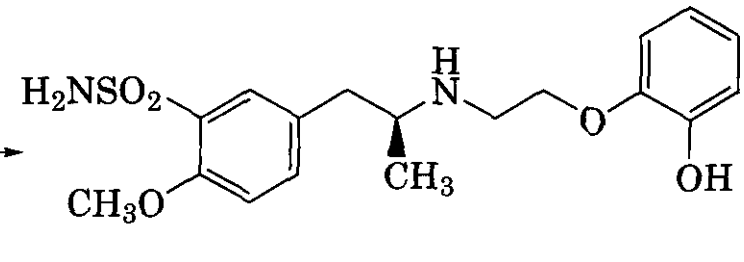

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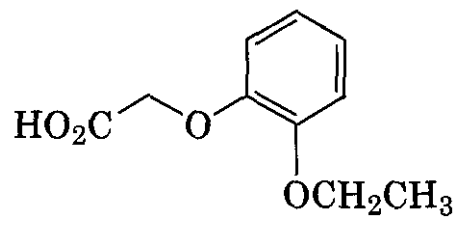

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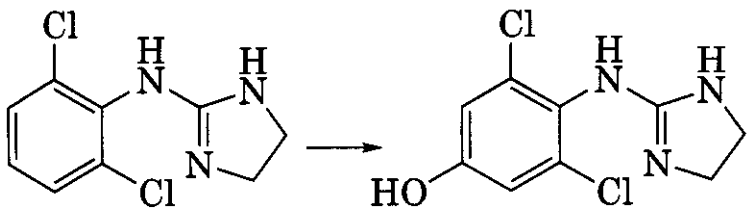

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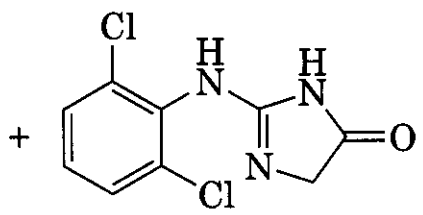

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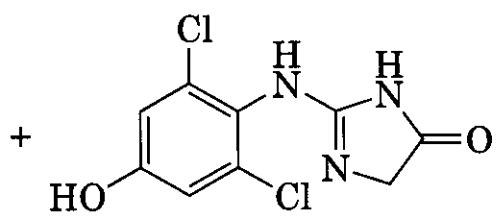

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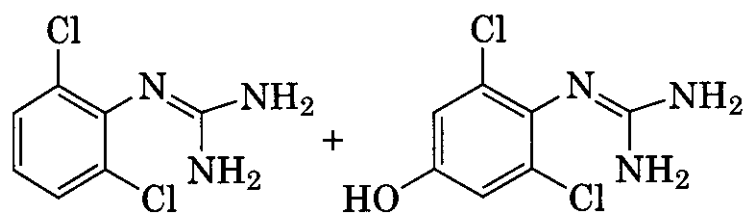


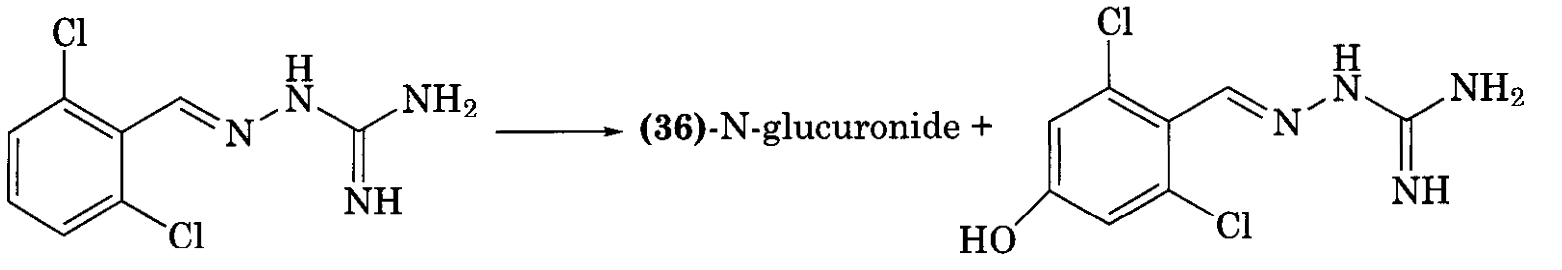
(80) (81)

guanabenz. About 15 other trace metabolites were detected by chromatography but not identified.

2.2.3 Metabolism of Representative Quinazolines. Terazosin (46) is completely absorbed, with little or no first-pass metabolism, and about 38% of administered terazosin is eliminated unchanged in urine and feces. The remainder is metabolized by hydrolysis of the amide bond to afford (84) and by *O*-demethylation to form the 6- and 7-*O*-demethyl metabolites (85) and (**86**), respectively (25). Diamine (87) has also been identified as a minor metabolite of terazosin, probably arising from oxidation and hydrolysis of the piperazine ring, although the intermediate products have not been identified.

Doxazosin (42) is well absorbed, with 60% bioavailability, but only about 5% is excreted unchanged. The major routes of metabolism are, like terazosin, 6- and 7-*O*-demethylation to afford (88) and (**89**), respectively (**26**). Hydroxylation at 6' and 7', to form (90) and (**91**), forms the other two identified metabolites.

2.2.4 Metabolism of Representative Aryloxypropanolamines. Propranolol (**57**), the first successful β-blocker, is also the most lipophilic, with an octanol/water partition coefficient of 20.2 (**27**), and is extensively metabolized. At least 20 metabolites of propranolol have been demonstrated (**28**), only a few of which are shown. The 4'-hydroxy metabolite (92) is equipotent with the parent compound (29). CYP2D6 is responsible for the 4'-hydroxylation and CYP1A2 for oxidative removal of the isopropyl group from the nitrogen to form (93) (30). The metabolites as well as the parent drug are extensively conjugated



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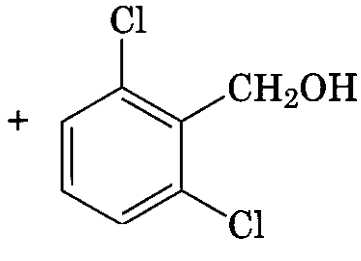

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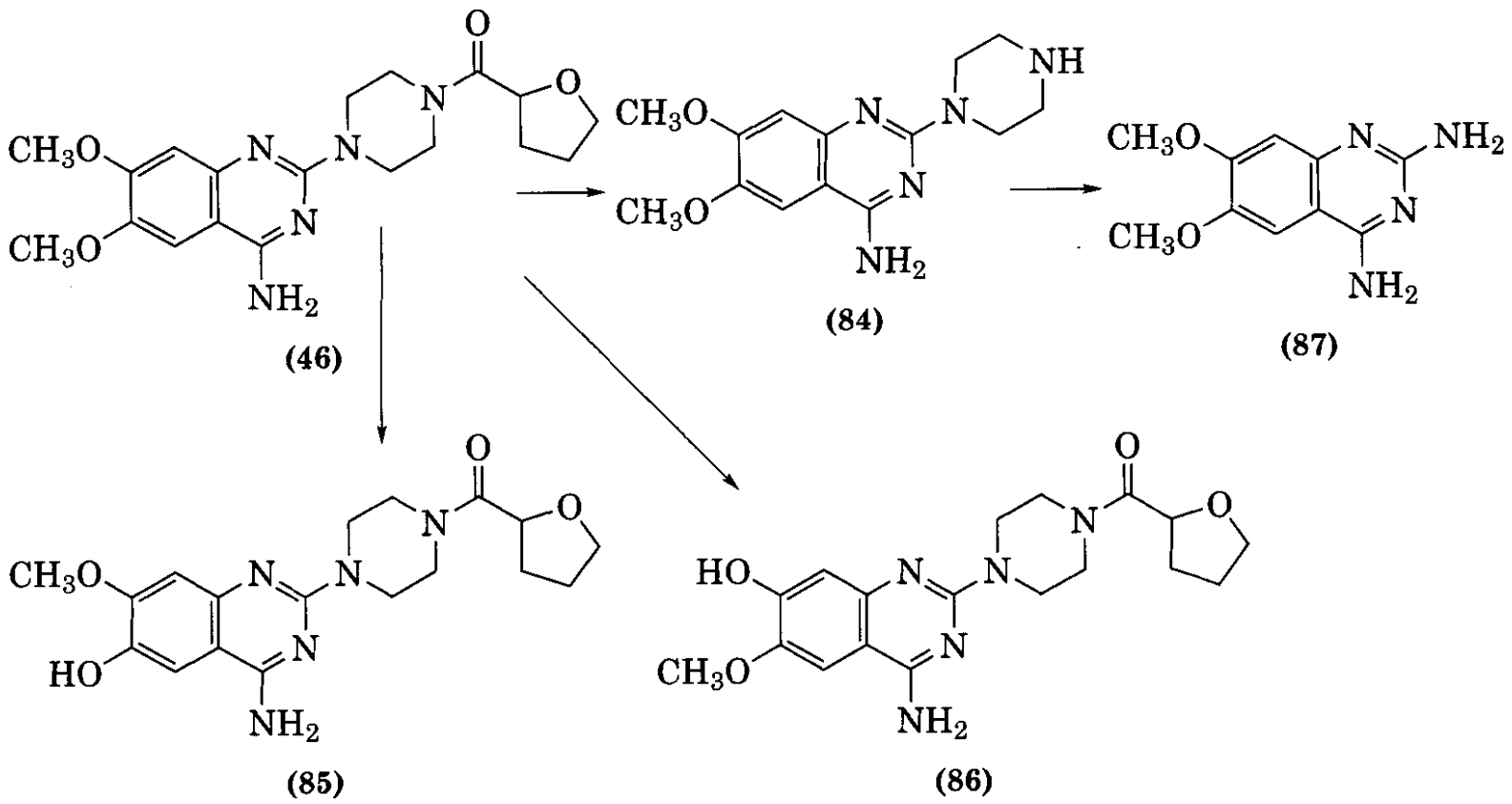

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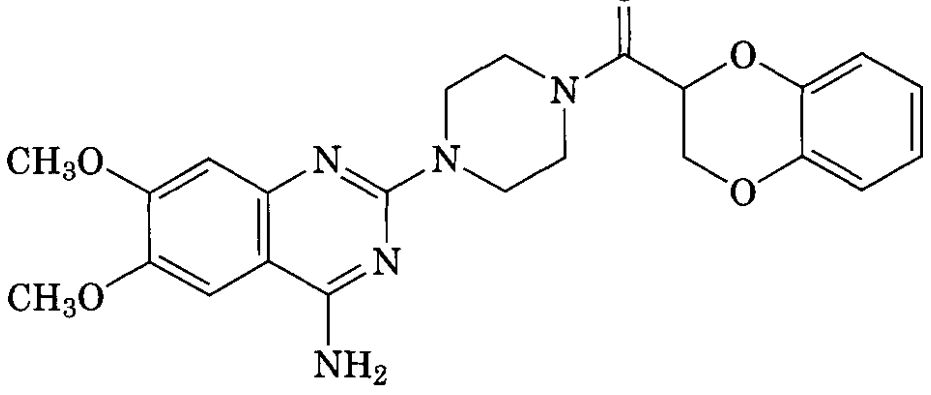

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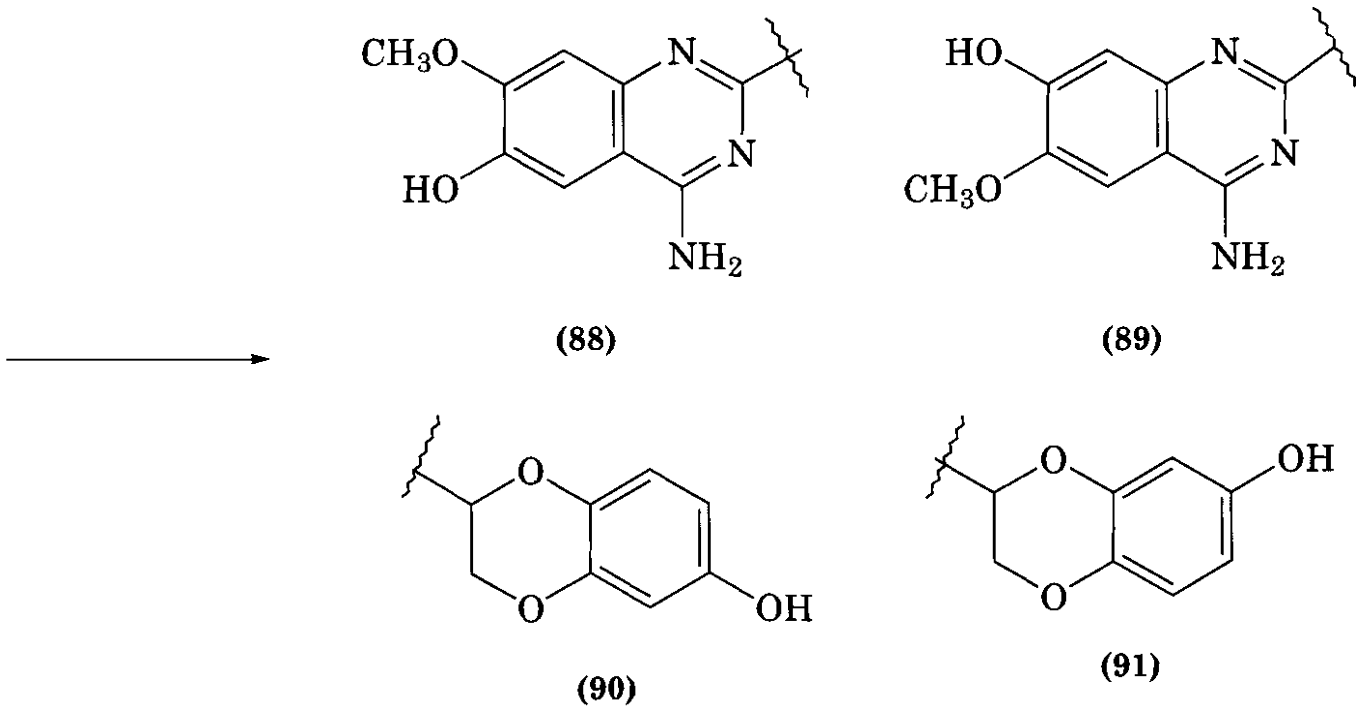
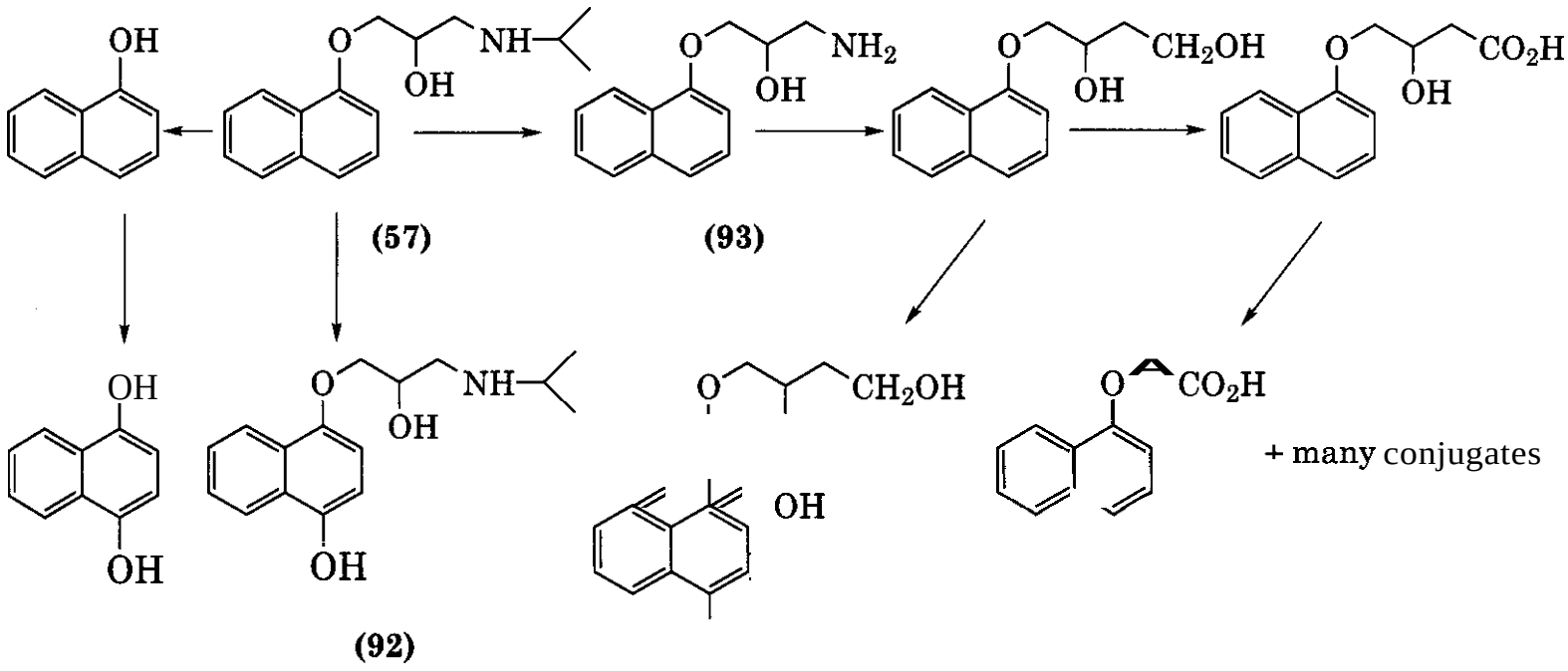
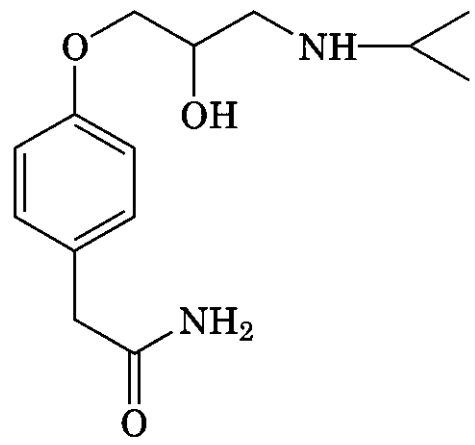


(88) (89) (90) (91)



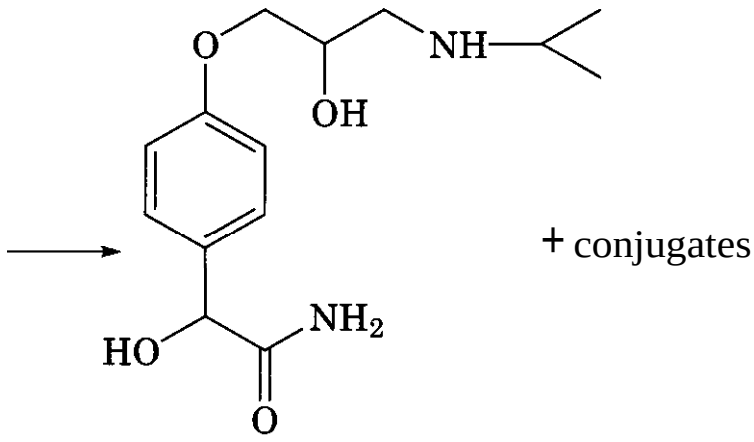
as sulfates and glucuronides. The high lipophilicity of propranolol provides ready passage across the blood-brain barrier and leads to the significant CNS effects of propranolol (27).

On the other hand, atenolol (46), with an octanol/buffer partition coefficient of 0.02 (27), does not cross the blood-brain barrier to any significant extent and is eliminated almost entirely as the unchanged parent drug in the urine and feces. Very small amounts of hydroxylated metabolite (94) and its conjugates have been identified (31), but well over 90% of atenolol is eliminated unchanged.



(46)

Metoprolol (53) is cleared principally by hepatic metabolism and is only 50% bioavailable because of extensive first-pass metabolism. The major metabolite (65%) is the carboxylic acid (95), produced by CYP2D6 *O*-demethylation followed by further oxidation (32–34). Benzylic oxidation CYP2D6 forms an active metabolite (96), which retains beta-blocking activity (35). The N-dealkylated product is a minor metabolite.



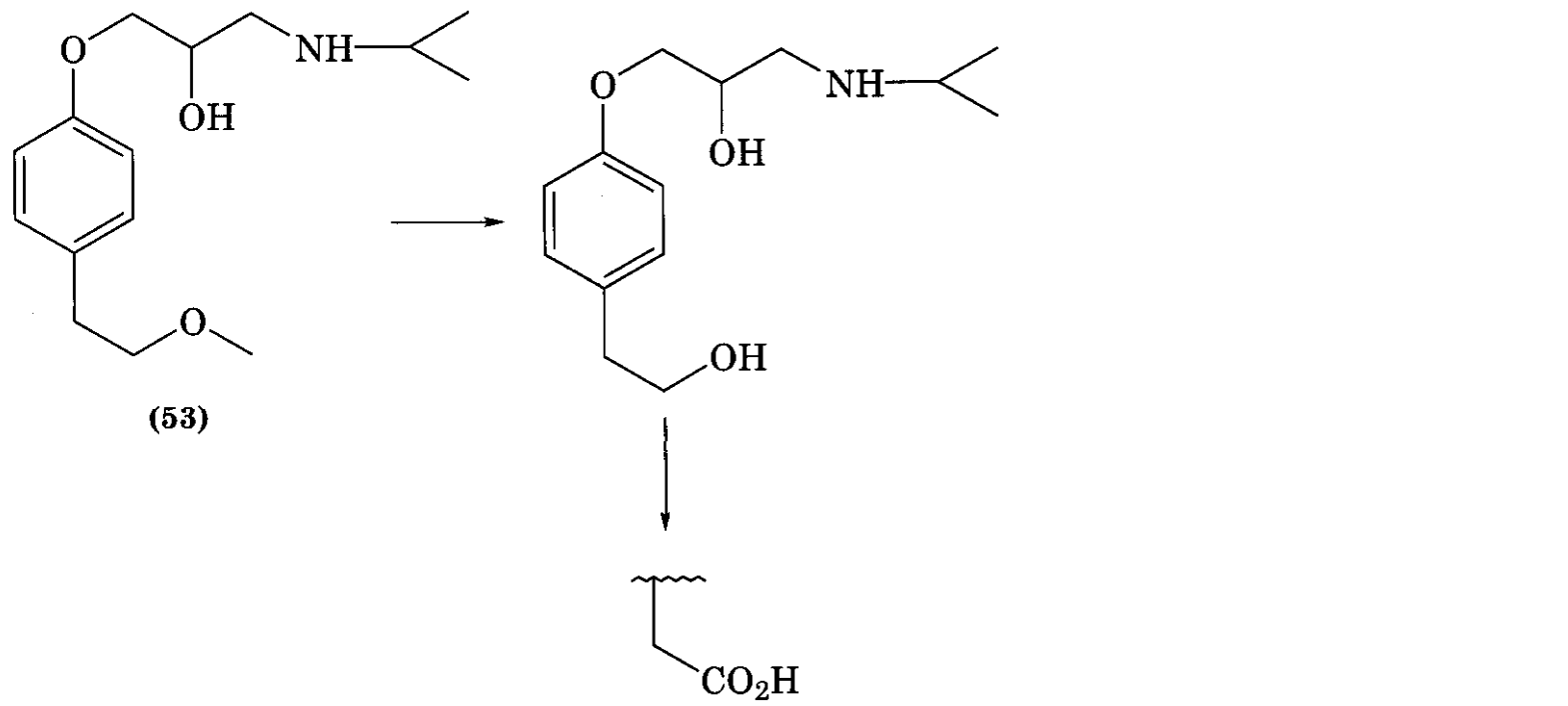
(94)

3 PHYSIOLOGY AND PHARMACOLOGY

The physiology and pharmacology of adrenergic and adrenergic-blocking drugs are well covered in standard pharmacology textbooks (36, 37).

3.1 Physiological Significance

Adrenergic and adrenergic-blocking drugs act on effector cells through receptors that are normally activated by the neurotransmitter norepinephrine (1, noradrenaline), or they may act on the neurons that release the neurotransmitter. The term ***adrenergic*** stems from the discovery early in the twentieth century that administration of the adrenal medullar hormone adrenaline (epinephrine) had specific effects on selected organs and tissues similar to the effects produced by stimulation of the sympathetic nervous system, which was



originally defined anatomically (38). Today the terms ***adrenergic nervous system*** and ***sympathetic nervous system*** are generally used interchangeably. The sympathetic nervous system is a division of the autonomic nervous system, which innervates organs such as the heart, lungs, blood vessels, glands, and smooth muscle in various tissues and regulates functions not normally under voluntary control. The effects of the sympathetic stimulation on a few organs and tissues of particular relevance to current pharmaceutical interventions are shown in Table **1.7** (39, 40). Excellent overviews of the adrenergic nervous system and its role in control of human physiology are provided in Katzung **(39)** and Hoffman and Palmer **(40)**.

3.2 Biosynthesis, Storage, and Release of Norepinephrine

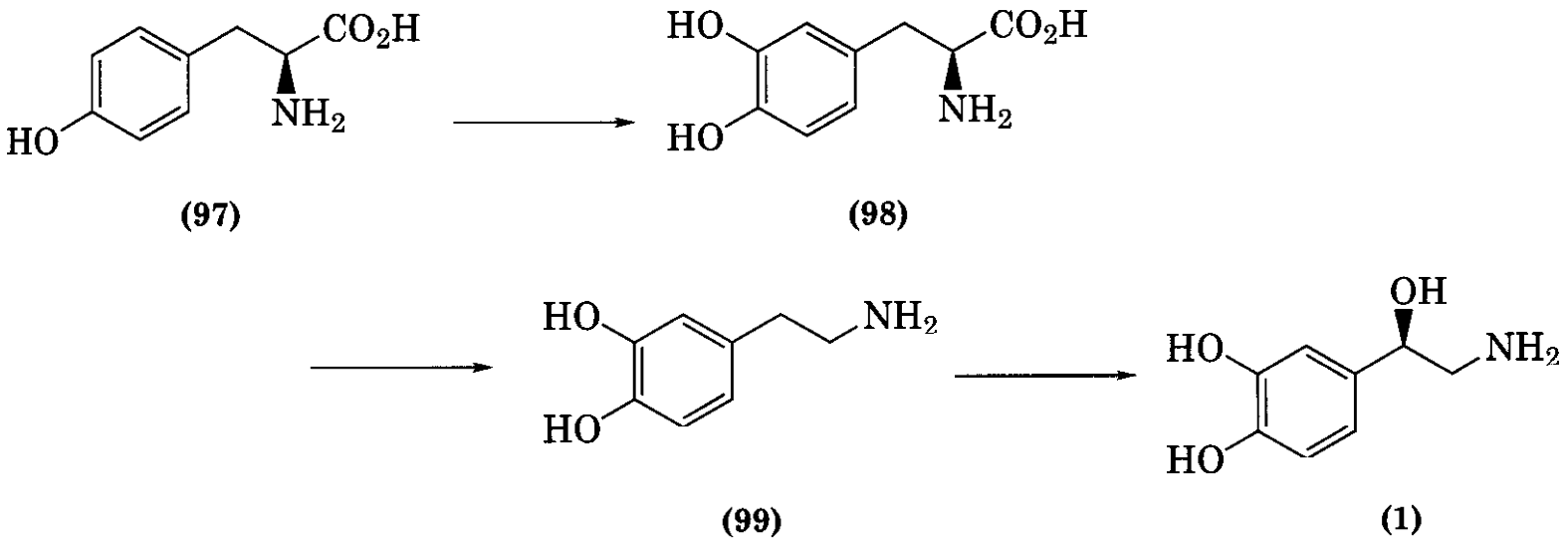
Biosynthesis of norepinephrine takes place within adrenergic neurons near the terminus of the axon near the junction with the effector cell. The amino acid L-tyrosine **(97)** is actively transported into the neuron cell (41), where the cytoplasmic enzyme tyrosine hydroxylase (tyrosine-3-monooxygenase) oxidizes the 3′-position to form the catechol-amino-acid L-dopa **(98)** in the rate-limiting step in norepinephrine biosynthesis (42). L-Dopa is decarboxylated to dopamine **(99)** by aromatic-L-amino acid decarboxylase, another cytoplasmic enzyme. Aromatic-L-aminoacid decarboxylase is more commonly known as dopa decarboxylase. Dopamine is then taken up by active transport into storage vesicles or granules located near the terminus of the adrenergic neuron. Within these vesicles, the enzyme dopamine β-hydroxylase stereospecifically introduces a hydroxyl group in the R absolute configuration on the carbon atom beta to the amino group to generate the neurotransmitter norepinephrine **(1)**. Norepinephrine is stored in the vesicles in a 4:1 complex, with adenosine

Table 1.7 Selected Tissue Response to Adrenergic Stimulation

Tissue	Principal Adrenergic Receptor	Effect
Heart	β_1 (minor β_2, β_3)	Increased rate and force
Blood vessels		
Skin, mucosa, visera	α_1	Constriction
Skeletal muscle	β_2	Dilation
Renal	α_1	Constriction
Lungs (bronchial muscle)	β_2	Relaxation
Eye		
Radial muscle, iris	α_1	Contraction (pupilary dilation)
Ciliary muscle	β_2	Relaxation
Uterus (pregnant)	β_2	Relaxation
Liver	α_1, β_2	Glycogenolysis, gluconeogenesis
Fat cells	β_3	Lipolysis
Kidney		
Renin release	β_1	Increased renin secretion
	minor α_1	Decreased renin secretion

triphosphate (ATP) in such quantities that each vesicle in a peripheral adrenergic neuron contains between 6000 and 15,000 molecules of norepinephrine (43). The pathway for epinephrine (2) biosynthesis in the adrenal medulla is the same, with the additional step of conversion of (1) to (2) by phenylethanolamine-N-methyltransferase.

Norepinephrine remains in the vesicles until it is released into the synapse during signal transduction. A wave of depolarization reaching the terminus of an adrenergic neuron triggers the transient opening of voltage-dependent calcium channels, causing an influx of calcium ions. This influx of calcium ions triggers fusion of the storage vesicles with the neuronal cell membrane, spilling the norepinephrine and other contents of the vesicles into the synapse through exocytosis. A summary view of the events involved in norepinephrine biosynthesis, release, and fate is given in Fig. 1.1. After release, norepinephrine diffuses through the intercellular space to bind reversibly to adrenergic receptors (alpha or beta) on the effector cell, triggering a biochemical cascade that results in a physiologic response by the effector cell. In addition to the receptors on effector cells, there are also adrenoceptors that respond to norepinephrine (a_2-receptors) or epinephrine (β_2-receptors) on the presynaptic neuron, which modulate the release of additional neurotransmitter into the synapse. Activation of presynaptic α_2-adrenoceptors by (1) inhibits the release of additional (1), whereas stimulation of presynaptic β_2-adrenoceptors by (2) enhances the release of (1), thus increasing overall sympathetic activation. Removal of norepinephrine from the synapse is accomplished by two mechanisms, reuptake and metabolism, to in-



(97) (98) (99) (1)

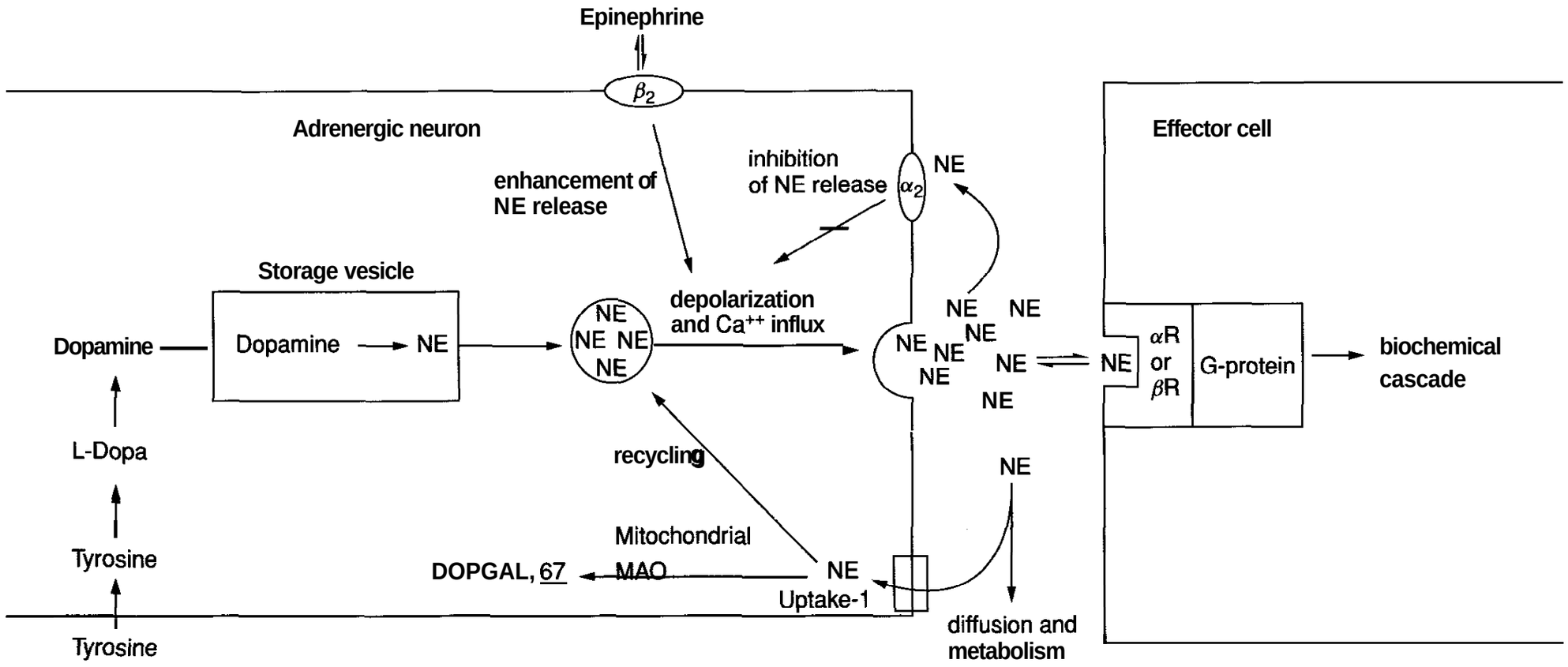


Figure 1.1. Diagram of synapse between an adrenergic neuron and its effector cell. NE, norepinephrine; αR, α-adrenoceptor; βR, β-adrenoceptor.

active compounds. The most important of these mechanisms is transmitter recycling through active transport uptake into the presynaptic neuron. This process, called uptake-1, is efficient and, in some tissues, up to 95% of released norepinephrine is removed from the synapse by this mechanism (44). Part of the norepinephrine taken into the presynaptic neuron by uptake-1 is metabolized by MAO through the same processes discussed earlier under norepinephrine metabolism, but most is sequestered in the storage vesicles to be used again as neurotransmitter. This uptake mechanism is not specific for (1) and a number of drugs are substrates for the uptake mechanism and others inhibit reuptake, leading to increased adrenergic stimulation. A less efficient uptake process, uptake-2, operates in a variety of other cell types but only in the presence of high concentrations of norepinephrine. That portion of released norepinephrine that escapes uptake-1 diffuses out of the synapse and is metabolized in extraneuronal sites by COMT. MAO present at extraneuronal sites, principally the liver and blood platelets, also metabolizes norepinephrine.

3.3 Effector Mechanisms of Adrenergic Receptors

Adrenoceptors are proteins embedded in the cell membrane that are coupled through a G-protein to effector mechanisms that translate conformational changes caused by activation of the receptor into a biochemical event within the cell. All of the β-adrenoceptors are coupled through specific G-proteins (G_s) to the activation of adenylyl cyclase (45). When the receptor is stimulated by an agonist, adenylyl cyclase is activated to catalyze conversion of ATP to cyclic-adenosine monophosphate (cAMP), which diffuses through the cell for at least short distances to modulate biochemical events remote from the synaptic cleft. Modulation of biochemical events by cAMP includes a phosphorylation cascade of other proteins. cAMP is rapidly deactivated by hydrolysis of the phosphodiester bond by the enzyme phosphodiesterase. The a,-receptor may use more than one effector system, depending on the location of the receptor; however, to date the best understood effector system of the a,-receptor appears to be similar to that of the β-receptors, except that linkage through a G-protein (G_i) leads to inhibition of adenylyl cyclase instead of activation.

The a,-adrenoreceptor, on the other hand, is linked through yet another G-protein to a complex series of events involving hydrolysis of polyphosphatidylinositol (46). The first event set in motion by activation of the α_1-receptor is activation of the enzyme phospholipase C, which catalyzes the hydrolysis of phosphatidylinositol-4,5-biphosphate (PIP,). This hydrolysis yields two products, each of which has biologic activity as second messengers of the a,-receptor. These are 1,2-diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP,). IP, causes the release of calcium ions from intracellular storage sites in the endoplasmic reticulum, resulting in an increase in free intracellular calcium levels. Increased free intracellular calcium is correlated with smooth muscle contraction. DAG activates cytosolic protein kinase C, which may induce slowly developing contractions of vascular smooth muscle. The end result of a complex series of protein interactions triggered by agonist binding to the α_1-adrenoceptor includes increased intracellular free calcium, which leads to smooth muscle contraction. Because smooth muscles of the wall of the peripheral vascular bed are innervated by a,-receptors, stimulation leads to vascular constriction and an increase in blood pressure.

3.4 Characterization of Adrenergic Receptor Subtypes

The discovery of subclasses of adrenergic receptors and the ability of relatively small molecule drugs to stimulate differentially or block these receptors represented a major advance in several areas of pharmacotherapeutics. An excellent review of the development of adrenoceptor classifications is available in Hiebel et al. (47).

The adrenoceptors, both alpha and beta, are members of a receptor superfamily of membrane-spanning proteins, including muscarine, serotonin, and dopamine receptors, that are coupled to intracellular GTP-binding proteins (G-proteins), which determine the cellular response to receptor activation (48). All G-protein–coupled receptors exhibit a common motif of a single polypeptide chain

that is looped back and forth through the cell membrane seven times, with an extracellular N-terminus and intracellular C-terminus. One of the most thoroughly studied of these receptors is the human β_2-adrenoreceptor (49). The seven transmembrane domains, TMD1-TMD7, are composed primarily of lipophilic amino acids arranged in a-helices connected by regions of hydrophilic amino acids. The hydrophilic regions form loops on the intracellular and extracellular faces of the membrane. In all of the adrenoceptors the agonist/antagonist recognition site is located within the membrane-bound portion of the receptor. This binding site is within a pocket formed by the membrane-spanning regions of the peptide. All of the adrenoceptors are coupled to their G-protein through reversible binding interactions with the third intracellular loop of the receptor protein.

Binding studies with selectively mutated β_2-receptors have provided strong evidence for binding interactions between agonist functional groups and specific residues in the transmembrane domains of adrenoceptors (50–52). Such studies indicate that Asp_{113} in transmembrane domain 3 (TMD3) of the β_2-receptor is the acidic residue that forms a bond, presumably ionic or a salt bridge, with the positively charged amino group of catecholamine agonists. An aspartic acid residue is also found in a comparable position in all of the other adrenoceptors as well as other known G-protein–coupled receptors that bind substrates having positively charged nitrogens in their structures. Elegant studies with mutated receptors and analogs of isoproterenol demonstrated that Ser_{204} and Ser_{207} of TMD5 are the residues that form hydrogen bonds with the catechol hydroxyls of β_2-agonists. Furthermore, the evidence indicates that Ser_{204} interacts with the *meta* hydroxyl group of the ligand, whereas Ser_{207} interacts specifically with the *para* hydroxyl group. Serine residues are found in corresponding positions in the fifth transmembrane domain of the other known adrenoceptors. Evidence indicates that the phenylalanine residue of TMD6 is also involved in ligand-receptor bonding with the catechol ring. Structural differences exist among the various adrenoceptors with regard to their primary structure, including the actual peptide sequence and length. Each of the adrenoceptors is encoded on a distinct gene, and this information was considered crucial to the proof that each adrenoreceptor is indeed distinct, although related. The amino acids that make up the seven transmembrane regions are highly conserved among the various adrenoreceptors, but the hydrophilic portions are quite variable. The largest differences occur in the third intracellular loop connecting TMD5 and TMD6, which is the site of linkage between the receptor and its associated G-protein. Sequences and binding specificities have been reported for numerous α- and β-adrenoceptor subtypes (47, 53–56). For purposes of drug design and therapeutic targeting, the most critical receptors are the a_{1} on prostate smooth muscle, a_{2} on vascular smooth muscle and in the kidney, a_{2} in the CNS, β_1 in heart, β_2 in bronchial smooth muscle, and β_3 in adipose tissue.

4 HISTORY

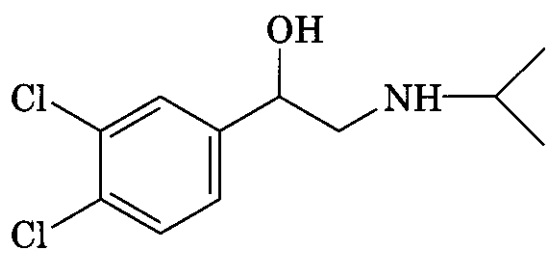
In 1895 Oliver and Schafer reported (57) that adrenal gland extracts caused vasoconstriction and dramatic increases in blood pressure. Shortly thereafter various preparations of crude adrenal extracts were being marketed largely to staunch bleeding from cuts and abrasions. In 1899 Abel reported (58) isolation of a partially purified sample of the active constituent (**2**), which he named epinephrine. Shortly thereafter von Fürth (59) employed an alternative procedure to isolate another impure sample of (**2**), which he named suprarenin, claiming it to be a different substance than that isolated by Abel. The pure hormone (2) was finally obtained in 1901 by both Takamine (60) and Aldrich (61). Takamine gave (2) yet a third name, adrenalin. Although the chemical structure was still not definitively known, a pure preparation of (2) was first marketed by Parke, Davis & Co. under the trade name Adrenaline (62, 63). Adrenaline eventually became the generic name employed outside the United States, whereas epinephrine became the U.S. approved name. By 1903 Pauly (64) had demonstrated that "adrenaline" was levorotatory and proposed two possible structures consistent with the available

data. The structure of racemic (2) was conclusively proved through nearly simultaneous synthesis by Stolz at Farbwerke Hoechst (65) and Dakin at the University of Leeds (66), but it had only one half the activity of the natural levorotatory isomer (67). The racemate was resolved by Flacher in 1908 (68).

The earliest major clinical application of (2) was the report in 1900 (69) of the utility of injected adrenal extracts in treating asthma attacks, followed in 1903 by a report (70) of the use of purified (2) for the same purpose. Injected epinephrine rapidly became the standard therapy for treatment of acute asthma attacks. A nasal spray containing epinephrine was available by 1911 and administration through an inhaler was reported in 1929. Also, early in the 1900s Hoechst employed the vasoconstrictor properties of epinephrine to prolong the duration of action of their newly developed local anesthetic procaine (63).

It had been recognized early on (71) that there were similarities between the effects of administration adrenal gland extracts and stimulation of the sympathetic nervous system. Elliot (72) suggested that adrenaline might be released by sympathetic nerve stimulation and over the years the term ***adrenergic nerves*** became effectively synonymous with ***sympathetic nerves.*** In 1910 Barger and Dale (73) reported a detailed structure-activity relationship study of epinephrine analogs and introduced the term ***sympathomimetic*** for chemicals that mimicked the effects of sympathetic nerve stimulation, but they also noted some important differences between the effects of administered adrenaline and stimulation of sympathetic nerves. It was not until 1946 that von Euler demonstrated that the actual neurotransmitter released at the terminus of sympathetic neurons was norepinephrine (1) rather than epinephrine (2) (74). In 1947 compound (**17**), the N-isopropyl analog of (**1**) and (**2**), was reported to possess bronchodilating effects similar to those of (2) but lacking its dangerous pressor effects. In 1951 (17) was introduced into clinical use as isoproterenol (isoprenaline) and became the drug of choice for treating asthma for two decades.

In the 1950s, dichloroisoproterenol (DCI, **100**), a derivative of isoproterenol, in which the catechol hydroxyls had been replaced by chlorines, was discovered to be a β-antagonist that blocked the effects of sympathomimetic amines on bronchodilation, uterine relaxation, and heart stimulation (75). Although DCI had no clinical utility, replacement of the 3,4-dichloro substituents with a carbon bridge to form a naphthylethanolamine derivative did afford a clinical candidate, pronethalol (**101**), introduced in 1962 only to be withdrawn in 1963 because of tumor induction in animal tests.



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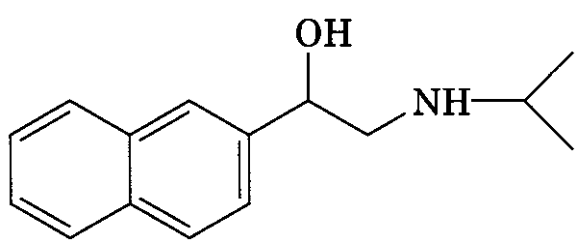


(101)

Shortly thereafter, a major innovation was introduced when it was discovered that an oxymethylene bridge, OCH_2, could be introduced into the arylethanolamine structure of pronethalol to afford propranolol (**57**), an aryloxypropanolamine and the first clinically successful β-blocker.

To clarify some of the puzzling differential effects of sympathomimetic drugs on various tissues, in 1948 Ahlquist (76) introduced the concept of two distinct types of adrenergic receptors as defined by their responses to (**1**), (**2**), and (**17**), which he called alpha receptors and beta receptors. Alpha receptors were defined as those that responded in rank order of agonist potency as (2) > (1) ≫ (17). Beta receptors were defined as those responding in potency order of (**17**) > (**2**) > (**1**). Subsequently, β-receptors were further divided into β_1-receptors, located primarily in cardiac tissue, and β_2-adrenoceptors, located in smooth muscle and other tissues, given that (**1**) and (2) are approximately equipotent at cardiac

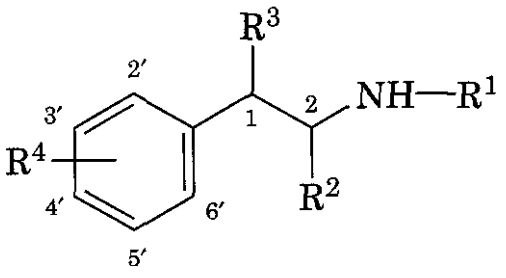
β-receptors, although (2) is 10 to 50 times more potent than (1) at most smooth muscle β-receptors (77). Alpha receptors were also subdivided into a_1 (postsynaptic) and a_2 (presynaptic) adrenoceptors (78). Development of selective agonists and antagonists for these various adrenoceptors has been thoroughly reviewed in Ruffolo et al. (79).

5 STRUCTURE-ACTIVITY RELATIONSHIPS

Comprehensive reviews of the structure-activity relationships (SAR) of agonists and antagonists of a-adrenoceptors (80) and β-adrenoceptors (81) are available, which thoroughly cover developments through the late 1980s. Only summaries of these structure-activity relationships are provided here.

5.1 Phenylethylamine Agonists

The structures of the phenylethylamine adrenergic agonists were summarized in Table 1.2. Agents of this type have been extensively



studied over the years since the discovery of the naturally occurring prototypes, epinephrine and norepinephrine, and the structural requirements, and tolerances for substitutions at each of the indicated positions have been well established and reviewed (79, 82). In general, a primary or secondary aliphatic amine separated by two carbons from a substituted benzene ring is minimally required for high agonist activity in this class. Tertiary or quaternary amines have little activity. Because of the basic amino groups, pK_a values range from about 8.5 to 10, and all of these agents are highly positively charged at physiologic pH. Most agents in this class have a hydroxyl group on C-1 of the side chain, β to the amine, as in epinephrine and norepinephrine. Given these features in common, the nature of the other substituents determines receptor selectivity and duration of action.

5.1.1 R' Substitution on the Amino Nitrogen. As R^1 is increased in size from hydrogen in norepinephrine to methyl in epinephrine to isopropyl in isoproterenol, activity at α-receptors decreases and activity at β-receptors increases. Activity at both a- and β-receptors is maximal when R^1 is methyl as in epinephrine, but a-agonist activity is dramatically decreased when R^1 is larger than methyl and is negligible when R^1 is isopropyl as in (17), leaving only β-activity. Presumably, the β-receptor has a large lipophilic binding pocket adjacent to the amine-binding aspartic acid residue, which is absent in the a-receptor. As R^1 becomes larger than butyl, affinity for α_1-receptors returns, but not intrinsic activity, which means large lipophilic groups can afford compounds with a,-blocking activity [e.g., tamsulosin (24) and labetalol (26)]. Tamsulosin (24) is more selective for a_{1A}, the a,-adrenoceptor subtype found in the prostate gland, over those found in vascular tissue. In addition, the N-substituent can also provide selectivity for different β-receptors, with a t-butyl group affording selectivity for &-receptors. For example, with all other features of the molecules being constant, (66) [the active metabolite of prodrug bitolterol (14)] is a selective β_2-agonist, whereas (17) is a general β-agonist. When considering its use as a bronchodilator, it must be recognized that a general β-agonist such as (17) has undesirable cardiac stimulatory properties (because of its β_1-activity) that are greatly diminished in a selective β_2-agonist.

5.1.2 R^2 Substitution α to the Basic Nitrogen, Carbon-2. Small alkyl groups, methyl or ethyl, may be present on the carbon adjacent to the amino nitrogen. Such substitution slows metabolism by MAO but has little overall effect on duration of action of catechols because they remain substrates for COMT. Resistance to MAO activity is more important in noncatechol indirect-acting phenylethylamines. An ethyl group in this position diminishes a-activity far more than β-activity, and is present in isoetharine (16). Substitution on this carbon introduces an asymmetric center,

producing pairs of diastereomers when an OH group is present on C-1. These stereoisomers can have significantly different biologic and chemical properties. For example, maximal direct activity in the stereoisomers of α-methylnorepinephrine resides in the *erythro* stereoisomer (65), with the (1*R*,2*S*) absolute configuration (83), which is the active metabolite of the prodrug methyldopa (12) (84). The configuration of C-2 has a great influence on receptor binding because the (1*R*,2*R*) diastereomer of a-methylnorepinephrine has primarily indirect activity, even though the absolute configuration of the hydroxyl-bearing C-1 is the same as that in norepinephrine. In addition, with respect to a-activity, this additional methyl group also makes the direct-acting (1*R*,2*S*) isomer of a-methylnorepinephrine selective for a,-adrenoceptors over a,-adrenoceptors, affording the central antihypertensive properties of methyldopa.

5.1.3 R^3 Substitution on Carbon-1. In the phenylethyamine series, a hydroxyl group at this position in the R absolute configuration is preferred for maximum direct agonist activity on both a- and β-adrenoceptors. If a hydroxyl is present in the *S* absolute configuration, the activity is generally the same as that of the corresponding chemical with no substituent. This is the basis for the well-known Easson-Stedman hypothesis of three-point attachment of phenylethanolamines to adrencoceptors through stereospecific bonding interactions with the basic amine, hydroxyl group, and aromatic substituents (85). A comprehesive and excellent review of the stereochemistry of adrenergic drug-receptor interactions was written by Ruffolo (86).

An example of a phenethylamine agonist lacking an OH group on C-1 is dobutamine (27), which has activity on both a- and β-receptors but, because of some unusual properties of the chiral center on R^1, the bulky nitrogen substituent, the overall pharmacologic response is that of a selective β_1-agonist (87). The (−)-isomerof dobutamine is an a,-agonist and vasopressor. The (+)-isomer is an α_1-antagonist; thus, when the racemate is used clinically, the a-effects of the enantiomers effectively cancel, leaving the β-effects to predominate. The stereochemistry of the methyl substituent does not affect the ability of the drug to bind to the a,-receptor but does affect the ability of the molecule to activate the receptor; that is, the stereochemistry of the methyl group affects intrinsic activity but not affinity. Because both stereoisomers are β-agonists, with the (+)-isomer about 10 times as potent as the (−)-isomer, the net effect is β-stimulation. Dobutamine is used as a cardiac stimulant after surgery or congestive heart failure. As a catechol, dobutamine is readily metabolized by COMT and has a short duration of action with no oral activity.

5.1.4 R^4 Substitution on the Aromatic Ring. The natural 3′,4′-dihydroxy substituted benzene ring present in norepinephrine provides excellent receptor activity for both α- and β-sites, but such catechol-containing compounds have poor oral bioavailability and short durations of action, even when administered intravenously, because they are rapidly metabolized by COMT. Alternative substitutions have been found that retain good activity but are more resistant to COMT metabolism. For example, 3′,5′-dihydroxy compounds are not good substrates for COMT and, in addition, provide selectivity for β_2-receptors. Thus, because of its ring-substitution pattern, metaproterenol (18) is an orally active bronchodilator having little of the cardiac stimulatory properties possessed by isoproterenol (17).

Other substitutions are possible that enhance oral activity and provide selective β_2-activity, such as the 3'-hydroxymethyl, 4'-hydroxy substitution pattern of albuterol (13), which is also not a substrate for COMT. A recently developed selective p,-agonist with an extended duration of action is salmeterol (21), which has the same phenyl ring substitution R^4 as that of (13)but an unusually long and lipophilic group R^1 on the nitrogen. The octanol/water partition coefficient log P for salmeterol is 3.88 vs. 0.66 for albuterol and the duration of action of salmeterol is 12 vs. 4 h for albuterol (88). There is substantial evidence that the extended duration of action is attributed to a specific binding interaction of the extended lipophilic side chain with a specific region of the β_2-receptor, affording salmeterol a unique binding mechanism (89). The long

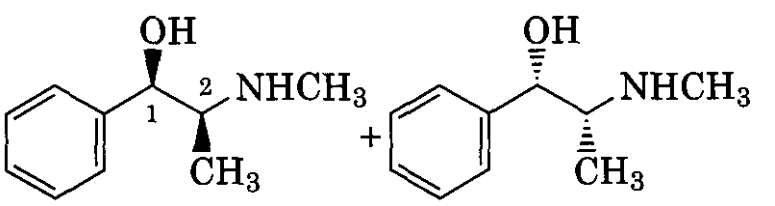
lipophilic nitrogen substituent of salmeterol has been shown, through a series of site-directed mutagenesis experiments, to bind to a specific 10 amino acid region of transmembrane domain 4 of the β_2-adrenoceptor. This region, amino acids 149–158, is located at the interface of the cyctoplasm and TMD4. Thus "anchored" by the side chain, the remaining part of the molecule can pivot and repetitively stimulate the receptor through binding to aspartate 113 of TMD3 and serines 204/207 of TMD5. This lipophilic anchoring is postulated to keep the drug localized at the site of action and produce the long duration of action of salmeterol.

At least one of the phenyl substituents must be capable of forming hydrogen bonds and, if there is only one, it should be at the 4'-position to retain β-activity. For example, ritodrine (20) has only a 4'- OH for R^4, yet retains good β-activity with the large substituent on the nitrogen, making it β_2 selective.

If R^4 is only a 3'- OH, however, activity is reduced at a-sites and almost eliminated at p-sites, thus affording selective a-agonists such as phenylephrine (11) and metaraminol (8). Further indication that a-sites have a wider range of substituent tolerance for agonist activity is shown by the 2',5'-dimethoxy substitution of methoxamine (9), which is a selective a-agonist that also has β-blocking activity at high concentrations.

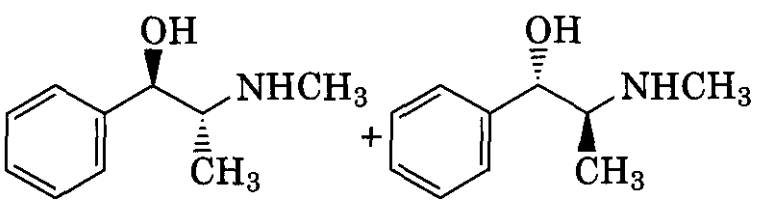
When the phenyl ring has no substituents (i.e., R^4 = H), phenylethylarnines may have both direct and indirect activity. Direct activity is the stimulation of a receptor by the drug itself, whereas indirect activity is the result of displacement of norepinephrine from its storage granules, resulting in stimulation of the receptor by the displaced norepinephrine. Because norepinephrine stimulates both a- and β-sites, indirect activity itself cannot be selective; however, stereochemistry of R^1, R^2, and/or R^4 may also play a role.

For example, ephedrine *erythro*-(5) and pseudoephedrine threo-(5) have the same substitution pattern and two asymmetric centers, so there are four possible stereoisomers. The drug ephedrine is a mixture of the *erythro* enantiomers (1*R*,2*S*) and (1*S*,2*R*); the threo pair of enantiomers (1*R*,2*R*) and (1*S*,2*S*) constitute pseudoephedrine (Ψ-ephedrine). Analogous to the catechol a-methylnorepinephrine (65, the active metabolite of methyldopa), the ephedrine stereoisomer with the (1*R*,2*S*) absolute configuration has direct activity on the receptors, both a and β, as well as an indirect component. The ephedrine (1*S*,2*R*) enantiomer has primarily indirect activity. Pseudoephedrine, the threo diastereomer of ephedrine, has virtually no direct activity in either of its enantiomers and far fewer CNS side effects than those of ephedrine.



(1R:2S) (1S:2R)

Ephedrine



(1R:2R) (1S:2S)

Pseudoephedrine

Other phenylethylamines, such as amphetamine and methamphetamine, which lack both ring substituents and a side chain hydroxyl, are sufficiently lipophilic to readily cross the blood-brain barrier and cause dramatic CNS stimulation, principally through indirect activity. The clinical utility of amphetamine and its derivatives is entirely based on CNS stimulant and central appetite suppressant effects.

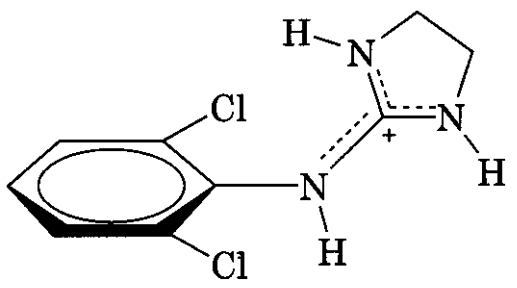
Thus, tamsulosin has no utility in treating hypertension, but far fewer cardiovascular side effects than those of terazosin and doxazosin in treating BPH.

5.1.5 Imidazolines and Guanidines. Although nearly all β-agonists are phenylethanolamine derivatives, α-adrenoceptors accommodate a far more diverse assortment of structures (80). Naphazoline (29), oxymetazoline (30),

tetrahydrozoline (**31**), and xylometazoline (**32**) are selective a,-agonists and thus are vasoconstrictors. They all contain a one-carbon bridge between C-2 of the imidazoline ring and a phenyl substituent; thus, the general skeleton of a phenylethylarnine is contained within the structures. Lipophilic substitution on the phenyl ring *ortho* to the methylene bridge appears to be required for agonist activity at both types of a-receptor. Bulky lipophilic groups attached to the phenyl ring at the ***meta*** or ***para*** positions provide selectivity for the a,-receptor by diminishing affinity for a,-receptors.

Closely related to the imidazoline a,-agonists are the aminoimidazolines, clonidine (**35**), apraclonidine (**33**), brimonidine (**34**); and the structurally similar guanidines, guanabenz (36) and guanfacine (37). Clonidine was originally synthesized as a vasoconstricting nasal decongestant but in early clinical trials was found to have dramatic hypotensive effects, in contrast to all expectations for a vasoconstrictor (90). Subsequent pharmacologic investigations showed not only that clonidine does have some α_1-agonist (vasoconstrictive) properties in the periphery but also that clonidine is a powerful agonist at a,-receptors in the CNS. Stimulation of central postsynaptic a,-receptors leads to a reduction in sympathetic neuronal output and a hypotensive effect. A very recent review thoroughly discusses the antihypertensive mechanism of action of imidazoline a,-agonists and their relationship to a separate class of imidazoline receptors (91).

Similar to the imidazoline α_1-agonists, clonidine has lipophilic *ortho* substituents on the phenyl ring. Chlorines afford better activity than methyls at a, sites. The most readily apparent difference between clonidine and the α_1-agonists is the replacement of the CH, on C-1 of the imidazoline by an amine NH. This makes the imidazoline ring part of a guanidino group, and the uncharged form of clonidine exists as a pair of tautomers. Clonidine has a pK_a value of 8.05 and at physiologic pH is about 82% ionized. The positive charge is shared over all three nitrogens, and the two rings are forced out of coplanarity by the bulk of the two *ortho* chlorines as shown.



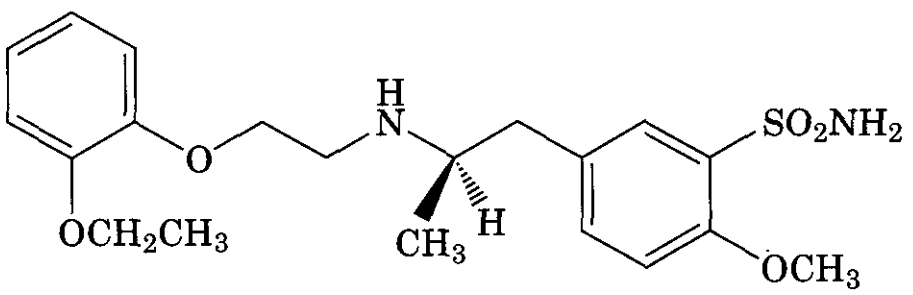
(35)

The other imidazolines, (33) and (**34**), were synthesized as analogs of (35) and were discovered to have properties similar to those of α_2-agonists. After the discovery of clonidine, extensive research into the SAR of central α_2-agonists showed that the imidazoline ring was not necessary for activity in this class. For example, two ring-opened analogs of (35) resulting from this effort are guanabenz (10) and guanfacine (37). These are ring-opened analogs of clonidine, and their mechanism of action is the same as that of clonidine.

Tolazoline (41) has clear structural similarities to the imidazoline a-agonists, such as naphazoline and xylometazoline, but does not have the lipophilic substituents required for agonist activity. Phentolamine (40) is also an imidazoline a-antagonist but the nature of its binding to a-adrenoceptors is not clearly understood.

5.1.6 Quinazolines. Prazosin (**43**), the first known selective a,-blocker, was discovered in the late 1960s (92) and is now one of a small group of selective a,-antagonists, which includes two other quinazoline antihypertensives, terazosin (44) (25, 93) and doxazosin (42). The latter, along with tamsulosin (**24**), was discovered to block a,-receptors in the prostate gland and alleviate the symptoms of benign prostatic hyperplasia (BPH).

The first three agents contain a 4-amino-6,7-dimethoxyquinazoline ring system attached to a piperazine nitrogen. The only structural differences are in the groups attached to the other nitrogen of the piperazine, and the differences in these groups afford dramatic differences in some of the pharmacokinetic properties of these agents. For example, when the furan ring of prazosin is reduced to form the tetrahydrofuran ring of terazosin, the compound becomes significantly more wa-



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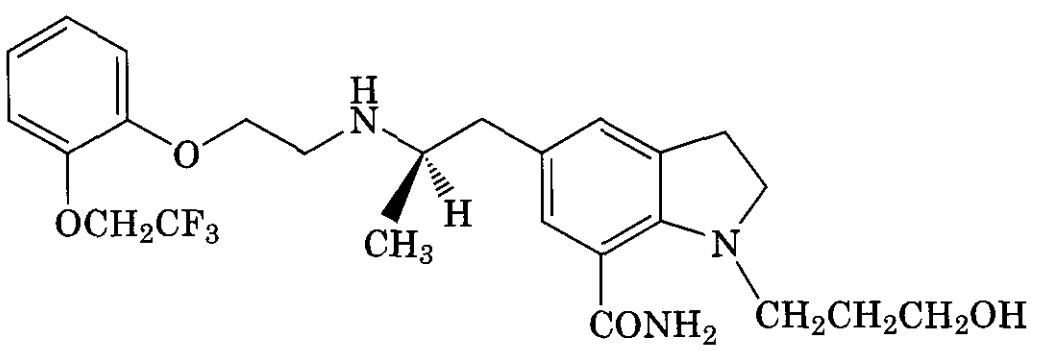

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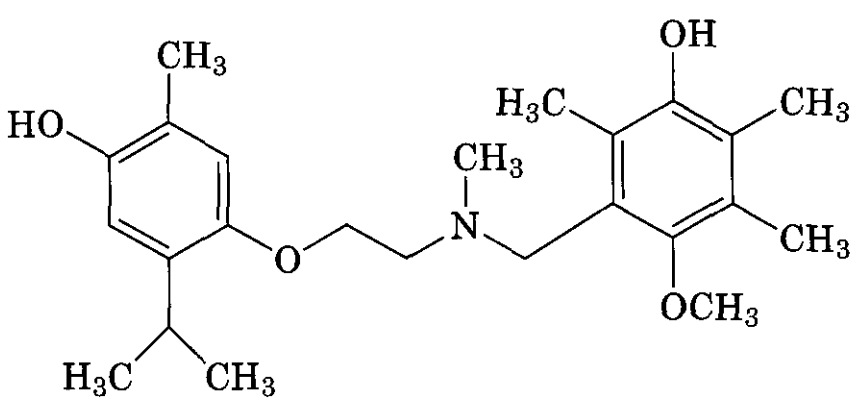


(103)

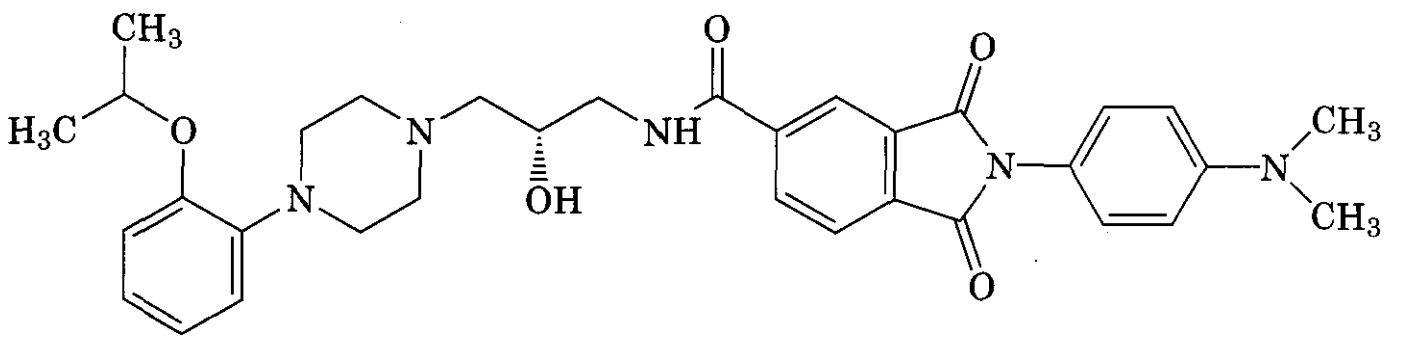
ter soluble (94), as would be expected, given tetrahydrofuran's greater water solubility than that of furan.

5.1.7 Aryloxypropanolamines. In general, the aryloxypropanolamines are more potent β-blockers than the corresponding arylethanolamines, and most of the β-blockers currently used clinically are aryloxypropanolamines. Beta-blockers have found wide use in treating hypertension and certain types of glaucoma.

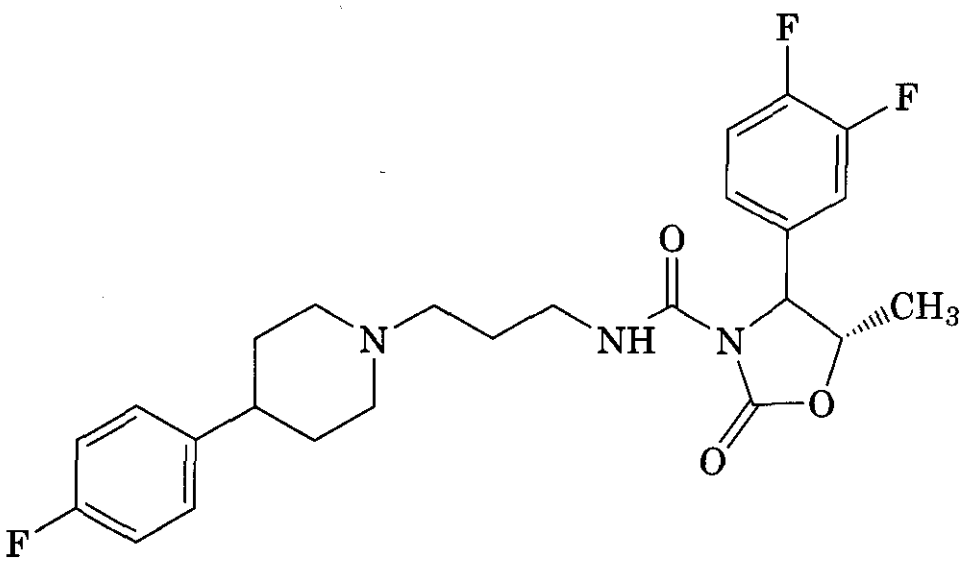
At approximately this same time, a new series of **4-substituted phenyloxypropanololamines** emerged, such as practolol, which selectively inhibited sympathetic cardiac stimulation. These observations led to the recognition that not all preceptors were the same, which led to the introduction of β_1 and β_2 nomenclature to differentiate cardiac β-receptors from others.

Labetalol (26) and **carvedilol (59)** have unusual activity, in that they are antihypertensives with α_1-, β_1-, and β_2-blocking activity. In terms of SAR, you will recall from the earlier discussion of phenylethanolamine agonists that, although groups such as isopropyl and t-butyl eliminated a-receptor activity, still larger groups could bring back a,-affinity but not intrinsic activity. Thus these two drugs have structural features permitting binding to both the α_1- and both β-receptors. The β-blocking activity of labetalol is approximately 1.5 times that of its a-blocking activity. The more recently developed carvedilol has an estimated β-blocking activity 10 to 100 times its a-blocking activity.

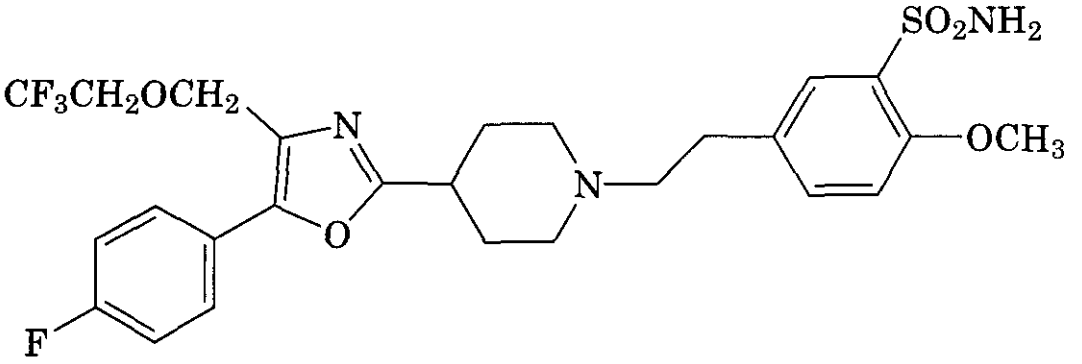
A physicochemical parameter that has clinical correlation is relative lipophilicity of different agents. Propranolol is by far the most lipophilic of the available β-blockers and en-



(104)



(105)



(106)

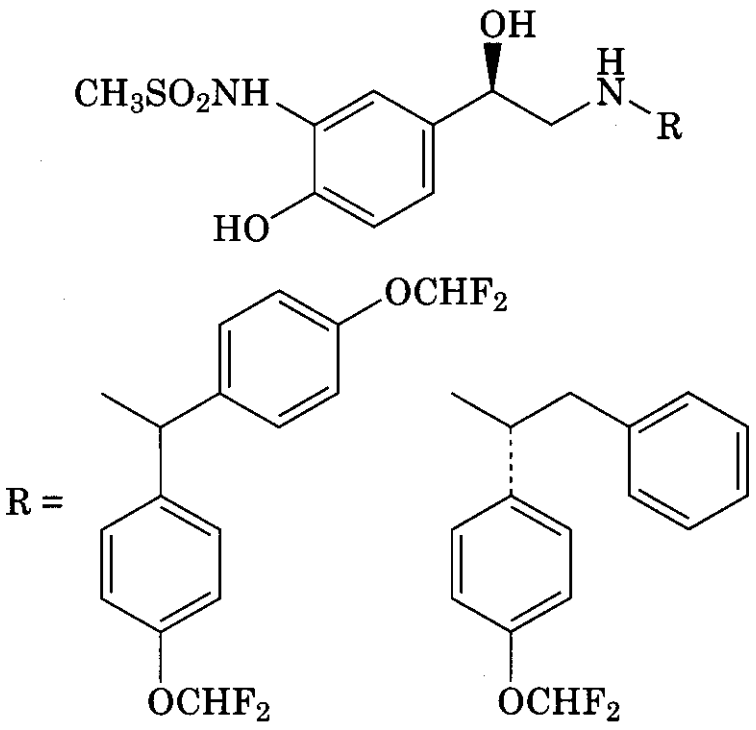
ters the CNS far better than less lipophilic agents, such as atenolol or nadolol. Lipophilicity as measured by octanol/water partitioning also correlates with primary site of clearance. The more lipophilic drugs are primarily cleared by the liver, whereas the more hydrophilic agents are cleared by the kidney. This could have an influence on choice of agents in cases of renal failure or liver disease (27).

6 RECENT DEVELOPMENTS

Recently, major research efforts in development of adrenergic drugs have focused largely on efforts to discover new selective a,,-antagonists for treatment of prostatic hypertrophy and to develop selective β_3-agonists for use in treating obesity and type 2 diabetes.

6.1 Selective α_{1A}-Adrenoceptor Antagonists

The successful application of tamsulosin (24) to the treatment of BPH with minimal cardiovascular effects has led to an extensive effort to develop additional antagonists selective for the a,,-receptor. Phenoxyethylamine (102, KMD-3213), a tamsulosin analog, has been reported to be in clinical trial in Japan, as has (**103**) (95).

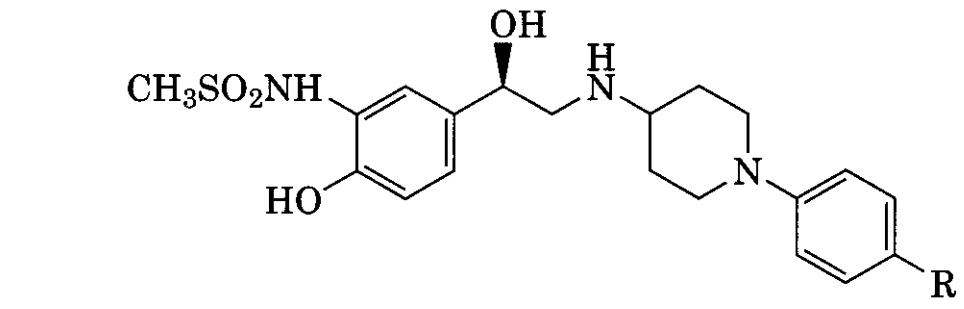


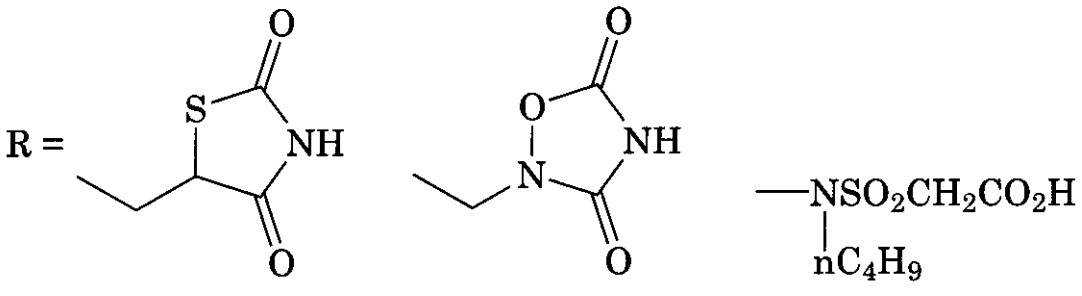
(107) (108)

Other series of highly selective α_{1A}-antagonists, and representative examples, are arylpiperazines, arylpiperidines, and piperidines, represented by (**104**), (**105**), and (**106**), respectively. Several compounds in these series have entered clinical trials, but little has been reported about the outcomes (95). In addition to the review by Bock (95), two other very thorough reviews of this field have recently been published (96, 97).

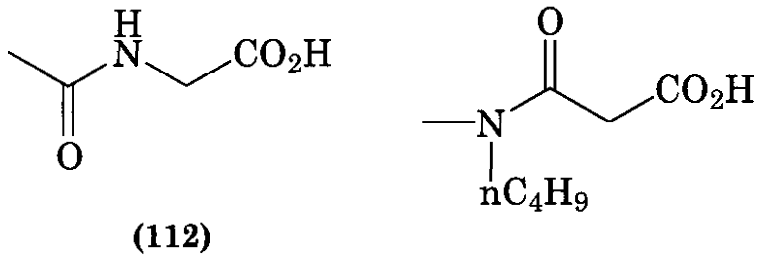
6.2 Selective β_3-Agonists

The other major area of recent emphasis in adrenergic drug research has been development of selective &-agonists to induce lipolysis in white adipose tissue. This area has been extensively reviewed (4, 7, 8, 55, 98). Because obesity and diabetes are reaching epidemic proportions in the United States, an effective weight reduction has enormous therapeutic and market potential (99). As a consequence, there is a veritable avalanche of potential new drugs being published. To date several compounds that looked promising in receptor assays and animal studies have entered clinical trials and failed. The reader should consult the listed reviews for extensive descriptions of the progress in this field through 2000. Some of the most promising recent candidates have been an extensive series of 3'-methylsulfonamido-4'-hydroxyphenylethanolamines prepared by competing groups. Compounds (**107**) (BMS-194449) and (108) (BMS-196085) have both gone into clinical trial but are reported to have failed (100,101).

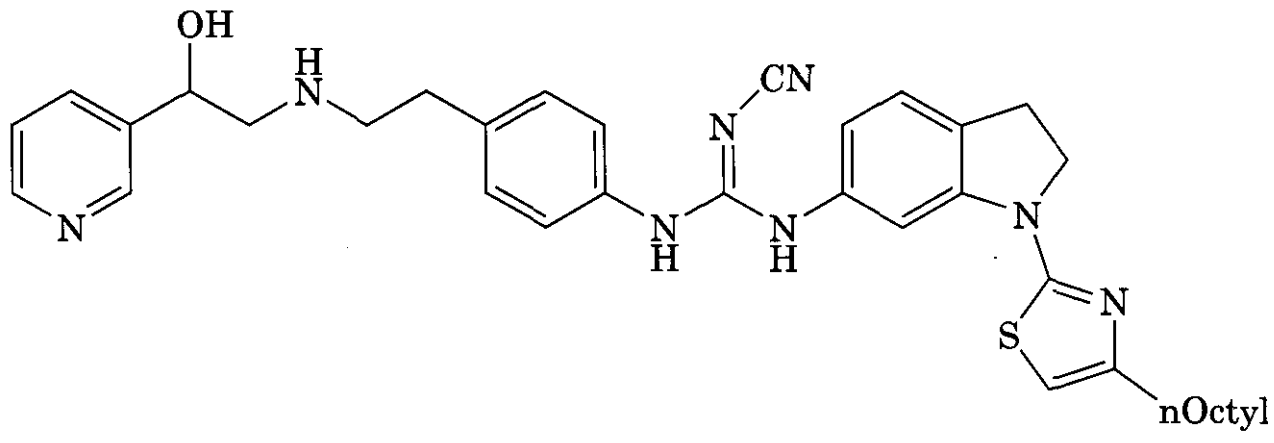




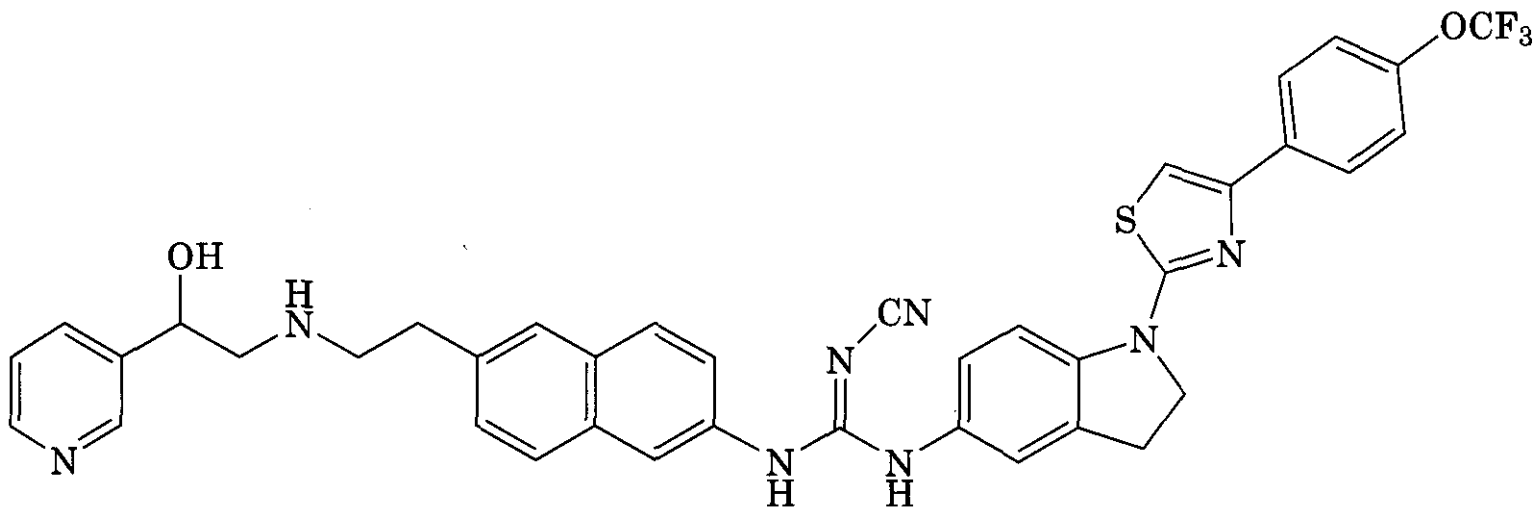
(109) (110) (111)



(112) (113)



(114)



(115)

In a second series, compounds (109–111) were reported as the most active derivatives in the compounds reported in each study (102–104). Finally, compounds (112) and (113) from the same publication were reported to be among the most potent and selective human β_3-agonists known to date (105).

Another group reported another series of very selective β_3-agonists in a series of cyanoguanidine compounds. The most potent and selective in the series were reported to be (114) and (115)(106).

The rate of publication in the two areas of selective α_{1A}-antagonists and selective β_3-agonists continues very high through the time this chapter was written. There is a large market for a successful drug(s) in either of these areas and the level of competition in these areas will continue to be intense.

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CHAPTER TWO

Cholinergics

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Contents

Burger's Medicinal Chemistry and Drug Discovery
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1 INTRODUCTION

The transmission of impulses throughout the cholinergic nervous system is mediated by acetylcholine (**1**), and compounds that produce

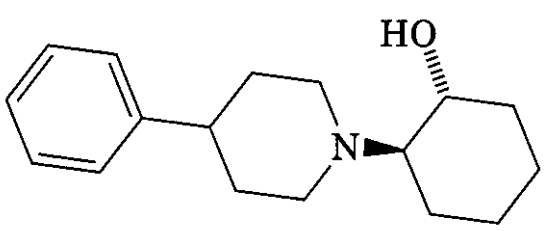
$$CH_3—CO—CH_2—CH_2—\overset{+}{N}(CH_3)_3$$

(1)

their pharmacologic effects by mimicking or substituting for acetylcholine are called cholinergics or parasympathomimetics.

Compounds that inhibit or inactivate the body's normal hydrolysis of acetylcholine by acetylcholinesterase in nervous tissue and/or by butyrylcholinesterase (pseudocholinesterase, cholinesterase) in the plasma are called anticholinesterases. The gross observable pharmacological effects of both types of compounds are quite similar. More recently, compounds have been found that enhance the release of acetylcholine from cholinergic nerve terminals, thus (like the anticholinesterases) producing cholinergic effects by an indirect mechanism.

Choline is taken into the nerve terminal from the synaptic cleft by a sodium-dependent, high-affinity active transport process, which is the rate-limiting step in the biosynthesis of acetylcholine in the nerve terminal (1). In the nerve terminal, choline reacts with acetylcoenzyme **A** in a process catalyzed by choline acetyltransferase. The acetylcholine thus synthesized is sequestered in the synaptic storage vesicles in the nerve terminal for future use as a neurotransmitter. The active transport of acetylcholine into the storage vesicles has been reviewed **(2).** Vesamicol (2) at micromolar concentrations blocks transport of acetylcholine into the vesicles (3, 4).



(2)

Therapeutic indications for cholinergics, anticholinesterases, and/or acetylcholine releasing agents in contemporary practice or those contemplated for future use include the following:

1. Relief of postoperative atony of the gut and the urinary bladder. In such conditions, cholinergicstimulation may relieve the stasis by stimulating peristaltic movements of the intestine and ureters and by constriction of the bladder.
2. Reduction of intraocular pressure in some types of glaucoma, by increasing the drainage of intraocular fluid through the canal of Schlemm.
3. Relief of muscular weakness in myasthenia gravis. This condition reflects a failure of an appropriate amount of acetylcholine to reach cholinergic receptors on the postmyoneural junctional membrane following rapidly repetitive nerve impulses. The reduced level of acetylcholine may result from excessive enzyme-catalyzed hydrolysis of it or from diminished production or release; the etiology of the disease usually involves an autoimmune response phenomenon, primarily to the acetylcholine receptor at the postjunctional end plate (5). However, in approximately **10%** of patients demonstrating the myasthenic syndrome, the cause is congenital and in these individuals, traditional cholinergictherapy is ineffective (5).
4. Relief of the symptoms of Alzheimer's disease and some other types of senile dementia. **A** deficiency of functional cholinergic neurons, particularly those extending from the lateral basalis, has been observed in patients with progressive dementia of the Alzheimer type (6). Cholinomimetic therapy has been directed at compensating for the inadequate cholinergic activity in these neurons. However, clinical results with cholinergics and anticholinesterases have often been disappointing or inconsistent due, in some instances, to the inability of quaternary ammonium drugs to penetrate

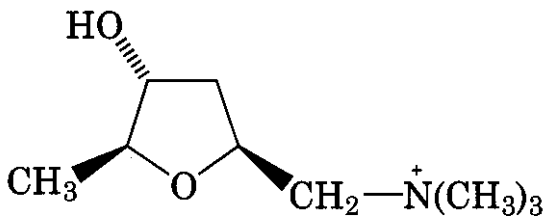
the blood-brain barrier or to a lack of specificity or selectivity of the drug for the cholinergic receptor(s) involved in the pathological condition. There continues to be great emphasis on the search for and study of nonquaternary ammonium molecules (having greater lipophilic character) that will penetrate the blood-brain barrier and interact with appropriate acetylcholine receptors in the brain. Thus older tertiary amine drugs such as pilocarpine and arecoline, which demonstrate only modest cholinergic activity and are classed as partial agonists, have been the subjects of intense structure-activity studies. Relief of Alzheimer symptoms by reinforcement of central cholinergic activity by inhibition of acetylcholinesterase has been the subject of a tremendous research effort (see Section 6). The utility of cholinergics in correction of other types of deficits in memory and learning has been investigated for many years (7), with largely inconclusive results. However, this remains a fascinating and a potentially significant area of research.

5. Relief from pain. Selected cholinergic agents have been found that display antinociceptive (analgesic) effects grossly similar to those of morphine. Most of these are nicotinic receptor agonists, but a modest number of muscarinic agonists have been found that also display antinociceptive effects.
6. Relief of symptoms of Parkinson's disease. Acetylcholine receptors of the nicotinic type have been implicated in the Parkinsonian syndrome (7–9).

2 CHOLINERGIC (ACETYLCHOLINE) RECEPTORS

Acetylcholine receptors have been subdivided into two major pharmacological types (muscarinic and nicotinic), based on their selective response to two alkaloids: muscarine (3) and nicotine (4).

Neither nicotine nor muscarine is a normal physiological component of the mammalian body; hence the muscarinic/nicotinic classification of acetylcholine receptors is artificial. Although it is well established that muscarine



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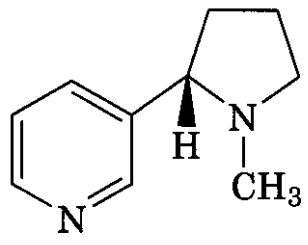


(4)

is a true cholinergic agonist, it is recognized that nicotine lacks agonist effect in some parts of the nervous system; some of its peripheral actions are indirect and probably involve presynaptic release of acetylcholine (10–14).

Muscarinic receptors occur peripherally at parasympathetic postsynaptic sites on glands and smooth (nonstriated) muscles, and they are involved in gastrointestinal and ureteral peristalsis, pupillary constriction, peripheral vasodilatation, reduction of heart rate, and promotion of exocrine glandular secretion (salivary glands, sweat glands, tear glands). Autonomic ganglia also contain muscarinic receptors. Peripheral nicotinic receptors are found postsynaptically on striated (voluntary) muscle fiber membranes and in all autonomic ganglia (sympathetic as well as parasympathetic). There are also nicotinic and muscarinic pathways in the central nervous system.

Muscarinic receptors are subcategorized as M_1, M_2, M_3, M_4, and M_5 (15). By definition, M_1 receptors occur especially in the cerebral cortex and hippocampus, where they are thought to play an important role in learning and memory processes (16). It has been assumed for some years that M_1 receptor agonists are the most likely candidates for treatment of cholinergic deficits involved in Alzheimer's disease (17). M_1 receptors are found peripherally in autonomic ganglia where they are involved in membrane depolarization, which is mediated by stimulation of phospholipase C and subsequent production of inositol-1,4,5-triphosphate and diacyl glycerol (18). M_2 re-

ceptors are present in the cerebellum, heart, smooth muscle, and at some potassium channels (15); M_3 receptors are found in secretory glands and smooth muscle. M_4 receptors are located in the basal forebrain and the striatum. M_5 receptors are found in the substantia nigra. M_1, M_3, and M_5 receptors modulate some potassium, chloride, and calcium channels, and M_2 and M_4 receptors modulate some calcium channels (19). All of these ion channel effects are indirect and complex. The central nervous system contains all known subtypes of muscarinic receptors (18). Both muscarinic M_2 (20) and nicotinic (21) receptors decrease in numbers with the progression of Alzheimer's syndrome, whereas the numbers of M_1 receptors do not decrease (22). Alzheimer patients also show reduced activity of acetylcholinesterase; of high affinity, sodium-dependent choline uptake; and of choline acetyltransferase (23). Contemporary and projected therapeutic roles for muscarinic receptors in the central nervous system have been reviewed (24).

Muscarinic receptors are glycoproteins with molecular weights of approximately 80,000. They are located on the outer surface of the cell membrane, and they are of the G-protein-linked type; M_1, M_3, and M_5 receptors couple with G_s proteins to stimulate phospholipase-C, whereas M_2 and M_4 receptors couple with G_i proteins to inhibit adenylate cyclase (25). The molecular basis of muscarinic receptor function has been reviewed (26). Nordvall and Hacksell (27) proposed a molecular model of the transmembrane domains of the M_1 receptor, to explain the three-dimensional interaction of the receptor with its ligands. However, the authors specified that the model is "primarily of qualitative value".

Postsynaptic nicotinic receptors in the peripheral nervous system are designated as N_1 (in autonomic ganglia) and N_2 (at myoneural junctions). Nicotinic receptors are of the ion channel type. They are pentameric proteins that are composed of one, two, or more distinct subunits, each of which contains multiple membrane-spanning regions, and the individual subunits surround an internal channel (28). Nicotinic receptors are highly heterogeneous, and subcategorization has been difficult; those in neuronal tissue are believed to have a similar pentameric structure, but they vary considerably in the nature of the subunit combinations (29).

At least some neuronal nicotinic subunits are homologous to those found in muscle. These have been designated as a if they contain vicinal cysteine residues analogous to Cys192-Cys193 of the Torpedo receptor, or β if they do not. Stimulation of these receptors produces depolarization (a result of cation channel opening) and firing of the postganglionic neuron. Nine homologous nicotinic receptor subunits have been identified thus far in mammalian nervous systems (30). Two main categories of nicotinic receptor pentamers have been identified in the brain, based on their high affinity for either nicotine or for the nicotinic receptor blocker a-bungarotoxin. The former are considered to be formed by α4- and β2-subunit receptors, and the latter are thought to be a7 or α7* nicotinic receptors (30). Nicotinic receptor glycoprotein has been isolated and extensively studied (31–33). Reviews of the classification, recommended nomenclature, and function of nicotinic receptor subtypes are available (29, 30, 34–36).

A family of presynaptic ion channel-type acetylcholine receptors in the brain modulates the release of acetylcholine, dopamine, and other neurotransmitters implicated in learning and memory processes (37). There is convincing evidence to implicate a deficit in nicotinic receptors in the symptomatology of Alzheimer's disease. Furthermore, the neurotoxin β-amyloid attenuates nicotine-induced release of acetylcholine and dopamine (37).

Although acetylcholine is optically inactive, its in *vivo* receptors exhibit discrimination between enantiomers of synthetic and naturally occurring cholinergic stimulants. Both central and peripheral muscarinic receptors are highly stereospecific; peripheral nicotinic receptors seem to be less so, although these usually show preference for one or the other member of enantiomeric pairs. Central nicotinic receptors frequently demonstrate a higher degree of stereoselective binding character than is noted with the peripheral receptors. Understanding of nicotinic receptor stereoselectivity and specificity is complicated by the liklihood that, as was mentioned previously, at some in *vivo* sites, some nicotinic

stimulant agents function indirectly by promoting presynaptic release of acetylcholine.

3 ACETYLCHOLINE AND ANALOGS

Acetylcholine has virtually no clinical uses. Its rapid rate of hydrolysis in the gastrointestinal tract precludes oral administration, and a similarly rapid hydrolysis by esterases in the blood and by acetylcholinesterase in the nervous tissue limits its usefulness.

The need for therapeutically satisfactory cholinergic agents coupled with the simple and easily synthesized structures necessary for cholinergic activity have stimulated preparation and study of a great number of analogs of acetylcholine. The following structural variations have been addressed:

1. Alteration of the quaternary ammonium head.
2. Replacement of the acetyl group by other acyl moieties.
3. Alteration of the ethylene bridge connecting the quaternary ammonium and the ester groups.
4. Substitution of another group for, or elimination of, the ester moiety.

The "five atom rule," first suggested by Alles and Knoefel (38) and stated more formally by Ing (39), proposes that, for maximum muscarinic activity, there should be attached to the quaternary nitrogen atom, in addition to three methyl groups, a fourth group with a chain of five atoms, as illustrated for acetylcholine: C-C-O-C-C-N. This empirical observation has been found to be valid for a large number of molecules, regardless of the precise nature of the five atoms involved.

Studies of a large number of compounds have supplied considerable information on structural requirements for cholinergic activity; however (especially in the older literature) these data must be interpreted with caution. They have been obtained using a variety of in *vivo* and in vitro testing procedures and biological preparations in a variety of animal species, and different biological responses associated with stimulation of the cholinergic nervous system were measured. Additionally, these older studies were performed before the exquisite heterogeneity of both muscarinic and nicotinic receptors was recognized; thus, many compounds were classified merely as "muscarinic" or "nicotinic." The observed effectiveness of a cholinergic agent in producing a biological response depends, inter alia, on its inherent potency and intrinsic activity as well as on the rate at which it is metabolically inactivated and/or excreted. Frequently, these individual factors were not separately and individually assessed. This problem has been cited (40) with regard to the lack of consistency among laboratories in the methods used to determine cholinergic receptor subtype selectivity. Therefore, in the following discussion of the relationship of chemical structure to cholinergic activity, frequently only generalized (and tentative) conclusions can be made, and these may have been based on a composite of the cholinergic activities for which the compound was tested.

3.1 Variations of the Quaternary Ammonium Croup

Two types of alterations of the quaternary head have been studied: replacement of the nitrogen by other atoms and replacement of the N-methyl groups by hydrogen, alkyl, nitrogen, or oxygen. Acetyl phosphonocholine (**5**) (39), acetylarsenocholine (6) (39), and acetylsulfonocholine (7) (**41**) exhibit muscarinic effects, but they are considerably less potent than acetylcholine.

$$CH_3—CO—CH_2—CH_2—R$$

(5) R = $^{+}P(CH_3)_3$ **(8)** R = $C(CH_3)_3$
(6) R = $^{+}As(CH_3)_3$ **(9)** R = $^{+}N(CH_3)_2NH_2$
(7) R = $^{+}S(CH_3)_2$ **(10)** R = $^{+}N(CD_3)_3$

Ing (39) noted that the potencies of acetylcholine analogs containing charged atoms other than nitrogen (phosphorus, arsenic, sulfur) are in inverse order to the volumes occupied by these atoms. The carbon isostere (**8**) of acetylcholine exhibits no cholinergic activity, but it is an excellent substrate for acetylcholinesterase (42). Studies of the role of nitrogen substituents in the acetylcholine molecule indicate that the *N,N,N*-trimethyl quaternary

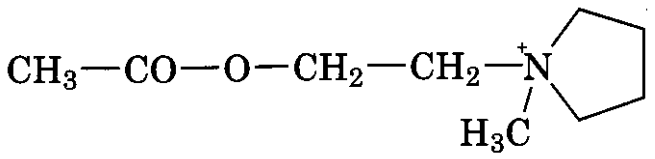
Table 2.1 Representative Esters of Choline

$$R{-}O{-}CH_2{-}CH_2{-}\overset{+}{N}(CH_3)_3$$

Number	R	Reference
(1)	HCO	61
(2)	$BrCH_2CO$	59
(3)	C_2H_5CO	61,62
(4)	H_2NCH_2CO	63
(5)	n-C_3H_7CO	61,62
(6)	i-C_3H_7CO	61
(7)	n-C_4H_9CO	61,62
(8)	C_6H_5CO	62
(9)	$C_6H_5CH_2CO$	62
(10)	$C_6H_5CH{=}CHCO$	62
(11)	$(C_6H_5)_2C(OH)CO$	64
(12)	$CH_3(CH_2)_{10}CO$	65
(13)	$CH_3(CH_2)_{14}CO$	65
(14)	$HOCH_2CO$	61
(15)	$CH_2{=}CHCO$	66
(16)	CH_3COCO	61
(17)	$CH_3CHOHCO$	67
(18)	O_2N	68
(19)	H_2NCO	69,70
(20)	$(CH_3O)_2PO$	71

ammonium pattern of acetylcholine itself is optimum for potency and activity. The acetate esters of *N,N*-dimethylethanolamine, N-methylethanolamine, and ethanolamine possess weak muscarinic activity, and they show no nicotinic activity (43). The tertiary amine congener of carbamyl choline (Table 2.1, 19) exhibits greatly diminished nicotinic and muscarinic effects compared with the *N,N,N*-trimethyl quaternary compound (44). These conclusions seem valid for cholinergic agents having, like acetylcholine, a high degree of molecular flexibility. In contrast, in certain acetylcholine congeners in which the nitrogen is a part of a relatively rigid ring system (pyrrolidine, morpholine, piperidine, quinuclidine), tertiary amine salts are more potent muscarinics than their quaternary derivatives (45). This enhanced activity of the tertiary amines has been rationalized on conformational grounds. It is assumed that the tertiary amines are protonated at their in *vivo* sites of action.

The pyrrolidine compound (11) is 20–33% as potent as acetylcholine (46); this compound can be viewed as a cyclic congener of acetyl

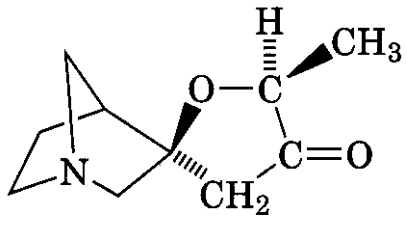


(11)

N-methyl,*N*,*N*-diethylcholine, and it is decidedly more potent than the diethylcholine ester.

However, in general, incorporation of the choline nitrogen into a heterocyclic ring markedly lowers the potency compared with acetylcholine (47, 48).

The report (49) that the tertiary amine (12) is a full and nonselective muscarinic agonist



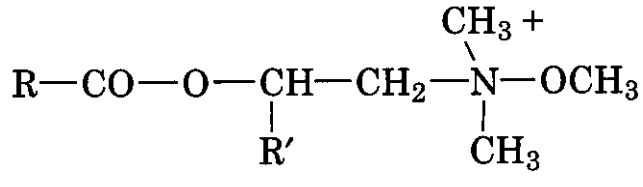
(12)

lends credence to proposed significance of molecular rigidity in muscarinic agonism.

Replacement of one N-methyl group of acetylcholine by ethyl permits retention of most of the cholinergic activity, but as more N-methyl groups are replaced by ethyl, there is a progressive loss of cholinergic effect (50). When one N-methyl is replaced by n-propyl or n-butyl, there is almost complete loss of cholinergic activity (41).

The hydrazinium congener (9), in which one N-methyl is replaced by NH_2, was less active than acetylcholine in all assays performed (51). The tris-(trideuteromethyl) congener (10) showed similar potency to acetylcholine in a dog blood pressure assay (52).

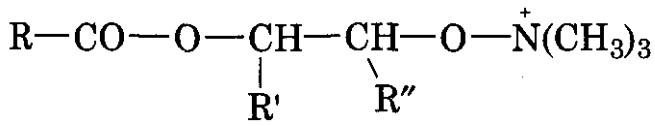
Replacement of one N-methyl in acetylcholine and in three congeners (13–16) by me-



(13) R = CH_3; R′ = H
(14) R = R′ = CH_3
(15) R = H_2N; R′ = H
(16) R = H_2N; R′ = CH_3

thoxyl permits retention of some cholinergic effects, and in certain compounds, nicotinic or muscarinic activities are enhanced over the parent *N,N,N*-trimethyl system (53).

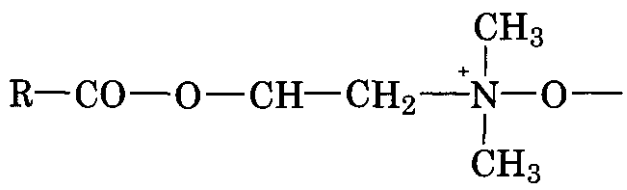
The reverse *N*-alkoxy systems (17) demonstrated only extremely weak muscarinic activ-



(17) R = CH_3 or NH_2
R', R = combinations of H, CH_3

ity (54). These compounds violate the five atom rule.

Amine oxide analogs of the cholinergic agonists (18–21) exhibit little or no cholinergic effect, and they are not substrates for cholinesterases (55).



(18) R = CH_3; R′ = H
(19) R = R′ = CH_3
(20) R = H_2N; R′ = H
(21) R = H_2N; R′ = CH_3

The observed biological effects of several variations of the quaternary head of acetylcholine and its congeners may be rationalized by invoking results of molecular orbital calculations (56), which indicate that in both muscarine and acetylcholine the nitrogen atom is nearly neutral and a large part (70%) of the formal charge is distributed among the three attached methyl groups, which form a large ball of spreading positive charge.

Furthermore, Kimura and coworkers (57) determined that chain extension of one alkyl group of the tetramethylammonium cation produces a great decrease in the charge density on the nitrogen, and they proposed that cholinergic agonist activity for a quaternary ammonium compound requires a minimum level of charge density on the nitrogen.

3.2 Variations of the Acyl Croup

Qualitatively, choline has the same pharmacological actions as acetylcholine, but it is far less active at most sites (58). However, choline has been reported (34) to be a full agonist at one nicotinic receptor subtype, and at some other nicotinic subtypes it can act as a partial agonist or a coagonist.

Acetylation of the alcohol function of choline greatly increases the potency. However, formylcholine is less potent than acetylcholine, and homologation of the acetate methyl group of acetylcholine generally produces compounds that are much less potent than acetylcholine (see Table 2.1). Polar groups such as OH (Table 2.1, **17**) and NH, (4) markedly decrease muscarinic potency, but a group (16) permits retention of considerable activity. Bromoacetylcholine (number 2) is a muscarinic and nicotinic agonist (59) and, under reducing conditions, it binds covalently to nicotinic receptors but not to muscarinic receptors (60).

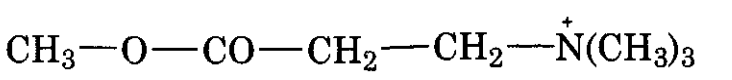
Acrylylcholine (Table 2.1, **15**), which has been isolated from tissues of a marine gastropod (72), has relatively high cholinergic activity. Higher fatty acid esters (12 and 13) were prepared for testing as hemolytic agents, but apparently they have never been evaluated for cholinergic activity. A study of acetylcholine congeners derived from relatively high molecular weight acids (73), most of which contained a benzene ring, revealed that as the molecular weight of the acid increases parasympathetic stimulant activity dimninishes, and there is a gradual change to atropine-like (muscarinic blocking) activity.

In general, carbamic acid esters of choline and its congeners are more potent and more toxic than the corresponding acetates. Carbamyl choline (19 in Table 2.1) is a potent muscarinic agent, and it demonstrates pronounced nicotinic stimulant effects at autonomic ganglia. It is likely that these ganglionic actions are due, at least in part, to release of endogenous acetylcholine from the terminals of cholinergic fibers (74).

Carbamyl choline is is a poor substrate for acetylcholinesterase and nonspecific cholinesterases (74). The nitrate ester (18, Table 2.1)

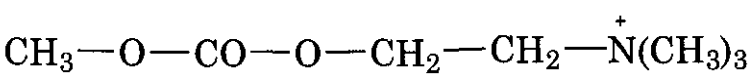
has marked nicotinic and muscarinic agonist effects, and it also displays an intense paralyzing nicotine action. The dimethylphosphate ester **(20,** Table 2.1) has powerful nicotinic action but little muscarinic effect.

The "reversed ester" congener (**22**) of acetylcholine exhibits weak muscarinic and no



(22)

nicotinic effects (75). In contrast, the carbonate congener **(23)** is a full agonist at muscarinic and nicotinic receptors, with an activity

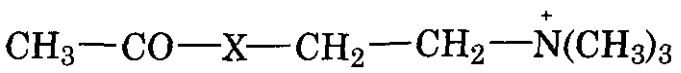


(23)

approximately one order of magnitude less than that of acetylcholine (76).

Acetylthiocholine **(24)** and acetylselenocholine **(25)** exert acetylcholine-like effects on the guinea pig ileum and on the frog rectus abdominis, but they are somewhat less potent than acetylcholine (77).

Unesterified thiocholine and selenocholine display a relatively high degree of acetylcholine-like potency and activity compared with their acetate esters, in contrast to the dramatic potency difference between choline and acetylcholine. The biological effects of these unesterified thiols and selenols have been suggested to be due to their oxidation to disulfide and diselenide derivatives **(77).** The amide congener **(26)** of acetylcholine has little or no

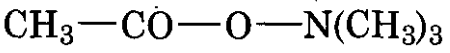


(24) X = S
(25) X = Se
(26) X = NH

cholinergic activity **(68).** Acetylthionocholine, in which the carbonyl oxygen of acetylcholine is replaced by sulfur, displayed some acetylcholine-like effects in an electroplax preparation (78).

3.3 Variations of the Ethylene Bridge

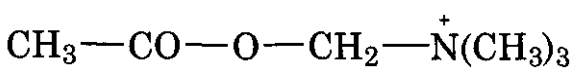
The distance between the ester moiety and the cationic head of acetylcholine seems to be critical. Acetoxytrimethylammonium (**27**), com-



(27)

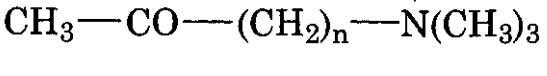
pletely lacking the ethylene bridge of acetylcholine, showed a pharmacological profile quite similar to acetylcholine (79), but it was much less potent.

Acetoxymethyltrimethylammonium **(28)** appeared to have little or no muscarinic effect



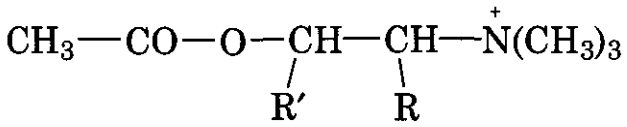
(28)

on a guinea pig ileum preparation **(76).** The profound instability of this compound in solution precluded collection of quantitative data. An older report **(68)** had indicated that structure **(28)** has "intense muscarinic action" and "marked nicotine stimulant action." Acetyl y-homocholine **(29)** is decidedly less potent/ active than acetylcholine **(73). 4-Acetoxybutyltrimethylammonium (30)** exhibits extremely weak muscarinic and nicotinic effects (80).



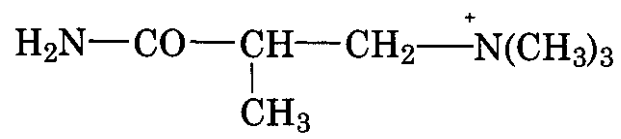
(29) n = 3
(30) n = 4

Replacement of one or more of the hydrogen atoms of the ethylene bridge with alkyl groups produces marked changes in potency and activity. Acetyl β-methylcholine **(31)** is equipotent to acetylcholine as a muscarinic agonist, but it has a much weaker nicotinic action **(74).** A factor in the observed potency of acetyl β-methylcholine is its slower rate of hydrolysis by acetylcholinesterase because of poor affinity of the compound for the enzyme's catalytic site **(81)** and its extremely high resistance to hydrolysis by nonspecific serum cholinesterases.



(31) R = H; R′ = CH_3
(32) R = CH_3; R′ = H
(33) R = R′ = CH_3

Acetyl a-methylcholine (32) is a more potent nicotinic than a muscarinic, but both effects are decidedly less than those of acetylcholine (82); it is hydrolyzed by acetylcholinesterase at a rate similar to that of acetylcholine (73). The carbarnate ester of (±)-β-methylcholine (**34**), bethanechol, is a useful



(34)

therapeutic agent, and acetyl β-methylcholine (31) has some limited diagnostic uses (84). The introduction of the C-methyl group into the acetyl a- and β-methylcholine molecules creates a chiral center, and the enantiomers exhibit different properties (Table 2.2).

S-(+)-Acetyl-β-methylcholine (the eutomer) is hydrolyzed by acetylcholinesterase at about half the rate of acetylcholine; the *R*-(−)-enantiomer is a weak inhibitor of the enzyme **(82).** Work of Ringdahl (85) suggests that the markedly lower pharmacological activity of *R*-(−)-acetyl-β-methylcholine is a result both of lower affinity for the muscarinic receptor(s) and lower intrinsic activity. The antipodes of carbamyl-β-methylcholine (bethanechol) (34) displayed a eudismic ratio of 740 at rat jejunum sites (86) and of 915 using guinea pig intestinal muscle (87); the S antipode is the eutomer.

(±)-*erythro*-Acetyl-α,β-dimethylcholine (33) exhibits 14% of the muscarinic potency of acetylcholine, and it is almost completely resistant to acetylcholinesterase; the (+)-threo isomer is inert as a cholinergic and is a poor substrate for acetylcholinesterase (88). These racemic mixtures have apparently never been resolved. gem-Dimethyl substitution of acetylcholine, either on the α,α or the β,β positions of the choline moiety, greatly reduces but does not abolish muscarinic activity (89). Both compounds are relatively poor substrates for bovine erythrocyte acetylcholinesterase. Replacement of the C-methyl groups in the ethylene bridge by longer chains causes an increase in toxicity and a reduction in muscarinic activity, e.g., the acetate esters of β-*n*-propyl- and β-*n*-butylcholines (90, 91).

3.4 Substitution of the Ester Group by Other Groups

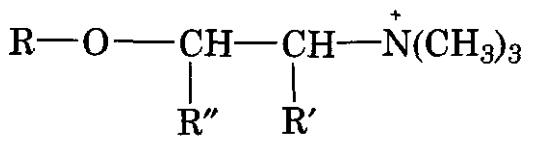
The ester moiety of acetylcholine does not appear to be essential for cholinergic activity. In general, alkyl ethers of choline and of thiocholine are less potent and less active than acetylcholine (47); thio ethers are less potent than the corresponding oxygen compounds. Contrary to some earlier literature reports, the vinyl ether of choline (36) is not a more potent muscarinic agent than the ethyl ether (35) (both are weak muscarinics), but it is a better nicotinic agent, displaying higher potency than acetylcholine (92).

a-Methyl substitution of choline in its ethyl and vinyl ethers (compounds 38 and 40) greatly diminishes muscarinic potencies but

Table 2.2 Muscarinic Activities of Acetyl C-Methylcholines[a]

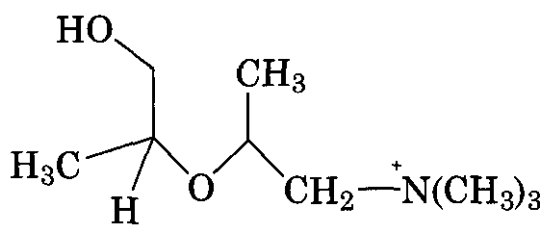
Substituent	Stereochemistry	Number Moles Equivalent to 1 Mole of AcCh as Agonist in Guinea Pig Ileum	Activity Ratio, (+)/(−)
α-CH_3	R S	**49**	
	S-(−)-	**232**	8
	R-(+)-(eutomer)	**28**	
β-CH_3	R S	**1.58**	
	S-(+)-(eutomer)	**1.01**	**240**
	R-(−)-	**240**	

[a]Adapted from Ref. 83. Courtesy of Plenum Press.



(35) R = C_2H_5; R′ = R′ = H
(36) R = CH_2 = CH; R′ = R″ = H
(37) R = C_2H_5; R′ = H; R″ = CH_3
(38) R = C_2H_5; R′ = CH_3; R″ = H
(39) R = CH_2 = CH; R′ = H; R″ = CH_3
(40) R = CH_2 = CH; R′ = CH_3; R″ = H

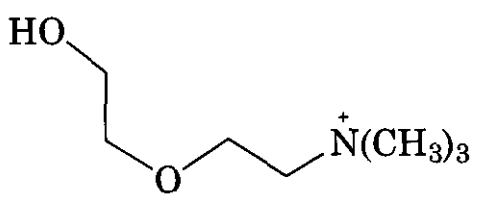
permits retention of potent nicotinic effects. β-Methyl substitution (compounds37 and 39) permits retention of some degree of muscarinic effect, but nicotinic effects are completely abolished (92). A series of open-chain congeners of muscarine, typified by structure (**41**), exhibited low muscarinic potency, which



(41)

was ascribed by Friedman (47) to the compounds' stereochemical heterogeneity.

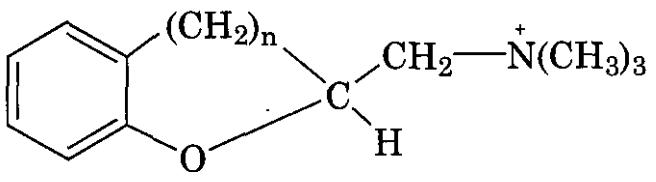
An open chain analog (42) of desmethylmuscarine lacking chiral centers exhibited extremely low muscarinic activity (93).



(42)

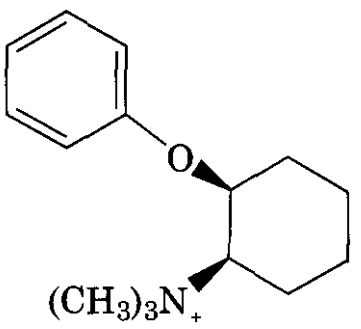
Some aromatic ethers of choline display marked nicotinic activity, but they are inactive at muscarinic sites (94). The o-tolyl ether of choline is a potent ganglionic stimulant (95), but the 2,6-xylyl ether of choline is inert as a nicotinic agent (96).

Additional ring-substituted phenyl ethers of choline were described by Hey (94). Clark and coworkers (97) studied conformationally restricted racemic bicyclic choline phenyl ethers (43) in which the choline moiety was a part of the ring system.



(43) n = 1–3

It was concluded that the nicotinic activity of choline phenyl ether and of choline o-tolyl ether is a reflection of the ability of the molecule to assume a "planar" conformation when interacting with the ganglionic nicotinic receptor. In contrast, the inactive 2,6-xylyl ether of choline cannot assume this planar disposition. Evaluation of additional conformationally restricted aryl choline ethers (44–47) revealed that only the piperidine derivative (47) is a ganglionic stimulant (98).



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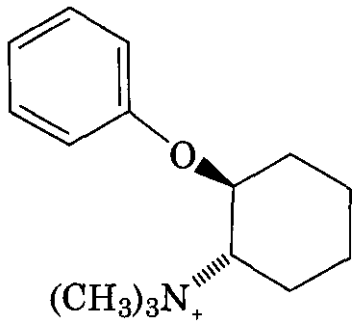
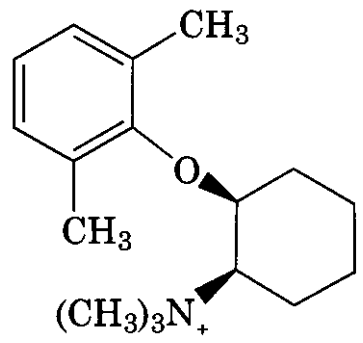


(45)

Ketonic systems (**48a–c**), carbon isosteres of the ester moiety, display weak activities, and they are predominantly more nicotinic than muscarinic (47).

The secondary alcohol analogs of these ketones are even weaker and the thio ketones are also weak (48).



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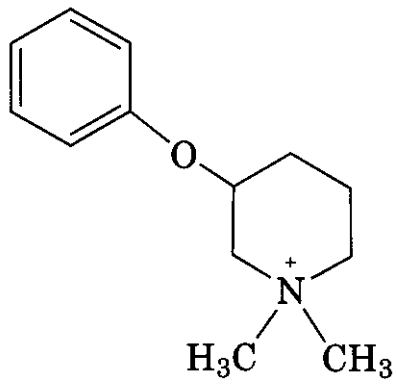

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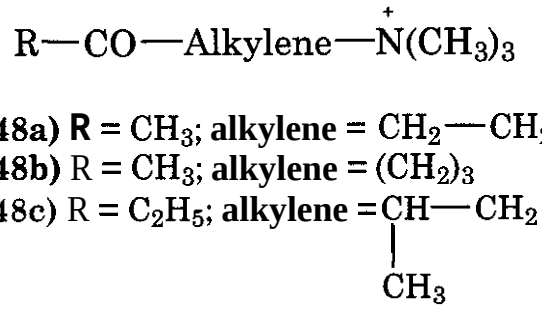
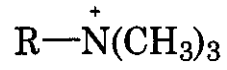


(48a) **R** = CH_3; **alkylene** = $CH_2—CH_2$
(48b) R = CH_3; **alkylene** = $(CH_2)_3$
(48c) R = C_2H_5; **alkylene** = $CH(CH_3)—CH_2$

In a series in which an **alkyl** chain (methyl through n-amyl) replaces the acetate ester moiety (**49**), minimum activity occurs in most assays when R = ethyl or n-propyl, and **maxi-**



(49)

mum muscarinic potency is demonstrated when R = *n*-amyl (47, 48), consistent with the five-atom rule.

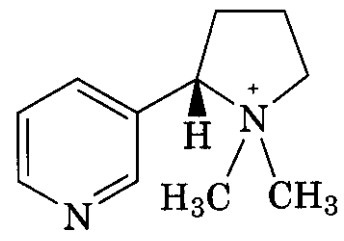
Above heptyl, the compounds become antagonists to acetylcholine. Numerous examples of ketones and N-alkyl congeners have been tabulated (47, 48).

An earlier review (47) descibes the pharmacology of additional analogs and congeners of acetylcholine.

4 CHOLINERGICS NOT CLOSELY RELATED STRUCTURALLY TO ACETYLCHOLINE

4.1 Nicotine, Its Analogs and Congeners, and Other Nicotinic Receptor Stimulants

Naturally occurring levorotatory nicotine (**4**) has the S- absolute configuration. Its **enantiomer**, R-(+)-nicotine, was decidedly less potent than the naturally occurring material in assays for peripheral effects (99). The **pyrrolidine** methyl quaternary derivative of *S*-(−)-nicotine (50) shows peripheral activity **com-**

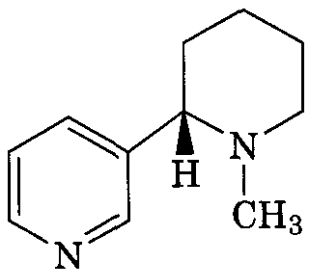


(50)

parable to that of nicotine itself (100) but it lacks the secondary blocking action of the tertiary **amine**. In contrast, N-alkylation of the pyridine ring nitrogen of S-nicotine produces quaternary compounds that are potent and selective antagonists at nicotinic receptor subtypes (101). The dimethiodide salt of *S*-nicotine has a low order of potency (100).

Nornicotine, in which the N-methyl is replaced by hydrogen, is somewhat less potent and active than nicotine in most assays (102). R- and S-nornicotine are equipotent with R-(+)-nicotine, the less active, unnatural **enantiomer**, in a rat brain membrane-binding assay (103). In that study, S-(−)-nicotinewas 13 times more potent than its R- enantiomer. Replacement of the N-methyl of nicotine with ethyl or n-propyl causes an exponential loss of peripheral nicotinic effect (102). Anabasine (**51**), a relatively minor alkaloidal constituent of tobacco, demonstrated approximately 1/10 the affinity of nicotine in a binding assay (104). **A** synthetic azetidine congener (52) of nicotine binds with the same affinity as nicotine to rat brain membrane tissue, and it displayed a greater potency than nicotine in a rat behavioral assay (105). Evaluation of a series of 6-substituted nicotine derivatives (53) led to the conclusion that affinity for rat brain

(without cerebellum) nicotinic receptors is related to the lipophilicity of the 6-substituent, as well as to the size of the substituent (106). Electronic factors seem to play a less significant role.



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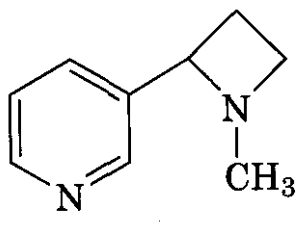
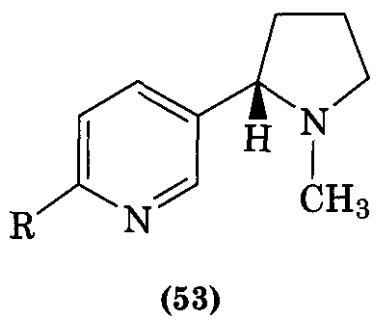
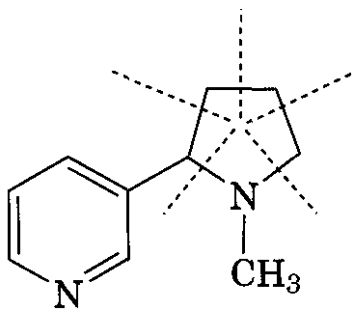


(±) – (52)



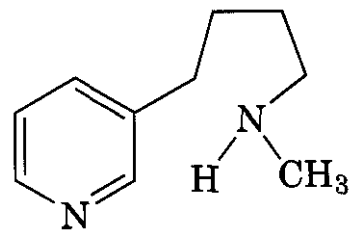
(53)

Synthetic compounds (**54**) representing structures in which each of the bonds of the pyrrolidine ring of nicotine is cleaved, one by one, produced a series of "seconicotines" (102).

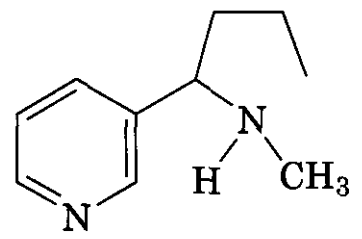


(54)

Only the open chain congeners (55) and (56) display nicotine-like activity. The potency of compound (55) is increased in its *N,N*-dimethyl congener; in contrast, the *N,N*-dimethyl derivative of compound (56) is inert.

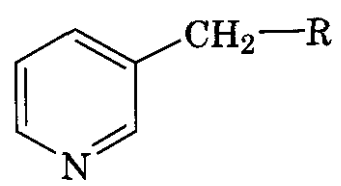


(55)

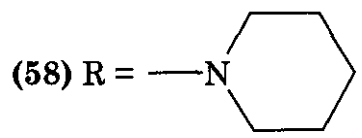


(56)

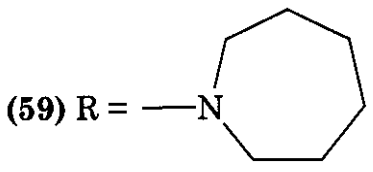
A nicotine structural isomer (57) retains a considerable degree of nicotine-like activity (102). The piperidine congener (58) is somewhat less potent and active, and the perhydroazepine congener (59) is inert. The pyrrolidine and piperidine ring N-methyl quaternary derivatives of compounds (57) and (58) are slightly less active than the corresponding tertiary bases (107). Glennon and coworkers (108) reported that the 6,7,8,9-tetrahydro-5*H*-pyrido[3,4-*d*]azepines (**60a** and **60b**) showed high affinity for nicotinic receptors in rat brain (without cerebellum). The pyrido[3,4-*c*]azepines (**61a** and **61b**) had much less affinity for the receptors (cf. compound 59). These workers (108) reported additional 3-substituted pyridine systems in which the substituent was ω-aminoalkyl, aminopropenyl, aminopropynyl, and aminoethoxy. Some of these derivatives bound to nicotinic receptors, but it was not possible to arrive at structure-activity conclusions from these data.



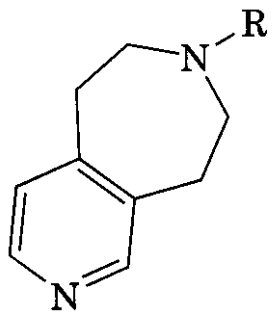
(57) R = —N



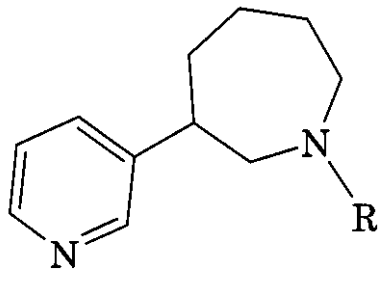
(58) R = —N



(59) R =

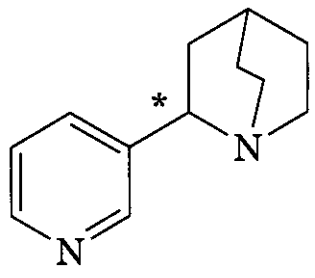


(60a) R = H
(60b) R = CH_3



(61a) R = H
(61b) R = CH_3

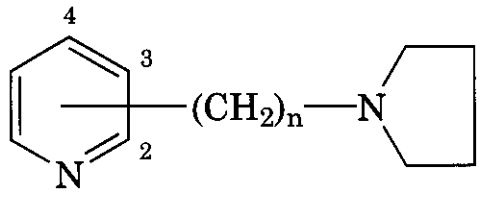
The R-enantiomer of the pyridylquinuclidine (62) has a slightly higher affinity for $\alpha_4\beta_2$ nicotinic sites than the S-enantiomer (109).



(62)

The S-enantiomer is 20 times less potent at ganglionic subtypes than is the R.

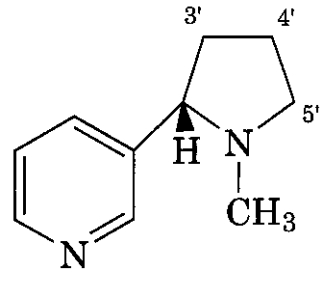
Further modifications of compound (**57**) are illustrated in structure (**63**).



(63)

When n = 2, all positions of attachment to the pyridine ring (carbon 2, 3, or 4) result in extremely low potency and activity. When n = 1, attachment to positions 2 and 4 produces practically inert compounds (102). Replacement of the pyridine ring of structure (**57**) by bioisosteric benzene, 2-thienyl, 2-furanyl, and 2-pyrrolyl ring systems abolishes almost all nicotine-like activity.

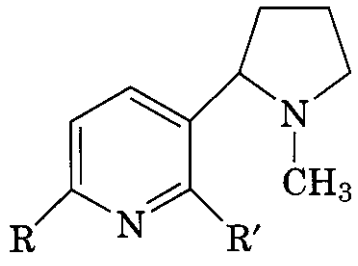
The findings that S-nicotine ameliorates some of the symptoms of the Alzheimer syndrome (110) and has a neuroprotective effect in animals (111) have stimulated increased interest in nicotine analogs and congeners. Members of a series of 3'-, 4'-, and 5'-substituted nicotine analogs (64) were evaluated as



(64)

ligands for a nicotinic receptor from rat brain membranes (112).

Only a small substituent is tolerated at position 4'; the (2′-*S*,4′-*R*)-methyl congener is the most potent of the entire series, but it is somewhat less potent than S-nicotine itself. None of the 3'- or 5'-substituted analogs approach this binding affinity. 2-Chloronicotine (65) is exponentially less potent than nicotine in an $\alpha_4\beta_2$ nicotinic receptor binding assay, whereas 6-chloronicotine (66) is twofold more potent

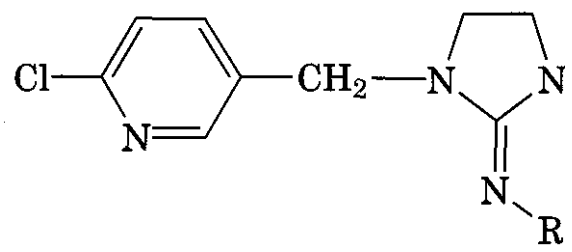


(65) R = H; R′ = Cl
(66) R = Cl; R′ = H

than nicotine (113). The stereochemistry of these chloronicotines was not addressed.

From **a** study of insecticidal nicotinic agents (114) imidacloprid (67) was reported to

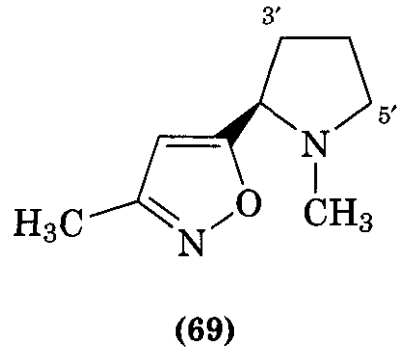
act selectively at the insect versus the mammalian nicotinic acetylcholine receptor(s). In contrast, the desnitro compound (**68**), a mam-



(67) R = NO_2
(68) R = H

malian minor metabolite of (**67**), is selective for the mammalian versus the insect nicotinic receptor(s) and it is similar to (−)-nicotine in potency in a mouse brain binding assay (115).

In a series of substituted 2-arylpyrrolidines in which the substituted aryl group is a bioisosteric replacement for the pyridine ring of nicotine, it was found that the isoxazole derivative (69) is a potent cholinergic channel activator (116).



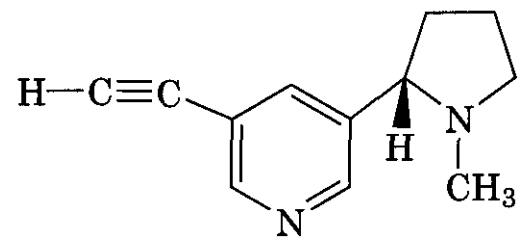
(69)

All substitutions on the pyrrolidine ring diminished the binding affinity compared with that of compound (**69**). The primary metabolism of compound (69) involves oxidation at the 5'-position. It was therefore unexpected that the 5'-methyl congener of compound (69) has in vitro half lives equivalent to or shorter than those of (69) itself.

Nicotine decelerates aging of nigrostriatal dopaminergic neurons (8) and it has been suggested that nicotine may relieve the symptoms of Parkinson's disease (7).

5-Ethynylnicotine (**70**) was designed as a potential anti-Parkinsonian agent (9).

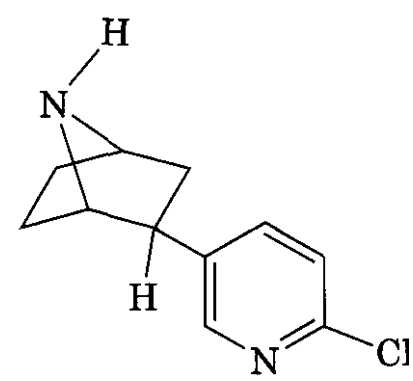
This compound was more potent than nicotine in releasing dopamine from rat striatal slices. The (*S*)-compound was the more potent



(70)

and active of the enantiomeric pair. Its effects were blocked by the nicotinic antagonist mecamylamine.

Antinociceptive (analgesic) activity of (*S*)-(−)-nicotine has been observed and documented in many animal species (117). (−)-Epibatidine (**71**), extracted from the skins of a



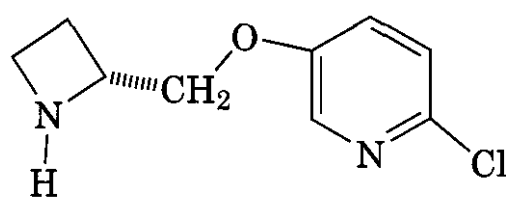
(71)

South American frog, Epipedobates tricolor, is 200–400 times more potent than morphine as an analgesic (118).

The stereochemistry of (−)-epibatidine (1*R*,2*R*,4*S*) has been established by several total syntheses, of which ref. 119 is representative. The antinociceptive effect of (−)-epibatidine is antagonized by pretreatment with mecamylamine (120) (a ganglionic blocking agent), but not by naloxone (121) nor, surprisingly, by hexamethonium (122). It is established (120,121) that epibatidine is a nicotinic receptor agonist. Both enantiomers of epibatidine [(+)-epibatidine is synthetic] had potent antinociceptive activity (121), and the only central receptors at which potent affinity was found were nicotinic. Epibatidine did not bind to μ, κ, or δ analgesic receptors, nor to muscarinic or 5-HT, receptors. There was no significant binding to adenosine, adrenergic, dopaminergic, GABA, substance P, cholecystokinin, NMDA, or σ receptors (121). Epibatidine has very high affinity for the major nicotinic subtype in the brain, $\alpha_4\beta_2$ (123).

However, the alkaloid is nonselective in its actions on nicotinic receptors, including the ganglionic ($\alpha_3\beta_x$) and neuromuscular ($\alpha_1\beta_1\delta\gamma\varepsilon$) subtypes, as a consequence of which there is a very narrow therapeutic index between beneficial analgesic actions and toxic and ultimately lethal actions on the cardiorespiratory system. Replacement of the chlorine atom of (+)-epibatidine with hydrogen resulted in retention of comparable affinity for nicotinic sites, whereas replacement with methyl or iodine lowered affinity (118).

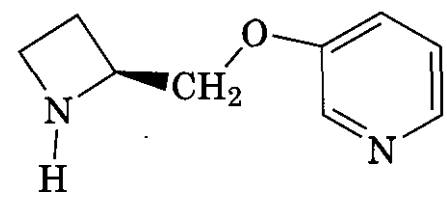
ABT-594 (**72**), described as an azetidine bioisostere of nicotine with the (*R*)-absolute



(72)

configuration, retains analgesic actions while showing a reduced propensity toward the toxic side effects seen with epibatidine (**123**).

ABT-594 was approximately 70 times more potent than morphine in a spectrum of acute and chronic nociceptive models, and unlike morphine, it showed no evidence of tolerance or opioid-like dependence liability, nor did it show effects on respiration or gastrointestinal motility. It is said (113) not to demonstrate the addictive effects of nicotine. The (*S*)-enantiomer of ABT-594 also showed potent analgesic activity and, like the (*R*)-enantiomer, it was active after intraperitoneal or oral administration (124). Structure-activity studies suggested that the N-unsubstituted azetidine moiety and the 2-chloro substituent on the pyridine ring are important contributors to the potent analgesic activity. The deschloro compounds (73) and (74) possess subnanomolar affinity for brain nicotinic receptors (**125**), but no analgesic testing data were reported for these compounds. Computational studies indicated that a reasonable superimposition of a low energy conformer of (73) with (*S*)-nicotine and (−)-epibatidine can be achieved. Further, it was concluded that the optimal internitrogen distance for binding of these types of compounds to nicotinic receptor(s) is 5.5 Å. Derivatives of (73) having a halogen atom at positions 5 or 6 in the 3-pyridyl fragment are potent nicotinic receptor ligands (126).



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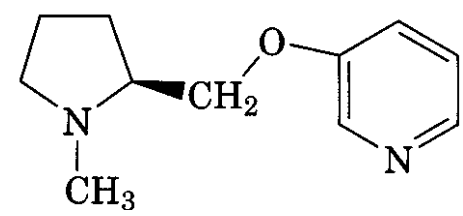
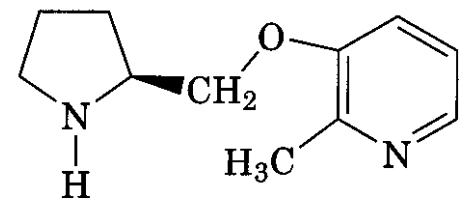


(74)

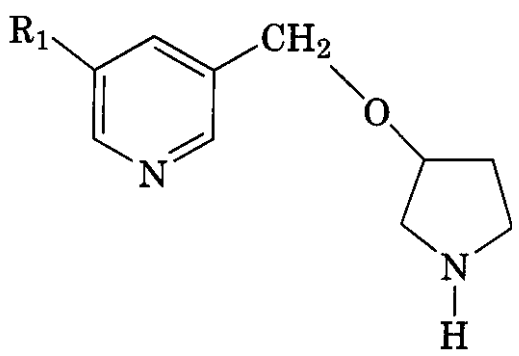
These as well as the 2-fluoro analog possess subnanomolar affinity for the receptors in rat forebrain. The 5-iodo compound showed the highest affinity, comparable to that of epibatidine. Additional 3-pyridyl ether congeners of (**73**)/(**74**) have been cited from the patent literature (109); some of these bind with high affinity to ^{3}H-cytisine and ^{3}H-epibatidine sites, but exhibit much lower affinity for ^{125}I-bungarotoxin sites. Compound (**75**), the 2-methyl congener of (**74**), was described (127) as a nicotinic receptor ligand for receptor subtypes having mainly a, and β_2 subunits. It showed positive effects in rodent and primate models of cognitive enhancement, and a reduced propensity (compared to nicotine) to activate peripheral ganglionic-like nicotinic receptors and to elicit seizures.



(75)

A series of pyridine-based nicotine congeners (76–80) in which R_1 and R_2 were H or a variety of substituents was subjected to 3D-QSAR analysis of their ability to bind to central $\alpha_4\beta_2$ receptors (128).

Bulky substituents at the 6-position of the pyridine ring reduce the affinity of the com-



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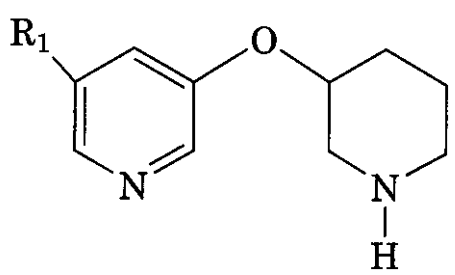

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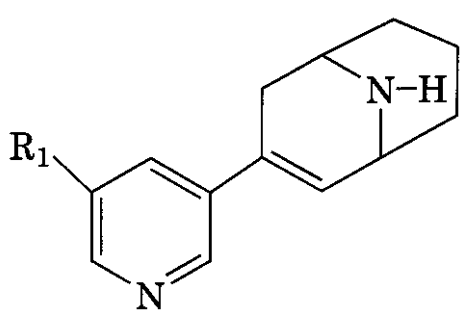

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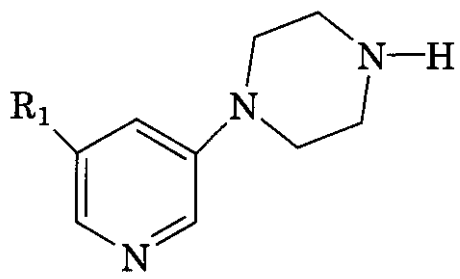

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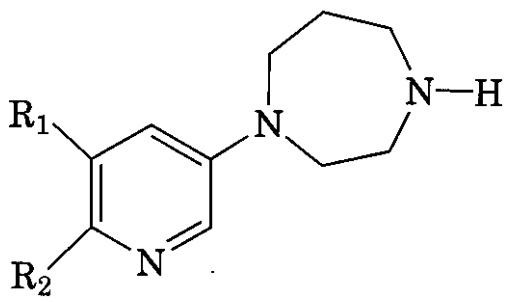

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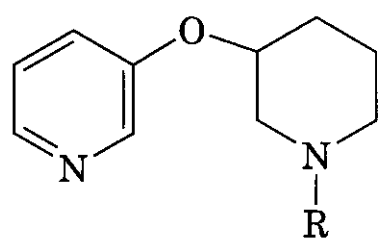

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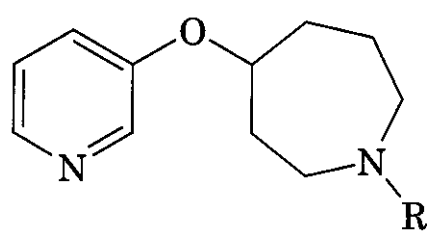


(82)

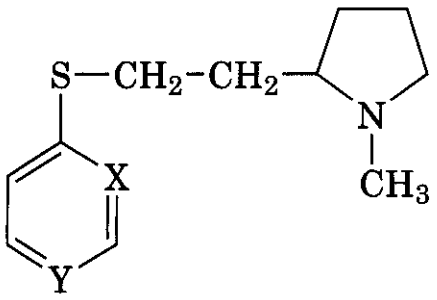
pounds, whereas bulky ring systems including an sp^3 nitrogen increase the affinity of the compounds, consistent with results reported earlier by Glennon et al. (129).

A series of pyridyl ethers, typified by (81) and (82), exhibited high binding affinity at ^{3}H-cytisine rat forebrain sites (cytisine is a nicotinic receptor partial agonist), but only moderate affinity at ^{3}H-epibatidine sites (109, 130).

A group of thio ethers (**83**), (**84**), (**85**), reported from the patent literature, was stated (109) to bind tightly to ^{3}H-nicotine receptors in rat cerebrum and to induce calcium flux through $\alpha_4\beta_2$ receptors with potency comparable to that of nicotine.

DBO-83 (86) showed powerful affinity for $\alpha_4\beta_2$ nicotinic receptors, and it demonstrated antinociceptive activity (123).

In a series of substituted 3,8-diazabicyclo[3.2.1]octane derivatives, described as structurally related to epibatidine (131), the most interesting was (**87**). This compound showed analgesic properties after subcutaneous injection, which were reversed by mecamylamine but not by naloxone. It had high affinity for binding at the $\alpha_4\beta_2$ nicotinic subtype, but it had no effect at the myoneural junction. The S-(−)-spiro compound (88) is a potent full agonist in vitro at the rat α_4 nicotinic receptor (132); it is highly selective over the α_3 subtype. However, even minor changes in structure result in significant loss in α_4 affinity. A synthetic analog (89) of the marine worm toxin anabaseine (90) is "functionally selective" for the α_4 nicotinic receptor



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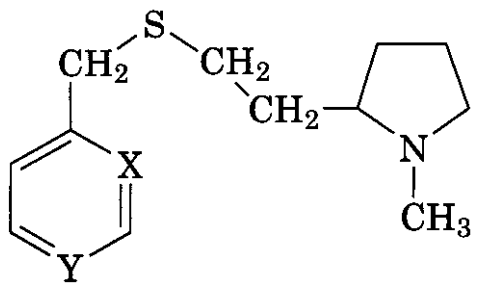

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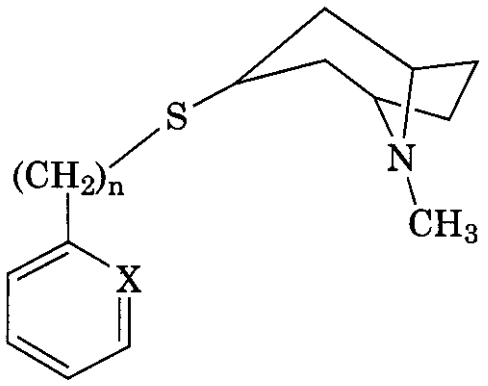
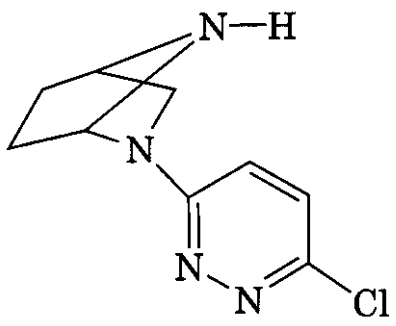


(85)

(83), (84), (85) X or Y = N



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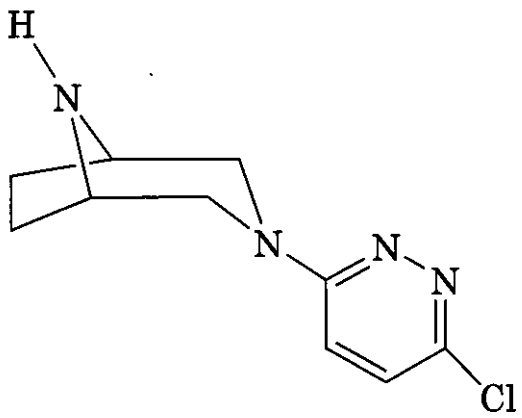
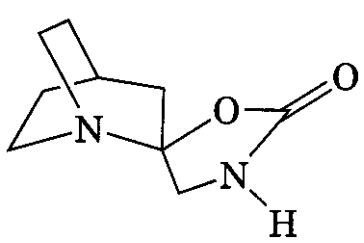
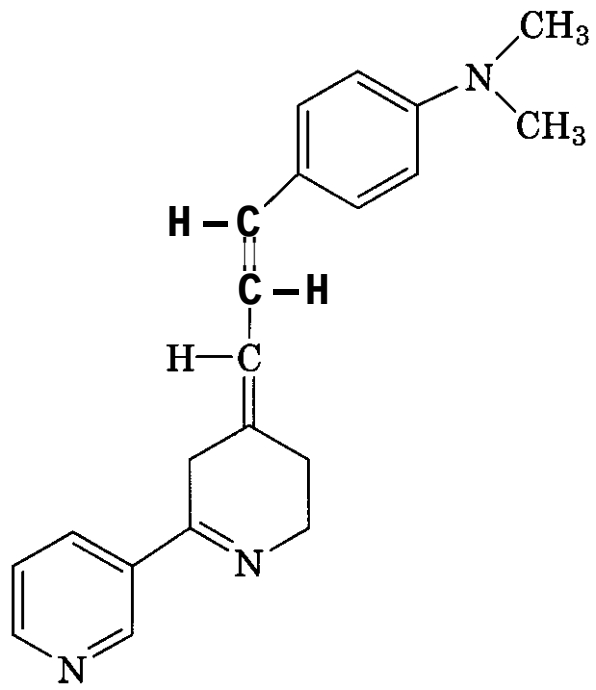


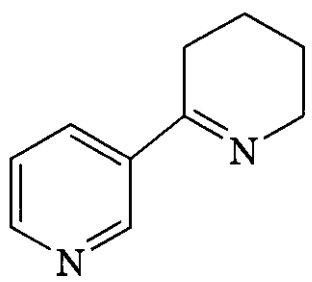
(87)



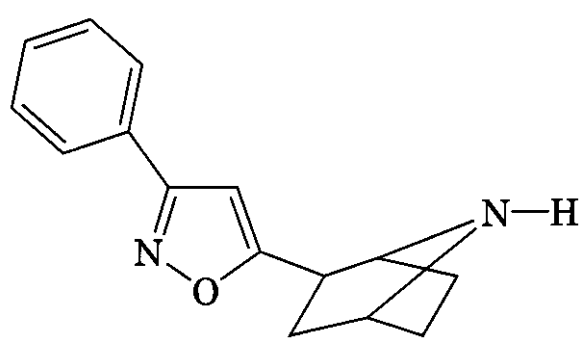
(88)



(89)



(90)



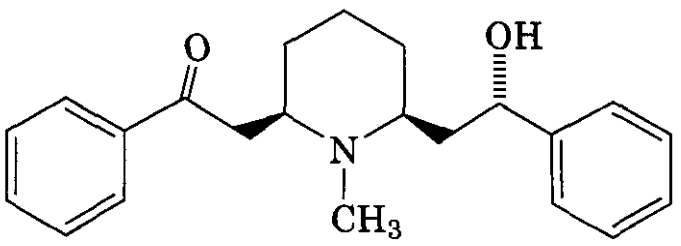
(91)

(133). The structural dissimilarity between the two a, receptor stimulants **(88)** and **(89)** is striking.

The phenylisoxazole **(91)** showed moderate binding affinity to ^{3}H-cytisine binding sites in

rat cortex, but it also demonstrated a markedly lower toxicity compared to epibatidine (109). Antinociceptive activity of this compound was not reported.

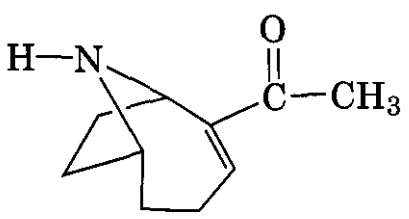
Lobeline (92), an alkaloid obtained from *Lobelia inflata*, binds with high affinity at some nicotinic receptors, and it produces some (but not all) of the effects of (−)-nicotine (134).



(92)

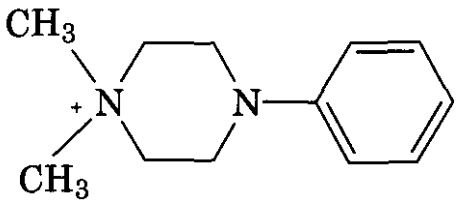
Lobeline also demonstrates antinociceptive effects, but these are complex (135): they are observed after intrathecal but not after subcutaneous administration. Subcutaneous administration of lobeline enhanced nicotine-induced antinociception in a dose dependent manner. Removal of one or both oxygen functions from lobeline permits retention of the analgesic potency and activity of lobeline itself (134). Moreover, removal of one or both oxygens diminishes, by at least 25 times, the affinity for nicotinic receptor(s) in rat brain homogenates. It was concluded that there is no direct relationship between neuronal nicotinic receptor (primarily $\alpha_4\beta_2$ type) affinity and analgesia as measured by the tail-flick assay.

(+)-Anatoxin-A (**93**), produced by a fresh water cyanobacterium, is more potent than nicotine or acetylcholine in stimulation of some subpopulations of nicotinic receptors (136). The compound also stimulates release of acetylcholine from hippocampal synaptosomes.



(93)

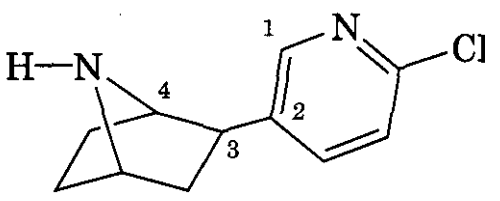
1,1-Dimethyl-4-phenylpiperazinium (DMPP) (**94**), like nicotine, is an autonomic ganglion



(94)

stimulant. However, unlike nicotine, the initial stimulation is not followed by a dominant blocking action (137).

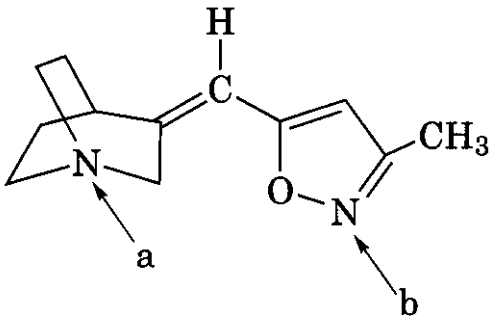
Applying an extension of techniques of distance geometry to analysis of a number of known nicotinic receptor agonists, Sheridan et al. (138) proposed a definition of the nicotinic agonist pharmacophore, the essential features of which were proposed to be: A, a cationic center; B, an electronegative atom; and C, an atom that forms a dipole with B. It was concluded that there is only one arrangement possible for superimposition of these essential groups: a triangle having dimensions A-B (4.8 Å), A-C (4.0 Å), and B-C (1.2 Å). Further consideration of this proposed nicotinic agonist pharmacophore by computational chemical studies of nicotine, epibatidine, and a series of oxazolyl-azabicycloalkanes (139) led to the conclusion that future modeling of nicotinic agonists should utilize the global energy *mimimum* of epibatidine as the reference structure, in which the torsion angle $\tau = 7°$ (structure **95**).



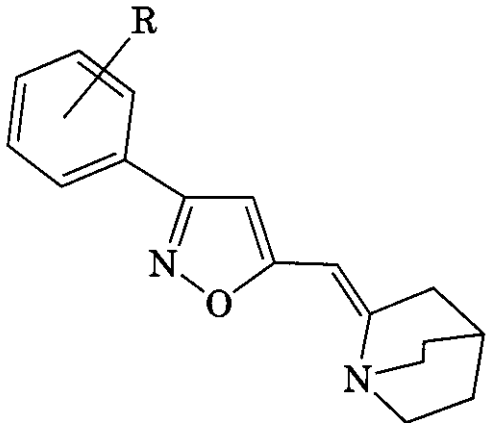
(95) $\tau = C_1—C_2—C_3—C_4$

With respect to the oxazolylazabicyclooctanes (structure **96**), the optimum a-b distance was concluded to be 7–8 Å. Additionally, it was concluded that the receptor area is sensitive to changes in steric bulk and electrostatic potential in the aliphatic area around the sp^3 nitrogen.

Numerous nicotinic ligands interact with muscarinic acetylcholine receptors, and vice versa (109). Compounds (97) and (98) are examples of this varying selectivity: the nicotinic/muscarinic selectivity ratio in these systems depends on the size of the substitution on



(96)



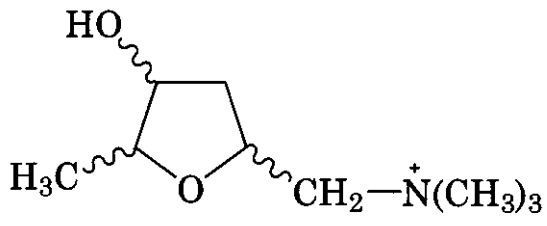
(97) R = 3,4-dihydroxyphenyl
(98) R = 3,4-di[OCH_2(4-methoxyphenyl)]

the hydrogen bond acceptor geometrically opposite to the cationic moiety. Smaller substitutions favor nicotinic binding, whereas larger substitutions favor muscarinic binding (140).

Additional recent advances in nicotinic molecular design and therapies are reviewed in Ref. 109.

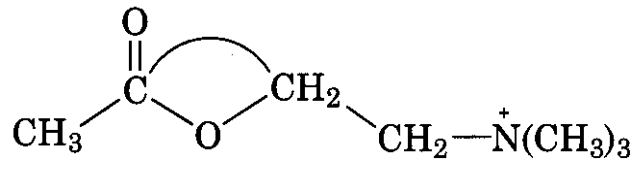
4.2 Muscarine, Muscarone, and Related Compounds

The muscarine molecule (3) may be viewed as a cyclic analog of acetylcholine in which the carbonyl and β-carbons are linked by a bimethylene bridge (cf. structures 99 and 100).



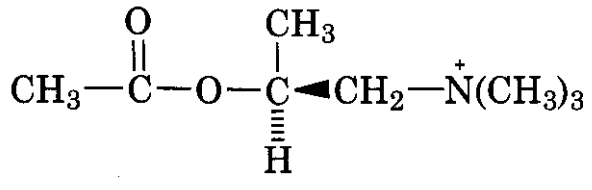
(99)

Naturally occurring (+)-muscarine is one of eight stereoisomers of structure (99). The (−)-enantiomerof (+)-(2*S*,3*R*, 5*S*)-muscarine



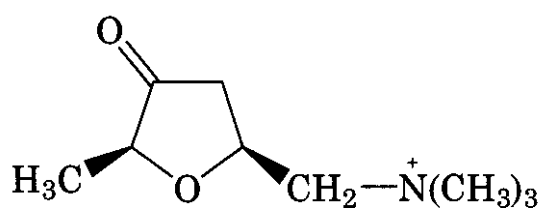
(100)

(3)is almost inert, as are both enantiomers of the other three diastereomers of structure (99) (epimuscarine, allomuscarine, and epiallomuscarine) (141). A point of interest is the absolute configurational identity of the C2 position of muscarine (3)and of the S-(+)-eutomer of acetyl β-methylcholine (101).



(101)

The oxidation product of (+)-(2*S*,3*R*,5*S*)-muscarine, muscarone (102), shows even more structural analogy to the acetylcholine



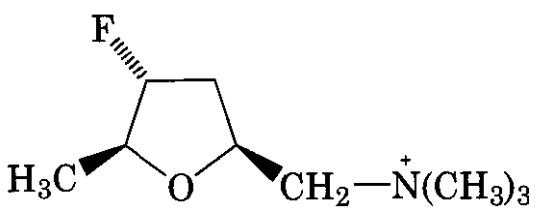
(102)

molecule; it is an active muscarinic agonist, and it also exhibits a nicotinic component of activity not possessed by muscarine.

For many years, the literature (e.g., Ref. 142) consistently presented misleading and/or incorrect information concerning the stereochemistry of the muscarone molecule, and there were accompanying hypotheses, based on this misinformation, rationalizing the seemingly confusing relationship of the absolute configuration of muscarone enantiomers to their pharmacological properties. This confusion was resolved by the proof (143) that the eutomer of muscarone has chirality at C2 and C5 (2*S*, 5*S*) identical with natural muscarine. The earlier literature (141,144) had reported small eudismic ratios (2.4–10.1) for the muscarone enantiomers, in contrast to the large

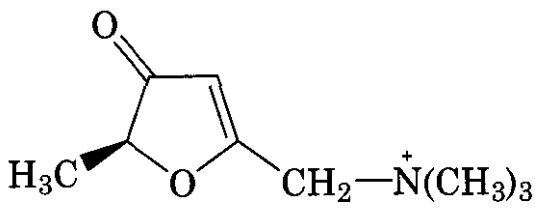
values established for muscarine enantiomers. This pharmacological inconsistency has been explained by De Amici and coworkers (145) on the basis of optical heterogeneity of the muscarone enantiomers used in the earlier studies. These workers performed enantiospecific syntheses to obtain the two muscarone enantiomers in >98% enantiomeric excess. In both binding and functional studies, (−)-(2*S*, 5s)-muscarone was the eutomer, and the eudismic ratios of the muscarone enantiomers were in the range of 280–440, which are quantitatively similar to those for muscarine.

Enantiomerically pure 4-deoxy-4-fluoromuscarines have been reported (146). The most active member of the series was the 2*S*,4*R*,5*S*-enantiomer (**103**), whose pharmacology is much like that of muscarine itself, except that the fluoro compound showed a one order of magnitude increase in affinity for cardiac M_2 receptors (those controlling rate).



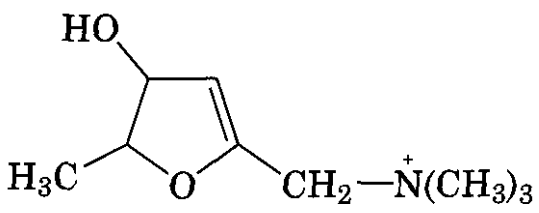
(103)

Beckett and coworkers (147) reported the approximate equivalence of muscarinic action shown by enantiomers of 4,5-dehydromuscarone (104).

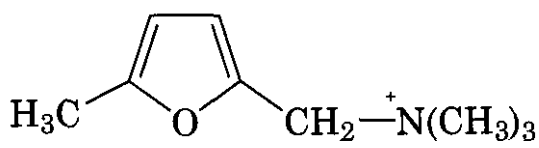


(104)

dl-Dehydromuscarine (**105**) retains considerable muscarinic agonist activity, but it shows no effects at nicotinic receptors (141). Incorporation of the elements of the muscarine structure into an aromatic ring has produced some systems, such as compound (**106**), which approach acetylcholine in muscarinic potency (41). Activity is lowered by changing



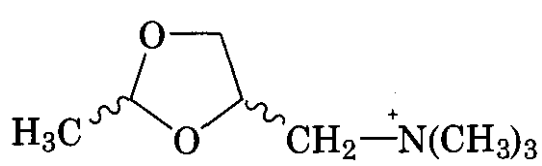
(105)



(106)

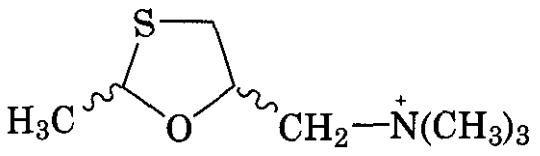
the C2 methyl of compound (**106**) to ethyl (148) or replacing the C2 methyl with hydrogen (41). The data on these furan derivatives are consistent with the rule of five.

Muscarinic activity of 2-methyl-4-trimethylammoniummethyl-1,3-dioxolane (107) resides in the cis isomer (149); stereospecific synthesis of the two enantiomers of the cis isomer revealed that the *L*-(+) enantiomer



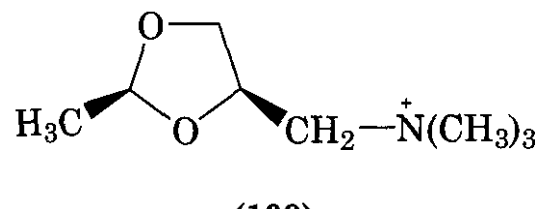
(107)

(C4 = *R*) is more than 100 times more potent than the *D*-(−) enantiomer (C4 = *S*), and is approximately six times more potent than acetylcholine in a guinea pig ileum assay. The more active L-(+) compound is related configurationally to the most potent muscarine stereoisomer (**3**), although it should be noted that several authors in the older literature incorrectly assigned the *S* absolute configuration to position 4 of the L-(+)-cis compound (**107**), apparently through misapplication of priority rules. 2,2-Dialkyl analogs of these dioxolanes are much weaker muscarinic agonists than the parent systems (**107**), and the difference in potency between the C4 R and *S* enantiomers diminishes sharply with increasing size of substituents at C2 (150). Both enantiomers of the cis- and trans-oxathiolane system (**108**), bioisosteres of the dioxolanes, were evaluated for nicotinic and muscarinic effects (151).



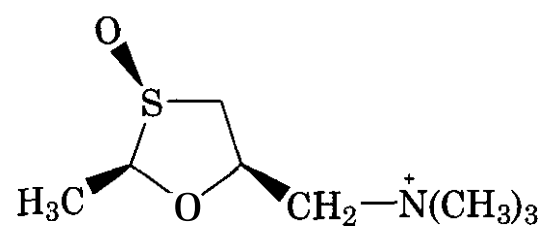
(108)

The (+)-cis isomer of (108) was the most potent muscarinic of the series. It demonstrated a eudismic ratio of the same high order of magnitude as that for muscarine and the dioxolanes. This (+)-cis enantiomer has the same absolute configuration as the muscarinically most active L-(+)-muscarine (2) and the (+)-*cis*-dioxolane (109). The other isomers represented by structure (**108**), although much less potent than the (+)-cis isomer, also demonstrated a degree of muscarinic agonist effect. All four isomers of structure (108) showed similar nicotinic potency and activity, close to that of carbamyl choline, and eudismic ratios were small.

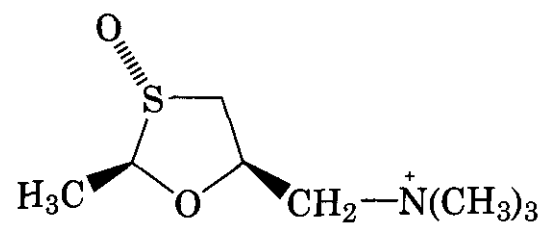


(109)

Studies of the diastereomeric *cis*-sulfoxides (110) (2*R*,3*S*,5*R*) and (**111**) (2*R*,3*R*,5*R*) indicated that compound (**111**), which has



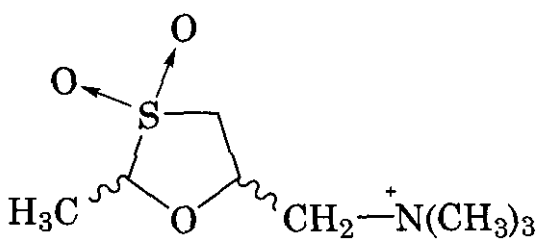
(110)



(111)

the same absolute configuration as (+)-(2*S*,3*R*,5*S*)-muscarine (3), is a potent and selective muscarinic agent with a large eudismic ratio (152). (Note that the presence of the sulfur atom reverses the R,S designations of the chiral centers, compared with muscarine.)

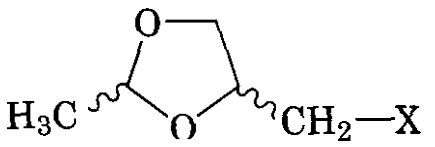
Compound (110) is exponentially less potent than (**111**) at muscarinic receptors. Both compounds demonstrate low nicotinic potency and activity. The sulfone congeners (**112**) of the enantiomers (2*R*,5*R* and 2*S*,5*S*) of the *cis*-structure are weak muscarinics with a eudismic ratios of unity.



(112)

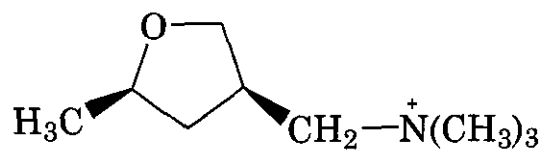
Neither is an extremely potent nicotinic, although the 2*R*,5*R* enantiomer is more potent than the 2*S*,5*S*.

The cis and trans isomeric mixtures of dioxolane congeners bearing sulfur, phosphorus, or arsenic cationic heads (113–115) display lower muscarinic effects than the corresponding nitrogen system (153).

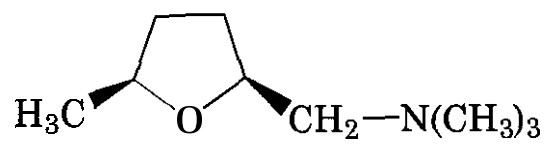


(113) X = $^{+}S(CH_3)_2$
(114) X = $^{+}P(CH_3)_3$
(115) X = $^{+}As(CH_3)_3$

Both of the racemic cis-1- and cis-3-desetherdioxolane compounds (**116** and **117**) demonstrate muscarinic activity not substan-



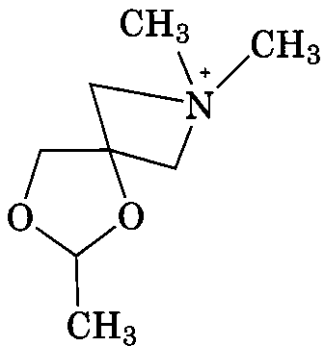
(116)



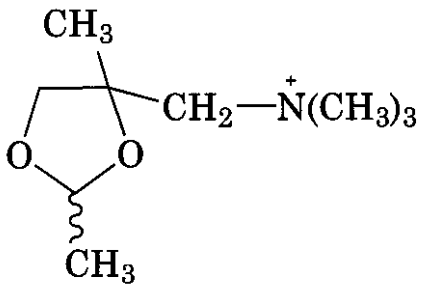
(117)

tially lower than that of the "supermuscarinic" *L*-(+)-*cis*-dioxolane (**109**) (153). It was suggested (154) that occupation of only one of the two receptor subsites proposed to be reacting with the ring oxygens of the cis-dioxolane (109) is sufficient to induce muscarinic activity.

The moderately high potency (1110 acetylcholine) of the spirodioxolane (118) compared with the low muscarinic potency (1/300 acetylcholine) of a mixture of isomers of a more flexible system (**119**), led Ridley and coworkers (155) to speculate that the three-dimensional geometry imposed by the molecular rigidity of compound (**118**) may approximate the shape of the L-(+)-cis-dioxolane (109) when it binds to muscarinic receptor(s).



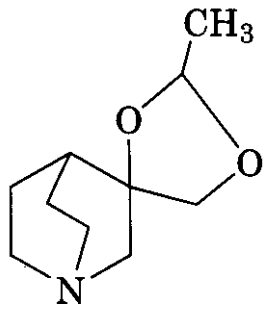
(118)



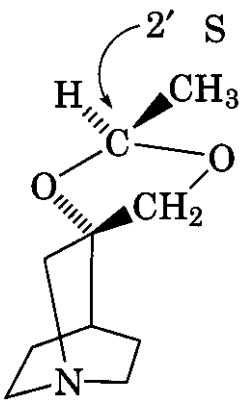
(119)

A more complex spirodioxolane molecule (**120**), bearing a tertiary amine rather than a quaternary moiety, was resolved (156). The 3*R*,2′*S* isomer (121) was more potent in binding studies, but the 3*R*,2′*R* isomer (**122**) displayed larger selectivity between M_1 receptors (ganglion) and M_2 receptors (heart).

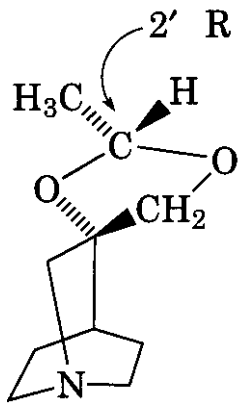
The 3R stereochemistry of the most potent isomers (121) and (122) is consistent with that of other potent 1,3-dioxolane derivatives. Extension of these studies (157) led to a racemic 1,2,4-oxadiazole derivative (**123**) that has high affinity and efficacy at central muscarinic receptors.



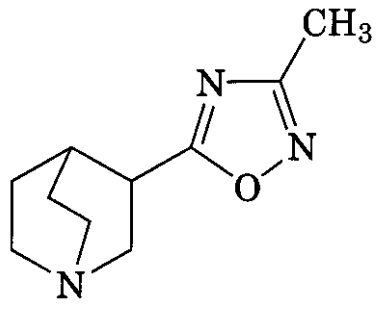
(120)



(121)

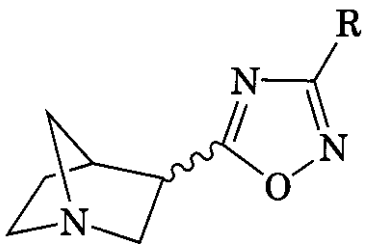


(122)

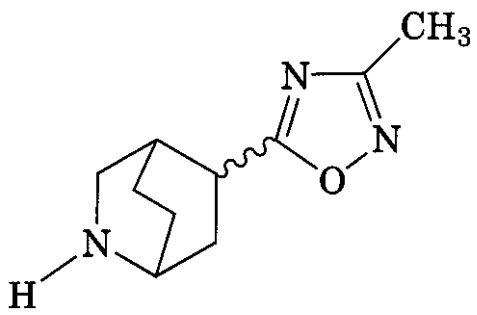


(123)

Additional 1,2,4-oxadiazole derivatives containing 1-azanorbornane (**124a** and **124b**) and isoquinuclidine (125) rings were studied (158). These compounds can exist as geometric isomers, and the exo-1-azanorbornane isomer (**124b**) was described as one of the most potent and efficaceous muscarinic agonists known.

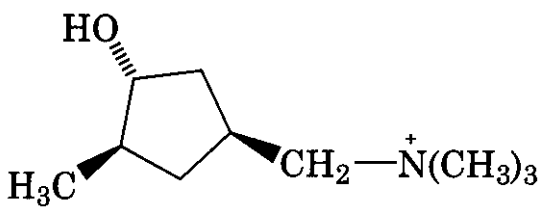


(124a) R = CH_3
(124b) R = NH_2



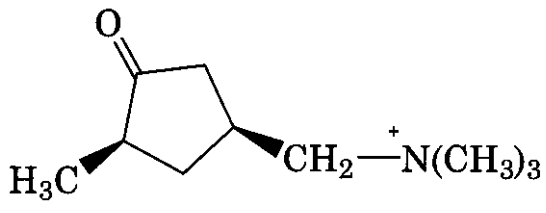
(125)

A carbocyclic muscarine analog, (±)-desethermuscarine (**126**), exhibits striking muscarinic effects (159), although the compound



(126)

is considerably less potent than was originally reported (160). The other three stereoisomers of desethermuscarine (epi-, allo-, and epiallo-) are weaker cholinergic agents (161,162). Two attempts (159, 163) to obtain (±)-desethermuscarone (**127**) resulted in inseparable mix-



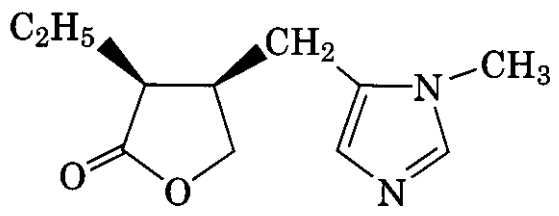
(127)

tures of epimers, which were reported (159) to be equipotent to acetylcholine in assays for nicotinic and muscarinic effects. The high potencies of desethermuscarine (126) and of the mixture of desethermuscarone epimers suggest that the ring oxygens in muscarine and muscarone may not play a critical role in agonist-muscarinic receptor interactions. Beckett and coworkers (147) had suggested the prime importance of the keto group of muscarone (compared with the ring oxygen), and the importance of the ring C-methyl group has been cited previously.

Certain cyclohexane analogs of desethermuscarine and desethermuscarone showed greatly diminished muscarinic activity (164). These compounds, however, lacked the presumably important ring C-methyl group.

4.3 Pilocarpine and Analogs and Congeners

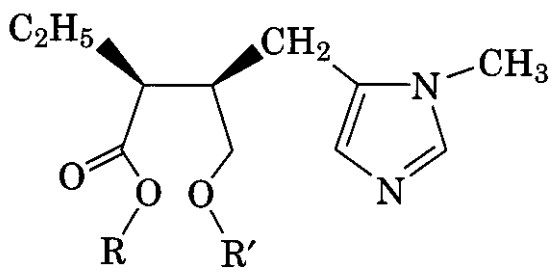
Pilocarpine (**128**), the chief alkaloid from the leaflets of shrubs of the genus *Pilocarpus*, has a dominant muscarinic action, but it causes



(128)

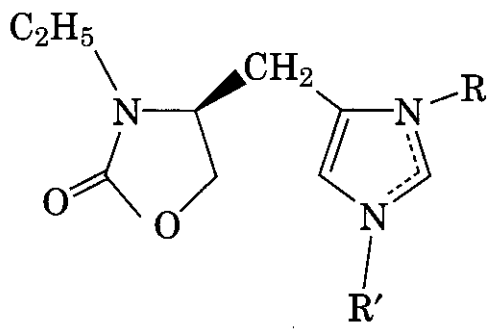
anomalous cardiovascular responses, and the sweat glands are particularly sensitive to the drug (165).

Its structure is distinguished by the lack of a quaternary ammonium head; however it is presumed that a nitrogen-protonated cation is the biologically active species. Pilocarpine has been described as a muscarinic partial agonist (166). Structural and conformational analogies and interatomic distance similarities between pilocarpine and muscarinic agonists such as acetylcholine, acetyl β-methylcholine, muscarine, and muscarone have been invoked (167) to rationalize pilocarpine's pharmacologic properties. The potential utility of pilocarpine in treatment of glaucoma is limited by its low ocular bioavailability. A double prodrug strategy (168, 169) involves hydrolytic cleavage of the lactone ring of pilocarpine and esterification of the freed carboxyl and alcohol groups (**129**) to produce derivatives with a greatly enhanced lipophilic character. In the presence of human plasma or rabbit eye homogenates, pilocarpine was reformed from these derivatives in quantitative amounts because of the action of tissue esterases.



(129)

Cyclic carbamate analogs (**130–132**) of pilocarpine were designed (170) to extend pilocarpine's duration of action, which is a reflection of its metabolic inactivation by hydrolytic cleavage of the lactone ring.



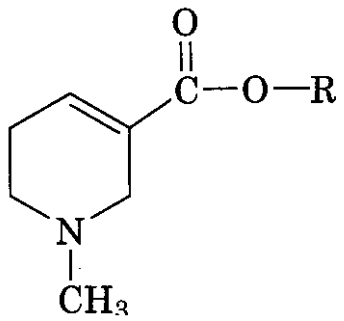
(130) R = CH_3; R′ = H
(131) R = R′ = H
(132) R = H; R′ = CH_3

Analog (**130**), having the same substitution pattern as pilocarpine, was equipotent to pilocarpine in a guinea pig ileum assay. In *vitro*, base-catalyzed epimerization of pilocarpine at the C-ethyl group position forms the diastereomer isopilocarpine in which pharmacologic activity is lost (171).

4.4 Arecoline and Analogs and Congeners

Arecoline (**133a**), an alkaloidal constituent of the seeds of Areca catechu, is a cyclic "reverse ester" bioisostere of acetylcholine (cf. compound 22).

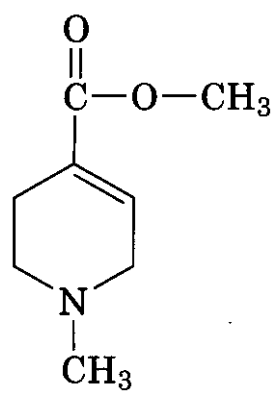
In contrast to pilocarpine, arecoline acts at nicotinic receptors as well as at muscarinic sites; it has been described (172) as a partial agonist at M_1 and M_2 receptors. Arecoline is equipotent to its quaternary analog, *N*-methylarecoline, as a muscarinic agonist (172). The secondary amine, norarecoline, is a somewhat weaker muscarinic agonist than arecoline (173,174). The muscarinic activity of esters of arecaidine (the free carboxylic acid derivative



(133a) R = CH_3
(133b) R = C_2H_5
(133c) R = n-C_3H_7
(133d) R = CH_2—CH=CH_2
(133e) R = CH_2—C≡CH

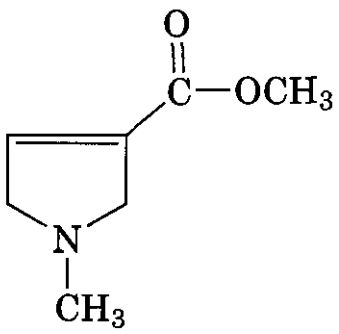
of arecoline) varies with the nature of the alcohol: from the methyl ester (**133a**) to the ethyl (**133b**), the affinity for the muscarinic receptor(s) increases (175), but there is a sharp drop in affinity and intrinsic activity with the n-propyl ester (**133c**). However, the allyl ester (**133d**) is more potent and active than the n-propyl (although less potent than the assay's reference standard compound, carbamyl choline); the propargyl ester (**133e**) is more active than carbamyl choline and indeed was described (175) as a more potent muscarinic agonist than acetylcholine. Data were presented suggesting that the triple bond of the propargyl ester contributes to receptor binding. Reduction of the ring double bond in the methyl (**133a**) (arecoline) and ethyl (**133b**) esters of arecaidine causes a 250 to 1000 times reduction in muscarinic receptor affinity.

The ester group positional isomer (**134**) is less potent and less active than arecoline in the guinea pig ileum assay (176), and the *N,N*-dimethyl quaternary derivative of (134) is slightly more potent than the tertiary amine.



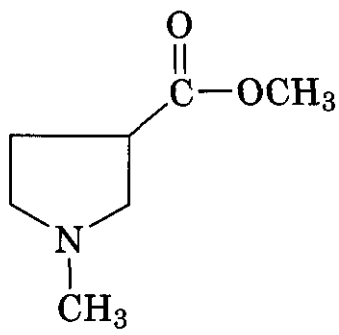
(134)

The five-membered ring congener (**135**) of arecoline is approximately 50% as **muscarinically** potent as arecoline in a guinea pig ileum assay, and the five-membered ring analog



(135)

(136)of dihydroarecoline is approximately 1% as potent as arecoline in this assay (175). The sulfur bioisostere (**137**) of arecoline (R = CH_3) is more potent and active than its *N,N*-dimethyl quaternary ammonium congener, being approximately equipotent to arecoline itself (176). The ester group positional isomer of the sulfur bioisostere (138)(R = CH_3) retains muscarinic effects, but it is somewhat less potent and less active than the 3-substituted compound (137). In both (137) and (138)the ethyl and n-propyl esters are inferior to the methyl.



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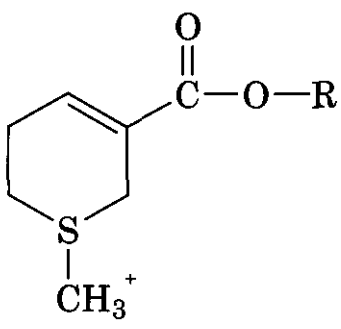
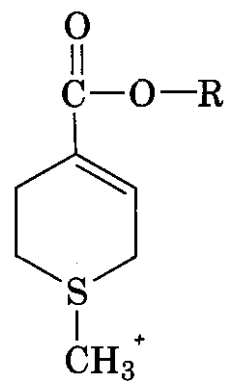


(137)

Further application of the bioisosterism strategy replaced the tetrahydropyridine ring of arecoline with tetrahydropyrimidine (**139a–e**) (177).



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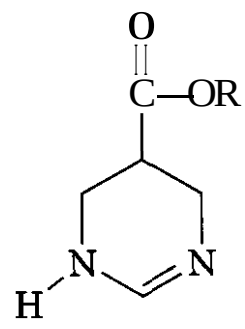
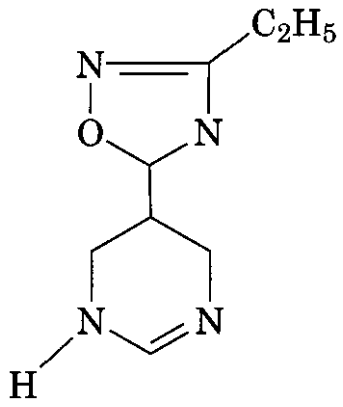


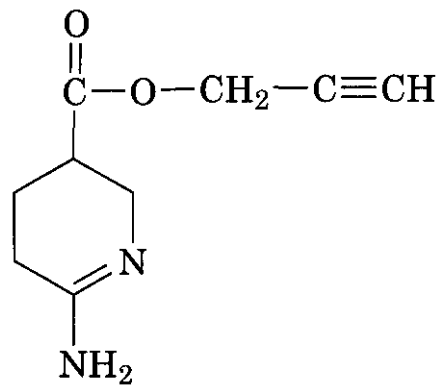
(139a) R = CH_3
(139b) R = C_2H_5
(139c) R = n-C_3H_7
(139d) R = CH_2—C≡CH
(139e) R = CH_2—C_6H_5

It was proposed that the amidine moiety of the tetrahydropyrimidine ring would be a suitable ammonium bioisostere, lacking the permanent cationic head present in a quaternary ammonium system. This might facilitate penetration of the blood-brain barrier. Of this series, the methyl ester (**139a**) shows high affinity for muscarinic receptors in rat brain, and it stimulates phosphoinositide metabolism in the rat hippocampus. It ameliorated memory deficits associated with lesions in the septohippocampal cholinergic system in rats. In a subsequent communication (178) it was reported that compounds (**139b**), (**139e**), (**140**), and (141) exhibited marked functional selectivity for M_1 vs. M_3 receptors.

The potential utility of arecoline in producing significant cognitive improvement in Alzheimer patients (179) and in enhancing learning in normal young humans and in aged nonhuman primates (180, 181) is largely negated by its short duration of action, which has been ascribed (182) to rapid in *vivo* hydrolysis of the ester group. This metabolic lability stimulated preparation of metabolically stable aldoxime derivatives (142) (183); compounds



(140)



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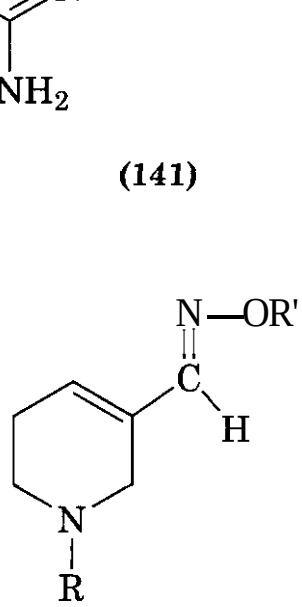
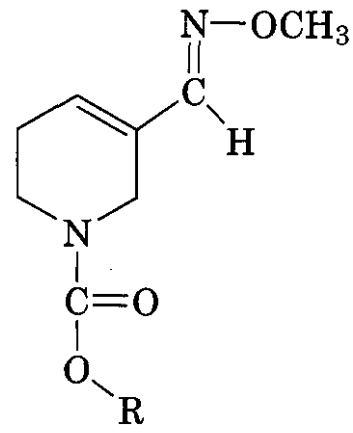


(142)

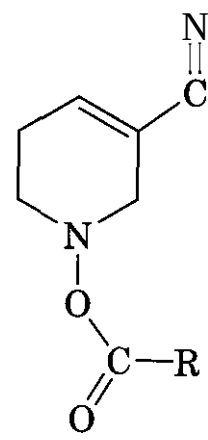
were prepared in which **R** and **R'** = alkyl, cycloalkyl, olefinic, or acetylenic groups.

Derivatives of structure (142) in which **R** = CH_3 and **R'** = CH_3 or propargyl are muscarinic agonists, both in vivo and in vitro. These compounds are two to three orders of magnitude more potent than arecoline, and they have a longer duration of action. They are orally effective. A large number of carbamate derivatives (**143**), where **R** = aryl or alkyl, were prepared as possible prodrugs to the aldoximes (142). The derivative where **R** = p-chlorophenyl had high potency and activity (181); it was more active in an assay for CNS effect than in an assay for peripheral cholinergic effect. Thus, this compound was pro-



(143)

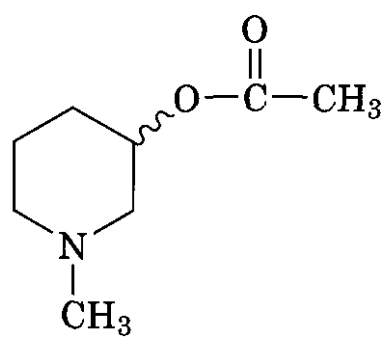
posed to demonstrate a separation of CNS from peripheral effects. Structural variations based on (**144**) in which **R'** = CH, and the R group is such that the carbonyl moiety is a



(144)

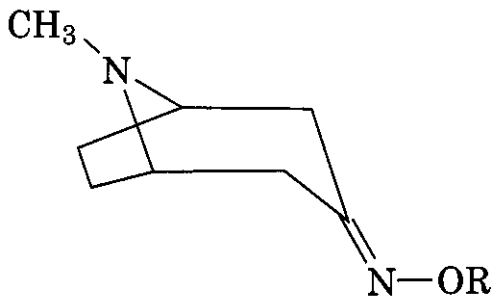
part of a carbonate, carbamate, or carboxylate ester provided compounds having little or no muscarinic activity. Some of the carbamate derivatives demonstrated in vitro cholinesterase inhibitory activity (184).

Both enantiomers of the reversed ester congener (145) of arecoline are (approximately equally) weak muscarinic agonists (175), as are their N-methyl quaternary derivatives.



(145)

Ketoxime ethers derived from structures (146)and (147) where R = H, methyl, propargyl, or C-alkyl-substituted propargyl showed M_3 agonist activity in a rat aorta preparation, but no M_2 agonist activity (185).



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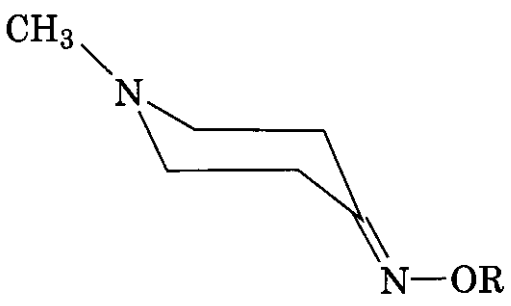
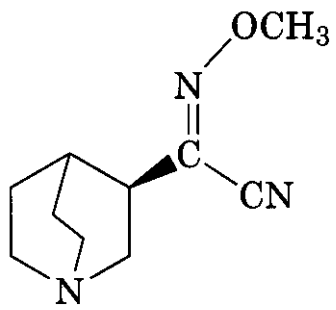


(147)

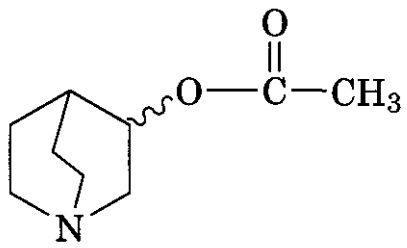
Modification of the aldoxime ether group by introduction of an electron withdrawing moiety led to the finding (186) that the *Z-N*-methoxyimidoyl nitrile group serves as a stable methyl ester bioisostere. An example of this molecular modification, compound (**148**),



(148)

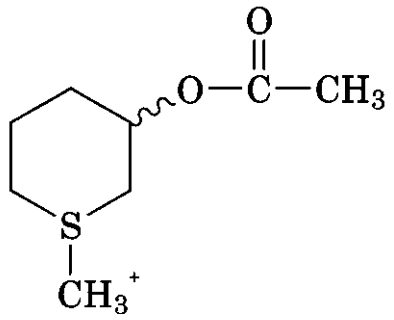
is a functionally selective M_1 partial agonist for which potential utility in treatment of Alzheimer's syndrome was suggested.

In contrast to the enantiomers of (**145**), both enantiomers of 3-acetoxyquinuclidine (aceclidine, 149) are potent muscarinics, and the S-enantiomer is only approximately one order of magnitude less potent than acetylcholine.



(149)

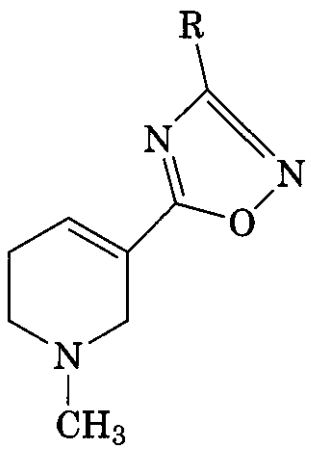
All four isomers of the thianium system (150) have been prepared and studied (175). The sulfur atom in sulfonium salts may form a chiral center, and the stereoisomers can be isolated, given that the energy barrier to pyramidal inversion is substantially higher (~100 kJ/mol) than it is in the case of the corresponding ammonium compounds (−38 kJ/mol). The (+)-trans-thianium isomer (150) demonstrated high muscarinic potency, slightly higher than that of the S-quinuclidine (**149**), but the (−)-*trans*-enantiomer (150) and the (±)-*cis*-isomer (150) demonstrated low potency. These data on the piperidine, quinuclidine, and thianium derivatives were rationalized (175) on conformational bases.



(150)

In an alternate strategy to provide resistance to in *vivo* ester cleavage of arecoline (187), bioisosteric replacement of the methyl ester groups of arecoline and norarecoline by a 3-alkyl-1,2,4-oxadiazole ring (151)was investigated (188).

Analogs of structure (**151**), where R = unbranched C_{1-8} alkyl, are muscarinic agonists, and most of these show strong affinity in two binding assays in rat brain membranes. Derivatives of structure (**151**), in which R = a branched alkyl chain or a cyclic system, are muscarinic antagonists. Analogs in which the R group contains an ether moiety (e.g., CH_2-O-CH_3) are also muscarinic agonists, but they have lower receptor binding affinity than the



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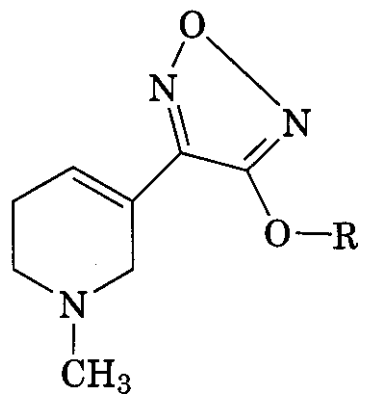

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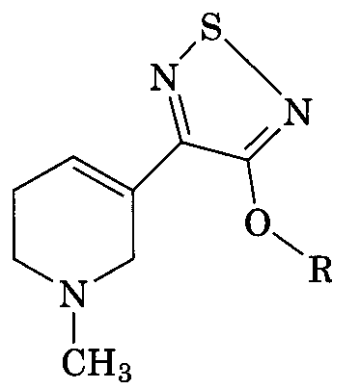
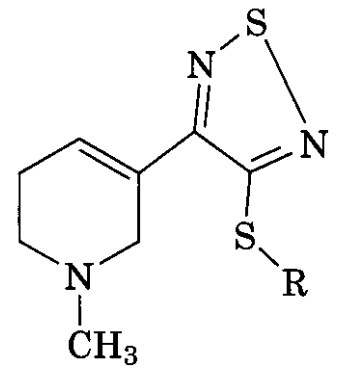


(155)

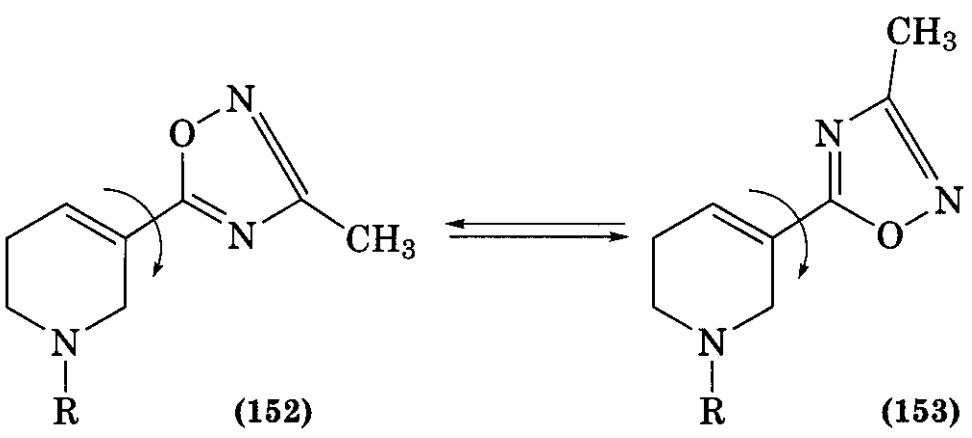


(156)

alkyl derivatives. Congeners of (**151**) in which the 1,2,5,6-tetrahydropyridine ring was replaced by quinuclidine or tropane, are potent antagonists with high affinity for central muscarinic receptors. Introduction of a methyl substituent at position 5 or 6 of the tetrahydropyridine ring of (151) (R = n-C_4H_9) destroyed agonist effect and produced a muscarinic antagonist in the single example reported. The N-desmethyl analog of (**151**), where R = n-butyl, retains potent muscarinic agonism. Additional members of the series of derivatives of (151) have been reported (189), and molecular mechanics calculations indicated a preference for the E rotameric form (152).

In continuation of efforts to identify M_1-selective muscarinic agonists capable of crossing the blood-brain barrier, the 3-carbomethoxy group of arecoline was replaced by bioisosteric 1,2,5-oxadiazole (154) or by 1,2,5-thiadiazole rings with oxygen ether substituents at position 3 (155) or with thioether substituents at position 3 (156) (190).

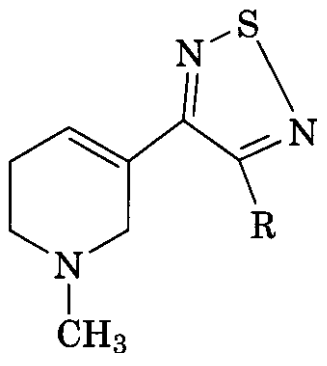
The ring-oxygen bioisosteres (**154**) (R = n-butyl or n-hexyl) show low affinity for central muscarinic receptors. However, all members of the thiadiazole oxygen ether series (**155**), where R varied from CH, through n-C_8H_{15}



(152) E Rotamer ⇌ (153) *Z* Rotamer

and also included some branched chain C_6 alkyl groups, demonstrated high potency in displacing tritiated oxotremorine-M (a nonselective muscarinic agonist) and tritiated pirenzepine (a selective M_1 antagonist) from rat brain membrane tissue. The n-butyloxy and n-pentyloxy substituents provided maximal pharmacological effects. A subsequent communication (191) reported that a derivative of (155) in which R = n-hexyl (xanomeline) is an M_1 agonist with potential value in treatment of Alzheimer's syndrome. The primary site of oxidative metabolism of xanomeline is the hexyloxy side chain (**192**), and a secondary metabolic process involves N-demethylation. To prevent this oxidative N-demethylation, the N-methyl group was incorporated into a series of azabicyclic and tricyclic systems, illustrated by (159) and (**160**).

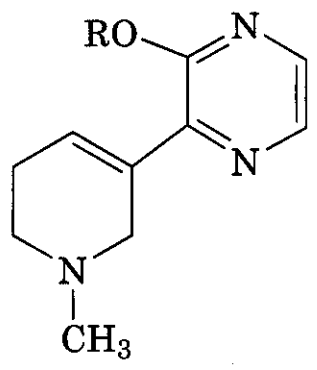
Some members of the series exhibited selectivity for M_1 receptor binding. The alkylthio analogs (156) demonstrated a similar structure-activity relationship to the alkoxy series (155); however, the thio ethers have higher receptor affinity and higher potency. Thus, these systems (155) and (156) show a higher degree of selectivity for M_1 receptors than for M_2. The unsubstituted system (**157**) (R = H) is a potent but nonselective muscarinic agonist. Derivatives of (**157**), where R = n-propyl, n-pentyl, n-heptyl, or n-octyl, have 10 to 100 times less affinity for central muscarinic receptors than the corresponding alkoxy and alkylthio derivatives.



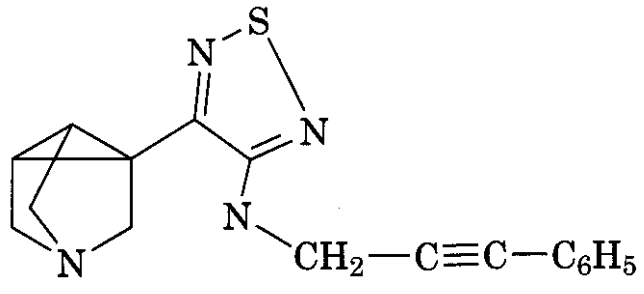
(157)

Compound (**156**) was reported (193) to be a potent antinociceptive agent, but there was little difference between an analgesic dose and one producing salivation. Analogs of (156) were prepared (194) in attempts to separate antinociceptive effects from undesirable muscarinic side effects (salivation, tremors). These efforts were unsuccessful. As a result of a concurrent study of rigid, conformationally restricted analogs of (**156**), it was proposed that the biologically active conformation of (156) has a torsion angle τ C3-C4-C3′-N2′ "close to 180°."

Study of a series of arecoline congeners (158) in which the carbomethoxy group is replaced by a pyrazine moiety (R = CH_3—C_7H_{15}) revealed that M_1 agonist activity is related to chain length, with n-hexyl providing maximum activity (195). A comparison of M_1 agonist efficacy of these pyrazines and related 1,2,5-thiadiazoles (155) and 1,2,5-oxadiazoles (154) suggested that M_1 efficacy may be related to the magnitude of electrostatic potential located over the nitrogens of the respective heterocycles. The heteroatom directly attached to the 3 position of the pyrazine or of the 1,2,5-thiadiazole markedly influences the M_1 efficacy of the compounds by determining the energetically favorable conformers for rotation about the bond connecting the tetrahydropyridyl ring and the heterocycle. A three-dimensional model for the M_1 agonist pharmacophore was proposed as a result of these studies.

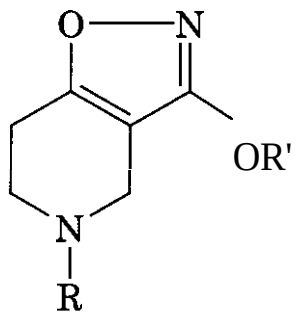


(158)



(159) X = O
(160) X = S

The tetrahydropyridine ring was fused with a 3-alkoxyisoxazole moiety in a series of compounds (**161a–g**) **(174).** Both (**161a**) and

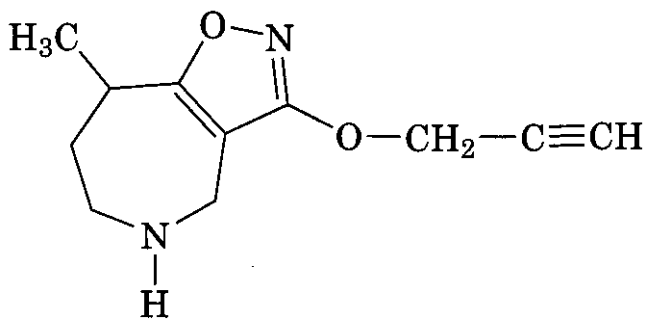


(161a) R = R′ = CH_3
(161b) R = H; R′ = CH_3
(161c) R = H; R′ = C_2H_5
(161d) R = H; R′ = n—C_3H_7
(161e) R = H; R′ = n—C_4H_9
(161f) R = H; R′ = CH_2—CH=CH_2
(161g) R = H; R′ = CH_2—C≡CH

(**161b**) are muscarinic agonists in a guinea pig ileum assay, and as with arecoline and norarecoline, the N-methyl tertiary amine congener (**161a**) is somewhat more potent than the nor derivative (**161b**).

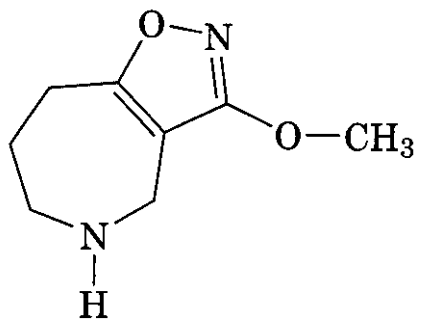
Variation of the *O*-alkyl (R′) group in the nor series (**161b–g**) produced pharmacological activities and potencies parallel to those described for the esters of arecaidine (**113a–e**): the *O*-*n*-propyl and n-butyl homologs (**161d**) and (**161e**), respectively, demonstrate only weak muscarinic agonist effect; however, the 0-ally1 and propargyl homologs displayed prominent muscarinic agonism, with the *O*-propargyl compound being one order of magnitude more potent that the *O*-allyl. However, there is no apparent correlation between the effects of the compounds on central and peripheral cholinergic receptors. It was speculated **(174)** that the effects observed in the ileum preparation are mediated primarily by M_2 receptors, whereas the rat brain membrane-binding data may represent a nondescriminate binding to all types of muscarinic binding sites.

The tetrahydroazepine congener **(162)** of the tetrahydropyridine isoxazole systems **(161)** is said to possess high affinity for the central M_1 receptor, coupled with only limited toxicity (196).



(162)

Another tetrahydroazepine congener (**163**) displays higher affinity for muscarinic receptors but somewhat lower efficacy than the analogous fused piperidine compounds **(161)** **(197);** it was described as a partial agonist. Studies (198) of sulfur analogs and congeners **(164-167)** of the isoxazolotetrahydropyri-



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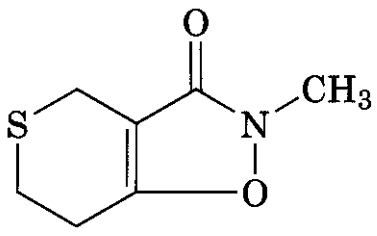
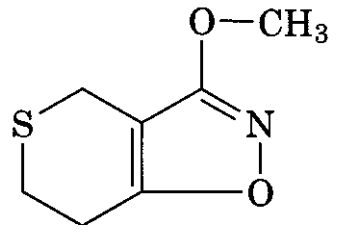


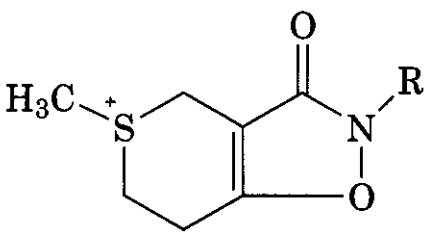
(164)

dines (**161**) demonstrated that the thiapyran derivatives **(164)** and **(165)** are inactive as muscarinic agonists. However, the S-methyl sulfonium derivative (**167a**) binds to brain and heart muscarinic receptors, albeit not as strongly as arecoline. Compound (**167a**) is

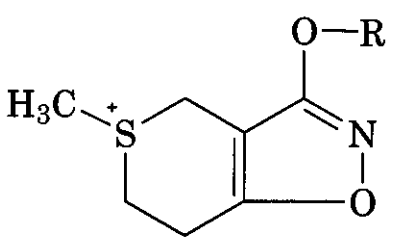


(165)

also inferior in potency and activity to "sulfoarecoline" (137). However, the numerically large ratio of agonist activity at M_1 receptors to that at M_2 receptors for compound (**167a**) is slightly greater than for arecoline or sulfoarecoline, which is a desirable parameter for therapy of Alzheimer's disease. The 0-ethyl homolog (**167b**) is a muscarinic antagonist, in contrast to its tetrahydropyridine bioisostere (**161c**), which is described as a muscarinic partial agonist (198). The *O*-isopropyl-*S*-methyl (**167c**) and *O*-propargyl-*S*-methyl (**167d**) sulfonium homologs demonstrate pharmacological properties similar to those of the 0-methyl homolog (**167a**). The single structure (**166a**) derivative tested was a weak muscarinic agonist in all assays, but it demonstrated a decided preference for M_1 receptors over M_2 receptors.



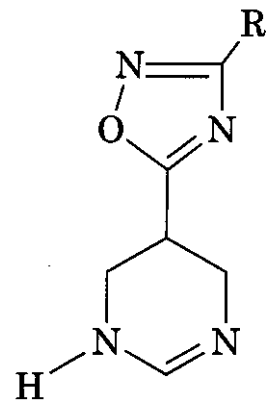
(166a) R = CH_3
(166b) R = C_2H_5
(166c) R = $CH(CH_3)_2$
(166d) R = CH_2—C≡CH



(167a) R = CH_3
(167b) R = C_2H_5
(167c) R = $CH(CH_3)_2$
(167d) R = CH_2—C≡CH

Further modification of the arecoline structure involved replacement of the ester group of tetrahydropyrimidine derivatives (as in structures **139a–f**) with a 1,2,4-oxadiazole ring (structures **168a–h**) (199).

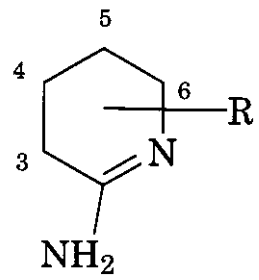
Each of the test compounds (**168a–h**) bound with high affinity to muscarinic receptors from rat brain. The 3-methyl homolog (**168a**) displayed high efficacy at muscarinic receptors coupled to phosphoinositide metab-



(168a) R = CH_3
(168b) R = C_2H_5
(168c) R = n-C_3H_7
(168d) R = n-C_4H_9
(168e) R = n-C_5H_{11}
(168f) R = n-C_6H_{13}
(168g) R = n-C_7H_{15}
(168h) R = n-C_8H_{17}

olism in rat cortex and hippocampus. Increasing the length of the alkyl substituents (**168b–h**) increased the affinity for muscarinic receptors, albeit not in a linear fashion, yet decreased activity in the phosphoinositide turnover assay. It was concluded that at low concentrations, compound (**168a**) selectively stimulates M_1 receptors.

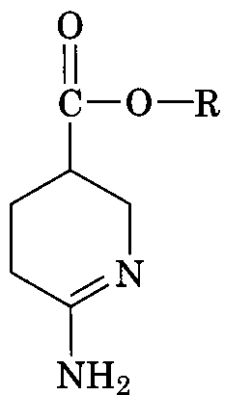
Regioisomers of an exocyclic amidine system (**169a–d**) bioisosteric with arecoline were tested as their racemic modifications (200).



(169a) R = 3-$COOCH_3$
(169b) R = 4-$COOCH_3$
(169c) R = 5-$COOCH_3$
(169d) R = 6-$COOCH_3$

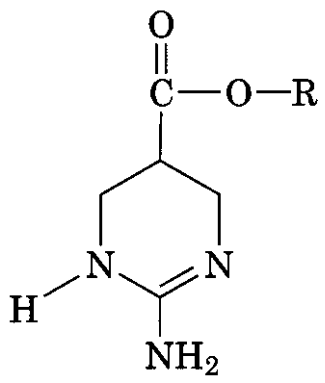
Only the 5-carbomethoxy isomer (**169c**) displayed high affinity and activity at muscarinic receptors coupled to phosphoinositide metabolism in rat cortex. Evaluation of other alkyl esters (**170a–c**) of the 5-carboxylic acid revealed that only the propargyl derivative (**170c**) retained substantial agonist activity.

In a series of cyclic guanidines, (2-amino-tetrahydro-pyrimidines, **171a–e**), all mem-



(170a) R = C_2H_5
(170b) R = n–C_3H_7
(170c) R = CH_2—C≡CH

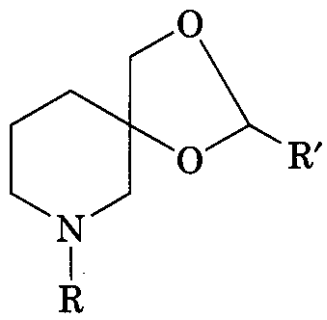
bers showed high binding affinities in a rat brain membrane assay (200). However, only the methyl and propargyl esters (**171a** and **171d**) showed high muscarinic agonist activity in a phosphoinositide metabolism assay. Computational chemical studies revealed a common minimum energy conformation for all of the muscarinically active members of several series (169, 170, 139, and **171**), which suggests that all of the subject compounds in this study interact with muscarinic receptors in a similar fashion. The utility of amidine systems as suitable replacements for the quaternary ammonium group of acetylcholine in designing ligands for M_1 receptors is suggested by the results of these studies.



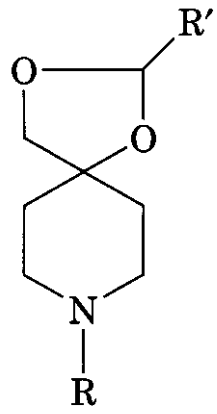
(171a) R = CH_3
(171b) R = C_2H_5
(171c) R = n–C_3H_7
(171d) R = CH_2–C≡CH
(171e) R = 2–C_3H_7

The spiropiperidine systems (**172a, 172b**) (diastereomers, stereochemistry unspecified) are hybrids of the arecoline molecule and of the spirodioxolanes (121 and 122).

Compounds (**172a**) and (**172b**) (spiro-3-piperidine derivatives) show weak binding ability in rat cortex (201); compounds (**173a** and (**173b**) (spiro-4-piperidine derivatives) showed marked muscarinic agonist effects in a phosphatidyl inositol turnover assay. Compounds (**173a**) and (**173b**) also showed moderate binding ability against [^{3}H]-*N*-methylscopolamine and [^{3}H]oxotremorine in rat cortex tissue. Compound (**173a**) compared favorably with arecoline in terms of receptor efficacy although it, like (**173b**), demonstrated much lower receptor affinity. The 2-ethyl homolog (**173c**) demonstrated decidedly lower receptor efficacy than (**172a**) or (**172b**), and it also demonstrated lower receptor affinity. Variations of structure (173) are represented by (174) and (**175**), which are agonists at rat central M_1 receptors (202).

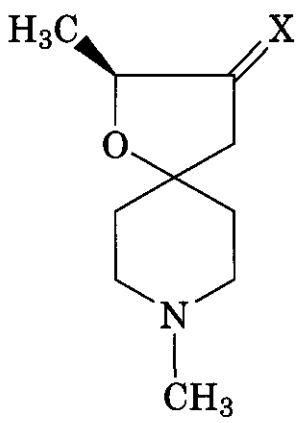


(172a) R = R′ = CH_3
(172b) R = H; R′ = CH_3

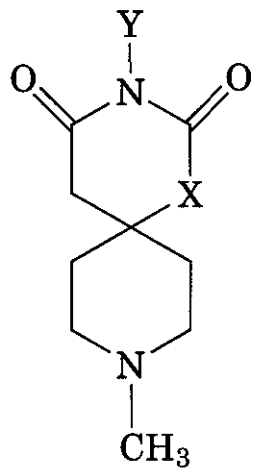


(173a) R = R′ = CH_3
(173b) R = H; R′ = CH_3
(173c) R = CH_3; R′ = C_2H_5

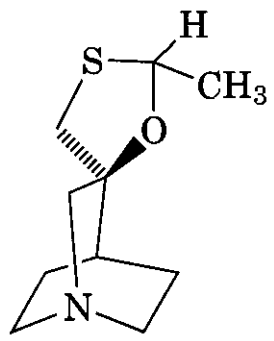
Studies of extended series of other spiropiperidine derivatives demonstrated that compound (176) is a partial muscarinic agonist that reverses carbon dioxide-induced impairment in mice (203), and that compound (**177**) is a muscarinic agonist with an affinity for cortical M_1 receptors (204). The (±)-spiroquinuclidine derivative (178) is a selective M_1 agonist (205).



(174) X = O
(175) X = CH_2



(176) X = CH_2; Y = OCH_3
(177) X = O; Y = C_2H_5

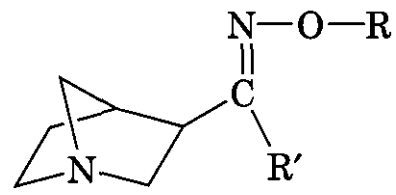


(178)

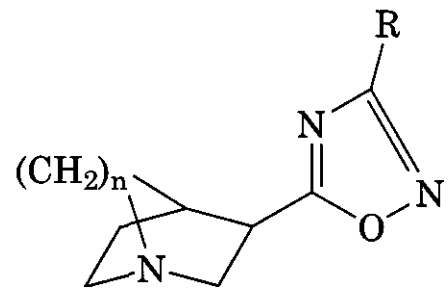
Some quinuclidine and azabornane derivatives bearing oximino or heterocyclic ring substituents at position **3** (e.g., 179 and **180)** demonstrate potent muscarinic agonism. Some compounds of these types are selective for M_1 and M_3 receptors (206–210).

4.5 Oxotremorine and Analogs and Congeners

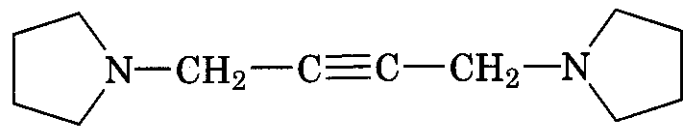
Tremorine (**181**), a synthetic compound with weak cholinergic activity **(211, 212)** is metabolized to the lactam oxotremorine (**182**),



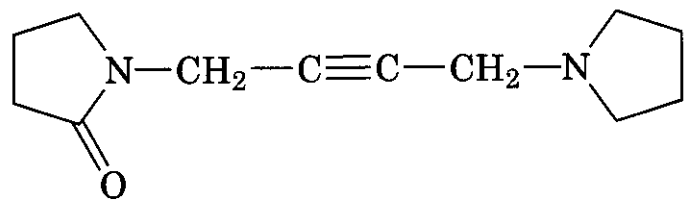
(179)



(180)



(181)



(182)

which is approximately equipotent to acetylcholine as a muscarinic agent but lacks nicotinic effects (213).

As with pilocarpine and arecoline, increased interest in pharmacotherapy of Alzheimer's disease and other memory deficit conditions has led to renewed and expanded studies of oxotremorine. This compound has little or no effect on serum or red cell butyrylcholinesterase. Oxotremorine has been described as a potent muscarinic partial agonist **(172).** However, an earlier report **(11)** presented evidence that oxotremorine has an indirect action in the CNS, perhaps by stimulation of choline acetyltransferase, resulting in elevation of acetylcholine levels. The peripheral actions of oxotremorine, including effects on cardiovascular mechanisms, have been ascribed **(172)** to preferential activation of M_1 receptors. Brimblecomb **(211, 212)** reported

pharmacological data on a large number of tremorine-oxotremorine derivatives, from which the following conclusions were drawn.

1. The carbonyl group of oxotremorine is essential.
2. The pyrrolidine nitrogen of oxotremorine may be replaced by a trimethylammonium quaternary group ("oxotremorine-M"), but replacement with a tertiary amine (dimethylamino or diethylamino) results in great loss of activity.
3. The acetylenic bond is essential; partial or complete saturation results in complete loss of activity.
4. Increase in the size of the lactam ring results in a change from agonism to antagonism.

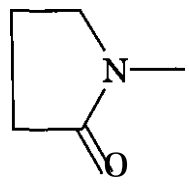
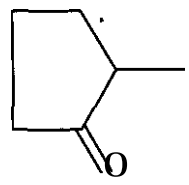
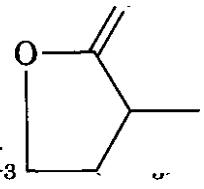
To assess the validity of conclusion 2 above, Brimblecomb (211) compared the trimethylammonium quaternary moiety with the 1-pyrrolidino group in a series of compounds (Table 2.3).

Variations are apparent in the activities of the quaternary ammonium salts, but the variation is not nearly as great as that demonstrated by the tertiary amines (pyrrolidines). The Brimblecomb group (214) described an additional series of some 23 tremorine-oxotremorine congeners, but none of these compounds showed significant muscarinic agonist effects in the guinea pig ileum or the cat blood pressure assay.

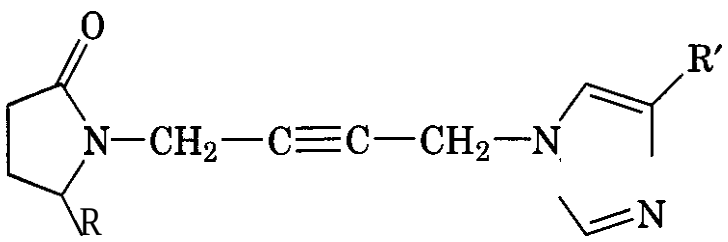
In a series of compounds (183a–c) in which the pyrrolidine ring of oxotremorine is replaced by imidazole, the parent compound

Table 2.3 Muscarinic Activities of Oxotremorine Congeners *and* Analogs Containing Trimethylammonium or Pyrrolidino Groups

$R—CH_2—C\equiv C—CH_2—R'$

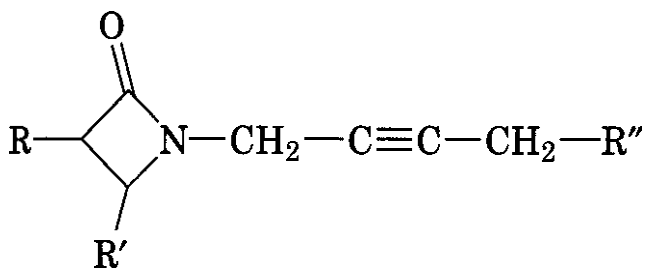
Compound Number	R	Muscarinic Activity (isolated guinea pig ileum, acetylcholine = 1)	
		$R' = \overset{+}{N}(CH_3)_3$	R' = 1-pyrrolidino
(1)		1.74	1.48
(2)		0.11	0.03
(3)		0.06	0.001
(4)	 CH_3	0.87	1.15
(5)	$(CH_3)_2NCON(CH_3)$–	0.03	0.04
(6)	$(CH_3)_2NCOO$–	0.15	0.004
(7)	CH_3COCH_2–	2.10	0.005
(8)	CH_3COO–	0.47	0.005

(183a) resembles oxotremorine in its muscarinic efficacy (215); addition of a methyl group to the imidazole ring (**183b**) greatly decreased muscarinic activity (216), whereas addition of methyl groups to both the imidazole and the pyrrolidone rings (**183c**) produced a potent muscarinic antagonist (215).



(183a) R = R' = H
(183b) R = H; R' = CH_3
(183c) R = R' = CH_3

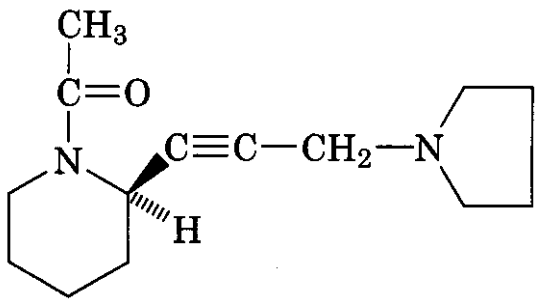
All members of a series of oxotremorine analogs (**184a–e**), in which the pyrrolidone ring iscontracted to a β-lactam moiety (217), demonstrated uniformly weak cholinergic effects.



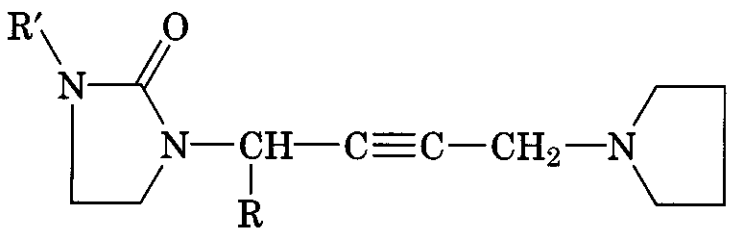
(184a) R = R' = H; R" = cNC_4H_8
(184b) R = CH_3; R' = H; R" = cNC_4H_8
(184c) R = H; R' = CH_3; R" = cNC_4H_8
(184d) R = H; R' = CH_3; R" = $N(CH_3)_2$
(184e) R = H; R' = CH_3; R" = $\overset{+}{N}(CH_3)_3$

Compound (**184a**) is the most potent muscarinic agonist of the series, being 6 times less potent than its pyrrolidone congener (182). Compound (**184b**) is a weak partial muscarinic agonist; (**184c**) is a muscarinic antagonist; (**184d**) and (**184e**) are muscarinic agonists, 220 times less potent than (**184a**). An N-acetylated piperidine derivative (**185**) is a potent muscarinic agonist (40).

Conversion of the pyrrolidone ring of oxotremorine and some congeners into an imidazolidone ring (**186**) produces compounds (generally of low potency) with a variety of effects at muscarinic receptors (218).

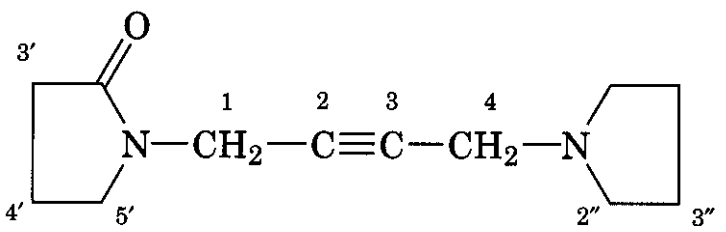


(185)



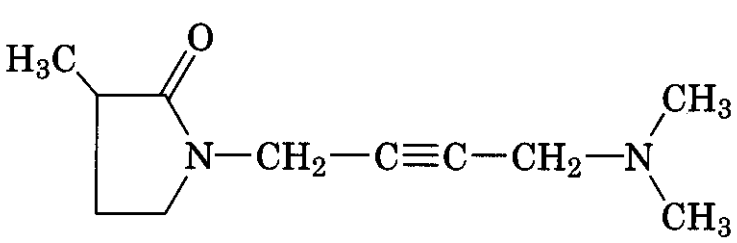
(186) R = H or CH_3; R' = H, CH_3, CH_3CO, or HCO

Studies (219,220) of methyl group substitution into the oxotremorine molecule (compounds (**187a–g**), all of which were tested as their racemates) revealed that the 3'-methyl isomer (**187a**) has weak muscarinic stimulant activity in intact mice.



(187a) 3′–CH_3
(187b) 4′–CH_3
(187c) 5′–CH_3
(187d) 3″–CH_3
(187e) 2″–CH_3
(187f) 1–CH_3
(187g) 4–CH_3

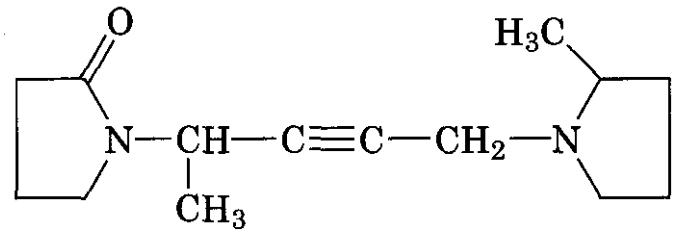
Similarly, the *N,N*-dimethyl congener (188) displays weak oxotremorine-like activity. The remaining isomers (**187b–g**) have



(188)

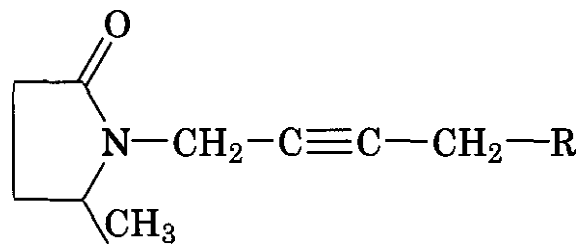
central atropine-like antimuscarinic effects but only weak peripheral parasympatholytic actions.

Compounds (**187d–f**), as well as the C1, C 2 dimethyl derivative (**189**), were resolved and

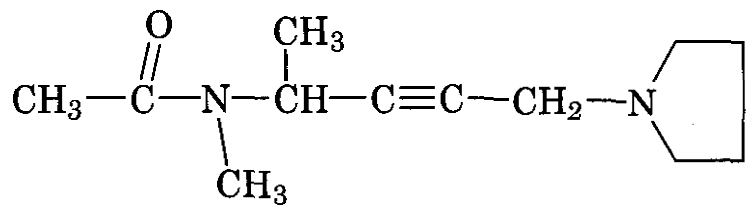


(189)

the enantiomers were evaluated (221). Some compounds are agonists, some are partial agonists, and some are antagonists at muscarinic sites. In some instances. the eudismic ratio is large, and in some instances the ratio is relatively small. Structure-activity conclusions cannot be drawn from these data. Arnstutz and coworkers (222) resolved the 5'-methyl pyrrolidone derivative (**187c**) as well as the *N,N*-dimethyl tertiary amine (**190a**) and its quaternary ammonium derivative (**190b**). The pyrrolidone derivative [(*R*)-**187c**] is a central and peripheral muscarinic antagonist, as has been reported earlier for the racemate. Compounds (*R*)-(**190a**) and (*R*)-(**190b**) are potent muscarinic agonists in the guinea pig ileum. Compound (*R*)-(**190a**) (the tertiary amine) shows both central muscarinic (hypothermia) and central antimuscarinic activity (antagonism of oxotremorine-induced tremor) in vivo. These central agonist-antagonist properties probably reflect interactions of the drug with different subpopulations of muscarinic receptors. For all three compounds in this study (**187c**, **190a**, **190b**), the (*R*)-enantiomers are considerably more potent than the (*S*)-, both in vivo and in vitro, irrespective of whether agonist or antagonist effects were measured. Substitution of a benzene ring into various positions of the oxotremorine molecule destroys muscarinic stimulant activity; these derivatives are competitive muscarinic antagonists (223). The N-methylacetamide congener (**191**), closely related to compound (4) in Table 2.3, was reported (224,225) to be a presynaptic antagonist and a postsynaptic agonist at muscarinic receptors in vitro and in vivo.

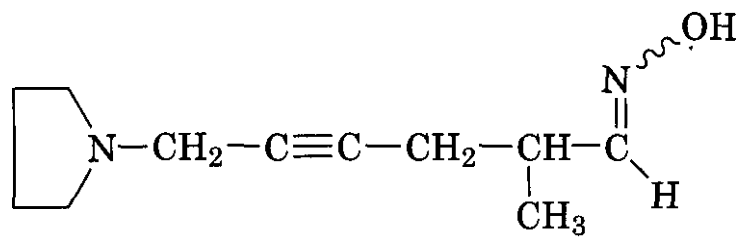


(190a) R = $N(CH_3)_2$
(190b) R = $\overset{+}{N}(CH_3)_3$



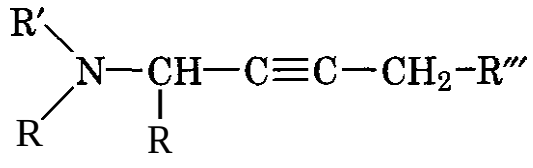
(191)

Racemic (**191**) (226), as well as its pure enantiomers (227), blocks oxotremorine-induced tremors. In other regions of the brain (e.g., those involved in analgesia and hypothermia), (191) acts as a muscarinic agonist (228). Replacement of the acetamido group of (191) with an oximino moiety and homologation of the chain (192) resulted in a fivefold greater selectivity for M_1 receptors (229).



(192)

A series of congeners (193) addressed replacement of the acetamide moiety of compound (191) by methanesulfonamido, trifluoroacetamido, methylsulfonimido, or acetimido; introduction of a methyl into the C1 position of

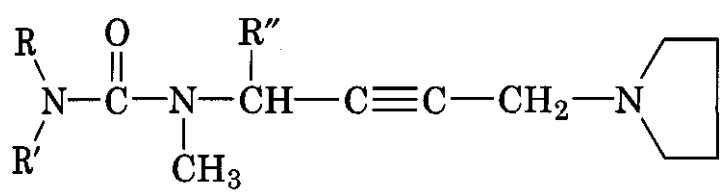


(193)

R = CH_3SO_2; CF_3CO; CH_3CO
R' = CH_3; CH_3SO_2; CH_3CO
R = H, CH_3
R = 1-pyrrolidyl; $N(CH_3)_2$; $N(C_2H_5)_2$; $^{+}N(CH_3)_3$

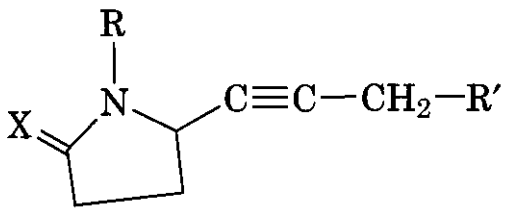
the 2-butyne chain; and variation of the C4 tertiary amino group or quaternary group (230).

Replacement of the acetyl group or the N-methyl group in (**191**) and its analogs by a methansulfonyl group abolishes efficacy and decreases affinity at guinea pig ileal receptors. The trifluoroacetamide analogs of (191) also exhibited diminished affinity and efficacy. Substitution of an acetyl group for the N-methyl group of (**191**) decreases efficacy, but has little effect on affinity for the receptor(~)Most of the tertiary amines showed central antimuscarinic effects. Bioisosteres of (191)bearing a urea moiety (194) in which the R, R', and R'' groups were combinations of CH, and H, displayed muscarinic agonism, partial agonism, or antagonism (218); a structure-activity relationship is not apparent in this series.



(194)

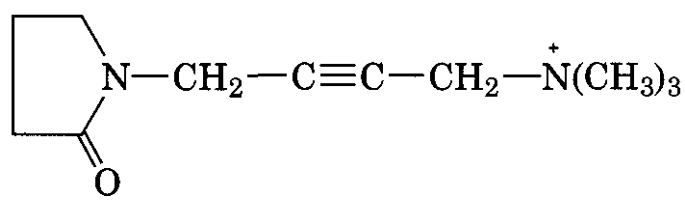
Conformationally restricted analogs of (191)and (**195a–i**) have been described (231).



(195a) R = CH_3; X = O; R' = cNC_4H_8
(195b) R = CH_3; X = O; R' = $N(CH_3)_2$
(195c) R = CH_3; X = O; R' = $^+N(CH_3)_3$
(195d) R = CH_3CO; X = O; R' = cNC_4H_8
(195e) R = CH_3CO; X = O; R' = $N(CH_3)_2$
(195f) R = CH_3CO; X = O; R' = $^+N(CH_3)_3$
(195g) R = CH_3CO; X = H_2; R' = cNC_4H_8
(195h) R = CH_3CO; X = H_2; R' = $N(CH_3)_2$
(195i) R = CH_3CO; X = H_2; R' = $^+N(CH_3)_3$

These structural modifications resulted in decreased affinity for rat cerebral cortex tissue and in most cases abolished efficacy at both central and peripheral muscarinic receptors. Other conformationally restricted analogs of (195) in which the amide moiety and the methyl group on the butynyl chain were joined to form a six- or seven-membered ring preserved affinity for muscarinic receptor(s), but abolished efficacy (232).

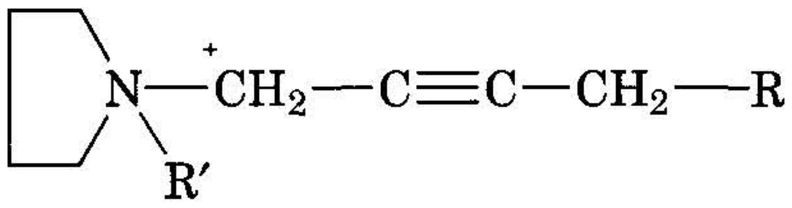
In a series of oxotremorine and oxotremorine-M (196) analogs (197, **198**), all compounds studied (tertiary and quaternary amines) demonstrated analgesic effects in neurogenic and inflammatory pain models (233). It was concluded that these compounds were acting as muscarinic analgesics. The antinociceptive effects were blocked by atropine but not by naloxone or mecamylamine. Members of the series varied as to classic muscarinic agonist effects (e.g., salivation).



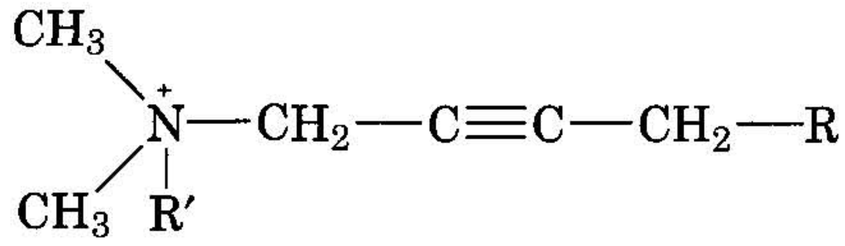
(196)

A nitrogen mustard congener (**199**) of oxotremorine is a potent and selective muscarinic agonist (234).

When (199) was administered to an intact animal, the signs of muscarinic stimulation were followed by a phase of long-lasting antimuscarinic effects (235). Both the stirnulatory and the blocking effects are elicited by the aziridinium ion (**201**) formed by *in vivo* cyclization of the parent 2-chloroalkylamine. This aziridinium system is closely related structurally to "oxotremorine-M" (**196**), which is an extremely potent muscarinic agonist (212). The blocking activity is correlated with alkylation (covalent bond formation) of the muscarinic receptor(s) by the aziridinium ion. The bromine-derived mustard (**200**) shows threefold greater *in uitro* muscarinic stimulant activity than the chloro compound (**199**), and this can be rationalized on the basis that bromine is a better leaving group, and aziridinium ion formation is more facile in (200) than in (199). Neither of these mustards displays a significant amount of effect at nicotinic receptors. These compounds may be useful in receptor inactivation studies. The slower rate of cyclization of the chloro compound (**199**) may permit its penetration of the blood-brain barrier before formation of the aziridinium



(197)



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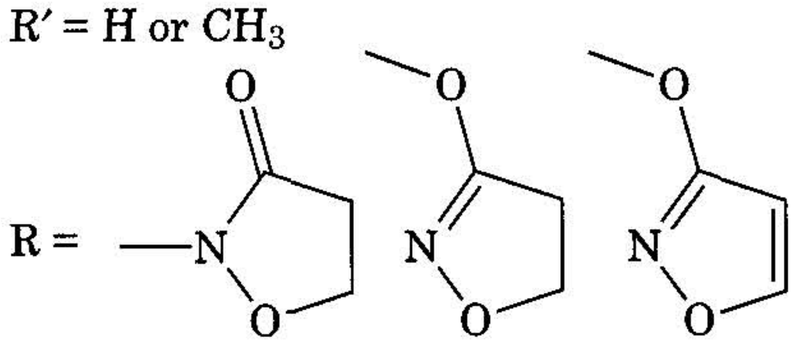
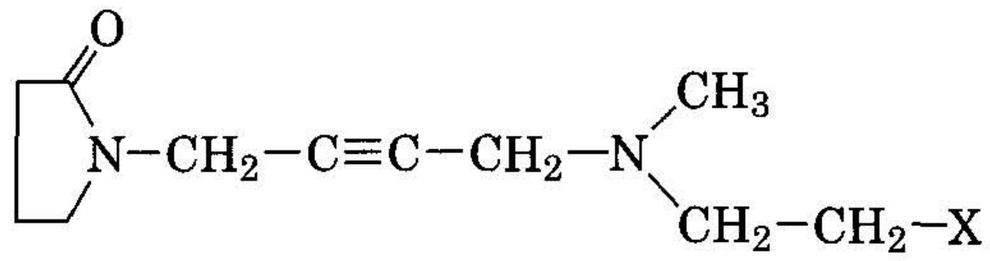


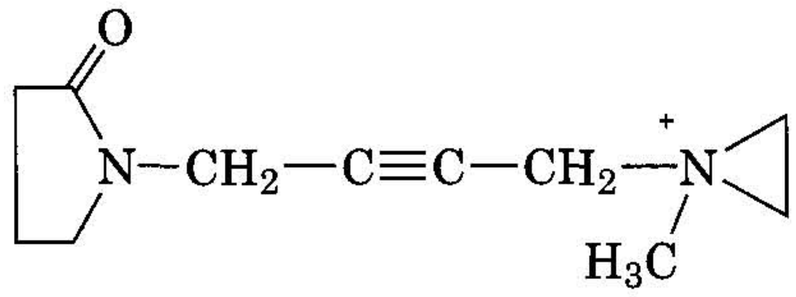
ion. The racemic C-methyl analogs (**202**) (X = Cl or Br) showed potent muscarinic effects (236), similar to those of (**198**) and (**199**), but Cl-(**202**) and Br-(**202**) were more potent.



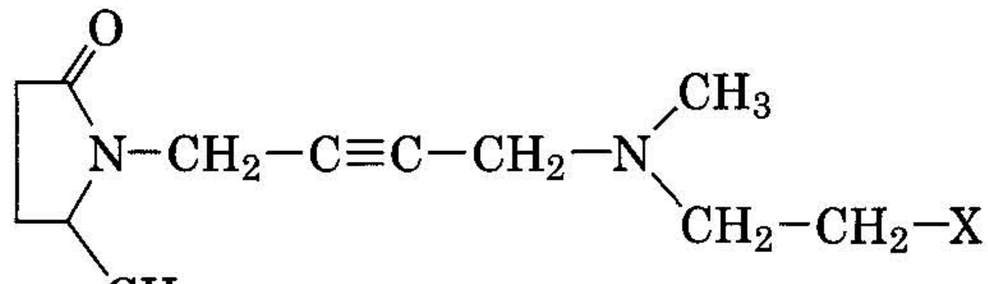
(199) X = Cl
(200) X = Br

This enhanced potency was ascribed to greater receptor affinity rather than to a greater rate constant for alkylation of the muscarinic receptors.

Tertiary 2-, 3-, and 4-haloalkylamine analogs of oxotremorine were investigated in mice as prodrugs of muscarinic agonists (237) (Table 2.4). The azetidinium cyclization product of the 3-halopropylamine moiety (compounds



(201)



(202)

Table 2.4 Apparent First-Order Rate Constants for Cyclization of ω-Haloalkyl Oxotremorine Congeners

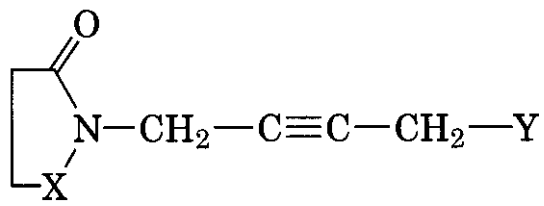
Number	*n*	*X*	k_1 (min^{-1})	$t_{1/2}$ (min)
(**1**)	2	Cl	0.019	36.5
(**2**)	2	Br	0.850 ± 0.035	0.8
(**3**)	3	Cl	0.0016 ± 0.0003	436
(**4**)	3	Br	0.0610 ± 0.0041	11.4
(**5**)	3	I	0.0491 ± 0.0055	14.1
(**6**)	4	Cl	>2	>0.4

3–5 in Table 2.4) and the pyrrolidinium product of the 4-halobutylamine moiety of compound (6) in Table 2.4 should be much less susceptible to nucleophilic attack *in vivo*, and hence these quaternary systems, unlike the aziridinium moiety, should have little or no tendency to bond covalently with the muscarinic receptor(s) to produce blockade. Central muscarinic effects (tremors, analgesia) of this series of compounds correlated well with the k_1 and $t_{1/2}$ data. The slow cyclization rate of compound (3) and the extremely rapid cyclization rate of compound (6) were reflected in weak or no CNS-related activities. As might be predicted, compound (3) showed relatively weak peripheral muscarinic activity (salivation) whereas compound (6) was potent. Compounds (4) and (5) were cited as meriting further study.

McN-A-343 (**203**) is a selective M_1 agonist and it has been reported to exhibit antinociceptive effects (238). It was suggested that postsynaptic M_1 receptors are involved in antinociception, and that presynaptic M_2 receptors may also be involved, on the basis that they participate in modulation of acetylcholine release.

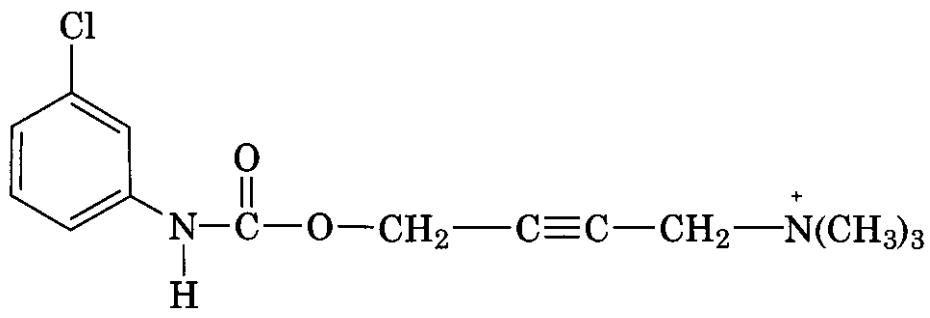
Of a series of congeners of compound (**203**), (±)-**204** and (±)-**205** were concluded to be muscarinic partial agonists, showing five- and 16-fold higher potency, respectively, than that of compound (**203**) (239). The (*S*)-enantiomers of (204) and (205) exhibit low eudismic ratios (**1.5** and 4.9, respectively). Compound (205) has been described (240) as one of the most potent M_1-selective agonists (sic) known.

Dimethylsulfonium (**206a** and **206b**) and thiolanium (**206c** and **206d**) analogs of oxotremorine demonstrate higher affinities for peripheral muscarinic receptors than the corresponding trimethylammonium and *N*-methylpyrrolidinium compounds (241).



(206a) X = CH_2; Y = $^+S(CH_3)_2$
(206b) X = CO; Y = $^+S(CH_3)_2$
(206c) X = CH_2; Y = $c^+SC_4H_8$
(206d) X = CO; Y = $c^+SC_4H_8$

However, the sulfur compounds have lower intrinsic activities than their nitrogen analogs. The sulfur compounds also demonstrate potent affinity for rat cerebrocortical tissue in a (−)-[^{3}H]-*N*-methylscopolamine displacement assay. Sulfonium congeners (207) bear-



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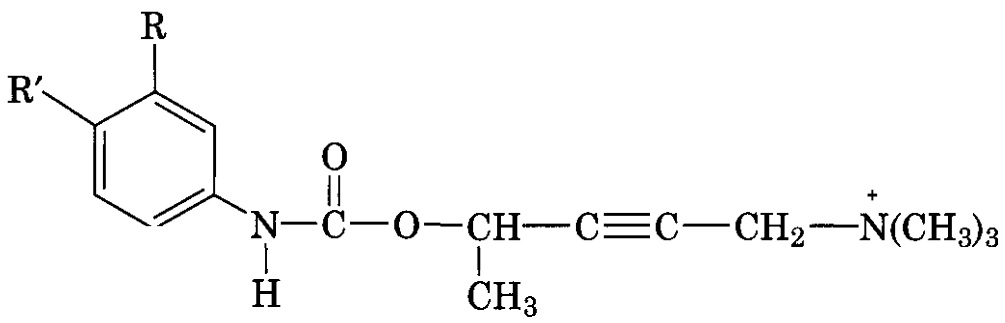
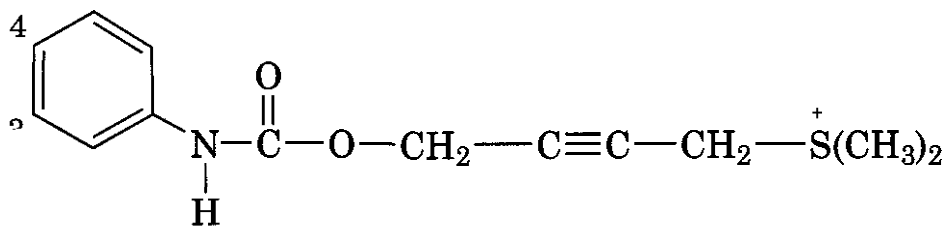


(204) R = Cl; R′ = H
(205) R = H; R′ = Cl



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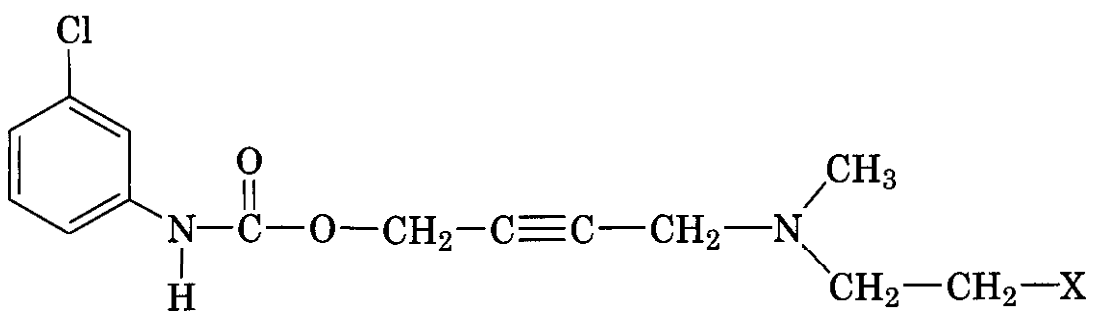
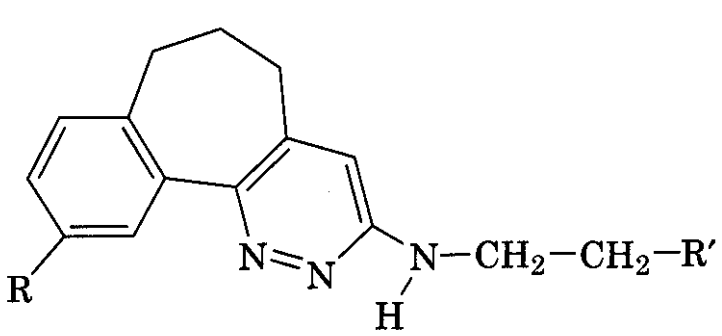


(208a) X = Cl
(208b) X = Br

ing chlorine or bromine at positions 3 or 4 of the benzene ring retain selectivity for ganglionic muscarinic receptors, but they were concluded to be partial agonists when compared with the nitrogen system (203) (242). Nitrogen mustard congeners (**208a** and **208b**) of (203) demonstrate effects analogous to those described previously for other oxotremorine-based nitrogen mustards (243).



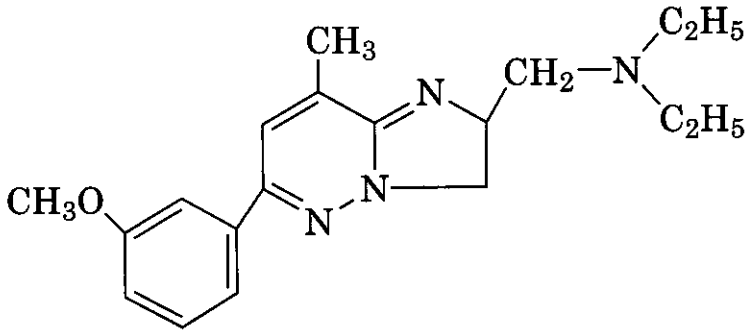
(210) **R** = H; R' = morpholino
(211) R = NO_2; R' = $N(C_2H_5)_2$

4.6 Miscellaneous, Structurally Unique Muscarinic Agonists

Several structurally unique muscarinic agonists have been identified.

Compound (209) is claimed to be highly selective for the M_1 receptor (196); (210) and (211) have been described as selective M_1 agonists devoid of classical muscarinic side effects (196,244).

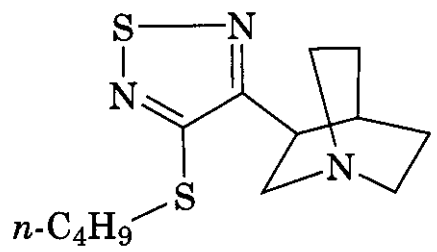
Evidence was presented many years ago (245) suggesting that some muscarinic agents



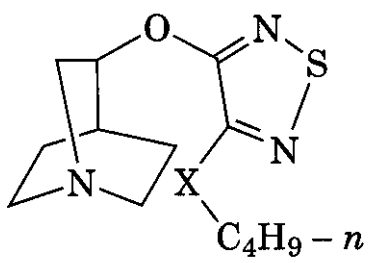
(209)

demonstrate analgesic activity. Shannon et al. (246), and references therein) presented a historical survey of muscarinic agonists that have been reported to demonstrate antinociceptive effects. These effects seem enigmatic, in that the involvement of acetylcholine in excitation of the pain sensation has been cited (247). Agonists at various G-protein–coupled muscarinic receptors have potent analgesic activity, but this is frequently accompanied by typical muscarinic side effects (123). Analgesic properties of the arecoline congener (156) were cited previously. Vedaclidine (212) appears to be the most prominent of these agents, having M_1 agonist and M_2/M_3 antagonist activity (123).

1,2,5-Thiadiazole ether analogs (213,214) of vedaclidine (**212**) have also been viewed as being analogs of the potent muscarinic agonist aceclidine (149) (248). These ether analogs are

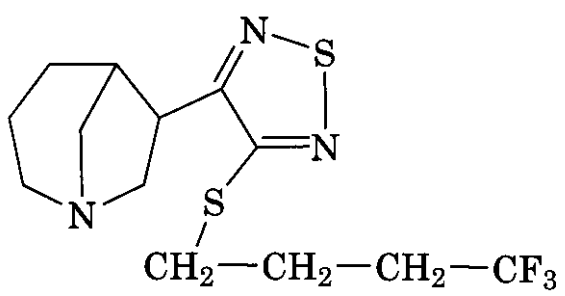


(212)



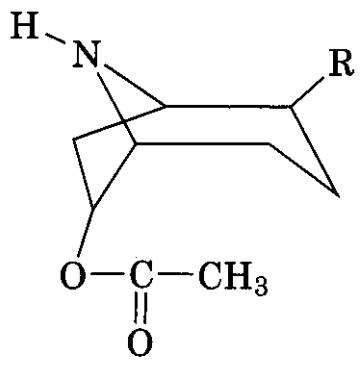
(213) X = O
(214) X = S

potent, efficaceous, and selective M_1 receptor agonists. Replacement of the 3-oxyquinuclidine moiety of (213) or (214) by ethanolamine, hydroxypyrrolidine, hydroxyazetidine, hydroxyisotropane, or hydroxyazanorbornane led to compounds with high muscarinic receptor affinity and/or M_1 agonist efficacy. A concomitant computational chemistry study led to the proposed description of an M_1 receptor pharmacophore. No mention was made of testing of this series of agents for antinociceptive effects. Compound (215) inhibited jejunal contraction in the ferret, as well as demonstrating potent analgesic effects in the mouse writhing test (249).



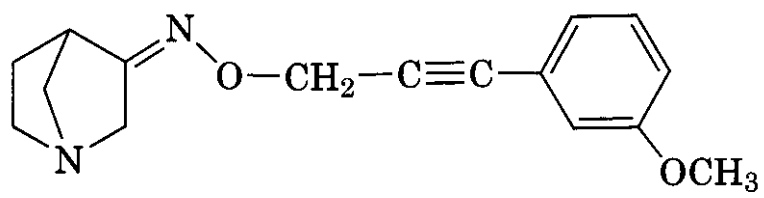
(215) 5 – *R*, 6 – *R*, exo

A natural product, baogongteng-A (216) was reported (250) to have agonist activity at muscarinic receptors. A series of congeners was prepared (251), of which (217) was found to be a potent muscarinic agonist with selectivity toward M_2 receptors.



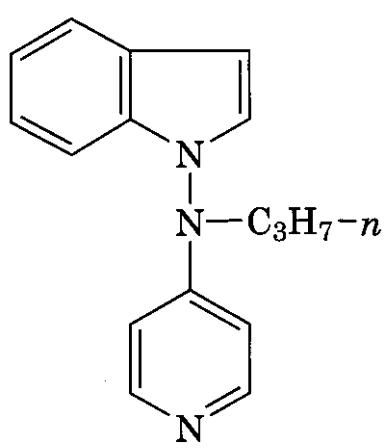
(216) R = OH
(217) R = H

The R-enantiomer of (218) is highly selective for M_1 receptors over M_2 (252). The synthetic route to (218) led to a 60:40 mixture of Z:E oxime stereoisomers (253); this mixture, on standing for 1 h at room temperature in methanolic hydrogen chloride changed to an 85:15 equilibrium mixture of *Z*:*E*. Evaluation of a series of azabicyclo[2.2.1]heptane analogs. of (218) revealed that with only a few exceptions, muscarinic activity resided in the Z-oximes, and that the E-isomers were inert or very weakly active.



(218)

An indole derivative, besipirdine (219), displays some cholinomimetic effects, in addition to prominent adrenergic actions. The mechanism of the cholinergic effects was stated to be unclear (254).



(219)

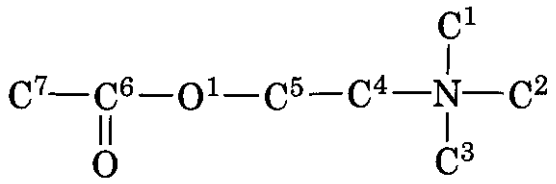
5 CONFORMATION-ACTIVITY RELATIONSHIPS OF SOME CHOLINERGIC AGONISTS

Conclusions about pharmacologically significant conformations of cholinergic agonists have been largely based on X-ray diffraction studies and NMR (chiefly ^1H) studies. Casy (255) noted that in the cholinergic field, NMR evidence complements the results of X-ray studies. Casy (256) proposed four questions to which conformational studies of cholinergic agonists must be addressed:

1. Does the "active" conformation of a cholinergic ligand correspond to its preferred stereochemistry or is an energetically less favored form bound to the receptor?
2. Is there a unique mode of ligand binding to cholinergic receptors or do multiple modes exist?
3. May the dual effects (nicotinic and muscarinic) of acetylcholine be explained in terms of conformational isomerism?
4. Do agonist and antagonist ligands occupy the same or different binding sites (with one or more features common to both)?

These challenging questions remain, some 25 years later, largely unanswered, despite a large body of chemical and biological work and a voluminous literature. Indeed, establishment of the existence of multiple subpopulations of both nicotinic and muscarinic receptors renders question 3 even more formidable.

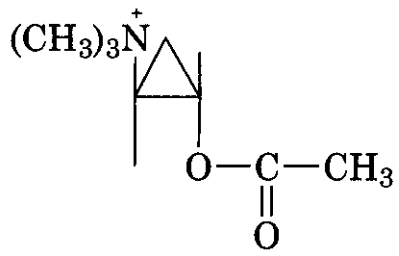
The three-dimensional steric disposition of the flexible acetylcholine molecule and those of its congeners can be defined on the basis of torsion angle τ. Summations and definitions of nomenclature incident to use of torsion angles are found in refs. 257 and 258. Structure (220) defines relevant torsion angles in the acetylcholine molecule.



(220)

$\tau_1 = C^5—C^4—N—C^3$
$\tau_2 = O^1—C^5—C^4—N$
$\tau_3 = C^6—O^1—C^5—C^4$
$\tau_4 = C^7—C^6—O^1—C^5$

The torsion angles τ_1 and τ_4 usually fall close to 180"; the values of τ_2 and τ_3 are more useful in defining pharmacologically significant conformations for acetylcholine and related molecules. From X-ray studies (259) it was concluded that in most cases τ_3 values fall in the range 180 ± 36" (antiplanar), placing the quaternary head and the acetyl group far apart, and the τ_2 angle commonly has a value of 73–94", so that the N and O functions are approximately synclinal (gauche). Many compounds with the O-C-C-N^+ moiety, where the oxygen-containing function is hydroxy or acyloxy, prefer the τ_2 synclinal (gauche) N/O disposition in the solid state: *L*-(+)-muscarine iodide (3), the (4*R*)-(+)-*cis*-dioxolane (107), and the furan derivative (106). NMR data suggested (260) that acetyl-β-methylcholine (31) exists in solution in a τ_2 synclinal conformation. However, there are many exceptions: in the crystal state the potent muscarinic agonists carbamoyl choline (number (19) in Table 2.1) and (+)-*trans*-ACTM (221) (259) prefer the τ_2 anticlinal τ_3 antiplanar conformations, as do the weakly active thio and seleno analogs of acetylcholine (24) and (25).



(221)

L-(+)-Muscarone (102) exhibits a τ_2 angle that is antiplanar (261).

The crystal structures of certain nicotinic agents, for example, acetyl a-methylcholine (32) and lactoylcholine (17 in Table 2.1), have torsion angle (τ_2 and τ_3) features similar to most muscarinic agents (262). In contrast, some cyclic analogs of aryl choline ethers exhibit maximum nicotinic effects when the τ_2 is antiplanar ("transoid") (98).

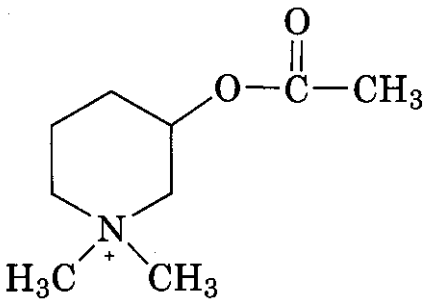
However, there is no assurance that any of the preferred conformations determined experimentally by X-ray or solution NMR methods, or by molecular orbital calculations (263),

represent the geometry of the agonist at cholinergic receptors; barriers to rotation in molecules such as acetylcholine are low (264, 265), and there is considerable rotational freedom in the muscarine and muscarone systems (256). It is well established as a broadly applicable working hypothesis that an agonist molecule may interact with its receptor(s) in other that its (the agonist's) lowest energy conformation. The energy expended by the agonist's assuming a higher energy conformation is compensated for by the energy advantage of the agonist-receptor interaction itself.

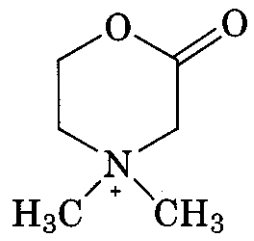
An alternate strategy is the study of conformationally restrained acetylcholine analogs in which the presumed pharmacologically significant portions of the molecule (the quaternary ammonium head and the ester function) are in relatively "frozen" positions, so that the three-dimensional geometry of the pharmacophoric moieties is known. This approach presents the disadvantage of frequently requiring molecules that are larger and more complex than the parent, with altered receptor affinity and different solvent partition characteristics a possible consequence. Casy (266) has discussed this difficulty in more detail.

Schueler (267) suggested that the muscarinic and nicotinic effects of acetylcholine are mediated by different conformers of the flexible molecule, and he evaluated structures (±)-(222) ("transoid") and (223) ("cisoid") as examples of analogs of conformational extremes of acetylcholine.

Both (222) and (223) exhibited only feeble cholinergic effects, and the difference in activity between the two was not great. Both of the enantiomers of (**222**) were far less potent than acetylcholine at muscarinic sites (268), but they have intrinsic activities (compared with acetylcholine) approaching unity. The *S*-enantiomer was somewhat more effective than the R-, demonstrating an S:R eudismic ratio of 13.4. However, because of the ability of the piperidine ring to undergo ring inversion (conformational flip) with concomitant change in the τ_2 torsion angle, the piperidine ring of compound (222) is not an ideal template for acetylcholine conformational investigation. Casy (269) has summarized literature conformational studies on compound (222).

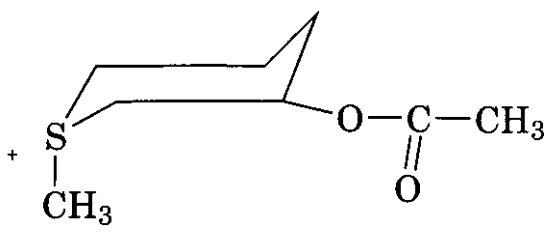


(222)



(223)

The trans-sulfur isostere (**224**) of the 3-acetoxypiperidinium system (**222**) is weak muscarinic agonist (6–10% as potent as acetylcholine); the cis-isomer (**225**) is inert (270).



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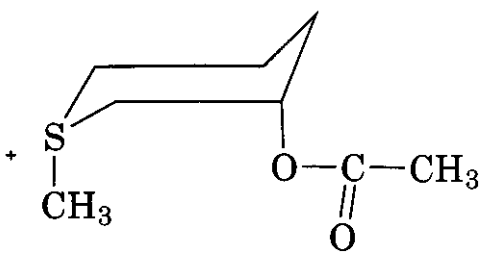
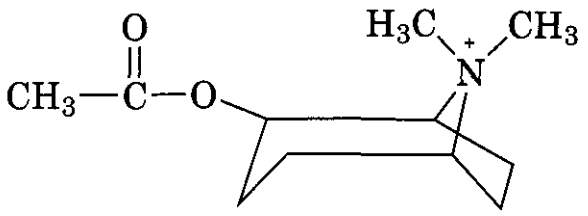


(225)

Both the cis and trans isomers (224 and 225) display prominent nicotinic action; the cis-isomer is approximately 20% as potent as acetylcholine and is approximately seven times as potent as the trans-isomer. In the trans-compound (**224**), the τ_2 angle (S-C-C-O) is described as anticlinal-antiplanar on the assumption that the chair conformer has an axial S-methyl and an equatorial acetoxy (270). However, the ability of the thianium ring to undergo ring inversion (175) presents the

same uncertainty of stereochemical interpretation that was cited for the piperidinium ring of compound (222).

The stereoisomeric tropyl acetates (226) and (**227**), in which a greater degree of molec-



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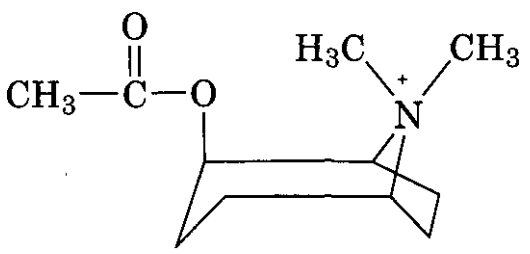
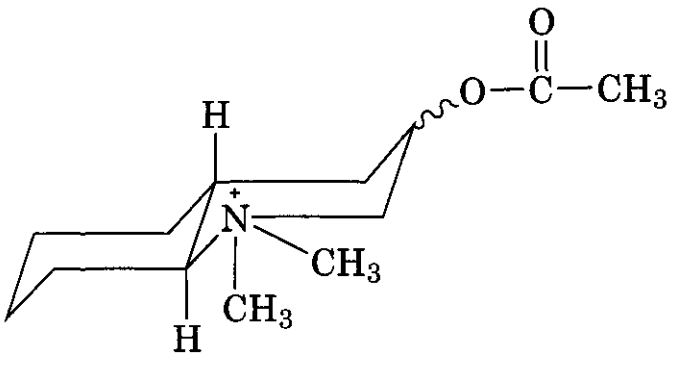


(227)

ular rigidity is imposed, are extremely weak muscarinics, but both compounds exhibited potent nicotinic effects (271).

However, as demonstrated by Hardegger and Ott (**272**), some tropane ring systems can assume a conformation in which the piperidine ring is a boat. Therefore, the possibility of ring inversion in (226) and (227) cannot be precluded, and as with the piperidine and thiane rings, conformational integrity is questionable.

Stereoisomers of the trans-decahydroquinoline (**228**) (273) and the trans-decalin (**229**) (88) displayed extremely low orders of muscarinic effect, with the 2,3-*trans*-diaxial isomer of (229) being the most potent of the four stereoisomers of this structure (0.06%the potency of acetylcholine).



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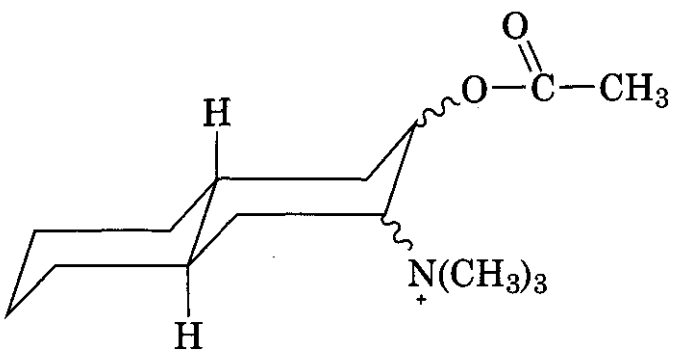
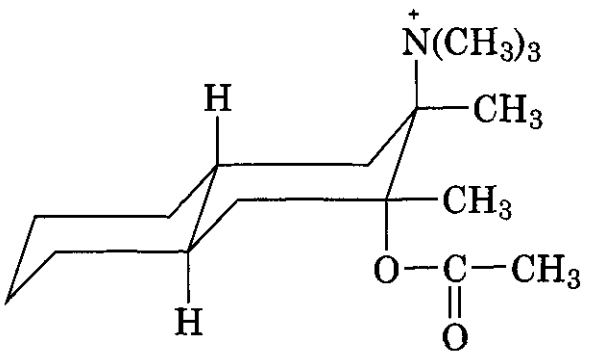


(229)

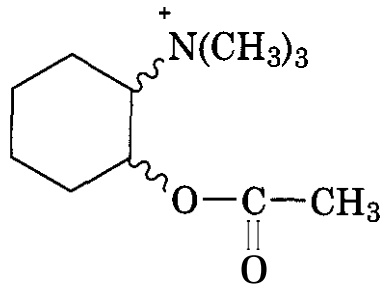
A possibly serious defect in the design of compounds such as the piperidinium (**222**), the morpholinium (**223**), the tropines (226) and (**227**), and the decahydroquinoliniums (**228**) is that these molecules do not bear the trimethylammonium cation characteristic of acetylcholine, but rather the quaternary head is a part of a ring system. It was indicated previously that incorporation of the nitrogen atom of acetylcholine itself into a ring is detrimental to cholinergic activity and potency.

In a series of C-methylated trans-decalin congeners of (**229**), the 2,3-dimethyl compound (230) was the most potent muscarinic in a guinea pig ileum assay [2% as active as acetylcholine (274)].



(230)

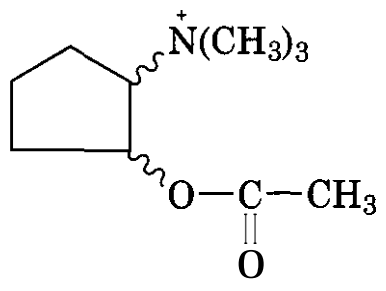
The most potent members of all of these series of decalin-derived molecules have antiplanar τ_2 stereochemistry. In the series of cyclohexane-derived compounds (**231**), the (1*R*,2*R*)-*trans* compound was an extremely weak muscarinic and the (±)-*cis*-isomer was completely inert (275). Casy (256) suggested that an energetically unfavored trans-diaxial conformer (antiplanar τ_2) for structure (231) may be the pharmacologically active form of the molecule. However, introduction of a t-butyl group into the cyclohexane system to sta-



(231)

bilize the 1,2-*trans*-diaxial geometry did not lead to greatly increased muscarinic effect (276).

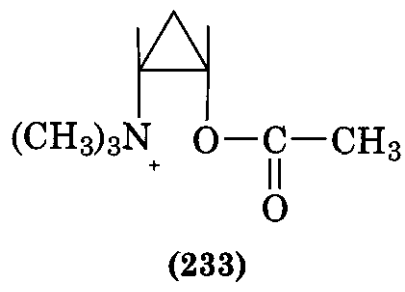
The cis- and trans-cyclopentane systems (232)have been described (256)as feeble spasmogenics with a τ_2 angle near anticlinal.



(232)

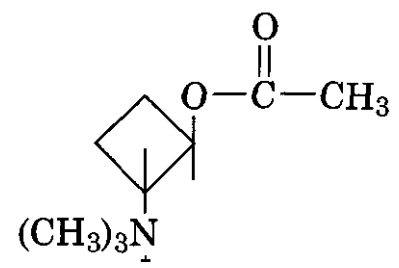
Congeners of acetyl y-homocholine (29) and 4-acetoxybutyltrimethylammonium (**30**), in which the amino alcohol entity is a part of cyclopropane or cyclobutane ring systems, exhibited feeble muscarinic and appreciable nicotinic effects (277, 278), but these results provide little insight into active conformations for acetylcholine. The low cholinergic potencies and activities of all of these preceding compounds probably preclude their being used as a basis for acetylcholine conformation-activity hypotheses, because "almost any compound with a quaternary nitrogen has some stimulant or inhibitory activity at cholinergic receptor sites" (279).

The cyclopropane ring has been exploited as the smallest system capable of conferring conformational rigidity on an acetylcholine analog (280, 281); (+)-*trans*-ACTM (221) equals or surpasses acetylcholine's muscarinic potency in two test systems, and it is an excellent substrate for acetylcholinesterase. The (−)-trans-enantiomer is several hundred times less potent, and the racemic *cis*-compound (**233**) is almost inert.



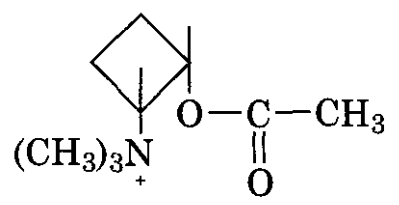
(233)

All stereoisomers of (**221**) and (233) are feeble nicotinics. X-ray analysis of (+)-trans-ACTM (221) (282) established the τ_2 angle as 137" (which is within the anticlinal range), and because of the rigidity of the cyclopropane ring, this value probably closely approaches the solution conformation. The (1*S*, 2*S*) absolute configuration of (+)-*trans*-ACTM superimposes on the equivalent centers in the potent muscarinic agonists (*S*)-(+)-acetyl-β-methylcholine (31) (see Table 2.2) and (2*S*,4*R*,5*S*)-(+)-muscarine (3) (283). A racemic cyclobutane analog (**234**) of trans-ACTM is much less potent than (±)-*trans*-ACTM (284).



(234)

No conformational study on structure (**234**) has been reported, and inspection of molecular models did not reveal any convincing structural or steric differences between the cyclopropane and cyclobutane systems. The racemic cis-cyclobutane isomer (235) is equipotent to the racemic trans-isomer (234) as a muscarinic receptor stimulant (285). Chothia and Pauling (282) defined the following molecular parameters for muscarinic agonism in acetylcholine congeners (cf. structure (**220**), based on conformational analysis of (+)-trans-



(235)

ACTM (221): $\tau_1 = 180°$; $\tau_2 = +73°$ to $+137°$; $\tau_3 = 180 \pm 35°$; $\tau_4 = 180°$ or $-137°$. Interatomic distances were defined as: N^+-O^1 = 360; N^+-C^6 = 450; N^+-C^7 = 540 pm. Low potency or inactivity of certain acetylcholine derivatives was attributed to deviation from one or more of these parameters. However, Casy (256) cited examples of deviations from these values in which high agonist activity is manifested.

It has not been possible to demonstrate unequivocally that acetylcholine assumes different conformations for interaction at nicotinic and muscarinic receptors and/or at the subpopulations of each major receptor subtype; neither has this theory been disproved by the body of chemical and biological data. It must be concluded that the relationship of acetylcholine's molecular geometry to its physiological roles is still not understood.

6 ANTICHOLINESTERASES

Symptoms resulting from an inadequate supply of acetylcholine may be relieved by blocking the body's acetylcholine-deactivating mechanism. Interest in this category of agents has increased greatly over the past several years, as a result of recognition of the potential value in therapy of Alzheimer's disease as well as of other defects in memory and learning. Giacobini (286) presented data confirming that a steady-state increase in acetylcholine resulting from cholinesterase (sic) inhibition in the brain results in improvement of cognitive function and mild-to-moderate cases of Alzheimer's syndrome. Sussman and coworkers (287) showed that the catalytic site of acetylcholinesterase is located at the bottom of a deep and narrow gorge surrounded by 14 aromatic amino acids. Moreover, these workers presented evidence that the quaternary ammonium moiety of acetylcholine does not interact with an anionic site on acetylcholinesterase, but rather it binds with the π electrons of Trp-84 (tryptophan). QSAR studies (288) of a series of nicotine analogs and congeners indicated that the cationic moieties of various nicotinic receptor ligands interact with aromatic groups on $\alpha_4\beta_2$ and α_7 nicotinic receptors to an extent proportional to their receptor binding coefficients.

Figure 2.1 is a simplified representation of the catalytic region of acetylcholinesterase

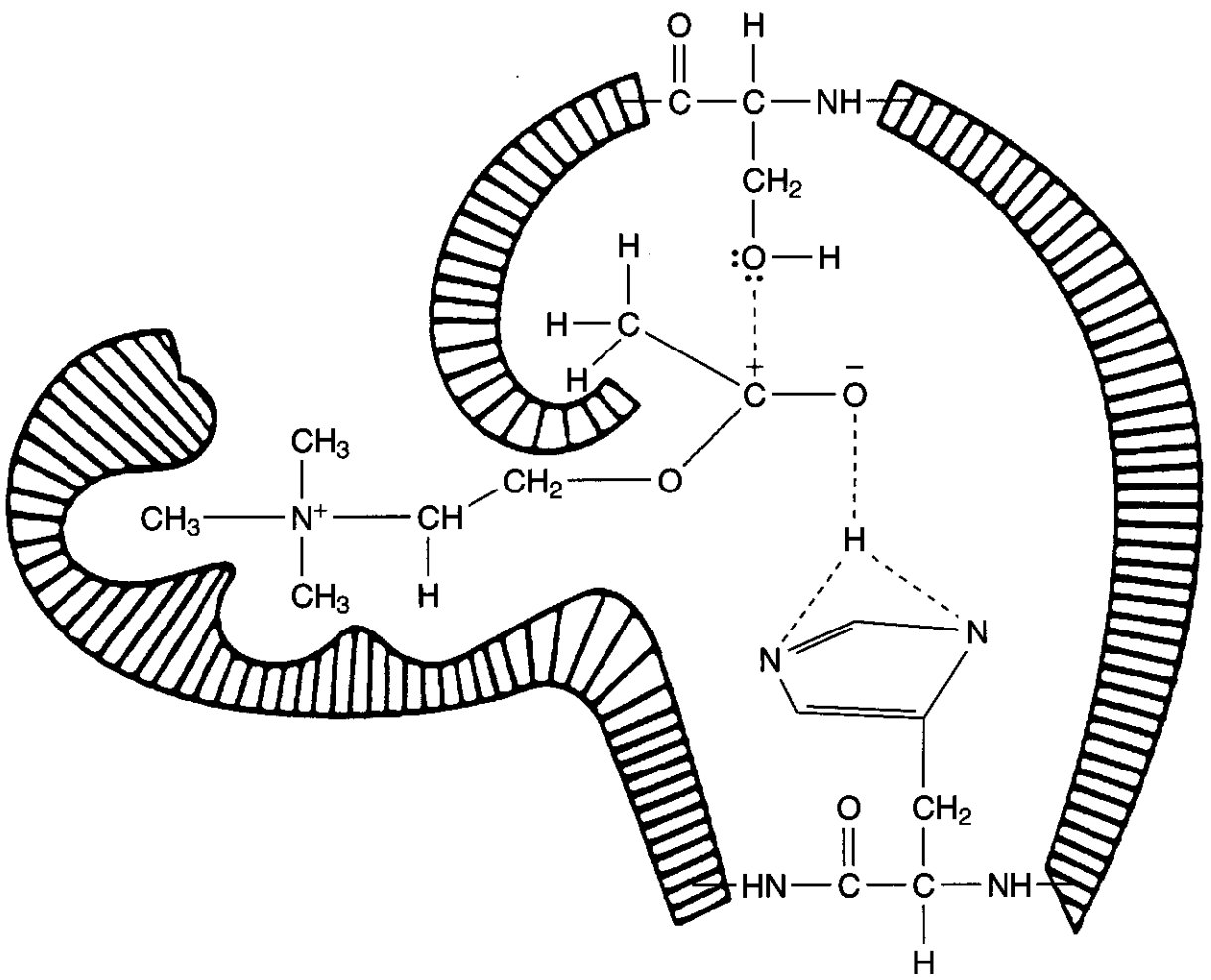


Figure 2.1.

(289). Significant features are the Trp-84 aromatic center that anchors the quaternary head of the substrate; a serine residue, the primary alcohol moiety of which participates in a transesterification reaction with acetylcholine, resulting in acetylation of the enzyme; and an imidazole ring (part of a histidine residue) that, as a neighboring group, participates in and facilitates the acetyl group transfer. The resulting acetylated serine moiety is extremely labile and rapidly undergoes spontaneous hydrolytic cleavage to liberate acetate anion and to regenerate the active catalytic surface.

Taylor (290) described three classes of acetylcholinesterase inhibitors, based on their mechanism of action:

1. Reversible inhibitors.
2. Agents having a carbamate ester moiety that is hydrolyzed by acetylcholinesterase, but much more slowly than acetylcholine.
3. Phosphoric acid- or phosphonic acid-derived inhibitors, which are true hemisubstrates for acetylcholinesterase.

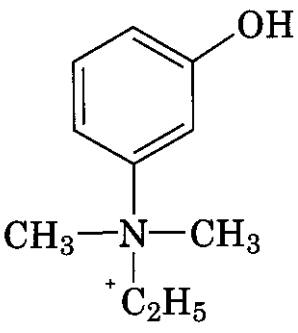
Both the carbamates and the phosphorus derivatives form a covalent (ester) bond with the serine OH of the enzyme in essentially the same manner as does acetylcholine. Taylor (290) stated that the terms reversible and irreversible as they have been applied to the carbamate and phosphorus-derived anticholinesterase agents, respectively, reflect only quantitative differences in rates of cleavage of the esterified enzyme, and not an actual difference in mechanism.

6.1 Quaternary Ammonium Reversible Inhibitors

Simple quaternary compounds such as tetramethylammonium cation combine with the substrate cation-binding site of the catalytic surface of acetylcholinesterase and thus deny acetylcholine's access to this site. These compounds have a short duration of action due to the facile reversibility of their binding and rapid renal elimination (290), and thus they have minimal therapeutic utility. Cohen and Oosterbaan (291) tabulated a comprehensive list of tetraalkyl quaternary ammonium acetylcholinesterase inhibitors. Homologation of some or all of the methyl groups on the tetramethylammonium molecule tends to increase potency (292). However, attempts to correlate biological test data with the calculated diameter of the unhydrated quaternary head led to inconclusive results.

Belleau (293) calculated entropies and enthalpies of binding to acetylcholinesterase for a homologous series of alkyltrimethylammonium compounds $RN^+(CH_3)_3$, where R = CH_3 through n-$C_{12}H_{15}$. The observed relative potencies in the series were rationalized on the basis of hydrophobic bonding phenomena coupled with the ability of the alkyl chains to displace ordered water from the acetylcholinesterase surface.

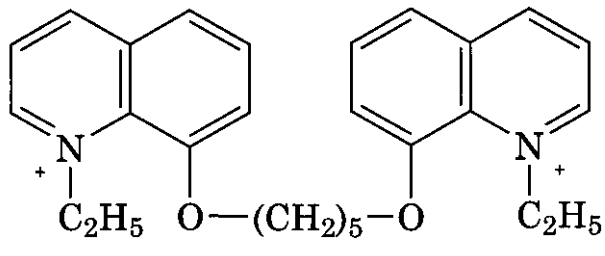
Edrophonium (236) combines a quaternary head for binding to the complimentary site of the enzyme with a phenolic OH, which presumably hydrogen bonds to a portion of the esteratic area.



(236)

However, even this compound displays a rapidly reversible inhibition of the enzyme and its duration of action is short (290).

Holmsted (294) tabulated an extended series of bis-quaternary ammonium compounds that have been evaluated for anti-acetylcholinesterase activity; compound (**237**) is representative of this category, which includes some of the most effective enzyme inhibitors.



(237)

Additional examples of anticholinesterase bis-quaternary ammonium compounds were

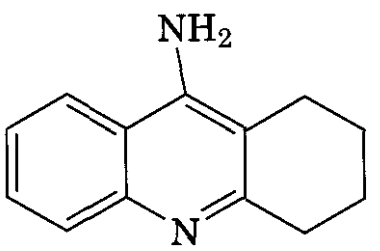
reported by Fulton and Mogey (295) and by Cavallito and Sandy (296), who noted that there is a gradual increase in antiacetylcholinesterase activity as the chain joining the two quaternary heads increases. The optimum connecting chain length was stated to be five or six carbons. In addition, enzyme inhibitory activity was maximal in those molecules in which the substituent(s) on the quaternary nitrogen were decidedly lipophilic. Later studies (297) demonstrated efficient bonding of decamethonium (238) to Torpedo acetylcholinesterase, contradicting earlier (296) reports.

$$(CH_3)_3\overset{+}{N}(CH_2)_{10}\overset{+}{N}(CH_3)_3$$

(238)

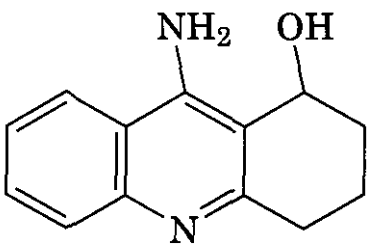
6.2 Reversible, Noncovalent inhibitors Related to 1,2,3,4-Tetrahydro-9-Aminoacridine

1,2,3,4-Tetrahydro-9-aminoacridine (239), THA, tacrine) was described in 1961 (298) as a reversible inhibitor of acetylcholinesterase and an even more potent inhibitor of butyrylcholinesterase. On the basis of X-ray crystal studies it was reported (299) that a tryptophan residue (Trp-84) at the catalytic surface of acetylcholinesterase is the binding site for the aromatic ring of (239). This is the same domain that is believed to bind the quaternary head of acetylcholine.



(239)

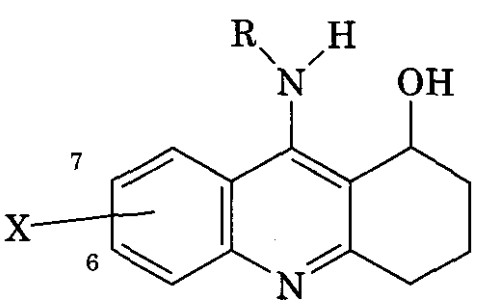
Clinical efficacy in relief of the symptoms of Alzheimer's disease was claimed (300) for THA, but this positive finding is tempered by its tendency to produce hepatotoxicity (301). It was speculated (302) that the hepatotoxicity of (239) might be related to its lipophilic character, and a (±)-1-hydroxy derivative (240) was designed in the hope that the OH group would serve as a metabolic "handle" for glucuronidation and subsequent facilitated elimination. Compound (240) is a somewhat less potent acetylcholinesterase inhibitor in vitro than THA. However, the two compounds are approximately equipotent in reversal of scopolamine-induced memory impairment in mice, a putative predictive model of activity in Alzheimer's disease. These data suggest that, in addition to acetylcholinesterase inhibition, there may be other biochemical components to the mechanism of action of (240). This specu-



(240)

lation, which may be applicable to THA itself and to others of its active analogs and congeners, is supported by a summation (303) of reported pharmacological effects of THA: blockade of potassium channels; inhibition of neuronal uptake processes; and inhibition of monoamineoxidase. In the design of THA-like anti-Alzheimer drugs, it has been emphasized that selectivity of inhibition of acetylcholinesterase as compared with inhibitory effect on butyrylcholinesterase is a highly desirable strategy for minimizing unwanted side effects.

A structure-activity study (304) addressed modifications of the THA molecule, illustrated in structure (241): X = H, 6-Cl, 7-Cl, 6-F, or 6-



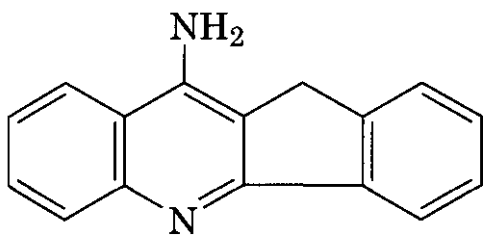
(241)

CF_3; R = H, alkyl, benzyl, ring-substituted benzyl, or ω-phenoxyalkyl.

Most of the compounds were inferior to (239) as inhibitors of acetylcholinesterase, but some were decidedly less toxic, and a few were equieffective or superior to (239) in an assay evaluating their ability to reverse sco-

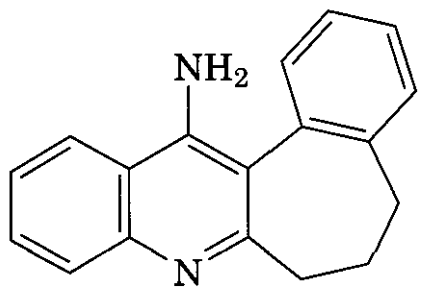
polamine-induced memory impairment. Recanatini et al. (305) reported a comprehensive structure-activity study of THA derivatives substituted on positions 6 and 7 of the ring system and bearing selected groups on the 9-amino group. QSAR studies and comparative molecular field analysis (CoMFA) of the THA analogs permitted some conclusions to be drawn with respect to the applicability of these analytical techniques to formulation of descriptive and predictive structure-activity relationships of THA derivatives.

Addition of a fourth ring to the THA system sometimes leads to compounds such as (242), which have marked selectivity for inhibition of acetylcholinesterase over butyrylcholinesterase (303).



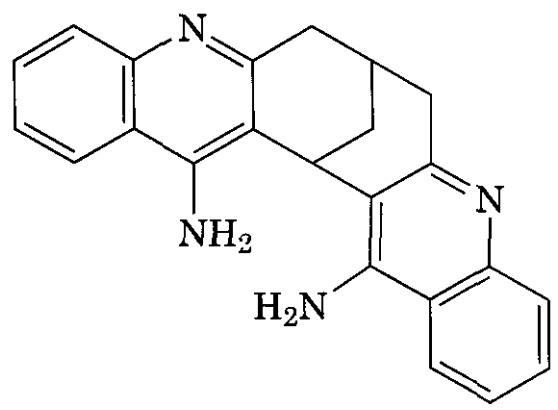
(242)

Compound (243) was reported to be 100–400 times more potent than THA in inhibition of neuronal uptake of serotonin. A series of



(243)

compounds related to (244) was designed by "molecular duplication" of the THA molecule (306). Some members of the series showed inhibitory action against acetylcholinesterase, but they were less potent than THA. One compound reversed cognitive deficits in middle aged rats. Compound (245) was designed taking into account that there are two binding sites for THA on acetylcholinesterase (307). The compound is 10,000 times more selective for acetylcholinesterase than for butyrylcholinesterase and is approximately 1000 times more potent than THA in inhibition of rat acetylcholinesterase. This preference of (245) for acetylcholinesterase and the fact that THA-like agents may exhibit unwanted side effects arising from peripheral actions accompanying desired central actions led to studies of a series of heterodimeric analogs (246) of (245) (297).



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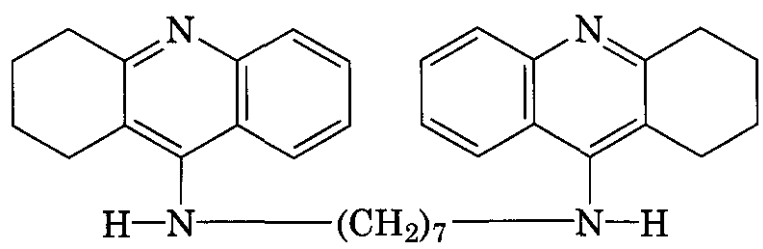
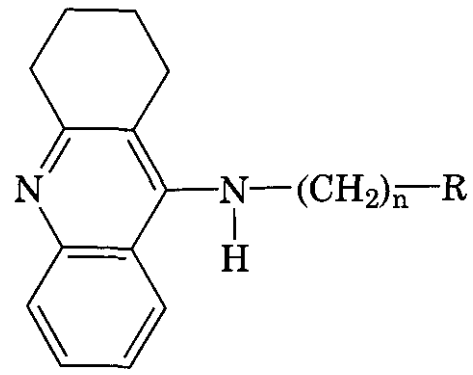
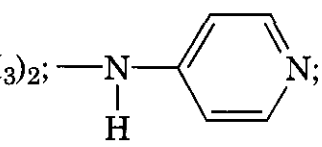
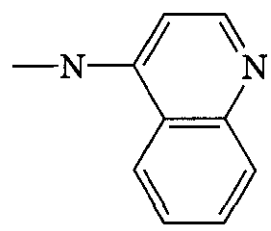


(245)



(246)

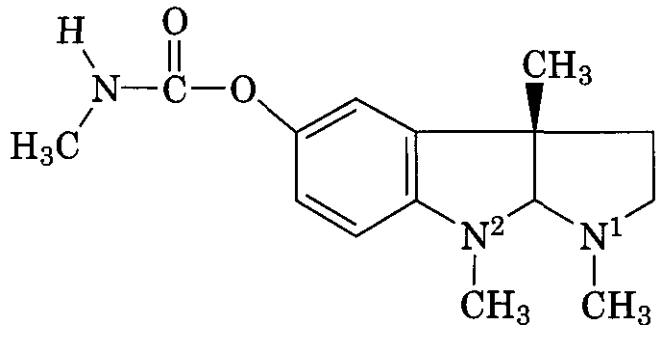
n = 7 or 8

R = —NH_2; —$N(CH_3)_2$;  ; 

Comparison of calculated desolvation free energies with IC_{50} values suggested the importance of ligand hydrophobicity (low desolvation free energy) for effective cation-π interaction of the homodimer (245) with peripheral site(s).

6.3 Carbarnate-Derived Inhibitors

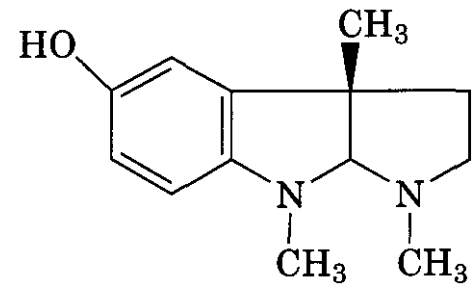
The prototype carbarnate-derived acetylcholinesterase inhibitor is physostigmine (**247**), an alkaloid isolated from the seeds of the Calabar bean, Physostigma venenosum. Physostigmine exhibits equal inhibitory activity against acetylcholinesterase and butyrylcholinesterase (308).



(247)

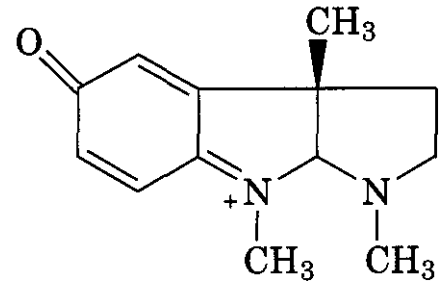
At the pH of the body fluids, a significant proportion of physostigmine molecules is protonated at N^1. This cationic species participates in binding of the molecule to the catalytic domain of acetylcholinesterase, to permit transfer of the N-methylcarbamyl moiety to the OH of the serine residue, analogous to the process described for the acetyl group of acetylcholine. The resulting carbamylated enzyme is much more stable than the acetylated enzyme ($t_{1/2}$ for hydrolysis of the carbamylated enzyme is 15–30 min compared to <1 ms for the acetylated serine moiety) (290). Maintenance of the enzyme in its carbamylated form prevents its catalytic hydrolysis of acetylcholine for a prolonged time. In *vivo*, the duration of observable enzyme inhibition by agents such as physostigmine is 3–4 h (290). Watts and Wilkinson (309) developed a kinetic scheme that was offered as a more adequate explanation for carbamate-acetylcholinesterase reactions and provides an explanation for the observed catalysis of ester cleavage of carbamylated acetylcholinesterase by excess carbamate. Physostigmine has been reported (310) to have memory enhancing effects in patients with Alzheimer's disease. However, for clinical use it has a relatively short half- life, variable bioavailability, a low therapeutic index, and a multiplicity of unwanted cholinergically related side effects.

(+)-Physostigmine, the enantiomer of the naturally occurring alkaloid, has little effect on acetylcholinesterase in *vitro* (311,312); it is a weak centrally acting cholinergic agonist. Eseroline (**248**), the ester cleavage product of



(248)

physostigmine, is devoid of action toward horse serum butyrylcholinesterase (313). Rubreserine (**249**), an oxidation product of the

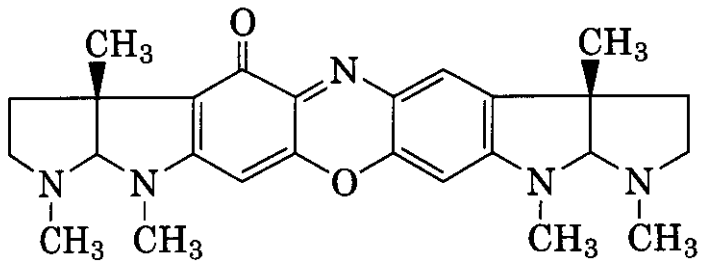


(249)

ester-cleaved physostigmine molecule, was reported to be approximately 1/100 as potent as physostigmine as an inhibitor of horse serum butyrylcholinesterase (314).

Eserine blue (**250**), formed from a reaction of rubreserine with ammonia (315), was reported to exhibit very low potency in a horse serum butyrylcholinesterase assay (314). A later communication (316) suggests total inactivity of these latter two physostigmine derivatives.

Brossi (317) prepared two short series of physostigmine homologs, one in which the substituent(s) on the nitrogen of the carbamate moiety was (were) varied, and one in which the substituent on N^1 was varied. Both

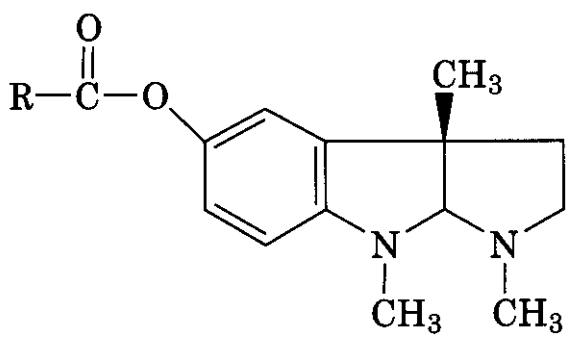


(250)

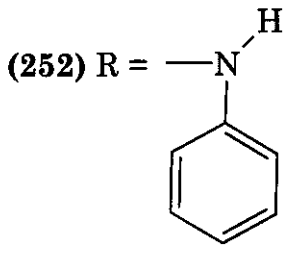
(+)- and (−)-enantiomers of the first series were prepared, but only the (−)-enantiomer of the second series was reported. Several of the (−)-enantiomers (same absolute configuration as physostigmine) showed high potency in inhibition of acetylcholinesterase and of butyrylcholinesterase from a variety of sources.

Heptylphysostigmine (**251**) is a more lipophilic homolog and is reported (318) to be less toxic than physostigmine, while retaining its in vitro acetylcholinesterase inhibiting potency.

Phenserine (**252**), a selective inhibitor of acetylcholinesterase with minimal effect on butyrylcholinesterase, was cited as a possible anti-Alzheimer drug (319).

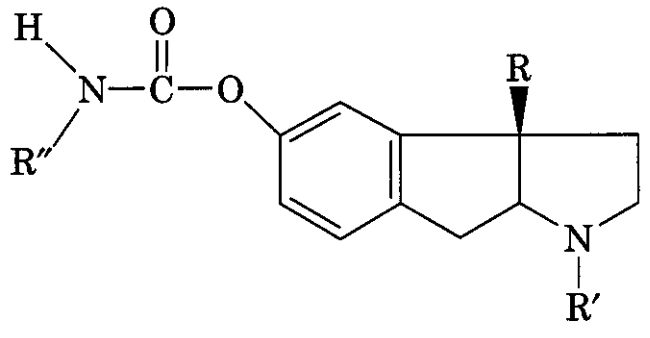


(251) R = —N(H)C_7H_{15}—*n*

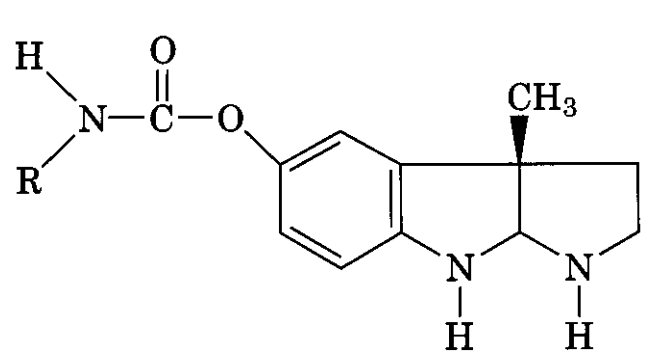


(252) R = —N(H)

It was postulated (320) that replacing the N^2 of the physostigmine molecule with a methylene group would increase the molecule's chemical and metabolic stability by conversion of the potentially less stable aminal group to a more stable amino group. A series of 8-carbaphysostigmine congeners (**253**) was studied, in which R was H or CH_3; R' was CH_3, C_2H_5, *n*-C_3H_7, or benzyl; and R" was a variety of C_1-C_7 alkyl chains, phenyl, or benzyl. All compounds were tested as their racemic modifications; selected ones were resolved, and both enantiomers were studied. Two (−)-enantiomers, R = CH_3, R' = C_2H_5, R" = n-C_7H_{15}; and R = CH_3, R' = C_2H_5, R" = *n*-C_6H_{13}, were more potent than physostigmine or (251) in inhibition of acetylcholinesterase, and they were six- to 10-fold more potent than their respective (+)-enantiomers. These more active enantiomers had the same absolute configuration as physostigmine itself. Two *bis*-noreseroline derivatives (**254a**, **254b**) showed marked antiacetylcholinesterase activity (316).



(253)

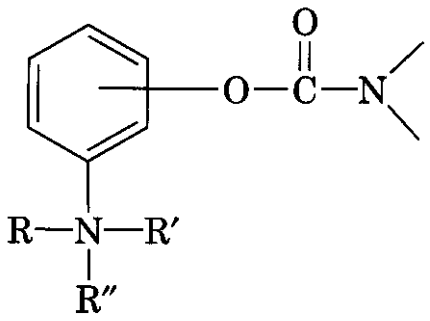


(254a) R = C_6H_5 (3a *S*)
(254b) R = CH_3 (3a *S*)

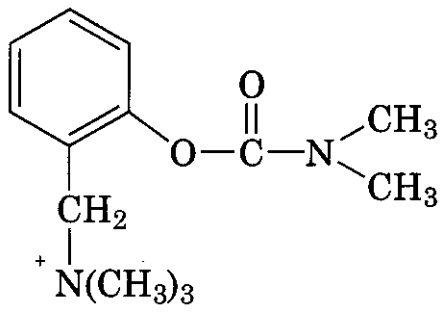
Compound (**254a**) had relatively little effect on butyrylcholinesterase, whereas (**254b**) was a potent inhibitor of both enzymes. The 3a *R* enantiomers were less potent but they showed the same selectivity as the 3a ***S*** compounds.

Studies aimed at incorporation of the carbamate ester moiety and the cationic site of physostigmine into simpler organic molecules have led to quaternary ammonium compounds based on structure (**255**).

The 3-substituted isomer of (255) is neostigmine. This compound is more stable than physostigmine in aqueous solution, it



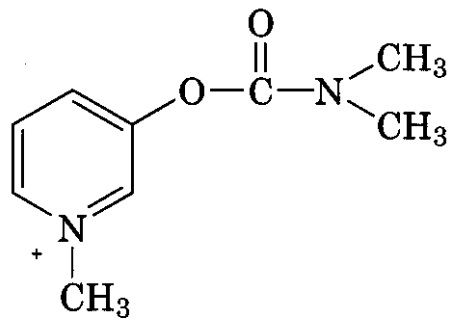
(255)



(257)

does not penetrate the blood-brain barrier, and also (unlike physostigmine) the pharmacological effects of neostigmine are pH independent (321). Neostigmine is extremely potent, and it has replaced physostigmine as the reference drug for carbamamate-derived inhibitors of acetylcholinesterase.

The position 3-substituted isomers of (255) and (**256**) frequently exhibit prominent miotic activity (which was taken as a reflection of anticholinesterase activity), whereas *ortho*- and para-substituted molecules are inert (313). Foldes and coworkers (322) concluded that the optimum N^+-to-C=O interatomic distance for compounds of the type (255) is 4.7 Å. The meta isomers of these compounds meet this requirement, as does pyridostigmine (**256**), which is used clinically. Molecular models



(256)

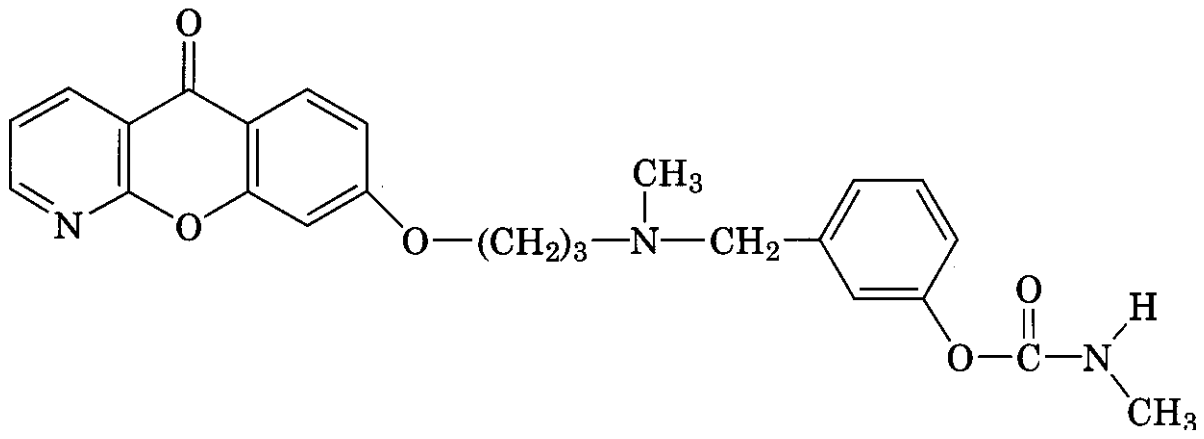
demonstrate that the active m-quaternary ammonium phenylcarbamate systems can assume reasonable conformations in which their cationic heads and C=O groups coincide with analogous groups in acetylcholine. Long (313) described the ortho-substituted compound (257) as a potent acetylcholinesterase inhibitor, whereas the meta- and para-isomers were much less active. Compound (257) conforms to the 4.7-Å N^+-to-C=O distance requirement, but its other two positional isomers do not. It is noteworthy that physostigmine and its congeners (e.g., compounds 251 and 253 deviate from the proposed 4.7 Å requirement; their N^+-to-C=O distance is considerably greater (on the order of 8–8.5 Å).

Compound (258) was reported to be 190 times more potent than physostigmine against acetylcholinesterase, and it was 60 times more selective for inhibition of acetylcholinesterase than for butyrylcholinesterase (323).

Most anti-Alzheimer drug design studies directed at enzyme inhibition have sought compounds that inhibit acetylcholinesterase, but have little or no effect on butyrylcholinesterase. However, based on cited literature suggestions that "inappropriate" butyrylcholinesterase activity increases the risk and/or progression of Alzheimer's disease, Brossi et al. (324) described physostigmine congeners (eseroline and N_1 noreseroline carbamates) having a high level of selectivity for butyrylcholinesterase rather than acetylcholinesterase. Possible therapeutic utility for these agents was proposed, but no test data were presented.

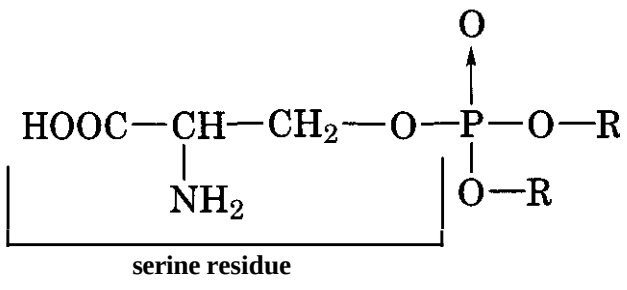
6.4 Phosphorus-Derived Inhibitors

Among the most powerful anticholinesterases (inactivating both acetylcholinesterase and plasma butyrylcholinesterase) are phosphorus-containing compounds, most commonly derivatives of orthophosphoric acid or of phosphonic acids. The entire category is frequently collectively called organophosphorus compounds although, strictly speaking, this nomenclature is inaccurate. Certain of these organophosphorus compounds are extremely toxic, and much of the developmental work in the area was done with the object of preparing chemical warfare agents ("nerve gases"). Compounds in this category are potent and useful insecticides, and have been used world-



(258)

wide for this purpose. The reaction between acetylcholinesterase and most organophosphorus inhibitors has been presumed to occur only at the esteratic site of the enzyme, and the reaction here is a transesterification, comparable to that involving the carbamate esters and acetylcholine itself. The reaction at the esteratic site of acetylcholinesterase is enhanced by the geometry of the tetrahedral phosphates, which resemble the transition state for acetate ester hydrolysis. The resulting serine-phosphorylated or -phosphonylated enzyme is extremely stable; if the R groups (structure 259) are methyl or ethyl, regeneration of the enzyme by hydrolytic cleavage requires several hours.

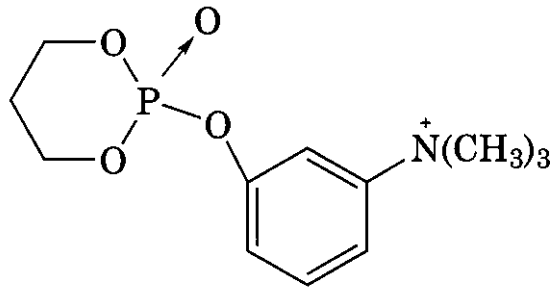


(259)

If the R groups are isopropyl, essentially no hydrolysis occurs and reestablishment of enzyme activity can occur only after de novo synthesis of the enzyme. A characteristic structural feature of the anticholinesterase phosphorus compounds is the grouping P-Z, where Z is an electronegative moiety, a good leaving group, and the cleavage of the P-Z bond is accompanied by liberation of a large amount of energy. The P-Z bond is eminently susceptible to attack by nucleophiles, such as the serine OH of the esteratic site of acetylcholinesterase. In general, the enzyme inhibitory potency of the organophosphorus compounds parallels the ease of nucleophilic attack on the phosphorus atom. Compounds in this category include ester and amide derivatives of orthophosphoryl halides, pyrophosphate esters and amides, alkyl and aryl phosphonic acid derivatives, and thiophosphoric acid derivatives. Representative compounds are shown in Table 2.5.

Holmsted (294, 325) and Hayes (326) have tabulated and discussed a large number of organophosphorus acetylcholinesterase inhibitors. Tabun, sarin, and soman (2, 3, and 4 in Table 2.5) are among the most toxic "nerve gases" known. OMPA (6) is inert as such, but it is metabolized to an N-oxide derivative that is the biologically active entity (327). Parathion (8) is inactive in inhibition of acetylcholinesterase in vitro; mixed-function oxidases (in human liver) convert parathion into its oxygen bioisostere paraoxon (9), the pharmacologically active metabolite (328). Echothiophate (7) is representative of inhibitors that bind initially to the agonist cation binding site of acetylcholinesterase as well as to the esteratic area. This compound is used clinically in the treatment of certain types of glaucoma.

The 1,3,2-dioxaphosphorinane (260) is representative of organophosphorus acetylcho-



(260)

Table 2.5 Some Phosphorus-Containing Acetylcholinesterase Inhibitors

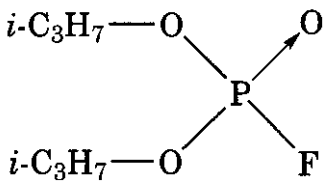
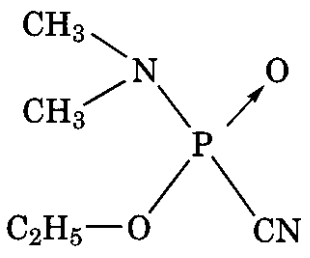
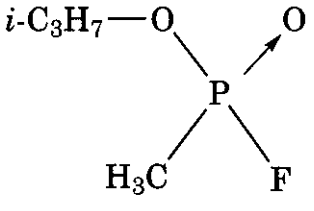
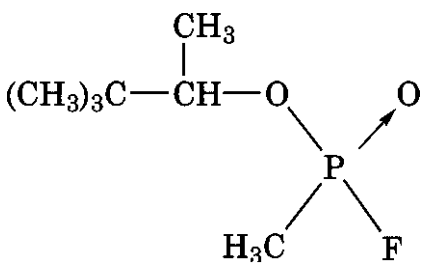
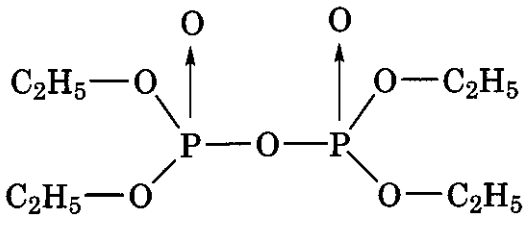
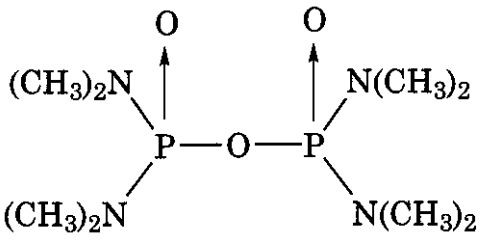
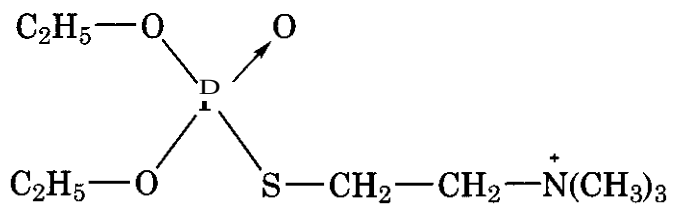
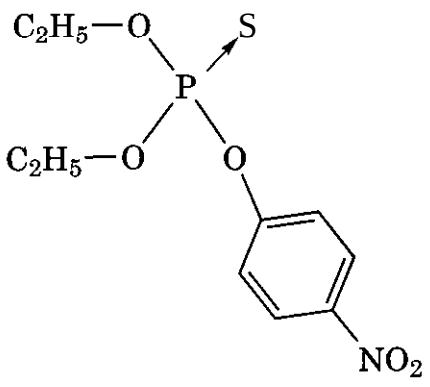
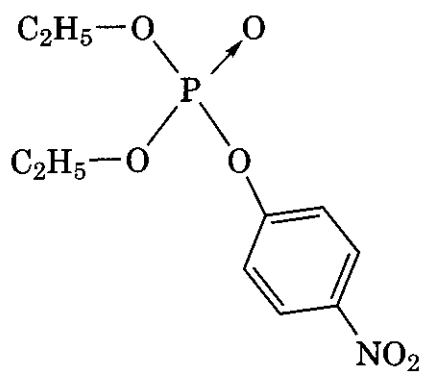
Number	Structure	Chemical, Proprietary, or Generic Name(s)
(1)		DFP, diisopropyl fluorophosphate, diisopropyl phosphofluoridate
(2)		Tabun, ethyl *N*-dimethyl phosphoroaminocyanidate
(3)		Sarin (GB), isopropyl methylphosphonofluoridate
(4)		Soman, pinacolyl methylphosphonofluoridate
(5)		Tetraethyl pyrophosphate (TEPP)
(6)		Octamethylpyrophosphoramide

Table 2.5 (Continued)

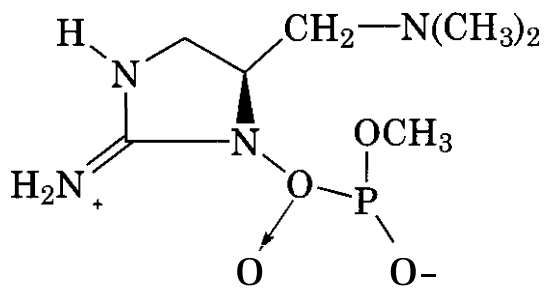
Number	Structure	Chemical, Proprietary, or Generic Name(s)
(7)		Echothiophate, diethoxyphosphorylthiocholine
(8)		Parathion
(9)		Paraoxon

linesterase inhibitors that were designed to have a short duration of action (329).

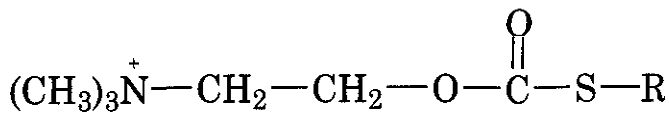
This compound inactivates acetylcholinesterase by formation of "an unstable covalent intermediate." The inhibited enzyme hydrolyzes spontaneously with $t_{1/2} \approx 10$ min. Compound (**260**) was proposed (329) to be a useful adjunct prophylactic agent against the insecticide paraoxon and chemical warfare agents such as soman.

A neurotoxic natural product, anatoxin-a(s) (**261**), was isolated from several biological sources, including a blue-green alga (330, 331).

Its high toxicity (LD_{50} = 20–40 μg/kg in mice) has been ascribed to anticholinesterase activity.



(261)

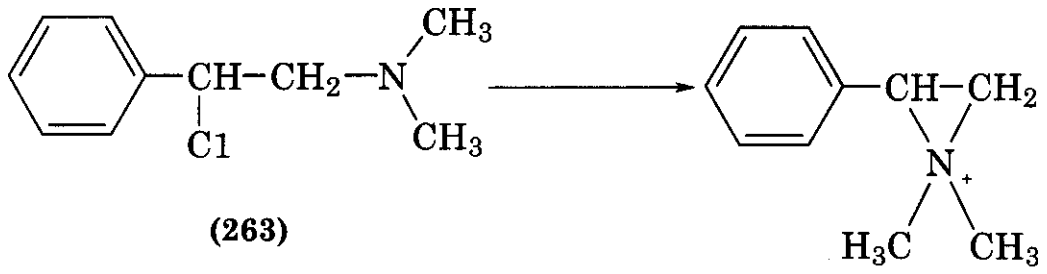


(262a) R = C_5H_{11}
(262b) R = C_7H_{15}

6.5 Miscellaneous Inhibitors

Thiocarbonate derivatives of choline (**262a**, **262b**) competitively inhibit acetylcholinesterase from various sources (332).

These compounds are not hydrolyzed by acetylcholinesterase. Possible utility in treatment of Alzheimer's syndrome was suggested. An α-chloro-β-phenethylamine (263) irrevers-

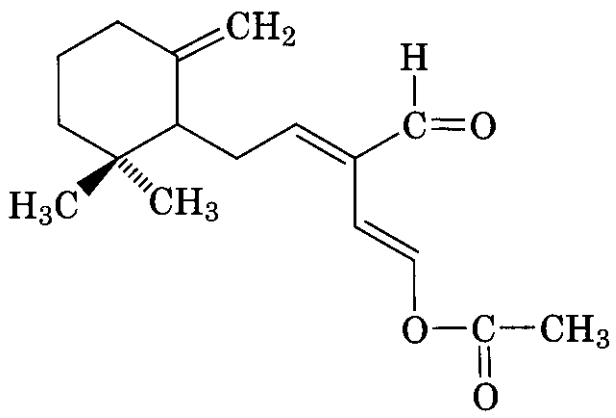


(263) (264)

ibly inactivates acetylcholinesterase (333);the active pharmacophoric species is the aziridinium cation (264).

The quaternary ion nature of the aziridinium cation allows for reversible complex formation with the cation binding site of the enzyme, which precedes slow alkylation of the nucleophilicsite (serineOH). Tetramethylammonium retards the irreversible inactivation of the enzyme by this compound.

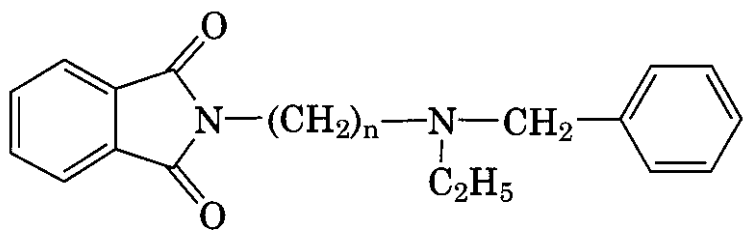
Onchidal (**265**) is the principal constituent of a secretion of glands of a mollusk (334). This



(265)

compound is an irreversible inhibitor of acetylcholinesterase. It is not a substrate for the enzyme, and its mechanism of action apparently does not involve acylation of the active site serine hydroxyl. It was speculated (334) that the α,β-unsaturated aldehyde moiety may be involved, through Michael addition, in covalent bond formation with the enzyme.

Based on a molecular design strategy derived from rationalizations concerning the topography of the catalytic area of acetylcholinesterase, inhibitors based on compounds (266) and (267) were identified (335, 336). Study of an extended series (structure 268 and congeners of 266 and 267) revealed that one compound (268: R = H; Y = H; n = 3 and attachment of the ketonic carbon to position 8 of the tetrahydrobenzazepine ring) demonstrated potent *in vitro* inhibition of acetylcholinesterase (337). It was active in a series of in *vivo* assays for CNS cholinergic effects, but it produced no significant peripheral cholinergic effects. Molecular modeling studies (docking analysis) of members of the series of compounds (266–268) indicated (338) that the *N*-benzyl group interacts with the same tryptophan residue (Trp-84) as the aromatic ring of THA (**239**). The other aromatic ring in these inhibitors interacts with another tryptophan residue (Trp-279) on the enzyme molecule, and hydrogen bonding interaction between the carbonyl group of the inhibitors (266–268) and a tyrosine (Tyr-121) hydroxyl on the enzyme seems to play an important role. These data should be useful for the future design of more potent, more specific inhibitors.



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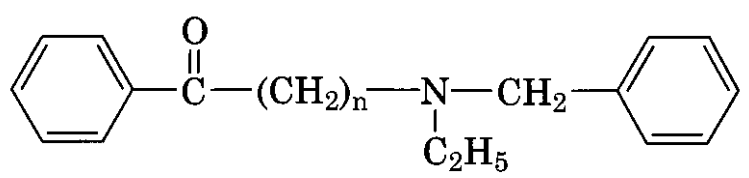
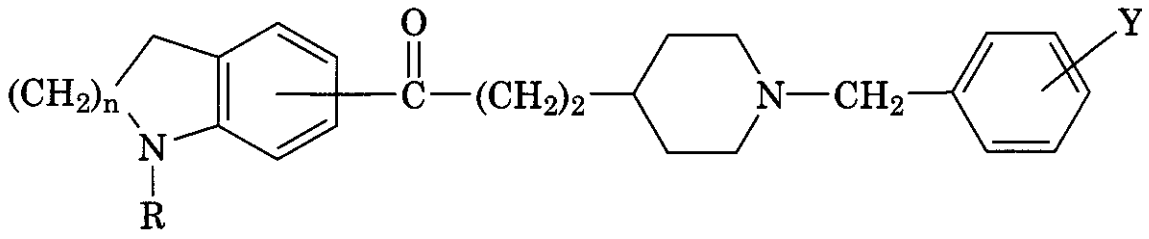
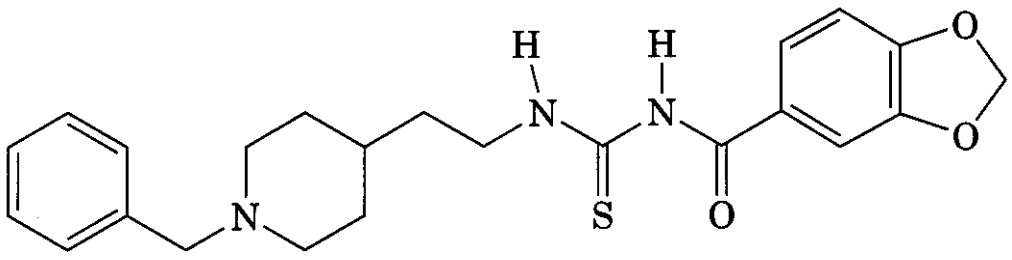


(267)

Members of a series of 1-aroyl-3-[1-(benzyl-4-piperidinyl)ethyl]thiourea derivatives (269) are potent (submicromolar range) acetylcholinesterase inhibitors (339).



(268)

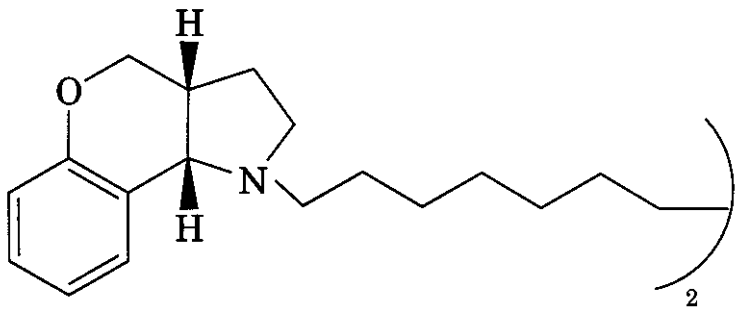


(269)

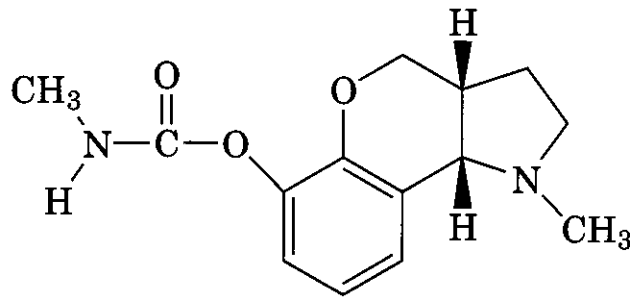
Comparable potency was retained by replacing the unsubstituted benzene ring with a bioisosteric 2-pyridyl group. The guanidine congener of (269) was almost inactive. In a passive-avoidance test in rats, compound (269) had maximal antiamnesiac activity at 0.03 mg/kg with a therapeutic ratio greater than 1000, and it displayed cholinergic side effects only at high doses. Its potential use as an antidementia agent was suggested (339). The linear diamide (270) (caproctamine) was described (340) as a more potent noncovalent inhibitor of acetylcholinesterase than of butyrylcholinesterase.

It was reported to interact at both the active site of the enzyme and at a second distal site. It also showed prominent inhibitory action at M_2 receptors, but it had only weak effect at M_1 and M_3 subtypes. In a search for less flexible analogs of caproctamine (**270**), the tricyclic system (**271**) was found to exhibit "good" acetylcholinesterase inhibiting activity (341).

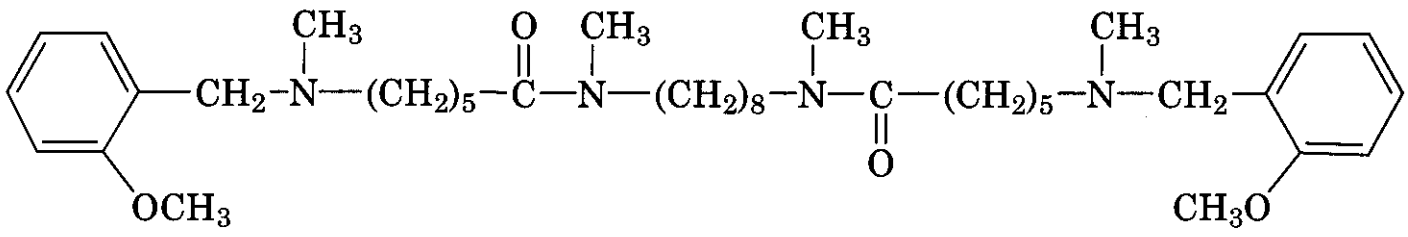
A carbamate derivative of the hexahydrochromenopyrrole moiety of (**271**), (±)-(**272**) (viewed as an analog of the physostigmine ring system), was almost as potent as physostigmine *in vitro* against human acetylcholinesterase and butyrylcholinesterase. Other ben-



(271)



(272)



(270)

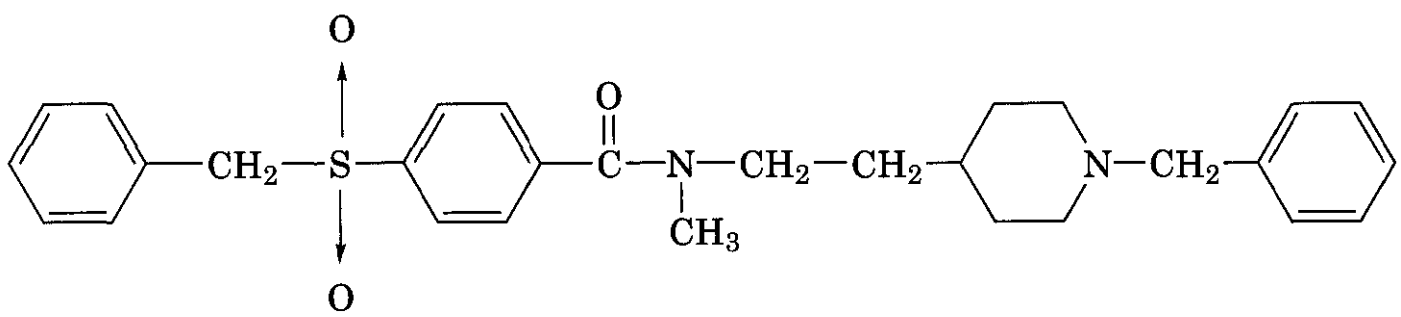
zene ring position isomers of the carbamate moiety were much less potent. The two enantiomers of (**272**) exhibited approximately the same potency as the racemic material.

Of an extensive series of 1-benzyl-4-[2-(*N*-benzoylamino)ethyl]piperidine derivatives, designed and evaluated as inhibitors of acetylcholinesterase, the sulfone derivative (273) was the most potent (342).

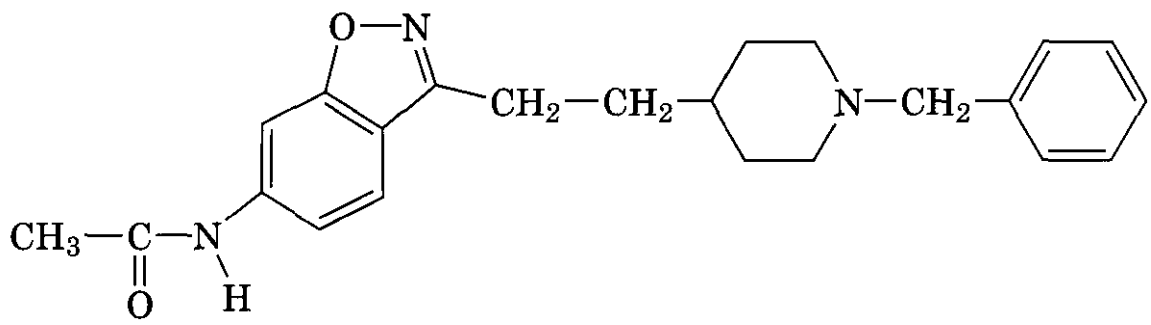
This compound showed an 18,000-fold preference for acetylcholinesterase over butyrylcholinesterase. It was a reversible inhibitor in a concentration-dependent manner.

Benzisoxazole derivatives (274) and (275) showed threefold inhibitory selectivity for acetylcholinesterase over butyrylcholinesterase (343).

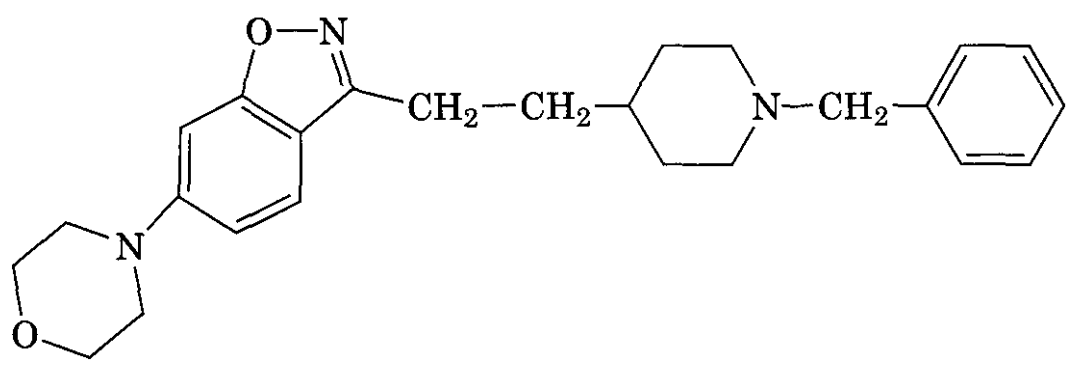
Compound (274) produced dose-dependent elevation of acetylcholine in mouse forebrain after oral administration. Possible palliative value in treatment of Alzheimer's syndrome was suggested. Donepezil (**276**) is a reversible, noncompetitive inhibitor of acetylcholinesterase (344). Its affinity for this enzyme is approximately 1250 times greater than that for butyrylcholinesterase. The compound produced a marked increase in the acetylcholine content of rat; cerebral cortex. Introduction of a fluorine atom at the 2-position of the indanone ring of (276) resulted in a compound with increased potency (345). Additionally, there was a significant difference in the anticholinesterase activity between the enantiomers of 2-fluoro-(276) compared to that of the nonfluorinated molecule. Docking simulations of fluorodonepezil with acetylcholinesterase have been reported (346). A "hypothetical binding site" for (276) at the acetylcholinesterase catalytic region was proposed (344). Of a series of aminopyridazines, (**277**) was the



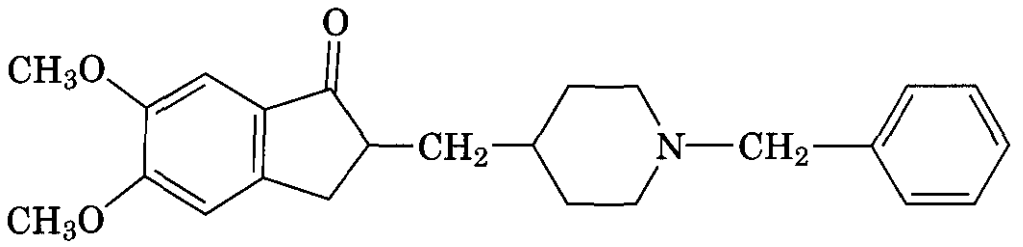
(273)



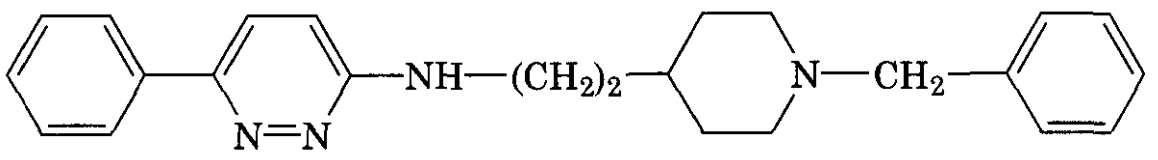
(274)



(275)



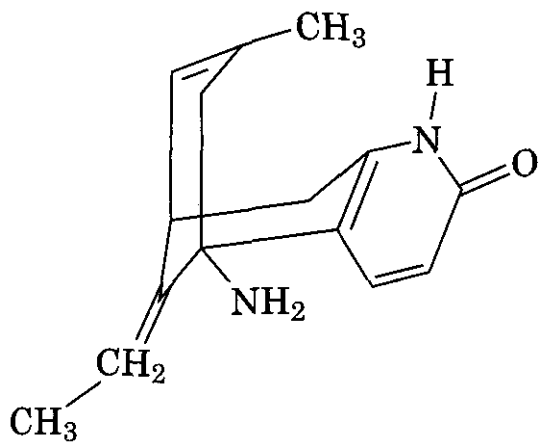
(276)



(277)

most potent inhibitor of electric eel acetylcholinesterase (IC_{50} = 0.12 m*M*) (347). It showed less activity against butyrylcholinesterase.

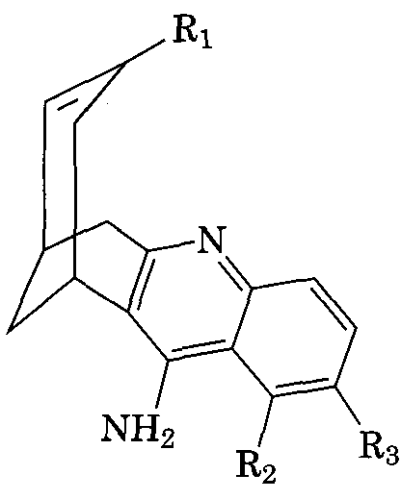
Huperzine-A (**278**), an alkaloid from *Huperzia* serrata Trev. (or Lycopodium serratum Thunb.) is three times as potent as physostig-



(278)

mine against acetylcholinesterase, but it is less potent against butyrylcholinesterase (348).

Several members of a series of hybrid molecules of THA and huperzine-A (**279**) were more active against acetylcholinesterase than (−)-huperzine-A, but they were not as selective for the enzyme (compared with butyrylcholinesterase) as huperzine-A (349). Molecular modeling of compounds in the series with acetylcholinesterase from Torpedo californica showed them to interact "as truly THA-huperzine-A hybrids." However, it was noted that acetylcholinesterase from Torpedo is somewhat different from that of humans. A subsequent paper (350) reported a study of predic-



(279)

R_1 = **n**– and branched chain alkyl, phenyl
R_2, R_3 = H, F, CH_3

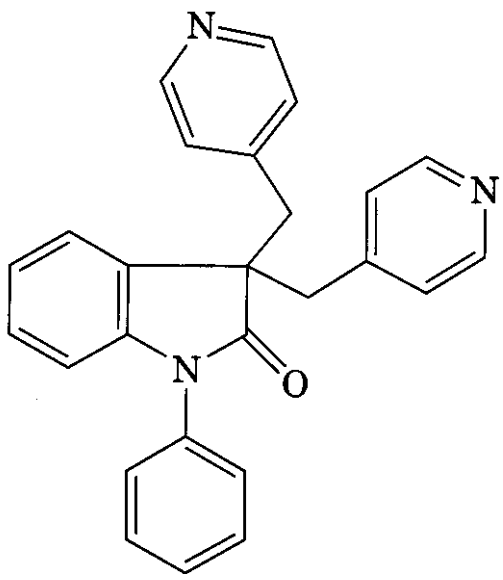
tion of binding free energies of tacrine-huperzine-A hybrids. Overall, the results were concluded to support the validity of the putative binding model described in ref. 349. Extension of the series of compounds (**279**) produced halogenated and/or alkylated congeners that were tight binding but reversible inhibitors of mouse brain acetylcholinesterase (351). These compounds showed ability to cross the blood-brain barrier. Molecular modeling simulations provided a basis to explain the differences in inhibitory activity in this series of compounds.

7 ACETYLCHOLINE-RELEASE MODULATORS

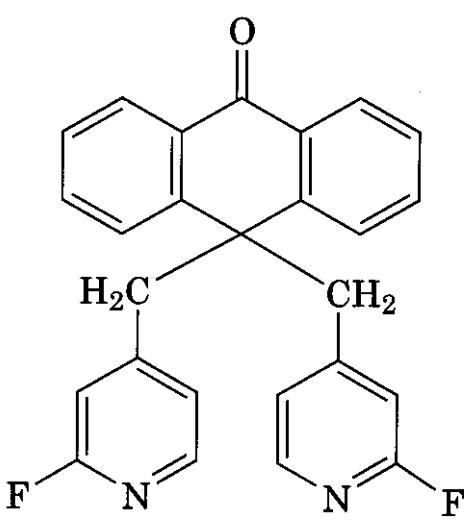
Structure-activity studies (352) of 3,3-disubstituted oxindoles led to DuP-996 (linopir-

dine) (**280**), which enhances potassium-evoked acetylcholine release in rat cortex, hippocampus, and caudate nucleus, ***in vitro*** (353,354).

This enhancement of acetylcholine release from nerve terminals occurs only when the release has been triggered (355). Compound (280) is reported (355) to exert significant effects on the human central nervous system. Dopamine and serotonin release are also enhanced by this agent, but release of glutamate, GABA, and norepinephrine is unaffected. Further structure-activity studies of 3,3-disubstituted oxindoles have been reported (356), and the potential utility of this category of compounds in treatment of cognitive and neurological deficiencies was stressed. Compound (281) was reported to be superior to (280) as an acetylcholine-releasing agent (23).



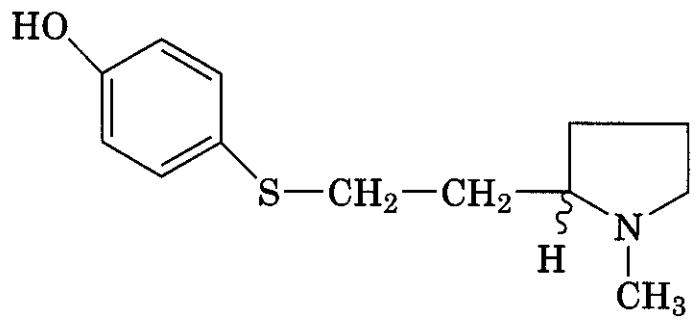
(280)



(281)

This newer compound is effective both ***in vivo*** and ***in vitro.*** Its mechanism of acetylcholine release was not established, but it was suggested that the blockade of certain K^+ channels may be involved.

Compound (282) stimulates the release of acetylcholine (and also of dopamine and norepinephrine) in brain regions involved in



(282)

memory and learning (37). This compound was resolved, and it was reported that neither the (*R*) nor the (*S*) enantiomer alone was as active as the racemate in any of a series of whole animal behavioral experiments.

One of the possible advantages to a therapeutic strategy for Alzheimer's disease or other deficits in memory and learning using acetylcholine-releasing agents is that such a process would seem to permit stimulation of both nicotinic and muscarinic receptors in the brain and to permit stimulation of all subgroups of acetylcholine receptors.

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CHAPTER THREE

Anticholinergic Drugs

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1 INTRODUCTION

The role of acetylcholine as a parasympathetic neurotransmitter and its effects on smooth muscle and glands are reviewed elsewhere. Typical parasympathetic effects, in addition to cardiac inhibition and vasodilation in certain areas, are miosis and increased gastrointestinal motion and secretion. It is believed that acetylcholine is the common factor in many of these processes. Electrical stimulation of parasympathetic nerves causes the appearance of acetylcholine at the neuromuscular junction; presumably, acetylcholine appears regularly during the spontaneous functioning of the postganglionic fibers of the parasympathetic nerves, and is regularly kept from accumulating by hydrolysis with acetylcholinesterase. Spontaneous release of acetylcholine at the parasympathetic nerve endings results in the involuntary contraction or spasm of the muscle. Therefore, the contractions of the stomach, intestinal tract, heart, certain blood vessels, and many other structures in various pathological situations are often attributed to the amounts of acetylcholine in excess of normal requirements. Gastric secretion, salivation, micturition, lacrimation, sweating, and miosis are influenced by acetylcholine. The rates of these activities can be controlled by certain anticholinergic drugs.

Anticholinergic drugs interfere with physiological functions that depend on cholinergic nerve transmission. These drugs do not prevent acetylcholine from being released at nerve endings, although they may compete with the liberated neurohormone for cholinergic receptor sites. Acetylcholine is the chemical transmitter at the postganglionic parasympathetic nerve endings, as well as at autonomic ganglia and somatic neuromuscular junctions. Acetylcholine is also a chemical transmitter at certain synapses in the central nervous system and drugs acting at central cholinergic sites are discussed elsewhere. Different types of anticholinergic drugs antagonize the actions of acetylcholine at the above-mentioned three types of peripheral synapses. Anticholinergic drugs that block somatic neuromuscular junction (curariform drugs) and autonomic ganglia (ganglionic blocking drugs) are described elsewhere. The pharmacological actions of anticholinergic drugs discussed in this chapter mimic the effects of cutting the parasympathetic nerve supply to various organs; therefore they are designated as parasympatholytics. Muscarine mimics the actions of acetylcholine on the structures innervated by parasympathetic nerves; it is relatively inactive at autonomic ganglia and the somatic neuromuscular junction. Parasympatholytics that antagonize the actions of muscarine are also known as antimuscarinic agents.

The classic parasympatholytic agent is atropine, and thus anticholinergic drugs used to be referred to as atropinic agents. Typical ef-

fects produced by atropine are mydriasis, tachycardia, decreased gastrointestinal peristalsis, and diminished secretions of gastric juice, saliva, and sweat. A large number of anticholinergic agents have been synthesized that have specific actions and uses. Although all anticholinergics could be considered as antispasmodics to different degrees, for convenience they are divided into three categories: (1) antispasmodics, which are specifically used to relieve spasms of the bowel (e.g., irritable colon, spastic colitis); (2) antiulcer agents, which reduce gastric secretion; and (3) mydriatics and cycloplegics, which relax the sphincter of the iris and the ciliary muscles.

1.1 Types and Selectivity of Antispasmodics

Substances patterned on atropine are widely used as antispasmodics of the gastrointestinal tract. Theoretically, any such substance that relaxes the acetylcholine-induced spasm of the smooth muscles in suitable doses can be termed an antispasmodic. In practice, not every anticholinergic agent can be used as an antispasmodic. The reason is that in addition to their spasmolytic action, anticholinergics influence the functions of other organs including heart, sweat and salivary glands, and iritic and ciliary muscles, producing side effects. Moreover, a number of them in small doses cause undesirable disorders in the central nervous system (CNS). The same antispasmodic is not suitable for the spastic states of all organs. Further, there are differences in the *in vitro* and *in vivo* efficacies of antispasmodics. Atropine abolishes the acetycholine-induced spasm of guinea pig ileum completely; however, it is a familiar clinical experience that atropine does not antagonize completely the spasm caused by increased tone of the intestinal vagus nerve.

A number of agents cause spasm of the gastrointestinal tract. The spasm may be induced not only by acetylcholine but also by histamine, 5-hydroxytryptamine, or barium chloride. Atropine and other anticholinergics are effective mostly against acetylcholine-induced spasm, and less against the remaining three spasmogens. Against a spasm induced by acetylcholine, atropine is effective at the lowest concentrations (e.g., 10^{-9} g/mL). Higher concentrations are necessary to antagonize 5-hydroxytryptamine spasm (10^{-7}), histamine spasm (10^{-6}), and Ba^{2+} spasm (10^{-5}). Thus, atropine is a highly specific anticholinergic neurotropic spasmolytic.

The barium ion acts on all smooth muscles, regardless of innervation, and is called a musculotropic spasmogen. Drugs that relieve the spasm produced by barium ions are called musculotropic spasmolytics. Papaverine and nitrites are typical members of this class. However, various drugs that resemble atropine manifest both kinds of spasmolytic action in widely varying situations.

The ideal atropine-like antispasmodic should be specific for the spasmogen, should have selectivity for smooth muscles, and should abolish completely the spasm induced by the stimulation of the parasympathetic nerve to the organ. Further, the atropine-like antagonist should be specific for the subtype of muscarinic receptor localized in the organ. None of the available antispasmodics satisfies all these requirements. However, a great many compounds have been synthesized with the hope of developing drugs that will exhibit more selective antispasmodic action and have fewer side effects than those of atropine.

Some of these antispasmodics show relative selectivity toward the subtype of muscarinic receptor localized in smooth muscle cells.

1.2 Gastric Secretion, Peptic Ulcer, and Anticholinergics as Antiulcer Agents

The pathophysiology of peptic ulcer is not fully known and, in the present state of knowledge, it is not possible to present the pertinent normal physiology briefly. For a detailed discussion on the physiology and chemistry of gastric secretion and the pathologic physiology of peptic ulcer, reference should be made to reviews on the subject (1–5). The following is a brief summary of the gastric secretion and its relationship to peptic ulcer, a knowledge of which is necessary to understand the problems of developing antiulcer agents.

Gastric juice contains a mixture of water, inorganic ions, hydrochloric acid, pepsinogens, mucus, various polypeptides, and the intrinsic factor. Pepsinogens are precursors of the proteolytic enzymes, pepsins. They are readily converted into the corresponding pepsins by either acid or pepsin itself. Conversion

by acid is instantaneous at pH 2.0. In humans, gastric juice contains hydrocholoric acid during the period of interdigestive secretion as well as during the period of digestive secretion. Although the mechanisms of interdigestive secretion are not known, they depend partly on the tonic activity of the vagus. The gastric secretory activity during the period of digestive secretion may be divided into three phases, cephalic, gastric, and intestinal. Each phase is named to denote the region in which the stimuli act to induce gastric secretion.

In the cephalic phase the stimuli are initiated in the central nervous system. The stimuli are the sight, smell, taste, and thought of food, which act through conditioned and unconditioned reflexes. The final efferent path is the vagus nerve. The impulses in the vagus nerve stimulate the secretory cells in the gastric glands. Acetylcholine, which is released from the postganglionic nerve endings, exerts a direct action on the secretory cells. Administration of atropine abolishes this phase. The secretion is high in acid and pepsinogens, and its concentration of mucus is lower than that of the basal secretion; mucus output rises 8–10 times as the volume increases.

The gastric phase of secretion begins copiously as soon as the food enters the stomach, and it may continue 3–4 h, with a total volume of 600 mL or more of strongly acid juice contain ng a high concentration of pepsinogens. The gastric phase of secretion is caused by local and vagal responses to distension and by the hormone gastrin, released by the musosa of the pyloric gland area. The local nerves of the pyloric area are confined to the mucosa and are cholinergic. Irrigation of the pyloric gland area with acetylcholine releases gastrin, and this liberation of gastrin is abolished by atropinization. There is a synergism between gastrin and acetylcholine at the target cells; the effect of injected gastrin on both acid and pepsinogen secretion is increased two- to eightfold by subthreshold parasympathomectic stimuli, and it is strongly inhibited by atropinization.

The intestinal phase that begins when chyme passes from the stomach to intestine, contributes about 10% of the total response to a test meal. Protein and its digestion products, milk, dilute alcohol, and acid itself are effective stimulants. Although there may be a nervous component, the intestinal phase includes humoral stimulation of secretion by unknown agents. Gastrin released from the small intestine may be involved. The response to whatever humoral agent comes from the intestine is greatly increased when subthreshold doses of cholinergic drugs are given.

A number of humoral inhibitors of gastric secretion arise in the small intestine. They are termed enterogastrones. An enterogastrone is present in the jejunum and duodenal mucosa. It is released in the presence of fat and inhibits gastric secretion and motility. The hormone secretin, which stimulates pancreatic secretion, is an enterogastrone. It is produced in the proximal duodenum and inhibits gastric secretion in the presence of acids. Cholecystokinin, which is the same as pancreozymin, and gastrin share the same terminal tetrapeptide. Given alone, cholecystokinin is only a mild stimulant of gastric acid secretion. It is a competitive inhibitor of the receptor for gastrin, which is a powerful stimulant of gastric acid secretion. Therefore, in the presence of gastrin, cholecystokinin decreases the total output of acid. Glucagon (and possibly enteroglucagon) reduces the gastrin-induced acid secretion by noncompetitive inhibition of the receptors to gastrin. A gastric inhibitory polypeptide (GIP) that is present in duodenal mucosa inhibits both histamine- and gastrin-induced acid secretion. A vasoactive intestinal peptide (VIP), which has been isolated from small intestinal mucosa, inhibits histamine-induced acid secretion. GIP and VIP are two possible enterogastrones whose significance has yet to be established.

Histamine, the exact role of which is not clearly understood, stimulates secretion of gastric juice that is rich in hydrochloric acid. Recently, histamine receptors have been divided into three types, H1, H2, and H3. Stimulation of H2 receptors by histamine results in increased gastric acid secretion. H2 receptor antagonists (burinamide, metiamide, cimetidine) inhibit histamine-induced gastric acid secretion in both humans and animals. In humans, H2 antagonists inhibit not only histamine- but also pentagastrin (a synthetic analog of gastrin)-stimulated gastric acid secretion. This suggests that, at least in humans, gastrin acts partially by histamine. Blockage of acetylcholine receptors by atropine and his-

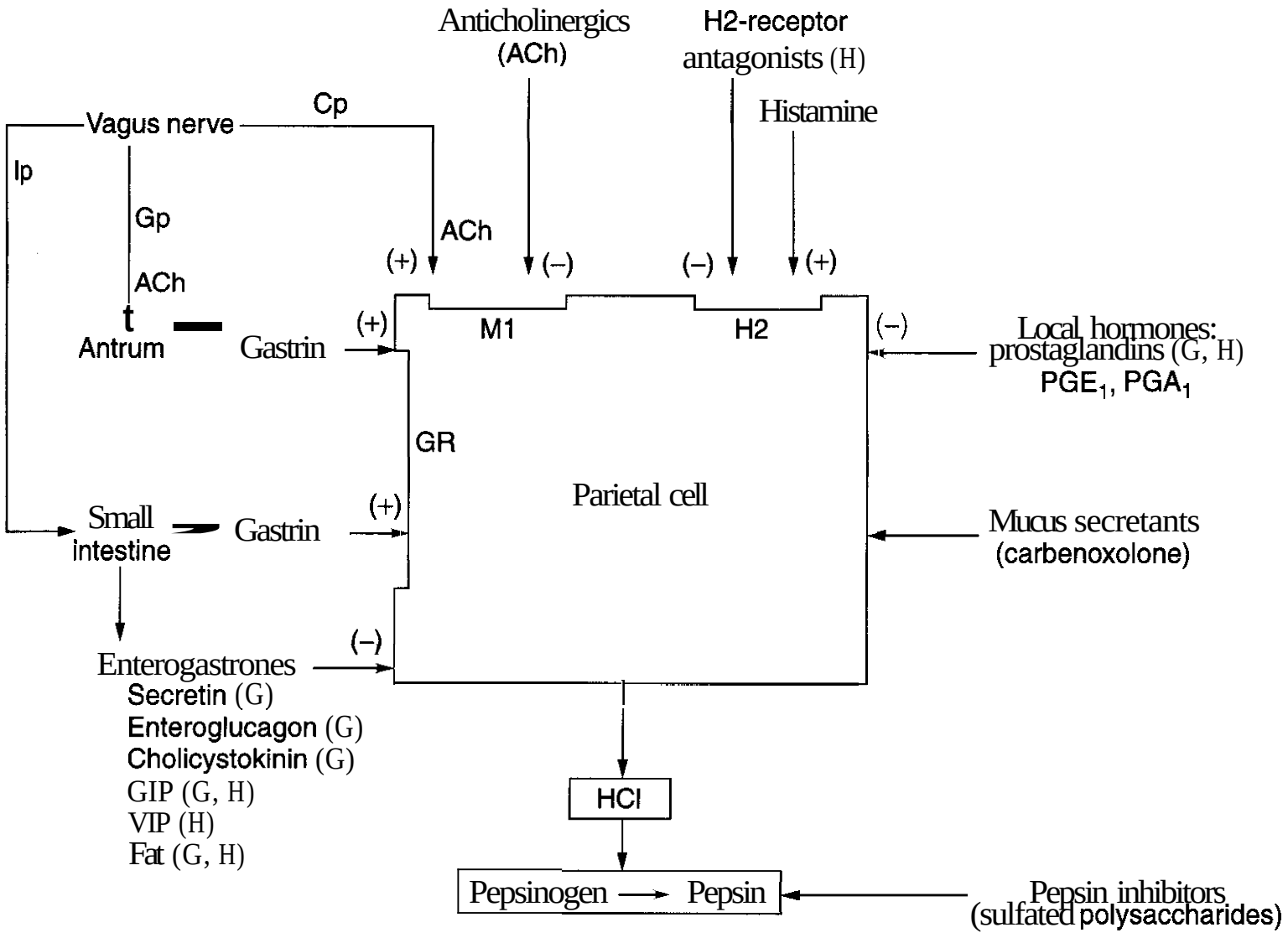


Figure **3.1.** Interactions among neuronal and hormonal factors and pharmacological agents during cephalic (Cp), gastric (Gp), and intestinal (Ip) phases of gastric acid secretion by parietal cell. ACh, acetylcholine; GIP, gastric inhibitory peptide; VIP, vasoactive intestinal peptide; MI, muscarinic receptor; H2, histamine H2 receptor; GR, gastrin receptor; (+), stimulation of acid secretion; (–), inhibition of acid secretion. In parentheses, next to the inhibitory agents, is indicated the blocked stimulant agent (ACh, acetylcholine; H, histamine; *G*, gastrin).

tamine receptors by H2 antagonists results in reduction of the effectiveness of gastrin to induce acid secretion.

Therefore, there seems to be a complex interaction among the three receptors, acetylcholine receptors, H2 receptors, and gastrin receptors involved in the acid secretion by parietal cells.

Among local hormones and messengers, prostaglandins (PGE,, PGA_1) adenosine 3′,5′-monophosphate (cyclic AMP, cAMP) inhibit both pentagastrin- and histamine-stimulated gastric secretion. According to present evidence, all hormones that reduce gastric acid secretion increase both adenylcyclase and intracellular cAMP activity. Conversely, all hormones that primarily stimulate gastric acid secretion reduce intracellular cAMP levels. Therefore, cAMP is involved in the final links of gastric acid secretion.

The interplay among various neuronal and hormonal factors in the gastric acid secretion by the parietal cell during cephalic, gastric, and intestinal phases are schematically shown in Fig. **3.1.** In addition to being inhibited by atropine-like agents and enterogastrones shown in Fig. **3.1,** the acid secretion is inhibited by gastrone in the mucus of human stomach, by urogastrone isolated from the urine of men and dogs, by strongly acid solutions in the duodenum, and by stimulation of the sympathetic nervous system.

Peptic ulcer occurs in the pyloric region of the stomach or the first few centimeters of the intestine. The gastroduodenal muscosa is exposed constantly to mechanical, physical, and chemical insults, some of which have already been described. A peptic ulcer does not develop without the presence of a pepsin-containing juice of such low pH that it can exert a peptic

influence on the gastric wall itself. The extent of this insult is determined by the number of acid- and pepsinogen-producing cells, their irritability, and/or the magnitude of the stimuli that reach them. These stimuli are partly nervous (vagal) and partly hormonal (gastrin, corticosteroids).

The healthy stomach does not digest itself. Counteracting the aggression are defensive factors such as buffering and dilution by food, inhibition of the secretion of gastric juice, and drainage of gastric contents. In addition, however, the local condition of the mucosa (the mucosal resistance) is also of importance. Some of the determinants of mucosal resistance are the mucous barrier, the local circulation, and the healing capacity of the mucosa. A peptic ulcer forms when the insult is more powerful than the defense. In the case of duodenal ulcers, the powerful irritation is often the important factor; in gastric ulcers it is the insufficient defense.

The ideal agent for the treatment of the peptic ulcer would be one that selectively inactivates pepsin or inhibits the output of hydrochloric acid so as to maintain the pH of the gastric contents at about 4.5 for long periods after its oral ingestion. It should produce no, or only minimal, side effects, induce no tolerance, and be inexpensive. It should be effective during all periods and phases of gastric secretion and prevent the formation of ulcers.

Atropine-like anticholinergics do not satisfy all requirements of an antiulcer agent. They block acetylcholine action at the neuroeffector junction of the vagus. They give relief to patients with a peptic ulcer by their antisecretory and antispasmodic effects. They decrease the basal hydrochloric acid and pepsin secretion, thereby allowing the healing of ulcers. The antispasmodic effects of atropine-like agents are as consistent as their antisecretory effects. Motor activity is closely related to ulcer pain, and the pain-relieving action of anticholinergic agents seems to be related to their effect on depressing motor activity (antispasmodic effect).

An "effective" atropine-like anticholinergic drug is capable of favorably influencing the excessive gastric secretion under certain conditions. It exerts a significant effect on acid secretion during the basal and interdigestive night secretion to the point of abolishing it completely for many hours (6). Its effect on secretion during the feeding of milk and cream is significant (7, 8). However, anticholinergic drugs do not effectively perform a "medical vagotomy" and they do not effectively reduce gastric acidity to the extent of achlorhydria when patients are fed (8, 9). The effective anticholinergic agent as an antiulcer drug should be selective for the subtype of muscarinic receptors localized on the secretory cells of gastric glands as well as mucosa of the pyloric gland area. Anticholinergics selective for muscarinic receptors of M1 subtype are useful in decreasing gastric acid secretion.

1.3 Anticholinergics as Mydriatics and Cycloplegics

The size of the pupil is determined by the balance of forces exerted by the dilator muscles fibers (sympathetically innervated and radially arranged) and the constrictor muscle fibers (parasympathetically innervated and circularly arranged) of the iris. Normally both sets of muscle fibers have a constant degree of tonus and act reciprocally to dilate or constrict the pupil. Any substance that paralyzes the constrictor muscle fibers (parasympatholytic) allows the unopposed tone of dilator muscle fibers to widen the pupil.

Acetylcholine is the transmitter between the constrictor muscle fibers and the parasympathetic nerve that innervates them. Therefore, acetylcholine and its congeners stimulate the constrictor muscle fibers of the iris and constrict the pupil. Atropine and related compounds paralyze the constrictor muscle fibers and cause widening or dilatation of the pupil.

The ciliary muscle is innervated by the parasympathetic nerve, and acts to decrease the tone on the supporting muscle fibers of the lens, and thus increases the accommodative power of the eye. Acetylcholine and its congeners constrict the ciliary muscle fibers, and atropine and related compounds paralyze the ciliary muscle.

Mydriatics are drugs that dilate the pupil, but have minimal effect on the ciliary muscle and thus on accommodation. Cycloplegics are drugs that partially or completely paralyze accommodation. Most of the anticholinergics have both properties to varying degrees. For mydriatics other than anticholinergics and for

drugs that constrict the pupil (miotics), the appropriate chapter should be consulted.

Mydriatics and cycloplegics are special types of antispasmodics. In clinical practice mydriasis is produced by local instillation of the chosen drug into the conjunctival sac. This enables one to produce the desired effects on the eye with minimal systemic effects. However, such compounds should possess properties that allow them to penetrate the cornea in effective concentrations. There are no significant differences between the muscarinic receptors of the guinea pig ileum or the rabbit iris, as judged by the binding characteristics of potent anticholinergic agents. Muscarinic receptors in both tissues are possibly of the M3 subtype. If anticholinergic drugs are available that are selective for muscarinic receptors on constrictor muscles and ciliary muscles, mydriatic and cycloplegic effects can be produced by different drugs.

1.4 Anticholinergic Drugs in Premedication during Anesthesia

Prevention of some undesirable side effects during anesthesia has been considered a function of premedication with anticholinergic drugs. For example, atropine is a popular anticholinergic agent that has been used for its antisialogogic, antibradicardia, and antiemetic effects (10). The emphasis of using anticholinergic drugs during premedication has been changing over the years because of the availability of inhalational anesthetic agents that are better than ether. An anticholinergic agent (e.g., atropine), although no longer regarded as an essential premedicant under all circumstances, does have specific applications for injured patients and children. Atropine (0.6 mg, i.v.) blocks the muscarinic actions of suxamethonium (succinylcholine), bradycardia, and salivation during crash induction of anesthesia in an injured patient (10). Administration of an anticholinergic drug to prevent bradycardia in children in response to suxamethonium or tracheal intubation is desirable.

1.5 Anticholinergic Activity as a Side Effect of Drugs and Anticholinergic Syndrome

Many of the drugs used in current medical practice, especially anesthetic drugs and other drugs used as adjuvants to anesthesia have properties that disturb patient recovery because of their anticholinergic effects on the CNS (11). These effects are termed central anticholinergic syndrome (CAS) and are discussed in different chapters on centrally acting drugs (volume 4). These effects can be reversed with physostigrnine, the centrally active cholinesterase inhibitor, which has a sparing effect on acetylcholine molecules at muscarinic receptor sites. An increased number of acetylcholine molecules displace the molecules of anticholinergic drug from the muscarinic receptor sites.

1.6 Classification of Anticholinergic Agents Based on Subtypes of Muscarinic Receptors

Acetylcholine produces its parasympathomimetic effects by binding at cholinergic receptors of the muscarinic type. The classical anticholinergic agent, atropine, binds to the same muscarinic receptors and prevents acetylcholine from binding to these receptors and eliciting muscarinic responses. Based on modern developments in the design of relatively sensitive antagonists for muscarinic receptors in different tissues (12–15), muscarinic receptors have been subdivided into three (possibly five) subtypes M1 to M5 (Table 3.1). All muscarinic receptors are glycoproteins of molecular weight of 80,000 and have seven membrane-spanning regions. All of the receptors have a slow response time (100–250 ms) and are coupled to G-proteins (13, 14). They act directly on ion channels or are linked to second-messenger systems, attenuation cAMP formation (16, 17), and formation of inositol triphosphate and diglyceride (16, 18). The final effect of activation of these receptors can be to open or close K^+ channels, Ca^{2+} channels, or Cl^- channels. These multiple channel activities lead to either depolarization or hyperpolarization of the cell membrane. The final responses are either excitatory or inhibitory. Atropine blocks all of these activities and does not distinguish subtypes. Selective muscarinic agonists and antagonists that will distinguish different subtypes are needed. Further, it will be a major advance to obtain information to indicate that each subtype performs a specific function. Then it will be possible to develop specific anticholinergic drugs that are useful only as (1) antispasmodic, (2) antisecretory, or (3) mydriatic agents. With certain anticholin-

Table 3.1 Provisional Division of Muscarinic Receptors (M), Their Agonists and Antagonists into Five Subtypes[a]

M Subtype	M1	M2	M3	M4	M5
Previous names	$M_{1\alpha}$	$M_{2\alpha}$ Cardiac M_2	$M_{2\beta}$ Glandular M_2	M_2(?)	?
Tissue location[b]	Lower esophageal sphincter, gastric glands, CNS ganglia	Heart	Glands, smooth muscle, CNS		
Selective agonists[c]	McN-A-343	—	—	—	—
Selective antagonists[d]	Pirenzepine (+)-Telenzepine	Methoctramine AF-DX 116 Himbacine	HHSID p-F-HHSID	— Himbacine (High Affinity)	—
Effector pathway[e]	IP_3/DG	cAMP ↓ K^+ channel ↑	IP_3/DG	cAMP ↓	IP_3/DG
Gene	m_1	m_2	m_3	m_4	m_5
Amino acids (human)	460	466	590	479	532

[a]Summarized from Refs. 12–18.
[b]CNS, central nervous system sites.
[c]Selective agonists for receptors M2–M5 are not available.
[d]HHSID, hexahydrosiladifenidol; p-F-HHSID: p-fluoro-hexahydrosiladifenidol.
[e]IP_3/DG, inositol-1,4,5-triphosphate/diglyceride.

ergic agents, some degree of selectivity (not specificity) has been attained to produce antispasmodic, antisecretory, or mydriatic effects (Table 3.2). No antagonist has a potency on one receptor subtype that is more than 10 times higher than its potency on other subtypes. All receptor subtypes have K_d values for (−)-*N*-methylscopolamine (NMS) and (−)-3-quinuclidinyl benzilate (QNB) of less than 1.0 n*M* (13). NMS and QNB are standard anticholinergic agents in addition to atropine to compare anticholinergic potencies at muscarinic receptors.

2 BIOCOMPARATIVE ASSAY OF ANTICHOLINERGICS

Many of the methods of obtaining experimental evidence for the antispasmodic, antiulcer, and mydriatic activities do not measure precisely and selectively only one type of pharmacological activity. However, the techniques that are available (19, 20), if used with an understanding of their scope and limitations, can provide useful information in the development of anticholinergic agents and their structure-activity relationships.

2.1 Antispasmodic Activity

In studying drugs more or less like atropine, it is customary to test their antispasmodic action on smooth muscles, such as the isolated guinea pig ileum, duodenum, or jejenum of rabbit, or rat intestine. Acetylcholine or any one of the cholinergics may be used as a spasmogen, and the ability of the antispasmodic to inhibit or abolish the cholinergic-induced spasm may be measured. Helical strips of blood vessels with intact endothelium (e.g., strips of rat aorta) can also be used to evaluate the antispasmodic activity of anticholinergic drugs (21). The antagonistic activities may be expressed as affinity constants or relative molar activities in relation to a standard antagonist. The selectivity of the antispasmodic activity may be determined by using different spasmogens (e.g., histamine, 5-hydroxytryptamine, nicotine, and barium chloride).

Thiry-Vella fistulas (19), prepared at various levels of the gastrointestinal tract, have been used in the conscious dog for determining motility by (1) placing an indigestible bolus in the oral end of the fistula and determining the traverse time before and after treatment with drugs; (2) placing a balloon

Table 3.2 Derivatives of Solanaceous Alkaloids and Their Semisynthetic Substitutes"

Generic Name	Trade Names	Dose or Preparation	Advantage, if any, of Molecular Modification	Therapeutic Use
Atropine sulfate USP		0.5 mg (oral i.v or s.c.); 0.5–1.0% ophthalmic solution		Mydriatic with long recovery period; preanesthetic medication to decrease secretions, treatment of Parkinsonism, and anti-ChE poisoning
Atropine tannate	Atratran	1–2 mg (tablet)	Slow absorption with sustained release of the alkaloid	Antispasmodic in ureteral and renal colic
Ipratropium[b] bromide	Atrovent	Inhaler	Low systemic absorption	Bronchodilator in asthma
Atropine N-oxide hydrochloride	X-tro Genatropine	0.5–1.0 mg (capsule)	Slow release of the alkaloid	Same as atropine for oral use
Hyoscyamine hydrobromide		0.25–1.0 mg	Possibly fewer central effects than atropine because of small doses administered	Same as atropine for oral use
Methylatropine bromide	Mydriasine	0.5–2% solution	Mydriatic with short recovery period	Mydriatic
Methylatropine nitrate	Metropine	1–5% solution	Same as above	Mydriatic
Scopolamine hydrobromide USP		0.6 mg (oral, (s.c.); 0.2% solution	Central depressant ("twilight sleep")	As a sedative during pre- or postoperative gynecologic care
Genescopolamine hydrobromide		1–2 mg	Gradual release of alkaloid	Same as above
Methscopolamine bromide NF	Pamine Lescopine	2.5–5.0 mg (oral); 0.25–1.0 mg (s.c. or i.m.)	Parasympatholytic without central effects	Antisecretory and antispasmodic in peptic ulcer
Methscopolamine nitrate NND	Skopolate Skopyl	24 mg (oral) 0.25–0.5 mg (s.c. or i.m.)	Same as above	Same as above
Homatropine hydrobromide USP		1–2% solution	Mydriatic with recovery period less than that of atropine (see Table 3.18)	Mydriatic
Homatropine methyl bromide NF	Novatropine Mesopin	5 mg (oral)	Parasppatholytic without central effects	Antisecretory and antisposmodic
Anisotropine methylbromide[c]	Valpin Endo	10 mg (oral)	Parasympatholytic without central effects	Antisecretory and antispasmodic

"For details of the preparations and their uses, standard references (97–99) in pharmacology should be consulted.

[b] 8-Isopropylnoratropine methobromide.

[c] 8-Methyl-3-(2-propylpentonoyloxy) tropinium bromide odatropine bromide.

containing water and attached to a kymographic recording system in the fistula and recording the pressure waves and their alterations by the action of drugs; or (3) placing a French catheter in the aboral end of the fistula, connecting it to a suitable recording system, and thus making a record of normal pressures and those occurring after treatment.

Other qualitative and quantitative methods to study the antispasmodics have been described (19). These include (1) the fluroscopic study of the gastrointestinal motility and (2) the use of an ingestible pressure-sensitive radio-telemetering capsule (Transensor) for measuring the pressure in the gastrointestinal tract.

The subtype of muscarinic receptor in the smooth muscle has been characterized as M3 by use of selective anticholinergics and different smooth muscle preparations from different species. These smooth muscle tissues include (1) trachea (22), ileum (23, 24), uterine artery (25), and submucosal arterioles of guinea pig (26); (2) aorta (27) and coronary artery of rabbit (28); and (3) trachea (29), aorta (30), and iris (31) of rat. Human uterine arteries (32), airways (33), and ciliary muscles (34) have also been shown to contain the M3 type of muscarinic receptors.

2.2 Antiulcer Activity

The problems encountered in testing drugs for antiulcer activity result in part from a lack of complete understanding of the physiological and biochemical mechanisms involved in the formation of ulcers, and in part from the testing of drugs for activity on normal or quasi-normal animal preparations, although they are ultimately applied to abnormal or pathological human states. The various methods differ in producing ulcers in experimental animals (19).

A preparation developed by Shay et al. (35) has been used to test for antiulcer activity on an all-or-none basis. The ligation of the pylorus of rats, previously fasted for 48–72 h, leads to the accumulation of acid gastric contents and ulceration of the stomach 17–19 h after the operation. The antiulcer agents are given subcutaneously or intraduodenally at the time of ligation of the pylorus, or orally 1 h before. The animals are killed 17–19 h after pyloric ligation and the stomach contents are collected for examination. The stomach is opened along the greater curvature and the ulcers are examined and scored by a suitable scheme such as 0 = normal, 1 = scattered hemorrhagic spots, 2 = deeper hemorrhagic spots and some ulcers, 3 = hemorrhagic spots and ulcers, and 4 = perforation. Variable results have been reported by investigators using this technique.

Production of chronic experimental peptic ulcers in dogs (or rats) by the Mann-Williamson procedure (36) is one of the standard methods. The gastric juice is diverted into the intestine some distance from the pancreatic and biliary secretions. The objective is achieved by isolating the duodenum from the pylorus and the jejunum. The oral end of the duodenum is closed and its distal end is anastomosed with a loop of ileum, so as to discharge the pancreatic and biliary secretions into the lower portion of the bowel. The cut end of the jejunum is then anastomosed to the pylorus. About 95% of dogs so prepared develop typical chronic peptic ulcers just distal to the gastric anastomosis with the jejunum. With similar operative procedures 85% of rats develop gastric, marginal, or jejunal ulcers.

The complete reversal of the duodenum in dogs produces chronic peptic ulcers in about 6 months (19). These animals maintain their weight until the development of ulcerations and might become a useful preparation for detecting and comparing antiulcer activity.

Stress produces ulcers in the rats, which could be used to test the antiulcer activity of drugs (37). Rats fasted for 48 h and immobilized in a galvanized screen cage under light ether anesthesia develop ulcers in the glandular region of the stomach after 4 h of restraint. The estimate of severity can be all or none, or may be coded in the same way as the Shay preparation.

One of the side effects of adrenocorticotropic hormone (ACTH) and corticoid therapy in humans is the development or reactivation of gastroduodenal ulcers. Daily subcutaneous administration of cortisol or Δ^1-cortisol to rats for 4 days results in the regular development of gastric ulcers (38). This procedure has been adapted to testing antiulcer activity (39). There are certain differences between steroid ulcers and "natural" ulcers in localization, rate of development, and severity (40).

The antisecretory activities of anticholinergics are as important as their antiulcer activities for their therapeutic usefulness. The Pavlov gastric pouch (41) with intact vagal and sympathetic nerve supply and a modified Heidenhain pouch (42), which is essentially denervated, are prepared from dog stomach and have been used for determining the action of drugs on gastric secretion. Histamine or a test meal is usually used as a stimulus. Similar methods for the preparation and use of chronic total gastric fistulas and chronic denervated gastric pouches have been described for determining drug action on gastric secretion in rats (43–45).

There are a significant number of reports in which antisecretory and antimobility effects of anticholinergic drugs have been evaluated in ulcer patients (9, 46). The antisecretory potency can be measured best in the duodenal ulcer patient in whom the acid output is already high. Ability of the drug to abolish or diminish acid output under histamine stimulation is a stringent test of activity, although the test has limited physiological relevance. The effect of the drug on the amount of acid secreted under ordinary clinical conditions is the most pertinent of all tests in relation to therapeutic application.

Despite extensive research, certain aspects of ulcer disease are not clearly elucidated. Because of the multiple processes that control acid and pepsin secretion and defense and repair of gastroduodenal mucosa, it is more likely that causes of ulceration differ among individuals (Section 3.5). Two other factors have been acknowledged as risk factors in the pathophysiology of peptic ulcers: nonsteroidal anti-inflammatory drugs (NSAIDs) and *Helicobacter* pylori infection (47–50). NSAIDs induce a significant number of gastric and duodenal ulcers, possibly because of inhibition of prostaglandin synthesis with consequent loss of protective effects. H. pylori has been recognized as a risk factor in the ulcerative process, similar to acid and pepsin. Duodenal ulcer is typified by H. pylori infection and duodenitis and possibly impaired duodenal bicarbonate secretion in the face of moderate increases in acid and peptic activity. Increased peptic activity with decreased duodenal buffering capacity possibly leads to enhanced mucosal injury and finally results in gastric metaplasia. In the presence of antral H. pylori, the gastric metaplasia becomes colonized and inflamed. The inflammation and infection disrupts mucosal defense and regenerating mechanisms, resulting in ulceration. The combination of inflammation, protective deficiencies, and moderate amounts of acid and pepsin may be enough to induce ulceration. Several groups of drugs including anticholinergic agents have been developed to antagonize risk factors causing ulcer disease. A good animal model, which incorporates all variable causes of ulcer disease, is yet to be developed.

The muscarinic receptors of the parietal cells are of the M1 subtype. The specific anticholinergic agents for M1 receptors are considered to be effective for the treatment of ulcer disease (51). The muscarinic receptors on the duodenal smooth muscle are possibly of the M3 subtype. Anticholinergics at M3 may partially decrease pain of duodenal ulcers by decreasing the motility of the duodenum (52).

2.3 Mydriatic and Cycloplegic Activities

A simple and relatively accurate test for mydriatic activity has been described (52). The method requires mice and a binocular microscope, magnifying about 10 times and provided with a scale in the eyepiece with which to examine and measure the diameter of the pupil of the mouse. A strong light shining into the eye of the mouse must be attached to the microscope. The diameter of the pupil is measured at the peak effect after administration of the anticholinergic agent by intraperitoneal injection. The duration of the effect is also important, given that one of the most characteristic and valuable properties of atropine and analogous compounds is the prolonged effect that they produce in the eye.

