Adrenergic Drugs

Overview

Overview

-- Adrenergic drugs exert their principal pharmacological and therapeutic effects by either enhancing or reducing the activity of the sympathetic division of the autonomic nervous system. Substances (drugs)that produce effects similar to stimulation of sympathetic nervous activity are known as sympathomimetics or adrenergic stimulants. Those that decrease sympathetic activity are referred to as sympatholytics, antiadrenergics, or adrenergic-blocking agents.

-

Overview

-- **Adrenergic agents either act on adrenergic receptors (adrenoceptors, ARs) or affect the life cycle of adrenergic neurotransmitters (NTs), including norepinephrine (NE, noradrenaline), epinephrine (E, adrenaline), and dopamine (DA). Normally these NTs modulate many vital functions, such as the rate and force of cardiac contraction, constriction and dilation of blood vessels and bronchioles, the release of insulin, and the breakdown of fat (table 16.1).**

Overview

TABLE 16.1 Most Commonly Used Adrenergic Prescription Drugs

Mechanism of Action	Drug	Major Indications
α_1-Agonists	Naphazoline (Privine)	Nasal & ophthalmic congestion
α_2-Agonists	Clonidine (Catapres)*	Hypertension
	Methyldopa (Aldomet)	Hypertension
α_1-Blockers	Prazosin (Minipress)	Hypertension & benign prostatic hyperplasia (BPH)
	Terazosin (Hytrin)*	Hypertension & BPH
	Doxazosin (Cardura)*	Hypertension & BPH
	Tamsulosin (Flomax)	BPH & hypertension
β_2-Agonists	Albuterol (Ventolin)*	Asthma
α_1-, β_1-, & β_2-Blockers	Labetalol (Normodyne)*	Hypertension
	Carvedilol (Coreg)	Hypertension & heart failure
β_1- & β_2-Blockers	Propranolol (Inderal)*	Hypertension, arrhythmias, & angina
	Nadolol (Corgard)*	Hypertension, angina, & hyperthyroidism
	Timolol (Timoptic)*	Glaucoma & hypertension
	Sotalol (Betapace)*	Arrhythmias
	Levobunolol (Betagan)	Glaucoma
β_1-Blockers	Acebutolol (Sectral)	Hypertension, angina, & hyperthyroidism
	Atenolol (Tenormin)*	Hypertension, angina, & hyperthyroidism
	Metoprolol (Lopressor)*	Hypertension
	Bisoprolol (Zebeta)*	Hypertension

Adrenergic NTs(structure and physicochemical properties)

-- NE, E, and DA are chemically catecholamines (CAs), which refer generally to all organic compounds that contain a catechol nucleus (ortho-dihydroxybenzene) and an ethylamine group (Fig. 16.1). In a physiological context, the term usually means DA and its metabolites NE and E. E contains one secondary amino group and three hydroxyl groups. Using calculated log p(-0.63) of E, one would expect the molecule is polar and soluble in water.

-
-

NTs

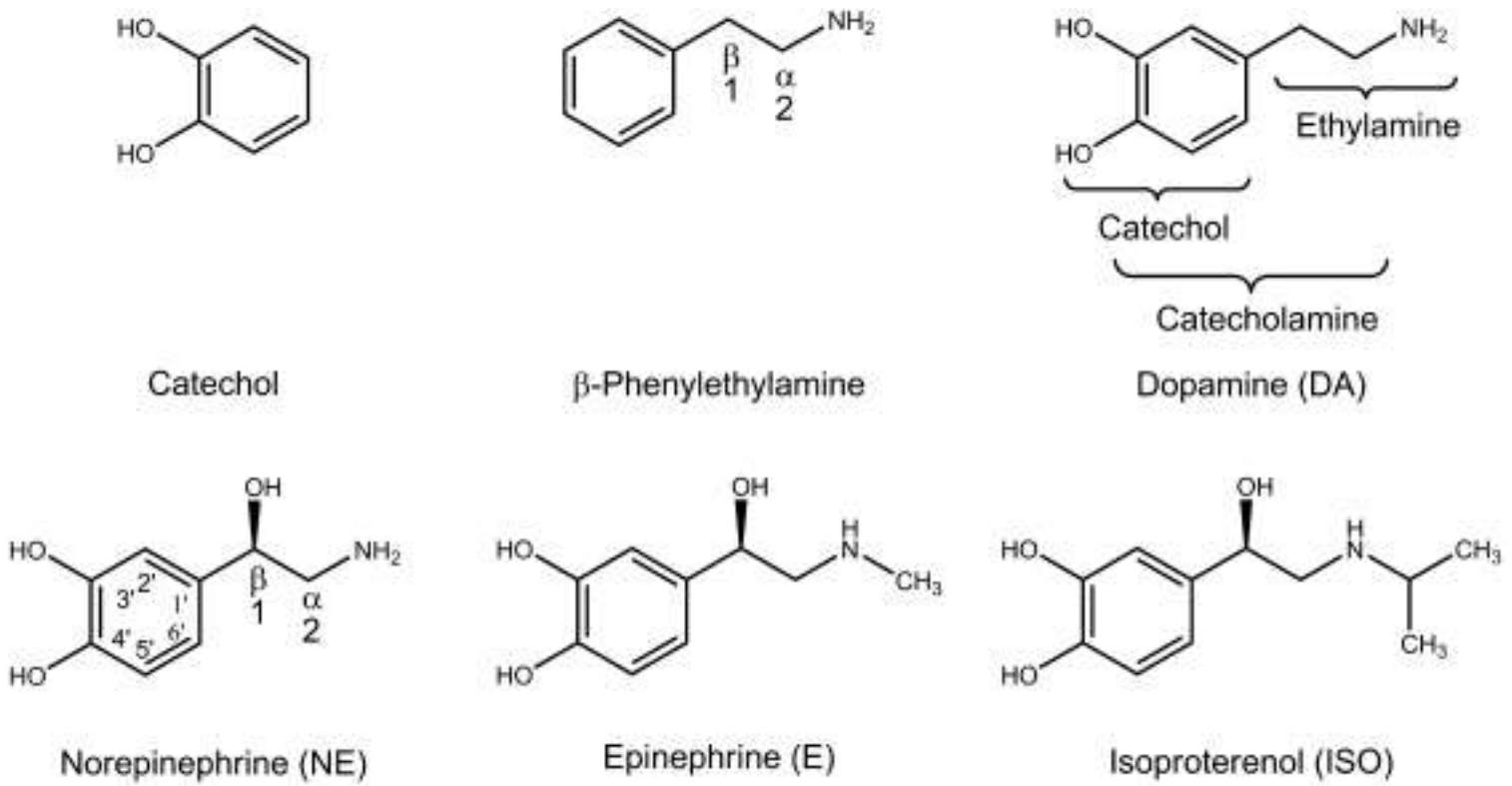


Figure 16.1 • Adrenergic neurotransmitters and related compounds.

NTs

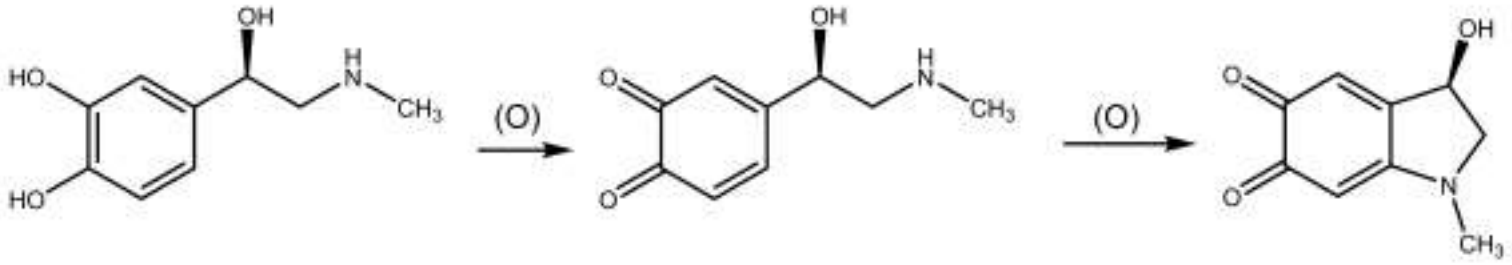
- -- E is a weak base (pKa = 9.9) because of its aliphatic amino group. It is also a weak acid (pK**a** =8.7) because of its phenolic hydroxyl groups. It can be predicted that ionized species (the cation form) of E at physiological pH is predominant (log D at pH 7 = -2.75). This largely accounts for the high water solubility of this compound as well as other CAs.

NTs

-- log p of 0-3 is optimum for absorption and we can predict that E. has poor absorption and poor central nervous system (CNS) penetration.
-- The catechol functional groups in E. and NE. are highly susceptible to facile oxidation in the presence of oxygen (air) or other oxidizing agents to produce a quinone analog, which undergoes further reactions to give adrenochrome(Fig. 16.2). Hence, solutions of these drugs often are stabilized by the addition of an antioxidant (reducing agent) such as ascorbic acid or sodium bisulfite.

-

NTs



Epinephrine (E) A quinone analog Adrenochrome

Figure 16.2 • Oxidation of Epinephrine (E).

NTs

-- E and NE each possess a chiral carbon atom; thus, each can exist as an enantiomeric pair of isomers. The enantiomer with the (R) configuration is biosynthesized by the body and possesses the biological activity. This (R) configuration of many other adrenergic agents also contributes to their high affinity to the corresponding adrenoceptors

-

Biosynthesis

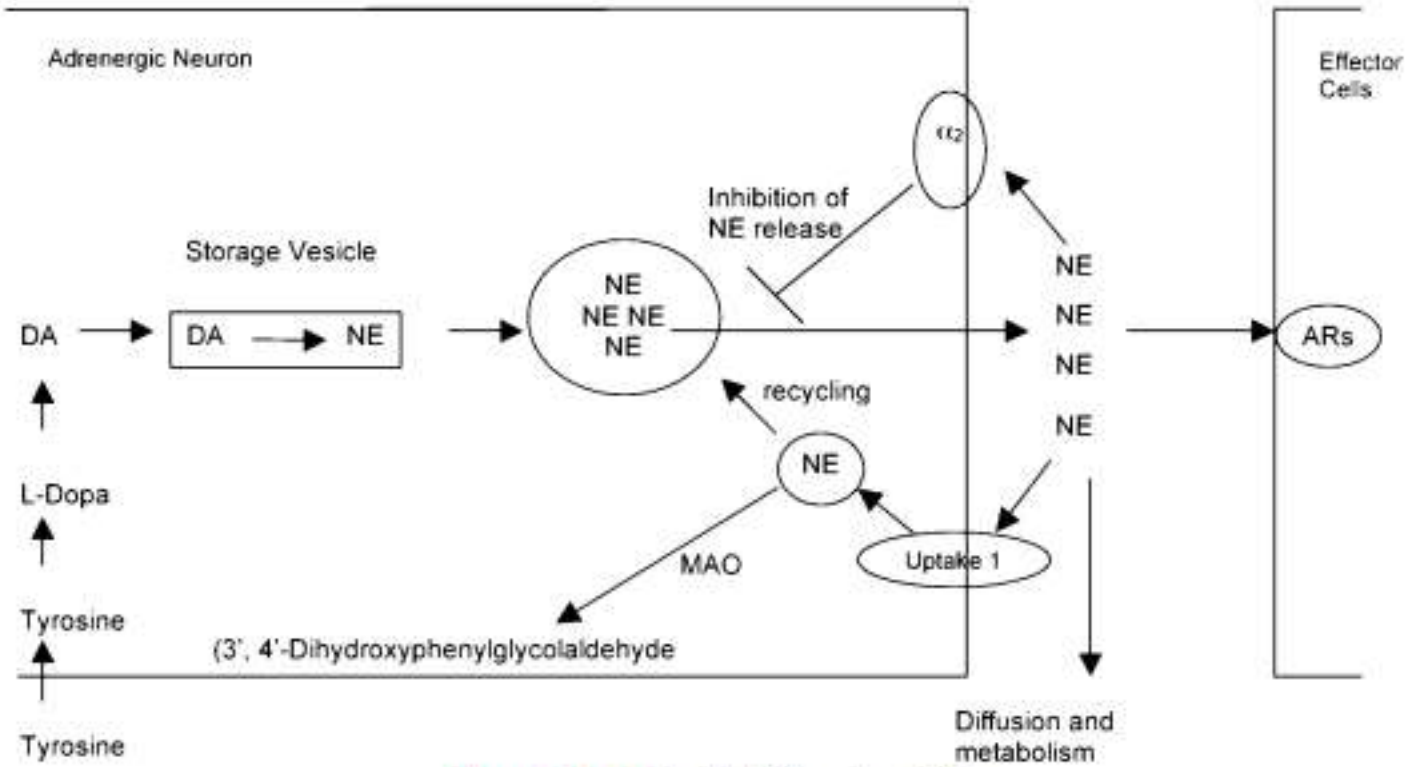


Figure 16.3 • Model of life cycle of NE.

Biosynthesis

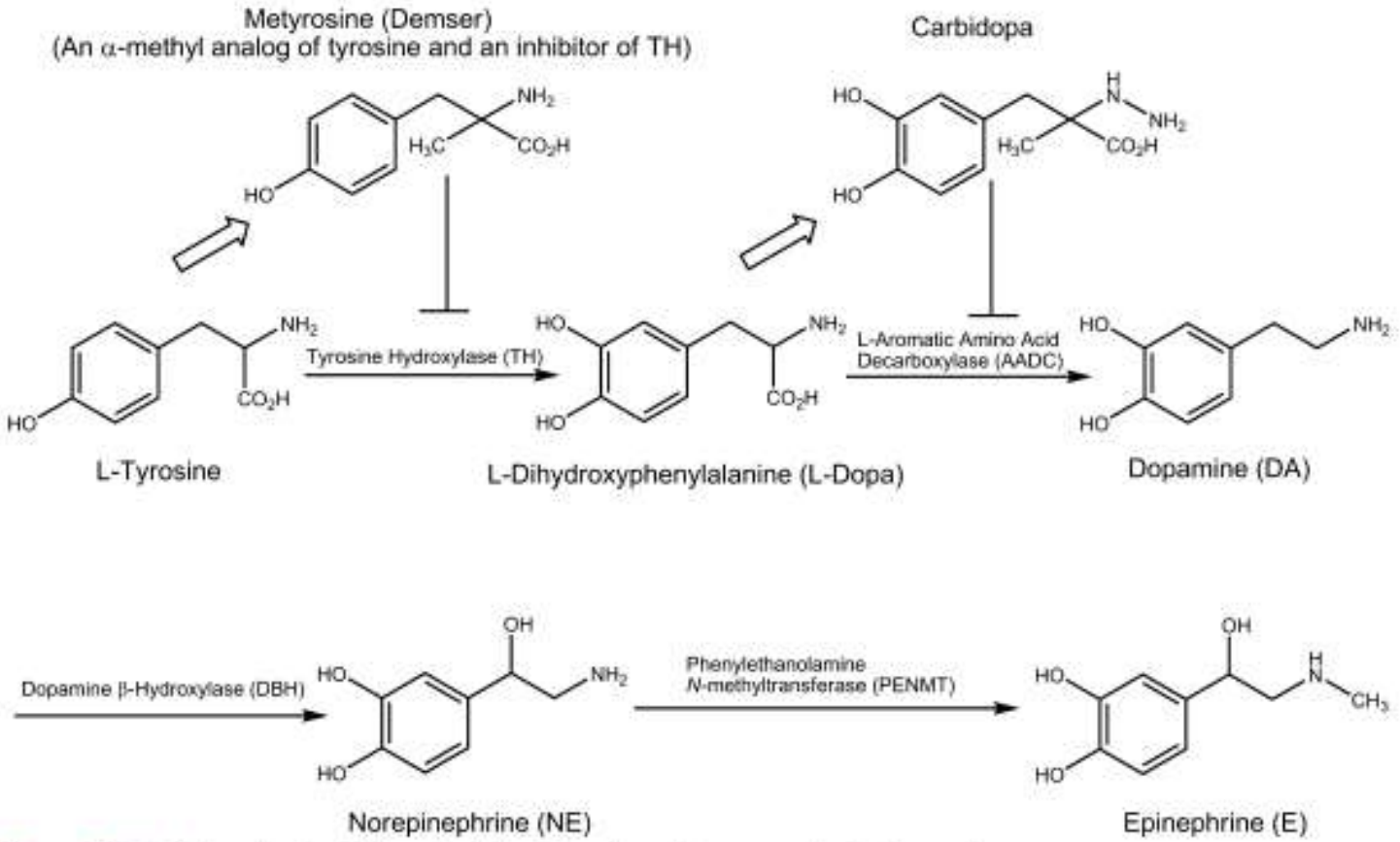


Figure 16.4 • Biosynthesis of the catecholamines dopamine, norepinephrine, and epinephrine.

Biosynthesis

-- L-Tyrosine is normally present in the circulation and transported actively into the adrenergic neuron. TH is stereospecific. As usual for the first enzyme in a biosynthetic pathway, The hydroxylation is the rate-limiting step in the biosynthesis of NE.

Biosynthesis

--Further proof that this step is rate limiting in CA biosynthesis is that inhibitors of TH markedly reduce endogenous NE and DA in the brain and NE in the heart, spleen, and other sympathetically innervated tissues. This enzyme plays a key role in the regulation of CA biosynthesis and is, therefore, the biological target of some drugs. The methylated analog of tyrosine are more potent than unmethylated ones .

-

Biosynthesis

--The second step in CA biosynthesis is the decarboxylation of L-DOPA to give DA, which is an important NT and a drug. The enzyme involved is DOPA decarboxylase . Although originally believed to remove carboxyl groups only from L-DOPA but this enzyme is more appropriately referred to as L-aromatic amino acid decarboxylase (AADC).

-

Biosynthesis

- -- Parkinsonism can be characterized as a DA deficiency in the brain. Direct parenteral DA administration is useless because the compound does not penetrate the blood-brain barrier (BBB). Oral dosing with L-DOPA (levodopa) could act as a prodrug because it entered the brain (on a specific carrier) and then was decarboxylated to DA there. Many adverse systemic effects were the result of the high doses needed to achieve the desired results. The main reason is the relatively higher concentration of AADC in peripheral system than in the brain. Inhibition of peripheral AADC activity by coadministration of L-DOPA with peripheral decarboxylase inhibitor such as carbidopa (charged at physiological pH), can markedly increase the proportion of levodopa that crosses the BBB.

Biosynthesis

-- The third step in CA biosynthesis is side-chain hydroxylation of DA to give NE. DA formed is hydroxylated stereospecifically at the β carbon to NE by dopamine β-hydroxylase (DBH, dopamine monooxygenase). The NE formed is stored in the vesicles until depolarization of the neuron initiates the process of vesicle fusion with the plasma membrane and extrusion of NE into the synaptic cleft.

-

Biosynthesis

- The last step in CA biosynthesis is the *N*-methylation of NE to give E . The reaction is catalyzed by the enzyme phenylethanolamine-*N*-methyltransferase (PNMT). The methyl donor (*S*-adenosyl methionine (SAM)) is required for the *N*-methylation of NE.

Storage

- -- A large percentage of the NE present is located or stored within highly specialized subcellular particles(synaptic vesicles but also referred to as *granules*) in sympathetic nerve endings and CNS. The concentration in the vesicles is maintained also by the vesicular monoamine transporter(VMAT). Depolarization of neuron initiates the process of vesicle fusion with the plasma membrane and extrusion of NE into the synaptic cleft.

Release

--The entrance of Ca^{+2} into these cells results in the extrusion of NE by exocytosis of the granules. Ca^{+2} - triggered secretion involves interaction of highly conserved molecular proteins leading to docking of granules at the plasma membrane and then NE is released from sympathetic nerve endings into the synaptic cleft, where it interacts with specific presynaptic and postsynaptic adrenoceptors on the effector cell triggering a biochemical cascade that results in a physiologic response by the effector cell.

Release

-- Indirectly acting (e.g. tyramine and amphetamines) and mixed sympathomimetics (e.g. ephedrine) are capable of releasing stored transmitter from noradrenergic nerve endings by a calcium-independent process. These drugs are poor agonists (some are inactive) at adrenoceptors but they are excellent substrates for VMAT. They are taken up into noradrenergic nerve endings by NE reuptake transporter (NET) responsible for NE reuptake into the nerve terminal. They are then transported by VMAT into the vesicles displacing NE which is subsequently expelled into the synaptic space by reverse transport via NET. Their action does not require vesicle exocytosis.

REMOVAL

- Once NE has exerted its effect at adrenergic receptors, there must be mechanisms for removing the NE from the synapse and terminating its action at the receptors. These mechanisms include (a) reuptake of NE into the presynaptic neuron (recycling, major mechanism) by NET and into extraneuronal tissues, (b) conversion of NE to an inactive metabolite, and (c) diffusion of the NE away from the synapse. The first two of these mechanisms require specific transport proteins or enzymes, and therefore are targets for pharmacologic intervention.

Uptake

-- The most important of these mechanisms is recycling the NE. This process is termed *uptake-1* and involves a Na/Cl-dependent transmembrane (TM) NET that has a high affinity for NE. This reuptake system also transports certain amines other than NE into the nerve terminal, and can be blocked by such drugs as cocaine and some of the tricyclic antidepressants.

-

Uptake

-- Some of the NE that reenters the sympathetic neuron is transported from the cytoplasm into the storage granules carried out by an H+ -dependent TM VMAT. Certain drugs, such as reserpine block this transport, preventing the refilling of synaptic vesicles with NE and eventually cause nerve terminals to become depleted of their NE stores. By this mechanism, reserpine inhibits neurotransmission at adrenergic synapses.

-

Uptake

In addition to the neuronal uptake of NE, there exists

- an extraneuronal uptake process, called *uptake-2* with relatively low affinity for NE.

Metabolism

-- The second mechanism of CA removal is metabolism by monoamine oxidase (MAO) and catechol-*O*-methyltransferase (COMT). Both E and NE are orally inactive and have short durations of action because of their high hydrophilicity, ionization, and extensive first-pass metabolic deactivation by COMT and MAO that have lacked substrate specificity . Drugs that are catechols are subjected to metabolism by COMT, whereas drugs with unsubstituted or secondary *N*-methyl-amino groups are often substrates for MAO.

Metabolism

-- MAOs oxidatively deaminate CAs to their corresponding aldehydes which are rapidly oxidized to the corresponding acid by the enzyme aldehyde dehydrogenase(AD). In some circumstances, the aldehyde is reduced to the glycol by aldehyde reductase (AR). •

Metabolism

- MAO inhibitors (MAOIs) prevent MAO-catalyzed deamination of NE, DA, and 5-HT following their reuptake into the nerve terminal from the synaptic cleft. As a result, higher concentration of the NTs will be stored in the vesicles and become available for release from the presynaptic terminals on demand. Antidepressants such as phenelzine (Nardil), isocarboxazid (Marplan), and tranylcypromine (Parnate) are MAOIs.

Metabolism

-- There are two types of MAOs and these exhibit different substrate selectivity.[8] MAO-B primarily metabolizes DA and thus MAO-B inhibitors would tend to preserve brain DA and be effective by themselves and/or potentiate levodopa.

- Selegiline (Eldepryl) is a selective type MAO-B inhibitor and does extend the duration and increase the efficacy of levodopa . MAO-A selectively deaminates NA and E.

Metabolism

-- The second enzyme of importance in the metabolism of CAs is COMT that *O*-methylates meta-or (3')-OH group of CAs and renders them inactive. For example, the action of COMT on NE and E gives normetanephrine and metanephrine respectively.

•

Metabolism

-- Drugs ,that are structural analogs of NE. tolcapone (Tasmar) and entacapone (Comtan),are COMT inhibitors presently available. •

Receptors

-- CAs released from either noradrenergic nerve terminals or the adrenal medulla are recognized by and bind to specific receptor molecules on the plasma membrane of the neuroeffector cells. These receptor-ligand interactions produce a physiological response.

Receptors

-- Many cells possess these receptors and the binding of an agonist will generally cause the organism to respond in a fight-or-flight manner . For instance, the heart rate will increase and the pupils will dilate, energy will be mobilized, and blood flow will be diverted from other organs to skeletal muscle.

Metabolism

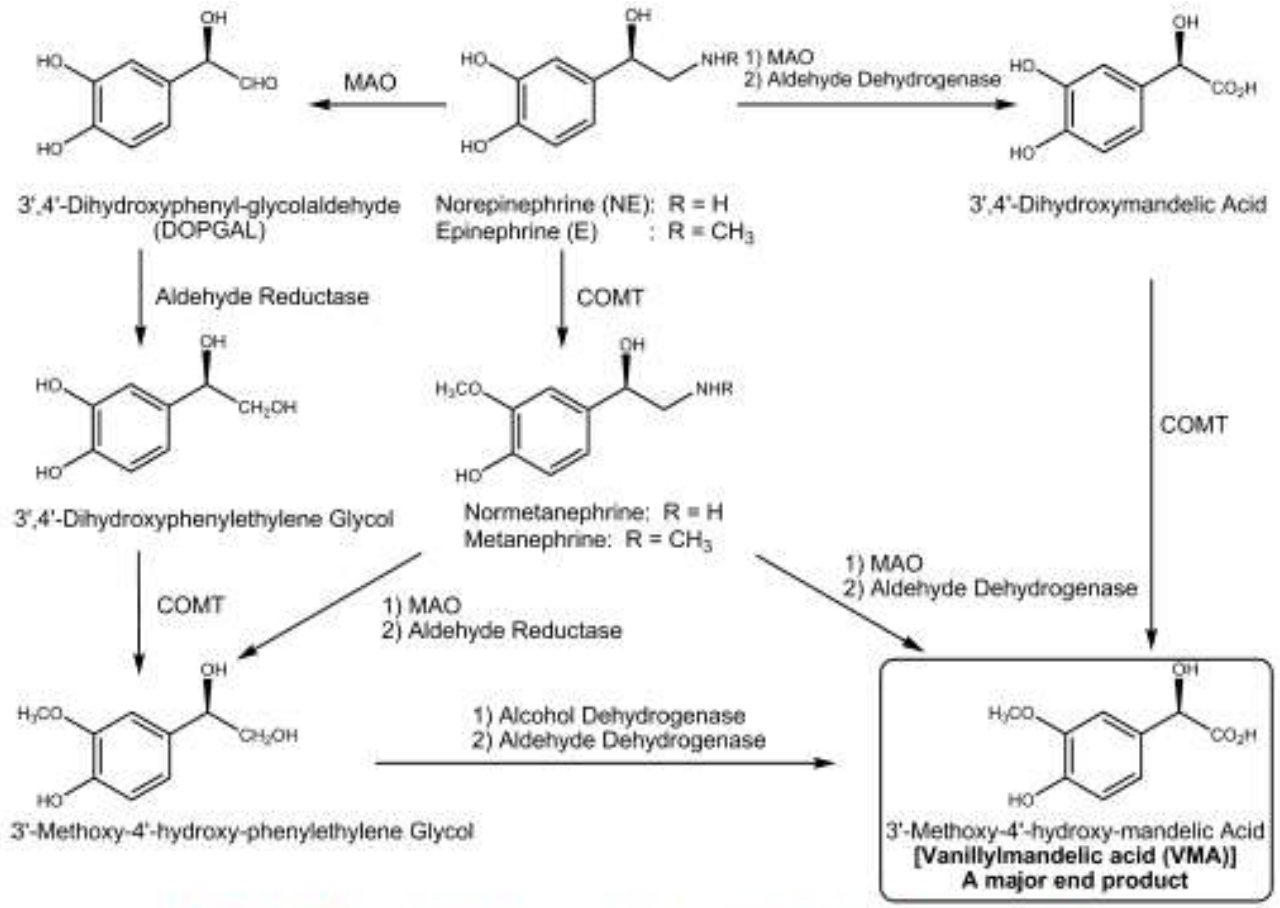


Figure 16.5 • Metabolism of norepinephrine and epinephrine by MAO and COMT.

Adrenergic Receptors(AR)

--Membrane receptors transfer information from the environment to the cell's interior. A few nonpolar signal molecules such as estrogens and other steroid hormones are able to diffuse through the cell membranes and enter the cell.

--Most signaling molecules such as CAs are too polar to pass through the membrane, and no appropriate transport systems are available. Thus, the information that they present must be transmitted across the cell membrane without the molecules themselves entering the cell. A membrane-associated receptor protein such as adrenergic receptors often performs the function of information transfer across the membrane.

(AR)

--Classifying α - and β-adrenoceptors is based on their apparent drug sensitivity. The diverse physiological responses of CAs are mediated via α1-,α2-,β1-,β2 and β3.

•

(AR)

--They all belong to the superfamily of guanine • nucleotide (G)-regulatory proteins i.e.(G-protein–coupled receptors (GPCR)). Different structurally related recepto-rs regulate distinct physiological process by controlling the synthesis or release of various second messengers. ***An important factor in the response of any cell or organ to adrenergic drugs is the density and proportion of α- and β-adrenoceptors.*** For example, NE has relatively little capacity to increase bronchial airflow, because the receptors in bronchial smooth muscle are largely of the β2-subtype. In contrast, isoproterenol (ISO) and E are potent bronchodilators

(AR)

- ***<u>The various adrenoceptor types and subtypes</u>*** are not uniformly distributed with certain tissues containing more of one type than another(Table 16.3). The clinical use of receptors-selective drugs becomes obvious when we consider the adrenoceptor subtypes and their locations.

AR

TABLE 16.3 Distribution and Effects of Adrenoceptors and Main Uses of the Adrenergic Drugs

Organ or Tissue	Predominant Adrenoceptors	Effect of Activation	Physiological Effect	Drugs	Therapeutic Uses
Blood vessels and skin	α_1	Vasoconstriction	↑ Blood pressure	α_1-Agonists	Shock, hypotension
Mucous membranes	α_1	Vasoconstriction		α_1-Agonists α_1-Antagonists	Nasal congestion Hypertension
Prostatic gland muscle	α_{1A}	Contraction	Prostatic hyperplasia	α_{1A}-Antagonists	BPH
CNS	α_2	↓ NE release	↓ Blood pressure	α_2-Agonists	Hypertension
Heart muscle	β_1 (minor β_2, β_3)	Muscle contraction	↑ Heart rate & force	β_1-Antagonists	Hypertension Arrhythmias
Bronchial smooth muscle	α_1	Smooth muscle contraction	Closes airways		
	β_2 (Bronchodilation)	Smooth muscle relaxation	Dilates & opens airways	β_2-Agonists	Asthma and COPD
Uterus (pregnant)	α_1	Muscle contraction			
	β_2	Smooth muscle relaxation	(−) Uterine contractions	β_2-Antagonists	Premature labor
Kidney	β_1	Increases rennin secretion	↑ Blood pressure		

(αAR)

A-The postsynaptic α-receptors be designated α1 and that presynaptic α-receptors be referred to as α2.

B- Later, a functional classification of the α-receptors was proposed wherein α1-receptors were designated as those that were excitatory, while α2-receptors purportedly mediated inhibitory responses.

C- Further developments revealed, however, that both α1- and α2-receptors could be either presynaptic or postsynaptic and either excitatory or inhibitory in their responses. However, the physiologic significance of postsynaptic α2-receptors is less well understood.

(αAR)

- Pharmacological and molecular biological methods have shown that it is possible to subdivide the α1- and α2-receptors into additional subtypes.

αAR

--The interaction of CAs or adrenergic drugs to the receptors alters the tertiary or quaternary structure of the receptor, including the intracellular domain.The information by CAs or drugs, which act as primary messengers, must be transduced into downstream activity that can alter the biochemistry of the cell. The signal–transduction mechanisms involve coupling to G proteins. G proteins of particular importance for adrenoceptor function include Gs, the stimulatory G protein of adenylyl cyclase (AC); Gi, the inhibitory G protein of AC; and Gq, the protein coupling receptors to phospholipase C (PLC).

αAR

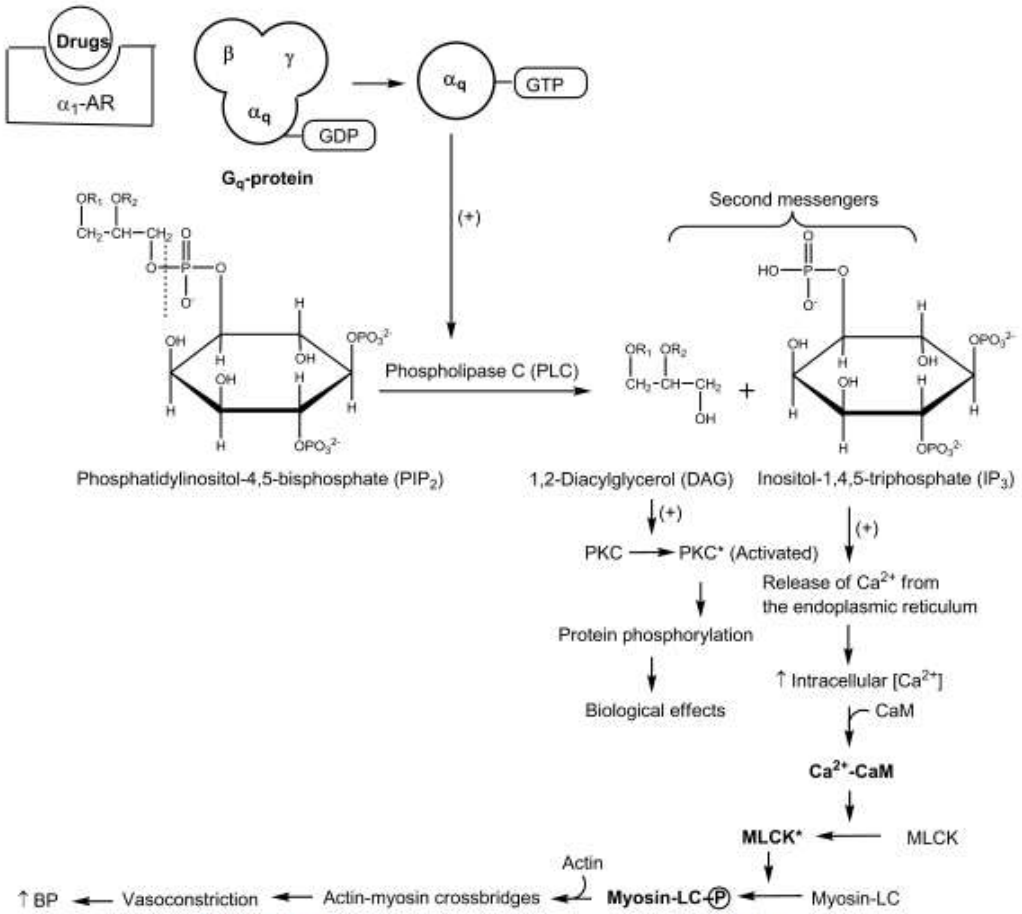


Figure 16.6 • Mechanism of α_1-receptor–mediated signal transduction.

(αAR)

- α-Receptors of the CNS and in peripheral tissues perform a number of important physiological functions. α-receptors are involved in control of the cardiovascular system. For example, constriction of vascular smooth muscle is mediated by α1-receptors. In the heart, activation of α1-receptors results in a selective inotropic response with little or no change in heart rate.
In contrast , the β1-receptor, which is the predominant postjunctional receptor in the heart, is mediating both inotropic and chronotropic effects.

αAR

--In the brain, activation of α2-receptors reduces sympathetic outflow from the CNS, which in turn causes a lowering of blood pressure.

- Interaction of the prototypical presynaptic α2- receptor with agonists such as NE and E results in inhibition of NE release from the terminal sympathetic neuron. The α2-receptors not only play a role in the regulation of NE release but also regulate the release of other NTs, such as acetylcholine and serotonin. Both α1-and α2-receptors also play an important role in the regulation of several metabolic processes, such as insulin secretion and glycogenolysis.

(βAR)

- Three β-receptor subtypes have been cloned, including β_1,β_2, and ,later, a third subtype in brown adipose tissue designated as the β_3-subtype.

(βAR)

- --α1,β1, β2 and β3receptors are postsynaptic and linked to stimulation of biochemical processes in the postsynaptic cell.

- --The β-adrenoceptor subtypes also differ in terms of the rank order of potency of the adrenergic receptor agonists NE, E, and ISO (Table 16.4):-

βAR

TABLE 16.4 Subtypes of Adrenoceptors and Their Effector Systems

Receptor	Agonists	Antagonists	G Protein	Main Biochemical Effectors
α_1	E ≥ NE >> ISO Phenylephrine Methoxamine	Prazosin Corynanthine	G_q	(+) PLC → ↑ IP_3 & DAG ↑ Ca^{2+}
α_2	E ≥ NE >> ISO Clonidine	Yohimbine	G_i G_i($\beta\gamma$ subunits)	(−) AC → ↓ cAMP ↑ K^+ channels
β-type	Isoproterenol	Propranolol	G_s	(+) AC → ↑ cAMP
β_1	ISO > E = NE Dobutamine	Metoprolol Betaxolol	G_s	(+) AC → ↑ cAMP ↑ L-type Ca^{2+} channels
β_2	ISO > E > NE Terbutaline	Butoxamine	G_s	(+) AC → ↑ cAMP

G_s, the *s* in subscript indicates the subunit's stimulatory role; G_i, the *i* in subscript indicates the subunit's inhibitory role; Gq, the *q* in subscript indicates the protein coupling receptors to phospholipase C.

βAR

- The β1-receptors are located mainly in the heart, where they mediate the positive inotropic and chronotropic effects of the CAs. They are also found on the juxtaglomerular cells of the kidney, where they are involved in increasing renin secretion. The β2-receptors are located on smooth muscle throughout the body, where they are involved in relaxation of the smooth muscle, producing such effects as bronchodilation and vasodilation. They are also found in the liver, where they promote glycogenolysis. The β3-receptor is located on brown adipose tissue and is involved in the stimulation of lipolysis.

βAR

--Cloning of the gene and complementary DNA (cDNA) for the mammalian β-receptor has made it possible to explore the structure–function relationships of the receptor.

-

βAR

--Specifically, an aspartic acid residue in transmembrane region III (TM-III) acts as the counterion to the cationic amino group of the adrenergic agonist.

- Two serine residues in TM-V, form H-bonds with the catechol OH groups of the adrenergic agonists.

The β-OH group of adrenergic agonists is thought to form an H-bond with the side chain of asparagine 293 in TM-VI, whereas the phenylalanine residue at position 290 in the same TM-VI is believed to interact with the catechol ring (Fig. 16.8).

Because the serine is in the fifth membrane-spanning region and the aspartic acid is in the third, it is likely that CAs bind parallel to the plane of the membrane, forming a bridge between the two TM-spanning regions.

βAR

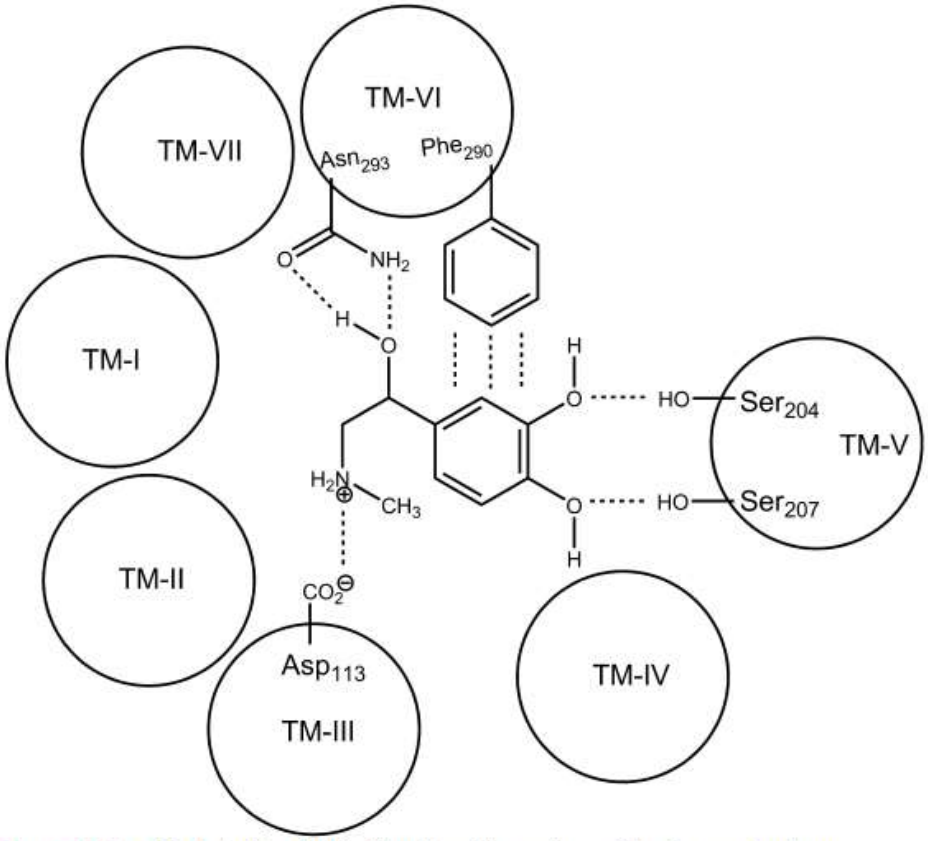


Figure 16.8 • Model of β_2-AR binding sites: Illustration of the Easson-Stedman hypothesis representing the interaction of three critical pharmacophoric groups of norepinephrine with the complementary binding areas on the adrenergic receptor as suggested by site-directed mutagenesis studies.

βAR

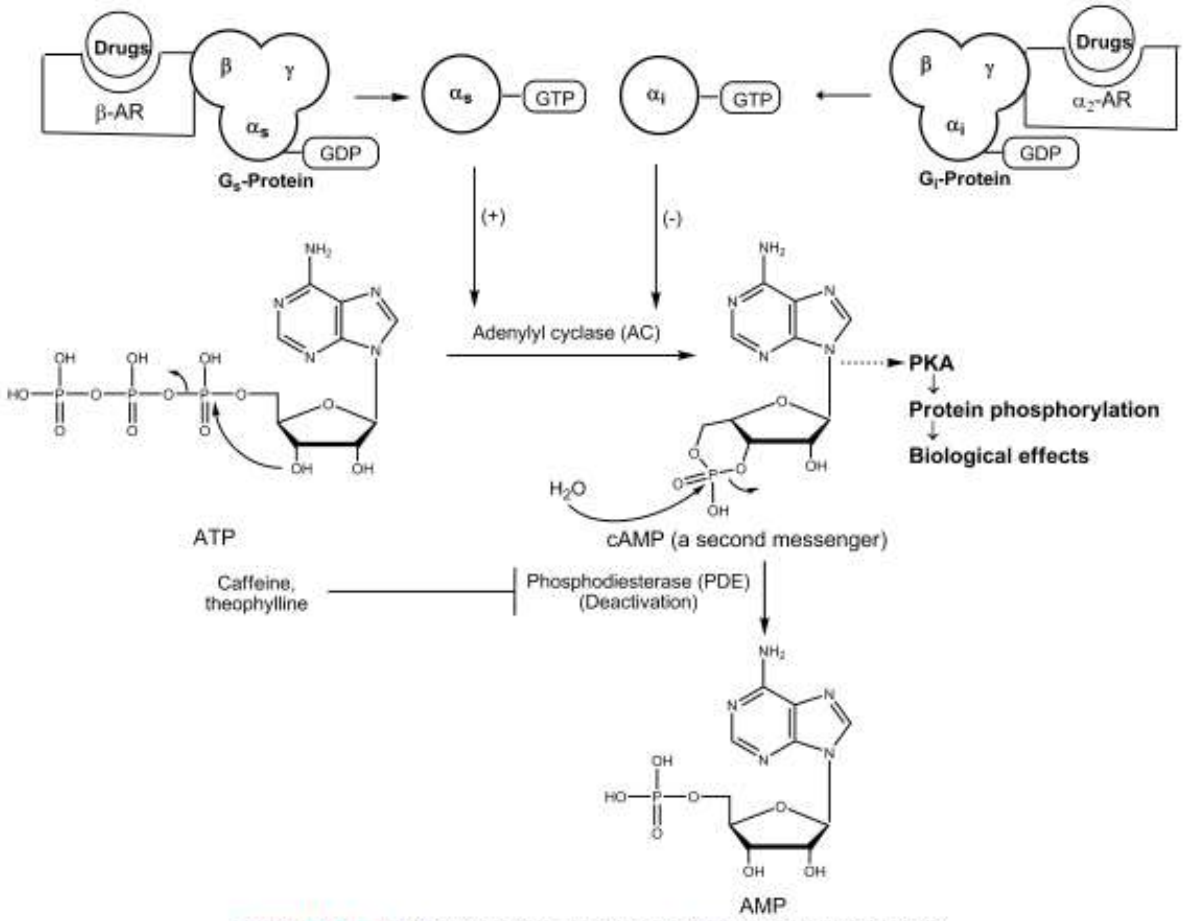


Figure 16.7 • Mechanism of β_2-receptor–mediated signal transduction.

βAR

- Molecular biological techniques have shown the existence of adrenergic receptor polymorphism for both the α-and β-receptors. It is postulated that such polymorphisms may be an important factor behind individual differences in responses to drugs acting at these receptors. In addition, there may be an association between the polymorphisms of adrenergic receptor, genes and disease states.

Drugs Affecting Adrenergic Neurotransmission(DAAN)

- Drugs Affecting CA Biosynthesis

DAB

- ***Metyrosine (α-Methyl-L-tyrosine).*** Inhibitors of the first and the rate-limiting enzyme TH would be the most effective. Metyrosine is a much more effective competitive inhibitor of E and NE biosynthesis. It is often possible to “fool” the enzymes into accepting structurally similar and unnatural substrates such as metyrosine. Metyrosine differs structurally from tyrosine only in the presence of an α-methyl group (Fig. 16.9). It is one example of a CA biosynthesis inhibitor in clinical use. Although metyrosine is used as a racemic mixture, it is the (-) isomer that possesses the inhibitory activity.

DAB

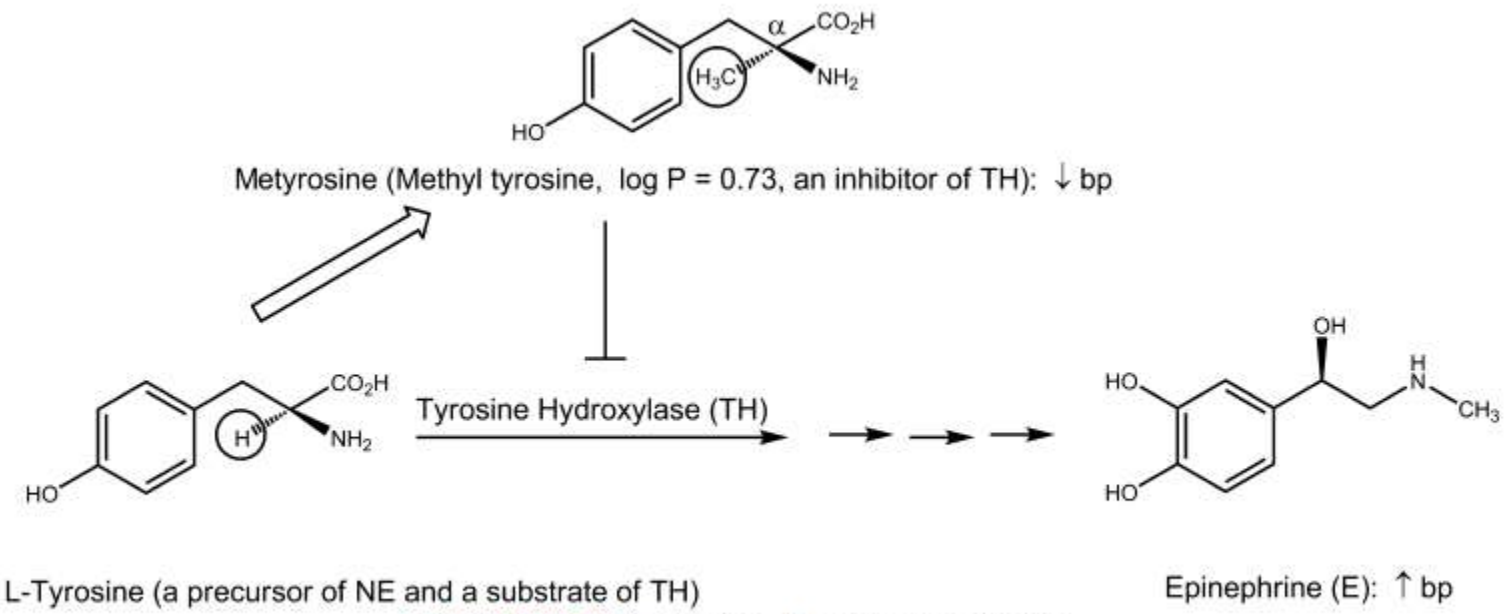


Figure 16.9 • Mechanism of action of metyrosine.

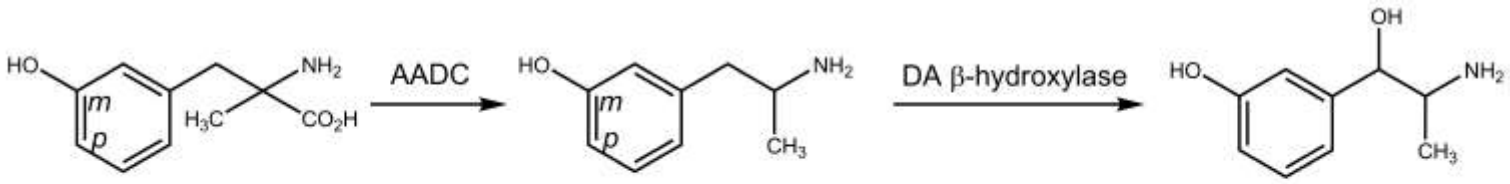
DAB

The drug is polar (log P=0.73) and excreted mainly unchanged in the urine. Because of its limited solubility in water caused by intramolecular bonding of the zwitterions, crystalurea occurred . Inhibitors of CA synthesis have limited clinical utility because such agents nonspecifically inhibit the formation of all CAs and result in many side effects. Sedation is the most common side effect of this drug.

DAB

- A similar example is the use of α-methyl-*m*-tyrosine in the treatment of shock. It differs structurally from metyrosine only in the presence of *m*-OH instead of *p*-OH in metyrosine. This unnatural amino acid is accepted by the enzymes of the biosynthetic pathway and converted to metaraminol (an α1-agonist) as shown (Fig. 16.10). Inhibitors of AADC (e.g., carbidopa) have proven to be clinically useful, but not as modulators of peripheral adrenergic transmission. Rather these agents are used to inhibit the metabolism of drug L-DOPA administered in the treatment of Parkinson disease..

DAB



α-Methyl-*m*-tyrosine α-Methyl-*m*-tyramine Metaraminol (α_1-agonist)

Figure 16.10 • Metabolic activation of α-methyl-*m*-tyrosine to metaraminol.

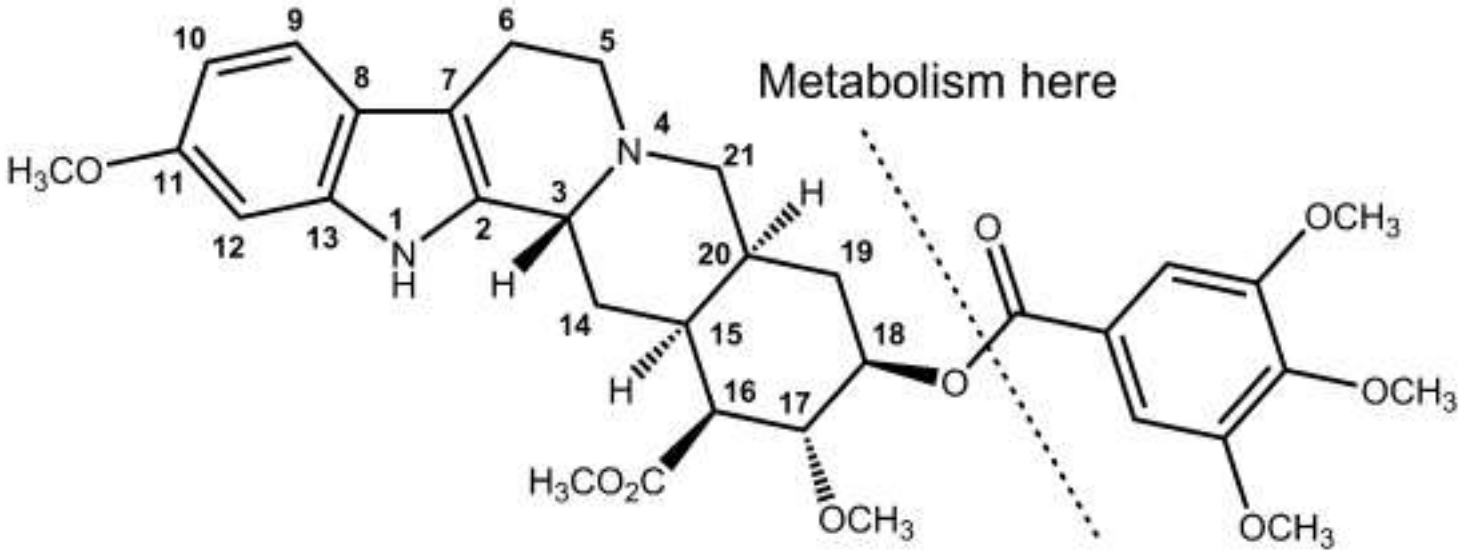
DAAN

- Drug Affecting Storage And Release Of CA

DASR

- **Reserpine (an NT Depleter).** Reserpine is an indole alkaloid obtained from the root of *Rauwolfia serpentina* found in India. As is typical of many indole alkaloids, reserpine is susceptible to decomposition by light and oxidation. Reserpine is extensively metabolized through hydrolysis of the ester function at position 18 and yields methyl reserpate and 3,4,5-trimethoxybenzoic acid. It not only depletes the vesicle storage of NE in sympathetic neurons in PNS, neurons of the CNS, and E in the adrenal medulla, but also depletes the storage of serotonin and DA in their respective neurons in the brain. Reserpine binds extremely tightly with and blocks VMAT that transports NE and other biogenic amines from the cytoplasm into the storage vesicles.

DASR



Reserpine (Log P = 4.37, Log D = 3.93)

DASR

- Thus in sympathetic neurons, NE, which normally is transported into the storage vesicles, is instead metabolized by mitochondrial MAO in the cytoplasm. In addition, there is a gradual loss of vesicle-stored NE as it is used up by release resulting from sympathetic nerve activity so that the storage vesicles eventually become dysfunctional. The end result is a depletion of NE in the sympathetic neuron.

DASR

A sustained effect up to several weeks is seen after the last dose has been given. This is because the tight binding of reserpine to storage vesicles continues for a prolonged time, and recovery of sympathetic function requires synthesis of new vesicles over a period of days to weeks after discontinuation of the drug. Most adverse effects of reserpine (log P = 4.37) are caused by CNS effects because it readily enters the CNS. Sedation and inability to concentrate or perform complex tasks are the most common adverse effects. More serious is the occasional psychotic depression that can lead to suicide, which support monoamine theory of pathology of depression. Agents with fewer side effects have largely replaced reserpine in clinical use.

-

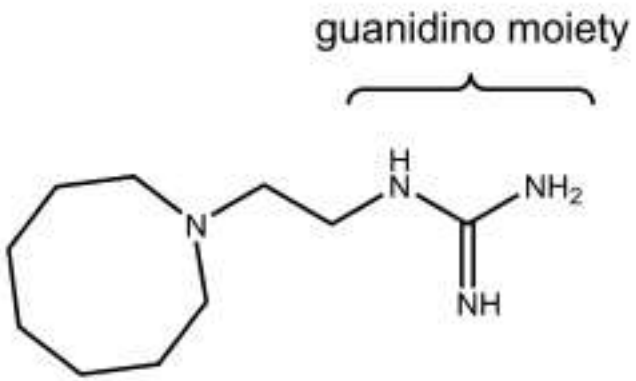
DASR

- ***Guanethidine (Ismelin) and guanadrel (Hylorel)*** are seldom used orally active antihypertensives. Drugs of this type enter the adrenergic neuron by way of the uptake-1 process and accumulate within the neuronal storage vesicles. There they bind to the storage vesicles and stabilize the neuronal storage vesicle membranes, making them less responsive to nerve impulses. The ability of the vesicles to fuse with the neuronal membrane is also diminished, resulting in inhibition of NE release into the synaptic cleft in response to a neuronal impulse and generalized decrease in sympathetic tone. Long-term administration of some of these agents also can produce a depletion of NE stores in sympathetic neurons.

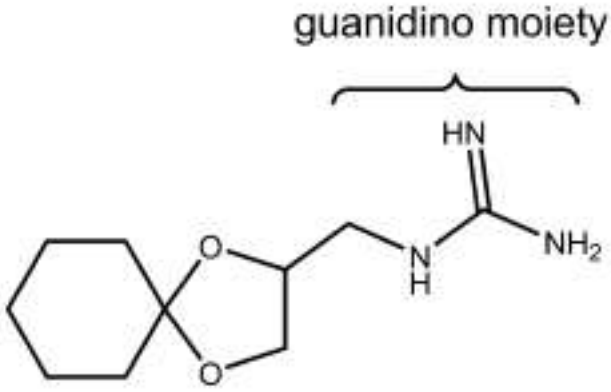
DASR

- Both neuronal blocking drugs possess a guanidino moiety which is attached to either a hexahydroazocinyl ring linked by an ethyl group as in guanethidine, or a ring linked by a methyl group as in guanadrel. The presence of the more basic guanidino group (pK_a =12) than the ordinary amino group in these drugs means that at physiological pH, they are essentially completely protonated. Thus, these agents do not get into the CNS. As a result, this drug has none of the central effects seen with many of the other adrenergic antihypertensive agents described. Guanethidine contains two basic nitrogen atoms with pK_a values of 9.0 and 13.43, and can therefore form guanethidine monosulfate ($C_{10}H_{22}N_4 . H_2SO_4$) or guanethidine sulfate [$(C_{10}H_{22}N_4)_2 . H_2SO_4$].