Entopic pupillometry is an accurate and practical method for measuring the size of the pupil in humans (53). With a Cogan entopic pupillometer, the normal size of the pupil and the near and far points before and after instillation of the drug in the conjunctival sac can be measured at different time intervals. The amount of light entering the eye is quite small and the movements of the eye during the measurement do not interfere with the test.

2.4 Miscellaneous Anticholinergic Activities

A number of other methods are available for comparing the activities of anticholinergic agents, of which the antitremor and antisalivary effects are widely used. Arecoline or pilocarpine may be used to induce tremor or salivation in a suitable species that can be blocked by an anticholinergic agent. There seems to be good correlation between anticholinergic and antitremor effects (54). Recovery of the salivary gland from cholinergic block may conceivably precede that of the gastric glands and the two effects may therefore not necessarily parallel each other in duration (9).

3 SOLANACEOUS ALKALOIDS

The older anticholinergic drugs are the various galenical preparations of belladonna, hyoscyamus, and stramonium, all of which are derived from plants of the potato family, the Solanaceae. The species used as drugs include Atropa belladonna, one of several plants known colloquially as "deadly nightshade"; *Hyoscymus* niger (black henbane); and Datura *stramonium* (jimsonweed, jamestown weed, or thorn apple). The active principles in all these plants consist mostly of (−)-hyoscyamine, with smaller variable amounts of (−)-scopolamine (hyoscine). Atropine is (±)-hyoscyamine.

3.1 History

The poisonous nature of solanaceous alkaloids has been known for many centuries (55). The toxic properties of deadly nightshade were evident when children ate the blackberries, which looked attractive in a fall hedgerow in England. The children became delirious and their eyes had widely dilated pupils. The deadly nightshade was used by the poisoners of the Middle Ages to induce obscure and often delayed poisoning. Therefore, Linné, in 1753, named the shrub Atropa belladonna after *Atropos*, the oldest of the Three Fates, who cuts the thread of life. "Belladonna" does not refer to Atropos, who is considered as a grim and awesome female. but to the Italian name ("handsome women") of the plant, which was used by Venetian ladies to give them dilated pupils ("sparkling eyes").

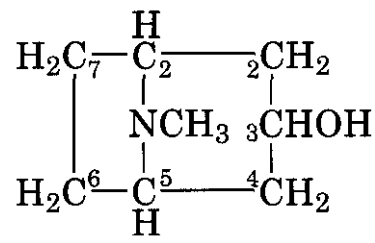
Datura has an ancient history, for it is said to have been used at the oracular shrine of Apollo in his temple at Delphi. Here the priestess of the god Pythia sat on a tripod uttering incoherent words in a divine ecstacy, in reply to the questions that were asked. Pythia was intoxicated by the fumes from burning datura leaves; her replies were interpreted by a priest in the form of a verse. The more common uses of datura were for robbery or conspiracy. Indian courtesans were known to place datura in their visitors' wine, so that they could be robbed without interference. As recently as 1908, there was a plan to poison the European garrison in Hanoi in Vietnam using datura. Those in the conspiracy intended to stupefy the soldiers, and then to kill them.

The pharmacological actions of atropine and related alkaloids are intimately connected with our knowledge of the organization and function of the autonomic nervous system. Schmiedeberg and Koppe (56) were the first in 1869 to focus attention on the similarity between a drug effect and electrical stimulation, when they pointed out that muscarine and vagus stimulation affected the heart in the same fashion and the actions of both were antagonized by atropine. Further, they recommended atropine as an antidote for mushroom poisoning. As early as 1887, Kobert and Sohrt (57) provided experimental proof for both similarities and dissimilarities between atropine, and scopolamine.

Atropine was isolated by Mein in 1831 (58), and since then the synthesis of both atropine and scopolamine has been achieved (59, 60). A biogenetic scheme for the synthesis of atropine-like alkaloids in datura species starting from ornithine has been described (61).

3.2 Chemical Structure

All the solanaceous alkaloids are esters of the dicyclic amino alcohol 3-tropanol (tropine, 1). Atropine is an ester of (+)-tropic acid and tropine. In scopolamine the organic base is sco-



(1)

pine. Scopine differs from tropine in having an oxygen bridge between C-6 and C-7.

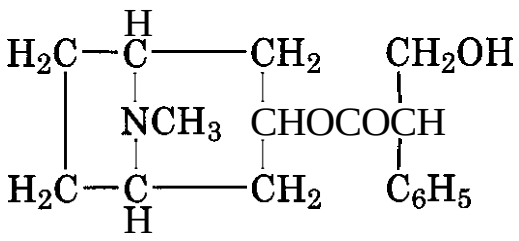
There are some other alkaloids that are members of the solanaceous alkaloids (e.g., apoatropine, noratropine, belladonnine) but they are not of sufficient therapeutic value to be discussed in this context.

The carbon α to the carboxyl group of tropic acid is asymmetric and is easily racemized during the isolation of the solanaceous alkaloids. Atropine and atroscine are racemic forms. The corresponding levo isomers, (−)-hyoscyamine and (−)-scopolamine (hyoscine), occur naturally in the solanaceous plants.

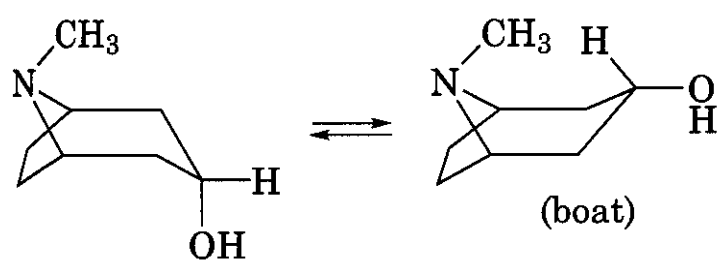
The absolute configuration of (−)-tropic acid has been established by its correlation with (−)-alanine (62). According to the Cahn-Ingold-Prelog convention (63), natural (−)-tropic acid possesses the (*S*) configuration. Accordingly, (−)- hyoscyamine and (−)-hyocine have an (*S*) configuration (64).

The piperidine ring system can exist in two principal conformations. Its chair form has the lowest energy requirement. However, the alternate boat form can also exist, because the energy barrier is not great. The formula of 3-hydroxytropine (1) indicates that, even though there is no optical activity because of the plane of symmetry, two stereoisomeric forms, tropine (2) and pseudotropine (3), can exist because of the rigidity imparted to the molecule through the ethane chain across the 1,5 positions (65a).

In tropine, the axially oriented hydroxyl group, ***trans*** to the nitrogen bridge, is designated as α or anti, and the alternate, equatorially oriented hydroxyl group as β or syn. It is generally considered that cycloheptane is fixed through an —N(CH_3)— bridge in the structures of tropine and pseudotropine. Therefore, a chair conformation is ascribed to the piperidine ring system in tropine and pseudotropine. However, there is only a seeming difference between the two conformations of tropane derivatives (66). The tropane system can be considered with equal justification as a piperidine twisted through the —CH_2CH_2— bridge or as a cycloheptane fixed through an —N(CH_3)— bridge. When the tropane system is structured by the chair form of piperidine, it also represents the boat form of cycloheptane. Similarly, the boat form of piperidine is at the same time a chair form of the cycloheptane ring. Therefore, it may be assumed that both forms are present in a state of equilibrium (65a)65. Based on the conformations of the tropane system, the structure of atropine (4) can be represented by (5) and (6), of which (5) is more generally accepted.

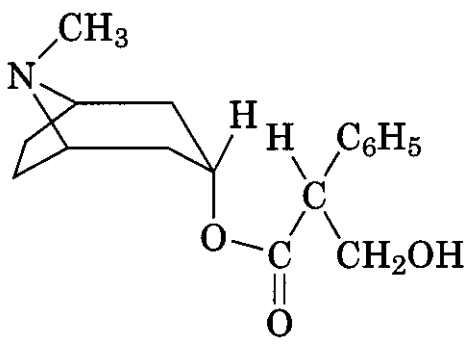


(4)

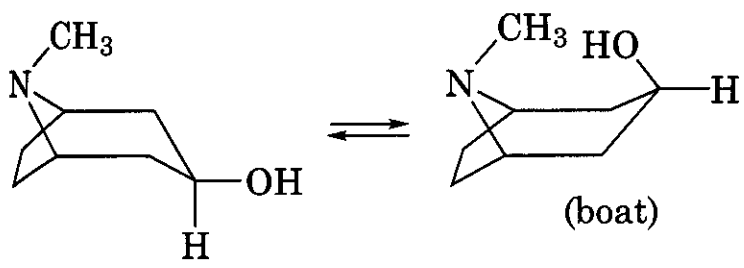


(chair) (boat)

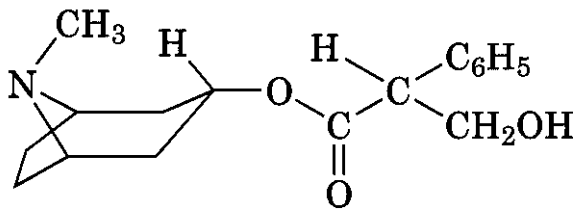
(2)



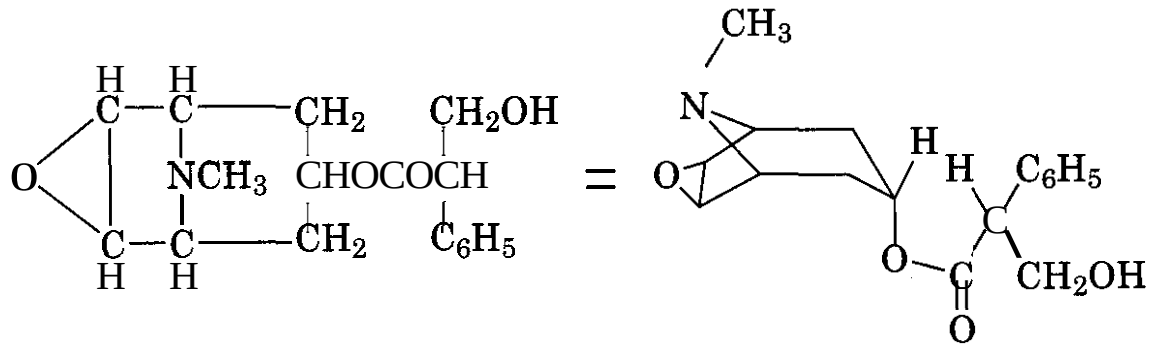
(5)



(3)

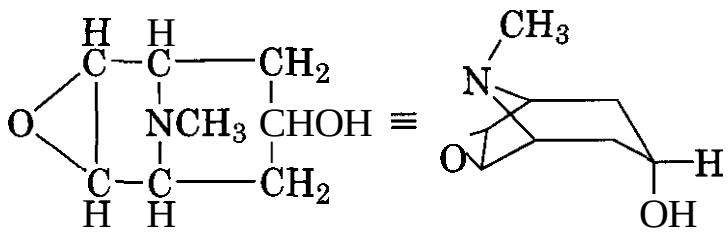


(6)



(7)

The amino alcohol derived from scopolamine (7), that is, scopine (8), has the axial orientation of the 3-OH group but in addition has a β-oriented epoxy group bridged across the 6, 7 positions.



(8)

3.3 Preparative Methods

Conventional methods of alkaloid isolation are used to obtain a crude mixture of atropine and (−)-hyoscyamine from the plant products. This crude mixture of alkaloids is racemized to atropine by refluxing in chloroform or by treatment with cold dilute alkali (67).

Atropine can be synthesized from tropinone and tropic acid as starting materials. Tropinone can be prepared by Robinson's synthesis (68) and reduced under proper conditions to tropine. (±)-Tropic acid can be prepared from ethyl phenylacetate (69, 70) or acetophenone (71). The *O*-acetyl derivative of tropyl chloride reacts with tropine to yield *O*-acetyl of atropine hydrochloride, from which the acetyl group hydrolyzes spontaneously in aqueous solution (72).

One of the commercial sources for (−)-hyoscyamine is Egyptian henbane (*Hyoscyamus muticus*) in which it occurs to the extent of 0.5%. Another method for extraction of the alkaloid uses Duboisia species. It is prepared from the crude plant material in a manner similar to that used for atropine and is purified as the oxalate. (?)-Tropic acid can be resolved through its quinine salt and the separated enantiomorph can be converted into (+)- and (−)-hyoscyamines.

(−)-Scopolamine (hyoscine) is isolated from the mother liquor remaining after the isolation of hyoscyamine, and is marketed as its hydrobromide. Scopolamine is readily racemized to atroscine, when subjected to treatment with dilute alkali.

The synthesis of scopolamine differs from that of atropine in the synthesis of the amino alcohol, scopine portion of the molecule. Fodór and coworkers (60, 73, 74) have synthesized scopine starting from 6-β-hydroxy-3-tropanone. Esterification of scopine with *O*-acetyltropyl chloride and mild hydrolysis of the acetylscopolamine give scopolamine.

3.4 Molecular Factors in the Absorption, Fate, and Excretion of Atropine and Related Compounds

The belladonna alkaloids are absorbed rapidly after oral administration (75). They enter the circulation when applied locally to the mucosal surfaces of the body. Atropine absorbed from inhaled smoke of medicated cigarettes can abolish the effects of intravenous infusion of methacholine in humans. The transconjunctival absorption of atropine is considerable. About 95% of radioactive atropine is absorbed and excreted following subconjunctival injection in the rabbit. The total absorption of quaternary ammonium derivatives (Section 3.5) of the alkaloids after an oral dose is only about 25%. The liver, kidney, lung, and pancreas are the most important organs that take up the labeled atropine. The liver probably excretes metabolic products of atropine by way of bile into the intestine (in mice and rats).

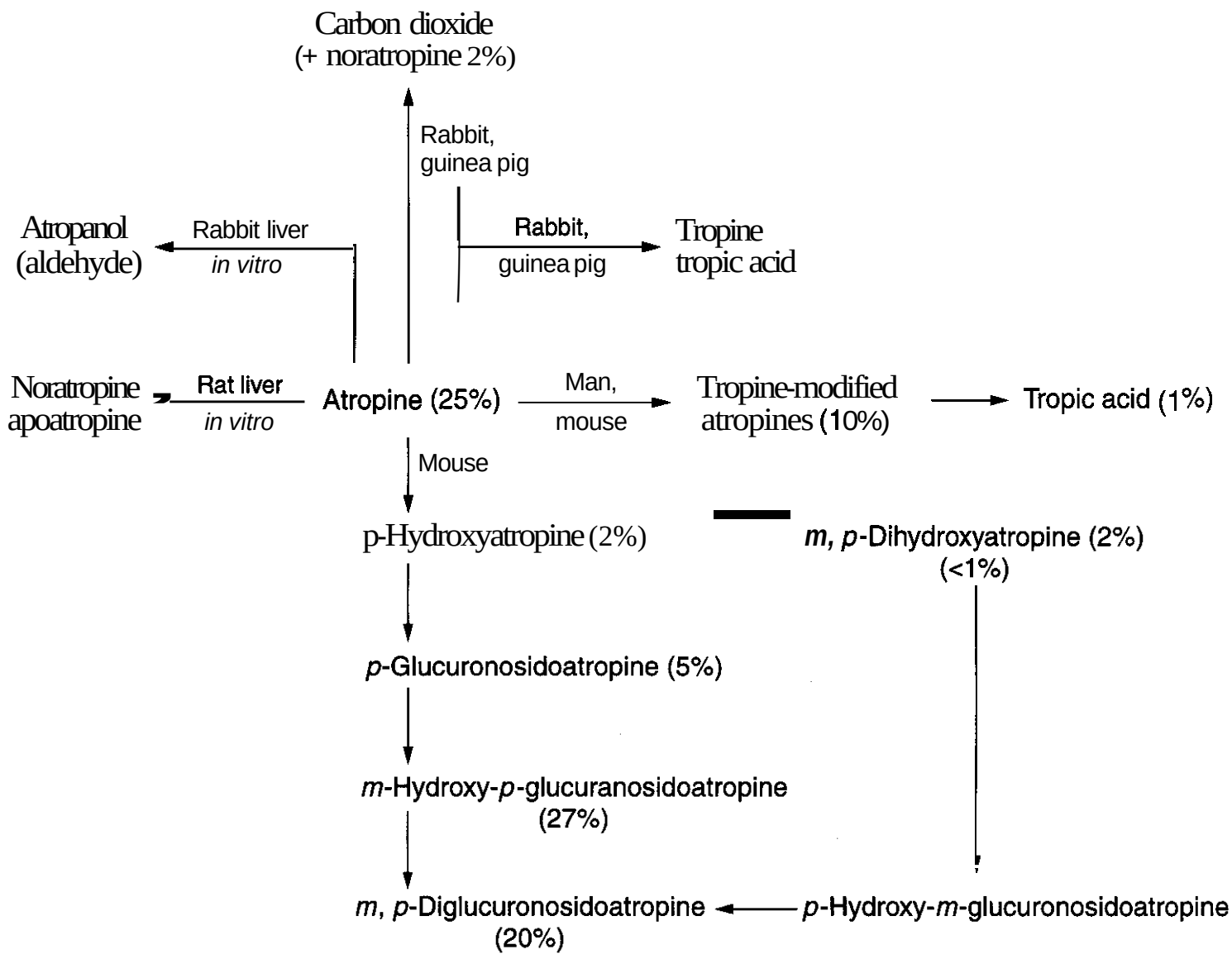


Figure 3.2. Metabolism of atropine and its variations in different species.

Because most of the synthetic antispasmodic and antiulcer agents are administered orally, their absorption through the gastrointestinal tract limits their therapeutic usefulness. There are striking diferences in the absorption of tertiary amines and quaternary ammonium compounds (76–78). The tertiary amines (e.g., noroxyphenonium, mepiperphenidol; Section 4) are absorbed completely from rat intestinal loops. The maximal absorption of the corresponding quaternary ammonium compounds is about one-fifth of the total dose. The poor absorption of quaternary ammonium compounds may be partly attributable to the positive charge that promotes the formation of a nonabsorbable complex with mucin. The ready absorption of tertiary amines may be explained partly by their permeability through lipid membranes (79).

Considerable species variations have been reported for the metabolic detoxification of atropine in mammals (80–91). These differences seem to be more quantitative than qualitative. At least four types of molecular modification6 occur for the urinary excretion of atropine (Fig. 3.2). Cleavage of the ester bond takes place in the rabbit and the guinea pig (84), whereas *para* and *meta* hydroxylation of the benzene ring of tropic acid occurs in the mouse and the rat (80, 82). The tropine moiety of atropine is also chemically modified for excretion in man and mouse and, though unidentified, "tropine-modified atropines" are excreted in humans and in mouse (83). Tropic acid itself does not undergo metabolic alteration for urinary excretion in all species mentioned above. The metabolic conversions of tropine itself are not fully investigated. However, demethylation of atropine-N-$^{14}CH_3$ (or tropine-N-$^{14}CH_3$) has been reported in a number of species with exhalation of $^{14}CO_2$ (90). The possible metabolic changes of atropine are schematically represented in Fig. 3.2.

After intravenous injection of atropine, approximately 25% of the dose is excreted in mouse urine as atropine, more than 50% as

conjugates with glucuronic acid, and the remaining 20–25% as intermediate oxidation products (probably p-hydroxyatropine and 3,4-dihydroxyatropine) and "tropine-modified atropines." Rats are known to metabolize atropine in a manner similar to that in mice. In humans, about 50% of the administered dose of atropine is excreted unchanged in the urine and about 33% as unknown metabolites that are esters of tropic acid. Neither hydroxylation of the tropic acid moiety nor glucuronide formation has been demonstrated in humans (83). Only less than 2% appears as tropic acid in urine.

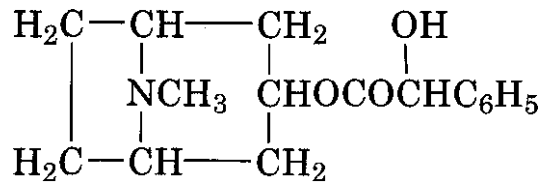
It has been known for more than a century that rabbits can tolerate large quantities of atropine (84, 85). The cause of this observation is the ability of the serum of some, but not all, rabbits to hydrolyze atropine into tropic acid and tropine. The hydrolysis is attributed to an enzyme, atropinesterase, which is found in most other tissues as well as the serum of these rabbits. The highest activities are found in the liver and intestinal mucosa; only the brain and aqueous humor of the eye contain no enzyme. The enzyme is also found in the liver of the guinea pig and accounts for the appearance of tropic acid in the urine of the rabbit or the guinea pig, but not other animals, following the administration of atropine.

The presence of atropinesterase in rabbits is inherited through an incompletely dominant gene (84). This gene is associated with another gene that influences the color of the fur, causing "extension of black pigment in the fur."

Atropinesterase can also hydrolyze homatropine and scopolamine. This enzyme is stereospecific for (*S*)-(−)-hyoscamine, which is split; the more inert (*R*)-(+) isomer is not readily hydrolyzed (84).

3.5 Semisynthetic Derivatives of Solanaceous Alkaloids

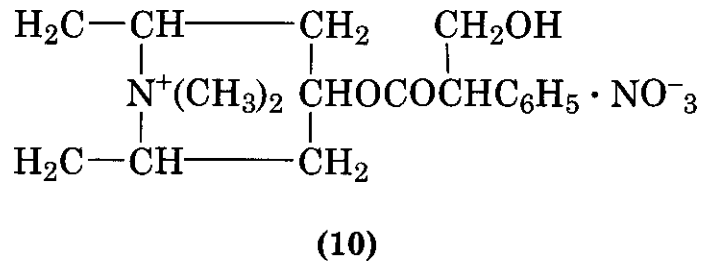
Early attempts to modfy the atropine molecule (4) were aimed at converting the solanaceous alkaloids containing the tertiary nitrogen into quaternary ammonium compounds and N-oxides. Later developments have been to retain the tropine (orscopine) portion of the molecule and substitute various acids for tropic acid. In this way a series of tropeines have been synthesized, among which a number of active compounds have been found (86–92). Of the tropeines, mandelyl tropeine (9,



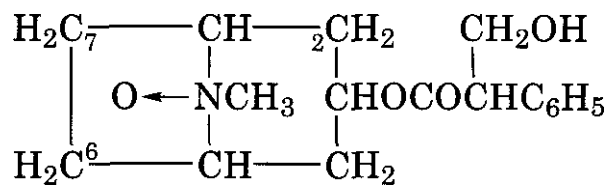
(9)

homatropine), has survived as a thrapeutic agent to the present.

Methylatropine nitrate (10)(or bromide) is a synthetic quaternary derivative of atropine. Atropine oxide (atropine N-oxide) is known as a genatropine (11)and may be prepared by oxidation of the alkaloid with hydrogen peroxide.

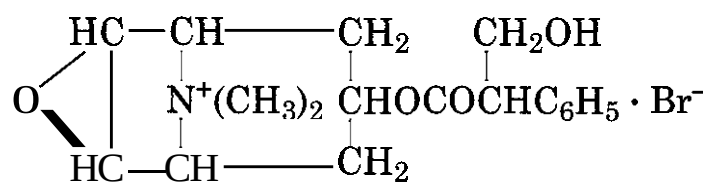


(10)



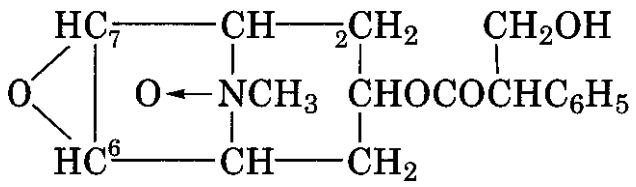
(11)

The derivatives of scopolamine (7) prepared by similar methods are available commercially. These include methscopolamine bromide (12), methscopolamine nitrate, and genoscopolamine (scopolamine N-oxide, 13).

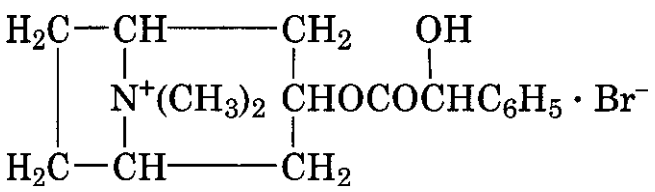


(12)

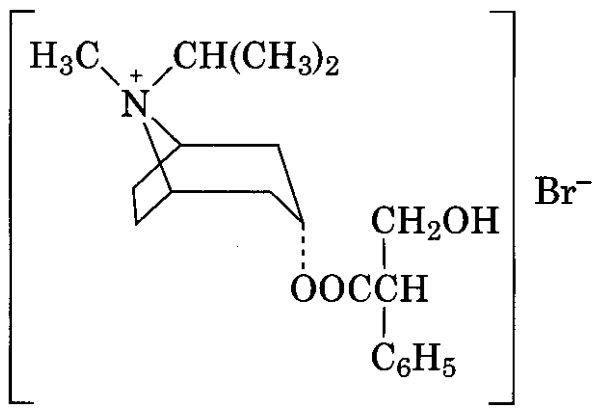
Homatropine (9) is prepared by evaporating tropine with mandelic and hydrochloric acids. Homatropine methylbromide (14) may



(13)



(14)



(15)

be prepared from homatropine by treating it with methyl bromide.

Quaternization may or may not increase all types of anticholinergic activity significantly. However, there are certain practical advantages to quaternization. The quaternary ammonium derivatives usually penetrate the CNS less readily than the corresponding tertiary amine analogs. Therefore, quaternization serves as a useful technique to avoid or minimize the side effects caused by the stimulation of the central nervous system by the tertiary amines, when the drugs are used for their peripheral actions. When a tertiary alkaloidal base is converted to the quaternary form, the latter is also less readily absorbed through the intestinal wall (93, 94; Section 3.2). This is not a disadvantage when the drug is used for its effects in the gastrointestinal tract. However, if the drug is used for its systemic or central actions, the effect becomes erratic and unpredictable because the absorption is poor after oral administration. The tertiary amines are preferred for ophthalmic use because they penetrate the cornea better than their quaternary ammonium derivatives. However, when a drug (e.g., atropine) has a long-lasting mydriatic effect, its recovery period can be shortened by quaternization. Therefore, the selection of the type of the derivative (Table 3.2) depends on the specific purpose for which it is used, the mode of its administration, and the duration of the desired effect.

Ipratropium bromide (15) is a useful quaternary derivative of atropine (95, 96). It is marketed as a metered-dose inhaler for treatment of bronchospasm associated with chronic obstructive pulmonary disease. Because it is administered as an aerosolized preparation, its droplets are deposited in upper airways and produce bronchodilation within minutes.

Only less than 5% of the drug enters the systemic circulation. Ipratropium is an example of developing a useful drug for a specific purpose by minor molecular modification of atropine.

The N-oxides are converted to the corresponding tertiary bases *in vivo*. Atropine N-oxide and scopolamine N-oxide are slowly reduced to atropine and scopolamine in the animal body. Therefore, N-oxidation is a convenient technique for prolonging the duration of the action of the alkaloidal bases. The *N*-oxides are said to be less toxic.

Solanaceous alkaloids have a wide and variable spectrum of anticholinergic activities and are widely used in therapeutics. The various derivatives of atropine, scopolamine, and homatropine are listed in Table 3.2. Because of their chemical differences and the resulting biological interactions, different derivatives are preferred for antispasmodic, antisecretory, mydriatic, or central effects. Scopolamine produces the same type of depression of the parasympathetic nervous system as do atropine and homatropine, but it differs markedly from atropine in its action in the central nervous system. Whereas atropine stimulates the CNS, causing restlessness, scopolamine can act dose dependently as a sedative. It also causes temporary amnesia ("twilight sleep") when used along with morphine in obstetric and gynecologic procedures. For a detailed discussion of the anticholinergic activities of solanaceous alkaloids and their related com-

pounds, the standard textbooks or review in pharmacology should be consulted (75, 97–99).

4 SYNTHETIC ANTICHOLINERGICS

Although atropine and its related alkaloids are potent anticholinergics, they have a wide spectrum of pharmacological activities. Therefore, therapeutic administration of these alkaloids to elicit a particular desired activity invariably results in some undesirable side effects. For this reason, the search for compounds possessing one or another of the specific desirable actions has been an active field of investigation in medicinal chemistry. The ideal specificity of action has not been attained in these attempts; perfect atropine substitutes with predominant antispasmodic, antisecretory, or cycloplegic actions have yet to be synthesized. However, some progress has been made since the discovery of multiple subtypes of functional muscarinic receptors (M1-M5) and the cloned muscarinic receptors (m_1–m_5) have been identified (Table 3.1). Several antagonists, which show selectivity to one subtype of muscarinic receptors over others, have been introduced and they have become useful in the delineation of subtypes of muscarinic receptors in various tissues. Some of these agents may become useful as antiulcer agents, antispasmodics, or mydriatics.

4.1 Analogs of Atropine

The synthetic anticholinergic drugs can be considered as analogs of atropine or antagonists of acetylcholine. Most of these compounds were designed using broad principles of molecular modification such as (1) scission of the atropine molecule into simpler molecules containing the essential pharmacophoric groups; (2) molecular modification by introducing "blocking" moieties into cholinergics; and (3) changes in other anticholinergics using principles of bioisosterism.

The structure of atropine has been the basis for a large number of synthetic anticholinergic agents. However, no significant changes have been made to affect the "ester-complex" grouping because of atropine-like properties. Another probable consideration is that it is far simpler to synthesize a fairly complex molecule from two halves by esterification than by any other method. Therefore, many esters of amino alcohols and carboxylic acids have been synthesized as atropine substitutes, in which the structures of either one or both halves have been changed. For example, in homatropine, the tropic acid moiety has been replaced by mandelic acid. The amino alcohol moiety (tropine, 1) of atropine has afforded unusually rich opportunities for the synthesis of anticholinergics (Fig. 3.3). Scission of its piperidine ring at point X gives the derivatives of hydroxyalkylpyrrolidines (16), and scission of its pyrrolidine ring at point Y makes it possible to proceed to derivatives of 4-hydroxypiperidine (17). The scission of both rings at Z leads to dialkylaminoalkanol derivatives (18). Furthermore, simplification and alteration of these three groups of amino alcohols has resulted in the synthesis of esters containing structural features more or less similar to those of atropine.

Antagonists of acetylcholine often have chemical structures resembling that of acetylcholine, although they differ from it by greater complexity of the molecule and higher molecular weight. Acetylcholine is a quaternary ammonium compound; atropine and tropine contain a tertiary nitrogen. Therefore, a number of atropine-like compounds having quaternary nitrogen atoms have been synthesized. In some of them, the acetyl group of acetylcholine has been replaced by acid moieties containing blocking groups (e.g., diphenylacetic acid).

The principles used in the design of antimetabolites have been applied to synthesize atropine-like compounds. The ester group in atropine-like compounds has been replaced by a thioester, an amide, an ether group, or a chain of methylene carbons (Table 3.3).

All synthetic anticholinergic agents have some structural features in common. In most, the molecule has bulky "blocking moieties," often cyclic radicals, linked by a chain of atoms of limited length, to a positively charged amine nitrogen (Fig. 3.4). The length and structure of the main chain have considerable influence on the anticholinergic activity of the substance. At the same time the chemical nature of the main chain determines the class of

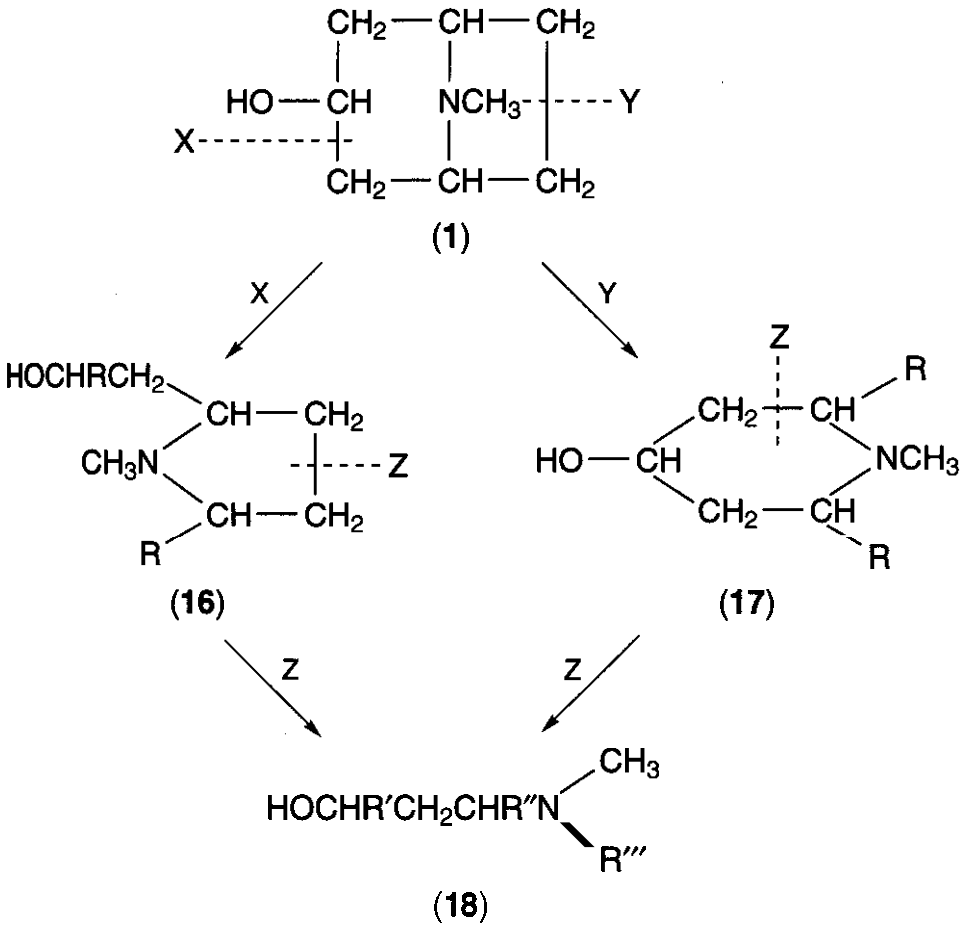
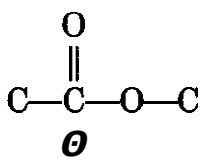
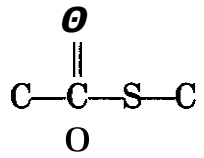
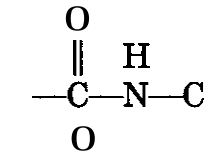
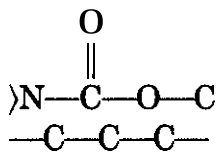
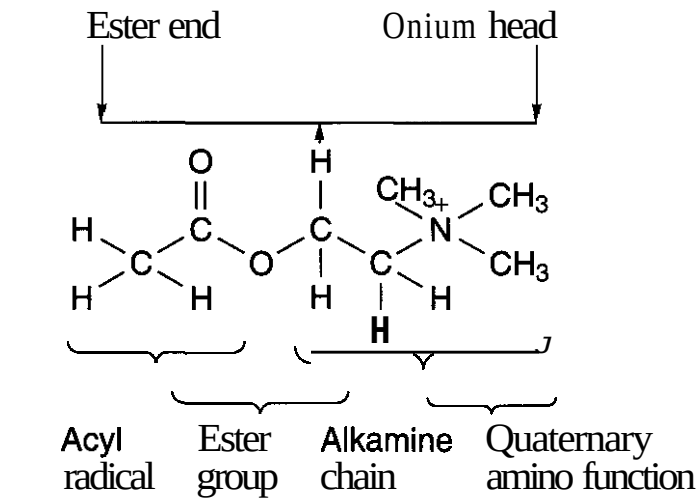


Figure 3.3. "Scissions" of tropane ring.

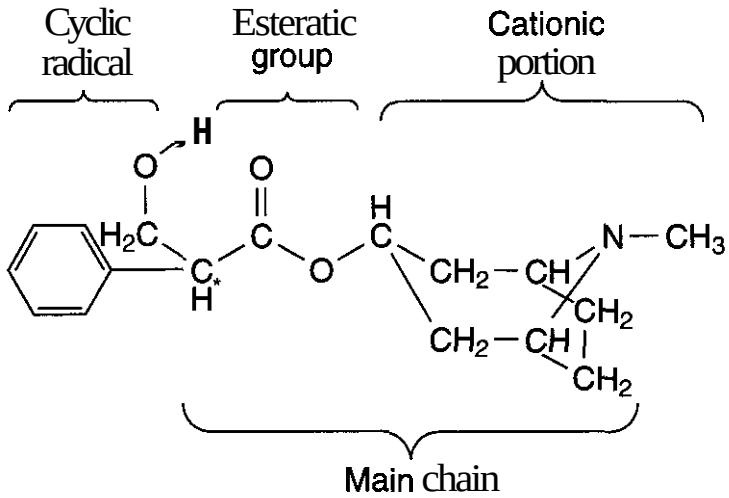
organic substances to which a given substance belongs. Therefore, the classification of synthetic anticholinergics in Table **3.3** is based on the structure of the main chain of the molecule, taking into consideration wherever necessary the presence or absence of any additional pharmacophoric groups (OH, $CONH_2$). It is beyond the scope of this text to consider all compounds that belong to each group. However, several examples from drugs used as therapeutic agents are discussed at appropriate places in the following pages. These compounds may be classified differently, and the same compound may be placed in more than one group. Each one of them may be considered as an agent with optimum anticholinergic activities among a series of structurally related compounds whose structure-activity

Table 3.3 Classification of Synthetic Anticholinergics

Group	Characteristic Group in the Main Chain	Atoms in the Chain	Additional Pharmacophoric Groups that May Be Present
1	Ester		–OH
2	Thioester		–OH
3	Amide		–OH
4	Carbamate		
5	Alkane	—C—C—C—	
	(a) Amino alcohols		–OH
	(b) Amides		—$CONH_2$
6	Alkene	—C=C—	



Acetylcholine (19)



Atropine (20)

Figure 3.4. Structural features of acetylcholine and atropine. The asymmetric carbon in atropine is marked with an asterisk (*).

relationships have been evaluated for different types of pharmacological effects. Compounds with the same or similar structural features may exhibit other pharmacological effects as side effects. For example, a large number of compounds have been synthesized containing an ether link in the main chain. These compounds are useful as antiparkinsonian drugs and antihistaminic agents.

4.2 Receptor-Subtype–Selective Anticholinergics

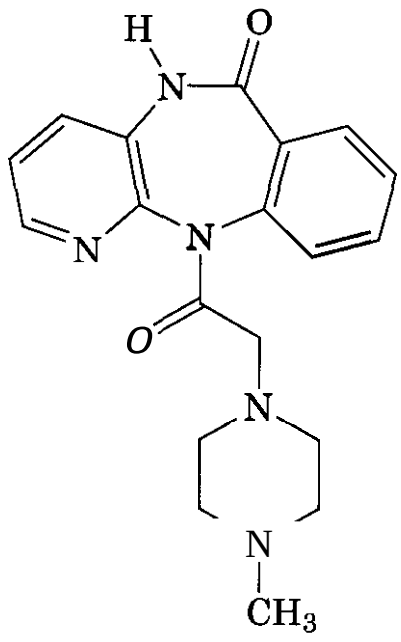
The muscarinic actions of acetylcholine can be either stirnulatory or inhibitory. Acetylcholine stimulates secretion and contraction of the gut, but it inhibits the contraction of the heart and relaxes the smooth muscle of blood vessels. Acetylcholine can inhibit adenylate cyclase and activate guanylate cyclase. In the cortical neurones, muscarinic agents cause a slow depolarization mediated by closing potassium channels. Acetylcholine opens potassium channels in the heart and causes hyperpolarization and a reduced rate of firing of the nodal tissue. In many tissues, calcium channels are opened and probably intracellular calcium is mobilized and, like many other transmitters, acetylcholine increases the turnover of phosphoinositides. Therefore, there is much expectation that selective agents will be found among muscarinic antagonists that will be useful to block one particular physiological or biochemical response to acetylcholine. Thus, several agents have been synthesized that have structural features similar to those of atropine-like agents, cyclic blocking moieties linked by a chain of atoms of limited length to a positively charged nitrogen atom.

Based on the cyclic-blocking moieties and other substituent groups, subtype-selective muscarinic antagonists can be classified into eight groups: (1) tricyclic benzodiazepines, (2) benzothiazepines, (3) quinuclidines, (4) polymethylene tetramines, (5) indenes, (6) siladifenidols, (7) diphenylacetyloxy derivatives, and (8) himbacine akaloids.

4.2.1 Tricyclic Benzodiazepines.

Significant side effects of tricyclic antidepressants, like imipramine, are antimuscarinic effects. Benzodiazepines also cause some antimuscarinic effects like dry mouth at therapeutic concentrations. Some of the well-known anticholinergic agents, Banthine, Probanthine, and Trest, have tricyclic bulky moieties at the end of their molecules. Some molecular features of these three types of phamacological agents are present in tricyclic pyrido-benzodiazepines. In these compounds, portions of benzene and pyridine (or other rings) are fused to a seven-membered diazepine ring in the middle. The trycylic bulky moiety containing benzene, diazine, and pyridine rings (or other rings) at the end of a molecule satisfies one of the requirements for an anticholinergic agent. Further substitutions on the imino-nitrogen atom have resulted in selective M1 receptor antagonists that are useful as antiulcer agents.

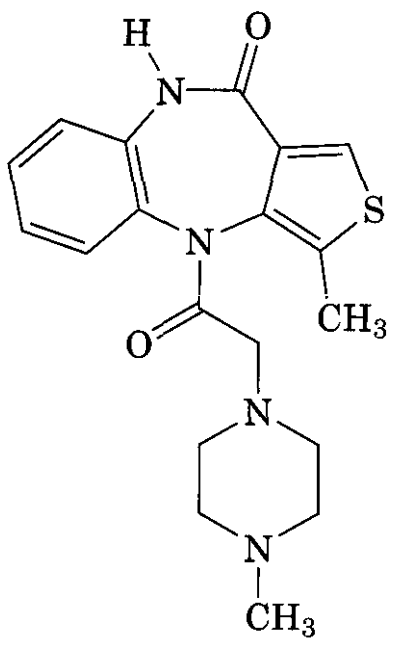
Pirenzepine (21) was the first M1 receptor antagonist shown to inhibit gastric secretion (100, 101). This drug (100–150 mg/day) is used in several countries to decrease gastric secretion and achieve maximal rates of ulcer



(21)

healing. At these doses, incidence of dry mouth and blurred vision are not significant. It has a low lipid solubility and limited permeabilty into the CNS, so it does not cause any CNS side effects.

Telenzepine (22) is an analog of pirenzepine and 4–10 times more potent for inhibition of gastric secretion.



(22)

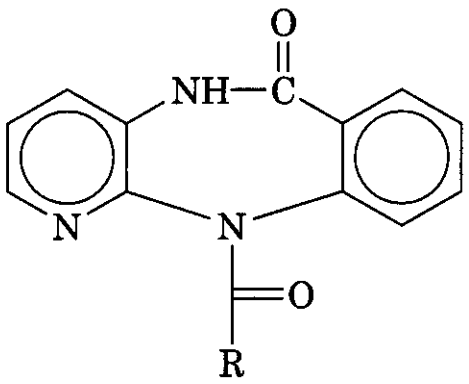
AF-DX-116 is an analog of pirenzepine that differs markedly in its profile of muscarinic activities (102,103). It has greatest affinity for cardiac M2 receptors. Its cardioselectivity is also observed in humans and may become useful in sinus bradycardia and AV block of vagal origin.

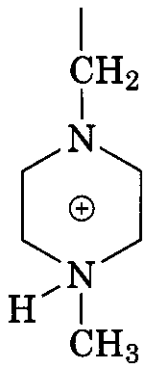
AX-RA 513 (**24**) is another analog of pirenzepine and exhibits selectivity toward M2 receptors (104). The spatial orientation of the protonated side-chain nitrogen atom in relation to the tricycle seems to be of major importance for M1/M2 selectivity.

AQ-RA 741 (25) is an analog of pirenzepine that exhibits higher affinity to chimeric m2 and M4 receptors than for m5 receptors (105). VH-AH-37 (**26**), a pirenzepine derivative, exhibits higher affinity to chimeric m5 receptors than to m2 receptors.

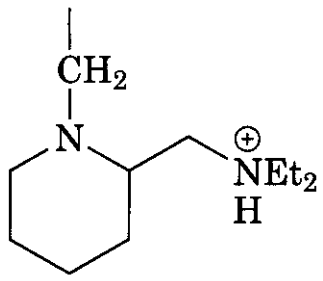
4.2.2 Benzothiazepines. These are closely related compounds to benzodiazepines. The nitrogen atom in the diazepine ring is replaced by a sulfur atom (**27**). Among these compounds BTM-1086 was found to be an M1 receptor antagonist (106). It is cis(−)-2,3-dihydro-3-(4-methylpiperazinyl)-2-phenyl-1,5-benzothiazepin-4(5*H*)-one monohydrochloride. It inhibits acetylcholine release from parasympathetic nerves and also gastric secretion.

4.2.3 Quinuclidine-Based Antagonists. A series of achiral3-heteroaryl substituted quinuclidin-2-ene derivatives (28–31) was synthesized by Hacksell et al. (107), who determined their dissociation constants (K_i) at different subtypes of muscarinic receptors. Among these compounds 2-benzoforanyl quinuclin-2-diene exhibits the highest affinity (K_i, 9.6 n*M*) at M1 receptors. Its affinity at M2 (K_i, 31 n*M*) or M3 (K_i, 59 n*M*) receptor is lower than that at M1 receptor. This antagonist is well accommodated within the defined model (108) of m1-receptor. The quinuclidin-2-ene ring will be located in an area of the receptor defined by val 102, ala 160, and val 385, where the quinuclidine ring of potent agonists bind (108). Substitution of the benzofuranyl group (28) by benzothienyl (**29**), benzoxzolyl (**30**), or benzothia-zoylyl (**31**) group decreased the affinity at the M1 receptor (Table 3.4). There is a good correlation between the magnitude of the electrostatic potential in the benzene nucleus and the M1 receptor affinity. Further, future work may yield more selective M1 antagonists that will be useful in the treatment of ulcers.

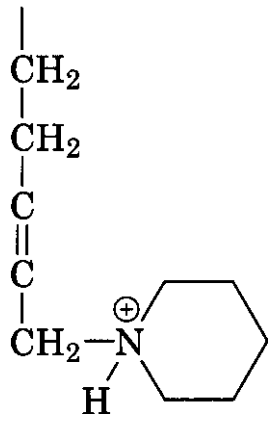




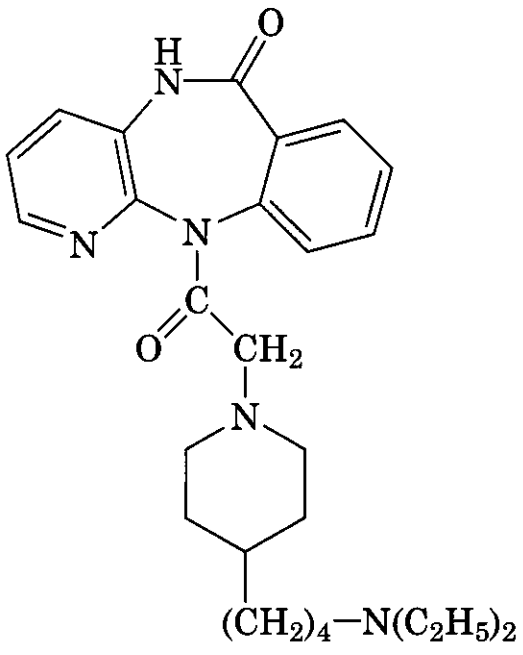
(21) (pirenzepine)



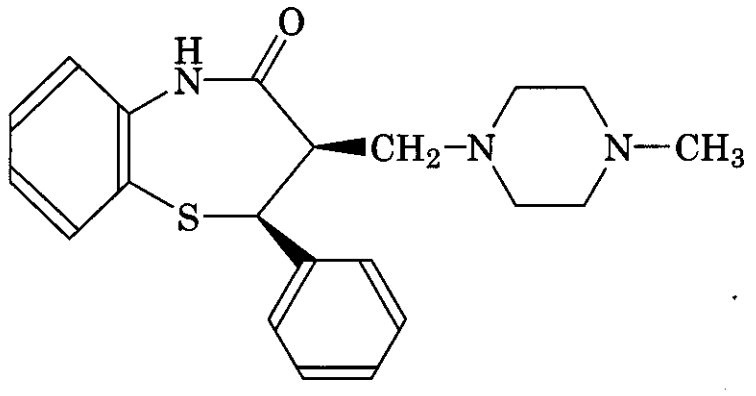
(23) (AF-DX 116)



(24) (AQ-RA 513)



(25) AQ-RA 741



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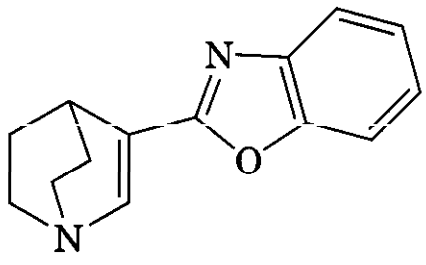

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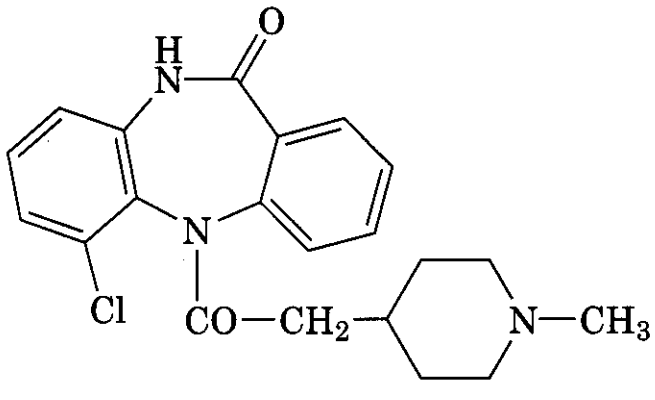
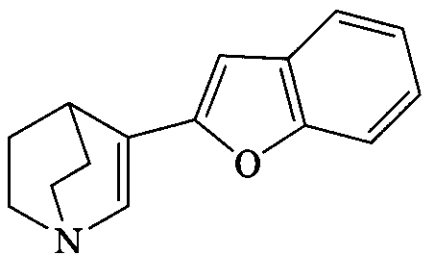


(26) UH-AH 37

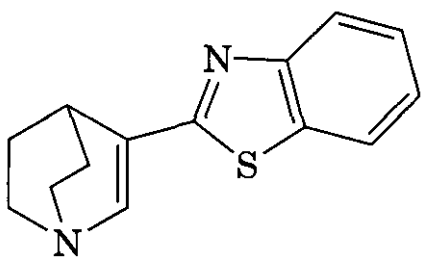


(29)

Table 3.4 K_1 Values of 2-R-Quinuclidin-2-enes at Muscarinic Receptors

	K_i (values)"		
R Group	M1	M2	M3
Benzofuranyl	9.6	31	59
Benzottienyl	81	270	420
Benzoxazolyl	100	400	720
Benzothiazoyl	170	600	1100

"Reciprocals of K_1 values give relative affinities at the receptors. Summarized from Hacksell et al. (107).



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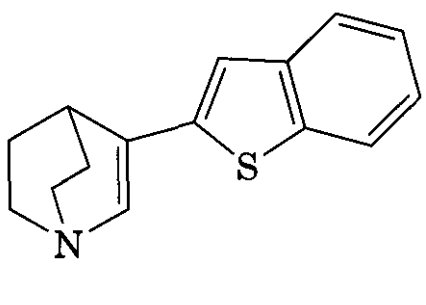
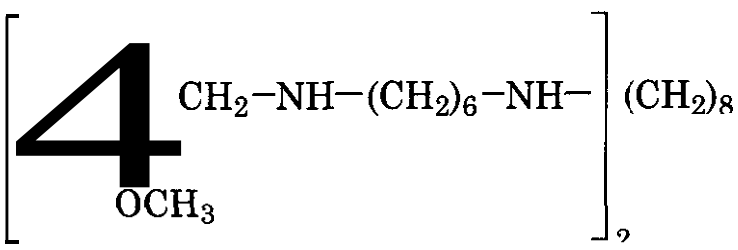


(31)

4.2.4 Polymethylene Tetramines. Several polymethylene tetramines were developed as antagonists of the M2 receptor (109, 110). Among these, methoctramine is a prototype compound (32). The selectivity and affinity of methoctramine-like compounds at M2 recep-



(32)

tors are dependent on a tetramine backbone and the nature of substituents on the terminal nitrogens (**33, 34**). This selectivity is improved by introduction of N-methyl groups into the tetramine backbone and introduction of a tricyclic system on the terminal nitrogens of the tetramine backbone. Among a series of tetramines, tripitramine (33) is a potent and selective M2 receptor antagonist (Table 3.5).

Table 3.5 Methoctramine-Related Tetramines and Their Selectivities at M2 and M3 Subtypes of Muscarinic Receptors[a]

	pA_2		Selectivity Ratio[b]
Antagonist	M2	M3	M2/M3
Methoctramine"	7.78	6.28	32[c]
Tripitramine"	9.69	6.50	1550[c]
4-DAMP	8.53	9.19	0.22[d]

"Test system for M2 receptors: guinea pig left atria. Test system for M3 receptors: guinea pig ileum.

[b]The selectivity ratio is the antilog of the difference between pA_2 values on two different systems.

'Data from Melchiorre et al. (109).

[d]Data from Tumialti et al. (110).

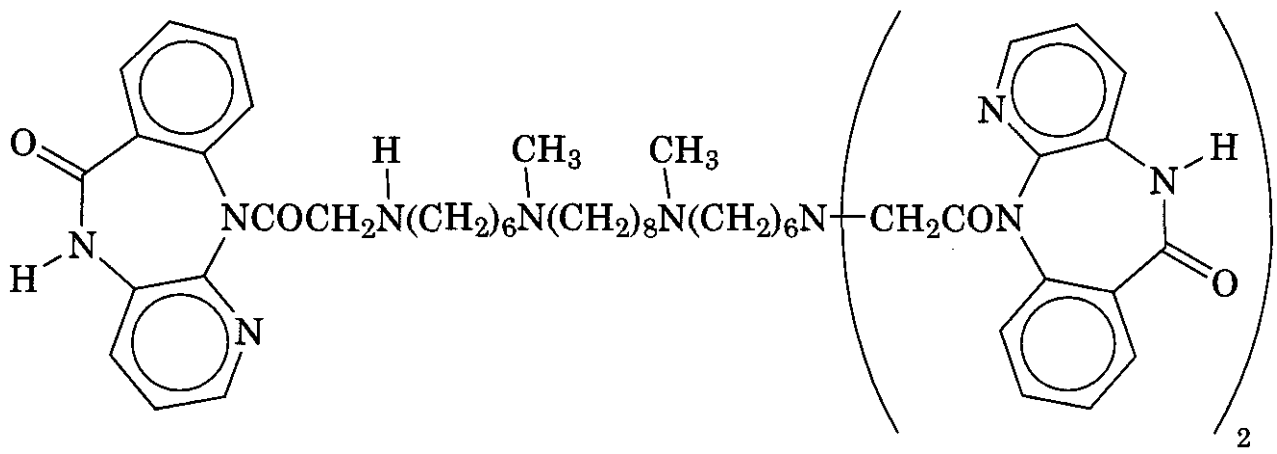
4.2.5 Indene Derivatives. Dimethylpyrindene (Dimethindene, 35) was first introduced as an H1 receptor antagonist. Subsequently, it was found to be an M2 receptor antagonist. Because of the presence of asymmetric carbon in the molecule, it occurs in two optical forms. In general, (*S*)-dimethindene is more potent than (*R*)-enantiomer at muscarinic receptor subtypes M1, M2, and M3. However, the stereoselectivity (31- to 41-fold) is greatest at M2 receptors (Table 3.6). (*S*)-Dimethindene is more specific for muscarinic receptors than at receptors of other biogenic amines (norepinepinephrine, dopamine, and 5-HT). It penetrates the blood-brain barrier in humans and

Table 3.6 Activities of Enantiomers of Dimethindene at Subtypes of Muscarinic Receptors

		pA_2 Values of Isomers	
Receptor Subtype"	Test System	(R)	(S)
M1	Rabbit vas deferens[b]	5.81	6.83
M1	Rat duodenum[b]	5.49	6.36
M2	Guinea pig atria	6.25	7.86
M2	Rabbit vas deferens	6.22	7.74
M3	Guinea pig ileum	5.61	6.92
M3	Guinea pig trachea	5.59	6.96

"Different selective agonists were used to stimulate the receptors.

[b]Some test systems contain more than one subtype of muscarinic receptor.



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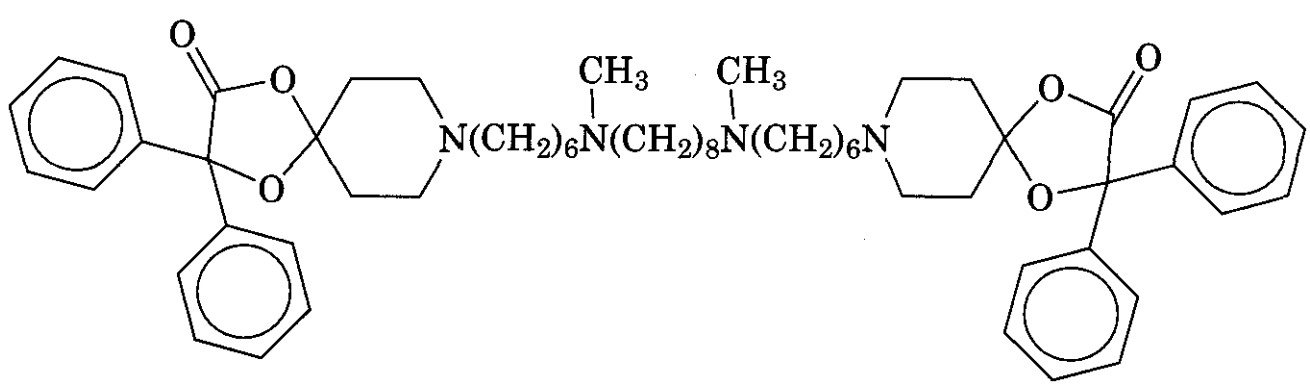

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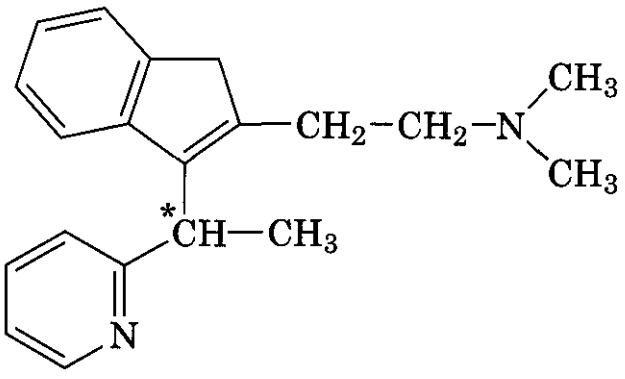

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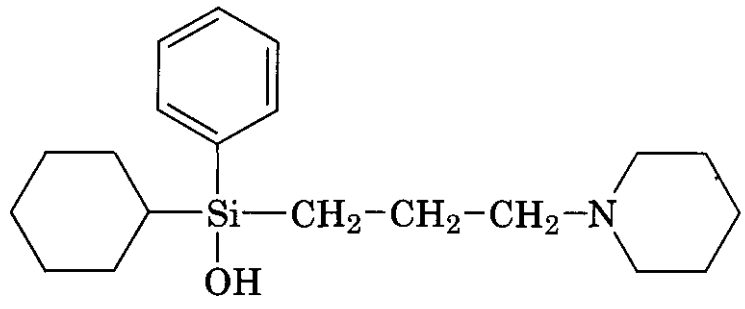
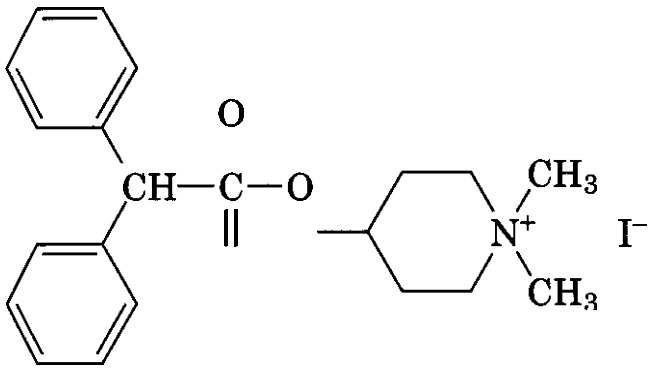


(36)

therefore may become a valuable tool in Alzheimer's disease or evaluation of M2 receptors of the CNS by PET studies.

4.2.6 Sila-difenidols. Studies on antocholinergic agents of procyclidine (Kemadrin, Table 3.3) and defenidol type have shown that substitution of the central carbon atom (R_3—C—OH) by the silicon atom (R_3—Si—OH) leads to drugs with increased antimuscarinic potency and increased selectivity for M3 receptors (**36**). Hexahydrosiladifenidol (HHSID) and its p-fluoro-derivative have been used to characterize M3 receptors in smooth muscle (112, 113). HHSID shows a 15- to 30-fold higher antimuscarinic potency at M3 receptors of guinia pig ileum and urinary bladder than at M2 receptors of the rat heart and vascular endothelium.

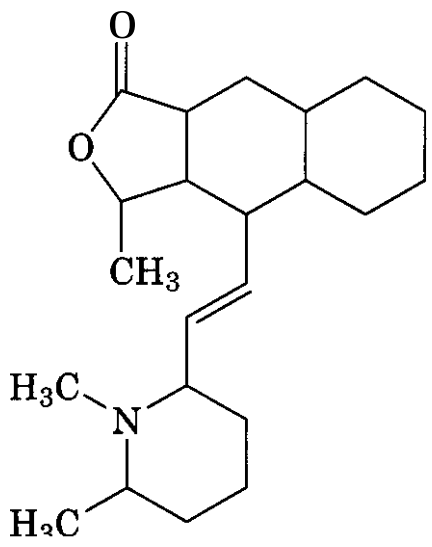
4.2.7 Diphenylacetyloxy Derivatives. Barlow and his collaborators (114) synthesized several muscarinic antagonists and tested them at the muscarinic receptors in the heart and the smooth muscle. One of these compounds, 4-[(diphenylacetyl)oxy]-1,1-dimethylpiperidium (**4-DAMP**, 37) was about 10 times more potent on M3 receptors of the smooth muscle than on M2 receptors of the heart (115). It has become very useful to identify M3



(37)

in several tissues, especially smooth muscles of trachea, ileum, vascular tissue, and cilia of different species.

4.2.8 Himbacine. This akaloid has a tricyclic structure (38). It is considered to be selective for cardiac M2 receptor (116). However, it



(38) Himbacine

binds to all five cloned muscarinic receptor subtypes in the following order of potencies (117): hM2 = hM4 > hM3 > hM1 > hM5. Its K_d values at these receptors are 4, 7, 59, 83, and 296, respectively. It is a potent blocker of oxotremorine-induced and muscarinic receptor-mediated cyclic AMP inhibition in rat striatum (K_d, 4.4 nM) and N1E-115 neuroblastoma cells (K_d, 10.6 nM) responses, which are considered to be mediated by M4 receptors. It inhibits oxotremorine-induced acetylcholine release from rat hippocampal tissue (K_d, 8.6 nM). The subtype of muscarinic receptor involved in this response is possibly the M2 or M4 type. At the postsynaptic putative M1 and M3 receptors involved in the phosphoinositide turnover in the rat cortex, himbacine has low activity and higher K_d (181 nM). It appears that himbacine is a potent muscerinic antagonist at M2 or M4 receptors compared to M1 or M3 receptors (116,117).

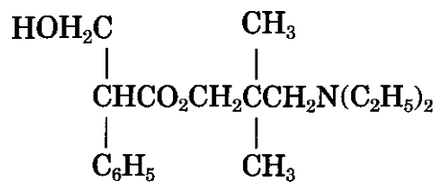
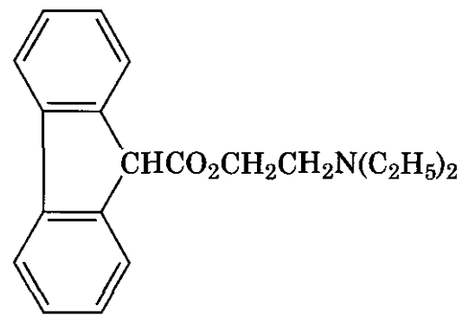
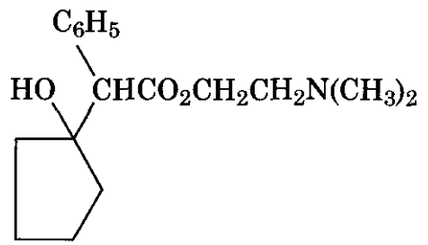
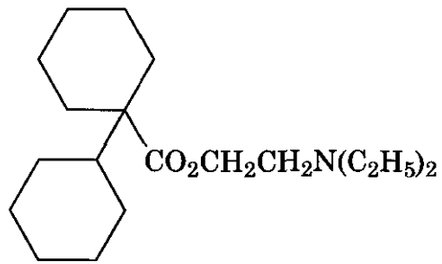
5 STRUCTURE-ACTIVITY RELATIONSHIPS

Although atropine-like agents are antagonists of acetylcholine at one type of cholinergic receptor (muscarinic receptor) that is specific for activation by L(+)-muscarine, they may demonstrate many other pharmacological properties (ganglionic blocking; neuromuscular blocking; musculotropic, central stimulant, or depressant activities). The following discussion of the relationships of structure to activity is limited to their inhibitory actions at the muscarinic receptors. Certain structural features are common in many anticholinergic agents that have been synthesized and evaluated pharmacologically (Fig. 3.4). Some of these features also appear in cholinergics (19). A typical atropine-like anticholinergic agent (20) contains a cationic head and a heavy blocking moiety (cyclic groups), which are connected by a chain of atoms of definite length (118–124). Their molecules include essential constituent groups (cationic head, cyclic radicals) as well as nonessential but contributing anchoring groups (e.g., hydroxyl). The steric factors that are related to the essential groups significantly influence the anticholinergic activity. Several anticholinergic agents incorporating the above structural features are listed in Table 3.7.

5.1 Cationic Head

The cationic head is an essential group in a large number of anticholinergic and cholinergic compounds. Ordinarily, this is a substituted ammonium group or, less frequently, a sulfonium or a phosphonium group. The mechanism of the cholinergic or anticholinergic action of substances has long been linked to such cationic groups (118–124). It is reasonable to assume that the cationic head with its positive charge is attracted by the negatively charged field (anionic center) of the muscarinic receptor. Thus, the cationic head seemingly starts the process of the adsorption of the substance at the receptor. Following the at-

Table 3.7 Synthetic Anticholinergics (Atropininic Agents)

Nonproprietary Name	Selected Proprietary Names	Chemical Name of Salt	Structure of Base	Reference for Synthetic Procedures
		Tertiary Amines—Characteristic Group in the Main Chain: Ester		
Adiphenine	Trasentine	2-Diethylaminoethyldiphenyl-acetate hydrochloride	$(C_6H_5)_2CHCO_2CH_2CH_2N(C_2H_5)_2$	125,126
Amprotropine	Syntropan	3-Diethylamino-2,2-dimethyl-propyl tropate phosphate		127, 128
Amino-carbofluorene	Pavatrine	2-Diethylaminoethyl-9-fluorene-carboxylate hydrochloride		129
Cyclopentolate USP	Cyclogyl	β-Dimethylaminoethyl (1-hydroxycyclopentyl)-phenylacetate hydrochloride		130
Dicyclomine NND	Bentyl	2-Diethylaminoethylbicyclo-hexyl-1-carboxylate hydrochloride		131

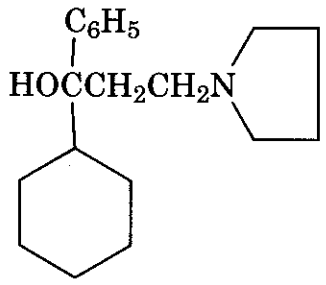
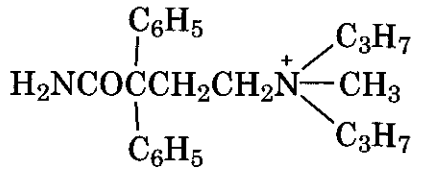
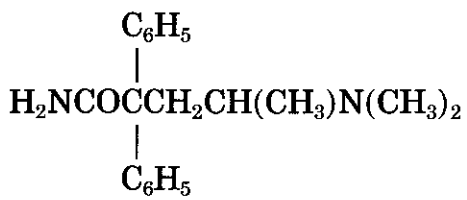
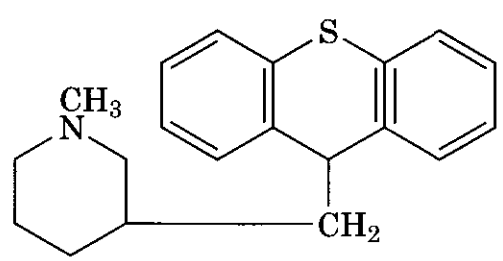
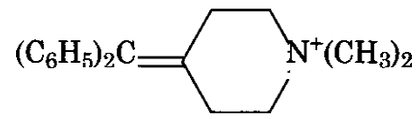
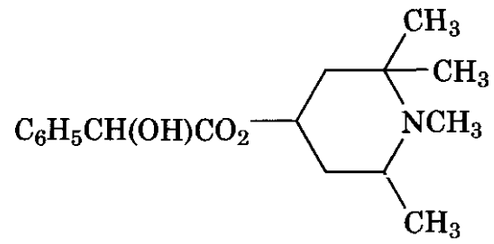
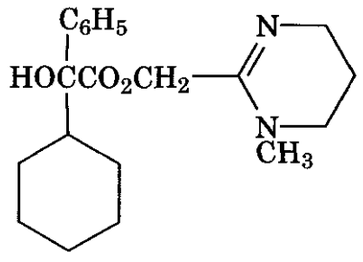
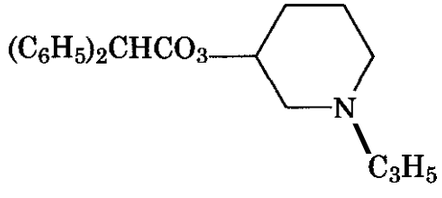
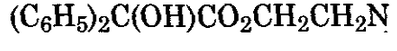
Amino alcohols containing tertiary nitrogen:				
Procyclidine	Kemadrin	1-Cyclohexyl-1-phenyl-3-pyrrolidino-1-propanol hydrochloride		155–157
Amino amides containing quaternary nitrogen:				
Isopropamide ND	Darbid	3-Carbamoyl-3,3-diphenyl-propyl)diisopropylmethyl-ammonium iodide		[illegible]0
Amino amides containing tertiary nitrogen:				
Aminopentamide	Centrine	α,α-Diphenyl-γ-dimethylaminovalermide		[illegible]1–[illegible]4
Miscellaneous:				
Methixene ND	Trest	1-Methyl-3-(thioxanthen-9-ylmethyl)piperidine hydrochloride hydrate		165

Table 3.7 (Continued)

Nonproprietary Name	Selected Proprietary Names	Chemical Name of Salt	Structure of Base	Reference for Synthetic Procedures
		Characteristic Group in the Main Chain: *Alkene*		
Diphemanil NF	Prantal	4-Diphenylmethylene-1,1-di-methylpiperidinium methylsulfate		166
Eucatropine	Euphthalmine	4-(1,2,2,6-Tetramethyl-piperidyl) mandelate hydrochloride		132,133
Oxyphencyclimine ND	Daricon, Vio-Thene	1-Methyl-1,4,5,6-tetrahydro-2-pyrimdylmethyl α-cyclo-hexyl-a-phenylglycolate hydrochloride		134
Piperidolate NND	Dactil	1-Ethyl-3-piperidyl diphenyl-acetate hydrochloride		135
Pipethanate	Sycotrol	2-(1-Piperidino)ethyl benzilate hydrochloride		136

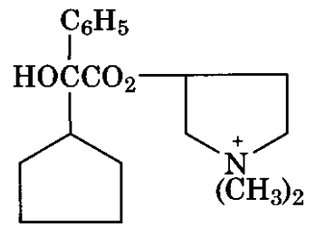
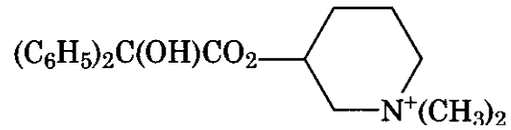
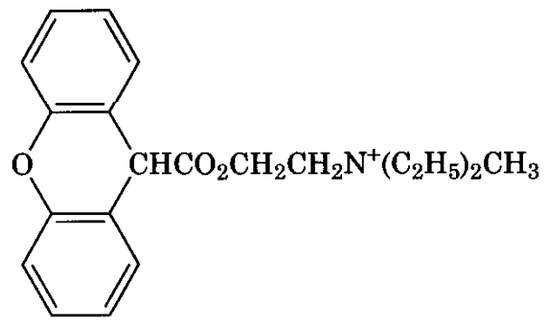
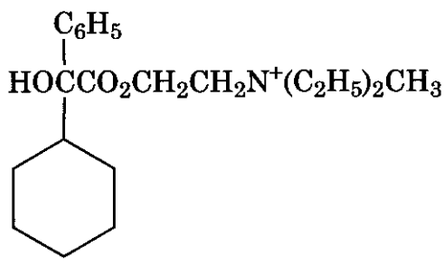
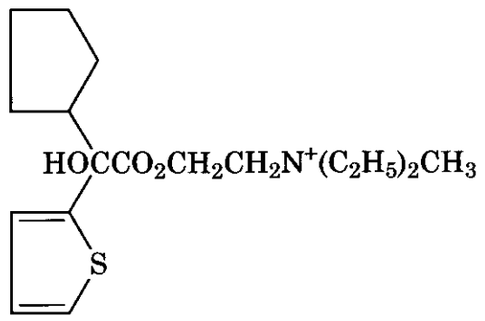
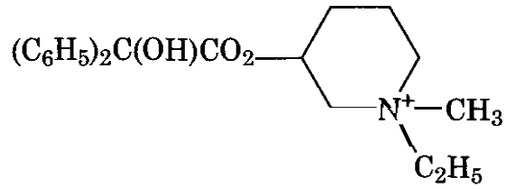
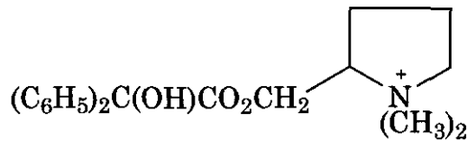
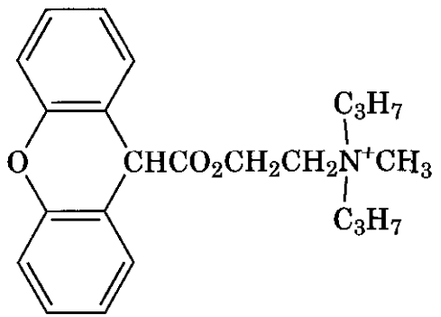
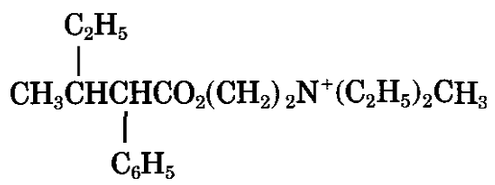
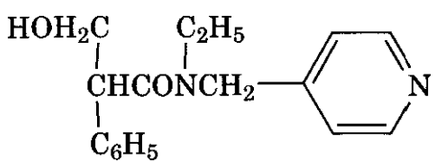
Glycopyrrolate ND	**Robinul**	3-Hydroxy-1,1-dimethyl-pyrrolidinium bromide a-cyclopentylmandelate		137
Mepenzolate ND	**Cantil**	N-Methyl-3-piperidyl benzilate methylbromide		138,139
Methantheline NF	Banthine	β-Diethylaminoethyl 9-xanthenecarboxylate methobromide		129
Oxyphenonium NND	**Antrenyl**	Diethyl(2-hydroxyethyl)-methylammonium-a-phenyl-a-cyclohexylglycolate bromide		140
Penthienate NF	Monodral	2-Diethylaminoethyl a-cyclopentyl-2-thiophene-glycolate methobromide		141, 142

Table 3.7 (Continued)

Nonproprietary Name	Selected Proprietary Names	Chemical Name of Salt	Structure of Base	Reference for Synthetic Procedures
Pipenzolate NND	Piptal	N-Ethyl-3-piperidyl benzilate methobromide		135
Poldine	Nadon	2-Hydroxymethyl-1,1-dimethylpyrrolidinium methylsulfate benzilate		143
Propantheline USP	Pro-Banthine	β-Diisopropylmethylaminoethyl 9-xanthenecarboxylate bromide		129
Valethamate ND	Murel	2-Diethylaminoethyl-3-methyl-2-phenylvalerate methobromide		144, 145

Characteristic Groups in the Main Chain: Thioester, *Amide,* or Carbarnate

Thioesters				
Thiphenamil	Trocinate	β-Diethylaminoethyldiphenylthiolacetate hydrochloride		146, 149
Amides				
Tropicamide ND	Mydriacyl	N-Ethyl-2-phenyl-N-(4-pyridylmethyl) hydracrylamide		150

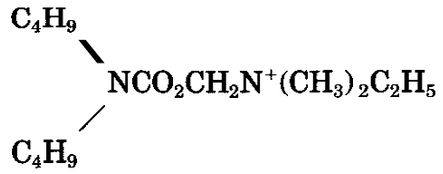
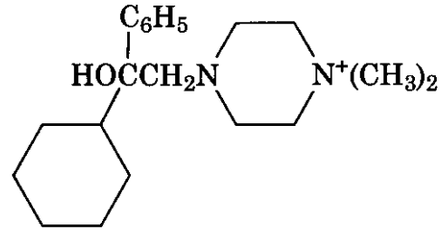
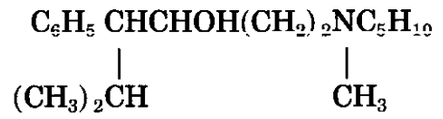
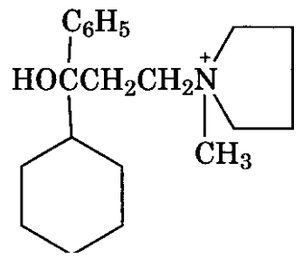
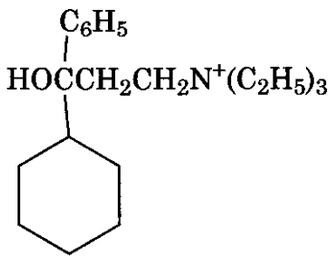
Carbamates				
Dibutoline NND	**Dibuline**	**Bis[dibutylcarbamate of ethyl-(2-hydroxyethyl)-dimethylammoniumlsulfate**		151,152
		Characteristic Groups in the Main Chain: Alkane		
Amino alcohols containing quaternary nitrogen				
Hexocyclium ND	**Tral**	**N-(β-Cyclohexyl-β-hydroxy-β-phenylethyl)-N′-methyl-piperazine methylsulfate**		153
Mepiperphenidol	**Darstine**	**5-Methyl-4-phenyl-1-(1-methylpiperidinium)-3-hexanol bromide**		154
Tricyclamol NND	**Elorine, Tricoloid**	**1-Cyclohexyl-1-phenyl-3-pyrrolidino-1-propanol methochloride**		155–157
Tridihexethyl NF	**Pathilon**	**3-Diethylamino-1-phenyl-1-cyclohexyl-1-propanol ethiodide**		158, 159

Table 3.8 Basicity and Anticholinergic Activity of Substituted Aminoethyl Esters of Benzilic Acid

$$(C_6H_5)_2C(OH)COOCH_2CH_2\overset{+}{N}(CH_3)_2R$$

R	Basicity, pK_a	Van der Waals Radius[a] of N–R (Å)	Dose[b] Required to Eliminate the Effects of Arecoline in Mice, μmol/kg	
			Salivation	Tremor
H	8.08	2.25	5.0	6.8 (6.7)
CH_3	10.87	3.09	0.48	—
OH	4.68	3.01	94.8	284.5
OCH_3	10.18	4.37	4.5	—

[a]The van der Waals' radius of N–R bond gives an estimate of the steric volume and, therefore, steric hindrance for the interaction of the cationic head at the muscarinic receptor.

[b]Doses are calculated from the values reported by Kuznetsov and Golikov (65).

traction of the oppositely charged groups, the weaker dipole-dipole, hydrophobic, and van der Valls forces go into action; if there are many of them, especially in the case of anticholinergics, they contribute to the stability of the drug-receptor complex. In such an interaction not only the charge of the cation head but also its size and shape are of vital importance.

The basicity of different amino derivatives, and consequently the degree of their ionization at physiological pH, varies over a broad range. The more ions of the anticholinergic ammonium compound or amine in solution, the greater the probability of their interaction with the anionic center of the muscarinic receptor to form the drug-receptor complex. In addition, the stability of the drug-receptor complex that has formed should depend on the basicity, given that the rate of hydrolysis of salts is inversely proportional to the base strength.

Thus, high basicity should favor the anticholinergic activity of a substance. Although the logic of this conclusion is simple, its proof involves great difficulties. In a series of anticholinergics, transition from one derivative to another is associated with stepwise changes in basicity, as well as steric factors. In this respect the N-oxides, which are obtained through the oxidation of the corresponding tertiary amines, have lower basicities and also lower anticholinergic activities (164–166). The N-oxides are closer to the corresponding quaternary ammonium compounds than to the tertiary ammonium ions in steric respect and partition between aqueous and organic phases. Alkylation of the oxygen atom converts the N-oxides into typical quaternary ammonium compounds. By this procedure, both the basicity and anticholinergic activity (Table 3.8) of the substance increase sharply.

The influence of a steric factor is more evident among compounds in which the size of the substituents at the nitrogen atom is varied both in the series of anticholinergic and cholinergic compounds. Progressive replacement of the N-methyl groups of acetylcholine with ethyl groups leads to a stepwise reduction in muscarinic activity (167). Likewise, maximal anticholinergic or blocking activity (Table 3.9) is obtained by replacing the N-methyl groups of β-dimethylaminoethyl benzilate methylchloride with ethyl groups (119). Further increases in size to butyl or larger alkyl groups reduce or abolish the activity (119, 168–172). Therefore, it seems that for stimulant activity, the small cationic head must fit into a definite space and must aid the neutralization of the charge of the anionic site of the receptor. The inhibitory action is obtained when large enough groups are substituted on the cationic portion to prevent close contact with the receptor and hence the neutralization of the charge (173,174). Thus, the cationic portion of the blocking agents provides the electrostatic forces necessary to orient the molecules toward the receptor and hold them in place.

The anticholinergic activity depends not only on the number and the molecular weight of the alkyl radicals that are connected to the

Table 3.9 Influence of the Number, Size, and Structure of **Alkyl** Groups in the **Cationic** Head **on** the **Anticholinergic** Activity

Compound Pair	Name or Structure of Compound[a] Series A	Series B	Test System	Activity Ratio[b] B/A	Ref.
1	$RN(CH_3)_2$	$RN(C_2H_5)_2$	Cat: salivation	1.63	119
			Cat: blood pressure	2.09	
	+	+	Mouse: mydriasis	0.45	
2	$RN(CH_3)_2nC_3H_7$	$RN(CH_3)_2isoC_3H_7$	Cat: salivation	2.00	119
			Cat: blood pressure	2.38	
	+	+	Mouse: mydriasis	4.09	
3	$RN(CH_3)_2nC_3H_7$	$RN(CH_3)_2nC_4H_9$	Cat: salivation	0.49	119
			Cat: blood pressure	0.52	
	+	+	Mouse: mydriasis	0.63	
4	$RN(CH_3)_2C_2H_5$ (lachesine)	$RNCH_3(C_2H_5)_2$	Cat: salivation	1.06	
			Cat: blood pressure	1.31	
	+	+	Mouse: mydriasis	0.60	
5	$RNCH_3(C_2H_5)_2$	$RN(C_2H_5)_3$	Cat: salivation	1.00	119
			Cat: blood pressure	0.79	
			Mouse: mydriasis	1.33	
6	Atropine (⟩NCH_3)	N-Ethylnoratropine	Cat: blood pressure	0.04	179
7	Homatropine	*N*-Isopropylnor-homatropine	Cat: blood pressure	0.12	179
8	Atropine	N-Allylnoratropine	Cat: blood pressure	0.04	180
			Rat: mydriasis	0.13	

[a] $R = (C_6H_5)_2C(OH)CO_2CH_2CH_2-$.

[b] In Tables 3.9–3.11, 3.13, and 3.15 the influence of the molecular modification on the pharmacological activity is expressed as activity ratios. An activity ratio represents the ratio of the relative molar activities of two substances, whose activities are compared with a standard substance. **A** ratio of 1.0 indicates that the molecular modification that converts the compound in series **A** to the corresponding compound in series B does not change the pharmacological activity. An activity ratio of greater than unity indicates that the molecular modification has increased the activity; when it is less than unity the molecular modification has decreased the activity.

nitrogen atom but also on their structure. In contrast to di-*n*-propylamino derivatives, diisopropylamino derivatives have an anticholinergic activity close to or higher than the activity of diethylamino derivatives (136, 175–178). The close correlation of the activities of the diethyl and diisopropyl derivatives could be related with the equal linear lengths (from the nitrogen atom) of these radicals.

In the case of cyclic amino alcohols where nitrogen enters into the composition of the cycle, the optimal anticholinergic effect is produced not by the N-ethyl, *N*-isopropyl, or *N*-allyl, but by N-methyl radical, as is apparent from a comparison of the esters of tropine (Tables 4–6). It may be that the elements of the cyclic structure occupy a sufficiently large space besides the nitrogen atom.

As a general rule, quaternization with a small alkyl group increases activity (Table 3.10), although a few exceptions have been reported (181, 182).

Besides the charge on the cationic head of anticholinergics (and cholinergics), other factors seem to contribute to the interaction between the muscarinic receptor and the anticholinergics. The substituents at the nitrogen atom apparently participate actively in the process. This is evident from the anticholinergic action of the 3,3-dimethylbutyl ester of benzilic acid, $(C_6H_5)_2C(OH)CO_2CH_2CH_2C(CH_3)_3$, which contains no nitrogen and consequently is not ionized, but which has in the corresponding position a t-butyl radical that sterically imitates the trimethylammonium group (191). **A** similar replacement of a trimethylammonium group with a t-butyl radical in acetylcholine leads to its "carbon analog," $CH_3CO_2CH_2CH_2C(CH_3)_3$, which is similar to acetylcholine in its behavior toward cholinesterase (192).

5.2 Cyclic Moieties

The introduction of two phenyl groups into a molecule of acetylcholine or a cholinergic

Table 3.10 Differences Between the Anticholinergic Activities of Tertiary and Quaternary Ammonium Compounds and Atropine-like Agents

Compound Pair	Series A: Tertiary Ammonium Compounds	Series B: Quaternary Ammonium Compounds	Test System	Activity Ratio B/A	Ref.
1	Atropine	Methylatropine	Guinea pig: ileum	2.10	119
			Mouse: mydriasis	2.30	122
2	(−)-Hyoscyamine	(−)-Methylhyoscyamine	Mouse: mydriasis	2.70	123
3	(−)-Scopolamine	(−)-Methscopolamine	Guinea pig: ileum	7.60	183–185
			Mouse: mydriasis	1.00	
4[a]	Tertiary analog of methantheline $XN(C_2H_5)_2$	Methantheline + $XN(C_2H_5)_2CH_3$	Rabbit: intestine	2.83	186, 187
5[b]	Tertiary analog of penthienate $XN(C_2H_5)_2$	Penthienate + $XN(C_2H_5)_2CH_3$	Rabbit: ileum	1.24	122
			Rabbit: salivation	30.80	
6	(±)-Procyclidine	(±)-Tricyclamol	Guinea pig: ileum	18.3	188, 189
			Mouse: mydriasis	13.5	
7	(±)-Benzhexol[c]	Methyl analog of (±)-benzhexol	Guinea pig: ileum	2.64	190
			Mouse: mydriasis	8.89	
8[d]	$RN(CH_3)_2$	$RN(CH_3)_3$	Cat: salivation	17.9	119
			Cat: blood pressure	10.3	
		+	Mouse: mydriasis	2.41	
9[d]	$RN(C_2H_5)_2$	$R(CH_5)_3$	Cat: Salivation	15.1	119
			Cat: blood pressure	9.06	
			Mouse: mydriasis	14.2	

[a]For complete structures see Table 3.7.
[b]For complete structures see Table 3.7.
[c]1-Piperidino-3-phenyl-3-cyclohexyl-propan-1-ol.
[d]$R = (C_6H_5)_2C(OH)CO_2CH_2CH_2^-$.

substance [i.e., $CH_3CO_2(CH_2)_2{}^+N(CH_3)_3$ or $CH_3(CH_2)_4{}^+N(CH_3)_3$] changes the compound to an anticholinergic agent [$(C_6H_5)_2CHCO_2(CH_2){}^+N(CH_3)_3$ and $(C_6H_5)_2CH(CH_2)_4{}^+N(CH_3)_3$, respectively].

Anticholinergics contain varied cyclic structures, the phenyl group being the most common (65c). Very often one encounters cyclohexyl and cyclopentyl radicals and the corresponding unsaturated groups (cyclohexenyl, cyclopentenyl). Substances containing *a-*, or, less frequently, β-thienyl radicals may possess high anticholinergic activity. Often unbranched (methyl, ethyl) or branched (isobutyl, isoamyl) groups are located at the same carbon atom together with one or two cyclic groups. The anticholinergic activities of substances that contain only aliphatic radicals are lower than those of the corresponding comounds with cyclic substituents.

The most common and, as a rule, the most active anticholinergics contain two cyclic substituents as blocking groups at the same carbon atom (Table 3.11) but a third cyclic substituent lowers the anticholinergic activity (193). When these cyclic groups are too large, such as biphenyl and naphthyl, the compounds have low anticholinergic activities. A sufficiently large number of anticholinergics that contain only one cyclic group on carbon are known; however, there is usually also an aliphatic group or, even better, a hydroxyl group present in such a case. Examples of such compounds are the esters of tropic acid. The introduction of a second benzene ring into the α-carbon of tropic acid lowers the anticholinergic activity of its aminoalkyl esters (65c).

It is difficult to assess which cyclic substituents contribute the most for the anticholinergic activity. It could be that the effect of one or another moiety depends on the substituents already present and on other characteristics of the substance. An overwhelming majority of the therapeutically most active anticholin-

Table 3.11 Influence of Cyclic Radicals on Anticholinergic Activity (Test System: Rabbit Intestine)

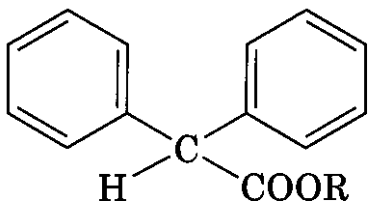
Compound Pair	Name or Structure of Compound[a]		Activity Ratio B/A	Ref.
	Series A	Series B		
1	$C_6H_5CH_2R$	$(C_6H_5)_2CHR$ (adiphenine)	6.7	193
2	$C_6H_5CH_2R$	$(C_6H_5)_3CR$	0.7	193
3	$CH_2(OH)R$	$C_6H_5CH(OH)R$	23.3	193
4	$C_6H_5CH(OH)R$	$(C_6H_5)_2C(OH)R$	114	193
5	Adiphenine (transentine)	$(C_6H_5)CH(C_6H_{11})R$ (transentine-H)	3.3	194,195
6	Adiphenine	Dicyclomine	10.0	196

[a] $R = CO_2CH_2CH_2N(C_2H_5)_2$.

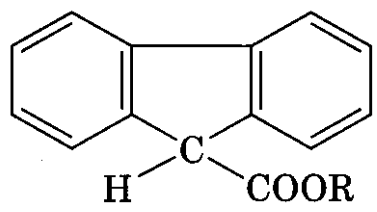
ergics contain at least one phenyl group (Table 3.7). The second cyclic group, where there is one, need not be a phenyl. It is even better if, for example, it is a cyclohexyl, cyclopentyl, or any other cyclic structure. Such unsymmetrical doubly substituted compounds have higher anticholinergic activities and lower toxicities (65, 194, 195). This is a situation similar to that in 5,5-disubstituted barbituric acid hypnotics and anticonvulsants.

A question might arise whether the aromatic (flat surface) nature of one of the cyclic radicals is essential for anticholinergic activity, because such a large number of anticholinergics contain a phenyl group. The sufficiently high activity of compounds in which both substituents are alicyclic (e.g., cyclohexyl or cyclopentyl) provides a basis for asserting that the aromatic nature of the substituents is not essential in anticholinergics (65c)65.

Not just the number and the character of the cyclic group but also the mode of linking of the substituents are important for anticholinergic activity. Two benzene rings are linked differently in 2-diethylaminoethyl esters of diphenylacetic acid (39), fluorene-9-carboxylic acid (40) and p-biphenylacetic acid (41). Of these, the diphenylacetic acid derivatives have the highest anticholinergic activity (193).



(39)



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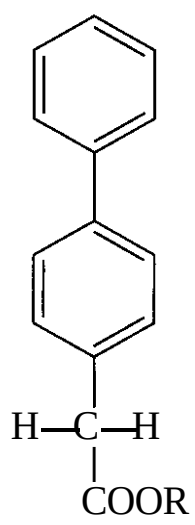
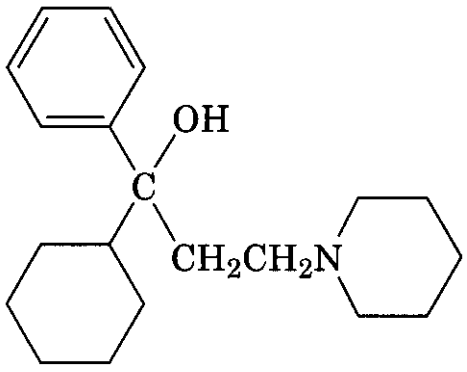


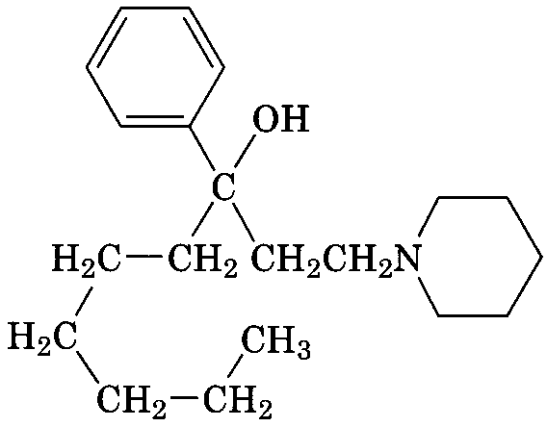
(41)

The importance of the cyclic nature of the substituent and not simply of its mass is evident from the comparison of the anticholinergic activities of 1-cyclohexyl-1-phenyl-3-piperidino-1-propanol (42) and 1-(*n*-hexyl)-1-phenyl-3-piperdino-1-propanol (43), of which (42) is an active anticholinergic, whereas (43) is not effective (174).

As far as the contribution of cyclic structures to anticholinergic activity is concerned, the introduction of cyclic groups into acetylcholine or a cholinergic compound leads to a change in the pharmacological properties that, without lowering and possibly even



(42)



(43)

strengthening its affinity for the muscarinic receptor, abolishes or blocks the action of the chemical transmitter. This phenomenon is similar to the transition from a metabolite to an antimetabolite. It has been suggested that the cyclic groups of the anticholinergic agent form an additional contact with the muscarinic receptor by hydrophobic or van der Walls forces; as a result, this contact is strengthened and the muscarinic receptors are protected from approaching molecules of acetylcholine (174, 197). Cyclic groups of substantial size can create a kind of protective screen that sterically hinders the approach of molecules of acetylcholine, not only to the given active site but also to the vicinity of the active sites of the receptor. Tricyclic anticholinergic agents may fall under this category.

5.3 Length of the Main Chain Connecting the Cationic Head and the Cyclic Croups

The presence of the cationic head and of cyclic groups is not sufficient for optimal anticholinergic activity of a compound. The activity depends on the mutual distribution of these groups. This establishes the basic requirements for a chain of atoms that connects the cationic head and the cyclic moieties; these apply to the length and form of the chain, lateral branching, and functional groups in the chain, if any.

A considerable number of the anticholinergics belong to the group of aminoalkyl esters of substituted acetic acids. In an overwhelming majority of cases, the substituted esters of β-aminoethanol are more active as anticholinergics than are the corresponding derivatives of γ-aminopropanol (119, 170, 175, 198). Further increase of the chain length of the amino alcohol leads to a decrease or disappearance of the anticholinergic activity. The arninoalkyl esters of diphenylacetic acid are more active anticholinergics than the corresponding aminoalkyl esters of β,β-diphenylpropionic acid (199). Therefore, in all these esters with high anticholinergic activity the main chain connecting the cyclic moieties and the cationic head contains five atoms (Table 3.12, series 1–3). In an homologous series of compounds in which the ester group is replaced by a chain of carbon atoms (Table 3.12, series 4–9), there are three atoms in the main chain in compounds with maximal anticholinergic activity. To explain the differences in the anticholinergic activities of different series of compounds, the ability of their structures to exist in different spatial conformations has to be taken into account.

Acetylcholine and related esters can exist in two conformations (Fig. 3.5), the skewed and extended forms (200) (e.g., 44 and 45, respectively, for acetylcholine). The skewed form (44) of acetylcholine is closely related to the structure of muscarine (46) (200). Similarly the substituted aminoethyl esters, which are anticholinergics, may exist in two conformations. The skewed forms of acetylcholine (44), muscarine (46), the skewed form of aminoethyl esters (47), and the extended form of aminopropane derivatives (48) all interact at the same muscarinic receptors. In the former two compounds the interatomic distance between the quaternary nitrogen and the ether oxygen atom is nearly the same, and both of them are agonists. In (48) the interatomic distance between the quaternary nitrogen and the carbon atom to which the cyclic radicals are attached is the same, and both of them are

Table 3.12 Chain Length Between Cationic Head and Cyclic Radicals Among Anticholinergics

No.	Series	Value of n for High Anticholinergic Activity	Total Number of Atoms in the Chain	Test System	Ref.
1	$(C_6H_5)_2C(OH)CO_2(CH_2)_nNC_5H_{10} \cdot HCl$	2[a]	5	Rabbit: mydriasis	198
	+				
2	$(C_6H_5)_2C(OH)CO_2(CH_2)_nNC_5H_{10} \cdot CH_3Br$	2[a]	5	Rabbit: mydriasis	198
	+				
3	$(C_6H_5)_2C(OH)CO_2(CH_2)_n(C_2H_5)(CH_3) \cdot CH_3Cl$	2[a]	5	Mouse: mydriasis	119
4	$(C_6H_5)(C_2H_5)C(OH)(CH_2)_nN(C_2H_5)_2 \cdot HCl$	2	3	Rabbit: ileum	158
5	$(C_6H_5)(C_2H_5)C(OH)(CH_2)_nNC_5H_{10} \cdot HCl$	2	3	Rabbit: ileum	158
6	$(C_6H_5)_2C(OH)(CH_2)_nNC_5H_{10} \cdot HCl$	2	3	Rabbit: ileum	158
7	$(C_6H_5)_2CH(CH_2)_nN(C_2H_5)_2 \cdot CH_3I$	2	3	Mouse: salivation	
8	$(C_6H_5)_2C(OH)(CH_2)_nN(C_2H_5)_2 \cdot HCl$	2	3	Mouse: salivation Mouse: tremor	
9	$(C_6H_5)_2C(OH)(CH_2)_nN(C_2H_5)_2 \cdot CH_3I$	2	3	Mouse: salivation	

[a] No exact values are available for esters with $n = 1$

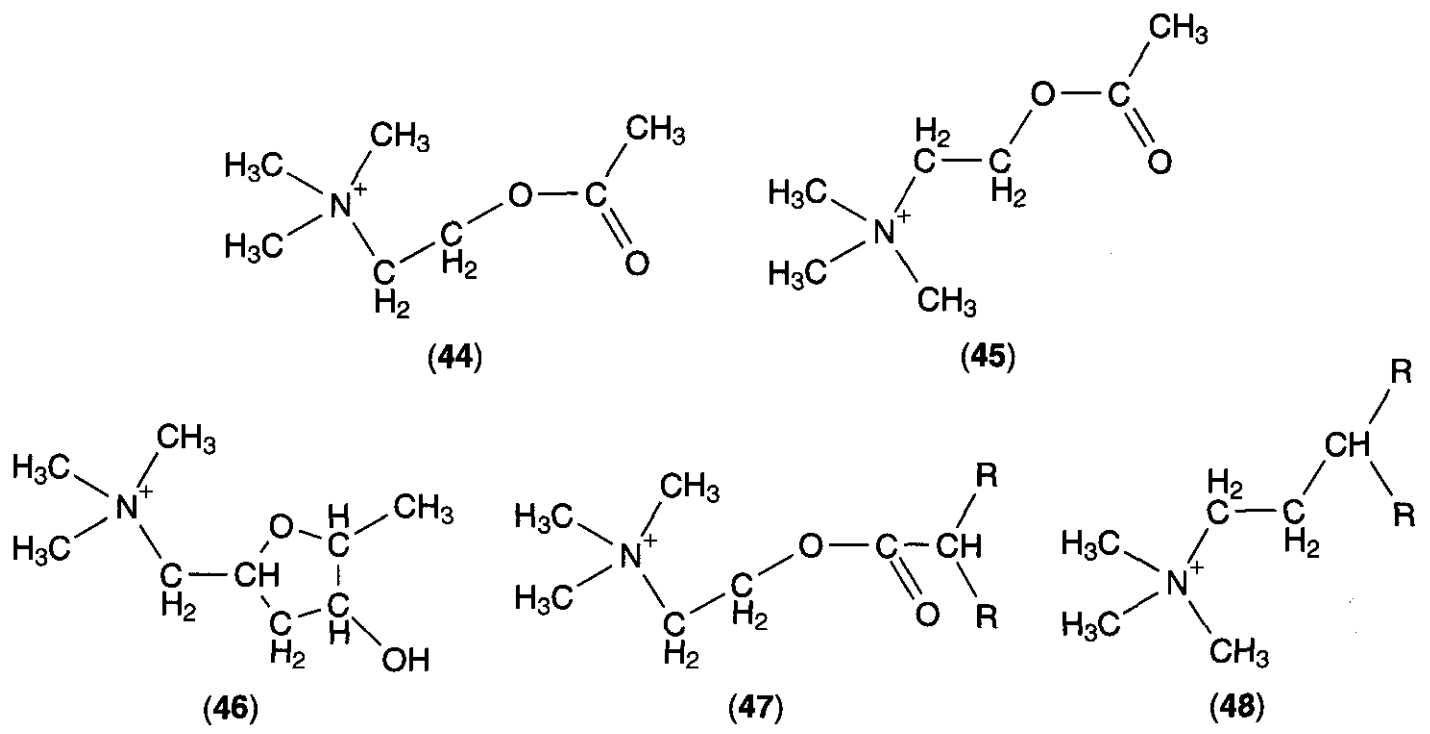


Figure 3.5. Conformations of cholinergics and anticholinergics.

antagonists (65d)65. Thus, anticholinergic activity depends not only on the length of the main chain of the molecule but also on its ability to adopt a certain conformation that is favorable for the interaction of the substance with the receptor.

There is some information about the influence of branching of the main chain on the anticholinergic activity. Esters with a methyl group α to the ester oxygen in the amino alcohol part are less active than compounds without the methyl group (175, 198, 201). Similarly, the derivatives of 1,3-aminopropanol, aminopropane, and γ-aminobutyronitrile (201, 203), which contain a branch at the carbon atom β to the nitrogen, are less active anticholinergics than the compounds without the branching. The negative influence of such a side chain has been explained by steric hindrance at the receptor (174).

The inclusion in the main chain of optimum length of other atoms such as oxygen, sulfur, nitrogen, and other functional groups changes any anticholinergic activity (65). However, such compounds are considerably potent (see Section 5.4).

5.4 Esteric Linkage

The question of the importance of complex ester grouping in anticholinergics, and even more of its role, has not been cleared up sufficiently. A great importance was attached to the complex esters in the initial period of the search for atropine-like substances, when active compounds were known only among the esters of amino alcohols and carboxylic acids. However, the presence of this grouping is not necessary for the manifestation of anticholinergic activity. Presently, a large number of substances are known that belong to different chemical structures and that possess high anticholinergic activity (Table 7).

The influence of an ester link can be assessed by comparing similar compounds that do not contain pharmacophoric groups other than the anchoring groups (amino nitrogen, cyclic radicals). Comparative data on the anticholinergic activities of the 2-diethylaminoethyl ester of diphenylacetic acid $[(C_6H_5)_2CHCO_2CH_2CH_2N(C_2H_5)_2]$ and 1,1-diphenyl-5-diethylaminopentane $[(C_6H_5)_2CHCH_2CH_2CH_2CH_2N(C_2H_5)_2]$ indicate that they are equally active (65d). Thus, the complex ester group is not essential for anticholinergic activity; however, it may contribute to optimal activity when it is present in atropine-like compounds (123). It may influence the conformation of a molecule that in turn determines the effectiveness of the interaction of the essential anchoring groups, the cationic head and the cyclic radicals (65d), with the muscarinic receptor.

5.5 Hydroxyl Croup

The anticholinergic compounds that contain a hydroxyl group in a certain position of a molecule possess considerably higher activity than similar compounds without the hydroxyl. That position is of great importance. For esters of amino alcohols and hydroxycarboxylic acids, maximum activity is achieved if the hydroxyl is β to the carboxyl group. Atropine is about 10 times more active than homatropine. However, esters with an a-hydroxyl also possess considerable anticholinergic activity. In anticholinergic amino alcohols the hydroxyl on the third carbon atom from the nitrogen gives optimal activity (Table 3.7). The location of the hydroxyl group in relation to the cyclic radicals is also of vital importance. In the great majority of anticholinergics they are located at the same carbon atom or at adjacent carbons.

The hydroxyl group in anticholinergics can be replaced by CN^- and $CONH_2^-$ groups while preserving some degree of activity. However, replacement of the hydroxyl by methoxy or an acetoxy group lowers the activity (204, 205).

The hydroxyl group may interact by hydrogen bonding with a site on the muscarinic receptor, which is rich in electrons. In support of this statement (65e), hydroxylated anticholinergics form complexes in solution with substances such as amines that contain electron-donor atoms. In a series of structurally related compounds the anticholinergic activity was proportional to their capacity for molecular association by way of the hydroxyl group. There is a direct relationship between the anticholinergic activity and the mobility of the hydrogen atom of the hydroxyl group as determined by the rate of acetylation. The contribution of the hydroxyl group to the free energy of adsorption is quite high, of the order of 2 kcal; it is apparently independent of the number of methyl groups attached to the cationic head (206).

Although a hydroxyl group increases the activity of an anticholinergic, it does not convert a cholinergic substance into an anticholinergic. Propionyl-, α-hydroxypropionyl-, and α,β-dihydroxypropionylcholines possess cholinergic properties (207). a-Hydroxy substitution decreases the original muscarinic activity to about 1/3, whereas the introduction of both a- and β-OH functions decreases the muscarinic activity to about one-tenth.

The introduction of a hydroxyl group into 2-diethylaminoethyl phenylacetate approximately doubles its activity (Table 3.13), whereas the same structural change in the corresponding ester of diphenylacetic acid increases its activity about 140 times. The positive influence of the hydroxyl group has been observed in a large number of anticholinergics (Table 3.7). The exceptions are those cases in which the cyclic groups are too large or are connected in such a way that they can sterically prevent the interaction of the hydroxyl with the surface of the muscarinic receptor.

5.6 Epoxy Group

The presence of an epoxy group seems to increase the mydriatic activity (Table 3.13). However, scopolamine (**7**), which contains an epoxy group, is a central depressant, as indicated by drowsiness, euphoria, amnesia, and dreamless sleep. Atropine (**4**), which does not contain an epoxy group, stimulates the medulla and higher cerebral centers. In clinical doses (0.5–1.0 mg), this effect is usually confined to mild vagal excitation. Toxic doses of atropine cause restlessness, disorientation, hallucinations, and delirium.

5.7 Stereoisomerism and Anticholinergic Activity

5.7.1 Opticalisomerism. Atropine (**4–6**) is the racemic form of hyoscyamine, which is the (*S*)-(−)-tropyl ester of 3α-tropanol (2). The carbon a to the carbonyl group is asymmetric. (*S*)-(−)-Hyoscyamine is more active than (*R*)-(+)-hyoscyamine as an anticholinergic (Table 3.11). The alkaloid scopolamine (**7**) is the (*S*)-(−)-tropyl ester of scopine (**8**); again the (*S*)-(−) isomer is more active than (*R*)-(+) isomer in its anticholinergic activities.

A considerable number of synthetic anticholinergic agents patterned after the structure of atropine contain an asymmetric carbon atom corresponding to the position of the asymmetric carbon in atropine. In all compounds examined, the asymmetric carbon is

Table 3.13 Influence of the Hydroxyl and Epoxy Groups on Anticholinergic Activity

Group	Name or Structure of Compound		Test System	Activity Ratio B/A	Ref.
	Series A	Series B			
Hydroxyl	$C_6H_5CH_2CO_2CH_2N(C_2H_5)_2$	$C_6H_5CH(OH)CO_2CH_2CH_2N(C_2H_5)_2$	Rabbit: intestine	2.3	193
	$(C_6H_5)_2CHCO_2CH_2CH_2N(C_2H_5)_2$	$(C_6H_5)_2C(OH)CO_2CH_2CH_2N(C_2H_5)_2$	Rabbit: intestine	143	193
Epoxy	(−)-Hyoscyamine	(−)-Scopolamine	Guinea pig: ileum	0.24	208
			Mouse: eye	2.70	
			Cat: eye	5.80	
			Cat: blood pressure	0.64	
			Cat: salivation	0.77	
	(−)-Methylhyoscamine	(−)-Methylscopolamine	Mouse: eye	1.00	208
			Cat: eye	3.33	
			Cat: blood pressure	0.80	
			Cat: salivation	0.80	

Table 3.14 Optical Isomerism and Anticholinergic Activity

No.	Compounds Whose (+) and (−) Isomers Are Tested	Test System	Position of the Asymmetric Carbon	Active Isomer	Isomeric Ratio[a]	Ref.
1	Hyoscyamine	Dog: salivation	a-carbon to the carbonyl group	(−)	30	208
		Cat: salivation		(−)	20	
		Cat: bloodpressure		(−)	23	
		Guinea pig: ileum		(−)	32	
		Rabbit: ileum		(−)	110	
2	Scopolamine	Dog: salivation	a-carbon to the carbonyl group	(−)	17	208
		Rabbit: intestine		(−)	15	
3	Tricyclamol	Guinea pig: ileum	Carbon with cyclic radical	(−)	160	209
				(−)	62	
4	Benzhexol	Guinea pig: ileum	Carbon with cyclic radical	(−)	10	209
		Rabbit: intestine		(−)	160	123
		Mice: mydriasis		(−)	5	209
5	Procyclidine	Guinea pig: ileum	Carbon with cyclic radical	(−)	49	209
		Mice: mydriasis		(−)	18	209
6	1,1-Diphenyl-3-piperidino-1-butanol hydrochloride	Rabbit: intestine	a to N	(+)	84	210 211
7	Methiodide of no. 6	Rabbit: intestine	a to N	(+)	3	210 211
8	Diphenylacetate of 3-quinuclidinol	Rabbit: intestine	β to N	(−)	24	212

[a]Activity ratio between the enantiomers.

located in the acyl moiety and is connected with the cyclic and the hydroxyl groups (directly or through a methylene group). The (−) isomers are often more active than (+) isomers (Table 3.14), indicating some apparent stereospecificity with respect to the carbon atom *a* to the carbonyl group of atropine.

The atropine-like activities of some compounds in which the asymmetric carbon atom is considerably closer to the amino group have been described. In 1,1-diphenyl-3-piperidino-1-butanol, the carbon *a* to the nitrogen is asymmetric. In this case the (+) isomer seems to be more active than the (−) isomer. In 3-quinuclidinyl diphenylacetate, the carbon atom β to the nitrogen is asymmetric; the (−) isomer has more atropine-like activity than does the (+) isomer.

5.7.2 Derivatives of Tropine and Pseudotropine. The configuration of the 3-OH group in the tropine part of the molecule has significant influence on the activity at the muscarinic receptor (Table 3.15). The derivatives of ψ-tropine (pseudotropine, **3)** are less active; the activity ratio for the ψ compound relative to the isomeric tropine varies from **2** to 13, but more information is needed on this point.

Table 3.15 Relative Anticholinergic Activities of the Esters of Tropine and Pseudotropine

Isomer Pair	Pair of Structural Isomers A	Pair of Structural Isomers B	Test System	Activity Ratio A/B	Ref.
1	Atropine	Tropyl-ψ-tropine	Cat (?): blood pressure	2	213
2	Benzoyl-tropine HCl	Benzoyl-ψ-tropine	Rabbit or Guinea pig: intestine	3	214
3	CH_3I of no. 2	CH_3I of no. 2	Guinea pig: intestine	13	214
4	C_2H_5Br of no. 2	C_2H_5Br of no. 2	Guinea pig: intestine	4	214

5.7.3 Stereochemical Configuration. The acetylcholine-like cholinergics and atropine-like anticholinergics contain similar pharmacodynamic groups. In various hypotheses, it has been assumed that both stimulant and blocking drugs interact with the muscarinic receptor through the essential pharmacodynamic groups. The tropic acid portion of atropine contains an asymmetric carbon, and the muscarinic receptor is stereospecific for the carbon a to the carbonyl group in anticholinergics. Acetylcholine does not contain such an asymmetric carbon atom. Lactoylcholine is an agonist that contains an asymmetric carbon (207), and the muscarinic receptor is stereospecific for the carbon *a* to the carbonyl group among lactoylcholine-like cholinergics (215). Because of the structural similarities in tropic and lactic acids, it has even been suggested that a lactoylcholine-like parasympathetic neurohormone may occur in animal tissues (207, 216). However, this has not been corroborated.

5.7.4 Dissociation Constants of Cholinergics and Anticholinergics. The absolute configuration [(*R*) and (*S*)] is self-consistent for a molecule in question and cannot be used to relate a series of compounds. The configuration in relation to a standard substance (D and L) is very useful to compare a series of compounds. For example, the pharmacological parameters of all D compounds in a series can be compared with those of the L compounds, provided each one of the compounds contains a single asymmetric carbon (217).

In a number of studies on structure-activity relationships, the pharmacological activities are expressed in terms of potencies or relative molar activities, which are derivatives of their ED_{50} values. The reciprocals of ED_{50} values do not give exact measures of affinities (218), which are required to make valid conclusions on the stereoisomer-receptor interactions and the nature of receptor surfaces. The differences in the potencies of a pair of stereoisomers may be attributed to the differences in their affinities or intrinsic efficacies. For these reasons, the following information is necessary to make definite conclusions for delineating receptor surfaces using stereoisomer-receptor interactions (217): (1) the dissociation constant of agonists (K_A) and antagonists (K_B) acting at the same receptors, (2) the absolute configuration of the compounds, and (3) the interrelationships between the configurations of agonists and antagonists acting at the same receptors.

The dissociation constants of some cholinergic agonists and antagonists have been determined (Table 3.16). D(+)- and L(−)-lactoylcholines are agonists at muscarinic receptors and there is no significant difference in their intrinsic efficacies. The K_A of D(+)-lactoylcholine is lower than the K_A of the L(−) isomer at the muscarinic receptor. Therefore, the D(+) isomer has a higher affinity to the muscarinic receptor than that of the L(−) isomer (219). Mandeloyl- and tropinoylcholines are competitive antagonists of acetylcholine and lactoylcholine at the muscarinic receptors (220–222). Among these anticholinergics, the D isomer has a higher affinity ($1/K_B$) than that of the corresponding L isomer. The above anticholinergics did not exhibit significant intrinsic efficacies at the muscarinic receptors. The carbon *a* to the carbonyl carbon of the ester group is asymmetric in agonists (lactoylcholines) and their competitive antagonists (mandeloylcholines and tropinoylcholines). Therefore, the D isomers have the preferred relative configuration that comes into definite spatial position with the muscarinic receptor. Similarly, D(−)-hyoscamine has a higher affinity to the muscarinic receptor than does the L(+) isomer and has the preferred configuration (223).

5.8 Compounds with Dual Action: Cholinergic and Anticholinergic Activities

In several groups of atropine-like agents, derived from acetylcholine-like compounds, agonist activity is replaced by partial agonist activity and eventually antagonist activity with increasing substitution (224–227). For example, a transition between cholinergic and anticholinergic properties occurs when the acyl group of acetylcholine is progressively lengthened. Cholinergic activity decreases from formylcholine to butyrylcholine, and the higher members of the series are anticholinergics (Table 3.17). It has been demonstrated that hyoscyamine and atropine at small dose levels exhibit cholinergic properties (228, 229).

Table 3.16 Dissociation Constants and Intrinsic Efficacies of Cholinergic and Anticholinergic Agents

Cholinergic or Anticholinergic and Configuration	Activity: Type of Receptor[a] (Test System)	Activity: Dissociation Constant[b] (K_A or K_B)	Activity: Relative Intrinsic Efficacy	Ref.
Acetylcholine	Muscarinic	1.08×10^{-6}	1.00	219
	Nicotinic	2.17×10^{-6}	1.00	219
(R)-D-(+)-Lactoylcholine	Muscarinic	7.3×10^{-5}	0.52	219
	Nicotinic	1.85×10^{-5}	1.07	219
(S)-L-(−)-Lactoylcholine	Muscarinic	3.02×10^{-4}	0.30	219
	Nicotinic	8.08×10^{-5}	1.15	219
(R)-D-(−)-Acetyl-β-methylcholine	Muscarinic	Inactive	—	224
(S)-L-(+)-Acetyl-β-methylcholine	Muscarinic (active isomer)	1.24×10^{-6}	—	224
(R)-D-(−)-Mandeloylcholine	Muscarinic	3.00×10^{-6}	NS[c]	220,221
(S)-L-(+)-Mandeloylcholine	Muscarinic	5.22×10^{-6}	NS	220,221
(S)-D-(−)-Tropinoylcholine	Muscarinic	2.15×10^{-8}	NS	220,222
(R)-L-(+)-Tropinoylcholine	Muscarinic	3.26×10^{-7}	NS	220,222
(S)-D-(−)-Hyoscyamine	Muscarinic	4.47×10^{-10}	NS	223
(R)-L-(+)-Hyoscyamine	Muscarinic	1.41×10^{-8}	NS	223

[a]Muscarinic activities are tested on the guinea pig longitudinal ileal muscle in all cases except acetyl-β-methylcholine, which is tested on the circular muscle from fundus of rabbit stomach. Nicotinic activities are tested on the frog rectus abdominis muscle.

[b]Moles/liter.

[c]Not significant.

6 INTERACTION OF ANTICHOLINERGICS AT THE MUSCARINIC RECEPTORS

It is generally accepted that acetylcholine and atropine interact with the same postganglionic muscarinic receptors. Whereas acetylcholine stimulates these receptors, atropine blocks them. Although considerable progress has been made in understanding these interactions of stimulant and blocking drugs, some aspects of drug-receptor interaction are not clear. For detailed discussions of cholinergic

Table 3.17 Cholinergic and Anticholinergic Activities of Choline Esters

$$RCO_2CH_2CH_2\overset{+}{N}(CH_3)_3 \cdot I^-$$

R	Intrinsic Activity α	pD_2	pA_2	Test System	Ref.
H	1	5.2		Rat: intestine	225
CH_3	1	7.0		Rat: intestine	
CH_3CH_2	1	5.3		Rat: intestine	
$CH_3(CH_2)_2$	0.5	5.1		Rat: intestine	
$CH_3(CH_2)_3$	0	—	4.7	Rat: intestine	
$CH_3(CH_2)_5$	—	—	4.7	Guinea pig: ileum	227
$CH_3(CH_2)_6$	—	—	4.7	Guinea pig: ileum	
$CH_3(CH_2)_7$	—	—	5.0	Guinea pig: ileum	
$CH_3(CH_2)_8$	—	—	5.5	Guinea pig: ileum	
$CH_3(CH_2)_9$	—	—	6.0	Guinea pig: ileum	
$CH_3(CH_2)_{10}$	—	—	6.5	Guinea pig: ileum	
	0		5.4	Rat: intestine	225

and anticholinergic drugs at the muscarinic receptors see original papers and reviews on the subject (230–236).

6.1 Kinetic Basis for the Mechanism of Action of Anticholinergics

The major action of a number of anticholinergics is a competitive antagonism to acetylcholine and other cholinergic agents. The antagonism can therefore be overcome by increasing the concentration of acetylcholine at receptor sites of the effector organs. Thus anticholinesterases partially reverse the antagonism of anticholinergics by sparing acetylcholine at the receptor sites. The anticholinergics can inhibit all muscarinic actions of acetylcholine and other choline esters. Responses to postganglionic cholinergic nerve stimulation may also be inhibited, but less readily than responses to administered choline esters. The differences in the ability of anticholinergics to block the effects of exogenous choline esters and the effects of endogenous acetylcholine liberated by the postganglionic parasympathetic nerves may result from the release of the chemical transmitter by the nerve at the receptors in relatively inaccessible sites where diffusion limits the concentration of the antagonist.

6.2 Specificity of Antagonism

Atropine is a highly selective antagonist of acetylcholine, muscarine, and other cholinergic agents on the smooth and cardiac muscles and glands. This antagonism is so selective for cholinergic agents that atropine blockade of the actions of other types of drugs has been taken as evidence for their actions through cholinergic mechanisms. For example, the smooth muscle of guinea pig ileum is stimulated by muscarine, 5-hydroxytryptamine, histamine, and barium chloride. Atropine is more specific in blocking the stimulant effects of muscarine and acetylcholine at lower dose levels than those of the other three stimulant agents.

6.3 Molecular Basis for the Interaction of Acetylcholine and Anticholinergics at the Muscarinic Receptors

Structure-activity relationships among muscarinic agents (or cholinergics) indicate the existence on the receptor of two active sites separated by a distance 3.2 ± 0.2 Å (219, 237–239). One of them is an anionic site with which the quaternary ammonium group interacts to induce stimulant or blocking actions. The ether oxygen of muscarine and the ester oxygen of acetylcholine interact with the second site. There are some similarities between the active sites on acetylcholinesterase and the muscarinic receptor. The amine portion of anticholinergics interacts at the same anionic site as the quaternary group of acetylcholine and atropine. Several facets of acetylcholine-atropine antagonism are well known:

1. One molecule of atropine blocks one molecule of acetylcholine. Atropine is a larger molecule than acetylcholine and either mechanically or electrostatically inactivates receptors engaged by it.
2. Atropine has greater affinity than acetylcholine for the receptor. Its intrinsic activity is not significant, whereas acetylcholine has high intrinsic activity. Substances with intermediate intrinsic activities behave either as cholinergics or as anticholinergics, depending on the nature of their influence on the receptor. Among such substances are partial agonists with "dual action"; cholinergic activity precedes the anticholinergic activity. The partial agonists can be detected in a homologous series by gradually proceeding from agonists to antagonists with increasing molecular weight.
3. Besides the cationic head, bulky cyclic groups are essential constituents of compounds with anticholinergic activity. It seems clear that the van der Waals or hydrophobid binding of the planar cyclic groups together with the binding of the amine group produce a strong drug-receptor complex, which effectively blocks the close approach of acetylcholine to the receptor.
4. Acetylcholine increases potassium efflux and causes depolarization of the membrane, both of which effects are blocked by atropine.
5. The receptor proteins on the membrane may undergo molecular disorientation during the interaction of acetylcholine with

Table 3.18 Mydriatic and Cycloplegic Activities of Anticholinergics in Man"

	Drug[b]	Strength of Solution, %	Mydriasis: Maximal, min	Mydriasis: Recovery, days	Cycloplegia: Maximal, h	Cycloplegia: Recovery, days
1	Atropine sulfate	1.0	30–40	7–10	1 3	8–12
2	Oxyphenonium bromide	1.0	3040	7–10	1 3	8–12
3	Scopolamine hydrobromide	0.5	20–30	3–5	½–1	1–2
4	Atropine methyl nitrate	1.0–5	30	2	1	2
5	Homatropine bromide	1.0	1030	¼ – 4	½–1 ½	½ -2
6	Cyclopentolate hydrochloride	0.5–1.0	30–60	1	½ -1	1
	Dibutoline sulfate	5.0–7.5	60	¼ – ½	1	¼ – ½
8	Tropicamide[c]	1.0	20–35	¼	½	2–6 h
9	Eucatropine hydrochloride	5–10	30	¼ – ½	None	—

"For details see Refs. 241–243. The values should be considered approximate.
[b]One instillation of one drop unless otherwise specified.
'Two drops at 5-min intervals.

the cholinergic receptor, and this change in the receptor proteins may be prevented by a suitable blocking agent (240). Given that five subtypes of muscarinic receptors have been isolated and their amino acid sequences have been determined, future investigations may reveal the molecular nature of interactions of anticholinergics with muscarinic receptors.

7 THERAPEUTIC USES OF ANTICHOLINERGICS

The chief use of most of the antispasmodic agents is as an adjunct in the management of the peptic ulcer; this group of drugs includes adiphenine, aminopentamide, amprotropine, dibutoline, diphemanil, glycopyrrolate, hexocyclium, homoatropine methylbromide, methscopolamine bromide, methscopolamine nitrate, oxphencyclimine, oxphenonium, penthienate, pipenzolate, piperidolate, pipethonate, propanthelin, tricyclamol, and trihexethyl (241). To this group of several M1 receptor antagonists that decrease acid secretion should be added pyrenzipine, which is a leading compound in this group of agents. The anticholinergic agents that are useful as adjuvants in the management of the functional disorders of the bowel (e.g., irritable colon, spastic colitis, ulcerative colitis, and diverticulitis) include dicyclomine, hexocyclium, mepenzolate, and valethamate.

The mydriatic and cycloplegic activities of anticholinergics in humans are listed in Table 3.18. Atropine is recommended in situations requiring complete and prolonged relaxation of the sphincter of iris and the ciliary muscle. Mydriatics, like cyclopentolate, eucatropine, and homatropine bromide, with a shorter duration of action, are usually preferred for measuring refractive errors because of the relative rapidity with which their cycloplegic effects are terminated.

Atropine and scopolamine are used for premedication before the administration of some inhalation anesthetics, to reduce excessive salivary and bronchial secretions. Atropine and related agents have been used in the treatment of renal colic and hyperhidrosis, and to control sweating that may aggravate certain dermatologic disorders. Atropine also may be used to counteract the toxicity of certain cholinergic drugs and anticholinesterase agents.

Certain drugs with anticholinergic effects are used for the symptomatic treatment of Parkinson's disease (paralysis agitans) and related syndromes of the extrapyramidal tracts. (Of the presently available drugs, none is useful in all cases of Parkinsonism.) Despite claims of superiority for newly introduced synthetic agents, none possesses outstanding efficacy and freedom from adverse side effects when compared clinically with atropine and scopolamine (241).

8 MOLECULAR BASIS FOR THE SIDE EFFECTS OF ANTICHOLINERGICS

The most widely used mode of approach in the design of anticholinergics is based on the use of tropine alkaloids as models of prototypes, from which congeners or homologs or analogs have been designed. Tropine alkaloids have many pharmacological activities and interact at many cholinergic sites. In drug design the main purpose is to increase one pharmacological action at one particular site of action while concomitantly suppressing other pharmacological activities at other sites. It is not always possible to abolish all pharmacological effects other than the desired activity by molecular modification. Though the desired activity is useful in its therapeutic applications, other pharmacological activities manifest themselves as side effects. For example, atropine, scopolamine, and cocaine are structurally related, each having atropine nucleus. They differ in some of their pharmacological activities. Atropine stimulates the CNS, scopolamine depresses the CNS, and cocaine is a local anesthetic and CNS stimulant.

By molecular modification, it has been possible to produce a series of anticholinergics having qualitative effects resembling those produced by parasympathectomy to a particular organ. Although these drugs exert specific therapeutic effects at one organ, they exert side effects at other organs. Recent developments on the design of anticholinergics selective for subtypes of muscarinic receptors and identification of subtypes of muscarinic receptors in a number of organs has partially provided a solution for this problem.

The untoward effects associated with the use of anticholinergics are manifestations of their pharmacological actions, and usually occur on excessive dosage. The effects include dryness of mouth, blurred vision, difficulty in urination, increased intraocular tension, tachycardia, and constipation. Most of these side effects are lessened when the quaternary anticholinergics are administered orally in the treatment of peptic ulcer because of low absorption into the systemic circulation. In the case of tertiary amines the central side effects of euphoria, dizziness, and delirium may be observed because the drugs can cross the blood-brain barrier.

Many synthetic quaternary ammonium compounds may block acetylcholine at ganglia at high doses. Ganglionic-blocking agents cause impotence as a side effect. High doses of methantheline may also cause impotence, an effect rarely produced by pure antimuscarinic drugs and indicating ganglionic blockade. Toxic doses of quaternary ammonium compounds (e.g., menthantheline, propantheline, and oxyphenonium) block acetylcholine at the somatic neuromuscular junction and paralyze respiration.

Adiphenine and amprotropine have local anesthetic activities, and anesthesia of the oral mucosa results when tablets of these drugs are chewed. It should be remembered that local anesthetic esters and amides exert their action by anticholinergic mechanisms, probably essentially at the nodes of Ranvier.

The central side effects have appeared among children even when cyclopentolate, tropicamide, and other anticholinergics are used as mydriatics. All anticholinergics increase intraocular pressure in most patients with simple glaucoma.

Some of the cyclic groups in anticholinergics are pharmacophoric moieties for other types of activities. For example, the compounds containing a phenothiazine nucleus exhibit central depressing and antihistaminic side effects. These side effects are of advantage in the treatment of Parkinson's syndrome. The side effects of certain drugs that result from their anticholinergic activities are prominent among some analgesics (e.g., meperidine), antihistamines (e.g., promethazine), psychosedatives (e.g., benactizine), and psychotomimetics (e.g., dexoxodrol).

9 PROFILE OF ANTICHOLINERGIC ACTIVITIES OF VARIOUS AGENTS

The relative anticholinergic activities of the well-known therapeutic compounds are listed in Table **3.19.** Although it is very difficult to justify collecting the results of a wide variety of experiments, it seems likely that the table gives some idea of their relative antisecretory,

antispasmodic, and mydriatic activities relative to atropine. Ratios less than unity indicate that they are more active than atropine.

Atropine itself is a very active substance in all three types of activities. (−)-Methscopolamine seems to be the most active of all compounds; it is about five times as active as atropine. None of the synthetic compounds is more active than (−)-methscopolamine, and very few of them are more active than atropine. The compounds with high antisecretory activities also exhibit some degree of antispasmodic and mydriatic activities. Therefore, there is no complete dissociation between the three types of anticholinergic activities. Compounds with only one type of anticholinergic activity have yet to be synthesized. Only among very weak compounds is there any dissociation between the antispasmodic and the mydriatic activities (e.g., propivane). However, this difference may be related to the mode of administration. Mydriatic activities are measured after instillation into the eye, whereas antispasmodic and antisecretory activities are measured after parenteral administration to the animal or on in vitro preparations. To establish a claim that one compound has only one type of anticholinergic activity, two types of data should be available: (1) all types of activities should be measured in the same animal after the drug is administered by the same route; (2) the exact concentrations of the drugs at the sites of their action should be known. Such information is not available for most compounds in published literature.

For their antispasmodic and antisecretory activities in humans, the drugs are administered orally. A comparison of their oral doses (micromoles) indicates that atropine is the most active compound. In clinical experience all three types of anticholinergic activities are exhibited by all compounds. The principal advantage of the available quaternary ammonium compounds lies in the fact that they are relatively free of any of the CNS effects that may be seen with atropine. This may permit the administration of sufficient quantities of the compounds to achieve a more fully effective peripheral anticholinergic action.

Studies on the effectiveness of anticholinergic agents in the ulcer disease complicated by Helicobacter pylori infection are hampered by the lack of suitable animal models (262). Marchetti et al (263) have developed a mouse model of H. pylori infection that mimics human disease. The pathogenesis of H. *pylori* infection in *vivo* was investigated using fresh clinical isolates of bacteria to colonize the stomachs of mice. The gastric pathology resembling human disease was observed with cytotoxin-producingstrains but not with noncytotoxin strains. Oral immunization with purified H. pylori antigens protected mice from bacterial infection. This model will be useful for the development of therapeutic regimens involving vaccines against H. pylori and anticholinergic agents.

10 NONANTICHOLINERGICS AS ANTIULCER AGENTS

The interplay of various neuronal, hormonal, and other factors in gastric acid secretion are shown in Fig. **3.1.** Pharmacological agents can be used to decrease gastric acid secretion by their action at different sites. So far, the principal medications other than anticholinergics and antiacids to treat peptic ulcer are limited. Experimental and clinical investigations are in progress on a number of agents that can decrease the volume and acidity of gastric secretion through mechanisms other than blockade of the cholinergic nervous system (264–267). These include (1) histamine H2-receptor antagonists (266), (2) gastrin inhibitors (265), (3) pepsin inactivators (265), (4) mucus producers (69), (5) prostaglandin analogs (69), (6) enterogastone and its analogs (265, 267), (7) noncholinergic antispasmodics (69, 264), and (8) gastric H^+/K^+-ATPase inhibitors.

Histamine H2 receptor antagonists are popular for the treatment of peptic ulcer (266). A single dose of cimitidine, an H2 receptor antagonist, has a maximum effect on nocturnal acid output in humans, and no further effect is obtained by adding poldine, an anticholinergic agent, to cimitidine (266c). Cimitidine is also

Table 3.19 Relative Activities of Anticholinergics[a]

No.	Drug[b]	Equipotent Molar Ratios Relative to Atropine[c]			Total Dose Per Day in Humans[e]		Ref.
		Antisecretory[d]	Antispasmodic[e]	Mydriatic	mg	μmol	
		Solanaceous Alkaloids and Semisynthetic Substitutes					
1	Atropine sulfate	1.0	1.0	1.0	0.8–2.0	2.3–5.8	
2	Methylatropine	0.48c 3.02r,a	0.47g	0.44m			208
3	(−)-Hyoscyamine	0.56c	0.31g	0.54m			
4	(+)-Hyoscyamine	11.0c	10.0g				
5	(−)-Methylhyoscyamine	0.25c	—	0.20m			
6	(−)-Scopolamine	0.73c	1.3g	0.20m			
7	(−)-Methscopolamine	0.29c	0.17g	0.21m	5–10	13–25	
8	(±)-Homatropine	0.44r,a 30d	8.5g	7.7c			244
9	(±)-Methylhomatropine	1.8rb	7.2rb	2.4m			245
		Synthetic Anticholinergics: Esters, Quaternary					
10	Glycopyrrolate	0.9r,a	1.0g	1.0m	2–6	5–15	246
11	Lachesine	0.39c	0.96rb	0.96m			119
12	Mepenzolate	—	—	—	100	238	
13	Methantheline	0.37c	0.48g	3.0m	400	952	247
14	Methyleucatropine	1.6c	—	36m			185
15	Oxyphenonium	1.0rb 1.0d,a	1.0g	1.0rb	40	93	248, 249
16	Penthionate	0.26rb	0.39rb		20–40	48–9	122
17	Pipenzolate		1.0g		20–25	46–58	250
18	Poldine methyl sulfate	1.0c	1.0g	1.0m	8–48	18–106	251
19	Propantheline	0.26c	0.40g	1.6m	75–240	167–536	247
20	Valethamate				30–80	78–207	
		Esters, Tertiary					
21	Adiphenine		42rb		300–600	865–1729	252
22	Amprotropine phosphate	20rb	55rb	20	200–400	494–988	252
23	Benactyzine	5.6c	3.5rb	17m	3–9	8.3–24.8	119
24	Carbofluorene	100d	7.5rb		500	1449	253
25	Cyclopentolate	—	—	—			
26	Dicyclomine	60r,a	8.0rb	8.8r	60–80	173–231	196

27	**Eucatropine**	29c		250m			119, 185
28	**Oxyphencyclimine**				20–50	53–132	
29	**Piperidolate**				200	557	
30	**Propivane**	40rb	29rb	5000c	400	1274	252 253
			Thioesters, Tertiary				
31	**Triphenamil**		6.0rb		800	2204	254
			Carbamates, Quaternary				
32	**Dibutoline sulfate**	10.8d,a 43h,a		7.7h	75–100	233–311	255
			Main Chain: Alkane, Quaternary				
33	**Hexocyclium methyl sulfate**				100	234	
34	**Isopropamide iodide**		0.85rb		10	21	256
35	**Mepiperphenidol**	0.37c	0.48g		200–500	541–1351	257, 258
36	**Tricyclamol chloride**		1.2g	2.3m	200–300	593–890	209
37	**Trihexethyl chloride**	1.0r,a	2.17rb		75–200	212–567	259
			Main Chain: Alkane, Tertiary				
38	**Aminopentamide**		2.1rb		2	6.8	260
39	**Benzhexol**		3.7g	16m			209
40	**Procyclidine**		22g	31m	20	62	
41	**Methixene**				3–6	8–16	
			Main Chain: Alkene, Quaternary				
42	**Diphemanil methyl sulfate**	0.74	8.0g		400–600	1026–1538	261

[a]No comparative studies of all anticholinergics in the same animal species or on the same test system are available. The above data were assembled or cross-calculated from information reported in a number of sources; therefore the activities are relative and approximate. However, the information is useful to compare the available anticholinergic agents.

[b]All quaternary salts are bromides, and all tertiary amines are listed as hydrochlorides unless otherwise specified.

[c]The compounds were tested in different species. The following abbreviations are used to indicate the species: c, cat; d, dog; h, human; g, guinea pig; m, mouse; r, rat; rb, rabbit. Values less than unity indicate that they are more active than atropine.

[d]Antisecretory activities are on salivation unless otherwise indicated. "a" after the species indicates inhibition of acid secretion.

[e]All antispasmodic activities are inhibition of the contraction of intestine using cholinomimetics as spasmogen.

[f]The total dose includes the initial dose, as well as maintenance dose used orally (except dibutoline, which is administered subcutaneously) in man.

an effective drug in healing gastric and duodenal ulcers. Anticholinergic drugs and antacids help to control symptoms, but they do not accelerate healing. Promethazine, an antihistaminic inhibits the release of gastrin in the dog and humans (265).

Pepsin inhibitors, sulfated amylopectin (Depepsin),and carrageenin decrease acid secretion in experimental animals and protect animals against histamine-induced ulcers (265). They have to be studied further adequately to ascertain their therapeutic usefulness.

Carbenoxolone and cimetidine are complementary in their contribution to the healing of peptic ulcers, and the use of both may be better than either singly for some patients. Carbenoxolone accelerates healing by helping the defense mechanisms of the body. It stimulates extramucus secretion and prolongs cell life in the gastric epithelium. Cimetidine reduces gastric acid secretion. It would be interesting to know whether anticholinergic drugs are more effective for the treatment of peptic ulcer in the presence of carbenoxolone.

Carbenoxolone (Biogastrone, Duogastrone) is the disodium salt of glycyrrhetinic acid hemisuccinate. It is prepared by hydrolysis of glycyrrhizic acid, a glycoside in licorice root. It increases the secretion of mucus and accelerates the healing of gastric ulcers. This drug is now under clinical investigation in the United States.

Pharmacological doses of several prostaglandins and their analongs inhibit gastric acid secretion. 15-(*R*)-Methyl-PGE_2, in small doses (100–200 μg, oral), reduces gastric acid secretion and output in humans and animals, and it is currently being studied in the treatment of peptic ulcer.

Among the nonanticholinergic antispasmodics, alverine citrate (Spacolin) and isometheptene (octin) hydrocholoride or mucate are available on the market. They relax smooth muscle by nonspecific actions. They exert little effect on gastric acid secretion. They are most useful in the symptomatic treatment of gastrointestinal disorders characterized by hypermotility and spasm.

11 ANTICHOLINERGICS DEVELOPED FOR SPECIFIC USES

Ipratropium (Atrovent) was first developed for the symptomatic treatment of obstructive (bronchospastic) disease (268). It is a derivative of atropine. Two chemical features distinguish it from atropine: *(1)* an isopropyl group replaces the N-methyl group of the atropine component; *(2)*the methyl quaternary group (as the second substituent on the N-atom) points toward the pyrrolidine part of the ring system and occupies an equatorial position.

These structural features allow the Ipratropium molecule to attain a relatively selective bronchodilator effect on the cholinergically innervated airways. By the inhalation route of application, this poorly absorbable quaternary ammonium derivative becomes selective to primarily affect the airway smooth muscle. Another advantage of ipratropium over atropine is that, in contract to the latter, it does not suppress mucociliary function.

A novel series of *N,N'*-disubstituted 6,7-diazabicyclo-(3,2,2)-nonane derivatives were synthesized and pharmacologically tested (268). Compounds belonging to the 3-α configuration were more potent than those of the 3-β configuration. The quaternary ammonium derivatives were also more potent than the tertiary amines in this series. Ba 679 Br, tiotropium bromide, a new azoniatricyclo nonare derivative has shown potent bronchodilator activity that significantly outlasts that of ipratropium (269–271). This special feature and its selectivity can be explained by its slower dissociation rate from M1 and M3 than from M2 muscarinic receptors. In patients with chronic obstructive pulmonary disease (COPD) tiotropium is significantly more effective than ipratropium. Several studies indicate that tiotropium is very useful for long-term maintenance treatment of patients with airflow obstruction resulting from COPD.

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CHAPTER FOUR

CNS Stimulants

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Contents

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1 INTRODUCTION

Natural products that have stimulant properties have been known for millennia, and their active species (including ephedrine and cocaine) are now well known. Stimulants, also called psychostimulants, are drugs that lead to increased arousal, improved performance on tasks of vigilance and alertness, and a sense of self-confidence and well-being. High doses can produce feelings of elation or euphoria. Because of their ability to produce elation and euphoria, the stimulants have reinforcing properties and can lead to dependency. That is, because they make users feel "good," they are sometimes taken for extended periods of time in an attempt to maintain an elevated mood.

Tolerance develops to the mood-elevating properties of psychostimulants, however, and more and more of the drug must be taken to maintain the effect. Increased doses also prevent sleep, and continued use can result in symptoms of psychosis. Cessation of the drug after one of these binges may lead to an emotional and physical "crash" (the result of poor nutrition, lack of sleep, and increased physical activity), and severely depressed mood. In dependent individuals, craving for the drug occurs, resulting in another period of extensive drug use with the subsequent crash. This cycle is repeated in chronic psychostimulant dependency.

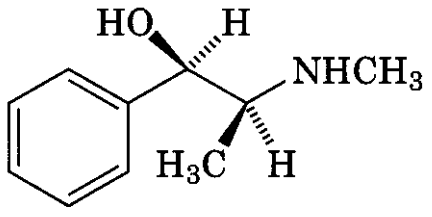
Many central nervous system (CNS) stimulants also have appetite-suppressant effects that led to their use in treating obesity. In short-term studies, amphetamine-like drugs have been shown to be more effective than placebo in promoting weight loss. Long-term (>20 weeks) weight loss has not been shown, however, unless the drug is taken continuously (1)At one time, stimulants were widely prescribed for appetite control, but they lose efficacy rather quickly through the development of tolerance. Thus, it was not uncommon for patients to become dependent on them, with symptoms of withdrawal upon abrupt cessation. The increased awareness of the addictive potential of stimulants, coupled with their widespread abuse, has led to much more extensive restrictions over their availability. These drugs are much more carefully controlled today, and are rarely used for weight control except in a few special instances.

There remain some important medical uses for this class of drug, yet as noted later, the therapeutic actions of psychostimulants must be balanced against their undesirable actions. Issues of dependency, tolerance, and potential abuse must be considered when deciding whether treatment with a psychostimulant is an appropriate therapy. On the other hand, new generations of drugs that have sprung from an understanding of the classic stimulants may open important therapeutic horizons for the future. Table 4.1 catalogs various data on psychostimulant and anorexigenic preparations.

1.1 Ephedra and Khat

The Chinese drug ma huang (Ephedra sinica Stapf) has been used in China for more than 5000 years. The alkaloid that is responsible for the CNS stimulant effects is ephedrine. The levorotatory erythro isomer (1)is the most active of the four possible stereoisomers with that structural formula. Khat (kat, or qat) or Abyssinian tea (*Catha* edulis Forskal) is the product from a small tree or shrub indigenous to tropical East Africa. Khat leaves are chewed habitually by peoples in East Africa and certain other Arabian countries, and produce a mild CNS stimulant effect (2). The principal

active component in Khat is a substituted phenethylamine derivative known as (−)-cathinone (2) (3).



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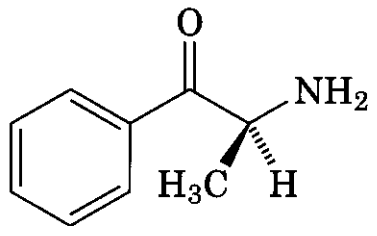
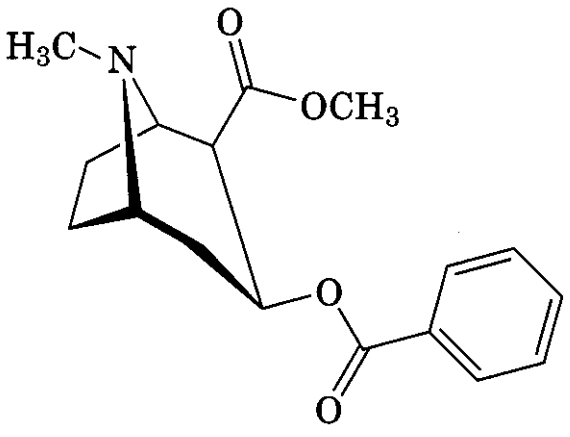


(2)

Both of these compounds possess a β-phenethylamine framework, a common structural theme that occurs in many related CNS stimulants. In general, these compounds have similar mechanisms of action.

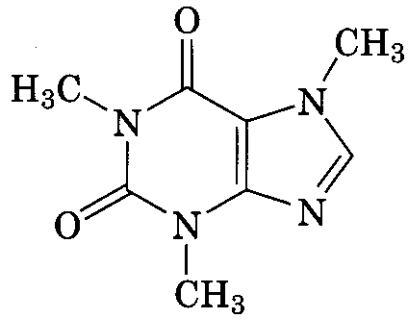
(−)-Cocaine (3) has a completely different structure and, as we shall see later, its mechanism of action is also somewhat different from the structurally simpler (1) and (2). Nevertheless, all of these natural prototype CNS stimulants have the common action of exerting powerful effects on brain pathways that utilize dopamine as the neurotransmitter.



(3)

1.2 Caffeine

From an economic standpoint, the most important CNS stimulant is caffeine (1,3,7-trimethylxanthine, 4). It occurs naturally and is a product of kola (cola) nuts (Cola nitida, where it occurs to the extent of about 3.5%, by weight), of coffee beans (Coffea arabica, where it constitutes about 1–2% by weight), and tea (Camellia sinesis, where it makes up 1–4% of the mass of dried leaves). The annual consumption of caffeine has been estimated at 120 million kilograms, the approximate equivalent of one caffeine-containing beverage per day for each of the world's six billion plus inhabitants. As a beverage, the worldwide consumption of tea is surpassed only by water. The structurally related dimethylxanthines, theophylline (5) and theobromine (6), have less of a CNS stimulant effect, and are principally important for their ability to relax smooth muscle. Cocoa and chocolate have little caffeine, but do contain theobromine.



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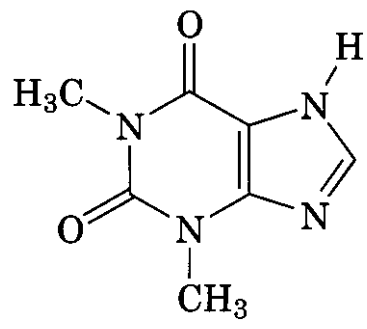

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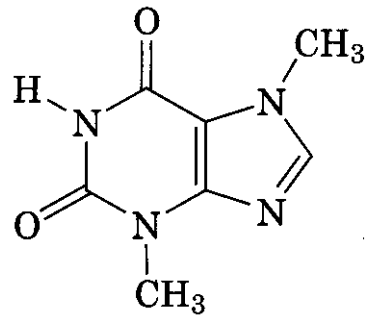


(6)

A regular cup of coffee contains between 40 and 176 mg of caffeine, with a mean content of about 85 mg. Tea contains less caffeine, with

Table 4.1 **Psychostimulant** and **Anorexigenic Preparations**

Generic Name (structure)	Trade Name	Originator	Chemical Class	Dose[a] (mg/day)
Psychostimulants				
Cocaine HCl (3)	Cocaine HCl powder	Mallinckrodt	Ecgonine methyl ester benzoate	NA
Amphetamine (7)	Adderall	Shire Richwood	Phenethylamine	5–30 mg
Amphetamine sulfate (7)	Amphetamine sulfate	Lannett		5 mg
Dextroamphetamine sulfate (10)	Dextroamphetamine sulfate	Various		5–10 mg
	Dexedrine	SmithKline-Beecham		
	Dextrostat	Richwood		
Methamphetamine HCl (11)	Desoxyn	Abbott		5 mg
	Desoxyn Gradumet	Abbott		5–10 mg
Methylphenidate HCl (8)	Methylphenidate HCl	Various	α-Phenyl-2-piperidineacetic acid methyl ester	5–20 mg
	Ritalin	Ciba-Geigy		
	Methylin	Mallinckrodt		
	Metadate ER	Medeva		10 mg, ER
	Concerta	Alza		18 mg, ER
Modafinil (44)	Provigil	Cephalon	Diphenylmethyl-sulfinyl-2-acetamide	100–200 mg
Pemoline (9)	Pemoline	Apothecan	2-Amino-5-phenyl-4(5*H*)oxazolone	18.75–75 mg
	Cylert	Abbott		
	PemADD	Mallinckrodt		
Caffeine (4)	Quick Pep; Caffedrine; NoDoz; Stay Awake; Vivarin; Stay Alert; Enerjets; Starbucks	Thompson; Thompson; Bristol-Myers; Major; SK-Beecham; Apothecary; Chilton	Trimethylxanthine	75–200 mg

Anorexiants				
Benzphetamine (**22**)	**Didrex**	**Upjohn**	Phenethylamine	25–50 mg
Diethylpropion (16)	Diethylpropion	various	Phenethylamine	25–75 mg
	Tenuate	Aventis	Phenethylamine	
Phendimetrazine (**21**)	Phendimetrazine	various	Phenethylamine	35 mg
	Bontril PDM	**Carnrick**		
	Plegine	Wyeth-Ayerst		
	Phendimetrazine	various		105 mg, SR
	Adipost	Jones		
	Bontril Slow-Release	Carnrick		
	Dital	**UAD**		
	Dyrexan-OD	**Trimen**		
	Melfiat-105 Unicelles	**Numark**		
	Prelu-2	Boehringer-Ingelheim		
	Rexigen Forte	ION Labs		
Phentermine (19)	Phentermine	Various	Phenethylamine	8–37.5 mg
	Ionamin	Medeva		
	Fastin	SmithKline-Beecham		
	Zantryl	Ion		
	Adipex-P	**Lemmon**		
	Obe-Nix 30-P	Holloway		
Decongestants and Bronchodilators				
Ephedrine sulfate (**1**)	**Pretz-D** 0.25% spray	Parnell	Phenethylamine	NA
	Ephedrine sulfate	West-Ward	Phenethylamine	25 mg
	Ephedrine sulfate	Various	Phenethylamine	50 mg/mL

[a] **Administered orally unless otherwise noted.**

an average of about 27 mg per cup, and an ounce of sweet chocolate typically contains between 75 and 150 mg of combined methylxanthines (4).

The reader should be aware that the use of the term CNS *stimulation* encompasses several physiological mechanisms of action and many different types of biologically active substances. A number of different agents, including caffeine (see below), affect these pharmacological mechanisms and cause CNS stimulation. Other diverse examples include strychnine (causing CNS stimulation by blockade of inhibitory glycine receptors) and benzodiazepine inverse agonists (causing CNS stimulation by decreasing the inhibitory effects of GABA on inhibitory chloride channels). It is not the intent of this chapter to provide an encyclopedic treatment of all the possible substances that can cause "CNS stimulation," but rather to focus primarily on the psychostimulants (i.e., drugs that affect brain monoaminergic systems).

2 HISTORY

The historical development of amphetamine and methamphetamine is described in interesting detail by Angrist and Sudilovsky (5). The discovery of psychostimulants differs somewhat from the usual drug discovery process because there was a long folkloric history of the use of khat, coca leaves, and *ma huang* (ephedra). Although there may not have been a formal pharmacological classification of CNS stimulants at that time, the ability of these agents to alleviate fatigue was well recognized.

Amphetamine itself was synthesized in 1887 and first studied as early as 1910, but its stimulant effects were not discovered until about 1930. Amphetamine was independently resynthesized in 1927 by the noted psychopharmacologist Gordon Alles in a program to develop synthetic substitutes for ephedrine, a drug then being used as a bronchodilator for the treatment of asthma (6). The central stimulant effects of amphetamine were probably noted about 1930, when it appeared in nasal inhalers in Germany. The first medical use for amphetamine was in the treatment of narcolepsy (7) and, by 1936, orally active Benzedrine tablets were available without prescription (8). By 1937 it was being used recreationally by the general population, with particular popularity among American college students (9).

It is not clear when or by whom methamphetamine was first synthesized. Various accounts indicate its first preparation somewhere between 1888 and 1934 (5). In any case, Hauschild (10) published the first studies of the pharmacology of methamphetamine in 1938, characterized its stimulant effects in animals, and also carried out a self-experiment.

3 CLINICAL USE OF AGENTS

3.1 Therapeutic Applications

Psychostimulants generally increase the level of activity, alleviate fatigue, increase alertness, and elevate mood (or cause euphoria in high doses). Unfortunately, the ability to produce euphoria leads these compounds to have a high potential for abuse and dependency. The principal clinical indications for psychostimulants are in the treatment of attention deficit hyperactivity disorder (ADHD) and the sleep disorder known as narcolepsy. A less commonly recognized use, but one that' is gaining importance, is in the treatment of depression in terminal patients or the chronically ill (11–13). There is also need for psychostimulants in certain occupations (e.g., in the military), as a countermeasure to fatigue from irregular or prolonged work hours, where a high level of vigilance and alertness must be maintained (14, 15). Some specific clinical applications include the following.

3.1.1 Attention Deficit Hyperactivity Disorder (ADHD). ADHD is a diagnosis applied mostly to children, but one that persists into adulthood for many people. It is reflected in a persistent pattern of inattention and/or hyperactivity-impulsivity that is more frequent and severe than typically observed in individuals at a comparable level of development (16). Inattention prevents ADHD patients from keeping their mind on one thing and focusing their attention; they are easily bored with a task after only a short while. They have no

difficulty devoting attention to activities that they enjoy, but find it hard to focus conscious attention to organizing or completing a task, or learning something new. They may forget to plan ahead and tasks are rarely completed, or are filled with errors.

Children with ADHD (particularly of school age) have great difficulty being still, they may be in and out of their seats, and talk incessantly. The inability to focus makes learning tasks boring, and exacerbates the desire to move around and become involved in distractions. ADHD children may squirm, shake their legs, touch everything, or make distracting noises. Hyperactive teens and adults may feel intensely restless, and may try to do several things at once, going from one activity to the next. Impulsivity is another characteristic of ADHD, with patients often acting without thinking about the consequences. They may have difficulty curbing their immediate reactions to situations, making inappropriate remarks without thinking what they are saying. They find it hard to wait for things they want or to wait to take their turn.

In normal subjects, psychostimulants can increase activity and talkativeness, especially at higher doses. Paradoxically, in ADHD sufferers, stimulants appear to have a calming effect, and allow an increased focus and attention to tasks. Although appearing paradoxical, it is now believed that the decreases in activity in ADHD are secondary to improvements in attention. This beneficial effect of low doses of the stimulants has led to a large number of children being prescribed methylphenidate (Ritalin) or various amphetamine preparations for the treatment of ADHD. This, in turn, led to great concern about the fact that these drugs were overprescribed for ADHD, and that children who are merely highly energetic were routinely being given them for behavior management. The reader should be aware of this social issue, but it requires no further comment in the context of this chapter.

3.1.2 Narcolepsy. Narcolepsy is a condition that includes as its predominant symptom, excessive daytime sleepiness (EDS), persistent drowsiness, and daytime sleep attacks that may occur without warning and are often irresistible. Another hallmark symptom of narcolepsy is cataplexy, which is a sudden loss of voluntary muscle control, often triggered by emotions such as laughter or surprise. Cataplexy occurs more frequently during stress or fatigue. The attack may involve only a feeling of weakness and limp muscles or it may result in total muscular collapse, during which the person can appear unconscious, but actually remains awake and alert. Attacks may be very brief or may last for tens of minutes. Another characteristic symptom of narcolepsy is hypnagogic hallucinations. These are vivid, realistic, and often frightening, reminiscent of nightmares, and are usually accompanied by sleep paralysis, a temporary inability to move. Whereas the psychostimulants can have a beneficial effect, they are likely to be supplanted by newer drugs that are more specific and have fewer side effects.

3.1.3 Use for Depression in Terminal Illness. Although this indication for psychostimulants is not as widely recognized, agents such as amphetamine and methylphenidate are preferred because they do not suffer from the weeks-long delay in onset of action that is characteristic of traditional antidepressant medications. Thus, a rapid antidepressant response can be achieved in severely ill patients, who in some cases may not survive long enough for a traditional antidepressant medication to begin to have an effect (11, 17–19).

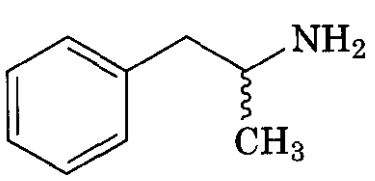
3.1.4 Use in Obesity. As noted earlier, many of the psychostimulants have also been used as anorectics (anorexics; anorexigenics), that is, as appetite suppressants. A few of them are still useful in this regard, but the high abuse potential of psychostimulants, coupled with the development of tolerance to their anorectic effects, has meant that prescribing psychostimulants for weight control has generally fallen into disfavor.

3.1.5 Apnea in Premature Infants. Apnea of prematurity (AOP) occurs in about 90% of premature neonates weighing less than 1 kg at birth, and in 25% of infants with a weight of less than 2.5 kg (20). The first-line pharmacological therapies for the management of AOP,

to stimulate respiration, are the methylxanthines, with theophylline (5) presently being most extensively used. Recent studies suggest, however, that caffeine (4) should be considered the drug of choice because of similar efficacy, longer half-life, fewer adverse effects, and better brain penetration than that of theophylline (21).

3.2 Side Effects, Adverse Effects, and Drug Interactions

Generally, psychostimulants like amphetamine (7) and methylphenidate (8) can be used safely with most classes of medications and with few contraindications (22). The acute adverse reactions to stimulants can generally be understood from the perspective of their pharmacology. Psychostimulants act as indirect sympathomimetic agents; they either directly release stored catecholamines, including those in peripheral adrenergic neurons responsible for vascular tone, or else block their reuptake. These actions affect the cardiovascular system in fairly predictable ways. In addition, cocaine produces a local anesthetic effect by the blockade of sodium channels (23). Although this would normally be the pharmacological basis for a class I antiarrhythmic drug, it paradoxically induces proarrhythmia (24).

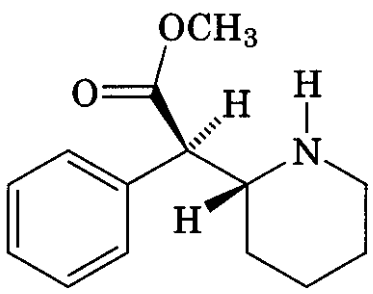


(7)

In addition to acute effects, however, prolonged usage of amphetamines (and other psychostimulants) can produce an "amphetamine psychosis." This syndrome was first clearly documented by Connell (25) and is regarded as very similar to paranoid schizophrenia, characterized by "paranoid psychosis with ideas of reference, delusions of persecution, auditory and visual hallucinations in a setting of clear consciousness" (25). The psychosis clears quickly after the drug is withdrawn. Psychosis has been induced experimentally in normal subjects by continuous amphetamine administration (26). Amphetamine psychosis has been recently discussed extensively by Angrist (27). Interestingly, psychostimulants can induce a psychotogenic response in schizophrenics, in doses that are subpsychotogenic in normal subjects, and methylphenidate was found to have greater potency in this regard (28). Activation of psychotic symptoms by methylphenidate was found to be a predictor of risk of relapse (29). These, and other studies, are all consistent with the dopamine hypothesis of schizophrenia.

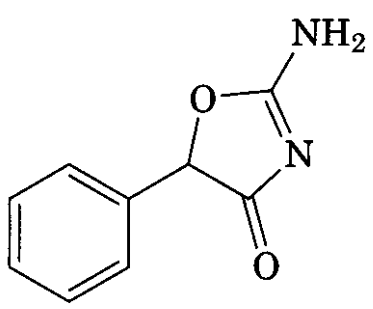
3.2.1 Methylphenidate. Methylphenidate (Ritalin, 8) is widely prescribed for the treatment of ADHD. Approximately 90% of children treated for ADHD are given methylphenidate (30), representing about 2.8% of all U.S. children aged 5–18 years (31). It is both well tolerated and efficacious in the treatment of attention deficit hyperactivity disorder, and is associated with few serious adverse effects (32). Although there are rare reports of drug interactions between methylphenidate and certain other drugs, they are so infrequent that there is no consistent pattern that can be identified. Toxic concerns with methylphenidate would principally revolve around the abuse of this drug to obtain a stimulant high, and the consequent possibility of developing dependency. A further concern with the long-term use of methylphenidate is the possibility that patients may be at increased risk for psychostimulant abuse. Although when taken orally methylphenidate has a low euphorigenic potential (33), when used intravenously it has an abuse pattern and symptoms of toxicity similar to those of cocaine and amphetamine (34). Recent studies in rats have also shown that animals treated with methylphenidate develop behavioral sensitization, suggesting that human users may have increased susceptibility to psychostimulant abuse (35).

3.2.2 Pemoline. Pemoline (9), an agent used in treatment of ADHD, has been associated with hepatotoxicity, with the majority of cases occurring in pediatric patients. From its marketing in 1975 up to 1989, 12 cases of acute hepatic failure and six deaths associated with pemoline hepatotoxicity had been reported to the FDA (36). Death generally oc-



(8)

curred within 4 weeks of the onset of signs and symptoms of liver failure. In two recent cases, pemoline-induced liver failure required liver transplantation (37).



(9)

Although the absolute number of reported cases is not large, the rate of reporting is 4–17 times higher than that expected in the general population. This estimate may be conservative because of underreporting and because the long latency between initiation of pemoline treatment and the occurrence of hepatic failure may limit recognition of the association. If only a portion of actual cases was recognized and reported, the risk could be substantially higher. By contrast, a meta-analysis of the literature by Shevell and Schreiber (38) suggests that the risk of acute hepatic failure may be an overestimate. Nevertheless, because of its association with life-threatening hepatic failure, pemoline should not ordinarily be considered as a first-line therapy for ADHD. In fact, pemoline has been withdrawn from the Canadian market as a result of this toxicity (39).

3.2.3 Cocaine. The coca plant is a small shrub or tree that is indigenous to South America, where for centuries the leaves have been chewed by the local native populations. The dried leaves of *Erythroxylum* coca Lamarck, or *E. truxillense* Rusby, commercially known as Huanuco coca, or Truxillo coca (4), respectively, serve as the raw material for the production of (−)-cocaine (3). The latter was first isolated in 1860 and became medically important as an excellent local anesthetic agent, but one that is a potent and highly addictive CNS stimulant. The acute toxicity of cocaine derives primarily from its intense sympathomimetic actions. In 1991 an attempt was made to assess the intrinsic toxicity of cocaine by computing the incidence of adverse health outcomes per population of drug abusers. The rates of emergency department visits and deaths were estimated at 15.1 and 0.5, respectively, per 1000 persons using drugs (40).

Cocaine can have marked effects on the heart and cardiovascular system. Adverse actions may include myocardial ischemia, cardiac arrhythmias, cardiotoxicity, hypertensive effects, cerebrovascular events, and a hypercoagulable state (24, 40). By 1997 more than 250 cases of myocardial infarction related to the recreational use of cocaine had been documented in the literature (41). Although less common, aortic dissection related to use of cocaine-free base ("crack cocaine") has also been documented (42). Seizures also can be associated with cocaine use (43).

Acutely, cocaine can cause anxiety or panic reactions. Used chronically, cocaine can induce a psychosis that closely resembles that produced by amphetamine. It is generally considered that amphetamine psychosis predominantly mimics the positive symptoms of schizophrenia, but in fact stimulant-induced psychosis can mimic a broad range of symptoms, including negative and bizarre symptoms (44). Paranoid behavior has been produced in experienced cocaine users by continuous (4 h) cocaine infusion (45).

In addition to these physiological toxicities, cocaine addicts suffer from a variety of social and economic problems that result in tremendous costs to society. Many of the estimated 1.5 million cocaine addicts in the United States (see http://www.nida.nih.gov), are underemployed, and if they are employed at all, they are likely to be involved in drug distribution activities, and typically perform marginal roles in the legal economic system (46, 47). Adults in such drug-using households rarely engage in conventional behaviors, and often

parent children by using conduct norms that are structured to produce individuals who have reduced chances to become conventional adults (48).

3.2.4 Caffeine. The psychostimulant action of caffeine generally is accepted as well established. Caffeine quickens reaction time and enhances vigilance, increases self-rated alertness, and improves mood. There is, however, little unequivocal evidence to show that regular caffeine use is likely to benefit substantially either mood or performance. Indeed, one of the significant factors motivating caffeine consumption appears to be "withdrawal relief" (49).

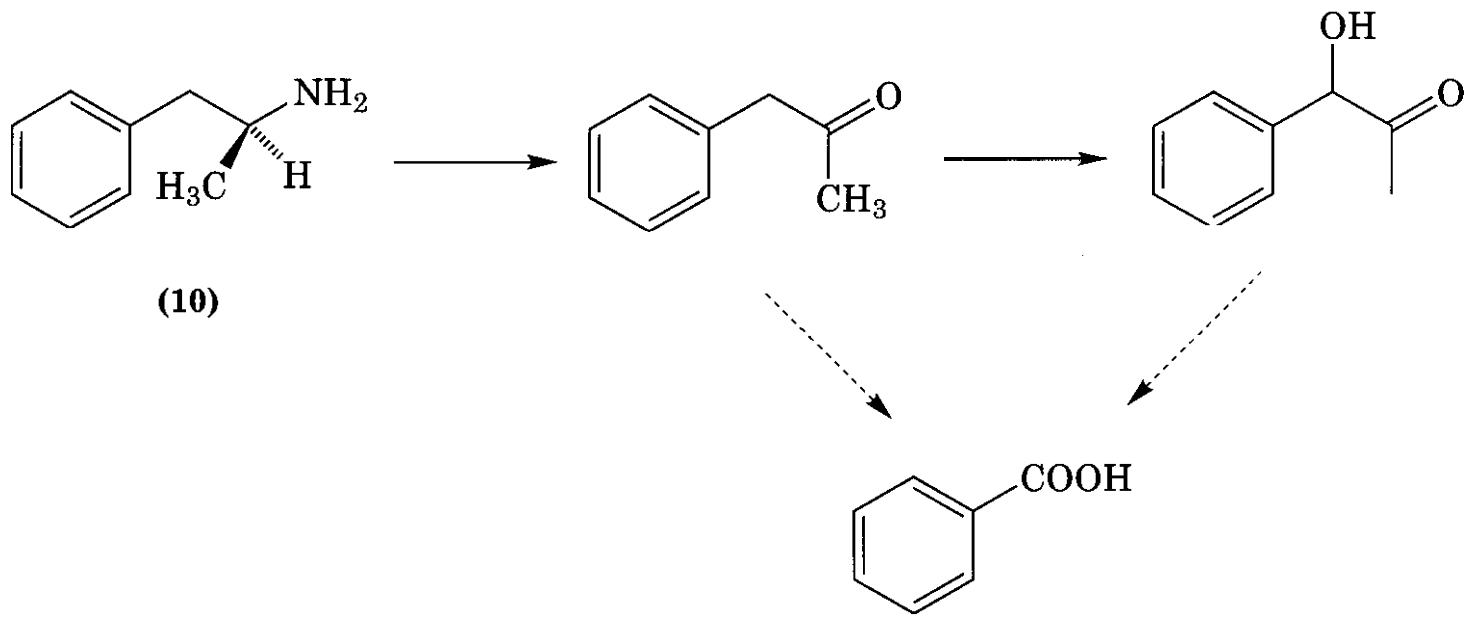
Caffeine can produce adverse and unpleasant effects if doses are increased. Caffeine has weak reinforcing properties, but with little or no evidence for upward dose adjustment, possibly because of the adverse effects of higher doses. Withdrawal symptoms, although relatively limited with respect to severity, do occur, and may contribute to continued caffeine consumption (50). Health hazards are small if any, and caffeine use is not associated with incapacitation (51). Acute intake of caffeine increases blood pressure, with the strongest pressor response in hypertensive subjects. Some studies with repeated administration of caffeine have shown a persistent pressor effect, whereas in others chronic caffeine ingestion did not increase blood pressure (52). Epidemiologic studies have produced contradictory findings regarding the association between blood pressure and coffee consumption. During regular use, tolerance to the cardiovascular responses develops in some people, and therefore no systematic elevation of blood pressure can be shown either in long-term or in population studies. Thus, regular caffeine consumption may be harmful to some hypertension-prone subjects (52). The hemodynamic effects of chronic coffee and caffeine consumption have not been sufficiently studied.

Finally, caffeine may provoke a panic attack in individuals who suffer from panic or anxiety disorders (53–56). Recently, an adenosine A_{2A} receptor knockout mouse has been developed that has behavioral symptoms that correspond to functional antagonism of this receptor, similar to the effects of caffeine (57).

3.3 Absorption, Distribution, Metabolism, and Elimination

All substituted amphetamines are strong organic bases, with pK_a values ranging from 9.5 to 10 (58). The pK_a of both cocaine and phenmetrazine is somewhat lower, at 8.5, and methylphenidate has a pK_a of 8.8 (58). Thus, these bases are all significantly protonated at physiological pH. Binding to their biological targets also probably occurs with the protonated species [e.g., (59)]. These drugs are all administered as their water-soluble salts, usually as hydrochlorides or sulfates. At physiological pH, of course, these bases exist in an equilibrium between the protonated ionized form and the unprotonated un-ionized species. The latter free bases are relatively lipid soluble and readily penetrate the brain, where they exert their CNS stimulant effects. Many of these drugs are eliminated in the urine unchanged because acidic urine leads to a higher fraction of protonated species, thus decreasing reabsorption of the unchanged drug in the renal tubules. Decreasing urinary pH by, for example, administering ammonium chloride leads to the anticipated increased urinary excretion and reduced duration of action (60). A comparison has been reported of the urinary excretion pattern of methamphetamine in humans, guinea pig, and rat (61). In humans, 23% of the dose was excreted unchanged. Ring-hydroxylated and N-demethylated metabolites were excreted as 18 and 14% of the dose, respectively.

3.3.1 Amphetamine Metabolism. The metabolism of (+)-amphetamine (10)is variable, depending on the species studied. Possible metabolic transformations involve hydroxylation at the a or β side-chain carbon atoms, the nitrogen atom, and the *para* position of the aromatic ring. These metabolites would then be further oxidized, or conjugated and excreted. One or more of these pathways predominates, depending on which animal species is being studied. In humans, the half-life of (+)-amphetamine has been reported as 7 h (62). About 30% of the dose of racemic amphetamine is excreted unchanged, and acidifi-



(10)

cation of the urine can decrease the half-life significantly (63, 64). In humans, the principal metabolite is benzoic acid (65). The details of the sequences of metabolic reactions of amphetamine that lead to benzoic acid have not been elucidated (62), but the β-hydroxylated metabolite, norephedrine, has also been identified as a metabolite in humans (66).

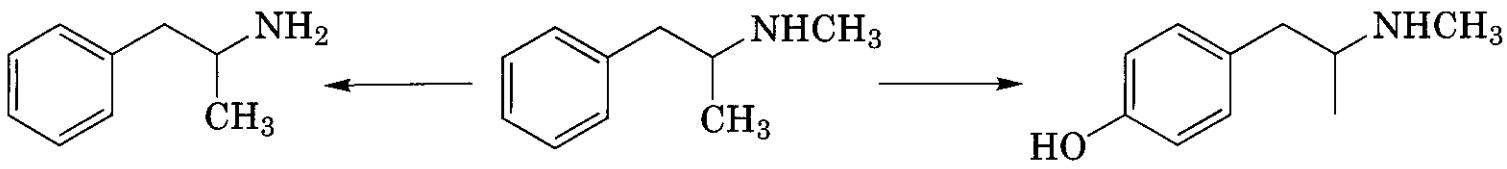
The metabolism of methamphetamine (11) involves both N-demethylation and ring hydroxylation. Caldwell et al. (61) reported that 23% of the administered dose was excreted as unchanged drug, 18% as the 4-hydroxylated compound, and 14% as the N-demethylated amphetamine.

3.3.2 Methylphenidate Metabolism. The metabolism and pharmacokinetics of methylphenidate have been studied extensively. Methylphenidate (8) is administered as the racemic threo isomer, but the (−)-threo enantiomer is more rapidly metabolized.

Methylphenidate is an ester, and the methyl ester is rapidly cleaved. The ester hydrolysis product, called ritalinic acid, comprises about 80% of the urinary metabolites after an orally administered dose (67). The lability of the ester function is probably the major factor limiting the oral bioavailability of methylphenidate to between 10% and 50% (68). Ritalinic acid is not pharmacologically active. A ring-hydroxylated ritalinic acid metabolite (2%) has also been identified. Other minor pathways involving oxidation of the piperidine ring (oxo-ritalin) and conjugation reactions represent less than about 1% of the administered dose (30).

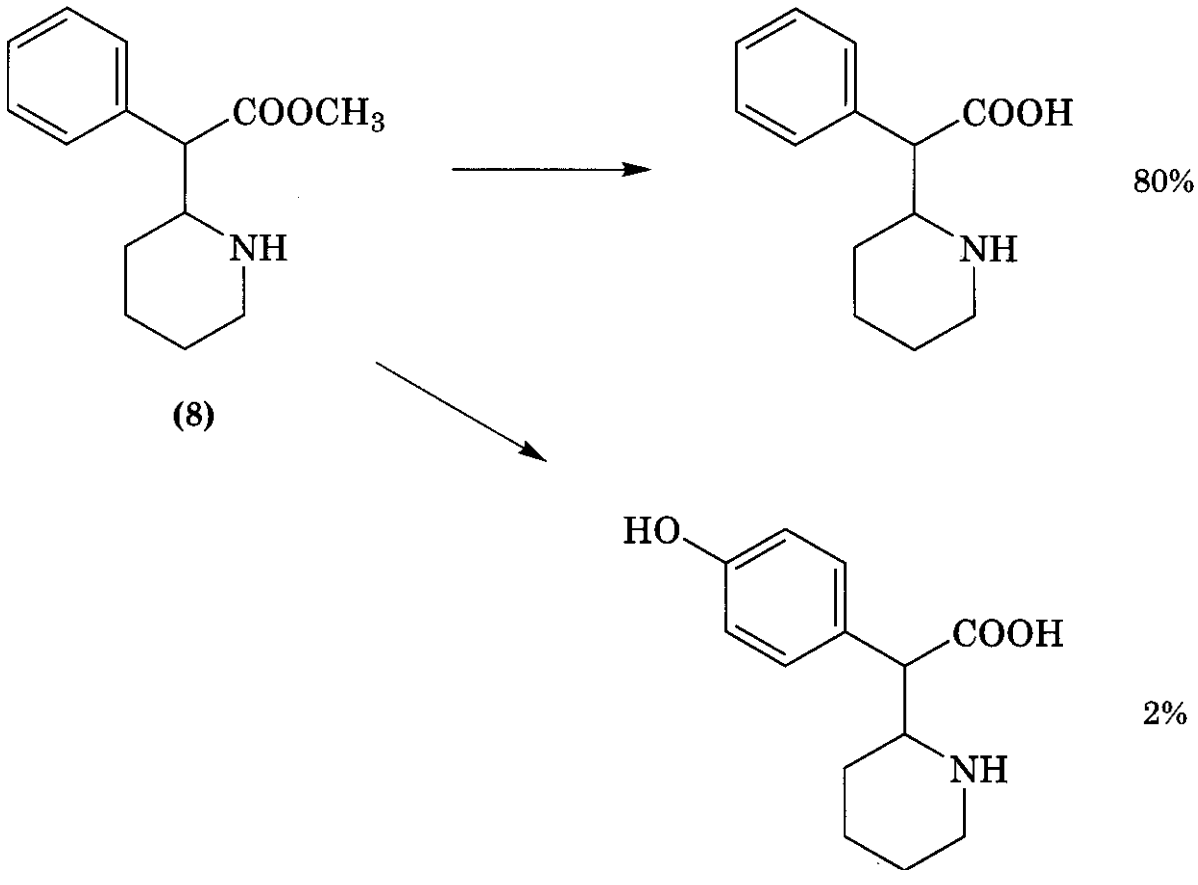
3.3.3 Cocaine Metabolism. Because cocaine (3) has two ester functions, both can be hydrolyzed *in vivo* to generate metabolites. Hydrolysis of the methyl ester leads to benzoylecgonine (12), and hydrolysis of the benzoyl ester leads to ecgonine methyl ester (13). Tropan-3β-ol-2β-carboxylic acid is known as ecgonine (14). In cocaine users who also consume significant amounts of ethanol, a transesterification product (cocaethylene, 15) is also detected. Cocaethylene is also a potent psychostimulant, with about four times higher potency as a local anesthetic than that of cocaine itself (69), and can enhance the cardiotoxicity associated with cocaine use.

3.3.4 Diethylpropion Metabolism. Diethylpropion (16) is used most extensively as an appetite suppressant. It possesses the core phenethylamine structure characteristic of

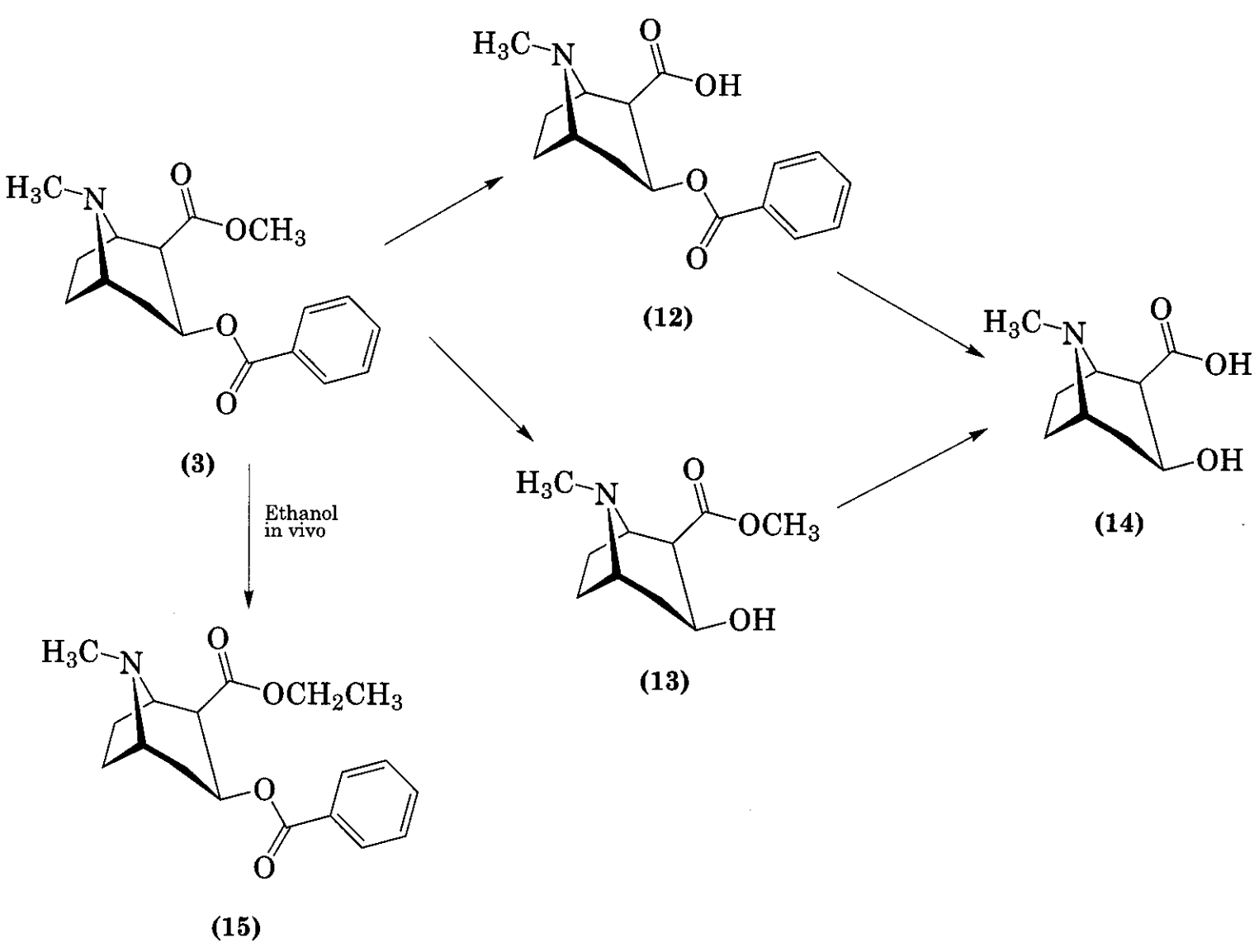


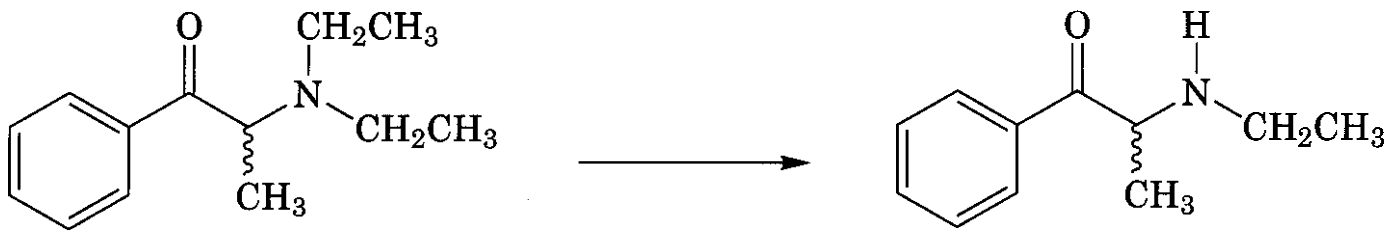
(11)

14% 23% 18%



many psychostimulants, but is a tertiary aminoketone. It is extensively metabolized in humans, with only about 3–4% of the drug excreted unchanged. a-Alkylaminoketones are apparently N-dealkylated readily and, thus, the principal metabolite is the N-deethylated compound (**17**), constituting about 35% of the administered dose. Reduction of the carbonyl





(16) (17)

is less important, with about *20%* of the dose going that route, to afford *N,N*-diethylnorephedrine. About *30%* of the dose cannot be accounted for as an amine product in the urine and is probably a deaminated metabolite (70). Studies by Yu et al. *(71)* found that the N-deethylated metabolite (**17**) was probably responsible for the pharmacological effects of diethylpropion (16). These workers reported that the N-monoethyl metabolite *(17)* was a substrate at the norepinephrine and serotonin transporters and an inhibitor at the dopamine uptake transporter, whereas (1*R*,2*S*) and (1*S*,2*R*)-*N*,*N*-diethylnorephedrine as well as diethylpropion itself were inactive in those assays.

4 PHYSIOLOGY AND PHARMACOLOGY

4.1 Where and How These Drugs Work

Neurons in the central nervous system communicate by chemical transmission. Of relevance to the present discussion are monoamine neurons that release dopamine, norepinephrine, or serotonin as one of their transmitters in response to an action potential. Reuptake transporter proteins embedded in the neuronal plasma membrane then clear the synapse of monoamines, typically taking up *70–80%* of the released transmitter. This reuptake is thought to be the major termination mechanism for the monoamine chemical signaling process.

All psychostimulants appear to elevate synaptic levels of dopamine and norepinephrine. In addition, cocaine and, to a lesser extent, some of the other agents also raise synaptic levels of serotonin. It is the current consensus that elevated dopamine levels lead to CNS stimulation and are responsible for the reinforcing properties of stimulants (72–78). Nevertheless, recent studies have begun to focus attention on glutamate systems as potential key components of the actions of psychostimulants. For example, Swanson et al. *(79)* have shown that repeated cocaine administration leads to long-term attenuation of group I metabotropic glutamate receptor function in the nucleus accumbens. In particular, this functional reduction was related to significantly reduced mGluR5 immunoreactivity in the medial nucleus accumbens. Even more exciting is the recent report that mGluR5 knockout mice do not display the reinforcing and locomotor effects of cocaine, in spite of the fact that cocaine administration increases extracellular dopamine in the nucleus accumbens of these mice to levels that do not differ from those of wild-type animals (80). In the near future, the role of glutamate systems in the actions of psychostimulants will no doubt be more fully elucidated, resulting in new approaches to the treatment of conditions that now respond to classical stimulants.

There are two principal mechanisms for increasing synaptic monoamine levels. One is to block the reuptake of neurotransmitter after its excitation-coupled release from the neuronal terminal. Thus, blocking the action of the uptake carrier protein prevents clearance of the neurotransmitter from the synapse, leaving high concentrations in the synaptic cleft that can continue to exert a signaling effect. This mechanism is the one invoked to explain the action of cocaine, a potent inhibitor of monoamine reuptake at the dopamine, serotonin, and norepinephrine transporters, and of methylphenidate, which is a reuptake inhibitor at the dopamine and norepinephrine transporters *(81)*. It should be noted, however, that methylphenidate also has the ability to induce the release of catecholamines stored in neuronal vesicles (82, 83).

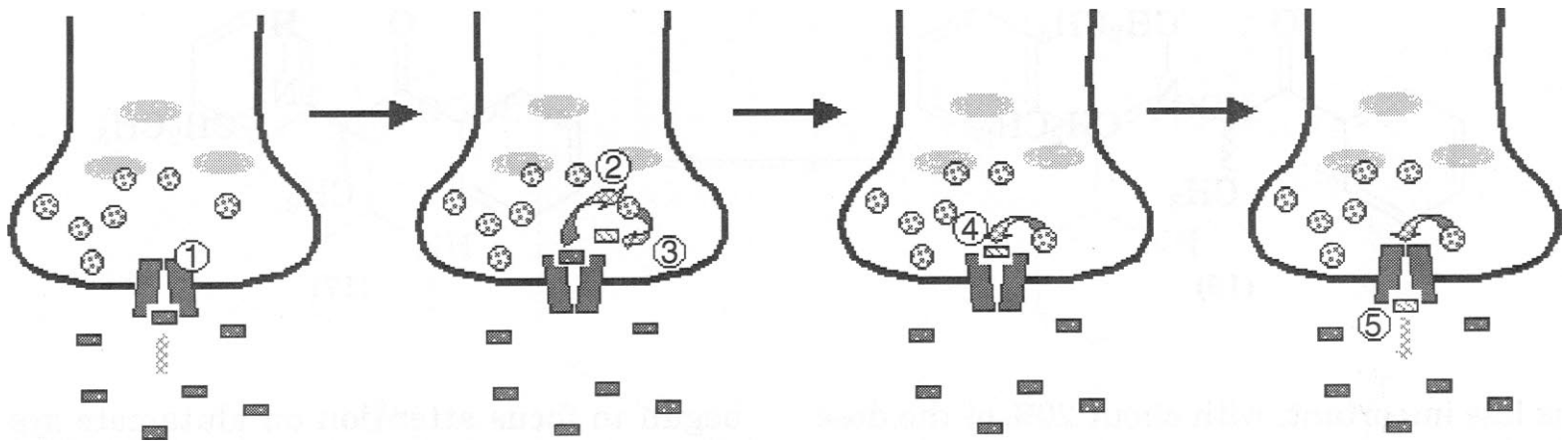


Figure 4.1. Amphetamine interacts with the dopamine transporter protein (1) and is transported inside. Na^+ and Cl^- are cotransported, and K^+ is countertransported in the process. After being transported inside the terminal, high concentrations of amphetamine can displace dopamine from vesicular storage sites (2), leading to elevated cytoplasmic levels of dopamine (3). After amphetamine dissociates on the intraneuronal surface, dopamine binds to the carrier (4). The carrier then transports dopamine to the extracellular face (5), driven by the favorable concentration gradient, where the dopamine dissociates and leaves the carrier available for another cycle.

The second mechanism is the one more relevant to the action of amphetamine and related agents. This mechanism is illustrated in Fig. 4.1. Amphetamine, and other small molecular weight compounds with similar structures, are substrates at the monoamine uptake carriers and are transported into the neuron. The uptake carrier has an extracellular and intracellular face, and after transporting a substrate (amphetamine, etc.) into the neuron, the intracellular carrier face can bind to dopamine and transport it back to the extracellular face. This exchange diffusion mechanism is calcium independent, and is capable of robustly increasing synaptic transmitter levels. This process is often described as a "reversal" of the normal uptake carrier process.

Whereas the CNS stimulant effects of these molecules depend on an action in the brain, uptake inhibitors and substrates at peripheral monoamine carrier sites can obviously exert other physiological effects. Cocaine is an excellent local anesthetic agent. Furthermore, its potent inhibition of norepinephrine reuptake leads to stimulation of a-adrenergic receptors, causing local vasoconstriction that delays the diffusion of the anesthetic agent out of the tissue. Similarly, users who chronically insufflate cocaine into their nasal passages often develop necrotic lesions as a result of the local vasoconstricting effect of cocaine, again arising from the blockade of norepinephrine reuptake. Not surprisingly, cocaine and amphetamines have effects on the cardiovascular system, by virtue of their ability to enhance indirect adrenergic transmission at peripheral sites. Knowledge of the physiology of the sympathetic nervous system and the functions of peripheral adrenergic nerve terminals allows a relatively straightforward prediction of the types of drug effects possessed by monoamine uptake inhibitors or releasing agents.

4.2 Biochemical Pharmacology: Receptor Types and Actions

The monoamine reuptake carrier proteins (targets of the psychostimulants) are members of a larger Na^+/Cl^- transporter family that includes a number of other proteins, including the GABA transporters, amino acid transporters, and orphan transporters (84). The primary amino acid sequence of the monoamine transporters is highly conserved, with several regions of these proteins having high homology. It is presently believed that all of the members of this family possess a membrane-spanning 12 α-helix motif, with a single large loop containing glycosylation sites on the external face of the membrane (Fig. 4.2). Members of this family of proteins have been identified not only in mammalian species, but also in eubacteria and archaebacteria, indicating their very early emergence in the evolution of life.

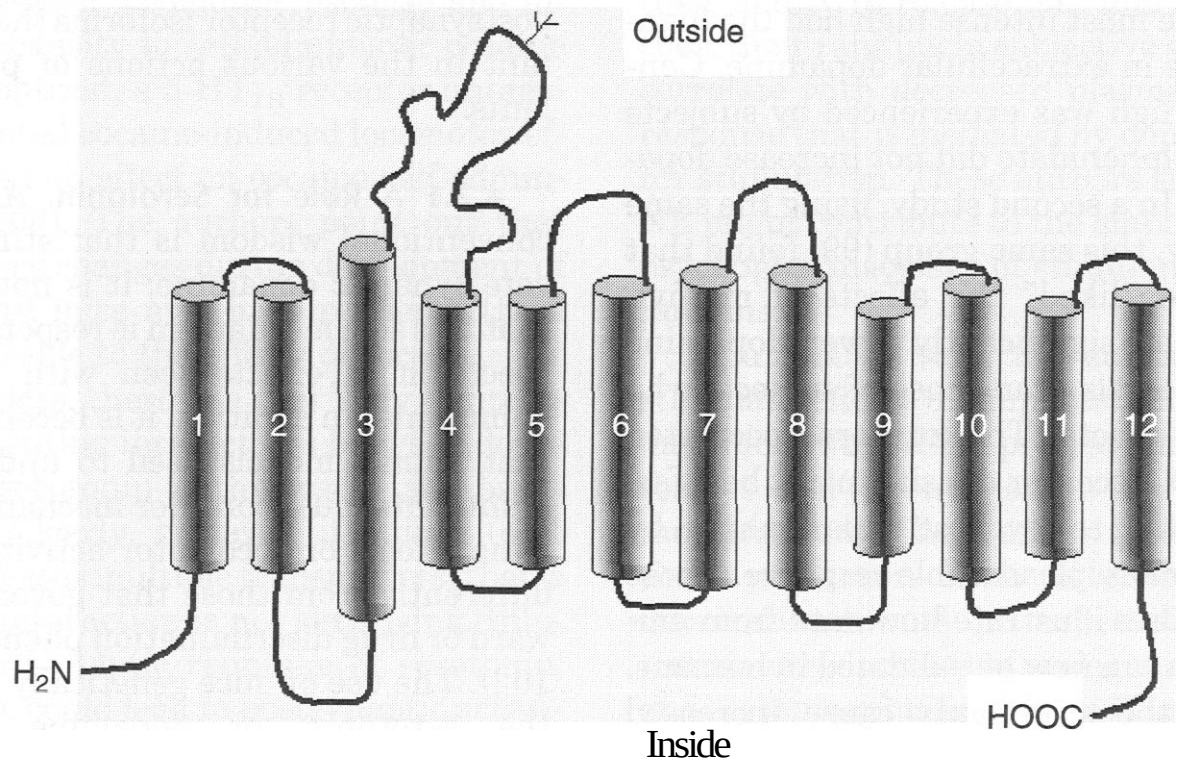


Figure 4.2. Representation of the 12-helix transmembrane transporter protein family. Both the amino- and the carboxyl-terminus are intracellular, with the second extracellular loop being larger, and possessing glycosylation sites. [Adapted from Nelson (84).]

The human norepinephrine uptake transporter was first sequenced and then expressed in HeLa cells in 1991 (85) and found to have properties identical to those of the native transporter. The cloning and sequencing of the dopamine transporter (86–88) and the serotonin transporter (89, 90) were reported in the same year. There are a number of excellent review articles written about monoamine transporters (84, 91–93).

Pharmacological studies of the mechanism of action for psychostimulants in animals have almost uniformly pointed to the importance of dopamine pathways for the increases of locomotor activity and reinforcing properties (94, 95). The conclusions of those studies have generally been extrapolated to humans, with little clinical evidence until recently to clearly support these ideas. In the past several years however, clinical studies of several stimulants, using *in vivo* brain imaging either with single photon emission computed tomography (SPECT), or positron emission tomography (PET) techniques, have provided evidence for elevated extracellular dopamine in response to psychostimulant administration. In essence, these studies employ either a single photon- or positron-emitting dopamine receptor antagonist. The labeled antagonist is administered both in the absence and in the presence of the stimulant drug of interest. The imaging technique then is used to determine how much of the labeled ligand has been displaced from its receptors by competition from increased extracellular endogenous dopamine. Based on the known affinity of the labeled ligand for its dopamine receptor, calculations can be used to determine the increased concentration of dopamine that must have been available at the receptors. These definitive studies have clearly established a role for dopamine in the effects of stimulants in humans (72, 74).

This type of approach has recently been applied to the study of methylphenidate. For example, Booij et al. (96) used SPECT imaging and an [^{123}I]benzamide dopamine D_2 receptor ligand antagonist ([^{123}I]IBZM) to measure significant displacement of the ligand by endogenous dopamine that had been released in response to administration of methylphenidate. In related work, Volkow et al. (74) used [^{11}C]-(+)-threo-methylphenidate to show that greater than 80% occupancy of the dopamine transporter was required to produce the stimulant "high." With the dopamine D_2 receptor antagonist [^{11}C]raclopride, Volkow et al. (97, 98) showed that the intensity of the methylphenidate "high" was quantitatively correlated with the levels of released dopamine and dopamine D_2 receptor occupancy. Subjects who

perceived the most intense high had the highest increases in extracellular dopamine. Conversely, no high was experienced by subjects when methylphenidate did not increase dopamine levels. In a second study, using the same methodology, this same group (99) found that subjects who "liked" the effects of methylphenidate had significantly lower dopamine D_2 receptor levels than those of subjects who disliked its effects. The authors speculated that lower D_2 receptor density might be a factor contributing to psychostimulant abuse, by providing a more pleasurable response. These imaging studies illustrate how data from animal research can now be validated in humans.

Because the stimulants cause increased synaptic levels of dopamine, and other monoamine neurotransmitters, they indirectly lead to stimulation of various postsynaptic receptors, through the increased concentrations of neurotransmitter. A large number of animal studies have been reported that used various agonists and antagonists to elucidate the role of different dopamine receptor isoforms. Until recently, however, only nonspecific ligands (i.e., with effects on both the D_1-like and D_2-like families) were available. In drug discrimination studies, rats have been trained to recognize and discriminate the interoceptive cue produced by injection of amphetamine or cocaine (100). Administration of a partial but selective D_1-receptor agonist SKF 38393 was partially recognized by these cocaine-trained rats, but not by amphetamine-trained animals. Yet, both amphetamine- and cocaine-trained rats discriminated the cue produced by a dopamine D_2 agonist bromocriptine as being similar to their training drugs. A dopamine D_3-selective agonist produced cocaine responses, but was only partially recognized by amphetamine-trained rats. Following additional experiments with dopamine receptor subtype selective antagonists, the authors concluded that the dopamine D_2 receptor played an essential role, but that both the D_1 and D_3 receptors might have some less important function. There is an extensive present research effort under way in many laboratories that is attempting to elucidate both the anatomical substrates and the specific postsynaptic receptor isoforms that are important in the various actions of psychostimulants.

4.2.1 A Role for Serotonin. Although the conventional wisdom is that stimulants elevate synaptic dopamine, it is not at all clear that this sole mechanism is responsible for the spectrum of effects seen with the psychostimulants. In addition, it is becoming evident that animal models used to understand the stimulants must consider mechanisms underlying effects on locomotor activity somewhat differently from those that govern either reward or drug discrimination phenomena (101, 102). The use of mice genetically deficient for the serotonin or dopamine transporter ("knockout mice") has produced some particularly interesting findings. For example, knockout mice lacking the DA transporter have high levels of extracellular dopamine, a condition that would presumably mimic the pharmacological action of cocaine and display spontaneous hyperlocomotion (103). Surprisingly, these mice still self-administered cocaine (104). Further experiments in these mice indicated the probable involvement of the serotonin transporter. In addition, conditioned place preference, another animal model of the reinforcing quality of a drug, could be established for cocaine in mice lacking either the dopamine transporter or the serotonin transporter (105). Place preference could also be established for methylphenidate, another stimulant that is thought to work through dopamine mechanisms, in mice lacking the dopamine transporter.

Experiments with knockout mice often produce unexpected results. It must be kept in mind, however, that when a key protein is missing during neural development, the offspring often have some type of adaptation that is not seen in the wild-type organism. Some caution, therefore, must be exercised in interpreting the results. For example, Belzung et al. (106) found that mice lacking the serotonin 5-HT$_{1B}$ receptor failed to display conditioned place preference. However, when these knockout mice were compared in studies using classical pharmacological antagonists of the 5-HT$_{1B}$ receptor, divergent results were obtained. The 5-HT$_{1B}$ receptor knockout mice

had an increased locomotor response and increased propensity to self-administer cocaine (107). By contrast, a 5-HT$_{1B}$ receptor antagonist attenuated cocaine-induced locomotor effects but had no effect on cocaine self-administration (108). The authors point out that compensatory changes during development of the knockout mice may have rendered them more vulnerable to the effects of cocaine.

Nonetheless, there is a vast body of literature documenting interactions between dopamine and serotonin pathways in the brain (109–111). Clearly, however, if a drug (e.g., cocaine) releases multiple transmitters, then a behavioral interaction is not surprising. Inhibition of presynaptic reuptake of serotonin, for example, could lead to postsynaptic activation of a variety of other receptors, some of which could modulate dopamine function. In addition to potential effects on 5-HT$_{1B}$ receptors, other studies have implicated serotonin 5-HT$_1$ receptors (112), 5-HT$_{2A}$ receptors (113), and 5-HT$_{2C}$ receptors (114). 5-HT$_{1B}$ receptors can also modulate the locomotor effects of cocaine (115).

4.2.2 A Role for Norepinephrine. Although the vast majority of studies of psychostimulants have focused on the role of dopamine and/or serotonin, the importance of norepinephrine (thought to be paramount 30 years ago) is generally now overlooked. Details of the mechanism of action of psychostimulants have been developed primarily through the use of animal models, in which dopamine seems to be the key player, and these results then have been extrapolated to humans. Yet, cocaine also is a potent NE uptake inhibitor, and the potency of amphetamine for norepinephrine release is similar to that for dopamine release. Indeed, in the rat prefrontal cortex, amphetamine and cocaine increased extracellular norepinephrine to an extent that was quantitatively similar to that of dopamine (116). Further, it appeared that the increase in prefrontal cortical norepinephrine was actually attributable to the blockade of the norepinephrine transporter by both drugs. Recently, Rothman et al. (117) reported that the oral doses of several stimulants required to produce amphetamine-like subjective effects in humans were most closely correlated with their ability to release NE, and not DA. Further, their ranking in subjective effects did not correlate with decreased plasma prolactin, a response that is mediated by dopamine receptor stimulation. These authors suggested that NE might contribute to the amphetamine-like psychopharmacology of stimulants, at least in humans.

In a related vein, the subjective psychostimulant effects of amphetamine were attenuated following a 2-h pretreatment with a tyrosine- and phenylalanine-free amino acid mixture (118). These amino acids are biosynthetic precursors of the catecholamines, and deprivation would be expected to produce transient reductions in endogenous dopamine and norepinephrine. The authors concluded that tyrosine depletion attenuates the release of dopamine required for the psychostimulant effect. Interestingly, the pretreatment did not reduce the subjective appetite-suppressant (anorectic) effect of amphetamine. The study authors attributed this latter finding to a continued release of norepinephrine by amphetamine. Tyrosine depletion, however, would also attenuate norepinephrine biosynthesis and it may be more reasonable to conclude that the anorectic effect might be related to the often-overlooked ability of amphetamine to release neuronal serotonin.

This chapter makes no attempt to review all the literature that focuses on the role of norepinephrine and serotonin in the actions of psychostimulants. At the time of this writing, the general consensus seems to be that effects on dopamine systems are necessary, but perhaps not sufficient, conditions to explain all the different actions of stimulants. There appears to be increasing awareness, spurred initially by studies of cocaine, that serotonin may be a much more important player than was heretofore recognized. In the next few years this role likely will be studied and elucidated in much greater detail.

The role of norepinephrine in the actions of stimulants has largely been overlooked, although a few studies suggest that this transmitter may be of major significance. On the other hand, until clinical studies are carried out using receptor blockers and specific norepinephrine transporter inhibitors, this area will remain muddy, at best. In virtually every

example, from amphetamine to cocaine, the compounds have significant effects at the norepinephrine transporter, in some cases equal to, or even greater than, those at the dopamine transporter. When behavioral or mood changes are correlated with levels of extracellular dopamine, and dopamine is highly correlated with changes in extracellular norepinephrine, one cannot be certain which underlying pharmacology is ultimately more important without experiments using specific blockers of both dopamine and norepinephrine transporters and receptors. It may be that effects on dopamine are necessary, but not sufficient, and that both norepinephrine and serotonin play modulatory roles. Because the stimulants have such diverse effects, including increasing activity, mood, appetite suppression, and so forth, it seems likely that serotonin and norepinephrine play more or less important modulatory roles, depending on which aspects of the specific drug's effects are being studied.

Caffeine and the other methylxanthines inhibit phosphodiesterases, the enzymes that degrade cAMP. For many years it was believed that the stimulant effect of caffeine was attributed to this enzyme inhibition. At the plasma concentrations obtained after two to three cups of coffee ($\sim$10 μM), however, antagonism of adenosine A_1 (and A_2) receptors in brain is believed to be the most relevant action to explain the stimulant effects of caffeine (119, 120). Perhaps not surprisingly, in view of earlier discussion in this chapter, caffeine administration has been shown to lead to elevated levels of brain dopamine (121, 122). It is thought that adenosine receptor stimulation facilitates GABA- or acetylcholine-mediated inhibition of dopamine receptors in striatopallidal and striatonigral neurons (123), with the end result of decreased dopaminergic function; adenosine antagonists would thus have a reverse action. Many studies have examined the interaction between adenosine A_{2A} receptors and dopamine receptors, both of which are highly concentrated and colocalized in the striatum and have reciprocal antagonistic interactions (124–127). There is abundant evidence for pre- and postsynaptic interactions between adenosine and dopamine receptors, by which adenosine inhibits doparninergic activity [e.g., (128)]. With respect to stimulation of locomotor activity in animal models, studies have implicated the dopamine D_2 receptor (129, 130). It has not been clear, however, whether effects mediated by striatal adenosine A_{2A} receptors absolutely depend on the presence of dopamine D_2 receptors. To study this problem, Chen et al. (131) employed genetic knockout mice deficient either in dopamine D_2 receptors or adenosine A_{2A} receptors, or a double knockout mouse deficient in both types of receptors. These studies found that A_{2A} receptors may affect neuronal activity in a manner that is partially independent of the presence of dopamine D_2 receptors, such that endogenous adenosine may be most accurately viewed as a facilitative modulator of striatal neuronal activity rather than simply as an inhibitory modulator of D_2 receptor neurotransmission.

These studies, and many others, conclude that the acute locomotor stimulant effects of caffeine in animal models are mediated in part by dopaminergic systems and dopamine receptors. Recent studies suggest that tolerance to the locomotor stimulant effects of chronic caffeine may also be related to specific changes in dopaminergic function (128). Thus, in spite of the fact that methylxanthines are structurally different from other psychostimulants, and do not directly affect dopamine transporters or receptors, in fact their stimulant action is derived from effects on central dopamine pathways.

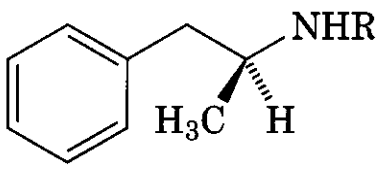
5 STRUCTURE-ACTIVITY RELATIONSHIPS

Examination of the structure-activity relationships (SARs) of several of the classic stimulants provides not only an understanding of the development of other drugs, but provides important clues as to the underlying mechanisms involved in interaction with the target protein(s). The following sections will hopefully illustrate both of these points.

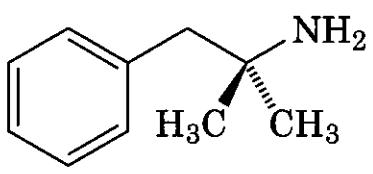
5.1 Amphetamine

There are a number of related structures that are often referred to as "amphetamines," although the name amphetamine refers to one specific molecular entity. Grouped in this class would be (+)-amphetamine (10), *N*-methyl-

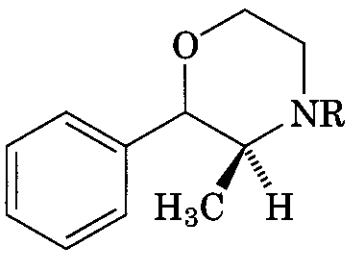
amphetamine [S-(+)-methamphetamine, **18**], phentermine (**19**), phenmetrazine (Preludin, **20**), and phendimetrazine (21). **Diethylpropion (Tenuate;** 16) is used as an appetite suppressant and, although it has the amphetamine skeleton, its effects are much weaker as a stimulant than those of the other structures listed here. The stereochemistry at the a **side-chain** methyl group is the same for the most potent enantiomer of each structure, although the pure enantiomer has not generally been marketed except for the cases of (+)-amphetamine (10)and (+)-methamphetamine (18).



(10) R = H
(18) R = CH_3

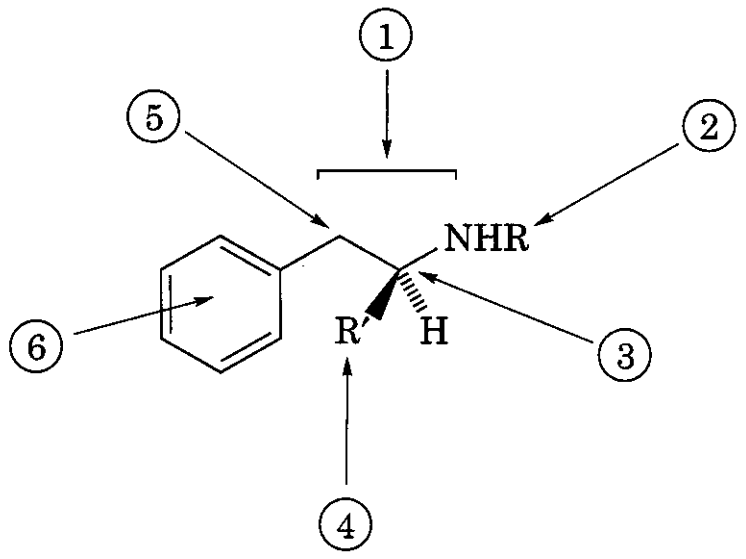


(19)



(20) R = H
(21) R = CH_3

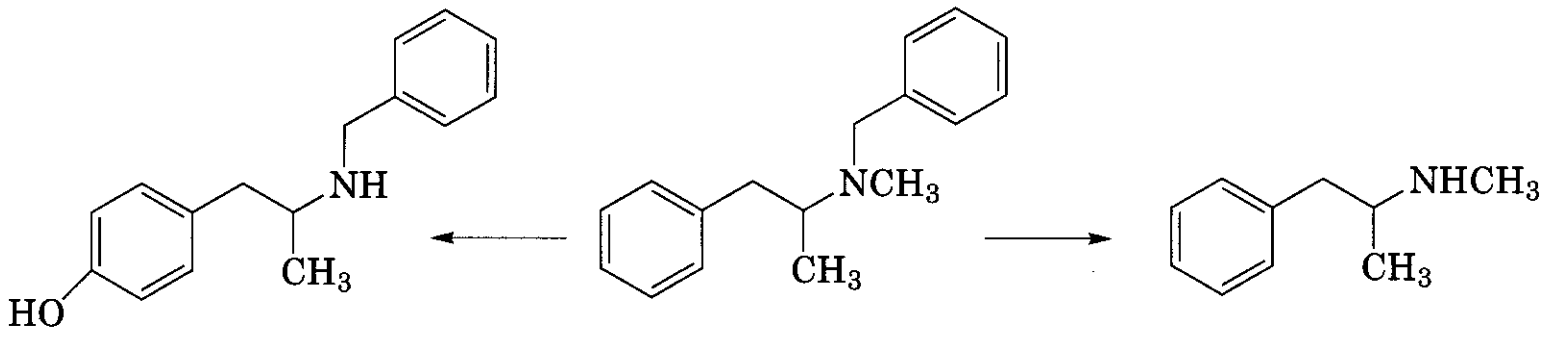
The structural requirements of the **dopamine** (and norepinephrine) transporter appear to be fairly rigid. There is very little molecular variation that is tolerated without significant loss of activity. The relatively limited information that is available, mostly from animal studies, can be summarized by considering the various areas of substitution for a general phenethylamine structure. These structure-activity relationships have been recently surveyed (**132**), and an extensive and comprehensive review by Biel and Bopp (133) covered the older literature.



5.1.1 Length of the Side-Chain. The length of the side-chain is limited to two carbon atoms (134, **135**). That is, for transporter substrates, the optimum pharmacophoric template appears to be a basic nitrogen two carbon atoms removed from an aromatic ring system. This observation of course is not too surprising, given that the transporter substrates **dopamine,** norepinephrine, and serotonin all bear this essential core.

5.1.2 Nitrogen Substituents. Nitrogen **substituents** are very limited. The primary amine (amphetamine)and the ***N*-methylamine** (methamphetamine) are the most potent compounds (**135**). An N-methyl increases the potency of both amphetamine and cathinone (2) (136). Larger alkyl groups (135, 137) or ***N,N*-**dialkylation, either dramatically attenuate or completely abolish stimulant activity (138). Nevertheless, ***N,N*-dimethylamphetamine** has appeared on the illicit market (139) and does appear to have behavioral effects in rats and monkeys similar to amphetamine (138, 140). The rapid onset of action suggested that the ***N,N*-dimethyl** compound itself had pharmacological effects, rather than the **N-demethylated** metabolite, methamphetamine, although the latter is one of the known metabolites of ***N,N*-dimethylamphetamine** (141).

Active metabolites may be much more important in ***N,N*-dialkylated** compounds that possess a **β-keto** function, as in cathinone (2). In that case, the ***N,N*-dimethyl** compound is nearly as active as the N-monomethyl compound (**142**). It is known, however, that the



(22)

alkyl groups of β-aminoketones are readily cleaved metabolically. Thus, the *N,N*-dimethyl cathinone analog is likely converted in vivo to the N-monomethyl compound methcathinone. This argument is based on evidence that for diethylpropion, the *N,N*-diethyl congener of cathinone, it is the N-monoethyl metabolite that is the active species (70, 71).

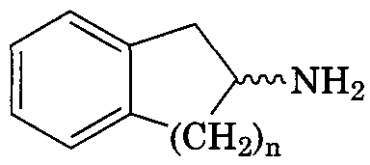
Although longer N-alkyl groups lead to less active compounds, one exception to this generalization is benzphetamine (Didrex), *N*-benzyl-N-methylamphetamine (22). Despite the *N,N*-dialkyl groups in benzphetamine, in humans it produces subjective effects characteristic of amphetamine-like drugs such as phenmetrazine (20) (143). Although *para*-hydroxy-N-benzylamphetamine is a major metabolite of benzphetamine, methamphetamine and amphetamine are also detectable in urine and hair following administration of benzphetamine (144–146). It is not clear from the literature whether the reinforcing effects of benzphetamine are attributable to metabolic formation of amphetamine or methamphetamine. Based on the studies with *N,N*-dimethylamphetamine by Witkin (138), however, one would predict that the parent molecule has some pharmacological activity.

5.1.3 Stereochemistrv at the a Carbon. The stereochemistry at the a carbon atom, when enantiomers exist, is homochiral to that of *S*-(+)-amphetamine (**10**), shown earlier. Both the releasing actions at dopamine and norepinephrine transporters in isolated rat brain slices (147) and the locomotor and stereotypic effects in rodents (148) are more potently affected by the S-(+) isomer of amphetamine than by the *R*-(−) isomer. In this latter study, the (+) enantiomer was about five times more potent than the (−) isomer, paralleling the potency difference found with the enantiomers in vitro, using rat brain striatal synaptosomes (149). The two isomers were of nearly equal potency in their effects on norepinephrine accumulation by rat hippocampal synaptosomes (149). This stereochemical requirement applies to p-keto derivatives as well; the corresponding active isomer has the *S*-(−) configuration (136).

5.1.4 The α-alkyl Substituent. The a-alkyl group cannot be much larger than a methyl. Phenethylamine itself, lacking the side chain a-methyl group, is inactive in *vivo* because of its rapid inactivation by monoamine oxidase. Addition of the a-methyl group retards metabolism by this route, leading to the orally bioavailable drug amphetamine. The uptake transporter, however, cannot tolerate large groups in this region and the a-ethyl analogs of both amphetamine and methamphetamine had markedly attenuated activity in a drug discrimination assay with rats trained to discriminate (+)-amphetamine (150). α,α-Dimethyl groups, as in phentermine (**19**), though giving an active compound, still reduce activity.

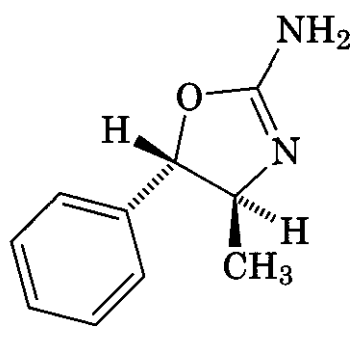
Attempts to incorporate the side chain into ring structures also led to compounds with attenuated activity. For example, in drug discrimination assays using rats trained to recognize the effect of (+)-amphetamine (**10**), compounds (23) and (24) either failed to produce amphetamine like effects, or had much lower potency (150, 151). When n = 3, the compound lacked any amphetamine-like action.

5.1.5 Other Side-Chain Substitutions. Limited substitution of the side chain is tolerated. A β-hydroxy group on methamphetamine



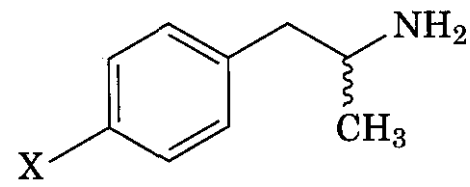
(23) n = 1
(24) n = 2

gives ephedrine (**1**), shown earlier. Although ephedrine is a CNS stimulant, its effects are much weaker than those of methamphetamine. Similarly, addition of a **β-hydroxy** to amphetamine gives phenylpropanolamine, a compound that is nearly devoid of CNS stimulant effects. One may speculate that the polar hydroxy group reduces the hydrophobicity of these compounds such that CNS penetration is much reduced. The N-methyl of ephedrine increases lipid solubility, so ephedrine has a greater CNS action than that of **phenylpropanolamine**. Addition of a keto function to the structure of amphetamine or methamphetamine gives cathinone (2) or its corresponding N-methyl derivative, methcathinone, the latter of which also has greater potency than that of the primary **amine** (142). It should be noted that an oxygen at the β position can be incorporated into a heterocyclic ring as in **phenmetrazine** (20) and phendimetrazine (**21**). Methyl **aminorex** (25) is also a potent stimulant that incorporates the essential features of the amphetamine template into an oxazoline ring. The **4*S*,5*S*-*trans*** isomer shown (25) is the most potent of the four possible **stereoisomers** (152, **153**).



(25)

5.1.6 Aromatic Ring Substitution. Simple ring substituents can change the targets of the amphetamines from one monoamine uptake carrier to another. The **dopamine** and norepinephrine uptake carrier proteins have the most stringent structural demands, and any substitution decreases their potency at these sites. The serotonin carrier is relatively promiscuous and tolerates a variety of ring **substituents**, many of which dramatically increase the potency at the serotonin carrier from that of amphetamine itself. No ring modifications are known that give rise to a substituted amphetamine that completely retains amphetamine-like psychostimulant activity. para- Fluoroamphetamine (26; *X*=*F*) has been reported to have effects in rats resembling those of amphetamine, but substitution with larger halogens (**e.g.**, chloro or **iodo**) leads to compounds that have significant serotonin releasing potency, and that produce behavioral effects that are different from those of amphetamine itself (**154**).

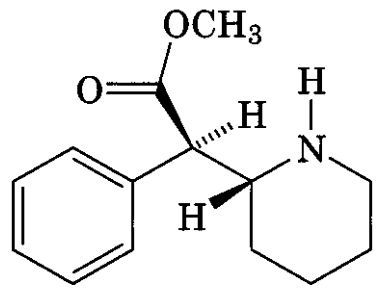


(26) X = F, Cl, I

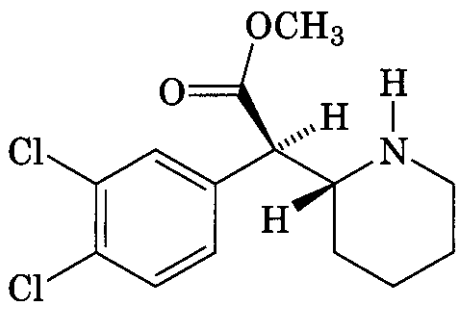
5.2 Methylphenidate

The ***R*,*R*-(+)-stereoisomer** of methylphenidate (8) is known to be the more active (**155**) and is often referred to as the active "threo" isomer. The (−)-enantiomer and the **erythro** stereoisomers are much less potent. One study has reported a series of aromatic **ring-substituted** analogs. The most potent compounds in that report were halogen substituted in the **3-** or **3,4-** positions of the ring. For example, the dichloro compound (27) was 32-fold more potent than methylphenidate itself in inhibiting **dopamine reuptake** (156). That finding parallels a recent report by Deutsch et al. (**157**), that replacing the phenyl ring with a **β-naphthyl** moiety (158) gave a compound with about eightfold higher affinity for the **dopamine** transporter. Those workers also reported that the corresponding a-naphthyl analog had only about one-tenth the potency of methylphenidate at the DAT. Taken together, these latter observations indicate that the DAT must have a hydrophobic region that generally extends from the **3,4-** positions of the aromatic phenyl ring of methylphenidate.

Deutsch et al. (157) also examined the effect of heterocyclic ring size. The **pyrrolidyl**



(+)-8



(27)

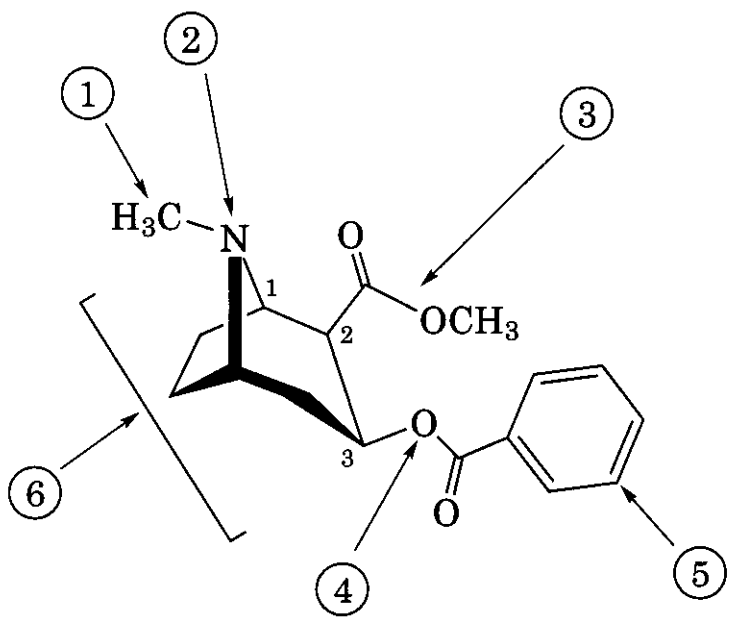
and azepino, as well as the azacyclooctane congeners, were significantly less potent than methylphenidate itself. That report also contained data for the morpholine analog of methylphenidate (158), which had an approximately 15 times lower affinity at the DAT. Beyond the studies cited here, very little additional SAR work has been done with methylphenidate.

5.3 Cocaine

Of all the psychostimulants, cocaine has probably been most studied, particularly within the last decade, as a result of its widespread abuse. Structure-activity studies have been carried out with numerous analogs, not only to elucidate the molecular requirements for interaction with the various monoamine transporters, but also in attempts to develop treatments that might be useful for cocaine addiction. Ideally, understanding the structure-activity relationships will be useful to understanding the functional topography of the binding site of the transporters, and, if a three-dimensional structure of the transporters can be developed, these features would map onto the binding site. Nevertheless, because the topic of this chapter is stimulants, and not the structure-activity relationships of monoamine transporters, an exhaustive summary of the more than 200 papers that have appeared on the SAR of cocaine and its analogs will not be presented. A useful perspective on the SAR of cocaine analogs as it was understood in 1992 has been presented by Carroll et al. (159), with more a recent update in 1997 (160).

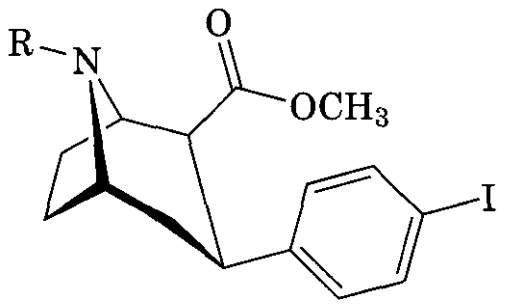
An attempt will be made here to distill down the essence of the SAR of cocaine as it relates to its stimulant properties. In many cases, compounds have been reported that have not been tested in vivo, but have only been compared for affinity at the monoarnine transporters or in an in vivo assay. Some of these data will be summarized if they are reported in the context of the stimulant effects of cocaine. Similarly, there have been numerous attempts to develop cocaine analogs that may bind to the dopamine transporter and actually block the stimulant or reinforcing effects of cocaine itself, in efforts to develop treatments for cocaine addiction. This chapter largely ignores many of those studies unless they contain in vivo data suggesting they are relevant to a discussion of stimulant effects. Nevertheless, because stimulant properties have been associated with binding to the DAT, a good deal of the SAR discussion here must be discussed in the context of in *vitro* DAT affinity.

A consideration of the structure-activity relationships of cocaine can focus on a number of key elements in the structure, as indicated below. Each of the following sections includes a discussion of the particular numbered structural element.



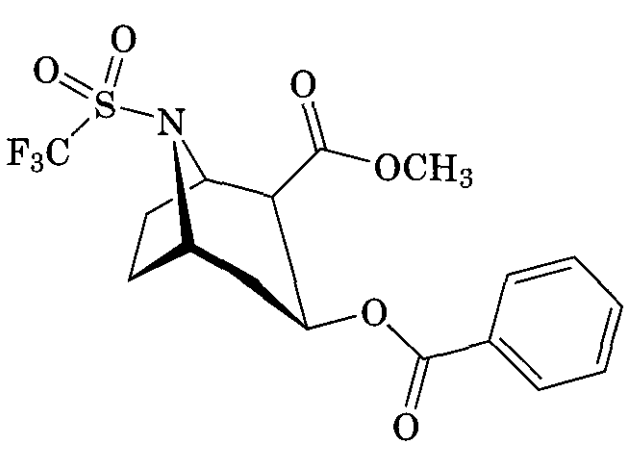
5.3.1 N-substituents. N-demethylation of cocaine has only a minor effect on affinity at

monoamine transporters **(161).** In phenyltropane analogs where the ester linkage has been removed (**28**), extensions of the N-alkyl out to n-butyl have no effect on dopamine transporter affinity **(162).** Effects at the serotonin transporter are variable, but affinity only decreases modestly. At the norepinephrine transporter, affinity drops about three times with the longer N-alkyl group.



(28) R = CH_3, n-C_3H_8, n-C_4H_9

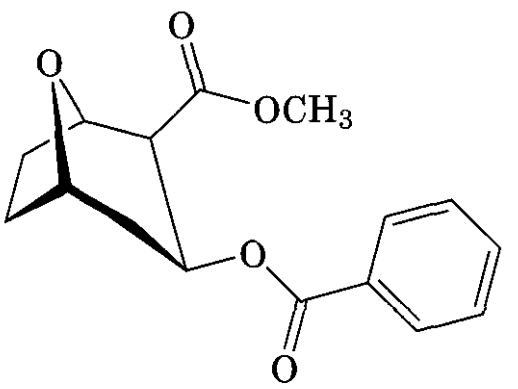
5.3.2 Basic Nitrogen Atom. For many years it was assumed that the basic nitrogen of cocaine was required for activity. It seemed logical to believe that the nitrogen, protonated at physiological pH, would interact with an anionic site such as an aspartate residue in the transporter **(163).** It was surprising, therefore, when nonbasic N-sulfonyl cocaine analogs such as **(29)** were found to possess high affinity for the dopamine transporter (164). These compounds are not protonated at physiological pH, and if hydrogen bonding were required for activity, these analogs could serve only as hydrogen bond acceptors. Even then, the low electron density remaining on a nitrogen with the powerfully electron-withdrawing trifluoromethylsulfonyl group attached, would suggest that this interaction should be very weak.



(29)

Replacement of the nitrogen atom with oxygen as in **(30)** gives compounds that retain high affinity for the dopamine transporter **(165).** This finding was accommodated by proposing that the oxygen atom could act as a hydrogen bond acceptor at the transporter (165), a conclusion that would at least be consistent with the activity of the N-sulfonated derivatives (**29**).

It was even more surprising, therefore, when the report appeared that even a polar oxygen was not required for good uptake inhibitors. Carbocyclic compounds such as (**31**) proved to have transporter affinities nearly equal to those of their amine-containing counterparts (166)!



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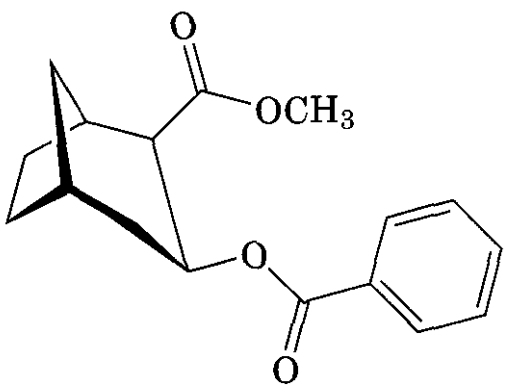
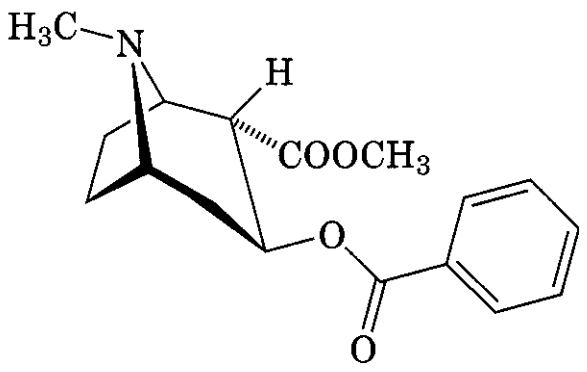


(31)

These authors postulated that there are various acceptor sites in the dopamine transporter, where an inhibitor may bind and cause dopamine uptake inhibition. The topography of these sites is probably different in the three monoamine transporters.

5.3.3 Substituent at C(2). Epimerization of the ester function to give pseudococaine (**32**)

results in about a 150-fold loss in affinity for the dopamine transporter (161). In compounds lacking the ester linkage (see section below) the effect is more dramatic, resulting in a more than 1000 times lower potency.

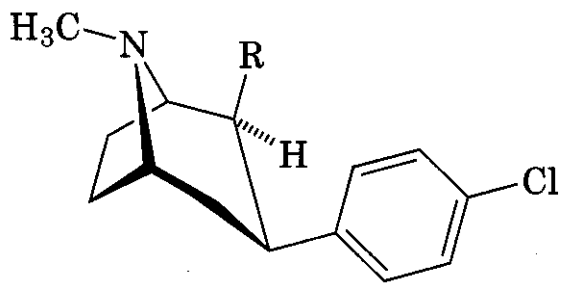


(32)

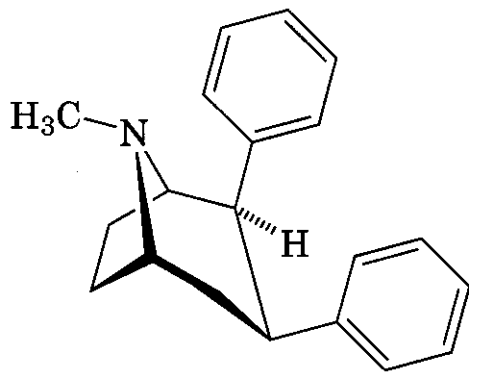
The ester is not an essential function. Replacement of the ester with an ethyl or vinyl group did not lead to significant loss of binding affinity, demonstrating that a polar function capable of hydrogen bonding was not essential (167, 168). Indeed, substitution at the 2β position with alkyl groups as long as n-butyl, 2-phenethyl, or 2-stryl gave compounds (e.g., 33) with exceptionally high affinities at the dopamine transporter (168).

Kelkar et al. (169) have extended the 2P-alkyl group to include a polar hydroxy or methyl ester function at the distal end of a three carbon chain, with no significant loss of affinity compared to that of a simple carbomethoxy function. They concluded that this region of the cocaine binding site must be either a large cleft in the transporter protein or exterior to the binding site. They also noted that this region is relatively insensitive to electrostatic interactions. Chang et al. (170), found that the 2P-phenyl analog (34) was equipotent to the 2-carbomethoxy compound, but had enhanced selectivity for the dopamine transporter over the serotonin transporter. These authors also concluded that a hydrophobic group at this region of the molecule might be a contributing factor for binding at the dopamine transporter.

Esters larger than a methyl are quite potent. In the 3-benzoyl series of tropane esters, both the isopropyl and phenyl esters had high affinity and selectivity for the dopamine transporter (171). The phenyl ester (35; RTI-15) dose-dependently substituted in the drug discrimination paradigm in rats trained to discriminate the effects of cocaine. In contrast, whereas cocaine increased locomotor activity in mice, RTI-15 had no effect on activity and at high doses even decreased this measure (172). Because this compound was a potent inhibitor of the dopamine transporter, it suggests that high selectivity for the dopamine transporter may lead to differential retention of cocaine-like effects.



(33) R = alkyl



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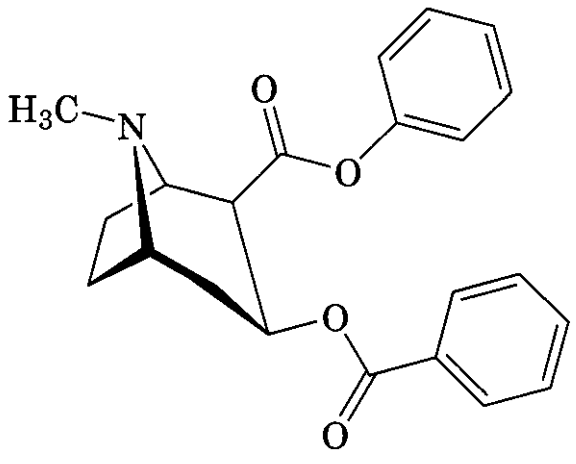
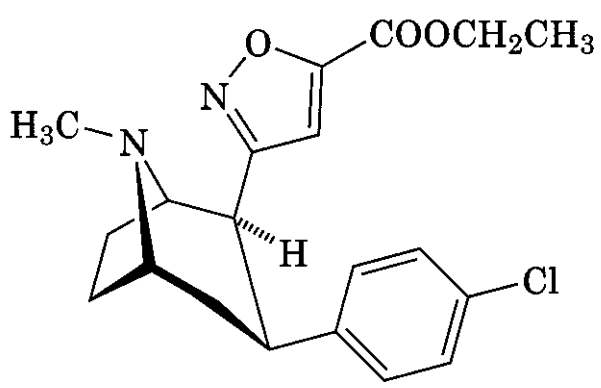


(35) (RTI 15)

The isopropyl and phenyl esters in the 3-phenyltropane series of analogs have higher affinity for the dopamine transporter than does the methyl ester (173). Similarly, tertiary amide analogs of cocaine and phenyltropane analogs are more potent than secondary or primary amides, and also have enhanced selec-

tivity for binding at the dopamine transporter over that of the norepinephrine or serotonin transporters (173). Replacement of the ester or amide function with a carboethoxy isoxazole substituted substituent gave (36), a highly potent inhibitor with selectivity for the dopamine transporter (174, 175). This compound had about twice the affinity of cocaine at the DAT.



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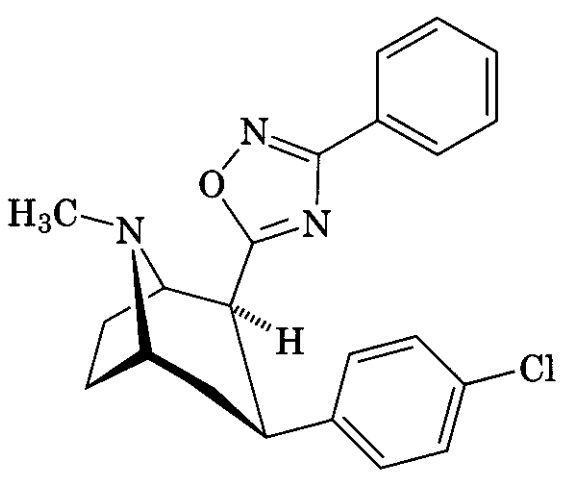
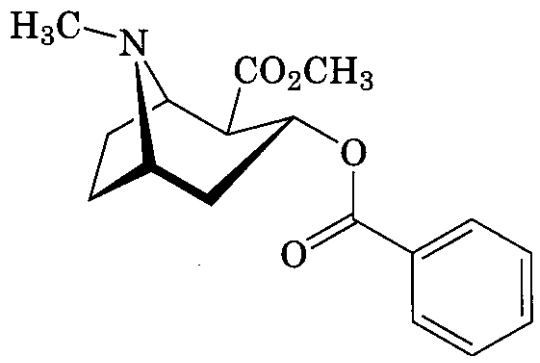


(37)

Similarly, Carroll et al. (176) reported that the 1,2,4-oxadiazoles (e.g., 37) that are bioisosteres of ester groups, are potent cocaine analogs. Compound (37) had low nanomolar affinity for the dopamine uptake transporter with greater than 100-fold selectivity for the dopamine transporter over the norepinephrine and serotonin transporters.

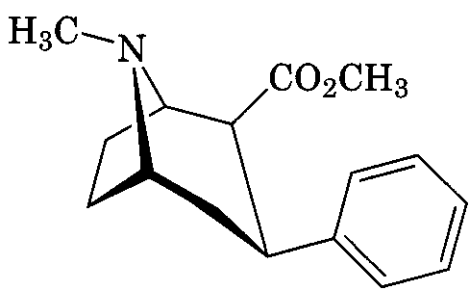
5.3.4 The Ester Linkage at C(3). In cocaine, the 3a epimer "allococaine" (38) has considerably reduced activity compared to that of cocaine itself (177). This structural change, however, causes the tropane ring to favor the



(38)

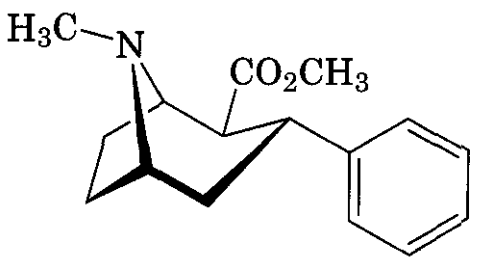
pseudochair, rather than the boat, conformation that occurs in natural (−)-3β-cocaine.

It was first reported by Clarke et al. (178) that removal of the ester linkage from cocaine, to give a compound with the phenyl ring directly attached to the tropane ring (WIN 35,065-2; 39), possessed higher affinity for the dopamine transporter than did cocaine itself. By contrast to benzoyl esters, however, the configuration at the 3 position is not so critical in phenyltropane compounds. That is, in the WIN series where the ester has been removed, the 3β phenyl orientation (39) was only about twofold more potent than the 3α phenyl (40) at the dopamine transporter. At the serotonin transporter, however, the 3β compound was significantly more potent (165). A similar trend was observed in the 8-oxa analogs, leading to the conclusion that the dopamine transporter is able to accommodate the 3-phenyl ring when the bicyclic ring is in either the boat or chair conformation, whereas the serotonin transporter is less accommodating.



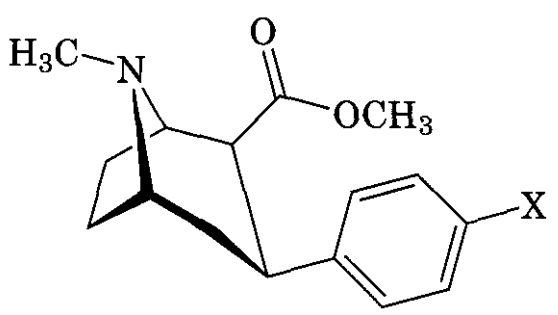
(39)

5.3.5 Substitutions on the Aromatic Ring at Position 3. In the phenyltropane analogs of cocaine, where the ester linkage has been removed and the phenyl ring is attached directly to the tropane ring (WIN and RTI compounds), substitution at the *para* ring position with halogens or a methyl group gave compounds (41) with increased affinities at the



(40)

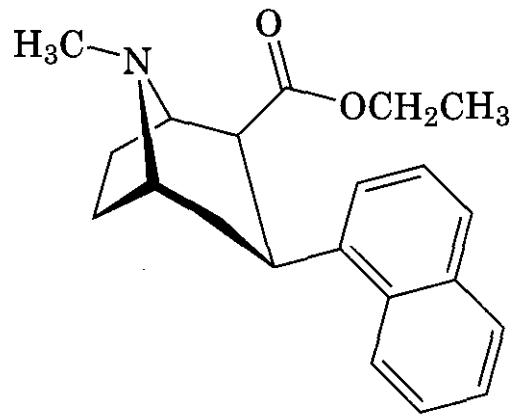
dopamine transporter compared with the unsubstituted compound, and with much increased affinity compared to that of cocaine itself (179). Behavioral potency paralleled the affinity increases, with all of the phenyltropanes being considerably more potent in elevating locomotor activity in mice (180) and in substituting for a cocaine stimulus in the drug discrimination paradigm in rats (181).



(41) X = F, Cl, Br, I, CH_3

The rank order of affinity for aromatic ring substituents in the WIN series was 3,4-Cl_2 > I > Cl > F > H, whereas in the 8-oxa (3β) analogs it was 3,4-Cl_2 > Br > Cl > I > F > H (165). Replacing the 3β-phenyl with either a 1- or 2-naphthyl substituent gave significantly enhanced affinity at all three monoamine transporters, with the 2-naphthyl (**43**) being about five- to sixfold more potent than the 1-naphthyl (42) (182). This result is parallel to similar findings reported by Deutsch et al. (157), where replacing the phenyl ring of methylphenidate with a 2-naphthyl moiety gave an analog with about 70-fold higher potency than when the phenyl ring was replaced with a 1-naphthyl.

5.3.6 Requirement for the Intact Tropane Ring System. We have seen earlier that there is no absolute requirement for the basic nitrogen in the tropane structure, and that even a polar oxygen isostere replacement is not needed for cocaine congeners to possess potent



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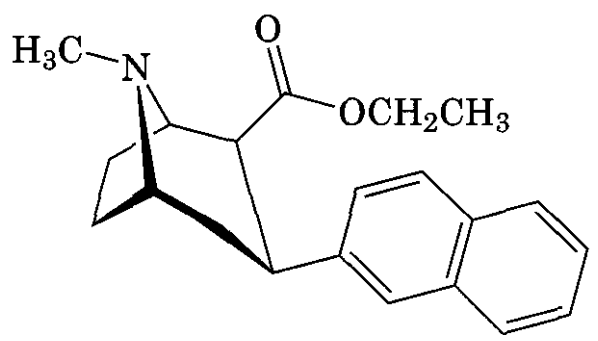
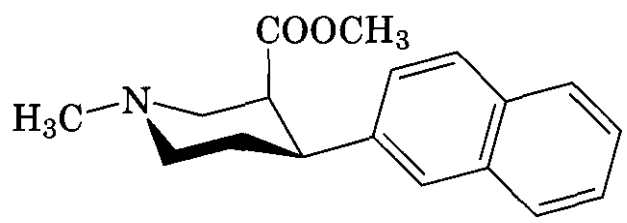


(43)

monoamine reuptake inhibition. It is perhaps not too surprising, therefore, that the bridged bicyclic tropane ring is not an essential structural feature. In a series of 4-arylpiperidine carboxylic acid methyl esters, several of the compounds were significantly better uptake inhibitors than cocaine (183). The most potent compound in the series, (**44**), was about 20 times more potent at the dopamine uptake transporter than cocaine.



(44)

6 RECENT AND FUTURE DEVELOPMENTS

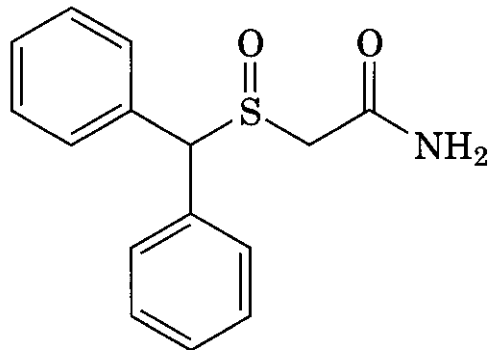
As reviewed above, the drugs that have been used for their stimulant properties were largely the result of compounds that were discovered empirically over many centuries. Understanding the active principles of these drugs has led to major advances in medicinal chemistry. For example, one of the most exciting recent findings has to do with understand-

ing the monoamine transporters at the molecular level. Whereas it has been known in pharmacology for many years that cocaine targeted an energy-dependent reuptake pump, the cloning and expression of these transporters has given access to high "purity" proteins that are amenable to more detailed study. These proteins have all been sequenced, and shown to be membrane bound with 12 membrane-spanning helical segments. A large number of site-specific mutations have been used to correlate specific residues in the protein with specific functions. Nevertheless, it will likely take a technical breakthrough to obtain a crystal structure of one of these transporters or one of their homologs, an accomplishment that will no doubt lead to much greater understanding of how transporters function. Inability to crystallize these proteins is not a problem that is unique to monoamine transporters, but continues to plague the study of all membrane-bound transporters and receptors, and other membrane-bound proteins.

Site-directed mutagenesis, cysteine-scanning accessibility methods, high field NMR, homology modeling, and continued development of structure-activity relationships will no doubt lead to better and better models of the monoamine transporters. These approaches are having the greatest impact on studies of uptake inhibitors, rather than studies of substrates.

Particularly interesting recent advances indicate that ligands from different chemical classes may bind in novel ways to the dopamine transporter. Using site-directed mutagenesis and photoaffinity labeling probes, investigators have produced results suggesting that the substrate uptake and cocaine binding sites are probably not identical (184). Indeed, it also now seems likely that different chemical classes of uptake inhibitors may even bind to distinct regions of the transporter (185, 186), leading to different overall conformations in the transporter protein and perhaps subtly altered mechanisms of inhibition. These different transporter conformations would explain the observed differences in the pharmacology of different chemical classes of DAT inhibitors. New derivatives that have selective affinity at these alternate binding sites may block the actions of cocaine without markedly affecting the normal transport function of the protein. Hence, there is presently intense interest in such compounds because they may provide new avenues for the treatment of cocaine and psychostimulant addiction.

There also is a need for improved drugs to replace existing CNS stimulants, as treatments for medical conditions like narcolepsy, ADHD, obesity, and for general attentional purposes. Yet, virtually all of the existing stimulants have the capacity to produce enhanced mood, or euphoria. This side effect means that they all possess abuse potential to a greater or lesser degree. Advances in medicinal chemistry and molecular neurobiology have provided hope, however, for new generations of drugs. For example, recently the nonamphetamine, nonstimulant drug modafinil (Provigil, 45) was approved for use in narcolepsy (see, e.g., Ref. 187). This drug has been shown to be more effective than amphetamine, with fewer side effects, although its mechanism of action has not yet been elucidated. The discovery that mutations of either the gene for the novel neuromodulator orexin, or the orexin receptor, can cause narcolepsy, leads to the hope for even better and more specific drugs to treat that disorder, as well as to the possibility of better treatments for obesity (188–191).



(45)

Even those actions of the stimulants that are absolutely dependent on activation of monoamine systems may also be amenable to breakthroughs in medicinal chemistry. For example, the beneficial effects of stimulants on ADHD may be attributable primarily to activation of only certain receptors. Recently, the anatomical and functional substrates of

attention, learning, and memory have begun to yield their secrets. This work has suggested that certain drugs [e.g., selective D_1 dopamine agonists (192, 193)] may provide all or much of the beneficial effects of the stimulants without the abuse potential. Finally, the cracking of the human genome, and the prediction of more than 100,000 human proteins, offers the hope for novel targets for future efforts in medicinal chemistry. Whereas these may be known neurotransmitter pathways (e.g., GABA or glutamate receptors), new targets may be novel proteins whose function is not understood today, but will be tomorrow, and current uses of psychostimulants may be no more than historical artifacts in a decade.

6.1 Web Site Addresses and Recommended Reading

- http://www.nida.nih.gov/DrugAbuse.html
- http://www.mentalhealth.com/
- http://www.psyweb.com/indexhtml.html
- http://www.fda.gov/medwatch/

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CHAPTER FIVE

Sedative-Hypnotics

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1 INTRODUCTION

People are becoming increasingly aware of the importance of sleep. One-third of our life is spent in sleep, which has a major influence on an individual's physical, social, and psychological well-being. Sleep has become the subject of extensive clinical and research interest.

At one time, wakefulness was considered as the active state and sleep as the passive state of all human functions. Today both wakefulness and sleep are considered to be active processes, with wakefulness and sleep being at two ends of a continuum.

Although wakefulness and sleep are two distinct functional states, they seem to influence each other. Disturbances during sleep may impair daytime function and problems experienced during wakefulness may adversely affect sleep.

As health professionals have become more aware of the importance of sleep, the treatment of sleep disorders has received increasing attention. As a result, new drugs have become available on the market and new information has become available on the role of sleep factors, the homeostatic regulation of sleep, circadian rhythm, chronotherapy, the role of immunology, and genetics of sleep disorders. The new knowledge will further enhance the ability of health professionals to develop new medicines for the treatment of sleep disorders.

1.1 Classification of Sedative-Hypnotics

Arbitrarily the sedative-hypnotics may be classified as follows:

1. Barbiturates
2. Benzodiazepines

3. Halogenated compounds (chloral hydrate, ethchlorvynol, carbromal)
4. Heterocyclic compounds (piperidinediones, thiazoles, pyrrolopyrazinones, imidazopyridines, pyrazolopyrimidines)
5. Antihistamines
6. Other sedative-hypnotics (valnoctamide, propofol, plant extracts, endogenous sleep factors, melatonin)

1.2 The Ideal Hypnotic Drug

The characteristics of an ideal hypnotic for the elderly have been summarized as follows (1):

- Induces sleep promptly after administration.
- Maintains sleep for an adequate period without undesired awakenings.
- Promotes a sleep state identical to a non–drug-induced or "natural" sleep.
- Leaves the individual feeling refreshed and well rested on awakening.
- Does not cause undesired daytime sedation or drowsiness.
- Causes no impairment of coordination and psychomotor function.
- Does not lose efficacy when taken repeatedly (for a number of consecutive nights or on a chronic basis).
- Does not accumulate in the body during chronic usage.
- Does not lead to dependence.
- Is not harmful if overdose is taken.
- Does not cause "rebound insomnia" when suddenly discontinued.
- Does not exacerbate sleep apnea or other conditions contributing to disturbed sleep.
- Causes no undesired reactions, such as cardiovascular or gastrointestinal distress.
- Does not cause enzyme induction or participate in other clinically important drug interactions.
- Is inexpensive.

Although a lot of progress has been made towards the goal of finding the ideal hypnotic, in the absence of having found such an agent, research towards the goal must continue.

2 CLINICAL USE OF AGENTS

2.1 Current Drugs on the Market

Current drugs on the market include barbiturates, benzodiazepines, halogenated sedative-hypnotics, heterocyclic sedative-hypnotics, antihistamines, and other sedative-hypnotics.

2.2 Side Effects, Adverse Effects, and Drug Interactions/Contraindications

2.2.1 Barbiturates. Barbiturates (1) (see Table 5.1) have a spectrum of deleterious side effects including lethargy, confusion, and depression. Chronic use of barbiturates is associated with a potential for addiction involving both physical and psychological dependence.

Tolerance and abstinence may develop with any of the barbiturates. It has been reported that abrupt withdrawal of secobarbital (given 0.8–2.2 g/d for 6 weeks) causes both minor symptoms (tremors, anorexia, insomnia, and apprehension) and major symptoms (seizures, delirium, and hypothermia) that can persist for up to 2 weeks after discontinuation. Tolerance to barbiturates can develop within 2 weeks of treatment because of induction of hepatic microsomal enzymes (2).

Barbiturates are lethal at about 10 times the hypnotic dose that is about 6–10 g of phenobarbital or 2–3 g of secobarbital, pentobarbital, or amobarbital (3).

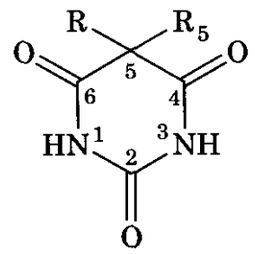
The adverse effects of the barbiturates include respiratory depression, allergic reactions, skin rashes, hepatitis, cholestasis, and photosensitivity.

As with other sedatives, paradoxical excitement and irritability may occur. This is common in children and the elderly.

The toxic effects of an overdosage result from profound central depression and may include coma, respiratory and cardiovascular depression with hypertension, and shock leading to renal failure. Withdrawal of the drug is more frequently a problem with barbiturates than with benzodiazepines. Withdrawal of barbiturates leads to rapid eye movement (REM) sleep rebound and rebound insomnia.

Drug interactions of barbiturates are pronounced because of the induction of hepatic microsomal enzymes caused by barbiturates.

Table 5.1 Sedative-Hypnotic Barbiturates in Clinical Use in the United States and Europe*



(1)

Barbituric Acid	Generic Name	Trade Name	R_5	R	Other Modifications	Duration of Action (h)	Onset of Action (h)	Average Adult Hypnotic Dose (g)
5-Ethyl-isopentyl	Amobarbital	Amytal	C_2H_5	$(CH_3)_2CHCH_2CH_2$		2-8	0.25-0.5	0.1–0.3
5-Allyl-5-isopropyl	Aprobarbital	Resedorm	$CH_2{=}CHCH_2$	$(CH_3)_2CH$		2-8	0.25–0.5	0.065-0.13
5,5-Diethyl	Barbital	Barbitalum	C_2H_5	C_2H_5		4–12	0.5–1	0.3-0.5
5-(1-Cyclohexen-1-yl)-1,5-dimethyl	Hexobarbital	Sombulex	CH_3	$(CH_2)_4CH{=}C-$	1–CH_3	1-4	0.25	0.25-0.4
5-Ethyl-1-methyl-5-phenyl	Mephobarbital	Mebaral	C_2H_5	C_6H_5	1–CH_3	1–4	0.25	0.2–0.4
5-Allyl-5-(1-methyl-2-pentynyl)	Methohexital	Brevital	$CH_2{=}CHCH_2$	$CH_3CH_2C{\equiv}CCH(CH_3)$	1–CH_3		0.08	0.05–0.12
5-Ethyl-5-(1-methylbutyl)	Pentobarbital	Nembutal	C_2H_5	$CH_3(CH_2)_2CH(CH_3)$		2–4	0.5	0.1–0.2
5-Ethyl-5-phenyl	Phenobarbital	Luminal	C_2H_5	C_6H_5		4–12	0.5–1	0.1–0.2
5-Isopropyl-5-ethyl	Probarbital	Ipral	C_2H_5	$(CH_3)_2CH$		4–12	0.5–1	0.12–0.25
5-Allyl-5-(1-methyl butyl)	Secobarbital	Seconal	$CH_2{=}CHCH_2$	$CH_3(CH_2)_2CH(CH_3)$		1–4	0.25	0.1–0.2
5-Allyl-5-*sec*-butyl	Talbutal	Lotusate	$CH_2{=}CHCH_2$	$CH_3CH_2CH(CH_3)$		2–4	0.5	0.12
5-Allyl-5-(1-methyl butyl)-2-thio	Thiamylal	surital	$CH_2{=}CHCH_2$	$CH_3(CH_2)_2CH(CH_3)$	2–S	4–12		0.075–0.15
5-Ethyl-5-(1-methyl butyl)-2-thio	Thiopental	Pentothal	C_2H_5	$CH_3(CH_2)_2CH(CH_2)$	2–S	4–12	30 Seconds	0.1–0.15

*The principal route of administration for barbiturates is oral. However many barbiturates (hexobarbital, methohexital, pentobarbital, thiopental, etc.) are also available in injectable formulation but indicated for anesthesia (not as sedative hypnotics).

Table 5.2 Sedative-Hypnotic Barbiturates in Clinical Use Outside the United States

Barbituric Acid	Generic Name	Trade Name	R_5	R	Other Modification	Duration of Action (h)	Onset of Action (h)	Average Adult Hypnotic Dose (g)
5,5-Diallyl	Allobarbital	Dial, Curral	$CH_2{=}CHCH_2$	$CH_2{=}CHCH_2$		2–8	0.25–0.05	0.1–0.3
5-(2-Bromoallyl-5-allyl	Brallo barbital	Vespoerone	$CH_2{=}C(Br)–CH_2$	$CH_2CH{=}CH_2$		2–4		0.15
5-see-Butyl-5-ethyl	Butabarbital	Butisol	C_2H_5	$CH_3CH_2CH(CH_3)$		2–4	0.5	0.1
5-Allyl-5-isobutyl	Butalbital	Sandoptal	$CH_2{=}CHCH_2$	$(CH_3)_2CHCH_2$		2–4	0.5	0.2–0.4
5-(2-Bromoallyl)-5-*sec*-butyl	Butallylonal	Pernoston	$CH_2{=}CBrCH_2$	$CH_3CH_2CH(CH_3)$		2–4	0.5	0.2
5-Butyl-5-ethyl	Butethal	Neonal	C_2H_5	n-C_4H_9		4–12	0.5–1	0.05–0.1
5-Crotyl-5-ethyl	Crotyl Barbital	Melidorm	C_2H_5	$CH_2–CH{=}CHCH_3$		2–4		0.25
5-(1-Cyclohexen-1-yl)-5 ethyl	Cyclobarbital	Phanodorm	C_2H_5	$(CH_2)_4CH{=}C–$		2–8	0.25–0.5	0.1–0.2
5-Allyl-5-(2-cyclopenten-1-yl)	Cyclopentenyl-allylbarbituric acid	Cyclopal	$CH_2{=}CHCH_2$	$(CH_2)_2CH{=}CHCH$		2–4	0.5	0.12–0.25
5-(1-Cyclohepten-1-yl)-5-ethyl	Heptabarbital	Medomin	C_2H_5	$(CH_2)_5CH{=}C–$		2–4	0.5	0.1–0.4
5,5-Diethyl-1-methyl	Metharbital	Gemonil	C_2H_5	C_2H_5	1–CH_3			0.1
5-(1-Methylbutyl)-5-[2-(methylthio)-ethyl]-2-thio	Methitural	Neraval	$CH_3SCH_2CH_2$	$CH_3(CH_2)_2CH(CH_3)$	2–S	4–12		0.075–0.15
1-Methyl-5-(2-bromoallyl)-5-isopropyl	Narcobarbital	Narcotal	$CH_2–C(Br){=}CH_2$	$(CH_3)_2CH$	1–CH_3	2–4		0.1–0.3
5-(2-Bromoally1)-5-isopropyl	Propallylonal	Noctal	$CH_2–C(Br){=}CH_2$	$(CH_3)_2CH$		2–4	0.5	0.1–0.2
5-Ethyl-5-(1-methyl-1-butenyl)	Vinbarbital	Delvinal	C_2H_5	$CH_3CH_2CH{=}C(CH_3)$		2–4	0.5	0.1–0.2
5-(1-Methyl-butyl)-5-vinyl	Vinylbital	Speda	$CH{=}CH_2$	$CH_3(CH_2)_2CH(CH_3)$		2–8		0.15

The rate of metabolism of several drugs (warfarin, phenytoin, tricyclic antidepressants, oral contraceptives, etc.) is increased.

Barbiturates are rarely used today, and when barbiturate treatment is initiated, it should be restricted to 2 weeks because of the risk of tolerance developing.

2.2.2 Benzodiazepines. Benzodiazepines **(2)** (see Table **5.3)** in low doses have a sedative effect; however, in higher doses, they induce sleep and may even lead to coma. The sedative effect of benzodiazepinesimpairs motor skills, attention, memory, judgment, and if severe, may lead to confusion and incoordination. The risk of accidents is increased, including an increase of road traffic accidents. Consumption of alcohol potentiates the effects of benzodiazepines.

Benzodiazepines also cause anti-anxiety, muscle relaxant, and anticonvulsant effects, amnesia, depression, and respiratory depression. Tolerance to benzodiazepines often develops but occurs less frequently than that with barbiturates.

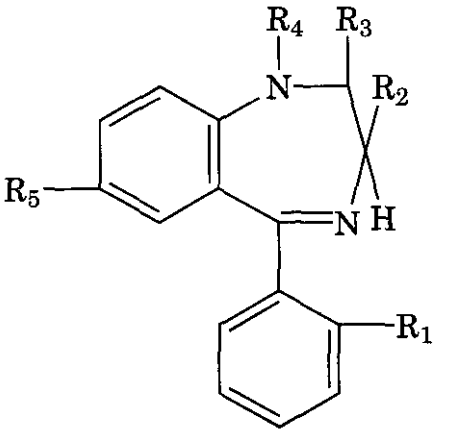
Withdrawal of benzodiazepines can cause rebound of the original symptoms. **A** transient worsening of these symptoms may occur especially if benzodiazepinesare used in high dose and for a long duration.

In 2001, Flunitrazepam has been subjected to the same prescribing restrictions as narcotics in several countries because of its continuing abuse by drug addicts.

The adverse effects of benzodiazepines include dizziness, vertigo, light-headedness, headache, changes in libido, ataxia, tremor, and urinary retention or incontinence.

The sedative and hypnotic effects of benzo diazepines are accentuated by other drugs acting on the gamma-aminobutyric acid, ($GABA_A$) receptor, especially barbiturates and alcohol. However, there are fewer drug

Table 5.3 Structures of Benzodiazepines



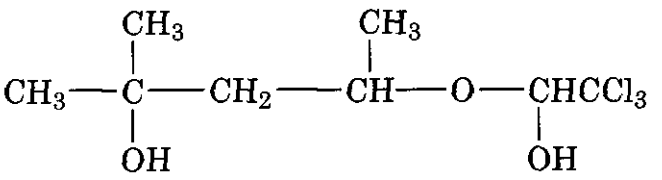
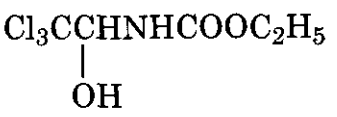
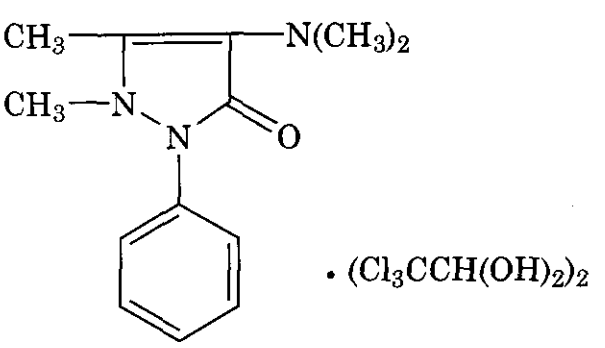
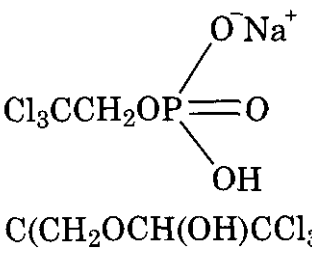
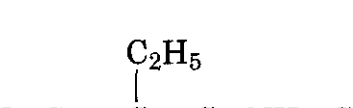
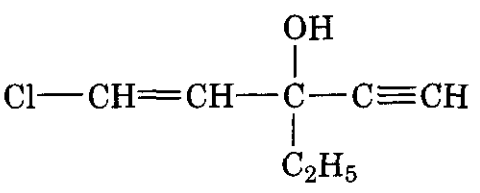
(2)

Benzodiazepine	R_1	R_2	R_3	R_4	R_5
Cinolazepam	F	OH	=O	$(CH_2)_2CN$	Cl
Delorazepam	Cl	H	=O	H	Cl
Doxefazepam	F	OH	=O	$(CH_2)_2OH$	Cl
Flunitrazepam	F	H	=O	CH_3	NO_2
Flurazepam	F	H	=O	$(CH_2)_2N(C_2H_5)_2$	Cl
Lorazepam	Cl	OH	=O	H	Cl
Lormetazepam	Cl	OH	=O	CH_3	Cl
Nimetazepam	H	H	=O	CH_3	NO_2
Nitrazepam	H	H	=O	H	NO_2
Nordazeparn	H	H	=O	H	Cl
Potassium clorazepate	H	COOK	OH OK	H	Cl
Quazepam	F	H	=S	CH_2CF_3	Cl
Temazepam	H	OH	=O	CH_3	Cl

Table 5.4 Benzodiazepines

No.	Generic Structure	Trade Name	Originator	Chemical Class	First w/w Launch	Dose (mg/day)
1.	Bromazepam	Lexotan	Roche	Pyridinyl-benzodiazepine	1973	3–6 p.o divided doses
2.	Brotizolam	Lendormin	Boehringer Ingelheim	Triazolo-diazepine	1974	0.25–0.50 p.o.
3.	Cinolazepam	Gerodorm	Gerot (Austria)	Benzodiazepine-propionitrile	1992	5–20 p.o
4.	Delorazepam	En	Ravizza (Italy) BASF/ Abbott	Chlordesmethyl diazepam	1980	0.5–2.0 p.o.
5.	Doxefazepam	Doxans	Searle Schiaparelli (Italy)	Hydroxyethyl-benzodiazepine	1988	5–20 p.o.
6.	Estazolam	Eurodin Esilgan	Takeda Abbott	Triazolo-benzodiazepine	1976	2–4 p.o.
7.	Flunitrazepam	Rohipnol	Roche	Nitro-benzodiazepinone	1974	1–2 p.o.
8.	Flurazepam	Dalmadorm Dalmane	Roche	Flurophenyl-benzodiazepinone	1968	10–30 p.o.
9.	Loprazolam	Dormonoct	Roussel (Aventis)	Piperazinyl-methylene benzodiazepine	1983	0.5–2 p.o.
10.	Lorazepam	Ativan	Wyeth-Ayerst	Chlorophenyl-benzodiazepine	1971	1–2.5 p.o. 25–50 μg/kg i.v.
11.	Lormetazepam	Noctamid Loramet	Schering A.G. Wyeth-Ayerst	Chlorophenyl-benzodiazepine	1980	0.5–2 p.o.
12.	Midazolam	Dormicum	Roche	Imidazo-benzodiazepine	1982	7.5–15 p.o. 2.5–7.5 i.v.
13.	Nimetazepam	Erimin	Sumitomo (Japan)	Nitro-benzodiazepine	1977	3–5 p.o.
14.	Nitrazepam	Mogadon	Roche	Nitro-benzodiazepine	1965	5–10 p.o.
15.	Nordazepam	Madar	Ravizza (Italy) BASF/Abbott	Benzodiazepinone	1975	2.5–10 p.o.
16.	Potassium Clorazepate	Tranxene	Sanofi/Abbott	Benzodiazepine carboxylate	1967	5–20 p.o.
17.	Quazepam	Quazium Doral	Schering-Plough (Italy) Wallace (US)	Trifluoroethyl-benzodiazepine	1985	15 p.o.
18.	Temazepam	Remestan Normison/ Restoril	Wyeth-Ayerst Novartis	Benzodiazepinone	1969	5–30 p.o.
19.	Triazolam	Halcion	Upjohn (Pharmacia)	Triazolo-benzodiazepine	1978	0.125–0.50 p.o.

Table 5.5 Halogenated Sedative Hypnotics

Structure	Structure Number	Chemical Name	Generic Name
$Cl_3CCH(OH)_2 \cdot (CH_3)_3\overset{+}{N}CH_2CO_2^-$	**(3)**	Chloral hydrate compound with betaine	Chloralbetain
		2-Methyl-2(2,2,2-trichloro-1-hydroxyethoxy)-2-pentanol	Chloralodol or chlorhexadol
		Ethyl *N*-(2,2,2-trichloro-1-hydroxyethyl carbamate	Carbochloral
		4-Dimethylamino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one compound with chloral hydrate	Dichloralphenazone
		2,2,2-Trichloro ethanol, di-H-phosphate, sodium salt	Triclofos
		Pentaerythritol hemiacetal with chloral	Petrichloral
		2-bromo-2-ethyl-butyrylurea	Carbromal
	**(4)**	1-chloro-3-ethylpent-1-en-4-yn-3-ol	Ethchlorvynol

interactions because benzodiazepines do not induce hepatic microsomal enzyme production.

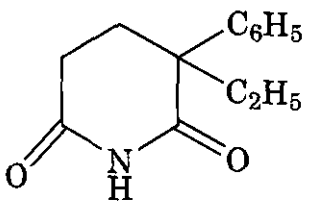
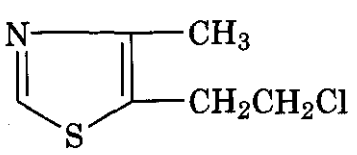
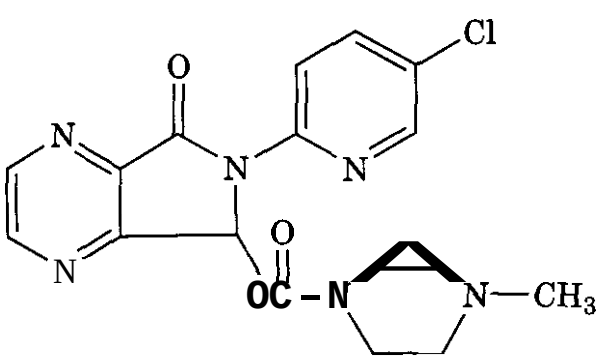
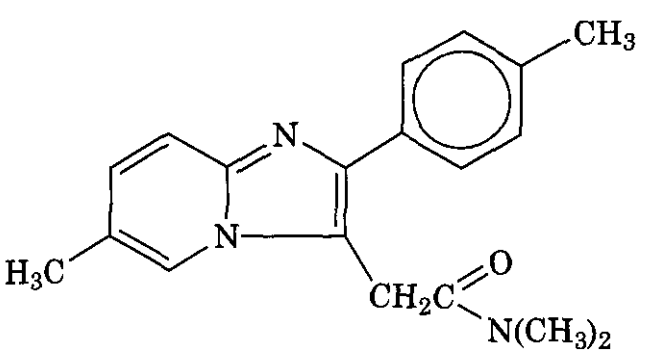
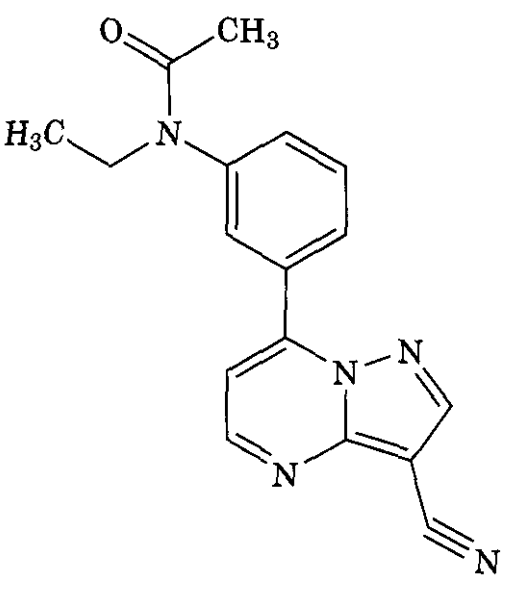
The advantages of benzodiazepines over older hypnotic drugs and especially over barbiturates are as follows: safer in overdose, less tendency to tolerance and dependency, less adverse potential, greater sedation-to-anxiolysis ratio, and fewer drug interactions.

2.2.3 Halogenated Sedative-Hypnotics. Most of these agents are only rarely used because of their high risk of causing tolerance and dependence and their danger in overdose. Chloral hydrate (3) (see Table 5.5) seems the least problematic with relatively low abuse potential; it is still used as an alternative to benzodiazepines. The adverse effects include gastric irritation, light headedness, ataxia, nightmares, excitement, confusion, allergic reactions, and skin rash. It also causes drowsiness and motor incoordination; therefore, chloral hydrate should not be used concomitantly with other CNS depressant drugs such as alcohol. The adverse effects of ethchlorvynol (4) (see Table 5.5) are similar to those of

Table 5.6 Halogenated Sedative Hypnotics

No.	Generic Name (Structure)	Trade Name	Originator	Chemical Class	Dose (mg/d)
1.	Chloral hydrate	Noctec	Squibb	2,2,2-Trichloro-1,1-Ethandiol	p.o. 500–1000
2.	Chloralodol or chlorhexadol	Lora	Wallace (USA)	2-Methyl-2(2,2,2-trichloro-1-hydroxy ethoxy)-2-pentanol	p.o. 800–1600
3.	Carbochloral	Prodorm	Parke-Davis	Ethyl *N*-(2,2,2-trichloro-1-hydroxyethyl carbamate	p.o. 400–800
4.	Dichloralphenazone	Welldorm Chloral01 Horner (Canada) Bonadorm Ferroson (Denmark)	Smith & Nephew (UK)	4-Dimethylamino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one compound with chloral hydrate	p.o. 225–700
5.	Triclofos	Triclos	Marion Merrell Dow	2,2,2-Trichloro ethanol, di-H-phosphate, sodium salt	p.o. 500–1000
6.	Petrichloral	Petrin	Parke-Davis	Pentaerythritol hemiacetal with chloral	p.o. 1200–2000
7.	Carbromal	Carbrital Somben Chinoin (Hungary) Talambrol Kwizda (Austria)	Parke-Davis	2-Bromo-2-ethyl-butyryl urea	p.o. 250–1000
8.	Ethchlorvynol	Placidyl	Abbott	1-Chloro-3-ethyl-pent-1-en-4-yn-3-ol	p.o. 100–500

Table 5.7 Heterocyclic Sedative Hypnotics

Structure	Chemical Name	Generic Name
	3-Ethyl-3-phenyl-2,6-piperidinedione	Glutethimide (5)
	5-(2-Chloroethyl)-4-methylthiazole	Clomethiazole (6)
	4-Methyl-1-piperazinecarboxylic acid ester of 6-(5-chloro-2-pyridy1)-6,7-dihydro-7-hydroxy-5H-pyrrolo {3,4-b jpyrazin-5-one	Zopiclone (7)
	(1) Imidazo[1,2-α]pyridine-3-acetamide,*N*,*N*,6-trimethyl-2-(4-methylphenyl)-,[*R*,-(*R**,*R**)]-2,3-dihydroxybutanedioate (2:1);	Zolpidem (8)
	*N*-{3-(3-Cyanopyrazolo[1,5-α] pyrimidin-7-ylphenyl)]-*N*-ethylacetamide	Zaleplon (9)

chloral hydrate. **Ethchlorvynol** is contraindicated in patients with porphyria, hepatic, and renal failures.

2.2.4 **Heterocyclic Sedative-Hypnotics.** The adverse effects of glutethimide (5) (see Table **5.7)** include nausea, headache, excitement, hangover, blurred vision, and skin rashes.

Overdose of glutethimide may cause even a greater danger to life than barbiturate overdosage (4); glutethimide has the highest mortality of drug-induced comas **(5).** Like barbiturates, glutethimide induces microsomal

Table 5.8 Heterocyclic Sedative-Hypnotics

No.	Generic Name	Trade Name	Originator	Chemical Class	First w/w Launch	Dose (mg/day)
1.	Glutethimide	Doriden Dorimide	Ciba-Geigy Cenci (USA)	Piperidinedione	1954	250600 p.o.
2.	Clomethiazole	Heminevrin	Astra (Sweden)	Methylthiazole	1957	192–384 p.o 8 mg/kg i.v.
3.	Zopiclone	Imovane	RPR	Pyrrolopyrazinone	1985	7.5 p.o.
4.	Zolpidem	Stilnox Ambien	Synthélabo Searle (USA)	Imidazopyridine	1988	5–10 p.o.
5.	Zaleplon	Sonata	Wyeth-Ayerst	Pyrazolopyrimidine	1999	10–20 p.o.

hepatic enzymes and can cause increased metabolism of anticoagulants and other drugs with reduced effects.

Clomethiazole **(6)** (see Table 5.7) may produce nasal irritation and sneezing on administration, conjunctival irritation, headache, gastrointestinal disturbances, nausea, vomiting, fever, cough, and tachycardia. It is contraindicated in patients with chronic pulmonary insufficiency, renal failure, or liver disease.

Zopiclone (7) (see Table 5.7) has a short half-life but can cause daytime sedation. However, it causes less morning-after drowsiness and fatigue than longer-acting hypnotics. Rebound phenomena following withdrawal have not proved a serious problem. Zopiclone causes minimal impairment of psychomotor performance and mental alertness the morning after night time administration (6).

Zolpidem (8) (see Table 5.7) rarely causes daytime sedation because of its short duration of action. However, it may cause nausea, vomiting, diarrhea, headaches, and dizziness. Tolerance to its use can develop, but this is less than with benzodiazepines. It may also cause mild respiratory depression. Withdrawal symptoms associated with zolpidem are unusual.

Zaleplon (9) (see table 5.7) is a very short-acting hypnotic that occasionally causes headaches and dizziness and may lead to respiratory depression. It rarely causes daytime sedation because of its short duration of action. Psychomotor impairment is unusual. The risk of tolerance is unknown; the withdrawal symptoms are rare, and dependency is unlikely.

2.2.5 Antihistamines. Antihistamines may produce gastrointestinal disturbances such as nausea, vomiting, diarrhea or constipation, anorexia, or increased appetite. They may also produce antimuscarinic effects such as blurred vision, dysuria, micturition, dryness of mouth, tightness of the chest, hypotension, muscular weakness, tinnitus, euphoria, and occasionally headaches. Antihistamines should not be given to neonates and are contraindicated during acute attacks of asthma. All antihistamines used as sedative-hypnotics belong to the class of H_1-receptor antagonists.

2.3 Absorption, Distribution, Metabolism, and Elimination

2.3.1 Barbiturates

2.3.1.1 Absorption. Barbiturates are well absorbed after oral administration usually given as sodium salts. They have a long duration of action, usually 6–10 h. Peak plasma levels occur in 2–12 h and are dose related.

2.3.1.2 Distribution. The lipid solubility (lipophilicity) of barbiturates varies and determines how readily they cross the blood-brain barrier.

It is possible to calculate the amount of barbituric acid in the undissociated form at various pH values from the equation:

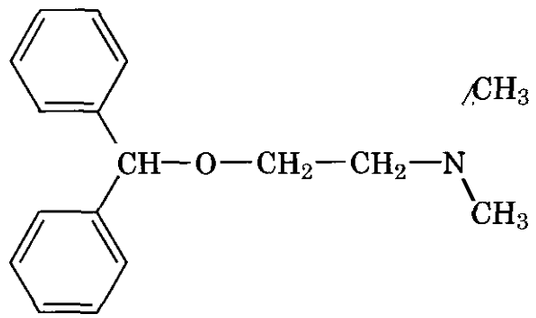
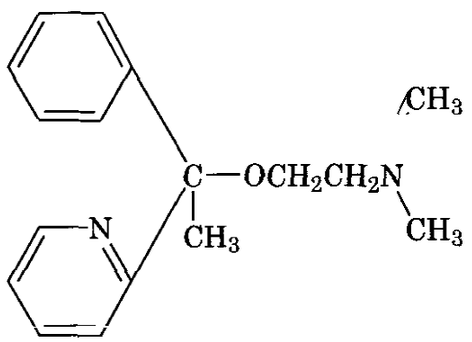
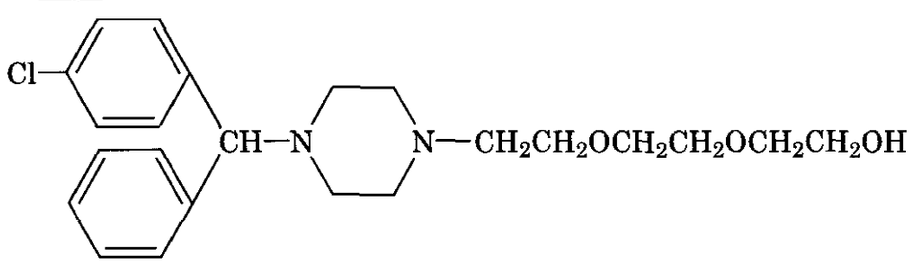
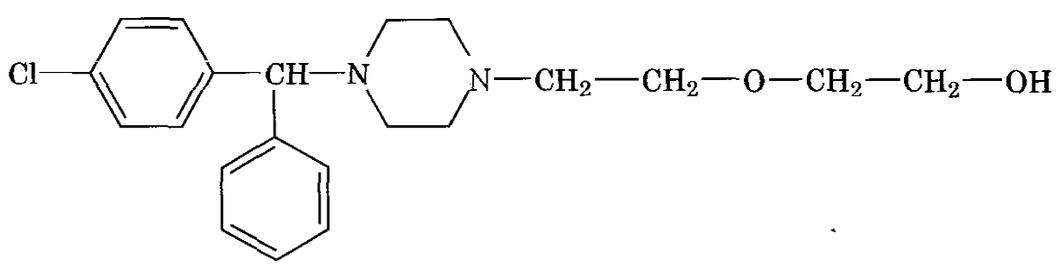
$$\log \frac{[A^-]}{[HA]} = \mathrm{pH} - \mathrm{p}K_a$$

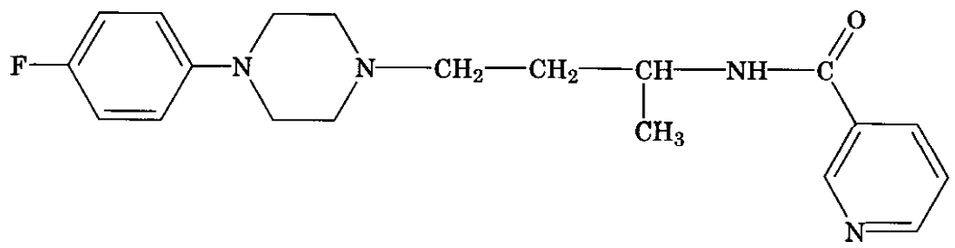
where [A-I = Concentration of the anion

[HA] = Concentration of the undissociated acid

The values of several examples are given in Table 5.13.

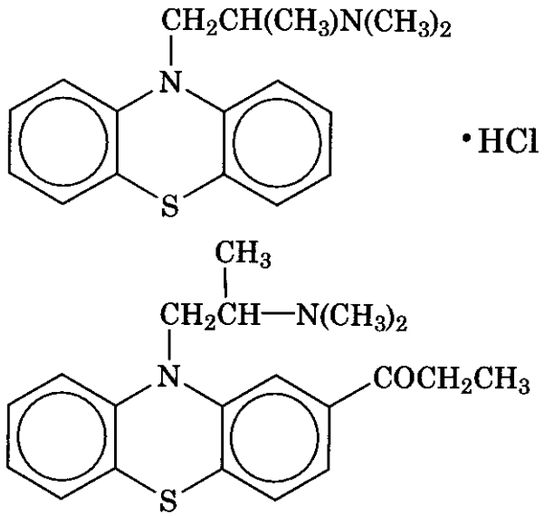
Table 5.9 Antihistamines

Structure	Chemical Name	Generic Name
	2-Benzhydryloxy-*N*,*N*,-dimethylamine	Diphenhydramine
	*N*,*N*-Dimethyl-2-[α-methyl-α-(2-pyridyl)benzyloxy]ethylamine	Doxylamine
	1-(*p*-Chloro-α-phenylbenzyl)-4-[2-[2-(2-hydroxy-ethoxy)ethoxy]-ethylldiethylenediamine	Etodroxizine
	2-[2-[4-[(4-Chlorophenyl)phenyl-methyl]-1-piperazinyl]- ethoxylethanol	Hydroxyzine



N-[3-[4-(*p*-Flurophenyl)-1-piperazinyl]-1-methyl-propyllnicotinamide

Niaprazine

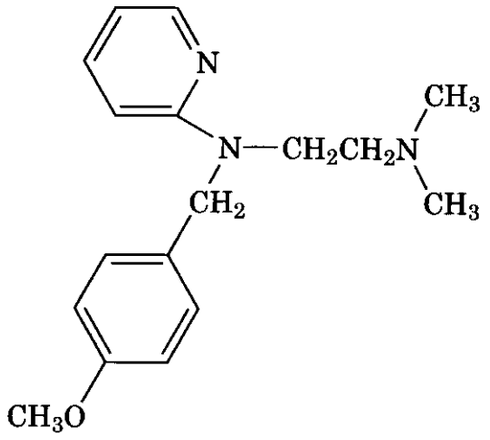


N,*N*α-Trimethyl-10H-phenothiazine-10-ethanamine

Promethazine

1-[10-[2-(Dimethylamino)propyl]-10H-phenothiazin-2-yl]-1-propanone

Propiomazine



2-[(2-Dimethyl-amino-ethyl)(p-methoxybenzyl)amino]pyridine

Pyrilamine

Table 5.10 Antihistamines

No.	Generic Name (Structure)	Trade Name	Originator	Chemical Class	Dose (mg/day)
1.	Diphenhydramine	Benadryl	Warner Lambert	Ethanamine	25–50 p.o.
2.	Doxylamine	Unisom	Pfizer	Ethanamine	25 p.o.
3.	Etodroxizine	Indunox	UCB (Belgium)	Piperazinyl-ethoxy-ethanol	12.5–50 p.o.
4.	Hydroxyzine	Atarax	UCB (Belgium) Roerig (USA)	Piperazinyl-ethoxy-ethanol	50–100 p.o.
5.	Niaprazine	Nopron	Sanofi-Synthélabo	Piperazinyl-pyridine carboxamide	2 mg per kg
6.	Promethazine	Phenergan	RPR	Phenothiazine	15–50 p.o.
7.	Propiomazine	Propavan	Pharmacia	Phenothiazine	25–50 p.o.
8.	Pyrilamine (Mepyramine)	Kriptin Anhisan	Whitehall-Robins RPR	Ethanediamine	25–50 p.o.

Among the given examples, only 5-ethyl-5-phenylbarbituric acid and 1-methyl-5-ethyl-5-phenylbarbituric acid have a proper ratio of dissociated forms present at physiological pH to enable them to cross the blood-brain barrier and exert an effect in the CNS.

Lowering serum pH increases the non-ionized (undissociated) portion in the serum, enhancing diffusion into tissue, whereas higher serum pH has the opposite effect (7).

Estimates of the apparent volume distribution for phenobarbital vary nearly twofold from 0.36 to 0.67 L/kg. Phenobarbital enters the brain in a manner reflecting blood flow patterns, and in steady state, it is evenly distributed in gray and white matter. The binding to plasma proteins of barbiturates plays a minor role in distribution.

2.3.1.3 ***Metabolism.*** The principal site of metabolic inactivation is in the liver. In the metabolism, the lipophilic character of the barbiturates decreases, which in turn decreases the ability of the barbiturates to penetrate into the CNS. There are four pri-

Table 5.11 Other Sedative-Hypnotics

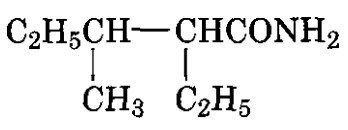
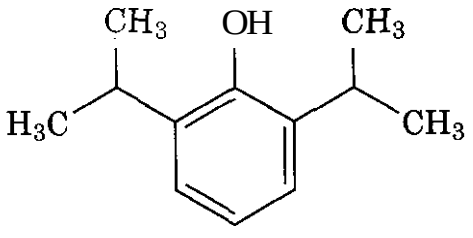
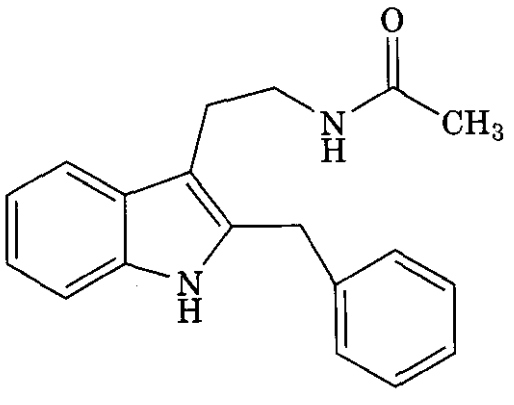
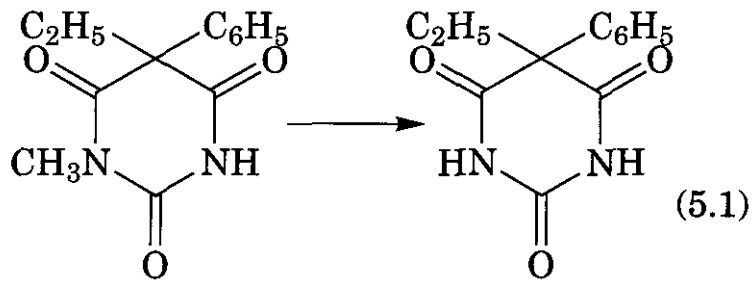
Structure	Chemical Name	Generic Name
	2-Ethyl-3-methylvaleramide	Valnoctamide
	2,6-Bis(1-methylethyl)phenol	Propofol
	*N*-[2-(5-Methoxy-1H-indol-3-yl)ethyl] acetamide	Melatonin

Table 5.12 Other Sedative-Hypnotics

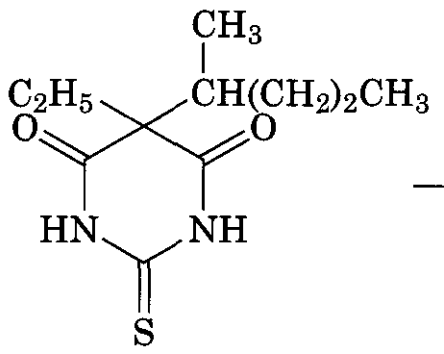
No.	Generic Name (Structure)	Trade Name	Originator	Chemical Class	Dose (mg/day)
1.	Valnoctamide	Axiquel nirvanil	McNeil (J&J) Recordati (Italy)	Valeramide	400–800 p.o.
2.	Propofol	Diprivan	Zeneca	Phenol	0.3–0.5 IV mg/kg/h
3.	Melatonin	Cronocaps melatol	Medix (Mexico) Elisium (Argentina)	Indolylethyl acetamide	1–3 p.o.

mary metabolic processes that may take place.

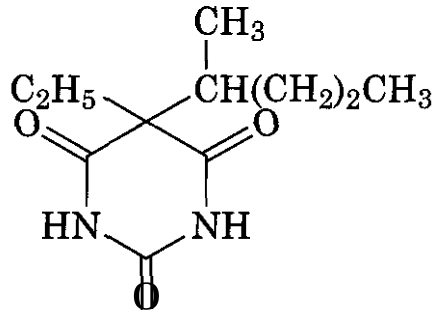
1. Oxidation of substituents attached to C5 is the most important pathway of metabolism for the barbiturates. The oxidative processes may yield alcohols, ketones, and carboxylic acids. For example, pentobarbital is oxidized to a hydroxy compound and a carboxylic acid (8) as shown in Fig. 5.2. The oxidative process may also yield phenols. If the barbiturate has a phenyl group attached to C5, by far the most important metabolic product is the p-hydroxyphenyl derivative, which has been shown to be formed through the intermediate epoxide (9). For example, phenobarbital is metabolized top-hydroxyphenobarbital (Fig. 5.3). The oxygenated metabolites (alcohols, phenols, ketones, and carboxylic acids) may be excreted in the urine in the free form or conjugated with glucuronic or sulfuric acid.
2. N-Dealkylation (N-demethylation) is an important metabolic pathway for *N*-substituted barbiturates (10). Mephobarbital (1-methyl-5-ethyl-5-phenylbarbituric acid) is metabolized to phenobarbital (5-ethyl-5-phenylbarbituric acid), which is subject to further metabolic processes.
3. Desulfurization of 2-thiobarbiturates is a common metabolic process. For example,



1-Methyl-5-ethyl-5-phenylbarbituric acid — 5-Ethyl-5-phenyl barbituric acid (5.1)



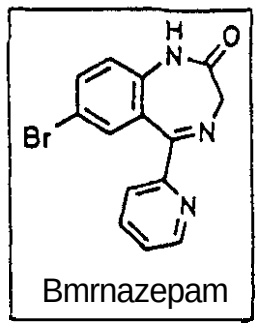
Thiopental (5.2)



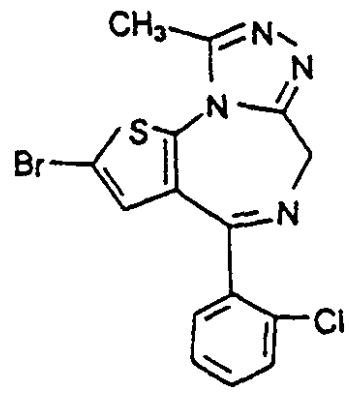
Pentobarbital

Table 5.13 Percentage of Various Barbituric Acids in Undissociated Form at Physiological pH (7.4)

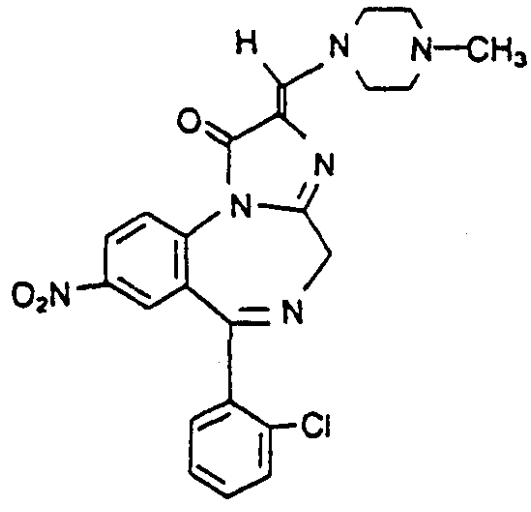
Compound	pK_a	Percentage of Undissociated Form
Barbituric acid	4.12	0.05
5-Phenylbarbituric acid	3.75	0.02
5-Ethyl-5-phenylbarbituricacid	7.29	43
1-Methyl-5-ethyl-5-phenylbarbituric acid	7.80	61
1,3-Diethyl-5-ethyl-5-phenylbarbituric acid		100



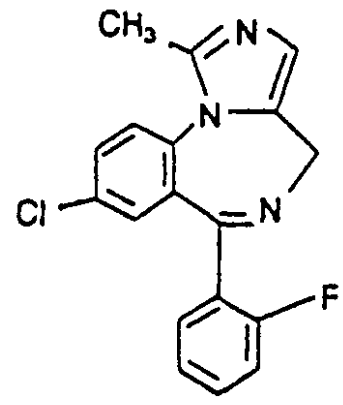
Bmrnazepam



Brotizolam

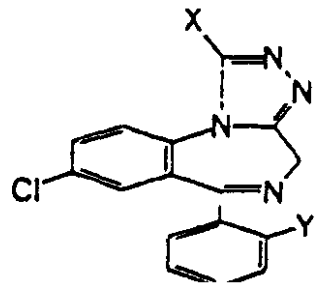


Loprazolam



Midazolam

Triazolo-benzodiazepines



Estazolam: X = H, Y = H
Triazolam: X = CH_3, Y = Cl

Figure 6.1. Benzodiazepine structures.

pentobarbital [5-ethyl-5-(1-methylbutyl) barbituric acid] is one of the metabolic products of thiopental [5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acid].

4. Ring scission of the barbituric ring leads to the formation of acetamides or acetyl urea derivatives. Both acetyl urea and acetamide derivatives are more hydrophilic than barbiturates. The biotransformations of barbiturates have been reviewed (11, 12).

2.3.1.4 ***Elimination.*** Barbiturates are eliminated from the body both by hepatic metabolism and by renal excretion. In the liver, phenobarbital is parahydroxylated and subsequently conjugated to glucuronic acid.

Both unmetabolized and parahydroxylated phenobarbital are excreted in the urine. The extent of glucuronide formation of phenobarbital varies widely. Phenobarbital elimination has first-order kinetics and thus is indepen-

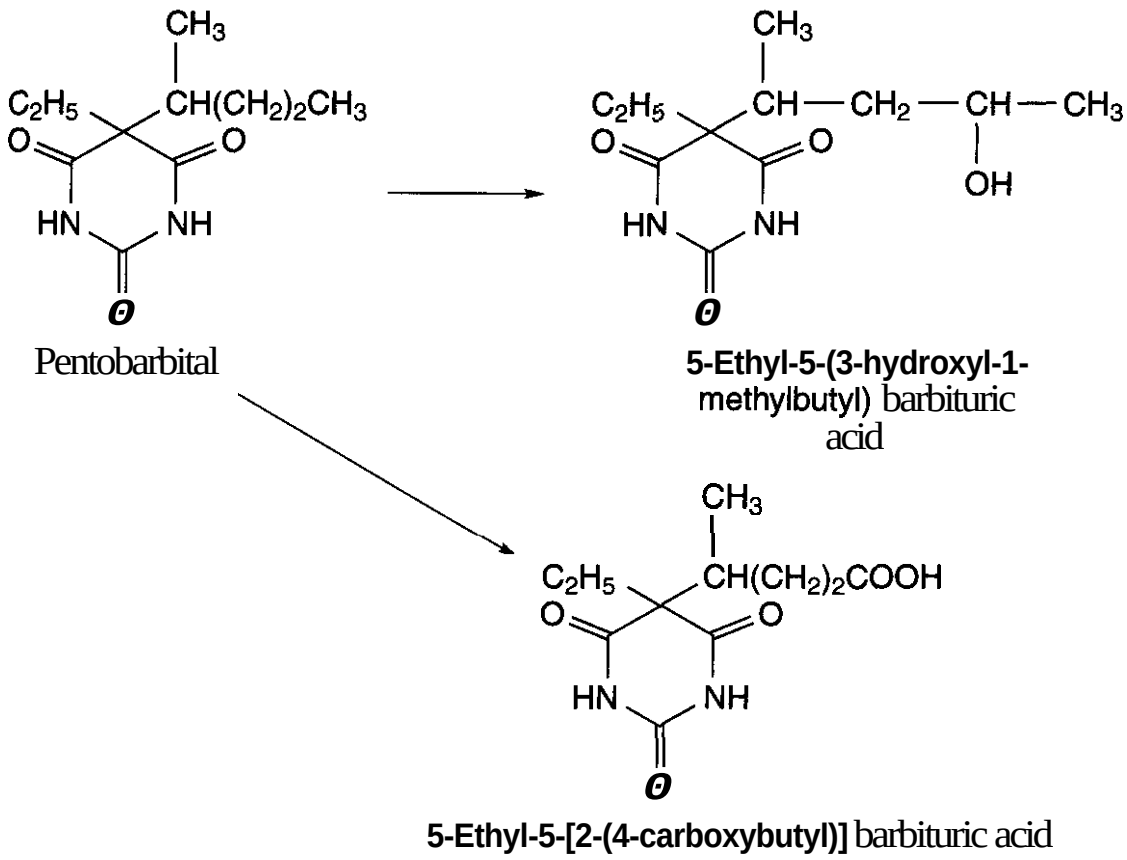
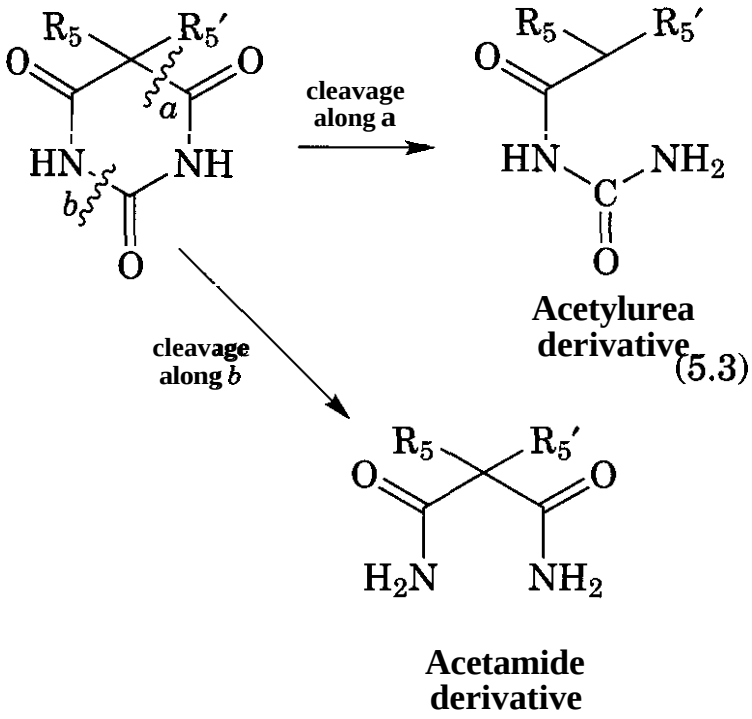


Figure 5.2. Metabolism of pentobarbital.



dent of concentration. From **11%** to **50%** of phenobarbital is eliminated from the body per day, corresponding to a half-life range of **24–140** h. Average half-lives after single doses range from **75** to **126** h and are not influenced by route of administration. Alkalization of urine increases phenobarbital excretion.

2.3.2 Benzodiazepines. The pharmacokinetic properties of benzodiazepines are most important in selecting a specific drug for the treatment of sleep disorders. In fact, pharmacokinetic parameters provide a rational method for selecting a benzodiazepine specifically as a hypnotic. It has been suggested that only those benzodiazepines are suited as hypnotics that are rapidly detoxified and excreted **(13).** The most important factors contributing to the pharmacokinetic profile of a compound are absorption, distribution, metabolism, and elimination/clearance.

2.3.2.1 Absorption. The rate of absorption from the gastrointestinal tract after oral dosage determines the speed of the onset of action of a benzodiazepine. For a quick onset of action, the benzodiazepine must dissolve completely in the stomach and cross the stomach mucosa into the systemic circulation. The different dissolution and absorption kinetics of benzodiazepines will affect their onset of action. Once the benzodiazepine is in the systemic circulation, it must also cross the blood-brain barrier to enter the **CNS.** Therefore, the lipophilicity of the benzodiazepine is important in determining the entry into the **CNS** and the onset of clinical action. Most benzodiazepines are highly lipophilic with the 3-hydroxy-substituted benzodiazepines (lormetazepam, lorazepam, temazepam, and doxefazepam); triazolam is the least lipophilic.

2.3.2.2 Distribution. Distribution is one of the important factors contributing to the duration of action of benzodiazepines. Benzodiazepines normally cross the blood-brain barrier quite easily and exert a pharmacodynamic

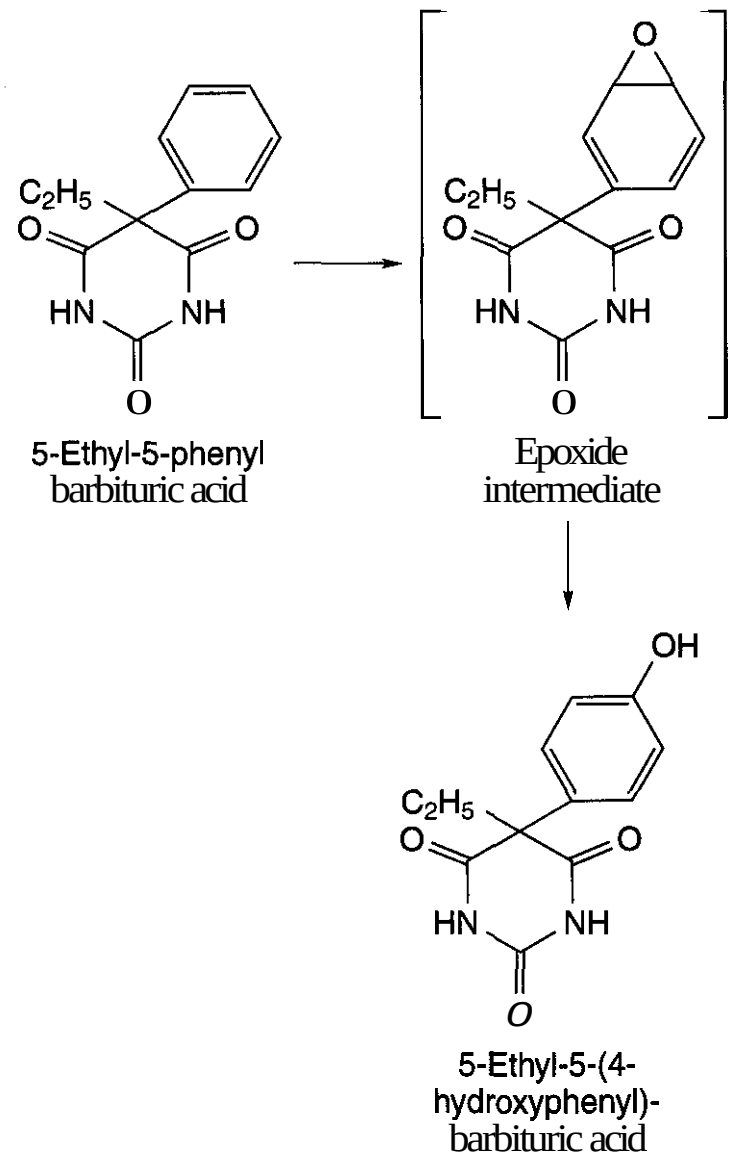


Figure 5.3. Metabolism of phenobarbital.

effect as long as their plasma concentration remains above a certain level. However, redistribution may influence plasma concentrations. Redistribution of a benzodiazepine may involve its reentry from the CNS across the blood-brain barrier into the systemic circulation and subsequent distribution into active storage sites. The phenomenon may explain the finding that the duration of clinical activity of a benzodiazepine is shorter than would be expected based on its elimination half-life.

Using the elimination half-life alone to predict duration of action of a benzodiazepine can be misleading (14). However, it can also be said that if elimination of a benzodiazepine is the rate-determining step and the absorption and distribution are comparable and fast, then the elimination half-life may provide a relative estimate of duration of action.

2.3.2.3 Metabolism. The role of decline in plasma concentration of benzodiazepines is determined by redistribution of the drug into inactive storage sites, elimination, and metabolism.

The presence of active metabolites contributes heavily to the duration of action of benzodiazepines. Some active metabolites have much longer plasma half-lives than their parent compounds. If the parent drug is not biotransformed into active metabolites, the duration of action is determined by the rate of elimination of the parent compound. The biotransformation pathways of several benzodiazepines are shown in Figs. 5.4, 5.5, 5.6, and 5.7.

Benzodiazepines may be divided into three classes based on their pharmacokinetic characteristics:

1. Short half-life drugs, such as triazolam, midazolam, and brotizolam, with elimination half-lives of up to 6 h.
2. Medium half-life drugs, such as temazepam, estazolam, lormetazepam, and loprazolam, with half-lives of 6–12 h.
3. *Long-acting* drugs, such as nitrazepam, flurazepam, and flunitrazepam, with half-lives over 12 h.

The elimination half-lives of a number of benzodiazepines and their metabolites are shown in Table 5.14.

2.3.2.4 Elimination. Elimination half-life indicates the rate of drug disappearance from plasma (and from brain) after distribution equilibrium has been reached and after the elimination curve has entered its terminal phase (also called the β phase). Clearance is the ratio of the rate of elimination over the arterial plasma concentration and would better describe the elimination process. However, it is more difficult to measure clearance than half-life; therefore, half-life is used most often.

Half-life is useful in describing the relative extent of drug accumulation. The longer the half-life, the greater the accumulation ratio. The accumulation ratio is defined as the difference of plasma concentration during 24 h following a dose after steady state has been reached compared with the plasma concentration during 24 h following the first dose.

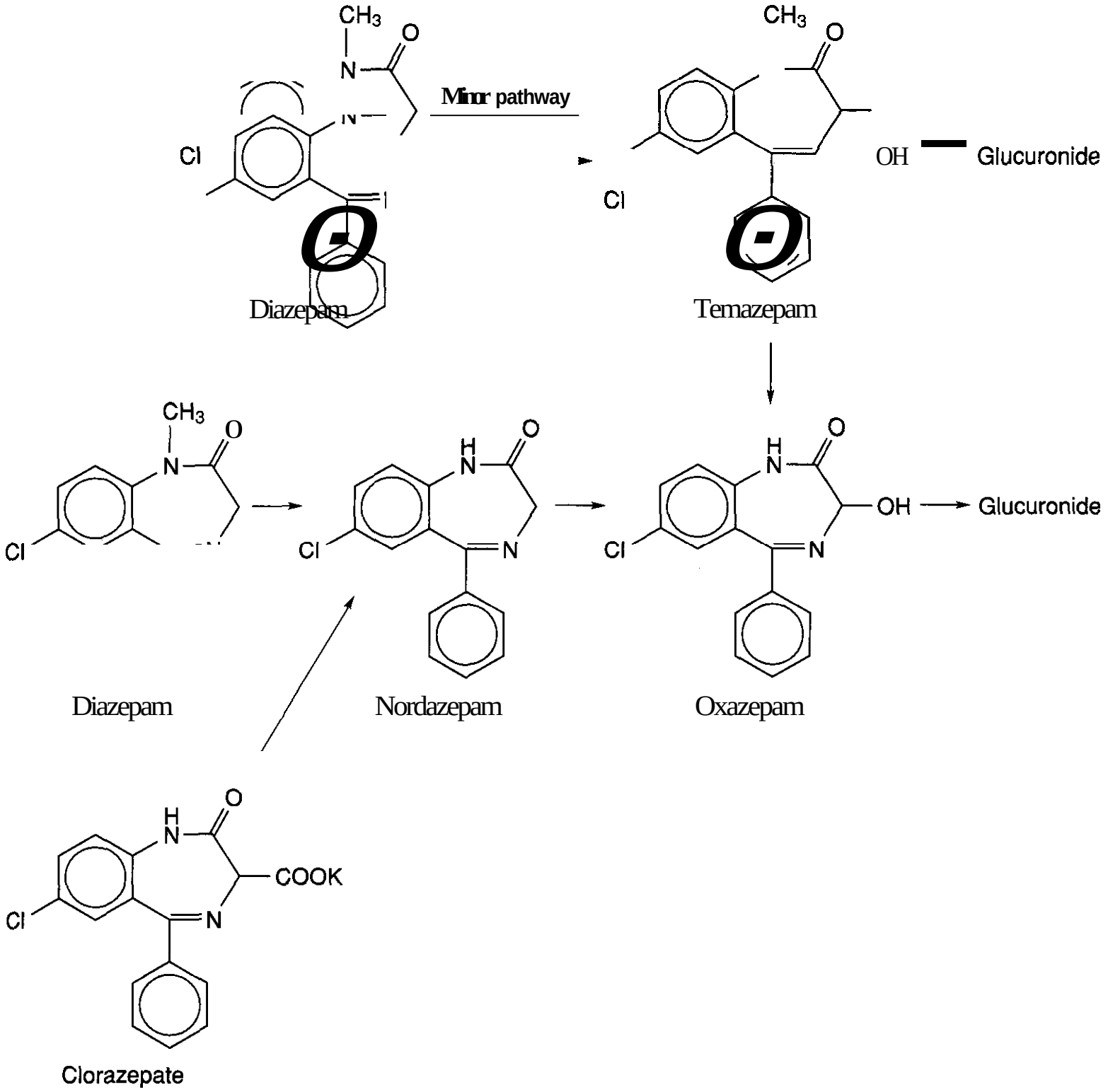


Figure 5.4. Biotransformation pathways of potassium clorazepate, diazepam, nordazepam, oxazepam, and temazepam.

Half-life also determines the time necessary to attain steady state. Long half-life hypnotics accumulate slowly but extensively, whereas short half-life benzodiazepines reach steady state rapidly and accumulate to a small extent.

Half-life also determines the time required for drug washout after multidose treatment is discontinued. Short half-life benzodiazepines disappear quickly, whereas long half-life compounds are eliminated slowly following the last dose.

These factors are important when considering the daytime drowsiness and psychomotor impairment produced next day after drug administration. In general, these residual effects occur more often after long-acting drugs (such as nitrazepam or flurazepam) than after intermediate (temazepam) or short-acting benzodiazepines (triazolam).

Drug dose is a critical issue in determining unwanted daytime sedation. Thus, 5 mg of nitrazepam produces few effects compared with 10 mg. Similarly, large doses (over 0.5 mg) of triazolam produce definite hangover effects.

After stopping benzodiazepines, patients may experience rebound insomnia or anxiety.

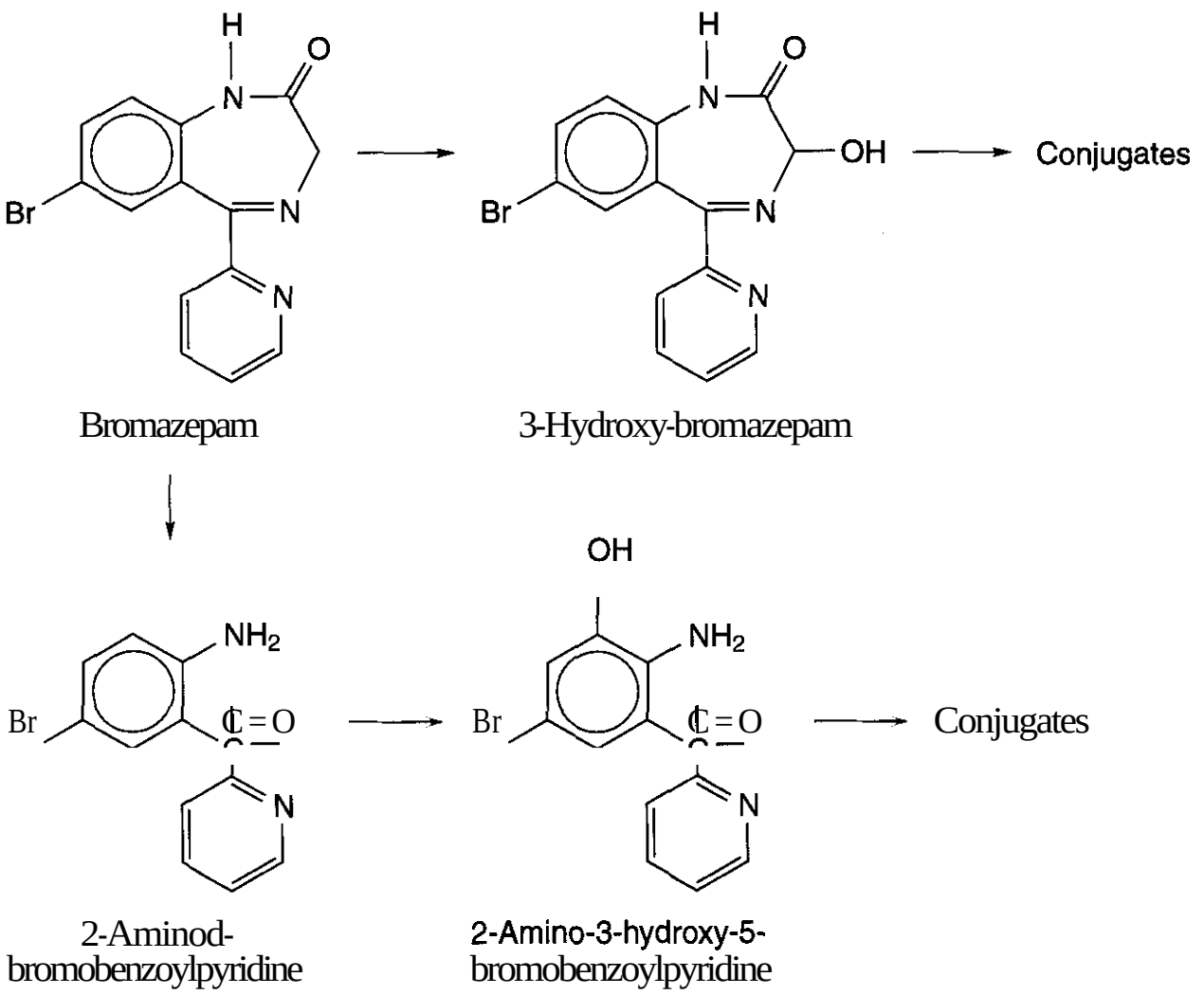


Figure 5.5. Biotransformation pathways of **bromazepam**.

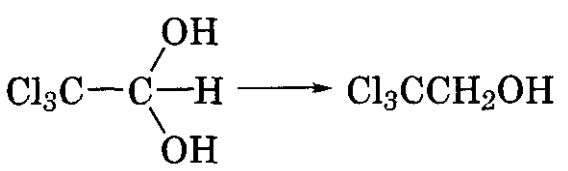
Rebound insomnia occurs more often with short-acting and intermediate-acting **benzodiazepines**, when given in high doses and withdrawn abruptly. Conversely, very long-acting benzodiazepines (such as **flurazepam** and **quazepam**) show milder rebound effects.

Ideally, a hypnotic drug should not produce unwanted drowsiness or **heavy-headedness** on awakening. Several studies in **humans** have indicated that **benzodiazepines** produce **morning**-after effects visible in EEG recordings. **Nitrazepam** (5–10 mg) and **flurazepam** (15–30 mg), for **example**, produce a shift to low voltage, high frequency activity in the EEG (15, 16), which is still present 12–18 h after a single dose of the drug. There is disagreement whether **benzodiazepine administration** also produces an **impairment** of **performance** of **intellectual** and motor function. Several investigators observed impaired performance (15–18), whereas others have not confirmed these findings (19).

It is generally agreed, however, that even though the **symptoms** of **hangover** may be recognized, **benzodiazepines, like all** other sedative-hypnotics, do produce psychomotor and EEG changes in many subjects on the **morning** after drug administration (13). Subjects given **benzodiazepines** should be warned of this fad.

2.3.3 Halogenated Sedative-Hypnotics

2.3.3.1 ***Chloral Hydrate.*** Chloral hydrate is rapidly absorbed from the stomach and starts to act within 30 min. However, no chloral hydrate blood levels are found in humans, because the metabolic conversion of chloral hydrate is very fast (Eq. 5.4).



$$Cl_3C-C(OH)(H)-OH \longrightarrow Cl_3CCH_2OH \qquad (5.4)$$

Chloral hydrate Trichloroethanol

The long-lasting hypnotic effect is caused by its metabolite, **trichloroethanol** (20).

Trichloroethanol passes into the cerebrospinal fluid and has a half-life of 7–11 h. In addition to **trichloroethanol**, chloral hydrate is also metabolized to **trichloroacetic** acid, which is inactive. Both **trichloroethanol** and **trichlo-**

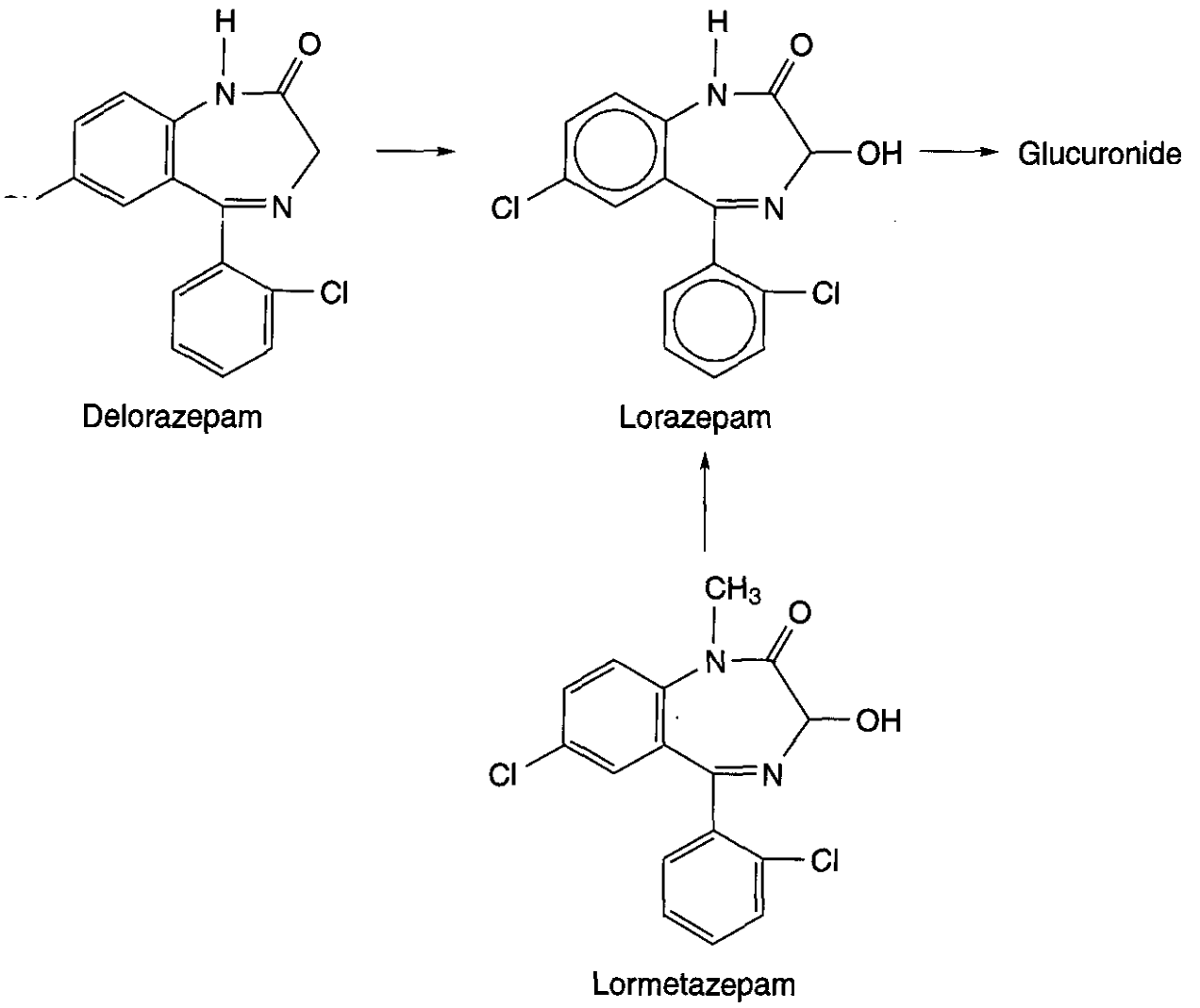


Figure 5.6. Biotransformation pathways of delorazepam, lormetazepam, and lorazepam.

roacetic acid are excreted in the urine and in the bile, in part, as glucuronides.

2.3.3.2 Ethchlorvynol. Ethchlorvynol is rapidly absorbed from the gastrointestinal tract and is extensively metabolized mainly in the liver and to a lesser extent in the kidney. The half-life of ethchlorvynol is biphasic with a rapid initial phase and a 10- to 25-h-long terminal phase. Only traces of unchanged drug appear in the urine. The metabolic half-life is 5.6 h, and it disappears from the plasma with a rapid α phase and a much slower β phase, because of extensive tissue redistribution.

2.3.4 Heterocyclic Sedative-Hypnotics

2.3.4.1 Glutethimide. Glutethimide is irregularly absorbed from the gastrointestinal tract and is extensively metabolized in the liver. It is about 50% bound to plasma proteins and has a biphasic plasma half-life. It is excreted in the urine with only up to 2% of unchanged drug and 98% as metabolites. Glutethimide is highly lipid soluble and may be stored in adipose tissue.

2.3.4.2 Clomethiazole. Clomethiazole is rapidly absorbed from the gastrointestinal tract and produces peak plasma concentrations in about 15–45 min after oral administration. It is broadly distributed in the body and is extensively metabolized in the liver and excreted in the urine with only very small amounts of unchanged drug appearing in the urine.

2.3.4.3 Zopiclone. Zopiclone is well absorbed from the gastrointestinal tract with peak plasma concentration reached within 2 h after administration. It has a half-life of 4–6 h and a duration of action of 6–8 h. It is metabolized in the liver to N-desmethylzopiclone and zopiclone N-oxide, and these two metabolites constitute about 36% of the dose excreted in the urine along with small amounts of zopiclone. Excretion of drug and metabolites was essentially complete 48 h after the final dose.

2.3.4.4 Zolpidem. Zolpidem is rapidly absorbed from the gastrointestinal tract and has a half-life of 1.5–2.5 h. It has a rapid onset of action that is detectable within 15–30 min. The peak plasma level is reached within 1–2 h

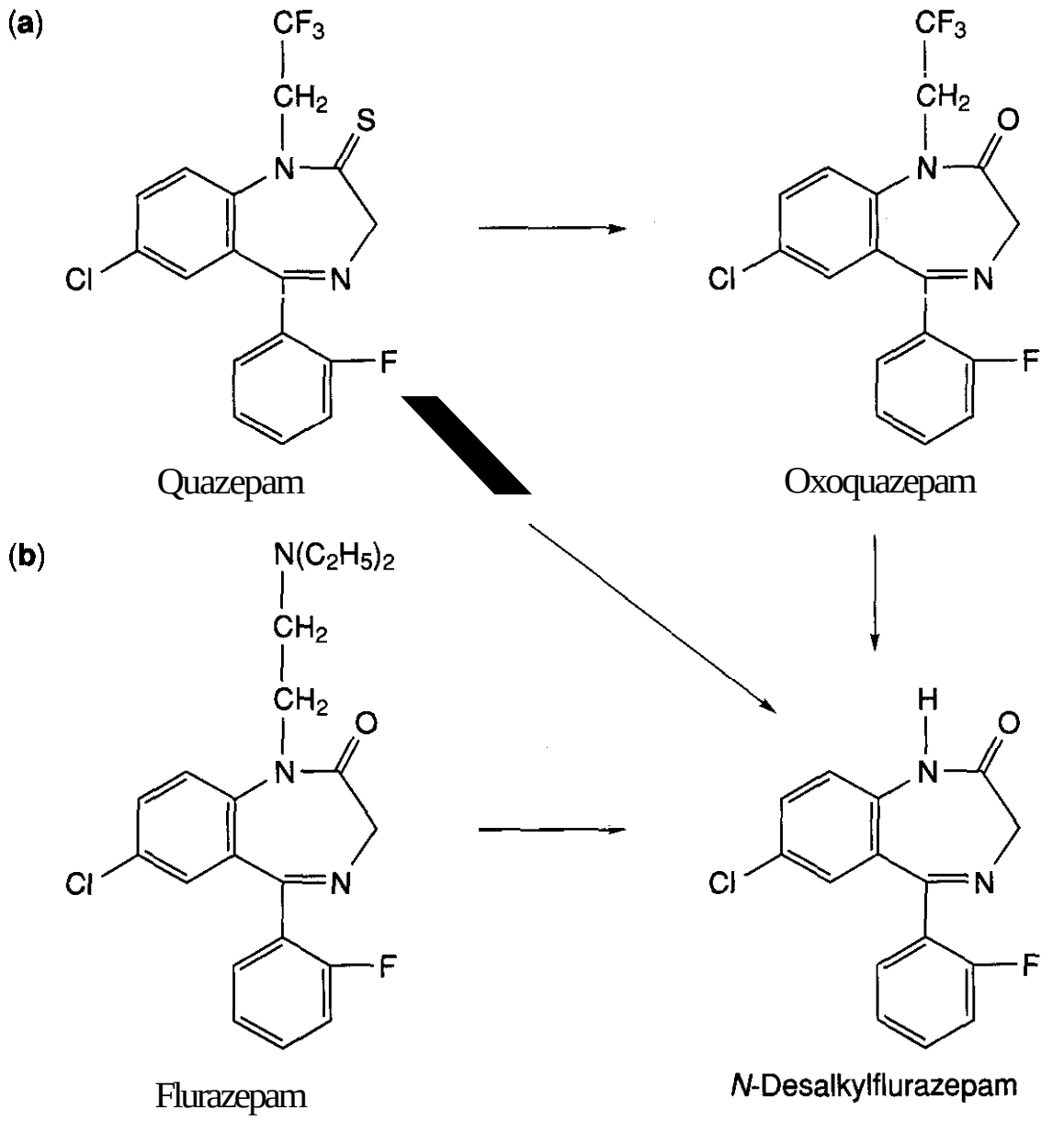


Figure 5.7. **Biotransformation** pathways of **quazepam** and ***flurazepam***.

after administration. Its clinical effect lasts 5–7 h. It is metabolized in the liver and gives rise to nitrogen demethylated and hydroxymethylated metabolites that are inactive and are eliminated primarily by renal secretion. The mean elimination half-life is 2.6 h. **Zolpidem** should not be administered with or immediately after a meal, because with food, the

Table 5.14 Elimination Half-Lives of a Number of Benzodiazepines and Their Metabolites

Drug	Elimination Half-Life of Parent Compound (h)	Active Metabolite	Elimination Half-Life of Metabolite (h)
Short half-life			
Brotizolam	5.0 (3–5)	1-Hydroxymethyl derivative	Short
Triazolam	2.3 (1.4–3.3)	1-Hydroxymethyl derivative	Short
Midazolam	2.5 (1–3)	1-Hydroxymethyl derivative	Short
Intermediate half-life			
Loprazolam	5.3 (4–8)	None	
Lormetazepam	9.9 (7–12)	None	
Temazepam	12.0 (8–21)	None	
Estazolam	12.00	None	
Long half-life			
Flunitrazepam	15.0 (9–25)	7-Amino derivative	23
Flurazepam	Very short	*N*-Desalkyl-flurazepam	87
Nitrazepam	28 (20–34)	None	
Clorazepate	1 (0.6–1.2)	Nordazepam (Desmethyldiazepam)	64
Quazepam		Oxoquazepam	40
		N-Desalkyl flurazepam	87

mean area under the curve and C_{max} were decreased by up to 25%.

2.3.4.5 ***Zaleplon.*** Zaleplon is rapidly absorbed from the gastrointestinal tract and reaches peak plasma level within 1 h after ingestion. It has a very fast onset of action. It has a half-life of 30 min and a short duration of action, less than 5 h. Zaleplon has a terminal phase elimination half-life of approximately 1 h. Zaleplon does not accumulate with once-daily administration, and its pharmacokinetics are dose proportional in the therapeutic range. The absolute bioavailability of zaleplon is approximately 30% because it undergoes significant presystemic metabolism. Zaleplon is a lipophilic compound. The blood-to-plasma ratio for zaleplon is approximately 1, indicating that zaleplon is uniformly distributed throughout the blood with no extensive distribution into red blood cells.

Zaleplon is primarily metabolized by aldehyde oxidase to form 5-oxo-zaleplon. Other metabolites include desethylzaleplon and 5-oxo-desethylzaleplon. Approximately 70% of the administered dose is recovered in urine within 48 h, almost all as zaleplon metabolites and their glucuronides. An additional 17% is recovered in feces within 6 days, mostly as 5-oxo-zaleplon. The effects of zaleplon on sleep onset are reduced if it is taken with or immediately after a high-fat or heavy meal.

2.3.5 **Antihistamines.** Antihistamines are rapidly absorbed from the gastrointestinal tract. After oral administration the onset of action of H_1-receptor antagonists is prompt, occurring within 0.5 h, although the peak histamine blockade may not occur until 5–7 h after oral administration.

Bioavailabiity has not been well studied because of the lack of intravenous formulations. Most first-generation histamine H_1-receptor antagonists readily cross the blood-brain barrier, which consists of the endothelial lining of the capillaries of the CNS. The first-generation antihistamines listed in Section 2.1.5 are used as sedative-hypnotics.

The second-generation H_1-receptor antagonists, such as fexofenadine, loratadine, and cetirizine, are relatively lipophobic and penetrate poorly into the CNS because of their larger molecular size or their electrostatic charge. The relative contribution of these factors is unknown.

Serum elimination half-life values differ greatly from one antihistamine to another. Most of the antihistamines are metabolized by the hepatic cytochrome P450 system and give rise to metabolites that are usually excreted in the urine.

3 PHYSIOLOGY AND PHARMACOLOGY

The clinical effects of sedative-hypnotics include sedation and sleep. Sedative-hypnotic drugs depress the function of the CNS and in a dose-dependent fashion produce drowsiness (sedation). Several sedative-hypnotic drugs, especially the older ones, produce sedation, sleep, unconsciousness, surgical anesthesia, coma, and ultimately may cause fatal depression of respiration and cardiovascular regulation.

Because these drugs facilitate the onset and maintenance of a state of sleep that resembles natural sleep, the physiology of sleep is of significant importance and is described below (Section 3.1). In the second part, the pharmacology is addressed (Section 3.2).

3.1 Physiology of Sleep

3.1.1 Wakefulness and Sleep. Sleep and wakefulness are two easily recognizable distinct functional states. The person in sleep shows a reduced awareness and responsiveness both to internal and external stimulation and his/her motor functions are inhibited. The sleeping person seems quiescent. However, the sleeping person exhibits some movements, indicating that sleep is an active process.

The cerebral activity of a human in wakefulness and in sleep display significant differences. In the human electroencephalogram (EEG), wakefulness is characterized by low amplitude waves and α rhythm. On the other hand, the EEG in sleep consists of high amplitude slow waves and spindles, indicating slow synchronized, idling neural activity. Electro-oculographic (EOG), electromyographic (EMG), and electrocardiographic (ECG) data have lent additional support to the finding that both wakefulness and sleep are active processes.

3.1.2 Sleep Studies. As early as 1907, Legendre and Pieron discovered the existence of endogenous sleep factors (21). Injection of blood serum from sleep-deprived dogs could induce sleep in dogs who were not deprived of sleep. The search for endogenous sleep factors is still continuing.

In the 1920s and 1930s, Kleitman studied the effects of sleep deprivation (22) and concluded that the build-up of endogenous sleep factors did not exceed certain limits, and humans became as impaired as they would get after approximately 2.5 days of wakefulness.

The first EEG recordings were reported by Berger in 1930 (23). He discovered differences in the electrical rhythms of subjects when awake or asleep.

In the late 1940s, sleep research in animals was conducted by implantable electrodes. This led to the designation of the ascending reticular activating system by Moruzzi and Magoun (24). They discovered that an important structure of the brain stem was interposed between sensory input and the higher centers of the brain. High frequency electrical stimulation of the brain stem reticular formation through implanted electrodes produced activation of the EEG, wakefulness, consciousness, and behavioral arousal. On the other hand, reticular deactivation produced by EEG synchronization leads to sleep and lack of consciousness.

In the early 1950s, Aserinsky and Kleitman (25) deployed the method of EOG for the measurement of eye mobility. Soon thereafter, Aserinsky and Kleitman (26) discovered that sleep onset is characterized by slow eye movement, which in time, changes to REM sleep. Subsequent research by Aserinsky and Kleitman led to the conclusion that REM sleep is associated with dreaming.

Dement and Kleitman (27) expanded their research in all-night sleep recordings using EEG and EOG techniques. They observed a sequence of patterns over the course of the night that revealed a basic sleep cycle.

3.1.3 States of Sleep. EEG studies have revealed that the activity of the brain is not constant during sleep. EOG and EMG data have lent additional support to the finding that sleep consisted of two distinct organismic states. As a result the following three physiological states exist:

1. Alert wakefulness
2. Nonrapid eye movement (NREM) sleep
3. REM sleep

3.1.4 Alert Wakefulness. In wakefulness, the EEG shows a high level of low voltage fast (LVF) frequency activity (9–10 Hz α activity mixed with low amplitude β activity). The EMG shows a high level of tonic activity and the EOG shows frequent eye movements.

3.1.5 NREM Sleep. This consists of four stages:

1. ***Stage 1 NREM Sleep.*** The first state of sleep (also called drowsy state). This is characterized by a 50% diminution of the α waves of wakefulness in the EEG and low amplitude, mixed frequency activities (in the range of 3–7 Hz) consisting of θ and some β waves. The muscular tone in the EMG becomes slightly relaxed, and slow rolling eye movements (SEM) are observed in the EOG. The state of drowsiness quickly changes to definite sleep.
2. ***Stage 2 NREM Sleep.*** The onset of stage 2 NREM sleep is observed by bursts of 12- to 16-Hz sleep spindles in the EEG. The EMG activity is less than in wakefulness and stage 1, and the EOG does not show eye movements. Stage 2 sleep lasts about 30–60 min.
3. ***Stage 3 NREM Sleep.*** Stage 3 sleep follows stage 2 sleep and is characterized by slow wave sleep. The EEG shows 20–50% of 6 waves of 2 Hz or less with amplitudes greater than 75 μV from peak to peak. The muscle tone in the EMG is more relaxed than before, and there are no eye movements in the EOG.
4. ***Stage 4 NREM Sleep.*** This stage is characterized by 6 waves of 2 Hz with amplitudes greater than 75 μV, which constitute more than 50% of the total. Toward the end of the deep sleep (δ sleep), sleep lightens, often abruptly to stage 2 NREM sleep, accompanied by body movements. After a

brief period of stage 2 NREM sleep, the first REM sleep occurs about 60–90 min after sleep onset.

3.1.6 REM Sleep. This is characterized by rapid eye movements. The slow wave pattern in the EEG is desynchronized and changes to a low amplitude mixed frequency θ including some α pattern and often displaying saw tooth waves. No sleep spindles are seen. The muscle tone below the chin is totally relaxed or abolished.

3.1.7 Sleep Cycle. The first REM period lasts a few minutes and changes to stage 2 and subsequently to stage 3 and stage 4 of NREM sleep before the second REM sleep period occurs. Accordingly, a full sleep cycle consists of a sequence of NREM sleep and REM sleep, and a cycle lasts about 90–110 min. Altogether, three to five REM periods occur during the night in 90- to 110-min cycles alternating with NREM sleep. However the various NREM sleep stages change in duration. The first two cycles are dominated by stages 3 and 4 NREM sleep. Subsequent cycles are dominated by stage 2 NREM sleep and stage 3 and 4 NREM periods are brief or do not appear at all.

Conversely, the REM sleep duration increases from the first to the last cycle. The last REM cycle is usually the longest REM cycle and may last as long as an hour. As a result, the first third of sleep is dominated by stages 3 and 4 NREM sleep and the last third of the sleep is dominated by REM sleep.

Sleep deprivation studies (28) have demonstrated the need for both NREM and REM sleep. Subjects deprived of sleep tend to spend more than a normal amount of time in NREM sleep when allowed to sleep. Similarly, if the subjects are awakened every time that REM sleep begins, they become selectively deprived of REM sleep, because every time they fall asleep again, the sleep cycle begins with NREM sleep. As a result, REM sleep occurs after shorter and shorter times. Furthermore, subjects deprived of REM sleep spend more than a normal amount of time in REM sleep when allowed to follow a normal pattern. This rebound affect is also observed after REM-sleep suppressing drugs are withdrawn (29).

Sleep laboratory studies are usually performed by applying the standardized methods developed by Rechtschaffen and Kales (30).

3.1.8 Neurotransmitters and Sleep. Based on the very elegant studies performed in cats, Jouvet postulated that there is a direct relationship between 5-hydroxytryptamine (5-HT, serotonin) and NREM sleep (31, 32).

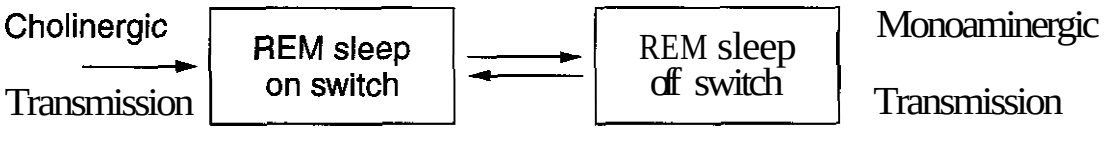
Correlations were also obtained for other neurotransmitters as well.

3.1.9 Neurotransmitters and REM Sleep in Humans. Neurotransmitters have both a direct and indirect action on REM sleep. The indirect action is manifested by a self-inhibitory feed back mechanism (Equation 5.5). Cholinergic transmission (acetylcholine) activate neurons in their pontine reticular formation (PRF) and produce REM sleep. REM sleep gradually activates REM-off monoaminergic neurons, which produce a self-inhibitory feedback and eventually terminates REM sleep. As the REM sleep period changes to NREM sleep, the REM-off neuronal activity gradually decreases during NREM sleep, and. is at minimum at the onset of REM sleep.

The role of REM sleep on and off switches are further accentuated by the effects of various drugs in humans in the NREM/REM sleep cycle (Equation 5.5 and Fig. 5.8).

3.1.10 Nonhypnotic Drugs Affecting REM On Switch

1. Muscarinic Agonists. The muscarinic/nicotinic agonist carbachol produces a long lasting REM sleep that is blocked by the administration of atropine. The same effect was obtained by using the selective muscarinic agonist bethanecol. Further studies revealed that M_2 muscarinic agonists are active, whereas the M_1 muscarinic agonists are without effects (33).



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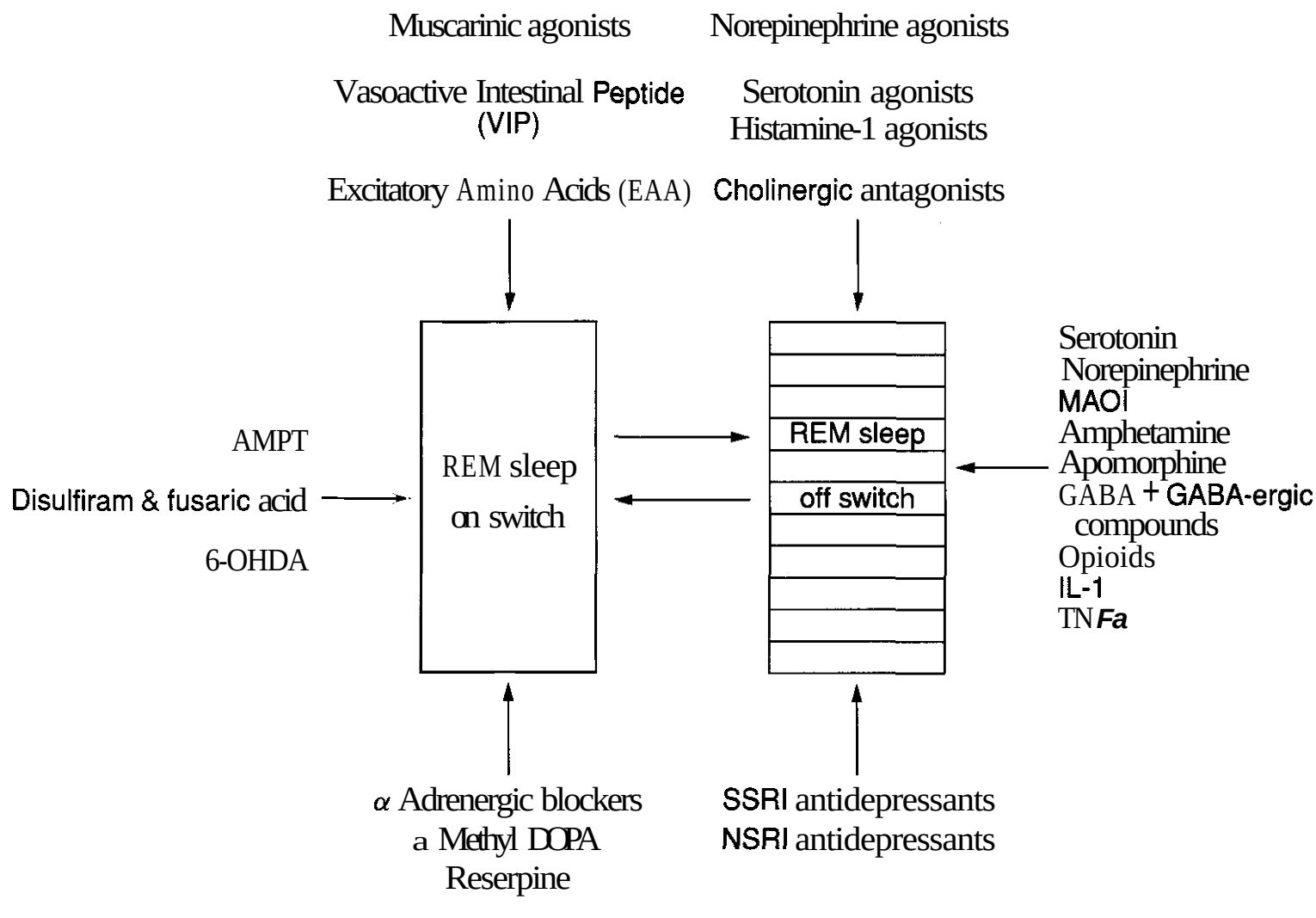


Figure 5.8. REM sleep on and off switches.

2. Excitatory Amino Acids (EAAs). Excitatory amino acids are claimed to be the principal excitatory transmitters in the reticular formation **(34).** EAAs including glutamate are involved in the positive feedback interaction between the cholinergic and the reticular neurons.
3. Vasoactive Intestinal Peptide. The peptide is found in the same neurons of the reticular formation where acetylcholine is also present. On injection, it enhances REM sleep.
4. a-Methylparatyrosine (AMPT). Administration of AMPT, an inhibitor of catecholamine synthesis, reduces both dopamine and norepinephrine brain levels and in turn, increases REM sleep.
5. Disulfiram and Fusaric Acid. Administration of these dopamine-β-hydroxylase inhibitors produces an increase in REM sleep several hours after the drug administration. This is caused by depletion of norepinephrine brain levels.
6. 6-Hydroxydopamine (6-OHDA). Administration of 6-OHDA destroys catecholamine-containing neurons and therefore decreases norepinephrine brain levels. In turn, REM sleep is increased.
7. Reserpine. Reserpine depletes monoamines (serotonin, dopamine, and norepinephrine) in the brain in a nonspecific manner. Depletion of these neurotransmitters in the brain facilitate REM sleep.
8. a-Methyldopa. Administration of α-methyldopa results in the synthesis of the false transmitter a-methylnorepinephrine, which selectively displaces norepinephrine from the vesicles. The decreased norepinephrine brain levels facilitate REM sleep.
9. a Adrenergic Blockers. Administration of phenoxybenzamine, thymoxamine, piperoxane, and yohimbine, all a, adrenoreceptor blockers, facilitates REM sleep. All these agents reduce norepinephrine concentration in the brain by enhancing norepinephrine release from the synaptic vesicles.

Interestingly, the β adrenergic receptor blockers in general have no effect on wakefulness, REM sleep, or NREM sleep, indicating that only a adrenergic receptors and not the β adrenergic receptors may be involved in the maintenance of sleep (35).

3.1.11 Nonhypnotic Drugs Affecting REM Off Switch

1. Cholinergic Antagonists. Atropine, which is a muscarinic antagonist that in some cases also blocks the effects of nicotinic agonists, suppresses REM sleep. The effects of the muscariniclnicotinic agonist carbachol, which facilitates REM sleep, are prevented by systemic administration of atropine. Similarly, scopolamine delays the appearance of REM sleep (35).
2. Histamine H_1 Receptor Agonists. Selective H_1 receptor agonists decrease both NREM and REM sleep stages. This action can be prevented by pretreatment with H_1 antagonists. In addition, it seems that blockade of CNS H_1 receptors leads to sedation accounting for the well-known sedative activity of antihistamines (36).
3. Monoamine Oxidase (MAO) Inhibitors. Inhibitors of the catabolic enzyme MAO increase both catecholamine and serotonin brain levels, which in turn produce significant and prolonged decrease in REM sleep. This is especially the case with phenelzine and nialamide nonselective MAO inhibitors and less pronounced with the reversible MAO-A inhibitor moclobemide. In normal subjects, moclobemide reduces REM sleep period during the first night, but by the third night, REM sleep returns to normal levels (37).
4. Amphetamine. Amphetamine facilitates dopamine release and inhibits norepinephrine reuptake, which results in increased postsynaptic norepinephrine levels. Amphetamine increases wakefulness and reduces REM sleep. These effects may be prevented by the preadministration of pimozide, a specific D_2 receptor antagonist, which inhibits presynaptic dopamine release. Propranolol, a p-adrenergic blocker, does not prevent the effect of amphetamine. In addition, the effect of amphetamine on sleep in cats is not attenuated by destruction of norepinephrine neurons, which seem to indicate that amphetamine-induced arousal and insomnia are mediated by dopaminergic mechanisms.
5. Apomorphine. This nonselective D_1/D_2 receptor agonist may interact with either presynaptic or postsynaptic receptors depending on the dose. Large doses of apomorphine delay and reduce REM sleep.
6. SSRI Antidepressants. Selective serotonin reuptake inhibitor (SSRI) antidepressants decrease REM sleep. This is ascribed to the increase in postsynaptic serotonin levels. Several drugs, including fluoxetine, that specifically inhibit the reuptake of serotonin induce wakefulness during sleep and increase alertness during the day.
7. NSRI Antidepressants. Nonselective serotonin reuptake inhibitors (NSRI)also block the reuptake of norepinephrine. Several of the old tricyclic antidepressants belong in this category. Imipramine and desipramine reduce REM sleep because of the increased norepinephrine levels at postsynaptic receptors caused by reduced reuptake of norepinephrine.
8. $GABA_A$ opioids, interleukin-1 (IL-1), and tumor necrosis factor a (TNFα) all inhibit REM sleep.

3.1.12 Neurotransmitters and NREM Sleep in Humans. Several agents that influence the brain levels of neurotransmitters have a profound effect on NREM sleep in humans. Most studies indicate that tryptophan, a precursor of serotonin, increases NREM sleep time and decreases sleep latency (38, 39). Tryptophan was used one time as a sleep-inducing agent, but it is no longer available because it causes eosinophilic myalgia syndrome.

Low and moderate L-DOPA dosages that are converted to doparnine produced no consistent effects on NREM sleep (40, 41), but high doses of L-DOPA suppress NREM sleep and total sleep, resulting in nearly total insomnia. Parkinson's syndrome patients treated with L-DOPA often complain of insomnia.

AMPT and a-methylphenylalanine(AMPA), both of which decrease catecholamine brain

levels, had very few measurable effects on NREM sleep. In general, GABA (inhibitory amino acid) promotes NREM sleep and influences sensory transmission and seems to have an opposite action to glutamate excitatory amino acid.

NREM On Switch Promoters	
Serotonin	DSIP
Melatonin	CCK
GABA	Insulin
Adenosine	IL-1
PGD_2	IFN
GHRH	TNF-α

CCK, cholecystokinin; DSIP, δ sleep inducing peptide; GHRH, growth hormone releasing hormone; IFN, interferons; IL-1, interleukin-1; TNF-α, tumor necrosis factor α. GABA, gamma-aminobutyric acid; PGD_2, prostaglandin D_2.

There is a mutual interaction of the factors controlling NREM and REM sleep. It is well documented that during selective REM sleep deprivation, there is also a significant reduction in the low frequency activity of the NREM sleep EEG (42). To explain the interaction between NREM and REM sleep, several models were proposed (43). The reciprocal interaction models on the NREM and REM sleep cycle (44) evolved from neurophysiological data obtained in animals. It is postulated that NREM-REM sleep cycle is caused by the reciprocal interaction of two neuronal systems in the brain stem with self-excitatory and self-inhibitory connections. For further details, recent monographs on sleep medicine should be consulted (45–47).

3.2 Pharmacology

Additional details are described in Section 5.3.

3.2.1 Evaluation of Sedative-Hypnotics. The methods usually employed in the evaluation of sedative-hypnotic drug candidates in small rodents (mouse or rat) involve measurements of the levels of CNS depression. These involve measurements of sleeping time (48), loss of righting reflex (49), performance on the rotarod (50), behavior in the activity cage (51–53), and finally, potentiation of other CNS depressants (54).

Recently more emphasis has been placed on the evaluation of drug candidates in the rat, cat, and monkey by measuring drug-induced changes in the sleep-wakefulness cycle. These studies have been reviewed (55).

The ultimate answer for the usefulness of a drug candidate is found in humans (56). Sleep laboratory studies in humans have been increasingly used recently in the evaluation of the effectiveness of hypnotic drugs. These studies have been reviewed (57, 58).

Briefly, evaluation of the effects of hypnotics on sleep physiology and sleep architecture, as measured by electroencephalography, has received increased attention. Continuous all-night electrophysiological measurement of sleep termed polysomnography (PSG) has made it possible to evaluate the action of hypnotics on sleep. PSG assessment as a means of evaluating the hypnotic effects of a drug candidate is now required by the U.S. FDA.

This method is used to determine sleep parameters including total sleep time, number of awakenings during sleep, sleep latency, sleep efficiency, effects on sleep stages, and sleep architecture (reduction of slow wave sleep or REM sleep, changes in the latency of REM sleep, etc.). Assessment of hypnotics on sleep include measures of nighttime sleep and daytime function, and quality of sleep, which is measured by changes in stage 1 sleep, which typically is increased in many insomnia conditions.

In addition to showing statistically significant improvement on various parameters (for example, latency of sleep, onset of sleep, or total sleep time in transient insomnia or maintaining sleep and reducing wakefulness in chronic insomnia) the adverse effects caused by hypnotics are also measured. These include effects on residual sedation, rebound insomnia (referring to increase in wakefulness or anxiety), amnesia, and adverse cardiopulmonary effects.

The goal of sleep research is to help find hypnotic drugs that will approximate the ideal hypnotic drug.

3.2.2 Pharmacologic Effects of Sedative-Hypnotic Drugs

3.2.2.1 Before Barbiturates. In ancient times, alcoholic beverages and herbal potions have been used to induce sleep.

The first chemical used specifically as a sedative and later as a hypnotic was bromide in the middle of the 19th century.

Bromides were followed by paraldehyde, urethane, and sulfonal. These medications were no great improvement because they could be addictive, and an overdose could kill. The launching of chloral hydrate was an improvement because chloral hydrate is a relatively safe hypnotic drug.

Pharmacologic effects of chloral hydrate:

- It induces sleep in 0.5 h.
- The sleep lasts about 6 h.
- It causes a relatively small reduction in **REM** sleep.
- It is used mainly in children and the elderly.
- It is most effective when used for only 1–3 nights to treat transient insomnia.

3.2.2.2 Barbiturates. A major event in the field was the launching of barbital (5,5-diethylbarbituric acid) in 1903 and phenobarbital (5-ethyl-5-phenylbarbituric acid) in 1912. The barbiturates dominated the field for nearly 50 years, until the launch of Librium (chlordiazepoxide) in 1960, the first of the benzodiazepine class drugs. Benzodiazepinescompletely overtook the barbiturates and had become The dominant class of sedative hypnotic drugs until the launch of retrocyclic sedative-hypnotics.

The pharmacologic effects of barbiturates are outlined as follows:

- Barbiturates are general CNS depressants. They depress the CNS at all levels in a dose-dependent fashion.
- Barbiturates decrease the amount of time spent in REM sleep.
- In sufficient doses, barbiturates are anticonvulsant and suppress convulsant activity.
- In sedative doses, barbiturates have little effect on the cardiovascular system. Toxic doses can cause circulatory collapse.
- Barbiturates depress respiration at any dose level.
- Barbiturates induce hepatic microsomal drug-metabolizing enzymes resulting in an increased degradation of barbiturates, ultimately leading to barbiturate tolerance.
- Because of their enzyme-inducing effects, barbiturates can cause increased inactivation of other compounds (anticoagulants, phenytoin, theophylline, digoxin, glucocorticoids, etc.). This may lead to serious problems with drug interactions.

3.2.2.3 Benzodiazepines, The launch of Librium in 1960 was quickly followed by the launch of diazepam in 1961, oxazepam in 1964, nitrazepam in 1965, clorazepate potassium in 1967, temazepam in 1969, lorazepam in 1971, and so on.

The benzodiazepines overtook the barbiturates because of the following disadvantages of barbiturates:

- They have a narrow therapeutic-to-toxic dosage range.
- They suppress REM sleep.
- Tolerance develops relatively quickly.
- They have a high potential for physical dependence and abuse.
- Drug interactions secondary to microsomal enzyme induction are frequent.

The pharmacological effects of benzodiazepines are outlined as follows:

1. Benzodiazepines exert multiple pharmacologic effects on CNS structures, including sedation, hypnosis, decreased anxiety, muscle relaxation, and anticonvulsant activity.
2. Benzodiazepinesare not general neuronal depressants as are barbiturates.
3. Benzodiazepines exert activity at various levels of the neuraxis, but some structures are affected to a much greater extent than are others.
4. Benzodiazepines have similar pharmacologic profiles, but the drugs differ in selectivity. The clinical usefulness of benzodiazepine drugs varies accordingly.
5. Benzodiazepines enhance GABAergic transmission in all CNS structures.

6. Benzodiazepines get attached to receptor sites that are highly specific. By combining with receptor sites, they elicit a pharmacologic effect.
7. All the effects of benzodiazepines that are mediated by receptors can be prevented or reversed by drugs that act as selective benzodiazepine antagonists.
8. Benzodiazepines show less tendency to tolerance and dependency than other older sedative-hypnotic drugs, especially barbiturates. Also, benzodiazepines produce less abuse potential.
9. Benzodiazepines are safer in overdose especially compared with barbiturates.
10. Benzodiazepines produce fewer drug interactions because they do not induce hepatic microsomal enzymes.

3.2.2.4 **Heterocyclic Sedative-Hypnotics.** Despite the advantages of benzodiazepines compared with other sedative-hypnotic drugs, tolerance and dependency do occur. Rebound insomnia may also occur either during each night of treatment with benzodiazepines or during withdrawal of treatment extended for several days. Cessation of benzodiazepines may also lead to recurrence of the original symptoms or even to transient worsening.

Heterocyclic compounds were synthesized and tested as sedative-hypnotics with the direct purpose of substituting them for both barbiturates and benzodiazepines (Table 5.7).

In quick succession, two heterocyclic non-benzodiazepine sedative/hypnotics were launched in 1985 by Rhone-Poulenc as zopiclone and in 1988 by Synthelabo as zolpidem. In 1999, Wyeth Ayerst launched another heterocyclic non-benzodiazepine sedative/hypnotic, zaleplon.

The pharmacologic effects of heterocyclic sedative-hypnotics are summarized as follows:

- All these drugs, zopiclone, zolpidem, and zaleplon, display affinities to the benzodiazepine receptor. However, problems with dependency and/or tolerance have not been reported.
- All these drugs elicit rapid onset of action.
- All these have a short half-life with no active metabolite.
- There is no objective evidence of rebound insomnia or tolerance in studies of up to 30–40 nights at recommended doses.
- All these have a favorable safety and tolerability profile.
- They generally preserve the stages of normal sleep.

3.2.2.5 **Antihistamines.** The pharmacologic effects of antihistamines are summarized as follows:

- Effects of first generation H_1-receptor antagonists in the CNS are mediated by blockade of endogenous histamine neurotransmitter.
- Conversely, antihistamines at high concentrations may inhibit the metabolism of histamine methyltransferase thus increasing the availability of histamine to act at CNS histamine receptor.
- These agents also block α-adrenergic receptors, serotonin receptors, and/or cholinergic muscarinic receptors, causing dysuria, micturition, dryness of the mouth, and tightness of the chest.
- High dose therapy may produce allergic reactions and cross-sensitivity to related drugs.
- Continuous use of antihistamine may produce tolerance, rendering the sleep-inducing effects essentially non-existent.

3.2.3 Clinical Pharmacology of Important Sedative-Hypnotics

3.2.3.1 **Benzodiazepines.** Bromazepam is usually administered orally between 3 and 18 mg daily in divided doses. In a clinical trial in children, bromazepam suppository and chloral hydrate suppository were compared as anesthetic premedication. Increasing doses of bromazepam were used in children. The group younger than 5 years were inadequately sedated compared with those receiving chloral hydrate. However, in the group 5 years and older, bromazepam and chloral hydrate were equally effective (59).

Brotizolam in 0.25- or 0.50-mg doses was compared in 38 clinical and epidemiological

studies with triazolam, zopiclone, zolpidem, midazolam, temazepam, lormetazepam, and loprazolam. A total of 5506 patients participated in the parallel-design and crossover studies. To provide clinically relevant comparisons, only studies using comparator agents in doses equipotent to the triazolam doses were included.

Two general findings emerged. First, significant CNS side effects such as excitement and violence were not observed with any of the hypnotic agents including brotizolam. With regard to other CNS side effects, depression, irritability, rebound insomnia, and early morning insomnia were observed for all hypnotics in these studies. Second, the authors claim that remarkable similarities were found among all these agents in terms of efficacy, side effects, and performance-related effects (60).

However, epidemiologic studies do not carry the same significance as direct comparative randomized double-blind studies conducted against placebo and a comparator drug.

Brotizolam (0.25 mg) in randomized double-blind studies was as effective as flunitrazepam (2 mg) (61) and triazolam (0.25 mg) (62). However, brotizolam produced markedly fewer adverse side effects than flunitrazepam. In addition, it was also reported (63) that the incidence of hangover and gastrointestinal problems was greater in patients treated with 2 mg flunitrazepam than in those treated with 0.5 mg brotizolam.

Brotizolam (0.5 mg) was as effective as 2 mg flunitrazepam in inducing sleep and was superior in maintaining sleep in a double-blind trial involving 40 patients due to undergo surgery (64).

Cinolazepam was evaluated in a double-blind placebo controlled trial in 20 normal subjects. The subjects were given either placebo or 40 mg cinolazepam orally 30 min before bedtime. A significant improvement in sleep maintenance was obtained with 40 mg cinolazepam, measured by sleep disruption using nocturnal traffic noise. Sleep architecture was only minimally affected, and sleep quality improved significantly (65).

Delorazepam in 2-mg doses in a controlled trial produced a hangover effect and behavioral impairment in humans, but no such effects were observed after a 1-mg dose or a 100-mg dose of amobarbital (66). The plasma levels of delorazepam correlated well with sleep-inducing effects. In a single-blind study, delorazepam in a 2-mg dose proved to be a highly effective hypnotic agent in 22 patients who suffered from long-lasting severe insomnia (67).

In 20 psychiatric patients, delorazepam (2 mg/d) was as effective as lorazepam (5 mg/d) in the improvement of anxiety and insomnia. No serious side effects were reported (68).

Doxefazepam in a 5-mg dose in a controlled clinical study in humans was equivalent to a 15-mg dose of flurazepam in terms of sleep induction time, sleeping time, and quality of sleep. Moreover, doxefazepam caused significantly less hangover effect compared with that caused by flurazepam (69).

In 40 patients, the efficacy of doxefazepam in a 20-mg dose did not diminish over 1 year, and no major side effects were reported (70).

In a double-blind study involving 1139 patients, estazolam in a 2-mg dose seemed to be equivalent to a 10-mg dose of nitrazepam in efficacy. A higher dose of estazolam (4 mg) was more effective but also caused more incidence of side effects than a 10-mg dose of nitrazepam (71). The incidence of unsteadiness caused by 2 mg of estazolam was the same as that caused by 5 mg of nitrazepam.

In a double-blind crossover study, estazolam (2 and 4 mg) was compared with nitrazepam (5 and 10 mg). Administration of 2 mg of estazolam prolonged total sleep time and decreased stage 1 and stage 2 sleep, whereas 4 mg of estazolam reduced stage 2 sleep and REM sleep. Nitrazepam (5 mg) produced prolongation of total sleep and a decrease in frequency of awakenings, whereas 10 mg of nitrazepam prolonged stage 2 sleep and reduced the total wakefulness during the night (72).

Flunitrazepam in a 2-mg dose was compared with zolpidem in a 20-mg dose. Forty-two insomniac female in-patients between 30 and 65 years of age were included in a double-blind, parallel group trial and were randomly allocated to the two treatments. Study duration was 9 days with 2 days of placebo run-in, 5 days of active medication, and 2 days of placebo withdrawal. Sleep latency, sleep dura-

tion, number of awakenings, and time spent asleep during the night were given an ordinal score; condition in the morning was evaluated by visual analogue scale (VAS), and the psychomotor performance was evaluated by the following tests: night-day for anterograde amnesia, digit span for verbal recall, and Grunberger's fine motor function test.

There was no difference between zolpidem and flunitrazepam for any of the variables; the drugs were significantly better than placebo baseline for all the sleep efficacy variables. The results of this study indicate that zolpidem is as effective as flunitrazepam in inducing and maintaining sleep, but it does not induce a sense of weakness in the morning and does not impair memory (73).

In a double-blind study of 102 patients with a mean age of 70 years, flunitrazepam was compared with zopiclone (74). The patients rated their sleep in a diary. There was no statistically significant difference between the relatively low dose of 5 mg zopiclone and 1 mg flunitrazepam for 11 of the 12 variables measuring subjective sleep quality and quantity. There was no difference between the drugs in regard to patients' feelings of being rested or alertness.

A multi-center, double-blind, randomized, placebo-controlled, parallel-group study compared the next-day residual effects, hypnotic efficacy, and sleep staging effects of flurazepam (30 mg) and zolpidem (10 and 20 mg) with those of placebo in patients with chronic insomnia.

As measured by objective and subjective criteria, both zolpidem and flurazepam were effective hypnotics. Sleep stages were affected more by flurazepam than by zolpidem. The incidence of treatment-emergent adverse events was approximately the same for zolpidem (10 mg), flurazepam, and placebo. The 20-mg dose of zolpidem (twice the therapeutic dose) was associated with a higher incidence of adverse effects. It was concluded that no next day residual effects are associated with nightly intake (three nights) of the recommended dose of zolpidem. At this dose, zolpidem was an effective and safe hypnotic (75).

Flurazepam (30 mg) was compared with estazolam (2 mg) and placebo in a multi-center, randomized, double-blind study for seven consecutive nights in insomniac patients. Both flurazepam and estazolam significantly improved sleep. There was no significant difference in hypnotic effect between flurazepam and estazolam.

The percentage of patients reporting any adverse effect was greatest for flurazepam followed by estazolam and placebo.

Estazolam and flurazepam effectively, and comparably, relieved insomnia when administered for seven nights in adult patients complaining of insomnia. Estazolam demonstrated a more favorable side effect profile than flurazepam (76).

Loprazolam (1mg) was compared with triazolam (0.25 mg) in a cross-over double-blind trial. The drugs were administered by a design of cross-over on the first two nights and continuation of the preferred treatment. Sixty-seven outpatients suffering from chronic insomnia took part in the study.

Both drugs provided improvement in sleep quality (decreased sleep latency, increased total duration of sleep, decreased number of night awakenings) and were equally well tolerated (77).

Lorazepam (1 mg) was compared with triazolam (0.25 mg), zolpidem (10 mg), zopiclone (7.5 mg), and placebo in a randomized double-blind study using 10 nocturnal polysomnograms with at least 72-h washout intervals. Six healthy middle-aged subjects who were normal sleepers (three men and three women) received single dose of the drugs both under basal and under perturbed conditions. For each individual, five recordings were carried out under basal conditions (sound pressure level not higher than 30 dB) and five recordings under acoustically perturbed conditions (continuous white noise at 55 dB). Sleep quality was assessed by visual analogue scale (VAS). Zolpidem produced the highest protective action of the four drugs during perturbation (78).

Lormetazepam (1mg) was compared with midazolam (15 mg) and zopiclone (7.5 mg) as night medication in patients scheduled for elective surgery the next morning. Sixty patients divided at random into three groups (double-blind) received the medication at one time.

The three hypnotics were equally effective as sleep medication for sleep onset latency, duration of sleep. and condition on awakening, whereas zopiclone provided significantly fewer spontaneous awakenings.

On the other hand. the lormetazepam group scored significantly better in an ocular imbalance test than the zopiclone group (79).

Midazolam (7.5 mg), flunitrazepam (1mg), and placebo were compared in a double-blind crossover trial to study the effects of drugs on sleep, nighttime respiration, and body movements in five elderly insomniac patients. No signs of increased respiratory resistance was seen with either of the drugs or placebo. There were no differences in the quality and quantity of sleep induced by either drug. Only the sleep onset latency was shorter with flunitrazepam compared with midazolam and placebo (80).

Nimetazepam is metabolized to nitrazepam. It is used as a hypnotic in 3- to 5-mg doses. The features and characteristics of nimetazepam are very similar to those of nitrazepam.

Nitrazepam in doses as low as 5–10 mg produces a hypnotic effect in humans comparable with a 100- to 200-mg dose of amobarbital (81–83), 50- to 200-mg dose of butabarbital (18, 83–85), 100- to 200-mg dose of secobarbital (86), and 250- to 500-mg dose of glutethimide (87). Nitrazepam suppressed REM sleep (88) in humans but the extent of suppression decreased with time (88).Sleep is longer lasting and less broken while using nitrazepam, and no tolerance was obvious after 2 months of nitrazepam use (89). After stopping nitrazepam, there is a rebound of REM sleep, which reaches a maximum in 1–2 weeks (90). Complete recovery after nitrazepam use takes 3–6 weeks (91). Nitrazepam also produces hangover effects with impairment of psychomotor performance and difficulty in falling asleep that may be longer lasting than those produced by sodium amobarbital (17).

Nordazepam is the principal metabolite of diazepam. It has been administered as a hypnotic in 7.5- to 15-mg daily doses (92).

The effects of nordazepam and a precursor, potassium clorazepate, on sleep were evaluated in humans (92). A dose of 5 or 10 mg nordazeparn or 15 mg of potassium clorazepate increased total sleep time and reduced the number of awakenings, and the time required to fall asleep was decreased. A dose of 5 mg of nordazepam had no effect on the duration of sleep stages. Nordazepam (10 mg) and potassium clorazepate (15 mg) decreased the duration of stage 2 sleep. During the recovery night, stage 1 sleep was reduced and stage 2 sleep was increased. No effects of stage 3 sleep were observed, but stage 4 sleep seemed to be suppressed.

Potassium clorazepate is decarboxylated rapidly at the pH of the stomach to form nordazepam (desmethyldiazepam), which is quickly absorbed. The peak plasma nordazepam concentration is obtained 45 min after clorazepate administration of 15 mg (93).

Potassium clorazepate is used in the United States in rather high doses of 15–60 mg daily in two to four divided doses or as a single dose at night. In the United Kingdom, a single dose of 15 mg potassium clorazepate is usually given at night or a dose of 7.5 mg is given three times a day.

Clorazepate in 22.5-mg daily doses administered for 8 days decreased REM sleep, stage 4 sleep, sleep latency, and total waking (94), whereas total sleep time was increased. During recovery, REM sleep, and sleep latency were increased (95).

Quazepam (15 mg) was compared with triazolam (0.5 mg) and placebo in 65 insomniac patients. The patients were treated with placebo for 4 days into the study, and if no amelioration of insomnia was observed, they were allocated randomly to the drugs with 32 patients receiving quazepam and 33 triazolam for 8 weeks and finally placebo for another week. The sleep quality, sleep efficacy, unwanted side effects, and the rebound effects had been assessed by specific evaluation. Quazepam had significantly less night awakenings. At the treatment's interruption, only the patients treated with triazolam had longer awakenings and rebound symptoms. In conclusion, quazepam has good hypnotic effect without inducing rebound effects (96).

Temazepam (15 mg) was compared with triazolam (0.125 mg), zolpidem (5 mg), and placebo. After a single-blind placebo screening weak 335 elderly insomniacs were randomized to 28 days of double-blind treatment with the

drugs or placebo followed by a 14-day single-blind placebo withdrawal period. The primary efficacy parameters were self-reported sleep latency (SSL) and self-reported total sleep duration (SSD); they were measured by responses on daily morning questionnaires.

Compared with placebo, zolpidem and temazepam produced significantly shorter SSL over the 4 treatment weeks, but triazolam did not. In the zolpidem group, SSL was significantly shorter than in the placebo group in all 4 treatment weeks; in the temazepam group, SSL was significantly shorter than in the placebo group at weeks 1, 3, and 4. SSD was increased above baseline levels in all groups. No tolerance to the subjective effects or rebound above baseline levels occurred in any of the treatment groups. Overall, the drugs were well tolerated. No difference was found among the placebo and treatment groups in overall adverse event incidence rates. However, compared with zolpidem and placebo, temazepam produced significantly higher incidences of drowsiness and fatigue, and triazolam produced a significantly higher incidence of nervousness than zolpidem (97).

Triazolam was compared with zolpidem and placebo in a double-blind randomized study in elderly insomniacs. The patients received zolpidem (5 mg; 70 patients), zolpidem (10 mg; 74 patients), or triazolam (0.25 mg; 77 patients). The 3-week active treatment period was preceded by 3 days and followed by 7 days of placebo administration. Both patients and clinicians evaluated sleep quality. The improvements between the end of placebo phase and the end of the active treatment phase were significant for all treatments. Overall evaluation indicated that zolpidem and triazolam are both effective in geriatric insomniac patients (98).

3.2.3.2 ***Heterocyclic Sedative-Hypnotics***

Zopiclone (7.5 mg) was compared to temazepam (30 mg) and placebo in a double-blind 3-week study in insomniac patients. The patients were assessed before and at the end of each of the 3 weeks active treatment phase and 1 week and 3 weeks posttreatment. The results indicated that zopiclone and temazepam possess a clinically significant hypnotic activity with no rebound insomnia or anxiety occurring during the week of drug withdrawal and that the two drugs are relatively safe and effective in the treatment of insomnia (99).

Zolpidem (10 mg) or triazolam (0.25 mg) were given to patients for 14 days in a randomized double-blind study. Data form 139 patients were used in the analysis. No statistically significant differences were found between the two groups regarding sleeping time, number of awakenings, or sleep quality. Morning feeling and day feeling were numerically better for zolpidem, although not statistically significant. There was no statistically significant difference in the number of patients experiencing side effects in the two treatment groups. On a short-term basis, administration of zolpidem (10 mg) seemed as effective and well tolerated as triazolam (0.25 mg) (100).