DASR



Guanethidine
pKa = 13.43
No CNS activity



Guanadrel
pKa = 12.76
No CNS activity

Sympathomimetic Agents

Sympathomimetic agents produce effects resembling those produced by stimulation of the sympathetic nervous system . They may be classified as agents that produce effects by a direct,indirect, or mixed mechanism of action. Direct-acting agents elicit sympathomimetic response by interacting directly with adrenergic receptors.

•

SA

- Indirect-acting agent exert their effects by inducing release of NE from adrenergic nerve terminals; the NE that is released by the indirect-acting agent activates the receptors to produce the response.
- Compounds with a mixed mechanism of action interact directly with adrenergic receptors and indirectly cause the release of NE. As described later, the mechanism by which an agent produces its sympathomimetic effect is related intimately to its chemical structure.

Direct-Acting Sympathomimetics

-

(SAR)

SA

- The parent structure for many of the adrenergic drugs is β-phenylethylamine (Fig.16.11). The manner in which β-phenylethylamine is substituted on the *meta*- and *para*-positions of the aromatic ring, on the amino (R_1), and on α-(R_2) and β-positions of the ethylamine side chain influences not only their mechanism of action and the receptor selectivity, but also their absorption, oral activity, metabolism, degradation, and thus duration of action (DOA). For the direct-acting sympathomimetic amines, maximal activity is seen in β-phenylethylamine derivatives containing (a) a catechol and (b) a (1*R*)-OH group on the ethylamine portion of the molecule. Such structural features are seen in the prototypical direct-acting compounds NE, E, and ISO. The SARs are supported by the model of β2- AR binding studies (Fig. 16.8).

SA

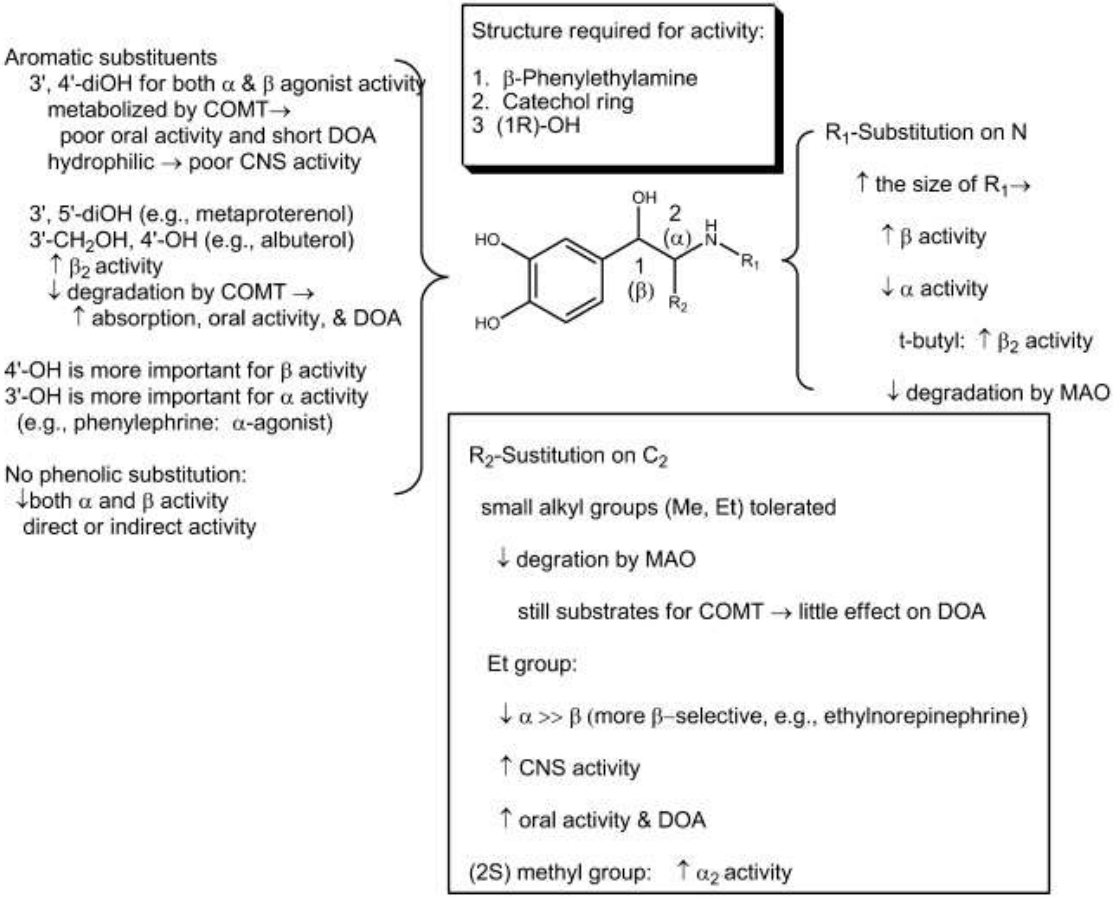


Figure 16.11 • Structure–activity relationship of adrenergic phenylethylamine agonists.

SA

- ***Optical Isomerism.*** A critical factor in the interaction of adrenergic agonists with their receptors is stereoselectivity. Substitution on either carbon-1 or carbon-2 yields optical isomers. (1*R*,2*S*) isomers seem correct configuration for direct-acting activity. For CAs, the more potent enantiomer has the (1*R*) configuration. This enantiomer is typically several 100-fold more potent than the enantiomer with the (1*S*) configuration. For all direct-acting phenylethylamine-derived agonists that are structurally similar to NE, the more potent enantiomer is capable of assuming a conformation that results in the arrangement in space of the catechol group, the amino group, and the (1*R*)-OH group in a fashion resembling that of (1*R*)-NE. This explanation of stereoselectivity is based on the presumed interaction of these three critical pharmacophoric groups with three complementary binding areas on the receptor and is known as the Easson-Stedman hypothesis. This three-point interaction is illustrated in(Figure16.8).

SA

Separation of Aromatic Ring and Amino Group.

By far, the greatest adrenergic activity occurs when two carbon atoms separate the aromatic ring from the amino Group with few exceptions to all types of activities.

R1, Substitution on the Amino Nitrogen Determines α- or β-Receptor Selectivity.

1- The amine is normally ionized at physiological pH. This is important for direct agonist activity, because replacing nitrogen with carbon results in a large decline in activity.

SA

- 2- The activity is also affected by the number of substituents on the nitrogen. ***Primary and secondary*** amines have good adrenergic activity, whereas tertiary amines and quaternary ammonium salts do not.
- 3- The nature of the amino substituent also dramatically affects the receptor selectivity of the compound. As the size of the nitrogen substituent increases, α-receptor agonist activity generally decreases and β-receptor agonist activity increases . Thus, ***NE*** has more α-activity than β-activity and ***E*** is a potent agonist at α1-, β1-, and β2-receptors. ***ISO***, however, is a potent β1- and β2-agonist but has little affinity for α-receptors

SA

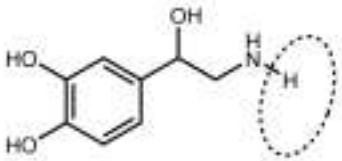
4- The nature of the substituents can also affect β1- and β2-receptor selectivity. In several instances, it has been shown that a β2 directing *N-tert*-butyl group enhances β2-selectivity. For example, ***N-tert-butylnorepinephrine (Colterol)*** is 9 to 10 times more potent as an agonist at tracheal β2-receptors than at cardiac β1-receptors. These results indicate that the β-receptor has a larger lipophilic binding pocket adjacent to the amine-binding aspartic acid residue than do the α-receptors.

•

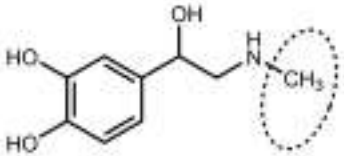
SA

- 5- Increasing the length of the alkyl chain offers no advantage, but if a polar functional group(adding a phenol group) to the end of alkyl chain results in a dramatic rise in activity, indicating that an extra polar-binding region has been accessed, which can take part in H-bonding. The activity of the ***extension analog*** is thereby increased by a factor of 800.
6- As R_1 becomes larger than butyl group, it can provide compounds with α1-blocking activity ***(e.g., tamsulosin and labetalol).*** Large substituents on the amino group also protect the amino group from undergoing oxidative deamination by MAO.

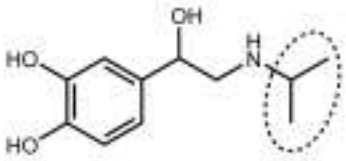
SA



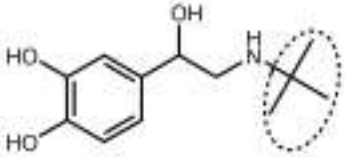
Norepinephrine (NE)
α > β agonist
α agonist



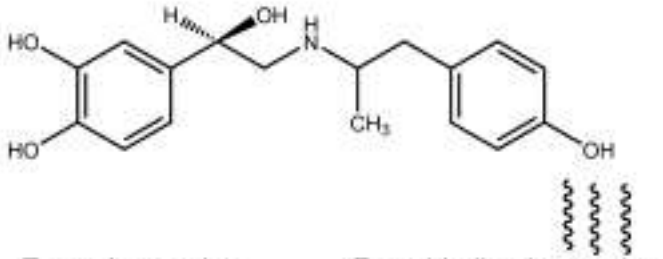
Epinephrine (E)
α, β_1 and β_2 agonist
nonselective α and β agonist



Isoproterenol (ISO)
β_1 and β_2 agonists
nonselective β agonist



N-*t*-Butylnorepinephrine (Colterol)
selective β_2 agonist



Extension analog

Extra binding interaction

SA

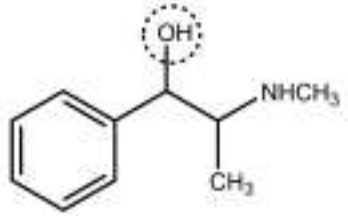
R2, Substitution on the α-Carbon (Carbon-2).

- 1- Substitution by (methyl or ethyl) slows metabolism by MAO but has little overall effect on DOA of catechols because they remain substrates for COMT. However, the resistance to MAO activity is more important in noncatechol indirect-acting phenylethylamine. Because addition of small alkyl group increases the resistance to metabolism and lipophilicity,such compounds often exhibit enhanced oral effectiveness and greater CNS activity than their counterparts that do not contain an α-alkyl group.
- 2- In addition, compounds with an α-methyl substituent persist in the nerve terminals and are more likely to release NE from storage sites. For example, ***metaraminol*** is an α-agonist and also exhibits a greater degree of indirect sympathomimetic activity.

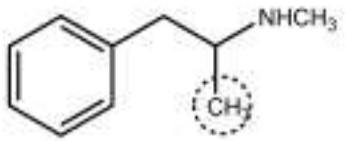
SA

- 3- Methyl or ethyl substitution on the α-carbon of the ethylamine side chain reduces direct agonist activity at both α- and β-receptors. α-substitution also significantly affects receptor selectivity. An ethyl group in this position diminishes α-activity far more than β-activity, affording compounds with β-selectivity (e.g., ***ethylnorepinephrine*** and ***isoetharine***). In the case of β-receptors, for example, α-ethyl substitution results in compounds toward the β2-selectivity, whereas in the case of α-receptors, α-methyl substitution gives compounds toward the α2-selectivity.
  4- α-substitution will lead to introduction of a chiral center, which has pronounced effects on the stereochemical requirements for activity. For example, with α-methylnorepinephrine, it is the *erythro* (1*R*,2*S*) isomer that possesses significant activity at α2-receptors.

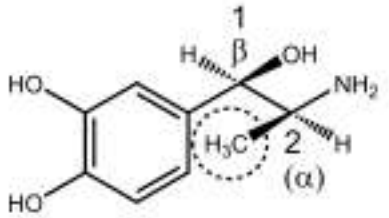
SA



Ephedrine (Log P = 1.05)
more α and β activity
less lipophilic → less CNS activity



Methamphetamine (Log P = 1.97)
less α and β activity
more lipophilic → more CNS activity



(1R, 2S)-α-Methylnorepinephrine
active isomer
selective α_2 agonist

SA

- ***OH substitution on the β-carbon (carbon-1) : 1-*** generally decreases CNS activity largely because it lowers lipid solubility.
2- Such substitution greatly enhances agonist activity at both α- and β-receptors. For example, ***ephedrine*** is less potent than methamphetamine as a central stimulant, but it is more powerful in dilating bronchioles and increasing blood pressure and heart rate.
3- Compounds lacking the –OH group (e.g. DA) have a greatly reduced adrenergic receptor activity. Some of the activity is retained, indicating that OH group is important but not essential.
4- The R-enantiomer of NE is more active than the S-enantiomer, indicating that the secondary alcohol is involved in an H-bonding interaction.

SA

- ***<u>Substitution on the Aromatic Ring</u>. 1-*** Maximal α- and β-activity also depends on the presence of 3’ and 4’ catechol OH groups.Tyramine, which lacks two OH groups, has no affinity for adrenoceptors, indicating the importance of the OH groups. Studies of β-adrenoceptor structure suggest that the OH groups on serine residues 204 and 207 probably form H bonds with the catechol OH groups at positions 3’ and 4’, respectively.

SA

- 2- Catechol moiety is an important structural feature for maximal adrenoceptor agonist activity, catechol moiety can be replaced with other substituted phenyl moieties to provide and design selective β2-agonists.

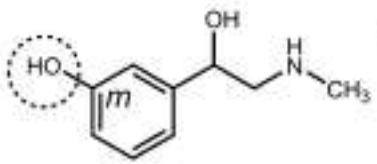
A- For example, replacement of the catechol function of ISO with the resorcinol structure gives a selective β2-agonist, ***metaproterenol***. Furthermore, because the resorcinol ring is not a substrate for COMT, β-agonists that contain this ring structure tend to have better absorption characteristics and a longer DOA than their catechol-containing counterparts.

B- In another approach, replacement of the *meta*-OH of the catechol structure with a hydroxymethyl group gives agents, such as ***albuterol***, which show selectivity to the β2-receptor.

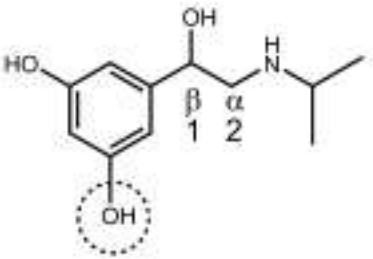
SA

- 3- Modification of the catechol ring can also bring about selectivity at α-receptors that the catechol moiety is more important for α_2-activity than for α_1-activity. For example, removal of the *p*-OH group from E gives ***phenylephrine*** which is selective for the α_1-receptor. Phenylephrine is less potent than E at both α- and β-receptors, with β_2-activity almost completely absent.
- 4- *m-OH* group can be replaced by other groups capable of interacting with the binding site by H-bonding. It can be replaced by CH_2OH, NHMe, NHCOR, NMe_2, or $NHSO_2R$ group.

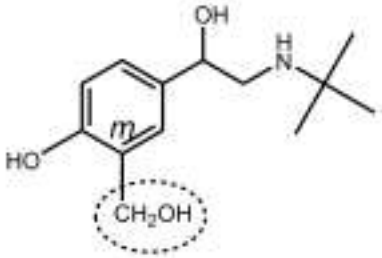
SA



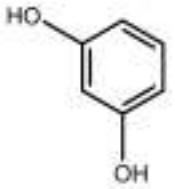
Phenylephrine
less α and β activity than NE
selective α_1 agonist
almost no β activity



Metaproterenol
selective β_2 agonist
not metabolized by COMT →
better absorption & longer DOA



Albuterol
selective β_2 agonist
not metabolized by COMT →
better oral bioavailability



Resorcinal

SA

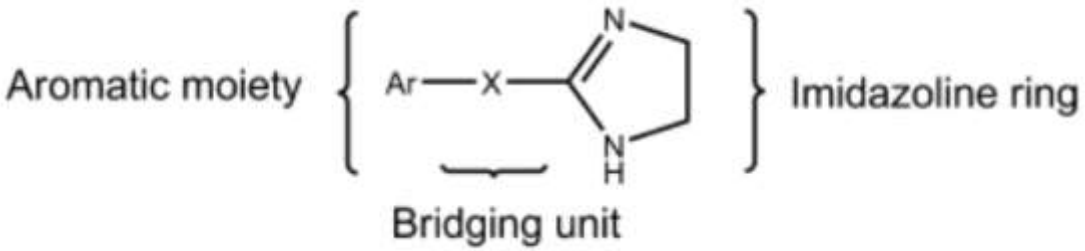
- ***Agents without OH Groups.*** Phenylethylamines that lack OH groups on the ring and the β-OH group on the side chain act almost exclusively by causing the release of NE from sympathetic nerve terminals and thus results in a loss of direct sympathomimetic activity. OH substituents on the phenylethylamine structure makes the resultant compounds less lipophilic whereas unsubstituted or alkylsubstituted compounds cross the BBB more readily and have more central activity. Thus, amphetamine and methamphetamine exhibit considerable CNS activity. CAs per oral have only a brief DOA and are almost inactive, because they are rapidly inactivated in the intestinal mucosa and in the liver before reaching the systemic circulation. In contrast, compounds without one or both phenolic
- OH substituents are, however, not metabolized by COMT, and they are orally active and have longer DOA.

SA

Imidazolines and α-Adrenergic Agonists. A second chemical class of α-agonists, the imidazolines, which give rise to α-agonists and are thus vasoconstrictors. These imidazolines can be nonselective, or they can be selective for either α_1- or α_2-receptors. Structurally, most imidazolines have their heterocyclic imidazoline nucleus linked to a substituted aromatic moiety via some type of bridging unit (Fig. 16.12). The optimum bridging unit (X) is usually a single methylene group or amino group. Modification of the imidazoline ring generally results in compounds with significantly reduced agonist activity. Agonist activity is enhanced when the aromatic ring is substituted with halogen substituents like chlorine (Cl) or small alkyl groups like methyl group, particularly when they are placed in the two *ortho* positions.

-
-

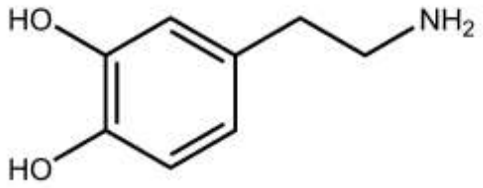
SA



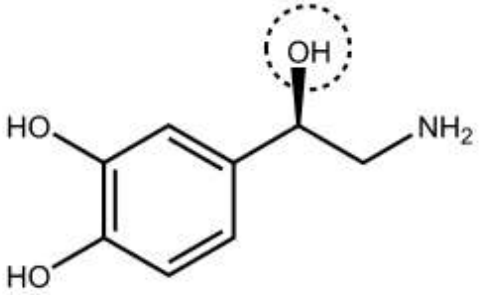
SA

- **ENDOGENOUS CATECHOLAMINES**: The three naturally occurring catecholamines DA, NE, and E are used as therapeutic agents.
- ***Dopamine.*** (DA, 3',4'-dihydroxyphenylethylamine) differs from NE in lacking of 1-OH group. It is a central NT particularly important in the regulation of movement. As a catechol and primary amine, DA is rapidly metabolized by COMT and MAO and has a short DOA with no oral activity. It is used intravenously in treatment of shock. In contrast with the NE and E, DA increases blood flow to the kidney as a result of its agonist action on the D1-DA receptor.

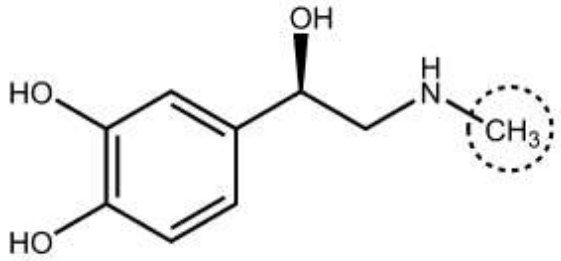
SA



Dopamine (DA, Log P = 0.12)



NE (Log P = -0.63)



E (Log P = 0.28)

All are polar and metabolized by both MAO and COMT → orally inactive and short duration of action

SA

- DA stimulates the β1-receptors of the heart to increase cardiac output and rate. Some of the effects of DA on the heart are also caused by NE release resulting in stimulation of α1-receptors, leading also to vasoconstriction and an increase in arterial blood pressure. DA should be avoided or used at a much reduced dosage if the patient has received an MAO inhibitor. Careful adjustment of dosage also is necessary in patients who are taking tricyclic antidepressants.

SA

- ***Norepinephrine (NE)*** differs from DA only by addition of a 1-OH substituent (β-OH-DA) and from E only by lacking the *N*-methyl group. Like DA, it is polar and rapidly metabolized by both COMT and MAO, resulting in poor oral bioavailability and short DOA (1 or 2 minutes even when given intravenously). It is a stimulant of α_1-, α_2-, and β_1-adrenoceptors (notice that lacking the *N*-methyl group results in lacking β_2- and β_3-activity). It is used to counteract various hypotensive crises, because its α-activity raises blood pressure and as an adjunct treatment in cardiac arrest because its β-activity stimulates the heart. It has limited clinical application caused by the nonselective nature of its activities.

SA

- ***Epinephrine (E, Adrenalin)*** differs from NE only by the addition of an *N*-methyl group.It is used in aqueous solution for inhalation as the free amine. Like other amines, it forms salts with acids, hydrochloride and the bitartrate being the most common.

SA

- Like NE, it lacks oral activity and has short DOA. It is much more widely used clinically than NE. E is a potent stimulant of all α1-, α2-, β1-, β2-, and β3- adrenoceptors, and thus it switches on all possible adrenergic receptors, leading to a whole range of desired and side effects. Particularly prominent are the actions on the heart and on vascular and other smooth muscle. It is a very potent vasoconstrictor and cardiac stimulant. E has greater β-activity caused by an additional *N*-methyl group. Therefore, E is used to stimulate the heart in cardiac arrest.

SA

The ability of epinephrine to stimulate β_2-receptors has led to its use by injection and by inhalation to relax bronchial smooth muscle in asthma and in anaphylactic reactions. It is also used in inhibiting uterine contraction. Because of its α-activity, E is used to treat hypotensive crises and nasal congestion, to enhance the activity of local anesthetics, and as a constrictor in hemorrhage.

SA

- In addition, E is used in the treatment of open-angle glaucoma, where it apparently reduces intraocular pressure by increasing the rate of outflow of aqueous humor from the anterior chamber of the eye. The irritation often experienced on instillation of E into the eye has led to the development of other preparations of the drug that potentially are not as irritating. One such example is dipivefrin.

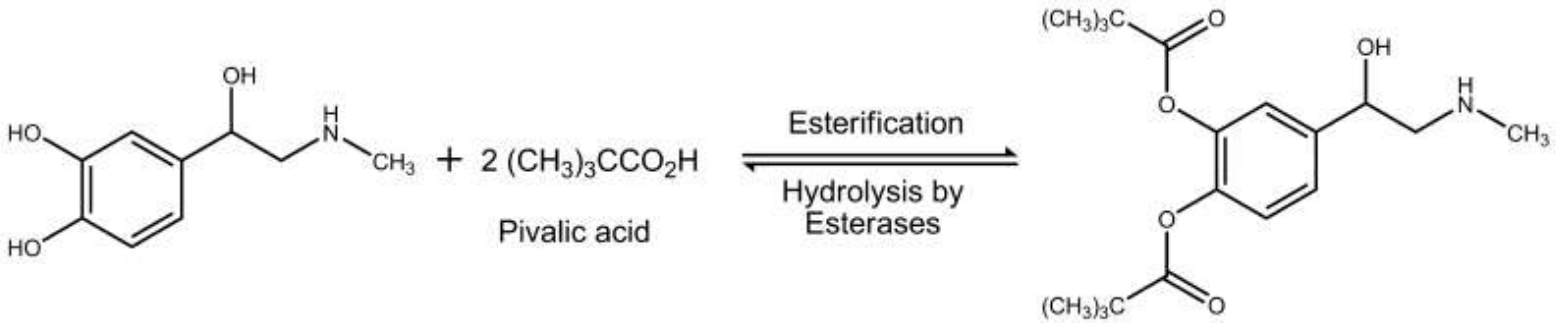
SA

- ***Dipivefrin .*** To overcome several of the pharmacokinetic and pharmaceutical shortcomings of E as an ophthalmic agent, the prodrug approach has been successfully applied. Dipivefrin is a prodrug of E that is formed by the esterification of the catechol OH groups of E with pivalic acid. Most of the advantages of this prodrug over E stem from improved bioavailability. The greatly increased lipophilicity allows much greater penetrability into the eye through the corneal epithelial and endothelial layer. The stroma in between requires hydrophilicity for penetration. Dipivefrin has that, too, due to the 1-OH group and cationic nitrogen (the eyedrops contain the hydrochloride [HCl] salt).

SA

- This dual solubility permits much greater penetrability into the eye than the very hydrophilic E hydrochloride. Increased DOA is also achieved because the drug is resistant to the metabolism by COMT.
- In addition to its increased in vivo stability, it is also less easily oxidized by air due to the protection of the catechol OH groups. After its absorption, it is converted to E by esterases slowly in the cornea and anterior chamber. Dipivefrin also offers the advantage of being less irritating to the eye than E.

SA



Epinephrine (E)
catechol: easily oxidized
less lipophilic than dipivefrin
poor absorption and poor penetration of the eye
irritating to eyes when used in treatment of glaucoma

Dipivefrin: Ester and prodrug of E
Noncatechol: less easily oxidazied
more lipophilic than E
better absorption and penetration of the eye
less irritating to the eye than E

α-ADRENERGIC RECEPTOR AGONISTS

All selective α1-agonists have therapeutic activity as vasoconstrictors. Structurally, they include (a) phenylethanolamines(phenylephrine , metaraminol and methoxamine .
(b) 2- arylimidazolines (xylometazoline, oxymetazoline , tetrahydrozoline and naphazoline).

SA

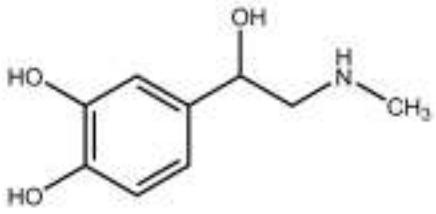
Phenylephrine. A prototypical selective direct-acting α1-agonist.

- differs from E only in lacking a *p*-OH group. It is orally active, and its DOA is about twice that of E because it lacks the catechol moiety and thus is not metabolized by COMT. However, its oral
- bioavailability is low because of its hydrophilic properties (log P= 0.3) and metabolism by MAO. It is less potent than E and NE. It is used similarly to metaraminol and methoxamine for hypotension or severe hypotension resulting from either shock or drug administration.

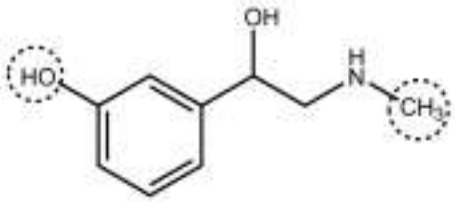
SA

- It also has widespread use as a nonprescription nasal decongestant in both oral and topical preparations. In the eye, it is used to dilate the pupil and to treat open-angle glaucoma. In addition, it is used in spinal anesthesia to prolong the anesthesia and to prevent a drop in blood pressure during the procedure. It produces little CNS stimulation. ***Metaraminol*** is just another example.

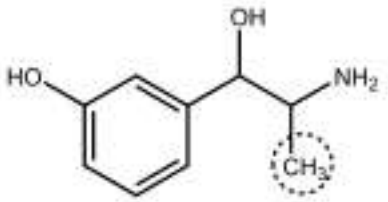
SA



Epinephrine (E)



Phenylephrine



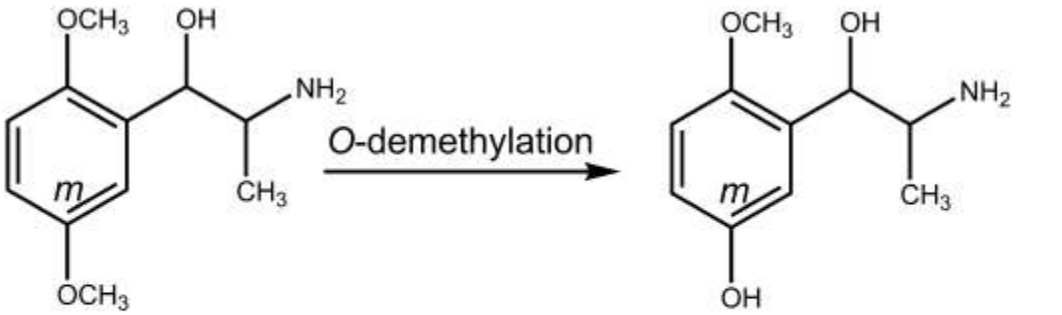
Metaraminol

SA

Methoxamine : α 1-agonist vasopressor. It is bioactivated by *O*-demethylation to an active *m*-phenolic metabolite. It is less potent than phenylephrine as a vasoconstrictor. Methoxamine is used primarily during surgery to maintain adequate arterial blood pressure, especially in conjunction with spinal anesthesia.Because it is not a substrate for COMT, its DOA is significantly longer than NE.

•

SA



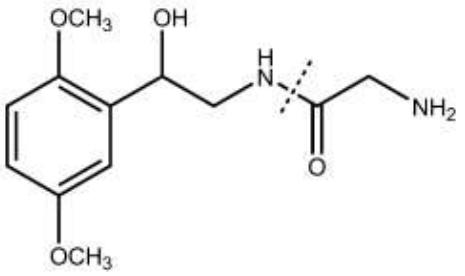
Methoxamine
Prodrug

An active *m*-phenolic metabolite
α_1 agonist and vasoconstrictor

SA

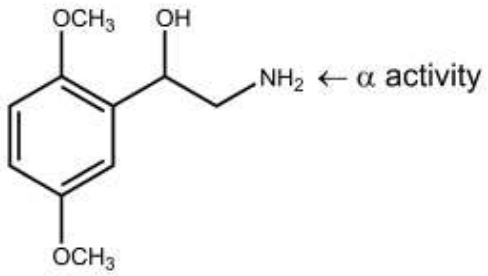
- ***Midodrine (ProAmatine)*** is the *N*-glycyl prodrug of the selective α1-agonist desglymidodrine. Removal of the *N*-glycyl moiety from midodrine occurs readily by amidases. Midodrine is orally active and represents another example of a dimethoxy--phenylethylamine derivative that is used therapeutically for its vasoconstrictor properties. Specifically, it is used in the treatment of symptomatic orthostatic hypotension.

SA



Midodrine: *N*-glycyl prodrug of desglymidodrine

amidases in the liver
and throughout the body



Desglymidodrine
α_1 agonist and vasoconstrictor
differs from methoxamine in lacking α-CH_3

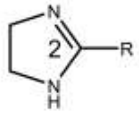
SA

- ***Naphazoline (Privine), tetrahydrozoline (Tyzine,Visine), xylometazoline (Otrivin), and oxymetazoline (Afrin)*** are 2-aralkylimidazolines α1 agonists. These agents are used for their vasoconstrictive effects as nasal and ophthalmic decongestants. All 2-aralkylimidazoline α1-agonists contain a one-carbon bridge between C-2 of the imidazoline ring and a phenyl ring, and thus a phenylethylamine structure feature is there. *Ortho*-lipophilic groups on the phenyl ring are important for α-activity.

SA

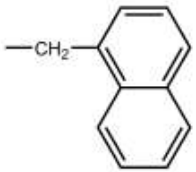
- *meta* or *para*-bulky lipophilic substituents on the phenyl ring may be important for the α1-selectivity. They have limited access to the CNS, because they essentially exist in an ionized form at physiological pH caused by the very basic nature of the imidazoline ring (pK_a=10–11). Xylometazoline and oxymetazoline have been used as topical nasal decongestants because of their ability to promote constriction of the nasal mucosa.

SA

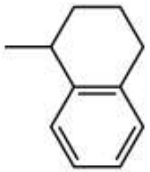


Imidazoline moity
pKa 9-10
Limited access to the CNS

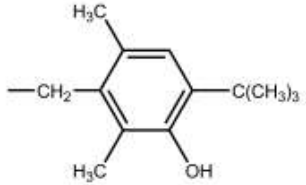
Naphazoline R =



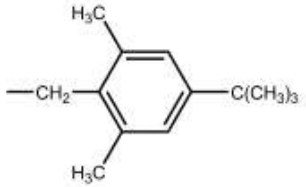
Tetrahydrozoline R =



Oxymetazoline R =



Xylometazoline R =



SA

- ***Clonidine (Catapress)*** differs from 2-arylimidazoline α1-agonists mainly by the presence of *o*-chlorine groups and a NH bridge. The *o*-chlorine groups afford better activity than *o*-methyl groups at α2 sites. Clonidine contains a NH bridge (aminoimidazolines) instead of CH_2 bridge in 2-arylimidazoline. The uncharged form of clonidine exists as a pair of tautomers. Clonidine is an example of the (phenylimino) imidazolidine derivatives that possess central α2-agonist selectivity.

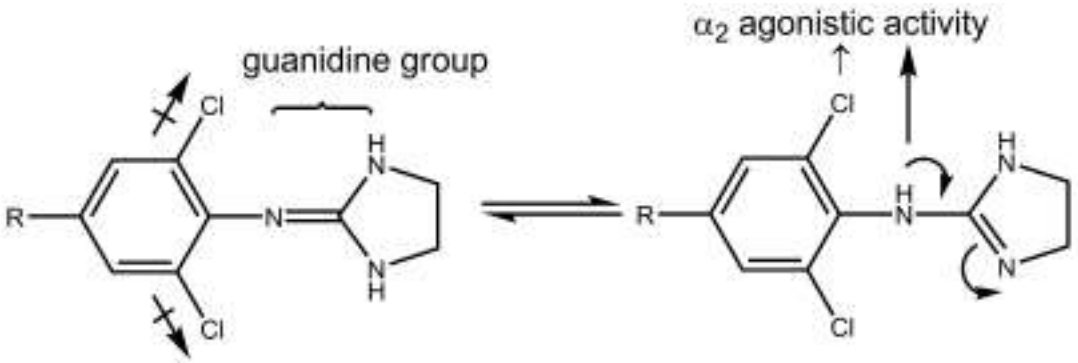
SA

- The ability of clonidine and its analogs to exert an antihypertensive effect depends on the ability of these compounds not only to interact with the α2-receptor in the brain but also to gain entry into the CNS. For example, in the case of clonidine, the basicity of the guanidine group (typically pK_a =13.6) is decreased to 8.0 (the pK_a of clonidine) because of the inductive and resonance effects of the dichloro phenyl ring. Thus, at physiological pH, clonidine will exist to a significant extent in the nonionized form required for passage into the CNS. It has an oral bioavailability of more than 90%.

SA

- Alkyl(methyl) substitutions can be placed at the two ortho positions of the (phenylimino) imidazolidine nucleus without affecting the affinity of the derivatives for α2-receptors but are much more readily metabolized to the corresponding acids (inactive) and thus have short DOA. Various halogen substituents such as chlorine seem to provide the optimal characteristics in this regard. One of the metabolites of clonidine, 4-hydroxyclonidine, has good affinity for α2-receptors, but because it is too polar to get into the CNS, it is not an effective antihypertensive agent.

SA



Clonidine (pKa = 8.0) : R = H some passage into the CNS

4-Hydroxyclonidine : R = OH no passage into the CNS

Apraclonidine (pKa = 9.22) : R = NH_2 no passage into the CNS

SA

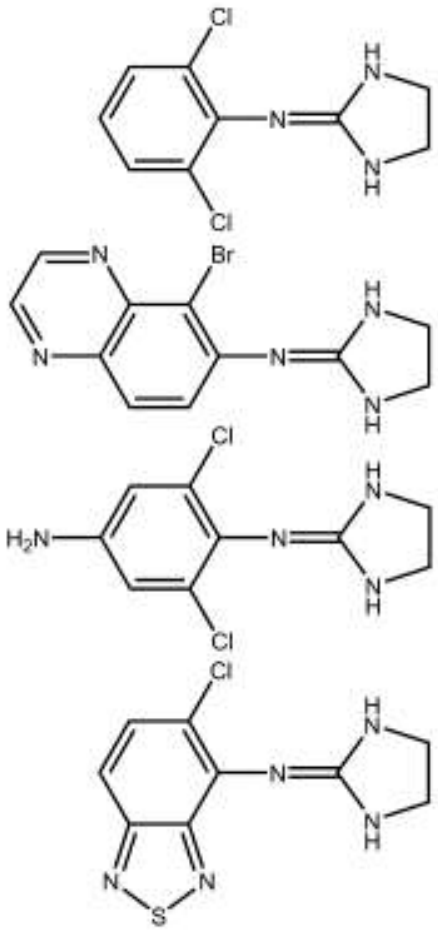
Apraclonidine (Iopidine) and Brimonidine (Alphagan).

Development of analogs of clonidine for specific use in some areas. Two of such examples are apraclonidine and brimonidine. Apraclonidine does not cross the BBB but brimonidine can cross the BBB and hence can produce hypotension and sedation, although these CNS effects are slight compared with those of clonidine. CNS effects of these drugs are correlated well to their log P, pK_a and thus log D value. Both apraclonidine and brimonidine are selective α_2-agonists.

SA

- Brimonidine is a firstline agent for treating glaucoma. Apraclonidines's mechanism of action may be related to a reduction of aqueous formation, whereas brimonidine lowers intraocular pressure by reducing aqueous humor production.
- Another example is ***tizanidine (Zanaflex***), which finds use in treating spasticity associated with multiple sclerosis or spinal cord injury. By stimulating α2-adrenergic receptors, it is believed to decrease the release of excitatory amino acid NTs from spinal cord interneurons.

SA



Drugs	Log P	pKa	Log D at pH 7	CNS activity
Clonidine	1.6	8.3	0.8	++
Brimonidine	0.9	9.63	-1.34	+
Apraclonidine	0.3	9.22	-1.91	--
Tizanidine	0.65	9.18	-1.47	+

SA

Guanabenz (Wytensin) and Guanfacine (Tenex) ***(Open-Ring Imidazolidines).*** The imidazoline ring was not necessary for central α2- agonist activity. Two clonidine analogs, guanabenz (pK_a =8.1) and guanfacine (pK_a=7), which are closely related chemically and pharmacologically, are also used as antihypertensive drugs. In these compounds, the 2,6- dichlorophenyl moiety found in clonidine is connected to a guanidino group by a two-atom bridge.

SA

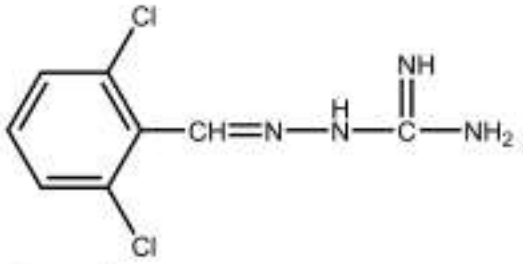
- In guanabenz, this bridge is an imine group, whereas for guanfacine, it is a —CH_2CO— moiety. For both compounds, conjugation of the guanidino moiety with the bridging moiety helps to decrease the pK_a of the basic group, so that at physiological pH a significant portion of each drug exists in its nonionized form. This accounts for their CNS penetration and high oral bioavailability (70%–80% for guanabenz and 80% for guanfacine).

SA

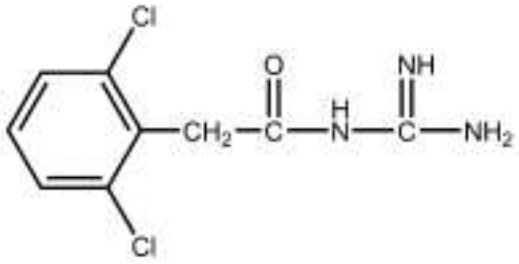
Guanfacine is more selective for α2-receptors than is clonidine. Their mechanism of action is the same as that of clonidine.

•

SA



Guanabenz
pKa = 8.1 → mainly nonionized→
penetrate the CNS
oral bioavailability = 70-80%



Guanfacine
pKa = 7 → mainly nonionized→
penetrate the CNS
oral bioavailability = >80%

SA

Methyldopa (L-α- methyldopa, Aldomet) Differs structurally from L-DOPA only in the presence of an α -methyl group. Originally synthesized as an AADC inhibitor, methyldopa ultimately decreases the concentration of DA, NE, E, and serotonin in the CNS and periphery. However, its mechanism of action is not caused by its inhibition of AADC but, rather, by its metabolism in the CNS to its active metabolite (α-methylnorepinephrine). Methyldopa is transported actively into CNS via an aromatic amino acid transporter, where it is decarboxylated by AADC in the brain to (1*R*,2*S*)α-methyldopamine. This intermediate, in turn, is stereospecifically β-hydroxylated by DBH to give the (1*R*,2*S*)α--methylnorepinephrine. This active metabolite is a selective α2-agonist because it has correct (1*R*,2*S*) configuration (Fig. 16.13).

SA

- α-methylnorepinephrine acts on α2-receptors in the CNS in the same manner as clonidine, to decrease sympathetic outflow and lower blood pressure. Absorption appears to involve an amino acid transporter. Absorption is thus affected by food. Methyldopa is used only by oral administration because its zwitterionic character limits its solubility. The ester hydrochloride salt of methyldopa, methyldopate (Aldomet ester), was developed as a highly water-soluble derivative that could be used to make parenteral preparations. It is converted to methyldopa in the body through the action of esterases (Fig. 16.13).

SA

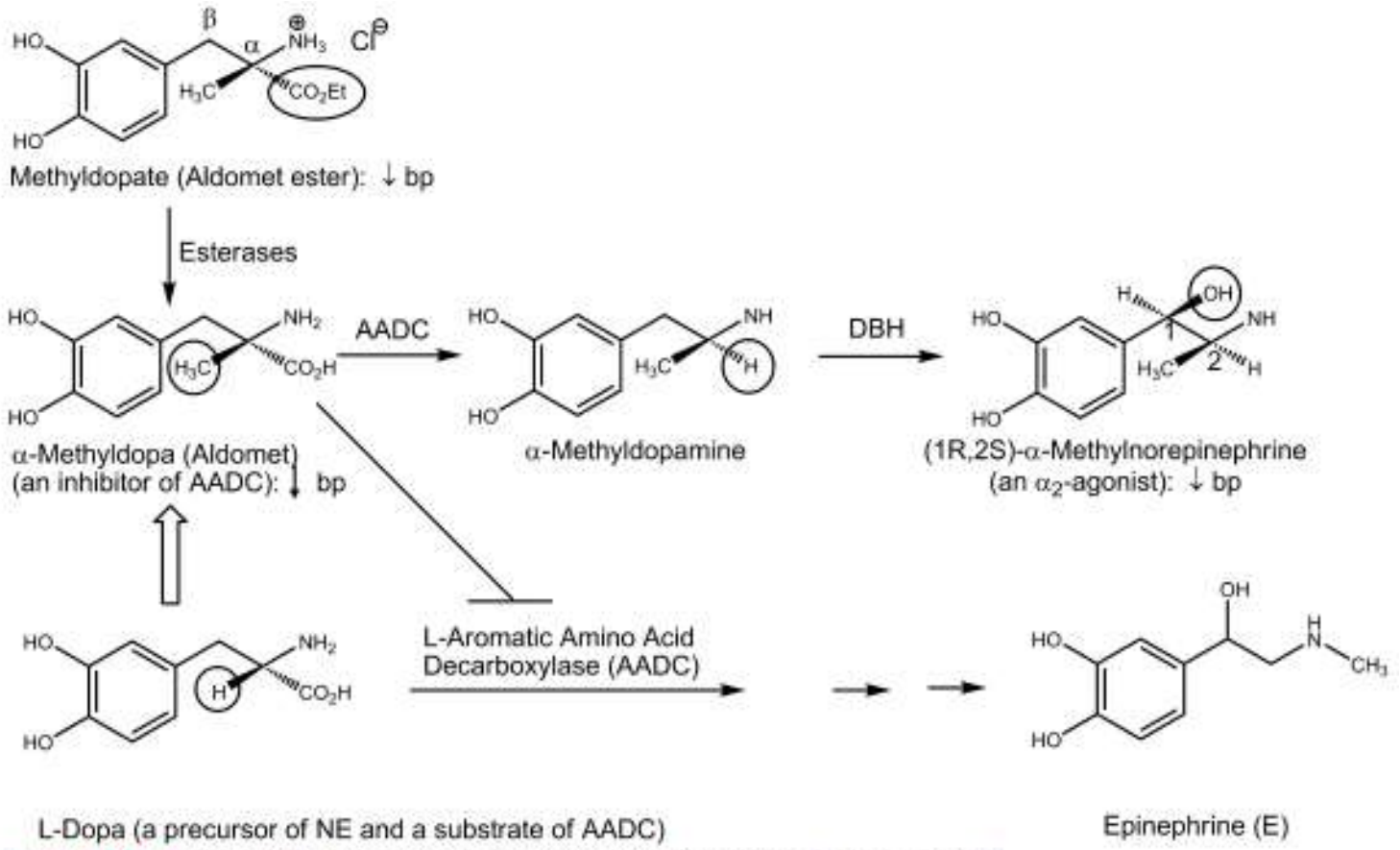


Figure 16.13 • Metabolic conversion of methyldopate and methyldopa to α-methyl-norepinephrine.

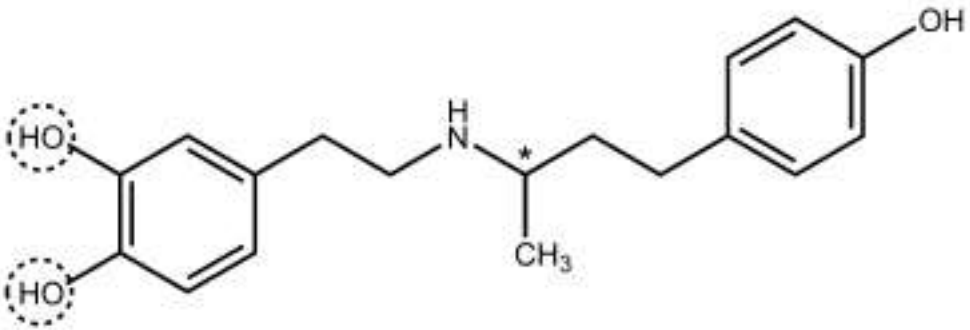
SA

- DUAL α - AND β-AGONISTS/ANTAGONISTS
- ***Dobutamine (Dobutrex)*** is a positive inotropic agent administered intravenously for congestive heart failure. It resembles DA structurally but possesses a bulky 1-(methyl)-3-(4 hydroxyphenyl) propyl group on the amino group. It possesses a center of asymmetry, and both enantiomeric forms are present in the racemic mixture used clinically. The (-) isomer of dobutamine is a potent α1-agonist, which is capable of causing marked pressor responses. In contrast,(+)-dobutamine is a potent β1-agonist.

SA

- Dobutamine contains a catechol group and is orally inactive and thus is given by intravenous infusion. Solutions of the drug can exhibit a slight pink color because of oxidation of the catechol function. It has a plasma half-life of about 2 minutes because it is metabolized by COMT and by conjugation, although not by MAO.

SA



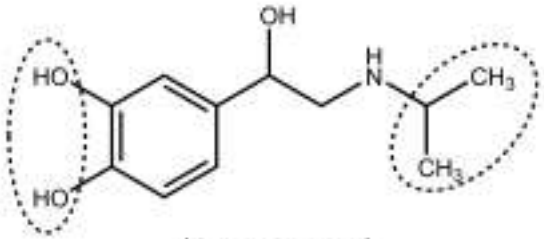
Dobutamine
oxidazed slightly by air
COMT metabolism and conjugation →
orally inactive and short DOA

β-Adrenergic Receptor Drugs

- ***Isoproterenol*** is a nonselective and prototypical β-agonist (β2/β1 =1). The principal reason for its poor oral absorption characteristics and relatively short DOA is its facile metabolism by sulfate and glucuronide conjugation of the phenolic OH groups and *O*-methylation by COMT. Because it is a catechol, it is sensitive to light and air. Aqueous solutions become pink on standing.

SA

Di-OH groups result in:
sensitive to air and light
metabolized by COMT, sulfate and glucuronide conjugation → poor absorption and short DOA



Isoproterenol

Isopropyl goup results in:
↑ β activity, virtually no α activity
resistant to MAO →

SA

It does not undergo oxidative deamination by MAO. The drug has DOA of 1 to 3 hours after inhalation. Because of an isopropyl substitution on the nitrogen atom, it has virtually no α-activity. However, it does act on both β1- and β2-receptors. It thus can produce an increase in cardiac output by stimulating cardiac β1 receptors and can bring about bronchodilation through stimulation of β2- receptors in the respiratory tract. It is one of the most potent bronchodilators available for use by inhalation and injection. Cardiac stimulation is an occasionally dangerous adverse effect. This effect of ISO on the heart is sometimes used in the treatment of heart block.

-

SA

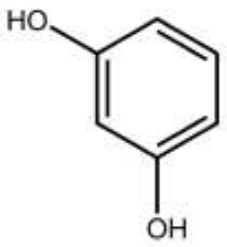
- The cardiac stimulation caused by its β1-activity and its lack of oral activity have led to its diminished use in favor of more selective β-agonists. The problems have been overcome at least partially by the design and development of several noncatechol selective β2-agonists. These agents relax smooth muscle of the bronchi, uterus, and skeletal muscle vascular supply. They find their primary use as bronchodilators in the treatment of acute and chronic bronchial asthma and other obstructive pulmonary diseases.

SA

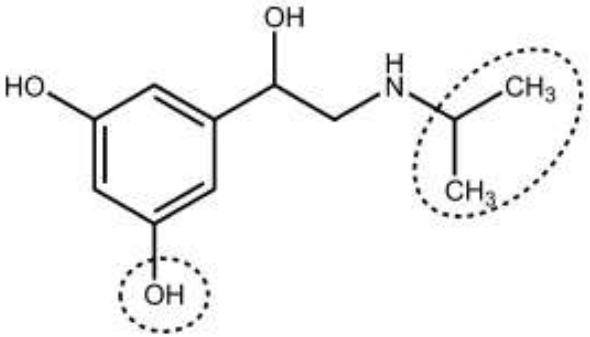
Metaproterenol, terbutaline and fenoterol (an investigational drugs)

- belong to the structural class of resorcinol bronchodilators that have 3',5'-diOH groups of the phenyl ring (rather than 3',4'-diOH groups as in catechols). 3',5'-diOH groups confer β2-receptor selectivity on compounds with large amino substituents. For example, metaproterenol (a resorcinol analog of ISO), terbutaline (an *N*-*t*-butyl analog of metaproterenol), and other similar compounds are resorcinol β2-selective agonists. They relax the bronchial musculature in patients with asthma but cause less direct cardial stimulation than do the nonselective β-agonists. Metaproterenol has a β-directing *N*-isopropyl group and it is less β2 selective than either terbutaline or albuterol (both have β2-directing N-t-butyl group).

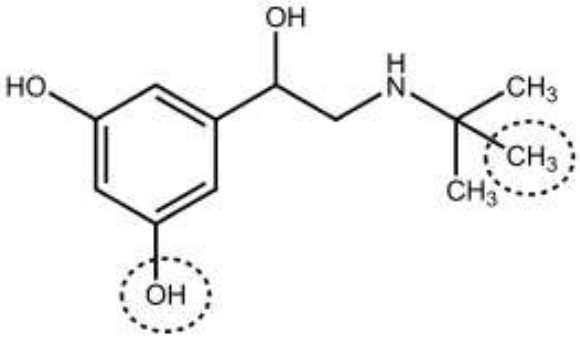
SA



Resorcinal



Metaproterenol
3, 5, Di-OH group result in:
↑ β_2 activity:
not metabolized by COMT
orally active and longer DOA



Terbutaline
Bulk *N*-R goup results in:
↑ β_2 activity & virtually no α activity
not metabolized by MAO
orally active and longer DOA

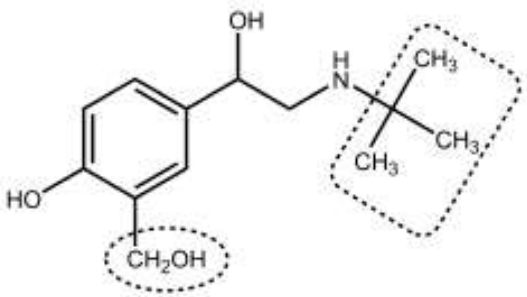
SA

- As a result metaproterenol is more prone to cause cardiac stimulation. Although these agents are more selective for β2-receptors, they have a lower affinity for β2-receptors than ISO but they are much more effective when given orally and they have a longer DOA. This is because they are resistant to the metabolism by either COMT or MAO. Instead, their metabolism primarily involves glucuronide conjugation. Although both metaproterenol and terbutaline exhibit significant β2-receptor selectivity , the common cardiovascular effects associated with other adrenergic agents can also be seen with these drugs when high doses are used.

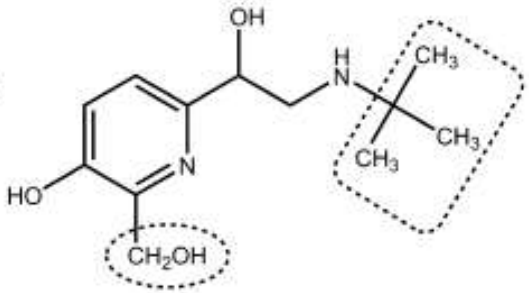
SA

Albuterol, pirbuterol and salmeterol are examples of selective β_2-agonists whose selectivity results from replacement of the *meta*-OH group of the aromatic ring with a hydroxymethyl moiety. Pirbuterol is closely related structurally to albuterol (β_2/β_1 =60); the only difference between the two is that pirbuterol contains a pyridine ring instead of a benzene ring. As in the case of metaproterenol and terbutaline, these drugs are not metabolized by either COMT or MAO. Instead, they are conjugated with sulfate. They are thus orally active, and exhibit a longer DOA than ISO. (*S*)- albuterol enhances bronchial muscle contraction, and this undesirable effect is completely avoided by using the pure (*R*)-albuterol, levalbuterol.

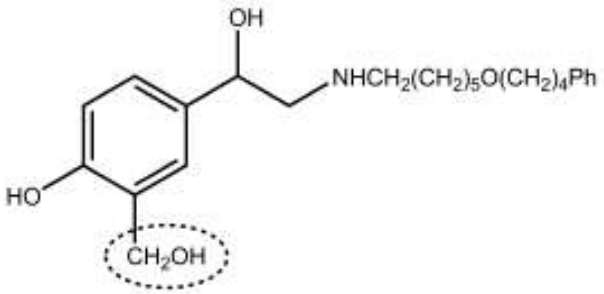
SA



Albuterol



Pirbuterol



Salmeterol

SA

- Salmeterol has an *N*-phenylbutoxyhexyl substituent in combination with a β-OH group and a salicyl phenyl ring for optimal direct-acting β2-receptor selectivity and potency. This drug associates with the β2-receptor slowly resulting in slow onset of action and dissociates from the receptor at an even slower rate.

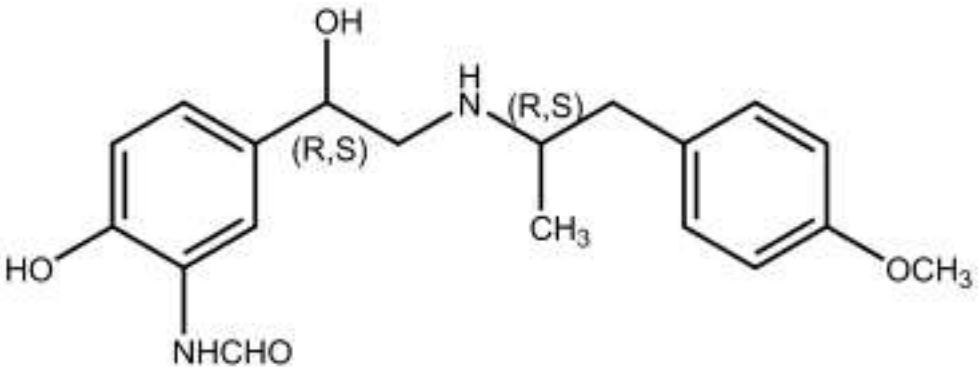
SA

- It is resistant to both MAO and COMT and highly lipophilic (log P =3.88). It is thus very long acting (12 hours), an effect also attributed to the highly lipophilic phenylalkyl substituent on the nitrogen atom, which is believed to interact with a site outside but adjacent to the active site.

SA

- ***Formoterol and Levalbuterol.*** Formoterol (Foradil) is also a lipophilic (log P=1.6) and long-acting β2-agonist. It has 3'-formylamino (β-directing) and 4'-OH groups on one phenyl ring and a lipophilic β-directing (1-(*p*-methoxyphenyl))-isopropyl group on the nitrogen atom. Its long DOA (12 hours) has been suggested to result from its association with the membrane lipid bilayer from which it gradually diffuses to provide prolonged stimulation of β2 receptors and its resistance to MAO and COMT. Formoterol has a much faster onset of action than does salmeterol as result of its lower lipophilicity. Both of these long-acting drugs are used by inhalation and are recommended for maintenance treatment of asthma, usually in conjunction with an inhaled corticosteroid.

SA



Formoterol (Log p = 1.6)

SA

All of the previously mentioned β2-agonists possess at least one chiral center and are used as racemic mixtures(except albuterol which is used as levalbuterol). Formoterol possesses two chiral centers and is used as the racemic mixture of the (R,R) and (S,S) enantiomers.

-

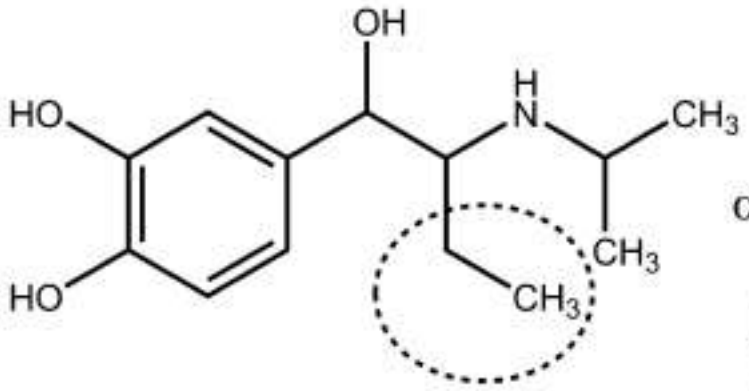
SA

- As mentioned previously, it is the (R) enantiomer configuration phenylethanolamines that possesses the pharmacological activity. There is no clinical advantage for using (*R*,*R*)- formoterol as bronchodilators compared with the racemic mixture because of its high potency and low dose but the inactive isomer may be responsible for some of the adverse effects. Levalbuterol the pure (*R*) isomer of racemic albuterol, represents the first attempt to address this issue as mentioned earlier.