Zaleplon was evaluated for efficacy and safety compared with zolpidem. After a 7-night placebo (baseline) period, 615 adult patients were randomly assigned to receive, in double-blind fashion, one of five treatments (zaleplon: 5, 10, or 20 mg; zolpidem: 10 mg; or placebo) for 28 nights followed by placebo treatment for 3 nights. Sleep latency, sleep maintenance, and sleep quality were determined from sleep questionnaires that patients completed each morning. The occurrence of rebound insomnia and withdrawal effects on discontinuation of treatment were also assessed. All levels of significance were $P \leq 0.5$. Median sleep latency was significantly lower with zaleplon (10 and 20 mg) than with placebo during all 4 weeks of treatment and with zaleplon (5 mg) for the first 3 weeks. Zaleplon (20 mg) also significantly increased sleep duration compared with placebo in all but week 3 of the study. There was no evidence of rebound insomnia or withdrawal symptoms after discontinuation of 4 weeks of zaleplon treatment. Zolpidem (10 mg) significantly decreased sleep latency, increased sleep duration, and improved sleep quality at most time points compared with placebo; however, after discontinuation of zolpidem treatment, the incidence of withdrawal symptoms was significantly greater than that with placebo and there was an indication of significant rebound insomnia for some patients in the zolpidem group compared with those in the placebo group. The frequency of adverse side events in

the active treatment groups did not differ significantly from that in the placebo group. Zaleplon is effective in the treatment of insomnia. In addition, zaleplon seems to provide a favorable safety profile, as indicated by the absence of rebound insomnia and withdrawal symptoms once treatment was discontinued (101).

4 HISTORY

4.1 Discovery of Important Hypnotic Drugs

4.1.1 Before Barbiturates. The introduction of ether and chloroform in the 1820s led to the synthesis of similar chemical compounds. The famous German chemist Justus Liebig synthesized chloral in 1831 by passing chlorine through alcohol (hence its name). This led to the development of chloral hydrate, the first synthetic potent hypnotic drug that was safe enough for routine use. Chloral hydrate was introduced in 1869.

In 1887, a number of new sulfur compounds were synthesized by Eugen Baumann at the University of Freiburg, Germany and evaluated by Alfred Kast. One of these, sulfonal, was acquired and launched by Bayer in Germany.

4.1.2 Barbiturates. Based on the finding that some compounds containing a quaternary carbon (sulfonal and amylene) displayed hypnotic properties, in 1903, Emil Fischer and J. von Mehring synthesized 5,5-diethylbarbituric acid. This was marketed by Bayer as Veronal in the early 1900s. Since 1903, hundreds of barbiturates have been synthesized, but only a few turned out to be useful. In addition, because of their side effects, the synthesis of non-barbiturate hypnotics has been undertaken.

4.1.3 Halogenated Sedative-Hypnotics. Attempts to develop effective halogenated sedative hypnotic drugs without bothersome side effects have not met with success and resulted in only marginally useful products.

4.1.4 Heterocyclic Sedative-Hypnotics

4.1.4.1 The First Phase. CIBA (Novartis) introduced Doriden (glutethimide) in 1954 and Roche followed with the introduction of Noludar (methyprylone) in 1955. However, these two sedative hypnotic drugs displayed many of the unwanted side effects of the barbiturates. Noludar is no longer marketed and the sales of Doriden are insignificant today.

4.1.4.2 The Second Phase. The second phase of the development of heterocyclic sedative-hypnotics was initiated after the development and launch of the benzodiazepine sedative-hypnotics. Although the 1,4-benzodiazepines are safe and effective drugs, they too have produced certain undesirable side effects. This compelled some companies to develop non-benzodiazepine sedative-hypnotic drugs.

These include Rhone Poulenc (Aventis), the developer of zopiclone (launched in 1985), Synthelabo (Sanofi Synthélabo) the developer of zolpidem (launched in 1988), and Wyeth Ayerst, the developer of zaleplon (launched in 1999).

All three compounds elicit rapid onset of action and a full night's sleep. They have a short half-life with no active metabolites. There is no objective evidence of rebound insomnia or tolerance in studies of up to 30–40 nights at recommended doses. They have a favorable safety and tolerability profile and generally preserve the stages of normal sleep.

Zopiclone, zolpidem, and zaleplon have made major inroads in capturing significant market shares of the sedative-hypnotic market segment.

4.1.5 Benzodiazepines. After the first phase of the launch of heterocyclic sedative-hypnotics, as exemplified by glutethimide and methyprylone, most significant milestones were reached by Roche. In 1960, Roche launched Librium (chlordiazepoxide) and in 1963, Valium (diazepam). These two compounds were the first two 1,4-benzodiazepine class compounds launched in the world. Subsequently a number of companies launched several other 1,4-benzodiazepines for a number of indications (sedative-hypnotic, anxiolytic, anticonvulsant, and muscle relaxant).

Nitrazepam (Mogadon) was the first benzodiazepine marketed in 1965 by Roche as a specific sedative-hypnotic drug. At that time it was already well known that diazepam mar-

keted in 1963 had also displayed sedative-hypnotic activity, but diazepam was marketed primarily as an anti-anxiety agent.

The benzodiazepines became widely used and dominated the market for approximately 25 years.

However, the contention that benzodiazepines have solved the problems usually associated with the use of barbiturates has been contraindicated. It has been pointed out that in humans, benzodiazepines produce a considerable reduction in REM sleep, and in addition, an appreciable reduction of stage **3** and stage 4 sleep. Furthermore, it has also been reported that a distinction between barbiturates and benzodiazepines on the basis of withdrawal effects on the sleep pattern as documented by EEG measurements seems unwarranted. On the other hand, it should also be noted that, although in humans larger dose of the benzodiazepines suppress REM sleep, the extent of REM sleep suppression is usually smaller than with most other types of hypnotic drugs.

Benzodiazepines drugs are often taken in suicidal attempts but have rarely been fatal following even a large overdose. In this respect, benzodiazepines possess tremendous advantage over barbiturates and several other classes of hypnotic drugs.

4.1.6 Antihistamines. A number of antihistamines display sedative activity. Some of these drugs have been employed as sedative-hypnotics. Several antihistamines are available on the OTC market, which explains their relative popularity.

4.2 Discovery of Zolpidem

In the early 1990s, the French company Synthblabo assembled a group of well-trained scientists to focus on research in the CNS area. The focus of the CNS department was to discover innovative drugs that act through novel mechanisms and meet unfulfilled therapeutic needs. Another goal was to create new drugs with improved efficacy or safety in the areas of anxiety, depression, schizophrenia, sleep disorders, and neurodegenerative diseases.

4.2.1 The Use of Benzodiazepines. In the area of sleep disorders, the launch of benzodiazepines represented a major step forward. They replaced the barbiturates, which were very effective but produced significant side effects. The biggest problem was the potential fatal respiratory depression caused by overdose of barbiturates, which led to the barbiturates being used in suicide attempts.

The launch of benzodiazepines eliminated the risk associated with using barbiturates. The benzodiazepines are well tolerated and produce only low toxicities. However, after extensive use of benzodiazepines in millions of patients, it became apparent that benzodiazepines also produce side effects, although to a considerably lower degree than barbiturates.

The most important side effects of benzodiazepines include the following:

1. Benzodiazepines disturb the natural architecture of sleep, shortening REM sleep, stage 3 and 4 (the deep sleep) periods, and lengthening stage 2 sleep.
2. Extensive use of benzodiazepines may lead to psychological and physical dependence, withdrawal syndromes, and rebound insomnia on discontinuation of benzodiazepine treatment.
3. Benzodiazepines may also cause memory loss (anterograde amnesia), apneas, disabling residual effects on alertness, and may potentiate the CNS depressing effects of alcohol.

The recognition that benzodiazepines possess these adverse effects caused Synthblabo scientists to initiate research to develop a new sleep-inducing drug that would be devoid of side effects.

4.2.2 The Development of Zolpidem. The fact that there are several benzodiazepine receptor (BZR) site subtypes preferentially located in certain areas of the brain (see Section 5.2.2) encouraged Synthblabo scientists to search for new hypnotics that would lack the unwanted pharmacologic activities generally associated with the hypnotic activity of benzodiazepines and that would not cause tolerance, dependence, or rebound phenomena when treatment was discontinued.

The next step involved the search for compounds that would display selective activity

for BZR subtypes. This research culminated in the discovery of zolpidem, a selective agonist of the BZ-1 subtype that turned out to have selective hypnotic activity free of the disadvantages of the benzodiazepines.

Synthélabo's strategy consisted of three stages:

1. Analysis of three-dimensional molecular models helped determine the affinity of the ligands for their receptors and elaborate the chemical concept.
2. Synthesis of large number of heterocyclic compounds corresponding to the chemical concept led to the identification of the imidazopyridine class of compounds.
3. Lead optimization of imidazopyridines displaying optimal hypnotic properties led to the selection of SL 80, 0750 (zolpidem).

Zolpidem showed powerful sleep-inducing activity in EEG tests in rats with a short duration of action. In addition, zolpidem did not alter the architecture of sleep and thus induced physiological sleep. Clinical trials in human with zolpidem began in 1983 and confirmed the results obtained in animals. Furthermore, in humans, zolpidem had a short half-life combined with fast elimination from the body. Zolpidem was first marketed in France in 1988 and subsequently it was marketed worldwide. The features of zolpidem are described elsewhere in the chapter.

5 STRUCTURE-ACTIVITY RELATIONSHIPS

5.1 Quantitative Structure-Activity Relationships (QSAR)

5.1.1 Barbiturates. QSAR studies by Hansch presented evidence that the hypnotic activity of barbiturates depends largely on their relative lipophilic character as determined by octanol-water partition coefficients (102–104). Employing Equation 5.6, a QSAR analysis was conducted on over 100 barbiturates as well as a number of non-barbiturates having hypnotic activity.

$$\log 1/C = -k(\log P)^2 + k'\log P + k'' \quad (5.6)$$

In Equation 5.6, C represents the moles of drug per kilogram of test animal producing hypnosis, P is the calculated partition coefficient (based on the measured value for 5,5-diethylbarbiturate), and k, k', and k'' are constants derived by the method of least squares. The barbiturates were clustered into eight groups depending on the species of test animal and the means of measurement of hypnosis, such as ED_{50} or MED, as reported in the literature from which the biological test data were taken. A constant termed $\log P_0$ was derived by setting the derivative $(d)\log(1/C)/d\log P$ equal to zero and solving for $\log P$. $\log P_0$ was defined as representing "ideal liphophilic character" for a set of congeners under specific test conditions. For the five groups of barbiturates for which confidence intervals could be calculated, there was good general agreement among the P_0 values with a mean value of 1.9. P_0 values of about 2 were also obtained for various non-barbiturate hypnotics (e.g., acetylenic alcohols, *N, N'*-diacylureas) and it was suggested that any organic compound with $\log P = 2$, which is not rapidly metabolized or eliminated, would possess some hypnotic properties.

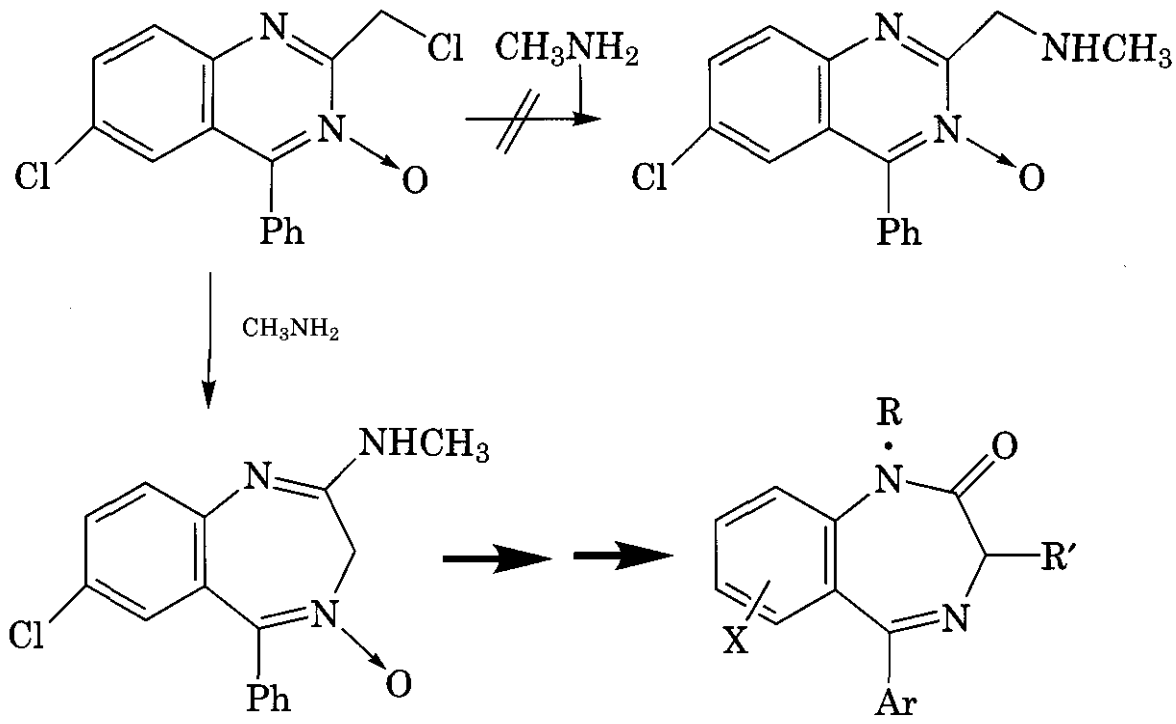
In a subsequent study, Hansch reported that the induction of cytochrome P450 by a series of 5,5-substituted barbiturates was directly related to their hydrophobicity (105). Analysis of the in vitro data for barbiturate-induced P450 in cultured chick hepatocytes yielded Equation 5.7, where C is the drug concentration that caused a 50% increase in cytrochrome P450 and P is the octanol-water partition coefficient.

$$\log 1/C = 1.02(\pm 0.16)\log P + 2.75(\pm 0.28) \quad (5.7)$$

$$n = 9, \quad r = 0.98, \quad s = 0.19, \quad F = 222$$

n = number of observations, r = correlation coefficient, s = standard deviation, F = statistical significance factor.

5.1.2 Benzodiazepines. Sternbach's discovery of the benzodiazepines, a major class of drugs having a diversity of therapeutically



(5.8)

chlordiazepoxide benzodiazepinones

useful pharmacological effects, must certainly rank among the landmark achievements in the annals of medicinal chemistry. Discovery of the prototype compound, chlordiazepoxide, was serendipitous in that it, rather than the simple substitution product, was formed by an unexpected ring enlargement from the treatment of a quinazoline N-oxide derivative with methylamine (Equation 5.8) (106, 107). After its structural elucidation, it was found that this compound possessed desirable pharmacological properties including anxiolytic, sedative, muscle relaxant, and anticonvulsant activities. Ensuing preclinical and clinical studies corroborated the initial findings and led to its introduction in 1960 as Librium. Synthetic investigations aimed at finding compounds with even better pharmacological profiles led to the conversion of N-oxides such as chordiazepoxide to the classical 1,4-benodiazepin-2-one chemotype (Equation 5.8); compounds of this type were found to have activity at least equivalent to that of chlordiazepoxide and some, such as diazepam, showed severalfold greater potency in various tests. Thus, alternate synthetic procedures were developed to obtain a variety of analogues and study SAR. A summary of the earlier qualitative SAR findings based exclusively on in *vivo* data is graphically shown in Fig. 5.9.

A QSAR study was carried out on over 50 1,4-benodiazepinones that were previously reported by Sternbach and that bore a variety of substituents at positions 7 and 2' (108). Using CNDO/2 methodology and calculated values for dipole moment (μ) and net charge on the carbonyl oxygen (qo), analyses of the data for several different in *vivo* tests were conducted. Equations 5.9 and 5.10 were derived from data for the pentylenemetrazole test (a measure of anticonvulsant activity) and the "Cat" test (a measure of sedative/muscle relaxing activity in cats), respectively.

$$\log 1/C = -0.50(\pm 0.089)\mu + 3.26(\pm 0.29) \quad (5.9)$$

$$r = 0.6206, \quad s = 0.8663, \quad F = 31.32$$

$$\log 1/C = -0.481(\pm 0.067)\mu + 4.24(\pm 0.19) \quad (5.10)$$

$$r = 0.7614, \quad s = 0.4855, \quad F = 51.03$$

Improvements in regression by qo were generally not significant, and the introduction of the Hansch lipophilic substituent constant failed to produce improvement. It was suggested that the negative term containing μ might be caused by a binding process involving dipole interactions that remove drug molecules from the active site.

In the decades after the first synthesis of the prototypical agents chlorodiazepoxide and

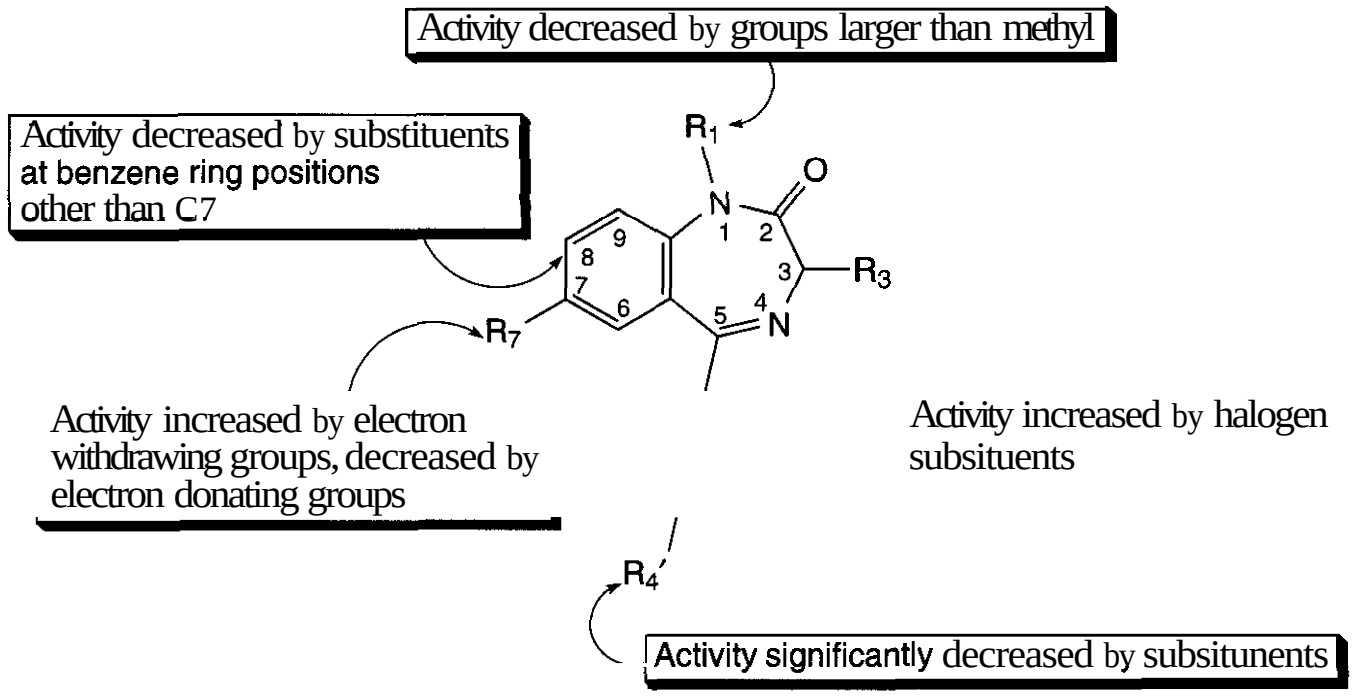


Figure 5.9. Qualitative structure activity relationships of benzodiazepin-2-ones.

diazepam, thousands of benzodiazepine congeners were synthesized and studied by numerous laboratories. The discovery and characterization of the BZR in the late 1970s led to the development of BZR radioligand binding assays, thus affording an in vitro method for the rapid screening of large numbers of compounds. The ensuing wealth of BZR binding data afforded the opportunity for further QSAR studies that have been reviewed (109, 110); a summary of a number of these analyses follows.

A Free-Wilson analysis by Borea of in *vivo* data of 55 benzodiazepines led to the following rank orders of contributions to activity of substituents at position 7 and 2' (as depicted in Fig. 5.9), thus showing the importance of electron withdrawing groups at these positions (111):

Position 7: NO, $> CF_3 >$ Br $>$ CN $>$ Cl $> N(CH_3)_2 > SOCH_3 >$ SBu $> SCH_3 >$ CH, $>$ H $> SO_2CH_3 >$ Ph $>$ F

Position 2': Cl $>$ F $>$ Br $>$ NO, $> CF_3 >$ H $> OCH_3 > CH_3$

Despite several obvious inconsistencies, the results indicate the importance of electron withdrawing groups at positions 7 and 2'. Another study by Borea employing the Free-Wilson method was based on the inhibition of [^{3}H]diazepam binding by a set of 39 benzodiazepines (112). In addition to corroborating the positive effect of electron withdrawing moieties at positions 7 and 2', the results of this analysis supported the importance of a carbonyl at position 2, the detrimental effect of groups larger than H or CH_3 at positions 1 and 3, and the negative impact of substituents at position 4'; the latter observation suggests that the pendant phenyl ring occupies a hydrophobic pocket in the receptor binding site and that the depth of the pocket cannot accommodate a para substituent. The importance of hydrophobic effects was demonstrated by a significant correlation between inhibition of BZR binding and lipophilic character (measured by chromatographically determined R_m values) as shown in Equation 5.11 (113).

$$-\log K_i = -5.542 + 4.751(\pm 0.720)R_m - 1.344(\pm 1.842)R_m^2 \quad (5.11)$$

$$n = 14,\ r = 0.934,\ s = 0.236,\ F = 37.48$$

Conformational and electronic properties of 21 benzodiazepines were calculated using empirical energy and semiempirical molecular orbital methods (114). Compounds that were either highly active or very weakly active in BZR binding (e.g., diazepam, $K_i = 8.9$ nM and medazepam, $K_i = 3850$ nM) were found to have very similar low energy conformations, thus indicating that conformational factors are not important for receptor recognition. However, mapping the electro-

static potential of the benzodiazepine molecule led to the postulation that interactions between electron withdrawing substituents at C7, the carbonyl oxygen at position 2 and the N4 imine nitrogen with three different cationic receptor sites are required for high-affinity analogs.

A distance geometry approach using a three-dimensional structure-directed QSAR method (REMOTEDISC) was employed to analyze the inhibition of [^{3}H]diazepam binding by 29 benzodiazepines (115). The results of the method, which uses three-dimensional structure, conformational energies, and atom-based physiochemical properties to model the receptor binding cavity were based on Equation 5.12.

$$E_{\text{calcd}} = -WE_c + \sum_{i=1}^{n_s}\sum_{j=1}^{n_p}\left[C_{ij}\sum_{k=1}^{n=0}P_{jk}\right] \qquad (5.12)$$

$$r = 0.980$$

In this Equation, E_c is the conformational energy with the weighting factor W, C values are the site pocket and physiochemical property-dependent coefficients determined by regression analysis, n_s is the number of site pockets, n_p is the number of ligand atoms occupying the site pocket, P_{jk} is the jth physicochemical property of the kth occupying atom of the ligand, and r is the correlation coefficient. The derived model, which was comprised of nine binding site pockets, suggested that N1 substituents should be small and hydrophilic; C7 substituents should be dispersive and hydrophilic; and the 4' position encounters steric repulsion. Except for C3, little role was found for hydrophobic interactions.

A study employing a combined QSAR-CoMFA (comparative molecular field analysis) used observed [^{3}H]diazepam pIC_{50} values, calculated HOMO and LUMO energies and total dipole moments; hydrophobic, steric, and field/inductive substituent effects were also considered (116). Equation 5.13 was derived for a set of 30 compounds that varied only in substitution at the C7, C2′, and N1 positions (the latter varied only between H and CH_3).

$$pIC_{50} = 0.87(\pm0.25)I_2 + 0.59(\pm15)\pi_7 - 0.038(\pm0.015)\text{E-LUMO} + 6.59(\pm0.27) \qquad (5.13)$$

$$n = 30, \quad r = 0.932, \quad s = 0.30$$

In the above equation, I, is an indicator value having a value of 1 or 0 for the presence or absence of a 2' substituent. The conclusions of the study were that the positive sign of the a, descriptor suggests that the C7 substituent interacts with a complementary hydrophobic pocket on the receptor and that binding to the receptor is enhanced by increasing negative values of E-LUMO, which are associated with decreased electron density in the fused benzene ring. It was postulated that a charge-transfer interaction occurs between the ligand and an electron-rich site of the receptor binding domain.

However no explanation was offered for the beneficial effect of a 2' substituent.

As shown in Equation 5.14, hydrophobicity of the C7 substituent was found to be an important factor in the correlation of binding data with the physicochemical parameters of benzodiazepines and β-carbolines (117).

$$-\log IC_{50} = 0.45(\pm0.14)\pi_7 + 1.11(\pm0.36)\sigma_7 + 2.17(\pm0.54)\sigma_{2'} - 0.87(\pm0.39)I_6 + 6.99 \qquad (5.14)$$

$$n = 50, \quad r = 0.91, \quad s = 0.31, \quad F = 52.38$$

A positive correlation was also established with the Hammett electronic parameters for positions C7 and C2′, but the negative correlation with the I, term shows that presence of a substituent at the 6 position decreases binding affinity.

As part of a review and commentary on the above-mentioned QSAR, Hansch reevaluated the earlier work and derived some new results (110). His analysis of the [^{3}H]diazepam binding data of over 70 benzodiazepines led to

Equation 5.15, which shows a positive contribution to binding affinity by both hydrophobicity (log$P$) and sterimol parameters, B_{1-7} and $B_{1-2'}$ for substituents at the 7 and 2' positions, respectively.

$$\begin{aligned}\log 1/C &= 1.3(\pm 0.37)\log P \\ &\quad - 2.3(\pm 0.59)\log(\beta 10^{\log P} + 1) \\ &\quad + 1.08(\pm 0.32)B_{1-7} \\ &\quad + 1.05(\pm 0.32)B_{1-2'} + 2.54(\pm 0.89)\end{aligned} \tag{5.15}$$

$$n = 74, \quad r=0.85, \quad s=0.39, \quad F=42.4$$

The significance of the B_{1-7} term was interpreted as pointing to a steric effect of the first atom of groups in the 7 position; i.e., the larger the atom attached to C7, the more effective the binding. It was suggested that atoms at this position produce a conformational change in the receptor that is conducive to ligand binding. The positive steric effect of C2′ substituents implies that receptor binding is enhanced by the twisting of the 5 phenyl ring out of the plane of the seven-membered ring. No role was found in Equation 5.15 for electronic parameters.

Hansch's reevaluation of the earlier QSAR study of *in vivo* data (108) resulted in Equation 5.16 for the pentylenetetrazole test. In contrast to Equation 5.9), which shows no role for hydrophobic effects, Equation 5.16 is positively correlated with both hydrophobicity and steric effects of the C7 and C2′ substituents as well as with qo (charge on the carbonyl oxygen).

$$\begin{aligned}\log 1/C &= 134.4(\pm 0.41)\mathrm{qo} + 1.04(\pm 0.53)B_{1-7} \\ &\quad + 0.95(\pm 0.48)B_{1-2} \\ &\quad + 1.72(\pm 1.3)\log P - 0.41(\pm 0.2) \\ &\quad \times (\log P)^2 + 43(\pm 15)\end{aligned} \tag{5.16}$$

$$n = 47, \quad r = 0.87, \quad s = 0.56, \quad = 6.73$$

The summary conclusion drawn was that overall ligand hydrophobicity is important for BZR binding and that for the classical benzodiazepinone chemotype the hydrophobic1 steric effects of moieties appended to positions C7 and C2′ are positively associated with receptor affinity.

The computational construction of artificial neural networks has also been applied to relate physicochemical parameters of benzodiazepines with their receptor affinity and to predict BZR properties and BZR ligand affinities. In a study by Maddalena and Johnston, back-propagation artificial neural networks were used to examine the QSAR between substituent constants at six positions on 57 benzodiazepinones with their empirically determined binding affinities (118). Among the findings of the study were the following:

- Position 7 is the most important location for enhancing BZR affinity; increases in substituent lipophilicty and electronic charge were found to be directly related to increases in receptor binding. The optimal C7 substituents from best to worst were determined to be $CH_2CF_3 > I > Br > CF_3 > Cl > C(CH_3)_3 > NO, > F > N_3 > CH{=}CH_2$.
- Substituents at position 2' are of second-most importance in positively influencing BZR affinity, and increases in the polar nature of these substituents were shown to be beneficial, although this effect was diminished if the groups were bulky.
- Substitution at positions 3 and 8 is disfavored, and electrostatic influences at these positions are important.

Figure 5.10 summarizes the findings of the various QSAR studies. It is somewhat disconcerting that there is considerable disagreement among the key conclusions drawn from several of the studies, e.g., some propose a hydrophobic interaction of C7 substituents, whereas others suggest that hydrophilic groups are favored or that electrostatic interactions of C7 groups with a cationic subsite of the receptor are important. However comparison of Figs. 5.9 and 5.10 shows that the various QSAR largely corroborate the earlier qualitative SAR reported by Sternbach. Because

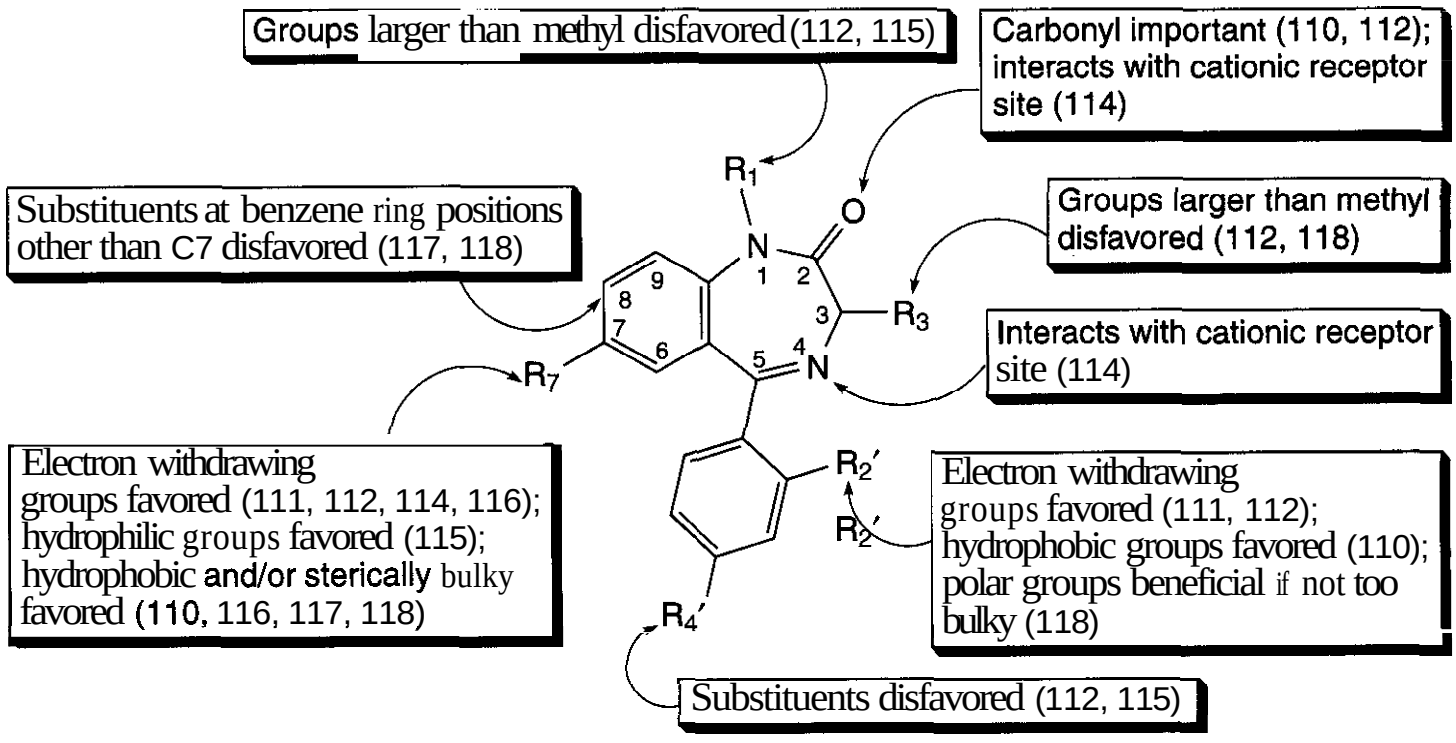
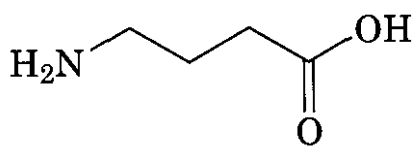


Figure 5.10. Summary of findings from QSAR studies of benzodiazepin-2-ones. References in parentheses.

the latter were based exclusively on ***in vivo*** data, whereas most of the QSAR studies have used in ***vitro*** binding data, the general agreement between the two suggests that the pharmacological effects of the benzodiazepines are due mainly, if not solely, to their BZR interactions.

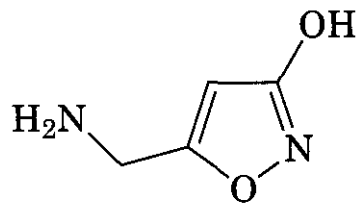
5.2 Receptor Interactions

Many of the pharmacological properties of barbiturates and benzodiazepines, including their therapeutic actions and their tolerance and dependence, are associated with their effects on GABAergic neurotransmission in the CNS. GABA (10) is a major inhibitory neuro-

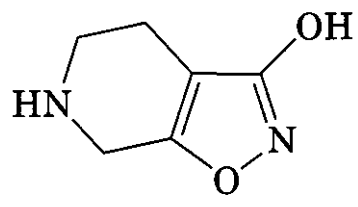


(10)

transmitter that mediates its effects through interaction with several different receptor subtypes. Activation of the $GABA_A$ receptor, a member of a superfamily of transmitter-gated ion channels, opens an ion channel allowing the entry of chloride ion with the result of dampening neuronal activity through hyperpolarization of cell membrane potential. The $GABA_A$ receptor complex bears distinct recognition sites of differing affinities for GABA (10)itself as well as for GABA agonists such as the natural product muscimol **(11)**and the



(11)



(12)

synthetic analog THIP (12, gaboxadol), in which incorporation of the basic nitrogen into a 6-membered ring results in much greater structural rigidity than in the conformationally flexible GABA molecule. The 3-hydroxyisoxazole moiety common to both (**10**) and **(11)** mimics the carboxyl group of GABA. Based on the equipotent agonist activity of GABA and THIP despite the disparity in their structural mobility, it has been suggested that GABA interacts at the receptor level in an extended near planar conformation (119). In addition to its GABAergic activity in preclinical

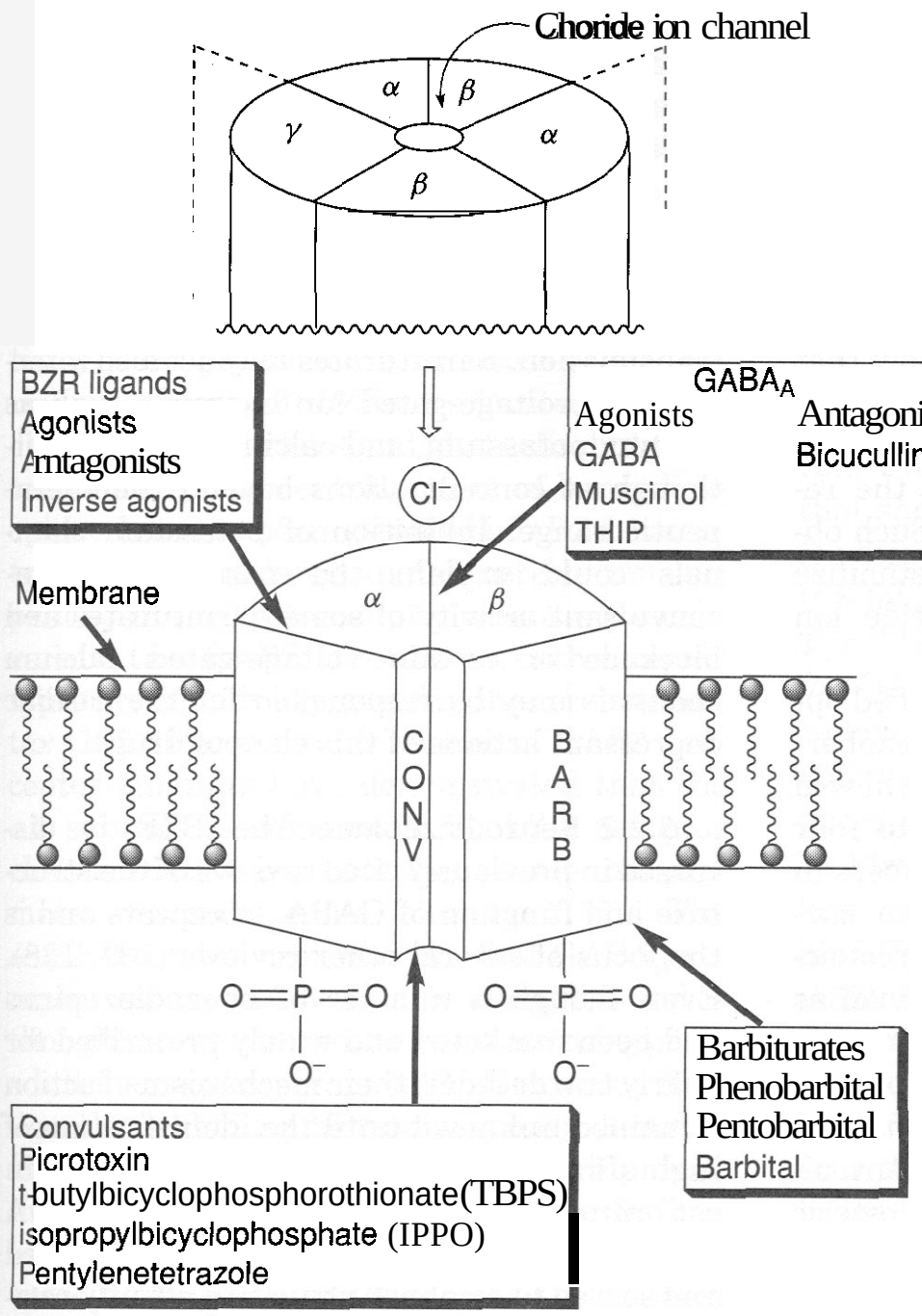


Figure 5.11. Schematic representation of the $GABA_A$ receptor complex. Reprinted with modification from P.A. Saunders and I.K. Ho, ***Prog. Drug Res.,*** 34, 261 (1990) with permission from Birkhauser' Verlag AG.

tests, THIP has been found to be an effective sedative-hypnotic in humans **(120–122).**

As shown in Fig. **5.11,** the $GABA_A$ receptor complex also has binding sites for drugs such as barbiturates and benzodiazepines as well as for certain convulsive agents such as picrotoxin which inhibit chloride channel activity. GABA receptors, their diversity, and pharmacology have been extensively reviewed (123–128).

Electron microscopy studies have revealed that, like acetylcholine receptors, the ion channel of the $GABA_A$ receptor is formed by the pentameric assembly of hetero-oligomeric subunits **(129);** each subunit has four transmembrane spanning domains and all five subunits are arranged so that their second transmembrane domains comprise the ion channel wall. Cloning of the subunits from vertebrates has resulted in nearly **20** cDNAs, which have been grouped into six classes based on sequence homology. The **16** human $GABA_A$ receptor subunits **(al–6,** β1–4, **yl–4,** δ, &) that have been cloned to date show approximately **30%** sequence identity among subunits and about **70%** homology among subunit subtypes (125). Despite the enormous number of possible arrays, it seems that a rather limited number of hetero-oligomeric combinations actually occur in nature and that a functional $GABA_A$ receptor requires both an **a** and β and one other subunit type. The pentameric assembly is illustrated in Fig. **5.11.**

5.2.1 Barbiturates. Barbiturate interactions with the $GABA_A$ receptor complex have been reviewed **(130,131).** Barbiturate effects on receptor-mediated chloride ion flux have been extensively studied employing electrophysiological techniques either in *vivo* or in isolated

or tissue-cultured neurons. Patch-clamp studies in cultured mouse spinal neurons have shown that barbiturates such as pentobarbital increase the duration of channel opening while causing a slight decrease in the frequency of opening (132). The increased opening occurs both in the presence and absence of $GABA_A$ agonists, and whereas synaptosomal ion flux studies employing [$^{36}Cl^-$] show that pentobarbital enhances the effect of GABA-stimulated ion flux, the maximal response of the combination is no greater than the response to pentobarbital alone (133). Such observations indicate that barbiturates stabilize the open conformation of the chloride ion channel (129).

A study of the effects of highly purified optical isomers of several barbiturates (hexobarbital, pentobarbital, and thiopental) showed the *S*-enantiomers to be about two to four times more potent than the R-enantiomers in the potentiation of GABA-induced ion currents (134). These results support a direct action of barbiturates at the receptor level as opposed to non-specific effects on membranes.

A direct action of barbiturates on the $GABA_A$ receptor complex has been further demonstrated by their chloride-dependent, picrotoxin- and bicuculline-sensitive allosteric enhancement of GABA and benzodiazepine receptor binding (135). The efficacy of compounds to act as benzodiazepine receptor agonists, inverse agonists or antagonists has been correlated with the ratio of their IC_{∞} versus [^{3}H]flunitrazepam binding in the presence and absence of pentobarbital (136). The "barbiturate shift" is >1 for agonists, <1 for inverse agonists, and -1 for antagonists. As will be subsequently discussed, the effects of various barbiturates on benzodiazepine binding may be associated with their functional activity.

The molecular pharmacology of barbiturate action at $GABA_A$ receptors remains to be elucidated. Neither specific amino acid point mutations within $GABA_A$ receptor subunits nor chimeras between $GABA_A$ and glycine receptors have unequivocally established the location of barbiturate binding sites, although some studies suggest that the barbiturate-receptor interactions may occur solely within the β subunit (135).

In addition to their interaction with the $GABA_A$ receptor complex, the pharmacological effects of barbiturates may in part be mediated by other mechanisms (135). For example, pentobarbital has been shown to depress responses to quisqualate and kainate but not NMDA, suggesting a receptor subtype-specific blockade of excitatory glutamatergic neurotransmission. Barbiturates may act also interact with voltage-gated ion channels such as sodium, potassium, and calcium channels, although at concentrations beyond the therapeutic range. Inhibition of potassium channels could explain the paradoxical proconvulsant activity of some barbiturates and blockade of certain voltage-gated calcium channels may be responsible for the cardiac depressant actions of this class of drugs.

5.2.2 Benzodiazepines. The BZR is discussed in previously cited reviews of the structure and function of $GABA_A$ receptors and is the focus of several other reviews (137, 138). Even though a number of benzodiazepines had been marketed and widely prescribed for nearly two decades, their mechanism of action remained unknown until the identification of high affinity benzodiazepine binding sites in rat brain tissue in the late 1970s (139,140). A plethora of BZR binding studies soon followed and served to establish structure-affinity relationships as discussed in Section 5.1.2.

Earlier investigations had indicated a diversity of CNS actions for benzodiazepines, including modulation of calcium and adenosine uptake, inhibition of the effects of excitatory amino acids, and alterations of sodium ion permeabilities; however, ensuing studies focused on the effects of benzodiazepines on GABA-mediated synaptic inhibition. There is now an abundance of empirical evidence that shows that compounds interacting as agonists at BZRs on the $GABA_A$ receptor complex cause an allosteric potentiation of GABA-gated chloride current intensity. Electrophysiological experiments have established that benzodiazepines, like barbiturates, increase chloride ion flux. However, unlike barbiturates, which cause a increase in the duration of chloride channel opening, benzodiazepines augment channel opening frequency (132, 141). Agents such as diazepam were found to potently en-

hance GABA responses at low nanomolar concentrations and the effects of diazepam were blocked by the benzodiazepine antagonist, flumazenil (Ro 15–1788). The sedative-hypnotic, zopiclone, also potentiated GABA responses, but at high nanomolar concentrations.

Two distinct sets of BZR, designated as BZ-I and BZ-II (alsocalled ω-1 and 0-2, respectively), are prevalent in the CNS (142). Peripheral type (0-3) BZRs are located in mitochondrial membranes and glial cells, but their relevance to the central action of benzodiazepines has not been established. BZ-I receptors have abundance in the cerebellum but paucity in the hippocampus, whereas the converse is true for BZ-II receptors; both receptor types are equally expressed in the cerebral cortex (125). Recombinant studies of $GABA_A$ receptor subunits have demonstrated that the $\alpha 1$ subunit is important for BZ-I receptor characteristics and that the $\gamma 2$ subunit promotes benzodiazepine binding (137). The $\alpha 1\beta 2\gamma 2$ combination was the first $GABA_A$ receptor subtype to be clearly identified and is thought to be the most abundant subtype in adult mammalian brain (143). Numerous BZR ligands, including benzodiazepines, β-carbolines, and imidazopyridines such as zolpidem, interact with this receptor subtype. Both a and γ subunits contribute to the benzodiazepine binding site, which seems to be situated at the interface of these subunits (144, 145). Mapping the benzodiazepine binding site by site-directed mutagenesis has revealed that alterations of certain amino acids within the N-terminal domains of the a and γ subunits have significant effects on the affinity and efficacy of ligands. One such site identified by alanine scanning of the a1 subunit is histidine 101 (rat numbering) or 102 (human/bovine numbering) (146). Various other substitutions of the histidine have been examined and the effects of several BZR ligands on the mutant $\alpha 1$ subunits co-expressed with $\beta 2$ and $\gamma 2$ subunits in *Xenopus* oocytes were evaluated by electrophysiological techniques (147). Substitution by Phe, Tyr, and Gln had little effect on the ability of flunitrazepam to potentiate GABA-induced currents, but other mutations (Lys and Glu) resulted in a drastic reduction of the flunitrazepam response. The importance of this amino acid residue for agonist recognition was further substantiated by the finding that His^{102} of the bovine $\alpha 1$ subunit is the major site of photoaffinity labeling by [^{3}H]flunitrazepam (148, 149). It was postulated that the residue interacts directly with the pendant phenyl group of diazepam and other 5-phenyl benzodiazepines. Other amino acids within the $\alpha 1$ subunit that may be part of the benzodiazepine binding site are Gly^{200}, Thr^{206}, and Tyr^{209} (150, 151). Replacement of Tyr^{209} by Ala, Phe, or Gln afforded mutant receptors that showed moderate to total loss of affinity for diazepam or flunitrazepam (Tyr^{209}Phe: 2- to 8-fold decrease, Tyr^{209}Ala: –40-fold decrease, Tyr^{209}Gln: no detectable affinity). The affinity for GABA of these *Xenopus* oocyte–expressed receptors was only slightly diminished, but the ability of flunitrazepam to stimulate GABA-induced currents was abolished.

Phe^{77} and Met^{130} within the $\gamma 2$ subunit have been shown to be necessary for high affinity binding of and/or modulation by BZR ligands (152). Receptors containing a $\gamma 2Phe^{77}$Ile mutation retained high affinity for flunitrazepam but not for other ligands such as flumazenil and methyl β-carboline-3-carboxylate. However the mutation prevented allosteric modulation of ion channel currents by flunitrazepam. It was suggested that Phe^{77} serves as a contact point for certain BZR ligands, although it is not essential for high affinity binding, the energy requirements for which are satisfied by other contact points. But in the absence of Phe^{77}, ligands may occupy the binding site in a conformation that is incapable of initiating the allosteric changes leading to channel modulation. A $\gamma 2Phe^{77}$Tyr modification resulted in significant loss of affinity for benzodiazpinones bearing a 5-phenyl moiety, e.g., diazepam, which had a K_i of 3 μM at the mutant receptor versus 12 nM at the wild-type receptor (153). High affinity was retained by BZR ligands lacking the phenyl moiety, thus implying that the tyrosine hydroxyl interferes with the phenyl group and that the latter is in close proximity to Phe^{77} when diazepam and like molecules bind to the wild-type receptor. Together the $\alpha 1His^{101}$, $\alpha 1Gly^{200}$, $\alpha 1Thr^{206}$, $\alpha 1Tyr^{209}$, $\gamma 2Phe^{77}$, and $\gamma 2Met^{130}$ residues may be involved in the formation of the binding pocket of BZR ligands (151).

Of particular relevance to sedative-hypnotic activity is the observation that zolpidem is a drug that shows high affinity and selectivity for $GABA_A$ receptors containing the a1 subunit (154). Whereas many BZR ligands bind potently to receptors bearing other a subunits, zolpidem interacts only with those bearing $\alpha 1$; for example, it exhibits high affinity for $\alpha 1\beta 2\gamma 2$ receptors but is inactive at $\alpha 5\beta 2\gamma 2$ receptors. To determine the structural elements of the $\alpha 1$ subunit that are essential for zolpidem's activity, chimeras of a1 and a5 were constructed in the putative extracellular N-terminal domain and transfected with $\beta 2$ and $\gamma 2$ subunits in HEK293 cells, and the affinities of the resulting combinations for zolpidem were measured (155). Chimeras that showed enhanced zolpidem binding relative to wild-type $\alpha 5\beta 2\gamma 2$ served to identify the regions of a1 that are of critical importance to the binding of this drug. Subsequent single amino acid replacements of various a5 residues by the corresponding a1 amino acids within these regions led to the finding that Thr^{162}, Gly^{200}, and Ser^{204} of the $\alpha 1$ subunit are important for zolpidem recognition. It was suggested that Thr^{162} and Gly^{200} play a conformational role and orient the binding pocket in a manner that is optimal for zolpidem binding, whereas Ser^{204} participates in hydrogen bond formation with the carbonyl group of the compound's acetamide moiety. Interactions between His^{101} and a nitrogen atom of the imidazole ring of zolpidem and hydrophobic interactions between the drug's aromatic ring and $\gamma 2Phe^{77}$ or $\gamma 2Met^{130}$ were also postulated.

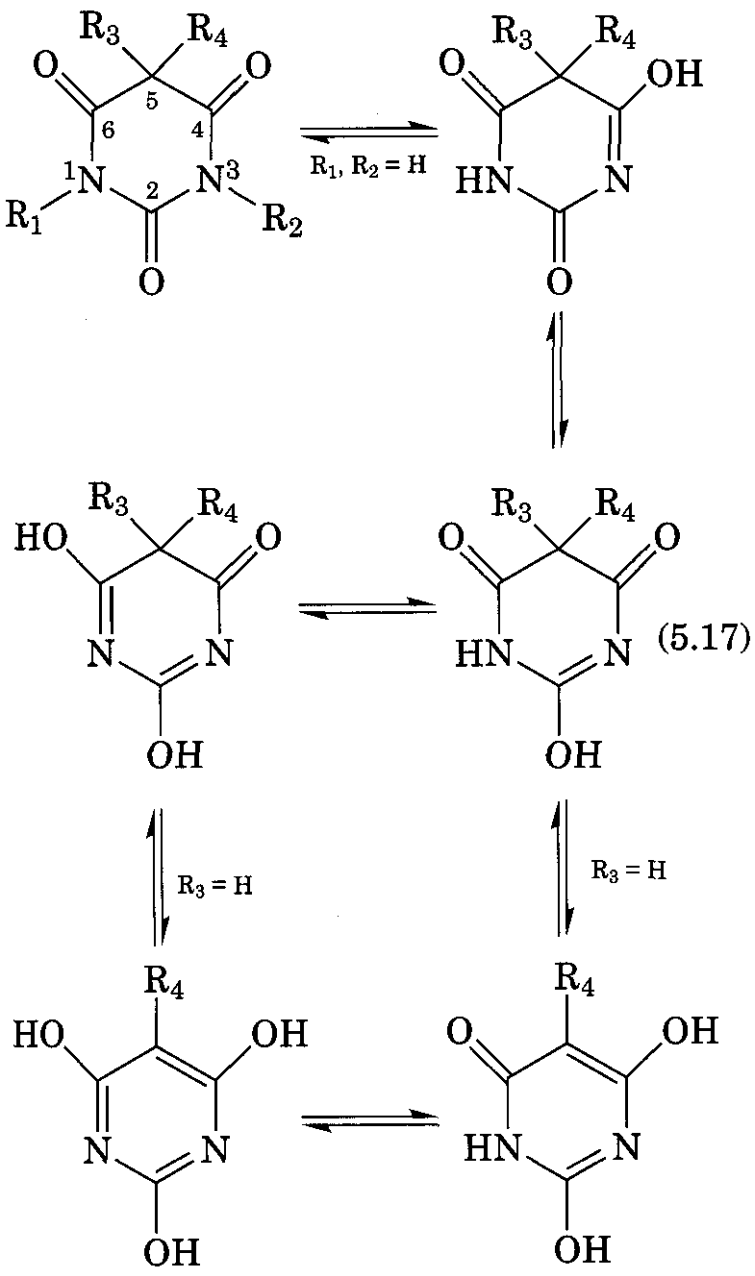
The cited reviews pertaining to GABA/benzodiazepine receptors discuss numerous other studies that employ chimeric receptors and site-directed mutagenesis to determine the key structural components of $GABA_A$ receptor subtypes comprised of various a, β, and γ subunit permutations.

5.3 Structure-Function Relationships

5.3.1 Barbiturates. Structural features of barbiturates that are required for hypnotic activity include the following:

1. Hypnotic activity increases with lipid solubility until the total number of carbon atoms at both C-5 substituents is between 6 and 10. Further increase in the sum of the number of carbon atoms decreases hypnotic activity despite increased lipophilicity, indicating that lipophilicity must remain within certain limits.
2. Within the same series, the branched chain isomer generally has greater lipid solubility, hypnotic activity, and shorter duration of action than the straight chain isomer. Stereoisomers have approximately equal potencies.
3. Within the same series the unsaturated allyl, alkenyl, and alkynyl derivatives are more hypnotic than the saturated analogs with the same number of carbon atoms. Compounds bearing alicyclic or aromatic substituents are more potent than those having aliphatic substituents with the same number of carbons.
4. Conversion of a 5,5-disubstituted barbituric acid to a 1,5,5-trisubstituted analog does not result in a significant change in hypnotic activity.
5. Introduction of polar substituents (OH, NH_2, COOH, CO, RNH, SO_3H) into an aromatic moiety at the 5-position decreases lipid solubility and potency.
6. Replacement of the oxygen at C-2 by a sulfur atom results in faster onset but shorter duration of hypnotic activity. Replacement of more than one carbonyl oxygen by sulfur causes a loss of activity, again indicating an upper limit to lipophilicity.

The pharmacological activity of barbiturates is also influenced by their acidity, which is attributed to the lactam-lactim tautomerism that can occur in all derivatives in which at least one of the ring nitrogens is unsubstituted; keto-enol tautomerism also takes place in 5-unsubstituted or mono-substituted compounds (Equation 5.17). Barbiturates must have acidity within certain limits to possess hypnotic activity (156). For example, barbituric acid (R_1, R_2, R_3, R_4 = H), which has a p*K*a of 4.1 and is >99% dissociated at physiological pH, and the neutral *N, N'*-disubstituted compound (R_1, R_2, R_3 = Et, R_4 = Ph) that is completely undissociated, are devoid of hypnotic



activity. In contrast, 5,5-disubstituted and 1,5,5-trisubstituted derivatives (e.g., R_1 = H, R_2 = H or CH_3, R_3 = Et, R_4 = Ph), which have p*Ka* in the range of 7–8 and are 40–60% dissociated, are capable of crossing the blood-brain barrier and exerting CNS effects, including sedation. It was shown that the ionized form of barbiturates can permeate liposomal bilayers provided that 5-substituents impart sufficient lipophilicity (157).

The dissociation of 5,5-disubstituted barbituric acids has been investigated on a thermodynamic basis, and it was found that increasing steric bulk of 5-substituents causes enthalpic changes that are acid-strengthening but that are offset by acid-weakening entropic effects (158). These findings were rationalized in terms of ion-dipole forces in the ionized species, which produce a solvation shell capable of hindering the rotation of substituents. It is apparent that the pharmacological activities of barbiturates are dependent on an appropriate balance between lipid solubility and ionic character and that these physicochemcial properties are influenced by the size and shape of substituents at the 5-position. Such properties determine the membrane permeability of the compounds and consequently their absorption and distribution and possibly their susceptibility to metabolism as well. But as mentioned in Section 5.2.1, the sedation/hypnosis and other drug actions (anticonvulsant, anesthetic) of barbiturates are unlikely to be caused by unspecific effects on cell membranes but instead on their interaction at recognition sites on the $GABA_A$ receptor complex. This, in turn, modulates the binding of both GABA and benzodiazepines. Numerous barbiturates have been shown to reversibly increase the affinity for equilibrium binding of [^{3}H]diazepam, and it has been shown that the efficacy of binding enhancement can be correlated with their structure/function categorization as agonists, partial agonists or antagonists (159). Compounds such as (±)-pentobarbital and its stereoisomers, (±)-secobarbital, amobarbital, and (+) and (−) pentobarbital, all of which enhance [^{3}H]diazepam binding to about the same maximal level (ca. 125% above baseline), are classed as full agonists and are CNS depressants with anesthetic/hypnotic activity. Another group of barbiturates bearing *N*-methyl substituents also potentiate [^{3}H]diazepam binding but to a lesser degree (ca. 35–75% above baseline) and reduce the pentobarbital enhancement down to their own maximal level, indicating that they function as partial agonists. A third group was found to lack the ability to enhance benzodiazepine binding and includes some derivatives that have *in vivo* excitatory activity and that reverse the binding enhancement of pentobarbital, thus classing them as functional antagonists.

5.3.2 **Benzodiazepines.** BZR ligands of different structural types span a spectrum of intrinsic functional activity from full agonist to antagonist to inverse agonist. Agonists, which are comprised mainly of the classical benzodiazepine chemotype, exert a positive cooperative effect on GABA-induced chloride ion currents, and this action at the molecular level translates into a variety of *in vivo* effects, including anxiolysis, anticonvulsant, and mus-

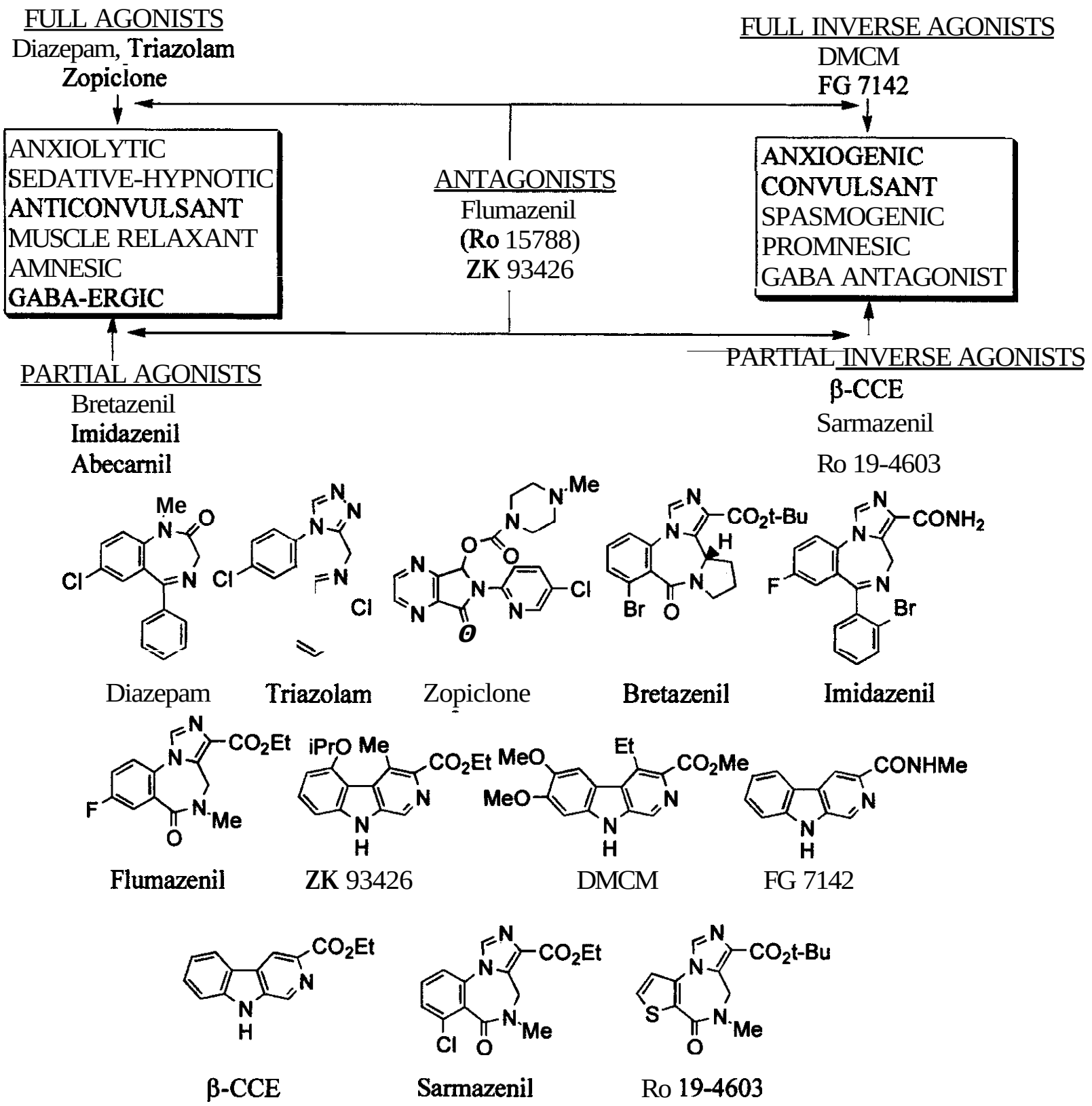


Figure 5.12. Functional activity of representative BZR ligands.

cle relaxing activity and sedation/hypnosis. Functional antagonists display no efficacy of their own, but their occupancy of the benzodiazepine binding site can block the action of both agonists and inverse agonists. The latter, which include some β-carboline and tricyclic azepine derivatives, exert a negative cooperative effect on GABA binding, and thus are anxiogenic and proconvulsant. Some compounds with intermediate activities can be classed as partial agonists or partial antagonists. BZR ligands of various functional types have been reviewed (160, 161), and Fig. 5.12 shows examples of a number of such compounds that are classified according to their pharmacological properties.

The mechanism of action of agents such as DMCM as inverse agonists has been explained in terms of a two-state model for the benzodiazepine-GABA receptor complex (162). The model proposes that the complex exists in equilibrium between interconvertable open and closed channel conformations. GABA and BZR agonists such as diazepam exhibit recip-

rocal cooperativity in that they both bind selectively to the open conformation and allow chloride ion flux. Conversely, an inverse agonist binds selectively to the closed conformation and in so doing exhibits negative cooperativity by allosterically inhibiting GABA binding. This rationale of inverse agonism is supported by the observation that GABA receptor stimulation reduces the BZR affinity of DMCM (163), and thus it may be assumed that DMCM reciprocally reduces the affinity of GABA for its receptor.

There is evidence that the functional activity of BZR ligands is caused by the interactions (hydrogen bonding, hydrophobic interactions) of their structural moieties with key amino acid residues that comprise the ligand recognition site. The previously mentioned mutations of His^{101} in the $\alpha 1$ subunit have significant effects on binding affinities, as well as altering the characteristic agonist, inverse agonist, or antagonist responses to various ligands as determined by electrophysiology (147). Introduction of a $His^{101}Arg$-mutated $\alpha 1$ subunit into mice has served to demonstrate that certain behavioral actions of benzodiazepines are mediated by specific $GABA_A$ receptor subtypes (164). $\alpha 1(His^{101}Arg)$ transgenic mice failed to show the sedative, amnesic, and in part, the anticonvulsant responses to diazepam, thus indicating that these behaviors are attributable to BZR agonist activation of the $\alpha 1$ containing $GABA_A$ receptor subtypes, which are localized mainly in cortical areas and thalamus. In contrast, the anxiolytic, myorelaxant, motor-impairing, and ethanol-potentiating effects of diazepam were retained and must be caused by the drug's interaction with nonmutated $GABA_A$ receptors in the limbic system ($\alpha 2$, $\alpha 5$), in monoaminergic neurons ($\alpha 3$), and in motor neurons ($\alpha 2$, $\alpha 5$). The finding that benzodiazepine-induced behavioral responses are mediated through distinct neuronal circuits has implication for drug design. That is, agonists acting upon $\alpha 2$-, $\alpha 3$-, and/or $\alpha 5$- but not on al-containing receptor subtypes could be nonsedative and nonamnesic anxiolytics.

Numerous pharmacophore models of the BZR have been proposed, and a number of these were reviewed about a decade ago (165). More recent examples include the mapping of the peripheral BZR (166) and development of a three-dimensional pharmacophore model for BZR ligands having anxiolytic activity (167).

Cook and co-workers have published extensively on their studies of BZR pharmacophore modeling (168–173). These studies have employed Comparative Molecular Field Analysis (CoMFA) of the structural parameters of a wide range of BZR ligands, many of which were designed and synthesized to probe the size, shape, and functional group tolerance of the benzodiazepine binding domain. An example of a derived model showing the comparative binding fit of an agonist (diazepam) and an antagonist/partial inverse agonist (β-CCE) is depicted in Fig. 5.13.

Of particular relevance to sedative/hypnotic agents is the development of a pharmacophore for the sedation endpoint (173). Complementary behavioral and computational studies of 21 structurally diverse BZR ligands that influence spontaneous motor activity (a behavioral indicator of sedation) were conducted. A five-component three-dimensional pharmacophore consisting of two proton-accepting moieties, a hydrophobic region, a ring with polar moieties, and an aromatic ring was derived and is represented in Fig. 5.14. The model was shown to accommodate the ligand structural requirements in the overlapping portion of the binding sites for agonists, inverse agonists, and antagonists that impart effects on the behavioral sedation endpoint.

Agonists decreased spontaneous motor activity, inverse agonists caused an increase, and antagonists, while lacking intrinsic activity of their own, blocked the effects of the agonist, flunitrazepam. The reliability of the model was evaluated in several ways: ligands without effect at the sedation endpoint did not accommodate the pharmacophore requirements; several BZR ligands that were not used in pharmacophore development but that were known to affect sedation satisfied the pharmacophore; and use of the pharmacophore parameters to search three-dimensional databases resulted in identification of additional BZR ligands known to have effects on the sedative endpoint. The model may thus be useful for the design of novel chemotypes having sedative/hypnotic activity.

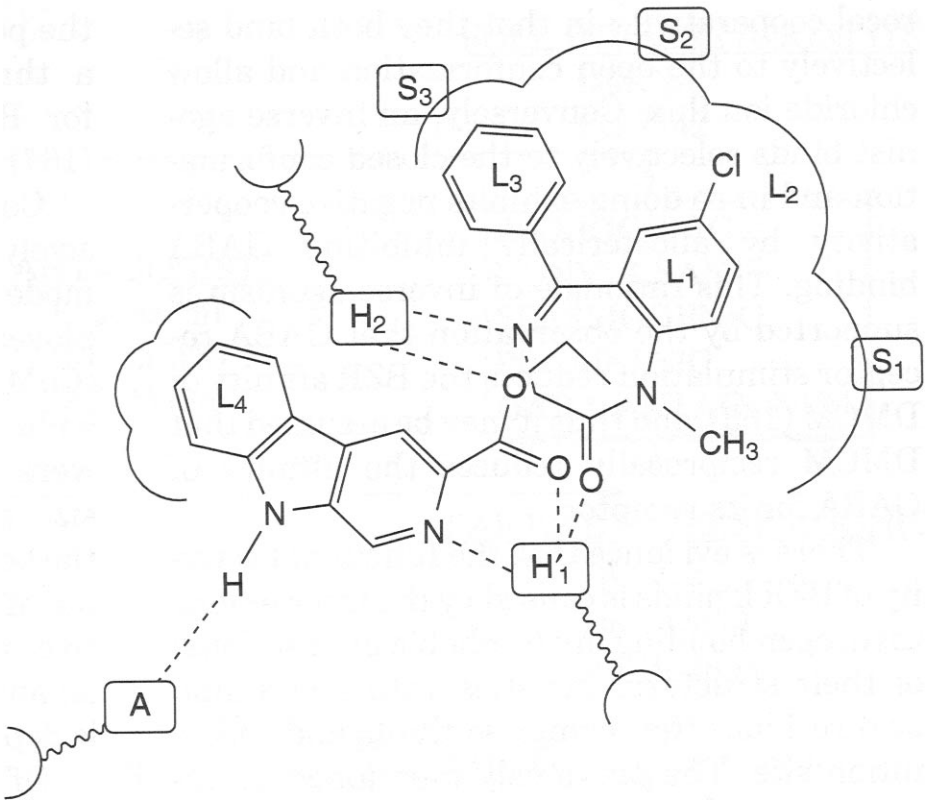


Figure 5.13. Pharmacophore/receptor model for the BZR showing overlap of diazepam (right) and p-CCE (left). Sites H_1 and H_2 designate hydrogen bond donor sites on the receptor protein complex, A represents a hydrogen bond acceptor site necessary for potent inverse agonist activity, L_1–L_4 are lipophilic regions in the binding pharmacophore and S_1–S_3 are regions of steric repulsion within the receptor ligand binding domain. Reprinted from Q. Huang, et al., ***Drug Design*** and Discovery, **16,** 55 (1999) with permission of Harwood Academic Publishers.

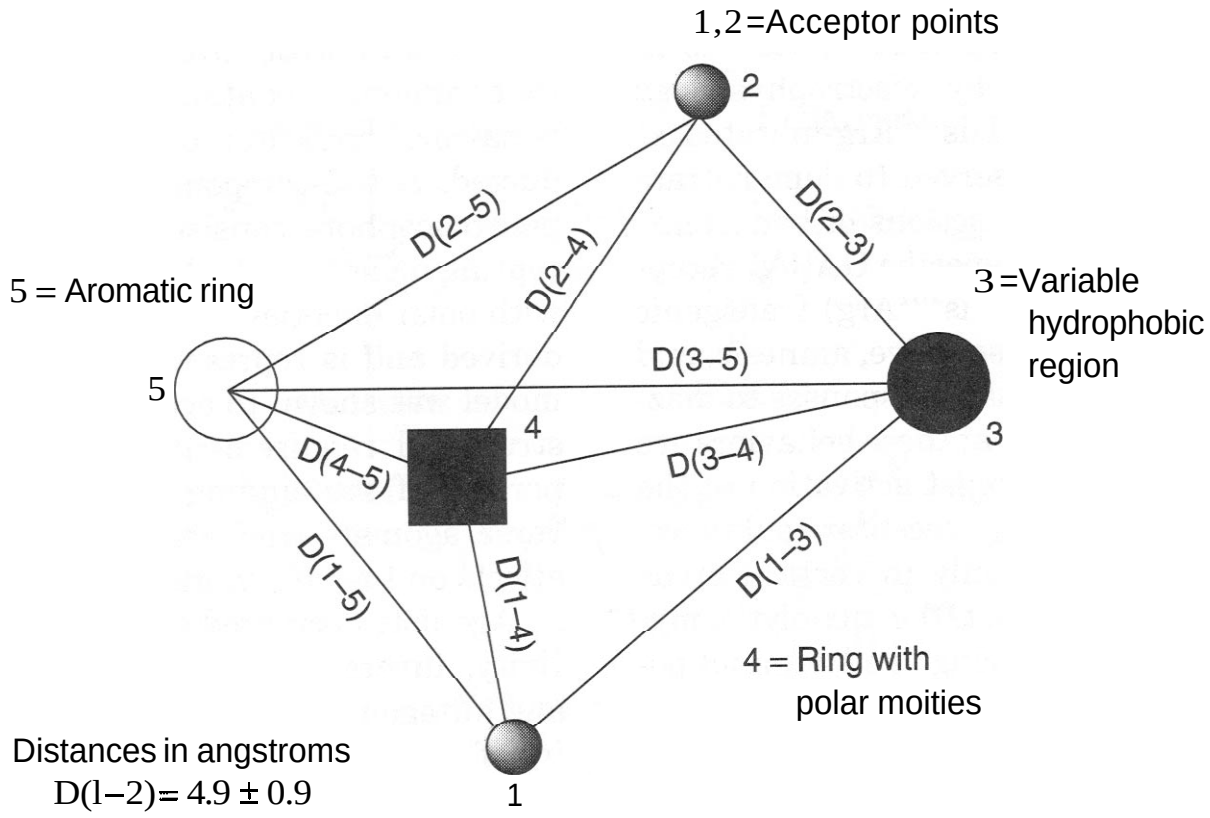
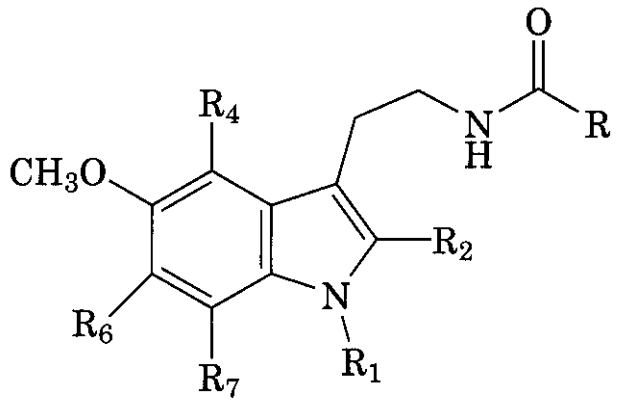


Figure 5.14. Five component 3D pharmacophore for ligand recognition of $GABA_A$/benzodiazepine receptors eliciting a response at the sedation endpoint. Zolpidem shown as one of the cbmpounds used in construction of the pharmacophore model. Reprinted from D.L. Harris, et al., Eur. J. Pharmacol. **401,** p. 271 (2000) with permission of Elsevier Science.

6 RECENT DEVELOPMENTS

6.1 Melatonergic Agents

Melatonin (**13**:R_1, R_2, R_4, R_5, R_7 = H; R = CH_3) is an endogenous hormone that is bio-



(13)

synthesized within and secreted by the pineal gland. It has a wide variety of biological effects in various species, but in humans its principal effect is the regulation of the body's circadian rhythm (sleep-wake cycle). It is well-established that the pineal gland produces and releases melatonin during the hours of darkness and that the functions of the pineal gland are controlled by light and changes in the duration of the photoperiod (174,175).

There is some evidence that the pharmacological actions of melatonin may in part be mediated through the GABA-BZR complex. Melatonin has been shown to inhibit [^{3}H]diazepam binding in human and bovine cortex, although only at high (ca. 50 μM) concentrations, and chronic injection of doses ranging from 1 to 5 mg/kg for 3 weeks in rats increased both high and low affinity GABA binding in forebrain membranes (176). Several behavioral effects of melatonin in rodents, including depression of locomotor activity, analgesia, and inhibition of seizure susceptibility, were blunted by the benzodiazepine antagonist flumanezil, thus suggesting that these effects of melatonin may involve central synapses employing GABA as a neurotransmitter (177). Significant increases in levels of the serotonin metabolite, 5-HIAA, in forebrain regions of pinealectomized rats suggests a nexus between the mode of action of melatonin and the serotonergic system (178).

However, it is most likely that central effects of melatonin are mediated primarily through its high affinity binding to melatonin receptors. Approximately 20 distinct melatonin receptor DNA sequences have been identified in a variety of species, including humans (179,180). Analysis of full-length receptor sequences indicates that they are members of the seven-transmembrane spanning superfamily of G-protein coupled receptors and phylogenetic analysis supports their division into three subtypes: mt_1 and MT_2 (formerly designated as mel$_{1a}$, mel$_{1b}$) and mel$_{1c}$ (181). Of these, only the first two subtypes are found in humans; the mel$_{1c}$ receptor seems to be confined to non-mammalian vertebraes. The interaction of melatonin with each of the subtypes, expressed in cell lines, attenuates forskolin-stimulated cyclic AMP accumulation, indicating that the receptors are functionally coupled to inhibitory G proteins (180).

Various studies have demonstrated that melatonin has sleep-enhancing properties in animals. For example IP administration of melatonin to rats caused a dose-related reduction in time to sleep and time spent awake without alteration of either normal EEG patterns or brain concentrations of serotonin, noradrenaline, or dopamine (182); Doses of 10 mg/kg, IP, potentiated pentobarbital sleep time in mice, an effect that, unlike that of diazepam, was not blocked by pretreatment with the BZR antagonist, flumazenil (183). These studies suggest that the sedative component of melatonin is probably mediated through its activation of melatonin receptors and not by its modulation of monoaminergic or GABAergic neurotransmission. Melatonin has rapid absorption, a short half-life, lack of toxicity, and manifests other pharmacological effects only at doses 10- to 20-fold higher than those producing sedation (184). These are highly desirable properties for a sedative-hypnotic agent.

Thus, there has been considerable interest in evaluating the use of exogenously administered melatonin to normalize sleep patterns, especially in cases where the normal secretion of the hormone has been disrupted, e.g., in jet lag, shift workers, and the elderly. Clinical studies have demonstrated the ability of exogenously administered melatonin to synchro-

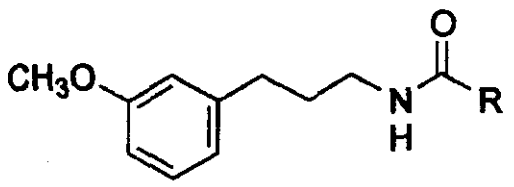
nize circadian rhythms. It was effective in phase-advancing the sleep of patients suffering from phase-delay syndrome (185), facilitating post-flight adaptation to jet lag (186), and synchronizing the sleep-wake cycle of blind patients (187). As high as 50% of elderly individuals suffer from sleep disorders that may be attributed to their diminished levels of endogenous melatonin. Results of several clinical studies indicate a beneficial effect of melatonin in elderly insomniacs. For example, 1-week treatment of conventional release melatonin (2 mg) was as effective as 2-month treatment with a 1 mg sustained release formulation on sleep initiation. Sleep maintenance (sleep efficiency and activity level) showed significant improvement only after the 2-month treatment with sustained release melatonin (188). A review of data on nighttime administration of melatonin to insomniacs with noncircadian sleep disturbances led to the conclusion that there is not convincing evidence of the therapeutic efficacy of the drug in this patient population (189). Nonetheless melatonin seems to be useful in improving sleep parameters in individuals whose biological clocks have been disrupted by normal aging or by work or travel habits.

There have been numerous reports of synthetic agents that exhibit high affinity for melatonin receptors. The great majority of such agents seem to be functional agonists and thus may mimic the pharmacological properties of the natural hormone, including effects on sleep. However, the wide disparity among the measurements of melatonin receptor binding (some employing various tissues from various mammalian and non-mammalian species and others using cloned receptors expressed in cell lines) make it very difficult to compare the potency of such agents.

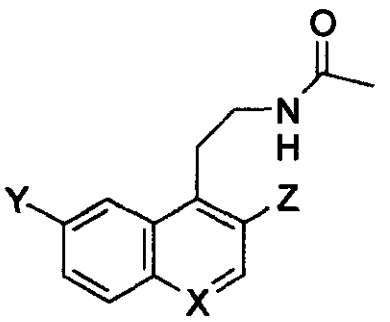
The indole-containing analogs of melatonin itself serve to establish a fairly tight SAR (181). The 5-methoxy group and the acylated amino ethyl side-chain are essential for melatonin receptor affinity; replacement of methoxy by H, OH, halogen, or even other alkoxy groups results in diminished affinity. Increasing the size of the R group in (13) to n-propyl enhances affinity, but further increase in size or branching is detrimental. Of the various ring positions, only the 2-position is tolerant of substitution, and in fact, introduction of 2-substituents as large as phenyl affords more potent ligands. Substitution at positions 1, 4, 6, and 7 generally leads to loss of affinity, although analogs where R_6 is either Cl or CH_3O have been reported to be MT, selective ligands with 50- to 60-fold higher affinity for the MT, versus the mt, receptor (190).

Many non-indolic melatonergic agents have been synthesized, thus demonstrating that the indole ring is not required for recognition at melatonin receptors. Some selected examples of these are shown in Fig. 5.15, whereas more comprehensive summaries are provided in several reviews (179, 180, 181, 191). Structure (14) represents the simplest chemotype to exhibit melatonin receptor affinity and homologs in which R is methyl and n-propyl have K_i values of 63 and 5.5 nM, respectively, in binding to the receptor in chicken brain (192). The side-chain carbon appended to the aromatic ring corresponds to C3 of melatonin's indole nucleus, and the flexibility of the side-chain permits the molecule to adopt a conformation that should closely approximate receptor-bound melatonin.

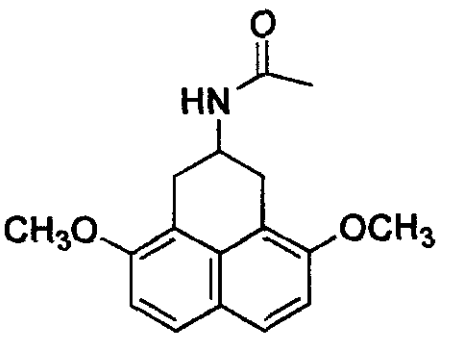
The naphthalene ring is an effective indole surrogate as exemplified by compound (15) (S-20098, agomelatine), which has been found to have essentially equivalent affinity (K_i .= 0.035 nM) to that of melatonin in binding to ovine pars tuberalis (193). Compound (15) has undergone extensive *in vivo* evaluations that have shown that it closely mimics the properties of melatonin. Several studies have shown agomelatine to entrain circadian rhythms in rats in a dose-dependent manner ($ED_{..}$ = 5.5 mg/kg) and to be as effective as melatonin in entrainment (194, 195). Agomelatine also resembled melatonin in that both compounds at 3 mg/kg IP reduced EEG power spectra in non-REM sleep without affecting vigilance states and brain temperature in rats (196). The compound has been taken into clinical trials and evaluated in healthy young men. Comparison of both drugs in a double-blind, placebo-controlled cross-over study showed them to be equally effective in inducing an earlier onset of the endogenous circadian nocturnal decline in core body temperature and heart rate (197). Single early evening doses of both melatonin (5 mg) and agomelatine (5 and 100



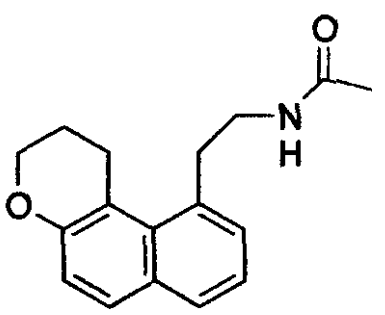
(14)



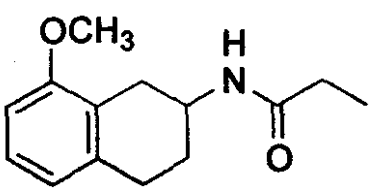
(15): X = CH, Y = OMe, Z = H
(16): X = CH, Y = H, Z = OMe
(17): X = CH, Y = Z = OMe
(18): X = N, Y = OMe, Z = H



(19)



(20)



(21)

Figure 5.15. Melatonin receptor ligands.

mg) increased sleep propensity and advanced sleep termination in the subjects without affecting the EEG patterns in either REM or non-REM sleep (198).

Other structural permutations of the naphthalene chemotype, as exemplified by (16–18), also retain potent melatonin receptor binding. The monomethoxy derivative (16) is less potent (K_i = 2.7 nM) in binding to ovine pars tuberalis receptors than its regioisomer (15), but addition of a second methoxy group increases the affinity (K_i = 0.7 nM) of (17) by an order of magnitude (199). Quinoline (18) is about as potent (K_i = 5.9 nM) as melatonin in binding to human mt, receptors expressed in CHO cells (200). The phenylene derivative (19) (201), in which the arnide-containing side-chain is conformationally constrained, and (20) (202), in which the alkoxy group is incorporated into a dihydropyran ring, also exhibit potent binding with K_i values of 0.7 and 0.1 nM, respectively, at the receptors in chicken brain and ovine pars tuberalis. The tetralin derivative (21) is a MT_2-selective ligand with 20-fold higher affinity for the MT, than the mt, receptor (190). Subtype-selective compounds may prove to be of value in elucidating the relative importance of the receptor subtypes in the pharmacology of melatonin.

Whether any synthetic melatonergic agonists will ever become approved drugs for the treatment of sleep disorders is a matter of speculation. A major obstacle they face is that the naturally occurring hormone is available on an over-the-counter basis in many countries including the United States; thus, any synthetic drug would have to have some distinct advantage over melatonin itself. Melatonin receptors have been identified in the vasculature and their activation by melatonin results in a vasoconstrictor response (181). Thus, melatonin could be contraindicated in individuals, particularly the elderly, who have cardiovascular conditions. Perhaps a receptor subtype-selective agent would maintain the beneficial effects of melatonin on sleep without melatonin's vasoconstrictor properties and other pharmacological effects.

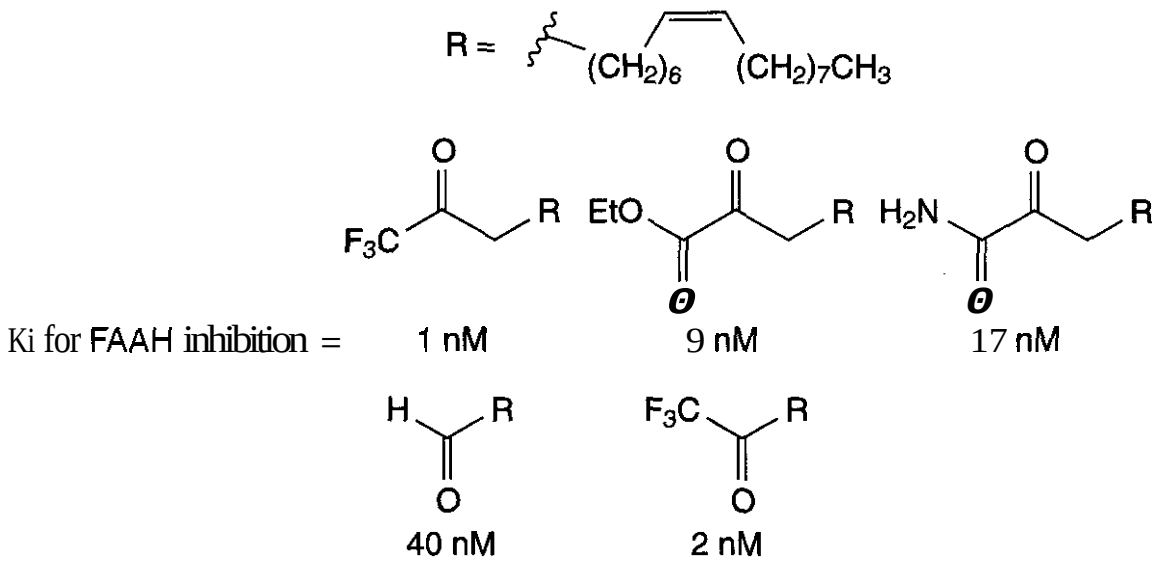
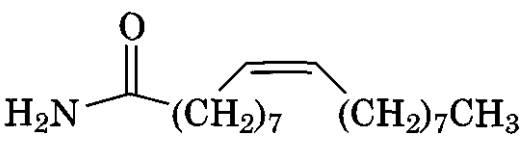


Figure 5.16. Inhibitors of FAAH.

6.2 Oleamide

In the mid-1990s a collaborative team of chemists and biologists at the Scripps Research Institute reported on the isolation and structural and pharmacological characterization of a substance that accumulated under conditions of sleep deprivation in cats (202–205). Clues to the structure of the material, which was obtained in only trace quantities from cat cerebrospinal fluid, were afforded by mass spectometry, which revealed an empirical formula of $C_{18}H_{35}NO$ and a lipid fragmentation pattern. Subsequent comparison of the isolated material with a large number of synthetic compounds having the correct formula and degree of unsaturation established its structure as 9(Z)-octadecanamide (22).



(22)

This material, referred to by the trivial name, oleamide, proved to be of interest owing to the fact that the synthetic compound was found to induce sleep in rodents and cats. Various measures of sleep parameters after oleamide administration showed the sleep to be of a physiological quality. The compound was found to dose-dependently increase the total time of slow wave sleep at the expense of waking and to lower body temperature, which is characteristic of physiological sleep. Furthermore intraventricular injection of oleamide (2.8 mg) into rats caused sleep analogous to its IP administration (5 or 10 mg/kg), thus indicating a direct action in the brain. These effects were found to be compound-specific because a number of close structural analogs including the ***trans*** isomer, compounds with the cis double bond migrated to other positions within the 18 carbon chain, a saturated analog, and the corresponding carboxylic acid were all weakly effective or ineffective at inducing sleep.

Studies of the degradation and regulation of oleamide revealed that it was hydrolyzed to oleic acid and ammonia by the action of a membrane-bound enzyme, which based on the inhibition of its activity, seemed to be a serine or cysteine protease. Isolation and sequencing of the protein led to the cloning of its cDNA and expression in COS-7 cells. The expressed enzyme was found to not only hydrolyze oleamide but a number of other fatty acid amides and was thus designated as fatty acid amide hydrolase or FAAH (206).

A number of inhibitors of FAAH have been synthesized (207), and the more potent of these are depicted in Fig. 5.16. The most potent compounds contain a highly electrophilic carbonyl as part of either an a-ketoester, α-ketoamide of trifluoromethyl ketone moiety, structural features common to serine/cysteine protease inhibitors. Several of these FAAH inhibitors caused sleep enhancement and lowering of body temperature in rats that were comparable with those induced by oleamide. Based on these findings, it has been suggested that FAAH may represent a therapeutic target for

the discovery of sleep aids that potentiate the effects of oleamide by blocking its degradation; some inhibitors (e.g., a-ketoamides) might be dual acting agents that mimic the action of oleamide as well as attenuating its metabolism (208). Of course, as with any class of enzyme inhibitors, the potential clinical use of FAAH inhibitors is contingent on their selectivity, because indiscriminate interaction with other proteases could result in undesirable side effects.

A detailed review of oleamide, including a discussion of its possible modes of action such as serotonin receptor modulation and gap junction inhibition, is available (208).

7 THINGS TO COME

Pharmaceutical company research on sedative-hypnotics rose and peaked within the several decades after the introduction of the benzodiazepines and has waned since then because CNS-related drug discovery efforts have focused instead on improved therapy for such disorders as depression and schizophrenia and the unmet medical need of stroke, Alzheimer's disease, and other neurodegenerative maladies. However, given the imperfections of available drugs and the high incidence of sleep disorders among the ever-growing elderly population, more efficacious and safer agents are certainly needed. The current stable of drugs typically act as CNS depressants that do not promote physiological sleep and that may cause cognitive and memory impairment, motor skills impairment (especially when ingested along with alcohol), and have potential abuse liability. Where then, should we look for better agents, agents that may be better classified as sleep normalizers rather than sedative-hypnotics? Use of pharmacophore models such as that shown in Fig. 5.14 could conceivably lead to the design and synthesis of patentably-novel compounds, but their pharmacological properties would most likely resemble those of the known sedative-hypnotics from whose structural parameters the models are constructed. As discussed in previous sections, development of both melatonin receptor agonists and FAAH inhibitors could prove to be fruitful approaches, although both have some caveats.

New opportunities for sleep therapy may be forthcoming from recent discoveries pertaining to the hypocretin/orexin ligand-receptor system. The hypocretinlorexin (Hcrt/Ox) gene, described in 1998, encodes two neuropeptides, hypocretin-1 (Hcrt1) and hypocretin-2 (Hcrt2), which are called orexin-A(Ox-A) and orexin-B (Ox-B), respectively (209, 210). Although the cell bodies in which the hypocretins are made are restricted to the perifornical and dorsal and lateral hypothalamic areas, they send projections to multiple neuronal systems throughout the brain including those containing neurons responsible for maintenance of the waking state (211). mRNAs for hypocretin receptors are differentially distributed in the brain with the highest levels of Hcrt1R mRNA occurring in ventromedial hypothalamic nuclei and locus coeruleus, whereas Hcrt2R mRNA are found predominantly in hypothalamic paraventicular nuclei and layer VI of the cortex (212). The hypocretin/orexin ligand-receptor system and its implications for sleep and sleep disorders have been reviewed, and a model for the involvement of the neuropeptides in arousal state control has been proposed (213). The model suggests that a balanced sleep-wake cycle may depend on modulation by hypocretinlorexin cell activation of monoaminergic neuronal populations in the locus coeruleus (noradrenergic), dorsal raphe nucleus (serotonerigic), and ventral tegmental area (dopaminergic), as well as of cholinergic cells in the basal forebrain. In support of such a model are findings that a mutation of the Hcrt2R gene resulting in nonfunctional receptors causes narcolepsy in dogs and that hypocretin knockout mice exhibit "behavioral arrest" and significantly increased levels of REM and non-REM sleep. If removal of the hypocretin/orexin ligand-receptor system results in a condition marked by excessive and uncontrollable sleep (i.e., narcolepsy), then overactivity of this system may cause excessive arousal and impaired sleep. It will be of interest to see if further research in this exciting area and the identification of small non-peptide molecules capable of selectively blocking hypocretin/orexin receptors will lead to novel agents for the effective and safe treatment of sleep disorders.

8 WEBSITES

The following are a number of selected websites that pertain to sleep disorders and/or sedative-hypnotics.

http://www.methodisthospitals.org/services/diagnostics/sleep/sleep4.html: diagnosis and therapy of sleep disorders

http://www.todoc.com/sleep/sleep.html: sleep disorders and their treatment

http://blueprint.bluecrossmn.com/article/iac/100547893: sleep disorders and their treatment

http://www.aafp.org/afp/20000501/2763.html: abuse of and intoxication by psychotropic drugs including sedative-hypnotics

http://www.acnp.org/G4/GN401000173/CH169.html: mechanism of action and pharmacology of barbiturates

http://www.aafp.org/afp/20000501/2763.html: abuse of and intoxication by psychotropic drugs including sedative-hypnotics

http://www.acnp.org/G4/GN401000173/CH169.html: mechanism of action and pharmacology of barbiturates

http://www.neuronic.com/neuronics/sleepand2.htm: abstracts of clinical studies on sleep and sleep disorders

http://www.extendedcare.com/library/sleepprint.html: sleep and sleep disorders in older adults

http://swdca.org/seniors.html: sleep disorders in the elderly

http://www.medinfosource.com/gt/g000809.html: sleep disorders in the elderly

http://www.ascp.com/public/pubs/tcp/1999/may/r_r.shtml: clinical study on treatment of sleep disorders in the elderly with sedative-hypnotic agents

http://www.theberries.ns.ca/archives/conscious sedation.html: administration of sedative-hypnotic agents to patients to achieve conscious sedation (e.g., preoperative and in dentistry)

http://www.nyspsych.org/cybercol/mar98/bialer.html: co-administration of protease inhibitors and psychotropic medications such as sedative-hypnotics to patients with HIV infection

http://www.hivdent.org/mentalh/mental_sleepd.htm: diagnosis and treatment of sleep disorders in HIV patients

http://www.well.com/user/woaJfsseda.htm: facts on sedative-hypnotics including abuse potential

http://www.hc-sc.gc.ca/hppb/alcohol-otherdrugs/pube/straight/sedative.html: commonly abused sedative-hypnotics

http://www.aafp.org/afp/20000501/2763.html: abuse of and intoxication by psychotropic drugs including sedative-hypnotics

http://www.acnp.org/G4/GN401000173/CH169.html: mechanism of action and pharmacology of barbiturates

http://www.csusm.edu/DandB/Sedatives.html: behavioral effects and mechanism of action of barbiturates

http://www.sonatasleep.corn/healthpro/about-sonatalabout_sonata.html: facts about zaleplon (Sonata)

http://www.laurushealth.com/Library/HealthGuide/DrugGuide/_showTopic.asp?topic_id=9482&sequence=1: facts about zolpidem

http://www.sonatasleep.comlhealthpro/pi/pi.htm: drug information on zaleplon (Sonata)

http://www.healthyplace.com/medications/triazolam.htm: pharmacology, indications, contraindications, etc., of triazolam (Halcion)

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CHAPTER SIX

Anticonvulsants

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1 INTRODUCTION

The central nervous system (CNS) constitutes the cerebral cortex, the limbic system, the midbrain, the brainstem, the cerebellum, and the spinal cord (1)Epilepsy is one of the most common disorders of the brain, affecting about 50 million individuals worldwide (1, 2). Epilepsy is a chronic and often progressive disorder characterized by the periodic and unpredictable occurrence of epileptic seizures that are caused by abnormal discharge of cerebral neurons (2). Epilepsy is not a disease, but a syndrome of different cerebral disorders of the CNS. This syndrome is characterized by paroxysmal, excessive, and hypersynchronous discharges of large numbers of neurons (3). These seizures may be identified on the basis of their clinical characteristics. These clinical attributes, along with their electroencephalographic (EEG) pattern, can be used to categorize seizures (4). Seizures are basically divided into two major groups: partial and generalized. Partial (focal, local) seizures are those in which clinical or EEG evidence exists to indicate that the disorder originates from a localized origin, usually in a portion of one hemisphere in the brain (4). Partial seizures may be further subdivided into simple partial, complex partial, and partial seizures evolving into secondarily generalized seizures. In generalized seizures, the evidence for a local origin is lacking. Generalized seizures may be further subdivided into absence (nonconvulsive), myoclonic, clonic, tonic, tonic-clonic, and atonic seizures.

A later Commission study expanded the classification of seizures that occur in patients with epilepsy. Patients are classified into appropriate types of epilepsy and epileptic syndromes characterized by different seizure types, etiologies, ages of onset, and EEG features (5). More than 40 distinct epileptic syndromes have been identified, making epilepsy an extremely diverse collection of disorders. The first major division of epilepsy is localization-related (i.e., focal, local, partial) epilepsies, which account for about 60% of all epilepsies. The remainder, about 40%, is composed of generalized epilepsies (2). An epilepsy, or epileptic syndrome, is either idiopathic, virtually synonymous with genetic epilepsy; or symptomatic, which is attributed to a structural lesion or major identifiable metabolic derangements (2). Both types of seizure patterns and epilepsy determine the choice and prognosis of therapy. As an example, the most common, and most difficult to treat, seizures in adult patients are complex partial seizures, whereas primary generalized tonic-clonic (formerly, "grand mal" epilepsy) seizures respond in most patients to treatment with anticonvulsants. However, for many seizure types and epilepsy syndromes, there is little information about the pathophysiological basis. However, on the other hand, and most fortuitously, insight into how partial seizures, generalized tonic-clonic seizures, and generalized absence seizures arise is substantial, given that these seizure types constitute about 90% of seizures (7).

In the absence of a specific etiologic understanding in any of the epilepsies or epileptic syndromes, approaches to drug therapy of epilepsy must of necessity be directed at the control of symptoms, that is, the suppression of seizures. Currently, all available drugs are anticonvulsant (i.e., antiseizure) rather than antiepileptic (2). The latter term should be used only for drugs that prevent or treat epilepsy and not solely its symptoms (2). The goal of therapy with an anticonvulsant agent is to have the patient seizure free without interfering with normal brain function. Thus, the selection of an anticonvulsant agent is based primarily on its efficacy for specific types of seizures and epilepsy (7, 8). Although seizure control is generally good in most patients, a significant proportion of patients with epilepsy suffer from intractable or drug-resistant epilepsy, despite early treatment and an optimum daily dosage of an adequate anticonvulsant agent (9–13). There is thus a need for new drugs with a greater benefit as related to side effects and tolerability, even at the expense of efficacy, when compared to the existing antiepileptic agents (14, 15).

2 CLINICAL APPLICATIONS

As previously indicated, EEGs have been used in the diagnosis of epilepsy. However, the EEG findings should also be correlated with the ictal event for an unequivocal determination (16). For a complete discussion of seizure types, refer to the previous edition of this chapter (8).

2.1 Current Drugs

The anticonvulsant agents may be conveniently grouped into three general categories (2):

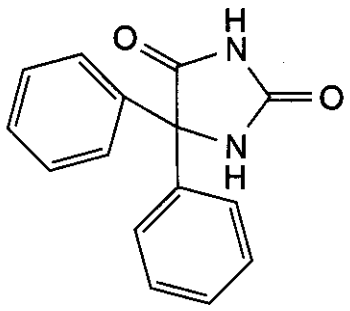
1. "First-generation" or older agents as exemplified by phenytoin (**1**), carbamazepine (**2**), valproate (**3**), the benzodiazepines (**4**), ethosuximide (**5**), phenobarbital (**6**), primidone (**7**), and trimethadione (**17**), all of which were introduced between 1910 and 1970 (see Fig. 6.1).
2. "Second-generation" or newer agents consisting of vigabatrin (**8**), gabapentin (**9**), felbamate (**10**), lamotrigine (**11**), oxcarbazepine (**12**), zonisamide (**13**), tiagabine (**14**), topiramate (**15**), and levetiracetam (16) (see Fig. 6.2).
3. "Third-generation" agents are those agents that are in preclinical or clinical development.

Table 6.1 provides the proprietary name, USP or nonpropietary name, chemical class, and the manufacturer of the nongeneric agent. The general therapeutic indications for the first- and second-generation agents are found in Table 6.2, whereas Table 6.3 lists the newer anticonvulsant agents for use in pediatric patients (17). The third-generation agents are found in Sections 6 and 7 of this chapter.

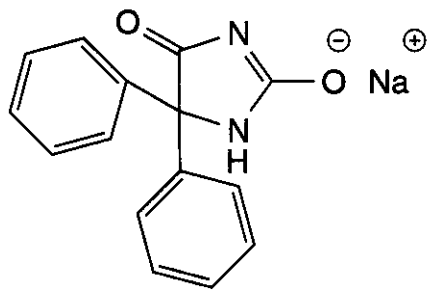
2.2 Side Effects, Adverse Effects, Drug Interactions/Contraindications

Table 6.4 provides the side effects, adverse effects, and drug interactions and/or contraindications for the listed anticonvulsants. Additional explanations as noted in the table are provided as follows.

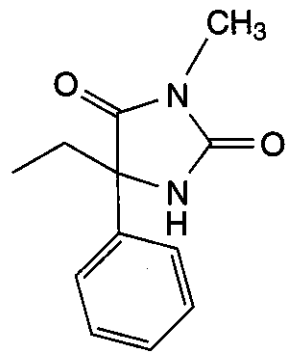
Phenytoin. As with all anticonvulsants, phenytoin included, their central side effects include drowsiness and/or dizziness. In addition, phenytoin also produces blurred vision that may be serious to those individuals who operate heavy machinery. The adverse effects with phenytoin can occur to the cardiovascular system when the agent is administered rapidly by the intravenous route. Atrial and ventricular conduction depression and ventricular fibrillation have occurred, particularly with the elderly or seriously ill patients (18). Gingival hyperplasia occurs frequently with this agent and may be reduced with good oral hygiene. Drug interactions with phenytoin are widely distributed and include valproic acid, which increases the effects of phenytoin by both inhibiting metabolism and displacing the bound phenytoin from plasma proteins, whereas the salicylates displace the drug from its plasma protein binding sites in a dose-dependent manner. Phenytoin concentrations may be decreased with the following drugs. Barbiturates exert a variable effect on the phenytoin concentration; thus the combination will present problems for the clinician, espe-



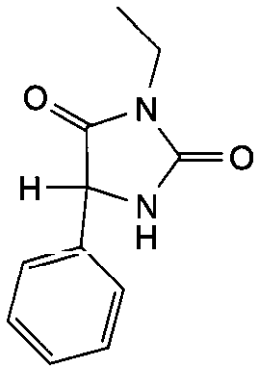
Phenytoin, (1)



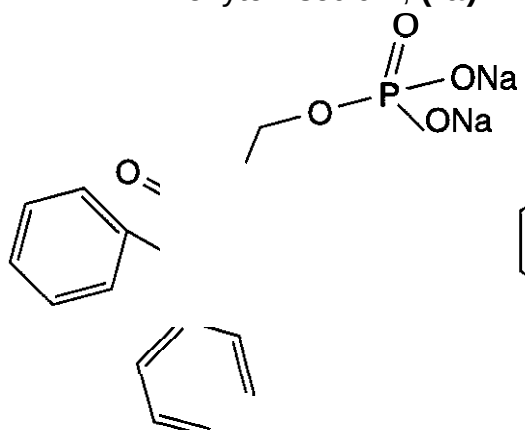
Phenytoin sodium, **(Ia)**



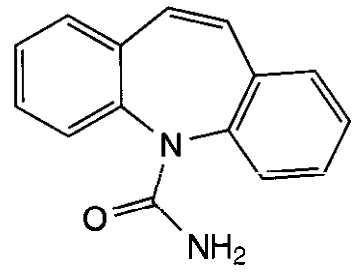
Mephenytoin, **(1b)**



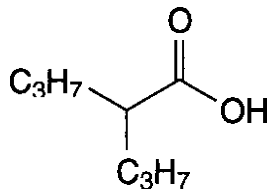
Ethotoin, (Ic)



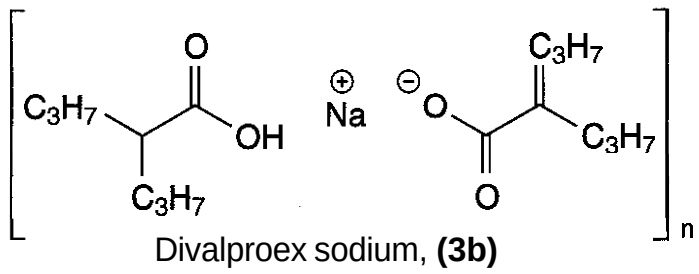
Fosphenytoin, **(Id)**



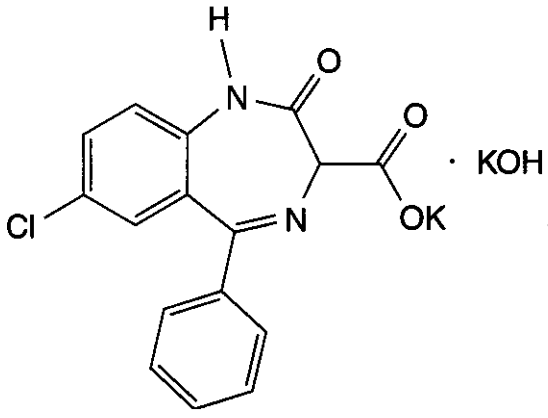
Carbamazepine, (2)



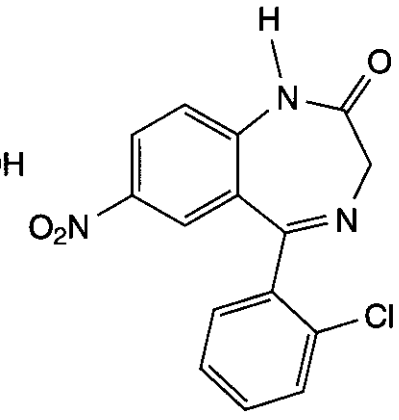
Valproic acid, **(3a)**



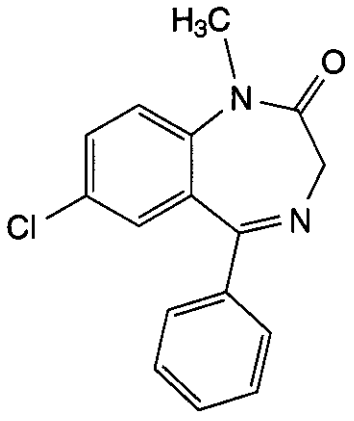
Divalproex sodium, **(3b)**



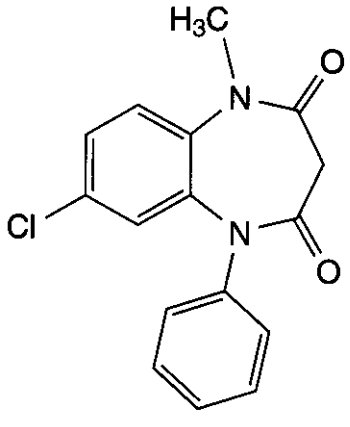
Clorazepate dipotassium, **(4a)**



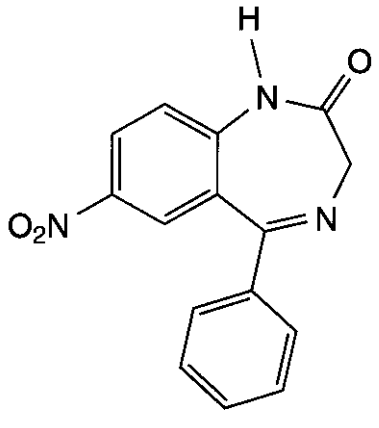
Clonazepam, **(4b)**



Diazepam, (**4c**)



Clobazam, **(4d)**



Nitrazepam, **(4e)**

Figure 6.1. "First-generation" anticonvulsants.

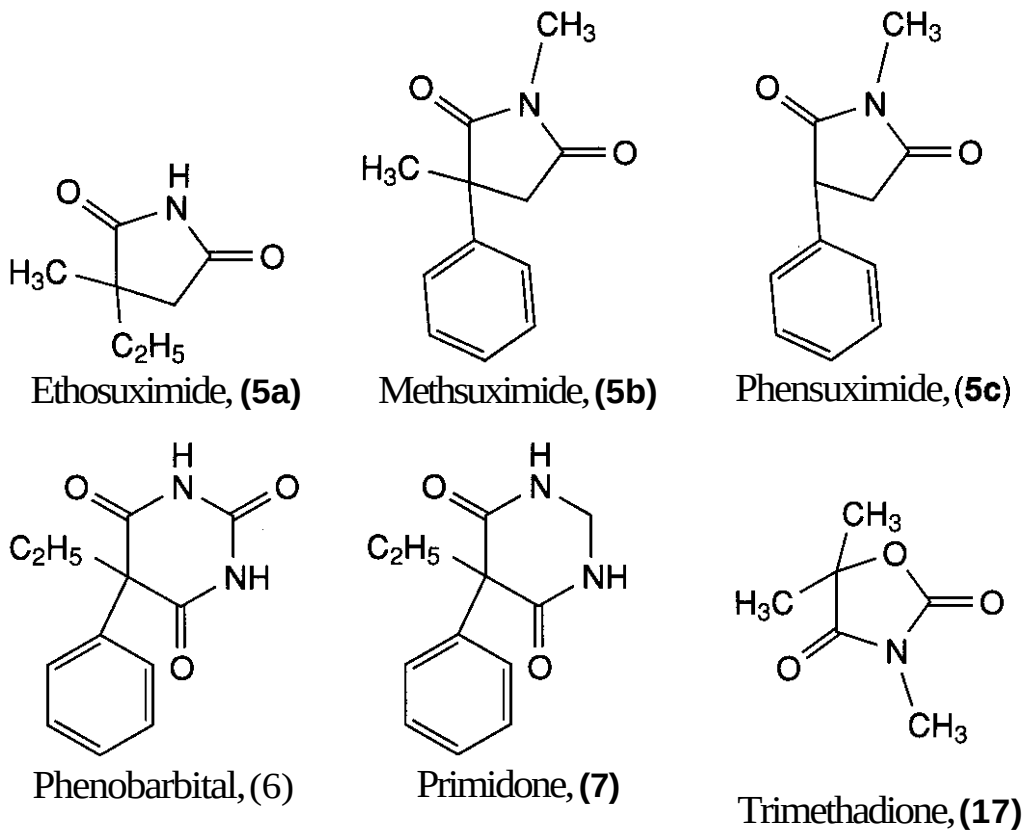


Figure 6.1. (Continued.)

cially when initiating or stopping either drug. Likewise, carbamazepine exerts a variable effect on phenytoin levels; conversely, carbamazepine serum levels may also be decreased. Folate deficiency has been noted with long-term phenytoin therapy, given that folate is a cofactor in the metabolism of phenytoin through hydroxylation (see metabolism). Similarly, influenza virus vaccine may increase, decrease, or have no effect on the total serum phenytoin concentrations (18).

Carbamazepine. The adverse effects with carbamazepine include the potential to produce aplastic anemia and agranulocytosis; however, it should be noted that the majority of patients presenting with leukopenia have not progressed to these more serious blood dyscrasias. Initial pretreatment hematological testing should be undertaken with all patients with carbamazepine. Drug interactions are focused on the 3A4 isoform of the P450 enzyme; inhibitors increase carbamazepine levels, whereas inducers produce the opposite effect. Special note should be taken with the carbamazepine-isoniazid interaction. Isoniazid inhibits carbamazepine metabolism, whereas carbamazepine may increase isoniazid metabolism to hepatoxic products (18).

Valproic Acid. An adjunctive agent, valproic acid bears three warning statements: (1) hepatotoxicity especially in children <2 years old who are on multiple therapy; (2) teratogenicity that includes neural tube defects; and (3) life-threatening pancreatitis.

Diazepam. The side effects of diazepam for intravenous administration are similar to those of phenytoin when administered by this route, that is, cardiovascular collapse when administered too rapidly.

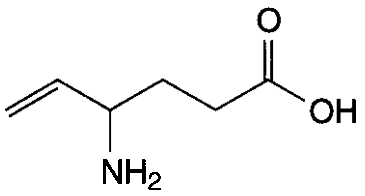
Clonazepam. Clonazepam interacts with phenytoin with the resultant decrease in plasma levels of the benzodiazepine.

Clobazam and Nitrazepam. Two new investigational benzodiazepines appear to be safe and effective as anticonvulsants. However, these agents have long elimination half-lives that increase the potential not only for drug accumulation but also for residual side effects.

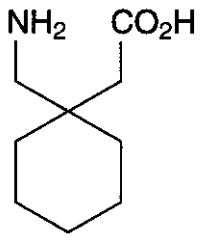
Ethosuximide. This succinimide is relatively safe from side effects and drug interactions.

Phenobarbital. The older barbiturate is to be used with caution in patients with patent liver or respiratory symptoms. Phenobarbital can cause fetal damage when administered to pregnant women.

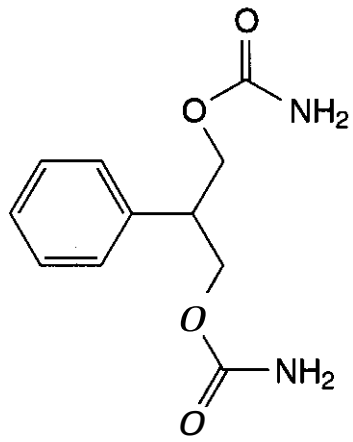
Primidone. As noted from the structure and biotransformation pathway, primidone is closely related to phenobarbital; thus patients who are hypersensitive to phenobarbital should



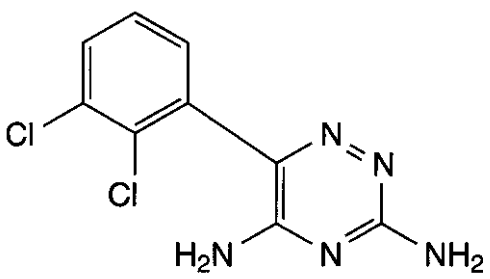
Vigabatrin, (8)



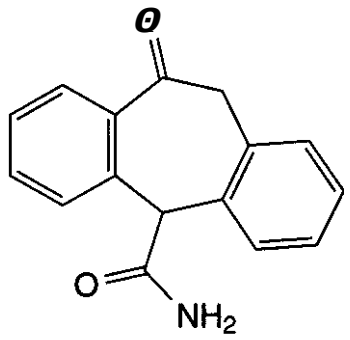
Gabapentin, (9)



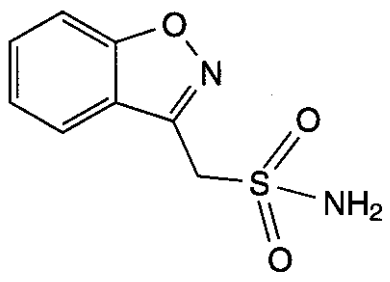
Felbamate, (10)



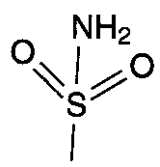
Lamotrigine, (11)

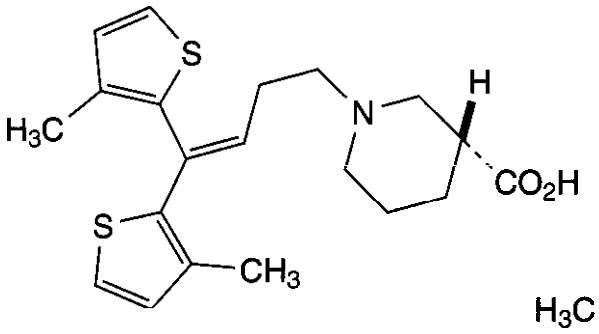


Oxcarbazepine, (12)

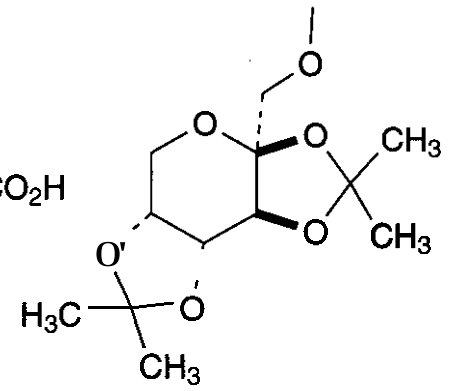


Zonisamide, (13)

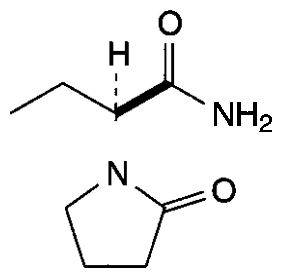




Tiagabine, (14)



Topiramate, (15)



Levetiracetam, (16)

Figure 6.2. "Second-generation"anticonvulsants.

not be given this agent. Because the parent compound is metabolized to two active metabolites (see Section 2.3), patients with impaired liver function should be carefully monitored.

Trimethadione. This agent contains the warning statement that, because of the potential to produce fetal malformations and serious side effects, trimethadione should be used only when other less toxic drugs have been found ineffective in controlling petit mal seizures.

Vigabatrin. A new investigational adjunctive agent in the United States, little is known about its side effects, drug interactions, or contraindications. However, a recent article from Sweden disclosed a strong relationship between visual field effects (VFDs) and vigabatrin treatment (19). These VFDs were related to the duration and total dose of the agent. Further, the VFDs were irreversible and, in a significant number of patients, progressive.

Gabapentin. This agent is used as an adjunctive agent and is relatively safe from side effects in nonpregnant adult patients. There was noted a small decrease in gabapentin excretion with concurrent cimetidine administration, which was not clinically significant. It is recommended that gabapentin be taken at least 2 h after antacid administration.

Felbamate. Felbamate is an adjuvant anticonvulsant, containing the warning that its use is associated with a marked increase in the incidence of aplastic anemia and that patients being started on the drug should have liver function tests performed before therapy is initiated. Animal studies have revealed a statistically significant increase in hepatic cell adenomas in high dose studies (18). It is postulated that this cancer was induced by toxic by-products urethane and methyl carbamate. Felbamate is not recommended as first-line therapy and is indicated for those patients who respond inadequately to alternative treatments and whose epilepsy is so severe that a substantial risk of aplastic anemia or liver failure is deemed acceptable in light of the benefits provided by its use.

Lamotrigine. This agent is an adjuvant anticonvulsant that bears the warning that serious skin rashes requiring hospitalization and cessation of treatment have been associated with its use. It is approved for use in pediatric patients $\geq$ 16 years of age who have seizures associated with the Lennox-Gastaut syndrome. Discontinue treatment at the first sign of rash, unless the rash is clearly not drug related. Visual problems (i.e. blurred vision) occurred more frequently with patients receiving both carbamazepine with lamotrigine.

Oxcarbazepine. Oxcarbazepine is a new, relatively safe anticonvulsant: however, cross-hypersensitivity was noted in patients previously hypersensitive to carbamazepine (25–30%). Asymptomatic hyponatremia has occurred in clinical trials during the first 3 months of therapy. Clinicians are advised to monitor serum sodium levels for patients during maintenance treatment.

Zonisamide. Zonisamide is a new sulfonamide anticonvulsant. In one study it was noted that 4% of the patients developed clinically possible or confirmed kidney stones (18). Patients should report the appearance of sudden back pain, abdominal pain, or blood in the urine that could indicate a kidney stone. In several clinical studies, zonisamide was associated with a statistically significant 8% mean increase from the baseline of serum creatinine and blood urea nitrogen (BUN). This was indicative of a decrease in the glomerular filtration rate (GFR). Therefore, do not use this agent in patients with renal failure (GFR $<$ 50 mL/min) because there is limited information on dosing and toxicity (18). Fatalities have occurred, although rarely, as a result of severe reactions to sulfonamides, including Stevens-Johnson syndrome, toxic epidermal necrolysis, fulminant hepatic necrosis, agranulocytosis, aplastic anemia, and other blood dyscrasias. Such a reaction may occur when a sulfonamide is readministered, regardless of the route of administration. If signs of hypersensitivity or other serious reactions occur, discontinue zonisamide immediately.

Tiagabine. Tiagabine is an adjunctive agent that does not present any reported drug interactions. However, this agent possesses clinically significant side effects related to the CNS. These are: (1) impaired concentration, speech or language problems, and confusion; and (2) somnolence and fatigue. These effects are mild to moderate and occurred to a greater frequency when used in combination with other anticonvulsants.

Topiramate. Topiramate is an adjunctive agent that possesses clinically significant side effects related to the CNS. These are: (1) psychomotor slowing, difficulty with concentration and speech, or language problems; and (2) somnolence and fatigue. These effects are mild to moderate and occurred to a greater frequency when used in combination with other anticonvulsants. Kidney stones occurred in 1.5% of patients exposed to topiramate and may be related to the drug's weak carbonic anhydrase inhibiting properties. Carbonic anhydrase inhibitors, such as acetazolamide or dichlorphenamide, increase kidney stone formation by decreasing citrate excretion and by increasing urinary pH. Thus, the combination of topiramate with carbonic anhydrase inhibitors should be avoided (18).

Levetiracetam. Levetiracetam is a new anticonvulsant agent that does not as yet present any drug interactions. Dizziness and somnolence occurred to a statistically significant extent (14.8% versus 8.4% of the placebo patients) (18). This side effect occurred more frequently with patients receiving levetiracetam in combination with other anticonvulsant agents.

Table 6.1 Proprietary Names, USP or Nonproprietary Name, Chemical Class, and Manufacturer of Antiepileptic Agents in Current Use[a]

Generic Name (USP or Nonproprietary Name)	Chemical Classification	Proprietary Name (Manufacturer)	Route of Administration/Dose
Phenytoin, USP, (1) (5,5-diphenylhydantoin; 5,5-diphenyl-2,4-imidazolidinedione)	Hydantoin	Dilantin (Parke-Davis)	Dilantin Infatab (50 mg chewable tablet) Dilantin-125 (oral suspension containing 125 mg/5 mL)
Phenytoin sodium, parenteral, (1a)	Hydantoin	Dilantin (Parke-Davis)	Injection: 50 mg/mL (=46 mg phenytoin)
		Phenytoin sodium (SoloPak; Elkins-Sinn)	
Phenytoin, Prompt, (1a)	Hydantoin	Phenytoin sodium (various)	Tablet (100 mg = 92 mg phenytoin)
Phenytoin sodium, extended, (1a)[b]	Hydantoin	Phenytoin sodium (various)	Capsules (100 mg = 92 mg phenytoin)
		Dilantin Kapseals (Parke-Davis)	Capsules (30 mg = 27.6 mg phenytoin; 100 mg = 92 mg phenytoin)
Mephenytoin, USP, (1b)	Hydantoin	Mesantoin (Sandoz)	Tablet (100 mg)
Ethotoin, USP, (1c)	Hydantoin	Peganone (Abbott)	Tablet (250 mg, 500 mg)
Fosphenytoin sodium, (1d)	Hydantoin	Cerebyx (Parke-Davis)	Injection (150 mg = 100 mg phenytoin; 750 mg = 500 mg phenytoin)
Carbamazepine, USP, (2)	Iminostilbene	Tegretol (Novartis)	Tablets (chewable, 100 mg; 200 mg) Suspension (100 mg/5 mL)
		Tegretol-XR	Tablets, extended release (100 mg, 200 mg, 400 mg)
		Cabatrol (Athena Neurosciences)	Capsules, extended release (200 mg, 300 mg)
			Tablets (200 mg)
		Atretol (Athena Neurosciences)	Tablets (200 mg)
		Epitol (Teva)	Tablets (chewable, 100 mg)
		Carbamazepine (Various)	Tablets (200 mg)
Valproic acid, USP, (3a)	Aliphatic acid	Depakene (Abbott)	Capsules (250 mg) Syrup [250 mg (as sodium valproate)/5 mL]
		Depakote, (3b) (Abbott)	Tablets, delayed-release [125 mg, 250 mg, 500 mg (as divalproex sodium)]
			Capsules, sprinkle [125 mg (as divalproex sodium)]
		Depakote ER (Abbott)	Tablets, extended release (500 mg)
		Depacon (Abbott)	Injection [100 mg/mL (as valproic acid)]
		Valproic acid (various)	Capsules (250 mg) Syrup [250 mg (as sodium valproate)]

Clorazepate dipotassium, USP, (4a)	Benzodiazepine	Tranxene-T (Abbott)	Tablets (3.75 mg, 7.5 mg, 15 mg)
		Tranxene-SD	Tablets (11.25 mg, 22.5 mg)
		Gen-Xene (Alra)	Tablets (3.75 mg, 7.5 mg, 15 mg)
		Clorazepate (various)	Tablets (3.75 mg, 7.5 mg, 15 mg)
Clonazepam, USP, (4b)	Benzodiazepine	Klonopin (Roche)	Tablets (0.5 mg, 1 mg, 2 mg)
		Clonazepam (various)	Tablets (0.5 mg, 1 mg, 2 mg)
Diazepam, USP, (4c)	Benzodiazepine	Valium (Roche)	Tablets (2 mg, 5 mg, 10 mg)
			Injection (5 mg/mL)
		Diazepam (various)	Tablets (2 mg, 5 mg, 10 mg)
		Diazepam (Roxane)	Solution (1 mg/mL)
		Diazepam Intensol (Roxane)	Solution (Intensol) (5 mg/mL)
		Diastat (Elan)	Gel, rectal (2.5 mg," 5 mg," 10 mg,[c] 10 mg,[d] 15 mg,[d] 20 mg[d])
Ethosuximide, USP, (5a)	Succinimide	Zarontin (Parke-Davis)	Capsules (250 mg)
			Syrup (250 mg/5 mL)
		Ethosuximide (Copley)	Syrup (250 mg/mL)
Methsuximide, USP, (5b)	Succinimide	Celontin Kapseals (Parke-Davis)	Capsules, half strength (150 mg)
			Capsules (300 mg)
Phensuximide, USP, (5c)	Succinimide	Milontin Kapseals (Parke-Davis)	Capsules (500 mg)
Phenobarbital, USP, (6)	Barbiturate; Ureide	Phenobarbital (various)	Tablets (15 mg, 16 mg, 16.2 mg, 30 mg, 60 mg, 90 mg, 100 mg)
			Capsules (16 mg)
			Elixir (15 mg/5 mL, 20 mg/5 mL)
		Phenobarbital sodium (various)	Injection (30 mg/mL, 60 mg/mL, 65 mg/mL, 130 mg/mL)
		Luminal Sodium (Sanofi Winthrop)	Injection (130 mg/mL)
Primidone, USP, (7)	Dihydrobarbiturate	Mysoline (Wyeth-Ayerst)	Tablets (50 mg, 250 mg)
			Oral Suspension (250 mg/5 mL)
		Primidone (various)	Tablets (250 mg)
Trimethadione, USP, (17)	Oxazolidinedione	Tridione (Abbott)	Dulcets (Tablets, chewable) (150 mg)
			Capsules (300 mg)
Vigabatrin, (8)	GABA analog	Sabril (Marion Merrell Dow)	—[e]

Table 6.1 (Continued)

Generic Name (USP or Nonproprietary Name)	Chemical Classification	Proprietary Name (Manufacturer)	Route of Administration/Dose
Gabapentin, (9)	Cyclohexaneacetic acid	Neurontin (Parke-Davis)	Capsules (100 mg, 300 mg, 400 mg)
Felbamate, (10)	Propanediol carbamate	Felbatol[e] (Wallace)	Tablets (400 mg, 600 mg) Suspension (600 mg/5 mL)
Lamotrigine, (11)	Triazine	Lamictal (GlaxoWellcome) Lamictal Chewable Dispersible Tablets (GlaxoWellcome)	Tablets (25 mg, 100 mg, 150 mg, 100 mg) Tablets, chewable (5 mg, 25 mg)
Oxcarbazepine, (12)	Iminostilbene	Trileptal (Novartis)	Tablets (150 mg, 300 mg, 600 mg)
Zonisamide, (13)	Sulfonamide	Zonegran (Elan Pharma)	Capsules (100 mg)
Tiagabine, (14)	Nipecotic acid analog	Gabitril Filmtabs (Abbott)	Tablets (4 mg, 12 mg, 16 mg, 20 mg)
Topiramate, (15)	Sulfamate analog	Topamax (Ortho-McNeil)	Tablets (25 mg, 100 mg, 200 mg) Capsules, sprinkle (15 mg, 25 mg, 50 mg)
Levetiracetam, (16)	Acetamide	Keppra (UCB Pharma)	Tablets (250 mg, 500 mg, 750 mg)

[a]See Fig. 6.1 for the structures.
[b]May be used for once-a-day dosing.
[c]Pediatric.
[d]Adult.
[e]Investigational drug.
[f]It has been recommended that use of this drug be discontinued if aplastic anemia or hepatic failure occurs unless, in the judgment of the physician, continued therapy is warranted.

Table 6.2 Anticonvulsant Effect of First- and Second-Generation Agents Against Different Types of Seizures in Human Epilepsy"

	Clinical Efficacy			
		Generalized Seizures		
Agent	Partial Seizures	Tonic–Clonic	Absence	Myoclonic
First-generation				
Carbamazepine	+	+	NE	NE
Phenytoin	+	+	NE	NE
Phenobarbital	+	+	NE	+
Primidone	+	+	+	+
Valproate	+	+	+	+
Benzodiazepines	+	+	+	+
Ethosuximide	NE	NE	+	±
Second-generation				
Lamotrigine	+	+	+	+
Topiramate	+	+	±	+
Oxcarbazepine	+	?	NE	NE
Felbamate	+	+	±	+
Vigabatrin	+	?	NE	NE
Tiagabine	+	?	?	NE
Gabapentin	+	?	NE	NE

"Effect is indicated by the following: +, effective; ±, inconsistent data; NE, not effective; ?, no data available (or found). Data are from Ref. 2. For a complete description of the seizure types, refer to previous edition (Ref. 8).

2.3 Absorption, Distribution, Metabolism, and Elimination

Table 6.5 provides the pharmacokinetic parameters noted for the listed anticonvulsants, which include, where possible, peak plasma levels, half-life, volume of distribution, and plasma protein binding. Additional explanations as noted in the table are provided as follows.

2.3.1 Hydantoins

Phenytoin. Phenytoin (**1**) is slowly absorbed from the small intestine. The rate, extent, and bioavailability vary because of the manufacturer's formulation process. Intramuscular injection tends to precipitate at the site of injection, resulting in erratic plasma levels; these levels are significantly lower than those obtained by the oral route. Phenytoin is metabolized in the liver to inactive hydroxylated metabolites (see Fig. 6.3) (20). For a complete discussion, the reader is referred to the earlier edition of this chapter (8). The metabolism of phenytoin is capacity limited and shows saturability. Because the elimination of the *p*-hydroxy glucuronide metabolite is rate limited by its formation from phenytoin, measurement of the metabolite in urine can be used to determine the rate of metabolism, patient compliance, or bioavailability (18).

Mephenytoin. Chemically, mephenytoin is 3-methyl-5-ethyl-5-phenylhydantoin (**1b**) (Fig. 6.1) and is dispensed as the racemate. The R and S forms undergo stereoselective oxidative biotransformation. The *S*-mephenytoin undergoes rapid para-hydroxylation (Fig. 6.3), whereas the R enantiomer is slowly demethylated to the active N-desmethyl metabolite (5-ethyl-5-phenylhydantoin, nirvanol), which is more potent than the parent compound (21). The metabolism of mephenytoin appears to be through cytochrome P450, specifically through the CYP2 family (24). The toxicity of this metabolite has limited its use (8).

Ethotoin. Chemically, 3-ethyl-5-phenylhydantoin, ethotoin (**1c**) undergoes two biotransformation pathways leading to inactive products: p-hydroxylation [pathway (1)] and deethylation [pathway (2)]. This product has relatively low potency compared to that of phenytoin. Like phenytoin, ethotoin displays saturable metabolism with respect to the formation of the two metabolites (18).

Table 6.3 New Antiepileptic Medications: Pediatric Indications and Effectiveness[a]

Agent (Initial Availability)	Seizure Type or Epilepsy Syndrome					
	CPS	PGS	Abs	JME	LGS	IS
Felbamate (1993)	Monotherapy, Adjunctive > 14 years	ENL	ENL	ENL	Adjunctive > 2 years	ENL
Gabapentin (1994)	Adjunctive > 3 years	ENL	NE	NE	?	?
Lamotrigine (1995)	ENL	ENL	ENL	ENL	Adjunctive > 2 years	?
Topiramate (1996)	Adjunctive > 2 years	Adjunctive > 2 years	ENL	ENL	ENL	ENL
Tiagabine (1997)	Adjunctive > 12 years	?	NE	NE	?	ENL
Levetiracetam (1999)	ENL	?	?	?	?	?
Oxcarbazepine (2000)	Adjunctive 4–16 years	?	?	?	?	?
Zonisamide (2000)	ENL	ENL	ENL	?	ENL	ENL

[a]CPS, complex partial seizures; PGS, primary generalized seizures; Abs, absence; JME, juvenile myoclonic epilepsy; LGS, Lennox–Gastaut syndrome; IS, infantile spasms; ENL, effective, not labeled (although no indication exists, studies support its use in pediatric patients); NE, not effective; ?, not studied (no well-performed pediatric studies exist for this indication). Data are from Ref. 17.

Fosphenytoin. The disodium phosphate ester of 3-(hydroxymethy)phenytoin (**1d**) is a water-soluble derivative of phenytoin, which is rapidly converted into phenytoin in the body.

2.3.2 Iminostilbenes

Carbamazepine. Carbamazepine(5*H*-dibenz-[bfazepine-5-carboxamide, 2) is reasonably well absorbed and varies with the dosage form (Table 6.5). Compared to the suspension, the extended release tablet showed 89% bioavailability. Plasma levels are variable and may bear no apparent relationship to the daily dose. Carbamazepine is 76% bound to plasma proteins and was similar for the active 10,11-epoxide (see Fig. 6.3) (22). It has been shown that the free fraction of carbamazepine is inversely correlated with the serum α_1-acid glycoprotein concentration (23). Transplacental transport is rapid (30–60 min) and the drug accumulates in fetal tissues (18). The drug is metabolized (Fig. 6.3) in the liver by the P450 3A4 isozyme to the active 10,11-epoxide, and subsequently to the inactive cis diol. As noted in Table 6.5, carbamazepine can also induce its own metabolism (autoinduction).

Oxcarbazepine. Chemically, oxcarbazepine is 10,11-dihydro-10-oxocarbamazepine (12), and is similar to carbamazepine in having the dibenzazepine nucleus with the 5-carboxamide substitution; however, it is different at the 10,11-bridge position and thereby differs from carbamazepine in its metabolic disposition (Fig. 6.3). After oral administration, oxcarbazepine is completely absorbed and extensively metabolized to the active enantiomeric secondary alcohols (Fig. 6.3) (24). There is a decreased incidence of allergic skin reactions with oxcarbazepine compared to carbamazepine (25). For patients on carbamazepine, replacement with oxcarbazepine requires no tapering off from the carbamazepine, which may be withdrawn at once (25). An additional advantage of oxcarbazepine is its reduced tendency to induce oxidative metabolism, especially in polytherapy, because it is easier to reach the therapeutic level of other antiepileptic agents during treatment with oxcarbazepine than with carbamazepine (26).

2.3.3 Aliphatic Acid

Valproic Acid. Valproic acid (VPA), is available in several chemical forms, including valproic acid, sodium valproate, and divalproex sodium, a stable coordination compound containing equal proportions of valproic acid and sodium valproate. In either of these forms, the dosage is expressed as valproic acid equivalents (Table 6.1) (18). Oral valproic acid derivatives are rapidly absorbed; the absolute bioavailability of divalproex extended-release (ER) tablets was about 90% relative to that of the intravenous infusion. The ER form had an average bioavailability of 81–89% compared to that of divalproex delayed-release tablets given twice daily. The relationship between plasma concentration and clinical response is not clear. This may be attributed to the nonlinear concentration-dependent protein binding of valproic acid, which in turn affects the clearance of the agent (18).

As noted in Fig. 6.3, valproic acid is extensively metabolized by way of separate Phase I pathways: β-oxidation, P450-dependent desaturation, P450-dependent w-hydroxylation, P450-dependent (ω-1)-hydroxylation, and P450-dependent (ω-2)-hydroxylation (27). Two factors in the pharmacokinetics/pharmacodynamics of valproic acid have led to the hypothesis of an active metabolite: (1) the anticonvulsant activity of valproic acid correlates poorly with steady-state valproic serum concentrations; and (2) the time course of anticonvulsant effect differs from that predicted from the pharmacokinetics of valproate, in that protection against seizures is not maximal until some time after steady-state concentrations of valproate are achieved and persists long after the parent drug has been cleared from the systemic circulation (28–30). Although (*E*)-2-ene VPA does show anticonvulsant activity, it was the only metabolite found in the brain after valproate therapy. Moreover, it was cleared from the brain and plasma more slowly than the parent agent, and was shown to provide relatively slow washout kinetics compared to that of valproate (31–33); the opposing view argues that this agent is not an active metabolite. It is questionable whether the amount of (*E*)-2-ene VPA formed that has not been further biotransformed can account for the majority of the anticonvulsant

Table 6.4 Side Effects, Adverse Effects, Drug Interactions/Contraindications of the Anticonvulsant Agents

Anticonvulsant Agent	Side Effects	Adverse Effects	Drug Interactions	Contraindications
Phenytoin	Drowsiness, dizziness, or blurred vision	IV administration: cardiovascular collapse," hypotension Oral administration: CNS: nystagmus, ataxia, slurred speech Dermatologic: rash Endocrine: diabetes insipidus, hyperglycemia Others: Gingival hyperplasia," thrombocytopenia, agranulocytosis, tinnitus, diplopia	Increased effects: a. Inhibition of metabolism with allopurinol, amiodarone, benzodiazepines, chloramphenicol, cimetidine, disulfiram, ethanol, fluconazole, isoniazid, metronidazole, miconazole, omeprazole, phenacemide, succinimides, sulfonamides, trimethoprim, valproic acid[a] b. Plasma protein displacement with salicylates,[a] tricyclic antidepressants, valproic acid[a] c. Unknown mechanism: chlorpheniramine, ibuprofen, phenothiazines Decreased effects: a. Increased metabolism with barbiturates," carbamazepine," diazoxide, ethanol, rifampin, theophylline b. Decreased absorption with antacids, charcoal, sucralfate c. Unknown mechanism: antineoplastics, folic acid," influenza virus vaccine," loxapine, nitrofurantoin, pyridoxine	Do not use in sinus bradycardia, sinoatrial block, second- and third-degree AV block, or in patients with Adams–Stokes syndrome.
Carbamazepine	Drowsiness, dizziness, or blurred vision	Hematologic: aplastic anemia, leukopenia, agranulocytosis, bone marrow depression CNS: dizziness, drowsiness, unsteadiness Other: nausea, vomiting	Increased effects (P450 3A4 inhibitors): cimetidine, danazol, diltiazem, erythromycin, troleandomycin, clarithromycin, fluoxetine, isoniazid, niacinamide, nicotinamide, propoxyphene, ketoconazole, itraconazole, verapamil, and valproate Decreased effects (P450 3A4 inducers): cisplatin, doxorubicin, felbamate, rifampin, phenobarbital, phenytoin, primidone, theophylline	History of bone marrow depression; hypersensitivity to carbamazepine and tricyclic antidepressants; concomitant use of monoamine oxidase (MAO) inhibitors. Discontinue MAO inhibitors for ≥14 days before carbamazepine administration.

Valproic acid	Dizziness, suicide ideation, hyperammonemia, nausea	CNS: asthenia, somnolence, dizziness, tremor GI: nausea, vomiting, abdominal pain Hematologic: thrombocytopenia Respiratory: infection Other: alopecia, headache	Increased effects with **chlorpromazine,** cimetidine, erythromycin, **felbamate,** salicylates Decreased effects with rifampin, carbamazepine, cholestyramine, phenytoin, lamotrigine, phenobarbital	Hepatic disease; hypersensitivity to valproate; pregnancy (FDA category **D**); children < 2 years (especially those on multiple antiwnvulsant therapy, those with congenital metabolic disorders, those with severe seizure disorders, and those with organic brain disease); **pancreatitis.**
Clorazepate dipotassium	Drowsiness, ataxia, and confusion			Hypersensitivity to benzodiazepines.
Diazepam	Drowsiness, ataxia, and confusion	IV administration: cardiovascular collapse CNS: sedation and sleepiness		Hypersensitivity to benzodiazepines; psychoses; acute narrow-angle glaucoma; children < 6 years; lactation; concomitant alcohol administration.
Clonazepam	Drowsiness, ataxia, and confusion		Decreased effects with phenytoin	Hypersensitivity to benzodiazepines; **clinical** or biochemical evidence of significant liver disease.
Clobazam	Drowsiness, hangover effects, dizziness, weakness, and lightheadedness		None reported	Hypersensitivity to benzodiazepines.
Nitrazepam	Fatigue, dizziness, lightheadedness, drowsiness, lethargy, mental confusion, ataxia		None reported	Hypersensitivity to benzodiazepines.
Ethosuximide	Drowsiness, ataxia	GI: nausea, vomiting	Ethosuximide increases phenytoin levels Ethosuximide decreases **primidone** and phenobarbital levels	Hypersensitivity to succinimides.

Table 6.4 (Continued)

Anticonvulsant Agent	Side Effects	Adverse Effects	Drug Interactions	Contraindications
Phenobarbital	Drowsiness, ataxia	Somnolence	Increased effect with MAO inhibitors, valproic acid Decreased effect with chloramphenicol, rifampin	Barbiturate sensitivity; alcohol; pregnancy (FDA category D); manifest or latent porphyria; severe respiratory disease when dyspnea or obstruction is evident; nephritic patients.
Primidone	Drowsiness, dizziness, GI upset,	CNS: ataxia and vertigo GI: nausea, anorexia, vomiting	Increased effect with isoniazid, nicotinamide, phenytoin Decreased effect with carbamazepine, ethosuximide	Lactation; pregnancy; porphyria; hypersensitivity to phenobarbital.
Trimethadione	Drowsiness, dizziness, sore throat, blurred vision	Systemic lupus erythematosus; skin rash (leading to exfoliative dermatitis or severe erythema multiforme); fatal aplastic anemia; fatal nephrosis has occurred		Hypersensitivity to oxazolidinediones; pregnancy (FDA category D); photosensitivity.
Vigabatrin	None reported in 75% of patients tested	Somnolence, fatigue (adults); agitation, insomnia (children)	Decreases phenytoin levels	Dose-dependent field effects."
Gabapentin	Drowsiness, dizziness, headache, viral infection, nausea, vomiting	Somnolence, dizziness, ataxia, fatigue, and nystagmus	Increased effect with cimetidine Decreased effect with antacids	Hypersensitivity to the drug; patients > 12 years of age; pregnancy (FDA category C).
Felbamate	Lymphadenopathy, leukopenia, agitation,	Aplastic anemia, liver failure, carcinogenic (in animal studies)," tachycardia	Decreased effect with phenytoin, phenobarbital, carbamazepine	Hypersensitivity to the drug or other carbamates; avoid prolonged exposure to sunlight or sunlamps; may cause photosensitivity; history of blood dyscrasias or hepatic dysfunction; pregnancy (FDA category C).

Lamotrigine	Dizziness, diplopia, ataxia, blurred vision," nausea, and vomiting	Rash, dizziness, headache	Increased effect with folate inhibitors, valproic acid Decreased effect with acetaminophen, primidone, phenobarbital, phenytoin, carbamazepine	Hypersensitivity to the drug; dizziness; pregnancy (FDA category C); children < 16 years; renal and/or hepatic function impaired patients.
Oxcarbazepine	Psychomotor slowing; dizziness and somnolence	Cross sensitivity from carbamazepine, hyponatremia	Decreased effect with (P450 3A4 inducers): carbamazepine, phenytoin, and phenobarbital; also decreased with verapamil and valproic acid	Hypersensitivity to the drug and to carbamazepine, alcohol. Recommend additional nonhormonal forms of contraception.
Zonisamide	Somnolence, anorexia, dizziness, headache, nausea, agitation/ irritability	Pruritus, vomiting, amblyopia, tinnitus, asthenia, kidney stones	Decreased effect with (P450 3A4 inducers): carbamazepine, phenytoin, and phenobarbital	Hypersensitivity to sulfonamides or zonisamide; skin rash; pregnancy (FDA category C); sudden back pain; abdominal pain; or blood in the urine." Not approved for pediatric use.
Tiagabine	Generalized weakness	Dizziness, somnolence, depression, confusion, asthenia	None reported	Hypersensitivity to the drug; pregnancy; lactation.
Topiramate	Psychomotor slowing; somnolence	Fatigue, headache, injury, anxiety, rash, palpitation, kidney stones	Increases the effect of alcohol and CNS depressants, carbonic anhydrase inhibitors Decreases the effect of oral contraceptives, digoxin	Hypersensitivity to the drug; use in children has not been established.
Levetiracetam	Dizziness	Somnolence, asthenia, infection, and dizziness[a]	None reported	Hypersensitivity to the drug; pregnancy (FDA category C).

"For a full discussion, see Section 2.2. Data are from Ref. 18.

Table 6.5 Pharmacokinetic Properties of Antiepileptic Agents[a]

Generic Name (USP or Nonproprietary Name)	Peak Plasma Levels Achieved (h)	$t_{1/2}$ (h)	Volume of Distribution (V_d, L/kg)	Metabolism/Excretion	Protein Binding (%)
Phenytoin sodium, Extended, (1a)	12	Dose-dependent	0.6	Liver/kidney; <5% of the unchanged drug remaining	87–93
Phenytoin, Prompt, (1a)	1.5–3.0				
Mephenytoin, (1b)		*(S) = 1* *(R) ~ 70*		Liver/kidney; —42% of unchanged drug remaining	No data
Desmethyl metabolite (active)		150–200			
Ethotoin, (1c)	2	3–9		Liver/kidney	No data
Carbamazepine, (2)	4–5 (tablets); —1.5 (suspension);~3–12 (extended release)	25–65 (initial); 3–24 (repeated administration)		Liver/kidney; metabolites are found in the urine (72%) and the feces (28%)	76
Epoxide metabolite (active)		3–24			
Oxcarbazepine, (12)		~2	0.8–2.0	Liver/kidney; >95% found in the urine, constituting <1% of unchanged drug; —80% as MHD, or conjugates	
Monohydroxy (MHD) metabolite (active)	3–13	~9	49		40
Valproic acid, (3a)		9–16	11 L/1.73 m^2	Liver/kidney	80–94
Clorazepate dipotassium, (4a)				Liver/kidney	97
Desmethyl metabolite (active)		55–100	1.0–1.8		
Diazepam, (4c)		20–50		Liver/kidney	97–99
Clonazepam, (4b)	1–4	18–60	1.5–4.4	Liver/kidney	50–85
Nitrazepam, (4e)		2	~2	Liver/kidney	
Clobazam, (4d)	1–4	18		Liver/kidney	
Desmethyl metabolite (active)		—77			
Ethosuximide, (5a)	3–7	30 (children);60 (adults)		Liver/kidney; 25% excreted unchanged in urine	0

Methsuximide, (5b)		<2		Liver; <1% excreted unchanged in urine	No data
Desmethyl metabolite (active)		40			
Phenobarbital, (6)		75–126	0.42-0.73	Liver; 25%excreted unchanged in urine	40-60
Primidone, (7)		5–15 (primidone); 53–140 (phenobarbital); 10–18 (PEMA)		Liver/kidney; –40%is excreted unchanged; metabolized to phenobarbital and P E W (both active)	20-25
Trimethadione, (17)	1.5–3.0	11–16			
Desmethyl metabolite (active)		6–13 days			
Gabapentin, (9)		5-7	0.65-1.04	Not appreciably metabolized; excreted in urine unchanged	<3
Felbamate, (10)	2–6	16–22	0.84-0.76	Liver/kidney; 40-50% unchanged in urine; 40%as unidentified metabolites and conjugates	22–25
Lamotrigine, (11)	1.4–4.8	26	0.9–1.3	Liver/kidney; 94% excreted in urine as conjugates, 2%in feces	–55
Zonisamide, (12)	2–6	–63 (plasma); 105 (erythrocytes)	–1.45	Liver; kidney	–40%
Tiagabine, (14)	–45 min	5.4–8.0		Liver; kidney	96
Topiramate, (15)	–2	21		Liver/kidney; 70%of unchanged drug in urine	13–17
Levetiracetam, (16)	~1	6–8		Liver/kidney; 66% unchanged drug	

"Data are from Ref. 18.

activity after a relatively large dose of valproate. Levels of (*E*)-2-ene VPA in the cerebrospinal fluid (CSF) (34) and the brain (35) in humans were determined to be too low to provide effective anticonvulsant protection.

Valproic acid is rapidly distributed and the plasma protein binding is concentration dependent (18). As previously noted, valproic acid is extensively metabolized, primarily in the liver, with about 30–50% of the drug excreted as the glucuronide (phase II metabolism) in the urine, about 30–40% by the phase I mitochondrial β-oxidation pathway, and about 10–20% by microsomal cytochrome P450-mediated hydroxylation/dehydrogenation of the side chain that provides the major phase I metabolites (36). The metabolites of valproic acid have been thought to be the cause of a rare, but fatal hepatotoxicity (35). The synthetic (*E*)-2,4-diene VPA has been shown to induce the same hepatic microvesicular steatosis seen in patients, in chronic administration studies in rats (36). The ultimate causative factor(s) of hepatoxicity of valproic acid currently remain undefined (28, 29).

2.3.4 Benzodiazepines

Clorazepate Dipotassium. Clorazepate dipotassium is a prodrug that is rapidly and almost quantitatively converted into the active decarboxylated analog, N-desmethyldiazepam [nordazepam (**4f**)] (37–40).

Diazepam. Like clorazepate, diazepam is converted into nordazepam (**4f**) by *N*-demethylation (Fig. 6.3) and to oxazepam (**4g**). Direct hydroxylation produces N-methyl-oxazepam (**4h**). The low concentrations of (**4g**) and (**4h**) preclude their role as active anticonvulsants (41). Unlike clorazepate, diazepam is bioactive. The transformation of diazepam to nordazepam is less complete, in that 62–73% of diazepam is excreted in the urine and about 10% is found in the feces (41). Diazepam is rapidly absorbed when taken orally (30–90 min) or rectally (10–60 min).

Clonazepam. Clonazepam, chemically 5-(2-chlorophenyl)-1,3-dihydro-7-nitro-2*H*-1,4-benzo-diazepin-2-one(**4b**), is closely related to nitrazepam (**4e**), differing only at position 5 with the o-chloro substituent. Only 0.5% of the original drug is recovered unchanged in the urine after 24 h, indicating extensive biotransformation to the 7-amino (**4i**) and 7-acetamido (**4j**) derivatives. Abrupt withdrawal of the drug has led to worsening of seizures with or without additional psychic symptoms such as dysphoria, restlessness, or autonomic signs (42).

Nitrazepam. As indicated previously, nitrazepam (**4e**), is similar in structure to clonazepam. Absorption after oral administration occurred within 1 h; however, in some cases relatively slow absorption (up to 4 h) has been reported (43–48). Rectal administration provided more rapid absorption (median peak time 18 min versus 38 min orally) (48). Although a good correlation was apparent between the volume of distribution and elimination half-life, when comparing the young, elderly, female, and male subjects, the differences observed in volume of distribution related to sex, age, and body weight seem to depend on the relative proportion of body adipose tissue (49). The metabolic pattern of nitrazeparn is similar to that of clonazepam, with the principal formation of the inactive 7-amino (41) and 7-acetamido (**4m**) compounds.

Clobazam. Clobazam (**4d**) differs from the previous benzodiazepines, given that it is a 1,5-benzodiazepine rather than a 1,4-derivative. It was demonstrated that N-desmethylclobazam (**4k**) possessed anticonvulsant activity (50, 51).

2.3.5 Succinimides

Ethosuximide. Ethosuximide (**5a**) is rapidly absorbed when administered orally (18). The drug is extensively metabolized principally to the inactive diasteromeric 2-(1-hydroxyethyl)-2-methylsuccinimide (**5d**) (Fig. 6.3) (52) and the inactive 2-hydroxyethyl isostere. About 20% of the drug is excreted unchanged in the urine.

Methsuximide. Like ethosuximide, methsuximide (**5b**) is rapidly absorbed. The drug is also rapidly N-demethylated to N-desmethylmethsuximide (**5d**), the active metabolite (53). Less than 1% of unchanged methsuximide is found in the urine.

2.3.6 Barbiturate and 2-Desoxybarbiturate

Phenobarbital. Phenobarbital (5-ethyl-5-phenylbarbituric acid, **6**) is relatively insolu-

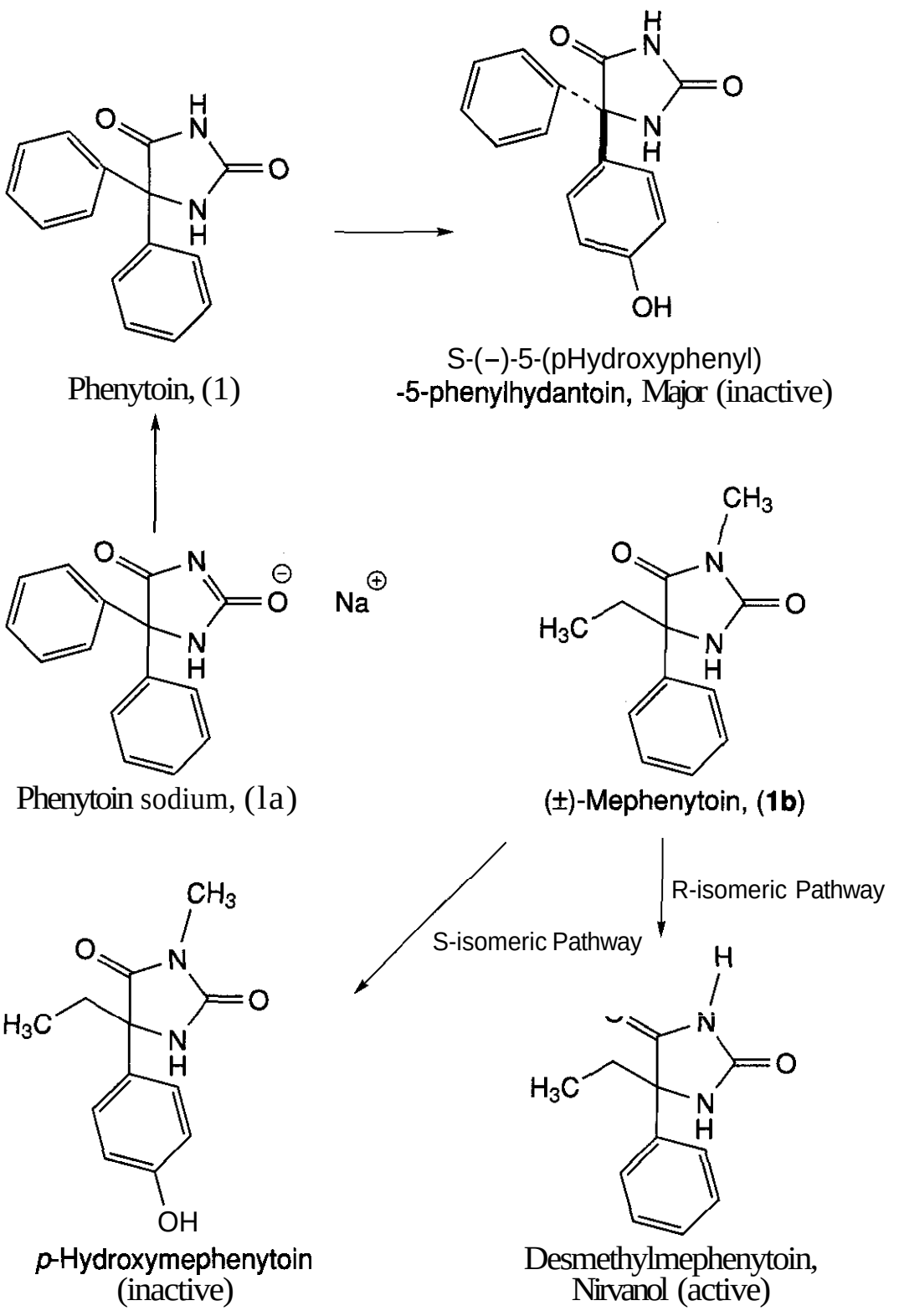


Figure 6.3. Metabolism of anticonvulsants.

ble in water (1g in 1000 mL), but readily soluble as the sodium salt (1g in 1 mL) (54). The drug is metabolized principally to the *p*-hydroxy metabolite (6a) (55). Absorption of the sodium salt is rapid and relatively complete (80–100%)(56, 57). It is distributed to all body tissues (58). The elimination of phenobarbital follows first-order kinetics, and thus is independent of concentration (59). Although the average half-life is not influenced by the route of administration, the rate of urine flow and urinary pH do influence the elimination rate (59). Increasing urine flow decreases resorption in the nephron and increases clearance, whereas increasing urinary pH also increases excretion (59).

Primidone. Primidone (2-desoxyphenobarbital, *7)* is readily absorbed from the gastrointestinal tract. As seen in Fig. 6.3, primidone undergoes two principal biotransformation pathways: (*1*) C_2 oxidation to form phenobarbital (6) and (2) C_2 ring cleavage to form phenylethylmalonamide (PEMA, 7a), both active. It has been shown that primidone is, in fact, active and not a prodrug of phenobarbital (60).

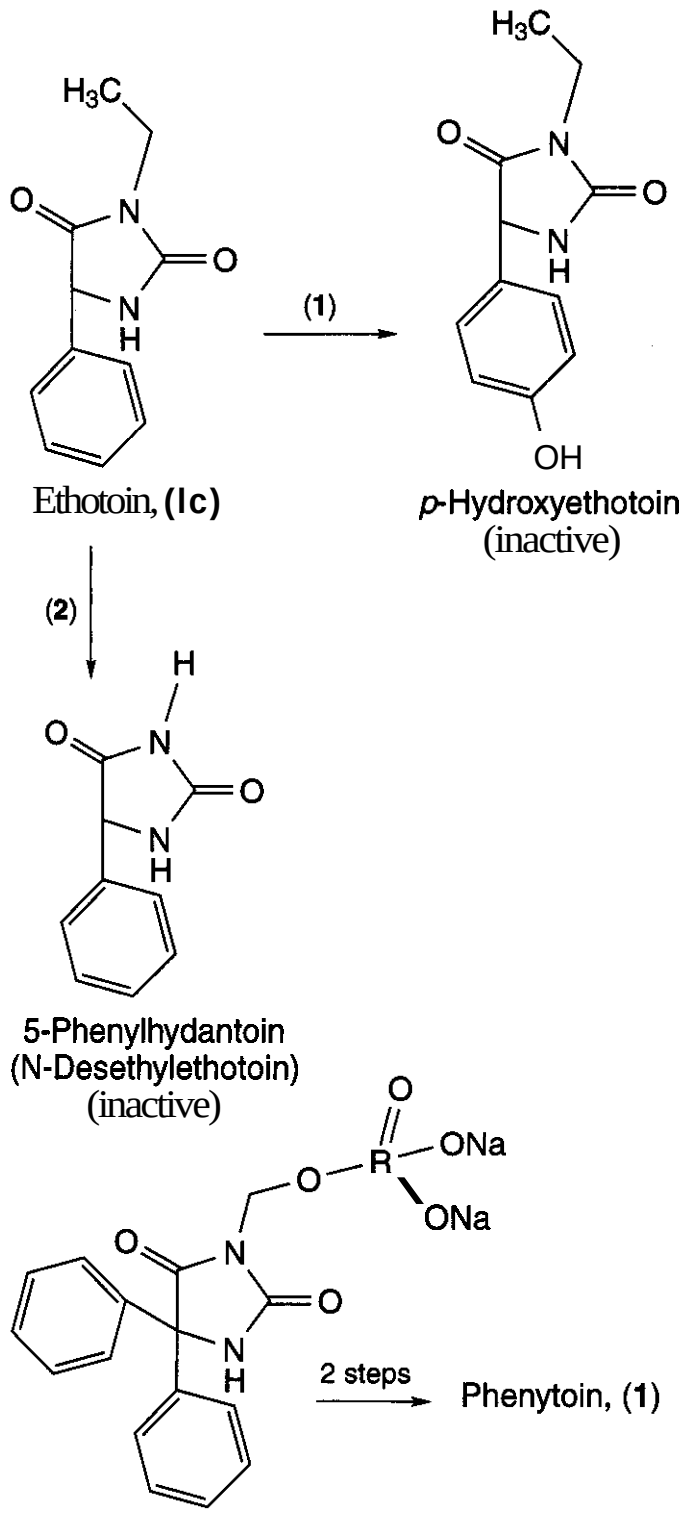


Figure 6.3. (Continued.)

2.3.7 Oxazolidinedione

Trirnethadione. Trimethadione, chemically 3,5,5-trimethyloxazolidine-2,4-dione (**17**), is rapidly absorbed after oral administration and is converted, through N-demethylation into the active dimethyldione (**17a**) (61). Trimethadione is extensively metabolized, with less than 5% appearing in the urine after oral administration (61).

2.3.8 CABA Analog

Vigabatrin. Vigabatrin, chemically 4-amino-5-hexenoic acid (**8**), exists as a racemic mixture of R-(−)- and S-(+)-isomers, where the R-(−)-isomer is inactive (62). In comparing the absorption, time of peak effect, and $t_{1/2}$ of the racemate and the separate enantiomers, the following was obtained (63). As noted in the following table, the peak plasma concentrations for both isomers were reached within the same time range. The kinetics of the S-(+)-isomer was not influenced by the *R*-(−)-isomer, given that the data for the racemate were similar to those of the S-(+)-isomer alone. It should be noted that. in contrast to other biologically active compounds wherein the inactive isomer is considered to be an impurity that can influence the kinetics and the action of the active isomer, with vigabatrin, the toxic effects of the racemate are exclusively attributed to the active S-(+)-isomer (63). This results from its mechanism of action as a GABA-T inhibitor (62). As noted in the table, the drug is rapidly absorbed and, because it is water soluble, it is readily distributed to all parts of the body. The volume of distribution in healthy volunteers is 0.8 L/kg (62). The metabolism and protein binding of vigabatrin are negligible; there was no chiral inversion of the *R*-(−)-isomer to the active S-(+)-isomer (63).

Pharmacokinetics of Vigabatrin[a]

Enantiomer	Peak Concentration (h)	$t_{1/2}$ (min)
Racemate	0.5–2	447[b]
S (+)	0.5–2	386
R (−)	0.5–2	485

[a]Data are from Ref. 63.
[b]For the S-(+)-isomer.

Gabapentin. Gabapentin, chemically 1-(aminomethyl)-cyclohexaneacetic acid (**9**), like vigabatrin, is neither metabolized nor protein bound (64–66). Gabapentin exhibits dose-dependent bioavailability, in that the plasma concentration of the drug is not directly proportional to the dose throughout the therapeutic range of dosage (67).

2.3.9 Carbarnate

Felbamate. Felbamate, chemically 2-phenyl-1,3-propanediol dicarbamate (**10**), is well absorbed after oral administration (68). Felbamate is distributed to all tissues and has been metabolized by several pathways in ani-

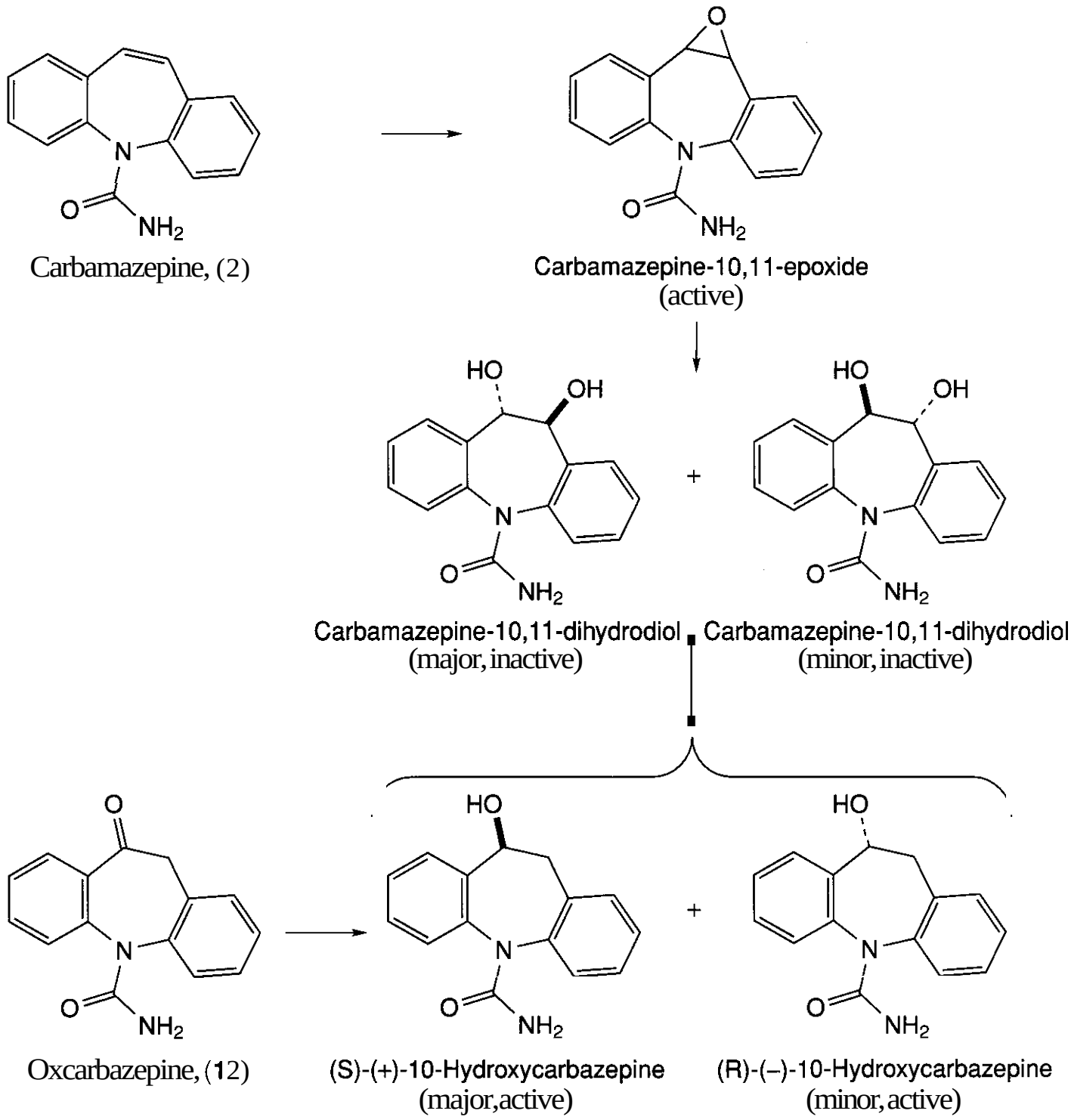


Figure 6.3. (Continued.)

mals (Fig. 6.3): (1) ring hydroxylation to form *p*-hydroxyfelbamate (**10a**); *(2)* aliphatic hydroxylation to form 2-hydroxyfelbamate (**10b**); and **(3)** hydrolysis to form the primary alcohol, 2-phenyl-1,3-diol monocarbamate (1-hydroxyfelbamate, **10c**) and further to the carboxylic acid (**10d**) (78). In humans, unchanged felbamate is found to the extent of 50% in the urine of male volunteers; the combined hydroxy metabolites (**10a**) and (**10b**) (10–15%) and the hydrolyzed (**10c**) (0.7–2.7%) constitute the remainder. The propionic acid derivative (**10d**) was not recovered. The metabolites are devoid of significant anticonvulsant activity (68).

2.3.1 0 **Triazine**

Larnotrigine. Lamotrigine, chemically 3,5-diamino-6-[2,3-dichlorophenyl]-1,2, 4-triazine (**11**), is rapidly and completely absorbed after oral administration with minimal first-pass metabolism. Lamotrigine is metabolized predominantly by glucuronic acid conjugation to an inactive 2-*N*-glucuronide (11a) (76%), and the 5-*N*-glucuronide (**11b**). Ten percent of unchanged drug was recovered in the urine (18). Following multiple administrations to healthy volunteers, lamotrigine induces its own metabolism, resulting in a 25% decrease (25.4 h) in half-life and a 37% increase in plasma clearance at steady state. The clearance was 25%

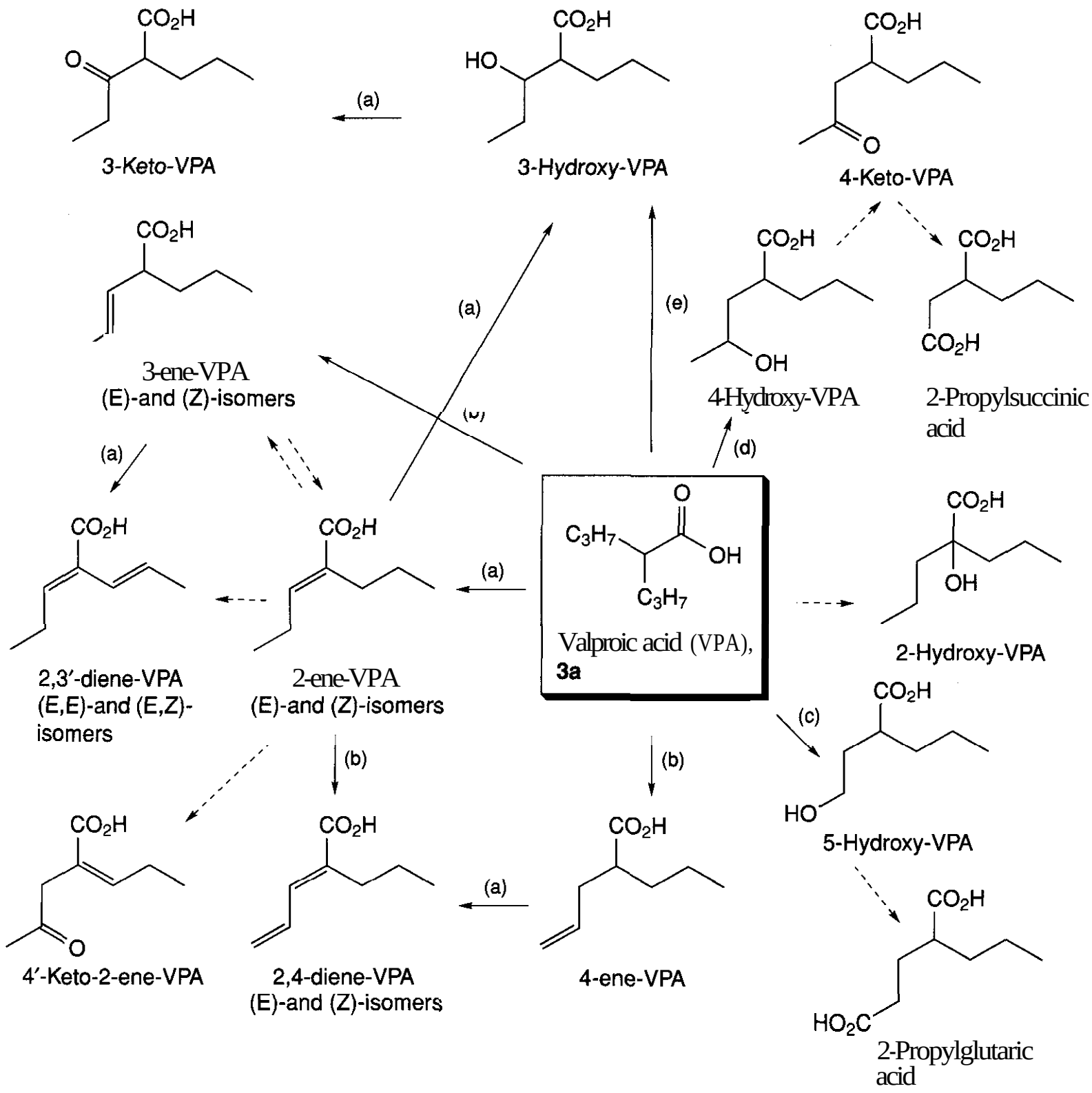


Figure 6.3. **(Continued.)** (a) β-oxidation; (b) P450-dependent desaturation; (c) P450-dependent ω-hydroxylation; (d) P450-dependent (ω-1)-hydroxylation; (e) P450-dependent (ω-2)-hydroxylation). The broken arrows indicate a metabolic route in which the details are not yet confirmed. (After Ref. 27.)

lower in non-whites than that in whites, but was not affected by gender (18).

2.3.11 Oxcarbazepine. Oxcarbazepine **(12)** was previously discussed *[see* Carbamazepine **(2)**].

2.3.12 Sulfonamide

Zonisamide. Zonisamide **(13)** is chemically 1,2-benzisoxazole-3-methanesulfonamide. It should be noted that zonisamide binds extensively to erythrocytes as well as plasma proteins, resulting in an eightfold higher concentration in red blood cells. It is excreted primarily in the urine as: **(1)** unchanged drug **(35%);** (2) inactive N-acetyl zonisamide (**13a**) **(15%);** and **(3)** as the *O*-glucuronide of the ring-opened 2-sulfamoyl-acetyl phenol (SMAP, **13b**) **(50%)** (18).

2.3.13 Nipecotic Acid Analog

Tiagabine. Tiagabine, chemically (*R*)-(−)-1-[4,4-bis(3-methyl-thienyl)-3-butenyl]-3-

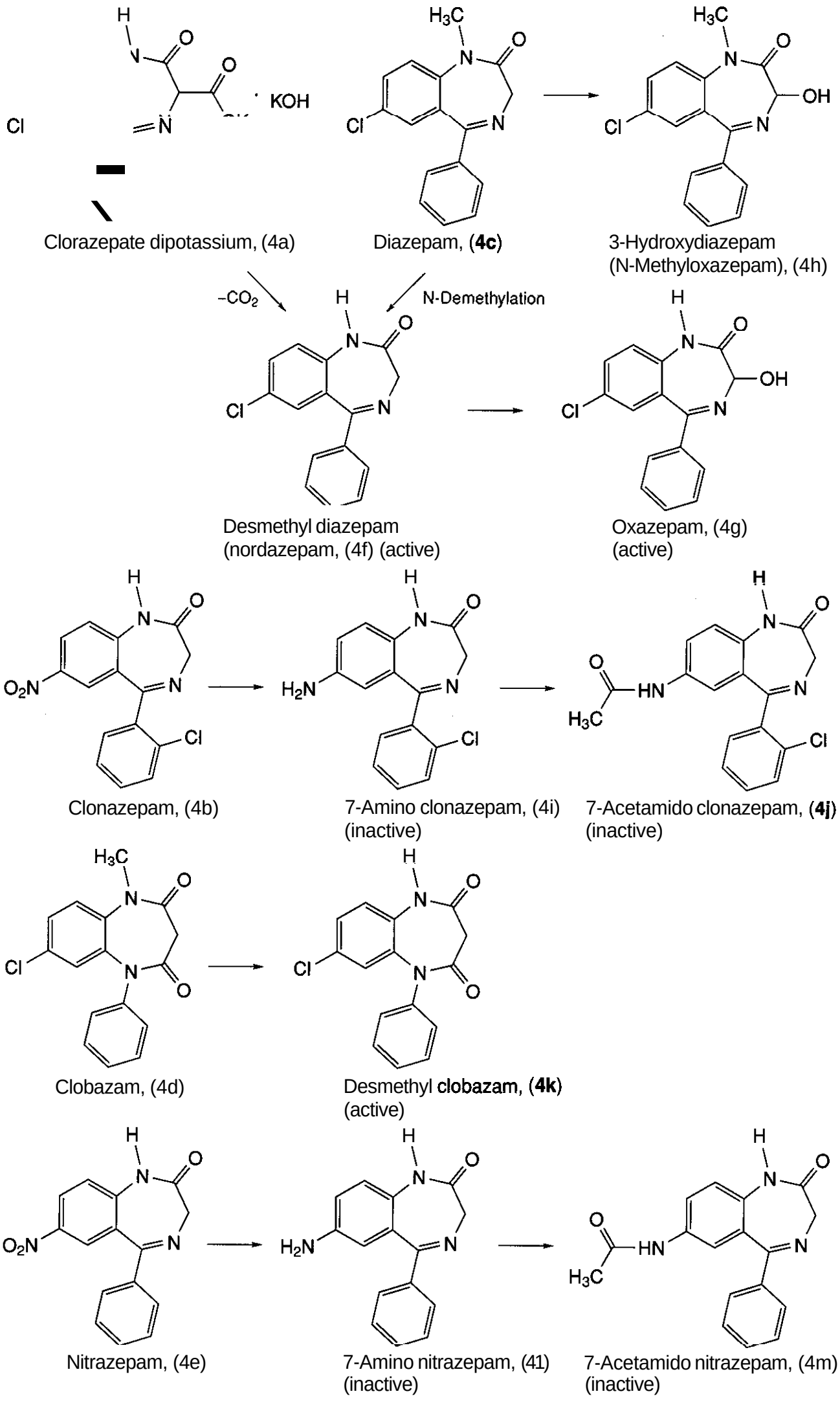
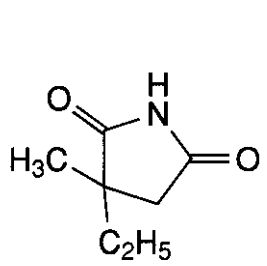
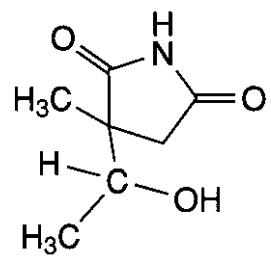


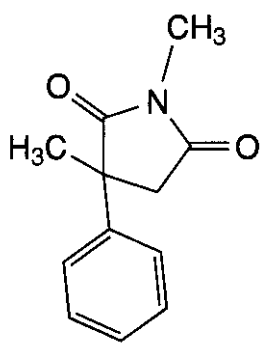
Figure 6.3. (Continued.)



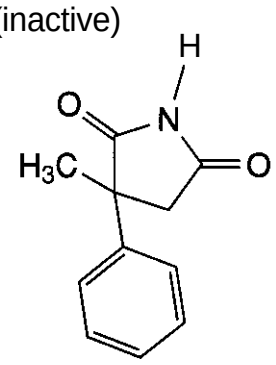
Ethosuximide, (**5a**)



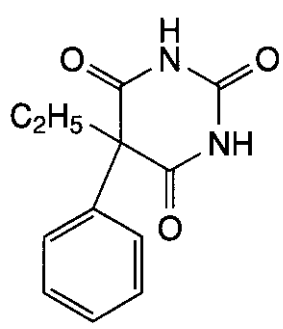
2-(1-Hydroxyethyl)-2-methylsuccinimide, (5d) (inactive)



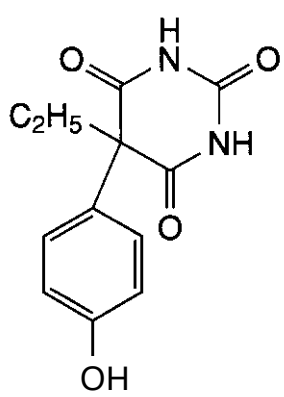
Methsuximide, (**5b**)



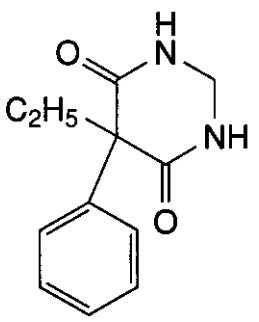
N-Desmethylmethsuximide, (**5e**)



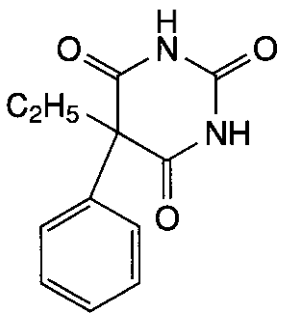
Phenobarbital, (6)



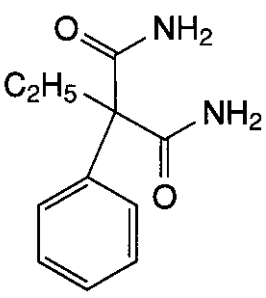
p-Hydroxyphenobarbital, (6a)



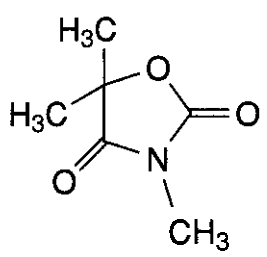
Primidone, (7)



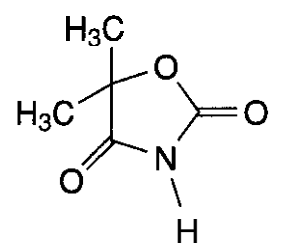
Phenobarbital, (6) (active)



Phenylethylmalonamide (PEMA), (7a) (active)

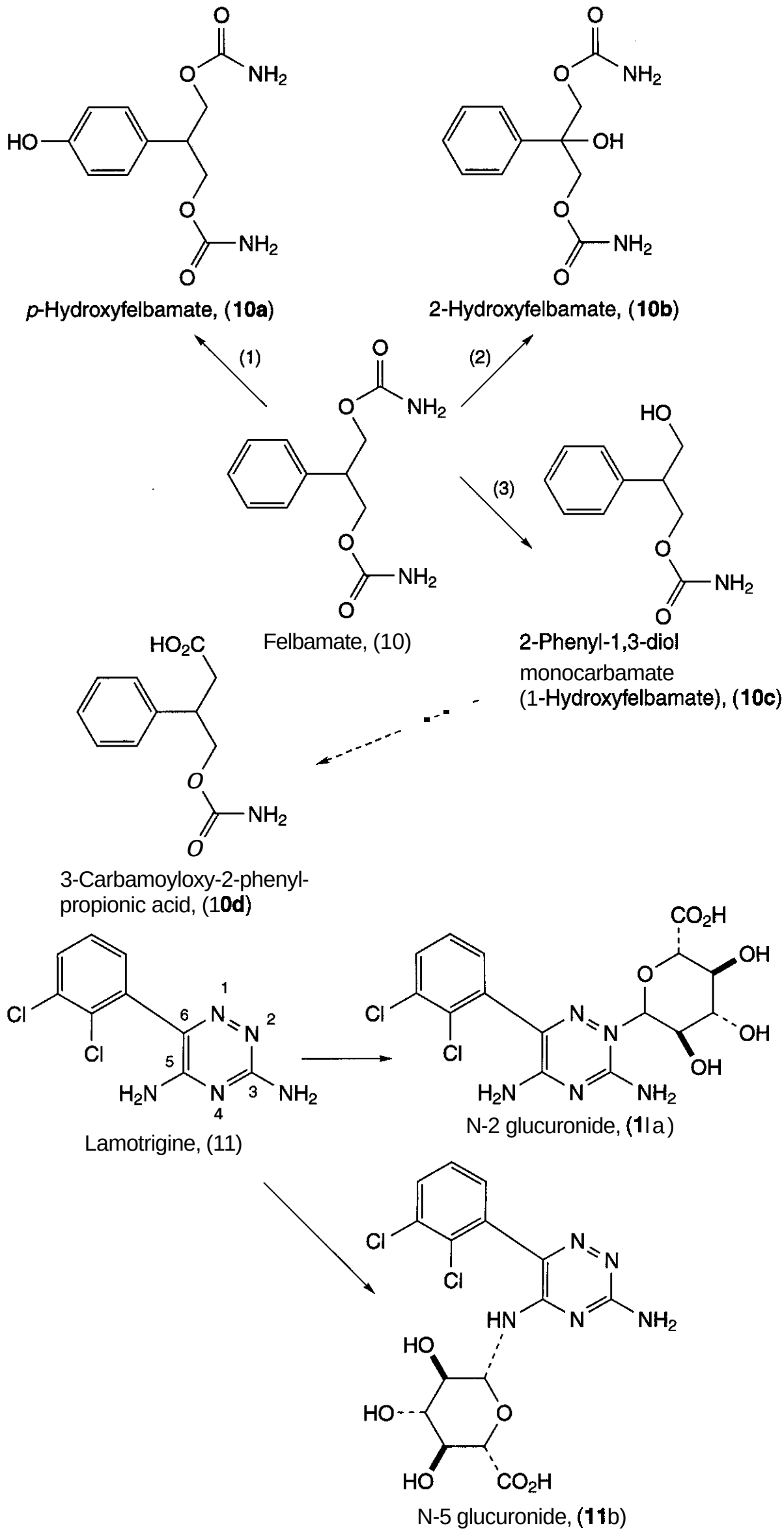


Trimethadione, (17)



Diimethadione, (**17a**)

Figure 6.3. (Continued.)



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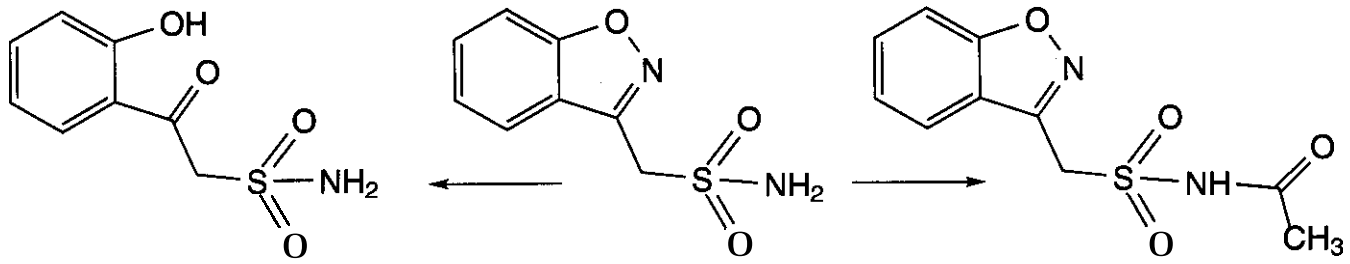
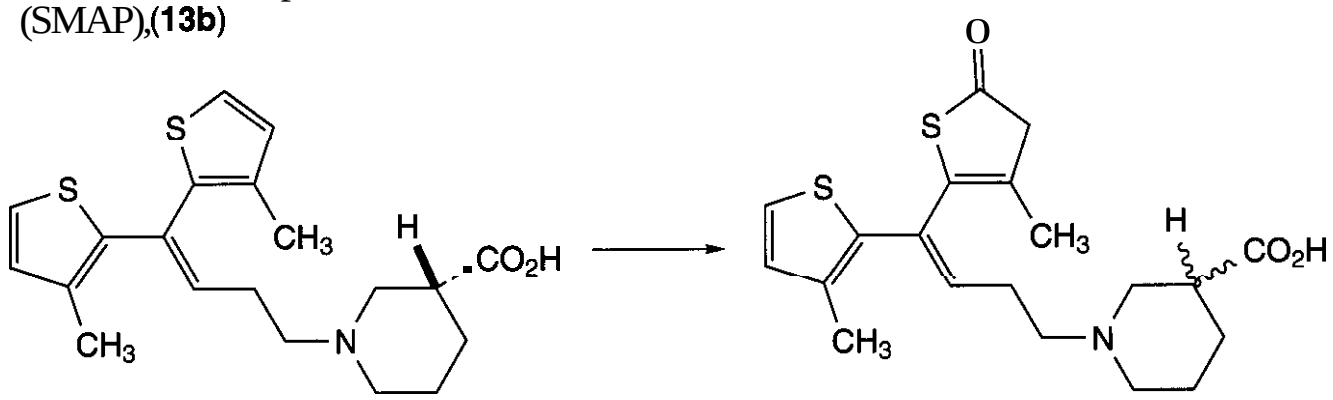


2-Sulfamoylacetyl phenol (SMAP),(**13b**)

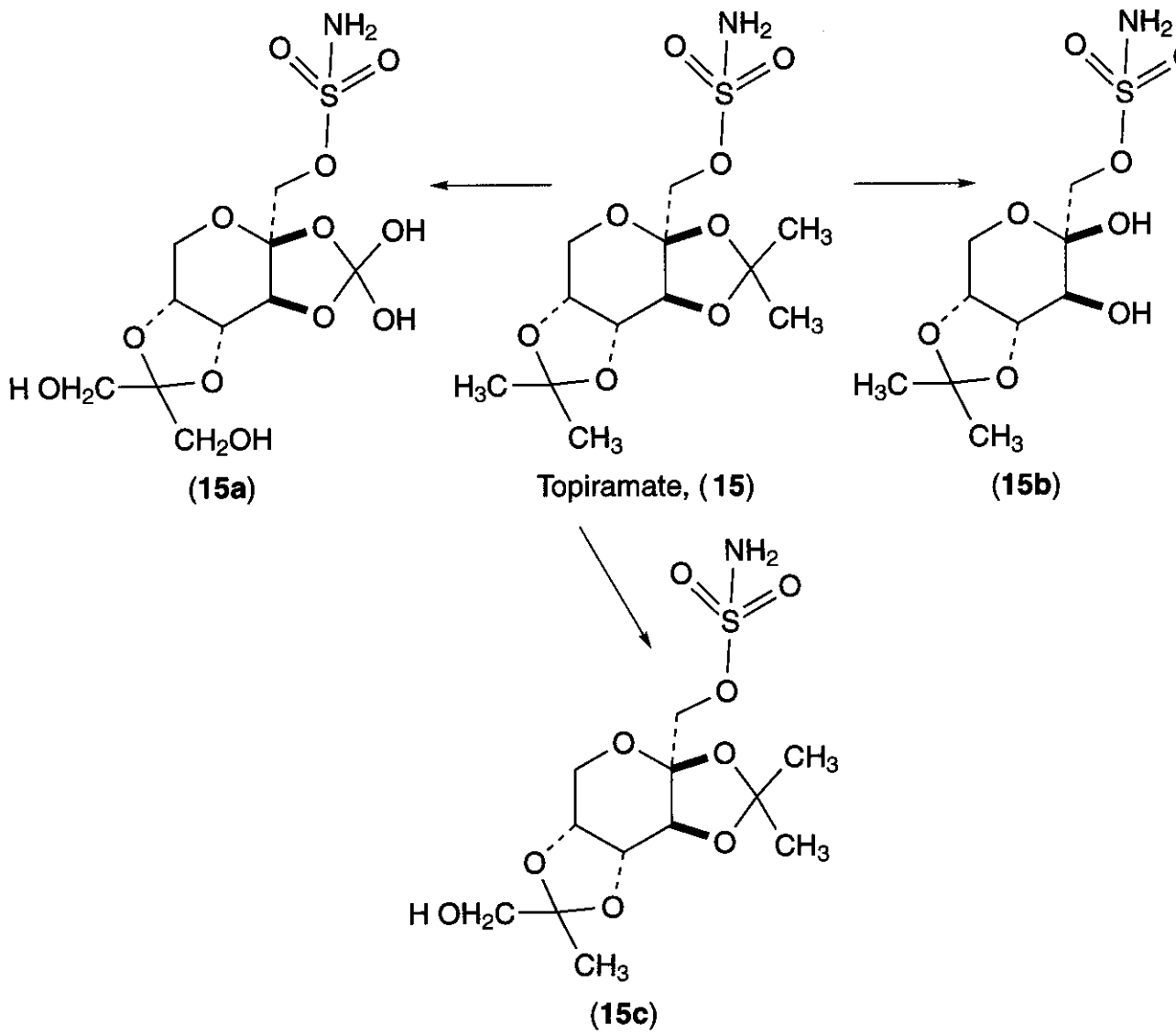
Zonisamide, (13)

N-acetylzonisamide, (13a)



Tiagabine, (14)

5-Oxo tiagabine, (**14a**)



(**15a**)

Topiramate, (**15**)

(**15b**)

(**15c**)

Figure 6.3. (Continued.)

piperidinecarboxylic acid hydrochloride (**14**), is rapidly and nearly completely absorbed (95%), with an absolute bioavailability of about 90% (18). The metabolism of tiagabine has not been fully elucidated, although two pathways have been identified in humans: (1) thiophene ring oxidation, forming inactive isomers of 5-oxo tiagabine (4–5% of the dose) (**14a**); and (2) carboxylic acid glucuronidation **(69).** Of the administered dose, 25% was ex-

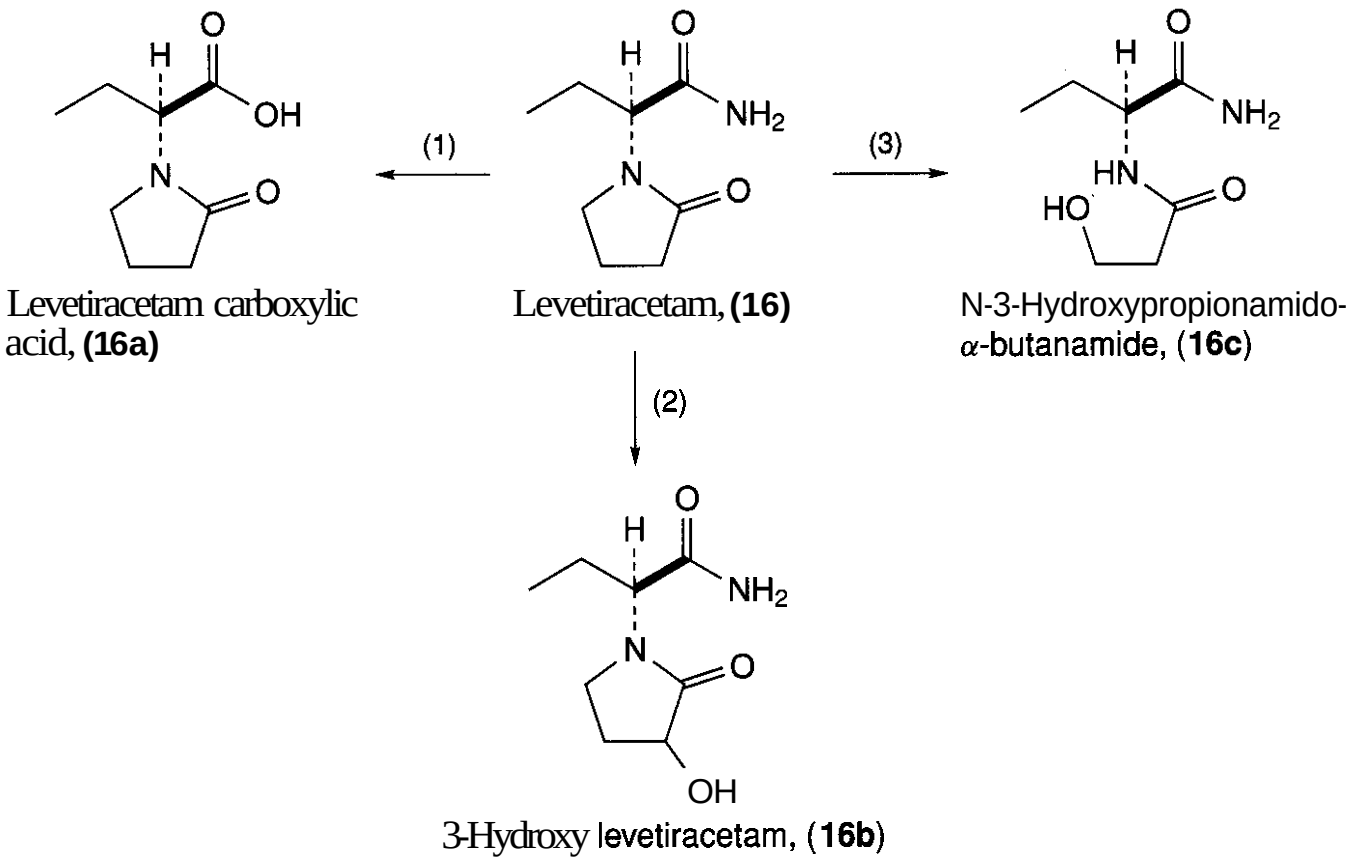


Figure 6.3. (Continued.)

creted in the urine, with 60% (~ 14% of the dose) identified as the isomers of (**14a**). The remaining dose was recovered in the feces 3–5 days after dosing (69). Two major, as yet unidentified metabolites were present in the feces, and accounted for >60% of the fecal residue. The mean half-life of the drug in healthy volunteers was independent of the administered dose, indicating linear elimination kinetics (69).

2.3.14 Sulfamate

Topiramate. Topiramate, chemically 2,3:4,5-bis-*O*-(1-methylethylidene)-β-D-fructopyranose sulfamate (**15**), is rapidly absorbed. The relative bioavailability of the tablet is about 80% of that of the solution (18). Metabolism of the drug is not extensive and ~70% of the administered dose is found in the urine. The metabolites (Fig. 6.3) result from w-oxidation, that is, di- (**15a**) and mono-hydroxymethyl (**15c**) derivatives of the isopropylidene carbons and ring cleavage (**15b**) as well as glucuronidation (81). None of the metabolites possessed significant activity. There is evidence of renal tubular reabsorption of topiramate.

2.3.15 Pyrrolidinone Acetamide

Levetiracetam. Levetiracetam is a single enantiomer, (−)-(*S*)-α-ethyl-2-oxo-1-pyrrolidine acetamide (16). The drug is rapidly absorbed, with peak plasma concentrations occurring in about 1 h after oral dosing (18). The oral bioavailability of the tablets is 100%. Levetiracetam produces one major metabolite, levetiracetam carboxylic acid (**16a**) (Fig. 6.3) in humans. The inactive carboxylic acid (**16a**) constitutes 24% of the dose. Two minor inactive metabolites, the diastereomeric 3-hydroxy levetiracetam (**16b**) (2% of the dose) and the ring-opened butanamide (**16c**) (1% of the dose) were also isolated. There was no enantiomeric interconversion of levetiracetam or its major metabolite (18). The plasma half-life of the drug in adults is 7 ± 1 h and is eliminated by renal excretion of unchanged drug (66%). The mechanism of excretion is glomerular filtration, with subsequent partial tubular reabsorption; the carboxylic acid metabolite (**16a**) is excreted by glomerular filtration and active tubular secretion.

3 PHYSIOLOGY AND PHARMACOLOGY

As stated in the previous edition (8), seizures in humans and laboratory animals result in rapid voltage changes in their EEG patterns. These changes are accompanied by an extracellular depolarizing shift caused by a large

excitatory postsynaptic potential. The exact biochemical mechanisms leading to these discharges and the resultant epileptic attack are still unknown. Several events, however, are known to occur. The EEG changes relate to the opening of specific ion channels in the neuronal membrane. At the onset of the hypersynchronous discharge, the extracellular Ca^{2+} concentration falls and the extracellular K^{+} concentration rises (3). Further, the excessive neuronal discharge may release large amounts of excitatory neurotransmitters at synapses that may result in an avalanche of stimulation. The current proposed cellular mechanisms by which the anticonvulsants exert their effect are indicated for the "first-generation" and "second-generation" agents in Tables 6.5 and 6.6, respectively. These mechanisms are summarized below.

3.1 Ion Channels

As noted in Tables 6.6 and 6.7, there is evidence that some classes of the older and newer anticonvulsants interact with voltage-dependent sodium channels (82–85). Voltage-gated sodium channels are responsible for the generation of action potentials of nerve fibers through fast, selective transport of sodium ions across the cell membrane, leading to the rapid depolarization of the cell network (85). The Hodgkins and Huxley model for the function of the sodium channel postulates that they exist in at least three different states, as shown in Fig. 6.4(86). These states are: (1) the resting (closed) or nonconducting state; (2) the activation state resulting from changes in the resting potential of the channel, which increases the ability of the channel to inwardly conduct Na+ across the cell membrane until an action potential is elicited; and (3) this open channel state exists for a short period and closes rapidly to the inactivated state, which terminates inward flow of Na^{+} and the resulting voltage change. The reactivation to the resting state is membrane potential dependent, given that repeated depolarizations delay the transformation back to the resting state (86).

Drugs that interact with sodium channels to block ion flux cause the channels to inactivate to a greater degree and with smaller depolarizations than normal (86). The relatively slow off-rate caused by anticonvulsants that act as sodium channel blocking agents provides an accumulated block after repeated depolarization (termed use-dependency). Thus, in seizures, the sodium channel-blocking agents are effective only if the depolarization lasts for at least 5 s. These agents normally do not interfere with the normal action potential or excitatory synaptic potentials that typically last less than 200 ms (86).

As noted in Tables 6.6 and 6.7, several first-generation and second-generation agents act by blockade of voltage-dependent Na+ channels; however, there are problems in explaining the clinical and experimental facts concerning these agents. As an example, carbamazepine and phenytoin are listed as Na^{+} channel-blocking agents (80); however, in the clinic, an epileptic patient found to be resistant to one of these agents may respond favorably to alternative treatment with the other of the two drugs, pointing out that these agents may act by more than one mechanism (87). Furthermore, in a subgroup of rats found resistant to phenytoin, they have responded well to carbamazepine (88, 89). Of the newer agents, lamotrigine, as noted in Table 6.1, is a broad-acting anticonvulsant acting against a variety of seizure types; however, as noted in Table 6.7, it does not effect either GABAergic potentiation or blockade of thalamic T-type Ca^{2+} channels, thus providing further doubt of these agents acting by a single mechanism. This problem was caused by the classical method of animal testing of anticonvulsants for generalized tonic-clonic seizures [i.e., the maximal electroshock seizure (MES) test]. This method was developed by Goodman and coworkers in 1944 and is still employed today (8, 90–96). The MES test has been found to be particularly sensitive to Na^{+} channel blockers (97).

3.2 GABAergic Mechanisms

GABA, γ- or 4-aminobutyric acid (19), formed by the decarboxylation reaction shown in Fig. 6.5, is present within a large proportion of the central nervous system, where it is the major inhibitory neurotransmitter controlling synaptic transmission and neuronal excitability (98, 99).

Table 6.6 Proposed Cellular Mechanisms of the "First-Generation" Agents[a]

Seizure Type	Anticonvulsant Agent	Blockade of Voltage/ Dependent Na+ Channels	Potentiation of GABAergic Mechanisms	Blockade of Thalamic T-type Ca^{+2} Channels	Blockade of Glutamatergic Mechanisms
A. Generalized tonic-clonic and partial seizures	Phenytoin	++	NE	NE	±
	Carbamazepine	++	NE	?	±
	Phenobarbital	+	+	NE	+
B. Broad spectrum	Valproate	++	+	NE	±
	Benzodiazepines	+	++	NE	NE
C. Absence seizures	Ethosuximide	NE	NE	+	NE

"Effect is indicated by +, ++, effective; ±, inconsistent data; NE, not effective in therapeutically relevant concentrations; ?, no data available (or found). Data are from Refs. 76, 77.

Table 6.7 Proposed Cellular Mechanisms of the "Second-Generation" Agents[a]

Seizure Type	Anticonvulsant Agent	Blockade of Voltage-Dependent Na^+ Channels	Potentiation of GABAergic Mechanisms	Blockade of Thalamic T-type Ca^{+2} Channels	Blockade of Glutamatergic Mechanisms
A. Generalized tonic-clonic and partial seizures	Vigabatrin	?	++	?	?
	Tiagabine	?	++	?	?
	Gabapentin	±	+	NE	±
B. Broad spectrum	Lamotrigine	++	NE	NE	±
	Oxcarbazepine	++	NE	?	?
	Topiramate	+	+	?	+
	Felbamate	+	+	?	++
	Zonisamide	++	NE	+	NE
C. Absence seizures	—	—	—	—	—

[a]Effect is indicated by +, ++, effective; ±, inconsistent data; NE, not effective in therapeutically relevant concentrations; ?, no data available (or found). Data are from Refs. 78-86.

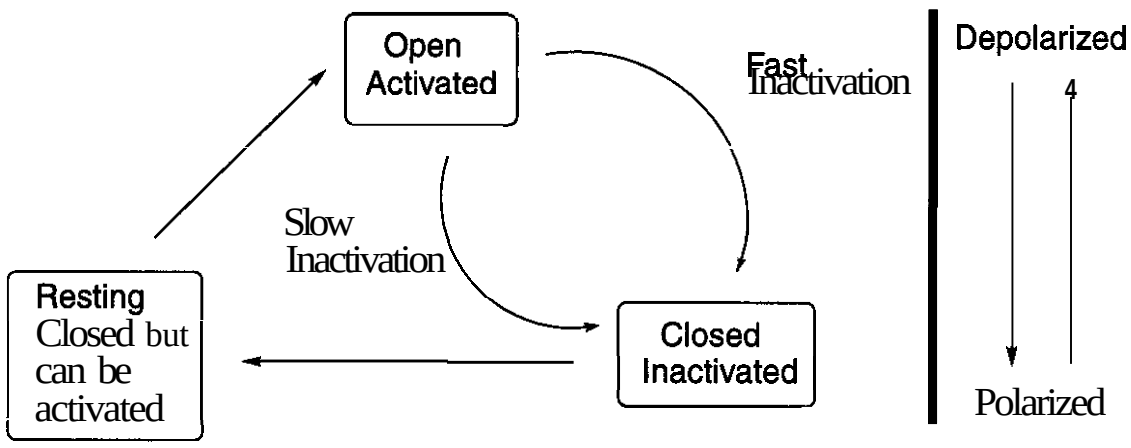
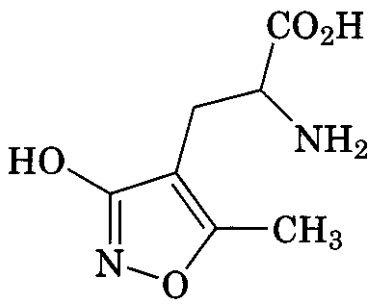


Figure 6.4. Relationship between open, closed, and resting state of Na^+ channels. (After Ref. 86.)

There are at present three known classes of GABA receptors, $GABA_A$, GABA„ and $GABA_C$, with distinctive binding properties and different functional responses to GABA, although each is involved with inhibition of the CNS (100). All GABA receptors are found as pre- and postsynaptic receptors, and as autoreceptors (100).

3.2.1 **$CABA_A$ Receptors.** Of the three, the postsynaptic receptors are responsible for inhibiting neuronal excitability (101, 102). $GABA_A$ receptors (Table 6.8) are major GABA receptors that are linked to chloride channels and are activated by isoguvacine (21) (Fig. 6.6), modulated by barbiturates and the benzodiazepines, and antagonized by bicuculline (101, 102). This receptor is termed a hetero-oligomeric complex and is composed of at least four types of multiple allosterically interacting binding sites (GABA, benzodiazepine, barbiturate, and picrotoxin sites), together with an intrinsic chloride ion channel (103). Each of the allosteric binding sites is thought to be physically distinct, and can be occupied simultaneously to induce their individual pharmacological effects through allosteric interaction. It is established that the $GABA_A$ receptor complex plays a significant role in the action of anticonvulsant agents (104). As noted in Tables 6.6 and 6.7, the older agents (i.e., phenobarbital, valproate, and the benzodiazepines) act through this mechanism, whereas the newer agents (i.e., topiramate, felbamate, vigabatrin, tiagabine, and gabapentin) are also GABAergic agonists, although by different mechanisms (105, 106). Topiramate, in addition to potentiating GABA, also prolongs inactivation of sodium channels. Felbamate, also a GABA agonist, like topiramate, also blocks the (*RS*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA, 25) as well as the *N*-methyl-D-aspartate (NMDA, 26) receptors (these latter compounds are discussed later) (107).



(25) AMPA

Vigabatrin acts by blocking GABA-T, the enzyme responsible for the breakdown of GABA (Fig. 6.5); tiagabine acts by inhibiting the reuptake of the neurotransmitter,

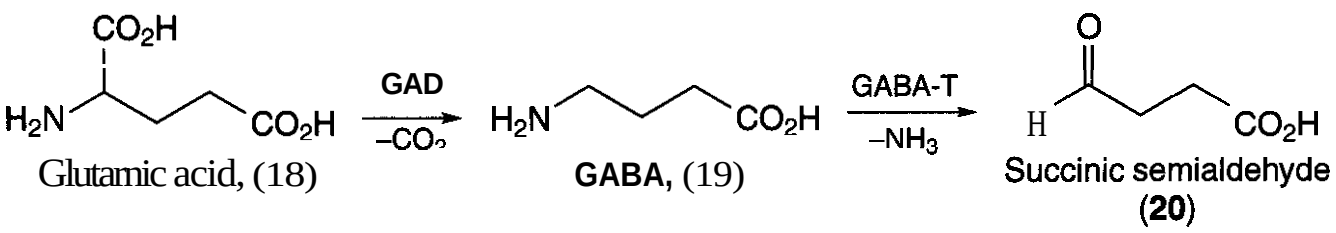
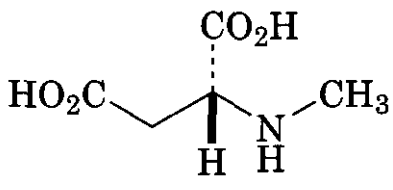


Figure 6.5. Major pathway for the synthesis and degradation of **GABA. GABA,** γ-aminobutyric acid; **GAD,** glutamic acid decarboxylase; **GABA-T, GABA** transaminase.

Table 6.8 The GABA Receptor Subtypes

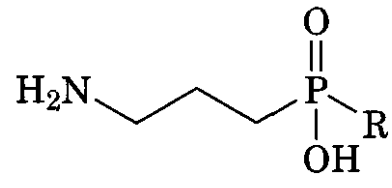
GABA Receptor Subtype	Linked	Activated	Modulated	Response to Bicuculline
$GABA_A$	Chloride channel	Isoguvacine, (21)	Barbiturates; benzodiazepines	Bicuculline-sensitive
$GABA_B$	Calcium or potassium channels	(−)-Baclofen, (22)	Protein kinase C	Bicuculline-insensitive
$GABA_C$	Chloride channel	cis-4-Aminocrotonic acid (CACA, 23); *cis*-2-(Aminomethyl)-*cyclo*-propane-carboxylic acid (CAMP, 24)		



(26) NMDA

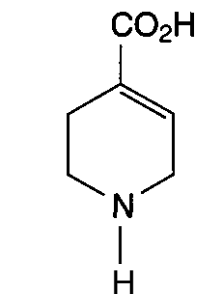
whereas gabapentin presumably acts by activation of GAD (108), with the resultant increase in GABA levels in patients (109). It should also be noted that gabapentin (see Table 6.7) exerts several other cellular actions not related to GABA (see Table 6.7 and Ref. 110).

3.2.2 CABA, Receptors. In theory, drug that depresses $GABA_B$ receptor-mediated inhibition should also be effective anticonvulsants (111). This has been demonstrated in some animal models, but not yet in humans (112). Several phosphinic acid analogs (**27**) of GABA have been synthesized and found to be selective, orally active $GABA_B$ agonists in animals (113); however, they have not been advanced to the market.

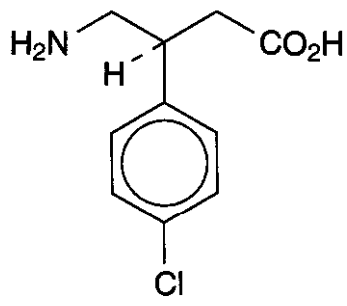


(27) Phosphinic acid analogs
R = C_2H_5; n-C_3H_7; $CH_2C_6H_5$; $CH_2C_6H_{11}$

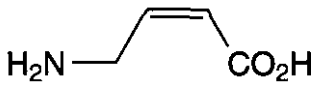
The $GABA_B$ agonist baclofen (22) has been shown to prolong spike-and-wave discharges in animal models, thus enhancing the amount of GABA available to the receptor, and $GABA_B$ antagonists block them (112, 114–117).



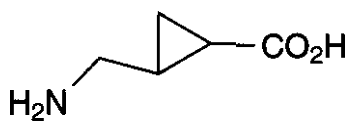
Isoguvacine, (21)



(–)-Baclofen, (**22**)



cis-4-Aminocrotonic acid (CACA, 23)

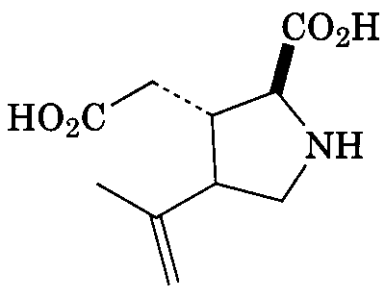


cis-2-(Aminomethyl)-cyclopropanecarboxylic acid (CAMP, 24)

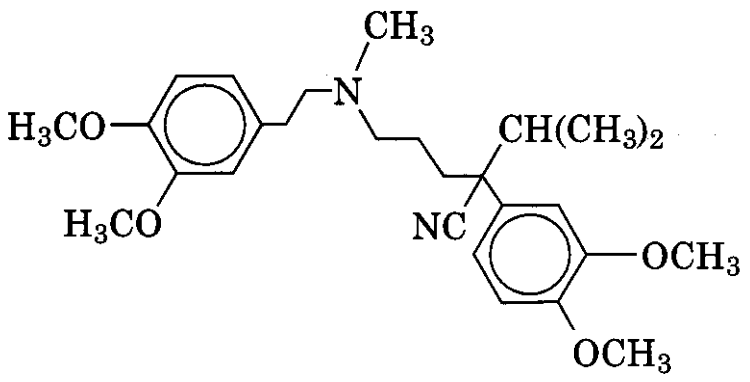
Figure 6.6. GABA activators.

3.3 Glutamate Receptors

(*S*)-Glutamic acid (18) the main excitatory neurotransmitter in the central nervous system and other excitatory amino acids operate through four different classes of receptors (118). In addition to the three heterogeneous classes of ionotropic excitatory amino acids (iGluRs) [i.e., AMPA (**25**), NMDA (**26**), and kainic acid (28) receptors (119–123)], a heterogeneous class of G-protein-coupled excitatory amino acid receptors (mGluRs) has been shown to have an important function in neuronal signaling processes (124, 125). It is now generally understood that both iGluRs and mGluRs play important roles in health and disease processes including epilepsy (119). It



(28) Kainic acid

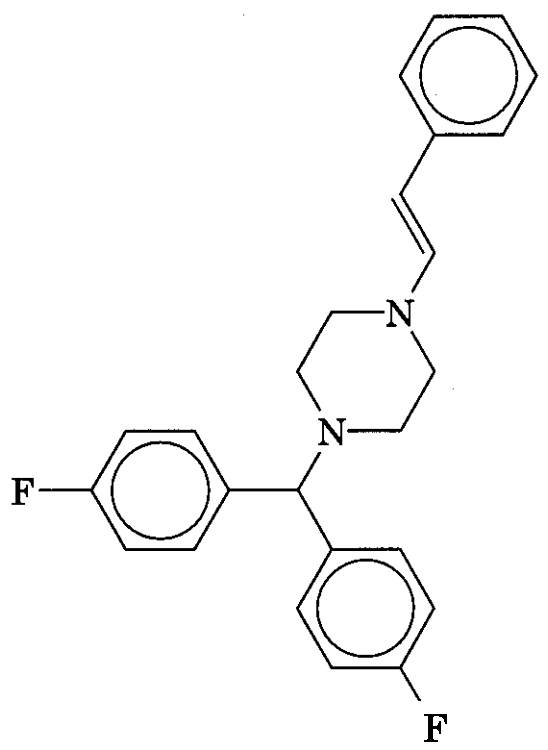


(29) Verapamil

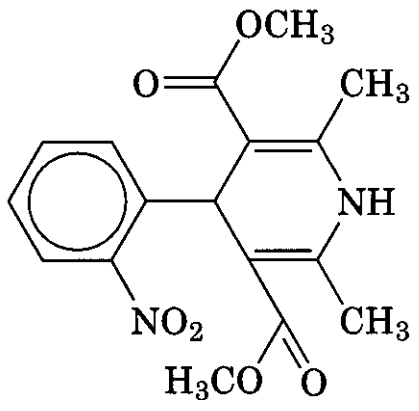
was previously noted that several of the recently marketed anticonvulsant agents act by new mechanisms (80). In this respect, inhibition of glutamatergic excitation, particularly mediated by the NMDA and non-NMDA type of glutamate receptors, has been suggested to play a significant role (80). As noted in Table 6.7, there is indeed evidence for an anti-glutamatergic action for the newer agents, whereas in Table 6.6, there is also evidence that the older drugs act through this mechanism as well.

3.4 T-Type Ca^{2+} Channels

Calcium channels have been classified into L-, N-, T-, P/Q-, and R-types on the basis of their pharmacological and/or electrophysiological properties (126, 127). The classification of voltage-dependent calcium channels divides these channels into three groups: high voltage-activated, which includes L-, N-, P-, and Q-types, intermediate R-type; and low voltage-activated, T-type (128). These channels are composed of a, β, and γ-subunits whose sequences are known (128, 129). The a,-subunit of voltage-sensitive Ca^{2+} channels has a secondary structure similar to that of the a-subunit of Na^{+} channels as well as some sequence homology (127, 129). Because of the homology between these two relevant sites, some Ca^{2+} channel-blockers, such as verapamil (**29**), flunarizine (**30**), nifedipine (**31**), and diltiazem (**32**), also act on Na+ channels (130). These agents, however, showed only low or absent anticonvulsant activity in controlled trials (78). These were all L-type channel blockers. In addition to T-type Ca^{2+} channel blockers on the market [i.e., ethosuximide (**5a**), zonisamide (**13**), and trimethadione (**17a**) (78)], a new series of anticonvulsant aroyl(aminoacyl)pyrroles (lead compound



(30) Flunarizine



(31) Nifedipine

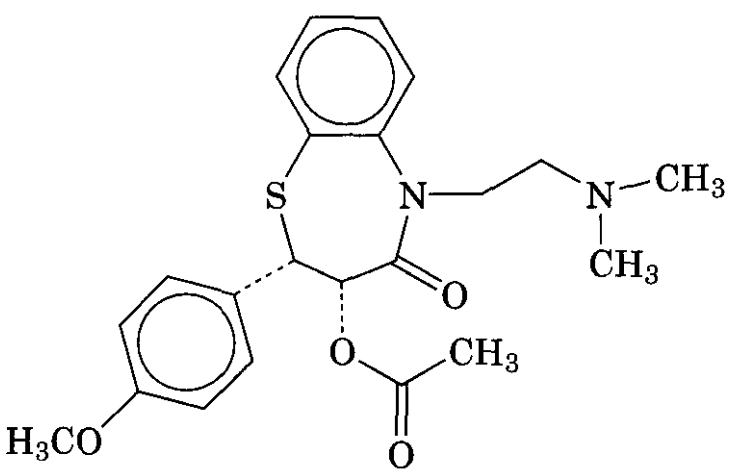
RWJ 37868, **33**) have been synthesized, which act by blocking Ca^{2+} influx into cerebellar cells (131).

4 HISTORY

The history of anticonvulsants has been based on serendipity (8). Table 6.9 indicates the rel-

Table 6.9 Historical Development of **Anticonvulsant** Drugs

Anticonvulsant Agent	Year(s)	Scientist(s)	Reference(s)
Phenytoin, (**1**)	1938	Introduced by Merritt and Putnam	132, 133
	1908	Synthesized by Biltz	134
Carbamazepine, (2)	1960	Schindler	135–137
	1899	Initial synthesis by Thiel and Holzinger	138
Valproic acid, (**3**)	1963	Introduced by Meunier et al.	139
	1882	Synthesized by Burton	140
Diazepam, (**4c**)		Found to be effective in seizure models by:	
	1964	1.Hernandez-Peon et al.	141
	1967	2.Kopeloff and Chusid	142
Ethosuximide, (**5a**)		Antiepileptic properties described by:	
	1958	1.Vossen	143
	1958	2.Zimmerman and Burgmeister	144
Phenobarbital, (**6**)	1912	Introduced by Hauptman	145
Primidone, (7)	1952	Introduced by Handley and Stewart	146
	1949	Synthesized by Bogue and Carrington	147
Trimethadione, (17)	1944	Introduced by Everett and Richards	148
	1944	Synthesized by Spielman	149
Vigabatrin, (**8**)	1977	Synthesized by Jung et al.	150
Gabapentin, (9)	1978	Synthesized by Satzinger and Hartenstein	151
Felbamate, (**10**)	1993	Synthesized by Berger, Ludwig et al.	152, 153
		Introduced by Brodie	154
Lamotrigine, (**11**)	1985	Developed by the Wellcome Research Laboratories, Beckenham, Kent	
		Initial clinical trial Cohen et al.	155
Oxcarbazepine, (**12**)	1987	Developed by Ciba-Geigy Ltd., Basel, Switzerland	
		Initial clinical trial Meinardi et al.	156
Zonisamide, (**13**)	1976	Discovered by Dainippon Research Laboratories, Japan; Clinical studies in the United States sponsored by Warner-Lambert/Parke-Davis	
		Synthesized by Uno et al.	157, 158
Tiagabine, (14)	1990	Developed by Novo Nordisk A/S, Denmark	
		Synthesized by Braestrup et al.	159
Topiramate, (15)	1980	Developed by R.W. Johnson Pharmaceutical Research Institute	
		Synthesized by Matyanoff et al.	160
Levetiracetam, (16)	1992	Developed by UCB S.A. Pharma Sector, Belgium	161



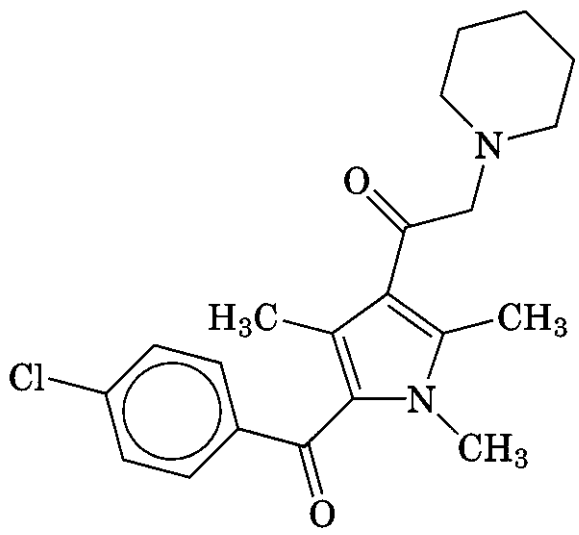
(32) Diltiazem

evant scientists involved in the development of these agents.

As indicated previously (8), the classical research on epilepsy began with the work by John Hughlings Jackson (162), who found that experimental seizures, analogous to the clinical manifestations, could be replicated in laboratory animals by electroshock and a variety of naturally occurring chemicals (163). The following chronology (Table 6.10) evolved from that time.

Table 6.10 **Historical Development of Drug Testing**

Year	Event (Reference)
1864	Convulsions were induced in dogs and rabbits with absinthe (164).
1870	Seizures were produced in animals by excessive electrical stimulation of the brain (165).
1875	Picrotoxin, a component of Anamirta *cocculus* and **A.** paniculata, was first used as a convulsant (166).
1882	Albertoni tested a variety of agents against electrically induced convulsions in dogs (167).
1925–1926	Pentylenetetrazol (34) was synthesized by Schmidt (168); Hildebrant reported its convulsant activity (169).
1937	Putnam and Merritt reported a method for determining the anticonvulsant property of chemical compounds and led to the discovery of phenytoin (1)(122).
1944	Richards and Everett reported on the anticonvulsant activity of trimethadione (17), originally synthesized as an analgesic), in seizures induced by pentylenetetrazol in rats (148).
1944	Goodman and coworkers (90–96) provided standards in the evaluation of potential anticonvulsant candidates. These included the maximal electroshock seizure (MES) test and the subcutaneous pentylenetetrazol (scMet, or scPTZ) test, two standards for drug evaluation used today.
1966	Establishment of the Epilepsy Branch within the National Institute of Neurological Disorders and Stroke (NINDS), a pharmacological testing service for academia and industry (170–173).



(33) RWJ 37868

Of the compounds listed in Table 6.9, only a few anticonvulsant agents were synthesized with a definite plan of attack. This is particularly true with the "first-generation" drugs, which were found by serendipity or screening (random screening of newly synthesized compounds of diverse structure, or structural variation of known anticonvulsant agents) (2, 8). Although the growing trend is toward rational drug design, the mechanisms of action for several of the newer agents remain obscure (174). As related earlier in this chapter, three principal mechanisms of action are seen for the second-generation agents:

1. Enhancement of inhibitory (principally GABA-mediated) processes
2. Reduction of excitatory (principally glutamate-mediated) processes
3. Modulation of membrane cation conductance (Na^+, Ca^{2+}, or K^+)

A review of the second-generation anticonvulsants reveals that screening or serendipity led to the development of felbamate (10), lamotrigine (11), zonisamide (13), topiramate (15), and levetiracetam (16); on the other hand, clobazam (4d) and oxcarbazepine (12) were developed by structural variation of known agents (78). Only three, vigabatrin (8), gabapentin (9), and tiagabine (14), were developed by mechanism-based rational development (78).

Vigabatrin. In recognition of the role of GABA-mediated mechanisms in the pathogenesis of epilepsy, vigabatrin (γ-GABA, 8) was designed as an irreversible inhibitor of GABA-T as seen previously in Fig. 6.5 (175). As noted in Fig. 6.5, blockade of GABA-T, the major pathway for the degradation of GABA, would lead to an elevation of GABA. This was found to be true in rodents (153); further, the

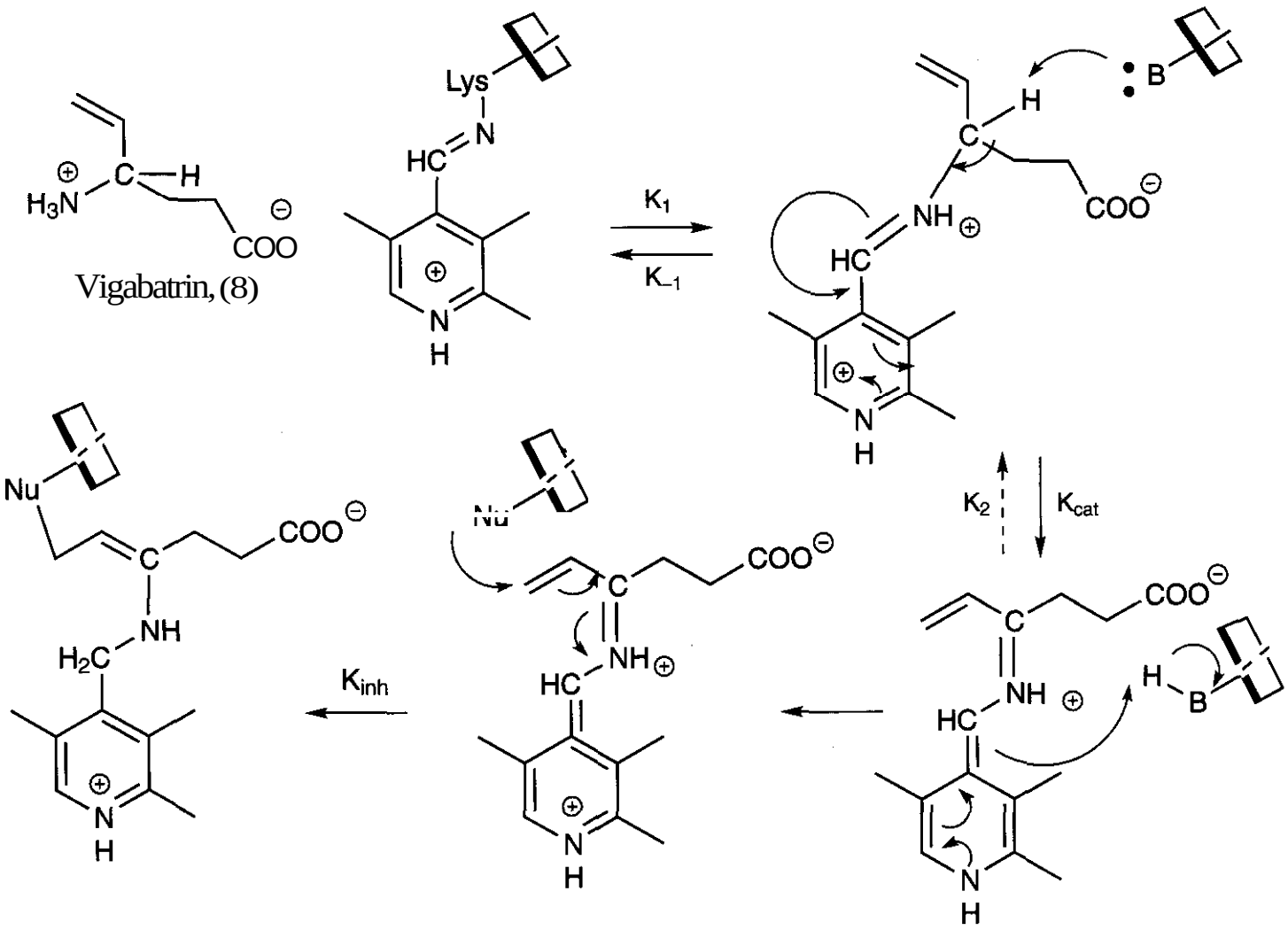
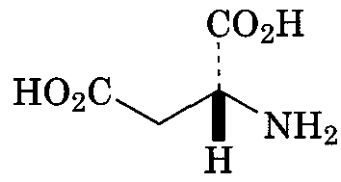


Figure 6.7. Proposed mechanism of action of vigabatrin (8) (after Ref. 175). B, base in enzyme active site; Nu, nucleophilic residue in enzyme active site.

increase occurred preferentially in the neuronal GABA-T sites (176). The proposed mechanism of vigabatrin is shown in Fig. 6.7 and is adapted from Jung et al. (175). GABA-T is a pyridoxal phosphate enzyme that forms a Schiff's base with the active site (K_1); the enzyme abstracts a proton from C4, forming an aldimine-ketimine equilibrium (K_{cat}-K_{-2}). The reactive C5, C6 unsaturated ketimine undergoes a Michael addition with a nucleophilic residue of the active site before it dissociates from the enzyme surface (175).

Although the action of vigabatrin is primarily by augmentation of GABA-mediated function, another mechanism has been suggested; that is, a decrease in brain levels of the excitatory amino acids glutamate (18) and aspartate (35) may also play a part (177).

Gabapentin. Like vigabatrin, gabapentin [(1-aminomethyl)cyclohexaneacetic acid, (9)] was designed as a lipid-soluble GABA analog, which would cross the blood-brain barrier, while retaining much of the chemical and physical properties of GABA (8, 178). However, the hypothesis of a direct GABA-mimetic action was contradicted in a series of biochemical studies (179). It has subsequently been shown that gabapentin limits Na^+-dependent action potentials of cultured mouse neurons in a manner that develops slowly with sustained exposure to the agent (174). This blockade is both voltage and frequency dependent and occurs at clinically effective concentrations of the drug. It is, however, not a sodium-channel blocker in the same manner as that previously noted with phenytoin and carbamazepine, but instead acts at a distinct site or indirectly (180). Further, gabapentin was found to increase GABA accumulation in discrete regions in rat brain, in a time course that parallels the anticonvulsant effect (181). There has



(35) Aspartic acid

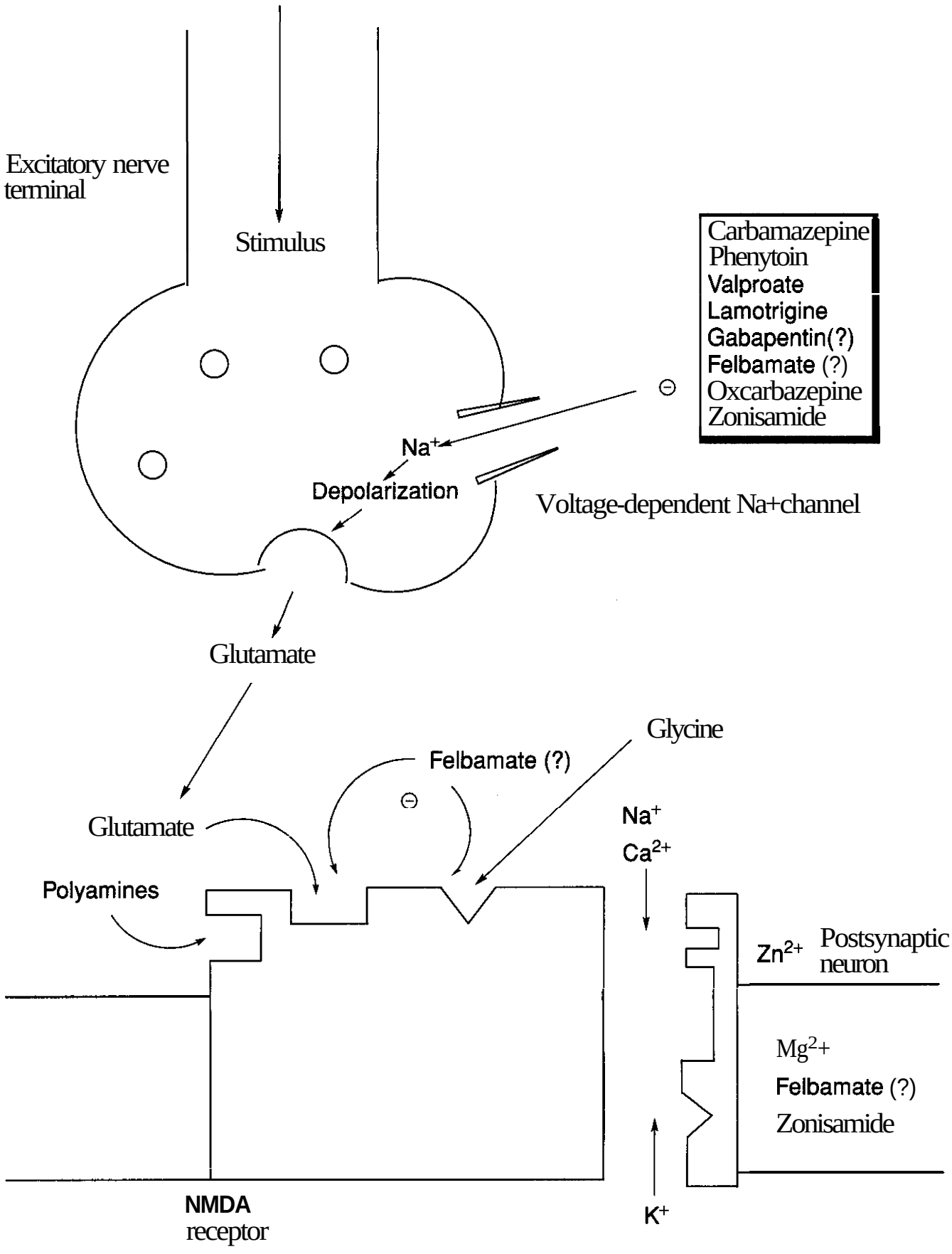
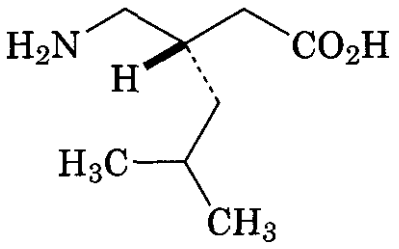
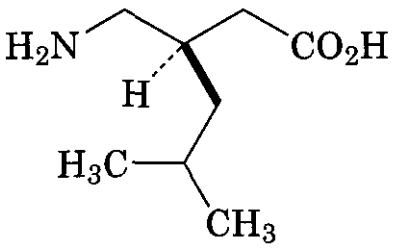


Figure 6.8. Possible sites of interaction of antiepileptic drugs on glutamate-mediated transmission (after Ref. 174). The NMDA receptor is associated with an ion channel permeable to Na^+ and Ca^{2+}, and is associated with a number of modulatory sites, including a strychnine-insensitive glycine-binding site. Glycine is an absolute requirement for the receptor-channel complex to enter the open state.

recently been found a specific binding site for a [^{3}H]gabapentin (182). It is localized in discrete areas in the brain of rats that are associated with excitatory input (183, 184). S-(+)-3-Isobutyl-GABA (**36a**) displaces [^{3}H]gabapentin binding and has anticonvulsant activity, whereas the R-(−) enantiomer (**36b**) was less active in each evaluation (185). These data support the conclusion that there is an association between the ability of gabapentin to interact with its binding site and the anticonvulsant effects (174).



(36a) S-(+)-3-Isobutyl-GABA

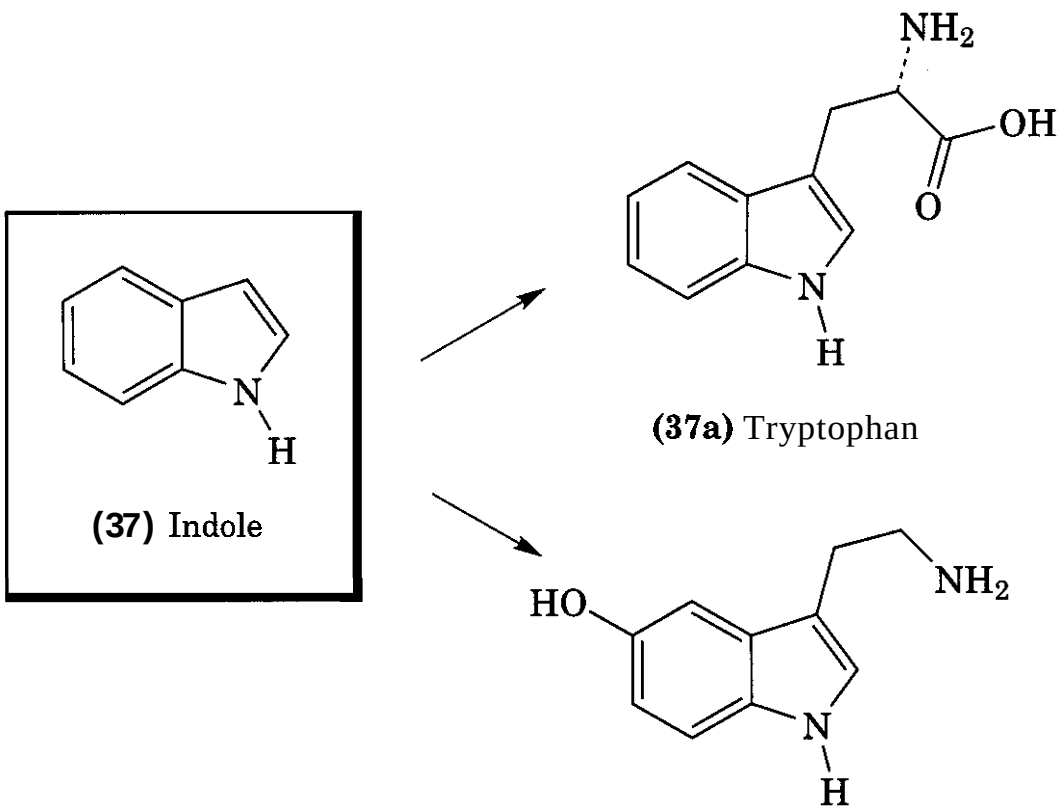


(36b) R-(-)-3-Isobutyl-GABA

Felbamate. In a recent report, it was noted that felbamate (2-phenyl-1,3-propanediol dicarbarnate, 10) can reduce sustained repetitive firing of spinal cord neurons at therapeutic concentrations (186). This provides indirect evidence of a blocking effect on voltage-dependent Na+ channels as a mechanism of action (187). However, felbamate also has been found to exert action on other systems as well. The NMDA site has been postulated as an additional target for felbamate. Felbamate has been shown to interact with strychnine-insensitive glycine-binding sites in concentrations within the therapeutic range (188). Further, it was shown that glycine can selectively reverse the anticonvulsant effects of felbamate in rodents and provides evidence that felbamate acts as an antagonist at the NMDA-associated glycine-binding site (189). The result would be a reduction in excitatory amino acid transmission (174). Additional research has shown that felbamate can block NMDA responses in a low affinity noncompetitive, or open channel manner (i.e., the ion channel has been previously opened by glutamate, or other agonist). These actions are summarized in Fig. 6.8. An additional study showed that felbamate can enhance $GABA_A$ receptor-mediated responses in therapeutic concentrations (189). Thus, it is clear that felbamate exerts multiple effects as an anticonvulsant.

Lamotrigine. Lamotrigine [3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine (**11**)] was the result of the observations of Reynolds and coworkers that chronic anticonvulsant treatment with phenytoin (1), phenobarbital (6), and primidone (7) led, in many instances, to a disturbance in folate metabolism and that there may be a relation between the antifolate effects of these drugs and their therapeutic actions (190). It was subsequently shown that folic acid and other folates were convulsant in laboratory animals when injected directly into the brain or after massive parenteral doses (191). However, lamotrigine has been shown to be a potent anticonvulsant, with only weak antifolate activity (8). Currently, it has been shown that lamotrigine primarily acts by stabilizing presynaptic neuronal membranes through a blockade of voltage-dependent Na^+ channels, thus preventing the release of excitatory neurotransmitters, principally glutamate (see Fig. 6.8) (192). Although this blockade is similar to that elicited by phenytoin and carbamazepine (193, 194), it differed in also blocking electrically and visually evoked afterdischarge tests, where only lamotrigine provided protection (191). This latter test is used as a model of local (partial) seizures and is useful in identifying drugs such as ethosuximide (191). It is therefore of interest to note that, although lamotrigine demonstrates an effect similar to that of phenytoin and carbamazepine in blocking Na+ channels [useful in protection against tonic-clonic seizures and some partial seizures (77, 174)], it should be noted that lamotrigine provides broader seizure protection as well.

Oxcarbazepine. Oxcarbazepine (10,11-dihydro-10-oxo-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide or 10,11,dihydro-10-oxo carbamazepine, 12) was designed to avoid the dose-dependent side effects noted in some patients (e.g., nausea, headache, dizziness, ataxia, diplopia) and to minimize enzyme induction and drug-drug interactions displayed by carbamazepine (195, 196). As shown previously (Fig. 6.3), the change in structure results in a difference in metabolism. Although both carbamazepine and oxcarbazepine are ultimately converted to the inactive *trans* diol, oxcarbazepine does not form the active 10,11-epoxide intermediate, but does form the active 10-hydroxy metabolite MHD (197). The mechanism of action of oxcarbazepine is very similar



(37) Indole

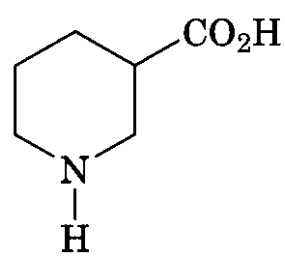
(37a) Tryptophan

(37b) Serotonin

to that of carbamazepine (i.e., MHD blocks voltage-dependent Na^+ channels), but unlike carbamazepine, produces a reversible, dose-dependent decrease in high voltage-activated Ca^{2+} channels, an effect not antagonized by nifedipine, and reduces glutamatergic transmission (198).

Zonisamide. Zonisamide (1,2-benisoxazole-3-methanesulfonamide, 13) was developed because of its isosteric similarity to indole (**37**), a structural skeleton of tryptophan (**37a**) and serotonin (**37b**) (8,157). Zonisamide prevents repetitive neuronal firing by blockade of voltage-sensitive Na+ channels. It also reduces voltage-dependent T-type Ca^{2+} channels, facilitates dopaminergic and seratoninergic neurotransmission, weakly inhibits carbonic anhydrase, and may protect neurons from free-radical damage, thus stabilizing neuronal membranes (199). Zonisamide does not potentiate the synaptic activity of **GABA** (198).

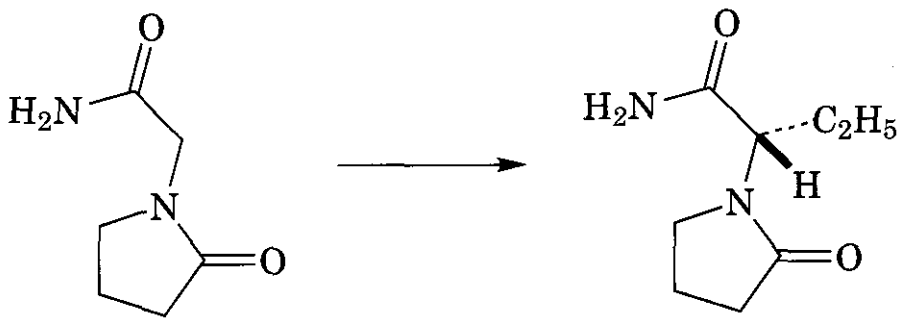
Tiagabine. Tiagabine {(*R*)-(−)-1-[4,4-bis(3-methyl-thienyl)-3-butenyl]-3-piperidinecarboxylic acid or *R*-(−)-*N*-[4,4-di(3-methylthien-2-yl)but-3-enyl]nipecotic acid) (14) was designed specifically as a **GABA reuptake** inhibitor, operating through a high affinity, sodium-dependent transport system (200). Earlier, it was observed that nipecotic acid (3-piperidine carboxylic acid, 38) possessed **GABA reuptake** blockade *in vitro*;



(38) Nipecotic acid

however, because of its polar nature it could not permeate the blood-brain barrier (8, 198). Tiagabine was thus designed using the nipecotic acid nucleus with the polar nitrogen atom attached to a bis lipophilic thienyl carrier linked by an aliphatic chain (8, 198). These modifications permitted rapid brain penetration, where it effectively inhibits **GABA** reuptake. Tiagabine possesses an anticonvulsant effect, which parallels elevation of **GABA** levels. Whether this is the only anticonvulsant mechanism awaits further investigation (201).

Topiramate. Topiramate (**15**), a unique sulfamate-substituted sugar moiety, possesses multiple proposed mechanisms of action: (*1*) blockade of voltage-activated sodium channels; (2) enhancement of **GABA** acting at the benzodiazepine-insensitive $GABA_A$ receptor; and **(3)** modest blocking action at the AMPA glutamate receptors (196). There are no reported effects at **NMDA** receptors. Although the agent possesses weak carbonic anhydrase



(39) Piracetam (16) Levetiracetam

inhibitory activity, this action, as it relates to anticonvulsant activity, remains problematic. However, it can explain the appearance of kidney stones in some patients resulting from the formation of the insoluble N-acetyl metabolite (Fig. 6.3), a phenomenon known for the sulfonamides.

Levetiracetam. Levetiracetam [(−)-(*S*)-α-ethyl-2-oxo-1-pyrrolidine acetamide (**16**)] is unique in that it failed the two primary evaluation screens, that is, the MES and the scMet (scPTZ) tests (198). In contrast, levetiracetam possesses strong seizure protection in animal models (i.e., genetic models and kindled rodent models), which are more indicative of the chronic disease state (201,202). Research on the mechanism of action has been ongoing and reveals the following: (1) the results on the GABA system lead to conflicting results; one laboratory reported that it is unlikely that the anticonvulsant action is mediated by action on the GABAergic system (203), whereas another laboratory reported that its action may in part be attributed to potentiation of GABAergic inhibition (204); (2) it is the first drug to demonstrate antiepileptogenic activity, which provides seizure protection long after termination of treatment (205); (3) a stereospecific binding site for [^{3}H]levetiracetam exists that is not displaced by phenytoin, carbamazepine, valproate, phenobarbital, or benzodiazepines (206); (4) the displacement of [^{3}H]levetiracetam binding occurred with amiloride, a T-type Ca^{2+} antagonist; however, levetiracetam is inactive in the T-channel assay (208). Thus, the mechanism of action remains unknown (198).

Of interest is the structural interrelationship between levetiracetam (16) and its precursor piracetam **(39)** (207). Both are 2-pyrrolidinones; however, the chirality of levetiracetam allows for a wider range of therapeutic activity as noted above. These actions/inactions are shown in Table 6.11 (208).

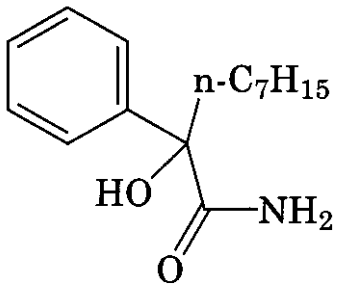
5 STRUCTURE-ACTIVITY RELATIONSHIPS

5.1 Hydantoins

In addition to an extensive summary provided previously on this moiety (8), Brouillette et al. (209) employed comparative molecular field analysis (CoMFA), a three-dimensional structure-activity technique, to provide a new potential anticonvulsant, 2-hydroxy-2-phenylnonanamide (**40**), whose Na+ channel inhibition ($IC_{50} = 9\ \mu M$) compared favorably to 40 μM for phenytoin (**1**). This study suggested that the hydantoin ring system is not necessary in Na+ channel binding. Research on water-soluble prodrugs of phenytoin has continued since the work by Stella, which led to the synthesis of fosphenytoin (**1d**) (8,209–215). A

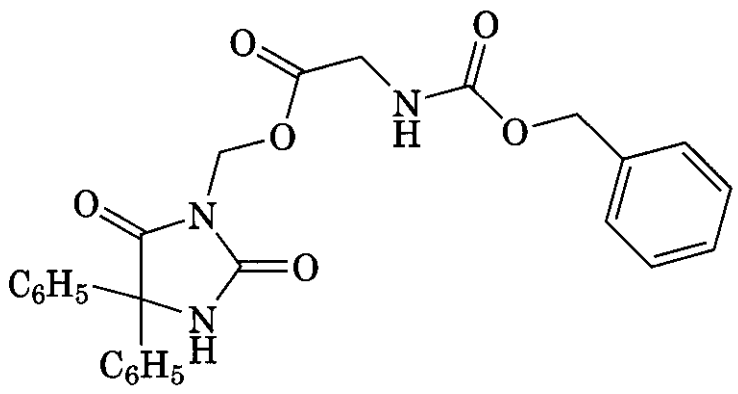
Table 6.11 2-Pyrrolidinones

Effect	Levetiracetam	Piracetam
Brain-specific stereoselective binding site	Yes	No
Improvement in learning and memory	Less effective	Excellent
Seizure prevention	Excellent	Poor
Therapeutic indication	Epilepsy (partial; possibly generalized and myoclonus)	Age-related cognitive disturbances; poststroke aphasia



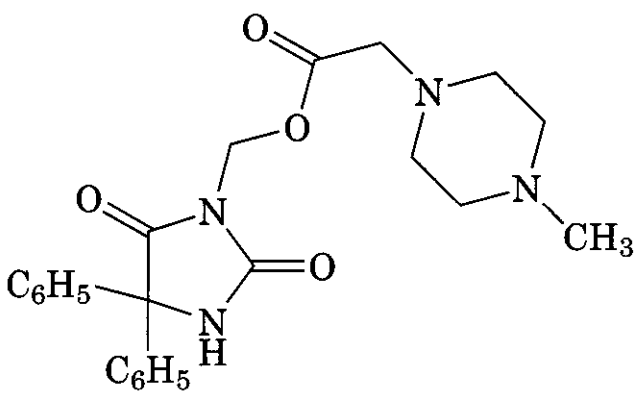
(40)

series of *N*-benzyloxycarbonyl-amino acid prodrugs of phenytoin, synthesized by Scriba and Lambert, led to (**41**), which provided a de-



(41)

creased median effective dose (ED_{50}) in the MES test and an increased median toxic dose (TD_{50}) compared to that of phenytoin (**216**). The piperazine prodrug (**42**), prepared by

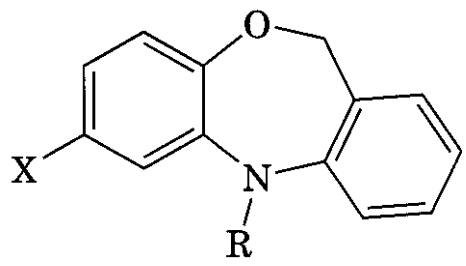


(42)

Bosch and coworkers, was hydrolyzed more rapidly than fosphenytoin under the same in vitro conditions (96.52 ± 7.15% at 2 min for 42 versus 11.63 ± 1.8% for fosphenytoin) (217).

5.2 Iminostilbenes

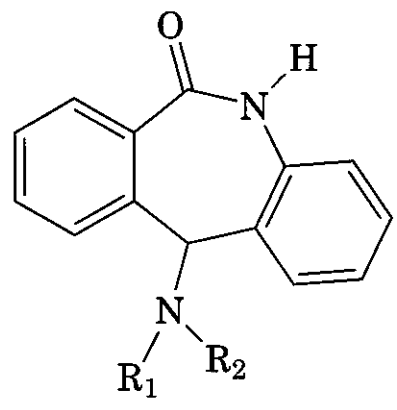
Several structure-activity studies were described. Yale synthesized a series of 5,11-dihydro[*b*,*e*] [1,4]oxazepine-5-carboxamides (43) and tested these analogs for anticonvulsant



(43)

activity as well as for activity in trigeminal neuralgia (218). Two compounds, (**43a**) (X = Cl, R = $CONH_2$) and (**43b**) (X = H, R = $CONH_2$), were compared to carbamazepine (2). In the oral MES evaluation (**43a**) and (**43b**) provided ED_{50} values of 14 and 29 mg/kg, respectively, compared to 19 mg/kg for carbamazepine. The three compounds were compared for their ability to reduce the evoked thalamic or trigeminal potentials in the cat. In these studies, (**43b**) was equipotent to carbamazepine, whereas (**43a**) was less potent than carbamazepine; however, both oxazepines possessed a longer duration of action, were less toxic, and provided a more selective effect on the trigeminal sensory system than that of carbamazepine.

A series of alkylamino-5,6-dihydro-6-oxomorphanthridinones (chemically, dihydrodibenz[*b*,*e*]azepines, 44) were synthesized and

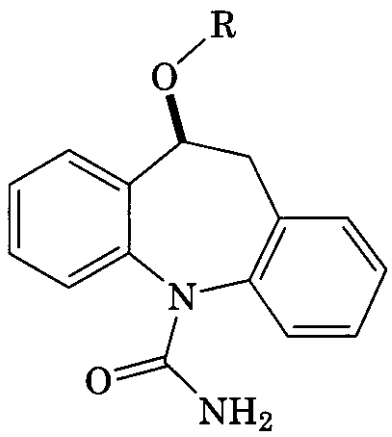


(44)

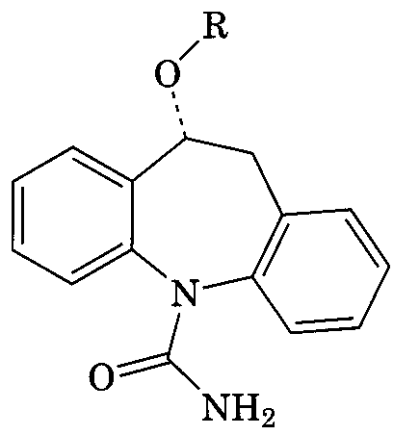
evaluated for anticonvulsant activity by Waring and Whittle (**219**). The most active compound, (**44a**) ($R_1 = R_2 = CH_3$; MES ED_{50} = 42 mg/kg; scMet ED_{50} = 38 mg/kg), was advanced to clinical trial in epileptic patients.

Research on derivatives of oxcarbazepine (12) has evolved. Soares-da-Silva and coworkers provided data from two studies. As indicated previously (Fig. 6.3) oxcarbazepine (**12**) is rapidly and extensively metabolized to a mixture of the active metabolites, the (10*S*)-

alcohol (**45a**) and its (10*R*)-enantiomer (**45b**), both appearing in plasma (220) and urine (221) in an approximately 4:1 ratio. The first study (222) evaluated the esters of the **mono-**



(45a) R = H; *S*(+)



(45b) R = H; *SR*(-)

hydroxy derivatives and it noted that the acetates (R = $COCH_3$) were the most active compounds. Of further interest was the fact that the acetate of (**45a**) was active in its own right and not a **prodrug** in the true sense. Comparative data are provided in Table 6.12. **As** noted, whereas carbamazepine was the most active, the acetate of (**45a**) was as active as oxcarbazepine. In addition, blockade of voltage-sensitive sodium channels was also evaluated, revealing that the acetate of (**45a**) had a statistically significant different IC_{50} of 138 ± 32 μM, compared to that of carbamazepine (210 ± 15 μM) and provided 95.6 ± 2.6% inhibition of sodium uptake at 300 μM (carbamazepine 64.8 ± 3.0% at 300 μM). In a follow-up study, they indicated that acetates (45) appear to be preferable to oxcarbazepine because they provide a clearer metabolic pathway (**223**). Therapeutically, acetate (**45a**) was found to be more appropriate as the *R*-(−)-isomer, which has a greater propensity to undergo inactivation to the trans-diol (**45c**) (222). The **biotransformation** of the esters is shown in Fig. 6.9. The predominant pathway is indicated with the heavy arrow.

The quadracyclic (±)-**5-aminocarbonyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine** (ADCI, **47**), results from the fusion of two active anticonvulsant compounds, MK 801 (dizocilpine, **46**), a potent noncompetitive NMDA antagonist (224, 225) and carbamazepine (2). The compound acts as a selective, low **affinity** channel blocker of the NMDA receptor and also possesses Na^+ **channel-blocking** activity (226). ADCI is devoid of the tendency to cause behavioral impairment as MK 801. ADCI is a racemate, although the (+)-enantiomer displays a four- to fivefold greater potency at the NMDA receptor and a greater than twofold potency for seizure models **in animals**. There was no enantioselectivity in the Na^+ channel evaluation, however. The (+)-enantiomer (SGB-017) is currently in Phase **II** of clinical development.

5.3 Barbiturates

Of renewed interest is the emergence of **etero-**barb (*N*,*N*′-**dimethoxymethyl** phenobarbital, 6b) (200). This agent possesses attenuated sedative and hypnotic properties compared to those of phenobarbital **(6).** Although

Table 6.12 Comparative Data **of** Carbamazepine **(2),** Oxcarbazepine **(12), and** Acetate **of** 45a **and** 45b[a]

Compound	% Protection	MES ED_{50} (mg/kg)	TD_{50} (mg/kg)	Protective Index[b]
Carbamazepine, (**2**)	**100 ± 0.0**	**3.4 ± 0.1**	**27.4 ± 0.1**	**8.1**
Oxcarbazepine, (12)	**68.3 ± 20.3**	**6.1 ± 0.1**	**40.1 ± 1.2**	**6.6**
Acetate of (**45a**)	**100 ± 0.0**	**6.3 ± 0.1**	**78.6 ± 4.2**	**12.5**
Acetate of (**45b**)	5.9 ± **1.8**	**18.0 ± 0.1**	**134.9 ± 3.5**	**7.5**

[a]**Values are means ± SEM of 5–8 rats/group.**
[b]**Protective index** = TD_{50}/ED_{50}.

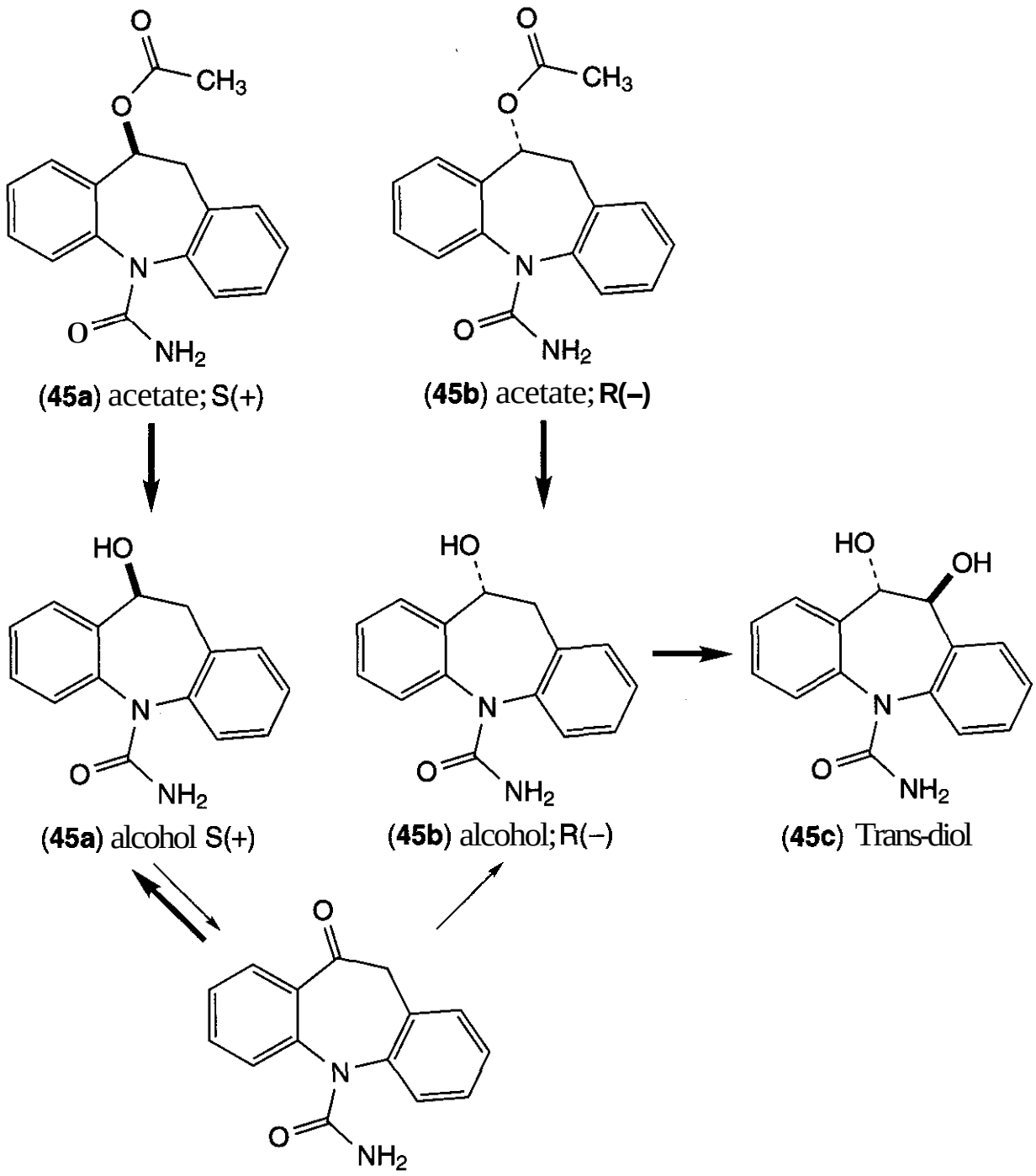
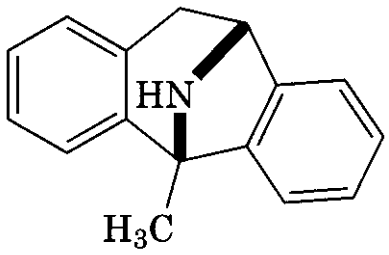


Figure 6.9. Proposed metabolism of acetates of oxcarbazepine (12). Dark arrows are the preferred pathway.

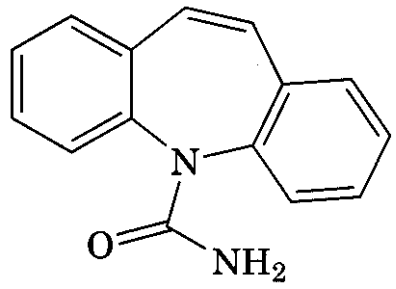
eterobarb is completely converted to phenobarbital, N-monomethoxymethyl phenobarbital (MMP, **6c**), the first step in the metabolism occurs more rapidly than the final step. Eterobarb does not enter the brain and may be considered a **prodrug** for MMP and phenobarbital, both active anticonvulsants (200). Eterobarb has the advantage of lacking sedative activity, a prominent side effect with phenobarbital. The metabolic conversion and interactions are shown below.

5.4 Benzodiazepines

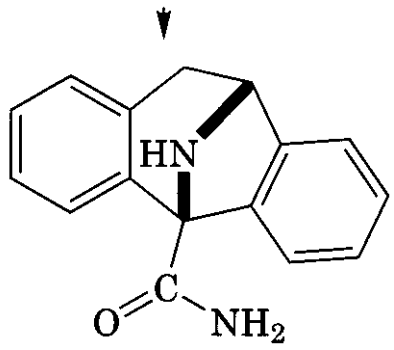
Benzodiazepines act by potentiating the GABAergic receptor, a high affinity, saturable binding site (8).The anticonvulsant activity of these agents *in vivo* was directly correlated to their *in vitro* binding effect (227–229). Of the drugs currently in the market, the anticonvulsant **1,4-benzodiazepines** (**48a**) are composed of clorazepate dipotassium ($R_1 = R_3$ = H, R_2 = CO_2K, R_4 = Cl, **4a**), clonazepam (R_1 = R, = H, R_3 = 2-Cl, R_4 = NO,, **4b**), diazepam (R_1 = CH_3, R, = R_3 = H, R_4 = Cl, **4c**), and nitrazepam (R_1 = R_3 = R_3 = H, R_4 = NO,, **4e**); whereas clobazam [**4d** (R, = CH,, R_2 = R_3 = H, R_4 = Cl)] possesses the isosteric **1,5-benzodiazepine** system (**48b**). Talampanel (**LY300164, 49**) is the most potent in a series of 5H, **2,3-benzodiazepines** (230).The compound

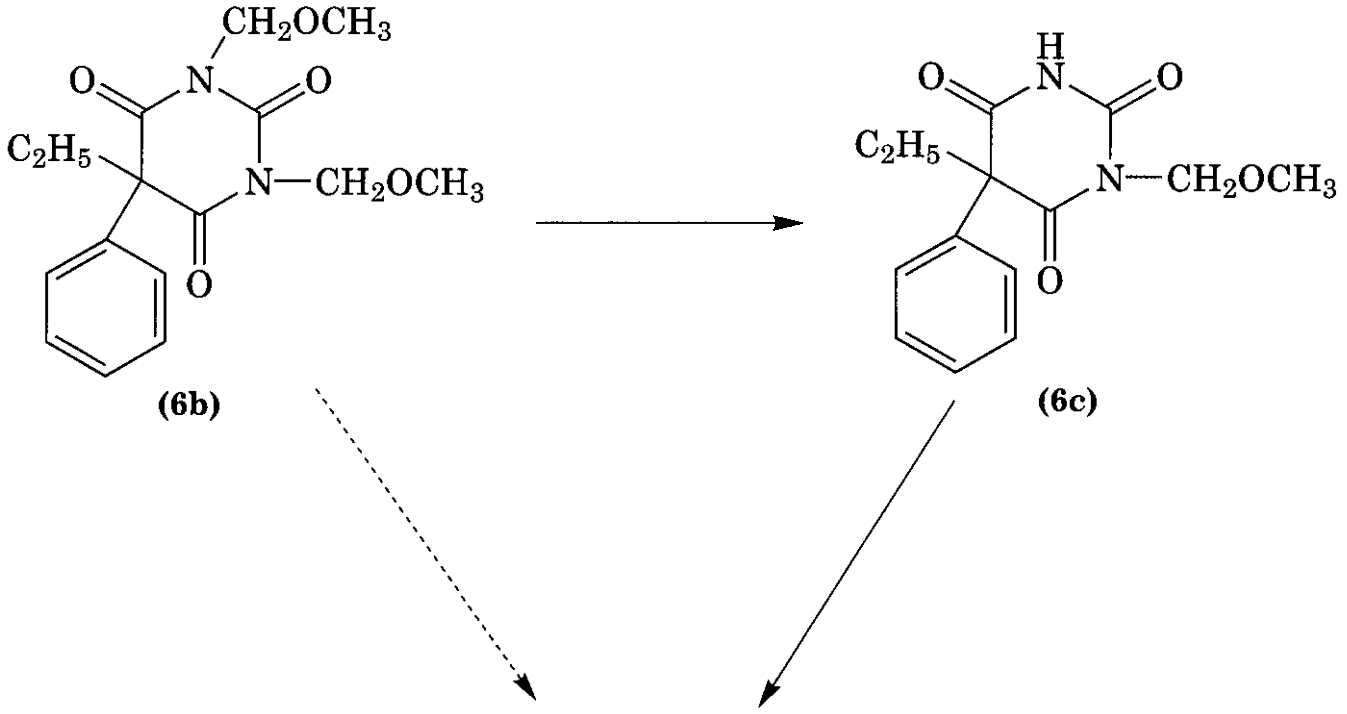


(46) Dizocilpine **(MK 801)**

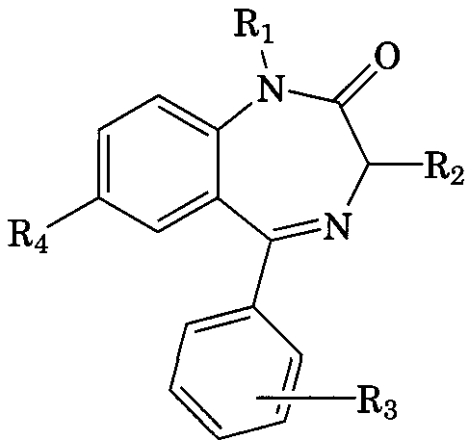


(2) Carbamazepine

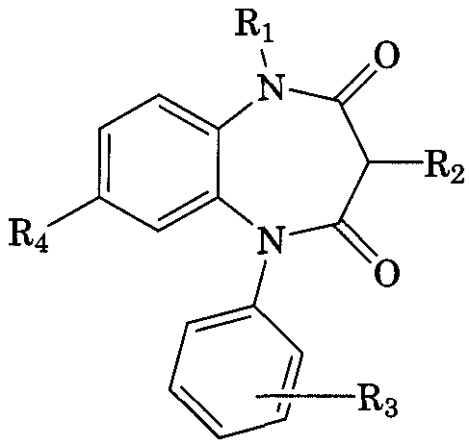




(6) Phenobarbital



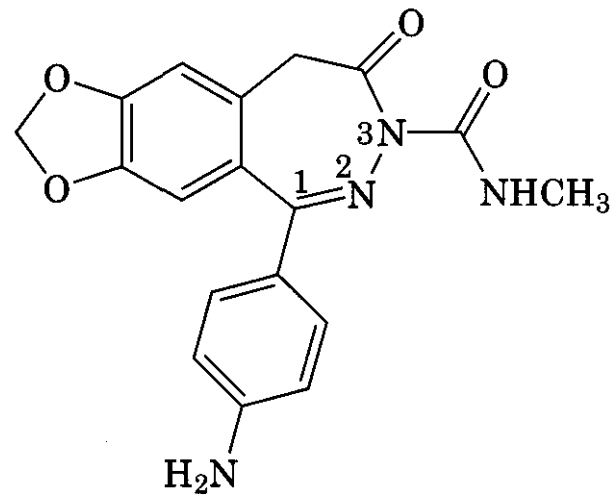
(48a)



(48b)

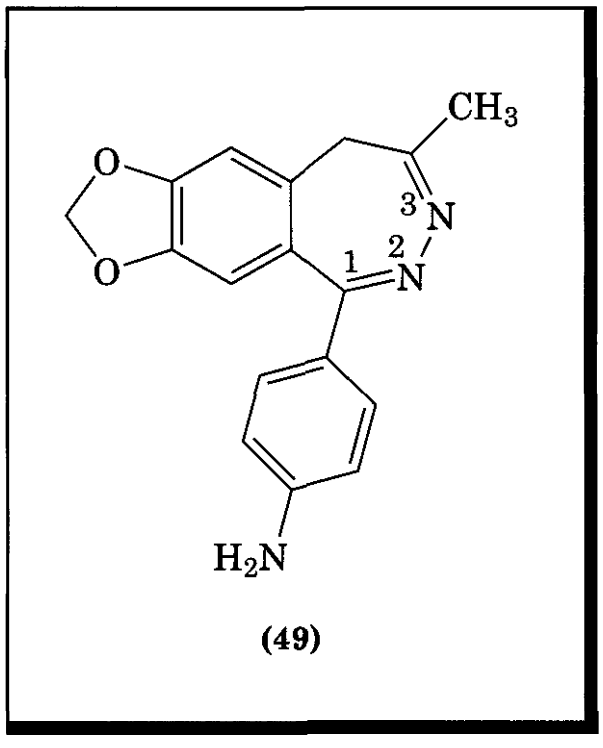
is a selective, noncompetitive antagonist of the AMPA receptor. From analysis of the metabolic products, it was observed that the C1 aniline moiety would lend itself to modification, given that the N-acetyl functionality was the primary route of metabolism in monkeys (231). The nitro, chloro, iodo, and proteo derivatives were found to be inactive in the MES model.

In a related study by Grasso et al., it was shown that 1-(4-aminophenyl)-3,5-dihydro-3-methylcarbamoyl-7,8-methylenedioxy-4*H*-benzodiazepin-4-one(**50**) was the most potent

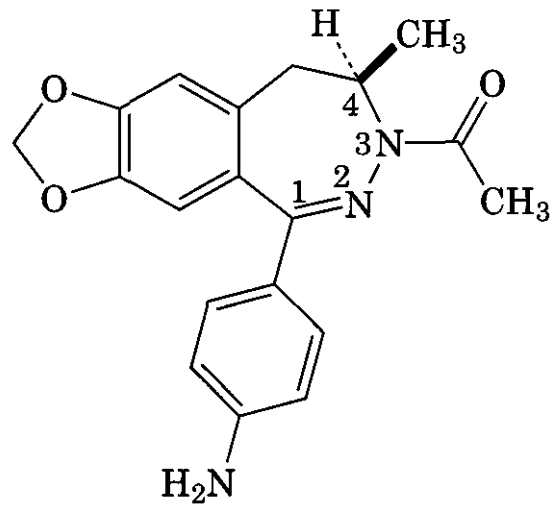


(50)

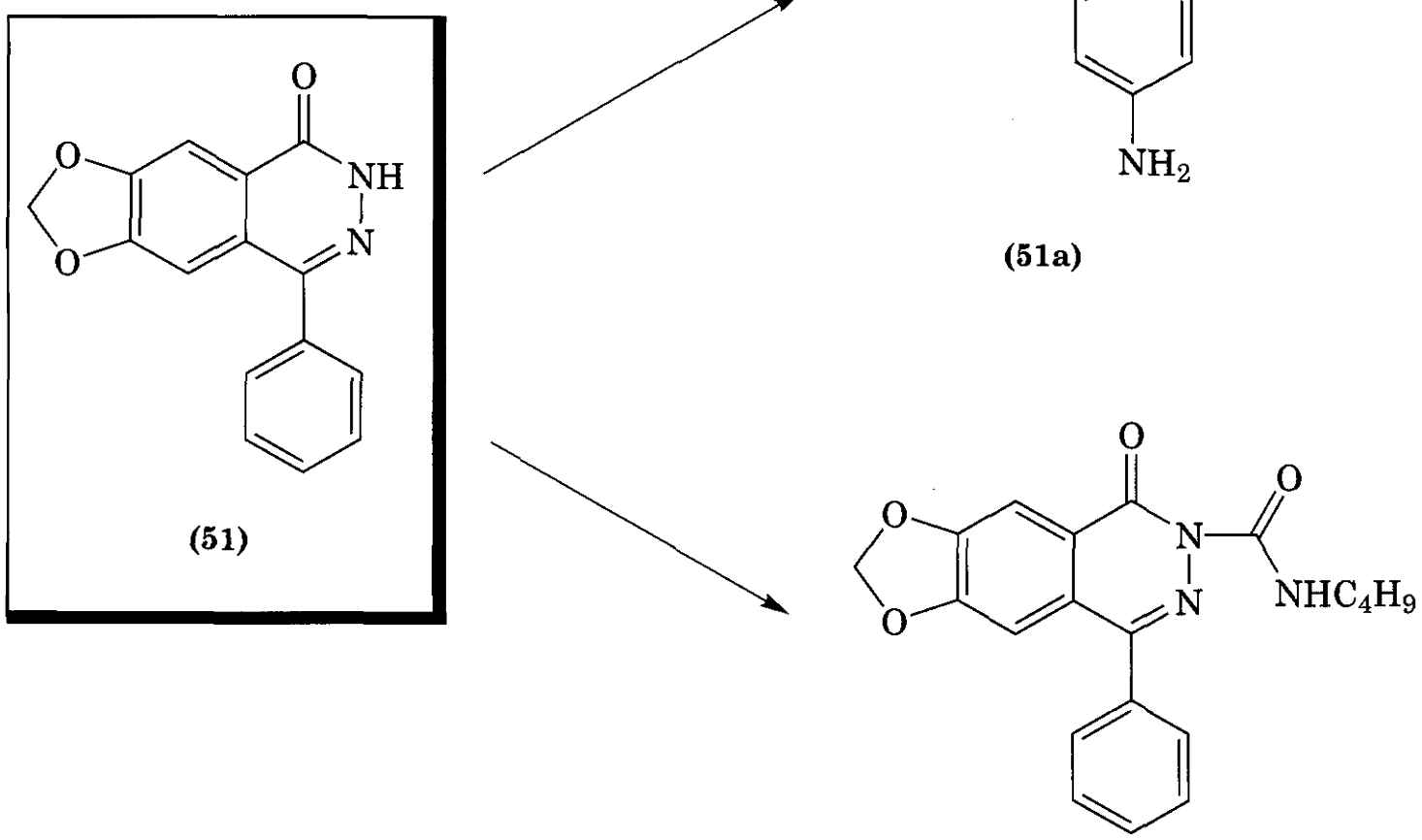
in a series of analogs (232). Because of its long duration of MES and kainic acid-induced seizure protection, (50)may become a useful tool in the mapping of the AMPA/kainic acid receptor. The Grasso group synthesized a novel series of 4-aryl-6,7-methylenedioxyphthalazin-1(2*H*)ones, the most active of which was (**51**); however, (**51a**) and (**51b**) were less active than (**49a**) (233).



(49)

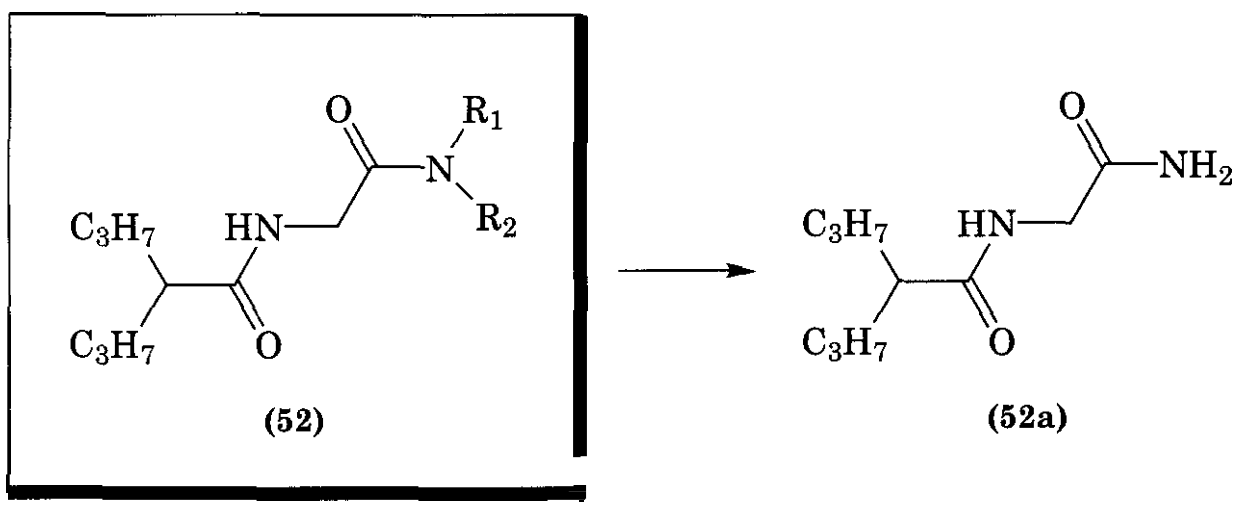


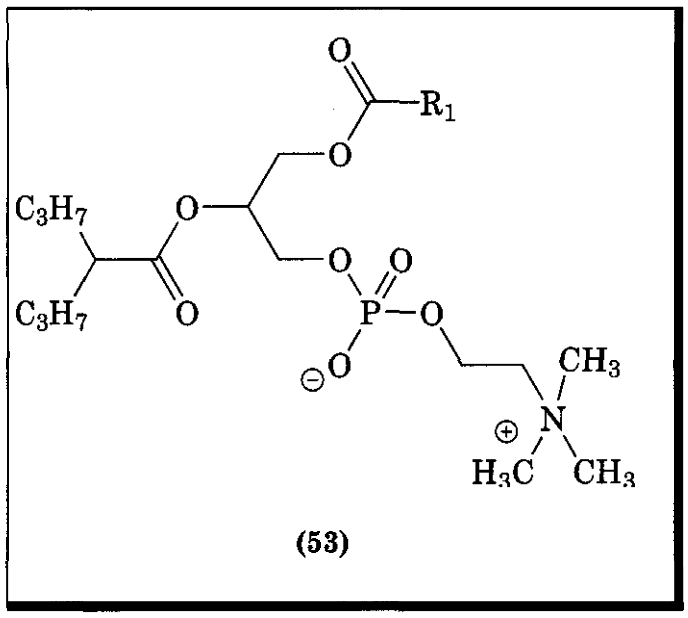
(49a)



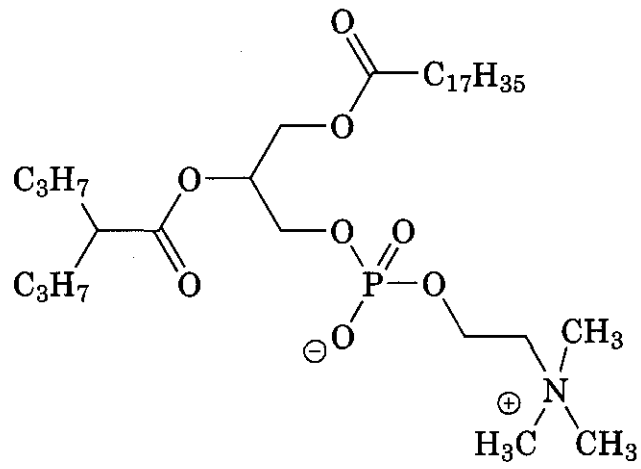
5.5 Valproate

In a study to evaluate the structure-pharmacokinetic-pharmacodynamicrelationships of a series of N-alkyl and *N,N*-dialkyl derivatives of valproic acid (**52**), N-valproyl glycinamide (valrocemide, TV 1901, **52a**) emerged as the most promising candidate (MES ED_{50} 152 mg/kg; scMet ED_{50} 127 mg/kg; TD_{50}, 369 mg/kg), compared to valproate (**3a**) (MES ED_{50} 272 mg/kg; scMet ED_{50} 149 mg/kg; TD_{50}, 426 mg/kg) (234,235). The drug combined glycine, a neuroinhibitory transmitter, as an amide, to valproate, an anticonvulsant. The polar nature of glycine would not permit it to pene-





(53)



(53a)

trate the blood-brain barrier; however, the conjugate product (**52a**) could serve as a chemical drug-delivery system for glycine or, alternatively, the combination could act as a drug on its own. It was found that the intact (**52a**) did, in fact, exert anticonvulsant activity. As expected, the major metabolic product was the carboxylic acid, valproyl glycine, and this compound was devoid of anticonvulsant activity. Only a small amount of valproate was detected in animals. Valrocemide is currently undergoing phase II clinical trials (198).

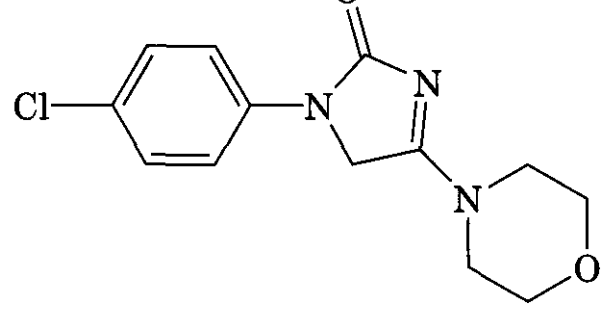
In another study, a series of phosphatidyl choline analogs (53) containing valproate were prepared. The agents were developed based on targeted drug-delivery technology and were directed toward delivering therapeutic levels of valproate in the vicinity of the epileptic focus (198). In theory, this process would limit the drug to a restricted area, thus limiting the overall systemic and side effects. The most active, DP-VPA (DP 16, **53a**) is composed of phosphatidylcholine linked to valproic acid as an ester. This linkage renders the molecule inactive. The cleavage of DP-VPA and local release of active valproic acid occurs selectively in response to epileptic activity in the brain signaled by elevation of phospholipase A_2. Once the neurons return to their normal state and enzymatic hyperactivity subsides, drug activation ceases, thus providing dual control over drug action (198). The drug has completed phase I studies and phase II studies are being planned.

6 RECENT DEVELOPMENTS

The following are drugs in development.

6.1 AWD 131–138

Chemically, AWD 131–138 is 1-(4-chlorophenyl)-4-morpholino imidazolin-2-one (54). This

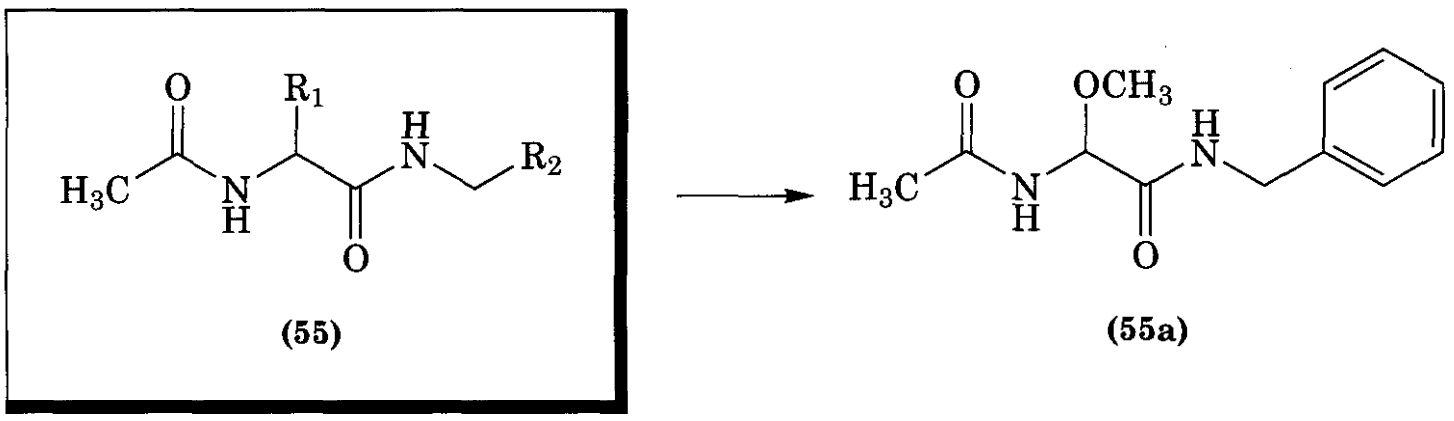


(54)

compound is currently in Phase I clinical development (198). It possesses a broad spectrum of anticonvulsant activity as well as anxiolytic action. Its mechanism of action is by blockade of the voltage-activated Ca^{2+} channel in a dose-dependent manner. The Ca^{2+} channel subtype is currently unclear. AWD 131–138 is rapidly absorbed in rats and dogs and displays a high metabolic stability under in vitro human liver slices (228).

6.2 Harkoseride

Chemically, harkoseride is (*R*)-2-acetamido-N-benzyl-3-methoxypropionamide(**55a**), part of a class of functionalized amino acid deriva-



(55) (55a)

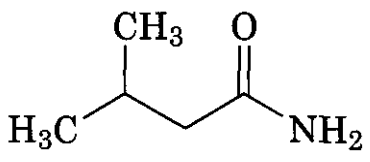
tives developed by Kohn and coworkers (236, 237) and Paruszewski (238,239). Harkoseride shows excellent anticonvulsant activity in several animal models, including two models of status epilepticus. It also provides neuroprotective effects in rat models of focal ischemia (198). It is currently undergoing phase II clinical evaluation. It was found to be rapidly and completely absorbed, was less than 1% plasma bound, and possessed a half-life of about 12 h. The drug is eliminated primarily by renal excretion and the metabolites have not been identified. Preliminary data indicate that harkoseride does not affect the blood levels of carbamazepine, phenytoin, or valproate (198).

6.3 LY 300164

LY 300164 was previously discussed (see Benzodiazepines, Section 5.4).

6.4 NPS 1776

Chemically, NPS 1776 is 3-methylbutanamide, or isovaleramide (**56**), a branched-



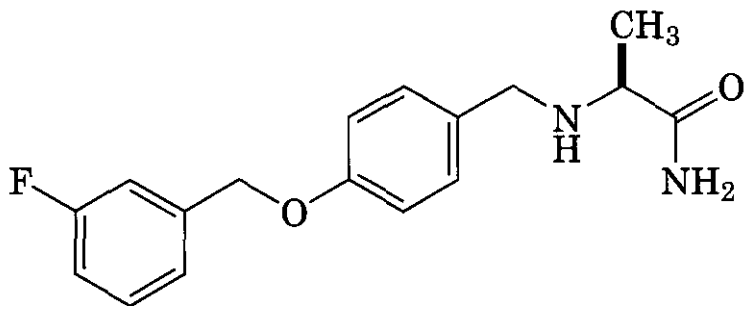
(56)

chained aliphatic amide that possesses a broad spectrum of activity similar to that of valproate (240). The mechanism of action is unknown; it was inactive in in *vitro* neurotransmitter binding or uptake assays (198). This suggests that its mechanism does not involve a direct receptor-mediated action. Being a small, neutral molecule, it is easily soluble in aqueous media and readily diffuses through biological membranes. It is thus rapidly absorbed and extensively distributed throughout body water. It is not bound to plasma proteins, but is extensively metabolized, with about 50% excreted in the urine of rats as the w (i.e., 4-hydroxy), and ω-1 (i.e., 3-hydroxy) oxidation products.

The drug has successfully completed Phase I clinical trials (198).

6.5 NW-1015

Chemically, NW-1015 is (*S*)(+)-2-[4-(3-fluorobenzyloxy)benzylamino]propanamide (57)

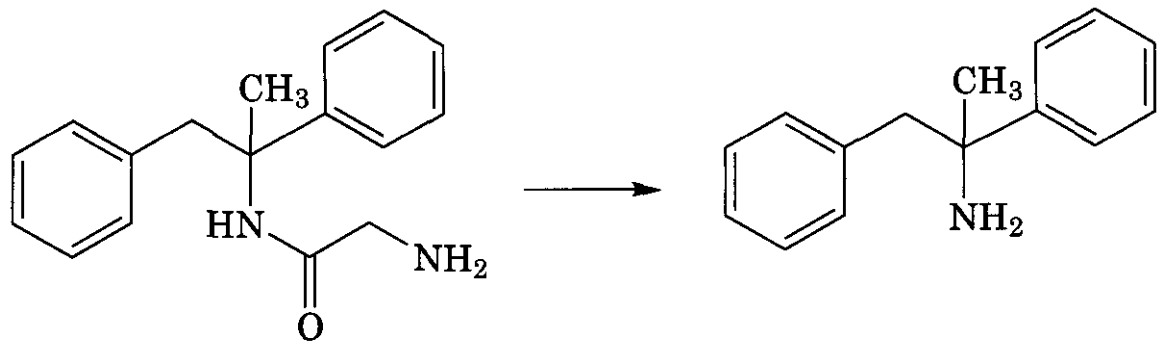


(57)

(241). The compound combines frequency and use-dependent blockade of Na^+ channels, Ca^{2+} channel modulation, inhibition of glutamate release, and monoamine oxidase B inhibition. A study in human volunteers was successfully completed. The findings of MAO-B inhibition at the dosages tested indicate the possibility of the potential use in Parkinson's disease.

6.6 Pregabalin (CI-1008)

This agent was previously discussed under gabapentin. As noted, pregabalin is the *S*-(+)-enantiomer of 3-aminomethyl-5-methylhex-



(58) (58a)

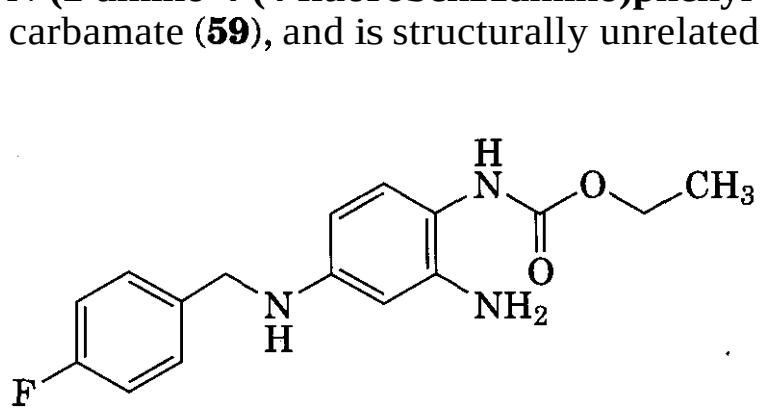
anoic acid (**36a**). The mechanism of action is unknown, but it is likely to differ from that of other anticonvulsants. Pregabalin does not appear to have any direct action at Na+ and Ca^{2}+ channels, and it does not seem to affect transmitter responses to glutamate, NMDA, or GABA. Additionally, it does not change neurotransmitter uptake (i.e., glutamate, GABA, monoamine, adenosine, cholinergic, or opiate receptors). Pregabalin, however, increases GABA content in neuronal tissues, binds to the $\alpha 2/\delta$ subunit of the Ca^{2+} channels, and enhances glutamic acid decarboxylase activity (185). Pregabalin is not significantly metabolized in humans. Studies in healthy volunteers indicate the drug has a 90% oral bioavailability and is not bound to plasma proteins. In clinical studies, pregabalin provided positive results against partial seizures. Additional studies are planned to evaluate its use as monotherapy and in pediatric patients (198).

6.7 Remacemide

Remacemide, which chemically is (±)-2-amino-*N*-(1-methyl-1,2-diphenylethyl)acetamide (**58**), and its principal active desglycinyl metabolite (**58a**), are low-affinity, noncompetitive NMDA receptor blockers and Na+ fast-channel blockers (242). Remacemide is rapidly absorbed on oral administration and achieves a peak plasma level in 1 h, whereas the active metabolite (**58a**) takes 2–3 h. The parent has a half-life of 3–4 h, compared to 12–15 h for the active metabolite (243). Comedication with enzyme-inducing anticonvulsants (i.e., phenytoin, carbamazepine, and phenobarbital) induces the metabolism of both remacemide and (**58a**), thus reducing their plasma concentration. The agent has been studied for its anticonvulsant effect, and because of its neuroprotective potential, trials have also been conducted for other indications, including Parkinson's disease and Huntington's disease. A phase III study in a monotherapy trial with carbamazepine, however, indicated that the efficacy of remacemide was inferior to that of carbamazepine (198).

6.8 Retigabine (D-23129)

Chemically, retigabine (D-23129) is ethyl **N-(2-amino-4-(4-fluorobenz1amino)phenyl-**carbamate (**59**), and is structurally unrelated

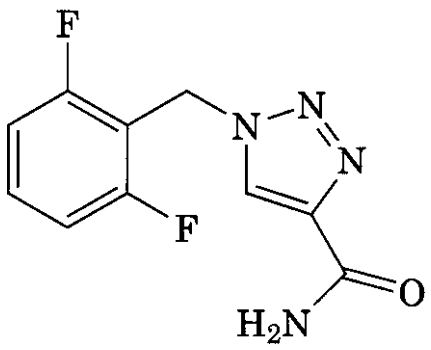


(59)

to currently marketed anticonvulsant agents. It shows a unique mode of action by increasing K^{+} conductance in neuronal cells (198). Phase I and II studies have shown good tolerability and efficacy trials are ongoing.

6.9 Rufinamide (CGP 33101)

Chemically, rufinamide (CGP 33101) is 1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazolo-4-carboxamide (**60**), which interacts with the inactivated state of the Na+ channel, limiting high frequency firing of action potentials in neurons. It does not significantly interact with the following neurotransmitter systems: GABA; adenosine; NMDA; monoaminergic; cholinergic binding sites; and other excitatory amino acid binding sites (244). Based on the broad-spectrum preclinical profile, favorable clinical

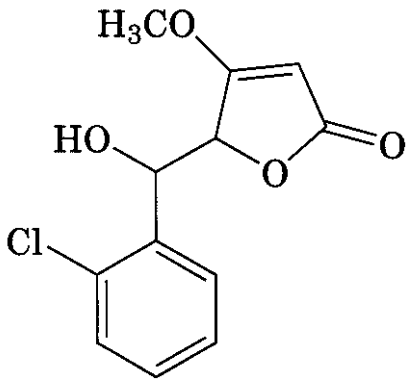


(60)

pharmacological characteristics, and efficacy and safety results from early clinical trials, phase III development procedures are being undertaken (198).

6.10 Losigamone (AO-33)

Chemically, losigamone (AO-33) is (±)-5(*R*,*S*), α-(*S*,*R*)-5-[(2-chlorophenyl)hydroxymethyl)]-4-methoxy(5*H*)-furanone (**61**), and belongs to

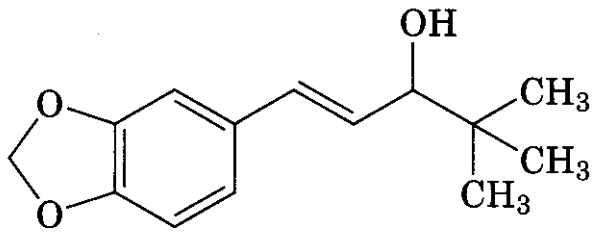


(61)

the group of β-methoxy-butenolides, which is found in a large number of natural products [e.g., the piperolides obtained from ***Piper sanctum*** (244)]. Losigamone exists as a racemic mixture of two ***threo*** enantiomers. There is evidence that the two isomers differ in anticonvulsant activity; the (+)-isomer (AO-242) is more potent than the (−)-isomer (AO-294), but the reverse may be true depending on the animal model (226). The toxicity profiles, however, are identical (244). The mechanism of action of losigamone is unclear at present. The agent is rapidly absorbed, with peak plasma concentrations occurring 2–3 h after an oral dose. It is bound to plasma proteins to the extent of 60%, and has a half-life of 4 h. Although the preceding data refer to the racemate, there are data that an enantioselective difference exist in the pharmacokinetics of the drug. When the individual isomers are given separately, the apparent oral clearance of the (−)-enantiomer is >10 times that of the (+)-enantiomer. Losigamone is eliminated primarily by oxidation. Biotransformation is stereoselective, with the (−)-enantiomer undergoing greater first-pass metabolism compared to that of the other isomer. It has undergone one clinical trial with no serious adverse events reported (226).

6.11 Stiripentol

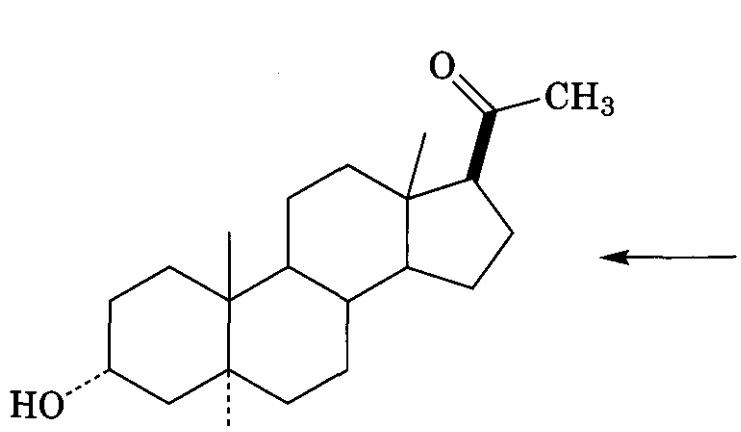
Chemically, stiripentol is 4,4-dimethyl-1-[(3,4-methylenedioxy)phenyl]-1-penten-3-ol (62). This agent is limited by its extensive metabolism (245). Phase II trials in Europe have demonstrated its efficacy in hard-to-treat partial epilepsies. Its effectiveness in partial seizures, however, is lower than that of the currently available agents (246).



(62)

6.12 Canaxolone (CCD 1042)

This steroid is a member of a novel class of neuroactive steroids, termed epalons, that allosterically modulate the $GABA_A$ receptor complex through a unique recognition site (226). This compound was developed after observations that endogengenously occurring metabolites of progesterone (**63a**) had significant anticonvulsant effects in animals. Although chemically related to progesterone, ganaxolone possesses no hormonal activity. The agent was successful in phase I and phase II studies in refractor infantile spasms. The safety and tolerability was generally good. Because of extensive first-pass metabolism, the development of a suppository dosage form is underway.



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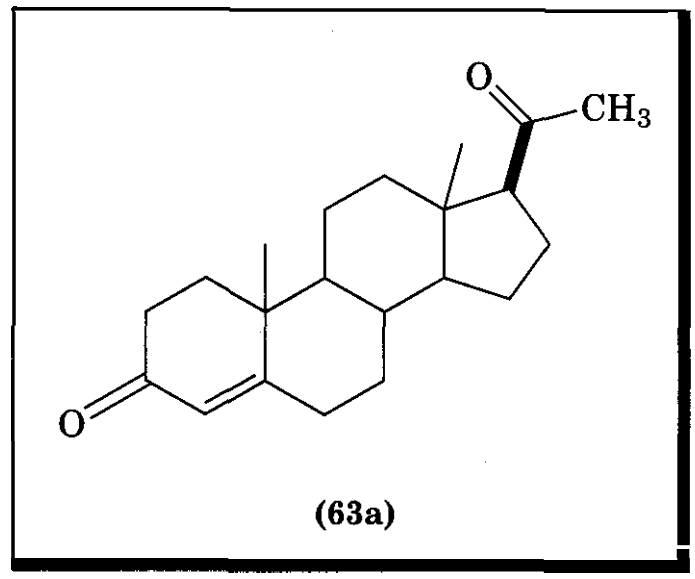
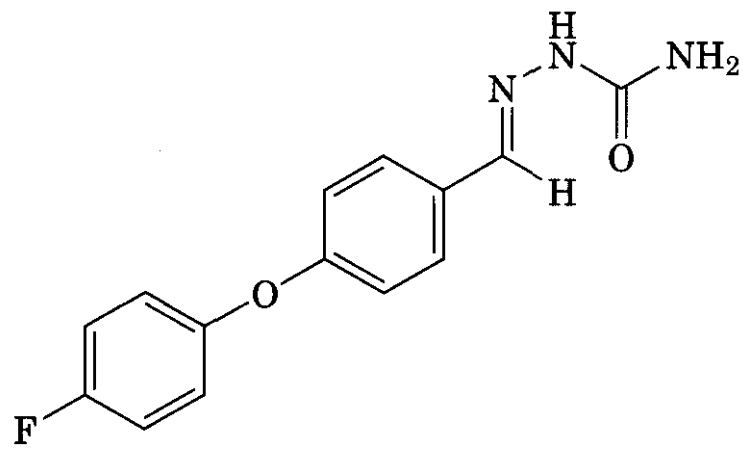


(63a)

6.13 Soretolide (D 2916)

Chemically, soretolide (D 2916) is *N*-(5-methyl-3-isoxazolyl)-2,6-dimethylbenzamide (**64**), a compound similar to carbamazepine in its activity profile (**229**). It was noted that the active hydroxymethyl metabolite, (**64a**), was formed preferentially in female rats; however, it is uncertain whether this species-specific effect is noted in humans (247). It is currently undergoing a multicenter study in refractory partial epilepsy (229).



(65)

7 THINGS TO COME

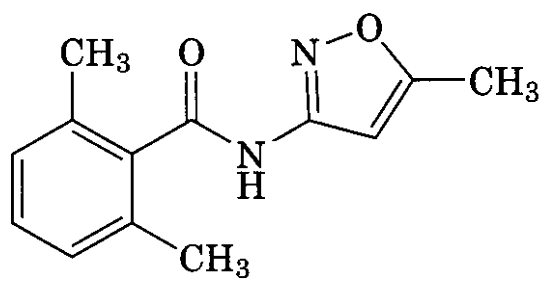
7.1 (Aryloxy)Aryl Semicarbazones

Dimmock et al. have prepared an extensive series of semicarbazones (248–261). The lead compound among the (aryloxy)aryl semicarbazones is 4-(4′-fluorophenoxy)benzaldehyde semicarbazone (**65**). Preclinical evaluations have been completed and an IND has been filed. The compound is a potent sodium channel blocker and it is planned to be developed for the treatment of neuropathic pain. Phase I clinical trials are scheduled in the near future.

Of further interest was the model Dimmock employed in determining the structure-activity relationship among the compounds in this series. This model is shown in Fig. 6.10 (251, 253).

7.2 AMP397A

Evolved from a series of *N*-phosphonoalkyl-5-aminomethylquinoxaline-2,3-diones (**262**), AMP397A (66) has emerged. This compound is an orally active, potent competitive AMPA receptor antagonist active in a broad spectrum of anticonvulsant tests. AMP397A combines a



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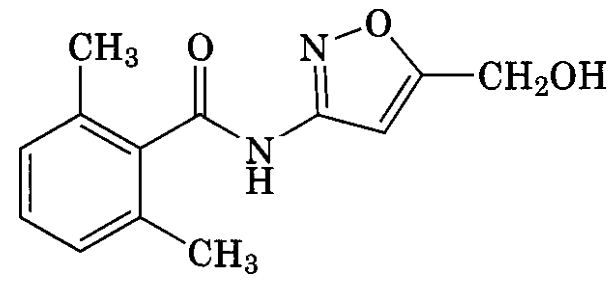


(64a)

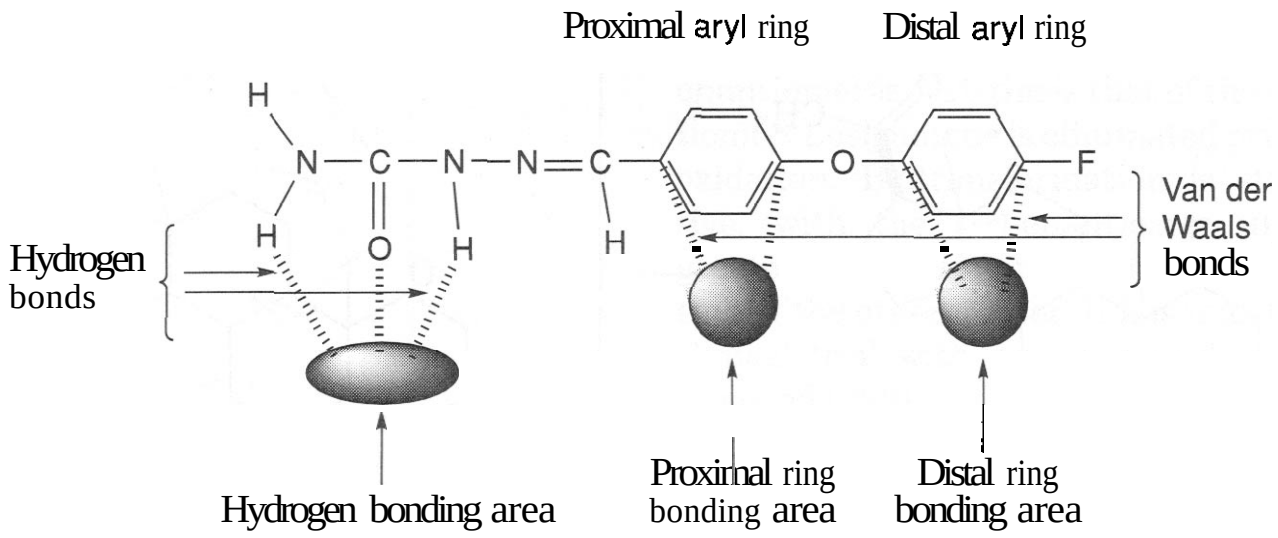
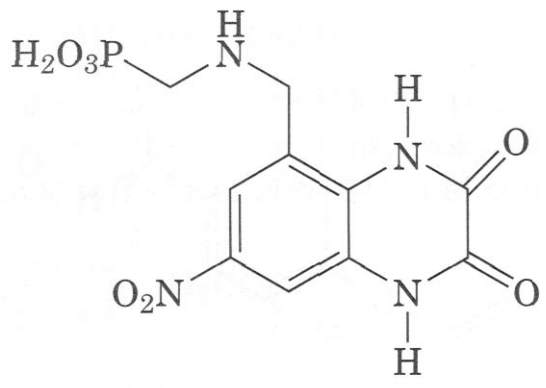


Figure 6.10. Postulated interaction of compound (65) at a binding site. (After Ref. 251.)



(66)

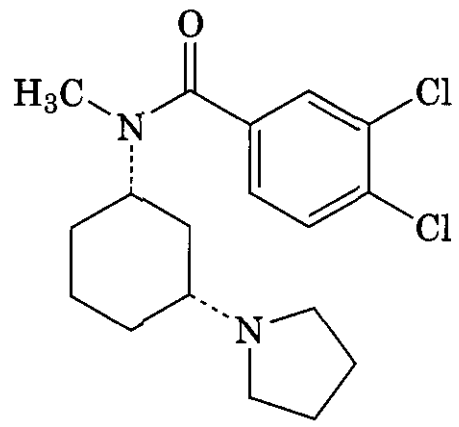
high affinity for native human AMPA receptors (IC_{50} = 11 n*M*) with moderate affinity for the competitive site of NMDA receptors (IC_{50} = 420 n*M*). The NMDA component does not contribute significantly to its antiepileptic properties. In addition to its broad anticonvulsant spectrum (MES, pentylenetetrazol, strychnine, and picrotoxin), it strongly decreases burst activities in genetically epilepsy-prone rats with absence-type seizures, suppresses kindling development, and decreases the severity of behavioral syndromes in kindled rats. As a result of preclinical results, it is expected to be active in patients with partial, generalized tonic-clonic, and myoclonic/absence type seizures (198).

7.3 U-594494A

U-594494A, chemically (±)-*cis*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-benzamide (**67**), is a potent, long-acting anticonvulsant without sedative or analgesic effects (263). It is not only effective in antagonism of electroshock seizures, but also effective against excitatory amino acids and Ca^{2+}-induced seizures (264). The drug is structurally related to a κ-opioid agonist, although it shows no binding affinity to this receptor. Its primary effect is with sodium channels; it blocks N1E-115 mouse neuroblastoma cells in a voltage- and use-dependent manner (265). The compound was found to be a long-acting anticonvulsant, but its brain levels could not account for its extended time course (266). It was found that two active metabolites, *cis*-*N*-(2-aminocyclohexyl)-3,4-dichlorobenzamide (**67a**) and cis-N-(2-methylaminocyclohexyl)-3,4-dichlorobenzamide (**67b**) were formed, each of which was an active anticonvulsant (263). The individual enantiomers were recently evaluated and the (−)-isomer was metabolized to a lesser extent than the (+)-isomer, which had a lower oral bioavailability as well (267). Fischer et al. independently evaluated U-54494A (268). It was observed in this study that there was considerable evidence to suggest that the stimulation of κ-receptors reduces the entry of Ca^{2+} into neurons or nerve terminals, which may be related to the closure of N-type Ca^{2+} channels. This action can result in a decrease of neuronal excitability and a reduction of transmitter release (269–271). Fischer concluded that it was thus difficult to draw definitive conclusions regarding the involvement of central κ-opioid receptor mechanisms in the anticonvulsant actions of U-54494A (268).

7.4 SB-204269

Chemically, (+)-(3*R*,4*S*) trans-4-(fluorobenzamido)-6-acetyl-3,4-dihydro-2*H*-benzo[*b*]



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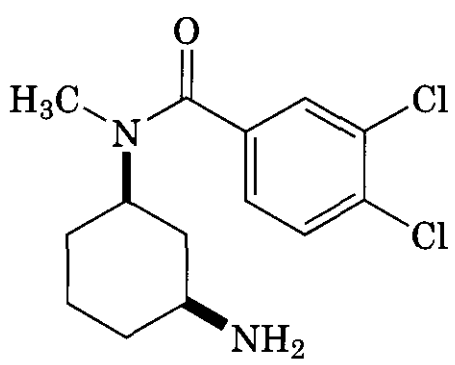
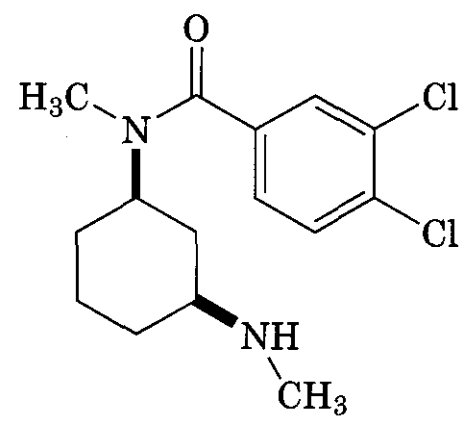


(67a)



(67b)

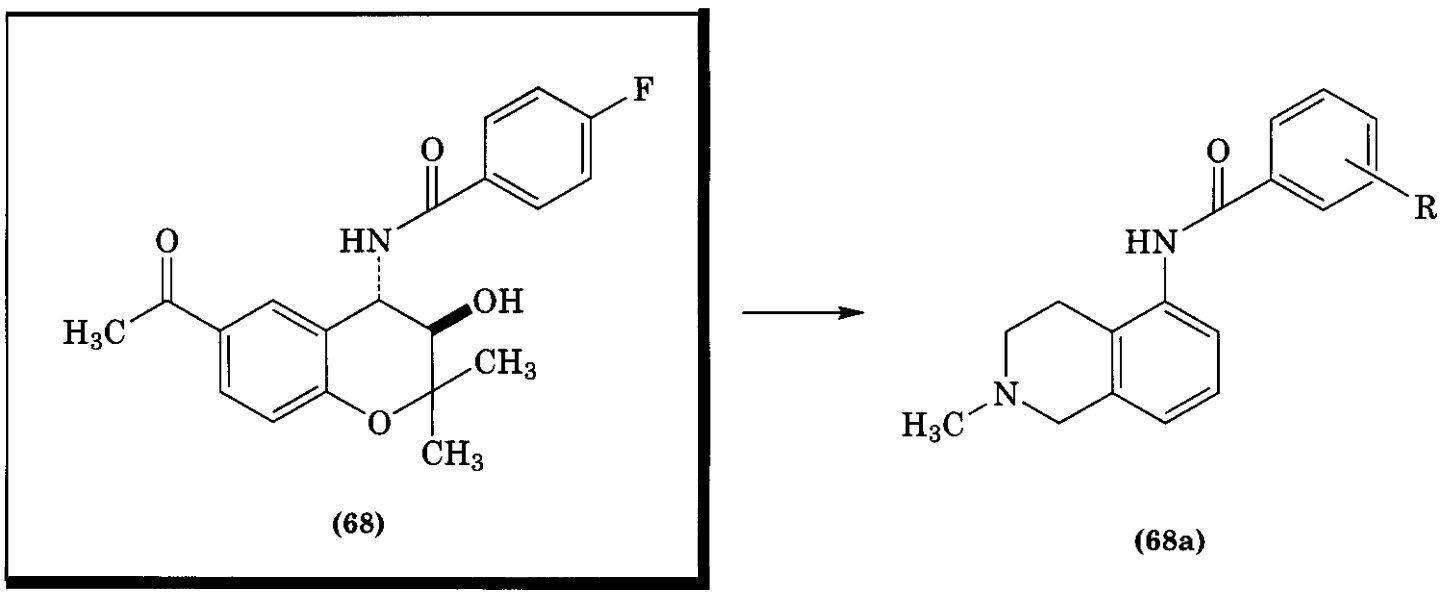
pyran-3-ol (**68**), SB-204269 showed good anticonvulsant activity in the MES evaluation and is currently undergoing clinical evaluation of epilepsy and has progressed to Phase II of clinical development (272–275). The stereochemistry was found to be necessary with the *trans*-4*S* configuration essential for activity. As with levetiracetam (**16**), this series were discovered with a unique [^{3}H]SB-204269 binding site assay (275). In a subsequent study, a series of alternative structural classes were prepared by high throughput screening of the SB compound library in the [^{3}H]SB-204269 binding assay and led to the following series of active 1,2,3,4-tetrahydroisoquinolinyl benzamides (**68a**) (276).

Data for the active compounds are presented in Table 6.13. As seen from the data, the structure-activity studies have led to a refinement of the original pharmacophore model (272). It should also be noted that these structures bear a close structural resemblance to (−)-levcromakalim (**69**), an antihypertensive ATP-sensitive potassium channel opener;

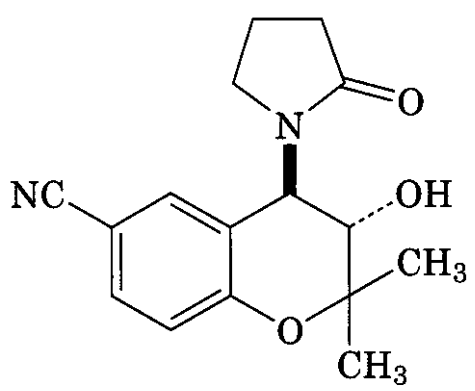
Table 6.13 Active 1,2,3,4-Tetrahydroisoquinolinyl Benzamides (68a) (276)

Compound	R	[^{3}H]SB-204269 Binding, pK_i	Mouse MES Data (% Increase in Seizure Threshold at 10 mg/kg po 1 h Postdose)
68a (1)	2-OCH_3, 4-OC_2H_5, 5-Cl	8.0	95*
68a (2)	3-Cl, 4-OCH_3	8.6	128**
68a (3)	3-Cl, 4-OC_2H_5	8.7	195**
68a (4)	3-Cl, 4-OC_3H_7-i	8.3	192**
68a (5)	3-Br, 4-OCH_3	8.8	163**

*$P < 0.01$; ** $P < 0.001$.



(68) (68a)

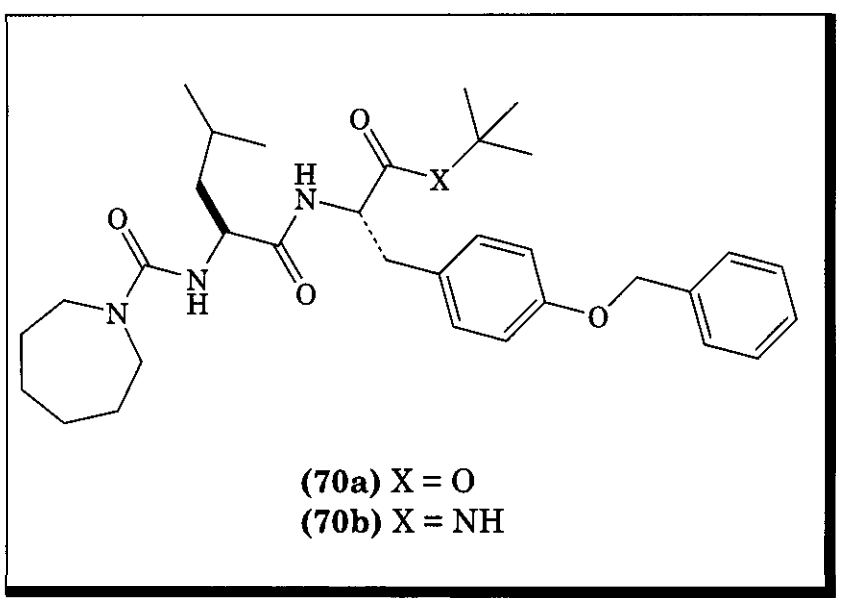


(69)

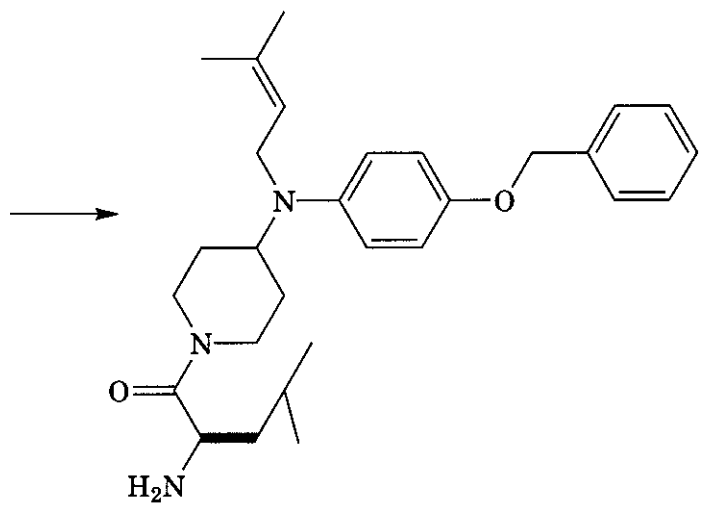
however, the 3*S*,4*R* stereochemistry abolished anticonvulsant activity (273).

7.5 N-Channel Ca^{2+} Blockers

Excessive calcium entry into depolarized neurons contributes significantly to neuronal injury. Voltage-sensitive calcium channels (VSCCs) regulate, among other functions, cellular excitability and neurosecretory activity, functions implicit in epileptogenic events (277). Wherease several anticonvulsant agents act by blocking T-type Ca^{2+} channels, Hu and coworkers investigated the N-type VSCCs (278,279). Based on a high throughput screening, PD 151307 (**70a**) was synthesized, which possessed significant N-type antagonistic activity (IC_{50} = 0.32 μM) in IMR-32 human neuroblastoma cells (279). Unfavorable physicochemical properties (i.e., high C log P and poor aqueous solubility) led to the synthesis of the amide (**70b**) (PD 167341), with similar Ca^{2+} blocking activity (IC_{50} = 0.59 μM), but with improved lipophilic and solubility properties. Modification of (**70b**) included: (1) plating the (4-*O*-benzyl)-phenyl group on the C-terminal end; (2) replacing the tert-butyl amide with a 3-methyl-2-butyenylamine to generate structure (71) (*S*)-2-amino-1-{4-[(4-benzyloxyphenyl)-(3-methylbut-2-enyl)-amino]-piperidine-1-yl}-4-methyl-pentan-1-one (280), a potent N-type Ca^{2+} antagonist



(70a) X = O
(70b) X = NH



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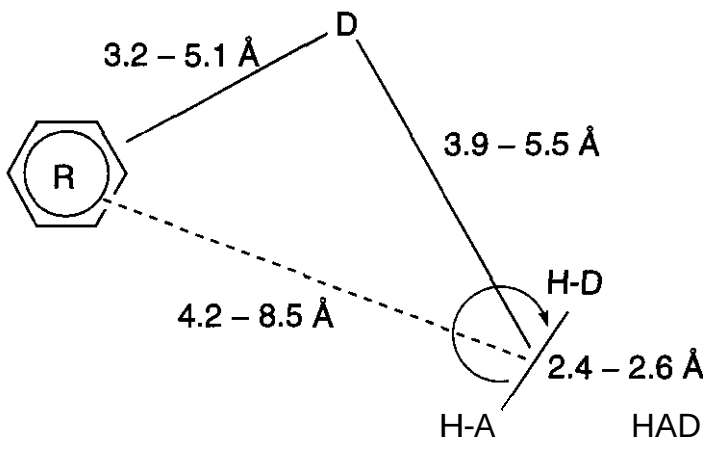

Figure 6.11. Suggested pharmacophore model for anticonvulsants acting at the voltage-dependent sodium channel on the basis of molecular dynamics simulations on phenytoin (**1**), carbamazepine (**2**), lamotrigine (**11**), zonisamide (**13**), and rufinamide (**60**). Remacemide (**58**) is discussed in the text. (After Ref. **281.**)

($IC_{50} = 0.67\ \mu M$), with anticonvulsant activity ($ED_{50} = 6$ mg/kg) in the audiogenic seizure model (8) as well as possessing significant analgesic activity.

7.6 Models of the Anticonvulsant Receptor

Several authors have provided insight into the putative MES receptor based on their structure-activity data. As noted by Unverferth et al. (281), there have been several attempts to postulate a general pharmacophore for the different anticonvulsant classes, all of which are anti-MES in animal studies and are, or have the potential to be, effective in generalized tonic-clonic seizures. These include: benzodiazepines (282); barbiturates (283); triazolines (284); semicarbazones (248–261); and enaminones (285–288), respectively; and for different compounds with similar anticonvulsant profiles (289–292). The Unverferth model (Fig. 6.11) provides an excellent representation of the current anticonvulsants phenytoin (**1**), carbamazepine (**2**), lamotrigine (**11**), zonisamide (**13**), and rufinamide (**60**). Remacemide (**58**) is also included as a possible candidate (Fig. 6.12).

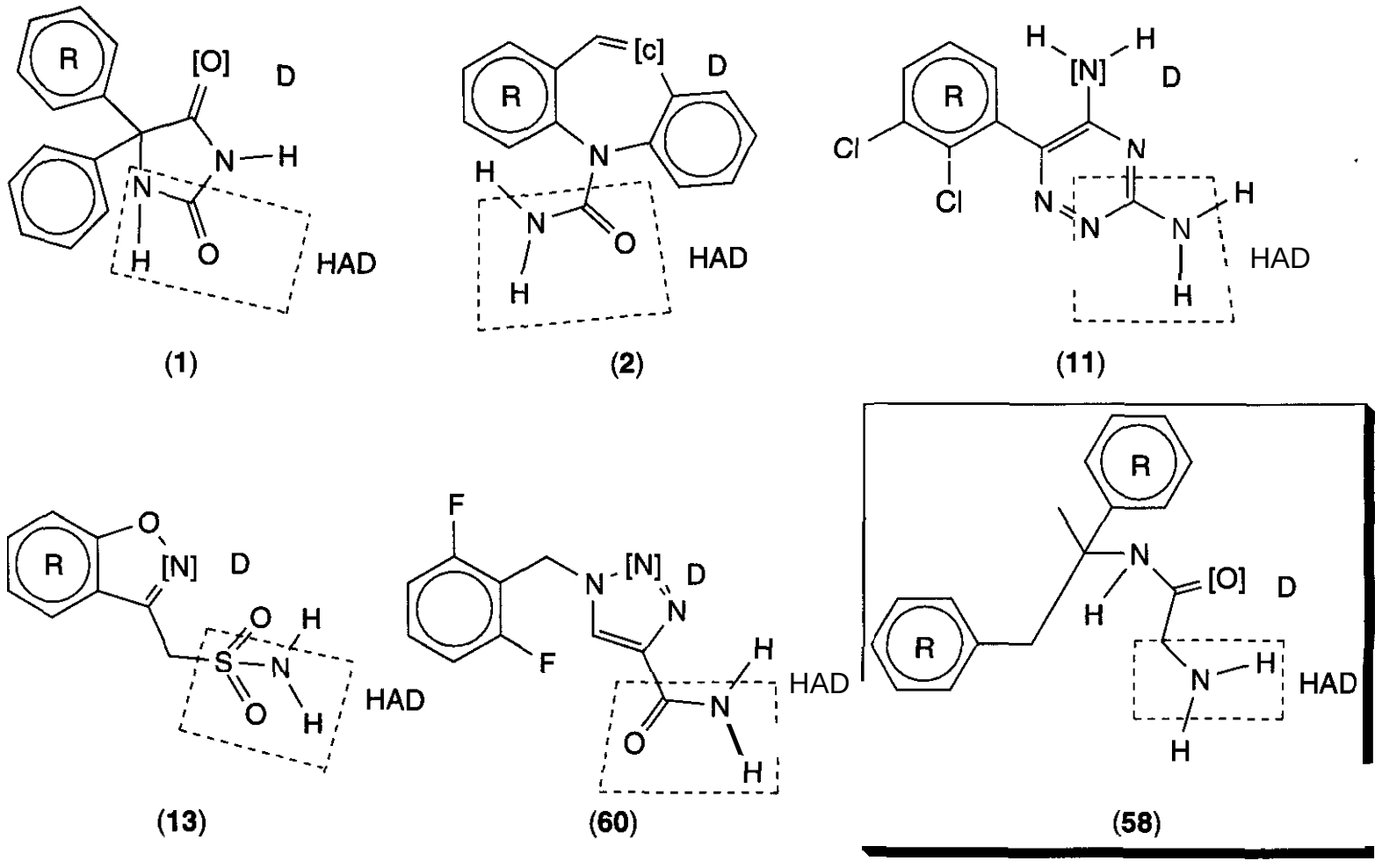

Figure 6.12. Selected anticonvulsants for the development of a pharmacophore model. The essential structural elements are indicated by dotted rectangles. **1** = phenytoin; **2** = carbamazepine; **11** = lamotrigine; **13** = zonisamide; **60** = rufinamide; inset, **58** = remacemide. R, hydrophobic unit; D, electron-donor group; HAD, hydrogen donor/acceptor unit. (After Ref. **281.**)

Features of commonality include: (*1*) at least one aryl ring (R); *(2)*one electron donor atom (D); and **(3)**a second donor atom in close proximity to the N-H group, forming a hydrogen bond acceptor/donor (HAD). In the structures listed in Fig. 6.12, the moieties are indicated with either a bracket or a dotted rectangle. Through molecular modeling techniques, each structure was minimized and superimposed (281). The deviation was <0.7 Å. In the case of remacemide (58), it contains the HAD function as the amide, but not at the correct position. Taking the carbonyl oxygen as the donor atom (D), the hydrogen-bond function would be represented by the amine function, which was different in the previous compounds. This model is not all inclusive and deviated from that proposed by Brouillette et al. (209, 293–295), in that the orientation of the aromatic ring was not in a specific conformation (the rings are rotated in relation to the R-D-HAD plane by 10–40"). Needless to say, research in this area is sorely needed. This problem is complicated by the fact that the three-dimensional structure of the sodium channel is unknown, and as stated by Madge et al. (75), it is likely to be many years before high resolution structures are available for these channels.

7.7 Porcine Embryonic GABAergic Cell Transplants

Historically, transplantation of fetal neurons and glia have been demonstrated to survive, integrate, and reduce functional deficits in animal models of Parkinson's disease and Huntington's disease (296, 297). In addition, surgical implants in humans have been successfully performed in pilot studies (298). An FDA-approved safety and feasibility study of transplantation of embryonic porcine lateral ganglionic eminence cells (NeuroCellTMFE, Diacrin, Inc.) into epileptogenic tissue in patients with surgically amenable temporal or frontal lobe onset seizures was initiated (198). Before transplantation, the cells were treated with anti-MHC I monoclonal antibodies, thus removing the need for treatment with the immunosuppressant cyclosporine. Preliminary evaluation in three patients with medically refractory partial-onset seizures have shown that the procedure is safe and well tolerated. More data in animal models with focal epilepsy are needed to determine long-term applicability (198).

7.8 A New Causative Agent for Epilepsy

A recent report indicated that cryptogenic epilepsy, the group of epilepsy syndromes for which an etiology is unknown, consisting of about 20% of all epilepsy syndromes, may be caused by *Toxoplasm* gondii (299). A statistically significant elevation of T. gondii antibodies was found compared to that of controls, suggesting that T. gondii infection with brain cysts may be a cause of the disease.

8 WEB SITE ADDRESSES AND RECOMMENDED READING FOR FURTHER INFORMATION

8.1 For Information on Anticonvulsant Evaluations

- NINDS ADD Program Webpage: http://www.ninds.nih.gov/asp.htm

8.2 For Information on Epilepsy

- Epilepsy Foundation of America Webpage: http://www.efa.org/
- Society for Neuroscience Webpage: http://www.sfn.org/
- American Epilepsy Society: http://www.aesnet.org/
- University of Pennsylvania: http://www.med.upenn.edu/health/hi_files/neurology/epilepsy/ep_seizures-.html

8.3 References on Animal Procedures

- The Neuronal Microenvironment (Neuromethods Series, Vol. 9), A. A. Boulton, G. B. Baker, and W. Walz. Humana Press, Totowa, NJ, 1988.
- Check the previous edition of this chapter also.

8.4 Sodium Channels

- The study reported by Madge et al. (75) is the most current on the topic.

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CHAPTER SEVEN

Narcotic Analgesics

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1 INTRODUCTION

Although treatment of mild to moderate pain can typically be accomplished with nonnarcotic analgesics such as acetaminophen or aspirin, treatment of severe pain often requires use of an opioid analgesic such as morphine. These drugs are associated with serious side effects, however, most notably addiction liability and respiratory depression, which limit their clinical usefulness. Therefore there has been an intensive effort to find new analgesics that retain the effectiveness of morphine, but do not have the undesired side effects. As a result, a wide variety of compounds with opioid activity have been identified and significant strides have been made in understanding the mechanisms of opioid action.

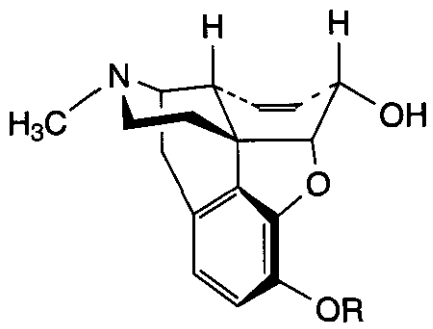
The effects of opium, from which morphine (1, Fig. 7.1) is isolated, have been known for thousands of years, but it is only within the twentieth century, and really within the last 25 years, that we have begun to understand the effects of opioid analgesics at a molecular level. Beckett and Casy in 1954 proposed that opiate effects were receptor mediated (1), but it was not until the early 1970s that the stereospecific binding of opiates to specific receptors was demonstrated in mammalian brain tissue (2–4). In the mid-1970s two other discoveries revolutionized our understanding of opioid analgesics and how they produce their effects. The characterization and classification of three different types of opioid receptors by Martin and coworkers in the mid-1970s (5, *6)* formed the foundation of our current understanding of opioid pharmacology. This discovery sparked renewed interest in attempting to separate analgesic activity from the undesired side effects associated with morphine and other opioid analgesics. At the same time the search for endogenous ligands for opioid receptors led to the discovery of peptides with opiate-like activity. The first peptides identified were the pentapeptides leucine and methionine enkephalin (3 and 4) (7), followed by the longer peptides dynorphin and β-endorphin. Because these peptides are structurally distinct from the alkaloid opiates, the term *opioid* was introduced to describe all compounds, both nonpeptide and peptide, with opiate-like activity. To understand the interactions of opioids with their receptors at a molecular level, knowledge of receptor structure was still needed. A major breakthough came when first the delta (δ) opioid receptors (8, 9), followed shortly thereafter by the mu (μ) (10) and kappa (κ) (11) receptors, were sucessfully cloned in the early 1990s.

Leucine enkephalin (3) Tyr-Gly-Gly-Phe-Leu

Methionine enkephalin (4)

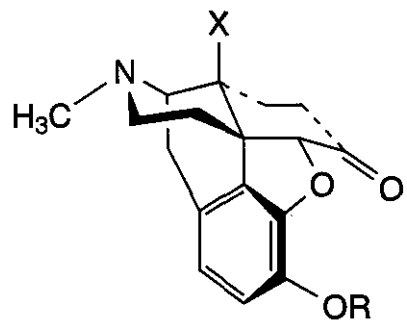
Tyr-Gly-Gly-Phe-Met

The focus of this chapter is on recent developments in the opioid field, with summaries of key features of the structure-activity relationships (SAR) of older compounds. Much of the early opioid SAR is discussed in detail in two comprehensive books on opioid analgesics published in 1986 (12, 13). Specific areas in which there has been considerable research in the last decade and which are discussed in this chapter include: the molecular biology of opioid receptors (see Section 3.2.4), the design



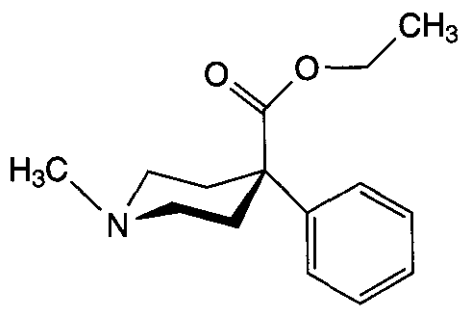
R = H (1) Morphine

R = CH_3 (2) Codeine

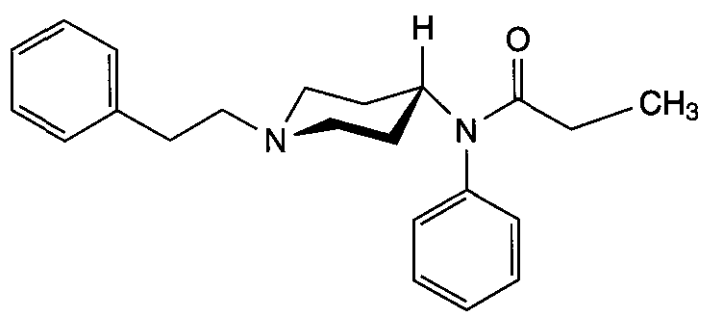


R = H, X = H (5) Hydromorphone

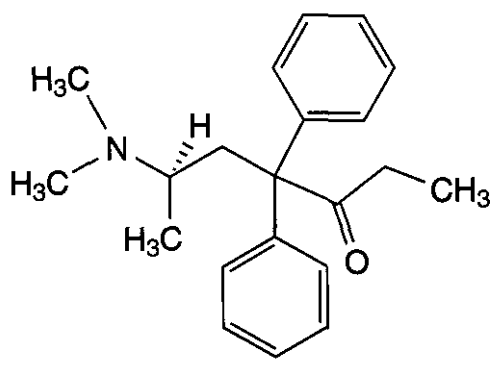
R = CH_3, X = OH (6) Oxycodone



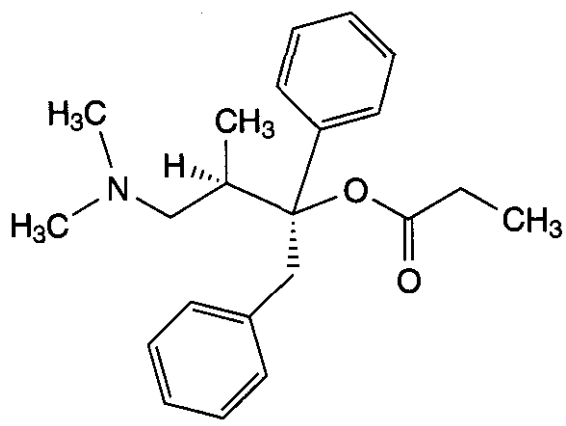
(7) Meperidine



(8) Fentanyl



(9) Methadone



(10) Propoxyphene

Figure 7.1. Structures of the most commonly used clinical agents.

and synthesis of agonists and antagonists selective for the different receptor types (Sections 5.3, 5.9, and 5.10), the design and synthesis of opioid peptide analogs (Section 6), and the recent characterization of the related opioid-receptor like (ORL1) receptor and its endogenous ligand orphanin FQ/nociceptin (Section 7.2). Before describing the different classes of nonpeptide analgesics and opioid peptides, the clinical use of analgesic agents (Section 2), opioid receptors, and the methods used to characterize the opioid activity of compounds (Section 3) are discussed.

2 CLINICAL USE OF AGENTS

Opioid analgesics are indispensable drugs in the management of cancer pain (14), and the American Academy of Pain Medicine and the American Pain Society also advocate the prudent use of narcotic analgesics for the treatment of chronic pain (15, 16). The widespread use of opioids in chronic, nonmalignant pain, however, is still somewhat controversial because of the lack of substantial evidence from long-term controlled studies demonstrating effectiveness in this setting (17). The clinical use of opioids in different types of pain and in different clinical settings has been reviewed in detail in a recent book (18).

The World Health Organization (WHO) introduced a three-tiered approach for the treatment of cancer pain (19) that also serves as a model for the management of acute and chronic pain. In this model, the first tier consists of acetaminophen (APAP) or a nonsteroi-

dal anti-inflammatory agent [e.g., aspirin (ASA) or ibuprofen] for mild to moderate pain. If the pain persists or increases, then treatment progresses to the second tier, where a narcotic analgesic is added to the regimen. Frequently, this is accomplished by use of a combination product such as ASA plus codeine that combines an opioid with a nonnarcotic analgesic. The third tier is reached when the pain escalates from moderate to severe. At this level the opioid may be used as a single agent, given that opioids do not have a ceiling to their analgesic effect as do the nonnarcotic analgesics such as ASA and APAP (20). Adjuvant drugs, such as tricyclic antidepressants or anticonvulsants, may be added to opioid therapy as a means to enhance the efficacy of opioids for pain relief (17, 21).

For continuous pain, analgesic agents are generally prescribed for use on a regular, around-the-clock basis by use of a long-acting analog. For acute pain or pain after surgery, often an immediate-release, short-acting opioid is used. In addition, short-acting opioids with rapid onset are used for "rescue" doses when breakthrough pain is problematic (21, 22).

2.1 Current Drugs on the Market

The structures for the most commonly used clinical agents are shown in Fig. 7.1. Some of the opioid agonists used clinically (Table 7.1) such as morphine may be used as the sole agent for analgesia. Because of their rapid onset and short duration of action, fentanyl and other 4-anilidopiperidines have been used extensively as adjuncts to anesthesia, whereas methadone and its analog levomethadyl acetate (LAAM) are used as maintenance agents for individuals who are addicted to narcotics. Other agents such as loperamide or diphenoxylate are used primarily for their constipating side effect to treat diarrhea. Some drugs are used extensively in combination products (Table 7.2) for treatment of pain. Most of the clinically used agents are agonists at μ opioid receptors. In contrast, mixed agonists/antagonists generally interact with two distinct opioid receptors to provide analgesic activity while exhibiting decreased potential for serious side effects such as respiratory depression and addiction (see Section 2.2 below). Pentazocine is a prototype for the mixed agonist/antagonist class and acts as an agonist at κ opioid receptors generating analgesia (see Section 3.2); its antagonist activity at μ opioid receptors significantly decreases or eliminates the potential for respiratory depression and addiction liability generated through μ receptor activation. The mixed agonists/antagonists shown in Table 7.3 find limited clinical utility, despite the analgesia resulting from activating κ opioid receptors. The analgesic effect produced reaches a maximum despite increased drug dose (analgesic ceiling). Further, these drugs exhibit a different side-effect profile, including dysphoria and hallucinations, which also appears to be a result of κ-agonist activity (23).

Opioid agonists also have an antitussive effect attributed to the depression of the cough reflex. Thus some opioids, typically codeine or one of its derivatives, are used for their antitussive activity, predominantly in combination products. The antitussive effect is in part the result of the interaction with opioid receptors at the cough center in the brain (23). The dose required for antitussive activity, however, is lower than that required for analgesia; the opioid receptors involved in blocking the cough reflex are less sensitive to naloxone than those responsible for analgesia (23).

Opioid antagonists (Table 7.4), predominantly naloxone, are used clinically to reverse the effects of opiates in overdose or postoperative sedation. Naltrexone, which has oral bioavailability, is used for the treatment of narcotic addiction and alcohol dependence. As discussed below (Section 2.2.2.1), peripherally selective antagonists are being evaluated for treatment of constipation and other gastrointestinal side effects associated with opioid agonist use.

2.2 Side Effects, Adverse Effects, Drug Interactions/Contraindications

In addition to analgesia, clinically used opioids display a plethera of biological effects. The most common side/adverse effects involve alterations of the nervous, respiratory, gastrointestional, and integument systems (see Refs. 23, 25 and references cited therein for more detailed discussions). The most serious side effects associated with the majority of opioid

Table 7.1 Opioid Agonists Used Clinically"

Chemical Class	Generic Name	Trade Name (manufacturer)	Route of Administration	Equal Aanalgesic Dose (mg)[b] i.m.	Equal Aanalgesic Dose (mg)[b] p.o.
4,5β-Epoxymorphinans	Morphine (1)	Available as generic	p.o., i.v., i.m., s.c., rectal	10	60
	Codeine (2)	Available as generic	p.o., i.v., i.m., s.c.	120	200
	Hydromorphone (5)	Dilaudid (Knoll), also available as generic	p.o., i.v., i.m., s.c., rectal	1.5	7.5
	Hydrocodone[c]				
	Oxymorphone	Numorphan (Endo Laboratories)	i.v., i.m., s.c., rectal	1	10[d]
	Oxycodone (6)	OxyContin (Purdue Pharma LP), also available as generic	p.o.		30
Morphinans	Levorphanol	Levo-Dromoran (ICN), also available as generic	p.o.	2	4
Phenylpiperidines	Meperidine (7)	Demerol (Sanofi-Synthelabo) also available as generic	p.o., i.v., i.m., s.c.	75	300
	Diphenoxylate[e]	Lomotil (Searle), also available as generic	p.o.		
	Difenoxin[f]	Motofen (Carnrick)	p.o.		
	Loperamide[g]	Imodium A-D (McNeil-CPC), also available as generic	p.o.		
4-Anilidopiperidines	Fentanyl (8)	Sublimaze (Taylor)	i.v.	0.1[h]	
		Fentanyl Oralet (Abbott)	Lozenges		
		Actiq (Abbott)	Lozenges on a stick		
		Duragesic (Janssen)	Transdermal patches		
	Alfentanil	Alfenta (Taylor)	i.v. with individualized dosing		
	Remifentanil	Ultiva (Abbott)	i.v. with individualized dosing		
	Sufentanil	Sulfentanil citrate (ESI Lederle), Sufenta (Taylor)	i.v. with individualized dosing	0.02[h]	
Acyclic analgesics	Methadone (9)	Available as generic	i.m., s.c., p.o. with individualized dosing	10	20
	Levomethadyl acetate	Orlaam (Roxane)	p.o. with individualized dosing		
	Propoxyphene (10)	Darvon-N (napsylate) (Eli Lilly), available as generic (HCl)	p.o.		130–200[i]

"Agents currently marketed in the United States. Unless otherwise noted these drugs are Class-I1 controlled substances. From Ref. 24.

[b]Based on short-term use for acute pain.

"Used only as an antitussive product.

[d]Rectal administration.

'Class V narcotic available only by prescription for treatment of diarrhea.

[f]Class IV narcotic available only by prescription for treatment of diarrhea.

[g]A noncontrolled substance available both by prescription and over the counter for treatment of diarrhea.

[h]i.v. dose.

[i]Dose of 130 mg for the HCl salt and 200 mg for the napsylate salt.

Table 7.2 Examples of Narcotic Agonists Currently Marketed as Oral Combination Products for Pain[a,b]

Trade or Common Name (manufacturer)	Narcotic Component	Nonnarcotic Component
APAP with codeine (generic)	Codeine	APAP
ASA with codeine (generic)	Codeine	ASA
DHC Plus (Purdue Frederick)	Dihydrocodeine	APAP, caffeine[c]
Synalgos-DC (Wyeth-Ayerst)	Dihydrocodeine	ASA, caffeine
Vicodin (Knoll)	Hydrocodone	APAP
APAP with hydrocodone (generic)		
Alor 51500 (Atley)	Hydrocodone	ASA
Vicoprofen (Knoll)	Hydrocodone	Ibuprofen
Percocet (DuPont)	Oxycodone	APAP
N A P with oxycodone (generic)		
Percodan (Du Pont)	Oxycodone	ASA
ASA with oxycodone (generic)		"
Mepergan Fortis (Wyeth-Ayerst)	Meperidine	Promethazine[d]
Darvocet-N (Eli Lilly)	Propoxyphene sapsylate	APAP
APAP with propoxyphene (generic)		"
Wygesic (Wyeth-Ayerst)	Propoxyphene HCl	APAP
APAP with propoxyphene (generic)	'	
Darvon	Propoxyphene HCl	ASA, caffeine[c]

"Products currently marketed in the United States. From Ref. 24.
[b]Codeine, hydrocodone, and hydromorphone are used in combination products as antitussives.
"May be beneficial in vascular headaches.
[d]Used for sedative effect.

analgesics are respiratory depression, addiction liability, and constipation that are associated with their agonist activity at μ opioid receptors.

2.2.1 Central Side Effects

2.2.1.1 Respiratory Depression. Mu opioids used for analgesia slow breathing, resulting in one of the most serious adverse effects **(22).** Respiratory depression is caused at least in part by interaction of opioids with the respiratory center in the brain stem, causing a decreased response to carbon dioxide and thus depression of breathing rate (23). Respiratory depression can occur at doses far lower than those that affect consciousness and increases

Table 7.3 Narcotic Agonist–Antagonists[a]

Chemical Class	Generic Name	Trade Name (manufacturer)	Controlled Substance Class	Route of Administration	Equivalent Dose (mg)[b]
6,14-*Endo*etheno opiates	Buprenorphine	Buprenex (Reckitt & Colman)	C-V	i.v., i.m.	0.3
4,5α-Epoxy-morphinans	Nalbuphine	Nubain (DuPont)	NA[c]	i.v., i.m., s.c.	10
Morphinans	Butorphanol	Stadol (Mead Johnson)	C-IV	i.v., i.m., nasal spray	2.5
Benzomorphans	Pentazocine[d]	Talwin (Sanofi Winthrop)	C-IV	i.v., i.m., s.c.	30
Aminotetralin	Dezocine	Dalgan (Astra)	NA[c]	i.v., i.m., s.c.	10

"Agents currently marketed in the United States. From Ref. 24.
[b]Parenteral dose equivalent to 10 mg morphine.
'Not applicable.
[d]Also available as oral combination products with ASA (Talwin Compound), APAP (Talacen), or naloxone (Talwin NX) (Sanofi Winthrop).

Table 7.4 Narcotic Antagonists[a]

Generic Name	Trade Name (manufacturer)	Route of Administration
Naloxone	Narcan (DuPont)	i.v., i.m., or s.c.
Naltrexone	ReVia (DuPont)	p.o.
	Depade (Mallinckrodt)	
Nalmefene	Revex (Ohmeda)	i.v., i.m., or s.c.

[a]Agents currently marketed in the United States. From Ref. 24.

progressively with increasing drug dose (23). Mortality from opioid overdose is almost always a result of respiratory arrest. Of important note is that the respiratory effects of sleep, which often accompanies pain relief, are additive with the depressant effects of the opioid analgesic on respiration (23). The most profound respiratory depression occurs within 5–10 min postintravenous (i.v.) administration of morphine, and this effect occurs more rapidly as the lipophilicity of the narcotic analgesic increases (23).

Opioid-naive patients with severe pain who require high doses of opioids are at highest risk for respiratory depression, whereas patients receiving chronic opioid therapy rarely experience this problem (20). Fortunately, the occurrence of respiratory depression can often be circumvented with appropriate titration of opioid dose (22) unless there is underlying pulmonary dysfunction such as emphysema or severe obesity (23).

2.2.1.2 Tolerance, Dependence, and Addiction Liability. Patients treated with long-term opioid therapy often develop tolerance and usually become physically dependent on narcotic analgesics as well. Tolerance results when exposure to a drug results in its decreased effectiveness with time and larger doses are required to achieve the same response (26). Physical dependence is also an adaptive state that is characterized by a specific constellation of withdrawal symptoms that occur upon abrupt cessation or significant reduction in the dose of the opioid or administration of an opioid antagonist (26). Addiction, however, is distinct from physical dependence, and "the term addiction should never be used when physical dependence is meant" (22). The American Academy of Pain Medicine, the American Pain Society, and the American Society of Addiction Medicine have written a consensus document that clearly outlines the recommended definitions for addiction, physical dependence, and tolerance related to the use of opioids for the treatment of pain (26). According to the consensus document definitions, addiction is "a primary, chronic, neurobiologic disease, with genetic,' psychosocial, and environmental factors influencing its development and manifestations. It is characterized by behaviors that include one or more or the following: impaired control over drug use, compulsive use, continued use despite harm, and craving." Physical dependence is defined as "a state of adaptation that is manifested by a drug class specific withdrawal syndrome that can be produced by abrupt cessation, rapid dose reduction, decreasing blood level of the drug, and/or administration of an antagonist" (26). There is, however, a very low addiction potential for opioids used for pain management (27), on the order of only 3 cases per 10,000 patients (see Ref. 22 and references cited therein). In the pain management patient it is important to distinguish between addiction and pseudoaddiction (22), which relate to the motivation for obtaining the narcotics. In pseudoaddiction uncontrolled pain is the motivating factor for the drug-seeking behavior that stops once relief is obtained (22). Pseudoaddiction may be attributed to tolerance or pseudotolerance where an increase in dosage needs results from other factors such as disease progression, a new disease process, or drug interactions (28).

The pharmacological mechanisms responsible for the euphoria and rewarding behavior associated with μ opioid analgesics and addiction liability remain uncertain (23). These effects are distinct from analgesia (29). Considerable evidence suggests that the rewarding effects result from interaction of the opioid with dopaminergic pathways, particularly in the nucleus accumbens (23). Activation of μ or δ opioid receptors results in the release of dopamine, which results in the motivational effect (30). In contrast, agonists interacting with κ opioid receptors, naloxone, and μ-selec-

tive antagonists inhibit dopamine release (31), producing aversion rather than motivation (32).

2.2.1.3 Sedation and Cognitive Impairment. The initiation or dose escalation of narcotic analgesics may cause drowsiness and impair cognitive function. Tolerance usually develops fairly quickly to these side effects, but other medications that induce somnolence will produce an additive effect when taken concomitantly. If sedation remains problematic, in order to achieve adequate analgesia a psychostimulant such as caffeine, dexamphetamine, or methylphenidate may be added to counteract the side effect (22).

2.2.1.4 Nausea and Vomiting. The most bothersome and unpleasant side effects for patients receiving opioids for pain are often the nausea and vomiting that have been associated with all clinically used μ agonists. Emesis predominantly results from direct stimulation of the chemoreceptor trigger zone, yet the degree of effect depends on the individual (23). Nausea and vomiting related to narcotic analgesics occur in 10–40% of patients (33). Tolerance often develops to these side effects, however, and they often vanish with long-term use (22). Nausea and vomiting can be treated by use of a variety of drugs such as transdermal scopolamine, hydroxyzine, or a phenothiazine for movement-induced nausea (20), or metoclopramide or cisapride for patients with nausea and vomiting stemming from delayed gastric emptying. If the nausea and vomiting persist, steroid therapy with dexamethasone may be initiated or a 5-HT, antagonist such as ondansetron may be used (22). Another alternative is to try a different opioid, given that there is significant individual variability in this side effect (20).

2.2.2 Other Side Effects

2.2.2.1 Constipation. The most common side effect of long-term narcotic analgesia is constipation plus other gastrointestinal (GI) effects collectively referred to as opioid bowel dysfunction. The frequency of these side effects is very high [40–50% or more in patients receiving opioids (34–36)] and can become the limiting factor in opioid use. These effects are mediated predominantly by μ receptors in the bowel (23). The effects begin with delayed food digestion in the small intestine and decrease in peristaltic waves in the large intestine, resulting in the retention of bowel contents. This is compounded by the enhanced tone of the anal sphincter and the reduction of the reflex relaxation in response to rectal distension. Tolerance does not usually develop to this side effect, and the patient on long-term opioid therapy remains chronically constipated.

Patients are generally started prophylactically on a regimen including a laxative such as bisacodyl or senna that increases bowel motility plus a stool softener like docusate (20, 22). In patients refractory to laxatives, oral naloxone (22) has been successfully used as a therapeutic alternative for constipation without loss of analgesia (37). Because of its central activity, however, higher doses of naloxone can decrease the analgesic effectiveness of the opiate and precipitate opioid withdrawal in some patients (37). Peripherally selective antagonists offer the advantage of reversing the gastrointestinal and other peripheral side effects of narcotic analgesics without the potential for decreasing their central analgesic activity. Two peripherally selective antagonists, the quaternary derivative of naltrexone N-methylnaltrexone bromide (methylnaltrexone, see Section 5.3.1) and the phenylpiperidine alvimopan (ADL 8–2698, LY246736; see Section 5.7.1), are undergoing clinical trials for opioid-induced constipation (34–36, 38). After both i.v. and oral administration methylnaltrexone reverses the opioid-induced delay in GI transit (34–36) and is effective in individuals receiving chronic opioid treatment (methadone users) as well as in healthy volunteers (34, 36). In clinical trials oral alvimopan reverses the delay in GI transit after the administration of exogenous opioids to both opioid naive individuals and patients receiving chronic opioid treatment (both pain patients and individuals taking methadone for opioid addiction) (38, 39); in addition it has been shown to speed the recovery of bowel function after abdominal surgery (40).

The constipating effect of orally administered opiates can be used for the treatment of diarrhea, as with camphorated tincture of opium (Paregoric or Parepectolin, which is a paragoric plus kaolin as an adsorbent and pec-

tin as a demulcent) (24). Two phenylpiperidine derivatives are used solely as antidiarrheal agents. Diphenoxylate, which is a congener of meperidine, is available only in combination with atropine, which has antispasmodic activity in the intestine. At therapeutic doses diphenoxylate does not show any central nervous system (CNS) effects, but at high doses it displays the typical opioid profile including euphoria. The carboxylic acid metabolite, difenoxylic acid (Motofen; Table 7.1) has activity similar to that of the parent (23). Unlike diphenoxylate, the second opioid used for diarrhea, loperamide, does not exhibit pleasurable CNS effects even at large doses (23); loperamide is a substrate for P-glycoprotein in the blood-brain barrier, which excludes this drug from the CNS (41, 42).

2.2.2.2 Itching. After administration of opioids there may be urticaria at the injection site or generalized itching because of degranulation of mast cells, resulting in histamine release. The itching is a common side effect and often one that results in severe patient distress (23). The histamine release may also be partially responsible for the pruritus and sweating after drug administration as well as flushing resulting from blood vessel dilation of the skin (23). Antihistamines may be used to combat the discomfort (20) or patients can be switched to either fentanyl or oxymorphone (43), which do not tend to cause histamine release. Opioid antagonists such as naloxone are effective in controlling the pruritus and can be used at low doses without loss of pain control (34, 44); the peripherally selective antagonist methylnaltrexone exhibits antipruritic efficacy without the potential to reverse morphine analgesia (45).

2.2.3 Contraindications. Contraindications include hypersensitivity to opioids, head trauma or increased intracranial pressure, severe respiratory depression or compromised respiratory function, and potentially, liver or renal insufficiency (46). Whether morphine or other opioids are used depends on the severity of the contraindication, and the potential benefits must be weighed relative to the risk. Anaphylactoid reactions have been reported after morphine or codeine administered i.v., although the reactions are rare (23). Morphine may induce or exacerbate asthmatic attacks; hence, fentanyl may be a better choice in asthmatic patients (23). Other relative contraindications to the use of narcotics also exist with respect to the potential for drug abuse (17). Although a history of substance abuse does not definitely preclude the use of opioids, it does necessitate careful vigilance. If the episode of abuse is active or recent, then another pain management strategy may be prudent. Consideration of the social network also requires consideration, especially if the patient lives with a substance abuser or has a home life conducive to enabling abuse.

2.2.4 Drug Interactions. The pharmacological activity of opioids can be affected by a number of other drugs, including amphetamines, antihistamines, antidepressants, and antipsychotics (see Ref. 23). Small doses of amphetamine significantly enhance the analgesic activity and euphoric effects of morphine and may counteract sedation. Diphenhydramine and hydroxyzine are antihistamines that exhibit modest analgesic activity themselves, and hydroxyzine has been shown to enhance the analgesic effects of low doses of narcotic analgesics (47). The depressant effects of some opioids may be exaggerated and prolonged by monoamine oxidase inhibitors, tricyclic antidepressants, and phenothiazines; the exact mechanism of action is not fully understood, but may involve metabolic or neurotransmitter alteration (23). The antidepressants desipramine (48) and nefazodone (49) appear to enhance morphine-induced analgesia. The phenothiazine antipsychotics can potentiate the analgesic effect of opioids, but also increase respiratory depression and sedation (23).

2.2.4.1 ***Interactions with Cytochrome P_{450} Enzymes.*** Drug interactions with opioid analgesics can also result from their interaction with cyctochrome P_{450} (CYP) isozymes, specifically 3A4 and 2D6 (Table 7.5) (50).

The CYP3A4 isozyme is responsible for the metabolism of a large number of endogenous compounds as well as a wide range of drugs (50). Fentanyl, alfentanil, and sufentanil are substrates for CYP3A4, and therefore drugs that inhibit this enzyme, such as erythromycin, HIV protease inhibitors, or cimetidine,

Table 7.5 Cytochrome P_{450} Isozymes and Opioid Substrates and Inhibitors

P_{450} Isozyme	Substrate	Inhibitor
1A2	Methadone	
2D6	Codeine	Codeine
	Hydrocodone	Methadone
	Meperidine	Propoxyphene
	Methadone	
	Morphine	
	Propoxyphene	
3A4	Alfentanil	Dextropropoxyphene
	Fentanyl	Propoxyphene
	Sufentanil	

may result in oversedation or increased respiratory depression as well as prolonged duration of action of the opioid (51). Conversely, more rapid metabolism of alfentanil or fentanyl may result when these agents are used in combination with rifampin, which is an inducer of 3A4 (51).

Genetic polymorphism in CYP2D6 results in varied drug metabolism (52), with the lack of this isoenzyme affecting between 5 and 10% of Caucasians and 1–3% of African-Americans and Asians (52). Individuals lacking 2D6 may display a larger response to some drugs and be at greater risk for toxicity because of their inability to metabolize certain drugs. Several opioids such as morphine, meperidine, and methadone are metabolized by 2D6 (Table 7.5), and interactions with drugs that induce 2D6 result in loss of opioid activity. Thus rifampin, phenytoin, phenobarbital, primadone, and carbamazepine can all result in a significant reduction in opioid concentrations and concomitant use may require increasing the opioid dose. This genetic defect is also problematic when metabolism to an active metabolite occurs. Because CYP2D6 can convert codeine to morphine (see Section 2.3.2 below), 2D6 inhibitors, especially quinidine, can significantly diminish the analgesic effects of codeine (51). Ritonavir and cimetidine also inhibit metabolism by 2D6 and therefore can significantly increase the concentration of opioids metabolized by 2D6 (Table 7.5), including meperidine, methadone, and propoxyphene, which may result in toxicity (51).

2.3 Absorption, Distribution, Metabolism, Elimination

2.3.1 Absorption and Distribution. Opioid analgesics are available for administration by a variety of routes, including by subcutaneous (s.c.) and intramuscular (i.m.) injections and rectal suppositories as well as by oral and intravenous (i.v.) administration (see Tables 7.1, 7.3, and 7.4). The least invasive and safest route that provides adequate analgesia should be chosen (22). Opioids are absorbed from the gastrointestional tract or the rectal mucosa and are also readily absorbed into the bloodstream after s.c. or i.m. injection. Some narcotics with increased lipophilicity may be absorbed through the nasal or buccal mucosa (butorphanol nasal spray and fentanyl lozenges, respectively). Fentanyl is sufficiently lipophilic to be absorbed through the skin (fentanyl transdermal patch) (23,241.

Intravenous administration of opioids results in a rapid onset of action. The more lipophilic drugs show more rapid onset of action after s.c. administration because of differing rates of absorption and penetration into the CNS across the blood-brain barrier (23). Intraspinal administration produces long-lasting analgesia, but the hydrophilicity of morphine can result in rostral spread of the drug in the spinal fluid. This may be problematic, given that respiratory depression can occur up to 24 h after the last administered dose as a result of the drug reaching the respiratory control center in the brain (23). Fentanyl and hydromorphone are highly lipophilic and are rapidly absorbed by spinal tissue, and thus the rostral spread is significantly reduced, resulting in a localized analgesic effect (23).

The binding of opioids to serum proteins is of considerable importance, given that it influences the distribution of the drug as well as metabolism and excretion (53). The protein binding of morphine is low (35%), moderate with meperidine (70%), and high for methadone (90%)(54). The high human plasma protein binding of methadone is well known (55); the highest binding is to β-globulin III (56) and albumin (53, 56). The extensive protein binding of methadone is an important factor because it significantly affects the amount of drug available in the plasma for penetration

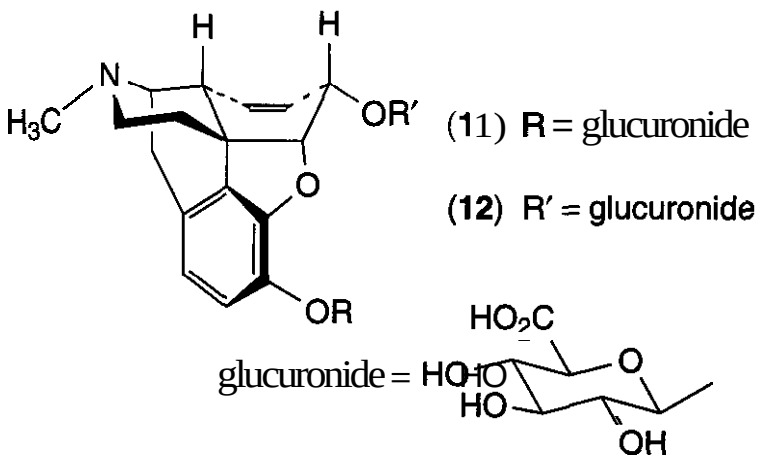


Figure 7.2. Major metabolites of morphine.

across the blood-brain barrier. The extensive protein binding may provide a reservoir for methadone to replenish the drug to the blood (57). It has been suggested that the high protein binding of methadone accounts for its mild but extended withdrawal symptoms (23). It is also fairly common for drug abusers (50% for heroin addicts) to have serum protein abnormalities, including elevated globulin and albumin levels, which may further complicate addiction treatment (57). In addition, there may also be liver dysfunction or disease stemming from concomitant alcohol abuse that may account, in part, for the abnormal serum protein levels (57).

2.3.2 Metabolism and Elimination of Morphine and Derivatives. Most opioids are subjected to significant first-past hepatic metabolism (see Ref. 23). Thus oral morphine has only approximately one-quarter bioavailability compared to that of a parenteral dose. The major route of metabolism is through glucuronic acid conjugation. Two major metabolites are formed with the conjugation of the glucuronic acid to either position 3 (M3G, 11, Fig. 7.2) (50%) or 6 (M6G, 12) (5–15%) of morphine, whereas only small amounts of the diglucuronide are formed (23, 25). M6G has pharmacological actions indistinguishable from those of morphine, yet it is twice as potent and is present in higher concentrations in plasma than in morphine (58). It has been suggested that M6G accounts for a significant portion of morphine's analgesic activity, especially with chronic use (59). Dose adjustment is not required in mild hepatic disease, but excessive sedation can occur in cirrhotic patients because of the accumulation of M6G (59). M3G has very low affinity for opioid receptors and does not contribute to analgesia, but it may contribute to neuroexcitatory side effects such as allodynia (60). M3G has been reported to antagonize morphine (61), but this effect has not been consistently observed (62). Metabolism by N-demethylation to normorphine is a minor metabolic pathway for morphine, whereas N-dealkylation is important to the metabolism of some morphine congeners (see Ref. 63 and references cited therein for an excellent overview of morphine metabolism and elimination). Codeine, levorphanol, oxycodone, and methadone have a high oral to parenteral potency ratio attributed to decreased first-pass hepatic metabolism (23).

A small but significant amount of codeine (−10%) is metabolized to morphine through *O*-demethylation by the 2D6 isozyme of hepatic cytochrome P_{450} (64). The resulting morphine is thought to be responsible for the analgesic activity because codeine has very low affinity for opioid receptors (23). Patients lacking CYP2D6, because of a genetic polymorphism, are unable to metabolize codeine to morphine (51). As noted above, there also appears to be variation in metabolic efficiency depending on ethnicity (65). The predominant metabolite of codeine is the 6-glucuronide (C6G), which is renally excreted. One controversial report suggests that codeine analgesia is a result of C6G and not the morphine metabolite (66). Hydrocodone is also metabolized to hydromorphone and oxymorphone is a minor active metabolite of oxycodone through hepatic P_{450} 2D6 metabolism.

Naloxone undergoes extensive hepatic first-pass metabolism through glucuronidation. Naltrexone is metabolized to the active metabolite 6-naltrexol, which is less potent but has a prolonged half-life compared to that of the parent drug.

The opioids are predominantly excreted renally (23). Morphine is eliminated by glomerular filtration from the kidney, predominantly as M3G or M6G (23). The majority of excretion (>90%) takes place on the first day, but a small amount of enterohepatic circulation accounts for the presence of small amounts of morphine and metabolites for several days after the last drug dose of drug is given (23).

Both the free and conjugated forms of codeine are excreted in the urine (23).

2.3.3 Metabolism and Elimination of Other Opioid Agents. Meperidine is also metabolized in the liver. It is either hydrolyzed directly to meperidinic acid or N-demethylated to normeperidine and then hydrolyzed to normeperidinic acid. The acid forms are conjugated, then excreted (23).

Fentanyl is also hepatically metabolized and renally excreted. However, the congener remifentanil is metabolically distinct when compared to other members in its chemical or pharmacological class. Remifentanil is metabolized by plasma esterases to remifentanil acid, which is approximately 3000-fold less potent than the parent opioid (67).

Methadone undergoes significant hepatic metabolism by N-demethylation and cyclization to form pyrrolidines and pyrroline (23). Propoxyphene is also hepatically metabolized predominantly by N-demethylation and renally eliminated. The metabolite norpropoxyphene is cardiotoxic and produces arrhythmias and pulmonary edema that have led to reports of cardiac arrest and death (59). This is especially problematic because of the long half-life of norpropoxyphene that accumulates with repeated doses of the parent drug. Methadone is excreted in the urine but also in the bile (23).

3 PHYSIOLOGY AND PHARMACOLOGY

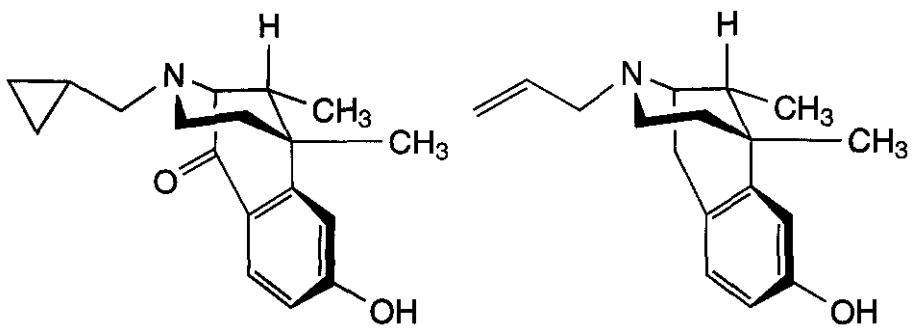
3.1 Opioid Effects in the Central Nervous System and the Periphery

Opioid receptors are found in both the CNS and in the periphery. In the CNS different types of opioid receptors (μ, κ, and δ receptors; see below) exhibit distinct anatomical distributions (see Refs. 68–70 for reviews), and there is considerable species variation in both relative receptor density and receptor distribution. Peripheral receptors mediate some effects of opioids, such as inhibition of gut motility, and for a number of years receptors from tissues such as the guinea pig ileum (GPI) formed the basis of standard bioassays used to assess compounds for opioid activity (see Section 3.2.3.3 below). Peripheral receptors have also been implicated in analgesia, particularly in cases of inflammation (see Refs. 71–74 for reviews). Readers are referred to a comprehensive two-volume series on opioids (75, 76) plus more recent reviews (18, 77, 78) for detailed reviews of opioid pharmacology and physiology.

3.2 Multiple Opioid Receptor Types

3.2.1 Discovery of Multiple Opioid Receptor Types and Current Nomenclature. Our understanding of opioid receptors has expanded considerably from the early assumption of a single opioid binding site to the characterization of multiple types of opioid receptors (see Refs. 79–81 for reviews). The initial proposal of opioid receptors by Beckett and Casy in 1954 (1) assumed a single opioid binding site. Multiple opioid receptors were postulated as early as the 1960s by both Portoghese (82) and Martin (83), but it was behavioral studies in the chronic spinal dog by Martin and coworkers in the mid-1970s (5, 6) that led to the classification of multiple opioid receptors. On the basis of the pharmacological profile of a variety of opioids, Martin proposed three types of opioid receptors, μ, κ, and σ receptors, with morphine (1, Fig. 7.1), ketocyclazocine (13), and SKF-10,047 (*N*-allylnorcyclazocine, 14), respectively, as the prototypical ligands (Fig. 7.3). The discovery of the enkephalins led to the proposal of a distinct opioid receptor type, the δ receptor, for these opioid peptides (84). The existence of three distinct opioid receptor types, the μ, κ, and δ receptors, has now been clearly established and these receptors have been cloned (see below). Sigma (σ) receptors, however, are not considered opioid receptors because effects associated with this receptor are not reversed by opioid antagonists such as naloxone (see Ref. 85). Other opioid receptor types have also been proposed (see Ref. 86), but these receptor types are not universally accepted.

During attempts to clone the opioid receptors (see Section 3.2.4 below), a related receptor with high sequence homology was identified by several research groups (see Refs. 87–90 for recent reviews). This receptor, referred to by Mollereau et al. as opioid-receptor-like 1 (ORL1) receptor (91), does not dis-



(13) Ketocyclazocine

(14) SKF 10,047
(N-allylnormetazocine)

Figure 7.3. Agonists used by Martin and coworkers (5, 6) to define κ and σ receptors. Morphine **(1)** was the prototypical ligand used to characterize μ receptors.

play affinity for classical opioid ligands, including naloxone. Although distinctly different from opioid receptors, the ORL1 receptor interacts with the opioid receptor system in the regulation of analgesia and other physiological effects. Details concerning the pharmacology of the ORL1 receptor and its endogenous ligand orphanin FQ/nociceptin (OFQ/N) (92, 93) are discussed below under Recent Developments (Section 7.2).