SA

- ***Isoetharine.*** (α-ethyl ISO) was the first β2-selective drug. Because of the presence of the β2-directing α-ethyl group and β-directing isopropyl group, isoetharine is a β2-agonist and is resistant to MAO. However, because it contains the catechol ring system, it is metabolized by COMT and *O*-sulfated quite effectively. Consequently, it has a short DOA similar to that of ISO and is used only by inhalation for the treatment of acute episodes of bronchoconstriction.

SA



α-ethyl group results in:

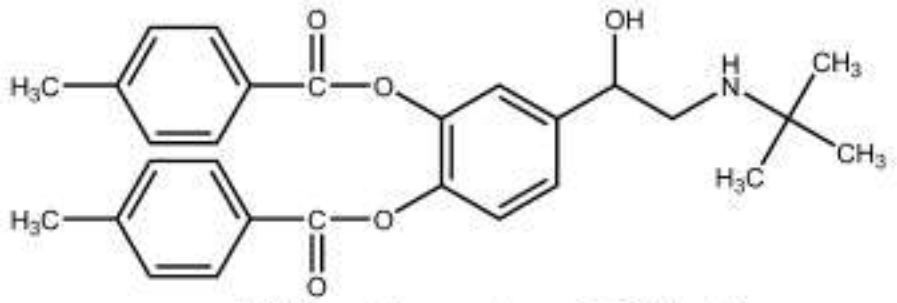
β_2 activity < ISO
resistant to MAO metabolism

Isoetharine

SA

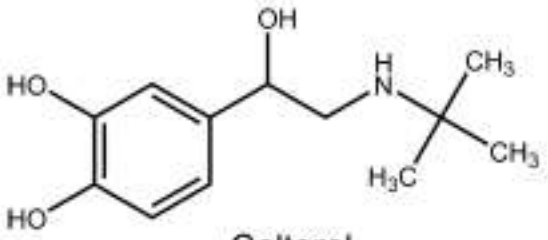
- ***Bitolterol (a Prodrug).*** Colterol (active metabolite of bitolterol) differs from ISO by replacing the β-directing *N*-isopropyl to β2-directing *N*-*tert*-butyl group, which results in the increased β2-selectivity. Bitolterol is a prodrug of colterol (a β2-selective agonist) in which the catechol OH groups have been converted to di-*p*-toluate esters, providing increased lipid solubility caused by the presence of the two lipophilic di-*p*-toluate esters in bitolterol. The presence of the bulky di-ester and bulky *N*-*tert*-butyl groups also prolong the DOA (8 hours) because it is resistant to COMT and MAO metabolism. Bitolterol is administered by inhalation for bronchial asthma and reversible bronchospasm. The highly bulky *p*-toluoyl groups
- apparently inhibit the efficiency of the esterases. After absorption,it is hydrolyzed by esterases slowly enough in the lung and other tissues to give the active product(colterol) affording sustained bronchodilatation.

SA



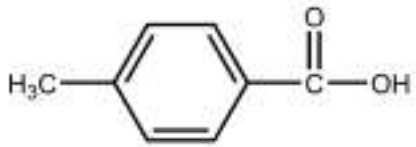
Bitolterol (a prodrug of Colterol)

esterases in the lung
and other tissues



Colterol

\+ 2



p-Toluic Acid

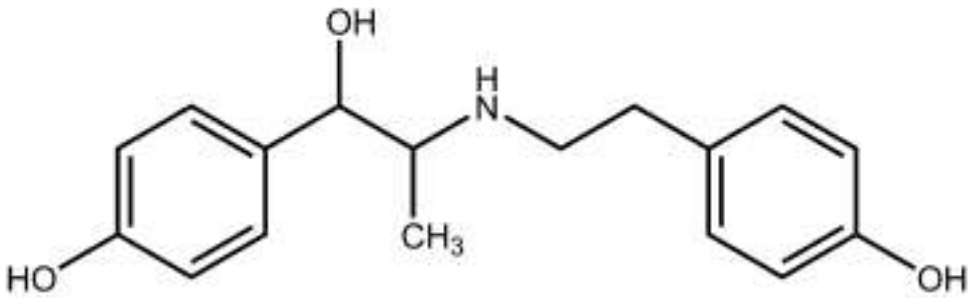
SA

- The DOA of a single dose of the prodrug bitolterol is twice that of a single dose of colterol, permitting less frequent administration and greater convenience to the patient. Colterol is then metabolized after pharmacological action by COMT and conjugation.

SA

- ***Ritodrine (Yutopar)*** is a selective β2-agonist that was developed specifically for use as a uterine relaxant but the cardiovascular effects usually associated with its administration are mild tachycardia and slight diastolic pressure decrease. Usually, it is administered initially by intravenous infusion to stop premature labor and subsequently it may be given orally.

SA



Ritodrine

SA

- ***β3-Adrenergic Receptor Agonists.*** The β3-receptor has been shown to mediate various pharmacological effects such as lipolysis, thermogenesis, and relaxation of the urinary bladder. Activation of the β3-receptor is thought to be a possible approach for the treatment of obesity, type 2 diabetes mellitus, and frequent urination. Therefore, it is recognized as an attractive target for drug discovery. Selective β3-agonists have been developed, but they have not been approved for therapeutic uses.

SA

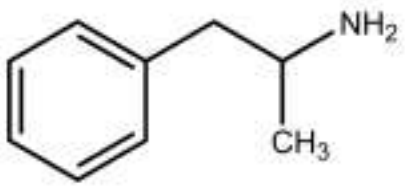
Continuing clinical studies will clarify the physiologic effects mediated by β3-receptors and elucidate the potential therapeutic uses of its agonists in humans.

-

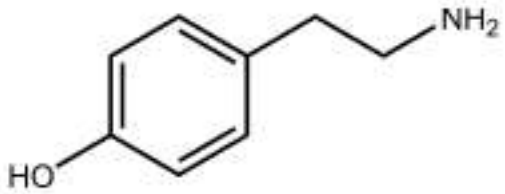
Indirect-Sympathomimetic Agents

As with the direct-acting agents, the presence of the catechol OH groups enhances the potency of indirect-acting phenylethylamines. However, the indirect-acting drugs that are used therapeutically are not catechol derivatives and, in most cases, do not even contain an OH moiety. In contrast with the direct-acting agents, the presence of a β–hydroxyl group decreases, and an α-methyl group increases, the effectiveness of indirect-acting agents. The presence of nitrogen substituents decreases indirect activity, with substituents larger than methyl groups rendering the compound virtually inactive. Phenylethylamines that contain a tertiary amino group are also ineffective as NE-releasing agents. Although amphetamine and *p*-tyramine are often cited as prototypical indirect-acting sympathomimetics but amphetamine-type drugs exert their primary effects on the CNS and discussed in CNS stimulants . The following agents are indirect sympathomimetics but acting in periphery.

ISA



Amphetamine
Log P = 2.81



p-Tyramine

ISA

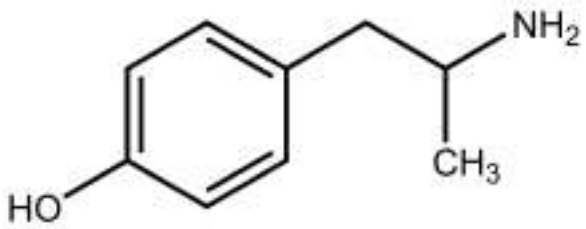
Hydroxyamphetamine :

It is an effective, indirect-acting sympathomimetic drug. It differs from amphetamine in the presence of *p*-OH group and so it has little or no CNS-stimulating action. It is used to dilate the pupil for diagnostic eye examinations and for surgical procedures on the eye. It is sometimes used with cholinergic blocking drugs like atropine to produce a mydriatic effect, which is more pronounced than that produced by either drug alone.

•

•

ISA

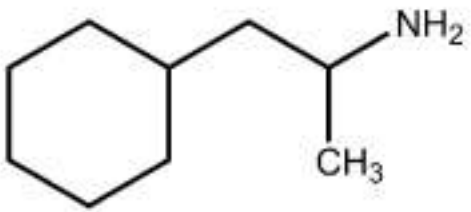


Hydroxyamphetamine
Log P = 1.07
pKa = 10.71

ISA

- ***Propylhexedrine (Benzedrex) :***
It is another analog of amphetamine in which the aromatic ring has been replaced with a cyclohexane ring. This drug produces vasoconstriction and a decongestant effect on the nasal membranes, but it has only about one half the pressor effect of amphetamine and produces decidedly fewer effects on the CNS. Its major use is for a local vasoconstrictive effect on nasal mucosa in the symptomatic relief of nasal congestion caused by the common cold, allergic rhinitis, or sinusitis.

ISA



Propylhexedrine

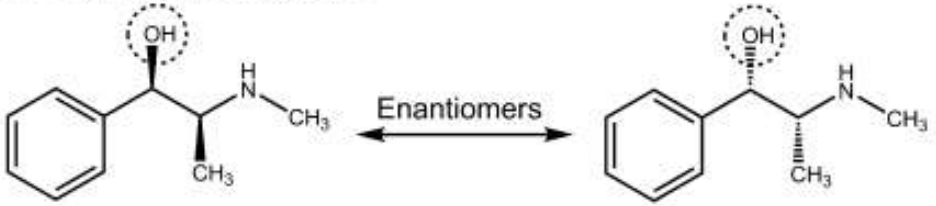
ISA

- ***L-(+)-Pseudoephedrine.*** Is the (*S*,*S*) diastereoisomer of ephedrine. Whereas ephedrine has a mixed mechanism of action, L-(+)-pseudoephedrine acts mostly by an indirect mechanism and has virtually no direct activity. The structural basis for this difference in mechanism is the stereochemistry of the carbon atom possessing the β-OH group. In pseudoephedrine, this carbon atom possesses the (*S*) configuration, the wrong stereochemistry at this center for a direct-acting effect at adrenoceptors. Although it crosses the BBB (log P=1.05, pK_a=9.38), L-(+)-pseudoephedrine's lack of direct activity affords fewer CNS effects than does ephedrine. It is a naturally occurring alkaloid from the *Ephedra* species. This agent is found in many OTC nasal decongestant and cold medications. Although it is less prone to increase blood pressure than ephedrine, it should be used with caution in hypertensive individuals, and it should not be used in combination with MAO inhibitors.

ISA

Ephedrine = *erythro* racemate
cis
Mixed mechanism of action

correct (1R, 2S) configuration



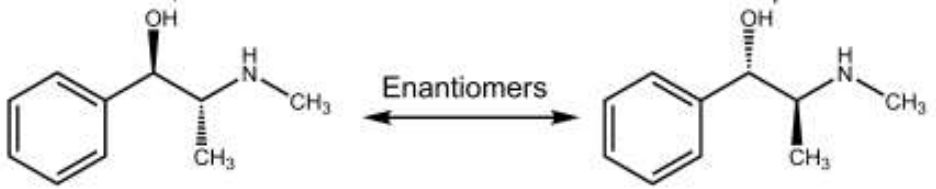
(1*R*,2*S*)-*D*-(-)-Ephedrine
Direct activity on α and β
Some indirect activity.
The most active of the four isomers as a pressor amine

(1*S*,2*R*)-*L*-(+)-Ephedrine
Mixed mechanism of action
but primarily indirect activity

Diastereomers

Diastereomers

Pseudoephedrine = *threo* racemate
trans
Principally, indirect-acting



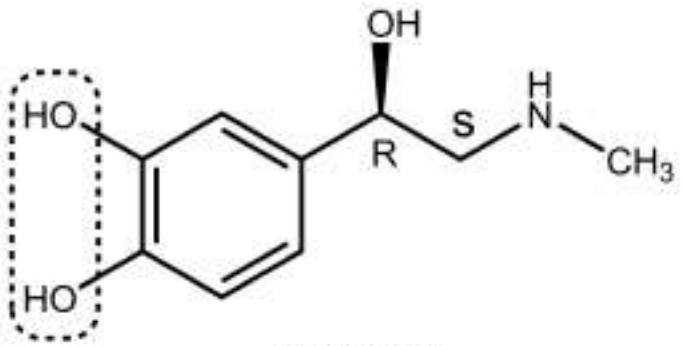
(1*R*,2*R*)-*D*-(-)-Pseudoephedrine

(1*S*,2*S*)-*L*-(+)-Pseudoephedrine
Virtually no direct activity
Mostly indirect activity

Mixed Active Sympathomimetics (MAS)

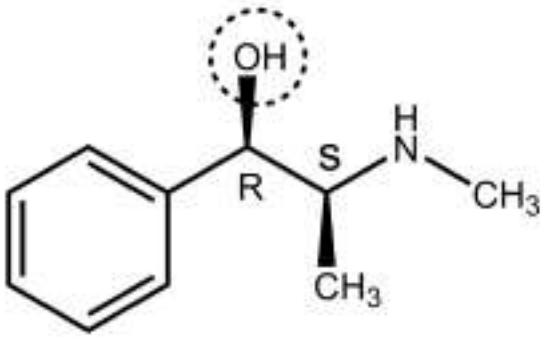
- Those phenylethylamines (phenylisopropylamines) are considered to have a mixed mechanism of action usually have no hydroxyls on the aromatic ring but do have a β-hydroxyl group.
D-(-)-Ephedrine. The pharmacological activity of (1*R*,2*S*)-D-(-)-ephedrine resembles that of E. The drug acts on both α- and β-receptors. Its ability to activate β–receptors probably accounted for its earlier use in asthma. It is the classic example of a sympathomimetic with a mixed mechanism of action. Lacking H-bonding phenolic OH groups, ephedrine is less polar (log P =1.05, pK_a=9.6) and, thus, crosses the BBB far better than do other CAs. Therefore, ephedrine has been used as a CNS stimulant and exhibits side effects related to its action in the brain. It causes more pronounced stimulation of the CNS than E.

MAS



Epinephrine
Log P = -1.63

correct (1R) (2S) configuration



D-(-)-Ephedrine
Log P = 1.05

MAS

- The drug is not metabolized by either MAO or COMT and therefore has more oral activity and longer DOA than E. A significant fraction of the drug is excreted unchanged in the urine although it can be *p*-hydroxylated and *N*-demethylated. Because it is a weak base, its excretion can be accelerated by acidification of the urine. Ephedrine has two asymmetric carbon atoms; thereby creating four optically active isomers. The *erythro* racemate is called *ephedrine*, and the *threo* racemate is known as *pseudoephedrine* (ψ-ephedrine). Natural ephedrine is the D (-) isomer, and it is the most active of the four isomers as a pressor amine. This is largely because of the fact that this isomer has the correct (1*R*,2*S*) configuration for optimal direct action at adrenergic receptors.

MAS

- Ephedrine decomposes gradually and darkens when exposed to light. The free alkaloid is a base, and an aqueous solution of the free alkaloid has a pH above 10. The salt form has a pK_a of 9.6. Ephedrine and its salts are used orally, intravenously, intramuscularly, and topically for various conditions, such as allergic disorders, colds, hypotensive conditions, and narcolepsy. It is used locally to constrict the nasal mucosa and cause decongestion and to dilate the pupil or the bronchi. Systemically, it is effective for asthma, hay fever, and urticaria.

MAS

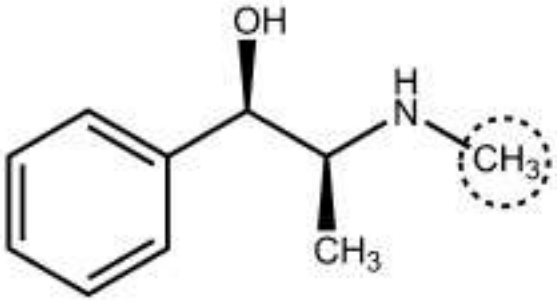
This drug is an alkaloid from *Ephedra*. Ma huang, the plant containing ephedrine. In recent years, various companies have begun marketing extracts of *Ephedra* shrubs for such purposes as weight loss and enhancement of athletic performance. Herbalists also market them as “alternative medicines” for cold and cough relief. It has been estimated that nearly one third of young, obese women have used a weight-loss supplement containing *Ephedra.*

-
-

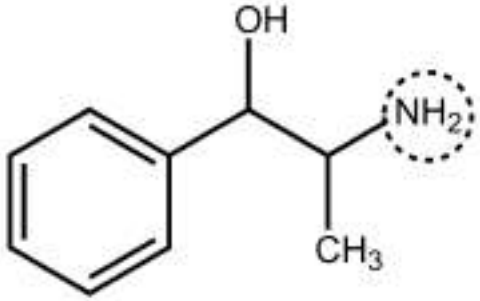
MAS

- ***Phenylpropanolamine*** is the *N*-desmethyl analog of ephedrine and thus has many similar properties. Lacking the *N*-methyl group, phenylpropanolamine is slightly more polar, and therefore does not enter the CNS. This modification gives an agent that has slightly higher vasopressive action and lower central stimulatory action than ephedrine. Its action as a nasal decongestant is more prolonged than that of ephedrine. It is orally active. Phenylpropanolamine was a common active component in OTC appetite suppressants and cough and cold medications until 2001 when the Food and Drug Administration (FDA) recommended its removal from such medications, because studies showed an increased risk of hemorrhagic stroke in young women who took the drug.

MAS



Ephedrine
Log P = 1.05

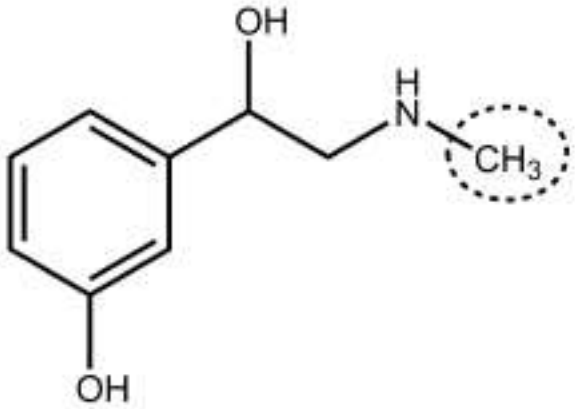


Phenylpropanolamine

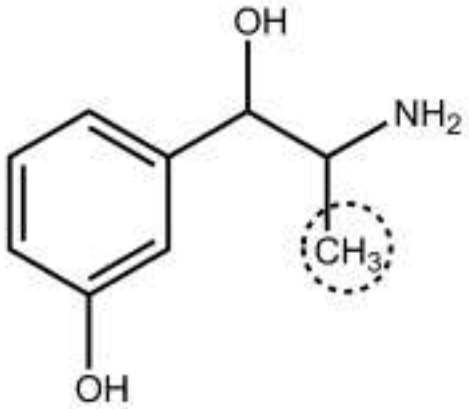
MAS

- ***Metaraminol*** is the *N*-desmethyl-α-methyl analog of phenylephrine. It possesses a mixed mechanism of action, with its direct-acting effects mainly on α1-receptors. It is used parenterally as a vasopressor in the treatment and prevention of the acute hypotensive state occurring with spinal anesthesia. It also has been used to treat severe hypotension brought on by other traumas that induce shock.

MAS



Phenylephrine



Metaraminol

Adrenergic Receptor Antagonist(Blockers)

α-Blockers

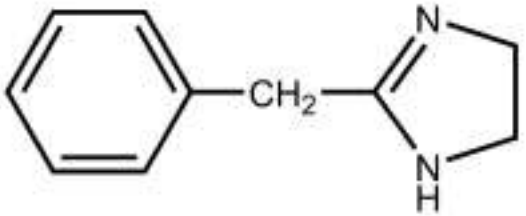
- Because α-agonists cause vasoconstriction and raise blood pressure, α-blockers should be therapeutically used as antihypertensive agents. Unlike the β-blockers, which bear clear structural similarities to the adrenergic agonists NE,E, and ISO, the α-blockers consist of several compounds of diverse chemical structure that bear little obvious resemblance to the α-agonists.

AB

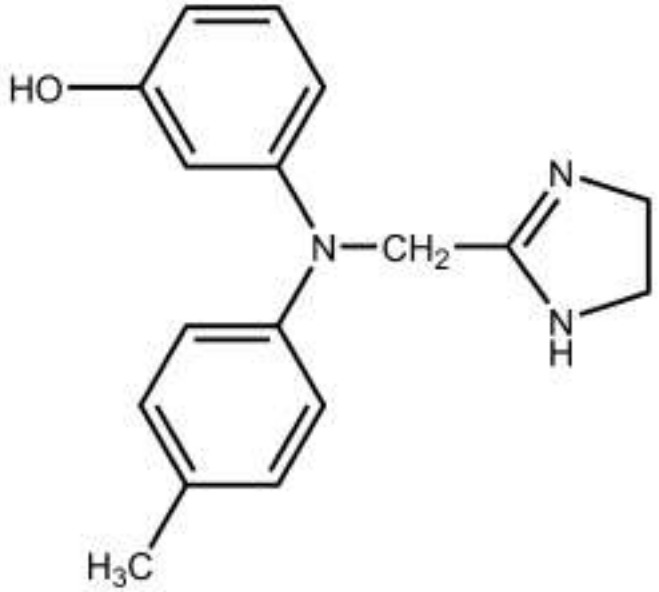
NONSELECTIVE (reversible) α -BLOCKERS

- ***Tolazoline and phentolamine*** are imidazoline competitive α-blockers. The structure of tolazoline is similar to the imidazoline α_1-agonists, but does not have the lipophilic substituents required for agonist activity. The type of group attached to the imidazoline ring thus dictates whether an imidazoline is an agonist or a blocker. Both agents have α1 and α2 blocking. Both have a direct vasodilatory action on vascular smooth muscle that may be more prominent than their α-blocking effects. Tolazoline has a histamine-like and acetylcholine-like agonistic actions probably contribute to its vasodilatory activity. Its histamine-like effects include stimulation of gastric acid secretion, rendering it inappropriate for administration to patients who have gastric or peptic ulcers.

AB



Tolazoline



Phentolamine

AB

Nonselective IRREVERSIBLE α-BLOCKERS •

Agents in this class produce a slowly developing, prolonged • adrenergic blockade that is not overcome by E. They are irreversible α-blockers, because β-haloalkylamines in the molecules alkylate α-receptors. Dibenamine is the prototypical agent in this class, but phenoxybenzamine is used therapeutically today.The initial step of alkylation involves the formation of an intermediate aziridinium ion (ethylene iminium ion). The positively charged aziridinium ion electrophile then reacts with a nucleophilic group on the α-receptor resulting in the formation of a covalent bond between the drug and the receptor. Unfortunately, these nonselective drugs alkylate not only α-receptors but also other biomolecules, leading to their toxicity. It is thus used only to relieve the sympathetic effects of pheochrom-ocytoma.

AB

- ***Phenoxybenzamine*** An old but powerful α-blocker, is haloalkylamine that blocks α_1- and α_2- receptors irreversibly. Phenoxybenzamine administration has been described as producing a “chemical sympathectomy” because of its nonselective blockade of the excitatory responses of the smooth muscles and of the heart muscle.

AB

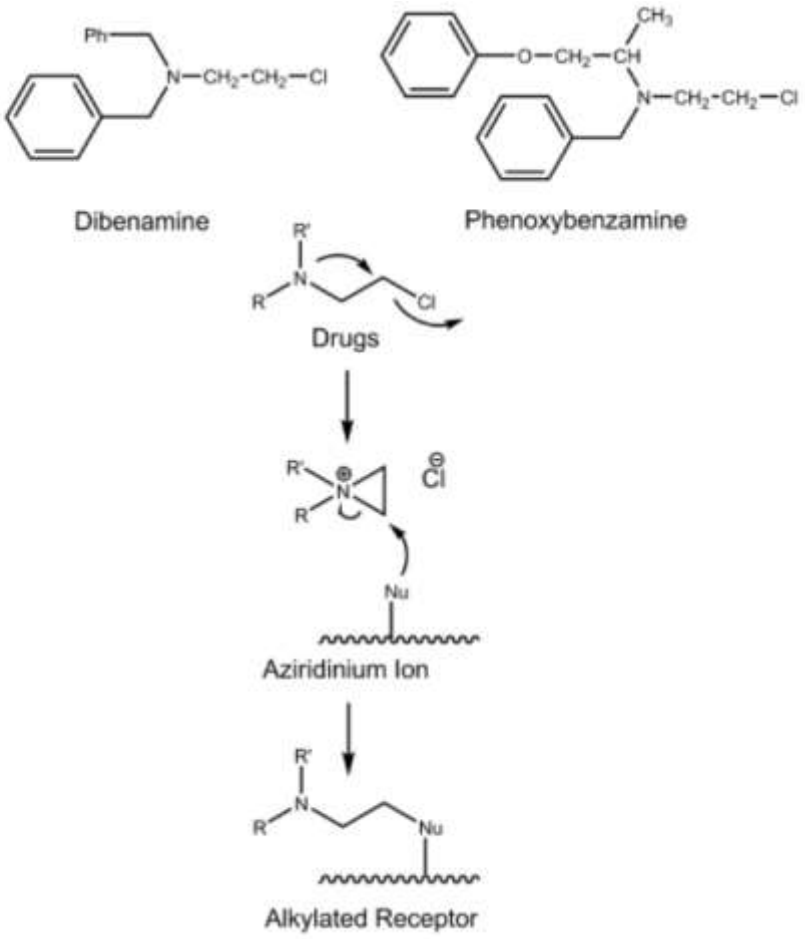


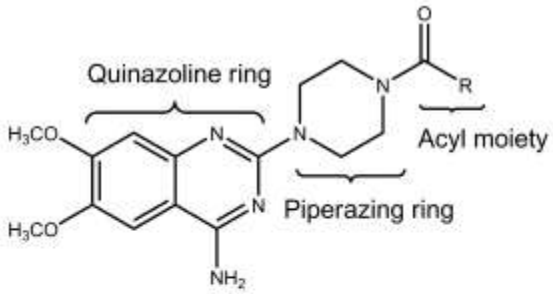
Figure 16.14 • Mechanism of inactivation of α-adrenergic receptors by β-haloalkylamines.

AB

SELECTIVE α_1-BLOCKERS

- ***Prazosin , terazosin , and doxazosin (Cardura)*** are quinazoline α_1-blockers. As a result of its greater α_1-receptor selectivity, the quinazoline class of α-blockers exhibits greater clinical utility and has largely replaced the nonselective haloalkylamine and imidazoline α-blockers. Structurally, these agents consist of three components: the quinazoline ring, the piperazine ring, and the acyl moiety. The 4-amino group on the quinazoline ring is very important for α_1-receptor affinity. Although they possess a piperazine moiety attached to the quinazoline ring, this group can be replaced with other heterocyclic moieties (e.g., piperidine moiety) without loss of affinity.The nature of the acyl group has a significant effect on the pharmacokinetic properties.

AB



Prazosin: R =

Terazosin: R =

Doxazosin: R =

AB

- These drugs dilate both arterioles and veins and are thus used in the treatment of hypertension. They produce peripheral vasodilation without an increase in heart rate or cardiac output. This advantage is attributed, at least in part, to the fact that prazosin blocks postjunctional α_1-receptors selectively without blocking presynaptic α_2-receptors.
- Contraction of the smooth muscle of prostate gland, prostatic urethra, and bladder neck is also mediated by α_1-adrenoceptors and blockade of these receptors relaxes the tissue. For this reason, these agents are also used in the treatment of BPH, where they help improve urination flow rates.

AB

The main difference between prazosin, terazosin, and doxazosin lies in their pharmacokinetic properties. As mentioned previously, these differences are dictated by the nature of the acyl moiety attached to the piperazine ring.

These drugs are metabolized extensively with the metabolites excreted in the bile. The fact that a single α_{1A}-adrenoceptor subtype is found in the prostatic and urethral smooth muscle cells led to the design of drugs with uroselectivity for this receptor subtype. Thus, alfuzosin and tamsulosin are uroselective α_1-blockers and first-line drugs for treatment of BPH without utility in treating hypertension.

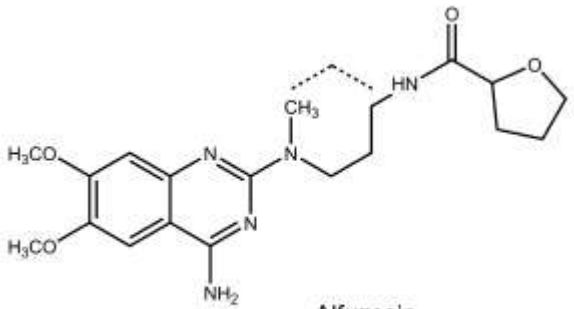
-
-

AB

- ***Alfuzosin (Uroxatral)*** is also a quinazoline α_1-blocker but differs from terazosin in replacing the piperazine ring in terazosin with an open piperazine ring (a rotatable propylenediamine group). Alfuzosin is more selective for the subtype of α_{1A}-receptor in the prostate gland than those in vascular tissue. Thus, it has been used extensively in treating BPH as a first-line drug with fewer cardiovascular side effects than terazosin and doxazosin.

AB

Terazosin

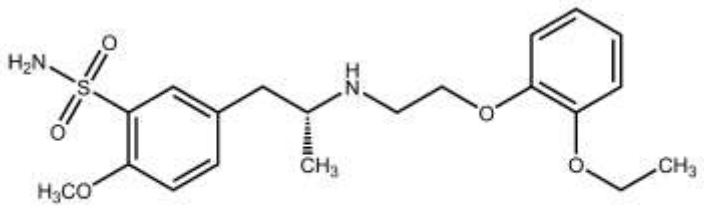


Alfuzosin

AB

- ***Tamsulosin (Flomax),*** a nonquinazoline benzensulfonamide, is the first in the class of subtype selective α_{1A}-blocker.It is many folds more selective for α_{1A}-receptors than for the other α_1-receptors. Tamsulosin is efficacious in the treatment of BPH with little effect on blood pressure. Orthostatic hypotension is not as great with this agent as with the nonselective quinazolines.

AB



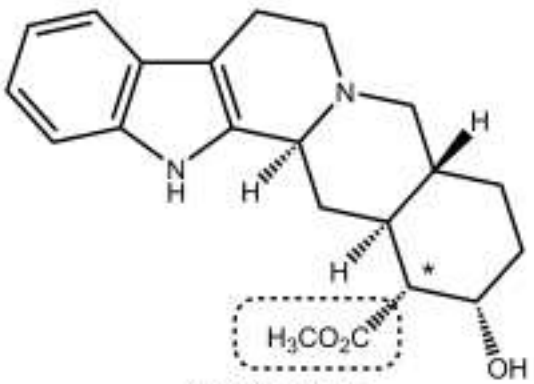
Tamsulosin

AB

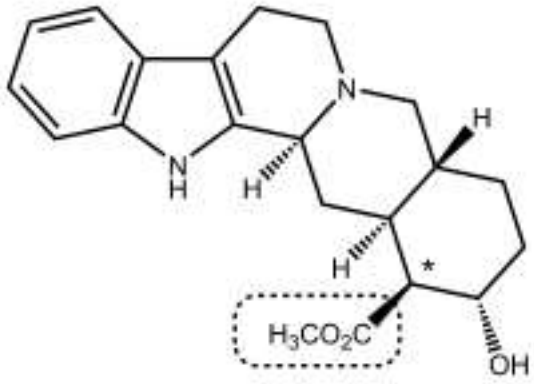
SELECTIVE α_2-BLOCKERS

- ***Yohimbine and Corynanthine.*** Yohimbine (Yocon) is a competitive and selective α_2-blocker. The compound is an indolealkylamine alkaloid and is found in the bark of the tree *Pausinystalia yohimbe* and in *Rauwolfia* root; its structure resembles that of reserpine. These isomeric indole alkaloids known as the yohimbanes exhibit different degrees of selectivity toward the α_1- and α_2-receptors depending on their stereochemistry.

AB



Yohimbine



Corynanthine

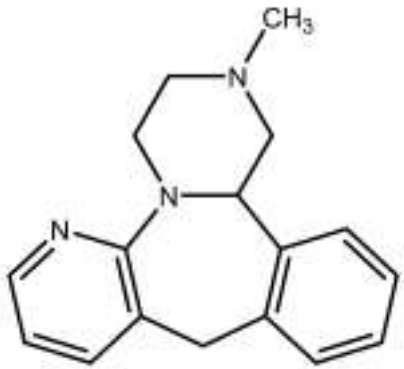
AB

- For example,yohimbine is a selective α_2-blocker, whereas corynanthine is a selective α_1-blocker. The only difference between these two compounds is the relative stereochemistry of the carbon containing the carbomethoxy substituent. In yohimbine, this group lies in the plane of the alkaloid ring system, whereas in corynanthine, it lies in an axial position and thus is out of the plane of the rings.[56]

AB

- Yohimbine increases heart rate and blood pressure as a result of its blockade of α2-receptors in the CNS. It has been used experimentally to treat male erectile impotence.
- ***Mirtazapine (Remeron)*** is another example of tetracyclic α2-blockers that shows selectivity for α2-receptors versus α1-receptors. Blockade of central α2-receptors results in an increased release of NE and serotonin. This has prompted its use as an antidepressant. This agent also has activity at nonadrenergic receptors. It is a potent blocker of 5-HT2 and 5-HT3 serotonin receptors and at histamine H1-receptors.

AB



Mirtazapine (Log D = 1.38)

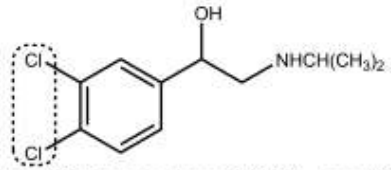
β –Blocker

SAR

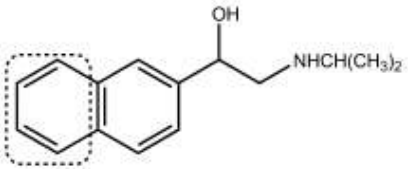
- β-Blockers are among the most widely employed antihypertensives
- and are also considered the first-line treatment for glaucoma.Most of β-blockers are in the chemical class of aryloxypropanolamines.

A- The first β-blocker was dichloroisoproterenol (DCI). DCI differs structurally from ISO in that the agonist directing 3',4'-di OH groups have been replaced by two chloro groups.This simple structural modification has provided the basis for nearly all of the approaches used in subsequent efforts to design and synthesize therapeutically useful β-blockers. Unfortunately, DCI is not a pure antagonist but a partial agonist. The substantial direct sympathomimetic action of DCI precluded its development as a clinically useful drug.

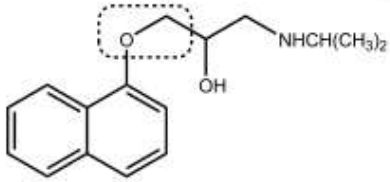
B-Blocker



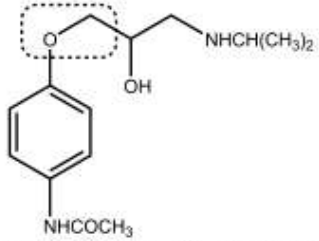
*Dichloro*isoproterenol (DCI, a partial β agonist)



Pronethalol (a β blocker with toxicity)



Propranolol (a prototype of β blockers)



Practolol (a prototype of β_1 blockers)

B-Blocker

B- β-blocking actions of propranolol, a close structural relative of pronethalol, has become one of the most widely used drugs. It is the standard against which all other β-blockers are compared.

B-Blocker

Propranolol belongs to the group of β-blockers known as *aryloxypropanolamines*. An ***-OCH_2- group*** has been incorporated into the molecule between the aromatic ring and the ethylamino side chain. Because this structural feature is frequently found in β-blockers, the assumption is made that the -OCH_2- group is responsible for the antagonistic properties of the molecule, but this group is present in several potent β-agonists.

B-Blocker

As conclusion the nature of the ***aromatic ring*** and its substituents is the primary determinant of β-antagonistic activity. The aryl group also affects the absorption, excretion, and metabolism of the β-blockers. Note that the side chain has been moved from C2 to the C1 position from the naphthyl ring.

-

B-Blocker

- The nature of the aromatic ring is also a determinant in their β1-selectivity. One common structural feature of many cardioselective -blockers is the presence of a **para-substituent** of sufficient size on the aromatic ring along with the absence of ***meta-substituents***.

C- Practolol is the prototypical example of a β1-blocker of this structural type. It was the first cardioselective β1-blocker to be used extensively in humans. Because it produced several toxic effects, however, it is no longer in general use.

B-Bocker

- Like β-agonists, ***β-directing tert-butyl and isopropyl groups***, are normally found on the amino function of the aryloxypropanolamine β-blockers. It must be a secondary amine for optimal activity.

B-Blocker

- For β-blockers, the β-OH-substituted carbon must be in the *S* absolute configuration for maximal β-blocking activity. Because of the insertion of an oxygen atom in the side chain of the aryloxypropanolamines, the priority of the substituents around the asymmetric carbon differs from the agonists. The pharmacologically more active enantiomer of β-blockers interacts with the receptor recognition site in same manner as that of the agonists. In spite of this fact, propranolol and most other β-blockers are used clinically as racemic mixtures. The only exceptions are levobunolol, timolol, and penbutolol, with which the (S) enantiomer is used.

B-Blocker

- Propranolol (log P =3.10) is the most lipophilic drug among the available β-blockers, and thus it enters the CNS much better than the less lipophilic drug such as atenolol (log P=0.10) or nadolol (log P=1.29). The use of lipophilic β-blockers such as propranolol has been associated with more CNS side effects, such as dizziness, confusion,or depression. These side effects can be avoided, however, with the use of hydrophilic drugs, such as atenolol or nadolol. The more lipophilic drugs are primarily cleared by the liver, and so their doses need to be adjusted in patients with liver disease. In contrast, the less lipophilic drugs are cleared by the kidney and so their doses need to be adjusted in patients with impaired renal function.

B-Blocker

NONSELECTIVE β-BLOCKERS (FIRST GENERATION)

- ***1- Propranolol (Inderal)*** is the prototypical.It blocks the β1- and β2-receptors with equal affinity, lacks ISA, and does not block α-receptors. Propranolol, like the other β-blockers discussed, is a competitive blocker whose receptor-blocking actions can be reversed with sufficient concentrations of β-agonists. Currently, propranolol is approved for hypertension, cardiac arrhythmias, angina pectoris,etc . In addition, because of its high lipophilicity (log P= 3.10) and thus its ability to penetrate the CNS, propranolol has found use in treating anxiety and is under investigation for the treatment of a variety of other conditions, including schizophrenia, alcohol withdrawal syndrome, and aggressive behavior.

B-Blocker

- By blocking the β1–receptors of the heart, propranolol slows the heart, reduces the force of contraction, and reduces cardiac output. Because of reflex sympathetic activity and blockade of vascular β2-receptors, administration may result in increased peripheral resistance. Because it exhibits no selectivity for β1-receptors, it is contraindicated in the presence of conditions such as asthma and bronchitis.

B-Blocker

A pharmacological effect of propranolol is MSA which is a nonspecific (not mediated by specific receptors). It is also referred to as a *local anesthetic effect*. Both enantiomers possess membrane stabilizing activity.

•

B-Blocker

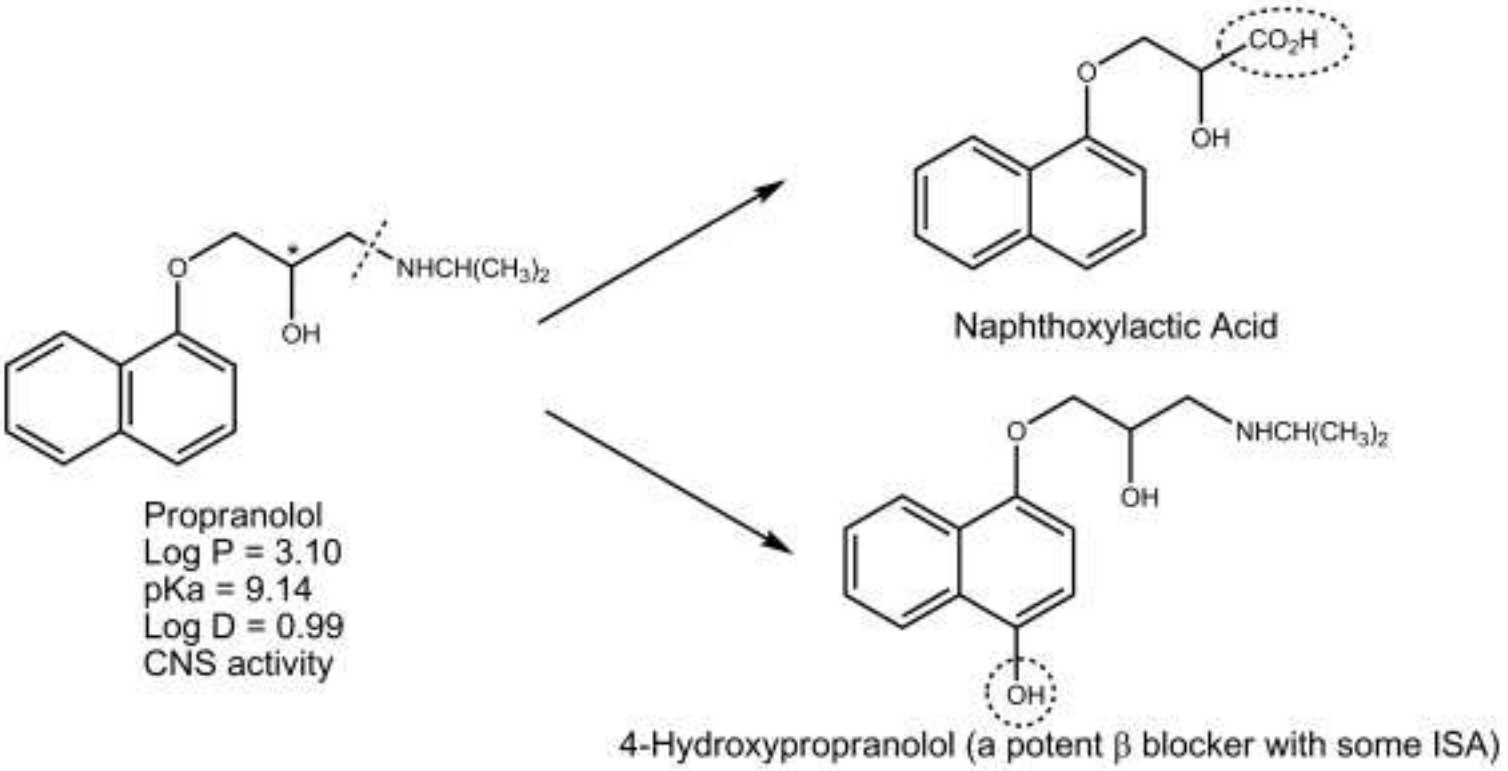
Lower doses are extracted more efficiently than higher doses, indicating that the extraction process may become saturated at higher doses. In addition, the active enantiomer is cleared more slowly than the inactive enantiomer.

B-Blocker

One of the major metabolites is naphthoxylactic acid. It is formed by a series of metabolic reactions involving *N*-dealkylation,deamination, and oxidation of the resultant aldehyde. Another metabolite of particular interest is 4-hydroxypropranolol, which is a potent β-blocker with some ISA. It is not well known that 4hydroxypropranolol makes to the pharmacological effects seen after administration of propranolol.

-
-

B-Blocker

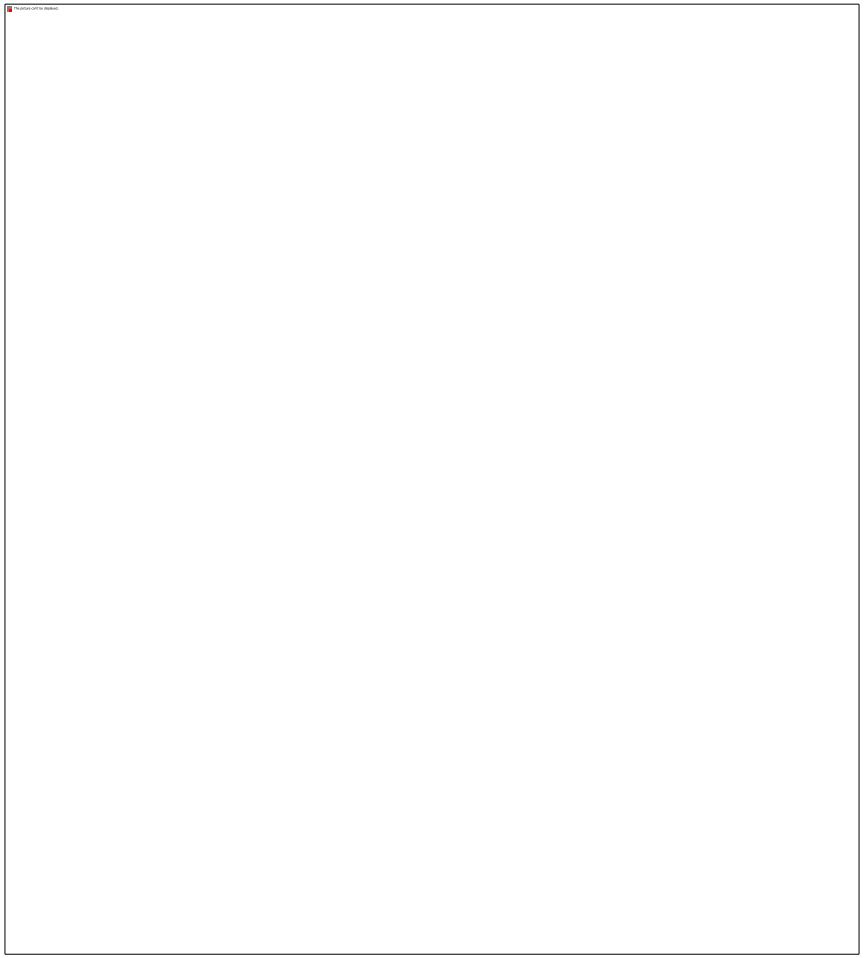

O
NHCH(CH3)2
OH
Propranolol
Log P = 3.10
pKa = 9.14
Log D = 0.99
CNS activity
CO2H
O
OH
Naphthoxylactic Acid
O
NHCH(CH3)2
OH
OH
4-Hydroxypropranolol (a potent β blocker with some ISA)

B-Blocker

2-Other Nonselective β-Blockers: Several other clinically used nonselective β-blockers include *nadolol* (Corgard), *pindolol* (Visken), *penbutolol*, *carteolol*, *timolol* (Timoptic), *levobunolol* (Betagan), *sotalol* , and *metipranolol* (Figure 16.15).

-

B-Blocker

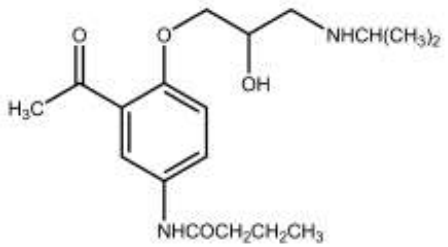


B-Blocker

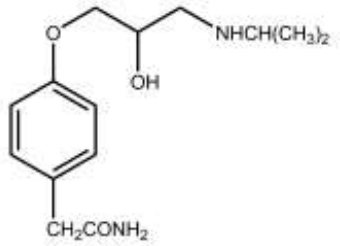
β1- selective blocker (second generation):

These are drugs have a greater affinity for the β1-receptors of the heart than for β2-receptors in other tissues. Such cardioselective agents should provide two important therapeutic advantages. The first advantage should be the lack of a blocking effect on the β2-receptors in the bronchi. Theoretically, this would make β1-blockers safe for use in patients who have bronchitis or bronchial asthma. The second advantage should be the absence of blockade of the vascular β2-receptors, which mediate vasodilation.This would be expected to reduce or eliminate the peripheral resistance that sometimes occurs after the administration of nonselective β-blockers. Unfortunately, cardioselectivity is usually observed with β1-blockers at only relatively low doses.

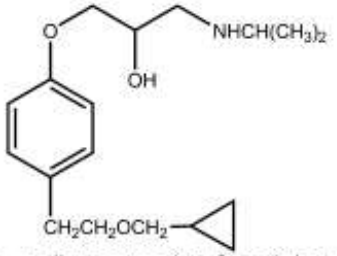
B-Blocker



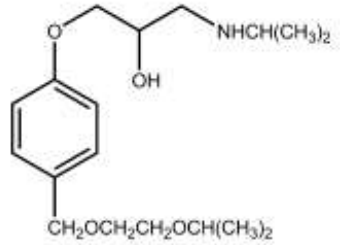
Acebutolol: antihypertensive



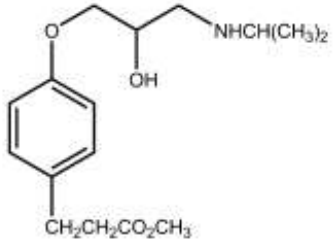
Atenolol: antihypertensive



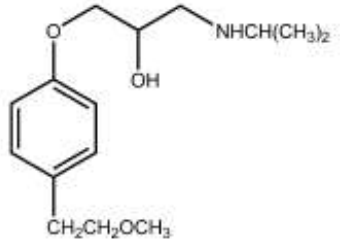
Betaxolol: antihypertensive & antiglaucoma



Bisoprolol: antihypertensive



Esmolol: short-acting antihypertensive



Metoprolol: antihypertensive

Figure 16.16 • β_1-Selective blockers.

B-Blocker

- At present, the following β1-selective blockers are used therapeutically: *acebutolol* , *atenolol* (Tenormin),*betaxolol* (Kerlone, Betoptic), *bisoprolol* , *esmolol* , and *metoprolol* (Lopressor).(Figure 16.16). All of these agents except esmolol are indicated for the treatment of hypertension.
- Esmolol was designed specifically to possess a very short DOA; it has an elimination half-life of 9 minutes.

B-Blocker

- The short DOA of esmolol is the result of rapid hydrolysis of its ester functionality by esterases present in erythrocytes (Fig. 16.17). The resultant carboxylic acid is an extremely weak β-blocker that does not appear to exhibit clinically significant effects. The acid metabolite has an elimination half-life of 3 to 4 hours and is excreted primarily by the kidneys. This agent is administered by continuous IV infusion for control of ventricular rate in patients with atrial flutter, atrial fibrillation, or sinus tachycardia. Its rapid onset and short DOA render it useful during surgery, after an operation, or during emergencies for short-term control of heart rates.

B-Blocker

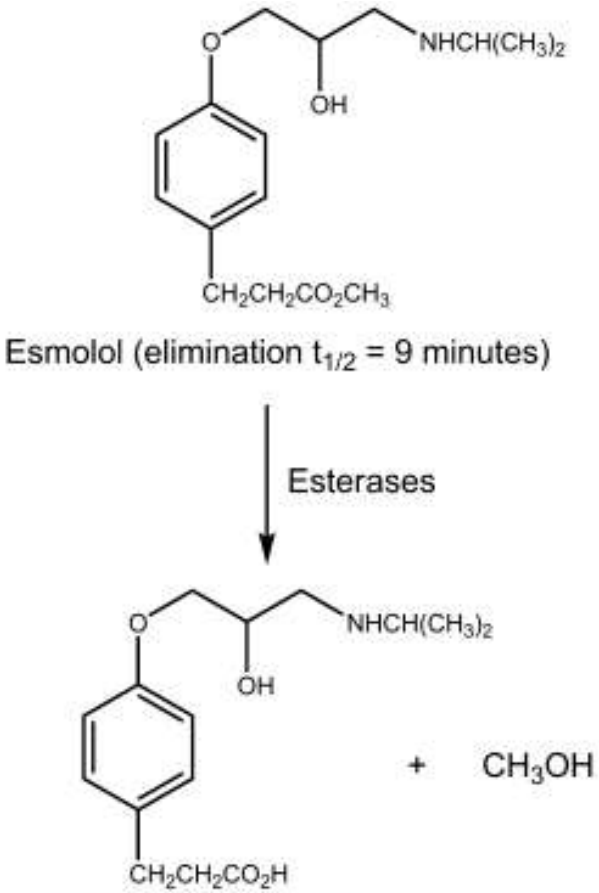


Figure 16.17 • Metabolism of esmolol.

B-Blocker

Betaxolol, with a half-life ranging between 14 and 22 hours, has the longest DOA of the β1-selective blockers.
Like propranolol,metoprolol has low bioavailability because of significant first-pass metabolism.

-

B-Blocker

Atenolol (log P=0.10), like nadolol (log P=1.29), has low lipid solubility and does not readily cross the BBB. In the case of bisoprolol, about 50% of a dose undergoes hepatic metabolism, whereas the remaining 50% is excreted in the urine unchanged.

B-Blocker

Acebutolol is one of the very few β-blockers whose metabolite plays a significant role in its pharmacological actions. This drug is absorbed well from the gastrointestinal tract, but it undergoes extensive first-pass metabolic conversion to diacetolol by hydrolytic conversion of the amide group to the amine, followed by acetylation of the amine (Fig. 16.18). After oral administration, plasma levels of diacetolol are higher than those of acebutolol.

-
-

Diacetolol is also a selective β_1-blocker with partial agonistic activity; it has little membrane-stabilizing activity.

B-Blocker

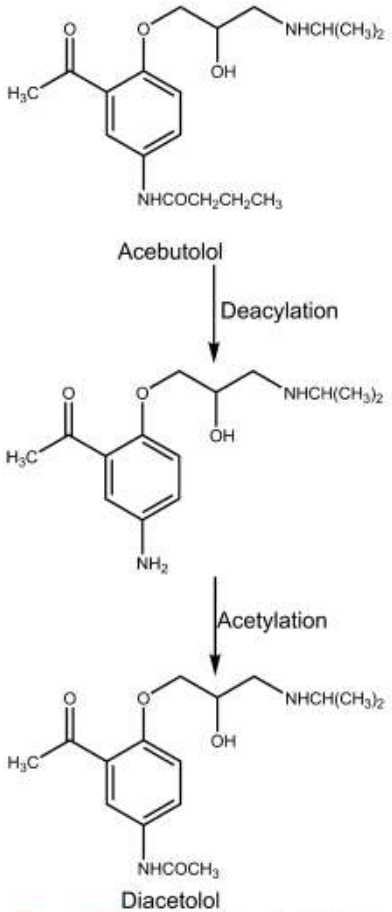


Figure 16.18 • Metabolism of acebutolol.

B-Blocker

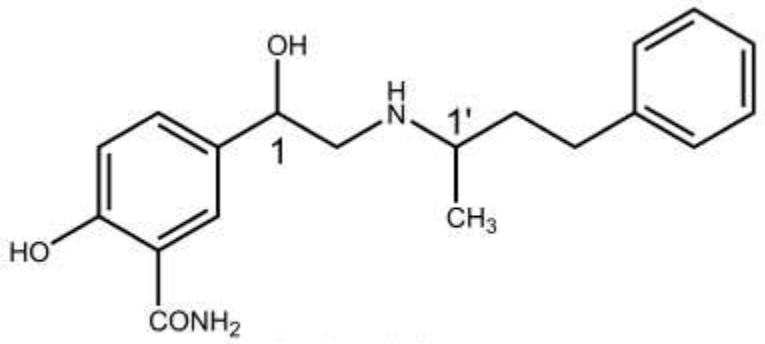
β-BLOCKERS WITH α1-ANTAGONIST ACTIVITY(THIRD GENERATION)

Several drugs have been developed that possess both β- and α-receptor blocking activities within the same molecule. Two examples of such molecules are labetalol and carvedilol. As in the case of dobutamine, the arylalkyl group with nearby methyl group in these molecules is responsible for its α1-blocking activity. The bulky *N*-substituents and another substituted aromatic ring are responsible for its β-blocking activity.

B-Blocker

- ***1- Labetalol*** a phenylethanolamine derivative, is a drug that acts as competitive blockers at α1-, β1-, and β2-receptors. It is a more potent β-blocker than α-blocker. Because it has two asymmetric carbon atoms (1 and 1’), it exists as a mixture of four isomers. It is this mixture that is used clinically in treating hypertension. The different isomers, however, possess different α- and β-blocking activities. The β-blocking activity resides solely in the (1*R*,1’*R*) isomer, whereas the α1-blocking activity is seen in the (1*S*,1’*R*) and (1*S*,1’*S*) isomers, with the (1*S*,1’*R*) isomer possessing the greater therapeutic activity. Labetalol is a clinically useful antihypertensive agent. The rationale for its use in the management of hypertension is that its α-receptor–blocking effects produce vasodilation and its β-receptor–blocking effects prevent the reflex tachycardia usually associated with vasodilation. Although labetalol is very well absorbed, it undergoes extensive first-pass metabolism.

B-Blocker



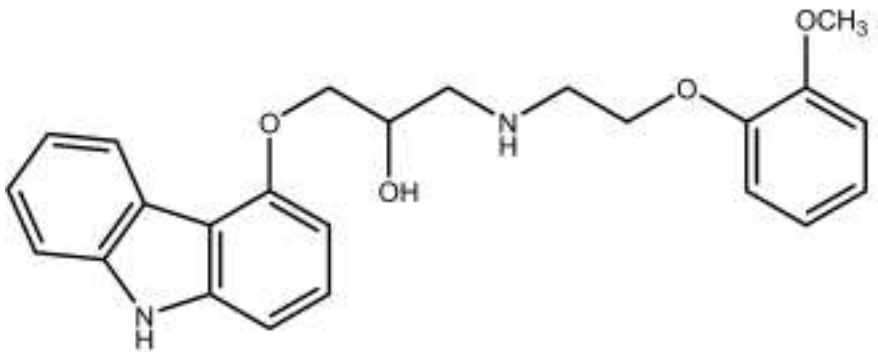
Labetalol

B-Blocker

2- Carvedilol

Like labetalol, it is a β-blocker that possesses α1-blocking activity . Only the (S) enantiomer possesses the β-blocking activity, although both enantiomers are blockers of the α1-receptor. Overall, its β-blocking activity is 10- to 100-fold of its α-blocking activity. This drug is also unique in that it possesses antioxidant activity and an antiproliferative effect on vascular smooth muscle cells. It thus has a neuroprotective effect and the ability to provide major cardiovascular organ protection.[65] It is used in treating hypertension and congestive heart failure.

B-Blocker



Carvedilol

Answer to problem on sodium hydroxide solution

a) The volume of 1.11 *N* hydrochloric acid solution used in titrating sodium hydroxide sample was for total alkali regardless of whether sodium carbonate is present as impurity or not; *i. e.,* the same volume of hydrochloric acid solution would be consumed if sodium carbonate was precipitated as barium carbonate (explain this fact by the related balanced chemical equations).

HCl NaOH

$N_1 \times V_1 = N_2 \times V_2$

$1.11 \times 4.5 = N_2 \times 10$

$N_2 = 0.4995$ the normality of the prepared sodium hydroxide solution

b) Any solid sodium hydroxide sample taken from the original container would contain sodium carbonate as impurity by 3% w/w. Thus, you have to calculate how much sodium hydroxide was used to prepare the 10 milliliters- sample. This is calculated from the volume of hydrochloric acid used for total alkali:

1.11 *N* HCl 1 *N* HCl

$N \times V = \acute{N} \times \acute{V}$

$1.11 \times 4.5 = 1 \times \acute{V}$

$\acute{V} =$ 4.995 mL of 1 *N* hydrochloric acid solution would be required for total alkali (V_3)

$wt = V_3 \times ch.factor$

$wt = 4.995 \times 0.04$

$wt =$ 0.1998 g of total alkali (sodium hydroxide and the impurifying sodium carbonate) calculated as sodium hydroxide was used originally to prepare 10 mL of solution

$\frac{3}{100} \times 0.1998 =$ 0.005994 g of sodium carbonate was present in the sample used to prepare 10 mL of solution

$wt = V_2 \times ch.factor$

$0.005994 = V_2 \times 0.053$

$V_2 = 0.113$ mL of 1 *N* hydrochloric acid solution was consumed by the amount of sodium carbonate present in the sample assayed

1.11 *N* HCl 1 *N* HCl

$N \times V = \acute{N} \times \acute{V}$

$1.11 \times V = 1 \times 0.113$

$\acute{V} = 0.1$ mL of 1.11 *N* hydrochloric acid solution would be consumed by the amount of sodium carbonate present in the sample assayed

It is important to note that the percent w/w is related to solid samples. If you were asked to calculate the percent w/v, then this is related to the prepared solution as follows:

for total alkali: $\frac{0.1998}{10} \times 100 = 1.998\ \%\ w/v$

for sodium carbonate: $\frac{0.005994}{10} \times 100 = 0.05994\ \%\ w/v$

Assay of sodium hydroxide solution

NaOH solution

- From B.p, NaOH solution contains not less than 97.5% w/w of total alkali (as NaOH) and not more than 2.5 % w/w Na_2CO_3.
- **Assay:**

➢ 10 ml of unknown(bulb pipette).

➢ 25 ml of distilled water.

➢ Add 2.5 ml of barium chloride solution.

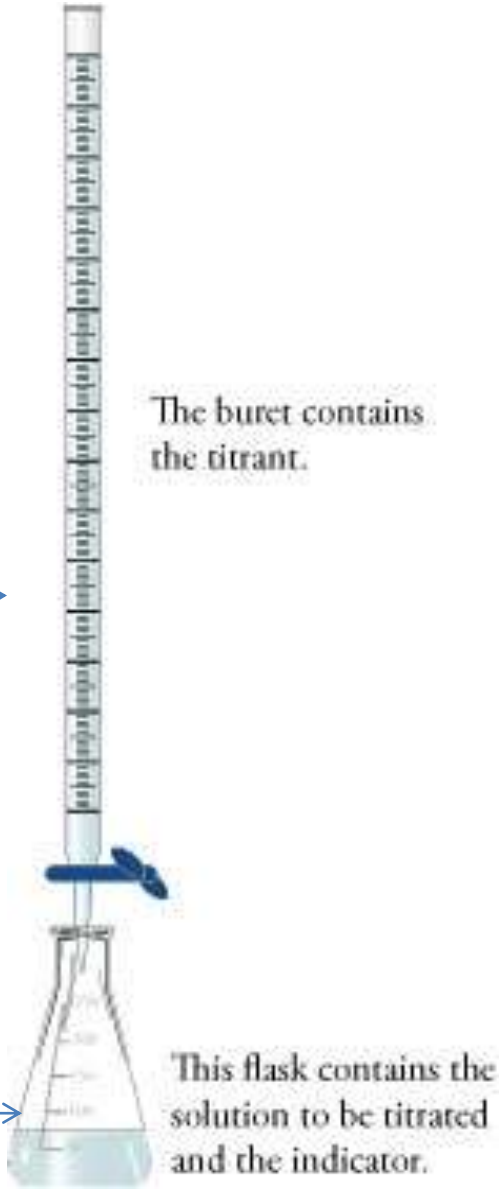
➢ Titrate with 1N HCl solution using 1-2 drops of phenolphthalein ind..

➢ The first end point from pink ⟶ colorless(turbid)

➢ To the turbid sol. add 5 drops of Bromophenol Blue ind. and complete titration with 1N HCl .

➢ The second end point bluish violet ⟶ yellowish green

1N HCl solution



10 ml of unknown
25ml of D.W
2.5 ml of $BaCl_2$ solution
1-2 drops of ph.ph. ind. (1^{st} titration)
5-6 drops of B.p.B ind. (2^{nd} titration)

Chemical principle:

- NaOH is strong base, absorbs CO_2

$$2NaOH + CO_2 \longrightarrow Na_2CO_3 + H_2O$$

- both NaOH and Na_2CO_3 react with HCl

$$NaOH + HCl \longrightarrow NaCl + H_2O$$

$$Na_2CO_3 + 2HCl \longrightarrow 2NaCl + H_2O + CO_2$$

When we assay a sample, we do the assay for total alkalinity contributed to NaOH and Na_2CO_3.

- Barium chloride ($BaCl_2$) is added to precipitate all carbonate

$$BaCl_2 + Na_2CO_3 \longrightarrow BaCO_3\downarrow + 2NaCl$$

soluble in alkaline

insoluble in alkaline

- ***1st titration***:

$$NaOH + HCl \longrightarrow NaCl + H_2O$$

Why HCl do not react with $BaCO_3$?

Why the end point is turbid?

- ***2nd titration***:

$$2HCl + BaCO_3 \longrightarrow BaCl_2 + H_2O + CO_2$$

definition of <u>chemical factor</u>: the weight of substance that is chemically equivalent to 1ml of std. solution.

- **Calculation of the *chemical factor*:**

a) From reaction of HCl with NaOH:

1Mwt of NaOH ≡ 1 Mwt HCl

1 Mwt of NaOH ≡ 1 eqwt HCL

1 ✖40 gm of NaOH ≡ 1 liter of 1*N* HCl

40/1000 gm NaOH ≡1ml of 1*N* HCl

0.04 gm of NaOH ≡ 1ml of 1*N* HCl of total alkalinity calculated as NaOH(chemical factor)

b) From reaction of 2HCl with Na_2CO_3

2Mwt of HCl $\equiv$1Mwt of $BaCO_3$ $\equiv$1Mwt Na_2CO_3

1Mwt Na_2CO_3 $\equiv$ 2Mwt of HCl

1Mwt Na_2CO_3 $\equiv$ 2 eqwt of HCl

½ Mwt Na_2CO_3 $\equiv$ 1 eqwt of HCl

106/2 gm Na_2CO_3 $\equiv$ 1liter of 1*N* HCL

53 gm Na_2CO_3 $\equiv$ 1liter of 1*N* HCL

53/1000 gm Na_2CO_3 $\equiv$ 1ml of 1*N* HCL

0.053 gm Na_2CO_3 $\equiv$ 1ml of 1*N* HCL (chemical factor)

- **Calculations** :

V_1 is the of HCl consumed in the 1st titration

V_2 is the of HCl consumed in the 2nd titration

$V_1+V_2= V_3$ total HCl consumed.

correct the V_3 according to this equation:

$V \times N= V' \times N'$

Corrected V_3 x 0.04= gm wt. of total alkali

Then the % w/v of total alkali

Corrected V_2 x 0.053= gm wt. of Na_2CO_3

Then the % w/v of Na_2CO_3 in the unknown

Hormone therapy

Glucose Metabolism Disorders
Diabetes

Insulin And Modified Insulin

- Type 1 diabetes must be treated with insulin replacement therapy.

Human insulin is a heterodimer (Fig. 20.1), consisting of two different peptide subunits linked covalently by two disulfide bridges. This heterodimer is produced from a single, continuous, and substantially larger (110-amino-acid) protein, namely preproinsulin. Among other reasons, production in this manner ensures proper three-dimensional (3D) folding and cross-linking of insulin. Preproinsulin is converted to proinsulin (86-amino-acid residues), from which insulin is produced by removal of a 35-amino-acid segment joining the two subunits.

Insulin

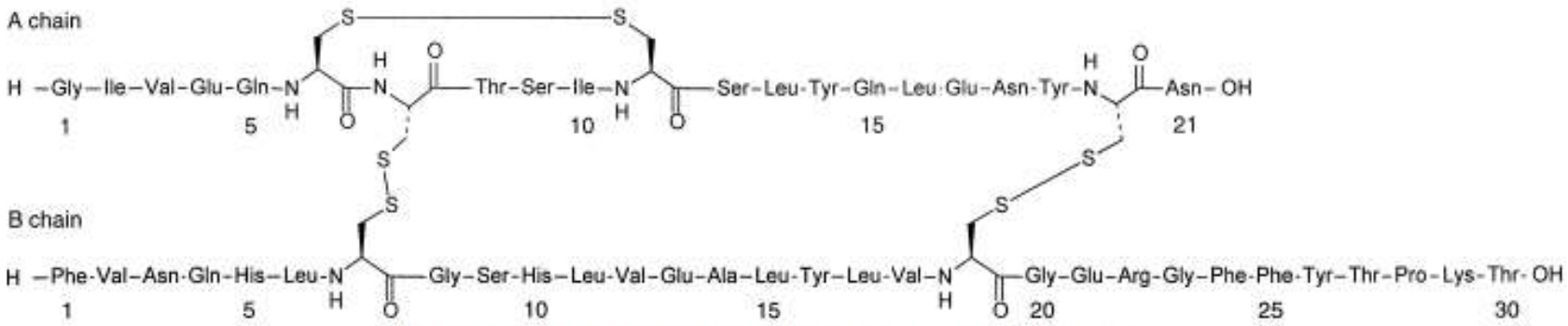


Figure 20.1 • The primary structure of human insulin.

TABLE 20.1 Modified Insulins[a]

	Trade Name	Structural Differences vs. Human Insulin	Characteristics Achieved
Bovine insulin		$Thr^{A8} \rightarrow Ala$, $Ile^{A10} \rightarrow Val$ $Thr^{B30} \rightarrow Ala$	
Porcine insulin		A chain same as hIns $Thr^{B30} \rightarrow Ala$	
Insulin aspart	NovoLog	A chain same as hIns $Pro^{B28} \rightarrow Asp$	Rapidity of onset increased, duration substantially decreased vs. regular human insulin[b]
Insulin lispro	Humalog	A chain same as hIns $Pro^{B28} \rightarrow Lys$, $Lys^{B29} \rightarrow Pro$	Rapidity of onset increased, duration substantially decreased vs. regular human insulin[b]
Insulin glulisine	Apidra	A chain same as hIns $Asn^{B3} \rightarrow Lys$, $Lys^{B29} \rightarrow Glu$	Rapidity of onset increased, duration substantially decreased vs. regular human insulin[b]
Insulin glargine	Lantus	$Asn^{A21} \rightarrow Gly$ Arg^{B31} and Arg^{B32} added to B chain *C* terminus	Extended action
Insulin detemir	Levemir	A chain same as hIns Lys^{B29} derivatized: N^{ε}-$CO(CH_2)_{12}CH_3$ Thr^{B30} removed	Soluble *and* long-acting

[a]See also Table 20.2 for other details and information pertaining to modified insulins.
[b]Solution dimerization is reduced or abolished, the onset rate is thus substantially increased because the monomer remains in solution; completion of release into the systemic circulation occurs substantially sooner because the molecules mostly stay in solution at the injection site.

Insulin

- Even though these differences are quite modest, and allow either bovine or porcine insulin to bind to and activate human insulin receptors comparably to human insulin, immunogenicity to the animal insulins can and does occur. (Although it is now realized that, historically, more incidences of immune intolerance were likely traceable to minor impurities in the insulin preparations than to the animal insulin itself.) Human recombinant insulin is produced commercially in Escherichia coli or yeast. Because proper folding of the final product must be achieved, and purification is nontrivial, the production processes for human recombinant insulin are highly complex and demanding. Human insulin had also been produced commercially by conversion of porcine insulin, which requires only the B30 Ala→Thr replacement, and can be done in such a way as to preserve 3D structure.

Insulin

- Insulin is monomeric in solution only at very low concentrations.
- In many pharmaceutical preparations, it is dimeric; that is, it exists in a form in which one molecule of A-B covalently linked heterodimer associates noncovalently with another A-B heterodimer. In solutions of pH near neutral, the addition of suitable amounts of Zn(+2)to such dimeric preparations results in the formation of hexamers (i.e., 6 A-B heterodimer). Hexamer formation also occurs without Zn(+2) in solutions having concentrations higher than 0.2 µm.

Insulin

- Pharmaceutical advantage has been taken of the formation of such noncovalent multimers, because solubilities of the various insulin species differ and,
- thus, release rates from the injection site can be considerably altered, and reliably so. Consequently, products such as protamine zinc insulin suspension, semilente insulin, and
- ultralente insulin were created.The B21→B30 sequence was found to play a crucial role in this

Insulin

noncovalent insulin dimerization resulting in such products as neutral protein Hagedorn (NPH) insulin, also known as isophane insulin.

Amino acid substitutions in the B21→B30 region, in some cases in conjunction with a further single amino acid substitution elsewhere in the A-B heterodimer, have, over the past decade, provided several therapeutically valuable “modified insulins,” including lispro insulin, insulin aspart, insulin glargine, insulin glulisine, and insulin detemir (see Table 20.2). In these products, enhanced solution stabilities, modified solubilities, increased rapidity of onset, and varied (shortened or extended) durations of action are achieved.

HORMONES

STEROID HORMONES

STEROIDS

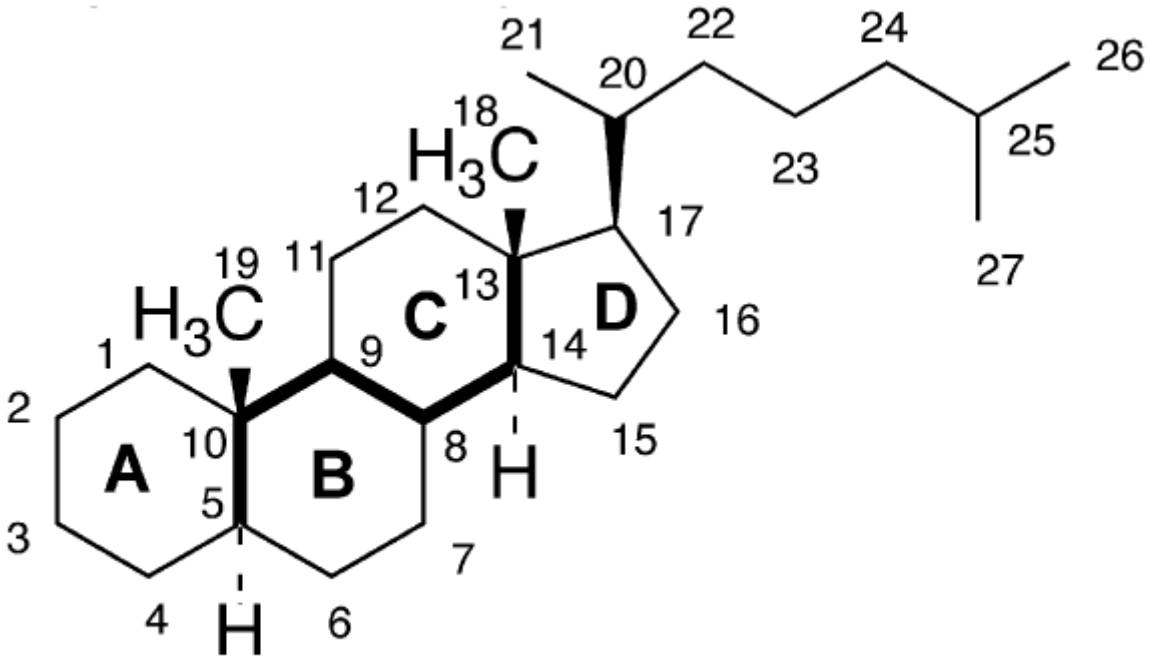
- These hormones and related drugs are chemically based on a common structural backbone, the steroid backbone.The variations in structures provide specificity for the unique molecular targets.Five groups of steroids are present: Estrogens,androgens,progestins,glucocorticoids(GCs) and mineralocorticoids(MCs).

STEROID NOMENCLATURE,STEREOCHEMISTRY AND NUMBERING

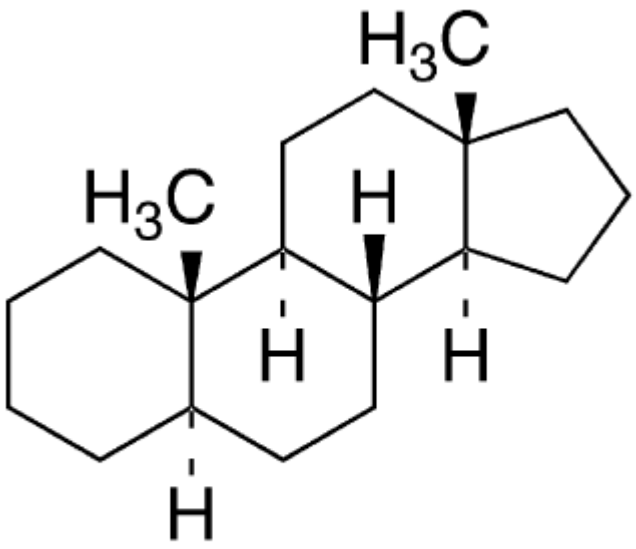
--All steroids are named as derivatives of cholestane,androstane,pregnane or estrane.The standard system of numbering is 5α-cholestane.

--The absolute stereochemistry of the molecule any substituents is shown with solid(β)and dashed(α)bonds.Most carbons have one β bond or one α bond,with β bond lying closer to the top or C18 and C19 methyl side.Both α and β-substituents may be axial or equatorial. This stereochemistry is shown with 5α-androstane.

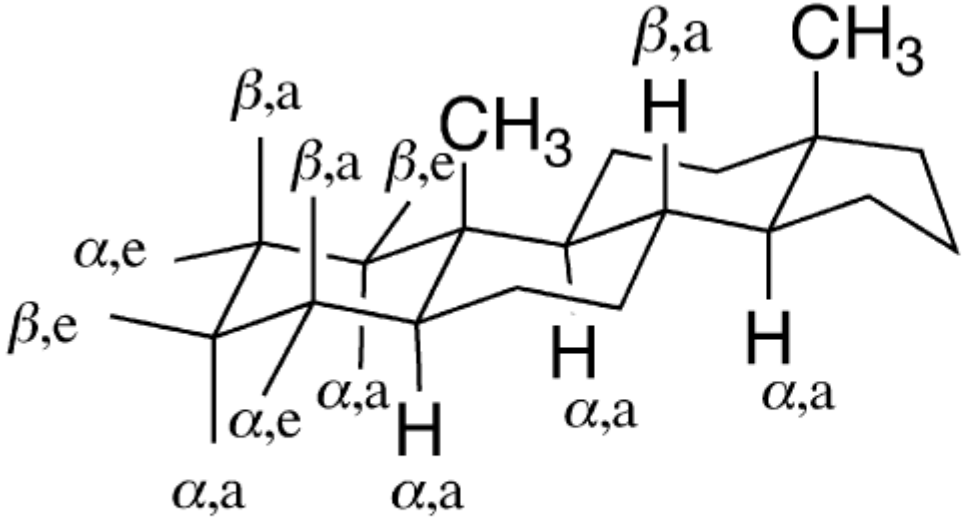
STEROIDS



5α-Cholestane



5α-Androstane



5α-Androstane

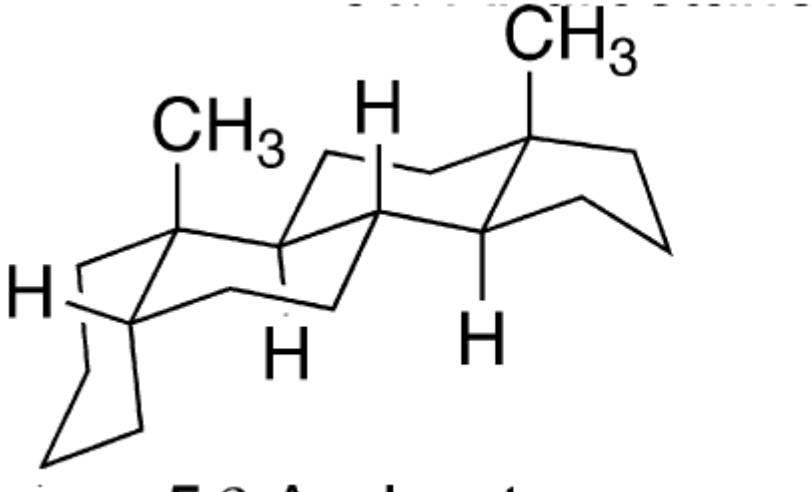
a = axial
e = equatorial
α = alpha bond
β = beta bond

STEROIDS

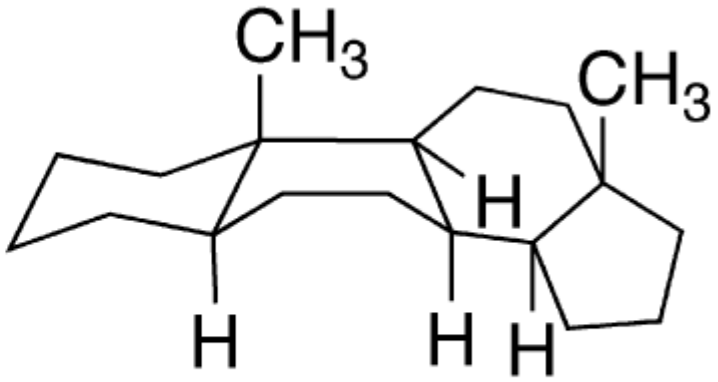
--The stereochemistry of the H at C5 is always indicated in the name,while of the other H atoms is not unless differs from 5α-cholestane. Changing stereochemistry of any of ring juncture or backbone carbons greatly changes the shape of molecule(steroid) as in 5α,8α-androstane and 5β-androstane.

-

STEROIDS



5β-Androstane



5α,8α-Androstane

STEROIDS

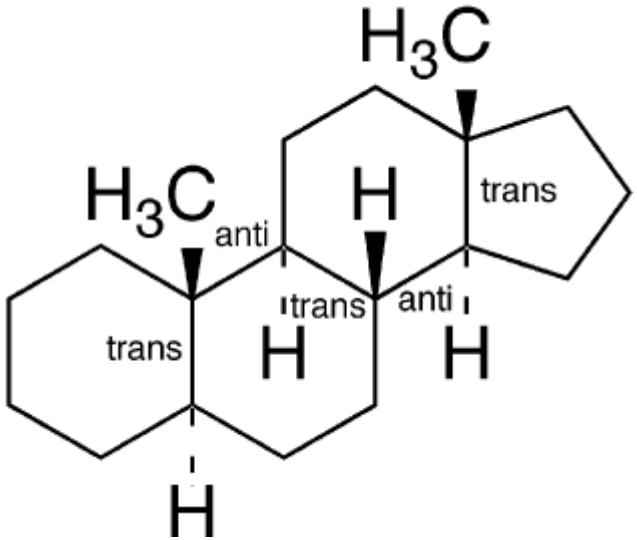
--Because that backbone stereochemistry has immense effect on the shape of molecule,the stereochemistry at all backbone carbones must be clearly shown.
--That is all hydrogens along the backbone should be drawn.
--When the stereochemistry is not known,a wavy line is used in drawing.Methyls are indicated as CH3.

-

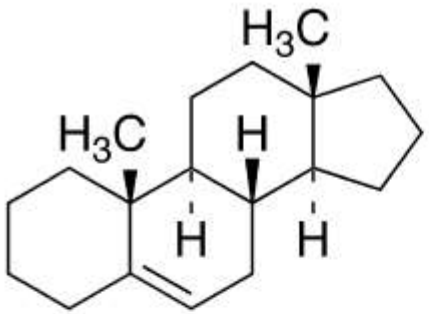
STEROIDS

--The terms cis and trans are occasionally used in steroid nomenclature to indicate backbone stereochemistry among rings.
--Also syn-and anti-are used for indicating stereo.in bonds connecting rings.

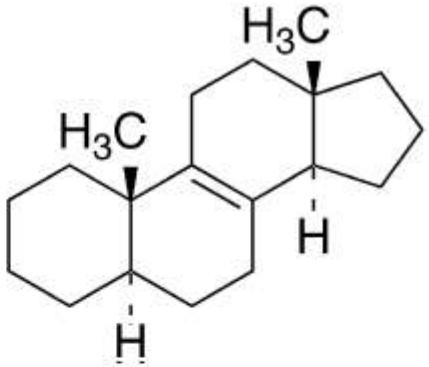
-



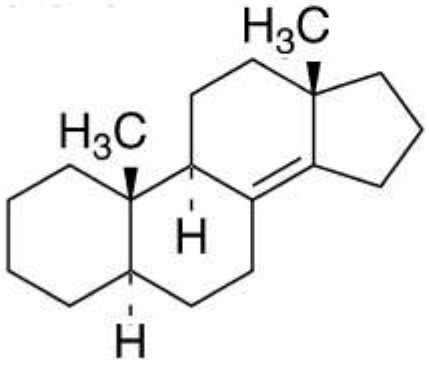
STEROIDS



5-Androstene or
Δ^5-Androstene or
Androst-5-ene



5α-Androst-8-ene or
5α-Δ^8-Androstene

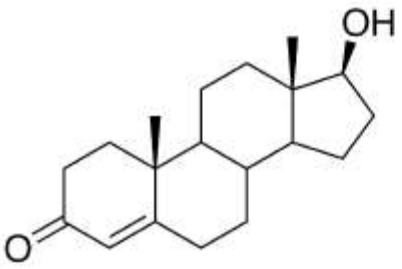


5α-Androst-8(14)-ene or
5α-$\Delta^{8(14)}$-Androstene

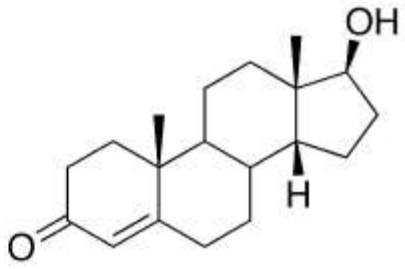
STEROIDS

- --common names testosterone and cortisone are much easier to use than long systematic names.Substituents must always have their position and stereo.clearly indicated when common names used(ex:17α-methyltestosterone,9α-fluorocortisone).
--Steroid drawings sometimes appear with lines instead of methyls and backbone stereo.is not indicated unless it differs from that of 5α-androstane.

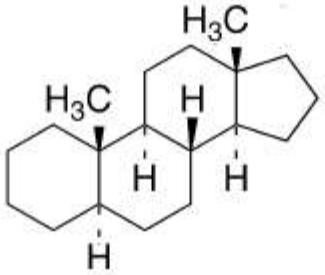
STEROIDS



Testosterone



14β-Testosterone



5α-Androstane

STEROID BIOSYNTHESIS

--In mammals are biosynthesized from cholesterol which in turn is made invivo from acetyl-coenzyme A via mevalonate pathway.
--Conversion of cholesterol to pregnenolone is the rate limiting step in steroid hormones biosynthesis.It is not the enzymetic transformation itself but the translocation of cholesterol to the inner membrane of mitochondria of steroid-synthesizing cells is the rate limiting.
--The enzymes involved in the transformation of cholesterol to the hormones are mainly are CTP450 and dehydrogenases.

CHEMICAL AND PHYSICAL PROPERTIES OF STEROIDS

--Steroids are mostly white crystalline solids (needles,leaflets,etc.).depending on the particular compound,solvents and luck of chemist.

- –Because steroids have 17 C or more,they tend to be water-insoluble.Addition of hydroxyls or other polargroups (or decreasing carbones) increases solubility slightly.Salts are the most water soluble.

STEROIDS

TABLE 25.1 Solubilities of Steroids

	Solubility (g/100 mL)		
	$CHCl_3$	EtOH	H_2O
Cholesterol	22	1.2	Insoluble
Testosterone	50	15	Insoluble
Testosterone propionate	45	25	Insoluble
Dehydrocholic acid	90	0.33	0.02
Estradiol	1.0	10	Insoluble
Estradiol benzoate	0.8	8	Insoluble
Betamethasone	0.1	2	Insoluble
Betamethasone acetate	10	3	Insoluble
Betamethasone $NaPO_4$ salt	Insoluble	15	50
Hydrocortisone	0.5	2.5	0.01
Hydrocortisone acetate	1.0	0.4	Insoluble
Hydrocortisone $NaPO_4$ salt	Insoluble	1.0	75
Prednisolone	0.4	3	0.01
Prednisolone acetate	1.0	0.7	Insoluble
Prednisolone $NaPO_4$ salt	0.8	13	25

CHANGES TO MODIFY PHARMACOKINETIC PROPERTIES OF STEROIDS

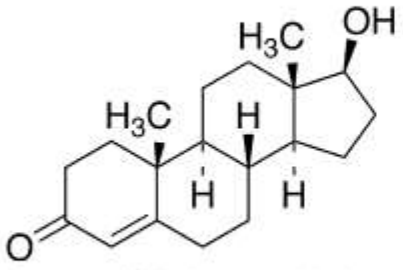
- --The steroids can be made more lipid or water soluble by making suitable ester derivatives of hydroxyl groups.
--Derivatives with increased lipid solubility are made to decrease rate of releasing drug from IM site of injecti-on (depot preparations).
--More lipid-soluble derivatives also are made to imrove skin absorption and prefered for dermatological preparations.
--Water-soluble derivatives are suitable for IV preparat-ions.

STEROIDS

- --Hydrolyzing enzymes are present throughout mammalian cells,especially liver,OH-esterification will not significantly modify the activity of most compounds.
--estradiol,progesterone and testosterone are particularly susceptible to rapid metabolism after absorption or rapid inactivation in GIT before absorption.A simple chemical modification can decrease the rate of inactivation and increase the drug’s half-life or make it possible to be taken orally.

STEROIDS

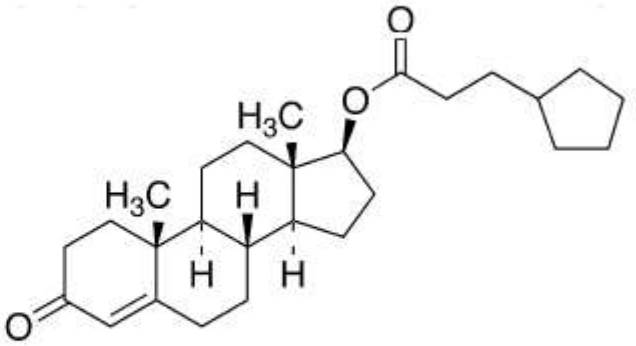
1. Increase Lipid Solubility (Slower rate of release for depot preparation; increase skin absorption)



(IM dose: 10-25 mg
2-3 times/week)

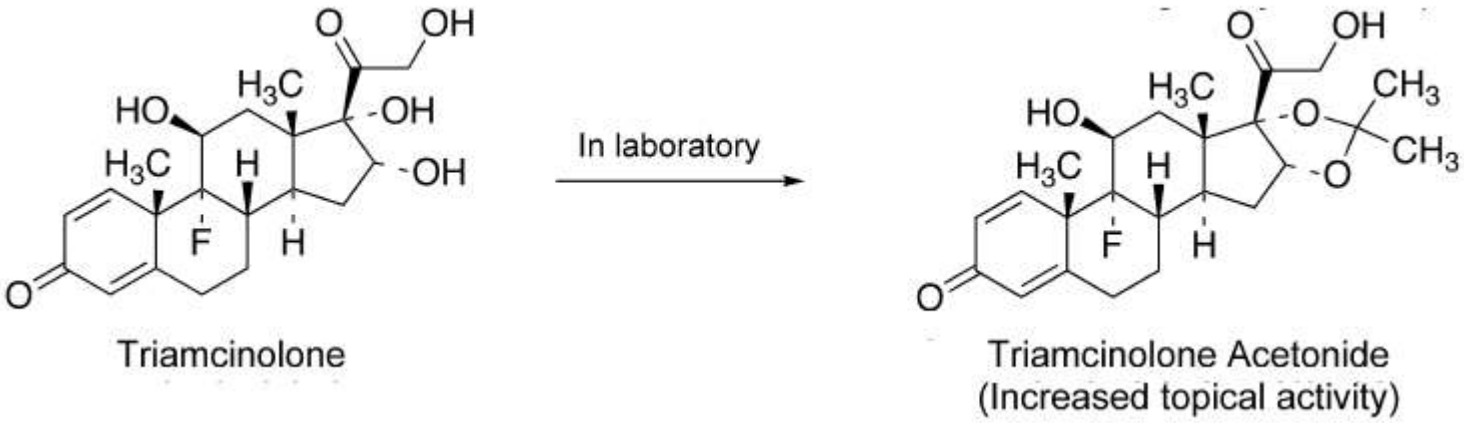
In laboratoy

In vivo



Testosterone Cyclopentylpropionate*
(Testosterone cypionate; IM dose: 200-400 mg every 4 weeks)

STEROIDS

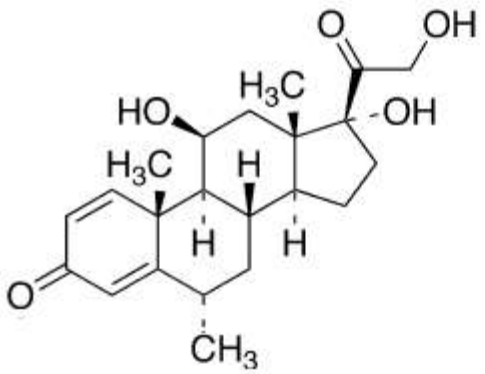


Triamcinolone

Triamcinolone Acetonide
(Increased topical activity)

STEROIDS

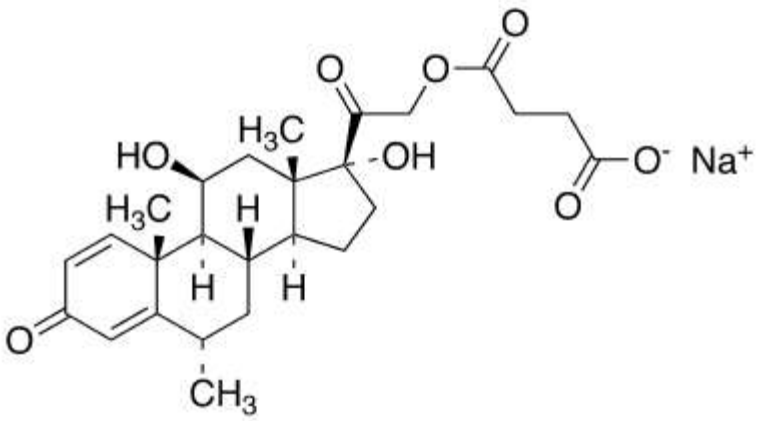
2. Increase Water Solubility (Suitable for IV use)



Methylprednisolone
(Not water-soluble)

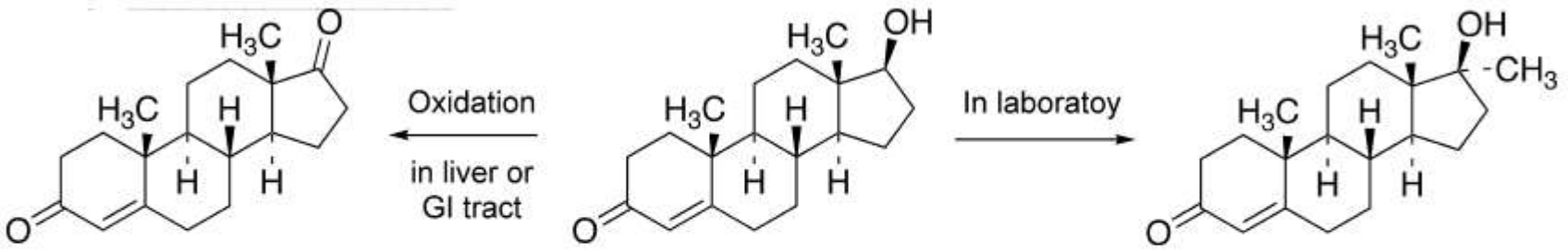
In laboratoy

In vivo



Methylprednisolone Sodium Succinate*
(Sufficiently water-soluble for IV)

3. Decrease Inactivation



Androstenedione
(Reduced activity relative to testosterone)

Testosterone
(Not orally active)

17α-Methyltestosterone
(Orally active – 17 oxidation not possible)

Figure 25.6 • Common steroid modifications to alter therapeutic utility. (*, prodrug.)

STEROID HORMONE RECEPTORS

--Steroid hormones regulate tissue-specific gene expression.The individual hormones exhibit remarkable tissue selectivity,even though their structural differences are relatively minor.Estrogens(estradiol)increase uterine cell proliferation but not prostate cell proliferation.Androgens such as testosterone do the reverse,but neither affect stomach epithelium.The basis of such selectivity is the presence of selective steroid hormone receptors in individual tissues.

- --The steroid receptors are key players in gene expression,but many other are also involved,ex:

STEROIDS

--Chaperone proteins help fold the receptor proteins into the proper three-dimensional shape for binding the steroid ligand.

-

STEROIDS

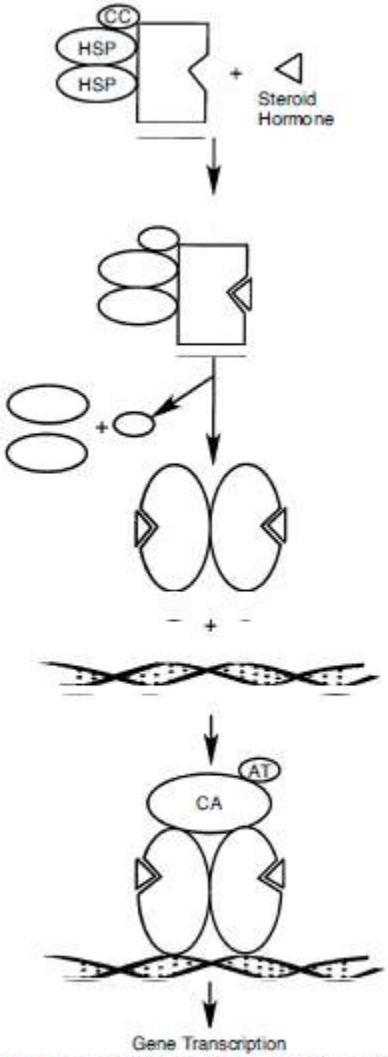


Figure 25.7 • Generic structural model of a steroid hormone–receptor complex and its activation for gene transcription. (AT, histone acetyltransferase; CA, coactivator; CC, cochaperone; HSP, heat shock protein; SHR, steroid hormone receptor.)

STRUCTURE OF STEROID HORMONE RECEPTORS

--The complementary DNAs(cDNAs) of all the major steroid hormone receptors have been cloned,giving the complete amino acid sequence of each.The organization of the domains for all types of steroid receptors is the same but the no.of amino acids for each receptors varies:

- 1-N-terminal(A/B)domain.Once steroid-receptor complex has bound to target genes,this domain activates the hormone response elements adjacent to the genes.

STEROIDS

- 2-DNA-binding(C)domain:This short section is made of about 65 amino acid organized into two zinc fingers important for recognition and binding to DNA response elements.
3-Hinge(D) domain involved with nuclear localization and transport(translocation)of steroid-receptor complex into nucleus.
4-C-terminal ligand-binding(E) domain(LBD): includes 250 amino acids.This section has the steroid-binding site.

STRUCTURE OF STEROID HORMONE-RECEPTOR COMPLEXES

--Steroid-receptor complexes include steroid receptor as well as other proteins(chaperone) proteins,cochaperones and immunophilins. Their role is to chaperone the correct conformation and folding of complex proteins.Without the chaperones,steroid hormone-binding site on receptor does not have the proper folding and conformation for optimal steroid binding.

-

Antibacterial Antibiotics

Alexander Fleming accidentally discovered penicillin in 1929. The numbers of antibiotics added to our therapeutics has grown largely. Antibiotics have the terms of treating infectious diseases. Because of the overuse of many of these agents and the biochemical fickleness of many bacteria , resistance to antibiotics has become a serious problem in the 21st century. Indeed, there are now organisms that cannot be arrested or killed by any of the common antibiotics. Clearly, new approaches are needed.

Substance is classified as an antibiotic if the following conditions are met:

1. It is a product of metabolism (but it may be even anticipated by chemical synthesis).

2. It is a synthetic product produced as a structural analog of a naturally occurring antibiotic.

3. It antagonizes the growth or survival of one or more species of microorganisms.

4. It is effective in low concentrations.

An antibiotic must possess other attributes. First, it must exhibit sufficient selective toxicity to be effective against pathogenic microorganisms or neoplastic tissue without causing significant toxic effects on the normal tissues. Second, an antibiotic should be chemically stable enough to be isolated, processed and stored for a reasonable length of time without deterioration of potency. Third, the rates of biotransformation and elimination of the antibiotic should be slow enough to allow a convenient dosing schedule, yet rapid and complete enough to facilitate removal of the drug and its

metabolites from the body soon after administration has been discontinued.

A-COMMERCIAL PRODUCTION

The commercial production of antibiotics for medicinal use follows a general pattern, differing in detail for each antibiotic. The general scheme may be divided into six steps: (a) preparation of a pure culture of the desired organism for use in inoculation (fermentation medium); (b) fermentation, during which the antibiotic is formed; (c) isolation of the antibiotic from the culture medium; (d) purification; (e) assays for potency, sterility, absence of pyrogens, and other necessary data; and (f) formulation into acceptable and stable dosage forms.

B-MECHANISMS OF ACTION

1-Antibiotics that interfere with the synthesis of bacterial cell walls have a high potential for selective toxicity. Some antibiotics structurally resemble some essential metabolites of microorganisms, which suggests that competitive antagonism may be the mechanism by which they exert their effects. Thus, cycloserine is believed to be an antimetabolite for D-alanine, a constituent of bacterial cell walls.
2-Many antibiotics selectively interfere with microbial protein synthesis (e.g., the aminoglycosides, tetracyclines, macrolides, chloramphenicol, and lincomycin) or nucleic acid synthesis (e.g., rifampin).
3-Others, such as the polymyxins and the polyenes, are believed to interfere with the integrity and function of microbial cell membranes. The mechanism of action of an antibiotic determines, in general, whether the agent exerts a bactericidal or a bacteriostatic action.

TABLE 8.1 Mechanisms of Antibiotic Action

Site of Action	Antibiotic	Process Interrupted	Type of Activity
Cell wall	Bacitracin	Mucopeptide synthesis	Bactericidal
	Cephalosporin	Cell wall cross-linking	Bactericidal
	Cycloserine	Synthesis of cell wall peptides	Bactericidal
	Penicillins	Cell wall cross-linking	Bactericidal
	Vancomycin	Mucopeptide synthesis	Bactericidal
Cell membrane	Amphotericin B	Membrane function	Fungicidal
	Nystatin	Membrane function	Fungicidal
	Polymyxins	Membrane integrity	Bactericidal
Ribosomes	Chloramphenicol	Protein synthesis	Bacteriostatic
50S subunit	Erythromycin	Protein synthesis	Bacteriostatic
	Lincomycins	Protein synthesis	Bacteriostatic
30S subunit	Aminoglycosides	Protein synthesis and fidelity	Bactericidal
	Tetracyclines	Protein synthesis	Bacteriostatic
Nucleic acids	Actinomycin	DNA and mRNA synthesis	Pancidal
	Griseofulvin	Cell division, microtubule assembly	Fungistatic
DNA and/or RNA	Mitomycin C	DNA synthesis	Pancidal
	Rifampin	mRNA synthesis	Bactericidal

C-CHEMICAL CLASSIFICATION

1-β-LACTAM ANTIBIOTICS

Antibiotics that possess the *β*-lactam (a four-membered cyclic amide) ring structure . The first antibiotic penicillin (penicillin G or benzylpenicillin) and then a close biosynthetic relative, phenoxymethyl penicillin (penicillin V) remain the agents of choice for the treatment of infections caused by most species of Gram-positive bacteria. The discovery of a second major group of *β*-lactam antibiotics, the cephalosporins, and chemical modifications of naturally occurring penicillins and cephalosporins have provided semisynthetic derivatives that are variously effective against bacterial species known to be resistant to penicillin, in particular, penicillinase-producing staphylococci and Gram-negative bacilli.

a-Mechanism of Action

The lethal antibacterial action of these agents has been attributed to a selective inhibition of bacterial cell wall synthesis. Specifically, the basic mechanism involved is inhibition of the biosynthesis of the dipeptidoglycan that provides strength and rigidity to the cell wall. Penicillins and cephalosporins acylate a specific bacterial D-transpeptidase,

thereby rendering it inactive for its role in forming peptide cross-links of two linear peptidoglycan strands by transpeptidation and loss of D-alanine. Bacterial D-alanine carboxypeptidases are also inhibited by *β*-lactam antibiotics.

Seven different functional proteins have been revealed, each with an important role in cell wall biosynthesis.These penicillin-binding proteins (PBPs) have the following functional properties:

• PBPs 1_a and 1_b are transpeptidases involved in peptidoglycan synthesis associated with cell elongation. Inhibition results in spheroplast formation and rapid cell lysis caused by autolysins (bacterial enzymes that create nicks in the cell wall for attachment of new peptidoglycan units or for separation of daughter cells during cell division).

• PBP 2 is a transpeptidase involved in maintaining the rod shape of bacilli. Inhibition results in ovoid or round forms that undergo delayed lysis.

• PBP 3 is a transpeptidase required for septum formation during cell division. Inhibition results in the formation of filamentous forms containing rod-shaped units that cannot separate.

• PBPs 4 through 6 are carboxypeptidases responsible for the hydrolysis of D-alanine–D-alanine terminal peptide bonds of the cross-linking peptides.

b-Nomenclature

Two numbering systems for the fused bicyclic heterocyclic system exist:

1- The Chemical Abstracts system initiates the numbering with the sulfur atom and assigns the ring nitrogen the 4-position. Thus, penicillins are named as 1-thia-4-azabicyclo heptanes.

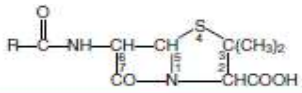
2-The system adopted by the USP is the reverse of the Chemical Abstracts procedure, assigning number 1 to the nitrogen atom and number 4 to the sulfur atom.

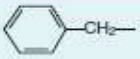
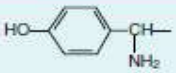
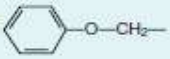
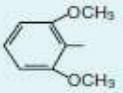
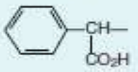
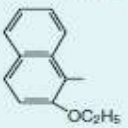
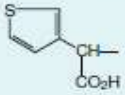
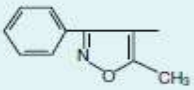
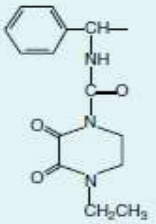
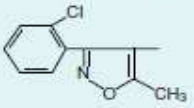
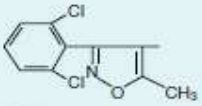
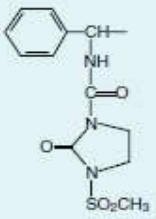
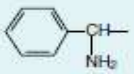
Three simplified forms of penicillin nomenclature have been adopted for general use:

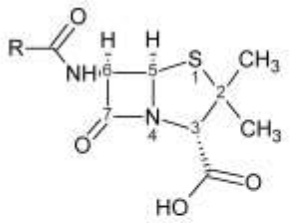
1-using the name "penam" for the unsubstituted bicyclic system with one of the foregoing numbering systems as just described. Thus, penicillins generally are designated according to the Chemical Abstracts system as 6-acylamino-2,2-dimethylpenam-3-carboxylicacids.

2-Using the name "penicillanic acid" to describe the ring system with substituents that are generally present (i.e., 2,2-dimethyl and 3-carboxyl).

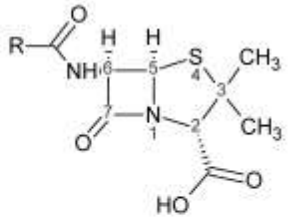
3-Followed in this chapter, to name the entire 6-carbonylaminopenicillanic acid portion of the molecule as penicillin and then distinguishes compounds on the basis of the R group of the acyl portion of the molecule. Thus, penicillin G is named benzylpenicillin, penicillin V is phenoxymethylpenicillin, methicillin is 2,6-dimethoxyphenylpenicillin, and so on.



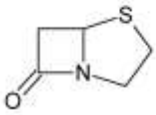
Generic Name	Chemical Name	R Group	Generic Name	Chemical Name	R Group
Penicillin G	Benzylpenicillin		Amoxicillin	D-α-Amino-*p*-hydroxybenzylpenicillin	
Penicillin V	Phenoxymethylpenicillin		Cyclacillin	1-Aminocyclohexyl-penicillin	
Methicillin	2,6-Dimethoxyphenyl-penicillin		Carbenicillin	α-Carboxybenzyl-penicillin	
Nafcillin	2-Ethoxy-1-naphthyl-penicillin		Ticarcillin	α-Carboxy-3-thienyl-penicillin	
Oxacillin	5-Methyl-3-phenyl-4-isoxazolylpenicillin		Piperacillin	α-(4-Ethyl-2,3-dioxo-1-piperazinylcarbonyl-amino)benzylpenicillin	
Cloxacillin	5-Methyl-3-(2-chlorophenyl)-4-isoxazolylpenicillin				
Dicloxacillin	5-Methyl-3-(2,6-dichlorophenyl)-4-isoxazolylpenicillin		Mezlocillin	α-(1-Methanesulfonyl-2-oxoimidazolidino-carbonylamino)benzyl-penicillin	
Ampicillin	D-α-Aminobenzyl-penicillin				



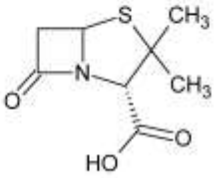
Chemical Abstracts



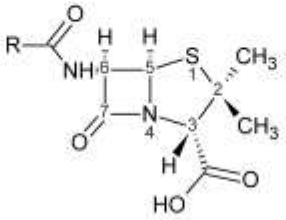
USP



Penam



Penicillanic Acid



Chemical Abstracts

c-Stereochemistry

The penicillin molecule contains three chiral carbon atoms (C-3, C-5, and C-6). All naturally occurring and microbiologically active synthetic and semisynthetic penicillins have the same absolute configuration about these three centers. The carbon atom bearing the acylamino group (C-6) has the L(R)

configuration, whereas the carbon(C-3) to which the carboxyl group is attached has the D(S) configuration. Thus, the acylamino and carboxyl groups are trans to each other, with the former in the α and the latter in the β orientation relative to the penam ring system. The atoms composing the 6-aminopenicillanic acid (6-APA) portion of the structure are derived biosynthetically from two amino acids, L-cysteine (S-1, C-5, C-6, C-7, and 6-amino) and L-valine (2,2-dimethyl, C-2, C-3, N-4, and 3-carboxyl). The absolute stereochemistry of the penicillins is designated 3S:5R:6R.

d-Synthesis

Attempts to synthesize chemically these compounds resulted in only trace amounts until adapted techniques developed in peptide synthesis to the synthesis of penicillin V but the last step of reaction series develops only 10 to 12 % penicillin. Therefore this procedure is not likely to replace the established fermentation processes.

Two other developments have provided additional means for making new penicillins, the isolation of 6-APA from a culture of *P. chrysogenum* . This compound can be converted to penicillins by acylation of the 6-amino group. Another route to synthetic penicillins by converting a natural penicillin, such as penicillin G potassium, to an intermediate (Fig. 8.1), from which the acyl side chain has been cleaved and which then can be treated to form biologically active penicillins with various new side chains.

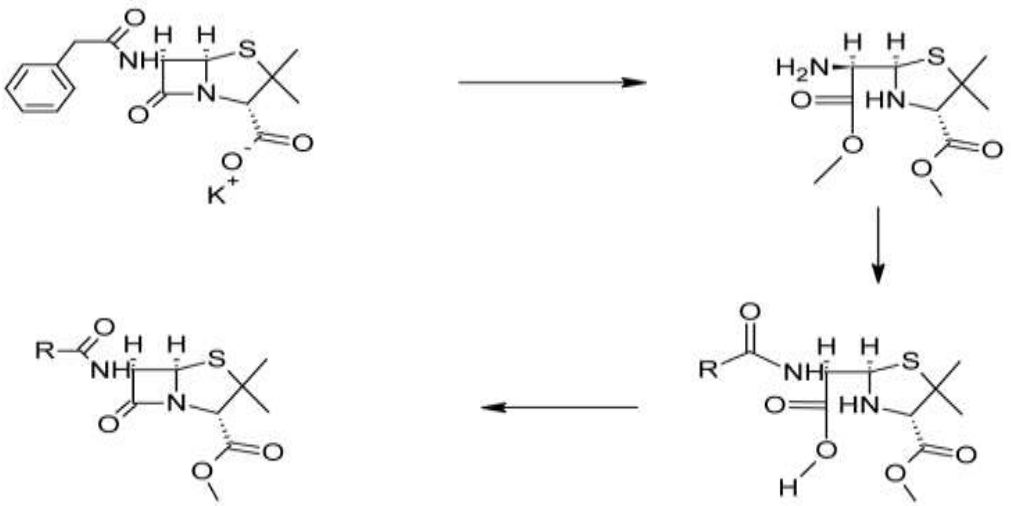


Figure 8.1 • Conversion of natural penicillin to synthetic penicillin.

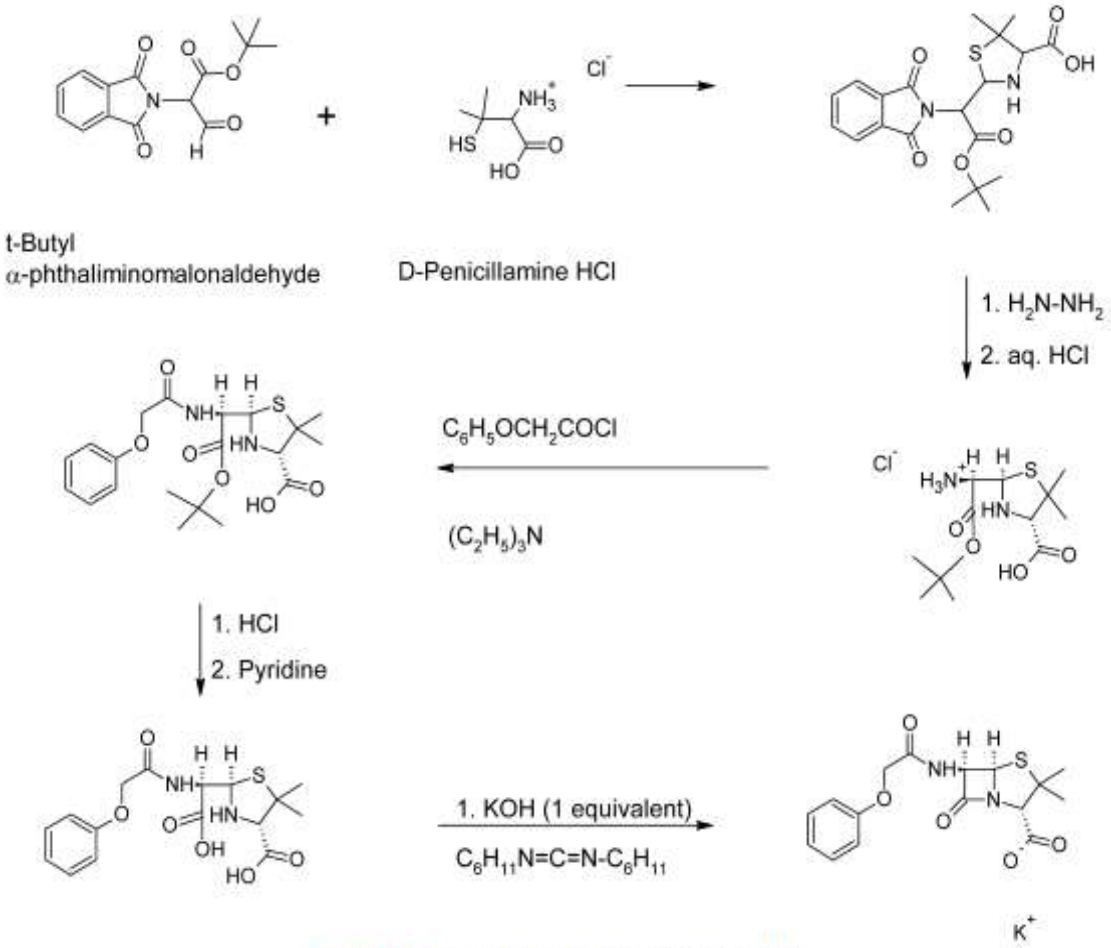


Figure 8.2 • Synthesis of phenoxymethylpenicillin.

e-Chemical Degradation

1-Crystalline penicillin must be protected from moisture.When kept dry, the salts will remain stable for years without refrigeration.
2-The solubility and other physicochemical properties of the penicillins are affected by the nature of the acyl side chain and by the cations used to make salts of the acid.
3-Most penicillins are acids with pKa values in the range of 2.5 to 3.0, but some are amphoteric.The free acids are not suitable for oral or parenteral administration. The sodium and potassium salts of most penicillins, however, are soluble in water and readily absorbed orally or parentally. Salts of penicillins with organic bases, such as benzathine, procaine etc... have limited water solubility and are, therefore, useful as depot forms to provide effective blood levels over a long period in the treatment of chronic infections. 4-The main cause of deterioration of penicillin is the reactivity of the strained lactam ring, particularly to hydrolysis. The *β*-lactam carbonyl group of penicillin *readily* undergoes nucleophilic attack by water or (especially) hydroxide ion to form the inactive *penicilloic acid*.Other nucleophiles, such as hydroxylamines, alkylamines, and alcohols, open the *β*-lactam ring to form the corresponding hydroxamic acids, amides, and esters. *One of the causes* of penicillin allergy may be the formation of antigenic *penicilloyl* proteins in vivo by the reaction of nucleophilic groups (e.g; *ε*-amino) on specific body proteins with the *β*-lactam carbonyl group. 5-By controlling the pH(6.0-6.8) and refrigerating the solutions, aqueous preparations of the soluble penicillins may be stored for up to several weeks.6-Oxidizing agents also inactivate penicillins, but reducing agents have little effect on them.7-Temperature affects

the rate of deterioration;prolonged heating inactivates the penicillins.