In 1996 the International Union of Pharmacology (IUPHAR) recommended that OP1, OP2, and OP3 be used as the accepted names for δ, κ, and μ receptors, respectively, (94) to replace the DOR, KOR, and MOR nomenclature typically used in the literature; OP4 was the proposed name for the related ORL1 receptor. This nomenclature has not gained widespread acceptance, however, and in 2000 the International Narcotic Research Conference (95) recommended a modified nomenclature DOP, KOP, MOP, and NOP for δ, κ, μ, and ORL1 receptors, respectively, which is consistent with the nomenclature requirements of IUPHAR.

3.2.2 Signal Transduction Mechanisms. There is considerable evidence that opioid receptors are coupled to G-proteins and produce their effects through these proteins (see Refs. 96, 97 for reviews of opioid receptors and G-proteins). The structure of cloned opioid receptors is consistent with their belonging to this receptor superfamily (see below). G-proteins are heterotrimers, consisting of a, β, and γ subunits, which bind guanine nucleotides to their a-subunit and catalyze the hydrolysis of GTP to GDP. G-proteins mediate the interaction of opioid and other receptors with a variety of effector systems, including adenylyl cyclase and ion channels. Numerous forms of G-proteins have been identified, including G_i and G_o that inhibit adenylyl cyclase and G_s that stimulates adenylyl cyclase. Pertussis toxin inhibits G_i and G_o by ADP-ribosylation of the a-subunit, whereas cholera toxin persistently activates G_s. Thus sensitivity to pertussis toxin is an indication of the involvement of G_i (or G_o) in the transduction mechanism, whereas sensitivity to cholera toxin is an indication of involvement of G,.

Of the effector systems that have been implicated in the transduction mechanisms for opioid receptors, the best studied is opioid inhibition of adenylyl cyclase (see Refs. 69, 97–99 for reviews). Thus binding of an agonist to opioid receptors inhibits the activity of adenylyl cyclase and decreases intracellular cAMP in a number of different tissues. Pertussis toxin sensitivity of opioid inhibition of adenylyl cyclase has been demonstrated in many systems, indicating the involvement of either G_i or G_o in the transduction mechanism. Agonist activation of all three types of cloned opioid receptors to inhibit adenyl cyclase has been demonstrated (see Ref. 100 and references cited therein). There is also some evidence that μ and δ opioid receptors can stimulate adenylyl cyclase in certain tissues (see Refs. 69, 97 for reviews). There are conflicting reports on whether κ opioid receptors stimulate or inhibit phosphatidylinositol turnover in some tissues (see Ref. 100); δ and μ receptors, however, do not appear to be coupled to phosphatidylinositol turnover in neuroblastoma cell lines NG108–15 and SK-N-SH (101).

Opioid receptors can also be coupled to ion channels through G-proteins (see Refs. 69, 98, 99, 102 for reviews). All three receptor types can decrease voltage-dependent Ca^{++} current. The coupling of opioid receptors to calcium channels involves a G-protein, and the actions of opioids on Ca^{++} current are blocked by pertussis toxin, indicating involvement of G_i or G_o. Activation of μ and 6 receptors can also increase K^+ conductance. Similar to the results found for calcium channels, potassium channel coupling to opioid receptors appears to involve a G-protein and is sensitive to pertussis toxin. Considerable evidence suggests that the effects on ion currents are attributed to direct coupling of opioid receptors to ion channels through G-proteins and are not related to inhibiton of adenylyl cyclase (see Ref. 102).

Agonist binding to opioid receptors also appears to activate the extracellular signal regulated kinase (ERK) cascade, which consists of three intracellular kinases: a mitogen-activated protein kinase (MAPK) kinase kinase, a MAPK kinase, and a MAPK homolog (see Ref. 100). This activation appears to be through G_i or G_o (see Ref. 100 and references cited therein).

3.2.3 Characterization of Opioid Receptors. Early evaluation of compounds for opioid activity relied on testing for antinociceptive activity in *vivo*. These pharmacological assays are often predictive of analgesic activity in humans, but the activity of compounds observed in these assays is affected by a variety of factors, including the route of administration of the compound, the ability of the compound to cross the blood-brain barrier into the CNS, the susceptability of the compound to metabolism and pharmacokinetics, the choice of noxious stimulus, and the animal species and strain used for the assay (see Ref. 103). The results of in vitro assays are not influenced by many of these factors that complicate in *vivo* assays. The pharmacological activity of opioids in vitro can still be complex, however, because more than one opioid receptor type is present in many tissues. Opioid receptors are present in a variety of peripheral tissues, and isolated tissue preparations, particularly the guinea pig ileum (GPI) and mouse vas deferens (MVD), have been routinely used to assess opioid activity. Radioligand binding assays for each of the opioid receptor types have been instrumental in the identification of selective opioids. With the cloning of the opioid receptors, assays for both opioid receptor affinity and efficacy can now be routinely performed by use of these cells that express only a single receptor type, greatly simplifying the interpretation of the results of the assays. Each of these types of assays and their utilization in characterizing compounds for opioid activity are discussed below.

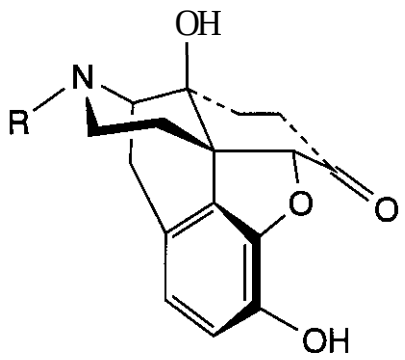
3.2.3.1 ***Ligands Used to Characterize Opioid Receptors.*** Since the identification of multiple opioid receptor types, considerable effort has focused on developing more selective ligands for each of the receptor types (see Figs. 7.4–7.6 and Sections 5.3, 5.9, 5.10, and 6 below). Ligands commonly used to study the different receptor types include both nonpeptides and peptides (see Table 7.6). Morphinans such as morphine and the antagonists naloxone (**16**), naltrexone (**17**), and cyprodime (18) (104) are used to study μ receptors (Fig. 7.4); naloxone and naltrexone retain significant affinity for 6 and κ receptors and therefore at higher concentrations these compounds will antagonize all three receptor types. Several peptides, including the enkephalin analog DAMGO ([D-Ala^2,MeNPhe^4,glyol]enkephalin, **15**), and CTOP and CTAP (19 and **20**), somatostatin analogs that antagonize μ receptors (105), are also used to characterize μ receptors. Early studies of 6 opioid receptors used the enkephalin analog DADLE ([D-Ala^2,D-Leu^5]enkephalin, **21**), but several more 6-selective enkephalin analogs, including DSLET and DTLET ([D-Ser^2,Leu^5,Thr^6]-, 22 and [D-Thr^2,Leu^5,Thr^6]enkephalin, 23) and particularly the cyclic analog DPDPE (*cyclo*[D-Pen^2,D-Pen^5]enkephalin, 24) (Fig. 7.5), are now used routinely to characterize δ receptors; the naturally occurring deltorphins (e.g., **25**), peptides isolated from frog skin (106), exhibit marked 6-receptor selectivity. The nonpeptide agonists BW373U86 (**26**), SNC 80 (**27**) (107), and TAN 67 (28) (108,109) also exhibit very high selective for δ receptors and are used frequently to study these receptors. Delta-receptor antagonists include the peptides ICI 174,864 (29) (110), TIPP (Tyr-Tic-Phe-Phe

Agonist

Tyr-D-Ala-Gly-MeNPhe-$NHCH_2CH_2OH$

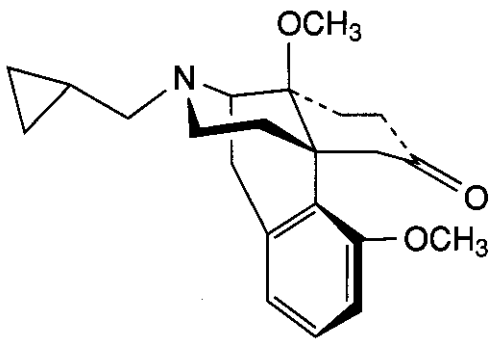
(15) DAMGO

Antagonists

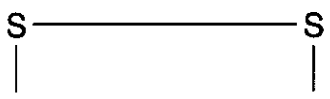


$R = CH_2CH{=}CH_2$ (16) Naloxone

$R = CH_2$–cyclopropyl (17) Naltrexone



(18) Cyprodime



D-Phe-Cys-Tyr-D-Trp-X-Thr-Pen-$ThrNH_2$

(Pen = penicillamine)

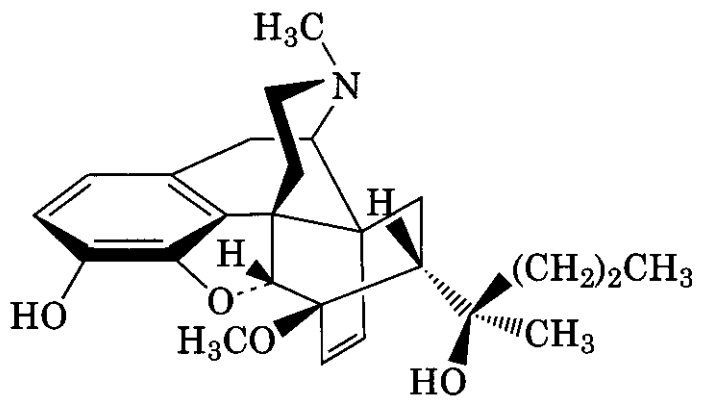
X = Om (19) CTOP

X = Arg (20) CTAP

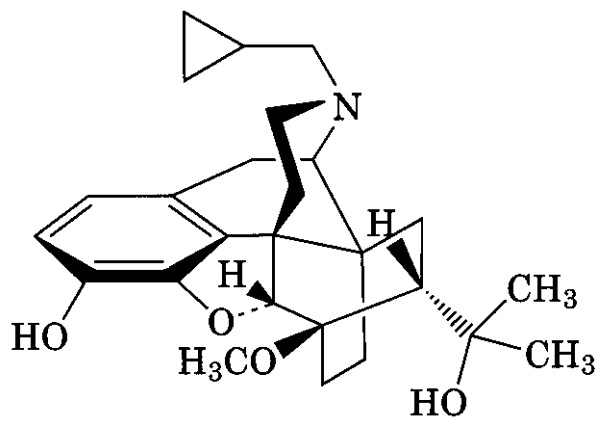
Figure 7.4. Ligands used to characterize μ opioid receptors.

OH, 30) (111), and TIPP[Ψ] (Tyr-TicΨ[CH_2NH]Phe-PheOH, 31) (112), and the nonpeptide naltrindole (32) (113). Recently, however, TIPP has been reported to exhibit agonist activity in adenylyl cyclase assays (114). Early studies of κ receptors used benzomorphans such as ethylketocyclazocine (EKC, 33), a close analog of ketocyclazocine, and bremazocine (34) (Fig. 7.6), but the selectivity of these ligands for κ receptors is very low (see Table 7.8 below). Kappa-selective agonists such as U50,488 (35) (115), U69,593 (36) (116), and CI-977 (37) (117, 118), and the κ-selective antagonist norbinaltorphimine (norBNI, 39) (119, 120)) are now available. Recently, Portoghese and coworkers described GNTI (41) (121–123), which is a more κ-selective antagonist than norBNI, and which should be a useful pharmacological tool to study κ receptors. The structure-activity relationships of these compounds are discussed in more detail in the sections below.

3.2.3.2 ***Radioligand Binding Assays.*** The demonstration of stereospecific binding of tritiated ligands to opioid receptors in the early 1970s (2–4) paved the way for the subsequent development of radioligand binding assays for each of the opioid receptor types, which have been instrumental in the identification of selective opioids. Early studies often used nonselective ligands such as [^{3}H]etorphine ([^{3}H]-42) or [^{3}H]naloxone, which labeled all types of



(42) Etorphine



(43) Diprenorphine

opioid receptors, but today tritiated ligands selective for each receptor type are available (see Table 7.7 and Refs. 69, 94). [^{3}H]DAMGO ([^{3}H]-15) is most often used for the radioligand binding assays for μ opioid receptors. [^{3}H]DADLE ([^{3}H]-21) was often used in early binding studies examining δ opioid receptors,

Agonists

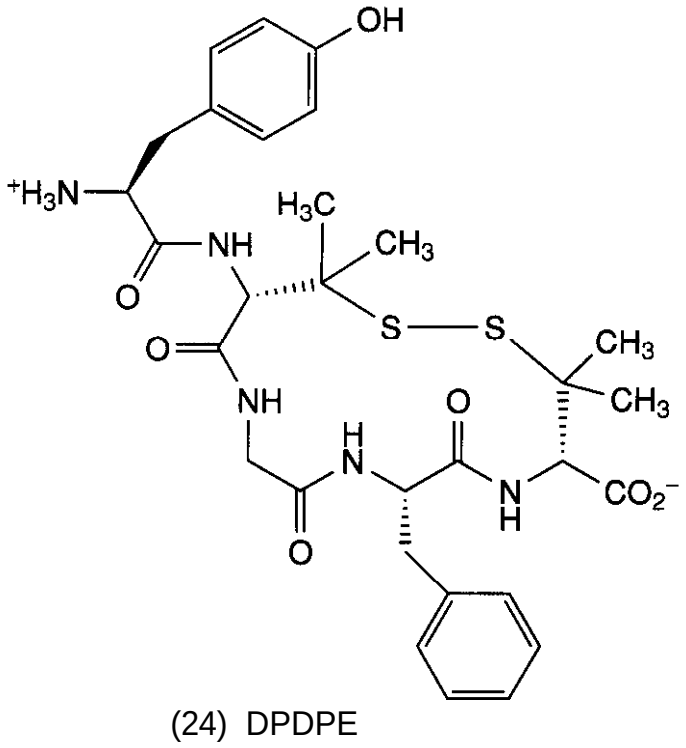
Tyr-D-Ala-Gly-Phe-D-Leu

(21) DADLE

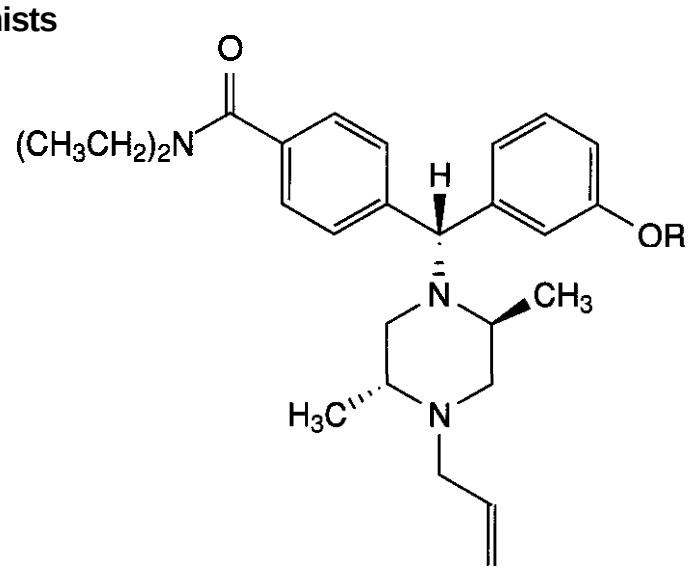
Tyr-X-Gly-Phe-Leu-Thr

X = D-Ser (22) DSLET

X = D-Thr (23) DTLET

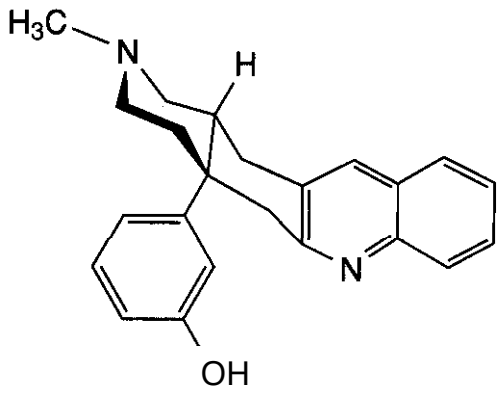


(24) DPDPE



R = H (26) (±) BW373U86

$R = CH_3$ (27) (+) SNC 80

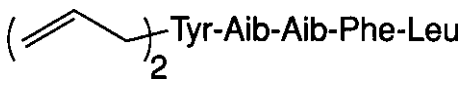


(28) TAN 67

Tyr-D-Ala-Phe-Glu-Val-Val-GlyNH_2

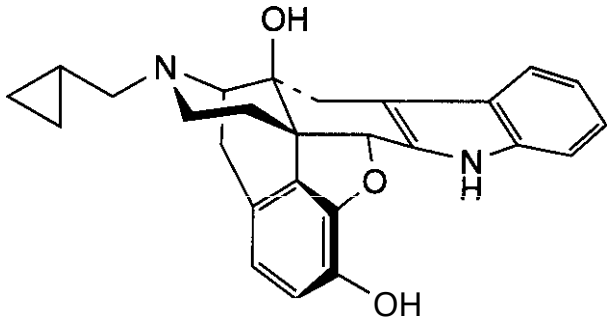
(25) [D-Ala^2]deltorphin II

Antagonists



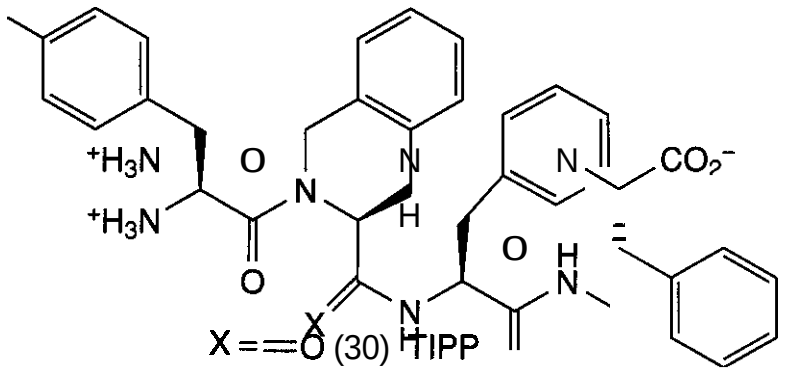
(Aib = -$NHC(CH_3)_2CO$-)

(29) ICI 174,864



(32) Naltrindole (NTI)

HO

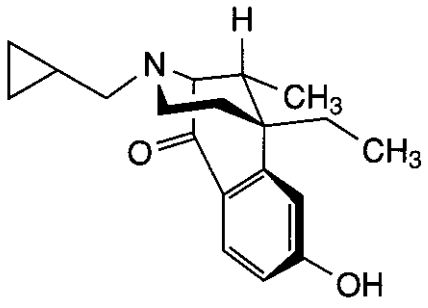


X = O (30) TIPP

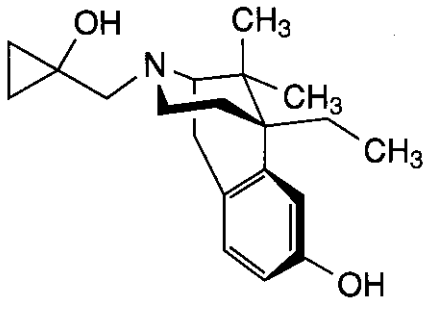
$X = H_2$ (31) TIPP[Ψ]

Figure 7.5. Ligands used to characterize δ opioid receptors.

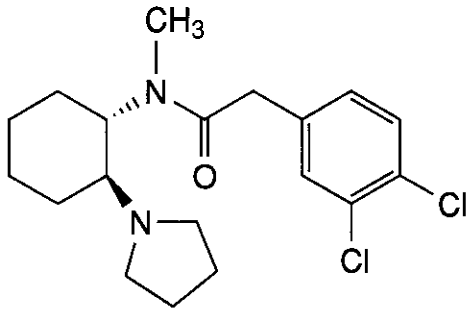
Agonists



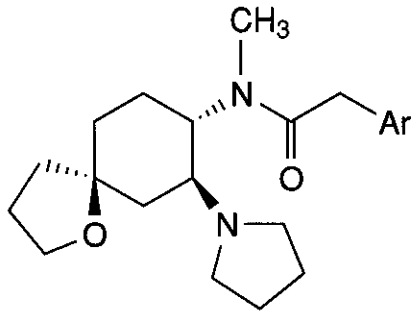
(33) Ethylketocyclazocine (EKC)

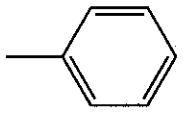


(34) Bremazocine

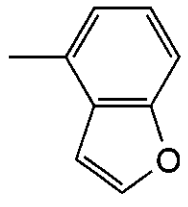


(**35**) U50,488



Ar = 

(**36**) (–) U69,593

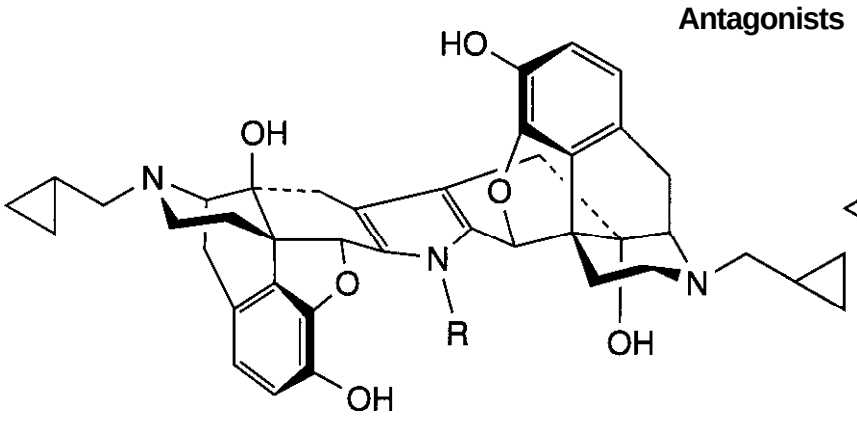
= 

(**37**) CI-977

Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln

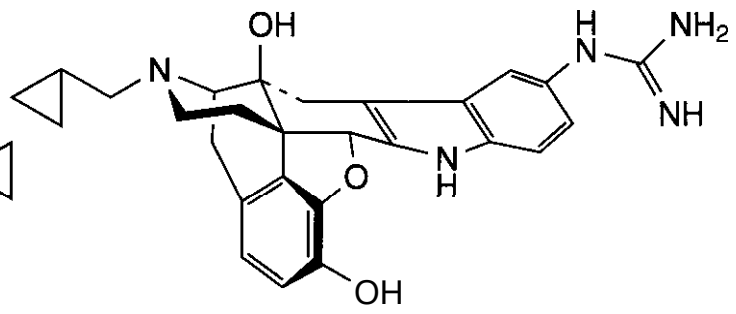
(38) Dynorphin A

Antagonists



R = H **(39)** norBNI

R = CH_3 **(40)** BNI



(41) GNTI

Figure 7.6. Ligands used to characterize κ opioid receptors.

but studies now commonly use the more 6-selective ligand [^{3}H]DPDPE ([^{3}H]-**24**). Early binding studies of κ opioid receptors were hampered by the low κ selectivity of available tritiated ligands and the low levels of κ receptors in rat brain (see below). One solution was to examine the binding of ligands such as [^{3}H]EKC ([^{3}H]-**33**) or [^{3}H]bremazocine ([^{3}H]-34) in the presence of unlabeled μ- and 6-selective ligands such as DAMGO and DPDPE to block μ and 6 receptors in the tissue preparation. Because the highly κ-selective ligand U69,593 ([^{3}H]-**36**) is now available in tritiated form (116), it is routinely used in κ-receptor binding assays; the κ-selective ligand CI-977 (37) (117, 118) is also available in tritiated

Table 7.6 Ligands Commonly Used to Study Different Opioid Receptor Types[a]

Receptor Type	Agonists	Antagonists
μ	Morphine (1) DAMGO (15)	(Naloxone, 16) (Naltrexone, 17) Cyprodime (18) CTOP, CTAP (19, 20)
6	DPDPE (24) DSLET, DTLET (22, 23) Deltorphins (25) (DADLE, 21) BW373U86 (26) SNC 80 (27) TAN 67 (28)	TIPP (30) TIPP[ψ] (31) ICI 174,864 (29) Naltrindole (32)
κ	U50,488 (35), U69,593 (36) CI-977 (37) (EKC, 33) (bremazocine, 34) Dynorphin A (38) and derivatives	norBNI (39) GNTI (41)

[a]Compounds with limited selectivity for a given receptor type are listed in parentheses (see Table 7.8 for affinities and opioid activities of these agents). See Figs. 7.1 and 7.4–7.6 for structures of these agents.

form. The affinity of a variety of opioids in radioligand binding assays for μ, δ, and κ receptors are given in Table 7.8.

Prior to cloning of the opioid receptors, affinity for these receptors was most commonly determined by the use of homogenates or membrane fractions from rat, guinea pig, or mouse brain, which contain all three types of opioid receptors. The relative amounts of different opioid receptor types vary between species, however, particularly for κ receptors. In rat brain κ opioid receptors constitute only about 10–15% of the total number of opioid receptor sites (124), whereas in species such as guinea pig they represent approximately 30% of the total opioid receptor population (125). Over 80% of the opioid receptors in the guinea pig cerebellum are κ receptors (126), so this tissue was frequently used in κ-receptor binding assays. In contrast the rabbit cerebellum contains predominantly (>70%) μ opioid receptors (127). Species differences also exist for S receptors, with mouse brain exhibiting substantially higher 6-receptor density than rat brain (128). NG108–15 cells contain only δ receptors and therefore have been used in radioligand binding assays for these receptors (129). With the cloning of opioid receptors (Section 3.2.4 below), binding assays are now typically performed using these cells that express only a single receptor type.

***3.2.3.3 In Vitro* Assays *for* Efficacy.** Until the cloning of the opioid receptors isolated tissue preparations, particularly smooth muscle preparations, were used extensively to characterize opioids (see Refs. 131, 132 for reviews). The electrically stimulated GPI myenteric plexus-longitudinal muscle and the MVD

Table 7.7 Commercially Available Tritiated Opioid Receptor Ligands[a]

Receptor Type			Nonselective
μ	6	κ	(or low selectivity)
Morphine (1) DAMGO (15)	DPDPE (24) [D-Ala^2]deltorphin II (25) [D-Ala^2,Ile^{5,6}]deltorphin II Naltrindole (32)[b] (DADLE, 21)	U69,593 (36) CI-977 (37) (Bremazocine, 34)	EKC (33) Diprenorphine (43)[b] Naloxone (16)[b]

[a]Compounds with limited selectivity for a given receptor type are listed in parentheses. Ligands commercially available from PerkinElmer Life Sciences, Amersham Biosciences, or Tocris. Iodinated derivatives of β-endorphin and Met(O) enkephalin are also available.

[b]Antagonists

Table 7.8 Opioid Receptor Affinities and Opioid Activity in the GPI and MVD of a Variety of Opioid Ligands Commonly Used to Study Opioid Receptors[a]

a. Agonists	K_i (nM)			K_i Ratio	IC_{50} (nM)	
Agonist	μ	δ	κ	$\mu/\delta/\kappa$	GPI	MVD
Nonselective agonists						
Etorphine (**42**)	1.0	0.56	0.23	4.3/2.4/1	0.08	0.40
(−)-EKC (**33**)	1.0	5.5	0.52	1.9/10.6/1	0.18[b]	4.4[b]
Agonists preferentially interacting with μ receptors						
Morphine (**1**)	1.8	90	317	1/50/175	28	478
Meperidine (7)	385	4,350	5,140	1/11/13	1,109	16,000
Fentanyl (**8**)	7.0	150	470	1/21/67	0.92	26
Methadone (9)	4.5	15	1,630	1/3.3/360	22	523
DAMGO (15)	1.9	345	6,090	1/180/3,200	4.5	33
Agonists preferentially interacting with 6 receptors						
BW373U86 (**26**)[c]	46[d]	0.92[d]	—	50/1	143	0.2
SNC 80 (**27**)[c]	2,470[d]	1.0[d]	—	2,300/1	5,460	2.7
TAN 67 (**28**)[e]	2,320	1.1	1,790	2,070/1/1,600	26,000	6.6
DPDPE (24)	710	2.7	>15,000	260/1/>5,500	2,350	2.8
DSLET (22)	39	1.8	6,040	22/1/3,350	110	0.59
DADLE (21)	14	2.1	16,000	6.7/1/7,600	8.9	0.73
[D-Ala^2]deltorphin II (25)	2,450	0.71	>10,000	3,450/1/>14,000	>3,000	0.32
Agonists preferentially interacting with κ receptors						
(−)-Bremazocine (34)	0.62	0.78	0.075	8.3/10.4/1	0.13[b]	1.98[b]
U50,488 (**35**)	435	9,200	0.69	630/13,300/1	16	11
U69,593 (**36**)	2,350	19,700	1.4	1,680/14,000/1	2.0	ND[f]
CI-977 (**37**)	100	1,040	0.11	910/9,450/1	0.09	ND
[D-Pro^{10}]Dyn A-(1–11)	0.56	2.3	0.029	19.3/79/1	3.3	ND

b. Antagonists	K_i (nM)			K_i Ratio	K_e (nM)		
Antagonist	μ	6	κ	$\mu/\delta/\kappa$	μ (GPI)	κ (GPI)	δ (MVD)
Nonselective antagonists							
Naloxone (16)	1.8	23	4.8	1/13/2.7	1.9	18	49
Naltrexone (17)	1.1	6.6	8.5	1/6.0/7.7	0.36	4.4	12
Diprenorphine (43)	0.24	1.0	0.14	1.7/7.1/1	0.31	0.5[g,h]	3.6
Antagonists preferentially interacting with μ receptors							
Cyprodime (**18**)	9.4	356	176	1/38/19	31	1,160	6,110
CTOP (19)	1.7	>1,000	>1,000	1/>590/>590	16	444	NA[i] (1 μM)
Antagonists preferentially interacting with 6 receptors							
Naltrindole (32)	11	0.12	18	92/1/150	22	100	0.27
ICI 174,864 (29)	29,600	190	65,400	155/1/345	>5,000[g]	>5,000[g]	17
TIPP (**30**)[j]	1,720	1.2	ND	1,43011	ND	ND	3.0–5.9
TIPP[ψ] (**31**)[k]	3,230	0.31	ND	10,500/1	ND	ND	2.1–2.9
Antagonists preferentially interacting with κ receptors							
NorBNI (39)	14	10	0.34	41/29/1	25	0.05	16
GNTI (**41**)[l]	22	46	0.18	125/257/1	30	0.20	NA[i] (100 nM)

[a]Data from Ref. 130 except where otherwise indicated.
[b]Antagonist at μ and δ receptors in the rat and hamster vas deferens, respectively.
[c]From Ref. 107.
[d]IC_{50}.
[e]From Ref. 109.
[f]ND, not determined.
[g]Determined in the MVD.
[h]Agonist in the GPI (IC_{50} = 1.4 nM).
[i]NA, not active at indicated dose.
[j]From Ref. 111.
[k]From Ref. 112.
[l]From Ref. 122.

preparations have been the tissues used most extensively. The predominant effect of opioids is to inhibit smooth muscle contraction, generally by inhibiting the release of neurotransmitters; in the GPI and MVD acetylcholine and norepinephrine, respectively, are the neurotransmitters effected (see Ref. 132). In contrast to radioligand binding assays, which measure only opioid affinity, these bioassays provide information on the intrinsic activity of the compounds tested. The activity of compounds in these assays can be complex, however, because both tissues contain more than one opioid receptor type. The GPI contains both μ and κ receptors, with little if any functional δ receptors, whereas the MVD contains all three opioid receptor types. The activity of a variety of opioids in these tissues are given in Table 7.8. GPI and MVD preparations enriched in a single receptor population have been prepared by use of the affinity labels β-chlornaltrexamine (β-CNA) and β-funaltrexamine (β-FNA) (133, 134) (see Section 5.11). Vas deferens from other species have been used to characterize opioids and appear to contain predominantly a single receptor population. The rabbit vas deferens appears to contain only κ receptors (135), whereas the hamster vas deferens contains only 6 receptors (136). In the rat vas deferens β-endorphin is a potent inhibitor, which led to the proposal that this tissue contains an additional type of opioid receptor, the ε receptor (137–139).

With the cloning of the opioid receptors, in vitro functional assays that used these receptors have been developed. These assays have used measurement of effects on signal transduction systems to determine the efficacy and potency of compounds being studied. Because opioid receptors are G-protein–coupled receptors, measurement of the stimulation of binding of the radiolabeled nonhydrolyzable analog of GTP [^{35}S]GTPγS (140, 141) following interaction of a compound with the receptor can be used to determine the efficacy and potency. Inhibition of adenylyl cyclase has also been used as a functional assay to evaluate the efficacy and potency of opioid ligands at opioid receptors (142,143).

3.2.3.4 ***in Vivo Evaluation of opioids.*** A variety of antinociceptive assays that use different noxious stimuli and different animal species have been used to examine the activity of potential analgesics. Animal models for pain include models of acute pain (e.g., hot plate, tail flick, paw pressure, and writhing assays), persistent pain (e.g., the formalin test), chronic pain (e.g., adjuvant-induced arthritis), and neuropathic pain (e.g., nerve ligation) (see Table 2.1 of Ref. 144). The different types of noxious stimuli commonly used in these antinociceptive assays include heat (e.g., hot plate and tail flick assays), pressure (e.g., tail pinch and paw pressure assays), chemical (writhing or abdominal constriction assay and the formalin test), and electrical (tail shock vocalization) stimuli. Among the most commonly used assays are the hot plate, tail flick, and writhing assays. In the hot plate test, the latency to various behavioral responses (e.g., forepaw or hindpaw licking) is measured when the animal is placed on a hot plate, typically set at 55°C, whereas the tail flick assay measures the time for the animal to flick its tail away from radiant heat focused on the tail. In the writhing or abdominal constriction assay the animal is injected intraperitoneally (i.p.) with an irritant (typically phenylquinone or acetic acid) and the dose of a test compound required to abolish the writhing syndrome determined. On the basis of a comparison of the potencies of standard opioids in several pain models, it was concluded that the mouse abdominal constriction assay (use of 0.4% acetic acid) was the most sensitive to opioids, whereas the mouse hot plate test (at 55°C) was the least sensitive; the rat tail flick test was intermediate (see Ref. 144). For a detailed discussion of these antinociceptive assays, readers are referred to excellent recent reviews (144, 145) that describe these procedures and discuss methodological issues and recent developments.

Activation of all three types of opioid receptors (μ, δ, and κ) can produce antinociception, but there are significant differences in the effects of activating different receptor types, depending on the noxious stimulus used and the animal species (see Refs. 103,146 for excellent reviews; see particularly Table 1 of Ref. 103 for a summary of supraspinal opioid receptor involvement in antinociceptive assays). Although μ agonists are active against all types of noxious stimuli, the activity of κ agonists

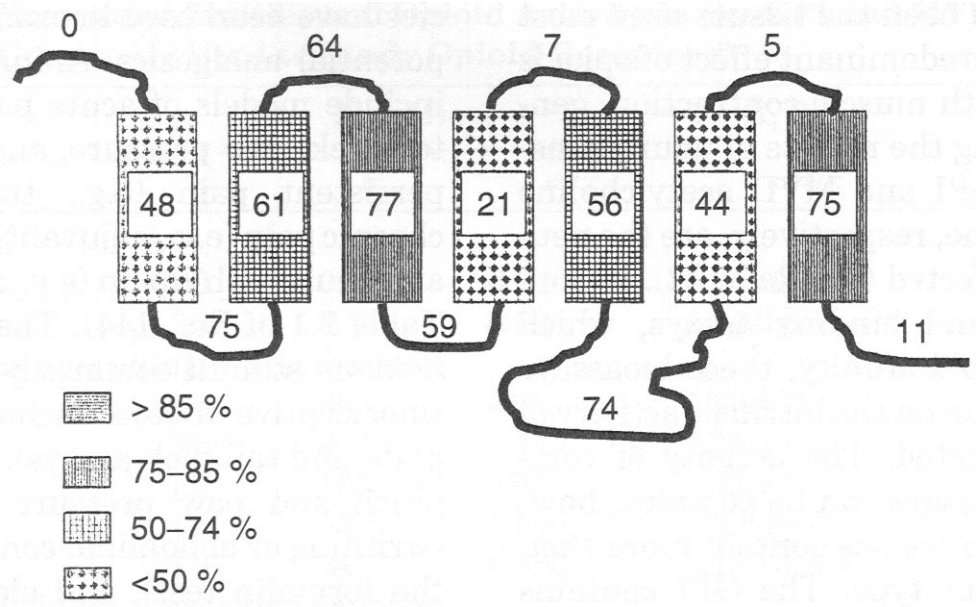


Figure 7.7. Schematic diagram of the protein structure of the three opioid receptors. Rectangles indicate transmembrane helices and numbers indicate the percentage identical residues among the three opioid receptors in that segment (from Ref. 100).

depends on the type of stimulus. Kappa agonists are active against chemical and pressure stimuli, but they are inactive against electrical stimulation and their efficacy against thermal stimuli is dependent on the intensity (147). Delta agonists may be active against all four types of stimuli in mouse, depending on the route of administration (see Ref. 103), but there are species differences. It has been reported that while δ agonists are effective in both the tail flick and hot plate assays in mice, in rats they are active in the hot plate, but not tail flick assays (148).

3.2.4 Opioid Receptor Structure and Molecular Biology. The first successful cloning of opioid receptors used a cDNA library from NG108–15 cells to clone δ receptors (8, 9). After expression of the cDNA library in COS cells, the cells were screened for [^{3}H] δ-ligand binding and the clone isolated. Yasuda et al. subsequently reported cloning of both mouse brain κ and δ receptors while trying to isolate cDNAs encoding somatostatin receptors (11), and Chen et al. reported cloning of rat μ receptor (10). Since then the cloning of all three opioid receptor types from several different species, including human, have been reported (see Refs. 79, 80, 94, 149–152 for reviews). The human μ, δ, and κ receptors exhibit 91–95% sequence homology to the corresponding type of receptor from rat and mouse (79). Studies with antisense oligodeoxynucleotides to each type of cloned opioid receptor and in knockout mice lacking individual opioid receptors have confirmed the involvement of the cloned receptors in analgesia mediated by *p,* δ, and κ receptors (see Refs. 149,153,154 for reviews).

The opioid receptors belong to the family of G-protein–coupled receptors. On the basis of the model for this family of receptors, the receptors contain extracellular regions including the N-terminus and extracellular loops, seven putative transmembrane (TM) regions,, and intracellular regions including the C-terminus and intracellular loops (Fig. 7.7). Comparison of the sequences (155) indicates the highest sequence homology between the κ, μ, and δ receptors in TM2, TM3, and TM7 (Fig. 7.7). TM2 and TM3 each contain a conserved Asp residue; the conserved Asp in TM3 is thought to interact with protonated amine groups on opioid ligands (see below). There are also high similarities in the intracellular loops; the third intracellular loop is thought to be involved in interactions with G-proteins. The second and third extracellular loop, TM1, and TM4–6 are less conserved. The largest structural diversity occurs in the extracellular N-terminus. Potential sites for possible post-translational modification have been identified on the receptors. Two potential glycosylation sites are located in the N-terminal sequence. Two possible sites for protein kinase C phosphorylation are located in the C-terminus plus a third site is found in the third

intracellular loop; a possible palmitoylation site is also located in the C-terminus. Conserved Cys residues in the first and second extracellular loops may be involved in a disulfide linkage.

3.2.4.1 Mutagenesis Studies of Opioid Receptors. A variety of chimeric receptors between different opioid receptor types have been prepared in attempts to identify regions of the receptors that are involved in ligand recognition and receptor-type selectivity (see Refs. 156, 157 for recent reviews). What has emerged from these studies is a complex picture of the possible roles of different regions of the receptors. It has been suggested that the extracellular loops may play an exclusionary role rather than a direct role in ligand binding, acting as filters to prevent ligands selective for other receptor types from binding (158). However, which loop serves this role can be different for different receptors excluding the same ligand. Thus the study of μ/δ chimeric receptors suggested that the first extracellular loop (EL1) causes the loss of high affinity binding of the μ opioid agonist DAMGO to 6 receptors (159,160); subsequent site-directed mutagenesis studies suggested that Lys^{108} in EL1 of the 6 receptor was the residue responsible (161). In contrast, results for μ/κ chimeric receptors suggest that EL3 may be responsible for the low binding affinity of DAMGO to κ receptors (162, 163); site-directed mutagenesis identified Glu^{297}, Ser^{310}, Tyr^{312}, and Tyr^{313} as the residues in the κ receptor responsible for discriminating against DAMGO (164). The involvement of different loops also appears to be ligand dependent (see Ref. 157). The major determinant for binding of the p-selective alkaloids appears to involve the region of TM5 through TM7 (160), and similarily the binding of the δ-selective peptide DPDPE (24) to 6 receptors appears to involve this region near the C-terminus of the receptor (160). EL3 also seems to be important for the selectivity of several other 6-selective compounds, both peptide and nonpeptide ligands, for 6 over μ receptors (165, 166); point mutations indicated that Trp^{284}, Val^{296}, and Val^{297} were the crucial residues in EL3 of the δ receptor for δ selectivity (166).

Because of the number of acidic residues in EL2, it has been suggested that this region contributes directly to the affinity of κ receptors for the opioid peptide dynorphin A through ionic interactions with basic residues in the C-terminus of this peptide. Examination of both κ/μ (167, 168) and κ/δ (169) chimeric receptors supported the importance of EL2 for the high affinity of dynorphin A for κ receptors. However, in a recent study in which three or four of the seven acidic residues in EL2 of κ receptors were mutated to the corresponding amides, the mutant receptors still bound dynorphin A with high affinity, raising questions about the role of these acidic residues in dynorphin A affinity for κ receptors (170). The study of chimeric receptors also suggests that different domains of a receptor may be involved in interactions with different ligands. Thus, in contrast to dynorphin A, the κ-selective nonpeptide agonists U50,488 and U69,593 appear to require the whole κ receptor except for EL2 (167, 168). U69,593 also appears to bind to κ receptors differently than does naloxone, suggesting that agonists and antagonists may bind differently to this receptor (171).

Site-directed mutagenesis has been used to examine the roles of individual residues [see Refs. 156, 157 and the Center for Opioid Research website (http:Nwww.opioid.umn.edu) for a complete listing of opioid receptor mutations with references]. Thus the conserved Asp in both TM2 and TM3 have been substituted with neutral residues (172–174) to explore their roles in opioid ligand binding. Results for the mutation of the Asp in TM2 suggested that agonists and antagonists may bind differently to their receptors. Substitution of the Asp in TM3 dramatically reduced the affinity of some, but not all, opioids (174), raising the possibility that this is not a universal counterion for opioid ligands. The His in TM6 has also been implicated in the binding of ligands to the μ receptor (173), and this residue is conserved across the three receptor types. Mutation of Glu^{297} at the top of TM6 in κ receptors was used to demonstrate the importance of this residue for the κ-receptor affinity and selectivity of the nonpeptide κ-selective antagonist norBNI (175); conversely, the single mutation of the corresponding residue Lys^{303} in the μ receptor to Glu imparts high affinity for norBNI (121). Indeed, the close simi-

larity between the opioid and ORL1 receptors (see Section 7.2) has been clearly demonstrated by the ability to convert the ORL1 receptor to an opioid receptor with the mutation of only four residues in TM6 and TM7 (176). Site-directed mutagenesis has also been used to examine possible points of attachment of the affinity label β-FNA (177) (see Section 5.11.1 below).

Thus chimeric receptors and site-directed mutagenesis have provided tremendous insight into receptor-structure and receptor-ligand interactions. However, the data from these studies must be interpreted with caution, particularly in cases involving loss of function (157). Changes in the primary sequence of a receptor could have significant effects on the protein secondary or tertiary structure, which in turn could affect the affinities of various ligands. Thus whether observed changes in ligand affinity are attributable to the direct effect of the mutations or indirect effects that result from altering the tertiary structure of the protein is not known.

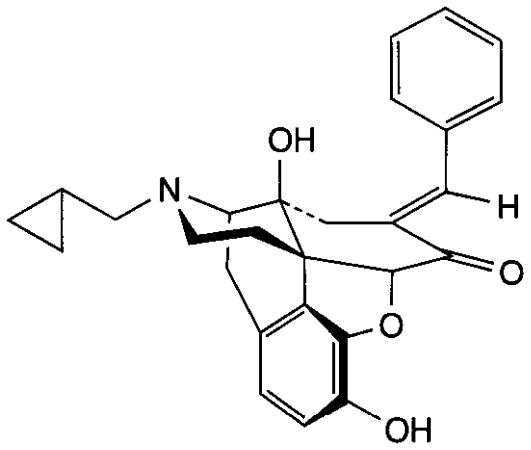
These studies demonstrate the complexities of ligand binding to opioid receptors and indicate that more research is required to more fully understand, at the molecular level, the interactions of different opioid ligands with their receptors.

3.2.4.2 Computational Models of Opioid Receptors. Because G-protein–coupled receptors are transmembrane proteins, until quite recently (178) structural information on these receptors was limited to low resolution (6–9 Å) structures from electron microscopy studies of rhodopsins. With the deduced amino acid sequences of the opioid receptors available, computational models of the three-dimensional structures of opioid receptors were developed based initially on these low resolution structures (166, 179–188). The rigid structures of many nonpeptide opioid ligands decreases the possible degrees of conformational freedom, decreasing the complexity of docking these compounds to opioid receptor models, and therefore several of the reports have described possible binding modes for these compounds. These include tifluadom-like benzodiazepine ligands at κ opioid receptors (179), naltrexone-derived antagonists at κ and δ receptors (180), morphinans including morphine to the μ receptor (182), arylacetamides to the κ receptor (182, 183, 186), fentanyl analogs at the μ receptor (182, 187), and piperazine and piperidine derivatives at δ receptors (182, 188). Modeling of the flexible opioid and opioid-like peptides bound to their receptors is more challenging, and therefore reports describing their receptor-binding modes have been more limited. Models have been developed for the binding of the conformationally constrained δ-selective peptides JOM-13 (Tyr-*cyclo*[D-Cys-Phe-D-Pen]OH) and DPDPE to δ opioid receptors (182), and the extracellular loops have been incorporated into computational models of κ receptors (181) and ORL1 receptors (189), so that possible binding modes of Dyn A-(1–10) and OFQN-(1–13) to these receptors could be examined. With the recent report of the X-ray structure of rhodopsin (2.8-Å resolution) (178), additional reports of computational models of opioid receptors derived from this structure are beginning to appear in the literature (see Refs. 156, 190).

3.2.4.3 Receptor Subtypes, Splice Variants, and Receptor Dimerization. Before cloning of the opioid receptors, subtypes of each of the three opioid receptors were proposed on the basis of evidence from pharmacological assays (see Refs. 69, 103 for excellent reviews), although opioid receptors identified by cloning have consistently represented only a single subtype for each receptor type. Currently, there is still considerable debate on the existence and nature of some of these receptor subtypes.

There is considerable evidence from both functional assays and binding studies supporting the existence of two types of δ opioid receptors (see Refs. 69, 191, 192). A key factor in the characterization of these δ-receptor subtypes was the availability of ligands selective for each of the proposed subtypes (Fig. 7.8). The δ_1 subtype was characterized by its preferential stimulation by the enkephalin analog DPDPE (24) and DADLE (21) and selective antagonism by the naltrindole analog BNTX (7-benzilidenenaltrexone, 44) (193) and the affinity label (see below) DALCE (45) (194). The δ_2 subtype was distinguished by its stimulation by [D-Ala^2]deltorphin II (25) and DSLET (22) and antagonism by the benzofuran naltrindole analog naltriben (46) (195)

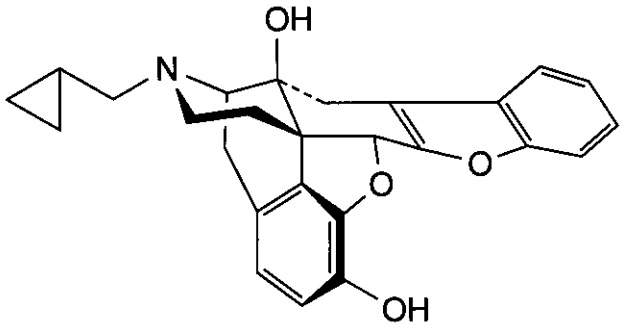
Delta receptor ligands:



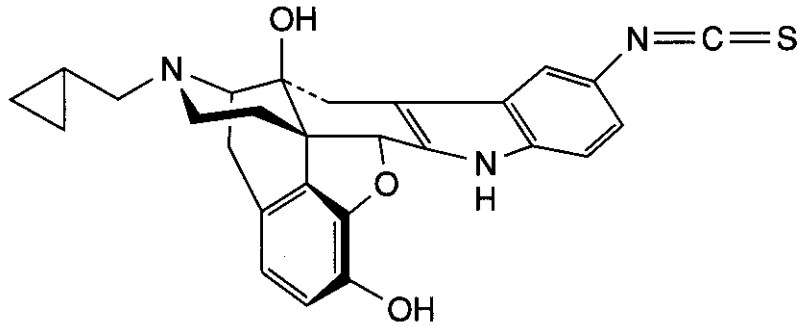
(44) BNTX

Tyr-D-Ala-Gly-Phe-Leu-Cys

(45) DALCE

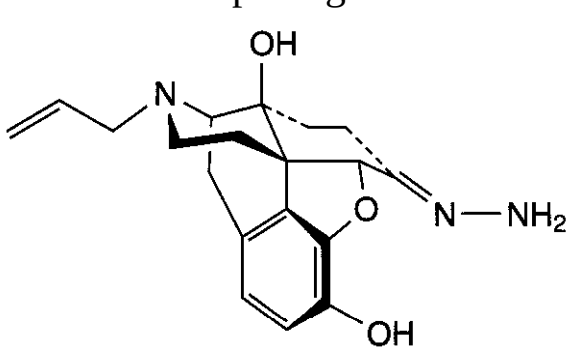


(46) Naltriben

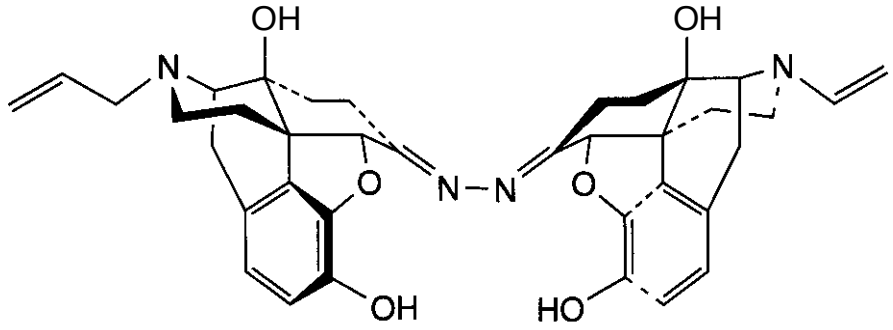


(47) Naltrindole isothiocyanate (NTII)

Mu receptor ligands:

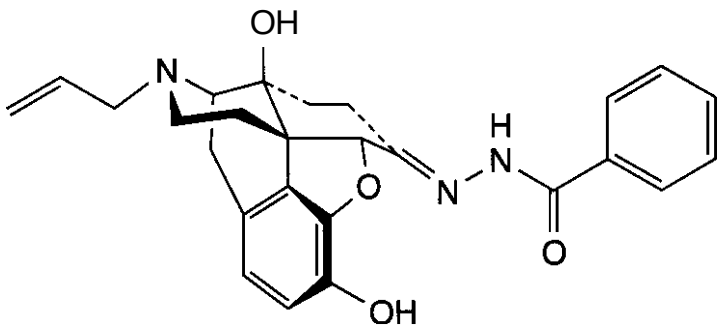


(48) Naloxazone



(49) Naloxonazine

Kappa receptor ligand:



(50) Naloxone benzoylhydrazone (Nal(Bzo)H)

Figure 7.8. Ligands used to characterize opioid receptor subtypes.

and the irreversible antagonist naltrindole isothiocyanate (NTII, 47) (196). Delta-receptor subtypes were also proposed by Rothman and coworkers which were differentiated by whether they were associated with a μ-δ receptor complex (δ_{cx}) or were not associated with such a complex (δ_{ncx}) (see Ref. 197 for a review). These researchers used the irreversible ligands (see Section 5.11) FIT (198) or (+)-transSUPERFIT (199) to selectively acylate δ_{ncx} receptors (200) and used the μ-receptor affinity label BIT (198) to deplete the membranes of δ_{cx} receptors (201). On the basis of binding and pharmacological studies Rothman and coworkers proposed that the δ_{ncx} and the δ_{cx} receptors corresponded to the δ_1 and 6,

receptors, respectively (202, 203). Pharmacological characterization of the cloned 6 opioid receptor is consistent with classification as the δ_2 subtype (204), and antisense oligonucleotides differentially affect the two subtypes (205). In δ-receptor knockout mice binding of both [^{3}H]-DPDPE and [^{3}H]-[D-Ala^2]deltorphin II is absent (206), indicating that the proposed subtypes are not due to different gene products; however, selective 6 agonists still retain analgesic potency, suggesting the existence of a second 6-like analgesic system (206).

Multiple types of μ opioid receptors have been proposed by Pasternak and coworkers. Two subtypes of μ receptors, the μ_1 site, which was suggested to be a common high affinity site for both nonpeptide opioids and opioid peptides, and the μ_2 site, which was proposed to correspond to the "traditional" p-binding site (207), were initially postulated by these researchers (see Refs. 69,208). The hydrazone derivative of naloxone, naloxazone (48) (209), and its dimeric azine derivative naloxonazine (49) (210) have been used to study the putative μ_1 receptors (see Ref. 69), which is thought to represent about 20% of the specific binding to rat brain membranes (208). Following the cloning of the opioid receptors, Pasternak and coworkers characterized multiple splice variants of the μ receptor (see Refs. 211, 212 for reviews).

Three or more subtypes of κ receptors have been postulated. In the early 1980s several groups reported differences between the binding of [^{3}H]EKC and [^{3}H]diprenorphine or [^{3}H]etorphine (after blockade of μ and δ receptors), suggesting that there might be κ-receptor subtypes; there was considerable debate, however, concerning the nature of these different binding sites (see Refs. 69, 213). Subsequently, the κ-selective arylacetamides U50,488 and U69,593 helped to clarify the definition of different κ-receptor subtypes, and two populations of binding sites, κ_1 versus κ_2, were differentiated on the basis of their sensitivity and insensitivity, respectively, to these arylacetamides (214). The cloned κ receptors appear to be the κ_1 subtype on the basis of pharmacological characterization (204) and the analgesic activity of U50,488 is abolished in κ-receptor knockout mice (215) (other effects of U50,488, hypolocomotion and dysphoria, are decreased in these animals). Mice deficient in each of the opioid receptors and animals lacking all three opioid receptors have been examined for residual κ_2 receptor bindingsites (216); all of the residual non-K, receptor labeling could be accounted for by μ and 6 receptors, and the triple receptor deficient mice exhibited no residual binding of [^{3}H]bremazocine, indicating that no other gene product was involved in binding this opioid ligand. A third κ-receptor subtype, κ_3, has also been proposed on the basis of studies that used naloxone benzoylhydrazone, Nal(Bzo)H (50) (217).

Recently, an alternative explanation for opioid receptor subtypes, receptor dimerization, has appeared in the literature that could explain why different gene products have not been identified for the proposed receptor subtypes in spite of the evidence from pharmacological assays for their existence. The details of these studies are discussed under Recent Developments (Section 7.1) below.

3.3 Physiology of Non-μ Opioid Receptors

Because of the side effects associated with analgesics that interact with μ opioid receptors, there has been considerable interest in developing opioid ligands that interact with other opioid receptors. In addition to analgesic activity, there are a number of other potential therapeutic applications of ligands, both agonists and antagonists, for non-μ opioid receptors.

3.3.1 Delta Receptors. There has been considerable interest in 6 opioid agonists because they exhibit antinociceptive effects without the side effects associated with μ opioid receptor agonists. Antinociceptive activity was first demonstrated with 6-selective opioid peptides (see Ref. 218 for a review), and more recently with nonpeptidic 6-selective agonists (see Refs. 219–222 for reviews). Of particular interest is the activity of δ agonists in inflammatory and neuropathic pain (220). Delta opioid receptors also modulate μ opioid receptors and, as discussed earlier, one classification of δ opioid receptor subtypes was based on their association with μ opioid receptors. There is now considerable evidence that interaction between the two receptor types can alter the activity of μ opioid agonists. Delta agonists

can potentiate the analgesic activity of μ agonists (223,224) and δ antagonists can decrease the development of tolerance and dependence to morphine (225, 226). Although the δ-selective antagonist naltrindole can block the development of tolerance to the analgesic effects of morphine (225), it does not block the beneficial development of tolerance to the respiratory depressant effects of morphine (227). Delta ligands can also reverse the respiratory depression caused by μ agonists, and interestingly, δ agonists as well as antagonists were reported to have similar effects on respiration (228). Thus δ receptor ligands, particularly δ antagonists, could have important therapeutic applications to minimize the deleterious effects of morphine. These findings have prompted the search for compounds that exhibit both μ agonist and δ antagonist activity, and peptidic ligands with this mixed activity have been reported by Schiller to produce potent analgesic effects without physical dependency and less tolerance than that of morphine (229). Nonpeptide ligands with mixed activity have also recently been reported (230) (see Section 5.3.3 below).

Delta receptors may also modulate responses to substances of abuse other than opioids, including cocaine, amphetamines, and alcohol (219, 220, 222). Several δ agonists have been reported to at least partially discriminate for cocaine (see Ref. 222). There are numerous reports that δ-receptor antagonists can attenuate a number of the effects of cocaine (see Refs. 219, 220, 222), and an antisense oligodeoxynucleotide to δ opioid receptors blocks cocaine-induced place preference in mice (231). Thus δ antagonists could potentially be used in the treatment of cocaine abuse. One study, however, found that the δ-receptor antagonist naltrindole can potentiate the lethal effects of cocaine; this was found only after intracisternal administration and not after i.v. administration, so it is not clear whether this would limit the application of δ-receptor antagonists in treatment of cocaine abuse (232). Similar results have been found for δ agonists and antagonists in methamphetamine-induced place preference (see Ref. 222); one important finding was that the δ agonist DADLE can protect against the neuronal damage caused by methamphetamine (see Refs. 233, 234 and references cited therein). There is considerable evidence that endogenous opioids and opioid receptors play important roles in the abuse of alcohol (235, 236), and the opioid receptor antagonist naltrexone has been used to treat alcoholism. The involvement of δ opioid receptors in alcohol abuse, however, remains unclear. Delta-selective receptor antagonists have been examined for their ability to decrease alcohol consumption in animal models, but the results have been mixed, with some studies reporting decreased alcohol consumption, whereas others observed no effects (see Refs. 222,236 for reviews). One possible explanation for this discrepancy could be differences in the genetic backgrounds of the animals studied.

Delta receptors are also involved in a number of other biological effects, including both centrally and peripherally mediated effects (see Refs. 219,220,222 for reviews). Delta agonists are effective in learned helplessness animal models, suggesting a potential therapeutic application in depression (237). Convulsant effects have been observed with nonpeptide δ agonists [particularly BW373U86 (see Section 5.10.1], however, which could prevent their clinical use unless these adverse effects can be separated from their desired action (e.g., antinociceptive activity; see Ref. 238). Opioids, including δ receptor ligands, have immunomodulatory effects (239). Delta agonists have been reported to stimulate immune responses, whereas δ antagonists can cause immunosuppression (see Refs. 219, 220). The immunomodulatory effects are still present in δ receptor knockout mice (240), however, indicating that these effects are not mediated by these receptors.

3.3.2 Kappa Receptors. Like μ and δ receptors, activation of κ opioid receptors can produce antinociceptive effects. However, the effectiveness of κ opioid agonists as antinociceptive agents varies in different types of pain (see Ref. 146), and κ agonists are less effective in thermal antinociceptive assays involving more intense stimuli.

Kappa agonists can also produce antinociceptive effects in inflammatory pain through interaction with peripheral κ opioid receptors. Kappa agonists have been shown to have anti-

arthritic activity (241, 242), and to produce antinociception in capsaicin-induced thermal allodynia through interaction with peripheral κ receptors (243, 244). Because many painful conditions are associated with inflammation, there is significant potential for the application of κ agonists as peripheral analgesics. Peripheral κ opioid receptors also appear to be involved in visceral pain and the activity of κ agonists is enhanced in the presence of chronic inflammation of the viscera (245, 246), suggesting potential therapeutic applications of peripherally selective agents without the side effects (e.g., dysphoria) associated with many centrally acting κ agonists.

In many cases activation of central κ opioid receptors opposes the activity of μ opioid receptors (see Ref. 247 for a recent review). Several studies have reported that κ agonists at doses that do not affect the baseline pain threshold can antagonize the analgesic activity of μ opioid agonists (see Ref. 247). The κ-opioid peptide dynorphin A-(1–13) has been reported to improve the memory impairment induced by the μ agonist DAMGO (248). Kappa agonists also reduce tolerance to morphine in a variety of antinociceptive tests, and dynorphin can inhibit morphine withdrawal symptoms in morphine-dependent animals (see Ref. 247 and references cited therein). Kappa agonists generally lack reinforcing effects and can abolish the reinforcing effects of morphine (see Ref. 247). This effect of κ agonists on morphine reward may be attributed to the opposing modulation of the mesolimbic dopamine system by μ and κ opioid receptors, where μ agonists increase dopamine levels while κ agonists decrease dopamine levels. Kappa agonists also generally have opposite subjective effects from μ opioid agonists (dysphoria for κ agonists versus euphoria for μ agonists). Chronic administration of morphine and other drugs with positive reinforcing effects increases brain levels of the endogenous κ opioid peptide dynorphin (see Ref. 249 and references cited therein). It has been proposed (249) that this produces an imbalance in abstinent μ opioid-dependent individuals and dysphoric mood states, which can result in the desire to take μ opioid agonists to normalize mood. Thus on the basis of this "κ overdrive" model, a κ-receptor antagonist could be useful to normalize this imbalance and treat opioid addiction (249).

The effects of κ opioid agonists on dopamine levels also has implications for the treatment of cocaine abuse. Cocaine blocks reuptake of dopamine, and considerable evidence suggests that cocaine's reinforcing effects are mediated by these increases in extracellular dopamine (see Refs. 250, 251 and references cited therein). Because κ agonists can decrease dopamine levels, they can act as functional antagonists of cocaine (250, 251). Several κ agonists have been shown to decrease cocaine self-administration (250–254; but see Ref. 255) and κ agonists can also attenuate many of the behavioral effects of cocaine (see Ref. 250).

Central κ opioid receptors also mediate other effects that could be therapeutically beneficial. Kappa agonists exhibit neuroprotective effects in stroke (256); these effects are observed in experimental models even several hours after blood vessel occlusion (see Ref. 257 and references cited therein), which is particularly promising for the potential therapeutic use of κ agonists in stroke. Dynorphin improves memory dysfunction in an animal model of amnesia (see Ref. 247). Kappa opioid agonists have also been shown to cause down-regulation of HIV expression in human microglial cells (258) and $CD4^+$ lymphocytes (259), which raises the intriguing possibility of using κ ligands as adjuvant therapy in HIV-infected individuals; whether this will have clinical applicability remains to be determined.

3.4 Endogenous Opioid Peptides

During the mid-1970s the search for endogenous ligands for opioid receptors led to the discovery of peptides with opiate-like activity. The first opioid peptides reported were the pentapeptides leucine and methionine enkephalin (3and 4) (7), followed shortly thereafter by dynorphin A (38) (260,261) and @endorphin (51)(262). Because these peptides are structurally distinct from the alkaloid opiates, the term *opioid* was introduced to describe all compounds, both nonpeptide and peptide, with opiate-like activity. These mammalian opioid peptides share a common N-terminal tetrapeptide sequence, but differ in their C-terminal residues (Fig. 7.9). They also differ in

Proenkephalin peptides

Leu-Enkephalin (3)	Tyr-Gly-Gly-Phe-Leu
Met-Enkephalin (4)	Tyr-Gly-Gly-Phe-Met
Met-enkephalin-Arg^6-Phe^7	Tyr-Gly-Gly-Phe-Met-Arg-Phe
Met-enkephalin-Arg^6-Phe^7-Leu^8	Tyr-Gly-Gly-Phe-Met-Arg-Gly-Leu

Prodynorphin peptides

Dynorphin A (38)	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln
Dynorphin B	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Gln-Phe-Lys-Val-Val-Thr
α-Neoendorphin	Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Arg-Pro-Lys
β-Neoendorphin	Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Arg-Pro

Pro-opiomelanocortin peptides

β_h-endorphin (51)	Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu

Endomorphins

Endomorphin-1 (52)	Tyr-Pro-Trp-PheNH_2
Endomorphin-2 (53)	Tyr-Pro-Phe-PheNH_2

Orphanin FQ/nociceptin and related peptides

Orphanin FQ/ Nociceptin (54)	Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln
Orphanin FQ 2	Phe-Ser-Glu-Phe-Met-Arg-Gln-Tyr-Leu-Val-Leu-Ser-Met-Gln-Ser-Ser-Gln
Nocistatin (human)	Pro-Glu-Pro-Gly-Met-Glu-Glu-Ala-Gly-Glu-Met-Glu-Gln-Lys-Gln-Leu-Gln

Figure 7.9. Mammalian opioid peptides and orphanin FQ/nociceptin.

the receptor types with which they preferentially interact. Although the enkephalins exhibit some preference for interacting with δ receptors (Table 7.9), the dynorphins preferentially interact with κ receptors; β-endorphin possesses high affinity for both μ and δ receptors. This led Goldstein to apply the "message-address" concept (see also Section 6.3) to the opioid peptides (263). In Goldstein's proposal the common N-terminal tetrapeptide sequence constituted the "message" sequence responsible for activating opioid receptors, whereas the unique C-terminal sequences functioned as "address" components to direct the peptides to particular opioid receptors.

Recently, a new class of opioid peptides, the endomorphins (52 and 53, Fig. 7.9), were discovered (264) that do not share the classical "message" sequence with other mammalian opioid peptides. In contrast to other mammalian opioid peptides, the endomorphins show high selectivity for their preferential receptor, the μ receptor (Table 7.9). Since their discovery the pharmacology of these new mammalian opioid peptides has been studied extensively (see Ref. 265 for a detailed review).

Table 7.9 Opioid Receptor Affinities and Opioid Activity in the GPI and MVD of Endogenous Opioid Peptides[a,b]

Peptide	K_i (nM)			K_i Ratio	IC_{50} (nM)	
	μ	6	κ	$\mu/\delta/\kappa$	GPI	MVD
Proenkephalin peptides						
Leu-enkephalin **(3)**	19	1.2	8,210	16/1/6,840	36	1.7
Met-enkephalin (4)	9.5	0.91	4,440	10/1/4,880	6.7	1.5
Met-enkephalin-Arg^6-Phe^7	3.7	9.4	93	1/2.5/25	10	5.3
Met-enkephalin-Arg^6-Gly^7-Leu^8	6.6	4.8	79	1.4/1/16	35	2.9
Prodynorphin peptides						
Dynorphin A **(38)**	0.73	2.4	0.12	6.1/20/1	0.29	0.91
Dynorphin **A-(1–8)**	3.8	5.0	1.3	2.9/17/1	4.9	9.2
Dynorphin B	0.68	2.9	0.12	5.7/24/1	0.25	2.1
β-Neoendorphin	6.9	2.1	1.2	5.7/1.8/1	3.3	9.9
α-Neoendorphin	1.2	0.57	0.20	6.0/2.8/1	3.0	7.7
POMC peptide						
β_h-Endorphin (**51**)	2.1	2.4	96	1/1.1/46	62	40
Endomorphins"						
Endomorph-1 **(52)**	0.36	1,506	5,428	1/4,183/15,077	3.6	—
Endomorphin-2 **(53)**	0.69	9,233	5,240	1/13,381/7,594	4	—

"Data from Ref. 130 except where otherwise indicated.
[b]See Section 7.2 for data on orphanin FQ/nociceptin.
'Data from Ref. 264.

After the identification of the ORL1 receptor, two groups isolated a 17-residue peptide (54, Fig. 7.9) as the endogenous ligand for this receptor (92, 93). This peptide was referred to as orphanin FQ by one group because it was the ligand for the orphan receptor (FQ are the N- and C-terminal residues, respectively, of the peptide) (92) and named nociceptin by the other group, since in the initial studies this peptide was reported to produce hyperalgesia (93). Although the N-terminal tetrapeptide sequence of orphanin FQ/nociceptin (OFQ/N) is similar to that of the classical opioid peptides, the presence of Phe rather than Tyr in position 1 is an important factor in the peptide's high selectivity for ORL1 over opioid receptors (see Section 7.2).

The classical mammalian opioid peptides are derived from three distinct precursor proteins (Fig. 7.10) (see Refs. 75,266 for reviews). Processing of the precursor proteins occurs at pairs of basic residues. β-Endorphin is derived from proopiomelanocortin (POMC), along with ACTH, a-MSH, and β-lipotropin (see Ref. 267 for a review). The enkephalins are derived from proenkephalin A (see Ref. 268 for a review). This protein contains four copies of Met-enkephalin flanked by pairs of basic residues along with one copy of Leu-enkephalin. In addition, extended Met-enkephalin derivatives Met-enkephalin-kg^6-Phe^7 and Met-enkephalin-Arg^6-Gly^7-Leu^8 and longer peptides (e.g., peptides E and F and BAM-20) are obtained from proenkephalin A. The dynorphins, which contain a number of basic residues in the C-terminus, are derived from prodynorphin (also called proenkephalin B; see Ref. 269 for a review). In addition to dynorphins A and B, longer dynorphins (e.g., dynorphin 32, which contains both dynorphin A and B) and a- and β-neoendorphins are obtained from prodynorphin B. The precursor protein of the endomorphins has yet to be identified. The OFQ/N precursor protein prepronociceptin (93) has been characterized and also contains additional biologically active peptides related to OFQ/N, orphanin/nociceptin 2 (270) and nocistatin (271) (Fig. 7.10).

4 HISTORY: IDENTIFICATION OF MORPHINE AND EARLY ANALOGS

The effects of opium have been known for thousands of years, with written references dating as far back as the third century B.C.

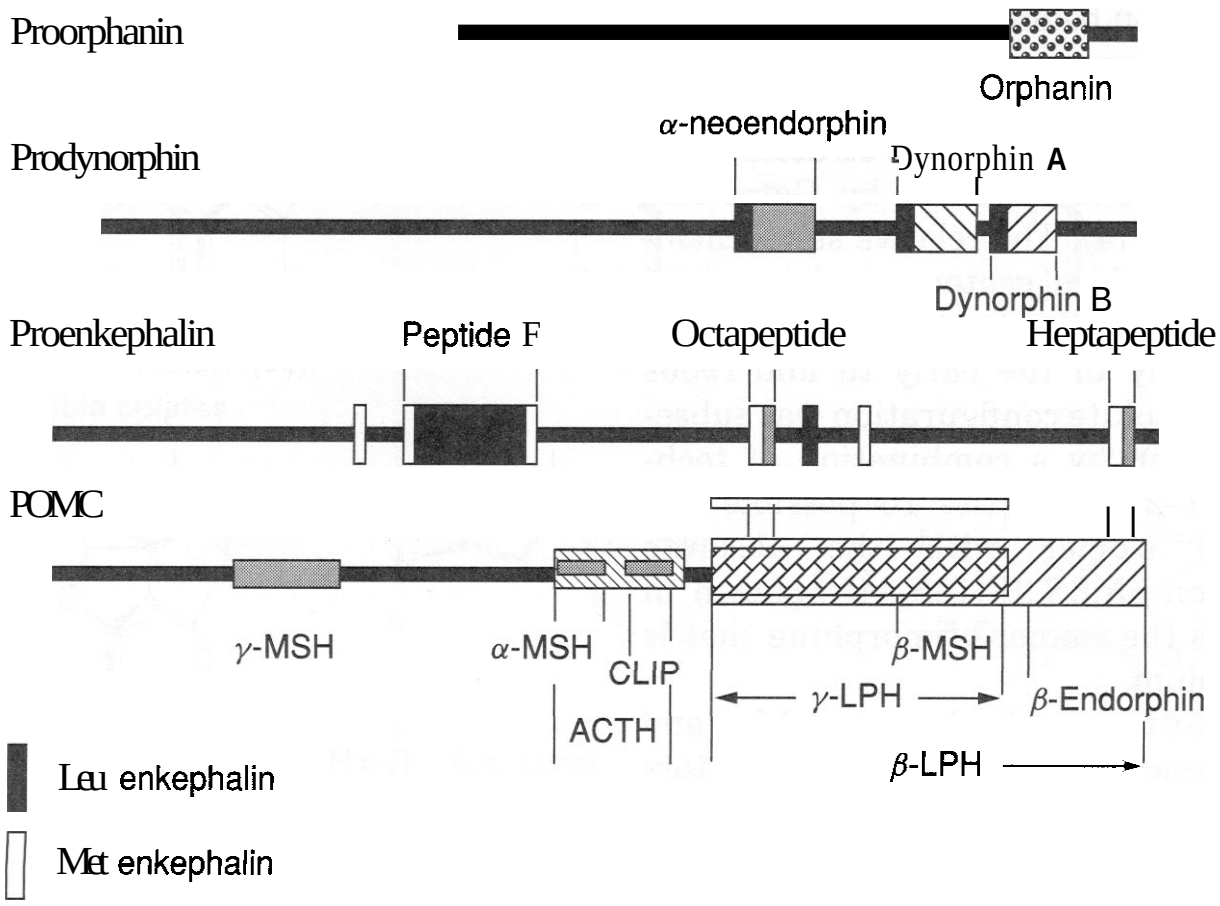
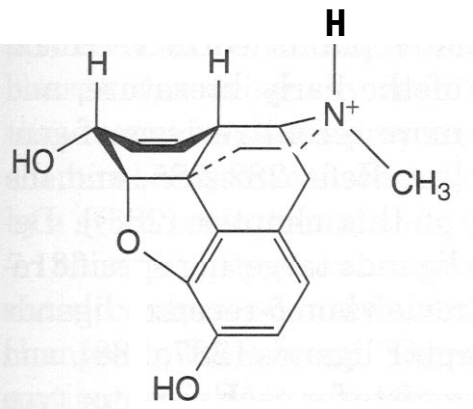
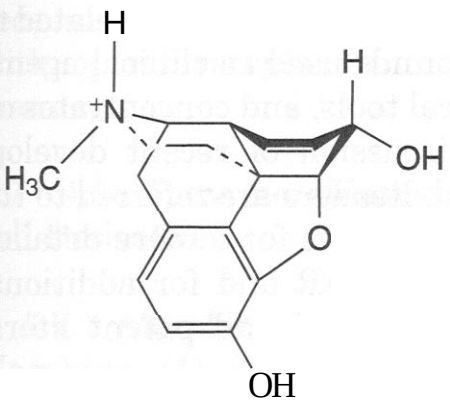


Figure 7.10. Peptide precursors and processing products (taken from Ref. 100).

Morphine was first isolated from opium in 1805 by Sertürner, who named it after Morpheus, the Greek god of sleep and dreams. The 3-methyl ether codeine (2, Fig. 7.1) was subsequently isolated from the poppy plant *Papaver somniferum* in 1832. By the mid-1800s the purified alkaloids morphine and codeine began to be used in place of crude opium preparations for medicinal purposes **(23).** Because of its complex structure, it took more than a century



(–)-Morphine(1)



(+)-Morphine

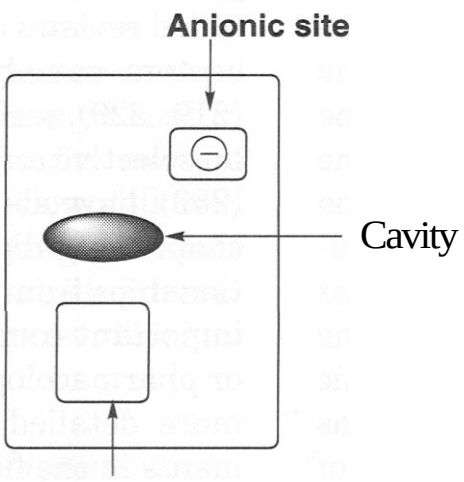
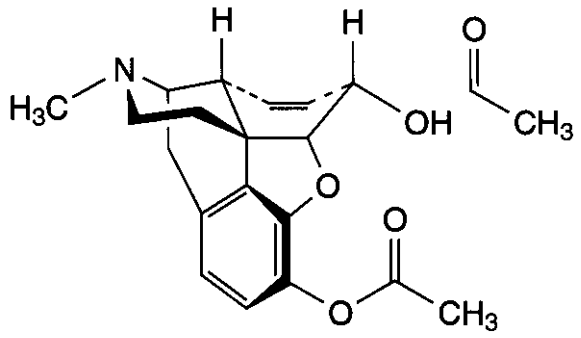


Figure 7.11. Beckett and Casy model for opioid receptor binding (adapted from Ref. 1**).**

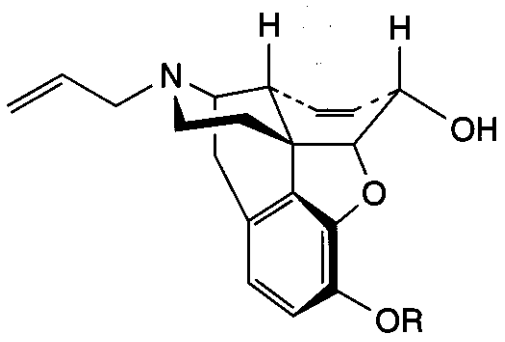
after its isolation before the correct structure of morphine was proposed by Gulland and Robinson in 1925 (272). It was not until the early 1950s that the proposed structure was confirmed by total synthesis by Gates and Tschudi (273, 274). The relative stereochemistry of the five chiral centers was determined by chemical synthesis (275) and X-ray crystallography in the early to mid-1950s (276). The absolute configuration was subsequently proven by a combination of techniques (277–279). Of the 16 possible isomers, one isomer with the absolute configuration 5*R*,6*S*,9*R*,13*S*,14*R*, shown in Fig. 7.11, is the isomer of morphine that is found in opium.

The earliest model for how morphine and other analgesics interact with opioid receptors was proposed by Beckett and Casy (Fig. 7.11) (1). Based on the SAR of analgesics available, this model consisted of three sites: an anionic binding site, which interacted with the protonated amine on the ligand; a flat surface that interacted with the aromatic ring of the analgesic; and a cavity to accommodate the piperidine ring of the rigid opioid alkaloids. This model explained the stereospecificity of opioid receptors; whereas the natural (−)-isomer of morphine fits all three sites, in the unnatural (+)-isomer the projecting ring would not fit properly into the cavity (Fig. 7.11).

Synthetic efforts to modify the structure of morphine began with the synthesis of 3,6-diacetylmorphine (heroin, 55, Fig. 7.12) in the late 1800s (280). The first narcotic antagonist N-allylnorcodeine (57) was prepared in 1915 (281). The subsequent demonstration of the antagonist activity of N-allylnormorphine (nalorphine, 56) in the 1940s stimulated the examination of other N-substituted morphine analogs for antagonist activity. The exploration of the SAR of morphine and its antagonist derivatives was actively pursued during the 1950s and 1960s, along with related synthetic morphinan and benzomorphan analogs. It was the differences observed in the patterns of pharmacological activity for different compounds that resulted in the proposal of multiple opioid receptors in the 1960s and their subsequent classification by Martin and coworkers in the mid-1970s (5, 6) (see Section 3.2.1).



(55) Heroin



R = H **(56)** Nalorphine

R = CH_3 **(57)** N-Allylnorcodeine

Figure 7.12. **Early** synthetic analogs of morphine.

5 STRUCTURE-ACTIVITY RELATIONSHIPS OF NONPEPTIDE OPIOID LIGANDS

5.1 Introduction

In a single chapter such as this it is impossible to discuss the SAR of all of the compounds with opioid activity in detail. Two comprehensive books on opiates published in 1986 (12, 13) cover much of the early literature, and there have been more recent reviews of general opioid SAR [see Refs. 282–285, and the previous edition of this chapter (286)]. Detailed reviews on ligands targeting specific receptors, namely, reviews on δ-receptor ligands (219, 220), κ-receptor ligands (287, 288), and on selective antagonists for each receptor type (289), have also recently been published. This chapter highlights key structure-activity relationships from the earlier literature related to important compounds used as clinical agents or pharmacological tools, and concentrates on more detailed discussion of recent developments in the field. Readers are referred to the above-mentioned reviews for a more detailed discussion of opioid SAR and for additional references to the journal and patent literature.

Compounds with opioid activity are structurally diverse, ranging from rigid multicyclic

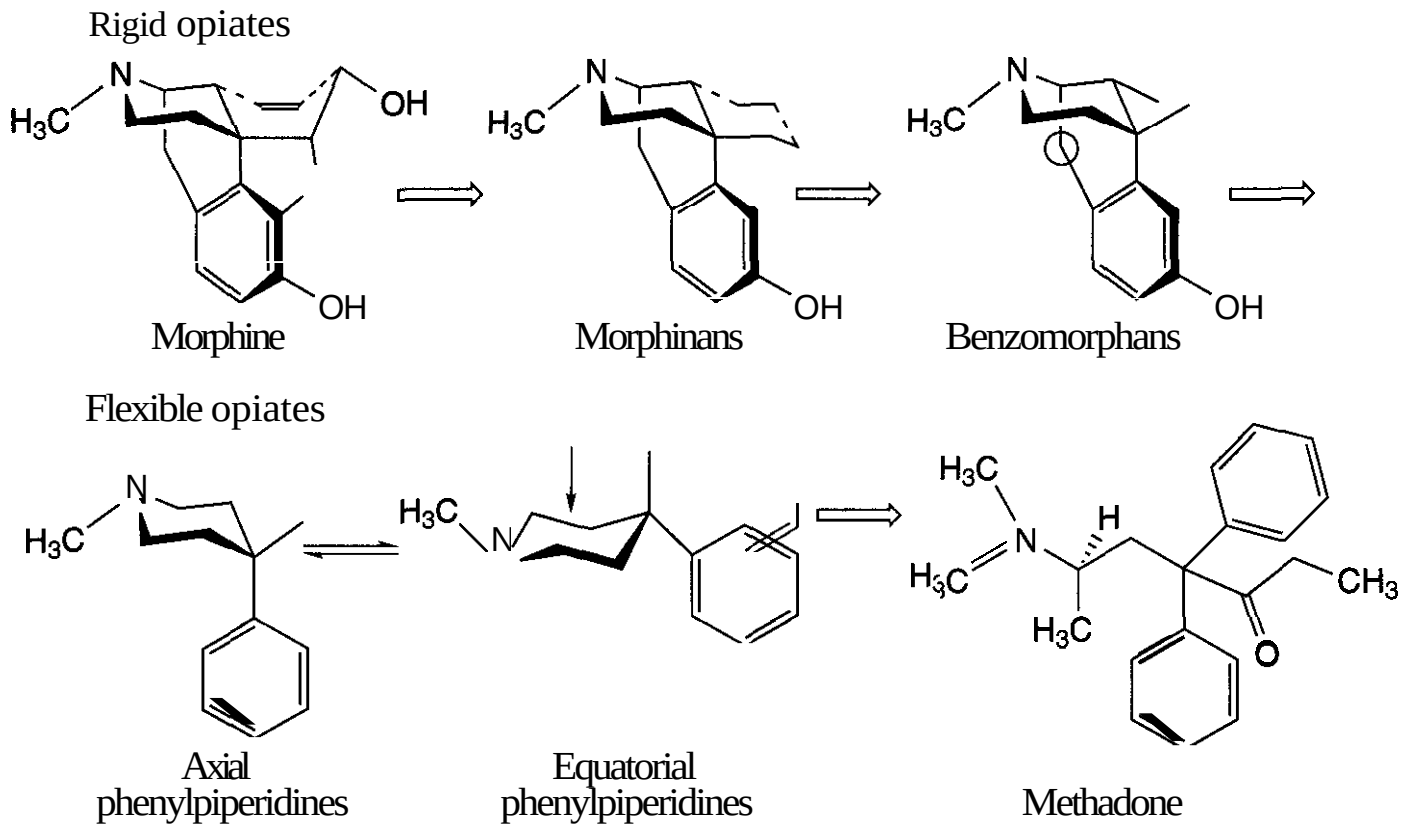


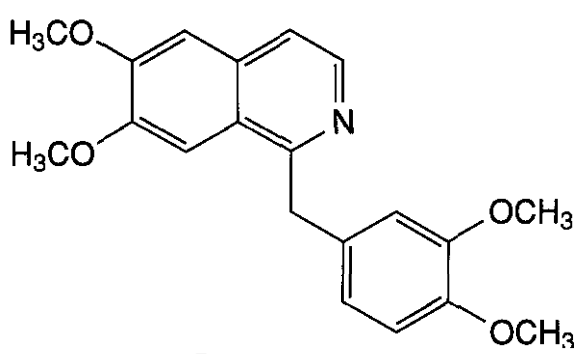
Figure 7.13. Systematic dismantling of morphine (adapted from Ref. 290).

compounds such as **morphine** to flexible acyclic analgesics such as methadone and the **opioid** peptides. The majority of the classical **nonpeptide** opioids fall into one of five chemical classes: the **4,5α-epoxymorphinans**, the **morphinans**, the **benzomorphans**, **phenylpiperidine** derivatives, and acyclic analgesics. **Although** not related in many cases to how the opioid activity of these different chemical classes was originally identified nor to how these compounds are prepared, these different chemical classes can be related conceptually by a systematic dismantling of morphine (Fig. **7.13).** As rings present in morphine are eliminated, conformational flexibility increases and changes in **SAR** occur. The effects on conformation and **SAR** are discussed for each of these chemical classes in turn below, followed by descriptions of agonists selective for κ and δ receptors and of affinity labels, which interact with opioid receptors in a **nonequilibrium** manner.

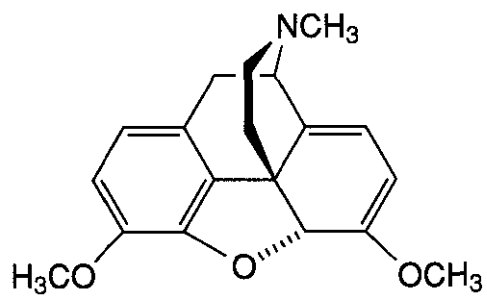
5.2 4,5α-Epoxymorphinans: Morphine and Derivatives

5.2.1 Morphine and Alkaloids from Opium.

Morphine **(1)** is the principal alkaloid in opium, which is derived from the seed capsules of the poppy plant *Papaver somniferum.* Opium contains over 50 alkaloids that fall into one of two chemical classes: the **phenanthrene** class, including **morphine** and related derivatives; and the benzylisoquinoline alkaloids, such as **papaverine** (58, Fig. 7.14; see Ref. 291 for a complete listing of alkaloids present in opium). In addition to morphine, which on average accounts for 10–20% of opium, other **re-**



(58) Papaverine



(59) Thebaine

Figure 7.14. Alkaloids found in opium. The structures of morphine **(1)** and codeine **(2)** are shown in Fig. 7.1.

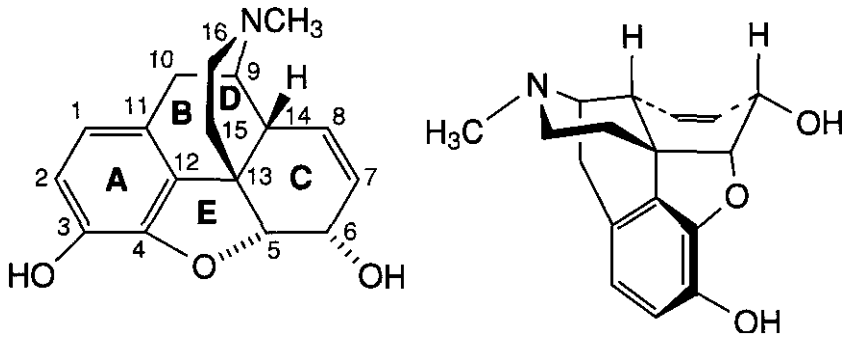


Figure 7.15. Structure **of morphine.**

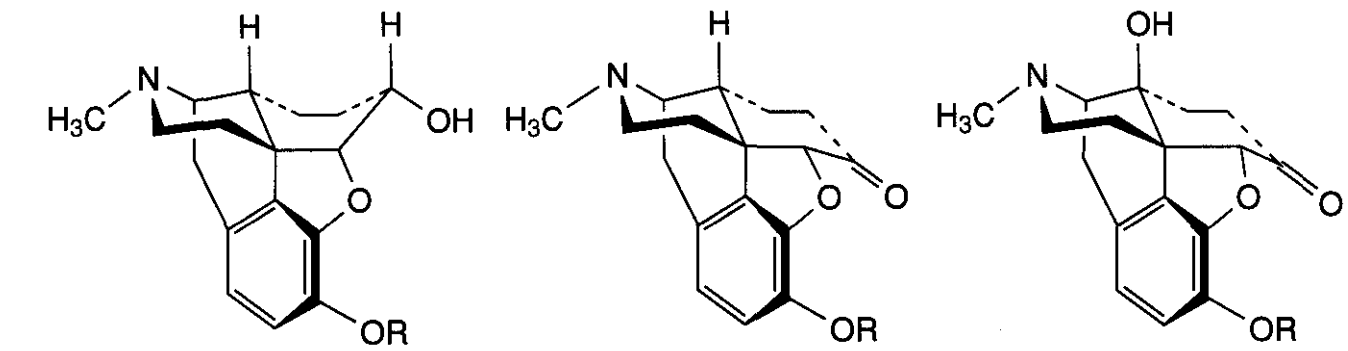
lated alkaloids to morphine found in opium include codeine (2) (0.5%) and thebaine (59) (0.3%). Whereas the latter is inactive as an analgesic, it is a key synthetic intermediate for the preparation of several potent analgesics (see Section 5.4 below); although present in low amounts in P. sornniferum, it is the principal alkaloid found in another species of poppy, *Papaver* bracteaturn (292).

One interesting finding has been the detection of morphine and related opiate alkaloids in vertebrates, including in a variety of mammalian tissues (see Refs. 58, 293–295 for reviews; see also Ref. 296 for a review of isolation techniques). The conversion of codeine and thebaine, which are known intermediates in the biosynthetic pathway in P. somniferum, to morphine in several tissues from rat supports the conclusion that the morphine found in animals is of endogenous origin. Endogenous morphine has been postulated to be involved in neural and immune regulation (see Ref. 295), but the levels detected in mammalian tissues are low [low pmol/g (295)] compared to those of the endogenous opioid peptide levels (297), so what physiological roles such endogenous opiate alkaloids play are still unclear.

The conformation of morphine was determined from X-ray studies (see Ref. 298 for a review). The overall shape of the molecule is a three-dimensional "T," with rings A, B, and E forming the vertical portion of the "T" and rings C and D forming the top of the "T" (Fig. 7.15). The piperdine (D) ring is in the energetically favored chair conformation, but the C ring of morphine is in a boat conformation, which places the 6α-hydroxyl in the equatorial position (see Ref. 298). (+)-Morphine, the enantiomer of the naturally occurring (−)-morphine, has been synthesized (see Refs. 283, 299) and is devoid of analgesic activity.

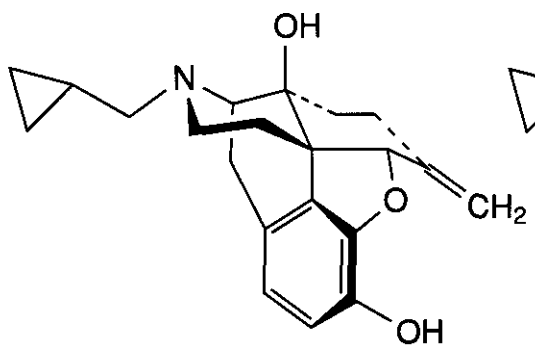
5.2.2 Morphine Derivatives. The synthesis of 3,6-diacetylmorphine (heroin, 55) in the late 1800s (280) marked the beginning of synthetic efforts to modify the structure of morphine, and since then numerous structural modifications have been made to morphine (see Refs. 299, 300 for detailed reviews). Although modifications have been made to all portions of the molecule, the focus has been in three regions: the phenol at position 3, the C ring, and the basic nitrogen. The phenol, although not critical for activity, enhances opioid receptor affinity (301). Masking of the phenolic hydroxyl group generally decreases opioid activity. Methylation of the 3-phenol of morphine gives codeine (2, Fig. 7.1), which has approximately 10–20% the potency of morphine. As noted in Section 2.3.2, a small but significant percentage (−10%) of codeine is *O*-demethylated to morphine, accounting for its analgesic activity. Heroin, on the other hand, is approximately twice as potent as morphine. Heroin penetrates the blood-brain barrier very rapidly [68% uptake of heroin versus unmeasurable uptake of morphine 15 s after carotid injection into rats (302)], and this may partially account for its greater potency. Heroin is rapidly deacetylated to give 6-acetylmorphine, which has potency similar to that of morphine. As discussed in Section 2.3.2, the 6-glucuronide metabolite of morphine (12) is about twice as potent as morphine (58) and may account for a significant portion of morphine's analgesic activity, especially with chronic use (59).

Much of the early synthetic efforts focused on modifications of the C ring. Changing the B-C ring juncture in morphine or codeine from cis to trans decreases potency 2- to 10-fold (303). This is understandable because such a change causes severe distortion in the C ring

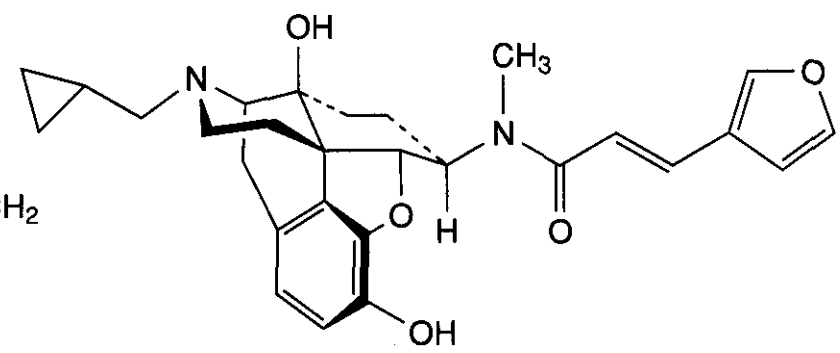


R = H **(60)** Dihydromorphine **(5)** Hydromorphone **(63)** Oxymorphone

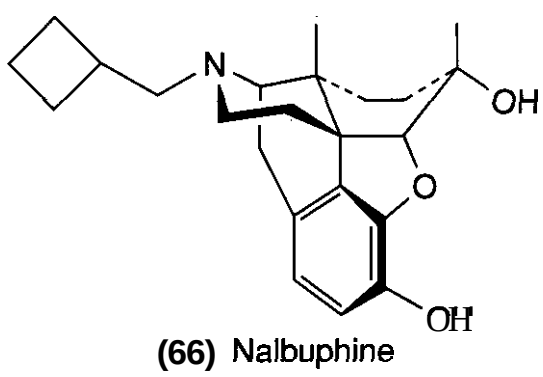
R = CH3 **(61)** Dihydrocodeine **(62)** Hydrocodone **(6)** Oxycodone



(64) Nalmefene



(65) TRK-820



(66) Nalbuphine

Figure 7.16. C-ring derivatives of morphine. Structures of hydromorphone **(5)** and oxycodone **(6)** from Fig. **7.1** are included for comparison. Nalmefene (64) is an antagonist, TRK-820 (**65**) is a κ-selective agonist, and nalbuphine **(66)** is a mixed agonist/antagonist.

of *trans-morphine*. Reduction of the C_7-C_8 double bond of morphine and codeine yields **dihydromorphine** (60, Fig. 7.16) and **dihydrocodeine** (**61**), respectively, which have similar to slightly increased (15–20%)potencies compared to those of their parent compounds. Oxidation of the C_6 **hydroxyl** in **morphine** decreases analgesic potency, but when this same modification is made to **dihydromorphine** or **dihydrocodeine** to give **hydromorphone** (**5**) and hydrocodone (**62**), respectively, opioid potency is enhanced. This modification also alters the conformation of the C ring of the opiate (see Fig. 7.16). Although the C ring of morphine and other derivatives containing a 6α-alcohol (60 and 61) is in a boat conformation, in **oxymorphone** (63)and the antagonist derivative naloxone (16) (see below), the C ring is in the chair conformation (see Fig. 7.16 and Ref. 298). Numerous additional modifications have been made to the 6 position of 4,5α-epoxymorphinans (see Refs. 299, 300). These include analogs that can bind irreversibly to opioid receptors and selective antagonists in which additional rings are attached at the 6 and 7 positions; these types of opiates are discussed in Sections 5.11 and 5.3, respectively, later in the chapter. **Zwitterionic** groups have also been attached at the 6 position to produce peripherally selective derivatives with decreased ability to penetrate the CNS (304). **A 14β-hydroxyl** group can be introduced into the opiate skeleton by oxidation of thebaine (see Refs. 299, 300) and this modification can enhance potency. The **14β-hydroxylated** derivatives **oxymorphone** (63)and **oxycodone** (6) are

potent analgesics, with oxymorphone exhibiting approximately 5–10 times the potency of morphine. The analgesic activities of these morphine derivatives, along with a variety of other opioids, are compared in Table 7.1 in Section 2.1 above.

5.3 N-Substituted 4,5α-Epoxymorphinans: Opioid Antagonists

5.3.1 introduction. A critical determinant of the type of activity observed for morphine derivatives is the identity of the nitrogen substituent, and variation in this group yields compounds ranging in activity from pure agonists to pure antagonists. Removal of the *N*-methyl of morphine to give normorphine decreases potency (the relative potency of normorphine, however, is dependent on both the route of administration and the species examined; see Ref. 305). Although some nitrogen substituents such as phenethyl enhance agonist activity, other nitrogen substituents, notably propyl, allyl, cyclopropylmethyl (CPM), and cyclobutylmethyl (CBM), impart antagonist activity to the compounds. The replacement of the N-methyl group of morphine and codeine by an allyl group yields *N*-allylnormorphine (nalorphine, **56**), and *N*-allylnorcodeine (57, Fig. 7.12), respectively. Although the first narcotic antagonist N-allylnorcodeine was prepared in 1915 (281), it was the demonstration of the antagonist activity of nalorphine in the 1940s that stimulated the examination of other N-substituted morphine analogs for antagonist activity (see Ref. 305). Nalorphine is inactive as an analgesic in animals, but it is an effective analgesic in humans (306). Although it was thought that mixed agonist/antagonists such as nalorphine were agonists at κ receptors and antagonists at μ receptors, these drugs may be more accurately described as partial agonists at both κ and μ receptors (see Ref. 307). Nalorphine produces intense psychotomimetic effects and dysphoria, which precluded its clinical use as an analgesic. However, it was used as an antidote for opioid overdose until the introduction of naloxone in the late 1960s.

Modification of the N-substituent of the potent morphine analog oxymorphone has led to several compounds with antagonist activity. The *N*-allyl derivative of oxymorphone, naloxone (16, Fig. 7.4), was the first example of a pure opioid antagonist essentially devoid of agonist activity, and is 7–10 times as potent an antagonist as nalorphine (see Ref. 305). Naloxone has a short duration of action and is used for the treatment of narcotic overdoses (see Section 2.1). Although naloxone exhibits some preference for μ receptors, it also antagonizes S and κ receptors as well (see Table 7.8), and sensitivity to antagonism by naloxone is routinely used as a criterion for opioid receptor involvement in an observed response. The N-CPM derivative naltrexone (17) is a pure antagonist that is more potent than naloxone, has a longer duration of action, and is orally active, making it more useful for the treatment of former opiate addicts. The two antagonists have different metabolic routes, which accounts for the differences in duration of action and doses required (see Section 2.3.2 and Ref. 305). The 6-methylene analog of naltrexone, nalmefene (**64**), is also used clinically as an antagonist (see Table 7.4) to reverse the effect of opioids and has a longer duration of action than naltrexone (24). Interestingly, at cloned μ opioid receptors nalmefene is an inverse agonist, whereas naloxone and naltrexone are neutral antagonists (the latter two compounds display inverse agonist activity if the cells were pretreated with morphine) (308). The 6β-fluoro analog of naltrexone, cyclofoxy, has been prepared in tritiated form (309).

Recently, the 6β-substituted naltrexamine derivative TRK-820 (65) was reported to be a κ-selective agonist [K_i = 3.5 n*M*, K_i (κ/μ) ratio = 15; see Table 7.10 below], which acts as a full agonist at κ receptors, partial agonist at μ receptors, and a weak antagonist at ORL1 receptors (see Section 7.2 below) (310,311). The N-CBM analog of naltrexone is a mixed agonist/antagonist, and the 6α-alcohol derivative, nalbuphine (**66**), is used clinically (see Table 7.3). Examination of diastereomeric pairs of nalorphine and naloxone quaternary ammonium salts (which have chiral nitrogens) found that the diastereomers with the *N*-allyl group equatorial were the more potent antagonists (312, 313).

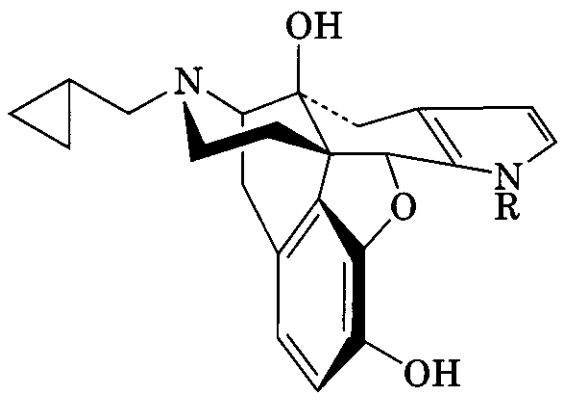
The quaternary derivatives of several narcotic antagonists have been used to explore the central versus peripheral actions of opi-

oids. These compounds generally have much lower affinity for opioid receptors and antagonist potency (1–12%) relative to that of the corresponding tertiary antagonists (314), and in some cases (e.g., N-methylnaloxone) the quaternary antagonist or an active metabolite appears to enter the CNS after peripheral administration (see Ref. 314). The peripheral selectivity of the quaternary antagonist methylnaltrexone (315) appears to be greater than that of N-methylnaloxone or *N*-methylnalorphine (316), most likely because of the higher resistance of methylnaltrexone to *N*-demethylation (317). The extent of this metabolic pathway varies among species; in humans no appreciable N-demethylation has been observed, which probably has a significant impact on the peripheral selectivity of this drug. Methylnaltrexone is currently being examined in clinical trials for the treatment of opioid-induced constipation (see Section 2.2.2.1).

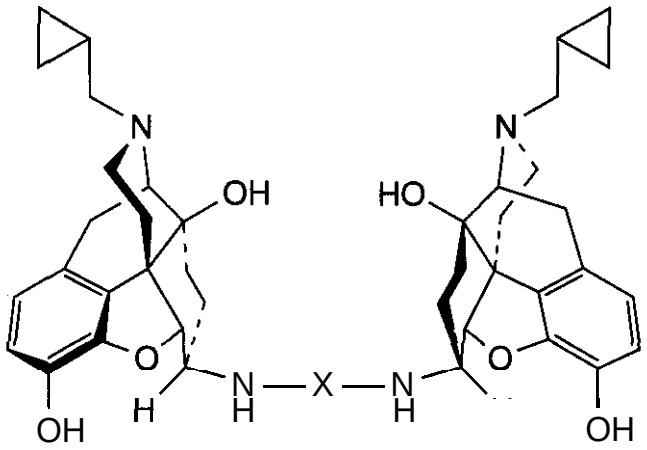
Although the above agents have been useful pharmacological tools as well as therapeutic agents, these compounds preferentially antagonize μ opioid receptors. Antagonists selective for all three opioid receptor types are valuable tools in understanding both the pharmacological effects of opioid agonists and the physiological effects of the endogonous opioid peptides. In the past decade Portoghese and coworkers have synthesized numerous naltrexone derivatives with additional groups and ring systems attached to the C ring. Depending on the modifications made, analogs selective for both κ and δ receptors have been prepared (see Refs. 289, 318–321 for reviews).

5.3.2 Kappa-Receptor Selective Antagonists. Portoghese and coworkers used the bivalent ligand approach to design κ-selective ligands. In this design strategy it was envisioned that two pharmacophores could bridge two neighboring opioid recognition sites if they were connected by an appropriate spacer. This should then lead to substantial increases in potency because of the close proximity of the second pharmacophore to the neighboring site and hence a large increase in the local concentration of the pharmacophore (see Ref. 319). The first κ-selective antagonist TENA (68, Fig. 7.17) was prepared by connecting two naltrexamine pharmacophores with a spacer obtained from triethylene glycol (322,323). Both the monovalent analog with a single pharmacophore and an analog with a longer spacer were much less potent κ antagonists, suggesting that TENA contained the appropriate spacer length for κ-receptor affinity and selectivity. Subsequently, glycyl units were used in the spacer, making it possible to easily vary the chain length of the spacer; a central succinyl (X = -Gly_n-$COCH_2CH_2CO$-Gly_n- in 67) (324) or fumaryl group (-Gly_n- COCH=CHCO-Gly_n- in 67) (325) were used in the spacer. Although the optimum length of the spacer for interaction with μ receptors was similar in the two series (two glycyl units, i.e., $n = 2$, in each half of the spacer), the optimum length of the spacer for interaction with κ receptors was different in the two series (325). In the series with the succinyl spacer, the shortest spacer (with no glycyl units, i.e., $n = 0$) yielded the most potent κ antagonist, whereas in the series with the more rigid fumaryl spacer a much longer spacer (two glycyl units, $n = 2$, in each half) was required for maximal κ-antagonist activity.

Incorporation of a rigid pyrrole spacer led to the synthesis of the κ-selective antagonists norbinaltorphimine (norBNI, 39, Fig. 7.6) and binaltorphimine (40) (119, 120). These compounds were much more selective for antagonism of κ receptors than TENA (120), and norBNI has been routinely used to determine whether κ opioid receptors are involved in an observed activity. A more detailed understanding of how these compounds interact with opioid receptors has come from examining their structure-activity relationships and molecular modeling. The monovalent ligand with only the pyrrole ring attached (73) is not selective for κ receptors, suggesting that the pyrrole ring func-

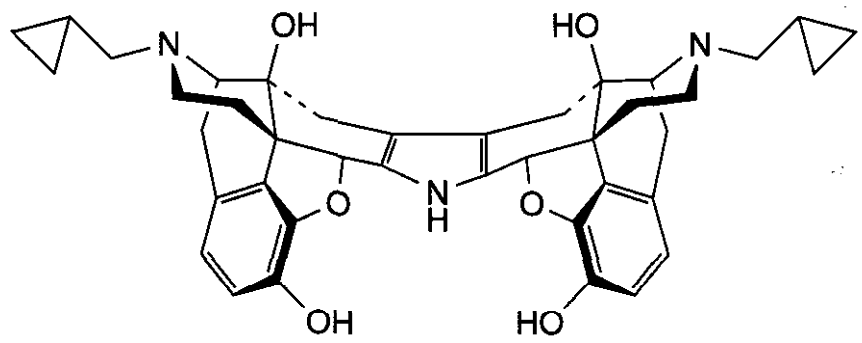


(73)

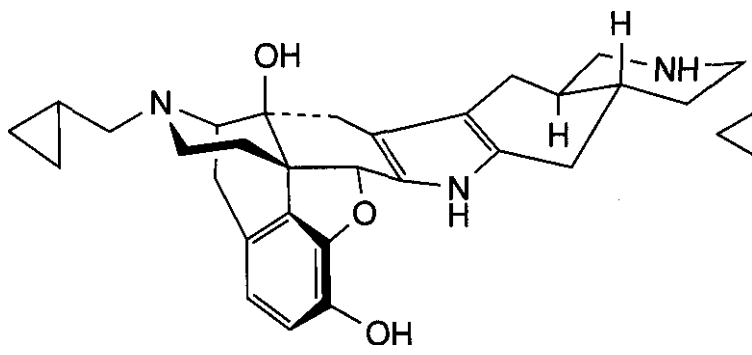


(67)

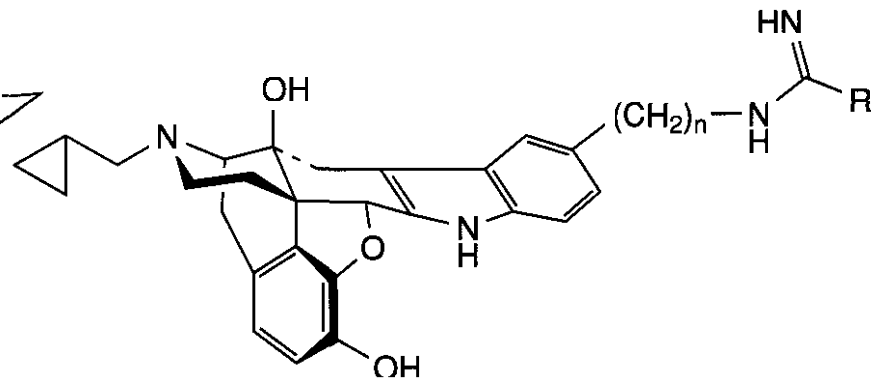
(68) X = -$(CH_2CH_2O)_2CH_2CH_2$- (TENA)



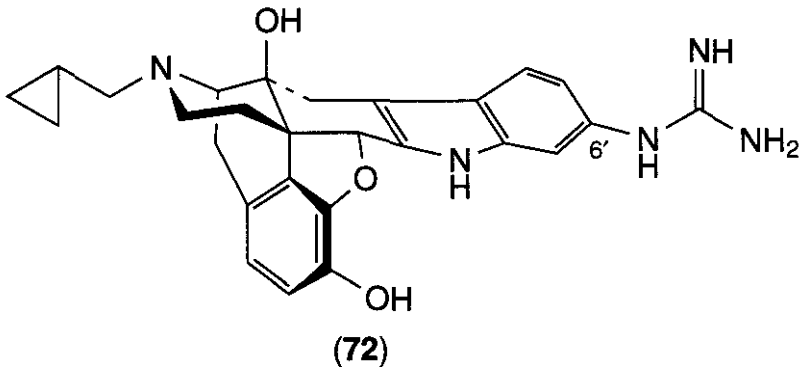
(69)



(70)



(71) $n = 1$ or 2
$R = CH_3$ or $(CH_2)_3CH_3$



(72)

Figure 7.17. κ-Receptor selective antagonists and related compounds. The structures of norBNI (39), binaltorphimine (40), and GNTI (41) are shown in Fig. 7.6.

tions as a spacer and does not contribute to κ selectivity **(326).** This conclusion is supported by comparison of different spacers; whereas the thiophene analog of norBNI in which a sulfur replaces the indole nitrogen, which has a very similar structure, retains κ-receptor selectivity, a pyran derivative with a very different spacer loses κ selectivity **(327).**

While the κ antagonists described earlier were designed using the bivalent ligand approach, subsequent studies revealed that these ligands do not appear to bridge two opioid binding sites, but that a basic amino group in the second pharmacophore interacts with a subsite on the κ opioid receptor. Thus the *meso* isomer of norBNI (**69**) was also found to be a potent κ antagonist, indicating that only one pharmacophore is required for interaction with κ receptors **(328).** This suggested that the second half of the bivalent ligand might be mimicking the basic C-terminal "address" sequence of dynorphin, which imparts κ-receptor affinity to the peptide, and that simpler derivatives could be used in place of the second pharmacophore. **A** simplified analog of norBNI that does not contain the second aromatic ring **(70)** is twice as selective as norBNI for κ receptors **(329).** The basicity of the second nitrogen is important for κ-receptor selectivity in both norBNI analogs **(330)** and the simplified analog **(70) (329).** Examination of the binding of norBNI to mutated κ receptors (175) suggests that this nitrogen interacts with Glu^{297} at the top of TM6 on the receptor; a significant (70-

fold) increase in affinity for the preceptor mutated at the corresponding position ([K303E]) (331) is consistent with this conclusion. A computational model of norBNI bound to κ receptors has been developed (180), and in this model the second nitrogen forms an ion pair with Glu^{297}. With the recent proposal that opioid receptors may exist as dimers (see Section 7.1 below), however, Portoghese is revisiting these bivalent ligands and how they may interact with dimeric receptors (321).

These results led to the preparation of analogs of the 8-selective antagonist naltrindole (see below) with basic alkylamidino groups (71) (122, 332, 333) and guanidinium groups (GNTI, 41) (121–123) attached to the indole ring as κ-selective antagonists. Like norBNI, the decrease in binding affinity of GNTI to [E297K] mutated κ receptors and increase in affinity for the corresponding [K303E] mutated μ receptors and the [W284E] mutated 8 receptors suggests that the guanidinium group of GNTI interacts with Glu^{297} on κ opioid receptors (121, 334). Interestingly, shifting the position of the guanidinium group from the 5' position in GNTI to the 6' position (72) results in a potent κ agonist (335).

5.3.3 Delta-Receptor Selective Antagonists and Related Compounds. Portoghese and coworkers used the "message-address" concept to design naltrexone derivatives selective for 6 receptors (see Ref. 336). The "message-address" concept, which was originally described by Schwyzer for ACTH (337), was applied to the opioid peptide dynorphin by Chavkin and Goldstein (263). In this model the "message" consists of the amino acids in the peptide that are responsible for activating the receptor and producing a biological response, whereas the "address" component is the portion of the molecule that enhances affinity for a given receptor type. Portoghese postulated that in the endogenous enkephalins the Tyr^1 residue functions as the "message" and the phenyl ring of Phe^4 functions as part of both the "message" and the "address"; the intervening Gly^2-Gly^3 then functions as a spacer. In naltrexone analogs, the naltrexamine moiety of these antagonists functioned as the "message" portion and a phenyl ring was attached to the pyrrole spacer as the "address." This led to the synthesis of naltrindole (NTI, 32, Fig. 7.5) (113, 338), the first nonpeptide δ-selective antagonist. In addition to being δ selective, this naltrexone analog is about 500 times as potent as the δ-selective peptide antagonist ICI 174,864 (29, Fig. 7.5) (338). In a computational model of NTI bound to δ opioid receptors (180), the indole moiety is directed toward a hydrophobic pocket formed by residues from TM6 and TM7, which includes two residues (Trp^{284} in TM6 and Leu^{zg9} in TM7 of the mouse δ receptor) unique to the δ opioid receptor. Site-directed mutagenesis studies of these positions in δ, μ, and κ receptors were consistent with these positions contributing to the δ receptor affinity and selectivity of NTI (334). Substitution of Ala in the position corresponding to Leu^{299} in μ and κ receptors increased the affinity for NTI by 21- and 96-fold, respectively (334). Substitution of Trp^{284} in the δ receptor had a smaller effect, with mutation to Glu (334) and Lys (166) (the residues found in the corresponding position in κ and μ receptors, respectively) decreasing the affinity of NTI 9- and 15-fold [mutation to Ala decreased NTI affinity fivefold (166)].

Fluorescent derivatives of naltrindole have been prepared. The fluorescent analog in which fluorescein was attached through a tetraglycine spacer to 7'-amino-NTI has been prepared (339); its fluorescence is blocked by NTI, indicating specific binding to δ receptors. Recently, a fluorogenic "reporter affinity label" derivative of naltrindole, PNTI, was prepared from 7'-amino-NTI that, in contrast to its reversible counterpart, is an agonist in the MVD (340) (see Section 5.11 below).

Naltrindole analogs containing other heterocyclic spacers have also been examined. The benzofuran analog naltriben (NTB, 46, Fig. 7.8) is also a δ-selective antagonist (195); as discussed earlier in Section 3.2.4.3, subsequent studies indicated that this analog is a selective δ_2 antagonist (341, 342).

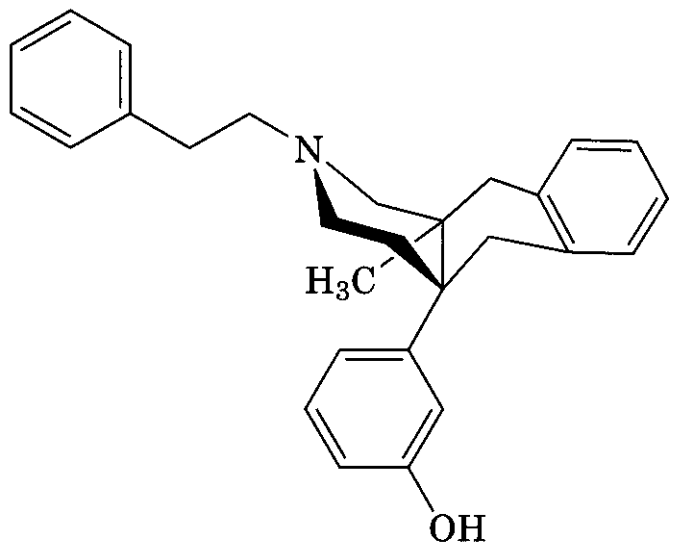
The aromatic ring in the "address" portion of naltrindole and its analogs is important for antagonist activity at δ receptors. The tetrahydroindole derivate is much less potent, as are several 6-aryl derivatives (195). The 7(*E*)-benzylidine analog of naltrexone, 7-benzylidenenaltrexone (BNTX, 44, Fig. 7.8), however, is a potent δ antagonist (193,342). It has

100-fold greater affinity for [^{3}H]DPDPE sites (δ_1) than for DSLET (δ_2) sites, and therefore is a selective 6, antagonist (193); it is also a selective antagonist for δ_1 receptors in the mouse spinal cord in antinociceptive assays (342). Substitution on the phenyl ring of BNTX with either o-methoxy or o-chloro resulted in analogs with increased antagonist potency and 6-receptor selectivity in smooth muscle assays, but in *vivo* in the tail flick assay these analogs exhibited similar selectivity but lower potency than BNTX (343). Both the *E*- and corresponding *Z*-isomers of a series of aryl analogs of BNTX have been prepared (344), and the *Z*-isomers were found to have higher 6-receptor selectivity; the (*Z*)-1-naphthyl derivative exhibited the highest 6-receptor affinity (K_i = 0.7 versus 6.2 n*M* for BNTX) and selectivity for 6 over μ receptors.

The relative position of the "address" aromatic ring in these analogs is important. Those analogs with the phenyl ring in the same region of space as the phenyl ring of the indole in NTI are active as 6 antagonists (345); a large decrease in activity was observed for indole regioisomers of NTI that have the aromatic ring in a different relative position (346). Attachment of the phenyl ring directly to the 7 position of the morphinan system resulted in analogs with decreased 6 receptor potency and selectivity (347). Peripherally selective naltrindole analogs were prepared by conjugating amino acids to 7'-carboxynaltrindole (74, Fig. 7.18); these derivatives are δ-selective antagonists in smooth muscle assays and 6,-selective antagonists in *vivo* (348). Benzylation of the indole nitrogen of NTI, by contrast, results in a selective and long-lasting 6, antagonist (75) (349). Attachment of fluoresceineamine through a thiourea linkage to the *para* position of the benzyl ring of *N*-benzylnaltindole resulted in an analog with potent antagonist activity in vitro. The inability to block the fluorescence by several &selective ligands, including NTI, however, suggested that the fluorescent analog exhibits high nonspecific binding, possibly because of the high lipophilicity of the fluorophore (350).

A number of other naltrindole analogs have been synthesized, including numerous ones by Rice and coworkers. Although most of the modifications examined decreased 6 opioid receptor affinity, in several cases [e.g., the *N*-2-methylallyl naltrindole analog (351)], the 3-methyl ether of naltrindole (352), and the corresponding ring-opened 4-hydroxy-3-methoxyindolemorphinan (353), the selectivity for δ receptors increased. Replacement of the cyclopropylmethyl (CPM) group by the 2-methylallyl in NTB and SoRI 9409 (see below), but not BNTX or SIOM (see below), resulted in compounds that retained reasonable 6-receptor affinity and selectivity (354). The 14-alkoxy derivatives, with or without a 5-methyl group, also exhibited high 6-receptor selectivity in the MVD assay (355,356). Interestingly, the N-cyclohexylethyl derivative (76) derivative is a p-selective agonist (357). Additional analogs of naltrindole have been reported in the patent literature (see Refs. 219, 220 for reviews).

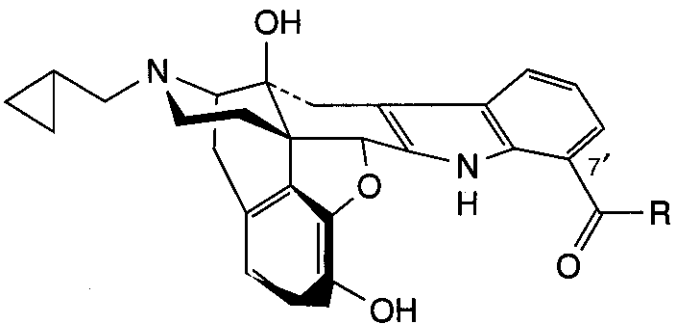
Fragmentation of the indolomorphinan structure of NTI by removal of the 4,5α-epoxy and 10-methylene groups resulted in a series of octahydroisoquinolines (see Refs. 219,358). The compounds that are the analogs of NTI with a five-membered ring spacer [SB 205588B (78) and SB 206848 (79, Fig. 7.18) are 6-receptor antagonists, whereas compounds with a six-membered ring as the spacer are 6 agonists (see Section 5.10.2 below). Interestingly, the related compound (84)



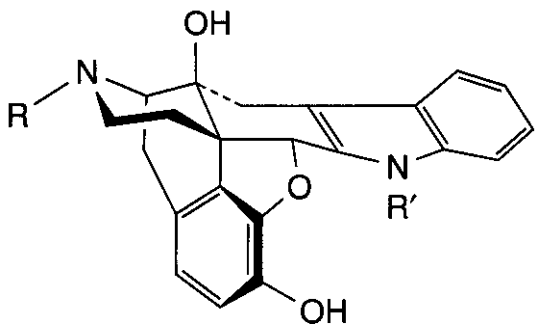
(84)

without the pyrrole ring spacer and the cis configuration of the ring juncture is an antagonist at μ and κ receptors (359).

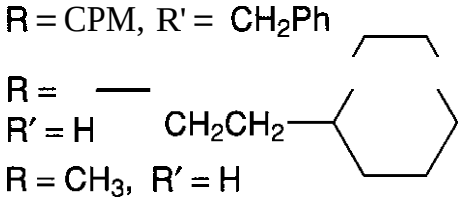
As discussed earlier in Section 3.3.1, 6-receptor antagonists can decrease the development of tolerance and dependence to mor-



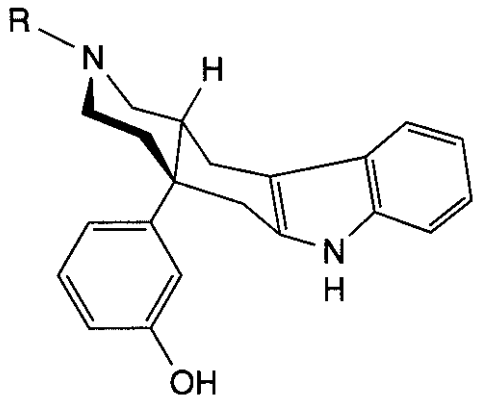
(74) R = Gly, Asp, etc.



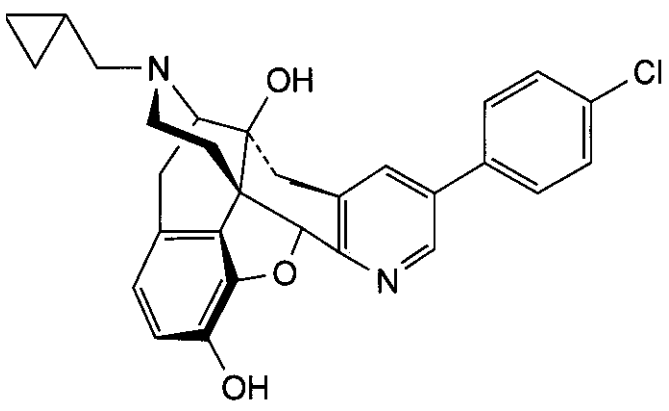
R = CPM, R' = CH_2Ph (75) N-Benzylnaltrindole



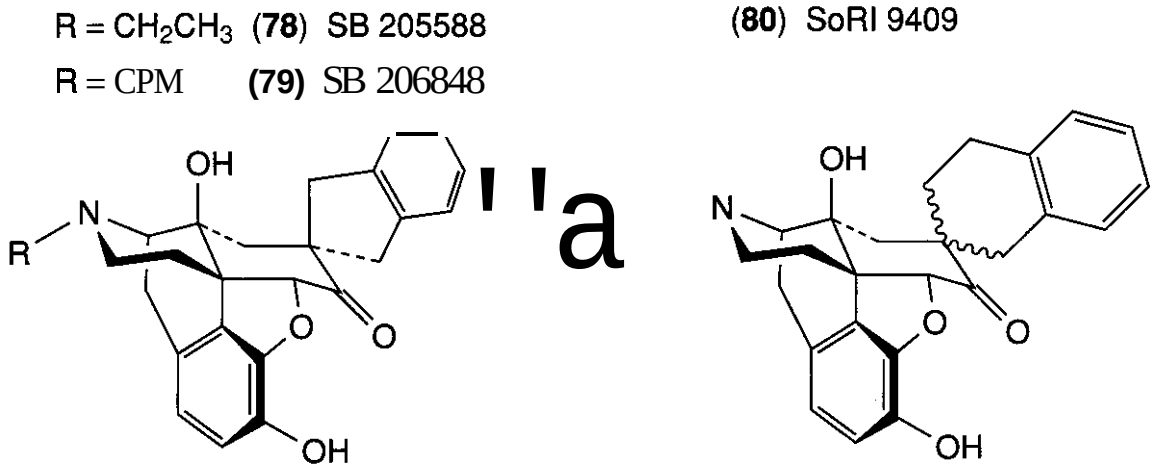
R = —
R′ = H (76)
R = CH_3, R′ = H (77) Oxymorphindole



R = CH_2CH_3 (78) SB 205588
R = CPM (79) SB 206848



(80) SoRI 9409



R = CH_3 (81) SIOM
R = CPM (82) SINTX

(83)

Figure 7.18. Delta receptor antagonists and agonists related to naltrindole (32). Other δ-selective ligands, including naltrindole, are shown in Figs. 7.5 and 7.8.

phine, and therefore there is considerable interest in the development of compounds exhibiting both 8 antagonist activity and μ agonist activity. Recently, Rice and coworkers identified naltrindole analogs with μ agonist activity as well as δ-receptor antagonist activity (230, 360). The 7′-phenoxy naltrindole derivative exhibits weak (IC_{50} = 450 nM) agonist activity in the GPI, while retaining potent 6-receptor antagonist activity (K_e = 0.25 nM) in the MVD (360). The naltrindole analog SoRI 9409 (80), bearing a phenyl ring attached to a pyridine rather than to an indole ring (230), is a 8-receptor antagonist, but unexpectedly also exhibits μ opioid agonist activity in the GPI assay (230); interestingly, the corresponding derivative without the chlorine is a preceptor antagonist. Further examination of SoRI 9409 in nociceptive assays indicated that the compound is a weak partial agonist in the high intensity (55°C)tail flick test and weak full agonist in the acetic acid writhing assay (230,361); studies in *vivo* were also consistent with activity as a mixed partial μ

agonist/δ antagonist. In contrast to morphine, repeated doses of SoRI 9409 did not produce tolerance (230, 361), and SoRI 9409 suppressed withdrawal signs when coadministered with naloxone to morphine-dependent animals. In [^{35}S]GTPγS assays, however, SoRI 9409 exhibits no μ-agonist activity and instead acts as an antagonist at μ and κ as well as at δ receptors; at this time the reason for this difference between the in vitro and in vivo results is not clear.

Attempts have also been made to prepare agonist analogs of NTI by modification of the basic nitrogen. The N-methyl analog oxymorphindole (OMI, 77, Fig. 7.18) is a partial agonist (IC_{50} = 100 n*M*) (338), whereas the unsubstituted and the N-phenethyl derivatives are full agonists (IC_{50} = 85–180 n*M*) in the MVD (362). These derivatives either did not exhibit any δ antagonist activity or were only weak δ antagonists in this smooth muscle preparation. In vivo, these analogs, along with naltrindole, exhibit antinociceptive activity (362, 363). On the basis of antagonism by selective antagonists, the antinociceptive activity of all of the compounds except the unsubstituted derivative appears to be mediated by κ rather than δ receptors (the unsubstituted derivative was not antagonized by any of the selective antagonists). At lower doses these compounds also exhibit antagonism at δ receptors in vivo. Modification of the indole structure of OMI to change the orientation of the phenyl ring yields the 7-spiroindane derivative, 7-spiroindanyloxymorphone (SIOM, 81), which is a δ_1 agonist (364). Portoghese et al. suggested that the phenyl ring of the indane ring system adopts a conformation similar to that of the phenyl ring of Phe^4 of the δ-selective enkephalin analog DPDPE (24). [Linking the C-terminal sequence Phe-LeuOMe of leucine enkephalin to oxymorphanone (but not to naltrexone) Also significantly increases affinity for δ receptors (365).] Like NTI, the results of site-directed mutagenesis of positions corresponding to Trp^{284} (in TM6) and Leu^{2g9} (in TM7) of the δ receptor in δ, μ and κ receptors were consistent with these positions being involved in the 6-receptor affinity and selectivity of SIOM (334). Replacing the N-methyl group of SIOM with a CPM group gives SINTX (82) which is a potent δ antagonist (345). The benzospiroindanyl derivative of SINTX exhibits improved selectivity for δ over μ receptors, although lower potency in vitro compared to SINTX and is a δ_1 antagonist in *vivo* (366). The 14-hydroxyl group contributes to both δ agonist and antagonist activity, and its removal from SIOM and NTI decreases agonist and antagonist potency, respectively (367). The 7-spirobenzocyclohexyl derivatives of SIOM are also potent δ agonists, but the corresponding analogs of SINTX (83, both a and β isomers) are p-selective antagonists (368).

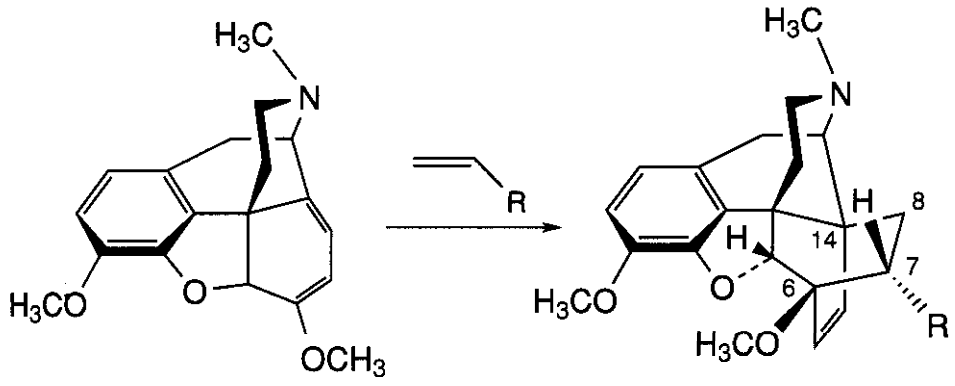
5.4 Diels-Alder Adducts

Thebaine (59), which is present in large amounts from the species of poppy *Papaver* bracteaturn (292), serves as the precursor for the 6,14-*endo*etheno opiates. Although the natural levorotatory isomer of thebaine is inactive as an analgesic, it was recently reported that the (+) isomer exhibits significant antinociceptive activity (369); both isomers exhibit some affinity for opioid receptors [the (+) isomer for μ receptors and the (−) isomer for δ receptors], but the affinities were very low (μ*M*).

The Diels-Alder reaction of thebaine with various electrophiles yields compounds (Fig. 7.19) that have extremely high potencies, over 1000-fold higher than morphine in some cases (see Refs. 370, 371). X-ray (372) and NMR (373) analysis of 19-propylthevinol, the 3-methyl ether of etorphine (42 above), indicates that the 6,14-etheno bridge is held "inside" (*endo*) the tetrahydrothebaine ring system and below the plane (α) of the C_7-C_8 bond (see Fig. 7.19 and Ref. 283); the C ring is held in a boat conformation by the 6,14-*endo*-etheno bridge.

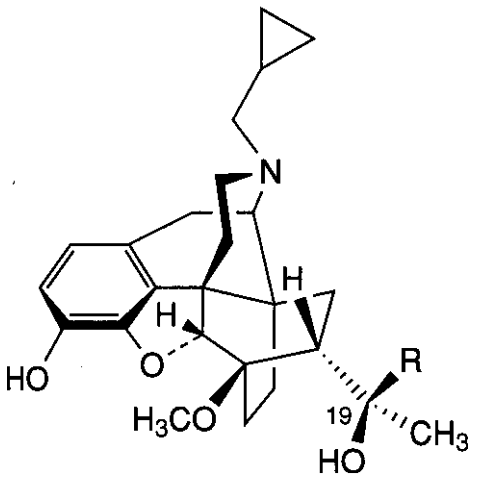
The C_7 substituent in these compounds is in the a configuration and in many cases contains a chiral center. The stereochemistry at C_{19} can have significant effect on potency of the derivatives (see Ref. 283); generally, the diastereomer with the R configuration at C_{19} is the more potent isomer, with the differences in potencies of the diastereomers sometimes exceeding 100-fold.

A variety of C_{19} alcohol derivatives have been prepared (see Refs. 370,3711, and three of these compounds are frequently used in opioid research. Etorphine (42) is a potent anal-



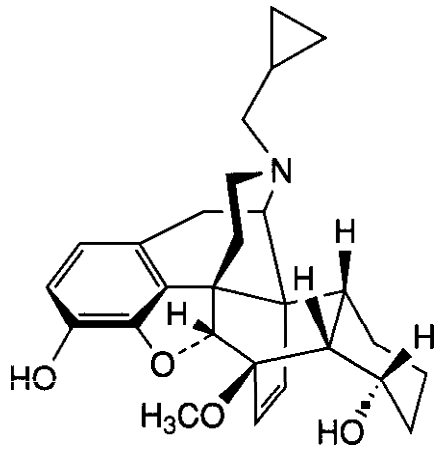
(59) Thebaine

endoetheno derivatives



$R = CH_3$ (43) Diprenorphine

$R = C(CH_3)_3$ (85) Buprenorphine



(86) BU48

Figure 7.19. Diels-Alder reaction to give 6,14-*endo*etheno derivatives of thebaine (**59**) and the structures of buprenorphine **(85)** and BU48 (**86**). The structure of diprenorphine (**43**) is included for comparison.

gesic, over 1000-fold more potent than morphine, which has been widely used to immobilize animals, including large game animals. It exhibits high affinity for all three opioid receptor types (see Table 7.8), and therefore [^{3}H]etorphine has been used as a universal tritiated ligand for opioid receptors (see Section 3.2.3.2). The *N*-cyclopropylmethyl 6,14-ethano derivatives diprenorphine (43) and buprenorphine (85, Fig. 7.19), which differ only in the identity of one of the alkyl groups attached to C_{19} (see Refs. 374, 375 for structural studies of these compounds), exhibit antagonist and partial agonist activities, respectively. Diprenorphine also has high affinity for all three opioid receptor types and its tritiated form has been used as a universal tritiated ligand for opioid receptors (see Section 3.2.3.2); it antagonizes μ, 6, and κ ligands in the MVD (see Table 7.8). Buprenorphine, which is used clinically, is a potent partial agonist at preceptors with antagonist (or partial agonist) activity at κ receptors (376). This very lipophilic agent dissociates slowly from opioid receptors, and its complex pharmacology is not completely understood (see Ref. 307). Its unique pharmacological profile offers several clinical advantages; it causes less severe respiratory depression than full agonists and has less abuse potential. It can suppress withdrawal symptoms in addicted individuals undergoing withdrawal from opiates and thus has been used in the maintenance of these patients. Because of its partial agonist activity, however, it can also precipiate withdrawal symptoms in those addicted to opiates. The 18,19-dehydro derivative of buprenorphine HS-599 exhibits higher affinity, selectivity, and potency at μ receptors than does the parent compound (377). A series of buprenorphine analogs were prepared in which C_{20} was constrained in a five-membered ring to assess the influence of the orientation of the C_{20} hydroxyl on activity; although the configuration of this hydroxyl did not affect the binding affinity, it did influence κ-receptor efficacy and potency (378). One of these novel ring-constrained analogs BU48 (86) exhibits an unusual pattern of pharmacological activity, producing 6 opioid receptor-mediated convulsions but not δ receptor-mediated antinociception (238).

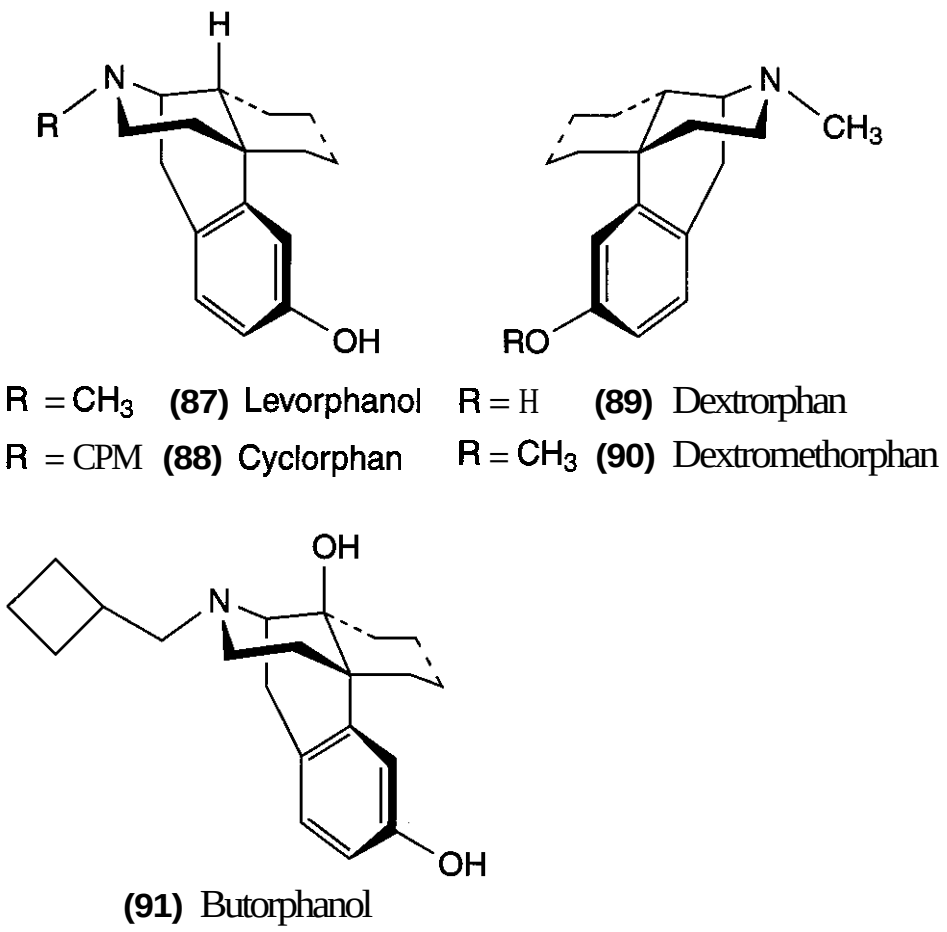
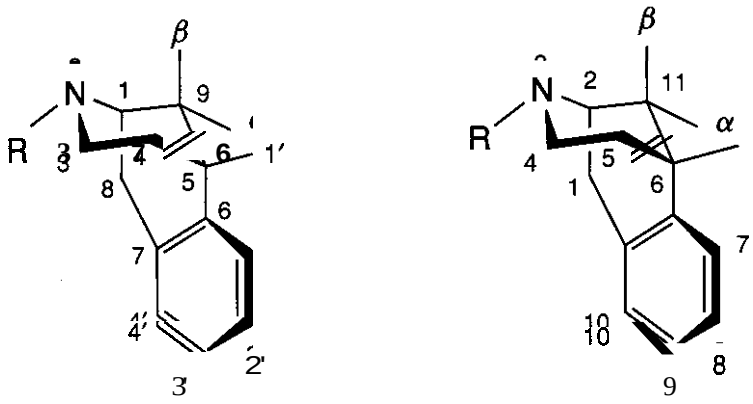


Figure 7.20. Morphinan **derivatives.**

5.5 Morphinans

Morphinans differ in structure from **morphine** and other **4,5α-epoxymorphinans** in that they lack the **4,5α-ether** bridge. Although the **4,5α-epoxymorphinans** are typically prepared from naturally occurring alkaloids, the **morphinans** are generally made from **racemic** materials and therefore must be resolved to obtain individual isomers. **Racemorphan** (379) was one of the first morphinans studied. After resolution the analgesic activity was found exclusively in the *levo* isomer, **levorphanol** (87, Fig. 7.20) (380), which has a configuration identical to that of morphine and is about 4–5 times **as** potent, whereas the *dextro* isomer, **dextrorphan** (**89**), has negligable opioid activity. Levorphanol and **dextrorphan** were used in one of the first attempts by Goldstein to demonstrate stereospecific binding to opioid receptors (381). **Dextrorphan,** and **particularly** its 3-methyl ether **dextromethorphan** (**90**), has significant antitussive activity. In derivatives with an unsubstituted 6 position such as **dextromethorphan** the C ring exists in the chair conformation (382). In contrast to the morphine derivatives, the **morphinans** with a ***trans*** B-C ring juncture are potent analgesics (see Ref. 283).

Substitution on the nitrogen of morphinans has similar effects on activity as found in derivatives of morphine (see Ref. 383). Thus replacement of the N-methyl with groups such as an **allyl** or CPM group to yield levallorphan and cyclorphan (**88**), respectively, imparts antagonist activity at μ receptors. **As** indicated earlier for **nalorphine,** these compounds were thought to be mixed **agonists/antagonists** with agonist activity at κ receptors, but may be more accurately described **as** partial agonists at both κ and μ receptors (see Ref. 307). **Neumeyer** and coworkers recently described the receptor binding affinities of a series of ***N*-substituted morphinans** as potential therapeutics for cocaine abuse (384,385). **A** comparison of **cyclorphan** with its cyclobutylmethyl analog ***in vivo*** illustrates the effect small changes in the N-substituent can have on the activity in this class of compounds. Interestingly, **cyclorphan** (88) produced antinociception through δ as well as κ receptors and was a preceptor antagonist; although the ***N*-cyclobutylmethyl** derivative was also a κ **agonist** in antinociceptive assays, it was an **agonist** at μ receptors but was without effect, either agonist or antagonist, at ti receptors (384).



(92)
6,7-Benzomorphan numbering

Benzazocine numbering
(Chemical Abstracts)

Figure 7.21. Numbering systems for benzomorphans based on benzomorphan and benzazocine nomenclature (used in Chemical ***Abstracts).*** The more common benzomorphan numbering is used in this chapter.

As is the case for morphine derivatives, 14-hydroxylation yields potent analogs. The *N*-cyclobutylmethyl derivative with a 14β-hydroxyl group, butorphanol (91) is one of the mixed agonists/antagonists used clinically in the United States (Table 7.3); in addition to a parental formulation, a nasal spray formulation of this drug was introduced in 1992. Because 4,14-dimethoxy-*N*-methylmorphinan-6-one proved to be a very potent agonist, Schmidhammer et al. prepared the corresponding N-CPM derivative cyprodime (18, Fig. 7.4) (104). Cyprodime is a pure preceptor antagonist without any agonist activity (104), which has enhanced preceptor selectivity compared to that of naloxone and naltrexone (see Table 7.8).

5.6 Benzomorphans

Further structural simplification by elimination of the C ring of the morphinan structure yields the benzomorphans (see Fig. 7.21), which are also known as benzazocines.

Although the benzomorphan ring system was first synthesized in 1947 by Barltrop (386), it was the synthesis of 2,5-dimethylbenzomorphan (92, R = R' = CH_3) by May and Murphy (387) that began the investigation into the synthesis and pharmacology of this structural family. As is the case for the morphinans, the benzomorphans are prepared synthetically and therefore are obtained as racemic mixtures. A number of these racemates have been resolved and the activities of the individual isomers examined (see Refs. 283, 388: for reviews, also see Ref. 389). The active isomers are the *levo* isomers, which have the same absolute configuration at the bridgehead carbons as that of morphine (i.e., 1*R*,5*R*; see Refs. 283, 388), although in some cases the dextro isomers retain weak activity.

The majority of the benzomorphan derivatives were prepared before the classification of multiple opioid receptors and were characterized by the use of ***in vivo*** assays. In the classification of multiple opioid receptors Martin and coworkers (5, 6) used the benzomorphan ketocyclazocine (13, Fig. 7.3) as the prototypical ligand to define κ receptors (see Section 3.2.1 above), and subsequent characterization indicated that a variety of benzomorphans had high affinity for κ receptors. Several of these benzomorphans, both agonists and antagonists, were important ligands in early studies of κ receptors. The selectivity of these benzomorphans for κ receptors is generally low, however (see Table 7.10). and much more selective agonists and antagonists [i.e., the arylacetamide agonists (Section 5.9) and antagonists norBNI and GNTI (Section 5.3.2)] are now available for studying κ receptors.

A variety of benzomorphans have been prepared with various combinations of alkyl substituents (methyl, ethyl, propyl) at the 5 and 9 positions (see Refs. 283,388). The alkyl group at the 9 positioncan be oriented either a, in which the substituents in the 5 and 9 positions are cis, or β, in which these substituents are ***trans*** (see 92, Fig. 7.21). The synthetically minor β isomers, which have the opposite stereochemistry from that of the corresponding position in morphine (C,), are more potent than the a isomers as antinociceptive agents (see Refs. 283, 388). Attachment of a 3-alkanone side chain at the 9β position of metazocine (94) yielded a series of potent compounds

Table 7.10 Opioid Receptor Affinities and Opioid Activity in the GPI and MVD of Benzomorphan Derivatives and TRK-820[a]

a. Agonist	K_i (nM)			K_i Ratio	IC_{50} (nM)	
Agonists	κ	μ	δ	κ/μ/δ	GPI	MVD
(−)-EKC (**33**)	0.52	1.0	5.5	1/1.9/10.6	0.18'	4.4[b]
(−)-Bremazocine (**34**)	0.075	0.62	0.78	1/8.3/10.4	0.13[b]	1.98[b]
Mr2034 (97)	0.45	0.66	5.8	111.4113	0.77'	20[b]
TRK-820 (**65**)[c]	3.5	53	1,200	1/15/340	0.0048	0.036

b. Antagonists	K_i (nM)			K_i ratio	K_e (nM)		
Antagonist	κ	μ	6	κ/μ/δ	μ (GPI)	κ (GPI)	δ (MVD)
(−)-Win 44,441 (93)	0.69	0.67	6.4	1/1/9.6	0.67	2.8	15
Mr2266 (98)	0.28	1.3	2.7	1/4.6/9.6	1.5	1.3	14

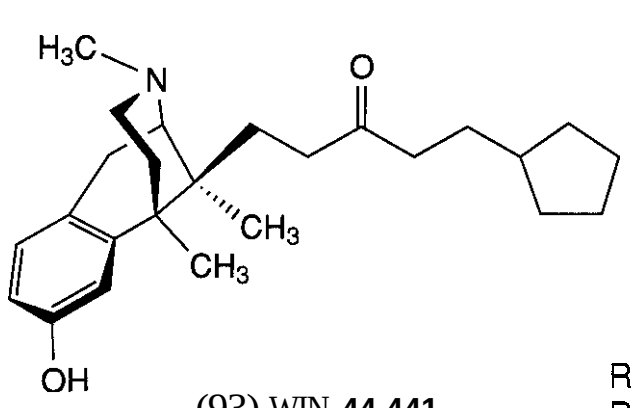
"Data for EKC and bremazocine from Table 7.8 are included for comparison. Data from Ref. **130** except where otherwise indicated.

[b]Antagonist at μ and δ receptors in the rat and hamster vas deferens, respectively.

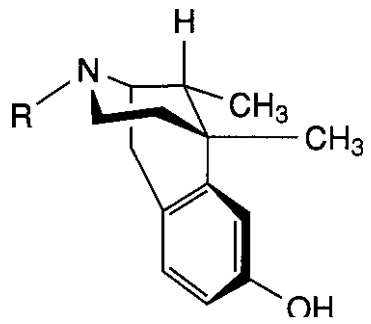
"From Refs. **310, 311.**

which range from pure agonists to pure antagonists, depending on the length of the side chain (390). One of these derivatives, Win 44,441 (93, Fig. 7.22) is a potent κ-receptor antagonist that has been used to characterize κ receptors; the active isomer is the *levo* isomer, Win 44,441–3 (391). It is also a potent antagonist at μ receptors, however, and does not exhibit selectivity for κ over μ receptors (see Table 7.10). In the benzomorphan derivatives introduction of a 9β-hydroxyl in the 9α-methylbenzomorphans, which corresponds to the 14-hydroxyl in oxymorphone and naloxone, does not enhance the agonist activity in the N-methyl derivatives (see Ref. 283), but in benzomorphans with other substituents on the nitrogen [e.g., cyclopropylmethyl or dimethylallyl (see below)], a 9β-hydroxyl enhances antagonist potency 3- to 10-fold (392).

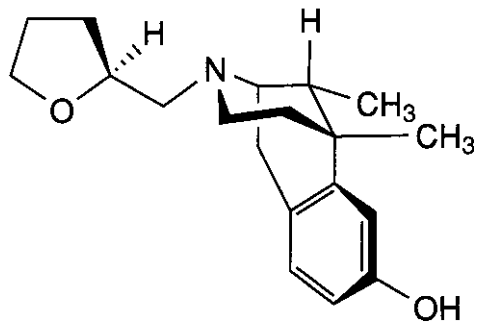
As in the morphinan series, a variety of substituents on the nitrogen have been examined (see Refs. 283,388,389). Metazocine (**94**), with an N-methyl substituent analogous to morphine, exhibits agonist activity, and the N-phenethyl analog phenazocine shows increased analgesic potency. Replacing the N-methyl with groups such as allyl or cyclopropylmethyl (CPM) led to compounds with antagonist or mixed agonist/antagonist activity (generally agonist activity at κ receptors and antagonist activity at other opioid receptors, similar to nalorphine; see Ref. 130). The orientation of the alkyl group at position 9 influences the relative agonist versus antagonist activities of these compounds, with the orientation of this group affecting antagonist potency less than agonist potency (see Ref. 283). A number of these derivatives exhibit dysphoric and psychotomimetic effects, limiting their clinical usefulness. Thus the *N*-allyl derivative N-allylnormetazocine (SKF 10,047, 14, Fig. 7.3) was the prototypical ligand used by Martin and coworkers (**5, 6**) to characterize σ receptors and exhibits psychotomimetic arid dysophoric effects in humans (see Ref. 393); based on animal studies, these adverse effects appear to reside in the dextro isomer (394). The N-dimethylallyl derivative pentazocine (**95**), however, produces considerably less dysphoria than does cyclazocine (96) (307) and is the only benzomorphan used clinically (see Table 7.3). The N-CPM analog cyclazocine (96) is a potent mixed opioid agonist/antagonist, but its considerable psychotomimetic effects prevent its clinical use (see Ref. 393). As indicated earlier, the 8-keto derivative ketocyclazocine (13) was used in the initial characterization of κ receptors by Martin and coworkers; the 5-ethyl, 8-keto derivative ethylketocyclazocine (EKC, 33, Fig. 7.6) has also been used in a variety of studies of κ receptors. The closely related benzomorphan (−)-bremazocine (34, Fig. 7.6) (**395**) exhibits some preference for binding to κ receptors [K_i (μ/κ) ratio = 8.2; see Table 7.101 and thus has been used in its tritiated form in radioligand-binding



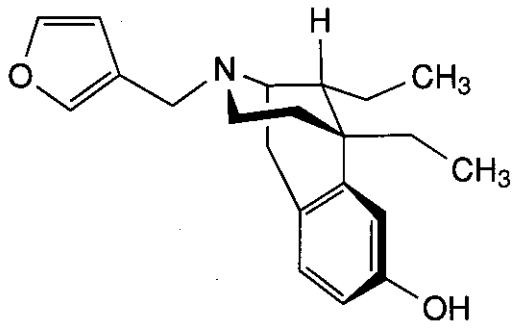
(93) WIN **44,441**



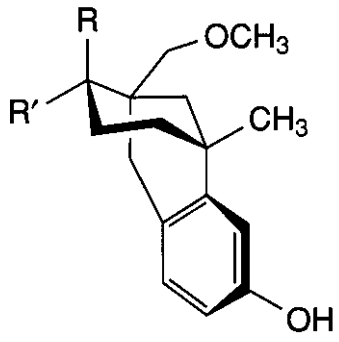
R = CH_3 (94) Metazocine
R = $CH_2CH{=}CH_2$ (14) **SKF** 10,047
R = $CH_2CH{=}CH_2(CH_3)_2$ (95) Pentazocine
R = CPM (96) Cyclazocine



(**97**) Mr2034



(**98**) Mr2266

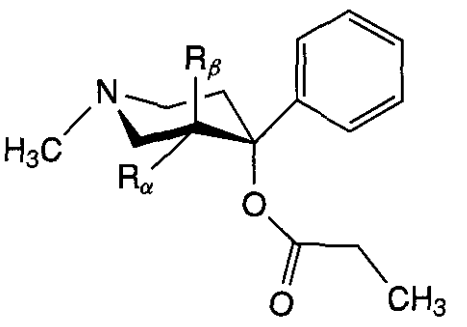


(**99**) R = NH_2, R′ = H
(**100**) R = H, R′ = NH_2

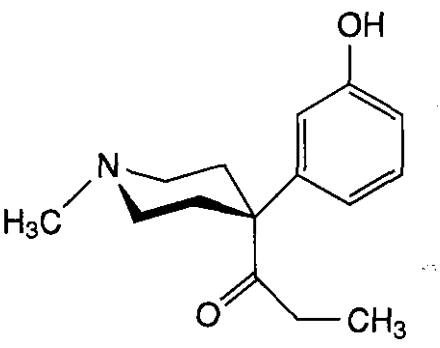
Figure 7.22. Benzomorphan derivatives.

assays for κ receptors (see Section 3.2.3.2); in smooth muscle assays these compounds exhibit mixed agonist/antagonist activity, with agonist activity at κ receptors and antagonist activity at μ and δ receptors (see Ref. 130).

A series of analogs with tetrahydrofurfuryl (396, 397) and furfuryl (398) groups on the nitrogen have been used as κ-receptor agonists and antagonists. The tetrahydrofurfuryl derivative Mr2034 [**97**, absolute configuration 1*R*,5*R*,9*R*,2″*S* (397)] exhibits high affinity for κ receptors and has been used as a κ agonist, but it binds equally well to μ opioid receptors (see Table 7.10); like other benzomorphan mixed agonist/antagonists, it exhibits agonist activity at κ receptors, but antagonist activity at μ and δ receptors in smooth muscle assays (see Ref. 130). Although this *levo* isomer is inactive at σ-PCP receptors, it also produces dysphoric and psychotomimetic effects in humans that are antagonized by naloxone, suggesting that in this case these adverse effects are mediated by κ receptors (393). In the case of the furyl-substituted analogs the chain length affects the type of activity observed; thus the *N*-2-furfuryl derivative exhibits antagonist activity, whereas the longer N-[2-(2-furyl)ethyl] derivative is a potent analgesic (398). Mr2266 (**98**), the *levo* isomer of the 5,9-diethyl *N*-2-furfuryl analog, has been used as a κ-receptor antagonist in a variety of studies, particularly in early studies when more κ-selective antagonists were not available. Similar to other benzomorphans, its selectivity for κ



R_α	R_β	
CH_3	H	(101) α-Prodine
H	CH_3	(102) β-Prodine
$CH_2CH{=}CH_2$	H	(103) α-Allylprodine
H	$CH_2CH{=}CH_2$	(104) β-Allylprodine



(105) Ketobemidone

Figure 7.23. Examples of piperidine analgesics with different substituents on C_4. The structures of meperidine (**7**) and fentanyl (**8**) are shown in Fig. 7.1.

receptors is low [K_i (μ/κ) ratio = 4.6; see Table 7.101 and in smooth muscle assays it antagonizes both μ and κ receptors at similar concentrations (see Table 7.10).

The requirement for a phenol for opioid receptor interaction was recently revisited in a series of cyclazocine analogs in which the hydroxyl was replaced by a primary, secondary or tertiary amino group (399) and several of these analogs retained high affinity for opioid receptors; the same modificationsto morphine decreased p-receptor affinity by at least 35-fold (400).

A series of analogs of the benzomorphans were prepared in which the amino group is exocyclic (99 and 100) (401). In contrast to the benzomorphans and other rigid opiates, the receptor binding of (99) and its analogs exhibits almost no stereoselectivity [K_i (μ) = 2.0 and 2.2 nM for the (+) and (−) isomers]; in *vivo*, both isomers of (99) were inactive. Racemic (100) exhibits an almost 10-fold increase in κ-receptor affinity (K_i = 6.6 nM) compared to that of the isomers of (99) while retaining nanomolar affinity for μ receptors (K_i = 2.0 nM); this compound is a full κ agonist in *vivo*.

5.7 Piperidine Derivatives

Further structural simplification by elimination of the B ring of the benzomorphans yields the phenylpiperidines (Fig. 7.13). This disrupts the fused three-ring system found in the morphinans and benzomorphans, resulting in much more flexible compounds. Thus, although the B ring in the rigid opioid alkaloids fixes the phenyl ring in an axial orientation relative to the piperidine (D) ring, without the B ring the phenyl ring can be either axial or equatorial, depending on the substitution pattern on the piperidine ring (see Fig. 7.13). Thus the structure-activity relationships for the phenylpiperidines can be complex, with differences in SAR observed for different groups within this structural family; these differences can partly be explained by conformational differences and the orientation of the phenyl ring.

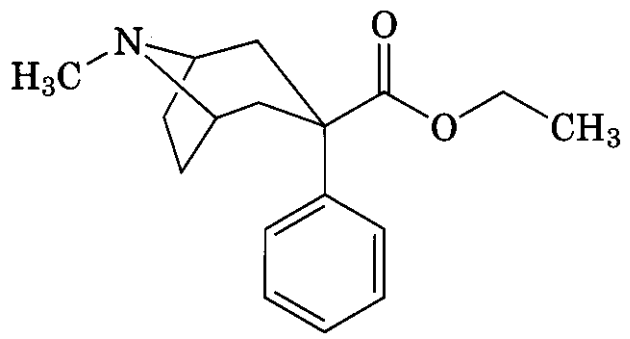
The prototype of this class meperidine (**7**, Fig. 7.1) was discovered serendipitously in 1939 during examination of compounds for antispasmodic activity (402). During the pharmacological testing in mice it was noticed that meperidine caused a Straub tail reaction, in which the animals carried their tail in an erect position and which is a characteristic effect of narcotic analgesics in this species. Subsequent examination of meperidine verified that it was an analgesic, with 10–20% the potency of morphine. This discovery stimulated research into this class of analgesics. Later it was found that meperidine's affinity for opioid receptors is very low (0.2% that of morphine); however meperidine penetrates into the brain more readily and reaches much higher (600-fold) concentrations in the brain than does morphine (403).

The piperidine analgesics can be classified into groups based on the substitution on C_4 (Fig. 7.23). In the case of the 4-arylpiperidines the second substituent can be attached to the piperidine ring either through a carbon (e.g., meperidine, 7) or an oxygen atom (e.g., α- and β-prodine, 101 and 102). Another group of piperidine analgesics are the extremely potent

4-anilidopiperidines (e.g., fentanyl, 8, Fig. 7.1). Meperidine, a-prodine, and fentanyl are all μ opioid agonists (404). All portions of the piperidine analgesics—the N-substituent, the phenyl ring, the piperidine ring, and the C_4 substituent—have been modified. Modifications made to the piperidine ring include substitutions, particularly 3-alkyl substitutions, that affect the preferred conformation of these compounds, introduction of conformational constraints to fix the conformation of this ring and the orientation of the 4-aryl ring, and expansion or contraction of the piperidine ring. It is not possible to cover all of the details of the structure-activity relationships of the piperidine analgesics in this chapter, and thus selected modifications are discussed here. More detailed discussions of the SAR are given in the comprehensive books on opiates (see Refs. 12, 405) and extensive reviews by Casy (283, 284, 406); the reviews by Casy discuss conformational studies in considerable detail.

5.7.1 4-Arylpiperidines with a Carbon Substituent at C_4 The carbon substituents at C_4 of the 4-arylpiperidines can be a carbalkoxy (e.g., meperidine, 7, Fig. 7.1), a ketoxy (ketobemidone, 105, Fig. 7.23) or alkyl substituent. In the case of meperidine (also called pethidine) examination by X-ray crystallographic (407, 408) and NMR (409) techniques indicates that the phenyl ring is equatorial in the major conformer, although in computational studies the calculated energy differences between the equatorial and axial phenyl conformations of meperidine is small (0.6–0.7 kcal/mol) (410).

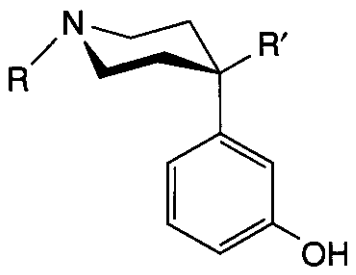
A large number of analogs of meperidine have been prepared (see Ref. 405). The 4-substituent in meperidine is optimal for analgesic activity; lengthening or shortening of the ester chain decreases activity, and except for ketobemidone, substitution of the ester by an amide or a ketone or hydrolysis to the acid reduces activity. Introduction of an m-hydroxyl on the phenyl ring of meperidine to give bemidone enhances activity. This substitution, however, can have complex effects on biological activity, and depending on the nitrogen substitution, both potent agonists and antagonists have been produced in this series (see Ref. 405). A wide variety of *N*-substituents have been examined (see Ref. 405). Several phenylalkyl groups [e.g., phenethyl (in pheneridine) andp-aminophenethyl (in anileridine)] increase potency; *N*-allyl or cyclopropylmethyl groups, however, do not generate antagonists in the meperidine series. Introduction of a 3-methyl group into the piperidine ring yields two isomers, with the 3P-methyl isomer (with the methyl and phenyl cis) 8–10 times more potent than the a isomer (411); the 3P-methyl group should increase the population of the the axial 4-phenyl conformer (283). In conformationally constrained tropane derivatives of meperidine (412, 413) the small difference in potency between the α- and β-phenyl isomers (the a-phenyl derivative 106 has only 3–4 times the potency of the



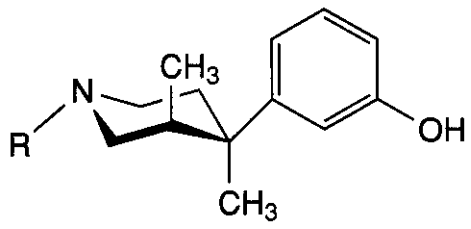
(106)

β-phenyl isomer) suggested that the analgesic. activity of meperidine analogs is not very sensitive to the conformation of the phenyl ring. In the a-phenyl analog (**106**), in which the phenyl ring adopts a pseudoaxial orientation (413), introduction of a m-hydroxyl group decreases potency (414), suggesting that, even though the phenyl ring in these meperidine derivatives adopts a similar conformation to that found in morphine, the interactions of the phenyl ring with the receptor are different for these two types of compounds.

A variety of 4-arylpiperidines without an oxygen functionality on the second substituent at C_4 (107, Fig. 7.24) have been prepared. All of the active derivatives contain a m-hydroxyl group on the phenyl ring (406). The 4-alkyl derivatives favor an axial aryl conformation (**107**) (409,415,416). Introduction of a 3-methyl group, however, can alter the preferred conformation, with the 3β-methyl isomer favoring the equatorial aryl conformation (108) (415, 416). The structure-activity rela-



(**107**)



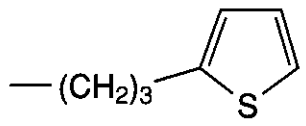
(**108**)

R = —CH_3 (**109**)

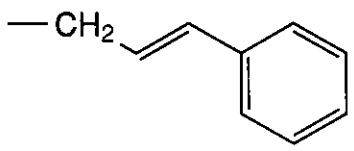
R = —$CH_2CH_2CH(OH)C_6H_{11}$ (**110**) LY255582

R = —CH_2—$CH(CONHCH_2CO_2H)(CH_2C_6H_5)$ (**111**) LY246736

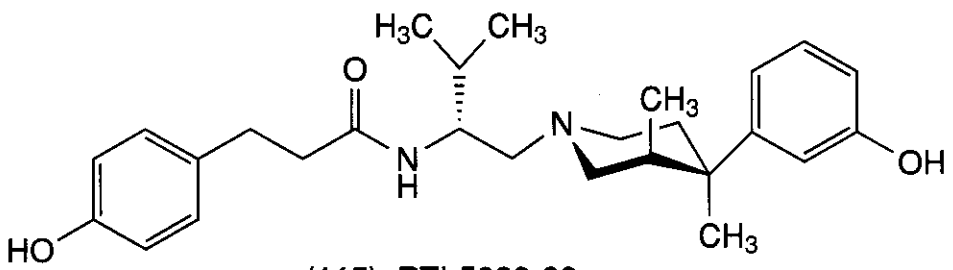
R = —$(CH_2)_3CH(CH_3)_2$ (**112**) LY227053



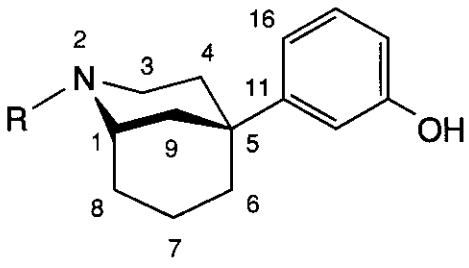
(**113**) LY253886

R = 

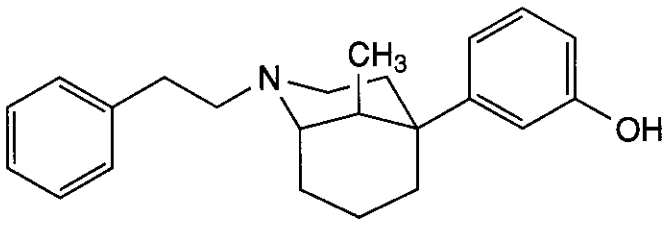
(**114**)



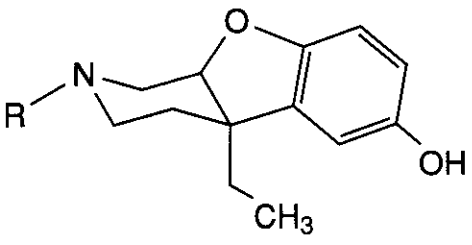
(**115**) RTI-5989-29



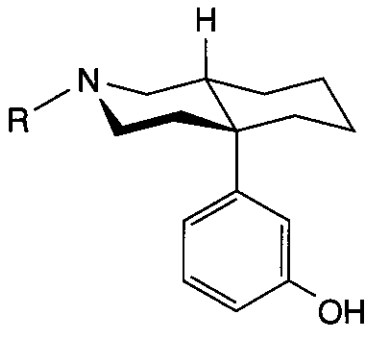
(**116**)



(**117**)



(**118**)



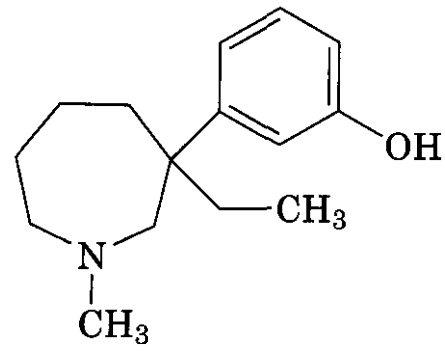
(**119**)

Figure 7.24. 4-Alkyl-4-arylpiperidines.

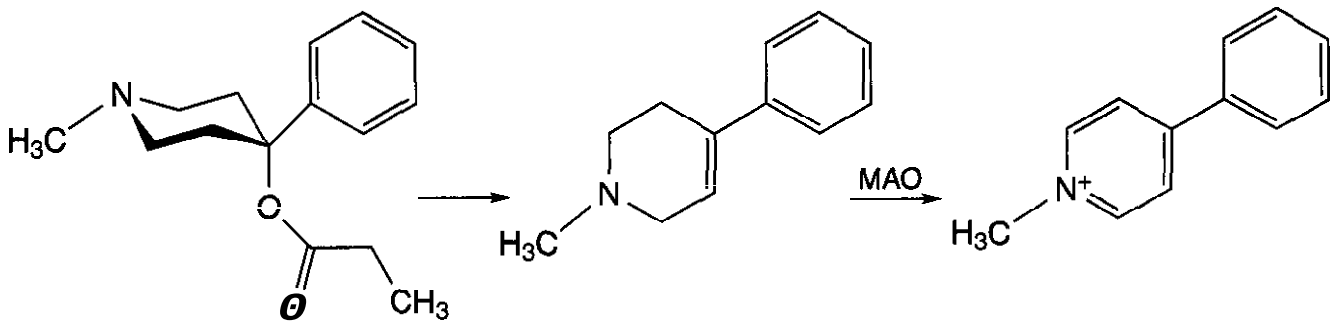
tionships found in the more rigid opiates, particularly with regard to the nitrogen substituent and its effects on agonist versus antagonist activity, do not hold in the 4-alkyl-4-arylpiperidine series. In the case of 4-alkylpiperidines, where the axial aryl conformation is preferred; for example, the *N*-allyl derivative of 4-propyl-4-(m-hydroxyphenyl)piperidine (107, R = allyl), are agonists with only weak antagonist activity (415,416). In contrast the 1,3β,4-trimethyl derivative (**109**) is an antagonist rather than an agonist (417).In this series the 3-methyl group is critical for antagonist activity and an *N*-allyl or cyclopropylmethyl group did not increase antagonist potency (417). Further variation of the nitrogen substituent of the *trans*-3,4-dimethylphenylpiperidines (108) has resulted in the synthesis of several potent analogs with antagonist activity at both μ and κ receptors (418–421), including compounds such as LY255582 (**110**), which has potent anorectant activity, and the peripherally selective antagonist LY246736 (**111**, now designated AD 8-2698 or alvimopan) (420), which is undergoing clinical trials for the treatment of gastrointestinal motility disorders (see Ref. 38 and Section 2.2.2.1). Potent antagonists that exhibit some selectivity for κ receptors [LY227053 (**112**), LY253886 (**113**) (421), and (115) (422)], along with p-selective antagonists (114) (423), have also been identified.

Conformationally constrained derivatives of the 4-alkyl-4-arylpiperidines, in which the aryl ring is constrained into both axial and equatorial conformations, have been prepared. Arylmorphans (116) are constrained analogs in which the aryl ring is equatorial (see Ref. 283,406). The N-methyl derivative is equipotent with morphine after subcutaneous administration to mice (424); the configuration of the more potent dextro enantiomer of this analgesic [the 1*S*,5*R* isomer (425)] is related stereochemically to the more potent enantiomer of 4-arylpiperidines such as a- and β-prodine (101 and 102, respectively) rather than to the rigid benzomorphan (−)-metazocine (283). The receptor affinities of both isomers for different receptor types have also been examined (426). Consistent with the stereochemical relationships, the *N*-allyl and *N*-CPM derivatives are agonists with little if any antagonist activity (427).Introduction of a 9β-methyl group, which is comparable to the 3-methyl substituent of the *trans*-3,4-dimethylphenylpiperidines, resulted in compounds with antagonist activity; the N-phenethyl derivative (117) was much more potent than the N-methyl analog (428). These derivatives still retain rotational freedom around the phenylpiperidine bond. Further constraint of the 5-arylmorphans, with an ether bridge between the aryl ring and the morphan ring system, yielded compounds with low affinity for opioid receptors (see Ref. 283), but the constrained benzofuro[2,3-*c*]pyridin-6-ols (**118**), in which the phenyl ring is constrained to a dihedral angle of 92° (426), retain μ opioid receptor affinity and are potent analgesics (429). In the trans-4a-aryldecahydroisoquinolines (**119**), in which the aryl ring is constrained to the axial conformation, the N-methyl derivative has twice the potency of morphine; interestingly, although the N-methyl derivative is selective for μ receptors, the N-CPM derivative exhibits a slight preference for κ receptors (430).

Active analogs were also prepared by shifting the alkyl and aryl substituents to the 3 position. All active derivatives of the 3-methyl-3-arylpiperines contain the m-hydroxyl on the phenyl ring (283). Although the N-methyl derivatives are weak analgesics, derivatives with an *N*-arylalkyl substituent are significantly more potent (12). Some derivatives with N-allyl or N-CPM groups behave as antagonists, similar to the morphinan series. Ring contracted and expanded analogs have also been prepared, including the mixed agonist/antagonist meptazinol (120). The pharmacological effects of this compound are somewhat unusual (see Ref. 431) and may involve both opioid and cholinergic mechanisms (432). It has



(120) Meptazinol



(121) MPPP **(122)** MPTP **(123)** MPP^+

Figure 7.25. Formation of MPTP and MPP^+ from MPPP.

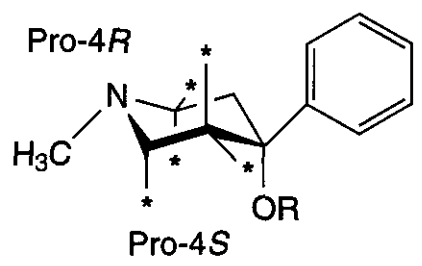
been proposed that the opioid actions of meptazinol are mediated by μ_1 receptors (431).

5.7.2 4-Arylpiperidines with an Oxygen Substituent at C_3 Reversal of the ester in meperidine gives MPPP (*N*-methyl-4-phenyl-4-propionoxypiperidine, 121) and increases analgesic activity 5- to 10-fold (see Ref. 405). First described in 1943 (433), this compound took on new significance in the late 1970s when the drug began appearing as a "designer drug" (a compound with a minor structural change that was prepared in attempts to evade laws regulating controlled substances) and sold on the streets as a heroin substitute. During the synthesis of MPPP in clandestine drug laboratories, however, dehydration occurred during the acylation of the 4-phenyl-4-piperidol, resulting in the formation of MPTP (*N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, 122; see Fig. 7.25). People who took the contaminated drug exhibited symptoms of parkinsonism, even in subjects in their twenties (434). The neurotoxic effects of MPTP are thought to be attributable to its metabolism to MPP^+ (123, N-methyl-4-phenylpyridinium) by monoamine oxidase and destruction of dopaminergic neurons, resulting in the parkinsonism-like symptoms (see Ref. 435).

A wide variety of modifications have been made to MPPP, including modification of the ester functionality, variation in the nitrogen substituent, substitution on the piperidine ring, etc. (see Ref. 405). The propionyl chain is the optimum length, and hydrolysis of the ester to the corresponding alcohol usually results in compounds with little if any activity. Several *N*-phenylalkyl substitutions such as phenethyl increase potency; in some cases [i.e., the anilinoethyl derivative, $(CH_2)_2NHC_6H_5$ (436)], the resulting compound can be more than 1000-fold more potent than meperidine.

A variety of C_3 alkylated derivatives of the reversed esters of meperidine were examined (see Ref. 405). Incorporation of a methyl group at C_3 yields *a*- and *β*-prodine (101 and 102, Fig. 7.23) (437). The *β* isomer (102) has five times the potency of the a isomer. X-ray crystallography and NMR indicate that the preferred conformation of the prodines is the chair form, with the phenyl ring equatorial (see Refs. 283, 438). Computational studies also indicate a preference for this conformer; in the case of the reversed esters the energy differences between the equatorial and axial conformers (1.9–3.4 kcal/mol) are much greater than the differences between the two conformers of meperidine *(7)* and ketobemidone (**105**) (410).

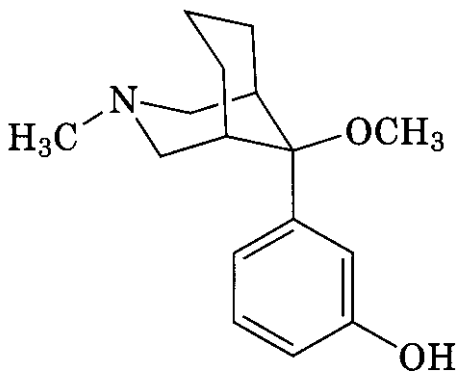
Larger alkyl groups (ethyl, propyl, allyl) are also tolerated at the 3 position, but for these derivatives the a isomer is more potent than the *β* isomer. For the a isomers the more potent enantiomer has the 3*R*,4*S* stereochemistry (439). This led to the suggestion that the prodine derivatives present the pro-4*R* face of the molecule to the receptor (see Fig. 7.26; also



(124)

Figure *7.26.* Pro-4R and pro-4*S* face of prodine derivatives. Positions that tolerate methyl substitution are indicated by an asterisk.

see Ref. 283); thus substitution on the 4*R* side interferes with drug-receptor interactions, whereas substituents on the 4*S* side are not involved in interaction with the receptor and are well tolerated. A variety of other methyl- and dimethyl-substituted derivatives of the reversed esters have been prepared, resulting in compounds with a wide range of potencies and with different orientations of the phenyl ring, depending on the substitution pattern (see Refs. 283, 405, 440); positions that tolerate methyl substitution are indicated in structure (124) (Fig. 7.26) by an asterisk. Very potent analogs have been prepared by bridging the 3 and 5 positions with a trimethylene chain (125, 126) (441). Both the a isomer



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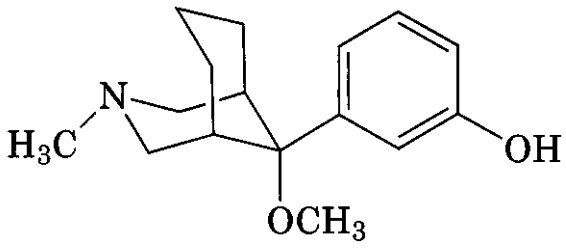


(126)

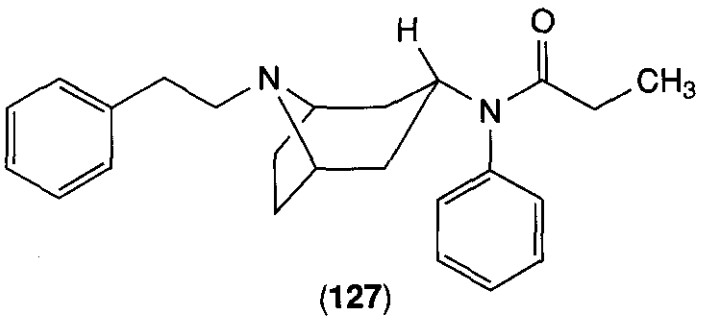
(125, in which the aryl ring is axial) and the β isomer (126, in which the aryl ring is equatorial) have similar potencies; introduction of a m-hydroxyl group on the phenyl ring results in a dramatic (400- to 1000-fold) increase in the potency of both isomers. The corresponding *m*-hydroxyphenyl-3,7-diazabicyclanes also exhibited significant antinociceptive activity (442).

In contrast to meperidine and other phenylpiperidines with a C_4 carbon substituent discussed earlier, introduction of a *m*-hydroxyl group on the phenyl ring of the allylprodines (103 and 104, Fig. 7.23) results in inactive compounds (443). This led Portoghese to propose an alternative mode of interaction to explain how a-allylprodine (103) and other phenylpiperidines in which the phenyl ring is equatorial can interact with the same receptors as morphine and other rigid opiates where the phenyl ring is axial. In the initial bimodal binding model proposed by Portoghese (82), the amine of different opiates was postulated to interact with a common anionic site and the rest of the molecule then would pivot around the nitrogen to bind in one of two possible orientations. The bimodal binding model was subsequently modified to include a second lipophilic site (443), where the equatorial phenyl ring of phenylpiperidines such as a-allylprodine (103) was proposed to interact.

A substantial reduction in analgesic potency was also observed for the rn-hydroxyl derivative of the 2α-methyl reversed ester in which the preferred conformation of the phenyl ring is axial (444), suggesting that there can also be differences in receptor interactions for the axial aryl moiety in this derivative and in morphine. This in turn may be attributable to differences in the relative orientation of the aryl rings in the rigid morphine versus the phenylpiperidine derivatives in which the aryl ring is free to rotate.

5.7.3 4-Anilidopiperidines. 4-Anilidopiperidines, in which an amido nitrogen functionality is positioned between C_4 of the piperidine ring and the phenyl ring, are extremely potent analgesics. Fentanyl (8, Fig. 7.1), the prototype of this class, has almost 500 times the potency of meperidine and 200–500 times the potency of morphine (see Ref. 445). Like meperidine, fentanyl is a μ agonist (404). Because of their rapid onset and short duration of action fentanyl and other 4-anilidopiperidines have been used extensively in anesthesia. In 1991 fentanyl became available as a transdermal patch for the treatment of chronic pain, and lozenge formulations have recently become available (see Table 7.1).

The preferred conformation of fentanyl and other 4-anilidopiperidines has been examined by X-ray crystallography, NMR, and computational methods (see Refs. 187, 284, 446). In the crystal structure the anilido moiety adopts an equatorial conformation (see 8, Fig. 7.1) (447). Several conformationally con-



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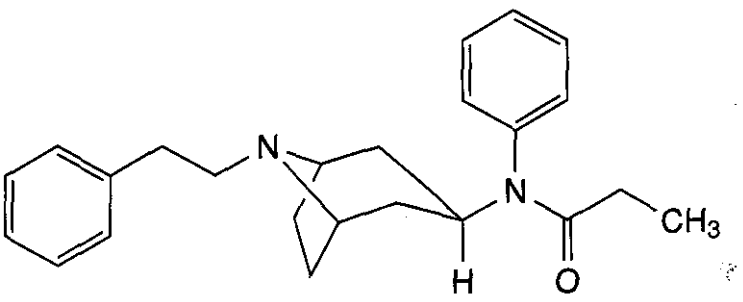

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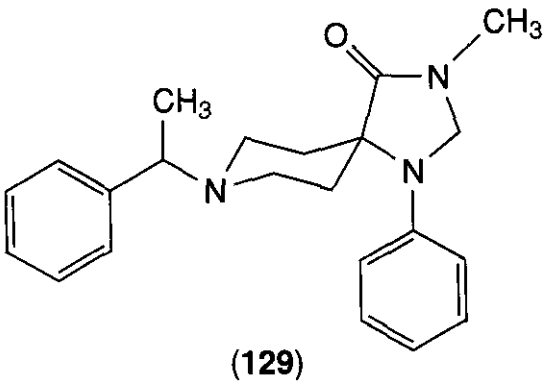

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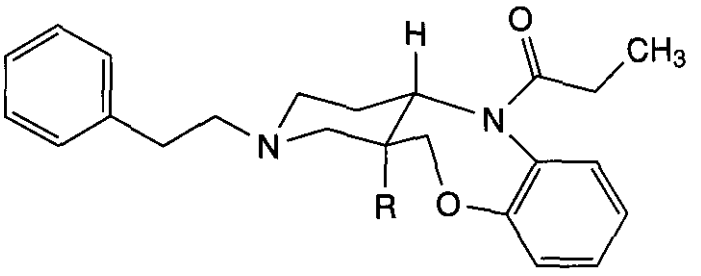
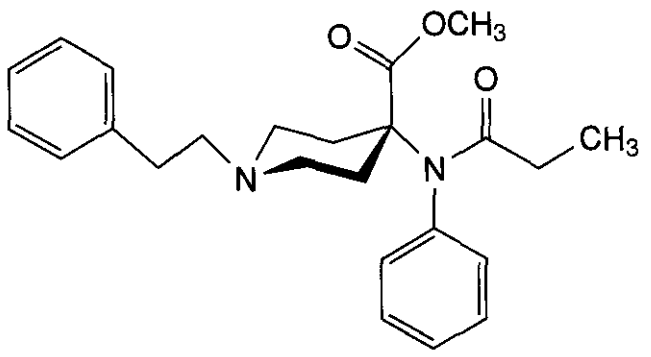
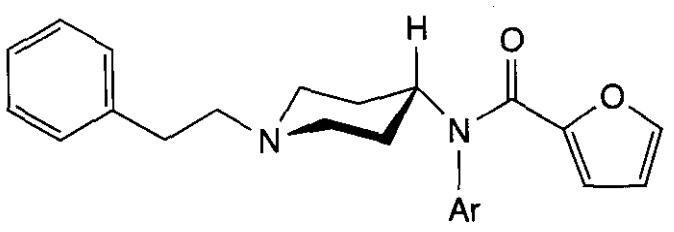
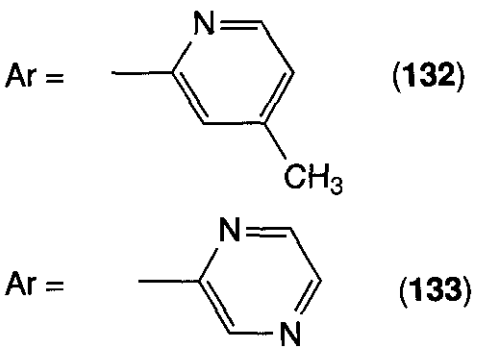


(130) R = H or CH_3



(131) Carfentanil





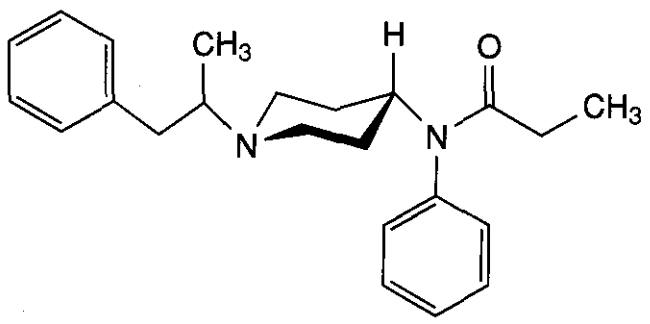
(132)

(133)

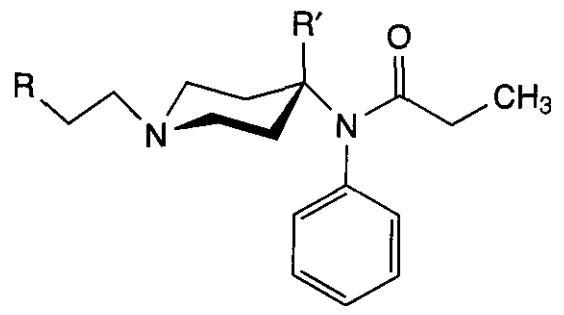
Figure 7.27. Fentanyl analogs.

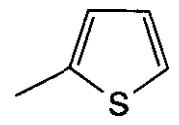
strained analogs of fentanyl have been prepared [see the previous edition of this chapter (286) for a detailed review]. Generally, conformationally constrained derivatives in which the phenyl is constrained in the β orientation are inactive (see Ref. 448), but in conformationally constrained tropane analogs of fentanyl the β analog (128, Fig. 7.27), in which the anilido group is equatorial, is more potent than the *a* isomer (**127**), in which the propananilido moiety is pseudoequatorial (449). The high potency of the conformationally constrained spirane derivative (129) provides evidence that the phenyl ring may be oriented α when fentanyl binds to opioid receptors. Additional conformationally constrained 1,5-benzoxazepine derivatives constrained through cyclization between the *ortho* position of the phenyl ring and the 3 position (**130**) are also potent analgesics (450).

A large number of modifications have been made to fentanyl (see Refs. 284,406,445,448). Unlike the phenylpiperidines a second substituent at C_4 is not required, but the addition of a polar carbon substituent (CO_2CH_3 or CH_2OCH_3) at this position to give, for example, carfentanil (**131**), enhances potency 27-fold over that of fentanyl and results in compounds 7800 times the potency of morphine (451). In the case of the anilido side chain, the



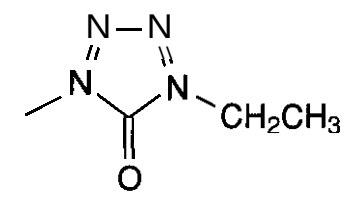
(134) α-Methylfentanyl



R = 

(135) Sufentanil

$R' = CH_2OCH_3$

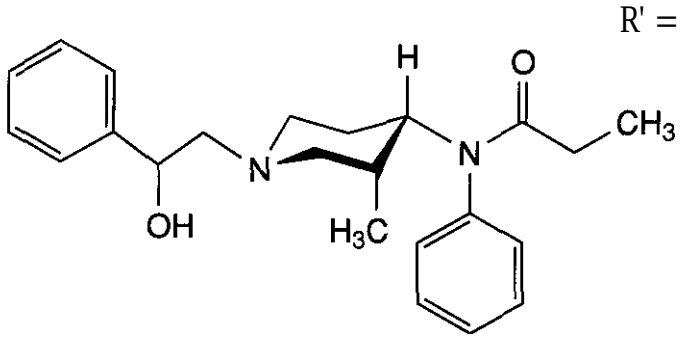


(136) Alfentanil

$R' = CH_2OCH_3$

$R = CO_2CH_3$
$R' = CO_2CH_3$

(137) Remifentanil



(138) Ohmfentanyl

Figure 7.27. (Continued.)

propionamide is the optimum length (448); the methoxyacetamides are also potent analgesics (see Ref. 406). Replacement of the propionamide with 2- or 3-fumaramide resulted in compounds with antagonist activity against both morphine-induced analgesia and respiratory depression (**132**) or against respiratory depression alone (**133**) (452); the flat aromatic furan ring appears to be important for antagonist activity. Previous attempts to produce antagonist derivatives of fentanyl by replacement of the piperidino phenethyl group with allyl or CPM were unsuccessful (**449, 453**), and thus it appears that changes should be made around the amide nitrogen rather than to the basic piperidino substituent to impart antagonist activity to fentanyl analogs (450).

A variety of changes to the nitrogen substituent on the piperidine ring have been made that produce potent analgesics. The branched-chain derivative a-methylfentanyl (134) has achieved notoriety as the street drug "China White" (454). The phenethyl substituent is the optimum length, but potency can be enhanced by replacement of the phenyl ring with a heterocycle such as the 2-thienyl ring (451). Combination of a heterocycle substitution on the piperidino chain and a polar carbon substituent attached to position 4 have yielded the clinically used agents sufentanil (**135**) and alfentanil (136) (Table 7.1), which like fentanyl are used as adjuncts in anesthesia. Although less potent than fentanyl and sufentanil, alfentanil has a faster onset and shorter duration of action than these other 4-anilidopiperidines. The rapid onset of action of alfentanil may be attributed to its physiochemical properties; alfentanil is a much weaker base ($pK_a = 6.5$) than fentanyl ($pK_a = 8.4$) and sufentanil ($pK_a = 8.0$), so that a higher propor-

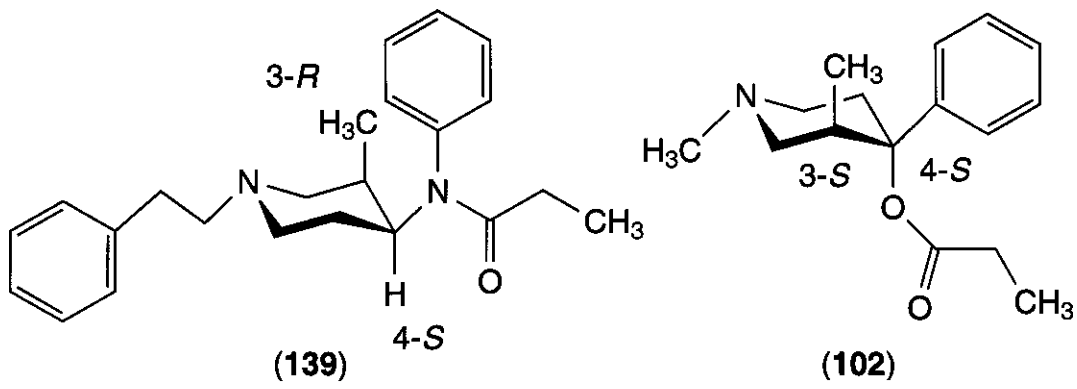


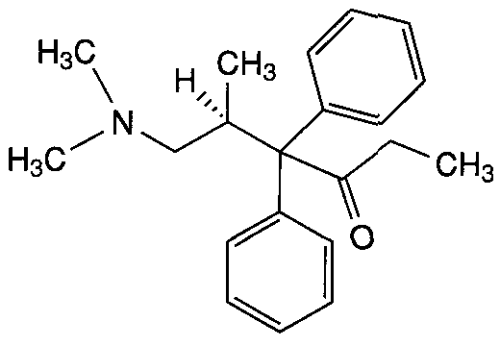
Figure 7.28. Comparison of the 3*R*,4*S* isomer of cis-3-methylfentanyl (139) and the 3*S*,4*S* isomer of β-prodine (102).

tion of the unprotonated amine would be available to penetrate the blood-brain barrier (406). Other heterocyclic substitutions have been made on the piperidino chain (450), resulting in some compounds with less respiratory depression. Incorporation of an ester functionality into the piperidino substituent to give remifentanil (137) (Table 7.1) results in a compound that is 30-fold more potent than alfentanil, and that has very rapid onset (1.6 min) and offset (5.4 min), which is independent of the duration of administration (455, 456). This is due to the rapid hydrolysis by esterases to the acid derivative, which has very low analgesic activity and is rapidly excreted.

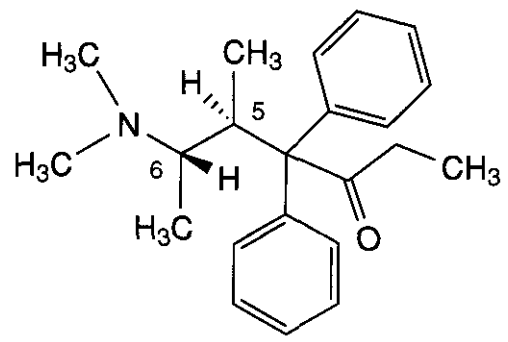
Introduction of a methyl substituent in the 3 position of the piperidine ring results in chiral compounds. The racemic cis derivative of fentanyl is more potent than the trans derivative (457). For the cis racemate the *dextro* isomer has 120 times the potency of the (−)-isomer; the absolute stereochemistry of the (+)-cis isomer is 3*R*,4*S* (458). The analog of cis-3-methylfentanyl with a hydroxyl group on the N-phenethyl chain ohmfentanyl (138) is extremely potent (7000 times morphine) in antinociceptive assays and exhibits remarkable selectivity for μ receptors [27,000-fold selectivity for μ versus δ receptors (459)]. Comparisons of the 3*R*,4*S* isomer of *cis*-3-methylfentanyl (139) and the 3*S*,4*S* isomer of β-prodine (102, Fig. 7.28) suggest that although these compounds are both μ agonists, they represent different classes of ligands that have different modes of interaction with opioid receptors (284) (there is an error in the stereochemistry at position 3 of β-prodine in Ref. 284, p. 504). Fentanyl derivatives containing propyl and allyl substituents at C_3 exhibit significant differences in SAR from the corresponding prodine analogs (see Ref. 284), supporting this conclusion. A series of *cis*/trans pairs of 3-methylfentanyl analogs was reported (460), and in some cases in which the anilido ring had an *ortho* substituent, the trans isomers were more potent than the cis isomers. This may be due to steric hindrance between the *ortho* substituent and the 3-methyl group in the cis isomers interfering with the phenyl ring adopting an α orientation (see above) (284). As discussed earlier, the conformationally constrained 1,5-benzoxazepine derivatives linked through the *ortho* position on the phenyl ring and the 3-methyl substituent (130) are active in antinociceptive assays (450).

Recently, two models of cis-(+)-3-methylfentanyl (139) and other 4-anilidopiperidines docked to μ opioid receptors were described (182, 187). The binding mode for *cis*-3-methylfentanyl in the two models is different, which has been attributed to different conformations of this compound used for docking to the receptor (187). Comparison of *cis*-3-methylfentanyl to *N*-phenethylnormorphine suggested that there was considerable overlap in the region of the receptor occupied by the N-phenethyl groups, but that there was no overlap in the region in the receptor binding pocket occupied by the N-phenyl ring of cis-3-methylfentanyl and the phenol ring of the morphine analog (see Fig. 7.5 in Ref. 187).

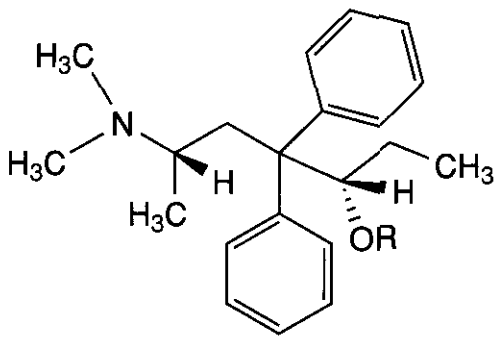
Affinity label derivatives of fentanyl and (+)-*cis*-3-methylfentanyl FIT (198, 461) and SUPERFIT (458), which have an isothiocyanate group on the phenyl ring of the piperidino substituent, have been prepared. Unlike the reversible parent compounds, which are μ agonists, these affinity labels irreversibly bind to 6 receptors. These compounds are discussed further in Section 5.11 on affinity labels.



(140) Isomethadone

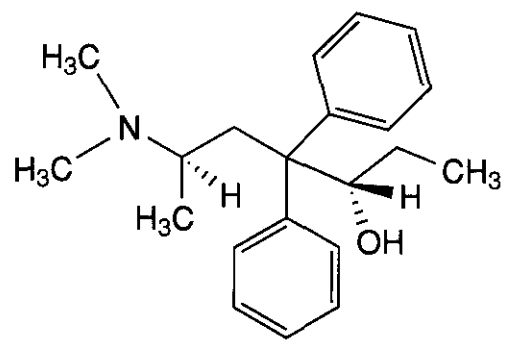


(141) *erythro*-5-Methylmethadone

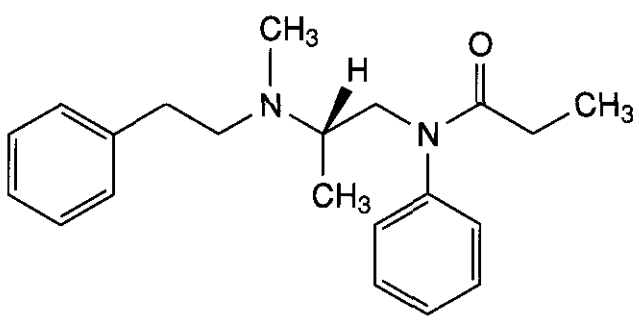


R = H (142) α-Methadol

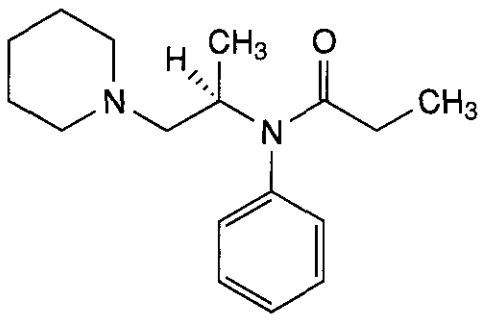
R = Ac (143) *levo*-α-Acetylmethadol (LAAM)



(144) β-Methadol



(145) Diampromide



(146) Phenampromide

Figure 7.29. Methadone analogs.

5.8 Acyclic Analgesics

Methadone (9, Fig. 7.1) and its analogs can be viewed as ring-opened derivatives of the phenylpiperidines. As was the case for meperidine, methadone was not discovered by this systematic structural approach, but was instead identified in Germany during World War II while investigators were looking for compounds with antispasmodic activity (462). These acycylic analgesics are highly flexible molecules capable of adopting a multitude of conformations (see Ref. 284). Methadone is a potent analgesic, and the racemate has approximately twice the potency of morphine and 5–10 times the potency of meperidine (see Ref. 463). The principal use of methadone, however, has been in the maintenance of individuals addicted to narcotics. It has relatively good oral activity and long duration of action, permitting once-daily dosing, and is reported to produce less euphoria than does morphine. The major metabolic pathway for methadone involves N-demethylation, although these derivatives are not stable and undergo cyclization to inactive metabolites through intramolecular Schiff base formation (see Ref. 463). Methadone is also metabolized to an active metabolite methadol (see below).

Methadone and its isomer isomethadone (140, Fig. 7.29), which was also obtained from early syntheses of methadone (see Ref. 463), each contain an asymmetric center. The more active isomers for both compounds are the (−) isomers (see Refs. 284,463). The (−) enantiomer of methadone, which has the R configuration, has 7–50 times higher potency in antinociceptive assays and greater than 10-fold higher affinity for opioid receptors than that of

the (+) isomer. In the case of isomethadone, which is slightly less potent than methadone, the more active (−) enantiomer has the S configuration and has 40 times the potency of the (+) isomer (see Refs. 284,463). NMR, circular dichroism (464), and molecular modeling studies (465) suggest that isomethadone may be less flexible than methadone because of the proximity of the methyl group to the phenyl rings in isomethadone.

These flexible analgesics exhibit their own distinct SAR (see Refs. 284, 463, 466). A variety of nitrogen substituents have been examined, and whereas larger acyclic groups markedly decrease or abolish activity, compounds containing a cyclic substituent, such as pyrrolidine, piperidine, or morpholine, on the nitrogen retain activity. The two-carbon chain length between the quaternary carbon and the nitrogen is the optimal length, and lengthening the chain abolishes activity. Removal of the methyl group from the alkyl chain to give the achiral compound normethadone decreases potency 6- to 10-fold relative to that of methadone and isomethadone, respectively. Introduction of a second methyl group at position 5 yields erythro- and *threo*-5-methylmethadone (467). The erythro form (141) has five times the potency of methadone, whereas the threo form is inactive. The more active *levo* isomer of erythro-5-methylmethadone has the 5*S*,6*S* configuration (468). Interestingly, one of the isomers of the inactive threo racemate, with the 5*S*,6*R* configuration, combines the configurations found in the more active enantiomers of methadone and isomethadone (467). NMR analysis suggests that the erythro form exhibits greater conformational flexibility than that of the threo form. The authors suggested that the marked difference in analgesic activity may be attributable to different conformational preferences of the threo and erythro forms (467), and that the conformation observed for *erythro*-5-methylmethadone in the solid state (469) is the active conformation of erythro-5-methylmethadone, as well as for (−)-methadone and (−)-isomethadone.

The ketone side chain is also important for activity (see Ref. 463) and changing the length of this chain decreases activity. Reduction of the ketone to the two possible alcohols, α- and β-methadol (142 and 144) decreases activity, but activity can be restored by acetylation (470); the resulting acetates are more potent than the parent ketones. The more active methadol isomers, (−)-α- and (−)-β-methadol [with absolute configurations 3*S*,6*S* and 3*S*,6*R*, respectively (471)], both have the same 3*S* configuration, suggesting that the stereochemistry around the alcohol is more important in these derivatives. The more active 3*S*,6*R* isomers of β-methadol and β-acetylmethadol are derived from the more active *R*-(−) enantiomer of methadone. An interesting reversal in enantioselectivity occurs in the a series; although the more potent 3*S*,6*S* isomer of α-methadol is derived from the less active *S*-(+) isomer of methadone, acetylation reverses the enantioselectivity, so that the more potent isomer is the 3*R*,6*R*-(+) isomer (471). *levo*-α-Acetylmethadol (LAAM, 143) has a longer duration than methadone, requiring dosing only once every 3 days, and is being used in the United States for maintenance of individuals addicted to narcotics. Like methadone, the major route of metabolism of this compound is *N*-demethylation. In the case of the methadols and acetylmethadols, these secondary amines-derivatives are active, with potencies similar to those of the parent tertiary amines, and probably contribute to the activity and longer duration of action of LAAM (see Ref. 463). The ketone of methadone has also been replaced with a variety of other functional groups, including esters, ethers, and amides (see Ref. 463). In the acyloxy series propionyloxyisomethadone shows significant activity (472); further modification of this compound yields propoxyphene (see below).

Most modifications of the phenyls in the diphenyl fragment of methadone result in substantial loss of analgesic activity (see Ref. 463). In normethadone analogs replacement of one of the phenyl rings by a benzyl group abolishes activity (473), but in the isomethadone analog with a propionoxy group in place of the ketone this modification results in propoxyphene (10, Fig. 7.1), which has modest analgesic activity (approximately 1/10 the potency of methadone 472; see Table 7.1). The replacement of one of the phenyl rings by a benzyl group introduces a second chiral center into this molecule. The active (+) isomer has the 2*S*,3*R* stereochemistry, which is the

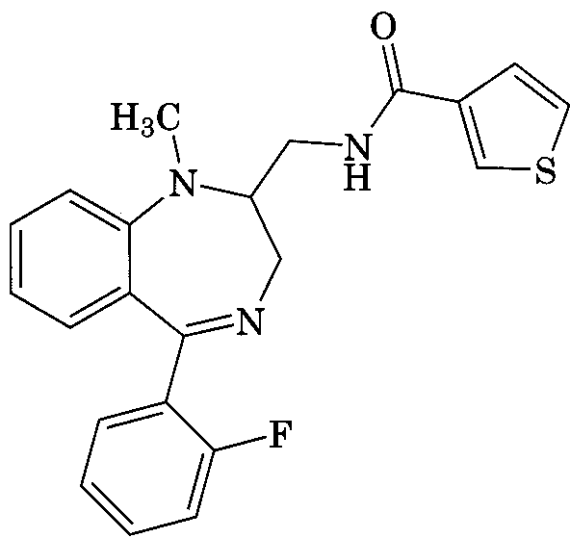
same absolute configuration at C_3 as in the active (−)-isomethadone (see Refs. 284, 463). N-Arylpropionamide analgesics such as the methadone analog diarnpromide (145) and the isornethadone analog phenampromide (146) contain a single aromatic ring attached to a nitrogen, analogous to the 4-anilidopiperidines, and are exceptions to the requirement for a second phenyl ring. Diampromide is somewhat more potent than phenampromide, with a potency between meperidine and morphine. The more active isomer of phenampromide is the R-(−) isomer (which has the same configuration as the more active (−)-isornethadone), whereas the more active isomer of diampromide has the *S* configuration, which is opposite that of (−)-methadone (see Refs. 284,463). This led Portoghese to suggest that methadone and diampromide probably differ in their modes of interaction with opioid receptors (82).

5.9 Kappa-Selective Agonists

There has been considerable interest in developing κ agonists as analgesics that would not have the side effects characteristic of morphine and other μ opiates (e.g., respiratory depression and addiction), and therefore numerous κ-selective compounds have been reported over the last two decades (see Refs. 287, 288, 474 for reviews). As noted earlier (Section 3.3.2), although κ receptors can mediate analgesia, there are differences between the effects mediated by μ and κ receptors (see Ref. 146 for a review). There has also been interest in κ-selective compounds as potential neuroprotective and anticonvulsant agents (see Refs. 256, 475 for reviews).

Although some compounds with κ agonist activity are used clinically as analgesics (e.g., pentazocine, nalbuphine, and butorphanol, Table 7.3 above), the use of many κ agonists has been severely limited by centrally mediated side effects, mainly dysphoria and sedation, associated with most of these compounds (146). The ability of κ agonists to produce analgesia in inflammatory pain through interaction with peripheral κ opioid receptors has stirred interest in the development of peripherally selective kappa agonists (see Section 5.9.2).

5.9.1 Centrally Acting Agonists. Benzomorphan ligands such as EKC (33) and bremazocine (34, Fig. 7.6) have been used to study κ opioid receptors, but these compounds exhibit low selectivity for these receptors [K_i ratio (μ/κ) = 1.9 for EKC and 8.3 for bremazocine; see Table 7.101. As discussed earlier (Section 5.3.1), the 4,5α-epoxymorphinan TRK-820 (65, Fig. 7.16) is also a potent agonist at κ opioid receptors, exhibiting modest selectivity for these receptors [K_i ratio (μ/κ) = 15; see Table 7.101. The benzodiazepine tifluadom (147) reported by Romer (476) is also a κ



(147) Tifluadom

opioid receptor agonist, but it too exhibits low selectivity for κ receptors [K_i ratio (μ/κ) = 5.4; see Table 7.111. Racemic tifluadom exhibits only very weak affinity for benzodiazepine receptors (IC_{50} = 4.1 @) (477); the (−) isomer has greater affinity for opioid receptors and is more selective for opioid over benzodiazepine receptors than the (+) isomer (477). Tifluadom also exhibits high affinity for peripheral CCK receptors [IC_{50} = 29 n*M* for the (−) isomer] and is an antagonist at these receptors (478). Additional analogs of tifluadom with comparable affinity and somewhat greater selectivity have been reported that are devoid of affinity for CCK receptors (179). A computational model of tifluadom bound to κ opioid receptors has also been described (179).

The first κ-selective nonpeptide derivative identified was the benzacetamide U50,488 (35, Fig. 7.6), which was found while investigators examined cycloalkane-1,2-diamines as antidepressants (115). This compound exhib-

Table 7.11 Opioid Receptor Affinities, κ Selectivity, and Analgesic Activity of κ Opioid Ligands[a]

Compound	Receptor Affinity (K_i, nM)		K_i Ratio	Analgesic Activity MPE_{50} (mg/kg)[b]		
	κ[c]	μ[d]	μ/κ	i.v.	p.o.	References(s)
Tifluadom (**147**)	4.1	22	5.4			130
U50,488 (35)	0.69	435	630	1.96	9.6	
(−) isomer	0.89					482
(+) isomer	299					482
U69,593 (**36**)	0.67	2,460	3,670	0.67	18.4	
Spiradoline (U62,066, **148**)	0.35	43.7	125	0.38	48.5	
(−) isomer	0.31	84	271			486
(+) isomer	1,360	9.8	0.0072			486
PD 117302 (**149**)	0.50	399	798	1.41	25.3	
(−) isomer	0.39	414	1,060			486
(−) CI-977 (**37**)	0.11	99.6	905	0.02	1.8	
DuP 747 (150)	6[e]	304[e]	50.6	0.46 s.c.[f]	6.2[f]	494
S,S isomer	7[e]	750[e]	107	0.15 s.c.[f]	5.2[f]	495
Niravoline (RU 51599,151)	0.41	699	1,700	—	—	533
BRL 52537A (**155**)	0.24	1,560	6,500	0.05 s.c.[g]	—	503
BRL 52656A (**156**)	0.57	2,340	4,100	0.11 s.c.[g]	—	503
GR 89696 (**158**)	1.15[h]	0.65[h]	0.56	0.0005 s.c.[i]	—	507, 511
R-84760 (159)	0.44[j]	297[j]	681	0.0013 s.c.[f]	0.013[f]	506
Apadoline (RP 60180,160)	0.55[k]	57.1	104[l]	—	—	514
ICI 197067 (161)	6.3[m]	11,800[m]	1,870	0.05 s.c.[n]	—	515
ICI 199441 (**162**)	6.9[m]	4,500[m]	652	0.004 s.c.[n]	—	515
HZ2 (**163**)	15[o]	>1,000[o]	>65	—	—	521

[a]Data from Ref. 118 except where otherwise indicated.

[b]Rat paw pressure test, MPE_{50}, is the dose required to produce 50% of the maximum possible analgesic effect, except where otherwise indicated.

[c]Inhibition of [^{3}H]U69,593 binding in guinea pig forebrain, except where otherwise noted.

[d]Inhibition of [^{3}H]DAMGO binding in guinea pig forebrain, except where otherwise noted.

[e]U50,488 K_i = 6 and 825 nM in κ and μ assays, respectively (494).

[f]Mouse phenylquinone writhing assay ED_{50} s.c. and p.o., respectively. ED_{50} for U50,488 = 1.2 mg/kg s.c. and 13 mg/kg p.o. (494); 0.47 mg/kg s.c. and 27 mg/kg p.o. (506).

[g]ED_{50} mouse tail flick assay. U50,488 ED_{50} = 1.9 mg/kg s.c.

[h]Binding determined in monkey cortical membranes.

[i]Mouse acetylcholine writhing assays ED_{50} s.c. ED_{50} for U50,488 = 0.41 mg/kg s.c.

[j]IC_{50} values for U50,488 = 7.59 and 571 nM for κ and μ binding, respectively.

[k]Inhibition of [^{3}H]EKC binding in guinea pig cerebellum.

[l]K_i (δ) = 1.6 nM ([^{3}H]EKC binding in NG 108–15 cells), K_i ratio (δ/κ) = 2.9.

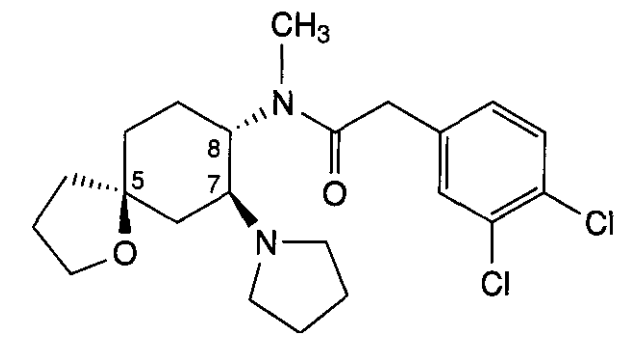
[m]IC_{50} values for inhibition of [^{3}H]bremazocine binding in guinea pig brain minus cerebellum and [^{3}H]naloxone binding in rat brain for κ and preceptors, respectively. U50,488 IC_{50} = 95.5 and 14,200 nM, respectively.

[n]ED_{50} in mouse acetic acid abdominal constriction assay. U50,488 ED_{50} = 1.1 mg/kg s.c.

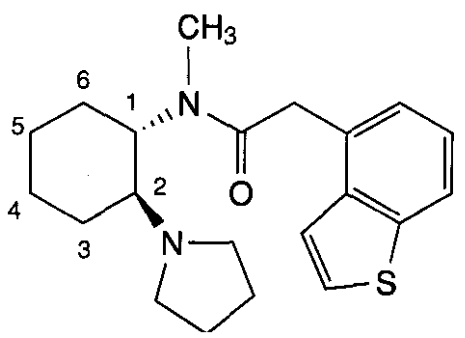
[o]Inhibition of [^{3}H]CI-977 and [^{3}H]naloxone binding in rat brain membranes for κ and preceptors, respectively.

its high κ selectivity in binding assays (see Table 7.11) and produces analgesia through κ receptors *in vivo* (479,480).

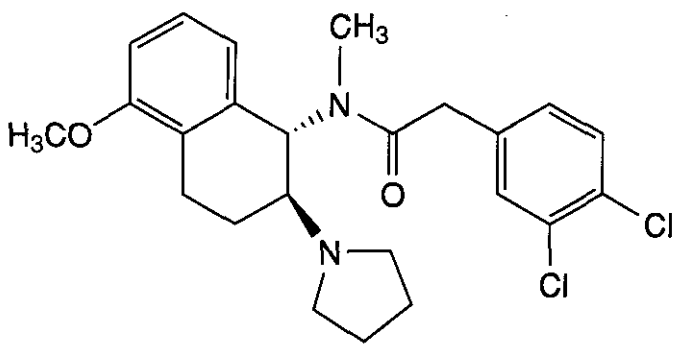
U50,488, which was initially characterized as the racemic mixture, was resolved (481), and the *levo* isomer found to have greater affinity for κ receptors (482) (see Table 7.11). The absolute stereochemistry of the *levo* isomer was determined by X-ray crystallography of an intermediate to be 1*S*,2*S* (481). The protective effects of U50,488 against the temporary bilateral carotid occlusion model of cere-



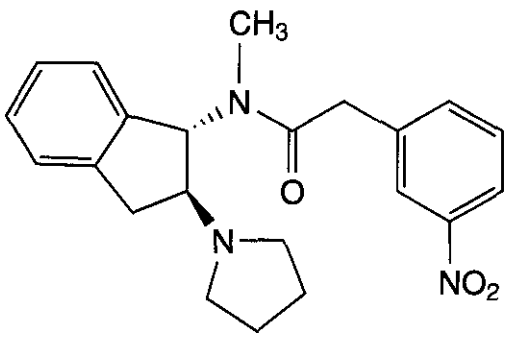
(148) Spiradoline
((±) U62,066)



(149) (±) PD 117302



(150) (±) DuP 747



(151) Niravoline
(RU 51599)

Figure **7.30.** κ-**Receptor selective** agonists. **The structures of** U50,488 (**35**), U69,593 (**36**), **and CI-977 (37) are given in Fig. 7.6.**

bral ischemia also reside predominantly in the *levo* isomer (**483**), consistent with the hypothesis that κ receptors may be involved in these protective effects.

Introduction of a spiro ether group on the cyclohexane ring was one of the earliest modifications to **U50,488** reported, **giving** (−)-**U69,593** (36, Fig. 7.6) (116) and spiradoline (**U62,066**, 148, Fig. 7.30) (484). **U69,593** exhibits improved κ-receptor selectivity over **U50,488** (116) (see Table 7.11). The tritiated form of **U69,593** was prepared by catalytic **tritiation** of the aromatic chlorines of **U62,066** (**116**), and this highly selective tritiated κ **ligand** has been used extensively in **radioligand**-binding assays (see Section 3.2.3.2). The X-ray structure of **U69,593** has been reported [see Ref. 485; however, as noted by **Rees** (**474**), there is a discrepancy between the X-ray structure as drawn in this paper (**5*R*,7*S*,8*S***) and that indicated in the title (**5*S*,7*S*,8*S***), which has led to some confusion concerning the absolute stereochemistry of **U69,593**]. **As** is the case with **U50,488,** the (−) isomer of spiradoline is much more potent (**>30-fold**) than the (+) isomer in analgesic assays (484) and possesses much greater affinity for κ receptors (see Table 7.11) (486); the (+) isomer displays preceptor selectivity (see Table 7.11) (486). Like **U50,488** spiradoline exhibits **neuroprotective** effects and is even more effective than **U50,488** in reducing postischemic necrosis of the vulnerable hippocampal CA_1 neurons (487). Further examination of C_4 **and/or** C_5 methyl ether and spiro tetrahydrofuran substituents indicated that optimal κ-receptor selectivity was obtained when the oxygen was in the equatorial (β) orientation at C_4 of the ring (**117**).

From the initial compounds it was clear that the spacing between the amide and the aromatic ring system in the N-methylamide side chain is critical for κ-receptor activity. Whereas phenylacetamide derivatives (**i.e.**, **-NCH_3COCH_2Ar**) such as **U50,488** and its analogs exhibit κ activity, **benzamide** derivatives (**i.e., -NHCOAr** and **-NCH_3COAr**) exhibit morphine-like effects (115,488). In both series *N*-methyl substitution increases potency over the **unsubstituted** secondary **amides** (115, 489).

Numerous variations have been examined in all portions of the **U50,488** and spiradoline structures [see Refs. 287,288,474 and the previous edition of this chapter (286) for detailed reviews]. Figure 7.31 shows the general **struc-**

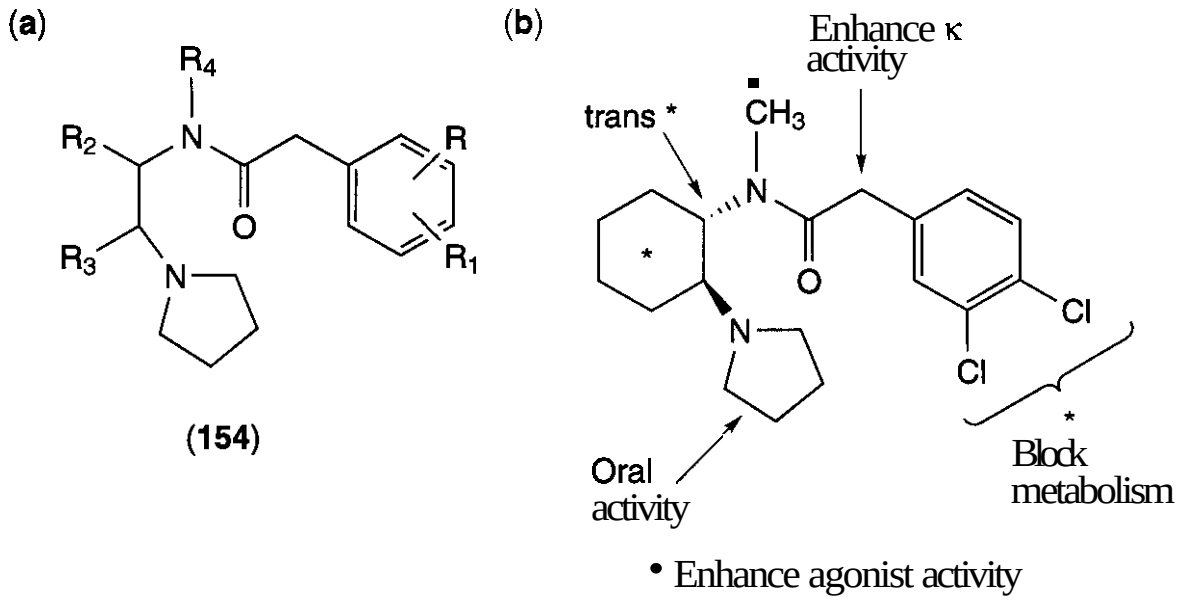
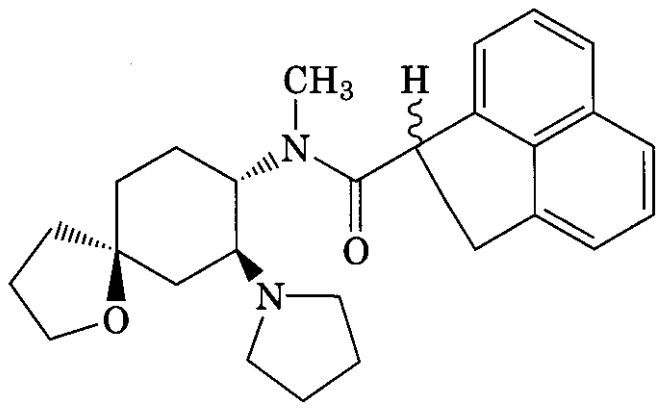
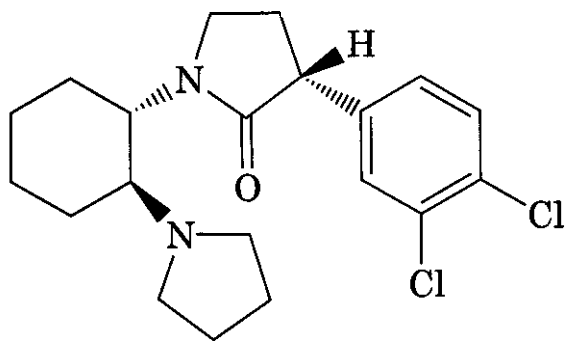


Figure 7.31. (a) General structure (154) common to most of the analogs of U50,488; (b) summary of SAR for analgesic activity of U50,488 (35) (from Ref. 287).

ture (154) common to most of the analogs and a summary of the SAR of U50,488 (287). In addition to varying the spacing between the amide and aromatic ring, other modifications in the N-methylamide side chain of U50,488 and spiradoline examined include replacement of the amide linkage, substitution on the methylene between the amide and the aromatic ring, and varying the identity of the aromatic ring system. The amide linkage appears to be important because substitution of this linkage with a variety of replacements (reversed amide, reduced N-methyl amide, or ester) all caused significant decreases in κ-receptor affinity (489); only the *N*-methylthioamide derivative retained significant affinity, and even this minor substitution of sulfur for oxygen resulted in approximately a 15-fold decrease in affinity. Whereas substitution of a methyl group a to the carbonyl results in a large (200-fold) decrease in κ-receptor affinity, conformational constraint involving linking the α carbon to either the aromatic group (152) (490) or the amide nitrogen (153) (491) resulted in analogs with high affinity for κ receptors.



(152)



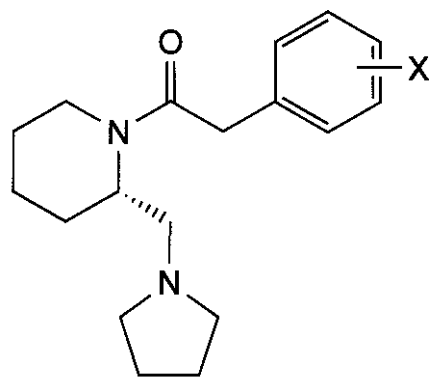
(153)

Variations in the aromatic ring system of the N-methylamide side chain led to two well-characterized agents. Replacement of the dichlorophenyl ring with either 4-benzo[*b*]thiophene to give (±) PD 117302 (149, Fig. 7.30) or 4-benzofuran (492) yields potent compounds with high κ-receptor selectivity (see Table 7.11). Again, the (−)-S,S enantiomer exhibits much greater affinity for κ receptors than the R,R isomer (see Table 7.11) and is the one exhibiting analgesic activity. Similar replacement of the phenyl ring of U69,593 with the 4-benzofuran ring system yields (−) CI-977 (enadoline, 37, Fig. 7.6) (117, 118), which is also one of the most potent and highly selective κ ligands (see Table 7.11) (117, 118). Tritiated CI-977 is commercially available (see Table 7.7) and is frequently used in radioligand-binding assays.

Numerous variations and substitutions on the cyclohexane ring have also been reported. The configuration of the 1,2-diamino amide on the cyclohexane ring is critical for κ-receptor affinity and activity. Kappa ligands such as U50,488 and its analogs all have the *trans* configuration; isomers with the cis configuration have weak affinity for κ receptors and instead have much higher affinity for the (+)-3-PPP [(+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine] or σ binding site (482). For the amine side chain the pyrrolidine ring is the optimal substituent; changing the ring size decreased κ-receptor affinity and selectivity and "opening" the ring to give the *N*,*N*-diethyl analog resulted in almost a 500-fold decrease in κ-receptor affinity (492). Attachment of aromatic rings to the cyclohexane ring has been examined by several groups (493–495). By combining the structures of U50,488 and 2-aminotetralin, researchers at DuPont Merck developed tetrahydronaphthyl analogs as κ agonists (494,495); a 5-methyl ether substituent gave optimal activity and led to the identification of DuP 747 (150) as a new κ analgesic. Analysis of the two enantiomers of racemic DuP 747 indicated that again the (+)-*S*,*S* isomer was the one with high κ-receptor affinity and analgesic activity (495). The related compound niravoline (RU 51599, 151), in which a cyclopentane ring replaces the cyclohexane ring (496, 497), has been studied for its diuretic effect in rats with cirrhosis (498–500) and in brain edema in animal models of ischemia and stroke, traumatic brain injury, and brain tumors (see Refs. 501,502).

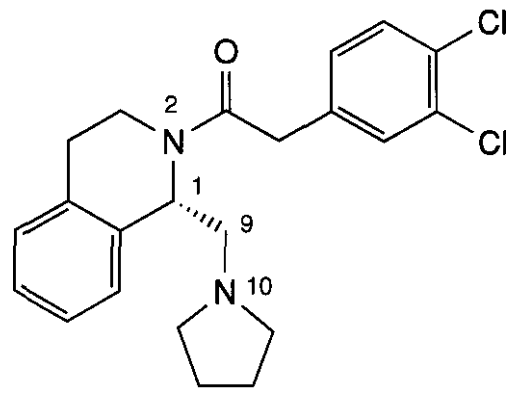
Alternative ring systems to the 1,2-diamine substituted cyclohexane skeleton have also been examined (Fig. 7.32). Researchers from Glaxo and SmithKline examined both piperidine and piperazine analogs as κ agonists (see Refs. 286–288 for detailed reviews). Researchers at SmithKline in Italy identified a common pharmacophore (-N-C-C-N-$COCH_2Ar$) in κ-selective ligands, with a torsion angle of 60° around the C-C bond (503), which led them to develop piperidine derivatives such as BRL 52537A (155) and BRL 52656A (156). The torsion angle found for the N_2-C_1-C_9-N_{10} pharmacophore by X-ray and NMR analysis of these compounds was approximately 60°, similar to that in the proposed pharmacophore (503). In both the piperidine and the related tetrahydroisoquinoline series (e.g., 157) the (−)-*S* isomer was the active enantiomer (503–505). A compound with the related thiazine ring system (R-84760, 159) (506) has also been reported to have κ opioid receptor activity. R-84760 is an extremely potent κ agonist, with a potency of 2.5–20 times that of CI-977 in several different assays; the stereochemistry of R-84760, the most active isomer, is 3*R*,1′*S*. Because of the "relative inaccessibility" of the 2,4-substituted piperidine ring system, the Glaxo group evaluated piperazine analogs where N_4 substituents could be more easily introduced (507). This led to the development of racemic GR 89696 and its R isomer GR 103545 (158) (507, 508). Neuroprotective effects have been demonstrated for GR 89696 (509). GR 89696 has been examined in the guinea pig hippocampus and reported to be an agonist at the κ_2-receptor subtype but an antagonist at κ_1 receptors (510). Recent studies in rhesus monkeys (511) found that GR 89696 exhibited considerable affinity for preceptors (Table 7.11) in binding assays, but low efficacy and potency (100-fold lower than at κ receptors) in [^{35}S]GTPγS assays. In *vivo*, the effects of GR 89696 were less sensitive than those of, U50,488 or U69,593 to antagonism by naltrexone and insensitive to norBNI, consistent with action through the postulated κ_2 receptors. Interestingly, substitution of a methyl group α to the pyrrolidine nitrogen had a marked effect on the affinity of the derivatives for μ opioid receptors, with the S,S isomer exhibiting subnamolar affinity for μ as well as κ receptors, whereas the S,R and R,S exhibited very low (>1 μ*M*) affinity for μ receptors (512). Addition of a 3-hydroxyl to the pyrrolidine ring led to compounds with significant peripheral selectivity (see below) (513). The phenothioazine derivative apadoline (RP 60180, 160) (514) exhibits 100-fold selectivity for κ over μ receptors, but it possesses only a small (threefold) selectivity for κ over δ receptors.

Compounds with κ receptor activity that do not contain the cyclohexane ring have also been identified (Fig. 7.32). Although replacement of the cyclohexane ring with an unsub-

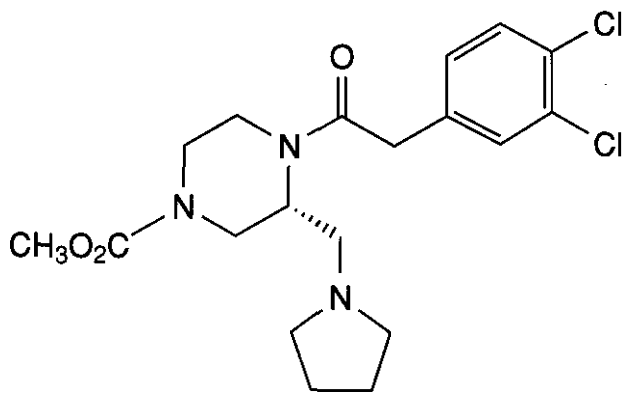


X = 3,4-diCl (155) (*S*) BRL 52537A

X = 4-CF_3 (156) (*S*) BRL 52656A

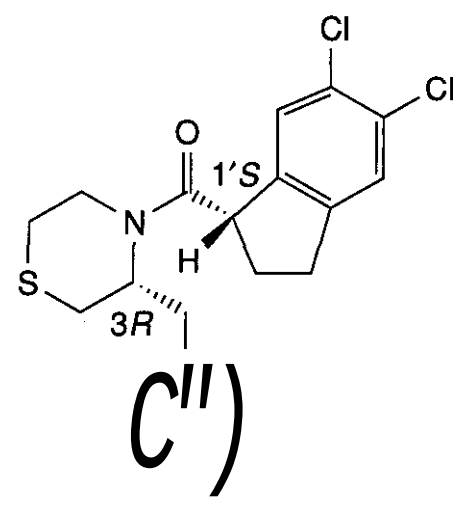


(**157**)

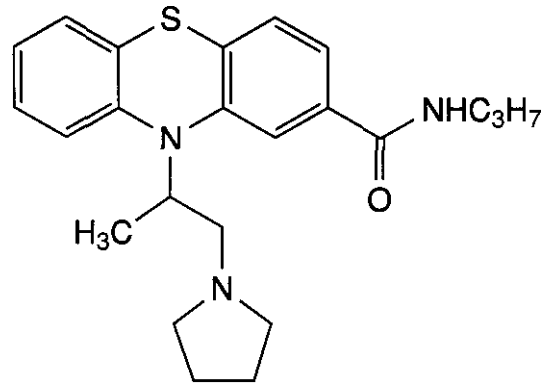


(158) (*R,S*) GR 89696

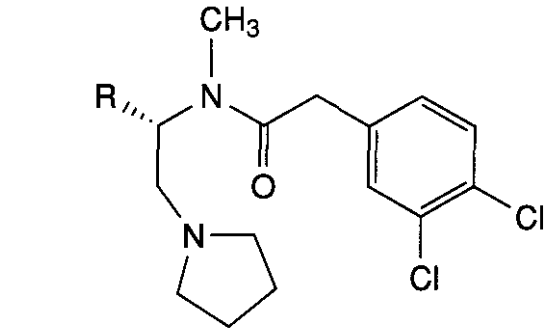
(*R*) GR 103545



(**159**) R-84760



(**160**) Apadoline
(RP 60180)



R = $CH(CH_3)_2$ (161) (*S*) ICI 197067

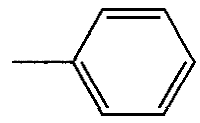
=  (**162**) (*S*) ICI 199441

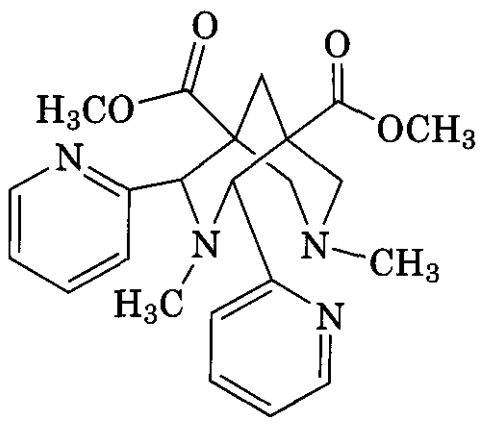
Figure 7.32. κ-Receptor selective agonists with alternative ring systems and open chain analogs of the 1,2-diamine substituted cyclohexane ring.

stituted ethyl chain results in compounds with weak κ-receptor affinity, a substituent α to the amide functionality yields compounds with high affinity for κ receptors (515,516). Examination of both isomers indicated that a methyl group at the 1 position with the *S* configuration was active and had reasonable affinity for κ receptors, whereas the isomer with the R configuration at this position was inactive (516). Conformational analysis suggested that only those compounds capable of adopting a conformation similar to that of U50,488 were κ agonists. The most potent analogs were the isopropyl (ICI 197067, 161) and phenyl derivatives (ICI 199441, 162, Fig. 7.32). Fluorescent derivatives of ICI 199441 have been prepared by attachment of fluorescein isothiocyanate by a spacer to the ***meta*** or ***para*** position of the central phenyl ring (517). Attachment of an acidic functionality to the ***meta***

position of the central phenyl ring of ICI 199441 gave ICI 204448, which has limited ability to penetrate the CNS (518) (see below).

U50,488 and its analogs are structurally distinct from the benzomorphan κ opiates and are considerably more flexible than are the rigid alkaloids. This raises questions concerning their bioactive conformation and how they bind to opioid receptors compared to the more rigid alkaloid opiates. Early studies (519–521) attempted to identify possible bioactive conformations of U50,488 and its congeners by conformational analysis of the ligands and superimposition of the arylacetamides with benzomorphans. With the cloning of opioid receptors and development of computational models of these receptors (see Section 3.2.4.2 above), several groups (179, 182, 183, 186) have proposed possible binding modes for the arylacetamides docked to the κ opioid receptor. Although all of the models of the arylacetamides docked to κ opioid receptors assumed an interaction of the protonated amine of the ligand with Asp^{138}, there are significant differences in other proposed receptor-ligand interactions in these models (see Ref. 186 for a detailed comparison of the models). These results illustrate the complexity of modeling these more flexible ligands and determining how they interact with their receptors.

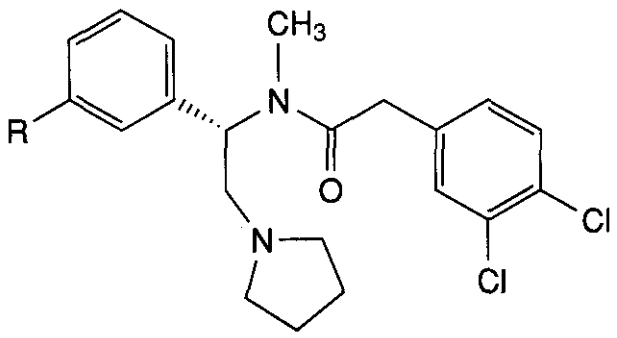
HZ2 (**163**), which is structurally unrelated to the arylacetamide κ-selective agonists, exhibits reasonable κ-receptor affinity (K_i = 15 nM) with low affinity for μ receptors (K_i > 1000 nM) (521). NMR and molecular modeling studies were performed to compare this novel bicyclononanone to the arylacetamides and ketocyclazocine (521). The compound is reported to have potent antinociceptive activity and, like other κ agonists, to be active against inflammatory pain (522). The quaternary methiodide derivative retains high κ-receptor affinity, and thus may be useful as a peripheral κ agonist (523).



(163) HZ2

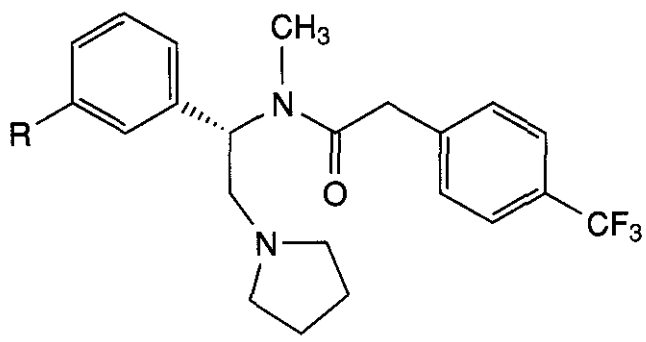
Several of the κ-selective ligands have undergone testing in humans (524–531; see Ref. 475 for a review). Side effects associated with κ receptors include sedation and dysphoric effects (146). The dysphoric effects are of particular concern and have severely limited the usefulness of the majority of centrally acting κ agents. Many of the older nonselective compounds possessed high affinity for σ and PCP sites, raising the possibility that these sites might contribute to the dysphoric effects. The benzomorphan Mr2034 (**97**), which is inactive at these sites (393), and the κ-selective agonist spiradoline (148) also produce naloxone-sensitive dysphoric effects (532), however, indicating that κ receptors mediate psychotomimetic effects. Clinical trials of spiradoline for the treatment of pain have been discontinued (475). Initial evaluation in humans of enadoline (CI-977, **37**, Fig. 7.6) for its diuretic effect found that the dysphoric effects attributable to the drug were minimal and not considered clinically significant (525), although in a subsequent study of its use in postsurgical pain adverse neuropsychiatric events led to early termination of the study (527). Enadoline has, however, been granted orphan drug status for the treatment of severe head injuries (475). Apadoline (RP 60180,160) has been evaluated in an experimental human pain model and reported to cause fewer side effects than the clinically used agent pentazocine (529). Niravoline (151) was examined for its aquaretic effect in patients with cirrhosis; the highest doses examined induced personality disorders, but moderate doses produced the desired aquaretic effect and were well tolerated (531). However, clinical development of this agent has been discontinued (see Ref. 475). Clinical investigation of several centrally acting κ-selective agents (apadoline, DuP 747, enadoline, and the 4,5α-epoxymorphinan TRK-820) has continued (288).

5.9.2 Peripherally Acting Agonists. Concern over centrally mediated side effects has

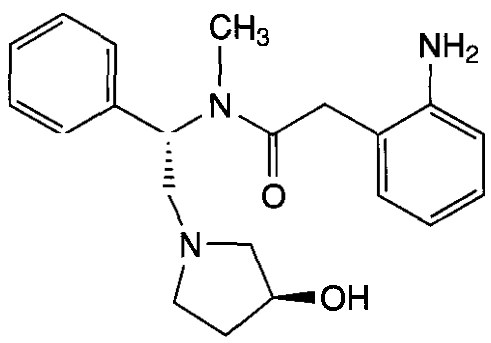


R = OCH_2CO_2H **(164)** (*R,S*) ICI **204448**

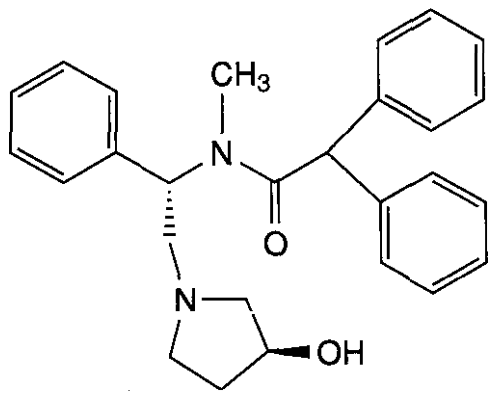
R = NH-L/D-Asp (**165**)



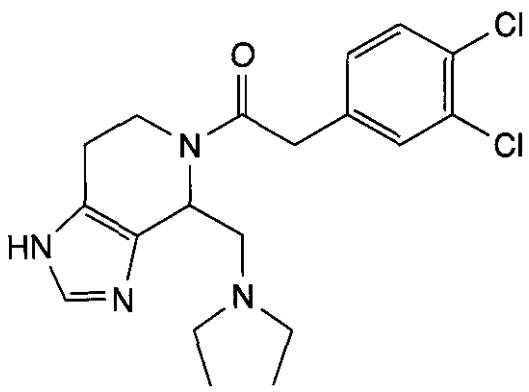
(166) R = OCH_2CO_2H, etc.



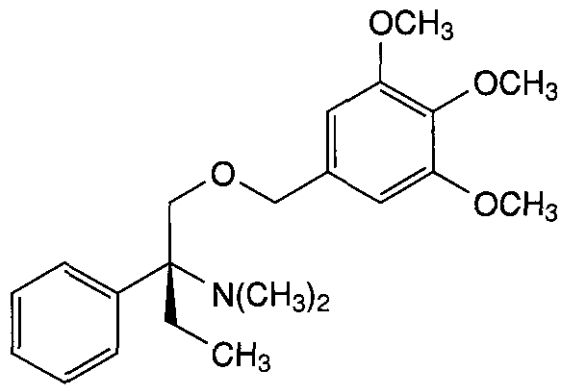
(167) EMD 60400



(168) Asimadoline **(EMD 61753)**



(169) BRL 52974



(170) Fedotozine

Figure 7.33. Peripheral κ-receptor selective agonists.

prompted attempts to develop peripherally selective κ opioid agonists. Peripheral opioid receptors can mediate analgesia, particularly in cases of inflammation (see Refs. 72–74 for reviews). To limit penetration of the blood-brain barrier (BBB), generally polar and/or charged functionalities have been introduced into the compounds (see Ref. 534 for a review). Several modifications to the κ-selective agonist ICI 199441 (162, Fig. 7.32) have been reported to restrict the compounds, access to the CNS. In ICI 204448 (164, Fig. 7.33) an acid functionality was introduced on the central phenyl ring (518), and Portoghese and coworkers prepared aspartic acid conjugates of ICI 199441 (165) (535) to decrease penetration into the CNS. Investigators at the Adolor Corporation tested various substituents on the phenylacetamide side chain phenyl ring and found that the 4-trifluoromethyl group resulted in an analog with high κ-receptor affinity and less central activity compared to that of the parent ICI 199441 (536); incorporation of additional functionalities into the central phenyl ring (e.g., 166) were examined to further restrict the compounds to the periphery (537). Researchers at Merck prepared EMD 60400 (167) (538) by introducing an amino substituent on the phenyl ring of the phenylacetamide side chain and a 3-hydroxyl group on the pyrrolidine ring; researchers at Glaxo prepared analogs of GR 103545 with reduced penetration of the BBB by the use of a similar approach (513). In the case of BRL 52974 (**169**) (539), an imidazole ring was attached to the piperidine ring to increase hydrophilicity.

In asimadoline (EMD 61753,168) (540) an additional phenyl ring was attached α to the amide on the phenylacetamide side chain, which increases lipophilicity. This compound is also a peripherally selective κ agonist (541); studies in knockout mice lacking P-glycoprotein indicate that asmidaloline is transported by P-glycoprotein, and that transport by this protein limits the compound's penetration of the BBB (542). As noted by Barber et al. (541), amphiphilic structures such as asimadoline generally have greater oral activity than hydrophilic compounds; consistent with this they found that asimadoline exhibits much greater potency by the oral route than did ICI 204448 (in pressure nociception in inflammatory hyperalgesia: ID_{50} for asimadoline = 0.2 mg/kg s.c. and 3.1 mg/kg p.o. versus ID_{50} for ICI 204448 = 0.8 mg/kg s.c. and 30 mg/kg p.o.). Oral absorption of asimadoline was not significantly altered in the P-glycoprotein knockout mice, indicating that the intestinal P-glycoprotein did not impede absorption after oral administration (542). Unexpectedly, increases in pain were reported in clinical trials of this compound in patients after knee surgery (543). Subsequent investigation of this compound in inflammation in the rat found that, although the analgesic effects of asimadoline were κ opioid receptor mediated, the adverse hyperalgesic and proinflammatory effects observed were not mediated by opioid receptors (543).

Fedotozine (Jo-1196, **170**), which is structurally related to the acyclic κ agonists, has in *vivo* antinociceptive effects on duodenal pain that appear to be mediated by peripheral κ opioid receptors, but the compound is inactive after central administration (544). In binding assays (in dog myenteric plexus), however, this compound exhibits similar affinity (K_i = 0.3–0.8 μM) for all three types of opioid receptors (545). Unlike other κ agonists, fedotozine does not induce diuresis after either s.c. or intracerebroventricular (i.c.v.) administration (546). Fedotozine also fails to substitute for either U50,488 or morphine in animals trained to discriminate these drugs (547). The main effects demonstrated for fedotozine have been in the gastrointestinal tract (see Ref. 548 for a detailed review of the pharmacology of fedotozine), and therefore this compound has been investigated clinically for the treatment of digestive disorders characterized by abdominal pain, namely, dyspepsia and irritable bowel syndrome (see Ref. 548 for a review). In Phase II/III studies for both disease states (549–551) significant improvement of symptoms in the patients treated with fedotozide compared to placebo was reported. Fedotozine also relieves visceral hypersensitivity to gastric and colonic distention, which are often observed in dyspepsia and irritable bowel syndrome, respectively (552, 553). Although clinical trials of fedotozine have been discontinued (see Ref. 475), peripherally selective κ agonists remain potentially important therapeutic agents for treatment of these digestive disorders.

5.10 Delta-Selective Agonists

5.10.1 BW373U86, SNC 80, and Analogs. Initially, all of the δ-selective agonists were peptide derivatives. The first nonpeptide agonist selective for δ receptors, BW373U86 (26, Fig. 7.5), was discovered by screening (554). The pharmacology of this compound has been examined in considerable detail (see Refs. 219, 221 for reviews). This compound has only modest selectivity for δ receptors in binding assays (see Table 7.12). In antinociceptive assays in mice it appears to function as a partial agonist at both δ and μ receptors. The activity of BW373U86 is highly dependent on the route of administration, with effects at the spinal level mediated by δ receptors and supraspinal effects involving interactions with μ receptors (555). In monkeys BW373U86 did not produce antinociceptive effects in the warm-water tail-withdrawal assay after subcutaneous administration (556). This compound also produces convulsant effects in both mice and monkeys (556–558), which appeared to be mediated by δ receptors.

BW373U86 is a racemic mixture and this could complicate its pharmacological profile. Therefore Rice and coworkers undertook the synthesis and characterization of isomers of BW373U86 (107). The isomers with the R configuration at the benzylic carbon exhibited greater affinity for δ receptors than did the isomers with the S configuration. One compound, SNC 80 (27, Fig. 7.5), the methyl ether

Table 7.12 Opioid Receptor Affinities, δ Selectivity, and Opioid Activity in the MVD of δ Opioid Agonists[a]

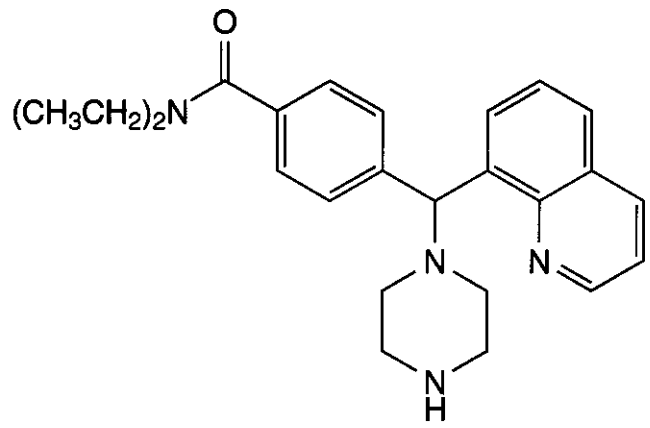
Agonist	*IC_{50} or K_i (nM) δ	μ	κ	IC_{50} Ratio μ/δ	IC_{50} (nM) MVD	Reference
BW373U86 (**26**)	0.92*	46*	—	50	0.2[b]	107
SNC 80 (**27**)	1.0*	2500*	—	2,300	2.7[b]	107
	2.9	2500	—	860	2.7	561
172	0.87*	3800*	7500*	4,370	—	568
(−) **SL-3111 (174)**	4.1*	7700*	—	1,900	360	569
(−) **175**	5.6	2620	1450	470	—	572
176	0.4	5000	—	14,000	—	575
177	1.2	1200	—	980	—	576
(±) **TAN 67** (**28**)	1.1	2300	1800	2,070	6.6[c]	109
(SB **205607**)	0.67	110	450	170	160	580
(−) **TAN 67** (SB **213698**)	0.47	70	270	150	62	580
(+) **TAN 67** (SB **213697**)	11	815	>1000	74	>1000	580
(−) **178**	0.9	129	1300	140	26	577

[a]Data for BW373U86, SNC 80, and TAN 67 fiwm Table 7.8 are included for comparison.
[b]IC_{50} in the GPI are 143 and 5500 nM for BW373U86 and SNC 80, respectively.
[c]IC_{50} in the GPI is 26,500 nM.

of (+)-BW373U86, exhibited marked selectivity for δ over μ receptors in binding and smooth muscle assays (see Table 7.12) (107, 559), making it the most δ-selective nonpeptide agonist reported. SNC 80 is a systemically active δ receptor selective agonist, with its antinociceptive actions produced through interaction with both δ_1 and δ_2, but not μ, opioid receptors (559). SNC 80 is more effective in nociceptive assays than BW373U86, and consistent with its higher selectivity for δ receptors, the antinociceptive effects of SNC 80 appear to be mediated only by δ receptors (see Ref. 221). Brief, nonlethal seizures were observed in mice only at very high doses of SNC 80 (100 mg/kg) (559); this may be attributable to metabolism of SNC 80 to a BW373U86-like compound (560). In monkeys SNC 80 does not cause convulsions at doses up to 32 mg/kg, suggesting it may be safer than BW373U86 (221).

Several groups have explored the SAR of SNC 80. Rice and coworkers examined substitutions for the methoxy group on the phenyl ring (561), modifications of the amide functionality (562), modifications to the piperazine ring (563), and different substitutions on N^4 of the piperazine ring (564). The methoxy group on the one phenyl ring of SNC 80 could be substituted with other groups with retention of δ-receptor affinity and selectivity; the derivative without a substituent at this position has higher δ affinity and selectivity [IC_{50} = 0.94 nM, IC_{50} (μ/δ) ratio > 26601 than that of SNC 80 (561). The amide functionality is particularly sensitive to modification, with the *N,N*-dialkylbenzamide derivatives having higher δ-receptor affinity than the monosubstituted or unsubstituted derivatives (562). This suggests that the amide group is an important structural feature for interaction with δ receptors.

Modifications can be made to the piperazine ring, including removal of the methyl groups and replacement of N^1 by carbon, with retention of reasonable δ-receptor affinity and selectivity (563, 565, 566). A series of simplified piperazine derivatives were prepared, including (171) (Fig. 7.34), with improved δ-receptor selectivity (IC_{50} ratio = 1240 versus 245 for SNC 80) and increased metabolic stability over that of SNC 80 (567). A piperidinylidene derivative of SNC 80 with a double bond to the benzylic carbon exhibits higher δ-receptor affinity than that of the corresponding piperidine derivative (563). Researchers at AstraZeneca examined an extensive series of these piperidinylidene analogs (568) and iden-



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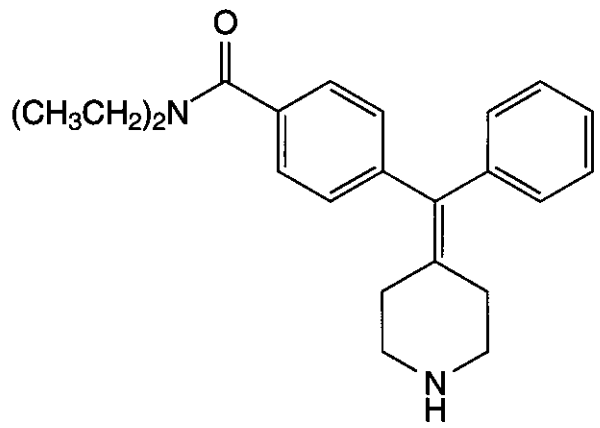

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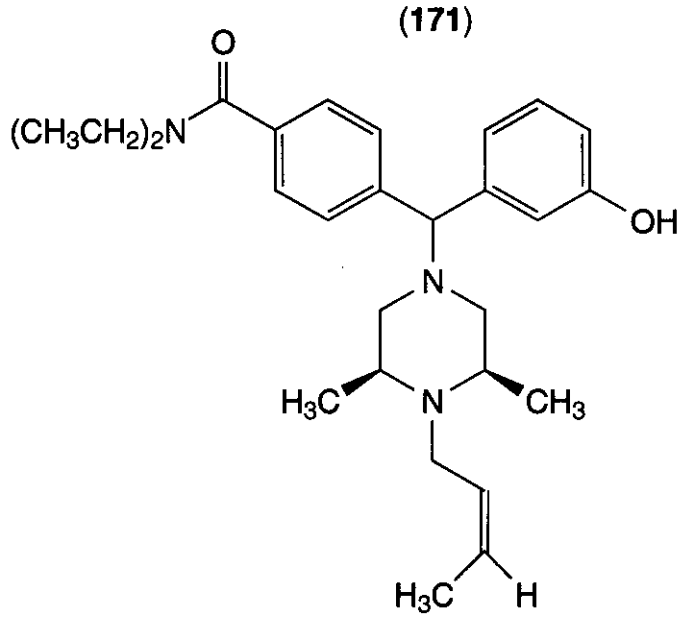
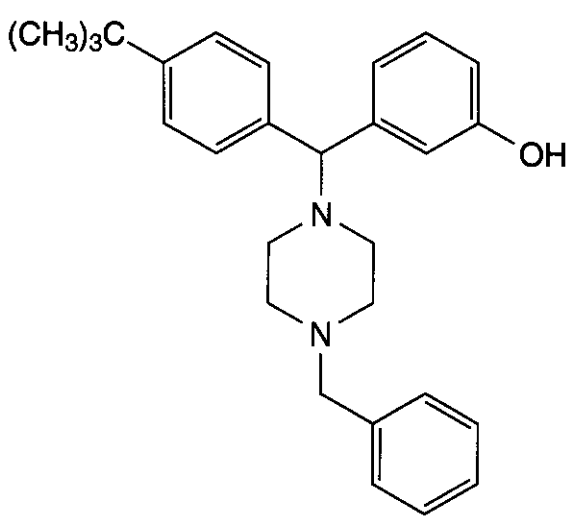
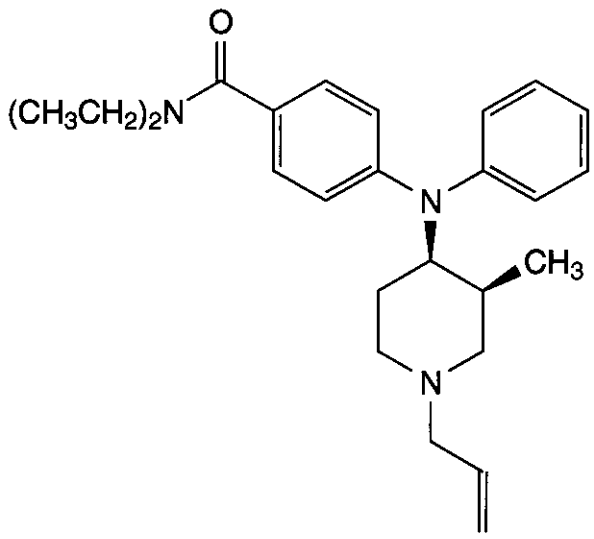


(173) DPI 2505



(174) SL-3111



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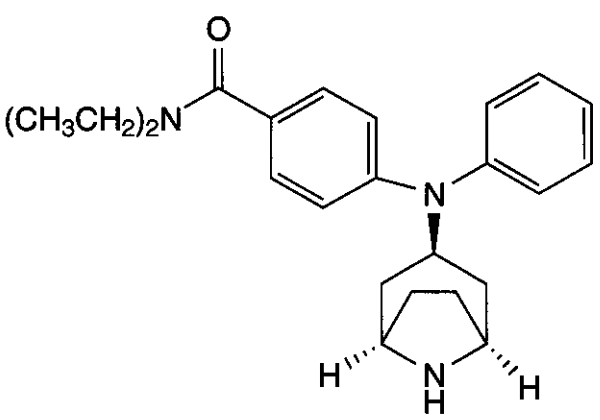

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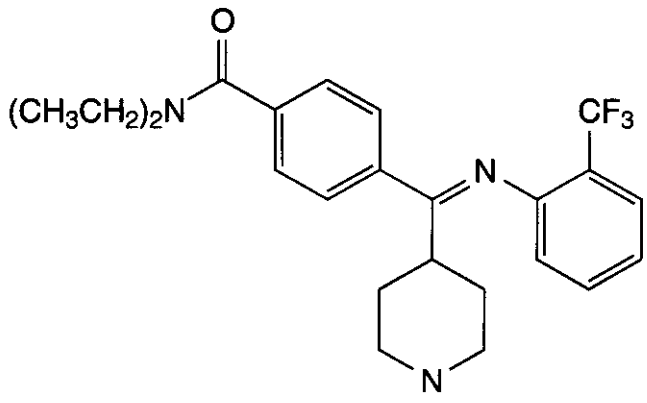


Figure 7.34. SNC 80 **analogs.**

tified derivatives (e.g., 172) that exhibited extremely high selectivity for 6 receptors (see Table 7.12) and were considerably more stable to degradation by rat liver microsomes than was SNC 80 (568). Interestingly, the analog DPI 2505 (173) in which the 2-methyl group is shifted to the 3 position has been reported to be an antagonist (228). The basic N^4 nitrogen is critical for 6 receptor binding, and opening the piperizine ring also results in large decreases (>60-fold) in δ-receptor affinity (563). A variety of alkyl substituents on N^4 of the piperazine ring are tolerated by the 6 receptor (564, 566), but SNC 80 analogs containing a saturated alkyl group exhibit decreased efficacy compared to SNC 80 in the [^{35}S]GTPγS assay; the N-cyclopropylmethyl analog is also a partial agonist (564). Thus the SAR of the nitrogen substituent of SNC 80 for δ-receptor affinity is distinctly different from the SAR of this group in the morphinans for interaction with μ receptors.

Hruby and coworkers designed a series of piperazine derivatives as peptidomimetic analogs of the δ selective peptide [(2*S*,3*R*)-Tmt^1]DPDPE (Tmt = 3,2′,6′-trimethyltyrosine) (569, 570), with SL-3111 (174) (see Table 7.12) as the lead compound. Although SL-3111 is structurally similar to SNC 80, the large decrease in δ-receptor affinity upon methylation of the phenol and the high affinity of SL-3111 for the [W284L] mutated human 6 receptor is in contrast to the results for SNC 80 and more closely parallel those for the peptide p-C1-DPDPE, suggesting that the binding profile of SL-3111 is more similar to that of the peptide than to SNC 80 (569).

A series of 4-aminopiperidine derivatives was designed by researchers at the Research Triangle Institute by transposition of the N^4 of the piperazine ring and the benzylic carbon (571–573), with the cis (3*S*,4*R*) isomer (175) exhibiting the highest affinity. This transposition decreased 6-receptor affinity while increasing preceptor affinity, such that the piperidines exhibited somewhat lower 6 selectivity than the piperazine derivatives (see Table 7.12). A large series of these 4-aminopiperidine analogs was also prepared by researchers at R. W. Johnson Pharmaceutical Institute and subjected to CoMFA analysis (188). Recently, a series of constrained 4-diarylaminotropane derivatives (176) were reported by two groups (574, 575), with the unsubstituted derivative exhibiting high δ-receptor affinity and exceptional 6 selectivity (see Table 7.12) (575); the *N*-allyl and related derivatives exhibit decreased efficacy and antagonist activity in the [^{35}S]GTPγS assay (574). In an alternative approach chosen to yield achiral δ agonists without the complicated stereochemistry of SNC 80, researchers at R. W. Johnson also prepared a series of piperazinyl benzamidines (576); the highest affinity ligand (177) exhibited affinity and selectivity similar to SNC 80 (see Table 7.12). A number of other SNC 80 analogs described in the patent literature were reviewed by Dondio (219, 220).

One question is how SNC 80 interacts with 6 opioid receptors compared to the more classical morphinan ligands. Dondio et al. (577) compared SNC 80 to the δ antagonist SB 205588 (78, Section 5.3.3). In this model the basic N^4 nitrogen and the oxygenated phenyl ring of SNC 80 were superimposed on the corresponding groups in SB 205588, with the centroid of the second phenyl ring of SNC 80 overlapped with the pyrrole ring of SB 205588. Based on this model, it was hypothesized that the amide group of SNC 80 might function as a nonaromatic 6 "address" and thus be responsible for the δ selectivity of SNC 80. Loew and coworkers in their pharmacophoric model for a wide range of δ receptor ligands also overlaid the basic N^4 nitrogen and oxygenated phenyl ring of SNC 80 with the corresponding groups in the epoxymorphinans, with the amide occupying the third site in the three-point model for δ-selective opioid recognition (578). Coop and Jacobson, however, developed a four-point pharmacophoric model based on a series of 4,5α-epoxymorphinans with high affinity for 6 receptors and found that SNC 80 did not fit the model, suggesting that SNC 80 does not bind to the 6 receptor in the same orientation as oxymorphindole (579).

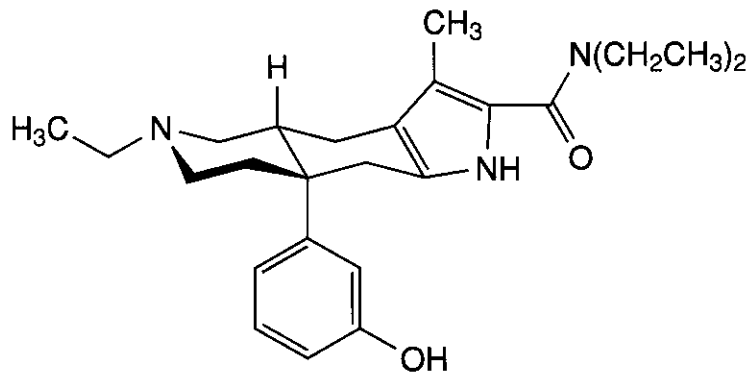
Mutational analysis of δ opioid receptors has found that three residues, Trp^{284} at the top of TM6 and Val^{2g6} and Val^{297} in the third extracellular loop, are crucial for the δ-receptor affinity of SNC 80 as well as other δ-receptor agonists (166). Computational models of BW373U86 (182) and 4-aminopiperidine ana-

logs of SNC 80 (188) docked to the δ receptor have been described. In the models the basic nitrogen and oxygenated phenyl ring of these compounds occupy similar regions in the receptors as the corresponding groups in the epoxymorphinans, although there are some differences in the exact location of the two types of compounds and orientation of their phenyl rings (see Ref. 182). In both models the benzamide ring occupies a region at the TM/extracellular interface, and interacts with one or more of the residues identified as critical from mutagenesis studies. Comparison of the docking of SIOM with the 4-aminopiperidine compounds suggested that the benzamide ring occupies a similar region to the spiroindane of SIOM, consistent with the pharmacophoric models, and thus functions as an "address" to target the compounds to the 6 receptor (188).

5.10.2 Other &Receptor Agonists. Attempts to identify δ-selective agonists related to the epoxymorphinans have concentrated in two areas. Modification of the nitrogen substituent of NTI resulted in the identification of SIOM as a δ_1 agonist (see Section 5.3.3 above). Other 6-selective agonists identified have been octahydroisoquinolines. As discussed earlier (Section 5.3.3), octahydroisoquinolines are either antagonists or agonists, depending on the ring size of the spacer. Although compounds with five-membered ring spacers are δ-receptor antagonists, introduction of a six-membered ring spacer, explored by both Japanese and Italian researchers, resulted in a new class of Breceptor agonists (108,358,580; see Refs. 109,219 for reviews), with TAN 67 (28, Fig. 7.5) (108, 109) being the most extensively studied. The rationale for the design of TAN 67 by the Japanese group involved making the phenol ring freely rotatable by removing the 4,5-epoxy and 10-methylene functionalities of the epoxyrnorphinans and using a heteroatom capable of forming a hydrogen bond with the receptor as an additional pharmacophoric group (109). Racemic TAN 67 shows high affinity and selectivity for 6 receptors (Table 7.12) (109), and is a potent agonist in cloned δ human cells (581), and in the MVD (109). *In vivo* racemic TAN 67 exhibits antinociceptive activity in the acetic acid writhing assay, but not the tail flick test, in normal mice [TAN 67 was active in the tail flick assay in diabetic mice (582)]; antagonism by BNTX but not naltriben suggested that TAN 67 produced its antinociceptive effects through δ_1 receptors (583).

The pharmacological effects of the two enantiomers of TAN 67 are distinctly different (see Ref. 584 for a review). (−) TAN 67 (also named SB 213698 by the Italian group), which has the same absolute configuration as that of morphine, shows high affinity and selectivity for 6 receptors (Table 7.12), and is a full agonist in the MVD (580), whereas the (+) isomer is inactive *in vitro*. *In vivo* (−) TAN 67 exhibits antinociceptive activity in the tail flick assay after both i.c.v. (585) and intrathecal (i.t.) (586) administration, which appears to be mediated by δ_1 receptors. Whereas (+) TAN 67 was inactive after i.c.v. administration (585), after i.t. administration (+) TAN 67 produced nociceptive behavior (586); interestingly, the effects of (+) TAN 67 were blocked by both naltrindole and (−) TAN 67 (584). (−) TAN 67 exhibits decreased affinity for the [W284L] mutated δ receptor compared to the wild-type receptor, but the magnitude of the decrease is less than that for SNC 80 (587). In contrast this mutation increases the intrinsic activity of SNC 80 (indicated by an increase in the maximum [^{35}S]GTPγS binding), but it decreases the intrinsic activity of (−) TAN 67, suggesting that these compounds may interact with different active receptor conformations (587). In a recent report of a pharmacophoric model for the 6 opioid receptor, low energy conformations of TAN 67 were identified in which the phenol, the basic amine, and the second aromatic amine could be superimposed on the corresponding groups in OMI and SIOM (588).

Based on their comparison of the antagonist SB 205588 and SNC 80 and the resulting hypothesis that the amide of SNC 80 might function as a nonaromatic "address", Dondio and coworkers prepared pyrrolooctohydroisoquinolines (**178**) (Fig. 7.35) lacking the second aromatic ring, which exhibit high 6-receptor affinity and selectivity (see Table 7.12) (577). The analog in which the pyrrole ring was in the opposite orientation (179) also retained high δ-receptor affinity (K_i = 3 n*M*), suggesting that this ring functions as a spacer (589).



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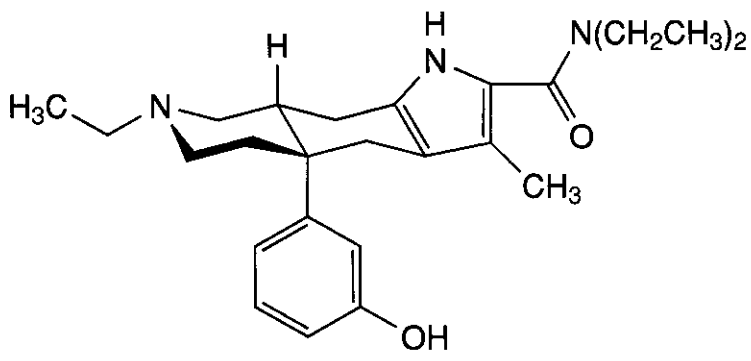

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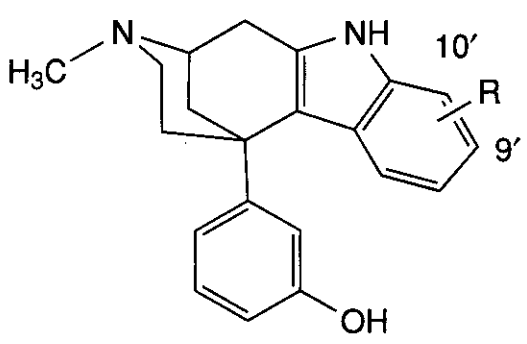
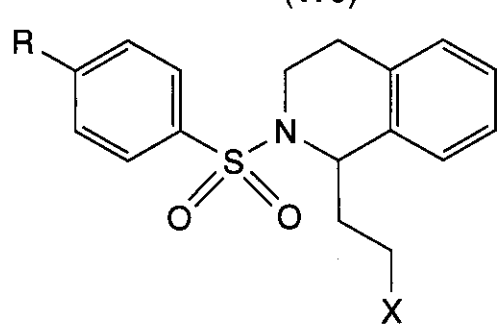
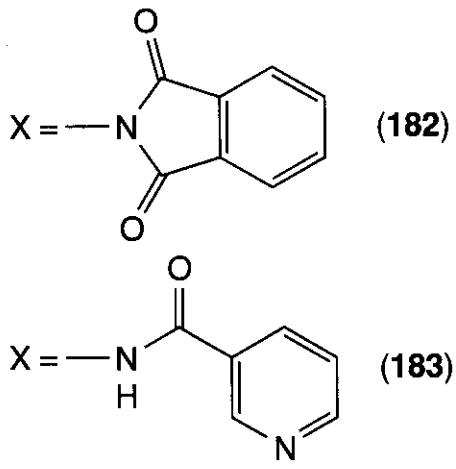


R = H (180)

R = OCH_3 (181)



R = CH_3, X = (182)



R = $(CH_2)_3CH_3$, X = (183)

Figure 7.35. Other nonpeptide δ-receptor selective agonists.

An attempt has also been made to convert 5-(3-hydroxyphenyl)-2-methylmorphan, which has negligable δ-receptor affinity, to 6-selective agonists by use of the "message-address" concept (590). Addition of an indole ring system to the morphan skeleton, to give (**180**), increased 6-receptor affinity more than 140-fold (IC_{50} = 7 n*M*); this structural change had little effect on preceptor affinity, however, so that the selectivity of this compound for 6 receptors was low [IC_{50} ratio (μ/δ) = 4.21. Based on differences between the SAR of SNC 80 and the indole phenylmorphan derivatives, Rice and coworkers postulated that the indole phenyl ring, not the phenol, of the indole phenylmorphans might be structurally analogous to the methoxyphenyl group on SNC 80. Therefore, they prepared a series of methoxy-substituted derivatives of (180); the C9′ and C10′ substituted derivatives (181, Fig. 7.35) exhibited somewhat higher Breceptor affinity and potency, but the selectivity for 6 receptors was still low [IC„ ratio (μ/δ) values of 18 and 7, respectively] (591).

Recently, a new type of nonpeptidic δ agonist (**182**), which lacks a basic nitrogen, was identified by high throughput screening (592). Further structural modification of this lead compound to improve its water solubility led to a series of amide derivatives (e.g., 183) with modest 8-receptor affinity (IC_{50} = 37–256 n*M*) and in *vivo* potency comparable to TAN 67 (593).

5.11 Nonpeptide Affinity Labels Used to Study Opioid Receptors

Affinity labels, ligands that interact with receptors in a nonequilibrium manner, are useful pharmacological tools to study opioid receptors and receptor-ligand interactions.

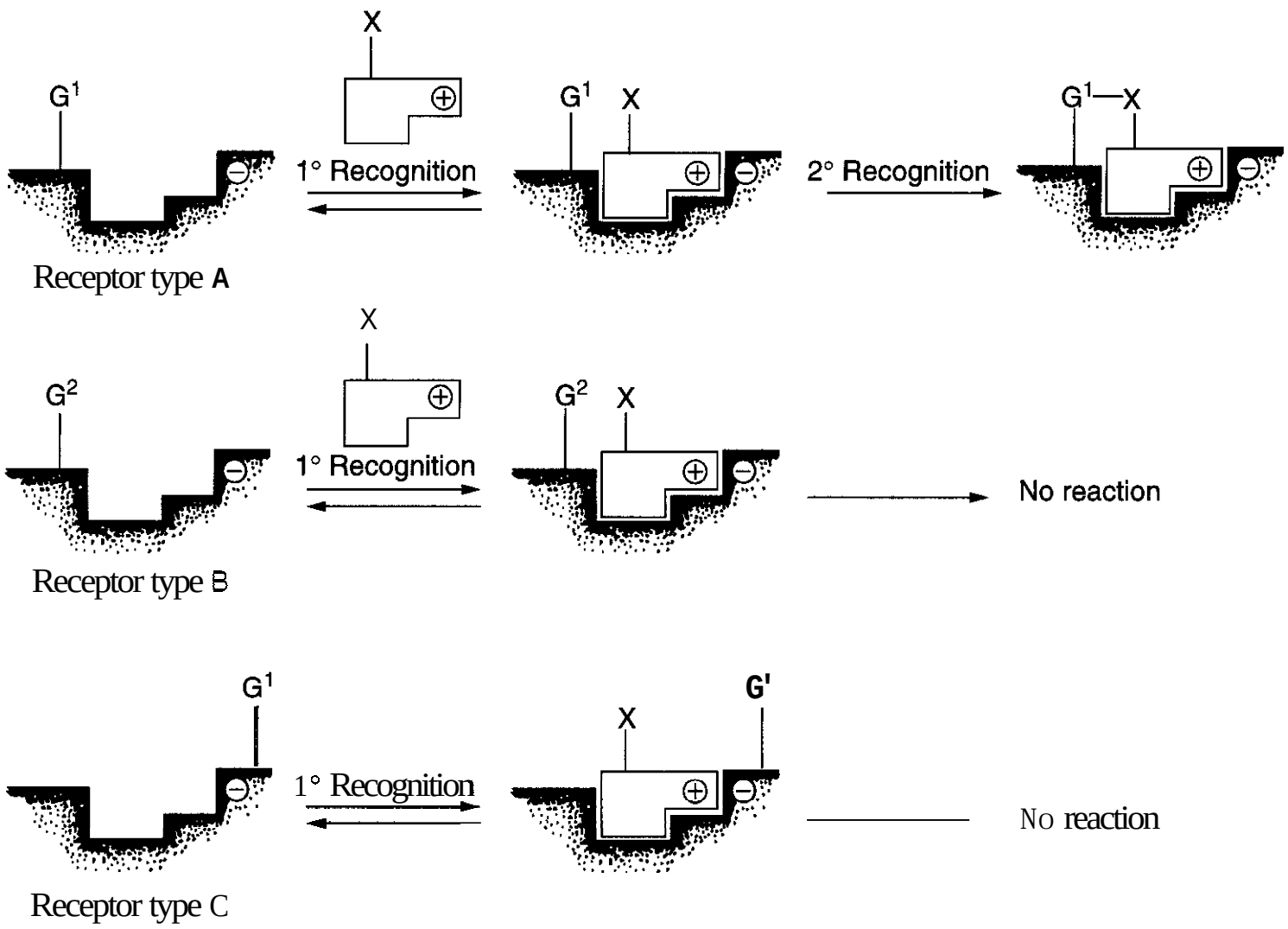
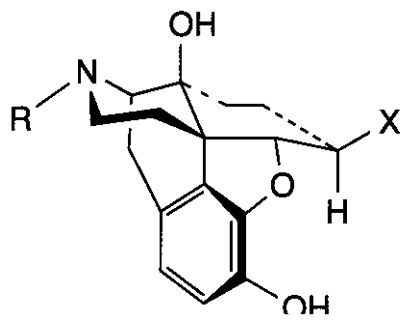


Figure 7.36. Two-step mechanism for covalent binding of an affinity label containing a selective electrophilic group X with receptor type A. Although receptor types A-C have similar topographical features that lead to reversible binding (1° recognition), they differ with respect to the reactivity of the receptor-based nucleophiles (G^1 and G^2) and their locations. Only with receptor type A is the nucleophile G^1 reactive with respect to X and within covalent binding distance (2° recognition) (from Ref. 319).

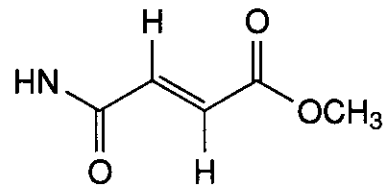
Tritiated affinity labels have been useful in receptor isolation and for determination of the molecular weights of solubilized receptors (see Refs. 86, 99 for reviews). These compounds can be used to irreversibly block one or more receptor type in tissues containing multiple receptor types, so that the remaining receptors can be studied in isolation. The covalent binding of affinity labels can be used to study receptor-ligand interactions. Thus Liu-Chen and coworkers have characterized the binding of the affinity labels β-funaltrexamine (β-FNA) and SUPER-FIT to μ and S receptors, respectively, by use of a combination of molecular biology approaches and protein isolation (see below).

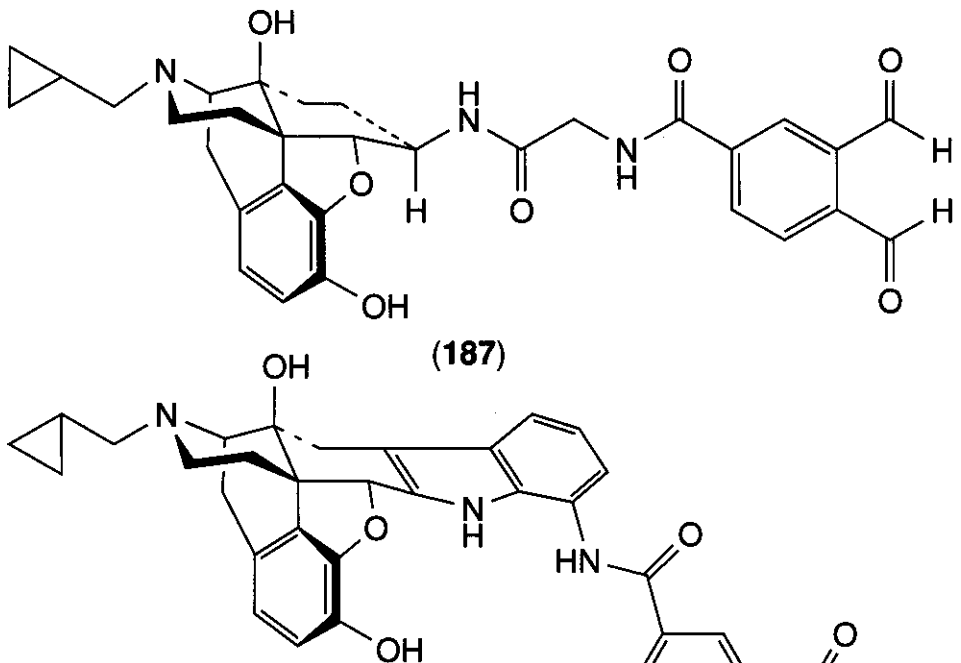
There are two types of affinity labels based on the type of reactive functionality. Electrophilic affinity labels contain an electrophilic group that can react with nucleophiles on the receptor. Photoaffinity labels are converted to highly reactive intermediates, most often a nitrene or carbene, upon exposure to light of the appropriate wavelength and these reactive species then can react covalently with the receptors. Reaction of affinity labels with receptors involves a two-step mechanism (Fig. 7.36) (319, 594). Initially the ligand binds to the receptor reversibly, followed in the second step by covalent bond formation between the reactive functionality on the ligand and a group on the receptor. Depending on the nature of the reactive functionality on the affinity label, the second step can enhance receptor selectivity. Whereas an electrophilic affinity label may bind reversibly to more than one receptor type, covalent bond formation requires the proper juxtaposition of an appropriate nucleophile on the receptor with the electrophilic group on the ligand (see Fig. 7.36), so that covalent binding can occur to only one type of



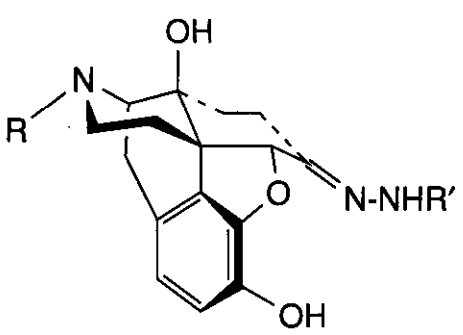
R = CH_2–cyclopropyl X = $N(CH_2CH_2Cl)_2$ (**184**) β-Chlornaltrexamine (β-CNA)

R = CH_3 X = $N(CH_2CH_2Cl)_2$ (**185**) β-Chloroxymorphamine (β-COA)

R = CH_2–cyclopropyl X =  (**186**) β-Funaltrexamine (β-FNA)



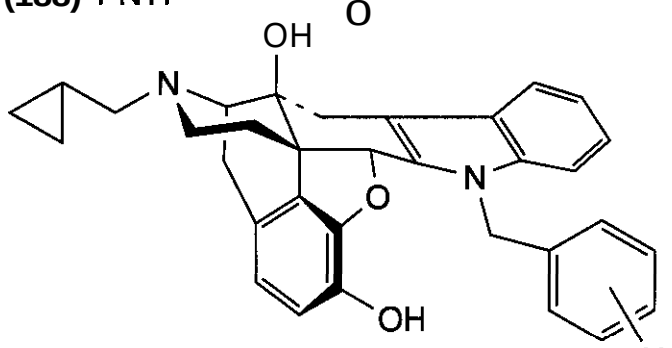
(**187**)



R = allyl, CPM, or CH_3

(**189**)

(**188**) PNTI



(**190**) X = $NHCOCH_2Br$ or N=C=S

Figure 7.37. Affinity label derivatives of naloxone, morphine, and naltrindole.

receptor; examples of electrophilic affinity labels that exhibit such enhanced selectivity are β-FNA (186, Fig. 7.37) and naltrindole isothiocyanate (NTII, 47, Fig. 7.8) (see below). In the case of photoaffinity labels, however, the reactivity of the photolyzed intermediate is so high that they can react with almost any residues on the receptor, and therefore the selectivity is determined only by the first reversible step in the mechanism.

A variety of affinity labels, mostly nonpeptide ligands, have been prepared for opioid receptors. Detailed reviews of the structure-activity relationships for affinity labels have been published [see the previous edition of this chapter (286) and Refs. 319, 594–5961. Therefore the discussion below focuses on those ligands that have been most useful in the characterization of opioid receptors, and on recent reports of new affinity label derivatives.

5.11.1 Morphine and Naltrexone Derivatives. A variety of morphine and naltrexone derivatives have been prepared that incorporate a reactive functionality, often at the 6 position (Fig. 7.37). Incorporation of a nitrogen mustard at the 6β position of naltrexamine by Portoghese and coworkers yielded β-chlornaltrexamine (β-CNA, 184) (597, 598), the first successful opioid antagonist affinity label. This compound is a potent affinity label that because of the reactivity of the nitrogen mustard blocks all opioid receptor types (see Ref. 594). It has been a useful tool in studying opioid receptors and has been used to single out a specific receptor type in tissues containing multiple receptor types. The desired receptor type for study can be protected by incubation with a ligand selective for that receptor and the tissue subsequently treated with β-CNA to irreversibly block the remaining opioid receptors (133, 134, 599). Only one of the chloroethyl groups of β-CNA is required for irreversible antagonist activity (600). The nitrogen mustard analog of oxymorphone, β-chloroxymorphamine (β-COA, 185) (601,6021, has also been prepared. It is a potent irreversible agonist in the GPI and appears to bind irreversibly to opioid receptors in *vivo*; in *vivo*, it initially produces analgesia followed by a long-lasting antagonism of morphine analgesia (602,603).

To obtain affinity labels selective for a single receptor type, Portoghese incorporated less reactive electrophiles at the 6β position leading to the preparation of P-FNA (186) (604). This compound illustrates how the second step in the mechanism of affinity labels can enhance selectivity; whereas β-FNA interacts reversibly with κ receptors, where it is an agonist, it is an irreversible antagonist at μ receptors. [There are conflicting reports on whether the effects of β-FNA on δ receptors are irreversible (605, 606).] The orientation and configuration of the fumaramide functionality are important for irreversible binding to μ opioid receptors; neither the 6β-maleimide with a cis double bond nor a-FNA with' the electrophile in the 6α position is an irreversible μ antagonist (605). Examination of the conformations of a- and P-FNA by X-ray diffraction (607) offers insight into the differences in reactivity of these two isomers. The conformations of the ring system in the two compounds are almost identical except for the C ring, which is in a twist-boat conformation for the a isomer and a chair conformation for P-FNA (see 186). The fumarate group is then equatorial in both compounds, which when the morphinan skeletons are superimposed places the fumarate double bond in a-FNA more than 2 Å away from the double bond in P-FNA and in the wrong orientation for reaction with a nucleophile on the receptor.

The binding of P-FNA to μ opioid receptors was examined by Liu-Chen and coworkers by use of a combination of molecular biology approaches and protein isolation. These studies illustrate the utility of using affinity labels to study receptor-ligand interactions. The binding of [^{3}H]β-FNA to μ/κ receptor chimeras suggested the region of the receptor from the middle of the third extracellulular loop (EL3) to the C-terminus was necessary for irreversible binding to μ receptors (608). Subsequent isolation and partial purification of the labeled receptor and digestion with CNBr, however, located the point of attachment of P-FNA to the EL2-TM5 region of the receptor; subsequent site-directed mutagenesis of residues in this region indicated that the point of attachment was Lys^{233} at the EL2-TM5 interface, which is a conserved residue among the opioid receptors (177). Thus the selectivity of P-FNA irreversible binding for μ opioid receptors appears to be due to differences in the tertiary structure of the receptor, not the primary sequence. This illustrates the subtleties of receptor-ligand interactions and the importance of examining receptor-ligand interactions directly.

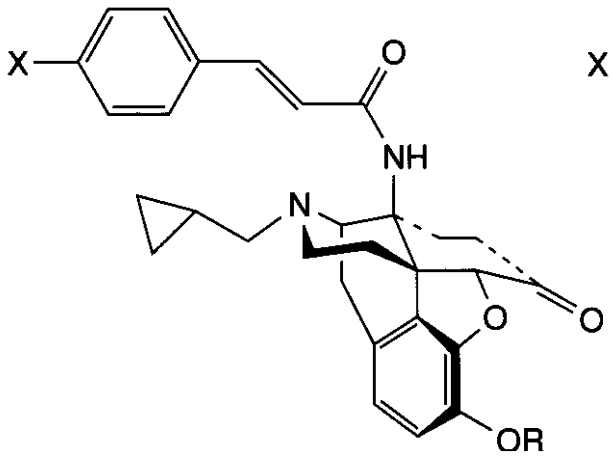
Recently, Portoghese and coworkers reported the phthalaldehyde derivatives of 6P-

naltrexamine (187) and naltrindole (188) as "reporter affinity labels" (340, 609). Reaction of the phthalaldehyde group with an amine and thiol (from Lys and Cys side chains, respectively, in the receptor) results in a fluorescent isoindole; detection of fluorescence indicates that covalent reaction has occurred. In contrast to naltrindole, the phthalaldehyde derivative PNTI (188) is an agonist in the mouse vas deferens, leading to the proposal that the covalent binding of PNTI to 6 opioid receptors results in a conformational change in the receptor and agonist activity (340).

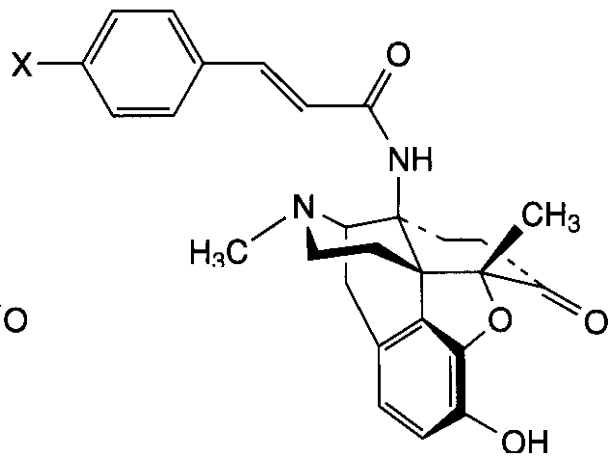
Pasternak and coworkers have prepared a series of hydrazone derivatives of the 6-ketone of naloxone, naltrexone and oxymorphone (189, Fig. 7.37) (209,210, 610). Prolonged actions in *vivo* and nonequilibrium binding in *vitro* have been reported for several of these compounds. The hydrazone naloxazone (48, Fig. 7.8) (209) and the corresponding azine naloxonazine (49) (210) have been used to characterize the postulated μ_1-receptor subtype (see Ref. 208); the azines are 20- to 40-fold more potent than the corresponding hydrazones (210). Studies with [^{3}H]naloxazone suggested that a portion of the binding may involve covalent interaction with μ_1-receptors (611); other researchers, however, have not found evidence for irreversible binding to opioid receptors (612, 613). The acylhydrazone naloxone benzoylhydrazone [Nal(Bzo)H, 50, Fig. 7.8] exhibits extremely slow dissociation from μ receptors ("pseudoirreversible" binding), which may be related to interactions with a G-protein (614); it also binds reversibly to κ receptors and the tritiated form has been used to characterize the proposed κ_3-receptor subtype (see Section 3.2.4.3) (217,615).

Portoghese and coworkers prepared derivatives of the 6-selective antagonist naltrindole containing reactive functionalities as affinity labels for 6 receptors. An isothiocyanate group was incorporated at the 5' position of naltrindole to give NTII (47, Fig. 7.8) (196), which is a potent and selective nonequilibrium δ-receptor antagonist. NTII antagonizes the antinociceptive activities of [D-Ala^2]deltorphin II and DSLET, but not that of DPDPE, and therefore was proposed to be a selective 6,-receptor antagonist (616). Electrophilic moieties, either an isothiocyanate or haloacetamide, have also been incorporated into the indole N-benzyl aromatic ring of the 6,-selective antagonist *N*-benzylnaltrindole (BNTI, 75) to give (190) (617). Interestingly, the *meta*-substituted isothiocyanate derivative was an irreversible 6 agonist in the MVD; the *ortho*- and *para*-substituted isothiocyanates and the haloacetamides were 6-receptor antagonists that exhibited time-dependent increases in antagonism consistent with covalent interaction with 6 receptors. In *vivo*, these compounds were less selective for 6, over δ_1 receptors than BNTI.

A number of 14β-amino substituted derivatives containing electrophilic groups (Fig. 7.38) were prepared by Archer and coworkers [see the previous edition of this chapter (286) for a detailed review]. Reactive functionalities that have been attached to the 14β-amino group include bromoacetamide, thioglycolamide, and cinnamoyl groups. The naltrexamine derivative clocinnamox or C-CAM (191) (618) has been the most extensively studied and is a potent long-lasting μ antagonist (619–621). Binding studies indicated that clocinnamox selectively decreases the density of μ receptors (621), but not of S or κ receptors (622), without affecting receptor affinity, as would be expected for an irreversible ligand. A subsequent examination of [^{3}H]clocinnamox binding to mouse brain membranes, however, (623) found that the binding was fully reversible, although half of the binding dissociated very slowly ($t_{1/2}$ = 11 h). The *p*-nitro-substituted derivative with a 5P-methyl group MET-CAMO (194) was the first N-methyl derivative reported with long-lasting p-receptor selective antagonist activity with no agonist activity (624); it appears to bind irreversibly to μ receptors (625,626). Like MET-CAMO, the corresponding p-chloro-substituted, 5β-methyl derivative MET-Cl-CAMO (195) is also a long-lasting p-receptor antagonist with no agonist activity, which appears to bind irreversibly to μ receptors (627). The p-methyl derivative of clocinamox, methocinnamox or M-CAM (193), has been reported to be a more μ receptor selective, long-lasting antagonist in *vivo* than clocinnamox or β-FNA (628), although in standard binding assays, like clocinnamox, its selectivity for μ receptors is very low (K_i ratio = 3–8). Recently, the relative importance of



R = H, X = Cl (191) Clocinnamox (C-CAM)
R = CH_3, X = Cl (192) MC-CAM
R = H, X = CH_3 (193) M-CAM



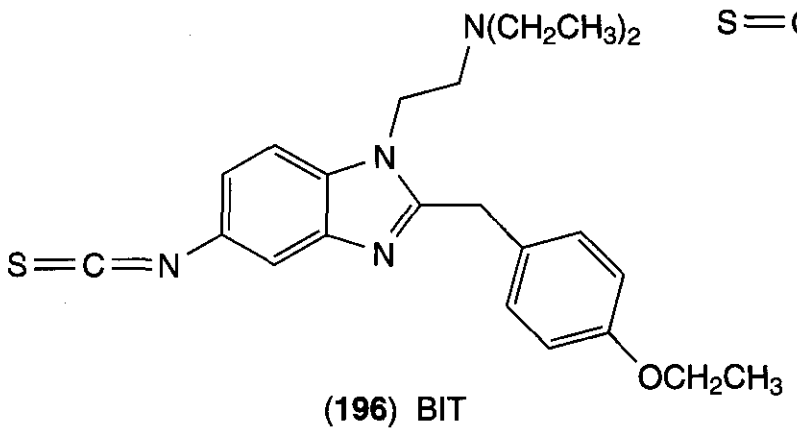
X = NO_2 (194) MET-CAM0
X = Cl (195) MET-Cl-CAMO

Figure 7.38. Affinity label derivatives of naloxone and morphine containing a reactive functionality at the 14β position.

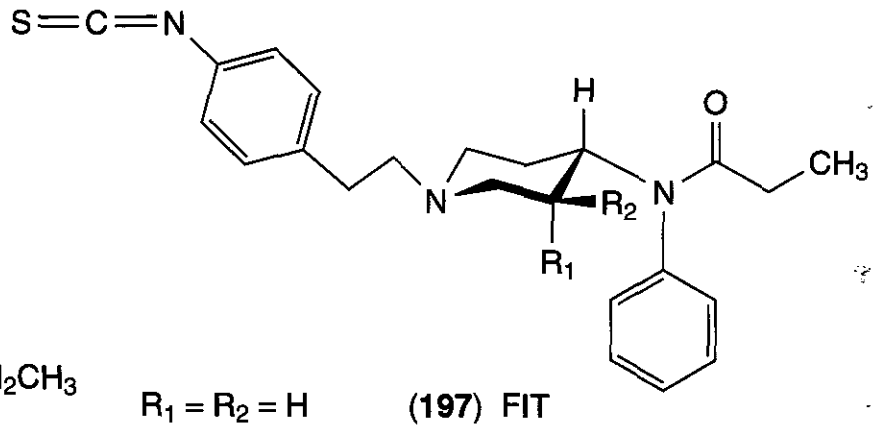
the 3-hydroxyl group to the opioid receptor affinity of clocinnamox was examined by preparing both a series of 3-alkyl ether derivatives (629) and the 3-deoxy analog (630). Interestingly, in the 3-alkyl ether derivatives the identity of the alkyl group affects efficacy. In *vivo*, the 0-methyl derivative MC-CAM (**192**) is a p-partial agonist, the propargyl ether is a potent agonist, and the cyclopropylmethyl analog is a long-lasting antagonist with little agonist activity; both the methyl and propargyl ethers exhibit delayed long-lasting antagonist activity. The 3-deoxy analog exhibits high μ opioid receptor affinity comparable to that of clocinnamox, indicating that the C_3-hydroxyl does not play a significant role in the binding of these 14β-cinnamoyl epoxymorphinans to opioid receptors; the deoxy derivative exhibits greater selectivity for μ over δ receptors than clocinnamox.

5.11.2 Other Nonpeptide Affinity Labels. Rice and coworkers prepared a variety of affinity labels on the basis of the structures of etonitazine, fentanyl, and oripavine (Fig. 7.39) (198, 461). The etonitazene derivative BIT (196) selectively inactivates μ receptors, whereas the fentanyl derivative FIT (fentanyl isothiocyanate, 197) and the oripavine derivative FAO (fumaramido oripavine, 200) selectively inactivate δ receptors. The selective alkylation of δ receptors by FIT, which is a derivative of the p-selective ligand fentanyl, illustrates how much the alkylation step can influence the receptor selectivity of affinity labels. Both BIT and FIT have been prepared in tritiated form (631) and [^{3}H]FIT used to specifically label a 58-kDa protein from NG108–15 cells (632). The enantiomeric pair of the cis-&methyl derivatives of FIT were synthesized and one of these isomers, the (+)-3*R*,4*S* enantiomer, SUPERFIT (**198**) was found to be a very potent (5–10 times the potency of FIT) and selective irreversible ligand for δ receptors (458). SUPERFIT was used to purify δ receptors from NG108–15 cells to apparent homogeneity (633). Liu-Chen and coworkers used similar approaches to those described above for β-FNA to study the binding of SUPERFIT to δ opioid receptors. The results for wash-resistant inhibition of binding to μ/δ chimeric receptors suggested that the segment from the start of the first intracellular loop to the middle of TM3 of δ receptors is important for the selective irreversible binding of SUPERFIT (634). The enantiomeric pair of the *trans*-3-methylfentanyl isothiocyanates have also been prepared (199). The (+)-3*S*,4*S* isomer was a Bselective acylating agent in vitro, with potency similar to that of SUPERFIT, and has been used to selectively deplete δ_{ncx} binding sites (see Section 3.2.4.3 above) (200).

Several derivatives of κ-selective compounds containing an isothiocyanate have been prepared as potential irreversible ligands



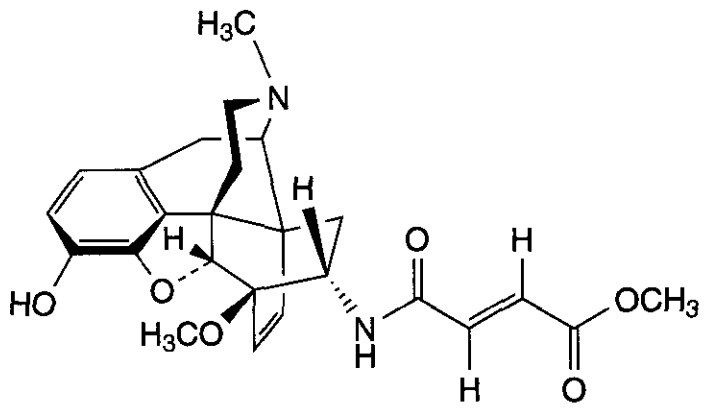
(**196**) BIT



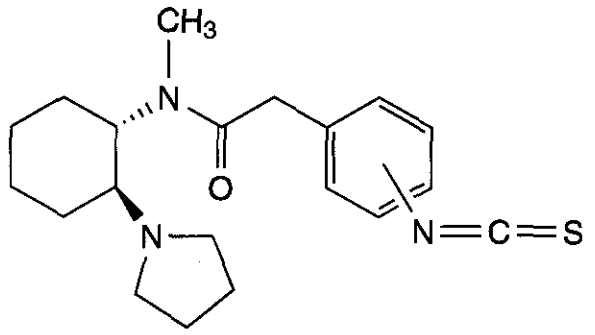
$R_1 = R_2 = H$ (**197**) FIT

$R_1 = CH_3$, $R_2 = H$ (**198**) SUPERFIT

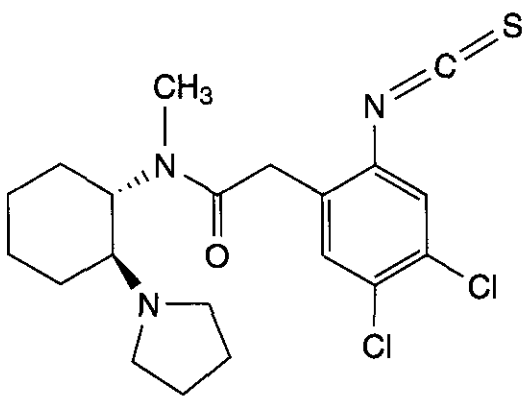
$R_1 = H$, $R_2 = CH_3$ (**199**) transSUPERFIT



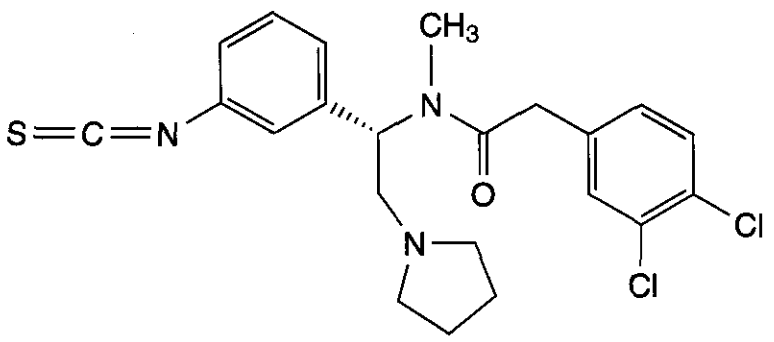
(**200**) FAO



(**201**)



(**202**) UPHIT



(**203**) DIPPA (*S*)

Figure 7.39. Other nonpeptide affinity labels.

for κ receptors (Fig. 7.39). de Costa et al. prepared analogs of U50,488 (**201**) and (**202**) (635–637). In the series of enantiomerically pure analogs of (**201**) the (−)-1*S*,2*S* isomers generally exhibited wash-resistant inhibition of binding of [^{3}H]U69,593 to guinea pig brain membranes, but none of the compounds irreversibly inhibited the binding of [^{3}H]bremazocine to either guinea pig or rat brain membranes (635, 637), supporting the conclusion of heterogeneity of κ receptors. The (−)-*o*-isothiocyanate isomer of (**201**) exhibited selective wash-resistant inhibition of [^{3}H]U69,593 binding, and was the most potent *in vitro*, but it failed to produce any irreversible inhibition of κ receptors after i.c.v. injection into guinea pig brain. This led de Costa and coworkers to prepare UPHIT (**202**), the chlorine-containing analog of (**201**), to improve affinity and selectivity (636). In contrast to (**201**), this compound inhibited binding to κ receptors after i.c.v. administration (636). *In vivo*, in mice UPHIT antagonizes antinociception produced by U69,593, but not by bremazocine, providing additional supporting evidence for κ-receptor subtypes (638).

Isothiocyanate derivatives of the κ-selective agonist ICI 199441 have also been described. Chang et al. prepared the *m*-isothiocyanate derivative DIPPA (**203**) (639, 640), which exhibits wash-resistant inhibition of [^{3}H]U69,593 binding and long-lasting κ re-

ceptor antagonism in vivo. Liu-Chen and coworkers examined the binding of the corresponding p-isothiocyanate derivatve to μ/κ chimeric receptors and determined that the region from TM3 to the C-terminus of the κ receptor is important for the binding of this compound (641). Nelson and coworkers incorporated the isothiocyanate in the phenylacetamide phenyl ring of ICI 199441 (642). These isothiocyanate analogs all exhibited wash-resistant inhibition of binding, whereas the parent compound with an unsubstituted phenylacetamide phenyl ring was completely removed by washing; the lead compound ICI 199441, with chlorines on this ring but without the isothiocyanate group, however, was not completely removed by the washing procedure.

A number of photoaffinity label derivatives of opiates have also been prepared [see the previous edition of this chapter (286) and Ref. 594 for more detailed reviews]. Azide derivatives of a number of different opiates, including etonitazene, carfentanil, and 6α- and 6β-substituted naltrexamine derivatives, have been prepared (see Ref. 286). A significant problem with using opioid photoaffinity labels is the sensitivity of opioid receptors to destruction by short-wavelength UV irradiation (643). Incorporation of a nitro group into the aromatic ring bearing the azide functionality shifts the absorption maximum so that photolysis can be conducted at longer wavelengths, where little if any photodestruction of opioid receptors occurs.

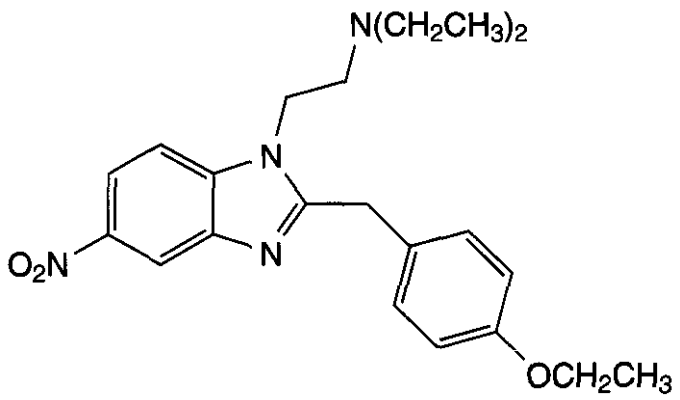
5.12 Miscellaneous Nonpeptide Opiates

A variety of compounds in other chemical classes have also been identified that have analgesic activity (see Ref. 466). In addition to the κ-receptor selective arylacetamides, such as U50,488 and related compounds, the benzodiazepine tifluadom (147) is a κ agonist (see Section 5.9). The benzimidazole etonitazene (204, Fig. 7.40) is a potent analgesic, having approximately 1500 times the potency of morphine in mice (644), and is a preceptor agonist. The aminotetralin dezocine (**205**) bears some resemblance to the benzomorphans and contains a phenol and basic amino group; in contrast to other opiates, however, the amine group is a primary amine. Dezocine is the *levo* isomer of the β epimer; the (+) isomer is inactive. This clinically used analgesic is a mixed agonist/antagonist (645) (see Table 7.3) with partial μ agonist activity; it may also have activity at κ receptors (see Ref. 307). The cyclohexane derivative tramadol (Ultram, 206) is an atypical analgesic (see Refs. 307, 646, 647 for reviews), which appears to produce analgesia through both opioid and nonopioid mechanisms (648). Tramadol antinociception appears to be mediated through both activation of μ opioid receptors, where it exhibits low affinity ($K_i = 2\ \mu M$), and by inhibition of monoamine uptake. Examination of the isomers of tramadol (649) found that although the (+) enantiomer had higher affinity for μ receptors ($K_i = 1.3\ \mu M$), the activities of the isomers were complementary and synergistic. Other recently reported compounds with opioid receptor affinity and activity include pyrrolidinylnaphthalenes, which are structurally related to heterosteroids (650). Highly constrained tricyclic piperazine derivatives (**207**) and (208) were prepared, which are structurally related to the 4-anilidopiperidines and which exhibit reasonable affinity (K_i values of 10 and 7 nM, respectively) and selectivity [K_i ratio ($\mu/\delta/\kappa$) = 1/230/300 and 1/71/110] for μ receptors; in *vivo*, (208) is a sixfold more potent analgesic than morphine (651). 3-Amino-3-phenylpropionamide derivatives were prepared as small molecule mimics of the peptide antagonists CTOP and CTAP and analogs identified with high affinity for μ and κ opioid receptors (e.g., 209) (652).

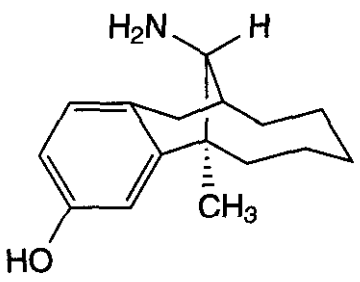
6 OPIOID PEPTIDE ANALOGS

6.1 Introduction

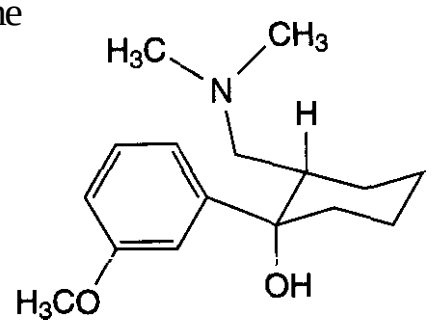
The identification of the opioid peptides in the mid-1970s opened up a whole new area for the development of opioid receptor ligands. The endogenous opioid peptides, both mammalian and amphibian (see below), have served as lead compounds that have been extensively modified to enhance potency, receptor type selectivity, stability, and/or decrease conformational flexibility. Peptide ligands selective for both μ and δ opioid receptors are found in more than one type of peptide sequence. Thus some enkephalin analogs, as well as the recently discovered endomorphins, analogs of



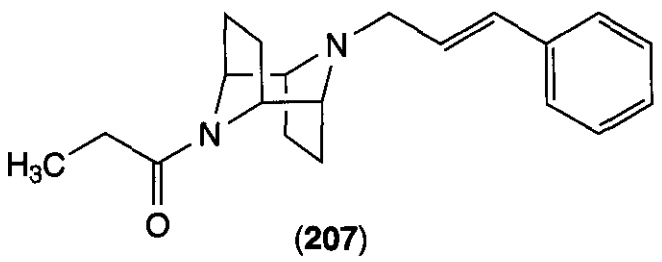
(204) Etonitazene



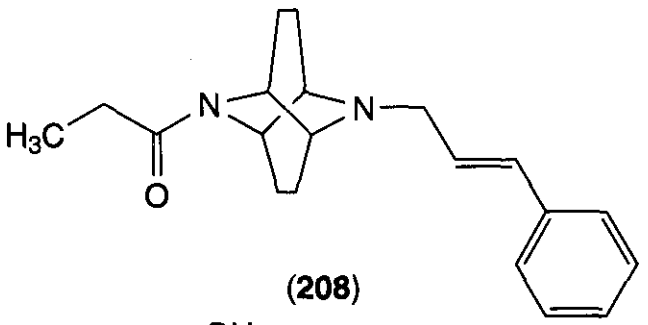
(205) Dezocine



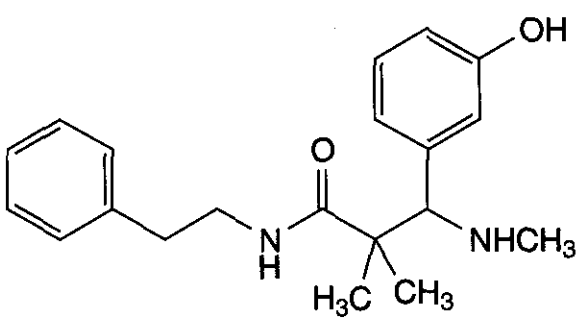
(206) Tramadol



(207)



(208)



(209)

Figure 7.40. Miscellaneous nonpeptide opiates.

the peptide β-casomorphin (derived from casein) and the amphibian peptide dermorphin, preferentially interact with μ opioid receptors (see Sections 6.2.1, 6.4.1, and 6.5.1 below). Other enkephalin analogs, as well as analogs of the amphibian peptides the deltorphins, preferentially interact with δ opioid receptors (see Sections 6.2.2 and 6.5.2 below). For κ receptors the endogenous opioid peptides identified to date have been limited to one class of peptides, the dynorphins (see Section 6.3 below). Peptides with affinity for opioid receptors, which have sequences completely different from those of the endogenous opioid peptides, have also been identified. Analogs of somatostatin, which are μ opioid receptor antagonists, have been prepared, and novel peptides with opioid receptor affinity have been identified through the use of combinatorial approaches (see Section 6.6). Affinity label derivatives of opioid peptides that bind irreversibly to opioid receptors have also been identified (see Section 6.7). In addition to preparing analogs of opioid peptides, inhibitors of opioid peptide metabolism have been developed as an indirect approach to using opioid peptides as potential therapeutic agents (see Section 6.8).

Much of the early SAR of the enkephalins has been discussed in a classic review by Morley (653) and in *The* Peptides, Volume 6 (654). Subsequent reviews of opioid peptides include those by Hruby (655, 656) and Schiller (657,

Dermorphin (212)	Tyr-D-Ala-Phe-Gly-Tyr-Pro-SerNH_2
Deltorphin (dermenkephalin, deltorphin A, 213)	**Tyr-D-Met-Phe-His-Leu-Met-AspNH_2**
[D-Ala^2]deltorphin I (deltorphin C, 214)	**Tyr-D-Ala-Phe-Asp-Val-Val-GlyNH_2**
[D-Ala^2]deltorphin II (deltorphin B, 25)	Tyr-D-Ala-Phe-Glu-Val-Val-GlyNH_2

Figure 7.41. Opioid peptides from frog skin.

658), which contain extensive tabular data. An issue of ***Biopolymers (Peptide Science)*** (Vol. 51, Number 6, 1999) is devoted solely to reviews of peptide and peptidomimetic ligands for opioid receptors. The reader is referred to these reviews for additional references to the literature.

In addition to the four classes of opioid peptides discussed earlier (Section 3.4), other peptides of mammalian origin with opioid activity have also been identified. β-Casomorphin (**210**), obtained by enzymatic digestion of the milk protein casein (659, 660), exhibits some selectivity for μ receptors. Human β-casomorphin (211) differs from the bovine sequence in two positions; the human β-casomorphin pentapeptide and tetrapeptide fragments are less potent than the corresponding bovine peptides (661). Other peptides with affinity for opioid receptors include peptides derived from hemoglobin (see Ref. 662).

Bovine β-casomorphin (**210**)

Tyr-Pro-Phe-Pro-Gly-Pro-Ile

Human β-casomorphin (211)

Tyr-Pro-Phe-Val-Glu-Pro-Ile

6.1.1 Opioid Peptides from Amphibian Skin. Based on their finding amphibian skin peptides, which were counterparts to other mammalian bioactive peptides, Erspamer and coworkers examined amphibian skin for opioid peptides (see Ref. 663 for a review). This led first to the isolation and characterization of dermorphin (212, Fig. 7.41), which is a μ-selective peptide (see Table 7.13), from the skin of South American Phyllemedusinae hylid frogs in the early 1980s (664). Inspection of the sequence of one of the cloned cDNAs for the precursor of dermorphin suggested the existence of another heptapeptide with a similar *N*-terminal sequence (665). This then led to the isolation of deltorphin (also called dermenkephalin or deltorphin A, 213, Fig. 7.41), the first δ-selective amphibian opioid peptide, from these frogs (666, 667). Synthesis confirmed that the amino acid in position 2 of deltorphin was D-methionine rather than L-methionine (666, 668, 669). Two additional peptides [D-Ala^2]deltorphin I (also referred to as deltorphin C, 214, Fig. 7.41) and [D-Ala^2]deltorphin II (also referred to as deltorphin B, 25, Fig. 7.5) were subsequently discovered (106) which exhibited even greater δ-receptor affinity and exceptional selectivity

Table 7.13 Opioid Receptor Affinities and Opioid Activity in the GPI and MVD of Peptides from Amphibian Skin[a]

	K_i (nM)[b]		K_i Ratio	IC_{50} (nM)	
Peptide	μ	δ	μ/δ/κ	GPI	MVD
Dermorphin (**212**)	0.70	62	1/89/>14,000	1.4	2.4
Deltorphin (**213**)	1,630	2.4	680/1/>4,000	5,000	1.4
[D-Ala^2]Deltorphin I (**214**)	3,150	0.15	21,000/1/>66,000	>1,500	0.21
[D-Ala^2]Deltorphin II (**25**)	2,450	0.71	3,400/1/>14,000	>3,000	0.32

[a] Data from Ref. 106.
[b] K_i > 10,000 nM for κ receptors for all of the amphibian skin peptides.

(see Table 7.13), making them the most selective of the naturally occurring opioid peptides. [D-Ala^2]deltorphin II has been used to study the proposed 8,-receptor subtype (see Section 3.2.4.3 above). The pharmacology of these amphibian opioid peptides has been discussed in a recent review (670).

The unique feature of these amphibian skin opioid peptides is the sequence between the important aromatic residues. In contrast to the enkephalins and other mammalian opioid peptides that contain the Gly-Gly dipeptide sequence between Tyr and Phe, the amphibian opioid peptides contain a single D-amino acid (see Fig. 7.41), which apparently arises from a post-translational conversion of the L-amino acid to its D isomer (665). The identification of these unusual opioid peptides expanded our understanding of the structural requirements for interaction with opioid receptors and provided new lead compounds for further modification (see Sections 6.5.1 and 6.5.2 below).

6.2 Enkephalin Analogs

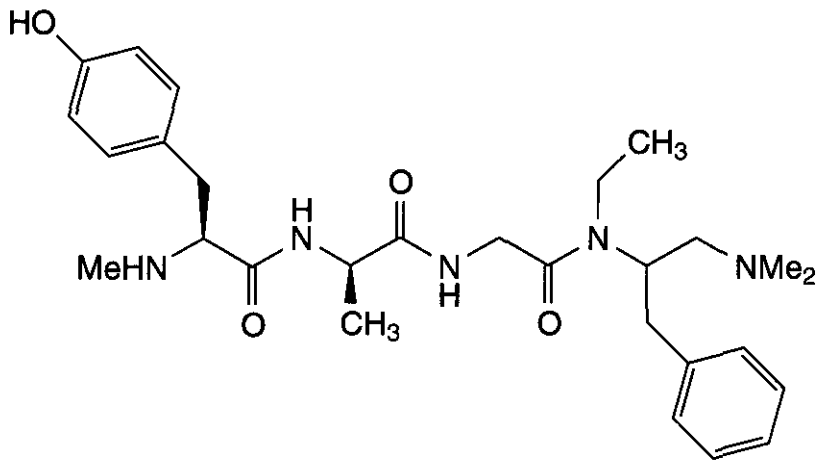
The enkephalins have been the most extensively modified of the opioid peptides, and thousands of analogs of these pentapeptides have been prepared (see Refs. 653–658 for reviews). The naturally occurring enkephalins exhibit some selectivity for 8 receptors (see Table 7.9), but these peptides are rapidly degraded by a variety of peptidases (see Section 6.8 below). Therefore one major goal of structural modification of these small peptides has been to increase metabolic stability. Depending on the nature of the modifications made, both μ- and δ-selective enkephalin derivatives have been prepared (enkephalin derivatives generally have very low affinity for κ opioid receptors). These derivatives have included both linear peptides and conformationally constrained derivatives. Conformational constraints have included cyclizations between residues in the peptide chain and local constraints by incorporation of an amino acid whose side-chain conformation is restricted.

Early SAR studies (see Ref. 653 for a review) identified important structural features of the enkephalins and which positions could be readily modified. The importance of Tyr^1 for opioid activity was apparent from the large decreases in potency when this residue was substantially modified. A D-amino acid in position 2 was one of the early modifications examined to decrease the cleavage of the Tyr-Gly bond by aminopeptidases (671). Incorporation of a D-amino acid at this position increases potency at both p and 6 receptors and is found in the vast majority of enkephalin derivatives; an L-amino acid at position 2 significantly decreases potency at both receptors. In the enkephalins the 3 position is very intolerant of substitution, and therefore enkephalin derivatives generally retain a glycine at this position. There are significant differences between μ and δ receptors, however, in the structural requirements for residues in positions 4 and 5, and frequently modifications in these positions have been used to impart selectivity for one of these opioid receptor types (see below).

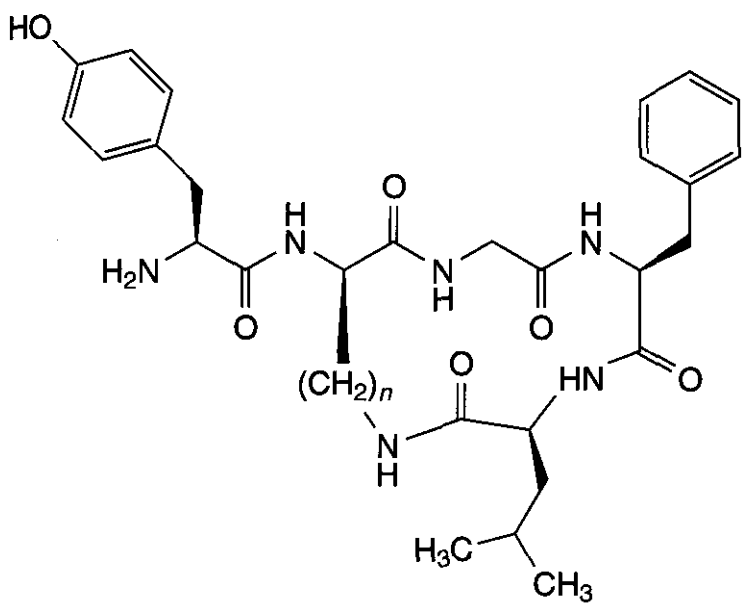
6.2.1 Mu-Selective Enkephalin Analogs

6.2.1.1 ***Linear Analogs.*** Enkephalin analogs with substantial structural changes from the endogenous peptides, particularly in the C-terminus, retain affinity for μ opioid receptors and can exhibit improved preceptor selectivity. Thus the aromatic moiety of Phe^4 is not an absolute requirement for interaction with μ receptors, and a cyclohexane ring (672) or leucine side chain (673, 674) is tolerated in this position. Significant variation is also tolerated in residue 5 and the C-terminus by μ receptors, and modifications in this region of the peptides have been very useful in differentiating μ versus 8 receptors. Thus, the C-terminus can be amidated, reduced, or eliminated completely with retention of p-receptor affinity and often with a substantial increase in preceptor selectivity.

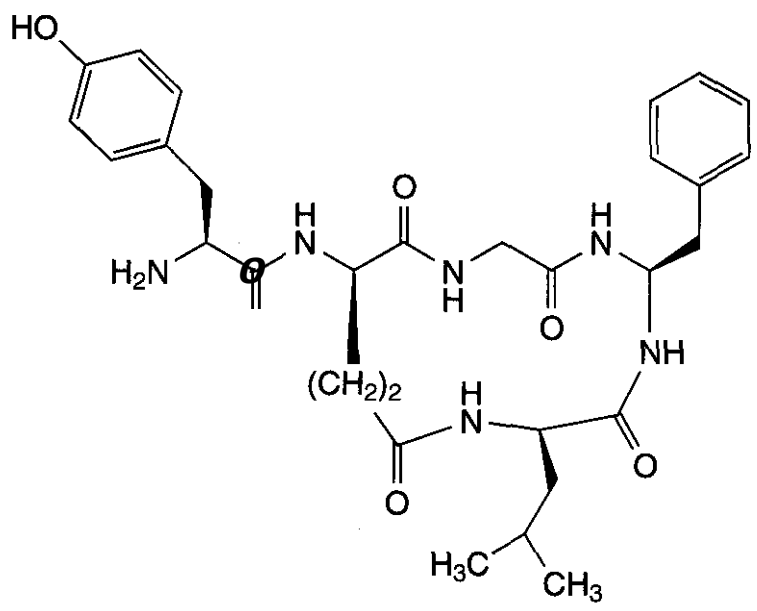
One of the most commonly used p-selective ligands DAMGO (15, Fig. 7.4) (675) contains a reduced C-terminus and is available in tritiated form. Substitution of the glyol functionality of DAMGO with Met(O)ol (to give FK 33824) increases 6-receptor affinity 10-fold, decreasing p-receptor selectivity (130). Other related tetrapeptide analogs with a modified C-terminus syndyphalin-25 (Tyr-D-Met-Gly-NMePheol) (676) and LY 164929 (NMeTyr-D-Ala-Gly-NEtPhe[Ψ(CH_2NMe_2)], 215, Fig. 7.42) with a reduced C-terminal amide (677) exhibit significantly higher selectivity for μ re-



(**215**) LY 164929



(**216**) $n = 2$
(**217**) $n = 4$



(**218**)

Figure 7.42. μ-Receptor **selective enkephalin analogs. The structure of** DAMGO **(15) is shown in Fig. 7.4.**

ceptors than DAMGO (Table 7.14). Shorter tripeptide **amide** analogs with a branched **alkyl** chain in place of the second phenyl ring also exhibit preceptor selectivity, although their potency in the GPI is significantly lower ($IC_{50} = 240–320$ nM) (678).

6.2.1.2 Conformationally Constrained Analogs. Local restriction of conformational freedom can be accomplished by incorporating conformationally constrained amino acids (see Refs. 679,680 for reviews). In the case of the enkephalins, incorporation of 2-amino-6-hydroxy-2-tetralincarboxylic acid (Hat, Fig. 7.43) in place of **Tyr**^{1} in [Leu^5]enkephalin methyl ester results in a μ-**selective** compound [IC_{50} ratio (**MVD/GPI**) = 5.11, whereas the analog containing 2-hydroxy-5-hydroxy-2-indane**carboxylic** acid (**Hai**) is virtually inactive (**681**). Incorporation of **2′,6′-dimethyltyrosine** (Dmt, Fig. 7.43) in position 1 of [Leu^5]enkephalin results in large increases in both μ and 6 affinity and an analog with some selectivity for μ receptors (**682**), whereas incorporation of 2′,6′-dimethylphenylalanine (**Dmp**, Fig. 7.43) in position 4 results in increased preceptor selectivity as a result of decreased **δ-receptor** affinity (683). Interestingly, combining the two modifications results in a weak antagonist ($pA_2 = 6.90$) with some selectivity (20-fold) for μ receptors (683).

Enkephalin derivatives selective for μ receptors have also been prepared by cyclization between a D-amino acid and the C-terminus. Tyr-*cyclo*[N$^{\gamma}$-D-Dab-Gly-Phe-Leu] (**216,** Fig. 7.42; Dab = **a,γ-diaminobutyric** acid) (684) exhibits both high potency and preceptor selectivity (Table **7.14**), whereas the corresponding linear **peptide** [D-Dab^2,Leu^5]enkephalinamide

Table 7.14 Opioid Receptor Affinities and Opioid Activity in the GPI and MVD of μ-Selective Enkephalin Analogs[a]

Peptide	K_i (n*M*) μ	K_i (n*M*) δ	K_i Ratio δ/μ	IC_{50} (n*M*) GPI	IC_{50} (n*M*) MVD	Reference
DAMGO (**15**)	1.9	345	180	4.5	33	130
Syndyphalin-25 (Tyr-D-NMet-Gly-NMePheol)	0.29[b]	1,250[b]	4,300	0.0025	—	694
LY 164929 (**215**)	0.6[b]	900[b]	1,500	—	—	677
Tyr-*cyclo*[D-X-Gly-Phe-Leu]						
X = D-Dab (**216**)	13.8	115	8.3	14.1	81.4	686
X = D-Lys (**217**)	12.4	14.6	1.2	4.8	141	686
Tyr-*cyclo*[D-Lys-Glyψ[CSNH]Phe-Leu]	4.55	654	44	24	186	692
Tyr-*cyclo*[D-Glu-Gly-gPhe-D-Leu] (**218**)	11.0	389	35	19.4	313	693

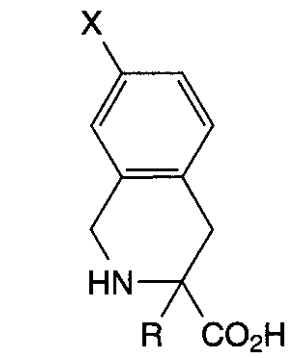
[a]Data for DAMGO from Table 7.8 are included for comparison.
[b]IC_{50} values.

is not preceptor selective (685). This was the first demonstration that receptor selectivity could be imparted by conformational restriction. Related analogs with D-Orn or D-Lys in position 2 also exhibit high potency for μ receptors but decreased selectivity in radioligand-binding assays (see Table 7.14) (686). The conformations of these peptides have been examined by both computational methods (687–690) and NMR spectroscopy (689, 691). As expected, the ring structure reduces conformational flexibility, but some flexibility in the ring remains, particularly for the larger ring sizes, and different intramolecular hydrogen bond patterns have been proposed for the cyclic structures. Modifications to the peptide bonds in these cyclic peptides, e.g., thioamide replacement of the Gly^3-Phe^4 peptide bond (692) or partial retro-inverso analogs of (**216**) e.g., Tyr-*cyclo*[D-Glu-Gly-gPhe-D-Leu] (218, Fig. 7.42) (693), can further enhance μ-receptor selectivity (see Table 7.14).

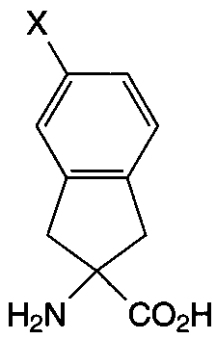
6.2.2 Delta-Selective Enkephalin Analogs

6.2.2.1 Linear Analogs. Differences in the SAR of enkephalin analogs for interaction with 6 versus μ opioid receptors have been exploited to develop Bselective derivatives. Thus more hydrophilic D-amino acids, such as D-Ser or D-Thr, can be incorporated into position 2 to impart δ-receptor selectivity, whereas μ opioid receptors prefer a more hydrophobic residue in this position (674). Whereas μ receptors can accommodate an aliphatic residue in position 4 of enkephalins, δ receptors generally require an aromatic moiety in this position. At the C-terminus δ receptors prefer a free carboxylic acid, and lengthening of the peptide with a residue such as Thr can result in increased 6 selectivity.

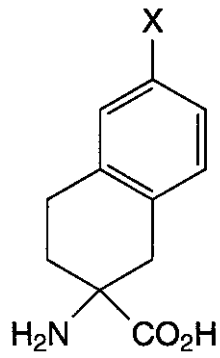
Several linear enkephalin analogs have enhanced selectivity for 6 opioid receptors (Tables 7.8 and 7.15). DADLE (21; see Fig. 7.5 and Table 7.8) (695) was an early analog prepared that shows a slight preference for 6 opioid receptors. Roques and coworkers prepared several [Leu^5,Thr^6]enkephalin analogs containing D-Ser, D-Thr, or a derivative in position 2, which have greater selectivity for these receptors. Thus incorporation of D-Ser in position 2, which yields DSLET (**22**) (696), and substitution of D-Thr in this position to give DTLET (**23**) (697) enhances δ-receptor selectivity. X-ray structures for both DADLE (698) and DTLET (699) have been reported; in both cases a single bend conformation is found in the crystals (see Ref. 700 for a review). The steric bulk of the residues in positions 2 and/or 6 was increased by incorporating the t-butyl ether derivatives of D-Ser and/or Thr. The resulting derivatives DSTBULET ([D-Ser(O^tBu)^2, Leu^5,Thr^6]enkephalin) and BUBU ([D-Ser(O^tBu)^2,Leu^5,$Thr(O^tBu)^6$]enkephalin) (696) exhibited decreased affinity for μ receptors and thus enhanced selectivity for δ receptors. Replacement of the ether in the side



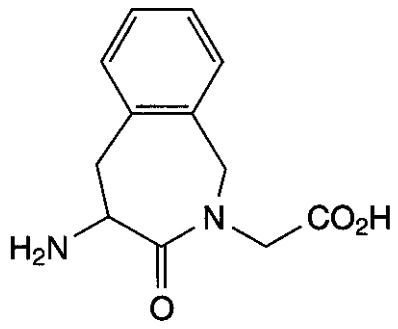
X = H, R = H Tic
X = H, R = CH_3 MeTic
X = OH, R = H Tic(OH)



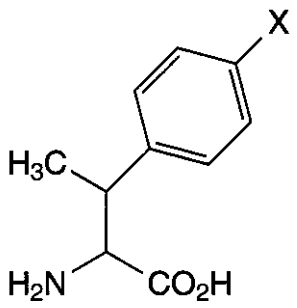
X = H Aic
X = OH Hai



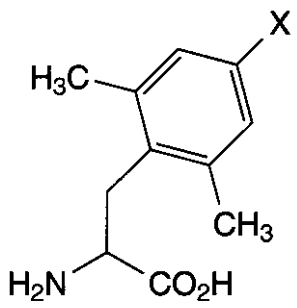
X = H Atc
X = OH Hat



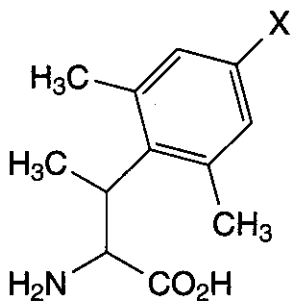
Aba-Gly



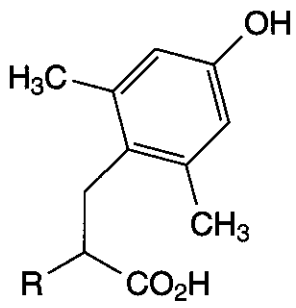
X = H ß-MePhe
X = OH ß-MeTyr



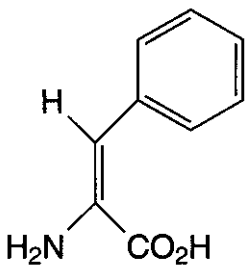
X = H Dmp
X = OH Dmt



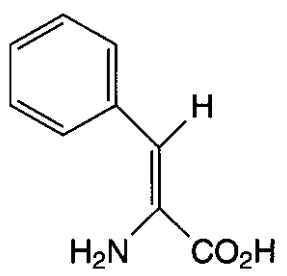
X = H Tmp
X = OH Tmt



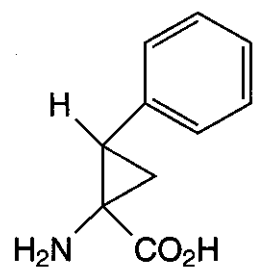
R = H Dhp
R = CH_3 Mdp



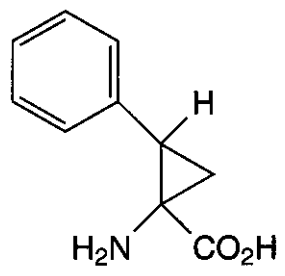
Δ^EPhe



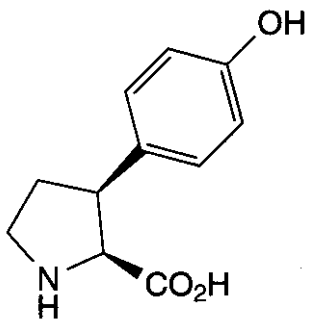
Δ^ZPhe



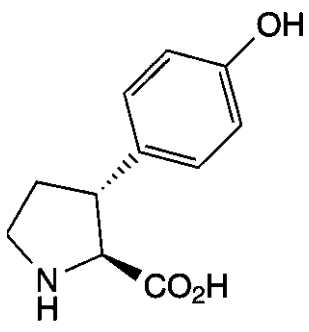
∇^EPhe



∇^ZPhe



c-Hpp



t-Hpp

Figure 7.43. Conformationally constrained phenylalanine and tyrosine analogs.

chain at position 2 by a sulfur to give BUBUC ([D-Cys(S*t*Bu)^2,Leu^5,Thr(O*t*Bu)^6]enkephalin) (701) further decreases affinity for μ receptors, resulting in an analog with a 1000-fold selectivity for δ over μ receptors (Table 7.15).

The penetration of the blood-brain barrier (BBB) by enkephalin analogs is relatively low and therefore their analgesic activity after systemic administration is relatively weak (702). Therefore modifications have been made to enkephalin analogs in an attempt to enhance BBB penetration. Attachment of D-glucose through an 0-linkage to Ser^6 in the DTLET analog Tyr-D-Thr-Gly-Phe-Leu-SerNH, resulted in a peptide that retains similar opioid receptor affinity and potency in smooth mus-

Table 7.15 Opioid Receptor Affinities and Opioid Activity in the GPI and MVD of δ-Selective Enkephalin Analogs[a]

Peptide	K_i (nM) ti	μ	K_i Ratio μ/δ	IC_{50} (nM) MVD	GPI	Reference(s)
Agonists						
DSLET (22)	1.8	39	22	0.59	110	
DTLET (23)	2.7	34	13	0.41	68	
DSTBULET	2.8	370	130	1.1	1,800	
BUBU	1.7	480	280	0.6	2,790	
BUBUC	2.9	2,980	1,030	0.05	13,300	701
[D-Thr^2,Leu^5]enkephalin-Ser(OR)^6						
R = H	2.4	4.0	1.7	2.7	25	702
R = D-glucose	3.4	8.2	2.4	1.6	34	702
DPDPE (24)	2.7	710	260	2.8	2,350	
DPLPE	2.8	660	235	4.1	3,000	
[Phe(*p*-Cl)^4]DPDPE	1.6	780	490	0.89	8,300	
cyclo[D-Pen^2,Cys^5]enkephalin-Phe^6	1.4[b]	280[b]	200	0.016	82.7	720
cyclo[D-Pen^2,Phe(*p*-X)^4,Pen^5]-enkephalin-Phe^6						
X = F	0.43[b]	1,650[b]	3,800	0.016	740	721
X = Br	0.20[b]	4,200[b]	21,000	0.18	3,400	721
[2′-MeTyr^1]DPDPE	0.89[b]	1,170[b]	1,320	4.5	430	726
[(2*S*,3*R*)-Tmt^1]DPDPE	5.0	4,300	850	1.8	antagonist[c]	727, 728
[(2*S*,3*S*)-Tmt^1]DPDPE	210	720	3	170	290	727, 728
[Hat^1]DPDPE	2.36'	1,440[b]	610	22	3,500	726
[(2*S*,3*S*)-β-MePhe^4]DPDPE	10	14,000	1,400	39	57,400	732
Antagonists						
ICI 174,864 (29)	190	29,600	155	17[d]	>5,000[d]	
N,*N*-Dibenzyl Leu-enkephalin	78	1,600	20	180[d]	NA[e]	737, 738
[(2*S*)-Mdp^1,D-Ala^2,Leu^5]enkephalin amide	12	190	16	28[d]	154[d]	739

"Data for DSLET, DTLET, DPDPE, and ICI 174,864 from Table 7.8 are included for comparison. Data from Ref. 130 except where otherwise indicated.

[b] IC_{50} values.

[c] IC_{50} = 5 μM.

[d] Antagonist, K_e values (nM).

"Not active.

cle assays to the parent peptide (see Table 7.15) (702). Glycosylation led to significant increases in both enzymatic stability and BBB permeability, resulting in significantly improved analgesia after i.v. administration (703).

6.2.2.2 Conformationally Constrained Analogs. Local restriction of conformation in the linear enkephalins has included incorporation of dehydroamino acids [*e.g.*, dehydrophenylalanine (ΔPhe, Fig. 7.43)] and cyclopropylmethylphenylalanine (∇Phe) into the peptides (see Ref. 704 for a review). In the case of the dehydrophenylalanine (ΔPhe^4) derivatives of [D-Ala^2,Leu^5]enkephalin, the *Z* isomer exhibits 150–260 times higher affinity for δ and μ receptors than the E isomer (705). For the peptides containing cyclopropylphenylalanine (∇Phe), one of the *Z* isomers exhibits high affinity for [^{3}H]naloxone binding sites in rat brain (706), whereas the 2*R*,3*S* isomer of V^EPhe exhibits high affinity (K_i = 13 nM) and selectivity (K_i ratio = 250) for δ opioid receptors (707); the latter compound, however, was essentially inactive in smooth muscle assays (IC_{50} = 2000–4000 nM).

Delta-selective enkephalin analogs were prepared by cyclization through disulfide bond formation. Cyclic enkephalin analogs containing D- or L-Cys residues in positions 2 and 5

were first synthesized by Sarantakis (708) and Schiller (709). These initial cyclic enkephalin analogs exhibit only a slight preference for 6 over μ receptors, but introduction of methyl groups on the β carbons of the cysteine residues by incorporation of penicillamine (Pen) in positions 2 and/or 5 markedly enhances &receptor selectivity (710–713). The most &selective compounds in the series were the bis-penicillamine derivatives DPDPE (*cyclo*[D-Pen^2,n-Pen^5]enkephalin, 24, Fig. 7.5) and DPLPE (*cyclo*[D-Pen^2,L-Pen^5]enkephalin) (713). The individual contributions of the β-methyl groups in residue 2 to δ-receptor affinity and selectivity were examined by preparing *cyclo*[(3*S*)Me-D-Cys^2,D-Pen^5]- and *cyclo*[(3*R*)-Me-D-Cys^2,D-Pen^5]enkephalin (714). The β-methyl groups in the 2 position had only a minor affect on 6 opioid receptor affinity, and the similar affinity for μ receptors of *cyclo*[(3*S*)Me-D-Cys^2,D-Pen^5]- and *cyclo*[D-Cys^2,D-Pen^5]enkephalin suggested that adverse steric interactions with the pro-R methyl group are responsible for the low μ-receptor affinity, and therefore δ-receptor selectivity, of DPDPE.

Additional modifications to DPDPE *can* further enhance Sreceptor affinity and/or selectivity (see Ref. 656 for a review, including tables of analogs). Halogen substitution on the *para* position of the phenylalanine ring in position 4 enhances both δ-receptor potency and selectivity (715), and [Phe(*p*-Cl)^4]DPDPE has been used in tritiated form in radioligand binding assays. Halogenation *can* also increase blood-brain barrier permeability (716, 717). [L-Ala^3]DPDPE was prepared and exhibits an unusual spectrum of activity (718). In the mouse vas deferens, [L-Ala^3]DPDPE is a potent (IC_{50} = 12 n*M*) δ-selective agonist, whereas at central 6 receptors it is a moderately potent 6,-receptor antagonist and weak agonist with no apparent affects on central 6,-receptors. The conformation of [L-Ala^{3}1DPDPE has been compared to that of DPDPE (719), and the differences in conformation around residues 2 and 3 were proposed to explain the differences in efficacy observed for the two analogs. A C-terminal extension of *cyclo*[D-Pen^2,L-Cys^5]enkephalin (DPLCE) with phenylalanine resulted in an analog with extremely high potency in the MVD (IC_{50} = 0.016 n*M*) (720). A similar extension of *cyclo*[D-Pen^2,L-Pen^5]enkephalin (DPLPE) had only a modest effect on &receptor affinity or potency in the MVD, but halogenation of the resulting DPLPE-Phe resulted in increased Sreceptor affinity and exceptionally high Sreceptor selectivity and potency (see Table 7.15) (721); the p-chloro and p-bromo derivatives also exhibited enhanced penetration of the BBB (717).

Glycopeptide derivatives of *cyclo*[D-Cys^2,Cys^5]enkephalin and DPDPE have been prepared by attachment of Ser(β-D-glucose)-GlyNH_2 to the C-terminus (722). After peripheral administration, the *cyclo*[D-Cys^2,Cys^5]enkephalin derivative exhibits significant analgesic activity that appears to be centrally mediated, indicating that the glycopeptide penetrates the blood-brain barrier. The penetration of the BBB was initially postulated to be through interaction with the glucose transporter GLUT-1, but subsequent studies have proved this to be incorrect (see Ref. 723 and references cited therein). In a series of *cyclo*[D-Cys^2,D-Cys^5,Ser^6,Gly^7]enkephalinamide analogs in which the sugar attached to Ser^6 was varied, the nature of the sugar affected analgesic potency after i.v. administration; the α-glucose derivative showed the highest analgesic potency and 6-receptor affinity of the glycosylated analogs examined (723). Recently, a prodrug derivative of DPDPE, in which PEG (polyethylene glycol) is attached to the *N*-terminus, has been reported to also exhibit enhanced analgesia compared to that of DPDPE after i.v. administration; this prodrug has very weak affinity for 6 opioid receptors, but is converted to DPDPE in *vivo* (724).

The conformation of DPDPE has been examined by NMR and computational methods (see Ref. 725 and references cited therein) and an X-ray structure has been obtained (725). There is generally good agreement between these studies on the conformation of the 14-membered ring, but there is still considerable conformational flexibility around Tyr^1 in DPDPE. This is evident in the crystal where three distinct structures, with essentially identical conformations for the 14-membered ring but with different conformations for Tyr^1, were found (725). The activity of the Hat^1, β-MeTyr^1, and Tmt derivatives (see be-

low) suggested that the preferred conformation of the side chain of residue 1 in DPDPE is trans (726–728). Based on the activity of the β-MePhe[4] derivatives (see below), the proposed side-chain conformation for Phe[4] is gauche (−) ($\chi_1 = -60°$) (729).

Therefore, other modifications to DPDPE have been made to incorporate additional conformational constraint into the aromatic residues in the peptide. Constrained Tyr analogs, including 2'-methyltyrosine, Dmt, β-methyltyrosine, 2′,6′,β-trimethyltyrosine (Trnt, Fig. 7.43), and Hat (726–728, 730), have been incorporated at position 1. Substitution of Dmt enhanced affinity at both δ and μ receptors and increased both in vitro and in vivo potency (730). Peptides containing 2′-MeTyr and Hat were also potent analogs with high 6-receptor selectivity (726) (see Table 7.15). Of the four peptides containing β-MeTyr, the (2*S*,3*R*)-β-MeTyr[1] derivative has the highest affinity for δ receptors and the greatest potency in the MVD (726). In the Tmt derivatives, which combine methyl groups on the phenyl ring with the β-methyl substitution, only the peptide containing the 2*S*,3*R* isomer of Tmt retains high δ receptor potency and selectivity (727, 728), whereas the peptide containing the diastereomer 2*S*,3*S* exhibits much lower δ-receptor affinity and selectivity (Table 7.15). Interestingly, in *vivo* [(2*S*,3*S*)-Tmt[1]]DPDPE is the more potent analog in the tail flick test after i.c.v. administration (731). The antinociceptive activity of both peptides is antagonized by β-FNA as well as by DALCE (731), suggesting that in contrast to DPDPE the antinociceptive activity of both these Tmt analogs is partially mediated by μ receptors as well as δ_1 receptors; these results are surprising for [(2*S*,3*R*)-Tmt[1]]DPDPE, given its high selectivity for δ receptors in binding assays.

Further conformational constraint in DPDPE has also been examined by incorporation of constrained residues in position 4. All four isomers of β-methylphenylalanine have been incorporated into DPDPE (732). [(2*S*,3*S*)-β-MePhe[4]]DPDPE (see Table 7.15) exhibits the highest 6-receptor affinity of the four peptides, although it is 6- to 10-fold less potent than DPDPE; it also exhibits the highest selectivity for δ over μ receptors. Incorporation of a p-nitro group into (2*S*,3*S*)-β-MePhe[4] increases 6-receptor affinity and potency 6- to 10-fold, so that [(2*S*,3*S*)-β-MePhe(*p*-NO_2)[4]]DPDPE exhibits potency at δ receptors similar to that of DPDPE. Further constraint of Phe[4] by incorporation of 2′,6′,β-trimethylphenylalanine (Tmp, Fig. 7.43), however, results in decreased δ receptor binding and selectivity; in the case of the 2*S*,3*S* isomer affinity for the μ receptor increases, resulting in a peptide with 10-fold selectivity for μ receptors (733).

6.2.2.3 Enkephalin Analogs with Antagonist Activity at δ Receptors. The first antagonists for δ opioid receptors were prepared by *N*,*N*-dialkylation of the N-terminus of enkephalins. The first δ-selective antagonists reported were *N*,*N*-diallyl Leu-enkephalin methyl ester and the derivative ICI 154,129 containing a thioether in place of the peptide bond between residues 3 and 4 (734), although the potency of these peptides was weak (K_e against Leu-enkephalin in the MVD = 254 n*M* for ICI 154,129). Substitution of Aib (α-aminoisobutyric acid) in positions 2 and 3 of *N*,*N*-diallyl Leu-enkephalin to give ICI 174,864 (29, Fig. 7.5, Table 7.15) enhanced potency approximately 10-fold and δ-receptor selectivity at least fivefold compared to that of ICI 154,129 (110), and so ICI 174,864 has been frequently used as a δ-selective antagonist in a number of pharmacological studies. The structure-activity relationships for the *N*,*N*-diallyl derivatives as δ antagonists are distinctly different from those of the unsubstituted agonist series (735). Thus the *N*,*N*-diallyl derivatives of a variety of potent enkephalin agonists such as DADLE are weak nonselective antagonists. Moreover, although ICI 154,129 is a selective δ antagonist, the corresponding [Gly[3]Ψ(CH_2S)-Phe[4],Leu[5]]enkephalin is virtually inactive as an agonist. Other modifications have been made to the *N*,*N*-diallyl enkephalins, including replacement of Gly[2]-Gly[3] with a rigid 4-aminobenzoic acid spacer (736), which yields a δ antagonist with potency similar to that of ICI 154,129. Substitution of benzyl groups for the allyl groups in *N*,*N*-diallyl Leu-enkephalin enhances δ-antagonist potency, whereas *N*,*N*-dialkylation with other alkyl groups (e.g., phenethyl) result in derivatives with agonist activity (737). Interestingly, although ICI 174,864 is more potent than *N*,*N*-

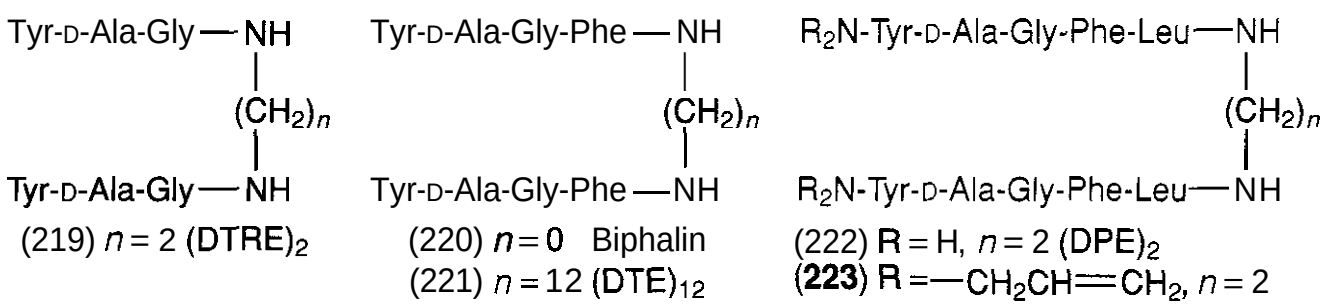


Figure 7.44. Dimeric enkephalin analogs.

dibenzyl Leu-enkephalin in the MVD (737), the reverse was true in binding studies that used cloned 8 receptors (738), suggesting possible differences in 8 receptors in the two preparations. ***N,N*-Dialkyl** Leu-enkephalin analogs bearing a reactive functionality recently have been described (738) (see Section 6.7 below).

Recently, the Leu-enkephalin analog containing a novel derivative of Dmt lacking a basic amine [(2*S*)-2-methyl-3-(2,6-dimethyl-4-hydroxyphenyl)propanoic acid, (2*S*)-Mdp, Fig. 7.431 was reported; this peptide is an antagonist in both the MVD and GPI smooth muscle preparations, with fivefold higher potency in the MVD (739).

6.2.3 Dimeric Enkephalin Analogs. A number of dimeric enkephalin analogs have been prepared (Fig. 7.44). Their receptor selectivity depends on the peptide sequences and the length of the spacer between the two peptides. The tripeptide dimer $(DTRE)_2$ [(Tyr-D-Ala-Gly-NH)$_2$$(CH_2)_2$, 219, Table 7.161 is μ selective, whereas the corresponding monovalent tripeptide Tyr-D-Ala-GlyNH$_2$ (TRE) is much less potent and only moderately μ selective (740). Replacement of the Tyr residue in one half of DTRE, with Phe or D-Tyr significantly decreased potency, which suggests that μ opioid receptors contain two similar sites that are in close proximity to one another (741).

In the tetrapeptide series biphalin [(Tyr-D-Ala-Gly-Phe-NH)$_2$ 2201, with only a hydrazine spacer, is not selective for μ or δ receptors (Table 7.16) (742). This peptide exhibits potent analgesic activity in *vivo* (742, 743), even though its potency in smooth muscle assays is modest (Table 7.16). Biphalin is equipotent with morphine, but produces little if any physical dependence after systemic (i.p.) administration. The truncated dimer Tyr-D-Ala-Gly-Phe-NHNH(Phe) retains high binding affinity and potency in smooth muscle assays, indicating that the entire sequence of biphalin is not required for activity (744). Modification of the residue in the 4 position can alter opioid receptor affinities and impart some selectivity for either μ or 8 receptors. Thus introduction of L-β-MePhe or naphthylalanine in this position results in analogs that preferentially interact with μ receptors (see Table 7.16) (745), whereas p-halogenation or nitration of the phenyl rings enhances 8-receptor affinity more than preceptor affinity (746); *para* chlorination also increases penetration across the BBB (747,748). In situ brain perfusion studies of [^{125}I-Tyr]biphalin suggest that part of the transport of this compound may use the large neutral amino acid carrier (749).

Other dimeric enkephalin derivatives have been prepared that exhibit 8-receptor selectivity. The dimer $(DTE)_{12}$ (221) with a long

Table 7.16 **Opioid Receptor Affinities and Opioid Activity in the GPI and MVD of Dimeric Enkephalin Analogs**

	K_i (n*M*)		K_i Ratio	IC_{50} (n*M*)		
Peptide	6	μ	δ/μ	MVD	GPI	Reference(s)
$(DTRE)_2$ (219)	14,000	34	410	2300	410	740,741
Biphalin (220)	5.2	2.8	1.8	8.8	27	745
[(2*S*,3*S*)-β-MePhe$^{4,4'}$]Biphalin	110	1.3	85	180	21	745
DTE_{12} (221)	10	38	0.26	38	1,000	750

spacer ($n = 12$) between the two tetrapeptides is a δ-selective ligand (the monomer Tyr-D-Ala-Gly-PheNH$_2$ is μ selective) (750). In the pentapeptide series (DPE)$_n$ the dimer with n = 2 (**222**) exhibits the greatest δ selectivity in this series (751), but the selectivity of this peptide is low [IC_{50} ratio (μ/δ) = 6.51. Dimeric analogs with antagonist activity were also examined. The dimeric derivative of the antagonist *N*,*N*-diallyl Leu-enkephalin (223) is approximately ninefold more potent than the monomeric *N*,*N*-diallyl-Tyr-Gly-Gly-Phe-LeuNHEt (752), but the antagonist activity of the truncated dimer *N*,*N*-diallyl-Tyr-Gly-Gly-Phe-Leu-NHCH$_2$CH$_2$NH(AcPhe-Leu) suggests that the enhanced activity of the full dimer is not due to bridging two 6-receptor binding sites.

6.3 Dynorphin Analogs

Dynorphin has not been nearly as well studied as other smaller opioid peptides. Although several peptides with high affinity for κ receptors are obtained from prodynorphin (see Section 3.4 above), SAR studies have focused on derivatives of dynorphin A (see Refs. 656, 753 for reviews). Dynorphin A is a heptadecapeptide, but dynorphin A-(1–13) accounts for essentially all of the activity of the larger peptide (754). Further truncation of dynorphin A-(1–13) from the C-terminus identified the basic residues Arg^7 and Lys^{11} as important for κ receptor potency and selectivity (263). Thus, typically, dynorphin A-(1–13) or A-(1–11) has been used as the parent peptide for further modification.

The possible conformations of dynorphin A have been studied by a variety of spectral techniques (see Ref. 753 for a review). Like other linear peptides, a variety of conformations have been observed for dynorphin A, which depend on the experimental conditions. Schwyzer (755) proposed a "membrane-assisted" model for dynorphin's interaction with κ receptors, in which the N-terminal "message" sequence adopts an a-helical structure from residues 1–9 when it binds to the receptor. NMR studies of dynorphin A bound to dodecylphosphocholine micelles (756, 757) observed a helical structure in the N-terminal portion of the peptide, supporting this proposal. This helical structure of dynorphin A has been docked to a computational model of κ opioid receptors (181).

Studies of dynorphin A have been complicated by its metabolic lability (758). In addition to inactivation by peptidase cleavage in the N-terminus, cleavage in the C-terminus yields shorter active peptides, which may have different receptor selectivity profiles from that of the parent peptide. Dynorphin A-(1–8), which is the predominant form of dynorphin A present in rat brain (759,7601,is less selective for κ receptors than the longer peptides (see Ref. 761). C-Terminal amidation enhances the metabolic stability of dynorphin A-(1–13) (758), and therefore this modification is typically incorporated into dynorphin A analogs. The peptide bonds in dynorphin A-(1–11)NH$_2$ have been replaced by reduced amide bonds to increase metabolic stability (762, 763); this modification was well tolerated by κ receptors in the C-terminal "address" sequence, but led to decreased opioid receptor affinity when incorporated in the "message" sequence. An analog of dynorphin A - 1 8 E2078 ([NMeTyr^1,NMeArg^7,D-Leu^8]dynorphin A-(1–8)NHEt) (764, 765), containing modifications to stabilize the peptide to metabolism has been studied extensively in *vivo*. It exhibits a slight preference for κ receptors in binding assays (see Table 7.17) and produces analgesia after both i.v. and s.c. administration (764–766), apparently by spinal κ receptors (767).

E2078 (**224**)

MeTyr-Gly-Gly-Phe-Leu-Arg-NMeArg-D-LeuNHEt

6.3.1 Linear Analogs. Early structural modifications were made in the C-terminal "address" sequence of dynorphin A (263) and focused on the nonbasic residues (see Refs. 656, 753). D-Pro was incorporated in place of Pro^{10} in both dynorphin A-(1–11) (768) and A-(1-13) (769) and was reported to enhance κ-receptor selectivity (see Table 7.17). Ala and D-Ala were substituted for Ile^5 with retention of κ-receptor affinity and selectivity (769, 770). Replacement of the basic residues individually by N^ϵ-acetylated lysine (771) indicated that

Table 7.17 Opioid Receptor Affinities and Opioid Activity in the GPI and MVD of Analogs of Dynorphin A (Dyn A)

Peptide	K_i (nM)			K_i Ratio	IC_{50} (nM)		Reference(s)
	κ	μ	δ	$\kappa/\mu/\delta$	GPI	MVD	
Agonist analogs							
Dyn A-(1–13)	0.15	1.3	4.1	1/8.6/27	1.7	78	772
Dyn Ia (**225**)	0.25	6.7	71	1/26/280	0.5	236	773
[D-Pro^{10}]Dyn A-(1–11)	0.032	2.0	7.5	1/62/230	—	—	768
E2078 (**224**)	1.9	4.5	27.2	1/2.4/14	0.3	7.4	764
[*N*-Allyl,D-Pro^{10}]Dyn A-(1–11)	0.049	11	450	1/220/9,160	18	—	777, 801
[*N*-CPM,D-Pro^{10}]Dyn A-(1–11)	0.020	9.6	560	1/480/28,000	2.2	—	777, 801
[*N*-Benzyl,D-Pro^{10}]Dyn A-(1–11)	0.029	31	175	1/1070/6,080	990	—	777, 801
[Ala^3]Dyn A-(1–11)NH_2	1.1	210	730	1/190/660	1.7	—	779
[D-Ala^3]Dyn A-(1–11)NH_2	0.76	260	1,000	1/350/1,300	8.1	—	779
[(*R*)-Atc^4,D-Ala^8]Dyn A-(1–11)NH_2	0.89	33	>10,000	1/37/>6,000	—	—	782
[(*S*)-Atc^4,D-Ala^8]Dyn A-(1–11)NH_2	9.5	88	>10,000	1/9/>1,000	—	—	782
cyclo[Cys^5,Cys^{11}]Dyn A-(1–11)NH_2	0.28	0.27	1.6	1/1/6	1080	420	785
cyclo[Cys^5,D-Pen^{11}]Dyn A-(1–11)NH_2	1.1	31	240	1/28/220	690	—	787
cyclo[D-Asp^2,Dap^5]Dyn A-(1–13)NH_2	0.22	0.49	10	1/2/46	0.16	—	791
cyclo[D-Asp^5,Dap^8]Dyn A-(1–13)NH_2	8.0	75	3,300	1/9/400	>5000	—	792, 793
cyclo[D-Asp^6,Dap^9]Dyn A-(1–13)NH_2	2.6	4.4	48	1/2/19	46	—	792, 793
cyclo[D-Asp^3,Lys^7]Dyn A-(1–11)NH_2	4.9	310	130	1/64/27	600	—	794
Antagonist analogs							
[*N*,*N*-Diallyl,D-Pro^{10}]Dyn A-(1–11)	21	135	350	1/6.5/17	190[a]	—	799
[*N*,*N*-DiCPM,D-Pro^{10}]Dyn A-(1–11)	0.19	3.9	166	1/21/880	—[b]	—	801
Dynantin	0.82	213	163	1/260/200	3.9, 0.63[c]	—	802
[Pro^3]Dyn A-(1–11)NH_2	2.7	5,700	8,800	1/2,100/3,260	244, 494[c]	—	781
Arodyn	10	1,700	5,800	1/170/580	—[b]	—	806
JVA-901 (venorphin)	20	250	5,300	1/13/270	—[b]	—	808

[a] K_e values against a dynorphin analog.
[b] Reverses the agonist activity of Dyn A-(1–13)NH_2 in an adenylyl cyclase assay using cloned κ receptors.
[c] K_e values against U50,488 and a dynorphin analog, respectively.

substitution of Arg^6 by a nonbasic residue is well tolerated, and that the basicity of Arg^6 is not required for interaction with κ receptors, but contributes to κ-receptor selectivity by decreasing preceptor affinity. Analogs in which Arg^6 or Arg^7 was replaced by norleucine also retained high κ-receptor affinity, but exhibited decreased κ selectivity (772). A C-terminal extended peptide dynorphin A-(1–13)-Tyr-Leu-Phe-Asn-Gly-Pro (dynorphin Ia, **225**), based on the structure of a dynorphin-related peptide purified from bovine adrenal medulla, was synthesized (773) and found to be more selective than dynorphin A-(1–13) for κ receptors; additional structural modifications (D-Leu^5 and/or N-methylation of Tyr^1) were

made to reduce the motor effects observed with dynorphin Ia (774). Labeled derivatives of dynorphin A were prepared by attaching functionalities to the C-terminus [DAKLI, [Arg^{11,13}]dynorphin A-(1–13)- Gly-NH $(CH_2)_5NH$-R, where R is fluorescein, ^{125}I-labeled Bolton Hunter reagent, or biotin (775)] or to the side chain of Lys^{13} (biotin) in dynorphin A-(1–13) amide (776).

Dynorphin Ia (225)

Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Tyr-Leu-Phe-Am-Gly-Pro

Several studies have focused on modifications in the N-terminal sequence of dynorphin A. Interestingly, the most κ-selective derivatives of dynorphin A have been prepared by modifications in this "message" sequence rather than in the C-terminal "address" sequence. The N-terminal monoalkylation of [D-Pro^{10}]dynorphin A-(1–11) with an allyl, cyclopropylmethyl, or benzyl group results in marked enhancement in κ-receptor affinity by decreasing preceptor affinity (Table 7.17) (777); *N,N*-dialkylation results in analogs with antagonist activity (see below). An alanine scan (770) verified the importance of Tyr^1 and Phe^4 in dynorphin A-(1–13) for potency in smooth muscle assays and receptor affinity. Although incorporation of D-amino acids in position 2 can enhance metabolic stability and yields analogs with high affinity for κ receptors, this modification significantly increases preceptor affinity and thus yields μ-selective analogs (263,778). In contrast, substitution of either Ala or D-Ala in position 3 of dynorphin A-(1–11)NH_2 markedly enhances κ-receptor selectivity (Table 7.17) (779); incorporation of other amino acids in this position, however, is generally less well tolerated (780). Recently, [Pro^3] dynorphin A-(1–11)NH_2 was reported to be a highly selective κ-receptor antagonist (see below) (781).

6.3.2 Conformationally Constrained Analogs. Local constraints have been incorporated into dynorphin A. The conformationally constrained phenylalanine derivative Atc (2-aminotetralin-2-carboxylicacid, Fig. 7.43) has been incorporated in [D-Ala^8]dynorphin A-(1–11)NH_2 (782). Interestingly, the peptide containing (??)-Atc, which corresponds to a conformationally constrained D-Phe analog, possesses higher affinity for κ and preceptors than the peptide containing the S isomer (Table 7.17), even though the D-Phe^4 analog exhibits relatively low affinity for these receptors (K_i values of 8.9 and 146 nM, respectively). Both peptides exhibit negligible affinity for δ receptors. A novel conformational constraint, 4-aminocyclohexanecarboxylic acid, has been incorporated into dynorphin A-(1–13) amide in place of Gly^2-Gly^3 (783). Although the affinities of the two peptides for κ receptors is significantly reduced compared to the parent peptide, it is interesting to note that the peptides containing the cis and trans isomers of 4-aminocyclohexanecarboxylicacid exhibit similar affinity for κ receptors.

Cyclic derivatives of dynorphin A have been prepared by formation of both disulfide and lactam linkages (see Ref. 656 for a review including extensive tables). The first cyclic dynorphin analog reported was *cyclo*[D-Cys^2,Cys^5]dynorphin A-(1–13) (784), which is a more potent agonist in smooth muscle assays than dynorphin A-(1–13). Subsequently, a variety of cyclic dynorphin analogs containing a disulfide linkage in the C-terminus were described (785–787; see Ref. 656 for a tabular summary of these analogs). Except for the analogs cyclized through a D-amino acid in position 5, these peptides with a constraint in the C-terminus retain high affinity for κ receptors in radioligand binding assays. Three analogs cyclized between positions 5 and 11 (*cyclo*-[Cys^5,D-Pen^{11}]-, *cyclo*[Pen$^{5.11}$]-, and *cyclo*-[Pen^5,D-Pen^{11}]dynorphin A-(1–11)NH_2) show increased κ-receptor selectivity in the radioligand-binding assays compared to that of the linear parent peptide (Table 7.17). Interestingly, a number of these cyclic disulfide-containing peptides, particularly those in which position 5 is involved in the disulfide linkage, show much lower potency in the GPI than expected from the binding assays; these discrepancies between the two assays were postulated to be due to different receptor subtypes present in the brain versus peripheral tissues (785–787). A conformational search of *cyclo*[Cys^5,Cys^{11}]- and *cyclo*[Cys^5,D-Ala^8,Cys^{11}]-dynorphin A-(1–11)NH_2 found a low energy

Dynantin	(2*S*)-Mdp-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-LysNH_2
TIPP-Dyn A	**Tyr-Tic-Phe-Phe-Leu-Arg-Arg-Ile-Arg-Pro-LysNH_2**
Extacet	AcArg-Phe-Met-Trp-Met-Arg-Arg-D-Ala-Arg-Pro-LysNH_2
Arodyn	AcPhe-Phe-Phe-Arg-Leu-Arg-Arg-D-Ala-Arg-Pro-LysNH_2
JVA-901 / venorphin	AcTyr-Lys-Trp-Trp-Leu-Arg-Arg-D-Ala-Arg-Pro-LysNH_2

Figure 7.45. Novel antagonist analogs of dynorphin A.

a-helical conformation for the disulfide-bridged ring in these peptides, suggesting that this conformation may be important for the potency of these peptides at central κ receptors (788). When *cyclo*[Cys^5,Cys^{11}]dynorphin A-(1–11)NH_2 bound to micelles was examined by NMR and molecular dynamics, conformations resulting from *cis-trans* isomerism around the Arg^9-Pro^{10} amide bond were observed (789), with the *trans* isoform exhibiting *a* β turn from residues Cys^5 to Ile^8, whereas the *cis* isoform contained a type III β turn from residues Arg^7 to Pro^{10}.

Conformational constraint through lactam formation has been examined in both the N-terminus and C-terminus of dynorphin A (790–794). The initially reported derivatives exhibited low K_e values for antagonism by naloxone (K_e = 1.5–4.5 n*M*) in the GPI, suggesting that these peptides preferentially interacted with μ receptors (affinities for κ receptors were not reported) (790). More recently, two series of dynorphin A-(1–13)amide analogs constrained through a lactam linkage between residues in either the *i* and *i* + 3 positions (791–793) or *i* and *i* + 4 positions (794) have been reported (Table 7.17). These constraints were chosen to be compatible with the helical conformation proposed by Schwyzer as the bioactive conformation for the *N*-terminal sequence of dynorphin A (755). For derivatives constrained between a D-Asp in position *i* and Dap (α,β-diaminopropionic acid) in position *i* + 3, the constraint is well tolerated by κ receptors between positions 2 and 5 (791) and also between positions 6 and 9 (792, 793), whereas moving the constraint to residues 3 and 6 markedly decreases affinity for opioid receptors (792, 793). Consistent with the results for the cyclic disulfide-containing analogs cyclized through position 5, *cyclo*[D-Asp^5,Dap^8]dynorphin A-(1–13)NH_2 is a weak agonist in the GPI, while retaining reasonable affinity for κ receptors in the radioligand-binding assays (792, 793). For the i to *i* + 4 derivatives (794) *cyclo*[D-Asp^3,Lys^7]dynorphin A-(1–11)NH_2 exhibited the highest κ-receptor affinity and selectivity (Table 7.17); the cyclic peptide *cyclo*[Lys^5,D-Asp^9]dynorphin A-(1–11)NH_2 also exhibited nanomolar affinity for both κ and μ receptors. The synthesis of novel *N*-terminal to side-chain cyclic dynorphin A analogs was recently reported (795,796).

6.3.3 Dynorphin A Analogs with Antagonist Activity. Early attempts to prepare κ-selective antagonists by modification of dynorphin A met with limited success, and the analogs reported generally exhibited weak antagonist activity, residual agonist activity, and/or low selectivity for κ receptors (797,798). In recent years antagonist analogs with improved pharmacological profiles have been identified. Modifications of Tyr^1 have resulted in dynorphin A analogs with antagonist activity. Incorporation of Phe in place of Tyr^1 in [D-Ala^8]dynorphin A-(1–11)NH_2 resulted in a peptide that antagonized dynorphin A (K_e = 30–65 n*M*) in the GPI, but still exhibited weak agonist activity (772). The *N*,*N*-terminal dialkylation of dynorphin A fragments with either allyl or cyclopropylmethyl (CPM) groups resulted in analogs with antagonist activity (142, 799–801); the *N*,*N*-di(cyclopropylmethyl) derivative exhibits higher κ-receptor selectivity than that of the NJV-diallyl peptide (142, 801). Recently, novel analogs of 2′,6′-dimethyltyrosine, which lack a basic amine (Dhp and Mdp, Fig. 7.43), have been incorporated in place of Tyr^1 in dynorphin A-(1–11)NH_2, resulting in [(2*S*)-Mdp^1]dynorphin A-(1–11)NH_2 (dynantin, Fig. 7.45), which is a highly κ-selective peptide that is a potent antagonist at κ opioid receptors (see Table 7.17)

(802). [Pro^3] dynorphin A-(1–11)NH_2 was also reported to be a highly selective, but relatively weak, κ-receptor antagonist (see Table 7.17) (781).

Chimeric dynorphin A analogs, in which the N-terminal "message" sequence of dynorphin A is replaced by small peptides with opioid antagonist activity (Fig. 7.45), have also been prepared as potential antagonists. Addition of the C-terminal "address" sequence in these peptides significantly enhances κ-receptor affinity, but the receptor preference of the N-terminal sequence still often predominates. Thus incorporation of the δ-receptor antagonist TIPP (Tyr-Tic-Phe-Phe) in place of the *N*-terminal sequence of [D-Pro^{10}]dynorphin A-(1–11)NH_2 or the corresponding acid resulted in peptides with affinity and antagonist activity at κ receptors, but which still preferentially bind to 6 receptors (803, 804). Incorporation of only Tic^2 in position 2 produced similar results for the dynorphin A-(1–11)NH_2 derivative (805) or a slight (twofold) preference for 6 over κ receptors for the [D-Pro^{10}]dynorphin A-(1–11) analog (804); incorporation of N-MePhe in position 2 of [D-Pro^{10}]dynorphin A-(1–11) resulted in a greater (eightfold) preference for κ over 6 receptors but little selectivity for κ over ρ receptors (804).

Attachment of the C-terminal "address" sequence from [D-Ala^8]dynorphin A-(1–11)NH_2 to the acetylated hexapeptide [Arg^6]acetalin, which is a preceptor antagonist, to give the peptide extacet increases κ-receptor affinity 65-fold, resulting in nanomolar affinity for κ receptors (K_i = 6.6 n*M*), but it still preferentially binds to preceptors (K_i = 1.1 n*M*) (804). Examination of analogs of this lead peptide by use of a combinatorial library led to the identification of arodyn, a novel dynorphin A analog with higher affinity, much higher selectivity, and antagonist activity at cloned κ receptors (see Table 7.17) (806).

A Boc-protected tetrapeptide derived from a sequence found in Philippine cobra venom has been reported to have weak antagonist activity at κ receptors (807). Attachment of the C-terminal "address" sequence of [D-Ala^8]-dynorphin A-(1–11)NH_2 to this sequence resulted in a novel acetylated dynorphin A analog, JVA-901 (now referred to as venorphin), which exhibits antagonist activity and greatly enhanced affinity for cloned κ receptors (808). In spite of the structural similarities to dynorphin A, the SAR of venorphin is completely different from that of dynorphin A, suggesting that these two peptides interact with κ receptors in different ways (809,810). In venorphin only Trp^3, and not Tyr^1 or a basic functionality, is required in the N-terminal "message" sequence for high affinity for κ receptors (809), and Arg^9, which is not important for the κ-receptor affinity of dynorphin A, is the most critical residue in the C-terminus (810).

6.4 Opioid Peptides with the Tyr-Pro-Phe Sequence

6.4.1 β-Casomorphin Analogs and the Endomorphins. β-Casomorphin was identified over 20 years ago, so a number of analogs of this peptide have been reported. The heptapeptide β-casomorphin (210), although exhibiting some selectivity for μ receptors, is a weak opioid agonist [IC_{50} in the GPI = 57 μ*M* (811)]. Shortening the peptide to the pentapeptide and tetrapeptide increases potency (811, 812), so generally analogs have been prepared of one of these smaller fragments. Conversion of the tetrapeptide to the C-terminal amide to give morphiceptin (226; see Table 7.18) (812) substantially increases both potency and p-receptor selectivity. Further modification of morphiceptin by incorporation of D-Pro at position 4 and N-methylation at position 3 yields PL017 (227) (813), which is significantly more potent than morphiceptin and more μ selective (see Table 7.18). D-Pro or D-Pip (pipecolic acid) in position 4 of β-casomorphin-(1–5) also enhances potency in the GPI (814).

Morphiceptin (226) Tyr-Pro-Phe-ProNH_2

PL017 (227) Tyr-Pro-NMePhe-D-ProNH_2

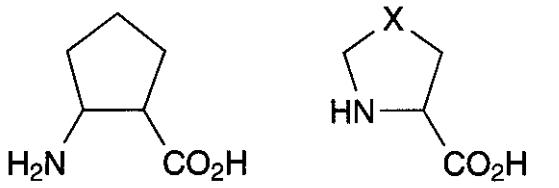
Cis/trans isomerization occurs around amide bonds involving the nitrogen of proline and other N-alkyl amino acid residues. NMR studies of both morphiceptin and PL017 found that, whereas the major isomers were the all-*trans* conformers, the second most common isomer (25%) had a *cis* arnide bond between

Table 7.18 Opioid Receptor Affinities and Opioid Activity in the GPI and MVD of μ-Selective Opioid Peptide Analogs

Peptide	K_i (nM) μ	K_i (nM) δ	K_i Ratio δ/μ	IC_{50} (nM) GPI	IC_{50} (nM) MVD	Reference
β-Casomorphin analogs						
Morphiceptin (**226**)	63	30,000	475	318	4800	813
PL017 (**227**)	11	7,250	660	34	240	130
Tyr-*cyclo*[D-Orn-Phe-D-Pro-Gly] (**232**)	0.88	13.2	15	2.1	4.9	825
Tyr-*cyclo*[D-Orn-2-Nal-D-Pro-Glyl (**233**)	5.9	17.2	2.9	384	K_e = 200–270''	825
Tyr-*cyclo*[D-Orn-2-Nal-D-Pro-D-Ala]	0.76	72	95	600	K_e = 5.4–6.0[a]	828
Dmt-*cyclo*[D-Orn-2-Nal-D-Pro-Gly] (**234**)	0.46	0.46	1	7.9	K_e = 2.1–3.3[a]	830
CHO-Dmt-*cyclo*[D-Orn-2-Nal-D-Pro-Glyl	218	33	0.15	K_e = 216[a]	K_e = 16''	829
Dermorphin analogs						
TAPP (Tyr-D-Ala-Phe-PheNH_2)	1.5	625	409	255	780	831
DALDA (**242**)	1.7	19,200	11,400	3.23	800	831
[Dmt[1]]DALDA (**243**)[b]	0.14	2,100	14,700	1.4	23	832
Tyr-*cyclo*[D-Orn-Phe-Asp]NH_2 (**244**)	10.4	2,220	213	36.2	3880	833
Tyr-*cyclo*[D-Orn-Phe-Glu]NH_2	0.98	3.21	3.3	1.2	1.1	834
Tyr-*cyclo*[D-Orn-Aic-Glu]NH_2	4.21	209	50	7.21	36.5	834
JOM-6 (246)	0.29	24.8	86	—	—	835
JH-54 ([Phe[1]]JOM-6, 247)	1.36	1,020	750	9.1	—	835
[D-Hat[1]]JOM-6 (248)	0.39	58	150	—[c]	—[c]	836

"Antagonist.
[b]K_i (κ) = 22 nM.
[c]EC_{50} = 1.4 and 1500 nM in GTPγS assays using cloned μ and δ receptors, respectively

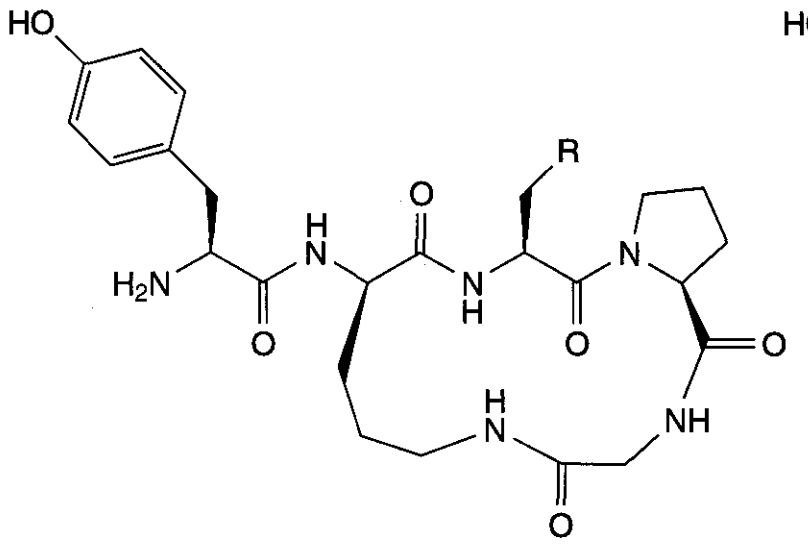
Tyr^1 and Pro^2 (815). Therefore, Goodman and coworkers incorporated 2-aminocyclopentane carboxylic acid (2-Ac^5c, 228, Fig. 7.46) into position 2 of morphiceptin analogs to eliminate possible *cis/trans* isomerization (816, 817). Of the four possible stereoisomers only morphiceptin analogs containing *cis*-(1*S*,2*R*)-2-Ac^5c, which exhibits a structure similar to the cis conformation of the Tyr-Pro amide bond, are potent opioids, whereas the analogs with *cis*-(1*R*,2*S*)-2-Ac^5c, which is similar to morphiceptin with the Tyr-Pro bond in a trans configuration, were inactive. This led Goodman and coworkers to propose that the cis conformation around the Tyr-Pro amide bond is required for the opioid activity of morphiceptin and its analogs (817). A more detailed model for the bioactive conformation of morphiceptin was developed based on the comparison of a series of active and inactive analogs (818).



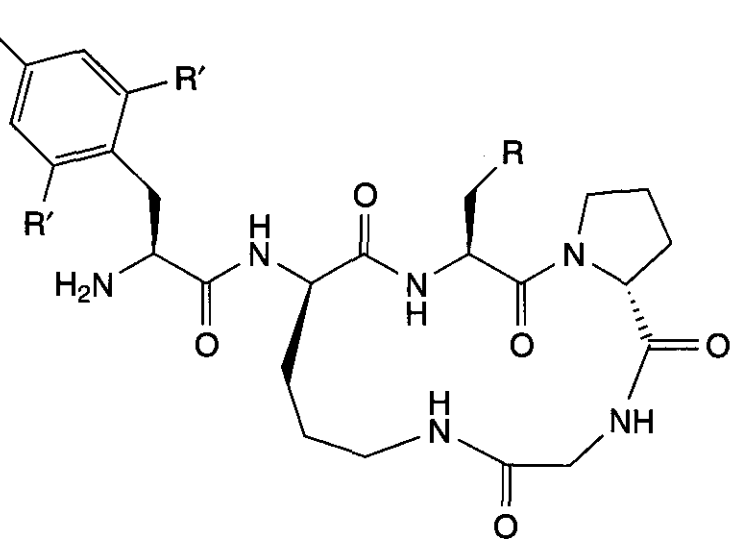
(**228**) 2-Ac^5c (**229**) X = S or O

Figure 7.46. Proline analogs.

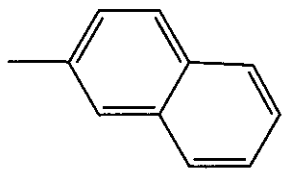
Like morphiceptin and PL017, *cis/trans* isomerization occurs around the Tyr-Pro amide bond in endomorphin-1, with similar populations (75%trans, 25%cis) found for the two conformations (819). Based on structural comparison of the cis and trans conformations of endomorphin-1 to other μ- and δ-selective opioid peptides, Podlogar et al. proposed that the trans conformation was the bioactive form (819). Recently, Schiller and coworkers reported endomorphin-2 and morphiceptin analogs containing a pseudoproline derivative in



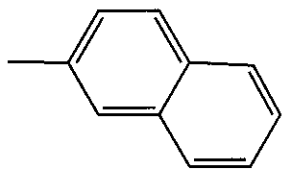
R = Ph
(**230**)



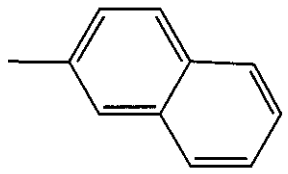
R′ = H, R = Ph
(**232**)

R = 

(**231**)

R′ = H, R = 

(**233**)

R′ = CH_3, R = 

(**234**)

Figure 7.47. Cyclic β-casomorphin analogs.

place of Pro^2 (820); analogs of both peptides containing the dimethylated pseudoprolines Xaa[$\Psi^{Me,Me}$Pro] (Xaa = Cys or Ser, 229, Fig. 7.46), which exist almost exclusively in the cis conformation, retain μ opioid receptor affinity and agonist activity, indicating that the cis conformation around the Tyr-Pro amide bond is the bioactive form. Pro has been proposed to function as a stereochemical spacer, and inversion of its stereochemistry in endomorphin-1 essentially abolishes agonist activity in the GPI (821). [D-Pro^2]endomorphin-2, however, was a much more potent analgesic than endomorphin-2 in the tail flick assay after i.c.v. administration; the analgesia produced by both peptides was reversed by naloxone (822).

Potent cyclic derivatives of β-casomorphin-(1–5) were prepared by cyclization between a D-amino acid in position 2 and the C-terminus. Tyr-*cyclo*[D-Orn-Phe-Pro-Gly] (230, Fig. 7.47) and Tyr-*cyclo*[D-Orn-Phe-D-Pro-Gly] (232) exhibit high affinity in μ receptor binding assays and are potent agonists in smooth muscle assays (Table 7.18) (823). Peptides Tyr-*cyclo*[D-X-Phe-D-Pro] (X = D-Orn or D-Lys) in which Gly^5 has been removed, resulting in a smaller ring size, exhibited potency in the GPI similar to that of the corresponding peptides with a larger ring, but decreased potency in the MVD (824). Substitution of Phe^3 with 2-naphthylalanine (2-Nal) in the D-Pro-containing peptides gave Tyr-*cyclo*[D-X-2-Nal-D-Pro-Gly] [X = D-Orn (233, Table 7.18) or D-Lys], which are agonists in the GPI but antagonists in the MVD (825), and thus are mixed μ agonists/δ antagonists. Examination of Tyr-*cyclo*[D-Orn-X-D-Pro-Gly] [X = Phe (232) or 2-Nal (**233**)] and Tyr-*cyclo*[D-Orn-2-Nal-Pro-Gly] (**231**) by NMR indicated that the overall backbone con-

Table 7.19 Opioid Receptor Affinities and Antagonist Activity in the GPI and MVD of TIPP and Selected Analogs[a]

Peptide	K_i (nM) 6	K_i (nM) μ	K_i Ratio μ/δ	K_e (nM) MVD	K_e (nM) GPI	Reference(s)
TIPP (30)	1.2	1,720	1,430	3.0–5.9	—	111
$TIPPNH_2$	3.0	79	26	**14-18**	IC_{50} = *1,700*[b]	111
Tyr-D-Tic-Phe-$PheNH_2$	7.3	520	71	IC_{50} = 454[b]	IC_{50} = 37[b]	111
TIPP[ψ] (**31**)	0.31	3,230	10,500	*2.1–2.9*	—	112
DIPP (**236**)	0.25	141	570	*0.15*	IC_{50} = *770'*	851
$DIPPNH_2$ (237)	0.12	1.2	10	*0.20*	IC_{50} = 18[b]	866
DIPP[ψ]-NH_2 (**239**)	0.45	0.94	2	*0.54*	IC_{50} = *7.7'*	866
TICP (Tyr-Tic-Cha-Phe)	0.61	3,600	5,900	*0.44*	—	847
TICP[ψ]	0.26	1,050	4,000	*0.22*	—	847
Dmt-TicOH	0.022	3,300	150,000	*5.7*	NA[c]	854
	1.6[d]	890[d]	560	—	—	857
(2*S*,3*R*)-Tmt-TicOH	9.3	35,000	3,800	1.8	—[e]	861
Dmt-D-$PheNH_2$	15	3.6	0.24	—	60–310	864
240[f]	9.35	0.22	0.023	—	—[f]	865
Tyr-Tic-$NHCH_2CHPh_2$	0.98	29	29	IC_{50} = 3.8[b]	IC_{50} = 3,600[b]	846, 866
Tyr-Tic-$NHCH_2CH(Ph)CO_2Et$	0.57	890	1,560	IC_{50} = 1.3[b]	—	846
N,*N*-Me_2-Dmt-Tic-NH-1-adamantane	0.16	1.1	9	0.87	IC_{50} = 16[b]	868
241[g]	0.17	62	360	—[g]	—[g]	858

[a]Data for TIPP and TIPP[ψ] from Table 7.8 are included for comparison. Data for a number of other analogs are given in Ref. 837.
[b]Agonist.
[c]Not active.
[d]IC_{50}.
[e]30% inhibition at 30 μM.
[f]K_i (κ) = **68** nM; EC_{50} = 0.18 nM for GTPγS binding at cloned preceptors.
[g]K_i (κ) = 1.3 nM; EC_{50} = 0.65 and 6.9 for GTPγS binding at cloned δ and κ receptors, respectively. Inactive at μ receptors.

formations and preferred side-chain conformations were roughly similar for these peptides (826). Interestingly, substitution of 1-naphthylalanine in position 3 of (**232**) yields a compound with full agonist activity in the MVD (825). Substitution with other bulky aromatic amino acids was generally well tolerated by δ receptors, but drastically decreased preceptor affinity (827). Replacement of Gly^5 in (233) with sarcosine or D-Ala significantly enhanced S-receptor affinity and antagonist potency (see Table 7.18) (828). Recently, derivatives of Dmt-*cyclo*[D-Orn-2-Nal-D-Pro-Gly] (234) lacking a basic N-terminus were reported (see Table 7.18); these peptides exhibit antagonist activity at both δ and μ receptors (829).

6.4.2 TIPP and Related Peptides. Exploration of a series of tetrapeptides consisting solely of aromatic residues led Schiller and coworkers to identify TIPP (Tyr-Tic-Phe-Phe, 30, Fig. 7.5) (111). TIPP is a potent δ antagonist in the MVD, with very high selectivity for S receptors (Table 7.19). [Recently, however, TIPP and its analog TIPP[Ψ] (31, Fig. 7.5, see below) were reported to exhibit agonist activity in adenylyl cyclase assays using cells containing both endogenous and transfected S opioid receptors (114).] The tripeptide derivatives TIP and TIP-NH_2 are also S-receptor antagonists in the MVD. In contrast, the tetrapeptide Tyr-D-Tic-Phe-Phe-NH_2 with D-Tic in position 2 is a potent agonist that is selective for μ receptors. The amide derivative of TIPP, in addition to 6-antagonist activity in the MVD, is a full agonist in the GPI (see Table 7.19), and was the first compound reported to be a mixed μ agonist/δ antagonist (111).

The conformations of TIPP were examined by molecular mechanics and X-ray crystallography (see Ref. 837 for a review). Examination

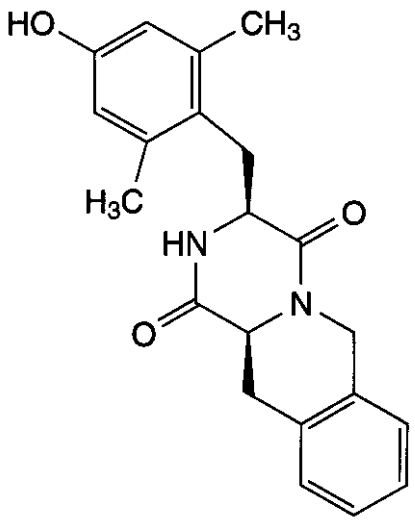
of the 6 antagonist TIP and the μ agonist Tyr-D-Tic-PheNH, by molecular mechanics found compact structures for both peptides, but with different patterns of aromatic ring stacking (838). Superimposition of low energy conformations of these two peptides found that the Phe^3 residues were on opposite sides of the plane defined by the Tic residue, providing a possible explanation for the differences in activity observed for the two peptides. Comparison of TIP to the nonpeptide 6 antagonist naltrindole found good spatial overlap of the N-terminal amine and aromatic rings in the peptide with the corresponding groups in the alkaloid. In this model the Tyr-Tic peptide bond is trans. In an alternative model based on the weak dipeptide 6 antagonist $Tyr\text{-}TicNH_2$ this bond is cis (839).

Further examination of the possible conformations of a series of TIPP analogs, including derivatives of TIPP[Ψ] (**31**), by molecular mechanics found low energy conformations consistent with the model containing a trans Tyr-Tic bond, but the model with a cis amide bond still remained plausible (840). Other modeling studies involving TIPP found conformations consistent with both the trans (841, 842) and the cis conformations (842). Therefore Schiller and coworkers prepared $Tyr\Psi[CH_2NH]$Tic-Phe-PheOH and $Tyr\Psi[CH_2NH]$MeTic-Phe-PheOH (MeTic = 3-methyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, Fig. 7.43) in which a cis amide bond between the first two residues is sterically forbidden (843). The δ-receptor affinity and antagonist activity of these peptides, although 20- to 40-fold lower than TIPP, is consistent with a trans conformation as the bioactive conformation for TIPP. Interestingly, these modifications substantially increased μ-receptor affinity, so that $Tyr\Psi[CH_2NH]$MeTic-Phe-PheOH was a nonselective antagonist at both μ and 6 receptors (< a two-fold difference in K_e values in the GPI and MVD).

During examination of TIPP by NMR in deuterated DMSO spontaneous degradation through diketopiperazine formation occurred (844). This led Schiller and coworkers to prepare TIPP[Ψ] (31, Fig. 7.5) and the tripeptide TIP[Ψ] (112) containing a reduced peptide bond between Tic^2 and Phe^3. These analogs exhibit increased 6-receptor antagonist potency in the MVD and higher δ-receptor affinity compared to the parent peptides and exceptional 6-receptor selectivity (see Table 7.19). This modification also enhances the metabolic stability of the peptides. Whereas the diketopiperazine of Tyr-Tic is inactive, the Dmt analog *cyclo*[Dmt-Tic] (235, Fig. 7.48) exhibits 6-receptor affinity (K_i = 9.6 nM) and weak antagonist activity (K_e = 3.98 μM) in the MVD (845).

A variety of structural modifications have been made to all positions of TIPP (see Refs. 837, 846 for recent reviews), and these can have a profound effect on the activity profile of these peptides. Numerous substitutions have been made in position 3, and the results indicate that an aromatic residue is not required in this position for 6 antagonist activity. Thus the cyclohexylalanine (Cha) analogs of both TIPP and TIPP[Ψ] (TICP and TICP[Ψ], respectively) are approximately 10-fold more potent as 6-receptor antagonists than the parent peptides (847) (see Table 7.19). Interestingly, incorporation of D-amino acids containing a β-methyl group can have a profound effect on the efficacy at 6 receptors (848,849). Thus, the (2*R*,3*R*)-β-$MePhe^3$ derivative of TIPPNH, is a 6 antagonist, whereas the (2*R*,3*S*)-β-$MePhe^3$ isomer is a 6 agonist, and incorporation of β-Me-D-Tic^2 derivatives in Tyr-D-Tic-Phe-$PheNH_2$ converts the peptide from an agonist to an antagonist. Iodination of Tyr^1 in TIPP converts the peptide from an antagonist to a 6-selective agonist (850); the $Tyr(3'\text{-}I)^1$ analogs of TIPP[Ψ], TICP, and TIP, however, are δ antagonists, thus illustrating the subtleties of the effects of modifications on efficacy at 6 receptors (837,846).

Substitution of Dmt in position 1 of TIPP and analogs yielded DIPP (Dmt-Tic-Phe-Phe, 236, Fig. 7.48) and related peptides. DIPP is an extremely potent 6 antagonist (Table 7.19) and like TIPPNH, is also a full agonist in the GPI (851, 852). As noted earlier the nonpeptide naltrindole can prevent the development of morphine tolerance and dependence in mice (225), and therefore compounds with mixed μ agonist/δ antagonist activity could have therapeutic potential. Thus, Schiller and coworkers also incorporated Dmt into position 1 of TIPPNH, and $TIPP[\Psi]NH_2$ to enhance μ agonist activity; the resulting $DIPP[\Psi]\text{-}NH_2$ (**239**) was the first compound with balanced μ



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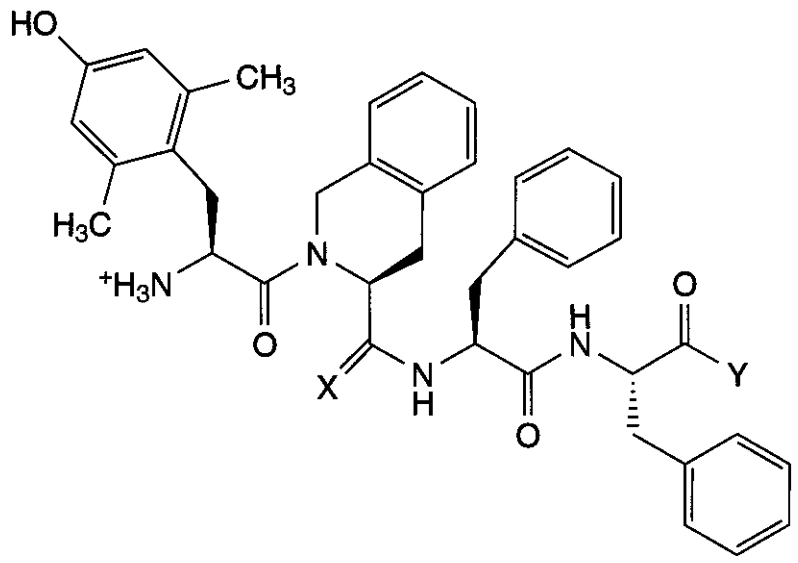
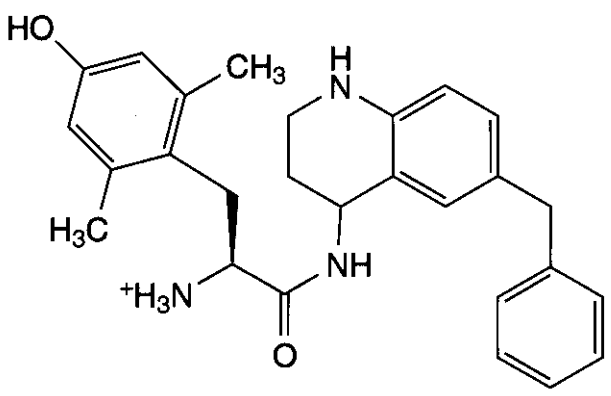
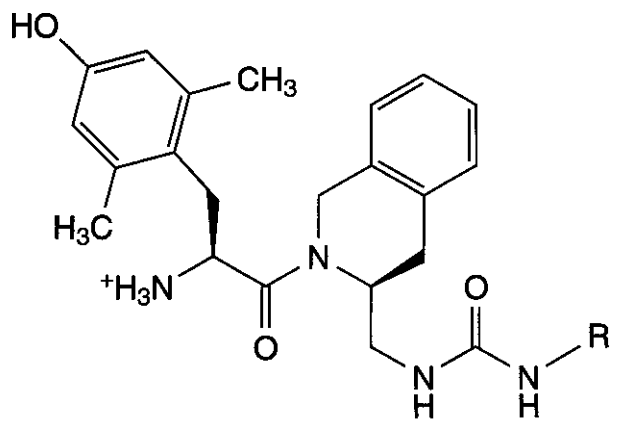


X = =O, Y = OH **(236)** DIPP
X = =O, Y = NH_2 **(237)** DIPPNH_2
X = H_2, Y = OH **(238)** DIPP[Ψ]
X = H_2, Y = NH_2 **(239)** DIPP[Ψ]NH_2



(240)



(241) R = *t*Bu

Figure 7.48. Analogs of Dmt-Tic and TIPP.

agonist/δ antagonist properties (229, 853). DIPP[Ψ]-NH_2 is a potent analgesic after i.c.v. administration (three times more potent than morphine) and produces less acute tolerance than morphine and no physical dependence with chronic administration (229). The tripeptide analogs Dmt-Tic-Ala-X (where X = OH or NH_2) also exhibit high affinity for 8 receptors and are potent antagonists in the MVD; the amide derivative retains appreciable μ-receptor affinity and is a weak agonist in the GPI (854). Replacement of Dmt^1 with *N*,2′,6′-trimethyltyrosine (NMeDmt) in DIPPNH_2 decreases efficacy at μ receptors, so that NMeDmt-Tic-Phe-PheNH_2 is a partial agonist and NMeDmt-TicΨ[CH_2NH]Phe-Phe-NH_2 is an antagonist in the GPI (229).

Based on the hypothesis that the "message" domain in these 8 antagonist peptides consisted of only the Tyr-Tic dipeptide rather than the tripeptide Tyr-Tic-Phe, Temussi and coworkers synthesized Tyr-L/D-Tic- NH, and Tyr-L/D-Tic-AlaNH_2 (839). Although they exhibited much lower potency than TIPP and TIP, the shorter peptides containing Tyr-L-Tic were δ-selective antagonists; peptides containing the Tyr-D-Tic sequence were nonselective agonists. Lazarus and coworkers explored the structure-activity relationships of Tyr-Tic in considerable detail (see Refs. 837,855,856 for reviews, including extensive tables of analogs). The dipeptide Dmt-TicOH was initially reported to have exceptionally high 8-receptor affinity and selectivity (854), although other researchers subsequently reported lower values (857, 858) (see Table 7.19). Modifications examined have included N-terminal alkylation, which enhances 8-receptor antagonism

(859). Thus, *N*,*N*-Me_2-Dmt-TicOH and *N*,*N*-Me_2-Dmt-Tic-AlaOH exhibit enhanced 6 antagonist potency in the MVD (K_e = 0.2–0.3 n*M*) (859); moreover, these derivatives cannot undergo diketopiperazine formation. In the GTPγS assay, *N*,*N*-Me_2-Dmt-TicNH$_2$ is a full inverse agonist, whereas Dmt-TicOH is a partial inverse agonist and Dmt-TicNH$_2$ is a neutral antagonist (860). (2*S*,3*R*)-Tmt-TicOH, with a β-methyl group added to Dmt (861), is also a full inverse agonist in this assay (862). Derivatives of Dmt-Tic with substitutions on Tic[2], with or without a C-terminal acid functionality, have also recently been reported (857, 863). Interestingly, substitution of Tic by D-Phe to give Dmt-D-PheNH$_2$ resulted in a p-receptor antagonist (although its selectivity was relatively low) (864), whereas substitution of Tic by 6-benzyl-1,2,3,4-tetrahydroquinoline-4-amine to give (240) resulted in a p-selective agonist (Table 7.19) (865).

The C-terminal extension of Tyr-Tic and Dmt-Tic can alter efficacy at 6 receptors, depending on the substitution. Thus the efficacy of the dipeptide amides Tyr-Tic-NH(CH$_2$)$_n$Ph depends on the length of the amide chain (antagonist for n = 1 or 3, agonist for n = 2) (846). In Tyr-Tic-NH(CH$_2$)$_2$Ph substitution on the phenyl ring by p-fluorine or chlorine or replacement of this phenyl ring by a cylohexyl ring converts the peptide from an agonist back to an antagonist (846). In contrast *ortho* substitution on the phenyl ring with chlorine enhances 6 agonist potency 10-fold (846, 866). Introduction of a second phenyl group and N-terminal methylation also enhance agonist potency, so that NMeTyr-Tic-NHCH$_2$CHPh$_2$ is an extremely potent 6 agonist (Table 7.19). Substitution of the β carbon of the phenethyl group in Tyr-Tic-NH(CH$_2$)$_2$Ph with CO_2Et results in the most 6 selective agonist within this class (Table 7.19) (846). Removal of the C-terminal acid functionality from Tyr-Tic-AtcOH converts the peptide from an antagonist to an agonist (846).

The C-terminal extension of Tyr-Tic and Dmt-Tic can also enhance p-receptor affinity. The dipeptide derivative Dmt-Tic- NH(CH$_2$)$_3$Ph is a 6-receptor antagonist and μ-receptor agonist (867). Interestingly, Dmt-Tic-NH-1-adamantane is a weak agonist at 6 receptors and potent agonist at **p** receptors, although its affinity for the two receptors is similar (868). *N*,*N*-Me_2-Dmt-Tic-NH-1-adamantane is both a potent δ-receptor antagonist and p-receptor agonist (Table 7.19) (868). Interestingly, modification of the C-terminus of Dmt-Tic to yield amine, urea, and thiourea derivatives (e.g., 241) results in compounds with nanomolar affinity for κ as well as μ "and 6 receptors, and agonist activity at all three receptor types (858).

6.5 Opioid Peptides with the Tyr-D-aa-Phe Sequence

6.5.1 Dermorphin Analogs and Related μ-Selective Peptides

6.5.1.1 Linear Analogs. As indicated earlier (Section 6.1.1), the amphibian heptapeptide dermorphin is a potent p-selective peptide, and thus it has served as the lead compound for structural modification (see Ref. 663 for a review). Dermorphin contains a C-terminal amide, and deamidation of dermorphin or its shorter fragments to the corresponding acids decreases affinity for μ receptors and potency in the GPI. Examination of shorter fragments indicated that the *N*-terminal tetrapeptide Tyr-D-Ala-Phe-Gly-NH$_2$ maintains significant opioid activity, and therefore a variety of tetrapeptide amide derivatives have been prepared (see below).. The further truncated tripeptide amide Tyr-D-Ala-PheNH$_2$ also retains significant opioid activity (25% of the potency of Met-enkephalin) (869), and could be further simplified by removing the C-terminal amide to give dipeptide aryl amides with maintenance of opioid activity. Tyr-D-Ala phenylpropylamide (DAPPA) was further modified by incorporation of Dmt in position 1 to give SC-39566 (870), which is orally active (871).

Extension of dermorphin with residues from the precursor sequence also yields potent p-selective peptides. Although introduction of the additional residues through the Glu[9] or Ala[10] residues decreases p-receptor affinity, apparently because of introduction of the acidic Glu[9] residue, further extension of the sequence increases p-receptor affinity as basic residues are incorporated (872); the resulting pentadecapeptide (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-Gly-Glu-Ala-Lys-Lys-Ile-Lys-Arg-NH$_2$) has exceptional affinity for μ receptors

(K_i = 2.0 pM), but this C-terminal extension does not enhance potency in the GPI. Dimeric derivatives of dermorphin fragments have been prepared by bridging two monomers with hydrazine or diamines of various lengths (873). Di-dermorphin, [(dermorphin-NH-)$_2$], in which two dermorphin molecules are linked by hydrazine, exhibits fivefold higher affinity for preceptors and similar p-receptor selectivity as dermorphin. Di-tetra-dermorphin, [(Tyr-D-Ala-Phe-Gly-NH-CH$_2$)$_2$] and other dimeric derivatives exhibit greater affinity for 6 receptors, and hence decreased p-receptor selectivity compared to that of the monomer.

Glycosylated derivatives of dermorphin were prepared by attaching β-D-glucose to the hydroxyl of a Ser or Thr, or galactose through a C-α linkage to Ala in position 7 (874–876). Although glycosylation decreased p-receptor affinity twofold, the penetration of the blood-brain barrier was significantly higher for the glycosylated derivatives and they exhibited twice the antinociceptive activity of dermorphin (875, 876). The enhanced BBB penetration by the C-α-galactoside analog suggested that the glucose transporter was not involved in the transport (875).

Amino acid substitutions have been examined in every position of dermorphin (see Ref. 663 for a review). An alanine scan of the peptide indicated that substitutions in positions 4, 6, and 7 are well tolerated, whereas substitution particularly in positions 1 or 2, but also in positions 3 or 5, results in large decreases in potency in the GPI (877). A D-amino acid in position 2 is important for activity, and the L-Ala^2 peptide is virtually inactive (<0.1% the potency of dermorphin). Tetrapeptide analogs containing D-methionine sulfoxide in position 2 are also potent p-selective agonists (878).

Substitutions for Gly^4 are well tolerated, particularly in the tetrapeptide derivatives. Sarcosine (NMeGly, Sar) at position 4 in tetrapeptide derivatives enhances opioid activity in antinociceptive assays (879). Substitution of Phe in position 4 of the tetrapeptide amide yields the dermorphin/enkephalin hybrid TAPP (Tyr-D-Ala-Phe-PheNH$_2$) (831), which is a potent p-selective agonist (see Table 7.18). This peptide can also be considered an analog of endomorphin-2, although TAPP was synthesized several years before the discovery of the endomorphins (264). TAPP analogs in which either Phe^3 or Phe^4 was nitrated were prepared to examine whether the Phe^3 aromatic ring of dermorphin interacts with a different subsite on opioid receptors than the Phe^4 aromatic ring of the enkephalins. Examination of Phe(p-NO$_2$)-containing analogs of dermorphin, morphiceptin, and various enkephalin analogs found that p-nitro substitution in Phe^3 of dermorphin and morphiceptin causes large decreases in potency in the GPI, whereas this substitution in Phe^4 of p- and δ-selective enkephalin analogs enhances potency in smooth muscle assays (880). Incorporation of Phe(p-NO$_2$) in position 3 of TAPP decreases receptor affinity, whereas incorporation in position 4 increases affinity, consistent with the concept of two distinct receptor subsites for these aromatic residues on opioid receptors (881). Bulky aromatic amino acids such as tryptophan or naphthylalanine are well tolerated in positions 3 and 4 of TAPP and yield even more lipophilic peptides (881).

Incorporation of D-Arg in position 2 of dermorphin and tetrapeptide analogs yields peptides that are potent opioids in antinociceptive assays in mice (879,882). The tetrapeptide derivative Tyr-D-Arg-Phe-Sar (TAPS) is a potent opioid in antinociceptive assays and causes respiratory stimulation, rather than respiratory depression, that is antagonized by naloxonazine (883); TAPS also antagonizes the respiratory depression caused by dermorphin. On the basis of these results TAPS has been postulated to be a μ_1 agonist and a μ_2 antagonist *in vivo* (883). In contrast, incorporation of L-Tic in position 2 of dermorphin converts the peptide to a δ-receptor antagonist (884).

Schiller and coworkers postulated that positively charged ligands should display μ-receptor selectivity (831) on the basis of Schwyzer's proposal that μ receptors are located in an anionic membrane compartment (885). Consistent with this concept they found that incorporation of a positively charged residue in position 4 of tetrapeptide dermorphin derivatives enhances p-receptor selectivity by decreasing affinity for 6 receptors (831). The combination of a D-Arg in position 2 with a second basic residue in position 4 yielded the polar peptide Tyr-D-Arg-Phe-LysNH$_2$ (DALDA, 242), which carries a net charge of +3 (831) and exhibits exceptional p-receptor

selectivity in binding assays (see Table 7.18). Quaternization of the side chain amine of Lys^4 in DALDA and related analogs is well tolerated, and the resulting analogs retain potent in *vivo* antinociceptive activity in the mouse writhing assay after s.c. administration (886). The antinoceptive effects of DALDA and the quaternary derivatives are substantially reduced by the quaternized antagonist *N*-methyllevallorphan, suggesting that these peptide analogs have a high degree of peripheral antinociceptive activity in this assay. The distribution of DALDA to the CNS is limited (887), and the antinociceptive activity of DALDA in the hot plate test after s.c. administration is low (888). The in vivo distribution of DALDA has been examined in pregnant sheep (889), and DALDA was not detected in any of the fetal plasma samples. This is in contrast to meperidine and morphine, which undergo rapid transfer across the placenta to the fetus, and suggested that DALDA could be a promising opioid for obstetrical use.

Replacement of Tyr^1 by Dmt results in a peptide [Dmt^1]DALDA (243) (832) with 10-fold higher affinities for both μ and S receptors and 200-fold higher affinity for κ receptors (890). [Dmt^1]DALDA is 220 and 3,000 times more potent than DALDA and morphine in the rat tail flick test after i.t. administration. [Dmt^1]DALDA also inhibits norepinephrine uptake in spinal cord synaptosomes, and this dual action may contribute to its antinociceptive potency. Both [Dmt^1]DALDA and DALDA exhibit longer durations of antinociceptive action than morphine after i.t. administration to rats (7 and 13 h, respectively, versus 3 h) (890). In sheep these peptides have longer elimination half-lives (1.5 and 1.8 h, respectively) than that of either morphine (20–30 min) or the much more hydrophobic p-selective peptide DAMGO (15 min) (891). In contrast to morphine and DALDA, [Dmt^1]-DALDA did not cause respiratory depression at the doses examined (832), suggesting that it could be a drug candidate for intrathecal analgesia.

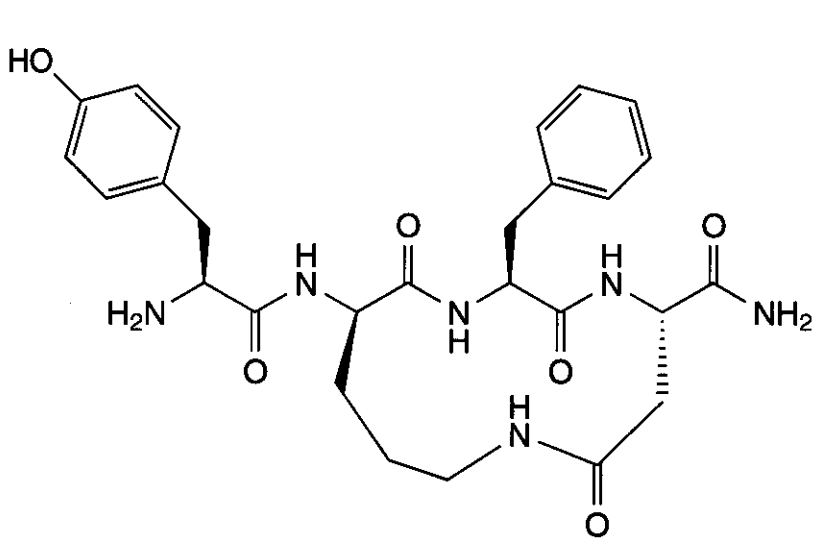
X-D-Arg-Phe-$LysNH_2$

DALDA (242)	X = Tyr
[Dmt^1]DALDA (243)	X = Dmt

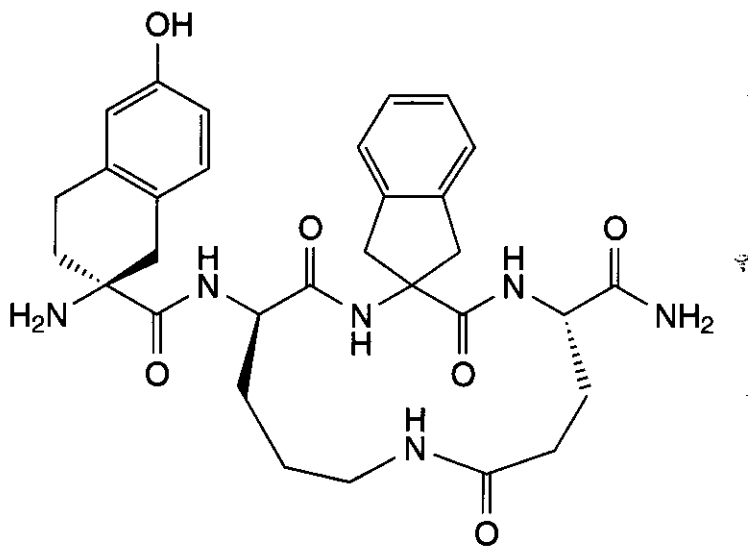
6.5.1.2 Conformationally Constrained Analogs. Dermorphin analogs containing a local constraint were prepared by incorporation of a dipeptide mimetic in place of Phe^3-Gly^4. The Aba^3-Gly^4 (see Fig. 7.43) analog of dermorphin retains high affinity at μ receptors and potency in the GPI, whereas this structural modification increases S-receptor affinity and potency in the MVD 17- to 25-fold (892). A number of heterocycles are also tolerated as bond replacements for the Phe^3-Gly^4 peptide bond (893).

Cyclic tetrapeptide analogs of dermorphin with the structure Tyr-*cyclo*[D-X-Phe-Y]NH_2 were prepared by Schiller and coworkers. The 13-membered ring cyclic peptide Tyr-*cyclo*[D-Orn-Phe-Asp]NH_2 (244, Fig. 7.49) exhibits high selectivity for μ receptors (see Table 7.18), whereas the more flexible peptide Tyr-*cyclo*[D-Lys-Phe-Glu]NH_2 with a 15-membered ring is nonselective (833). Peptides Tyr-*cyclo*[D-Asp-Phe-Orn]NH_2 (894) and Tyr-*cyclo*[D-Asp-Phe-Dab]NH_2 (Dab = a,γ-diaminobutyric acid) (895) in which the lactam linkage is reversed also exhibit high affinity and μ selectivity. The antiparallel cyclic dimers (Tyr-D-Orn-Phe-AspNH_2)$_2$ and (Tyr-D-Asp-Phe-OrnNH_2)$_2$, obtained as by-products during the synthesis of the cyclic monomers, have similar affinities for both μ and δ receptors (894).

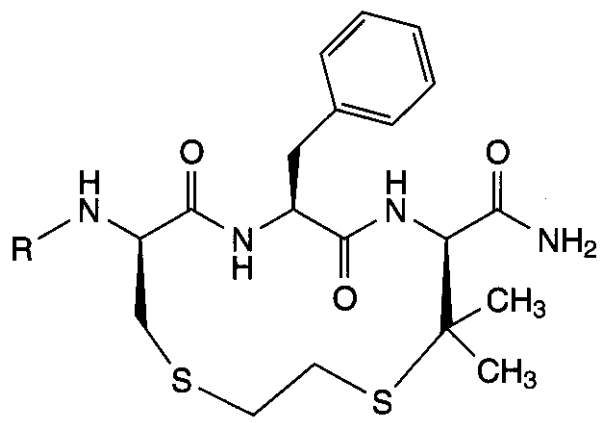
Various modifications to Phe^3 in these cyclic peptides have also been examined. Many of the modifications (e.g., p-nitro or N-methyl substitution, or shortening the side chain) decrease preceptor affinity by 25-fold or more (895). Although the cyclic structure restricts the conformation of the peptide backbone, there is still considerable conformational flexibility around the Tyr^1 and Phe^3 side chains (896). Conformational constraint of Phe^3 by incorporation of 2-aminoindan-2-carboxylic acid (Aic, Fig. 7.43) into the relatively nonselective peptide [K_i ratio (δ/μ) = 3] Tyr-*cyclo*[D-Orn-Phe-Glu]NH_2 markedly enhances μ-receptor selectivity (see Table 7.18) (834). This is a direct consequence of conformational restriction because incorporation of the acyclic derivatives a-methylphenylalanine or 2′-methylphenylalanine in position 3 does not significantly change preceptor selectivity. Interestingly, the peptides containing L- and D-2-



(244)



(245)



R = Tyr (246) JOM-6
R = Phe (247) JH-54
R = D-Hat (248)

Figure 7.49. Conformationally constrained dermorphin analogs.

aminotetralin-2-carboxylic acid (**Atc,** Fig. 7.43) in position 3 had similar preceptor affinity and high p-receptor selectivity (834). The restricted conformation of the side chain of residue 3 in the **Aic** analogs was apparent from comparisons of molecular dynamics simulations for the Phe^3 versus Kc^3-containing peptides (897). Further conformational constraint of Tyr-*cyclo*[D-Orn-Aic-Glu]NH_2 by replacement of Tyr^1 with L- or D-Hat yields peptides with only two freely rotatable bonds (245, Fig. 7.49) (897); interestingly, both diastereomers exhibit reasonable preceptor affinity and selectivity [K_i (μ) = 20–30 nM, K_i ratio (δ/μ) = 12–19].

Cyclic pentapeptide derivatives of **Tyr-*cyclo*[D-Dab-Phe-Phe-**(D- or L-)**Leu]** containing retro-inverso modifications were prepared by Goodman and coworkers (898). The parent peptides **Tyr-*cyclo*[D-Dab-Phe-Phe-D/L-Leu]** are potent agonists in the GPI with relatively low p-receptor selectivity [IC_{50} ratio (MVD/GPI) = 4.4 and 12 for the L-Leu and D-Leu analogs, respectively]. The analog **Tyr-*cyclo*[D-Dab-Phe-gPhe-S-mLeu]** containing **a** reversed amide bond between Phe^4 and Leu^5 is also a p-receptor selective agonist. Interestingly, reversal of **a** second amide bond in the side chain lactam linkage to give **Tyr-*cyclo*[D-Glu-Phe-gPhe-rLeu]** results in a **δ-selective** derivative [IC_{50} ratio (GPI/MVD) = 11], which exhibits a considerably different conformation from that exhibited by the other retro-inverso analogs that preferentially interact with μ receptors (899).

In the case of peptides cyclized through a disulfide or dithioether linkage, the receptor selectivity depends on the linkage. The cyclic disulfide analog Tyr-*cyclo*[D-Cys-Phe-Cys]-NH_2 exhibits 10-fold greater affinity for S receptors than the corresponding lactam **Tyr-*cyclo*[D-Asp-Phe-Orn]-NH_2** and is therefore less p-receptor selective [IC_{50} ratio (δ/μ) = 34] (895). The cyclic tetrapeptide JOM-13 Tyr-cy-

clo[D-Cys-Phe-D-Pen]OH is a δ-selective agonist (900) (see Section 6.5.2.2 below), but incorporation of an ethyl group between the two sulfurs and amidation of the C-terminus result in the p-selective peptide JOM-6 (246, Table 7.18) (900). Comparison of JOM-13 and its p-selective amide derivative JH-42, which contains E-dehydrophenalanine in position 3, docked to computational models of δ and μ receptors, respectively, found key differences (182). The Phe^3 side chain of the peptide adopts the gauche (−) (~ −60°) conformation and interacts with Leu^{3m} in TM7 in the S receptor, whereas the presence of Trp in the corresponding position in the μ receptor causes a shift of the entire peptide within the binding pocket and reorientation of the Phe^3 side chain from gauche to the trans conformation. The C-terminal acid in JOM-13 forms an ion pair with Lys^{214} in TM5 of the S receptor in the model, but because of the shift in the peptide in the binding site, its C-terminus is in close contact with Glu^{229} in the preceptor and therefore amidation of the C-terminus removes unfavorable electrostatic repulsion. The shift of the peptide lengthened the distance between the phenol of Tyr^1 of the peptide and His^{297} in the preceptor binding site compared to the corresponding distance in the S binding pocket, suggesting that the hydrogen bond between these two groups may be less important for binding to μ receptors. Mosberg and coworkers therefore prepared JH-54 (247), the Phe^1 analog of JOM-6, and found that it retained high affinity for μ receptors but greatly reduced affinity for δ receptors (Table 7.18), consistent with the expected results from the modeling (835); this peptide is a potent full agonist in the GPI. Even the aromaticity of the residue in position 1 was not critical, and the Cha^1 analog of JOM-6 retains moderate p-receptor affinity (K_i = 32 n*M*) and agonist potency (EC_{50} = 59 n*M* in the GTPγS assay) (901). Incorporation of conformationally restricted tyrosine analogs in position 1 resulted in potent μ agonists (see [D-Hat^1]-JOM-6, 248, Table 7.18), but the potency changes in the GTPyS assay did not always correlate with affinity, suggesting that the conformations required for receptor activation versus binding were different (836).

6.5.2 Deltorphin Analogs and Related Peptides

*6.5.2.1 **Linear Analogs.*** Among the naturally occurring deltorphins, [D-Ala^2]deltorphin I and II are more potent and more δ-selective than deltorphin (see Table 7.13) and have a distinctly different C-terminal sequence (106). Like dynorphin, the deltorphins have been divided into two domains, the N-terminal "message" region and C-terminal "address" sequence. There are differences in the structure-activity relationships for deltorphin and [D-Ala^2]deltorphin I and II, particularly in the C-terminal "address" domain, and therefore the SAR in this region of the peptides is discussed separately below. [D-Ala^2] deltorphin I and II are also metabolically more stable than deltorphin, apparently because of the branched amino acid present in position 5 of the former peptides (902).

A number of studies have examined possible conformations of the deltorphins (see Refs. 903–905 for reviews). A β turn in the *N*-terminus has been observed for both the p-selective peptide dermorphin and the δ-selective deltorphins, while differences in the conformations of the C-terminal sequences have been described (see Refs. 903–905 and references cited therein). These studies are complicated, however, by the inherent conformational flexibility of linear peptides, and very different. conformations can be observed for a given peptide (see, e.g., Ref. 906).

Amino acid substitution has provided information on the contributions of different residues in the deltorphin sequence to δ-receptor affinity and selectivity. A variety of substitutions have been made in each position of the deltorphins, resulting in hundreds of analogs reported in the literature. Only selected analogs and structural modifications are discussed here to illustrate key SAR; for more detailed discussion readers are referred to recent detailed reviews (904,9051, which include extensive tables of analogs.

One of the first structural modifications that was examined in the deltorphins was the chirality of residue 2. Along with the initial descriptions of the deltorphin sequence, the peptides with both D-Met and L-Met in position 2 were synthesized to determine which was the naturally occurring sequence (666, 668, 669). Whereas the peptide containing D-Met

Table 7.20 Opioid Receptor Affinities and Opioid Activity in the GPI and MVD of Deltorphin Analogs[a]

Peptide	K_i (nM)		K_i Ratio	IC_{50} (nM)		Reference
	δ	μ	μ/δ	GPI	MVD	
[D-Ala^2]Deltorphin I (**214**)	0.15	3,150	21,000	>1,500	0.21	
[D-Ala^2]Deltorphin II (**25**)	0.71	2,450	3,400	>3,000	0.32	
[Dmt^1,D-Ala^2]deltorphin II	0.13	1.0	7.7	300	—	924
[(2*S*,3*S*)-Tmt^1,D-Ala^2]deltorphin I	3.0	17,000	5,740	3,840	0.66	728
[Tic^2]deltorphin I	6.49	9,230	1,420	>65,000	22.8[b]	907
[D-Ala^2,Nle^3]deltorphin I	10	1,020	100	—	—	925
[D-Ala^2,D/L-Atc^3]deltorphin I	5.36	670	125	1,820	0.115	907
[D-Ala^2,D/L-Atc^3]deltorphin I	6.52	1,410	215	1,380	0.178	907
[D-Ala^2,Phe^4]deltorphin I	1.5	3.9	2.6	—	—	919
[*N*-*n*BuGly^6]deltorphin	0.04	820	18,000	580	0.56	922
JOM-13	0.74	54	70	460	4.2	926
[*t*-Hpp^1]JOM-13	0.66	110	170	770	1.6	926
[*c*-Hpp^1]JOM-13	2.4	720	300	12,000	75	926
[Δ^Z-Phe^3]JOM-13	2.4	780	330	—	—	927
cyclo[D-Cys^2,Cys^5]deltorphin I	0.87	5.5	5.9	2.98	0.23	928
cyclo[D-Cys^2,Pen^5]deltorphin I	2.2	3,760	1,700	1,100	0.25	928
cyclo[D-Pen^2,Pen^5]deltorphin I	3.7	26,000	7,000	68,000	6.30	928

[a]Data for [D-Ala^2]deltorphin I and II from Table 7.13 are included for comparison.
[b]Partial agonist.

exhibited high 8-receptor affinity and selectivity, the analog with L-Met had very low affinity and potency in the MVD, indicating the importance of a D-amino acid in this position. As was found for deltorphin, the D-Ala in position 2 of [D-Ala^2]deltorphin I is also important for δ-receptor affinity and selectivity, and the L-Ala^2 analog has extremely weak activity in the MVD ($IC_{50} > 1\ \mu M$) (106). The exception to the requirement for a D-amino acid in position 2 is [Tic^2]deltorphin I, which retains high affinity and exhibits increased selectivity for 8 receptors (Table 7.20) (907); this substitution decreases efficacy, however, and in the MVD this analog is a partial agonist. In deltorphin substitution of D-Ala, as is found in [D-Ala^2]deltorphin I and II, for D-Met is well tolerated at 8 receptors, although it increases preceptor affinity and potency in the GPI (908, 909). In contrast, substitution of D-Met in position 2 of [D-Ala^2]deltorphin I or II causes a large decrease in 8-receptor affinity and selectivity (908, 909).

Modifications to the aromatic residues in the N-terminal "message" sequence have been examined, mostly in [D-Ala^2]deltorphin I or II. Replacing either Tyr^1 or Phe^3 by a D-amino acid causes a large decrease in 8-receptor affinity (909). As with most opioid peptides, other modifications of Tyr^1 are also generally not tolerated, except for substitutions by conformationally restricted derivatives of Tyr (see Section 6.5.2.2 below). A variety of structural modifications have been examined at position 3 of [D-Ala^2]deltorphin I, including incorporation of aromatic, heterocyclic, and nonaromatic amino acids (see Ref. 904) and conformationally constrained derivatives (see Section 6.5.2.2 below). The effects of substitution on the phenyl ring of Phe^3 vary with the substituent. para-Substitution with a halogen, but not an amine, nitro, hydroxyl, or methyl group, in [D-Ala^2]deltorphin I is well tolerated by δ receptors, and p-bromo substitution enhances 8-receptor selectivity in both binding and smooth muscle assays (910–912). The heterocyclic phenylalanine analog 3-(2-thienyl)alanine is well tolerated in position 3 by 8 receptors, but other heterocyclic aromatic amino acids, such as the pyridylalanine derivatives or His, significantly decrease 8-receptor affmity (912). An aromatic residue is not required in position 3 of [D-Ala^2]deltorphin I, and the analogs with Cha and even Leu or

norleucine (Nle) exhibit δ-receptor affinity only six- to sevenfold lower than that of the parent peptide (Table 7.20). QSAR analysis of the effect of substitution in position 3 suggested that the binding site around this side chain is very similar for 6 and μ receptors (913). Substitution of either the a-carbon or nitrogen of Phe^3 with a methyl group, which can alter the conformation of the peptide backbone, is not tolerated (911).

The C-terminal truncation of the deltorphins can affect both &receptor affinity and selectivity. Deamidation of both [D-Ala^2]deltorphin I and II causes significant decreases in &receptor affinity (25- to 50-fold) and 6 selectivity (908, 914, 915). Although there is one report of a large (90-fold) decrease in 6-receptor affinity upon conversion of deltorphin to the C-terminal acid (914), there are other reports that deamidation of this heptapeptide causes only a small decrease (916), or even a slight increase (915), in δ-receptor affinity. Shortening of either [D-Ala^2]deltorphin I or deltorphin from the C-terminus causes progressive decreases in &receptor affinity and selectivity and potency in the MVD (908, 915, 916). The hexapeptide and pentapeptide derivatives of [D-Ala^2]deltorphin I retain 6-receptor selectivity (908); the corresponding deltorphin fragments, although retaining high affinity for 6 receptors, are nonselective (916). The *N*-terminal tetrapeptide amide derivative of deltorphin is selective for preceptors (917,918) [the tetrapeptide acid exhibits low affinity for both μ and 6 receptors (918)], indicating that the C-terminal "address" sequence is capable of changing receptor type selectivity. Indeed, swapping the C-terminal tripeptide sequence of dermorphin and deltorphin reverses the receptor selectivity profile [i.e., [Leu^5,Met^6,Asp^7]dermorphin is δ selective (916)]. The N-terminal tetrapeptide amide fragment of [D-Ala^2]deltorphin I (Tyr-D-Ala-Phe-$AspNH_2$) shows a slight preference for μ receptors (908, 915), but its affinity (K_i = 100–195 n*M*) and selectivity for μ receptors is much lower compared to that of the *N*-terminal tetrapeptide amide fragment of deltorphin [Tyr-D-Met-Phe-$HisNH_2$; K_i (μ) = 8.0 n*M*].

In the C-terminal "address" sequence of [D-Ala^2]deltorphin I and II considerable attention has focused on the role of the acidic residue in position 4. Replacement of Asp and Glu in this position with Asn and Gln, respectively, results in compounds that maintain δ-receptor affinity, but have enhanced p-receptor affinity and thus significantly decreased 6-receptor selectivity (908, 914, 915). A variety of other amino acid substitutions in this position are well tolerated by 6 receptors but significantly enhance p-receptor affinity (908, 919). Thus the negative charge at position 4 is not necessary for &receptor interaction, but contributes significantly to 6-receptor selectivity by interfering with interaction with μ receptors. Consistent with this, substitution of Asp/Glu by His, the residue found in this position of deltorphin, does not affect δ-receptor affinity but increases preceptor affinity (908,919). Replacement of Asp/Glu by hydrophobic residues [e.g., Phe (see Table 7.20), α-aminobutyric (Abu), or α-aminoisobutyric acid (Aib), and the conformationally restricted 1-aminocycloalkane derivatives1 substantially increases p-receptor affinity, resulting in analogs with nanomolar affinity for both δ and μ receptors, which has been referred to as "opioid infidelity" (903). QSAR analysis of substitutions in this position has also been performed (913), and suggests that the receptor binding site for this residue is quite different for 6 versus preceptors. Analysis of binding to δ receptors suggested at least a partial positive charge in the binding pocket, with both electrostatic and van der Waals forces, but not hydrogen bonding, contributing to receptor binding.

In positions 5 and 6 of [D-Ala^2]deltorphin II replacement of Val^5 and Val^6 individually by Ala suggested that Val^5 is more important than Val^6 for δ-receptor selectivity (920). Replacement of valine in both positions by norleucine, Ile, or γ-methylleucine enhances both 6-receptor affinity and selectivity four- to eightfold (920). Replacing Gly^7 in [D-Ala^2]-deltorphin I with Asp, which is found in this position in deltorphin, decreases affinity for both 6 and μ receptors approximately 10-fold (919).

Amino acid substitutions in the C-terminus of deltorphin have also focused mainly on the charged residues His^4 and Asp^7. As was found for Asp^4/Glu^4 in [D-Ala^2]deltorphin I and II, His^4 appears to play an important role in the

&receptor selectivity, but not affinity, of deltorphin. Thus a variety of amino acid substitutions for His^4, including Gly and aromatic amino acids, are well tolerated by 6 receptors, but they enhance preceptor affinity and thus decrease 6-receptor selectivity (915). Interestingly, substitution by Lys markedly decreases &receptor affinity (915), and substitution by Asp, as is found in [D-Ala^2]deltorphin I, decreases both 6- and preceptor affinity (908, 914, 915). Replacement of His^4 by D-His only decreases 6-receptor affinity and selectivity 4–6-fold, whereas des-His^4 analogs have markedly lower &receptor affinities and selectivities (909), suggesting that His^4 may play a role in maintaining the necessary spatial orientation of the C-terminal sequence of deltorphin relative to the N-terminus. Further modifications of His^4 have been examined (921). N-Methylation on either nitrogen of the imidazole enhances δ-receptor selectivity, whereas N^{α}-methylation of His^4 decreases both δ-receptor affinity and selectivity. Substitution of the conformationally constrained residue Tic for His^4 is well tolerated by 6 receptors, but decreases 6-receptor selectivity (921). The charge on Asp^7 also does not appear to be critical for interaction with 6 receptors, but contributes to 6-receptor selectivity by adversely affecting preceptor affinity. Thus replacement of Asp^7 by a nonacidic amino acid decreases 6-receptor affinity by less than two- to threefold, but enhances preceptor affinity so that 6-receptor selectivity decreases (914, 915,919).

A number of residues have been incorporated in position 5 of deltorphin, and generally the 6 receptor affinities of these analogs correlate with the hydrophobicity of the residue in this position (see Ref. 904). Branched hydrophobic amino acids Val, Ile, and γ-methylleucine have been incorporated into this position of deltorphin to enhance metabolic stability; these analogs have Breceptor affinities and selectivities similar to those of the parent peptide (902). N-Alkyl amino acids were also introduced at position 6 to decrease metabolism of the Leu^5-Met^6 bond; these analogs possess the desired stability to degradation by both rat brain and plasma, and the N-nBuGly^6 analog exhibits exceptional selectivity for δ receptors (Table 7.20) (922). A variety of other substitutions for Met^6 in deltorphin are well tolerated (see Ref. 904), suggesting that residue 5 is more important for receptor interaction. However, incorporation of an additional acidic residue in position 6 decreases δ-receptor affinity (914,923).

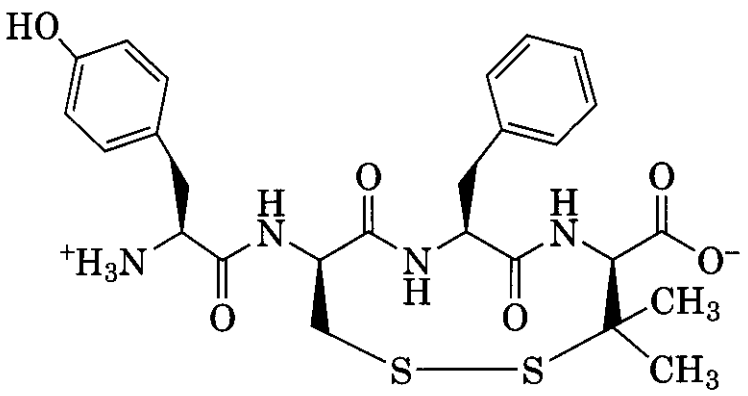
6.5.2.2 Conformationally Constrained Analogs. The conformationally restricted tyrosine analogs Dmt and Tmt (Fig. 7.43) have been incorporated into position 1 of [D-Ala^2]deltorphin I or II. Substitution of Dmt for Tyr in [D-Ala^2]deltorphin II markedly enhances μ-receptor affinity, resulting in a compound with nanomolar affinity for both receptors (Table 7.20) (924). In contrast [D-Ala^2]deltorphin I analogs containing either of the 2*S* isomers of Tmt, which has the additional β-methyl group, exhibited high Breceptor affinity and selectivity; the 2*S*,3*S* isomer exhibited greater 6-receptor selectivity than that of the parent peptide (IC_{50} ratio = 5,740 versus 3,500) (728).

Linear peptides containing conformationally constrained Phe analogs in position 3 have also been prepared. The conformationally restricted phenylalanine analogs Aic and both isomers of Atc (Fig. 7.43) are well tolerated at this position by 6 receptors, although the Aic^3 analog is less selective for 6 receptors than are the Atc^3 derivatives of [D-Ala^2]deltorphin I (907, 911). The similar potencies of both diastereomers of [D-Ala^2,Atc^3]deltorphin I are in sharp contrast to the large (13- to 50-fold) decrease in potency of the D-Phe^3 analog compared to that of [D-Ala^2]deltorphin I, suggesting that the receptor binding mode of the D-Atc^3 analog may be different from that of the D-Phe^3 derivative (907). The configuration of Atc in the diastereomeric peptides was not determined in these initial studies, although it was in subsequent derivatives of [D-Ala^2,$Ile^{5,6}$]deltorphin I and II (929).

The four isomers of β-MePhe were incorporated into both [D-Ala^2]deltorphin I and deltorphin (930). The peptides containing the 2*S* isomers exhibit higher Breceptor affinity and agonist activity in the MVD than the analogs with the 2R isomers for both [D-Ala^2]deltorphin I and deltorphin, consistent with the preference for L- over D-Phe in this position. The stereochemistry of the methyl group at the β position, however, had different effects on the 6 affinity and agonist

activity of the two peptides, with the 3*S* isomer preferred in [D-Ala^2]deltorphin I and the 3R isomer preferred in deltorphin. Substitution with the dipeptide mimetic Aba^3-Gly^4 is also reasonably well tolerated in [D-Ala^2]-deltorphin II (IC_{50} = 5.0 n*M*) (892). Other constrained residues in position 3 e . Tic or NMePhe) result in drastic decreases in δ-receptor affinity (907,911).

As in the case of cyclic enkephalin analogs, conformationally constrained δ-selective peptides with the sequence Tyr-D-X-Phe were also prepared by cyclization through a disulfide bond. Cyclization between D-Cys in position 2 and D-Pen in position 4 yielded the tetrapeptide derivative Tyr-*cyclo*[D-Cys-Phe-D-Pen]-OH (JOM-13, 249), which contains a fairly



(249) JOM-13

rigid 11-membered cyclic structure and which exhibits high affinity and selectivity for δ receptors (Table 7.20) (900). Conformational analysis by NMR, X-ray crystallography, and computational analysis (931) indicated a single preferred conformation for the cyclic tripeptide portion of JOM-13, but the key pharmacophoric elements, the exocyclic Tyr^1 and the Phe^3 side chains, still exhibit conformational flexibility.

A variety of substitutions have been made for Phe^3 in JOM-13 (932–935). Comparison of Phe^3 substitutions in JOM-13 to the same substitutions for Phe^4 in DPDPE found that modifications that would be expected to affect the conformation of the peptide backbone (e.g., incorporation of NMePhe or D-Phe) had deleterious effects on δ-receptor affinity and potency in the MVD in both series (932); the effects of these substitutions were generally greater in the pentapeptide series, however, which may be due to the greater rigidity, and therefore reduced susceptibility to conformational perturbation, of the tetrapeptide. Other substitutions that affect only the side chain of residue 3, particularly homophenylalanine (Hfe), had significantly different effects on &receptor affinity in the two series. Hfe substitution does not adversely affect δ-receptor affinity or potency in the MVD when it is incorporated into JOM-13 (although it does increase preceptor affinity 20-fold so that δ-receptor selectivity decreases), but it decreases δ-receptor affinity and potency in the MVD 100- and 25-fold when incorporated into DPDPE (932). *para*-Substitution of Phe^3 with fluorine, chlorine, or a nitro group in JOM-13 is well tolerated by δ receptors, similar to the results found for DPDPE analogs (933). An aromatic residue at position 3 is not essential for δ-receptor interaction, and incorporation of Cha at position 3 of JOM-13 causes less than a threefold decrease in δ-receptor affinity (934); not surprisingly, this modification also enhances μ-receptor affinity so that [Cha^3]JOM-13 exhibits only a fourfold lower K_i value for δ receptors than for μ receptors.

The conformation of residue 3 has been restricted by incorporation of p-MePhe (935) and dehydrophenylalanine (ΔPhe) (927) in this position. Both the 3*S* and 3R diastereomers of L-β-MePhe were compatible with interaction with δ receptors, with the peptide containing the 2*S*,3*S* isomer exhibiting approximately eight-fold higher affinity than that of the peptide containing the 2*S*,3*R* isomer. Surprisingly, the peptide containing the 2*R*,3*R* isomer of D-β-MePhe also exhibits high affinity and selectivity for δ receptors. Examination of this latter peptide by molecular modeling suggested a side chain conformation of Phe^3 in JOM-13 with χ_1 about –60" when the peptide interacts with δ receptors (935). On the basis of these results, it was predicted that the JOM-13 analog containing A^{Z}Phe (Fig. 7.43), but not A^{E}Phe, could be superimposed on the resulting proposed bioactive conformation of JOM-13; as predicted, [Δ^{Z}Phe^3]JOM-13 exhibited nanomolar affinity and higher δ-receptor selectivity than JOM-13, whereas incorporation of A^{E}Phe in position 4 resulted in a 60-fold decrease in δ-receptor affinity (927).

Mosberg and coworkers also examined conformationally constrained tyrosine derivatives in position 1 of JOM-13 (926,936). Incorporation of trans- and cis-3-(4'-hydroxyphenyl)proline (t-Hpp and c-Hpp, respectively; Fig. 7.43) yielded the analogs with the highest Greceptor affinity and selectivity (Table 7.20), with the peptides containing Hat and Hai (2-hydroxy-2-aminoindan-2-carboxylic acid) exhibiting modest 6-receptor affinities and selectivities. Molecular modeling of the active Tyr^1-substituted analogs suggested that in the receptor-bound conformation the side chain of residue 1 is in the trans (χ_1 − 180'') conformation and that the exocyclic peptide group is in an extended conformation (Ψ_1 and Φ_2 − 160°) (926); a second low energy conformation differing in Φ_2 (−70'') also still remained a possibility.

These results led to a pharmacophoric model of the bioactive conformation of JOM-13 (926, 935). To distinguish between the two remaining possible bioactive conformations for JOM-13, these conformations were then compared and superimposed with DPDPE and other δ-receptor ligands (TIPP, and the alkaloids SIOM and OMI) (841). Similar arrangements in space of the key pharmacophoric elements (the tyramine moiety and the second aromatic ring) were found with the other ligands for JOM-13 in the proposed bioactive conformation (with Φ_2 − 160°), but not the alternative (Φ_2 − 70'') conformation. Different orientations were observed for the second aromatic ring in agonists versus antagonists, but the same orientation of this aromatic ring was found for both peptide and alkaloid ligands with the same type of activity (i.e., agonists JOM-13, DPDPE, and SIOM). Subsequently, JOM-13 was docked to a computational model of the S receptor (937); in contrast to the modeling based on superimposition of different ligands, only the alternative conformation (Φ_2 − 70°), and not the proposed conformation with Φ_2 about 160°, was compatible with the receptor-binding pocket. These results are another indication that the receptor interactions may differ for different ligands, and caution against a simplistic view of a single common binding mode for different ligands (937).

Deltorphin and [D-Ala^2]deltorphin I and II analogs cyclized by a disulfide linkage between residues 2 and 5 have also been reported. *cyclo*[D-Cys^2,Cys^5]deltorphin I exhibits affinity for δ receptors similar to that of [D-Ala^2]-deltorphin I, but the cyclic peptide exhibits 400-fold higher preceptor affinity and thus very low 6-receptor selectivity (Table 7.20) (938). Substitution of Pen in position 5 or preparation of the [D-Pen^2,Pen^5] derivative in the deltorphin I series decreases preceptor affinity 730- and 5000-fold, respectively, while decreasing S-receptor affinity by only 3.5- to 6-fold (928), thus restoring high δ-receptor selectivity (Table 7.20). A series of *cyclo* [D-Cys^2,L/D-Pen^5] derivatives of [D-Ala^2]deltorphin II and deltorphin were also prepared (939); these analogs, including some with D-Pen in position 5, exhibit nanomolar affinity for δ receptors, but their selectivity for S over preceptors is much lower (generally <60-fold) than the [D-Pen^2, Pen^5] peptides.

Schiller and coworkers prepared derivatives of the p-selective lactam Tyr-*cyclo*[D-Orn-Phe-Asp] with the C-terminal Val-Val-GlyNH$_2$ sequence from [D-Ala^2]deltorphin (907). This C-terminal extension reduces μ-receptor affinity and increases 8-receptor affinity 10-fold, resulting in similar affinities for the two receptors. The peptide Tyr-*cyclo*[D-Lys-Phe-Asp]-Val-Val-GlyNH, with a slightly larger ring size exhibits eightfold higher δ-receptor affinity and a slight preference for δ over μ receptors.

6.6 Other Peptides with High Affinity for Opioid Receptors

6.6.1 Mu-Receptor Antagonists Derived from Somatostatin. Potent p-opioid antagonists have been identified that are derivatives of somatostatin rather than of an opioid peptide (see Ref. 656 for a review). Somatostatin exhibits low affinity for opioid receptors, and the potent somatostatin analog SMS-201,995 (D-Phe-*cyclo*[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr-ol) was found to be an antagonist at μ opioid receptors (940). Further structural modification yielded a series of peptides with the general structure D-Phe-*cyclo*[Cys-Tyr-D-Trp-X-Thr-Pen]-ThrNH,, where X = Lys, Om, or Arg in CTP, CTOP (19, Fig. 7.4), and

Table 7.21 Opioid Receptor Affinities and Antagonist Activity in the GPI and MVD of Peptide Antagonists[a]

Peptide	K_i (nM) μ	K_i (nM) δ	K_i Ratio δ/μ	pA_2 GPI (μ)
CTP	3.7	1,150	310	7.1
CTOP (19)	4.3	5,600	1,300	6.4
CTAP (**20**)	2.1	5,310	2,530	7.1
TCTP	1.2	9,320	7,770	8.1
TCTOP	1.4	20,400	11,400	7.4
TCTAP	1.2	1,270	1,000	8.7

[a]Data **from Ref. 944.**

CTAP (**20**), respectively (105, 941), which exhibit greatly reduced affinity for somatostatin receptors and enhanced affinity and selectivity for μ receptors. Analysis of CTP by NMR suggested that the central Tyr-D-Trp-Lys-Thr sequence adopts a type II′ β turn (942). The conformation of these peptides was further restricted and preceptor selectivity further enhanced by incorporation of the constrained amino acid D-Tic in position **1;** the resulting peptides TCTP, TCTOP, and TCTAP are potent p antagonists with exceptional selectivity for μ receptors (Table 7.21) (943,944). Incorporation of either isomer of β-Me-D-Phe in position **1** decreases p-receptor affinity and selectivity 10- and 40-fold, respectively (945).

Other p-receptor antagonists have been identified from combinatorial libraries (see below).

6.6.2 Peptides and Peptidomimetics from Combinatorial Libraries. Mixture-based combinatorial peptide libraries have been extensively explored by Houghten and coworkers and have led to the identification of a variety of peptides with affinity for opioid receptors (see Ref. 946 for a review). Early hexapeptide libraries, deconvoluted by binding to p opioid receptors and either iterative deconvolution (947) or positional scanning (948; see Ref. 946 for a description of these deconvolution techniques), identified sequences related to the enkephalins. Other nonacetylated peptide libraries have identified more varied peptides, some that resemble opioid peptides and some that do not. Iterative deconvolution of a hexapeptide library resulted in identification of both Tyr-Pro-Phe-Gly-Phe-XNH, (X = one of 20 natural amino acids), reminiscent of the sequence of β-casomorphin, and the unrelated peptides Trp-Trp-Pro-Lys-His-X-NH, (949) with affinity for μ receptors. Hexapeptide libraries have also been examined for affinity for δ receptors, resulting in the identification by positional scanning of several peptides that share some similarity to the enkephalins [Tyr-X-Y-Z-Leu-ValNH,, where X-Y-Z = Gly-Met-His, His-Gly-Trp (950), and Gly-Phe-His (946)].

A variety of novel acetylated peptides with sequences unrelated to known opioid peptides have been identified for all three opioid receptors from combinatorial libraries (see Ref. 946). Initially, Houghten and coworkers used p-receptor binding as the screening assay to identify peptides from an acetylated hexapeptide amide library with affinity for μ receptors. These peptides, termed acetalins (**250**), are potent p-receptor antagonists in the GPI (951). Further exploration of the acetylated hexapeptide library resulted in the additional identification of AcPhe-Arg-Trp-Trp-Tyr-X-NH_2 and AcArg-Trp-Ile-Gly-Trp-X-NH_2; AcPhe-Arg-Trp-Trp-Tyr-MetNH_2 is an agonist, whereas AcArg-Trp-Ile-Gly-Trp-Arg-NH_2 is an antagonist at μ receptors (949). Screening of an acetylated hexapeptide library of all D-amino acids led to the identification of Ac-arg-phe-trp-ile-asn-lys-NH, (D-amino acids indicated by use of all lowercase letters for the amino acid) as a ligand for μ opioid receptors; interestingly, this peptide is a potent agonist that produces analgesia after peripheral administration (952). A nonacylated, all D-amino acid hexapeptide library also yielded peptides (ile-phe-trp-tyr-argNH_2 and ile-met-ser-trp-trp-glyNH_2) with affinity for μ opioid receptors (946). An acetylated decapeptide library was

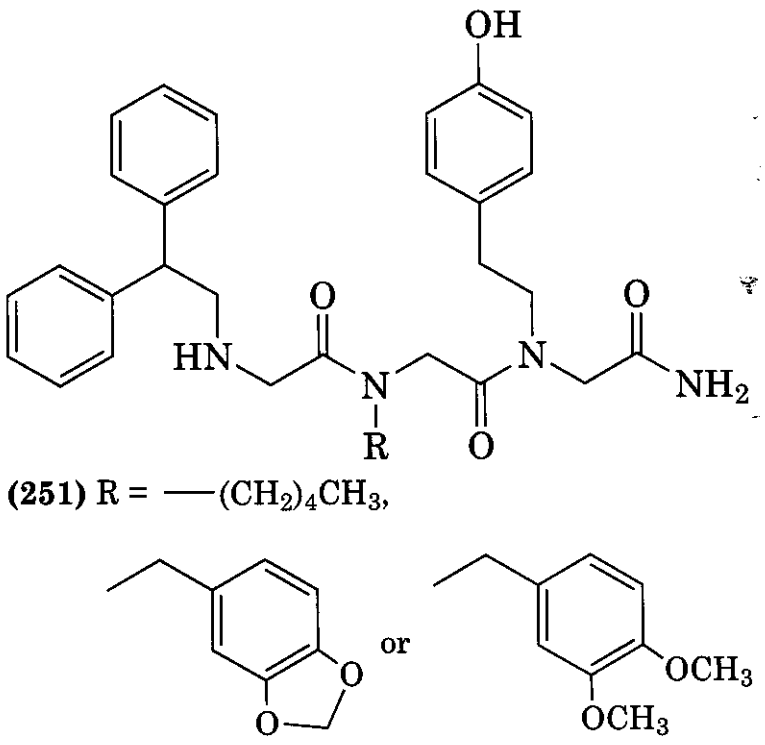
used to identify AcTyr-Arg-Thr-Arg-Tyr-Arg-Tyr-Arg-Arg-$ArgNH_2$ and AcArg-Gly-Trp-Phe-His-Tyr-Lys-Pro-Lys-$ArgNH_2$ as ligands for κ opioid receptors (946); like dynorphin A, these peptides have a number of basic residues in their C-terminal sequences.

AcArg-Phe-Met-Trp-Met-X-NH_2

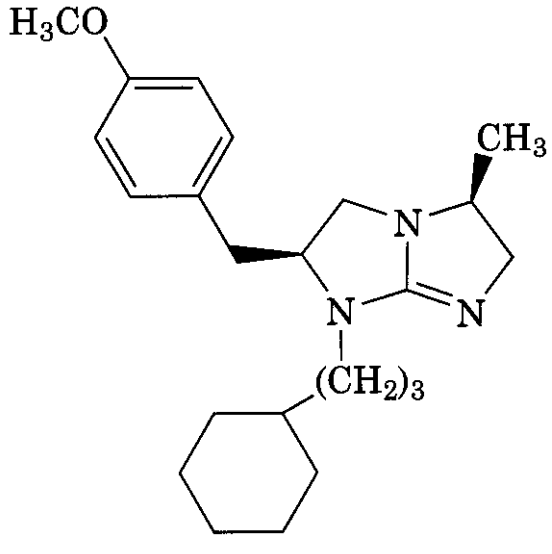
Acetalins (250) X = Arg, Lys, or Thr

A single tetrapeptide library containing both L- and D-amino acids, including a number of unnatural amino acids (50 amino acids total), has been screened by positional scanning for affinity for all three types of opioid receptors (143). This led to the identification of peptides with nanomolar affinity for each receptor type. Based on screening of the mixtures, an aromatic L-amino acid (Tyr for μ receptors, and Tyr or Trp for δ receptors) was incorporated in position 1 in the individual peptides prepared for these two receptor types. In the 4 position, an aromatic L-amino acid was also incorporated in peptides with affinity for μ receptors, whereas either Arg or Trp was found in this position in peptides with 8-receptor affinity. Interestingly, some of the peptides prepared as ligands for the δ receptor exhibited higher affinity for μ receptors. The most unusual results were those obtained for the κ receptor. In this case at each position the mixtures exhibiting the highest affinity contained a D-amino acid in the defined position, so that the resulting peptides contained all D-amino acids. The peptides with the highest affinity for μ and K receptors were tested in adenylyl cyclase assays and found to be full agonists.

Peptidomimetic ligands for opioid receptors have also been identified from combinatorial libraries. Screening of an *N*-(substituted)glycine peptoid library for affinity for μ opioid receptors yielded novel structures (**251**) with high affinity for these receptors (K_i = 6–46 n*M*) (953). A library of dipeptide amides with alkyl substituents on both the interior and C-terminal amides were prepared, and high affinity agonists for all three opioid receptors identified from the library (see Ref. 946). Peptide libraries can also be further modified ["libraries from libraries" (954)] to yield new potential ligands for receptors. Thus an acetylated hexapeptide library was subjected to exhaustive reduction, and the resulting polyamine library was screened by positional scanning for affinity for μ opioid receptors (955). The polyamine with the highest affinity was the fully reduced heptamine of AcTyr-Tyr-Phe-Pro-Thr-MetNH_2 (IC_{50} = 14 n*M*). The fifth and sixth residues could be truncated to yield the pentamine without loss of affinity; this derivative is an antagonist in both the GPI and MVD (K_e values of 13.6 and 163 n*M*, respectively). Further modifications were made to the pentamine to enhance preceptor affinity (see Ref. 946). A bicyclic guanidine library was also prepared, and after screening for binding at γ receptors, a ligand with modest affinity (252, IC_{50} = 37 n*M*) was identified (956).



(251) R = $-(CH_2)_4CH_3$,



(252)

6.7 Peptide Affinity Label Derivatives

Peptide-based affinity labels have been principally photoaffinity labels, including the azide derivatives of several enkephalin analogs and CTP (see Refs. 286,594 for detailed reviews). As noted earlier (Section 5.11.2), the use of photoaffinity labels, however, has been limited because opioid receptors are susceptible to inactivation by UV irradiation (643).

The preparation of opioid peptide derivatives containing electrophilic affinity labels has been limited to a few compounds. The chloromethyl ketone DALECK (Tyr-D-Ala-Gly-Phe-LeuCH_2Cl) (957, 958) is one of the best studied electrophilic peptide derivatives. It selectively alkylates μ opioid receptors (959, 960), and [^{3}H]DALECK has been used to label and characterize these receptors (959, 961, 962). DAMK (Tyr-D-Ala-Gly-Me$Phe CH_2Cl$), the chloromethyl ketone derivative of DAMGO, was also prepared and binds selectively to μ opioid receptors (963). The chloromethyl ketone of dynorphin A-(1–10) (964) inhibits binding to frog brain membranes in a wash-resistant manner, although the affinity of this peptide for κ receptors is relatively weak (apparent IC_{50} for irreversible blockade ~10 μM). The C-terminal maleamide derivative of DSLET was recently reported to exhibit wash-resistant inhibition of binding to 6 receptors at micromolar concentrations (965). Enkephalin analogs containing melphalan (Mel), the nitrogen mustard derivative of *p*-aminophenylalanine, were also prepared (see Refs. 286, 966). Recently, the first isothiocyanate derivatives of opioid peptides were described (738, 967); the Phe(*p*-N=C=S)^4 derivative of *N*,*N*-dibenzyl enkephalin and both the Phe(*p*-N=C=S)^3 and Phe(*p*-N=C=S)^4 derivatives of TIPP exhibited wash-resistant inhibition of binding to cloned 6 opioid receptors. The Phe(*p*-bromoacetamide)^4 derivative of TIPP is even more potent than the *p*-isothiocyanate derivative at inhibiting binding to cloned 6 opioid receptors in a wash-resistant manner (968).

Thiol-containing derivatives of opioid peptides have been prepared that potentially can form disulfide linkages with cysteine residues on opioid receptors. DALCE ([D-Ala^2,Leu^5,Cys^6]enkephalin,45, Fig. 7.8) (194) binds with high affinity to 6 receptors and has been used to characterize 6-receptor subtypes (see Section 3.2.4.3). 3-Nitro-2-pyridinesulfenyl (Npys)-containing derivatives of cysteine were incorporated into opioid peptides to yield potential affinity label derivatives (969–972). S-Activated enkephalin analogs containing the Npys group attached to the C-terminus label μ opioid receptors in a dose-dependent manner (969), whereas the Npys-protected derivative of DALCE reacts with 6 receptors (971). Incorporation of Cys(Npys) into position 8 of dynorphin A-(1–9) yields a peptide that decreases the B_{max} value, but does not affect the K_d value, for binding to κ receptors (970); recovery of binding after treatment with dithiothreotol suggests that the dynorphin analog binds to κ receptors through a disulfide bond. [D-Ala^2,Cys(Npys)^8]-dynorphin A-(1–9)-NH_2 is reported to label all three opioid receptors, whereas [D-Ala^2,Cys(Npys)^{12}]dynorphin A-(1–13)NH_2 apparently labels μ and δ, but not κ, receptors (972).

6.8 Peptidase Inhibitors

All of the ligands discussed so far in this chapter interact directly with opioid receptors. An alternative approach to producing analgesia is to inhibit the metabolism of the endogenous opioid peptides, thus increasing their concentration and occupancy of opioid receptors (see Refs. 973–976 for reviews). The two major enzymes involved in metabolism of the opioid peptides are the aminopeptidases, especially the bestatin-sensitive aminopeptidase-N [APN (EC 3.4.11.2)], and the neutral endopeptidase-24.11 [NEP or neprilysin (EC 3.4.24.11) (974)], which cleaves at the Gly-Phe bond. NEP has been cloned, and the crystal structure of the extracellular domain of the enzyme complexed with the inhibitor phosphoramidon has recently been reported (977). Both of these enzymes have a broad substrate specificity and cleave other peptides in addition to the opioid peptides. NEP particularly exhibits much lower activity toward longer opioid peptides, and thus the opioid effects resulting from inhibition of this enzyme are probably mainly due to interfering with the metabolism of the enkephalins and possibly the heptapeptide Met-enkephalin-Arg^6-Phe^7 (973). Other minor metabolic routes include

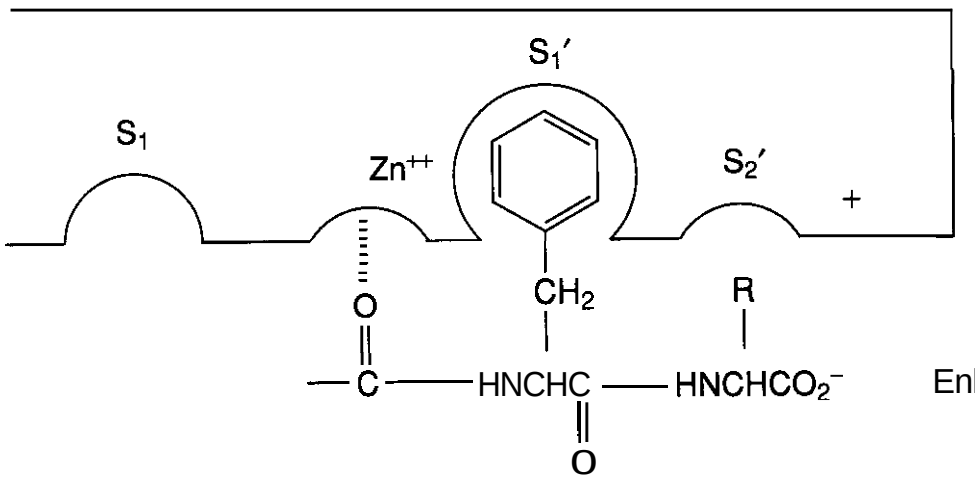
cleavage of the Gly-Gly bond by dipeptidylarninopeptidase (DAP).

Studies of peptidase inhibitors in brain slices indicate the importance of inhibiting both enzymes in order to significantly increase the concentrations of the endogenous opioid peptides (see Refs. 973–976). Thus, inhibiting NEP with thiorphan decreases the formation of [^{3}H]Tyr-Gly-Gly from [^{3}H]Met-enkephalin, but increases the production of [^{3}H]Tyr, whereas the opposite results were found with the APN inhibitor bestatin. Both APN and NEP, as well as the other enzymes involved in enkephalin metabolism, are zinc metallopeptidases. Thus it is possible to design mixed inhibitors capable of blocking multiple enzymes that more effectively protect the opioid peptides from metabolism (see below).

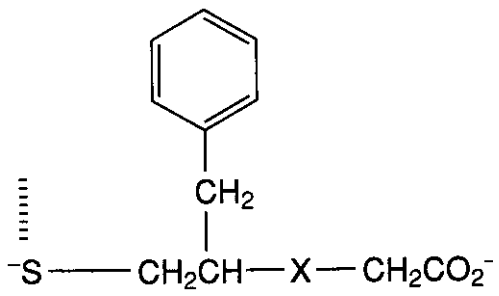
Because these enzymes are zinc metallopeptidases, all of their inhibitors contain a metal coordinating group such as a thiol, carboxylic acid, or hydroxamic acid. The first potent synthetic inhibitor of NEP, thiorphan (253;K_i = 4.7 nM) (978), was based on a model of the binding of enkephalins of the active site of NEP (Fig. 7.50). Thiorphan exhibits antinociceptive activity in mice that is blocked by naloxone. This inhibitor also inhibits angiotensin converting enzyme (ACE, K_i = 150 nM), so a variety of modifications to thiorphan were made in attempts to increase selectivity for NEP (see Refs. 973, 974 and references cited therein). Structural modifications of the P_1' and P_2' moieties generally led to mixed inhibitors of NEP and ACE, so changes were then made to the P_1'-P_2' amide bond. Retroinversion of this amide bond yields retrothiorphan (254) (979), which has similar affinity for NEP (K_i = 6 nM) as thiorphan, but greatly reduced affinity for ACE (K_i > 10,000 nM). The bioavailability of thiorphan is improved by protecting the thiol and carboxylic acid functionalities to give the prodrug acetorphan (255) (980), which is rapidly hydrolyzed to thiorphan by esterases. Acetorphan was the first NEP inhibitor examined in clinical studies, but it had no effect on flexion reflexes or pain sensation in humans (981), emphasizing the importance of inhibiting both NEP and APN to obtain analgesia. Acetorphan produces naloxone-reversible antidiarrheal effects (982), and is in clinical use for this indication in France (983).

In order to more completely inhibit enkephalin degradation, mixed inhibitors that can inhibit more than one of the metabolizing enzymes have been designed. Roques and coworkers developed hydroxamate mixed inhibitors, based on the hypothesis that the strength of the hydroxamate coordination to the zinc could overcome less than perfect fit of the inhibitor side chains to the active sites of the different metallopeptidases (973, 974). This led to the preparation of kelatorphan (256), the first virtually complete inhibitor of enkephalin metabolism (984). Kelatorphan, which is the R,S isomer, potently inhibits NEP, DAP, and APN (IC_{50} values of 1.8, 0.9, and 380 nM, respectively), whereas its S,S isomer is a potent selective inhibitor for NEP (IC_{50} = 1.8, 100, and 29,000 nM for NEP, DAP, and APN, respectively) (985). An analog of kelatorphan RB38A (257) exhibits similar potency for NEP and DAP, but greater potency against APN than that of kelatorphan (IC_{50} = 0.9, 2.5, and 120 nM, respectively) (986).

Although kelatorphan and related inhibitors exhibit nanomolar potency for both NEP and APN, these derivatives have high water solubility and do not readily cross the blood-brain barrier (973–975). When attempts to improve the bioavailability of these inhibitors met with little success, an alternative strategy was explored. In this approach potent inhibitors of NEP- and APN-containing thiol groups were linked by a disulfide bond (987). The disulfide bond, although stable in plasma, is readily cleaved in the brain. The dual inhibitor prodrug RBlOl (259) is very active in antinociceptive assays after either i.v. or s.c. administration at low doses (988). The RBlOl analog RB120 (258) has been reported to be orally active (989). The antinociceptive response to RBlOl is potentiated by a CCK-B (cholecystokinin) antagonist, presumably due to reversal of the physiological antagonism of the opioid system by CCK (975, 976). Unlike morphine and preceptor analgesics, both RB101 and kelatorphan are devoid of respiratory depressant effects (990). RB120 at analgesic doses also does not produce a discriminative response

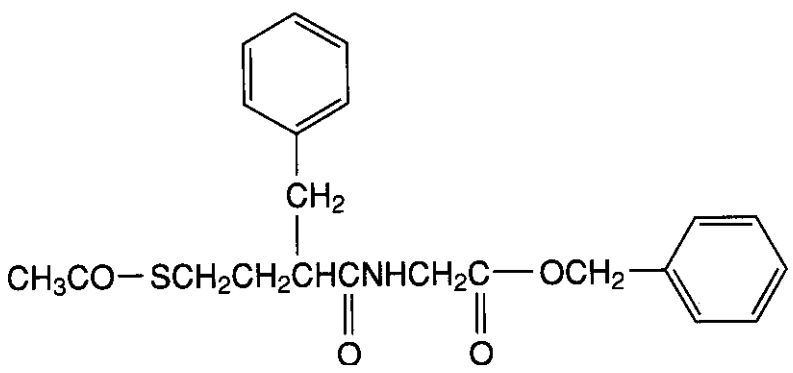


Enkephalin (enzyme substrate)

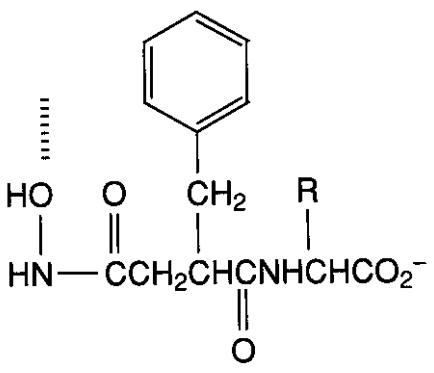


X = O ‖ CNH **(253)** Thiorphan

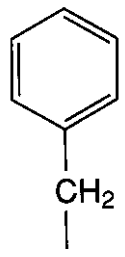
X = O ‖ NHC **(254)** Retrothiorphan

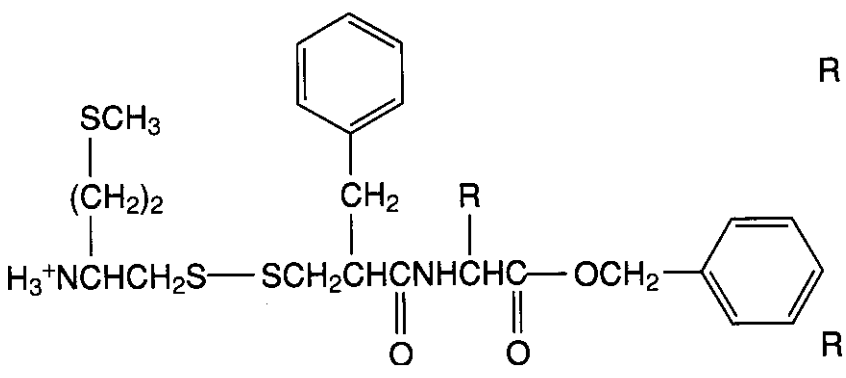


(255) Acetorphan



R = CH_3 **(256)** Kelatorphan

R =  **(257)** RB38A



R = CH_3 **(258)** RBI20

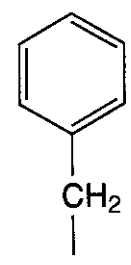
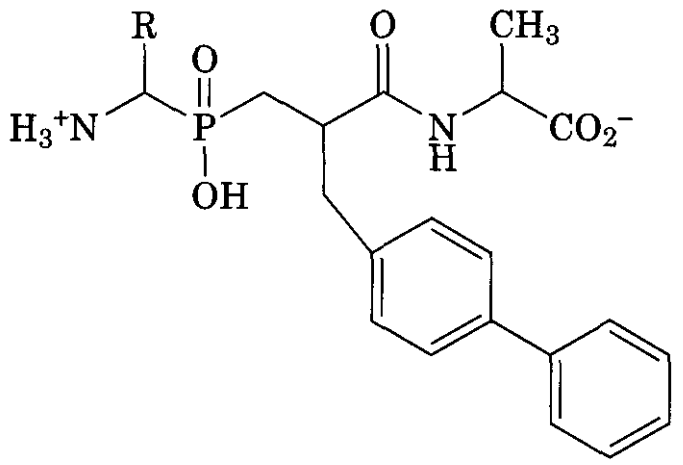
R =  **(259)** RBI01

Figure 7.50. Inhibitors of opioid peptide metabolism.

and does not generalize to morphine, suggesting that this inhibitor may have low abuse potential (991). These major differences in the side effect profile of these enzyme inhibitors compared to morphine has been attributed to the low tonic release of the endogenous enkephalins in brain regions relevant to these side effects of morphine (see Refs. 975, 976 and references cited therein).

Recently, a-aminophosphinic derivatives (260) have been identified as both selective



(260)

APN inhibitors (992) and dual inhibitors of both APN and NEP (993–995). The phosphinic moiety binds to the zinc in the active site and mimics the tetrahedral transition state formed during peptide bond cleavage. Optimization of the groups in the P,, P_1', and P,' sites resulted in the first true dual inhibitors of APN and NEP with low nanomolar affinity for both enzymes (993,994). These compounds are much more potent antinociceptive agents than kelatorphan after i.c.v. administration ($ED_{,,}$ = 12–20 nmol versus 158 nmol in the hot plate test), most likely as a result of the more efficient inhibition of APN; they are also more potent than RB101 ($ED_{,,}$ = 72 nmol), illustrating the advantage of a single compound with dual action (993). Large doses (100 mg/kg), however, were required to produce an antinociceptive response after i.v. administration (995), which was attributed to poor penetration of the blood-brain barrier by these polar compounds. Therefore prodrug derivatives were prepared by protecting both the carboxylic acid and phosphinic functionalities with lipophilic groups (995). These derivatives produced a long-lasting (−2 h) antinociceptive response after i.v. or i.p. administration. The kinetics for the hydrolysis of the two lipophilic groups were different, with the carboxylate ester undergoing much more rapid hydrolysis in plasma than in rat brain membranes; the deprotection of the phosphinate group was slower, resulting in approximately 55 and 80% of the completely deprotected compound after a 1-h incubation with plasma and rat brain membranes, respectively (995).

7 RECENT DEVELOPMENTS

7.1 Opioid Receptor Structure: Receptor Dimerization

As discussed earlier in Section 3.2.4.3, although pharmacological studies have suggested multiple subtypes of each of the opioid receptors, cloned opioid receptors consistently represent only a single subtype for each receptor type. Recently, an alternative explanation for opioid receptor subtypes has appeared in the literature, which could explain these discrepancies (see Ref. 996). Receptor dimerization has been described for both cloned 6 (997) and κ receptors alone (998) (homodimers), as well as for heterodimers between κ and 6 receptors (998) and between μ and 6 receptors (999, 1000). The κ-6 opioid receptor heterodimers exhibit a ligand binding profile that is virtually identical to that previously reported for the proposed κ_2-receptor subtype (998). The μ-δ heterodimers exhibit a distinct binding profile (999, 1000), binding some 6 receptor ligands (the $-selective antagonist BNTX and the 6,-selective agonist deltorphin II) but not others (the 6,-selective agonist DPDPE and the 6,-selective antagonist naltriben) (999). Receptor dimerization has been observed for other G-protein–coupled receptors and may be a universal phenomenon for receptor modulation (1001, 1002); this fascinating area was the subject of a recent symposium summarized in a special supplemental issue of Neuropsychopharmacology (vol. 23, number S4, 2000). Based on results for adrenergic-muscarinic chimeric receptors (1003), Reynolds and coworkers proposed that the dimerization interface includes transmembrane helices 5 and 6 (see Ref. 1004 and references cited therein), although these studies cannot distinguish between the contact dimer

and domain-swapped dimer modes of dimerization (1004). The growing evidence for dimerization of opioid receptors prompted Portoghese to revisit some of his earlier work on bivalent ligands and their possible interactions with dimeric receptors (321). Computational models of possible dimeric opioid receptors are only beginning to appear in the literature (321).

7.2 Opioid-Receptor-Like 1 (ORL1) Receptor and Its Endogenous Ligand Orphanin FQ/Nociceptin (OFQ/N)

During attempts to clone different opioid receptor types, several laboratories isolated a cDNA for a receptor with high homology to opioid receptors (see Refs. 87, 89, 90 for recent reviews). Because this receptor, referred to by Mollereau et al. as opioid-receptor-like 1 (ORL1) receptor (91), did not display affinity for classical opioid ligands, it was classified as an "orphan receptor." Subsequently, two groups isolated a 17-residue peptide as the endogenous ligand for this receptor (see Fig. 7.9) (92, 93). This peptide was referred to as orphanin FQ by one group because it was the ligand for the orphan receptor (F and Q are the N- and C-terminal residues, respectively, of the peptide) (92) and named nociceptin by the other group, since in the initial studies this peptide was reported to produce hyperalgesia (93). The OFQ/N precursor protein prepronociceptin (93) contains additional biologically active peptides related to OFQ/N. Nocistatin (Fig. 7.9) (271) blocks a number of the effects of OFQ/N (see Ref. 1005 for a review), but nocistatin does not interact with ORL1 receptors. A second 17-residue peptide, referred to as orphanin/nociceptin II, is also found in prepronociceptin (270), along with a longer peptide $OFQ/N_{160-187}$ (1006); these peptides, however, have not been as well characterized as OFQ/N (see Ref. 87 for a recent review).

Like opioid receptors, the ORL1 receptor is a G-protein–coupled receptor, consisting of seven transmembrane (TM) regions plus extracellular and intracellular domains. The ORL1 receptor exhibits high sequence homology to opioid receptors, with 60–62% identity for the whole transmembrane domain (residues 52–342 in the human ORL1 receptor) (90) and higher homology in TM2, 3, and 7. There is also high sequence homology with opioid receptors in the intracellular loops, consistent with ORL1 receptors coupling to the same G-proteins as opioid receptors. Splice variants of the ORL1 gene have been reported (see Refs. 87, 90 for reviews), and the results of some pharmacological studies have suggested receptor heterogeneity (see Ref. 87), but the existence of subtypes of the ORL1 receptor has not been firmly established.

The ORL1 receptor exhibits high selectivity for its endogenous ligand, and has very low affinity for most opioid ligands. Site-directed mutagenesis and chimeric receptor studies have examined possible structural reasons for this selectivity of the ORL1 receptor. Point mutations of only four residues in TM6 [VQV276–278IHI] and TM7 [T302I] of the ORL1 receptor were sufficient to impart binding affinity for Dyn A fragments without affecting the affinity or potency of OFQ/N (176). The additional mutation of a residue in TM5 [A213K] enhanced affinity for several opioid alkaloids, particularly antagonists (1007), but this mutant no longer bound OFQ/N. Alanine mutation of several TM residues that are conserved with opioid receptors yielded mutant receptors with reduced affinity for OFQ/N (1008), suggesting that the binding pocket in the ORL1 receptor may be similar to that found in opioid receptors. Alanine replacement of Gln^{286} at the C-terminus of TM6 in hORL1 results in a mutant to which OFQ/N still binds with high affinity, but which cannot mediate inhibition of forskolin-stimulated cAMP formation (1008), implicating this residue in ORL1 receptor signal transduction.

OFQ/N and the ORL1 receptor exhibit the greatest similarity to dynorphin A and κ opioid receptors, respectively, but OFQ/N exhibits very low affinity for κ receptors and, conversely, Dyn A exhibits low affinity for ORL1 receptors. To study the structural reasons for this selectivity, chimeric receptors have been constructed between ORL1 and κ receptors (1009, 1010). Replacement of the N-terminal region of the κ receptor up through the top of TM3 with the corresponding sequence of the ORL1 receptor imparted affinity for OFQ/N, but low potency in an adenylyl cyclase assay, without affecting the binding or potency of Dyn A. Further incorporation of extracellular

loop 2 (EL2) from the ORL1 receptor restored efficacy for OFQ/N, again without affecting the ability of Dyn A to bind and activate the receptor. Thus, EL2 appears to be required for activation of ORL1 receptors, but not κ opioid receptors.

Based on the experimental data, a computational model of OFQ/N bound to ORL1 receptors has been proposed (189). In this model the N-terminal sequence containing the two Phe residues binds in a highly conserved pocket formed by TM3, 5, 6, and 7, which is similar to that proposed for opioid receptors. Residues 5–7 (Thr-Gly-Ala) of OFQ/N are then positioned at the TM-EL2 interface in a largely nonconserved region; unfavorable side-chain interactions in this region of the receptor are then used to explain the selectivity of the ORL1 receptor for OFQ/N over Dyn A. The positively charged C-terminus of the peptide is proposed to make multiple contacts with the highly acidic EL2.

7.2.1 Physiological and Pharmacological Effects. Since their discovery, interest in this receptor and its endogenous ligand has increased exponentially. There have been a number of excellent reviews covering the complex pharmacology of this system (see Refs. 87–89 for recent reviews), including a special issue of the journal *Peptides* (Vol. 21, Number 7, 2000) devoted solely to the ORL1 receptor and OFQ/N.

Consistent with the sequence similarities between the ORL1 and opioid receptors, activation of the ORL1 receptor triggers the same signal transduction mechanisms as used by the opioid receptors. Thus activation of ORL1 receptors inhibits both forskolin-stimulated adenylyl cyclase and Ca^{++} currents and activates several other effectors, including inward rectifying K^+ channels, protein kinase C, mitogen-activated protein kinase (MAP kinase) and phospholipase C (see Ref. 89 for a review).

There has been considerable controversy over the roles of ORL1 receptors and OFQ/N in response to painful stimuli (see Refs. 87, 89, 1011 for recent reviews). When administered by intracerebroventricular (i.c.v.) injection OFQ/N was initially reported to produce hyperalgesia (92, 93), hence the name nociceptin for the endogenous peptide ligand. These effects, however, were subsequently classified as antianalgesic effects (89, 1011) based on re-evaluation of the controls and the effects of stress-induced analgesia accompanying the. experimental procedures. Effects reported for OFQ/N administered i.c.v. have ranged from hyperalgesia, analgesia, or anti-analgesic activity to a combination of these effects (87, 89, 1011). A similar range of activities have been. reported after spinal (intrathecal) administration (87, 89, 1012). A number of factors appear to influence these often contradictory findings, including the noxious stimuli studied, the species and strain of animal used, the dose of OFQ/N, stress, and the physiological state of the animal (see Ref. 87 for a detailed discussion). The most robust and consistently observed effects of OFQ/N are the anti-analgesic effects after supraspinal administration. OFQ/N acts as a functional antagonist of opioid receptors and blocks analgesia produced by a wide variety of opioids (see Refs. 87, 89). At the spinal level the predominant effect of OFQ/N appears to be inhibitory, resulting in analgesia and/or anti-hyperalgesia/anti-allodynia (see Refs. 87, 89, 1012). Several studies have reported anti-hyperalgesic or anti-allodynic activity for OFQ/N in rat models of inflammation and nerve injury (89, 1012). Because morphine appears to have reduced effectiveness in treating neuropathic pain after nerve injury, the activity of OFQ/N in this type of pain could have important therapeutic implications. OFQ/N has also been implicated in the development of tolerance to morphine (see Refs. 87, 89).

OFQ/N and the ORL1 receptor are also involved in a number of other physiological effects (see Refs. 87,891. One of the most significant effects is the anxiolytic activity of OFQ/N (1013), which has been postulated to be one of OFQ/N's most fundamental actions, and may help explain the effects of OFQ/N on other phenomena [e.g., locomotion, reward, and feeding (87)]. A small molecule ORL1 agonist has also demonstrated anxiolytic activity (1014), demonstrating an important potential therapeutic application of these compounds. Like opioids, OFQ/N inhibits electrically induced contractions in the GPI and MVD smooth muscle preparations; these effects are

not naloxone reversible, indicating that they are not mediated by opioid receptors (see Ref. 89 for a review).

7.2.2 Structure-Activity Relationships of OFQ/N and Other Peptidic Ligands for the ORL1 Receptor. Shortly after the identification of the endogenous ligand, several research groups began exploring the SAR of this peptide. Several recent reviews (88, 1015, 1016) have described the details of the SAR of OFQ/N, so the discussion here focuses on some of the key findings.

Truncation studies have been performed to identify the minimum sequence required for ORL1 affinity and activation. These studies revealed that, like opioid peptides, the N-terminal aromatic residue was important for biological activity (1017, 1018), although one study (1017) reported that the further N-terminal truncated fragments OFQ/N-(6–17) and OFQ/N-(12–17) retain affinity and activity for ORL1 receptors. In contrast to dynorphin A, where shorter fragments retain opioid activity (263), 13 of the 17 residues of OFQ/N appear to be required for ORL1-receptor affinity and activation (1017, 1018). The amide derivative of OFQ-(1–13) is a considerably more potent agonist in the mouse vas deferens assay than the acid derivative, apparently because of decreased metabolism (1016); therefore this fragment is typically used as the parent structure in further SAR studies (see below). OFQ/N-(1–11), however, has been reported to be active both in *vitro* (1019) and *in vivo* (1020), despite its low affinity for cloned ORL1 receptors in binding assays, resulting in the proposal of receptor heterogeneity for OFQ/N (1019). Results from binding studies have also suggested receptor heterogeneity (see Ref. 87).

A number of analogs of OFQ/N have been examined for their pharmacological activity (see Refs. 88, 1015, 1016 for recent reviews). Shortly after identification of the endogenous ligand the results of an alanine scan of OFQ/N were reported, identifying Phe^1, Phe^4, and Arg^8 as critical residues in the sequence (1017, 1021). Phe^1 can be replaced by tyrosine (1018, 1021), resulting in an analog that retains affinity and potency at ORL1 receptors, but also exhibits increased affinity and activity at opioid receptors, particularly κ and μ receptors (see Refs. 1015, 1016). Unlike in the opioid peptides, an aromatic amino acid is not required in position 1, and Phe^1 can be replaced by the aliphatic residues cyclohexylalanine and leucine (1022). In contrast, an aromatic residue is required in position 4, and replacement of Phe^4 with an aliphatic residue results in loss of activity (1022). An Arg in position 8 appears to be critical, and replacement with Lys results in large decreases (>100-fold) in affinity and potency (1016). Incorporation of Tyr in place of Leu^{14} was used to obtain an analog that could be radioiodinated or tritiated for use in radioligand binding assays (92); both labeled derivatives are commercially available.

A series of chimeric peptides between OFQ/N and Dyn A were prepared to explore the structural reasons for the selectivity of OFQ/N for ORL1 over κ opioid receptors (1023). The results from this study suggested that residues Thr^5 and Gly^6, in addition to Phe^1, are responsible for the activity and selectivity of OFQ/N for ORL1 receptors; the chimera Dyn A-(1–5)/OFQ/N-(6–17) (261) was able to bind and activate both ORL1 and κ opioid receptor.

Dyn A-(1–5)/OFQ/N (6–17) (**261**)

Tyr-Gly-Gly-Phe-Leu–Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Am-Gln

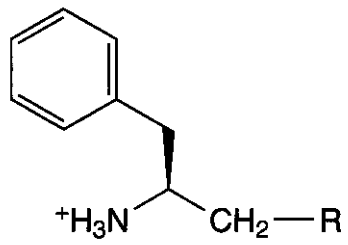
Early studies of the pharmacology of the ORL1 system were hindered by the lack of antagonists for this receptor. Therefore there was considerable excitement in the field when the first report of an antagonist appeared in the literature (1024). The reduced amide derivative of OFQ/N [$Phe^1\psi(CH_2NH)Gly^2$]OFQ/N-(1–13)NH_2 (262, Fig. 7.51; referred to as [F/G]NC(1–13)NH_2 by Calo and coworkers), which was synthesized to protect the peptide from metabolism by aminopeptidases, was initially reported to be an antagonist of OFQ/N-(1–13)NH_2 in smooth muscle preparations (1024). Subsequent examination of this compound in a variety of assays, however, indicated that the activity observed depended on the assay and that although (262) was an an-

tagonist in some assays, it was a partial or full agonist in a number of other assays, including forskolin-stimulated cAMP accumulation in Chinese hamster ovary cells expressing cloned ORL1 receptors (see Refs. 88, 1015 for detailed reviews). Subsequently, the N-substituted glycine analog [Nphe^1]OFQ/N-(1–13)NH_2 (263) was reported to be an ORL1-receptor antagonist (1025). Although the potency of the compound is weak ($pA_2 < 6$ in most assays), it is an antagonist in all of the assays examined to date (see Refs. 88, 1015 for reviews).

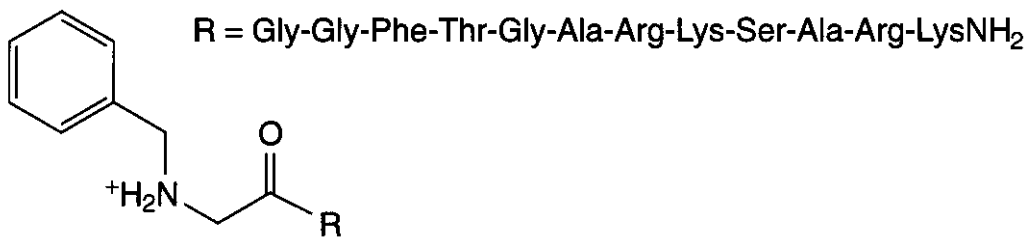
The use of combinatorial libraries has resulted in the identification of peptidic and peptidomimetic ligands for the ORL1 receptor that are not structurally related to OFQ/N. Houghten and coworkers identified acetylated hexapeptides with high affinity for the ORL1 receptor, with Ac-RYYRXK-NH_2 (264; X = W or I) having the highest affinity (1026); in most assays these peptides exhibit partial agonist activity (see Ref. 1015). A combinatorial library of conformationally constrained peptides resulted in the identification of III-BTD (265) that exhibits moderate affinity (K_i = 24 nM) but only modest selectivity [K_i ratio (ORL1/μ/κ/δ) = 1/4.6/6.1/22] for ORL1 receptors; this compound acts as an antagonist at ORL1 receptors, whereas it exhibits partial agonist activity at opioid receptors (1027). The related conformationally constrained peptide III-Haic (266) was nonselective, exhibiting modest affinity for ORL1 and the three opioid receptors (K_i = 50–125 nM) (1027).

7.2.3 Nonpeptide Ligands for the ORL1 Receptor. Because of the potential therapeutic applications of ORL1 receptor ligands, there has been considerable interest in identifying nonpeptidic compounds that interact with this receptor. Several opiates have been reported to exhibit some affinity for ORL1 receptors (see Refs. 1015, 1016) with some opiates, most notably the fentanyl analog lofentanyl (1018), naloxone benzoylhydrazone [Nal(BzO)H, 50] (see Refs. 1015, 1016), the naltrexamine derivative TRK-820 (65, Fig. 7.16) (311), and buprenorphine (1028), exhibiting reasonable affinity and/or potency. Reports by groups from the pharmaceutical industry of both nonpeptidic selective agonists and antagonists have begun to appear in the scientific and patent literature, with leads identified from screening assays (see Refs. 1015, 1016, 1029 for recent reviews). Starting from a lead (267, Fig. 7.52), identified by high throughput screening as a nonselective ORL1 ligand, a group from Hoffmann-La Roche explored a number of 1-phenyl-1,3,8-triaza-*spiro*[4.5]decan-4-ones to identify more selective and potent ORL1 agonists (1030–1033). The pharmacology of one of these compounds, Ro 64-6198 (**268**), has been examined in considerable detail (1014, 1033–1036), including determination of the affinities of the different stereoisomers for opioid receptors as well as ORL1 receptors (1033). The stereochemistry at positions 1 and 3a has significant effects on ORL1-receptor affinity, whereas the affinities for the opioid receptors are comparable for the different isomers; the 1*S*,3a*S* isomer exhibits the highest affinity for ORL1 receptors (pK_i = 9.41) and therefore the highest selectivity for ORL1 over opioid receptors (1033). This compound is a full agonist at ORL1 receptors and produces dose-dependent anxiolytic effects in several rat models of anxiety, with an efficacy and potency after systemic administration comparable to those of benzodiazepine anxiolytics such as diazepam (1014).

Researchers from the Banyu Tsukuba Research Institute reported the first nonpeptide antagonist for ORL1 receptors (1037). Starting from a lead (269) from their chemical library, which again exhibited reasonable affinity but poor selectivity for ORL1 receptors, these researchers identified J-113397 (**270**), which exhibits high affinity (K_i = 1.8 nM) and high selectivity for ORL1 receptors over opioid receptors (1037–1039). This compound is an antagonist of OFQ/N *in uitro* and *in vivo* after subcutaneous administration (1038, 1039). A series of 4-aminoquinolines (271) has also been examined for antagonist activity, based on the weak affinity of the lead compound (**271**) in a random screen (1040). JTC-801 (**272**) was selected because it showed the best bioavailability. After oral administration this compound was shown to antagonize the effects of OFQ/N and to produce an analgesic effect that was not antagonized by naloxone; this compound is reported to be undergoing clinical trials (1040).



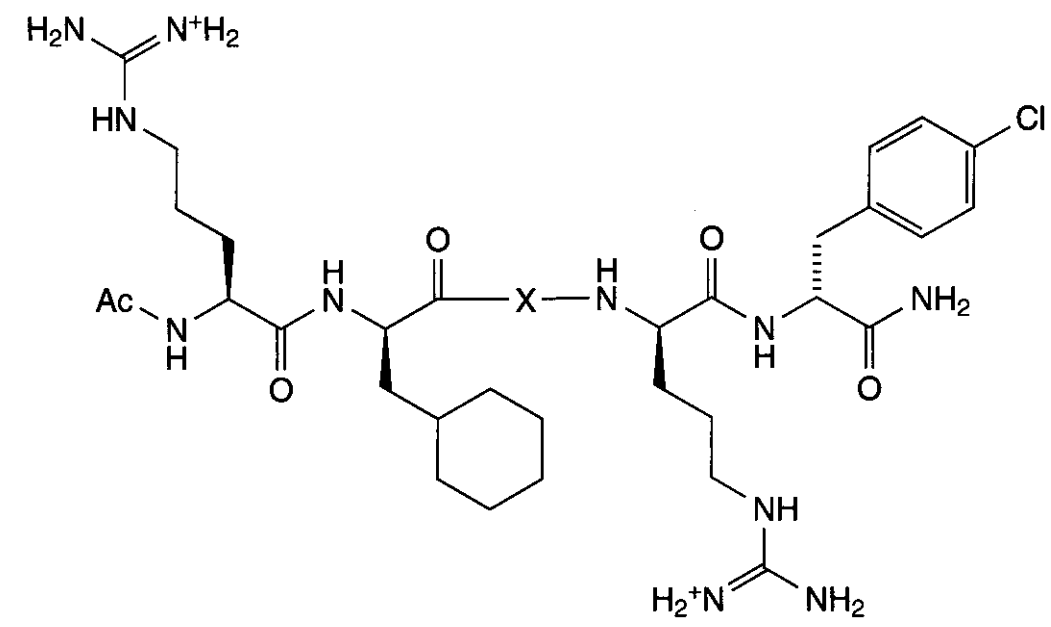
(**262**) [F/G]NC(1–13)NH_2



(**263**) [Nphe^1]OFQ/N (1–13)NH_2

Ac-Arg-Tyr-Tyr-Arg-X-LysNH$_2$

(264) X = Trp or Ile



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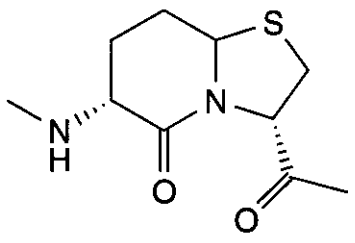

(265) III-BTD

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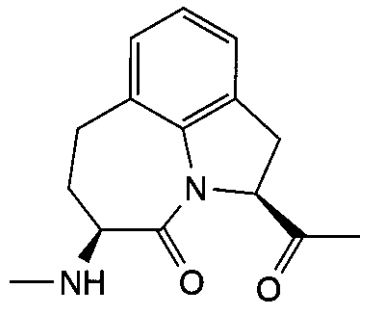
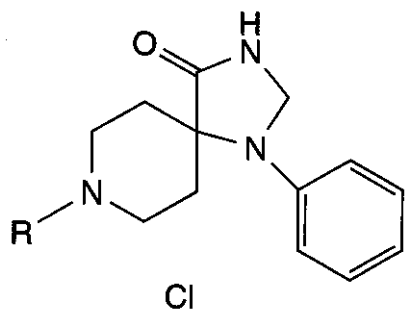


(266) III-Haic

Figure 7.51. Peptidic ligands for ORL1 receptors.

8 THINGS TO COME

Several advancements in opioid pharmacology during recent years could have **a** significant impact on the types of compounds used as narcotic analgesics in the future. The involvement of peripheral opioid receptors in inflammatory pain (see Section **3.3)** has resulted in the search for peripherally selective analgesics (see Section 5.9.2), which would be free from serious centrally mediated side effects. The challenge in this area may be to obtain com-



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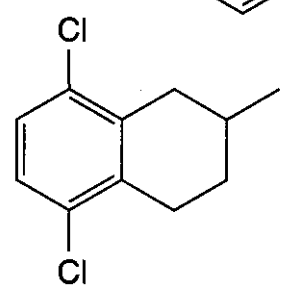

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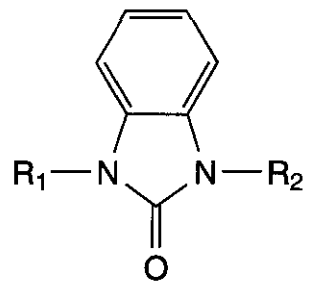
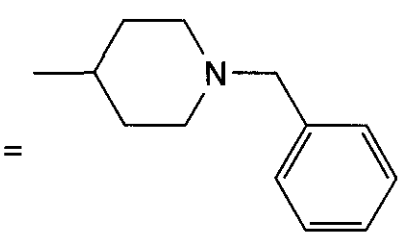


R_1 = H, R_2 =



(269)

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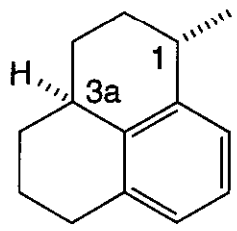

(268) Ro 64-6198

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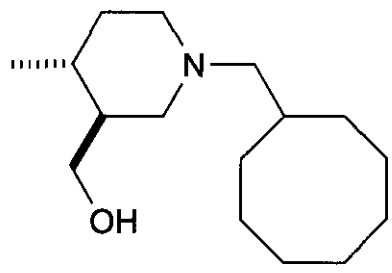
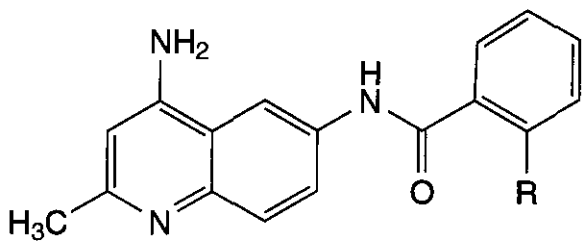
R_2 =

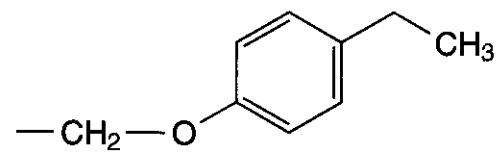


(270) J-11397



(271) R = **Ph**

R =



(272) JTC-801

Figure 7.52. Nonpeptidic ligands for ORL1 receptors.

pounds that do not cross the blood-brain barrier, but that are still orally bioavailable. The peripherally selective antagonists **methylnaltrexone** and alvimopan are currently undergoing clinical trials for treatment of **opioid-induced** constipation and related GI side effects.

The ability of 6-receptor antagonists to decrease the development of tolerance and dependence to morphine (see Section 3.3.1) has considerable therapeutic potential and has prompted the search for compounds with both μ agonist and δ antagonist activity. Although **peptide** derivatives with both activities have been studied in some detail (see Section **6.4.2**), nonpeptide opioids with this activity profile have been reported only recently. This approach of using δ antagonism together with μ agonism, either combined in a single drug or by coadministering two agents, to minimize the side effects of μ **agonists** is very exciting. This approach is still in its infancy, however, so its therapeutic application in clinical trials still remains to be demonstrated.

There has also been considerable advancement in the identification of agents that produce analgesic effects through different mechanisms than interaction with opioid receptors (see Refs. 1041–1044 for reviews). Thus NMDA (*N*-**methyl-D-aspartate**) antagonists, GABA (**γ-aminobutyric** acid) agonists, nicotinic acetylcholine receptor agonists (**e.g., epibatadine**), antagonists of substance P at NK-1 receptors, and a number of other compounds targeting different receptors exhibit **antinociceptive** activity in animal models. These compounds do not cause the side effects associated with the clinically used opiates, but they have their own distinct side effect profiles, which in some cases (**e.g.,** NMDA antagonists, which cause psychotomimetic effects) have resulted

in termination of clinical studies (see Ref. 1042). Also, the promising antinociceptive activity observed in animal models has not always translated into clinical efficacy in humans. Thus a number of NK-1-receptor antagonists are highly effective in animal models of pain and exhibit excellent pharmacokinetic profiles in humans, but have failed in phase II clinical trials for treatment of pain and migraine (see Ref. 1042). Thus, whether any of these novel targets will ultimately result in clinically used agents for pain remains to be determined.

Clearly, one very exciting target for the development of potential therapeutic agents is the ORL1 receptor (Section 7.2). Because of the complexity of ORL1 receptor involvement in pain perception, however, it is not clear at this time whether ligands for this receptor will prove to be clinically useful analgesics. The anxiolytic activity of ORL1 agonists, however, represents a promising approach for the development of novel clinically useful agents for this indication.

A major unmet therapeutic need is effective treatments for substance abuse, including abuse of opioids, cocaine, and amphetamines. Results for ligands interacting with either δ or κ receptors hold some promise in this area (see Section 3.3), but whether such agents will be clinically useful remains to be demonstrated. Although the results of some initial studies in humans have been promising [e.g., an improved positive response of opioid-dependent individuals to a "functional" κ antagonist (buprenorphine in the presence of naltrexone to block μ-agonist activity) as compared to naltrexone alone (249)], other studies have been disappointing [although the κ-selective agonist enadoline reduced the positive subjective effects of cocaine, neither enadoline nor the mixed agonist butorphanol modified self-administration of cocaine in humans (255)].

The goal of identifying potent analgesics free of the side effects of morphine and other narcotics has remained elusive. As more information has become available about opioid receptor structure, opioid pharmacology, and related systems (i.e., the ORL1 receptor), this has provided new challenges to medicinal chemists to prepare compounds with unique pharmacological profiles. With the diversity of structures exhibiting affinity for opioid receptors, there is still ample opportunity for the development of new therapeutic agents that, hopefully, will bring us closer to the goal of identifying optimal analgesics.

9 WEB ADDRESSES AND RECOMMENDED READING FOR FURTHER INFORMATION

9.1 Clinically Used Agents

- Drug Facts and Comparisons, Wolters Kluwer, St. Louis, 2001. May be accessed at www.factsandcomparisons.com
- C. Stein, Ed., Opioids in Pain Control: Basic and Clincial Aspects, Cambridge University Press, Cambridge, 1999. This book contains several chapters discussing the clinical use of opioids in different types of pain and clinical settings in addition to chapters discussing the pharmacology of opioids.

9.2 Pharmacology

- H. B. Gutstein and H. Akil in J. G. Hardman, L. E. Limbird, and A. G. Gilman, Eds., Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th ed., McGraw-Hill Medical Publishing Division, New York, 2001, pp. 569–619.
- A. Herz, H. Akil, and E. J. Simon, Eds., *Opioids I* and Opioids *II*, Handbook of Experimental Pharmacology, Vols. 104/I and 104/II, Springer-Verlag, Berlin, 1993. Two volumes containing comprehensive reviews of opioid pharmacology.

9.2.1 Opioid Receptor Structure and Molecular Biology

- University of Minnesota Center for Opioid Research and Design (CORD), http://www.opioid.umn.edu. This site includes computational models of the opioid receptors, a complete list of chimeric receptors and point mutations in opioid receptors with pharmacological data and literature references, and links to other relevant sites.
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9.2.2 Dimerization of Opioid and Other G-Protein–Coupled Receptors

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o G. R. Lenz, S. M. Evans, D. E. Walters, and A. J. Hopfinger, Opiates, Academic Press, Orlando, FL, 1986.

The above two comprehensive books contain detailed reviews of the early literature.

- A. F. Casy and G. H. Dewar, The Steric Factor in Medicinal Chemistry: Dissymmetric Probes of Pharmacological Receptors, Plenum Press, New York, pp. 429–501 and 503–548.

9.4 Delta and Kappa Opioid Receptors and Selective Ligands

9.4.1 Delta Opioid Receptor Pharmacology and 6-Receptor-Selective Ligands

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o G. Dondio, S. Ronzani, and P. Petrillo, Nonpeptide δ Opioid Agonists and Antagonists (Parts I and II), Expert Opin. Ther. Patents, 7,1075–1098 (1997); 9,353–374 (1999).

9.4.2 Kappa-Receptor-Selective Ligands

- A. Barber and R. Gottschlich, Novel Developments with Selective, Non-Peptidic Kappa-Opioid Receptor Agonists, Expert Opin. Invest. Drugs, 6,1351–1368 (1997).

- G. Giardina, G. D. Clarke, M. Grugni, M. Sbacchi, and V. Vecchietti, Central and Peripheral Analgesic Agents: Chemical Strategies for Limiting Brain Penetration in Kappa-Opioid Agonists Belonging to Different Chemical Classes, Farmaco, 50, 405–418 (1995).

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9.5 Opioid Peptides

- P. W. Schiller, Ed., Peptide and Peptidomimetic Ligands of Opioid Receptors, *Biopolymers*, 51, 377–458 (1999). This issue of Biopolymers contains reviews covering opioid receptor structure as well as peptide and peptidomimetic ligands for these receptors:

9.6 ORL1 Receptor and Orphanin FQ/Nociceptin

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CHAPTER EIGHT

Antidepressants

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Contents

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1 INTRODUCTION

The term depression refers to a depression of mood than can be a transient but, more commonly, is a chronic condition. The symptoms are distressing; patients report an ineffable and all-pervading depth of despair and hopelessness far beyond normal experience **(1,** 2). Depressed mood is typically accompanied by low energy or fatigue and sleep disturbances. Physical, social, and personal functioning are greatly impaired, leading to at least as much handicap as such physical illnesses as diabetes or arthritis. The risk of suicide is greatly increased. Depression is an important and common disorder: each year about 100 million people worldwide develop depression (2, 3), with the prevalence in women being about twice that in men. Depression can also be associated with other psychiatric disorders, such as anxiety disorders, or with physical illnesses that can worsen the prognosis. Although a partial remission of symptoms rather than full recovery is the usual outcome of drug treatment, the availability of safe and effective antidepressants is a major achievement of twentieth century psychopharmacology. Worldwide sales of antidepressants already exceed US$10 billion annually, making them the single most important group of psychopharmaceuticals, and include some of the most widely used prescription drugs (e.g., fluoxetine; Prozac).

The new wave of safer antidepressants introduced in the 1990s, led by fluoxetine (Prozac),is dominated by drugs that are serotonin-selective reuptake inhibitors (SSRIs). The SSRIs exhibit some adverse side effects, notably in impaired sexual function in both men and women, but because they are relatively safe physicians have been less inhibited about using them. This has extended the clinical recognition and treatment of depression to a much greater number of people than before. At the same time, new indications have been demonstrated and officially approved for the SSRIs, including syndromes associated with anxiety and panic, extending still further the wide use of these agents in psychopharmacology. There is even a growing veterinary use of such agents for companion animals who appear depressed!

2 CLINICAL USE

2.1 Available Agents and Classification

Table 8.1 lists the ***26*** antidepressant drugs currently registered for use in the United States and Europe. All are administered orally, usually on a once-a-day dosing regime. **A** typical course of treatment lasts 3 months. Why do we have so many antidepressants, and why does there still exist a need for additional agents? There are four primary reasons.

1. The initial selection of an antidepressant for the treatment of depressed patients depends to a large extent on diagnostic criteria. Often, however, the first agent selected is not the most therapeutically beneficial or best tolerated by the patient. Subsequent selection is empirical and involves trials with different agents from different classes (and sometimes even from within the same class), to identify the most suitable agent (i.e., the most effective agent with the fewest undesirable side effects) for a given patient.
2. Although effective, many of the presently available agents, particularly the tricyclic antidepressants (TCAs), produce undesirable side effects. Patients who initiate ther-

Table 8.1 Antidepressants: Drugs Currently on the Market (6, 7)

Generic Name	Proprietary Name: United States	Proprietary Name: Europe	Manufacturer	Daily Dose Range (mg)
Amitriptyline (1)	Elavil	Lentizol	Astra Zeneca	50–200
Amoxapine (2)	Asendin	Asendis	Wyeth Ayerst	100–250
Bupropion (3)	Wellbutrin	Wellbutrin	GlaxoSmithKline	300456
Citalopram (4)	Celexa	Cipramil	Lundbeck	20–60
Clomipramine (5)	Anafranil	Anafranil	Novartis	30–150
Desipramine (6)	Norpamin	—	HMR	100–300
Dothiepin (7)	—	Prothiaden	Knoll	75–150
Doxepine (8)	Sinequan	Sinequan	Pfizer	10–100
Fluoxetine (9)	Prozac	Prozac	Eli Lilly	20–60
Fluvoxamine (10)	Luvox	Faverin	Solvay	100–200
Imipramine (11)	Tofranil	Tofranil	Novartis	50–200
Lofepramine (12)	—	Gamanil	Merck	140–210
Maprotiline (13)	Ludiomil	Ludiomil	Novartis	25–75
Mirtazapine (14)	Remeron	Zispin	Organon	1545
Moclobemide (15)	—	Manerix	Roche	300–600
Nefazodone (16)	Serzone	Dutonin	BMS	100400
Nortriptyline (17)	Aventyl	Allegron	King	75–150
Paroxetine (18)	Paxil	Seroxat	GlaxoSmithKline	20–60
Phenelzine (19)	Nardil	Nardil	Parke Davis	30–45
Protriptyline (20)	Vivactil	—	Merck	15–60
Reboxetine (21)	Edronax	Edronax	Pharmacia/Upjohn	8–12
Sertraline (22)	Zoloft	Lustral	Pfizer	50–200
Tranylcypromine (23)	Parnate	Parnate	GlaxoSmithKline	20–30
Trazodone (24)	Desyrel	Molipaxan	HMR	150–300
Trimipramine (25)	Surmontil	Surmontil	Wyeth Ayerst	150–300
Venlafaxine (26)	Effexor	Effexor	Wyeth Ayerst	75–150

apy on an SSRI, for example, are more likely to complete a course of adequate dose and duration of antidepressant therapy than patients who initiate therapy with a TCA (4). In a review of more than 100 cases, patients receiving SSRIs tended to discontinue medication less often than those on TCAs or heterocyclic antidepressants. The difference was statistically significant when SSRI use was compared with use of TCAs, but the differences were not as significant when the SSRIs were compared to the heterocyclic antidepressants. The findings were attributed more to the side effects associated with the TCAs than with the therapeutic efficacy of the agents (5).

3. Most antidepressants have a delayed time of onset; currently available antidepressants require administration for at least 2–4 weeks before effects are evident. Newer agents with shorter onset times, or newer strategies for enhancing the onset time of existing agents, are required to overcome this problem.
4. Finally, there is a certain population of patients who are resistant to current therapies.

Most antidepressants in clinical use today act by enhancing the neurotransmission of serotonin [5-hydroxytryptamine (5-HT)], norepinephrine [NE; noradrenaline (NA)], or both. They do so either by blocking the reuptake (transport) of neurotransmitter, blocking the metabolism of neurotransmitter [i.e., monoamine oxidase (MAO) inhibitors], or by direct action on a neurotransmitter receptor. Hence, the antidepressants can be classified on the basis of their putative mechanisms of action (Table 8.2 and Figs. 8.1–8.4). Agents that block neurotransmitter reuptake can be further divided into those that are nonselective (e.g., tricyclic antidepressants with mixed action), serotonin-selective reuptake

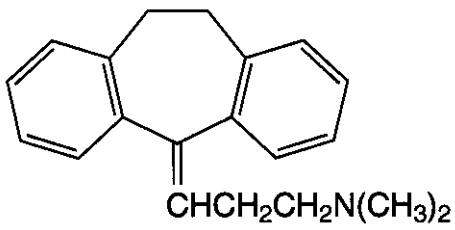
Table 8.2 Mechanistic Classification of Antidepressants and Other Types of Agents/Mechanisms Currently Being Explored for the Treatment of Depression

Transport Blockers (reuptake inhibitors)	
Tricyclic antidepressants (TCAs)	
Mixed 5-HT/NE	Amitriptyline (1) Arnoxepine (2) Clomipramine (5) Dothiepin (7) Doxepin (8) Imipramine (11) Trimipramine (25)
NE-Favoring	Desipramine (6) Maprotiline (13) Nortriptyline (17) Protriptyine (20) Lofepramine (12)
Serotonin-selective reuptake inhibitors (SSRIs)	Citalopram (4) Fluoxetine (9) Fluvoxamine (10) Paroxetine (18) Sertraline (22) Venlafaxine (26)
Norepinephrine-selective reuptake inhibitors (NSRIs; NaRIs)	Reboxetine (21)
Monoamine oxidase (MAO) inhibitors	
Irreversible MAO inhibitors	Phenelzine (19) Tranylcypromine (23)
Reversible (RIMAs)	Moclobemide (15)
Serotonergic agents	Nefazodone (16) Trazodone (24)
Other agents	Bupropion (3) Mirtazepine (14)

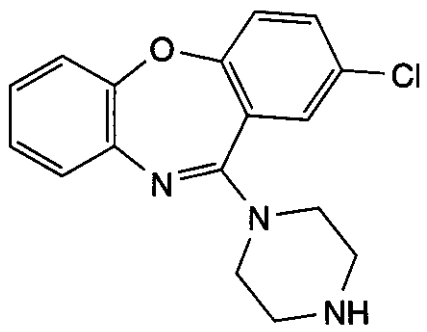
inhibitors (SSRIs), and norepinephrine-selective reuptake inhibitors (NSRI; referred to in Europe as noradrenergic-selective reuptake inhibitors or NaRIs). Nomenclature has recently become a bit unwieldy, in that the newer nonselective agents are referred to as serotonin- and norepinephrine-selective reuptake inhibitors (SNRIs), to distinguish them from the nonselective tricyclic antidepressants. Furthermore, some investigators now classify the norepinephrine-favoring tricyclic antidepressants as NSRIs; however, this latter nomenclature will not be used here to refer to the tricyclic antidepressants. A newer category of antidepressants is the "heterocyclic antidepressants," to differentiate them from other known antidepressants (most of which also happen to be heterocyclic!). For the most part, their mechanism(s) of action is not known with certainty; there is evidence, however, that many of these agents interact directly with serotonin receptors in the brain. The term "heterocyclic antidepressants" will not be used herein. Finally, some newer agents, with potentially unique mechanisms of action, are currently being explored; some of these agents are discussed.

2.2 Clinical Efficacy of Antidepressants

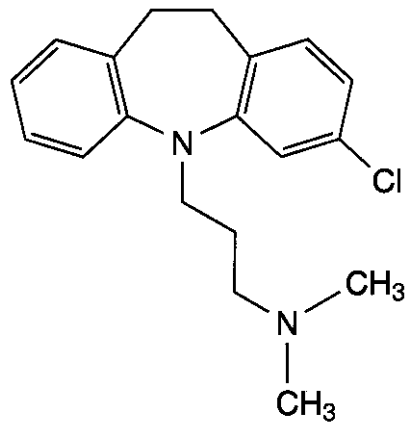
Antidepressants are given orally usually for extended periods of treatment ranging from a few weeks to many months in duration.



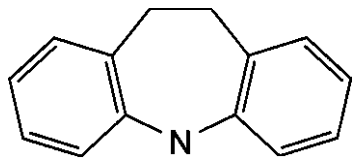
Amitriptyline (1)

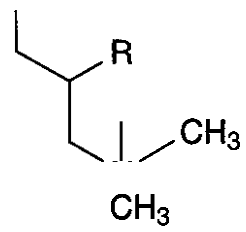


Amoxepine (2)

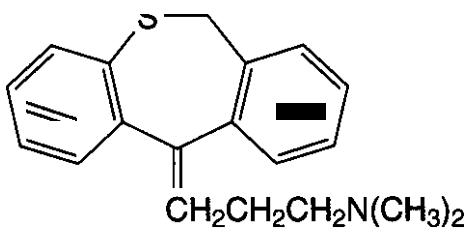


Clomipramine (5)

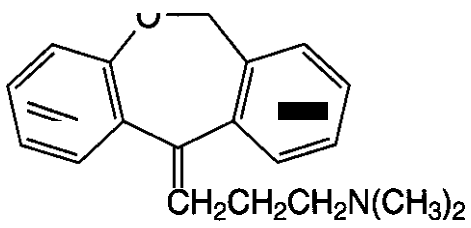




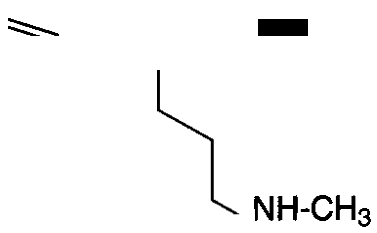
Imipramine R = –H (11)
Trimipramine R = –CH_3 (25)



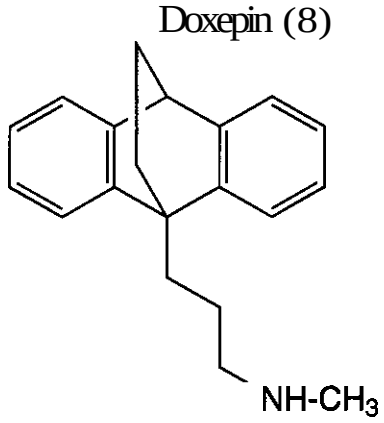
Dothiepin (7)



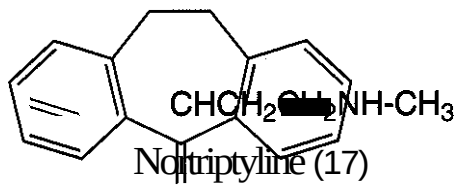
Doxepin (8)



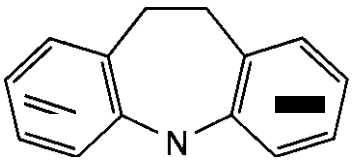
Desipramine (6)

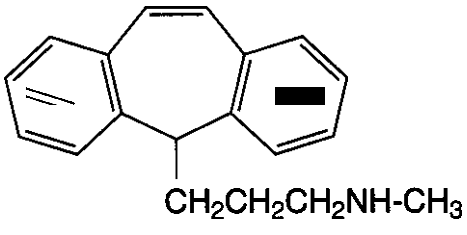


Maprotiline (13)

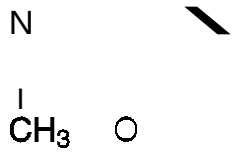


Nortriptyline (17)





Protriptyline (20)

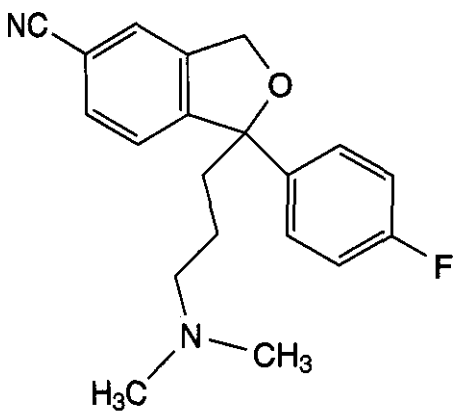


Lofepramine (12)

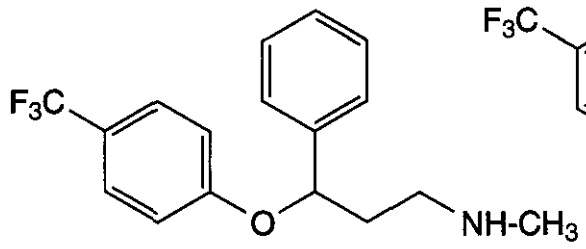
Figure 8.1. Structures of tricyclic antidepressants.

Clinical trials invariably employ self-reporting of symptoms, using standardized questionnaires, the tool most often used being the 17- or 21-item Hamilton Rating Scale for Depression (HAM-D). A positive response to drug treatment is usually defined as a decrease of at least 50% in the baseline HAM-D score.

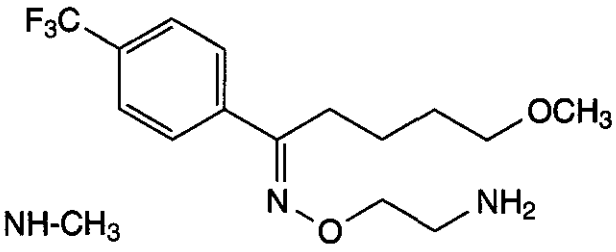
There are several puzzling features of antidepressant drug action. The first is that re-



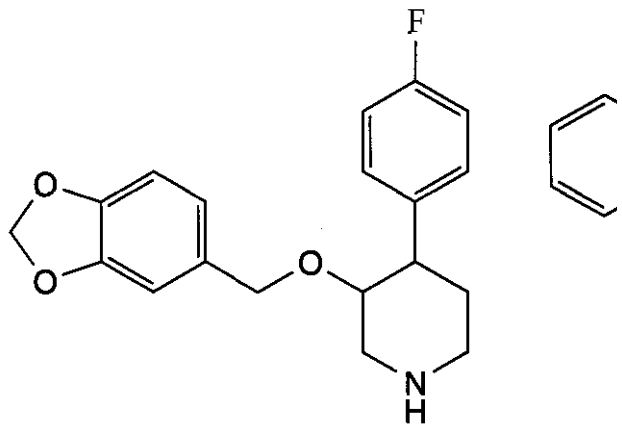
Citalopram (4)



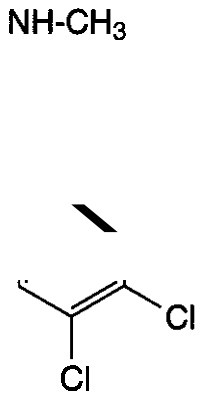
Fluoxetine (9)



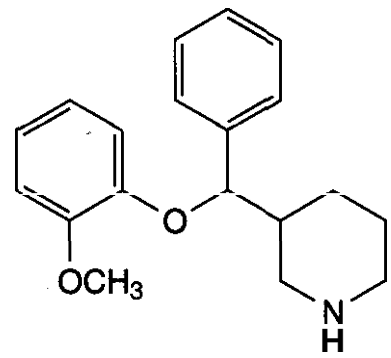
Fluvoxamine (10)



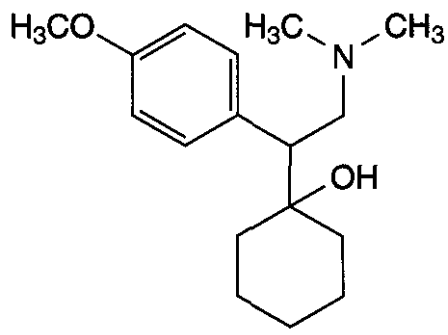
Paroxetine **(18)**



Sertraline **(22)**

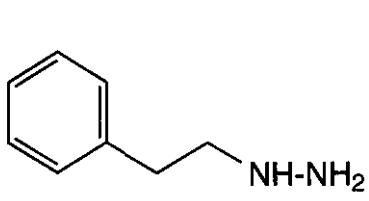


Reboxetine **(21)**

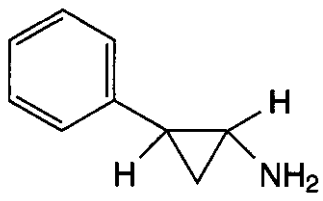


Venlafaxine **(26)**

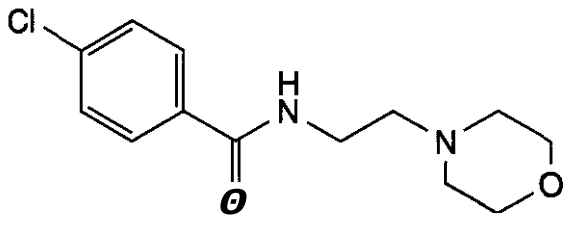
Figure 8.2. Structures of the SSRIs (citalopram, fluoxetine, fluvoxamine, paroxetine, sertraline), the NSRI reboxetine, and the SNRI venlafaxine.



Phenelzine **(19)**



Tranylcypromine **(23)**



Moclobemide **(15)**

Figure 8.3. Structures of common monoamine oxidase (MAO) inhibitors.

gardless of which drug is used, one-third or more of those treated fail to show any significant response. All drugs seem effective in about 60–70% of those treated, but placebo response rates range from 30% to 50%. Thus the true efficacy of antidepressant drugs may only be seen in less than 50% of those treated (8). The reasons for the high placebo response are partly because some patients show a spontaneous remission from their depression during the 6–8 weeks of the drug trial, but partly also because of the genuine power of the placebo effect, which is particularly noticeable in the treatment of psychiatric illnesses. The magnitude of the placebo effect is exceptionally large in trials of antidepressants, at least as great as that attributable to the antidepres-

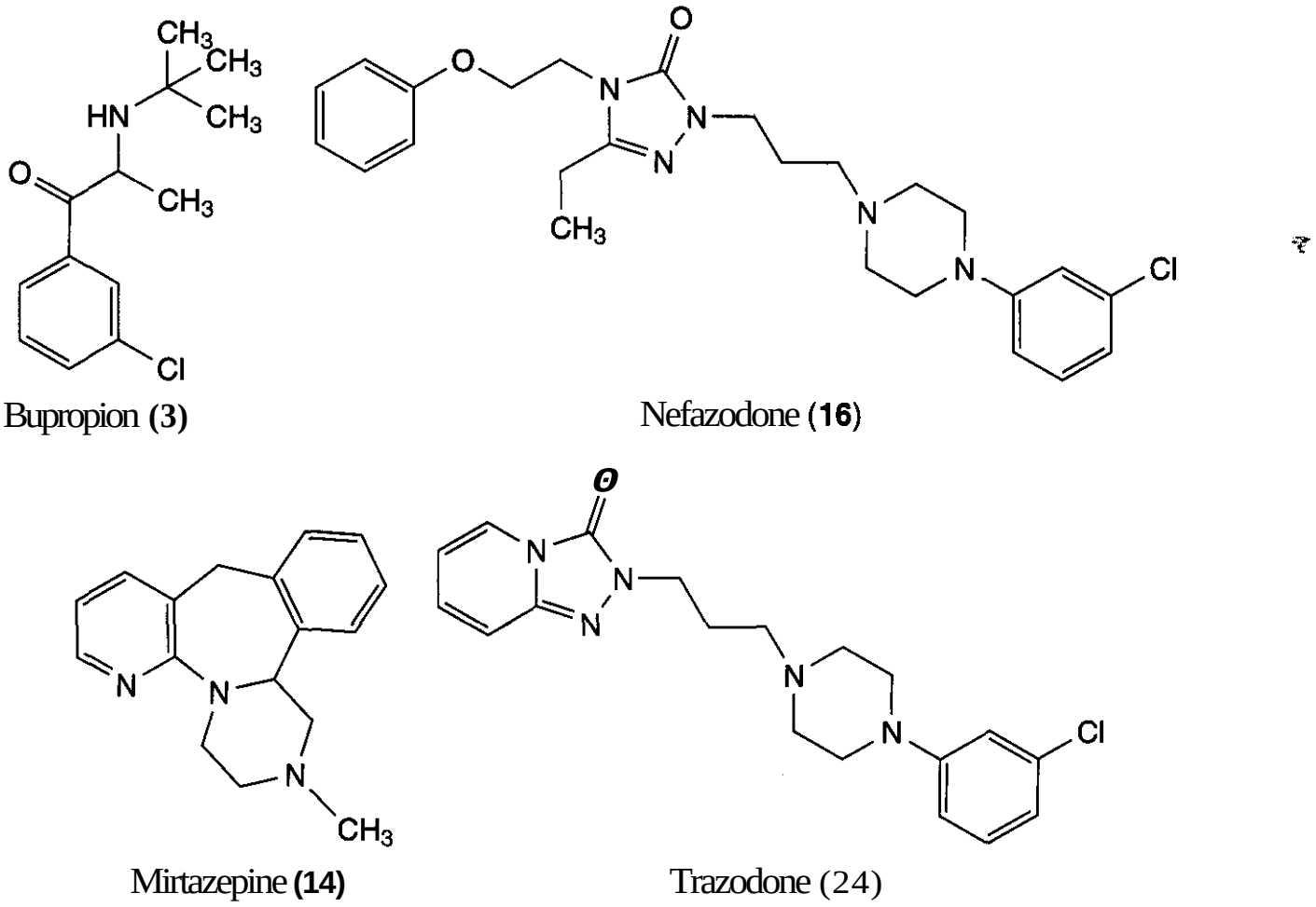


Figure 8.4. Structures of some of the newer atypical antidepressants: bupropion, mirtazepine, nefazodone, and trazodone.

sant drug (9). The placebo effect is variable; it is larger, for example, in patients with milder depression than in those with more severe forms of the illness. It is not uncommon for clinical trials of antidepressant drugs to fail to show a statistically significant difference between the drug-treated and placebo groups. The various classes of antidepressant drugs exhibit few differences in their clinical efficacy; the advantages of the newer compounds are related to their improved side-effect profiles rather than to a more powerful anti-

Table 8.3 Efficacy of Antidepressants Compared with Placebo in Controlled Clinical Trials (11)

Antidepressant	Drug Treated % Responders	Placebo % Responders	Drug-Placebo % Treated
Tricyclics (n = 3327)			
Amitriptyline (1)	60	25	35
Amoxepine (2)	67	49	18
Imipramine (11)	68	40	28
SSRIs (n = 2463)			
Paroxetine (18)	45	23	22
Fluoxetine (9)	60	33	27
Fluvoxarnine (10)	67	39	28
Sertraline (22)	79	48	31
MAO inhibitors (n = 1944)			
Phenelzine (19)	64	30	34
Moclobemide (15)	64	24	40
Other (n = 277)			
Mirtazapine (14)	48	20	28

Table 8.4 Additional Indications for SSRI Antidepressants

Agent	Additional Indications Approved by U.S. Food and Drug Administration
Fluoxetine (9)	Bulimia nervosa, obsessive compulsive disorder (OCD), panic disorder
Fluvoxamine (10)	OCD
Paroxetine (18)	OCD, panic disorder, social anxiety disorder
Sertraline (22)	OCD, panic disorder, posttraumatic stress syndrome

depressant action (8, 10). The results of a meta-analysis of antidepressant drug trials illustrate this point (Table 8.3).

A meta-analysis of 186 randomized controlled trials with amitriptyline (1) indicated that although this drug is less well tolerated than other tricyclic or SSRI antidepressants there was a small but significant 2.5% higher proportion of responders compared to that of the other drugs (12). A systematic search of 108 other meta-analyses suggested that combined serotonin/norepinephrine reuptake inhibitors have slightly superior efficacy to that of the SSRIs (13). Recent studies of the norepinephrine-selective reuptake inhibitor reboxetine (21) showed it to have comparable efficacy to that of other antidepressants, but it improved social functioning more than did the SSRIs (14, 15).

A second unexplained feature of antidepressant drug action is that the maximum clinical benefit is not seen until treatment has been continued for several weeks (10). Although a significant improvement in HAM-D scores can sometimes be detected after 2 weeks of treatment, it takes 4–6 weeks to obtain the maximum response. Boyer and Feighner (16) performed a meta-analysis of six trials to determine the predictive value of nonresponse to medication early in a clinical trial. They found that patients who failed to achieve at least a 20% reduction in HAM-D scores at any point during the first 4 weeks of a study had less than a 5% chance of becoming a "responder," as defined by a 50% or more reduction in HAM-D score by the end of the 6-week trial. The authors concluded that a full 6 weeks' trial of antidepressant medication is usually not justified if patients fail to respond during the first 4 weeks. A number of explanations have been proposed to explain the delayed clinical response to antidepressant drugs, and these are discussed below.

Depression is often associated with anxiety or other forms of psychiatric disorder, and the SSRIs in particular have come to be used increasingly in a variety of conditions other than major depression. Table 8.4 summarizes the uses for which SSRIs have been approved, based on the finding of significant beneficial effects in controlled clinical trials. In addition, agents in this class in controlled trials have shown usefulness in premenstrual dysphoria, borderline personality disorder, obesity, smoking cessation, and alcoholism (17).

The remarkable success of the SSRIs has prompted the question of whether genetic defects in the serotonin (SERT) transporter gene or in the regulation of its expression might explain the etiology of mood disorders. The gene coding for human SERT is localized on chromosome 17q11.2. It spans over 35 kilobases and is organized in 14 introns. No genetic variations have been found in the coding region of the SERT gene in depressed patients, but a number of studies have found that certain variants in a polymorphic region flanking the 5' region or in the second intron are associated with depressive illness, anxiety-related personality traits, or suicidal alcoholism (6).

2.3 Adverse Side Effects

2.3.1 introduction. The antidepressants include a wide range of compounds with differing modes of action (Table 8.2). It is not surprising to find that they display a plethora of differing side effects (18, 19). These range from adverse effects that can be unpleasant but relatively harmless, to rarer and often unpredictable serious adverse reactions. Particularly in the older, so-called first-generation antidepressants, these can be life threatening. Because depressed patients are often suicidal, it is not surprising that these drugs were often implicated in deaths resulting from intentional overdose (20, 21). Some antidepressants caused rare, idiosyncratic adverse effects that

were, nevertheless, so severe as to lead to withdrawal of the drugs from the market (e.g., nomifensine, zimelidine).

2.3.2 Monoamine Oxidase (MAO) Inhibitors. The first-generation MAO inhibitors tended to cause hypotension (often causing dizziness), headache, and mild anticholinergic effects such as dry mouth, constipation, blurred vision, and difficulty in micturition. Phenelzine (19) can cause mild sedation, but tranylcypromine (23) is more likely to act as a psychostimulant with mild amphetamine-like properties, thereby leading to agitation and insomnia (18). These effects are dose dependent and tend to lessen in severity with time. In fact, although antidepressants are not typically associated with abuse potential, 16 of 21 case reports of antidepressant abuse involved tranylcypromine (22). More serious adverse effects are related to the fact that the first-generation MAO inhibitors are compounds that irreversibly inhibit the enzyme in the brain and other organs. With chronic drug treatment, inhibition of enzyme activity is cumulative and may become almost complete. The enzyme is abundant in the liver, where it serves the function of detoxifying a variety of pharmacologically active organic amines that are absorbed from the diet.

Inhibition of liver MAO leaves the patient vulnerable to the so-called wine-and-cheese syndrome, with adverse cardiovascular effects caused by absorption of such vasoactive amines as tyramine into the general circulation [for review, see Blackwell et al. (23)]. The syndrome can include severe headache and hypertension and may lead to cerebral hemorrhage and death. Although this is a real risk, it seems likely that fears of MAO-food interactions may have been grossly exaggerated. Pare (24) reviewed the evidence in 1985 and noted that despite the widespread use of MAO inhibitors in the previous decade there had only been 17 reports of food interactions with phenelzine (19) and none of these proved fatal. With tranylcypromine (23) seven deaths had been reported, but in only two of these could a definite relationship with diet be established.

MAO inhibitors when taken alone in overdose can be fatal, with death usually resulting from pulmonary and cerebral edema. Despite the relatively low risk of food interactions, a wide range of foodstuffs is prohibited to patients taking MAO inhibitors, including mature cheeses, meat or yeast extracts, mature fish, pickled fish, smoked foods, and broad bean pods. Because of the long-lasting inhibition of MAO caused by these irreversible enzyme inhibitors, the dietary precautions have to be maintained for at least 14 days after cessation of drug treatment. These restrictions make these drugs unpopular with both patients and physicians. At least as important as the food-interaction risk is that of drug interactions. MAO inhibitors are dangerous to use in conjunction with a number of other clinically important drugs (18, 24). Serious interactions (usually hypertensive crises) may occur with pethidine, levodopa, sympathomimetic amines such as amphetamine and ephedrine, and other antidepressants including the tricyclics and SSRIs.

The second-generation MAO inhibitor moclobemide (15) was designed to lessen the risk of the food and drug interactions seen with earlier MAO inhibitors. Moclobemide selectively targets one form of the enzyme, monoamine oxidase A, leaving monoamine oxidase B in the liver active and capable of detoxifying tyramine and other dietary vasoactive amines (25). Moclobemide is also a reversible inhibitor of the enzyme, so its effects are not cumulative and are more rapidly reversible on termination of drug treatment. Adverse effects are few and infrequent, with dropouts because of side effects in clinical trials uncommon. Nausea, insomnia, headache, and dizziness occurred in some patients taking the drug, and others experienced agitation and restlessness (25, 26). Despite the improved selectivity of moclobemide, there have been a small number of reports of hypertension when moclobemide was combined with tyramine-rich foods (27). Moclobemide exhibits fewer adverse drug interactions than first-generation compounds, although combination with SSRIs is not recommended because of the danger that a "serotonin syndrome" could result (28). Moclobemide does, however, appear to be safe when given in conjunction with sympathomimetic amines, found in many common cough and cold remedies. The drug appears to be safe if

taken in overdose. Moclobemide represents a real advance in terms of its safety profile over the earlier MAO inhibitors, but the class as a whole remains largely out of favor.

2.3.3 Tricyclic Antidepressants. The older antidepressant drugs in this class exhibit a variety of adverse side effects, most of which are related to their secondary pharmacological actions on targets other than the monoamine transporters (29, 30). Although some of these side effects are uncomfortable but not serious, others are life threatening, and the tricyclic antidepressants have only a narrow therapeutic index. Serious toxicity *can* occur at doses that are only 2–6 times therapeutic. Thus, a single bottle of tablets *can* prove lethal. Given the increased risk of suicide in depressed patients, it is not surprising that tricyclic overdose was among the commonest causes of drug-related death in the United States in the early 1980s (20). Leonard (21) analyzed the number of deaths attributable to antidepressant overdose in England and Wales for the period 1977–1983, a total of more than 1500 deaths. It was possible to calculate an estimated death rate per million patients treated for each antidepressant. The rates were arnitriptyline = 166, dothiepin = 147, maprotiline = 115, imipramine = 105, doxepine = 99, trimipramine = 93, and clomipramine = 34. To put these data into perspective, however, the deaths associated with tricyclic antidepressants represent only approximately 10% of the risk of death through overdose with barbiturates, which were then widely used as hypnotics (31). The most serious effects of the older drugs are attributed to direct quinidine-like actions on the heart, interfering with normal conduction and causing prolongation of the QRS or QT interval. Death is most commonly the result of cardiac arrhythmia and arrest. Other toxic effects include respiratory depression, delirium, seizures, shock, and coma. It is worth noting that the newer tricyclic antidepressant lofepramine (12) has very little cardiotoxicity. In a review of fatal poisonings associated with antidepressants in England and Wales for the period 1993–1995, it was associated with fatal overdose in only 4.3 per million treatment episodes (of 3 months' duration), comparable to the fatalities associated with SSRIs (2.4 per million treatment episodes) (32, 33).

In therapeutic doses, the tricyclic antidepressants also cause a variety of less serious unwanted side effects. Many of these are associated with the ability of many of these drugs to act as antagonists at various monoamine receptors (see Table 8.6 below). Antidepressants interact with a large number of neurotransmitter receptors. Some of the targets listed in Table 8.6 represent multiple subtype; for example, "5-HT2" is in fact a composite of effects of 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$. (For a more complete and up-to-date summary of drug interactions with neurotransmitter receptors visit the online database http://pdsp.cwru.edu/pdsp.asp.) Several of the tricyclic agents bind with high affinity at muscarinic receptors, and the blockade of these cholinergic receptors causes a range of side effects; again, there are five different subtypes of muscarinic receptors. Anticholinergic side effects include dry mouth, blurred vision, constipation, urinary retention, and sinus tachycardia. The most potent effects of all are seen at histamine H1 receptors, where several tricyclic drugs bind with subnanomolar affinities. Blockade of these receptors probably contributes to the sedative effects of some of these agents. Sedation is also a side effect caused by blockade of alpha-adrenergic receptors, another common feature in this class of drum. Orthostatic hypotension is another side effect related to alpha,-adrenergic blockade, leading to dizziness on rising in young adults, but possibly causing syncope and falls in the elderly. Another unwanted feature of the older drugs is their tendency to cause significant weight gain, which may reduce patient compliance with the drug treatment regime. Tricyclic antidepressants also reduce seizure threshold, and this can lead to drug-induced seizures in 0.1–0.5% of patients, usually early in treatment (19). CNS side effects include a propensity to cause mania or hypomania in patients suffering from bipolar depression. Sexual dysfunction may be associated with tricyclic use. Decreased libido and delayed orgasm can be seen in men and women, and men may experience erectile dysfunction. These effects, however, are much more common in patients treated with the newer SSRIs (see Table 8.5).

The tricyclic drugs are extensively metabolized in the liver by the cytochrome p450 en-

Table 8.5 Summary of Side Effect Profiles of Antidepressant Drugs (34)

Generic Name	Sedation	Anticholinergic Effects	Hypotension	Cardiac Effects	Weight Gain
Amitriptyline (1)	+++	++++	+++	+++	+++
Arnoxepine (2)	+	++	+	++	+
Bupropion (3)	0	0	0	+	0
Citalopram (4)	+	+	0/+	0/+	0
Clomipramine (5)	++	+++	++	+++	+
Desipramine (6)	+	+	+	++	+
Doxepine (8)	+++	++	+++	++	++
Fluoxetine (9)	0/+	0	0	0	0
Fluvoxamine (10)	0	0	0	0	0
Imipramine (11)	+++	++	+	+++	++
Maprotiline (13)	++	++	++	+	+
Mirtazapine (14)	+++	+	0/+	+	+
Moclobemide (15)	0	0	0	0	0
Nefazodone (16)	+	0	+	0/+	0/+
Nortriptyline (17)	+	+	+	++	+
Paroxetine (18)	+	+	0	0	0
Phenelzine (19)	+	0	+++	0	++
Protriptyline (20)	0/+	++	+	+++	+
Sertraline (22)	0	0	0	0	0
Tranylcypromine (23)	+	0	+	0	0/+
Trazodone (24)	+++	0	++	0/+	+
Trimipramine (25)	+++	+++	++	+++	++
Venlafaxine (26)	0/+	0/+	0	+	0

zymes. Consequently, other drugs that induce such enzymes or compete with the tricyclic antidepressants for metabolism may alter their actions. Calcium channel blockers, cimetidine, phenothiazines, haloperidol, methylphenidate, glucocorticoids, oral contraceptives, and most SSRIs may inhibit the metabolism of the tricyclics. This will tend to exacerbate the adverse side effects of the tricyclics. Conversely, carbamazepine, phenytoin, barbiturates, primidone, and alcohol may induce liver cytochrome p450 enzymes and accelerate metabolism, making tricyclics less effective (8,19).

2.3.4 Serotonin-Selective Reuptake Inhibitors. Since their introduction in the mid-1980s SSRIs have become the most widely used of all antidepressants. This is largely because of their improved safety and tolerability in clinical use. Although the SSRIs are no more efficacious or rapid in onset of action than the tricyclics, they lack most of the serious toxicity and adverse side effects associated with the first-generation drugs. The relative absence of cardiac toxicity makes the SSRIs relatively safe in overdose (36). Fatal overdose has, however, been reported in six patients taking citalopram (4) (37), although the cause of death was disputed (38). The symptoms of SSRI overdose include nausea, agitation, seizures, and sometimes loss of consciousness (18). The relative safety of the SSRIs has led to their being prescribed more freely than the earlier antidepressants, and their use in a number of indications in addition to the treatment of major depression (Table 8.4).

There are, however, some hazards associated with the use of these drugs. SSRIs decrease serotonin uptake from the blood by platelets. Because platelets cannot synthesize serotonin, which is involved in platelet aggregation, SSRIs may impair platelet function. A case-controlled study found that the risk of gastrointestinal bleeding was three times greater in SSRI users than in controls (39). This conclusion was confirmed by a retrospective cohort study of 317,824 SSRI users, which emphasised that the risk of gastrointestinal bleeding was particularly important for elderly patients (40).

The SSRIs exhibit only low affinities for muscarinic and most other monoamine recep-

tors (Table 8.6). Consequently at therapeutic doses they are relatively free from the cholinergic side effects associated with the TCAs, and are less likely to cause sedation and drowsiness or hypotension. Instead of promoting weight gain, the SSRIs tend to suppress appetite and this can lead to weight loss. The most common side effect in the acute use of SSRIs is nausea, which clinical trial data indicate affects about 20% of patients taking fluoxetine (9), fluvoxamine (10), paroxetine (18), and citalopram (4) (18). The risk of nausea is reduced if the SSRIs are taken with a meal. Most SSRIs tend to cause CNS "activation," leading to insomnia, agitation, or anxiety. Paradoxically, paroxetine (18) tends to cause sedation and drowsiness to such an extent that in several countries the drug is prescribed with a warning not to drive (18). All SSRIs tend to cause sexual dysfunction, including loss of libido, erectile dysfunction, and delayed or absent orgasm. Both men and women are affected, and the incidence of these side effects is quite high. Although initial clinical trial data suggested that only a small proportion of patients suffered sexual dysfunction, more recent reports suggest that these side effects may occur in as many as 50–70% of patients taking SSRIs, and 30–40% of all those on antidepressant medication of any kind (41, 42). Patients are reluctant to volunteer information on sexual dysfunction, but when asked specifically a truer picture emerges.

A debate has raged for several years over the alleged association between the use of SSRIs and the occurrence of suicidal thoughts and suicide. However, meta-analysis of the available clinical data has failed to show any such association (43, 44). Nevertheless, the UK Medicines Control Agency as recently as October 2000 advised manufacturers of SSRIs to include a warning in patient information leaflets about the possibility of suicidal thoughts when they begin taking the products, before the antidepressant effects become apparent (45).

SSRIs are metabolized by cytochrome p450 enzymes in the liver. Most SSRIs inhibit CYP2D6, fluvoxamine (10) inhibits CYP1A2, and fluoxetine (9) inhibits CYP3A4. Consequently, these drugs may interfere with the metabolism of a number of other agents. Given concurrently with TCAs they may cause serious adverse effects. Fluvoxamine may raise levels of caffeine and theophylline, and fluoxetine can interfere with the metabolism of clozapine, cyclosporin, and tefenadine (18, 46). SSRIs should never be given with MAO inhibitors because a fatal "serotonin syndrome" has been reported with fluoxetine in this combination (18).

2.3.5 Other Agents

Bupropion. Bupropion (3) is a weak inhibitor of dopamine reuptake that has not been widely used outside the United States as an antidepressant. It has received a new worldwide lease on life as an effective means of treatment for tobacco smoking cessation (47). It has little sedative, cholinergic, hypotensive, or cardiotoxic properties (48). There are some adverse side effects, however, related to the ability of this drug to enhance dopaminergic function. These include insomnia, agitation, nausea, weight loss, and sometimes psychosis (49). The drug decreases seizure threshold and so should not be given to those at risk of seizures (49). In overdose acute toxicity is less serious than that seen with tricyclics (50). Bupropion (3) should not be given with MAO inhibitors, levodopa, or dopaminergic receptor agonists (49).

Venlafaxine. Venlafaxine (26) is an inhibitor of serotonin and norepinephrine uptake, but unlike the TCAs it has little affinity for muscarinic, histamine, or alpha-adrenergic receptors. Consequently, it does not exhibit the cholinergic, sedative, or hypotensive side effects seen with the earlier compounds (18, 19). Nevertheless, the side-effect profile includes headache, dry mouth, sedation, and constipation in up to 15% of patients (51). During the first 2 weeks of treatment as many as 30% of patients receiving the drug may experience nausea (52). At high doses close monitoring of blood pressure is needed, given that the drug tends to cause hypertension (52). Venlafaxine (26) appears to have little cardiac toxicity and seems to be safe in overdose, although seizures have been reported (53).

Trazodone. Trazodone (24) is a weak inhibitor of serotonin uptake and is an antagonist at 5-HT and alpha,-adrenergic receptors (Table 8.5). These properties appear to be related

Table 8.6 Affinities of Antidepressant Drugs for Human Monoamine Receptors"

Generic Name	Histamine H l	Muscarinic	α_1-Adrenergic	α_2-Adrenergic	Dopamine D2	5-HT_{1A}	5-HT,
Amitriptyline	**1.1**	**18**	**27**	940	1000	450	18
Amoxepine	25	1000	50	2600	**160**	—	—
Bupropion	6600	48,000	4600	81,000	—	>35,000	>35,000
Citalopram	470	2200	1900	15,300	—	—	—
Clomipramin	31	**37**	38	3200	**190**		
Desipramine	110	198	130	7200	3300	6400	350
Dothiepin	3.6	**25**	470	2400	—	—	—
Doxepine	**0.24**	80	**24**	**1100**	2400	**276**	**27**
Fluoxetine	6200	2000	5900	13,000	—	32,400	280
Fluvoxamine	>100	24,000	7500	15,000	—	—	—
Imipramine	11	90	90	3200	2000	5800	150
Lofepramine	360	67	100	2700	2000	4600	200
Maprotiline	**2**	570	90	9400	**350**	—	—
Nefazodone	24,000	11,000	48	**640**	910	**80**	**26**
Nortriptyline	10	150	60	2500	1200	**294**	**41**
Paroxetine	22,000	108	4600	17,000	32,000	>35,000	19,000
Protriptyline	25	**25**	130	6600	2300	—	—
Reboxetine	1400	3900	10,000	4300	9000	—	—
Sertraline	24,000	630	380	4100	10,700	>35,000	9900
Trazodone	350	>100	**36**	**490**	3800	**96**	**25**
Trimipramine	**0.2**	58	**24**	**680**	**180**	—	—
Venlafaxine	>35,000	>35,000	>35,000	>35,000	>35,000	>35,000	>35,000

"Equilibrium dissociation constants are nanomolar. Data were obtained from binding studies using the following: Histamine **H1**: **^{3}H-doxepin** (29) or **^{3}H-pyrilamine** (35); **Muscarinic**: **^{3}H-quinuclidinyl benzilate**; **α_1-Adrenergic**: **^{3}H-prazosin**; **α_2-Adrenergic**: **^{3}H-rauwolscine**; **Dopamine** D2: **^{3}H-spiperone**; **$5HT_{1A}$**: **^{3}H-8-OH-DPAT**; 5-HT;: **^{3}H-ketanserin**. Data are from Refs. **29, 30,** and 35. The four compounds with the highest affinity for each receptor are highlighted in bold type. For a more complete and up-to-date summary of drug interactions with neurotransmitter receptors see http://pdsp.cwry.edu/pdsp.asp.

to the side effects of sedation and hypotension (leading to dizziness) seen with the drug, and common at high doses (54). Trazodone lacks cardiac toxicity, although dysrhythmias have been reported (18); the drug appears safe in overdose. Trazodone sometimes causes an unusual type of sexual dysfunction, that can include increased libido, priapism, and spontaneous orgasm (55, 56). These symptoms, although rare, are dramatic and have received considerable attention. Combination with SSRIs or MAO inhibitors should be avoided because of the risk of "serotonin syndrome" (18).

Nefazodone. Nefazodone (16) is chemically related to trazodone but acts in a different manner, largely through inhibition of serotonin uptake and antagonism at 5-HT, receptors. Adverse effects are mild and infrequent. They include sedation, dry mouth, and dizziness in around 10% of patients (57). The drug causes less hypotension than does trazodone, and is unlikely to cause sexual dysfunction (18). It is considered safe to use in epilepsy and there appears to be no overdose risk (18). Nefazodone is a potent inhibitor of cytochrome p450 CYP3A4 and so should not be given with alprazolam, astenizole, terfenadine, cisapride, or cyclosporin (58).

Reboxetine. Reboxetine (21) is a norepinephrine-selective reuptake inhibitor that lacks affinity for most of the monoamine receptors. It thus does not exhibit the typical side-effect profile of the tricyclics. Nevertheless, side effects include increased sweating, postural hypotension (leading to dizziness), dry mouth, constipation, blurred vision, impotence, and dysuria. Tachycardia and urinary retention have also been reported (59). There is no evidence of cardiotoxicity and sexual dysfunction seems to be rare. In contrast to some of the earlier tricyclics that are sedative, reboxetine is nonsedating and can cause insomnia (60, 61)..

2.4 Pharmacokinetics

2.4.1 Tricyclic Antidepressants. The tricyclic antidepressants are, by and large, well absorbed after oral administration, although time to peak plasma concentration can vary from 1 to 12 h according to both the drug and the individual. Most of these compounds have long half-lives, and many are metabolized by demethylation in the liver, to yield biologically active desmethyl metabolites, which further extend their duration of action (62, 63) (Table 8.7). For example, imipramine (11) is metabolized to desipramine (6). In the case of lofepramine (12), its metabolite, desipramine (6), plays an important role in the overall actions of the drug, in that desipramine has a considerably longer elimination half-life than that of the parent compound (27, 34) (Table 8.7). The drugs are extensively metabolized in the liver by demethylation, hydroxylation, and glucuronide conjugation of the hydroxy metabolites. The lipid-soluble drugs are thus converted to water-soluble conjugates that are readily excreted by the kidney (64). There is increased renal clearance in children and decreased clearance in older people, factors that need to be taken into account in determining optimum dosage levels. Clearance is also reduced in patients with compromised liver or kidney function.

There is considerable individual variation in the metabolism of the tricyclics, and this is largely attributed to genetically determined differences in liver enzymes. Some 7 to 9% of Caucasians are classified as "slow metabolizers," measured by the rate of hydroxylation of the drug debrisoquin. This is caused by genetic polymorphism in the cytochrome p450 enzyme CYP2D6 (65). This enzyme plays an important role in the aromatic hydroxylation of tricyclic antidepressants. The tricyclic drugs have a narrow therapeutic window, so individual variations in drug metabolism can be important in determining the correct therapeutic dose and avoiding toxic overdose (66, 67).

2.4.2 SSRI. All the SSRIs are well absorbed and most have long half-lives, compatible with their use as once-a-day drugs (68–70). The formation of biologically active desmethyl metabolites is again a factor in prolonging the duration of action of some of these drugs. This is particularly important for fluoxetine (9), which is metabolized in part to form norfluoxetine, an active metabolite that has a half-life of 4–16 days (71, 72). The desmethyl metabo-

Table 8.7 Pharmacokinetic Parameters for Antidepressant Drugs[a]

Generic Name	Time to Peak Plasma Concentration (h)	Elimination Half-Life (h)	Percentage Plasma Protein Binding	Important Metabolite.
Amitriptyline **(1)**	1–5	10–26	94	Nortriptyline (**17**)
Amoxepine **(2)**	1–2	8–30	90	8-Hydroxyamoxapine
Bupropion **(3)**	3	10–21	85	BP-threoamino-alcohol
Citalopram **(4)**	1–6	33	80	Desmethylcitalopram
Clomipramine **(5)**	2–6	21–31	97	Desmethylclomipramine
Desipramine **(6)**	3–6	11–31	90	2-OH-desipramine
Dothiepin (**7**)	n.a.	14–24	n.a.	Desmethyldothiepin
Doxepine (**8**)	1 4	11–23	80	Desmethyldoxepine
Fluoxetine **(9)**	4-8	24–120	94	Norfluoxetine
Fluvoxamine **(10)**	2–8	15–26	77	None
Imipramine **(11)**	1–3	11–25	92	Desipramine **(6)**
Lofepramine* **(12)**	n.a.	4–6	n.a.	Desipramine **(6)**
Maprotiline **(13)**	4–12	28–58	88	Desmethylmaprotiline
Mirtazapine (**14**)	2–3	2 0 4 0	85	None
Moclobemide (**15**)	1–1.5	1.4	n.a.	Numerous
Nefazodone (**16**)	1	2 4	99	*m*CPP **(28)**
Nortriptyline **(17)**	3–12	18–44	92	10-OH-nortriptyline
Paroxetine (**18**)	5–7	24–31	95	None
Phenelzine **(19)**	2 4	n.a.	n.a.	n.a.
Protriptyline **(20)**	6–12	67-89	93	None
Reboxetine** **(21)**	2 4	12	97	Various
Sertraline **(22)**	6–8	27	99	*N*-Desmethylsertraline
Tranylcypromine **(23)**	1.5–3	1.5–3.5	n.a.	n.a.
Trazodone **(24)**	1–2	6–11	92	*m*CPP (**28**)
Trimipramine **(25)**	3	9–11	95	None
Venlafaxine **(26)**	2	5	30	*O*-Desmethylvenlafaxine

"Data are from Refs. **29,** *27, **81, and **82; n.a.**, data not available.

lite of sertraline (**22**), although less potent than the parent drug (**68**), also has an extended half-life (73, 74). The desmethyl metabolite of citalopram (4) is formed in relatively small amounts and appears to contribute less importantly (34, 75). The long duration of action of fluoxetine (9) and sertraline (22) make long drug-free periods necessary when switching patients to other drugs. All of the SSRIs are metabolized by cytochrome p450 CYP2D6 in the liver, so individual genetically determined differences exist in rates of drug clearance and there is the potential for interaction with other drugs that are metabolized by this enzyme (69, 70). Citalopram (4) and fluvoxamine (10) are also substrates of p450 CYP 2C19, which exhibits a particularly high rate of genetic polymorphism in Asians (76). The pharmacokinetics of fluoxetine and citalopram are complicated by the fact that they are racemic compounds and the individual enantiomers may be metabolized and eliminated differently (70, 77).

2.4.3 MAO **Inhibitors.** The MAO inhibitors are rapidly absorbed and are extensively degraded by first-pass metabolism in the liver. For the irreversible enzyme inhibitors phenelzine (19) and tranylcypromine (23) the elimination half-lives are relatively unimportant, given that enzyme inhibition persists for many days after the drug has been eliminated. A minimum of 7–14 days is needed after stopping treatment with these drugs before it is safe to switch to other agents. The reversible MAO inhibitor moclobemide (15) is rapidly absorbed and extensively metabolized in the liver. It has an elimination half-life of approximately 12 h (78). Because enzyme inhibition is reversible, the time to recover after stopping moclobemide treatment is much shorter, 16–24 h (79).

2.4.4 Other Antidepressants. The other antidepressant drugs in Table 8.7 are rapidly absorbed and vary in their half-lives, with some requiring multiple daily dosing. Trazodone and nefazodone (16) are metabolized in part to **m-chlorophenylpiperazine** (m-CPP; (**28**), a compound that acts as an agonist at some serotonin receptors (80). The metabolite may, thus, contribute to the biological action of these drugs. m-CPP (28) also has a longer half-life than the parent drugs and readily penetrates the CNS.

The norepinephrine selective uptake inhibitor reboxetine (21) is rapidly and completely absorbed, and is metabolized mainly by the cytochrome p450 3A4; because it does not interact with CYP 2D6 there is less risk of interactions with other drugs (81–83). Mirtazepine (14) also shows little interaction with p450 cytochrome isozymes and there is only a low risk of drug interactions (84). Mirtazepine is a racemate, and the two enantiomers are eliminated at different rates, with a twofold higher rate of elimination of the (*S*)-enantiomer than of the (*R*)-enantiomer (84).

3 PHYSIOLOGY AND PHARMACOLOGY

3.1 Monoamine Transporters

3.1.1 Discovery. The majority of both old and new antidepressants act by virtue of their ability to inhibit monoamine transporter mechanisms in the brain. The concept that neurotransmitters are inactivated by uptake of the released chemical into the nerve terminal from which it had been released or into adjacent cells is less than 40 years old. Before this it was generally assumed that the inactivation of norepinephrine and the other monoamine neurotransmitters after their release from nerves was likely to involve rapid enzymatic breakdown, akin to that seen with acetylcholinesterase. The degradation of monoamines by the enzyme monoamine oxidase was known early on, and in the 1950s a second enzyme catechol-0-methyl transferase (COMT) was discovered and was thought to play a key role in inactivating norepinephrine and other catecholamines.

It was not until high specific activity tritium-labeled radioactive catecholamines became available in the late 1950s, however, that experiments could be performed using quantities of monoamine small enough to mimic the very low concentrations of epinephrine or norepinephrine normally encountered in body fluids. When the first experiments were performed in the Axelrod laboratory at the National Institutes of Health with ^{3}H-epinephrine (85) and later with ^{3}H-norepinephrine (86), they yielded an unexpected result. Although in laboratory animals most of the injected dose of labeled catecholamine was rapidly metabolized (mainly by COMT), a substantial proportion of the injected monoamine (30–40%) was removed from the circulation by a rapid uptake into tissues, where it remained for some time unchanged. A key observation was that the uptake of ^{3}H-norepinephrine into the heart was virtually eliminated in animals in which the sympathetic innervation had been destroyed by surgical removal of the superior cervical ganglion (87). This led Hertting and Axelrod (88) to propose that the reuptake of norepinephrine by the same nerves from which it had been released might represent a novel mechanism for inactivating this neurotransmitter.

The discovery of norepinephrine uptake was followed by the finding that similar but distinct transporters were involved in the inactivation of 5-HT and dopamine, and that similar mechanisms existed for the inactivation of the amino acid neurotransmitters GABA, glycine, and L-glutamate (89, 90). Research interest has focused on these mechanisms, including in recent years the identification and cloning of the genes encoding the transporter proteins involved and the development of knockout strains of genetically engineered mice lacking one or other of these gene products. The family of neurotransmitter transporters has turned out to be far more extensive than previously imagined, with more than 20 different members (for review, see Ref. 91).

3.1.2 Monoamine Transporters. The norepinephrine transporter (NET) was cloned by Pacholczyk et al. in 1991 (92) and this soon led to the discovery of other related members of

the transporter gene family. Separate transporters exist for serotonin (SERT) and dopamine (DAT). The monoamine transporters are dependent on sodium and chloride ions for their function. They use the electrochemical gradient of sodium between the outside and inside surfaces of the cell membrane to provide the thermodynamic energy required to pump neurotransmitters from low concentrations outside the cell to the much higher concentrations inside the cell. Chloride ions accompany the entry of neurotransmitter and sodium, and there is a net movement of positively charged ions into the cell, although not in sufficient amounts to appreciably alter the resting membrane potential of the cell.

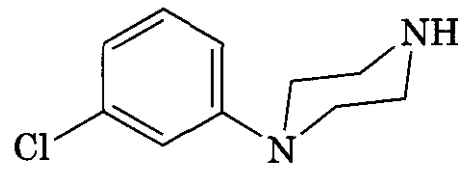
The vesicular neurotransmitter transporters represent another family (91) whose function is to maintain the very high concentrations of monoamine and amino acid neurotransmitters in storage vesicles. They use the proton gradient that exists across the vesicular membrane as the motive force. The vesicular monoamine transporters (VMAT) recognize serotonin, dopamine, norepinephrine, epinephrine, and histamine. VMAT-1 is present chiefly in amine-containing endocrine and paracrine cells in peripheral organs, whereas VMAT-2 is the predominant form found in monaminergic neurons in the CNS. It is also expressed in the histamine-containing cells of the stomach, and in the adrenal medulla and in blood cells. The Na^+/Cl^+-dependent transporters and the vesicular transporters are membrane proteins consisting of a single polypeptide chain of 5–600 amino acid residues, with a 12 a-helical membrane-spanning domain (91). The molecular mechanisms underlying the function of the neurotransmitter transporters remain unclear. Unlike flux through an open ion channel, there must be a gating cycle every time solute is transported, although the exact molecular details of this are not understood. No doubt selective mutations of amino acids in the transporter molecules will throw light on these questions in the future (93).

Immunocytochemical and *in situ* hybridization techniques have been used to study the cellular distribution of the transporters (91). Whereas NET and DAT are expressed exclusively in monoaminergic neurons SERT is expressed in both neurons and glia.

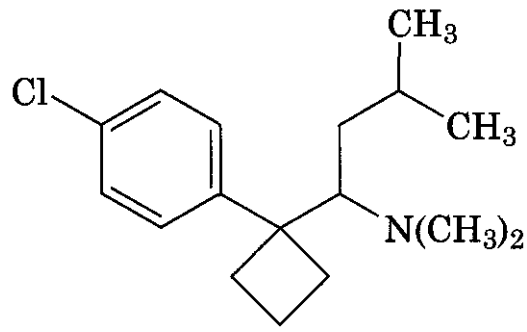
3.1.3 Drugs as Inhibitors of Monoamine **Transporters.** By far the most important group of CNS drugs that target the NE and serotonin neurotransmitter transporters (i.e., NET and SERT, respectively) is the tricyclic antidepressants and their modern counterparts. The discovery by the Axelrod group in 1961 (94) that imipramine (11) potently inhibited the uptake of norepinephrine led to the first understanding of the mechanism of action of the first-generation tricyclic antidepressants. After the discovery of the serotonin uptake system in the brain it soon became apparent that the classical tricyclic drugs imipramine (11) and amitriptyline (1) were potent as inhibitors of both NE and 5-HT uptake (Table 8.2). This reinforced the monoamine hypothesis of depression as a monoamine-deficiency state, and stimulated much further research in the pharmaceutical industry to discover new inhibitors of monoamine uptake. The debate as to whether inhibition of either NE or 5-HT was the more important in conferring antidepressant efficacy has swung one way and the other over the past 40 years and there is no definitive answer to this question. An early effort to improve the selectivity of antidepressants was made in the 1970s by scientists at the Ciba-Geigy Company in Switzerland (now Novartis), who developed the selective NE uptake inhibitor maprotiline (13) (95). This proved to be clinically effective as an antidepressant but it was not a great success commercially and had few clear advantages over the classical TCAs. This idea was also swept away by the wave of enthusiasm for serotonin-selective reuptake inhibitors (SSRIs) that started with the success of fluoxetine (96, 97). Table 8.8 summarizes the affinities of currently used antidepressants on cloned human monoamine transporters expressed in tissue culture cell lines (98). The availability of the human transporter proteins for screening represents a considerable advance. Although there are many published accounts of the effects of antidepressants on monoamine transporter mechanisms, most of these employed animal tissues and there are few reported

studies in which a large number of drugs were tested under the same experimental protocols.

Ironically, some of the most recently introduced antidepressants hark back to the nonselective compounds of the earlier era. Thus **venlafaxine** (26) is described as a drug that combines both NE and serotonin **reuptake** inhibition (99)[although **in** *vitro* binding data show that **venlafaxine** binds with more than 100 times higher affinity to human SERT than to NET (98) (Table 8.7)]. The compound **sibutramine** (29)is also an inhibitor of both NE and serotonin uptake, but it has been approved for use as an antiobesity agent rather than an antidepressant (102). At the same time reboxetine (21) is the **first** antidepressant **drug** in a new class of NET-selective inhibitors **(35).** Reboxetine is **reported** to be as effective as the **SSRIs** or older **tricylics,** but is not associated with sexual dysfunction (60, 61). It is claimed to be more effective than fluoxetine in improving the social adjustment of depressed patients (101). The older antidepressant bupropion (3), acts as a weak inhibitor of NE and **dopamine** uptake, with little effect on serotonin uptake, but it and some of its



(28) *m*CPP



(29) Sibutramine

Table 8.8 Antidepressants: Inhibition of Human Serotonin(SERT), Norepinephrine (NET), and Dopamine (DAT) Transporters[a]

Generic Name	Human SERT, K_d (n*M*)	Human NET, K_d (n*M*)	Human DAT, K_d (n*M*)	Selectivity: SERT vs. NET
Amitriptyline **(1)**	4.3	35	3250	8
Amoxepine (**2**)	58	16	4310	0.3
Bupropion (**3**)	9100	52,000	520	5.7
Citalopram **(4)**	1.2	4070	28,100	**3500**
Clomipramine **(5)**	0.3	38	2190	130
Desipramine **(6)**	17.6	0.8	3190	0.05
Dothiepin **(7)**	8.6	46	5310	5.3
Doxepine **(8)**	68	29.5	12,100	0.4
Fluoxetine (9)	0.8	240	3600	**300**
Fluvoxamine **(10)**	2.2	1300	9200	**580**
Imipramine (11)	1.4	37	8500	27
Lofepramine **(12)**	70	5.4	18,000	0.08
Maprotiline **(13)**	5800	**11.1**	1000	**0.002**
Mirtazapine (14)	>100,000	4600	>100,000	—
Nefazodone (**16**)	200	360	360	1.8
Nortriptyline **(17)**	18	4.4	1140	0.24
Paroxetine **(18)**	0.13	40	490	**300**
Protriptyline (20)	19.6	1.4	2100	0.07
Reboxetine* (**21**)	129	**1.1**	—	**0.008**
Sertraline (**22**)	0.29	420	25	**1400**
Trazodone **(24)**	160	8500	7400	53
Trimipramine **(25)**	149	2450	780	16
Venlafaxine (26)	8.9	1060	9300	120

"Data are from Refs. 98 and *35. The results are equilibrium dissociation constants (K_d) in n*M,* using ^{3}H-imipramine binding to human serotonin transporter, ^{3}H-nisoxetine binding to human norepinephrine transporter, and ^{3}H-WIN35428 binding to human **dopamine** transporter (98), or for reboxetine (35), ^{3}H-citalopram binding to human serotonin transporter and ^{3}H-nisoxetine binding to the human norepinephrine transporter.

metabolites may indirectly activate noradrenergic mechanisms (see Section 5.4 below). The compound has had little success as an antidepressant, but has been approved in the United States and Europe as an aid to smoking cessation (47).

What are we to make of these twists and turns? How *can* drugs that are selective NE reuptake inhibitors be equally effective as those that selectively target SERT? In practice it is difficult to know how selective the monoamine uptake inhibitors are in *vivo.* None of the antidepressants is completely selective for either NET or SERT. The SSRIs have some affinity for NET, and some (e.g., paroxetine) are quite potent inhibitors of NET (102). In some cases the formation of active metabolites alters the drug selectivity profile. Thus the nonselective compound imipramine (11) and the partially NET-selective compound lofepramine (12) are extensively metabolized to desipramine (6), a highly potent and selective NE reuptake inhibitor. Similarly, whereas amitriptyline (1) has little selectivity for either NET or SERT, the metabolite nortriptyline (17) is a selective NET inhibitor. It seems likely that both NET-selective agents and SSRIs exert their effects through some common final pathway in the brain. Perhaps the SSRIs act indirectly to modulate noradrenergic function (103,104). Experimental data from animal experiments using microdialysis probes showed increased levels of extracellular norepinephrine in rat hippocampus after chronic treatment with paroxetine (18) (103). The original monoamine hypothesis of depression as formulated by Schildkraut in 1965 (105) stated:

> "Some, if not all, depressions are associated with an absolute or relative deficiency of catecholamines, particularly norepinephrine, at functionally important adrenergic receptor sites in the brain. Elation conversely may be associated with an excess of such amines."

European opinion currently seems to be swinging back in support of the view that an upregulation of noradrenergic function may be the key element underlying the efficacy of antidepressant drugs (103, 104), but most American researchers continue to emphasize the importance of serotonin. There is some evidence that NET-selective drugs are subtly different in their clinical profiles from those of SSRIs. Healy and McMonagle (106) have suggested that these drugs affect overlapping clinical domains. They suggest that NET-selective agents tend to promote levels of energy and interest, whereas SSRIs affect impulse control and both categories of drug treat mood, anxiety, and irritability.

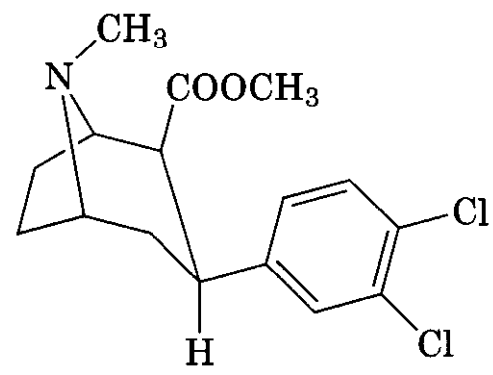
The molecular mechanisms in the brain that are triggered by the antidepressants, however, remain obscure. The fact that all drugs require a period of several weeks before they become fully effective suggests that they modify gene expression in the brain and that the resulting altered biochemical state takes a long time to become stabilized. Many theories have been proposed, including alterations in the expression of alpha- and beta-adrenergic receptors, changes in transcription factors and/or neurotrophic factors, and even morphological alterations in the connectivity of monoaminergic nerves.

It is possible that antidepressant drugs have other targets in addition to their actions at cell surface monoamine transporters. Al-Damluji and Kopin (107) have described a novel amine uptake process in peptide-containing hypothalamic neurons, which they named "transport-P." Like the vesicular transporters this process is driven by a proton gradient, but it is distinct from the vesicular transporters in being insensitive to reserpine, but sensitive to a variety of tricyclic antidepressants at micromolar concentrations (108). It is not clear, however, what role if any transport-P plays in the inactivation of the monoamine neurotransmitters.

Some antidepressants, notably mazindol and bupropion (3), inhibit the dopamine transporter (DAT) as well as NET or SERT. The DAT is best known, however, as one of the principal sites of action of the psychostimulant drug cocaine. Mice that are genetically engineered to knock out the expression of the DAT gene are profoundly hyperactive and fail to show any further stimulation of activity in response to cocaine or (+)-amphetamine (109). Such animals, nevertheless, will continue to self-administer cocaine (110), suggesting that the rewarding properties of the

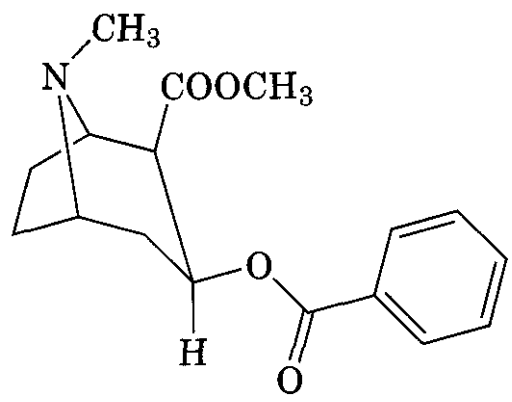
drug cannot be explained entirely by its ability to inhibit DAT. Cocaine (30) is also a potent inhibitor of both serotonin and NE reuptake. It retains some rewarding properties even in combined SERT and DAT knockout mice (111), suggesting that inhibition NE reuptake may also contribute importantly to its pharmacology. A corollary of the understanding that cocaine owes important parts of its overall CNS profile to mechanisms other than inhibition of DAT is that more selective inhibitors of dopamine reuptake might be useful and free of dependency liability. One such compound, brasofensine, is in clinical development for the treatment of Parkinson's disease (112). Other selective DAT inhibitors have been proposed for the treatment of the withdrawal phase of CNS drug abuse. On the other hand, the structure of cocaine (30) has been modified in such a manner [e.g., (31) and (32)] that the resulting agents behave primarily as selective 5-HT reuptake inhibitors (113, 114).

Some have suggested that a supersensitivity of central dopaminergic mechanisms may play an important role in the actions of antidepressant drugs (115). Animals treated chronically with antidepressants become sensitized to the behavioral stimulant effects of dopaminergic drugs, and there is evidence for increased dopamine release in the brain (115).

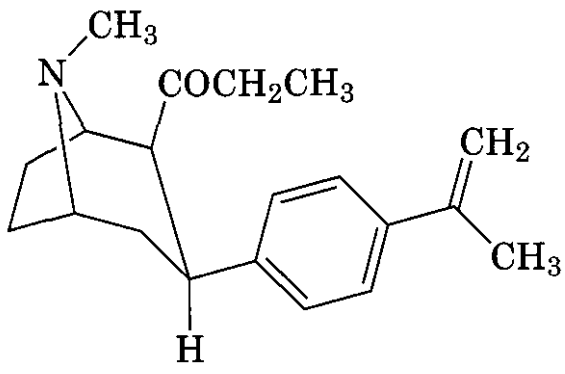


(32)

The neurotransmitter transporter family has provided many valuable targets for psychopharmacology. There is every prospect that this will continue. It might seem that the monoamine transporters had already been fully exploited, but the reemergence of NET-specific antidepressants and the possible applications of selective inhibitors of DAT suggest that there may still be room for innovation even in such a crowded field.



(30) Cocaine

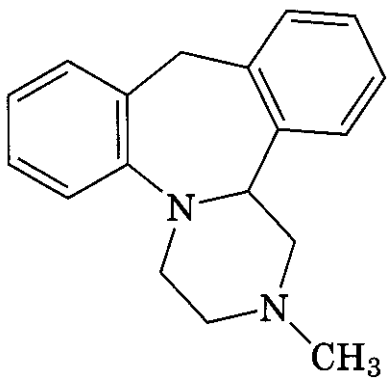


(31)

3.2 Serotonergic Agents

3.2.1 Receptor Populations. Seven major families or populations of serotonin receptors have been identified: 5-HT_1–5-HT_7 receptors. Several of these populations are divided into subpopulations (119–121). For example, 5-HT, (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT,, 5-HT_{1F}) and 5-HT, (5-HT,, 5-HT,, 5-HT_{2C}) receptors represent serotonin receptor populations for which subpopulations exist. With the exception of the 5-HT, receptors, which are directly linked to an ion channel, all the other 5-HT receptors belong to the G-protein-coupled superfamily of receptors. 5-HT, receptors are negatively coupled to an adenylate cyclase second-messenger system, whereas the 5-HT,, 5-HT,, and 5-HT, receptors are positively coupled. 5-HT, receptors are coupled to a phosphatidylinositol second-messenger system. There is evidence that 5-HT, receptors and 5-HT, receptors are involved in depression. Certain of the other 5-HT receptors may also play a role in depression and there is evidence for functional interactions between 5-HT receptors.

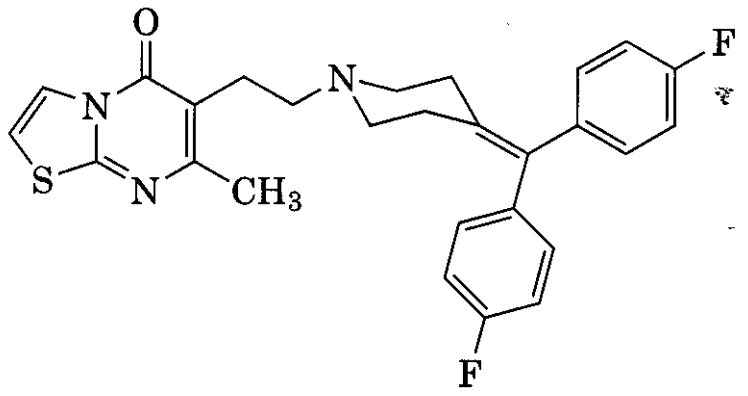
3.2.2 5-HT, Receptors. The exact mechanism of action of some antidepressants is currently unknown. For example, the atypical antidepressant trazodone (24) is a weak 5-HT reuptake inhibitor, whereas nefazodone (16) is a weak inhibitor of both 5-HT and NE reuptake (Table 8.7). Inhibition of neurotransmitter reuptake does not seem to account for the antidepressant actions of these two agents. Both bind at 5-HT, receptors with high ($K_i \approx 25$ nM) affinity (30) and are 5-HT, antagonists (122) (Table 8.6). A plausible mechanism of action for these drugs is that they enhance noradrenergic and serotonergic function by their ability to block presynaptic 5-HT receptors that normally exert an inhibitory effect on monoamine release in the brain, or by blockade of postsynaptic 5-HT, receptors (see below). Mianserin ("Tolvan") (27) is a nonselective 5-HT, and



(27) Mianserin

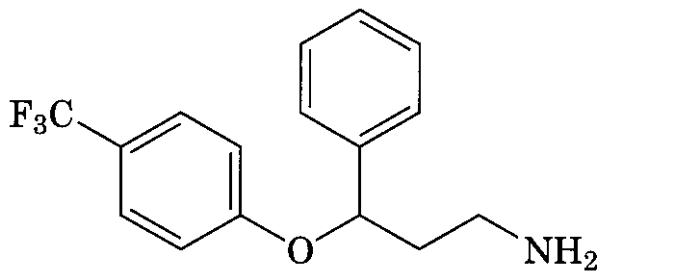
5-HT, antagonist with antidepressant activity (123); it binds with high affinity ($K_i < 10$ nM) at 5-HT, receptors (124). Hence, trazodone (**24**), nefazodone (**16**), and mianserin (27) represent atypical antidepressants that have in common a high affinity for 5-HT_{2A} receptors and 5-HT_2 antagonist action. It might be noted that certain tricyclic antidepressants also bind at 5-HT, receptors; imipramine (**11**), desipramine (**6**), nortriptyline (**17**), and maprotiline (**13**), for example, bind with submicromolar K_i values (124) (Table 8.6).

5-HT receptors are upregulated in depression. Hence, agents that downregulate 5-HT receptors might be of benefit in the treatment of this disorder. According to receptor adaptation theory, neurotransmitter antagonists should upregulate neurotransmitter receptors. However, paradoxically, 5-HT_2 antagonists generally downregulate 5-HT, receptors (e.g., Ref. 125). Mechanistically, then, it is reasonable that 5-HT, antagonists display antidepressant activity. Ritanserin (**33**) is a newer



(33) Ritanserin

example of a 5-HT, antagonist. In humans, ritanserin has been found to be as effective as amitriptyline (**1**), and superior to trazodone (**24**), as an antidepressant (126). The SSRI fluoxetine (9) is metabolized to norfluoxetine (34). (−)-Norfluoxetine retains antidepressant activity and displays only slightly lower affinity for 5-HT„ receptors than it displays for the 5-HT transporter (127). Results from an animal behavioral model predictive of antidepressant activity (the forced swim test) suggest that compounds that activate 5-HT„ receptors have antidepressant-like profiles (128). m-Chlorophenylpiperazine (*m*CPP; (**28**), which has significant affinity as an agonist at 5-HT„ receptors, was among the compounds that were positive in this test. Because *m*CPP is an important and long-lasting metabolite of both trazodone (24) and nefazodone (**16**), it may also contribute to their antidepressant profiles.

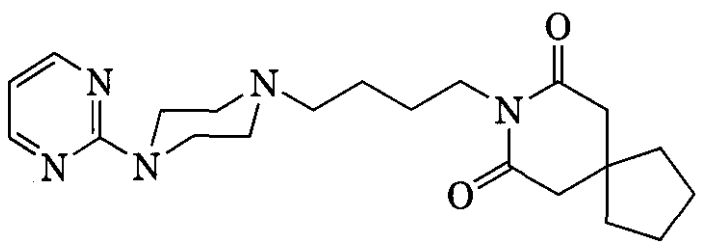


(34)

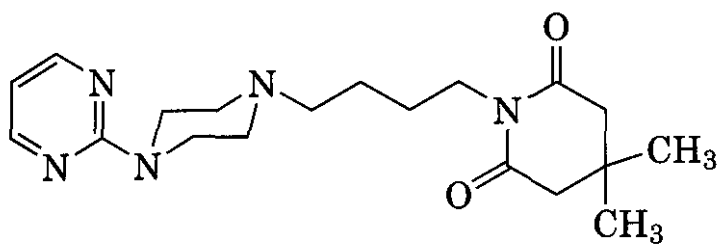
3.2.3 5-HT„ Receptors. 5-HT_{1A} receptors have been implicated as playing roles both in depression and in anxiety (129). Postsynaptic 5-HT„ (partial) agonist effects may be more

important for antidepressant action, whereas agonist effects at presynaptic 5-HT$_{1A}$ receptors may be more important for antianxiety activity (130–132). Furthermore, postsynaptic 5-HT$_{1A}$ receptors have been shown to be hypersensitive in depressed patients, whereas presynaptic receptors are hyposensitive (133). Also, electroconvulsive therapy has been demonstrated to upregulate cortical 5-HT$_{1A}$ receptors (134).

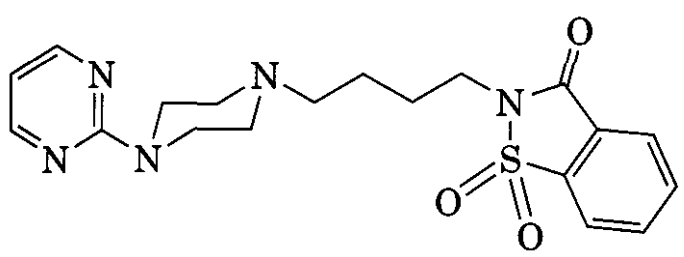
Certain long-chain arylpiperazines (LCAPs) (135) have been demonstrated to possess both anxiolytic and antidepressant actions. For example, the anxiolytic agents buspirone (**35**), gepirone (36) (**136**), and ipsapirone (37) (**137**) showed antidepressant activity in clinical studies. These agents are full agonists at presynaptic 5-HT$_{1A}$ receptors, but partial agonists at postsynaptic 5-HT$_{1A}$ receptors (126). An alternative approach is to develop postsynaptic 5-HT$_{1A}$ agonists with greater efficacy than that of those currently available. Flesinoxan (**38**), an example of such an agent, is currently in clinical trials. A newer agent of this type is exemplified by (39) (**139**), which binds at 5-HT$_{1A}$ receptors with high affinity ($K_i = 1$ nM).



(35) Buspirone

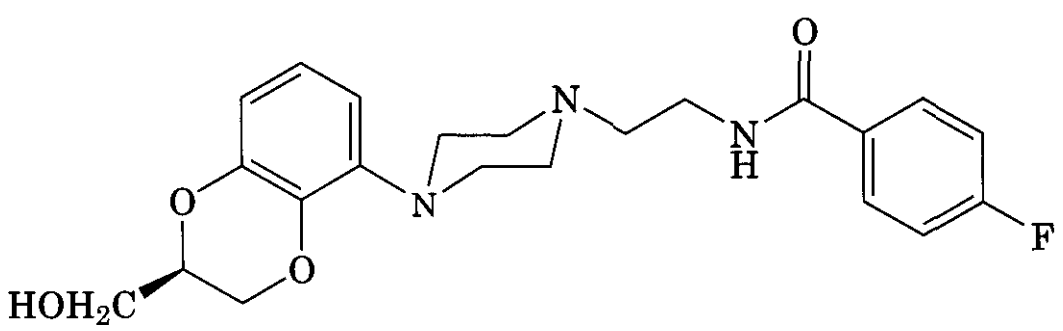


(36) Gepirone

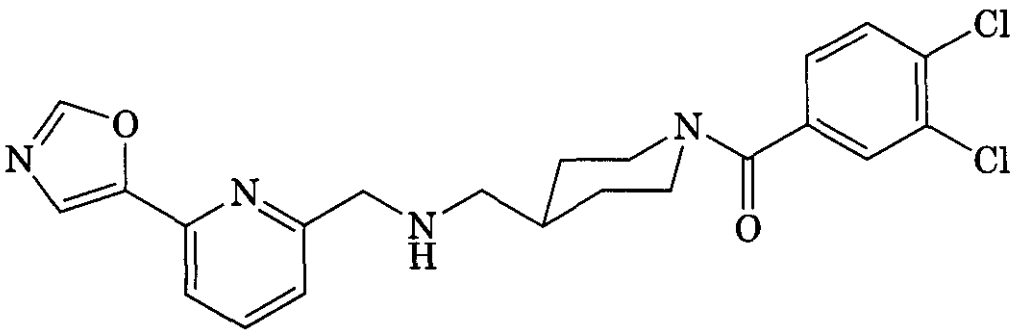


(37) Ipsapirone

A problem associated with many antidepressants is their delayed onset of action. Agents typically require 1–3 or more weeks before effects are realized. It has been hypothesized that the delay might be related, in part, to the initial elevation in synaptic 5-HT levels, which reduces the firing of serotonergic neurons by activating autoreceptors, mainly of the 5-HT$_{1A}$ subtype (139). During treatment with antidepressants these autoreceptors are desensitized and proper firing of 5-HT neurons is restored; many believe this to be one of the key changes elicited by antidepressant drugs of many different categories. 5-HT$_{1A}$ serotonin receptors are found both presynaptically and postsynaptically. Agents that behave as antagonists at presynaptic 5-HT$_{1A}$ receptors (i.e., somatodentritic autoreceptors) could, in theory, shorten the time of onset of those antidepressants that act by increasing synaptic levels of serotonin. In animal studies the combination of acute treatment with an SSRI together with a 5-HT$_{1A}$ antagonist led to a larger increase in 5-HT release, as predicted (140). Although no 5-HT$_{1A}$ antagonists are available for human use, the beta-blocker pindolol has appreciable affinity as an antagonist at 5-HT$_{1A}$ receptors (K_d value of approximately 10 nM). To date, the results of 15 placebo-controlled clinical trials, involving some 800 patients, using pindolol in treating de-



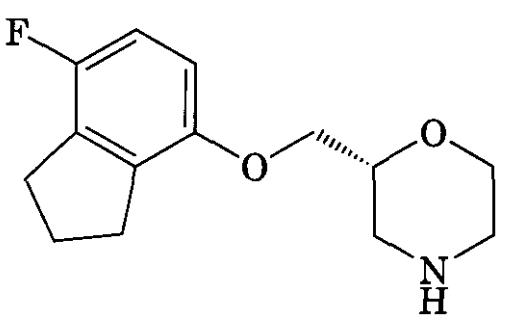
(38) Flesinoxan



(39)

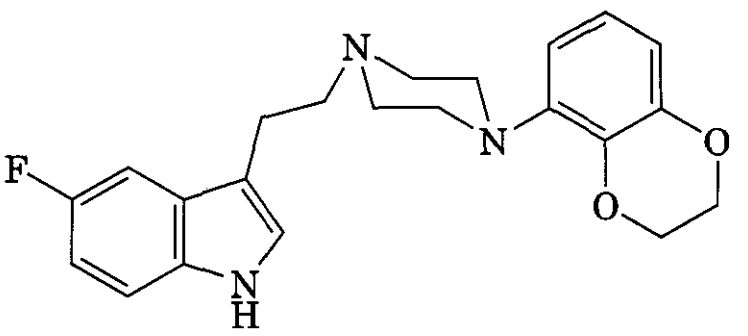
pression have been published (141, 142). Pindolol significantly accelerated the onset of action of SSRIs in five out of seven trials designed to test this concept. The combination of pindolol with fluoxetine (9), for example, reduced the median period required to obtain a clinical response (50% reduction in baseline score) from 29 to 19 days (142). A similar acceleration of rate of onset has been shown with a combination of pindolol and paroxetine (18), and citalopram (4) (143–146 and references therein). A role for β-adrenergic involvement in these actions of pindolol has been ruled out (147).

3.2.4 Mixed-Function Ligands. Recently, attempts have been made to incorporate multiple actions into the same molecule. For example, compound (**40**) (**YM-35992**) is a 5-HT,



(40)

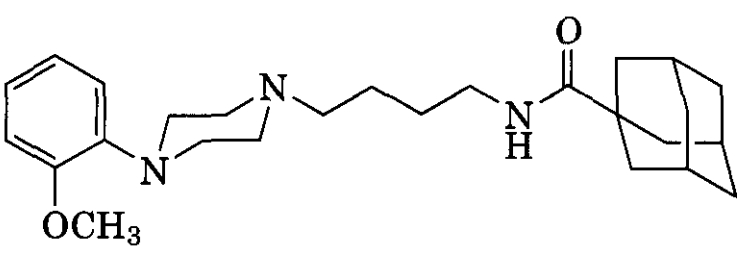
antagonist (IC_{50} = 86 nM) and a 5-HT reuptake inhibitor (SERT IC_{50} = 20 nM) (148). Compound (41) is a 5-HT$_{1A}$ (K_i = 2.3 nM) antagonist with high affinity for the 5-HT transporter (K_i = 12 nM) (149), whereas compound (42) displays somewhat higher affinity for



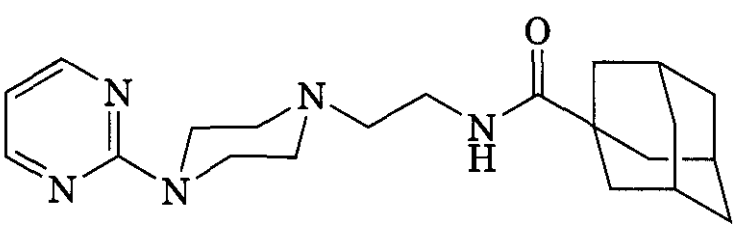
(42)

both sites (5-HT$_{1A}$ IC_{50} = 4.6 nM; SERT IC_{50} = 1.7 nM) (150).

Compounds combining high affinity both for 5-HT$_{1A}$ and 5-HT, receptors would be potentially useful for the treatment of depression. RK-153 (43) (151, 152) and adatanserin (44) (153) are examples of such agents. RK-153 (43) binds at 5-HT$_{1A}$ and 5-HT$_2$ receptors with high affinity (K_i values of 0.4 and 34



(43) RK-153



(44) Adatanserin

nM, respectively) and displays reduced affinity for α_1-adrenergic receptors ($K_i > 1000$ nM) but high affinity for dopamine D2 receptors ($K_i = 2.7$ nM), whereas adatanserin (**44**) binds at 5-HT$_{1A}$ and 5-HT$_2$ receptors with comparable affinity (K_i values of 1 and 73 nM, respectively), but displays substantially reduced affinity for dopamine D2 receptors (K_i = 166 nM). Both agents are 5-HT$_{1A}$ partial agonists, and adatanserin (44) has been demonstrated to be a 5-HT$_{2A}$ antagonist and is being developed as an antidepressant (153).

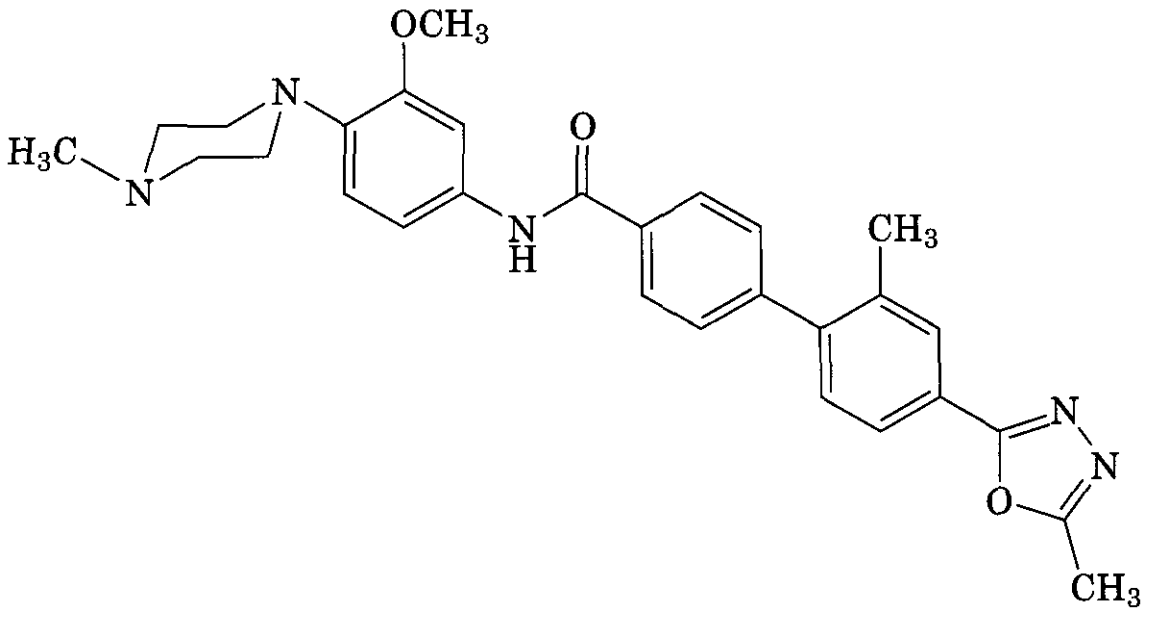
3.2.5 Other 5-HT Receptors. Although somatodendritic serotonin receptors are of the 5-HT$_{1A}$ type, terminal autoreceptors are of the 5-HT$_{1D/1B}$ type. These latter receptors are involved in the modulation of serotonin release, and blockade of these receptors has been proposed as a possible means of developing novel antidepressants or of enhancing the effects of SSRIs (reviewed in Refs. 154, 155). SSRIs require 1 or more weeks before their antidepressant actions are evident; this delay corresponds to the time required to desensitize 5-HT$_{1A}$ and 5-HT$_{1D/1B}$ receptors (156). Hence, a 5-HT$_{1D/1B}$ autoreceptor antagonist might hasten the onset of effects of an SSRI by mimicking its desensitizing action. Compound (**45**), a 5-HT$_{1D/1B}$ antagonist, has been demonstrated to augment citalopram-induced effects in rat ventral hippocampus and provides some support for this concept (157).

A population of receptors that has yet to receive extensive investigation from an antidepressant perspective are the 5-HT$_7$ receptors. 5-HT$_7$ receptors are known to be involved in the release of several neurotransmitters, including norepinephrine, serotonin, and dopamine, and there are preclinical data indicating 5-HT$_7$ antagonists might play a role in anxiety and, to a lesser extent, in depression (reviewed in ref. 158). It might be noted that mirtazepine (**14**), although its antidepressant properties have been attributed primarily to its antagonist action at α_2-adrenergic receptors and 5-HT$_2$ serotonin receptors, is also a 5-HT$_{1A}$ agonist and a 5-HT$_7$ antagonist (159).

Two of the most recently discovered populations of 5-HT receptors are the 5-HT$_5$ and 5-HT$_6$ receptors. Of interest is that certain typical and atypical antidepressants, as well as typical and atypical antipsychotic agents, bind at both populations of receptors (160, 161). A role for either receptor population in the actions of these agents has yet to be firmly established, but the nanomolar affinity of the agents for these receptors has heightened interest in them.

3.3 Alpha Adrenergic Receptors

Although several antidepressant drugs have weak affinity for alpha-adrenergic receptors (Table 8.6) only one compound is thought to owe its antidepressant activity to such an interaction. This is mirtazepine (**14**), which has nanomolar affinity as an α_2-adrenergic receptor antagonist (162, 163). The α_2-adrenergic receptors are located presynaptically on both noradrenergic and serotonergic nerve endings



(45)

and cell bodies, and exert an inhibitory effect on monoamine neuronal firing and monoamine release. Administration of mirtazepine to animals has been reported to increase the spontaneous rate of firing of noradrenergic neurons in rat locus coeruleus, and serotonin neurons in the raphe nucleus (164), and to increase levels of 5-HT release in hippocampus (165), although this was not confirmed in another publication (166). Clinical trials have shown that mirtazepine is equivalent to amitriptyline and other tricyclics in antidepressant efficacy (167). Some of these studies showed a clinical improvement within the first week of treatment (167). Mianserin (27) is another agent whose effects, at least in part, might involve a,-adrenergic antagonism (168). Many adrenergic agents possess an imidazoline ring. Imidazolines have been found to bind at nonadrenergic imidazoline binding sites and this has led to speculation that such sites might play a role in depression (see Section 5.4).

3.4 Monoamine Oxidase

Monoamine oxidase (MAO) is an enzyme located in the outer mitochondria1 membrane. As its name implies, it catalyzes the oxidative deamination of a variety of monoamines. The enzyme is particularly abundant in liver, where it serves to detoxify a variety of amines that are absorbed from the diet; these include the vasoactive monoamines tyramine, octopamine, phenylethanolamine, and phenylethylamine. The enzyme is also present in monoamine-containing neurons, where it serves to regulate levels of cytoplasmic monoamine neurotransmitters (169). There are two forms of MAO: MAO-A and MAO-B (170). Both are present in the liver, and in most monoaminergic neurons, although MAO-A is predominant in norepinephrine and dopamine-containing neurons, and MAO-B in serotoninergic cells. Both forms of the enzyme have a wide and overlapping range of substrates, but MAO-A shows some preference for the catecholamines norepinephrine and epinephrine, and serotonin, and MAO-B for tyramine, phenylethylamine, phenylethanolamine, and benzylamine. Both enzymes metabolize dopamine and tryptamine (169–171).

The first-generation MAO inhibitors phenelzine (19) and tranylcypromine (23) act as substrates for MAO-A and MAO-B but are converted by the enzyme to highly reactive intermediates that then react irreversibly with the enzyme to cause an irreversible inhibition of activity. Recovery of MAO activity after exposure to these MAO inhibitors requires the synthesis of new enzyme protein, a process that takes some weeks to completely restore activity (173, 174). A clinical antidepressant response is associated with an inhibition of platelet MAO activity of approximately 80%, and measurement of platelet MAO activity can be used to monitor treatment dose regimes (175).

A new series of MAO inhibitors are selective for MAO-A and cause reversible inhibition of the enzyme, thus leaving MAO-B in the liver intact to detoxify dietary amines, and showing rapid recovery of enzyme activity after discontinuation of drug treatment. The only drug of this type so far available for human use is moclobemide (15).

3.5 Other Proposed Mechanisms of Action

Although many lines of evidence point to a common mode of action of antidepressant drugs by an enhanced release of the monoamine norepinephrine and serotonin in the brain, many questions remain unanswered. If the monoamines themselves were responsible for regulating mood, why do depressed patients not feel an improvement immediately after receiving the first dose of antidepressant? The fact that the clinical response is delayed by several weeks suggests that the immediate effects of the drugs on monoaminergic mechanism in turn trigger longer-term changes in the brain, probably involving alterations in gene expression. One possibility already discussed is that the early effects of increased monoamine release are counteracted by brain mechanisms that downregulate monoamine release in response to the immediate effects of the drugs. Thus, receptor desensitization is required before the full effect of the antidepressants on monoamine release can be seen.

However, there are other alternative explanations for the delayed clinical response. There have been many studies of the neuro-

chemical changes caused in animal brain by chronic treatment with antidepressants. One of the changes elicited by many antidepressants is a downregulation in the expression of β-adrenergic receptors (176–178). This is of interest because one of the most consistent findings in depressed patients has been that β-adrenergic receptors are upregulated in peripheral lymphocytes and in the brains of suicide victims (179). It was proposed that the downregulation of β-receptors represents a marker of antidepressant efficacy (176, 177). The validity of this concept was soon challenged, however, because it was found that the newer SSRIs did not consistently downregulate β-adrenergic receptors, and citalopram (4)actually caused an increase (146). β-Adrenergic receptors are coupled to cyclic AMP formation but, although the receptors may be downregulated by antidepressant drugs, other components of cellular signaling that are regulated by cyclic AMP are upregulated, notably the cyclic AMP response element protein (CREB), a prominent transcription factor in the brain (180).Another way of increasing levels of cyclic AMP is to inhibit its degradation by phosphodiesterases.

The compound rolipram is a phosphodiesterase inhibitor and has been found to have clinical antidepressant activity (181). Although rolipram is not well tolerated because it causes severe nausea. it is possible that inhibitors with more selectivity for phosphodiesterase subtypes could be of future interest. In animals chronic treatment with SSRIs leads to increased expression of the PDE4A and PDE4B subtypes (183). Among the many genes that may be regulated by CREB are those encoding neurotrophic factors (183). An important new finding is that chronic treatment with antidepressants leads to increased expression of the neurotrophic factor BDNF (brain-derived neurotrophic factor) in rat hippocampus, and this may underlie the recent finding that chronic antidepressant treatment causes a proliferation of progenitor cells in the rat hippocampus (180, 184, 185). Neuroimaging and postmortem histological studies have reported reductions in neuronal and glial densities in dorsolateral prefrontal cortex in patients with mood disorders (185a,b). Compounds that targeted neurotrophic factor mechanisms could provide a novel approach to antidepressant drug discovery in the future (186).

Another possibly important change in receptor sensitivity associated with chronic treatment with antidepressants concerns receptors for glucocorticoids. Antidepressant treatment produces an improvement in the function of the hypothalamic-pituitary axis, in which several neuroendocine responses are blunted in depressed patients (187, 188).

4 HISTORY

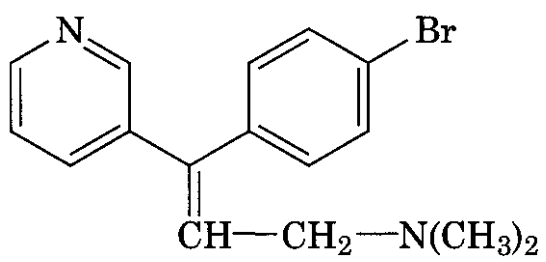
4.1 Discovery of the First Antidepressants

Before 1954, except for the use of electroconvulsive therapy, there were no effective treatments for depression. The two major classes of antidepressants, the monoamine oxidase inhibitors and inhibitors of monoamine transport, were discovered by accident in the 1950s. The drug iproniazid used for the treatment of tuberculosis was found to have a mood-elevating property (189) and clinical studies by George Crane and Nathan Kline in the United States showed it to be effective in treating major depression (190, 191). Its actions were traced to its ability to inhibit the monoamine-degrading enzyme MAO (192). Although this and other subsequently developed MAO inhibitors proved highly effective in the treatment of depression, the possible dangers associated with their use led to their being largely replaced by the safer inhibitors of monoamine transport. The first examples of the latter drugs to be widely used were imipramine (11) in Europe and amitriptyline (1)in the United States. Imipramine was synthesized originally by the Swiss company Geigy as a chlorpromazine-like molecule with potential as an antipsychotic drug. The Swiss psychiatrist Roland Kuhn, however, found it to be an effective antidepressant (193, 194). On the other side of the Atlantic, Merck first made amitriptyline also as a chlorpromazine-like molecule. It was shown subsequently to be an antidepressant by Frank Ayd (195). For a detailed and entertaining account of the history of the discovery of antidepressant drugs see Healy's ***The Antidepressant Era*** (1997) (196).

4.2 Case History: Fluoxetine (Prozac)

One of the earliest theories of how antidepressant drugs work was that they caused an increased availability of serotonin in the brain. This was supported by data from the British psychiatrist Alec Coppen, that combining an MAO inhibitor with the serotonin precursor tryptophan was a more effective antidepressant treatment than the MAO inhibitor alone (197), a result repeated by the Dutch psychiatrist Herman van Praag. The serotonin hypothesis was largely lost sight of, however, after the discovery that imipramine **(11)** and related tricyclic antidepressants were potent inhibitors of norepinephrine uptake **(11)** and the idea that norepinephrine was the key player in antidepressant drug actions dominated thinking, particularly in the United States (198). The norepinephrine-selective uptake inhibitor desipramine **(6)** from Merck proved highly successful, and in Europe the Swiss company Ciba launched its norepinephrine-selective uptake inhibitor maprotiline (**13**) (**95**).

Nevertheless, the "mixed" norepinephrine1 serotonin uptake inhibitors imipramine and amitriptyline continued to be very popular, particularly in Europe. In Sweden, the neuropharmacologist Arvid Carlsson, originally a champion of the norepinephrine hypothesis, became interested in the idea of developing selective inhibitors of serotonin uptake. Having failed to interest any major pharmaceutical company in this idea, he collaborated with the chemist Hans Corrodi at the Swedish company Astra. They produced the first SSRI, zimelidine (**46**), which was more potent than



(46) Zimelidine

clomipramine (**5**), the best SSRI then available, and unlike clomipramine zimelidine did not break down in the body to form a norepinephrine-selective active metabolite. Astra reported the first positive clinical trials with zimelidine as an antidepressant in 1980 (199). Zimelidine was initially successful in Europe and was to be marketed in the United States by Merck, who had completed extensive clinical trials and submitted the results to the FDA in 1983, although the drug was withdrawn, shortly thereafter following reports of drug-induced Guillain-Barré syndrome (peripheral nerve damage) in Europe. Carlsson also took the SSRI idea to the Danish company Lundbeck, which subsequently launched the highly selective and potent compound citalopram (4) in 1986. Meanwhile at Ciba-Geigy in Switzerland, Peter Waldmeier and colleagues had been working on the serotonin idea since the early 1970s and discovered several potent SSRIs, but were unable to persuade the company to develop any of them further (**95**). David Wong and colleagues at Eli-Lilly were more fortunate, but only after a long delay (96, 97). They had also been stimulated by Carlsson's ideas and started screening for SSRIs in 1972. They discovered fluoxetine (9) and reported its biochemical profile in 1974 (**96**), but Lilly was not at all clear what the drug would be used for. At one of the meetings of clinical experts that the company convened, Alec Coppen suggested that it might be tested in depression, only to be told that this was definitely not the target the company had in mind!' (196). It was only in the 1980s, when the antidepressant profile of zimelidine was reported, that Lilly began to speed up the development of fluoxetine (**9**), and it was 1985 until the first positive clinical trial results in depression were reported (**97**), followed by registration in the United States in 1987.

Several other SSRIs were registered: fluvoxamine (10) in 1983, sertraline (22) in 1990, and paroxetine (**18**) in 1991. It was fluoxetine (**9**), however, that captured the public imagination and became the single most important psychopharmaceutical product of the late twentieth century. Physicians liked it because it was safe in overdose, the dosage regime was simple (not requiring any gradual titration) and the side-effect profile was an improvement over that of the earlier tricyclics. Prozac was on the cover of *Time* and *Newsweek* and it gained a number of additional medical uses (Table 8.4). Some people also used it for non-medical purposes, just to make them feel bet-

ter, or to enjoy the opera more! A whole literature was spawned around the drug, most famously in Peter Kramer's book, Listening to *Prozac* (200). A new market was even found for the drug in the treatment of depression in companion animals. By the turn of the century, sales of Prozac were earning Lilly in excess of $2 billion annually.

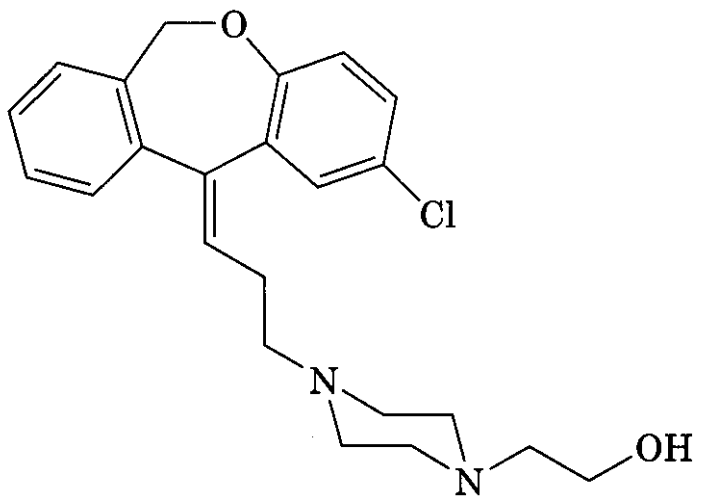
5 STRUCTURE-ACTIVITY RELATIONSHIP AND METABOLISM

5.1 Reuptake Inhibitors

The SAR of the tricyclic antidepressants has been studied for over 40 years and numerous reviews are readily available on these and related agents (168, 201–203). Only the highlights of some of the pertinent SAR studies are presented here. Tricyclic antidepressants have in common a tricyclic ring structure consisting of a six-membered or, more commonly, a seven-membered ring flanked, typically, by two benzene rings. The exact composition and orientation of the central ring is relatively inconsequential (see Fig. 8.1). Tricyclic antipsychotics and tricyclic antidepressants appear to exist on a structural continuum. For example, introduction of an electron-withdrawing group to the aromatic ring typically enhances the antipsychotic profile of the tricyclic agent. Replacement of a ring nitrogen atom (as in the phenothiazines) or an sp^2-hybridized carbon atom (as with the thioxanthenes) to which the aminopropyl substituent is attached, with an sp^3-hybrized carbon atom detracts from the antipsychotic profile but can enhance the antidepressant profile of an agent. That is, a ring nitrogen atom or an sp^2-hybridized carbon atom is not required at this position for antidepressant activity; for example, compare the structures of nortriptyline (17)and protriptyline (20).

The tricyclic system is generally attached to a basic terminal amine by a three-atom chain. The chain can be shortened to two atoms with retention of antidepressant activity and a decrease in antipsychotic character. The terminal amine is typically a secondary or tertiary amine, and the amine can be part of an alicyclic ring. In general, tertiary amines are more nonselective with respect to inhibition of 5-HT and NE reuptake, whereas the secondary amines are typically more selective at inhibiting NE reuptake. In *vivo*, however, one of the major routes of metabolism of the tertiary amines is by demethylation to a secondary amine.

Doxepine (8)is related in structure to the antipsychotic agent pinoxepin (47). However,



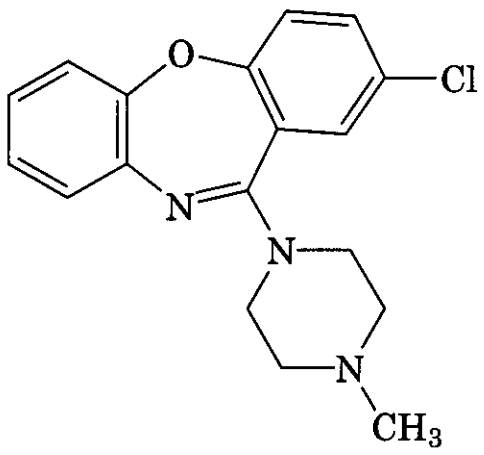
(47) Pinoxepin

because doxepine lacks the N-hydroxyethyl group of pinoxepin (a functionality known to enhance antipsychotic activity) and the electron-withdrawing chloro group, it behaves more as an antidepressant than as an antipsychotic agent.

Because the presence of a ring nitrogen atom or an sp^2-hybridized carbon atom in the central ring of these tricyclic structures is not a requirement for antidepressant activity, protriptyline (20) retains antidepressant action. Certain tricyclic antidepressants with a six-membered central ring have been shown to possess an antidepressant profile. If the central ring is capable of aromatization, by oxidation for example, to afford a completely planar structure, the resulting compound is inactive. If aromatization can be prevented, however, antidepressant activity is retained. Maprotiline (13) is an example of a tricyclic agent where aromatization is prevented by introduction of an alkyl bridge. Aromatization is prevented according to Bredt's rule. Because it is a secondary amine, maprotiline is fairly selective for inhibition of NE reuptake. Note that maprotiline does not possess either a nitrogen atom or sp^2-hybridized carbon atom in the central ring. Although originally considered as a member of a new class of antidepressants––

the "tetracyclic antidepressants"—maprotiline is now classified as a TCA.

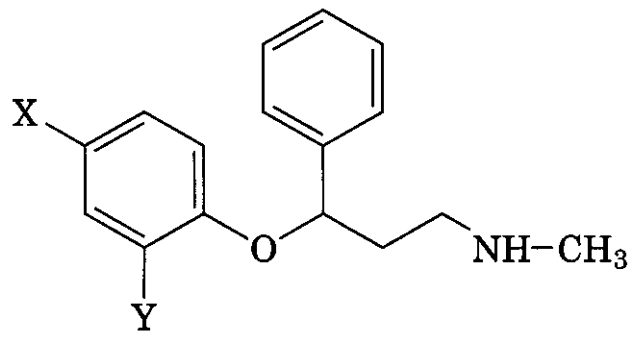
Amoxepine is a structurally interesting agent because of the introduction of an electron-withdrawing group. As already mentioned, the orientation of the tricyclic system does not seem crucial to antidepressant (or antipsychotic) activity. Loxapine (**48**), for example, is an antipsychotic agent. Arnoxepine is the N-desmethyl analog of loxapine. Because secondary amines show a greater antidepressant profile than that of tertiary amines, amoxepine possesses greater antidepressant character than that of loxapine. However, amoxepine also possesses some antipsychotic character attributed to the presence of the electron-withdrawing chloro group.



(48) Loxapine

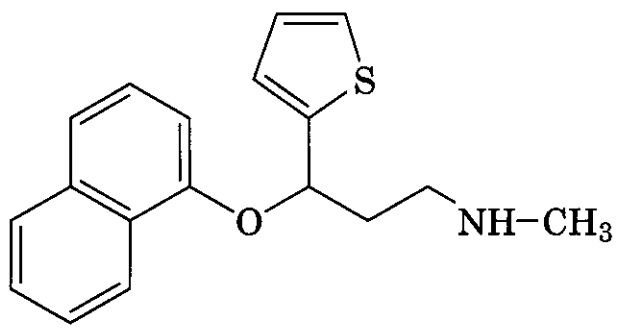
The tricyclic antidepressants typically undergo multiple routes of metabolism. The most common, depending on the particular ring system, are N-demethylation of the terminal amine, aromatic hydroxylation, and benzylic or "bridge" hydroxylation (see Table 8.7). In general, with the exception of the secondary amine metabolites of *N,N*-dimethylamino TCAs, the metabolites are usually less active or inactive as antidepressants.

Remarkably little has been published on the structure-activity relationships of reuptake inhibitors. Close inspection of these inhibitors reveals that many of them are related in structure to ring-opened tricyclic antidepressants. Indeed, small structural changes can result in shifts in selectivity. For example, fluoxetine (9) is a serotonin-selective reuptake inhibitor, whereas nisoxetine (49) is a norepinephrine-selective reuptake inhibitor.

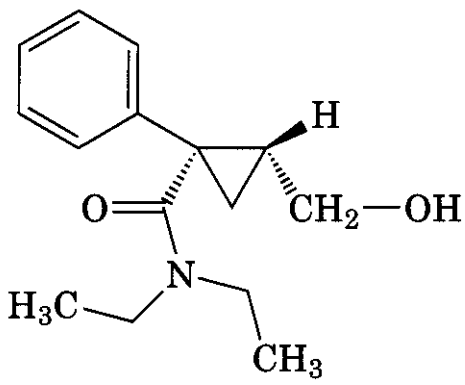


(9) Fluoxetine X = $-CF_3$, Y = –H
(49) Nisoxetine X = –H, Y = $-OCH_3$

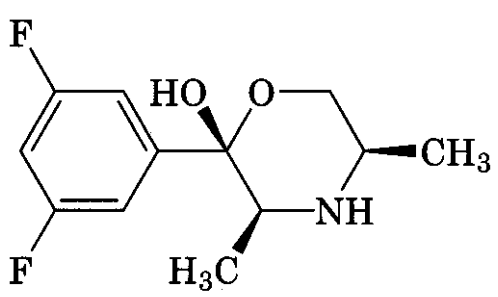
Newer norepinephrine-selective reuptake inhibitors currently in clinical trials include duloxetine (50) and milnacipran (51). An analog of a bupropion metabolite, (**52**), has been shown to act as a norepinephrine-selective reuptake inhibitor (204).



(50) Duloxetine



(51) Milnacipran

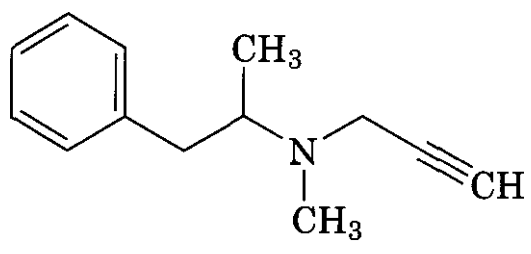


(52)

Sertraline and fluoxetine undergo metabolic demethylation. Unlike the metabolites of most other SSRIs, the desmethyl metabolite of fluoxetine, norfluoxetine (34), retains the ability to inhibit serotonin reuptake (205). Hence, fluoxetine takes longer to achieve steady-state levels, and it retains activity after metabolism. The half-life of fluoxetine is approximately 1 day, but elimination of norfluoxetine is prolonged (7–15 days) (205). In addition to its actions as an SSRI, norfluoxetine also binds at 5-HT, receptors (127).

5.2 Monoamine Oxidase Inhibitors

Because of undesirable side effects associated with monoamine oxidase inhibitor therapy (see section 2.3.2), pharmaceutical companies nearly abandoned research on new analogs in the 1960s. The early MAO inhibitors were nonselective and irreversible. Today, efforts toward the development of monoamine oxidase inhibitors are focused on selective MAO-A or MAO-B inhibitors. Selective MAO-B inhibitors are being examined in the treatment of, for example, schizophrenia, Alzheimer's disease, and Parkinson's disease. MAO-B inhibitors might be effective in the treatment of depression, but relatively little work has been done in this area. Selegiline (53) or (−)-deprenyl, a selective irreversible

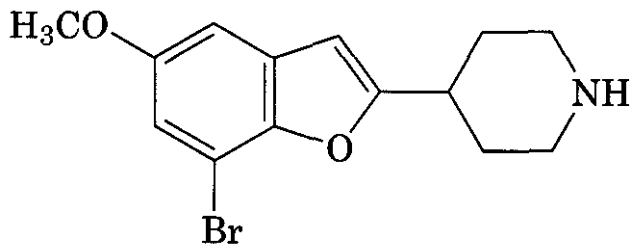


(53) Selegiline

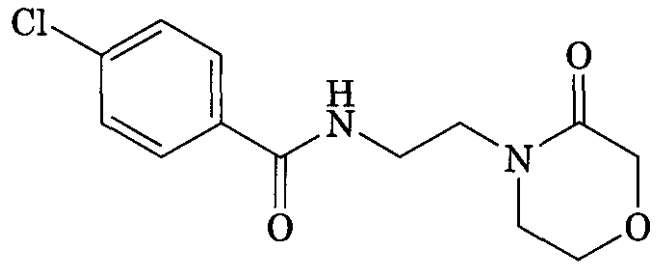
MAO-B inhibitor, is one of the few agents that has been examined in this regard and the clinical results are mixed (reviewed in ref. 206).

Of greater application to the treatment of depression are the reversible inhibitors of monoamine oxidase-A or RIMAs. Brofaromine (54) is a "tight-binding" but reversible inhibitor of MAO-A with approximately 100-fold selectivity vs. MAO-B. Moclobemide (15) is a RIMA with about five- to 10-fold selectivity for MAO-A. Although both agents display micromolar (or lower) affinity for adrenergic, serotonin, and most other receptors, brofaromine, interestingly, binds at the 5-HT and NE transporters with modest affinity (IC_{50} values of 150 and 500 n*M*) (207). For comparison, the half-life for disappearance of MAO-A inhibition in brain is phenelzine (19), 11 days; tranylcypromine (23), 2.5 days; brofaromine (54), 12 h; and moclobemide (15), 6 h (reviewed in ref. 207). Another advantage of RIMAs over the older MAO inhibitors is that a shorter washout time is required; whereas the older MAO inhibitors typically require about 2 weeks for washout, the washout period for moclobemide is about 48 h (208). This is an important consideration when switching antidepressant therapies from a MAO inhibitor to, for example, a TCA or SSRI.

Moclobemide undergoes multiple routes of metabolism with formation of a lactam (55)

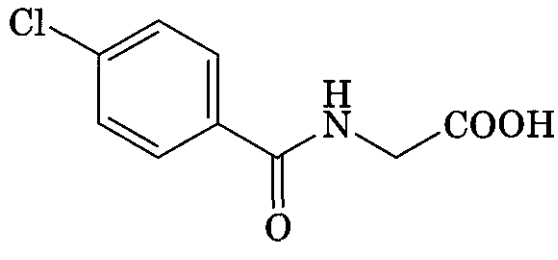


(54) Brofaromine



(55) Moclobemide lactam

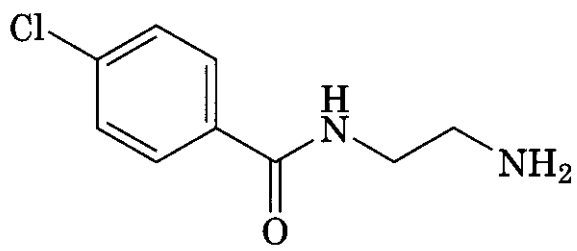
being a major metabolite. Other metabolites include 3-hydroxymoclobemide, meclobemide N-oxide, and compounds (56) and (57) (209). Lactam (55) is devoid of activity as a MAO inhibitor, whereas the primary amine (Ro 16–



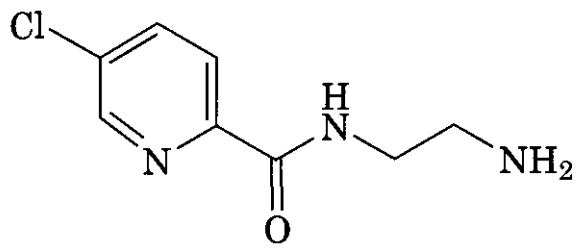
(56)

6491, 57) and several other minor metabolites are inhibitors of MAO-B (207). Analogs of the latter compound have been developed, including the selective MAO-B inhibitor Ro 19–6327 (58) and the selective MAO-A inhibitor Ro 41–1049 (59) (203).

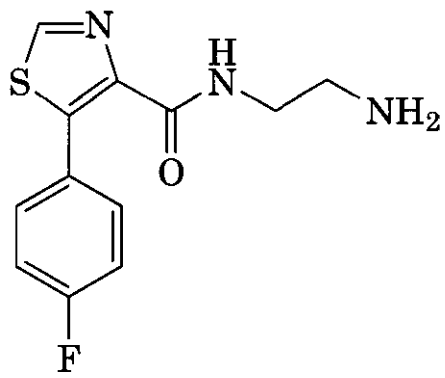
Newer RIMAs include the brofaromine analog sercloramine (60) (which, incidentally,



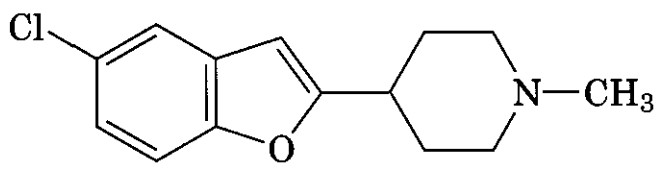
(57) Ro 16-6491



(58) Ro 19-6327

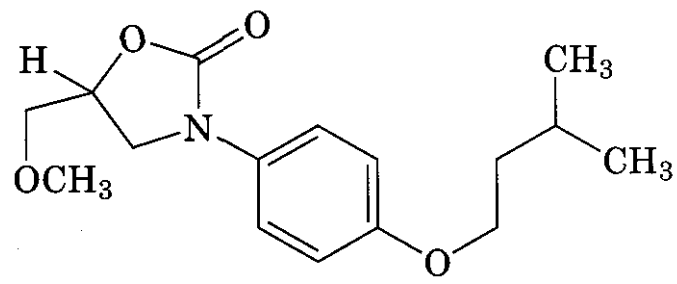


(59) Ro 41-1049

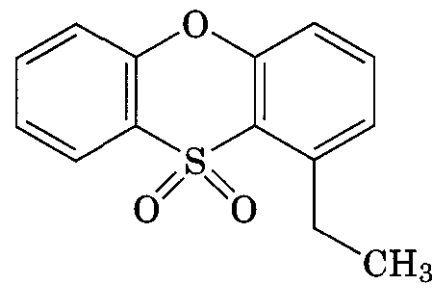


(60) Sercloramine

also behaves as a 5-HT reuptake inhibitor), befloxatone (61), and BW 1370U87 (62) (168). The latter two agents are somewhat more MOA-A selective than moclobemide and the relative order of potency is befloxatone > brofarornine > moclobemide > BW 1370U87,



(61) Befloxatone



(62) BW 1370U87

whereas duration of action decreases in the order brofaromine > BW 1370U87 > befloxatone > moclobemide (207).

5.3 Serotonergic Agents

The two populations of 5-HT receptors that have been definitely linked to depression are the 5-HT, and the 5-HT„ receptors. Numerous 5-HT, antagonists are available and belong to a multitude of chemical classes; structure-activity relationships are well beyond the scope of this chapter and several reviews are available (e.g., 151,210,211). 5-HT, receptors consist of three families: 5-HT„ 5-HT_{2B}, and 5-HT„. An important issue at this time is which one (or more) of these subpopulations is most involved in depression. This question remains to be answered. Another issue is that 5-HT„ receptors have been linked to cardiac valvulopathy (212). Hence, future development of 5-HT, ligands might wish to avoid agents with high affinity for 5-HT„ receptors.

5-HT„ receptor involvement is complicated by the issue of functional activity; what is more important, agonist actions, partial agonist actions, or antagonist actions? Evidence suggests that presynaptic 5-HT„ antagonism and postsynaptic 5-HT„ agonism might be the most desirable features for agents to target the treatment of depression. The structure-activity relationships of 5-HT_{1A} agents

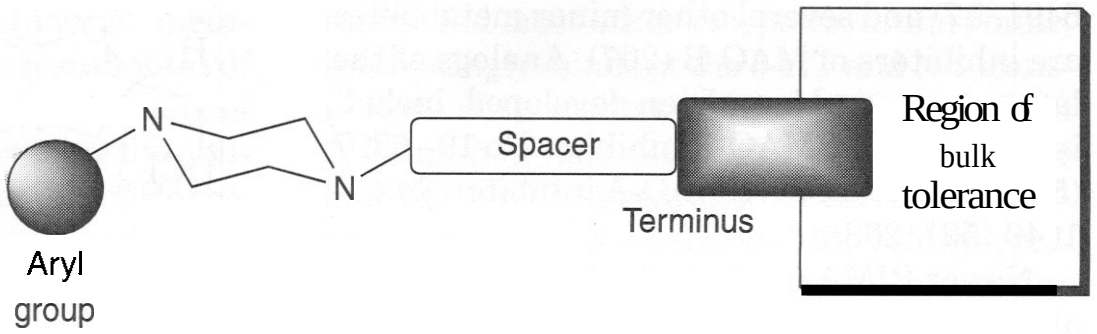
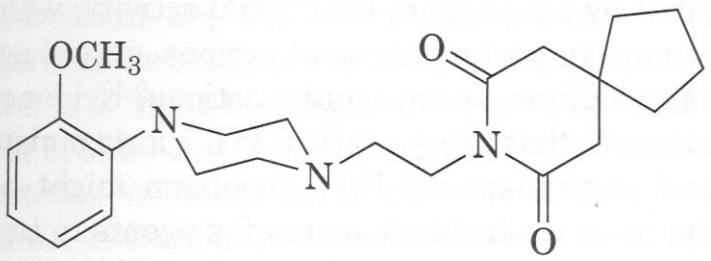


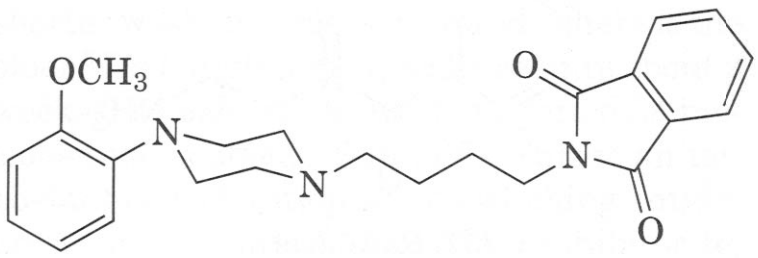
Figure 8.5. General structural features of long-chain arylpiperazines (LCAPs). (Adopted from Refs. 135 and 214.)

have been reviewed (120,151,213). Of particular interest are the arylpiperazines and, more specifically, the long-chain arylpiperazines (LCAPs) (Fig. 8.5). Simple arylpiperazines, those bearing only a small or no N_4-substituent, bind with modest affinity at 5-HT_{1A} receptors and display little to no selectivity, whereas the LCAPs, arylpiperazines with elaborated N_4 substituents, bind with higher affinity at 5-HT,, receptors and can display considerable selectivity (135, 151).

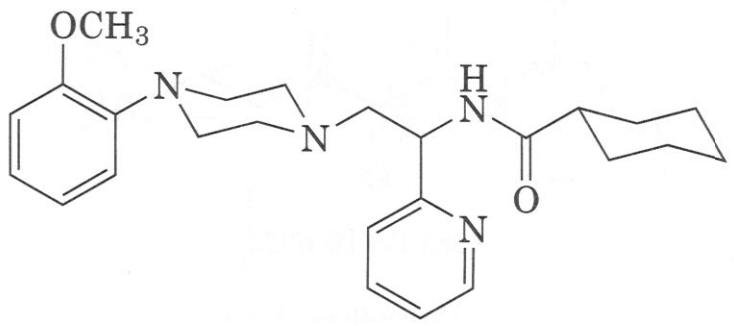
The aryl portion of the LCAPs (Fig. 8.5) can be widely varied, and the unbranched or branched *spacer* can be of two to five (or more, depending on the nature of the terminus) atoms in length. The ***terminus*** is usually an aromatic, heteroaromatic, imide, or amide function, and is associated with a region of considerable bulk tolerance (135). Given this pharmacophore model, hundreds, if not thousands, of possible analogs can be envisioned. Many have been synthesized and evaluated (e.g., reviewed in Ref. 151). Advantage has been taken of the region of bulk tolerance to develop some very bulky analogs (214). Inspection of the structures of buspirone (**35**), gepirone (**36**), and ipsapirone (37) shows that they meet these criteria. Alteration of the substituents modifies functional activity, but this has not yet been investigated in a systematic manner. For example, buspirone, gepirone, and ipsapirone are partial agonists;



(63) BMY-7378



(64) NAN-190



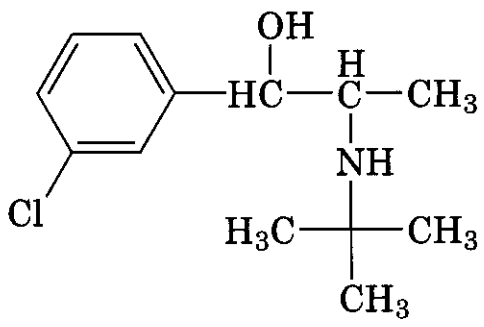
(65) WAY-100,635

BMY-7378 (63) and NAN-190 (64) were the first examples of LCAPs with antagonist actions (i.e., they are very low efficacy partial agonists); and WAY-100,635 (65) was the first example of a 5-HT,, "silent antagonist" (reviewed in Ref. 214).

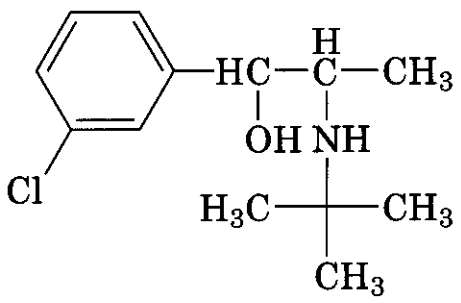
Structural modification, particularly with respect to the spacer and terminus groups, also influences selectivity; LCAPs show varying degrees of affinity toward several receptor populations, notably, 5-HT,, 5-HT,, dopamine D2, and α-adrenergic receptors (135, 151, 214). This has led to attempts to develop agents selective for each of these receptor populations by the appropriate structural modification. RK-153 (43) and adatanserin (44) are just two examples of such agents. Continued exploration of LCAPs should prove bountiful for the development of agents with the desired mix of selectivity and functional activity.

5.4 Other Agents

Several antidepressants work via mechanisms that are not yet fully understood. Perhaps the best example of this is bupropion (3). Although the mechanism of action of bupropion is most commonly attributed to inhibition of dopamine reuptake, its actions are diverse (215).In addition, one or more bupropion metabolites also seem to be active. In humans, bupropion is metabolized to two amino alcohols, the racemic threo amino alcohol (*R,R*-threo; 66) and the racemic erythro amino alcohol (*R,S*-erythro; 67), threohydroxybupropion and erythrohydroxybupropion, respectively; and a morpholinol, hydroxybupropion (68; BW 306U). The levels and half-lives of



(66)



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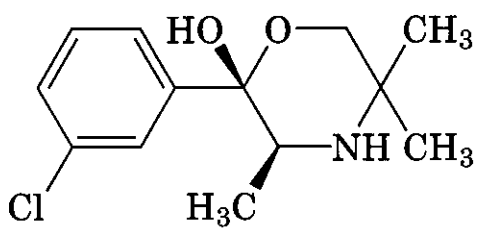


(68) Hydroxybupropion

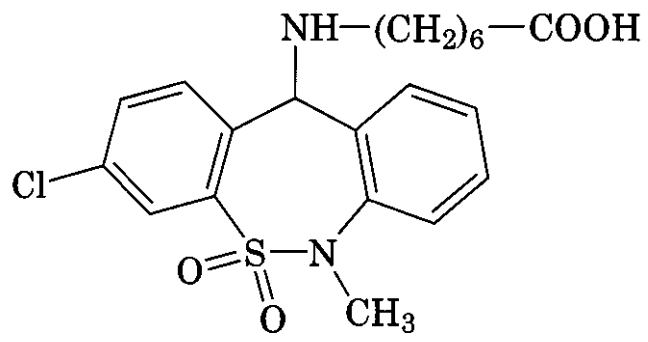
these metabolites have been quantitated in healthy volunteers (216–218).Hydroxybupropion (68) is the major metabolite in depressed patients (219). Interestingly, all three metabolites predominated over the parent compound in plasma and cereobrospinal fluid at steady state, and high levels of these metabolites were suggested to be associated with poor clinical outcome resulting from toxic effects possibly involving the dopaminergic system (219). In contrast, bupropion metabolites showed various degrees of activity in several animal models of antidepressant activity (220). However, the routes of metabolism in rodents and dogs are known to be somewhat different from those in humans (221–222); in fact, routes of metabolism in mice differ from those in rats (223). Nevertheless, at least hydroxybupropion is considered to be an active metabolite of bupropion (223). Both threohydroxybupropion (66) and hydroxybupropion (68)inhibit norepinephrine reuptake with potencies comparable to that of bupropion but are 10 to 25 times less potent than bupropion with respect to inhibition of dopamine reuptake (215).

Although there is evidence for dopaminergic involvement in the antidepressant actions of bupropion as determined by measuring, for example, extracellular levels of dopamine in the striatum and nucleus accumbens in rats (224), or by examining the increase in dopamine release in striatal synaptosomes upon treatment with bupropion (225), alternative mechanisms have been proposed. Bupropion and its metabolites produced noradrenergic-like effects on the firing rates of noradrenergic neurons located in the locus coeruleus of rat (226) and it has been suggested that bupropion might work through some yet unidentified adrenergic mechanism (reviewed in ref. 215). More recently, it has been shown that sustained administration of bupropion by the use of minipumps decreases the firing rate of norepinephrine neurons arising from increased activation of somatodendritic α_2-adrenoceptors, suggesting that the actions of bupropion are mainly attributable to enhancement of norepinephrine release, not to reuptake inhibition (227).This latter study also showed that bupropion can act on the serotonergic system.

One of the latest theories to account for the action of bupropion is its influence on imidazoline I, receptors. Agmatine (decarboxylated arginine) may be an endogenous ligand for I, sites. Evidence indicates that plasma agma-

tine levels are significantly elevated in depressed patients relative to those in healthy controls and that treatment with bupropion normalized these levels (228, 229 and references therein). Peripheral norepinephrine neurons possess nonadrenergic imidazoline binding sites that mediate inhibitory effects on the release of norepinephrine (230). Platelet I, receptors are also downregulated after antidepressant treatment; this has led to conjecture that elevation in brain imidazoline receptors might lead to greater inhibition of norepinephrine release (229).

Another curious agent is tianeptine (69). In vitro, neither tianeptine nor any of its major



(69) Tianeptine

metabolites has any effect on monoamine reuptake, release, or neurotransmitter binding; the biochemical effects (acute or chronic) of this agent in vivo indicate enhanced serotonin uptake in the cortex and hippocampus (230, 231).

Tianeptine has now been evaluated in over 3000 patients and has been found to be at least as effective as the TCAs (e.g., amitriptyline, imipramine, maprotiline), SSRIs (e.g., fluoxetine, sertraline), and mianserin (231, 232).

6 RECENT DEVELOPMENTS AND THINGS TO COME

6.1 Monoaminergic Drugs

All of the existing antidepressant drugs act through monoaminergic mechanisms, and further refinements are still possible in this arena. The concept of developing dual norepinephrine/serotonin uptake inhibitors, claimed for venlafaxine, has been adopted by other companies. Compounds with this profile are being developed by Eli-Lilly [duloxetine (**50**)] (235,236) and Pierre Fabre [milnacipran (**51**)] (237,238). The Danish company NeuroSearch has taken one step further and is developing a triple inhibitor of norepinephrine/serotonin and dopamine uptake with GSK (NS2389). Another idea is to separate the individual enantiomers of existing racemic antidepressant drugs, in the hope that the individual isomers may show some improvement over the parent drug. A number of existing antidepressants are racemates, which include fluoxetine (**9**), citalopram (**4**), mirtazepine (**14**), mianserin (27) and reboxetine (21). Lundbeck appears to have been successful in developing the active (*S*)-enantiomer of citalopram (**4**), escitalopram, which is reported to be slightly more efficacious and to have fewer side effects and a faster onset of action than that of the parent compound (239, 240). This approach will not always succeed, however. Lilly's attempt to develop (*R*)-fluoxetine failed because the compound caused small increases in cardiac QT intervals (241).

The ability of pindolol to accelerate the onset of action of antidepressant drugs has prompted a search for more selective antagonists or partial agonists acting at the 5-HT_{1A} receptor. The compound EMD 68843, from Merck KgaA, combines activity as an SSRI with a partial agonist profile at the 5-HT_{1A} receptor, and is in development as an antidepressant (242,243). The compound YM-35992 (40) from Yamanouchi, which combines an SSRI profile with blockade of 5-HT_{2A} receptors, represents another variant on this theme (244).

In view of the good clinical safety profile of the reversible MAO-A-selective MAO inhibitor moclobemide (**15**), it is worth noting that a second reversible MAO inhibitor befloxatone (61) is in development (245). In animal studies a combination of befloxatone with pindolol greatly accelerated the action of the MAO inhibitor in increasing serotonergic neuron discharge in rat brain (246), suggesting that this combination may be of clinical interest.

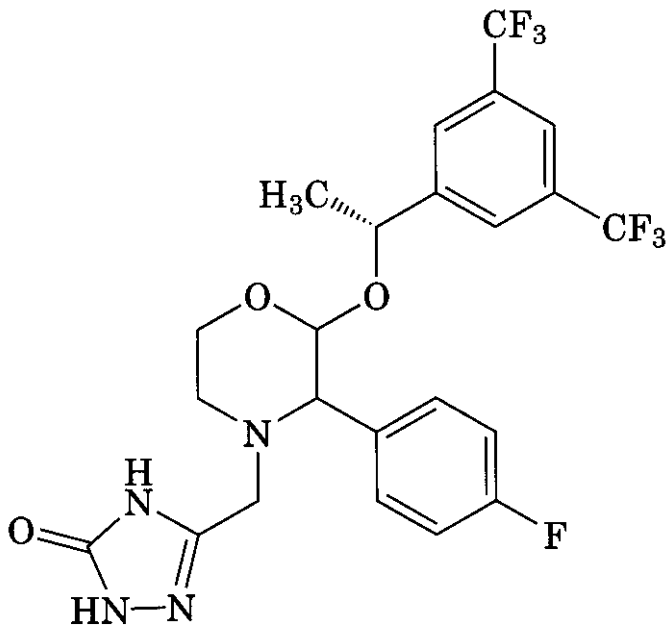
6.2 NMDA Receptor Antagonists

Since the early 1990s evidence has accumulated to show that a variety of drugs that block the glutamate receptor of the NMDA subtype have antidepressant-like profiles in behavioral

tests thought to predict antidepressant activity (247, 248). Chronic treatment of animals with NMDA antagonists causes a downregulation of β-adrenergic receptors, as seen with many antidepressant drugs (249). Skolnick and colleagues further showed that treatment of animals with clinically active antidepressants almost invariably downregulated NMDA receptor function, by reducing the affinity of glycine for an important modulatory site on the receptor (250,251). It is possible that this reflects a change in the pattern of expression of the various different subunits of which the NMDA receptor is composed (252). The changes in NMDA receptor function induced by chronic treatment with antidepressants may be regulated by the neurotrophic factor BDNF, whose expression is known to be upregulated in response to antidepressant exposure (see Section 3.4) (247). Unfortunately, the NMDA antagonist drugs that are currently available are not well tolerated in humans; they cause a range of serious adverse effects, including psychosis (253, 254). It is possible, however, that novel drugs that targeted particular NMDA subunit combinations could prove more benign and potentially useful as antidepressants (247).

6.3 Drugs Acting at Neuropeptide Receptors

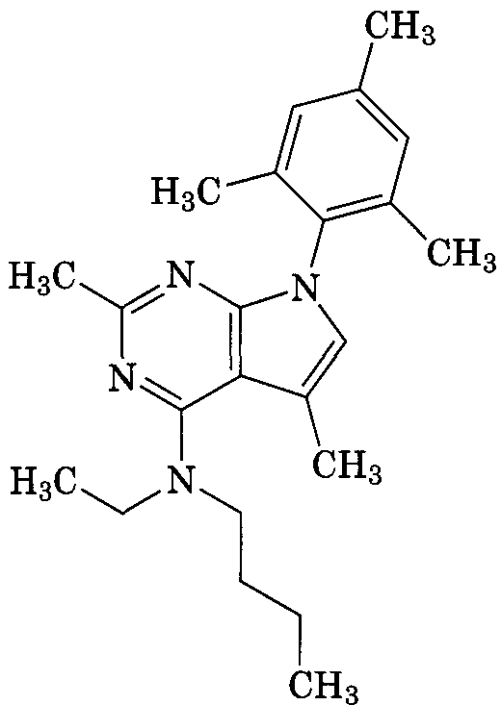
6.3.1 Substance P-NK1 Receptor Antagonists. Substance P is a neuropeptide that is widely distributed in small sensory nerve fibers and in a variety of neural pathways in the brain. During the 1990s a range of potent nonpeptide drugs were developed that act as potent and selective antagonists at the NK1 receptor, which recognizes substance P. Although these drugs were targeted initially at the treatment of pain, clinical trials in this indication proved disappointing (255). A breakthrough came when a team at Merck discovered that NK1 antagonists were active in animal behavioral models predictive of antidepressant action, and undertook a successful clinical trial (256). The results showed the NK1 antagonist MK-869 (70) to be as effective as the SSRI paroxetine (18), and to be associated with fewer sexual dysfunction side effects. This result triggered a high level of interest in what is potentially the first series of



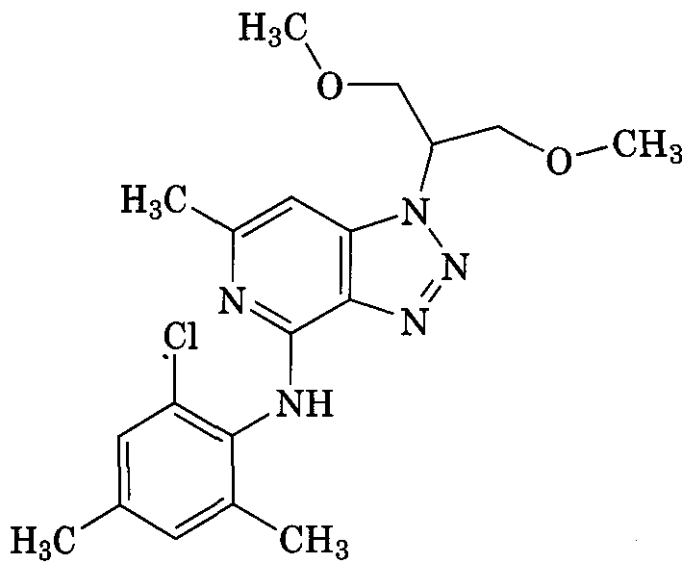
(70) MK-0869

antidepressant drugs that are not directly related to monoaminergic function (257). Nevertheless, there are intimate connections between substance P-containing pathways in the brain and the monoaminergic neurons, so it is possible that alterations in monoamine function may still represent a final common pathway for the actions of NK-1 antagonists (255).

6.3.2 CRF Antagonists. Corticotrophin releasing factor (CRF, or CRH) is a peptide secreted by hypothalamic neurons to control the release of ACTH from the anterior pituitary, but in addition, is present in a variety of neural pathways within the brain (258). A number of animal experiments suggest that increased release of CRF within the brain and from the hypothalamus may represent a final common pathway in mediating the effects of stress on the brain and body (258–260). CRF elicits behavioral signs of fear and anxiety when administered into the brain, and CRF antagonists conversely have anxiolytic/antidepressant profiles in behavioral tests (257). The chronic administration of antidepressant drugs leads to a downregulation of CRF expression in rat brain (261). These findings have led to an increased interest in the potential use of CRF antagonist drugs as agents to combat stress, anxiety, and depression. A variety of small molecule nonpeptide antagonists have been discovered (71 and 72) and shown to possess antidepressant activity in animal behavioral tests (259,260,262).



(71) CP-164,526



(72) DMP-696

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CHAPTER NINE

Antianxiety Agents

KEVIN S. CURRIE
Neurogen Corporation
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Contents

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1 INTRODUCTION

Anxiety is a normal emotion that all people experience to some extent. Indeed, anxiety is an essential part of the adaptive response to stressful or threatening stimuli. An appropriate amount of anxiety heightens caution and awareness in potentially dangerous or stressful situations, and so constitutes part of the "fight or flight" response. The anxious state is somewhat difficult to define, but may be described as a sense of unpleasant anticipation and excessive mental tension. Most people accept occasional anxiety as an integral part of normal life, but a significant number experience anxiety that is chronic, uncontrollable, and completely out of proportion to the generating stimulus. In such cases anxiety has ceased to serve an adaptive purpose: it has become pathological, and these individuals suffer from an anxiety disorder. The psychological symptoms include overwhelming worry and apprehension, coupled with feelings of helplessness and inability to cope. These are frequently accompanied by somatic symptoms such as tachycardia, palpitations, sweating, dizziness, nausea, insomnia, irritability, and impaired concentration. The impact on the patient can be debilitating. The anxiety associated with certain everyday activities can result in avoidance behaviors that cause extensive disruption of daily life and serious impairment of productivity and social functioning. Indeed, recent epidemiological studies show that anxiety is second only to depression in causing significant workplace absenteeism and reduced productivity (1).

Anxiety disorders, as a group, are among the most common psychiatric conditions, with lifetime incidence rates ranging between 16% (2) and 25% (3). The latter figure, reported in the U.S. National Comorbidity Survey (NCS), exceeds the 19.3% incidence rate for mood disorders such as depression (3). In any given year, almost 1 in 5 people will suffer from an anxiety disorder.

Anxiety is not a single disorder but rather a group of discrete conditions with their own epidemiology and symptoms, the current diagnostic criteria for which are set forth in the Diagnostic and Statistical Manual of Mental Disorders, volume four (DSM-IV) (4). The effectiveness of various treatments also varies from one disorder to another (5), so an accurate diagnosis is important before therapy is commenced. In general, a diagnosis of an anxiety disorder requires that the anxiety is not attributable to a coexisting medical condition or an ingested substance (e.g., caffeine).

1.1 The Anxiety Disorders

Generalized anxiety disorder (GAD) (6) is defined as excessive anxiety and worry occurring more days than not for a period of at least 6 months. The anxiety is accompanied by at least three of the following symptoms: restlessness, fatigue, impaired concentration, irritability, muscle tension, and sleep disturbance. The anxiety is uncontrollable and causes clinically significant distress. GAD has a lifetime prevalence of 6–10% (7), and the NCS study (3) indicates a high comorbidity with other psychiatric disorders, especially depression and panic disorder.

Social phobia (SP) or ***social anxiety disorder*** (SAD) (8–10) is characterized by a significant and persistent fear of social situations from which embarrassment may result (e.g., public speaking), or of situations where one is being observed or scrutinized by others (e.g.,

job interviews). Exposure to the feared situation induces pronounced anxiety, and the situation is either totally avoided or is endured only with extreme distress. The avoidance behaviors and associated anxiety cause significant disruption of the patient's normal social and occupational functioning. SP has been classified into two subtypes: public speaking only and generalized social phobia (11). Recent epidemiological studies reveal lifetime prevalence rates around 10% (12), making social phobia one of the most common anxiety disorders.

The essential feature of panic disorder (PD) (13) is the occurrence of repeated, unexpected panic attacks. There is a marked worry about the consequences of the attack and the possibility of having a future attack. The persistent anxiety evoked by the panic attacks causes major behavioral changes and intrusion into normal life. Around 50% of panic disorder patients also suffer from agoraphobia. A lifetime prevalence of 3.5% has been estimated (3), and a high comorbidity with depression and other anxiety disorders is observed.

Obsessive-compulsive disorder (OCD) involves recurrent obsessions and compulsions, which are severe enough to cause marked distress and major functional impairment. The sufferer is aware that the obsessions and compulsions are unreasonable, but is powerless to stop them. Obsessions are recurrent, unwanted thoughts or images, whereas compulsions are repetitive acts or rituals. The individual typically feels compelled to perform compulsions to alleviate the anxiety associated with an obsession, or to prevent the occurrence of some dreaded event. The lifetime prevalence of OCD is estimated at 2.3% (14).

In posttraumatic stress disorder (PTSD) (15), a characteristic set of symptoms develops following exposure to an event that induces extreme fear or terror (e.g., war, rape). These symptoms include a persistent and intrusive reexperiencing of the event through flashbacks or nightmares, and an intense distress at exposure to cues that are reminiscent of the event. The sufferer deliberately avoids such stimuli and may become detached, withdrawn, and emotionally numb. Additional symptoms include insomnia, impaired concentration, and unprovoked anger. Prevalence rates of 9–13% in the general population have been reported (16), but this can increase to over 70% in high risk populations (e.g., combat veterans, serious accident victims) (17).

Specific phobias are perhaps the most familiar of the anxiety disorders, and are characterized by a disproportionate fear of an object (e.g., spider, snake) or situation (e.g., flying, receiving an injection). When the stimulus is confronted it elicits an intense anxiety reaction, which the sufferer recognizes to be excessive but is unable to moderate. Prophylactic use of anxiolytics for predictably stressful situations is helpful, but in the long term, medication is less successful than behavioral therapy in the treatment of specific phobias.

Anxiety disorders are chronic conditions and drug therapy may need to continue for months or even years, particularly for the more intractable disorders such as OCD and PTSD. Response rates are often incomplete, and relapse upon discontinuation of treatment is common. For disorders with multiple symptoms (e.g., PTSD) only some of the symptoms are relieved. Coexistence of anxiety with other mental disorders, particularly depression (18, 19), is very common and such comorbidity is predictive of a poorer treatment outcome.

2 CLINICAL APPLICATIONS

2.1 Current Medications

Throughout history, humankind has used a variety of different agents to relieve anxiety. The oldest one of these is probably alcohol, which has served as an anxiolytic for thousands of years and is still widely used for the self-medication of anxiety today. In the past century, a number of CNS-depressant medications have been introduced, beginning with ethanol surrogates such as chloral hydrate. The barbiturates, carbarnates (e.g., meprobamate), and bromide salts were also used, although these agents were associated with a significant sedative effect, as well as more serious drawbacks such as the risk of dependency, high toxicity in overdose, and a potentially fatal interaction with alcohol.

At present, a number of drugs with various mechanisms of action are available for the

pharmacotherapy of anxiety disorders (20–24). Those with the most widespread current clinical use are listed in Table 9.1, and their chemical structures are shown in Figure 9.1.

2.1.1 Monoamine Oxidase Inhibitors and Tricyclic Antidepressants. In the 1960s, studies on the monoamine oxidase inhibitors (MAOIs) iproniazid and phenelzine (25) indicated that this class of drugs might be useful in the treatment of anxiety disorders. It has since been shown that phenelzine is particularly effective in social phobia (26–28), and may be useful in panic disorder (29), PTSD (30, 31), or OCD that is refractory to other treatments. Another class of antidepressant medications to show anxiolytic activity is the tricyclic antidepressants (TCAs) (32). Imipramine was the first member of this class proposed to have anxiolytic activity (33). The TCAs were the first antidepressants widely used for panic disorder, and their efficacy is well established (34, 35). Clomipramine is the most effective agent, and was long considered to be the gold standard in the treatment of panic disorder. Clomipramine has also shown significant efficacy in OCD (36–39), although at higher doses than those used for depression. Clomipramine was the first agent approved for OCD and its efficacy provided the earliest indications that serotonergic dysfunction may play a role in this disorder. Imipramine (40) and amitriptyline (41, 42) have been shown to be effective at alleviating some, but not all, PTSD symptoms, while desimipramine was ineffective (43). Imipramine has also demonstrated efficacy approaching that of the benzodiazepines in GAD (44, 45), although with a slower onset of action. TCAs have not been widely studied in social phobia, but one study was unable to differentiate imipramine from placebo (46).

There is ample clinical evidence for the efficacy of the TCAs and MAOIs in almost all of the anxiety disorders, and these medications are perhaps most useful when such anxiety is comorbid with depression. In spite of this, patient acceptance and medical enthusiasm for these agents is muted by their slow onset of action and malignant side-effect profile (see below). Accordingly they are generally considered, at best, second-line medications.

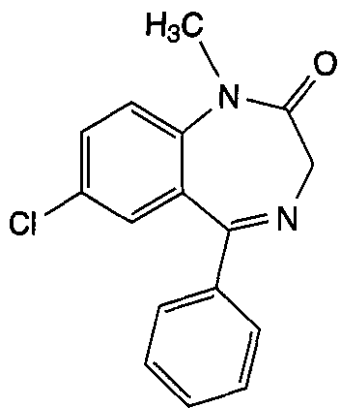
2.1.2 Benzodiazepines. In light of the unfavorable side-effect profiles of barbiturates, MAOIs, and TCAs, the discovery of the benzodiazepines (BZs) in the 1950s was to revolutionize the treatment of anxiety. In 1960 the first member of this class to be marketed was Librium (chlordiazepoxide), and for the next 40 years benzodiazepines were the mainstay of anxiety treatment. In addition to their anxiolytic properties, benzodiazepines also possess sedative, hypnotic, anticonvulsant, and muscle relaxant actions, and the sedative and muscle relaxant component of benzodiazepine activity may augment the anxiolytic effect. This overlap of pharmacodynamic activities blurs the distinction between sedatives and true anxiolytics, and the classification of a benzodiazepine as an anxiolytic is based heavily on pharmacokinetic considerations, with long-and short-lived benzodiazepines being used as anxiolytics and hypnotics, respectively. In the years since their discovery, over 30 benzodiazepines have entered into clinical practice for a variety of indications. Over the last 40 years the benzodiazepines have been by far the most widely prescribed anxiolytic agents and, although this dominance is now being challenged by alternative medications, they are still extremely popular (47).

Although the BZs show a robust anxiolytic effect, many of the clinical trials were conducted before the currently used divisions between specific anxiety disorders became available (4). As a result, knowledge of their efficacy in discrete anxiety disorders is incomplete. In clinical practice (48) BZs are widely used for GAD and as prophylactics in situational anxiety, with diazepam (1) historically being the most popular choice. Others in common use are chlordiazepoxide (2), clorazepate (3), lorazepam (4), alprazolam (5), oxazepam (6), bromazepam (7), and clonazepam (8). Response rates are high and the onset of therapeutic effect is immediate. This is an important contrast to the MAOIs, TCAs, and SSRIs, where an anxiolytic effect is not seen for several weeks. Although not specifically approved for this disorder. BZs are also effective in social phobia, with clonazepam (49) showing a superior response rate to that of alprazolam (50). Alprazolam and clonazepam are the only BZs approved for the treatment of panic disor-

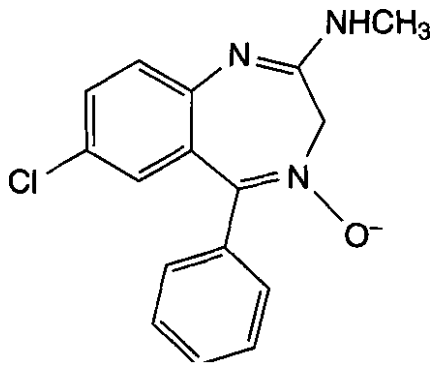
Table 9.1 Antianxiety Agents in Common Clinical Use

Generic Name (structure)	Trade Name	Originator	Mode of Action[a]	Therapeutic Indication[b]	Total Daily Dose (mg)	Dosing Schedule
Diazepam (1)	Valium	Roche	BZR agonist	Anxiety"	5–40	2–10 mg, 2–4 times daily
Chordiazepoxide (2)	Librium	Roche	BZR agonist	Anxiety	15–100	5–25 mg, 3-4 times daily
Clorazepate (3)	Tranxene	Abbott	BZR agonist	Anxiety"	22.530	Once daily
Lorazepam (4)	Ativan	Wyeth-Ayerst	BZR agonist	Anxiety"	2-3	2–3 mg, 2–3 times daily
Alprazolam (5)	Xanax	Pharmacia & Upjohn	BZR agonist	GAD, PD	0.5–1.5 (GAD) 5–9 (PD)	0.25–0.5 mg, 2-3 times daily
Oxazepam (6)	Serax	Wyeth-Ayerst	BZR agonist	Anxiety	30–120	10–30 mg, 3-4 times daily
Bromazepam[d] *(7)*	Lexotan	Roche	BZR agonist	Anxiety	4.5–9	1.5–3 mg, 3 times daily
Clonazepam (8)	Klonopin	Roche	BZR agonist	PD[c]	0.5–1	0.25–0.5 mg, twice daily
Buspirone (9)	BuSpar	Bristol-Myers Squibb	5HT-1A agonist	GAD	20–30	7.5 mg, 2-3 times daily
Paroxetine (10)	Paxil	SmithKline Beecham	SSRI	GAD, OCD, PD, SAD[e]	40 (OCD, PD) 20 (GAD, SAD)	Once daily
Sertraline (11)	Zoloft	Pfizer	SSRI	OCD, PD, PTSD[e]	50	Once daily
Fluvoxamine (12)	Luvox	Solvay	SSRI	OCD[e]	100-300	1–2 times daily
Fluoxetine (13)	Prozac	Eli Lilly	SSRI	OCD[e]	20–60	1–2 times daily
Citalopram (14)	Celexa	Forest	SSRI	PD[e]	20–40	Once daily
Venlafaxine (15)	Effexor	Wyeth-Ayerst	SNRI	GAD"	75–225	Once daily

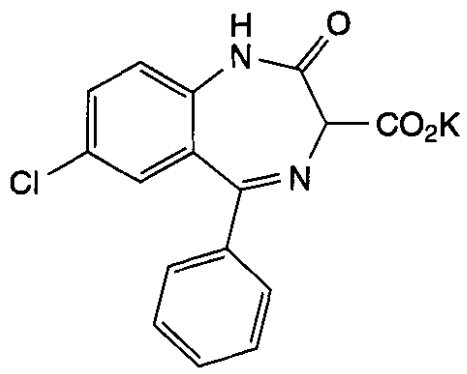
[a]BZR, benzodiazepine receptor; SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin and norepinephrine reuptake inhibitor.
[b]GAD, generalized anxiety disorder; PD, panic disorder; OCD, obsessive–compulsive disorder; SAD, social anxiety disorder; PTSD, posttraumatic stress disorder.
'Also indicated as an anticonvulsant.
[d]Not available in the United States.
'First indication is depression.



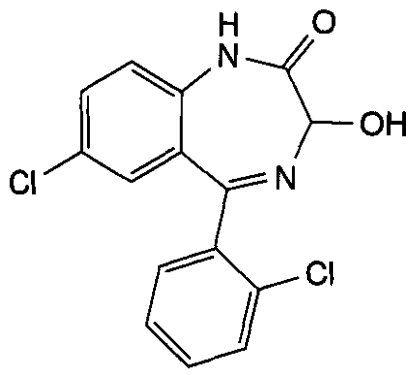
(1) Diazepam



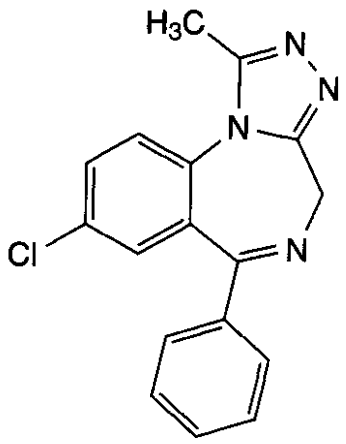
(2) Chlordiazepoxide



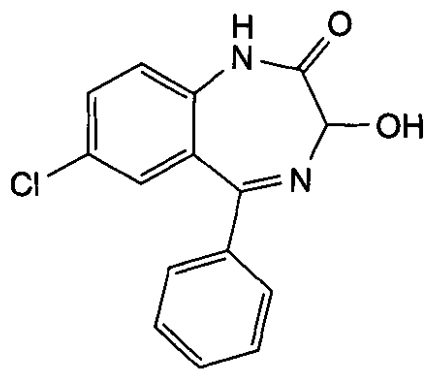
(3) Clorazepate



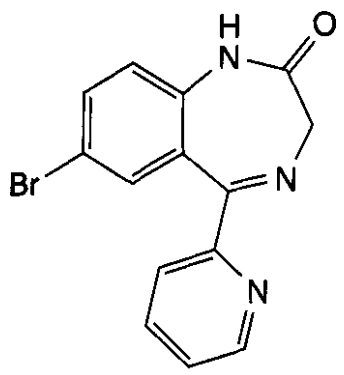
(4) Lorazepam



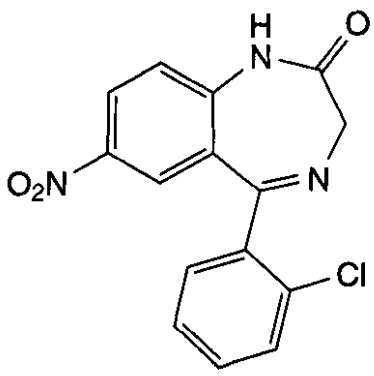
(5) Alprazolam



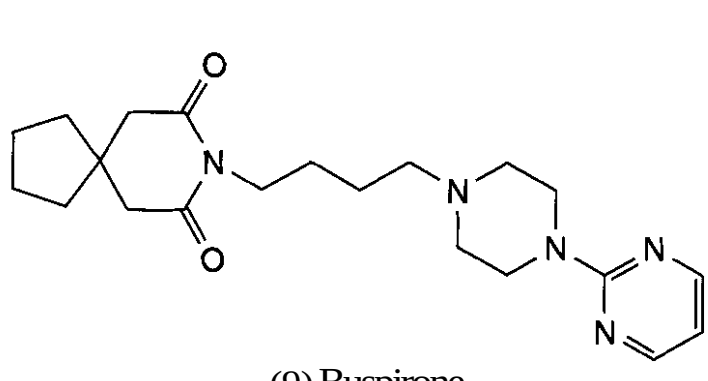
(6) Oxazepam



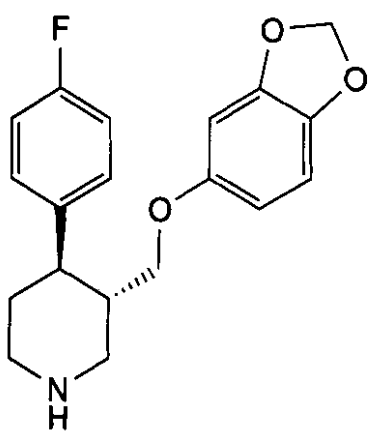
(7) Bromazepam



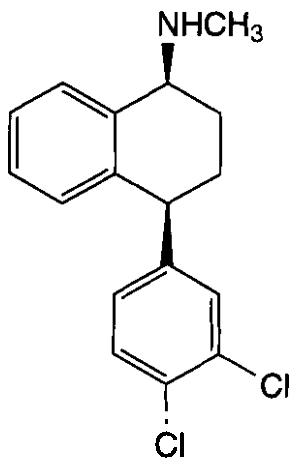
(8) Clonazepam



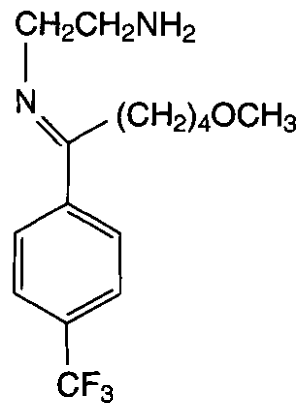
(9) Buspirone



(10) Paroxetine



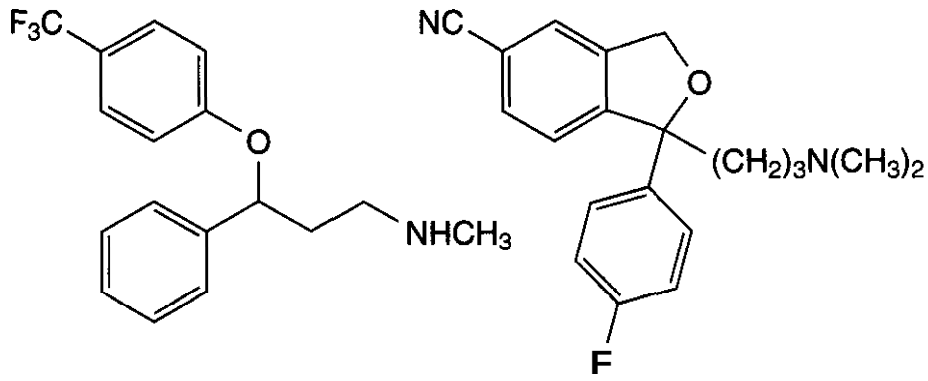
(11) Sertraline



(12) Fluvoxamine

Figure 9.1. Anxiolytics in clinical use.

der, although other high potency benzodiazepines (e.g., lorazepam) have shown a similar effect. Lower potency BZs (e.g., diazepam) require higher doses for panic disorder, and even alprazolam requires higher doses than those for GAD, reflecting the greater resistance of panic disorder to pharmacotherapy. There is little evidence for the efficacy of benzodiaz-

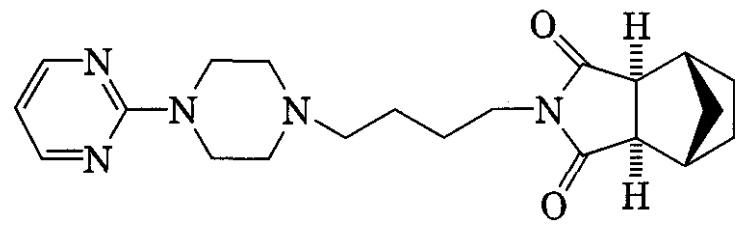


(13) Fluoxetine **(14)** Citalopram **(15)** Venlafaxine

Figure 9.1. (Continued.)

epines in OCD or PTSD, and none has been approved specifically for these disorders. In addition to the most common anxiolytic benzodiazepines shown in Table 9.1, a number of others are available (51), including pinazepam, camazepam, 2'-chlorodesmethyldiazepam, cloxazolam, ketazolam, and oxazolam. The use of these agents is much less widespread, however, and they are not available in the United States. Benzodiazepines have the potential to produce withdrawal symptoms and dependency upon prolonged administration. As a result they are indicated primarily for the short-term relief of anxiety and are increasingly not recommended for chronic dosing. The increasing awareness and negative reaction to the withdrawal, dependency, and abuse liabilities of the benzodiazepines has led to a significant reduction in their use, despite their proven efficacy.

2.1.3 Buspirone. The dominance enjoyed by the benzodiazepines is illustrated by the fact that no new anxiolytics were launched until buspirone (9) reached the market in 1986. Buspirone is a member of the azapirone class, and produces its therapeutic effects through partial agonism at 5HT-1A receptors. Buspirone is indicated and widely used for GAD, but has failed to show significant efficacy in other anxiety disorders (52). Although perhaps less consistently effective than the BZs, buspirone's improved safety profile provides the main point of differentiation between the two therapies. Buspirone causes less sedation, motor and cognitive impairment, and does not appear to be associated with any withdrawal syndrome. Drawbacks include a delayed onset of action (several weeks), and a reduced effect in patients who have recently used benzodiazepines (53). Buspirone is the only member of the azapirone class to be marketed in the United States, although tandospirone (16) is registered in Japan.



(16)

2.1.4 Serotonin Reuptake Inhibitors. Although there have been no launches of novel first-indication anxiolytics since buspirone in 1986, the last 10 years have seen a significant shift in the treatment of anxiety disorders. During this time it has become increasingly clear that the selective serotonin reuptake inhibitors (SSRIs), originally launched as antidepressants, *are* effective in a range of anxiety disorders (54–56). This finding has coincided with the realization of the withdrawal and dependency liability of the benzodiazepines, meaning that the SSRIs have comparable efficacy with an improved safety profile. The net result is that the SSRIs *are* now considered first-line treatments for panic disorder, OCD (57), social phobia (58), and PTSD, and *are* gaining popularity for GAD. Unlike the benzodiazepines or buspirone, the SSRIs have the added advantage of effectively treating depression, which is frequently comorbid with anxiety disorders (59).

Paroxetine (10) (60), is the most widely used SSRI in the treatment of anxiety. It is approved for the treatment of OCD and panic disorder, and is currently the only SSRI indicated for use in social phobia and GAD. Parox-

etine has also shown efficacy in one small trial for specific phobia (61). Fluvoxamine (12) (62) is used in OCD, and was the first SSRI to gain approval for this indication. Fluoxetine (13) (63), and sertraline (11)(64, 65) are also indicated for OCD. In addition, sertraline (66, 67) is approved for treatment of panic disorder and recently became the first and only medication approved for PTSD. In the United States, citalopram (14) (68) is indicated for use in OCD and has been used to prevent panic attacks for several years in Europe. Paroxetine and sertraline are marketed as single enantiomers, whereas citalopram and fluoxetine are available as racemic mixtures, and fluvoxamine is achiral. Venlafaxine (15) is a dual serotonin and norepinephrine reuptake inhibitor (SNRI) that has recently been approved for use in GAD (69, 70). The major drawback of the SSRIs in comparison to the BZs is their delayed onset of action. As with their antidepressant effect, it takes 2–4 weeks before anxiolytic efficacy is seen and this can cause patients to discontinue the drug. In this scenario, the benzodiazepines can be a useful adjunct to anxiolytic therapy with SSRIs. The immediate anxiolysis produced by the benzodiazepine serves as a bridge until the efficacy of the SSRI appears after 3–4 weeks, and the BZ is then tapered off slowly to avoid withdrawal, leaving the SSRI as a long-term monotherapy.

2.2 Adverse Effects

The monoamine oxidase inhibitors are associated with a number of undesirable side effects including weight gain, postural hypotension, sexual dysfunction, and insomnia. The most serious side effect is the risk of tyramine-related hypertensive crisis, often referred to as the "cheese effect," which can be fatal. To avoid this situation patients taking MAOIs must limit their tyramine intake, and the restrictive diet required to accomplish this leads to low patient compliance. A similar interaction occurs when switching patients from MAOI to SSRI therapy, and a minimum 2-week washout period before commencement of SSRI therapy is essential to allow MAO levels to return to normal. The therapeutic effects of the TCAs derive from their inhibition of serotonin and norepinephrine uptake, although they also act at muscarinic acetylcholine receptors, histamine H1 receptors, and α1-adrenergic receptors. Common anticholinergic side effects include dry mouth, constipation, urinary retention, and blurred vision. Blockade of adrenergic receptors can cause orthostatic hypotension and dizziness, and actions at histamine receptors lead to sedation and weight gain. The therapeutic effect of the TCAs does not appear until after 2–4 weeks of therapy, and during this period there can be an initial exacerbation of anxiety symptoms, which may lead to patient termination of therapy. The unfavorable side-effect profiles of the MAOIs and TCAs have largely relegated these drugs to last resort cases where other medications have failed.

As a class, the benzodiazepines have demonstrated a remarkable safety profile and their high tolerability has been a major factor in their widespread acceptance and phenomenal success. The most commonly reported side effects with benzodiazepine anxiolytics are daytime sedation ("hangover" effect), sleep disturbances, cognitive impairment, motor incoordination, and ataxia. The latter symptoms are of greater importance in the elderly, who constitute a significant population of BZ users, in whom they are associated with an increased incidence of falls and fractures (71). Many patients develop a tolerance to these side effects over time, however, while rarely developing tolerance to the anxiolytic effects. The greatest problems with the benzodiazepines are withdrawal symptoms, dependency liability, and abuse potential, the separation of which is not always obvious (72). Abrupt discontinuation of benzodiazepine therapy commonly leads to a "withdrawal syndrome" characterized by insomnia, anxiety, fatigue, irritability, light-headedness, headache, and gastrointestinal upset. These symptoms must be differentiated from rebound phenomena (i.e., recurrence of the original anxiety), which may also occur upon termination of therapy. Short-acting benzodiazepines (e.g., lorazepam) tend to have more severe withdrawal symptoms than those of long-acting ones (e.g., diazepam) because of the more pronounced fluctuations in blood levels.

To minimize the risk of withdrawal effects. benzodiazepine therapy is usually discontin-

ued by a gradual tapering of the dose. Nevertheless, an estimated 25% of patients find withdrawal difficult and fail to discontinue therapy (73, 74), and such patients may be considered to have a low dose dependency on benzodiazepines. On the other hand, high dose dependency, and abuse, is characterized by drug-seeking behaviors and the consumption of elevated, nontherapeutic doses. The risk of withdrawal and dependency increases with dose and length of treatment, but clinical experience shows that anxious patients have little tendency to escalate the dose of benzodiazepines, and such abuse is typically found in patients who have a history of alcohol and/or substance abuse (75, 76). It is noteworthy, however, that substance abuse is often comorbid with anxiety disorders. The increasing public and medical concern over the dependency and abuse liabilities of benzodiazepines has considerably reduced their use, and many countries now have restrictions on their dispensation. This may be an overreaction, however, which in many cases has likely led to the substitution of benzodiazepines by less effective medications. Recently, it has been argued that the risk-to-benefit ratio of benzodiazepines be reassessed and their clinical utility reevaluated (77, 78).

Buspirone is a well-tolerated drug, the most commonly reported side effects being transient dizziness, light-headedness, headache, and gastrointestinal disturbances. Other limitations of buspirone are its delayed onset of action (few days to a few weeks) and a significant drug interaction with MAOIs.

The SSRIs (paroxetine, fluoxetine, sertraline, fluvoxamine, citalopram) and SNRI (venlafaxine) have an impressive side-effect profile, and this has contributed to their widespread use. Possible adverse effects include nausea, insomnia, and agitation, but these are generally manageable and diminish over time. More significant is the association of the SSRIs with sexual dysfunction. in both men and women. These effects are longer lasting, and can occur in up to 40% of patients (79). A withdrawal syndrome has also been observed with the SSRIs, characterized by dizziness, headache, and irritability upon abrupt discontinuation. This is much less serious than that observed with benzodiazepines, however, and the symptoms typically resolve after a few days. Like buspirone, the SSRI/SNRI class has a delayed therapeutic effect and a potentially serious drug interaction with MAOIs.

2.3 Drug Metabolism

The metabolic profile of anxiolytic agents has important ramifications for their clinical use. This section discusses the metabolism of the benzodiazepines and buspirone in some detail. The metabolism of the SSRIs, TCAs, and MAOIs is covered in the chapter on antidepressants.

2.3.1 Benzodiazepines. To varying degrees, all benzodiazepines produce five clinical effects: anxiolysis, sedation, muscle relaxation, seizure protection, and memory impairment. In terms of the pharmacology underlying these effects there is little to choose between the benzodiazepines, in that they all act as nonselective agonists at the benzodiazepine receptor (BZR). The classification of benzodiazepines into therapeutic indications is therefore based largely on their pharmacokinetic profile. Thus, benzodiazepines with long half-lives and/or long-lived active metabolites are used as anxiolytics, whereas those with short half-lives are more appropriately used as sedative-hypnotics. Elimination of the benzodiazepines is by hepatic metabolism and excretion of conjugated metabolites in the urine (80–83). During metabolism, many benzodiazepines give rise to metabolites that often have comparable pharmacological activity to, but a longer half-life than, the parent compound. The metabolic profile is therefore an important determinant of the ultimate in *vivo* biological activity.

In general the first, and most rapid, step in 1,4-benzodiazepine metabolism (Fig. 9.2) is the oxidative removal of the N-1 alkyl substituent, if present (82). Benzodiazepines with a methyl group at N-1 are the most stable and the rate of dealkylation increases with the size of the N-1 alkyl group. For example, the dealkylation half-life for diazepam is 20–30 h, but for prazepam, which bears a cyclopropylmethyl group at N-1, this half-life is approximately 1 h (82). Substituents elsewhere in the molecule have little impact on the rate of deal-

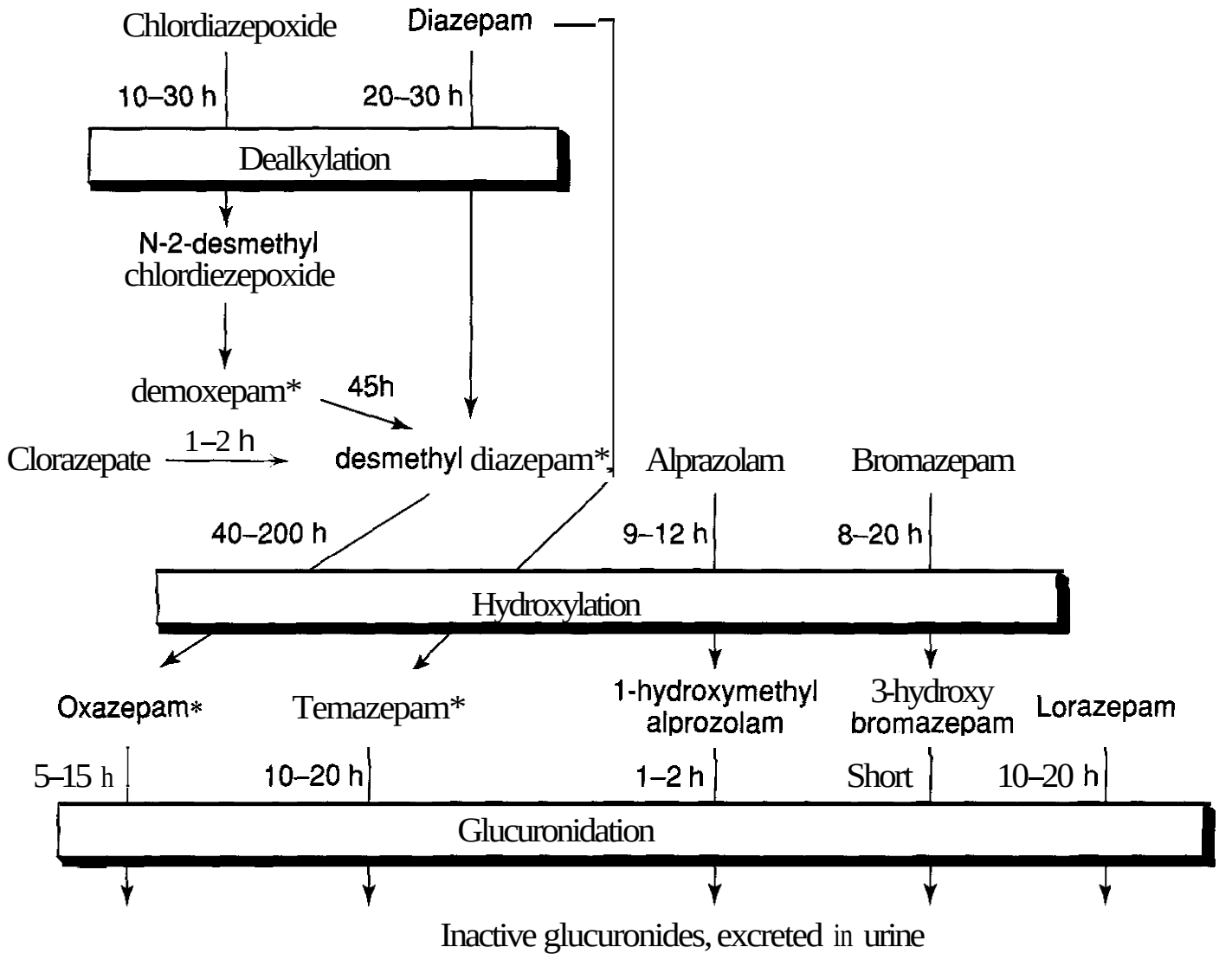


Figure 9.2. Metabolic pathways for common anxiolytic benzodiazepines. Capitalized names are marketed drugs. *Active metabolite.

kylation. Following dealkylation, the next step is hydroxylation at the 3-position. The hydroxylation half-lives (typically approaching or exceeding 100 h) are longer than the dealkylation half-lives, resulting in an accumulation of the pharmacologically active N-1 dealkylated metabolites, so these compounds contribute significantly to the overall biological activity. The rates of hydroxylation are relatively independent of the substituent in the C-ring, with the exception of bromazepam. The 3-hydroxy metabolites are then glucuronidated and excreted in the urine. The glucuronides are pharmacologically inactive, and this fact has been exploited in drugs such as oxazepam, lorazepam, and temazepam. As 3-hydroxybenzodiazepines, these agents are glucuronidated directly and their clinical use is not complicated by the formation or accumulation of active metabolites. Further, the rate of glucuronidation (84, 85) appears to be much less impacted than the rate of dealkylation or hydroxylation (82) by aging or by liver disease, making these drugs more suitable for use in elderly patients and those with liver dysfunction. The limited structural diversity of the benzodiazepines means that the same active metabolites can be generated from several different drugs. For example, oxazepam, which is marketed as an anxiolytic in its own right, is generated *in vivo* from diazepam, clorazepate, prazepam, and chlordiazepoxide. In the case of the triazolo- (e.g., alplrazolam) and imidazo- (e.g., midazolam) benzodiazepines, hydroxylation of the C-1 methyl group is the principal metabolic pathway.

Alprazolam (5) is rapidly absorbed after oral dosing, reaching peak plasma levels in less than 1 h with an oral bioavailability exceeding 90% (86). Alprazolam has a lower protein binding (68%) than that of most benzodiazepines (87), reflecting the increased polarity imparted by the triazole ring. Although the principal metabolite 1-hydroxymethylalprazolam is a potent BZR ligand (K_i = 4.2 nM) (88), it does not contribute significantly to the clin-

ical effect because it is rapidly conjugated and plasma levels never exceed 10% of the parent drug (89). The elimination half-lives of alprazolam and 1-hydroxymethylalprazolam are 9 to 12 h and 1 to 2 h, respectively.

Chlordiazepoxide (2) is completely absorbed upon oral administration and reaches peak plasma concentrations in 1–2 h (90). As a highly lipophilic molecule it is 94% bound to plasma proteins (91) and readily penetrates the brain, with CSF levels paralleling unbound plasma levels (92). Chlordiazepoxide has a mean half-life of 15 h. It is metabolized first by oxidative removal of the N-2 methyl group (to give N-desmethylchlordiazepoxide), followed by hydrolysis to the lactam (demoxepam), and reduction of the N-5 oxide to give desrnethyldiazepam. All of these metabolites are pharmacologically active.

Clorazepate (3) is readily decarboxylated in the stomach to generate desrnethyldiazepam, which is then rapidly absorbed with peak plasma levels occurring 0.5 to 2 h postdose. Clorazepate itself is poorly absorbed (93) and acts only as a prodrug of desmethyldiazeparn.

Diazepam (1) is completely and rapidly absorbed following oral administration, reaching peak plasma levels after 30–90 min. As a result of its lipophilic character it is highly protein bound (98–99%) and is widely distributed, readily crossing the blood-brain barrier and placenta, and also passing into breast milk. Metabolism of diazepam gives rise to three active metabolites. N-1 demethylation produces desmethyldiazepam, which has a longer half-life than that of diazepam (40–200 h compared to 20–30 h) (82). On multiple dosing, plasma desrnethyldiazepam levels exceed diazepam levels. Hydroxylation of desrnethyldiazepam then produces oxazepam (6), which is conjugated and eliminated as the glucuronide. Direct hydroxylation at the 3-position of diazepam yields temazepam, which is also directly conjugated and excreted. Of these metabolites, desmethyldiazepam contributes more to the overall pharmacodynamic effect because of its longer half-life and resultant accumulation.

After oral dosing bromazepam (7) is well absorbed, with a bioavailability of 84–98% and a rather variable T_{max} of 0.5 to 8 h (94). It has an elimination half-life of 8–19 h and, compared to other benzodiazepines, has a relatively high free fraction (30%). Bromazepam is metabolized principally by hydroxylation at the 3-position. The pyridyl ring at position 5 facilitates this reaction, the hydroxylation' half-life for bromazepam being considerably shorter ($t_{1/2}$ = 8–19 h) than that for other benr zodiazepines (94). A second pathway opens the diazepine ring to give the pharmacologically inactive 2-(2-amino-5-bromobenzoy1)pyridine-(95). Bromazepam metabolism does not produce any metabolites with a longer half-life than that of the parent compound.

Clonazepam (8) is almost completely absorbed after oral dosing (96) with an average T_{max} of 2–4 h. As with other 7-nitro benzodiazepines, the major metabolic pathway for clonazepam is reduction of the nitro group, acetylation of the resulting amine, and elimination of the acetamide. Hydroxylation of clonazepam or of 7-amino clonazepam to give the 3-hydroxy derivatives represents minor metabolic pathways. The elimination half-life of clonazepam is 20–30 h (97), and no active metabolites are produced.

Lorazepam (4) is well absorbed, with oral bioavailabilty close to 100% after a typical 2 mg dose, and peak levels are obtained within 2 h of administration. Lorazepam is conjugated at the 3-hydroxy group and the resulting inactive glucuronide is excreted in the urine with a mean elimination half-life of approximately 14 h (82).

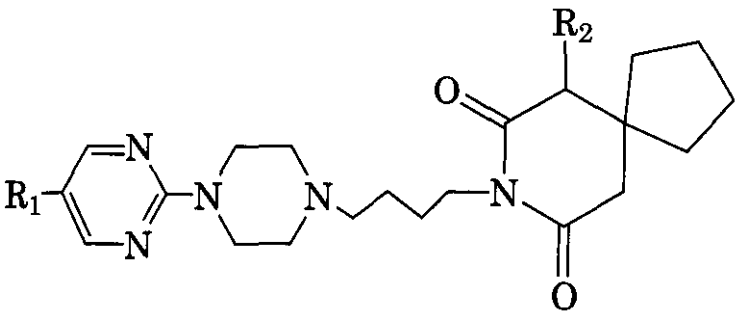
Oxazepam (6) is formed during the metabolism of many other benzodiazepines, but its own metabolic profile is relatively simple. Like lorazepam, the major metabolic pathway is glucuronidation at the 3-hydroxy group followed by urinary excretion. Up to 80% of the dose is recovered from the urine as the glucuronide. The mean half-life of oxazepam is approximately 9 h (98).

As previously mentioned, the choice of benzodiazepine for a given indication is governed in large part by its plasma half-life. This half-life, however, does not always predict the duration of the pharmacological effect. Using sedation (99) or psychomotor impairment (100) as endpoints, lorazepam was shown to have a longer lasting effect than that of diazepam, even though its plasma half-life is about half as long. For diazepam, the rate of elimination

from the CSF is equivalent to the rate of elimination from plasma (101), but lorazepam has a longer half-life in CSF than in plasma. Lorazepam has a greater affinity for the BZR than does diazepam (lorazeparn, $K_i = 1$ nM; diazepam, $K_i = 27$ nM) and dissociates from the receptor more slowly (102). Thus the release of lorazepam from the receptor has been suggested as the rate-limiting step in its elimination from CSF, resulting in a longer pharmacodynamic effect than would be expected based on the plasma half-life.

2.3.2 **Buspirone.** Buspirone is completely absorbed after oral dosing, but exhaustive fist-pass metabolism limits the absolute bioavailability to only 4% (103). The pharmacokinetics are linear and metabolism is not saturated in the therapeutic dose range (10–40 mg) (104). First-pass metabolism is decreased when buspirone is taken after a meal but absorption is not, leading to increased drug exposure (105). This effect is also seen with other drugs that undergo extensive first-pass metabolism (106). Age has little impact on buspirone pharmacokinetics, but hepatic cirrhosis extends the elimination half-life by 50% (105). The mean half-life of buspirone in normal subjects ranges from 2 to 11 h, averaging at around 4 h, and this short half-life accounts for one of buspirone's drawbacks, that is, the need for three times daily dosing. Buspirone is a lipophilic compound and is highly bound to plasma proteins (>95%), predominantly albumin (105).

The extensive oxidative metabolism of buspirone, mediated by CYP450 3A4, produces a number of hydroxylated metabolites including 1-(2-pyrimidinyl)piperazine (1-PP), 5'-hydroxybuspirone (17) and 6-hydroxybuspirone (18) (107). After a 20-mg dose of buspirone,



(17) R_1 = OH, R_2 = H
(18) R_1 = **H**, R_2 = OH

5'-hydroxybuspirone plasma levels (both free and conjugated) are up to six times greater than buspirone levels, indicating the importance of this metabolic pathway. Plasma levels of 1-PP are approximately fourfold greater than those of buspirone (105). 5-Hydroxybuspirone shows no biological activity, in keeping with the structure-activity relationship (SAR) for this series of compounds as it relates to affinity for the 5HT-1A receptor (see section 5.4.1); however, 1-PP has up to 20% of the potency of buspirone in the Vogel anticonflict model (105). It also has some affinity (IC_{50} = 95 nM) for the adrenergic a2 receptor, although it has no activity at the 5HT-1A receptor. Given its significantly higher plasma and brain levels relative to those of buspirone, 1-PP may contribute to the overall anxiolytic effect.

Until recently, it was believed that 1-PP was the only buspirone metabolite to possess any biological activity. Under the assumption that buspirone was the active agent, dosing schedules were designed to optimize buspirone exposure at the expense of metabolites. Bristol-Myers Squibb, the manufacturer of buspirone, recently carried out clinical studies on a buspirone transdermal patch (108) with the aim of increasing buspirone levels, and hence the anxiolytic effect, by circumventing first-pass metabolism. Surprisingly, no significant activity was seen with this delivery system, prompting a reevaluation of buspirone and its metabolites. Remarkably, almost 15 years after the launch of buspirone, it was discovered that 6-hydroxybuspirone (18) not only possessed anxiolytic activity, but is apparently responsible for most, if not all, of buspirone's clinical effects (109). (**18**) has significant affinity for the 5HT-1A receptor (K_i = 57 nM), approaching that of buspirone (K_i = 31 nM). Furthermore, (18) is the second most abundant human metabolite of buspirone, having blood levels 40 times greater than that of buspirone and severalfold greater than that of 1-PP after oral dosing. The initial preclinical work on buspirone revealed no in *vivo* anxiolytic activity for (**18**) in animal models, but more recently, using the rat pup ultrasonic vocalization model, (**18**) was found to have comparable activity to that of buspirone (ID_{50} = 0.13 and 0.10 mg/kg, respectively) (109).

Subsequent human pharmacokinetic studies have demonstrated that plasma levels of buspirone after oral dosing are minimal in comparison to those of (18), leading to the conclusion that (18)mediates the clinical anxiolytic effect of buspirone. These surprising discoveries were disclosed by Bristol-Myers in a U.S. patent (109) (the so-called 365 patent), the timing of which was remarkable. The "365" patent was granted on November 21, 2000, only one day before the expiration of buspirone's patent protection, and was subsequently listed by the FDA in the Approved Drug Products with Therapeutic Equivalence Evaluations list (the "Orange Book"). This move effectively granted Bristol-Myers a further 20 years exclusivity for buspirone, a drug that generated $700 million in sales in 2000. Several generic companies, who were already in production of buspirone in anticipation of the November 22 deadline, filed suit against the FDA, charging that the "365" patent had been improperly listed. At the time of writing, the courts have ruled against the FDA (110), the "365" patent has been removed from the Orange Book, and generic forms of buspirone are now available. The future development of 6-hydroxybuspirone, if any, is unknown.

3 PHYSIOLOGY AND PHARMACOLOGY

It is evident from section 2.1 and Table 9.1 that the clinically effective anxiolytics have several distinct modes of action and interact with a number of important neurotransmitter systems (111–114). The efficacy of the benzodiazepines clearly implicates GABA in anxiety, whereas the clinical utility of buspirone and the SSRIs point to an important role for serotonin. The TCAs exert their therapeutic effects through inhibition of serotonin and norepinephrine reuptake, and their efficacy suggests the involvement of both systems in anxiety disorders. Although there are no clinically available drugs that modulate the cholecystokinin (CCK) and corticotrophin-releasing factor (CRF) systems, increasing evidence argues for their participation in the regulation of anxiety.

Anxiety is actually a cluster of several different conditions, and it is highly unlikely that such a group of complex emotional disorders can be reduced to the dysregulation of a single neurotransmitter system. In reality, all of the aforementioned systems are intertwined with complex and dynamic interactions existing between them, and the exact role and relative contribution of each one in the pathology of anxiety remains unknown (115, 116). The function of a given neurotransmitter receptor may vary between receptor subtype and anatomical region, and responses to acute and chronic therapy may differ. The multitude of overlapping pathways that contribute to the regulation of anxiety makes it difficult to elucidate the pathology of the various disorders, but also offers numerous opportunities for pharmacological intervention. The following section discusses the roles of the aforementioned neurotransmitter systems in the pathophysiology of anxiety.

3.1 GABA

GABA (γ-aminobutyric acid) is the major inhibitory neurotransmitter in the brain and is estimated to be present at 30–50% of all CNS synapses. Consequently, many important neurotransmitter systems are under the inhibitory influence of GABA, including the serotonin, norepinephrine, CCK, and dopamine systems. Three types of GABA receptors have been identified (117): $GABA_A$, $GABA_B$, and GAB&. $GABA_A$ receptors belong to the superfamily of ligand-gated ion channels, and mediate fast synaptic inhibition. GABA, receptors are G-protein-coupled receptors that can mediate inhibition or excitation, and $GABA_C$ receptors appear to be similar to $GABA_A$ receptors but are localized predominantly in the retina. GABA exerts its inhibitory effects in the central nervous system by binding to the $GABA_A$ receptor and opening the chloride channel at the center of the receptor. The resulting influx of chloride ions hyperpolarizes the neuron and reduces its sensitivity to incoming stimuli. Within the $GABA_A$ receptor complex, and in close proximity to the GABA binding site, there is an allosteric site, the benzodiazepine receptor (BZR), through which the benzodiazepines produce their effects. Up to 10 additional modulatory sites on the $GABA_A$ receptor have been proposed, in-

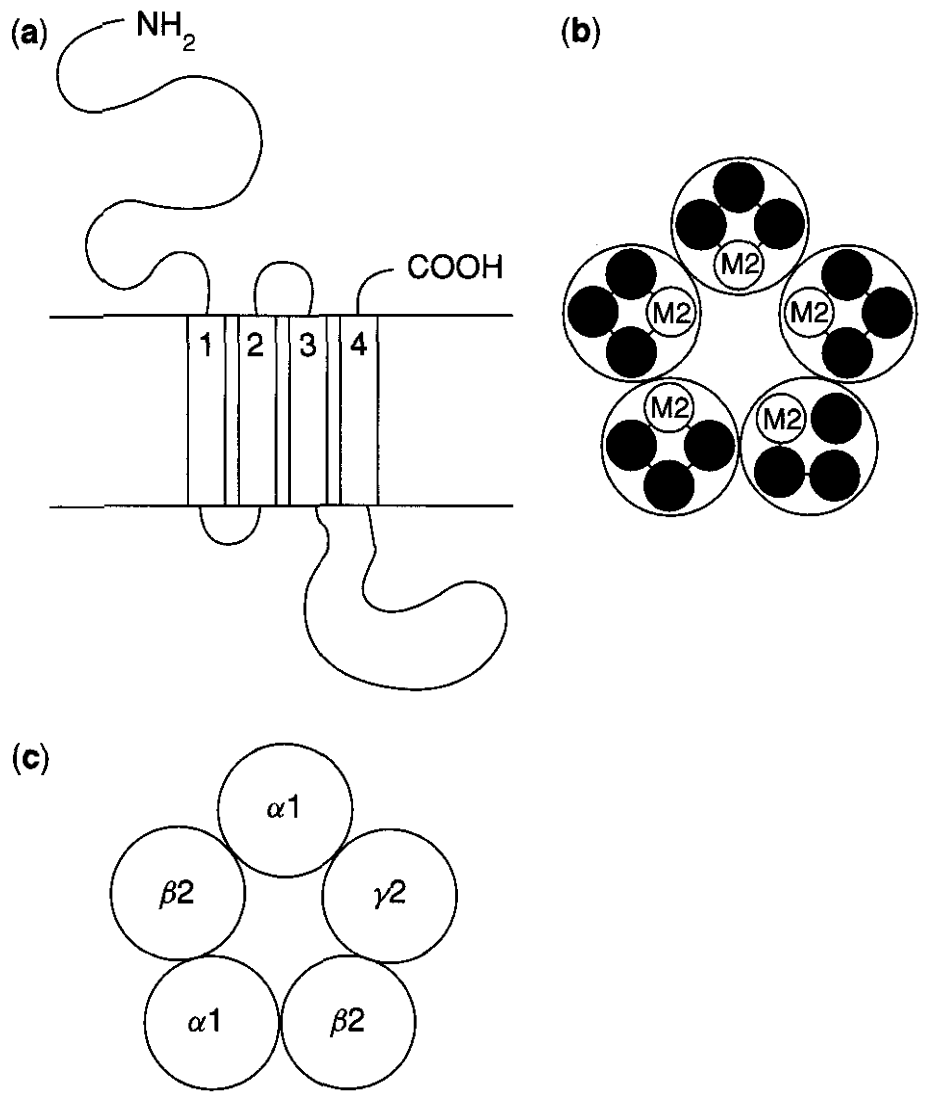


Figure 9.3. Fkpresentations of GABA-activated ion channels. a: general structure of one of the five protein subunits showing four membrane spanning regions; b: pentameric arrangement of the protein subunits showing the second membrane spanning region lining the pore of the ion channel; c: heteromeric makeup of the GABAa receptor with two α1, two β2, and one γ2 subunit. (Reprinted with permission from J. Med. *Chem.,* 43, 1427–1447, 2000. Copyright 2000 American Chemical Society.)

cluding those for barbiturates, neurosteroids, picrotoxinin, inhalation anesthetics, and ethanol (118).

Studies have shown a reduced density of lymphocyte BZ receptor sites (119) and platelet BZ sites in GAD patients, which was increased following diazepam treatment (120). These peripheral receptors are pharmacologically distinct from the central $GABA_A$ receptors, so the relevance of these studies to anxiety is unclear. More relevant may be the study of central BZR activity in anxiety patients. Reduced BZR sensitivity has been shown in GAD patients by measuring saccadic eye movements (121, 122). This effect was even more pronounced in patients with panic disorder (123), but was absent in OCD (124). These studies suggest that there may be a reduced BZ receptor function in some anxiety disorders.

3.1.1 The $GABA_A$/Benzodiazepine Receptor Complex. The $GABA_A$ receptor (125–127) is a transmembrane protein belonging to the superfamily of ligand gated ion channels (128). The receptor consists of five subunits arranged round a central chloride channel (Fig. 9.3). To date, 21 receptor subunits have been cloned (six a, four β, four γ, one 6, one ϵ, one π, one 8, and three *p),* although the ϵ and p subunits appear to be associated with $GABA_C$ rather than $GABA_A$ receptors. Immunohistochemical studies indicate that the α, β2/3, and γ2 subunits are most frequently coassembled to form $GABA_A$ receptors in uiuo, with a stoichiometry of 2α:2β:1γ (129). Even with this restriction, the differential combination of these subunits could potentially lead to thousands of receptor subtypes. Fortunately, the $GABA_A$ receptor population appears to be dominated by only a handful of receptor subtypes, differentiated mainly by the identity of the a subunit. These subtypes have been shown to possess a distinct but regionally overlapping distribution within the brain (128, 130). α1-containing subunits are the most abundant, accounting for over 50% of all $GABA_A$ receptors, and are distributed throughout the brain (131–133). a2 and α3 subtypes together constitute a further 30–35% of $GABA_A$ receptors, with a2 isoforms located in limbic structures and a3 receptors

found mainly in the cortex (134). a5 subtypes represent only 5% of the total $GABA_A$ receptor population and are localized in the hippocampus (135).

As shown in Fig. 9.3 the $GABA_A$ receptor has a topology characteristic of the nicotinoid superfamily of receptors. Each subunit spans the membrane four times, and the second transmembrane domain of each subunit forms the wall of the ion channel. The GABA binding sites, of which there are two per receptor, are located at the two homologous α/β interfaces. Point mutation studies have identified several key amino acid residues on both subunits that contribute to GABA binding (136), and both GABA sites must be occupied to induce opening of the chloride channel. The benzodiazepine receptor site (BZR) (137) is located at the interface of the a- and y-subunits, and there is one BZR per GABA receptor (129). Point mutation and photoaffinity labeling experiments have revealed at least 11 amino acid residues in the a- and y-subunits that contribute to ligand binding and efficacy at the BZR (138), the most significant of which is the histidine at position 102 (101 in rat) of the α-subunit. The α1-, α2-, α3-, and α5-containing receptors carry a histidine at position 102 and are benzodiazepine sensitive, but the a4 and a6 subtypes, which bear arginine at this position, are benzodiazepine insensitive (139). The γ2 subunit is more abundant in the brain than y1 or γ3 subunits and is important for ligand binding, given that the $GABA_A$ receptors of mice lacking the gene for γ2 subunits are insensitive to benzodiazepines (140). The nature of the β-subunit has a lesser influence on BZR pharmacology (141). This fact, coupled with the consistent expression of γ2 subunits in various subtypes, means that the identity of the a -subunit is the major determinant of BZR pharmacology (128, 135). Accordingly, the dominant benzodiazepine-sensitive $GABA_A$ subtypes in the brain are often referred to simply as the a1, α2, α3, and a5 $GABA_A$ receptors.

Along with an increased understanding of the molecular diversity and anatomical localization of different $GABA_A$ receptor subtypes has come the notion that specific clinical effects may reside in specific subtypes. The selective affinity of the hypnotic agent zolpidem (Ambien) has implicated the a1 subtype in sedation, whereas the localization of the a5 subunit in the hippocampus suggests its involvement in memory processes. The presence of α2 and a3 subtypes in limbic structures points to a role for these subtypes in anxiety and emotional behavior. Recent work with transgenic animals is confirming, for the first time, some of these theories (142). Mutation of the critical His-102 of the a subunit to an arginine abolishes the binding affinity of benzodiazepines (143) at the mutated subtype, but does not affect the response to GABA. Mice expressing a1, α2, or a3 receptors containing this knock-in mutation develop normally and show no overt anxious phenotype, arguing against the long-held notion that there are endogenous ligands for the BZR that regulate anxiety.

Evaluation of these animals in behavioral paradigms shows that the sedative and amnestic effects of diazepam are mediated through the a1 subtype (144–146). Although the a1 subtype appears to mediate sedation, the sleep latency and sleep pattern were unchanged in the mutant mice, indicating that different subtypes mediate the sedative and hypnotic effects of diazepam (147). Conditional knock-in mice wherein the a2 and a3 subtypes are rendered benzodiazepine insensitive have also been generated. In the elevated plus maze and light/dark choice models, a loss of diazepam anxiolytic activity is observed in the a2 knock-in animals, but not with the a3 knock-ins, implicating the a2 subtype in the mediation of benzodiazepine anxiolytic effects (148). Given this evidence, it is tempting to speculate that the ideal anxiolytic, one that is clinically effective but free from sedation, amnesia, dependency, and withdrawal effects, will be realized in a compound possessing the appropriate subtype selectivity. It is probable, however, that a given subtype is associated with more than one clinical effect. Indeed the a2 subtype, although clearly associated with the anxiolytic actions of diazepam, has also been shown, using a2 and a3 knock-in mice, to contribute to the myorelaxant actions of diazepam (149). Thus, simple subtype selectivity alone may not be enough to yield a true side effect-free anxiolytic. More fruitful may be the combination of subtype selectivity *and* partial ago-

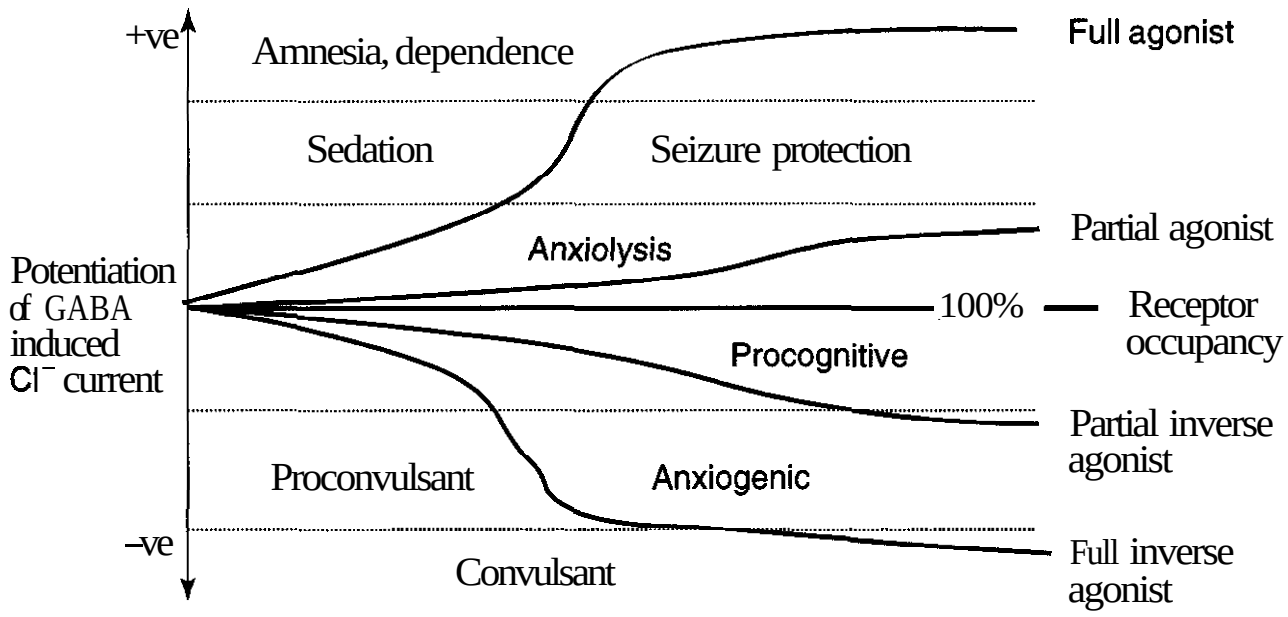


Figure 9.4. Anxiolysis via partial agonism.

nism, and this hypothesis underlies the most promising area of current anxiolytic research.

Ligands at the BZR are allosteric modulators of GABA function, and they have no effect in the absence of GABA. This allosteric modulation can be positive (enhancement of GABA function, increased neuronal inhibition) or negative (inhibition of GABA function, reduction in neuronal inhibition). Ligands effecting maximum positive modulation are known as full agonists, or as compounds with high intrinsic efficacy; that is, they produce the maximal response at low receptor occupancy. This profile is characteristic of the classical benzodiazepines. Ligands that induce maximum negative modulation are full inverse agonists, whereas antagonists have no effect on GABA function. In between these extremes, on a continuum of intermediate efficacy, lie partial agonists and partial inverse agonists. Partial agonists can be defined as ligands that induce a smaller response than that of a full agonist, at the same receptor occupancy (Fig. 9.4). In principle, a partial agonist might never produce the maximum potentiation of a full agonist, even at 100% receptor occupancy. Because anxiolysis requires less receptor occupancy than sedation and other side effects (150), a number of partial agonists have been investigated as potential nonsedating anxiolytics. The modulation of GABA response can span a tremendous range, from increases in GABA-induced Cl^- current approaching 800% to inhibition of 60%. It should be noted that the degree to which the GABA response is modulated by a given ligand is dependent on the GABA concentration used in the assay. When this variability is superimposed on the subunit heterogeneity and differential anatomical distributions of $GABA_A$ receptors a complex picture emerges, but it is one that offers numerous possibilities for improved therapeutic modulation of central GABA function.

Based on SAR data for a variety of structural classes of ligands, several models of the benzodiazepine binding site have been advanced (151–162). These models, although sharing many common features, have generally been slightly different for inverse agonists, antagonists, and agonists, as well as for diazepam-sensitive and diazepam-insensitive subtypes. The pharmacophoric descriptors for all these models have now been incorporated into an inclusive pharmacophore model for the BZR (163) that rationalizes ligand-receptor interactions at the molecular level for inverse agonists, antagonists, and agonists and for all major subtypes (α1–α6) (Fig. 9.5). This model identifies three important interactions that anchor the ligand through hydrogen bond donor (H1, H2) and hydrogen bond acceptor (A1) points on the receptor. L1, 2, 3, and Di represent lipophilic regions, the occupation of which influences the affinity, efficacy, and selectivity of the ligand. S1, S2, and S3 are regions of steric intolerance that restrict the size of BZR ligands. Region LDi appears to be larger in α1 subtypes than in the others, and occupation of this region may thus confer α1

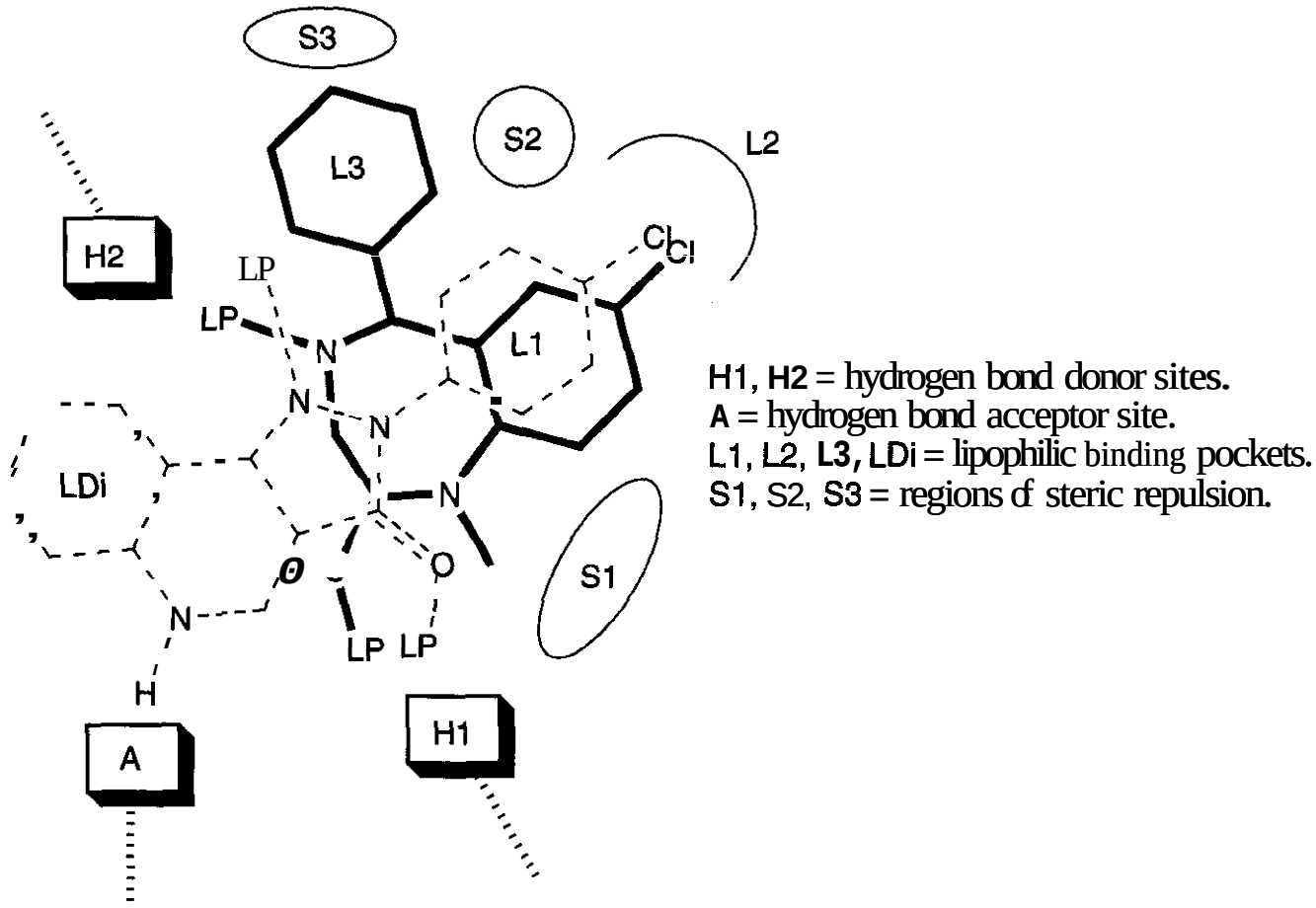


Figure 9.5. Diazepam (1) (heavy line) and CGS9896 (67) (dotted line) fitted to a schematic representation of the inclusive pharmacophore/receptor model for the BZR. Adapted from Ref. 163, copyright Overseas Publications Association, NV, with permission from Taylor and Francis Ltd.

selectivity (161, 162). Occupation of region L3 (e.g., by the C-5 phenyl ring of the benzodiazepines) is commonly associated with agonist activity. Recently, the inclusive pharmacophore model has been integrated with data from point mutation and photoaffinity-labeling experiments to provide an enhanced picture of the benzodiazepine binding site (164). Another study has used behavioral endpoints for different ligands to construct a model of ligand-BZR interactions that contribute to an anxiolytic effect (165).

3.2 Serotonin

Both depression and anxiety appear to be associated with a dysregulation of serotonergic function at some level, a hypothesis supported by the clinical efficacy of the SSRIs in both disorders. On the other hand, the benzodiazepines have no clinical utility as antidepressants. It has therefore been suggested that, although serotonin has a role in both disorders, different serotonergic pathways and receptor subtypes are responsible for the modulation of anxiety and depression (166, 167). Some of the major 5HT neuronal pathways originate in the raphe nucleus (168). They innervate a number of brain regions including the limbic system, and in particular the septohippocampal system and the amygdala. It has been proposed (169) that anxiety is a result of excessive serotonin activity in these specific brain regions. The serotonergic neurons arising from the dorsal raphe nucleus innervate the periacqueductal gray (PAG) and the amygdala, and these may regulate adaptive responses to acute stress (166). On the other hand, 5HT neurons originating in the median raphe nucleus, which innervate the hippocampus, mediate resistance to chronic stress and failure of this pathway may contribute to depression.

Through their widespread blockade of the serotonin transporter, the SSRIs are known to increase serotonin levels at virtually every synapse in the CNS, yet the exact neuronal pathways and receptors through which their anxiolytic effect is ultimately produced remain unclear (166). To date, 15 different 5HT receptor subtypes have been identified (170). Of these, seven subtypes have been implicated to a greater or lesser degree in anxiety: the 5HT-1A, 5HT-1B/D, 5HT-2A/B/C, and 5HT-3

receptors (171, 172). A survey of preclinical research into serotonergic agents as potential anxiolytics (173) shows that 50% of studies over the past two decades involve 5HT-1A ligands, whereas 5HT-2 and 5HT-3 compounds account for 15 and 13% of studies, respectively, giving a rough guide to the presumed importance of each receptor in anxiety.

3.2.1 5HT-1 Receptors. Of all the serotonin receptors implicated in anxiety, the 5HT-1A receptor (174) has been the subject of the most study. The only marketed anxiolytic acting directly on a serotonin receptor is buspirone (9), a 5HT-1A partial agonist. The 5HT-1A receptor is a member of the G-protein-coupled receptor family, and is found in brain structures known to be involved with emotional behavior, such as the hippocampus, amygdala, raphe nuclei, and hypothalamus. In the raphe nucleus, 5HT-1A receptors are presynaptic autoreceptors, whereas in the other brain regions they are postsynaptic. Activation of the postsynaptic receptors leads to neuronal inhibition in some limbic structures. Activation of presynaptic autoreceptors suppresses the firing rate of serotonin raphe neurons, thereby reducing serotonin turnover in the terminal fields (175, 176). Both modes of action are presumed to contribute to the therapeutic effects of buspirone (177). Mice lacking the 5HT-1A receptor have been generated by homologous recombination (178, 179), and these animals show an overt anxious phenotype in several anxiety models, supporting a role for the 5HT-1A receptor in the regulation of anxiety.

5HT-1B/D receptors, best known as the site of action of popular antimigraine drugs such as sumatriptan, have also been associated with anxiety (180), although the evidence for their involvement is less compelling than that for the 1A subtype. The 5HT-1/B/D receptors are found in the basal ganglia, hippocampus, and cortex (181), where they exist as autoreceptors on serotonergic neurons and as heteroreceptors on nonserotonergic neurons. 5HT-1B receptor agonists show an anxiogenic effect in various animal models (182), including the elevated plus maze (183) and social interaction (184) tests. Furthermore, selective 5HT-1B/D antagonists have shown an anxiolytic effect in the mouse light/dark box model, and this effect was attenuated by a 5HT-1B agonist and also by the benzodiazepine antagonist flumazenil (185). The latter phenomenon suggests a functional interaction between 5HT-1B/D receptors and the GABA system in the modulation of anxiety. Indeed, the 5HT-1B/D heteroreceptor is known to exert an inhibitory influence on the release of a number of neurotransmitters, including GABA (186). Thus, antagonists at the 5HT-1B/D receptors may produce an anxiolytic effect, at least in part, through a secondary enhancement of GABA function. 5HT-1B knockout mice (187) exhibit behaviors consistent with reduced anxiety in some animal models but not in others (188–190). 5HT-moduline is an endogenous tetrapeptide that influences serotonergic activity through antagonism at 5HT-1B receptors (191, 192). Centrally administered 5HT-moduline desensitizes 5HT-1B receptors, resulting in increased serotonin release (193). Consistent with this observation, specific 5HT-moduline antibodies have shown anti-anxiety effects in animal models (194), suggesting the involvement of this peptide in the regulation of anxiety. Clearly, further study is needed to fully characterize the role of 5HT-1B/D receptors in anxiety and to ascertain the potential, if any, of 5HT-1B/D antagonists as anxiolytic drugs.

3.2.2 5HT-2 Receptors. It has been suggested that the pathology of anxiety disorders may involve a hypersensitivity of central 5HT-2 receptors (171). This is supported by the anxiogenic effect of the nonselective 5HT-2 agonist m-chlorophenylpiperazine (*m*-CPP) in animal models of anxiety (195). Further, administration of m-CPP, which is the principal metabolite of the antidepressant nefazodone, elicited anxiety in panic disorder patients (196) and induced a transient exacerbation of symptoms in OCD patients (197). Together, these data raise the possibility of 5HT-2 receptor antagonists as anxiolytics. All three of the 5HT-2 subtypes (2A, 2B, 2C) have been associated with anxiety to some degree, the 2A and 2C isoforms being the most frequently linked.

In the CNS, 5HT-2A and 2C receptors are found postsynaptically on nonserotonergic

neurons (198). 5HT-2C receptors are found in the choroid plexus, cerebral cortex, hippocampus, and amygdala (199), and this localization is supportive of a role in affective disorders. As well as in anxiety, 5HT-2C receptors have been implicated in depression (200) and in the modulation of feeding behavior (201). 5HT-2A receptors are more widespread, with higher levels in cortical regions (198), and antagonists at this subtype may also possess antipsychotic activity. In behavioral studies there is considerable inconsistency, in that 5HT-2 antagonists have been shown to produce anxiolytic effects, no effects, or even anxiogenic effects in various animal models (202). Administration of a nonselective 5HT-2 antagonist (mianserin) or a selective 5HT-2B/C antagonist, but not a selective 5HT-2A antagonist, produced anxiolytic effects in animal models, implicating the 2B/C subtypes in anxiety modulation (203). The available evidence paints a confusing picture of 5HT-2 receptors in anxiety, and knowledge of the exact function and potential clinical applications of the various 5HT-2 subtypes remains incomplete. Further behavioral studies with subtype-selective ligands would help delineate the properties of the 5HT-2 subtypes, but the high degree of homology among the 5HT2 receptors (204, 205) has made this approach difficult. The pharmacology of this class of receptors is. further complicated by the unusual down-regulation of 2A and 2C receptors upon exposure to antagonists (206).

3.2.3 5HT-3 Receptors. 5HT-3 receptors are best known as the site of action of clinically useful antiemetics, such as ondansetron (Zofran). Although 5HT-3 receptors are found predominantly in the medulla oblongata (207), their presence, albeit at lower levels, in the amygdala and hippocampus raises the possibility of some role in emotional behaviors such as anxiety (208, 209). In rats, 5HT-3 antagonists have shown anxiolytic effects when injected into the amygdala but not when injected into the dorsal raphe nucleus (210). A similar anatomical pattern was observed for the anxiogenic effects of 2-methyl-5-hydroxytryptamine, a 5HT-3 receptor agonist. Central 5HT-3 receptors may influence the release of other neurotransmitters in the brain, including norepinephrine, dopamine, and CCK (211). Overall, 5HT-3 antagonists display erratic efficacy in animal models of anxiety and have failed to demonstrate a significant anxiolytic effect in the clinic (173,208).

3.2.4 GABA/Serotonin Interactions. The uncertainty surrounding the relative roles of GABAergic and serotonergic pathways in the regulation of anxiety is compounded by the interactions of the GABA system with the serotonergic system. The inhibitory influence of benzodiazepines on serotonin turnover in the brain was first noted over 30 years ago (212) and led to the theory that the therapeutic effects of the BZs may be mediated, in part, by their potentiation of GABAergic inhibition of 5HT neuronal firing and subsequent reduction in serotonin release.

Genetically modified mice lacking the 5HT-1A receptor are inherently anxious, and are insensitive to the anxiolytic effect of diazepam (213) in the elevated plus maze and open field tests. In addition, there is altered $GABA_A$ receptor expression in the brains of these 5HT-1A knockout mice. Expression of the a2 subunit was reduced to 59% of wild type levels in the amygdala, and to 47% of wild-type levels in the cortex, whereas no reduction in $\alpha 3$ expression was found. In addition, no subunit. reduction of any kind was found in the hippocampus and raphe nucleus. Given that the knockout mice lack both pre- and postsynaptic 5HT-1A receptors, the relative influence of each on $GABA_A$ receptor expression remains unclear. These results, and the fact that benzodiazepines are known to mediate fast inhibitory transmission in the amygdala by interaction with $GABA_A$ receptors (214), point to an intimate relationship between the 5HT-1A receptor and $GABA_A$ a2 receptors. This is particularly interesting in light of recent work with genetically altered mice containing mutations in GABA, receptor subtypes (148), which suggests that the anxiolytic effect of diazepam is mediated specifically by the a2 subunit.

3.3 Norepinephrine

The majority of noradrenergic neurons in the brain are located in the locus ceruleus (LC), and electrical stimulation of this region in an-

imals leads to behaviors typical of an anxiety state. Increased norepinephrine (NE) levels are also associated with the somatic symptoms of anxiety, such as dry mouth, rapid heart rate, and elevated blood pressure.

In the resting state, no differences in epinephrine and norepinephrine levels were found between GAD patients and normal controls (215). A different picture emerges using challenge paradigms, which explore the dynamic properties of the adrenergic system in its response to stress. A blunted growth hormone response to the adrenergic a2 partial agonist clonidine in GAD patients suggests a reduced sensitivity of $\alpha 2$ adrenergic receptors (216). Furthermore, a decrease in platelet a2 receptor sites has been reported in GAD patients (217). Stressful events are known to increase NE release in brain regions intimately involved with emotional behavior including the hypothalamus, amygdala, and LC, and this increase is attenuated by diazepam (218). Moreover, the attenuating effect of diazepam was blocked by the benzodiazepine antagonist flumazenil, whereas benzodiazepine inverse agonists (β-carbolines) facilitate NE release in these brain areas. Thus, inhibition of NE release in specific brain regions subsequent to $GABA_A$ receptor activation may contribute to the clinical action of the benzodiazepines. The present data leave the exact role of norepinephrine in the neurobiology of anxiety unresolved. It appears that in resting situations the noradrenegic system functions normally, but under challenge GAD patients may exhibit signs of reduced adrenergic receptor sensitivity.

3.4 Neuropeptides

3.4.1 Cholecystokinin. Cholecystokinin (CCK) is one of the most abundant neurotransmitters in the central nervous system. Two CCK receptor subtypes have been cloned to date, that is, CCK-A (Alimentary), which are primarily located in the periphery, and CCK-B (Brain) (219). CCK-B receptors are widely distributed throughout the brain, but have particularly high densities in the hypothalamus, limbic system, basal ganglia, hippocampus, and brain stem (219). CCK-4 (a tetrapeptide) and CCK-8S (a sulfated octapeptide) are important neurotransmitters in the central CCK system, and may have a role in the mediation of anxiety responses in animals and humans (220). Administration of CCK-4, a CCK-B agonist, promotes arousal and fear responses in a variety of animal models (221, 222), and this agonist-induced anxiogenic response can be blocked by pretreatment with CCK-B antagonists such as L-365,260 and CI-988 (222). Furthermore, administration of CCK-4 or pentagastrin induced panic attacks at a much higher rate in panic disorder (223), OCD (224) and GAD (225) patients than that in normal controls. These studies are indicative of a role for CCK in anxiety (226).

In addition, the CCK system interacts with other neurotransmitter systems that have established roles in anxiety. CCK is found in GABA neurons in the cortex, hippocampus, and amygdala, and discontinuation of long-term diazepam therapy produces an upregulation of CCK-8 binding in rat cortex (219). Additionally, clinically relevant concentrations of benzodiazepines have been shown to inhibit CCK-8-induced activation of rat hippocampal cells (227). A CCK/GABA relationship has yet to be demonstrated in human studies, however. In one study, the benzodiazepine receptor antagonist flumazenil failed to reduce the panicogenic effect of CCK-4 in healthy volunteers (228). In rats, the inhibition of exploratory behavior induced by CCK agonists can be blocked by pretreatment with the 5HT-3 antagonist ondansetron (229). A CCK/norepinephrine interaction has also been proposed. Selective neurotoxin destruction of noradrenergic nerve terminals in projections from the LC results in increased CCK receptor binding density in brain regions that receive noradrenergic input from the LC (219). CCK may also have a modulatory role in the HPA axis, in that CCK-B agonists have been shown to increase ACTH and cortisol secretion (230). In addition, CCK-8 administration stimulates the release of ACTH and of hypothalamic CRF (231).

3.4.2 Corticotrophin-Releasing Factor (CRF). CRF, a 41 amino acid peptide, mediates ACTH release from the hypothalamus and is intimately involved in the stress response. CRF is

also located in brain regions associated with anxiety, including the LC and the amygdala. The modulatory effects of CRF are mediated through CRF-1 and CRF-2 receptors, both members of the GPCR superfamily. CRF-1 receptors are the dominant form in the CNS and hence the most frequently associated with neuropsychiatric disorders such as anxiety, depression, and stress disorders (226, 232–234). CRF also interacts with the soluble CRF-binding protein, which influences CRF neurotransmission as well as the ability of CRF to activate the HPA axis (235).

Application of CRF directly into the LC stimulates neuronal firing, enhances NE release (236, 237), and produces anxiogenic effects in behavior models (238, 239). Alprazolam reduces CRF concentrations in both the LC and the amygdala (240), suggesting that suppression of CRF stimulation of noradrenergic neurons in the LC may contribute to the anxiolytic effect of the benzodiazepines. In various rodent models, including the elevated plus maze (241), social interaction (242), and Geller-Seifter conflict (243) paradigms, administration of CRF elicits behaviors consistent with an anxiogenic effect (244,245). CRF also potentiates the acoustic startle reflex, and this potentiation can be blocked by treatment with chlordiazepoxide (246). Genetically altered mice that overproduce CRF have been described and these animals exhibit a heightened state of anxiety in animal models (247, 248). Conversely, mice lacking the CRF-1 receptor display reduced anxiety in many behavioral models (249, 250). The behavioral responses to CRF in animals are therefore suggestive of a role for CRF in human anxiety disorders. This proposal is supported by the elevated cerebrospinal fluid levels of CRF found in OCD (251) and PTSD (252,253) patients, although this was not the case in panic disorder (254) or GAD (255) patients. Similarly, urinary free cortisol levels were no different in social phobia patients compared to those of normal controls (256), suggesting normal function of the HPA axis in this disorder.

The fact that CRF enhances the behavioral responses to stressful situations in animals indicates that it may play a role in the development of anxiety in humans (257). There is adequate evidence that excessive CRF secretion or activity is associated with at least some of the anxiety disorders (233,234,2581,and CRF receptor antagonists are therefore valid anxiolytic targets.

3.4.3 Neuropeptide Y. Of the neuropeptide Y (NPY) receptors identified to date, Y-1 is the most strongly linked to the regulation of anxiety. This receptor is found in high densities in the brain, particularly in the cortex, thalamus, and amygdala, and Y-1 receptors in the latter structure are believed to mediate the anxiolytic effect of NPY (226,259,260). In rats, administration of antisense oligonucleotides corresponding to the Y-1 receptor significantly increased anxiety in behavioral models compared to normal controls (261). Postmortem examination of the brains of the treated animals revealed a 60% decrease in Y-1 receptors and no change in Y-2 receptors.

NPY has shown an anxiolytic effect in several animal models (262, 263), although the mechanism through which this anxiolysis is produced is unclear. In rats, the anxiolytic effect of NPY could be blocked by treatment with an adrenergic a2 antagonist (idazoxan), but not by an a1 antagonist (prazosin) or a benzodiazepine receptor antagonist (flumazenil) (262, 264). These results might suggest a selective interaction between NPY and noradrenergic transmission, and indeed NPY is extensively colocalized with norepinephrine in the CNS (265). Yohimbine, an adrenergic a2 antagonist known to increase anxiety, significantly increases circulating NPY levels in human subjects, again suggesting that the anxiety-modulating effects of NPY are, in part, related to noradrenergic transmission (266). Other clinical data regarding this proposal are inconclusive, however. Low CSF levels of NPY have been correlated with higher anxiety scores among depressed patients (267), yet higher levels of NPY were found in panic disorder patients than those in normal controls (268). Another study failed to find any differences in plasma NPY levels between normal volunteers and patients with panic disorder or social phobia (269). Although current evidence suggests a linkage to the noradrenergic system, NPY is an abundant transmitter in the CNS and interactions with a variety of sys-

tems are likely. For example, NPY is also frequently colocalized with GABA and somatostatin, offering further pathways through which NPY may modulate anxiety. In mice, chronic treatment with benzodiazepine receptor agonists has been shown to increase Y-1 receptor expression in the amygdala (270). Additionally, it has been suggested that NPY could reduce anxiety by counteracting the anxiogenic effects of stress-induced CRF release from the amygdala (271).

3.5 Glutamate Receptors

The glutamate system is responsible for most of the brain's excitatory neurotransmission. Glutamate interacts with a number of receptors, including the NMDA, AMPA, kainate, and metabotropic glutamate receptors. The latter receptor is coupled to G-proteins, whereas the others are directly linked to ion channels. In addition to the ion channel, the NMDA receptor contains a number of modulatory sites, including sites for glycine and glutamate. Just as enhancement of GABA's inhibitory action decreases anxiety, it might be expected that inhibition of glutamate's excitatory functions would produce the same effect. Indeed, the direct injection of NMDA antagonists into discrete brain regions has provided evidence that functional antagonism of specific populations of these receptors produces an anxiolytic effect. Competitive antagonists (272), as well as glycine site antagonists and partial agonists (273, 274), are active in the elevated plus maze when microinjected into the dorsal periaqueductal gray matter (DPAG). In addition, intrahippocampal administration of competitive and uncompetitive NMDA antagonists and glycine partial agonists was reported to significantly increase punished responding (275, 276). Complementary to these results indicating that blockade of glutamate receptors is anxiolytic, other studies shave shown activation of NMDA receptors to be anxiogenic. Thus NMDA increases distress calls in the rat pup isolation model (277), decreases social interaction in rats, and decreases the time spent on the open arms of the elevated plus maze (278), behaviors characteristic of increased anxiety. Additionally, injection of glycine into the DPAG produced an anxiogenic response in the elevated plus maze test (279,280). Furthermore, NMDA was able to substitute as a discriminative stimulus in pigeons trained to recognize the anxiogenic benzodiazepine receptor inverse agonist β-CCE (281). The BZR antagonist flumazenil blocked the ability of β-CCE, but not NMDA, to act as a cue, suggesting that anxiety is the common stimulus produced by both compounds.

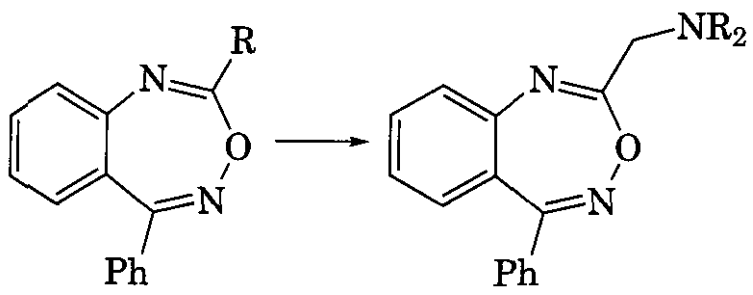
The foregoing evidence suggests that the excitatory glutamate system could have a complementary role to that of the inhibitory GABA system in anxiety. Thus activation of glutamate receptors or inhibition of GABA receptors is anxiogenic, whereas inhibition of glutamate receptors or activation of GABA receptors is anxiolytic.

4 HISTORY

The anxiolytic agents currently on the market owe much to serendipity as well as rational design in their genesis. With the exception of buspirone, all first indication anxiolytics were introduced before 1975, before many of the modern techniques of medicinal chemistry such as radioligand binding assays, molecular modeling, and pharmacokinetic screening were introduced into common practice.

4.1 Discovery of the Benzodiazepines

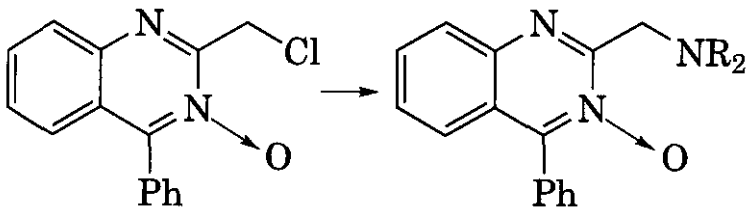
The benzodiazepines represent the single most important advance in the treatment of anxiety, and the story of their discovery is a truly remarkable one (282,283). In the 1950s, a major research effort was the search for new tranquilizers that would overcome the shortcomings of the medications in use at the time, such as the barbiturates and the phenothiazines. In the chemistry laboratories at Hoffman-La Roche in New Jersey, Dr. Leo Sternbach approached this task by turning to a series of compounds, the "benzoheptoxdiazines" (19), which he had previously investigated as dyestuffs during his research at the Jagiellonian University in Kraków, Poland. Sternbach considered, then rejected, the possibility of making modifications to known natural or synthetic tranquilizers and, having no knowledge of any discrete receptor target, he decided to pursue the benzoheptoxdiazines



(19) **(21)**
(20) R = CH_2Cl

simply out of his interest and familiarity with their chemistry. In today's world of directed and target-based medicinal chemistry, it would surely be difficult to justify basing one's research strategy on such a personal preference. Then, as now, there was an appreciation that a significant number of CNS agents were organic bases, and the essence of the research strategy was to introduce basic groups into the molecule by reaction of the chloride (20) with secondary amines. The synthesis of numerous compounds of type (21) followed, none of which showed any interesting biological activity.

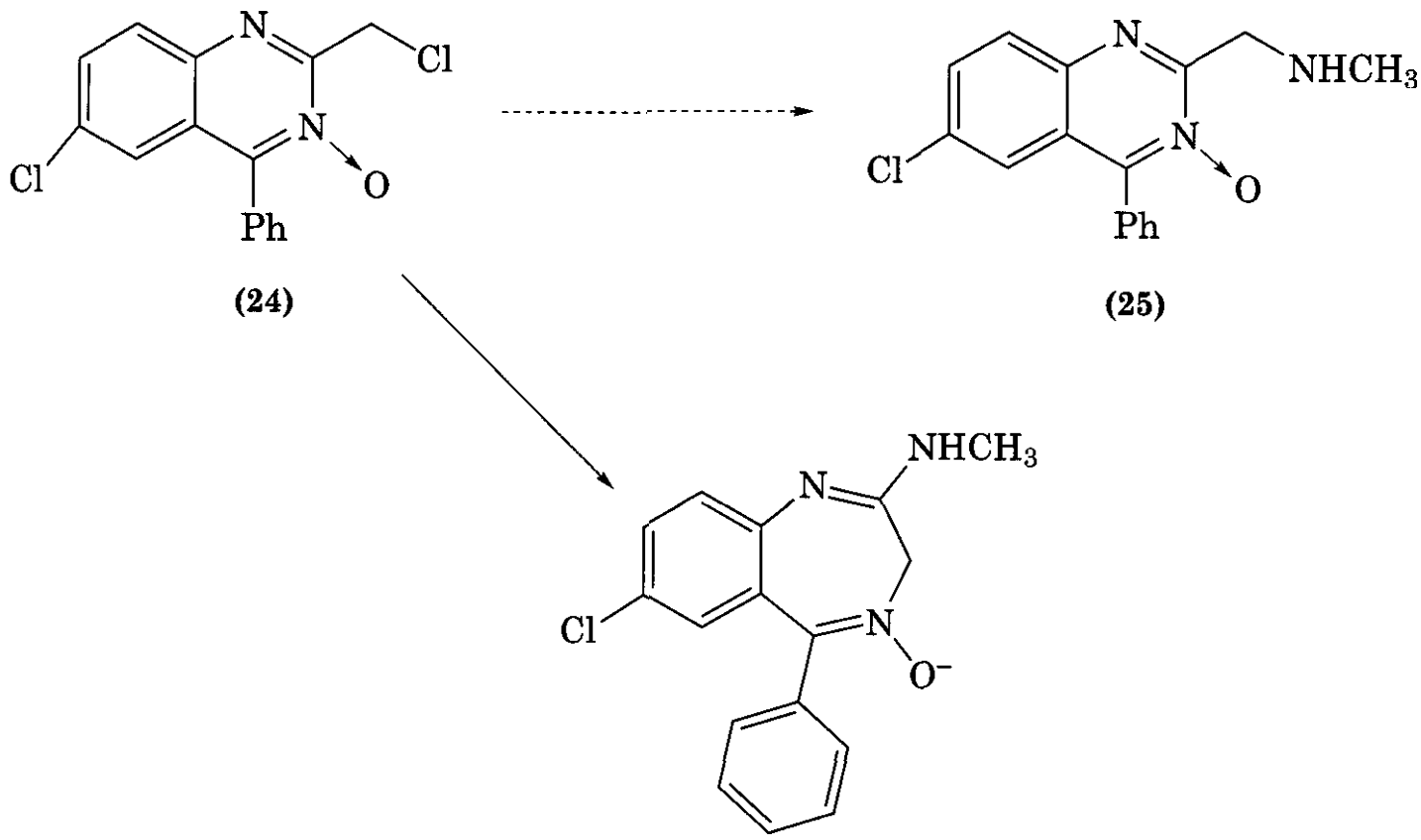
Then, studies on the chemistry of these compounds revealed that the structures of the presumed benzoheptoxdiazines (20) had been erroneously assigned, and that they were in fact quinazoline N-oxides (**22**). The reaction products were then reassigned structure (**23**), rather than structure (21).



(22) **(23)**

At this point the demands of other projects forced the abandonment of this synthetic work, and that would have been the end of the story but for a remarkable stroke of good fortune almost 2 years later. During a lab cleanup in 1957 Sternbach's assistant, Earl Reeder, came across a small quantity of a crystalline compound that had been formed from the reaction of the quinazoline N-oxide (24) with methylamine in 1955, but had not been sent for biological testing. To tie up loose ends the compound was duly submitted, with everyone involved unaware that this decision was the first step of a revolution in the treatment of anxiety disorders. A few days later, Sternbach and his colleagues were amazed to hear that this compound possessed an impressive biological activity profile, including sedative, muscle relaxant, anxiolytic, and anticonvulsant properties, that were comparable to, or greater than, the prevailing reference compounds of the time. Excitement grew when subsequent studies revealed the low toxicity of the compound, which was a real advantage over the current medications. The intensive chemistry effort inspired by this discovery was to provide yet another twist in the tale. It was found that just like the starting materials, the structure of this exciting compound had been incorrectly assigned! Instead of the expected substitution product (**25**), an unusual ring-expansion reaction had produced the 1,4-benzodiazepine (**2**). This compound, named chlordiazepoxide, progressed quickly through clinical studies with outstanding results and was subsequently approved by the FDA. In what seems an impossibly short timeframe to the scientist in modern drug discovery, chlordiazepoxide was launched as Librium in 1960, a mere 2% years after pharmacological studies on the compound had begun. Further SAR studies of this new class of compounds improved upon the potency of chlordiazepoxide and 3 years later, in 1963, Valium (diazepam) was launched and went on to become one of the most popular drugs in history. These successes created an intense and widespread interest in the benzodiazepines, and during the next two decades over 30 members of this class reached the marketplace as anxiolytics, sedative-hypnotics, and anticonvulsants.

Even in today's more rational drug discovery environment, it may be said that serendipity is one of the medicinal chemist's best friends. Of course, serendipity alone cannot provide a drug; it requires someone to recognize the opportunity and capitalize on it. As demonstrated by Sternbach and his colleagues, when chance discoveries fall into the hands of open-minded and persistent scientists, the results can be remarkable.



(2) Chlordiazepoxide

5 STRUCTURE-ACTIVITY RELATIONSHIPS

5.1 Animal Models of Anxiety

Regardless of the biological target with which it interacts, any potential anxiolytic agent must demonstrate efficacy in animal models to justify further development. Historically, the benzodiazepines have exerted a strong influence on the development of such models. The majority of in *vivo* anxiety models have been designed to detect benzodiazepine-like effects, and have been optimized and validated using benzodiazepines and benzodiazepine receptor ligands. With the evolution of non-benzodiazepine anxiolytics such as those acting on the serotonin or neuropeptide systems, the generality and predictive validity of many of these models is coming into question. For example, benzodiazepines and serotonergic agents display very different behavioral profiles when examined in a range of anxiety models (208). As already noted, anxiety is a complex disorder with a multitude of contributory and possibly overlapping mechanistic pathways, and differences in behavioral effects may reflect the extent to which the model in question actually represents the anxiety pathway being targeted (284). Accordingly, it is now well accepted that all models are not equivalent in their sensitivity, and novel anxiolytic agents must be subjected to a battery of behavioral tests for their activity to be determined with any confidence.

There are over 30 animal tests reported to model anxiety and anxiolysis (202), and those most commonly used are shown in Table 9.2.

Table 9.2 Animal Models of Anxiety

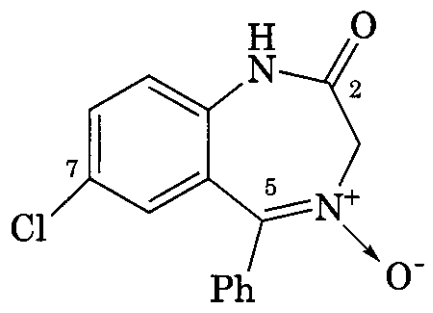
Unconditioned Models			Conditioned Models	
Exploratory Behavior	Social Behavior	Other	Conflict	Other
Elevated plus maze	Social interaction	Marble burying	Geller–Seifter	Active/passive
Light/dark box	Ultrasonic	Cork gnawing	Vogel	Avoidance
Open field test	Vocalization		Pigeon conflict	Fear potentiated startle
Zero maze	Human threat Social competition		Monkey conflict	Acoustic startle

These models are classified into two major groups: those based on unconditioned behavior and those based on conditioned behavior. The former tests employ responses controlled by operant conditioning procedures, and are typified by the traditional Geller-Seifter and Vogel conflict tests, whereas the latter models rely on the natural aversive reactions of animals to novel stimuli, such as an unfamiliar environment (elevated plus maze) or another animal (social interaction). Although it is imperative to use a variety of animal models, tests that do not involve unnatural responses or punishment are increasingly favored.

5.2 Benzodiazepines

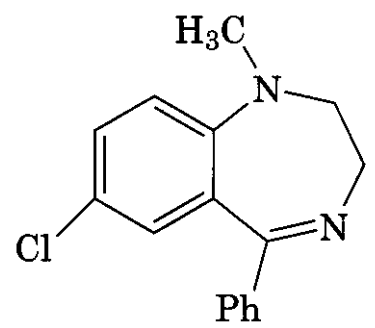
In the study of compounds acting at the BZR, modern researchers have at their disposal an array of powerful *in vitro* assays that provide a detailed pharmacological profile of any new ligand prior to *in vivo* behavioral testing. This environment is in sharp contrast to that which produced most of the classical 1,4-benzodiazepines. The clinical success and structural novelty of chlordiazepoxide and diazepam sparked a massive chemical investigation of this class of compounds, but in the 1960s and 1970s no *in vitro* assays existed for the pharmacological profiling of these new compounds. Indeed their mode of action was a mystery, the benzodiazepine receptor had yet to be discovered, and the concepts of intrinsic activity and subtype selectivity were many years away. Nevertheless, the productivity of this period was remarkable, with thousands of analogs being prepared and examined for tranquilizing activity. Consequently, many of the key SAR observations on the benzodiazepines are to be found in the older chemical literature (for reviews, see 285, 286.) These SAR observations were initially built around *in vivo* testing for tranquilizing and anticonvulsant activity rather than pharmacological properties such as affinity and intrinsic efficacy.