9-*Acid-catalyzed degradation* in the stomach contributes strongly to the poor oral absorption of penicillin. Thus, efforts to obtain penicillins with improved pharmacokinetic and microbiological properties have focused on acyl functionalities that would minimize sensitivity of the *β*-lactam ring to acid hydrolysis while maintaining antibacterial activity:

Substitution of an electron-withdrawing group in the *α* position of benzylpenicillin markedly stabilizes the penicillin to acid-catalyzed hydrolysis. Thus, phenoxymethylpenicillin, *α*-aminobenzylpenicillin, and *α*-halobenzylpenicillin are significantly more stable than benzylpenicillin in acid solutions. The increased stability imparted by such electron-withdrawing groups has been attributed to decreased reactivity (nucleophilicity) of the side chain amide carbonyl oxygen atom toward participation in *β*-lactam ring opening to form *penicillenic* acid. Obviously, *α*-aminobenzylpenicillin (ampicillin) exists as the protonated form in acidic (as well as neutral) solutions, and the ammonium group is known to be powerfully electron-withdrawing.

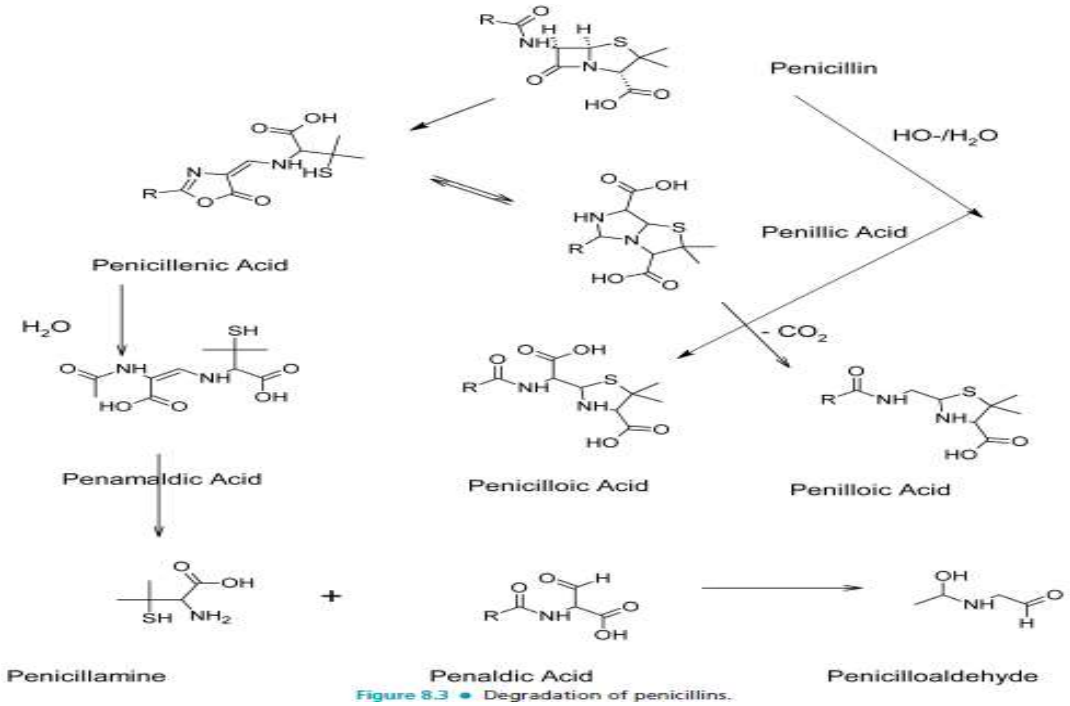


Figure 8.3 • Degradation of penicillins.

f-BacterialResistance

***1.*The most important biochemical mechanism of penicillin resistance is the bacterial enzymes that inactivate penicillins. Such enzymes, which have been given the nonspecific name *penicillinases*, are of two general types: *β*- lactamases and acylases. The more important of these are the *β*-lactamases, enzymes that catalyze the hydrolytic opening of the *β*-lactam ring of penicillins to produce inactive *penicilloic acids*. Synthesis of bacterial *β*-lactamases may be under chromosomal or plasmid R factor control and may be either constitutive or inducible (stimulated by the presence of the substrate), depending on the bacterial species. The resistance among strains of *Staphylococcus aureus* may be because of the production of an inducible *β*-lactamase. Resistance among Gram-negative bacilli, however, may result from constitutive *β*-lactamase elaboration. Specific acylases (enzymes that can hydrolyze the acylamino side chain of penicillins).These enzymes find some commercial use in the preparation of 6-APA for the preparation of semisynthetic penicillins. 6-APA is less active and hydrolyzed more rapidly (enzymatically and nonenzymatically) than penicillin.**

***2-*Another important resistance mechanism, especially in Gram-negative bacteria, is decreased permeability to penicillins. The cell envelope in most Gram-negative bacteria contains an outer membrane not present in Gram-positive bacteria, which creates a physical barrier to the penetration of antibiotics, especially those that are hydrophobic. Small hydrophilic molecules, however, can traverse the outer membrane through pores formed by proteins called *porins*. Alteration of the number or nature of porins in the cell envelope also could be an important mechanism of antibiotic resistance. *3-* Bacterial resistance can result from changes in the affinity of PBPs for penicillins. Altered PBP binding has been demonstrated in non–*β*-lactamase-producing penicillin-resistant *Neisseria gonorrhoeae* and methicillin-resistant *S. aureus* (MRSA).**

4-Certain strains of bacteria are resistant to the lytic properties of penicillins but remain susceptible to their growth inhibiting effects. Thus, the action of the antibiotic has been converted from bactericidal to bacteriostatic. This mechanism of resistance is termed *tolerance* and apparently results from *impaired autolysin* activity in the bacterium.

g-Penicillinase-Resistant Penicillins

In general, increasing the steric hindrance at the *α*-carbon of the acyl group increased resistance to *β*-lactamase, with maximal resistance being observed with quaternary substitution. The observation about antibacterial potency that the *α*-acyl carbon could be part of an aromatic (e.g., phenyl or naphthyl) or heteroaromatic (e.g., 4-isoxazolyl) system. Substitutions at the *ortho* positions of a phenyl ring (e.g., 2,6-dimethoxy [methicillin]) or the 2-position of a 1-naphthyl system (e.g., 2-ethoxyl [nafcillin]) increase the steric hindrance of the acyl group and confer more *β*- lactamase resistance than shown by the unsubstituted compounds or

those substituted at positions more distant from the *α*-carbon. Bulkier substituents are required to confer effective *β*-lactamase resistance among five-membered–ring heterocyclic derivatives. Thus, members of the 4-isoxazolyl penicillin family (e.g., oxacillin, cloxacillin, and dicloxacillin) require both the 3-aryl and 5-methyl (3-methyl and 5-aryl) substituents for effectiveness against *β*- lactamase–producing *S. aureus*.

Increasing the bulkiness of the acyl group is not without its price, however, because all of the clinically available penicillinase-resistant penicillins are significantly less active than either penicillin G or penicillin V against most non–*β*-lactamase-producing bacteria normally sensitive to the penicillins. The *β*-lactamase–resistant penicillins tend to be comparatively lipophilic molecules that do not penetrate well into Gram-negative bacteria. The isoxazoyl penicillins, particularly those with an electronegative substituent in the 3-phenyl group (cloxacillin, dicloxacillin, and floxacillin), are also resistant to acid-catalyzed hydrolysis of the *β*-lactam, for the reasons described previously. Steric factors that confer *β*-lactamase resistance, however, do not necessarily also confer stability to acid.

h-Extended-Spectrum Penicillins

Introduction of an ionized or polar group into the *α*-position of the side chain benzyl carbon atom of penicillin G confers activity against Gram-negative bacilli. The introduction of an *α*-amino group in ampicillin (or amoxicillin) creates an additional chiral center. Extension of the antibacterial spectrum brought about by the substituent applies only to the D-isomer, which is 2 to 8 times more active than either the L-isomer or benzylpenicillin (which are equiactive) against various species of the mentioned Gram-negative bacilli.

The basis for the expanded spectrum of activity associated with the ampicillin group is not related to *β*-lactamase inhibition. Hydrophilic penicillins, such as ampicillin, penetrate Gram negative bacteria more readily than penicillin G, penicillin V, or methicillin. This selective penetration is believed to take place through the porin channel of the cell membrane.

An α-hydroxy substitution also yields"expanded-spectrum" penicillins but they are about 2-5 times less active than their corresponding α-aminobenzyl counterparts and not very stable under acidic conditions.

Incorporation of an acidic substituent at the *α*-benzyl carbon atom of penicillin G also imparts clinical effectiveness against Gram-negative bacilli and, furthermore, extends the spectrum of activity to include organisms resistant to ampicillin. Thus, *α*-carboxybenzylpenicillin (carbenicillin) is active against ampicillin-sensitive Gram-negative species and additional Gram-negative bacilli of the genera *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Proteus*..... The potency of carbenicillin against most species of penicillin G-sensitive Gram-positive bacteria is several lower than that of either penicillin G or ampicillin, presumably because of poorer penetration of a more highly ionized molecule into these bacteria. (Note that *α*-aminobenzylpenicillins exist as zwitterions over a broad pH range and, as such, are considerably less polar than carbenicillin.) This increased polarity is apparently an advantage for the penetration of carbenicillin through the cell envelope of Gram-negative bacteria via porin channels.

Carbenicillin is active against some *β*-lactamase–producing strains of Gram negative bacteria. Because it is a derivative of phenylmalonic acid, carbenicillin *readily decarboxylates* to benzylpenicillin in the presence of acid; therefore, it is not active (as carbenicillin) orally and must be administered

parenterally. Esterification of the *α*-carboxyl group (e.g., as the 5-indanyl ester) partially protects the compound from acid-catalyzed destruction and provides an orally active derivative that is hydrolyzed to carbenicillin in the plasma.

A series of *α*-acylureido–substituted penicillins examplified by piperacillin exhibits greater activity against certain non-β-lactamase Gram-negative bacilli than carbenicillin. *β*-lactamases hydrolyze these penicillins. More facile penetration through the cell envelope of these particular bacterial species is the most likely explanation for this greater potency. The acylureidopenicillins, unlike ampicillin, are unstable under acidic conditions; therefore, they are not available for oral administration.

i-Protein Binding

Penicillins with polar or ionized substituents in the side chain exhibit low-to-intermediate fractions of protein binding. Accordingly, ampicillin and amoxicillin experience 25% to 30% protein binding and carbenicillin and ticarcillin show 45% to 55% protein binding. Those with nonpolar, lipophilic substituents (nafcillin and isoxazolyl penicillins) are more than 90% protein bound. The penicillins with less complex acyl groups (benzylpenicillin, phenoxymethylpenicillin, and methicillin) fall in the range of 35% to 80%.

j-Allergy to Penicillins

Allergic reactions to various penicillins constitute the major problem associated with the use of this class of antibiotics. Estimates place the prevalence of hypersensitivity to penicillin G throughout the world between 1% and 10% of the population.

Evidence suggests that penicillins or their rearrangement products formed in vivo (*e.g., penicillenic acids*) react with lysine ε-amino groups of proteins to form *penicilloyl* proteins, which are major antigenic determinants. Ampicillin is known to undergo polymerization reactions (especially in concentrated solutions) that involve nucleophilic attack of the side chain amino group of one molecule on the *β*-lactam carbonyl carbon atom of a second molecule, and so on. The high frequency of antigenicity shown by ampicillin polymers (in some ampicillin preparations) supports the theory that they can contribute to ampicillin-induced allergy.

k-Classification

Various designations have been used to classify penicillins, based on their sources, chemistry, pharmacokinetic properties, resistance to enzymatic spectrum of activity, and clinical uses (Table 8.3). Thus, penicillins may be biosynthetic, semisynthetic, or (potentially) synthetic; acid-resistant or not; orally or (only) parenterally active; and resistant to *β*-lactamases (penicillinases) or not. They may have a narrow, intermediate, broad, or extended spectrum of antibacterial activity and may be intended for multipurpose or limited clinical use.

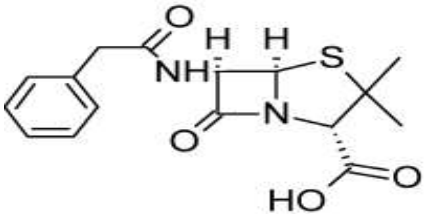
TABLE 8.3 Classification and Properties of Penicillins

Penicillin	Source	Acid Resistance	Oral Absorption (%)	Plasma Protein Binding (%)	β-Lactamase Resistance (*S. aureus*)	Spectrum of Activity	Clinical Use
Benzylpenicillin	Biosynthetic	Poor	Poor (20)	50–60	No	Intermediate	Multipurpose
Penicillin V	Biosynthetic	Good	Good (60)	55–80	No	Intermediate	Multipurpose
Methicillin	Semisynthetic	Poor	None	30–40	Yes	Narrow	Limited use
Nafcillin	Semisynthetic	Fair	Variable	90	Yes	Narrow	Limited use
Oxacillin	Semisynthetic	Good	Fair (30)	85–94	Yes	Narrow	Limited use
Cloxacillin	Semisynthetic	Good	Good (50)	88–96	Yes	Narrow	Limited use
Dicloxacillin	Semisynthetic	Good	Good (50)	95–98	Yes	Narrow	Limited use
Ampicillin	Semisynthetic	Good	Fair (40)	20–25	No	Broad	Multipurpose
Amoxicillin	Semisynthetic	Good	Good (75)	20–25	No	Broad	Multipurpose
Carbenicillin	Semisynthetic	Poor	None	50–60	No	Extended	Limited use
Ticarcillin	Semisynthetic	Poor	None	45	No	Extended	Limited use
Mezlocillin	Semisynthetic	Poor	Nil	50	No	Extended	Limited use
Piperacillin	Semisynthetic	Poor	Nil	50	No	Extended	Limited use

l-Products

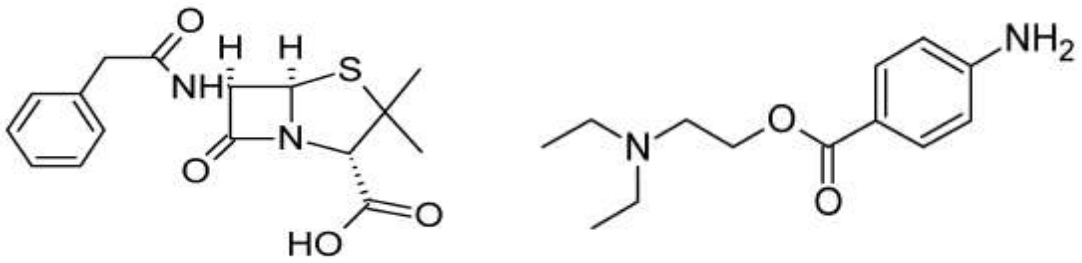
Penicillin G

Or benzylpenicillin. Sodium and potasium salts of penicillin G are inactivated by the gastric juice and are not effective when administered orally unless antacids, such as calcium carbonate is added. Because penicillin G is absorbed poorly from the intestinal tract, oral doses must be very large, about five times the amount necessary with parenteral administration. The water-soluble potassium and sodium salts can be used orally and parenterally to achieve high plasma concentrations of penicillin G rapidly. The more water-soluble potassium salt usually is preferred when large doses are required. Situations in which hyperkalemia is a danger as in renal failure. It requires use of the sodium salt; the potassium salt is preferred for patients on salt-free diets or with congestive heart conditions.



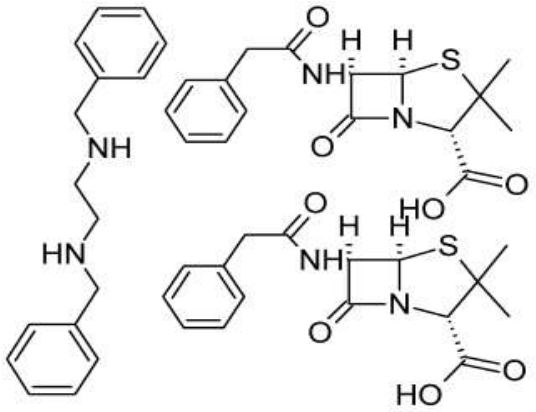
Penicillin G Procaine

Penicillin G procaine can be made readily from penicillin G sodium by treatment with procaine hydrochloride. This salt is considerably less soluble in water than the alkali metal salts. Some commercial products are mixtures of penicillin G potassium or sodium with penicillin G procaine; the water-soluble salt provides rapid development of a high plasma concentration of penicillin, and the insoluble salt prolongs the duration of effect.



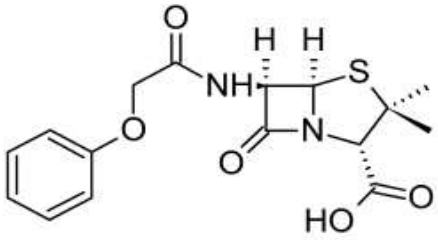
Penicillin G Benzathine

Since penicillin G benzathine is the salt of a diamine, 2 moles of penicillin are available from each molecule. It is very insoluble in water. This property gives the compound great stability and prolonged duration of effect. At the pH of gastric juice, it is quite stable, and food intake does not interfere with its absorption. It is available in tablet form and in several parenteral preparations.



Penicillin V

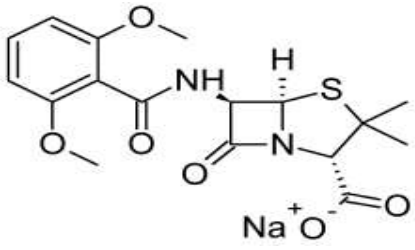
Phenoxymethylpenicillin, as a biosynthetic product, is resistant to hydrolysis by gastric juice and it is active orally. For parenteral solutions, the potassium salt is usually used. This salt is very soluble in water. The salt of phenoxymethylpenicillin with organic amine base provides a very long-acting form of this compound.



Methicillin Sodium

Reacting 2,6-dimethoxybenzoyl chloride with 6-APA forms methicillin.

Methicillin sodium is particularly resistant to inactivation by the penicillinase found in staphylococci. Methicillin and many other penicillinase resistant penicillins induce penicillinase formation, an observation that has implications concerning use of these agents in the treatment of penicillin G-sensitive infections. Clearly, the use of a penicillinase-resistant penicillin should not be followed by penicillin G. The absence of the benzyl methylene group of penicillin G and the steric protection afforded by the 2- and 6-methoxy groups make this compound particularly resistant to enzymatic hydrolysis.

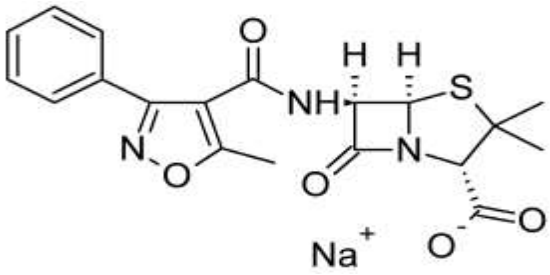


Oxacillin Sodium

Oxacillin sodium monohydrate is the salt of a semisynthetic penicillin that is highly resistant to inactivation by penicillinase. Apparently, the steric effects of the 3-phenyl and

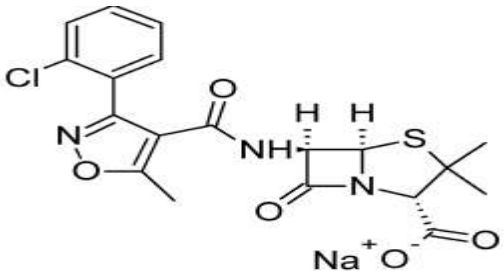
5-methyl groups of the isoxazolyl ring prevent the binding of this penicillin to the *β*-lactamase active site and, thereby, protect the lactam ring from degradation. It is also relatively resistant to acid hydrolysis and, therefore, may be administered orally with good effect.

The use of oxacillin and other isoxazolylpenicillins should be restricted to the treatment of infections caused by staphylococci resistant to penicillin G.



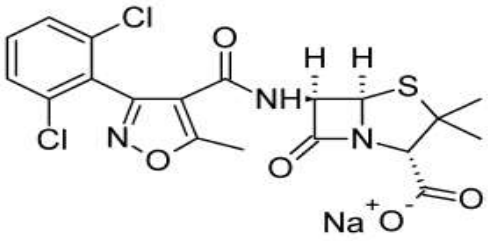
Cloxacillin Sodium

The chlorine atom *ortho* to the position of attachment of the phenyl ring to the isoxazole ring enhances the activity of cloxacillin sodium monohydrate over that of oxacillin not by increasing its intrinsic antibacterial activity but by enhancing its oral absorption, leading to higher plasma levels. In almost all other respects, it resembles oxacillin.



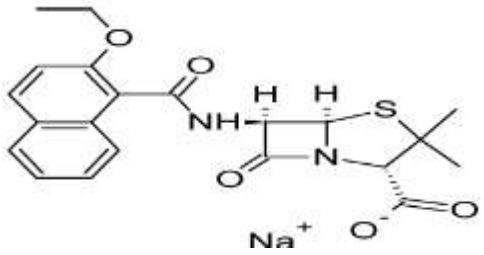
Dicloxacillin Sodium

The substitution of chlorine atoms on both carbons *ortho* to the position of attachment of the phenyl ring to the isoxazole ring is presumed to enhance further the stability of the oxacillin congener dicloxacillin sodium monohydrate. Progressive halogen substitution, however, also increases the fraction bound to protein in the plasma, potentially reducing the concentration of free antibiotic in plasma and tissues. Its medicinal properties and use are the same as those of cloxacillin sodium.



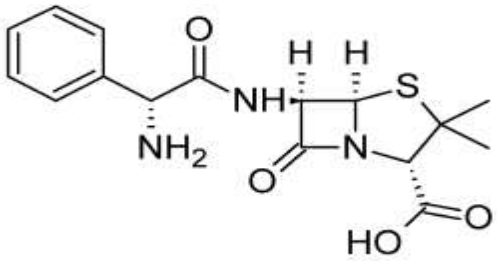
Nafcillin Sodium

Nafcillin sodium, another semisynthetic penicillin, is penicillinase-resistant compound. Nafcillin has substituents in positions *ortho* to the point of attachment of the aromatic ring to the carboxamide group of penicillin. The ethoxy group and the second ring of the naphthalene group play steric roles in stabilizing nafcillin against penicillinase.



Ampicillin

Ampicillin, D-*α*-amino benzyl-penicillin. This product is active against the same Gram-positive organisms that are susceptible to other penicillins, and it is more active against some Gram-negative bacteria and enterococci than are other penicillins. Obviously, the *α*-amino group plays an important role in the broader activity. It has been suggested that the amino group confers an ability to cross cell wall barriers that are impenetrable to other penicillins. D-(-)-Ampicillin, prepared from D-(-)-*α*-aminophenylacetic acid, is significantly more active than L-(+)-ampicillin.



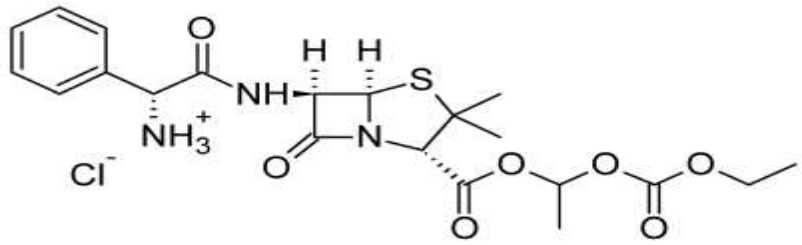
Ampicillin is not resistant to penicillinase . Ampicillin, together with probenecid to inhibit its active tubular excretion, has become a treatment of choice for gonorrhea in recent years.

Ampicillin is water soluble and stable in acid. The protonated *α*-amino group of ampicillin has a pKa of 7.3, and thus it is protonated extensively in acidic media.

Bacampicillin Hydrochloride

Bacampicillin hydrochloride is the hydrochloride salt of the 1-ethoxycarbonyloxyethyl ester of ampicillin. It is a prodrug of ampicillin with no antibacterial activity. After oral absorption, bacampicillin is hydrolyzed rapidly by esterases in the plasma to form ampicillin.

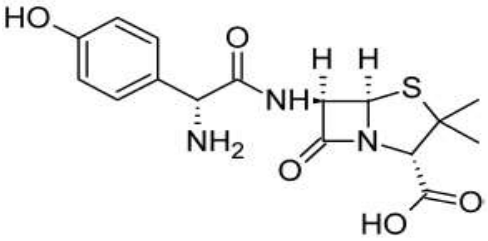
Oral absorption of bacampicillin is more rapid and complete than that of ampicillin and less affected by food. Effective plasma levels are sustained for 12 hours, allowing twice-a-day dosing.



Amoxicillin

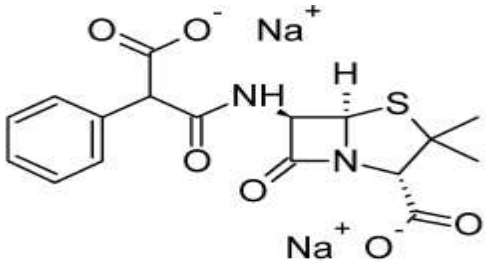
Amoxicillin, a semisynthetic penicillin, is simply the *p*-hydroxy analog of ampicillin, prepared by acylation of 6-APA with *p*-hydroxyphenylglycine.

Its antibacterial spectrum is nearly identical with that of ampicillin, and like ampicillin, it is resistant to acid, susceptible to alkaline and *β*-lactamase hydrolysis, and weakly protein bound. Early clinical reports indicated that orally administered amoxicillin possesses significant advantages over ampicillin, including more complete GI absorption to give higher plasma and urine levels, less diarrhea, and little or no effect of food on absorption. Amoxicillin is reportedly less effective than ampicillin in the treatment of bacillary dysentery, presumably because of its greater GI absorption. It is available in various oral dosage forms. Aqueous suspensions are stable for 1 week at room temperature.



Carbenicillin Disodium, Sterile

Carbenicillin disodium, disodium *α*-carboxybenzylpenicillin , is a semisynthetic penicillin. Examination of its structure shows that it differs from ampicillin in having an ionizable carboxyl group rather than an amino group substituted on the *α*-carbon atom of the benzyl side chain. Carbenicillin has a range of antimicrobial activity broader than any other known penicillin attributed to the unique carboxyl group. It has been proposed that the carboxyl group improves penetration of the molecule through cell wall barriers of Gram-negative bacilli compared with other penicillins.



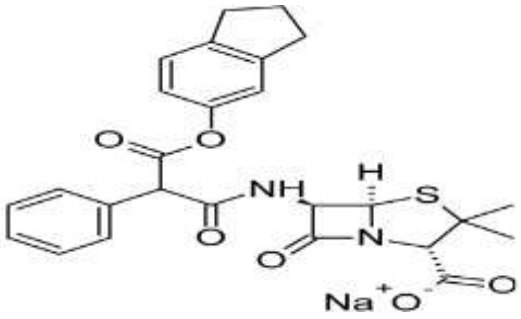
Carbenicillin is not stable in acids and is inactivated by penicillinase. It is a malonic acid derivative and, as such, decarboxylates readily to penicillin G, which is acid labile. Solutions of the disodium salt should be freshly prepared but, when refrigerated, may be kept for 2 weeks. It must be administered by injection and is usually given intravenously.

Carbenicillin has been effective in the treatment of systemic and urinary tract infections caused by *P. aeruginosa* and indole-producing *Proteus* spp. which are resistant to

ampicillin. The low toxicity of carbenicillin, with the exception of allergic sensitivity, permits the use of large dosages in serious infections. Most clinicians prefer to use a combination of carbenicillin and gentamicin for serious pseudomonal and mixed coliform infections. The two antibiotics are chemically incompatible, however, and should never be combined in an intravenous solution.

Carbenicillin Indanyl Sodium

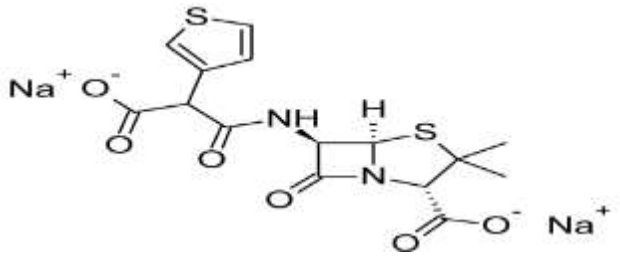
Efforts to obtain orally active forms of carbenicillin led to the eventual release of the 5-indanyl ester carbenicillin. After absorption, the ester is hydrolyzed rapidly by plasma and tissue esterases to yield carbenicillin. Indanyl carbenicillin thus provides an orally active alternative for the treatment of carbenicillin-sensitive systemic and urinary tract infections caused by *Pseudomonas* spp., indole-positive *Proteus* spp., and selected species of Gram-negative bacilli. It is stable in acid. It should be protected from moisture to prevent hydrolysis of the ester.



Ticarcillin Disodium, Sterile

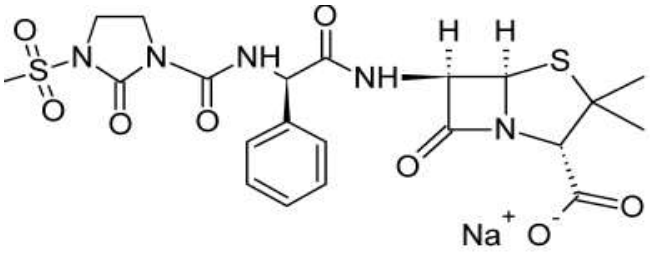
Ticarcillin disodium, *α*-carboxy-3-thienylpenicillin (Ticar), is an isostere of carbenicillin in which the phenyl group is replaced by a thienyl group. This semisynthetic penicillin derivative, like carbenicillin, is unstable in acid and, therefore, must be administered parenterally. It is similar to carbenicillin in

antibacterial spectrum and pharmacokinetic properties. Two advantages for ticarcillin are claimed: (a) slightly better pharmacokinetic properties, including higher serum levels and a longer duration of action; and (b) greater in vitro potency against several species of Gram-negative bacilli, most notably *P. aeruginosa* and *Bacteroides fragilis*. These advantages can be crucial in the treatment of serious infections requiring high-dose therapy.



Mezlocillin Sodium, Sterile

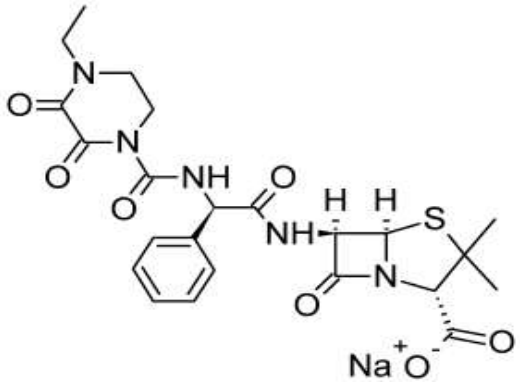
Mezlocillin (Mezlin) is an acylureidopenicillin with an antibacterial spectrum similar to that of carbenicillin and ticarcillin.It is much more active against most *Klebsiella* spp., *P. aeruginosa*, anaerobic bacteria, and *H. influenzae*. It is recommended for the treatment of serious infections caused by these organisms. Mezlocillin is not *generally* effective against *β*- lactamase–producing bacteria, nor is active orally.



Piperacillin Sodium, Sterile

Piperacillin (Pipracil) is the most generally useful of the extended-spectrum α-acylureidopenicillins. It is active against susceptible strains of Gram-negative aerobic bacilli, such as *P. aeruginosa*..etc. Piperacillin is also active against anaerobic

bacteria, especially *B. fragilis* and *S. faecalis* (enterococcus). *β*-Lactamase–producing strains of these organisms are, however, resistant to piperacillin which is hydrolyzed by *S. aureus* *β*-lactamase.



Piperacillin is destroyed rapidly by stomach acid; therefore, it is active only by intramuscular or intravenous administration.

β-LACTAMASE INHIBITORS

Early attempts to obtain synergy against *β*-lactamase–producing bacterial strains by using combinations consisting of a *β*- lactamase–resistant penicillin (e.g., methicillin or oxacillin) as a competitive inhibitor and a *β*-lactamase–sensitive penicillin (e.g., ampicillin or carbenicillin) to kill the organisms, met *with limited* success. Factors that may contribute to the failure of such combinations to achieve synergy include (a) the failure of most lipophilic penicillinase-resistant penicillins to penetrate the cell envelope of Gram-negative bacilli in effective concentrations, (b) the reversible binding of penicillinase-resistant penicillins to *β*-lactamase, requiring high concentrations to prevent substrate binding and

hydrolysis, and (c) the induction of *β*-lactamases by some penicillinase- resistant penicillins.

The naturally occurring, mechanism based inhibitor clavulanic acid, causes potent and progressive inactivation of *β*-lactamases (Fig. 8.4) and has created renewed interest in *β*-lactam combination therapy.

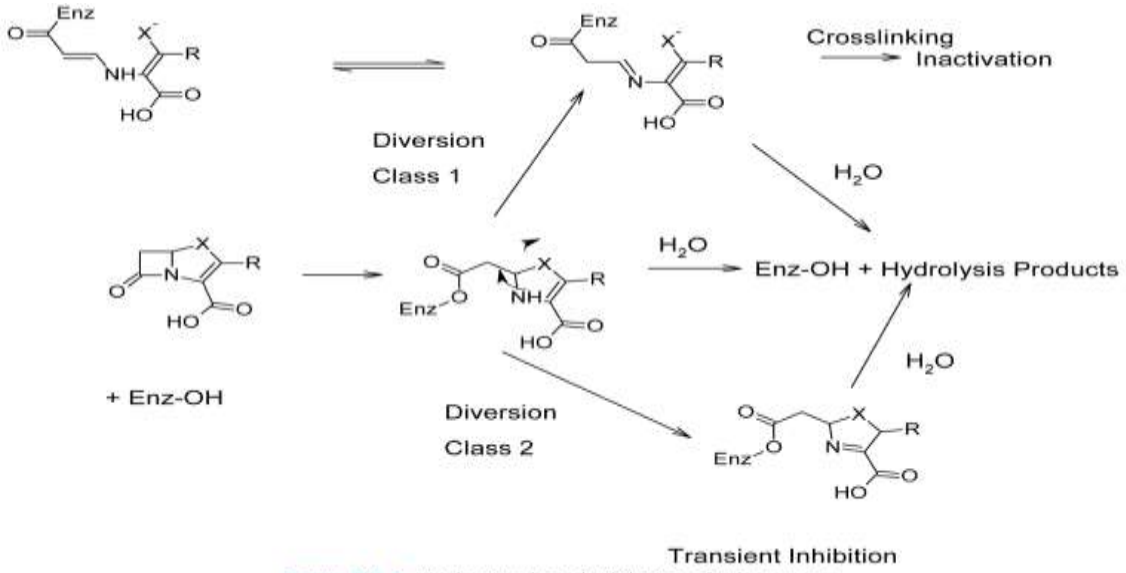


Figure 8.4 ● Mechanism-based inhibition of β-lactamases.

About the chemistry of *β*-lactamase inhibition, Knowles has described two classes of *β*-lactamase inhibitors: class I inhibitors that have a heteroatom leaving group at position 1 and class II inhibitors that do not. *With the β-lactamases,* an acyl-enzyme intermediate is formed by reaction of the *β*-lactam with an active-site serine hydroxyl group of the enzyme. *For normal substrates*, the acyl-enzyme intermediate readily undergoes hydrolysis, destroying the substrate and freeing the enzyme to attack more substrate. The acyl-enzyme intermediate (formed when a mechanism-based inhibitor is attacked by the enzyme) is diverted by tautomerism(class II) to a more stable imine form that hydrolyzes more *slowly* to eventually free the enzyme (transient inhibition) or, for a class I inhibitor, a second group on the enzyme may be attacked to inactivate it. Because these inhibitors are also substrates for

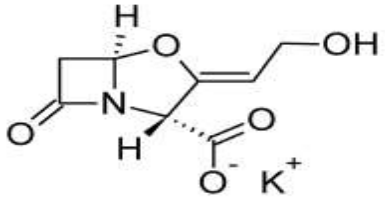
the enzymes that they inactivate, they are sometimes referred to as "suicide substrates."

Because class I inhibitors cause prolonged inactivation of certain *β*-lactamases, they are particularly useful in combination with extended-spectrum *β*-lactamase–sensitive penicillins to treat infections caused by *β*-lactamase–producing bacteria. Three such inhibitors are clavulanic acid, sulbactam and tazobactam. A class II inhibitor, the carbapenem derivative imipenem, has potent antibacterial activity in addition to its ability to cause transient inhibition of some *β*-lactamases.

Products

Clavulanate Potassium

Clavulanic acid is isolated from *Streptomyces clavuligeris*. Structurally, it is a 1-oxopenam derivative lacking the 6-acylamino side chain of penicillins but possessing a 2-hydroxyethylidene moiety at C-2. Clavulanic acid exhibits very weak antibacterial activity comparable with that of 6-APA and, therefore, is not useful as an antibiotic. It is, however, a potent inhibitor of *S. aureus β*-lactamase and *β*-lactamases elaborated by Gram-negative bacilli.

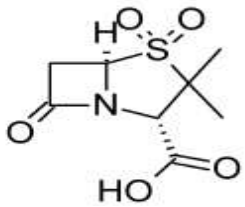


Combinations of amoxicillin and the potassium salt of clavulanic acid are available (Augmentin) with good oral bioavailability to be intended for the treatment of skin, respiratory, ear, and urinary tract infections caused by *β*-lactamase–producing bacterial strains which are resistant to amoxicillin alone. Clavulanic acid is acid-stable. It cannot

undergo *penicillenic acid* formation because it lacks an amide side chain. Potassium clavulanate and the extended-spectrum penicillin ticarcillin have been combined in an injectable form for the control of serious infections caused by *β*-lactamase–producing bacterial strains.

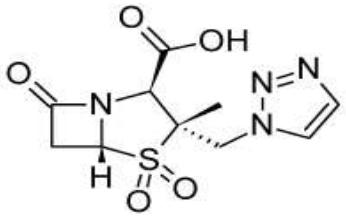
Sulbactam

Sulbactam is penicillanic acid sulfone or 1,1-dioxopenicillanic acid. This synthetic penicillin derivative is a potent inhibitor of *S. aureus* *β*-lactamase as well as many *β*-lactamases elaborated by Gram-negative bacilli. Sulbactam has weak intrinsic antibacterial activity but potentiates the activity of ampicillin and carbenicillin against *β*-lactamase–producing *S. aureus* and members of the Enterobacteriaceae family. It does not, however, synergize with either carbenicillin or ticarcillin against *P. aeruginosa* strains resistant to these agents. Failure of sulbactam to *penetrate* the cell envelope is a possible explanation for the lack of synergy.



Tazobactam

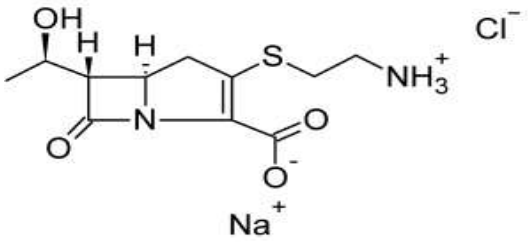
Tazobactam is a penicillanic acid sulfone that is similar in structure to sulbactam. It is a more potent *β*-lactamase inhibitor than sulbactam and has a slightly broader spectrum of activity than clavulanic acid. It has very weak antibacterial activity. Tazobactam is available in injectable combinations with piperacillin, a broad-spectrum penicillin.



CARBAPENEMS

Thienamycin

Thienamycin is a novel *β*-lactam antibiotic isolated and identified from fermentation of cultures of *Streptomyces cattleya*. Two structural features of thienamycin are shared with the penicillins and cephalosporins: a fused bicyclic ring system containing a *β*-lactam and an equivalently attached 3-carboxyl group. The bicyclic system consists of a carbapenem containing a double bond between C-2 and C-3 (i.e., it is a 2-carbapenem, or Δ^2-carbapenem system). The double bond in the bicyclic structure creates considerable ring strain and increases the reactivity of the *β*-lactam to ring opening reactions. The side chain is unique in two respects: it is a simple 1-hydroxyethyl group instead of the familiar acylamino side chain, and it is oriented to the bicyclic ring system rather than having the usual *α* orientation of the penicillins and cephalosporins. The remaining feature is a 2-aminoethylthioether function at C-2. The absolute stereochemistry of thienamycin has been determined to be 5*R*:6*S*:8*S*.

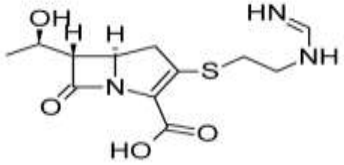


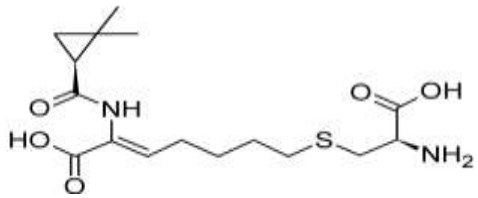
1-It is highly *active* against most aerobic and anaerobic Gram-positive and Gram negative bacteria, including *S. aureus*, *P. aeruginosa*, and *B. fragilis*. 2-Furthermore, it is *resistant to inactivation by most β-lactamases* elaborated by Gram-negative and Gram positive bacteria and, therefore, is effective against many strains resistant to penicillins and cephalosporins. Resistance to lactamases appears to be a function of the *α*-1-hydroxyethyl side chain because this property is lost in the 6-nor derivative and epithienamycins with *S* stereochemistry show variable resistance to the different *β*-lactamases.

It is more susceptible to hydrolysis in both acidic and alkaline solutions than most *β*-lactam antibiotics, because of the strained nature of its fused ring system containing an endocyclic double bond. This inactivation is believed to result from intermolecular aminolysis of the *β*- lactam by the cysteamine side chain of a second molecule. Another shortcoming is its susceptibility to hydrolytic inactivation by renal dehydropeptidase-I (DHP-I), which causes it to have an unacceptably short half-life in vivo.

Imipenem–Cilastatin

Imipenem is chemically stable derivative of thienamycin in which the primary amino group is converted to a nonnucleophilic basic function. Cilastatin is an inhibitor of DHPI. The combination (Primaxin) provides a chemically and enzymatically stable form of thienamycin. Imipenem retains the extraordinary broad-spectrum antibacterial properties of thienamycin. Its bactericidal activity results from the inhibition of cell wall synthesis associated with bonding to PBPs 1b and 2. Imipenem is very stable to most *β*-lactamases. It is an inhibitor of *β*-lactamases from certain Gram-negative bacteria resistant to other *β*-lactam antibiotics.





NEWER CARBAPENEMS

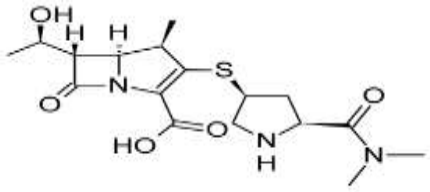
The improvements desired include: 1-stability to hydrolysis catalyzed by DHP-I, 2- stability to bacterial metallo-*β*-lactamases ("carbapenemases") that hydrolyze imipenem 3-activity against MRSA, and 4-increased potency against *P. aeruginosa*, especially imipenem-resistant strains.

Structure–activity studies established the importance of the Δ^2 position of the double bond, the 3-carboxyl group and the 6(*α*-1-hydroxyethyl) side chain for both broad spectrum *antibacterial activity* and *β-lactamase stability* in carbapenems. Modifications, therefore, have concentrated at positions 1 and 2 of the carbapenem nucleus. A-The incorporation of a *β*-methyl group at the 1-position gives the carbapenem stability to hydrolysis by renal DHP-I. B-Substituents at the 2-position, however, appear to affect primarily the spectrum of *antibacterial activity* of the carbapenem by influencing penetration into bacteria. The capability of carbapenems to exist as zwitterionic structures ,(as exemplified by imipenem and biapenem) resulting from the combined features of a basic amine function attached to the 2-position and the 3-carboxyl group, may enable these molecules to enter bacteria via their charged porin channels.

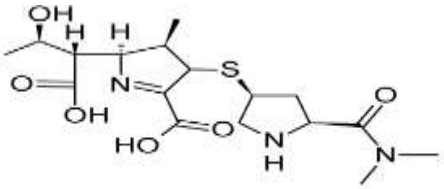
Meropenem

Meropenem is a second-generation carbapenem . It has recently be used for treating serious infections like septicemia, meningitis etc. Meropenem exhibits greater potency against Gram -ve and anaerobic bacteria than does

imipenem but it is slightly less active against most Gram +ve species. Meropenem is not hydrolyzed by DHP-I and is resistant to most β-lactamases, including a few carbapenemases that hydrolyze carbapenem.



Meropenem

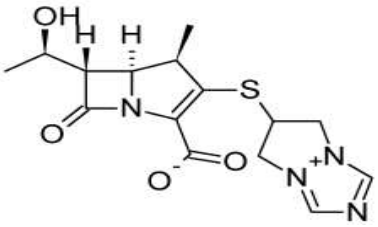


Meropenem metabolite

Meropenem is not active orally. 30% of dose is converted to the inactive metabolite formed by hydrolytic cleavage of the β-lactam ring. The lower incidence of nephrotoxicity of meropenem (compared with imipenem) has been correlated with its greater stability to DHP-I and the absence of the DHP-I inhibitor cilastatin in the preparation.

Biapenem

Biapenem is a newer second-generation carbapenem with chemical and microbiological properties similar to those of meropenem. Thus, it has broad-spectrum antibacterial activity that includes most aerobic Gram-negative and Gram-positive bacteria and anaerobes. Biapenem is stable to DHP-I and resistant to most β-lactamases. It is not active orally.

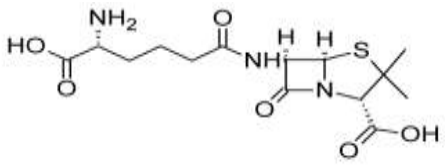


CEPHALOSPORINS

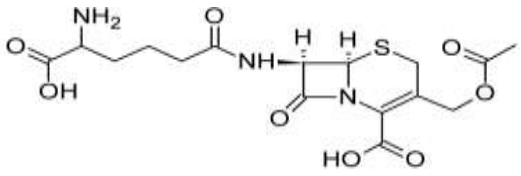
The cephalosporins are *β*-lactam antibiotics isolated from *Cephalosporium* spp. or prepared semisynthetically. In 1948, cultures of the fungus isolated principal antibiotic components penicillin N and cephalosporin C.

Penicillin N was 6-(D-4-amino-4-carboxybutyl-carbonyl)aminopenicillanic acid. The amino acid side chain confers more activity against Gram-negative bacteria, particularly *Salmonella* spp., but less activity against Gram-positive organisms than penicillin G. It has been used successfully in clinical trials for the treatment of typhoid fever but was never released as an approved drug.

Cephalosporin C is a close congener of penicillin N, containing a dihydrothiazine ring instead of the thiazolidine ring of the penicillins. The *α*-aminoadipoyl side chain could be removed to efficiently produce 7-aminocephalosporanic acid (7-ACA) which prompted investigations that led to semisynthetic cephalosporins of medicinal value.



Penicillin N



Cephalosporin C

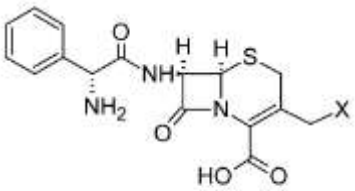
Nomenclature

***Chemical Abstracts* as 5-thia-1-azabicyclo oct-2-ene. Another system gives the saturated bicyclic ring system with the lactam carbonyl oxygen is named *cepham* (cf., *penam* for penicillins). According to this system, all commercially available cephalosporins and cephamycins are named *3-cephems* (or Δ^3-cephems) to designate the position of the double bond.**

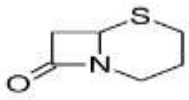
Semisynthetic Derivatives

The more useful semisynthetic modifications of the basic 7-ACA nucleus have resulted from acylations of the 7- amino group with different acids or nucleophilic substitution(reduction) of the acetoxyl group. The presence of an allylic acetoxyl function in the 3-position provides a reactive site at which various 7-acylaminocephalosporanic acid structures can easily be varied by nucleophilic displacement reactions. Reduction of the 3-acetoxymethyl to 3-hydroxymethyl to prepare 7-aminodesacetylcephalosporanic acid (7-ADCA) derivatives can be accomplished by catalytic hydrogenation.

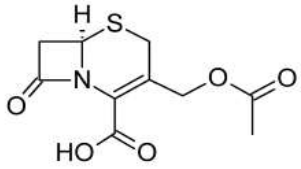
In the preparation of semisynthetic cephalosporins, the following improvements are sought: (a) increased acid stability, (b) improved pharmacokinetic properties, particularly better oral absorption, (c) broadened antimicrobial spectrum, (d) increased activity against resistant microorganisms (as a result of resistance to enzymatic destruction, improved penetration, increased receptor affinity, etc.), (e) decreased allergenicity, and (f) increased tolerance after parenteral administration.



Cephalosporin



Cepham



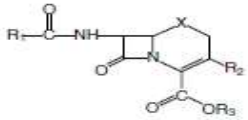
Cephalosporanic Acid

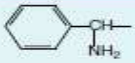
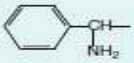
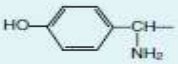
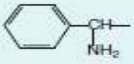
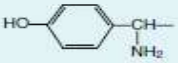
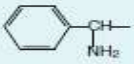
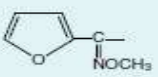
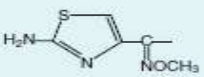
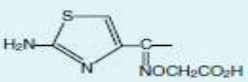
Chemical Degradation

Cephalosporins experience various hydrolytic degradation reactions (Table 8.4). Among 7-acylaminocephalosporanic acid

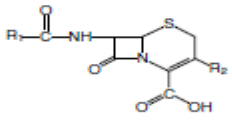
derivatives, the 3-acetoxylmethyl group is the most reactive site. *In addition* to its reactivity to nucleophilic displacement reactions, the acetoxyl function of this group readily undergoes solvolysis in strongly acidic solutions to form the desacetylcephalosporin derivatives. The latter lactonize to form the desacetylcephalosporin lactones , which are virtually inactive. The 7-acylamino group of some cephalosporins can also be hydrolyzed under enzymatic (acylases) and, possibly, nonenzymatic conditions to give 7-ACA (or 7-ADCA) derivatives. Following hydrolysis or solvolysis of the 3-acetoxymethyl group, 7-ACA also lactonizes under acidic conditions (Fig. 8.5). The reactive functionality common to all cephalosporins is the *β*-lactam. Hydrolysis of the *β*-lactam of cephalosporins is believed to give initially cephalosporoic acids.

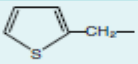
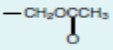
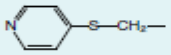
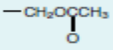
ORAL CEPHALOSPORINS

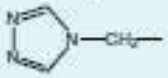
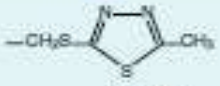
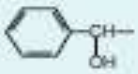
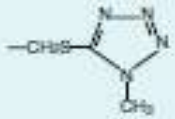
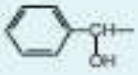
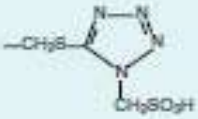
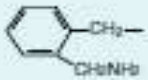
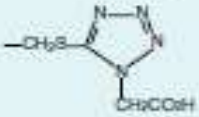
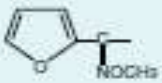
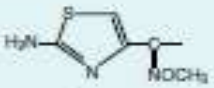
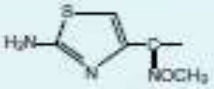
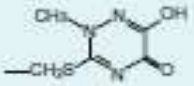
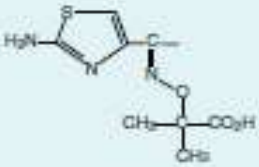
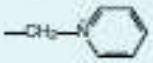
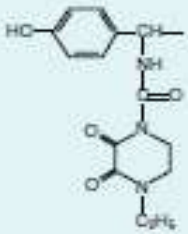
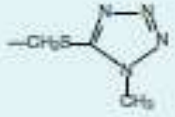


Generic Name	R_1	R_2	R_3	X
Cephalexin		—CH_3	—H	—S—
Cephradine		—CH_3	—H	—S—
Cefadroxil		—CH_3	—H	—S—
Cefachlor		—Cl	—H	—S—
Cefprozil		—CH=$CHCH_3$	—H	—S—
Loracarbef		—Cl	—H	—CH_2—
Cefuroxime axetil		—CH_2OCNH_2	—$CHOCCH_3$	—S—
Cefpodoxime proxetil		—CH_2OCH_3	—$CHOCOCH(CH_3)_2$	—S—
Cefixime		—C=CH_2	—H	—S—

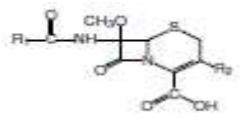
PARENTERAL CEPHALOSPORINS

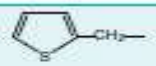
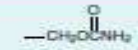
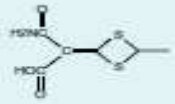
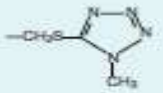
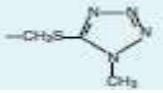


Generic Name	R_1	R_2
Cephalothin		
Cephapirin		

Generic Name	R_1	R_2
Cefazolin		
Cefamandole		
Cefonicid		
Ceforanide		
Cefuroxime		
Cefotaxime		
Ceftizoxime		
Ceftriaxone		
Ceftazidime		
Cefoperazone		

PARENTERAL CEPHAMYCINS



Generic Name	R_1	R_2
Cefoxitin		
Cefotetan		
Cefmetazole	$NCCH_2SCH_2$	

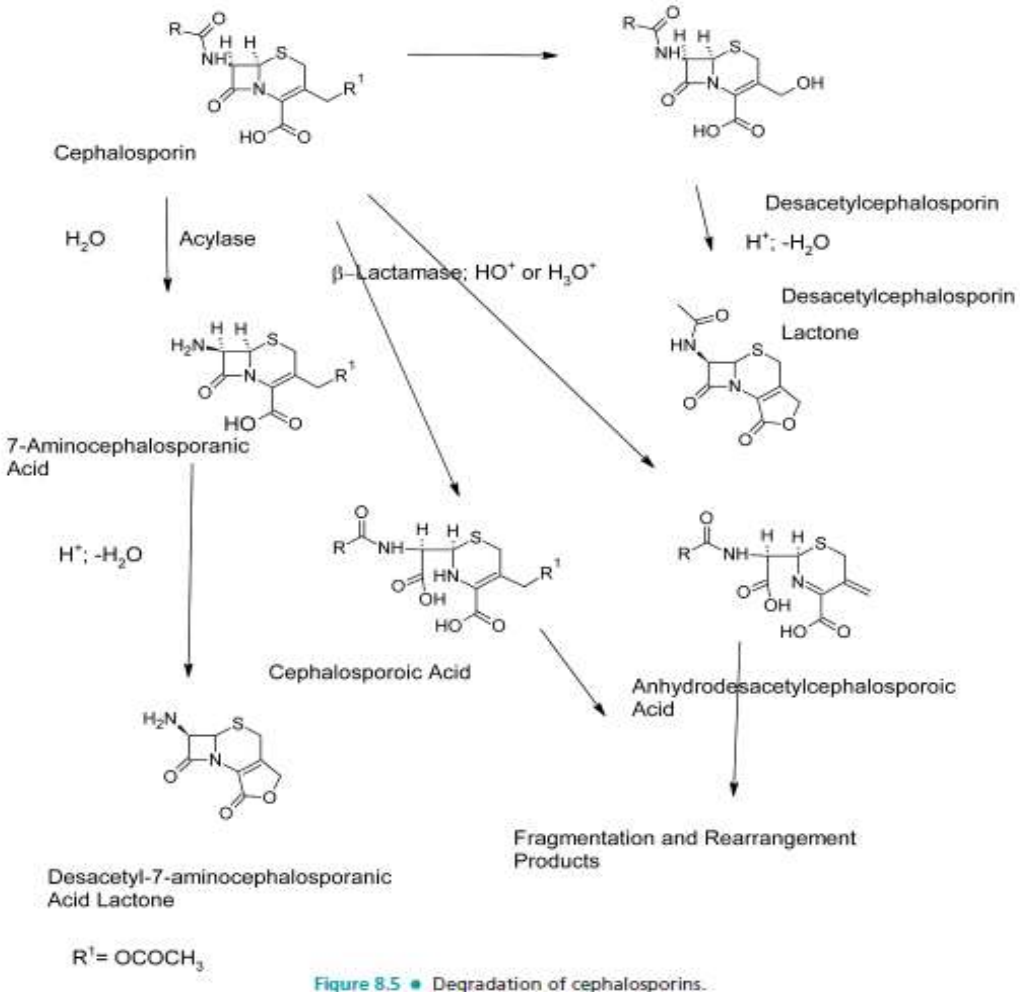


Figure 8.5 ● Degradation of cephalosporins.

Oral Cephalosporins

The oral activity conferred by the phenylglycyl substituent is attributed to increased acid stability of the lactam ring, resulting from the presence of a protonated amino group on the 7-acylamino portion of the molecule. Carrier mediated transport of these dipeptide-like, zwitterionic cephalosporins

is also an important factor in their excellent oral activity. Also important for high acid stability (and, therefore, good oral activity) of the cephalosporins is the absence of the leaving group at the 3-position.Cephalosporanic acid derivative, cephaloglycin, is poorly absorbed orally, presumably because of solvolysis of the 3-acetoxyl group in the low pH of the stomach. The resulting 3-hydroxyl derivative undergoes lactonization under acidic conditions. The 3-hydroxyl derivatives and, especially, the corresponding lactones are considerably less active in vitro than the parent cephalosporins.

Oral activity can also be conferred in certain cephalosporins by esterification of the 4-carboxylic acid group to form acid-stable, lipophilic esters that undergo hydrolysis in the plasma. Cefuroxime axetil and cefpodoxime proxetil are two *β*-lactamase–resistant alkoximino- cephalosporins that are orally active ester prodrug derivatives of cefuroxime and cefpodoxime, respectively, based on this concept.

Parenteral Cephalosporins

Hydrolysis of the ester function, catalyzed by hepatic and renal esterases, is responsible for some in vivo inactivation of parenteral cephalosporins containing a 3-acetoxymethyl substituent (e.g., cephalothin, cephapirin, and cefotaxime). Parenteral cephalosporins lacking a hydrolyzable group at the 3-position are not subject to hydrolysis by esterases. Cephradine is the only cephalosporin that is used both orally and parenterally.

Spectrum of Activity

The cephalosporins are considered broad-spectrum antibiotics with patterns of antibacterial effectiveness comparable to that of ampicillin. Several significant differences exist, however. Cephalosporins are much more resistant to inactivation by *β*-

lactamases, particularly those produced by Gram positive bacteria, than is ampicillin. Ampicillin, however, is generally more active against non–*β*-lactamase-producing strains of Gram-positive and Gram-negative bacteria which is also sensitive to cephalosporins.

***β*-Lactamase Resistance**

1-Cephalosporins are significantly less sensitive than all except the *β*-lactamase–resistant penicillins to hydrolysis by the enzymes . The "penicillinase" resistance of cephalosporins appears to be a property of the bicyclic cephem ring system rather than of the acyl group.
2-The different cephalosporins exhibit considerable variation in rates of hydrolysis by the staphylococcal β-lactamase.
3-The same acyl functionalities that impart *β*-lactamase resistance in the penicillins *unfortunately* render cephalosporins virtually inactive against *S. aureus* and other Gram-positive bacteria.

4-Most of β-lactamases hydrolyze penicillin G and ampicillin faster than the cephalosporins . Some *β*-lactamases belonging to group C are "cephalosporinases," which hydrolyze cephalosporins more rapidly.

5-The introduction of polar substituents in the aminoacyl moiety of cephalosporins appears to confer stability to some *β*-lactamases ,like an *α*-hydroxyphenylacetyl (or mandoyl) group in cefamandole or an *o*-aminomethylphenyl acetyl group in ceforanide are resistant to a few *β*-lactamases.
6-Steric factors also may be important because cefoperazone, an acylureidocephalosporin that contains 4-ethyl-2,3-dioxo-1-piperazinylcarbonyl group , is resistant to many *β*-lactamases.

7-Two structural features confer broadly based resistance to *β*-lactamases among the cephalosporins: *(a)* an alkoximino function in the aminoacyl group example

cefotamixe,ceftriaxone,cefuroxime and ceftizoxime and *(b)* a methoxyl substituent at the 7-position of the cephem nucleus having *α* stereochemistry. *β*-Lactamase resistance is enhanced modestly if the oximino substituent also features a polar function, as in ceftazidime, which has a 2-methylpropionic acid substituent on the oximino group. Both steric and electronic properties of the alkoximino group may contribute to the *β*-lactamase resistance conferred by this functionality since *syn*-isomers are more potent than *anti*-isomers. *β*-Lactamase–resistant 7*α*-methoxylcephalosporins, also called cephamycins because they are derived from cephamycin C (an antibiotic isolated from *Streptomyces* spp.), are represented by cefoxitin, cefotetan, cefmetazole...etc.

7-Base- or *β*-lactamase–catalyzed hydrolysis of cephalosporins containing a good leaving group at the 3-position is accompanied by elimination of the leaving group.

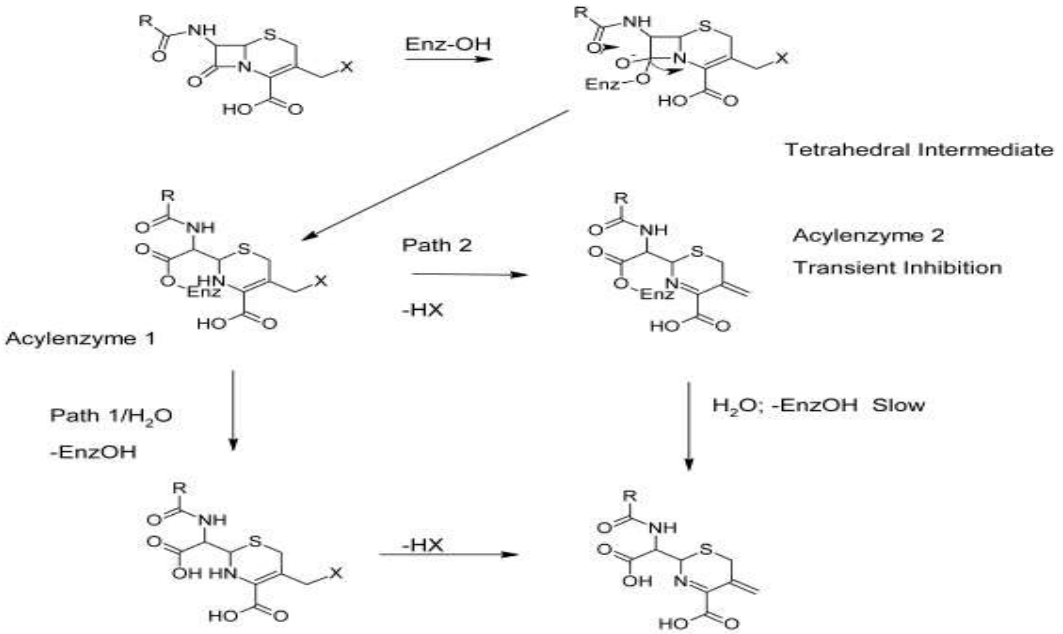


Figure 8.6 • Inhibition of β-lactamases by cephalosporins.

Antipseudomonal Cephalosporins

The primary mechanisms of resistance to β-lactams appear to involve destruction of the antibiotics by *β-lactamases* and/or *interference with their penetration through the cell envelope*.

Apparently, not all *β*-lactamase–resistant cephalosporins penetrate the cell envelope of *P. aeruginosa*, as only cefoperazone, moxalactam, cefotaxime, ceftizoxime, ceftriaxone, and ceftazidime have useful antipseudomonal activity. Two cephalosporins, moxalactam and cefoperazone, contain the same polar functionalities (e.g., carboxy and *N*-acylureido) that facilitate penetration into *Pseudomonas* spp. by the penicillins.

Adverse Reactions and Drug Interactions

The cephalosporin antibiotics are comparatively nontoxic compounds that exhibit highly selective toxicity toward bacteria. The most common adverse reactions to the cephalosporins are allergic and hypersensitivity reactions. These vary from mild rashes to life-threatening anaphylactic reactions. The issue of crosssensitivity between the two classes of *β*-lactams is very complex, but the incidence is considered to be very low (estimated between 3% and 7%).

Cephalosporins containing an *N*-methyl-5-thiotetrazole (MTT) moiety at the 3-position (e.g., cefamandole, cefotetan, cefmetazole, moxalactam, and cefoperazone) have been implicated in a higher incidence of hypoprothrombinemia than cephalosporins lacking the MTT group. This effect, which is enhanced and can lead to severe bleeding, is apparently because of inhibition of vitamin K–requiring enzymes. Cephalosporins containing the MTT group should not be administered to patients receiving oral anticoagulant or heparin therapy because of possible synergism with these drugs.

The MTT group has also been implicated in the intolerance to alcohol associated with certain injectable cephalosporins: cefamandole, cefotetan, cefmetazole, and cefoperazone. Thus, disulfiram-like reactions, attributed to the accumulation of acetaldehyde and resulting from the inhibition of aldehyde

dehydrogenase–catalyzed oxidation of aldehyde by MTT-containing cephalosporins, may occur in patients who have consumed alcohol before, during, or shortly after the course of therapy.

Classification

Cephalosporins are divided into first-, second-, third-, and fourth-generation agents, based roughly on their time of discovery and their antimicrobial properties (Table 8.5). In general, progression from first to fourth generation is associated with a broadening of the Gram-negative antibacterial spectrum, some reduction in activity against Gram-positive organisms, and enhanced resistance to *β*-lactamases.

TABLE 8.5 Classification and Properties of Cephalosporins

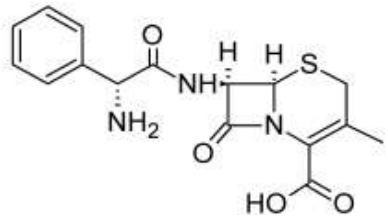
Cephalosporin	Generation	Route of Admin.	Acid Resistant	Plasma Protein Binding (%)	β-Lactamase Resistance	Spectrum of Activity	Antipseudomonal Activity
Cephalexin	First	Oral	Yes	5–15	Poor	Broad	No
Cephradine	First	Oral, parenteral	Yes	8–17	Poor	Broad	No
Cefadroxil	First	Oral	Yes	20	Poor	Broad	No
Cephalothin	First	Parenteral	No	65–80	Poor	Broad	No
Cephapirin	First	Parenteral	No	40–54	Poor	Broad	No
Cefazolin	First	Parenteral	No	70–86	Poor	Broad	No
Cefaclor	Second	Oral	Yes	22–25	Poor	Broad	No
Loracarbef	Second	Oral	Yes	25	Poor	Broad	No
Cefprozil	Second	Oral	Yes	36	Poor	Broad	No
Cefamandole	Second	Parenteral	No	56–78	Poor to avg.	Extended	No
Cefonicid	Second	Parenteral	No	99	Poor to avg.	Extended	No
Ceforanide	Second	Parenteral	No	80	Average	Extended	No
Cefoxitin	Second	Parenteral	No	13–22	Good	Extended	No
Cefotetan	Second	Parenteral	No	78–91	Good	Extended	No
Cefmetazole	Second	Parenteral	No	65	Good	Extended	No
Cefuroxime	Second	Oral, parenteral	Yes/No	33–50	Good	Extended	No
Cefpodoxime	Second	Oral	Yes	25	Good	Extended	No
Cefixime	Third	Oral	Yes	65	Good	Extended	No
Cefoperazone	Third	Parenteral	No	82–93	Avg. to good	Extended	Yes
Cefotaxime	Third	Parenteral	No	30–51	Good	Extended	Yes
Ceftizoxime	Third	Parenteral	No	30	Good	Extended	Yes
Ceftriaxone	Third	Parenteral	No	80–95	Good	Extended	Yes
Ceftazidime	Third	Parenteral	No	80–90	Good	Extended	Yes
Ceftibuten	Third	Oral	Yes	?	Good	Extended	No
Cefepime	Fourth	Parenteral	No	16–19	Good	Extended	Yes
Cefpirome	Fourth	Parenteral	No	–	Good	Extended	Yes

Products

Cephalexin

Cephalexin (Keflex), was designed purposely as an orally active, semisynthetic cephalosporin. *The oral inactivation of cephalosporins has been attributed to two causes: instability*

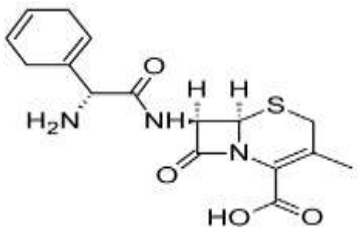
of the β-lactam ring to acid hydrolysis and solvolysis or microbial transformation of the 3-methylacetoxy group. **The *α*-amino group of cephalexin renders it acid stable.**



It is resistant to acid, and absorbed well orally. Because of minimal protein binding and nearly exclusive renal excretion, cephalexin is recommended particularly for the treatment of urinary tract infections.

Cephradine

Cephradine is the only cephalosporin derivative available in both oral and parenteral dosage forms. It closely resembles cephalexin chemically (it may be regarded as a partially hydrogenated derivative of cephalexin) and has very similar antibacterial and pharmacokinetic properties.

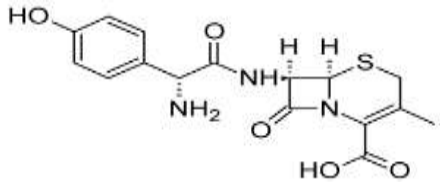


Cephradine is stable to acid . It is minimally protein bound. It is recommended for the treatment of uncomplicated urinary tract and upper respiratory tract infections caused by susceptible organisms.

Cefadroxil

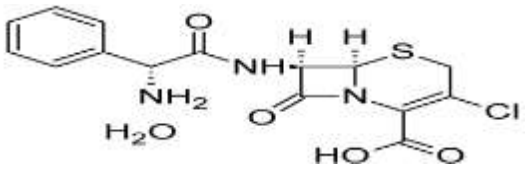
Cefadroxil is an orally active semisynthetic derivative of 7-ADCA, in which the 7-acyl group is the p-hydroxylphenylglycyl

moiety. The main advantage claimed for cefadroxil is its somewhat prolonged duration of action, which permits once-a-day dosing. The prolonged duration of action of this compound is related to relatively slow urinary excretion of the drug compared with other cephalosporins,



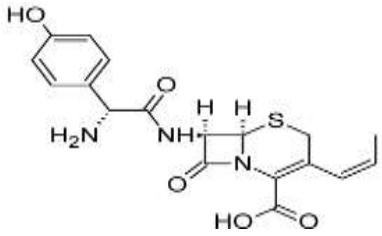
Cefaclor

Cefaclor differs structurally from cephalexin in that the 3-methyl group has been replaced by a chlorine atom. It is moderately stable in acid and achieves enough oral absorption to provide effective plasma levels. The antibacterial spectrum of activity is similar to that of cephalexin, but it is claimed to be more potent against some species sensitive to both agents.



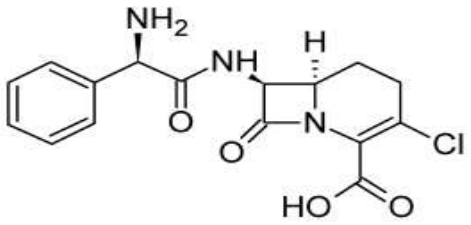
Cefprozil

Cefprozil (Cefzil) is an orally active second-generation cephalosporin that is similar in structure and antibacterial spectrum to cefadroxil. Oral absorption is excellent .



Loracarbef

Loracarbef (Lorabid) is the first of a series of carbacephems. Carbacephems are isosteres of the cephalosporin (or Δ^3-cephem) antibiotics in which the 1-sulfur atom has been replaced by a methylene (CH_2) group. Loracarbef is isosteric with cefaclor and has similar pharmacokinetic and microbiological properties. Thus, the antibacterial spectrum of activity resembles that of cefaclor. Loracarbef is chemically stable in plasma.



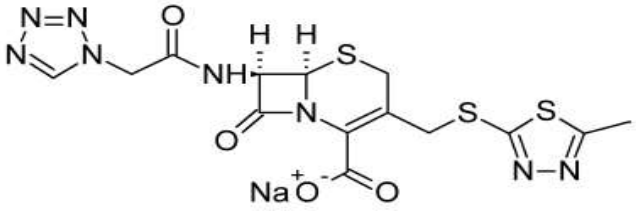
Cephalothin Sodium

Its spectrum of activity is more similar to that of ampicillin. Unlike ampicillin, cephalothin is resistant to penicillinase produced by *S. aureus* and provides an alternative to the use of penicillinase- sensitive penicillins .

Cephalothin is absorbed poorly from the GI tract and must be administered parenterally for systemic infections.

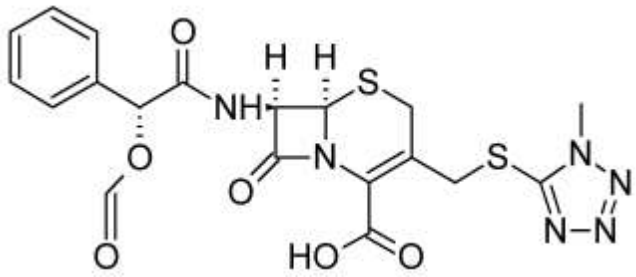
Cefazolin Sodium, Sterile

Cefazolin is one of a series of semisynthetic cephalosporins in which the C-3 is a thiol-containing heterocycle—here, 5-methyl-2-thio-1,3,4-thiadiazole. It also contains tetrazolylacetyl .



Cefamandole Nafate

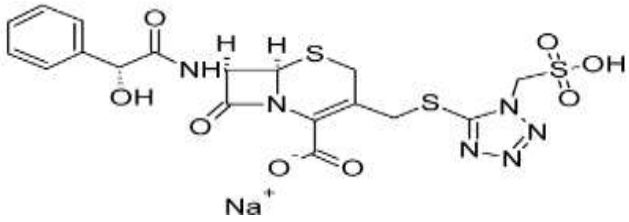
Cefamandole nafate is the formate ester of cefamandole, a semisynthetic cephalosporin that incorporates D-mandelic acids as the acyl portion and a thiol-containing heterocycle (5-thio-1,2,3,4-tetrazole) on the C-3 methylene carbon atom. Esterification of the *α*-hydroxyl group of the D-mandeloyl function overcomes the instability of cefamandole in solid-state dosage forms and provides satisfactory concentrations of the parent antibiotic in vivo through spontaneous hydrolysis of the ester at neutral to alkaline pH.



The D-mandeloyl moiety of cefamandole appears to confer resistance to a few *β*-lactamases. The L-mandeloyl isomer is significantly less active than the D-isomer.Cefamandole nafate is very unstable in solution and hydrolyzes rapidly to release cefamandole and formate. Cefamandole contains MTT group that may lead to bleeding and alcoholism side effects.

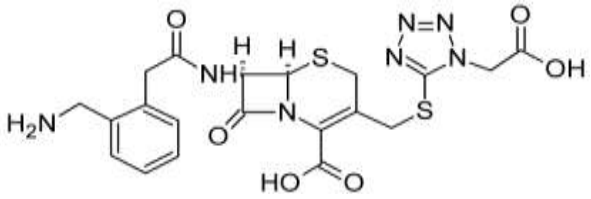
Cefonicid Sodium, Sterile

Cefonicid Sodium (Monocid) is a second-generation cephalosporin that is structurally similar to cefamandole, except that it contains a methane sulfonic acid group attached to the N-1 position of the tetrazole ring.



Ceforanide, Sterile

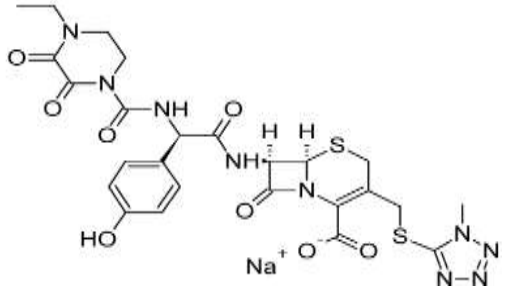
Ceforanide is classified as a second-generation cephalosporin.



Cefoperazone Sodium, Sterile

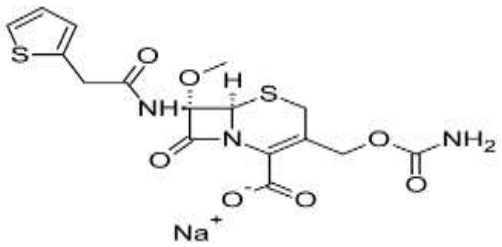
Cefoperazone (Cefobid) is a third-generation, antipseudomonal cephalosporin that resembles piperacillin chemically and microbiologically. Like piperacillin, cefoperazone is hydrolyzed by many of the *β*-lactamases that hydrolyze penicillins. Unlike piperacillin it is resistant to some (but not all) of the *β*-lactamases that hydrolyze cephalosporins.It contains MTT ring

that may induce adverse reactions as bleeding and alcoholism.



Cefoxitin Sodium

Cefoxitin (Mefoxin) is a semisynthetic derivative obtained by modification of cephamycin C, a 7*α*-methoxy-substituted cephalosporin. Cefoxitin is effective against certain strains of *E. coli* that is resistant to cephalothin and cefamendole. It is also effective against penicillin-resistant *S. aureus* and *N. gonorrhoeae*.

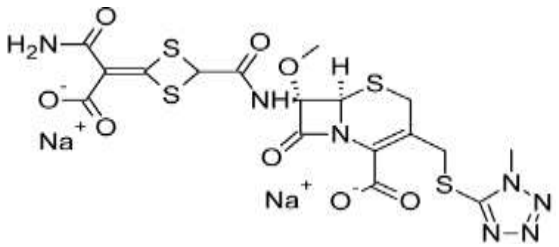


The activity of cefoxitin and cephamycins, in general, against resistant bacterial strains is because of their resistance to hydrolysis by *β*-lactamases conferred by the 7*α*-methoxyl substituent. Cefoxitin is a potent competitive inhibitor of many *β*-lactamases. It is also a potent inducer of chromosomally mediated *β*-lactamases.

The principal role of cefoxitin in therapy seems to be for the treatment of certain anaerobic and mixed aerobic–anaerobic infections. It is also used to treat gonorrhea caused by *β*-lactamase–producing bacteria. It is classified as a second generation agent.

Cefotetan Disodium

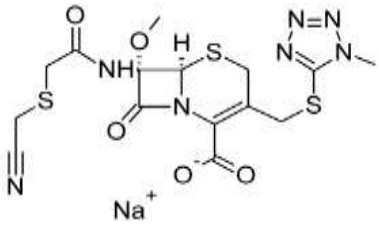
Cefotetan (Cefotan) is a third-generation cephalosporin. Cefotetan is resistant to destruction by *β*-lactamases. It is also a competitive inhibitor of many *β*-lactamases and causes transient inactivation of some of these enzymes.



Cefotetan contains the MTT group that has been associated with hypoprothrombinemia and alcohol intolerance. Another cephalosporin that lacks these properties should be selected for patients at risk for severe bleeding or alcoholism.

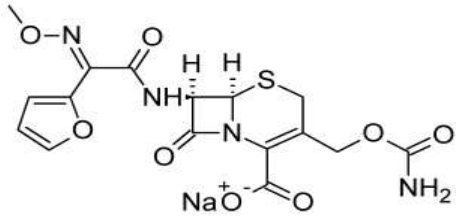
Cefmetazole Sodium

Cefmetazole is a semisynthetic, third-generation, parenteral cephalosporin of the cephamycin group. Like other cephamycins, the presence of the 7*α*-methoxyl group confers resistance to many *β*-lactamases. It is highly active against *N. gonorrhoeae*, including *β*-lactamase–producing strains. <u>In common with other cephamycins</u>, cefmetazole is ineffective against *Pseudomonas* spp. Cefmetazole has the MTT moiety associated with increased bleeding in certain high risk patients.



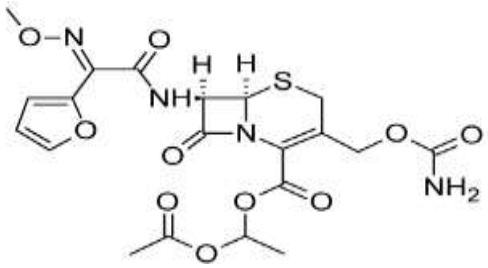
Cefuroxime Sodium

Cefuroxime is the first of a series of *α*-methoximinoacyl–substituted cephalosporins that constitute most of the third-generation agents available for clinical use. A *syn* alkoximino substituent is associated with *β*-lactamase stability in these cephalosporins. Cefuroxime is classified as a second generation cephalosporin . It is, active against *β*-lactamase–producing strains. Other important Gram-negative pathogens *P. aeruginosa* are resistant. It penetrates inflamed meninges in high enough concentrations to be effective in meningitis caused by susceptible organisms.



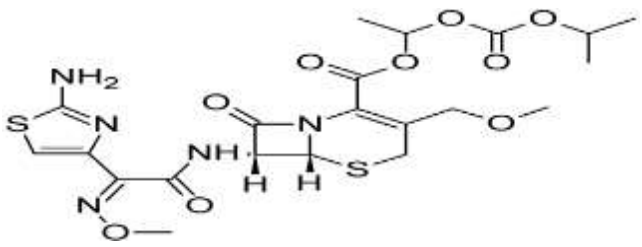
Cefuroxime Axetil

Cefuroxime axetil is the 1-acetyoxyethyl ester of cefuroxime. During absorption, this acid-stable, lipophilic, oral prodrug derivative of cefuroxime is hydrolyzed to cefuroxime by intestinal and/or plasma enzymes. The axetil ester improves an oral bioavailability of 35% to 50% of cefuroxime. Oral absorption of the ester is increased by food but decreased by antacids and histamine H2-antagonists. The latter effect may be because of spontaneous hydrolysis of the ester in the intestine because of the higher pH created by these drugs. Axetil is used for the oral treatment of non–life-threatening infections caused by bacteria that are susceptible to cefuroxime.



Cefpodoxime Proxetil

Cefpodoxime proxetil is the isopropyloxycarbonylethyl ester of the third-generation cephalosporin cefpodoxime. This orally active prodrug derivative is hydrolyzed by esterases in the intestinal wall and in the plasma to provide cefpodoxime. The oral bioavailability of cefpodoxime from the proxetil is estimated to be about 50%. Administration of the prodrug with food enhances its absorption.

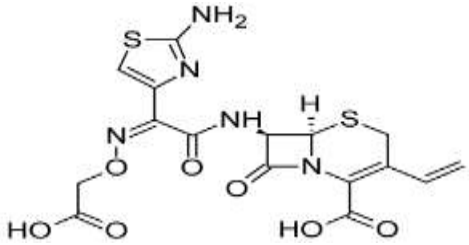


Cefpodoxime is a broad-spectrum cephalosporin with useful activity against a relatively wide range of Gram-positive and Gram-negative bacteria. It is also resistant to many *β*-lactamases. It thus finds use in the treatment of upper and lower respiratory infections, such as pharyngitis, bronchitis, otitis media, and community-acquired pneumonia. It is also useful for the treatment of uncomplicated gonorrhea.

Cefixime

Cefixime (Suprax) is the first orally active, third-generation cephalosporin that is not an ester prodrug to be approved for therapy in the United States. Oral bioavailability is surprisingly high, ranging from 40% to 50%. Facilitated transport of

cefixime across intestinal brush border membranes involving the carrier system for dipeptides may explain its surprisingly good oral absorption.

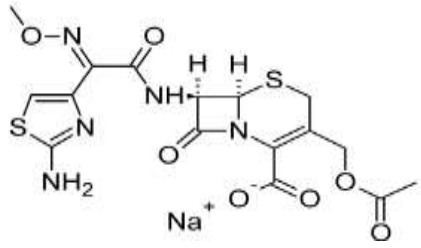


Cefixime is a broad-spectrum cephalosporin that is resistant to many *β*-lactamases. It is particularly effective against Gram-negative bacilli, including *E. coli*. Most *Pseudomonas* are resistant.

Cefixime is used for the treatment of various respiratory tract infections (e.g., acute bronchitis, pharyngitis, and tonsillitis) and otitis media. It is also used to treat uncomplicated urinary tract infections and gonorrhea caused by *β*-lactamase–producing bacterial strains.

Cefotaxime Sodium, Sterile

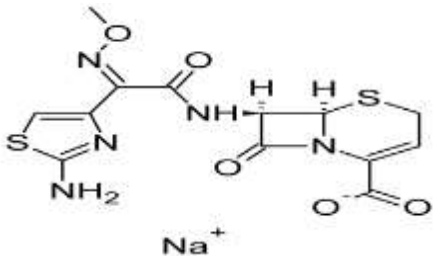
Cefotaxime (Claforan) was the first third-generation cephalosporin to be introduced. It possesses excellent broad-spectrum activity against Gram-positive and Gram-negative aerobic and anaerobic bacteria. Many *β*-lactamase–producing bacterial strains are sensitive to cefotaxime, including *N. gonorrhoeae* and *S. aureus*. Some, but not all, *Pseudomonas* strains are sensitive. The *syn*-isomer of cefotaxime is significantly more active than the *anti*-isomer against *β*-lactamase–producing bacteria. This potency difference is, in part, because of greater resistance of the *syn*-isomer to the action of *β*-lactamases. The higher affinity of the *syn*-isomer for PBPs, however, may also be a factor.



The parent drug reaches the cerebrospinal fluid in sufficient concentration to be effective in the treatment of meningitis. Solutions of cefotaxime sodium should be used within 24 hours. If stored, they should be refrigerated. Refrigerated solutions maintain potency up to 10 days.

Ceftizoxime Sodium, Sterile

Ceftizoxime (Cefizox) is a third-generation cephalosporin. This *β*-lactamase–resistant agent exhibits excellent activity against the Enterobacteriaceae and *Proteus* spp. Its activity against *P. aeruginosa* is somewhat variable and lower than that of either cefotaxime or cefoperazone.



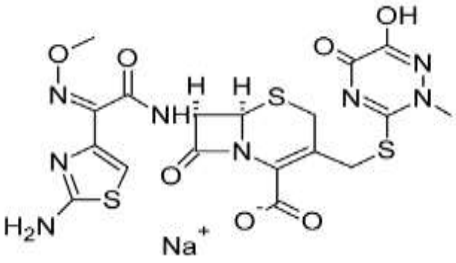
Adequate levels of the drug are achieved in the cerebrospinal fluid for the treatment of Gram-negative or Gram-positive bacterial meningitis. It must be administered on a thrice-daily dosing schedule because of its relatively short half-life.

Ceftriaxone Disodium, Sterile

Ceftriaxone (Rocephin) is a *β*-lactamase–resistant cephalosporin with an extremely long serum half-life. Once-daily dosing suffices for most indications. Two factors

contribute to the prolonged duration of action of ceftriaxone: high protein binding in the plasma and slow urinary excretion. Despite its comparatively low volume of distribution, it reaches the cerebrospinal fluid in concentrations that are effective in meningitis.

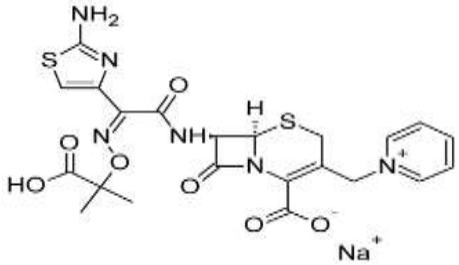
Ceftriaxone contains a highly acidic heterocyclic system on the 3-thiomethyl group. This dioxotriazine ring system is believed to confer the unique pharmacokinetic properties of this agent.



It is resistant to most *β*-lactamases. Solutions of ceftriaxone sodium should be used within 24 hours. They may be stored up to 10 days if refrigerated.

Ceftazidime Sodium, Sterile

Ceftazidime is a *β*-lactamase–resistant third-generation cephalosporin that is noted for its antipseudomonal activity. It is active against some strains of *P. aeruginosa* that are resistant to cefoperazone and ceftriaxone. Ceftazidime is also highly effective against *β*-lactamase– producing strains of the Enterobacteriaceae family.



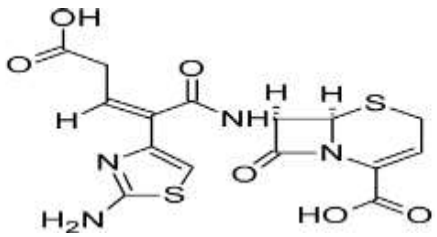
The structure of ceftazidime contains two features: (a) a 2-methylpropionicoximinoacyl group that confers *β*-lactamase resistance and, possibly, increased permeability through the porin channels of the cell envelope and (b) a pyridinium group at the 3-position that confers zwitterionic properties on the molecule. Ceftazidime is administered parenterally 2 or 3 times daily.

NEWER CEPHALOSPORINS

New cephalosporins fall into two categories: (a) orally active *β*-lactamase–resistant cephalosporins and (b) parenteral *β*-lactamase–resistant antipseudomonal cephalosporins.

Ceftibuten

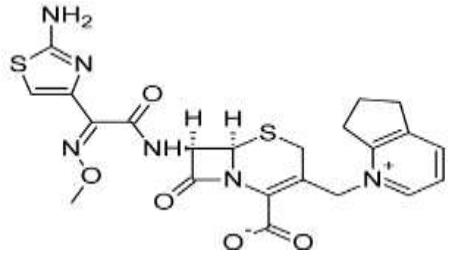
Ceftibuten is a recently introduced, chemically novel analog of the oximino cephalosporins in which an olefinic methylene group ($C=CHCH_2$-) with *Z* stereochemistry has replaced the *syn* oximino (C=NO-) group. This isosteric replacement yields a compound that retains resistance to hydrolysis catalyzed by many *β*-lactamases, has enhanced chemical stability, and is orally active. Oral absorption is rapid and nearly complete. It has the highest oral bioavailability of the third-generation cephalosporins.



Ceftibuten possesses excellent potency against most members of the Enterobacteriaceae family.

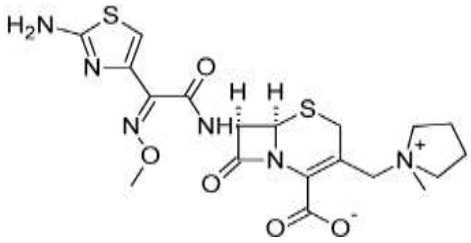
Cefpirome

Cefpirome is a newer parenteral, *β*-lactamase–resistant cephalosporin with a quaternary ammonium group at the 3-position of the cephem nucleus. Because its potency against Gram-positive and Gram-negative bacteria exceeds that of the first-generation and third-generation cephalosporins, respectively, cefpirome is being said as the first fourth generation cephalosporin. It is broad spectrum. Its efficacy against *P. aeruginosa* is comparable with that of ceftazidime.



Cefepime

Cefepime is a parenteral, *β*-lactamase–resistant cephalosporin that is chemically and microbiologically similar to cefpirome. It also has a broad antibacterial spectrum. Cefepime is also a fourth-generation cephalosporin.



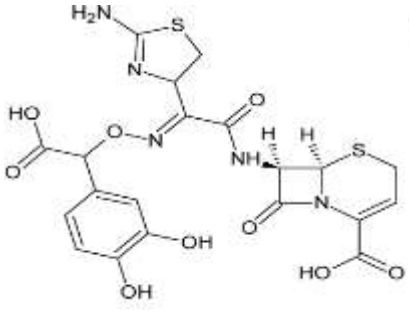
Future Developments in Cephalosporin Design

Recent research efforts in the cephalosporin field have focused primarily on two desired antibiotic properties: (a) increased permeability into Gram-negative bacilli, leading to enhanced efficacy against permeability-resistant strains of Enterobacteriaceae and *P. aeruginosa*, and (b) increased

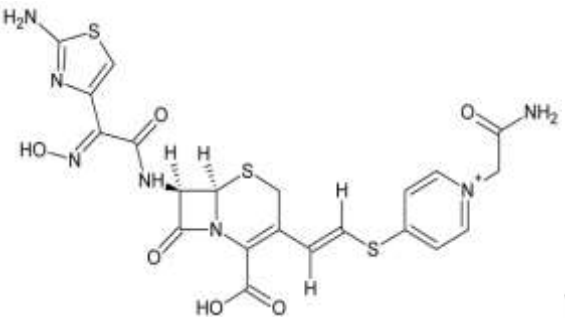
affinity for altered PBPs, in particular the PBP 2a (or PBP 2') of MRSA.

A catechol-containing cephalosporin that exhibits excellent in vitro antibacterial activity against clinical isolates is GR-69153. GR- 69153 is a parenteral *β*-lactamase–resistant cephalosporin with a broad spectrum of activity against Gram-positive and Gram-negative bacteria.

The antibacterial spectrum of GR-69153 includes most members of the Enterobacteriaceae family, *P. aeruginosa*, *H. influenza* and *N. gonorrhoeae*.



GR-69153



TOC-039

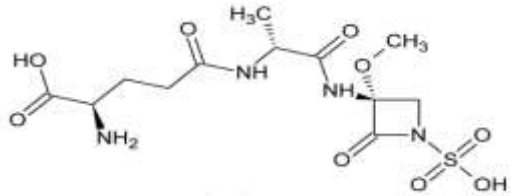
An experimental cephalosporin that has exhibited considerable promise against MRSA in preclinical evaluations is TOC-039.

TOC-039 is a parenteral, *β*-lactamase–resistant, hydroxyimino cephalosporin with a vinylthiopyridyl side chain attached to the 3-position of the cephem nucleus. It is a broad-spectrum agent that exhibits good activity against most aerobic Gram-positive and Gram-negative bacteria but inactive against *P. aeruginosa*.

MONOBACTAMS

The development of useful monobactam antibiotics began with the isolation of sulfazecin (SQ 26,445) and other monocyclic *β*-

lactam antibiotics from saprophytic soil bacteria in Japan and the United States. Sulfazecin was found to be weakly active as an antibacterial agent but highly resistant to *β*-lactamases.



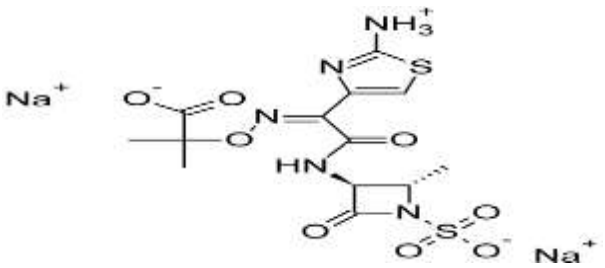
Sulfazecin

It has useful properties as an antibacterial agent. The 3-methoxy group, which was in part responsible for *β*-lactamase stability in the series, contributed to the low antibacterial potency and poor chemical stability of these antibiotics. A 4-methyl group, however, increases stability to *β*-lactamases and activity against Gram-negative bacteria at the same time. Unfortunately, potency against Gram-positive bacteria decreases. 4,4-Gem-dimethyl substitution slightly decreases antibacterial potency after oral administration.

Products

Aztreonam Disodium

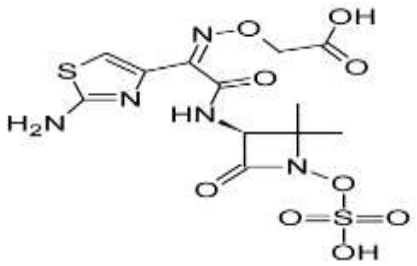
Aztreonam (Azactam) is a monobactam that binds with high affinity to PBP 3 in Gram-negative bacteria only. It is inactive against Gram-positive bacteria and anaerobes. *β*-Lactamase resistance is like that of ceftazidime, which has the same isobutyric acid oximinoacyl group. Aztreonam does not induce chromosomally mediated *β*-lactamases.



Aztreonam is particularly active against aerobic Gram-negative bacilli, including *E. coli* and *P. aeruginosa*. It is used to treat urinary and lower respiratory tract infections, intra-abdominal infections, and gynecological infections, as well as septicemias caused by these organisms.

Tigemonam

Tigemonam is a newer monobactam that is orally active. It is highly resistant to *β*-lactamases. The antibacterial spectrum of activity resembles that of aztreonam. It is very active against the Enterobacteriaceae, including *E. coli*, *Klebsiella*, *Proteus*..etc. It also exhibits good potency against *H. influenzae* and *N. gonorrhoeae*. Tigemonam is not particularly active against Gram-positive or anaerobic bacteria and is inactive against *P. aeruginosa*.



AMINOGLYCOSIDES

The discovery of streptomycin, the first aminoglycoside antibiotic to be used in chemotherapy. This success stimulated worldwide searches for antibiotics from the actinomycetes and, particularly, from the genus *Streptomyces*. Among the many antibiotics isolated from that genus, several are compounds closely related in structure to streptomycin. Six of them—kanamycin, neomycin, paromomycin, gentamicin, tobramycin, and netilmicin.

All aminoglycoside antibiotics are absorbed very poorly (less than 1% under normal circumstances) following oral administration, and some of them (kanamycin, neomycin, and

paromomycin) are administered by that route for the treatment of GI infections. Because of their potent broadspectrum antimicrobial activity, they are also used for themtreatment of systemic infections. Their undesirable side effects, particularly ototoxicity and nephrotoxicity, have restricted their systemic use to serious infections or infections caused by bacterial strains resistant to other agents.

Chemistry

Aminoglycosides are so named because their structures consist of amino sugars linked glycosidically. All have at least one aminohexose, and some have a pentose lacking an amino group (e.g., streptomycin, neomycin, and paromomycin). Additionally, each of the clinically useful aminoglycosides contains a highly substituted 1,3-diaminocyclohexane central ring; in kanamycin, neomycin, gentamicin, and tobramycin, it is deoxystreptamine, and in streptomycin, it is streptadine. The aminoglycosides are thus strongly basic compounds that exist as polycations at physiological pH. Their inorganic acid salts are very soluble in water. They distribute well into most body fluids but not into the central nervous system, bone, or fatty or connective tissues. They tend to concentrate in the kidneys and are excreted by glomerular filtration. Aminoglycosides are apparently not metabolized in vivo.

Spectrum of Activity

Although the aminoglycosides are classified as broadspectrum antibiotics, their greatest usefulness lies in the treatment of serious systemic infections caused by aerobic Gram-negative bacilli. The choice of agent is generally between kanamycin, gentamicin, tobramycin, netilmicin, and amikacin. Aerobic Gram-negative and Gram-positive cocci (with the exception of staphylococci) tend to be less sensitive; thus, the *β*-lactams and other antibiotics tend to be preferred for the treatment of infections caused by these organisms. Streptomycin is the

most effective of the group for the chemotherapy of TB, brucellosis, tularemia, and *Yersinia* infections. Paromomycin is used primarily in the chemotherapy of amebic dysentery. Under certain circumstances, aminoglycoside and *β*-lactam antibiotics exert a synergistic action in vivo against some bacterial strains when the two are administered jointly. For example, carbenicillin and gentamicin are synergistic against gentamicin-sensitive strains of *P. aeruginosa* and several other species of Gram-negative bacilli, and penicillin G and streptomycin (or gentamicin or kanamycin) tend to be more effective than either agent alone in the treatment of enterococcal endocarditis. The two antibiotic types should not be combined in the same solution because they are chemically incompatible. Damage to the cell wall caused by the *β*-lactam antibiotic is believed to increase penetration of the aminoglycoside into the bacterial cell.

Mechanism of Action

The aminoglycosides act directly on the bacterial ribosome to inhibit the initiation of protein synthesis and to interfere with the fidelity of translation of the genetic message. They bind to the 30S ribosomal subunit to form a complex that cannot initiate proper amino acid polymerization. The binding of streptomycin and other aminoglycosides to ribosomes also causes misreading mutations of the genetic code, apparently resulting from failure of specific aminoacyl RNAs to recognize the proper codons on messenger RNA (mRNA) and hence incorporation of improper amino acids into the peptide chain.

Microbial Resistance

The development of strains of Enterobacteriaceae resistant to antibiotics is a well-recognized, serious medical problem. Nosocomial (hospital acquired) infections caused by these organisms are often resistant to antibiotic therapy. Resistance is transferred from one bacterium to another by extra

chromosomal R factors (DNA) that self-replicate and are transferred by conjugation (direct contact). Strains carrying R factors for resistance to these antibiotics synthesize enzymes that are capable of acetylating, phosphorylating, or adenylylating key amino or hydroxyl groups of the aminoglycosides.

Resistance of individual aminoglycosides to specific inactivating enzymes can be understood, in large measure, by using chemical principles. First, one can assume that if the target functional group is absent in a position of the structure normally attacked by an inactivating enzyme, then the antibiotic will be resistant to the enzyme. Second, steric factors may confer resistance to attack at functionalities otherwise susceptible to enzymatic attack.

Aminoglycoside-inactivating enzymes include (a) aminoacetyl transferases (designated AAC), which acetylate the 6'-NH2 of ring I, the 3-NH2 of ring II, or the 2'- NH2 of ring I; (b) phosphotransferases (designated APH), which phosphorylate the 3'-OH of ring I or the 2"-OH of ring III; and (c) nucleotidyl transferases (ANT), which adenylate the 2"-OH of ring III, the 4'-OH of ring I, or the 4"-OH of ring III.

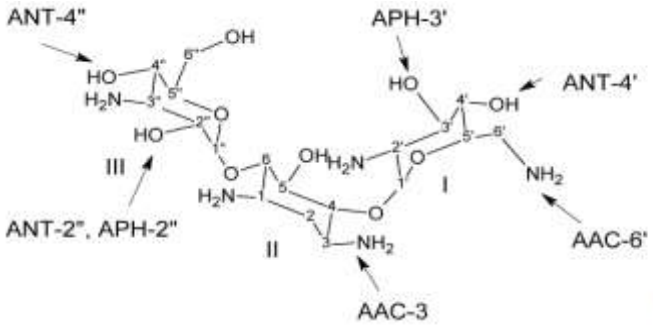


Figure 8.7 • Inactivation of kanamycin B by bacterial enzymes.

The gentamicins and tobramycin lack a 3'-hydroxyl group in ring I (see the section on the individual products for structures) and, consequently, are not inactivated by the phosphotransferase enzymes that phosphorylate that group in the kanamycins. Gentamicin C1(but not gentamicins Cla or C2

or tobramycin) is resistant to the acetyltransferase that acetylates the 6-amino group in ring I of kanamycin B. All gentamicins are resistant to the nucleotidyltransferase enzyme that adenylylates the secondary equatorial 4-hydroxyl group of kanamycin B because the 4-hydroxyl group in the gentamicins is *tertiary* and is oriented axially. Removal of functional groups susceptible to attacking an aminoglycoside occasionally can lead to derivatives that resist enzymatic inactivation and retain activity. For example, the 3- deoxy-, 4-deoxy-, and 3,4-dideoxykanamycins are more similar to the gentamicins and tobramycin in their patterns of activity against clinical isolates that resist one or more of the aminoglycoside-inactivating enzymes.

The most significant breakthrough yet achieved in the search for aminoglycosides resistant to bacterial enzymes has been the development of amikacin, the 1-*N*-L-(-)-amino-*α*-hydroxybutyric acid (L-AHBA) derivative of kanamycin A.

The cause of amikacin's resistance to enzymatic inactivation is unknown, but it has been suggested that introduction of the L-AHBA group into kanamycin A markedly decreases its affinity for the inactivating enzymes.

Bacterial susceptibility to aminoglycosides requires uptake of the drug by an energy-dependent active process. Uptake is initiated by the binding of the cationic aminoglycoside to anionic phospholipids of the cell membrane. Electron transport– linked transfer of the aminoglycoside through the cell membrane then occurs. Divalent cations such as Ca_2^+ and Mg_2^+ antagonize the transport of aminoglycosides into bacterial cells by interfering with their binding to cell membrane phospholipids. The resistance of anaerobic bacteria to the lethal action of the aminoglycosides is apparently because of the absence of the respiration-driven active-transport process for transporting the antibiotics.

Structure–Activity Relationships

It is convenient to discuss sequentially aminoglycoside SARs in terms of substituents in rings I, II, and III. Ring I is crucially important for characteristic broadspectrum antibacterial activity, and it is the primary target for bacterial inactivating enzymes. Amino functions at $6^{'}$ and $2^{'}$ are particularly important as kanamycin B ($6^{'}$-amino, $2^{'}$-amino) is more active than kanamycin A ($6^{'}$-amino, $2^{'}$- hydroxyl), which in turn is more active than kanamycin C ($6^{'}$-hydroxyl, $2^{'}$-amino). Methylation at either the $6^{''}$-carbon or the $6^{'}$-amino positions does not lower appreciably antibacterial activity and confers resistance to enzymatic acetylation of the $6^{'}$-amino group. Removal of the $3^{'}$-hydroxyl or the $4^{'}$-hydroxyl group or both in the kanamycins (e.g., $3^{'}$,$4^{'}$- dideoxykanamycin B or dibekacin) does not reduce antibacterial potency. The gentamicins also lack oxygen functions at these positions, as do sisomicin and netilmicin, which also have a $4^{'}$,$5^{'}$-double bond. None of these derivatives is inactivated by phosphotransferase enzymes that phosphorylate the $3^{'}$-hydroxyl group. Evidently, the $3^{''}$ phosphorylated derivatives have very low affinity for aminoglycoside-binding sites in bacterial ribosomes.

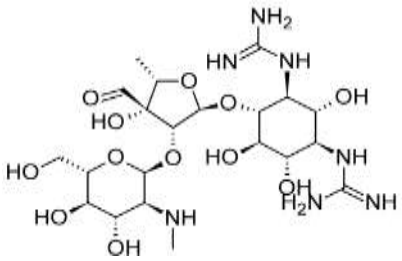
Few modifications of ring II (deoxystreptamine) functional groups are possible without appreciable loss of activity in most of the aminoglycosides. The 1-amino group of kanamycin A can be acylated (e.g., amikacin), however, with activity largely retained. Netilmicin (1-*N*-ethylsisomicin) retains the antibacterial potency of sisomicin and is resistant to several additional bacteria-inactivating enzymes. $2^{''}$-Hydroxysisomicin is claimed to be resistant to bacterial strains that adenylate the $2^{''}$-hydroxyl group of ring III, whereas 3-deaminosisomicin exhibits good activity against bacterial strains that elaborate 3-acetylating enzymes.

Ring III functional groups appear to be somewhat less sensitive to structural changes than those of either ring I or ring II. Although the 2″-deoxygentamicins are significantly less active than their 2″-hydroxyl counterparts, the 2″-amino derivatives (seldomycins) are highly active. The 3″-amino group of gentamicins may be primary or secondary with high antibacterial potency. Furthermore, the 4″- hydroxyl group may be *axial* or *equatorial* with little change in potency.

Products

Streptomycin Sulfate, Sterile

Streptomycin sulfate is freely soluble in water, forming solutions that are slightly acidic or nearly neutral. It is very slightly soluble in alcohol and is insoluble in most other organic solvents. Acid hydrolysis yields streptidine and streptobiosamine, the compound that is a combination of L-streptose and *N*-methyl-L-glucosamine.



Streptomycin acts as a triacidic base through the effect of its two strongly basic guanidino groups and the more weakly basic methylamino group. The solutions decompose if sterilized by heating, so sterile solutions are prepared by adding sterile distilled water to the sterile powder. The early salts of streptomycin contained impurities that were difficult to remove and caused a histamine-like reaction. By forming a complex with calcium chloride, it was possible to free the streptomycin from these impurities and to obtain a product that was generally well tolerated.

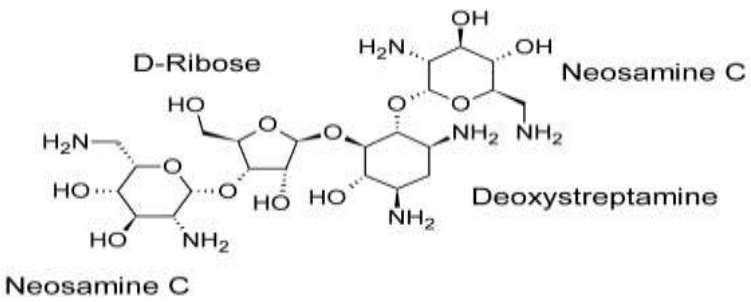
The term *streptomycin A* has been used to refer to what is commonly called streptomycin, and mannisidostreptomycin has been called *streptomycin B*. Hydroxystreptomycin differs from streptomycin in having a hydroxyl group in place of one of the hydrogen atoms of the streptose methyl group. Mannisidostreptomycin has a mannose residue attached in glycosidic linkage through the hydroxyl group at C-4 of the *N*-methyl-L-glucosamine moiety.

Clinically, a problem that sometimes occurs with the use of streptomycin is the early development of resistant strains of bacteria, necessitating a change in therapy. Other factors that limit the therapeutic use of streptomycin are chronic toxicities. Neurotoxic reactions have been observed after the use of streptomycin. These are characterized by vertigo, disturbance of equilibrium, and diminished auditory perception. Additionally, nephrotoxicity occurs with some frequency.

As a chemotherapeutic agent, streptomycin is active against numerous Gram-negative and Gram-positive bacteria. One of the greatest virtues of streptomycin is its effectiveness against the tubercle bacillus, *M. tuberculosis*. The possible development of damage to the otic nerve by the continued use of streptomycincontaining preparations has discouraged the use of such products. It remains one of the agents of choice, however, for the treatment of certain "occupational" bacterial infections, such as brucellosis, tularemia, bubonic plague, and glanders. Because streptomycin is not absorbed when given orally or destroyed significantly in the GI tract, at one time it was used rather widely in the treatment of infections of the intestinal tract. For systemic action, streptomycin usually is given by intramuscular injection.

Neomycin Sulfate

In a search for antibiotics less toxic than streptomycin, it is considered one of the most useful antibiotics for the treatment of GI infections, dermatological infections, and acute bacterial peritonitis. Also, it is used in abdominal surgery to reduce or avoid complications caused by infections from bacterial flora of the bowel. It has broad-spectrum activity against various organisms and shows a low incidence of toxic and hypersensitivity reactions. It is absorbed very slightly from the digestive tract, so its oral use ordinarily does not produce any systemic effect. The development of neomycinresistant strains of pathogens is rarely reported in those organisms against which neomycin is effective.

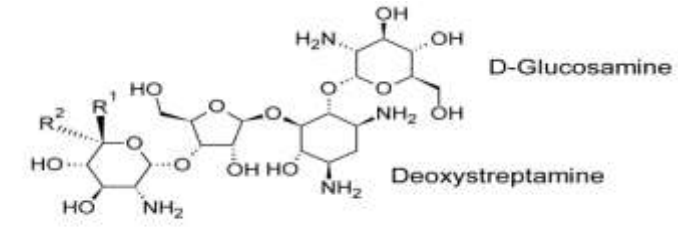


Neomycin, as produced by *S. fradiae*, is a mixture of closely related substances. Included in the "neomycin complex" is neamine (originally designated *neomycin A*) and neomycins B and C. *S. fradiae* also elaborates another antibiotic, the fradicin, which has some antifungal properties but no antibacterial activity.

Neamine may be obtained by methanolysis of neomycins B and C, during which the glycosidic link between deoxystreptamine and D-ribose is broken. Therefore, neamine is a combination of deoxystreptamine and neosamine C, linked glycosidically (*α*) at the 4-position of deoxystreptamine. Neomycin B differs from neomycin C by the nature of the sugar attached terminally to D-ribose. That sugar, called *neosamine B*, differs from neosamine C in its stereochemistry.

Paromomycin Sulfate

Paromomycin, however, more closely resembles neomycin and streptomycin in antibiotic activity than it does oxytetracycline, the antibiotic obtained from *S. rimosus*. It to consist of two fractions, paromomycin I and paromomycin II. The structure of paromomycin is the same as that of neomycin B, except that paromomycin contains D-glucosamine instead of the 6- amino-6-deoxy-D-glucosamine found in neomycin B. The same structural relationship is found between paromomycin II and neomycin C.



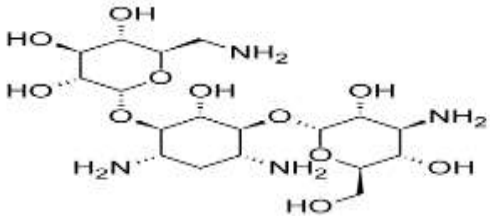
Paromomycin I: R^1=H; R^2=CH_2NH_2

Paromomycin II: R^1=CH_2NH_2; R^2= H

Paromomycin has broad-spectrum antibacterial activity and has been used for the treatment of GI infections caused by *Salmonella* and *Shigella* spp., and enteropathogenic *E. coli*.

Kanamycin Sulfate

Kanamycin was from *Streptomyces kanamyceticus*. Its activity against mycobacteria and many intestinal bacteria, as well as several pathogens that show resistance to other antibiotics.



Chromatography showed that *S. kanamyceticus* elaborates three closely related structures: kanamycins A, B, and C. Commercially available kanamycin is almost pure kanamycin A, the least toxic of the three forms. The kanamycins differ only in the sugar moieties attached to the glycosidic oxygen on the 4- position of the central deoxystreptamine. The kanamycins do not have the D-ribose molecule that is present in neomycins and paromomycins. Perhaps this structural difference is related to the lower toxicity observed with kanamycins. The kanosamine fragment linked glycosidically to the 6-position of deoxystreptamine is 3- amino-3-deoxy-D-glucose (3-D-glucosamine) in all three kanamycins. They differ in the substituted D-glucoses attached glycosidically to the 4-position of the deoxystreptamine ring. Kanamycin A contains 6- amino-6-deoxy-D-glucose; kanamycin B contains 2,6-diamino-2,6-dideoxy-D-glucose; and kanamycin C contains 2-amino-2-deoxy-D-glucose.

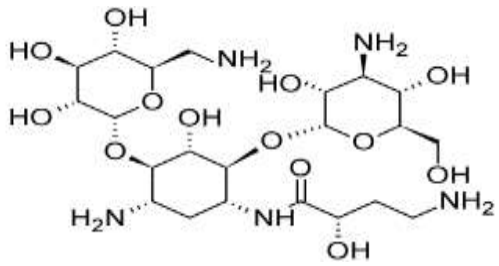
Kanamycin is basic and forms salts of acids through its amino groups. It is water soluble as the free base, but it is used in therapy as the sulfate salt, which is very soluble.

The use of kanamycin in the United States usually is restricted to infections of the intestinal tract (e.g., bacillary dysentery) and to systemic infections arising from Gramnegative bacilli (e.g., *Klebsiella*, *Proteus*, *Enterobacter*, and *Serratia* spp.). It is absorbed poorly from the intestinal tract; consequently, systemic infections must be treated by intramuscular or (for serious infections) intravenous injections. The use of kanamycin in the treatment of TB has not been widely

advocated since the discovery that mycobacteria develop resistance very rapidly.