5.2.1 1,4-Benzodiazepine-2-Ones: General Trends. The large number of compounds synthesized in the few years following the launch of chlordiazepoxide quickly identified some general SAR trends in the benzodiazepine series. Chlordiazepoxide (2) was found to be unstable in acid solution, hydrolyzing to the lactam (**26**), which possessed superior anxiolytic

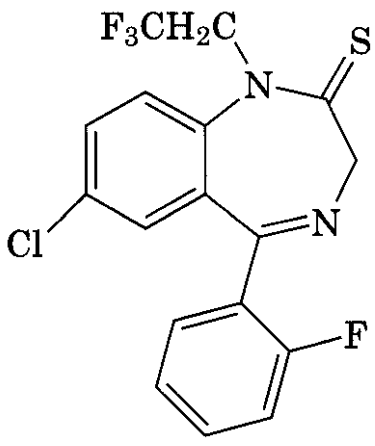


(26)

potency. In general, a carbonyl group is the optimum substituent at the 2 position, the analogous 2-amino derivatives being somewhat weaker. Thus the basic side chain, the introduction of which formed the basis of the original research strategy, turned out to be unnecessary, indeed detrimental, to the desired activity. An exception to this generalization is medazepam (**27**), which lacks any substituent at position 2. In humans, medazepam is metabolized to give a number of pharmacologically active benzodiazepines, however, including diazepam, desmethyldiazepam, and oxazepam (287, 288), all of which contribute to the anxiolytic effect. Quazepam (Doral, 28) ex-

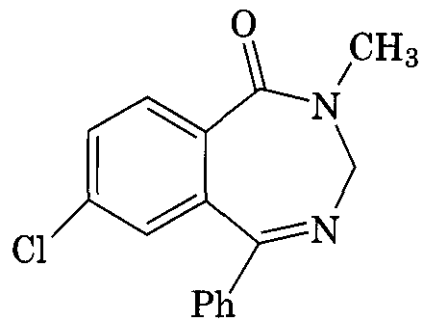


(27)



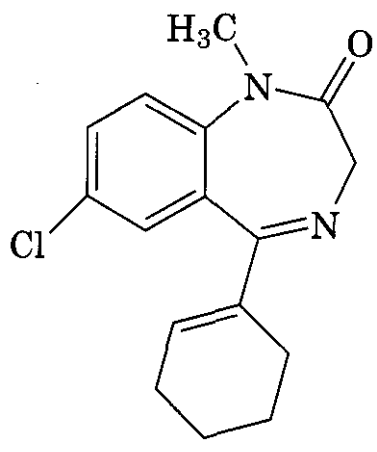
(28)

emplifies another variation at the 2 position, where the benzodiazepin-2-one has been converted to the thiolactam. The reverse lactam of diazepam (29)shows comparable activity in anticonvulsant tests (289).



(29)

Some of the fundamental structure-activity relationships established in the early years of benzodiazepine research have held true for virtually all benzodiazepines prepared since then. It was quickly found that methylation of chlordiazepoxide at N-1 enhanced the potency severalfold (282). N-Methyl benzodiazepines are typically more potent than the N-H analogs, and substituents larger than methyl generally diminish activity. In the A-ring, electronegative substituents such as halogen (especiallychlorine and bromine), nitro, or trifluoromethyl at position 7 are required for significant biological activity (290). Electron-releasing groups (e.g., methyl, methoxy) at this position decrease activity, as does substitution of any kind at position 6, 8, or 9. An aromatic substituent at C-5 is usually required for useful biological activity, although tetrazepam **(30)** provides a rare example where this is not the case. The C-5 phenyl ring is not tolerant of structural modification, and only *ortho* substitution with fluorine or chlorine is allowed. These substituents strongly potentiate the anxiolytic effects, however, giving compounds significantly more potent than the unsubstituted analogs (282,290). Although much less common than the C-5 phenyl benzodiazepines, some heteroaryl derivatives, of which bromazepam (7) is an example, have shown significant biological activity. Bromazepam is available as an anxiolytic and a sedative and is reported to have a similar in *vivo* activity profile to that of diazepam (291). Other heterocycles at C-5 (e.g., pyrimidine, pyrazine) show less activity than that of the 2-pyridyl group (290). Within the 1,4-benzodiazepine template, the effects of favorable substituents in different parts of the molecule appear to be synergistic. Flunitrazepam (31), which com-



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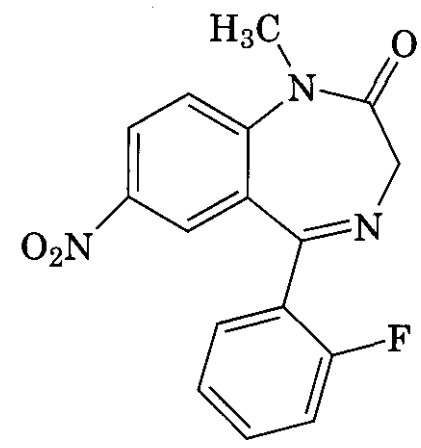


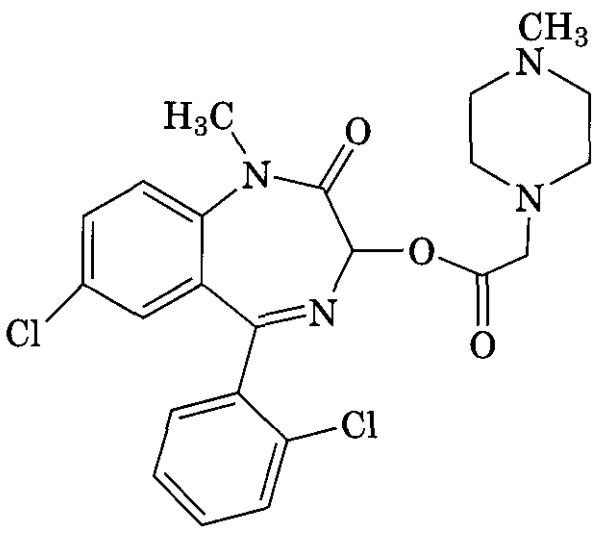
(31)

bines the optimal pharmacophoric groups at N-1, C-2, C-7, and C-5 (292), is among the most active of all the benzodiazepines. Flunitrazepam (Rohypnol) is a powerful sedative-hypnotic with significant memory-impairing and muscle-relaxant effects, all of which are strongly potentiated by alcohol. In recent times, these effects have earned Rohypnol notoriety as a "date-rape" drug, and have resulted in its removal from the market in many countries.

3-Hydroxybenzodiazepines represent active metabolites of many clinically useful benzodiazepines (e.g., diazepam), and consequently a large number of 3-substituted derivatives have been prepared and evaluated as anxiolytics in their own right. These compounds often show comparable potency to that of the parent benzodiazepines, but have a dramatically different metabolic profile. In hu-

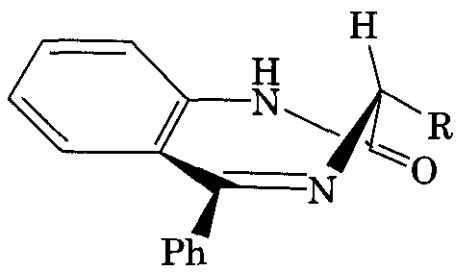
mans, diazepam gives rise to several active metabolites that contribute to buildup of active agent in the blood over time. One of these metabolites, oxazepam, is itself a marketed anxiolytic (293). Oxazepam is directly conjugated at the 3-hydroxy group and is excreted in the urine as the pharmacologically inactive glucoronide (294,295). By virtue of this small structural change, the clinical use of oxazepam is not complicated by the buildup of active metabolites, a factor known to contribute to the "hangover" effect seen with some benzodiazepines. Other 3-hydroxybenzodiazepines in clinical use include lorazepam Ativan (4), and the hypnotics temazepam (Restoril) and lormetazepam (Loramet).

The &position, by way of the hydroxy group, has been modified in an attempt to increase the water solubility of benzodiazepines, which are generally highly lipophilic compounds. For example, the amino ester derivative (32) of lorazepam had excellent water sol-



(32)

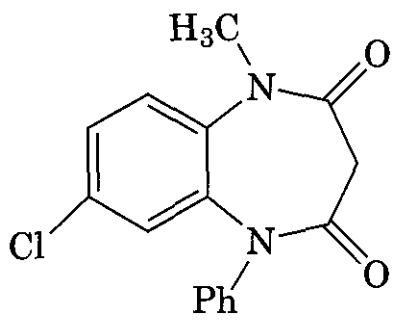
ubility (as the methanesulfonate salt), and showed anxiolytic efficacy comparable to that of the parent compound (296). The carboxylic acid at the 3-position of clorazepate (3) imparts high water solubility (297). Administered as its dipotassium salt, the anxiolytic activity of chlorazapate derives from its rapid decarboxylation in *vivo* to give desmethyldiazepam (298). The stereochemistry of the 3-substituent impacts on both the affinity and intrinsic efficacy at the BZR, the 3-(*S*) isomers generally having greater affinity and higher intrinsic efficacy than those of the 3-(*R*) isomers. For example, the 3-(*S*) enantiomer of 3-oxazepam hemisuccinyl ester produced potentiations of GABA induced chloride flux almost twofold greater than that of the 3-(*R*) enantiomer (299). Likewise the *3-(S)* enantiomer of 3-methyldesmethyldiazepam is twice as potent as, and is a stronger agonist (GABA shift = 2.7) than, the 3-(*R*) isomer (GABA shift = 1.5) (300). This stereochemical bias is believed to arise from a preferential stabilization of conformation (33) by the 3-(*S*) antip-



(33)

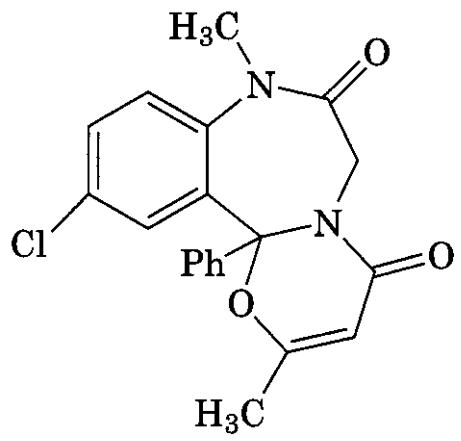
odes, in which the substituent occupies a quasiequatorial orientation (301). This conformation is believed to be preferred by the receptor (302), and is disfavored for the 3-(R) enantiomers. The commercially available 3-hydroxybenzodiazepines are marketed as racemic mixtures because their rapid racemization in *vivo* renders a chiral formulation impractical and unnecessary.

The N-4 atom may be replaced with a carbonyl group, as in clobazam (Frisium, 34),



(34)

which has clinical anxiolytic and anticonvulsant properties. The carbonyl group presumably participates in a hydrogen bond interaction with the receptor in a similar fashion to that of the imine nitrogen in the typical 1,4-benzodiazepines. Other modifications in this region include the fusion of heterocyclic rings across the 4,5 bond. Oxazolam, cloxazolam, and ketazolam (35) are examples of this variation that are in limited clinical use outside

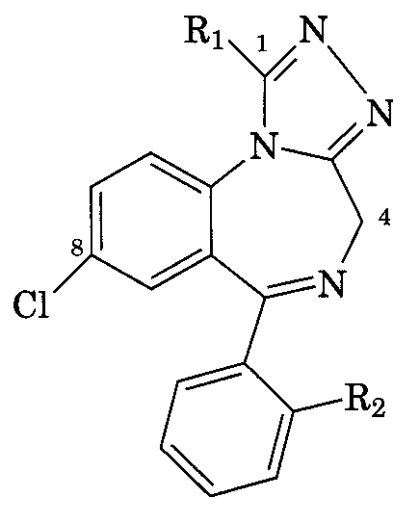


(35)

the United States as sedative anxiolytics (51). The fused heterocyclic ring is removed during metabolism to generate the parent benzodiazepine (303, 304), which contributes significantly to the activity. Ketazolam (**35**), for example, is converted into the active metabolites desmethylketazolam and desmethydiazepam, both of which have longer half-lives than that of the parent compound. Ultimately, 80% of a single oral dose (30 mg) of ketazolam is recovered as oxazepam or oxazepam-glucoronide in the urine (305).

5.2.2 1,2-Ring-Fused 1,4-Benzodiazepines. In the early 1970s it was found that the fusion of a triazole ring (306, 307) across the 1,2 positions of the benzodiazepinering significantly enhanced the in *vivo* potency (up to 10-fold) compared to that of the corresponding lactam (308). Subsequently, a range of five- and six-membered heterocycles has been annelated at this position (285), the most interesting being the triazole and imidazole variants.

Of the three possible triazole isomers that maintain a nitrogen at position 1 of the benzodiazepine ring, only the 4H-[1,2,4]-triazolo[4,3-*a*]-1,4-benzodiazepine analogs, exemplified by alprazolam (**5**), appear to have useful activity (285). Alprazolam is clinically effective in GAD and is approved for use in panic disorder. Other members of this class in clinical use include the hypnotics estazolam (36)and triazolam (**37**). A methyl group at the C-1 position is optimal, and replacement with hydrogen, larger alkyl groups, or aromatic rings reduces activity in animal models predictive of anxiolysis (285, 308, 309). The C-1 hydroxymethyl compounds (**38**), which are significant metabolic products of this chemotype,



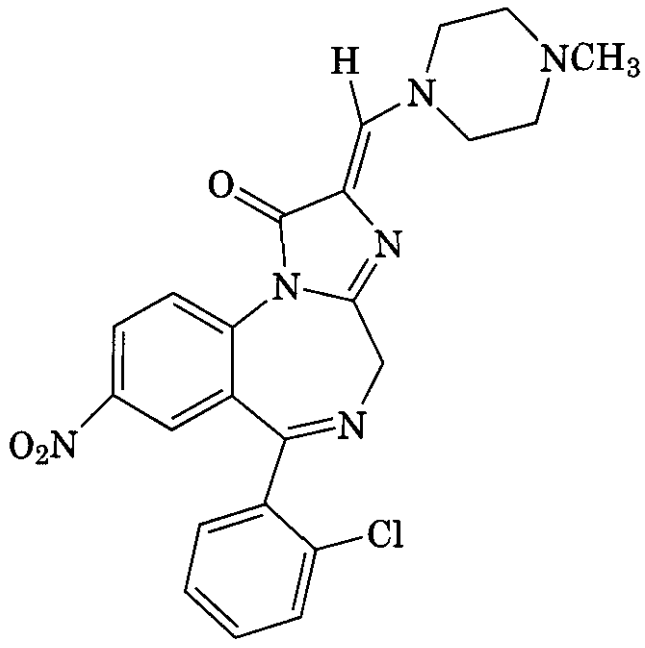
(5) $R_1 = CH_3$, R_2 = H (alprazolam)
(36) $R_1 = CH_3$, R_2 = Cl (triazolam)
(37) R_1, R_2 = H (estazolam)
(38) $R_1 = CH_2OH$, R_2 = H
(39) $R_1 = CH_2N(CH_3)_2$, R_2 = H

do show appreciable activity, however (310, 311). The related C-1 aminoalkyl derivatives, typified by adinazolam (Deracyn, **39**), have been reported to show anxiolytic or antidepressant activity in animal models, depending on the length of the chain linking the amino group to C-1 (312). With a single methylene linker, activity was seen in anxiolytic and antidepressant endpoints, and lengthening this chain abolished anxiolytic but maintained antidepressant activity. Increasing the size of the dimethylamino group was detrimental to both activities. For adinazolam, the pharmacological effects are largely mediated by the N-desmethyl metabolite. Repeated administration of (39) downregulates 5HT-2 receptors, and this may underlie its antidepressant properties (313). An NDA was filed for adinazolam following demonstration of anxiolytic efficacy in clinical trials (314, 315), but was subsequently withdrawn.

In keeping with the SAR of the 1,4-benzodiazepine-2-ones, the biological activity of the triazolobenzodiazepines is enhanced with electron-withdrawing groups (Cl, Br, NO_2, CF_3), and decreased with electron-donating groups (e.g., NH_2, SMe), at the C-8 position (285). Activity is potentiated by an *ortho* chloro or fluoro substituent in the C-6 phenyl ring. The N-5 oxides (316) and the 4-hydroxy derivatives (317) exhibit less anxiolytic activity than that of the parent compounds. This is in contrast to the benzodiazepin-2-one series, where the analogous 3-hydroxy derivatives

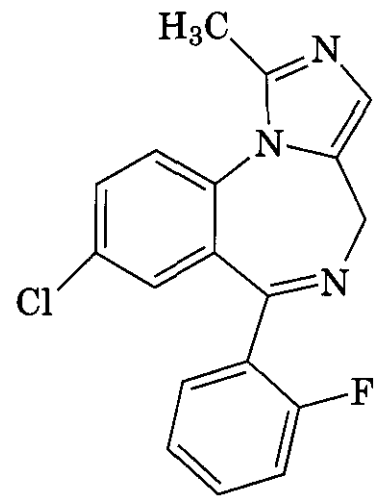
show comparable activity to that of the parent compounds. In general, 4-substitution in the triazolobenzodiazepine series is detrimental to activity (285, 318).

Imidazo[1,2-*a*][1,4]benzodiazepines generally show inferior biological activity to that of the analogous triazolo [1,2,4][4,3-*a*] derivatives, but some success has been achieved with a related series of amino-substituted imidazolone derivatives. This series is exemplified by loprazolam (**40**), which possesses potent



(40)

hypnotic activity (319), and is available under the trade name Dormonoct in Europe. The most active compounds of type (40) contain a methyl, ethyl, or propyl group at the N-4 position of the piperazine ring. The activity is further enhanced with a chlorine or fluorine at the 2-position of the C-6 phenyl ring, but is generally insensitive to the nature of the electronegative substituent at C-8 (320). More robust activity is observed with the isomeric imidazo[1,5-*a*][1,4]benzodiazepines, typified by midazolam (**41**). Midazolam is a highly potent compound possessing strong hypnotic, anxio-

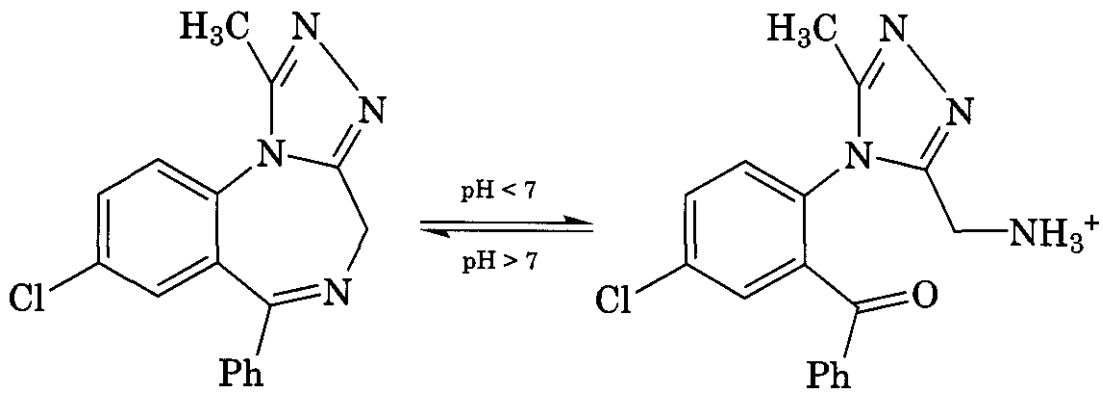


(41)

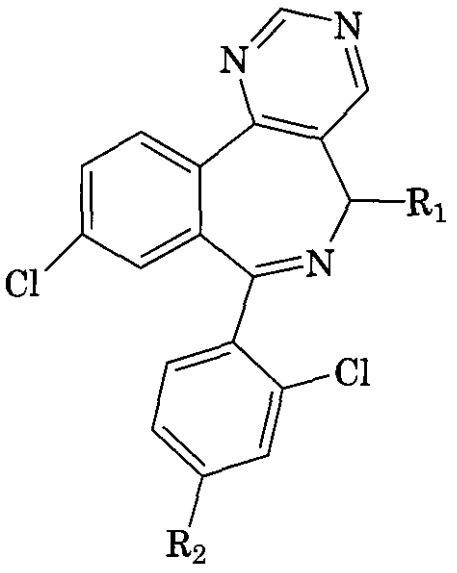
lytic, and memory-impairing effects. This activity profile has made midazolam (Versed) a popular choice as a premedication for surgical anesthesia.

Under acidic conditions many of the 1,2-heteroaryl fused benzodiazepines, including alprazolam (321) and triazolam (322), exist in the ring-opened form (e.g., 42, for alprazolam). Above pH 7 the reaction reverses to generate the benzodiazepine ring, meaning that this is the active form under physiological conditions.

The majority of 1,2-ring fused benzodiazepines maintain a nitrogen atom at position 1 of the benzodiazepine ring This nitrogen has been removed altogether to give a large number of 2-benzazepine derivatives, of which the 5H-pyrimido[5,4-*d*][2]benzazepine class shows the most interesting activity. Ro22-3245 (43) has a comparable BZR affinity to that of diazepam, but is up to eightfold more potent in animal anticonflict tests (323). The 9-chloro substituent is necessary for signifi-



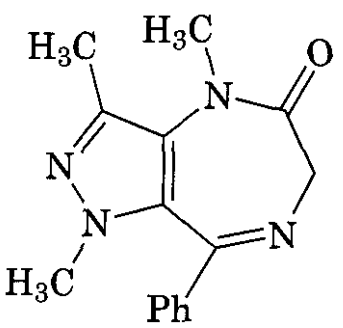
(5) **(42)**



(43) $R_1, R_2 = H$ (Ro22-3245)
(44) $R_1 = OH, R_2 = H$
(45) $R_1 = H, R_2 = OH$

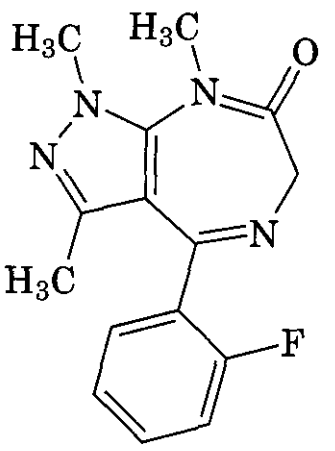
cant antianxiety activity, and the activity was potentiated by a chlorine or fluorine at the *ortho* position of the C-7 phenyl ring. Two of the metabolites of **Ro22-3245** are the 5-hydroxy (44) and the 4'-hydroxy (45) compounds. In keeping with established **benzodiazepine** SAR, (44) shows comparable activity to that of the parent compound and (**45**) is inactive (324).

5.2.3 **Heteroaryl[*e*][1,4]Diazepin-2-Ones.** A number of chemical classes with anxiolytic activity have been produced by replacement of the fused phenyl ring of the **1,4-benzodiazepines** with heterocycles. Ripazepam (**46**)

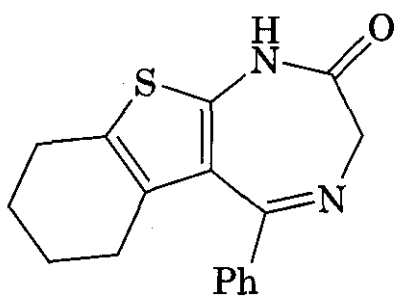


(46)

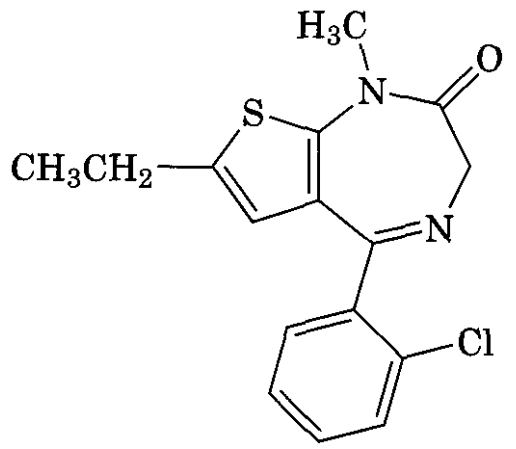
(**325**, 326) and zolazepam (**47**) (327) are among the most active members of a series of **6,8-dihydropyrazolo[3,4-*e*][1,4]benzodiazepin-2-ones**. Of the three possible isomeric thieno[1,4]diazepine-2-ones (**328**), only the **[2,3-*e*]** derivatives are reported to possess significant anxiolytic activity. This chemotype is **typified** by bentazepam (48) (329) and **clotiazepam** (49) (**330**), both of which showed **antianxiety** effects in human studies. **Lopirazepam** (**50**) appears to be the most interesting member of the pyridodiazepin-2-one family, and is said to have potentially useful **antianxiety** activity (331).



(47)

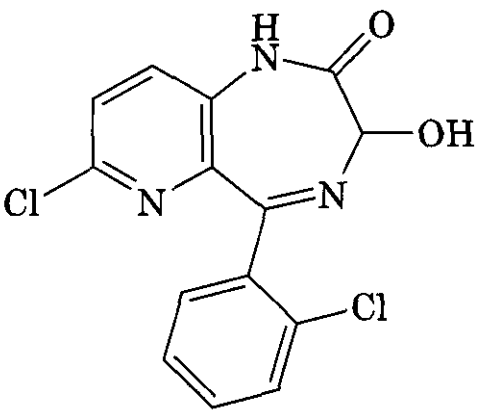


(48)



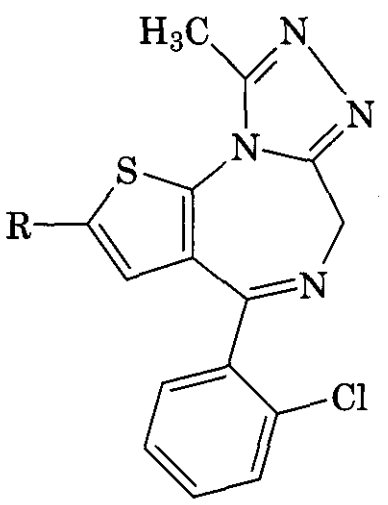
(49)

5.2.4 Bis-Heteroaryl[*a,f*][1,4]Diazepines. Not surprisingly, the A-ring modifications of the **benzodiazepin-2-ones** (Section 5.2.3) have been extended to many of the more potent **1,2**-ring fused benzodiazepines described in Section 5.2.2 to give a large number of **bis-heteroaryl** derivatives (**285**). Of the many variants



(50)

investigated, the thieno[3,2-*f*][1,2,4]triazolo-[4,3-*a*][1,4]diazepines, of which brotizolam (51)and Y7131 (52)are prominent examples, have the greatest biological activity. At least one substituent, usually alkyl, on the thio-



(51) R = Br
(52) R = Et

phene ring is required for significant anxiolytic activity, and any activity observed is enhanced with an *ortho* chloro or fluoro substituent on the C-6 phenyl ring (332). Brotizolam has been introduced as a hypnotic, and Y7131 (etizolam) is reported to have interesting anxiolytic activity (333, 334).

5.3 Non-Benzodiazepine Ligands at the BZR

Initially it was believed that the 1,4-benzodiazepine structure was an essential requirement for high affinity binding to the BZR, but a wide range of non-benzodiazepine chemotypes have since been shown to bind to the BZR with high potency, rendering the term "benzodiazepine receptor" no longer strictly appropriate. The major driving force behind the investigation of new ligands at the BZR has been the development of compounds that retain the therapeutic effects of the benzodiazepines, but are devoid of their unwanted side effects. This objective has been approached in two ways, through partial agonism and subtype selectivity.

5.3.1 Partial Agonism and Subtype Selectivity. The benzodiazepines act as full agonists (positive allosteric modulators) at the BZR, and they are able to induce maximal receptor response with low receptor occupancy. It has been suggested that anxiolysis requires less receptor occupancy than sedation (150) and other benzodiazepine side effects. In addition, a downregulation of receptors has been demonstrated upon prolonged treatment with full BZR agonists, a phenomenon that may underlie the development of tolerance and dependency. Based on this theory that different levels of receptor activation produce different clinical effects, compounds that act as partial agonists (i.e., compounds that produce less receptor activation than a full agonist at a comparable receptor occupancy) have become the focus of much attention (335). It is important to note that high *affinity* must be maintained in a partial agonist; only the functional activity is moderated. The intrinsic activity of BZR ligands may be determined in a number of ways. tert-Butylbicyclophosphorothionate (TBPS) is a ligand at the picrotoxinin side of the $GABA_A$ receptor whose binding affinity is modified in the presence of BZR ligands, and the TBPS shift (ratio of TBPS binding for the test drug to that of a known standard, such as diazepam) is used to estimate the intrinsic activity (336). This is a relative measurement: full agonists have a TBPS shift of 1; antagonists, 0; and inverse agonists have negative shifts. Partial agonists have values intermediate between 0 and 1 (337).

A second method describes intrinsic activity in terms of the GABA shift (338). This method relies on the bidirectional nature of the allosteric coupling between the BZR and the GABA site. Just as the activity of GABA at its receptor site is enhanced by ligands at the BZR, the affinity of BZR ligands for the benzodiazepine site is enhanced in the presence of

Table 9.3 Preferred Profiles of Partial Agonists and Profiles of Reference Drugs

	a1	a2	a3	α5	Reference
Preferred profile	<20%	>30%	>30%	<20%	344
Preferred profile	<10%	nd	40–>60%	nd[a]	345
Alprazolam (5)	326%	355%	787%	264%	127
Diazepam (1)	186%	290%	568%	157%	127
Lorazepam (4)	328%	233%	421%	181%	127
Bretazenil (83)	40%	37%	76%	64%	127

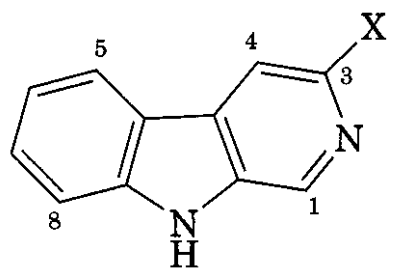
[a]nd, not disclosed,

GABA. This coupling, and hence affinity enhancement, is stronger for full agonists than for partial agonists, and is nonexistent for antagonists. Thus the GABA shift (ratio of IC_{50} without GABA/IC_{50} with GABA) is loosely predictive of the relative degree of intrinsic efficacy. This estimate is based on comparison with known full agonists such as diazepam, which has a GABA shift of 2.2–2.9. Accordingly, inverse agonists have values less than 1, full agonists have values of 2 or more, and partial agonists lie between 1 and 1.5 (339,340). A more accurate and meaningful assay is the direct measurement of the potentiation of GABA-induced chloride currents by a BZR ligand. This is determined in single oocytes expressing the desired recombinant receptor subtype, using a two-electrode voltage-clamp technique (341–343). A fixed concentration of GABA is applied (typically enough to produce 10–20% of the maximal current), and the potentiation of this current by the drug is measured over a full dose-response range. The maximal potentiation (%) and the EC_{50} value describe the efficacy and the potency, respectively. This assay directly measures the receptor activation by the ligand, unlike the TBPS and GABA shift assays that provide only relative efficacies.

A second approach to improve the side-effect profile has grown from the increasing knowledge of the diversity of $GABA_A$ receptor subtypes. Each of the four major diazepam-sensitive subtypes exhibits a distinct, although perhaps overlapping, pharmacology, anatomical distribution, and physiological function. Recent work with conditional knock-in animals strongly implicates the α1 and α2 subtypes in the mediation of the sedative and anxiolytic actions of diazepam, respectively (144–149). Accordingly, there has been an intensive effort to identify "anxioselective" compounds that selectively modulate GABA function at specific receptor subtypes. Although they are attractive proposals, partial agonism or subtype selectivity on their own may not be enough to produce the desired clinical profile. A given subtype (e.g., α2) may be associated with more than one clinical effect, depending on receptor activation, and so a full agonist selective for this subtype would be of little value. Similarly, a nonselective partial agonist may be unable to differentiate between effects requiring low activation of only one subtype. In recent times both these approaches have coalesced into the search for subtype-selective partial agonists, and this effort dominates the current medicinal chemistry and preclinical work in the area. With increasing knowledge of the physiological roles of $GABA_A$ subtypes guiding the subtype selectivity profile, the next challenge in this approach is the determination of the "right amount" of partial agonism. Based on preclinical animal models, some companies have revealed their preferred partial agonist profile for anxiolytics (344,345) (Table 9.3). It is clear from Table 9.3 that these profiles are dramatically different from those of the classical benzodiazepines, and are more selective than the prototypical partial agonist bretazenil.

An exhaustive review of the non-benzodiazepine ligands at the BZR is beyond the scope of this chapter, and the following section focuses only on those templates that have produced advanced anxiolytic candidates. In addition to the compounds described below, a wide range of small molecules bind with high affinity to the BZR. The reader is invited to consult recent reviews on this topic for more extensive coverage (346–351).

5.3.2 β-Carbolines. The isolation of the potent BZR ligand p-CCE (53) from human

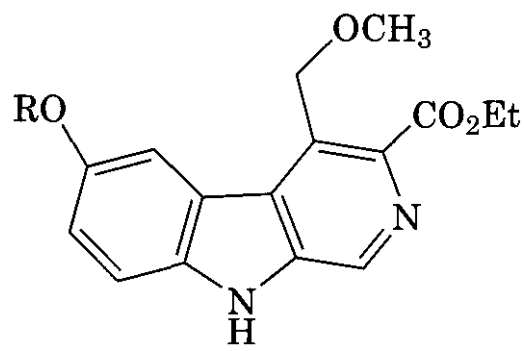


(53) X = CO_2Et (β-CCE)
(54) X = CO_2CH_3 (β-CCM)
(55) X = CO_2-*n*-Pr
(56) X = alkoxy

urine in 1980 (352) prompted a great deal of interest in p-carbolines as possible endogenous ligands for the BZR. Although the presence of β-CCE in urine was subsequently shown to be an artifact of the extraction procedure (353), interest in this series continued because of their high potency and unusual pharmacology. β-CCE and β-CCM (54) were the first compounds to exhibit inverse agonism at the BZR, and p-carbolines have become the most widely studied class of BZR ligands after the benzodiazepines themselves.

The pyridyl nitrogen atom is essential for affinity and is presumed to participate in a critical hydrogen bonding interaction with the receptor (354,355). A free NH at position 9 is also required, as affinity is drastically reduced in the N-methyl analogs (356). Computer modeling suggests that this loss of affinity results from a negative steric interaction between the N-methyl group and the receptor rather than the removal of a hydrogen bond donor site. Most of the early p-carbolines were inverse agonists but, through appropriate modification of substituents, compounds spanning the entire continuum of intrinsic activity up to full agonist can be obtained. Increasing the size of the 3-substituent increases the intrinsic activity. Thus extending the ester alkyl group from methyl (54) to n-propyl (55) changes the profile from inverse agonist to antagonist (357). The β-carboline ester group is hydrolytically labile and it can be replaced with alkoxy groups (56) to enhance stability and water solubility (358). The rank order of potency is methoxy < n-butyloxy < ethoxy < n-propyloxy (356,357). Branching is tolerated α to the oxygen, but not β or γ to the oxygen, suggesting a well-defined and limited lipophilic pocket in the receptor (354). In the C-4-substituted p-carbolines this change causes a greater loss in affinity, possibly because of a steric interaction between the C-3 and C-4 groups and consequent disruption of hydrogen bonding at N-2 (356). A 3-amino substituent abolishes binding affinity (356) because of insufficient lipophilic interaction with the receptor and the existence of the amino group in the imino tautomer, which would prevent hydrogen bond formation with N-2.

The size of the substituent at position 6 also has a major impact on the intrinsic efficacy, but less so on receptor affinity. Thus compound (57) has an IC_{50} value of 0.5 n*M* for

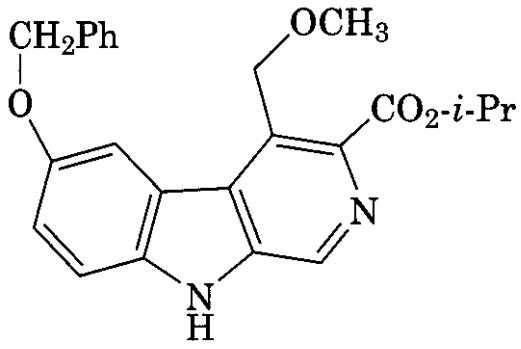


(57) R = CH_3
(58) R = Pr
(59) R = CH_2Ph

the BZR, antagonized the anticonvulsant effects of diazepam, and showed no anticonvulsant effects of its own, consistent with an antagonistic profile (359). The corresponding n-propyl compound (58) has an IC_{50} value of 8 n*M*, and at 20 mg/kg showed comparable efficacy to that of diazepam (2.5 mg/kg) in the elevated plus maze. Further, (58) showed no myorelaxation activity and antagonized diazepam-induced myorelaxayion, indicating partial agonist properties. Increasing the size of the 6-substituent further gives ZK93423 (59), which behaves as a full agonist. Modeling studies suggest that the influence of the 6-substituent on intrinsic efficacy derives from its full occupation of a lipophilic pocket (L3) of the receptor. The partial agonist (58) only partially occupies this region compared to the full agonist (59). Moving the 6-benzyloxy group from the 6- to the 5-position lowers the activ-

ity to that of a partial agonist (360, 361), further underlining the importance of this interaction in the agonist response. The C-5 phenyl ring of the benzodiazepines is also believed to confer agonist activity by interacting with the L3 region (see Fig. 9.5). Agonist and inverse agonist β-carbolines probably bind at the same site, but have slightly different interactions with various receptor regions. The presence of a methoxyrnethyl substituent at C-4 is proposed to favor the "agonist" alignment of the ligand through formation of a hydrogen bond with the receptor. This substituent does indeed confer agonist properties, as evidenced by the anxiolytic, anticonvulsant, and myorelaxant activities of (59). The corresponding 4-ethyl analog, which lacks this hydrogen bonding capability, is five- to 10-fold weaker at the BZR and is devoid of any such agonist activity (356). The affinities of numerous β-carboline derivatives at recombinant receptor subtypes reveal nonselective binding to a1, α2, α3, and a5 subtypes (356). In the absence of efficacy measurements at these subtypes, the clinical relevance of even the best selectivity (~20-fold) remains questionable.

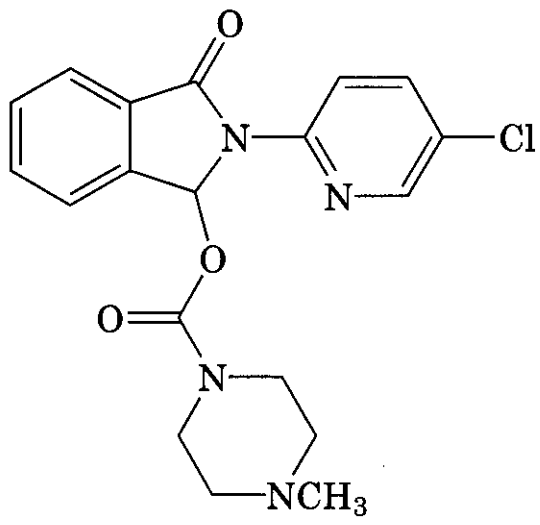
The conversion of the ethyl ester of (59) to an isopropyl ester gives abercarnil (60) the



(60)

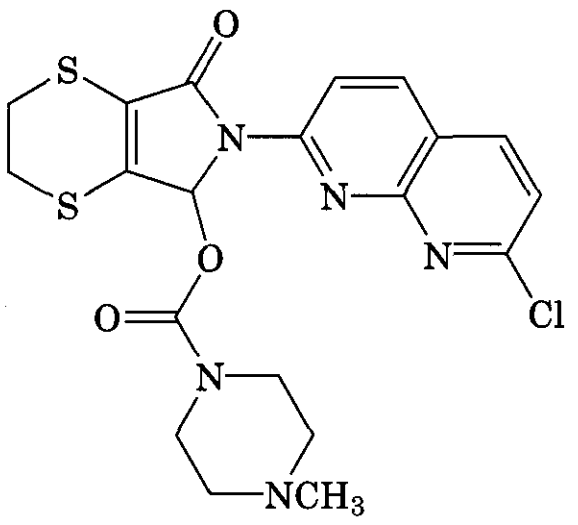
most clinically advanced β-carboline to date. Abecarnil is a potent BZR ligand (IC_{50} = 0.8 n*M*) and binds with equal affinity to all diazepam-sensitive subtypes (362). Abercarnil has a GABA shift of 1.2–1.4, compared to 2.8 for diazepam, indicating partial agonist character. In preclinical models, abercarnil showed anxiolytic (362) and anticonvulsant (363) activity, and appeared to be devoid of significant sedative and myorelaxant properties (362, 364). In addition, abercarnil exhibited little alcohol interaction (362) or tendency to induce symptoms of withdrawal, tolerance, and dependency (364–367), although the latter symptoms have been observed in other studies (368). In clinical trials, abecarnil was found to be well tolerated (369) and effective in GAD at doses of 3–9 mg per day with minimal side effects or discontinuation symptoms (370), although the therapeutic effect diminished after 6 weeks of dosing (371). At higher doses, however, side effects typical of a full agonist were observed including sedation, amnesia, and unsteady gait. Subsequent studies have shown that abercarnil potentiates GABA-induced chloride currents at the a3 subtype to the same degree as the full agonist flunitrazepam (372), and to the same degree as diazepam in rat cerebellar slices (373). These results suggest that abercarnil possesses neither the optimal subtype selectivity nor the optimal partial agonist properties for a side effect-free anxiolytic, and its clinical development has been terminated.

5.3.3 Cyclopyrrolones. The cyclopyrrolones were among the earliest non-benzodiazepine structures shown to have high affinity for the BZR. The best-known member of this series is zopiclone (61), which is available as a



(61)

hypnotic (Imovane, Rhone-Poulenc Rorer) in Europe and Canada. Several members of the cyclopyrrolone family have progressed into clinical trials as anxiolytics, including suriclone (62), pazinoclone (63), and pagoclone (64). Cyclopyrrolones are highly potent BZR ligands with affinities often in the subnanomolar range. The pyrrolone moiety is common



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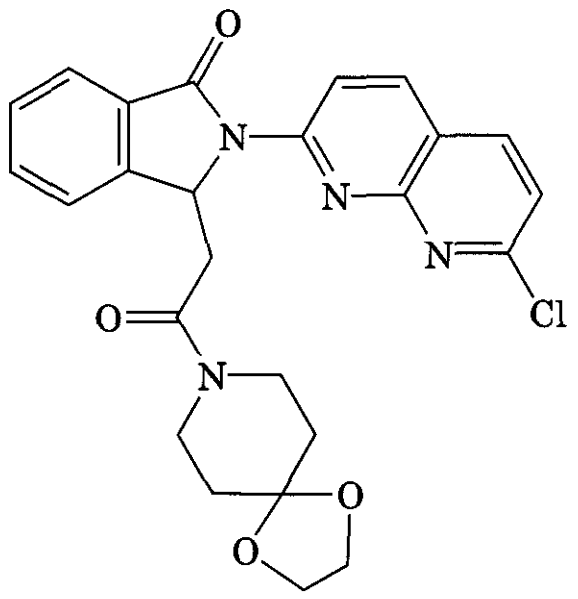

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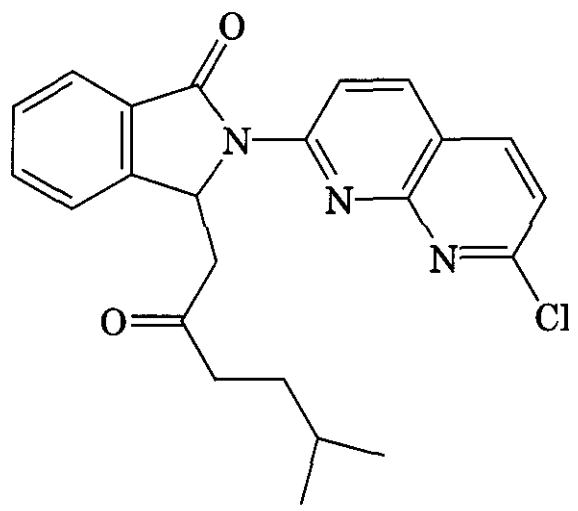


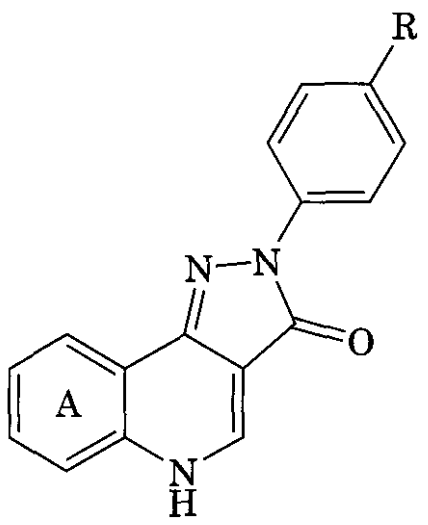
(64)

to all these structures, and modifications of the N-2 and C-3 substituents give rise to differences in intrinsic efficacy.

Suriclone (62) has shown positive anxiolytic efficacy in human trials (374) at daily doses of 0.3–1.2 mg, but was withdrawn from development before approval. Pazinaclone (DN-2327, Takeda Industries, (**63**)) has an IC_{50} value of 0.4 n*M* for the BZR and exhibits a number of properties that indicate partial agonist character. It is active in a range of, conflict and nonconflict anxiety models with **med's** ranging from 2.5 to 10 **mg/kg**, po (375), and these anxiolytic effects could be blocked by flumazenil. (63) showed little propensity to cause sedation or muscle relaxation (375,376) and did not affect sleep structure in cats at doses up to 20 **mg/kg** (377). Although (63) showed less memory impairment than that of diazepam (378), the two drugs were able to substitute for each other in drug discrimination tests (379). In Phase I trials, however, pazinaclone (single doses of 2–32 mg) produced similar deficits in psychomotor performance and short-term memory to alprazolam (0.25–2 mg) (380–382). It appears that pazinaclone may not possess the necessary selective partial-agonist profile to improve significantly on the benzodiazepines, and its development has been discontinued.

Pagoclone (64) is the most clinically advanced of the anxiolytic cyclopyrrolones, and has reached late-stage clinical trials for GAD and panic disorder. (64) has an IC_{50} of 0.4 n*M* at the BZR and shows no selectivity among the benzodiazepine-sensitive subtypes (383). Pagoclone shows ***in vivo*** anxiolytic activity at low oral doses (~0.1 **mg/kg**). In animal studies, (64) is 30 times more potent than diazepam in anxiety models but 10 times less potent in sedative paradigms (384). Although intrinsic efficacy data are not available, pagoclone's behavioral profile is that of a partial agonist, and accordingly it is claimed to have a greater separation of anxiolytic effects and side effects than those of standard anxiolytics (385). Phase I studies showed pagoclone to be well tolerated and revealed no significant effects on sleep, psychometric, or withdrawal measures (384, 386, 387). Drowsiness, a characteristic benzodiazepine action, was one of the most commonly reported side effects, however, especially at doses exceeding 1 **mg/day**. Clinical studies to date confirm pagoclone's effectiveness in panic disorder. At daily doses of 0.3 **mg/kg** (0.1 **mg/kg**, tid), (64) reduced panic attacks by 40–73% (387–389).

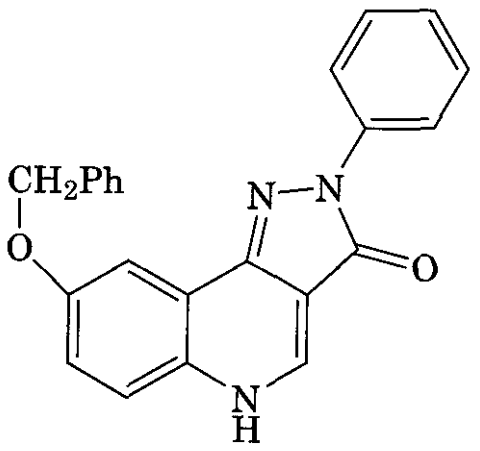
5.3.4 Pyrazoloquinolines. The pyrazoloquinoline series, originally developed at Ciba-Geigy, provides highly potent BZR ligands that span the entire continuum of intrinsic efficacy from full inverse agonist to full agonist. The freely rotating phenyl ring at N-2 plays a critical role in determining the intrinsic efficacy of the molecule, and minor changes in this ring induce dramatic shifts in efficacy. In anticonvulsant and anticonflict tests CGS8216 (**65**) behaves



(65) R = H
(66) R = OCH_3
(67) R = Cl

as an inverse agonist, and the 4-methoxy analog (**66**) as a weak partial agonist/antagonist. Replacing the methoxy group with chlorine gives CGS9896 (**67**), a more potent partial agonist (390) that displays robust anxiolytic and anticonvulsant activity in animal models (391). The relative intrinsic activities of these compounds are also indicated by their GABA shifts (392). Moving the methoxy- or chloro-substituent from the *para* to the meta position of the phenyl ring has little effect on affinity but increases the degree of agonism, as indicated by the change in the GABA shifts (from 1.07 and 0.94 to 1.7 and 1.1, respectively). The ortho-substituted derivatives are weak or inactive, possibly because of disruption of the coplanar arrangement between the phenyl ring and the pyrazoloquinoline nucleus. The N-2 phenyl ring may be replaced by thiophene (393). These compounds are potent BZR ligands ($K_i < 3$ nM), and different thiophene isomers possess different levels of intrinsic efficacy. Also, the introduction of substituents of increasing size (H, methyl, ethyl, n-propyl, *n*-butyl) in the thiophene ring leads to a shift in efficacy from inverse agonist, through partial agonist, to agonist (394). Incorporation of unsubstituted heterocycles at N-2 (pyrazine, pyridine) maintains affinity and gives inverse agonist compounds (395). The influence of the N-2 aromatic ring on the intrinsic activity is in keeping with molecular modeling studies, which place this ring in a lipophilic binding pocket (L2) responsible for agonist activity (396) (see Fig. 9.5) and identify N-1 and the C-3 carbonyl group as the key hydrogen bond acceptors. Differential occupation of the L2 region by various substituents is proposed to produce varying degrees of agonism.

In the A-ring, small substituents (e.g., OH, OCH_3, Cl, ethynyl) are tolerated at all four positions (395, 396), and have less impact on intrinsic activity than that of substituents in the N-2 phenyl ring. Large substituents are tolerated only at the 8-position, as indicated by the high affinity of the benzyloxy derivative (**68**) (396). The other three positional isomers



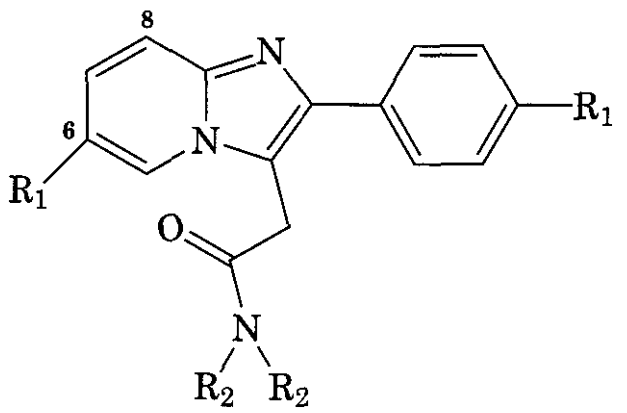
(68)

are inactive. The large benzyloxy group also confers agonist character on the molecule, which has a GABA shift of 1.3 (compared to the unsubstituted analog 65, a full inverse agonist).

Pyrazoloquinolines frequently possess subnanomolar affinity for the BZR, but show no subtype selectivity in their binding (396). Unlike the benzodiazepines, some pyrazoloquinolines also bind to the diazepam-insensitive receptor subtypes (a4 and α6). Although several compounds show partial agonist and anxioselective profiles (e.g., CGS9896) in preclinical models, there is no evidence to suggest any

subtype selectivity in these compounds. Poor solubility is another drawback that has hindered their development.

5.3.5 Imidazopyridines. The best known member of the imidazopyridine class is **zolpidem** (**Ambien, 69**), the world's most popular

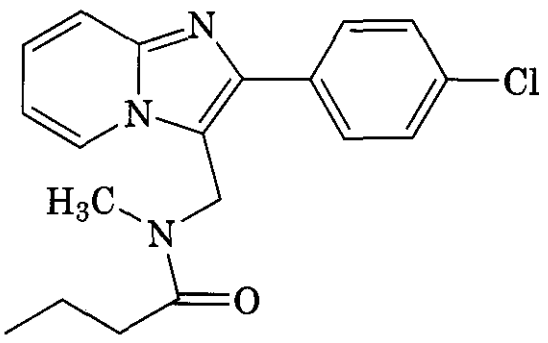


(69) $R_1 = CH_3$, $R_2 = CH_3$
(70) $R_1 = Cl$, $R_2 = n$-Pr

hypnotic drug. The close analog **alpidem** (70) is the most prominent anxiolytic of this class. **Alpidem** is active in a number of anxiolytic models but is reported to be less effective than classical benzodiazepines such as diazepam and alprazolam (397, 398). This difference may be **a** consequence of the subtype selectivity of the imidazopyridines. Both **alpidem** and zolpidem are selective for the $\alpha 1$ subtype of the BZR, having intermediate affinity at a2 and a3 sites and low affinity for a5 sites. The original anxiolytic imidazopyridine research strategy was based on al-selective ligands (**399**), but it is now believed that the a2 subtype mediates the anxiolytic effects of BZR ligands and so $\alpha 1$ selectivity is clearly a **nonoptimal** profile for an anxiolytic. The picture is further complicated by the fact that **alpidem** has an affinity for the peripheral benzodiazepine receptor (PBZR) comparable to that for the central BZR (K_i = 1–28 and 0.5–7 nM, respectively) (400). Further, the BZR antagonist flumazenil completely blocks the anxiolytic and anticonvulsant effects of benzodiazepines, but only partially blocks the same actions of **alpidem** (401). Thus it is possible that the anxiolytic actions of **alpidem** may result from a combination of direct action at the central BZR and an indirect action at the peripheral BZR (402–404). **Alpidem** progressed into clinical trials, where it was shown to be an efficacious anxiolytic, but was withdrawn from development because of the emergence of liver toxicity.

Selectivity for the CBZR over the PBZR is highly sensitive to small structural changes in the imidazopyridine nucleus and zolpidem, despite its similarity to **alpidem**, has no affinity for the PBZR. At the 6-position of **imidazopyridines**, chlorine is the optimal substituent and is fivefold more potent (IC_{50} = 86 nM) than the unsubstituted analog at the CBZR (405). The order of CBZR potency for 6-substituents is **Cl** > Br > CH, > I > H ≫ OCH, ≫ NO,, but PBZR affinity is largely unaffected by the nature of this substituent. Incorporation of substituents at the 8-position of the imidazopyridine nucleus eliminates affinity for the CBZR but maintains, and may enhance, affinity for the PBZR (405, 406). A 4-chloro substituent in the C-2 phenyl ring potentiates the affinity of imidazopyridines for both the CBZR and PBZR (**406**). In the **acetamide** side chain, the optimum N-alkyl groups are methyl, ethyl, and **propyl** (**399**). Increasing the length of the alkyl chain, or the introduction of branching, reduces affinity. Secondary **amides** are generally more potent than tertiary amides, and replacement of the amide moiety of zolpidem and **alpidem** with esters of similar size virtually eliminates affinity for both BZRs (405). Removal of the methylene linker between the carbonyl group and the imidazo ring, or extension of this linker to two carbons, dramatically reduces affinity at both BZRs (405). This suggests that there is a critical spatial relationship between the two hydrogen bond acceptor sites (the imidazole nitrogen and the carbonyl oxygen) that cannot be disrupted. Indeed, most BZR receptor models demand the formation of two hydrogen bonds at specific locations.

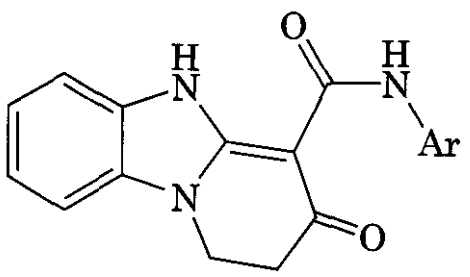
Molecular modeling studies have suggested that the acetamide side chain of **alpidem** is more important for PBZR binding than for CBZR binding, whereas the hydrogen bonding capability of the imidazole nitrogen is more important for CBZR than for PBZR **affinity** (407). The $\alpha 1$ selectivity of imidazopyridines has also been rationalized through **conformational** analysis and molecular calculations (408). Zolpidem is selective for **BZ1**($\alpha 1$) sites, whereas saripidem (71) has equal affinity for



(71)

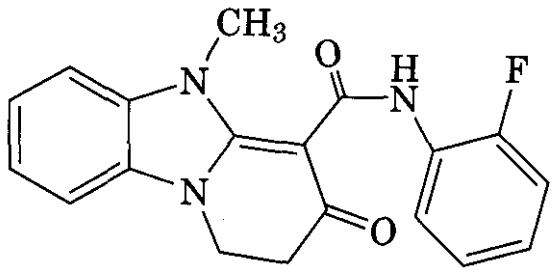
BZ1 and BZ2 (α2, α 3, and α5) sites. A two-dimensional NMR analysis (409) found that zolpidem possessed only one family of conformations, in which the acetamide moiety was positioned 2 Å above the plane of the imidazopyridine ring. This is consistent with the crystal structure of alpidem (399). Saripidem also exists in this conformation, which is presumed to impart BZ1 affinity. However, saripidem may exist in a second conformation in which the acetamide group is in the plane of the imidazopyridine system, and this conformation may confer BZ2 affinity.

5.3.6 Pyridobenzimidazoles. The pyridobenzimidazoles (72) (for a review, see Ref.



(72)
(73) Ar = 2-OCH_3-Ph
(74) Ar = 2-Cl
(75) Ar = Ph
(76) Ar = 2-F-Ph
(77) Ar = CH_2Ph
(79) Ar = 4-pyridyl

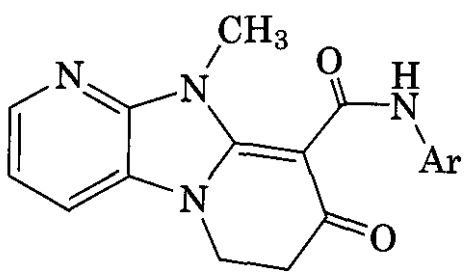
410), developed at the R.W. Johnson Research Institute, are a relatively new class of benzodiazepine receptor ligands that range in efficacy from antagonist to full agonist. SAR studies on the amide portion of the molecule showed that only the secondary amides are active, and that substituents in the 4-position of the phenyl ring generally lower the BZR affinity (340). The effect of 2-substitution is variable: the 2-methoxy compound (73) showed poor affinity (IC_{50} = 990 n*M*), the 2-chloro derivative (74) was comparable (12 n*M*) to the unsubstituted anilide (75), and the 2-fluoro derivative (76) was the most active (1.7 n*M*). (76) also possessed partial agonist character, as evidenced by its GABA shift of 1.2, and was active in conflict models (1mg/kg, ip). The benzylamide (77) was also active (IC_{50} = 12 n*M*), but with a GABA shift of 4.6 (compared to 2.2 for diazepam, a full agonist) it is clearly a full agonist itself. Methylation of the imidazole nitrogen in the 2-fluoro compound (76), to give (78), enhances the affinity (0.47



(78)

n*M*), raises the intrinsic efficacy (GABA shift = 1.6), and greatly enhances the in *vivo* potency (conflict med = 0.03 mg/kg, ip) (340). In both the NH and N-methyl cases, the introduction of the *ortho* fluoro substituent decreased the GABA shift relative to that of the unsubstituted anilide. Efforts to improve the poor oral bioavailability of this series led to RWJ-38293 (79), but only at the expense of in *vitro* (IC_{50} = 160 n*M*) and in *vivo* (conflict med = 3 mg/kg, ip and 10 mg/kg, po) potency (411).

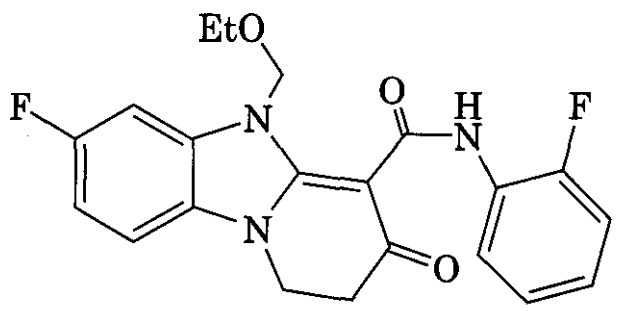
On the other hand the A-ring pyridyl derivatives (e.g., 80) have substantial water solubil-



(80)

ity and promising oral anxiolytic activity (conflict med = 3 mg/kg, po) (412). Substitution of the phenyl A-ring at the 6 and/or 7 positions with chlorine or fluorine provided the most active compounds in the conflict model. The sum of these SAR investigations has led to the

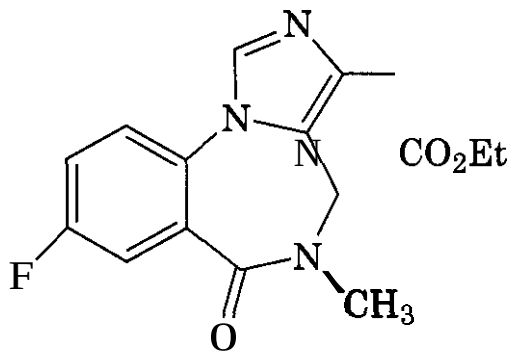
identification of RWJ-51204 (**81**), which combines favorable substitutions in the A-ring, the anilide, and at N-5, and is currently in



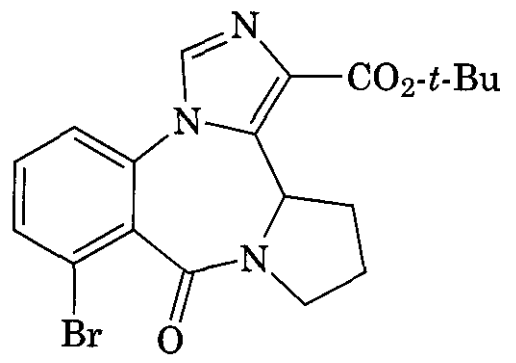
(81)

clinical development as an anxiolytic (410). (81) is an extremely potent partial agonist (IC_{50} = 0.3 n*M*, GABA shift = 1.17), with a preferential affinity for a1 and a2 subtypes. Because the reported GABA shift data are based on a heterogeneous population of native $GABA_A$ receptors it gives no information on subtype selectivity in terms of efficacy, and the clinical relevance of the binding selectivity remains unclear. (81) is reported to be highly active in animal models of anxiety with little tendency to induce sedation, motor incoordination, or withdrawal symptoms. In a monkey conflict model, RWJ-51204 was 10 times less potent (ED_{50} = 0.4 mg/kg, po) than lorazepam, alprazolam, and clonazepam, and was equipotent with diazepam. When side effects are considered, however, RWJ-51204 has a significantly wider therapeutic window than these reference benzodiazepines. Phase I studies in healthy volunteers revealed no adverse effects and clinical development is continuing. A safe and cost-effective large-scale synthesis of this compound has been reported (413).

5.3.7 Imidazobenzodiazepines. The imidazobenzodiazepines constitute a large and important series of high affinity BZR ligands that are structurally distinct from the classical benzodiazepines, and have produced inverse agonists, antagonists, and partial agonists. This family is exemplified by flumazenil (82) and bretazenil (**83**). Flumazenil is a potent BZR antagonist that is used clinically (Romazicon) to reverse benzodiazepine-induced sedation and for the management of benzodiazepine overdose. Tritiated flumazenil is also widely used as the radioligand in BZR binding



(82)

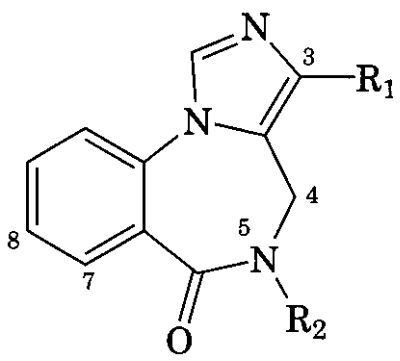


(83)

assays. Bretazenil is the most significant anxiolytic candidate to emerge from this series. (83) is a potent BZR ligand (K_i = 2 n*M*) and has a GABA shift of 1.4, compared to 2.9 for diazepam, indicating partial agonist character (414). This has also been demonstrated by *in vitro* experiments relating BZR receptor occupancy to the potentiation of GABA-stimulated chloride flux (415). Bretazenil was found to induce a maximal potentiation of Cl^- current of only 20%, compared to a 50% potentiation for diazepam. Diazepam produced 25% potentiation at 35% receptor occupancy, but bretazenil did not reach 25% potentiation even at receptor saturation. Electrophysiological measurements further confirm bretazenil's partial agonist character (Table 9.3) (127), although these data also reveal a lack of subtype selectivity. Comparison of *in vivo* receptor occupancy and behavioral effects shows that bretazenil requires higher occupancy than that of diazepam to produce comparable anxiolysis (416). Because sedation and myorelaxation require even higher receptor occupancy, it might be expected that these effects would be minimized with bretazenil. Indeed. in animal models bretazenil is an effective anxiolytic that induces less sedation than that of classical benzodiazepines (416), and can an-

tagonize the sedative and myorelaxant effects of benzodiazepines. Clinical trials showed bretazenil to be an effective anxiolytic, but also revealed side effects including sedation, amnesia, and performance impairment. The development of bretazenil was discontinued in 1997, and the results with this compound suggest that nonselective partial agonism alone may not be sufficient to provide a side effect-free anxiolytic.

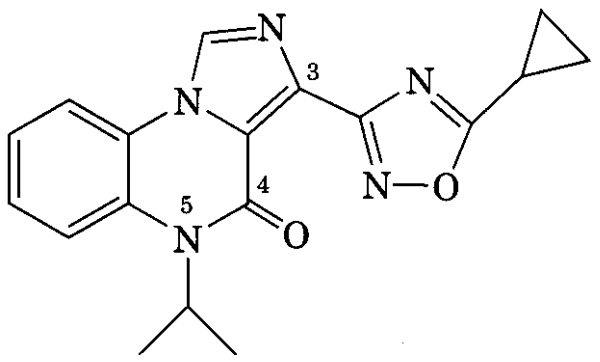
Many members of the imidazobenzodiazepine class display potent affinity for diazepam-insensitive (DI) subtypes (α4, α6) as well as the diazepam-sensitive (DS) subtypes (α1, α2, α3, α5) of the BZR, and numerous SAR studies have examined the requirements for DS/DI selectivity. Overall, these studies suggest that the binding sites for DS and DI subtypes are similar and that selectivity is governed by structural modifications at positions 3, 7, and 8 of the imidazobenzodiazepine structure (84). DS subtypes are responsible for the typical benzodiazepine actions (anxiolysis, sedation, etc.), and DI subtypes have been implicated in mediating some of the behavioral effects of ethanol (417–419).



(84)

At the N-5 position, a methyl group appears to be optimal for DS binding, given that the NH, N-propyl, and *N*-aryl analogs are inactive (420). The N-benzyl derivatives do show comparable affinity (2–14 n*M*) to that of the *N*-methyl derivatives, however. Cyclization of the N-5 substituent onto the 4-position [as in bretazenil (83)] is tolerated and tends to increase intrinsic activity relative to that of the N-methyl analogs. The presence of an ester at the 3-position is essential for potent DS and DI binding (421), and the DI subtypes are much more sensitive to the size of this group than are the DS subtypes (422–424). A variety of heterocycles have been investigated as potential replacements for the metabolically labile ester group, of which the 3- and 5-alkyl-1,2,4-oxadiazole derivatives appear to possess the best affinity (420). As a general rule, the oxadiazole derivatives have a higher intrinsic efficacy than that of the corresponding ester analogs. The SAR of the 3-substituent closely parallels that of the 3-substitent in the β-carbolines, suggesting that this region of both templates occupies the same binding pocket (420). The substitution pattern in the phenyl ring also has implications for DS/DI affinity and selectivity. Substitution with electron-withdrawing groups (e.g., halogens) at position 7 enhances affinity for the DS subtype more than for the DI subtype, whereas 8-substitution preferentially enhances affinity for the DI subtype (421–423). The net result of 7-substitution is therefore an enhancement of DS selectivity. Substitution at C-8 and C-9 of the phenyl ring dramatically reduces affinity for DS and DI isoforms (422). Among the four DS subtypes, a lipophilic substituent at C-8 has been found to confer selectivity (425), but no selectivity for subtypes implicated in anxiolysis (α2) has been reported.

5.3.8 Imidazoquinoxalines. Panadiplon (85) is the most advanced member of this se-

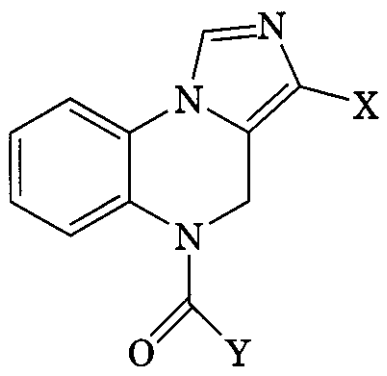


(85)

ries, having reached phase I trials as an anxiolytic. (85) behaves as a partial agonist *in vitro* and *in vivo* (337), is active in numerous anxiety models, and appears to possess fewer side effects than those of typical benzodiazepines, at least in animal models. However, the drug was withdrawn from the clinic because of evidence of dose-dependent hepatotoxicity, preventing a full assessment of its efficacy and side-effect profile in humans. This toxicity is

mediated by cyclopropane carboxylic acid, which is formed by metabolism of the oxadiazole moiety (337).

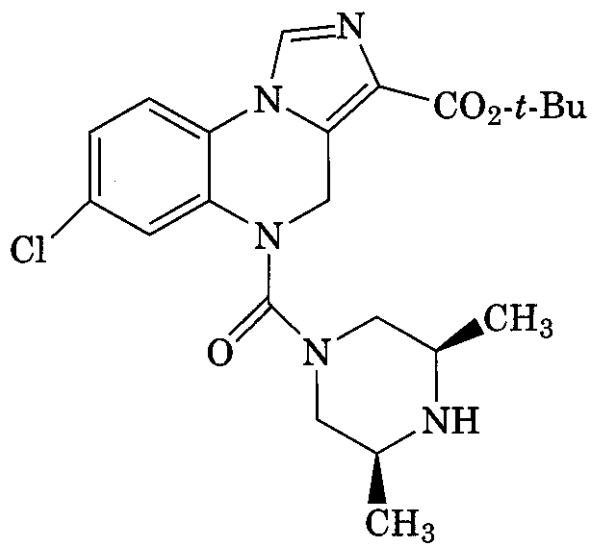
Given the toxicity associated with the oxadiazole at the 3-position of panadiplon, a number of alternative substituents were investigated at this site of the imidazoquinoxalin-4-ones. Esters have similar potency to, and lower intrinsic efficacy than, the oxadiazoles, but are less stable toward hydrolysis. Aryl groups at position 3 reduce affinity by up to 24-fold, and this deleterious effect is exacerbated by substitution of the aromatic ring (426). In this series there was little, if any, affinity or efficacy selectivity between $\alpha 1$ and $\alpha 3$ and any selectivity observed was in favor of $\alpha 1$ (426,427). In a closely related series of imidazoquinoxalines the carbonyl group now resides on the N-5 nitrogen, giving urea (**86**),



(86) Y = NR_2
(87) Y = alkoxy
(88) Y = alkyl, aryl

carbamate (87), and amide (88) derivatives. In this series the 3-oxadiazole and 3-aryl derivatives were equipotent. The 3-phenyl derivatives were active in physical-dependency models, whereas the oxadiazoles generally were not, implicating the 3-phenyl group in the mediation of this side effect (426). The nature of the N-5 substituent has little impact on affinity (generally <15 nM) but strongly influences intrinsic efficacy. The amide and carbamate derivatives tended to be full agonists, the degree of agonism increasing with the size and lipophilicity of the carbamate group (337, 428), whereas the urea analogs usually gave partial agonist profiles. In addition, the urea derivatives showed more consistent *in vivo* activity (anticonvulsant) than that of the others. As with the quinoxalin-4-one series, no useful subtype selectivity was observed in terms of affinity or efficacy (TBPS shift). Nevertheless, several compounds in this series showed desirable anxiolytic efficacy (Geller-Cook conflict and Vogel punished licking models) with minimal ethanol potentiation, although early tests showed physical dependency to be a common liability (337).

A curious finding among certain imidazoquinoxalines is a discrepancy in the efficacy results from the TBPS shift assay and direct measurement of chloride currents, the latter method consistently giving higher intrinsic efficacy (426). Further investigation revealed that some compounds have a biphasic dose-response curve in the chloride current assay. For example, (89) potentiates chloride current



(89)

at concentrations up to 1 μM and reduces it thereafter, returning to baseline at around 10 μM (429). Given that the TBPS assay was run at 5 μM and the chloride current assay at 0.5 μM, the latter method gives a higher, but more relevant, result. This phenomenon is limited specifically to the piperazine ureas and thus appears to be intimately related to the presence of a basic nitrogen in the urea moiety. Other ureas, amides, carbamates, and the **imidazoquinoxalin-4-ones** show no biphasic properties. This phenomenon may result from the interaction of ligands such as (89) with two binding sites at the BZR, one high affinity and one low affinity (426). Such behavior led to the hypothesis that ureas such as (**89**) would antagonize their own agonistic effects at high concentrations and so limit their abuse liability, although acute dependency studies have failed to support this theory.

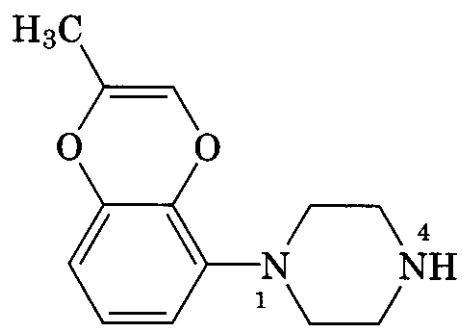
Molecular modeling studies show that the imidazoquinoxalin-4-ones, despite their obvi-

ous structural similarity to the imidazobenzodiazepines (e.g., **83**), adopt quite different three-dimensional conformations (428). For the imidazoquinoxalines (e.g., 86) the N-5 substituent lies out of the plane of the rigid tricyclic nucleus, and may occupy the same agonistic receptor pocket as the C-5 phenyl of the benzodiazepines and the piperazine side chain of zopiclone (**61**). Such an orientation accounts for the strong influence of this group on the intrinsic efficacy.

5.4 5HT-1A Ligands

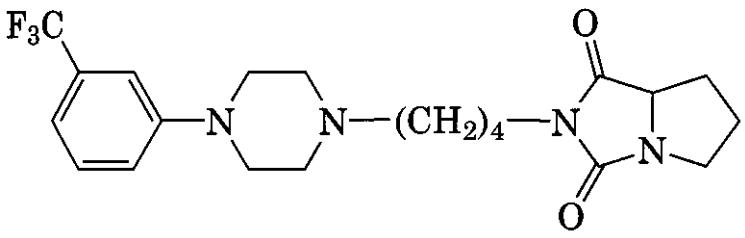
A number of chemical classes are known to provide potent 5HT-1A ligands (174, 430) including the aminotetralins (e.g., 8–OH DPAT), indolealkylamines (serotonin mimics), ergolines (e.g., LSD), and arylpiperazines. In terms of potential antianxiety agents, by far the most important of these chemotypes are the arylpiperazines, also referred to as the azapirones. In 1986 buspirone (9) became the first azapirone to be approved for the treatment of GAD, and tandospirone (16) has recently been launched for the same indication, although only in Japan. Other azapirones to reach late-stage clinical trials (431) for anxiety are gepirone, flesinoxan, and ipsapirone, although development of the latter two compounds has been discontinued. Buspirone itself has potent affinity (IC_{50} = 31 n*M*) for the 5HT-1A receptor, but also has significant dopamine D2 receptor binding (IC_{50} = 250 n*M*) (432). Indeed, buspirone was originally investigated as an antipsychotic and the dopaminergic properties were initially believed to underlie its unexpected anxiolytic effects. Many azapirone compounds possess significant D2 and adrenergic a1 activity in addition to the desired 5HT-1A affinity, and much of the medicinal chemistry work has been directed toward improving the selectivity for the 5HT-1A receptor. Modifications of the azapirone structure can be broken down into three regions of the molecule: the N-1 aromatic ring, the linker between N-4 and the imide, and the imide substructure.

5.4.1 Azapirones. Numerous SAR studies have shown that the N-1 aryl substituent plays a key role in determining affinity and selectivity for the 5HT-1A receptor, both among simple phenyl piperazines and azapirone derivatives. 1-Pyrimidinylpiperazine, the parent piperazine of buspirone, has negligible affinity for 5HT-1A receptors and 1-phenylpiperazine has only weak affinity (~500 n*M*) (433, 434), but appropriate substitution of the phenyl ring produces phenylpiperazines with potent 5HT-1A activity. Substitution at the 2- or 3-position is generally favorable for 5HT-1A affinity, but 4-substitution is not, and a Cl or CF_3 substituent at the 3-position enhances affinity twofold over that of 1-PP (434). 2-Methoxyphenyl is consistently one of the most active substitution patterns among simple aryl piperazines. A methoxy group at the 2- or 3-position increases affinity by three- and 1.5-fold, respectively, relative to that of 1-phenylpiperazine, and the 4-methoxy derivative is inactive (433). The 2,3-dimethoxy compound is weaker (1 μM) than either of the monomethoxy derivatives, but constraining the methoxy groups into a ring system such as benzodioxan increases affinity dramatically (40 n*M*). The 2-methylbenzodioxene derivative (90) is more potent still, with an affinity (5



(90)

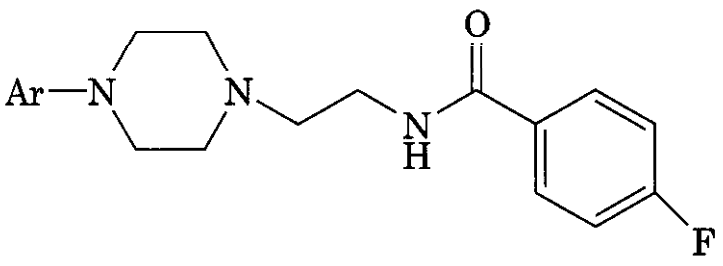
n*M*) comparable to that of &OH-DPAT. 2,3-Fusion of other rings such as furan, thiophene, and phenyl gave similarly active compounds. These results indicate that the azapirone N-4 substituent is not required for potent 5HT-1A affinity. The phenylpiperazine SAR also applies to N-4 substituted azapirones, with 2- and 3-substitution favored and 4-substitution being detrimental to receptor affinity (435, 436). In a bicyclohydantoin series, the 3-trifluoromethyl derivative (91) was the only compound to show any selectivity (27-fold) over that of α1 receptors (435). A positive relationship between the van der Waals volume of the meta substituent and the 5HT-1A/α1 selectivity has been noted (437). In



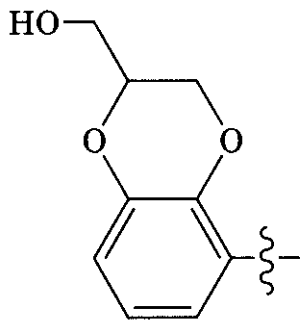
(91)

agreement with this observation, computational analysis of a series of arylpiperazines predicts that the 5HT-1A active site is able to accommodate bulky groups at this position, whereas the a1 receptor is not (438).

In a series of flesinoxan analogs, the 5HT-1A/D_2 selectivity was found to be predominantly modulated by the N-1 aryl substituent rather than by the N-4 substituent (439). The 2-methoxy derivative (92) exhibited high af-



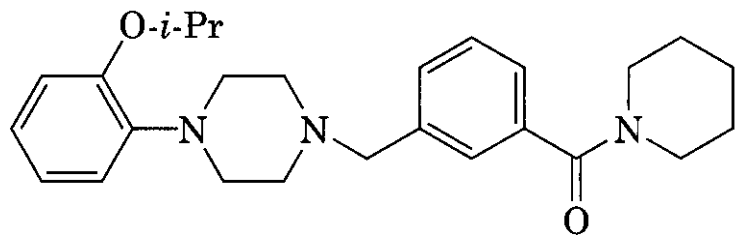
(92) Ar = 2-OCH_3-Ph



(93) Ar =

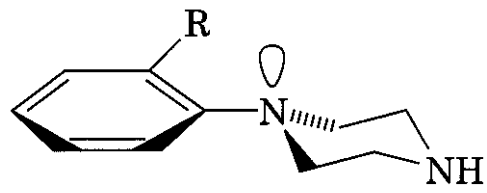
finity at both receptors (<5 n*M*) and was only fivefold selective for 5HT-1A. Constraining the methoxy group into a fused ring such as furan or benzodioxan enhanced 5HT-1A affinity and reduced D_2 affinity, thus enhancing the 5HT-1A/D_2 selectivity. The selectivity was improved further by substitution of the dioxan ring at the 2-position with a hydroxymethyl group to give flesinoxan itself (**93**), which has excellent affinity for 5HT-1A receptors (1.7 n*M*) and is 82-fold selective for 5HT-1A over D2 sites. Flesinoxan reached Phase III trials having shown efficacy in GAD and depression, but studies in panic disorder revealed a lack of efficacy and a possible panicogenic effect (440). The development of flesinoxan was recently terminated.

Mazapertine (94) is a highly potent, nonselective 5HT-1A ligand that also possesses high



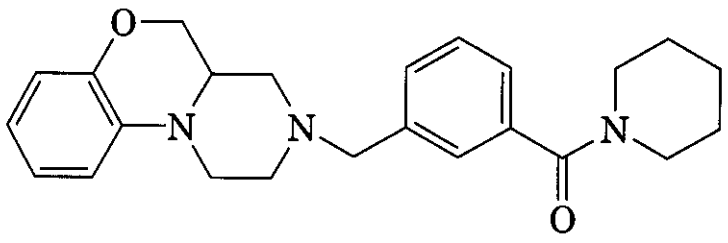
(94)

affinity for D_2, D_3, D_4 and a1 receptors (K_i values of 3 n*M*). In accord with other studies, 2-alkoxyphenyl derivatives were found to be the most potent 5HT-1A ligands in the mazapertine series (436). Increasing the size of this substituent from OCH_3 to OEt, to O-*i*Pr had little effect on the 5HT-1A or α1 affinity, but enhanced D_2 affinity, thus leading to diminished 5HT-1A/D_2 selectivity. Electron-withdrawing groups (CF_3, Cl, F, CN) at the 2-position also showed high 5HT-1A and α1 affinity (K_i < 20 n*M*), but with some selectivity over D_2 receptors (K_i > 60 n*M*). At the 3-position, the same substituents showed greater selectivity over D_2 (>300-fold) and a1 (>200-fold) sites. Molecular modeling suggests that a favorable conformation for phenyl piperazines, including those with single *ortho* substituents, places the phenyl and piperazine rings in a near coplanar arrangement (plane angle ~30°), with the nitrogen lone pair almost perpendicular to this plane, as in (95) (433,441–



(95)

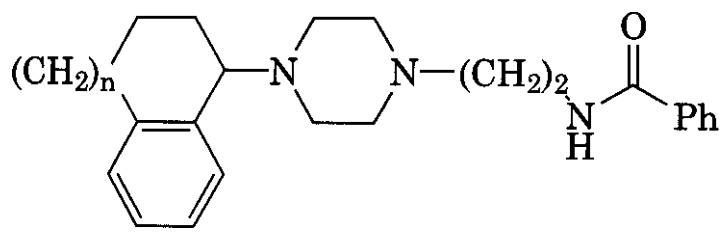
443). This is in agreement with the X-ray crystal structure of mazapertine, which shows the isopropoxyphenyl ring to be 35" out of plane with the piperazine ring (436). The notion that this represents the active conformation of azapirones is supported by the potent affinity (12 n*M*) of the tricyclic mazapertine analog (96) (444), in which the phenyl and piperazine rings are constrained in a near coplanar ar-



(96)

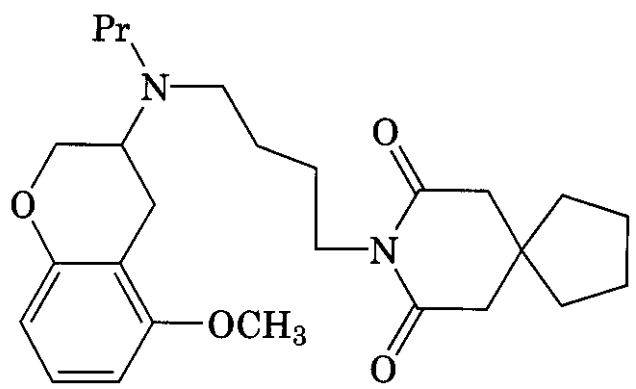
rangement by the $-CH_2O$-bridge. In contrast to mazapertine, (96) showed minimal affinity for $\alpha 1$, D_2, D_3, or other 5-HT receptors, suggesting that the multireceptor activity of arylpiperazines results from their ability to adopt a variety of active conformations, and that selectivity for the 5HT-1A receptor can be achieved by forcing the arylpiperazine into a coplanar conformation.

Replacement of the pyrimidine ring of buspirone with other heterocycles, such as pyrazine, pyridazine, or tetrazole, results in a loss of affinity (445). The N-1 position need not be directly attached to an aromatic ring, as shown by the benzocycloalkyl piperazines (97)



(97)

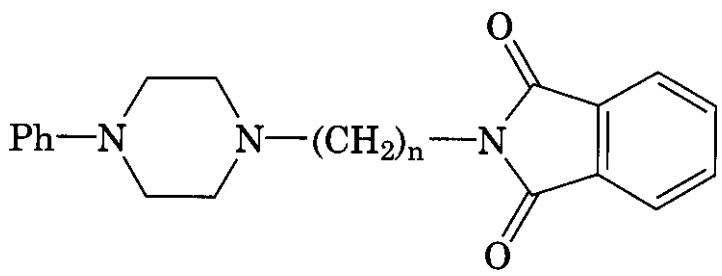
(446). Optimum affinity was obtained when the cycloalkyl ring was six membered. The (−) enantiomers were 10–50 times more potent than their (+) counterparts, and exhibited superior selectivity (>100-fold) over $\alpha 1$ and D_2 receptors. Elements of the tetralin and azipirone pharmacophores are combined in alnespirone (98), which has advanced to phase



(98)

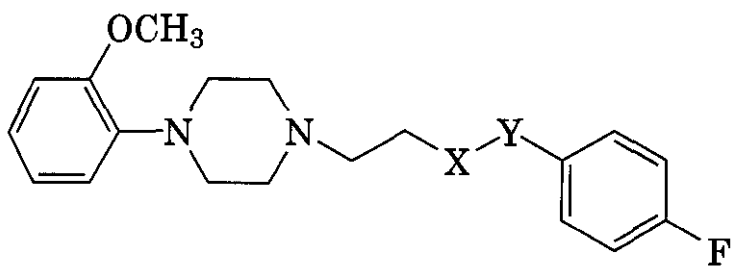
II trials as an anxiolytic. SAR studies showed that the 5-methoxy group, a tertiary amine, and a four carbon linker provide the optimum compromise between 5HT-1A affinity and selectivity vs. adrenergic and dopaminergic sites (447). The aminobenzopyran moiety, which is derived from the prototypical 5HT-1A ligand 8-OH-DPAT, contributes more to the 5HT-1A affinity, whereas the carbon linker has a greater impact on selectivity than on affinity. The (+) enantiomers are consistently more potent and selective than the (−) antipodes. Alnespirone binds with higher affinity (0.3 n*M*) and selectivity than that of either buspirone or ipsapirone (447, 448), is a full agonist at pre- and postsynaptic 5HT-1A sites, and is active in animal models at low doses (<4 mg/kg, ip) with no sedative effects.

Overall, the SAR data for the linker between N-4 and the imide group show that this region does not provide any interactions with the receptor and merely acts as a spacer. Some studies point to a role for this region in the modulation of the intrinsic activity of the arylpiperazines and of their selectivity for the 5HT-1A receptor. The general observation is that potency is optimized with a four carbon linker and drops off significantly as the chain is shortened, as exemplified by the phthaloyl derivatives (99) (434). This preference is further underlined by the tetralin derivatives (97), in which the benzamidoethyl derivatives were the only ones to be active (446). In the bicyclohydantoin series, the chain length was an important factor in determining the 5HT-1A/$\alpha 1$ selectivity (435,449).



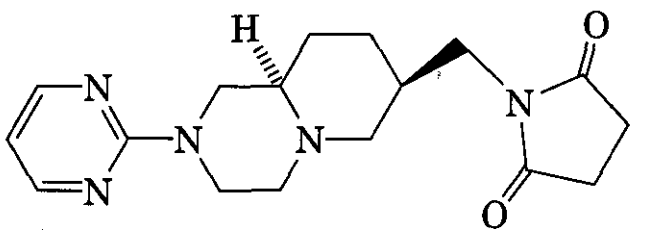
(99) n = 2, Ki > 10 μM
n = 3, Ki = 200 nM
n = 4, Ki = 10 nM
n = 5, Ki = 8.5 nM

In the flesinoxan series (100), replacement of the amide NH with a methylene to give the butyrophenone derivative (101) had no effect



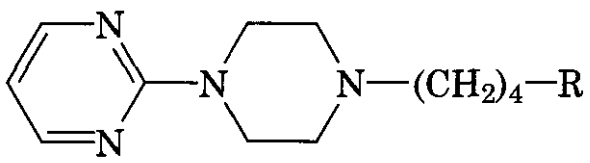
(100) X = NH, Y = (C = O)
(101) X = CH_2, Y = (C = O)
(102) X = CH_2, Y = CHOH
(103) X = CH_2, Y = CH_2

on 5HT-1A or D_2 affinity, indicating that the NH does not contribute a hydrogen bond interaction (439). Likewise, reduction of the carbonyl group in (100) to the alcohol (102) or the methylene (103) had only minor effects on the affinities for both receptors. In contrast to its minimal effects on receptor affinity, the nature of the linker has a significant impact on agonist/antagonist character (450). The amide (100) is a full agonist, whereas the buytrophenone analog (101) is a partial agonist, and the n-butyl compound (103) has a predominantly antagonistic profile. These studies maintained the length of the spacer at 4 atoms and showed that changes in the electrostatic or conformational nature of this chain had no effect on 5HT-1A or D2 affinity, but did modulate the functional characteristics of the compounds. The linker need not be a linear chain, as shown by the high affinity of mazapertine (**94**), which incorporates a phenyl ring (**436**), and sunepitron (**104**), which contains a bicyclic piperazine linker. Sunepitron is an agonist at 5HT-1A autoreceptors and also has appreciable affinity (35 n*M*) for a2 adrenergic receptors, where it acts as an antagonist (451). (**104**) was effective in the Vogel conflict test with an med of 1.78 mg/kg, ip, and a maximal effect greater than that of gepirone and comparable to that of diazepam (452). The combination of 5HT-1A agonist and a2 antagonist activities may account for the enhanced efficacy relative to gepirone. Sunepitron is currently in phase II clinical studies for anxiety.

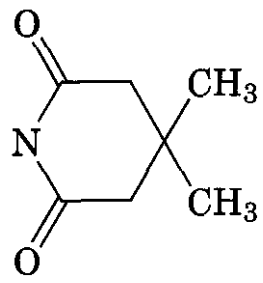


(104)

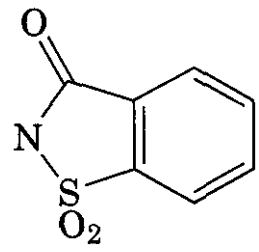
The substituent at the terminus of the linker chain is tolerant of a number of changes without a loss in potency. Indeed, the nature of this moiety provides the only point of differentiation between buspirone (**9**), tandospirone (**16**), gepirone (**105**), ipsapirone (**106**), and zalospirone (**107**), five of the most clini-



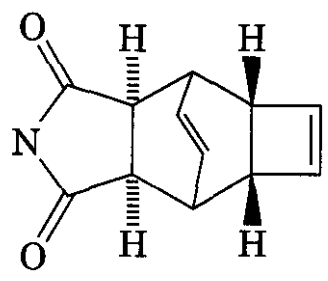
(105) R =



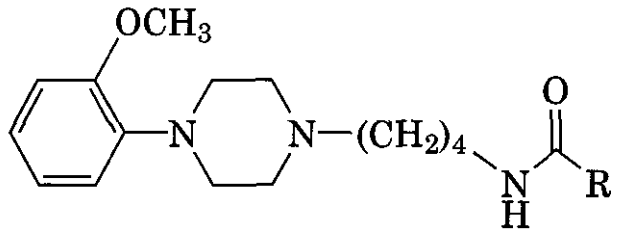
(106) R =



(107) R =



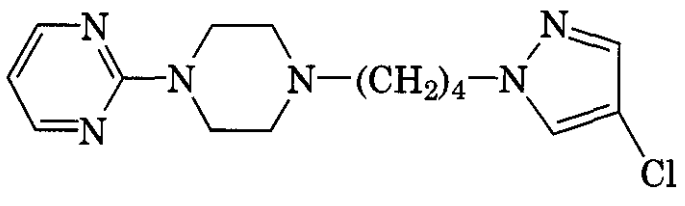
cally advanced azapirones. In a series of buspirone analogs, a gradual enhancement of affinity was seen as the log P of the molecule was increased through variation of the imide (445), indicating a positive relationship between the lipophilicity of this group and 5HT-1A receptor affinity. The imide of buspirone may be replaced by hydantoin derivatives, as in (**99**) (435, 449), diketopiperazines (437), phthaloyl groups, amides, or even a simple phenyl ring (434, 439) without disruption of affinity. In the amide series, a preference for a lipophilic group was suggested by the fact that the acetamide analog (**108**) was 25 times



(108) R = CH_3, Ki = **800** nM
(109) R = **Ph,** Ki = **1.3** nM

less potent at the 5HT-1A receptor than was the benzamide (109). Replacement of the phenyl ring in this series with thiophene maintains 5HT-1A and D_2 affinity, but substitution for more polar rings, such as furan, pyrrole, pyridine, or pyrimidine, reduces affinity 10–100 times at both receptors. Evidently the 5HT-1A and D_2 receptors both favor nonpolar, lipophilic groups at this position, and consequently little 5HT-1A selectivity is observed. This conclusion was supported by an excellent correlation of the measured log P of the compounds with their 5HT-1A and D_2 affinity (correlation coefficients of 0.96 and 0.93, respectively) (439).

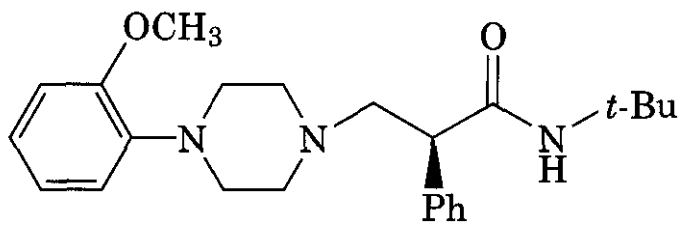
Lesopitron (**110**), a buspirone analog with a chloropyrazole as the terminal moiety, has moderate affinity for the 1A receptor (101 n*M*), where it acts as a pre- and postsynaptic agonist (453,454). In an important distinction



(110)

from buspirone, (110) has negligible activity at adrenergic and dopaminergic sites. In animal models of anxiety, such as the mouse light/dark box and the rat social interaction test, (110) was effective at low doses (<0.5 mg/kg, ip) and produced no sedative effects (453). Drug metabolism studies in rats suggest that, like buspirone, lesopitron may be subject to extensive first-pass metabolism (455). Phase I studies showed lesopitron to be well tolerated in human subjects up to 50 mg/day (456). In a lorazepam controlled phase II trial in patients with GAD, lesopitron (4–80 mg/day), showed signs of efficacy, although a clear endpoint was not achieved.

In the field of 5HT-1A receptor ligands agonists and partial agonists abound, but pure antagonists remain scarce. The first such compound to become available was WAY-100135 (**111**)(457), which incorporates a lipophilic

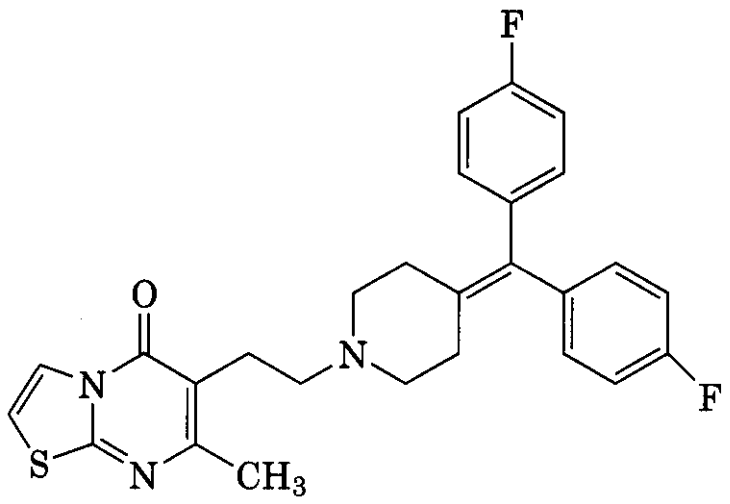


(111)

tert-butyl amide in the position normally occupied by the imide. The affinity resides primarily in the (*S*) isomer, which is 30 times more potent than its enantiomer. This compound is highly selective for 1A receptors, with negligible affinity for various dopamine, adrenergic, or 5HT sites, and is an antagonist at pre- and postsynaptic 5HT-1A receptors.

5.5 5HT-2 Ligands

Activity at the 5HT-2 receptor is an important feature of many atypical antipsychotic drugs (e.g., risperidone, olanzapine, sertindole), but the body of knowledge on 5HT-2 ligands in anxiety is more limited (173). Nonselective 5HT-2 antagonists, such as ritanserin (**112**), mianserin (**113**), and ketanserin (**114**), have been shown to produce anxiolytic effects in less than half of the preclinical studies conducted (202). The clinical data for ritanserin in human subjects are likewise inconclusive (458, 459). The mixed results obtained with



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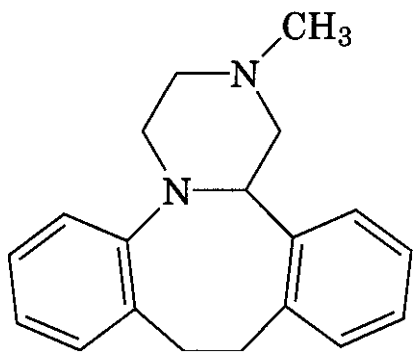

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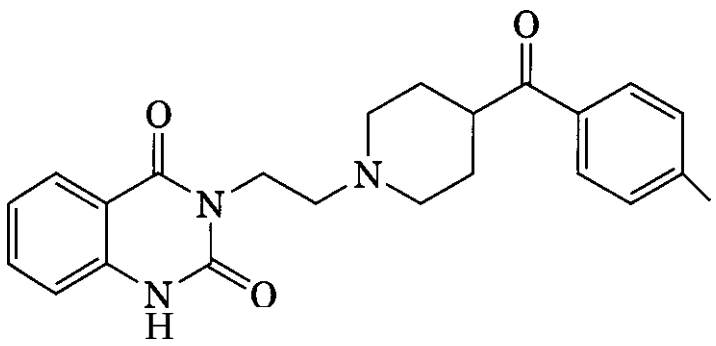
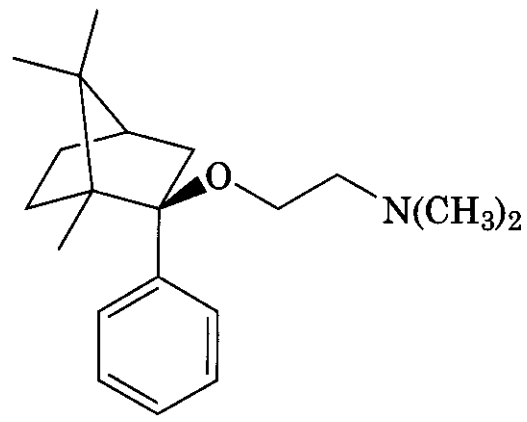


(114)

these nonselective compounds may reflect the fact that the various 5HT-2 receptor subtypes (2A, 2B, 2C) play different, perhaps even opposing, roles in the modulation of anxiety. Deramciclane (115) has high affinity for 5HT-2A (K_i = 18 nM) and 2C (K_i = 30 nM) receptors with antagonistic properties at both sites (460, 461). It is reported to elicit anxiolytic effects in animal models and to possess minimal sedative or muscle relaxant side effects (462). Although the preclinical behavioral data are promising, a true assessment of deramciclane's anxiolytic potential awaits the completion of ongoing clinical trials (463). Eplivanserin (116) is one of the few anxiolytic



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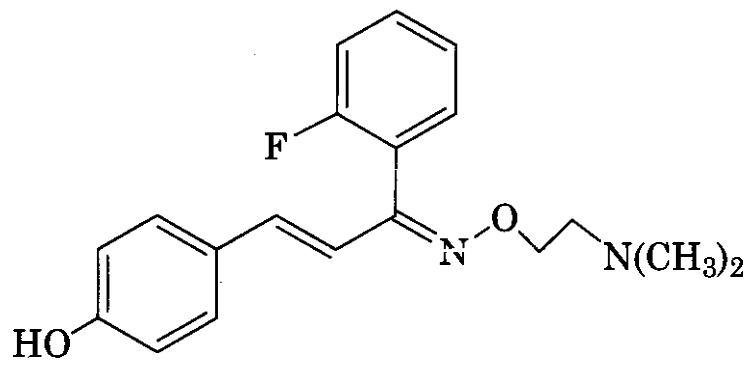
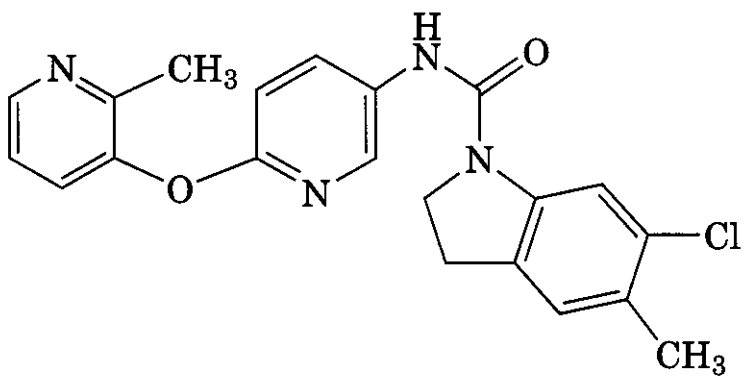


(116)

candidates to exhibit some preference between 5HT-2 subtypes, having 20-fold selectivity for the 5HT-2A (IC_{50} = 5.8 nM) over the 5HT-2C receptor (464). Eplivanserin showed anxiolytic activity in the elevated plus maze in rats (465) as well as in phase I human trials (167). Clinical anxiety studies with this compound appear to have been terminated, however. SB-242084 (117) (466) is the most selec-

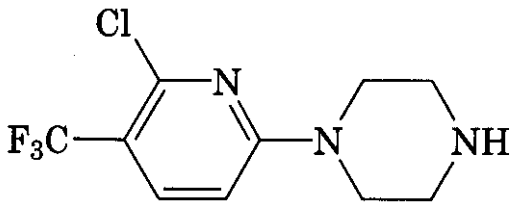


(117)

tive 5HT-2C antagonist known, having 160- and 100-fold selectivity over 5HT-2A and 5HT-2B receptors, respectively (467–470). Ligand-receptor modeling studies suggest that steric differences among the receptor subtypes may underlie this selectivity (469). (117) shows anxiolytic activity in several animal

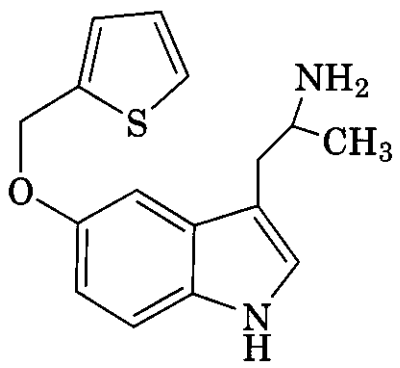
models including the Geller-Seifter and social interaction paradigms (470).

In addition to the 5HT-2 antagonists described above, some agonists have also shown anxiolytic effects. Org-12962 (118) is a pyri-



(118)

dylpiperazine derivative with agonist activity at 5HT-2A and 2C receptors that is undergoing clinical investigation for anxiety and depression. In a DPAG stimulation model of panic in rats, (118) was reported to have antipanic efficacy intermediate between that of clonazepam and fluoxetine (471). The 5HT-2B agonist BW-723C86 (119), whose structure is

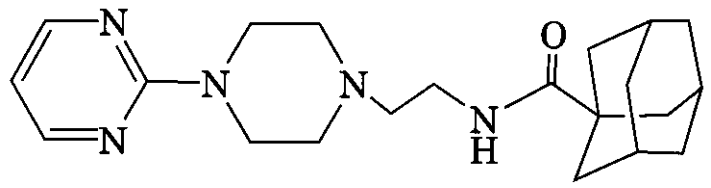


(119)

based on that of serotonin, showed anxiolytic activity in conflict models as well as in the rat social interaction test, with the magnitude of effect in the latter test approaching that of chlordiazepoxide (472,473). The observed anxiolytic effect was blocked by a 5HT-2B/2C antagonist, but not by a selective 5HT-2C antagonist, implicating the 5HT-2B subtype in the production of anxiolysis.

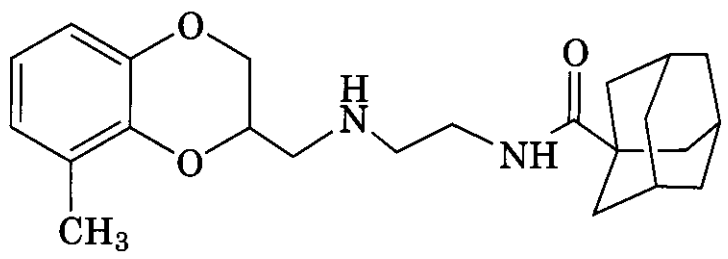
Overall, the utility of 5HT-2 antagonists in the treatment of anxiety remains in question, given the mixed history of these compounds in animal and human studies. The fact that 5HT-2 agonists and antagonist have both been reported to show anxiolytic effects leaves the relative roles of the 5HT-2 subtypes in anxiety unclear. A fuller understanding of the contributions of the 2A, 2B, and 2C receptors to anxiety is clearly desirable, but the 5HT-2 receptors form a very closely related group (475) and this objective has been hampered by the lack of subtype selective ligands.

A number of compounds with mixed 5HT-1A agonist and 5HT-2 antagonist properties have been investigated, seeking to combine the anxiolytic effects of both mechanisms (476). Molecular modeling studies have been used to study the receptor interactions that may contribute to 1A and 2A affinity among a series of buspirone analogs (477). Adatanserin (120) is a modified azapirone derivative with



(120)

affinity for 5HT-1A sites ($K_i = 1$ nM), where it acts as a partial agonist, and 5HT-2 sites ($K_i =$ 66 nM) where it acts as an antagonist (478–480). It is an effective anxiolytic in a range of anxiety models (481,482) predictive of clinical anxiolysis. In a pigeon conflict model adatanserin was the only mixed 5HT-1A agonist/5HT-2 antagonist to show significant effects, and elicited larger anxiolytic-like effects than those of either a 5HT-1A agonist or a 5HT-2 antagonist alone (481). HT-90B (121) is an-



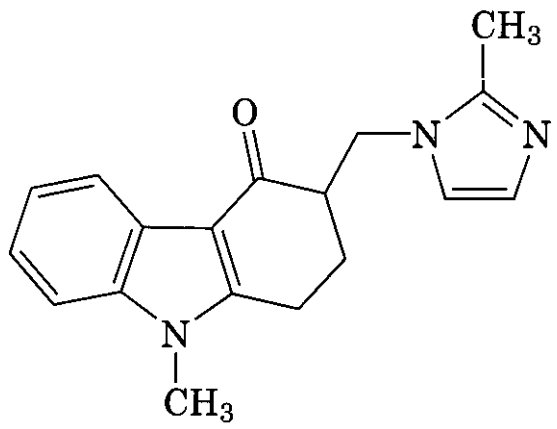
(121)

other mixed 5HT-1A agonist/5HT-2 antagonist that shows anxiolytic, as well as antidepressant, activity in preclinical models (483). The development of adatanserin and HT-90B has been discontinued.

5.6 5HT-3 Ligands

The main clinical action of 5HT-3 antagonists (484) is antiemesis, and several compounds have achieved considerable commercial suc-

cess in this indication. It has also been suggested that 5HT-3 antagonists may have useful antianxiety properties (209,485). The vast majority of clinical data in this respect concerns ondansetron (**122**) (486), a widely pre-



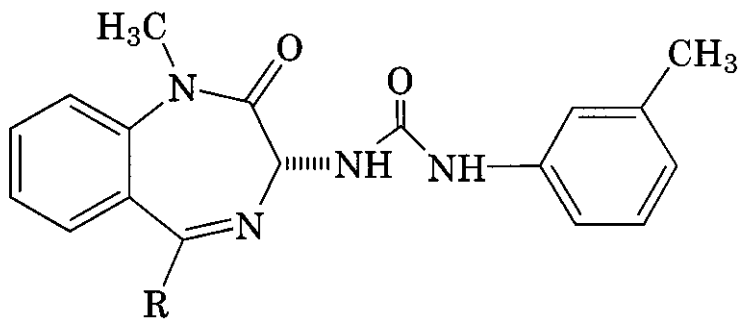
(122)

scribed antiemetic. In one placebo controlled trial (487,488) comparing ondansetron (1or 4 mg, tid) with diazepam (2 mg, tid), a statistically significant effect was observed, with the low dose being slightly more effective than the higher dose. Other studies (489,490) failed to demonstrate any significant effect in patients with GAD, and a study in panic disorder patients was inconclusive (491). In preclinical models the anxiolytic activity of 5HT-3 antagonists is highly variable (208), and consistent activity is seen only in a limited range of unconditioned models such as the light/dark exploration test. Ondansetron showed anxiolytic-like effects in only 63% of the reported preclinical studies (202), mainly in those based on exploration. The antianxiety activity of ondansetron, and of 5HT-3 antagonists in general, is marginal at best and this group appears unlikely to provide an advance in the treatment of anxiety.

5.7 Neuropeptide Receptor Ligands

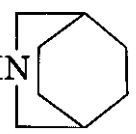
5.7.1 CCK-B Receptor Antagonists. Cholecystokinin (CCK), a 33 amino acid peptide, was isolated in 1968 (492), and since that time there has been great interest in developing ligands that interact with this important neuropeptide. The early CCK ligands were themselves peptide derivatives (493,494) and were thus of little value as drug candidates, particularly for CNS applications such as anxiety. The discovery of the first nonpetide CCK antagonist, the natural product asperlicin, in 1985 (495) shifted the medicinal chemistry focus from peptides to small molecule antagonists (496,497). CCK-B receptors are the dominant isoform in the brain, and antagonists at this subtype are potential anxiolytics. Many CCK-B antagonists also modulate gastric acid secretion through peripheral CCK-B (gastrin) receptors.

The most significant structural class derived from the asperlicin structure is the 1,4-benzodiazepines, exemplified by L-365,260 (**123**). The most striking aspect of the SAR in



(123) R = Ph

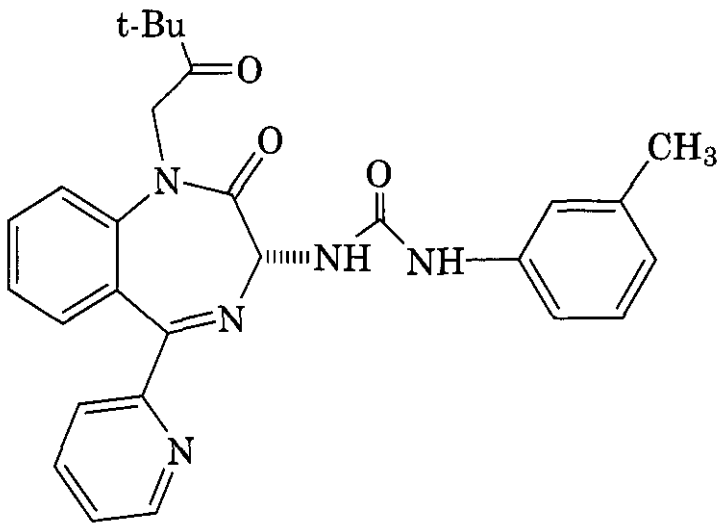
(124) R = HN



this series is the profound influence of the stereochemistry at C-3 on the binding profile: the (*R*) configuration is essential for potent and selective CCK-B affinity, the (*S*) configuration provides potent ligands selective for the CCK-A receptor. In healthy volunteers L365,260 was shown to relieve anxiety induced by the CCK agonists CCK-4 (498) and pentagastrin (499), although other studies failed to show efficacy on chronic dosing (500). The inconclusive evidence for the anxiolytic efficacy of (**123**) may be ascribed to its extremely low aqueous solubility and resultant formulation issues (501). Consequently, a major objective in this area became the enhancement of physical properties, by incorporation of acidic or basic solubilizing groups. Incorporation of acidic functions (e.g., carboxylic acid, tetrazole) in place of the methyl group at the 3-position of the ureido phenyl ring (502) maintained, or enhanced, CCK-B affinity (IC_{50} = 0.1–6 n*M*) and selectivity (500- to 10,000-fold), and increased aqueous solubility

by three orders of magnitude. However, in contrast to L-365,260, these and other acidic derivatives had very low brain penetration (503, 504), which precluded their development as anxiolytics. Replacement of the C-5 phenyl ring in (**123**) with cyclohexyl improves the CCK-B potency (30-fold) and selectivity (70-fold) (505). This modification also offered the possibility of improving solubility through the corresponding cyclic amine analogs, exemplified by L740,093 (124). These amidine derivatives have excellent CCK-B affinity and selectivity (IC_{50} = 0.1 n*M*, CCK-A/CCK-B = 16,000 for 124) along with improved water solubility and physical properties (506).

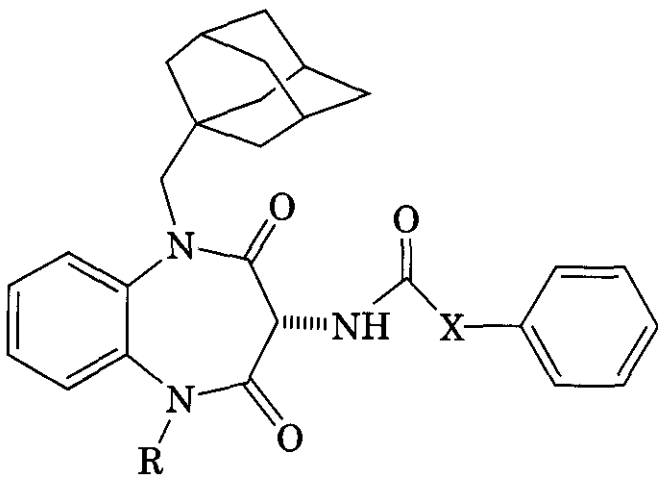
The N-1 methyl group may be replaced by aroylmethyl or methylene ester substituents, as in (125). High affinity is maintained and



(125)

CCK-B selectivity is improved over that of L-365,260 (507, 508). The pyridyl group in (**125**) provides a significant enhancement in oral bioavailability relative to the phenyl analog. Replacement of the 3-methyl group in (**125**) with basic moieties (NHMe, NMe_2) likewise maintains the receptor binding profile and enhances oral bioavailability (509).

In a related series of 1,5-benzodiazepinedione compounds (e.g., 126) a large alkyl group at N-1 is important for high affinity, the 1-adamantylmethyl group being optimal (510). At the 3-position, ureido (511) and carbamate (512, 513) substituents are tolerated, the former class having superior affinity and selectivity. As with the 1,4-benzodiazepine series, the absolute configuration at C-3 is a critical determinant of CCK-A/B selectivity (514).

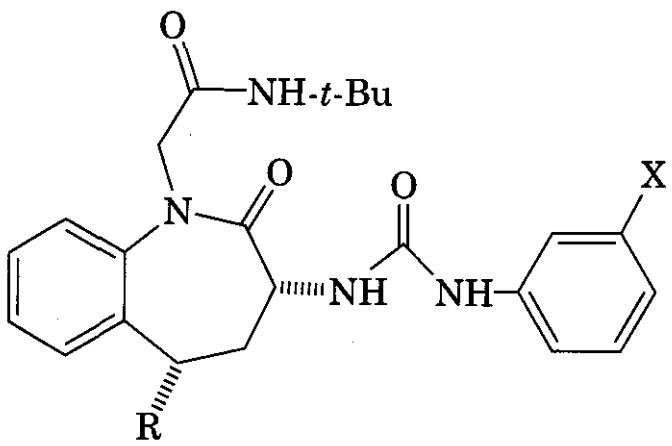


(126) R = Ph, Cyclohexyl: X = NH, O
(127) R = Ph, X = NH

(128) R = CH_2CH_2—N O

The most promising anxiolytic candidate from this series is GW-150013 (**127**) (504), currently in Phase II trials. In preclinical studies, (127) was active in anxiety models at exceptionally low doses, with an oral ED50 value of 0.05 μg/kg in the mouse lightldark box model. Furthermore, a 0.3 μg/kg oral dose of (127) produced a longer-lasting anxiolytic effect than a 1 mg/kg oral dose of diazepam (515). (127) has also shown significant anxiolytic efficacy in rats (social interaction test) and marmosets (human threat model) (516). The aqueous solubility of GW150013 is improved by replacement of the C-5 phenyl with a morpholinoethyl substituent, giving GW191869 (128) (506, 517). (**128**) exhibits anxiolytic efficacy over a wide dose range in several models, and surpasses the activity of (127) in the mouse light/dark box model (ED_{50} = 0.002 μg/kg, po). The issue of the absolute stereochemistry at C-3 is circumvented in a series of symmetrical 1,5-benzodiazepinediones (518) that have identical substituents at N-1 and N-5. These compounds contain a plane of symmetry and are thus achiral. Excellent affinity and selectivity are seen in the *N*-1,5-bis-carbonylmethyl derivatives, but the corresponding bisalkyl derivatives are generally less potent (519).

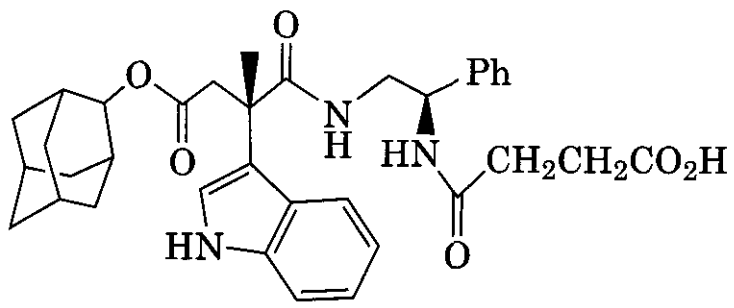
CP-212,454 (**129**) (520) is representative of a series of benzazepin-2-ones that possess high affinity and CCK-B selectivity. (129) was selected for clinical study as an anxiolytic but, not uncommonly among CCK-B antagonists,



(129) R = Ph, X = Cl
(130) R = cyclohexyl, X = CO_2H

solubility, bioavailability, and formulation issues terminated its development (521). The related carboxylic acid derivative CP-310,713 (**130**) (522) displayed enhanced potency (IC_{50} = 0.1 n*M*) and selectivity (14,000-fold) for the CCK-B receptor, as well as dramatically increased (24,000-fold) water solubility relative to that of (129). In monkeys, however, unexpectedly large doses of (130) were required to block CCK-4 induced panic attacks, presumably because of low brain penetration (521).

A series of dipeptoids based on the CCK-4 structure have been developed at Parke-Davis, culminating in CI-988 (131) (523).



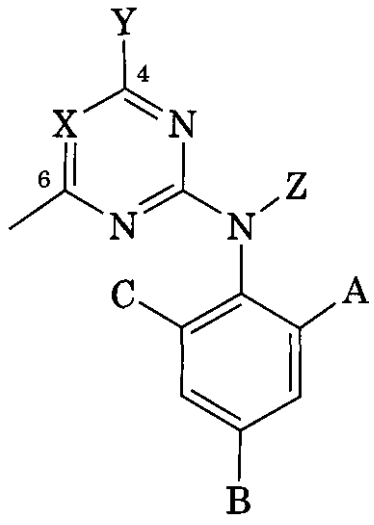
(131)

(131) is active in various anxiety models including the elevated plus maze and social interaction tests. In keeping with other CCK-B antagonist anxiolytics, exquisite potency is observed: the med in the mouse light/dark box test is 100 ng/kg, sc (524). Once again, however, poor brain penetration and low bioavailability limited the clinical efficacy (499). Clinical studies found that high doses (100 mg, po) of CI-988 were required to antagonize the panicogenic effects of CCK-4 in healthy volunteers (499), and other studies failed to find any efficacy in GAD (525, 526) or panic disorder (527) patients. The SAR work in the dipeptoid family reveals that the Tre and Phe residues are the essential components of the CCK-4 structure required for binding (528). Accordingly, the indole moiety is essential for the receptor affinity of CI-988 (529). Bulky substituents are preferred at the N-terminus, the 2-adamantyl group being optimal. Modifications at the C-terminus have resulted in enhanced affinity and have produced orally available anxiolytics with improved brain penetration, such as CI-1015 (514).

Other CCK-B antagonist series to show some anxiolytic activity include the quinazolines, also derived from asperlicin, developed at Eli Lilly (530), and "hybrids" (531–534), which combine important components of the 1,4-benzodiazepine series (the phenylurea) and the quinazolines.

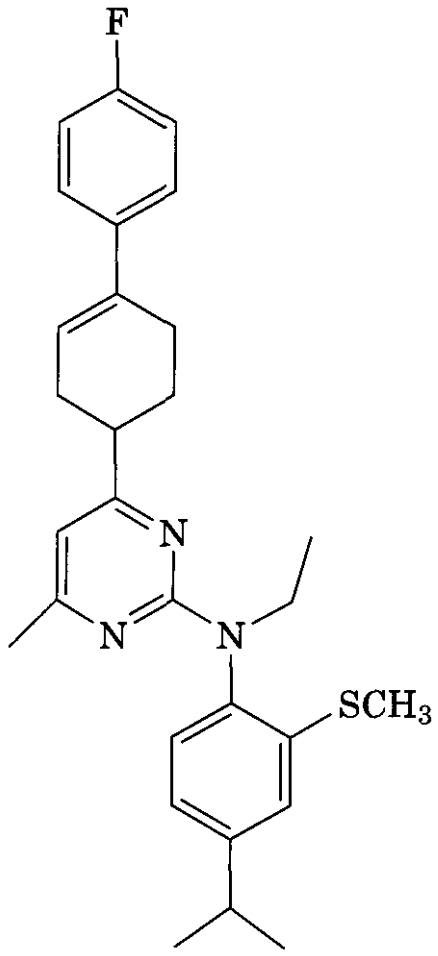
5.7.2 CRF-1 Receptor Antagonists. Antianxiety activity has been demonstrated with peptide CRF-1 receptor antagonists, such as a-helical CRF_{9-41}, D-Phe-CRF_{12-41}, and astressin, but this activity is evident only after central administration as the peptidic nature of these compounds prohibits brain penetration. The first patent for a nonpeptidic CRF antagonist was issued in 1991 and covered a series of oxopyrazolines that had only micromolar affinity for the CRF receptor (535). Since that time great strides have been made in the medicinal chemistry of CRF antagonists, and a number of potent and selective CRF-1 antagonists with oral efficacy are now available (258, 536–540).

The anilinopyrimidines and triazines, generalized in (**132**), constitute the first major class of small molecule CRF-1 antagonists (541, 542). On the heterocyclic ring, a methyl group is optimal at the 6-position, whereas the 4-position tolerates a range of substituents including dialkylamino, cyclic amino, phenyl, and tetrahydropyridyl (543–545). Compound (**133**) has an IC_{50} of 12.7 n*M* for the rat CRF-1 receptor (rCRF1) and an oral med of 0.3 mg/kg for reduction of CRF-induced anxiety in the elevated plus maze (546). The nitrogen at position 1 of the heterocycle is essential for binding affinity, and small alkyl groups (methyl, ethyl) are preferable on the central (aniline) nitrogen. On the phenyl ring, *ortho* substitu-



X = CH, N
Y = substituted amino, phenyl, tetrahydropyridyl
Z = small alkyl
A, B = medium size lipophilic groups
C = H or medium size lipohilic groups

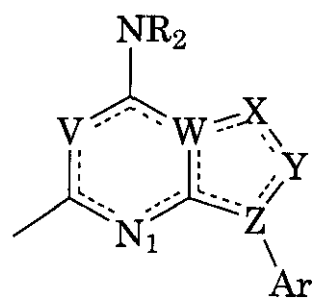
(132)



(133)

tion with a medium size lipophilic group (e.g., Br, I, SCH_3) enhances affinity for the human CRF-1 receptor (hCRF1), and lipophilic groups at the 4-position (e.g., isopropyl, methoxy) further increase activity. Consequently, a 2,4-disubstituted or 2,4,6-trisubstituted phenyl ring is a ubiquitous feature of potent CRF-1 antagonists. Conformational studies of the anilinopyrimidines and triazines (547) by the use of variable temperature NMR showed that there is restricted rotation about the two bonds connecting the central nitrogen to the aromatic rings, and that the phenyl ring is nearly orthogonal to the plane of the heterocyclic ring. This is in agreement with the positive effects of substituents on the central nitrogen and at the *ortho* position of the phenyl ring on receptor affinity, in that these modifications should reinforce the preferred conformation.

Given the conformational insights just described, it is not surprising that conformationally restricted analogs have been intensively investigated. Cyclization of the anilino nitrogen in (**132**) onto the heterocyclic ring leads to an extensive series of bicyclic CRF antagonists, many of which have improved potency and pharmacokinetics relative to the monocyclic derivatives such as (**132**). Numerous variants of the 6,5-bicyclic system containing a total of two to four nitrogen atoms, generalized in (**134**), have been explored, such as pyrrol-

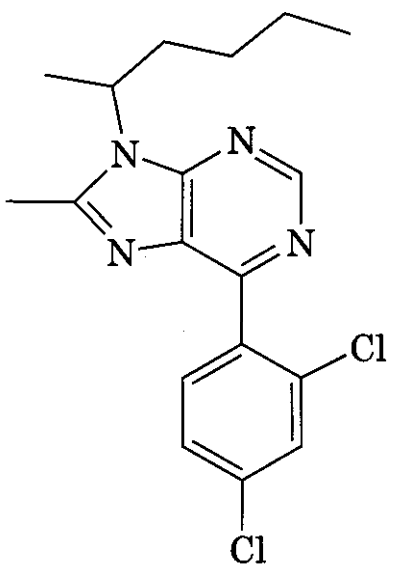


(134) V–Z = C or N; (N_1 + V–Z) = **2–4** atoms
(135) Pyrrolopyrimidines–V, Z = N; W, X, Y = C
(136) Pyrazolotriazines–V, W, **X** = N; Y, Z = C
(137) Pyrrolopyridines–Z = **N;** V, W, X, Y = C

opyrimidines (135) (548, 549), pyrazolotriazines (**136**) (550–552), and pyrrolopyridines (137) (553), among others (538–540), and a pharmacophore model for this template has been developed (540).

Although the number and location of nitrogen atoms within the bicyclic core vary among these chemotypes, in all cases the SAR of the appended substituents (i.e., the phenyl ring, the methyl group, and the alkylamino group) remain essentially the same. Thus the choice of a particular bicyclic system may be made for pharmacokinetic rather than pharmacological

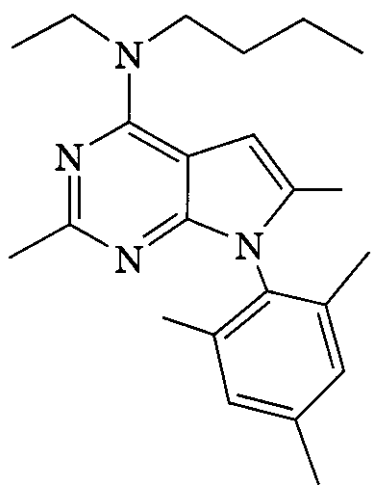
reasons. As with the monocyclic series the N-1 atom is required for receptor binding, and small substituents are required at the 6-position. On the phenyl ring, *ortho* substituents enhance affinity by encouraging a non-coplanar relationship between the phenyl ring and the bicyclic nucleus. At the ***para*** position, substituents up to a certain size are beneficial, again leading to the preferred 2,4- or 2,4,6-substituted phenyl ring. At the 4-position of the heterocyclic ring, acyclic amines, particularly unsymmetrical ones, are preferable to cyclic amines in terms of hCRF1 affinity. Inversion of the 6,5 ring system leads to a series of 5,6-bicyclic derivatives (554–557) (e.g., **138**),



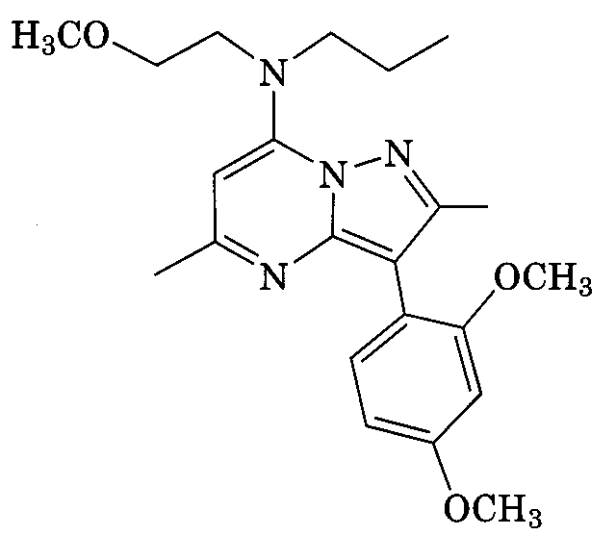
(138)

in which the imidazole nitrogen now provides the critical binding interaction. In addition 6,6-(558–560) and 5,5-(538) bicyclic derivatives have been reported but few data are available.

The 6,5-bicyclic class has provided the most interesting anxiolytic compounds to date. CP-154,526 (**139**), a pyrrolopyrimidinederivative, is a potent (IC_{50} = 2.7 n*M*) and selective hCRF-1 antagonist that is able to reverse the CRF-induced acoustic startle and fear potentiated acoustic startle responses in rats (561–563). In other models of anxiety, however, such as conflict paradigms and the elevated plus maze, (139) was inactive. This again highlights the need to evaluate potential anxiolytics with a novel mechanism of action in a wide variety of behavioral paradigms. NBI-30545 (140) is a functional antagonist with excellent hCRF1 affinity (2.8 n*M*) (538, 564).

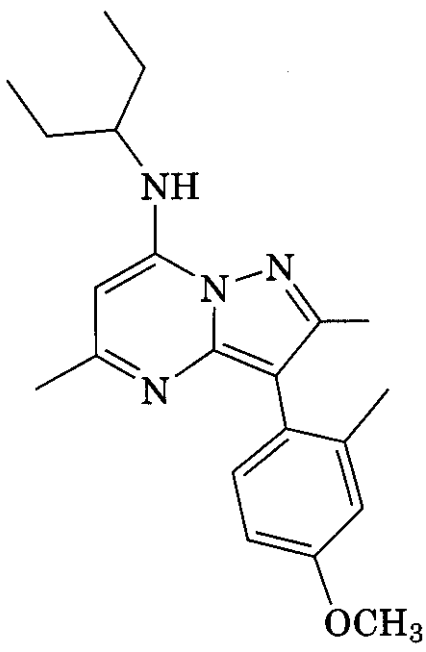


(139)



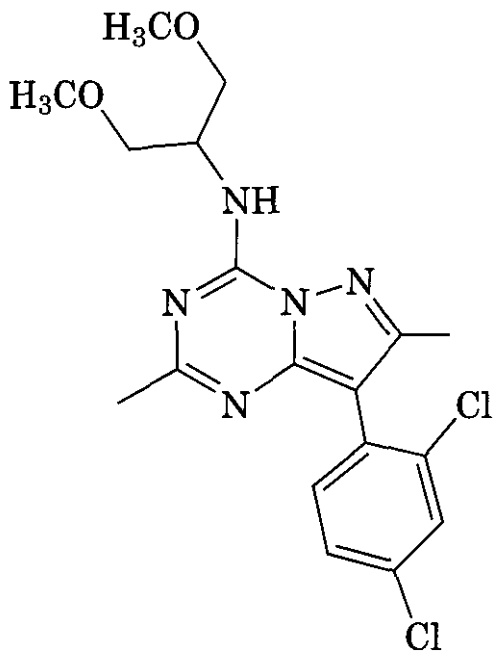
(140)

NBI-30545 has shown anxiolytic activity upon oral administration in the rodent elevated plus maze test, albeit at relatively high doses (20 mg/kg). Another pyrazolopyrimidine, DMP-904 (141) has high affinity (1 n*M*) and



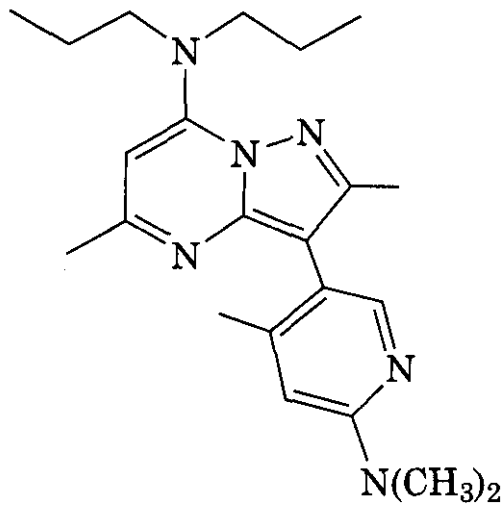
(141)

selectivity for the hCRF1 receptor, is a potent functional antagonist (10 n*M*) in the hCRF1 adenylate cyclase assay, and is orally available (565). (141) was orally efficacious in the rat light/dark open field (med = 0.3 mg/kg) and elevated plus maze (med = 5 mg/kg) models of anxiety. The closely related pyrazolotriazine, DMP-696 (142) also has high hCRF1 affinity



(142)

(1.7 n*M*), potent functional antagonism (IC_{50} = 82 n*M*) in *vitro,* and excellent selectivity for the CRF-1 receptor (566). This compound has a favorable pharmacokinetic profile in several species and has a greater oral bioavailability in the dog than that of DMP-904. (142) was less effective than DMP-904 in the rat bright/dark open field test, however, with a med of 3 mg/kg (po) and a maximally effective dose of 10 mgkg (po). Although the maximal effects of DMP-904 and DMP-696 in the situational anxiety test were slightly smaller than those of 20 mg/kg (po) chlordiazepoxide (57, 63, and 72%, respectively), the CRF antagonists showed no sedative or ataxic effects at doses up to 100 mg/kg (po) (565, 566), indicating a greatly improved side-effect profile compared to that of the benzodiazepine. Replacement of the substituted phenyl ring with pyridine (567), in an effort to improve water solubility, culminated in R-121919 (143), a potent CRF-1 antagonist with good brain penetration and reasonable bioavailability. (143) recently completed phase I clinical studies showing preliminary signs of antidepressant and antianxiety



(143)

effects (568), but development was discontinued because of liver enzyme elevation in a small number of patients.

It is evident from the preceding discussion that the CRF1 antagonists reported by a number of different companies share a remarkable structural similarity, testifying to the narrow range of small molecules that have potent affinity for this receptor.

6 CURRENT DEVELOPMENTS AND THINGS TO COME

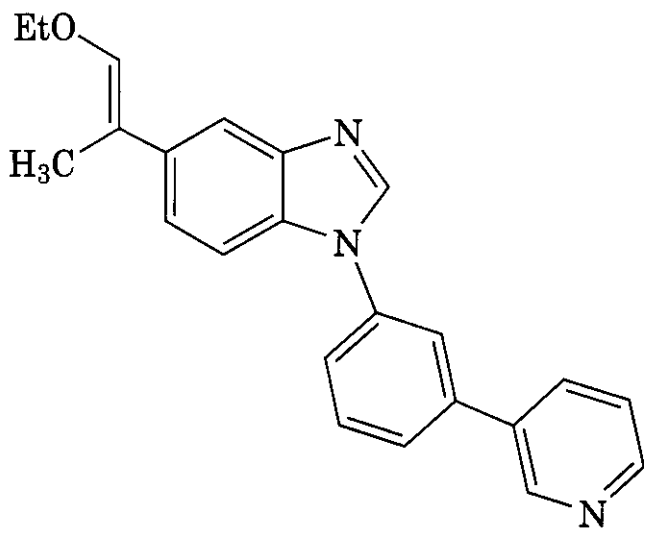
An ideal anxiolytic would be orally efficacious with a rapid onset of action, and be devoid of side effects such as tolerance, withdrawal, dependency, sedation, motor and cognitive impairment, interaction with CNS depressants, and toxicity in overdose. Such an agent would be effective in different anxiety disorders and would treat all the core symptoms of each disorder with a high response rate. Despite the progress made in anxiolytic research, the currently available antianxiety agents fall some way short of this perfect profile. This fact, coupled with the prevalence of the anxiety disorders, their impact on society, and the consequent market size, ensures a continued intense interest within the pharmaceutical industry in the development of new anxiolytics that come closer to the ideal profile. Currently, there are more biological mechanisms under investigation as potential targets than ever before (569). The most advanced of these approaches are those involving the GABA, serotonin, CCK, and CRF neurotransmitter systems, all of which have produced clinical candidates (570–572).

As already discussed, anxiety constitutes a complex group of emotional disorders, the development, regulation, and pharmacomodulation of which involves a number of the brain's major neurotransmitter systems. A clearer understanding of the influence of each of these systems on the genesis, development, and treatment of anxiety is central to the discovery of new and improved anxiolytics. In this respect, molecular biology, genetics, and pharmacology are throwing light on the intricate neurobiology of anxiety. Transgenic animal studies, for example, are identifying for the first time the individual $GABA_A$/BZR receptor subtypes that mediate the anxiolytic and other effects of the benzodiazepines. With the *a2* subtype already implicated as mediating the anxiolytic effects of diazepam, future work to determine the subtypes involved in serious benzodiazepine side effects such as withdrawal and dependency will be of tremendous value in the design of truly selective and side effect-free GABAergic anxiolytics. Things will not be as simple as engineering a subtype-selective compound, however, because it is likely that a given subtype will participate in more than one clinical effect. The a2 subtype, for example, is known to contribute to both the anxiolytic and myorelaxant actions of diazepam. Thus, to obtain real clinical selectivity, a degree of partial agonism must be superimposed on the subtype selectivity. "What is the right subtype selectivity?" and "What is the right degree of partial agonism?" are questions that are only now being posed in the clinic.

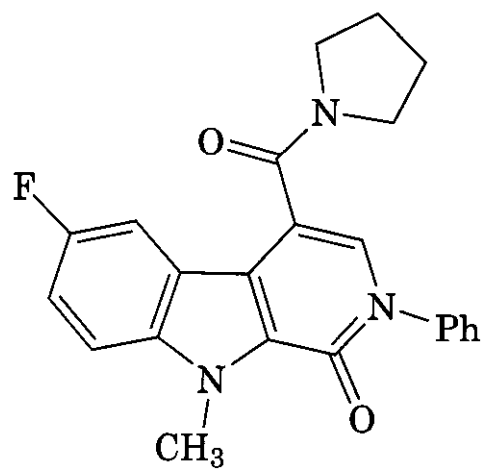
The serotonin system is intimately involved in anxiety, and up to seven 5HT receptor subtypes have been implicated to varying degrees. Different 5HT receptors and receptor subtypes may play overlapping, or even opposing, roles in emotional behavior, and the same receptor may have different functions in different brain regions. The sheer complexity of this transmitter system is underlined by the fact that in over 30 years of active research linking serotonin and anxiety, only one anxiolytic acting directly on 5HT receptors (buspirone) has been launched. From a better understanding of the role of each implicated subtype will surely come improved serotonergic anxiolytics (573, 574). As molecular biology reveals the regional expression of these receptors, techniques such as positron emission tomography (PET) scanning may reveal the involvement, or otherwise, of each in human anxiety. To design serotonergic anxiolytics, it is important to gain a fuller understanding of the various 5HT receptors at the molecular level, particularly in the case of closely related subgroups such as the 5HT-1B/D and 5HT-2A/B/C receptors. The involvement of neuropeptide systems in anxiety is less well established than GABA or serotonin, but antagonists at CRF-1 and CCK-B receptors have shown promising results and have exciting clinical potential. As the neurobiology of each pathway implicated in anxiety is investigated, every result seems to bring more questions. It is increasingly obvious that one neurotransmitter, far less one receptor or receptor subtype, does not act in isolation in regulating emotional behavior. The complex interrelationships between all of the neurotransmitter systems associated with anxiety makes it extremely difficult to extricate the true causative mechanism. Nevertheless, the existence of so many receptor targets that are able to modulate anxiety in some fashion offers numerous possibilities for new drug development.

With over 40 years of outstanding clinical success behind it, and a continuing research effort today, the modulation of GABAergic transmission through the BZR remains the mechanism most likely to provide the next advance in the treatment of anxiety. The efficacy of the benzodiazepines as antianxiety drugs is without question, but the current challenge is the improvement of what has become increasingly regarded as an undesirable side-effect profile. The subtype-selective partial agonist approach holds the most promise in this regard, but the extent of partial agonism required to maintain efficacy and optimize the therapeutic index remains an open question. Thus far, anticipated optimal profiles for anxiolysis in humans have been extrapolated from animal studies, and a number of studies with partial agonists currently in progress should soon reveal the applicability, or otherwise, of these profiles in the clinic. The most advanced candidate is pagoclone (64), currently in Phase III trials, which has already demon-

strated efficacy in panic disorder. In a placebo controlled trial pagoclone was found to be efficacious in GAD at low doses (0.3–1.2 mg/day) while causing minimal sedation (575). This could not be replicated in larger trials, however, and Pfizer is no longer pursuing this compound. Other compounds in the clinic include NGD91-3 (Neurogen) (576), NS-2710 (144)(Neurosearch) (577–580), and RWJ-51204 (81). SL-65,1498 (145) (Sanofi-Synthélabo) is

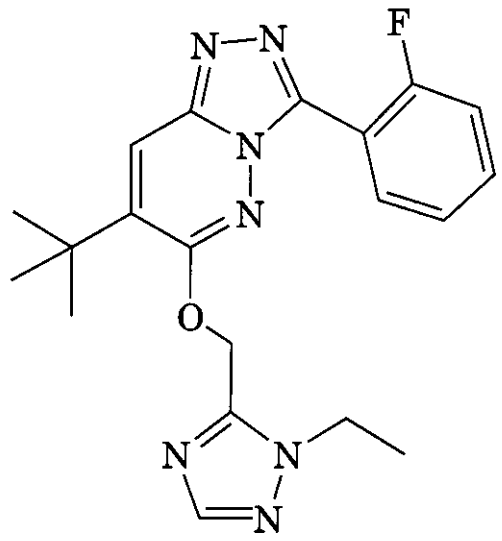


(144)



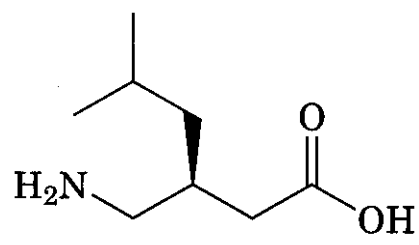
(145)

the lead compound from a series of pyridoindole-4-carboxamides. In electrophysiological studies SL-65,1498 exhibits functional selectivity for the $\alpha 2/3$ subtypes, and is claimed to be an effective anxiolytic in animal models with a favorable separation from sedative, muscle relaxant, amnestic, withdrawal, or dependency effects (581). As of August 2001, this compound was in phase IIA trials. Merck has advanced an $\alpha 2/3$ agonist in to phase II clinical studies (582). The recent heavy patent activity (583,584) from Merck, Sharpe, and & Dohme around $\alpha 2/3$ selective partial agonist 1,2,4-triazolopyridazines (e.g., 146) suggests the



(146)

clinical candidate is from this or a closely related series. At the time of writing, NGD91-3 had shown a trend toward efficacy, but failed to reach statistical significance in a Phase IIA placebo and Xanax controlled study in GAD patients (585). Thus the issue of finding the correct degree of partial agonism, and indeed the subtype-selective partial agonist concept in general, still awaits verification in the clinic with other compounds. Pregabalin (147)



(147)

(Pfizer) is a lipophilic GABA analog (586) that is under development for a number of indications, including anxiety. (147) has shown clinical efficacy in late stage social phobia (587) and GAD (588) studies. Pregabalin, and its close relative gabapentin, do not act at GABA receptors but likely mediate their anxiolytic effects through interactions with voltage-gated calcium channels (586).

At present, buspirone remains the only serotonin receptor ligand available for the treatment of anxiety The clinical development of several 5HT-1A agonists (ipsapirone, zalospirone, flesinoxan), 5HT-2 antagonists (eplivanserin, ritanserin), and mixed 5HT-1A agonist/

5HT-2 antagonists (adatanserin) as anxiolytics has been discontinued in recent years, perhaps reflecting that a fuller understanding of the roles of different 5HT receptors is required to exploit their modulation in the treatment of anxiety. A number of other compounds have reached various stages in development, however, including gepirone (105) (5HT-1A, Organon/Fabre-Kramer), alnespirone (98) (5HT-1A, Servier), sunepitron (104) (5HT-1A, Pfizer), lesopitron (110) (5HT-1A, Esteve), deramciclane (115) (5HT-2, Egis), and Org-12962 (118) (5HT-2, NV Organon), and it is hoped these will prove more successful.

In the meantime, it can be expected that the currently available SSRI antidepressants will continue to gain additional indications in the various anxiety disorders. Several neuropeptide modulators are currently in the clinic, in what will be important proof of concept studies for this rapidly expanding field. The CCK-B receptor antagonists L-740,093 (123) (Merck) and GW-150,013 (127) (GlaxoSmithKline) are potential anxiolytics, and the latter compound is currently in phase II trials. The first CRF-1 antagonists brought to the clinic for investigation in anxiety and depression are NBI-30775/R121919 (143) (Neurocrine) and DPC-368 (DuPont). Although clinical development of (143) has been discontinued because of liver enzyme elevation, the preliminary clinical data are encouraging for future compounds. Although the sample size was small (20 patients) and lacked a placebo control, (143) was found to decrease both anxiety and depression ratings (568). A follow-on compound has now completed phase I testing with no untoward effects and a pharmacokinetic profile appropriate for once-a-day dosing (589). DPC-368 has also successfully completed phase I trials, showing a half-life suitable for once-daily dosing. The impending phase II efficacy studies for these first-in-class CRF-1 antagonists will be of critical importance in establishing the clinical relevance of this mechanism.

Several other neurobiological targets and their associated ligands are postulated to have application in anxiety, such as the glutamate (LY-354,740, Eli Lilly & Co.), neurokinin (L-754,030, Merck) (590), and NPY systems. These are at an earlier stage in development, however, and their application in anxiety remains to be validated in the clinic.

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CHAPTER TEN

Antipsychotic Agents

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1 INTRODUCTION

Antipsychoticagents constitute a diverse class of drugs that are effective in the treatment of major psychoses, including those associated with schizophrenia (Table 10.1). These agents were originally known as "neuroleptics" because of their ability to lessen the reactivity to emotional and physical stimuli in highly agitated and/or psychotic patients, with little or no effect on consciousness. Antipsychotic agents have also been referred to as "major tranquilizers" to differentiate them from "minor tranquilizers," such as meprobamate and the benzodiazepines. The latter drugs, also referred to as "tranquilosedatives" or "anxiolytics," in contrast to the antipsychotic agents, are more frequently employed to calm patients suffering from anxiety.

It had been widely believed that the occurrence of acute extrapyramidal symptoms (EPS), including pseudo-Parkinsonism, dystonias, and akathisia, was an expected consequence of antipsychotic drug therapy. Indeed, all of the classical (or "typical") antipsychotic agents in clinical use in the United States cause EPS to varying degrees (1,2). Furthermore, the positive correlation between neuroleptic-induced EPS and the emergence of the biochemically very different, long-lasting syndrome of tardive dyskinesia (TD) reinforced the belief that motor system side effects were necessary correlates of antipsychotic efficacy. Given these expectations, the ability of clozapine to treat schizophrenia without the appearance of acute EPS or TD (3, 4, 6, 17) was a huge step forward in the treatment of this devastating psychiatric condition.

Table 10.1 **Antipsychotic Pharmaceuticals**

Chemical Name (Structure)	Trade Name(s)	Manufacturer	Chemical Class (Amine Type)	Equiv. Single Dose (mg)	Daily Dose Range (mg)	Route(s) of Administration
Promazine HCl (6)	Sparine	Wyeth-Ayerst	Phenothiazine (aliphaticamine)	200	40–1200	PO
Chlorpromazine HCl (1)	Thorazine	SmithKline Beecham	Phenothiazine (aliphaticamine)	100	30–800	PO, IM, IV, rectal (base)
Trifluprornazine HCl (9)	Vesptin	Apthecon	Phenothiazine (aliphaticamine)			IM
Thioridazine HCl (95)	Mellaril	Novartis	Phenothiazine (piperidine)	100	150–800	PO
Mesoridazine Besylate (96)	Serentil	Boehringer-Ingelheirn	Phenothiazine (piperidine)	50	30–400	PO, IM
Prochlorperazine Maleate (25)	Compazine	SmithKline Beecham	Phenothiazine (piperazine)	15	15–150	PO, IM (edisylate)
Trifluoperazine HCl (97)	Stelazine	SmithKline Beecham	Phenothiazine (piperazine)	5	2–40	PO, IM
Fluphenazine HCl (98)	Permatil, Prolixin	Schering, Apothecon	Phenothiazine (piperazine)	2	0.5–40	PO
Fluphenazine Enanthate	Prolixin Enanthate	Apothecon	Phenothiazine (piperazine)			IM
Fluphenazine Decanoate	Prolixin Decanoate	Apothecon	Phenothiazine (piperazine)			IM
Perphenazine (26)	Trilafon	Schering	Phenothiazine (piperazine)	10	12–64	PO, IM
Thiothixene (99)	Navane	Fberig	Thioxanthene (piperazine)	4	8–30	PO
Haloperidol (55)	Haldol	McNeil	Butyrophenone (piperidine)	2	1–15	PO, IM (lactate)
Haloperidol Decanoate	Haldol Decanoate	McNeil	Butyrophenone (piperazine)			IM
Pimozide (86)	Orap	Gate	Diphenylbutylamine		1–10	PO
Molindone (91)	Moban	Gate	Indolone (aliphaticamine)	10	15–225	PO
Risperidone (84)	Risperdal	Janssen	Benzisoxazole (piperidine)		2–8	PO
Ziprasidone (105)	Geodon	Pfizer	Benzisothiazole (piperazine)		40–160	PO, IM
Loxapine Succinate (100)	Loxitane	Watson	Dibenzoxazepine (piperazine)	15	20–250	PO, IM (HCl)
Clozapine (50)	Clozaril	Novartis	Dibenzdiazepine (piperazine)	50	300–900	PO
Olanzepine (52)	Zyprexa	Eli Lilly	Thienobenzdiazepine (piperazine)		5–20	PO
Quetiapine (53)	Seroquel	Zeneca	Dibenzthiazepine (piperazine)		100–800	PO

Unfortunately, potentially fatal agranulocytosis appears in 1–2% of patients treated with clozapine (7). This necessitates frequent blood monitoring, which can be inconvenient and expensive. Furthermore, despite its low potential for causing EPS and TD, clozapine causes other, dose-related side effects that can limit its effectiveness in some patients. The precise pharmacological actions of clozapine responsible for its clinical effectiveness are still being debated. Attempts to duplicate elements of its complex pharmacological profile have led to the discovery of several new atypical antipsychotic drugs that have been approved in the United States for the treatment of schizophrenia.

2 CLINICAL APPLICATIONS

2.1 What Is Schizophrenia?

Schizophrenia is a severe, life-long, idiopathic psychiatric disorder with a polygenic component. It is composed of severe thought disorders, termed psychoses, which are characterized by illogical, delusional, or paranoid thoughts. Schizophrenia typically has its onset in early adulthood with remissions and exacerbations throughout life.

The disorder afflicts approximately 1% of most populations. The signs and symptoms of schizophrenia usually begin in late adolescence or early adulthood and are manifested in a highly diverse and complex constellation of clinical presentations. These have been subdivided into two broad categories, positive and negative signs and symptoms. The positive components are typically the first to draw attention to the disorder and constitute the more overt manifestations of psychosis. These include false perceptions including hallucinations (usually auditory), in which the patient's internal dialogue is perceived to originate from others or from inanimate sources such as radios or cell phones. Delusions, bizarre and often repetitive behavior patterns that are inappropriate to setting, and disorganized speech characterize other manifestations of schizophrenia. The negative components are less spectacular, although more enduring and in many respects the more disabling of the characteristics of the disorder. These include alogia, anhedonia, avolution, blunted affect, disorganized thoughts, and social withdrawal. Impaired cognitive function, including memory and attention defects, may also occur.

2.2 Biochemical Basis for Schizophrenia

The concept that schizophrenia is a neurochemical disturbance is primarily supported by the fact that the clinical symptoms of the disorder can be diminished or exacerbated by medications that exert their actions through specific CNS receptors. It is clear, however, that schizophrenia is an illness of multiple symptom-defined domains, each with their own biology that coexist in individualized combinations in patients. Expanding efforts in basic research, including large-scale mRNA analyses, have identified other receptors, neurotransmitters, and structural components which contribute to the disease (8). From these many leads, novel targets have appeared for medicinal chemistry and clinical development. While these may be loosely grouped as the monoamine (dopamine, adrenergic, serotonin) and amino acid (primarily glutamate) neurotransmitter systems, the interconnectivity of these systems and increasing role of structural and synaptic deficiencies within the CNS make it difficult to lay the causative mantle at the feet of any one receptor.

2.2.1 Dopamine. The oldest and most enduring hypothesis for the etiology of schizophrenia is that it results from a hyperfunctioning of CNS dopaminergic systems. This hypothesis originated with Carlsson and Lindquist (9), who found that dopamine turnover as measured by homovanillic acid (HVA) levels was increased in laboratory animals after the administration of neuroleptic drugs. Subsequent research has supported the dopamine hypothesis and is principally based on the psychotomimetic effects of drugs that augment dopamine function in the CNS, the psychotolytic effects of dopamine-depleting drugs such as reserpine, the D_2 receptor antagonism of all antipsychotic drugs, and the increases in dopamine release and D_3 receptors in the schizophrenic brain.

Individuals who chronically self-administer the dopamine-releasing drug amphetamine or the dopamine precursor levodopa

(10) often present psychotic symptoms of untreated paranoid psychosis (11). Increases in D_3 receptors (15, 16, 342, 343) in the ventral striatum of the schizophrenic brain and increases in amphetamine-stimulated dopamine release in the striata of schizophrenic patients (14, 707) provide clinical evidence for heightened limbic striatal dopamine transmission in schizophrenia. It is not surprising therefore that essentially without exception, all drugs that treat schizophrenia are potent D_2/D_3 receptor antagonists (12). A preferential antagonism of serotonin $5HT_{2A}$ or D4 receptors, or a more rapid dissociation from the dopamine D2 receptor (699) may account for the atypical profile of drugs such as quetiapine, aripiprazole, olanzapine, and clozapine. Most of typical and atypical antipsychotics occupy 70–80% of striatal D_2 (and probably D_3 receptors) at clinically effective doses (13). The antipsychotic ineffectiveness of drugs that share many receptor features of effective antipsychotics *except* D_2 antagonism, including $5HT_{2A}$ antagonism (666) (see Section 8.2.1.1), implicates the D_2 receptor in the prime mechanism of their action. On the other hand, increased CNS levels of dopamine are not an absolute predictor for psychosis; many other agents that stimulate dopamine release such as ethanol or opiates do not produce the affective states seen in schizophrenia. Even the effects of amphetamine are not reliably psychotogenic. Lower doses can enhance focused attention and skilled motor performance, processes which are diminished in advanced schizophrenia.

Developmental malformations, impaired neuronal innervation, diminished parenchymal mass, metabolic compromise, and diminished dopamine tone in the frontal cortex typify schizophrenia but have little to do with dopamine receptors. The diminished transcription of genes for synaptogenesis, myelination, metabolism, and development in the schizophrenic brain (8) may explain these impairments and are beginning to provide a novel set of targets for therapeutic intervention.

2.2.2 Serotonin. Even before the dopamine hypothesis of schizophrenia became established, a role for overactive serotonin neurotransmission was suspected. This was based on the psychotogenic and hallucinogenic properties of the partial serotonin $5\text{-}HT_{2A}$ receptor agonist, lysergic acid diethylamide (LSD) (17) and on reports of abnormal CSF and circulating levels of serotonin in schizophrenics (18). The validity of the latter finding fell into question as these and subsequent measurements of cerebrospinal fluid (CSF) or peripheral serotonin activity in schizophrenics were inconsistent and probably of no relevance to CNS serotonergic function. Clinical trials with either 5-HT precursors or depleting agents were also inconclusive (19, 20).

Noting the structural resemblance of serotonin to the hallucinogenic indoles dimethyl tryptamine (DMT) and bufotenin, researchers proposed that psychotic symptoms were caused by these or similar compounds generated in schizophrenics by the abnormal transmethylation of endogenous indoleamines (21). Unfortunately, studies were unable to confirm increases in methylated indole amines in the plasma or CSF of schizophrenic patients versus controls (22). The transmethylation hypothesis is also questioned by the recognition that LSD-induced psychosis differs significant ways from the signs and symptoms of schizophrenia (23).

Renewed interest in serotonin's role in schizophrenia was initiated by three major findings. First, clozapine, thioridazine (24), and newer atypical antipsychotics (25) were found to more potently antagonize $5\text{-}HT_{2A}$ receptors than D_2 receptors. Second, the identification of 14 serotonin receptor subtypes provided new candidates for antipsychotic etiology and targets for drug development (26). A $5\text{-}HT_{1A}$ partial agonist activity of clozapine and newer atypical antipsychotics can be readily reconciled with their superior clinical profiles. Third, allelic variations of genes for the $5\text{-}HT_{2A}$ receptor have been associated with the diagnosis of schizophrenia (27) and the clinical response to atypical antipsychotics, for example, as shown by Arranz et al. for clozapine (28).

2.2.3 Clutamate. Glutamate and its modulatory receptors have been implicated in schizophrenia and potentially in its treatment with novel agents (29). After identification

and characterization of the phencyclidine (PCP) binding site, it was suggested that an endogenous PCP-like substance could be the causative agent (30). The noncompetitive *N*-methyl-D-aspartate (NMDA) antagonists PCP and MK-801 produce psychotomimetic states similar to those seen in schizophrenic patients, and the related drug, ketamine, can precipitate psychotic episodes in schizophrenics (31). To date, however, neither endogenous psychotogens nor changes in the NMDA receptor have been found in schizophrenia (32).

A second hypothesis suggests that excessive release of glutamate increases intracellular oxidation and gradually kills the target neurons (33), leading to psychosis. Several studies do show reduced neuron numbers in schizophrenia versus normal tissue (34–36). However, the reductions are relatively small compared with Parkinsonism, Alzheimer's dementia, or Huntington's disease, which are free of psychosis in all but the late stages. Also, a hypothesis of enhanced glutamate toxicity is inconsistent with the decreases in glutamate found in schizophrenia.

A role for decreased glutamate function in schizophrenia was first proposed by Kim et al., who found glutamate to be decreased in the CSF of schizophrenics (37). They postulated that hyperactive dopamine neurons in the ventral tegmental area (VTA) stimulate D_2 receptors, which in turn inhibits glutamate release and the activity of cortical neurons. D_2 antagonism would mitigate the effects of excessive dopamine release in schizophrenia (14, 707) and thereby disinhibit cortical glutamate release.

The hypoglutamatergic hypothesis of schizophrenia is attractive because it is consistent with the lack of changes in D_2 number in schizophrenia, and the increases in dopamine release in schizophrenia. They are also consistent with the ability of the noncompetitive NMDA antagonists PCP and ketamine to induce behaviors reminiscent of the positive symptoms of schizophrenia in normals and precipitate these episodes in patients. Ketamine was administered to schizophrenic patients acutely in a blinded, placebo-controlled trial (31). The drug caused a dose-related initiation of positive psychotic symptoms that were not blocked by haloperidol. The patients described their experiences with ketamine as being similar to those that they experienced during the florid periods of their illness (31). In addition, postmortem studies have found increased densities of NMDA and non-NMDA glutamate receptors and decreased production and/or release of glutamate in the neocortex (38). Also, N-acetylaspartylglutamate (NAAG), the precursor to glutamate, and *N*-acetyl-a-linked acidic dipeptidase (NAALADase), the enzyme that coverts NAAG to glutamate, are decreased in the hippocampus and frontal cortex of schizophrenics (39). Reduced NAALADase activity could account for reductions in glutamate-mediated neurotransmission and a secondary up-regulation of glutamate receptors in the neocortex.

Converging lines of research indicate diminished glutamate neurotransmission in schizophrenia. Decreases in cortical glutamate function would disrupt the balance between glutamate and D_2 receptors, which controls signaling between the basal ganglia, thalamus, and neocortex (40). A similar control loop involving 5-HT„ and glutamate receptors has been postulated (41). The complexity and diversity of excitatory amino acid receptors provides many drug targets for pharmaceutical intervention. No drug that combines activity at glutamate, D_2, and 5-HT_{2A} receptors has appeared.

3 ANTIPSYCHOTIC AGENTS

3.1 Therapeutic Indications

3.1.1 Schizophrenia and Psychoses. Antipsychotic drugs are primarily used to treat psychotic symptoms associated with schizophrenia, schizoaffective disorder, and to control acute mania in patients with manic-depressive disorder. Most antipsychotics are more effective in controlling the positive rather than the negative symptoms of schizophrenia. Some of the side effects associated with their use, particularly with the use of typical agents, may be mistaken for negative symptoms. This confounds the assessment of their effectiveness and can mislead the less-experienced practitioner to administer higher drug doses. The atypical agents are relatively

free of neuroleptic-induced deficit symptoms (NIDS) (42) and perhaps only for this reason seem to be superior to typical agents for treating negative symptoms of schizophrenia. Compared with even the atypical antipsychotics, clozapine has been shown by Kane et al. to be effective for residual positive symptoms in the treatment of refractory patients (2, 706). With the success of the atypical agents, newer therapies are expected to show improvements in positive and at least some negative symptoms, particularly deficits in cognitive functioning (43, 701). Clozapine treatment has been shown to improve several measures of cognitive function, especially attention and verbal fluency, whereas other cognitive functions such as memory are impaired by clozapine (702). Risperidone may improve working memory of schizophrenic patients. Overall, however, the evidence shows that the improvement in cognitive function with typical or atypical antipsychosis is small and of variable clinical relevance (702, 703) (see Section **3.2.6).** Similarly, atypical agents such as clozapine have little significant benefit on negative symptoms of schizophrenia (704, 705).

3.1.2 Other Indications. Some of the phenothiazines such as chlorpromazine (CPZ), trifluprornazine, prochlorperazine, and perphenazine are also used as antiemetic drugs. CPZ and haloperidol are effective in controlling intractable hiccups. Haloperidol and pimozide are approved, and other antipsychotics are used, to control tics associated with Tourette's disorder and the agitation associated with dementia. CPZ, thioridazine, risperidone, and haloperidol have been used in children to treat behavioral problems, including aggressive outbursts, hyperactivity, and stereotypies associated with conduct disorder, attention deficit hyperactivity disorder (ADHD), and autism, respectively. Antipsychotics are also used to treat tics produced by methylphenidate or amphetamine prescribed for those with ADHD. CPZ was also used long ago to treat symptoms of migraine, preoperative anxiety (the first use of CPZ in man), vascular headaches, tension headaches, and as an adjunct to control convulsions in tetanus and to treat acute intermittent porphyria. A variety of newer, safer agents have supplanted the use of CPZ in these indications.

A number of unapproved uses of antipsychotic drugs also exist. CPZ and haloperidol were used early on to treat phencyclidine (PCP)-induced psychosis. Psychoses associated with depression, bipolar disorder, and Alzheimer's disease are commonly treated with haloperidol, risperidone, or olanzapine. Psychotic symptoms in Parkinson's disease patients caused by levodopa and/or dopaminergic agonists have been alleviated with quetiapine, because EPS-prone typical neuroleptics contraindicated in Parkinson's disease.

3.2 Adverse Effects and Precautions

Antipsychotic drug therapy, particularly with typical agents, is associated with a wide range of unwanted side effects (Table 10.2). Such side effects can be troubling for patients, diminish compliance with their treatment, and in some cases, create serious health and safety risks. The most frequently observed effects result from dose-dependent actions on the central nervous system (EPS, sedation, and cognitive impairment), the autonomic nervous system (anticholinergic symptoms) including dry mouth, the cardiovascular system (tachycardia, postural hypotension, cardiac arrhythmias, QT, prolongation), the endocrine system (elevated prolactin release and decreased sexual function), and metabolism (weight gain). The frequency and severity of dose-dependent side effects with individual antipsychotic drugs can, in general, be correlated with potencies at D_2, a-1, muscarinic, and cholinergic and other receptors (44–47) (see Table 10.2). Other adverse effects that are more serious, less predictable, but fortunately less frequent include tardive dyskinesia, neuroleptic malignant syndrome, blood dyscrasias, and impaired hepatic function. Dermatologic and other allergic reactions and ophthalmic changes are also less frequent.

3.2.1 Extrapyramidal Symptoms. Acute extrapyramidal symptoms (EPS) consists of drug-induced parkinsonism (DIP), akathisia, and dystonia. Because of a common link to diminished CNS dopamine function at D_2 receptors, DIP and idiopathic Parkinson's disease are indistinguishable. As in Parkinson's

Table 10.2 Receptor Binding and Side Effects of Selected Antipsychotic Drugs

	Receptor Binding, pK_D[a]												Side Effects[b]				
	D_2-Dopaminergic[c,d]			5-HT_2[c,e]		Muscarinic[f,g]		$_1$-Adrenergic[h,i]			H_1-Histaminic[j,k]						
Drug	l,c	m,c	n,d	l,c	n,e	m,f	n,g	l,h	i	n,i	l j	m j	n,k	EPS	Weight Gain	Anti-Muscarinic	Orthostatic Hypotension	Sedation
Promazine	6.55	6.80		6.89		6.82		8.03	8.22		7.60	8.70		+2		+3	+2	+2
Chlorpromazine	7.60	7.72		7.74		7.15		8.37	8.59		7.55	8.05		+2	+2	+2	+3	+3
Thioridazine	7.20	7.59		7.80		7.74		8.15	8.30		7.60	7.80		+1	+3	+3	+3	+3
Mesoridazine		7.72				7.16			8.70			8.74		+1	+3	+3	+2	+3
Prochlorperazine	8.15	8.15				6.27			7.62			7.72		+3		+1	+1	+2
Trifluoperazine	8.36	8.59		7.62		6.18		7.17	7.62		6.87	7.21		+3	0	+1	+1	+1
Perphenazine		8.85				5.82			8.00			8.10		+2	+2	+1	+1	+2
Fluphenazine	8.43	9.10		7.60		5.72		7.89	8.05		7.24	7.68		+3	+1	+1	+1	+1
Thiothixene	8.35	9.35		7.44		5.54		7.96	7.96		7.43	8.22		+3	+2	+1	+2	+1
Haloperidol	8.36	8.40	8.69	7.35	6.52	4.05	5.46		8.21	7.58	4.66	5.72	5.92	+3	+1	+1	+1	+1
Pimozide	8.44			7.60				7.70			5.96			+2	–1	+2	+1	+2
Molindone		6.92				3.41			5.60			3.91		+2	0	+1	+1	+2
Risperidone			8.39		9.39		<5			8.61			7.48	+1	+1	0	+1	+1
Ziprasidone			8.17		8.68		5.61			7.88			7.19	+1	0	0		
Loxapine		7.15				6.35			7.55			8.31		+2	+1	+1	+2	+3
Clozapine	7.42	6.74	6.75	7.54	8.20	7.92	7.48	7.77	8.05	7.65	7.55	8.55	8.97	0/+1	+3	+3	+2	+3
Olanzepine			7.20		8.63		7.26			7.24			8.92	+1	+3	+3	+1	+1
Quetiapine			6.14		6.56		5.97			7.28			8.08	0/+1	+1	0	+2	+2

[a] Negative logarithm of the dissociation constant, K_D.
[b] +1 mild, +2 moderate, +3 severe.
[c] ^{3}H-Spiperone displacement.
[d] ^{3}H-Haloperidol displacement.
[e] ^{3}H-Ketanserin displacement.
[f] ^{3}H-QNB displacement.
[g] ^{3}H-Dexetimide displacement.
[h] ^{3}H-WB4101 displacement.
[i] ^{3}H-Prasocin displacement.
[j] ^{3}H-Mepyramine displacement.
'' H-Doxepin displacement.
'Ref. 45.
'' Ref. 46.
''Ref. 47.

disease, the cardinal signs of DIP are bradykinesia, muscle rigidity, and resting tremor frequently accompanied by a stooped posture, an unsteady gait, seborrheic dermatitis, and excessive salivation. Akathisia is described by patients as an inner restlessness and an inability to remain still while seated or standing. Acute dystonia is characterized by involuntary, sustained muscle contractions that may temporarily cease, only to be repeated in a slow writhing motion. Dystonic reactions may be manifested as oculogyric crises, torticollis, tongue thrusting, cramping of the hands or arms, and laryngeal spasm.

EPS associated with antipsychotic therapy tends to lessen with dosage reduction. The conjoint use of the same anticholinergic agents employed as adjuncts in the treatment of Parkinson's disease tends to ameliorate DIP and dystonia, but has less effect on akathisia. The frequency and intensity of EPS correlate with the antagonist potencies of the typical neuroleptics at dopaminergic D_2 receptors. Thus, the high potency agents (like fluphenazine and haloperidol) cause a higher incidence of EPS, while the lower potency agents (particularly thioridazine and mesoridazine, which also antagonize muscarinic receptors) cause a lower incidence of EPS. Clozapine and other atypical agents generally do not cause EPS. Dose-dependent EPS has been reported to occur with the putative atypical antipsychotic drug risperidone, but only at levels above the recommended daily dosage of 2–10 mg daily (48, 694). The absence of EPS seen with the atypicals has been attributed to several mechanisms and will be discussed in Sections 6.4 and 9.3.

3.2.2 Tardive Dyskinesia. Tardive dyskinesia (TD) is a late-appearing and sometimes irreversible syndrome that may occur after prolonged treatment with antipsychotic medications. It occurs more frequently in older patients and is characterized by involuntary, quick, repetitive movements of the face, eyelids, mouth (grimaces), tongue, extremities, and trunk. These disturbing choreiform movements may be accompanied by slow, twisting movements of the body and dystonic postures. The appearance of late developing symptoms of tardive dystonia and akathisia are generally considered to be less typical variants of TD. TD most commonly manifests during periods of dose reduction or abrupt withdrawal of neuroleptic medication. In the early days of antipsychotic therapy, it was an accepted clinical practice to allay the symptoms of TD by increasing the dose of a typical antipsychotic drug, or by changing from a less potent to a more potent drug (e.g., CPZ to fluphenazine or haloperidol).

The precise biochemical mechanisms giving rise to TD are poorly understood, but probably include effects on GABAergic neurons within the substantia nigra (44,261). Dopaminergic D_2 receptor antagonists (i.e., typical neuroleptics) alleviate the symptoms for a time, whereas dopaminergic D_2 receptor agonists and cholinergic muscarinic antagonists worsen the symptoms of TD. A direct correlation between the tendency of antipsychotic drugs to cause acute EPS in the short term and the risk of TD in the long term has been established (49). Thus, the incidence of TD is much higher with typical, than with atypical, antipsychotic drugs. The ability of clozapine to ameliorate TD caused by other antipsychotic drugs (50) demonstrates active protective mechanisms of its action beyond D_2 antagonism.

3.2.3 Neuroleptic Malignant Syndrome. Neuroleptic malignant syndrome (NMS) is a rare, but potentially lethal, adverse effect that has been reported with virtually all typical antipsychotic drugs in current clinical use. NMS is a complex syndrome consisting of hyperthermia, frequently associated with severe EPS (dystonia and parkinsonism), autonomic nervous system instability, myoglobinuria, and increased serum creatinine phosphokinase (CPK) levels. NMS has most frequently been reported in agitated male patients receiving high and rapidly escalating intramuscular doses of antipsychotic drugs (51). Comorbid medical conditions, such as agitation and dehydration, seem to play a role in the manifestation of NMS. A rechallenge of patients who have recovered from NMS with the same or a different antipsychotic drug is generally not associated with a reoccurrence of NMS symptoms. The elucidation of the pathophysiological basis for NMS has been confounded by the

complexity of symptomology. Guerra has offered a hypothesis for the etiology of NMS involving dysregulated sympathetic nervous system hyperactivity (52).

3.2.4 Seizures. The lowering of the threshold to seizures in susceptible patients has long been known to be a property of antipsychotic drugs. The aliphatic phenothiazines seem to present a slightly higher risk than do the piperazine phenothiazines or haloperidol. However, the overall incidence of actual seizures provoked by the typical antipsychotics is estimated to be less than 1%, so there is some disagreement about comparative risk (53). Clozapine, on the other hand, was associated by Welch et al. with a dose-related seizure incidence of 3% or greater (54). A correlation between seizure-inducing propensity and the central antimuscarinic potencies of antipsychotic drugs has been proposed (55), but other equally plausible mechanisms should also be explored. In general, concern about the possible occurrence of drug-induced seizures should not represent a contraindication to antipsychotic medication use. The risk can usually be minimized by the appropriate choice of antipsychoticagent, dosage reduction, and the concomitant use of anticonvulsant drugs, if needed.

3.2.5 Sedation. Conventional antipsychotics cause sedation to varying degrees. Drug-induced sedation can cause daytime somnolence and can facilitate the induction of sleep day or night. In the early use of CPZ for the treatment of agitated psychotic patients, sedation was initially seen as a beneficial effect. For the long-term treatment of schizophrenia, however, drug-induced sedation is undesirable, can contribute to negative symptoms, and be mistaken for the impaired cognition frequently associated with the disorder. Sedative effects correlate with the H_1-antihistaminic and possibly α_1-antiadrenergic potencies of the classical antipsychotic drugs. Thus, agents with low D_2 potency are more sedating than the high potency agents, because the relative contribution of H_1 and α_1 antagonism are more significant relative to D_2 potencies. Among the atypical agents, clozapine and olanzapine are particularly sedating, in keeping with their significant H_1-antihistaminic potencies. Less sedation is generally seen with risperidone. Fortunately, the sedative effects of these drugs tend to abate with prolonged use.

3.2.6 Cognitive Impairment. Studies attempting to determine the cognitive effects of antipsychotic drugs in schizophrenic patients have yielded conflicting results. The use of conventional neuroleptics has shown either no impairment, some impairment, or even improvement of cognition (56–59) and the issue is even somewhat controversial for the atypical agents (702, 703). Side effects of typical antipsychotic drugs can potentially impair performance in tasks intended to measure cognitive effects. EPS, in particular akathisia, caused by the more potent neuroleptics, can impair performance requiring motor responses. Sedation can interfere with performance in tasks requiring attention and vigilance. The addition of anticholinergic drugs to minimize EPS associated with conventional neuroleptics disrupts short-term memory in schizophrenic patients (58). This is consistent with the well-known, adverse effects of anticholerinergic agents on memory and cognitive performance in normal persons (60). In a review of adverse neurobiological effects arising from long-term use of typical neuroleptics, Jeste et al. (61) conclude that persistent cognitive impairment associated with their chronic use has not been clearly established.

3.2.7 Anticholinergic Effects. Antipsychotic drugs having relatively high affinities for blocking muscarinic cholinergic receptors can cause a variety of atropine-like side effects, such as dry mouth, blurred vision, constipation, urinary retention, and tachycardia. Such annoying side effects are usually transient and seldom dangerous. Elderly patients, however, are more susceptible to problems of constipation, paralytic ileus, and urinary retention. Patients with cardiovascular disease may be compromised by tachycardia induced by these drugs. The piperidine substituted phenothiazines, thioridazine, and mesoridazine, and to a lesser extent CPZ, cause the most anticholinergic effects among the classical antipsychotics. Clozapine and olanzapine have the highest

incidence of anticholinergic side effects among the atypical antipsychotic drugs.

3.2.8 Cardiovascular Effects. Many of the cardiovascular side effects of antipsychotic medications result from their actions on the autonomic nervous system. For example, the tachycardia seen with certain drugs is because of their antimuscarinic effects (see Section 3.2.7). Postural hypotension caused by some agents is believed to be caused by α_1-adrenergic blockade. The lower potency phenothiazines, CPZ, thioridazine, and mesoridazine, cause the highest incidence of postural hypotension, whereas this side effect rarely occurs with haloperidol. Among the atypical agents, clozapine and risperidone cause a higher incidence than do olanzapine, quetiapine, or aripiprazole. The dizziness experienced by most patients is usually transient and not serious, especially if the antipsychotic dosage is titrated gradually. However, postural hypotension, possibly coupled with sedation, can pose a serious risk of hip fracture in elderly patients.

Certain antipsychotic drugs exert direct quinidine-like actions on the heart (62). These dose-dependent changes, which are readily observed in the electrocardiograms (ECGs) of patients include the following: QT_c prolongation, abnormal T-wave morphology, and widening of the QRS complex. Drug-induced prolongation of the QT_c interval can trigger the initiation of torsade de pointes, a polymorphic ventricular tachycardia that is often fatal (63, 64). Abnormal ECG effects in psychiatric patients were first observed with the phenothiazines, CPZ and thioridazine (65). Based on case reports, thioridazine seems to be the worst offender, having been implicated in several instances of sudden death attributed to ventricular tachycardia (66; 695). A close analog, mesoridazine, can also cause ventricular arrhythmias. Chlorpromazine and trifluoperazine seem to cause fewer ECG abnormalities. There is no association of alterations in QT intervals with the use of olanzapine, quetiapine, or risperidone (695). Ziprasidone modestly prolongs the QT interval, but there is no evidence to suggest that this leads to torsade de pointes or sudden death (695). Nevertheless, its potential effects on ventricular repolarization warranted Caley and Cooper to recommend its use only as a second-line treatment in patients with comorbid cardiovascular risks (697). Torsade de pointes has also been associated with the use of intravenous haloperidol (67). It is likely that many of the unexplained deaths of younger patients in Great Britain in the 1980s attributed to high dose pimozide may have been caused by its arrhythmogenic effects (68).

Torsade de pointes is known to be triggered by hypokalemia and by the class III antiarrhythmic drugs that interfere with potassium channels. Potassium channel blockers prolong the QT_c interval by inhibiting the rapid component of the delayed rectifier current, I_r (69). Recently the human gene HERG (human ether-a-go-go-related gene) that encodes for a protein associated with I_r, has been transfected into mammalian cell lines (70) and employed to screen antipsychotic drugs for cardiotoxic effects (71–73). Haloperidol (71), thioridazine (72), and the otherwise promising atypical antipsychotic drug sertindole (73) are all high affinity antagonists for the HERG potassium channel protein. These antipsychotics bear more than a casual structural relationship to certain other non-cardiac drugs known to cause torsade de pointes arrhythmias (63, 64), such as the H_1-antihistaminic drugs, terfenadine and astemizole, and the 5-HT, agonist and prokinetic drug, cisapride.

3.2.9 Sexual Side Effects and Hyperprolactinemia. Clinical evaluation of the sexual function effects of antipsychotic agents are fraught with methodological difficulties including reduced sexual performance in unmedicated schizophrenics. The few well-controlled studies that have appeared have involved male subjects (74, 75).

Some of the effects of antipsychotic drugs on sexual function have been attributed by Aizenberg et al. to increased prolactin secretion by the anterior pituitary (74, 76), other effects may result from their specific autonomic actions (75). Prolactin secretion by the anterior pituitary is tonically inhibited by the hypothalamus, with dopamine acting as the prolactin release-inhibiting factor (PIF). Thus, conventional neuroleptics cause dose-related increases in serum prolactin levels (hy-

perprolactinemia) and corresponding prolactin-induced side effects. In women, such side effects include menstrual irregularities, breast swelling and tenderness, galadorrhea, and decreased sexual desire. Men can experience hypospermatogenesis, impotence, and loss of libido because of increased serum prolactin. Atypical neurolepticscause minimal effects on serum prolactin. Risperidone causes hyperprolactinemia only at the upper end of the therapeutic dose range. Other troubling side effects in male subjects, such as erectile dysfunction, priapism, and ejaculatory dysfunction are likely caused by a-adrenergic blockade (75).

3.2.10 Weight Gain. Increased appetite and weight gain can occur with many antipsychotic medications (77). Significant weight gain can affect patient compliance and can also pose serious risks for adult onset type II diabetes and a variety of cardiovascular diseases in older patients. Clozapine and olanzapine seem to cause more weight gain and incidence of type II diabetes than the phenothiazines, risperidone, or thiothixene (75). Clozapine and olanzapine elevate serum triglycerides and diminish insulin sensitivity, resulting in hyperglycemia. These actions can precede and contribute to the effect of weight gain. Haloperidol, loxapine, ziprasidone, and aripiprazole seem to have minimal effects on body weight or glucose metabolism. Several neurotransmitter systems have been implicated in the enhanced appetite and weight gain associated with antipsychotic drugs (78). Drugs that facilitate serotonin (5-HT) neurotransmission have been found to decrease appetite (satiety) and thereby promote weight loss (79). Conversely, drugs that antagonize serotonin at 5-HT, receptors increase food consumption and promote weight gain (79, 80). α_1-Adrenergic receptors are implicated in neuroleptic drug-induced weight gain, potentially through effects on energy use. The connection between histamine receptors and weight gain is based on the observations that many classical H_1-antihistaminic drugs cause weight gain and that the antipsychotic drugs associated with the greatest weight gains have the highest binding affinities at H_1-histaminergic receptors (79).

Endocrine actions of antipsychotic drugs could also contribute to weight gain. It has been suggested that hyperprolactinemia induced by typical neuroleptics could promote weight gain through an effect on gonadal steroidogenesis (81). In a relatively small study (81), significant weight gains along with decreased androgen levels were observed in men, but were not observed in women, who experienced reduced estrogen levels. Because the atypical antipsychotic drugs confer minimal effects on prolactin secretion, it is very unlikely that weight gain caused by clozapine or olanzapine can be attributed to their effects on gonadal steroids. On the other hand, both clozapine and olanzapine increase plasma levels of triglycerides (82, 83) and leptin (84), the lipid-regulating hormone secreted by adipocytes (85).

3.2.11 Hematologic Side Effects. The principal effects of antipsychotic drugs, notably clozapine, on the hematologic system were shown by Lieberman et al. (86) to involve leukocytosis and leukopenia. These usually occur during the first few months of therapy and frequently resolve within a few days after removal of clozapine (86). The more serious and frequently fatal complication of agranulocytosis is a very rare occurrence with antipsychotic drugs except clozapine. Early reports of clozapine-induced agranulocytosis in Finland (87) were so serious that they effectively prevented its approval in the United States until the use of the drug in treatment-resistant patients became evident (2, 706). An extensive survey of psychiatric patients treated with clozapine in Great Britain and Ireland found that 2.9% developed neutropenia and 0.8% developed agranulocytosis (88). Fatalities rarely occurred with proper hematologic monitoring (88). Elucidation of the mechanism of clozapine-induced agranulocytosis has attracted considerable interest (86, 89–94). Clinical observations suggested that an immune-mediated hypersensitivity reaction was responsible (86). This suggestion was later supported by the discovery of a serum factor in patients with clozapine-induced agranulocytosis (89).

3.2.12 Hepatic Effects. Liver abnormalities have been observed with many antipsy-

chotic drugs since the introduction of CPZ (95). Mild-to-moderate increases in transaminase enzymes are frequently seen during the first few weeks of therapy. Such findings rarely justify discontinuation of therapy because the levels usually return to normal. A cholestatic-like jaundice, accompanied by abdominal pain and idiosyncratic fever and chills, affects up to 2% of patients taking CPZ (95). This non–dose-dependent cholestasis has also been reported with haloperidol (96) and has rarely been reported for clozapine.

3.2.1 3 Dermatologic Side Effects. Allergic skin reactions associated with antipsychotic drugs range from urticaria and macropapular rashes to erythremia multiforme. Such reactions seem to be uncommon with the newer atypical agents. Photocontact urticaria (100), photosensitivity (sunburn) (101), and deposits in the lens (700) have been observed with CPZ and other phenothiazines including mesoridazine. The occurrence of hyperpigmentation of the skin seems to be associated with higher doses of phenothiazine-type neuroleptics in chronically treated patients (102, 103). It has been suggested that light-generated free radical species, possible interacting with melanin in the skin, may be responsible for both the hyperpigmentation (102,103) and photosensitivity phenomena (101).

4 ANIMAL MODELS OF EFFICACY AND SIDE EFFECTS

Because of its genetic complexity and uncertain etiology, schizophrenia, like so many neuropsychiatric disorders, has resisted the development of suitable animal models. Early progress in the field was hampered because of the prevailing belief that schizophrenia was a social or psychological disorder rather than a neurodevelopmental brain disorder.

4.1 Use of Animal Models

After the introduction of the first neuroleptic drugs, many animal models were developed to screen new compounds for potential antipsychotic activity. These models predict both antipsychotic efficacy and side effect liability in humans (104–106). Other animal models attempt to mirror specific symptoms of schizophrenia. Of these behaviorally isomorphic models (107), the few that have face validity are likely to become particularly useful for identifying atypical antipsychotic drugs (105, 106, 108, 109).

The concept of schizophrenia as a neurodevelopmental disorder has inspired attempts to create adverse and early postnatal events in animals to model the psychopathological processes underlying the disorder (109, 110). These neurodevelopmental models include prenatal malnutrition, viral infection and hypoxia, disrupted neurogenesis by X-ray irradiation or neurotoxins in *utero,* adverse postnatal experiential factors such as maternal deprivation and social isolation, and postnatal brain damage created by hippocampal, neocortical, or thalamic lesions (109–111). With the possible exception of maternal deprivation and social isolation, these models have not been sufficiently characterized pharmacologically to be used for antipsychotic drug screening.

4.2 Prediction of Antipsychotic Efficacy

4.2.1 Behavioral Effects of Psychostimulants. The psychosis-inducing properties of the psychostimulants amphetamine (121) and PCP (115) in human volunteers are well known. Both drugs also exacerbate psychotic symptoms in schizophrenic patients (115, 121, 122). However, the constellation of symptoms elicited by the two psychostimulants differ significantly. Amphetamine induces predominantly positive symptoms such as paranoia in volunteers, and tends to worsen such symptoms in schizophrenics. Certain negative symptoms may actually be improved by amphetamine (123). PCP induces positive and negative psychotic symptoms in normal volunteers and worsens a broad spectrum of psychotic symptoms in schizophrenics (122). Because of the psychotomimetic effects of amphetamine and PCP, both drugs have been used as animal models against which novel antipsychotic drug action could be predicted.

4.2.1.1 Behavioral Models of Dopaminergic Overactivity. The initial behavioral animal models for identifying potential antipsychotic drugs were based on the dopamine theory of

schizophrenia (9). The antagonism of increased locomotor and stereotypic behaviors induced by dopamine stimulants like apomorphine and amphetamine, the induction of catalepsy, and the disruption of the conditioned avoidance response each result from the blockade of D_2/D_3 dopaminergic receptors (108, 112). The concept of limbic selectivity (10, 11) further resolves these models, whereby antipsychotic activity results from the blockade of mesolimbic D_2 receptors, while EPS is produced by mesostriatal D_2 blockade. The phenothiazine derivative, thioridazine, and clozapine were the first neuroleptics to exhibit some degree of functional limbic selectivity on chronic administration (113,114). Increased locomotor activity induced by low (0.5 mg/kg) doses of amphetamine is largely mediated in the nucleus accumbens (124, 125). All typical and atypical antipsychotics antagonize hyperactivity in rats induced by low dose amphetamine (126, 127, 112), making this simple animal model a useful initial screen for potential antipsychotic drugs. This model is not entirely selective, however, because drugs lacking antipsychotic activity in humans, such as the GABA agonist muscimol, also antagonize amphetamine-induced locomotion (128).

4.2.1.2 Behavioral Models of Glutamaterigic Overactivity. The observation that psychotic-like symptoms can be induced in human volunteers by the psychomimetic drug phencyclidine (PCP) (115) led to an interest in the examination of PCP-induced behaviors in animals as models for screening potential antipsychotic drugs (116) (see Section 8.2.2). The potent noncompetitive NMDA receptor antagonist properties of PCP provided an additional hypothesis for the etiology of schizophrenia (117). This hypothesis has been tested in a wide range of PCP-induced behavioral effects in rodents, including increased locomotor and stereotyped behaviors (116–118), decreased social interaction (118, 119), and deficits in prepulse inhibition (120).

Hyperlocomotion and stereotyped behaviors induced in rodents by noncompetitive NMDA antagonists such as PCP and dizocilpine are antagonized by a wide range of antipsychotics (116, 117, 129–133). PCP can model in rats the negative symptomatology of social interaction (119, 134, 135). Chronic treatment with clozapine, and to a more partial degree, remoxipride and sertindole, reverses deficits in social interaction in rats induced by PCP (109,119, 134, 135). Haloperidol, chlorpromazine, and risperdal are not active. This is in keeping with the unique efficacy of clozapine in ameliorating negative symptoms in schizophrenic patients (2). The effectiveness of olanzapine only at higher doses (119,135) was confirmed by more recent studies that, however, failed to demonstrate an effect for the newer antipsychotic drug quetiapine (136).

The behavioral despair of mice forced to swim without escape is measured by their immobility during a retest under the same conditions. Immobility in the forced swim test (FST) is reversed by antidepressant drugs (137), whereas PCP enhances the immobility. Pretreatment with clozapine and risperidone, as well as the 5-HT, antagonists, mianserin and ketanserin, attenuated the PCP-induced increase in immobility, whereas haloperidol and antidepressants have no effect (138,139). The PCP enhancement of the FST may provide a more selective animal model for the negative symptoms of schizophrenia.

PCP and other NMDA antagonists increase cortical glutamate efflux (140, 141). This prompted the suggestion that the psychotogenic action of PCP may result from a potentiation of glutamatergic effects at non-NMDA receptors, in addition to their block of NMDA receptors. Several metabotropic glutamate receptor subtypes regulate glutamate efflux (142). Moghaddam and Adams (143) have shown that the group II metabotropic glutamate agonist LY 354740 [(+)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate] prevents PCP-induced locomotion and stereotypy and prevents the disruptive effects of PCP on working memory. These effects were achieved at doses of LY 354740 that did not affect spontaneous locomotion or dopaminergic neurotransmission, suggesting that metabotropic glutamate agonists might represent a new class of antipsychotic drugs.

4.2.2 Prepulse Inhibition. Abnormalities in information processing and attention mechanisms have long been recognized as hallmark characteristics of schizophrenia. Prepulse in-

hibition (PPI) is the reduction in the startle reflex response produced by a weak (non-startling) sensory stimulus given just before the startling stimulus. PPI is a measure of sensorimotor gating or information processing and is reliably impaired in schizophrenic patients (144, 145). Deficits in PPI are believed to reflect an inability to adequately inhibit irrelevant sensory information leading to sensory overload and cognitive fragmentation. The cross-species nature of PPI supports its use as an animal model with potential face, predictive, and construct validity (146). PPI in animals is impaired by the psychotomimetic agents amphetamine (144), PCP (147), and other NMDA antagonists (148, 149), and by 5HT,, agonists (150, 151).

Most antipsychotics, but also many non-antipsychotic drugs, reverse deficits in PPI induced by amphetamine or apomorphine (152, 153). In contrast, D_2 receptor antagonists such as haloperidol fail to reverse deficits in PPI induced by PCP or dizocilpine (153–155), while such deficits are, at least in part, reversed by clozapine (156), remoxipride (157), olanzapine (158), and quetiapine (155). Risperidone fails to reverse the effect of PCP (155), but it antagonizes the disruption of PPI induced by the $5\text{-}HT_{2A}$ agonist DOI (150,153).

Rats reared in isolation after weaning also experience deficits in PPI (159) and decreased social interaction. This effect has been attributed to enhanced dopaminergic activity (160). Isolation rearing deficits are maximal at puberty (161, 162) and thus parallel the ontogeny of schizophrenia in humans. The disruption of PPI in young rats reared in isolation is reversed by a broad spectrum of antipsychotic drugs including haloperidol, risperidone, clozapine, olanzapine, and quetiapine (153,163).

4.2.3 Latent Inhibition. Latent inhibition (LI) is like PPI but includes a greater cognitive component. It is a retarded acquisition of a conditioned response that occurs when a subject is exposed to the conditioning stimulus before the conditioning trials (164,165). Deficits in LI reflect the inability of the subject to ignore irrelevant stimuli and have been demonstrated in many (164–167), but not all (168), studies of schizophrenic patients. Like PPI, latent inhibition has been demonstrated in experimental animals and in humans. Consequently, the LI paradigm has been proposed as an animal model of schizophrenia to predict antipsychotic activity (165, 169).

Amphetamine disrupts the LI response in rats (170, 171), and this is reversed by haloperidol, clozapine, and sertindole (172, 173) and the $5\text{-}HT_{2A}$ antagonist MDL 100,907 (174). The 5-HT, agonist DOI, when administered in the pre-exposure phase only, disrupts LI and this disruption is prevented by haloperidol, clozapine, risperidone, and MDL 100,907 (175). This disruption could be because of effects of DOI on state-dependent learning rather than on attentional processes (175).

Potentiation of the LI response in rats has been consistently demonstrated for classical antipsychotics (169, 176) and for clozapine (169, 172, 173, 177, 178), remoxipride (178), sertindole (179), and MDL 100,907 (174). Because very few of the newer antipsychotic drugs have been investigated for their effects on LI, and MDL 100907 is not an effective antipsychotic, further studies with these drugs are required to validate the predictiveness of the LI model.

Although LI and PPI both reflect the ability of the organism to ignore irrelevant stimuli, they differ in many respects. PPI is a sensorimotor gating process that does not require conditioning and relies on mechanisms that filter exteroceptive stimuli for their physiological or cognitive significance. LI is a cognitive process, requiring preconditioning, and reflects a subject's ability to adjust behavior to changing conditions. In contrast to PPI, LI is not disrupted by PCP (180).

4.2.4 Conditioned Avoidance Response. Perhaps the oldest animal model to predict potential antipsychotic drug efficacy is the conditioned avoidance response (CAR) (108, 112, 181, 182). In the conditioned reinforcement model, experimental animals are trained to perform a certain response to avoid a mild shock. Trained avoidance responses may be active (pressing a lever, climbing a pole, or jumping out of a box) or passive (remaining in the darker of two compartments). Classical antipsychotic drugs and benzodiazepines reduce avoidance responding at doses that do

not impair natural (untrained) escape responding (183); antidepressants impair both escape and avoidance responding (184).

CAR is inhibited by a wide variety of structurally different D_2 antagonists (185–188). There is a positive and significant correlation between anti-avoidance activities of various antipsychotic drugs in rats and their D_2 receptor blockade (186)and their average daily clinical dose in treating schizophrenia (189). Somewhat conflicting results have been obtained for clozapine in CAR tests in different animal species (186, 190, 191). A biphasic effect has been reported in mice and in squirrel monkeys, wherein lower doses of clozapine actually increase avoidance, while higher doses antagonize the response (190). Studies in rats have shown that clozapine blocks CAR at doses that interfere (191) or do not interfere with motor function (186, 189). Remoxipride (189), risperidone (192), olanzapine (193), quetiapine (194), and ziprasidone (195) are all active in the CAR test.

The complexity of the CAR test is evident from the variety of drugs that either suppress or enhance responses. Wadenberg and Hicks (196) conclude that CAR is a sensitive test for potential antipsychotic drugs that act by various receptors, particularly those in the nucleus accumbens shell. α1-Adrenergic receptor antagonists inhibit CAR (197). This may contribute to the antipsychotic effectiveness of many neuroleptics, because almost all of them bind and probably antagonize the α1 receptor. Suppression of CAR by cholinergic potentiators like arecoline, pilocarpine, and physostigmine was observed many years ago (198, 199). The muscarinic cholinergic antagonist scopolamine attenuates the impairment of CAR induced by D_2 antagonists, but enhances the suppression of CAR caused by the selective D_1 antagonist SCH 23390 (200), suggesting a very different mechanism for the effect of D_1 receptors in the CAR paradigm. Partial muscarinic agonists including PTAC and RS86 inhibit CAR (201,202). PTAC exhibits a similar pharmacological profile to that seen with atypical neuroleptics, including a limbic-selective inhibition of dopamine cell firing and inhibition of amphetamine induced c-fos expression in the nucleus accumbens (201). Preliminary receptor selectivity data suggest that PTAC is a partial agonist at M_2 and M_4 receptors and an antagonist at M_1, M_3, and M_5 receptors (201). AMPA/kainate receptor antagonists also cause a neuroleptic-like suppression of CAR (203).

4.2.5 Drug Discrimination. Drug discrimination has been used for many years to detect the predominant receptor activities occurring *in vivo*. These inferences are based on whether behavioral responses in humans or animals that are sustained by exposure to a reference compound are also maintained by the test compound, such as one with antipsychotic properties (204). Drug discrimination studies can identify antagonist agents that block conditioned responses, and agonist miomics that sustain conditioned responses. Such tests can identify antipsychotics with similar pharmacological properties but are not necessarily predictive for the treatment of schizophrenia.

Systemic administration of classical antipsychotics or clozapine block amphetamine-induced discriminative stimulus responses in the rat (205,206). The amphetamine response can also be blocked by direct injection of the drug into the nucleus accumbens (207). While classical antipsychotics completely inhibit the response, clozapine and olanzapine are somewhat less active, risperidone and remoxipride are weakly active, and sertindole and quetiapine were inactive (208, 209). Based on these findings, it has been proposed that the antagonism of the amphetamine discriminative stimulus response may be an indication of neuroleptic-induced deficit properties (209). Antipsychotic drugs also seem to inhibit the discriminative stimulus effects of hallucinogenic 5-HT agonists like DOI, DOM, and LSD in proportion to their 5-HT$_2$ antagonist potencies (209–211).

Early studies indicated that PCP-induced discriminative stimulus responses are not antagonized by haloperidol (212, 213), and conflicting results have been obtained with NMDA antagonists including dizocilpine (214), whereas the metabotropic glutamate agonist LY 354740 is inactive (215).

Drug discrimination has also been used to compare antipsychotic drugs that act through different receptor mechanisms. The close structural analogs olanzapine and quetiapine

generalize to the clozapine discriminative stimulus in rats, whereas haloperidol, remoxipride, chlorpromazine, and fluphenazine are ineffective (216–218). Seroquel, haloperidol, and thioridazine partially generalized, but risperidone, sertindole, and amisulpiride, a D_2/D_3-antagonist (219), failed to generalize (220). In a study conducted in squirrel monkeys, quetiapine fully, and olanzapine partially, generalized to the clozapine stimulus, whereas risperidone, sertindole, and remoxipride failed to show generalization (221). Olanzapine itself also elicits a discriminative stimulus in rats (222). The olanzapine response generalized to clozapine, chlorpromazine, and thioridazine, as well as to the 5-HT$_2$ antagonist ritanserin and to scopolamine. A full generalization to the clozapine response was achieved with muscarinic antagonist scopolamine (220), reflecting the prominent antimuscarinic action of clozapine. A partial generalization occurred with the α1-adrenergic antagonist prazosin and the α2-adrenergic antagonist idazoxan (220). Given the most complex receptor pharmacology of clozapine and its chemical analogue olanzapine, it is not surprising that many antipsychotic drugs generalize to their discriminative stimulus cues (220,223).

4.3 Prediction of Side Effect Liability

While EPS and catalepsy in experimental animals are no longer considered to be necessary consequences of antipsychotic drug action (224, 225), even the newest atypical antipsychotics block D_2 receptors to varying degrees (12, 13), and with the exception of quetiapine, can induce EPS, catalepsy, and increase serum prolactin at high doses (226–228). Thus, tests for such liabilities remain in most antipsychotic screening batteries.

4.3.1 Catalepsy. CPZ and reserpine were the first neuroleptics found to create a state of tonic immobility, or "catalepsy," in experimental animals. In the catalepsy test, rats or mice are placed in an unusual position and the time required for the animal to correct this unusual position is determined. All typical antipsychotics induce catalepsy (229), whereas clozapine does not (230), and it can even reverse catalepsy induced by typical antipsychotics (50). The induction of catalepsy reflects the potential of a drug to cause EPS. Many of the newer antipsychotics, for example, the benzamides (188), risperidone (192), olanzapine (193), ziprasidone (231), and aripiprazole (232, 233), induce catalepsy only at high doses. Some drugs lacking neuroleptic activity, such as opioids (234) and muscarinic agonists (235), potently induce catalepsy. Muscarinic antagonists, on the other hand, reverse the cataleptogenic actions of haloperidol (236).

4.3.2 Stereotyped Behaviors Induced by Amphetamine and Apomorphine. Stereotypy responses in rodents consist of repetitive sniffing, licking, biting, and gnawing (237)because of excessive activation of striatal D$_2$ receptors (226,228). The EPS potential of antipsychotic drugs is also evaluated by their ability to antagonize stereotypy induced in rats by relatively high doses of amphetamine (5 mg/kg) or apomorphine (1mg/kg) (229, 238–240). Classical antipsychotics and the selective D_1 antagonist SCH 23390 fully and potently antagonize the stereotyped behaviors induced by amphetamine or apomorphine (238,239,241). Consistent with their atypical classification, thioridazine (239, 240), clozapine (239, 240), sertindole (242), quetiapine, and ziprasidone are ineffective even at the highest doses tested, whereas remoxipride, risperidone, and olanzapine antagonize amphetamine-induced stereotypy only at relatively high doses.

4.3.3 Paw Test. In the paw test, rats are placed on a platform with their fore- and hindlimbs lowered into four separate holes. The paw test determines the effects of drugs on the spontaneous retraction of extended forelimbs and hindlimbs (243). The test was originally developed to study the effects of GABAergic drugs on motor behavior mediated in the dorsal striatum (243). It was soon discovered that classical antipsychotics such as haloperidol and CPZ increase the time for retraction of the hindlimb (HRT) and the forelimb (FRT) with equal potencies, whereas thioridazine and clozapine increase HRT at lower doses than were required to increase FRT (244). It was proposed that FRT models the EPS effects of neuroleptics, whereas HRT models their thera-

peutic effects (244). In addition to thioridazine and clozapine, the D_2-selective antagonist benzamides, the D_1-selective antagonists SCH 23390 and SCH 39166, and the mixed receptor antagonists risperidone, olanzapine and sertindole, all increase HRT at significantly lower doses than those required to increase FRT (245,246). The predictive validity of the paw test is questioned (112) because SCH 39166 is not an effective antipsychotic (248). In general, however, drugs lacking neuroleptic activity were inactive in the paw test. These included opiates, benzodiazepines, antihistamines, and tricyclic antidepressants (246, 247). Scopolamine antagonizes the increase in FRT induced by haloperidol, but not its increase in HRT, supporting the hypothesis that FRT is related to EPS (244).

4.3.4 EPS in Primates. The ability of antipsychotic drugs to induce acute dystonia and other neurological side effects in nonhuman primates closely mirrors these responses in humans. *Cebus apella* monkeys are sensitized by long-term treatment with haloperidol to produce a nonhuman primate model of chronic EPS resulting in tardive dyskinesia (TD) (249–252). Some of the haloperidol-treated monkeys develop a prolonged dyskinetic syndrome resembling clinical TD, and these are used to study the potential of antipsychotic and other drugs to cause or treat TD (251, 252). Clozapine does not elicit dystonia in sensitized monkeys and is virtually free of EPS in humans. Quetiapine is also devoid of EPS potential in the dystonia model, after both oral (194) and intraperitoneal (253) administration. A large separation between predicted EPS and clinically effective doses was found for sertindole, whereas olanzapine, quetiapine, and remoxipride gave an intermediate separation. Haloperidol, risperidone, and ziprasidone had little separation (254–256), suggestive of EPS liability. The strength of the *Cebus* monkey dystonia model of TD is that the symptoms closely parallel the symptoms of TD in humans, including the individual vulnerability factor wherein some monkeys develop TD while others do not (257). Limitations are mainly the expense and ethical considerations of the model because of the use of monkeys and the long-term duration of their treatment (257).

4.3.5 Vacuous Chewing Movements. The chronic administration of classical antipsychotic drugs to rats induces oral dyskinesias, termed vacuous (or purposeless) chewing movements (VCMs) (258–260). This behavior has been proposed as a model for orifacial TD in humans. Chronic treatment with clozapine produces far fewer VCMs than does haloperidol (261,262). Rats treated for 6 months with clinically effective doses of olanzapine or sertindole produced very few VCMs (263). Notwithstanding the limited number of new antipsychotic drugs that have been investigated, the VCM model seems to have use for the preclinical assessment of potential new drugs for their TD-inducing liability (257), and without limitations inherent in the sensitized primate model.

4.3.6 Prolactin Response. Classical antipsychotic drugs stimulate prolactin release from the pituitary gland through the blockade of D_2 receptors innervated by tuberoinfundibular dopamine neurons (264). The selective D_1 antagonist SCH 23390 does not stimulate prolactin release (265), consistent with the sole presence of D_2 receptors in the pituitary. The correlation between serum prolactin increases and antipsychotic potencies in humans (266) is explained by the common D_2 mechanism for both actions. Although clozapine (265) and aripiprazole (267) can increase serum prolactin in rats, neither antipsychotic elevates prolactin in humans (268–270). More minor effects on human serum prolactin typify the newer antipsychotic drugs, particularly aripiprazole, risperidone, olanzapine, and ziprasidone (42), and these will be described in greater detail in the following sections dedicated to these drugs.

4.3.7 Cognitive Effects. Along with the overt psychotic symptoms that characterize schizophrenia, deficits in cognitive function are commonly manifested by impairments in attention, information processing, and memory (14, 701–703). While antipsychotic drugs can ameliorate impaired attention and information processing, their effects on memory

deficits are less clearly established (see Section 3.2.6). In fact, some studies in rats have shown that classical antipsychotics actually induce short-term memory deficits (271,272). In the Morris water tank model of spatial memory (273), low doses of haloperidol and risperidone decrease spatial memory in a dose-dependent fashion. These drugs also decrease swimming speed, suggesting that impairment of motor function related to D_2 antagonism (274) may be a contributing factor. Ziprasidone and olanzapine impair spatial memory at relatively high doses but do not affect swimming speed (275). Clozapine slightly impairs cognitive performance initially but is without effect in later trials. Sertindole and quetiapine have no effect on either spatial memory.

The same group of antipsychotic drugs have been evaluated for their effects on working memory (276,277) in a visual spatial version of the delayed non-matching to position paradigm (278, 279). Haloperidol and risperidone exhibit marked inhibitory effects in low doses, whereas considerably higher doses of clozapine, olanzapine, and ziprasidone are required to produce similar effects (276). Sertindole and quetiapine are inactive at the doses tested (276). Long-term treatment reveals a continued memory impairment of memory by haloperidol, the development of tolerance to the effects of clozapine on memory, and continued lack of impairment by sertindole (277).

5 DISCOVERY OF PROTOTYPE ANTIPSYCHOTICS

5.1 Discovery of Chlorprornazine

In the late 1940s, Laborit administered the antihistaminic phenothiazine derivative, promethazine, before surgery to ameliorate the symptoms of surgical shock. He observed that promethazine decreased the patients' concerns and fears of the impending surgery. In 1950, optimization of this early lead produced chlorpromazine (CPZ) a drug that demonstrated significant efficacy in reducing preoperative fears. It is this kind of indifference to impending stress that may form the basis for the predictive nature of the conditioned avoidance response (CAR) test for antipsychotics (see Section 4.2.4). CPZ was also used by Laborit to calm patients afflicted with schizophrenia who had been treated primarily by social isolation or physical restraints in mental institutions. Their unprecedented improvement heralded a revolution in the treatment of this terrible disease. CPZ and related drugs enabled many institutionalized patients to reenter society and home, something that had been the exception rather than the rule. This led to a marked decrease in the number of hospitalized patients, from 500,000 in 1955 to 222,000 in 1983.

Most patients who returned to society were able to function moderately well, but if they stopped their medication their condition deteriorated. Also, CPZ and compounds related to this phenothiazine series produced motoric side effects, including extrapyramidal side effects (EPS) that resemble Parkinson's disease. These symptoms could be severe and developed in up to 90% of patients on typical antipsychotic drugs. This condition often progressed to irreversible tardive dyskinesias, involuntary movements of the limbs and facial muscles that resemble the symptoms of Huntington's disease. In addition, such typical antipsychotics, although they were effective in treating the positive or florid symptoms of schizophrenia, did not ameliorate the negative symptoms of the disease.

5.2 Discovery of Haloperidol

Haloperidol was discovered in the Janssen Laboratories in 1958 (280,315,320,322). It is a butyrophenone derivative and was pursued because of its ability to block the behavioral activating effects of amphetamine in rat models. The amphetamine models were used as screening tools, because symptoms observed in paranoid schizophrenia were noted to be similar to those that developed in chronic amphetamine abusers. The behavioral profile of haloperidol was qualitatively similar to that of CPZ, but haloperidol required up to 50-fold lower doses to exert the same behavioral effects as CPZ. Around this time, the term "major tranquilizer" began to be replaced by the term "neuroleptic." The compound was pursued very vigorously, and the initial clinical results were published only 10 months after the initial synthesis of the compound. Haloperidol is still one of the most widely pre-

scribed neuroleptics in the United States (281), partly because of its inexpensive cost, its potency, and availability as a decanoate preparation for intramuscular depot administration that provides long-term efficacy and increased compliance (282).

5.3 Advent of Atypical Antipsychotics

Classical neuroleptics of the phenothiazine, butyrophenone, and thioxanthene type cause undesirable side effects, which at one time were considered to be an inevitable consequence of their potent antagonism of D_2 receptors. The search for atypical antipsychotic compounds focused initially on maintaining the generally favorable efficacy while decreasing the severity or incidence of these side effects, particularly the EPS. A large number of structurally diverse compounds have been investigated to this end. These compounds have varying degrees of receptor affinities in addition to a high D_2 affinity for serotonergic, histaminergic, muscarinic, cholinergic, and non-D_2 dopamine receptors. The tricyclic antipsychotic, clozapine, and the benzamide, amisulpiride, are associated with fewer EPS and preferentially increase dopamine turnover in the mesolimbic system, unlike the classical antipsychotics, such as haloperidol and CPZ, that elevate dopamine turnover to the same degree in mesolimbic and striatal brain areas. This finding indicates that the limbic and prefrontal areas of the brain may be antipsychotic target sites, whereas dopamine receptor blockade in the striatum is thought to be more responsible for the induction of EPS.

5.4 Discovery of Clozapine

Classical antipsychotic agents induce EPS on chronic administration and also do not treat up to 30% of psychotic patients. Clozapine, a tricyclic benzodiazepine derivative, was the first antipsychotic drug to display a dramatically different pharmacological profile and did so both in animals and clinically. This profile created the concept of a new class of "atypical" antipsychotic drugs.

Clozapine has had a checkered history from its initial discovery in the late 1960s by German and Austrian clinicians (283) to its rediscovery in the United States in the 1980s. It was synthesized initially as part of an effort to understand the relationships between chemical structure and biological effectiveness after the discovery by Kuhn in 1957 of the first tricyclic antidepressant drug imipramine. This sparked major interest in a number of pharmaceutical companies to investigate variants of such structures. Although somewhat simplistic, the hypothesis developed was that in such linear tricyclic systems, all of which contained a basic side-chain, if the two aromatic rings were in the same plane, i.e., flat, as was the case with 6:6:6 systems, the resulting molecule was most likely to exhibit antipsychotic properties, whereas in the 6:7:6 systems where the two aromatic rings are "twisted" or non-planar, the molecule was more likely to have antidepressant properties. Indeed, such an approach led to the discovery of the imipramine analogs, clomipramine and amitriptyline, and also to the discovery of amoxapine (284). However, surprisingly, clozapine was found not to be an antidepressant but an effective antipsychotic agent devoid of extrapyramidal side effects. The drug was successfully introduced in some European countries in 1968, but the finding of agranulocytosis in a Finnish study, where eight patients died, nearly ended the further development of the drug. The incidence of agranulocytosis in schizophrenic patients treated with clozapine is 1–2% (285). Consequently, clozapine was only used as second line therapy when at least two other standard antipsychotic drugs had failed. It was not until the 1980s in the United States that clozapine began to find acceptance, as evidence mounted that it was effective against both positive and negative symptoms of schizophrenia. The careful blood monitoring of patients treated with clozapine has dramatically decreased the lethality from agranulocytosis in the United States. The search for clozapine-like compounds (286) with comparable effectiveness but without the toxicological problems has been a quest for the past 20 years. The success of this search has required the painstaking elaboration of mechanisms by which clozapine exerts its therapeutic and toxicological effects (287).

5.5 Discovery of Amisulpride

With the discovery of atypical agents such as clozapine and thioridazine, companies began

searching for other classes of compound that would be effective antipsychotics devoid of the EPS problems of the classical drugs and also the toxicological liabilities of clozapine. A series of benzamides was developed starting from the structure of the local anesthetic, procainamide, that led progressively to the antiemetic compound, metoclopramide, and the observation that it demonstrated some weak antipsychotic properties. Further optimization of this benzamide structure led to the development of sulpiride, which possessed significant antipsychotic activity and had a lower incidence of EPS than classical agents (288). It was also found somewhat later that members of this class compound were generally highly selective for the dopamine D_2 family of receptors (289, 290), although varying degrees of selectivity across the D_2 receptor subtypes (D_2, D_3, and D_3) were found. Indeed, structural features of the substituted benzamides were later incorporated into compounds designed to specifically block the dopamine D_4 receptor (see Section 8.1.1). Although extensive variants of sulpiride have been investigated (291), only racemic sulpiride and amisulpride have been marketed widely outside the United States. The active enantiomer of amisulpride, S-amisulpride, is the only analog still under active clinical development. A highly promising analog, remoxipride (292, 293), produced good efficacy against both positive and negative symptoms of schizophrenia and no troublesome anticholinergic effects. Unfortunately, remoxipride produced unacceptable toxicity of aplastic anemia, and clinical trials were discontinued in 1993. However, this class of compound has provided some excellent pharmacological "tools" and radioligands because of their high selectivity for the D, receptor (294).

6 STRUCTURE-ACTIVITY RELATIONSHIPS

6.1 Drug Classes

Antipsychotic drugs currently approved for clinical use in the United States are summarized in Table 10.1. Drugs classified as typical include the following: several phenothiazine derivatives (**1–9**), the thioxanthene, thiothixene (**10**), the butyrophenone, haloperidol (**11**), the diphenylbutylamine, pimozide (**12**), the indoline, molindone (**13**), and the dibenz[b,f]-(1,4)oxazepine, loxapine (**14**). Drugs generally classified as atypical include: the benzisoxomole, risperidone (**15**), the benzisothiazole, ziprasidone (**16**), the dibenz[b,f](1,4)diazepine, clozapine (**17**), the thienobenz[b,f](1,4)azepine, olanzapine (**18**), and the dibenz[b,f](1,4)thiazepine, quetiapine (19) (Table 10.3).

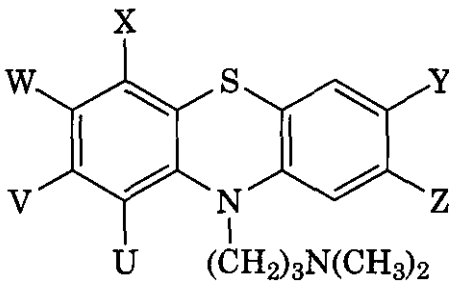
6.2 Tricyclic Neuroleptics

After the introduction of chlorpromazine (**1**) (CPZ) as a treatment for schizophrenia, thousands of new agents based on its tricyclic topology were prepared and examined pharmacologically. The neuroleptic potential of these agents was determined through a number of standardized animal tests, most notably those of motor activity and conditioned response as described in Section 5.3. These assays served to rank order the neuroleptic "potency" of new agents relative to CPZ. This classification became known as the "chlorpromazine index," and the biological potencies of compounds within a selected behavioral paradigm were expressed relative to CPZ whose index was unity. Although receptor binding theory and analysis were a number of years off, these tests were often remarkably accurate at predicting the rank order of affinity for the dopamine D_2 receptor subtype.

The tricyclic antipsychotics may be dissected into three substructures (Fig. 10.1), those being the pendent amine functionality (Site A), the diaryl heterotricyclic (Site C), and the intervening alkyl chain (Site B) (295). When considering the effect of structure on neuroleptic activity, it is informative to examine each of the three substructures of the tricyclic~in isolation to see how individual changes within each of these regions impacts the pharmacological properties of the resultant compound.

The distance between Sites A and C is critical for neuroleptic activity, with a three carbon chain being optimal. Shortening the chain to two carbons has the effect of amplifying the anticholinergic and antihistaminic properties. The amino ethyl derivatives diethazine (2) has proven useful in the treatment of Parkinson's disease, while promethazine (3) is effective as an antihistamine.

Table 10.3 Effects of Aryl Substitution in 10-(3-Dimethylaminopropyl)phenothiazines



No.	U	V	W	X	Y	Z	Chlorpromazine Index Block of Conditioned Response in Rats
(6)	H	H	H	H	H	H	0.07
(7)	Cl	H	H	H	H	H	>0.1
(1)	H	Cl	H	H	H	H	1
(8)	H	H	Cl	H	H	H	0.18
(9)	H	CF_3	H	H	H	H	1.7
(10)	H	H	CF_3	H	H	H	0.43
(11)	H	H	H	CF_3	H	H	0.06
(12)	H	OCH_3	H	H	H	H	0.07
(13)	H	CH_3	H	H	H	H	0.28
(14)	H	CO_2CH_3	H	H	H	H	0.49
(15)	H	Cl	H	Cl	H	H	1
(16)	H	2-$CH(CH_3)_2$	H	H	H	H	0.32
(17)	H	Cl	H	CH_3	H	H	1
(18)	H	CF_3	H	H	OCH_3	H	0.3
(19)	H	CF_3	H	H	H	Cl	0.2
(20)	H	Cl	H	Cl	Cl	H	>0.1

Whereas small alkyl substituents such as methyl are tolerated at the C2 carbon, larger substituents (i.e., R = phenyl) that restrict the free rotation decrease neuroleptic potency. Additionally, if rotation is restricted through ring formation as in (4), the chlorpromazine index is greatly reduced **(295).** Rotation restriction through ring formation at the 3-posi-

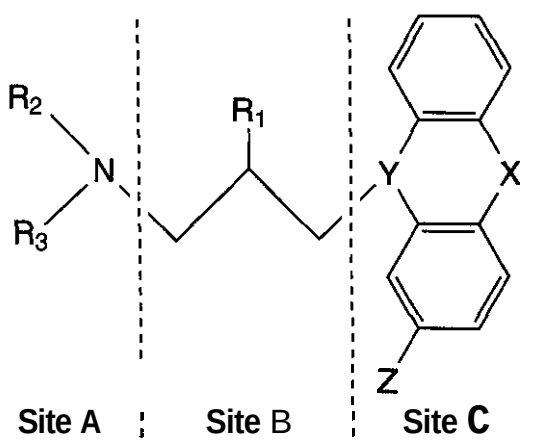
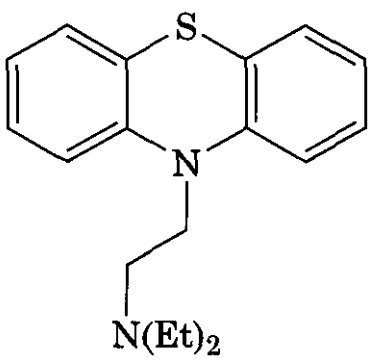
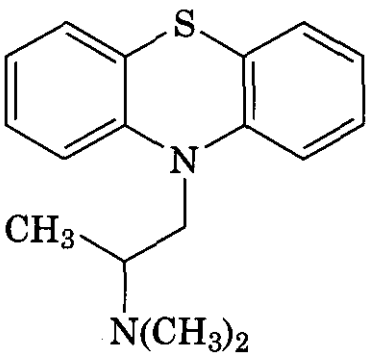


Figure 10.1. Subtructures of the tricyclic antipsychotics.

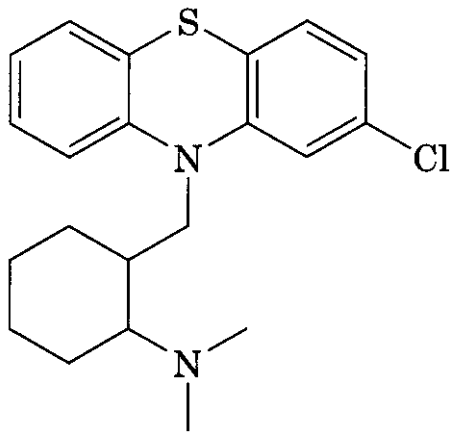


(2)

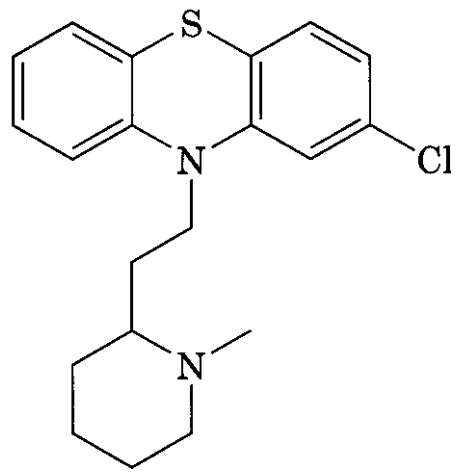


(3)

tion of the aminopropylon side-chain provides thioridazine (**5**), which is equipotent to chlorpromazine (**1**)(297).



(4) Chlorpromazine index <0.1



(5) Chlorpromazine index 1

Effect of Aromatic Substitution Within the Tricyclic System. The influence of aromatic substitution on the pharmacological profile of the tricyclic antipsychotics is fairly straightforward. For the purposes of this discussion we will examine the effect of varying substituents within the 10-(3-dimethylaminopropyl) phenothiazines (Table 10.3), although these influences are applicable across the various classes of tricyclic neuroleptics.

All other factors being equal, substitution at position 2 provides compounds of enhanced potency in blocking conditioned response in rats relative to both the parent compound and also compounds having the identical substituent at positions 1, 3, or 4. The electronic nature of the substituent also plays a role in determining the efficacy in this model. Electron withdrawing groups such as chloro (**1**) and trifluoromethyl (9) show superior conditioned response blocking activity relative to the corresponding alkyl (13 and 14) or alkoxy (12) derivatives (298.299).

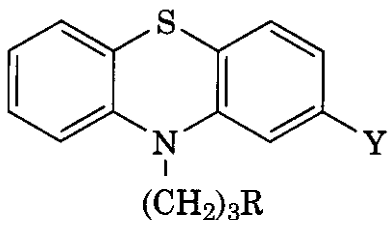
A number of polysubstituted derivatives have been examined (16–20). In each of these cases, a marked decrease in neuroleptic potency was reported (300,301).

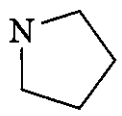
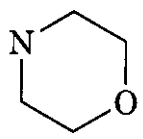
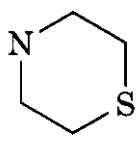
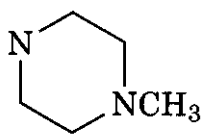
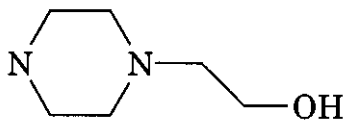
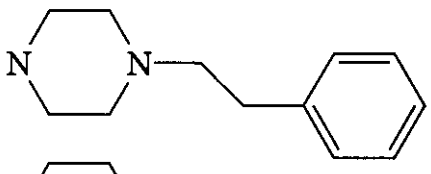
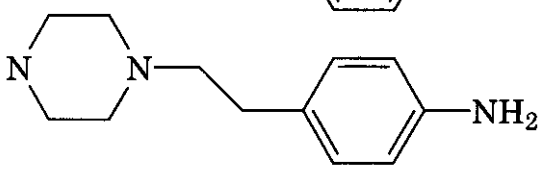
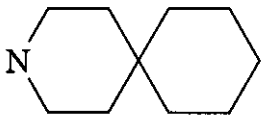
Nature of the Amino Group. The size and nature of the basic amino group has considerable influence on the behavioral profile of the phenothiazine neuroleptics (Table 10.4). A tertiary amine is optimal, both mono and desmethylchlorpromazine are several times less active than the parent molecule in rat conditioned response (302). In many cases, larger N-alkyl groups decrease neuroleptic potency (Table 10.4). The diethyl (**21**), pyrrolidinyl (**22**), morpholinine (**23**), and thiomorpholine (24) analogs of CPZ show decreased chlorpromazine indices in the rat conditioned response assay. In contrast, the *N*-methylpiperazine compound prochlorperazine (25) has enhanced potency, as do a number of other piperazines (26–28) (296). The azaspiranyl analog (29) produced pronounced CNS depression and sedation lasting more than 48 h (303).

Variations Within the Tricyclic Topology. With an eye toward maintaining the diaryl nature of the tricyclic site C, a wide variety of creative variations of CPZ were prepared. Introduction of other group VI elements in place of the sulfur in the phenothiazines produced the corresponding phenoxazine and phenoselenazine derivatives. As shown in Table 10.5, the phenoxazines are markedly less active than the phenothiazines (304a,b), whereas the phenoselenazines fall somewhere in between (305). The diminished activity of the phenoxazines may be a result of the shortened oxygen-carbon bond length, which would tend to pull the two aromatics closer together and at a tighter angle. The carbon (acridan) derivative (35) resemble the phenoselenozines, although the disubstituted acridan (36) is essentially inactive (306).

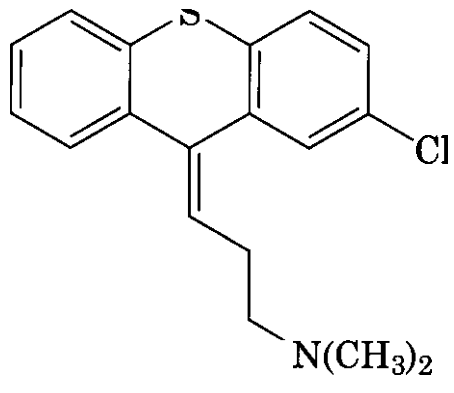
A unique series of neuroleptics results from the replacement of the nitrogen within the phenothiazine ring system with a methine carbon. The introduction of the double bond within the propylamino chain provides for geometric isomers. The geometry, wherein the

Table 10.4 Effect of Variation of the Basic Amino Group of 10-(3-Aminopropyl)phenothiazines



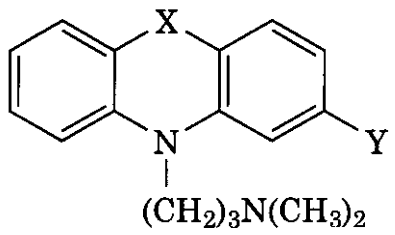
No.	R	X	CPZ Index Blockade of Conditioned Response in Rats
(21)	$N(Et)_2$	Cl	0.8
(22)		Cl	0.7
(23)		Cl	0.2
(24)		Cl	0.05
(25)		Cl	2.7
(26)		Cl	9.0
(27)		CF_3	3.0
(28)		CF_3	9.0
(29)		Cl	

2-substituted aromatic ring and the aminoethyl substructures are on the same side of the double bond, is referred to as *"cis,"* and the isomers having this configuration have been shown to possess enhanced neuroleptic potency relative to the *"trans."* For instance, chlorprothixine (**37**), the direct *cis* thioxanthene analog of chlorpromazine, showed similar activity to CPZ in conditioned behavioral tests in rats. The *trans* isomer (**38**) displayed

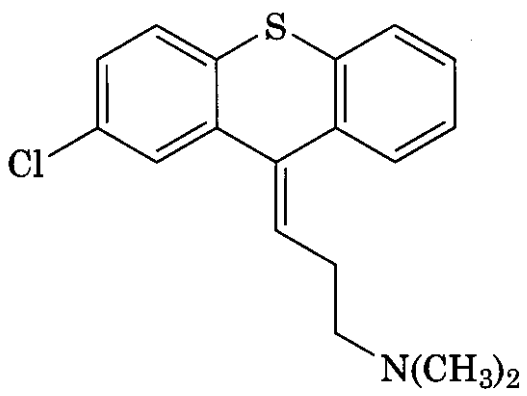


(37)

Table 10.5 Effect of Modifying Heteroatom at Position 10 of Phenothiazines

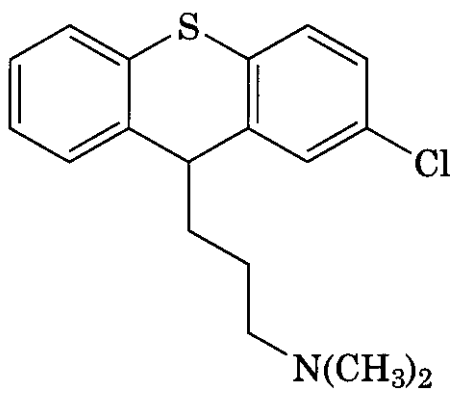


No.	X	Y	Chlorpromazine Index Blockade of Conditioned Response in Rats
(1)	S	Cl	1
(30)	S	CF_3	1.7
(31)	O	Cl	0.38
(32)	O	CF_3	0.29
(33)	Se	Cl	0.1
(34)	Se	CF_3	1.1
(35)	CH_2	Cl	~0.1
(36)	$C(CH_3)_2$	Cl	>0.1



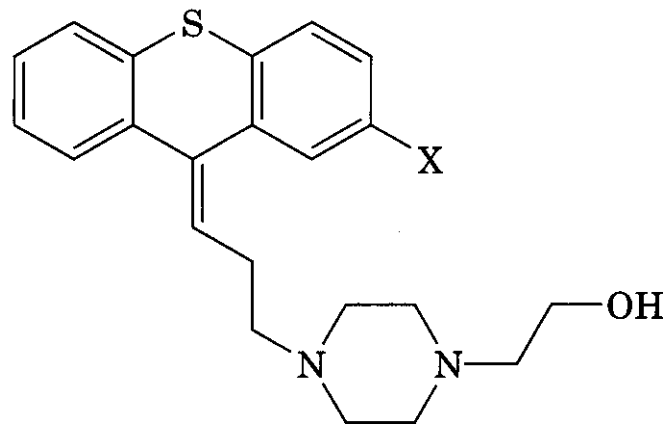
(38)

much less activity (307). Similarly, decreased activity was observed for the reduced compound (39)(308).



(39)

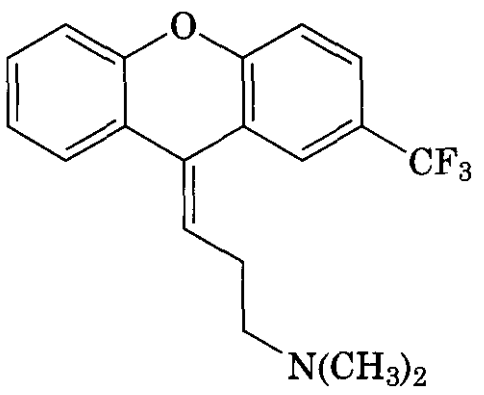
The structure-activity relationships of the thioxanthenes mimic that of the phenothiazines. Aromatic substitution at the 2 position enhances neuroleptic potency (309). Replacement of the dimethyl amino function by selected piperazine derivatives as in clopenthixol (40) and flupentixol (41) could produce compounds with high potency relative to chlorpromazine (310). In contrast to



(40) X = Cl
(41) X = CF_3

the diminished activity of phenoxazines relative to phenothiazines, within this tricyclic template, the xanthenes seem equipotent to the corresponding thio derivative. The 2-trifluoromethyl derivative (42) presented conditioned response blocking potency similar to that of CPZ. This latter point brought into question the postulate that sulfur at the 9 position optimally positioned the two aromatics relative to each other.

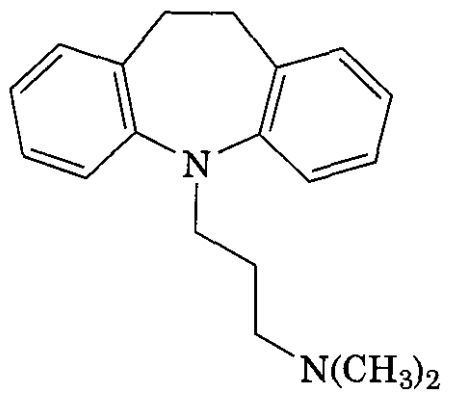
The phenothiazines and thioxanthenes have the characteristic of two phenyl groups fused to a central six-member ring giving a so-called 6–6-6 system. An ethylene is a commonly used bioisostere for a thio linkage in medicinal chemistry. Continued research in



(42)

the area of the tricyclics led to the use of this and other two-atom linkages to provide the **6:7:6** ring system.

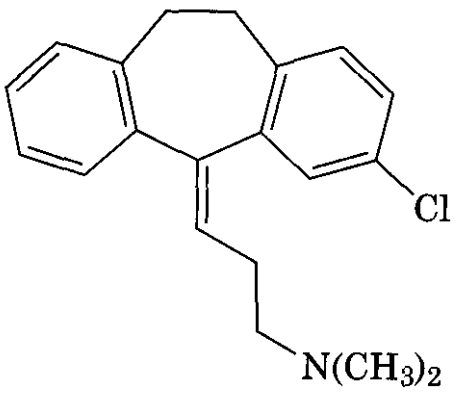
During clinical studies of dibenzazepine derivatives, it was observed that imipramine (**43**), unlike the thioxanthenes, was a rela-



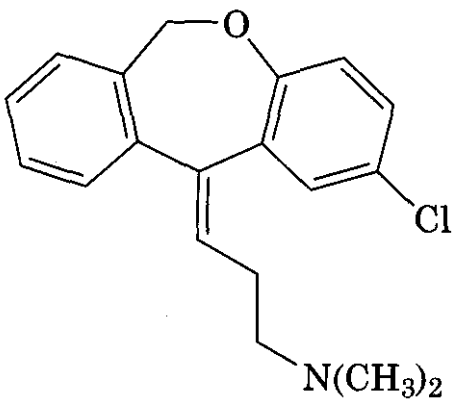
(43)

tively ineffective antipsychotic agent but seemed to have efficacy in the treatment of depression. Indeed this was found to be a general property of aminoalkylated derivatives of **6**:7:**6** compounds. While unsubstituted aromatic compounds of this class showed almost exclusively as antidepressants, they display central depressant activity in rats, whereas their deschloro analogs are much weaker. Two-substituted derivatives had observable neuroleptic actions. The dibenz[b,e]oxepin derivatives, (45) and (46) (pinoxepine), showed chlorpromazine indices of **1** and **6,** respectively (311,312).

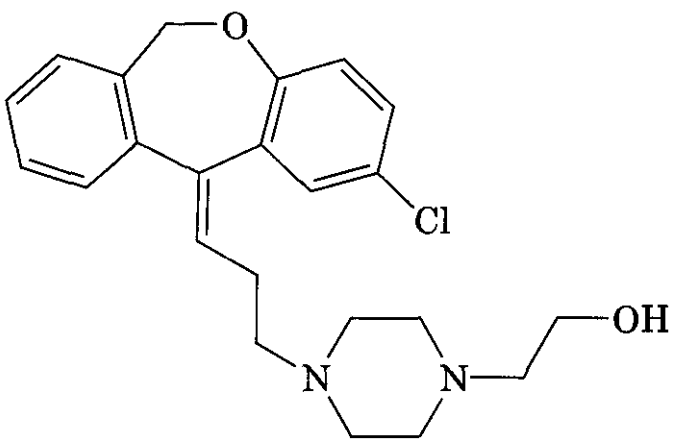
An entirely new class of neuroleptic agents based on the **6–7-6** template are the dibenz [b,f]thiepins. Representative of this class is octoclothiepine (**47**), which is approximately six times more potent than chlorpromazine in depressing spontaneous motor activity in the rotorod test (313).



(44)

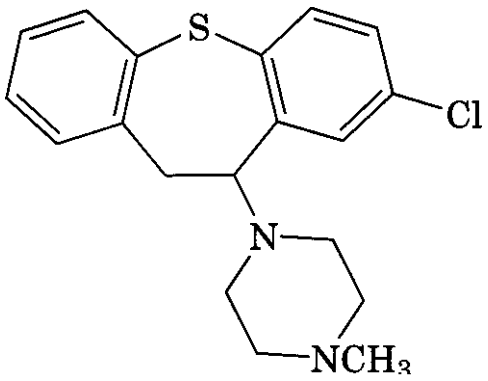


(45)

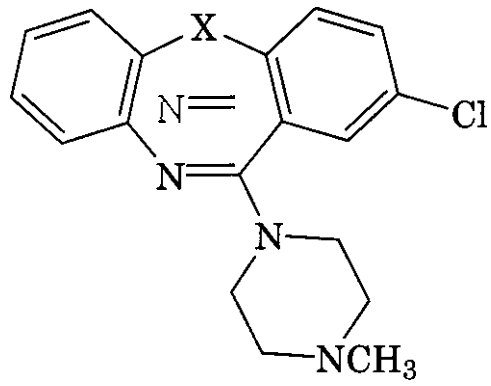


(46)

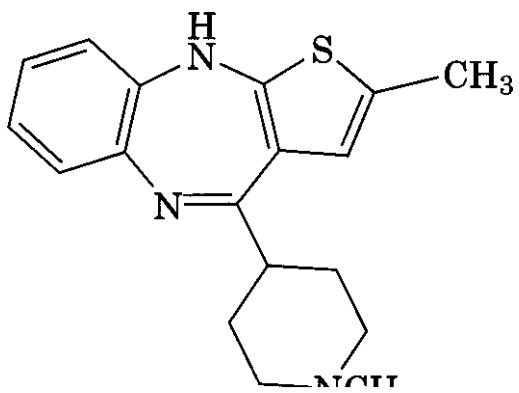
Another important class of neuroleptics is the dibenzazepines, which are represented graphically in Fig. 10.2. N-Methylpiperazinodibenzo[b,f](1,4)diazepines and related azepines, oxazepines and thiazepines, have all been tested clinically and have been shown to be effective in schizophrenic patients (314, 315) Compounds of this class produce sedation, as well as antiadrenergic and anticholinergic effects. Pronounced extrapyramidal symptoms are also generally observed with this family of agents. An important exception is the dibenzodiazepine clozapine (**50**), which is an unusual antipsychotic agent in that it



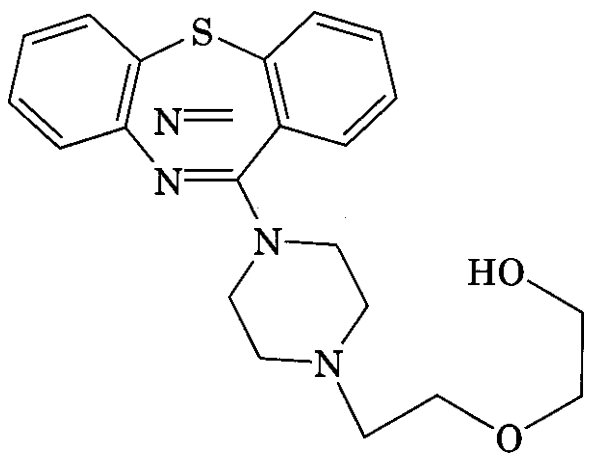
(47)



(48) X = **O** oxyclozapine
(49) X = S clotiapine
(50) X = NH clozapine
(51) X = CH_2 perlapine



(52)



(53)

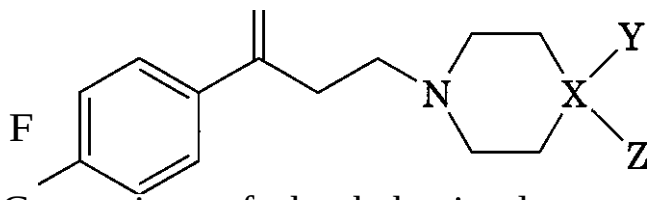
Figure 10.2.

does not produce extrapyramidal side effects. Indeed, clozapine has been used to ***suppress*** the motor effects of **tardive dyskinesia** (316). The reasons for this unique profile are a matter of continuing debate and research.

Two drugs related to clozapine include the almost identical compound, olanzapine (**52**), and quetiapine (53).Both confer similar **anti**psychotic profiles and a minimal propensity to elicit extrapyramidal side effects (317, 318). Olanzapine has also found use in the **treat**ment of **tardive dyskinesia** (319).

6.3 Butyrophenones

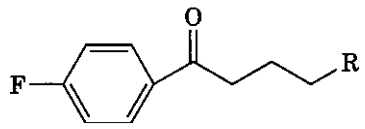
The butyrophenone antipsychotics were discovered during studies of modified meperidine derivatives (320). These compounds, which possess a tertiary amine at the fourth carbon of the **butyral** chain, could be made to possess minimal analgesic activity by the addition of a substituent at the 4-position of the aromatic ring. Many of the clinically tested compounds of this class showed high neuroleptic potency relative to chlorpromazine. The most effective compounds of this class are of the following general structure.



Comparison of the behavioral potencies and **dopamine** D_2 receptor binding of some of these compounds are displayed in Table 10.6. The first of these drugs to be introduced for treatment of psychosis was haloperidol (54). The relatively low toxicity of haloperidol relative to chlorpromazine resulted in its wide acceptance and it remains an important drug.

Related to haloperidol are methylperidol (**55**), trifluperidol (**56**), and compound (57). Comparison of these analogs shows that **po-**

Table 10.6 Behavioral and Dopamine D_2 Binding Data for Selected Butyrophenones



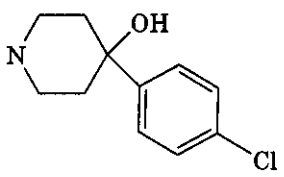
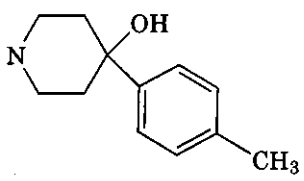
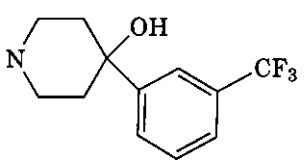
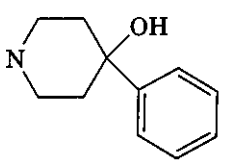
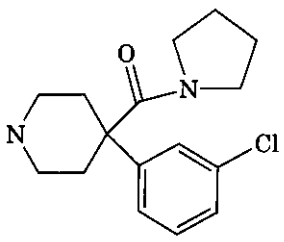
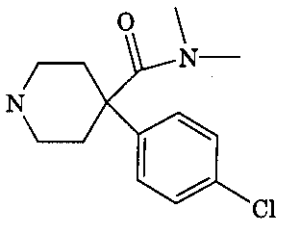
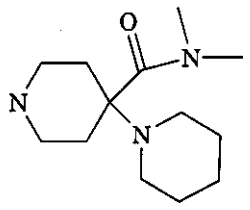
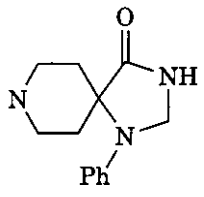
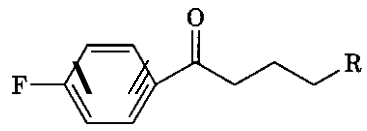
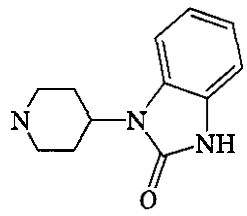
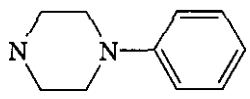
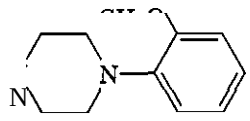
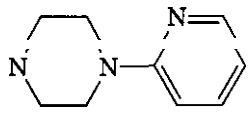
No.	Name	R	Dog Jumping Box Test[a] (Refs. 314, 320)	Rat: Jumping Box Test[a]	Rat: Amphetamine Stereotypy[b] (Ref. 321)	[^{3}H] Haloperidol Binding IC_{50} (nM) (Ref. 322)
(1)	**Chlorpromazine**		1	1	1	29
(54)	Haloperidol		50	17	35	1.5
(55)	Methylperidol		17	10	33	1.9
(56)	**Trifluperidol**		50	33	33	2.1
(57)	**Peridol**					47[c]
(58)	Haleperidide		8	5	10	
(59)	Paraperidide		50	5	10	
(60)	**Floropipamide**		2.5	0.1	0.2	
(61)	Spiperone		500	100	33	0.25

Table 10.6 (Continued)



No.	Name	R	Dog Jumping Box Test[a] (Refs. 314,320)	Rat: Jumping Box Test[a]	Rat: Amphetamine Stereotypy[b] (Ref. 321)	[^{3}H] Haloperidol Binding IC_{50} (nM) (Ref. 322)
(**62**)	Benperidol		500	33	33	0.33
(**63**)	Butropipazone		1.6	0.5	1	
(64)	Fluoanisone		5	3	3	3.8
(65)	Azaperone		**ND**	**ND**	**9**	10

"Animals are trained to jump to avoid an electric shock prepulsed by an auditory signal. Potency in inhibiting this avoidance relative to chlorpromazine

[b]Stereotypical gnawing and chewing in response to 10 mg/kg amphetamine I.V.

[c][^{3}H] spiperone binding, datum from Ref. 323.

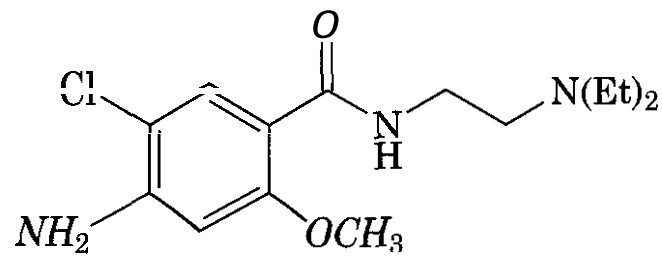
tency and dopamine binding are enhanced by substitution on the piperidino phenyl ring. Replacement of the tertiary hydroxyl of haloperidol by amides as in **(58)** and **(59)** produced effective compounds, as did simultaneous replacement of the aromatic by piperidine (**60**).

A cyclic variation of the 4-amino-4-carboxamido pattern can be seen in spiperone (**61**), which possess high affinity for the D_2 receptor. Similar *activity is observed for benzimidazalone* (**62**).

N-Aryl piperazine derivatives (X = N) tend to show activities that are significantly attenuated relative to the piperidines. A substituent (**64**) or an electron lone pair (**65**) adjacent to the point of attachment to the piperazine is beneficial.

6.4 Benzamides

The origin of the benzamide antipsychotics arose from the astute observation that rodents who were administered the antiemetic agent metoclopramide **(66)** exhibited behavioral properties consistent with antagonism of dopamine receptors **(325).** Metoclopramide initi-

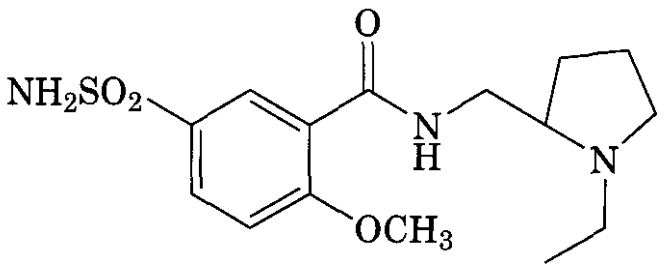


(66)

ated an acute dystonic reaction in a subset of patients taking the drug (326), a phenomenon that could be explained by dopamine blockade within the striatum.

Medicinal chemistry efforts in this area quickly determined that the 2-methoxybenzamide substructure was crucial to the elicitation of behavioral effects in rodents. The 2-me-

thoxybenzamide antiemetics metoclopramide, clebopride (**67**) and sulpiride (**68**) were compared for their ability to act as dopamine antagonists in rodents against tigan (**69**), which

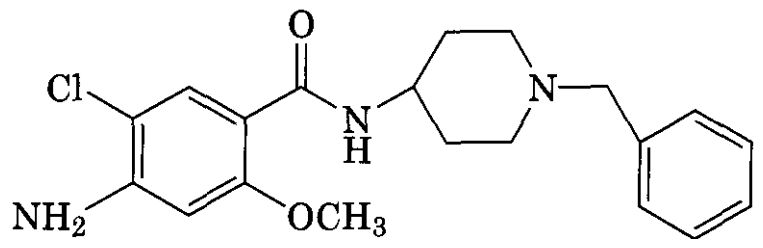


(67)

lacks this substituent. While all of these compounds inhibited apomorphine induced circling in animals with unilateral nigrostriatal dopamine lesions, tigan did so only at doses 7–100 times higher than the other agents. Similarly, the doses of tigan required to elevate the dopamine metabolite homovanillic acid (HVA) in striatal and limbic area were 8–200 times higher (327).

Benzamides with 2-pyrrolidinylmethyl side-chains were an area of intense investigation. Some representative examples are shown in Table 10.7. Optimal 1-substituents of the pyrrolidine ring were small alkyl, cycloalkyl, or benzyl. In the case of alkyl substituents, the (*S*)-enantiomers were more active at blocking D_2 receptors, whereas the reverse was true for the 1-benzylpyrrolidine derivatives (327). Removal of the N-substituent resulted in diminished activity.

One of the earliest benzamides to be put into clinical practice was sulpiride. A classic screening method for selection of compounds having a preference for mesolimbic over striatal D_2 receptors is the determination of a high ratio of blockade of DA agonist-induced stereotypies (striatal) versus agonist-induced hyperactivity (limbic). Based on this behavioral



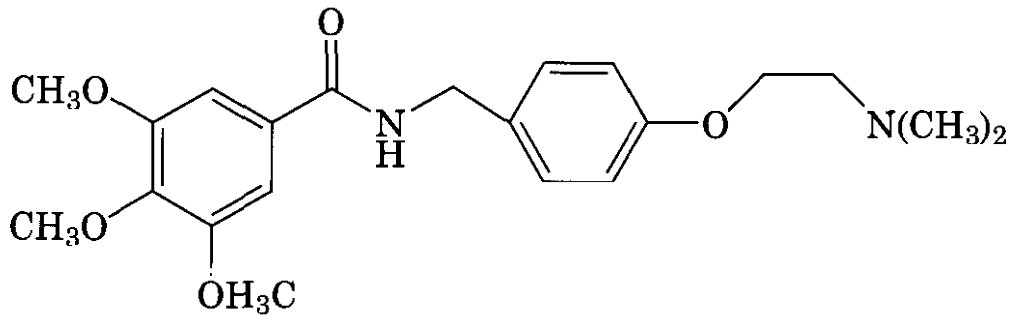
(69)

screen, sulpiride displayed an atypical profile in that the stereotypy/hyperactivity ratio for (*S*)-sulpiride was approximately three versus a value of one for haloperidol.

Although atypical, sulpiride had relatively low antipsychotic potency (328), possibly because of low bioavailability and poor brain penetration (329). These liabilities, coupled with the apparent atypical profile of sulpiride, spurred research in the benzamide area.

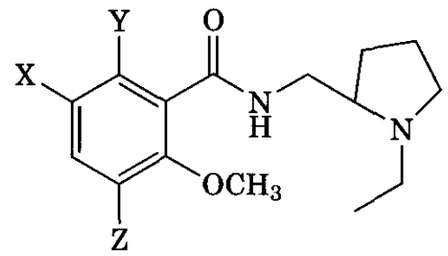
An immediate improvement in the drug properties could be obtained by the replacement of the sulfamido group with a more lipophilic group such as halogen. Compound (70) inhibited apomorphine-induced stereotypy in rats at a 30-fold lower dose than sulpiride (330). Whereas the racemic 2,6-dimethoxy derivative (71) was equipotent with sulpiride against apomorphine mediated behaviors, the S-enantiomer of the 3-bromo-2,6-dimethoxy derivative (remoxipride) (72) closely resembled haloperidol in its ability to block apomorphine-induced hyperactivity. The sevenfold ratio of stereotypy to hyperactivity for remoxipride suggested a low propensity to produce EPS in the clinic. Remoxipride was briefly used in clinical practice, but the development of aplastic anemia in a minority of patients led to its eventual withdrawal (331).

The dibromo derivative (**74**) showed increased activity over remoxipride, although the latter showed a better separation in the



(68)

Table 10.7 Behavioral Properties and D_2 Binding Affinities of 2-Methoxybenzamides



No.	Isomer	X	Y	Z	Blockade of Apomorphine ED_{50} (μmol/kg I.P.) Hyperactivity	Stereotypy	Ratio Stereotypy/ Hyperactivity	[^{3}H]Spiperone Binding: (IC_{50}, n*M*)
(68)	S	SO_2NH_2	H	H	65.6	212	5.8	233
(70)	S	Br	H	H	3.0	6.3	2.1	46
(71)	RIS	H	OCH_3	H	71	101	1.4	15,300
(72)	S	Br	OCH_3	H	0.86	6.5	7.6	1570
(73)	R	Br	OCH_3	H	120	>196	>1.6	>10,000
(74)	S	Br	OCH_3	Br	0.44	2.1	4.8	—
(75)	RIS	Cl	OCH_3	H	30.8	42.7	1.4	—
(76)	RIS	Cl	OCH_3	Cl	3.1	5.4	1.74	—
(77)	S	Br	OH	H	0.06	0.32	5.3	12
(78)	S	H	OH	Br	6.2	6.6	1.07	56
(79)	S	Cl	OH	H	0.20	0.87	4.4	39
(80)	S	H	OH	Cl	0.87	2.5	3.1	64
(81)	S	Cl	OH	Cl	0.13	1.80	13.8	32
(82)	S	Et	OH	Cl			−7	0.09
Haldoperidol					0.29	0.27	0.93	12

stereotypy/hyperactivity ratio. Substitution of chlorines for bromine in both cases led to diminished activity and a more typical behavioral profile (75 and 76).

Analysisofthehuman metabolites of remoxipride identified two products resulting from demethylation (332). The 3-bromo-6-methoxy salicylamide (77) (FLA 797) showed a much greater affinity for the D_2 receptor than did either its isomer (FLA 908) (78) or the parent compound. This result lead to speculation that the neuroleptic activity of remoxipride was caused by this metabolite.

The 100-fold increase in affinity of FLA 797 over remoxipride was puzzling. Determination of the logP values for remoxipride and FLA 797 found them to be nearly identical (2.1 versus 2.0), indicating a similar partitioning between octanol and water. This result is somewhat surprising because the free hydroxyl group of (77) would be expected to associate with water. A larger difference was found for logP where FLA 797 was found to be more lipophilic than remoxipride (1.7 versus 0.7). However, this difference seems unlikely to fully explain the difference in in uitro activity.

X-ray crystal structures of both compounds were performed and indicated major differences in the conformations of the two benzamides (333). For remoxipride, the phenyl group and the amide carbonyl are oriented almost perpendicular to each other. This orientation precludes the formation of a hydrogen bond between the amide hydrogen and the ortho methoxy group. Such hydrogen bonds have been noted in other types of ortho benzamides (334).

The 6-methoxysalicylamide displayed a planar conformation wherein a pseudo-ring is stabilized by a hydrogen bond between the phenol and the carbonyl oxygen. As depicted in Fig. 10.3, this conformation also allows for an additional interaction between the amide hydrogen and the remaining ortho methoxy. Such additional hydrogen bonds have been found in the related 2-methoxysalicylamides raclopride (81) (335) and eticlopride (82) (336). The exceptional dopamine D_2 affinity of these compounds relative to remoxipride suggests that this planar orientation of the benzamide substructure is the preferred conformation for binding of the benzamide neuroleptics to the receptor.

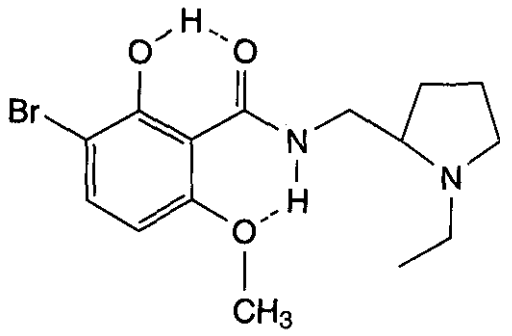
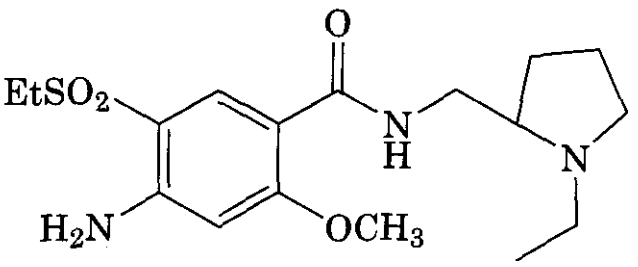


Figure 10.3. Solid state conformation of FLA 797.

Raclopride presents an exceptional separation between striatal- and limbic-mediated behaviors. Although it has been suggested that raclopride is able to differentiate between regional subclasses of D_2 receptors (337), this has never been clearly substantiated in the laboratory for this or any antipsychotic at the level of receptor binding.

Another benzamide of interest is amisulpiride (83). Clinical trials have shown



(83)

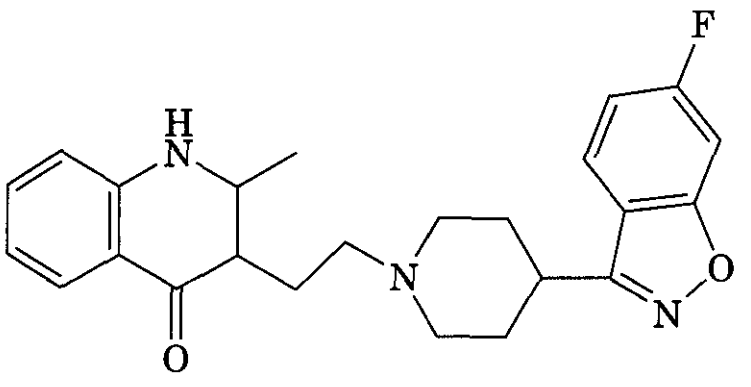
amisulpiride to be efficacious against the positive symptoms of schizophrenia at doses that have only a low propensity to induce EPS effects (338). In accordance with other benzamide neuroleptics such as sulpiride and raclopride, amisulpiride is highly selective for the D_2 and D_3 receptor subtypes for which it, like most antipsychotics, has similar affinity ($-2\,nM$). The high selectivity of (83) for the D_2 receptor family, coupled with its clinical profile, bring into question previous theories that the atypical nature of drugs such as clozapine is a result of their interaction with one or a number of other non-dopaminergic receptors (339,340).

Considerable evidence exists that D_3 receptors are presynaptic autoreceptors that are preferentially localized in limbic area (341–343). The atypical nature of amisulpiride

has been speculated to result from its preference for limbic presynaptic autoreceptors at doses below those that produce significant postsynaptic D_2 receptor occupancy (344). Its atypical profile and that of other drugs may also be derived from an ability to block limbic D_3 receptors, which are increased in schizophrenia and normalized by a variety of antipsychotics (341).

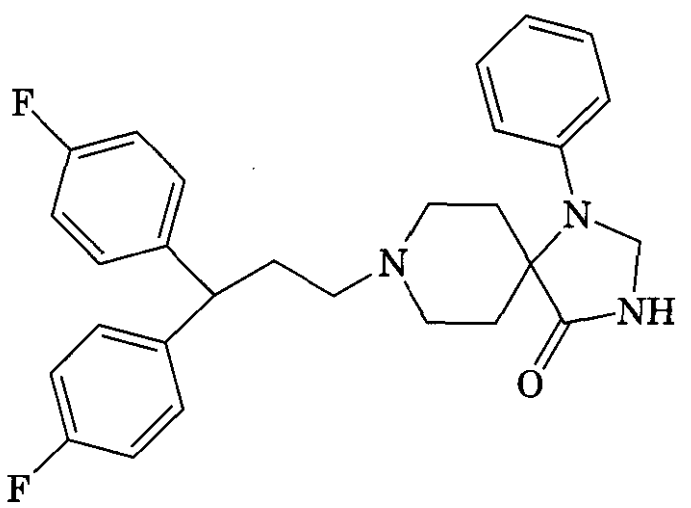
6.5 Miscellaneous Classes

In addition to the tricyclics, the butyrophenones, and the benzamides, a variety of other structural types have been exploited for their neuroleptic potential. Risperidone (84) is representative of a series of conformationally restricted butyrophenones (345).



(84)

A primary metabolic pathway for the butyrophenone class of compounds is the reduction of the carbonyl group. Modification of this functionality led to the development of the diphenylbutylpiperidines as long-acting antipsychotics. Fluspirilene (85) and pimozide (86) are the direct analogs of spiperone and benperidol.



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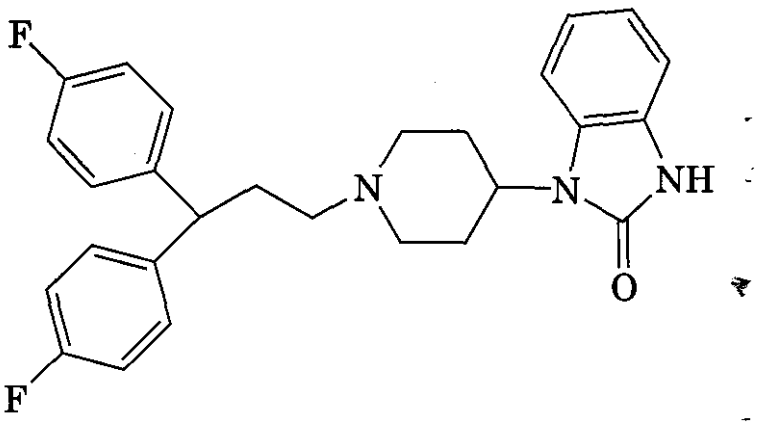
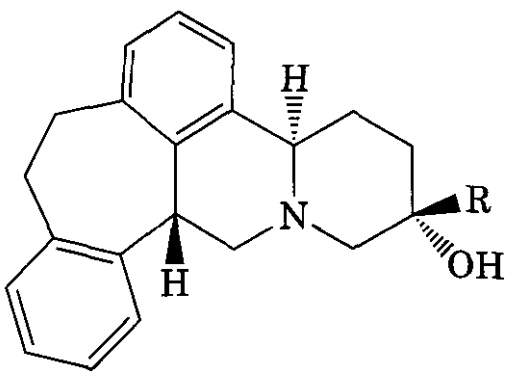


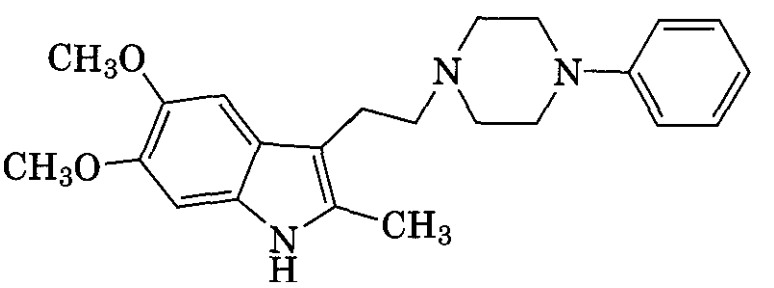
(86)

Butaclamol (87) and dexclamol (88) are effective neuroleptics that are distantly related to the tricyclics. Their use is limited by a high propensity to initiate EPS.

The clinical effectiveness of oxypertine (89) led eventually to the evaluation of the tetralone derivative (90)(346). A blending of the indole and tetralone structures of (89) and



(87) R = t-butyl
(88) R = isopropyl



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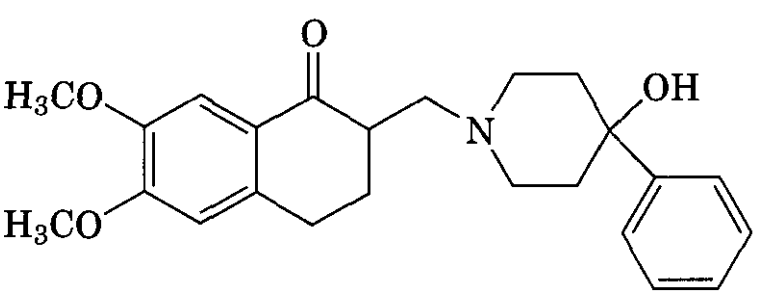
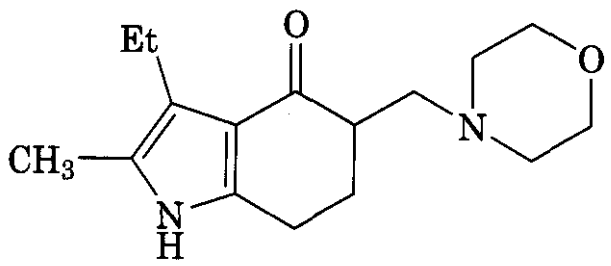
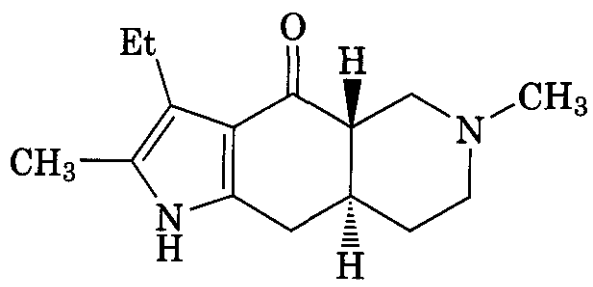


(90)

(90)produced a series of Mannich bases of pyrrole ketones, such as molindone (**91**), which displays a typical neuroleptic profile (347). The conformationally restricted analog (92)



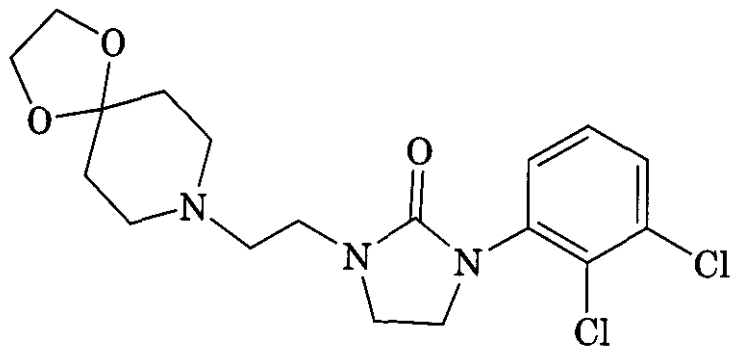
(91)



(92)

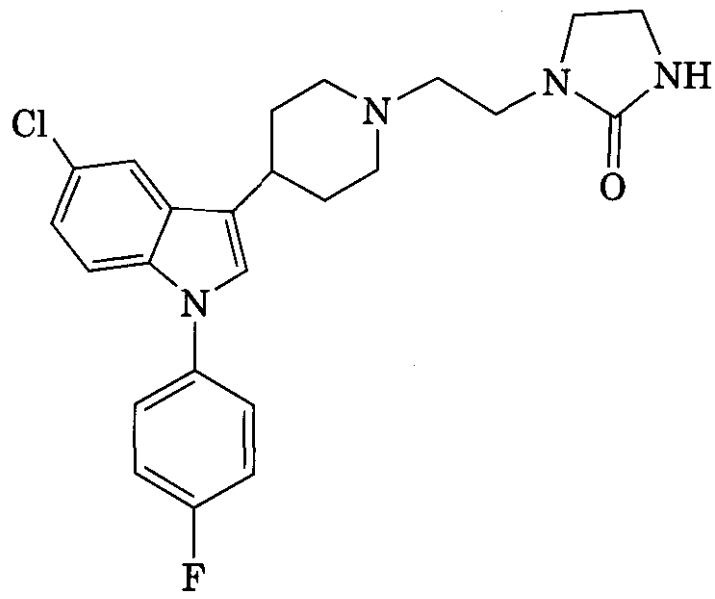
has antipsychotic activity in the avoidance test in rats in the potency range of haloperidol and over five times the potency of molindone (348). This result establishes the special requires of the binding pharmacophore within this series.

Imidazalones presented as a novel structural class of neuroleptic agents when AL 1965 (93) was investigated in the treatment of



(93)

schizophrenic psychosis (349). Although (93) resembled haloperidol in efficacy, a high incidence of extrapyramidal effects were reported. Another oxindole, sertindole (**94**), has been shown to be active against both positive and negative symptoms (350).



(94)

7 PHARMACOKINETICS, BIOTRANSFORMATION, AND DRUG INTERACTIONS

7.1 General Considerations

The antipsychotic drugs represent a chemically diverse group of compounds with varied pharmacokinetic properties (351–353). Most antipsychotics are highly lipophilic molecules (354, 355) that cross lipoidal membranes readily. They tend to distribute rapidly to tissues that are well supplied with blood, especially the lungs, skeletal muscle, liver, and brain. When administered orally, most antipsychotic drugs are well absorbed, but many undergo substantial first pass metabolism. Consequently, systemic bioavailabilities (F) for antipsychotics vary over a broad range (F = 10–80%). The comparatively high fraction of drug bound to plasma proteins (f) = 75–99% does not markedly affect the pharmacokinetics of most antipsychotic drugs, because of their comparatively large volumes of distribution (V_d = 20 L/kg; range, 30–60 L/kg) and the low affinity of binding to plasma proteins. Systemic clearances of these drugs are generally high (approximately 30–60 L/h) as are their hepatic extraction ratios. Most antipsychotic drugs exhibit biphasic (or polyphasic) pharmacokinetics with relatively short plasma half-lives (t_{max} = 2.5 h; range, 1–8 h) and long elimination half-lives ($t_{1/2}$ = 24 h; range, 8–150 h). The renal excretion of unchanged drug is negligible for most clinically useful antipsychotic drugs.

The main modes of biotransformation of antipsychotic drugs involve oxidative processes mediated by genetically controlled microsomal oxidases (phase I), followed by conjugative (phase II) reactions in many cases. Enterohepatic recycling may occur for certain phase II metabolites, especially glucuronides or ring-hydroxylated metabolites. Although a majority of phase I metabolites are inactive, or considerably less active than the parent drug, some ring hydroxylated and N-dealkylated metabolites have significant activity (356359) and may contribute to the overall pharmacological properties of the administered drug. The existence of active metabolites, each with distinct pharmacokinetic properties compared with the parent drug, complicates attempts to correlate assays of parent drug in the plasma with therapeutic responses and adverse reactions.

The steady-state concentrations of antipsychotic drugs after multiple dosing have been measured to establish a relationship between plasma concentrations and clinical efficacy or to monitor adverse effects (360–363). The majority of drugs in the class seem to exhibit linear pharmacokinetics, despite the wide interindividual variations in pharmacokinetic properties observed for specific agents. Linear pharmacokinetics allows the dosage to be readily adjusted if the steady-state plasma concentration is in the sub-therapeutic or toxic range.

7.2 Phenothiazines

The prototypical antipsychotic drug chlorpromazine (CPZ) has been the focus of more pharmacokinetic and biotransformation studies than any other antipsychotic drug. The pharmacokinetic parameters for CPZ were shown by Yeung et al. to display high interindividual variability even in healthy human volunteers (364). Thus, wide between-subject variations in half-life, volume of distribution, steady-state volume of distribution, and mean residence time values are observed, whereas systemic clearance is less variable (364). CPZ experiences extensive first pass metabolism leading to a systemic oral bioavailability of less than 10% relative to a single IV dose (365) or an average of around 30% relative to a single IM dose (366). Approximately 91–99% of CPZ in plasma is estimated to be protein-bound (367).

The net effect of first pass metabolism on the therapeutic efficacy of CPZ is difficult to evaluate because of the formation of numerous metabolites, some of which possess biological activity (356, 357). Numerous sites of attack by microsomal oxygenases are possible, and indeed most of these reactions occur in experimental animals and/or humans. In fact, of the 168 possible metabolites postulated for CPZ (368), more than 45 have been isolated from various human body fluids (357).

Phase I reactions of chlorpromazine that have been reported include the following: oxidative N-demethylation to yield the corresponding primary and secondary amines (368–371); aromatic hydroxylation to yield phenols (369–376); N^3-oxidation to yield the N-oxide (371, 372, 376, 377); S-oxidation to yield sulfoxide (369–373) and sulfone (378); oxidative deamination of the amino propyl side-chain (probably preceded by *N*-demethylation) to yield the carboxylic acid (371, 379); N^1-dealkylation to yield chlorophenothiazine and its oxidized metabolites (373); *N*-oxidation of the demethylated amines to yield hydroxylamines (380, 381); and both oxidative (373,375) and reductive (382,383) dechlorinations. Direct N-glucuronidation of CPZ to form the quaternary N-glucuronide, a phase II reaction, has also been reported (384,385).

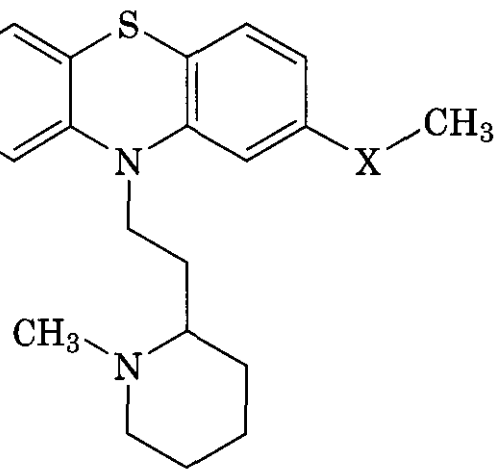
Ring hydroxylation occurs mainly at the 7 position of CPZ (369–375) with lesser amounts of 3-hydroxy (371, 374, 375) and trace amounts of 8-hydroxy metabolites also formed. Further hydroxylation of the 3- and 7-hydroxy metabolites to form 3,7- and 7,8-dihydroxy derivatives may also occur (376, 386). Hydroxy metabolites of CPZ are excreted in the urine largely as their *O*-glucuronides.

Numerous experimental animal studies have been carried out on several major and minor metabolites of CPZ to determine if its therapeutic and/or toxic actions can be attributed to one or more metabolites. These studies are described in detail in the previous edition of this series (387) and will not be recounted here.

Despite the keen interest in CPZ metabolites, only 7-hydroxy-CPZ has been evaluated

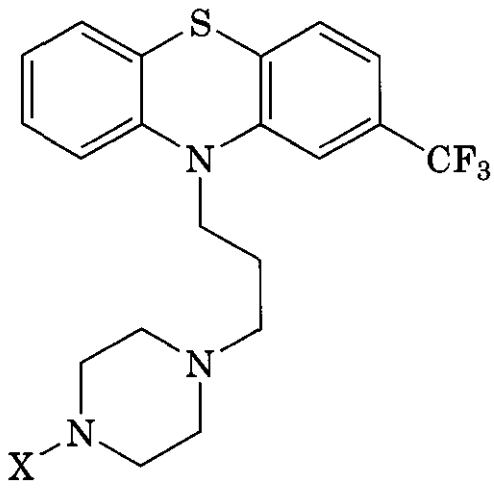
and found to be effective in schizophrenic patients (388). This metabolite has also been found in the CSF of CPZ treated patients in concentrations comparable with that of the parent drug (389). CSF concentrations of *N*-demethyl chlorpromazine in patients treated with CPZ are lower (390). Chetty et al. (391) suggest that six CPZ metabolites achieve sufficient plasma concentrations to influence the overall therapeutic response. The ratio of 7-hydroxy-chlorpromazine to chlorpromazine sulfoxide was found to be greater in the plasma of responding than in non-responding schizophrenic patients (392, 393).

In general, other phenothiazine antipsychotic agents seem to undergo biotransformations similar to those that occur with CPZ (370,394–404).Some differences exist, based on specific ring or side-chain substituents. Thioridazine (95) is oxidized on the 2-thio-



(95) X = S
(96) X = SO

methyl group to form the sulfoxide, mesoridazine (96)(397–400), a clinically employed antipsychotic drug in its own right, and the sulfone, sulforidazine (397–400). The side-chain piperidine ring is oxidized to form lactam metabolites. The asymmetric center present in the piperidinyl ethyl side-chain allows the formation of diastereoisomeric ring sulfoxide (404,405) and N-oxide (405) metabolites. Piperazinylpropyl-substituted phenothiazines such as prochlorperazine (**25**), trifluoperazine (**96**), perphenazine (**26**), and fluphenazine (96) form the same 10-(3-aminopropyl) phenothiazine metabolites that result from *N*,*N*-demethylation of the corresponding promazines; apparently, this is a result of successive oxidations on the piperazine ring (406–408).



(97) X = CH_3
(98) X = CH_2CH_2OH

After oral administration of thioridazine to schizophrenic patients, the 5-oxide achieves the highest plasma level, followed by mesoridazine, sulforidazine, and N-desmethyl thioridazine (356. 413). On the other hand. concentrations of thioridazine in postmortem brain samples were higher than any of its metabolites in schizophrenics treated with the drug (414). Thioridazine is bound to plasma proteins to a higher degree than are either of its active metabolites (415).Individual patient responses and side effect profiles of thioridazine may be influenced by debrisoquine hydroxylator status (mediated by the 2D6 isoform of cytochrome P450, CYP2D6), because higher plasma levels of thioridazine and its inactive 5-sulfoxide metabolite were seen in Door, compared with extensive, debrisoquine metabolizers among healthy human volunteers administered a single oral dose of thioridazine (416). Conversely, lower plasma levels of the active metabolites mesoridazine and thioridazine were observed in poor metabolizers than in extensive metabolizers (416). Both 2- and 5-sulfoxidation of thioridazine seem to be mediated by cytochrome P450 oxygenases, whereas *N*-oxidation is catalyzed by a flavin monooxygenase (417). Drug interaction studies of thioridazine with the serotonin selective reuptake inhibitor (SSRI)antidepressants fluoxetine and fluvoxamine, known inhibitors of CYP oxidations, indicate the potential for clinically significant interactions (418,419).

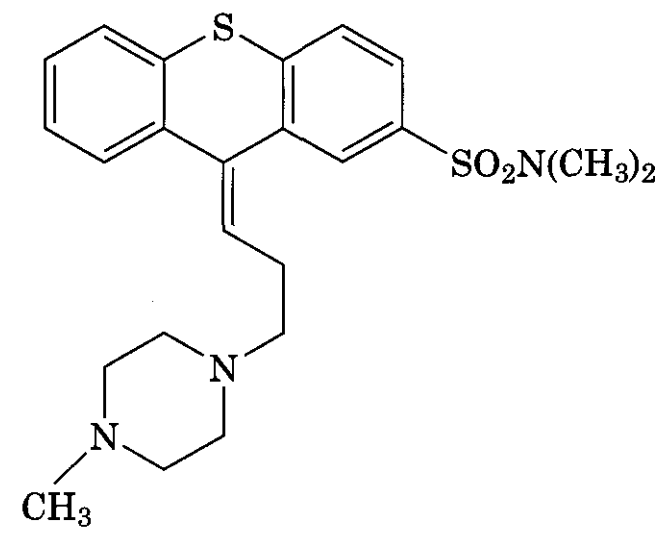
Of the numerous phenothiazines possessing piperazinylpropyl side-chains, fluphenazine has been the most studied in terms of its pharmacokinetic properties. The apparent reason for the interest in fluphenazine is that it is available in both oral and depot injectable dosage forms. Oral fluphenazine experiences extensive first pass metabolism (419–422) to form numerous metabolites, all of which are less active than the parent drug (410, 420–426). A distribution study of orally administered fluphenazine using radioimmunoassay established that brain concentrations of fluphenazine exceeded plasma concentrations by 10 to 27 times (420). The inactive sulfoxide was the major metabolite found in brain, while brain levels of 7-hydroxy-fluphenazine and fluphenazine-N-oxide were very low (420). It was concluded that metabolites do not contribute to the pharmacology of fluphenazine and that only the plasma level of the parent drug needs be monitored for correlation to therapeutic response.

A comparison of biotransformation patterns in patients receiving oral fluphenazine versus intramuscularly injected fluphenazine decanoate revealed a significant first pass effect (422,427). Thus, plasma ratios of fluphenazine sulfoxide and 7-hydroxy-fluphenazine to fluphenazine were much higher after oral administration compared with intramuscular administration of fluphenazine decanoate. The plasma ratios of fluphenazine-N-oxide to fluphenazine did not differ between the two dosage forms. In a study measuring fluphenazine serum levels during reduced dose fluphenazine decanoate maintenance therapy at two dose levels. it was determined that serum levels could be monitored at maintenance doses (429,430).

The hepatic clearance of perphenazine (26) was found to depend on the CYP2D6 genotype (430). Pollock et al. observed that elderly patients treated with perphenazine who were efficient CYP2D6 metabolizers developed fewer EPS than patients who were poor 2D6 metabolizers (431). They hypothesized that the principal perphenazine metabolite, N-dealkyl perphenazine (DNPZ) might be responsible (431). Consistent with this hypothesis, the CNS side effects of perphenazine were potentiated in another study by the coadministration of paroxetine, an SSRI that inhibits CYP2D6 (434). Indeed, DNPZ exhibited a more favorable $5HT_2/D_2$ ratio of receptor potencies, suggestive of atypical activity (432), than did perphenazine or 7-hydroxy-perphenazine (433).

7.3 Thioxanthenes

Thiothixene (99) is the only thioxanthene derivative that continues to be marketed for the treatment of psychotic disorders in the United



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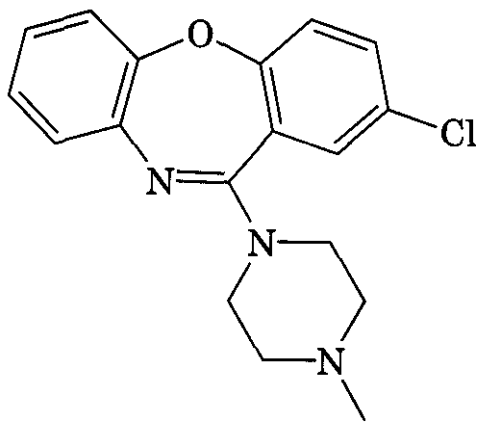
States. The two principal metabolites, thiothixene sulfoxide and N-desmethyl thiothixene, are formed by CYP 450 oxidations (435). The CYP isozymes involved are not known with certainty. However, the hepatic clearance of thiothixene was increased by the CYP3A4 inducer carbamazepine and decreased by the CYP3A4 inhibitor cimetidine (436). The CYP2D6 inhibitor paroxetine, however, had no effect on thiothixene clearance (437).

7.4 Dibenz(1,4)Oxazepines

The pharmacokinetics of loxapine (100) have been reviewed along with its pharmacodynamic properties (438). The drug is rapidly absorbed after oral absorption, with peak plasma levels being achieved within 1–2 h. No oral bioavailability data are available for loxapine, but it is evident that it is extensively metabolized in the liver and rapidly distributed into the tissues.

Loxapine undergoes several phase I oxidations (439, 440), including the following: *N*-demethylation to form amoxepine, a clinically



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useful antidepressant drug (358); aromatic hydroxylation to form 7-hydroxy- and 8-hydroxy-loxapines; and N-oxidation on the 4-piperidinyl nitrogen atom to form the N-oxide. The phenolic metabolites are excreted in the urine as glucuronide and sulfate conjugates (439, 440). Some 8-hydroxy-loxapine is converted to the methyl ether (439, 440). Direct *N*(4)-glucuronidation to form the quaternary glucuronide also occurs (441).

7.5 Dibenzo(1,4)Diazepines

The pharmacokinetics of clozapine (50) have been studied extensively (451–453). After single and multiple oral doses, plasma concentrations of clozapine reach peak levels in 4 h and decline in a biphasic manner consistent with first order absorption and elimination (453). The systemic bioavailability of clozapine is approximately 50% after oral administration and results from a moderate amount of first pass metabolism. The elimination half-life of clozapine ranges from 6 to 33 h (average, 16 h) (452). The drug is 90–95% bound to plasma proteins. Clozapine is extensively metabolized, predominantly through the cytochrome p450 enzymes 3A4 and 1A2 (698), resulting in only 2–5% of unchanged drug appearing in the urine and feces. Urinary excretion of metabolites accounts for approximately 50% of an orally administered dose of clozapine, with about 30% recovered as metabolites in the feces (451).

Clozapine is metabolized by a number of oxidative pathways (454–457). The most important of these are N-demethylation and *N*-oxidation on the N^4-piperazinyl nitrogen to give the major metabolites, desmethyl clozapine and clozapine-N-oxide (454–457). Oxidation at the piperazine 3-position to form the lactam (178), aromatic hydroxylation (454, 455, 457), and presumed oxidative displacement of the 3-chloro substituent by hydroxyl and thiomethyl groups (456) have also been reported. The structures of phenolic metabolites are not known with certainty, but are suggested to be 2-hydroxy-clozapine and 7-hydroxy-clozapine based on chemical synthesis and radioimmunoassay (455). Three unidentified polar metabolites, presumed to be phenolic and constituting up to 20% of total metabolites, were formed in human liver cell preparations *in vitro* (457). In a more recent study using ^{14}C-labelled clozapine in human volunteers (458), the major metabolic pathways were found to involve N-demethylation, N-oxidation, aromatic oxidation at the 7- and 8-positions, and sulfate and glucuronide conjugations. The major urinary components were 8-hydroxy-desmethyl clozapine, its glucuronide, 7-hydroxydesmethyl clozapine, and its sulfate, and clozapine-N-oxide. The primary fecal metabolite was clozapine-N-glucuronide. In a separate *in vivo* study, a relatively small amount (about 3%) of the *N*(4)-piperazinyl glucuronide of clozapine was identified (441). Olanzapine (52) is extensively metabolized by a combination of oxidation and glucuronidation reactions in animals and in man (468–470). Only about 10% of unchanged drug is excreted in the urine and feces. A novel tertiary amine glucuronide, olanzapine-10-N-glucuronide, is the principal urinary (13%) and fecal (7%) metabolite in humans (468,470). Considerably lesser amounts of the quaternary 4-N-glucuronide are also formed. Oxidative N-dealkylation, formation of the 4'-N-oxide, and oxidation of the thienylmethyl group to give the 2-hydroxymethyl- and 2-carboxylic acid derivatives also occur (468). The metabolic profiles of olanzapine in various animal species were similar to that in humans in many respects (469). The major differences were that the 10-*N*-glucuronide was not detected in any other species and that aromatic hydroxylation to form various 7-hydroxy derived metabolites was a significant pathway in some species (469).

Some of the oxygenases involved in specific olanzepine oxidations have been determined (472). Oxidative demethylation of olanzepine

is apparently catalyzed by CYP1A2, because this transformation is enhanced by cigarette smoke and the inducer carbamazepine and inhibited by fluvoxamine. The formation of the 2-hydroxy-methyl metabolite catalyzed by CYP2D6 is considered to be a minor pathway and not likely to lead to clinically significant drug interactions (472). A flavin nonooxygenase (FM03) is thought to be responsible for the 4-N-oxide (472).

Oxidative metabolism of clozapine was found to correlate with caffeine metabolism (462) and is thus largely carried out by cytochrome P4501A2 (CYP1A2). No correlation with CYP2D6 polymorphism was found (461, 462). Several interaction studies of clozapine with SSRI antidepressants have been reported (465–469). Fluvoxamine increased plasma concentrations of clozapine in schizophrenic patients (463, 464), presumably through inhibition of CYP1A2 catalyzed N-demethylation (465). Fluoxetine was found to increase the plasma concentrations of clozapine and its major metabolites, suggesting that this SSRI must interfere with pathways other than *N*-demethylation and N-dealkylation (466). Two other SSRIs, paroxetine (463) and citalopram (467), had no apparent effects on clozapine levels.

The biotransformation (468–470) and clinical pharmacokinetics (471) of olanzapine have been investigated extensively. The drug exhibits dose proportional linear pharmacokinetics in therapeutic dose ranges (471). Olanzapine is bound to both albumin and α-1-acid glycoproteins in the plasma. The mean elimination half-life in healthy adults was 33 h and the plasma clearance averaged 26 L/h.

7.6 Dibenz(1,4)Thiazepines

Quetiapine (53) is the only dibenz(1,4)thiazepine derivative currently available in the United States. Very few publications have been devoted to its biotransformation and pharmacokinetics (473, 474). Much of the following information has been obtained from data on file from the manufacturer (475).

Quetiapine exhibits dose proportional linear pharmacokinetics within the clinical dose range with accumulation that is predictable on multiple dosing. Clearance of quetiapine is largely through hepatic metabolism with a mean terminal half-life of about 6 h. Oral absorption of the drug is rapid with peak plasma levels achieved within 1.5 h. Quetiapine is widely distributed throughout the body with an average volume of distribution of 10 L/kg. The extent of plasma protein binding is 83% at therapeutic blood concentrations.

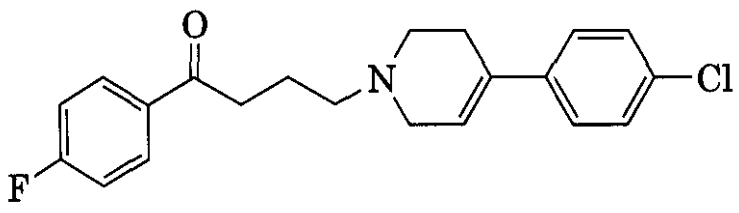
Quetiapine is extensively metabolized by cytochrome P450 oxidation. Less than 5% of unchanged drug is excreted. The major pathway involves CYP3A4 oxidation to the sulfoxide (473, 475). Other important reactions include oxidation of the side-chain alcohol to the carboxylic acid, aromatic oxidation to the phenolic 7-hydroxy-metabolite, and oxidative *N*-dealkylation of the entire side-chain (473, 475). Inducers of CYP3A4 (phenytoin and carbamazepine) significantly increased quetiapine clearance, whereas ketoconazole, an inhibitor of CYP3A4, significantly increased plasma levels of the drug (475).

7.7 Butyrophenones

The member of the butyrophenone class of antipsychotic drugs that has been studied the most extensively is haloperidol (476). The drug is rapidly and almost completely absorbed after oral administration. Significant first pass metabolism in the liver largely accounts for the systemic bioavailability of around 60% for haloperidol by the oral route (477). Haloperidol is 90–92% bound to plasma proteins. Peak plasma levels of haloperidol are achieved in 1.7–3.2 h, and the drug is rapidly distributed to well-perfused tissues such as the lungs, skeletal muscle, and brain, with a terminal half-life ranging from 6 to 33 h. Redistribution of haloperidol to adipose tissue apparently does not occur significantly (478).

Haloperidol undergoes extensive metabolism to form a myriad of primary and secondary metabolites (479–486). The principal phase I reactions involve the following: oxidative N-dealkylation to initially form 4-fluorophenylbenzoylpropionaldehyde (which is rapidly oxidized to the acid) and 4-(4-chlorophenyl)-4-hydroxypiperidine (479, 480, 483–485); N-oxidation to form haloperidol-*N*-oxide (485); and reversible reduction of the carbonyl group to the alcohol, or reduced haloperidol (481, 487). A phase II metabolite, haloperidol-*O*-glucuronide, accounts for as much

as 60% of total haloperidol metabolites excreted in the urine (486). Reduced haloperidol is also hydroxylated at the 2'-fluorophenyl ring position and this phase I metabolite is excreted in the urine as the sulfate and glucuronide conjugates (486). The quaternary *N*-glucuronide of haloperidol apparently does not form (441). Formation of the dehydration product of haloperidol, i.e., the 1,2,5,6-tetrahydroperidine derivative (**101**), is thought to



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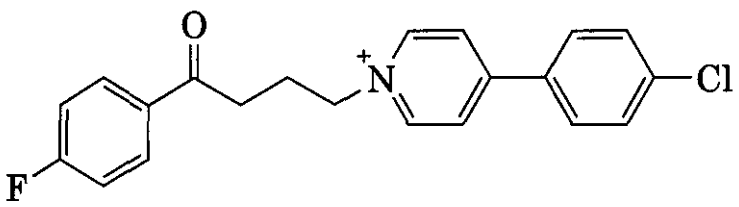
occur spontaneously **in** *vivo* (482–485). This metabolite, together with its N-oxide, are believed to be the sources of the corresponding pyridinium species (102) that has been identi-

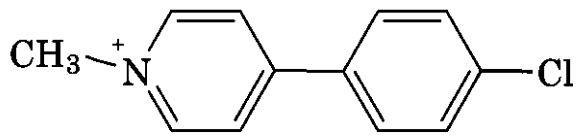


(102)

fied as a minor metabolite of haloperidol in animals and humans (482–485).

The biotransformation of haloperidol is decreased in poor, compared with extensive, metabolizers of debrisoquine (497). Plasma levels of reduced haloperidol on the other hand, are higher in poor metabolizers than in extensive metabolizers (497), presumably in part because of reduced oxidation of reduced haloperidol by CYP2D6 in poor metabolizers (488, 497). Other CYP2D6-mediated oxidations of haloperidol or reduced haloperidol may also contribute to the higher levels of reduced haloperidol in poor metabolizers. Systematic evaluations of the cytochrome P450 isozymes involved in the oxidative metabolism of haloperidol revealed a dominant role for CYP3A4 in the N-dealkylation process (498–501) and an important role in the oxidation of reduced haloperidol (498). CYP2D6 apparently is less important (498,500).

The structural similarity of the pyridinium metabolite (102) to 1-methyl-4-phenylpyridinium (MPP^+) (**103**), the toxic metabolic



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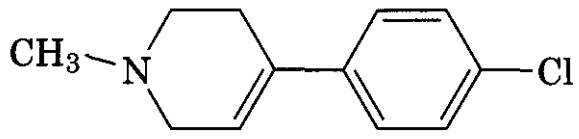
product responsible for the Parkinson's disease-inducing agent 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (**104**), prompted the

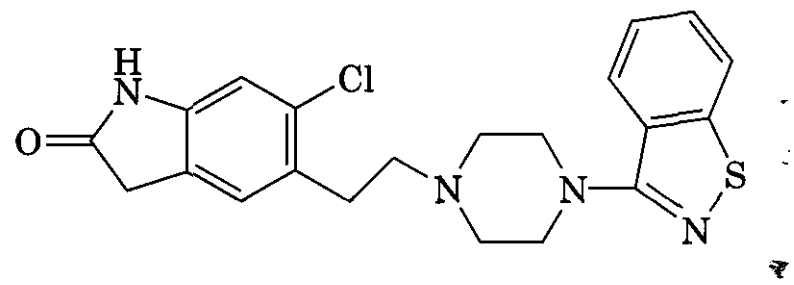


(104)

fascinating suggestion that this metabolite might be responsible for the persistence of EPS even after the discontinuation of haloperidol (484, 485, 502, 503). Indeed, (101) was found to be toxic to dopaminergic neurons in rat brain through inhibition of mitochondrial electron transport, a mechanism shared with MPP^+ (502). Both MPP and (102) exhibited comparable toxicity to serotonergic neurons, but MPP^+ was more toxic to DA-neurons (502). The pyridinium metabolite (**102**), like MPP^+, was also toxic to dopaminergic neuroblastoma cells in culture (503), but differed from MPP^+ in the time course of its toxicity.

Because (**102**) is found in rat brain after administration of haloperidol or its dehydrated metabolite (101) (504, 505), it is likely that despite its positive charge, (102) formed by hepatic metabolism crosses the blood brain barrier. It is also possible that some (102) is formed in the brain. In fact, (101) and (102) were found in postmortem brain tissue from schizophrenic patients treated with haloperidol (506). However, the presumed precursor (**101**), unlike MPTP, is not a substrate for MAO B, but is instead an irreversible inhibitor of the enzyme in human platelets (507). Several haloperidol metabolites, including (101) and (**102**), are potent inhibitors of ^{3}H-dopamine and ^{3}H-norepinephrine uptake in rat striatal and hippocampal slices (508). The pyridinium metabolite (102) also seemed to

enhance dopamine release, suggesting that it may induce amphetamine-like neurotoxicity. Halliday et al. showed that baboons treated with (101) developed urofacial dyskinesia that could be correlated with the destruction of neurones in the nucleus basilis of Meynert (509).



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7.8 Benzisoxazoles

The pharmacokinetics of risperidone, the first member of this structural class to be marketed worldwide, has been studied in experimental animals (510) and in humans (511). Risperidone (84) is well absorbed after oral administration and rapidly distributed to the tissues (70% absolute oral bioavailability). It is extensively metabolized in the liver at three different sites in the molecule (510, 511). Hydroxylation at the 9 position is mediated by CYP2D6, while oxidative N-dealkylation is apparently not affected by debrisoquine hydroxylator polymorphism (511,512). Opening of the isoxazole ring also occurs in humans, but is a less important pathway (511). In the rat, some oxidation also occurs at the 7 position of risperidone to provide minor amounts of 7,9-dihydroxy- and 7-keto-9-hydroxy metabolites (510). In humans, peak plasma levels of 9-hydroxyrisperidone, the major metabolite, are reached in 3 h with extensive metabolizers and in 17 h with poor metabolizers. The terminal half-lives of risperidone averaged 3 h in extensive metabolizers and 20 h in poor metabolizers. The average terminal half-lives of 9-hydroxyrisperidone are 21 h and 30 h in extensive and poor metabolizers, respectively. The fractions of risperidone and 9-hydroxyrisperidone bound to proteins in the plasma are 90% and 77%, respectively. Because the pharmacological activity of 9-hydroxyrisperidone is nearly equivalent to that of risperidone, the steady-state levels of antipsychotic species are similar in poor and extensive metabolizers.

7.9 Benzoisothiazoles

The atypical antipsychotic drug ziprasidone (105) (514) was approved for the treatment of schizophrenia in the United States in early 2001. Studies of the biotransformation of this benzoisothiazole derivative reveal that it is extensively metabolized in rats (515,516) and in humans (517) through aldehyde oxidase (697). Four principal routes of metabolism were identified: N-dealkylation of the arylethyl side-chain attached to the 4'-piperazinyl nitrogen; oxidation of the sulfur, resulting in the sulfoxide and the sulfone; reductive cleavage of the benzoisothiazolyl ring; and hydrolytic cleavage of the C=N bond of the isothiazolyl ring.

Eleven days after the administration of a single 20-mg oral dose of ^{14}C-labeled ziprasidone (517) to human volunteers, 20% was recovered in the urine and 66% in the feces. The absorption was rapid and the C_{max} for free drug and metabolites occurred at 2–6 h postdose. The mean peak serum concentration of unchanged ziprasidone was 45 ng/mL with a mean area under the curve (AUC) of 336 ng/h/mL. On the basis of AUC values, approximately 46% of the circulating radioactivity was attributed to unmetabolized ziprasidone. Less than 5% of the administered dose was excreted as unchanged drug.

Metabolites apparently do not contribute to the pharmacological activity of ziprasidone in *vivo* because the sulfone and sulfoxide metabolites exhibit low affinities at 5-HT, and D_2 receptors (517). Limited investigations of cytochrome P450 isoforms responsible for the primary oxidative metabolic reactions for ziprasidone indicate a major role for CYP3A4 oxidation to the sulfone, shown by Prakash et al. (518), and virtually no role for CYP2D6 (518,519).

7.10 Diarylbutylamines

The pharmacology and pharmacokinetics of pimozide (86), the most important member of this class of neuroleptics, have been reviewed (520). Pimozide is well absorbed after oral administration and is widely distributed into the tissues. It has an elimination half-life of around 55 h, despite extensive hepatic metab-

olism (521, 522). Phase I oxidative *N*-dealkylations occur on the bis(4-fluorophenyl)-butyl side-chain of pimozide to form bis(4-fluorophenyl)butyric acid and *N*-4-piperidinylbenzimidazolin-2-one, and at the 4-position of the piperidine ring of pimozide to form 1-[bis(4-flurophenyl)-butyl]piperidonol and benzimidazolin-2-one. None of the metabolites of pimozide that have been isolated from experimental animals or humans seem to be pharmacologically active (520).

The principal cytochrome P450 isoform involved in pimozide oxidation is CYP3A4 catalyzed N-dealkylation (523). CYP1A2 seems to also be involved. Pimozide may also interfere with the metabolism of substrates by CYP2D6. Potentiation of pimozide-induced cardiotoxicity (prolongation of the QT interval) in patients by clarithromycin-induced inhibition by CYP3A4 N-dealkylation has been reported (524).

7.11 Molindone and Related Compounds

The clinical pharmacodynamics and pharmacokinetics of molindone (91) have been reviewed (525). The drug is reputed to be rapidly absorbed after oral administration and rapidly metabolized. Only 2–3% of the unchanged drug can be recovered in the urine and feces. Molindone has a very short half-like (1.5–2 h) and is 1.5–1.7 times more bioavailable after intramuscular, rather than oral, administration (526). Molindone is less lipophilic than most antipsychotic drugs and has a lower fraction (around 75%) that is bound to proteins in the plasma (527). Clinical studies indicate that the antipsychotic effectiveness of molindone lasts more than 24 h (525,528,5291, suggesting that one or more active metabolites may contribute to its actions in *vivo*.

8 STRATEGIES FOR DRUG DISCOVERY

8.1 Dopamine Receptor Subtype Approaches

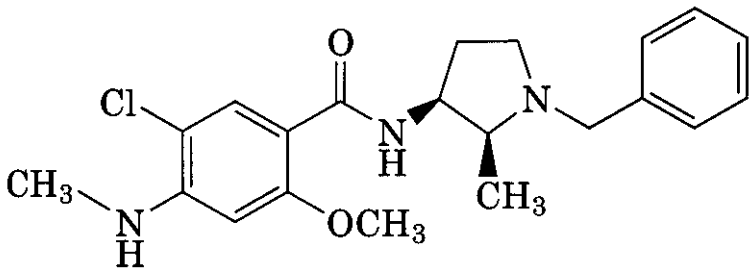
Dopamine receptors were first classified into D_1 and D, subtypes (530). As a testament to the validity of this initial classification, the five subsequently cloned dopamine receptors fall into structural and functional groupings commensurate with the D_1 or D, subtypes. The D_1-like receptors include D_1 and D_5, while the D_2-like receptors encompass D_2, D_3, and D_4 (531, 532). The D, receptor subtype has a short (D_{2S}) and long (D_{2L}) protein form that results from alternative transcription of the D_{2L} gene. The difference between the two receptors is a 29 amino acid peptide coded by an exon that has been spliced out of the third intracellular loop of D_{2L}. The D_3 receptor also has several variants, and the D_4 receptor has multiple variants that are characterized by a different number of repeating units also located in the third cytoplasmic loop of the receptor protein (533).

8.1.1 D_4 Selective Compounds. In general, the typical antipsychotics interact with the D_{2S}, D_3, and D_4 receptor subtypes but are more potent in their affinities at the D, receptor, with K_i around 1–5 nM and about 10-fold less potency at the D_4 and D_3 receptors. Clozapine, however, has its greatest affinity at the D_4 receptor subtype, with a K_i of 21 nM, in contrast to its 230 nM potency at the dopamine D, receptor (534). In addition, 10–30% increases in D_2 and D_3 receptors (535) and sixfold increases in the D_4 receptor subtype (536) have been reported in schizophrenic brains, compared with normals, although the latter finding remains controversial. Autoradiographic analyses with the D_4 selective ligand ^{3}H-NGD 94–1 show D_4 sites to be dense in rat and human hippocampus, hypothalamus, and neocortex, among other brain regions, and to be absent in the striatum (537). These findings suggested that a D_4-specific compound might treat schizophrenia as effectively as clozapine but without the D_2 antagonist-mediated EPS or the clozapine constellation of side effects.

Given these initial technical hurdles, a major effort was undertaken to discover potent, specific D_4 receptor antagonists (538). Whereas the D_4 hypothesis may yet prove correct, it remains unclear as to whether antipsychotic effects can be obtained with selective D_4 antagonists such as NGD 94–1, L-745–870, or sonepiprazole, or the mixed 5-HT_{2A}/D_4 antagonist fananserin. Each of these compounds have failed to show efficacy in the treatment of schizophrenia. However, the unique polymorphism of the D_4 receptor gene have lead to numerous attempts to associate this receptor

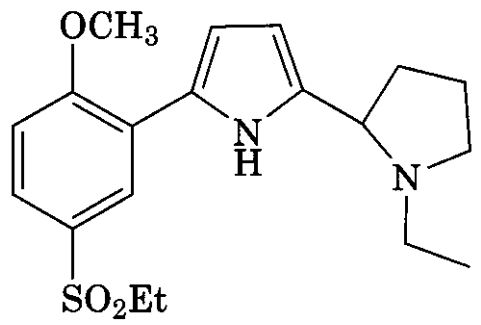
with other CNS disorders. Associations seem to be with ADHD, substance abuse (heroin but not alcohol), and novelty- or risk-seeking traits (539) but not with obsessive compulsive disorders, Parkinson's disease, schizophrenia, psychoses, mood disorders, or Tourette's syndrome.

The quest for D_4-selective ligands (540–544) began with the knowledge that the sulpiride class of atypical antipsychotics bound specifically to members of the D_2 family of receptors. One early investigation showed that conformationally restricted benzamide analogs of sulpiride-like molecules retained dopaminergic selectivity after replacement of the carboxamide with a pyrrole ring. This was shown in the case of the 2-phenyl pyrroles (545) and the corresponding 1-phenyl pyrrole derivatives (540). This series is closely related to NGD 94–1, the first potent and specific D_4 antagonist to be developed clinically. NGD 94–1 binds to the D_4 receptor with a 3.6 n*M* affinity (541). Clinically, NGD 94–1 is subject to relatively rapid (<1 h) hydroxylation in the pyrimidine ring to produce the corresponding 5-hydroxy pyrimidine derivative. This metabolite is also a potent D_4-specific ligand, but has partial D_4 agonist properties (546). [^{3}H]NGD 94–1 was synthesized (547) and used to localize and map D_4 receptors in the brains of rats, and normal and schizophrenic humans (548–550). Attempts were made to block the 5-hydroxylation by incorporation of a fluorine substituent at the 5-position of the pyrimidine. While this was successful, development of the resulting compound NGD 94–2/Sch-66712 was discontinued because of a potent inhibition of human liver cytochrome P450 2D6 (CYP2D6) (551). Some compounds like nemonapride (106) bound equally at all three



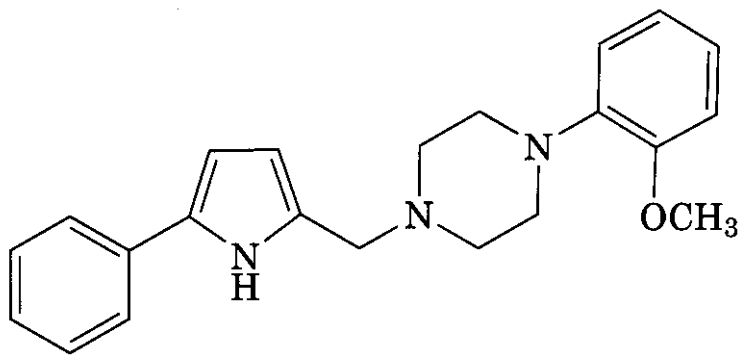
(106)

receptor subtypes, whereas amisulpiride (219, 552) and YM-43611 (553) bound about equally to the D_2 and D_3 but not D_4 receptors. Isosteric replacement of the amide functionality with a pyrrole as in (107) lea to a related series



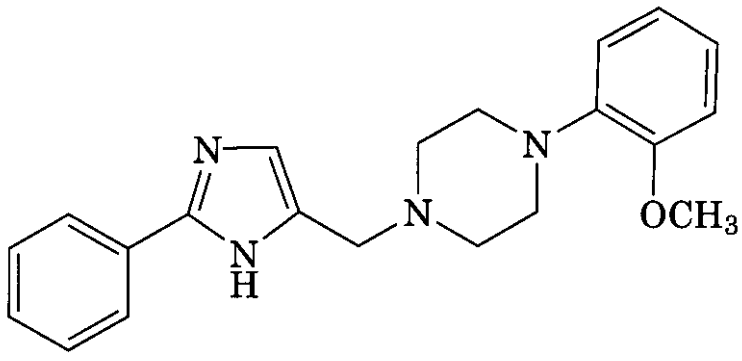
(107)

that maintained many of the pharmacological characteristics of the benzamides. Extension of this strategy to the butyrophenones provided (108) with high affinity for D_2 receptors



(108)

(545). The analogous 2-phenylimidazole compound (109) also showed strong affinity for D_2 as well as D_4 receptors (554). When the piperazine linked aromatic was either 2-pyrimidyl (110) (NGD 94–1) or 2-pyridyl (111), the first true selectivity for the D_4 receptor subtype emerged (541). Compounds (110) and (111) displayed both high affinity and selectivity for D_4 receptors (K_i of D_4 = 4 n*M*, K_i of D_2/D_4 = 590 n*M* and K_i of D_4 = 8 n*M*, K_i of D_2/D_4 = 260



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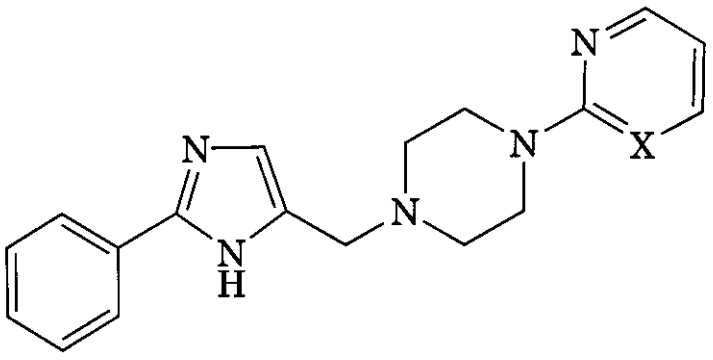
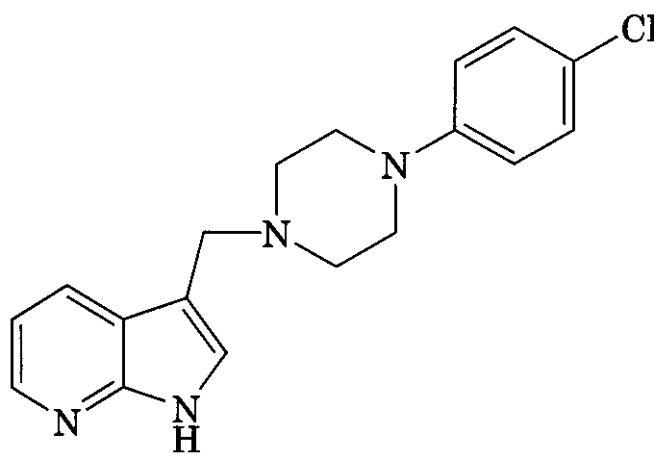


(110) X = N
(111) X = CH

n*M*, respectively). Attenuation of quinpirole-induced inhibition of forskolin-stimulated cAMP production in D_4-expressing CHO cells identified (110) as a functional antagonist at the receptor.

The structural diversity of D_4 selective ligands is illustrated further by the examples of compounds (112–118)(555–562). While a true



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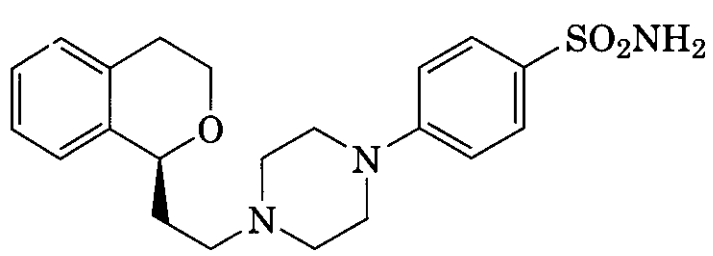
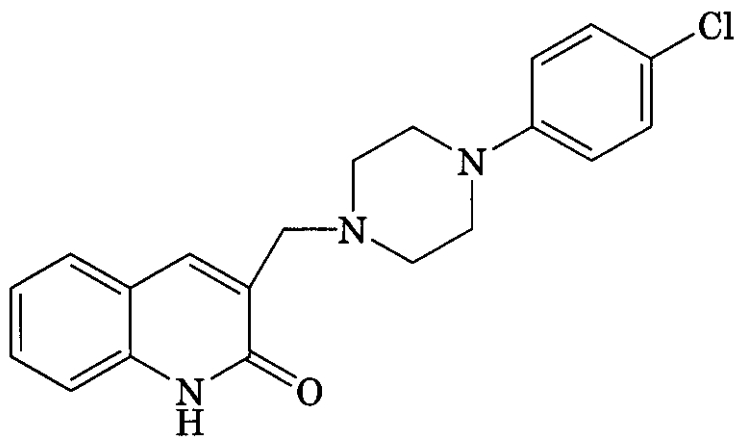


(113)

pharmacophore for the D_4 receptor is still emerging, aryl or arylalkyl piperazine and piperidine substructures are highly represented within this set of examples.

Compound (112) (L-745,870) possessed high affinity for the receptor ($K_i = 0.5$ n*M*) and selectivity over D_2 ($D_2/D_4 = 2200$) (555). Behaviorally, (**112**) did not antagonize amphetamine-induced hyperactivity in rats. In the



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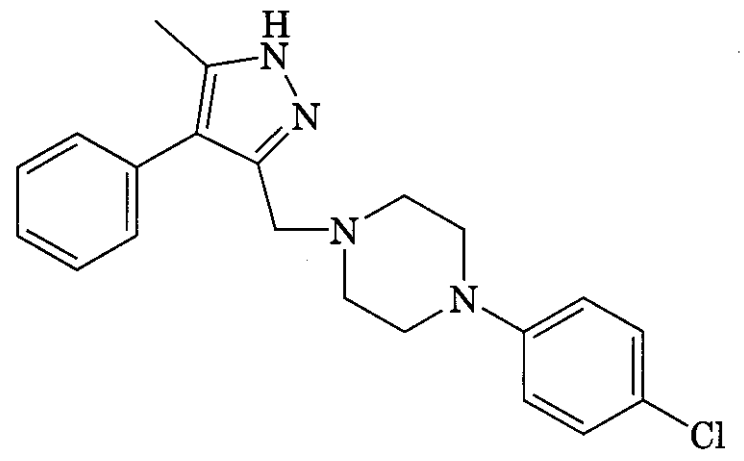

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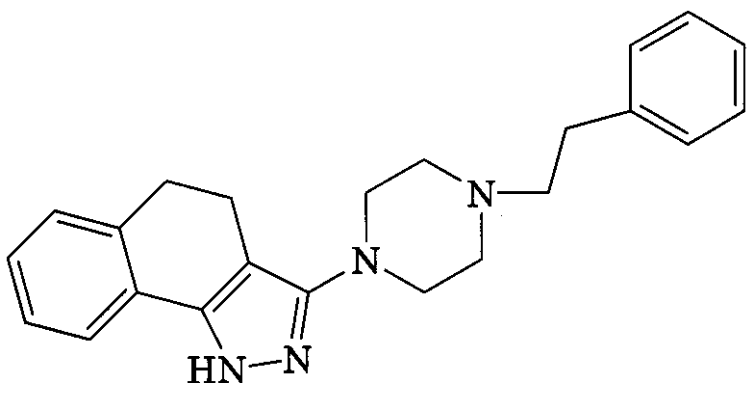

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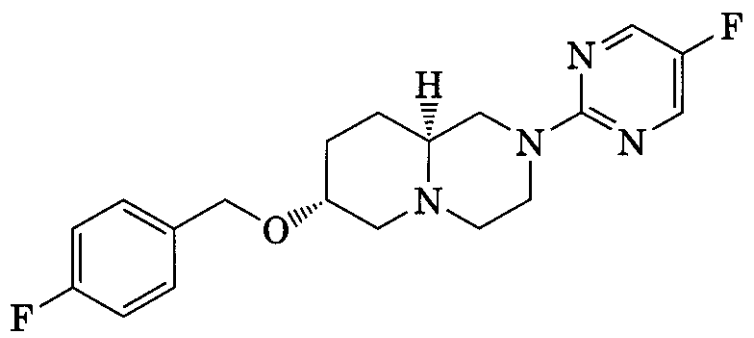

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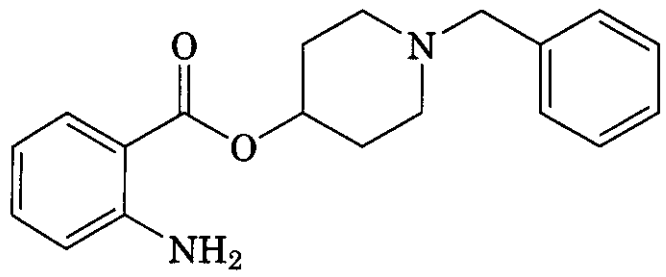
side effect models, (112) did not induce catalepsy or modify apomorphine stereotypy. While the lack of motoric side effects was en-

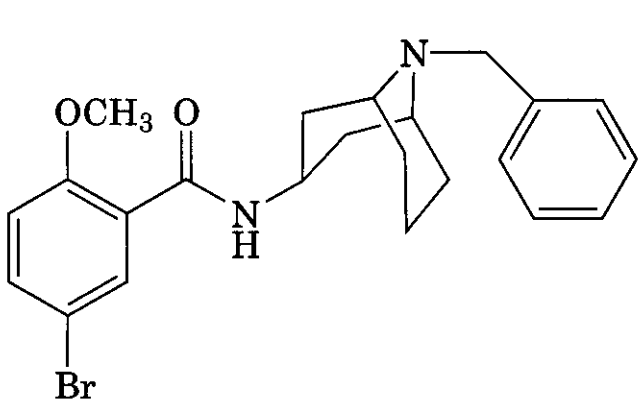


(118)

couraging, the behavioral transparency of (112) in the classic efficacy model placed the mechanism in question. Clinical trials with (112) in schizophrenic patients showed the compound to be ineffective (563). Clinical results of sonepiprazole (113) were similarly disappointing.

8.1.2 D_3 Selective Compounds. Essentially, all clinically effective antipsychotic drugs are antagonists of D_2 and D_3 receptors (564). It has been hypothesized that the blockade of dopamine D_2 and D_3 receptors in the nucleus accumbens and olfactory tubercle may be associated with antipsychotic effects and that blockade of the D_2 receptors in the caudate putamen, where D_3 receptors are relatively sparse, is responsible for the EPS side effects (565). D_3 receptors mediate the reinforcing effects of the psychostimulant cocaine (566), and thus, D_3 antagonists may block cocaine dependency. Thus, a preferential D_2/D_3 receptor antagonist may represent a superior target for the development of antipsychotic agents with a lower risk of EPS (567, 568).

As with the D_4 selective compounds, the search for D_3-specific ligands began with the benzamide, or sulpiride class of drugs that have selective, nanomolar affinity for D_2 and D_3 receptors. The aza bicyclononane (**119**) displayed a sixfold preference for D_3 over D_2 receptor sites (569). Efforts focused on the replacement of the substituents on the benzamide aromatic ring and on the modifications of the N-phenylalkyl group on the basic nitrogen, which lead to analogs that were more selective for D_2 than D_3 receptors. Variation of the benzamide to the corresponding naphthamides also produced D_2 rather than D_3 selective ligands (570). However, the halogenated phenylpiperazinonaphthamide (**120**)



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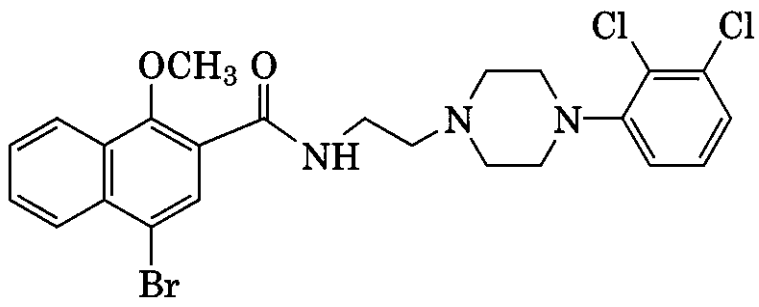
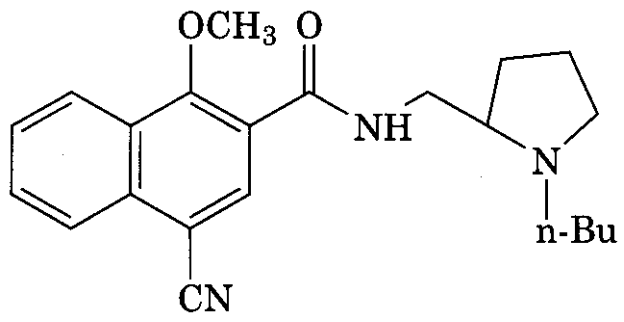


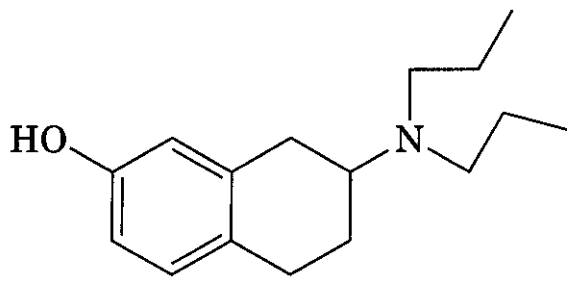
(120)

was identified as a partial D_3 agonist with a high selectivity for D_3 versus D_2 receptors (571). A related compound, the methoxynaphthamide nafadotride (**121**), is a D_3 selective antagonist with a D_2/D_3 K_i ratio of 10 (572).



(121)

N,N-Di-n-propyl derivatives of 5- or 7-hydroxy-2-aminotetralin, e.g., (*R*)-(+)-7-OH-DPAT (122) and (*S*)-(−)-5-OH-DPAT, and the corresponding indane derivatives, exemplified by U 99194 (**123**), were identified as potent and relatively selective D_3 agonists (575). However,



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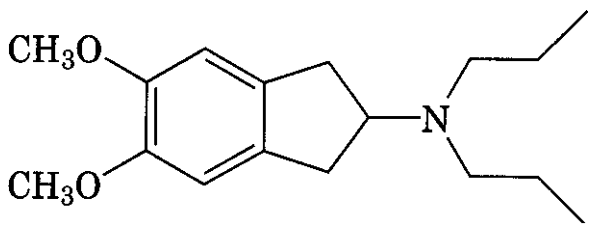

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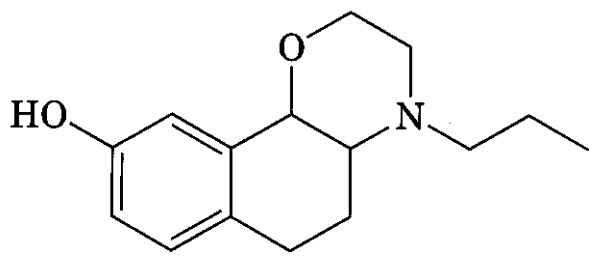
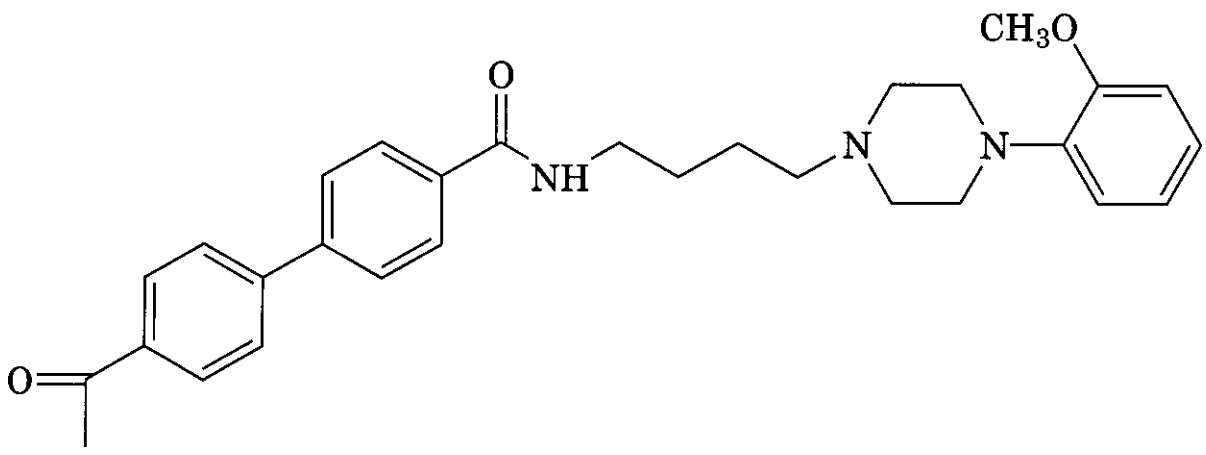


(126)

these agonists lacked sufficient selectivity to allow a clear discrimination between D_3 and D, receptor function in *vivo.* The first compounds to show a high degree of D_3 specificity were the biphenylamides exemplified by GR 103691 (**124**) (576). It has a moderately high affinity for 5-HT,, and a-, receptors, has a high D_3 affinity (K_i 5 n*M*), and exhibits a 100-fold selectivity over D_1, D_2, and D_4 receptors. The constrained fluorene amide derivative, NGB 2904 (125) (577), possesses high affinity (K_i 1.5 n*M*) at the D_3 receptor where it is a full antagonist, and greater than a 150-fold selectivity over all other dopamine, 5-HT,, and a-, adrenergic receptors. An extension of the amino tetralin approach lead to a series of constrained benzopyranopyrrolidine derivatives, such as (**126**), that displayed a comparable degree of D_3/D_2 selectivity to the original amino tetralins, and whereas appropriate replacement of the hydroxy substituent increased the D_3/D_2 selectivity somewhat, incorporation of the biphenylcarboxamidobutyl type substitution found in GR10361 produced highly potent and specific D_3 receptor antagonists, exemplified by S 33084 (127) (578). This, in turn, formed the basis for the discovery of the 4-quinoline carboxamide derivative SB-277011 (**128**), reported to be a potent and selective D_3 receptor antagonist with high oral bioavailability and CNS penetration (579). The inability of even high doses of SB-277011 to induce catalepsy or elevate serum prolactin supports the hypothesis that a selective dopamine D_3 antagonist would have a reduced liability to induce EPS or hyperprolactinemia. Its fate regarding clinical trials is unclear. Selective D_3 antagonists represent a new, but still unproven, approach to the treatment of



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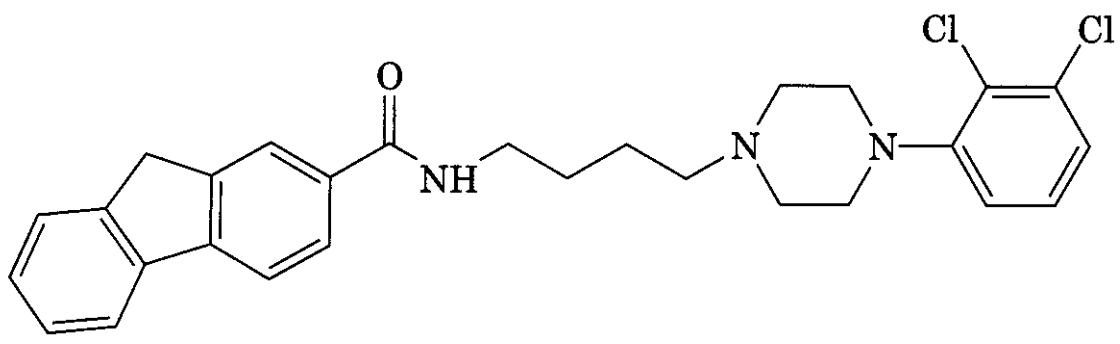

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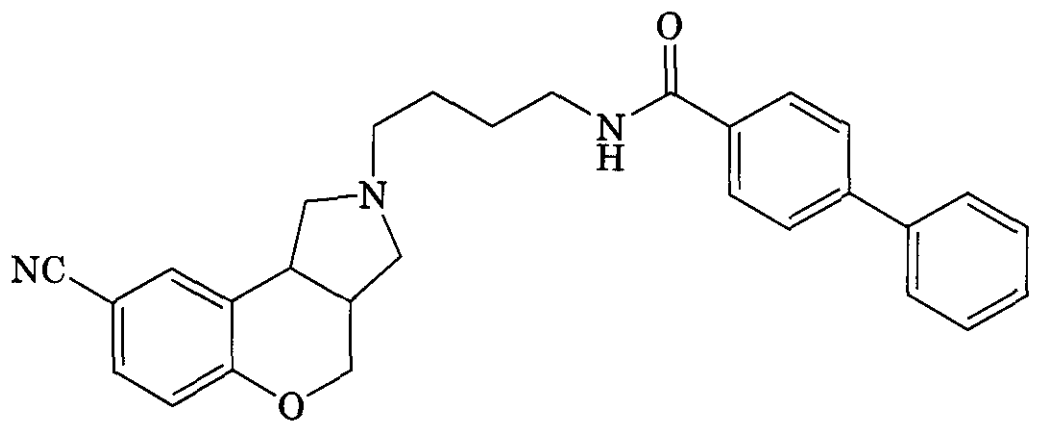


(127)

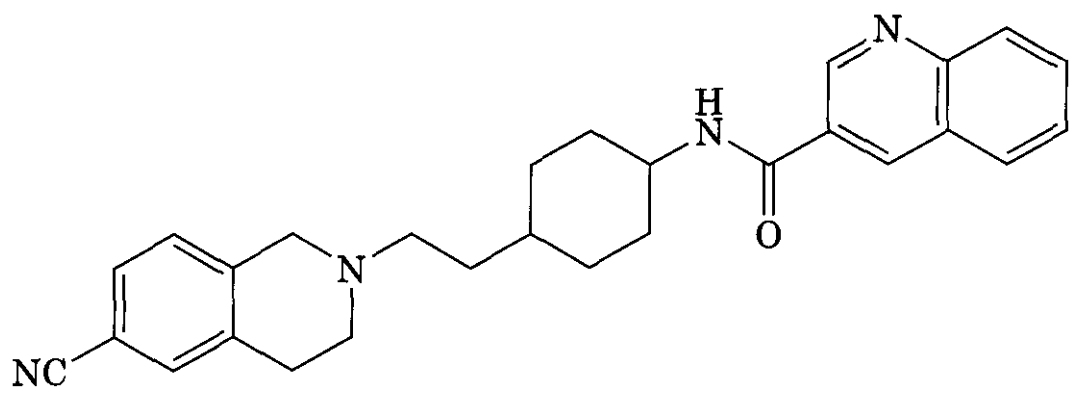
schizophrenia and related disorders. Functional synergy between D_2 and D_3 antagonism has been hard to study given the lack of D_2- and D_3-selective compounds. The use of such newly discovered compounds will include evaluations of the hypothesis presented here that preferential D_3/D_2 antagonism is a useful strategy for treating psychoses including those associated with schizophrenia.

8.1.3 Dopamine "D_2-Plus" Agents. As noted in Section 8, all antipsychotics except amisulpiride interact with a wide array of receptors. The potent D_2 antagonism of all antipsychotics including amisulpiride has kept open the exploration of novel D_2 antagonists that bind to other receptors, such as D_3, adrenergic or serotonergic receptors. This constitutes the "D_2 plus" approach for novel, atypical antipsychotics.

8.1.4 5-HT_{2A}/D_2 Antagonists. The most validated D_2-plus approaches are the ones that involve dopamine and serotonergic receptors. These include 5-HT_{2A}/D_2 receptor antagonism (the "SDA hypothesis"), the 5-HT_{1A} partial agonist/D_2 antagonist model, and the 5-HT_{1A} antagonist/5-HT_{1A} partial agonist/D_2 antagonist model (coined here as the "5HT-1A/2A/D2" model).

Autoradiographic studies conducted *in uitro* (580) were the first to identify the high affinity binding of [^{3}H]spiroperidol to 5-HT_2 receptors in the frontal neocortex, an area responsible for executive cognitive functioning. This and subsequent studies (24, 585, 586), including *in uiuo* radioligand binding (587), confirmed the SDA hypothesis with a broad variety of antipsychotic drugs by showing that 5-HT_{1A} receptors were preferentially occupied over D_2 receptors by atypical antipsychotics, whereas the converse was true for typical antipsychotics (584). The SDA hypothesis has been confirmed with human brain imaging studies (699), and postulates that this activity confers atypical properties by virtue of diminished EPS. The ratio of potencies at these receptors is more relevant than potency alone, because several atypicals, e.g., clozapine show moderate and low affinity at these sites, but still favor $5HT_{2A} > D_2$.

Consistent with these findings, 5-HT_{2A} antagonists reverse catalepsy induced by typical antipsychotics such as haloperidol (585).



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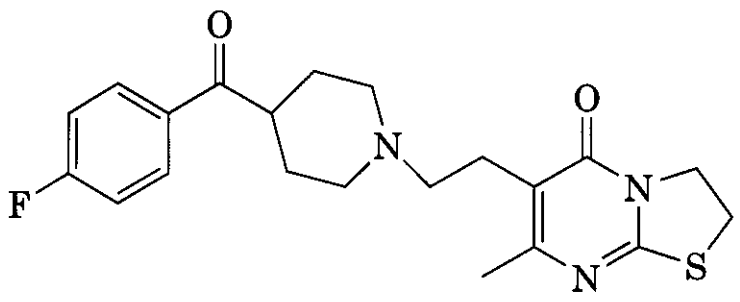

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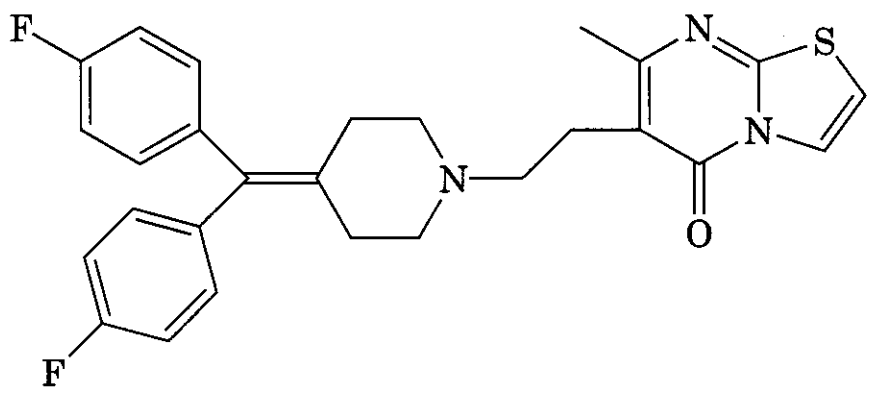
Chronic clozapine treatment reduces 5-HT$_2$ receptor densities in the neocortex without affecting striatal D_2 receptor densities, emphasizing its preferential interaction with the 5-HT$_2$ receptor in *vivo* (586). 5-HT$_2$ receptor down-regulation is produced by 5-HT$_{2A}$ agonists as well as antagonists like clozapine, and is caused by the internalization and degradation of the 5-HT$_2$ receptor complex (582).

8.1.5 **Aryl Piperazines/Piperidines.** There are two major structural classes that confer concomitant 5-HT$_2$ and D_2 antagonism. These are the **aryl piperidines/piperazines** and some tricyclic systems. One of the first clinical studies to assess the role of serotonergic blockade in antipsychotic therapy was conducted on setoperone (129), a 5-HT$_2$/D_2 antagonist that showed promising results but was later discontinued because of bioavailability issues (588).

Risperidone (84) has a D_2/5-HT$_{2A}$ ratio of 20 (K_i for D_2 and 5-HT$_2$ 3.1 and 0.16 nM,



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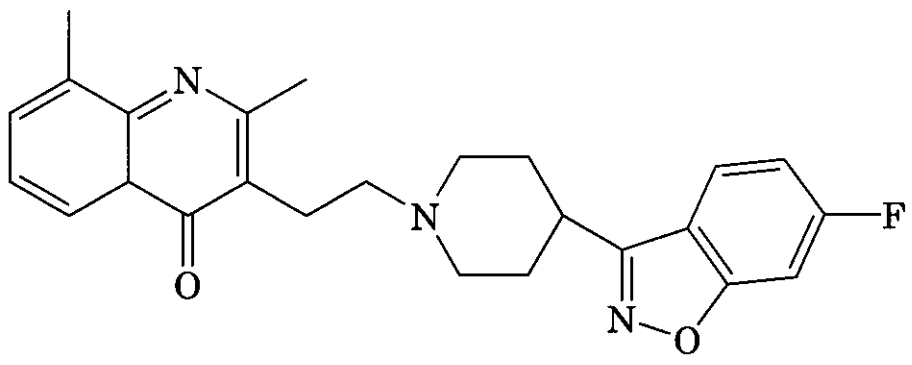

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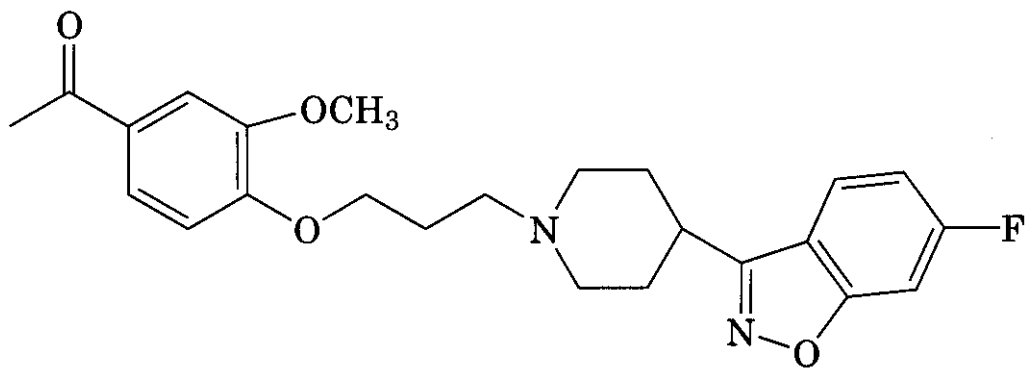


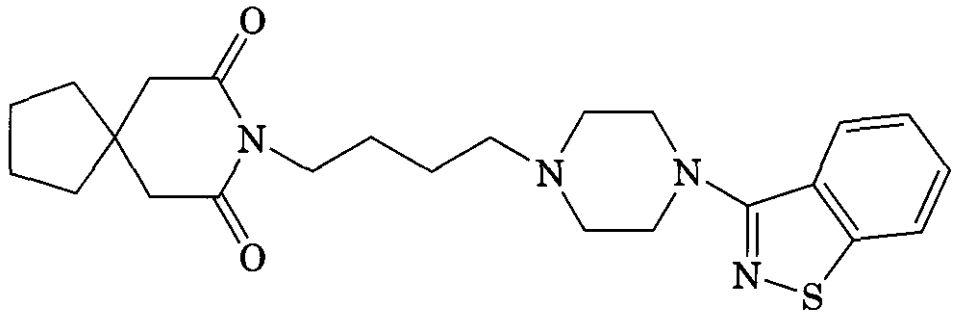
(132)

respectively). It arose from a structural hybridization of the selective 5-HT$_2$ antagonist ritanserin (**130**), where the fused pyrimidine side-chain was retained, and a 1,2-benzisoxazole-3-yl piperidine, reminiscent of the aryl piperidines in the potent D$_2$ blocker, haloperidol, was substituted. Clinical experience with risperidone has confirmed its antipsychotic efficacy with reduced propensity to induce EPS (589). Similar effects are observed with ocaperidone (**131**), a compound structurally related to risperidone but with a $D_2/5\text{-}HT_2$ ratio of only 5 (590).

Another member of the benzisoxazolyl piperidine class is HP-873 (**132**), which has a favorable $D_2/5\text{-}HT_2$ ratio of 17. In contrast with the typical antipsychotics, HP-873 also increases social interaction in rats, suggesting an ability to ameliorate negative symptoms (591).

Sertindole (94) was selected from a series of N-phenylindolyl-piperidines and has K_i's of 0.39 and 4.1 nM at the 5-HT$_2$ and D$_2$ receptors, respectively, and a favorable ratio of 10.5. Sertindole blocks the hyperactivity in rats induced by dopamine infusion into the nucleus accumbens without causing the rebound hyperactivity after cessation of treatment seen with typical antipsychotics. Although sertindole has a low propensity to induce catalepsy, both the direct analog in which the chloro substituent is replaced by cyano and also the corresponding piperazine and tetrahydropyridine analogs have more classical $D_2/5\text{-}HT_2$ ratios favoring D$_2$ affinity and thus potently induce catalepsy (592).

A series related to risperidone, which was not designed to incorporate 5-HT$_2$ antagonist activity, resulted in tiaspirone (133). This analog has a $D_2/5\text{-}HT_2$ ratio of 21, similar to that found for risperidone. The behavioral profile of tioperone was closer to clozapine than to the typical antipsychotic, haloperidol. Further evidence for the value of a high $D_2/5\text{-}HT_2$ affinity ratio in this series of compounds may be found with a metabolite of tiasperone (593), where hydroxylation of the six-membered imide ring showed somewhat decreased D_2 receptor affinity relative to tiasperone but had similar 5-HT$_2$ receptor affinity. This compound exhibited good inhibition of the conditioned avoidance response in rats and did not induce catalepsy. A series of thieno[3,2-c]pyridine and furo[3,2-c]pyridine compounds structurally related to tiosperone have similar profiles and demonstrate that a higher 5-HT$_2$ affinity can compensate for high D$_2$ affinity (594). These compounds block conditioned avoidance responses in rats despite weak D$_2$ affinity (K_i 115 nM) provided that potent 5-HT$_2$ affinity was present. They also did not induce catalepsy, again suggesting an atypical profile. Using a variant of the spiro-imide side-chain in tiosperone, the effect of varying the aryl group of the arylpiperazine demonstrated that a 1,2-benzisothiazole group was superior over other aryl groups in both *in vitro* and *in vivo* testing and retained a favorable $D_2/5\text{-}HT_2$ affinity ratio of 7. The structurally related compound in which the 1,2-benzoisothiazole nitrogen has been replaced by C—H to yield the corresponding benzothiophene, showed a similar *in vivo* profile with 5-HT$_2$ affinity (K_i = 20 nM) and a $D_2/5\text{-}HT_2$ affinity ratio of 26 (595). SM-9018 (**134**) is another variant with the 1,2-benzothiazolylpiperazine imide structure and shows affinity for D$_2$ (K_i = 5 nM) and 5-HT$_2$ (K_i = 0.61 nM) receptors, *in vivo* blockade of dopamine-mediated behaviors, and a reduced tendency to induce catalepsy (596). Because the compound also has potent 5-HT-$_1$ receptor affinity (K_i = 2.9 nM), it is possible that this also contributes to the *in vivo* profile (597, 585).



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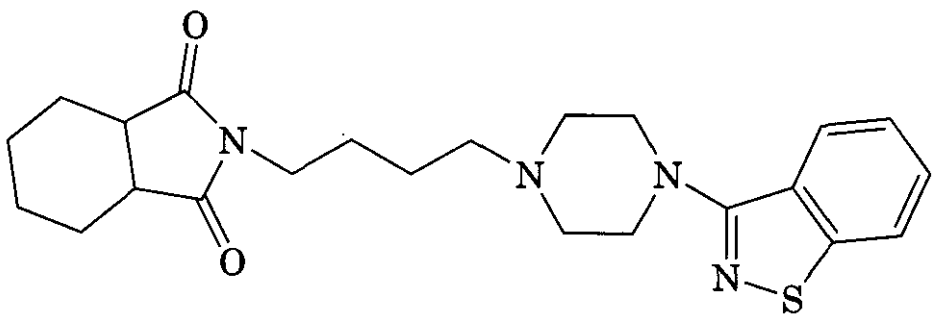
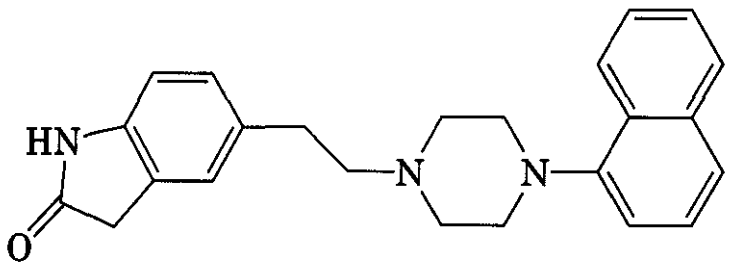


(134)

Of particular interest are 5-HT-1A/-2A/D2 compounds (with 5-HT$_1$ partial agonist, 5-HT$_{2A}$ antagonist, and D_2 antagonist affinities). These include a series of 1-naphthylpiperazines (**135**) (598) and the related and



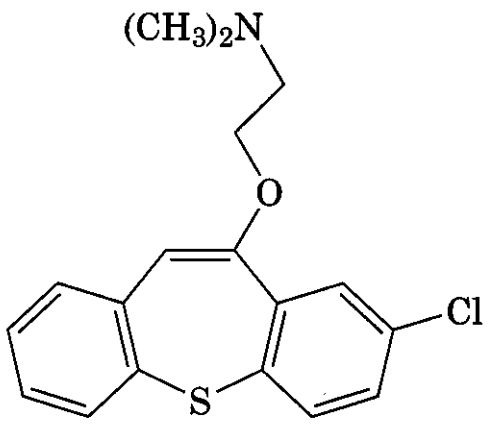
(135)

far more potent 1,2-benzothiazolylpiperazine analog, ziprasidone (105) (599). Ziprasidone has a favorable D_2/5-HT$_2$ ratio of 11.4 based on K_i values of D_2 = 4.8 nM and 5-HT$_2$ = 0.42 nM (600). Ziprasidone is also a potent, partial agonist at 5-HT$_{1A}$ receptors (601). Thus, the structurally very different 1,2-benzisoxazole– and 1,2-benzothiazole–based compounds, with their two side-chain variants of heterocycles linked by two atoms or imides linked by four atoms, seem to have validated the SDA hypothesis and added an important role for partial agonism at the 5-HT$_{1A}$ receptor (602, 603). Ziprasidone has been marketed in Sweden since 2000 and in the United States since 2001 as an oral and injectable atypical antipsychotic. The EPS that would otherwise be expected from its high D_2 antagonist affinity is probably minimized by its potent antagonist affinity at 5-HT$_{2A}$ receptors (599,602) and its potent, partial agonist affinity at 5-HT$_{1A}$ receptors (604,605).

8.1.6 Arylpiperazines/Piperidines. Risperidone, a potent SDA, is a highly successful atypical antipsychotic drug introduced to clinical practice in the mid-1990s. It treats the positive and negative symptoms of schizophrenia. Risperidone also has a potent affinity at α_1-adrenoreceptors and is an antagonist at the H_1 histamine receptor. Adverse EPS side effects, such as tardive dyskinesia and involuntary tremors, are less common with risperidone than with other typical antipsychotics, particularly haloperidol. Risperidone is more effective than conventional antipsychotics at improving overall cognitive function, particularly working memory, attention, and alertness (606). However, not all medical experts agree with this conclusion but suggest that depressive symptoms may be more amenable to treatment with risperidone, possibly because of its 5-HT$_{2A}$ antagonism.

Sertindole, a D_2 antagonist with prominent activity in the limbic system, also has potent and long-lasting affinity at 5-HT$_{2A}$ and α_2-adrenergic receptors. It is practically devoid of EPS side effects but had to be withdrawn from the market in the UK because of reports of cardiac arrhythmias and sudden death. This is presumably because of sertindole's effect at voltage-gated I_{Kr} ion channels that control heart rythm (607). Sertindole is contraindicated in patients with a long QT interval or those receiving drugs known to prolong the QT interval (608,609). Development of sertindole has been suspended in most countries, and it remains unclear whether sertindole will be approved in the United States or reintroduced in Europe.

A series of 6-aminoalkyltetrahydroindol-4-ones related to molindone (**91**) show potent affinities for D_2 and 5-HT$_{2A}$ receptors (610). Molindone exhibits many similarities to typical neuroleptics, including D_2 antagonism, antipsychotic efficacy, and EPS (611). Zotepine (136) is a potent D_2 and 5-HT$_{2A}$ antagonist with high affinity for the 5-HT$_2$ receptor.



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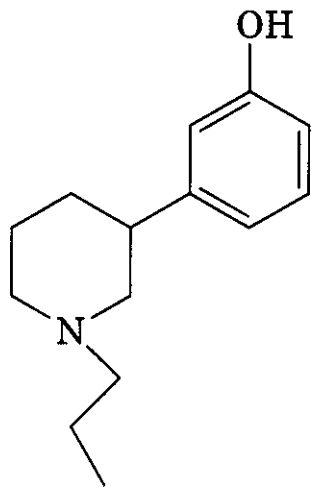

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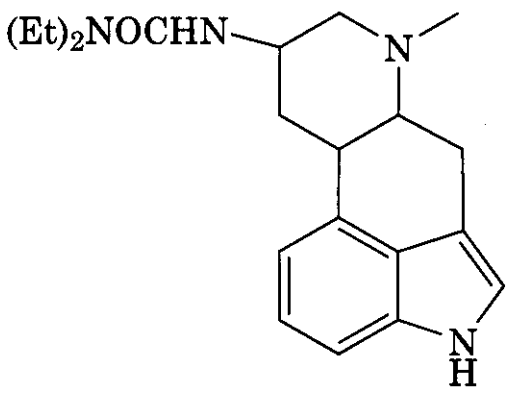

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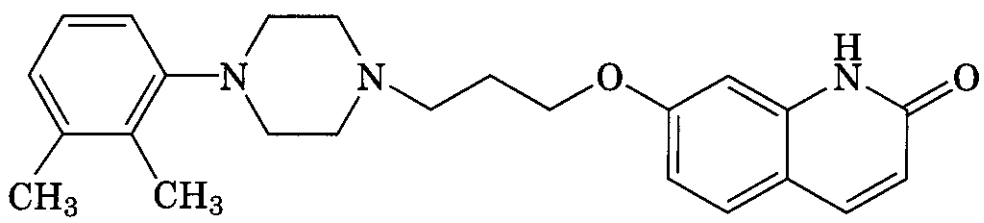
Zotepine has been launched in the UK and Japan and seems particularly effective in patients with negative symptoms (612). Studies suggest a less cataleptogenic profile than the butyrophenone antipsychotics.

8.1.7 Dopamine D_2 Partial Agonists. There is now evidence to support the original proposal of Arvid Carlsson et al. (613, 614) that partial D_2 agonists can treat psychosis by activating presynaptic D_2 autoreceptors and decreasing dopamine synthesis and release. In support of this bold proposal, D_2 agonists, and even partial agonists, have been shown to improve negative symptoms of schizophrenia. Unfortunately, except for (−)3PPP (137), in which modest efficacy was seen (617), most of these drugs also exacerbate positive symptoms and show diminished efficacy with chronic administration. This has been observed for the full agonists bromocriptine (615) and N-propylnorapomorphine (616), and the partial agonists terguride (138)(618) and OPC-4392 (139) (619). Nevertheless, these studies provided valuable clinical evidence that excess D_2 (and probably D_3) receptor stimulation can contribute to positive symptoms of schizophrenia and even ameliorate negative symptoms.

As described in Section 2.2.1, the positive symptoms of schizophrenia are believed to arise from an excess of dopaminergic activity in mesolimbic circuits, while the negative symptoms may arise from too little dopaminergic activity in mesocortical circuits. The excessive postsynaptic signaling can be directly blocked by traditional postsynaptic D_2 antagonists. In theory, a similar therapeutic result could occur by decreasing the amount of dopamine that is released from the presynaptic neuron. The electrical activity, biosynthesis, and release of dopamine from such neurons is under the negative control of D_2 autoreceptors that reside on the dopaminergic neuron cell body and axon terminal (620–622). Dopamine itself released from presynaptic neurons, or dopamine agonist drugs, stimulate these D_2 autoreceptors. Because of a preferential sensitivity of the autoreceptor to



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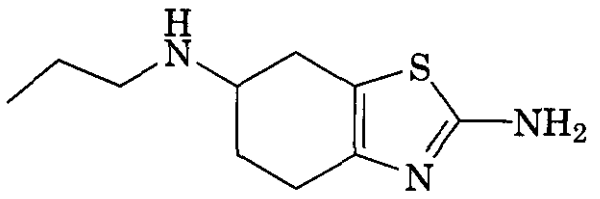

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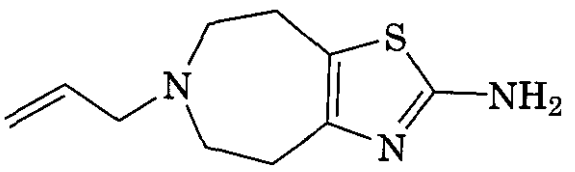

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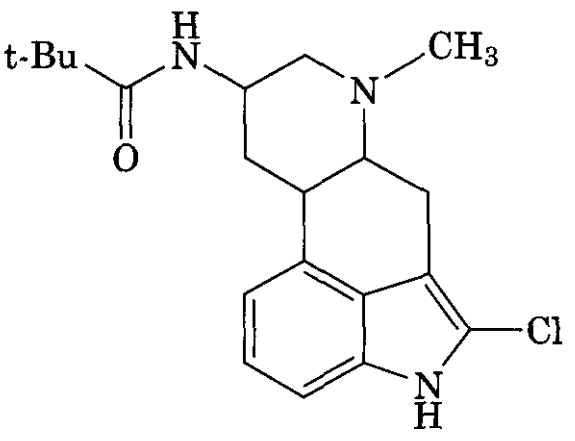
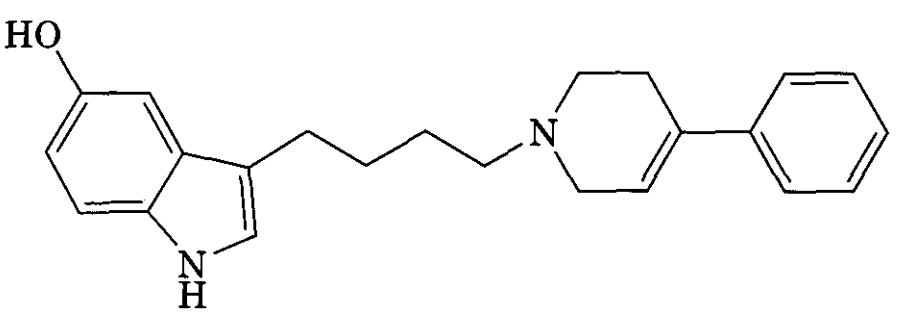


(143)

activation by partial agonist signaling, partial D_2 agonists can activate the feedback system while producing little if any agonism at postsynaptic D, receptors.

A number of compounds have been synthesized with the goal of such autoreceptor agonist properties. These include OPC-4392 (619), UH232, (−)-3-PPP (623–625), pramipexole (140), roxindole (141), and talipexole (142). The partial agonists terguride (618) and SDZ HDC 912 (143) (626) have shown partial effectiveness on the negative but not positive aspects of schizophrenia. Most commonly, such agents exacerbate the positive symptoms, as seen with apomorphine, (−)3-PPP, and OPC-4392. Another such compound, DU 127090 (134) (627) is in phase II clinical trials for schizophrenia. DU 127090 is a partial agonist at the D_2 family of dopamine receptors that can block high levels of dopamine signaling but also stimulate D, receptors when dopamine tone is low. Its potency at D_3 (1 nM) > D_4 = D_2 > 5-HT1A (10 nM) > 5-HT„ (0.5 μM). As determined by the reversal of cAMP accumulation, DU 127090 produces a potent, partial agonist effect that is below that of the partial agonist terguride (627). The suppression of dopamine release by DU 127090 was stronger than expected for such a partial agonist, because it was with aripiprazole, which produces little if any change in striatal or cortical dopamine release (640) and metabolism (641). This suggests that another mechanism, such as more complete D_2 agonism, or partial 5-HT„ agonism (629), may contribute to the action of DU 127090 or aripiprazole. While DU 127090 produced a favorable ratio of activity in the conditioned avoidance response (CAR) test versus the induction of catalepsy, it induced about one-half the dystonia in haldol-sensitized monkeys produced by haloperidol (630).

Another novel antipsychotic, aripiprazole (OPC-14597) (144) (619, 631) is a potent and partial D_2 agonist (632). While structurally very different from DU 127090, aripiprazole has a remarkably similar profile to DU 127090. Aripiprazole binds potently to both the human D_2 receptor (K_i = 1 nM) (633) and to the human 5-HT„ receptor (K_i = 1.65 nM) (628), and displays potent, but partial agonist effects at both (628, 633, 634). Aripiprazole is an effective, rapid-acting antipsychotic that induces little or no EPS (635–637). In animal models of presynaptic dopamine function, aripiprazole decreases dopamine synthesis (619) and the firing rate of mesolimbic dopa-



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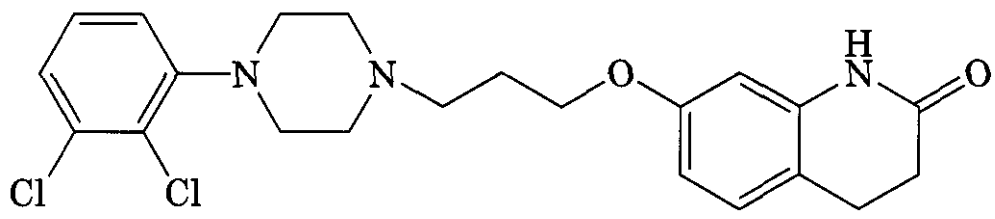


(144)

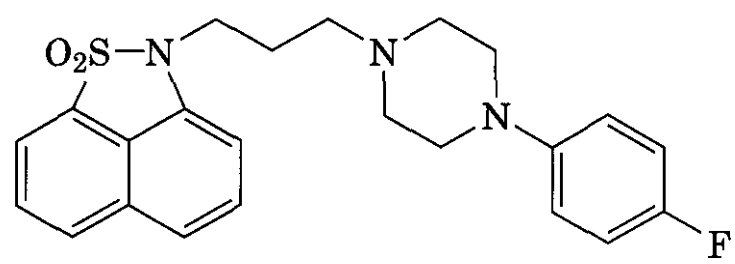
mine neurons (638). Unlike other dopamine agonists, it decreases spontaneous locomotion at both low and high doses. In models of postsynaptic striatal function, aripiprazole shows little propensity to induce rotation in animals with unilateral nigrostriatal dopamine lesions, nor does it induce stereotypy or increase locomotion in reserpinized or haloperidol-sensitized rats (632). Nevertheless, it inhibits stereotypy, locomotion, and rotation induced by a full agonist, such as apomorphine, and does so at lower doses than those required to induce catalepsy or ptosis (639). As a result of this partial agonist action at postsynaptic D_2 receptors, aripiprazole behaves like a functional antagonist when it competes with exogenously administered full D, agonists or dopamine. The potent, partial agonism of aripiprazole at D_2 receptors may account for the modest changes in cortical or striatal dopamine release, and the subtle increases in dopamine metabolism in these regions determined by in vivo microdialysis (640) and brain tissue measurements (641). Aripiprazole produces little or no change in prolactin release in rat (642) or human (635) studies. As evidenced by the small increases in dopamine metabolism, and other antagonist-like activities shown previously in vivo (632, 643, 644), aripiprazole can exert competitive antagonist properties as a result of its potent D_2 receptor binding but low D_2 intrinsic agonist activity. Other receptor actions of aripiprazole, particularly its equally potent, partial agonist activity at 5-HT$_{1A}$ receptors (628), may also be explained by the small or ameliorating effects of 5-HT$_{1A}$ agonism on dopamine synthesis (645), metabolism (629), release (646, 647), and catalepsy (648, 649).

8.2 Non-Dopaminergic Approaches

8.2.1 Serotonergic

8.2.1.1 **5-HT2A Antagonists.** As described in Section 8.1.4, the discovery that clozapine has a high affinity for 5-HT$_2$ receptors rekindled interest in the potential involvement of this receptor in the action of atypical antipsychotics and in the etiology of schizophrenia. 5-HT$_2$ receptors are comprised of A, B, and C subtypes. Most medicinal chemistry efforts have been directed toward combining 5-HT$_{1A}$ antagonism with a somewhat lower potency for D_2 receptor antagonism. Selective 5-HT$_{1A}$ antagonists relatively devoid of D_2 affinity were also made to treat schizophrenia, as efficacy, and not simply diminished side effects as initially proposed (24), were seen as potential actions of 5-HT$_{1A}$ antagonism. The first compound to have a reasonably selective 5-HT$_{1A}$ affinity was ritanserin (650), a mixed 5-HT$_{2}$ antagonist. Clinical studies with ritanserin indicated that it is effective in anxiety (651) and alcohol dependence (652). Clinical trials in schizophrenic patients, however, gave conflicting results (653). No effects were observed using ritanserin as an add-on medication to neuroleptic therapy (654).

A radical extension of the SDA concept produced the naphthsultam series, which was typified by fananserin (655,656). Fananserin (**145**) is a potent and selective antagonist at



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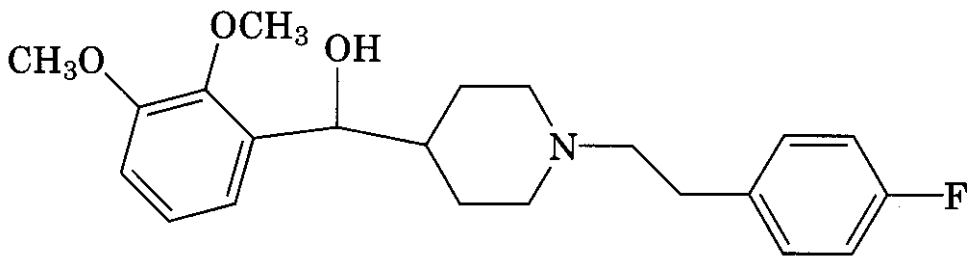
the human 5-HT$_{2A}$ receptor (K_i = 0.26 nM) with moderate affinity for the α_1 receptor (K_i = 38 nM) and little or no D_2 receptor affinity ($K^1 > 1$ μM) (657). Subsequently, fananserin was found to have potent affinity at the D_4 receptor (K_i = 2.9 nM) (658), and in vivo, to reduce behavior elicited by DA agonists such as apomorphine and amphetamine. It is also



(146)

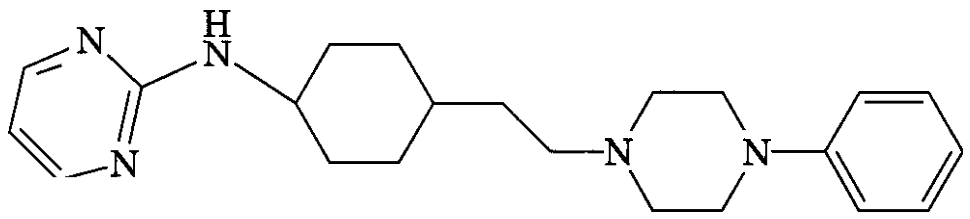
inactive in models of EPS liability (659). The ineffectiveness of fananserin (2) as well as the selective 5-HT$_{2A}$ antagonist ritaneserin as antipsychotics would tend to rule out 5-HT$_{2A}$ and D_4 antagonism as sufficient targets by themselves for antipsychotic drug discovery, and reinforces the critical presence of D_2 antagonism.

One sufficiently selective agent has arisen to test the hypothesis that 5-HT$_{2A}$ receptor antagonism alone can treat psychosis. MDL 100907 (146) is an N-phenethylpiperidine derivative with at least 100-fold higher affinity (1.5 n*M*) at human 5-HT$_{2A}$ receptors than at human D_2 and 5-HT$_{2C}$ receptors (660). MDL100907 exhibited an antipsychotic profile in a number of animal models for schizophrenia. As with atypical antipsychotics, MDL100907 decreased hyperactivity induced by amphetamine (661) and by PCP (662). It also decreased the deficits in prepulse inhibition induced by MK-801 (663) and failed to induce catalepsy, normally a predictor for EPS. Positron emission tomography (PET) studies conducted in humans by Wong et al. showed that low plasma levels of MDL100907 caused by a single oral 20 mg dose produced the desired high degree of cortical 5-HT$_{2A}$ receptor occupancy (664).

In an initial confirmation of the preclinical findings, a phase II clinical trial using 10–40 mg of MDL 100907 per day for 6 weeks showed improved Parkinson rating scale (BPRS) scores relative to placebo and no EPS liability (665). A larger phase III trial showed some minor improvement in positive symptoms at the 10-mg/day dose, but this effect disappeared at the 20-mg/day dose, compared with placebo. Functional PET scans of selected patients in the trial showed minor metabolic changes in the frontal cortex at the 10 but not 20 mg dose. Clinical trials of MDL100907 have been halted, presumably because of limited efficacy (666). Between the findings with ritanserin, fananserin, and MDL 100907, it seems that 5-HT$_{2A}$ receptor antagonism by itself is insufficient to produce robust antipsychotic effects. The ability of 5-HT$_{2A}$ antagonism to diminish EPS induced by D_2 blockade and treat anxiety associated with the disease (24, 580, 584, 667, 668) are likely components of atypical antipsychotic agents.

8.2.1.2 ***5-HT1A Partial Agonists.*** Like 5-HT$_{2A}$ antagonism, 5-HT$_{1A}$ receptor agonism can produce anxiolytic effects and diminish the catalepsy induced by D_2 antagonists in rodents (111, 597, 646) and primates (3). The specific role of 5-HT$_{1A}$ agonism in these effects was confirmed by the ability of selective 5-HT$_{1A}$ antagonists to block the 8-OH,DPAT-induced reversal of catalepsy (585, 597). The median raphe nucleus seems to be a critical site for this action, because infusions of 8-OH,DPAT into the raphe but not into striatal projection areas can reverse the catalepsy induced by the D_2 antagonist raclopride (645). Elevations in serum prolactin produced by haloperidol can also be attenuated by partial 5-HT$_{1A}$ agonists (669). Intrinsic 5-HT$_{1A}$ receptor agonist activity is a property of mainly the newest antipsychotic drugs. These include nemonapride (597), ziprasidone (195, 670, 671), and aripiprazole (628), and the original 5-HT$_{1A}$ partial agonist, clozapine (672). Another compound, PD 158771 (147) (673), was shown by Akunne et al. to be a D2/D3 partial agonist and 5-HT$_{1A}$ agonist with preclinical properties that are very similar to those of aripipirazole. This profile is consistent with the lack of elevations in serum prolactin seen in clinical trials with the mixed D_2/5-HT$_{1A}$ partial agonist aripiprazole (269,270) and the D_2 antagonist/5-HT$_{1A}$ partial agonist ziprasidone (195, 670, 671, 674).

Among these drugs, clozapine, aripiprazole, and PD 158771 can decrease dopamine and serotonin synthesis in the neostriatum, and most of these drugs share a similar behav-



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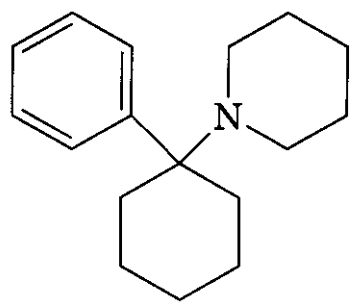
ioral profile suggestive of low EPS liability (645, 673, 675). Again consistent with partial 5-HT$_{1A}$ agonism (and 5-HT$_{2A}$ antagonism), some of these compounds are anxiolytic in preclinical tests including the Vogel conflict test, social contact test, and the elevated plus maze. As with aripiprazole (232), most of these compounds are also weaker at inducing catalepsy or blocking apomorphine-induced stereotypy than in preventing apomorphine-induced climbing. It may be that the potent, partial 5-HT$_{1A}$ agonism of these compounds mitigates against D_2 antagonist effects, including the propensity to produce EPS (597,647,649, 675) and elevation of serum prolactin (669). Partial 5-HT$_{1A}$ agonism may also lessen the depression and anxiety that frequently accompany psychosis (662, 668, 672). The effectiveness of ziprasidone or clozapine against the negative symptoms of schizophrenia may also derive from their intrinsic partial 5-HT$_{1A}$ agonism, which mediates the selective increases in frontal cortex dopamine release produced by either drug (647).

8.2.2 Glutamatergic Approaches. As described in Section 4.3 and reviewed by Goff and Coyle (676), deficiencies in the excitatory amino acid glutamate have been implicated in the etiology of schizophrenia, and thus its receptors and biosynthetic enzymes provide novel drug targets. The identification of numerous ionotropic and metabotropic glutamate subtypes, their multiple subunit combinations, and allosteric binding sites increase the number of targets that could be involved in schizophrenia.

8.2.2.1 ***Inotropic Glutamate Receptors.*** Ionotropic NMDA receptors are formed from combinations of the NR1, NR2, and NR3 subunits. NR1 has eight isoforms (A–H), NR2 has four (A–D), and NR3 is unique. The NR1 subunit is absolutely required for a functional NMDA receptor (677). NMDA receptors can be constructed from either doublets (NR1x/NR2y) or triplets (NR1x/NR2y/NR2z or NR1x/NR2y/NR3) of these subunits (678). A glycine modulatory site resides on the NR1 subunit, and its occupation is required for receptor activation (679). A inhibitory site, identified by the binding of ifenprodil, is present on receptors containing the NR2 subunit (680).

The noncompetitive NMDA antagonist ion channel blockers such as PCP (148) and the competitive antagonists such as CGS 19755 (149) are both psychotomimetic in humans



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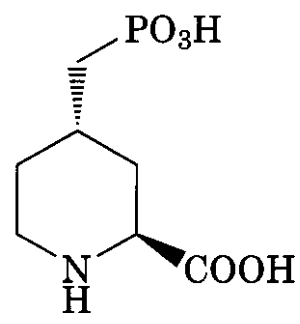
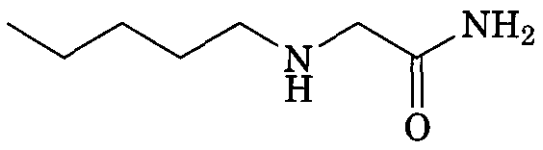


(149)

(681). Thus, it seemed reasonable that compounds that facilitated ionotropic glutamate receptor function had the potential to be antipsychotic. The discovery that the glycine modulatory site on NR1 must be occupied simultaneously with glutamate to affect excitatory neurotransmission, led to efforts to produce specific glycine agonists for this site. Glycine itself is poorly transported into the CNS and has some toxicity on its own. Thus, glycine

mimetics such as the partial agonist D-cycloserine and the glycine prodrug milacemide (**150**) have been examined in clinical trials



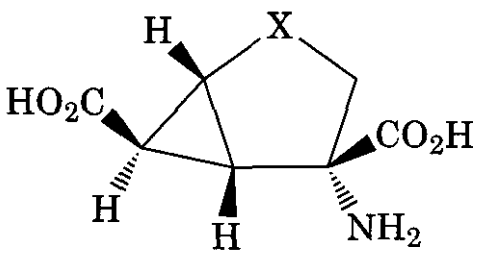
(150)

(682). The partial agonist properties of D-cycloserine (683) might be responsible for the ambiguous results obtained from the clinical trial.

An alternative possibility, of increasing glycine levels by inhibiting glycine uptake, was tested by treating rats with the glycine reuptake inhibitor glycyldodecylamide (684), which is well absorbed by the rat brain. Behaviorally, glycyldodecylamide attenuated PCP-induced hyperactivity in rats, a marker of antipsychotic potential. This result supports the idea that agents that facilitate glutamate neurotransmission may have roles as antipsychotic agents.

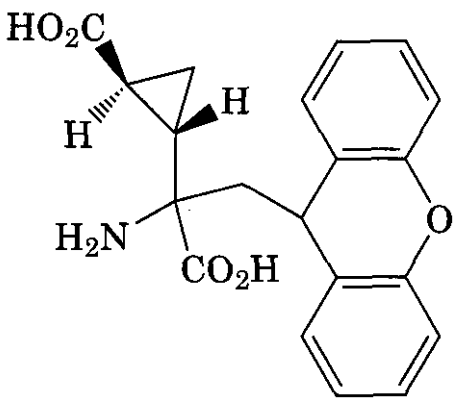
8.2.2.2 Metabotropic Glutamate Receptors. Unlike the classic ion-gated NMDA receptors, there exists eight related "metabotropic" glutamate (mGlu) receptors, termed mGlu1–8. These subtypes are grouped according to their agonist/antagonist pharmacology and their secondary messenger systems. Group I mGlu receptors (mGlu1 and mGlu5) are coupled to phospholipase C activation, whereas group II (mGlu2 and mGlu3) and group III receptors (mGlu4 and mGlu6–8) are negatively coupled to adenylate cyclase.

A large number of specific mGlu agonists and antagonists exist to enable antipsychotic drug discovery (685, 686). Group II metabotropic glutamate receptors seem to be the most promising because they are richly expressed in the prefrontal cortex and hippocampus (687,688). Excessive glutamatergic transmission in these areas is suspected to promote anxiety (689) and seizure disorders (690). mGlu2 receptors are presynaptic autoreceptors that dampen glutamatergic excitability in a use-dependent manner (691). Investigation into the role of group II receptor autoreceptors has been greatly facilitated by the identification of metabotropic glutamate



(151) X = CH_2
(152) X = O

2/3 receptor agonists such as LY354740 (151) and LY379268 (152) and the selective mGlu 2/3 receptor antagonist LY341495 (**153**) (685, 686).



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Group II mGlu agonists such as LY354740 and LY379268 attenuate PCP-evoked ambulations and fine motor movements, as do clozapine and haloperidol. Consistent with a mechanism involving mGlu2/3 receptors, inhibition of these PCP precipitated behaviors by LY379268, but not clozapine or haloperidol, were reversed by LY341495 (691). In contrast, clozapine and haloperidol potently block amphetamine-stimulated increases in locomotion and stereotypical movements, whereas doses of LY354740 and LY 379268 that block PCP-evoked behaviors in rats had less consistent effects on amphetamine-induced behaviors.

The treatment of the negative symptoms of schizophrenia has always proved challenging. The reduced cognitive abilities and social withdrawal that typify negative symptoms and contribute in a major way to the adequate function of schizophrenics (701). Negative functions are thought to occur from decreased dopamine neurotransmission in the prefrontal cortex. Recent findings show this to be associated with the inheritance of an allele for catechol-o-methyltransferase (COMT), which increases the thermal stability and thus activ-

ity of COMT. This hastens the rate of dopamine degradation and decreases synaptic concentrations of dopamine (708). Clozapine, the agent that best treats these symptoms, increases extracellular dopamine levels as well as the dopamine metabolites homovanillic acid and DOPAC in the prefrontal cortex (692).Similar brain microdialysis studies have shown a very similar profile for LY379268 (693).

These studies reveal the unique pharmacological and behavioral profiles of metabotropic glutamate agonists. In contrast to the strategies investigated for ionotropic glutamate receptors that sought to facilitate glutamate neurotransmission, it seems that the mixed mGlu2/3 autoreceptor agonists exert their efficacy within the models by attenuating glutamate neurotransmission in selected brain regions. Additionally, these agents seem to have modulatory effects on subsets of dopaminergic pathways. This presents the concept that group II metabotropic glutamate agonists may show promise as antipsychotic agents that act independently of the D_2/D_3 receptors antagonized in one way or another by essentially all antipsychotics. Whether any compound will successfully treat psychosis without antagonizing at least one member of the D_2 family of dopamine receptors will be an important question to answer in the next Burger's review of antipsychotic medicinal chemistry.

9 WEBSITE ADDRESSES

Communication technology has advanced dramatically since the publication of Burger's 5th Edition in 1995. Accordingly, we have included some appropriate website addresses together with a brief description of what may be found there.

- http://www.schizophrenia.com—this site provides patients, family members, and care givers with information on schizophrenia and related mental illnesses
- http://www.schizophrenia.com/research/research/html—schizophrenia information for researchers and professionals
- http://www.schizophrenia.co.uk—provides information linking principles and practice in schizophrenia
- http://www.mentalhealth.com—Internet mental health site is a free encyclopedia of mental health information
- http://www.mentalhealthfacts.com/druginfo/psychdrugs1.html—contains psychiatric drug information sheets
- http://www.merck.com/pubs/mmanual—The Merck Manual of Diagnosis and Therapy—see Section 15, Chapter 193
- http://www.nimh.nih.gov/publicat/schizoph.htm—The National Institutes of Mental Health information resource on schizophrenia

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CHAPTER ELEVEN

Investigative Agents for Use in Neurodegenerative Conditions

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1 INTRODUCTION

This chapter discusses strategies for the development of drugs to treat stroke, ruptured berry aneurysm, traumatic brain injury, and similar conditions. The strategies of interest to this chapter involve inhibition of radical formation, enhancement of repair of damaged DNA, augmentation of cellular energetics, and inhibition of excitotoxic mechanisms. Nitric oxide synthase (NOS) inhibitors and *N*-methyl-D-aspartate (NMDA) receptor antagonists can inhibit free-radical production and are being used or investigated in several neurodegenerative conditions. DNA repair can be influenced by poly(adenosine 5'-diphosphate-ribose) polymerase (PARP) inhibitors that are being actively investigated in stroke. PARP inhibitors, nicotinamide adenine dinucleotide (NAD) glycohydrolase inhibitors, and NAD precursors can influence cellular energetics and may be important in the treatment of stroke. Inhibition of excitotoxic mechanisms involves inhibitors of NMDA receptors and nitric oxide synthase. Many of the agents discussed in this chapter are being investigated in several neurodegenerative conditions.

1.1 Alzheimer's Disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis, Multiple Sclerosis, and Other Neurodegenerative Conditions

This chapter does not extensively discuss Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis, or some rare neurodegenerative diseases because they have been discussed previously (1). Alzheimer's disease is still poorly understood. For instance, it could be caused by plaque formation, tangle formation, or free-radical formation. Alzheimer's disease is widely treated in Europe with ginkgo preparations. Gingko produces a small improvement in cognitive function in patients. Structure-activity correlations in neurodegenerative models for ginkgolides, or flavonoids found in ginkgo, have not been published. In addition, there have been few attempts to synthesize new ginkgolides or flavonoids that may be more active than natural forms. It is not clear whether ginkgo preparations improve cognitive function through antioxidant or other mechanisms. However, several antioxidants, including vitamin E, have been suggested to be effective in the treatment of the disease. Alzheimer's disease is also treated with acetylcholinesterase inhibitors. The newer highly potent, reversible acetylcholinesterase inhibitors, such as donepezil, improve cognitive measurements in about 25% of patients by 24 weeks of treatment.

Parkinson's disease is caused by the oxidative stress-induced loss of dopaminergic neurons and can be effectively treated with levodopa in combination with dopa decarboxylase inhibitors such as carbidopa or catechol-*O*-methyltransferase inhibitors such as tolcapone. Levodopa is well known to increase the life spans of patients with Parkinson's disease. It may do this by enhancing brain dopamine levels and inhibiting tyrosine hydroxylase, which produces oxygen radicals. Several dopamine receptor agonists are available for use in Parkinson's disease and are extensively used in patients suffering from the adverse effects of levodopa. Anticholinergics such as trihexyphenidyl are also used in Parkinson's disease.

Amyotrophic lateral sclerosis is currently treated with riluzole, which improves survival in 30% of patients. Riluzole, an NMDA receptor antagonist, is discussed later in this chapter. A clinical trial of gabapentin, a (γ aminobutyric acid (GABA) agonist, in amyotrophic lateral sclerosis demonstrated little efficacy. Antioxidants have also not demonstrated significant efficacy so far.

Multiple sclerosis is treated with interferon beta, glatiramer acetate, and steroids. Glatiramer is a random copolymer of L-alanine, L-glutamate, L-lysine, and -tyrosine in a ratio of 6/1.9/4.7/1. About 30% of patients using glatiramer experience fewer relapses than do controls. Interferon beta appears to abolish relapses in about 10% of patients. Steroids are used to treat acute exacerbations. A wide range of immunomodulatory drugs are either used or being investigated, including azathioprine, cladribine, cyclophosphamide, immu-

Recent Citations for Drugs Used in Alzheimer's Disease, Amyotrophic Lateral Sclerosis (ALS), and Multiple Sclerosis (MS). Ache Refers to Acetylcholinesterase.

Agent	*First Author*	*Citation*
Ginkgo	*B. S. Oken*	*Arch. Neurol.,* 56, 1409–1415 (1998)
Ginkgo	*P. L. Le Bars*	*Dementia Geriat. Cognit. Disord.,* 11,230–237 (2000)
Antioxidants	*D. Pratico*	*Am. J. Med.,* 109,577–585 (2000)
Ache inhibitors	*P. Camps*	*Mol. Pharmacol.,* 57,409417 (2000)
Ache inhibitors	*W. J. Krall*	*Ann. Pharmacother.,* **33**, 441–450 (1999)
Ache inhibitors	*D. L. Bai*	*Curr. Med. Chem.,* 7,355–374 (2000)
ALS *drugs*	*O. Hurko*	*J. Neurol. Sci.,* **180**, 21–28 (2000)
Glatirarner acetate	*M. Filippi*	*Neurology,* 57,731–733 (2001)
Steroids	*F. Brusaferri*	*J. Neurol.,* **247**, 435–442 (2000)
MS drugs	*J. Bryant*	*J. Neurol. Neurosurg. Psychiat.,* 70,574–579 (2001)

noglobulin, methotrexate, and mitoxantrone. Mitoxantrone was approved for use in multiple sclerosis in November 2000. All of the agents used in this disease modify the immune system, especially T-cell responses.

1.2 Stroke and Other Ischemic Conditions

The major clinical approach to stroke currently is prevention. Stroke can be prevented in some patients by cessation of lifestyle problems such as smoking, excessive alcohol consumption, and obesity. These lifestyle problems lead to heart disease and atherosclerosis that can lead to stroke. Adequate treatment of hypertension, heart disease, hypercholesterolemia, and coagulation problems may be of some benefit in the prevention of stroke in other patients. Drugs such as aspirin, anticoagulants, antihypertensives, and lipid cholesterol-lowering agents, such as statins and niacin, have been recommended for use in stroke prevention. However, these drugs may be of little value for decreasing infarction in a patient who has already suffered from a stroke. This chapter focuses on agents that may be of use in decreasing infarction in stroke patients.

Treatment of neurodegenerative conditions presents some unique challenges. First of all, a drug must penetrate into the brain. Uptake into the brain can involve active uptake processes, such as the uptake of certain vitamins into the brain. This uptake usually is mediated by transport proteins that bind the drug and facilitate the penetration across the blood-brain barrier. Drugs that do not cross into the brain by active uptake must be adequately lipophilic. Lipophilicity may allow a drug to penetrate through the blood-brain barrier and remain in the brain. However, a drug must not be too lipophilic. Drugs that are too lipophilic do not penetrate rapidly into the brain. These drugs may be transported in the blood bound to lipoproteins, vitamin E for instance. Such drugs may gain access only to the endothelial lining of the brain vasculature. It is possible that these drugs may then slowly penetrate into the brain.

Another concern with drugs used to treat conditions such as stroke is that patients may be unconscious. Therefore, the drug must be delivered intravenously by injection or intragastrically by feeding tube. Intravenous drugs must be adequately soluble in aqueous solvents to allow injection into the blood. Drugs administered intragastrically must not cause vomiting, which is dangerous in unconscious patients.

Drugs used in the treatment of neurodegeneration must not greatly interfere with normal neurotransmission. In other words, normal neurotransmission should continue or should not be greatly altered in the presence of the drug. This is a concern with atropine-like drugs that can induce hallucinations at high doses because of inhibition of cholinergic neurotransmission. Some of the powerful NMDA receptor antagonists, similar to phencyclidine, disrupt normal neurotransmission and cause psychotic episodes in some patients that prevent the drugs from being useful.

A major concern is the treatment of the patient, without necessarily focusing on one mechanism of action of a drug. For instance, if cooling a patient down improves outcome,

why not use a drug that induces hypothermia? Hypothermia is routinely used in open chest surgery, where patients are mechanically cooled to enhance survival. Intracranial pressure may increase in patients after stroke. It is known that increased intracranial pressure is dangerous in patients suffering from traumatic brain injury. It is routine to monitor intracranial pressure in traumatic brain damage patients and to decrease intracranial pressure by withdrawing cerebrospinal fluids. Yet, it is not routine to monitor or correct intracranial pressure in stroke patients. There are drugs available that are known to decrease intracranial pressure. Yet, they are not used in the treatment of stroke. The point here is that it is the patient that counts. Too often therapeutic strategies are neglected because it is decided that the drug therapy induces an artifactual effect that is not an intended effect. For instance, if a drug inhibits excitotoxic mechanisms and also induces hypothermia, the drug may be neglected because the hypothermic effect is considered artifactual. Yet, such a drug could be a real benefit to patients. It is important to realize that it is the survival of the patient that is important.

The overall goal of therapeutics with these drugs is to prevent the death of cells in the brain. Of course it is important to protect neurons because they do not normally regenerate well. However, stem cells in the brain may allow some neuronal regeneration. Endothelial cells maintain the blood-brain barrier and must be protected if the brain is to survive. Astrocytes support neuronal activity by deactivating neurotransmitters, providing trophic factors and other activities. Therefore, astrocytes must be protected if the brain is to survive. Oligodendrocytes make myelin that is vital for axonal survival. Therefore, oligodendrocytes must be protected. It is obvious that all of the cells of the brain are vital to brain survival and should be protected if possible.

2 NECROSIS AND APOPTOSIS

Infarction of the brain is involved in stroke, ruptured berry aneurysm, traumatic brain damage, and other conditions and may be caused by temporary interruption of blood flow to areas of the brain. Infarction produces a large area of dead tissue that eventually may form an extensive glial scar. In stroke, infarction involves necrotic and apoptotic cell death (2, 3). The majority of the cell death in the core of the infarction is necrotic. However, apoptotic cell death also occurs in the core and is more prominent in the limit area surrounding the core, sometimes called the penumbra. The term *limit* will be used in this chapter. Penumbra is a term originally used to refer to an area that is not as ischemic as the core and that reperfuses more readily than does the core. The penumbra disappears during reperfusion. Eventually, the inner part of the penumbra may become part of the core of the infarction. The outer penumbra becomes the limit area where apoptosis is prominent. Necrosis is also seen in the limit area. The point here is that necrosis must be prevented in order to prevent extensive infarction. However, apoptosis prevention may also be important.

Cells die in the brain by two main processes, necrotic and apoptotic. However, some authors find other forms of cell death in the brain, such as oxytosis. Necrosis is a rapid process in which the defense mechanisms of the cell are overwhelmed. The cell nucleus swells and ruptures. Cellular mitochondria may swell. The cytoplasm swells and forms small vacuoles. The cell membrane may eventually rupture. Necrosis may be maximal in the brain at about 6–12 h after an insult.

Apoptosis is delayed and may be maximal at 24–48 h after an insult. However, apoptotic cells can be seen even within 6 h of an insult, but are not common. Apoptosis involves condensation of the nucleus. Condensation of the cytoplasm occurs with large vacuole formation. Some mitochondria may condense. Very uncommonly, some mitochondria swell in apoptosis. The cell splits up into membrane-bound apoptotic bodies that can be seen in the limit areas surrounding the core of an infarction. Apoptosis is a programmed form of cell death that occurs following a small but sufficient amount of damage to a cell. This initiates a program that eventually kills the cell.

3 DNA DAMAGE AND REPAIR

3.1 DNA Damage

DNA is damaged within minutes of oxygen free-radical generation in the brain. This damage must be repaired for the cell to survive. Even neurons need DNA. Although neurons are terminally differentiated, they have very active protein synthesis to maintain neurotransmission. Without intact DNA, neurons cannot continue normal neurotransmission. In addition, neurons have only two copies of DNA in the nucleus, unlike the liver and some other cells that have many copies of DNA in the nucleus. Extensive DNA damage leads to necrosis. A small, but critical, amount of DNA damage leads to apoptosis. Repair of neuronal DNA is critical to survival of patients suffering from stroke and similar conditions.

DNA damage is induced by oxygen radicals, necrotic events, and apoptotic events. Oxygen radicals are well known to cause DNA fragmentation and other damage to DNA. Necrosis involves the detachment of histones from DNA and the random cleavage of DNA by endonuclease. Apoptosis involves the cleavage of DNA, with the histones still attached, into nucleosome fragments by endonuclease.

DNA damage by oxygen radicals can stimulate DNA repair processes. DNA damage involved in either necrotic or apoptotic events may also stimulate DNA repair processes. Whether the cell can be rescued from DNA damage depends on how much DNA damage has occurred and when the DNA damage occurred. For instance, if necrotic events are already well under way, it may be too late to reverse the necrosis.

3.2 DNA Repair

Several enzymes are involved in DNA repair and are potential targets for drug therapy in neurodegeneration (4). DNA damage must first be recognized. This recognition depends on several poorly defined enzymes. Next, the damaged DNA must be excised, usually by a specific endonuclease. Then new DNA must be synthesized by a DNA polymerase. Finally, the new DNA is joined to the old DNA by DNA ligase that requires ATP. There are several distinct DNA repair processes: base-excision repair, nucleotide-excision repair, mismatch repair, and double-strand break repair. Each type of repair process involves slightly different enzymes.

Base-excision repair involves removal of a damaged base by DNA glycosylase. The damaged area is removed by an endonuclease. DNA polymerase beta resynthesizes DNA. This is called short-patch base-excision repair. However, a long-patch repair process also occurs. This involves recognition of the damage by PCNA (proliferating cell nuclear antigen), which activates DNA polymerases delta and epsilon. The new strand is joined to the old strand by Fen 1 (a structure-specific nuclease).

Nucleotide-excision repair involves PCNA, DNA polymerase epsilon, and several accessory proteins, such as RF-C (replication factor C), RP-A (replication protein A), and Lig I (DNA ligase I). It could be that these proteins form an aggregate DNA repair machine. PCNA is a hollow circular protein that encircles DNA. PCNA binds and activates many proteins involved in DNA repair. Mismatch repair is less well characterized, but involves DNA polymerase delta.

Double-strand breaks, nicks, and similar DNA damage activate PARP. Once the damage is recognized by PARP, there is a rapid. activation of PARP that quickly activates several enzymes involved in DNA repair. PARP activates transcription factors that regulate the transcription of proteins that may be involved in DNA repair.

3.3 PARP and DNA Repair

PARP contains two zinc fingers that bind the two broken stands of DNA and stabilize the structure. PARP uses NAD as a substrate to poly(ADP)-ribosylate proteins, including itself. Early on, there is a small amount of self-poly(ADP)-ribosylation that activates PARP by causing it to homodimerize. This dimerization allows the activated PARP to bind to four strands of fragmented DNA, stabilize the structure, and facilitate repair. PARP also poly(ADP-ribosylates) endonuclease, which deactivates endonuclease and protects DNA from cleavage.

PARP is composed of three domains (5). The N-terminal contains the two zinc fingers

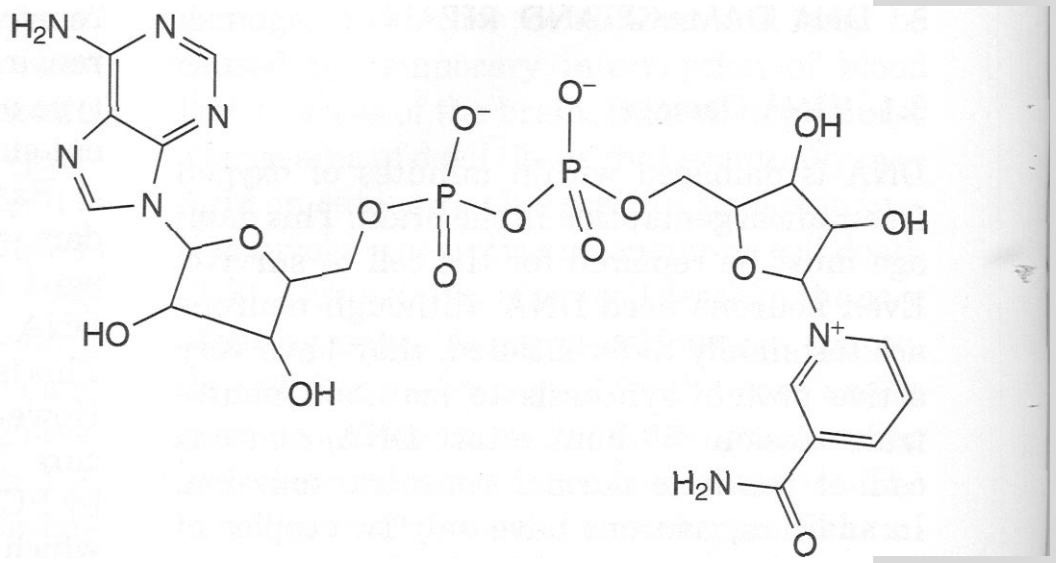


Figure 11.1. NAD.

and the nuclear location signal. The middle section contains the automodification area. The C-terminal contains the active site that binds NAD (Fig. 11.1). The active site has a nicotinamide-binding site and an adenine-binding site.

The crystal structure of the active site of PARP has been described (5). It is made up of a five-stranded antiparallel beta sheet and a four-stranded mixed beta sheet (Fig. 11.2). The two beta sheets are connected by two hydrogen bonds. The beta sheet structure is supported by a surrounding protein structure made up of five alpha helices, three 3_{10} helices, and beta sheet excursions. The active site is very similar to those found in the bacterial toxins that are mono(ADP-ribosyl) transferases (6).

Several amino acids are involved in the active site of PARP: Glu988, Tyr896, Ala898, Lys903, His862, Ser904, Gly863, and Tyr907 (6). A crystal structure for NAD bound to PARP is not available because the complex is unstable. However, a model system of NAD bound to diphtheria toxin, a mono(ADP-ribosyl) transferase, has yielded a useful crystal structure (7). NAD binds with the ribose in a 3'-endo conformation and the nicotinamide in a syn position (Fig. 11.3). Several hydrogen bonds and hydrophobic interactions occur be-

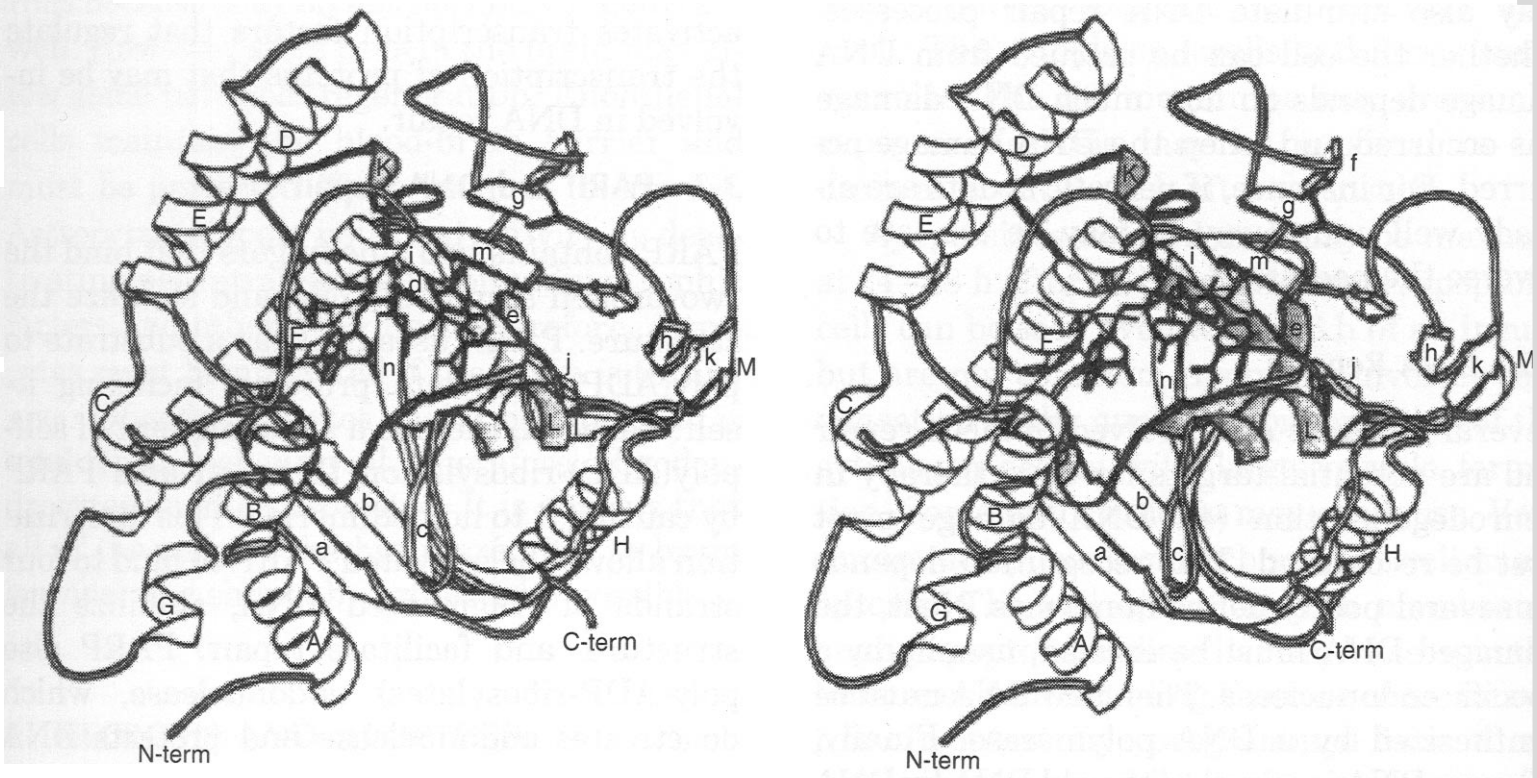


Figure 11.2. Ribbon representation of the PARP active site. A strongly conserved motif that binds NAD (black) is shown in gray. This figure is from Ref. 5 and is used with permission.

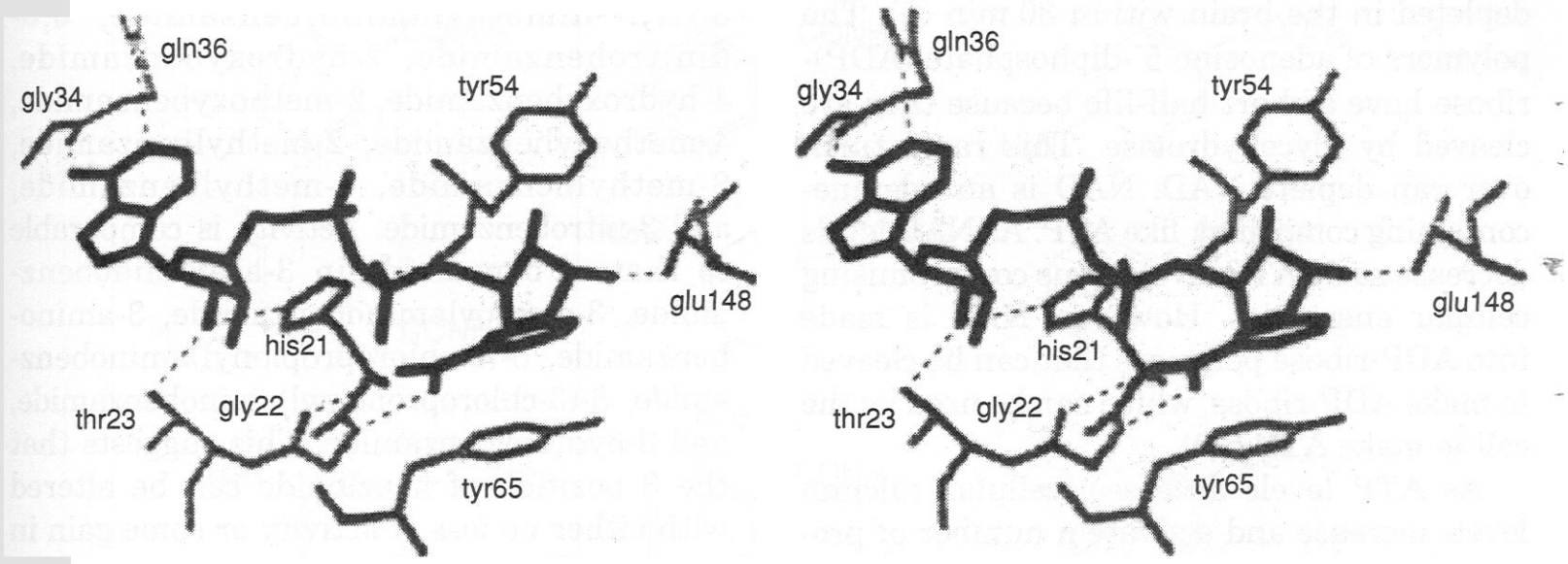


Figure 11.3. Stereo view of atomic interactions of NAD and a model of the PARP site. NAD is shown in black. The PARP site amino acids are shown in gray. Hydrogen bonds are shown as dashed lines. This figure is from Ref. 7 and is used with permission.

tween the enzyme and the substrate. The hydrogen bonds are as follows: Gly876, Asp770, and Arg878 bond to the adenine; Ser864 or His562 bonds to the ribose connected to the adenine; Asp766, Gln763, and Tyr896 bond to the phosphates; Gly863 or Ser904 bond to the nicotinamide; and Tyr907 and Glu988 bond to the ribose connected to the nicotinamide. Hydrophobic bonds are as follows: Leu877 and Ile872 bond to adenine; Tyr907 bonds to nicotinamide; and Tyr896, which may bond to the ribose connected to the nicotinamide. Glu988 may be involved in the catalytic mechanism by polarizing the NAD and the ADP-ribose acceptor through hydrogen bonding. This polarization would stabilize the NAD transition state and increase the nucleophilicity of the acceptor. The NAD transition state involves a ribose oxocarbenium ion that leads to cleavage in an S_N2 mechanism.

DNA can be stabilized under potentially damaging conditions by PARP inhibition. When DNA damage occurs, PARP is activated and binds to DNA, thus stabilizing it. PARP inhibitors cause PARP to remain bound to DNA, thereby providing long-term stabilization, even when oxygen radical generation occurs. After the generation of oxygen radicals has ceased, the PARP inhibitor can dissociate from PARP, allowing the DNA to be repaired.

After enough DNA repair has occurred, PARP causes the detachment of the repair machinery and of PARP itself from the DNA. PARP does this by poly(ADP)-ribosylating proteins, making them anionic. This establishes an electrostatic repulsion from negatively charged DNA.

PARP is a family of enzymes including PARP-1, PARP-2, PARP-3, tankyrase, and V-PARP (8). PARP-1 is perhaps the most important enzyme involved in DNA repair in stroke. However, if PARP-1 is deactivated, perhaps the other enzymes can take its place. The PARP inhibitors described so far in the literature are PARP-1 inhibitors. It is not known whether these compounds also inhibit the other forms of PARP.

4 CELLULAR ENERGETICS

During ischemia, oxygen levels diminish, thus shutting down some mitochondrial functions and greatly decreasing adenosine 5'-triphosphate (ATP) levels. When oxygen reperfuses into the brain, ATP levels rise to normal levels within about 5 min. ATP is required for cellular energetics, for oxidized glutathione (GSSG) reduction, for DNA repair, and for many other functions. Therefore, without ATP, DNA repair cannot proceed. The cell must turn to other ways to make ATP until oxygen returns and mitochondrial function returns.

PARP depletes cellular energy resources. It does this by using NAD as a substrate. When DNA damage is severe, such as during ischemia and reperfusion, NAD levels can become

depleted in the brain within 30 min (9). The polymers of adenosine 5'-diphosphate (ADP)-ribose have a short half-life because they are cleaved by glycohydrolase. This rapid turnover can deplete NAD. NAD is an adenine-containing wmpound, like ATP. As NAD levels decrease so do ATP levels, thus compromising cellular energetics. However, NAD is made into ADP-ribose polymers that can be cleaved to make ADP-ribose, which can be used by the cell to make ATP (10).

As ATP levels decrease, cellular calcium levels increase and activate a number of proteases, endonucleases, and other enzymes. Caspase enzymes also become activated. Caspase-3 rapidly cleaves PARP not bound to DNA, and to a lesser extent DNA-bound PARP. When PARP is cleaved, DNA is vulnerable to the actions of endonuclease, which is part of the apoptotic program.

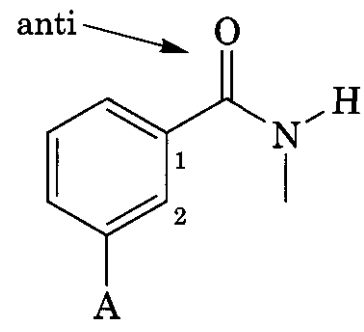
PARP inhibitors can be used to maintain cellular energetics. They do this by preventing NAD depletion, which helps prevent ATP depletion. In addition, inhibited PARP remains bound to DNA and is less vulnerable to the actions of caspase-3. This helps prevent necrosis and apoptosis.

41 PARP Inhibitors

PARP inhibitors have been synthesized (11) and are shown in Figs. 11.4 and 11.5. Some of them have been tested in stroke models with good results in terms of preventing infarction (12–18). Most of the inhibitors are based on the structure of nicotinamide and therefore bind to the nicotinamide site of PARP. Several inhibitors are well known, such as nicotinamide, benzamide, and 3-aminobenzamide (19, 20). However, new quinazolinone, phenanthradinone, and other inhibitors are being investigated. Nicotinamide has a K_i of about 15 μM for PARP (19). Activity is decreased in 6-aminonicotinamide, isonicotinamide, 1-methylnicotinamide, 5-methylnicotinamide, 8-methylnicotinamide, and thionicotinamide (Table 11.1).

Benzamide has a K_i of about 2 μM for PARP (19). Activity is decreased in 2-acetamidobenzamide, 2-aminobenzamide, 4-aminobenzarnide, 2-halobenzamide, 3-halobenzamide, 4-halobenzamide, 2,6-difluorobenzamide, 3-(*N*,*N*-dimethylamino)benzamide, 3,5-dinitrobenzamide, 2-hydroxybenzamide, 4-hydroxybenzamide, 2-methoxybenzamide, 4-methoxybenzamide, 2-methylbenzamide, 3-methylbenzamide, 4-methylbenzamide, and 3-nitrobenzamide. Activity is comparable to that of benzamide in 3-acetamidobenzamide, 3-acryloylaminobenzamide, 3-aminobenzamide, 3-(2-chloropropionyl)aminobenzamide, 3-(3-chloropropionyl)aminobenzamide, and 3-hydroxybenzamide. This suggests that the 3 position of benzamide can be altered with either no loss of activity or some gain in activity. These 3-position substituents (A) may interact with the ribose nucleoside binding domain of the active site. Substituents at the 3 position can improve water solubility of the agents, compared to hydrophobic benzamide. Several benzamide inhibitors of PARP are shown in Fig. 11.6 and are good inhibitors of PARP with significant inhibition at concentrations of 10 μM or less (21).

PARP inhibitors that are based on nicotinamide have an electron-rich aromatic ring, a carbonyl substituent in the anti conformation that contains a nitrogen and a free hydrogen, and an aromatic ring substituent (A) such as hydroxy, methoxy, or amino. The carbonyl group is usually a carboxamide, but can also be a thiocarbamoyl group. The oxygen in the carboxamide may serve as an electron donor and the carbon as an electron acceptor in PARP interactions. The carbonyl oxygen should be in a conformation anti to the 1,2-bond of the aromatic ring (22). The nitrogen of the carboxamide must not be alkylated because alkylation abolishes activity. This suggests that a hydrogen of the carboxamide nitrogen is involved in hydrogen bonding to the active site. The aromatic ring can be one ring or a polycyclic aromatic system. Heterocyclic rings can also be active.



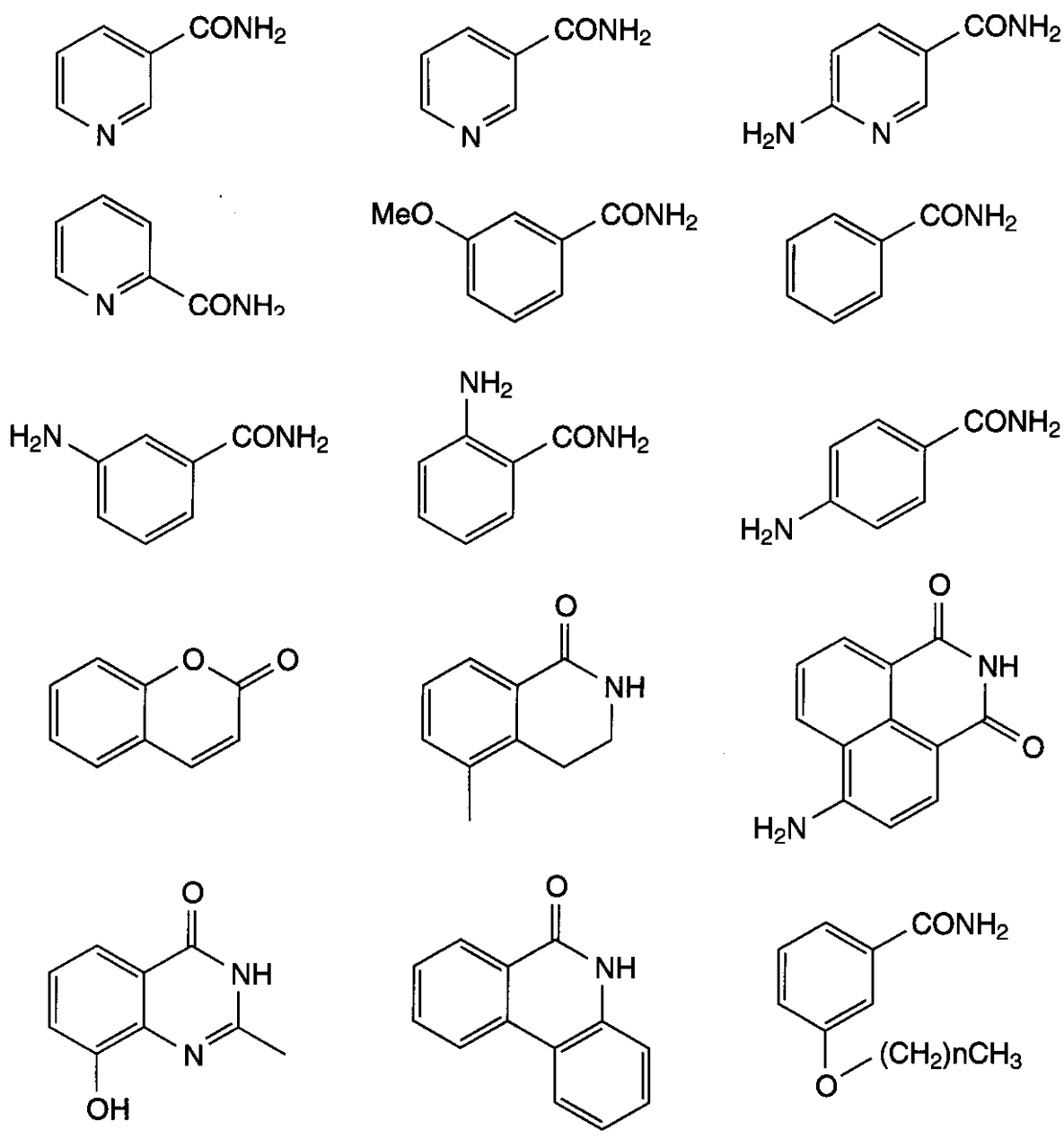


Figure 11.4. PARP inhibitors: nicotinamide, 5-methylnicotinamide, 6-aminonicotinamide, picolinamide, 3-methoxybenzamide, benzamide, 3-aminobenzamide, 2-aminobenzamide, 4-aminobenzamide, coumarin, 3,4-dihydro-5-methylisoquinolinone (PD 1287631, 4-amino-1,8-naphthalimide, 8-hydroxy-2-methyl-3-hydroquinazolin-4-one, phenanthridinone, and 3-*O*-alkylbenzamides. This figure is redrawn from Ref. 33 and is used with permission.

Several inhibitors are known that bind to the adenosine site of PARP (19). These inhibitors include 5-bromo-2′-deoxyuridine, caffeine, 5-bromouracil, diadenosine-tetraphosphate, 1-methyladenine, 5-nitrouracil, theophylline, theobromine, thymidine, and other compounds. These compounds are not as well studied as the nicotinamide analogs. In addition, it is not known whether these compounds can interact with the adenosine (A,) receptors that are involved in modulation of synaptic transmission and neuroprotective effects.

Inhibitors based on the structure of nicotinamide or benzamide have been reported to have toxicity problems arising from the fact that some of them may be antimetabolites for NAD synthesis or interact with other NAD biochemical pathways (22). Recent attempts to make more specific and more potent inhibitors of PARP have centered around making conformationally restricted analogs. For instance, the 5-substituted dihydroisoquinolinones below are restricted to anti conformations and are PARP inhibitors. However, the 7-substituted dihydroisoquinolinonesare restricted to syn conformations and are inactive. 5-Methyl-3,4-dihydroisoquinolinone (PD128763) is being investigated for use in stroke and other conditions. Several isoquinolinones appear to be good inhibitors of PARP (21), with significant inhibition at concentrations of 10 μM or less (Fig. 11.7).

Benzoxazoles have become of interest because they may have intramolecular hydrogen

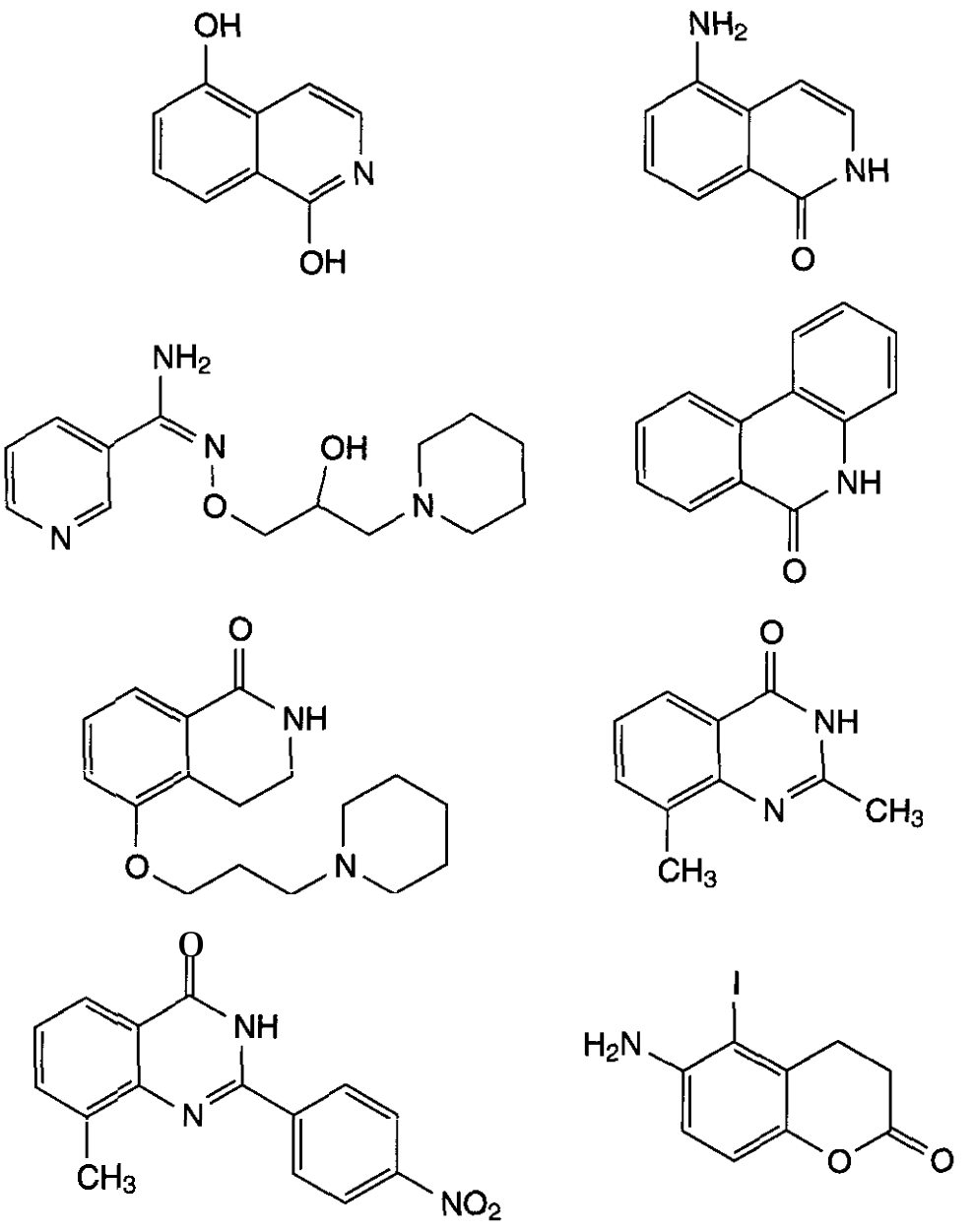
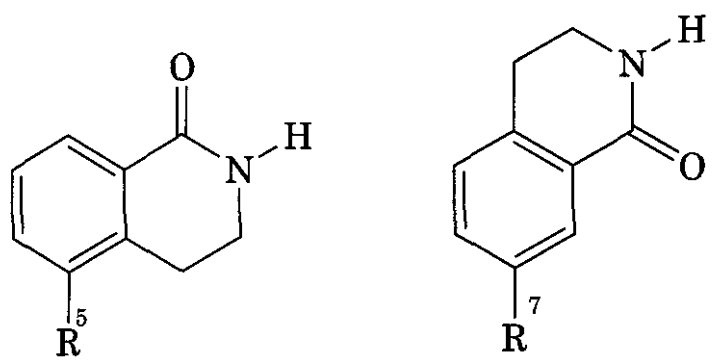
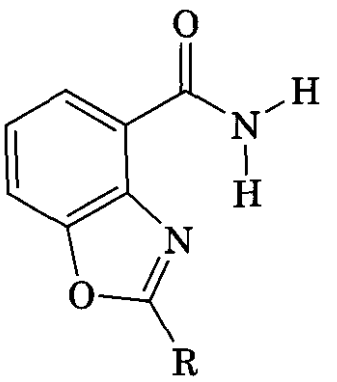


Figure 11.5. PARP inhibitors: 1,5-dihydroxyisoquinoline, 5-aminoisoquinolin-1(2H)-one, *O*-(2-hydroxy-3-piperidinepropyl)pyridine carbonic acid amidoxime, 6(5*H*)-phenanthridinone, 3,4-dihydro-5-[4-(1-piperidinyl) butoxy]-1(2*H*)-isoquinoline, 2,8-dimethyl-3-hydroquinazolin-4-one,8-methyl-2-(*p*-nitrophenyl)-3-hydroquinazolin-4-one, 5-iodo-6-aminocoumarin. These inhibitors are from Ref. 11 and have been **redrawn** with permission.



R = H, OH, OCH_3, NO_2, NH_2

bonding that may keep them in anti conformations. Attempts to make benzoxazoles have resulted in quinazolin-4-(3*H*)-one synthesis as a result of rearrangements during synthesis **(23).** However, some of the quinazolinones have been found to be good PARP inhibitors (Table 11.2). The quinazolinones are restricted to anti conformations. At least one hydrogen must be available on the nitrogen in the 3 position or activity is lost. In general, for substituents at the 8 position (X), a methyl or hydroxy group provides more activity than that of a methoxy group. This may be attributable to steric constraints in the active site. The addition of a 2-phenyl to the molecule (C) enhances activity if electron-withdrawing or electron-donating substituents are added in the ***para*** position.



Benzoxazole

Thiophenecarboxamides, thienopyridinones and thienopyrimidinones have been synthesized that are sulfur containing analogs of

Table 11.1 Effects of Various Compounds on Poly(ADP-ribose) Polymerase Activity[a]

Compound	K_i (μM)	IC_{50} (μM)
m-Acetamidoacetophenone	—[b]	930
2-Acetamidobenzamide	—	1000
3-Acetamidobenzamide	0.4	—
3-Acetamidobenzamide	—	12
8-Acetamidocarsalam[c]	—	1400
3-Acetamidosalicylamide[c]	—	2000
5-Acetamidosalicylamide	—	45
Acetophenone[c]	—	2300
3-Acryloylaminobenzamide	3.14	—
m-Aminoacetophenone	—	1900
2-Aminobenzamide	71	—
2-Aminobenzamide	—	100,650
3-Aminobenzamide	1.8–12	—
3-Aminobenzamide	—	5.4–33
4-Aminobenzamide	65–75	—
4-Aminobenzamide	—	400–1800
6-Amino-1,2-benzopyrone	47	—
6-Amino-1,2-benzopyrone[c]	—	850
2-Amino-3-chloro-1,4-naphthoquinone[c]	—	820
4-Amino-1,8-naphthalimide[c]	—	0.18
6-Aminonicotinamide	—	1100
5-Aminosalicylamide	—	100
A2′pA2′pA	50	—
Apigenin[c]	—	<1500[d]
Arachidonic acid[c]	—	44
Benzamide	1.0–39	—
Benzamide	—	3.3–22
1,2-Benzopyrone	—	47
1,2-Benzopyrone[c]	—	2800
1,4-Benzoquinone	—	400
Benzoyleneurea[c]	—	8.1
2-Bromobenzamide	—	2900
3-Bromobenzamide[c]	—	55
4-Bromobenzamide[c]	—	2200
5-Bromo-2'-deoxyuridine	—	15
3-(3-Bromopropionyl)aminobenzamide	1.73	—
5-Bromouracil	—	160
5-Bromouridine	—	210
Caffeine	244	—
Caffeine	—	1400
Carsalam[c]	—	460
2-Chlorobenzamide	—	1000
3-Chlorobenzamide	—	22
4-Chlorobenzamide	—	300
N-(2-Chloroethyl)-1,8-naphthalimide[c]	—	<1800
3-(2-Chloropropionyl)aminobenzamide	1.9–2.0	—
5-Chlorosalicylamide[c]	—	190
5-Chlorouracil	—	270
Chlorthenoxazin[c]	—	8.5
4-Chromanone[c]	—	720
Chromone-2-carboxylic acid	—	560
Coenzyme Q_0	—	3900

Table 11.1 *(Continued)*

Compound	K_i *(ELM)*	IC_{50} *(ELM)*
Cyclohexanecarboxamide	—	620
trans-I-Decalone'	—	4300
Diadenosine 5′,5‴-p1, p2-diphosphate	—	—
Diadenosine 5′,5‴-p1,p4(p1, N6-ethenyl)tetraphosphate	13.9	≈20
Diadenosine 5′,5‴-p1,p4(p1, p2-methylene)tetraphosphate	22.6	≈20
Diadenosine 5′,5‴-p1, p4-tetraphosphate	5.1–7.7	—
4-Diazoniobenzamide-dGMP	150	—
3,5-Dibromosalicylamide[c]	—	560
2,3-Dichloro-1,4-naphthoquinone[c]	—	260
2,6-Difluorobenzamide	—	180
10,11-Dihydrodibenz[*b*,*f*][1,4]-oxazepin-11-one[c]	—	<2300
1,5-Dihydroxyisoquinoline	—	0.39
1,3-Dihydroxynaphthalene[c]	—	1300
3,5-Dimethoxybenzamide[c]	—	1200
3-(*N*,*N*-Dimethylamino)benzamide	—	120
3,5-Dinitrobenzamide	—	2500
Ethidium bromide	143.8	—
Flavone[c]	—	22
2-Fluorobenzamide[c]	—	120
3-Fluorobenzamide	—	20
4-Fluorobenzamide[c]	—	200
Formycin B	69–75	—
Harmine hydrochloride	—	<3500
m-Hydroxyacetophenone	—	600
2-Hydroxybenzamide	—	82
3-Hydroxybenzamide	1.0	—
3-Hydroxybenzamide	—	9.1
4-Hydroxybenzamide	—	280
4-Hydroxy-1,2-benzopyrone[c]	—	570
1-Hydroxyisoquinoline	—	7.0
4-Hydroxy-2-methylquinoline[c]	—	74
N-Hydroxynaphthalimide sodium salt	—	450
4-Hydroxypyridine	—	2300
4-Hydroxyquinazoline	—	9.5
4-Hydroxyquinoline[c]	—	80
Hypoxanthine	—	1700
1-Indanone[c]	—	810
5-Iodouracil	—	71
5-Iodouridine	—	43
Isatoic anhydride[c]	—	<3900
3-Isobutyl-I-methylxanthine	—	3100
Isoluminol[c]	—	290
Isonicotinamide	—	990
Isonicotinate hydrazide	—	4800
Juglone[c]	—	250
Kynurenic acid[c]	—	670
Lawsone[c]	—	330
Linoleic acid[c]	—	48
Linolenic acid[c]	—	110
γ-Linolenic acid[c]	—	120
Luminol[c]	—	23
Menadione[c]	—	420
Menadione sodium bisulfite	—	720

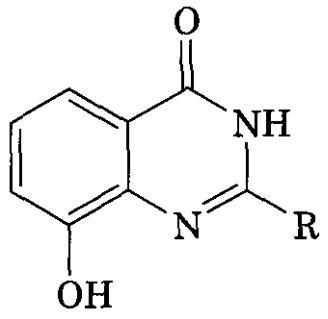
Table *11.1 (Continued)*

Compound	K_i (μM)	IC_{50} (μM)
2-Mercapto-4(3*H*)-quinazolinone[c]	—	44
2-Methoxybenzamide	—	20
3-Methoxybenzamide	0.6–2.9	—
3-Methoxybenzamide	—	3.4–17
4-Methoxybenzamide	—	1100
1-Methyladenine	226.6	—
2-Methylbenzamide	—	1500
3-Methylbenzamide	—	19
4-Methylbenzamide	—	1800
2-Methylchromone	—	45
I-Methylnicotinamide	—	1700
5-Methylnicotinamide	30–200	—
5-Methylnicotinamide	—	70350
8-Methylnicotinamide	—	7800
1-Methylnicotinamide chloride	—	3800
2-Methyl-4(3*H*)-quinazolinone[c]	—	5.6
NADH	55	—
NADP	460	—
1,8-Naphthalimide[c]	—	1.4
1,4-Naphthoquinone[c]	—	250
Nicotinamide	5.6–52	—
Nicotinamide	—	31–210
3-Nitrobenzamide	9.8	—
3-Nitrobenzamide	—	160
2-Nitro-6(5*H*)-phenanthridinone[c]	—	0.35
3-Nitrophthalhydrazide[c]	—	72
4-Nitrophthalhydrazide[c]	—	510
3-Nitrosalicylamide[c]	—	1600
6-Nitroso-1,2-benzopyrone	40	—
5-Nitrouracil[c]	—	430
Norharman	—	4700
Novobiocin	—	2200
Oleic acid[c]	—	82
Palmitoleic acid[c]	—	95
6(5*H*)-Phenanthridinone[c]	—	0.3
Phthalamide	—	1000
m-Phthalamide[c]	—	50
Phthalazine	—	150
1(2*H*)-Phthalazinone[c]	—	12
Phthalhydrazide[c]	—	30
a-Picolinamide	—	≈100–250
Plumbagin'	—	700
pppA2′pA2′pA	5	≈10
Pyrazinamide	—	130
Pyridoxal 5-phosphate[c]	—	4250
Quinazoline	—	2000
Reserpine[e]	—	790
trans-Retinal[c]	—	450
Showdomycin	107.8	—

Table *11.1 (Continued)*

Compound	K_i (μM)	IC_{50} (μM)
α-Tetralone[c]	—	310
Theobromine	15.2	—
Theobromine	—	110
Theophylline	29.8	—
Theophylline	—	46
Thiobenzamide	—	620
Thionicotinamide	—	1800
Thiophene-3-carboxamide	—	–50
Thymidine	13.3–140	—
Thymidine	—	43–290
2-Trichloromethyl-4(3*H*)-quinazolinone[c]	—	2200
Trp-P-1 (3-Amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole)	—	220
Trp-P-2 (3-Amino-1-methyl-5*H*-pyrido[4,3-*b*]indole)[c]	—	2200
Vitamin K_1[e]	—	520
Vitamin K_5[c]	—	1300
Xanthurenic acid[c]	—	190
$ZnCl_2$	—	10–77

[a]Table modified from Ref. 19 with permission.
[b]—, not determined.
[c]2% (final) Me_2SO.
[d]Maximum value estimated under conditions of limited solubility.
[e]10% (final) Me_2SO.



Quinazolinone

isoquinolinones and quinazolinones known to inhibit PARP (24). These compounds (Fig. 11.8) are potent inhibitors of PARP with significant inhibition found at concentrations of 10 μM or less. The possible neuroprotective activities of these compounds has not been reported.

4.2 NAD Clycohydrolase Inhibitors

ADP-ribose polymers and NAD are catabolized by glycohydrolase enzymes, such as NAD glycohydrolase. Some of the PARP inhibitors, such as nicotinamide, also inhibit glycohydrolase. The inhibition of NAD glycohydrolase could be another way to boost cellular energetics by slowing NAD turnover. Unfortunately, this approach has been neglected in the treatment of neurodegeneration.

NAD glycohydrolase is a member of the ADP-ribosyl transferase family of enzymes. The catalytic mechanism of NAD glycohydrolase is similar to the PARP mechanism and involves an oxocarbenium ion intermediate of NAD (25). Like the bacterial mono(ADP-ribose)transferase toxins, NAD glycohydrolase has a Rossman fold that binds NAD in an extended conformation and may prevent the synthesis of polymers of ADP-ribose. Therefore, NAD glycohydrolase can function only in the cleavage of NAD and perhaps the transfer of single ADP-ribose units to other proteins.

Several inhibitors of NAD glycohydrolase are known. Nicotinarnide, 3-aminobenzamide, and gallotannin (26) have been tested *in vivo* and were found to be neuroprotective. Novobiocin and meta-iodobenzylguanidine have been tested in an *in vitro* model of traumatic brain injury and were found to be protective (27). Inhibitors of NAD glycohydrolase include: 3-aminobenzamide, arachidic acid, arachidonic acid, benzarnide, bromodeoxyuridine, diethylamino(benzylidineamino)guanidine, meta-iodobenzylguanidine, isoniazid, novobiocin,

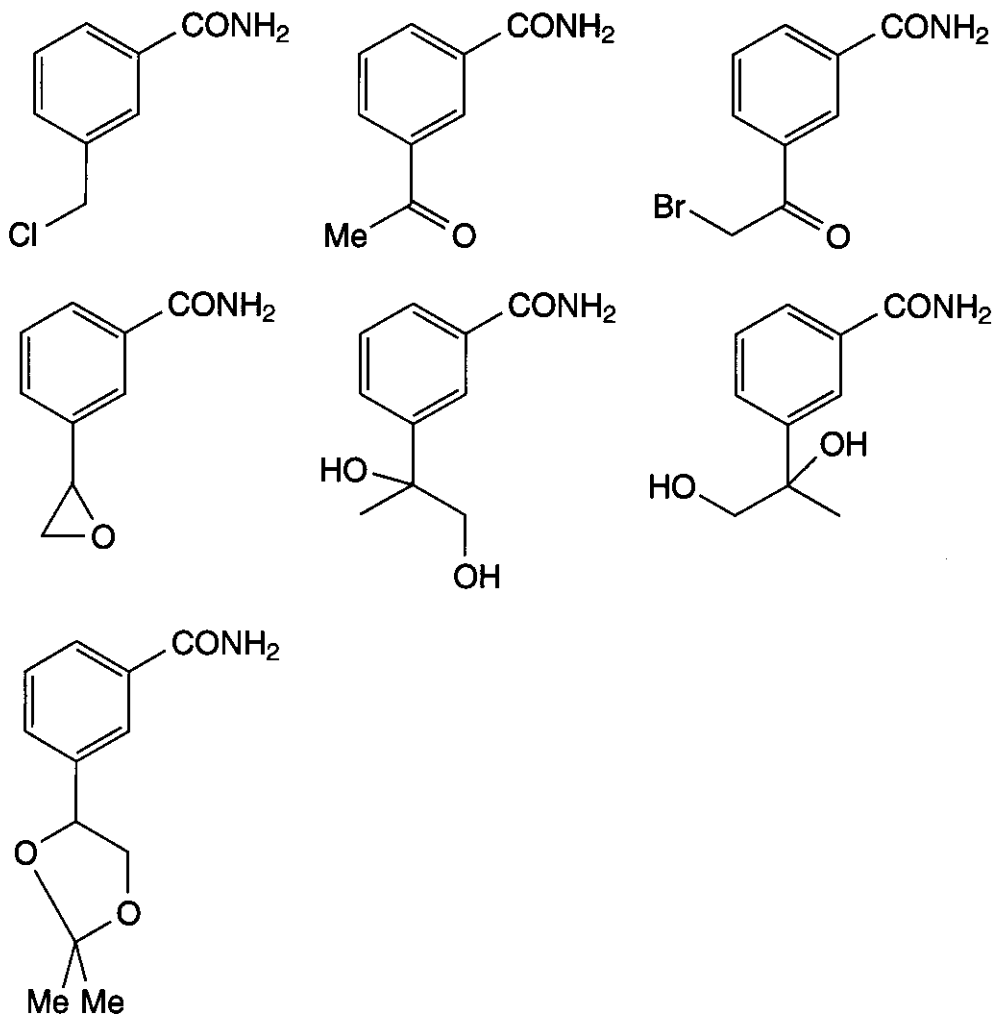


Figure 11.6. Benzamide inhibitors of PARP from Ref. **21.**

palmitic acid, palmitoleic acid, stearic acid, thymidine, theophylline, linoleic acid, vitamin K2, and vitamin K1 (25, 27, 28, 29).

4.3 NAD Precursors and Cellular Energetics

The most potent neuroprotective agent used clinically is nicotinamide, used in the treatment of pellagra. Before nicotinamide, the mortality from pellagra was 30% in the United States. About 10,000 deaths occurred every year, mostly caused by neurodegeneration. After the introduction of nicotinamide in 1938 (30), the mortality from pellagra decreased to nearly 0%. Of course, pellagra is caused by nicotinamide, or niacin, deficiency. Both nicotinamide and niacin are called vitamin B3. Pellagra is a disease of NAD deficiency.

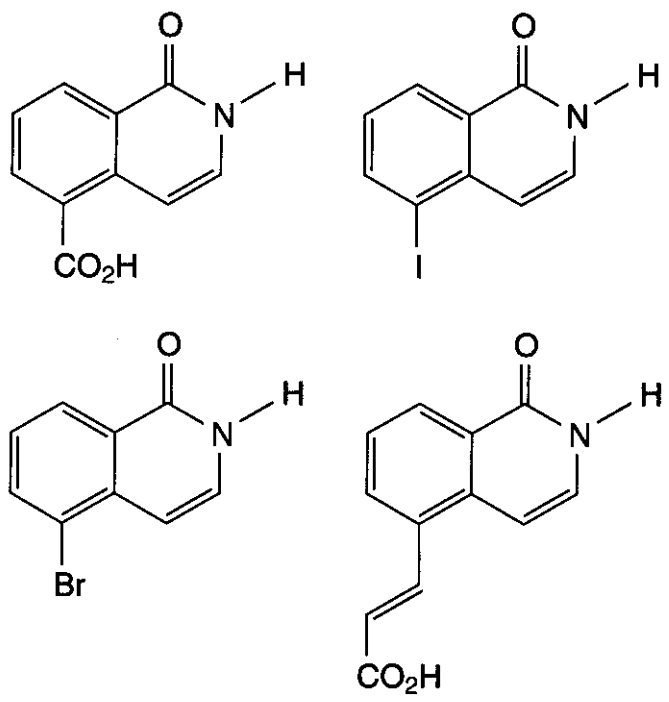
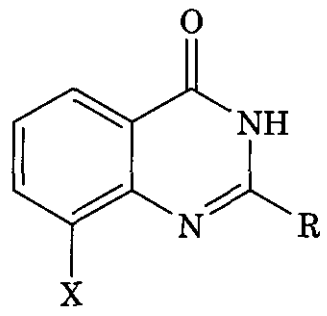


Figure 11.7. Isoquinolinone inhibitors of PARP from Ref. **21.**

Nicotinamide, but not niacin, is taken up into the brain by an active uptake process (31) and is converted into NAD (Fig. 11.9). Niacin released in the brain from catabolic processes is converted into nicotinamide as shown. Nicotinamide can increase brain levels of NAD by 50% or more (32). By increasing brain NAD levels, nicotinamide prevents ATP depletion (9) and protects cellular DNA (32). Therefore, nicotinamide maintains cellular energetics in the presence of oxidative stress.

Many compounds have been investigated that may maintain cellular energetics, especially in pellagra (Fig. 11.10). All but one of the compounds found to be active can be converted into NAD in the body (33). This is important because compounds that make NAD

Table 11.2 Selected Quinazolinones Tested for PARP Inhibition[a]



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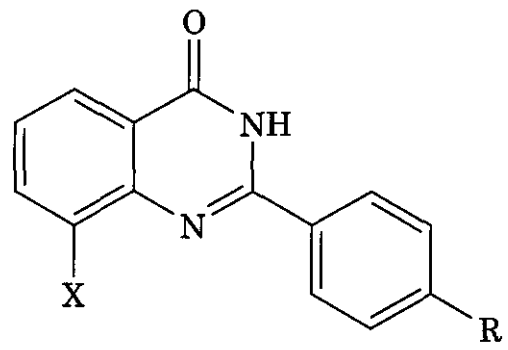


C

Compound[a]	Structure	R	X	IC_{50} (μM)
3AB				19.1
3HB				8.0
QN	(B)	H	H	15.8
PD128763				0.4
	(B)	Me	OH	0.4
	(B)	Me	Me	0.4
	(B)	Me	Orne	0.8
	(C)	H	Me	0.9
	(C)	H	Orne	4.2
	(C)	NO_2	Me	0.1
	(C)	NO_2	Orne	0.9
	(C)	CF_3	Me	>10
	(C)	CF_3	Orne	39
	(C)	CN	Me	0.3
	(C)	CN	Orne	1.3
	(C)	OMe	Me	0.2
	(C)	OMe	Orne	2.0
	(C)	N_3	Orne	1.9
	(C)	NH_2	Me	0.4
	(C)	NH_2	Orne	>0.3
	(C)	CO_2Me	Me	4.8
	(C)	H	OH	1.1
	(C)	NO_2	OH	0.2
	(C)	OH	OH	0.3
	(C)	OH	Me	0.2
	(C)	NH_2	OH	0.5

"Data are used with permission from Ref. 23 (© 1998 American Chemical Society).

[b]3AB is 3-aminobenzamide; 3HB is 3-hydroxybenzamide; QN is quinazolin-4(3*H*)-one; PD128763 is 5-methyl-3,4-dihydroisoquinolinone.

analogs in the body may be toxic and interfere with normal biochemical processes. However, compound **(21)** may make NAD analogs in the body and has been reported to be active in NAD deficiency. Perhaps the NAD analogs from (21) are not toxic and can substitute for NAD in normal biochemical processes. This seems unlikely, given that the 4-position of the NAD pyridine ring is involved in hydride transfer reactions catalyzed by several enzymes. The other active compounds can be converted in the body into nicotinamide by esterases, amidases, decarboxylation, and methyl oxidation to form carboxyl, N-dealkylation, and other processes. Tryptophan can be converted into nicotinamide in the body. The pathway is tryptophan, kynurenine, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, 1-amino-4-formyl-1,3-butadiene-1,2-dicarboxylic acid, quinolinic acid, niacin, and nico-

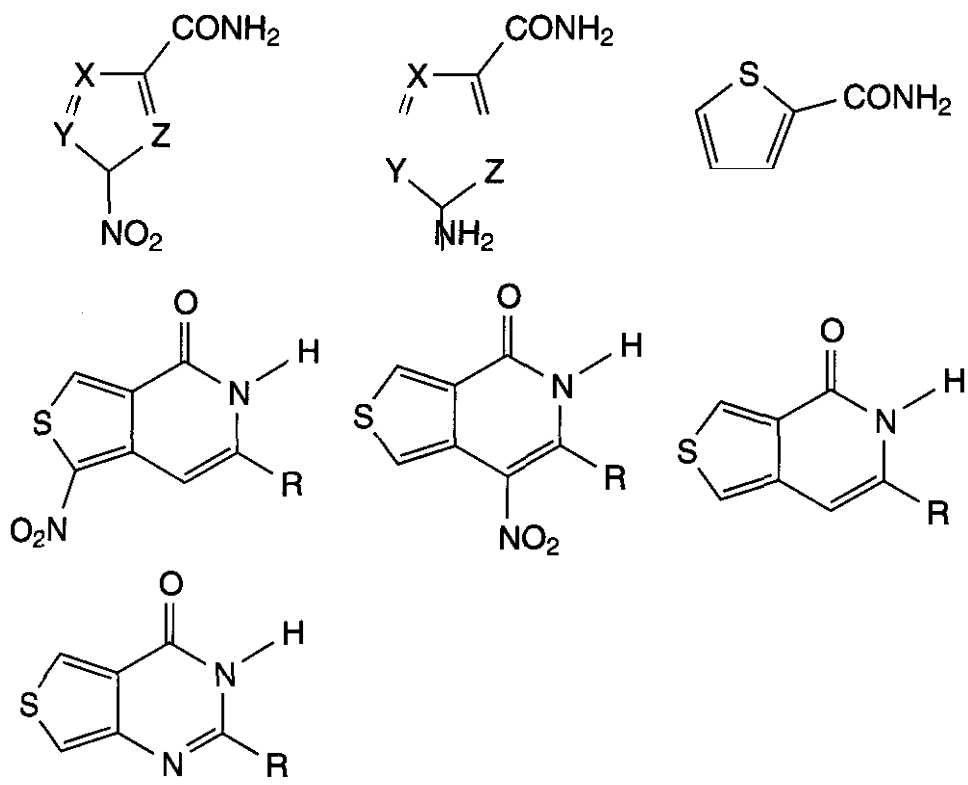


Figure 11.8. Thiophenecarboxamides, thienopyridinones, and thienopyrimidinone inhibitors of PARP. For the thiophenecarboxamides there are three series of inhibitors: (a) X = S; Y, Z = CH; (b) X, Z = CH; Y = S; (c) X, Y = CH; Z = S. For the thienopyridinones and thienopyrimidinones, R = Me or Ph. Adapted and redrawn from Ref. 24.

tinamide. Oral NAD is active because it is cleaved in the gut to make nicotinamide.

5 NMDA RECEPTOR ANTAGONISTS

NMDA receptors are ligand-gated ion channels that allow the influx of calcium upon ligand binding. The receptors contain at least two subunits, NR1 and NR2, and multiple binding sites for ligands and inhibitors. The receptors are glutamate receptors. Glutamate is released during ischemia such that activation of NMDA and other excitatory amino acid receptors may be involved in damage caused by ischemia and reperfusion. The binding sites include sites for AMPA, glycine, phencyclid-

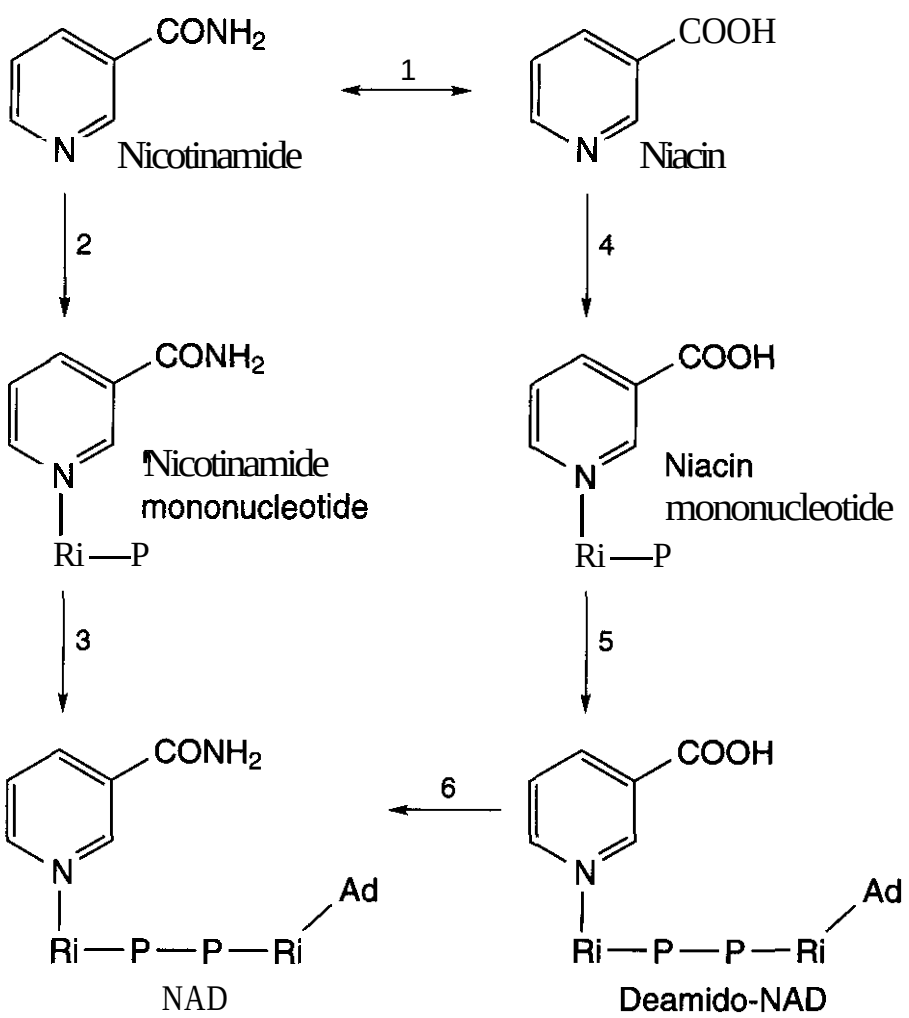


Figure 11.9. NAD synthesis pathway from nicotinamide or niacin: (1) nicotinamidase (E.C. 3.5.1.19); (2) nicotinamide phosphoribosyl transferase, which requires ATP; (3) NMN adenyl transferase, which requires ATP; (4) nicotinic acid phosphoribosyl transferase, which requires ATP; (5) NMN adenyl transferase, which requires ATP; (6) NAD synthetase, which requires ATP. Ri, ribose; P, phosphate; Ad, adenine; NMN, nicotinarnide mononucleotide. This figure is from Ref. 33 and is used with permission.

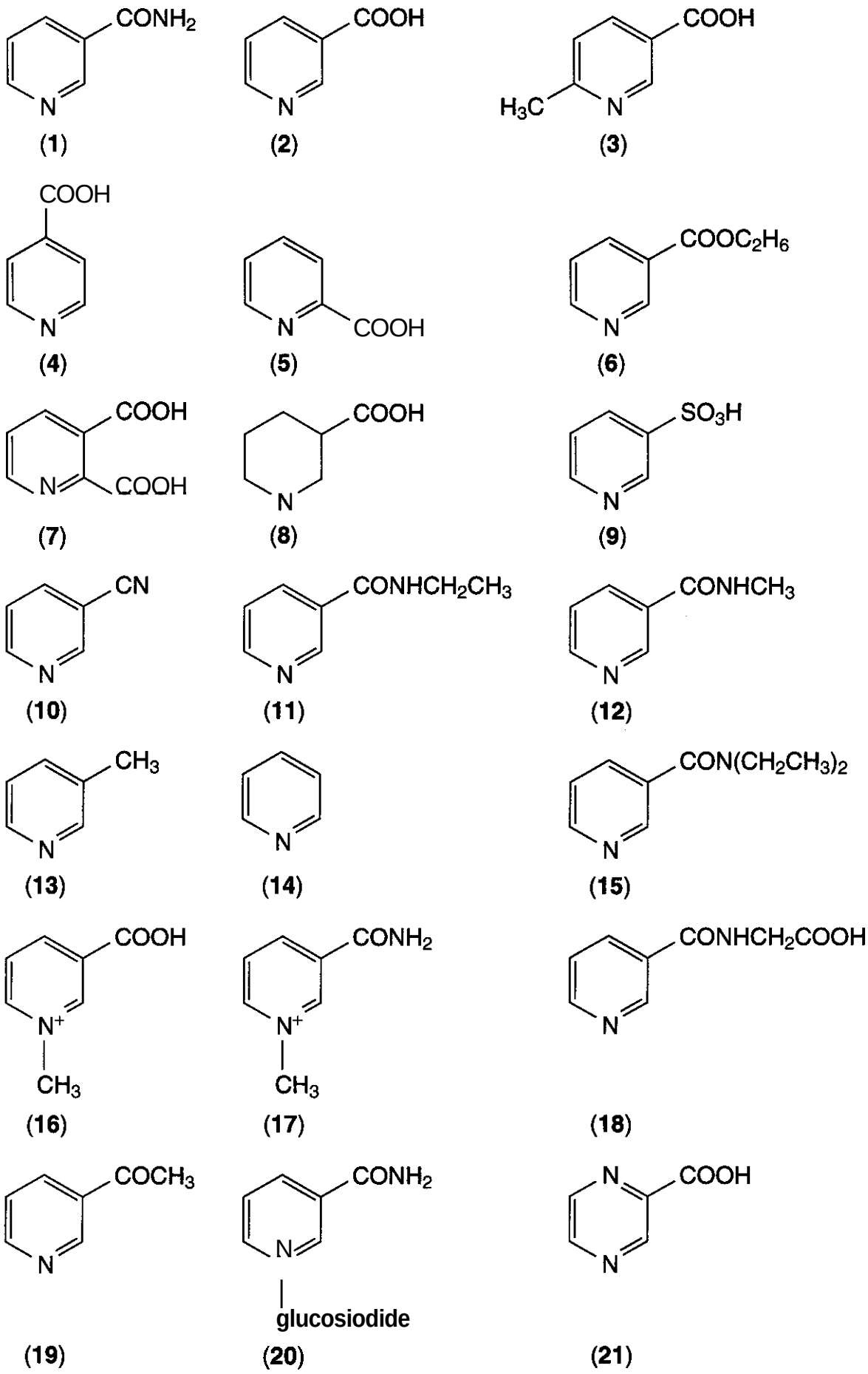


Figure 11.10. Nicotinamide analogs tested in pellagra or animal models of pellagra. The active compounds include **(1, 2, 6, 11, 12, 13, 15, 18,** and **20).** Compounds active in some models or in pellagra include (**7, 17, 21**), **pyrazine-2,3-dicarboxylic** acid, NAD, pyridyl-3-aldehyde, pyridyl-3-carbinol, tryptophan, and 3-hydroxyanthranilic acid. The inactive compounds are (**3, 4, 5, 8, 9, 10, 14, 16, 19**), 3-arninopyridine, thiazole-5-carboxylic acid, 2-methylpyridine, 3-methylpyridine, **2,6-dimethyl-**pyridine-3,5-dicarboxylic acid, pyridine-3,5-dicarboxylic acid, kynurenine, 3-hydroxykynurenine, and formylkynurenine. This figure is from Ref. 33 and is used with permission.

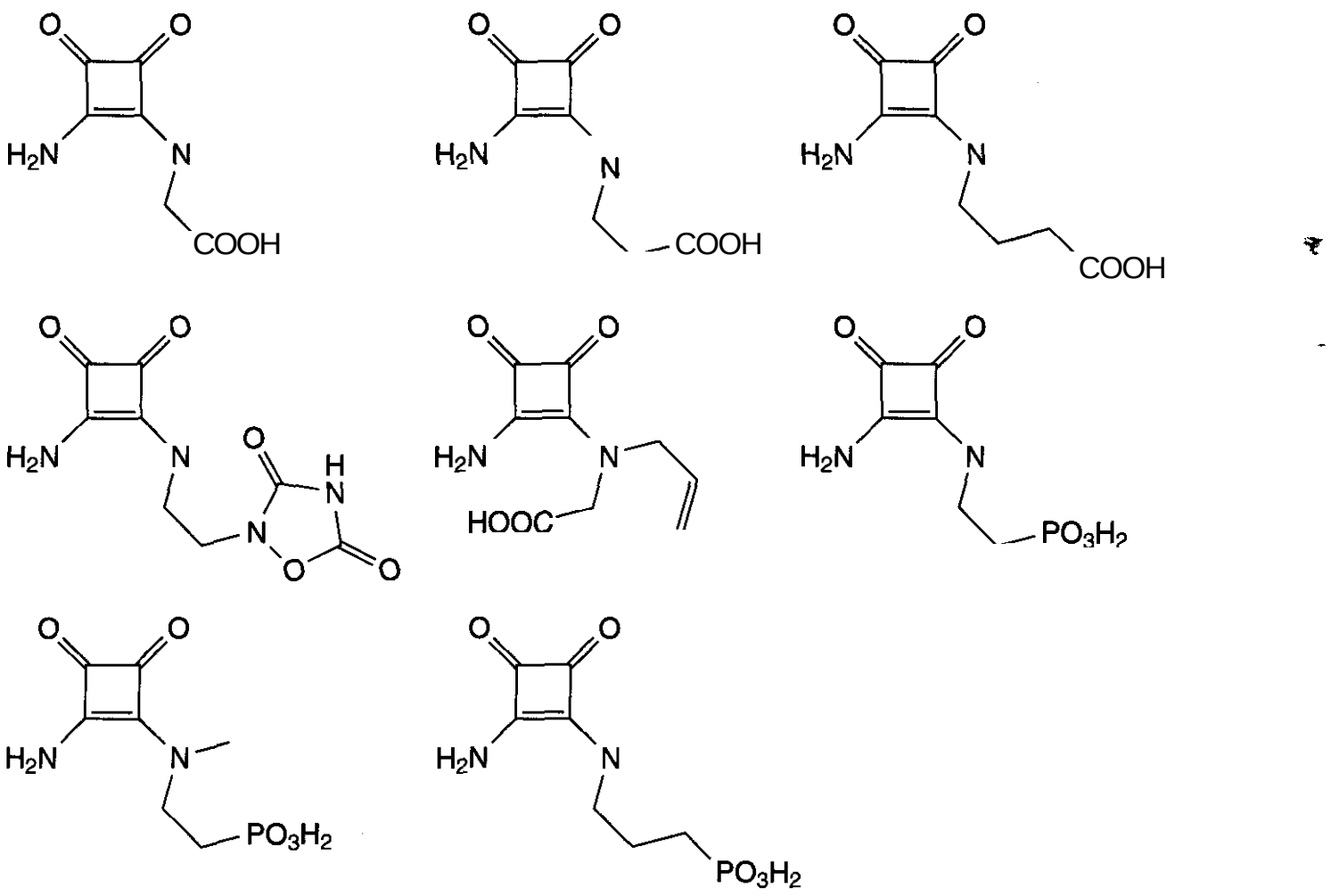


Figure 11.11. 3,4-Diamino-3-cyclobutene-1,2-diones redrawn from Ref. 34.

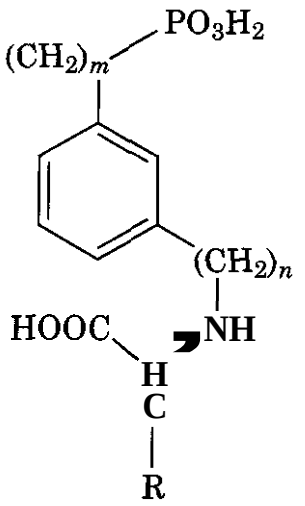
ine, kainic acid, polyamines, and other binding sites. AMPA is DL-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid. Compounds that alter glutamate release and thereby alter NMDA channel activation are known. Activation of NMDA receptors is associated with oxygen radical formation and activation of nitric oxide synthase.

Several NMDA receptor antagonists have been synthesized and tested in stroke models, epilepsy models, and in clinical trials. Many of the antagonists are limited by side effects such as hemodynamic abnormalities, hypotension, neuronal vacuolation, memory disturbances, cognitive disturbances, motor dysfunction, seizures, hallucinations, unpleasant dreams, psychotomimetic episodes, and other effects. This has made the search difficult for NMDA receptor antagonists that can be used as neuroprotective agents.

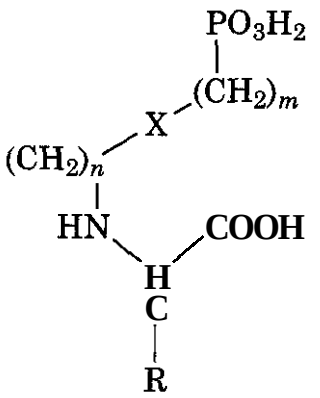
Many NMDA receptor antagonists have been synthesized that are based on the structure of glutamate, NMDA, and AMPA. Several 3,4-diamino-3-cyclobutene-1,2-dione derivatives (34) have been synthesized and found to be good inhibitors of the NMDA receptor (Fig. 11.11). These compounds were synthesized because it was noticed that several competitive inhibitors of the NMDA receptor had alpha-amino carboxylic acid and phosphonic acid functionalities separated by 3–5 carbons. All of the inhibitors shown in Fig. 11.11 have K_i values for inhibition of the NMDA receptor of 10 μM or less. In general, nitrogen substituents larger than those shown decrease receptor affinity. In addition, substitution on the second nitrogen decreases affinity.

A series of N-phosphonoalkyl and N-(phosphonoalkyl)phenyl-spaced alpha-amino acids have been explored as NMDA receptor antagonists (35). The compounds are competitive inhibitors and are shown in Table 11.3. A folded conformation of the inhibitors seems to be favored that places the phosphonic acid and the alpha-carboxylic acid moieties in close proximity in the receptor. The phosphonoalkyl group must be long and flexible enough to allow the phosphonic acid to stretch across the molecule and fit into the acidic binding site of the receptor. Addition of too much length creates excessive bulk that hinders the fit of the molecule into the receptor.

Table 11.3 *N*-(Phosphonoalkyl)phenyl-Spaced and *N*-(phosphonoalkyl) Alpha-Amino Acid Inhibitors of the NMDA Receptor"



A



B

Structure[b]	Position, X[b]	*n*	m	R	% Inhib[c]	IC_{50} (μM)
(A)	o	0	0 (COOH)[1]	H		4
(A)	o	0	0	H	27	
(A)	o	0	1	H	48	
(A)	o	0	2	H	0	
(A)	o	0	2 (E)[2]	H	9	
(A)	o	1	1	H	21	
(A)	o	1	1	CH_3	4	
(A)	m	0	2	H	0	
(A)	m	0	3 (E)[2]	H	13	
(A)	m	0	3	H	5	
(A)	m	1	0	H	21	
(A)	m	1	1	H		2.4
(A)	m	1	1	CH_3	31	
(A)	*m*	1	1	$CH(CH_3)_2$	0	
(A)	m	2	1	H		13.7
(A)	P	1	1	H		2.3
(B)	—	2	0	H	14	
(B)	—	3	0	H	3	
(B)	—	4	0	H	17	
(B)	CH=CH(E)[2]	1	1	H		1.0
(B)	CH=CH(E)[2]	1	1	CH_3		42.4
(B)	CH=CH(ZE)[2]	2	0	H		2.3
(B)	S	2	1	H	60	
(B)	S(O)	2	1	H	4	

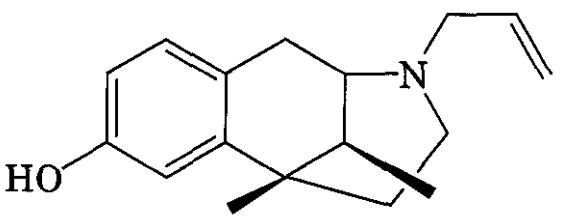
"Data are adapted from Ref. 35 with permission (© 1992 American Chemical Society).

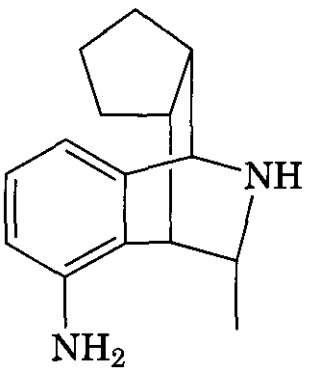
*Position, X refers to, for structure A, the position of the phosphate substituent on the phenyl ring, or for structure B, the nature of X.

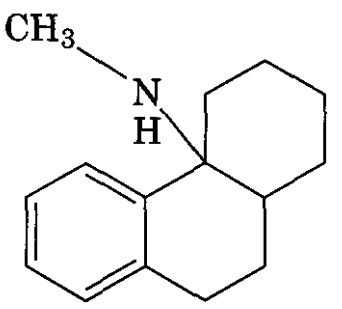
[c]% Inhib refers to the percentage inhibition at 100 μM. (1) The phosphate is replaced by COOH; (2) the geometry of the double bond is entgegen or a combination of entgegen and zuzammen.

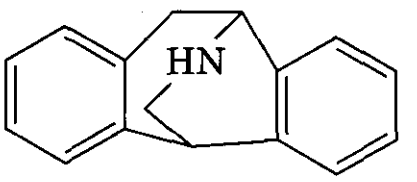
Many benzo[b]quinolizinium cations have been synthesized and found to inhibit the NMDA receptor (36, 37). These compounds tend to have high affinity for the open NMDA channel and are uncompetitive inhibitors (Table 11.4). They seem to be selective for the phencyclidine site of the NMDA receptor. Many of these compounds are neuroprotective in cell-culture systems. Of course, because they are permanently charged, they are not expected to penetrate the blood-brain barrier well. However, several uncharged compounds, below, have been synthesized that are non-competitive NMDA receptor antagonists and

may be of interest in clinical trails against stroke. These compounds are based on the structures of benzo[b]quinolizinium cations and dizocilpine, which have been clinically tested against stroke.





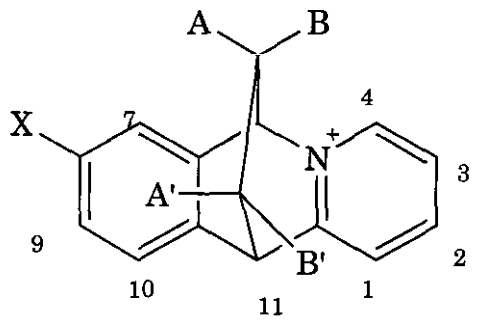




A family of peptides has been found in marine snails of the Conus genus that are NMDA receptor antagonists by virtue of their interactions at the polyamine site (38). These peptides apparently can cross the blood-brain barrier and cause ataxia, respiratory paralysis, and death. They are noncompetitive inhibitors of polyamine responses at NMDA receptors. However, the peptides do not produce the same effects as those of other polyarnine inhibitors, such as arcaine, ifenprodil, and 1,10-diaminodecane, which implies that the peptides act at a novel polyamine site on the NMDA receptor. Conantokin-G is the model peptide in this series and is Gly-Glu-Gla-Gla-Leu-Gln-Gla-Asn-Gln-Gla-Leu-Ile-Arg-Gla-Lys-Ser-Asn-NH_2. The peptide contains several gamma-carboxyglutamate (Gla) residues that make it resistant to peptidases. Conantokin-G has an IC_{50} of 0.2 μM in NMDA receptor assays. Many peptides were synthesized with one amino acid difference compared to that of conantokin-G. Only the peptide with an Ala at position 7 instead of Gla had enhanced activity (IC_{50} = 0.05 μM). All other amino acid substitutions either did not enhance or decreased activity. Although conantokin-G possesses alpha-helicity, there was no apparent correlation between activity and peptide tertiary structure. The ability of these peptides to modulate the NMDA receptor suggests there may be endogenous peptides that perform the same function.

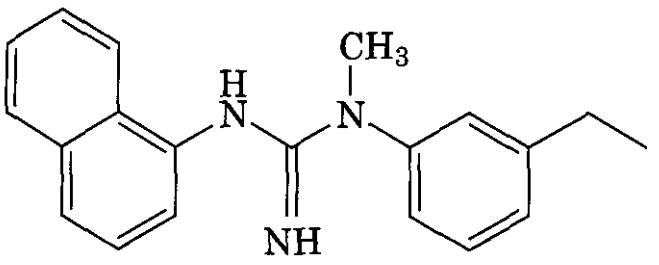
Bis(phenyl)guanidines have been explored as NMDA receptor antagonists (39). These compounds are similar to *N*-1-naphthyl-*N'*-(3-ethylphenyl)-*N'*-methylguanidine (aptiganel, below) that has been tested in clinical trails for traumatic brain damage and stroke. Many of these compounds are potent NMDA receptor antagonists, but are also sigma receptor antagonists. The sigma receptor is not well understood. Some sigma receptor antagonists may block calcium entry into cells and prevent apoptosis (40). These receptors may modulate NO synthase activity and appear to bind neurosteroids. However, the effects of sigma receptor antagonists may be dose related, causing potentiation of NMDA receptor activity at low doses and inhibition of NMDA receptor activity at high doses (41). Some sigma receptor antagonists have been found to be neuroprotective. However, several of the bis(phenyl)guanidines were found to have higher affinity for the NMDA receptor than for the sigma receptor (Table 11.5). For R1 substituents, iPr and F do not produce NMDA receptor-selective agents. In general, for R2 and R3, one or both of the two substituents should be SMe or CF, to retain NMDA receptor selectivity.

Table 11.4 Benzo[*b*]quinolizinium Cations Found to Inhibit the Phencyclidine Site of the NMDA Receptor"



X	A′	B′	A	B	K_i (n*M*)
H	OEt	Oet	H	Me	115
H	OEt	Oet	Me	H	34
H	OEt	Oet	Me	Me	8 (±)
H	OPr	Opr	Me	Me	12
H	OEt	Oet	Et	Et	11
9-Br	OEt	Oet	Me	Me	52
9-OMe	OEt	Oet	Me	Me	4
10-OMe	OEt	Oet	Me	Me	13
10-OH	OEt	Oet	Me	Me	8
11-Me	OEt	Oet	Me	Me	11
H	3-C_4H_3O	3-C_4H_3O	H	H	2
6-Me	3-C_4H_3O	3-C_4H_3O	H	H	1.2
10-OMe	3-C_4H_3O	3-C_4H_3O	H	H	6
10-OH	3-C_4H_3O	3-C_4H_3O	H	H	1.8
6-CN	3-C_4H_3O	3-C_4H_3O	H	H	10
9,10-CH_2O	3-C_4H_3O	3-C_4H_3O	H	H	2.7
9-OH	3-C_4H_3O	3-C_4H_3O	H	H	2.1
9-F	3-C_4H_3O	3-C_4H_3O	H	H	3.6
H	C_6H_5	C_6H_5	H	H	8.2

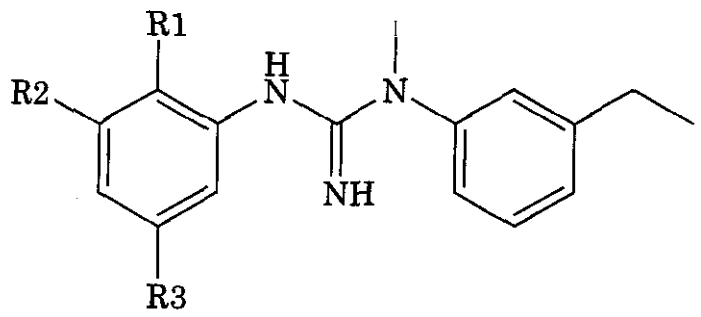
"Data are adapted from Refs. 36 and 37 with permission (© 1994, 1995 American Chemical Society).



Indole-2-carboxylates have been found to inhibit glycine binding to the NMDA receptor (42). At least one of these compounds decreased infarct volume after ischemia and reperfusion. Several of the analogs are able to inhibit NMDA-induced convulsions. Binding affinity decreases when bulky groups are substituted at R2 (Table 11.6). This implies that steric restrictions to binding may exist. The exception is THP (tetrahydropyranyl), which may be more flexible than phenyl substituents, perhaps avoiding steric restrictions to binding. Replacement of the urea with thiourea decreases affinity, which may indicate the importance of oxygen in hydrogen bonding or other interactions.

5-Amino-quinoxaline-2,3-diones can be powerful inhibitors of the AMPA receptor and the glycine site of the NMDA receptor (43). Several of the compounds are also anticonvulsants. None of them has been tested as a neuroprotective agent. For the carboxylic acid derivatives (Table 11.7), shortening of the spacer between the amine and the carboxylic acid increases AMPA affinity. However, large substituents on the amino acid side-chain decrease AMPA receptor affinity. N-Methylation improves selectivity for the AMPA receptor. Esterification decreases affinity for AMPA and NMDA receptors. The most potent glycine site (MDL) antagonist is a brominated amino acid derivative. Among the phosphonic acid derivatives the nitro-D-phosphoalanine derivative is a potent anticonvulsant and glycine

Table 11.5 Bis(phenyl)guanidines that Are Selective for Inhibition of the NMDA Receptor[a]

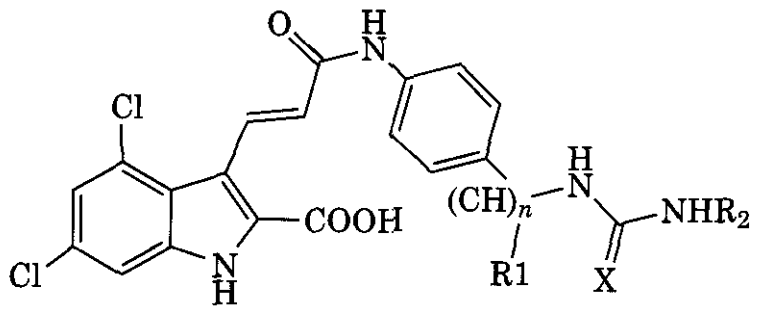


R1	R2	R3	K_i^b (n*M*)
H	SMe	H	6.4
Br	Et	Et	13
H	Et	Srne	6.4
Cl	Et	Srne	5.1
Br	Et	Srne	2.5
Cl	CF_3	Srne	6.9
Br	CF_3	Srne	4.3
Cl	SMe	Et	9.7
Br	SMe	Et	3.9
Cl	SMe	Srne	1.9
Br	SMe	Srne	1.7
Br	SMe	Br	4.1
Br	OCF_3	Et	12.3
Cl	Br	Srne	3.9

"Adapted from Ref. 39 with permission (© 1997 American Chemical Society).

[b]These compounds have K_i values for inhibition of the NNDA receptor that are at most 10 times lower than their IC_{50} values for inhibition of the sigma receptor.

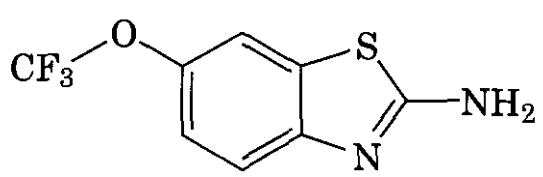
Table 11.6 Indole-2-carboxylate Inhibitors of the Glycine Site of the NMDA Receptor"



R1	R2	N, X	pK_i
H	H	0, O	8.80
H	H	1, O	8.67
H	H	2, O	8.20
H	Et	1, O	8.62
H	Et	1, S	7.91
H	CH_2COOH	1, O	8.57
H	c-C_3H_7	1, O	8.25
H	4-THP	1, O	8.15
H	C_6H_5	1, O	7.70
H	4-OCH_3-C_6H_5	1, O	7.69
H	3-C_5H_5N	1, O	7.83
R-(Me)	H	1, O	8.20
S-(Me)	H	1, O	7.93

"Adapted from Ref. 42 with permission (© 1999 American Chemical Society).

site antagonist. However, more potent glycine site antagonists are the brominated and the trifluoromethylated phosphoalanine derivatives. In general, increasing the size of the R substituent tends to decrease activity at the glycine site.



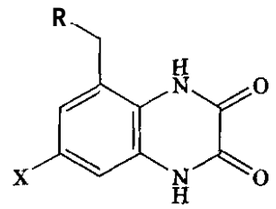
Riluzole

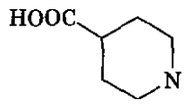
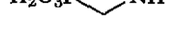
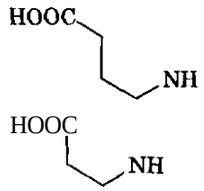
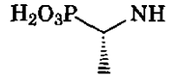
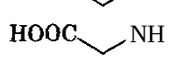
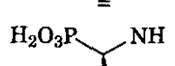
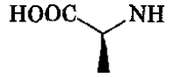
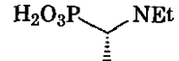
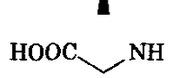
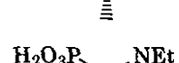
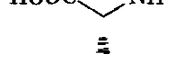
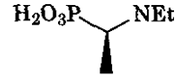
Riluzole is an NMDA antagonist with actions at the pre- and postsynaptic levels. It is currently being used in amyotrophic lateral sclerosis patients. Riluzole also inhibits glutamate-induced convulsions. Several riluzole analogs have been synthesized and tested for inhibition of glutamate-induced seizures (44). The trifluoromethoxy side-chain can be replaced with several other side-chains with retention of activity, such as methyl, ethyl, propyl, butyl, pentyl, and hexyl. These side-chains must be unbranched, given that branched side-chains may have no activity. This implies steric restrictions exist in the receptor for these side-chains. A variety of fluorinated hydrocarbon side-chains can be added in place of trifluoromethoxy, with retention of activity. These side-chains must be at the 6-position to be active. Perhaps a lipophilic receptor site exists that can interact only with 6-substituents. Many different substituents can be placed on the ring nitrogen with retention of antiseizure activity. It is very possible that many of these side-chains are metabolically removed upon injection into animals.

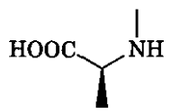
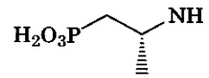
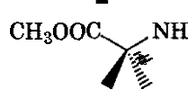
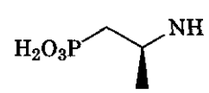
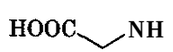
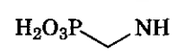
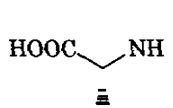
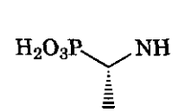
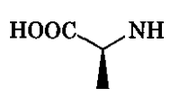
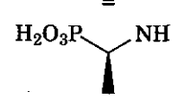
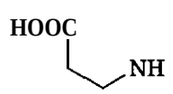
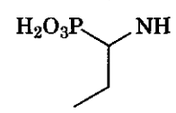
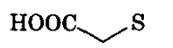
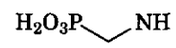
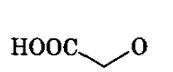
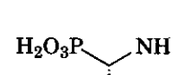
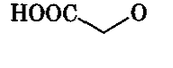
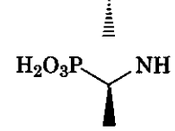
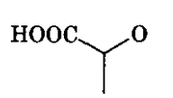
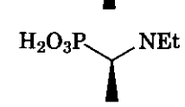
6 NITRIC OXIDE SYNTHASE INHIBITORS

Nitric oxide synthase (NOS) is a family of enzymes consisting of at least three isoforms, inducible (iNOS) found in macrophages and other cells, neuronal (nNOS) found in neurons, and endothelial (eNOS) found in endo-

Table 11.7 5-Amino-quinoxaline-2,3-diones Found to be Active at AMPA (IC_{50} in μM) and Glycine (MDL, IC_{50} in μM) Sites and Found to Have Anticonvulsant Activity (ESM in mg/kg)[a]



Carboxylic Acid Derivatives						Phosphonic Acid Derivatives					
	X	R	AMPA	MDL	ESM		X	R	AMPA	MDL	ESM
(8a)	NO_2		**0.07**	**3.9**	**44**	**(1a)**	NO,		**0.29**	**1.0**	**7**
(8b)	NO_2		**0.61**	**9%**	**n.t.**	**(1b)**	NO,		**0.12**	**24%**	**18**
(8c)	NO_2		**0.38**	**19%**	**35**	**(1c)**	NO,		**0.17**	**0.032**	**3**
(8d)	NO_2		**0.16**	**0.76**	**32 (15 min)**	**(1d)**	NO,		**0.38**	**51%**	**8**
(8e)	NO_2		**0.31**	**1.7**	**19, 0% (1 h)**	**(1e)**	NO,		**0.31**	**25%**	**26**
(8f)	NO_2		**0.38**	**1.3**	**0%**	**(1f)**	NO,		**0.08**	**13%**	**7, 18 (2 h)**
(8g)	NO_2		**0.29**	**−9%**	**0%**	**(1g)**	NO,		**1.3**	**31%**	**n.t.**
(8h)	NO_2		**1.2**	**34%**	**0%**	**(1h)**	NO,		**0.64**	**8%**	**18**

(**8i**)	NO_2		0.34	22%	0%	(**1i**)	NO_2		0.20	6%	9
(**8j**)	NO_2		1.9	−3%	n.t.	(**1j**)	NO_2		0.20	15%	10
(**9a**)	Br		4.7	0.04	0%	(**2a**)	Br		2.4	0.1	0%
(**9b**)	Br		4.5	0.12	n.t.	(**2b**)	Br		3	0.006	12, 18 (2 h)
(**9c**)	Br		4.3	2.2	n.t.	(**2c**)	Br		1.4	0.37	43
(**9d**)	Br		3.8	37%	n.t.	(**2d**)	Br		16%	0.045	20%
(**14b**)	Br		22%	0.12	0%(1h)	(**7a**)	CF_3		2.0	0.3	30
(**17**)	Br		4%	0.11	0%(1h)	(**7b**)	CF_3		1.2	0.006	8
(**18a**)	NO_2		2.0	0.31	n.t.	(**7c**)	CF_3		0.54	0.59	20
(**18b**)	NO_2		1.3	0.42	n.t.	(**7d**)	CF_3		0.068	4	10

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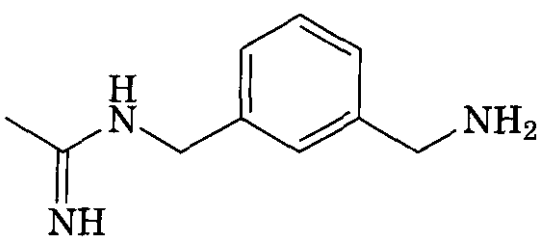
thelial cells throughout the body. Induction of iNOS depends on exposure to cytokines and other stimuli and is important in the immune response and other defense mechanisms. Blood flow to inflamed tissues may be controlled by iNOS. Blood pressure regulation and antithrombosis responses involve eNOS. Neuromodulation involves nNOS. Both eNOS and nNOS are constitutively expressed isoforms.

NOS is a homodimer and requires dimerization involving calmodulin for activation. However, iNOS contains tightly bound calmodulin, such that exogenous calmodulin binding is not required for activation. The enzyme contains a cytochrome P450 reductase domain and an oxygenase, heme protein domain. There are binding sites for NADPH, FAD, FMN, and tetrahydrobiopterin. The oxygenase domain oxidizes arginine with the formation of citrulline and nitric oxide. Activation of the enzyme requires dimerization because the flow of electrons from the reductase occurs to the *trans* oxygenase. Tetrahydrobiopterin facilitates dimerization, stabilizes the enzyme, shifts the iron to a high spin state, and facilitates electron transfer (45). NADPH supplies electrons to the reductase. Electron transfer in the reductase involves FAD and FMN. NOS makes NO that is a reactive radical, especially after interaction with oxygen when peroxynitrite is formed. Peroxynitrite can damage DNA and other macromolecules.

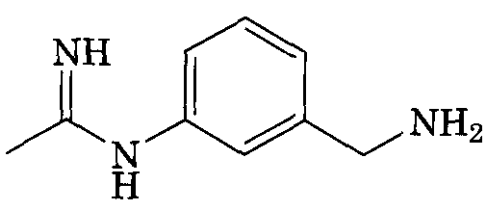
Many inhibitors have been synthesized based on the structure of L-arginine. These inhibitors are not isoform specific. Specificity may be important in the treatment of neurodegenerative disorders. It is possible that inhibition of nNOS could be very important in the inhibition of neurodegeneration. However, NO can diffuse long distances in the brain. For this reason, it is possible that eNOS and iNOS are important in neurodegeneration. Inhibitors based on the structure of arginine include N^G-methyl-L-arginine, N^G-nitro-L-arginine and its methyl ester, N^G-amino-L-arginine, N-δ-(iminoethyl)-L-ornithine, and others.

N-Phenylamidines are known to reversibly inhibit nNOS (46). Slight structural variations can switch isoform specificity with these compounds as seen with the two acetamidine structures shown below. N-(3-(Aminomethyl)-phenyl)acetamidine, below, was modified by changing the aminomethyl side-chain (Table 11.8). Ethylamino, methylaminomethyl, and dimethylaminomethyl side-chains retain nNOS specificity. A methanol side-chain produces an nNOS-specific inhibitor. However, acetyl and modified acetyl side-chains abolish activity.

Modification of the acetamidine functionality also produces nNOS-specific inhibitors



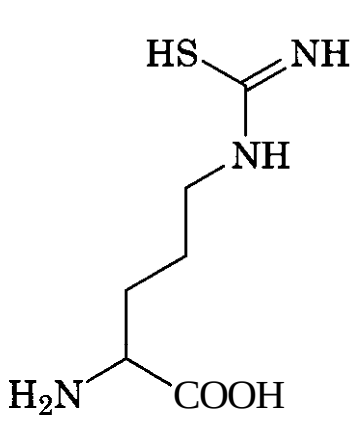
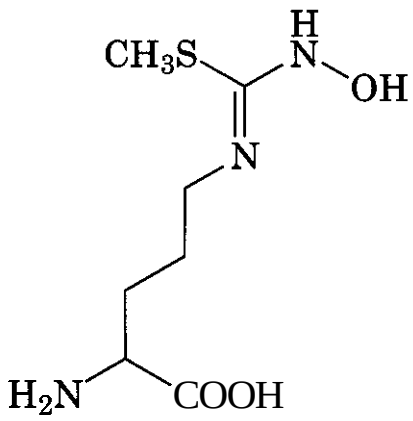
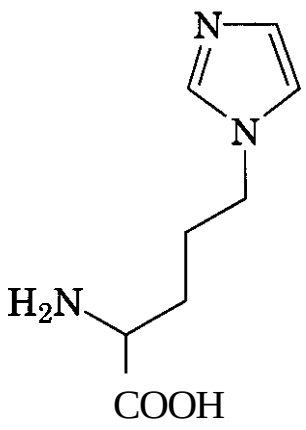
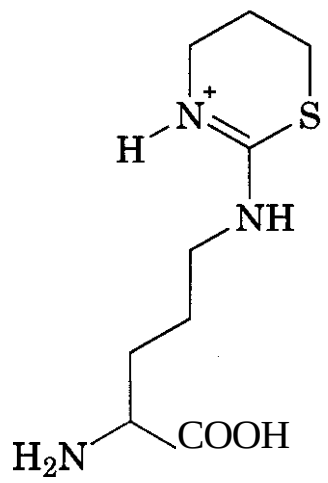
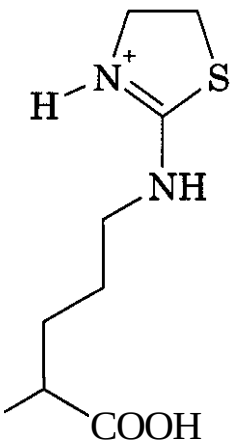
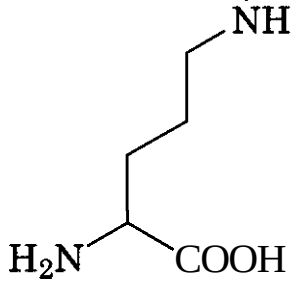
iNOS selective



nNOS selective

(Table 11.9). Several alkyl side-chains were found to be effective, including aminomethyl, fluoromethyl, 2-pyridyl, 2-furanyl, and 2-thienyl. This may imply that the acetamidine side-chain is involved in hydrophobic interactions with the enzyme that can be enhanced by aliphatic and aromatic groups. The 2-furanyl and 2-thienyl groups may be able to bind in the hydrophobic pocket created by Pro336, Val338, and Phe355 in the active site of nNOS.

Thiocitrulline and other citrulline analogs have been found to be good inhibitors of NOS (47, 48). The inhibitors reported so far do not seem to have isoform specificity. Thiocitrulline is the most potent inhibitor in the series. However, its N-hydroxy analog and other analogs, below, are nearly as potent. The inhibitors shown inhibit NOS with IC_{50} values in the range of 10 μM. It has been proposed (48) that the sulfur or imidazole nitrogen binds to the oxygenase heme iron. Nearby on the enzyme is a cationic site that binds the amino

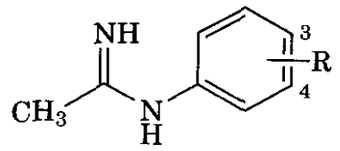







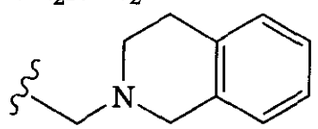
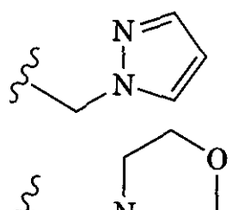
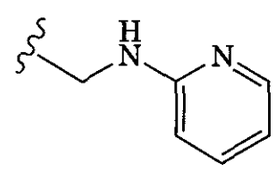
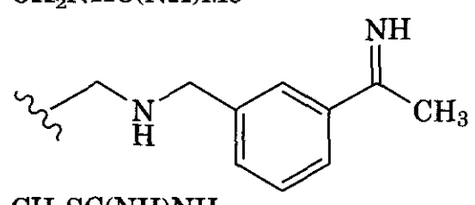
acid nitrogen. An anionic binding site may bind the carboxylic acid. An H-bonding site may bind the amino hydrogen.

Several dipeptides have been synthesized that incorporate isothiocitruline into the peptide (49). Some of these dipeptides are selective for inhibition of nNOS and iNOS but not eNOS. These peptides include S-methyl-L-**isothiocitrullinyl-L-phenylalanine,** S-methyl-L-**isothiocitrullinyl-L-leucine,** S-methyl-L-isothiocitrullinyl-L-tryptophan(-CHO), **S-methyl-isothiocitrullinyl-L-phenylglycine, S-methyl-L-isothiocitrullinyl-L-glycine,** *S*-methyl-L-isothiocitrullinyl-glycine, S-methyl-isothiocitrullinyl-L-tyrosine, and S-methyl-L-isothiocitrullinyl-4-nitro-L-phenylalanine. All of these dipeptides inhibit nNOS with IC_{50} values of about 10 μM, and they inhibit iNOS equally well. However, none of the dipeptides inhibits NOS as potently as S-L-isothiocitrulline (IC_{50} = 0.06 μM for nNOS). The ability of these dipeptides to inhibit NOS suggests that there may be endogenous peptide regulators of NOS.

Imidazole-containing amino acids can make good NOS inhibitors, given that imidazole ligates heme (50). The inhibitors display some isoform specificity (Table 11.10) in that at least two of them inhibit nNOS with affinities at least 10 times greater than their affinities for eNOS. The inhibitors with odd numbers of carbons in the spacer between the imidazole and amino acid are the most selective inhibitors. However, when a phenyl substituent is added to the imidazole, all isoform specificity is lost. Perhaps hydrophobic interactions exist between the enzyme and the hy-

Table 11.8 *N*-Phenylacetamidine Inhibitors of NOS[a]



Compound	R	Isomer	Salt	K_i (μM) iNOS	K_i (μM) eNOS	K_i (μM) nNOS	Selectivity eNOS/nNOS	Selectivity iNOS/nNOS
(14)	CH_2NH_2	3	2HCl	2.0	6.2	0.04	155	50
(33)	CH_2NH_2	4	2HBr	2.5	>50	1.5	>33	1.7
(34)	NH_2	3	2HBr	46	27	14	1.9	3.3
(35)	$CH_2CH_2NH_2$	3	2HCl	10	33	1	33	10
(36)	CH_2NHMe	3	2HBr	2.5	1.9	0.056	34	44
(37)	CH_2NMe_2	3	2HBr	6.1	9.1	0.17	54	36
(38)		3	2HBr	>50	>50	4.6	>11	>4.5
(39)		3	2HBr	>50	46	14	3.3	>3.6
(40)		3	2HBr	10	13	2.8	4.6	3.6
(41)		3	2HBr	>50	>50	11	>4.5	>4.5
(42)	CH_2NHOH	3	2HBr	6.3	>50	2.1	>24	3
(43)	$CH_2NHC(NH)Me$	3	2HBr	2.0	9.5	0.37	26	5.4
(44)		3	3HBr	1.6	3.0	0.042	71	38
(45)	$CH_2SC(NH)NH_2$	3	2HBr	4	17	0.36	47	11
(46)	CH_2OH	3	HCl	>50	15	0.6	25	>83

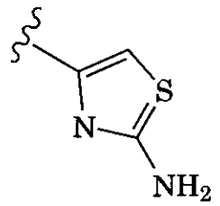
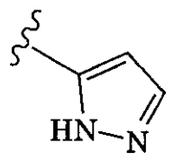
(47)	CH(OH)Me	3	HBr	>50	17	39	0.4	>1.3
(48)	CH_2CO_2H	3	HBr	>50	>50	>50		
(49)	CH_2CO_2Me	3	HCl	>50	>50	>50		
(50a)	CO_2tBu	3	HCl	>50	>50	>50		
(51)	$CH_2C(O)$-*N*-morpholinyl	3	HBr	>50	>50	>50		
(52)	$CH_2NHC(O)$-*N*-morpholinyl	3	HBr	>50	46	46	>1	>1
(53)	H		HBr	>50	11	6	1.8	>8
(54)	F	3	HCl	>50	26	8	3.3	>6
(55)	Br	3	HCl	>50	>50	13		>3.8
(56)	NO, ·	3	HCl	>50	>50	13		>3.8
(57)	CN	3	HCl	>50	43	10	4.3	>5
(58)	NMe_2	3	2HBr	>50	>50	33		>1.5
(59)	SMe	3	HCl	>50	39	13	3.0	>3.8
(60)	OMe	3	HCl	>50	38	>50	<0.8	
(61)	OH	3	HBr	20	14	>50	0.3	<0.3
(62)	C(O)Me	3	HCl	>50	44	5.6	7.9	7.9
(63)	CO_2H	3	HCl	>50	>50	>50		
(64)	$C(O)NH_2$	3	HCl	>50	>50	7.2	>6.9	>6.9
(65)	SO_2NH_2		HBr	46	8	29	0.3	1.6
(66)			2HBr	>50	>50	7.5	>6.7	>6.7
(67)		3	HBr	>50	27	4.6	5.9	>10
(68)	NHNH,	3	2HBr	4.6	7.9	1.1	7.2	4.2
(69)	$C(NH)NH_2$	3	2HCl	15	36	0.57	63	26
(70)	NHC(NH)Me	3	2HBr	17	38	1.5	25	11

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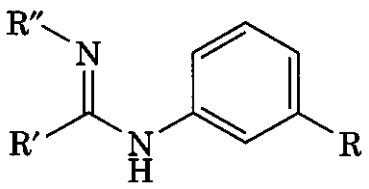
Table 11.9 Modified Amidine Inhibitors of NOS[a]



					K_i (μM)			Selectivity	
Compound	R	R′	R"	Salt	iNOS	eNOS	nNOS	eNOS/ nNOS	iNOS/ nNOS
(14)	CH_2NH_2	Me	H	2HCl	2.0	6.2	0.04	155	50
(71)	CH_2NH_2	Me	Me	2HBr	>50	>50	>50		
(72)	CH_2NH_2	CH_2NH_2	H	3HBr	3.4	13	0.19	68	18
(73)	CH_2NH_2	CH_2SMe	H	2HBr	0.021	0.32	0.011	29	2
(74)	CH_2NH_2	CH_2F	H	2HBr	0.48	1.1	0.011	100	44
(75)	CH_2OH	CH_2F	H	2HBr	17	3.8	0.30	13	57
(76)	CH_2NH_2	2-Pyridyl	H	2HBr	37	13	0.33	39	112
(77)	CH_2NH_2	2-Furanyl	H	2HBr	0.16	0.35	0.0063	56	25
(78)	CH_2NH_2	2-Thienyl	H	2HBr	0.12	0.40	0.0087	46	14

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drocarbon spacer. An odd number of carbons in the spacer may provide the optimal hydrophobic interactions, while keeping the amino acid in the proper position for binding to the anionic and cationic sites. The phenyl analogs may be too bulky for binding to the site with the best orientation of the amino acid portion of the inhibitor.

Several nitroarginine dipeptides have been found to be very selective inhibitors of nNOS (51). These peptidomimetic agents, below, appear to fit much better into the nNOS active site than into the iNOS or eNOS sites. The amide side-chain containing compound below has K_i (μM) values as follows: 0.13 nNOS, 25 iNOS, and 200 eNOS; the compound without the amide side-chain has K_i values of 0.54 nNOS, 100 iNOS, and 199 eNOS; the compound without the amide or the carbonyl has K_i values of 0.12 nNOS, 39 iNOS, and 314 eNOS. Elongation of the amine side-chain tends to decrease potency and isoform selectivity. All of these compounds are very selective for nNOS. The fact that the amide and carbonyl can be eliminated with retention of nNOS inhibitory activity and isoform selectivity shows that these functionalities, although not critical to nNOS binding, may be more important for binding to iNOS and eNOS. In addition, elimination of the carbonyl converts the amide nitrogen to a basic, secondary amine. The amine may be able to bind in the nNOS active site better than in the eNOS active sites.

Phenyl-2-aminopyridines have been explored as potential nNOS inhibitors (52). These compounds have some selectivity for nNOS over eNOS. They point out that the nNOS active site appears to be less sterically hindered than the eNOS active site. When the side-chain in the compound below is quinoline, the IC_{50} values (μM) for NOS inhibition are 0.21 nNOS and 0.83 eNOS; for the $PhCOCH_2$ side-chain, the values are 0.14 nNOS and 0.89 eNOS; for the $PhCH_2CO$ side-chain the values are 0.14 nNOS and 0.69 eNOS; for the $PhCH_2CH2$ side-chain the values are 0.26 nNOS and 0.45 eNOS, demonstrating almost complete loss of isoform selectivity. For the iBu side-chain the values are 0.35 nNOS and 0.37 eNOS, demonstrating no isoform selectivity. For the

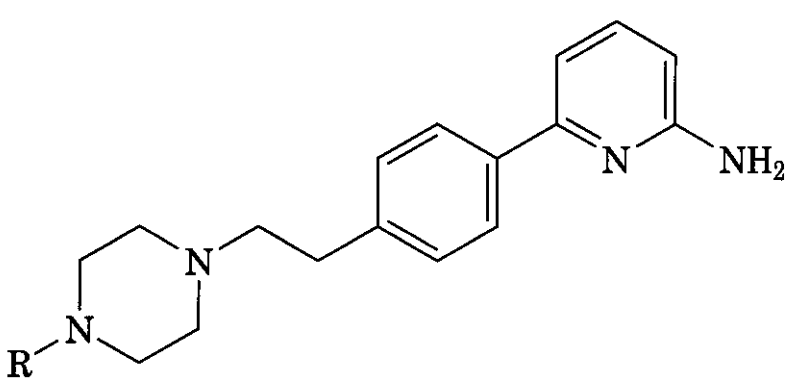
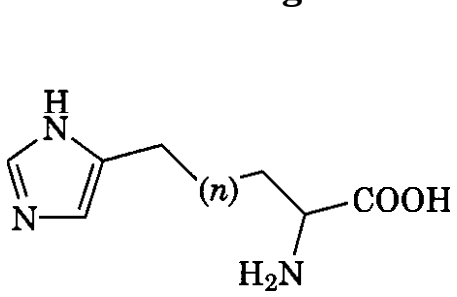
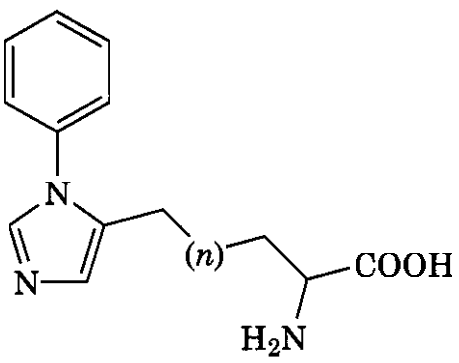


Table 11.10 Imidazole Containing Amino Acid Inhibitors of NOS[a]

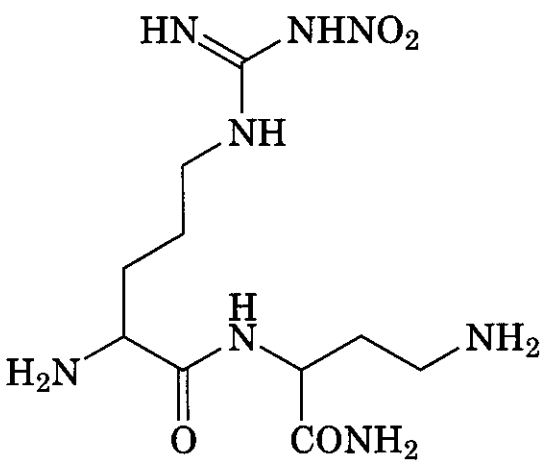


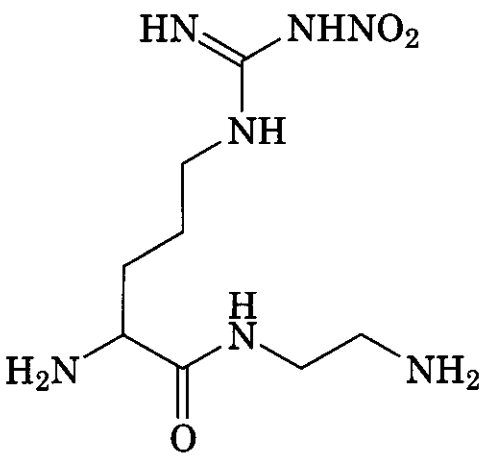


n = 0:1a
n = 1:1b n = 1:2a
n = 2:1c n = 2:2b
n = 3:1d n = 3:2c
n = 4:1e

	K_i (μM)		
Compound	iNOS	nNOS	ENOS
(**1a**)	950	170	500
(**1b**)	10	2	33
(**1c**)	35	65	150
(**1d**)	8	2	50
(**1e**)	40	150	250
(**2a**)	100	80	50
(**2b**)	50	100	17
(**2c**)	120	70	350

"**Data** are from Ref. 50, used **with** permission from **Elsevier** Science.





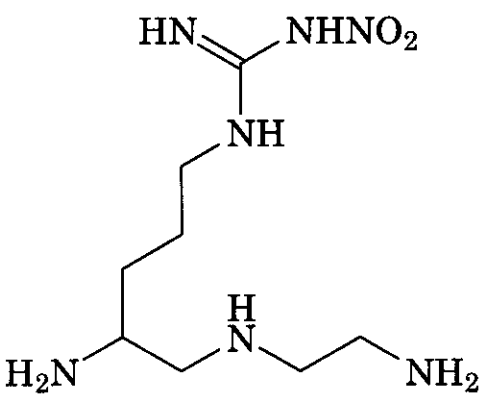
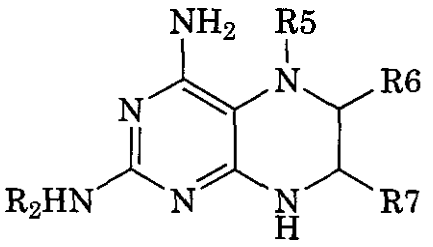
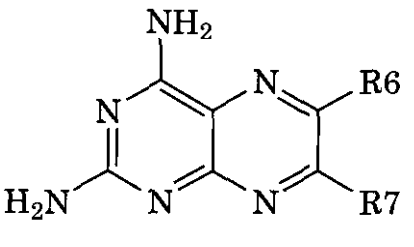


Table 11.11 Pteridine Inhibitors of nNOS[a]



A



B

Compound	R2	R4	R5	R6	R7	IC_{50} (μM)
(A)	H	H	H	$(CH_2OH)_2Me$	H	6
(A)	H	H	H	H	Ph	24
(A)	H	H	H	Ph	H	6
(A)	H	H	H	CH_2Oet	H	30
(B)	H	H	H	CH_2Odec	H	30
(B)	H	H	H	$CH_2OCOEtPhCoPh$	H	48
(B)	H	Me_2	H	4-MeOPh	H	74
(B)	H	Et_2	H	4-MeOPh	H	45
(B)	H	Dibenzyl	H	Ph	H	3
(B)	H	Dibenzyl	H	4-MeOPh	H	5
(B)	H	$(C_2H_4)_2O$	H	Ph	H	82
(B)	H	$(C_2H_4)_2O$	H	4-MeOPh	H	34
(B)	H	$(CH_2)_5$	H	Ph	H	62
(B)	H	$(CH_2)_5$	H	4-MeOPh	H	50

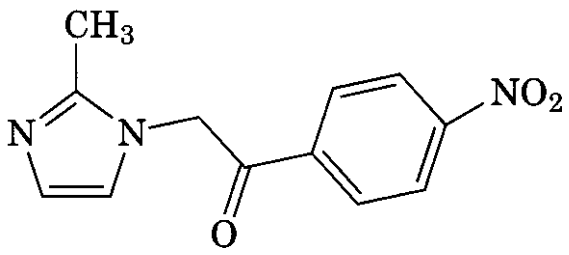
[a]Data are from Ref. 53, used with permission (© 1999 American Chemical Society).

$iPrNHCOCH_2$ side-chain the values are 0.33 nNOS and 0.82 eNOS. Apparently, an aryl or carbonyl functionality with sp^2 characteristics should be adjacent to the terminal piperazine to retain isoform selectivity.

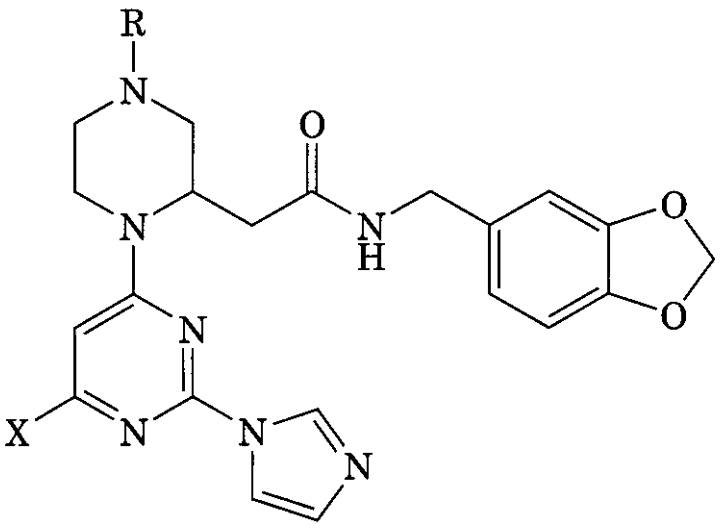
4-Amino-5,6,7,8-tetrahydropteridines and 2,4-diamino-pteridines have been synthesized and tested as nNOS inhibitors (53). These inhibitors are designed to fit into the tetrahydrobiopterin site of nNOS (Table 11.11). A binding site model has been proposed for these compounds. The nitrogen in the 3-position, the exocyclic nitrogen at the 4-position, and R4 may bind to the heme side-chain. Arg375 may bind to the exocyclic nitrogen, R4 and N5. R6 and R7 may bind to a hydrophobic environment made up of Phe470 and Ser112. The pteridine ring may be involved in π electron stacking with Trp457. In general, R2 substituents other than hydrogen do not enhance activity. Perhaps bulky groups in this position inhibit binding to the heme. Similarly, R5 substituents other than hydrogen are not beneficial in nNOS binding, perhaps because of prevention of binding to Arg375. R6 substituents that are hydrophobic enhance nNOS inhibition, perhaps by increasing affinity for the hydrophobic binding site. Substituents other than hydrogen at R7 may not be beneficial.

Several imidazoles have been found to inhibit NOS, including 1-phenylimidazole, 2-phenylimidazole, and 4-phenylimidazole. These imidazoles bind heme in NOS and other enzymes. A search for isoform-specific inhibitors based on an imidazole structure has led to the discovery of 1-(trifluoromethylphenyl) imidazole, N-(4-nitrophenacyl) imidazole, and **N-(4-nitrophenylacy1)-2-methyl-imidazole,** below (54). The nitrophenylacylimidazoles are selective for nNOS rather than eNOS inhibition. They appear to bind to the tetrahydrobiopterin site and are competitive inhibitors of tetrahydrobiopterin binding. They are noncompetitive inhibitors of arginine binding. It appears that electron-withdrawing N-1 substituents enhance activity and nNOS selectivity.

Some imidazoles are selective inhibitors of iNOS and could be useful in the treatment of stroke. Polymorphonuclear leukocytes and other inflammatory cells are prominent in the limit area within 24 h or less of ischemia and



reperfusion. This inflammatory response might be attenuated with iNOS inhibitors, and might be beneficial to patients. A series of imidazoles has been found that bind to the heme at the sixth axial position and adopt a U-shaped conformation in the region of the arginine active site (55). This binding disrupts a protein-protein interaction involving the 7a helix containing Glu371 and inhibits protein dimerization. The crystal structure of the protein inhibitor complex has been presented (55). Three inhibitors have been found and are shown below. They inhibit iNOS with K_i values of about 1–30 nM and are about 5 times less potent for nNOS inhibition and 1000 times less potent for eNOS inhibition. Active compounds are A: R = H, X = Cl; B: R = COOMe, X = H; and C: R = COEt, X = H.



Thiouracil antithyroid agents have been found to inhibit NOS with modest selectivity for nNOS inhibition (56). These compounds are essentially thiocitrulline analogs and are competitive inhibitors with respect to arginine and tetrahydrobiopterin. 6-Propylthiouracil, 6-methyl-2-thiouracil, and S-methylthiouracil were found to be NOS inhibitors with K_i values of 15, 14, and 60 μM, respectively. Dithiouracil, thiobarbituric acid, 5-carboethoxythiouracil, 2-mercaptopyridine, 2-mercaptothiazoline, 2-mercaptoimidazole, and ergothioneine were found to be poor NOS inhibitors. The sulfur of the inhibitors may bind to the heme. Hydrogen bonding may be possible with the carbonyl oxygen. A hydrophobic interaction may be possible with the propyl or methyl groups of the inhibitors. Of course, it is not proposed to use antithyroid drugs in the treatment of neurodegeneration because inhibition of thyroid function would not be recommended in these patients.

6-Nitrodopamine and 6-nitronorepinephrine have been shown to inhibit nNOS with K_i values of about 50 μM (57). These compounds appear to be endogenous and are produced by NOS nitration of dopamine and norepinephrine. It is unclear whether these compounds could ever reach sufficient concentrations, under physiological or disease conditions, to inhibit nNOS. The nitrocatecholamines inhibit nNOS competitively with respect to arginine and tetrahydrobiopterin and inhibit dimerization of the enzyme.

Suicide inhibitors of NOS are known and include N^5-(1-iminoethyl)-L-ornithine and N-(3-(aminomethyl)benzyl)acetamidine ((58), below). These amidines bind to Glu363 but cannot donate a proton to the ferric hydroperoxy intermediate. The hydroperoxy group attacks and hydroxylates the alpha-meso-heme carbon and produces biliverdin IXalpha. This leads to NOS inactivation with loss of biliverdin from the enzyme. A scheme (below) has been proposed for other mechanism-based inhibitors such as N-allyl-L-arginine and N^G-monomethyl-L-arginine that involves hydroxylation of the guanidino nitrogen, oxidation to produce the nitrosated analog, and elimination of NO with alkylation of the heme. These agents are not recommended for use in patients suffering from neurodegeneration because accumulation of abnormal heme degradation products would be detrimental.

Isothioureas have been explored as eNOS inhibitors (59). Bisisothioureas, below, bind to the guanidino site of eNOS participating in hydrogen bonds with Glu363 and Trp358. They then rotate into twisted configurations to position the second ureido group near the heme propionate of the pyrrole D ring for hydrogen bonding. The twisted conformation allows the bridge group between the two ureides to bond in nonpolar interactions with Val338.

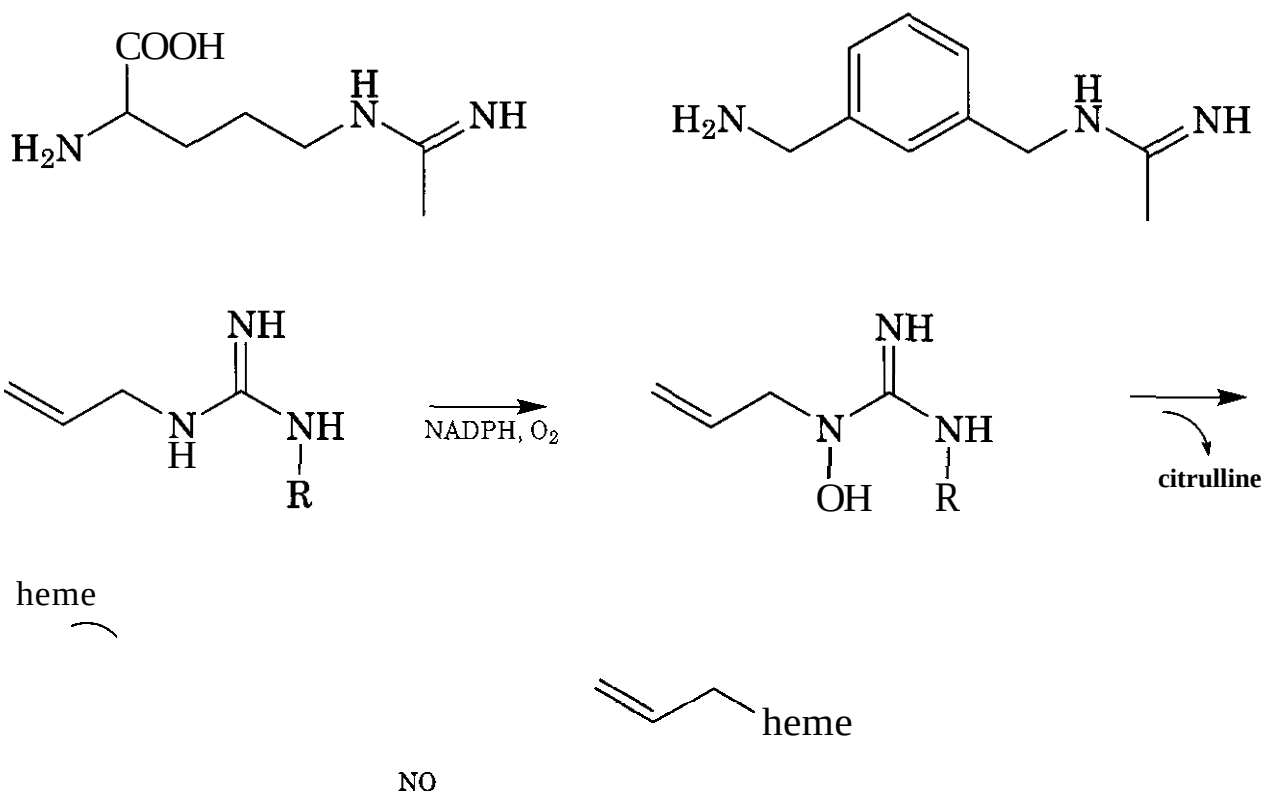
The S-ethylthiourea above the H bonds with both nitrogens to Glu363 in the active site. The ethyl and phenyl groups make nonpolar contacts with the protein at Pro336 and Val338, respectively. Methylation of either of the two nitrogens abolishes NOS inhibitory activity, demonstrating the importance of hydrogen bonds with this compound. In addition, the pocket bordered by Glu363 and Trp358 is too narrow to accommodate methylated nitrogens. H-Bonding in this pocket with drugs containing N appears to be favored over either S or O.

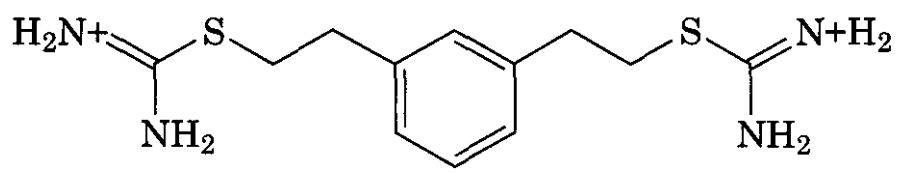
Extended chain compounds such as the bis-isothiourea, below, bind through one isothiourea to the chirality-specific pocket of the arginine binding site. The molecule extends along a long access channel that connects from the bulk solvent to the heme and pterin binding sites. The second isothiourea binds to Glu271 and Asp480. This compound is a competitive inhibitor of arginine binding and inhibits electron transfer from the reductase domain of the enzyme.

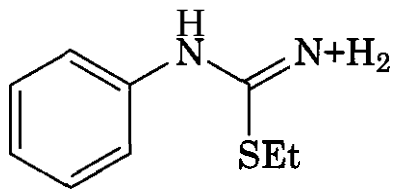
Bulky inhibitors such as ARL17477, below, and similar compounds, may bind to the access channel because of their bulk. There are amino acid differences between the access channels of the isoforms that may be useful in the design of isoform-specific inhibitors. ARL17477 is an nNOS-specific inhibitor. A similar compound, BN80933, contains an *N*-phenyl-thiopheneamidine linked to trolox by a piperidine bridge. This compound is even more bulky than ARL17477 and is also an nNOS-specific inhibitor.

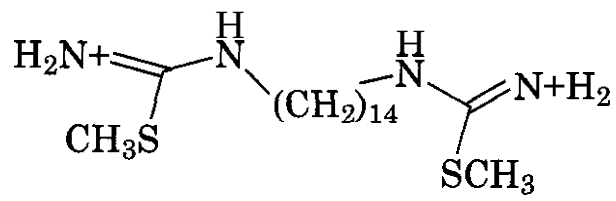
Several N-phenylisothioureas have been shown to be selective nNOS inhibitors (60). The bulk of the S-alkyl substituent is important, with ethyl providing the proper bulk, and smaller or larger groups decreasing nNOS inhibition (Table 11.12). N-Methylation of either isothioruea nitrogen decreases nNOS inhibition. The N-phenyl ring enhances inhibition. The para substitution with electron-withdrawing groups decreases iNOS inhibition without significantly altering nNOS inhibition; the para substitution with electron-donating groups can decrease nNOS inhibition. The bulk of the para substituent is important with trifluoromethyl providing the optimal bulk and larger groups decreasing inhibition. The *para* position of the phenyl ring makes direct contact with the heme propionates (59). This contact seems to confer isoform selectivity.

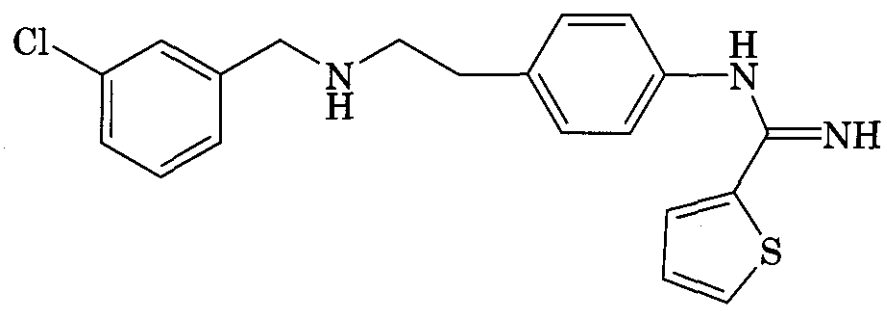
Several general comments about NOS inhibitors can be made. An inhibitor that inhibits electron transfer from the reductase or directly inhibits the reductase domain might





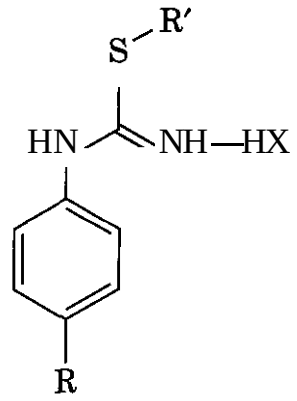






ARL 17477

Table 11.12 *N*-Phenylisothiourea Inhibitors of nNOS[a]



			K_i (μM)		
R	R′	X	iNOS	ENOS	NNOS
2-Cl	Et	Cl	2.9	1.8	0.17
4-OCF3	Et	Cl	55	17	0.70
4-OPh	Et	I	3.1	4.0	0.19
4-OBenz	Et	I	6.6	2.8	0.21
4-iPr	Et	Cl	25	7.7	0.33
2-CF3	Et	Cl	22	18	1.4
4-CF3	Et	Cl	37	9.4	0.32
4-NO2	Et	I	54	9.1	0.66
Ar=4-pyridyl		—	22	16	0.8

[a]Data adapted from Ref. 60 with permission (© 1997 American Chemical Society).

also inhibit cytochrome **P450** reductase and similar reductases. This would be detrimental to patients, in that drug metabolism might be inhibited by these compounds. NOS inhibitors that bind to heme might inhibit cytochrome **P450** and other heme enzymes, leading to inhibition of drug metabolism. NOS inhibitors that inhibit tetrahydrobiopterin binding

might inhibit other tetrahydrobiopterin enzymes such as tyrosine hydroxylase. This might lead to drug-induced parkinsonism. These concerns will be addressed in future research.

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CHAPTER TWELVE

Therapeutic and Diagnostic Agents for Parkinson's Disease

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1 INTRODUCTION

Parkinson's disease (PD) was first described by British physician James Parkinson in 1817 as the "shaking palsy" (1). One of the most common neurodegenerative disorders, PD is characterized by tremor, disturbances of posture, and paucity of volitional movement. The primary etiology of PD remains unknown, but the neuropathology is marked by progressive degeneration of monoamine-producing neurons, particularly the dopamine (DA)-containing neurons of the nigrostriatal system projecting from cell bodies in the midbrain substantia nigra to the extrapyramidal motor control centers of the caudate-putamen of the basal ganglia. Modern treatment has been based on rational attempts to replace lost DA by giving large doses of its immediate metabolic precursor, L-dopa, or substituting for lost DA with synthetic agonists. However, the pharmacotherapy of the disorder remains palliative, and limited in both effectiveness and tolerability, especially late in the progression of the illness. Improved symptomatic and hoped-for curative pharmacotherapy awaits better understanding of the fundamental pathogenesis of PD. This chapter summarizes the medicinal chemistry and salient neuropharmacology of currently available antiparkinsonism drugs and discusses ligands emerging for use in the neuroradiological diagnosis of PD.

2 PARKINSON'S DISEASE

PD affects about 1% of the general population, or more than one million persons in North America alone (2). Typically, the disorder presents in mid- or late life, with onset ranging from 40 to 80 years, and most likely at 55–65 years; cases of early onset before age 40 are less common, and rare juvenile cases have also been reported (3). PD presents as a classic tetrad of signs: (1) resting tremor that improves with voluntary activity, (2) bradykinesia or slowness of initiating voluntary movements, (3) rigidity of muscle and joint motility, and (4) postural disturbances including falls. These signs vary in their early intensity, combinations, and progression among individuals (2). Some cases also show dyscontrol of autonomic functions mediated by the potentially affected central noradrenergic sympathetic nervous system, with losses of norepinephrine neurons of the locus coeruleus (4). There can also be variable late neuropsychiatric disturbances that sometimes progress to a debilitating state and eventual fatality (2, 5, 6). Dementia is about six times more frequent in elderly patients with PD than without it (5). Major depression also occurs in PD, and psychosis is sometimes associated with DA agonist therapy (5–7). Mortality is 2–5-times higher among PD patients than in age-matched nonaffected persons, and life expectancy is greatly reduced (8).

Parkinsonism is a syndrome that represents the clinical outcome of etiologically diverse conditions. These can include idiopathic degenerative disorders (including idiopathic Lewy body dementia and multiple system atrophy with dysautonomia), infections (including postencephalitic parkinsonism of von Economo's encephalitis lethargica arising from the influenza epidemics of 1917 and following years), effects of varied neurotoxins (including heavy metals, certain phenylpyridines, and 2-amino-3-[methylaminol-propanoic acid [BMAA]), and as adverse effects of certain centrally active drugs (particularly neuroleptics and other DA D_2 receptor antagonists or DA-depleting agents) (9, 10). However, the designation PD is currently applied to the idiopathic disorder, to distinguish it from other parkinsonian syndromes.

Neuropathologically, PD is a slowly progressive neurodegenerative disorder of unknown cause that selectively affects the extrapyramidal DA nigrostriatal pathway. The disease is characterized by gradual destruction of DA-containing neurons in the pars compacta component of the pigmented midbrain substantia nigra, leading to a deficiency of the neurotransmitter in DA nerve terminals of the corpus striatum, particularly in the putamen (11). Degenerative changes in the pigmented nuclei of the noradrenergic locus coeruleus region of the midbrain-pons also are typical, and remaining catecholamine cells typically acquire fibrillar intraneuronal inclusions (Lewy bodies), whose development and significance remain unclear (5, 12). The dis-

covery of DA deficiency in postmortem brain tissue of PD patients a half-century ago, with then-emerging knowledge of DA biosynthesis and metabolism, led to the rational prediction that L-dopa (**1**), the immediate metabolic precursor of DA (**2**), would be an effective palliative agent in PD (13, 14).

2.1 Pathophysiology

The "basal ganglia" of the brain consist of five interconnected subcortical nuclei that span the telencephalon (forebrain), diencephalon, and mesencephalon (midbrain). These nuclei include the corpus neostriatum (caudate and putamen), globus pallidus, thalamus, subthalamic nucleus, and midbrain substantia nigra (pars compacta and pars reticulata). Medium-sized spiny neurons that produce the major inhibitory amino acid transmitter γ-aminobutyric acid (GABA) are principal neurons in the caudate-putamen. They receive much of the heavy input from descending corticostriatal projections mediated by the principal excitatory amino acid neurotransmitter glutamic acid, as well as a prominent dopaminergic input from the midbrain substantia nigra zona compacta. The GABA-producing inhibitory neurons, as well as intrinsic acetylcholine-producing interneurons, of the caudate-putamen respond to DA input through several DA receptors.

DA receptors are grouped into excitatory D_1-type (D_1 and much less prevalent D_5 receptors), and inhibitory D_2-type (D_2 with splice variants, and less abundant D_3 and D_4) subfamilies of membrane proteins. These peptides, composed of 387–477 amino acids, are typical of the superfamily of GTP-binding protein (G-protein)-associated membrane proteins that include most monoaminergic receptors and other physiologically important membrane proteins. Their structure is characterized by seven relatively hydrophobic, putative transmembrane regions linked by four extracellular and four intracellular loop segments, starting from an extracellular amino terminus, and extending to an intracytoplasmic carboxy end of the receptor polypeptide chain (15, 16). The third intracellular loop and carboxy-terminus segment vary most among D_1 and D_2 receptor subtypes, and probably interact critically with excitatory or inhibitory G-proteins and intraneuronal molecular components of effector mechanisms, including adenylyl cyclase and phospholipase C (15, 16).

The inhibitory spiny GABAergic neurons send projections by two major pathways whose net effect appears to exert balanced regulatory influences on the ascending thalamocortical circuits that mediate control of voluntary movement through the descending corticospinal projections to the spinal ventral-horn motoneurons that innervate skeletal muscles. A widely accepted, but tentative model of the basic anatomical connections of this complex is summarized schematically in Fig. 12.1 (12, 15, 17–20).

The output pathways from neostriatum are usually considered to include a direct or striatonigral pathway consisting of GABAergic neurons that project directly to the pars reticulata of the substantia nigra, as well as a prominent projection to the internal (medial) portion of the globus pallidus in human brain. Another *indirect* or striatopallidal pathway involves efferent GABAergic projections from striatum that communicate through the external (lateral) globus pallidus to the subthalamic nucleus. This inhibitory influence on the subthalamic nucleus is balanced against an excitatory glutamatergic input from cerebral cortex. In turn, the subthalamic nucleus exerts an excitatory glutamatergic influence on the substantia nigra reticulata.

Modified by both of these direct and indirect descending influences, the internal (medial) globus pallidus and pars reticulata send inhibitory GABAergic projections to modulate activity of ascending thalamic neurons, particularly in the ventral (mainly anterior and lateral) thalamic nuclei. The thalamic nuclei project ascending glutamatergic excitatory efferent to motor cerebral cortex, thus exerting a major regulatory influence over the descending corticospinal motor output pathway that controls the cholinergic spinal ventral motor horn cells innervating skeletal muscle (18).

DA modulates the activity of local and efferent inhibitory GABAergic neurons as well as acetylcholinergic interneurons of caudate-putamen (12, 15, 17). Excitatory DA D_1-type receptors, together with the neuropeptides substance P and dynorphin, are mainly expressed by the striatonigral GABAergic neurons in the

Circuitry of the Basal Ganglia

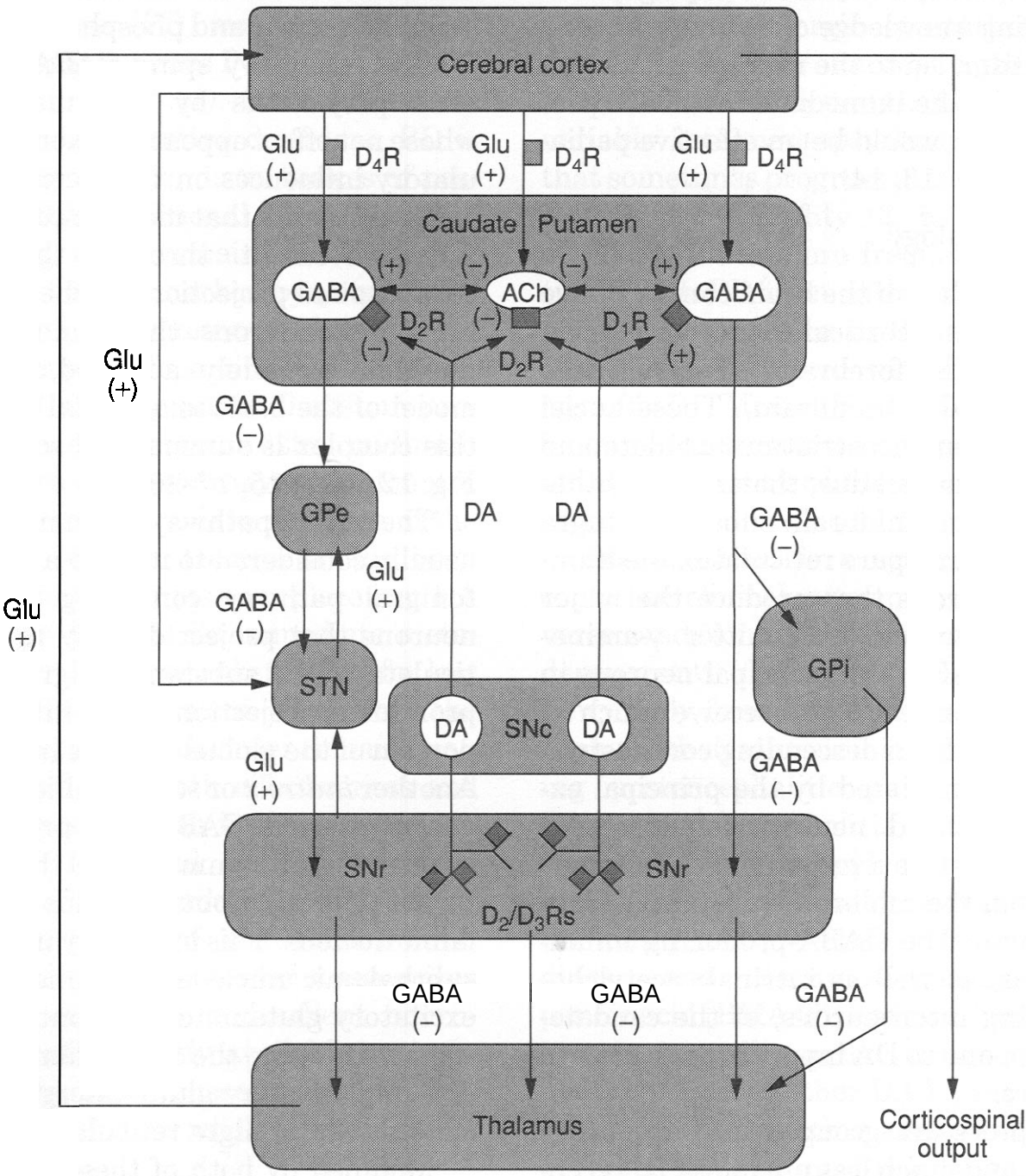


Figure **12.1.** Circuitry of the basal ganglia. Shown are major relationships to the neostriatum (caudate nucleus-putamen complex) with its prominent dopaminergic innervation (particularly of putamen in man) from the midbrain substantia nigra zona compacta (SNc), as well as descending control by corticostriatal glutamatergic projections. Dopamine exerts excitatory effects through D_1 receptors on efferent medium spiny neurons that are GABAergic and inhibitory to the substantia zona reticulata (SNr) and the internal portion of the globus pallidus (GPi), thus limiting a secondary inhibitory influence of nigrothalamic and pallidothalamic GABA neurons (that also express substance P [SP] and dynorphin [DYN] peptides) to *facilitate* the ascending excitatory glutamatergic (Glu) thalamocortical circuits. This short outflow loop from the neostriatum is paralleled by a long loop that involves D_2 dopamine receptors inhibitory to generally excitatory acetylcholinergic (ACh) interneurons (which are inhibited by GABA neurons) as well as to GABA efferents to the external portion of globus pallidus (GPe) and co-express enkephalins (ENK). The globus projects GABAergic neurons that tonically inhibit the excitatory glutamatergic neurons of the subthalamic nucleus (STN) that stimulate an inhibitory influence of nigra, internal globus pallidus, and pedunculopontine nucleus on the thalamus (including its ventral lateral nucleus) to yield a net *reduction* of thalamocortical activation. In Parkinson's disease (PD) the nigrostriatal dopamine projections degenerate and dopaminergic influences in the neostriatum are initially compromised and eventually lost. Loss of dopamine in PD leads to reduced influences through both the direct and indirect pathways to result in a net decrease in thalamocortical stimulation, with clinical bradykinesia. Excessive dopaminergic stimulation encountered in the treatment of PD increases thalamocortical activation, with clinical dyskinesias. This traditional model remains tentative and incomplete, and does not include simultaneous D_1 and D_2 influences that may occur on some GABAergic neurons in neostriatum. More inclusive models have been proposed (9).

direct output pathway. In contrast, inhibitory DA D_2-type DA receptors, along with the neuropeptide enkephalin, are predominantly localized to striatopallidal GABAergic efferents of the ***indirect*** output pathway. These relationships lead to a complex role for DA in the basal ganglia. By stimulating excitatory D_1 receptors, DA appears to have a net facilitatory effect on the direct GABAergic pathway to internal (medial) globus pallidus and midbrain, which can diminish their inhibitory connections to thalamus, to ***increase*** ascending excitatory thalamocortical activity. In contrast, activation of inhibitory D_2 receptors in the indirect pathway inhibits GABAergic neurons projecting to external (lateral) globus pallidus, and reduces its inhibitory influence on the subthalamic nucleus. These effects result in a net disinhibition of an excitatory glutamatergic link from the subthalamic nucleus to midbrain that increases nigral GABAergic inhibition of thalamus. The outcome is to ***decrease*** thalamocortical stimulation, opposite to the effect initiated by DA through the direct pathway (Fig. 12.1).

In sum, the overall effect of DA is to facilitate cortical excitation by thalamocortical glutamatergic projections through the direct pathway, but to decrease thalamocortical stimulation through the indirect pathway. Accordingly, in PD striatal DA deficiency alters the modulation of excitatory outflow from ventral thalamus to motor cortex (9, 12, 16). Presumably, a balance of D_1- and D_2-mediated dopaminergic function would be optimal in restoring the functional losses that follow degeneration of the DA-producing neurons. Neurochemically, striatal DA deficiency seems to account for the major motor symptoms of PD, particularly bradykinesia. The mainstay of pharmacological treatment (8) continues to be replacement therapy with the α-amino acid, L-3,4-dihydroxyphenylalanine (L-dopa), the immediate biochemical precursor of DA, discussed below, which should produce nearly physiological agonism of both D_1 and D_2 DA receptors.

2.2 Etiology

2.2.1 Genetic Factors. Although the neuropathology of PD is well defined, the primary cause remains unknown, thus limiting rational development of effective therapeutic and prophylactic pharmacotherapies. Several potentially convergent hypotheses have been proposed regarding the cause of PD. They consider: (1) genetic factors; (2) neurodevelopmental factors associated with advancing age, including oxidation of DA and dysfunction of oxidative metabolism; and (3) effects of environmental or endogenous neurotoxins.

Several neurodegenerative disorders affecting motility are genetically determined. Striking progress in defining the genetic basis of Huntington's chorea greatly increased interest in genetic contributions to other neurodegenerative disorders, including PD (21). Epidemiological studies have found that, apart from advancing age, a family history of PD is a predictor of increased risk of the disorder, suggesting a genetic contribution (22). Recent studies strongly indicate a genetic contribution in the disorder, although the majority of cases of PD are considered sporadic, given that genetic factors account for only a minority of cases, predominantly those of early onset (23, 24).

A specific genetic hypothesis has considered mutation of the gene for the protein α-synuclein in an autosomal dominant form of familial PD. This molecule is a highly conserved, relatively abundant, 140-amino acid. polypeptide of unknown function that is expressed mainly in presynaptic nerve terminals in the brain. It is controlled by a gene in the long-arm (4q) of human chromosome 4 (25). The gene for a-synuclein can be expressed as distinct structural variants that have been associated with some pedigrees involving PD (26, 27), but not in a larger number of other families (28, 29) or in sporadic cases (27). Other genetic findings have implicated mutations in the gene coding for ubiquitin carboxy-terminal hydrolase in another autosomal dominant form of PD, and other genetic factors associated with autosomal recessive forms of the disorder (30). However, these specific genetically based disorders, collectively, do not account for the great majority of evidently sporadic cases of PD.

Nevertheless, a large proportion of the uncommon cases of early-onset PD appear to be inherited by a mechanism involving abnormal molecular processing of intracellular neuronal

proteins by the process of conjugation with the protein ubiquitin required for the orderly degradation of intracellular proteins by proteases (23, 24). A specific autosomal dominant genetic defect produces a mutant form of the protein parkin. Normally, parkin enables ubiquitin ligase-mediated polyubiquitination of an O-glycosylated form (αSP22) of the protein a-synuclein. Because polyubiquitin conjugation is required for normal degradation of a-synuclein by proteasomes, ubiquitin-unconjugated αSP22 accumulates in neurons, possibly contributing to their degeneration (31).It remains unclear why such mechanisms might occur selectively in DA neurons, or whether they pertain to the more prevalent, nonfamilial forms of PD. The neuropathology of spontaneous PD, in contrast to the less common familial forms, is characterized by prominent accumulation of Lewy bodies rich in polyubiquitnated αSP22 and other proteins, presumably through insufficient degradation by proteases that remains unexplained.

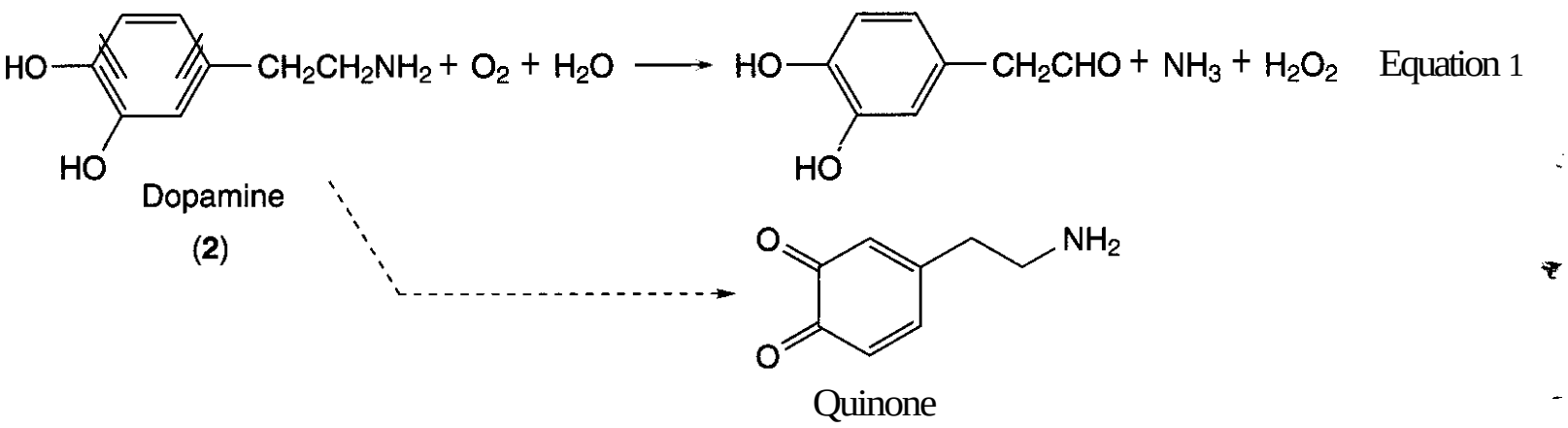
The proposal that an increase of a-synuclein may be responsible for PD has inspired new laboratory models of PD involving transgenic mice (32) or the fruit fly Drosophila (33) made to express the human wild-type a-synuclein and recreate certain features of PD. In both models, cytoplasmic inclusions of a-synuclein resembling Lewy bodies were detected, but these were fibrillar in structure, as in clinical PD, only in the fruit fly model. Mechanisms by which abnormal a-synuclein oligomers might selectively cause dysfunction and death of DA neurons remain unclear (34). Possibilities considered include abnormal cellular distribution of DA, with an accumulation of toxic by-products, and an effect of DA on a-synuclein oligomerization (31). Support for the second possibility arose from recent screening of a large number of compounds for effects on a-synuclein fibrilization; 15/169 compounds inhibited the process, and 14 of these were catechols, including L-dopa and DA (35). DA underwent oxidative ligation to a-synuclein and selectively inhibited the conversion of protofibrils to fibrils, causing accumulation of the a-synuclein protofibril. These findings suggest a basis for DA-neuron selectivity and may open a novel line for developing novel therapeutic approaches to PD (35).

2.2.2 Developmental Factors and Oxidation of Dopamine. DA itself has also been implicated in the disease process of PD through production of chemically reactive products of oxidation, including reactive quinones, peroxides, and free radicals (33). Toxic effects of these compounds may contribute to the apparently normal progressive loss of DA neurons with maturation and aging, at the rate of about 13%/decade (36, 37). Clinical symptoms of PD emerge as losses of DA neurons exceed 60–70% (38). However, the significance of this trend toward spontaneous, possibly genetically programmed (apoptosis) or degenerative, but evidently normal loss of DA neurons with aging for the pathoetiology of PD is not clear (39, 40).

Among products of the metabolism of DA, the monoamine oxidase (MAO)-catalyzed oxidation of this and other monoamine neurotransmitters generates hydrogen peroxide (Equation 1 of Fig. 12.2). The peroxide can undergo a redox reaction with superoxide in the Haber-Weiss reaction (41) to form the extremely cytotoxic free hydroxy ($HO^{\cdot}$) radical (Equation 2 of Fig. 12.2). Moreover, auto-oxidation of DA *can* yield the corresponding cytotoxic quinone (Fig. 12.2) (42). Manganese ion *can* catalyze oxidation of DA, and the resulting quinones have been implicated in manganese neurotoxicity, an environmentally determined form of parkinsonism (43). Additional evidence for a role of oxidative stress in PD includes increased levels of oxidized by-products of nucleic acids such as 8-hydroxyguanosine in cerebrospinal fluid or plasma (44).

Auto-oxidation of catecholamines also leads to formation of the polymeric pigment neuromelanin that increases with age and is responsible for the dark coloration of DA-producing cells in the substantia nigra and the norepinephrine neurons of the locus cœruleus (42). A physiological role for this polymer is not established, and there is no evidence to support its involvement either in normal loss of DA neurons with aging or in the pathophysiology of PD.

Conceivably, the symptoms of PD may result from two processes: a specific disease-related insult combined with changes attributed to normal aging that may include accumulation of endogenous neurotoxins. Such a two-factor



Equation 1

$$H_2O_2 + [O_2^{\bullet -}] \longrightarrow O_2 + [OH^- + OH^{\bullet}]$$

Equation 2

Figure 12.2. Toxic derivatives of dopamine. These include hydrogen peroxide, the free hydroxy radical, and the quinone.

pathophysiology might explain why PD is usually a progressive disorder of late onset (45).

2.2.3 Environmental Toxins. Even in disorders with strong familial association, a role of shared environmental exposures and other indirectly related factors must be considered. Studies of familial but idiopathic forms of PD-like neurodegenerative disorders in the Western Pacific do not support genetic or infectious etiologies, but leave open the possibility of toxic factors (46, 47).

There is some evidence to suggest that environmental toxins may cause some types of PD, or at least, parkinsonism syndromes (48). This hypothesis seems consistent with the fad that PD is now the second most common degenerative neuropsychiatric disorder (after the dementias), in contrast to its evident rarity in previous centuries (48). A striking example of a probably neurotoxic disorder is the PD-like syndrome characterized by tremor and bradykinesia as well as dystonia and psychiatric disturbances found among manganese miners in the Andes (49). Another example involves a correlation between a remarkably high incidence of PD and use of certain pesticides in an agricultural region of Quebec (50).

One of the best characterized epidemiological findings in PD is its lower incidence in cigarette smokers than in nonsmokers (51, 52). Chemical components of cigarette smoke, including nicotine, may protect against an environmental or endogenous toxin relevant to the neuropathology of PD. For example, carbon monoxide in cigarette smoke may detoxify potentially neurotoxic free radicals from environmental or endogenous sources, including DA. Compounds present in cigarette smoke or their metabolites might also inhibit MAO, the main enzyme responsible for metabolism of monoamine neurotransmitters including DA, producing hydrogen peroxide in the process, as noted above (53).

Overall, neither neurotoxin nor genetic models account for the majority of apparently spontaneous or sporadic cases of PD (48). Nevertheless, the discovery that a specific pyridine, MPTP, can produce a severe, acute parkinson syndrome in humans and some laboratory species stimulated unprecedented interest in both neurotoxic hypotheses concerning the etiology of PD and in developing a very useful experimental model for the disorder that has supported a great deal of recent research aimed at developing innovative treatments for the disease. This compelling model of a neurotoxin-induced form of the parkinsonism syndrome is described next.

2.2.4 Parkinsonism and MPTP. The tertiary amine N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 3, Fig. 12.3) is a potent and selective neurotoxin to DA-producing cells of the midbrain (51, 54). MPTP exposure induces a form of parkinsonism in humans and monkeys that is similar in neuropathology and motor abnormalities to idiopathic PD (55–58). Its significance emerged from a serendipitous series of events. In 1977, a young college student developed acute parkinsonian symptoms with severe rigidity, bradykinesia, and mutism (58). The abrupt and early onset of symptoms was so atypical that the patient ini-

tially was thought to have catatonic schizophrenia. Subsequent diagnosis of parkinsonism was substantiated by his therapeutic response to L-dopa. When he admitted having synthesized and used several illicit drugs, his psychiatrist visited his home and collected glassware that had been used for chemical syntheses.

Chemical analysis of the glassware revealed several pyridines, including MPTP (**3**), formed as by-products in synthesizing MPPP (*N*-methyl-4-propionoxy-4-phenylpiperidine), (4) and also known as "designer heroin" or "synthetic heroin." MPPP is also the *N*-desmethyl analog of a structurally similar narcotic analgesic, alphaprodine (**5**), and is the reverse ester of the analgesic meperidine (Demerol, **6**). It was unclear initially whether MPTP or other constituents of the injected mixture accounted for the neurotoxicity. The patient continued to abuse drugs, died of an overdose, and autopsy revealed degeneration of his substantia nigra, but without the Lewy bodies typical of the neuropathology of idiopathic PD (58).

Other persons exposed to preparations of MPTP (**3**) or MPPP (4) were later identified after presenting with similar acute parkinsonian symptoms. In several patients, MPTP was the principal or sole compound implicated, supporting the hypothesis that MPTP is a parkinsonism-producing neurotoxin. Although more than 400 people are known to have self-administered MPTP, only a few have developed parkinsonian symptoms, though the incidence may increase as they age. Both the clinical and neuropathological features of MPTP-induced parkinsonism resemble idiopathic PD more closely than any other model elicited by toxins, metals, viruses, genetic manipulation, or other means. Accordingly, understanding the molecular pathophysiology of MPTP neurotoxicity was aggressively pursued to clarify neurodegenerative mechanisms that might also occur in idiopathic PD.

The chemical structure of MPTP suggests that the compound should be relatively inert chemically, given that no reactive functional group is present, and that MPTP might undergo metabolic activation to a more reactive derivative. Researchers discovered that brain MAO-B (mainly in glial cells) catalyzes a two-electron oxidation of MPTP at the allylic a-carbon, to give the intermediate, 1-methyl-4-phenyl-2,3-dihydropyridinium species ($MPDP^+$, 7; see Fig. 12.3). This unstable charged substance undergoes further two-electron oxidation to the more stable, reactive species 1-methyl-4-phenylpyridinium (MPP^+, **8**) by auto-oxidation, disproportionation, and enzyme-catalyzed mechanisms (**59–61**). Inhibitors of MAO-B can prevent MPTP-induced parkinsonism in primates (**62**), further supporting the currently accepted view that MPP^+ (**8**) is probably the major toxic metabolite of MPTP (3) responsible for destruction of DA neurons, although a role for the unstable dihydropyridinium species $MPDP^+$ (7) has not been ruled out.

The relationship of MAO and MPTP has neurobiological relevance beyond MPTP neurotoxicity. Types A and B MAO catalyze the a-carbon oxidative deamination of monoamine neurotransmitters and other aromatic amines. These genetically dissimilar isozymes show differential selectivity for specific substrates and inhibitors (63). Most intraneuronal MAO is mitochondrial and type A, and oxidizes primary amines to aldehydes, which are then converted by aldehyde reductases to alcohols or carboxylic acids, including dihydroxyphenylatic acid (DOPAC) from DA. Additional 3-0-methylation yields the major final human metabolite of DA, homovanillic acid (HVA; see Fig. 12.4).

In contrast, MAO-B is found in other cell types, including glia in the nervous system. Oxidation of the heterocyclic tertiary-amine MPTP by MAO-B is an unprecedented reaction that suggests a novel physiological role for this enzyme. For example, MAO-B may regulate the oxidation state of pyridine systems, such as those involving nucleic acids and NADH (63). In turn, oxidative systems are implicated in MPTP neurotoxicity (see below). Interesting, too, is evidence that cigarette smokers have been found to have 40% less postmortem brain MAO-B activity than nonsmokers or former smokers (64), as well as a lower incidence of PD (64, 65). That cigarette smokers reportedly have depressed MAO-B activity (64) may be a potential link to their decreased risk of PD (65). Nicotine is not a potent inhibitor of MAO, and may even in-

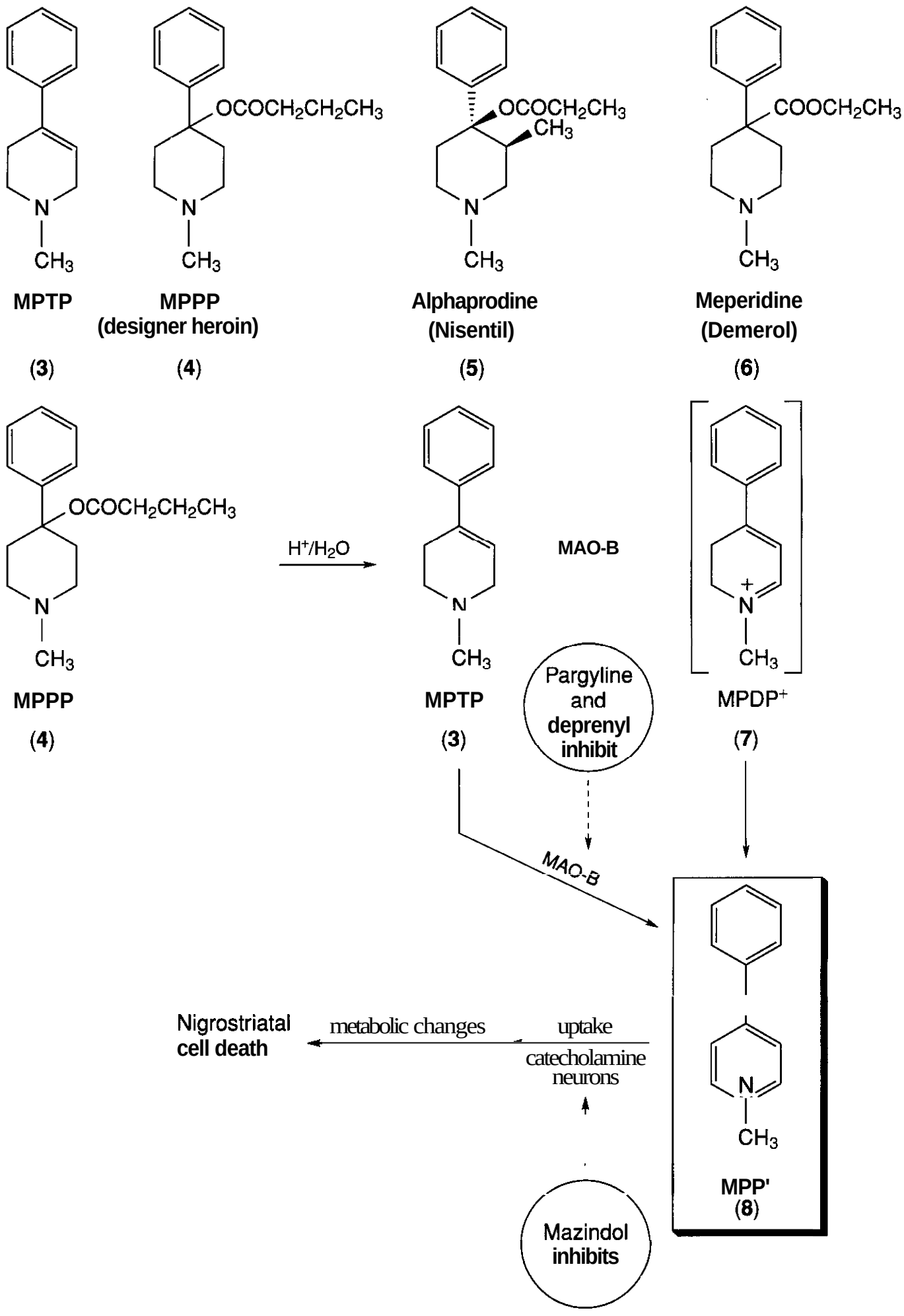


Figure 12.3. Phenylpiperidine analgesics and metabolic activation of MPTP. In efforts to synthesize the meperidine-like analgesic agent MPPP ("designer heroin,") (**4**), MPTP **(3)** can be formed. It is converted selectively by monoamine oxidase type **B (MAO-B,** inhibited by agents including deprenyl (selegiline) and pargyline to $MPDP^{+}$ (**7**), and thence to MPP^{+} **(8)** the proposed toxic species that accumulated in dopamine neurons to result in disruption of their cellular respiration and death.

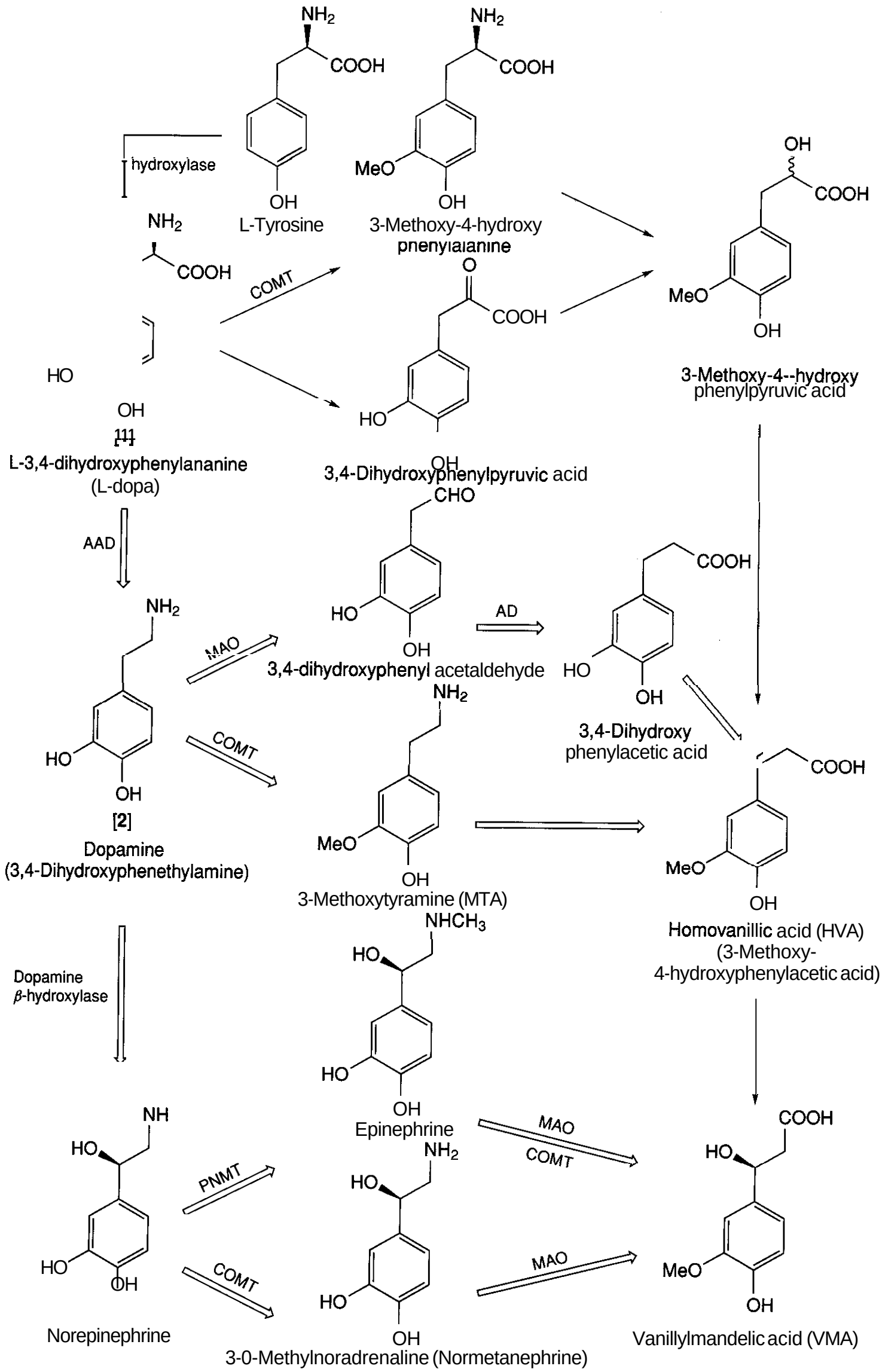


Figure 12.4. Pathways in the metabolism of L-dopa (**1**) and its major decarboxylated product dopamine (**2**). Major (heavy arrows) and minor (light arrows) reactions are indicated. AD, aldehyde dehydrogenase; AAD, aromatic L-amino acid decarboxylase; COMT, catechol-*O*-methyltransferase; DH, dopamine β-hydroxylase; MAO, monoamine oxidase; PNMT, phenylethanolamine-N-methyltransferase.

crease MPTP neurotoxicity (66). Other compounds in cigarette smoke, however, do inhibit MAO and cigarette smoke protects against MPTP-induced depletion of striatal DA in mice (67).

Extensive investigation indicates that nigrostriatal degenerative properties of MPTP are mediated by the MAO-B-derived metabolite MPP^+ (8). First, MPTP (3) binds selectively to MAO-B, which is highly concentrated in glial cells in human substantia nigra and corpus striatum (68), and is oxidized to MPTP (3) to $MPDP^+$ (7). Several factors may account for selective damage of nigrostriatal DA neurons by MPTP (69; Fig. 12.3). Notably the MPP^+ (3) produced from MPTP (3) is selectively accumulated by DA transporters into nigral DA cells and striatal DA nerve terminals (69). Within the DA nigral cells, MPP^+ binds to neuromelanin and may be gradually released in a depot-like fashion, maintaining toxic intracellular concentrations that inhibit mitochondrial respiration in DA neurons. MPP^+ can also displace DA from presynaptic vesicles into the cytoplasm, and oxidative byproducts of the DA displaced may contribute further to degeneration of DA neurons. Another critical mechanism is that MPP^+ concentrates in neuronal mitochondria, where it selectively inhibits complex I of the electron transport chain, inhibiting NADH oxidation and eventually depleting the nigrostriatal cell of ATP (70, 71). Depletion of ATP is probably the main mechanism of nigrostriatal cell death induced by MPP^+ derived from MPTP (72).

The serendipitous discovery and subsequent scientific investigation of the mechanism of parkinsonism produced by MPTP greatly stimulated study of the pathogenesis of idiopathic PD, and drew specific attention to intraneuronal oxidative metabolism. One result is growing evidence for a functional defect in the mitochondrial respiratory biochemistry in idiopathic PD, including a specific deficiency of NADH-coenzyme Q1 reductase, reflected in a 30–40% lower mitochondrial complex I activity in the substantia nigra of postmortem brains of PD patients compared to normal controls (73, 74). In general, mitochondrial dysfunction and oxidative metabolism (including oxidation of DA to electrophilic quinone-type species; see Fig. 12.2) are critical components of most theories of nigral cell degeneration in PD (8). Moreover, discovery of the selective ability of MPTP to induce nigral cell death has stimulated broad interest in identifying potential environmental or endogenous compounds as potential causative agents in PD, even though there is no evidence that MPTP itself is involved in idiopathic PD.

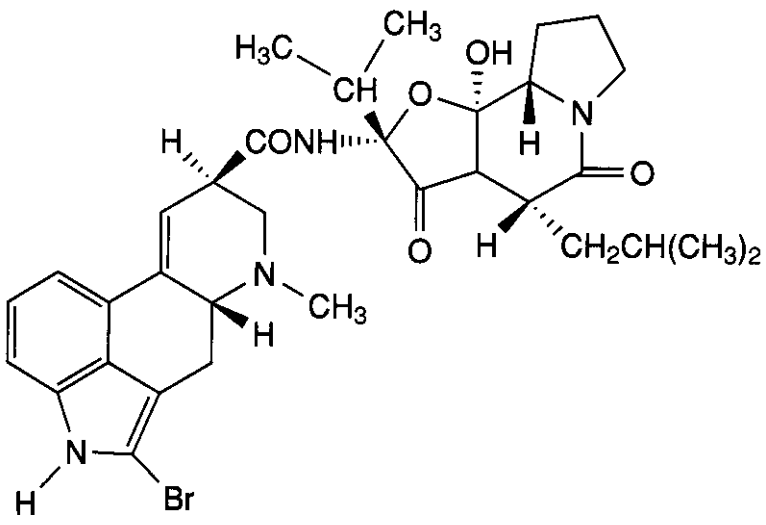
Another example of a relevant toxin effect is evidence that chronic, systemic inhibition of complex I by the lipophilic pesticide rotenone causes highly selective degeneration of nigrostriatal DA neurons that can produce hypokinesia and rigidity, as well as accumulation of fibrillar cytoplasmic inclusions that contain ubiquitin and a-synuclein (75). These findings lend additional support to the view that environmental toxic factors may contribute to risk of PD.

3 TREATMENTS FOR PARKINSON'S DISEASE

3.1 Dopaminergic Treatments

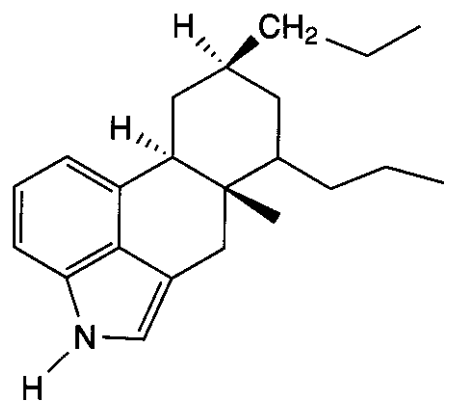
Available pharmacotherapy for PD continues to be palliative or symptomatic, involving partial compensation the DA deficiency in neostriatum, usually by direct stimulation of DA receptors, or by enhancing its synthesis or decreasing its catabolism. No treatment has been proved to stop or even slow the progressive neurodegeneration in PD (11, 76). However, evidence of a lesser rate of loss of DA neurons has recently been reported during 4 years of treatment with the direct DA agonist pramipexole (see 17 in Fig. 12.5) compared to L-dopa, which are discussed in the following sections (77). Encouraged by the discovery of parkinsonism produced by MPTP, and of the ability of MAO-B inhibitors to prevent it, selective MAO-B inhibitors or antioxidants were hypothesized to slow progression of idiopathic PD. Clinical evidence, however, has not been encouraging regarding the effectiveness of such treatments nor for their ability to limit the progression of idiopathic PD (11, 78, 79).

3.1.1 L-Dopa Metabolism and Therapy. Nearly 40 years after its introduction, levodopa or L-dopa (1; Fig. 12.4) remains an effective pharmacotherapy in PD (8, 11, 13, 80, 81).



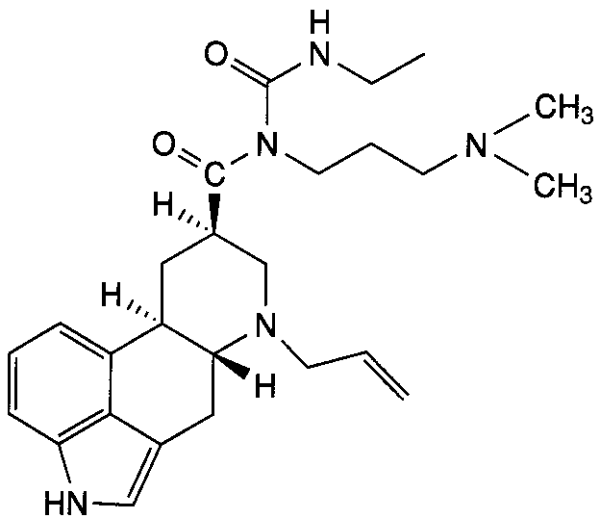
Brornocriptine (Parlodel)

(**11**)



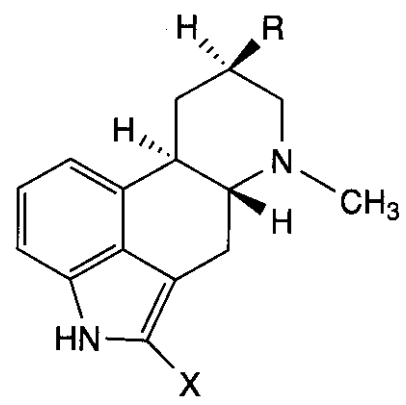
Pergolide (**Permax**)

(**12**)



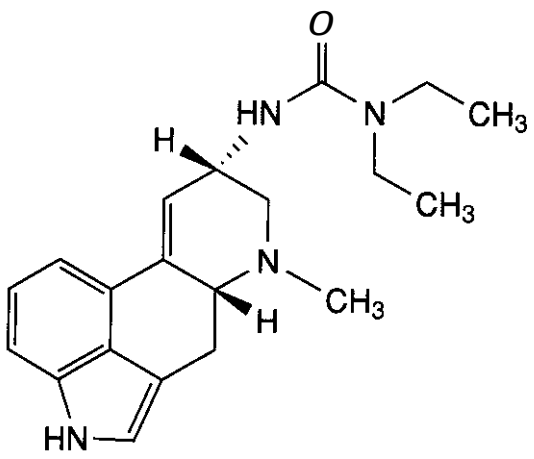
Cabergoline (Dostinex)

(**13**)



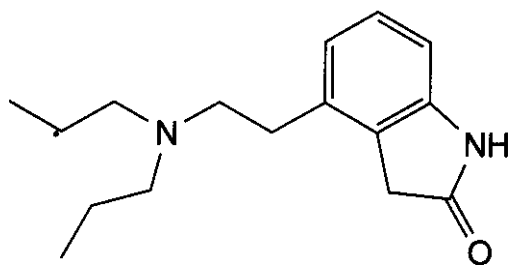
(**14a**) Lergotrile, R = CH_2CN, X = Cl

(**14b**) Pergolide, R = CH_2SCH_3, X = H



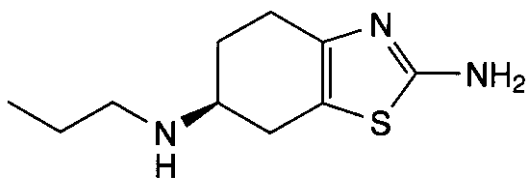
Lisuride

(**15**)



Ropinirole (**Requip**)

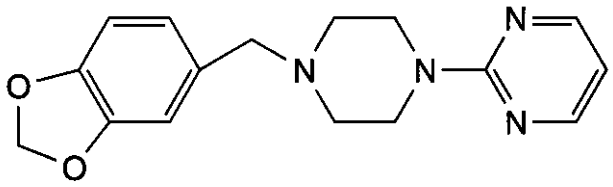
(**16**)



Pramipexole (Mirapex)

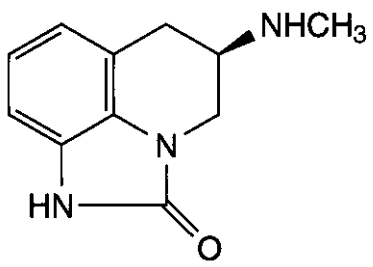
(**17**)

Figure 12.5. Representative D_2 partial agonists.



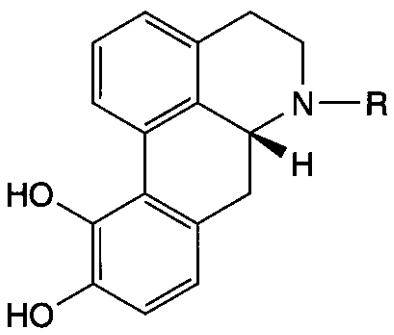
Piribedil

(**18**)



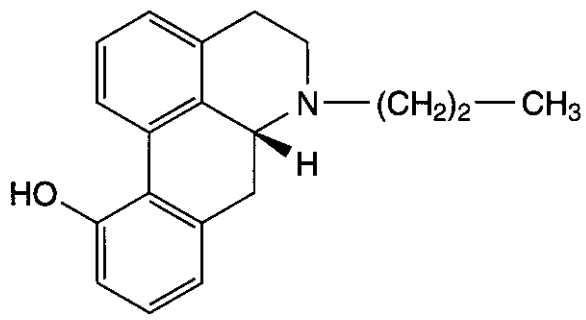
PNU-95666E

(**19**)



(20) Apomorphine, R = CH_3

(21) R(–) N-*n*-propylnorapomorphine (NPA), R = *n*-C_3H_7



R(–) N-*n*-propyl-11-hydroxynoraporphine

(**22**)

Figure 12.5. (Continued.)

Despite controversy regarding long-term efficacy, adverse effects, and even potential **neurotoxicity** of this amino acid precursor of DA, most PD patients derive a substantial benefit from L-dopa throughout their illness. Moreover, L-dopa increases life expectancy among patients with PD, particularly if instituted early in the course of PD (82).

In 1960 **Ehringer** and **Hornykiewicz** assayed DA in the brains of patients dying with PD and found that DA levels in the corpora **striata** of many of these patients averaged only 20% of normal (83). The **signs** of illness in PD patients resembled **behavioral** changes in rats treated with reserpine or other **amine-depleting** agents. These findings led **Birkmeyer** and **Hornykiewicz** to administer high oral doses of **racemic** dopa to PD patients in Vienna in 1960 (84). Subsequent clinical trials led by **Barbeau** in Montreal in the early 1960s and by **Cotzias** in New **York** in the late 1960s confirmed this effect of **racemic** dopa (**85**). **Barbeau** in Montreal, and later Cotzias, also demonstrated the greater potency and safety of the physiological levo **enantiomer** (**80, 81**).

Development of L-dopa (**1**; Larodopa; Fig. 12.4) as a therapeutic agent in PD is a rare example of a rationally predicted and logically pursued clinical treatment in a neurological disorder, based on **neurochemical** pathology and basic pharmacological theory (**13, 14**). The effectiveness of L-dopa treatment requires its penetration into the central nervous system (**CNS**) and local **decarboxylation** to DA. DA does not cross the blood-brain diffusion barrier because its amino moiety is **protonated** under physiological **conditions** (pK_a 10.6), **making** it excessively **hydrophilic** (86). However, its precursor amino acid L-dopa is less basic (pK_a 8.72) and polar at physiological pH, and more able to penetrate the CNS, in part facilitated by transport into brain with other aromatic and neutral aliphatic amino acids (86–88).

L-Dopa is normally a trace intermediary metabolite in the biosynthesis of **catecholamines**, formed from **L-tyrosine** in a **rate-limiting** **hydroxylation** step by **tyrosine hydroxylase**, a phosphorylation-activated cytoplasmic mono-oxygenase. L-Dopa is readily **decarboxyated** by the cytoplasmic enzyme **L-aromatic** amino acid decarboxylase ("dopa **decarboxylase**") to form DA (2). The effects observed after systemic administration of L-

dopa have been attributed to its peripheral and cerebral metabolites, mainly DA, with much less conversion to norepinephrine by β-hydroxylation, or epinephrine formed by N-methylation of norepinephrine by phenylethanolamine-*N*-methyltransferase (86, 87) (Fig. 12.4). A small amount of L-dopa is O-methylated to L-3-0-methyldopa (L-3-methoxytyrosine), which accumulates in the CNS because of its long half-life. However, most exogenous L-dopa is rapidly decarboxyated to DA in peripheral tissues, including liver, heart, lung, and kidney. Because only about 1% of an administered dose reaches the brain, L-dopa, by itself, has very limited dose effectiveness (89). In humans, appreciable quantities of L-dopa enter the brain only when administered by itself in doses (3–6 g daily) high enough to overcome losses caused by peripheral metabolism.

Inhibition of peripheral decarboxylase activity by coadministration of L-dopa with a hydrophilic, peripheral decarboxylase inhibitor (see Fig. 12.8 below) such as carbidopa (9; Lodosyn; combined with levodopa in Sinemet and Sinemet-SR) or benserazide (10; combined with levodopa in Prolopa) markedly increases the proportion of L-dopa that reaches the brain, where it can be converted to DA by widely available aromatic amino acid decarboxylase and replace its deficiency associated with PD. Doses of L-dopa required are correspondingly much lower (typically only 0.2–1.2 g/day), and most commonly attained with 25/100 mg doses of standard preparations of carbidopa/levodopa, or 15/200 mg of SR products, although preparations with other dosage ratios are available (20). Patients with PD are typically started on one of the combination products, either alone or with other adjunctive agents discussed below. The extended-release preparations should theoretically provide more sustained benefits with less "wearing off' of benefit after several hours, but the bioavailability of these products is variable. In general, tissue uptake of L-dopa is highly dependent on competition with other aromatic and neutral aliphatic amino acids, and can be decreased substantially by a protein meal.

Pyridoxine (vitamin B_6) is the cofactor for aromatic amino acid decarboxylase. It can reverse the therapeutic effects of L-dopa by increasing decarboxylase activity, with more peripheral conversion of the amino acid to DA to make less L-dopa available to the CNS. However, blockade of peripheral decarboxylation with carbidopa minimizes this effect of pyridoxine.

DA itself is relatively rapidly metabolized to its principal inactive excretion products of MAO (largely by MAO-A in mitochondria of aminergic nerve terminals) and by extraneuronal catechol-*O*-methyltransferase (COMT). Tissue concentrations of the methyl-donor cofactor of methylpherases, S-adenosyl-L-methionine (SAMe), can be depleted with large doses of L-dopa (90). The main by-products of DA are deaminated 3,4-dihydroxyphenylacetic acid (DOPAC) and deaminated, 3-*O*-methylated homovanillic acid (HVA; 3-methoxy-4-hydroxyphenylacetic acid; see Fig. 12.4).

A common adverse effect of L-dopa therapy is nausea and vomiting, possibly because of a combination of gastrointestinal irritation as well as stimulation by DA (and perhaps L-dopa) of the chemoreceptor trigger zone (CTZ) in the area postrema of the brainstem, an emesis-inducing center. The blood-brain barrier is poorly developed in area postrema, making the CTZ accessible to circulating emetics. An important advantage of combining L-dopa with a peripheral decarboxylase inhibitor. in association with the 75–80% reduction of the required doses of L-dopa, is less risk of emesis or other adverse effects associated with peripheral formation of excess DA. These can include activation of peripheral adrenergic and DA receptors, in part by releasing endogenous adrenergic catecholamines (88), with a variety of cardiovascular effects. Theoretically, vasoconstriction and hypertension might occur by stimulation of peripheral α-adrenoceptors, tachycardia by stimulation of cardiac β-adrenoceptors, and direct renal and mesenteric vasodilatation by DA. However, such effects are rarely encountered clinically with the use of a peripheral decarboxylase inhibitor with L-dopa (20).

After about 5 years, at least half of L-dopa-treated PD patients develop fluctuating motor responses, and nearly three-quarters do so by 15 years (90). These fluctuations include "off' periods of immobility, and "on" periods with abnormal involuntary movements or dyskine-

sias (see Fig. 12.1). This "on-off' phenomenon may reflect progression of the disease with more severe striatal nerve terminal degeneration and further loss of DA, along with increased sensitivity of its receptors.

Psychiatric disturbances such as hypersexuality, mania, visual hallucinations, and paranoid psychosis also are quite common adverse responses to treatment with L-dopa or direct DA agonists. These psychiatric disturbances are widely proposed to reflect excessive stimulation of DA receptors in mesolimbic or mesocortical DA systems. They can greatly complicate clinical management of depression commonly associated with PD, and of dementia that sometimes arises in late stages of the disease. Modern antidepressants usually are well tolerated, with inconsistent and probably minor risk of worsening bradykinesia with serotonin-enhancing antidepressants (91). Use of antipsychotic drugs, however, is limited to those with minimal risk of worsening bradykinesia and other aspects of extrapyramidal motor dysfunction (7). Clozapine is best tolerated; quetiapine and low doses of olanzapine are sometimes tolerated; and the new atypical antipsychotic agent ziprasidone has not been evaluated in PD patients with psychotic reactions, although the chemically related risperidone and older neuroleptic D_2 antagonists are not tolerated by PD patients (7, 92–95).

3.1.2 Clinically Used Dopamine D_2 Receptor Agonists. The nigrostriatal neurodegeneration underlying PD reduces the number of striatal nerve terminals available to decarboxylate L-dopa to DA, but also increases sensitivity of DA receptors as well as loss of the inactivation of DA by neuronal reuptake at DA transporters virtually expressed only by DA neurons and their terminals. Drugs that act directly to stimulate DA receptors do not require functioning DA nerve terminals or endogenous synthesis of DA, and can be particularly useful in managing late-stage PD. Several agents with direct DA-agonist activity have been used in the treatment of PD (20, 96–109). All are primarily agonists or partial agonists of the D_2 family of DA receptors. No agents with selective D_1 agonist activity, or well-balanced D_1 and D_2 agonist actions have been developed for clinical application, though apomorphine has some D_1 as well as potent D_2 agonist effects (110).

Bromocriptine (Parlodel, 11; Fig. 12.5) is an ergot alkaloid-peptide that acts as a weak partial agonist (or antagonist) at D_1-type and a partial agonist at D_2-type DA receptors, with moderate intrinsic activity. It was the first direct DA agonist to be employed in the treatment of PD, after its development as a prolactin inhibitor (101, 111, 112). Bromocriptine inhibits prolactin release from anterior pituitary mammotrophic cells that express D_2 DA receptors selectively. These receptors respond to DA produced in the arcuate nucleus of the hypothalamus and released at the median eminence into the hypophysioportal blood vessels, and carried to the pituitary to act as a prolactin-inhibitory hormone. Bromocriptine is an effective prolactin inhibitor at low doses (typically 1–5 mg/day), for which it is used to treat hyperprolactinemia associated with pituitary adenomas, or to suppress prolactin output in prolactin-sensitive metastatic carcinoma of the breast. The partial-agonist acts as an agonist at pituitary D_2 receptors that are normally in a high sensitivity state. At higher doses (typically 10–20 mg/day), bromocriptine and other D_2 partial-agonist ergolines act as D_2 agonists with antiparkinson, and perhaps mood-elevating, effects. This agonism evidently reflects the supersensitized status of denervated DA receptors in PD (94, 113).

The peptide component of bromocriptine evidently is not necessary for its dopaminergic activity, and pergolide (Permax, 12; see Fig. 12.5) was the first nonpeptide ergoline used successfully in the treatment of PD, as well to inhibit release of prolactin from the pituitary (96, 103, 107, 108, 111). Pergolide shows greater agonist effects at both D_2- and D_1-type DA receptors than does bromocriptine. Several additional experimental ergolines have been developed with a range of D_2 agonist or partial-agonist actions, including cabergoline (13), lergotrile (14a), pergolide (14b), and lisuride (15)(11, 95, 96, 101, 104, 105, 109).

Other small molecules are direct D_2 agonists or partial agonists, and are effective in the treatment of PD. They include ropinirole hydrochloride (Requip 16), and pramipexole dihydrochloride (Mirapex, 17) (11, 102, 103,

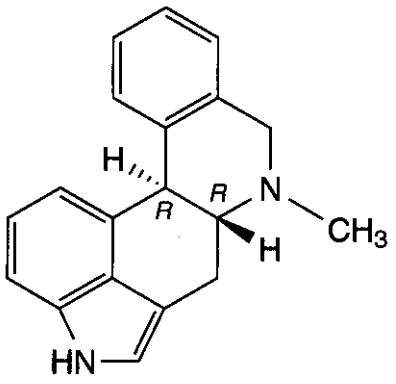
107, 108), as well as **piribedil** (**18**)(98) and the experimental agent R-PNU-95666-E (19)(99, 100) (Fig. 12.5). In contrast to the ergolines, these compounds are more selective in their interactions with DA receptors, and have much less effect at nondopaminergic sites.

Currently, ropinirole (**16**) and pramipexole (**17**) are among the most commonly prescribed direct DA agonists for PD in the United States (**20**). They were introduced primarily for use in advanced stages of PD to limit fluctuations in response to L-dopa therapy and as a "rescue" therapy when L-dopa became less effective. However, their relative tolerability, coupled with clinically unresolved concern that L-dopa therapy might contribute to additional toxic damage to DA neurons through formation of reactive oxidized by-products, has led to their growing use as a first-line treatment option, sometimes before L-dopa is added. An additional advantage of these agents is that they have a relatively prolonged dopaminergic action, to provide more sustained clinical benefit with less risk of fluctuation than with L-dopa, even as modified by cotreatment with inhibitors of its peripheral metabolism by **decarboxylase** and COMT. This impression is supported by controlled comparisons of the two forms of treatment (101–103). A recent study in patients initially treated with pramipexole (17) demonstrated a reduction in loss of striatal (^{123}I)-β-CIT (54, see Fig. 12.11) a marker of DA neuron degeneration, compared with those initially treated with L-dopa during a 46-month period (77).

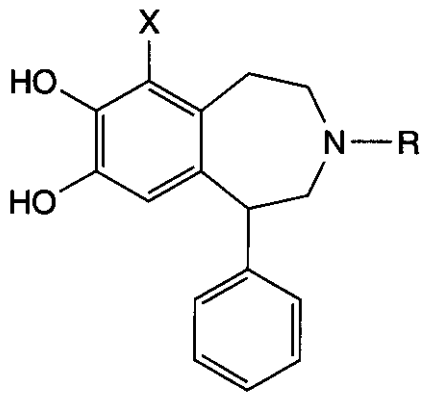
The direct DA agonists generally produce similar adverse effects, including initial nausea and vomiting, postural hypotension, and fatigue. These effects are more likely with the ergolines, which are started in low doses and increased slowly, **as** tolerated, whereas **ropinirole** and pramipexole can usually be dosed more rapidly to clinically effective levels. Additional risks include psychotic reactions when the DA agonists are given alone or with L-dopa. These reactions include hallucinations, delusions, and confusion, suggesting delirium, which is most likely to occur in elderly PD patients with mild dementia. Treatment is similar to psychotic reactions to L-dopa therapy, and usually includes use of low doses of clozapine or quetiapine (6, 7, 91–94). Adverse peripheral and central dopaminergic effects, including nausea, hypotension, and agitation also are encountered with ropinirole and pramipexole. They also can produce paradoxical somnolence as well as edema, and have been associated with uncommon **narcolepsy**-like sleep attacks during the daytime, with potential risk during driving (114).

R-(−)-**apomorphine** (20; Fig. 12.5) is an acid-rearrangement product of morphine employed in experimental neuropharmacology since the late nineteenth century (110, 115). Apomorphine is an agonist for both D_2 and D_1 DA receptors. With a pK_a of about 9, it is mostly protonated at physiological pH, but sufficiently lipophilic to cross the blood-brain barrier readily. Apomorphine hydrochloride was resurrected as a useful adjunct in the therapy of PD a decade ago (116), following years of neglect after promising early observations (85, 117). Lack of oral bioavailability, short duration of action, and potent central emetic action discouraged its clinical use. Nevertheless, in 1993, apomorphine received regulatory approval in the UK for control of refractory motor dysfunction and wide fluctuations in responses ("on-off' syndrome) to L-dopa or DA agonists (118–120). Improved motility in response to an acute challenge dose of apomorphine can also predict responsiveness to L-dopa treatment (121–123). Apomorphine can be administered subcutaneously by intermittent self-administration with a small self-injector (Penject), or continuous infusion with a portable **minipump** (115, 120). **Catechol-diester** and methylenedioxyaporphine **prodrugs** limit first-pass metabolic inactivation, while retaining much of the activity of **catecholaporphines** (124). The **11-monohydroxy** congener of the potent DA agonist *R*-(−)-*N*-*n*-propylnorapomorphine (21), *R*-(−)-*N*-*n*-propyl-11-hydroxynoraporphine (22; Fig. 12.5), retains the critical free hydroxyl group analogous to the meta-3-hydroxy substituent of DA, has potent dopaminergic activity, and is orally **bio**available (124–127).

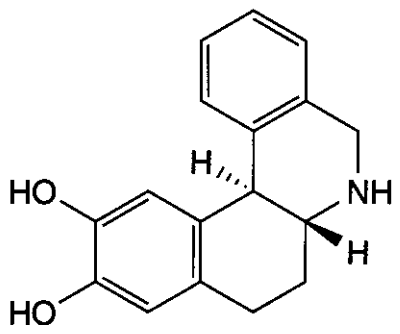
3.1.3 Experimental Dopamine D, Receptor Agonists. The opposing actions of the direct and indirect pathways in the basal ganglia (see Fig. 12.1) suggest that coordinated movement requires neurotransmission to be activated in



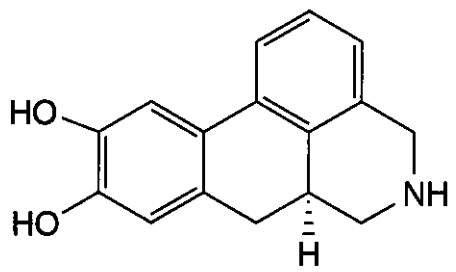
(**23**) CY-208-243



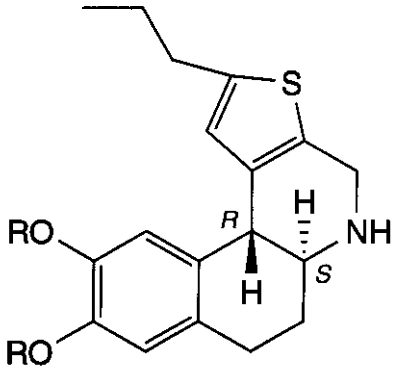
(**24**) **X** = **R** = H: SKF-38393
(**25**) X = CI, **R** = H: SKF-81297
(**26**) X = Br, R = $CH_2CH{=}CH_2$: Br-APB
(**27**) **X** = Cl, **R** = $CH_2CH{=}CH_2$: SKF-82958, CI-APB



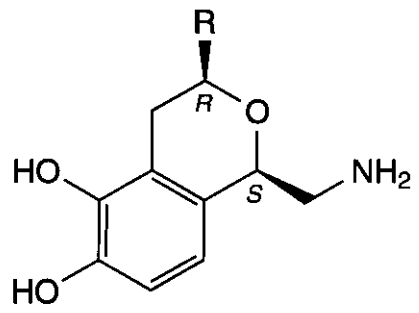
Dihydrexidine
(**28**)



Dinapsoline
(**29**)



(**30a**) R = H: A-86929
(**30b**) R = CH_3CO: ABT-431



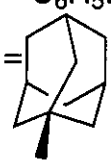
(**31**) R = C_6H_5: A-68930
(**32**) R =  : A-77636

Figure 12.6. Representative experimental D_1 agonists. These agents have unproved or untested clinical utility in the treatment of Parkinson's disease.

the direct pathway and attenuated in the indirect pathway. DA in the striatum may achieve such neuromodulation by stirnulatory actions at D_1-type receptors and inhibitory actions at D_2-type receptors. Consistent with a role for the D_1 receptor as a regulator of the direct output pathway, stimulation of D_1 receptors represents a plausible pharmacotherapeutic approach in PD. Initial clinical trials with the first selective D_1 partial agonists such as the benzergoline CY-208-243 (23; Fig. 12.6) and the phenylbenzazepine SKF-38393 (24) showed that these drugs were either short-acting or lacking in efficacy (11,128, 129). Other phenylbenzazepines are also short-acting D_1 partial agonists (**25–27**). It was later hypothesized that adequate testing of D_1 agonist utility in PD required a full, not a partial, agonist.

The benzophenanthridine dihydrexidine (28) was the first full-efficacy D_1 agonist to be developed, though it also has some D_2-type activity (130, 131). In MPTP-lesioned monkeys, dihydrexidine essentially eliminates all parkinsonian signs, and this effect was fully blocked by the D_1 antagonist SCH-23390 but not by the D, antagonist remoxipride, consistent with a D_1 mechanism of action (132). Moreover, continuous administration of dihydrexidine to rats for 2 weeks produced minimal or no changes in either D_1 receptor density or D_1 receptor-mediated DA-stimulated adenylyl cyclase activity, suggesting that tolerance should not develop to its antiparkinson effects. In PD patients, however, dihydrexidine has a narrow therapeutic index and dose-limiting adverse effects including flushing, hypotension, and tachycardia after single intravenous doses (133).

The D_1 receptor pharmacophore model developed for dihydrexidine subsequently was used to design novel molecular structures as full-efficacy D_1 agonists (134). One such compound is dinapsoline (29) (135). Numerous other analogs have since been produced with structural elements of both dihydrexidine and dinapsoline (29). For example, the dihydrexidine isostere A-86929 (**30a**) and its diacetyl prodrug ABT-431 (**30b**) are full D_1 agonists with sustained antiparkinson effects in MPTP-lesioned monkeys (136). In patients with PD, ABT-431 was highly effective against bradykinesia, but produced dyskinesias (137).

Several full-efficacy *R*-(+)-phenylbenzazepine D_1 agonists have been developed based on SKF-38393 (**24**), including SKF-81297 (25; Fig. 12.6) and its 6-halo derivatives, 6-Br-APB (26) and 6-CI-APB (SKF-82958, 27) (138, 139). In MPTP-lesioned monkeys, 6-Cl-APB (27) produced antiparkinson effects (140), but its utility was limited by very brief action (<1 h; 141) and severe adverse effects (142). Moreover, SKI?-81297 (25) and dihydrexidine (28) (141) showed beneficial results only in monkeys with severe parkinsonism, supporting the suggestion that D_1 agonists may be most useful in late stages of PD, if tolerable agents with prolonged action can be developed (143).

Several isochromans are also full D_1 agonists. The first compound in this series to be tested, A-68930 (31; Fig. 12.6), produced seizures, but an analog, A-77636 (**32**), showed antiparkinson effects without inducing seizures in MPTP-treated marmosets (144). However, A-77636 showed rapid desensitization to its beneficial effects (145), possibly related to its prolonged action (>20 h) (142).

3.1.4 Structural Modeling for Dopamine Agonists. Rational molecular design of DA agonists for treating PD is limited by a lack of validated three-dimensional models of interactions of DA with critical amino acid residues at DA binding sites within DA receptor peptide chains. Moreover, structural models that would differentiate the known DA D_1-type (D_1, D_5) or D_2-type (D,, D_3, D_4) receptors are also lacking, and the physiology of the low-abundance subtypes, including D_3, D,, and D_5 receptors, remains highly tentative, particularly at the level of motor control (15). Development of selective DA receptor agents continues to be guided by quantitative structure-activity relationships (QSAR) based on lead molecules, discovery of which in turn remains highly empirical, based largely on affinities to receptor subtypes in brain tissue or expressed in genetically transfected cultured cells, all labeled with receptor-selective radioligands (15, 146).

Because the side-chain of DA possesses unlimited flexibility and unrestricted rotation about the β-carbon-phenyl bond, little information can be obtained concerning conformational requirements for activation of DA receptors using the endogenous ligand itself. Accordingly, compounds in which the catechol ring and the aminoethyl side-chain of DA are held in rigid conformation have been synthesized. Several groups of such heterocyclic agents have contributed important insights into SAR requirements of DA agonists. Notably, aporphines, aminotetrahydronaphthalenes (aminotetralins), and aminobenzoquinolines have yielded D_2-like agonists, and the phenylbenzazepines and isochromans have provided D_1-like agents (146, 147).

Structural models of the DA-agonist *R*-(−)-enantiomers of apomorphine (20) and its more potent congener, N-n-propylnorapomorphine (21; Fig. 12.5) have been proposed, based on their X-ray crystal structure (148). Such anal-

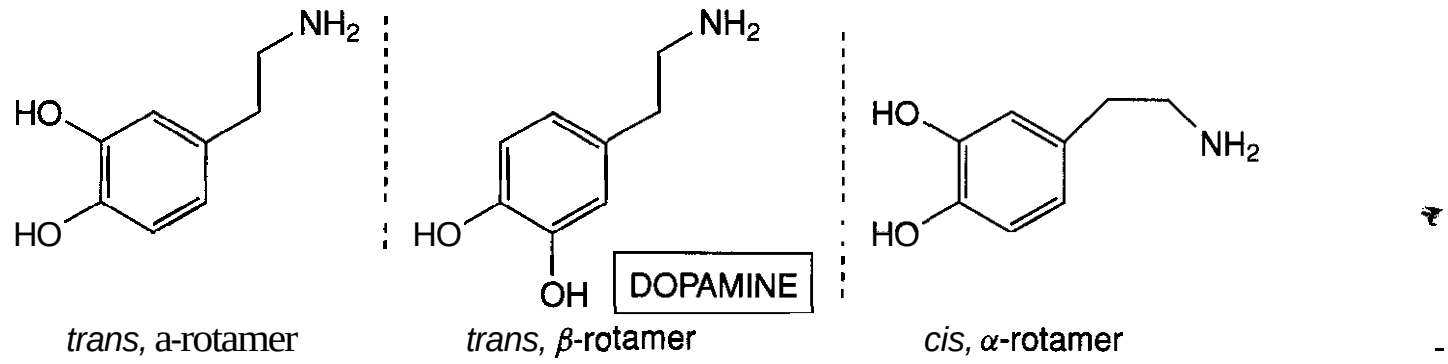


Figure 12.7. Rotameric forms of dopamine, a flexible β-phenethylamine.

yses show that these tetracyclics contain molecular features in common with DA in its *trans*-α-rotamer conformation (Fig. 12.7).

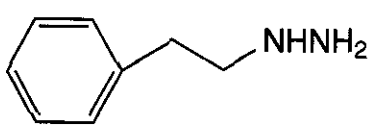
Experimentation with rigid dopaminomimetic agents strongly suggests that the preferred conformation of D_2-like DA agonists models DA in its extended *trans*-α-conformation. The *trans*-α-rotameric conformation of DA is also likely to be an important determinant of agonist activation of D_1-type receptors, based largely on consideration of a series of phenylbenzazepines that also contain the elements of DA within a rigid heterocyclicsystem (149) (Fig. 12.6).

Computational chemistry methods such as comparative molecular field analysis (CoMFA) also have been applied to elucidate quantitative three-dimensional structure-activity requirements (3D-QSAR) for activation of DA receptors. One such analysis considered 16 structurally diverse, prototypical template D_1 and D_2 DA agonists (150). Interactions of agonists with DA receptors were best described by a pharmacophore consisting of one protonated nitrogen (at physiological pH) and at least one electronegative center able to participate in hydrogen bonding (e.g., catechol, critically positioned hydroxy, or equivalent electronegative moieties). The pharmacophore maps for D_1- and D_2-type receptors differed primarily in requiring a higher position of the amino-nitrogen atom above the plane of the electronegative hydrogen bonding group D_1 receptor activity. These analyses indicate that the cationic nitrogen moiety is more important than the rotameric conformation of the electronegative hydroxy or other hydrogen-bonding groups in determining pharmacophore selectivity for D_1 and D_2 receptors.

3.1.5 Dopamine-Potentiating Agents. Several agents have been used in the treatment of PD that limit the metabolism of catecholamines. They include the MAO inhibitor selegiline (37) and several inhibitors of COMT. Because active neuronal transport ("reuptake") mediated by the DA transporter is the principal means of inactivating DA in the synaptic region (151), it is also plausible to expect that DA transport-inhibiting agents might also potentiate DA available in remaining dopaminergic neurons in early PD, and in potentiating DA produced from L-dopa. Although stimulant agents have some beneficial effects in mild PD, they are rarely used currently and evidently have not been evaluated as a means of potentiating L-dopa (114).

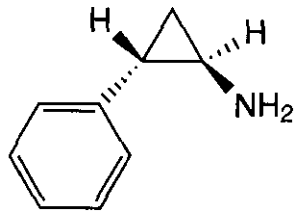
3.1.5.1 ***Monoamine Oxidase Inhibitors.*** Among MAO inhibitors, those selective for the MAO-A in monoaminergic nerve terminals might be expected to potentiate DA. These include the long-acting, irreversible, nonselective MAO-A/B inhibitors phenelzine (Nardil; 33; Fig. 12.8) and tranylcypromine (Parnate; 34) (136,152). There are also short-acting, reversible inhibitors selective for MAO-A, which include the antidepressant moclobemide (Manerix, 35 and the experimental agent brofaromine 36; Fig. 12.8) (153). Presumably because of their short and reversible actions, they have less risk of inducing hypertensive crises when combined with indirect sympathomimetic amines that release endogenous catecholamines, though they have some risk of inducing cerebral intoxication when given with serotonin-potentiating agents including serotonin (5-hyroxytryptamine) reuptake inhibitor antidepressants, meperidine, and other agents (136,152). Combinations of long-

MAO inhibitors



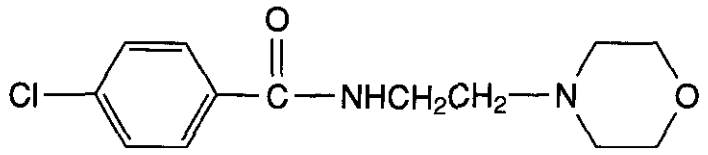
Phenelzine (Nardil)

(33)



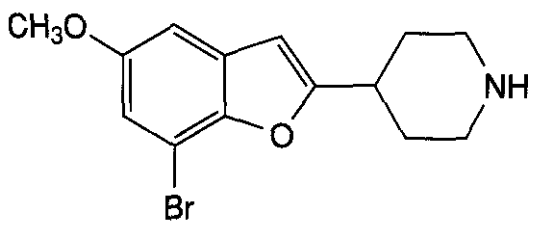
Tranylcypromine (Parnate)

(34)



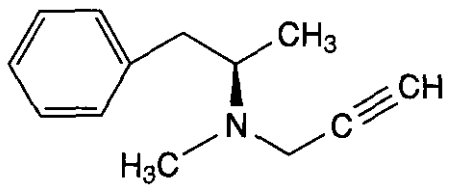
Moclobemide (Manerix)

(35)



Brofaromine

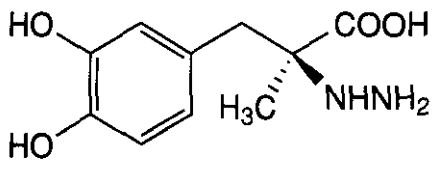
(36)



Selegiline (R(–)-Deprenyl, Eldepryl)

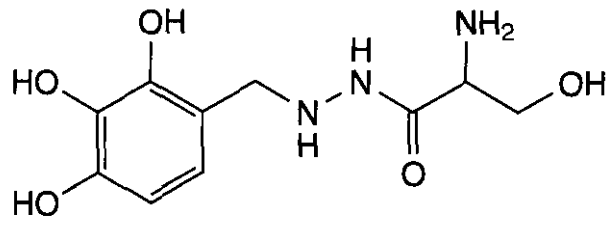
(37)

Aromatic amino acid decarboxylase inhibitors (peripheral)



L(–)-Carbidopa(Lodosyn)

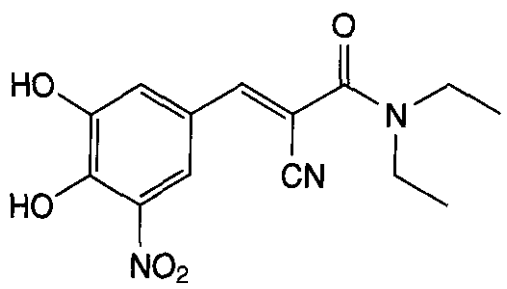
(9)



Benserazide

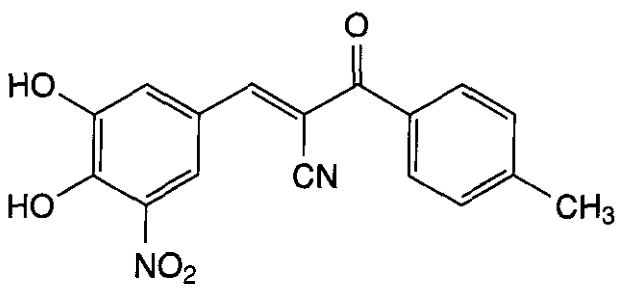
(10)

COMT inhibitors



Entacapone (Comtan)

(38)



Rolcapone (Tasmar)

(39)

Figure 12.8. Dopamine potentiating or protective agents. These include MAO inhibitors (only selegiline is used clinically to treat PD), inhibitors of the peripheral decarboxylation of L-dopa, and inhibitors of the 0-methylation of dopamine and L-dopa.

acting MAO-A/B inhibitors like phenelzine and tranylcypromine with L-dopa are contraindicated because of the risk of inducing hypertensive crises and delirium (20).

Selegiline hydrochloride (Eldepryl; L-deprenyl; *R*-(−)-*N*,2-dimethyl-*N*-2-propynylphenethylamine, 37; Fig. 12.8), at low doses (a10 mg/day), is a selective inhibitor of mainly nonneuronal MAO-B, which oxidizes MPTP to neurotoxic by-products (see Fig. 12.3). Probably by protecting DA, selegiline can increase the potency and duration of action of L-dopa, and do so safely provided that doses are kept low (153, 154). However, at doses above 20 mg/day, selegiline has an inhibitory effect on both MAO-A and MAO-B. In addition, it can be converted to methamphetamine or similar metabolic by-products, inhibit neu-

ronal transport of monoamines, and release DA (153). In this way, selegiline resembles traditional stimulants that release DA and other monoamines (114). It had also been hoped that use of an MAO-B inhibitor like selegiline might prevent formation of neurotoxic oxidation products of DA or perhaps MPTP-like compounds and slow the progressive neurodegeneration in PD, but evidence for such a neuroprotective effect of this agent or of antioxidants including vitamin E is lacking, and selegiline has only limited clinical benefits in early or mild PD (75, 76, 155).

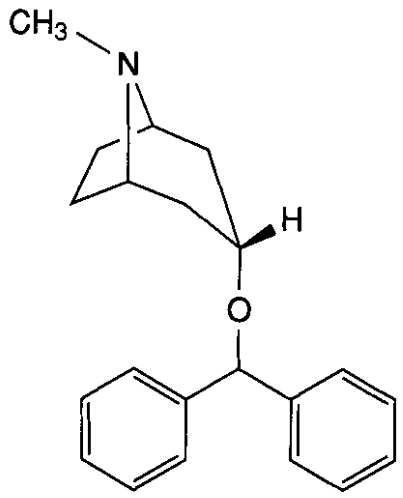
3.1.5.2 *Catechol-O-Methyltransferase* Inhibitors. The peripheral metabolism of L-dopa given alone leads to very limited access of the amino acid to the CNS. It is rapidly decarboxylated by L-aromatic amino acid decarboxylase and 3-*O*-methylated by COMT. In addition to potentiating L-dopa with peripheral decarboxylase inhibitors including carbidopa and benserazide, there are also inhibitors of COMT. This methyl transferase, with its methyl-donor cofactor S-adenosyl-L-methionine (SAMe), converts L-dopa and catecholamines preferentially to their m-methoxy derivatives (see Fig. 12.4). These include 3-0-methyl-dopa and 3-0-methyldopamine (3-methoxytyramine, 3-methoxy-4-hydroxyphenethylamine), as well as the 3-*O*-methylated, deaminated compound homovanillic acid (HVA), the major metabolite of DA in humans. Treatment with L-dopa can reduce tissue concentrations of SAMe (87), with uncertain consequences that should be limited by cotreatment with a COMT-inhibitor. COMT acts in both periphery and CNS, though the use of COMT inhibitors to potentiate L-dopa is most effective in peripheral tissues (20, 140, 156–158).

COMT inhibitors in current clinical use include entacapone (Comtan; 38) and tolcapone (Tasmar; 39; Fig. 12.8). Although these drugs lack beneficial effects on PD themselves, they potentiate L-dopa and prolong its actions by inhibiting the metabolic inactivation of L-dopa and DA. Entacapone is more widely used, acts only in the periphery, and is relatively short-acting (−2 h). Tolcapone is longer acting, but has about 2% risk of elevating hepatic transaminases, and has been associated with several cases of hepatic failure, leading to its preferred use when other options are not effective, and with clinical monitoring of hepatic functioning (156–159). Other adverse effects of these agents include severe diarrhea and risk of dyskinesias and psychosis in PD patients when combined with L-dopa (160).

3.2 Agents Acting on Nondopaminergic Systems

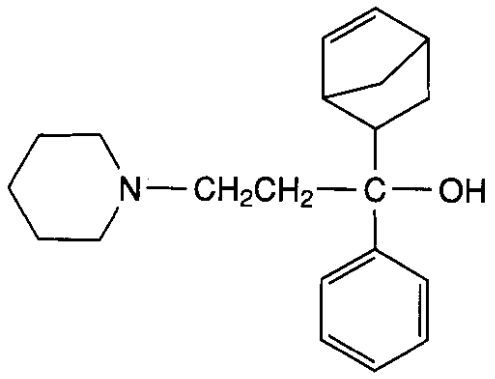
3.2.1 Anticholinergic Agents. Cholinergic interneurons in the striatum exert mainly excitatory effects on GABAergic output from the striatum (see Fig. 12.1). Drugs that increase cholinergic neurotransmission (e.g., the cholinesterase inhibitor physostigmine and the direct agonist carbachol) have long been known to aggravate parkinsonism in humans, whereas centrally active muscarinic antagonists (such as the belladonna alkaloids, including atropine), have moderately beneficial effects (20, 161–164). Accordingly, before the discovery of L-dopa, drug therapy for parkinsonism depended primarily on the limited efficacy of the natural belladonna alkaloids and newer synthetic antimuscarinic alkaloids, as well as antihistamines that also exert central antimuscarinic actions (Fig. 12.9). Synthetic central anticholinergic agents include benztropine mesylate (40; Cogentin and others), biperidin (41; Akineton), diphenhydramine (42; Benadryl and others), ethopropazine (43; Parsidol), orphenadrine (44; Disipal and others), procyclidine (45; Kemadrin), and trihexylphenidyl (46; Artane and others). Such drugs continue to be used to control parkinsonism and other adverse extrapyramidal neurological effects of the potent D_2-receptor antagonist neuroleptic antipsychotic agents, for which they are quite effective (20, 91).

In contrast, central antimuscarinic agents have limited therapeutic benefit in PD. They also exert a range of undesirable adverse effects because of their blockade of peripheral parasympathetic function. These include dry mouth, impaired visual accommodation, constipation, urinary retention, and tachycardia. Adverse CNS effects include delirium of varying severity, marked by confusion, memory impairment, and psychotic symptoms. Despite their relatively unfavorable benefit/risk ratio, these agents are still sometimes employed in



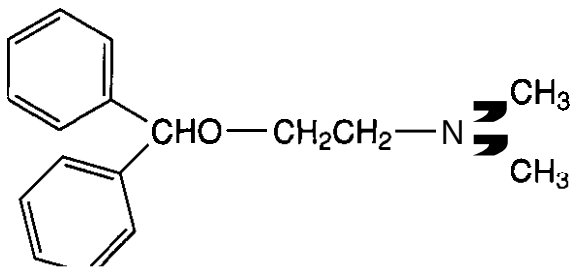
Benztropine mesylate (Cogentin)

(**40**)



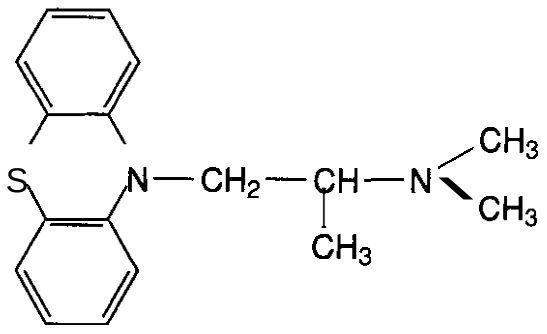
Biperiden (Akineton)

(**41**)



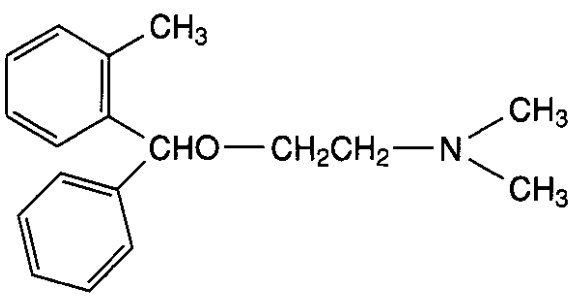
Diphenhydramine (Benadryl)

(**42**)



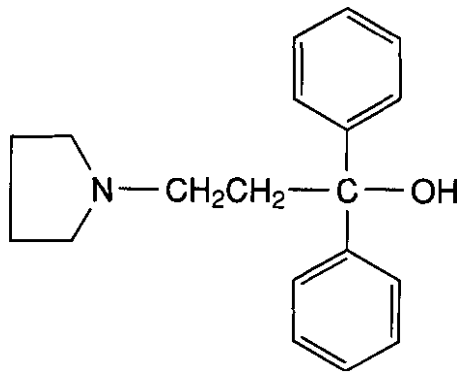
Ethopropazine (Parsidol)

(**43**)



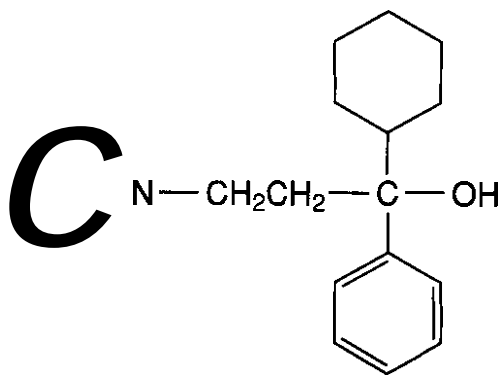
Orphenadrine (Disipal)

(**44**)



Procyclidine (Kemdrin)

(**45**)



Trihexyphenidyl (Artane)

(**46**)

Figure 12.9. Agents with central **antimuscarinic** activity sometimes used to treat idiopathic or **neuro**leptic drug-induced parkinsonism.

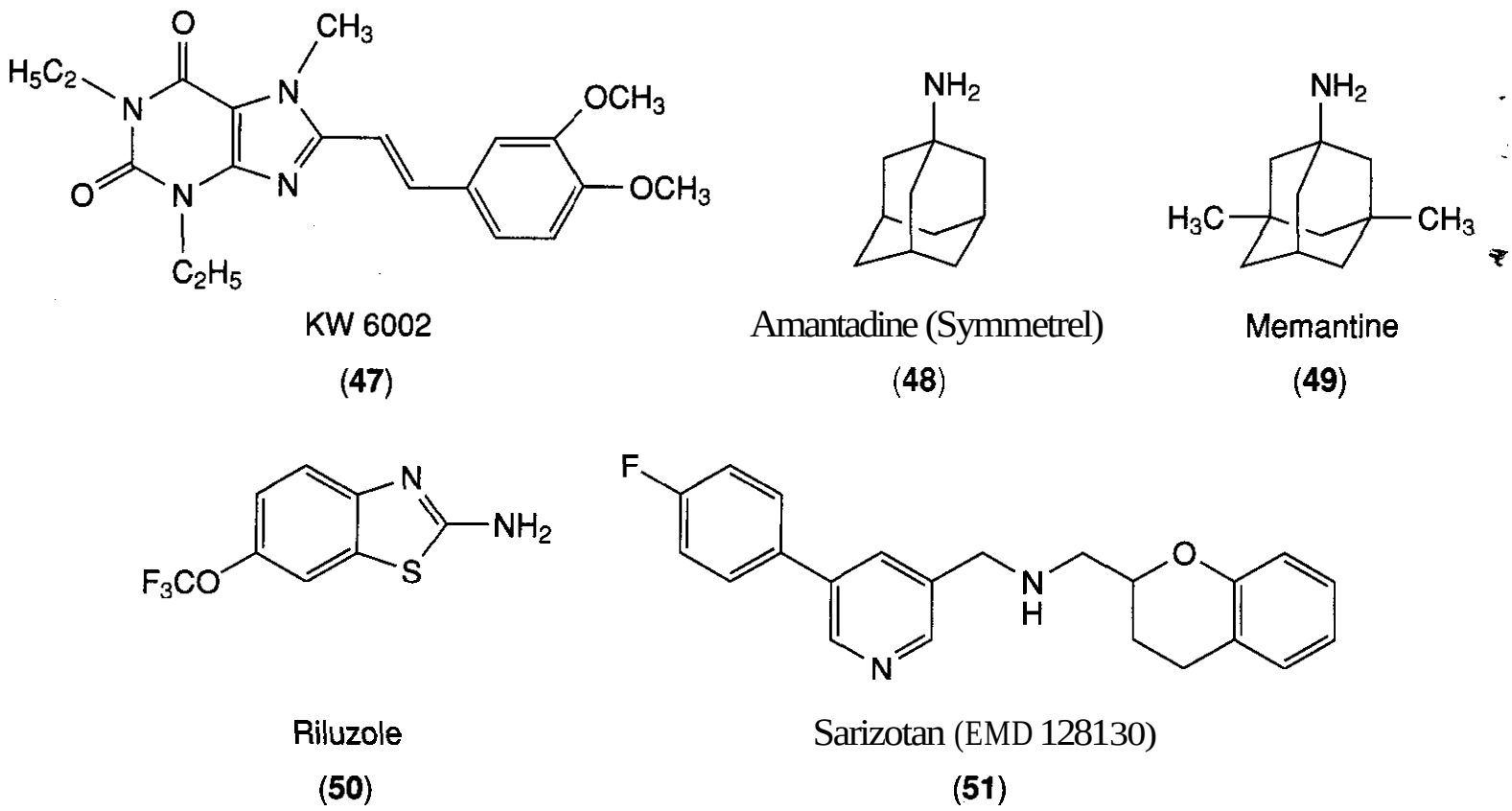


Figure 12.10. Agents acting on nondopaminergic systems. These include agents that act on adenosine, glutamate, and serotonin systems.

the treatment of PD in combination with L-dopa, particularly to help control tremor (163, 164).

3.2.2 Adenosine Antagonists. KW 6002 (46; Fig. 12.10) is a potent and selective antagonist at adenosine A_{2A} receptors (165, 166) currently in clinical trials for treatment of PD after improving motor disability in primate models of PD (167, 168). The adenosine A_2 receptor is one of four cloned adenosine receptors that are members of the seven-transmembrane, G-protein-coupled receptor superfamily. A_{2A} receptor mRNA is highly concentrated in the striatum, nucleus accumbens, and olfactory tubercle, and colocalizes with D_2 receptor mRNA in these brain regions (169). Activation of A_{2A} receptors inhibits GABA release in striatum and reduces GABA-mediated inhibition of striatal medium spiny output neurons (170). Thus, antagonism of A_{2A} receptors is expected to increase GABA-mediated inhibition of the medium spiny output neurons to help compensate for the loss of DA D_1 receptor-stimulated GABA release and D_2 receptor-mediated inhibition of these neurons in PD (171) (see Fig. 12.1). Adenosine A_2 receptors also oppose the actions of D_1 and D_2 receptors on gene expression (172) and second-messenger systems (173) and reduce the binding affinity of DA for D_2 receptors (173). An A_{2A} antagonist presumably would block these A_{2A} receptor-mediated inhibitory effects on DA neurotransmission and perhaps provide benefit in PD. Activation of A_{2A} receptors also stimulates release of acetylcholine in striatum (174). Because muscarinic acetylcholine receptor antagonists can ameliorate some signs of PD, A_{2A} receptor antagonists may exert additional benefits in PD by reducing striatal cholinergic neurotransmission (171).

3.2.3 Glutamate Antagonists. Amantadine (Symadine, Symmetrel, 48; Fig. 12.10) is an adamantane with an unusual cagelike structure, originally developed as an antiviral agent. Amantadine, as a primary amine with a pK_a of 10.8, is mainly in the protonated form at physiological pH, but it can enter the brain because of its unusual structure that not only increases its lipophilicity, but also prevents its catabolism by oxidative enzymes so that most of it is excreted in the urine unchanged. Amantadine has some ability to release DA and norepinephrine from intraneuronal storage sites and block reuptake of DA, and was initially considered a DA-potentiating agent for use in mild PD (172, 173, 175–177). How-

ever, in addition, amantadine and its congener, memantine (1-amino-3,5-dimethyladamantane, 49; Fig. 12.10) have some activity as NMDA (and possibly AMPA) glutamate receptor antagonists that might also provide neuroprotective effects (178). Both have been used to treat PD (20,178). Amantadine has moderately beneficial effects early in PD, can enhance the effects of L-dopa, and perhaps limit the severity of dyskinesias induced by L-dopa therapy (179). Also, memantine has been used as a spasmolytic agent in the treatment of both PD and dementia (180). The potential that such agents might afford long-term neuroprotective effects that might include slowing the progression of PD remains speculative.

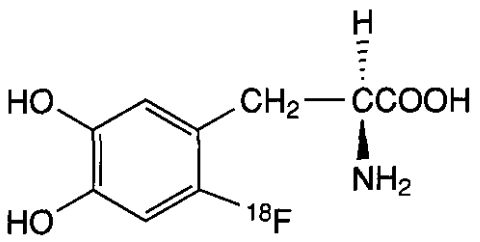
Riluzole is a benzothiazolamine (50) with sodium channel blocking activity that interferes with glutamatergic neurotransmission by blocking glutamate release in the subthalamic nucleus, an area of increased neuronal activity in PD (181). It is currently given to patients with amyotrophic lateral sclerosis (ALS) in an attempt to slow the progression of this degenerative disorder of motor neurons of the spinal anterior horn by inhibiting glutamate neurotoxicity (182). In the MPTP-lesioned monkey model of PD, riluzole delayed appearance of parkinsonian motor abnormalities by a mechanism not involving decreased formation of MPP^+, the neurotoxic metabolite of MPTP (183,184). Recently, riluzole was assessed for safety, tolerability, and efficacy in treatment of PD patients who had L-dopa–induced dyskinesias (185). Riluzole was effective in lessening dyskinesias in these patients, was well tolerated, did not interfere with L-dopa, and is undergoing further trials in PD.

3.2.4 Serotonin Agonists. Dysfunction of neurotransmission mediated by 5-hydroxytryptamine (5-HT; serotonin) occurs in the basal ganglia of patients with PD, and excessive serotonergic transmission may contribute to dyskinesias associated with dopaminergic treatments (186). 5-HT_{1A} receptors are expressed presynaptically on 5-HT terminals, where they limit serotonin release as autoreceptors (187). Their activation should decrease 5-HT release and perhaps alleviate dopaminergic dyskinesias in PD (188). Because 5-HT_{1A} receptor stimulation can reverse parkinsonism-like catalepsy induced by haloperidol (189), 5-HT_{1A} receptor activation might also counteract losses of nigrostriatal DA neurotransmission in PD (188). Moreover, in patients with advanced PD, intact striatal 5-HT terminals are an important site of decarboxylation of exogenous L-dopa to DA (190). A 5-HT_{1A} agonist might act at striatal serotonergic terminals to limit release of DA produced by L-dopa treatment and released from 5-HT terminals as a "false transmitter" (85).

Sarizotan (EMD-128130, 51; Fig. 12.10) is an arninomethylchroman derivative with potent central 5-HT_{1A} agonist activity. Given by itself to MPTP-lesioned monkeys, sarizotan had no effect on the severity of motor deficits or on beneficial responses to L-dopa, but it reduced L-dopa-induced choreiform dyskinesias by more than 90% (187). It is in clinical trials for the treatment of PD and of dyskinesias resulting from L-dopa therapy. The lack of interaction of sarizotan with L-dopa in MPTP-lesioned monkeys (187) is unsupportive of concern that it might limit release of DA from 5-HT terminals. Although sarizotan also has weak DA D_2 receptor affinity (10 times less than its 5-HT_{1A} affinity), its beneficial effects in parkinsonian monkeys seem specific to 5-HT_{1A} agonism, in that they were reversed by a selective 5-HT_{1A} antagonist (187).

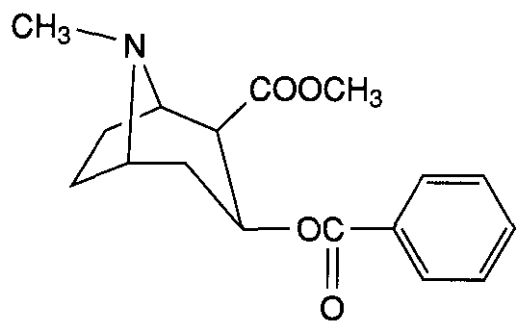
4 DIAGNOSTIC AGENTS FOR PARKINSON'S DISEASE

Even for an experienced clinical neurologist, diagnosis of PD can be difficult to confirm, especially in the early stages of this disease. Signs of PD vary markedly among patients and in the same person over time; disability can fluctuate dramatically, and progression of the disorder is unpredictable. In addition, a number of conditions mimic PD disease, and vary in their responses to antiparkinson drugs (191). Given these difficulties, brain imaging techniques are increasingly applied to both diagnostic and neuropharmacological studies of brain function in PD patients. Positron emission tomography (PET) and single photon emission computed tomography (SPECT) are sensitive methods employed in such studies.



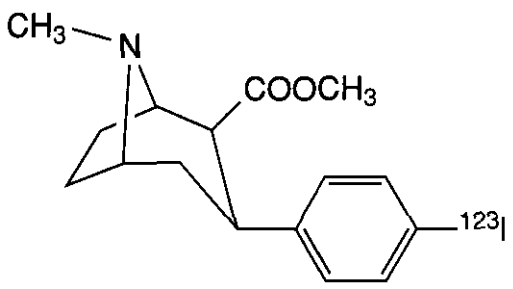
(^{18}F) L-6-fluoro-DOPA

(52)



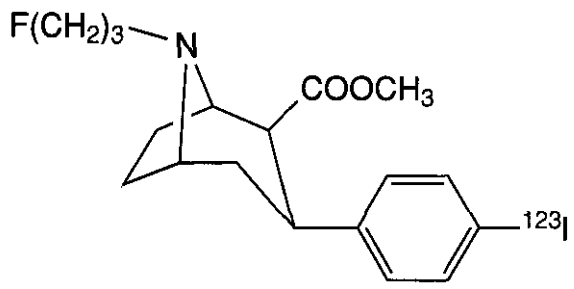
Cocaine

(53)



(^{123}I)-β-CIT
DOPAScan

(54)



(^{123}I)-FP-CIT (loflupane)
DATScan

(55)

Figure 12.11. Radioligands for dopamine neurons. These include [^{18}F]-labeled dopa and nonhydrolyzable, long-acting phenyltropane analogs of cocaine, which bind selectively to the dopamine transporter proteins and are highly specific markers of dopamine neurons. These agents are useful for imaging with [^{18}F] for PET and [^{123}I] for SPECT.

Spatial resolution is greater with PET, but SPECT technology is less expensive and more widely accessible in many clinical settings. In addition, positron-emitting nuclides used in PET imaging have very short half-lives (^{11}C, 20 min; ^{18}F, 109 min) and usually require an on-site cyclotron for their production. SPECT nuclides have longer half-lives (^{123}I, 13 h; ^{99m}Tc, 6 h), often can be supplied commercially, and [^{99m}Tc]-labeled radioligands can be prepared locally as needed. Specifically, quantitative assessment of nigrostriatal presynaptic DA nerve terminal function by PET using [^{18}F]-labeled L-6-fluorodopa ([^{18}F]dopa, 52; Fig. 12.11) has proved useful for the early diagnosis of PD (192).

Additional radioligands have recently been developed for probing the DA transporter proteins that are highly characteristic gene products of DA neurons and nerve terminals in the basal ganglia. Cocaine (53; Fig. 12.11) binds to the DA transporter (DAT) and other monoamine transporters, but radiolabeled cocaine is rapidly hydrolyzed at its benzoyl ester function, making it an impractical candidate for use in imaging (193). However, linking the phenyl ring of cocaine directly to the tropane system yields nonhydrolyzable, long-acting phenyltropanes. Many such compounds have proved to be potent stimulants and some have high affinity and varying selectivity for DA transporters in the brain. Some compounds of this type have been prepared as clinically useful radiopharmaceuticals (Fig. 12.11).

The first such agent was *p*-[^{123}I]phenyl-labeled 2-β-carbomethoxy-3β-(4-iodophenyl)-tropane (54 [^{123}I]β-CIT or RTI-55; DopaScan), although this agent requires about 8 h for peak uptake before imaging, thus limiting its practicability (77, 191, 194). However, the radioiodinated N-3-fluoropropyl analog of β-CIT [N-3-fluoropropyl-2-P-carbomethoxy-3β-(4-iodophenyl)-tropane, (**55**), [^{123}I]FP-CIT or [^{123}I]ioflupane (DATScan)] has the advantage of more favorable kinetics for clinical use because patients can be imaged 1–2 h after

injection of the radioligand (195). Moreover, a radioligand suitable for PET imaging is obtained by replacing the fluorine atom with [^{18}F] in FP-CIT (196). [^{123}I]-FP-CIT is also being studied in patients with PD and other neuropsychiatric disorders, and is commercially available for clinical application.

5 FUTURE DIRECTIONS

Research related to PD has been directed toward developing more effective and better-tolerated new treatments, largely guided by the central role of DA in the pathophysiology of the disorder. However, recent research has increasingly included efforts to clarify the pathophysiology of PD and to define its primary causes, which remain obscure. Research related to PD has recently emphasized pathogenesis. This trend is increasing understanding of the mechanisms of cell death, including apparently genetically programmed death (apoptosis)of catecholaminergic cells with advancing age (197), the role of environmental toxins, and the identification of specific genetic correlates, if not causes, of PD.

Guided by improved understanding of the neuroanatomy and neuropathophysiology of PD, important advances in therapy have been developed, with variable success to date. Innovative methods include application of deep brain stimulation, a neurosurgical treatment of PD (198), and neuronal transplantation. The first double-blind controlled trial of neuronal grafting in PD patients was reported in 2001 (199). Although the results were disappointing, alternative sources of donor tissue (such as stem cells) are being tested in laboratory animals (200) as candidates for clinical trials.

Increasing use of clinical neuroimaging with PET, SPECT, and functional MRI techniques using brain-imaging agents to detect losses of DA neurons in PD *in vivo* is likely to aid early diagnosis and enable monitoring of the progression of the disease. It also encourages development of novel treatments aimed at slowing the progression of PD.

Finally, the search for improved medicinal agents for the treatment of PD will continue, greatly stimulated by the broadening range of leads discussed above, and by gradual progress toward a better molecular understanding of the primary pathoetiology of the disease. In all of these efforts, medicinal chemists working in collaboration with basic and clinical biomedical research colleagues will continue to play a central role.

6 ACKNOWLEDGMENTS

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CHAPTER THIRTEEN

Alzheimer's Disease: Search for Therapeutics

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Contents

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1 INTRODUCTION

Alois Alzheimer (1864–1915) was the first to describe the specific unique pattern of neuropathologic changes in the brain of a victim of what we now call Alzheimer's disease (AD) (1). Emil Kraepelin (1855–1926) named the disease after him but to this day the precise definition of AD, the most common (50–70%) form of dementia in the elderly, is far from complete. Clinically, AD overlaps with many other conditions leading to dementia, advanced age in particular, making accurate diagnosis difficult if not impossible (2–5). Even at necropsy, macroscopic examination of AD brains again shows an extensive overlap with changes encountered in normal aging (6). Thus, at present, AD can be diagnosed positively only at necropsy on microscopic examination using silver impregnation stains, amyloidophilic dyes, or antibodies for visualization (6). Characteristic, but not unique to AD, is the observed presence and abundance of (1) so-called intracellular neurofibrillary tangles (NFT) and *(2)* extracellular amyloid in the form of meningeal vascular amyloid, cortical microvascular amyloid, and senile (neuritic) plaques (SP) in the affected parts of dissected brain. At present, SP, extracellular spherical abnormalities of about 0.2 mm diameter consisting of a core of amyloid β-peptides (Aβ) of various densities surrounded by dystrophic neurites, are at the center of AD research. The highest accumulation of the NFTs and SPs is observed in the hippocampus and the associative regions of the cortex and the formation of the latter precedes the formation of the former. The concentration of Aβ was shown by Greengard et al. to correlate with the severity of cognitive decline (7).

Earlier, six stages of AD propagation were proposed based on severity of NFT pathology (8, 9). The initial involvement of transentorhinal subdivision of the hippocampal cortex represents stages I and II (clinically silent cases). The disease propagation to limbic and neocortical sites represents stages III and IV (incipient AD) and V and VI (fully developed AD). A longitudinal MRI study of presymptomatic representatives of families with known autosomal dominant mutations leading to AD illustrated in real time the points of origin and the progress of the disease (10). By the use of voxel compression mapping of serial MRI images over the period of 5–8 years, the study reveals progressive atrophy in presymptomatic individuals with posterior cingulate and neocortical temporoparietal cortical losses, and medial temporal-lobe atrophy. A shorter 3-year longitudinal study of normal elderly has shown the predictive value of MRI guided 2-[^{18}F]fluoro-2-deoxy-D-glucose/positron-emission tomography (11). In that study the baseline metabolic reduction in the entorhinal cortex predicted future longitudinal memory and temporal neocortex metabolic reductions. Patients who eventually declined into a mild cognitive impairment (MCI) and AD showed memory impairement and hypometabolism in temporal neocortex and hippocampus.

It is established from early studies that in patients with diagnosed AD the widespread atrophy includes the primary motor and sensory cortices and cerebellum. The cholinergic innervation in these parts of brain is disrupted; choline acetyl transferase, high affinity nicotinic acetylcholine receptor (nAChR) binding, and choline transporter sites' decreased (12–15). The observed deposition of fibrils of the misfolded into β-sheet Aβ in the SP places AD in the group of over 20 clinically defined amyloidoses. The short-term memory impairment represents the earliest observable symptom of the disease. Further impairment of memory interferes with the determination of visuospatial orientation and causes severe mood alterations and depression combined with language disturbance, and loss of both judgment and reasoning ability (16, 17). The

average duration of AD is 6–10 years, and intercurrent infection is the most common cause of death (6).

2 EPIDEMIOLOGY

Given the available demographics, AD should be one of the most thoroughly researched diseases. Regretfully, this is not the case. An author may be tempted to paraphrase Charles Maurice de Talleyrand-Périgord (1754–1838): "You will kindly observe, ladies and gentlemen, that I neither condemn nor defend, I merely narrate." According to Brookmeyer et al. (18, 19) the estimated prevalence of AD in the United States in 1999 was 2.44 million with the range of 1.15 to 4.78 million. Over the next 50 years the incidence of AD is expected to triple from about 420,000 new cases in 1999 to 1.32 million per year. Assuming no successful intervention, the prevalence of AD could be expected to rise over the next 50 years by a factor of about 3.7 to 8.94 million with the range of 4.55 to 15.81 million in the United States alone. With the exception of early-onset familial AD (FAD) the disease is age correlated and its incidence grows exponentially with age. In part, because of their increased longevity (294), women represent and will continue to represent the majority (68% in 1997) of the affected population. If one adds the year 2000 U.S. census figures to the population of the European Union (EU-15) and Japan, the prevalence of AD in "the major pharmaceutical markets" for that year could be estimated as close to 8.7 million (20).

3 ETIOLOGY

Although a few genetic determinants and a number of genetic and nongenetic (called interchangeably medical, metabolic, lifestyle, environmental, etc.) risk factors are recognized, or suspected as causative, the etiology of AD remains unclear. Only a small percentage of AD cases can be segregated within families as early-onset (<60 years of age) or late-onset (>60 years of age) FAD representing the autosomal dominant mutation cases of high penetrance and therefore considered a genetic disease. The prevailing rest, lacking the clear genetic etiology, reflecting perhaps the effects of several genes, is classified as sporadic or late-onset AD (SAD or LOAD). When the age at clinical onset is considered, over 90% of AD develops after the age of 65 (21). By definition, in all FAD, LOAD, and SAD groups the pathological changes observed postmortem are associated with the formation of SP and NFT in the affected neurons (22). That association lends credence to Kakizuka's (23) hypothesis of protein precipitation as the common etiology of neurodegenerative disorders.

3.1 Genetic Determinants

At present it has been postulated that between 30–50 and 95% of the population risk for development of AD may be attributed to genetic factors (21, 24). However, when the autosomal dominant mutation cases of high penetrance are considered, that percentage drops to less than 5% (21). The most comprehensive and current lists of all mutations that may be considered as AD genetic determinants or risk factors are maintained by Online Mendelian Inheritance in Man (OMIM) (25) and Alzheimer Disease Mutation Database (ADMB) (26).

3.1.1 Presenilins 1 and 2. Mutations in the genes coding for the membrane proteins presenilin 1 (PS1) and presenilin 2 (PS2) are associated with the early-onset FAD (27). Mutations in the PS1 gene on chromosome 14 are responsible for 30–50% of early-onset FAD and AD with the onset before the age of 55 years. Some mutations in the PS2 gene on chromosome 1 have also been observed but are not as highly penetrant as those of PS1. It appears that intracellular binding of the amyloid precursor protein (APP) to either PS1 or PS2 leading to cell-cell adhesion (28) represents a part of their normal function. The presenilins and their mutated forms participate in APP processing leading to production, among others, of 4-kDa β-amyloid peptide (Aβ) of varied, predominantly 40–42 (39–43 overall) amino acid (aa) lengths (29). It was postulated that the PS1 mutations represent a "gain-in-function" effect and contribute to or are responsible for an aberrant increase in production of amyloidogenic (having greater tendency to misfold into a β-sheet and to form amyloid fibrils aggregating into amyloid) Aβ of

42-aa length (30). PS1 and PS2 may also affect other intracellular pathways linked to cell death (27). At present at least 35 to 50 different, mostly missense, and two splicing defect mutations in PS1 and two in PS2 have been identified in AD patients (21, 31). The PS1, PS2, and APP mutations linked to FAD were reported to increase secretion of Aβ (299).

3.1.2 Amyloid Precursor Protein (APP). Mutations in localized on the chromosome 21 gene for APP have also been connected to a limited number of early-onset FAD cases (32). At least 16 homologous amyloid-like proteins (APLP) and APP species have been isolated and characterized (32). From the identified major APP isoforms of 695, 751, and 770 aa, the 695-aa form is expressed preferentially in the neuronal tissue. Alternative splicing of APP provides a total of eight isoforms, with lengths of 677, 695, 696, 714, 733, 751, 752, and 770 amino acids (33). Seventeen single-aa and one two-aa (Swedish $APP_{K670N/M671L}$) mutations of APP have been identified so far (25). APP, a type 1 cell surface glycoprotein, is produced in many cells and processed through the secretory or endosomal-lysosomal pathways (34). Processing of APP is carried out by the proteolytic enzymes named secretases. When the cleavage takes place in the presence of distinct PS1 or PS2 mutations regulating the y-secretases cleavage sites, it may lead to an increase in the production of amyloidogenic Aβ42 (Aβ1–42). Under "normal" conditions, in the brains of nondemented elderly individuals, Aβ42 is actually the ubiquitous form (37). Although Aβ42 is considered "amyloidogenic," one should keep in mind that it is Aβ42 that is a major component of the SPs that are of diffused "preamyloid" nature and present in the old but otherwise AD-free individuals (296). It is the Aβ40 that dominates in the more compact, dense, "mature" SP, considered by some to be of greater inflammatory potential at a 1:10 ratio of Aβ42/Aβ40 (35–37). The diffuse, "preamyloid" SPs that can be observed throughout the brains of normal, aged individuals are therefore considered an age-related phenomenon (6). The question of why, when, and how the diffuse SPs mature into the dense AD-characteristic SPs remains unanswered. The normal function of intact APP in cells appears to involve the static cell-substrate adhesion and/or neurite outgrowth (38), synaptogenesis, synaptic plasticity, and promotion of neuronal cell survival (39). APP and an APP-binding protein FE65 are involved in the regulation of cell movement (40). Exposure of cortical neurons to monoclonal antibody, which binds to the extracellular domain of APP (human, rat, or mouse), leads to neurite degeneration followed by caspase-dependent apoptosis (41). One wonders whether the AD-associated neuronal cell death is attributable to aberrant processing of APP overproducing neurotoxic Aβ aggregates or to APP deprivation.

3.2 Genetic Risk Factors

3.2.1 Apolipoprotein E (APOE). Although the APP and PS1 mutations represent a clear autosomal-dominant Mandelian trait of high penetrance, their participation in all AD cases is minuscule (<5%). The case of PS2 remains far from being clarified but its participation is negligible. Nevertheless, the determined mutations provide, after expression in cell and animals, useful tools for *in vitro* and *in vivo* studies.

The polymorphism of the ApoE gene is considered a major, best-documented, genetic susceptibility risk factor for the late-onset AD (21). The last decade observed the emergence of ApoE as a dominating factor in aging-related diseases like cardiovascular disease (CVD) or dementia and longevity in general (42). Besides being synthesized in liver (90% of ApoE in circulation), lung, ovary, muscle, spleen, and kidneys, ApoE is also synthesized in the central nervous system (CNS) by glia, macrophages, and neurons (43). ApoE and its low density lipoprotein (LDL) receptors are employed in transport and metabolism of lipids, and in neural tissue repair after injury. Located on chromosome 19, the ApoE gene occurs in three natural allelic variants (ε2, ε3, ε4). The mature protein of 34.2 kDa is secreted in mono- or disialylated form but most of the sialyl is removed in circulation. The most ubiquitous allele, ε3, is found in approximately 78% of the Caucasian population (44). The presence of the ε4 allele (allelic frequency ε4 = 0.15) is considered a major genetic risk

factor for the late-onset FAD, SAD (45), CVD, and longevity. The ε4 heterozygous individuals have a three- to fourfold increased risk of AD, which doubles for the ε4 homozygotes (19). The ε2 allele (allelicfrequency ε2 = 0.08) seems to indicate the opposite (46).

The allelic frequency varies in different populations, with race being only one factor (47). The evident North-South allelic gradient in Europe (30–35% of Scandinavians are ε4 carriers) might have a greater impact on longevity than the Mediterranean diet of red wine, olive oil, and feta cheese (47–49). Patients carrying at least one ε4 allele were reported to have an increased density of SP and Aβ deposition. The exact role of polymorphism of this 299-aa, 34-kDa protein is unknown and probably very complex. The structural differences are relatively small. The most ubiquitous isoform ApoE3 has cysteine (Cys) and arginine (Arg) in positions 112 and 158, respectively. In the most common variant of ApoE2, the amino acid Arg^{158} is replaced by Cys. In ApoE4 Argreplaces the Cys^{112} residue. ApoE binds to the usual AD suspects, Aβ and the microtubule-associated protein tau, and has been localized in the SP. The purified, nonlipidated ε4 isoform was reported to have higher affinity than ε3 to Aβ (50), suggesting it may act as a pathological chaperone (51, 52) stabilizing the β-sheet structure of Aβ fibrils, and impairing Aβ clearance. The more recent data on binding of ApoE isoforms and Aβ suggest that the native lipid-associated ε3 isoform binds to Aβ with two- to threefold higher affinity than that of lipid-associated ε4 (53).

These findings may indicate that perhaps ApoE is involved in the clearance or routing out of Aβ from the CNS and that the presence of ε4 impairs the process, thus leading to its accumulation. Either alone or in complex with Aβ, ApoE seems to have no effect on APP processing (52). A critical and isoform-specific role of ApoE in SP formation has been demonstrated in a mouse model (54). In that model of FAD, APP^{V717F} transgenic mice expressing mouse, human, or no ApoE, the neuritic degeneration was virtually absent in $ApoE^{-/-}$ mice, although significant Aβ deposition was observed. When the mice expressing ApoE4 were compared with those expressing ApoE3, the former showed a 10-fold greater fibrillar Aβ deposition. The model thus confirmed a critical and isoform-specific role for ApoE in (1) Aβ trafficking and (2) SP formation. Conversely or additionally the binding of ApoE isoforms to tau may affect phosphorylation of that protein and lead to NFT formation (52, 55). The connection between ApoE isoforms, their serum concentration, high intake of dietary cholesterol, and/or high cholesterol blood levels and an increased risk of AD, cardiovascular disease, and longevity is visible and rational but remains to be proved (47).

The reports of observably lower incidence of SAD among the patients prescribed statins (56), the differentiated cholesterol response to statin treatment dependent on the allele present (57), and the surprising response of ε4 carriers with CVD to statin in a myocardial infarction survival study (58) emphasize the need for large enrollment studies of these links. An additional link between the ApoE allele and AD comes from the increased CSF cortisol as a function of ApoE genotype (59). The highest levels of cortisol that may contribute to neuronal degeneration were observed in ε4/ε4 individuals with AD.

3.2.2 Susceptibility Locus on Chromosome 10. As mentioned above 95% of AD cases show no clear pattern of inheritance and out of that group only 50% carry ApoE ε4 allele(s). A two-stage genomewide screen, used to locate additional genetic susceptibility risk factors (60, 61), located additional AD locus on chromosome 10. From the presented evidence the chromosome 10 locus modifies risk of AD not linked to the presence of ApoE genotype (60). It appears that the locus increases the risk of AD by elevating Aβ42 in carriers, as might be evidenced by its high blood levels (61).

3.2.3 Susceptibility Locus on Chromosome 12. Another genomic screen of families with LOAD (62) revealed AD susceptibility locus on chromosome 12 also not linked to the presence of ApoE ε4 allele. The α2-macroglobulin and lipoprotein receptor related protein (LRP) genes present on this chromosome were initially considered as suspects (63).

It has been proposed that a genetic variation in a transcriptional factor LBP-1c/CP2/LSF gene and not the LRP gene is the suscep-

tibility factor for LOAD (63). The search is far from over and one can expect additional loci to be discovered as the result of genomewide screening.

3.2.4 Tau Protein. Intracellular NFTs, consisting mostly of paired helical filaments (PHFs), occurring in the selected populations of nerve cells before degeneration carry the subunit protein: the microtubule-associated protein tau in the hyperphosphorylated, insoluble form (64). A number of familial neurodegeneratory diseases grouped into the so-called "frontotemporal dementia and parkinsonism linked to chromosome 17" (FTDP-17) show tau protein pathology indistinguishable from that of AD (64). Although no pathogenic mutations in tau protein have yet been associated with the AD, the abundance of NFTs correlates with the degree of neurodegeneration (65). The prevailing opinion at present is that the presence of NFTs in AD manifests a "downstream" response to the pathological events initiated by that disease. In transgenic mice expressing the Swedish mutation of APP ($APP695_{K595N/N596L}$), the $A\beta$ amyloidosis induces the initial stage of tau accumulation (66). Nevertheless, given the large number of neurodegenerative disorders with tau pathology, the mechanism of NFT formation is subject to detailed studies (67).

3.3 Nongenetic Risk Factors

Although a much higher percentage (30–50 to 95%) of genetic risk factors has been postulated, the genetic determinants of high penetrance listed above and linked to FAD represent only a small (<5%) percentage of total clinical manifestations of AD. FAD, LOAD, SAD, and the process of aging show many similarities, whereas the differences appear to be of a quantitative, chronological, and kinetic nature. It is highly unlikely that AD pathology can originate in response to environmental challenge alone. The elucidation of interdependency between genetic and nongenetic risk factors implicated in AD should benefit from the completion of long-term, multicenter, large-enrollment clinical studies, planned or under way at the present, and from exhaustive epidemiological investigations. For example, the already mentioned notorious genetic susceptibility factor, the ApoE genotype, has been implicated as a modulator of the environmental/metabolic risk factors, such as head injury, stroke, hypertension, arteriosclerosis, and thrombosis, serum cholesterol, or estrogen replacement therapy to mention a few (68). The putative environmental risk factors, considered among the others, are diet (high cholesterol and/or high caloric intake), smoking, alcohol consumption, depression, chronic or oxidative stress, and the like, affecting the vascular or endocrine equilibrium (69). What is hoped for is to establish a link between a genetic susceptibility factor, or the lack of it, and an environmental trigger (e.g., head injury) (66). The overwhelming number of factors, which appear to contribute to AD, encouraged attempts to formulate a unified hypothesis including or explaining all of them. Particularly of interest is the unifying hypothesis of Heininger (70–73), which advances the concept of AD as a metabolic disease. The genetic and environmental/metabolic factors, according to the hypothesis, could contribute to the deterioration of homeostasis of the calcium ion-energy-redox triangle and disrupt the cerebral reserve capacity under metabolic stress. The brain in AD would then attempt to adapt to energetic stress by releasing soluble $A\beta$ as an agent mediating the metabolic switch that preserves glucose for anabolic needs and promoting the oxidative use of ketone bodies. From that point of view, AD should be considered as a progeroid systemic disease and all the factors leading to it as progeroid factors (73).

Factors for which the anecdotal threshold has been crossed are briefly discussed below. Unsurprisingly, they link to the ApoE genotype.

3.3.1 Traumatic Head Injury (THI). A number of studies suggest a likely link between a risk of developing AD and THI acting as an environmental trigger (74). It is evident in the pig model of a brain injury that even a mild trauma with full recovery leads to the accumulation of $A\beta$ and formation of neurofilament inclusions (75). Similarly, an overexpression of APP was observed in the brains of gunshot survivors (76) and in head-injured sheep (77). Regretfully, studies involving large groups of participants, like the MIRAGE (78) and the

Rotterdam (79)studies, provide contradictory results. The presence of ApoE ε4 allele(s) is associated with worse neurological impairment in head injury or stroke patients, suggesting that ApoE4 might actually impair neural tissue repair (80).

3.3.2 Hypercholesterolemia and High Density Lipoprotein (HDL) Cholesterol. In the beginning, the relationship between ApoE genotype, total serum cholesterol, its high and low density lipoprotein fractions, and AD was the subject of narrow epidemiological studies in old and very old. A small Finnish study inferred that, independent of genetic status, high serum cholesterol represents an independent risk factor (81). The conclusion of the equally modest in size PAQUID study suggested that, independent of genetic and other risk factors, elevated high density lipoprotein cholesterol (HDLC) significantly reduced the risk of dementia of AD and vascular type (82). The same study reported that age, low HDLC, the presence of apoE4 allele, low education, and low wine consumption were significantly associated with AD. Studies of patients taking cholesterol level-lowering 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins)confirmed its, perhaps pivotal, role in the pathophysiology of AD (56, 83, 84). The reports indicated that the patients on statins did show a reduction in AD incidence (56, 83).

Epidemiological observations are beginning to be confirmed in vitro. For example, in APP751-transfected HEK 293 cells the elevated, by various means, level of cholesterol caused a dramatic reduction in secretion of the soluble APP fragment sAPPα (84). At the cellular level it is by now proved that cholesterol depletion (by 70%) in cultured neurons by combination of lovastatin treatment and methyl-β-cyclodextrin extraction reduces production of Aβ below detectable levels (85) by shifting the APP processing to a-secretase (86).

4 PREVALENT HYPOTHESIS: AMYLOID CASCADECUM-CHOLESTEROL ET INFLAMMATION

The delineation of the AD pathogenic cascade, leading to neuronal death by apoptosis, should provide an atlas of therapeutic targets of opportunity a la arachidonic acid cascade. The amyloid cascade hypothesis (29, 87) is so widely circulated and accepted that it leads one to recall a classic Russian proverb: "На безптиче и жопа соловей" ("In birdless field, even . . ·"). It has also awakened a vocal opposition rejecting β-amyloidocentrism and the Church of Holy Amyloid (CHA) (88).Proposed by Hardy and Allsop (89), and modified ever since, it may be coarsely summarized (Figs. 13.1 and 13.2) as follows.

APP is expressed in neurons and glia, where it is synthesized and cotranslationally inserted into the endoplasmic reticulum (ER) (90). Of all the APP mutations identified, most of them take place in the immediate vicinity of the N- and C-terminal of the Aβ fragment or in the middle of its β-turn area (Fig. 13.1).

Two distinct paths carry out the proteolysis of APP. Both appear to be regulated by numerous factors including stimulation of acetylcholine, serotonin, glutamate, and neuropeptide receptors (91, 92). The dominant, nonamyloidogenic a-pathway involving a-secretase, cleaves APP between Lys16 and Leu 17 of the Aβ domain in the extracellular space, and produces a soluble APPα fragment (sAPPα) and αC-terminal fragment (αCTF). αCTF (also known as C88 for 88 aa) is in turn cleaved by y-secretases to produce fragments p3 (from its 3-kDa molecular weight, synonymous with some Aβx-40 and Aβx-42). Most (90%) of the APP is processed that way. One should keep in mind that the activity of y-secretase indicates a relatively distance-specific [from the transmembrane domain (TM)], not aa-specific, (93) enzyme.

The second amyloidogenic β-pathway employs β-secretase [beta-site APP cleaving enzyme (BACE1), Asp2, memapsin2], which is largely present in the distal Golgi membrane (94). β-Secretase is responsible for cleaving APP at the N-terminus of Aβ between Asp1 and Met−1. Thus, the generated soluble APPβ fragment (sAPPβ) leaves the scene, whereas the membrane-bound βC-terminal fragment (βCTF or C99) is in turn cleaved by y-secretases to produce Aβ of 40- or 42-aa length. The y-secretase cleavage of C99 leaves an unstable CTFγ of 57- to 59-aa residues (95). Both Aβ40 and Aβ42 are produced in normal

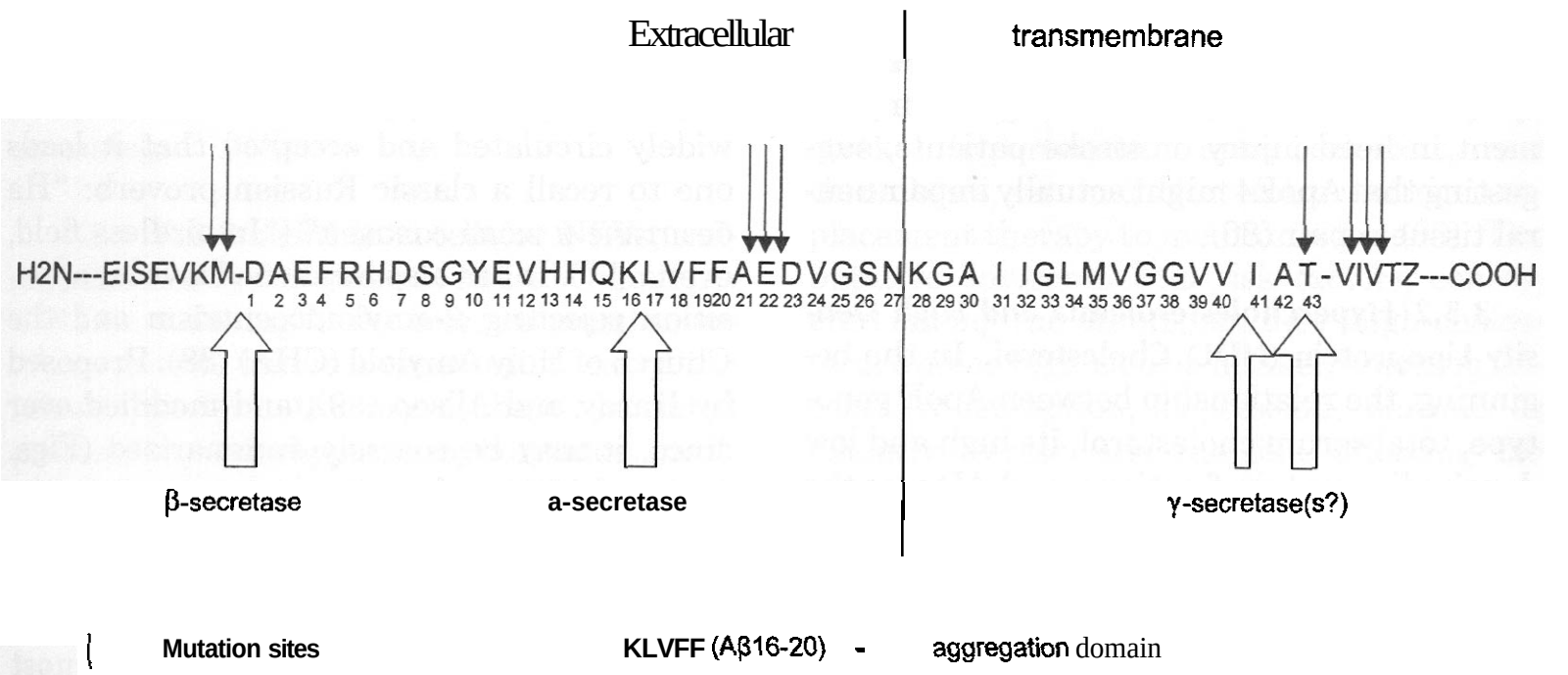


Figure 13.1. Human Aβ domain of APP: processing and mutation sites.

and SAD individuals but in different proportions (37). The ratios differ, with Aβ40/Aβ42 ratio of 10 in SAD. In nondemented geriatric controls Aβ42 was dominant. The soluble Aβ are present in cerebrospinal fluid (CSF) of normal and AD individuals at similar levels (10^{-8}–10^{-10} M) (96). Their serum and CSF levels were suggested (97), but do not appear to be indicative of the presence of AD (98). Naturally occurring antibodies to Aβ were reported to be significantly lower in the CSF and plasma of AD patients (99). The sites of production of Aβ of various lengths and its N-truncated fragments were the subject of numerous investigations. According to Greenfield et al. (90), APP translocated into the lumen of ER forms N-truncated Aβx-42, which stays in the ER in an insoluble form. The unprocessed APP moves through the Golgi apparatus (GA) to the trans-Golgi Network (TGN)

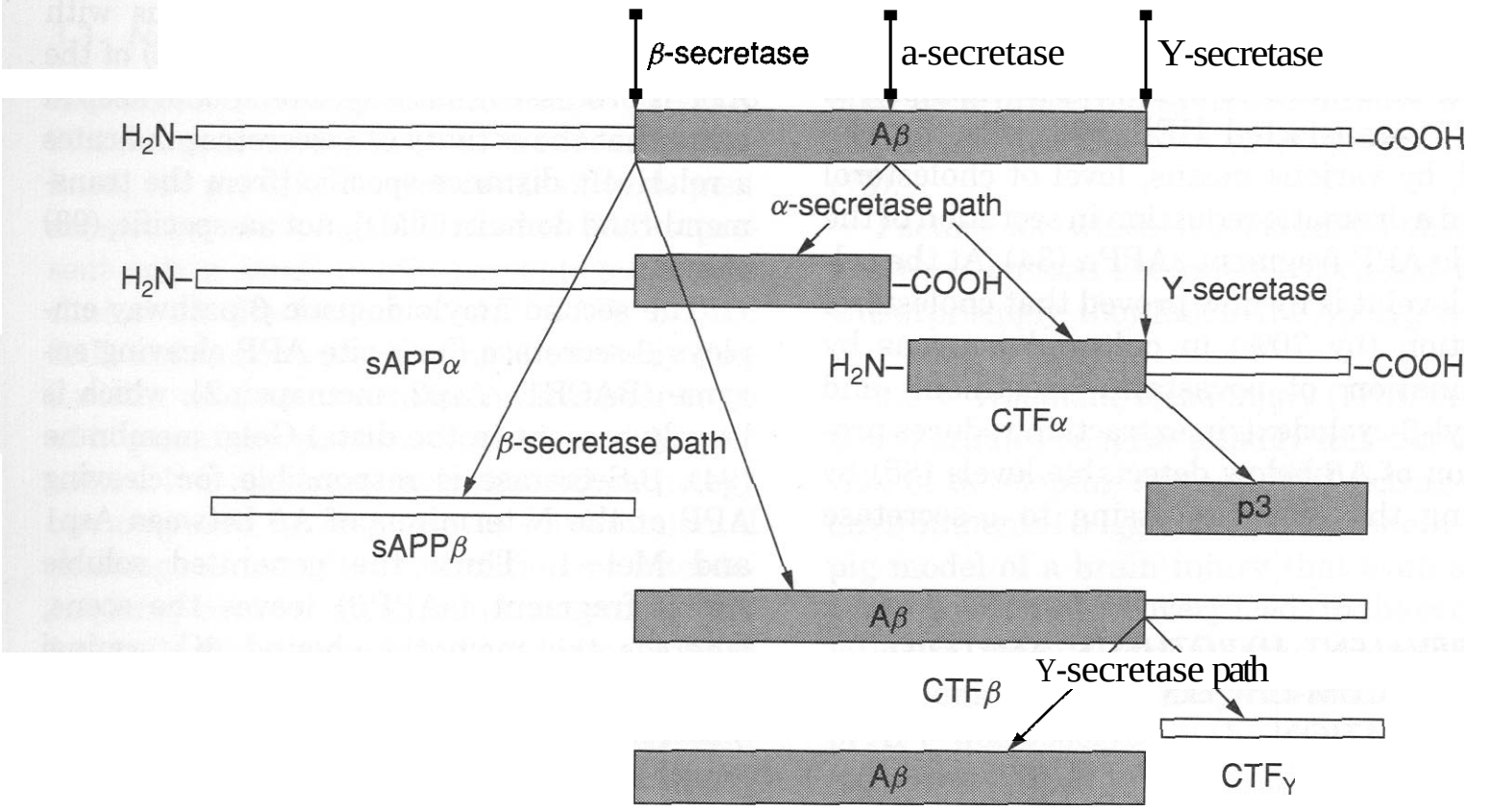


Figure 13.2. Amyloid cascade.

where Aβ40, Aβ42, and their N-truncated Aβx-40 and Aβx-42 peptides are generated. The unprocessed APP, βCTF (C99), and soluble Aβ are packaged for release into post-TGN secretory vesicles. When released they can travel to the surface of neuronal cells, where a-secretase generates the bulk of sAPPα (100), secreted together with Aβ40, Aβ42, and the N-truncated fragments. The unprocessed APP and the a- and β-CTFs can be internalized to the endosome or lysosome and returned to TGN for additional processing. That pathway was confirmed when production of Aβ40 and Aβ42 was abolished by preventing C99 from leaving ER (101).

The sAPPα fragment was reported to have neurotrophic and neuroprotective (against glucose deprivation and glutamate toxicity) properties, to stimulate neurite outgrowth, and to regulate synaptogenesis (39, 102). The memory-enhancing properties of sAPPα were reported in normal and amnestic mice (103). Recently, the sAPPα fragments originating from APP751 and APP695 were identified as ligands for the class A scavenger receptor (SR-A), suggesting a possible way for their clearance (104). Evidently, the Kunitz-type protease inhibitor (KPI) domain present on sAPPα originating from APP751 is not required for binding.

The biological activity, if any, of sAPPβ is so far unknown. The role or contribution of N-truncated Aβ (p3, for example) to AD is the subject of a hotly contested debate, entitled the shorter amyloid cascade hypothesis (87,105).

A mutation in either APP or PS1 or even PS2 (29) alters the way APP is processed, either in favor of increasing the ratio of amyloidogenic Aβ42 (which predominates in the immature, diffused SPs) or simply increasing the production of Aβ40. Indeed, some FAD-linked mutations in PS1(Δ9) lead to formation of higher levels of Aβ42 in TGN-to-plasma membrane transport vesicles (106). Presenilin is necessary for production of Aβ as it complexes with the CTFs (C99 and C83) (107). The evidence exists of a physical interaction between PS1 and Notch receptors, Notch being a proteinlike APP, a large single-transmembrane protein important in development and differentiation of adult self-renewing cells (108, 109). Both proteins have an atypical characteristic of being processed in the transmembrane domain (108, 110–113). That discovery presents the seekers of y-secretase inhibitors with a predicament of selectivity, how to prevent processing of APP without affecting the fate of Notch.

One of the remaining questions is how the Aβs, generated inside the cell, pass through or detach from the cell membrane and leave for extracellular space where the SPs are formed. To that effect, an ATP-binding cassette (ABC) transporter, a p-glycoprotein (p-gp), was proposed as an Aβ efflux pump (114). Equally important is the question of degradation of the generated Aβ. One of the candidate enzymes was reported to be neprilysin, one of the major endopeptidases in the brain (115) and the fastest to degrade both Aβ40 and Aβ42 (116). In the brains of neprilysin-deficient mice the elevated Aβ located in the same order and regions as reported for humans (hippocampus > cortex > thalamus/striatum > cerebellum).

Once secreted, the Aβ may aggregate spontaneously, with more hydrophobic Aβ42 showing greater rate of assembly (96,117). The length of Aβ is not the only determinant of the rate of aggregation. The studies of a pathogenic mutation of APP, the "Arctic" mutation (E693G), was reported to produce Aβ, which forms protofibrils at a much greater rate than does the wild-type (118). To form the core of SP after being generated through proteolytic cleavage of APP, Aβ have to undergo a continuous process of structural transformation (117, 119). The released Aβ initially stay in solution as a random coil or a-helix (120, 121). The transition from random coil or a-helix to β-sheet conformation begins the process of oligomerization and fibril formation, with Aβ42 assembling more readily than Aβ40 (122). A stable Aβ dimer appears to be the building block of the Aβ filament. The growth of the fibrils proceeds by the addition of Aβ dimers and tetramers (123). The amyloid fibrils thus formed are straight, unbranching fibers of 70–120 Å in diameter and of indeterminate length (124).

The *in vitro* studies of that process using synthetic Aβ40 at low pH suggested a kinetic model in which (1) a nucleation step is required, (2) nuclei are produced from seeds and from Aβ micelles, and (3) fibril elongation is achieved by irreversible binding of Aβ mono-

mers to the fibril ends (125). Concentration of Aβ appears as a critical parameter for micelle and fibril formation. Either AB monomer or dimer polymerization models could be used to determine the rate of fibril formation under given conditions (126). The maximum rate of formation was given as $t_{1/2}$ for Aβ42 of approximately 18 min and for Aβ40 as approximately 6 h. ApoE and antioxidants decreased the final amount of fibril formed in a dose-dependent way, although only ApoE extended the time to proceed to equilibrium (126). These findings are in agreement with the observation that free radicals, some perhaps generated by the Aβ fragments (127), appear to promote Aβ fibril formation (126). As always, there are also reports that Aβ does not form spontaneously free radicals (128). The other problem with the fibrillogenesis models is that a number of Aβx-40/42 N-terminal fragments have been reported in the SPs and they appear to aggregate much faster than do the full-length Aβ (129). The identified pyroglutamyl *N*-terminal fragments Aβ3pE and Aβ11pE, by aggregating faster and being more resistant to proteolysis than the full-length Aβ, would make perfect seeds for amyloid formation (130). From in vitro assays a number of compounds have been identified to affect that continuous process of assembly. A conclusion was also drawn that the processes of fibril assembly and disassembly appear to be distinct (119). The process of Aβ fibrillogenesis is still waiting for its Melvin Calvin to unravel it.

Secreted Aβ and their fragments in various oligomeric forms are considered to be, to a different degree, cytotoxic and to induce apoptosis in neuronal cells (131).

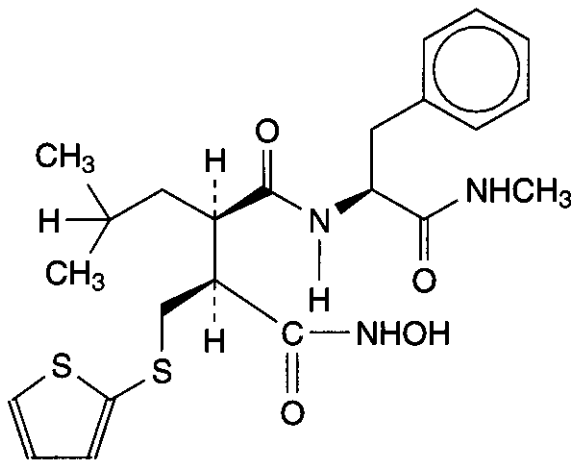
The transformation of immature, diffuse SPs composed almost exclusively from amyloidogenic Aβ42 into the mature, neuritic SPs is poorly understood. It appears to be a continuous process, with numerous intermediates observable in the postmortem brains. The construction and composition of mature, neuritic SPs are fairly complex processes, in which the core consists mainly of Aβ40/42 and Aβx-40/42 fragments and is surrounded by dystrophic neurites, activated microglia, and reactive astrocytes (96). In neuronal cultures conditioned by Aβ-treated astrocytes, but not directly in contact with Aβ, the astrocytosis was evidently doubling the number of apoptotic cells (132).

The presence of reactive microglia, macrophage-like phagocytic cells, associated with the mature, neuritic SP is one of the characteristics of AD. The presence of these main inflammatory response cells in AD represents the foundation of the inflammation hypothesis. The question whether reactive microglia are there to help or to harm is still open. Another question is whether they appear at the beginning or at the end of development of AD (133). It is the fibrillar Aβ that induce strong cellular activation in primary cell culture (134). Small aggregates of fibrillar Aβ and soluble Aβ, despite minor differences in interaction with microglia, do not show a great degree of degradation after internalization (96). Some of the fibrillar Aβ might be initially degraded by microglia but the degradation stops after 3 days. The soluble Aβ is released rapidly after internalization and very little is degraded. Almost accepted is the hypothesis that the neurotoxicity of Aβ is not linked to its monomeric forms or insoluble amyloid precipitate but to its soluble intermediate oligomers of full-length Aβ and its fragments (131, 135, 136).

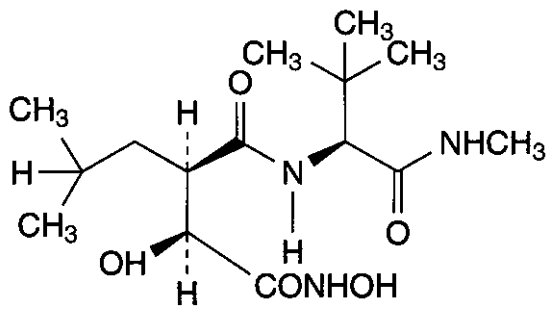
All the above-described events happen in the environment sensitive to cholesterol levels linked to or influenced by the genetic risk factor ApoE. The presence of the ApoE4 allele, ε4/ε4 in particular, indeed correlates with the degree of Aβ deposition (119,137–139).

4.1 Inhibitors of Aβ Production

4.1.1 α-Secretase Inhibitors. Proteolysis of APP by a-secretase takes place at the cell surface, manifesting an end to its processing by the cell (100). Inhibition of a-secretase would serve no purpose in AD but, as one of three enzymes or groups of enzymes processing APP, it deserves attention. Although not isolated as yet, it appears to belong to a group of zinc metalloproteases and to be closely related, by inhibition profile, to angiotensin-converting enzyme (ACE) secretase and to collagenase (100, 140). Some of the investigated (140) a-secretase inhibitors, such as batimastat (1)and marimastat (2), initially devel-



(1)



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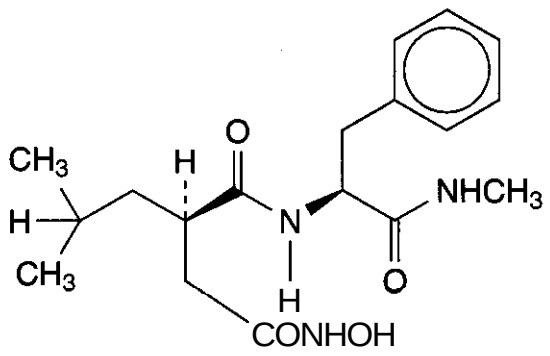

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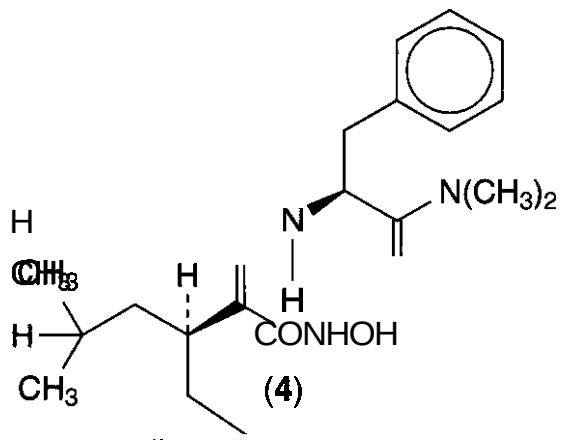

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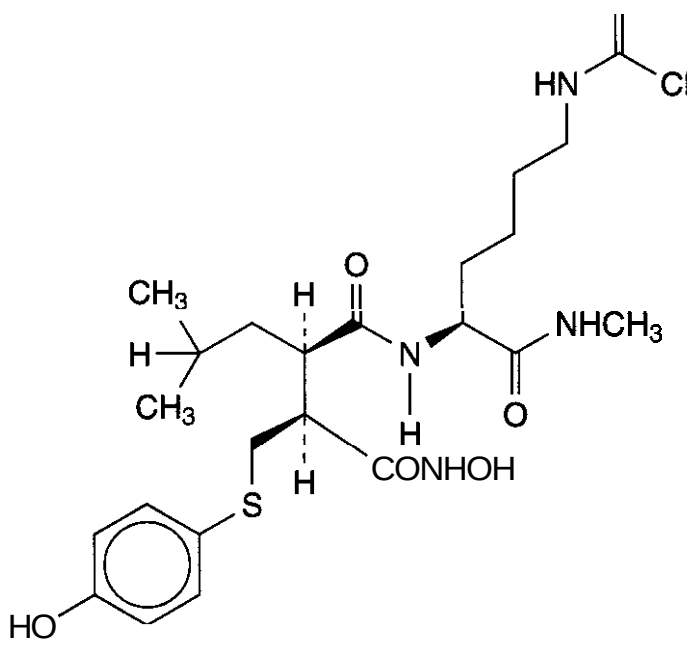
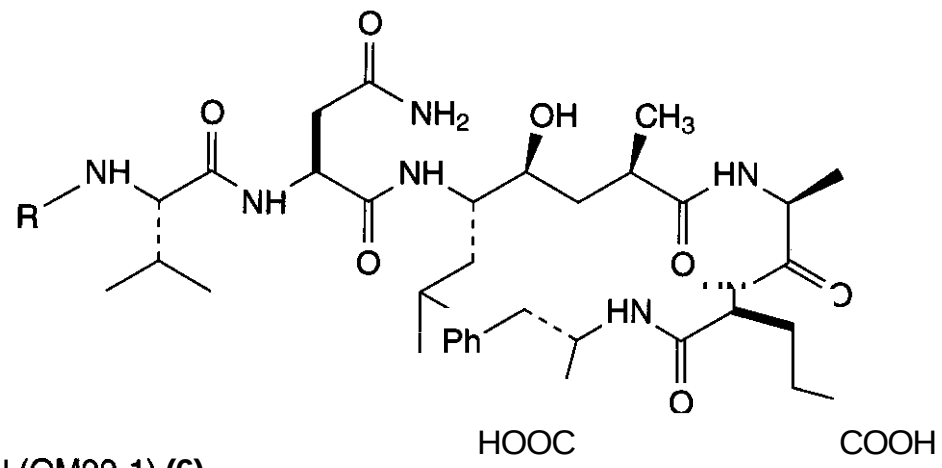


(5)

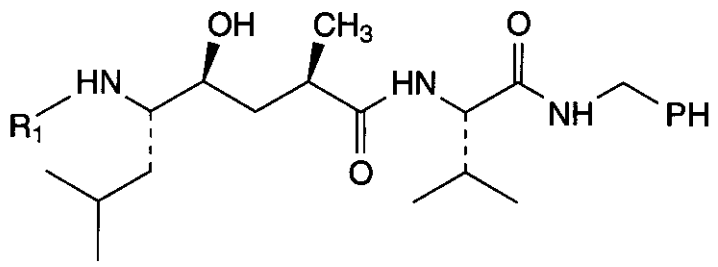
Figure 13.3. Inhibitors of a-secretase.

oped as antineoplastic agents, and their analogs can be seen in Fig. 13.3.

4.1.2 β-Secretase inhibitors. β-Secretase, also known as memapsin 2 or β-site APP cleaving enzyme (BACE1) [BACE2 was mapped to Down syndrome (141)], the primary membrane-anchored aspartic protease in mammalian brain, does not appear to be of importance in mammals (142). The loss of β-secretase activity in BACE knockout mice produced no profound phenotypic defects with simultaneous reduction of Aβ (142). The fact that the knockout mice developed normally and showed no consistent differences with their wild-type littermates makes β-secretase a perfect target of opportunity for the development of AD therapeutics. The observed absence of astrocytic BACE immunoreactivity in young transgenic Tg2576 mice (143) and the consecutive expression at later stages as a possible result of astrocytes activation should en-

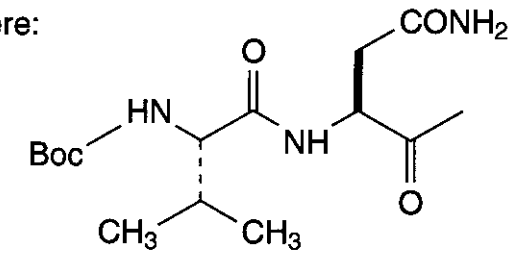


R = H (OM99-1) **(6)**
R = Glu (OM99-2) (**7**)

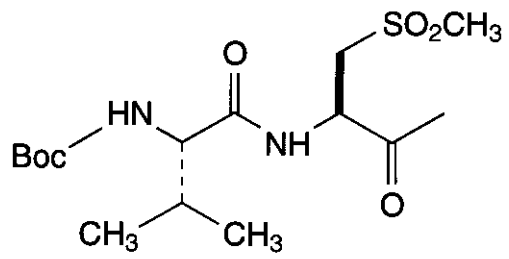


Where:

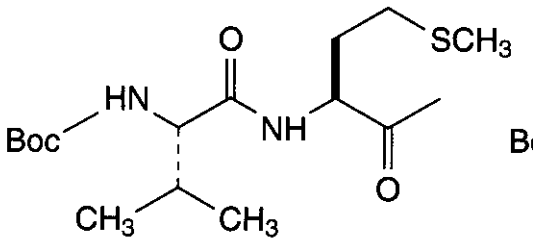
R_1 =



(**8**)



(**9**)



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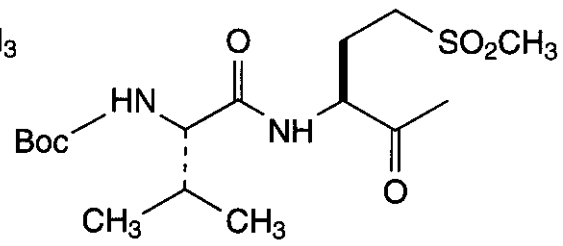


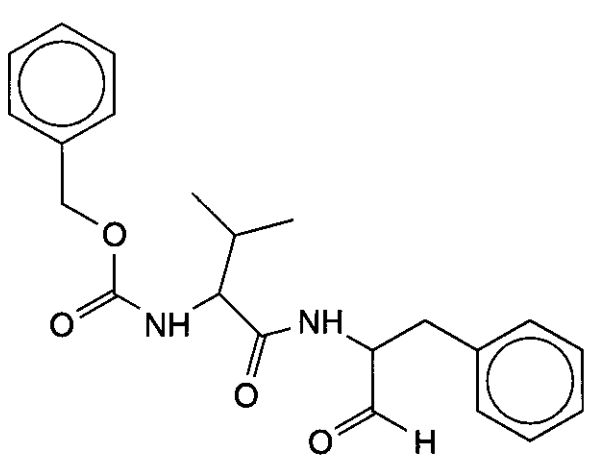
(**11**)

Figure 13.4. Inhibitors of β-secretase.

courage vigorous pursuit of that enzyme. The accumulated knowledge and experience with the approved peptidomimetic HIV protease inhibitor drugs is available. Yet, despite the encouraging experiments with the knockout mice, the trepidation persists that there might be a role for β-secretase in normal physiology and that the selectivity against other proteases might be difficult to attain.

The first reported p-secretase inhibitors, OM991 and OM992 (144) (6 and 7, Fig. 13.4), were designed by use of the template of the Swedish mutant APP. The Swedish mutation site of β-secretase action [SEVNL/DAEFR instead of SEVKM/DAEFR wild-type (see Fig. 13.111 was found to be a perfect substrate for recombinant memapsin 2 (144). X-ray crystallography studies of (7) bound to memapsin 2 permitted the design of inhibitors of lower molecular weight (145). The most potent of them (8–11, Fig. 13.4) have K_i values between 2 and 10 nM.

4.1.3 γ-Secretase Inhibitors. PS1-associated y-secretases participating in processing of APP are also needed for proteolysis of another transmembrane protein, Notch1 (146, 147). Both proteins appear to be competitive substrates: APP, or rather its C99 fragment, for Aβs and Notch1 for a signaling molecule



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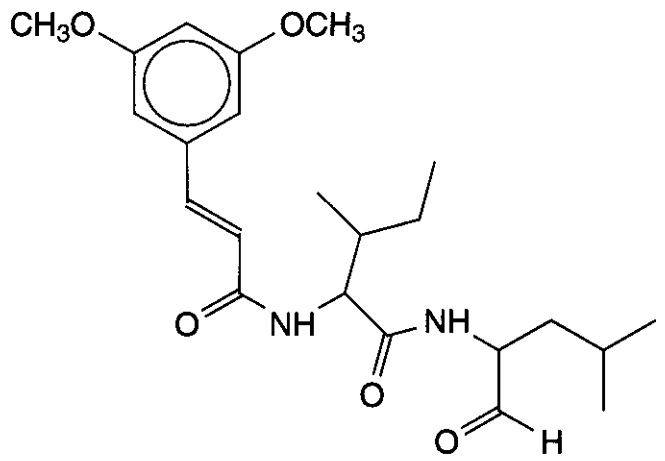

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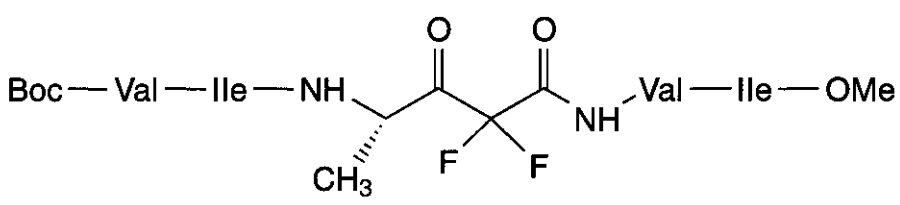

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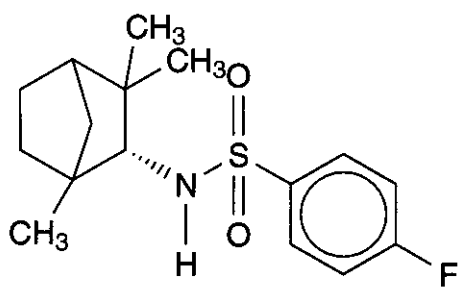

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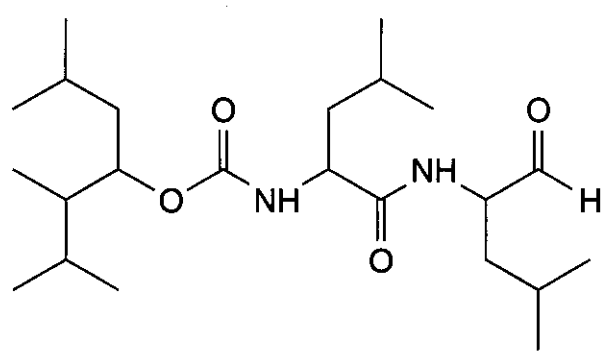


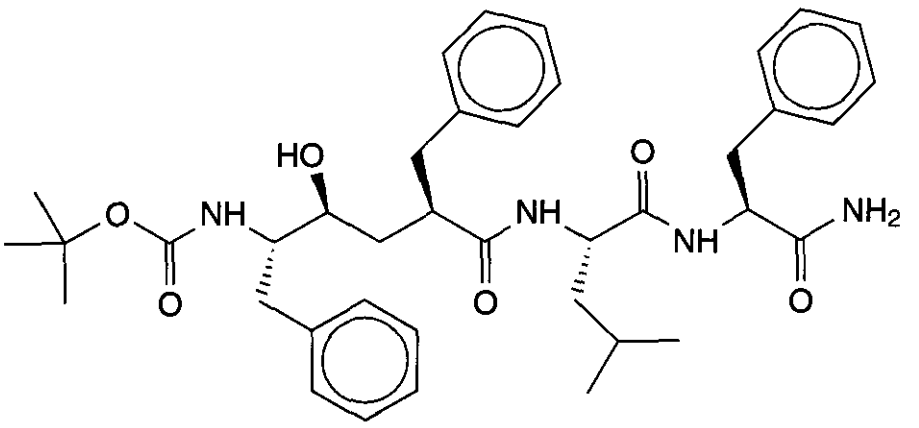
(15)

Figure 13.5. Inhibitors of y-secretase.

Notch C-terminal domain (NICD) (148). The dipeptide aldehyde (12, Fig. 13.5) (149) served as a lead to a group of inhibitors, with one compound (13) showing 10-fold improvement in IC_{50} values (150). A study of difluoro ketone peptidomimetics developed from a lead (14) provided a valuable insight into the mechanism of the action of y-secretases (151–153). At a micromolar level these compounds blocked the formation of Aβ40 and Aβ42 and increased formation of Aβ42 at a subinhibitory level (152,153). A similar group of dipeptide aldehydes (15) confirmed the findings (154). A group of fenchylamine sulfonamides (16) was reported to inhibit y-secretases at micromolar concentrations in HEK293 cells transfected with the double-mutant form of human APP (155).

The first peptidomimetic y-secretase inhibitors and aspartate mutations in PS1 were reported to impair Notchl proteolysis and nuclear translocation but to preserve Notchl signaling (156). This was further confirmed in fetal thymus organ cultures, where they interfered in T-cell development, indicating loss or reduction of Notchl function (109). An aspartyl protease transition state mimic, L-685,458 (17) was reported to be a potent (IC_{50} = 17 n*M*) inhibitor of y-secretase (157). One of the calpain inhibitors, MDL28170 (18), was reported to block production of Aβ40 but not Aβ42 (158). The search has been recently enhanced by introduction of a y-secretase assay based on detection of the elusive and unstable CTFγ (95).

The first group of y-secretase inhibitors free of interfering with Notch processing (19–21, Fig. 13.6) or endoproteolysis of presenilins reduced Aβ40 and Aβ42 in favor of C99 and C83 CTFs arising, respectively, from β- and a-secretase action (159). Their design was based on observations that (1) disruption of



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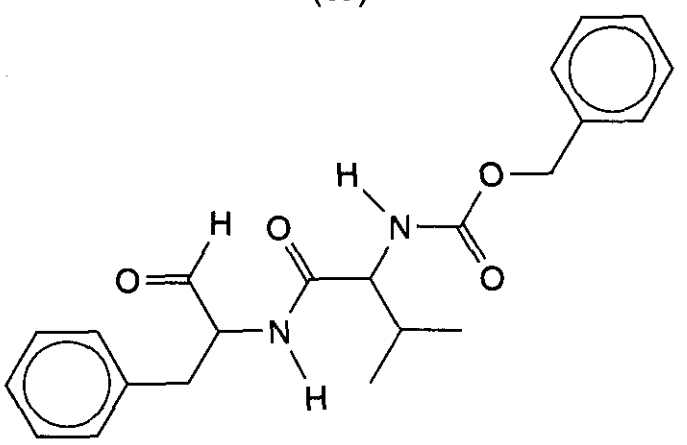

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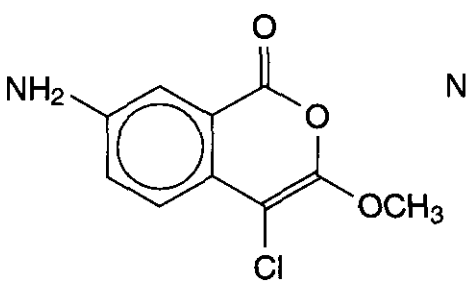

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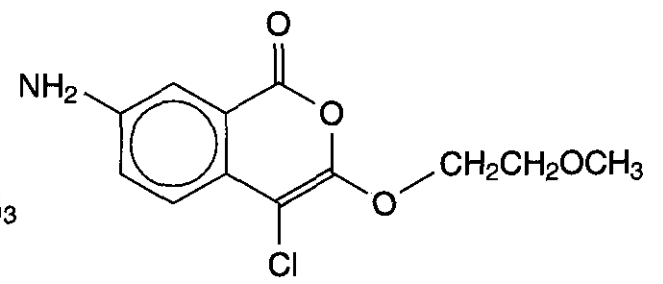

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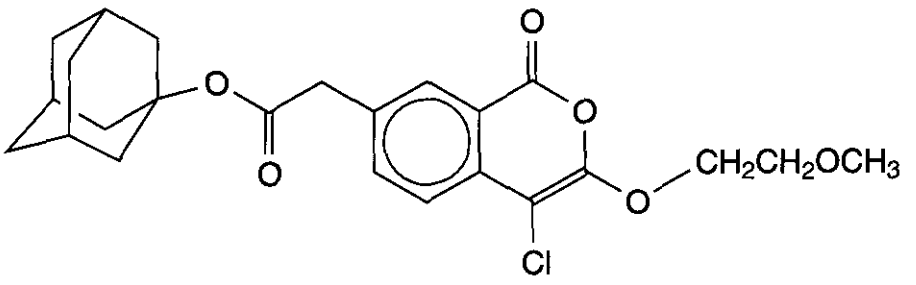


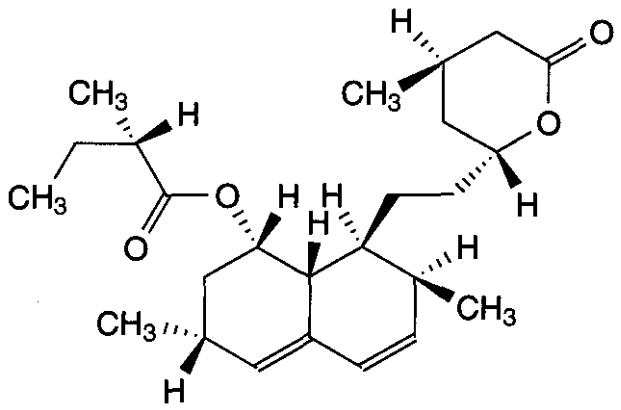
(21)

Figure 13.6. Inhibitors of γ-secretase.

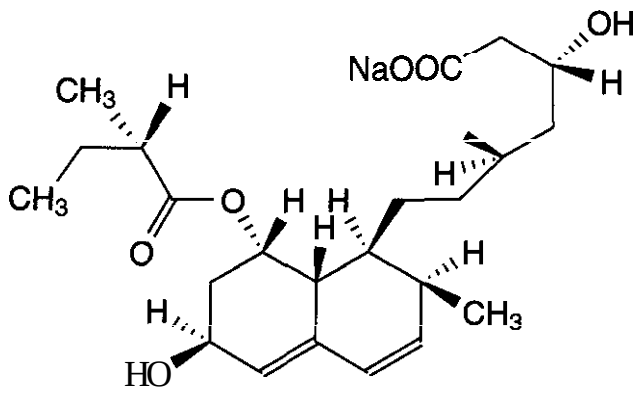
the chymotrypsin-like activity of the proteasome correlates with the inhibition of Aβ secretion (160) and (2) that the serine-protease inhibitor AEBSF modulates Aβ recovery (161). At 100 μM concentration, compounds (**19**), (**20**), and (21) achieved 70–80% inhibition of total Aβ40/42 recovered.

4.2 Statins

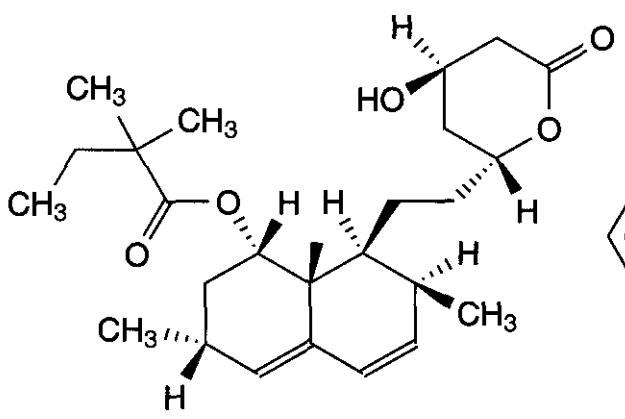
Developed to control elevated low density lipoprotein cholesterol, statins [the inhibitors of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase] are prescribed chronically to the elderly (162). The initial success of statins was impressive and the FDA approved six of them (22–27, Fig. 13.7) (163). Although all approved statins have been associated with a very low incidence of rhabdomyolysis, only one, cerivastatin (**27**), was withdrawn from the U.S. market because of it (164, 165). The ability of statins to reduce the levels of Aβs in *vitro* and *in vivo* was reported (85, 86, 166, 167) but at doses exceeding the approved human dosage. A cyclohexylalanine-based statin



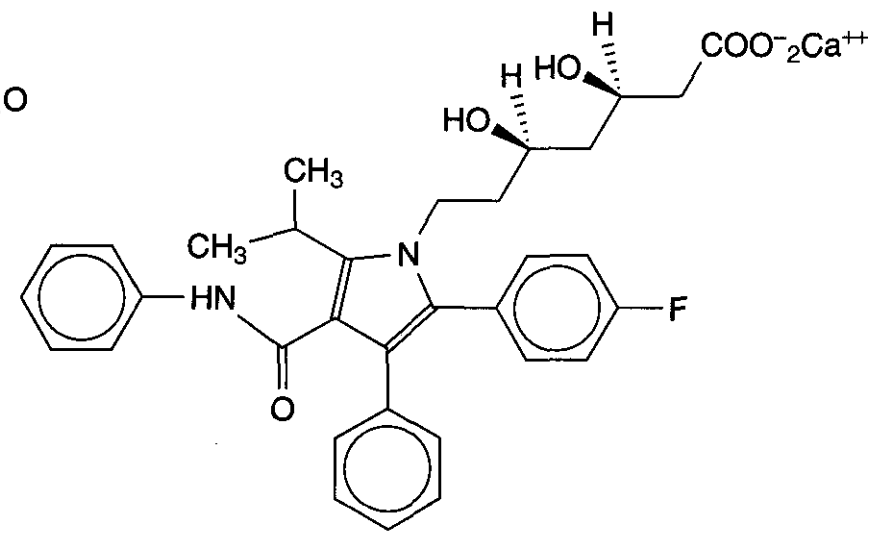
(22) Lovastatin [mevacor] (1987)



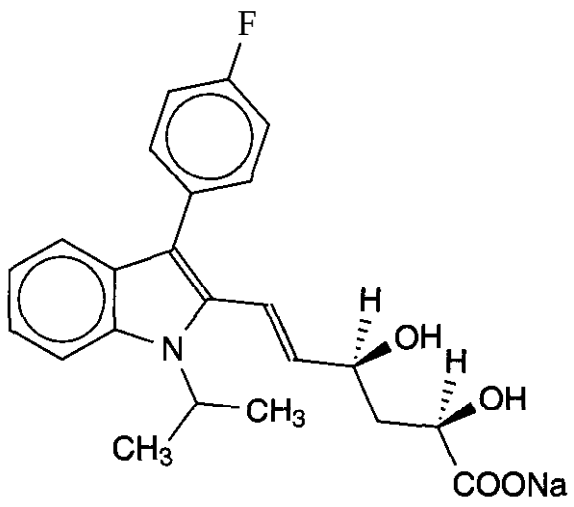
(23) Pravastatin sodium [pravachol] (1991)



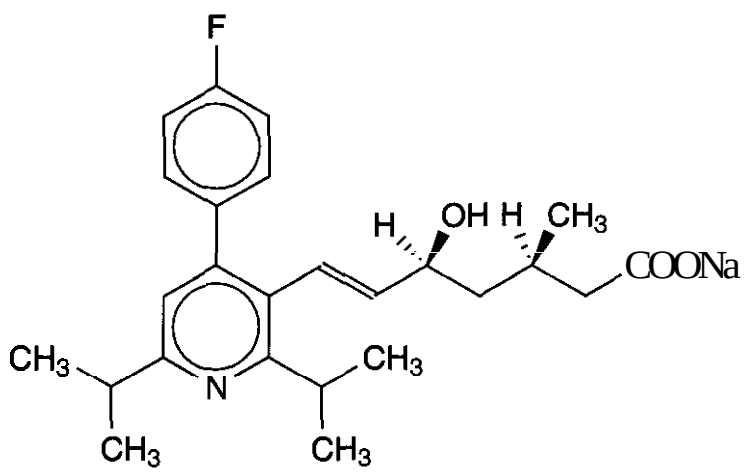
(24) Simvastatin [zocor] (1991)



(25) Atorvastatin calcium [lipitor] (1996)



(26) Fluvastatin [lescol] (1993)



(27) Cerivastin sodium [baycol] (1997–2001)

Figure 13.7. Statins.

(28, Fig. 13.8) was trawled out from the patent literature along a lipophilic tetraline (29) as an inhibitor of Aβ formation in cell culture (155). A small study of age- and gender-matched asymptomatic Japanese patients, either on pravastatin or simvastatin or naïve for an unknown length of time, did not show any observable difference in serum Aβs levels (168). One should remember that the Aβ levels in serum or CSF are not considered the best measure of therapeutic progress. To do justice, the cited study did not show any difference in total cholesterol and HDL cholesterol either.

The actual mechanism of how the statins affect AD remains unknown. For amyloid cascade enthusiasts, it is possible that the reported reduction in the AD prevalence in patients on statins (56, 83) reflects the cumulative effect of small but effective decreases in Aβ production over a long period of chronic administration. The explanation of

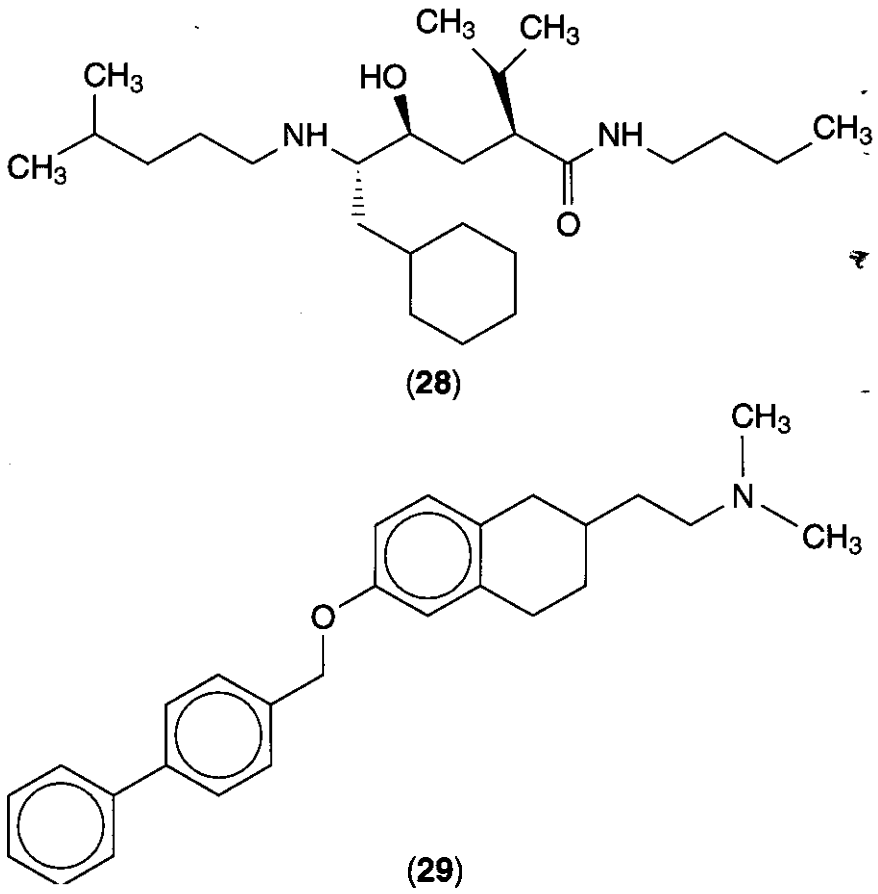


Figure 13.8. Inhibitors of Aβ formation in cell culture.

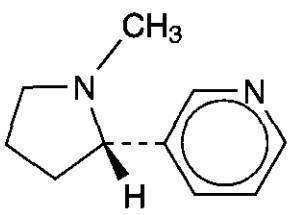
experimentally observed shift, favoring y-secretase processing of APP, attributed to lowering of membrane cholesterol, might be the peculiar nature of y-secretase (84). The y-secretase site of APP processing belongs to a very limited number of intrarnembrane proteolytic cleavage sites, requiring perhaps a cholesterol-rich environment such as a lipid raft domain (84, 169). Fluorescence microscopy studies of PC-12 and SH-SY5Y cell lines exposed to exogenous Aβ revealed an inverse correlation between membrane cholesterol level and Aβ cell surface binding and subsequent cell death (170). At present the debate continues (171a,b) while the clinical trials of statins for AD indication proceed (172). New, safer, and significantly superior statins, which reduce total cholesterol, low density lipoprotein cholesterol, and apolipoprotein B, and increase high density lipoprotein cholesterol more effectively than the presently available five, might be in the NDA pipeline (http://www.ndapipeline.com). Their effectiveness to reduce the incidence of AD may be hoped to similarly improve.

4.3 Compounds Affecting Fibril Formation

At physiological pH Aβ carries seven positive and seven negative charges in addition to two hydrophobic domains (123). That kind of molecule will self-assemble or complex with similarly attractive molecules. Aggregation (self-assembly) of Aβs and their neurotoxicity are intimately linked but may be affected by the different groups of compounds. If the SPs are responsible for initiation of neurodegeneration, prevention of formation of Aβ fibrils, or their disassembly, should have a therapeutic effect. If the SPs are just the deposits of assembled Aβ and cellular debris, taking them apart might do the opposite (173). The division of compounds affecting the Aβ fibril formation into inhibitors of Aβ aggregation (self-assembly) and accelerators of Aβ disaggregation is spurious, but practiced by many.

4.4 Inhibitors of Aβ Aggregation

To inhibit Aβ aggregation into amyloid is to prevent misfolding of soluble Aβ. Once the misfolding took place the aggregation is only a matter of time and far more difficult to control (174). So far only nicotine (30, Fig. 13.9) and melatonin (31) appear to be affecting the conformation of soluble Aβ (175–177). Antioxidants, such as antituberculosis antibiotic rifampicin (32) or a-tocopherol, were reported to inhibit aggregation and neurotoxicity of Aβ *in vitro* (178–180). A monoamine oxidase inhibitor, selegiline (33, Fig. 13.9), may also be



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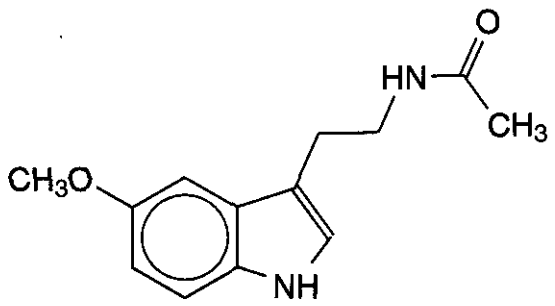

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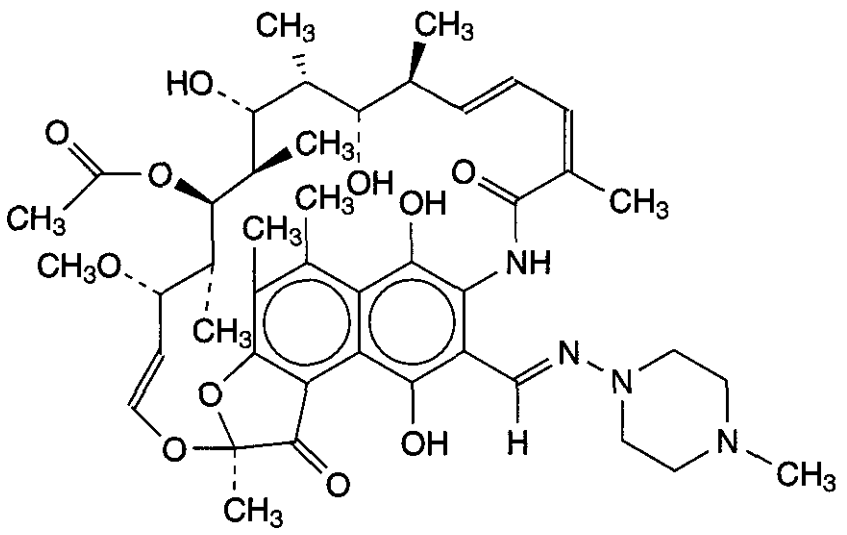

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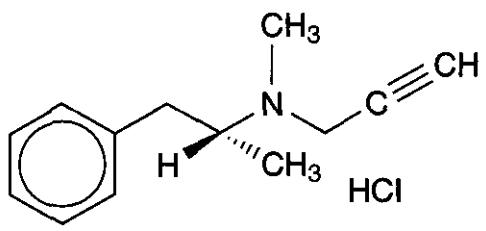

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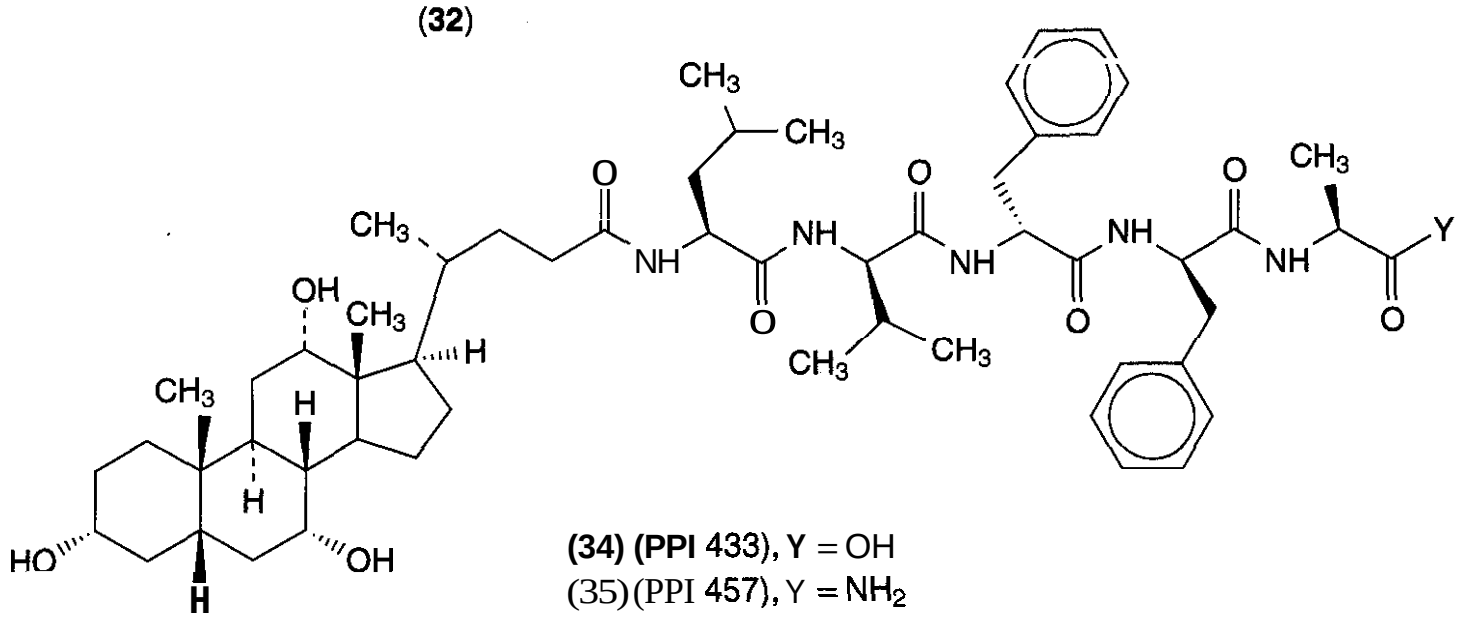


(34) (PPI 433), Y = OH
(35) (PPI 457), Y = NH_2

(36) NH_2-K(Me-L)V(Me-F)F(Me-A)E$CONH_2$

Figure 13.9. Inhibitors of Aβ aggregation and accelerators of Aβ fibril disaggregation.

considered to belong to that group. The role of ApoE as antioxidant and fibril formation inhibitor is still under investigation. It appears that it has a biphasic effect that inhibits fibril formation at lower concentrations, while accelerating at higher micromolar Aβ concentrations (126, 181). A constituent of high density lipoprotein complexes, apolipoprotein A-I (ApoA-I), has a higher affinity to ApoE than to Aβ, which it binds in a saturable, specific, and reversible manner with K_d of 6 n*M* (182). Laminin and entactin, the components of basement membrane, were reported to inhibit Aβ fibril formation by inducing a random coil structure (183, 184). Like ApoA-I, laminin binds Aβ with high nanomolar affinity.

The review by Findeis (185) and the work of Bohrmann et al. (117) start a very diversified list of compounds affecting aggregation of Aβ *in vitro* at micromolar concentrations.

Given the high affinity of Aβ for itself it was used as a lead to a number of D-aa analogs, providing stable compounds such as (34) and (35)(186).

4.5 Accelerators of Aβ Fibril Disaggregation

Once an Aβ fibril is formed and becomes a component of SP, it may be impossible to disaggregate it and to return it to its original random coil conformation. Although the process of assembly of Aβ fibril appears distinct from the process of disassembly, the same or similar groups of compounds may affect them both. The simplest of disaggregation agents 2,4-dinitrophenol and 3-nitrophenol were shown to cause, at micromolar concentrations, complete disaggregation and to block neurotoxicity of Aβ to rat hippocampal neurons in culture (187). In *vivo*, coinjection of these nitrophenols with Aβ into rat hippocampus reduced (86 +- 17%) the volume of amyloid deposits. According to the authors, the hydrophobic interaction of CTF nonpolar aa sequences responsible for fibril formation is disrupted by the hydrophobicity of nitrophenoles.

The N-methylated congeners of hydrophobic core domain Aβ16–22 (36, Fig. 13.9) were reported to inhibit fibrillogenesis and also disassemble preformed fibrils (188).

4.6 Aβ Catabolism and Removal

One assumes that even a slight shift in the anabolic-catabolic steady state of Aβ production and removal, persisting over a prolonged period of time, will eventually produce a pathological change. The hypothesis that the differential vulnerability of distinct parts of brain to Aβ deposition and neuronal destruction is based on altered metabolism of Aβ was behind the extensive search for the specific endopeptidase catabolizing Aβ. Howell et al. (189) reported extensive catabolism of Aβ by a neutral endopeptidase that leaves the APP intact. A thermolysin-like zinc metalloendopeptidase, neprilysin (NEP) (190), was determined to be involved in Aβ degradation by the use of multiple radiolabeled synthetic Aβ42 injected into rat hippocampus (191). Catabolism of endogenous Aβ42 was arrested by infusion of NEP inhibitor and led to pathological deposition. NEP mRNA levels were measured in the brain and peripheral organs of AD and control cases (192). Simultaneously measured were levels of the mRNA for the neuronal marker microtubule-associated protein 2. Its levels were constant in all brain areas and were not decreased with AD. That observation led to the conclusion that the lower levels of NEP mRNA observed in hippocampus and the temporal gyrus were not related to neuronal loss but were preordained. Both areas are the high SP areas of AD brain. In the NEP gene-disrupted mice, the degradation of exogenous and endogenous Aβ was affected in a gene dose-dependent manner (115). The highest levels of AB in NEP-deficient mice were in hippocampus and cortex and the lowest in cerebellum, reflecting the AD pattern. Immunohistochemical localization of NEP in postmortem human brain tissues indicated the same pattern (193). The hippocampus and the association cortices, the sites most affected in AD, showed the lowest NEP immunoreactivity compared to that of the primary somatosensory and visual cortices. The question remains whether the observed downregulation of NEP activity reflects aging or is particular to AD. The upregulation of NEP activity in the affected areas of Aβ deposition without affecting the NEP involved in the metabolism of numerous regulatory peptides of the nervous, cardiovascular, inflammatory, or immune systems presents another question. Another metalloprotease, endothelin-converting enzyme (ECE-1), was identified with similar properties of degrading Aβ (194).

Learning and memory deficits developed with aging in Aβ overproducing SAMP8 mice have been reversed with intracerebroventricular (i.c.v.) or even i.v. injections of Aβ antisense phosphorothiolate oligonucleotide (195).

4.7 Modulators of Aβ Neurotoxicity

Aβs, soluble as either random coil or α-helix, do not show any neurotoxicity (196, 197), which appears to be related to the degree of their aggregation (198). The inhibition of various cholinergic neurotransmitter functions by Aβ takes place at the very low concentrations below the neurotoxic treshold. The fibrils of Aβ are toxic to cultured neuronal cells and lead to their death, probably by observed rapid production of hydrogen peroxide and lipid peroxidation (199). Oxidative stress appears to be a proximal event in the AD (200) and the oxidative damage appears to be the greatest in the beginning of the disease and

decreases with the disease progression and formation of SP and NFT (201).

The recognition sequence of Aβ leading to aggregation consists of residues 16 to 20, that is, KLVFF (135). The residue itself had no effect on aggregation kinetics and only some effect on Aβ neurotoxicity. Coupling of the recognition sequence with a disrupting lysine hexamer (KLVFF-KKKKKK) dramatically accelerated Aβ aggregation, thus generating insoluble Aβ precipitate, and significantly improved protection against Aβ neurotoxicity (135, 136). The inference from these findings is that the insoluble Aβ aggregate is not the molecule to be blamed for toxicity but, rather, a soluble intermediate oligomer generated in the process (136).

The observation that the N-terminal part of Aβ is not associated with the fibril formation and remains outside of the fibril core (202, 203) led to generation of specific monoclonal antibodies (mAbs) against that part of Aβ (204–206). Used on PC12 cells the mAbs were shown to disaggregate Aβ fibrils, maintain Aβ solubility, and prevent neurotoxic effects (207).

4.8 APP Gene-Knockdown Agents

Gene knockdown, unlike gene knockout, permits fine-tuning of gene expression. So far only site-directed antisense oligonucleotides, directed at the Aβ region of APP, were tried in a strain of mice spontaneously overexpressing APP (208). Administered by i.c.v. injection, with or without antibody, the antisense phosphorothiolated oligonucleotides directed at the midregion of Aβ reversed deficits in learning and memory. Given alone, these oligonucleotides reduced APP levels by 43–68% in the arnygdala, septum, and hippocampus. It has been further proved that the phosphorothiolated oligonucleotides can cross the blood-brain barrier, and can be given in effective doses by i.v. route (195).

4.9 α-Secretase Shift

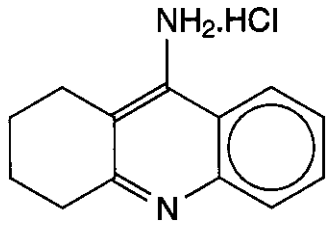
The a-secretase-processing route is dominant under normal conditions and it is unknown whether and how that process is impaired in favor of β-secretase in AD. Whether the increase in production of Aβ40 and/or Aβ42 is achieved at the expense of the a-secretase process is also unknown. In addition, the processing of APP in human neurons appears to differ considerably from rodent CNS primary neuron cultures and continuously dividing cell types (34). Different sites of a-, β-, and y-secretase activity require codistribution of APP, with respective secretases to affect the relative utilization (209). The removal of APP from compartments with β-secretase activity to compartments with a-secretase activity should increase the release of sAPPα and, it is hoped, decrease the release of Aβs (210–212). Phorbol esters acting by activation of protein kinase C (PKC) enhance the release of sAPPα from cortical synaptosomes (213, 214). The observation that muscarinic agents may act in a similar manner (215) provides for collateral efficacy of acetylcholine esterase inhibitors. A phosphatidylinositol 3-kinase inhibitor wortmannin decreased release of both Aβ and sAPPα in N2a neuroblastoma cells expressing either wild-type APP or the Swedish FAD mutant variant (209). Testosterone was reported to increase the secretion of sAPPα without affecting the total amount of cellular APP (216).

5 ATTENUATION OF CHOLINERGIC TRANSMISSION

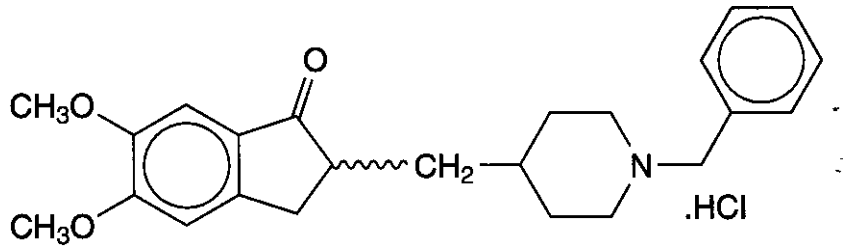
A failing cholinergic transmission may be attenuated either by *(1)* prevention of ACh destruction, *(2)* supplementation of diminishing amounts of ACh released with exogenous, or **(3)** blocking of feedback mechanism regulating the release or synthesis of ACh.

5.1 Acetylcholine Esterase Inhibitors

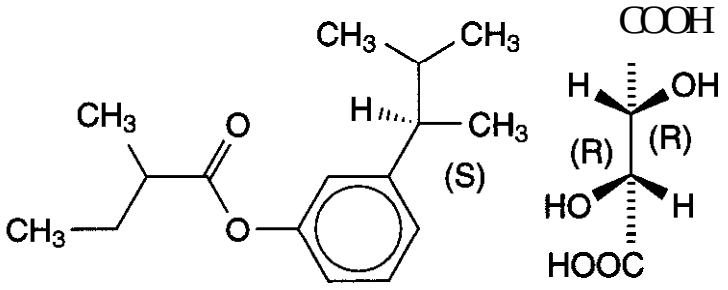
Acetylcholine esterase inhibitors are the first and so far the only group of drugs approved for AD indication. With the advancing destruction of the cholinergic network, the overall quantity of ACh released decreases below the level necessary for transmission. Inhibition of acetylcholine esterase (AChE) may prolong the life of Ach in the synapse, modulating the strength and the duration of the signal. The U.S. FDA has so far approved four AChE inhibitors. The first on the market, tacrine hy-



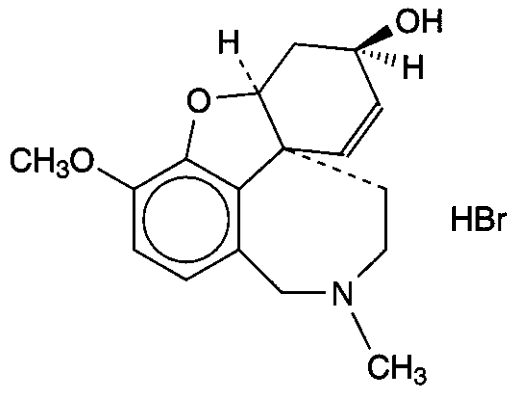
(37) Tacrine (cognex) (1993)



(38) Donepezil (aricept) (1997)



(39) Rivastigmine tartrate (exelon) (2000)



(40) Galantamine hydrobromide (reminyl) (2001)

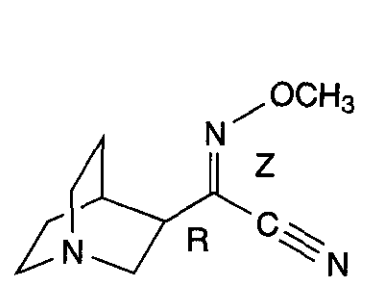
Figure 13.10. Approved acetylcholine esterase inhibitors.

drochloride (Cognex, 37, Fig. 13.10), was approved in 1993. It did inhibit the AChE and butylcholine esterase but the hepatotoxicity limited its use. Donepezil hydrochloride (Aricept, 38) approved in 1997, has a longer half-life time that permits once-a-day dosing and lower hepatoxicity. Rivastigmine tartrate (Exelon, 39), approved in 2000, has a similar profile. Galantamine hydrobromide (Reminyl, 40), initially extracted from daffodils, was approved in 2001. All four AChE inhibitors have shown in well-controlled trials a statistically significant improvement of at least three points on the Alzheimer's Disease Assessment Scale, measuring independently the cognitive (ADAS-Cog) and noncognitive function. The ADAS-Cog part of the scale scores between 0 and 70 points, with zero meaning the patient made no errors at all and 70 meaning the patient is profoundly demented. The normal individuals will usually make some errors, scoring between 5 and 10. One can therefore conclude that the relief provided by this group of drugs is modest. Thus, the results obtained by the approved AChE inhibitors are comparable to those produced by the porcine brain-derived hydrolysate, marketed outside the United States as Cerebrolysin (217).

The future of therapy with these drugs is in question. Although they prevent ACh from rapid hydrolysis, eventually the saved ACh activates the presynaptic autoinhibitory M2 receptors, if any are left, thus reducing the release of endogenous ACh. Because of their nonselective site of action their use eventually leads to M3 AChR stimulation and untoward gastrointestinal effects (nausea and vomiting). They do provide some improvement when prescribed to patients with early to mild AD. Their clinical benefits in advanced AD remain to be proved and seem doubtful. Thus, given the substantial investment in taking these drugs to the market, the extended pre- and postapproval search for collateral efficacy of these drugs, such as in affecting APP processing or allosteric sensitization of nicotinic receptors, seems justified (218–221).

5.2 Muscarinic Acetylcholine Receptor Agonists and Antagonists

An M1-selective agonist would be preferred for two reasons: (1) to activate M1 receptors in the affected areas and (2) to activate α-secretase, shifting the processing of APP from production of Aβ, as M1 muscarinic agonists are reported to do (222, 223). The problem with the muscarinic agents is their selectivity. A muscarinic agonist could be selective to a point in a functional assay and completely nonselective in a binding study. Every known muscarinic agonist tried in preclinical studies possessed an agonist, partial agonist, and antagonist portfolio that was impossible to un-



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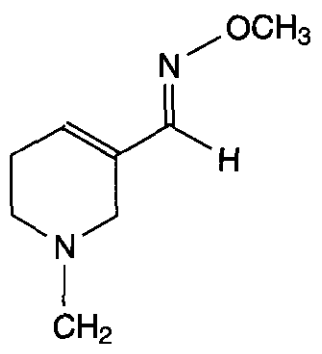

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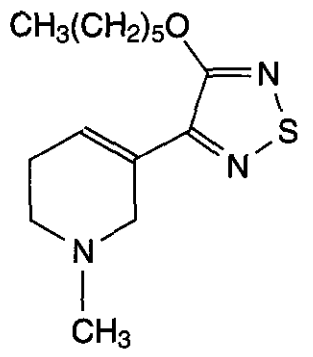

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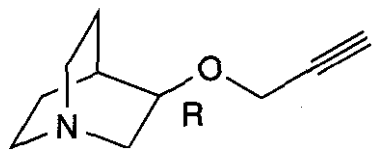

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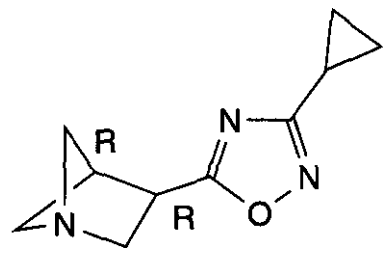

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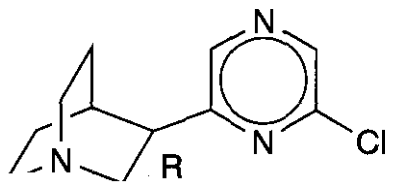

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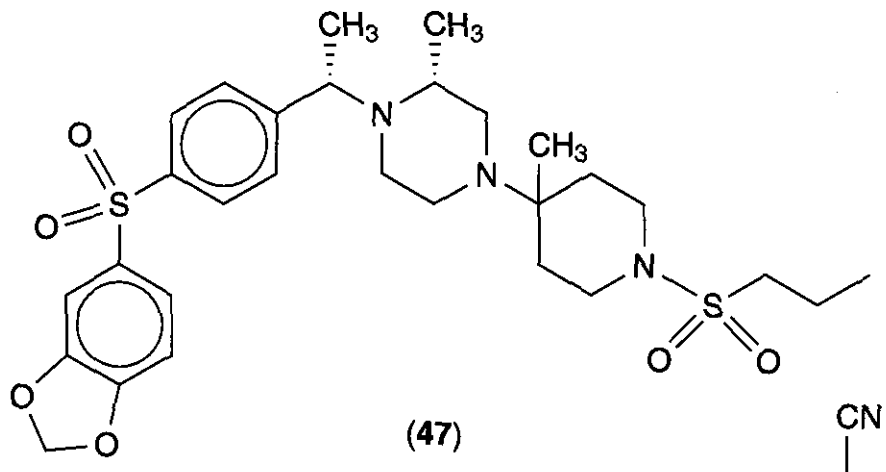

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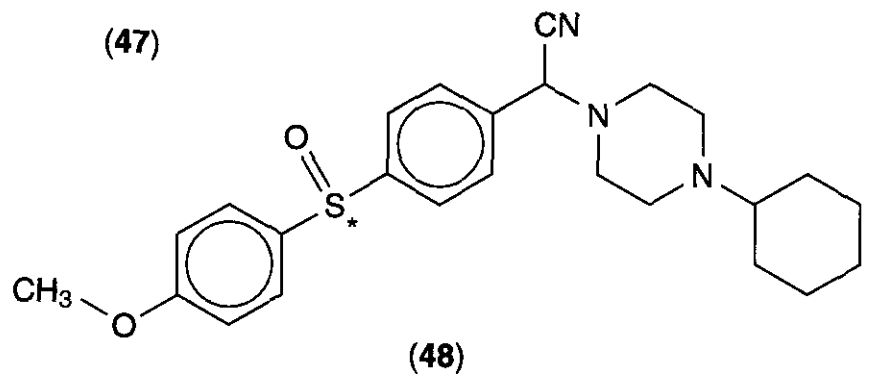

(48)

Figure 13.11. M1-selective agonists, M1/M2 agonist/antagonist, and M2 antagonists.

tangle and was thus dose limiting. Many of them, such as sabcomeline (SB-202026, 41, Fig 13.111, milameline (RU35926, **42**), xanomeline (LY246708, **43**), or talsaclidine (WAL 2014, **44**), have been tried in the clinic and failed. The reasons for failure were insufficient efficacy (lack of statistically significant improvement of cognitive functions) and side effects. Even the most selective M1 agonist talsaclidine was withdrawn from clinical studies, thus placing the muscarinic approach to AD in doubt (224).

Even more desired would be a combination of an M1-selective agonist with M2 antagonist properties, provided that the compound could be made CNS selective. Otherwise, the M2 AChR in the cardiovascular system would be affected. A number of compounds claim to possess that combination. For example, L-687,306 (**45**) and L-689,660 (46) were reported to be M1 agonists and M2 antagonists in functional studies (225) and nonselective in binding studies (221).

The antagonists SCH72788 (47) and SCH57790 (48) were reported to have greater affinity to M2 versus M1 AchR, and improved performance in rodent models of cognition (226,227).

5.3 Nicotinic Acetylcholine Receptor Agonists

Alfred Dunhill's book, The Gentle Art of Smoking, can be found only in the old bookstores, although the legend that smoking tobacco provides some degree of protection from AD persists (http://www.forest-on-smoking.org.uk/factsheets/factsalzheim.htm). It is recognized that nicotine and therefore the nicotinic ACh receptors (nAChRs) are involved in attention, verbal learning, spatial memory, and psycho-motor speed (228). The loss of nicotinic rather than mACh receptors is evident from imaging and postmortem autoradiographic and histochemical studies (229, 230). The first to go are nAChR in the upper cortical layers of the frontal cortex and in the temporal cortex (229). That reduction is far more pronounced than the loss of muscarinic acetylcholine receptors (mAChR), including M2 receptors (230). Particularly affected in AD appear to be neurons with high affinity nAChR of $\alpha4\beta2$ subtype, whereas the low affinity a7 homopentamer nAChR appears to be left intact (231). Epidemiological studies of the smoking population are hampered by the fact that the smokers, as perfect citizens, expire ahead of their cohort and the survivors may therefore belong to a more select group than their nonsmoking peers. The Rotterdam Study, a population-based follow-up study of elderly initially free of dementia concluded that the smokers have a higher risk of dementia and AD (232). Of interest is the finding of that study that the smoking carriers of ApoE $\varepsilon4$ allele had no increased risk, only the carriers of the other two "good" allelic forms. The effect of smoking may, of course, be explained away as being more than just a delivery of nicotine.

What is truly frightening is the report that nicotine alone, acting through nicotinic acetylcholine receptors (nAChR), stimulates angiogenesis and promotes tumor growth and atherosclerosis at concentrations that are pathophysiologically relevant (233).

Nevertheless. collateral functions for nicotine have been found to (1) promote an a-secretase shift, leading to release of neurotropic and neuroprotective sAPPα, and (2) prevent Aβ and CTFβ neurotoxicity (234, 235). Thus, given the remarkable heterogeneity of nAChR [17 known subunits forming multiple functional homopentamers and heteropentamers (236)] and their importance in the CNS, one may hope these adverse affects might be "designed out." From a family of nicotinic agonists based on marine animal toxin anabaseine (49, Fig. 13.12), a selective agonist of a7 nAChR, GTS-21 (50), was developed and tested (231). Its 4-hydroxy metabolite was shown to be equally efficacious on human and on rat a7 receptors. On the other hand, RJR-2403 (51) was equipotent with nicotine in CNS, although it was 15–30 times less potent in peripheral (237). In human nAChR subtypes expressed in *Xenopus* oocytes MR-2403 (51) was reported to be a selective agonist of human $\alpha4\beta2$ nAChR (238). Its close analog, TC-2559 (52), demonstrated CNS selectivity by improving cognition without affecting locomotor activity (239). One way to compensate for the loss is the search for allosterically potentiating ligands (APL) of nAChRs, which increase the probability of channel opening in-

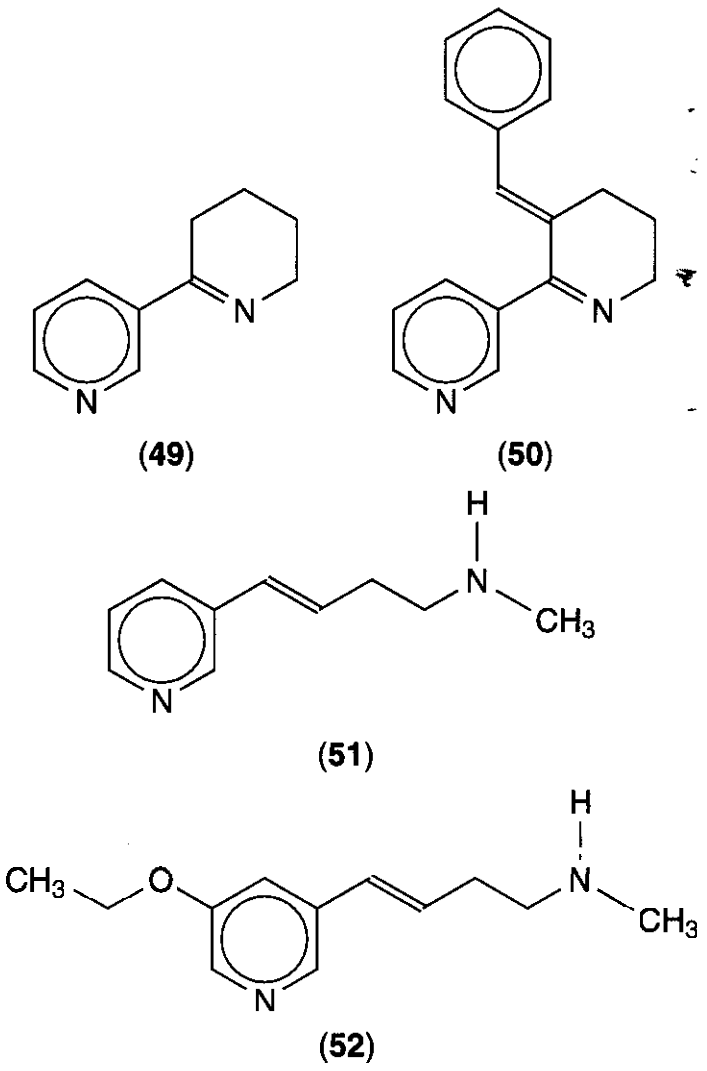

Figure 13.12. CNS-selective nicotinic agonists.

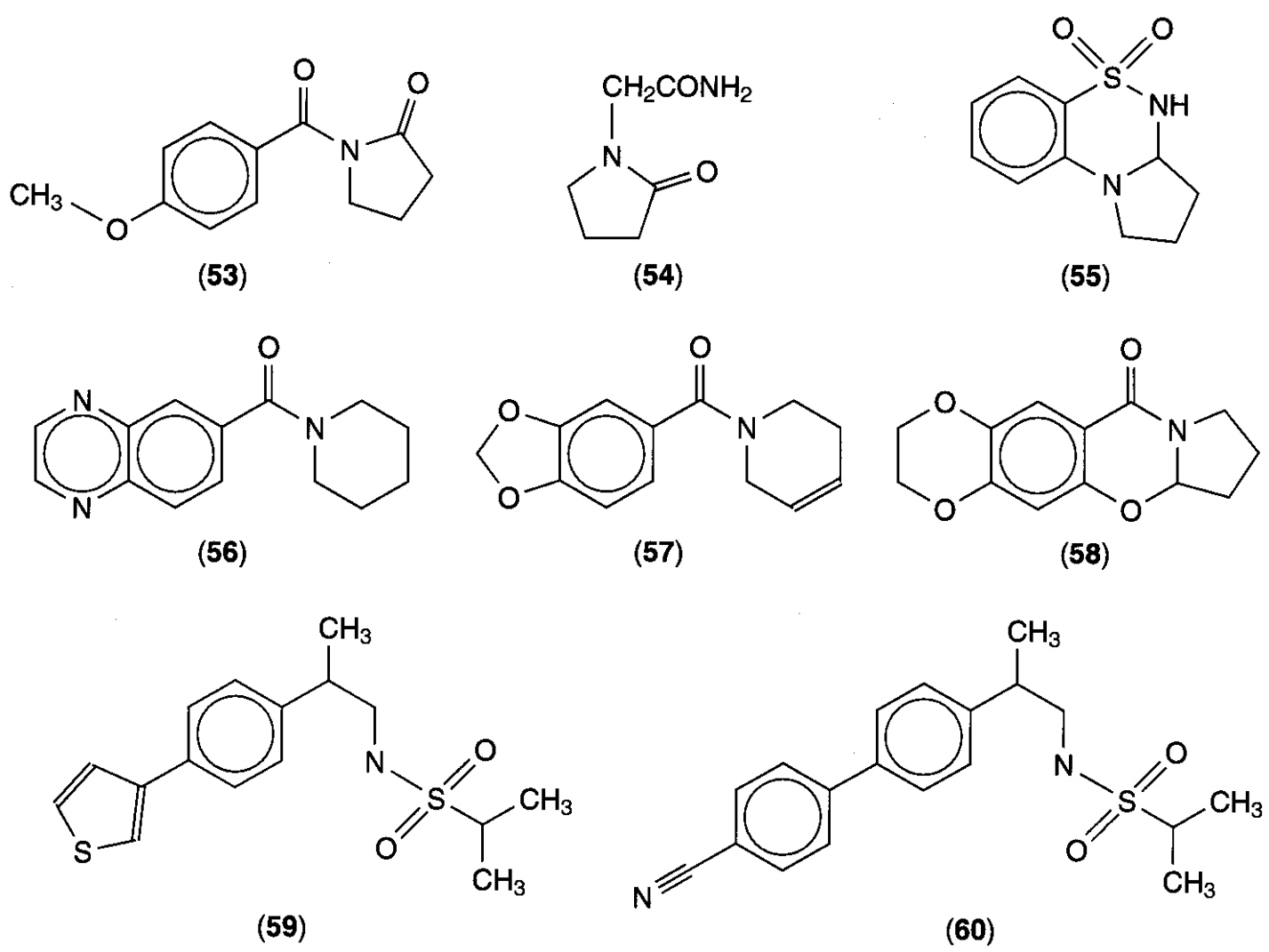

Figure 13.13. Modulators of AMPA responses.

duced by Ach and nicotinic agonists, and also decrease the receptor desensitization (230).

6 MODULATION OF AMPA RESPONSES

Episodic, short-term memory is the first to go with the progression of AD and other neurodegenerative diseases (240). Compounds such as aniracetam (53, Fig. 13.131, piracetam (54) or S18986–1 (55) (241), CX516 (56) (242), CX509 (**57**) (243), or CX614 (**58**) (244), and LY392098 (**59**) (245) or LY404187 (60) (246) positively modulate α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors. In addition S18986–1 enhances AMPA-mediated release of noradrenaline (247). The term nootropic agents is often applied to these compounds and one group was even registered as Ampakines (241, 248). Compounds such as aniracetam, piracetam, or S18986–1 positively modulate the AMPA receptors by suppressing the desensitization process (249). The Ampakines also facilitate the induction of NMDA-dependent, long-term potentiation in hippocampus in *vivo* (250). The mechanism of action of far more potent LY392098 and LY404187 was suggested to be different from that of Ampakines, in that the magnitude of the enhancement is time dependent, increasing with continued agonist exposure (246). In either case, despite the frequent forward-looking statements, compounds like the AChE inhibitors are nothing more than palliatives free of disease-modifying properties.

7 ESTROGENS AND ANDROGENS

The belief that estrogen, and especially the postmenopausal estrogen replacement therapy (ERT), may reduce the risk of AD is very strong. The idea was advanced by many short-term, open-label studies but rejected by the well-controlled, long-term studies (251–254). The discrepancy between the in vitro promises and the in *vivo* hopes is glaring (255,256). In cultured neurons 17β-estradiol reduced secretion of Aβ40/42 (257) and protected them from

Aβ toxicity (258), providing a molecular basis for the claim of ERT as an AD-preventive measure. Regretfully, longer-term ERT studies provided negative results (252). The use of estrogen for treatment of mild to moderate AD, in women who had hysterectomy, for a period of over 1 year also did not slow the disease progression (253). The results of the Rotterdam Study (254) did not support the hypothesis that a longer reproductive period reduces the risk of dementia. The awaited results of truly long term studies will hopefully provide an answer, although prognosis is not optimistic (259).

Like the estrogen levels, so the total testosterone level and the bioavailable testosterone level, in particular, decline in both sexes with age. Testosterone supplementation was reported to protect orchiectomized, aging male rats from osteoporosis (260), increase nerve growth factor levels in rat brain (261), or improve depression in aging men (262). The treatment of N2a cells and rat primary cerebrocortical neurons with testosterone increased secretion of sAPPα and diminished the release of Aβs (263). In a similar manner, treatment of GT1–7 rat hypothalamic cells with testosterone stimulated the α-secretory pathway by increasing the secretion of sAPPα, without affecting the total amount of cellular APP (216). In a group of six men treated for adenocarcinoma of the prostate with hormonal suppressants, the precipitous drop in their levels of testosterone and estradiol correlated with the increase of Aβ40 plasma levels (264). The therapeutic implications of observations like these remain uncertain.

8 IMMUNOTHERAPY

Very low levels of autoantibodies to Aβ were detected in the elderly, although they do not appear to be indicative of either the presence of AD or protection against developing that disease (265). In PDAPP transgenic mice overexpressing APP_{V717F} immunization with Aβ42 was reported to provide, depending on the time of immunization, protection from development of AD-like neuropathologies or reduction of their extent and progression (266, 267). The overall levels of Aβ were not affected. Peripheral administration of Aβ antibodies suggests their ability to cross the blood-brain barrier and reduce the amyloid burden (268). What is of interest is that the antibodies trigger microglia to clear amyloid deposits through cell-mediated phagocytosis, something they do not do alone (96). The synthetic, aged, and fibrillar Aβ42 (AN-1792) was used with adjuvant as an immunization tool in clinical trials (269). When some of the patients (5 of 360) showed brain inflammation, the trial was prudently suspended (297).

Given the fact that the Aβ42 does cross the blood-brain barrier in both directions, and may begin to form fibrils, immunization with a nontoxic, nonammmyloidogenic, homologous peptide K6Aβ1–30-NH_2 was suggested as a safer approach (270). A remarkable (89 and 81%) reduction of cortical and hippocampal brain amyloid burden was reported. Another approach reported was the production of highly specific antibodies locking the epitope EFRH of Aβ (271). One should remember that, unlike transgenic mice, the AD patients usually (298) do not show elevated levels of Aβ in either blood or CSF (a measure of success) and that their immune response may not match the expectations (272).

9 IMMUNOSUPPRESSION

Given the evidence presented, the AD manifests itself with the reactive microglia clustering and attacking SPs (273). The SP stimulate the microglia to become not only reactive but also neurotoxic (274). Whether an immunosuppressive therapy aimed at the inflammatory glia endogenous to the CNS has a chance of success remains an open question. Neuroimmunophilin ligands like cyclosporin A or FK-506 (tacrolimus) prevented overexpression of APP and Aβ and stimulated release of neuroprotedive sAPPα (275).

10 INHIBITORS OF APOPTOSIS

A large family of cysteine proteases has been identified as responsible for apoptosis of neuronal cells. Activation of caspase-3 in CNS was observed in stroke, spinal cord trauma, head injury, and AD and proposed as a target of

therapeutic intervention (276). The inhibition of neuronal loss in animal models of stroke and head injury by peptide-based inhibitors of caspase may provide leads for future systemically active agents. However, to do that the etiologic role of caspase in AD must be further investigated and proved (277).

11 ANTI-INFLAMMATORY AGENTS

A comparison of positron emission tomography (PET) and MRI scans of AD patients and normal individuals suggests activation of microglia in the early stages of the disease (278). The C^{11} tracer indicated increased binding in areas, which are known to be affected by the disease. Over a period of 12–24 months, the repeated MRI scans correlated the areas of the highest binding with the areas of highest rate of atrophy. In a mouse model of AD, ibuprofen suppressed plaque pathology in mice fed chow containing 375 ppm for 6 months (279). Numerous studies of risk and progress of AD among the users of anti-inflammatory drugs suggested a time of use-related decrease of risk (280), but could not prove their therapeutic usefulness (281). The selection of the right anti-inflammatory agent might be needed. At least two of the approved nonsteroidal anti-inflammatories (NSAIDs), ibuprofen (61, Fig. 13.14) and naproxen (**62**), and one cyclooxygenase (COX)-2 inhibitor, rofecoxib (**63**), are listed in IND trials for AD indication. So far the NSAIDs, steroids, or even antimalarials do not seem to work as therapeutics after the AD has been diagnosed. In a small, well-controlled study the antimalarial hydroxychloroquine showed no difference in the disease progress between the treated and the placebo groups (282).

A proteinase inhibitor characteristic of acute-phase inflammation, α1-antichymotrypsin (ACT) has been identified in SP and reported to be elevated in AD patients (283). The level of ACT depended on ApoE genotype and was the highest in ε3/ε3 homozygotes and correlated with cognitive impairment and duration of the disease. The ε4 genotypes have shown a lower level of ACT and no correlation with impairment or duration. One should keep in mind that the same ACT inhibits Aβ

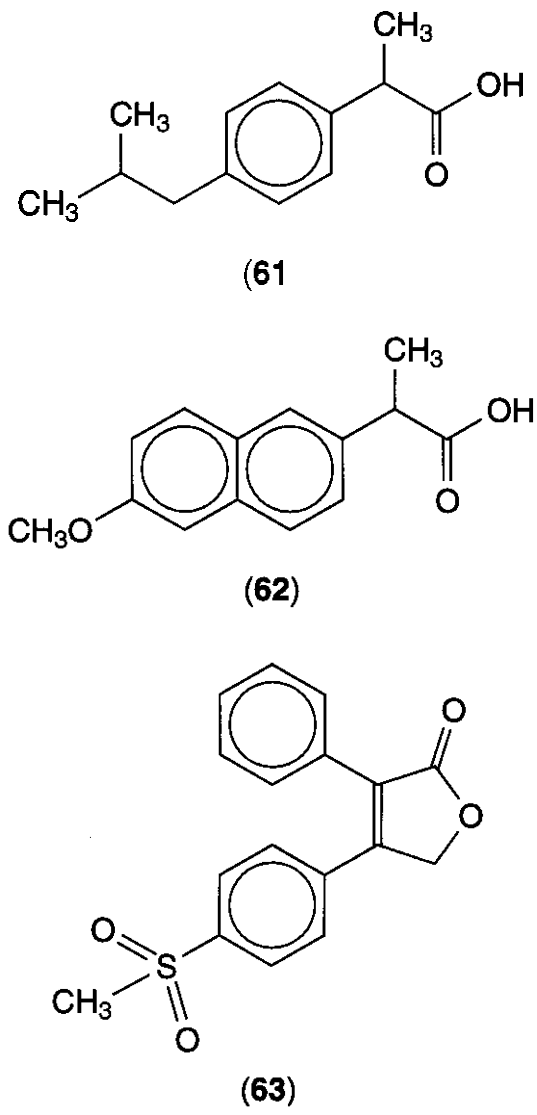

Figure 13.14. Anti-inflammatory agents in IND trials for AD indication.

degradation ***in vitro*** and ***in vivo*** (284) and catalyzes β-sheet aggregation in a transgenic mouse (285).

12 ANTIOXIDANTS

Evidence of selective oxidative damage, resulting from redox imbalance, involving neurons tied to AD is accumulating. The advanced glycation end products, nitration, lipid peroxidation adduction products, carbonyl-modified neurofilament protein, and free carbonyls belong to the list (200). The question remains whether the oxidative damage is caused by Aβ, known to possess oxidative and hydrolytic properties (286, 287), or by other factors, whereas Aβ is released as an antioxidant in response to oxidative stress (200). As in other groups of potential AD therapeutics there is a noticeable difference between their behavior ***in vitro*** and ***in vivo***. A number of potential antioxidants are or have been in clinical trials

as potential therapeutics: vitamin E (288), estrogen (251–254), ginkgo biloba (289), selegiline (290), idebenone (291), acetyl-L-carnitine (292), or melatonin. So far the results are less than promising.

13 CONCLUSIONS AND PERSPECTIVE

When faced with a disease. science has a number of options. It can look for means to prevent or delay the onset, reverse the damage (cure), or at least slow down the progress of disease. When the opportunities to do so do not present themselves, it can look for means to improve the quality of life of the victim while the disease progresses. The soundness of search for treatment of choice depends on what is known and what is suspected about the cause and course of the disease. The as yet uncertain etiology of AD precluded the attempts to prevent its onset. Cholesterol, immune response, and oxidation damage appear to precede the deposition of Aβ and formation of SP and NFT. This is why the epidemiology and in *vitro* studies fail to point to right therapies. At present the statins in drinking water are not an option. The statins, antioxidants, anti-inflammatory agents, estrogens, or testosterone may belong to preventive measures but may fail as therapeutics. The imprecise diagnostic tools, except for brain necropsy or prohibitively expensive longitudinal scanning with MRI or FDG/PET, hamper to this day the development of drugs that may delay the onset or reverse the damage or slow down the progress of disease. Thus the only option available initially was the search for improving quality-of-life palliatives. In working with the available tools, this appeared to be the most sensible approach, using the secondary change in symptoms as the measure of success. A more daring approach, an attempt to develop the treatment of putative causes of the disease with the simultaneous search for indicators of progress, is being adopted in synchrony with the progress in etiology.

One should not forget, with the exemption of limited FAD cases. AD is the disease of old age. Aging, amyloidosis, and neurodegeneration appear to be invariably linked.

A recent, visible increase in the number of AD publications may awake false hope, given the percentage of forward-looking statements appearing simultaneously: that numerical growth is still to convert into a feasible hypothesis and a successful line of therapeutics. This will not be achieved by reviewing each other's reviews. An expensive, aggressive approach, in which every hypothesis, every lead, no matter how anecdotal, should be pursued to exhaustion by the pharmaceutical stalwarts and the boutiques alike. Otherwise, the promised "bulging pipeline" of AD drugs will remain empty save for spinach (293).

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CHAPTER FOURTEEN

Cognition Enhancers

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1 INTRODUCTION

1.1 Dementia

The average life expectancy has increased in modern times, and with it so has the percentage of the world's population reaching old age. The elderly are at increased risk to suffer from dementia, which has the clinical features of impairments in cognition, memory, language, and visuospatial skills. The need for effective treatments in Alzheimer's disease (AD), age-associated memory impairment (AAMI), multi-infarct dementia (MID), vascular dementia, and Parkinsonian dementia is fast becoming acute (1,2). Although successful intervention has been achieved for a few reversible cognitive deficits, such as those induced by nutritional deficiencies (3), depression (4), acute drug toxicity (5–7), and some metabolic disorders (8, 9), formidable challenges remain.

2 ASSESSING COGNITION

2.1 Animal Models of Cognition

Although there are several accepted methods for assessing cognitive function in humans, this presents a more complex problem preclinically. Experimental paradigms vary enormously. Different animal species are used, such as nonhuman primates, rodents, and pigeons. Substrains in any given species (especially mice) often provide conflicting results, as can the age of the animal. Nearly 100 behavioral tasks used to assess various aspects of cognition have been estimated (10). Furthermore, protocols for behavioral assays that are commonly employed, such as passive avoidance (11) or the Morris water maze (12), can vary widely between investigators, often providing contradictory results (10). This variability has precluded all but the most generalized comparisons of the efficacy for different cognitive-enhancing compounds in animal models.

Another major obstacle in developing effective therapeutic strategies for cognitive enhancement is a lack of knowledge about how complex processes like learning and memory occur. Preclinical studies have revealed functional properties of neuronal plasticity that have intuitive appeal as potential substrates for memory encoding. The best studied of these is long-term potentiation (LTP) (13, 14). LTP is a long-lasting, use-dependent increase in synaptic strength that was first described in the hippocampus, a brain region critical for the formation of new long-term memories in humans (15). It has been hypothesized that the plasticity that occurs during LTP may provide a molecular basis for learning and memory. As such, electrophysiological tests to determine the effects of drugs on LTP have often been used as a way to identify cognitive enhancers. However, despite decades of dedicated research, the relationship between LTP and learning or memory is still actively debated (16–20). For this reason, and because this topic has been widely reviewed (21), studies assessing LTP will not be considered in this chapter.

2.2 Measurements of Human Cognition

Animal studies are used routinely for predicting the efficacy of cognitive-enhancing pharmaceutics in humans, but this has its limitations. For example, every marketed agent for the treatment of dementia has been shown to enhance cognition in some animal model, but the converse is not always true. Many agents that produce positive effects in animal models fail in clinical trials (22, 23). Increasing the predictiveness of preclinical behavioral tests would greatly improve the development of effective treatments for dementia. Presently, there is no single behavioral test or animal model that increases the likelihood of identifying an effective cognitive-enhancing compound in humans, although strategies have been proposed for achieving this goal (24).

A fundamental problem with attempting to quantify cognitive function is that cognition is multifaceted. This is especially evident in the preclinical setting, where the individual elements of attention, learning, and memory cannot be adequately measured in a single behavioral test. However, more extensive tests have become somewhat standardized to measure cognition in humans. The Mini-Mental State Examination [MMSE (25)] and the cognitive subscale of the Alzheimer's Disease Assessment Scale [ADAS-Cog (26, 27)] are used in virtually all clinical evaluations of cognitive enhancers. Use of these protocols allows reasonable comparisons to be made between drugs.

3 COGNITION-ENHANCING THERAPIES

3.1 Therapeutic Approaches

Alzheimer's disease is the best known age-related disorder for which the primary symptom is dementia. Advances have been made in understanding the etiology of AD, but much remains to be discovered. Clearly, there are documented neuropathological changes in the brain. For example, there is a broad spectrum of neurotransmitter dysfunction, where acetylcholine (ACh), serotonin [5-hydroxytryptamine (5-HT)], norepinephrine, dopamine (DA), and glutamate levels are reduced (28–31). Understandably, therapies that affect these neurotransmitter levels have been the focus of intense research.

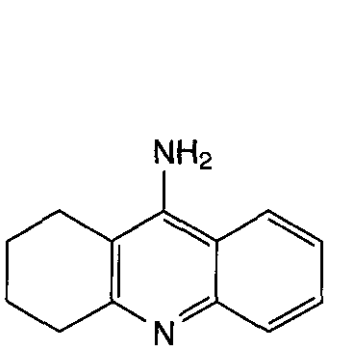
3.2 Clinically Approved Agents

Palliative treatment of Alzheimer's disease, the most common cause of dementia, has been the primary focus of research in cognitive enhancement. However, despite these efforts, effective pharmacological interventions remain elusive. The most fruitful pharmacological strategy pursued in AD research to date has focused on the relief of cognitive and memory deficits that are attributed to cholinergic dysfunction.

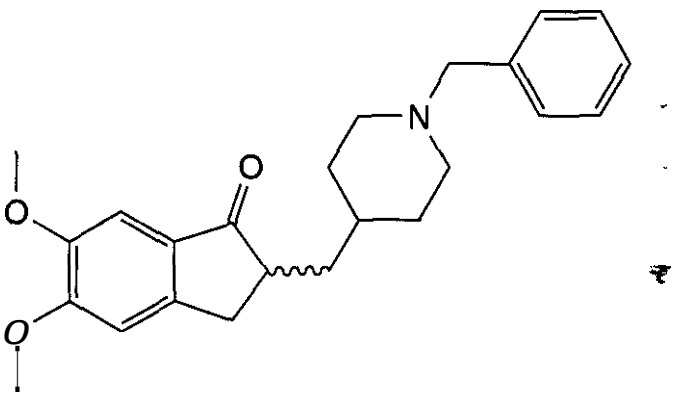
3.2.1 Acetylcholinesterase Inhibitors (AChEI). There is substantial rationale for exploring cholinomimetic therapies for the treatment of the symptoms of dementia (32–38). Central cholinergic depletion is a hallmark of AD and experimentally induced cholinergic dysfunction produces cognitive deficits both preclinically and clinically (39). Acetylcholinesterase (AChE) inhibitors suppress the normal breakdown of acetylcholine from the synaptic cleft, thereby increasing the overall level of ACh available to the relevant postsynaptic receptors. As such, AChE inhibitors represent a valid approach in the development of cognitive-enhancing compounds. Indeed, acetylcholinesterase inhibitors, such as tacrine (1), (Cognex; Warner-Lambert Co.), donepezil (2), (Aricept, E2020; Eisai Co. Ltd.), rivastigmine (3), (Exelon, Novartis), and galantamine (4), (Reminyl; Janssen) are the only U.S. FDA-approved drugs currently marketed in the United States for the symptomatic treatment of AD (Fig. 14.1). It should be noted, however, that tacrine is no longer widely used because of the recent advent of safer AChE inhibitors (e.g., Aricept). The evidence to date indicates that these agents provide only short-term relief, in part by slowing the progression of the disease (40–43).