Amikacin

Amikacin, 1-*N*-amino-_-hydroxybutyrylkanamycin A (Amikin), is a semisynthetic aminoglycoside first prepared in Japan. The synthesis formally involves simple acylation of the 1-amino group of the deoxystreptamine ring of kanamycin A with L-AHBA. This particular acyl derivative retains about 50% of the original activity of kanamycin A against sensitive strains of Gram-negative bacilli. The LAHBA derivative is much more active than the D-isomer. The remarkable feature of amikacin is that it resists attack by most bacteria-inactivating enzymes and, therefore, is effective against strains of bacteria that are resistant to other aminoglycosides, including gentamicin and tobramycin. In fact, it is resistant to all known aminoglycoside-inactivating enzymes, except the aminotransferase that acetylates the 6-amino group and the 4-nucleotidyl transferase that adenylylates the 4-hydroxyl group of aminoglycosides.

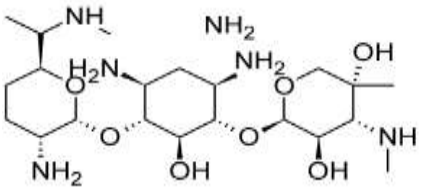


Preliminary studies indicate that amikacin may be less ototoxic than either kanamycin or gentamicin. Higher dosages of amikacin are generally required, however, for the treatment of most Gram-negative bacillary infections. For this reason, and to discourage the proliferation of bacterial strains resistant to it, amikacin currently is recommended for the treatment of serious infections caused by bacterial strains resistant to other aminoglycosides.

Gentamicin Sulfate

Gentamicin (Garamycin) belongs to the streptomycinoid (aminocyclitol) group of antibiotics. It has a broad spectrum of activity against many common pathogens, both Gram-positive and Gram-negative. Of particular interest is its strong activity against *P. aeruginosa* and other Gram-negative enteric bacilli.

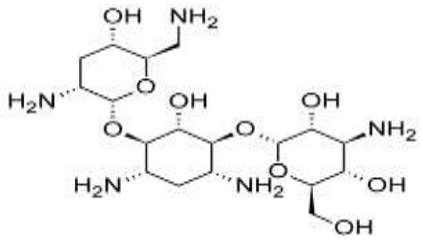
Gentamicin is effective in the treatment of various skin infections for which a topical cream or ointment may be used. It is recommended that topical gentamicin be reserved for use in such infections and in the treatment of burns complicated by pseudomonemia. An injectable solution containing 40 mg of gentamicin sulfate per milliliter may be used for serious systemic and genitourinary tract infections caused by Gram-negative bacteria, particularly *Pseudomonas*, *Enterobacter*, and *Serratia* spp. Gentamicin has been used for the treatment of hospital-acquired infections caused by such organisms. Resistant bacterial strains that inactivate gentamicin by adenylylation and acetylation.



Gentamicin sulfate is a mixture of the salts of compounds identified as gentamicins C_1, C_2, and C_{1a}. Coproduced, but not a part of the commercial product, are gentamicins A and B. Their structures were reported by Maehr and Schaffner and are closely related to those of the gentamicins C. It is chemically incompatible with carbenicillin, and the two should not be combined in the same intravenous solution.

Tobramycin Sulfate

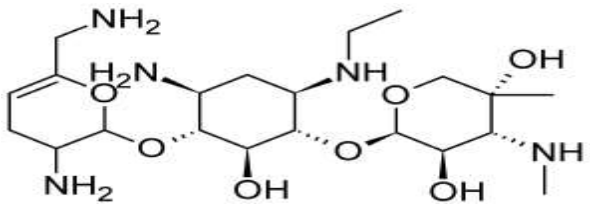
Five members of the nebramycin complex have been identified chemically.



The most important property of tobramycin is its activity against most strains of *P. aeruginosa*, exceeding that of gentamicin by twofold to fourfold. Some gentamicin-resistant strains of this troublesome organism are sensitive to tobramycin, but others are resistant to both antibiotics. Other Gram-negative bacilli and staphylococci are generally more sensitive to gentamicin. Tobramycin more closely resembles kanamycin B in structure (it is 3'-deoxykanamycin B).

Netilmicin Sulfate

Netilmicin sulfate, 1-*N*-ethylsisomicin (Netromycin), is a semisynthetic derivative prepared by reductive ethylation of sisomicin, an aminoglycoside antibiotic obtained from *Micromonospora inyoensis*. Structurally, sisomicin and netilmicin resemble gentamicin Cla, a component of the gentamicin complex.

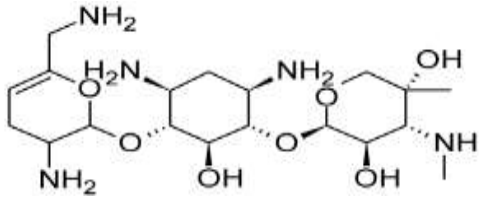


Against most strains of Enterobacteriaceae, *P. aeruginosa*, and *S. aureus*, sisomicin and netilmicin are comparable to gentamicin in potency. Netilmicin is active, however, against many gentamicin-resistant strains, in particular among *E. coli*,

Enterobacter, ***Klebsiella***, **and** ***Citrobacter*** **spp. The potency of netilmicin against certain gentamicin-resistant bacteria is attributed to its resistance to inactivation by bacterial enzymes that adenylylate or phosphorylate gentamicin and sisomicin. Evidently, the introduction of a 1-ethyl group in sisomicin markedly decreases the affinity of these enzymes for the molecule in a manner similar to that observed in the 1-*N*-ε-amino-α-hydroxybutyryl amide of kanamycin A (amikacin). Netilmicin, however, is inactivated by most of the bacterial enzymes that acetylate aminoglycosides, whereas amikacin is resistant to most of these enzymes.**

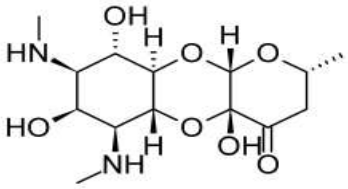
Sisomicin Sulfate

Although sisomicin has been approved for human use in the United States, it has not been marketed in this country. Its antibacterial potency and effectiveness against aminoglycoside- inactivating enzymes resemble those of gentamicin. Sisomicin also exhibits pharmacokinetics and pharmacological properties similar to those of gentamicin.



Spectinomycin Hydrochloride, Sterile

The aminocyclitol antibiotic spectinomycin hydrochloride (actinospectocin). It occurs as the white, crystalline dihydrochloride pentahydrate, which is stable in the dry form and very soluble in water. Solutions of spectinomycin, a hemiacetal, slowly hydrolyze on standing and should be prepared freshly and used within 24 hours. It is administered by deep intramuscular injection.



Spectinomycin is a broad-spectrum antibiotic with moderate activity against many Gram-positive and Gramnegative bacteria. Spectinomycin interferes with the binding of transfer RNA (tRNA) to the ribosomes and thus with the initiation of protein synthesis. Currently, it is recommended as an alternative to penicillin G salts for the treatment of uncomplicated gonorrhea. Many physicians prefer to use a tetracycline or erythromycin for prevention or treatment of suspected gonorrhea in penicillin-sensitive patients because, unlike these agents, spectinomycin is ineffective against syphilis.

TETRACYCLINES

Chemistry

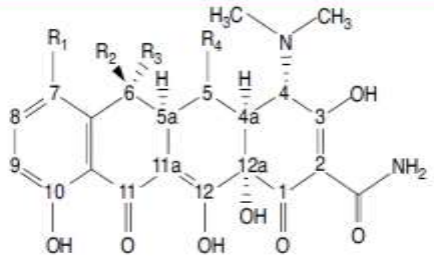
Among the most important broad-spectrum antibiotics are members of the tetracycline family. Nine such compounds—tetracycline oxytetracycline, chlortetracycline, doxycycline, and minocycline—have been introduced into medical use. The important members of the group are derivatives of an octahydronaphthacene, a hydrocarbon system that comprises four annulated six-membered rings.

The stereochemistry of the tetracyclines is very complex. Carbon atoms 4, 4a, 5, 5a, 6, and 12a are potentially chiral, depending on substitution. Oxytetracycline and doxycycline, each with a 5*α*-hydroxyl substituent, have six asymmetric centers; the others, lacking chirality at C-5.

Structure of the Tetracyclines

The tetracyclines are amphoteric compounds, forming salts with either acids or bases. In neutral solutions, these substances exist mainly as zwitterions. The hydrochloride salts are used most commonly for oral administration and usually are encapsulated because they are bitter. Water-soluble salts may be obtained also from bases, such as sodium or potassium hydroxides, but they are not stable in aqueous solutions. Water-insoluble salts are formed with divalent and polyvalent metals.

TABLE 8.6 Structures of Tetracyclines



	R_1	R_2	R_3	R_4
Tetracycline	H	OH	CH_3	H
Chlortetracycline	Cl	OH	CH_3	H
Oxytetracycline	H	OH	CH_3	OH
Demeclocycline	Cl	OH	H	H
Methacycline	H	CH_2		OH
Doxycycline	H	CH_3	H	OH
Minocycline	$N(CH_3)_2$	H	H	H

The unusual structural groupings in the tetracyclines produce three acidity constants in aqueous solutions of the acid salts.

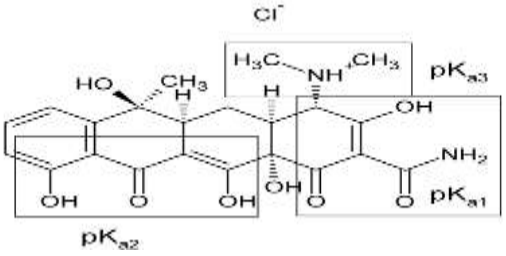
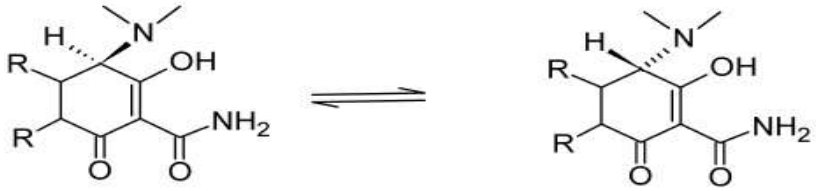


TABLE 8.7 pK_a Values (of Hydrochlorides) in Aqueous Solution at 25°C

	pK_{a1}	pK_{a2}	pK_{a3}
Tetracycline	3.3	7.7	9.5
Chlortetracycline	3.3	7.4	9.3
Demeclocycline	3.3	7.2	9.3
Oxytetracycline	3.3	7.3	9.1
Doxycycline	3.4	7.7	9.7
Minocycline	2.8	7.8	9.3

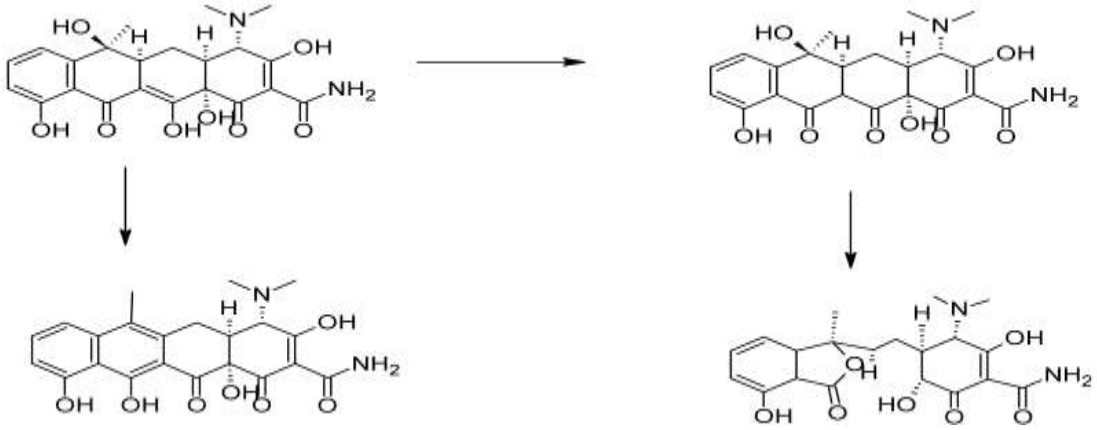
An interesting property of the tetracyclines is their ability to undergo epimerization at C-4 in solutions of intermediate pH range. These isomers are called *epitetracyclines*. Under acidic 1 day equilibrium approximately equal amounts of the isomers are present. The 4-epitetracyclines have been isolated and characterized. They exhibit much less activity than the "natural" isomers, thus accounting for the decreased therapeutic value of aged solutions.



Epi Natural

Strong acids and strong bases attack tetracyclines with a hydroxyl group on C-6, causing a loss in activity through modification of the C ring. Strong acids produce dehydration through a reaction involving the 6-hydroxyl group and the 5a-hydrogen. The double bond thus formed between positions 5a and 6 induces a shift in the position of the double bond between C-11a and C-12 to a position between C-11 and C-11a, forming the more energetically favored resonant system of the naphthalene group found in the inactive anhydrotetracyclines. Bases promote a reaction between the 6- hydroxyl group and the ketone group at the 11-position, causing the bond between

the 11 and 11a atoms to cleave, forming the lactone ring found in the inactive isotetracycline.



Stable chelate complexes are formed by the tetracyclines with many metals, including calcium, magnesium, and iron. Such chelates are usually very insoluble in water, accounting for the impaired absorption of most (if not all) tetracyclines in the presence of milk; calcium-, magnesium-, and aluminum-containing antacids; and iron salts. Soluble alkalinizers, such as sodium bicarbonate, also decrease the GI absorption of the tetracyclines.

The affinity of tetracyclines for calcium causes them to be incorporated into newly forming bones and teeth as tetracycline–calcium orthophosphate complexes. Deposits of these antibiotics in teeth cause a yellow discoloration that darkens (a photochemical reaction) over time. Tetracyclines are distributed into the milk of lactating mothers and will cross the placental barrier into the fetus. The possible effects of these agents on the bones and teeth of the child should be considered before their use during pregnancy or in children younger than 8 years of age.

Mechanism of Action and Resistance

Tetracyclines are specific inhibitors of bacterial protein synthesis. They bind to the 30S ribosomal subunit and, thereby, prevent the binding of aminoacyl tRNA to the mRNA–

ribosome complex. Both the binding of aminoacyl tRNA and the binding of tetracyclines at the ribosomal binding site require magnesium ions. Tetracyclines enter bacterial cells by two processes: passive diffusion and active transport. The active uptake of tetracyclines by bacterial cells is an energydependent process that requires adenosine triphosphate (ATP) and magnesium ions.

Three biochemically distinct mechanisms of resistance to tetracyclines have been described in bacteria: (a) efflux mediated by transmembrane-spanning, active-transport proteins that reduces the intracellular tetracycline concentration; (b) ribosomal protection, in which the bacterial protein synthesis apparatus is rendered resistant to the action of tetracyclines by an inducible cytoplasmic protein; and (c) enzymatic oxidation.

Spectrum of Activity

The tetracyclines have the broadest spectrum of activity of any known antibacterial agents. They are active against a wide range of Gram-positive and Gram-negative bacteria, spirochetes, mycoplasma, rickettsiae, and chlamydiae. Their potential indications are, therefore, numerous. Their bacteriostatic action, however, is a disadvantage in the treatment of life-threatening infections such as septicemia, endocarditis, and meningitis; the aminoglycosides and/or cephalosporins usually are preferred for Gram-negative and the penicillins for Gram-positive infections. Because of incomplete absorption and their effectiveness against the natural bacterial flora of the intestine, tetracyclines may induce superinfections caused by the pathogenic yeast *Candida albicans*. Resistance to tetracyclines among both Gram-positive and Gram-negative bacteria is relatively common. Superinfections caused by resistant *S. aureus* and *P.*

aeruginosa **have resulted from the use of these agents over time.**

Structure–Activity Relationships

The simplest tetracycline derivative that retains the characteristic broad-spectrum activity associated with this antibiotic class is 6-demethyl-6-deoxytetracycline. Many of the precise structural features present in this molecule must remain unmodified for derivatives to retain activity.

A-ring substituents can be modified only slightly without dramatic loss of antibacterial potency. The enolized tricarbonylmethane system at C-1 to C-3 must be intact for good activity. Replacement of the amide at C-2 with other functions (e.g., aldehyde or nitrile) reduces or abolishes activity. Monoalkylation of the amide nitrogen reduces activity proportionately to the size of the alkyl group.

The dimethylamino group at the 4-position must have the *α* orientation: 4-epitetracyclines are very much less active than the natural isomers. Removal of the 4-dimethylamino group reduces activity even further. Activity is largely retained in the primary and *N*-methyl secondary amines but rapidly diminishes in the higher alkylamines. A *cis*-A/B-ring fusion with a *β*-hydroxyl group at C-12a is apparently also essential. Esters of the C-12a hydroxyl group are inactive, with the exception of the formyl ester, which readily hydrolyzes in aqueous solutions. Alkylation at C-11a also leads to inactive compounds, demonstrating the importance of an enolizable *β*-diketone functionality at C-11 and C-12. The importance of the shape of the tetracyclic ring system is illustrated further by substantial loss in antibacterial potency resulting from epimerization at C-5a. Dehydrogenation to form a double bond between C-5a and C-11a markedly decreases activity, as does aromatization of ring C to form anhydrotetracyclines.

In contrast, substituents at positions 5, 5a, 6, 7, 8, and 9, representing the largely hydrophobic "northern and western" faces of the molecule, can be modified with varying degrees of success, resulting in retention and, sometimes, improvement of antibiotic activity. A 5-hydroxyl group, as in oxytetracycline and doxycycline, may influence pharmacokinetic properties but does not change antimicrobial activity. Acid-stable 6-deoxytetracyclines and 6- demethyl-6-deoxytetracyclines have been used to prepare various monosubstituted and disubstituted derivatives by electrophilic substitution reactions at C-7 and C-9 of the D ring. The more useful results have been achieved with the introduction of substituents at C-7. Oddly, strongly electron-withdrawing groups (e.g., chloro [lortetracycline] and nitro) and strongly electron-donating groups (e.g., dimethyl`lamino [minocycline]) enhance activity.

The most fruitful site for semisynthetic modification of the tetracyclines has been the 6-position. Neither the 6*α*-methyl nor the 6*β*-hydroxyl group is essential for antibacterial activity. In fact, doxycycline and methacycline are more active in vitro than their parent oxytetracycline against most bacterial strains. The conversion of oxytetracycline to doxycycline, which can be accomplished by reduction of methacycline giving a 1:1 mixture of doxycycline and epidoxycycline (which has a β-oriented methyl group).

6-Deoxytetracyclines also possess important chemical and pharmacokinetic advantages over their 6-oxy counterparts. Unlike the latter, they are incapable of forming anhydrotetracyclines under acidic conditions because they cannot dehydrate at C-5a and C-6. They are also more stable in base because they do not readily undergo *β*-ketone cleavage, followed by lactonization, to form isotetracyclines. Although it lacks a 6-hydroxyl group, methacycline shares the instability of the 6-oxytetracyclines in strongly acetic conditions. It suffers prototropic rearrangement to the anhydrotetracycline

in acid but is stable to *β*-ketone cleavage followed by lactonization to the isotetracycline in base. The greater lipid solubility of the 6-deoxy compounds has important pharmacokinetic consequences. Hence, doxycycline and minocycline are absorbed more completely following oral administration, exhibit higher fractions of plasma protein binding, and have higher volumes of distribution and lower renal clearance rates than the corresponding 6-oxytetracyclines.

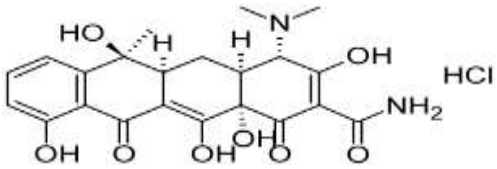
Polar substituents (i.e., hydroxyl groups) at C-5 and C-6 decrease lipid versus water solubility of the tetracyclines. The 6-position is, however, considerably more sensitive than the 5-position to this effect. Thus, doxycycline (6-deoxy-5-oxytetracycline) has a much higher partition coefficient than either tetracycline or oxytetracycline. Nonpolar substituents for example, 7-dimethylamino, 7-chloro, and 6-methyl, have the opposite effect.

The poorer oral absorption of the more water-soluble tetracycline and oxytetracycline can be attributed to several factors. In addition to their comparative difficulty in penetrating lipid membranes, the polar tetracyclines probably experience more complexation with metal ions in the gut and undergo some acid-catalyzed destruction in the stomach. Poorer oral absorption coupled with biliary excretion of some tetracyclines is also thought to cause a higher incidence of superinfections from resistant microbial strains.

Products

Tetracycline

Chemical studies on chlortetracycline revealed that controlled catalytic hydrogenolysis selectively removed the 7-chloro atom and so produced tetracycline

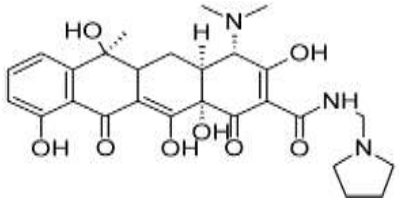


It is found in higher concentration in the spinal fluid than chlortetracycline and oxytetracycline. Also, an insoluble tetracycline phosphate complex.

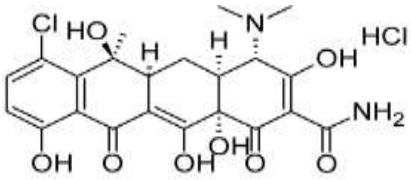
Tetracycline hydrochloride is also available in ointments for topical and ophthalmic administration. A topical solution is used for the management of acne vulgaris.

Rolitetracycline

Rolitetracycline, *N*-(pyrrolidinomethyl)tetracycline , was introduced for use by intramuscular or intravenous injection. This derivative is made by condensing tetracycline with pyrrolidine and formaldehyde in the presence of *tert*-butyl alcohol. It is very soluble in water (1 g dissolves in about 1 mL) and provides a means of injecting the antibiotic in a small volume of solution.



Chlortetracycline Hydrochloride

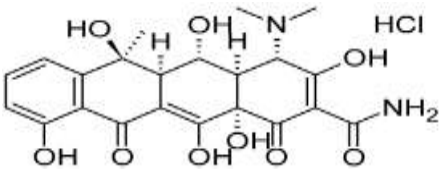


Oral and parenteral forms of chlortetracycline are no longer used because of the poor bioavailability and inferior

pharmacokinetic properties of the drug. It is still marketed in ointment forms for topical and ophthalmic use.

Oxytetracycline Hydrochloride

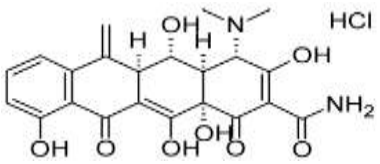
This compound was soon identified as a chemical analog of chlortetracycline that showed similar antibiotic properties.



The hydrochloride salt is more bitter than free base. It is much more soluble in water, 1 g dissolving in 2 mL, and more soluble in alcohol than the free base. Both compounds are inactivated rapidly by alkali hydroxides and by acid solutions below pH 2. Both forms of oxytetracycline are absorbed rapidly and equally well from the digestive tract, so the only real advantage the free base offers over the hydrochloride salt is that it is less bitter. Oxytetracycline hydrochloride is also used for parenteral administration (intravenously and intramuscularly).

Methacycline Hydrochloride

It has an antibiotic spectrum like that of the other tetracyclines but greater potency; about 600 mg of methacycline is equivalent to 1 g of tetracycline. Its particular value lies in its longer serum half-life.

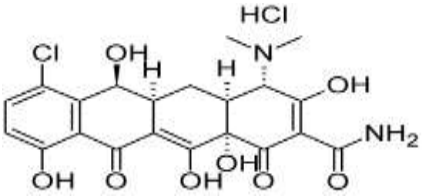


The greater stability of methacycline, both in vivo and in vitro, results from modification at C-6. Removal of the 6-

hydroxy group markedly increases the stability of ring C to both acids and bases, preventing the formation of isotetracyclines by bases. Anhydrotetracyclines still can form, however, by acid-catalyzed isomerization under strongly acidic conditions.

Demeclocycline

Demeclocycline is the 7-chloro-6-demethyltetracycline (Declomycin). Thus, it differs from chlortetracycline only in the absence of the methyl group on C-6.



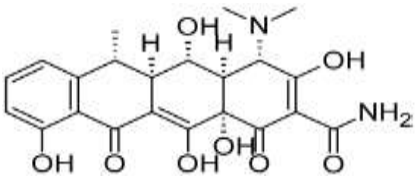
The incidence of discoloration and mottling of the teeth in youths from demeclocycline appears to be as low as that from other tetracyclines.

Doxycycline

A more recent addition to the tetracycline group of antibiotics available for antibacterial therapy is doxycycline, *α*-6-deoxy-5-oxytetracycline (Vibramycin). It was obtained first in small yields by a chemical transformation of oxytetracycline, but it is now produced by catalytic hydrogenation of methacycline or by reduction of a benzyl mercaptan derivative of methacycline with Raney nickel. The latter process produces a nearly pure form of the 6*α*-methyl epimer. The 6*α*-methyl epimer is more than 3 times as active as its *β*-epimer.

Absence of the 6-hydroxyl group produces a compound that is very stable in acids and bases and that has a long biological half-life. In addition, it is absorbed very well from the GI tract, thus allowing a smaller dose to be administered. High tissue

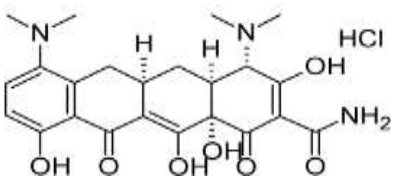
levels are obtained with it, and unlike other tetracyclines, doxycycline apparently does not accumulate in patients with impaired renal function. Therefore, it is preferred for uremic patients with infections outside the urinary tract. Its low renal clearance may limit its effectiveness, however, in urinary tract infections.



The hydrate form is sparingly soluble in water and is used in a capsule; the monohydrate is water insoluble and is used for aqueous suspensions, which are stable for up to 2 weeks when kept in a cool place.

Minocycline Hydrochloride

Minocycline, 7-dimethylamino-6-demethyl-6-deoxytetracycline (Minocin), the most potent tetracycline currently used in therapy, is obtained by reductive methylation of 7-nitro-6-demethyl-6-deoxytetracycline. Because minocycline, like doxycycline, lacks the 6-hydroxyl group, it is stable in acids and does not dehydrate or rearrange to anhydro or lactone forms. Minocycline is well absorbed orally to give high plasma and tissue levels. It has a very long serum half-life, resulting from slow urinary excretion and moderate protein binding. Doxycycline and minocycline, along with oxytetracycline, show the least in vitro calcium binding of the clinically available tetracyclines.



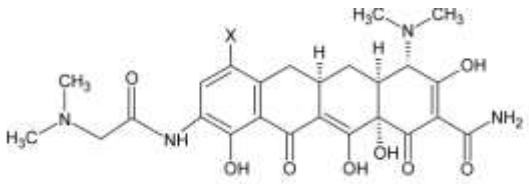
Minocycline has been effective against staphylococcal strains that are resistant to methicillin and all other tetracyclines, including doxycycline.

Minocycline has been recommended for the treatment of chronic bronchitis and other upper respiratory tract infections. Despite its relatively low renal clearance, partially compensated for by high serum and tissue levels, it has been recommended for the treatment of urinary tract infections.

NEWER TETRACYCLINES

The glycylcyclines, a class of 9-dimethylglycylamino-(DMG)-substituted tetracyclines exemplified by DMG-minocycline (DMGMINO), and DMG-6-methyl-6-deoxytetracycline (DMGDMDOT) were discovered.

The first of these to be marketed was tigecycline. The glycylcyclines retain the broad spectrum of activity and potency exhibited by the original tetracyclines against tetracycline-sensitive microbial strains and are highly active against bacterial strains that exhibit tetracycline resistance mediated by efflux or ribosomal protection determinants. If ongoing clinical evaluations of the glycylcyclines establish favorable toxicological and pharmacokinetic profiles for these compounds, a new class of "second-generation" tetracyclines could be launched.

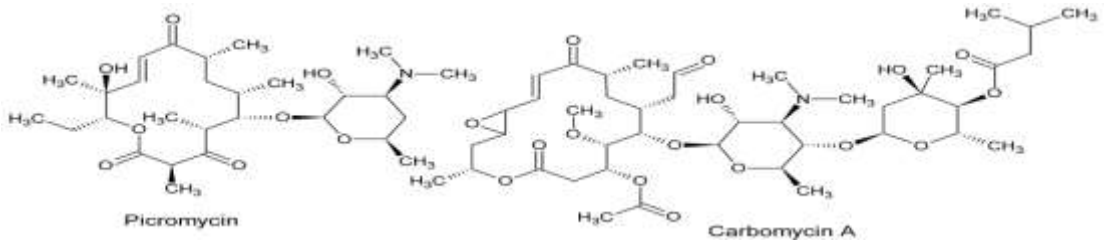


X = $N(CH_3)_2$ 9-(Dimethylglycylamino)minocycline (DMG-MINO)

X = H 9-(Dimethylglycylamino)-6-demethyl-6-deoxytetracycline (DMG-DMDOT)

MACROLIDES

Among the many antibiotics isolated from the actinomycetes is the group of chemically related compounds called the *macrolides*. picromycin, the first of this group to be identified as a macrolide compound. Erythromycin and carbomycin were reported as new antibiotics, and they were followed in subsequent years by other macrolides. Currently, more than 40 such compounds are known, and new ones are likely to appear in the future. Of all of these, only two, erythromycin and oleandomycin, have been available consistently for medical use in the United States. In recent years, interest has shifted away from novel macrolides isolated from soil samples (e.g., spiramycin, josamycin, and rosamicin), all of which thus far have proved to be clinically inferior to erythromycin and semisynthetic derivatives of erythromycin (e.g., clarithromycin and azithromycin), which have superior pharmacokinetic properties because of their enhanced acid stability and improved distribution properties.



Picromycin

Carbomycin A

Chemistry

The macrolide antibiotics have three common chemical characteristics: (a) a large lactone ring (which prompted the name *macrolide*), (b) a ketone group, and (c) a glycosidically linked amino sugar. Usually, the lactone ring has 12, 14, or 16 atoms in it, and it is often unsaturated, with an olefinic group conjugated with the ketone function. Because of the dimethylamino group on the sugar moiety, the macrolides are bases that form salts with pKa values between 6.0 and 9.0. This feature has been used to make clinically useful salts. The free bases are only slightly soluble in water but dissolve in somewhat polar organic solvents. They are stable in aqueous

solutions at or below room temperature but are inactivated by acids, bases, and heat.

Mechanism of Action and Resistance

Some details of the mechanism of antibacterial action of erythromycin are known. It binds selectively to a specific site on the 50S ribosomal subunit to prevent the translocation step of bacterial protein synthesis. It does not bind to mammalian ribosomes. Broadly based, nonspecific resistance to the antibacterial action of erythromycin among many species of Gram-negative bacilli appears to be largely related to the inability of the antibiotic to penetrate the cell walls of these organisms.

A highly specific resistance mechanism to the macrolide antibiotics occurs in erythromycin-resistant strains of *S. aureus*. Such strains produce an enzyme that methylates a specific adenine residue at the erythromycin-binding site of the bacterial 50S ribosomal subunit. The methylated ribosomal RNA remains active in protein synthesis but no longer binds erythromycin. Bacterial resistance to the lincomycins apparently also occurs by this mechanism.

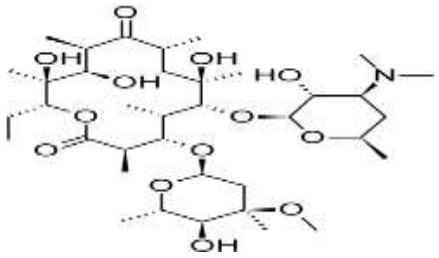
Spectrum of Activity

They are frequently active against bacterial strains that are resistant to the penicillins. The macrolides are generally effective against most species of Gram-positive bacteria, both cocci and bacilli, and exhibit useful effectiveness against Gram-negative cocci, especially *Neisseria* spp . In contrast to penicillin, macrolides are also effective against *Mycoplasma*, *Chlamydia*, *Campylobacter*, and *Legionella* spp. Their activity against most species of Gram-negative bacilli is generally low and often unpredictable, though some strains of *H. influenza* and *Brucella* spp. are sensitive.

Products

Erythromycin

erythromycin was isolated from *Streptomyces erythraeus*. It achieved rapid early acceptance as a well-tolerated antibiotic of value for the treatment of various upper respiratory and soft-tissue infections caused by Gram-positive bacteria. It is also effective against many venereal diseases, including gonorrhea and syphilis, and provides a useful alternative for the treatment of many infections in patients allergic to penicillins. More recently, erythromycin was shown to be effective therapy for Eaton agent pneumonia (*Mycoplasma pneumoniae*), venereal diseases caused by *Chlamydia*, bacterial enteritis caused by *Campylobacter jejuni*, and Legionnaires disease.



The commercial product is erythromycin A, which differs from its biosynthetic precursor, erythromycin B, in having a hydroxyl group at the 12-position of the aglycone. The amino sugar attached through a glycosidic link to C- 5 is desosamine, a structure found in several other macrolide antibiotics. The tertiary amine of desosamine (3,4,6- trideoxy-3-dimethylamino-D-*xylo*-hexose) confers a basic character to erythromycin and provides the means by which acid salts may be prepared. The other carbohydrate structure linked as a glycoside to C-3 is called *cladinose* (2,3,6- trideoxy-3-methoxy-3-C-methyl-L-*ribo*-hexose) and is unique to the erythromycin molecule.

As is common with other macrolide antibiotics, compounds closely related to erythromycin have been obtained from culture filtrates of *S. erythraeus.* Two such analogs have been

found, erythromycins B and C. Erythromycin B differs from erythromycin A only at C-12, at which a hydrogen has replaced the hydroxyl group. The B analog is more acid stable but has only about 80% of the activity of erythromycin. The C analog differs from erythromycin by the replacement of the methoxyl group on the cladinose moiety with a hydrogen atom. It appears to be as active as erythromycin but is present in very small amounts in fermentation liquors.

Erythromycin may be used as the free base in oral dosage forms and for topical administration. To overcome its bitterness and irregular oral absorption (resulting from acid destruction and adsorption onto food), various enteric coated and delayed-release dose forms of erythromycin base have been developed. Erythromycin has been chemically modified with primarily two different goals in mind: (a) to increase either its water or its lipid solubility for parenteral dosage forms and (b) to increase its acid stability (and possibly its lipid solubility) for improved oral absorption. Modified derivatives of the antibiotic are of two types: acid salts of the dimethylamino group of the desosamine moiety (e.g., the glucoheptonate, the lactobionate, and the stearate) and esters of the 2′-hydroxyl group of the desosamine (e.g., the ethylsuccinate and the propionate, available as the lauryl sulfate salt and known as the estolate).

The stearate salt and the ethylsuccinate and propionate esters are used in oral dose forms intended to improve absorption of the antibiotic. The stearate releases erythromycin base in the intestinal tract, which is then absorbed. The ethylsuccinate and the estolate are absorbed largely intact and are hydrolyzed partially by plasma and tissue esterases to give free erythromycin.

Superior oral absorption of the estolate is attributed to its both greater acid stability and higher intrinsic absorption than

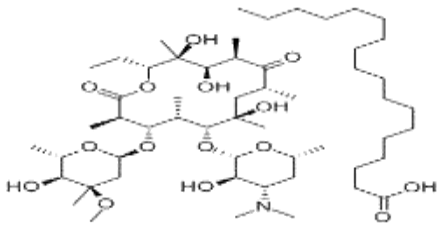
the ethylsuccinate. Also, oral absorption of the estolate, unlike that of both the stearate and the ethylsuccinate, is not affected by food or fluid volume content of the gut.

The water-insoluble ethylsuccinate ester is also available as a suspension for intramuscular injection. The glucoheptonate and lactobionate salts, however, are highly watersoluble derivatives that provide high plasma levels of the active antibiotic immediately after intravenous injection. Aqueous solutions of these salts may also be administered by intramuscular injection.

Erythromycin inhibits cytochrome P450-requiring oxidases, leading to various potential drug interactions. Thus, toxic effects of theophylline, the hydroxycoumarin anticoagulants, the benzodiazepines alprazolam and midazolam, carbamazepine, cyclosporine, and the antihistaminic drugs terfenadine and astemizole may be potentiated by erythromycin.

Erythromycin Stearate

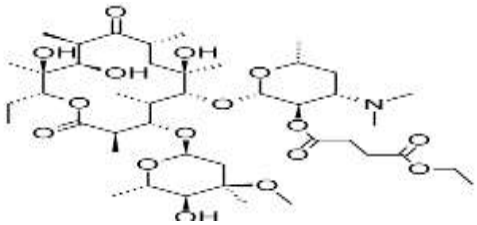
Erythromycin stearate is the stearic acid salt of erythromycin. Like erythromycin base, the stearate is acid labile. It is film coated to protect it from acid degradation in the stomach. In the alkaline pH of the duodenum, the free base is liberated from the stearate and absorbed.



Erythromycin Ethylsuccinate

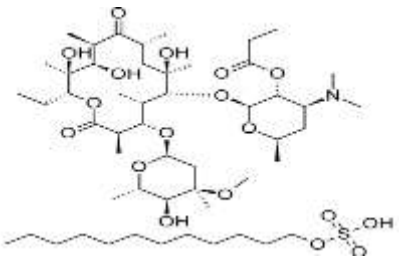
Erythromycin ethylsuccinate is the ethylsuccinate mixed ester of erythromycin in which the 2'-hydroxyl group of the

desosamine is esterified. It is absorbed as the ester and hydrolyzed slowly in the body to form erythromycin. It is somewhat acid labile, and its absorption is enhanced by the presence of food.



Erythromycin Estolate

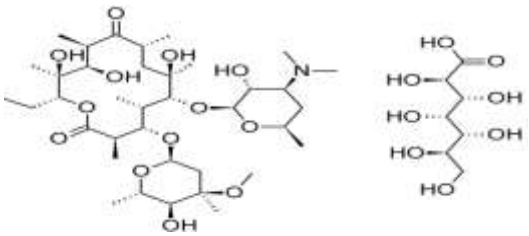
Erythromycin estolate, erythromycin propionate lauryl sulfate , is the lauryl sulfate salt of the 2^{-} propionate ester of erythromycin. Erythromycin estolate is acid stable and absorbed as the propionate ester. The ester undergoes slow hydrolysis in vivo. Only the free base binds to bacterial ribosomes. Some evidence, however, suggests that the ester is taken up by bacterial cells more rapidly than the free base and undergoes hydrolysis by bacterial esterases within the cells. The incidence of cholestatic hepatitis is reportedly higher with the estolate than with other erythromycin preparations.



Erythromycin Gluceptate, Sterile

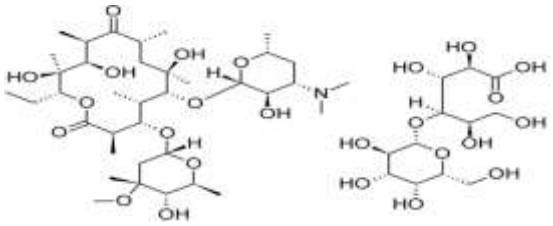
Erythromycin gluceptate, erythromycin glucoheptonate , is the glucoheptonic acid salt of erythromycin. It is a crystalline substance that is freely soluble in water and practically

insoluble in organic solvents. Erythromycin gluceptate is intended for intravenous administration for the treatment of serious infections, such as Legionnaires disease, or when oral administration is not possible. Solutions are stable for 1 week when refrigerated.



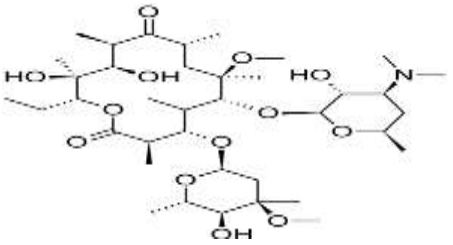
Erythromycin Lactobionate

Erythromycin lactobionate is a water-soluble salt prepared by reacting erythromycin base with lactobiono-*δ*-lactone. It occurs as an amorphous powder that is freely soluble in water and alcohol and slightly soluble in ether. It is intended, after reconstitution in sterile water, for intravenous administration to achieve high plasma levels in the treatment of serious infections.



Clarithromycin

Clarithromycin is the 6-methyl ether of erythromycin. The simple methylation of the 6-hydroxyl group of erythromycin creates a semisynthetic derivative that fully retains the antibacterial properties of the parent antibiotic, with markedly increased acid stability and oral bioavailability and reduced GI side effects associated with erythromycin.



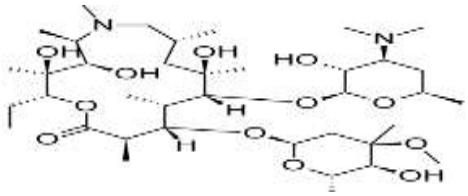
Clarithromycin is well absorbed following oral administration. Its oral bioavailability is estimated to be 50% to 55%. The presence of food does not significantly affect its absorption. Extensive metabolism of clarithromycin by oxidation and hydrolysis occurs in the liver. The major metabolite is the 14-hydroxyl derivative, which retains antibacterial activity. The amount of clarithromycin excreted in the urine ranges from 20% to 30%, depending on the dose, whereas 10% to 15% of the 14-hydroxy metabolite is excreted in the urine. Biliary excretion of clarithromycin is much lower than that of erythromycin.

Some of the microbiological properties of clarithromycin also appear to be superior to those of erythromycin. It exhibits greater potency against *M. pneumoniae*, *Legionella* spp., *Chlamydia pneumoniae*, *H. influenzae*, and *M. catarrhalis* than does erythromycin. Clarithromycin is significantly more active than erythromycin against group A streptococci, *S. pneumoniae*, and the viridans group of streptococci in vivo because of its superior oral bioavailability. Clarithromycin, like erythromycin, inhibits cytochrome P450 oxidases and, thus, can potentiate the actions of drugs metabolized by these enzymes.

Azithromycin

Azithromycin (Zithromax) is a semisynthetic derivative of erythromycin. It is a prototype of a series of nitrogen-containing, 15-membered ring macrolides known as *azalides*. Removal of the 9-keto group coupled with incorporation of a weakly basic tertiary amine nitrogen function into the

macrolide ring increases the stability of azithromycin to acid-catalyzed degradation. These changes also increase the lipid solubility of the molecule, thereby conferring unique pharmacokinetic and microbiological properties.



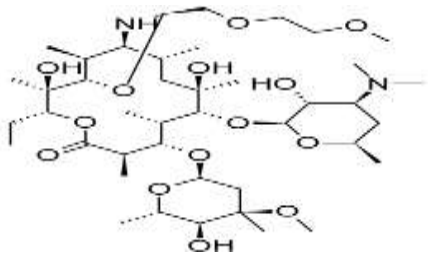
The oral bioavailability of azithromycin is good, nearly 40%, provided the antibiotic is administered at least 1 hour before or 2 hours after a meal. Food decreases its absorption by as much as 50%. The pharmacokinetics of azithromycin are characterized by rapid and extensive removal of the drug from the plasma into the tissues followed by a slow release. Tissue levels far exceed plasma concentrations, leading to a highly variable and prolonged elimination half-life of up to 5 days. Azithromycin apparently is not metabolized to any significant extent. In contrast to the 14-membered ring macrolides, azithromycin does not significantly inhibit cytochrome P450 enzymes to create potential drug interactions.

The spectrum of antimicrobial activity of azithromycin is similar to that observed for erythromycin and clarithromycin but with some interesting differences. In general, it is more active against Gram-negative bacteria and less active against Gram-positive bacteria than its close relatives. The greater activity of azithromycin against *H. influenzae*, *M. catarrhalis*, and *M. pneumoniae* coupled with its extended half-life permits a 5-day dosing schedule for the treatment of respiratory tract infections caused by these pathogens. The clinical efficacy of azithromycin in the treatment of urogenital and other sexually transmitted infections caused by *Chlamydia trachomatis*, *N. gonorrhoeae*, and *Ureaplasma urealyticum* suggests that

single dose therapy with it for uncomplicated urethritis or cervicitis may have advantages over use of other antibiotics.

Dirithromycin

Dirithromycin (Dynabac) is a more lipid-soluble prodrug derivative of 9*S*-erythromycyclamine prepared by condensation of the latter with 2-(2-methoxyethoxy) acetaldehyde. The 9*N*,11*O*-oxazine ring thus formed is a hemi-aminal that is unstable under both acidic and alkaline aqueous conditions and undergoes spontaneous hydrolysis to form erythromycyclamine. Erythromycyclamine is a semisynthetic derivative of erythromycin in which the 9-keto group of the erythronolide ring has been converted to an amino group.



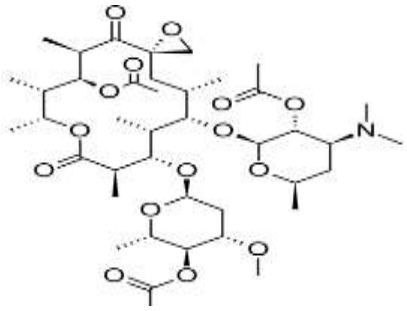
Erythromycyclamine retains the antibacterial properties of erythromycin in vitro but exhibits poor bioavailability following oral administration. The prodrug, dirithromycin, is provided as enteric-coated tablets to protect it from acidcatalyzed hydrolysis in the stomach. Orally administered dirithromycin is absorbed rapidly into the plasma, largely from the small intestine. Spontaneous hydrolysis to erythromycyclamine occurs in the plasma. Oral bioavailability is estimated to be about 10%, but food does not affect absorption of the prodrug.

Preliminary studies indicate that dirithromycin and erythromycyclamine do not interact significantly with cytochrome P450 oxygenases. Thus, the likelihood of interference in the oxidative metabolism of drugs such as phenytoin, theophylline, and cyclosporine by these enzymes may be less with dirithromycin than with erythromycin.

Dirithromycin is recommended as an alternative to erythromycin for the treatment of bacterial infections of the upper and lower respiratory tracts, such as pharyngitis, tonsillitis, bronchitis, and pneumonia, and for bacterial infections of other soft tissues and the skin. The once-daily dosing schedule for dirithromycin is advantageous in terms of better patient compliance.

Troleandomycin

Oleandomycin, as its triacetyl derivative troleandomycin, triacetyloleandomycin (TAO), remains available as an alternative to erythromycin for limited indications permitting use of an oral dosage form. The oleandomycin structure consists of two sugars and a 14-member lactone ring designated an *oleandolide*. One of the sugars is desosamine, also present in erythromycin; the other is L-oleandrose. The sugars are linked glycosidically to the positions 3 and 5, respectively, of oleandolide.



Oleandomycin contains three hydroxyl groups that are subject to acylation, one in each of the sugars and one in the oleandolide. The triacetyl derivative retains the in vivo antibacterial activity of the parent antibiotic but possesses superior pharmacokinetic properties. It is hydrolyzed in vivo to oleandomycin. Troleandomycin achieves more rapid and higher plasma concentrations following oral administration than oleandomycin phosphate, and it has the additional advantage of being practically tasteless.

Troleandomycin is the most potent inhibitor of cytochrome P450 enzymes of the commercially available macrolides. It may potentiate the hepatic toxicity of certain anti-inflammatory steroids and oral contraceptive drugs as well as the toxic effects of theophylline, carbamazepine, and triazolam. Several allergic reactions, including cholestatic hepatitis, have also been reported with the use of troleandomycin.

Approved medical indications for troleandomycin are currently limited to the treatment of upper respiratory infections caused by such organisms as *S. pyogenes* and *S. pneumoniae*. It may be considered an alternative to oral forms of erythromycin. It is available in capsules and as a suspension.

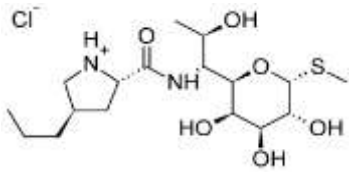
LINCOMYCINS

The lincomycins are sulfur-containing antibiotics isolated from *Streptomyces lincolnensis*. Extensive efforts to modify the lincomycin structure to improve its antibacterial and pharmacological properties resulted in the preparation of the 7-chloro-7-deoxy derivative clindamycin. Of the two antibiotics, clindamycin appears to have the greater antibacterial potency and better pharmacokinetic properties. They are primarily active against Grampositive bacteria, particularly the cocci, but are also effective against non–spore-forming anaerobic bacteria, actinomycetes, mycoplasma, and some species of *Plasmodium*. Lincomycin binds to the 50S ribosomal subunit to inhibit protein synthesis. Its action may be bacteriostatic or bactericidal depending on various factors, including the concentration of the antibiotic. A pattern of bacterial resistance and cross-resistance to lincomycins similar to that observed with the macrolides has been emerging.

Products

Lincomycin Hydrochloride

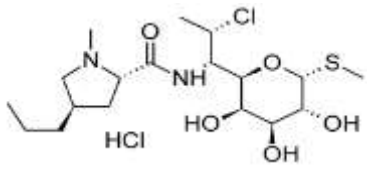
Lincomycin hydrochloride (Lincocin), which differs chemically from other major antibiotic classes. The structure contains a basic function, the pyrrolidine nitrogen, by which water-soluble salts with an apparent pKa of 7.6 may be formed. When subjected to hydrazinolysis, lincomycin is cleaved at its amide bond into *trans*-L-4-*n*-propylhygric acid (the pyrrolidine moiety) and methyl *α*-thiolincosamide (the sugar moiety).



Lincomycin is used for the treatment of infections caused by Gram-positive organisms, notably staphylococci, *β*-hemolytic streptococci, and pneumococci. It is absorbed moderately well orally and distributed widely in the tissues. Effective concentrations are achieved in bone for the treatment of staphylococcal osteomyelitis but not in the cerebrospinal fluid for the treatment of meningitis. At one time, lincomycin was considered a nontoxic compound, with a low incidence of allergy (rash) and occasional GI complaints (nausea, vomiting, and diarrhea) as the only adverse effects. Recent reports of severe diarrhea and the development of pseudomembranous colitis in patients treated with lincomycin (or clindamycin). In any event, clindamycin is superior to lincomycin for the treatment of most infections for which these antibiotics are indicated. It is degraded slowly in acid solutions but is absorbed well from the GI tract. Lincomycin diffuses well into peritoneal and pleural fluids and into bone. It is excreted in the urine and the bile. It is available in capsule form for oral administration and in ampules and vials for parenteral administration.

Clindamycin Hydrochloride

Magerlein et al. reported that replacement of the 7(*R*)-hydroxy group of lincomycin by chlorine with inversion of configuration resulted in a compound with enhanced antibacterial activity in vitro. Improved absorption and higher tissue levels of clindamycin and its greater penetration into bacteria have been attributed to a higher partition coefficient than that of lincomycin. Structural modifications at C-7 (e.g., 7(*S*)-chloro and 7(*R*)-OCH3) and of the C-4 alkyl groups of the hygric acid moiety appear to influence activity of congeners more through an effect on the partition coefficient of the molecule than through a stereospecific binding role. Changes in the *α*-thiolincosamide portion of the molecule seem to decrease activity markedly.



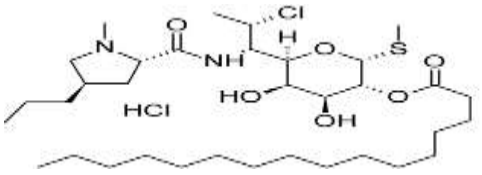
Clindamycin is recommended for the treatment of a wide variety of upper respiratory, skin, and tissue infections caused by susceptible bacteria. Its activity against streptococci, staphylococci, and pneumococci is indisputably high, and it is one of the most potent agents available against some non–spore-forming anaerobic bacteria, the *Bacteroides* spp. in particular. An increasing number of reports of clindamycin-associated GI toxicity, which range in severity from diarrhea to an occasionally serious pseudomembranous colitis. The colitis, which is usually reversible when the drug is discontinued, is now believed to result from an overgrowth of a clindamycin-resistant strain of the anaerobic intestinal bacterium *Clostridium difficile*. The intestinal lining is damaged by a glycoprotein endotoxin released by lysis of this organism. The

glycopeptide antibiotic vancomycin has been effective in the treatment of clindamycin-induced pseudomembranous colitis.

Clindamycin should be reserved for staphylococcal tissue infections, such as cellulitis and osteomyelitis, in penicillin-allergic patients and for severe anaerobic infections outside the central nervous system.

Clindamycin Palmitate Hydrochloride

Clindamycin palmitate hydrochloride (Cleocin Pediatric) is the hydrochloride salt of the palmitic acid ester of cleomycin. The ester bond is to the 2-hydroxyl group of the lincosamine sugar. The ester serves as a tasteless prodrug form of the antibiotic, which hydrolyzes to clindamycin in the plasma. The salt form confers water solubility to the ester, which is available as granules for reconstitution into an oral solution for pediatric use.

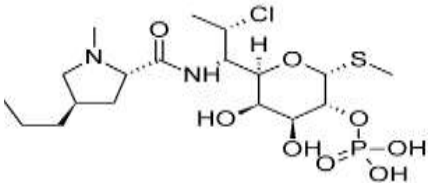


Although absorption of the palmitate is slower than that of the free base, there is little difference in overall bioavailability of the two preparations. Reconstituted solutions of the palmitate hydrochloride are stable for 2 weeks at room temperature.

Clindamycin Phosphate

Clindamycin phosphate (Cleocin Phosphate) is the 2-phosphate ester of clindamycin. It exists as a zwitterionic structure that is very soluble in water. It is intended for parenteral (intravenous or intramuscular) administration for the treatment of serious infections and instances when oral administration is not feasible. Solutions of clindamycin

phosphate are stable at room temperature for 16 days and for up to 32 days when refrigerated.



POLYPEPTIDES

Many of them have

Antineoplastic Agents

Antineoplastic agents describe the chemistry, use, metabolism and adverse effect profiles for the alkylating agents, antibiotics, natural products, antimetabolites and tyrosine kinase (TK) inhibitors used in the treatment of cancer.

The American Cancer Society defines cancer as a group of diseases characterized by uncontrolled growth, and the spread of abnormal cells that left untreated may lead to death. Related to this definition is the term *neoplasia*, which is the uncontrolled growth of new tissue, the product of which is known as a *tumor*, and these tumors may be either malignant or benign. Malignant tumors have the capability of invading surrounding tissues and moving to distant locations in the body in a process known as *metastasis*; characteristics that benign tumors do not possess. Treatment of malignant tumors or cancer has generally involved initially surgical removal followed by radiation and/or chemotherapy, if necessary. The term chemotherapy refers to drugs that are used to kill cells but it is often used to refer exclusively to anticancer agents(antineoplastics).

Cancer cells manifest four characteristics that distinguish them from normal cells: Uncontrolled proliferation, Dedifferentiation and loss of function, Invasiveness and Metastasis .

One way of classifying different types of cancer is based on the affected tissue as outlined below :

Carcinomas: These are the most common cancers (80-90% of cases) that originate in epithelial tissue, which includes skin and covering and lining of organs and internal passageways. Examples of carcinomas are breast cancer, lung cancer, and colon cancer.

Sarcomas: These start in connective tissue such as bones, tendons, cartilage, muscle, and fat. Example bone cancer and Kapposi sarcoma.

Leukaemia's and Lymphomas: These encompass all the other types of cancers. These constitute about 8% of all the human cancers and develop in the bone marrow and lymph systems, which manufactures blood .

The approach to treatment depends on the extent of the disease and the so-called stage.

The primary risk factor for most cancers is age. There are certainly other risk factors such as exposure to environmental toxins. This is related to the genetic component of cancer so that an aberrant cancer cell may arise as a result of mutation or translocation of DNA so that the chances increase with an increasing number of cell divisions and, hence, age.

Normal cellular growth and proliferation are generally driven by factors external to the cell. Mitogenic signals such as hormones and growth factors direct the cell to undergo mitosis and are generally released from other tissues. ***In cancer cells***, this system may become disrupted in several ways.1-For example, **some cells** may acquire the ability to synthesize their own growth factors in a process known as *autocrine signaling*.2-It is also possible for cells to lose the requirements for growth factors altogether but still be

capable of proliferating. Central to this idea is the concept of oncogenes (cancer-producing genes) the products of which may be responsible for these types of alterations. Oncogenes themselves are not normally present in the genome; however, precursors known as *proto-oncogenes* are commonly seen. Alterations in these proto-oncogenes by mutation or translocation may result in their transformation to an oncogene and the development of cancer.

The process of **cell division** occurs through a series of phases that collectively are known as the *cell cycle*. Starting in the G1 for gap 1 or growth 1 phase, the enzymes necessary for the replication of DNA are synthesized (Fig. 10.1). Alternatively, cells may enter the G0 phase in which they do not prepare for cell division but carry on normal metabolic processes. Entry into G0 (sometimes referred to as *senescence*) is not an irreversible process;

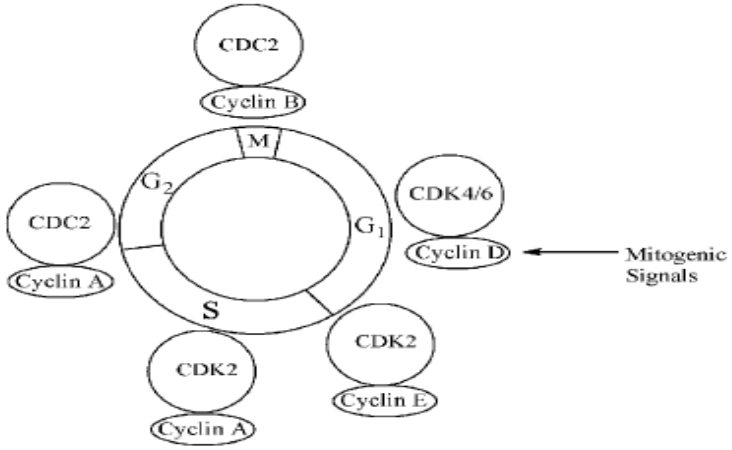


Figure 10.1 • Cyclins and the cell cycle.

If the cell is to undergo division, it will progress to the S phase where DNA is replicated. This is followed by the G2 phase, during which additional protein synthesis occurs including the formation of the microtubules. The M phase follows in which the DNA is segregated and cell division occurs. During the entire cycle, movement from one phase to the next is driven by proteins known as cyclins and their associated cyclin-dependent kinases (CDKs). There are several subtypes of cyclins with their CDKs, the concentrations of which change or cycle as the cell moves from G1 through M phase, and this concentration change is associated with moving the cell into the next phase. The D-type cyclins paired with CDK4/6 are in high concentration during the G1 phase, and their formation is under the control of external growth factors. Subsequent to this, the D-type cyclins help drive the formation of cyclin E with its CDK2, which drives the cell from the G1 to the S phase. This is followed by formation of A- and B-type cyclins with their CDKs, which push the cell into G2 and subsequently into M, respectively.