3.3 Exploratory Approaches

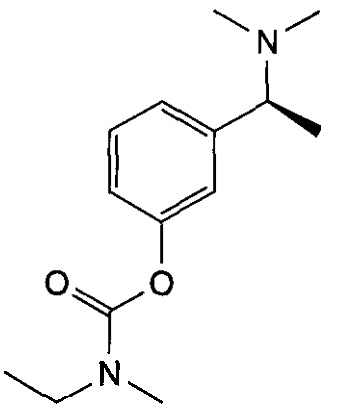
Many other pharmacological approaches are currently being examined to reduce the cognitive impairments seen in AD or in other dementia. These approaches include agents that act at glutamate receptors [e.g., AMPAkines



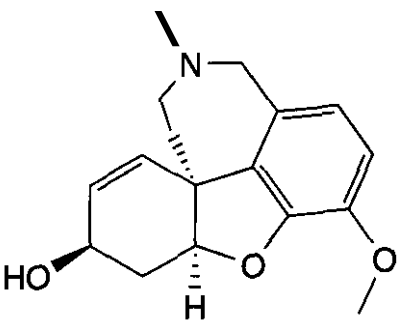
(1)tacrine



(2) donepezil



(3) rivastigmine



(4) galantamine

Figure 14.1. Acetylcholinesterase inhibitors on the market in the United States.

(44–46), NMDA receptor modulators (47–50)], monoarnine oxidase B (MAO B) inhibitors (51–55), antioxidants (52, 56–59), nootropics (41, 60), lipid-lowering agents (e.g., statins) (61), insulin (62), anti-inflammatory agents (63–67), and estrogen supplementation (63, 65, 66, 68, 69). Because several other reviews are available that compare these therapeutic approaches (70–77), the remainder of the present chapter focuses on ion channel modulation approaches to cognition enhancement.

4 ION CHANNEL MODULATION OF NEUROTRANSMISSION

In the remainder of this chapter, we briefly review the status of efforts that have in common the potential to enhance the activity of multiple neurotransmitter systems through the modulation of gated ion channels (also see reviews in Refs. 10, 78–80). Specifically, we discuss recent advances in the areas of: (1) y-aminobutyric acid subtype A/benzodiazepine receptor ($GABA_A$/BzR) inverse agonists; (2) nicotinic acetylcholine receptor (nAChR) agonists; (3) serotonin subtype 3 receptor ($5\text{-}HT_3R$) antagonists; and (4) potassium M-channel inhibitors.

4.1 $GABA_A$/Bz Receptor Complex

4.1.1 Physiology and Pharmacology of the $GABA_A$/Bz Receptor Complex. The neurotransmitter y-aminobutyric acid (GABA) and its associated receptors constitute the major inhibitory pathway in the brain (81). Attenuation of postsynaptic excitatory responses is achieved when GABA interacts with its receptors to stimulate ion conductances that lead to localized membrane hyperpolarization. In particular, the conductance of Cl^- ions is controlled through the ligand-gated $GABA_A$ and $GABA_C$ (82–84) receptor subtypes, whereas conductance of Ca^{2+} and K^+ ions occurs through the $GABA_B$ receptor subtype (85). The majority of GABA's inhibitory effect in the CNS is mediated by the $GABA_A$ receptor subtype (86).

$GABA_A$ receptors belong to the superfamily of ligand-gated ion channels that include serotonin subtype 3 ($5\text{-}HT_3$) receptor, nico-

tinic acetylcholinergic (nACh) receptors, and strychnine-sensitive glycine receptors (81, 87). $GABA_A$ receptors are believed to be hetero-oligomers assembled from eight protein subunits, drawn from several classes (α_{1-6}, β_{1-4}, γ_{1-4}, δ, ε, π, θ, and ρ_{1-3}) (88–92). The subunits of the $GABA_A$/BzR are classified according to the degree of sequence homology. Each subunit class is defined to exhibit amino acid homologies of about 60 to 80%, whereas the amino acid homology between the various subunit classes is approximately 30–40% (93, 94).

Various combinations of these subunits result in different $GABA_A$ channel isoforms that display differential pharmacology, CNS distribution, and developmental pattern (95). The number of different functional isoforms in *vivo* is unknown, but estimated to be between 10 (87) and 150 (96, 97). The genetic loss or mutation of a $GABA_A$ channel subunit can have profound neurological consequences. For example, it has recently been shown that disrupting the mouse gabrb3 gene, responsible for coding the β_3 subunit, produces EEG abnormalities, seizures, poor motor skills, and cognitive deficit (98). This research suggests that disruption of the analogous human *GABRB3* gene may contribute to the pathology of Angelman syndrome (99), a severe neurological disorder characterized by cognitive deficits caused by deletionslmutations of maternal chromosome 15qll-q13 (100, 101). Positron emission tomography (PET) studies with the high affinity BzR ligand [^{11}C]flumazenil on three Angelman syndrome patients with maternal deletion of 15qll-q13 found a decreased number of $GABA_A$/Bz receptors in the frontal, parietal, hippocampal, and cerebellar regions, which could partially underlie the cognitive deficits of this disorder (102).

The $GABA_A$ ion channel is formed by a pentameric assembly of hetero-oligomeric subunits, with each subunit having four transmembrane-spanning domains (93, 103). The recombinant $GABA_A$/BzR complex assembled from two a,, one β_2, and two γ_2 subunits most closely resembles the biochemical, electrophysiological, and pharmacological profile of native $GABA_A$/Bz receptors of the mammalian brain (104–107).

The $GABA_A$ ion channel can be modulated through ligand interaction with several receptors (see review in Ref. 108). The $GABA_A$/Bz receptor complex has traditionally been differentiated into two general subtypes, based on their affinities for the prototypical 1,4-benzodiazepines (Bz) flunitrazepam (23) and diazepam (22) (109). Diazepam (22) has high affinity for the $GABA_A$/Bz receptors composed of the α_{1-3} and a, subunits and are collectively termed the "diazepam-sensitive" (or "DS") binding sites. The DS binding sites are further categorized as being either Type I or Type II BzRs. The Type I receptor has pharmacological properties similar to those of the $\alpha_1\beta_2\gamma_2$ isoform, whereas the Type II BzR is associated with the $\alpha_{2,3,5}\beta_2\gamma_2$ subunit-containing $GABA_A$ isoforms. Diazepam (22) does not have high affinity for the a, and β_6 subunit-containing $GABA_A$/BzR isoforms, collectively termed "diazepam-insensitive" (or "DI") sites (110, 111).

The interaction between the subunits and the Bz receptor ligand provides for a wide range of allosteric regulation of chloride ion flux within the associated ion channel (112, 113). Agonists (e.g., prototypic 1,4-benzodiazepines), potentiate GABA-induced Cl^- flux and are typically characterized by sedative, anxiolytic, and anticonvulsant effects (114). Partial agonists, antagonists, and inverse agonists are also well known (115, 116).

Inverse agonists of the $GABA_A$ receptor retard GABA-induced Cl^- flux, thereby indirectly potentiating the propagation of excitatory signals. Typically, this type of modulation produces anxiogenic, somnolytic, and proconvulsant features (117–121). It deserves mention that the anxiogenic pharmacological property attributed to inverse agonists has recently been hypothesized to be a downstream result of hyperattentional impairments stemming from the behavioral assays that involve fear- or anxiety-related stimuli (122). Nevertheless, $GABA_A$/Bz receptor inverse agonists, because of their unique ability to reduce the inhibitory effects of GABAergic neurotransmission, continue to be investigated as therapies for several disorders, including dementia (123–125) and alcohol addiction (126–129).

One of the important features of AD's neuropathology is the degeneration of cholinergic

cells within the nucleus basalis of Meynert (NBM) with associated loss of afferents to the neocortex and amygdala (130, 131). The importance of these cholinergic neurons for learning and memory in experimental animals has been well established (132–139). Because NMB cholinergic neuronal activity is influenced by local GABAergic interneurons (140, 141), numerous experiments have been undertaken to investigate BzR inverse agonist effects on cortical ACh release and performance in cognitive tasks. It has been postulated that this form of intervention would enhance synaptic transmission while preserving informational fidelity (123).

4.1.2 Pharmacophore Model for the $GABA_A$/Bz Receptor Complex. The Bz receptor, sometimes referred to as the ω binding site, is located at the interface between the a and γ subunits (95, 121, 142–144). The identification of conserved residues necessary for drug-receptor interaction has been difficult, but progress has been made using site-directed mutagenesis, photoaffinity labeling, and chimeric subunits, as discussed below.

Most notable among the α_1 subunit residues to be identified from mutation analyses as being intricately involved with the $GABA_A$/Bz receptor is histidine 101 (rat numbering) or 102 (human/bovine numbering) (145,146). Photoaffinity labeling of the bovine αHis102 occurred preferentially with the agonist [^{3}H]flunitrazepam rather than with the inverse agonist [^{3}H]Ro 15-4513, where the photoincorporation is associated with an a, receptor fragment between residues 104 and the carboxyl terminus (147, 148). This supports the view that these structurally distinct BzR ligands interacted with different amino acid residues in the binding domains (147–149). It was further postulated from these studies that α_1H102 contributes to the L_3 lipophilic region, where the pendant phenyl group of diazepam (22) and other 5-phenyl benzodiazepines are believed to occupy (149).

Other amino acids within the α_1 subunit associated with the Bz binding site, affecting binding affinities by more than 10-fold, are Tyr-159, Gly-200, Thr-206, and Try-209 (145, 150–154). Site-directed mutagenesis of the a, subunit found amino acid Ile-215 strongly associated with the high affinity binding of RY80 (29), an imidazobenzodiazepine selective for the $GABA_A$ receptors containing the a, subunit (155). In another study using chimeric α_1/α_5 subunits and site-directed mutagenesis of the a, subunit, residues α_5Ile215 and α_5Thr208 were identified with the high affinity binding of L-655,708 (31), another a, subunit-selective ligand (156).

Within the γ_2 subunit, site-directed mutagenesis has revealed two residues, Phe77 and Met130, to be necessary for high affinity binding of BzR ligands (157, 158). Additional investigations using γ_2/α_1 chimeric subunits identified two domains of the γ_2 subunit, Lys-41/Try-82 and Arg-114/Asp-161, which together are necessary for high affinity binding (159). Mutagenesis within the γ_2 Lys-411 Trp-82 chimera further identified Met-57, Tyr-58, and Ala-79 as important for this binding (160).

Many laboratories have postulated pharmacophore models to account for the relationship between BzR ligand interaction and intrinsic activity to modulate the $GABA_A$ receptor function [see Codding (161, 162); Cook (121, 163–172); Crippen (173, 174); Filizola (175); Frier (176); Gardner (177); Gilli and Borea (178); Loew (179, 180); and Wermuth (181)]. Among these was a model for understanding binding affinity and pharmacological action of BzR β-carboline ligands, developed using three-dimensional quantitative structure-activity relationship comparative molecular field analysis (3D QSAR CoMFA) (163) and refined employing a procedure termed GOLPE (generating optimal linear PLS estimates) *ab* initio calculations (166). This model was later incorporated into a unified pharmacophore/receptor model that proposed only one binding cleft with multiple sites for interaction by agonist, antagonist, and inverse agonists (167, 182).

The unified pharmacophore/receptor model proposed by Cook and coworkers (167, 182) employed CoMFA of the structural parameters of 136 different BzR ligands, selected from 10 structurally diverse classes of compounds. The model described the BzR site as consisting of several key recognition elements, including two hydrogen bond donor sites

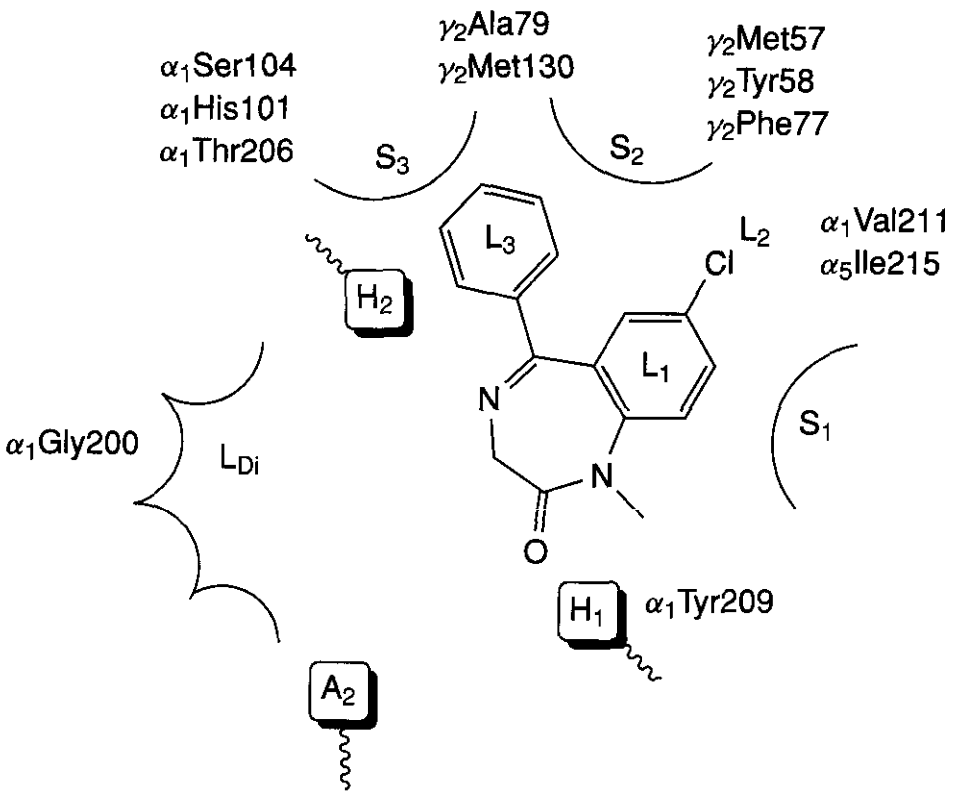


Figure 14.2. Pharmacophorelreceptor model of the $GABA_A$/BzR with ligand diazepam (22). Preliminary locations of the receptor's key amino acids are indicated. (Modified from Ref. 90.)

termed H_1 and H,; a hydrogen bond acceptor site termed A_2; four lipophilic regions termed L_1, L_2, L_3, and L_{Di}; and three regions of negative steric repulsion described as S,, S_2, and S_3 (Fig. 14.2). Another common pharmacophore/receptor model for the α_1-, α_2-, α_3-, α_5-, and a,-containing $GABA_A$ isoforms using 19 nonselective BzR ligands was recently published, which identified similar receptor/ligand interactions (175).

The Cook group further refined their unified pharmacophore/receptor model to provide additional insight into the different binding domains of the $GABA_A$/BzR subtypes $\alpha_1\beta_3\gamma_2$, $\alpha_5\beta_3\gamma_2$, and $\alpha_6\beta_3\gamma_2$. This analysis was based on the affinities of 151 BzR ligands from nine different structural families at five distinct ($\alpha_{1-3,5,6}\beta_3\gamma_2$) recombinant $GABA_A$/BzR subtypes (183).

Cook and coworkers undertook the difficult task of correlating the data between the amino acids believed to be involved in ligand binding and the specific region of their unified pharmacophore/receptor model (90). Some of the amino acid residues likely associated with BzR ligand binding are indicated in the unified pharmacophore/receptor model presented in Fig. 14.2. There are several key protein-ligand interactions that deserve mention, but in the author's words, it is clear that "much work remains at the molecular level to determine the exact location and function of the amino acids in question" (90).

The unified pharmacophore/receptor model (167, 182, 183) is the most advanced to date and will be used in the following discussion for comparing $GABA_A$/Bz receptor affinities and published in *vivo* pharmacological profiles between closely related classes of ligands. Detailed data for $GABA_A$/BzR ligand affinity at the various $GABA_A$ isoforms have only recently been available, and remain largely incomplete for all the many BzR ligands reported to date. Far fewer data have appeared in the literature for a $GABA_A$/BzR ligand's ability to modulate individual $GABA_A$ isoforms.

It is difficult to predict the in *vivo* pharmacology of a $GABA_A$/Bz receptor ligand based solely on its in vitro binding affinity and modulating effect for several different $GABA_A$ isoforms (114). For example, the in *vivo* inverse agonist DMCM (16) is an in *vitro* negative modulator at the $\alpha_{1-3}\beta_3\gamma_2$ and $\alpha_5\beta_3\gamma_2$ isoforms and a positive modulator at the $\alpha_5\beta_1\gamma_1$ isoform (184–187) (Table 14.1). Also, the classic in *vivo* antagonist flumazenil (Ro 15-1788, (26) is an in vitro weak positive modulator at the $\alpha_1\beta_3\gamma_2$ and $\alpha_2\beta_3\gamma_2$ isoforms, a positive modulator at the $\alpha_3\beta_3\gamma_2$ and $\alpha_4\beta_3\gamma_2$ isoforms, a weak negative modulator at the $\alpha_5\beta_3\gamma_2$ isoform, and a positive modulator at the $\alpha_6\beta_2\gamma_2$ isoform (187, 188).

Table 14.1 Reported BzR Ligand Modulation of GABA-Induced $GABA_A$ Isoform Function

Ligand	Modulation of GABA-Induced Function Positive (+); Negative (−); or Not Determined (ND)						Reference[a]
	α_1	α_2	α_3	α_4	α_5	α_6	
β-Carboline series							
Abercanil (5)	+	+	+	+	+	ND	$(w/\beta_3\gamma_2)^1$
	ND	+	ND	ND	ND	ND	$(w/\beta_1\gamma_2)^{2,6}$
	ND	+	ND	ND	ND	ND	$(w/\beta_1\gamma_1)^{2,6}$
DMCM (16)	−	−	−	−	−	ND	$(w/\beta_3\gamma_2)^1$
	−	−	−	ND	−	ND	$(w/\beta_1\gamma_2)^{2,6}$
	+	+	+ weak	ND	+	ND	$(w/\beta_1\gamma_1)^{2,6}$
	ND	ND	ND	ND	−	ND	$(w/\beta_2\gamma_2)^7$
β-CCM (14)	−	−	−	−	−	ND	$(w/\beta_3\gamma_2)^1$
	−	−	−	ND	−	ND	$(w/\beta_1\gamma_2)^{2,6}$
	+	+	−	ND	− weak	ND	$(w/\beta_1\gamma_1)^{2,6}$
Benzodiazepine series							
Flunitrazepam (23)	+	+	+	+	+	ND	$(w/\beta_3\gamma_2)^1$
	+	ND	ND	ND	ND	−	$(w/\beta_2\gamma_2)^3$
Diazepam (22)	+	+	+	ND	+	ND	$(w/\beta_3\gamma_2)^1$
	+	+	+	+	+	ND	$(w/\beta_1\gamma_2)^{2,6}$
	+	+	+	+	+	ND	$(w/\beta_1\gamma_1)^{2,6}$
Imidazobenzodiazepine series							
Flumazenil (26)	+ weak	+ weak	+	+	− weak	NA	$(w/\beta_3\gamma_2)^1$
(Ro 15-1788)	−	ND	ND	ND	ND	+	$(w/\beta_2\gamma_2)^3$
	ND	+	ND	ND	ND	ND	$(w/\beta_1\gamma_2)^{2,6}$
	ND	+	ND	ND	ND	ND	$(w/\beta_1\gamma_1)^{2,6}$
Ro 15-4513 (27)	− weak	− weak	+ weak	+	− weak	NA	$(w/\beta_3\gamma_2)^1$
	−	ND	ND	ND	ND	+	$(w/\beta_2\gamma_2)^3$
	ND	−	ND	ND	ND	ND	$(w/\beta_1\gamma_2)^{2,6}$
	ND	+	ND	ND	ND	ND	$(w/\beta_1\gamma_1)^{2,6}$
L-655,708 (31)	−	−	− weak	ND	−	ND	$(w/\beta_3\gamma_2)^{4,5}$
RY24 (30)	ND	ND	ND	ND	−	ND	$(w/\beta_2\gamma_2)^7$
Pyrazoloquinolinone series							
CGS9895 (34)	+ weak	+	+	+	− weak	ND	$(w/\beta_3\gamma_2)^1$
CGS8216 (39)	−	− weak	− weak	+	−	ND	$(w/\beta_3\gamma_2)^1$
	ND	+	ND	ND	ND	ND	$(w/\beta_1\gamma_2)^{2,6}$
	ND	+	ND	ND	ND	ND	$(w/\beta_1\gamma_1)^{2,6}$
Other series							
L-792782 (47)	− weak	+	− weak	ND	−	ND	$(w/\beta_3\gamma_2)^4$

[a]Ref. 1: (187); Ref. 2,6: (184, 185); Ref. 3: (188); Ref. 4,5: (156, 186); and Ref. 7: (189).

4.1.3 Structure-Activity Relationships with $GABA_A$/BzR Inverse Agonists

4.1.3.1 β-Carbolines and Pyridodiindoles. In 1979 β-carboline-3-carboxylic acid ethyl ester (BCCE) (**15**) was isolated as an artifact from human urine and found to have high affinity for the central BzR (190). Since that time, many laboratories have developed β-carboline analogs with different affinities for $GABA_A$/BzR isoforms (see references in Ref. 191) and abilities to modulate BzR-mediated GABAergic transmission (192). The β-carbolines have intrinsic activities ranging from full agonists [e.g., ZK 93423 (6) and abecarnil (**5**)] (193–196) to antagonists [e.g., BCCT (**8**)] (197) to weak/full inverse agonists [e.g., BCCE (**15**), DMCM (**16**), ZK 93426 (**12**), and FG 7142 (**18**)] (117–121, 193, 194, 198). The binding affinities and pharmacological profiles for several representative β-carbolines and their

close structural analogs, the dihydropyridodiindoles, at the $GABA_A$/Bz receptor are shown in Table 14.2. Where available, the affinities for specific $GABA_A$/Bz receptor isoforms are listed.

Most β-carbolines have good affinity for receptor isoforms containing the a,, a,, α_3, and a, subunits. Some selectivity is generally observed for the a,-containing isoform and there is usually little affinity for the isoform having an a, subunit. The exceptions for this are BCCT (8)and BCCE (**15**), which exhibit moderate affinity for the a, subunit (183).

As shown on the left in Fig. 14.3, β-carbolines with agonist activity, such as 6-PBC (**5**), were proposed to bind into the BzR so as to orient the centroid of their A-ring into the L_1 region, whereas the pyridyl N(2) nitrogen and the carbonyl oxygen at C3 formed a three-centered hydrogen bond with hydrogen bond donor site H_1 (**167**). Ligands with a C4 alkoxy moiety, such as 6-PBC (**7**), are able to form an additional hydrogen bond with donor site H_2 and can fully occupy the L_3 region, believed essential for potent agonist activity (191,201).

Inverse agonist of the β-carboline series also tend to have a vertical alignment when modeled into the BzR. However, it was hypothesized that for inverse agonist activity *in vivo* that these ligands bind into the BzR differently, as shown with BCCE (15) on the right side of Fig. 14.3, orienting the centroid of their A-ring into the L_{Di} binding pocket. To accomplish this, hydrogen bonds must form between the indole N(9)-H and the A, hydrogen bond acceptor site, and between the pyridyl N(2) nitrogen and the hydrogen bond donor site H_1 (163). Additional hydrogen bonding to H_1 was thought to be derived from the ligand's carbonyl oxygen. The ligand's C3 side chain was also proposed to interact with the hydrogen bond donor site H,.

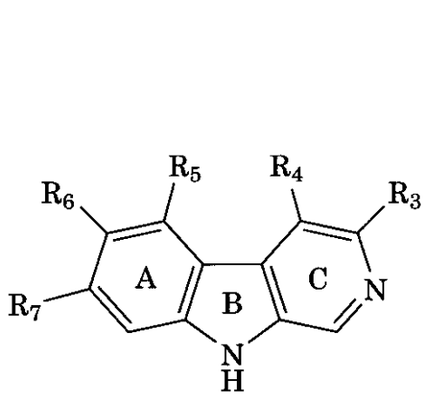
Both the β-carboline and the structurally related pyridodiiodole BzR ligands require the indole nitrogen of the B-ring to remain unsubstituted for interaction with the receptor's hydrogen bond acceptor site A_2 (121). For example, replacement of the indole N(9)-H on β-CCM (14) with a methyl group is accompanied by a decrease in binding affinity [IC_{50} = 5.0 n*M* for β-CCM (14) and $IC_{50} > 50{,}000$ for 9-methyl-β-CCM] (202). The binding affinity of the pyridodiindole PRI (19) is similarly decreased when its indole N(7)-hydrogen atom is replaced with a methyl group (IC_{50} = 4–1163 n*M*) (121).

Additional insight into the BzR pharmacophore was gained through the study of a series of rigid β-carboline analogs, the dihydropyridodiindoles (121,203). These BzR ligands helped to define the active conformation and-binding alignment of the parent β-carboline series. The unsubstituted pyridodiindole PRI (**19**), an inverse agonist, was believed to bind into the BzR active site in much the same way as BCCE (15)(Fig. 14.3) with the centroid of the E-ring occupying lipophilic region L_1 and its N5 nitrogen lone pair of electrons interacting with H_1 (167, 191).

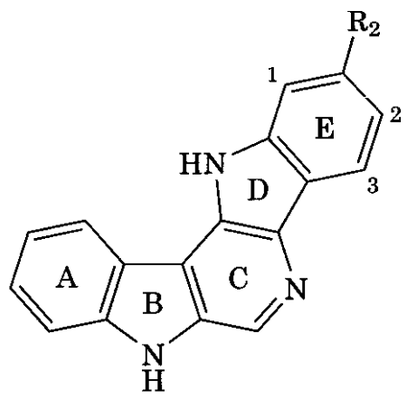
Substitutions at the 1-, 2-, or 4-positions were accompanied by diminished affinity, resulting from the steric constraints of the binding domain (165). Substitution at the 3-position helped to define the boundary of the repulsive region S_1. Several 3-position analogs [e.g., PRI2Me (**21**), PRI2MeO (**20**), and PRI2Cl (**11**)] had very good BzR affinities, with pharmacological profiles of the 3-methyl and 3-methoxy adducts maintained as inverse agonist, but the 3-chloro adduct shifting to an antagonist (167).

High affinity binding β-carbolines require moieties at the 3-position that are capable of accepting hydrogen bonds from the receptor's hydrogen bond donors H_1 and H,. Several β-carbolines with esters, amides, and alkoxy groups at the 3-position have been developed with excellent BzR affinities (Table 14.2). The shape and lipophilicity of the 3-position substituent has a direct effect on $GABA_A$ isoform selectivity. For example, the t-butyl ester BCCT (8)is very selective (20-fold) for the α_1-containing $GABA_A$ isoform (K_i = 0.72 n*M*), thought to be derived from the y-branching of the ester (**197**), but still maintains some affinity for the a,-containing $GABA_A$ isoform (K_i = 111 n*M*). The 3-ethoxy analog 3-EBC (**10**), developed as a long-lived, water-soluble replacement for the metabolically labile 3-position esters (**121**), also binds tightly with the α_1-containing $GABA_A$ isoform (K_i = 6.43 n*M*), but has little affinity for the a,-containing $GABA_A$ isoform (K_i = 826 n*M*) (191).

Table 14.2 **Binding Affinity and In *Vivo* Pharmacological Profile of Several $GABA_A$/BzR β-Carboline and Pyridodiindole Ligands**



β-Carboline



Pyridodiindole

(19) R_2 = H; PRI
(21) R_2 = CH_3; PRI2CH_3
(20) R_2 = OCH_3; PRI2CH_3O
(11) R_2 = Cl; PRI2Cl

	Ligand					K_i (nM)[a]					
	R_3	R_4	R_5	R_6	R_7	α_1	α_2	α_3	α_5	α_6	Ref.[b]
Agonist											
Abercanil (5)	CO_2i-Pr	CH_2OMe	H	OBn	H	12.4	15.3	7.5	6	>1000	1
ZK 93423 (6)	CO_2Et	CH_2OMe	H	OBn	H	4.10	4.20	6.0	4.5	>1000	1
Partial agonist											
6-PBC (7)	CO_2Et	CH_2OMe	H	OPr	H	0.49	1.21	2.2	2.39	1340	1
Antagonist											
BCCT (8)	CO_2t-Bu	H	H	H	H	0.72	15.0	18.9	110.8	>5000	1
BCCP (9)	CO_2n-Pr	H	H	H	H			IC_{50} = 3.0 nM			3
3-PBC (10)	OPr	H	H	H	H	5.30	52.3	68.8	591	>1000	1
PRI2Cl (11)						3.9	12.2	24.4	210	>10,000	1
Antagonist/weak inverse agonist											
ZK 93426 (12)	CO_2Et	Me	Oi-Pr	H	H			IC_{50} = 0.4 nM			2
BC6OBz (13)	CO_2Et	H	OBn	H	H	7.2	169	284	271	>10,000	1/6
Inverse agonist											
β-CCM (14)	CO_2Me	H	H	H	H	2.4	7.4	72	44	ND	4
BCCE (15)	CO_2Et	H	H	H	H	1.20	4.9	5.7	26.8	2700	1
DMCM (16)	CO_2Me	Et	H	OMe	OMe	5.70	8.3	4.0	1.04	134	1
3-EBC (17)	OEt	H	H	H	H	6.43	25.1	28.2	826.0	>1000	1
FG 7142 (18)	CONHMe	H	H	H	H			IC_{50} = 444 nM			5
PRI (19)						1.1	1.2	1.1	40.3	>10,000	1
PRI2MeO (20)						3.4	11.7	11.0	225	>10,000	1
PRI2Me (21)								IC_{50} = 10 nM			3

[a] Specific affinity data provided when available for a_1, a_2, a_3, a_5, and a_6 subunits coexpressed with $\beta_3\gamma_2$ in human cell lines.
[b] Ref. 1: (183); Ref. 2: (199); Ref. 3: (163); Ref. 4: (187); Ref. 5: (200); and Ref. 6: reported as having an "inactive" profile in (169).

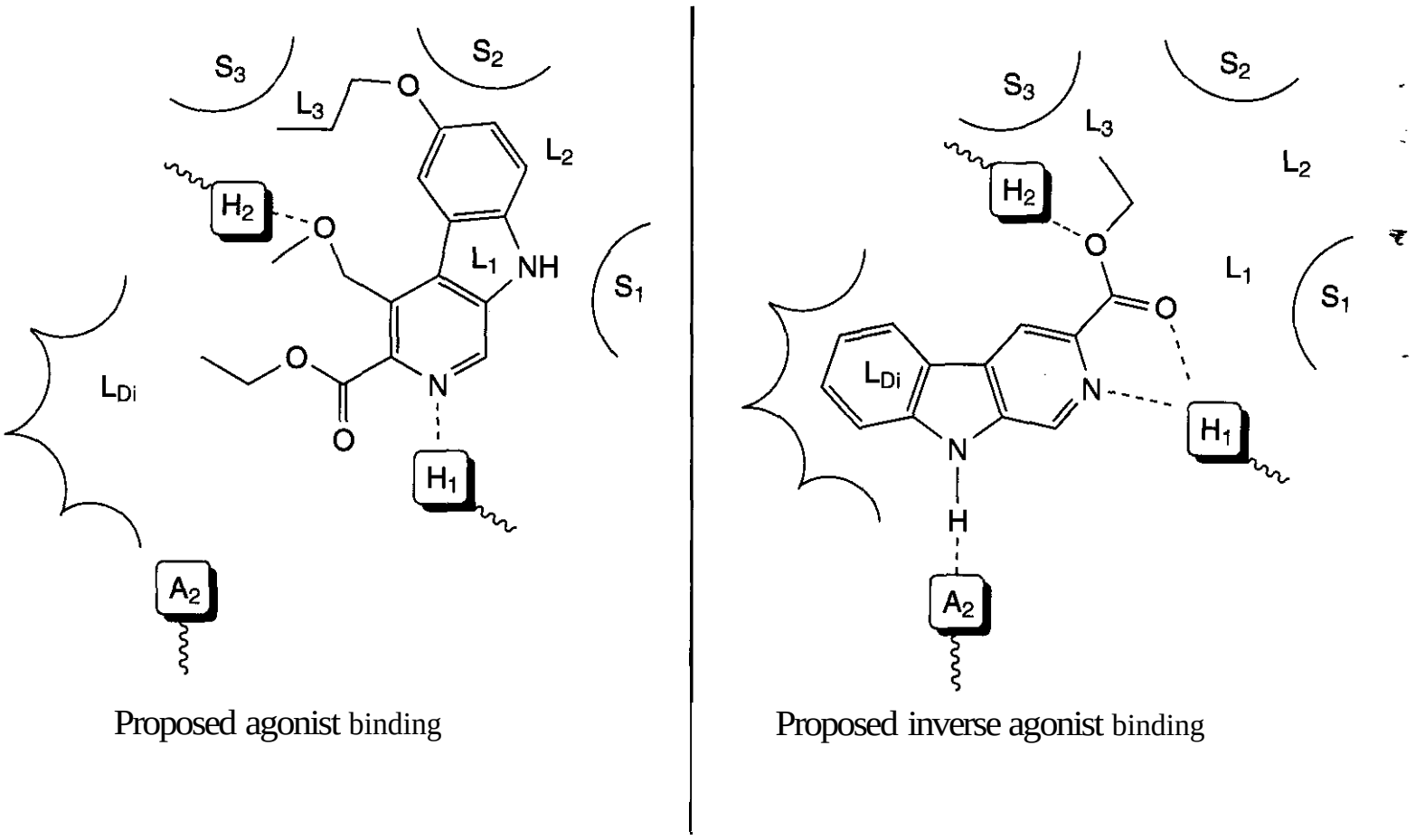


Figure 14.3. Binding conformation of partial agonist 6-PBC (**7**), left, and inverse agonist BCCE (**15**), right, in the $GABA_A$/BzR **pharmacophore/receptor** model. (Modified from Ref. 167.)

The pharmacological profile of the **β-carbolines** also varied with structural changes in the 3-position group. **Ligands** with smaller **3-position** groups, such as **β-CCM** (**14**), BCCE (**15**), and 3-EBC (**17**), were more selective for the a,-containing $GABA_A$ isoform and maintained inverse **agonist** profiles. The N-methyl **carboxamide** analog FG 7142 (18)is also an inverse agonist, but with diminished **BzR** affinity (195, 200). The ***n*-propyl** ester BCCP (9) and the t-butyl ester BCCT (8) provided ligands with overall antagonist profiles (121, 204, **205**). BCCT (**8**) was later found to be one of the most selective ligands for the a,-containing $GABA_A$ **isoform** (197,201). In contrast to 3-EBC (**17**), the 3-n-propoxy-P-carbolineanalog, 3-PBC (**10**) was made as a set of compounds to further investigate the strict steric requirements **of** the lipophilic binding region L_1 (121, 163). The affinity for this ligand was diminished by more than an order of magnitude and found to have an antagonist profile (166).

Substitution of the 6-position of inverse **agonist** BCCE (15)with a benzyloxy group gave **BC6OBz** (**13**). This **adduct** had an equivalent **affinity** for only the a,-containing $GABA_A$ **isoform** (K_i = 7.2 n*M*) (183) and a diminished inverse agonist profile (169). When inverse **agonist** **β-CCM** (14) was modified with methoxy groups at the 6- and 7-positions and an ethyl group at position 4, the ligand DMCM (16)was produced, which maintained **binding** affinity and an inverse agonist profile (163). This observation supported the hypothesis that the L_{Di} binding region of the a,-containing $GABA_A$ isoform was larger in size than the same region of the other **isoforms** (**191**).

Addition of a methoxyrnethyl group at the 4-position of **BC6OBz** (**13**) gave ZK-93423 (**6**), with greatly improved affinity for all $GABA_A$ **isoforms** (201). ZK-93423 (**6**), with a full **agonist** profile, was believed to orient differently in the **BzR** in such a way as to permit a hydrogen bond between the methoxymethyl group's oxygen atom and the hydrogen bond donor site H,. This new orientation would permit the benzyloxy moiety to fully occupy region L_3 of the receptor, believed necessary for the agonist pharmacology (Fig. 14.3). The ligand 6-PBC (**7**) was made with the smaller propoxy group at the 6-position to test this hypothesis, and resulted in a ligand with increased selectivity for the a, isoform and a partial agonist pharmacological profile (170, 171).

4.1.3.2 Imidazobenzodiazepines. Imidazobenzodiazepines have been a long-studied series of $GABA_A$/BzR ligands (167, 189, 206–213) that bind with good affinity to the α_1, α_2, α_3, α_5, and α_6 subunits containing $GABA_A$ isoforms, and often with excellent selectivity at the α_1-containingreceptors (189) (Table14.3).

The imidazobenzodiazepine Ro 15-4513 (27) is a partial inverse agonist with about 10-fold selectivity toward the α_5-containing receptor (189). In contrast, substituting a fluorine atom in the &position on Ro 15-4513 (27) generates the antagonist flumazenil [Ro 15-1788 (**26**)], and selectivity is lost. This observation led researchers to hypothesize that the lipophilic region L_2 may be smaller in the $\alpha_1\beta_2\gamma_2$-containing receptor than the related $\alpha_5\beta_2\gamma_2$-containing receptor, and that selectivity might be achieved with the correctly sized 8-position substituent (215). Consequently, several α_5 subsite-selectiveimidazobenzodiazepines were synthesized [e.g., RY24 (30)and RY80 (**29**)] that supported this hypothesis (215).

Molecular modeling with RY24 (30) and RY80 (29) in the unified pharmacophore/receptor model suggested that these ligands bind in an orientation that directs their 8-position substituent into the L_2 region, giving them selectivity toward the α_5-containing receptor (Fig. 14.4) (189, 215). Both RY24 (30) and RY80 (29) were assayed *in vivo* and found to have an inverse agonist profiles (215).

Based on the above findings, an investigation was launched into the modification of the classic BzR agonist diazepam (22) that investigated A-ring substituents directed toward the L_1 region (216,217). This led to the derivative QH-11-66 (**24**), the first benzodiazepine selective for the α_5-containing receptor. In contrast to RY24 (**30**) and RY80 (**29**), this BzR ligand was reported to have an agonist pharmacological profile.

Further insight into the topography of the Bz receptor came from a series of chiral, framework-constrained 4,5-substituted pyrroloimidazobenzodiazepines and azetidinylimidazobenzodiazepines (218, 219). Only the (*S*) enantiomers of these series of ligands bound to the BzR subtypes with high affinity, suggesting that the conformational topography at the five recombinant receptor subtypes was well conserved (218). The aliphatic ring of the 4,5-pyrroloimidazobenzodiazepine, as in L-655,708 (**31**), is believed to play an important role in maintaining an active *anti* conformation of the 3-position ester (183). Of particular interest from this series is the inverse agonist L-655,708 (**31**), a ligand that has at least 50-fold selectivity for the α_5-containing isoform and has been investigated preclinically by Merck for its cognition-enhancing properties (186). Additionally, L-655,708 (**31**) has been radiolabeled with tritium and used as a research tool for identification of α_5-containing $GABA_A$ receptors (214).

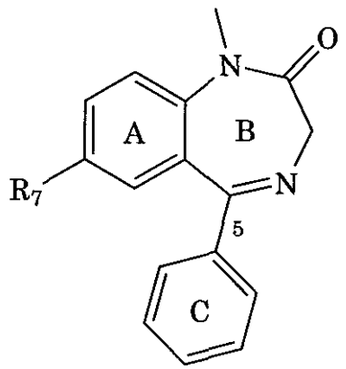
4.1.3.3 Pyrazoloquinolinones. The pyrazoloquinolinone series of BzR ligands have become generally known as the CGS series because of their discovery in the laboratories of Ciba-Geigy in the early 1980s (220). Like the β-carbolines, this series has been well explored and ligands with a continuum of intrinsic activities have been discovered (166, 209, 220–223) (Table 14.4).

The pyrazoloquinolinones have unique pseudoplanar topographies and high receptor affinities. These properties helped to systematically probe the structure-space of the BzR and greatly aided the development of a unified pharmacophore/receptor model (167,183). As illustrated in Fig. 14.5 with agonist CGS-9896 (**33**), the lone pair of electrons of N1 form a hydrogen bond with donor site H_2 and the lone pair of electrons *of* the C3 carbonyl oxygen align to form a hydrogen bond with donor site H_1. An additional hydrogen bond is believed to form between the proton on the N5 nitrogen and the acceptor site A_1. The centroid of the D-ring was believed to occupy the lipophilic region L_1.

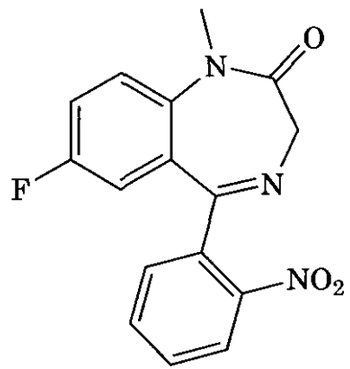
In the course of investigating substitution patterns of the pyrazoloquinolinones, it was observed that substituents at both the 6- and 7-positions generally had a negative effect on receptor affinity, presumably because of steric interactions in the binding domain of region L_{Di}. However, substituents only at the 7- or 8-position were better tolerated and helped to map out the steric constraints of region L_{Di} (224,226).

The pendant D-ring is the most amenable toward manipulation in regard to affinity and *in vivo* pharmacology (167). In contrast to the

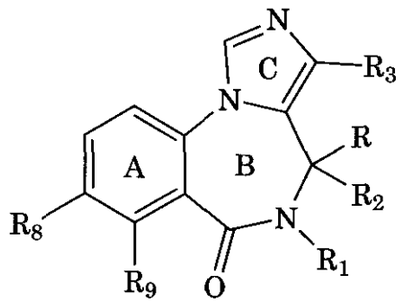
Table 14.3 **Binding Affinity and *In Vivo* Pharmacological Profile of Several $GABA_A$/BzR Benzodiazepine and Imidazobenzodiazepine Ligands**



(22) R_7 = Cl; Diazepam
(24) R_7 = acetylenyl; QH-11-66



(23) Flunitrazepam



Imidazobenzodiazepine

Ligand						K_i (nM)[a]					
	R	R_1	R_2	R_3	R_8/R_9	α_1	α_2	α_3	α_5	α_6	Ref.[b]
Agonist											
Diazepam (22)						14	20	15	11	>3000	1
Flunitrazepam (23)						2.2	2.5	4.5	2.1	>2000	1
QH-11-66 (24)						76.3	42.1	47.4	6.8	>3000	1
Partial agonist											
Bretazenil (25)	CO_2t-Bu	$-CH_2CH_2CH_2-$		H(S)	H/Br	0.35	0.64	0.2	0.5	12.7	1
Antagonist											
Flumazenil (26) (Ro 15-1788)	CO_2Et	Me	H	H	F/H	0.8	0.9	1.1	0.6	148	1
Partial inverse agonist											
Ro 15-4513 (27)	CO_2Et	Me	H	H	N_3/H	3.3	2.6	2.5	0.3	3.8	1
Sarmazenil (28) (Ro 15-3505)	CO_2Et	Me	H	H	H/Cl	(DS) K_i = 0.2 nM; (DI) K_i = 20 ± 2.4 nM					2
Inverse agonist											
RY80 (29)	CO_2Et	Me	H	H	C≡CH/H	28.4	21.4	25.8	0.49	28.8	1
RY24 (30)	CO_2t-Bu	Me	H	H	C≡CH/H	26.9	26.3	18.7	0.40	5.1	1
L-655,708 (31) (MSD; FG8094)	CO_2Et	$-CH_2CH_2CH_2-$		H(S)	OMe/H	48.5	27.4	24.5	0.45	83.2	1.3

[a]Specific affinity provided when available for a_1, a_2, a_3, a_5, and a_6 subunits coexpressed with $\beta_3\gamma_2$ in human cell lines.
[b]Ref. 1: (183); Ref. 2: (210); and Ref. 3: (214).

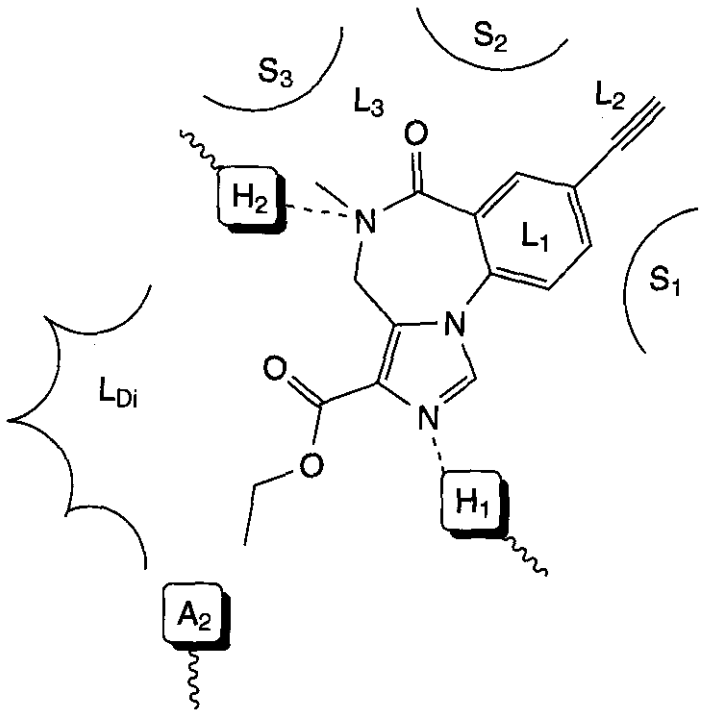


Figure 14.4. Binding conformation of inverse agonist RY80 (29) in the $GABA_A$/BzR pharmacophore/receptor model. (Modified from Ref. 189.)

inverse agonist CGS-8216 (**39**), partial agonists are the result of substitution at the 4′-position with either a chloro, to provide CGS-9896 (**33**), or a methoxy group, to provide CGS-9895 (34) (187,220). The binding affinities for a series of these 4'-substituted ligands suggest that electronegative substituents at the 4'-position favor selectivity at the α_1-containing isoforms because of a decrease in affinity at the α_5-containing isoforms (224). Substituents at the 2′- and 3'-positions of the D-ring can also provide for good receptor affinity, such as the electron-releasing 3'-methoxy adduct APQ3′OMe (37) and the 3'-chloro adduct APQ3′Cl (**38**), although the pharmacological activity shifts to that of an antagonist compared to that of the parent CGS-8216 (39) (212).

The D-ring can itself be replaced with a 2- or 3-thienyl moiety [e.g., 2TPQ (40) and 3TPQ (**42**), respectively] (225) and maintain good affinity and inverse agonist activity. However, simple methyl substitution on the thiophene can shift the pharmacological profile from an inverse agonist, observed for the 5-methylthien-3-yl analog S-135 (**43**), to a partial agonist, as is the case for 2TPQ5′Me (**35**) and 2TPQ4′5′diMe (**36**), again showing the sensitivity of the receptor's region L_2 toward small structural changes in the ligand (225). The inverse agonist S-135 (43) was selected from this research for further in *vivo* investigation of its potential as a cognition-enhancing agent (227, 228).

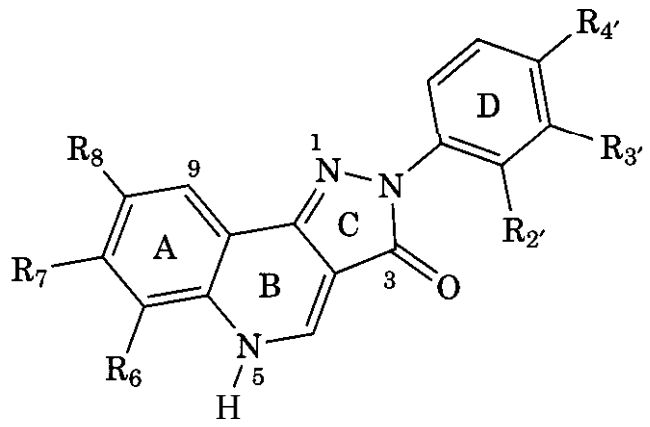
A closely related BzR ligand series are the imidazoquinolines, designed as pyrazoloquinoline bioisosteres, in which the C-ring pyrazolo has been substituted with an imidazo moiety (223). The imidazo analog of CGS-8216 (**39**) had a lower binding affinity than that of the parent series (K_i = 22 n*M* versus 0.22 n*M* for [^{3}H]diazepam displacement from rat cerebral cortex), but affinities increased when the D-ring was exchanged for an isoxazolyl moiety. The A-ring of this series could also be changed to a nonaromatic ring and maintain binding affinity. For example, the A-ring pyrano derivative generated from this research, S-8510 (**44**), had a moderate inverse agonist profile and was selected as a therapeutic candidate for the treatment of senile dementia (223).

4.1.3.4 Miscellaneous Chemotypes. Dainippon Pharmaceutical has recently disclosed a series of 1,6-naphthridine agents with activity at the benzodiazepine binding site, but no specific binding affinity data were provided (229) (Table 14.5). From this series, SX-3933 (49) was described to have inverse agonist activity and is currently under preclinical evaluation for cognition enhancement. In mice, SX-3933 (0.003–1 mg/kg, p.o.) attenuated scopolamine-induced memory deficits in a dose-dependent manner (229). SX-3933 was also reported to prevent impairment induced by dizocilpine (the noncompetitive NMDA receptor antagonist, MK-801) in both a spontaneous alternation test (ED_{50} range = 0.1–3 mg/kg, p.o.) and a novel object recognition test (ED_{50} range = 0.1–10 mg/kg, p.o.) (229).

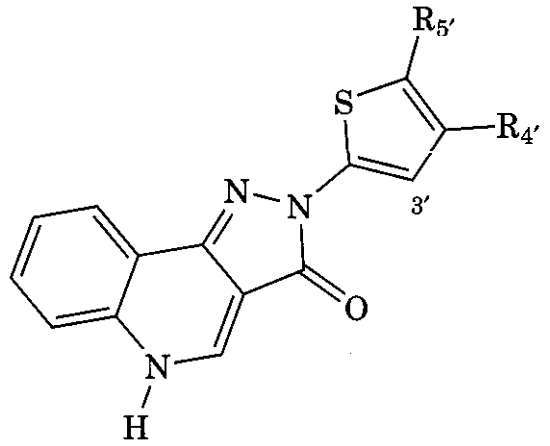
Merck researchers have recently disclosed a series of 3-phenyltriazolopyridazines with excellent selectivity for the a,-containing $GABA_A$ isoform (186) (Table 14.5). Selectivity for the a, subunit was maintained within the series when the A-ring was saturated or had alternative ring fusion. The inverse agonist L-792782 (47) was selected from this series and evaluated for cognition enhancement, discussed below.

A series of piperazine imidazoquinoxaline ureas was recently reported by Pharmacia & Upjohn to have high affinity for the

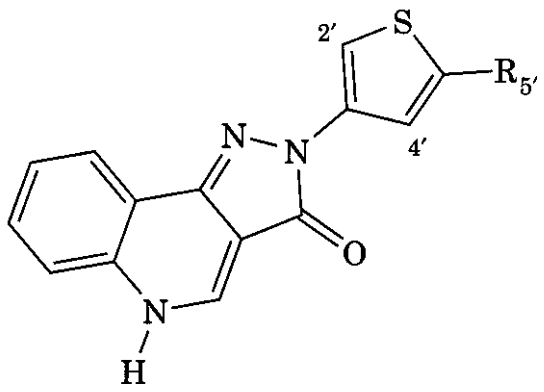
Table 14.4 **Binding Affinity and *In Vivo* Pharmacological Profile of Several $GABA_A$/BzR Pyrazoloquinolinone Ligands**



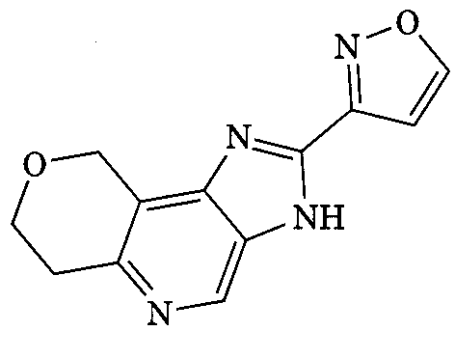
Pyrazoloquinolinone



(40) $R_{4'} = R_{5'} = H$; 2TPQ
(35) $R_{4'} = H$; $R_{5'} = CH_3$; 2TPQ5′CH_3
(41) $R_{4'} = CH_3$; $R_{5'} = H$; 2TPQ4′CH_3
(36) $R_{4'} = R_{5'} = CH_3$; 2TPQ4′5′diCH_3



(42) $R_{5'} = H$; 3TPQ
(43) $R_{5'} = CH_3$; S-135



(44) Imidazoquinoline S-8510

	Ligand				K_i (nM)[a]					
	$R_{2'}/R_{3'}/R_{4'}$	R_6	R_7	R_8	α_1	α_2	α_3	α_5	α_6	Ref.[b]
Agonist										
APQ3′Cl (32)	**H/Cl/H**	H	H	H		IC_{50} = 3.9 nM				**2**
Partial agonist										
CGS-9896 (**33**)	**H/H/Cl**	H	H	H		IC_{50} = 0.6 nM				5
CGS-9895 (34)	**H/H/OMe**	H	H	H	0.32	**1.1**	0.28	0.96	ND	7
2TPQ5′Me (35)						K_i = 0.3 nM				**3**
2TPQ4′5′diMe (36)						K_i = 0.7 nM				**6**
Antagonist										
APQ3′OMe (37)	**H/OMe/H**	H	H	H		IC_{50} = 0.5 nM				**2**
APQ2′Cl (38)	**Cl/H/H**	H	H	H		IC_{50} = 70 nM				**2**
Inverse agonist										
CGS-8216 (39)	**H/H/H**	**H**	**H**	**H**	0.05	0.08	0.12	0.25	17	**1**
2TPQ (40)						K_i = 0.4 nM				**3**
2TPQ4′Me (41)						K_i = 0.5 nM				**3**
3TPQ (42)						K_i = 0.73 nM				**3**
S-135 (43)						K_i = 0.32 nM				**3**
S-8510 (44)						K_i = 3 nM				**4**

[a]Specific affinity data provided for a_1, α_2, a_3, a_5, and a_6 subunits coexpressed with $\beta_3\gamma_2$ in human cell lines.
[b]Ref. **1: (224);** Ref. **2: (221);** Ref. **3: (225);** Ref. **4: (223);** Ref. **5: (220);** Ref. **6: (222);** and Ref. **7:** (187).

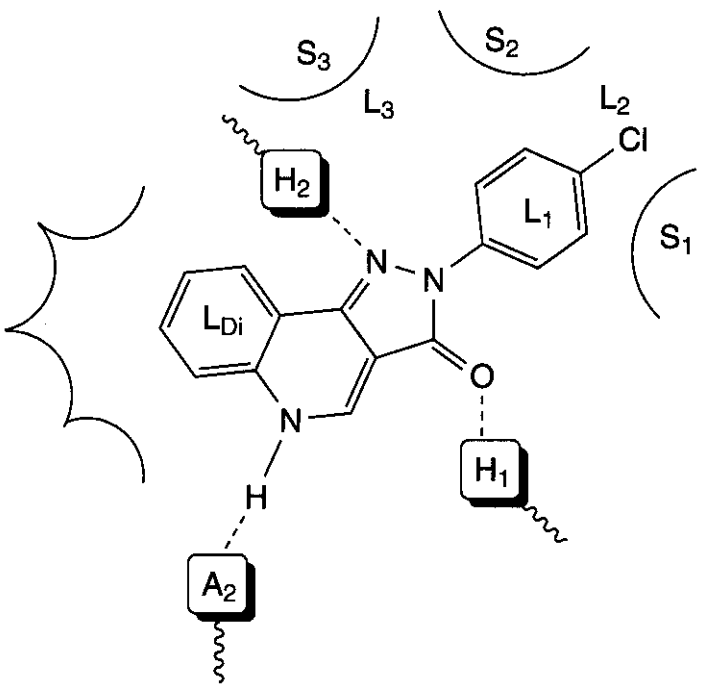


Figure 14.5. Binding conformation for agonist CGS-9896 (33) in the $GABA_A$/BzR pharmacophore/ receptor model. (Modified from Ref. 167.)

$GABA_A$/Bz receptor with a full continuum of intrinsic activities (230). This BzR ligand series is structurally similar to the 3-phenyltriazolopyridazines mentioned above, and have some similarity to the a,-selective hypnotic drugs zolpidem (45) and zopiclone (**46**), marketed by Sanofi-Synthelabo and Rhone-Poulenc Rorer, respectively, for sleeping disorders. Urea (48) represents one of the most potent inverse agonist from this series, with a K_i value of 6.67 n*M*. Manipulation of the 5-, 6-, and 7-positions generated analogs of widely varying intrinsic efficacy. In contrast to urea (**48**), substitution on the A-ring with a bulky group at the 7-position resulted in analogs that were positive modulators of the $GABA_A$ channel (230). No preclinical data for cognition enhancement have been reported from this series.

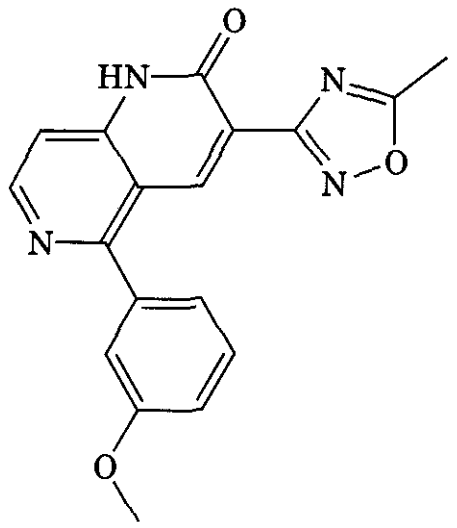
4.1.4 Cognition-Enhancement Experiments. As mentioned above, the intrinsic activity of $GABA_A$/BzR ligands was historically determined by observing behavior after the administration of BzR ligands. BzR inverse agonists are well known for their proconvulsant and anxiogenic effects in animals, actions opposite those of classic agonists for this site. As such, the classification of agonist, inverse agonist, or antagonist was often made after observation of response in behavioral assays designed to assess convulsant or anxiety-related behaviors. Although multiple approaches are now used to assess efficacy and functional activity both in *vitro* and in *vivo*, it is notable that in addition to the proconvulsant and anxiogenic effects of these compounds, BzR inverse agonists have also been shown to reliably enhance cognition in preclinical tests.

One way to potentially capitalize on the cognitive-enhancing effects of BzR inverse agonists without producing overt proconvulsant or anxiogenic behavior is to target receptors disproportionately localized in brain areas involved in cognitive processes. In this regard, expression of $GABA_A$ receptors containing the a, subunit is highly specific to the hippocampus in the rat and human and constitutes approximately 20% of these receptors (231). As such, compounds that specifically interact with the a,-containing isoform of the $GABA_A$/BzR have been an area of high interest. In the following, examples of specific and nonspecific BzR inverse agonists are discussed.

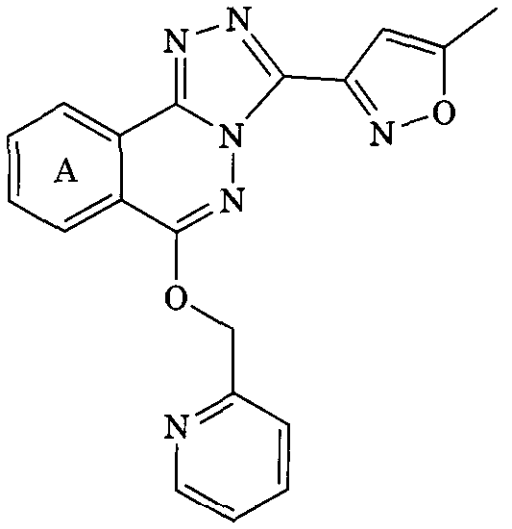
*β-Carbolines: β-CCM (**14**), ZK **93426** (**12**),* and *FG* 7742 (18). Direct injection of the BzR inverse agonist β-CCM (14) into the rat NBM enhanced performance in a two-trial recognition task (232), similar to the positive effects observed earlier for the peripheral administration of the same agent (233). Further development of β-CCM (14) and DMCM (16) was never considered because of their rapid esterase-mediated hydrolysis in *vivo*, poor solubility even as HCl salts, and toxicity (166,234).

In experiments with other β-carbolines, the BzR weak inverse agonist ZK 93426 (12) and partial inverse agonist FG 7142 (18) injected into the forebrain were observed to provide transient, task-dependent potentiation of cortical ACh efflux (125). Behavioral studies with FG 7142 have found that it improved working memory in normal rats (124,235). In addition, FG 7142 was shown to improve passive avoidance retention in both rats (236, 237) and mice (238). However, other investigators have reported impaired working memory performance in both rats and rhesus monkeys (239,240). In a clinical study, five healthy males were challenged with oral doses of FG 7142 increasing to 100–200 mg (and, in one case, 400 mg). In two of the 12 trials with doses of 100 mg or more, subjects experienced severe

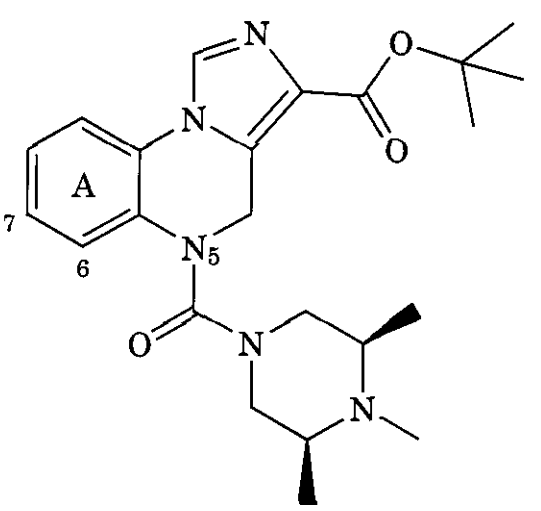
Table 14.5 Binding Affinity and *In Vivo* Pharmacological Profile of Various $GABA_A$/BzR Ligands



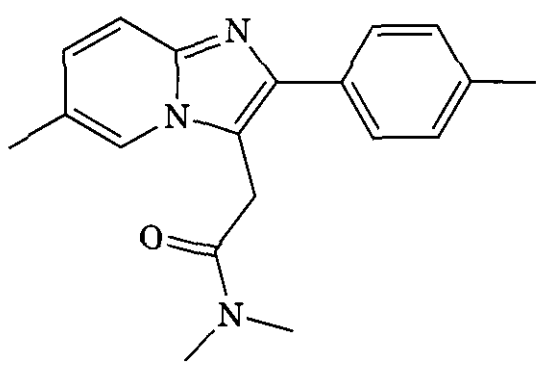
(49) SX-3933



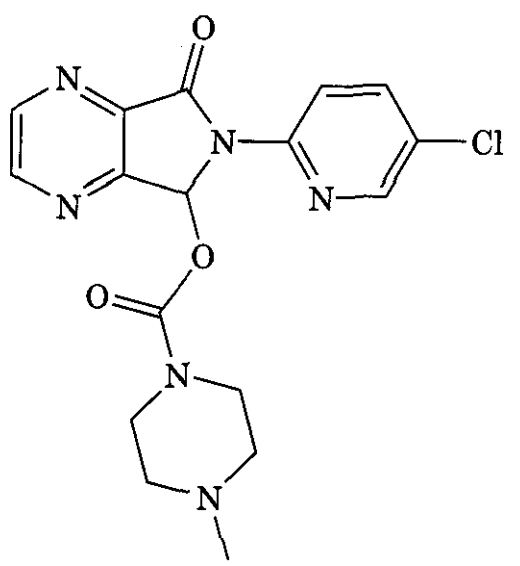
(47) L-792782



(48) Imidazoquinoxaline piperazine urea



(45) Zolpidem



(46) Zopiclone

	K_i (nM)[a]					Ref.[b]
	α_1	α_2	α_3	α_5	α_6	
Agonist						
Zolpidem (45)	26.7	156	383	>10,000	>10,000	1
Zopiclone (46)	28	64	29	46	ND	4
Inverse agonist						
L-792782 (47)	1.4	2.7	1.4	0.8	ND	2
Urea (48)			K_i = 6.67 nM			3
SX-3933 (49)			Not reported			5

[a] Specific affinity data provided when available for α_1, α_2, α_3, and α_6 subunits coexpressed with $\beta_3\gamma_2$ in human cell lines.
[b] Ref. 1: (191); Ref. 2: (186); Ref. 3: (230); Ref. 4: (187); and Ref. 5: (229).

anxiety and the study was terminated (119). Nevertheless, FG 7142 remains a valuable preclinical research tool.

In a single clinical study, ZK 93426 (12) was evaluated for its effect on scopolamine-induced cognitive impairments and was found to partially antagonize scopolamine's effects on memory and attention (241). No further development is expected.

Imidazobenzodiazempines Ro 15-3505 (28); Sarmazenil; Roche Holding AG), RY24 (30), and L-655708 (31); FG8094; Merck); and the Triazo-

Iopyridazine L-792782 ***(47;*** *Merck).* Ro 15-3505 (28) demonstrated convincing efficacy in preclinical measures of arousal and cognitive behavior such as T-maze and delayed match-to-position assays (242–245). However, this compound also demonstrated anxiogenic activity that precluded its clinical development for cognitive impairment (246, 247).

An entry into the area of a,-selective ligands is represented by the imidazobenzodiazepine RY24 (30). RY24 has been reported to show cognitive-enhancing effects after direct hippocampal injections (Helmstetter et al., unpublished, as discussed in Ref. 183). However, at similar concentrations this compound also produced an increase in freezing behavior indicative of anxiogenic activity. Note that no proconvulsant effects were seen up to the highest concentration tested (10 μg/μL). Interestingly, the procognitive and anxiogenic effects were seen only at lower concentrations and were absent at higher concentrations. This combined with the fact that no procognitive effects were noted after peripheral administration indicates that, at higher concentrations, this compound may possess agonist activity at additional subtypes in multiple brain regions that may have led to its anxiogenic profile.

Merck has recently disclosed a series of BzR inverse agonists with greater selectivity for $GABA_A$ receptors containing the α_5 subunit (248). A lead compound, L-655708 (**31**), demonstrated good brain-penetrating properties and receptor occupancy (156). However, there is a paucity of data with regard to its activity in behavioral assays of cognitive activity. Subsequent chemistry efforts led to the 3-phenyltriazolopyridazine, L-792782 (**47**), which produced a marked improvement in a water-maze match-to-position test (186). In this test, rats were placed in a standard water-maze apparatus with a hidden platform. After animals had "found" the platform on a first trial, a second trial followed at either a 0- or 4-h intertrial interval. L-792782 markedly decreased the time to find the platform on the second trial regardless of intertrial interval.

Imidazoquinoline S-8510 ***(44)*** *and Pyrazoquinolinones CGS-8216 (****39****).* The fused imidazopyridine S-8510 (44) is a $GABA_A$/Bz receptor weak inverse agonist that has demonstrated positive results in animal models of cognition (249, 250). S-8510 was shown to ameliorate the memory impairments of scopolamine-treated or basal forebrain lesioned rats in a water maze and mice in passive-avoidance behavioral paradigms (251, 252). EEG measurements confirmed the activating effects of S-8510 on rat brain function (253) and to enhance LTP in *vitro* (254). It was also disclosed that S-8510 did not induce anxiety or convulsions, even at 10–100 times the therapeutically effective dose (255). An additional, interesting feature of this compound is that it also displays antidepressant properties in mice (254). This finding is made particularly interesting, in that there remains a high degree of comorbidity between depression and dementia in geriatric populations (see, e.g., Ref. 256). S-8510 has been reported to be in phase 2 clinical trials in Japan as a potential treatment for senile dementia (257) and, according to a recent press release (PR Newswire, July 25,2001) has recently been included in a joint development venture between Shionogi & Co. Ltd. and GlaxoSmithKline.

The pyrazoloquinoline CGS-8216 (39) was reported to be an inverse agonist (258, 259) and to have cognition-enhancing properties for mice in a T-maze behavioral paradigm (260).

4.1.5 Future Direction. The only nonagonist $GABA_A$/Bz receptor ligand in clinical use is the antagonist flumazenil (26, Ro 15-1788, Hoffman-LaRoche). Flumazenil has been clinically described as an agent with few intrinsic properties and is used to reverse the effects of benzodiazepines in conscious sedation, general anesthesia, and the management of suspected benzodiazepine overdose. Reports from new clinical studies, however, show that flumazenil (26) does possess intrinsic activity and has significant negative effects on cognition, cardiovascular physiology, and mood (261).

Continued research into the identification of subtype-selective $GABA_A$/Bz receptor ligands can be expected to provide agents with more specific physiological activity. This may result in new treatments for a variety of disorders, including cognitive deficits, with decreased potential for adverse side effects.

4.2 Nicotinic Acetylcholine Receptor

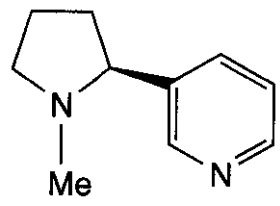
4.2.1 Physiology and Pharmacology of the nACh Receptor. Acetylcholine (ACh) controls neurotransmission through interaction with both nicotinic and muscarinic types of receptors. The nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels (LGIC)that regulate the flux of ions (Na^+, K^+, Ca^{2+}) through the neuronal membrane (262–264), whereas the muscarinic receptors are members of the G-protein–coupled receptor superfamily (GPCR). nAChRs are found on skeletal muscle at the neuromuscular junction, in autonomic ganglia of the peripheral nervous system, on sensory nerves and some peripheral nerve terminals, and at numerous sites in the spinal cord and brain. The nAChRs are further subdivided into neuronal nAChRs and the nicotinic receptors of the neuromuscular .iunction. The neuronal nAChRs involved in CNS neurotransmission are distinct from those of the skeletal muscle (265) and sympathetic ganglia (266). All nAChRs are thought to be homo- or hetero-oligomeric assemblies of five subunits, drawn from several classes (267,268). In mammalian or avian species, muscle nAChRs consist of a,, β_1, y, and δ subunits, whereas neuronal receptors are composed of a, to a, and β_2 to β_4 subunits (262, 269–271). In the rodent CNS, the predominant nAChR suptypes are $\alpha_4\beta_2$ and the homoligomer a, (272), and these subtypes have been implicated in the functions of learning and memory (273). Numerous nAChRs with distinct biophysical and pharmacological properties can be generated by the expression of a single a-type subunit (homomeric) or by coexpression of a- and β-subunits (heteromeric). The composition and distribution of discrete subtypes of nAChRs in the brain still remain largely unknown, although it has been established that certain subunits will preferentially combine to form functional channels (263,269, 274).

Considerable attention has been given to two nAChR subtypes: the heteromer containing $\alpha_4\beta_2$ subunits and the a, subunit homomer. Both of these receptors have been shown to participate in fast excitatory transmission by way of postsynaptic mechanisms (275, 276). In addition, recent experiments have shown that nAChR agonists can act presynaptically to facilitate neurotransmitter release. For example, nAChR agonists applied to cells expressing either the $\alpha_4\beta_2$ nAChRs [nicotine, carbachol/atropine, anatoxin-a, and epibatidine in mouse thalamus slices (277)] or a, nAChRs [nicotine in cultured rat hippocampal neurons (278, 279)], in the absence of high external Ca^{2+}, evoked the release of GABA, ACh, and glutamate.

The neurotransmitter release mediated by the a, nAChR subtype was shown to be predominantly the result of Ca^{2+} influx through the activated a, nAChR channel (278–281). Potentiation of neurotransmission mediated through the $\alpha_4\beta_2$ nAChR subtype was more complicated, in that it was facilitated through an intracellular increase in Ca^{2+} as a result of Ca^{2+} influx from either the $\alpha_4\beta_2$ nAChR or from voltage-dependent Ca^{2+} channels activated by $\alpha_4\beta_2$ nAChR-elicited depolarization (277).

The binding affinity to the $\alpha_4\beta_2$ site is frequently evaluated in homogenized rodent brain tissue using radioligands such as [^{3}H]cytisine, [^{3}H]nicotine, [^{3}H]acetylcholine, [^{3}H]methylcarbamylcholine (MCC), and [^{3}H]epibatidine. The regulation and functional activities have been investigated through several *in vitro* expression systems. Transient expression of chick, rat, and human $\alpha_4\beta_2$ nAChRs in *Xenopus* oocytes, and stable expression systems using chick $\alpha_4\beta_2$ in a mouse cell line (M10 cells) and human $\alpha_4\beta_2$ in a human cell line (K177 cells) have been used. Studies on endogenous $\alpha_4\beta_2$ nAChRs have been carried out in rodent thalamic tissues, and the responses in these systems have been measured, either as flux of radioactive ions (e.g., $^{86}Rh^+$ or $^{22}Na^+$) or as calcium influx detected with calcium-sensitive dyes (273).

Early drug discovery efforts in the nAChR area were achieved largely through observation of the behavioral effect of (*S*)-(−)-nicotine [hereafter designated simply as nicotine (50) in Fig. 14.61 and its resulting behavioral effects in humans. Acute treatment with nicotine improves cognitive performance in rodents (282, 283) and primates (284) for a variety of behavioral paradigms. Nicotine is also known to generate anxiolytic effects in humans (285). Recent clinical studies have



(50) (*S*)-(-)-nicotine

K_i: 1.1 n*M*[a]

Figure 14.6. "**Binding data: rat** brain/[^{3}H]cytisine (299).

suggested that nicotine may be useful for the palliative treatment of attention deficit associated with Alzheimer's disease (286, 287). Moreover, epidemiological studies on cigarette smoking suggest that nicotine has protective effects in AD patients. However, nicotine also produces undesirable effects on the cardiovascular, gastrointestinal, endocrine, and reward systems (288), as well as having poor oral bioavailability and a short duration of action (289, 290). Therefore, nicotine itself represents a poor choice for the safe and effective treatment of Alzheimer's dementia in an elderly population.

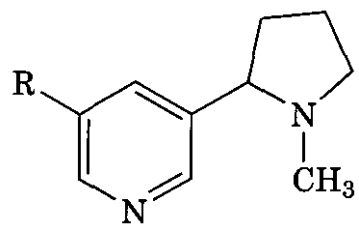
Nicotine is highly selective for $\alpha_4\beta_2$ versus *a,* with respect to binding affinity (binding K_i = 1.05 n*M* versus 4 μM) and moderately selective with respect to functional activity (functional EC_{50}: 4 μM **versus** 54 μM). Nicotine also binds with micromolar affinity to muscle-type receptors, and affects activation in the mid to high micromolar range. Moreover, nicotine binds with micromolar affinity to ganglionic receptors and elicits functional responses in the high nanomolar to micromolar range (291). Because ganglionic-type nAChRs are believed to mediate, at least partially, the gastrointestinal and cardiovascular liabilities of nicotine, significant pharmaceutical research efforts have focused on the development of, novel neuronal nAChR modulators with high selectivity for central versus ganglionic nAChRs. Subtype-selective nAChR modulators with activity for either the *a,* or $\alpha_4\beta_2$ subtypes have demonstrated the potential to become valuable therapeutic agents for a variety of disorders (291, 292). Described below are several important classes of neuronal nAChR agonists and their structure-activity relationships (see reviews in Refs. 10, 273, 291, 293–298).

4.2.2 Structure-Activity Relationships for nAChR Agonists

4.2.2.1 Nicotine Derivatives. *A* large number of nicotine analogs have been reported, and the SAR of nicotine derivatives has been presented in a recent review by Tonder et al. (295). Described herein are two classes of nicotine derivatives: 5-substituted nicotine analogs (e.g., SIB-1508Y, 51) and pyrrolidine ring-opened nicotinoids (e.g., RJR-2403, **57**).

4.2.2.1.1 5-Substituted Nicotine Derivatives. Substitution at the &position of nicotine's pyridyl ring is generally tolerated, but bulky groups such as phenyl reduce activity (Table 14.6) (300, 301). The analog with an ethynyl group at the 5-position, SIB-1508Y (**51**), displayed the same binding activity as that of

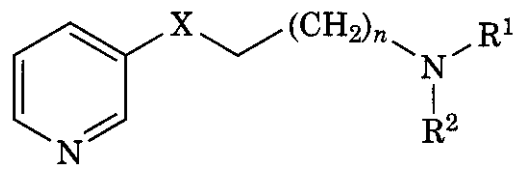
Table 14.6 Binding Data of 5-Substituted Nicotines



Compound	Stereochemistry	R	K_i (n*M*)
(51)(SIB-1508Y)	(*S*)	**Ethynyl**	3"
(**52**)	(*R*)	**Ethynyl**	75"
(**53**)	(*R,S*)	***Me***	1.5[b]
(**54**)	(*R,S*)	**Et**	11[a]
(**55**)	(*R,S*)	***Br***	19"
(**56**)	(*R,S*)	**Ph**	37[c]

"Rat cortex/[^{3}H]nicotine *[K_i* **(nicotine):4** n*M*] (300).
[b]Rat brain/[^{3}H]nicotine **(304).**
[c]Rat cortex/[^{3}H]nicotine **(302).**

Table 14.7 Binding Data of trans-Metanicotines



Compound	X	R^1	R^2	n	K_i (nM)[a]
(**57**) (RJR-2403)	(*E*)–CH=CH–	H	Me	1	26
(**58**)	(*E*)–CH=CH–	H	H	1	119
(**59**)	(*E*)–CH=CH–	Me	Me	1	4500
(**60**)	(*E*)–CH=CH–	H	*i*-Pr	1	270,000
(**61**)	(*E*)–CH=CH–	H	H	0	46,619
(**62**)	(*E*)–CH=CH–	H	H	2	1955
(**63**)	(*Z*)–CH=CH–	H	Me	1	354
(**64**)	–C≡C–	H	Me	1	58
(**65**)	$-CH_2CH_2-$	H	Me	1	910

[a] Rat brain/[^{3}H]nicotine (322).

nicotine. Compound (**52**), the enantiomer of SIB-1508Y, exhibited less affinity for the [^{3}H]nicotine binding site and was less efficacious in a measure of DA release from rat striatal slices (55% relative to nicotine, cf. 163% for SIB-1508Y) (300). Saturation of acetylene (54) as well as replacement of the ethynyl moiety in SIB-1508Y with bromine (55) resulted in modest reduction of the binding affinity but significantly decreased efficacy in the DA release assay [28% and 69% relative to nicotine for (54) and (**55**), respectively]. Worthy of note is that replacement of the ethynyl moiety in SIB-1508Y with a phenyl group (e.g., 56) shifted the subtype selectivity in the calcium flux assay using human recombinant nAChRs from a tendency to activate β_2-containing recombinant nAChRs to p,-containing receptors, although both compounds exhibited good potency at displacing [^{3}H]nicotine from rat cortical membranes (302). This observation led to the discovery of SIB-1553A (**111**) (see Fig. 14.11) as a selective nAChR agonist, discussed later. An enantioselective synthesis of SIB-1508Y (51) has recently been reported (303).

4.2.2.1.1.1 ***SIB-1508Y*** (51; Altinicline). SIB-1508Y (51 in Table 14.6), is a partial agonist of the human $\alpha_4\beta_2$ nAChR expressed in HEK293 cells (EC_{50} = 1.8 ± 0.7 μM) (305). However, SIB-1508Y is also more efficacious than nicotine in increasing DA release from rat striatal slices (305). Preclinical studies using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys (a model for Parkinson's disease) trained to perform a delayed matching-to-sample task have shown cognitive improvement after SIB-1508Y treatment (306,307). Of note is that this compound has also been shown to elicit antidepressant-like effects in the learned helplessness rat model of depression (308). SIB-1508Y had reached phase II clinical trials as a potential treatment for Parkinson's disease, but no further development has been reported.

4.2.2.1.2 Pyrrilidine Ring-Opened Nicotinoids. The nicotinoids, characterized as having an opened pyrrolidine ring, have been discussed in several reviews (273,293,295). Described herein is a series of trans-metanicotine analogs as nAChR agonists.

R. J. Reynolds Tobacco Co. researchers disclosed a series of trans-metanicotine analogs as nAChR agonists (309–313). Of the analogs with good binding affinity to the $\alpha_4\beta_2$-type receptor, most showed low activity at the muscle- and ganglionic-type receptors. Alkyne (64) is the only compound with significant activation of the ganglionic-type nAChR. Studies on the structure-activity relationship have been reported and are summarized in Table 14.7. First, secondary amines bind much more tightly than either primary or tertiary amines. For example, binding affinity follows the trend 2° (RJR-2403) > 1° (**58**) > 3° (**59**). Second, bulky substituents on the aliphatic nitrogen

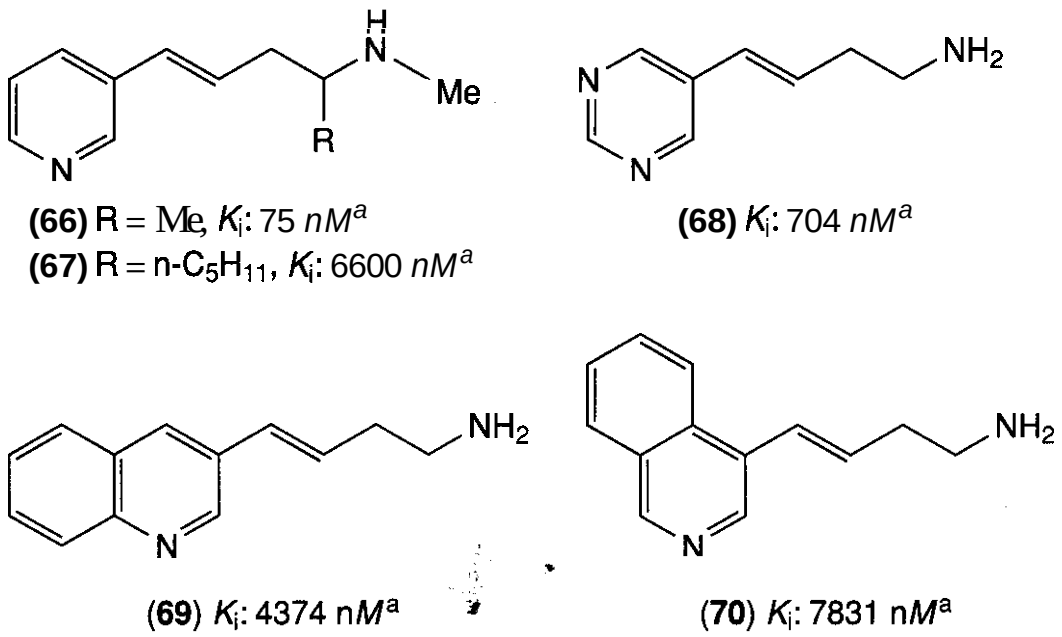


Figure 14.7. "Binding data: rat brain/[^{3}H]nicotine (322).

significantly reduce binding affinity (60 versus FUR-2403). Third, substitution on the olefinic amine side chain also reduces binding affinity. Comparison of the binding affinities of RJR-2403 with (66) and (67) (Fig. 14.7) reveals a 3 and 254 times reduction in binding affinity when a methyl and n-pentyl groups are introduced, respectively, on the carbon adjacent to the aliphatic nitrogen. Fourth, a chain length of four carbon atoms between heterocycles and side-chain nitrogen is optimal for high affinity binding (58 versus 61 and 62). Fifth, the trans double-bond geometry provides the best binding activity. Binding affinity follows the trend trans olefin (RJR-2403) > alkyne (64) > cis olefin (63) > saturated analog (65). Finally, the nature of the heteroaryl moiety is critical to the binding activity. In general, the binding affinity is in the order of pyridinyl (RJR-2403) > pyrimidinyl (68) > quinolinyl (69) > isoquinolinyl (70) (Fig. 14.7).

4.2.2.1.2.1RJR-2403 (57; Metanicotine). RJR-2403 (57) was selected for further development because of its high binding affinity for the $\alpha_4\beta_2$ nAChR in rat cortex [K_i = 26 nM (314)] and equivalent efficacy to that of ACh for human $\alpha_4\beta_2$ nAChRs expressed in *Xenopus* oocytes, with fourfold greater potency (EC_{50}: FUR-2403 = 16 ± 4.6 μM; ACh = 57 ± 13 μM) (315, 316). The compound was about 9 times less potent and slightly less efficacious than nicotine in stimulating DA release from rat striatum (314). However, in *vivo* microdialysis experiments have demonstrated that RJR-2403 stimulates the release of ACh, DA, NA, and 5-HT as effectively as nicotine in rat neocortex (317).

RJR-2403 has been described as equal to or better than nicotine as a cognitive enhancer in behavioral assays (274). This compound ameliorated scopolamine-induced impairments in passive avoidance as well as working and reference memory impairments in a radial arm maze task caused by NBM lesions in rats (318). Later studies using the radial maze showed that the positive cognitive effects of FUR-2403 were long lasting (360 min postoral administration) (319). RJR-2403 also showed significant antinociceptive effects in mice and rats in a variety of pain models (320). Preliminary pharmacokinetics studies showed that RJR-2403 is metabolically unstable and the major metabolic pathway in rats is the oxidation of the carbon a to the basic nitrogen (321). Positive results were reported for clinical trials of RJR-2403. However, further clinical development was suspended, presumably because of issues surrounding patent protection.

4.2.2.1.3 Isoxazole and Isothiazole Derivatives. Bioisosteric replacement of the pyridine ring in nicotine generated a series of novel isoxazole compounds that are selective and potent neuronal nAChR agonists, as exemplified by ABT-418 (71) (Table 14.8) (299). Among the variety of substituents examined at C3 of the isoxazole, methyl turns out to be optimal, even though other substituents, such as C_2-C_4 linear alkyl, CF_3, Br, and benzyl (not phenyl), still provide potent analogs. The 3-des-methyl

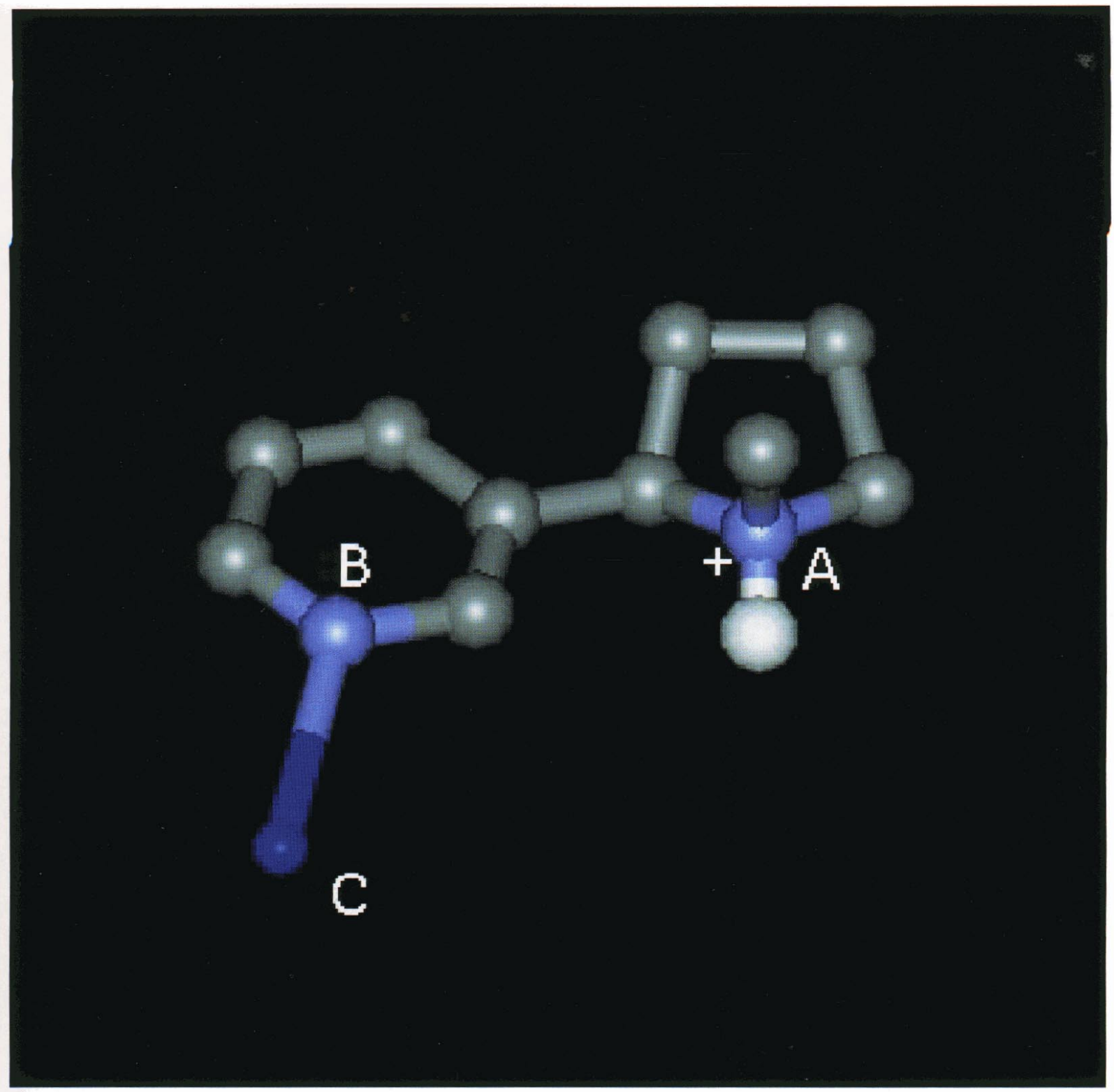
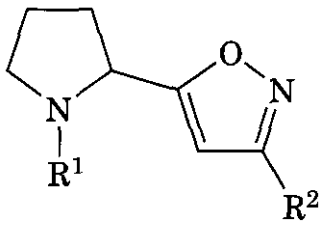


Figure 14.14. Example of pharmacophoric element selection for molecular modeling of nicotine. Pharmacophoric elements **A** and B are nitrogens and element **C** is on the nAChR receptor with which element B would optimally connect. Carbon, green; nitrogen, light blue; hydrogen attached to the basic nitrogen, white; putative nAChR binding site, dark blue.

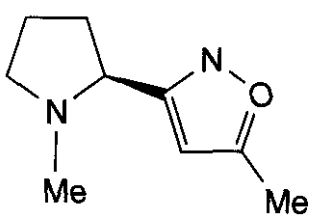
Table *14.8* Binding Data of Isoxazoles



Compound	Stereochemistry	R^1	R^2	K_i (n*M*)[a]
(71)(ABT-418)	(*S*)	Me	Me	4.2[b]
(72)	(*R*)	Me	Me	52.9[b]
(73)	(*S*)	*H*	Me	333[b]
(74)	(*R*)	H	Me	7.4[b]
(75)	(*S*)	Me	*H*	210[c]

"Rat brain/[^{3}H]cytisine.
[b]Ref. 299.
'Ref. 294.

analog of ABT-418, (**75**), was shown to possess 50 times lower binding affinity than that of ABT-418 for the [^{3}H]cytisine binding site (294). Substitution of the pyrrolidine moiety in ABT-418 generally reduces activity (323). The enantiomer of ABT-418, (**72**), has 13 times less binding affinity than that of ABT-418, compared with the 14-fold difference in binding affinity for the corresponding nicotine analogs. ABT-418 has approximately 80-fold more potent binding affinity than that of the desmethyl analog (**73**), whereas nicotine has a binding affinity 15-fold greater than that of the desmethyl analog. Also of note is that the opposite trend is observed with the two (*R*)-enantiomeric isoxazole counterparts. The (*R*)-NH-isoxazole (74) has a binding affinity sevenfold greater than that of the N-methyl analog (**72**), but binds with an affinity 2 times less than that of ABT-418. Isoxazole (**74**) also demonstrated comparable efficacy to that of ABT-418 and nicotine in stimulation of DA release from striatal synaptosomes.



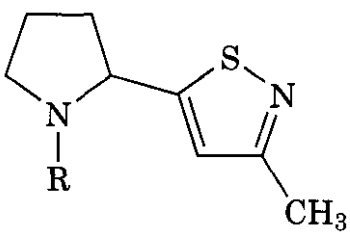
(**76**) K_i: 1333 n*M*[a]

Figure *14.8.* "Binding data: rat brain/[^{3}H]cytisine ***(299).***

The binding activity of the reverse isoxazole (76) is significantly reduced relative to that of ABT-418 (Fig. 14.8). Replacement of oxygen in ABT-418 with sulfur generates isothiazole (79) with reduced binding affinity (Table 14.9). It is interesting to note that the N-unsubstituted isothiazole (77) is sixfold more active than the isoxazole counterpart (**73**), but still 12 times less active than ABT-418, in terms of binding activity, and also less efficacious with respect to stimulation of dopamine release from striatal synaptosomes.

4.2.2.1.3.1 ***ABT-418*** (71). From a series of isoxazole analogs, ABT-418 (**71**), was selected for further evaluation (324–326). It is a full agonist at the brain $\alpha_4\beta_2$ nAChR, with reduced affinity for ganglionic-like nAChRs (327, 328). In ***vitro,*** ABT-418 stimulated the

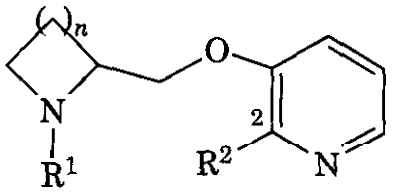
Table *14.9* Binding Data of Isothiazoles



Compound	Stereochemistry	R	K_i (n*M*)[a]
(77)	(*S*)	H	51
(78)	(*R*)	H	228
(79)	(*S*)	Me	222
(80)	(*R*)	Me	207

"Rat brain/[^{3}H]cytisine (299).

Table 14.10 Binding Data of 3-Pyridyl Ethers



Compound	n	Stereochemistry	R^1	R^2	K_i (nM)[a]
(**81**) (A-85380)	1	(*S*)	H	H	0.052
(**82**)	1	(*R*)	H	H	0.05
(**83**)	1	(*S*)	Me	H	0.45
(**84**)	1	(*R*)	Me	H	3.5
(**85**)	2	(*S*)	H	H	0.16
(**86**)	2	(*R*)	H	H	0.14
(**87**) (A-84543)	2	(*S*)	Me	H	0.15
(**88**)	2	(*R*)	Me	H	19.7
(**89**) (ABT-089)	2	(*S*)	H	Me	16.7
(**90**)	2	(*R*)	H	Me	39
(**91**)	2	(*S*)	Me	Me	28
(**92**)	2	(*R*)	Me	Me	3000
(**93**)	3	(*S*)	Me	H	73

[a]Rat brain/[^{3}H]cytisine (336).

release of [^{3}H]ACh from rat hippocampal synaptosomes (EC_{50} = 2.6 μM; nicotine EC_{50} = 1 μM) (328) and [^{3}H]dopamine from rat striatal slices (EC_{50} = 380 nM; nicotine EC_{50} = 40 nM) (327). Like nicotine, ABT-418 has poor oral bioavailability in dogs and monkeys (<5%) and moderate oral bioavailability in rats (27%) (329). ABT-418 was shown to be effective in animal behavioral models designed to access cognition enhancement (reviewed in Ref. (328)). Positive effects were observed for both mice and rats in passive avoidance and Morris water-maze tasks. Studies with nonhuman primates have demonstrated positive effects on cognition using the delayed matching-to-sample task (330) and a task designed to measure distractibility (331). An initial clinical trial of ABT-418 in AD patients yielded positive results in both selective reminding tasks (332) and nonverbal learning tasks (333). A controlled clinical trial also suggested that ABT-418 may be potentially useful in the treatment of attention deficit hyperactivity disorder (ADHD) (334). Phase II clinical trials with oral ABT-418 were reported to have had positive effects in enhancing acute attention in AD patients, but the compound's development was terminated for undisclosed reasons (335). More recently, clinical studies using transdermal administration of ABT-418 have been reported to be in progress (78).

4.2.2. I.*4 Pyridyl Ether Derivatives.* A series of 3-pyridyloxymethyl heterocyclic ether compounds have been identified with subnanomolar affinity for brain nAChRs (336) (Table 14.10). Of particular note are A-85380 (81) and A-84543 (87). A-85380 exhibits high binding affinity (K_i = 52 pM), for $\alpha_4\beta_2$ nAChRs, comparable to that of epibatidine, the most potent nAChR ligand reported to date. This compound stimulated ion flux at the human $\alpha_4\beta_2$ nAChR subtypes at 163% relative to nicotine.

A-85380 (**81**) is also a full nicotine agonist at recombinant human *a,* nAChRs and yet possesses potent activity at ganglionic-like nAChRs, as evidenced by 113% of the nicotine response in an assay examining cation flux through IMR-32 cells. The IMR-32 assay serves as a model for activity at human peripheral ganglionic receptors, which are believed to partially mediate undesired cardiovascular and gastrointestinal effects of nicotine. Not surprisingly, a radioiodinated analog of A-85380 has demonstrated utility as a research tool for in *vivo* studies of central nAChRs (337–339).

A-84543 (**87**), another full agonist at human $\alpha_4\beta_2$ (K_i = 150 pM), has been shown to have 32-fold selectivity to stimulate ion flux at

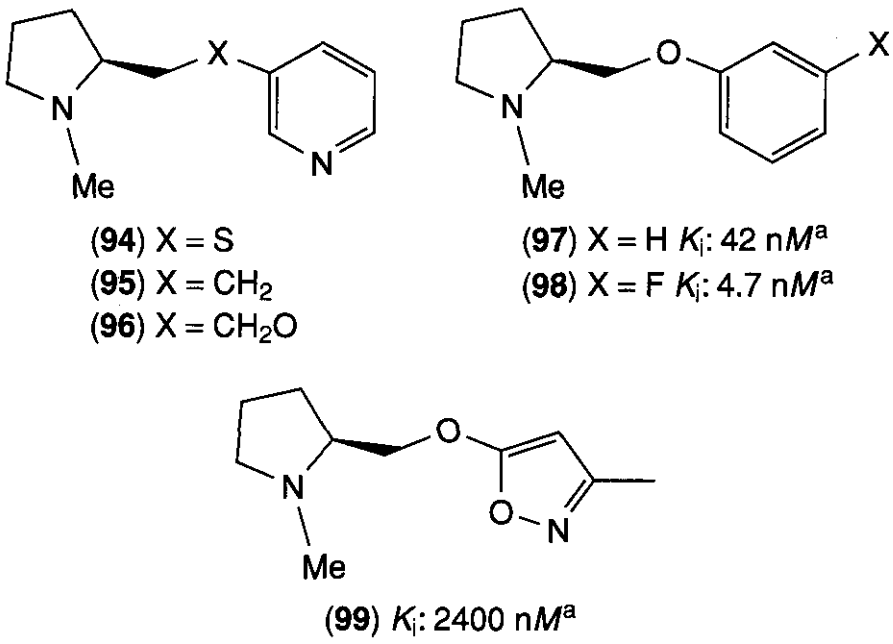


Figure 14.9. "Binding data: rat braid [^{3}H]cytisine (294).

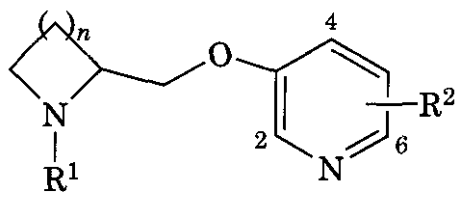
human $\alpha_4\beta_2$ nAChRs compared to human ganglionic nAChRs, and less efficacy (77% of nicotine response) at human sympathetic ganglionic nAChRs (340). Various substituents at the C5 position of the pyridyl moiety of A-84543 have been evaluated. According to the binding data, large substituents are well tolerated at the C5 position. However, functional activity can change from agonist to antagonist by means of varying the substituents on the pyridine ring (341).

It should be noted that the N-methylation for the pyridyl ether series has a different impact from that in the nicotine series (Table 14.10). For example, the N-H pyrrolidine analog (85) possesses comparable affinity to that of the corresponding N-methyl analog A-84543 (87). In the case of corresponding azetidines, the unsubstituted analog A-85380 is ninefold more potent than the N-methyl counterpart (83). With regard to stereochemistry, the *N*-methyl compounds with (*S*) configuration exhibit more potent binding affinity than that of their (*R*) counterparts, whereas the unsubstituted N-H enantiomers have similar affinity regardless of the stereochemistry. Structure-activity studies on the methyleneoxy moiety in A-84543 revealed that replacement of the oxygen atom with –S– (**94**), –CH_2– (**95**), and –CH_2O– (96) (Fig. 14.9) reduces affinity at the rat $\alpha_4\beta_2$ nAChR by about 2000-, 150-, and 120-fold, respectively. Replacement of the pyridine moiety in A-84543 (87) with a phenyl ring (97) decreases binding affinity by 280 times, but the corresponding m-fluorophenyl derivative (98, K_i = 5 n*M*) is about eightfold more potent than the unsubstituted analog (**97**) (Fig. 14.9). Both (**97**) and (**98**) are less efficacious than A-84543 (<40% of nicotine response) at human recombinant $\alpha_4\beta_2$ and ganglionic-type receptors.

SAR studies on the heteroaryl moiety in A-84543 (87) revealed that the pyridyl nitrogen in the 3-position is important, and that additional nitrogens in the ring are generally detrimental to activity. It is interesting to note that the 3-methyl-5-isoxazole moiety, which served as a bioisostere for pyridine in ABT-418, is a poor substitute for pyridine in A-84543 (see 99, Fig. 14.9). With respect to the N-methyl analogs, the pyrrolidinyl analog (**87**) is threefold more active than azetidine (83) and 480-fold more active than piperidine (93).

Extensive SAR studies were conducted with the aim of reducing propensity for peripheral ganglionic-like nAChRs, and it was discovered that the 2-methyl substitution of pyridine moiety results in the reduced activity at both ganglionic-like and human $\alpha_4\beta_2$ receptors. For example, both (85) and (88) possessed efficacy comparable to that of nicotine in the IMR-32 assay, whereas their respective 2-methyl analogs, (89) and (**92**), generated only 8% and 11% response, respectively, compared with that of nicotine. The 2-methyl pyridine analogs also have 100 to 300 times lower affinity than those lacking the 2-methyl substituent for native $\alpha_4\beta_2$ nAChRs and much reduced efficacy at human recombinant $\alpha_4\beta_2$ receptors. The pattern of binding affinities regarding stereochemistry and N-methylation parallels that observed for the corresponding 2-desmethyl series.

Table 14.11 Binding and *In* Vivo Data of 3-Pyridyl Ethers



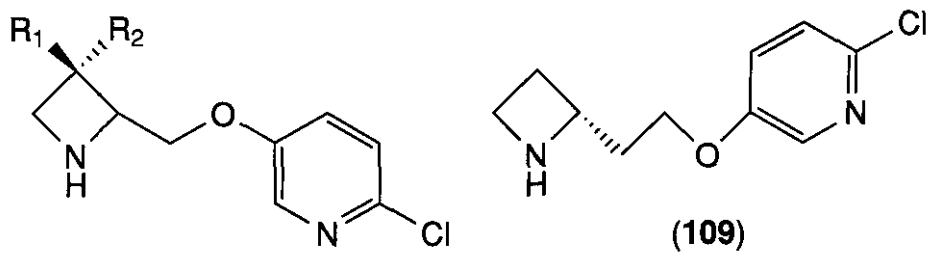
Compound	n	Stereochemistry	R^1	R^2	K_i (n*M*)[a]	MED (μmol/kg)[b]
(**81**) (A-85380)	1	(*S*)	H	H	0.05	>6.2
(**82**)	1	(*R*)	H	H	0.05	>6.2
(**100**) (A-98593)	1	(*S*)	H	6-Cl	0.04	0.62
(**101**) (ABT-594)	1	(*R*)	H	6-Cl	0.04	0.62
(**102**)	1	(*R*)	H	6-Br	0.17	0.62
(**103**)	1	(*R*)	H	5-Cl	0.12	62
(**104**)	1	(*R*)	Me	6-Cl	1.6	>6.2
(**105**)	2	(*S*)	H	6-Cl	0.09	>6.2
(**106**)	2	(*R*)	H	6-Cl	0.45	>6.2

[a]Rat brain/[^{3}H]cytisine.
[b]Minimum effective dose (MED) in mouse hot-plate assay, i.p. (294, 342).

Of potential relevance to the SAR of these compounds for cognition enhancement is their SAR in antinociceptive assays. In that regard, it is important to note that A-85380 (81)(Table 14.10), which possess affinity for $\alpha_4\beta_2$ receptors comparable to that of (?)-epibatidine, failed to demonstrate a significant antinociceptive effect in the mouse hot-plate model at doses up to 6.2 μmol/kg (Table 14.11), whereas epibatidine shows a robust activity at 0.1 μmol/kg. Compound (**82**), an enantiomer of A-85380, also did not show a significant analgesic activity at doses up to 6.2 μmol/kg, despite its high affinity for central $\alpha_4\beta_2$ nAChRs (Table 14.11) (342). In contrast, the 6-chloro analogs (100) and ABT-594 (101) not only maintained high affinity in a [^{3}H]cytisine displacement assay but also demonstrated analgesic effects at 0.62 μmol/kg comparable to that of epibatidine. Like epibatidine, both enantiomers have similar analgesic activity. As shown in Table 14.11, the binding affinity to the [^{3}H]cystine site (i.e., the putative $\alpha_4\beta_2$ nAChR) does not necessarily correlate well with analgesic activity. For example, both ABT-418 and ABT-594 are $\alpha_4\beta_2$ agonists, but profound analgesic-like effects were not observed for ABT-418, whereas ABT-594 demonstrated significant analgesic activity (343). The difference in activity between these two agonists may reflect interactions with different $\alpha_4\beta_2$ nAChR states, but this has yet to be verified (344,345). It has also been postulated that a different nAChR subtype is responsible for the analgesic activity, although other factors such as species differences and pharmacokinetic properties cannot be ruled out.

With respect to the activation of cation flux in IMR-32 cells, compounds (100) and ABT-594 were shown to be more potent and (in the case of ABT-594) more efficacious than the corresponding deschloro analog. The 6-bromo analog (**102**) maintains both binding activity and analgesic activity, whereas the 5-chloro analog (**103**) failed to demonstrate analgesic activity despite a binding affinity similar to that of compound (102). The N-methyl analog of ABT-594, (**104**), dramatically reduces both binding and analgesic activity.

The SAR studies on both the azacycle ring size and azetidine N-methylation have been carried out (Fig. 14.11) (342). Pyrrolidine analogs (105) and (106) failed to demonstrate significant activity in the hot-plate assay, even at doses 10-fold higher than that at which (100) and ABT-594 (101) showed robust effects. The in vitro tests showed only a modest decrease in potency with the increased ring size. N-Methylation of ABT-594 gave (**104**), with substantially reduced the [^{3}H]cytisine binding affinity and ganglionic-like activity, and loss of analgesic activity. Also of note is that one or two methyl substituents at the 3-position of the azetidine ring in ABT-594 (107 and 108) (Fig. 14.10) as well as one-carbon homologation of the linkage (**109**) signifi-



(109)

(107) R^1 = Me, R^2 = *H*; K_i: 7.6 *nM*[a]; hot plate MED: >62 mmol/kg[b]
(108) R^1 = R^2 = Me; K_i: 37 *nM*[a]; hot plate MED: >62 mmol/kg[b]
(109) K_i: 11 *nM*[a]; hot plate MED: >62 mmol/kg[b]

Figure 14.10. "Binding data: rat braid [^{3}H]cytisine; [b]minimum effective dose (MED) in mouse hot-plate assay (346).

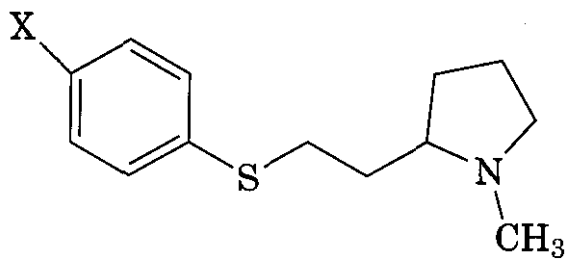
cantly reduces binding affinity and analgesic activity in the mouse hot-plate assay. It will be of interest to determine whether a similar SAR will be found when examining the potential efficacy of these compounds in behavioral models of cognitive function.

4.2.2.1.4.1 ABT-089 (89). ABT-089 ***(89)*** was identified as the backup compound to ABT-418. The agonist ABT-089 is nearly as potent as nicotine for stimulating ACh release from rat hippocampal tissue. However, ABT-089 was 25% less potent and only 70% as efficacious as nicotine in stimulating dopamine release from rat striatal tissue (314). ABT-089 has also been characterized as a weak agonist at the human *a*, nAChR subtype (1.5% response at 1 μM) and to inhibit the response to ACh (IC_{50} = 48 μM) (347). In *vivo* studies with ABT-089 demonstrated cognition enhancement in a variety of rat and monkey behavioral models (348, 349) and has recently been shown to reduce distractibility in adult monkeys (331). As previously described, ABT-089 is a relatively poor activator of ganglionic nAChRs, suggesting a reduced propensity for negative side effects at high doses (350). ABT-089 showed good oral bioavailability (30–70% across rat, dog, and monkey), superior to both ABT-418 and nicotine (294). It is noteworthy that the secondary amine functionality serves to enhance the oral bioavailability compared to the corresponding N-methyl analog (91) (62% versus 6% in dog), presumably enhancing its stability to first-pass metabolism (336). ABT-089 is currently in phase I clinical trials as a potential treatment for Alzheimer's disease, schizophrenia, and attention deficit hyperactivity disorder.

4.2.2.1.4.2 ABT-594 (101). A-98593 (**100**) was originally selected for further development and was later replaced with ABT-594 (101) because the latter shows less cardiovascular toxicity in dogs (294). The separation between antinociceptive doses and lethal doses in mice is fivefold greater than that for epibatidine (351). However, a recent study on the analgesic and toxic effects of ABT-594, nicotine, and epibatidine indicates that the acute safety profile of ABT-594 is not significantly improved over that of other nicotinic analgesics (351). ABT-594 has the affinity for $\alpha_4\beta_2$ neuronal nAChRs comparable to that of (±)-epibatidine, but its affinity for neuromuscular nAChRs is 4000 times less than that of (±)-epibatidine. Moreover, this compound is about 30 times less potent and less efficacious at ganglionic-like nAChRs in IMR-32 cells. The high selectivity for neuronal $\alpha_4\beta_2$ nAChRs is responsible for an improved therapeutic index over that of (±)-epibatidine. ABT-594 is also less potent than epibatidine in assays of acute and persistent pain and in the rotarod assay. However, it displays a clear separation between its motor and analgesic effects (352). ABT-594 shows good oral bioavailability across species (30–SO%), with oral half-lives ranging from 1.4 to 4.2 h. ABT-594 rapidly enters the brain, exhibiting a brain-to-plasma ratio of about 2 within 1.5 h (294,342). Recent studies on ABT-594 showed an increase in FGF-2 expression in various rat brain regions, suggesting a therapeutic significance in neurodegenerative disorders (353), but no preclinical data for cognitive enhancement have been reported. ABT-594 is currently in phase III trials as an antinociceptive agent (292, 314, 354).

4.2.2.1.5 Phenylthioether Derivatives

4.2.2.1.5.1 SIB-1553A (111). Based on the shift of nAChR subtype selectivity from SIB-1508Y (51) to ligand (**56**) (Table 14.6), Merck (then SIBIA Neuroscience) scientists designed



(110) X = H
(111) X = OH (SIB-1553A)

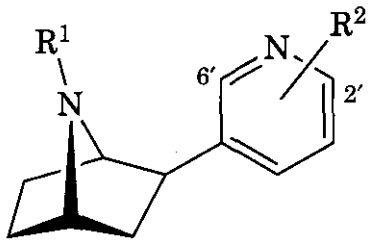
Figure 14.11.

compound (**110**), incorporating the phenyl and pyrrolidine rings of (56) but lacking the pyridine ring of nicotine and SIB-1508Y (Fig. 14.11) (302). Compound (110)did show selectivity over β_4-containing nAChR cell lines, but it lacked potency and efficacy in stimulating the release of neurotransmitters from brain slices of different regions in rat brain. Extensive SAR studies culminated in the discovery of SIB-1553A (Fig. 14.11) as a potent and efficacious nAChR agonist. A functional assay employing cell lines stably expressing human recombinant nAChR subtypes confirmed the selectivity for β_4-containing nAChR subtypes. Electrophysiological recording of current responses in Xenopus oocytes expressing recombinant human nAChRs showed that SIB-1553A was most efficacious on $\alpha_4\beta_4$ and had weak activity on $\alpha_3\beta_4$ and α_7 receptors. With respect to its ability to displace [^{3}H]nicotine from rat cortical membranes, this compound is significantly less potent than nicotine, indicating that SIB-1553A binds preferentially to a less abundant endogenous nAChR subtype than to the most abundant subtype, presumably composed of α_4 and β_2 subunits. SIB-1553A was more efficacious than SIB-1508Y in stimulating rat hippocampal ACh release (51) or nicotine (355). For example, subcutaneous administration of racemic SIB-1553A as well as both enantiomers at 40 mg/kg resulted in a 10- to 12-fold increase of acetylcholine from hippocampi of freely moving rats, and this effect could be attenuated by nAChR antagonists such as mecamylamine.

SIB-1553A has been evaluated for potential cognitive-enhancement effects in an animal model. When tested in a sequential spontaneous alternation task, this compound was found to reverse the working memory deficits of aged mice. In this paradigm, SIB-1553A was more efficacious and better tolerated than nicotine. SIB-1553A has been reported to reverse working and reference memory deficits associated with age, drug-induced cholinergic dysfunction, and specific lesion of cholinergic neurons in rodents and monkeys (356). Additionally, SIB-1553A caused dose-dependent improvements in performance on the delayed matching-to-sample task in MPTP-impaired monkeys (306). It is interesting that neither (*S*)- nor (*R*)- alone was as active as the racemate in any of these behavioral experiments. On January 7, 1999, Sibia issued a press release announcing that SIB-1553A had entered phase II Clinical trials as a potential treatment for AD.

4.2.2.2 ***Epibatidine Derivatives.*** Epibatidine (112 in Table 14.12), a trace alkaloid discovered from skin extracts of an Ecuadorian frog, was reported to be a nonopioid analgesic agent with a potency 200-fold greater than that of morphine in mice (357, 358). The absolute configuration of the natural epibatidine was assigned as (1*R*, 2*R*, 4*S*). Epibatidine remains the most potent naturally occurring nAChR ligand reported to date, with potency in many pharmacological and behavioral assays a few 100-fold greater than that of nicotine. The activity of epibatidine as a potent nonopioid analgesic agent has attracted significant attention (359). However, it remains unknown which subtype(s) is responsible for the antinociceptive effects of epibatidine, although central action appear to be involved (360). In contrast to nicotine, both enantiomers of epibatidine are highly potent at several nAChR subtypes. Also unlike nicotine, epibatidine is ineffective in models of cognitive performance. Further, epibatidine administration has been associated with adverse effects such as hypertension, convulsions, and respiratory depression (361, 362). It should be noted that nicotine has not been developed as an analgesic agent because of its poor spectrum of antinociceptive activity, low intrinsic activity compared with that of the opioids, and the poor side-effect profile (360, 363, 364). It has been suggested that the $\alpha_1\beta_1\delta\gamma(\varepsilon)$ and $\alpha_3\beta_4$ variant nAChR subtypes found at the neuromuscular junction (265) and sympathetic gan-

Table 14.12 Binding and *In Vivo* Data of Epibatidine Analogs



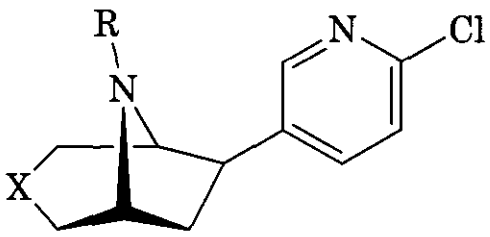
Compound	R^1	R^2	K_i (n*M*)	$ED_{,,}$ (nmol/mouse)[a]
(112)(1*R*, 2*R*, 4*S*), *natural*	*H*	*2'-C1*	0.045[b]	*0.5*
(113)(1*S*, 2*S*, 4*R*)	*H*	*2'-C1*	0.058[b]	*0.6*
(114)(racemic)	*H*	*2'-C1*	*0.027"*	
(115)(racemic)	*H*	*2'-F*	0.027[d]	*0.31*
(116)(racemic)	*H*	*2'-Br*	0.023[d]	*0.33*
(117) (racemic)	*H*	*2'-I*	0.070[d]	*Not done*
(**118**) (1*R*, 2*R*, 4*S*)	*H*	*2'-Me*	0.13[b]	
(**119**) (racemic)	*H*	*2'-OH*	107[d]	*52.4*
(120)(racemic)	*H*	2'-NH_2	1.3[d]	*27% @ 112*
(121)(racemic)	*H*	*2'-NMe,*	26.4[d]	*10% @ 32.5*
(**122**) (racemic)	*H*	2'-CF_3SO_3	8.5[d]	*19.2*
(123)(racemic)	*H*	*2'-H*	0.020[d]	*0.2*
(124)(1*R*, 2*R*, 4*S*)	*Me*	*2'-C1*	0.26[b]	
(125)(1*S*, 2*S*, 4*R*)	*Me*	*2'-C1*	0.11[b]	
(126)(racemic)	*Et*	*2'-C1*	*13.6"*	
(**127**) (racemic)	*H*	*6'-C1*	33[e]	

"Tail-flick test (366).
[b]Binding data: rat brain/[^{3}H]nicotine (358).
'Binding data: rat brain/[^{3}H]epibatidine (368).
[d]Binding data: rat brain/[^{3}H]epibatidine (366).
'Binding data: rat brain/[^{3}H]methylcarbamylcholine (MCC) (367).

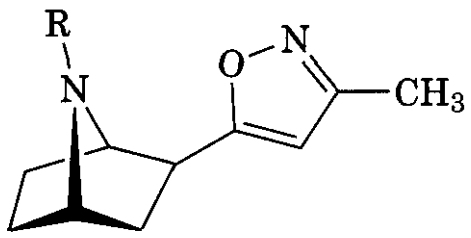
glia (266) mediate many of the undesired functional effects of (±)-epibatidine. Nevertheless, the antioceptive properties of epibatidine have led to a search for novel agents with enhanced selectivity for the $\alpha_4\beta_2$ receptor subtype over that of other nicotinic receptor subtypes, and these efforts culminated in the discovery of ABT-594 as a novel antinociceptive agent (294). It remains to be determined whether these compounds will display efficacy as cognitive-enhancing agents.

N-Methylation of natural epibatidine (**112**) and its enantiomer (113) reduced affinities for rat $\alpha_4\beta_2$ nAChRs by 6 and 2 times, respectively. With respect to functional assays, the impact of N-methylation on the activities of the two enantiomers is relatively small but differential, and the N-methyl products (124 and 125) showed modest enantioselectivities (358) (Table 14.12). N-Methyl (±)-epibatidine (**114**) demonstrated the analgesic activity in the mouse tail-flick model similar to that observed with (±)-epibatidine (365). The deschloro derivative of epibatidine (123) is comparable to epibatidine in terms of binding affinity for $\alpha_4\beta_2$ nAChRs and functional activity in models of ganglionic (PC12) and muscle (TE671) nAChR function, but its analgesic potency is significantly reduced in the formalin test but comparable to epibatidine in the tail-flick model (366, 367). N-Ethylation (126) reduces the binding activity by 486 times (368). A number of substituents at the 2'-position have been evaluated. In general, electron-withdrawing groups and small alkyl groups (115, 116, 117, 118) maintain activity, whereas electron-donating groups (OH, NMe_2) (**119**, 121) reduce activity with the exception of NH_2 (120). The 6'-chloro analog (127) is 55 times less active in terms of binding affinity (367).

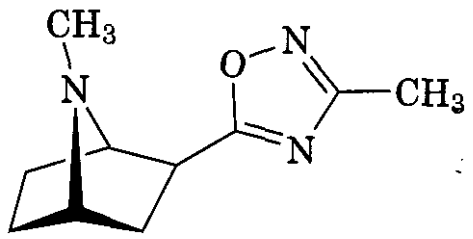
The racemic 8-azabicyclo[3.2.1]octane homoepibatidine derivatives were found to have analgesic activity in the hot-plate model (369, 370) (Fig. 14.12). Compound (128) was shown



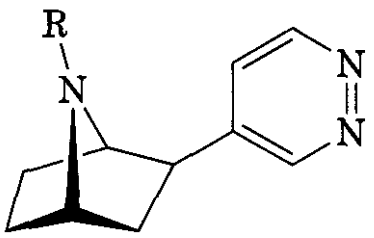
(128) X = CH_2, R = H
(129) X = CH_2, R = CH_3
(130) X = CH_2, R = i-Pr
(131) X = C(O), R = H
(132) X = $(CH_2)_2$, R = H



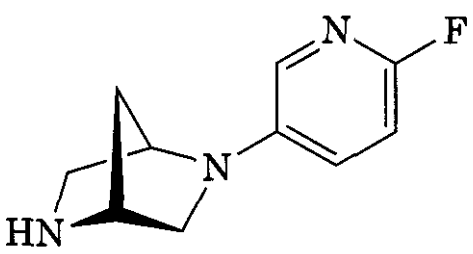
(133) R = H
(134) R = CH_3



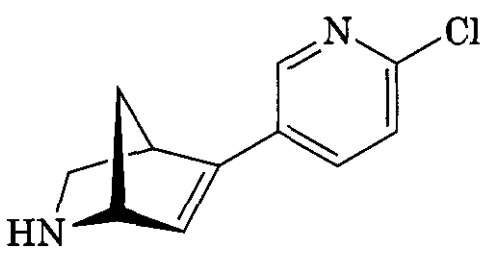
(135)



(136) R = H
(137) R = CH_3



(138)



(139)

Figure 14.12.

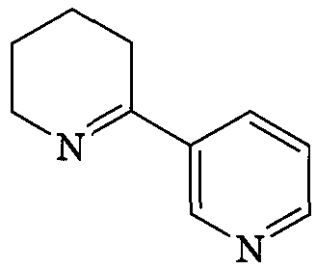
to have the same activity as that of epibatidine at fourfold higher dosage. The N-methyl analog (129) possesses activity comparable to that of epibatidine, but the N-isopropyl derivative (**130**) is 15 times less potent. The tropinone analog (**131**) was shown to be inactive even at high doses. The synthesis of the **bis-homoepibatidine** derivative (132) was reported, although its biological activity has not yet been disclosed (371).

Replacement of the chloropyridyl ring of **(±)-epibatidine** with methylisoxazole provided (?)-epiboxidine (133) as a potent **nAChR** agonist (372) (Fig. 14.12). This analog is 10 times less active than natural epibatidine and about 17-fold more potent than ABT-418 in inhibiting **[^{3}H]nicotine** binding to $\alpha_4\beta_2$ **nAChRs** in rat cerebral cortical membranes. Although **(±)-epiboxidine** exhibits potent activity at ganglionic-type **nAChRs** in PC12 cells, it is less toxic than epibatidine in mice.

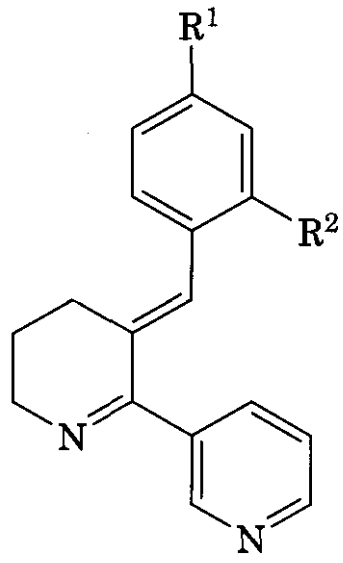
Bioisosteric replacement of the **chloropyridinyl** moiety in epibatidine with pyridazine generated (136) (Fig. 14.12). Both (136) and its N-methyl derivative (137) maintained much of the potency of natural epibatidine, but the selectivity between $\alpha_4\beta_2$ and $\alpha_3\beta_4$ subtypes was enhanced by eight- and 25-fold, respectively. However, the selectivity between $\alpha_4\beta_2$ and $\alpha_1\beta_1\delta\gamma(\varepsilon)$ was relatively unchanged. The ***in vivo*** studies of these pyridazine analogs have not yet been reported (373). Recently, identified epibatidine analogs include (138) [K_i = 0.03 nM (rat **brain/[^{3}H]cytisine**) (**374**)] and (139) [K_i = 0.26 nM (rat **brain/[^{3}H]epibatidine**) (**375**)] (Fig. 14.12).

4.2.2.3 ***Anabaseine Derivatives.*** The natural product anabaseine (140) (Fig. 14.13) was isolated from the marine worm ***Hoplonemetines hydrobiologia*** (376). ***In vitro*** analysis found anabaseine to have approximately 20 times lower **affinity** than that of nicotine for rat brain **nAChRs**, with a fivefold selectivity for the $\alpha_4\beta_2$ subtype over the a, subtype (377, 378). In ***Xenopus*** **oocytes**, however, **anabaseine** had a twofold higher intrinsic potency than that of nicotine at the expressed a, **nAChR** subtype.

DMAB-anabaseine and GTS-21 (141 and 142, respectively) are derivatives of **anabaseine** (**140**), obtained through condensation with the appropriate benzaldehyde (379,380) (Fig. 14.13). **DMAB-anabaseine** was more potent than nicotine, but had similar efficacy, at the a, **nAChR** subtype. Studies in rat brain indicate GTS-21 binds 20-fold more potently than nicotine to the $\alpha_4\beta_2$ **nAChR** subtype (K_i = 19 ± 4 nM) and 6 times less potently than



(140) anabaseine



(141) DMABA $R^1 = N(CH_3)_2$, $R^2 = H$
(142) GTS-21 $R^1 = OCH_3$, $R^2 = OCH_3$
(143) 40H-GTS-21 $R^1 = OH$, $R^2 = OCH_3$

Figure 14.13.

nicotine to the a, nAChR subtype (K_i = 650 ± 34 n*M*) (381). In clonal human K177 cells, GTS-21 had a 100-fold binding selectivity for the $\alpha_4\beta_2$ nAChR subtype over the a, nAChR subtype (K_i = 20 n*M* and 2 μM, respectively) (381). Interestingly, GTS-21 has been characterized as an a,-selective agent, having partial agonist activity at the a, nAChR subtype (—28% of ACh response from rat a, nAChR expressed in *Xenopus* oocytes), while showing negligible activity at the $\alpha_4\beta_2$ nAChR subtype (378). GTS-21 also failed to activate the human $\alpha_4\beta_2$ nAChR subtype expressed in K177 cells (381).

In animal models, both DMAB-anabaseine (**140**) and GTS-21 (142) improved reference memory in aged rats using a 17-arm radial maze (382) and normalized impaired auditory gating in mice, suggesting the a, nAChR has a role in sensory gating (383). GTS-21, but not DMAB-anabaseine, was effective for enhancing acquisition in both one-way active avoidance and Lashley III maze training (382). In NBM-lesioned rats, GTS-21 improved both passive-avoidance behavior and performance in the Morris water maze (384). GTS-21 has also been shown to enhance classical conditioning of aged rabbits in an eye-blink task (385–387) and to improve learning performance for normal monkeys in the delayed matching-to-sample task (381). Positive neuroprotective effects of GTS-21 have also been described (388–391). Psychological tests of GTS-21 on healthy young male subjects indicate a positive effect on some measures of cognition (392). GTS-21 is currently in phase I clinical trials as a potential therapy for Alzheimer's disease (393,394).

The ***in vitro*** activity attributed to GTS-21 (142) may not completely reveal its ***in vivo*** pharmacology. GTS-21 is primarily metabolized in humans to 3-(4-hydroxy, 2-methoxybenzylidene)-anabaseine, or 40H-GTS-21 (143 in Fig. 14.13) (395,396). This metabolite was later shown to be a selective partial agonist for both human and rat a, nAChR subtypes, demonstrating good potency (EC_{50}: human = 26 ± 7 μM; rat = 10 ± 2 μM) and efficacy compared to that of ACh (human = 50%; rat = 40%) (397). Some of the physiological and behavioral effects of GST-21 may be attributable to the actions of this primary metabolite. Both GTS-21 and 40H-GTS-21, however, appear to be very weak agonists of the human $\alpha_4\beta_2$ nAChR subtype, further supporting a role of α_7 nAChRs in cognitive processes.

4.2.3 nACh Receptor Pharmacophore Model.

Several pharmacophore models proposed over the years are presented in a recent review (295). The classic Beers and Reich model (Fig. 14.14) proposes that the essential elements of the nicotinic pharmacophore are a protonated or quaternized nitrogen atom (A) (e.g., protonated pyrrolidine nitrogen of nicotine) and an electronnegative atom capable of formation of a hydrogen bond (B) (e.g., the pyridine nitrogen of nicotine) (398). The distance between the center of charge (A) and the center of the van der Waals surface of the pyridine nitrogen was 5.9 Å (the Beers-Reich distance). Subsequently, Sheridan performed ensemble distance geometry with several known nicotinic agonists and proposed a pharmacophore with an additional element to the Beers and Reich model: a dummy point (C) or an atom to define a line along which the hy-

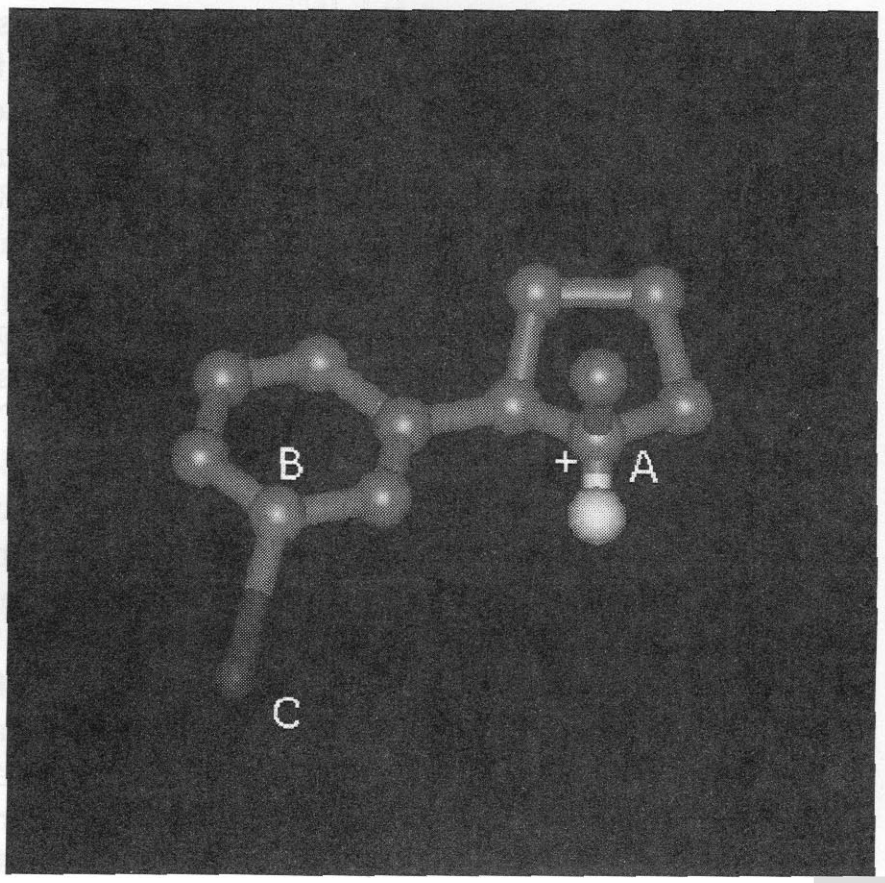
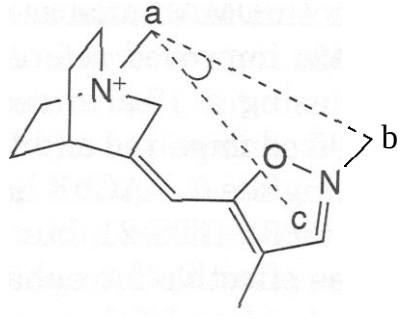


Figure 14.14. Example of pharmacophoric element selection for molecular modeling of nicotine. Pharmacophoric elements **A** and **B** are nitrogens and element **C** is on the nAChR receptor with which element **B** would optimally connect. Carbon, green; nitrogen, light blue; hydrogen attached to the basic nitrogen, white; putative nAChR binding site, dark blue. See color insert.

drogen bond may form (399). This element can be exemplified by the pyridine ring centroid or the carbonyl carbon.

The Sheridan model suggested the optimal distances between the three elements: A-B (internitrogen distance), 4.7 ± 0.3 Å; A-C, 4.0 ± 0.3 Å; B-C, 1.2 Å. The Sheridan models also suggested the Beers-Reich distance of **5.9** Å. The discovery of epibatidine as an exceptionally high affinity ligand for nAChRs led to the proposal that the optimal internitrogen distance for high affinity binding may be close to 5.5 Å as the lowest energy conformer of epibatidine had an internitrogen distance of 5.51 Å (400). A-85380, which possesses affinity similar to that of epibatidine, showed an internitrogen distance of **6.1** Å in the minimum energy conformer (340). However, based on the argument that the receptor-bound conformation of ligands may not be the same as the lowest energy conformations either in a vacuum or in solution, Koren et al. conducted computational studies on higher than minimum energy conformers of compounds such as epibatidine and A-85380. These studies show that the internitrogen distances for these stable conformers with relative energies not exceeding 0.42 kcal/mol are about 4.4 Å, which is close to the proposed ranges in the Sheridan model (401). Therefore, it can be concluded that epibatidine and the high affinity A-85380 based nAChR ligands possess stable conformers featuring similar spatial arrangement of the Sheridan pharmacophoric elements.

The most recent model developed by Olesen et al. suggests the essential groups are as follows: (*1*) site point a, corresponding to the protonated nitrogen atom; (*2*) site point **b,** corresponding to the electron negative atom capable of forming a hydrogen bond; **(3)** site point c, which is the center of a heteroaromatic ring or a C=O bond (Fig. 14.15). The site points a and b are placed 2.9 Å from the corresponding atoms in the direction of the lone pairs. Optimal pharmacophoric parameters were estimated as follows: a-b, 7.3–8.0 Å; a-c, 6.5–7.4 Å; Aabc, 30.4–35.8°. This three-element model was able to explain **65%** of the variation seen in the $p(IC_{50})$ values (295). It should be emphasized that it is oversimplified to assume that the binding affinity of nAChR



a–b: 7.3–8.0 Å
a–C: 6.5–7.4 Å
Aabc: 30.4–35.8

Figure 14.15. Olesen nicotinic pharmacophore model. (Modified from Ref. 295.)

ligands be determined by the limited set of distance or angle parameters as discussed above. Nevertheless, the proposed pharmacophore models have proved to be useful in rationalizing the activity data as well as in the design of new nAChR ligands.

With respect to the binding mode of nAChR agonists, Dougherty et al. reported a strong correlation between the cation-π binding abilities and the EC_{50} values for acetylcholine at the receptor for a series of tryptophan derivatives incorporated at the a-Trp-149 site in the nAChR. This study suggests that the a149 side chain accounts for the possible π-cation interaction between the quaternary ammonium group of acetylcholine and the nAChRs (402). Studies on the interaction of aromatic amino acid side chains with the cation-containing heterocyclic ring fragments of nicotinic ligands also showed that the tryptophan side chain was most pronounced in terms of interaction with the cation fragments, followed by tyrosine and phenylanine (403).

Because a crystal structure of nAChR is not available, the nAChR agonist binding site remains unknown. Nevertheless, the pharmacophore models and π-cation studies should provide valuable guidance in the design of more selective and potent nAChR agonists for the treatment of CNS-related diseases.

4.2.4 Future Direction. Significant pharmaceutical research efforts have gone into the search for novel neuronal nAChR modulators with selectivity for central versus ganglionic nAChRs, and these efforts have culminated in the identification of: (1) ABT-418, GTS-21, RJR-2403, and ABT-089 as cognition-enhancing agents; (2) SIB-1508Y for the treatment of Parkinson's disease; and (3) ABT-594 as an analgesic agent. Through the discovery of these novel agents, significant knowledge on nAChR has been gained. However, this is still insufficient to provide predictive in *vitro* receptor screens. Therefore, future studies should be directed at further understanding which of the nAChR isoforms mediate specific pharmacological functions of interest. As the crystal structure of the nAChR becomes available, structure-based design of nAChR agonists can be realized. Continued pharmaceutical research into the development of subtype-selective nAChR agonists is expected to generate agents with improved physiological activities and safety profiles.

4.3 5-HT, Receptor

4.3.1 Physiology and Pharmacology of the 5-HT, Receptor. The neurotransmitter serotonin [5-hydroxytryptamine (5-HT)] activates at least seven distinct (5-HT, to 5-HT,) receptors in the central and peripheral nervous systems to produce important modulatory effects (404). With the exception of the 5-HT, receptor, all 5-HT receptors are members of the G-protein-coupled receptor family that function through adenylyl cyclase or phospholipase C second messengers. The 5-HT, receptor, however, is a member of the superfamily of ligand-gated ion channels and serves to moderate neuronal depolarization by increasing the flux of Na^+, K^+, and Ca^{2+} (404–408). The structure of the 5-HT, receptor closely resembles that of other members of the ligand-gated ion channel family, especially the nAChRs (409, 410), and is thought to be a pentameric structure assembled from structurally distinct subunits. Currently, two subunits [$5\text{-}HT_{3A(a)}$ and 5-HT,, (411)] and an alternatively spliced variant [$5\text{-}HT_{3A(b)}$ (408, 412)] have been identified, although it is speculated that additional 5-HT, subunits may exist and could explain differences between Ca^{2+} permeability observed in native and recombinant 5-HT, receptor channels (406,413,414).

5-HT, receptors are generally localized in presynaptic nerve terminals or fibers (415). Experiments with rat striatal synaptosomes have demonstrated that presynaptic 5-HT, receptors have a high permeability for Ca^{2+} (416). Increased intracellular Ca^{2+} has been observed in isolated nerve terminals (synaptosomes)treated with a 5-HT, receptor agonist, a phenomenon that could facilitate action potential-dependent neurotransmitter release (417). In the mammalian brain, 5-HT, receptor agonists have been observed to stimulate the release of (1) ACh from rat dorsal hippocampus (418); (2) dopamine in the corpus striatum (419) and in the olfactory tubercle (420); (3) GABA and glutamate in the solitary tract nucleus (421); and (4) cholecystokinin in the cerebral cortex and nucleus accumbens (422). In contrast, 5-HT, re-

Table 14.13 Shared Pharmacological Properties Between 5-HT$_3$ and nACh Receptors

5-HT$_3$ Receptors	nACh Receptors	
	Agonist	Antagonist
Agonist	N-Methylbufotenine Iodide (432,433) (at ganglionic nAChR)	Ethanol (440), Halothane (434) and Isoflurane (434,435) (at the α_7 nAChR) Serotonin (5-HT) (443,444)
Antagonist	Acetylcholine (ACh) (442) (+) and (−) nicotine (442) Choline (Ch) (442) Epibatidine (442) DMPP (442) GTS-21 (442) Quipazine (442) Tropisetron (445) (all at the a, nAChR)	Chlorpromazine (436) QX-222 (436) *d*-Tubocurarine (439) (at the α_1 nAChR) Tropisetron (437) (at the α_9 nAChR)

ceptors have been observed to mediate inhibition of ACh release in human cerebral cortex synaptosomes (423), in rat cortical synaptosomes (424,4251, and in the rat frontal cortex (418).

The in *vitro* affinity of compounds for central 5-HT, receptor sites is often determined by radioligand binding assay, or more specifically, by the ability to displace radioligands from 5-HT, sites of rat entorhinal cortex. Several radioligands have been used, including [^{3}H]LY278584 (426), [^{3}H]GR 65630, and [^{3}H]BRL 43694 (427). The in *vitro* antagonistic activity at the 5-HT, receptors is carried out on the rat isolated vagus nerve (RVN) or guinea pig isolated ileum (GpI). 5-HT, receptor agonists cause a rapid depolarization of the vagus nerve. Antagonists cause parallel, rightward displacements of the agonist concentration response curve. The functional activity is expressed by the pA_2 value, which is the negative logarithm of the molar concentration of an antagonist that necessitates the doubling of the agonist dose to counteract the effect of that antagonist and restore the original response (428, 429). The most frequently used method for assessing the in *vivo* activity of 5-HT, receptor is accomplished through monitoring a transient, dose-dependent reflexive fall in heart rate and blood pressure (von Bezold-Jarisch reflex) evoked by 5-HT, or a 5-HT, receptor agonist, in urethane-anesthestized rats. This effect can be blocked by prior administration of a 5-HT3 antagonist such as ondansetron.

Recently, the nicotinic α_4 receptor subunit was found to coassemble with the 5-HT$_3$ subunit in *Xenopus* oocytes to form a Ca^{2+} permeable channel (430). However, analysis of native 5-HT, receptors purified from porcine cerebral cortex failed to reveal the presence of nACh receptor subunits (a, to a, or β_2) (431). Nevertheless, the prevalence and regional distribution of this subunit coassembly remain to be determined.

The structural similarity between 5-HT, and nACh receptors provides for an interesting but complex pharmacological overlap (Table 14.13). An overlap of agonist activity is known with N-methyl bufotenine iodide [the quaternary salt of serotonin; 5-HT$_3$, K_i = 75 n*M* (432, 433)]. General anesthetics such as isoflurane increase the apparent agonist affinity for both 5-HT, and nACh receptors, but also cause channel blockade of the nACh receptor (434, 435). Mutual receptor antagonism has been observed with chlorpromazine and quaternary linocaine derivative QX222 (436), tropisetron (437, 438), and also with *d*-tubocurarine (439). Ethanol has been found to potentiate agonist activity at the 5-HT, receptor (440) while inhibiting the nACh receptor (441). The nACh receptor agonists ACh, (*S*)- and (*R*)-nicotine, epibatidine, 1,1-dimethyl-4-phenylpiperazine (DMPP), and GTS-21 (142 in Fig. 14.13) have been shown to function as 5-HT, antagonists (442). 5-Hydroxytryptamine (5-HT), the endogenous agonist of the 5-HT, receptor, is an antagonist of nACh re-

ceptors (443,444). The 5-HT, antagonist quipazine and tropisetron have been reported to be an agonist of the a, nACh receptor subtype (445). It seems likely that further examples of pharmacological overlap will continue to be uncovered.

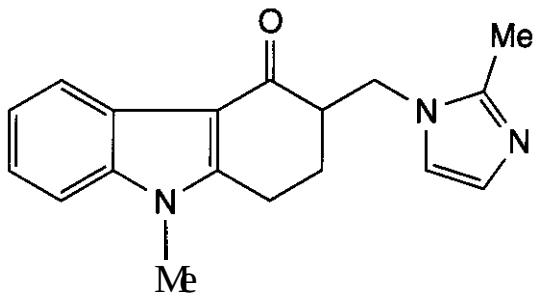
Currently, 5-HT, antagonists have found their greatest therapeutic value in the treatment of cancer chemotherapy-induced emesis (406). Release of 5-HT from the enterochromaffin cells in the gastrointestinal track often results from cancer chemotherapy with cytotoxic agents, such as cisplatin (446, 447). Blockade of 5-HT, receptors in the CNS or on peripheral vagal afferent fibers prevents the initiation of the vomit reflex.

There is a considerable literature indicating that 5-HT, agonists impair, whereas 5-HT, antagonists facilitate, learning and memory (448–451). The mechanisms by which 5-HT, antagonists achieve their positive effects on cognition are not clear. It would be reasonable to hypothesize that this is attributable to positive modulation of acetylcholine release in the neocortex (but not hippocampus), where such agents would be expected to have no effect or inhibit ACh release at behaviorally effective doses (418). Another alternative is that 5-HT, antagonists achieve their effects on cognition indirectly, through inhibition of GABAergic interneurons that regulate the release of many neurotransmitters, including glutamate (451–453). However, as mentioned earlier, there is growing evidence that multiple 5-HT, receptor subtypes exist that may have distinct distribution or functional properties, which may account for their effects. Several 5-HT, receptor antagonists are described that have been reported to improve cognition in various behavioral models.

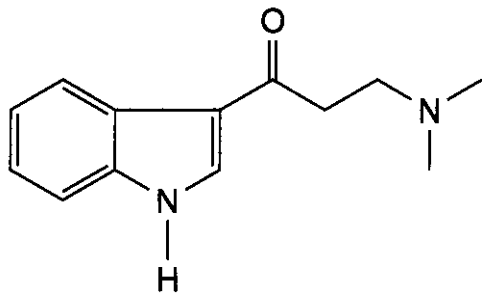
4.3.2 Structure-Activity Relationships for $5HT_3r$ Antagonists

4.3.2.1 Imidazolyl Indolyl Derivatives. The SAR studies of imidazolyl tetrahydrocarbazolones, exemplified by ondansetron (144) as 5-HT, antagonists, were presented in a review by Oxford et al. (428) (Fig. 14.16). The discovery of ondansetron began with the indolylpropanone (**146**), which was identified as a weak antagonist of $5\text{-}HT_3$-induced depolarization of rat vagus nerve ($pA_2 = 6.5$) through selective screening. The N-imidazolyl (147) was found to be at least 10-fold more potent ($pA_2 = 7.61$) than the parent dimethylamino derivative (146) and became a template for further elaboration. The 1-methylindole analog (**148**), which was marginally more potent than (**147**) in vitro, demonstrated oral activity in the BJ test in the rat ($ED_{50} = 0.11$ mg/kg). However, it potentiated the pentobarbitone sleeping time in the mouse, presumably because of the imidazole moiety, which can bind to and inhibit the hepatic cytochrome P450 oxidase system. This side effect was overcome by the introduction of a methyl group at the 2-position of the imidazole moiety. The resulting 2-methyl derivative (149) maintained the in vitro activity (pA_2 7.61), but its oral activity ($ED_{50} = 2.5$ mg/kg in the BJ test in the rat) was substantially reduced, presumably attributable to first-pass metabolism. Incorporating the side chain into the tetrahydrocarbazolone system generated the conformationally restrained compound, ondansetron (144, GR 65630) ($pA_2 = 8.6$), which was more potent than the acyclic ketone (**148**) on rat vagus nerve and had much improved oral activity in the rat ($ED_{50} = 7$ g/kg, p.o.). Like its acyclic counterpart (**148**), it had no effect on the cytochrome P450 oxidase system. Ondansetron displayed more than 1000-fold selectivity for the 5-HT, receptor over any other receptors examined, including $5\text{-}HT_2$- and $5\text{-}HT_1$-like receptors. Ondansetron is a racemic compound and both of its enantiomers have similar in vitro activity. The quaternary derivative (145) maintained activity, suggesting that the imidazole moiety is protonated in the binding interaction with the receptor.

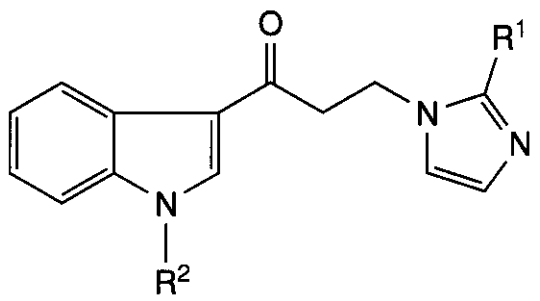
Considerable efforts have gone into the SAR studies on tetrahydrocarbazolones. First, substituting the indole nucleus at C6 (e.g., **151**, **152**), a potential site of metabolism, generally reduces potency, with the exception of the 6-F derivative (150). Second, substitution at the 9-position is well tolerated (e.g., 153, **154**), but some long-chain lipophilic substituents reduce activity (e.g., 155). Third, the carbonyl function in this series is critical to potent 5-HT, antagonist activity. The corresponding alcohols (156, 157) and the tetrahydrocarbozole (158) are less potent than the



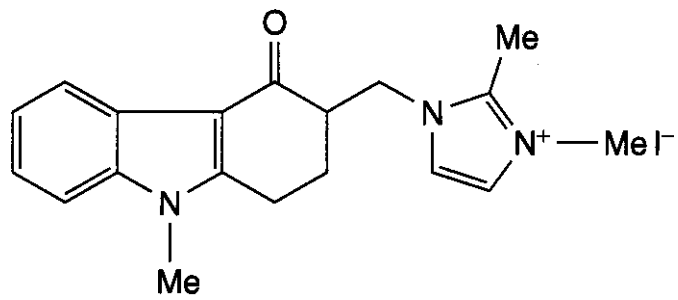
(144) ondansetron
pA_2 8.6 K_i 1.6



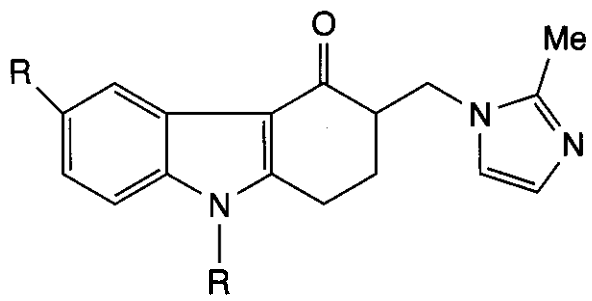
(146) pA_2 6.5



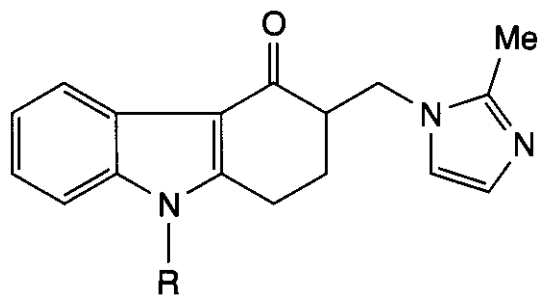
(147) R^1 = H, R^2 = H, pA_2 7.61
(148) R^1 = H, R^2 = Me, pA_2 7.67
(149) R^1 = R^2 = Me, pA_2 8.0



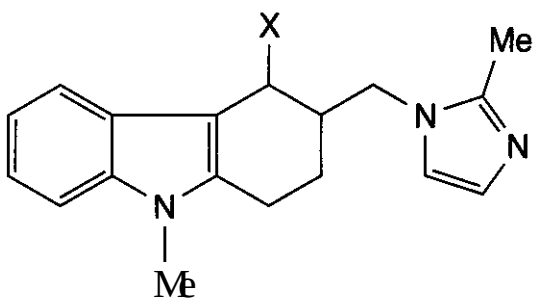
(145) pA_2 8.4



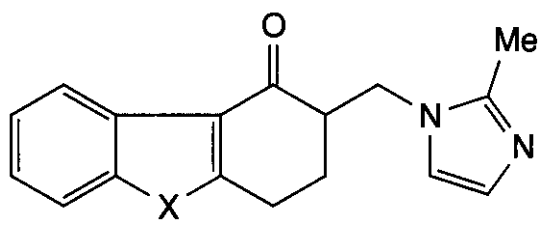
(150) R = F, pA_2 8.6
(151) R = Br, pA_2 7.5
(152) R = OMe, pA_2 6.1



(153) R = Et, pA_2 8.7
(154) R = CH_2Ph, pA_2 8.2
(155) R = $(CH_2)_3Ph$, pA_2 6.8



(156) X: β OH; pA_2 6.9
(157) X: α OH; pA_2 7.1
(158) X: H; pA_2 6.4



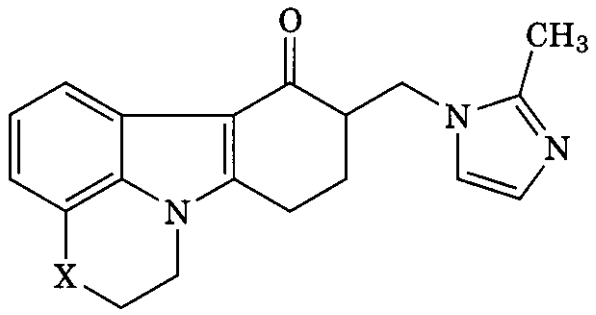
(159) X: O; pA_2 7.5
(160) X: S; pA_2 7.3

Figure 14.16. pA_2 values (428): K_i, **rat** brain **cortex/[^{3}H]Gr-65630** (429).

tetrahydrocarbazolone (144) by about 30 and 100 times, respectively. Finally, the **indole** nucleus is superior to the benzofuran (159) and benzothiophen (160) moieties.

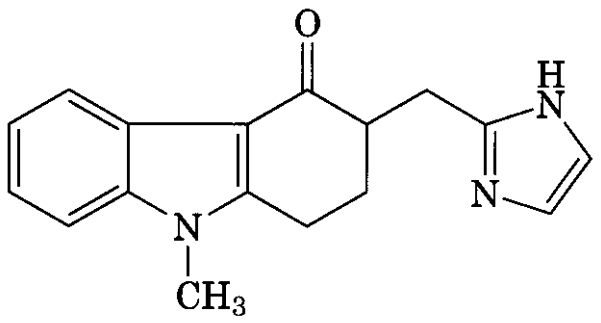
Researchers from Solvay reported a series of **1,7-annelated indole** derivatives of ondansetron as potent and selective **5-HT,** antagonists (Table 14.14) (429). Similar to **ondansetron,** the **1,7-annelated indoles** show little **stereoselectivity.** The (−)-isomers are only slightly more potent than the (+)-isomers. In this series the five- and six-membered ring analogs,

Table 14.14 Binding Data of **1,7-Annelated Indole** Derivatives

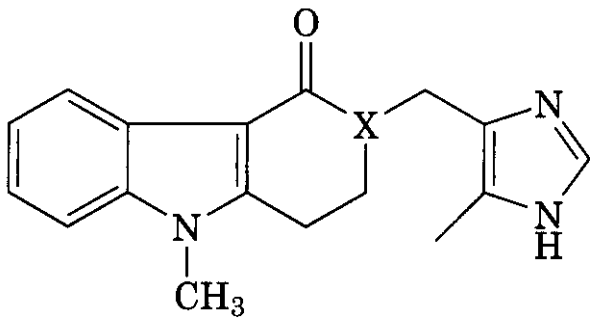


Compound	X	K_i^a
(±)-**161**	nil	0.23
(±)-**162**	CH_2	0.25
(+)-**162**	CH_2	1.40
(−)-**162** (cilansetron)	CH_2	0.19
(±)-**163**	$(CH_2)_2$	0.75
(+)-**163**	$(CH_2)_2$	0.93
(−)-**163**	$(CH_2)_2$	0.64
(±)-**164**	$(CH_2)_3$	1.10
(±)-**165**	O	1.30
(±)-**166**	S	0.77

"Rat brain cortex/[^{3}H]GR-65630 (K_i for (±)-ondansetron: 1.6 n*M*) (429).



(167) pA_2 7.7



(168) X = CH pA_2 10.2
(169) X = N, K_i 0.16 n*M* (alosetron)

Figure 14.17.

(161) and (**162**), are about sevenfold more potent and the azepine (**163**) twofold more potent than ondansetron (**144**), whereas azocine (**164**), morpholino (**165**), and thiomorpholino (166) exhibit a binding affinity comparable to that of (144).

From a series of 1,7-annelated indole derivatives, cilansetron [(−)-**162**] was selected for further pharmacological profiling both *in vitro* and *in vivo* (Table 14.14) (454). In the RVN and GpI, cilansetron [pA_2 9.94 (RVN); 7.80 (GpI)] is about 10-fold more potent than ondansetron [pA_2 8.99 (RVN); 6.80 (GpI)]. In the von Bezold-Jarisch reflex test (BJR) in unrestrained conscious rats, cilansetron (ED_{50} = 26 μg/kg, p.o.) is orally active at a dose 6 times lower than that of ondansetron (ED_{50} = 165 μg/kg, p.o.). Solvay announced Phase III studies for cilansetron for the treatment of diarrhea-predominant irritable bowel syndrome, in July 2001 (455).

The C-linked imidazole derivatives were also prepared (Fig. 14.17). The 2-imidazolylmethyl analog (167) is less potent than ondansetron, but compound (**167**) is a highly potent 5-HT, antagonist and has been used as the radioligand [^{3}H]GR 67330 in binding experiments. Replacement of the α carbon attached to the side chain imidazole moiety with a nitrogen provided alosetron (**169**), which is achiral and yet 10-fold more potent than ondansetron in terms of the binding affinity.

4.3.2.1.1 Ondansetron (144; Zofran; GR 65630). Ondansetron is the best-characterized 5-HT, antagonist [IC_{50} = 103 p*M* in recombinant h5-HT_{3A} receptor (456)] in behavioral studies (144 in Fig. 14.16). Although ondansetron has been occasionally shown to enhance memory in normal adult animals in avoidance (457, 458), spatial (451), or discrimination (459) tasks, the majority of studies in unimpaired animals have found no effect (460–462). However, ondansetron has been routinely shown to antagonize the impairing effect of scopolamine, a muscarinic cholinergic antagonist (461–465). In addition, ondansetron relieved deficits in spatial learning in young rats caused by destruction of basal forebrain cholinergic neurons (466) or in aged rats (464), and in a visual discrimination task in aged rhesus monkeys (467).

A clinical trial on young healthy individuals was conducted with ondansetron (144) to measure its effects on scopolamine-induced cognitive, behavioral, and physiological responses (468). This study found that a single dose of ondansetron only minimally attenu-

ated the scopolamine-induced changes. In another pilot study from the same investigators, elderly humans demonstrated no cognitive enhancement from ondansetron alone, nor had cognitive deficits induced by scopolamine treatment attenuated (469). A recent study conducted in 189 patients with dementia of the Alzheimer's type, ondansetron (10 or 50 μg bid for 24 weeks) failed to show any cognitive improvement over placebo (470). Ondansetron is currently not approved by the FDA for use in dementia, but is marketed as an antiemetic.

4.3.2.1.2 ***Alosetron*** (*169;* GR-68755). Alosetron (169 in Fig. 14.17) is a potent (K_i = 0.16 nM) and selective 5-HT, antagonist (471), and it has a longer duration of action and a wider effective dose range than that of ondansetron (472). Studies in the marmoset have shown alosetron to improve cognitive performance in an object discrimination reversal task (10 ng/kg, s.c. bid) (473) and to attenuate scopolamine-induced impairment in the acquisition of an object discrimination task (1mg/kg, i.p.) (474). In healthy humans, alosetron (10 and 250 μg) showed significant improvement in reducing spatial and verbal memory deficits induced by scopolamine (475). In another clinical study, alosetron (20 mg orally) enhanced accuracy on a computerized attention task but failed to reverse d-amphetamine-induced deficits in healthy male subjects (476).

Alosetron was approved for U.S. use in February 2000 for the treatment of irritable bowel syndrome (IBS) in women whose predominant bowel symptom is diarrhea, although the product was withdrawn in November 2001 because of serious side effects, particularly ischemic colitis. Earlier studies in men for anxiety disorder and schizophrenia, and Phase II studies for nonulcer dyspepsia, have also been discontinued.

4.3.2.2 Other Chemotypes. Several other 5-HT, antagonists have demonstrated cognition-enhancing effects in animal models. However, data from human studies have yet to be reported. (*R*)-Zacopride (170 in Fig. 14.18; Synthelabo) is undergoing clinical development as a potential treatment for cancer chemotherapy-induced emesis. In atropine-treated rats, (*R*)-zacopride significantly attenuated memory impairments in a spatial navigation task (477). However, no improvement in spatial working/short-term memory was observed in untreated adult rats (478).

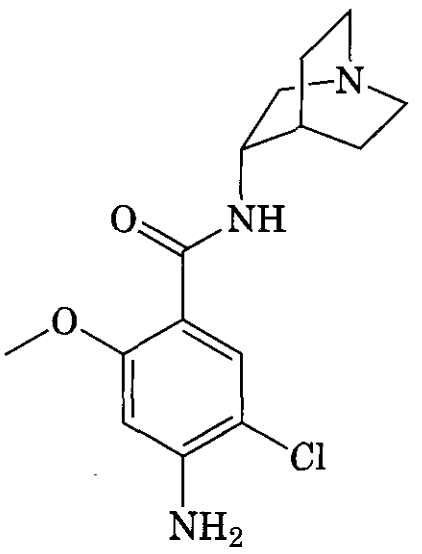
Itasetron (171; DAU 6215; Boehringer Ingelheim Corp.) is a selective and high affinity antagonist of the 5-HT, receptor (K_i = 3.75 nM) currently in clinical trials for the treatment of emesis (452). In scopolamine-impaired rats, acute administration of itasetron attenuates memory deficits in the Morris water maze and the passive-avoidance task (438, 479, 480). Positive effects for aged rats were also observed in an avoidance task (479,481) and in the Morris water maze (479,482).

Tropisetron [ICS-205,930, Novoban, (172) in Fig. 14.18; Novartis] is a potent 5-HT, receptor antagonist (K_i = 0.38–3.1 nM) (483–485) that has recently been found to possess *a*, nAChR subtype antagonist activity (437) (see Table 14.13) and *a*, nAChR partial agonist activity (445). In rat behavioral studies, tropisetron attenuated scopolamine-induced memory deficits in the passive-avoidance test (438), and spatial navigation deficits in the Morris water-maze task (463), and improved the retention of a conditioned response in rats with p-chloroamphetamine-induced deficits (486). Tropisetron is currently marketed for the treatment of nausea and vomiting associated with chemotherapy treatments (485).

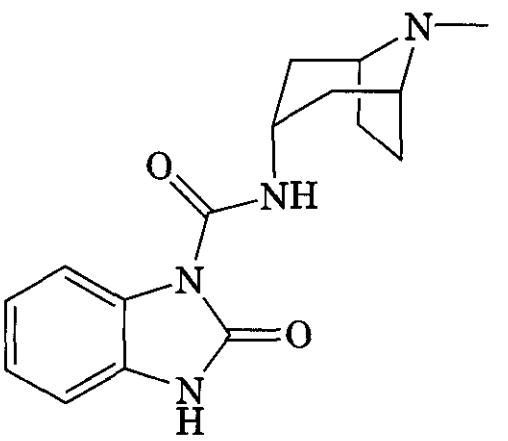
GYKI-46903 (173; Egis Gyogyszergyar) is a potent 5-HT, antagonist (K_i = 20 ± 2 nM) reported to be under development as a potential treatment for dementia (487,488). In rats, GYKI-46903 was shown to attenuate scopolamine-induced memory impairments in the stepdown passive-avoidance task and the eight-arm radial maze (489).

Mirisetron (174; SEC-579; WAY-100579; American Home Products Corp.) is a 5-HT, antagonist (IC_{50} = 1.2 nM) (490) that improved spatial learning of lesioned rats in the water maze (466) and enhanced acquisition of a visual object discrimination for aged monkeys (467). This compound had reached phase I Clinical trials for use as a potential treatment for anxiety, but no further development has been reported.

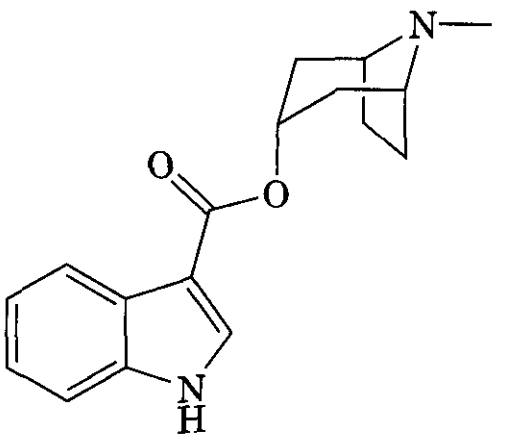
4.3.3 Future Direction. 5-HT, receptor antagonists were initially hailed as potential treatments for anxiety, Alzheimer's disease,



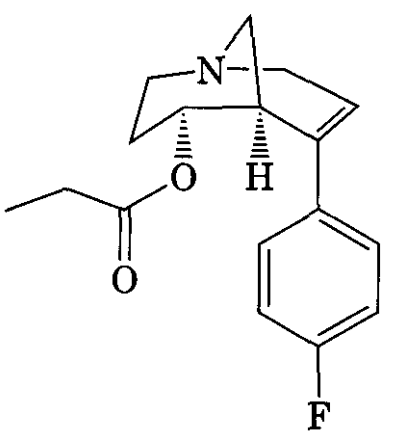
(170) (*R*)-zacopride



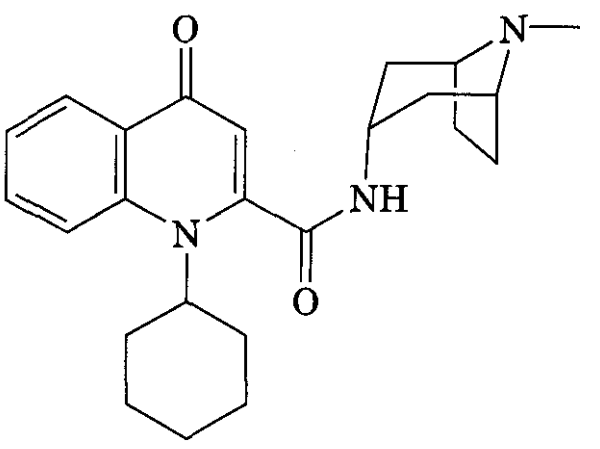
(171) itasetron



(172) tropisetron



(173) GYKI-46903



(174) mirisetron

Figure 14.18.

schizophrenia, pain, and drug dependency. Despite some early encouraging results, no 5-HT, antagonists have been approved for the treatment of cognitive dysfunction. It would be important to know whether 5-HT, antagonists produced any significant improvement over placebo in the clinical trials. At present, their therapeutic application for cognition enhancement remains unknown.

4.4 Potassium M-Channel

4.4.1 Physiology and Pharmacology. Potassium (K^+) channels serve a modulatory function in excitable and nonexcitable cells by regulating K^+ conductance (491,492). Within the CNS, many types of K^+ channels are known with differential patterns of distribution. Opening of neuronal K^+ channels generally acts to dampen neuronal excitability by pre- or postsynaptic mechanisms. Nonspecific K^+ channel blockers, such as 4-aminopyridine (4-AP) and 3,4-diaminopyridine (3,4-DAP), were initially evaluated for cognition enhancement in AD, but gave poor results (493). New compounds that are selective inhibitors of the K^+ M-current, or the channels that underlie this current, appear to offer better promise as cognitive enhancers.

The M-current [$I_{K(M)}$] is an outward K^+ conductance through M-type voltage-gated K^+ channels. This current, which is induced by membrane depolarization and is relatively slowly activating and deactivating, modulates the subthreshold electrical excitability of neurons in response to synaptic inputs (494). M-current is so named because it was inhibited by activation of muscarinic acetylcholine receptors (495). Inhibition of M-current results in enhancement of the release of excitatory neurotransmitters and their postsynaptic effects (496,497). This net amplification of signals that originate through normal synaptic

activity makes the M-channel an attractive target for the development of cognitive enhancers (123,498).

The molecular identity underlying the M-current had been established as the coassembly of channel subunits (499, 500), KCNQ2 (501) and KCNQ3 (502), and it now appears that KCNQ5 may play an additional role in the generation of an M-type current (503). Mutations in the genes encoding either of these channel subunits can result in inactive channels, causing a form of idiopathic generalized epilepsy (500–502, 504).

Several endogenous molecules are known to inhibit the M-current, including acetylcholine, uridine and adenosine triphosphate (505), bradykinin (506–509), angiotensin II (510), endothelin 1 (511), substance P, and luteinizing hormone-releasing hormone (512). On the other hand, M-current is increased (potentiated) by somatostatin and β-adrenoreceptors through a cAMP-dependent process (513). Recently, the novel anticonvulsant retigabine has been shown to activate human KCNQ2 and KCNQ2/3 channels expressed in CHO cells, perhaps contributing to the pharmacology of this compound (514).

Of the five known classes of muscarinic ACh receptors, M-current is preferentially inhibited by m1 or m3 subtypes (515, 516). Experiments with mice lacking the m1 receptor exhibit a loss of muscarinic regulation of M-current and are resistant to pilocarpine-induced seizures (517). Recently, specific antisense plasmids have been used to strongly suggest the G-protein $G\alpha_q$ as the primary transducer of muscarinic inhibition of $I_{K(M)}$ (518).

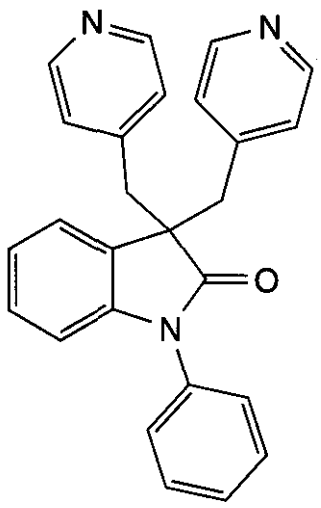
M1 mAChR agonists (that likely inhibit M-current. in addition to their other actions) have received considerable attention as possible cognitive enhancers (519–521). Although many m1 agonists have produced positive results in animal models (522–525), most of these compounds generate unacceptable side effects in humans (e.g., 526). It is possible that the cognition-enhancing properties of m1 agonists (and, perhaps, cholinesterase inhibitors) are the consequence of a downstream M-current inhibition, whereas the side effects result primarily from muscarinic receptor mediated over stimulation of the parasympathetic nervous system. If true, agents that selectively inhibit M-current by directly interacting with the M-channel may enhance cognition without the adverse side-effect profile typically observed with direct muscarinic agonists or acetylcholinesterase inhibitors. Several candidate M-channel modulators are discussed below.

4.4.2 Structure-Activity Relationships for M-Channel Blockers

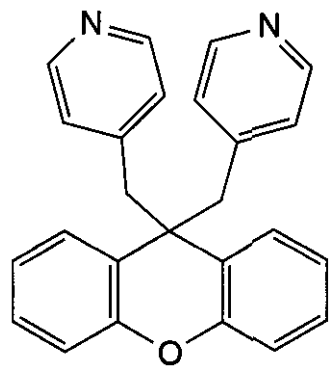
*Linopirdine (DuP 996; **175**), **XE991 (180)**, and **DMP 543 (181).*** The M-current blocker linopirdine (3,3-bis(4-pyridinylmethyl)-1-phenylindolin-2-one; DuP 996; 175) is one of a series of cognition-enhancing agents developed by DuPont-Merck (527, 528) that has been the focus of intense investigation (Fig. 14.19) (reviewed in Refs. 496, 529). In slices from rat brain cerebral cortex, hippocampus, and caudate nucleus, linopirdine was found to increase ACh (EC_{50} value of 4.5 μM) and other neurotransmitters in response to a depolarizing stimulus, but had no effect on basal release (530,5311,suggesting use dependency. In *vivo* microdialysis studies have shown an increase in the release of ACh from rat hippocampus at a dose of 10 mg/kg, s.c. (532).

Mechanistic studies have indicated that linopirdine (175) inhibits M-current ($I_{K(M)}$) by channel blockade from the extracellular side (529,533,534). Interestingly, linopirdine's effect on neurotransmitter release was demonstrated to be brain region specific, indicative of a complex in *vivo* mode of action (535). The mechanism underlying the effects of linopirdine on neurotransmitter release, in particular the potentiation of ACh release from basal forebrain cholinergic neurons, is not known, but could be explained by an action on presynaptic KCNQ2 and/or KCNQ3 channels (536).

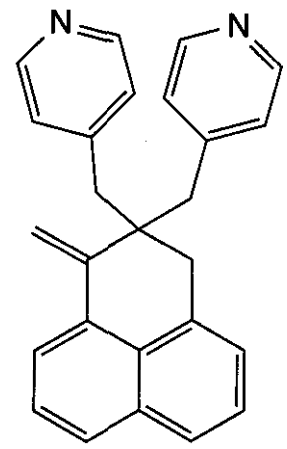
Several substitutions were made for linopirdine's phenylindolinone "core" to identify more active analogs (530). This was done in relation to three regioisomer variations of the pyridinylmethyl "pendent" moiety, while attempting to maintain asymmetry in the constructs. Two compounds worth noting are xanthene (176) and acenapthenone (177) derivatives that were found to be approximately equipotent with linopirdine for their ability to enhance depolarization-induced ACh release



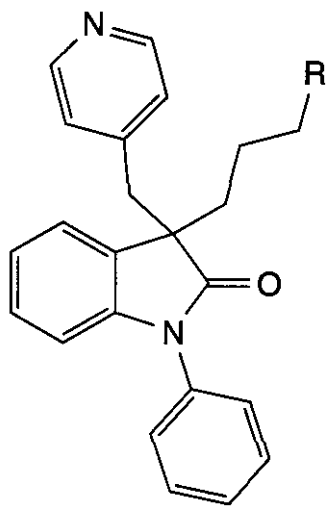
(175) linopirdine



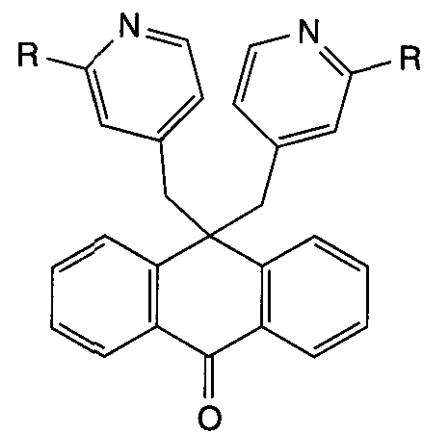
(176)



(177)



(178) R = CO_2Et
(179) R = CH_2CN



(180) R = H; XE991
(181) R = F; DMP-543

Figure **14.19.** Potassium M-current blockers.

from rat brain hippocampal slices. Further SAR studies focused on substitution of only one of the pair of pyridinylmethyl groups while maintaining the phenylindolinone core (537). Of the racemic compounds generated, the (±)-ethylbutyrate (178) and (±)-valeronitrile (**179**) derivatives deserve mention. Each analog replaced one of the two pendant groups with an isostere capable of acting as a hydrogen-bond acceptor and was found to be as good as, or superior to, linopirdine as enhancers of ACh release. Furthermore, when the racemic ethylbutyrate derivative (**178**) was resolved, the biological activity of the (+)-isomer was found, surprisingly, to be only slightly higher than that of the racemic mixture. The (−)-isomer was inactive and reported not to be an antagonist to ACh release.

Further research to develop a second-generation clinical candidate with improved pharmacokinetic and pharmacodynamic properties has focused on core and pendant group modifications of linopirdine (175) (530). The core structure of linopirdine was substituted with an anthrone moiety to provide XE991 (**180**), a more lipophilic analog. XE991 showed approximately 10-fold greater potency than that of linopirdine in inhibiting M-current in cultured superior cervical ganglion neurons and in KCNQ2/3 channels expressed in *Xenopus* oocytes (297). Further structural modification was done to reduce *in vivo* N-oxidation by replacing the pendant groups with 2-fluoro-4-pyridinylmethyl groups, generating DMP 543 (181). Both compounds were superior to linopirdine for enhancement of ACh release from rat brain slices (EC_{50}: linopirdine = 4.2 μM; XE991 = 490 nM; DMP 543 = 700 nM) and had greater *in vivo* potency and duration of action (538). An improved synthesis of DMP-543 was later reported (539).

4.4.3 Cognition-Enhancement Experiments. Linopirdine (175) has been assayed in a wide range of animal models for cognitive enhancement. In mice, linopirdine enhanced perfor-

mance in an active avoidance task (540) and improved working memory deficits caused by ethylcholine aziridium (AF64A) in the T-maze (541). In rats with and without hypoxia-induced memory deficits, linopirdine improved performance in the passive-avoidance task, suggesting augmentation of task acquisition and consolidation of information skills (540, 542). Linopirdine was also reported to improve the spatial learning of septal lesioned rats in the Morris water maze (543), but has produced conflicting results for untreated rats in similar tasks (543–545). In contrast, linopirdine did not improve performance in matching-to-sample paradigms using pigeons or squirrel monkeys (546). Initial clinical trials conducted with linopirdine in AD patients have been reported to show trends toward cognitive improvement (78). However, in phase III clinical trials linopirdine failed to show consistent efficacy (547) and continued development was terminated (548).

Phase I clinical studies with DMP 543 (181), up to a single dose of 55 mg, demonstrated that it was well tolerated and had an improved mean elimination half-life of 30–57 h (linopirdine was 0.4–3.2 h) (549). To date, preclinical studies describing cognitive enhancement with these compounds have not been published. The development of DMP 543, after reaching phase II clinical trials as a potential therapy for the treatment of Alzheimer's disease, has now been discontinued (DuPont Pharm, 2000 Annual Report).

4.4.4 Future Direction. Potassium channels, especially the KCNQ family, are widely involved with the physiology and pathophysiology of the human body. Research into their biology will continue to blossom as more cloned KCNQ subunits are available, along with selective agents to modify their action.

An additional area of potentially fruitful research is in the area of central Ca^{2+} channel modulation. Nimodipine, a centrally acting Ca^{2+} channel antagonist marketed in the United States by Bayer AG for the treatment of subarachnoid hemorrhage, is known to protect against the cognitive impairment that normally follows cerebral insult (550). This effect is thought to be attributed to the ability of nimodipine to normalize the function of intracellular Ca^{2+} dynamics. As such, this class of compounds may normalize the normally pathological levels of Ca^{2+} seen after cerebral insult, which may otherwise lead to cell death and cognitive dysfunction. One compound from this class, MEM 1003, is currently in late preclinical trials and may enter European clinical trials in 2002 (SCRIP, September 20, 2001).

5 CONCLUSION

As the world's population ages, effective treatment for the cognitive impairments of dementia becomes an increasingly important goal. The limited success of currently marketed acetylcholinesterase inhibitors may be partially attributed to factors such as potency, selectivity, or brain penetration. However, it is also likely that the potential of these drugs is limited because of their focused targeting of only one of the several neurotransmitter systems known to be disturbed in dementing illnesses. An additional potential limitation of these medicaments is that they do not necessarily increase the fidelity of the signaling event, but instead simply increase the overall background level of central ACh. This approach remains viable only until a critical number of ACh producing neurons degenerates, at which time a rapid decrease in central ACh can be expected.

The purpose of the present review has been to consider alternative, ion channel-based approaches to the development of cognitive enhancers. Existing preclinical data indicate that these approaches are at least as effective as cholinesterase inhibitors in enhancing cognition. The agents described herein all facilitate synaptic transmission in several neurotransmitter systems, primarily through enhancement of release. Thus, they work as positive modulators of normal brain circuit functioning, rather than affecting neurotransmitter levels without regard for temporal parameters that are likely critical to meaningful signaling. Another potential advantage of the mechanisms discussed above is that they present a multifaceted secondary pharmacotherapy that would seem to be more appropriate for treating the complex neuropathology of

dementia. Perhaps the ability to affect the activity of multiple neurotransmitter systems will provide an advance toward developing successful treatments for this debilitating condition.

6 WEB SITES FOR FURTHER READING

- http://www.alzheimers.org/index.html
- http://www.centerwatch.com
- http://www.alzheimers-support.com/
- http://www.mc.uky.edu/adreview/
- http://dementia.ion.ucl.ac.uk/
- http://www.agelessdesign.com
- http://www.healthandage.com
- http://www.kenes.com/vascular/index.html
- http://www.zarcrom.com/users/alzheimers/odem/al-d.html
- http://www.alz.uci.edu/

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CHAPTER FIFTEEN

Drugs to Treat Eating and Body Weight Disorders

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Contents

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1 INTRODUCTION

Eating and body weight disorders are a diverse group of endocrine, metabolic, and psychiatric diseases that are associated with atypical eating behaviors and/or significant changes in body weight. Eating behavior is an essential physiological activity that is required to furnish nourishment and energy and is one of many factors that determine body weight and appearance. Eating behavior is quite variable, but becomes pathological when it leads to serious health consequences such as malnutrition or excess adiposity. Major eating disorders, which occur predominantly in young women, include anorexia nervosa, bulimia nervosa, and binge eating disorder. Body weight also varies considerably between individuals. A healthy body weight depends on a range of genetic factors that govern metabolic processes that seek to maintain a state of energy homeostasis. Extreme variations from a "normal" body weight are associated with increased risks of developing numerous comorbidities. A decrease in body weight, especially in lean or fat-free body mass, occurs secondarily in many diseases and is often a noticeable symptom of some cancers and other adverse conditions. An increase in body weight, particularly fat mass, reflects a positive change in energy balance, in which energy intake exceeds energy expenditure and excess energy is deposited in adipose tissue. Prototypical body weight disorders include obesity and cachexia.

The biochemical and pharmacological mechanisms that underlie eating and body weight disorders are complex. These diseases are rarely caused by any single genetic factor, although defects in certain well-defined molecular targets or pathways can enhance the onset of these conditions, especially under certain environmental stimuli and in the presence of certain behaviors. These diseases are characterized by perturbations in numerous physiological systems, but often as a consequence, not a cause, of the initial pathology. The central nervous system (CNS) plays a critical role in the origin and progression of the diseases, with the hypothalamus regulating the interactions between various neurotransmitters and neuropeptides that are involved in maintaining energy homeostasis and with other brain regions controlling serotonergic function, which plays an important role in the development of psychiatric comorbiditiesassociated with many eating disorders.

Significant advances have recently been made in the treatment of these disorders. Combinations of pharmacological and nonpharmacological approaches, including counseling, cognitive behavioral therapy, lifestyle changes, and drug therapy, are generally required to successfully manage these diseases. Pharmacotherapy, however, is limited by the availability of safe and efficacious drugs and is rarely a stand-alone treatment for any of these diseases. Several classes of drugs, some of which have been available for decades, have been approved for the treatment of obesity,

Table 15.1 Classification of Body Composition by Body Mass Index (BMI) and Risk of Associated Comorbidities

BMI (kg/m^2)	Classification	Risk of comorbidities
<18.5	Underweight	Increased
18.5 to 24.9	Normal	Average
25 to 29.9	Overweight	Somewhat increased
≥30	Obese	
30 to 34.9	Obese, Class I	Moderately increased
35 to 39.9	Obese, Class II	More severe
≥40	Obese, Class III	Most severe

bulimia nervosa, and cachexia, although there is still a great medical need for new agents with improved properties.

This chapter describes (1) the physiology and pharmacology of the most common forms of eating and body weight disorders; (2) clinical guidelines for treatment; (3) current drugs and their mechanisms of action, adverse effect profiles, structure-activity relationships, and metabolic degradation pathways; and (4) new agents in development.

1.1 Eating and Body Weight Disorders: Definitions, Causes, Comorbidities

1.1.1 Obesity. Obesity is a condition of abnormal body weight resulting from an accumulation of extra adipose tissue, generally in response to a state of positive energy balance that occurs when energy intake exceeds energy expenditure. Obesity is characterized by an elevated body mass index (BMI), a commonly used descriptor of body composition that is defined as the weight of an individual in kilograms divided by the square of the height in meters, or kg/m^2 (1). Three discrete classes of obesity have been established based on BMI, depending on the risks of associated comorbidities (see Table 15.1). A preobese state is called overweight, with above-average risks of comorbidities. Morphological features of obesity are usually distinct and often gender specific, although there is considerable individual variability. Adiposity in men forms mostly in the abdomen, resulting in a unique "apple" shape; in women, excess fatty tissue generally accumulates in the upper body and/or in the hips and the buttocks, giving a unique "pear" shape.

The incidence of obesity has dramatically increased over the past decade, reaching epidemic proportions in industrialized countries throughout the world (2). In the United States, the disease varies significantly by age, race, and gender and is more prevalent in certain socioeconomic groups that are predominantly poor, less educated, and less affluent (3). The proportion of obese American men and women has increased by approximately 50% over the past decade, from 12% of the population in 1991 to 17.9% in 1998 (3). A majority of adult Americans and a significant number of children are now considered either overweight or obese (4). Interestingly, more people in the world are classified as overweight and obese rather than as malnourished, according to the World Health Organization.

Excess adiposity and above-average body weight, two distinguishing hallmarks of obesity, are associated with numerous health risks, resulting in the designation of obesity as a serious disease (5, 6). According to recent epidemiological data, obesity increases susceptibility to a wide range of both cardiovascular and metabolic diseases (7, 8), including hypertension (9), non-insulin-dependent diabetes mellitus or NIDDM (10, 11), and hyperlipidemia (12), and is an independent risk factor for certain cancers, including breast, colorectal, and endometrial cancers (13, 14). High BMIs also cause debilitating musculoskeletal (15) and respiratory/sleep disorders (16) that result in diminished quality of life (17). Because of these comorbidities, the risk of mortality for individuals with BMIs greater than 30 kg/m^2 is nearly twice that for individ-

Table 15.2 Common Comorbidities Associated with Obesity

Cardiovascular Diseases	Metabolic/Endocrine Diseases	Joint Diseases	CNS
Congestive heart failure	Diabetes	Osteoarthritis	Depression
Hypertension	Insulin resistance	Rheumatoid arthritis	Pain
Deep vein thrombosis	Glucocorticoid imbalance	Bone fractures	
Coronary artery disease	Gall bladder disease	Lower back pain	
Stroke	Hypercholesterolemia	Carpal tunnel syndrome	
	Hypertriglyceridemia		
	Gout		
	Pancreatitis		
	Liver disease		
Cancers	**Respiratory diseases**	**Immune impairments**	**Other**
Breast cancer	Asthma	Impaired wound healing	Infertility
Colorectal cancer	Chronic obstructive pulmonary disease (COPD)	Impaired immune response	Incontinence
Renal cell cancer	Chronic bronchitis	Renal failure	Renal failure
Endometrial cancer	Sleep apnea		

uals with normal BMIs (18). Obesity is now the second leading cause of preventable death in the United States after smoking, with approximately 300,000 deaths a year directly attributable to the disease or its comorbidities (19). A comprehensive listing of the comorbidities associated with obesity is provided in Table 15.2.

The causes of obesity are complex, but a combination of behavioral, genetic, hormonal, economic, and environmental factors all play critical roles in the origin and progression of the disease. Expression changes in genes and single-nucleotide polymorphisms can predispose individuals to become overweight or obese. However, lifestyle choices (i.e., low physical activity), food preferences (i.e., high fat diets, energy-dense foods), and certain behaviors (i.e., overeating) hasten the actual pathogenesis, generally when caloric intake exceeds energy output. The body has developed numerous methods for managing physiological states of negative energy balance and enhancing survival, but few mechanisms for coping with sustained states of positive energy balance and preserving long-term health. It should be noted that only a small percentage of individuals develop obesity as a result of specific genetic defects in essential metabolic processes (20).

1.1.2 Wasting/Cachexia Secondary to Other Diseases. Wasting diseases such as cachexia are body weight disorders that are characterized by excessive loss of lean body mass, especially muscle tissue, resulting in profound weight loss. The decrease in body weight that occurs with these diseases fails to stimulate an adaptive response that would normally enhance appetite. Profound loss of lean body mass ultimately leads to organ failure and death. Wasting diseases can be caused by a variety of factors such as change in taste perception, decreased food intake, increased satiety, or malnutrition, and often occur secondarily in diseases such as AIDS and cancer or with certain injuries such as severe burns. Some eating disorders such as anorexia nervosa and several forms of cancer (i.e., lung cancer) tend to cause severe wasting, which requires separate therapeutic management apart from the primary disease.

1.1.3 Eating Disorders. Eating disorders such as anorexia nervosa, binge eating disorder, bulimia nervosa, and night-eating syndrome are primarily behavioral diseases that are associated with irregular eating patterns and/or weight control. The two major eating disorders, anorexia nervosa and bulimia nervosa, are often caused by unrealistic percep-

Table 15.3 Symptoms Used to Define Anorexia Nervosa and Bulimia Nervosa as Determined by the American Psychiatric Association[a]

Anorexia nervosa
1. Refusal to maintain weight greater than the lowest weight considered normal for age and height.
2. Intense fear of gaining weight or becoming fat, even though underweight.
3. Distorted body image in women.
4. Three consecutive missed menstrual periods without pregnancy.
Bulimia nervosa
1. Recurrent episodes of binge eating (minimum average of two binge-eating episodes a week for at least 3 months).
2. A feeling of lack of control over eating during the binges.
3. Regular use of self-induced vomiting, laxatives, diuretics, or vigorous exercise to prevent weight gain.
4. Persistent preoccupation with body shape and weight.

[a]Ref. 5.

tions of ideal body shape and body weight. Anorexia nervosa results from extreme food restriction (starvation) and less frequently from other behaviors such as extensive exercise, and is characterized by a body weight that is less than 85% of expected relative weight (21). Binge eating disorder consists of episodic compulsive eating behavior that results in body weight gain (22). Bulimia nervosa is a binge eating disorder that is accompanied by inappropriate compensatory actions such as vomiting, laxative use, or less frequently, excessive exercise (21). Night-eating syndrome occurs with many sleeping disorders (apnea) and results in excessive caloric intake during nighttime periods of wakefulness (23). Anorexia nervosa, bulimia nervosa, and binge eating disorder occur frequently with other diseases of the CNS such as depression, anxiety, and various personality disorders and are listed as psychiatric/behavioral disorders in the Diagnostic and Statistical Manual of Mental Diseases, 4th edition (24). Several reviews describe these illnesses in great detail (21, 25).

Anorexia nervosa (AN) is a chronic disease that is associated with many life-threatening comorbidities (26). This disorder, which affects predominantly young women, often starts in middle adolescence, with a mean onset of action of 17 years of age, and is likely to occur in approximately 0.5% of women over the course of their lifetime. The clinical criteria used to define AN are listed in Table 15.3. Body weight is controlled by restriction of food intake, although extensive exercise is sometimes used as a substitute. The disease is often caused by excessive fears of body weight or fat gain, leading to dieting and self-imposed starvation. AN is difficult to diagnose in clinical practice because of the extreme measures patients undertake to hide the disease. Loss of body weight, especially body fat, leads to amenorrhea, which results in decreased estrogen production and diminished bone mineral density. Several other side effects of AN include hypotension, hypothermia, pancreatitis, and metabolic abnormalities associated with protein and electrolyte insufficiencies. The death rate from AN is 12 times higher than that for other age-matched, young women in the population. Leading causes of mortality include ventricular tachyarrhythmias that result from electrolyte imbalance (hypokalemia) or protein malnutrition (cachexia).

Bulimia nervosa (BN) is an eating disorder that is becoming increasingly prevalent in Western societies, often occurring in approximately 1–3% of women over the course of their lifetime. Unlike anorexia nervosa, BN is not associated with an abnormal state of body weight, in that most women with the disease are within 15% of their ideal body weight. Common symptoms of BN are listed in Table 15.3. Risk factors for BN include a previous experience with anorexia nervosa, low self-esteem, and certain personality traits. In the earliest stages of the disease, patients begin to diet, but the diet-induced hunger cannot be controlled. This failure then leads to binge eating behavior, followed by purging actions (vomiting, laxative, or diuretic use) or nonpurging behavior (excessive exercising, fasting) to remove excess calories. As the disease

progresses, binge eating and the compensatory behavior(s) become mechanisms for coping with and seeking temporary relief of comorbidities such as depression or anxiety (26). A typical binge eating episode consists mostly of high carbohydrate meals. The average duration of a meal can be greater than 1 h, with more than 4000 kcal ingested in a single meal. In extreme instances, the amount of food ingested at a single setting can be more than 20,000 kcal and can last for hours (27). The physical complications of bulimia nervosa tend to be limited to hypokalemia, metabolic alkalosis, arrhythmias, and gastric and esophageal rupture and rarely result in death (25).

Binge eating disorder is the only major eating disorder that is found appreciably in both sexes (35% men; 65% women) (28). A majority of binge eaters are overweight and experience one or more symptoms of classical depression. Binge eating behavior occurs alone and appears to be uncontrollable, although it may be soon followed by feelings of embarrassment or guilt.

1.2 Guidelines for Treatment of Eating and Body Weight Disorders

1.2.1 Obesity. An expert panel of physicians has published several recommendations for the treatment of obesity (29, 30). The guidelines call for a 5–10% decrease in body weight by first decreasing total caloric intake and increasing physical activity. This overall decrease in body weight, although small in magnitude, is normally adequate to restore "metabolic fitness" by decreasing blood pressure, blood glucose, and serum triglyceride/cholesterol levels. It is recognized that exercise and diet often prove difficult to implement on a routine basis and often prove ineffective over the long term in causing weight loss, primarily because weight loss by diet alone results in compensatory increases in energy efficiency and decreases in basal metabolic rate. In such cases where lifestyle changes fail to produce the desired reductions in body weight, antiobesity pharmacotherapy is subsequently recommended. Surgery is an option for patients with clinically severe obesity (i.e., BMIs > 40 kg/m^2 or BMIs > 35 kg/m^2 with coexisting conditions) who do not respond to any treatment program. The successful maintenance of lower body weight generally requires rigid adherence to strict behavioral modifications (31).

Several drugs for weight loss and weight maintenance are currently available. These drugs are recommended for patients with BMIs greater than 30 kg/m^2 without additional risk factors or with BMIs greater than 27 kg/m^2 with two or more risk factors (29). Obesity is now considered a chronic disease that must be managed accordingly.

1.2.2 Eating Disorders/Wasting Disorders. Few clinical guidelines have been established for the treatment of eating and wasting disorders. Disease-associated cachexia is usually managed with nutritional therapy and pharmacotherapy involving either anabolic agents or appetite stimulants. Eating disorders are treated with a combination of nutritional therapy, pharmacotherapy, and/or cognitive behavioral therapy (CBT). For anorexia nervosa, oral or parenteral nutritional therapy is first used to restore a healthy normal weight. This is followed by behavioral therapy to remove unhealthy preoccupations with weight loss and to change perceptions about body fat and appearance. Severe cases of anorexia, especially after onset of cachexia, are best managed within an in-patient hospital setting. Pharrnacotherapy is generally used to ease fear of food intake, enhance nutritional absorption, and protect against comorbidities, although it is not very successful in treating the disease itself. For bulimia nervosa, the initial goal of treatment is to remove the underlying cause of any overeating behavior such as diet-induced hunger or depression and eliminate the psychological need for and dependency on compensatory behaviors. Pharmacotherapy has proven remarkably effective in the treatment of this disease. The management of binge eating disorder requires behavioral therapy to restore restraint in eating behavior ("self-denial") and pharmacotherapy to address the underlying psychiatric components of the diseases (32). Because a majority of patients with binge eating disorder are also overweight, lifestyle changes are necessary to stimulate weight loss. For all eating disorders, prevention of relapse is still a major challenge.

The role of drug therapy in the treatment of eating disorders has been reviewed elsewhere (21, 33).

2 CLINICAL APPLICATIONS

2.1 Overview: Current Drugs

A limited number of safe and efficacious drugs are available for the pharmacological treatment of eating and body weight disorders. Compounds (1–9) are used with varying frequencies as antiobesity drugs, generally as part of a comprehensive treatment program involving lifestyle and behavioral changes (see Table 15.4). These drugs generally do not modulate the pathophysiology of obesity because cessation of therapy usually results in the return of any lost weight. Several antiobesity agents have been removed from the market or are now no longer prescribed as weight loss drugs, primarily because of safety concerns. These include thyroid hormone (10) and appetite suppressants such as dexamphetamine (11), aminorex (12), fenfluramine (13), dexfenfluramine (14), and phenylpropanolamine (15)(see Table 15.5).

Few drugs are available to specifically treat eating disorders, in part because these diseases are so complex, with features of eating, psychiatric, and body weight disorders. In general, antidepressant medications such as (16–24) are used to treat the underlying psychiatric symptoms associated with bulimia nervosa, binge eating disorder, and, less frequently, anorexia nervosa, and to prevent relapse of many of these disorders (Table 15.6). For anorexia nervosa, a variety of different drugs are used to overcome the side effects generally associated with malnutrition and the fears associated with food intake and body weight gain (see 25–29, Table 15.6).

Several drugs (30–33) have recently been approved as appetite stimulants or anabolic/growth-promoting agents in wasting diseases, generally as short-term treatments until a normal body weight is achieved (see Table 15.7). These agents are often prescribed in conjunction with other drugs used to treat the primary illness such as cancer or AIDS.

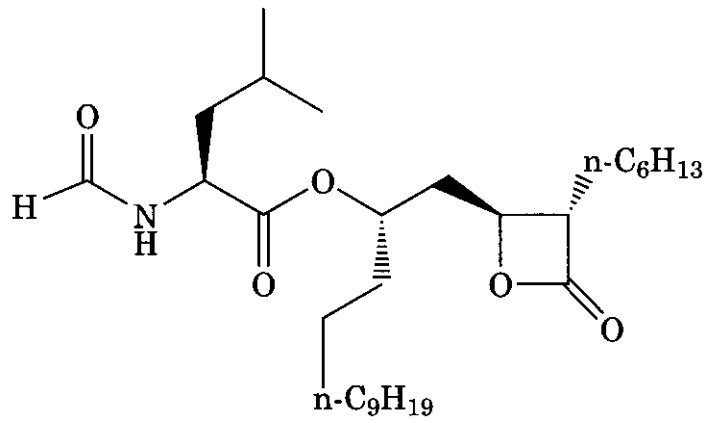
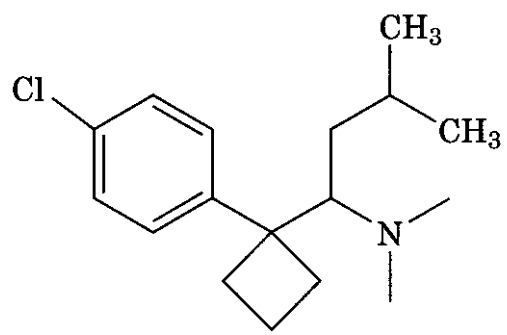
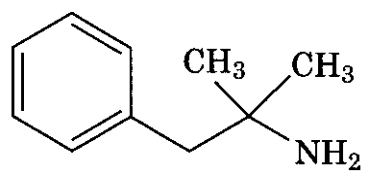
2.1.1 Drugs to Treat Obesity. Three classes of antiobesity drugs are currently used in weight loss therapy, including nonsystemic fat absorption inhibitors, appetite suppressants, and thermogenic/lipolytic agents (see Table 15.4). These drugs can either decrease energy expenditure or enhance fat metabolism. They generally decrease body weight by producing, negative states of energy balance. Fat absorption inhibitors and lipolytic agents act primarily at molecular targets in the periphery, whereas appetite suppressants generally mediate their effects in the central nervous system (CNS). Most centrally acting appetite suppressants have low molecular weights and are highly lipophilic, properties that promote transcellular diffusion across the blood-brain barrier into the brain.

Several recent reviews describe in greater detail the different pharmacological treatments for obesity and the clinical efficacies of both old and new agents (34–36). Worldwide and U.S. sales of selected drugs used for the treatment of eating and body weight disorders are presented in Table 15.8 (37).

2.1.1.1 ***Fat-Absorption Inhibitors.*** Orlistat (1, Ro 18–0647, tetrahydrolipstatin) is a nonsystemic fat absorption inhibitor approved for chronic use as a weight loss treatment. It prevents the breakdown of triglycerides in the gastrointestinal tract and blocks the intestinal absorption of fatty acids (38). It acts as an irreversible inhibitor of gastric and intestinal lipases by reacting with specific serine residues found in the catalytic sites (39). Orlistat is modestly effective at reducing both body weight and decreasing frequency of comorbidities such as diabetes, hypertension, and hyperlipidemia. In one 2-year clinical trial, obese patients with BMIs greater than 30 kg/m^2 lost 5–10% of their body weight in their first year on orlistat therapy and showed significantly less weight regain in their second year of the study (40).

2.1.1.2 ***Appetite Suppressants.*** Appetite suppressants induce satiety mechanisms and, less frequently, increase energy expenditure by interacting with sympathetic pathways in the CNS. These drugs decrease food intake by activating dopaminergic, sympathomimetic (adrenergic), and/or serotonergic systems in the brain. Modulations of these pathways occur through direct and indirect mechanisms. Indirect-acting drugs increase concentrations of

Table 15.4 Antiobesity Drugs

Generic Name	Structure	Trade Name(s)	Originator(s)	Class	DEA Class	Dose (mg/day)
Orlistat (**1**)		Xenical	Roche	Fat absorption inhibitor	No label	360
Sibutramine (**2**)		Meridia	Knoll (Abbott)	Appetite suppressant	IV	5–15
Phentermine (**3**)		Adipex; Ionamin	Teva Pharmaceuticals; Medeva Pharmaceuticals	Appetite suppressant	IV	18.75–37.5

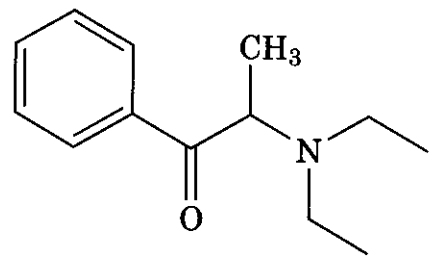
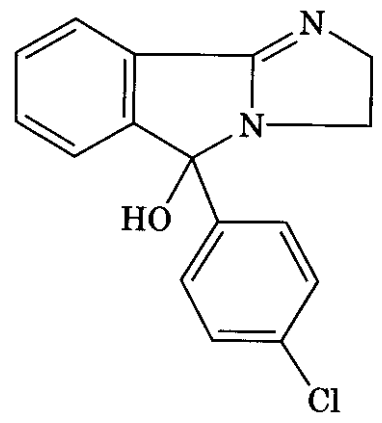
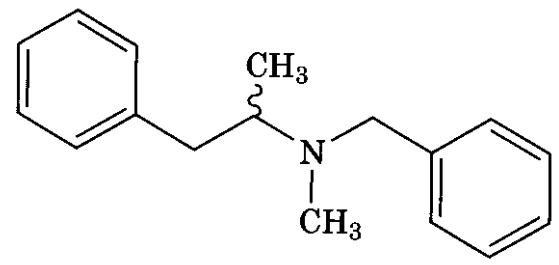
Diethylpropion (**4**)		Tenuate Propion	Aventis	Appetite suppressant	IV	75
Mazindol (**5**)		Sanorex	Novartis	Appetite suppressant	IV	3–12
Benzphetamine (**6**)		Didrex	Pharmacia	Appetite suppressant	III	25–150

Table 16.4 (Continued)

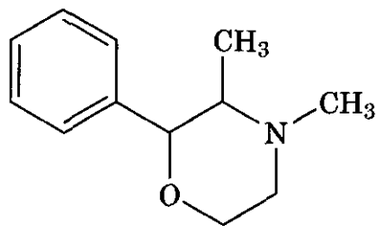
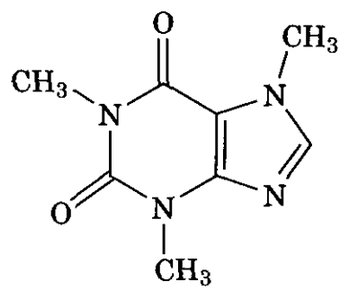
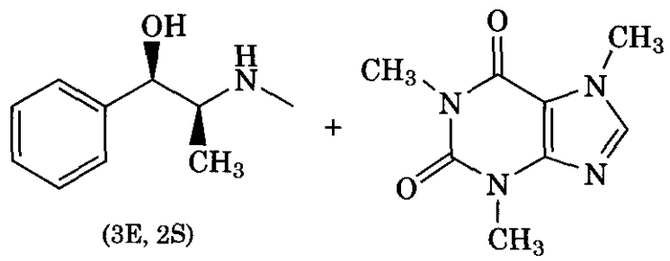
Generic Name	Structure	Trade Name(s)	Originator(s)	Class	DEA Class	Dose (mg/day)
Phendimetrazine (**7**)		Bontril; Plegine	Amarin Pharmaceuticals; Wyeth	Appetite suppressant	III	70–210
Caffeine (**8**)				Lipolytic agent	No label	
D-(−)-Ephedrine (**9**)/caffeine	 (3E, 2S)			Thermogenicl lipolytic agent	No label	75

Table 15.5 Miscellaneous Antiobesity Drugs

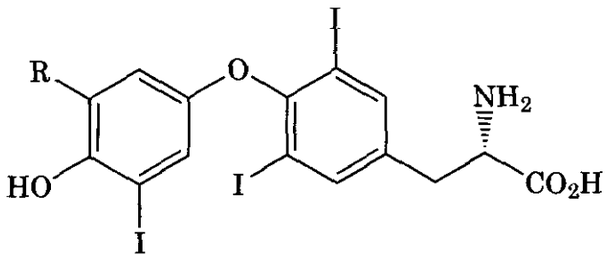
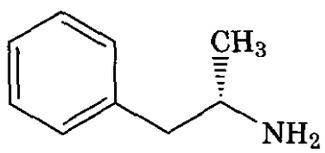
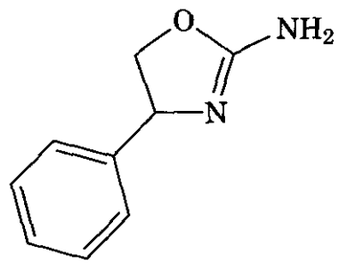
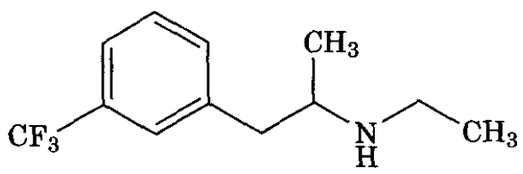
Generic Name	Structure	Class	Status
Thyroid hormone (**10**)	 R = I, T4, L-thyroxine (prohormone) R = H, T3, L-triiodothyronine (hormone)	Thermogenic agent	Rarely prescribed as weight loss agent because of hyperthyroidism
Dexamphetamine, Dexedrine (**11**)		Appetite suppressant	Rarely prescribed as weight loss agent, DEA Class II agent
Aminorex (**12**)		Appetite suppressant	Withdrawn from market because of risk of primary pulmonary hypertension
Fenfluramine (**13**)		Appetite suppressant	Withdrawn from market because of risk of cardiac valvular lesions (valvulopathy)

Table 15.5 (Continued)

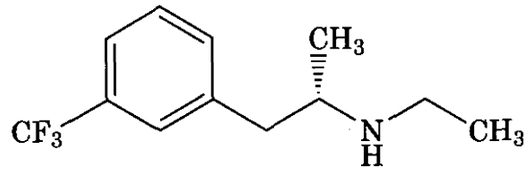
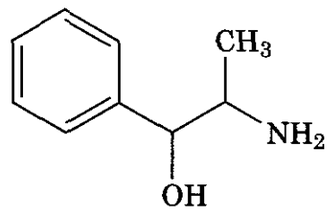
Generic Name	Structure	Class	Status
Dexfenfluramine (**14**)		Appetite suppressant	Withdrawn from market because of risk of valvulopathy
Phentermine (**3**)/**fenfluramine** (13)		Appetite suppressant	Withdrawn from market because of risk of valvulopathy
Phentermine (**3**)/**dexfenfluramine** (14)		Appetite suppressant	Withdrawn from market because risk of valvulopathy
Phenylpropanolamine (15, PPA)	 (*D*, *L*)	Appetite suppressant	Removed from market because of risk of hemorrhagic stroke

Table 15.6 Agents for Treatment of Anorexia Nervosa (AN), Bulimia Nervosa (BN), and Binge Eating (BE)

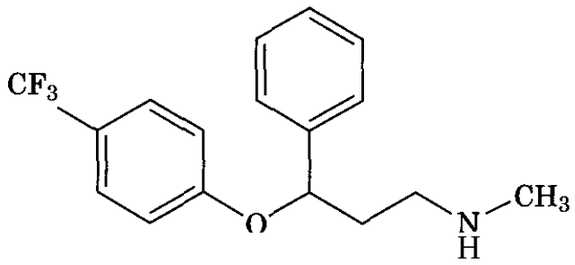
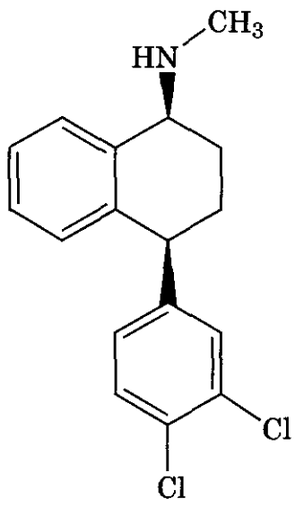
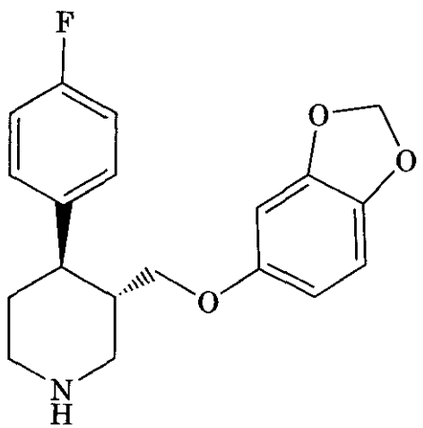
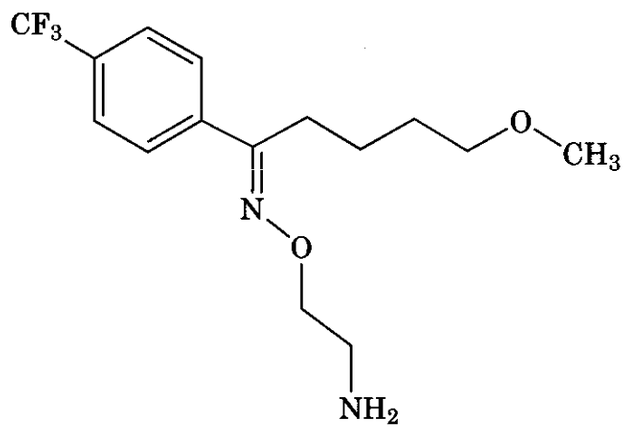
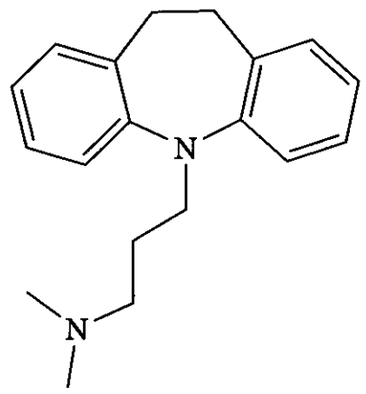
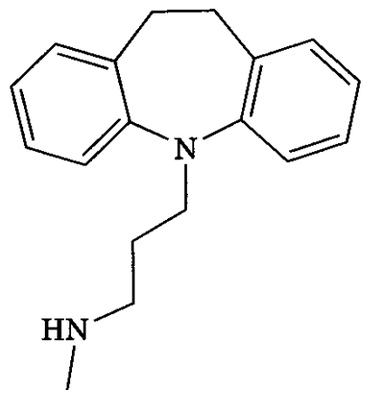
Generic Name	Structure	Trade Name	Originator	Indication	Class[a]	Dose (mg/day)
Fluoxetine (16)		Prozac	Lilly	AN[b] BN BE[c]	Antidepressant (SSRI)	60(BN) 20(A)
Sertraline (17)		Zoloft	Pfizer	BN[c] BE[d]	Antidepressant (SSRI)	
Paroxetine (18)		Paxil	GSK	BN[c]	Antidepressant (SSRI)	

Table 15.6 (Continued)

Generic Name	Structure	Trade Name	Originator	Indication	Class[a]	Dose (mg/day)
Fluvoxamine (19)		Luvox	Solvay	BN[c]	Antidepressant (SSRI)	
Imipramine (**20**)		Tofranil	Novartis BE[c,d]	BN[c,d]	Antidepressant (TCA)	150–300
Desipramine (**21**)[b]		Norpramin	Aventis	BN[c,d] BE[c,d]	Antidepressant (TCA)	150–300

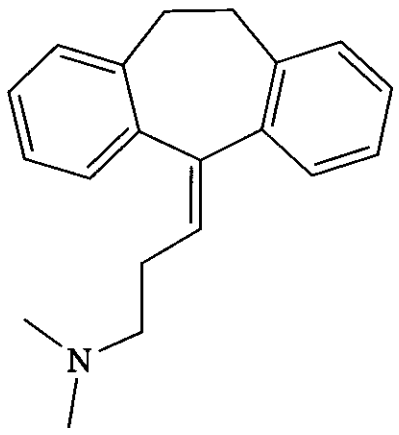
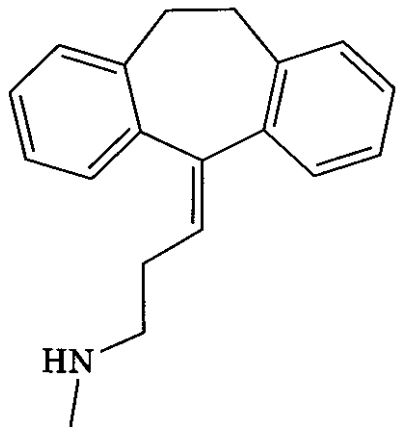
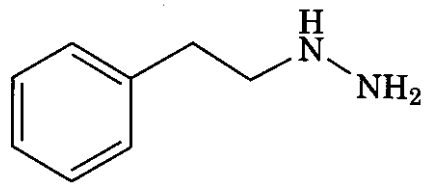
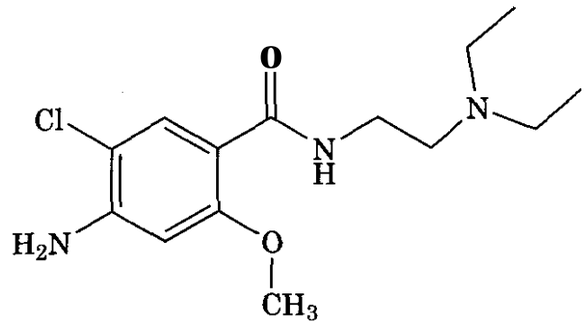
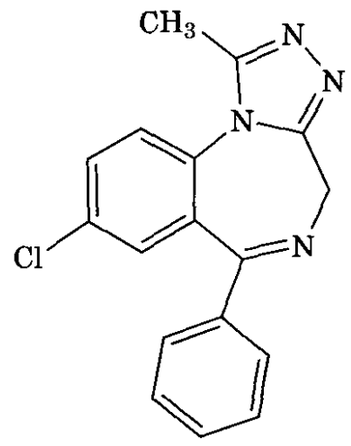
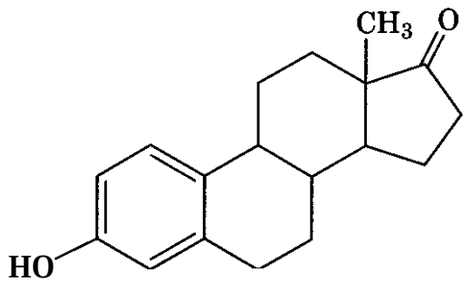
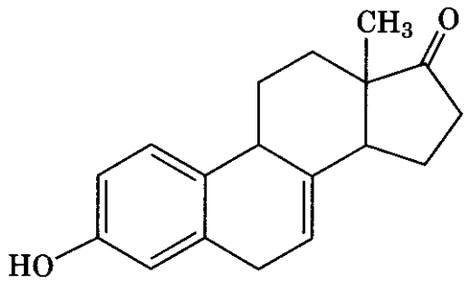
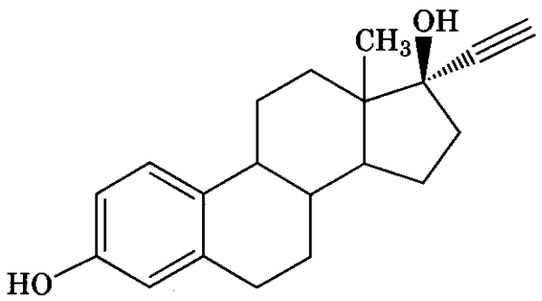
Amitriptyline (**22**)		Elavil; Limbitrol	AstraZeneca; ICN	BN[c,d]	Antidepressant (TCA)	50–300
Nortriptyline (**23**)		Aventyl; Pamelor	Lilly; Novartis		Antidepressant (TCA)	
Phenelzine (**24**)[b]		Nardil	Pfizer	BN[c,d]	Antidepressant (MAOI)	60–90

Table 15.6 (Continued)

Generic Name	Structure	Trade Name	Originator	Indication	Class[a]	Dose (mg/day)
Metoclopramide (**25**)		Reglan	Robins	BN[c]	Prokinetic agent	40–60
Alprazolam (**26**)		Xanax	Pharmacia	AN[c]	Anxiolytic agent	0.5–1.5

Conjugated estrogens: **Estrone (–60%):**  (**27**)	Premarin	Wyeth	AN[c]	Estrogenic agent	
Equilin (–30%):  (**28**)					
Ethinyl estradiol  (**29**)			AN[c]	Estrogenic agent	0.01-0.05

[a]SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic amine antidepressant; MAOI, monoamine oxidase inhibitor.
[b]With symptoms of depression.
"Off-label.
[d]Second-line therapy.

Table 15.7 Drugs for Treatment of Wasting Diseases and Cachexia

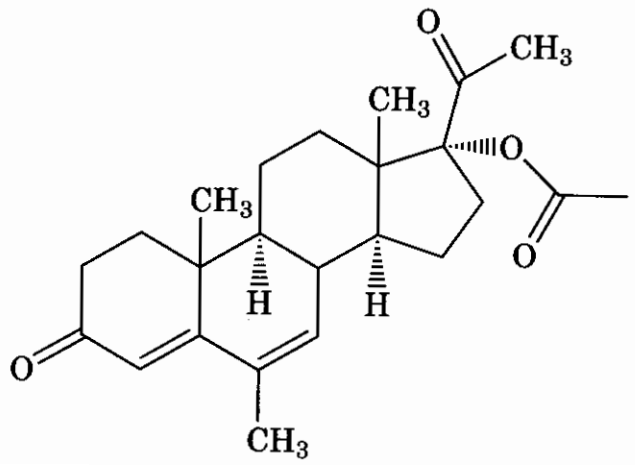
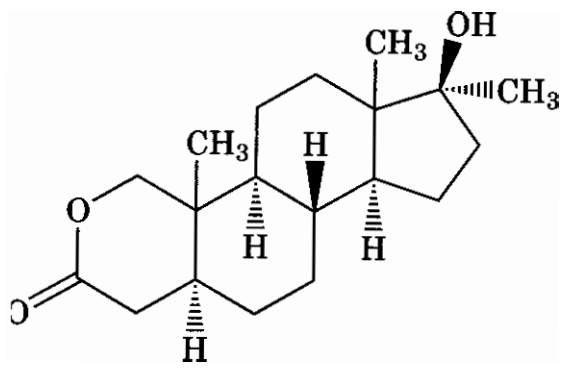
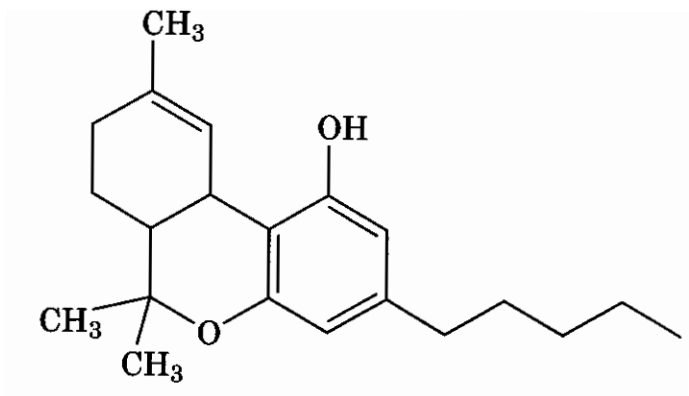
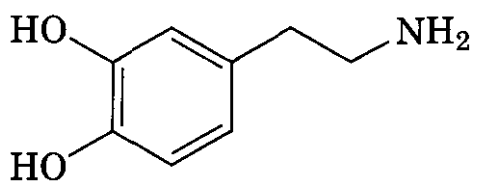
Generic Name	Structure	Trade Name	Originator	Dose (mg/day)
Megestrol oral suspension (**30**)		Megace	Bristol-Myers Squibb	800
Oxandrolone (**31**)		Oxandrin	BTG Pharmaceuticals	5–10
Dronabinol (**32**)		Marinol	Unimed Pharmaceuticals	5
Recombinant human growth hormone (**33**, rhGH)		Serostim	Serono	6

Table 15.8 Worldwide and U.S. Sales in Year 2000 of Selected Drugs used for the Treatment of Eating and Body Weight Disorders[a]

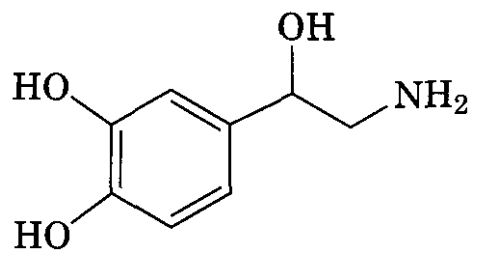
Drug	Indication	Worldwide Sales ($, millions)	U.S. Sales ($, millions)
Sibutramine	Obesity (OB)	184	94
Phentermine	OB	112	103
Phendimetrazine	OB	4	4
Orlistat	OB	539	202
Mazindol	OB	12	<1
Ephedrine	OB	32	17
Diethylpropion	OB		7
Oxandrolone	Cachexia (C)	40	38
Megestrol[b]	C	157	155

[a]Adapted from IMS Health Global Services, 2001.
[b]Most of the sales for Megestrol were for other indications.

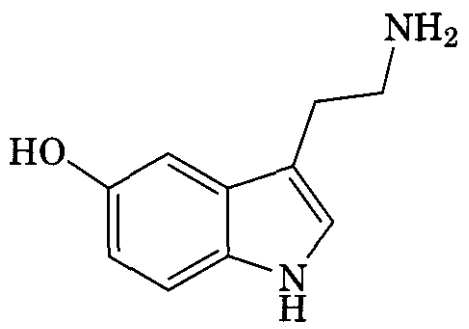
dopamine (DA, **34**), norepinephrine (NE, **35**), and serotonin [5-hydroxytryptamine (5-HT), 361 at postsynaptic neuronal receptors by (1)



(34) Dopamine (DA)



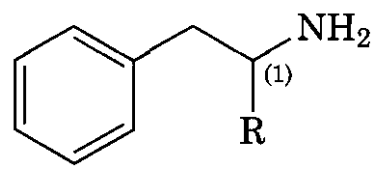
(35) Norepinephrine (NE)



(36) Serotonin (5-hydroxytryptamine – 5-HT)

stimulating the release of these neurotransmitters stored in intracellular granules or (2) inhibiting the uptake of synaptic levels of these neurotransmitters by membrane transporters. Direct-acting drugs, in contrast, act on pre- or postsynaptic receptors to affect appetite. Sympathomimetic drugs have been historically classified as either direct- or *indirect*-acting agents.

The majority of appetite-suppressant drugs are congeners of 2-phenethylamine (β-phenethylamine, **37**), a compound with close structural similarity to many biogenic amines such as NE and DA. The prototypical 2-phenethylamine derivatives are amphetamine **(38)** and its dextrorotatory isomer dexamphetamine (**11**), both of which stimulate NE and DA release and inhibit NE and DA reuptake. These drugs exhibit potent anorectic activity, but they also have extensive central excitatory properties (euphoria, restlessness) that restrict their general utility. Compound **(11)** is no longer prescribed as a weight loss agent because of its small therapeutic index, although it is still routinely prescribed for other conditions such as attention deficit hyperactivity disorder (ADHD).



(37) 2-Phenylethylamine, R = H
(38) Amphetamine, R = CH_3

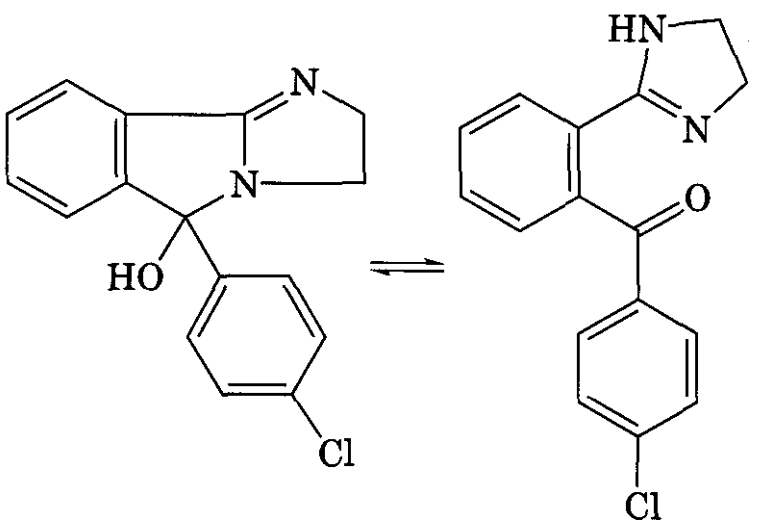
Two amphetamine/phenethylamine analogs, sibutramine (2)and phentermine (**3**), exhibit minimal central excitatory activities. Sibutramine, a combined NE and 5-HT reuptake inhibitor, is the only amphetamine-like derivative that is currently approved for

long-term use in weight loss therapy (41). In clinical trials, sibutramine has been shown to reduce total daily energy intake, decrease fat intake, and lower feelings of hunger (42). It also has been reported to increase energy expenditure, although the data are somewhat conflicting (43). Sibutramine has been found to prevent body weight regain after weight loss (44). The long-term effectiveness of sibutramine in reducing obesity-related comorbidities has yet to be conclusively established, but sibutramine has been shown to decrease cholesterol and triglyceride levels after 2 years of therapy (44). An extensive review of the human clinical trials with sibutramine was recently published (45).

Phentermine (3), a close-in analog of amphetamine with an extra methyl group adjacent to the primary amino group, is approved for short-term weight-loss therapy, generally lasting up to 3 months. Phentermine stimulates synaptic NE release, without affecting dopamine release or reuptake. Phentermine was often combined off-label with either fenfluramine (13) or dexfenfluramine (14), stimulators of 5-HT release and inhibitors of 5-HT reuptake, to give extremely effective weight-loss combinations called "fen/phen" or "d-fen/phen." These combinations, which never received U.S. Food and Drug Administration (FDA) approval, exhibited greater efficacy than any of the agents alone, probably because they targeted two important pathways involved in the regulation of food intake. Both "fen/phen" and "d-fen-phen" were associated with cardiac side effects (valvulopathy), partially attributed to the fenfluramine or dexfenfluramine part of the combination. Fenfluramine and dexfenfluramine have been removed from the market, but phentermine is still used as monotherapy for weight loss, although generally as an adjunct to diet and exercise. The anorectic effects of phentermine generally decrease with time.

Benzphetamine (6), diethylpropion (4), and phendimetrazine (7) are amphetamine-like drugs that have been available for several decades, but that are now considered second-tier agents for weight loss. These drugs produce modest reductions in body weight and are indicated for short-term use only. Benzphetamine contains a bulky N-benzyl group that reduces the central excitatory activity associated with the 2-phenethylamine class of appetite suppressants, without affecting anorectic activity. Diethylpropion is recommended for obese patients with elevated blood pressure or cardiovascular diseases. These drugs all decrease appetite by increasing NE release from nerve terminals.

Mazindol (5) is the only non-2-phenethylamine indirect-acting syrnpathomimetic drug approved as a short-term appetite suppressant. The drug was the principal anorectic agent used in many countries outside the United States until the introduction of Orlistat and Sibutramine. Mazindol is a weak inhibitor of NE reuptake and a potent inhibitor of both 5-HT and DA reuptake, although other activities may be partially responsible for the anorectic effect. This drug is postulated to bind at the "cocaine site" on the DA transporter, yet it exhibits few of the undesirable pharmacological activities associated with cocaine administration (46). Mazindol exists in two tautomeric forms, with the open-ring benzophenone tautomer (39) preferred under acidic conditions and the cyclic form preferred under neutral conditions.



(5) (39)

The biological activities of the different appetite suppressants are summarized in Table 15.9. Because these drugs can increase synaptic levels of multiple neurotransmitters, they often exhibit complex polypharmacology by interacting with entire families of biogenic amine receptors that often have multiple functions.

Table 15.9 **Comparative Summary of Pharmacological Mechanisms of Weight-Loss Drugs and Selected Drugs to Treat Eating Disorders**[a]

Compound	5-HT Reuptake Inhibition	5-HT Release	NE Reuptake Inhibition	NE Release	DA Reuptake Inhibition	DA Release
Sibutramine (2)	+		++		+	
Phentermine (3)				+		
Dexfenfluramine[b] (14)	+	+++				
Diethylpropion (4)				+		
Mazindol (5)	+		+		++	
Benzphetamine (6)				+		
Phendimetrazine (7)				+		
Dexamphetamine (11)			+	++	+	++
Fluoxetine (16)	+++					
Sertraline (17)	+++					
Paroxetine (18)	+++					
Fluvoxamine (19)	+++					
Imipramine (20)	++		+			
Desipramine (21)			++			

[a](+), weak activity; (++), moderate activity; (+++), potent activity.
[b]No longer available.

2.1.1.3 Lipolytic and Thermogenic Agents. Caffeine (**8**), a methylxanthine derivative occurring naturally in coffee and tea, is a central stimulant occasionally prescribed for short-term weight reduction. Caffeine has been shown to increase energy expenditure and stimulate lipolysis. The lipolytic activity of caffeine is mediated through antagonism of adenosine receptors (mainly A_1-R) in adipose tissue. Some of the pharmacological activities of caffeine may also be attributed to inhibition of phosphodiesterases, which increase intracellular concentrations of a cell-signaling molecule called cyclic adenosine 3′,5′-monophosphate (cAMP). Caffeine is often combined with D-(−)-ephedrine (**9**), a naturally occurring direct-acting sympathomimetic agent found in the plant Ephedra equisedina, which is available as an over-the-counter drug. Ephredrine is a weak β_2-adrenergic receptor agonist that increases intracellular cAMP levels. The caffeine/ephredine combination has been shown to significantly increase energy expenditure (thermogenesis) and fat metabolism in both obese women and adolescents (47–49). This combination is more efficacious than either agent alone, possibly because it has synergistic effects on the stimulation of CAMP.

2.1.2 Drugs to Treat Eating Disorders. Classical antidepressants drugs, such as selective 5-HT reuptake inhibitors, tricyclic amines, and monoamine oxidase inhibitors, are used to treat selected eating disorders, manage the underlying psychiatric symptoms associated with most atypical feeding behaviors (depression, anxiety), and to prevent relapse of all eating diseases after treatment (see Table 15.6). These agents are generally used in conjunction with cognitive behavioral therapy and nutritional counseling. Selective serotonin reuptake inhibitors (SSRIs) prevent reuptake of synaptic 5-HT by transporters located on neuronal membranes. The SSRI fluoxetine (**16**) is approved for the treatment of bulimia nervosa (50) and is used off-label in the treatment of binge eating disorders (51). Other marketed SSRIs such as sertraline (**17**), paroxetine (**18**), and fluvoxamine (19) are considered therapeutically equivalent to fluoxetine and sometimes used interchangeably with fluoxetine to treat bulimia nervosa, with the choice depending on the adverse effect profile of the particular SSRI. Sertraline has been shown to decrease frequency of binge eating disorder behavior (52); fluvoxamine has been found to prevent relapse of bulimia nervosa after psychotherapy (53). SSRIs are generally

not effective in treating anorexia nervosa, primarily because malnutrition and low body weight cause drastic reductions in synaptic levels of 5-HT (21, 54). SSRIs are efficacious in preventing relapse of anorexia nervosa after weight regain and normalization of 5-HT levels (55).

Tricyclic amine antidepressants (TCAs) are nonselective 5-HT and NE reuptake inhibitors. Two TCAs, imipramine (20) and desipramine (21), are used to treat bulimia nervosa, primarily as second-line therapies. Imipramine, a tertiary amine, is a 5-HT reuptake inhibitor with weak NE reuptake activity. Desipramine, a secondary amine and the des-ethyl analog of imipramine, is a NE reuptake inhibitor. Other TCAs used less frequently to treat BN include amitriptyline (22) and its N-desmethyl analog nortripyline (23).

Monoamine oxidase inhibitors are a class of antidepressant drugs that inactivate enzymes responsible for degrading biogenic amines, thus elevating extracellular or synaptic concentrations of neurotransmitters such as NE and 5-HT. Two forms of the enzyme are known: monoamine oxidase (MAO) A, which is found within nerve terminals and is highly selective for NE and 5-HT; and monoamine oxidase B, which is found extracellularly, primarily in platelets, and is highly specific for nonphenolic 2-phenethylarnine derivatives (56). Phenelzine (24) is a non-selective MAO inhibitor that forms an irreversible adduct with the enzyme through a phenethyl radical intermediate. It is used occasionally to treat bulimics, generally when all other therapies fail.

Several drugs are used to restore a normal body weight in anorectic patients and to prevent onset of comorbidities (26, 57). Metoclopramide (25), a prokinetic dopamine receptor antagonist and a muscarinic agonist, decreases bloating during nutritional therapy by increasing gastric emptying, thereby enhancing nutrient absorption (33, 58). Anxiolytic agents such as alprazolam (26) are helpful in easing fear of food intake and body weight gain and in reestablishing normal eating patterns. Estrogen replacement therapy through use of conjugated estrogens (estrone, 27; equilin, 28) or ethinyl estradiol (29) is often prescribed to increase bone mineral density, decrease risk of fractures, and prevent adult onset of osteoporosis, a potential concern for young, anorectic women with prolonged episodes of amenorrhea and decreased endogenous estrogen production.

The pharmacological mechanisms of the prototypical drugs used to treat eating disorders are summarized in Table 15.9.

2.1.3 Drugs to Treat Wasting Diseases. Several centrally and peripherally acting appetite stimulants and anabolic agents are used in the treatment of wasting diseases (see Table 15.7). A progesterone derivative, megestrol (30), has been shown to enhance caloric intake and weight gain in anorectic and cachectic AIDS patients. The exact mechanism by which this agent stimulates appetite is not yet known. An anabolic steroid and testosterone analog called oxandrolone (31, a class III controlled substance) has been found to stimulate weight gain, improve physical strength, and increase lean body mass in anorectic AIDS patients (59). Dronabinol [32, Δ^9-tetrahydrocannabinol (A^9-THC)], a synthetic derivative of one of the active components in *Cannabis sativa* L (marijuana), stimulates appetite through activation of central cannabinoid receptors, most likely the cannabinoid receptor 1 (CB_1-R) subtype, which is found almost exclusively in the brain. Dronabinol has been approved for use in body wasting disorders in AIDs patients. Recombinant human growth hormone (33, rhGH, somatotropin) is also used to stimulate protein anabolism and increase body weight gain (lean body mass), with one marketed rhGH preparation called serostim, approved for the treatment of AIDS wasting (60). Somatotropin mediates many of its pharmacological effects through a hormone synthesized by the liver, insulin-like growth factor 1 (IGF-1).

2.2 Adverse Effects and Precautions

Side-effect profiles of many antiobesity drugs have led to high attrition rates, lessening the overall appeal of pharmacotherapy as a treatment option. In general, anti-obesity drugs should have fairly mild and non–life-threatening side effects, because of the potential off-label use of such compounds for cosmetic purposes in relatively healthy individuals.

Orlistat can cause a number of unpleasant gastrointestinal events such as oily spotting, flatus discharge, fecal urgency, fatty/oily stool, and fecal incontinence that are related to its mechanism of action (inhibition of triglyceride breakdown) and its nonsystemic site of action (GI tract) (61). These side effects are generally more pronounced in obese patients on a high fat diet. Despite the GI effects, orlistat is generally considered one of the safest antiobesity drugs, primarily because it shows low systemic exposure, which generally minimizes the likelihood of general compound-related organ toxicity.

The adverse effects of amphetamine and related sympathomimetic appetite suppressants are well documented. All of these agents are classified by the U.S. Drug Enforcement Administration (DEA) as controlled substances (classes II-IV) according to their potential for causing addiction (see Table 15.4). Class II agents such as amphetamine are highly abused, with prescription restricted to special circumstances; class IV anorectic drugs such as sibutramine, phentermine, diethylpropion, and mazindol have minimal abuse potential.

Sibutramine is contraindicated in obese patients with preexisting cardiovascular diseases because it causes a small but significant increase in both systolic blood pressure and supine heart rate (62). Sibutramine elevates synaptic levels of NE, resulting in activation of sympathomimetic pathways involved in blood pressure regulation. Sibutramine does not appear to cause valvular heart disease, unlike other agents that potently stimulate 5-HT release like dexfenfluramine.

The principal side effects of phentermine are insomnia, restlessness, and euphoria. Some patients rapidly develop tolerance to this agent, resulting in discontinuation of therapy. The combination of phentermine with fenfluramine or dexfenfluramine was associated with increased incidences of both primary pulmonary hypertension (PPH) and cardiac valvulopathy, but it is unlikely that phentermine alone causes these same problems. Phentermine, nonetheless, contains a warning label listing PPH and cardiac valve lesions as possible adverse events.

Other sympathomimetic appetite suppressants (benzphetamine, diethylpropion, mazindol, and phendimetrazine) are associated with insomnia, restlessness (i.e., frequent awakening), and occasional mild euphoria, primarily because of their central stimulant properties. Toleration usually develops to many of these side effects. Drug therapy with these agents is restricted to 12 weeks.

Caffeine causes several centrally mediated side effects including nervousness, irritability, and sleeplessness. Caffeine also acts on kidneys to increase diuresis. Convulsions and increased heart rate can occur with particularly high doses of the drug.

The SSRIs used in the treatment of eating disorders such as bulimia nervosa are generally well tolerated. In clinical trials with fluoxetine, patients reported increased incidences of dry mouth, anxiety, nervousness, insomnia, and sexual dysfunction. Interestingly, two commonly reported side effects of fluoxetine treatment are anorexia and weight loss (63). However, fluoxetine is not approved as a therapy for the treatment of obesity, primarily because such SSRI-induced weight loss cannot be maintained. In one controlled trial with fluoxetine, severely obese patients (BMIs > 35.6 kg/m^2) reportedly lost significant body weight during the first 20 weeks of the study, but regained most of the lost weight in the second 20 weeks, despite continuation of drug treatment (64).

The tricyclic amine antidepressants such as desipramine and imipramine have been relegated to second-line therapy, mainly because they cause hypotension, tachycardia, blurred vision, dry mouth, constipation, fatigue, and sedation. attributed in part to their adrenergic, anticholinergic, and antihistaminergic properties. The monoamine oxidase inhibitor phenelzine causes numerous side effects resulting from its irreversible inhibition of both brain and liver monoamine oxidases and its nonselective inhibition of other enzymes. The liver activity of phenelzine prevents degradation of sympathomimetic amines in food products, leading to elevated levels of potentially toxic norepinephrine-like pressor substances. Adverse effects of phenelzine include severe hypertensive crises, nausea, and vomiting.

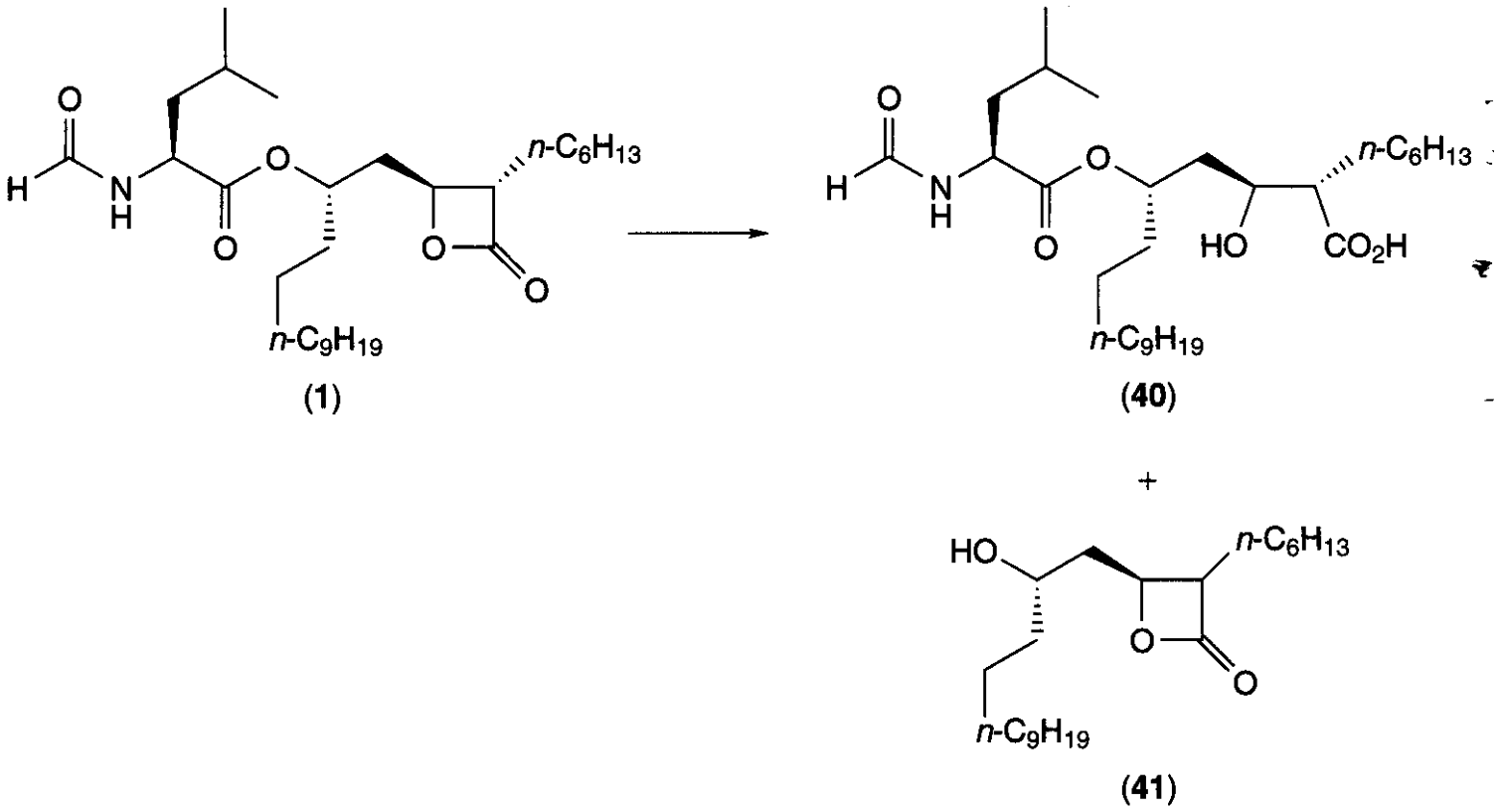


Figure 16.1. Plasma metabolites of orlistat.

These side effects can last for weeks, depending on the rate of resynthesis of new enzyme.

Drugs used in the treatment of wasting diseases exhibit several side effects, some of which are mechanism related. Oxandrolone is an anabolic steroid with potent androgenic activity that disrupts the hypothalamo-pituitary-gonadal axis. It can adversely affect growth and sexual development in children and suppress gonadotrophic function in adults. It also causes masculinization (i.e., hoarseness, hair growth) in women; acne and decreased spermatogenesis in men; and edema, jaundice, and cholestatic hepatitis in both sexes (65). Dronabinol (32) causes a wide variety of psychotropic effects including dysphoria, anxiety, and hallucination that are attributed to activation of cannabinoid receptors within the CNS. This drug can also cause tachycardia and orthostatic hypotension (66). Megestrol is associated with respiratory ailments, elevated liver enzymes, and hyperglycemia (66).

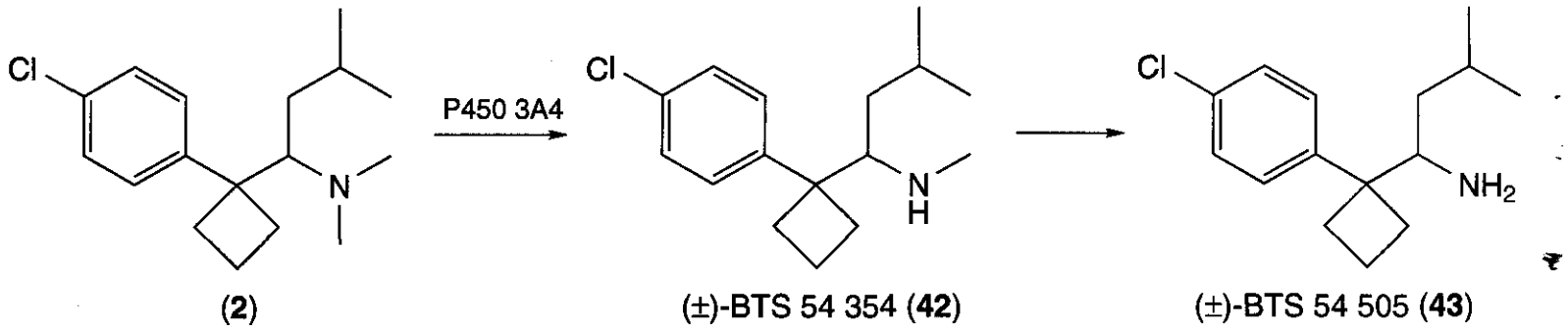
3 DRUG METABOLISM

The absorption and metabolic profiles of antiobesity drugs and appetite stimulants in humans are described below. Several novel biotransformation pathways in rodent species are also illustrated. The pharmacokinetic properties and metabolic degradation pathways for antidepressant drugs that are used in the treatment of eating disorders (i.e., SSRIs, TCAs, MAOIs) have been summarized in previous volumes (67).

3.1 Absorption/Metabolism of Antiobesity Drugs

3.1.1 Orlistat. Orlistat acts in the GI tract, where it inhibits pancreatic lipases located in the lumen. Systemic exposure of the drug is not required for pharmacological activity. With negligible oral bioavailability ($F < 5\%$), orlistat is primarily excreted unchanged in the feces. However, several metabolites including (40) and (41) have been identified in the plasma of both normal and obese volunteers. These compounds are formed by hydrolysis of both the N-formyl leucine ester moiety and the lactone ring and do not exhibit any inhibitory activities toward pancreatic lipases (68) (see Fig. 15.1).

3.1.2 Sibutramine. Sibutramine is readily absorbed into the systemic circulation, reaching maximum levels within 2.5 to 3.5 h (69). It undergoes rapid biotransformation by the cytochrome P450 family of isoenzymes in the liver (primarily P450 $3A_4$), to give the des-



(2) (±)-BTS 54 354 (42) (±)-BTS 54 505 (43)

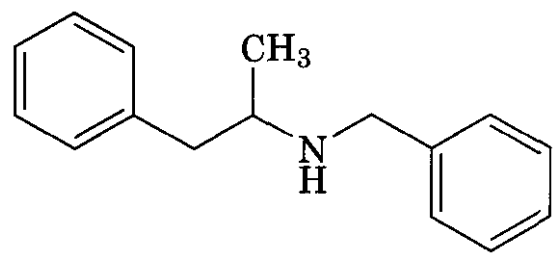
Figure 15.2. Biotransformation of sibutramine in humans.

methyl compound BTS 54 354 (42) and the di-desmethyl analog BTS 54 505 (43; see Fig. 15.2) (70). The R- and the S-enantiomers of both sibutramine and BTS 54 354 are demethylated at different rates, to give a complex plasma mixture of both primary and secondary 2-phenethylamine metabolites (44–47). The half-lives of the R-enantiomers of the two desmethyl and di-desmethyl metabolites (44) and (46) are considerably longer than the half-life of the parent drug. The two enantiomers of BTS 54 505 (46), (47) are further deactivated through oxidation and conjugation, to give compounds that are cleared through the kidney.

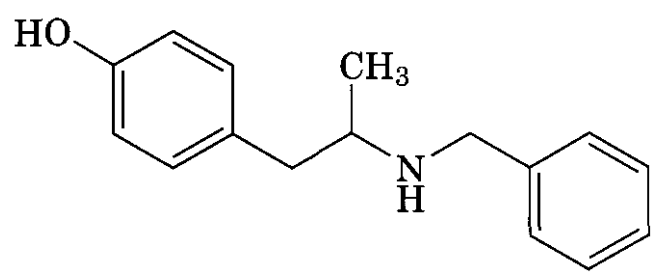
The pharmacological activities of sibutramine metabolites have been evaluated. The R-enantiomers of BTS 54 354 and BTS 54 505 (44 and 46) show potent NE, 5-HT, and DA reuptake inhibitory activities, with IC_{50} values less than 13 and 140 n*M* for NE reuptake inhibition and 5-HT reuptake inhibition, respectively (see Table 15.10) (71). The S-enantiomer of BTS 54 354 (45) exhibits weak inhibitory activity for inhibition of uptake of NE, 5-HT, and DA, whereas the S-isomer of BTS 54 505 (47) displays good activity for inhibition of NE and DA reuptake. Sibutramine metabolites may be primarily responsible for the potent anorectic activity of the parent drug.

3.1.3 Phentermine. Phentermine is an amphetamine analog with a quaternary carbon atom adjacent to the primary amino group. Unlike amphetamine, which is primarily metabolized first to phenylacetone (49) through a-hydroxlyation and then to benzoic acid (50), as shown in Fig. 15.3 (72), phentermine undergoes N-oxidation and aryl ring hydroxylation in different animal species (see Fig. 15.4). In humans, after an oral dose, phentermine is converted into the N-hydroxy and *N*-nitroso derivatives (52) and (53), whereas in rats, phentermine is oxidized to 4-hydroxyphentermine (54), which is then conjugated and excreted (73, 74).

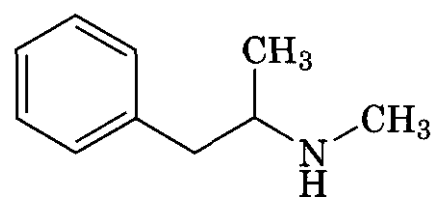
3.1.4 Benzphetamine. The short-acting anorectic benzphetamine is first metabolized in humans to norbenzphetamine (55) through N-demethylation and then to 1-(4-hydroxyphenyl)-2-(*N*-benzylamino)propane (56) through phenyl ring oxidation (75). Methamphetamine (57) and amphetamine (38) are



(55) Norbenzphetamine

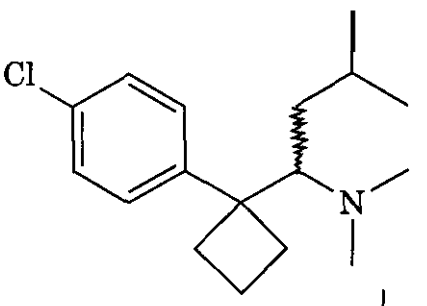
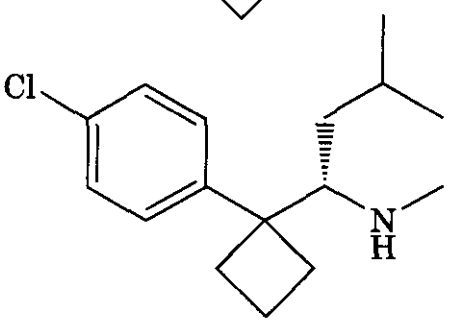
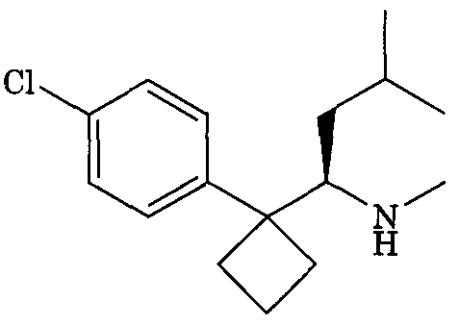
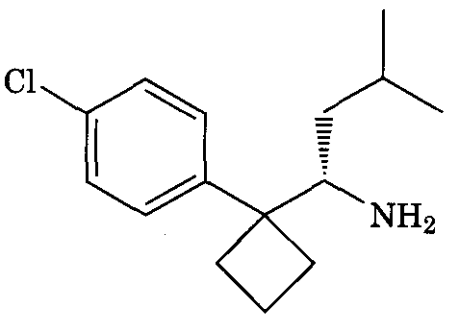
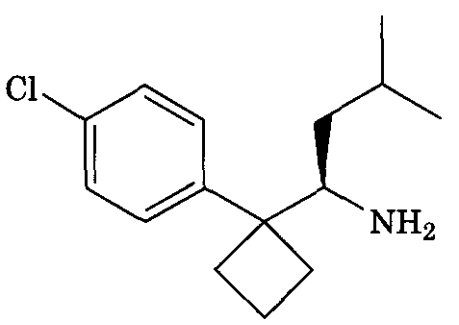


(56)



(57) Methamphetamine

Table 15.10 Pharmacological Activities of Sibutramine Metabolites in Rat Synaptosomes from Male Wistar Hypothalamus[a]

Compound	Structure	NE Reuptake Inhibition, K_i (nM)	5-HT Reuptake Inhibition, K_i (nM)	DA Reuptake Inhibition, K_i (nM)
Sibutramine		350	2800	1200
(*R*)-(+)-BTS 54 354 (**44**)		4	44	12
(*S*)-(−)-BTS 54 354 (**45**)		870	9200	180
(*R*)-(+)-BTS 54 505 (**46**)		13	140	9
(*S*)-(−)-BTS 54 505 (**47**)		62	4300	12

[a] Ref. 71.

also formed, but only as minor metabolites. Interestingly, only the D-enantiomers of (**57**) and **(38)** are detected in urine (**76**). In hepatic microsomes obtained from rats pretreated with phenobarbital, benzphetamine initially undergoes demethylation to give **norbenzphetamine** (**55**), as shown in Fig. 15.5 (77). The secondary amino group in (55) is then oxidized further to give the N-hydroxyl derivative (**58**), which is converted by a flavoprotein **mixed-function** amine oxidase into the nitrone derivative (59). Degradation of the nitrone metabolite, followed by oxidation yields 2-**nitroso-1-phenylpropane** (**60**).

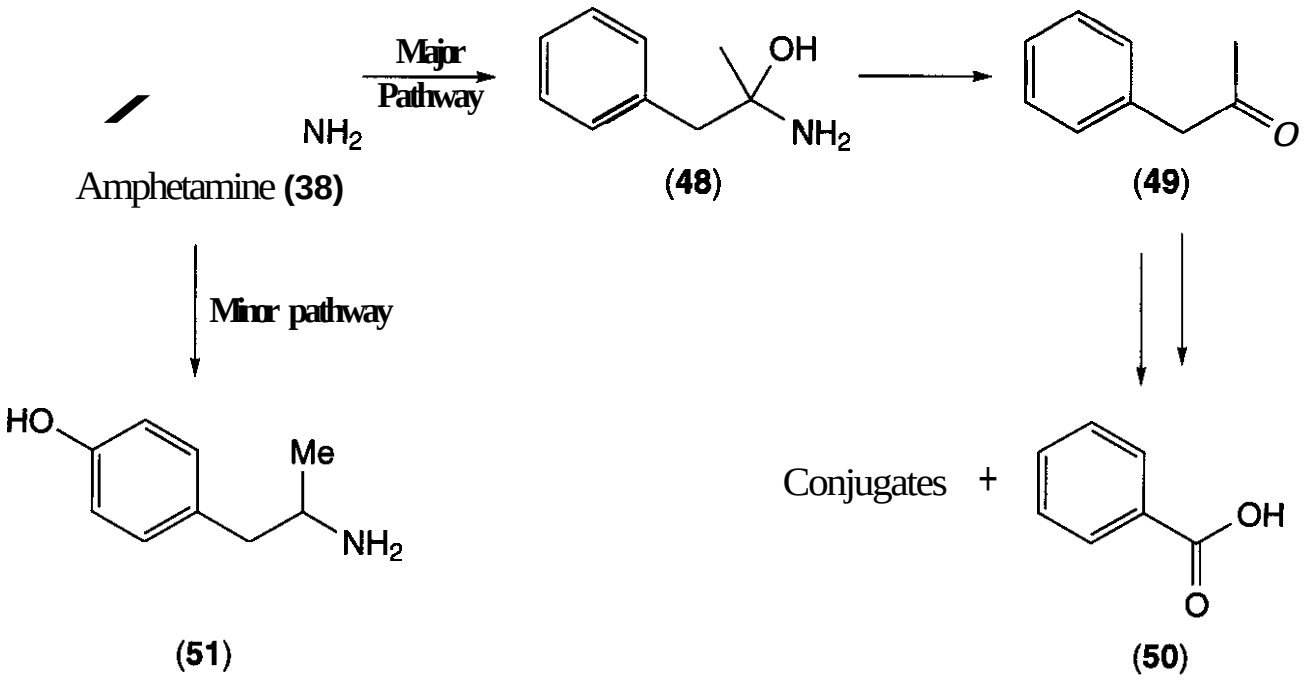
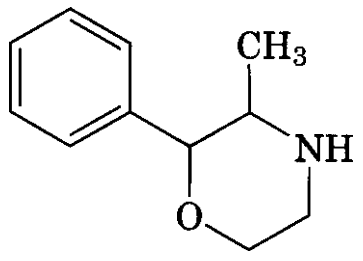
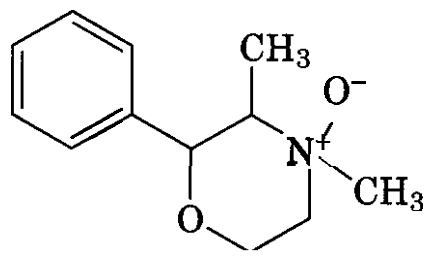


Figure 15.3. Metabolism of amphetamine.

3.1.5 Phendimetrazine. Phendimetrazine is readily absorbed from the GI tract after an oral dose in humans. It exhibits a short plasma half-life ($t_{1/2} = 1.9$ h), primarily attributable to high clearance, and requires qid dosing to maintain adequate plasma levels (78). A special slow-release drug capsule has been developed that extends the half-life to 9.8 h. Two primary metabolites, phenmetrazine (61) and phendimetrazine N-oxide (**62**), are formed by N-dealkylation and N-oxidation and are cleared through the kidney (79).



(61) Phenmetrazine



(62) Phendimetrazine N-Oxide

3.1.6 Diethylpropion. Diethylpropion undergoes P450-mediated mono-N-de-ethylation, to give an active metabolite called ethylpropion (63; see Fig. 15.6). Compound (63) is a

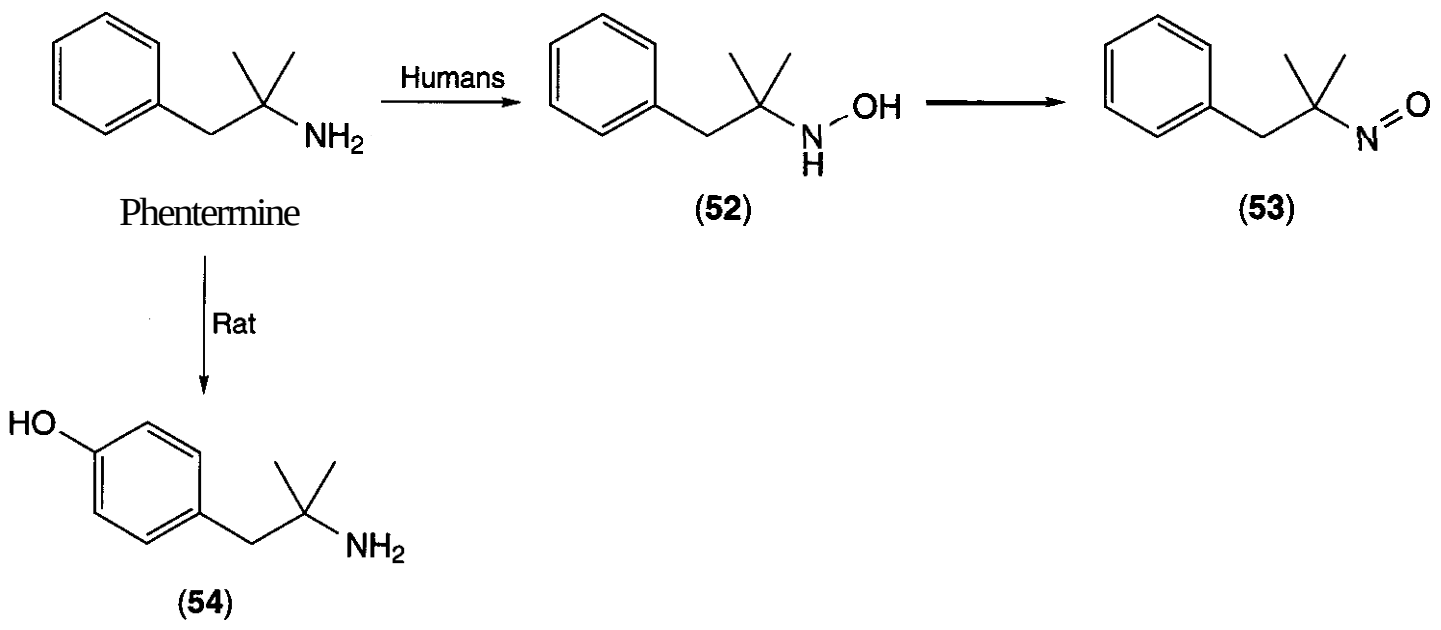


Figure 15.4. Biotransformation of phentermine in rats and humans.

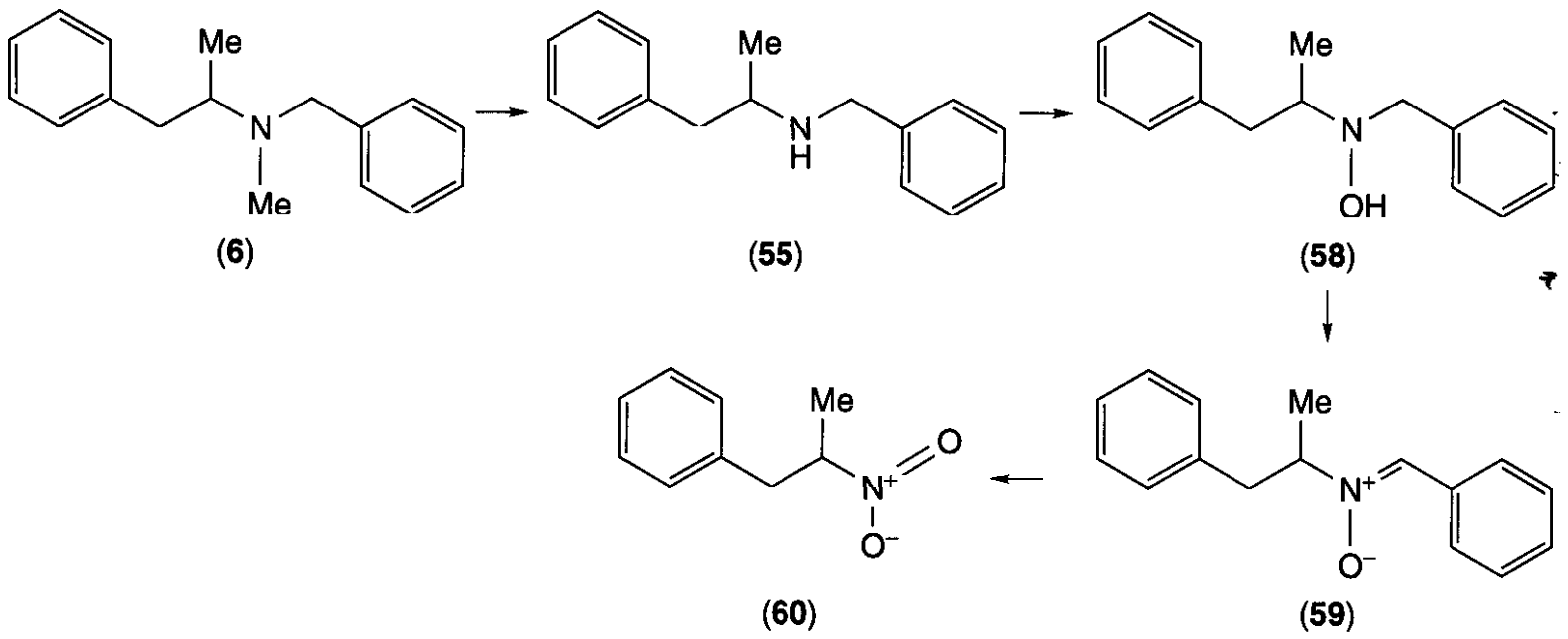


Figure 15.5. Metabolism of benzphetamine in activated rat liver microsomes.

moderately potent substrate for the NE transporter (IC_{50} = 99 n*M*) and an inhibitor of NE uptake (IC_{50} = 360 n*M*), in contrast to diethylpropion, which shows only weak activity in these assays (see Table 15.11) (80). Ethylpropion is further metabolized by P450 enzymes to the di-des-ethyl derivative (64) and benzoic acid (**50**), which exists in plasma as a glycine conjugate called hippuric acid (65) (81). A proposed pathway for the conversion of (64) to (**65**) is shown in Fig. 15.6. In one study, norephedrine analogs (66) and (67) were found in only neglible quantities in urine, suggesting reduction of the keto group is not a major route of elimination for compound (64) (see Table 15.11) (82). These norephedrine an-

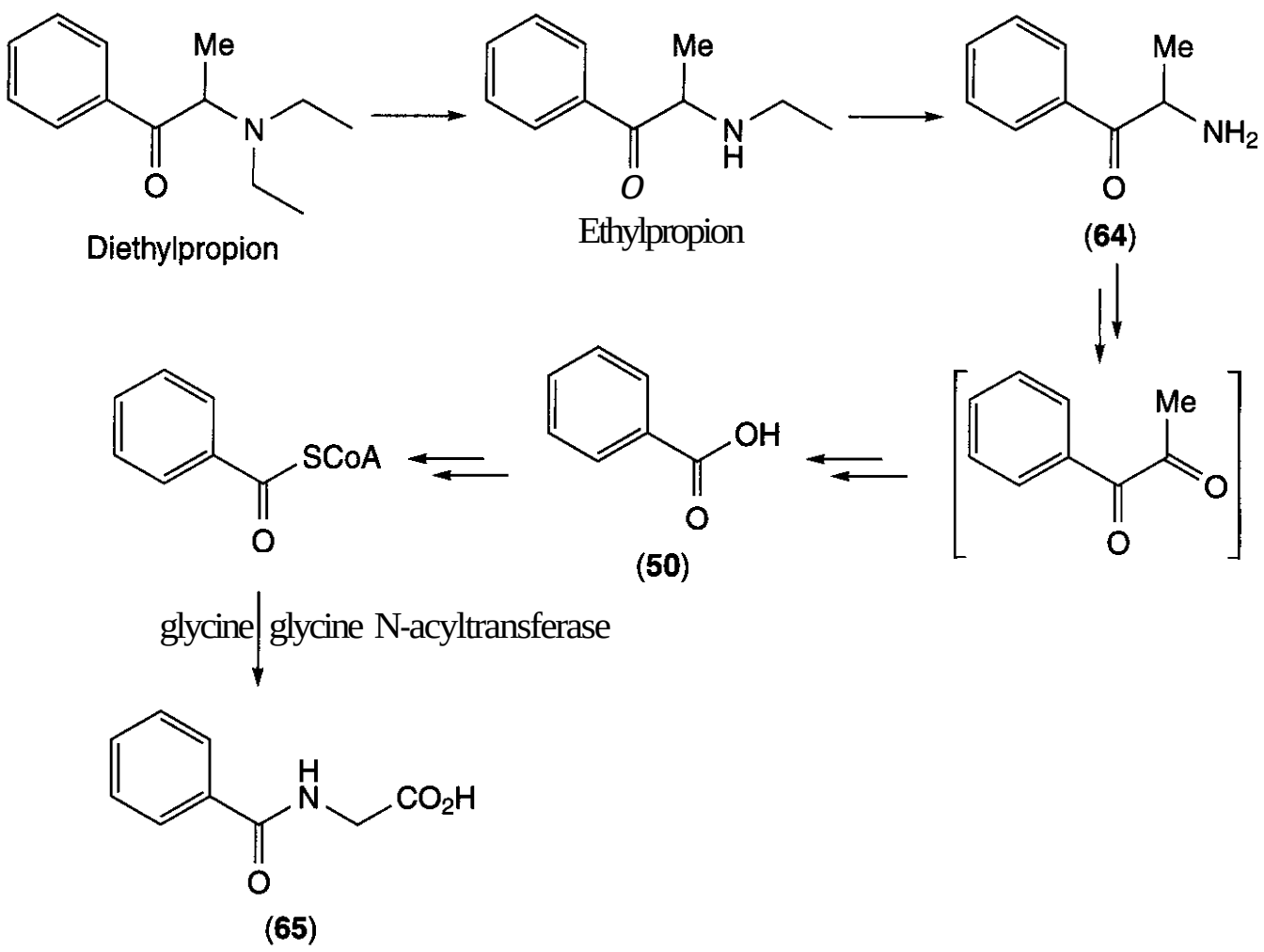
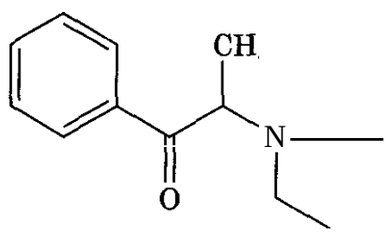
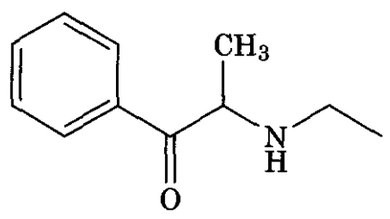
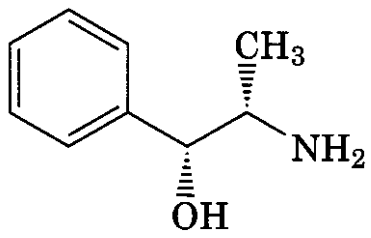


Figure **15.6.** Metabolism of diethylpropion to hippuric acid.

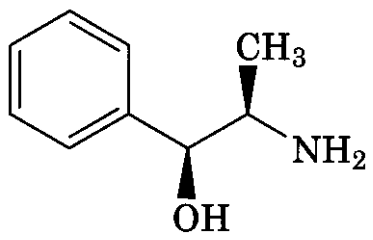
Table 15.11 Pharmacological Activities of Diethylpropion and Ethylpropion[a]

Compound	Structure	DA Uptake IC_{50} (n*M*)	DA Release IC_{50} (n*M*)	5-HT Uptake IC_{50} (n*M*)	5-HT Release IC_{50} (n*M*)	NE Uptake IC_{50} (n*M*)	NE Release IC_{50} (n*M*)
Diethylpropion (**4**)		>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Ethylpropion (**63**)		1014	>1000	3840	2118	360	99

[a]Ref. 80.



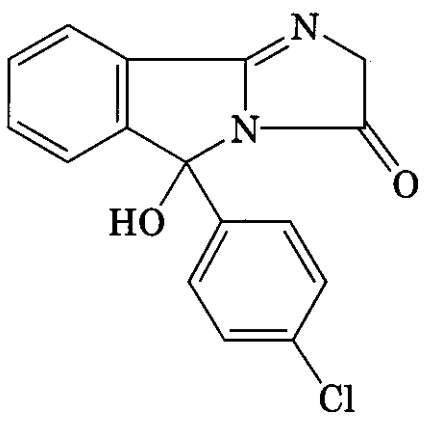
(66) (1R, 2S)



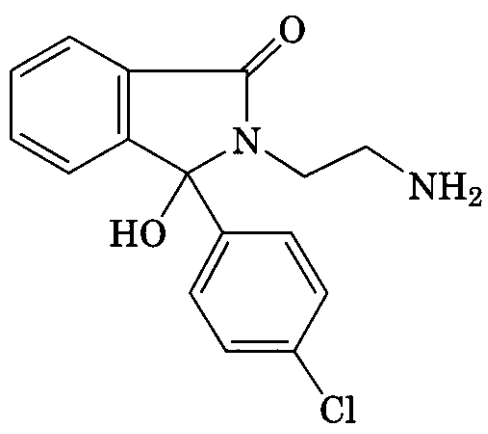
(67) (1S, 2R)

alogs do not inhibit DA, 5-HT, or NE uptake or stimulate DA, 5-HT, or NE release (80).

3.1.7 Mazindol. Mazindol exhibits a rapid onset of action and a long duration of action in humans, primarily because of its slow absorption and elimination (83, 84). The majority of drug is excreted unchanged in urine, although two metabolites, (68) and (**69**), have been identified that are formed by oxidation of the



(68)

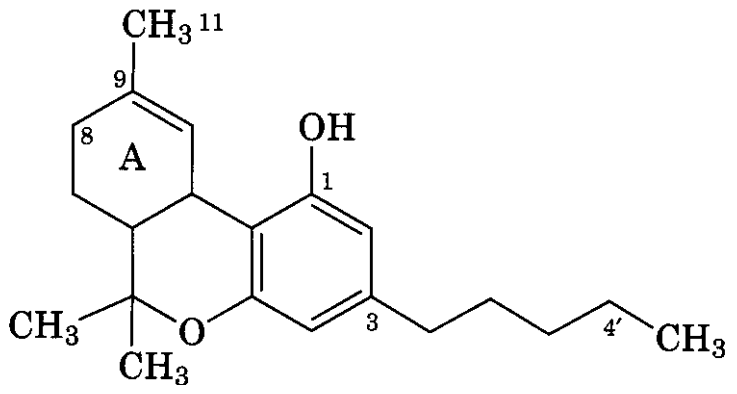


(69)

tricyclic heterocyclic template and hydrolysis of the imidazoline ring (85).

3.2 Metabolism of Appetite-Stimulant Drugs

Dronabinol (32) is an orally active **cannabinoid** derivative with low bioavailability (10–



(32) Dronabinol - Pyran Numbering System

20%), attributed in part to high first-pass metabolism (86). In humans, dronabinol undergoes allylic oxidation of the terpene ring, to give 11-hydroxy-Δ^9-tetrahydrocannabinol (**70**). This metabolite is oxidized by alcohol dehydrogenase enzymes in the liver to yield **11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic** acid (**71**), which occurs as a conjugate in plasma (see Fig. 15.7) (**87**). The C-3 pentyl chain in (71) undergoes additional oxidation, to give 4′-hydroxy-11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (**72**), which is degraded further to the dicarboxylic acid derivative (**73**). Several other metabolites (74, 75) are formed in small amounts through allylic oxidation of the C8 position on the dronabinol A ring, followed by oxidative carboxylation of the C-3 side chain (88). An interesting metabolite of A^S-THC is formed in mice through aromatization of the terpene ring, to give cannabinol (76) (see Fig. 15.8) (89). The biological activities of many of these metabolites have not been fully described in the literature.

Megestrol (30) is metabolized into three products in humans, as shown in Fig. 15.9, although the majority of drug is excreted unchanged. Megestrol undergoes allylic oxidation, hydroxylation adjacent to the carbonyl group on the A-ring, and conjugation, to give metabolites (**77**), (**78**), and (79) (90).

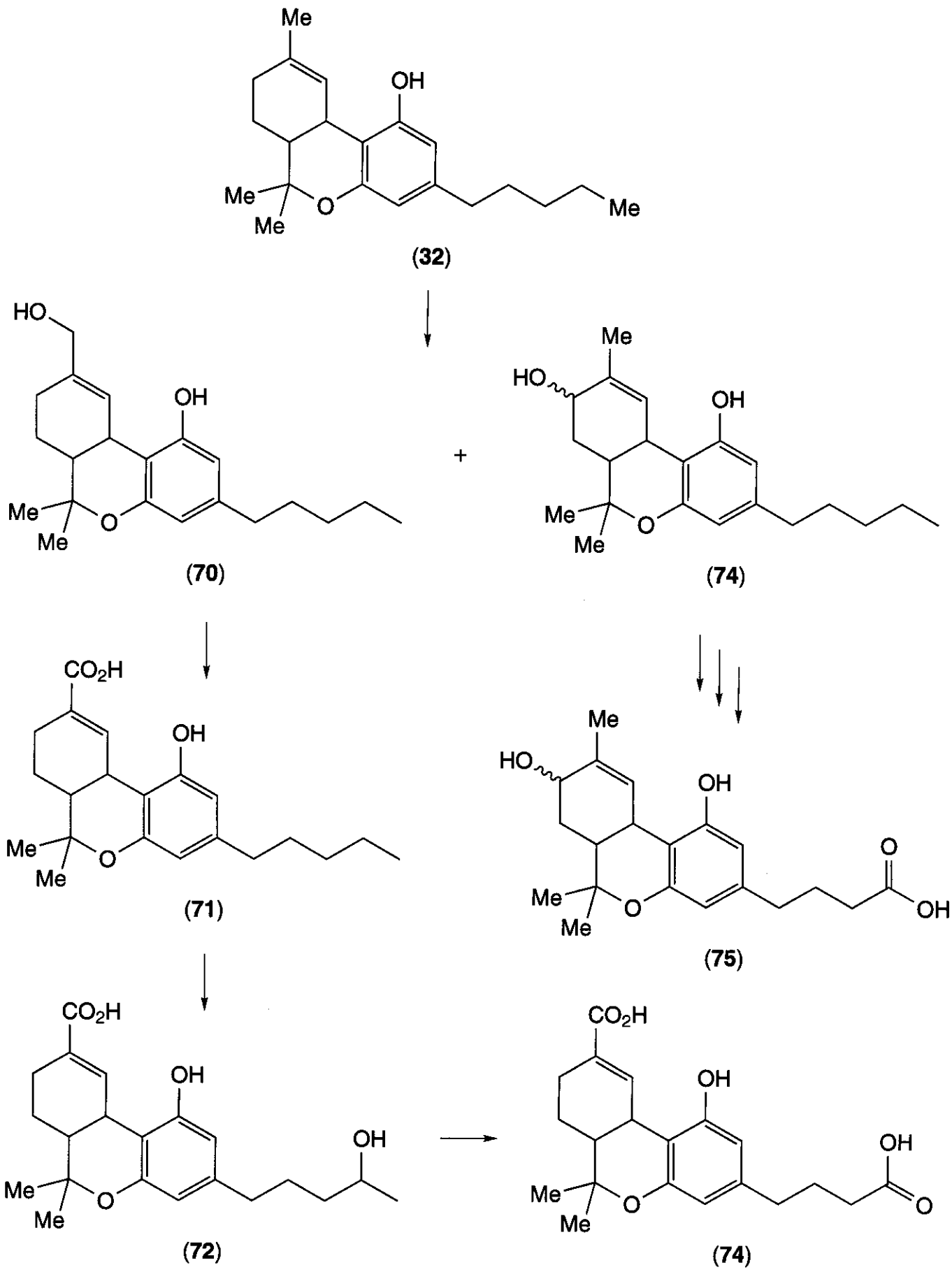


Figure 15.7. Metabolic degradation of dronabinol.

Oxandrolone (31)is rapidly absorbed into the systemic circulation after an oral dose. It undergoes oxidation on the cyclopentyl ring, to give a diol (80) called 16β-hydroxy-oxandrolone (see Fig. 15.10). The major plasma metabolites are glucuronide conjugates of both (31)and (80)(91).

4 PHYSIOLOGY AND PHARMACOLOGY

41 Body Weight

Body weight disorders, especially obesity, are complex physiological processes that result from disruptions to endogenous systems involved in the regulation and maintenance of

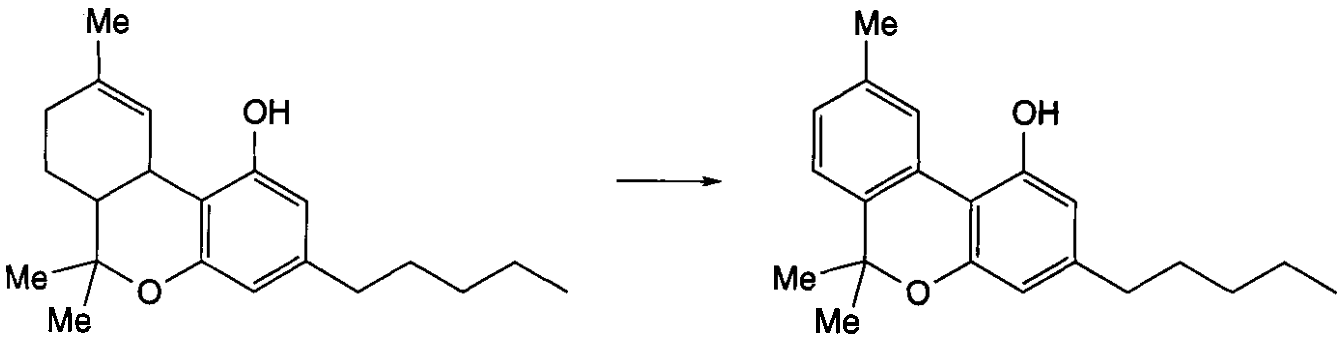
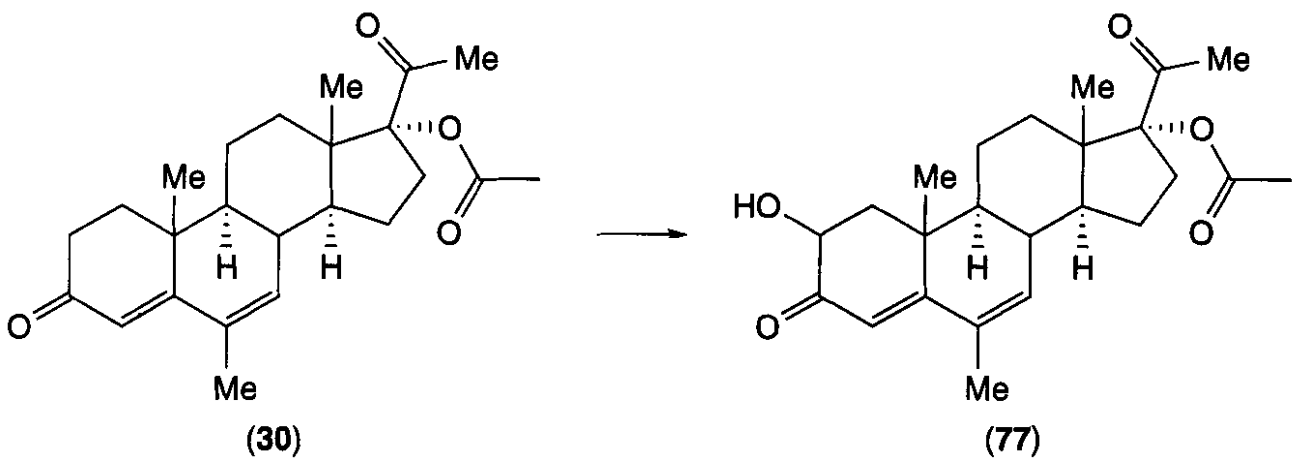


Figure 15.8. Conversion of dronabinol in mice to cannabinol.



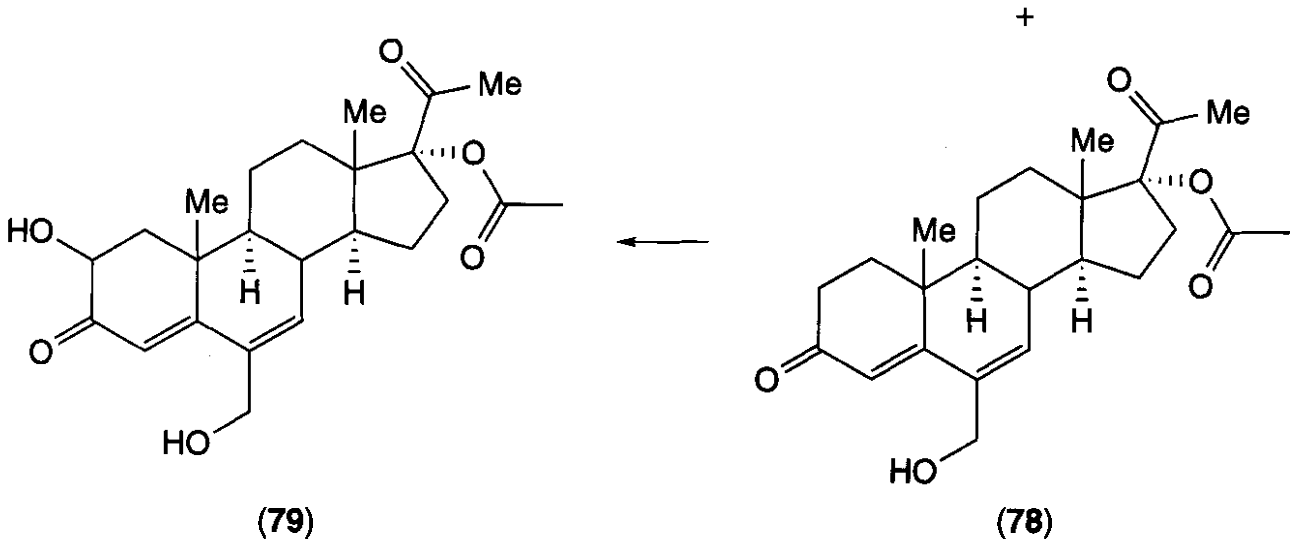


Figure 15.9. Proposed oxidative biotransformation of megestrol.

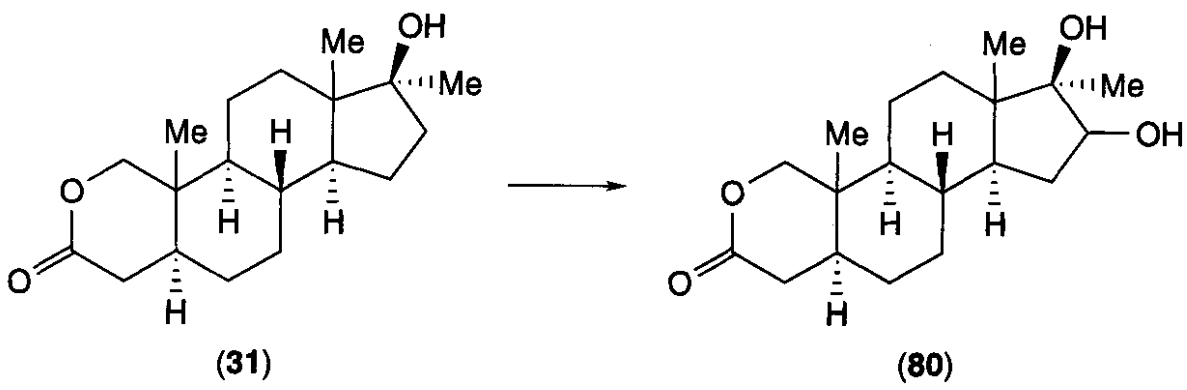


Figure 15.10. Metabolic hydroxylation of oxandrolone.

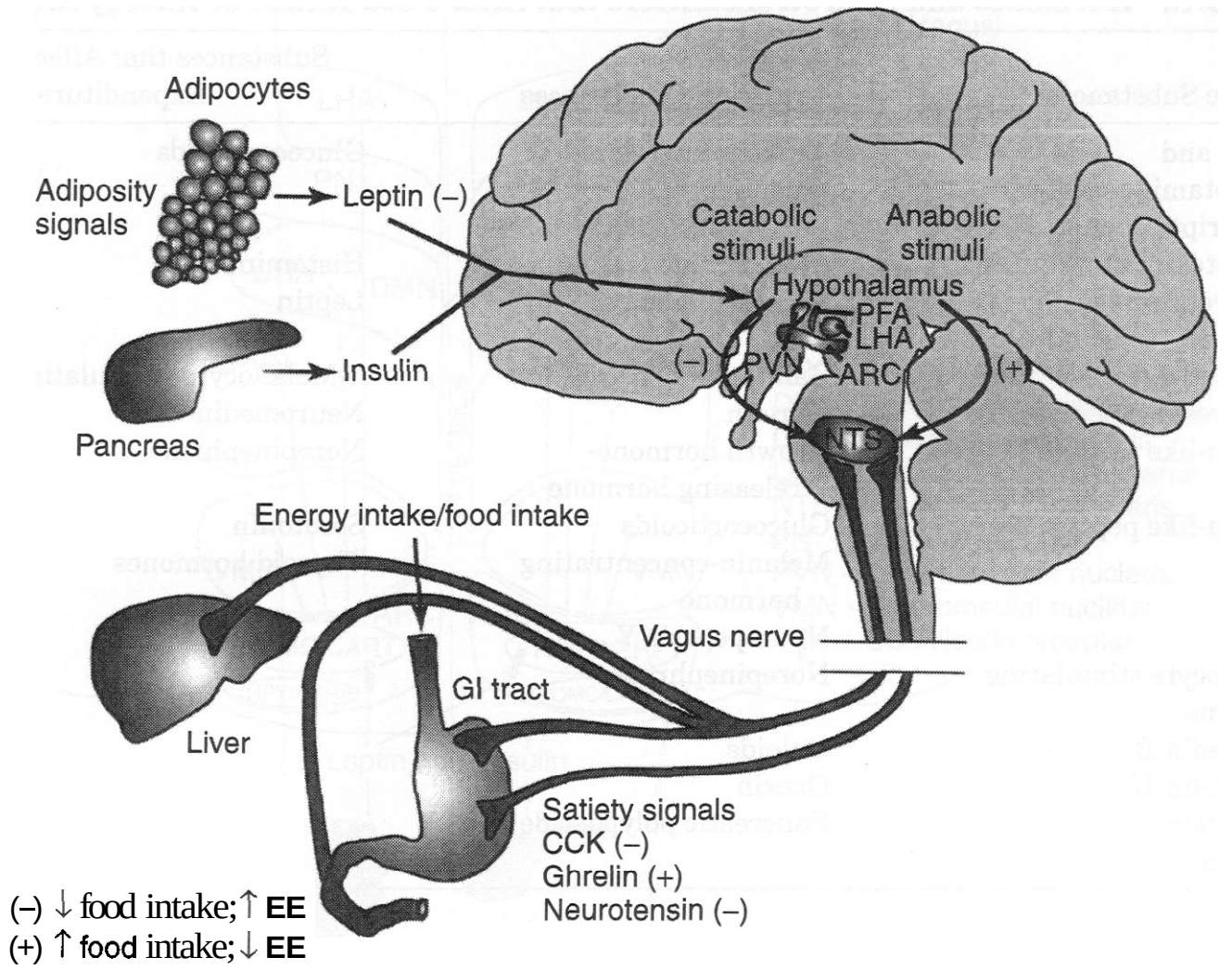


Figure 15.11. Pathways in the regulation of energy homeostasis.

energy homeostasis. Elucidation of the underlying mechanisms involved in obesity and related disorders has come primarily from receptor and hormone knockout mice and transgenic animal models (92, 93). Body weight is controlled through an integrated process that balances energy intake with energy expenditure. This system encompasses many overlapping interactions between adipose tissue, muscle, the adrenal glands, the GI tract, and the CNS (hypothalamus, nucleus tractus solitaris) (see Fig. 15.11). It also includes numerous satiety, anorectic, and orexigenic factors, several neurotransmitters (5-HT, NE, DA), and various circulating nutrients (glucose, lipids) (see Table 15.12). As a central integrator of these signals, the hypothalamus responds to the body's varied energy needs by shifting between anabolic and catabolic pathways, that is, stimulating food intake and increasing energy reserves or inhibiting food intake and decreasing body weight. The intricate mechanisms that control these processes can sometimes be disrupted. When energy input exceeds energy output for long periods of time, the excess calories are stored as adipose tissue, leading to obesity. Several recent reviews describe the regulation of body weight in extensive detail (94–96).

4.1.1 Neuroendocrine Regulation of Energy Homeostasis by the Hypothalamus. The hypothalamus, a collection of neuronal bodies located near the thalamus at the base of the brain, coordinates signals from the autonomic nervous system and regulates many bodily functions, including metabolism and temperature. The role of the hypothalamus in the control of energy balance was discovered through neuronal lesion studies: ablation of neurons located in the lateral hypothalamic area (LHA) was shown to cause leanness, whereas destruction of neurons in the ventromedial nucleus (VMN) was found to result in obesity (97). Several hypothalamic regions have now been shown to be involved in the regulation of energy homeostasis, including the LHA, VMN, and arcuate nucleus (ARC), located adjacent to the third ventricle. The ARC contains a discrete set of neurons that

Table 15.12 Hormones and Neurotransmitters that Alter Food Intake or Energy Expenditure

Anorectic Substances	Orexigenic Substances	Substances that Affect Energy Expenditure
Cocaine- and amphetamine-related transcript product	Agouti gene-related peptide	Glucocorticoids
Cholecystokinin	Anandamide	Histamine
Corticotropin-releasing hormone	γ-Butyric acid	Leptin
Enterostatin	Galanin	α-Melanocyte–stimulating hormone
Gastrin release peptide	Ghrelin	Neuromedin U
Glucagon-like peptide 1	Growth hormone-releasing hormone	Norepinephrine
Glucagon-like peptide 2	Glucocorticoids	Serotonin
Insulin	Melanin-concentrating hormone	Thyroid hormones
Leptin	Neuropeptide Y	
α-Melanocyte stimulating hormone	Norepinephrine	
Neuromedin B	Opioids	
Neuromedin U	Orexin	
Neurotensin	Pancreatic polypeptide	
Serotonin		

coexpress two orexigenic hormones, called *agouti* gene-related peptide (AGRP) and neuropeptide Y (NPY), and another grouping of neurons that coexpress anorectic hormones such as a-melanocyte-stimulating hormone (a-MSH) and peptidic hormone derived from cocaine and amphetamine-related transcript (CART) (see Fig. 15.12). a-MSH is a proopiomelanocortin (POMC)-derived peptide in the melanocortin (MC) family. Neurons in the ARC project to other sites within the hypothalamus, including the dorsomedial nuclei (DMN), paraventricular nucleus (PVN), perifornical area (PFA), and LHA, and to other regions of the brain. The hypothalamic pathways involved in food intake and energy homeostasis have been reviewed (98–100)

Several hypothalamic regions outside the ARC also synthesize neuropeptides involved in the regulation of energy homeostasis or contain receptors that respond to ARC hormones (see Figs. 15.13 and 15.14). The PVN contains multiple subtypes of receptors for NPY and POMC-derived peptides such as a-MSH. Neurons in the PVN synthesize and release corticotrophin-releasing hormone (CRH), an important central hormone that is involved in the regulation of many endocrine, immune, and stress responses. Both the PVN and the VMH contain mRNA for a CRH receptor subtype, termed CRH_2-R, that is involved in decreasing food intake and increasing energy expenditure in rats (101). Neurons in the LHA secrete an orexigenic hormone called melanin-concentrating hormone (MCH) that acts at specific receptors in the PVN. A newly discovered 33 amino acid neuropeptide, called orexin A, is synthesized in the PFA and LHA and may mediate its orexigenic activities through receptor subtypes found in the PVN and the VMN. More complete descriptions of the anatomical distribution of hypothalamic neurons that secrete neuropeptides and their receptors have been described elsewhere (97, 100).

A functional melanocortinergic system in the hypothalamus is essential for maintaining normal body weight. This system consists of two opposing hormones, AGRP and a-MSH, and two MC receptor subtypes, MC_3-R and MC_4-R (see Fig. 15.12). The neuropeptide a-MSH, an endogenous agonist for both MC_3-R and MC_4-R, decreases food intake and increases energy expenditure in rodents. AGRP, an inverse agonist of MC_3-R and MC_4-R, stimulates food intake by antagoniz-

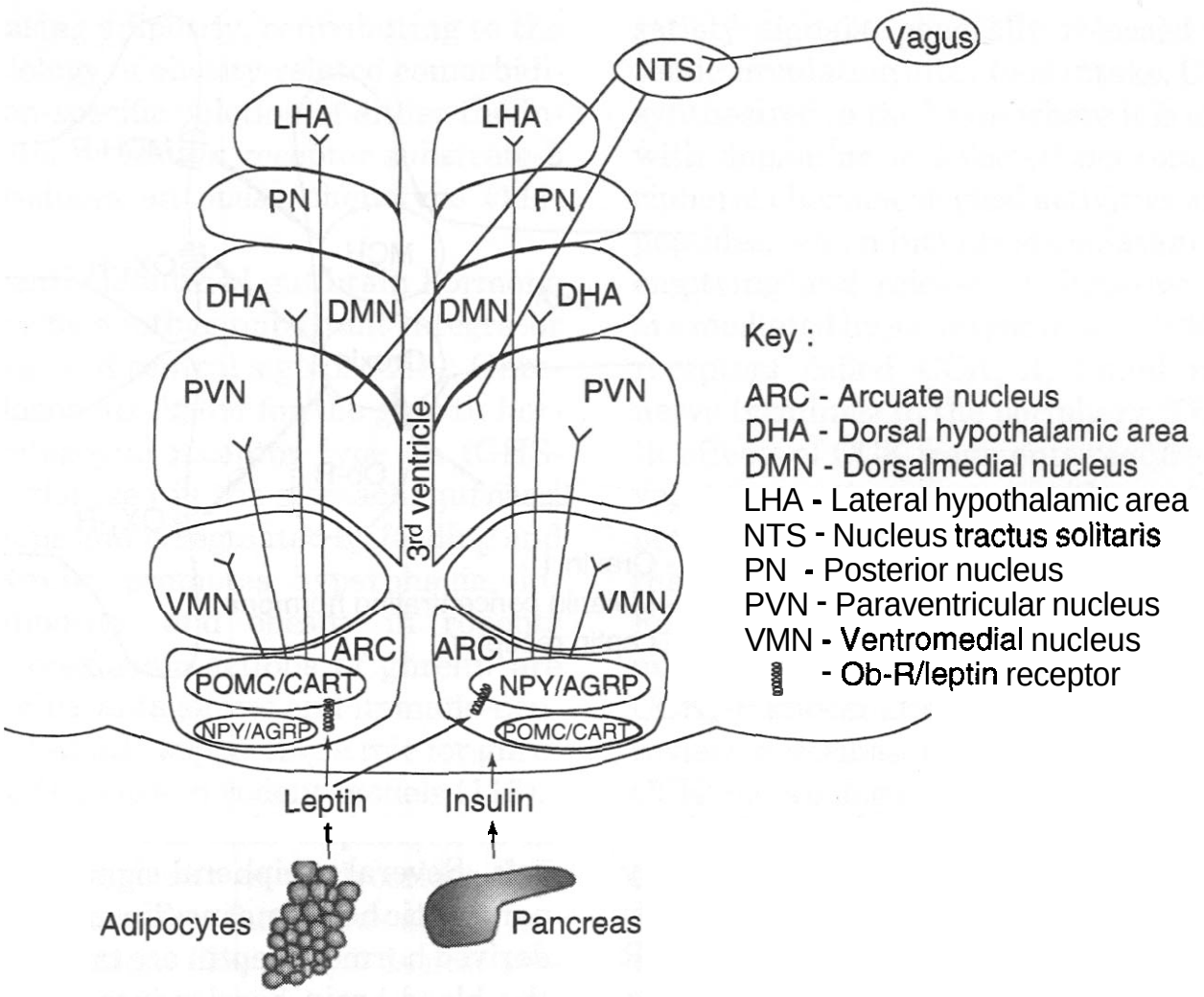


Figure 15.12. Projections of ARC neurons to other hypothalamic regions.

ing the actions of a-MSH (102). AGRP transgenic animals, POMC gene KO, MC_3-R KO, and MC_4-R KO mice are all obese. Interestingly, the obesity observed in the POMC knockout mice can be reversed by peripheral administration of a low dose of a-MSH. The obese phenotypes in MC_3-R and MC_4-R KO mice are caused by distinct mechanisms. The

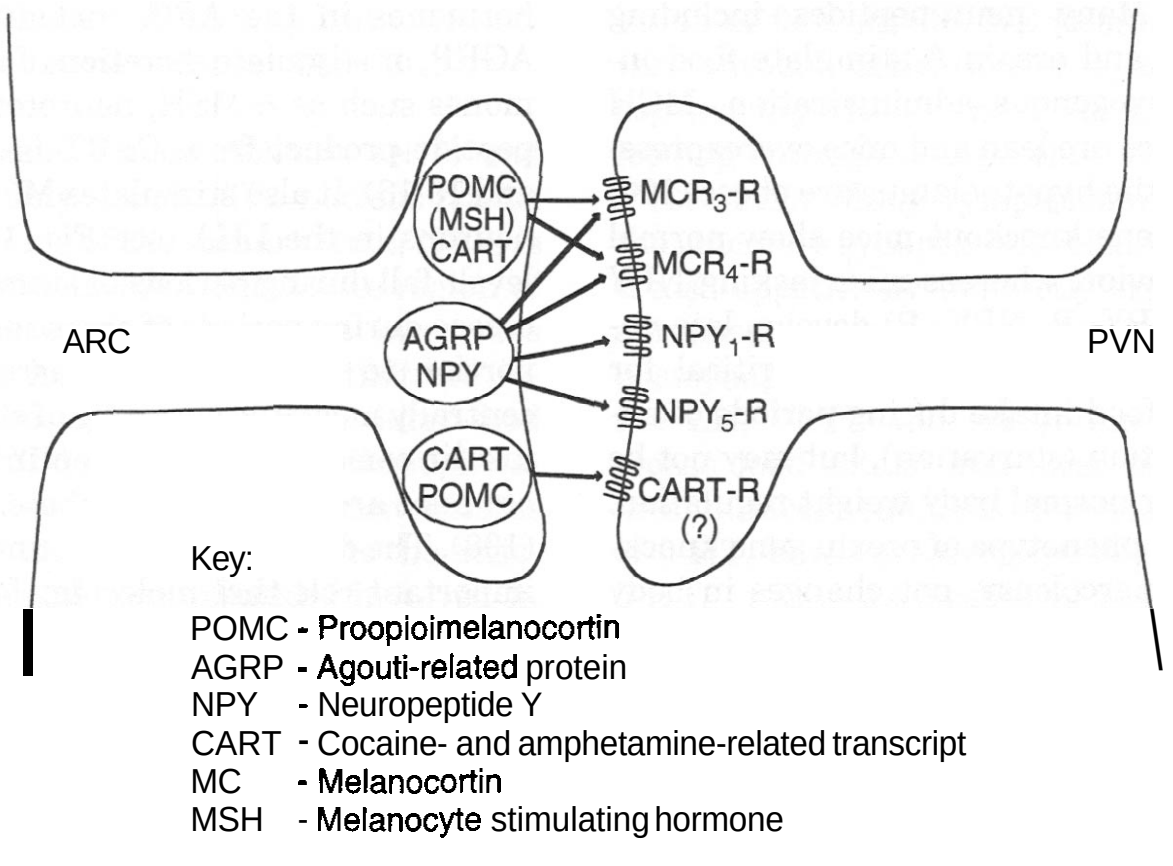


Figure 15.13. ARC and PVN neuron innervations.

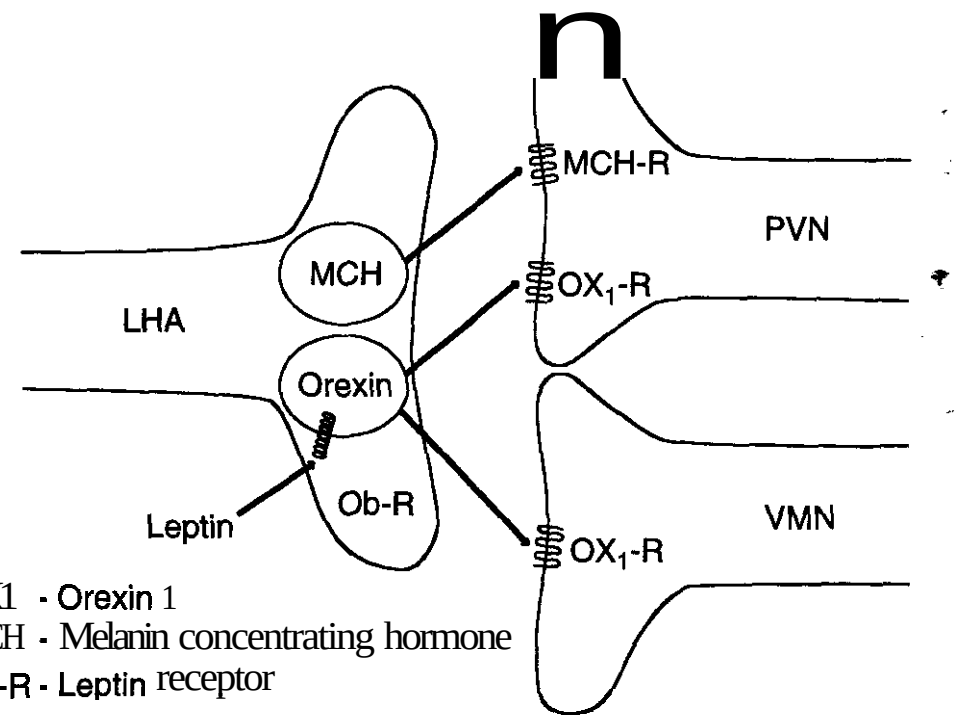


Figure 15.14. LHA neuronal projections.

morbid obesity in MC_4-R knockout mice is attributed to hyperphagia, whereas the obesity associated with MC_3-R knockout mice is attributed to metabolic deficiencies. MC_3-R knockout mice have decreased lean body mass in conjunction with increased adipose mass; however, they are not hyperphagic (103). AGRP transgenic mice are similar to MC_4-R KO mice.

The functional importance of other hypothalamic neuropeptide systems in the physiological regulation of body weight in humans is less clear. Many neuropeptides including MCH. NPY. and orexin A stimulate food intake upon exogenous administration. MCH knockout mice are lean and mice overexpressing MCH in the hypothalamus are obese (104, 105). NPY gene knockout mice show normal feeding behavior, whereas mice lacking NPY receptors (NPY_1-R, NPY_5-R) develop late-onset obesity. NPY appears to be critical for stimulating food intake during periods of energy deprivation (starvation), but may not be necessary for normal body weight regulation. The primary phenotype of orexin gene knockout mice is narcolepsy, not changes in body weight. However, icv administration of antibodies to orexin A or antagonists to the OX,-R decrease food intake (106, 107). Studies with selective peptide or nonpeptide pharmacological probes are necessary to fully decipher the complex roles of these neuropeptides and their receptors.

4.1.2 Peripheral/Central Adiposity Signals. Several peripheral signals including the pancreatic hormone insulin and the adipocyte-derived hormone leptin are transported across the blood-brain barrier into the arcuate nucleus and play important roles in the regulation of energy balance. Leptin provides a critical connection between energy reserves (adipose tissue) and the brain (108). Leptin mediates its effects through specific receptors (Ob-R) on ARC neurons (109) that either inhibit synthesis/secretion of orexigenic neurohormones in the ARC, including NPY and AGRP. or stimulate secretion of anorectic hormones such as a-MSH, neurotensin, and the peptide product from CART (see Figs. 15.12 and 15.13). It also stimulates MCH and orexin neurons in the LHA (see Fig. 15.14). Leptin levels fall during periods of starvation and increase during periods of excess energy, in proportion to adiposity. Any disruption in the centrally mediated signaling of either leptin or insulin can cause obesity. Leptin-deficient (*ob*/ob) mice are hyperphagic, obese, and diabetic (108). The discovery of leptin underscored the important role that molecular factors play in regulation of body weight homeostasis and set off the search for other candidate genes.

The pancreatic hormone insulin is primarily involved in the regulation of glucose homeostasis. However, insulin inhibits food intake when administered directly into the brain (110). Furthermore, insulin levels rise

with increasing adiposity, contributing to the pathophysiology of obesity-related comorbidities. Neuron-specific deletion of either the insulin receptor or insulin receptor substrate-2 in mice produces an obese phenotype (111, 112).

The recently identified gut/brain hormone ghrelin may be another important integrator of peripheral and central signals (113). Ghrelin, an endogenous ligand for the growth hormone secretagogue receptor type 1a (GHS-R1a), is synthesized in the stomach, gut, and hypothalamus and is regulated by feeding and fasting. Ghrelin promotes hyperphagia, increased adiposity, and obesity in rodents (114). The orexigenic actions of ghrelin are blocked by NPY antagonists and immuno-neutraliiation of AGRP, supporting a role for ghrelin in energy balance in rodent models (115).

Adipose tissue was once considered to be primarily a storage site of triglycerides that could be released as free fatty acids upon sympathetic stimulation. However, it is now recognized that adipose tissue is an important endocrine organ that secretes many hormones and cytokines that are involved in energy homeostasis, including leptin and other factors such as angiotensinogen, resistin, adipsin, ACRP30, tumor necrosis factor a, IL-6, and plasminogen activator inhibitor 1. The roles of these adipogenic hormones in the regulation of energy balance and carbohydrate metabolism have been reviewed elsewhere (116).

4.1.3 Nucleus Tractus Solitaris (NTS) and Peripheral Satiety Signals. The nucleus tractis solitaris (nucleus of the solitary tract, NTS) is a collection of neurons found in the region of the brain stem (hindbrain) that integrates many signals from the GI tract that are released into the circulatory system after food intake. The NTS receives signals through vagal nerve afferents and then communicates with the hypothalamus to modulate the activities of important neuropeptides involved in stimulating or inhibiting food intake. The NTS is also activated by the neurotransmitters NE and 5-HT, which can stimulate satiety mechanisms and increase energy expenditure.

Cholecystokinin (CCK) and related peptide fragments such as CCK-33 and CCK-8 are a class of gut peptides that act as short-term satiety signals, generally released into systemic circulation after food intake. CCK is also synthesized in the brain where it is colocalized with dopamine in selected neurons. The peripheral pharmacological activities of the CCK peptides, which include stimulation of gastric emptying and release of digestive enzymes, are mediated by a subtype of the CCK family of receptors called CCK_a-R, found mainly in nerve terminals in the periphery. The anorectic effects of CCK fragments require an intact vagal nerve that serves as the main conduit between the stomach and the brain, primarily the NTS. Otsuka Long-Evans Tokushima Fatty (OLETF) rats lack CCK_a-R and are hyperphagic and obese (117). Interestingly, CCK_a-R knockout mice are not obese. A recent review describes the biological activities of CCK and analogs (118).

Several other gut peptides such as enterostatin or gastrin-releasing-peptide (and other bombesin-like peptides) are also involved in initiating satiation responses after food intake. The roles of these peptides in regulating food intake have been reviewed elsewhere (34).

4.1.4 Adrenergic and Serotonergic Regulation of Food Intake. Both the hypothalamus and NTS/brain stem are innervated with NE and 5-HT fibers that are involved in the regulation of food intake. NE stimulates food intake through activation of a,-adrenergic receptors (α_2-AR) in the PVN and decreases feeding through α_1-ARs that are also in the PVN (119). Many sympathomimetic agents that increase synaptic NE release actually decrease appetite by indirectly stimulating a_1-adrenoceptor pathways. Serotonin acts on postsynaptic 5-HT_{1b} and 5-HT,, receptors in the LH, VMH, and NTS/brain stem, to decrease food intake and alter meal size and meal number (120, 121). The anorectic effects of 5-HT, however, are primarily mediated by 5-HT,, receptors located in the brain stem. For example, fourth ventricle administration of small molecule 5-HT,, receptor agonists is sufficient to mediate all the anorectic effects of such agents (122). Synaptic levels of 5-HT are controlled by a variety of signals, including such peripheral satiety factors as CCK. Activation of hypothalamic 5-HT receptors results

in a downregulation of NPY secretion (121). Serotonergic pathways involved in food intake appear to operate independently of leptinergic pathways.

4.1.5 Genetics. Several examples of monogenic single-nucleotide polymorphisms (SNPs) or mutations in human genes have been identified that lead to obesity or a predisposition to an obese phenotype. The most extensively characterized SNP is the $TRP_{64} \rightarrow ARG_{64}$ mutation in the human &-adrenergic receptor, which has been associated with an increased prevalence of obesity or diabetes in certain populations (123). It is not clear whether there are actually functional differences between the two isoforms (124, 125). A SNP in the CCK_a-R promoter region is associated with increased fat mass in humans (126). Mutations that inactivate the gene encoding the melanocortin-4 receptor subtype are found in approximately 5% of all morbidly obese humans and represent one of the commonest known monogenic causes of human obesity (127).

Several other monogenic mutations are found in humans but are much less common. These include the leptin, leptin receptor, POMC, and prohormone convertase I genes. Patients with Prada-Willi syndrome also have a gene deletion.

4.2 Eating Disorders/Cachexia

Although signals involved in the control of food intake are well known, the pathophysiology of anorexia nervosa and other wasting diseases such as drug or disease-mediated cachexia is less understood. Anorexia nervosa, binge eating disorder, and bulimia nervosa are in part eating, body weight, and psychiatric diseases. In all these conditions, symptoms of depression, obsessive compulsive behavior, and anxiety are common. These psychiatric symptoms are generally associated with decreased central serotonergic activity. In anorexia nervosa, for example, cerebrospinal fluid (CSF) levels of a metabolite of 5-HT called 5-hydroxyindoleacetic acid are low, but return to normal levels after weight regain (25). Several neurotransmitters, neuropeptides, and peripheral hormones are also known to be up- or downregulated in eating disorders, although these effects appear to be secondary to the pathogenesis of the diseases. In anorexia nervosa, for example, plasma levels of stress hormones such as ACTH and cortisol are elevated compared to controls, suggesting increased hypothalamic levels of CRH, a catabolic hormone (128). Plasma leptin levels are also decreased, although this is probably related to depletion of adipose tissue, whereas NPY and ghrelin levels are increased, probably as an adaptive response to decreased food intake (129, 130). In bulimia nervosa, CCK release is blunted because of delayed gastric emptying caused by stomach enlargement (131).

Cachexia may be driven by the increases in various cytokines that are observed in many wasting disorders (132). Cytokines such as IL-1 and IL-6 can promote negative nitrogen balance and protein wasting by modulating the activities of various hypothalamic neuropeptides involved in the regulation of energy homeostasis, including NPY. These cytokines can also increase leptin synthesis in adipose tissue and stimulate downstream leptinergic pathways in the CNS, thus leading to severe anorexia (133). Recent evidence suggests that elevated 5-HT levels may play an important role in the anorexia of certain cancers and other wasting disorders (134).

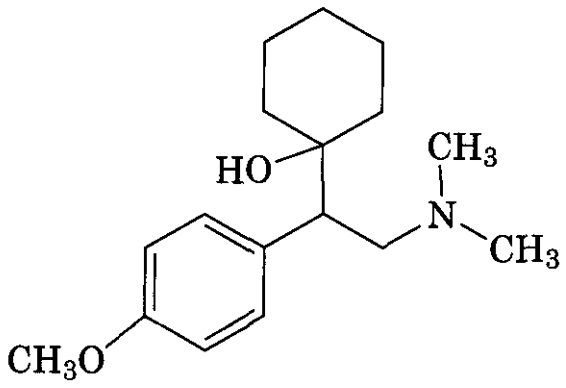
5 HISTORY

5.1 Discovery of Sibutramine

The anorectic effects of sibutramine, one of only two weight-loss drugs currently approved for long-term use, were serendipitously discovered during clinical trials. The compound was originally developed as an antidepressant medication because of its potent NE and 5-HT reuptake inhibitory activities. However, the drug failed to show any efficacy in Phase II human trials, despite demonstrating good activity in animal models of depression. Anorexia was observed as a side effect in many of the depressed patients, which then led to a change in the clinical program (135). The anorectic effects of sibutramine were later confirmed in animal models after both single and chronic dosing (136,137). The decrease in food intake was shown to occur through both NE and 5-HT pathways, given that it was partially

blocked by administration of centrally active serotonin receptor antagonists (metergoline), a,-receptor antagonists (prazosin), and &-receptor antagonists (metoprolol) (138).

Sibutramine failed to show antidepressant activity in humans, but was found to be a potent anorectic agent. A structurally distinct compound, venlafaxine (**81**), with similar *in vitro*



(81) Venlafaxine

pharmacology at both the NE and 5-HT reuptake transporters, proved to be an efficacious antidepressant drug, without displaying significant anorectic activity in humans. Venlafaxine has been shown to inhibit food intake in rodent models, but only at very high doses. The reasons for the disparities in the pharmacological activities between the two structurally different compounds are not yet fully known.

6 STRUCTURE-ACTIVITY RELATIONSHIPS

Structure-activity relationships (SARs) for several drugs used in the treatment of body weight disorders, including some 2-phenethylamine derivatives and dronabinol, have been described in the literature. For other drugs such as sibutramine and diethypropion, the only close-in compounds for which biological data exist are metabolites of the parent drug (see Section 3). Analogs of sibutramine are described in the patent literature, but no data are provided (139). The SARs of antidepressant medications have been described elsewhere in this series.

6.1 SAR of 2-Phenethylamines

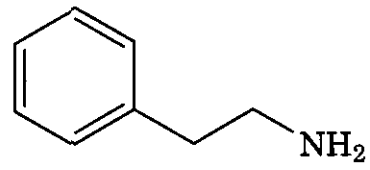
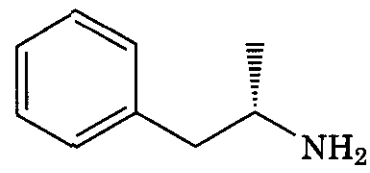
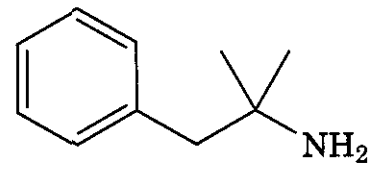
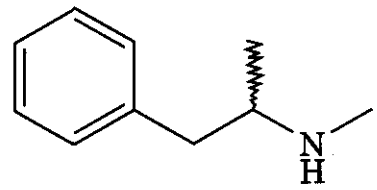
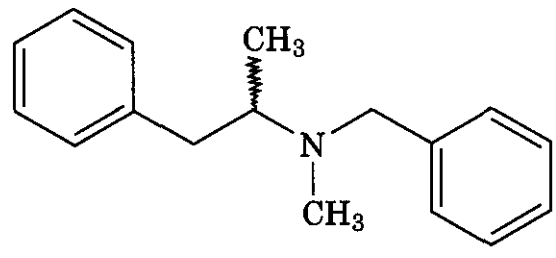
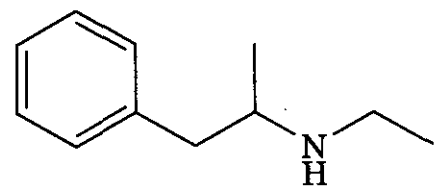
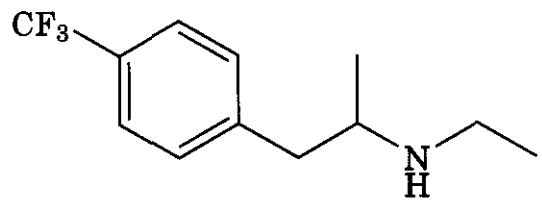
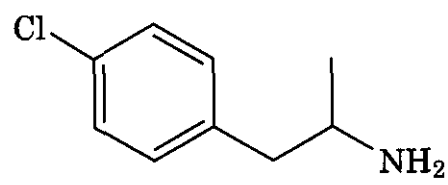
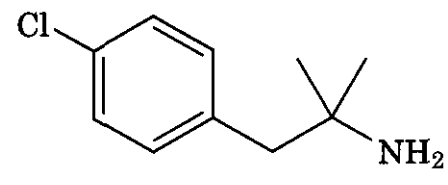
Appetite suppressants such as amphetamine, fenfluramine, and sibutramine stimulate 5-HT, NE, and/or DA release or inhibit their reuptake by membrane transporters. Such compounds all contain a 2-phenethylamine moiety, in which a basic amino group is separated from a lipophilic aryl ring by an alkyl spacer consisting of two carbon atoms. The unsubstituted derivative, 2-phenethylamine, shows weak sympathomimetic and serotonergic activity and exhibits no CNS exposure because of its rapid metabolism by MAOs. Introduction of one or two alkyl substituents at the C1 position adjacent to the amino group decreases metabolism by MAOs and enhances CNS *exposure* (140). Both amphetamine **(38)** and phentermine (3), which differ from 2-phenethylamine by the addition of one or two methyl groups, exhibit *good* CNS exposure.

The structure/activity relationships of 2-phenethylamines on serotonin uptake inhibitory activity have been investigated using platelet-rich plasma from human volunteers (141). The uptake activity of amphetamine is enhanced by alkyl substituents on the amino group and by the incorporation of methyl substituents adjacent to the nitrogen atom (see Table 15.13). Tertiary amines such as sibutramine exhibit weak 5-HT uptake activity. Introduction of lipophilic electronegative substituents (i.e., Cl, CF_3) on the aryl ring results in a significant increase in activity, whereas introduction of polar groups (i.e., OH) leads to a decrease in activity. The dextrorotatory isomers are more active than the corresponding levorotatory isomers.

Hansch and Caldwell have analyzed the quantitative structure/activity relationships (QSAR) of a series of amphetamine and 2-phenethylamine analogs, to discern the role of steric and hydrophobic aryl substituents on the inhibition of 5-HT uptake (142). From the biological data of 19 compounds, including those in Table 15.13. and some additional analogs, the following equation was derived for inhibition of uptake activity, where C is the IC_{50} concentration, MR_4 is the molar refractivity value of the aryl substituent scaled by 0.1, and π_3 is the hydrophobicity of the *meta* substituent on the aryl ring:

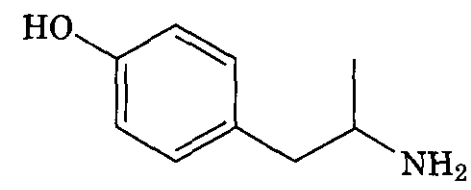
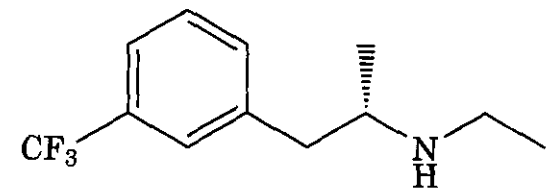
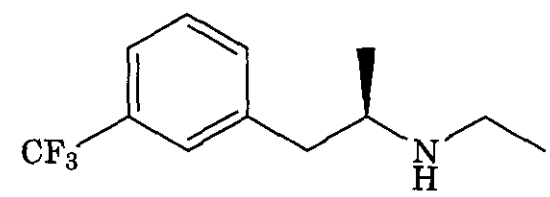
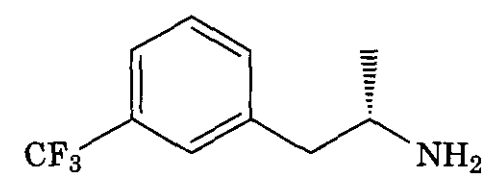
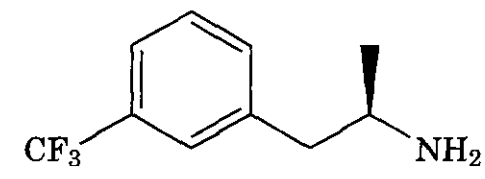
$$\text{Log } 1/C = 2.76(\pm 0.55)\text{MR}_4 + 0.89(\pm 0.32)\pi_3 + 0.48(\pm 0.26)I + 3.49(\pm 0.23) \quad (16.1)$$

Table 15.13 **Inhibition of Human Platelet 5-HT Uptake by 2-Phenethylamine Derivatives**[a]

Compound	Structure	5-HT Uptake Inhibition IC_{50} ($\times 10^{-5}/M$)
2-Phenethylamine (**37**)		28
(+)-Amphetamine (**11**)		5.51
(−)-Amphetamine(**82**)		28
Phentermine (**3**)		13.52
(±)-Methamphetamine (**57**)		5.41
(±)-Benzphetamine (**6**)		5.90
(+)-*N*-Ethylamphetamine (**83**)		1.66
(±)-*p*-Fenfluramine (**84**)		0.23
(±)-4-Chloromethylamphetamine (**85**)		0.32
Chlorphentermine (**86**)		0.26

An indicator variable **I** was added to the above equation to differentiate substituted 2-phenethylamine derivatives such as amphetamine from unsubstituted 2-phenethylamine compounds. The correlation coefficient (r) for the equation was high (r = 0.965), whereas s, the standard deviation, was low (s = 0.219). The data suggest that lipophilic substituents at the ***meta*** position on the **aryl** ring in phenethylamine analogs fit into an important hydrophobic pocket in the **5-HT** transporter and that the introduction of such groups should enhance the inhibitory activity.

Table 15.13 *(Continued)*

Compound	Structure	5-HT Uptake Inhibition IC_{50} ($\times 10^{-5}/M$)
(±)-4-Hydroxyampetamine (**87**)		1.98
(+)-Fenfluramine (**14**)		0.41
(−)-Fenfluramine (**88**)		1.3
(+)-Nor-fenfluramine (**89**)		0.7
(−)-Nor-fenfluramine (**90**)		3.19

"Ref. *141.*

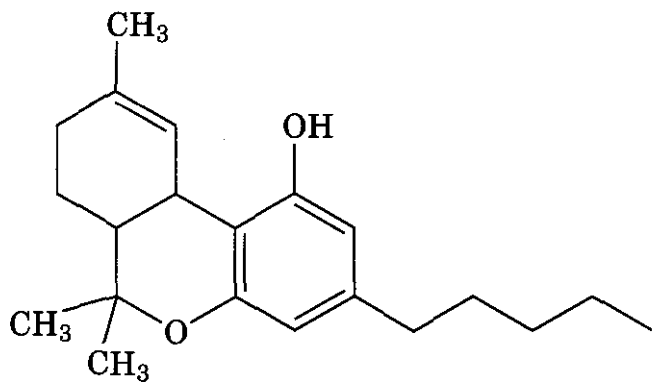
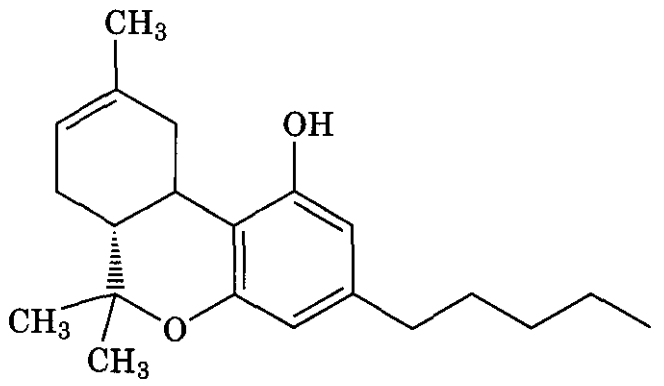
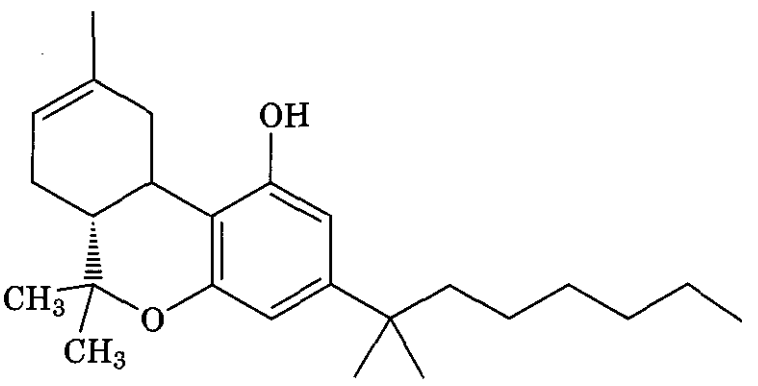
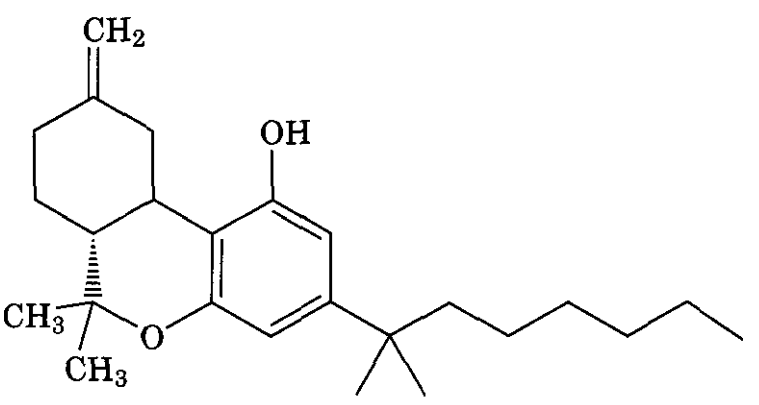
The SAR for norepinephrine uptake inhibition by amphetamine analogs is similar to that for inhibition of 5-HT reuptake. The protypical unsubstituted derivative, 2-phenethylamine, is a weak uptake inhibitor in isolated rat heart membranes (ID_{50} = 1.1 μM) (143). Introduction of a methyl group at the C1 position adjacent to the amino group results in a 10-fold increase in potency (i.e., dexamphetamine, (**8**), ID_{50} = 0.18 μM) (143). Sibutramine, a tertiary amine, shows moderate NE uptake activity (K_i = 350 nM), but its desmethyl and di-desmethyl metabolites, (*R*)-BTS 54 354 (**44**) and (*R*)-BTS 54 505 (**46**), exhibit potent activity (K_i values <20 nM) (see Table 15.10) (71).

The SAR for NE and 5-HT releasing activity of amphetamine derivatives is less well defined (144). NE release is increased by the presence of hydroxyl groups on the aryl ring and decreased by substitution with halogen or CF, groups. Phenolic and catechol derivatives of 2-phenethylamine, however, generally undergo rapid glucuronidation and thus show poor CNS exposure. Substitution of the terminal amino group with one alkyl group decreases NE activity and substitution with two alkyl groups essentially eliminates activity (see Table 15.11). In general, as the size of the alkyl group on the basic nitrogen group increases, the indirect syrnpathomimetic activity decreases. Serotonin release, in contrast, is generally enhanced by electron-withdrawing substituents on the phenyl ring (see Table 15.9). Thus, fenfluramine shows no NE releasing activity but is a potent 5-HT releasing agent. Likewise, amphetamine and phentermine do not stimulate 5-HT release but show weak to moderate activity for stimulating NE release. Sibutramine has little effect on either NE or 5-HT release.

6.2 SAR of Dronabinol Analogs

The structure-activity relationships of dronabinol (32) on cannabinoid receptor activity have been investigated (see Table 15.14). Both the phenolic hydroxyl group and the C3 alkyl chain in (32) are critical for binding affinity.

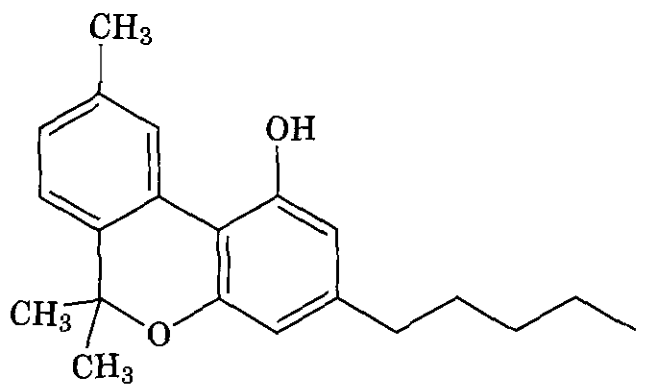
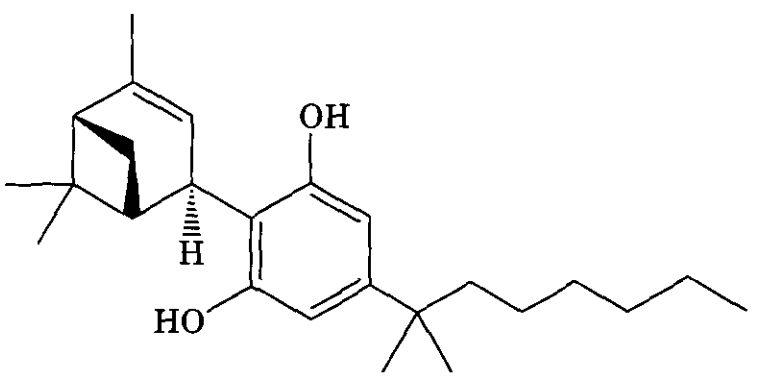
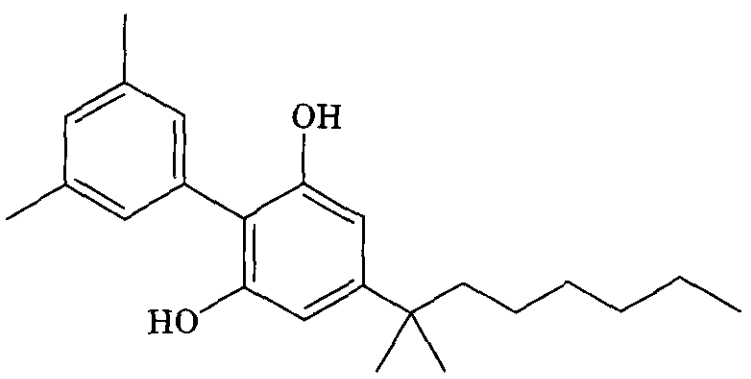
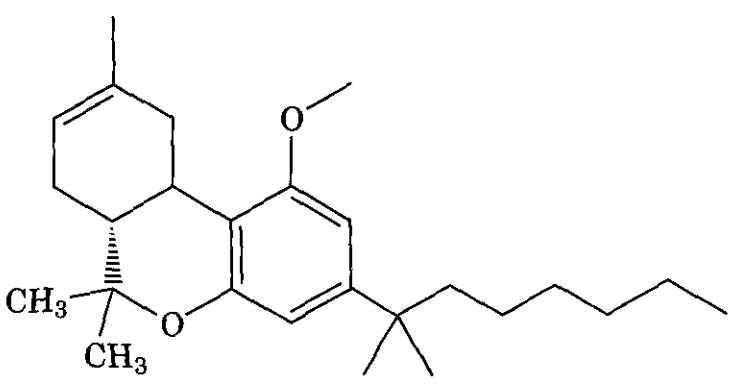
Table 15.14 Structure-Activity Relationships of Dronabinoid on CB_1-R Binding Activity

Compound	Structure	Name	CB_1-R, K_i (n*M*)	CB_2-R, K_i (n*M*)
(**32**)		Δ^9-THC	41[a]	36[a]
(**91**)		Δ^8-THC	126[a]	
(**92**)		A^8-THC-DMH	*0.83'*	0.83[b]
(**93**)			1.82[b]	0.58[b]

Transposition of the olefin from the Δ^9-position to the A^S-position as in compound (91) (Δ^8-THC) results in a threefold loss of potency (145). Compound (92) with a C3 dimethylheptyl (DMH) chain is 100-fold more potent than the analogous compound with a C3 pentyl chain. The binding affinities of compounds with either endocyclic or exocylic olefin groups in the cyclohexane ring of the tricyclic core template in the DMH series are similar. Aromatization of the terpene ring in A^S-THC does not lead to a significant drop in potency. Several bicyclic compounds such as (94) and (95) have been prepared that exhibit moderate activity.

Dronabinol (**32**) and analog (92) exhibit little selectivity for CB_1-R vs. CB_2-R. These two receptors show 44% sequence homology, with modest homology in the transmembrane domains (68%). Replacement of the phenolic hydroxyl group in the aromatic ring of (92) by a phenyl methyl ether moiety as in L-759633

Table 16.14 (*Continued*)

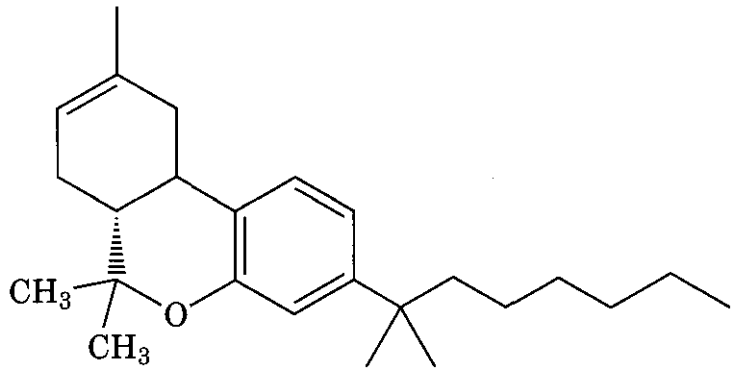
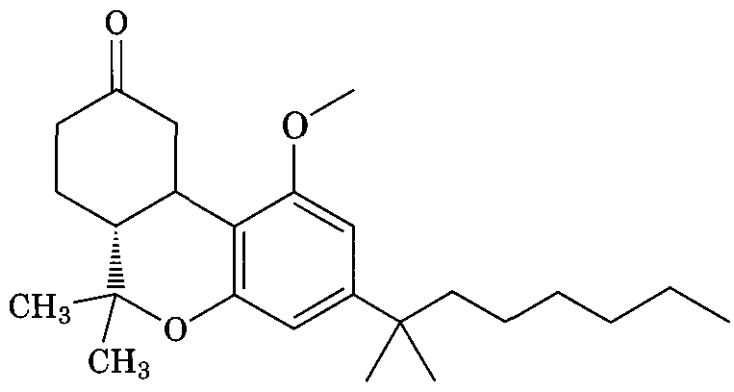
Compound	Structure	Name	CB_1-R, K_i (nM)	CB_2-R, K_i (nM)
(**76**)		**Cannabinol**	**2.49"**	1.98[a]
(**94**)			350[b]	41[b]
(**95**)			79[b]	2[b]
(**96**)		L-759633	15,850[b]	20[b]

(**96**) results in a 10,000-fold decrease in CB_1-R activity, but only a 40-fold loss of CB_2-R affinity, providing CB_2-R-preferring compounds (146). CB_2-R-selective compounds may show anti-inflammatory and immunosuppressive properties.

Fichera et al. used a series of 20 different cannabinoid compounds from several different chemical series to develop 3D-QSAR models for activities at CB_1- and CB_2-receptors (147). The QSAR study was performed by use of GOLPE methodology (148). Seven spatial regions were found in the CB_1-R, where steric, electronic, and lipophilic interactions between ligands and receptor appeared to be critical for activity. Additional interaction regions were identified that conferred selectivity for one of the receptors. All compounds used in the study, including the endogenous cannabinoids such as anandamide, were aligned against dronabinol.

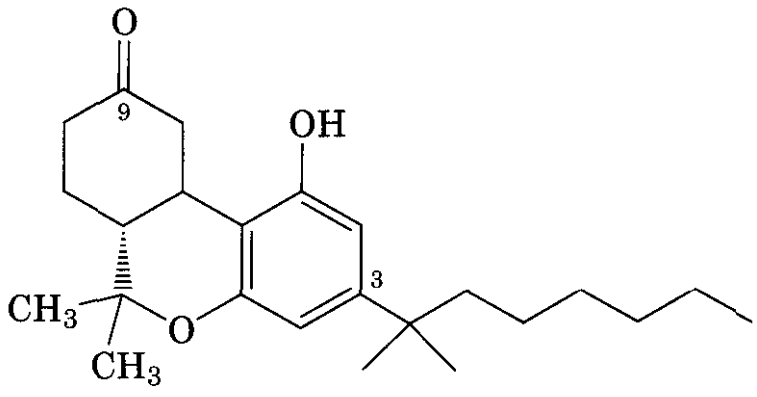
Several natural and synthetic dronabinol analogs have been clinically evaluated as safer alternatives to A^S-THC, although primarily as

Table 15.14 (Continued)

Compound	Structure	Name	CB_1-R, K_i (nM)	CB_2-R, K_i (nM)
(**97**)			250[b]	20.8[b]
(**98**)			621[b]	132[b]

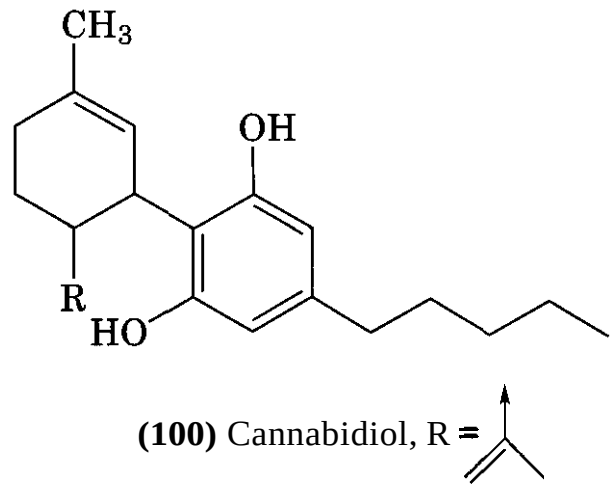
[a]Ref. 145.
[b]Ref. 146.

treatments for neurogenic pain and cancer-induced emesis (vomiting and nausea after chemotherapy). Nabilone (**99**), a nonselective



(99) Nabilone, CB_1-R **Ki** = 2.19 nM
CB_2-R Ki = 1.84 nM

CB_1-R and CB_2-R agonist, contains a carbonyl group at the C9 position and a C3 dimethylheptyl group. Cannabidiol (**100**), a bicyclic derivative of (32)in which the central pyran ring has been opened up, is a natural constituent of *Cannabis sativa* L that exhibits no psychotropic properties, although it shows neuroprotective activities.

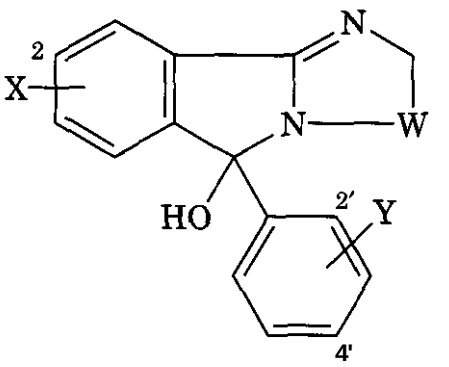


(100) Cannabidiol, R =

6.3 Mazindol Analogs

Mazindol is a potent inhibitor of both dopamine and serotonin uptake that was first described in 1975 (149). The compound exists in two tautomeric forms, although it is believed that most of the pharmacological activity lies in the closed, tricyclic form where the hydroxyl group can act as both a hydrogen-bond acceptor and donor. The open form has been shown to exhibit much weaker anorectic activity in rat models compared to that of the closed form tautomer (150).In one report on the SAR of Mazindol analogs, replacement of the C4′-Cl substituent on the B-ring with a C4′-F

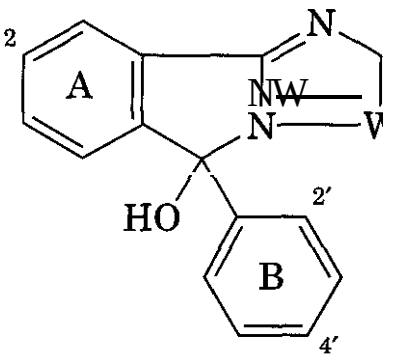
Table 15.15 Mazindol Structure–Activity Relationships



Compound	W	X	Y	IC_{50}, n*M* [^{3}H]Dopamine Uptake[a]
(**101**)	CH_2	H	H	124 ± 37
Mazindol (**5**)	CH_2	H	C4′-Cl	8.4 ± 1.3
(**102**)	CH_2	H	C4′-F	25.4 ± 2.7
(**103**)	CH_2	H	C4′-Br	8.6 ± 3.5
(**104**)	CH_2	H	C4′-I	18 ± 11
(**105**)	CH_2	H	C2′-Cl	770 ± 159
(**106**)	CH_2	C2-Cl	H	55 ± 17
(**107**)	$(CH_2)_2$	H	C4′-Cl	1.4 ± 0.35
(**108**)	$(CH_2)_3$	H	C4′-Cl	3.4 ± 2.3

[a] IC_{50} values measured in rat striatal tissue.

group results in a decrease in [^{3}H]dopamine uptake activity, although there is no change in activity when the C4′-Cl group is replaced by either C4′-Br or C4′-I groups (see Table 15.15) (46). An electronegative halogen substituent on the B-ring is necessary for activity. Interestingly, the dopamine uptake activity decreases if the B-ring is substituted with a C2′-Cl group or if the A-ring is substituted with a C2-Cl group. Inserting one or two methylene groups into the imidazoline ring to give six- and seven-membered rings further improves the inhibitory activities of the compounds (46). These homologs, moreover, show increased selectivity for the dopamine transporter over the serotonin or NE transporters.



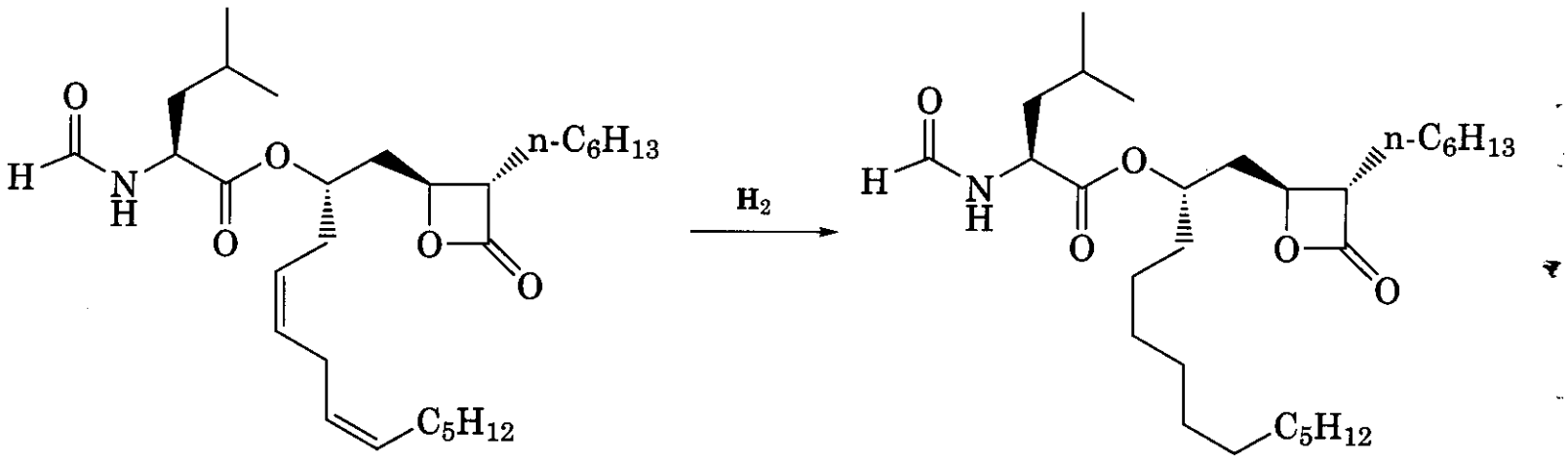
W = $(CH_2)_n$ where n = 1, 2, 3

6.4 Orlistat Derivatives

Lipstatin (109) is a natural product isolated from Streptomyces toxyticini, which shows moderate pancreatic lipase inhibitory activity (IC_{50} = 0.14 μg/mL) (151). Hydrogenation of (**109**) to give orlistat (**1**) results in only a slight loss of activity (151). However, replacement of the β-lactone ring in the drug with a 2-methyleneoxetane group leads to a significant drop in potency (152).

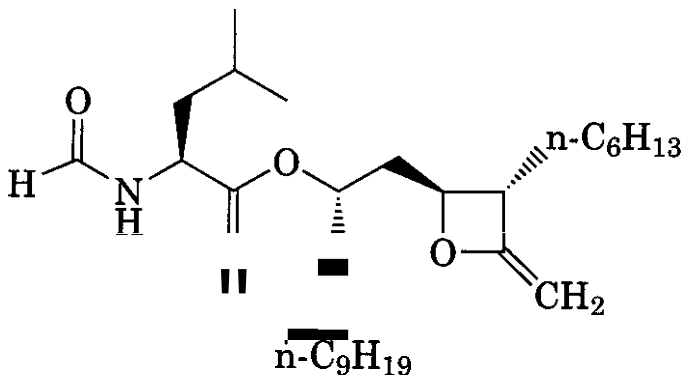
7 FUTURE DIRECTIONS

Several novel CNS and peripherally acting drugs are currently undergoing clinical evaluation (Phases I-III) for the treatment of obesity and select eating disorders (see Tables 15.16–15.18). These drugs exhibit varied mechanisms of action, most of which are clinically unprecedented, such as decreasing food intake through stimulation of gastrointestinal satiety pathways, increasing energy expenditure through thermogenesis of brown adipose tissue, or preferentially enhancing fat oxidation over carbohydrate oxidation. One new drug may be able to modulate specific path-



(109) Lipstatin, IC_{50} = 0.14 μg/mL

(1) Orlistat, IC_{50} = 0.4 μg/mL



(110) Compound, IC_{50} = 1.7 μg/mL

ways involved in reward mechanisms, which may prove effective in changing behaviors such as overeating that often result in obesity. A recombinant protein under development prevents the body's adaptive response to starvation that occurs after weight loss.

New weight loss drugs must meet certain guidelines established by the FDA before receiving marketing approval **(153).** These guidelines require that weight-loss agents

Table 15.16 **Antiobesity Drugs in Late-Stage Clinical Development**

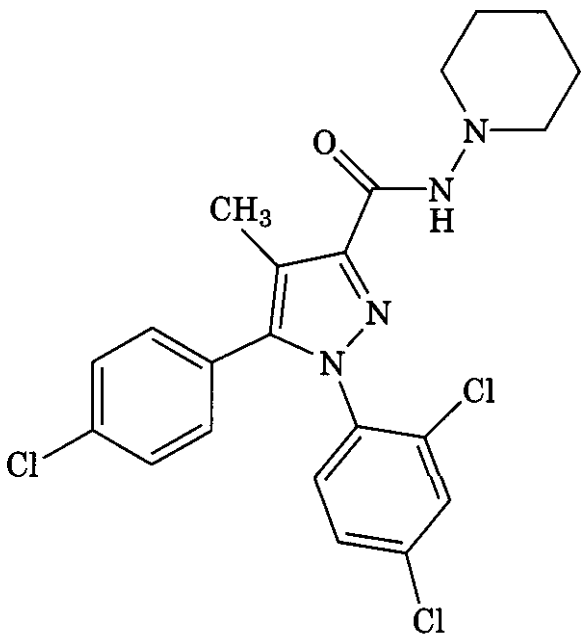
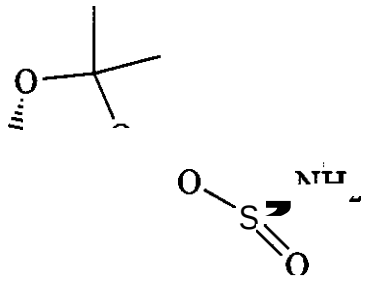
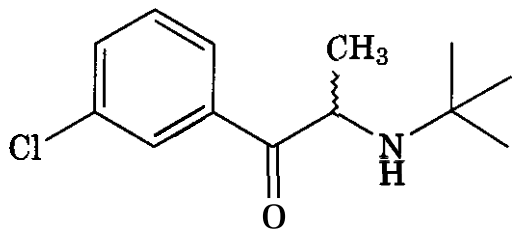
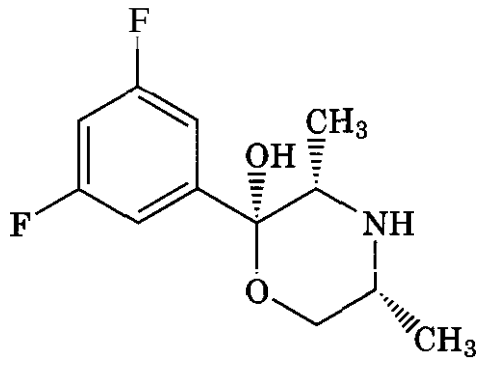
Name	Structure	Mechanism of Action	Development Stage
Axokine **(111)**	1-185-ciliary neurotropic factor (CNTF)	Activation of STAT signaling	Phase III
Rimonabant, SR-141716A **(112)**		CB_1-R antagonism	Phase III
Topiramate, Topimax **(113)**		Unknown mechanism	Marketed; Ph III—obesity

Table 15.17 **Centrally Acting Anorectic Drugs in Early Clinical Development**

Name	Structure	Mechanism of Action	Development Stage
Wellbutrin (**114**)		DA/NE reuptake inhibition	Marketed; Ph II—obesity
BW1555U88 (**115**)		DA/NE reuptake inhibition	Ph I
Leptin (**116**)	163 a.a.	Ob-R (leptin receptor) agonism	Ph I/Ph II

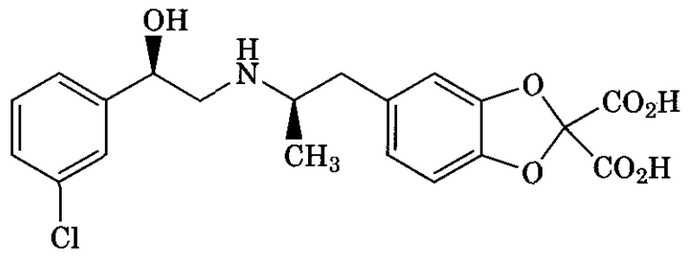
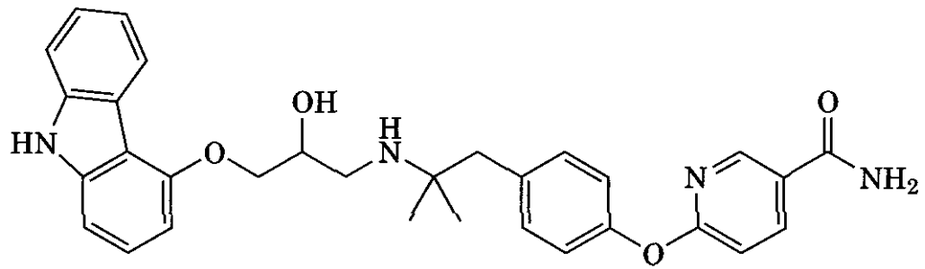
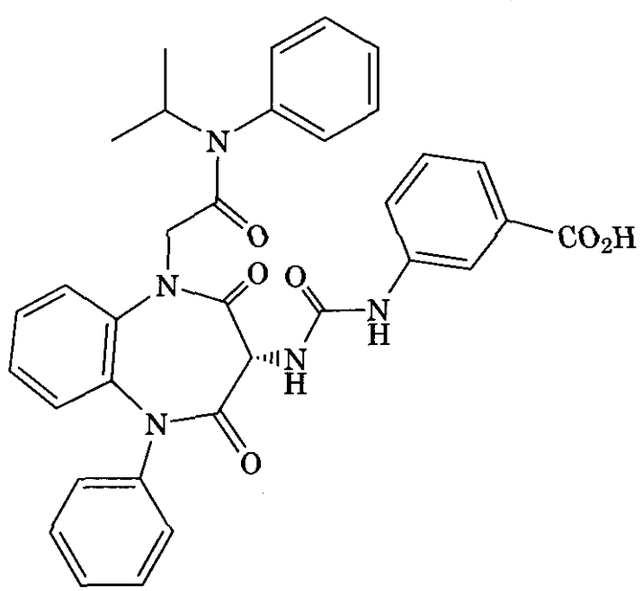
demonstrate efficacy for at least 1 year, defined as greater than 5% weight loss vs. placebo, generally in double-blind clinical trials in patients with BMIs greater than 30 kg/m^2 or BMIs greater than **27** kg/m^2 with at least one associated comorbidity. The clinical trials should preferably last at least **2** years, although the second year need not be blinded. The guidelines also require that such drugs reduce comorbidities, especially in overweight patients with BMIs less than 30 kg/m^2. Any drug used for the chronic treatment of a disease must also exhibit an exceptional safety profile, with acceptable benefit-to-risk profiles.

7.1 Antiobesity Compounds and New Drugs to Treat Binge Eating in Clinical Development

7.1.1 Axokine. Axokine **(111)** is a recombinant form of ciliary neurotrophic factor (CNTF), a 200 amino acid member of the **cytokine** family, which also includes proteins such as leptin, **gp130, interleukins,** prolactin, and growth hormone (154). CNTF promotes weight loss in several different animal models of obesity including *ob/ob* mice, diet-induced obesity, and *fa/fa* Zucker rats (155). Interestingly, CNTF appears to change the set point for body weight in both rodents and humans, given that withdrawal of treatment does not result in any rebound weight gain as is observed with most other weight-loss agents. In a 3-month clinical trial, obese patients who were administered **2** μg kg^{-1} day^{-1} reportedly lost 10 extra pounds compared to the placebo group (156). This weight loss was equivalent to that observed for sibutramine and **orlistat** over the same time period. In earlier trials for another indication, amyotrophic atherosclerosis, lean patients reportedly lost 14% of their body weight. However, the dose in this trial was much greater than that used in the obesity trial (15or 30 $\mu g/kg$ three times per week vs. a maximum dose of **2** μg kg^{-1} day^{-1}) (157).

The precise mechanism of action of CNTF in the promotion of weight loss has not been defined, but its effects appear to be mediated by various hypothalamic pathways. The weight loss from a low dose of CNTF in rodents and humans does not appear to be caused by cachectic cytokines such as **interleukin-1** because no muscle wasting was observed in the CNTF-treated groups. CNTF may mediate some of its effects by coupling to downstream signaling events in the leptin pathway. In a diet-induced model of obesity, CNTF, but not leptin, was shown to stimulate **phosphorylation** of the cytoplasmic transcription factor

Table 15.18 Peripheral Antiobesity Drugs

Name	Structure	Mechanism of Action	Development Stage (status)
CL-316243 (**117**)		β_3-AR agonism	Ph II (abandoned)
LY-377604 (**118**)		β_3-AR agonism	Ph I
GI 181771 (**119**)		CCK_a-R agonism	Ph I

called signal transducer and transcription factor 3 (STAT-3) in the arcuate nucleus and thereby activate downstream signaling pathways. In lean control animals, however, both CNTF and leptin stimulated phosphorylation of STAT-3. These data support a hypothesis that diet-induced obesity causes "leptin resistance" and that CNTF may overcome the resistance observed in this model of obesity.

7.1.2 Rimonabant. The centrally-acting selective cannabinoid-1 receptor (CB_1-R) receptor antagonist rimonabant (112, SR-141716A) has been reported to decrease body weight in Phase II clinical trials in a dose-dependent manner (158). The weight loss was comparable to that observed with marketed weight-loss agents such as either orlistat or sibutramine. In preclinical models, rimonabant was found to inhibit food intake in normal rats and NPY gene knockout mice, but not in CB_1-R-deficient mice (159,160). The preclinical and clinical data with rimonabant confirm a role for endogenous cannabinoids such as anandamide in the regulation of food intake. Recent studies have shown that hypothalamic levels of anandamide and 2-arachidonoyl glycerol, two of the endogenous ligands of the cannabinoid receptors, are decreased in leptin-treated (hypophagic) rats (160).

Rimonabant may also alter reward pathways that promote addictive behaviors such as overeating. This drug has been shown to attenuate the rewarding effects of alcohol in a strain of alcohol-preferring rats (161) and may similarly diminish the pleasurable effects of food and overeating that can often play important roles in the etiology of obesity.

7.1.3 Topiramate. Topiramate (113, Topamax) is a sulfamate-substituted D-fructose derivative that is currently marketed as an antiepileptic drug (AED). Topiramate promotes weight loss, whereas AEDs in general increase body weight gain. Topiramate has progressed into Phase III clinical trials for the treatment of both binge eating disorder and obesity. In an open-label trial involving 13 patients diagnosed with a DSM IV binge eating behavior, topiramate caused an average weight loss of 12% among all patients and decreased binge eating symptoms in 75% of the patients (162). The mechanism by which topiramate decreases body weight is unknown, although its anticonvulsant properties are attributed in part to increasing gamma-aminobutyric acid (GABA), a neurotransmitter that inhibits excitation of nerve cells in the brain (163). Topiramate may alter both energy intake and energy expenditure, decrease food intake, and increase energy utilization (164).

7.1.4 Combined Norepinephrine/Dopamine Reuptake Inhibitors. Bupropion (114, Wellbutrin, Zyban), a close-in congener of diethylpropion, is a weak and nonselective dopamine and norephinephrine uptake inhibitor that is currently marketed for depression and smoking cessation (165). It is also reportedly a noncompetitive nicotinic antagonist at some, but not all, of the nicotinic receptor subtypes (166). The drug is currently undergoing clinical evaluation as an antiobesity agent. In an 8-week study in 50 obese women, bupropion was found to be more effective than placebo in promoting weight loss. The bupropion group, for example, lost 6.2% of body weight, whereas the placebo group lost only 1.5% of body weight (167). The pharmacological mechanisms by which this drug decreases food intake are not full understood, but drugs that increase dopamine levels oftens decrease appetite (168).

BW1555U88 (115), a 3,5-difluorophenyl-3,5-dimethyl-2-morpholinol derivative, is in early clinical development for both smoking cessation and weight loss (169). This compound is significantly more potent than bupropion as both a NE and DA reuptake inhibitor and has been shown to decrease food intake and body weight in rodent models of obesity (170).

7.1.5 Leptin. Leptin (116), the product of the *ob* gene, is a member of the cytokine family of hormones. Leptin plays a critical role in the regulation of body weight, given that its complete absence in both animals and humans leads to profound morbid obesity (108, 171). Initial clinical results from small placebo-controlled studies have been disappointing, with moderate weight loss of 6 kg in 24 weeks reported for obese subjects on high doses of drug (0.3 mg kg^{-1} day^{-1}) (172). Recently, it has

been shown that many obese patients actually have elevated plasma, but low CSF levels of leptin, suggesting defects in leptin transport' across the blood-brain barrier, possibly because of saturation of the transporter (173).

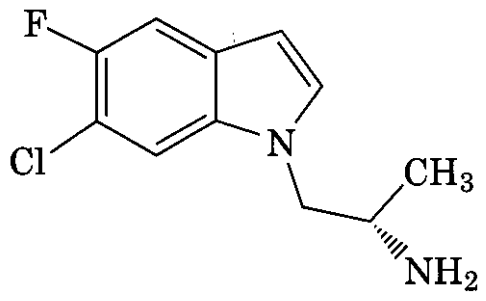
7.1.6 β_3-Adrenergic Receptor Agonists. β_3-Adrenergic receptor agonists (β_3-AR) are peripherally acting thermogenic agents that are currently undergoing clinical evaluation for the treatment of obesity and diabetes. β_3-AR agonists increase thermogenesis by activation of β_3-ARs on brown adipocytes, which leads to increases in intracellular cAMP levels, stimulation of uncoupling proteins, and increases in fatty acid acid oxidation and nonshivering therrnogenesis. These compounds also show a pronounced insulin-sensitizing, antidiabetic effect in diabetic rodent models, an effect that is independent of weight loss. A first-generation β_3-AR agonist, CL-316243 (**117**), has been reported to increase glucose disposal, whole body fat oxidation and plasma free fatty acids after 4 weeks in lean males (174). CL-316243 also has been found to increase whole body lipolysis in obese subjects after 3 months. No increase in thermogenesis, however, was observed in the study. Another β_3-AR agonist, LY-377604 (**118**), has been shown to increase energy expenditure and decrease respiratory quotient. The decrease in respiratory quotient indicated a shift from carbohydrate oxidation to fat oxidation. Several β_3-AR agonists also reported to increase energy expenditure in monkeys (175).

7.1.7 CCK_a Receptor Agonists. CCK is a short-term satiety signal that is released upon food ingestion by endocrine cells in the small intestine. In human studies, exogenous administration of CCK has been shown to reduce food intake in both lean and obese subjects (176, 177). A nonpeptidic CCK_a-R agonist, GI 181771 (**119**), is reportedly undergoing clinical evaluation as an anorectic agent. No long-term studies have been reported for CCK or CCK_a-R agonists in humans. A possible side effect of this class of anorectic agents is pancreatitis, or inflammation of the pancreas.

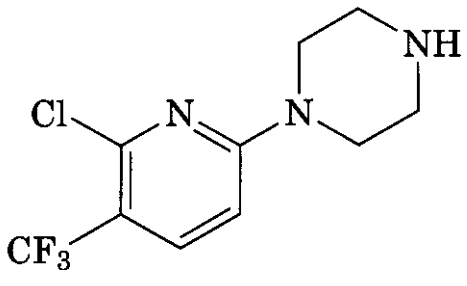
7.2 New Targets

In the past decade, many new genetic targets involved in regulation of energy homeostasis, especially in rodent models, have been identified. A number of these molecular targets are considered "druggable" or suitable for pharmacological modulation, including the 5-HT_{2c}-R that mediates the anorectic effects of fenfluramine, the NPY_1- and NPY_5-receptor subtypes that are involved in the stimulation of food intake, the melanocortin-4 receptor subtype, and the orexin-1 receptor subtype. Stimulation of NPY_1-R, NPY_5-R, and OX_1-R may be useful in the treatment of wasting diseases (anorexia nervosa, cachexia), whereas inhibition of these receptors may show utility in the management of body weight disorders such as obesity. Activation of the melanocortin system, in particular the MC_4-R subtype, should decrease food intake and antagonism of the system should increase food intake.

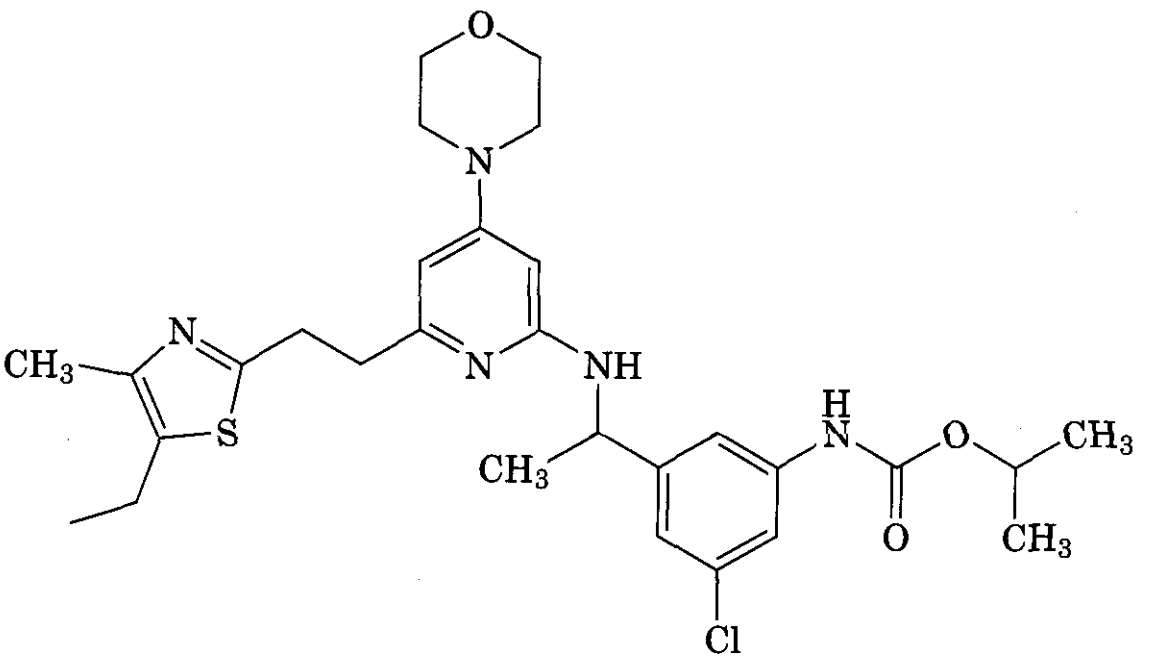
Selective ligands for 5-HT$_{2c}$, NPY, and orexin receptors are currently undergoing preclinical and clinical evaluation for the treatment of body weight disorders, primarily obesity. Ro 60–0175 (120) and ORG-12962 (121) are potent 5-HT$_{2c}$ receptor agonists currently in preclinical development. J-115814 (122) is a selective NPY Y_1-R antagonist with reportedly good brain exposure that decreases food intake in *db/db* and C57BL6 mice after



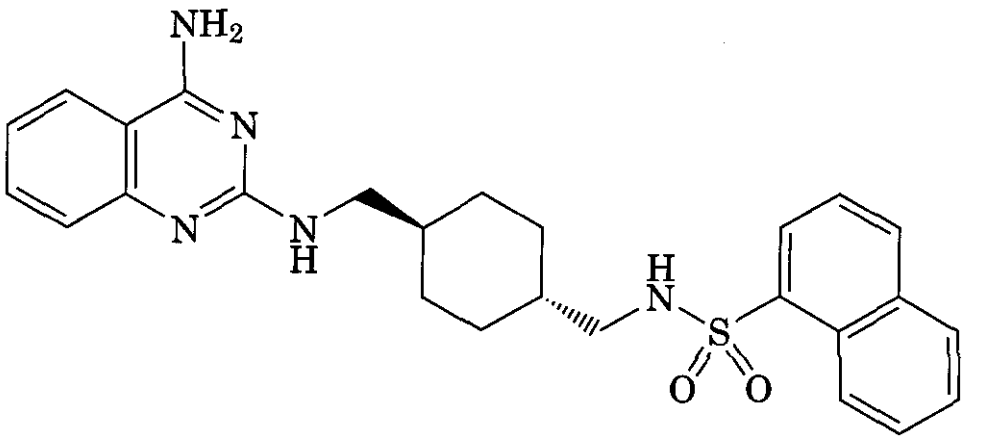
(120) Ro 60-0175



(121) ORG-12962

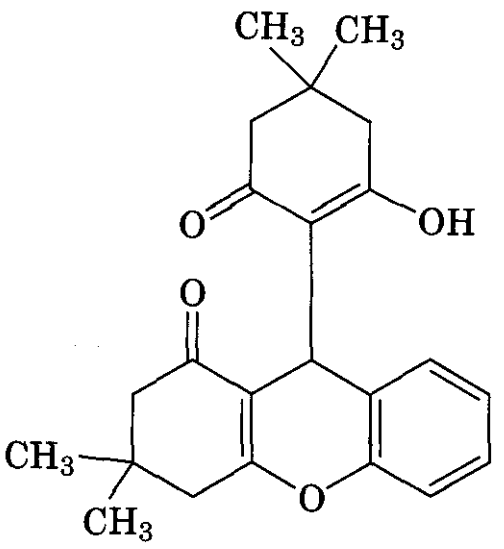


(122) J-115814

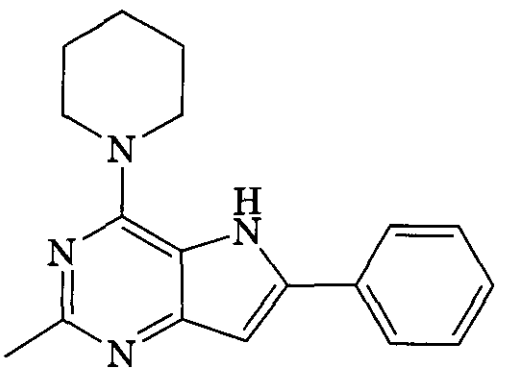


(123) CGP 71683A

intraperitoneal administration, but does not affect food intake in NPY_1-R knockout mice **(178).** CGP 71683A **(123)** and **L-152804 (124)** are potent NPY Y_5-R antagonists, although CGP 71683A is not particularly selective, with activity at several receptors that may influence food intake **(179-181). L-152804** inhibits the orexigenic effects of NPY_5-R, preferring peptidic agonists, but not the effects of NPY itself. **An** interesting series of **pyrrolo[3,2-*d*]pyrimidine** derivatives such as compound (**125**) have been reported as Y_5-R antagonists, with nanomolar affinity for the receptor **(182). A** selective OX,-R antagonist, **SB-334867-A**

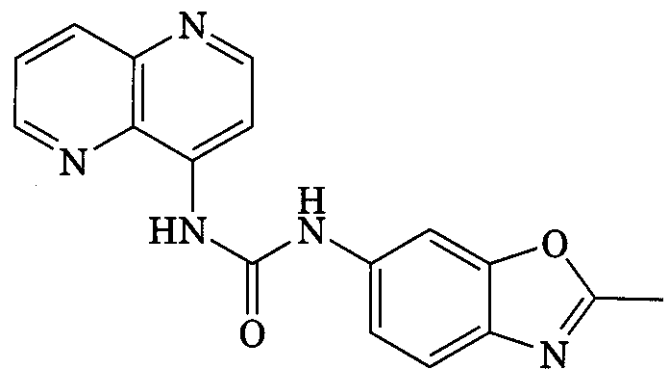


(124) L-152804



(125)

(126), has been identified that decreases food intake in rat models (107,183).



(126) SB-334867-A

8 CONCLUSIONS

The direct and indirect health costs associated with eating and body weight disorders are rapidly growing, especially as these diseases become more prevalent in the general population. The demand for successful therapies has increased as awareness of the comorbidities associated with these diseases has grown. These disorders are currently managed by use of a combination of pharmacotherapy, lifestyle changes, nutritional counseling, and/or cognitive behavioral therapy. At the present time, drug therapy plays only an adjunct role, usually initiated after lifestyle or behavioral changes have failed to produce desirable outcomes. Current drugs used to treat body weight disorders such as obesity are associated with high attrition rates, primarily because of the lack of efficacy, lack of specificity, development of tolerance, or the presence of side effects. Drugs used to treat eating disorders are limited primarily to antidepressant medications, which are especially effective at treating the psychiatric comorbidities that frequently cause abnormal feeding patterns. The importance of pharmacotherapy in the management of all these diseases will only increase insofar as safer, more selective, and more efficacious agents become available. Already, many novel agents with clinically unprecedented mechanisms of action and possibly improved safety profiles are in various stages of development, providing new hope for the many patients who have failed to fully respond to existing therapies.

9 ABBREVIATIONS

AN	anorexia nervosa
BE	binge eating
BMI	body mass index
BN	bulimia nervosa
DA	dopamine
5-HT	5-hydroxytryptamine
MAO	monoamine oxidase
NE	norepinephrine
SSRI	selective serotonin reuptake inhibitor
TCA	tricyclic amine antidepressants

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