If problems are encountered, the cell may be slowed from progressing or undergo programmed cell death also known as *apoptosis*. Regulation of the cell through the cell

cycle is the function of the tumor suppressor proteins such as retinoblastoma protein (Rb) p21 and p53.

The process of apoptosis can occur by both an intrinsic and extrinsic pathway (Fig. 10.2). Proapoptotic p53 products(Bad, Bax, and Bid) and antiapoptotic(Bcl-2 and Bcl-XL proteins). Apoptotic protease activating factor-1 (apaf-1) molecules.

The extrinsic pathway is activated when ligands of the tumor necrosis factor family of proteins (TNF-_, FasL, TRAIL) interact with the so-called death receptors (FAS, DR3-5, TNFR1) present on the cell surface.In this process, the proapoptotic Bid is activated by caspase-8. Bid opens the mitochondrial channels to release cytochrome c which again continues with the same intrinsic pathway.

The ability of drugs to kill cancer cells is generally believed to be because of their ability to induce the process of apoptosis. In high-dose therapy, cell death may occur by necrosis but this is also toxic to the patient.

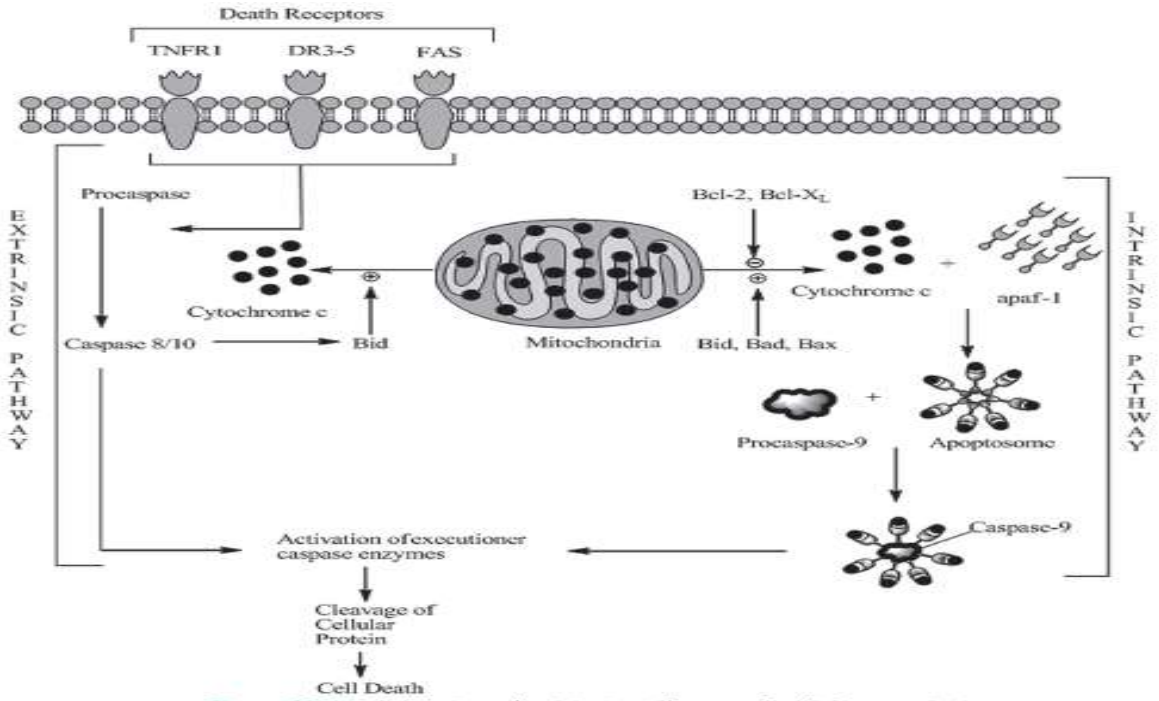


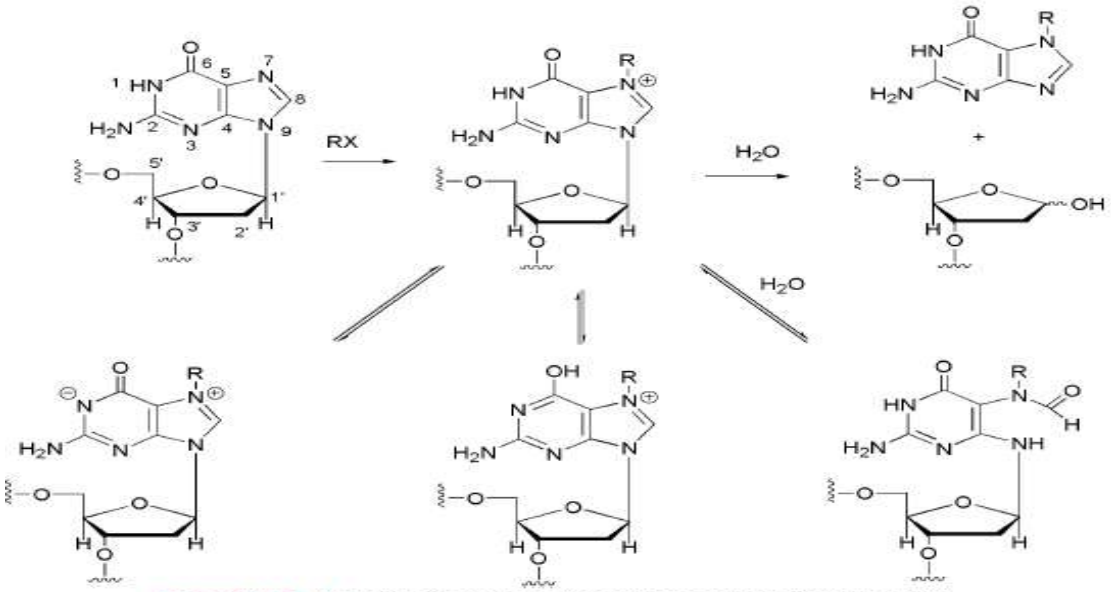
Figure 10.2 • Intrinsic and extrinsic pathways of cellular apoptosis.

DRUG CLASSES

Alkylating Agents

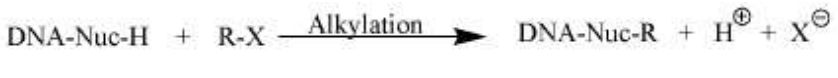
The alkylating agents are a class of drugs that are capable of forming covalent bonds with important biomolecules. The major targets of drug action are nucleophilic groups present on DNA (especially the 7-position of guanine);but also proteins and RNA among others may also be alkylated. Alkylation of DNA is thought to lead to cell death. Although the exact mechanism is uncertain but the potential mechanisms of cell death include activation of apoptosis caused by p53 activation and disruption of the template function

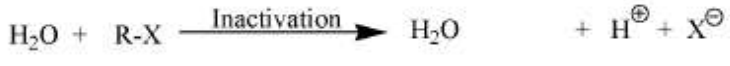
of DNA. ***In many cases, the cancer cells*** have dysfunctional p53 so that even though the cell has been unable to replicate DNA error free, cell death via apoptosis does not occur. Disruption of the template function of DNA may have several effects. There are several potential nucleophilic sites on DNA, which are susceptible to electrophilic attack by an alkylating agent (N-2, N-3, and N-7 of guanine, N-1, N-3, and N-7 of adenine, 0–6 of thymine, N-3 of cytosine). The most important of these for many alkylating agents is the N-7 position of guanine whose nucleophilicity may be enhanced by adjacent guanine residues:-**1-**Alkylation converts the base to an effective leaving group so that attack by water leads to depurination and the loss of genetic information if the resulting depurination is not repaired by the cell (Scheme 10.1).**2-**Additionally, alkylation has been proposed to result in altered base pairing away from the normal G-C: A-T hydrogen bonds because of alterations in tautomerization.**3-**The alkylation also leads to increased acidity of the N-1 nitrogen reducing the pKa from 9 to 7 to 8 giving rise to a zwitterionic form that may also mispair.



Scheme 10.1 • Alkylation of guanine N-7 and subsequent depurination of DNA.

The general mechanism for alkylation involves nucleophilic attack by –N= , $–NH_2$, – OH, $–O–PO_3H$ of DNA and RNA, while additional nucleophiles (–SH, COOH, etc.) present on proteins may also react (Scheme 10.2).





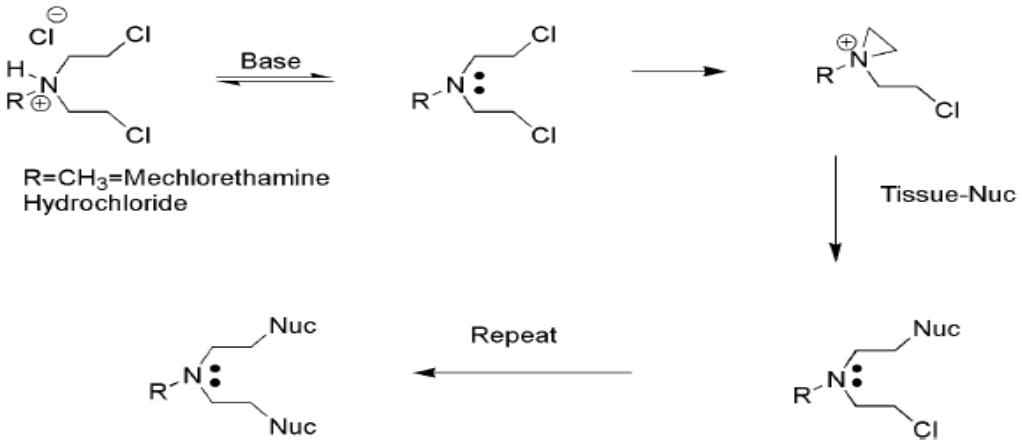
Scheme 10.2 • General reaction for alkylation and inactivation of alkylating agents.

Where X = a leaving group

Mechanisms by which cells may become resistant to these agents are thought to be similar. The alkylating agents are thought to be effective from G0-M and are, therefore, not considered cell cycle–specific agents.
Many of the toxicities seen with the various agents are similar. Myelosuppression and gastrointestinal (GI) disruption, which often present as nausea and vomiting.

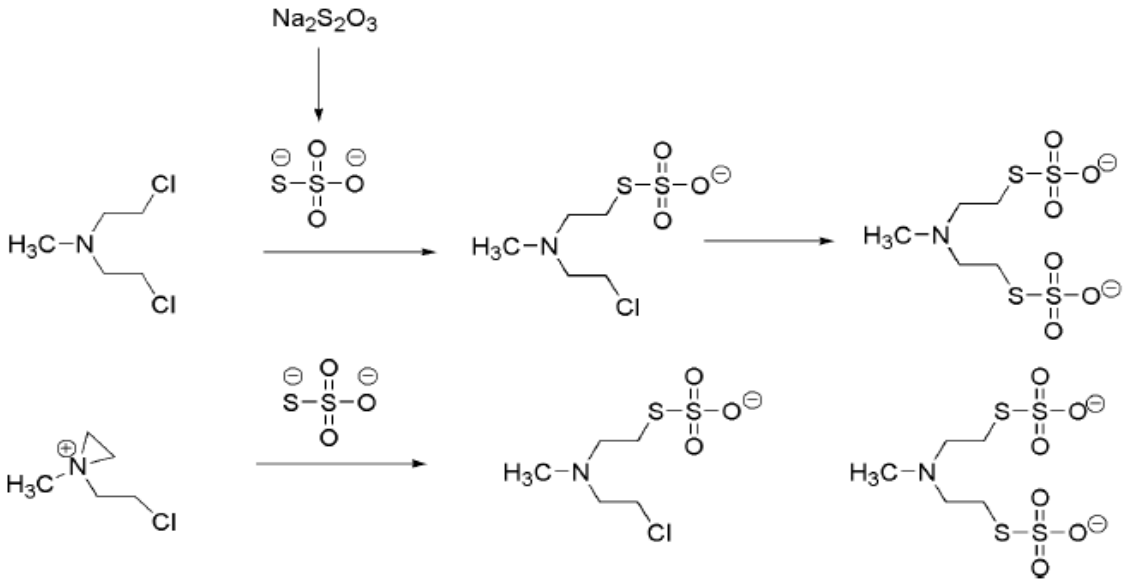
NITROGEN MUSTARDS

The nitrogen mustards are compounds that are chemically similar to sulfur mustard or mustard gas developed and used in World War I. Investigation of sulfur mustard revealed that it possessed antineoplastic properties but because the compound existed as a gas at room temperature, handling and administration of the material were difficult. Conversion of the sulfide to a tertiary amine allowed for the formation of salts, which exist as solids at room temperature allowing for easier handling and dosing. Mustards such as mechlorethamine are classified as dialkylating agents in that one mustard molecule can alkylate two nucleophiles. The initial acid–base reaction is necessary to release the lone pair of electrons on nitrogen, which subsequently displaces chloride to give the highly reactive aziridinium cation (Scheme 10.3). Nucleophilic attack can then occur at the aziridinium carbon to relieve the small ring strain and neutralize the charge on nitrogen. This process can then be repeated provided a second leaving group is present.



Scheme 10.3 ● Alkylation of nucleophilic species by nitrogen mustards.

Mechlorethamine is highly reactive, in fact, too reactive and therefore nonselective, making it unsuitable for oral administration and necessitating direct injection into the tumor. In cases of extravasation (drug escapes from the tumor into the underlying tissue), the antidote sodium thiosulfate (Na2S2O3), a strong nucleophile, may be administered. It is capable of reacting with electrophilic sites on the mustard, and once reaction has occurred, the resulting adduct has increased water solubility and may be readily eliminated (Scheme 10.4).



Scheme 10.4 • Thiosulfate inactivation of mechlorethamine.

The lack of selectivity of mechlorethamine led to attempts to improve on the agent. One rationale was to reduce the reactivity by reducing the nucleophilicity of nitrogen, thereby slowing aziridinium cation formation. This could be accomplished by replacement of the weakly electron-donating methyl group with groups that were electron withdrawing. This is seen in the case of chlorambucil and melphalan by attachment of nitrogen to a phenyl ring (Fig. 10.3).

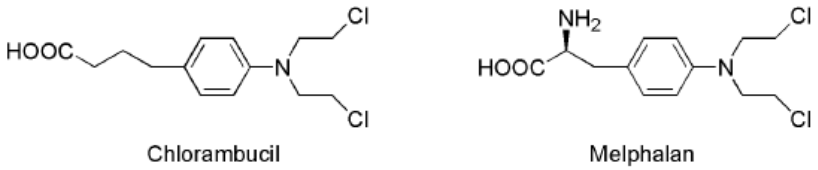


Figure 10.3 • Structure of chlorambucil and melphalan.

Reactivity was reduced such that these compounds could be administered orally. In the case of melphalan, attachment of the mustard functionality to a phenylalanine moiety was not only an attempt to reduce reactivity but also an attempt to increase entry into cancer cells by utilization of carrier-mediated uptake.

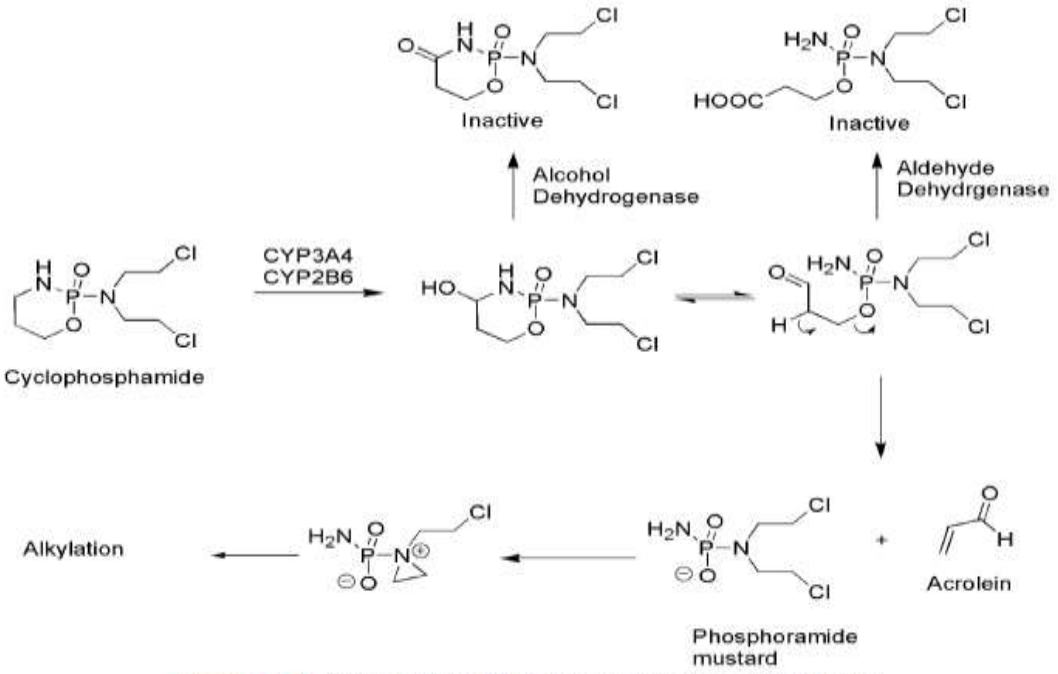
Attachment of more highly electron-withdrawing functionalities was utilized in the case of **cyclophosphamide** and **ifosfamide** (Fig. 10.4).

Figure 10.4 • Structures of cyclophosphamide and ifosfamide.

In these cases, aziridinium cation formation is not possible until the electron-withdrawing function has been altered . The drug was activated by cytochrome P450 (CYP) isozymes

CYP2B6 and CYP3A4/5 to give a carbinolamine that could undergo ring opening to give the aldehyde.

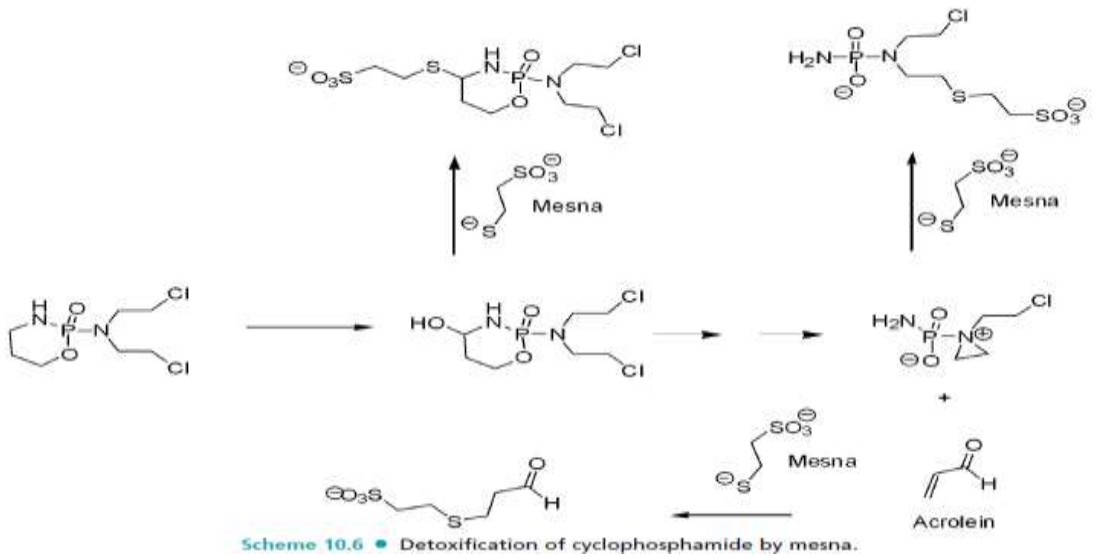
The increased acidity of the aldehyde *alpha* hydrogen facilitates a retro-Michael decomposition (Scheme 10.5). The ionized phosphoramide is now electron-releasing via induction and allows aziridinium cation formation to proceed. Acrolein is also formed as a result of this process, which may itself act as an electrophile that has been associated with bladder toxicity. Alternatively, the agent may be inactivated by alcohol dehydrogenase–mediated oxidation of the carbinolamine to give the amide or by further oxidation of the aldehyde intermediate to give the acid by aldehyde dehydrogenase.



Scheme 10.5 • Metabolic and chemical activation of cyclophosphamide.

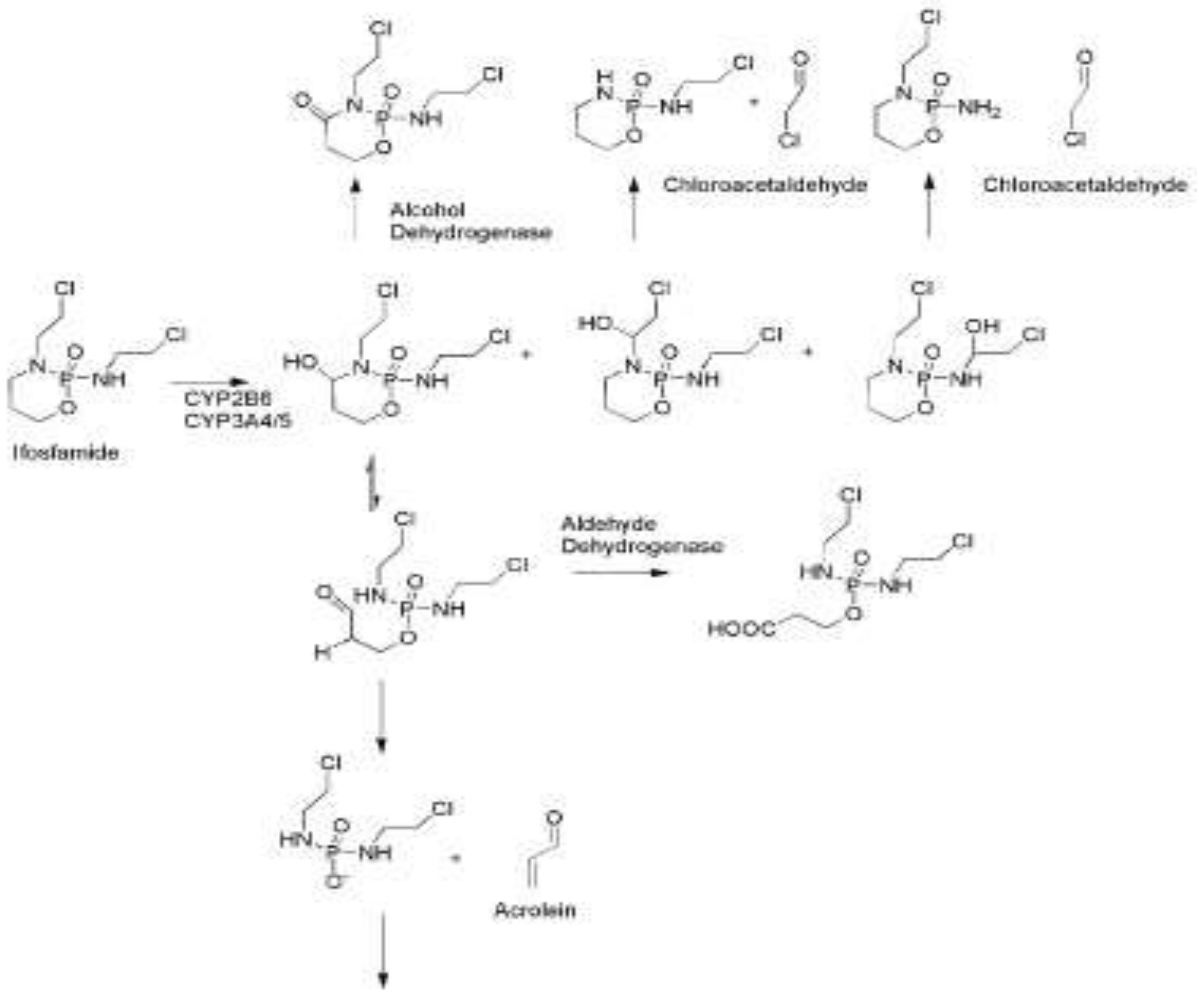
To decrease the incidence of kidney and bladder toxicity, the sulfhydryl (MSH) containing agent mesna may be administered and functions to react with the electrophilic species that may be present in the kidney. The sulfonic acid functionality serves to help concentrate the material in the urine, and the nucleophilic sulfhydryl group may react with the carbinolamine, aziridinium cation, the chloro substituents of cyclophosphamide, or ***via conjugate addition with acrolein*** (Scheme 10.6). This inactivation and detoxification may also be accomplished by other thiol-containing proteins such as

glutathione. Increased levels of these proteins may occur as cancer cells become resistant to these alkylating agents.



Scheme 10.6 • Detoxification of cyclophosphamide by mesna.

Ifosfamide contains similar functionality and also requires activation by CYP2B6 and CYP3A4/5 (Scheme 10.7). Although the agents are similar, there are differences in the metabolism and activity of the agents. Both are administered as racemic mixtures as a result of the presence of a chiral phosphorus atom. There appears to be little difference in the metabolic fate of the *R*- and *S*-isomers of cyclophosphamide, but in the case of ifosfamide, the *R*-isomer is converted to the required 4-hydroxy-ifosfamide 2 to 3 times faster than the *S*-isomer. The *S*-isomer undergoes preferential oxidation of the side chain to give *N*-dechloroethylation, which removes the ability of the agent to cross-link DNA and also produces the neurotoxic and urotoxic chloroacetaldehyde. An additional difference between cyclophosphamide and ifosfamide is the larger alkylating species that ultimately results after metabolic activation of ifosfamide.

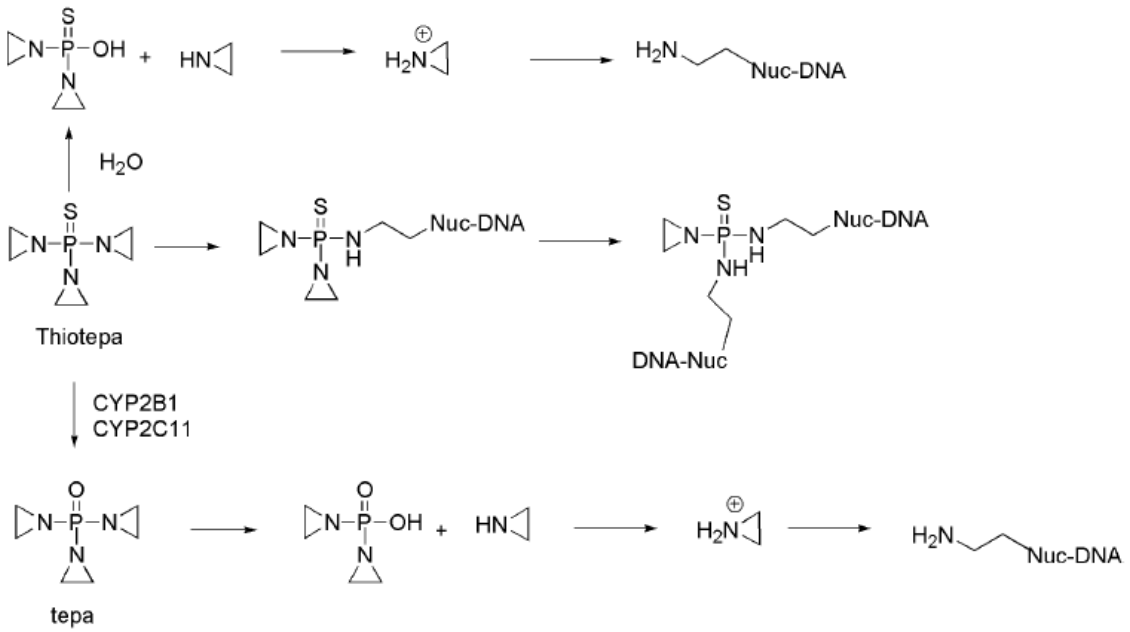


Scheme 10.7 • Metabolic and chemical activation of ifosfamide.

THIOTEPA

Thiotepa containing the thiophosphoramide functionality was found to be more stable than the oxa-analog (TEPA) but is metabolically converted to TEPA by desulfuration in vivo. Thiotepa incorporates a less reactive aziridine ring compared with that formed in mechlorethamine. The adjacent thiophosphoryl is electron withdrawing and, therefore, reduces the reactivity of the aziridine ring system. (Scheme 10.8).Monoalkylation is also possible as a result of aziridine formation via hydrolysis of thiotepa. Thiotepa is also metabolized by oxidative desulfurization mediated by CYP2B1 and CYP2C11.The decreased stability of the resulting TEPA undergoes hydrolysis to give aziridine, which may function to monoalkylate DNA. The conclusion that aziridine is the active alkylating agent once thiotepa has been converted to TEPA is based on the fact that when TEPA is incubated with DNA, no crosslinks are formed and only monoadducts are generated. The

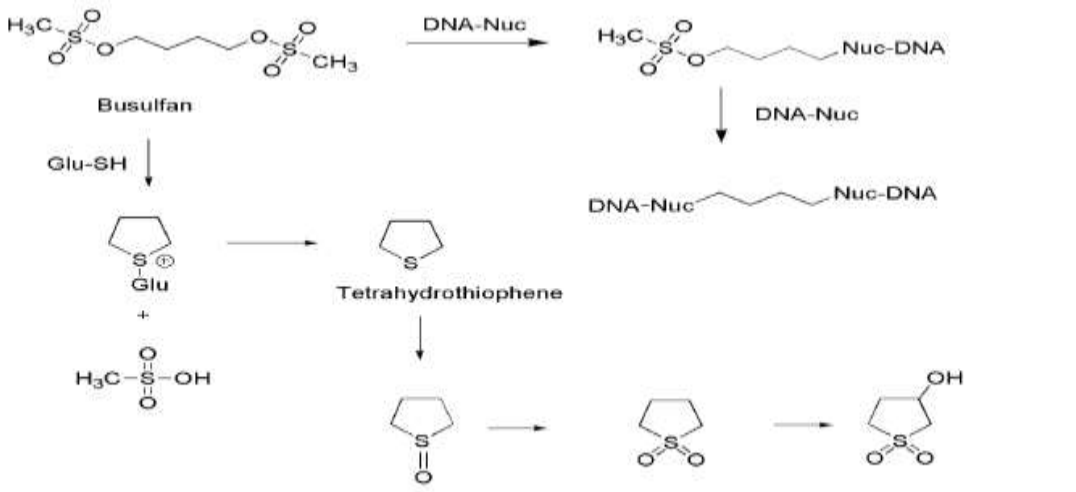
reactivity of aziridine generated by either route may be somewhat enhanced within cancer cells, where the pH is normally reduced 0.2 to 0.4 pH units resulting in an increase in reactivity toward nucleophilic attack.



Scheme 10.8 ● Metabolic and chemical interactions of thiotepa with DNA.

BUSULFAN

As an alternative to utilizing aziridines as electrophilic species, it was found that simply utilizing a carbon chain terminated at both ends by leaving groups gave compounds capable of acting as cross-linking agents (Scheme 10.9). Busulfan utilizes two sulfonate functionalities as leaving groups separated by a four-carbon chain that reacts with DNA to primarily form intrastrand cross-link at 5^{-}-GA-3^{-} sequences.The sulfonates are also subject to displacement by the sulfhydryl functions found in cysteine and glutathione, and metabolic products are formed as a result of nucleophilic attack by these groups to generate sulfonium species along with methane sulfonic acid. This is followed by conversion to tetrahydrothiophene, and further oxidation products are subsequently produced to give the sulfoxide and sulfone. The cyclic sulfone known as *sulfolane* may be further oxidized to give 3-hydroxysulfolane.



Scheme 10.9 • Metabolic and chemical reactions of busulfan.

ORGANOPLATINUM COMPOUNDS

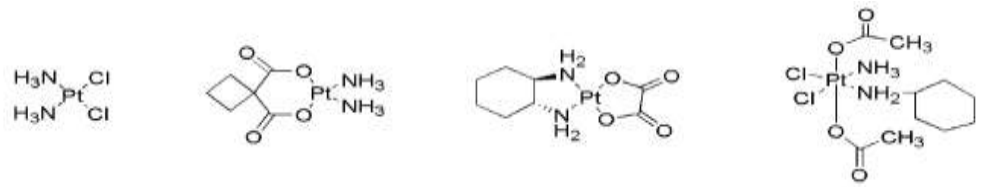
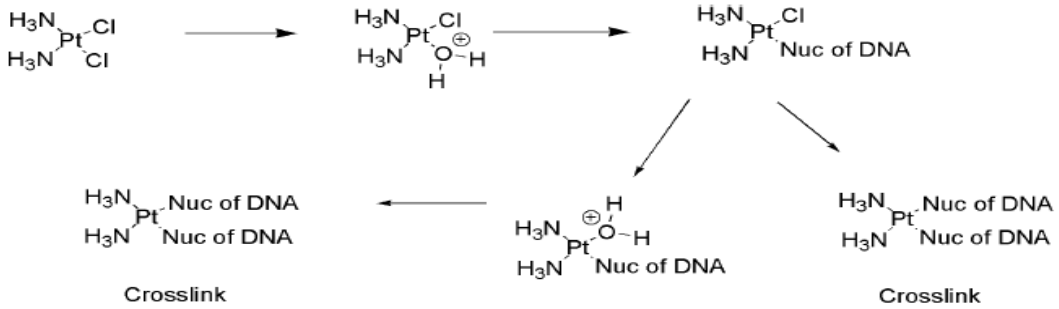


Figure 10.5 • Platinum-containing antineoplastics.

The first of these, cisplatin cis-[PtCl2(NH3)2] as the active species and mechanistic studies revealed that after administration of the agent to mammals, the dichloro species is maintained in the blood stream as a result of the relatively high chloride concentration (Scheme 10.10). Movement into the tumor cells is accomplished by passive diffusion or carrier-mediated transport. Once inside the tumor cell, the drug encounters a lower chloride concentration and one chloro group is substituted by a water molecule in a process known as aquation. This serves to "trap" the molecule in the cell as a result of ionization. Reaction with DNA occurs preferentially at the N-7 of guanine of two adjacent guanine residues resulting in primarily (95%) intrastrand cross-links.



Scheme 10.10 • Mechanism of cisplatin activation and formation of DNA adducts.

Platinum (II) is considered to be a "soft" electrophile and as a result, its complexes are subject to attack by "soft" nucleophiles such as thiol groups found on proteins. This can

result in significant protein binding (88%–95%) and inactivation caused by the presence of thiols in albumin, glutathione, and other proteins. Cisplatin administration is also associated with significant nephrotoxicity and neurotoxicity that is dose limiting. These factors lead to the development of lessnt nephrotoxicity and neurotoxicity that is dose limiting. reactive platinum compounds such as carboplatin and oxaliplatin in which the leaving group was incorporated into a chelate. More recently, there has been interest in the development of Pt(IV) compounds, which are much less reactive and believed to function as prodrugs requiring reduction to the Pt(II) species prior to reaction with nucleophiles. One such agent, satraplatin is currently in clinical trials. One advantage of these agents is the possibility of oral administration. **Satraplatin** has shown similar activity when given orally to that of cisplatin given by injection.

Tumor cells may become resistant to the platins by mechanisms that are seen with other chemotherapeutic agents such as decreased uptake, increased inactivation by thiol containing proteins and increased DNA repair. However, a deficiency in a type of DNA repair known as ***mismatch repair*** **(MMR)** has also been implicated in resistance to cisplatin and carboplatin. The process of MMR involves several enzymes that are responsible for maintaining the integrity of the genome, and interest has focused on the interaction of these enzymes with repeating units found throughout DNA known as *microsatellites*. When MMR processes are not operating, these microsatellites may become longer or shorter and this is known as *microsatellite instability*. This can result in frame shift errors such that tumor suppressor genes may become less effective, and the tumor cells therefore fail to undergo apoptosis even if alkylation has occurred. The binding of cisplatin- and carboplatin- DNA adducts by the MMR enzymes results in increased cytotoxicity of these agents. Several rationales have been put forward as to why this occurs, including the involvement of MMR enzymes in downstream signaling that activates apoptosis. A second rationale involves the ability of MMR enzymes to remove replication errors that occur past the point of adduct formation, and in the process of removing these errors, gaps in the DNA are created, which lead to cell death. When there is a deficiency in the MMR enzymes, cells may be resistant to cisplatin and carboplatin because both of these agents produce the same DNA adduct. The bulkier oxaliplatin-DNA adduct does not seem to be recognized by the same enzymes and does not depend on MMR enzymes for its cytotoxicity, and several cell lines that are resistant to cisplatin and carboplatin are still susceptible to oxaliplatin. These two compounds both form primarily intrastrand links between adjacent guanine residues or adjacent guanine–adenine residues.

NITROSOUREAS

It was found that activity of nitrosoureas could be enhanced by attachment of a 2-haloethyl substituent to both nitrogens (Fig. 10.6).

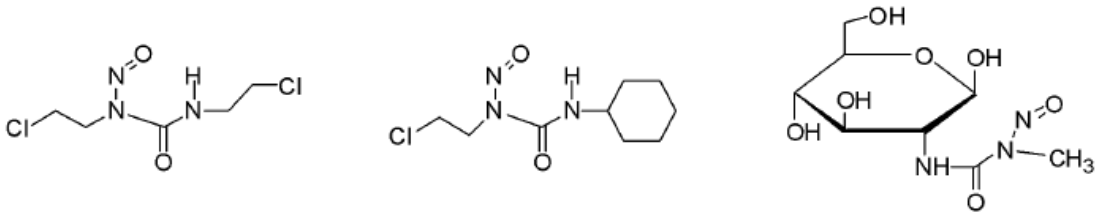
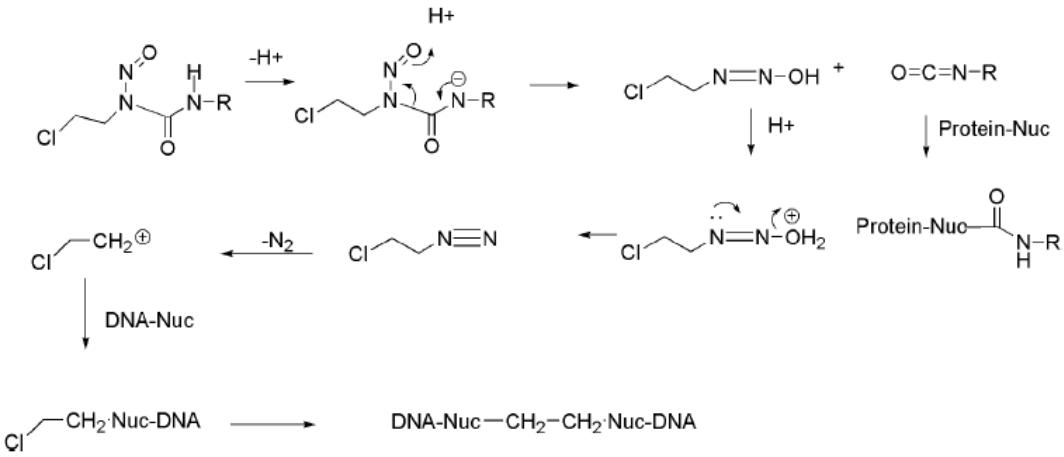


Figure 10.6 • Nitrosoureas antineoplastics.

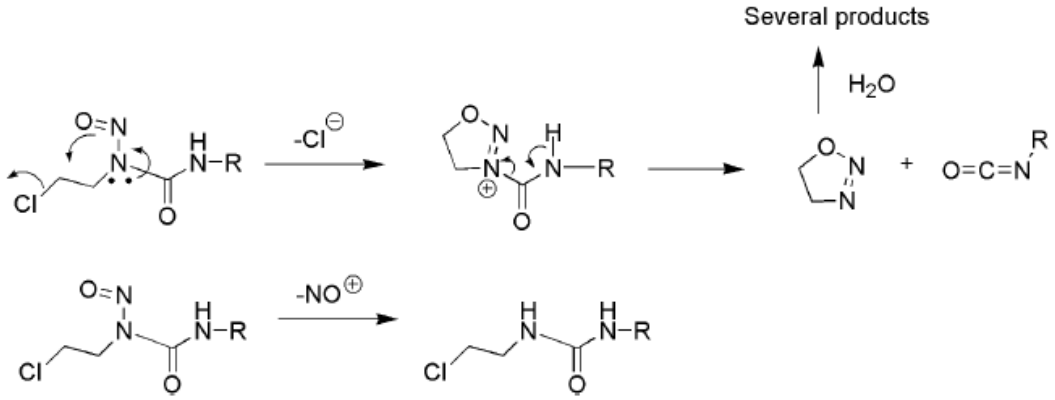
These compounds are reasonably stable at pH _ 4.5 but undergo both acid and base catalyzed decomposition at lower and higher pH, respectively. There are several pathways of decomposition that are possible for these compounds, but the one that appears to be most important for alkylation of DNA involves abstraction of the NH proton, which is relatively acidic (pKa _ 8–9), followed by rearrangement to give an isocyanate and a diazohydroxide. The diazohydroxide, upon protonation followed by loss of water, yields a diazo species that decomposes to a reactive carbocation (Scheme 10.11). The isocyanate functions to carbamylate proteins and RNA, whereas the carbocation is believed to be the agent responsible for DNA alkylation.

Alternative mechanisms of decomposition have also been proposed involving formation of chlorovinyl carbocations. In those cases where there is a chloroethyl moiety attached to the *N*-nitroso urea functionality, crosslinking of DNA occurs. Alkylation occurs preferentially at the N-7 position of guanine with minor amounts of alkylation at guanine O-6.



Scheme 10.11 • Mechanism of DNA alkylation by nitrosoureas.

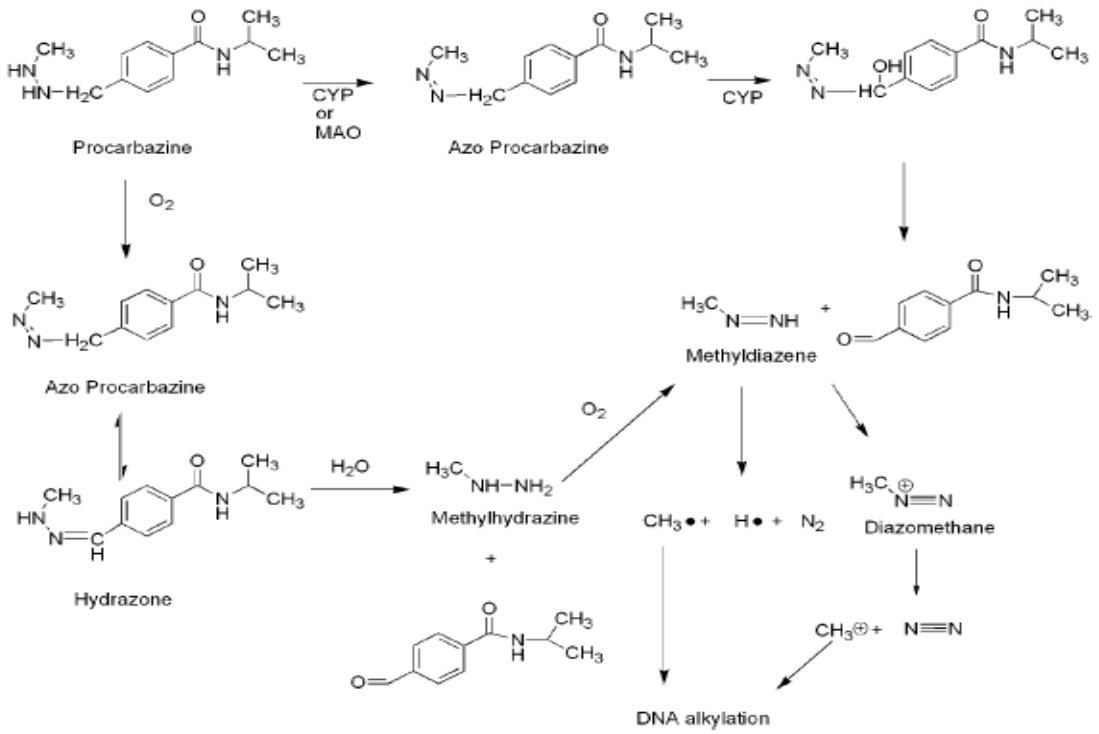
Two major routes of inactivation have been identified and are indicated in Scheme 10.12. The first of these involves dechlorination, which is facilitated by CYP participation and involves cyclization to give 4,5-dihydro-[1,2,3]oxadiazole and the isocyanate, which is still capable of carbamylating proteins. The oxadiazole can be further degraded by hydrolysis to give several inactive products. The second route involves denitrosation, which in the case of BCNU (carmustine) has been shown to be catalyzed by CYP monooxygenases and glutathione-*S*-reductase.



Scheme 10.12 • Metabolic and chemical inactivation of nitrosoureas.

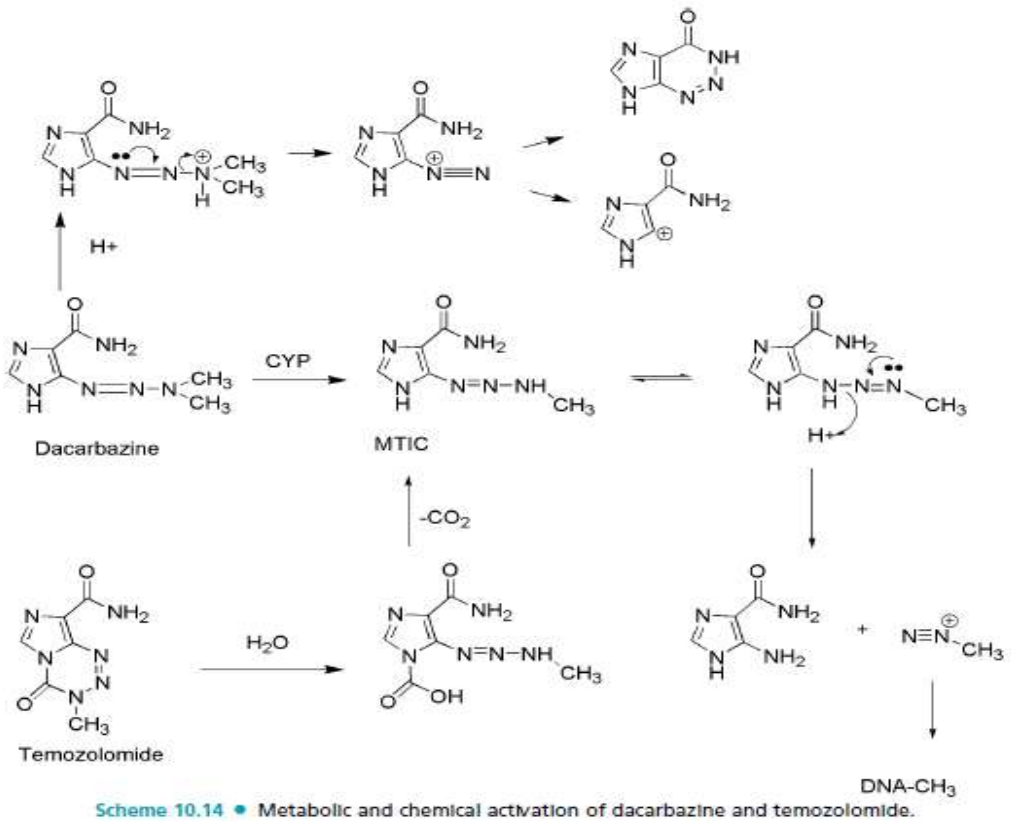
PROCARBAZINE, DACARBAZINE, AND TEMOZOLOMIDE

Procarbazine is an antineoplastic agent that was originally developed as a result of efforts to find new inhibitors of monoamine oxidase. Subsequent screening revealed antineoplastic activity. Metabolism studies revealed that oxidation of procarbazine does occur in the liver and is mediated by CYP and monoamine oxidase to give azo-procarbazine. This compound may also be generated nonenzymatically in an aerobic environment (Scheme 10.13). There are several chemical and metabolic pathways that azo-procarbazine may then undergo, and there is some disagreement regarding the exact structure of the active alkylating species. One such route involves CYP-mediated oxidation of the benzylic methylene carbon with subsequent decomposition to give methyldiazine and the aldehyde. The methyldiazine may then decompose by homolytic bond cleavage to give methyl and hydrogen radicals along with nitrogen gas or be further oxidized to give the diazo compound, which can decompose to give the methyl carbocation. Methyldiazine may also be produced by a minor route involving isomerization of azo-procarbazine to give the hydrazone, which subsequently undergoes hydrolysis to give the aldehyde and methylhydrazine. Methylhydrazine may react with oxygen to give methyldiazine, which then decomposes as before. Studies utilizing radiolabeled procarbazine indicated that the terminal methyl group was found covalently bound to the N-7 position of guanine especially on tRNA disrupting its function and preventing protein, RNA, and DNA synthesis. There was also a small amount of methylation at the O-6 position of guanine. Hydrazines are also capable of inhibiting monoamine oxidase as seen with isocarboxazid and phenelzine; however, procarbazine is only a weak inhibitor of this enzyme. The agent is also capable of inhibiting aldehyde dehydrogenase and producing a disulfiram-like reaction.



Scheme 10.13 • Metabolic and chemical activation of procarbazine.

Somewhat related is dacarbazine, which was initially thought to act as an inhibitor of purine biosynthesis, but latter was shown to be an alkylating agent. Activation of the agent occurs through the action of CYP (isozymes 1A1, 1A2, and 2E1) to give the demethylated product monomethyl triazeno imidazole carboxamide (MTIC) (Scheme 10.14). Tautomerization allows for decomposition to give the aminocarboxamido-imidazole and diazomethane, which is capable of alkylating DNA. An alternative pathway involves acid catalyzed or photoinduced loss of dimethylamine to give an alternative diazo compound (diazo-IC), which may not only generate a carbocation but also undergoes internal cyclization to give 2-azo-hypoxanthine.46 Formation of diazo-IC has been associated with pain at the injection site, which is often seen during dacarbazine administration.47 Methylation of DNA occurs at N-7, N-3 and O-6 of guanine among other sites. Dacarbazine proved to be more active against murine tumors than against human tumors. This was attributed to the enhanced ability of mice to metabolize the agent to MTIC and the subsequent conversion to a methylating species. Building on this idea was temozolomide, which undergoes conversion to the same intermediate, MTIC, as dacarbazine, but it does not require metabolic activation to do so. Hydrolysis of temozolomide gives the carboxy-triazene, which spontaneously loses CO_2 to give MTIC. Dacarbazine must be administered intravenously; however, the related temozolomide may be administered orally.



Scheme 10.14 • Metabolic and chemical activation of dacarbazine and temozolomide.

ANTIMETABOLITES

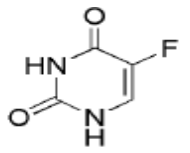
Antimetabolites are compounds closely related in structure to a cellular precursor molecule, Most antimetabolites are effective cancer chemotherapeutic agents via interaction with the biosynthesis of nucleic acids. Therefore, several of the useful drugs used in antimetabolite therapy are purines, pyrimidines, folates, and related compounds.

The antimetabolite drugs may exert their effects by several individual mechanisms involving enzyme inhibition at active, allosteric, or related sites. Most of these targeted enzymes and processes are involved in the regulatory steps of cell division and cell/tissue growth. An antimetabolite and its transformation products may inhibit several different enzymes involved in tissue growth. These substances are generally cell cycle specific with activity in the S phase.

The purine and pyrimidine antimetabolites are often compounds incorporated into nucleic acids and the nucleic acid polymers (DNA, RNA, etc.). The antifolates are compounds designed to interact at cofactor sites for enzymes involved in the biosynthesis of nucleic acid bases. Classic examples of pyrimidine and purine antimetabolites are 5-fluorouracil and 6-mercaptopurine, respectively, and the classic antifolate is methotrexate.

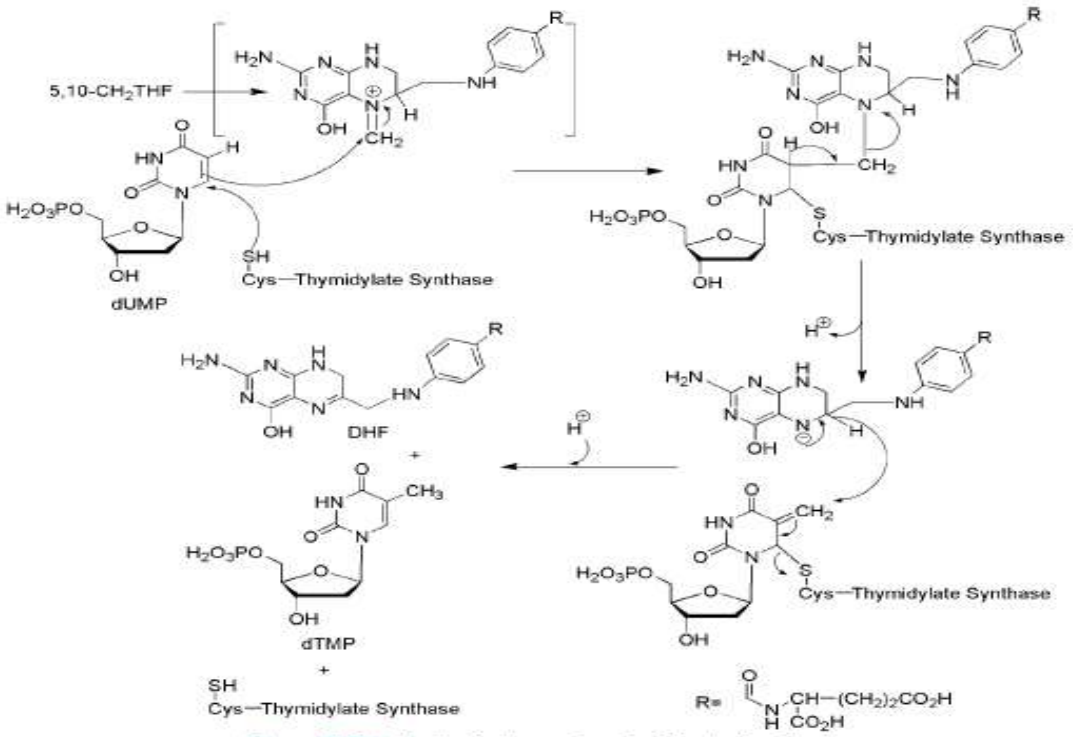
Pyrimidine Drugs

The anticancer drugs based on pyrimidine structure . The pyrimidine derivative 5-fluorouracil (5-FU) was designed to block the conversion of uridine to thymidine. The normal biosynthesis of thymidine involves methylation of the 5-position of the pyrimidine ring of uridine.



5-Fluorouracil

The replacement of the hydrogen at the 5-position of uracil with a fluorine results in an antimetabolite drug, leading to the formation of a stable covalent ternary complex composed of 5-FU, thymidylate synthase (TS), and cofactor (a tetrahydrofolate species). The normal pathway for the formation of thymidine from uridine catalyzed by the enzyme TS is shown in Scheme 10.17. Anticancer drugs targeting this enzyme should selectively inhibit the formation of DNA because thymidine is not a normal component of RNA.



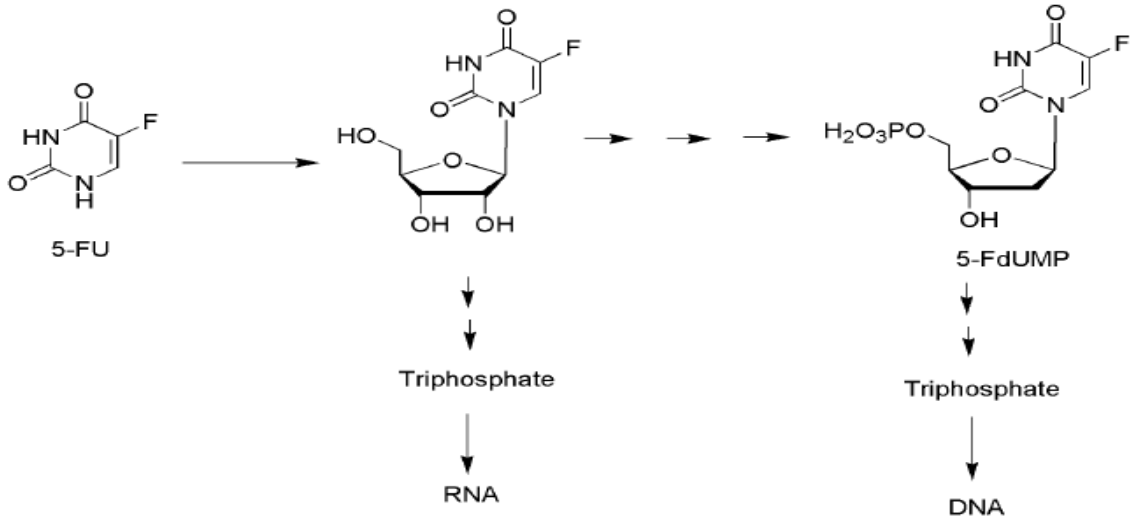
Scheme 10.17 • Biochemical conversion of uridine to thymidine.

TS is responsible for the reductive methylation of deoxyuridine monophosphate (dUMP) by 5,10-methylenetetrahydrofolate to yield dTMP and dihydrofolate.Because thymine is unique to DNA, the TS enzyme system plays an important role in replication and cell division. The tetrahydrofolate cofactor species serves as both the one-carbon donor and the hydride source in this system. The initial step of the process involves the nucleophilic attack by a sulfhydryl group of a cystine residue at the 6-position of dUMP. The resulting

enolate adds to the methylene of 5,10- CH2-THF perhaps activated via the very reactive *N*-5- iminium ion (see Scheme 10.17). The iminium ion likely forms at N-5 and only after 5,10-CH2-THF binds to TS .

The iminium ion is likely formed at N-5 because it is the more basic of the two nitrogens, whereas N-10 is the better leaving group. The loss of the proton at the 5-position of dUMP and elimination of folate yields the exocyclic methylene uracil species. The final step involves hydride transfer from THF and elimination to yield the enzyme, DHF, and dTMP.

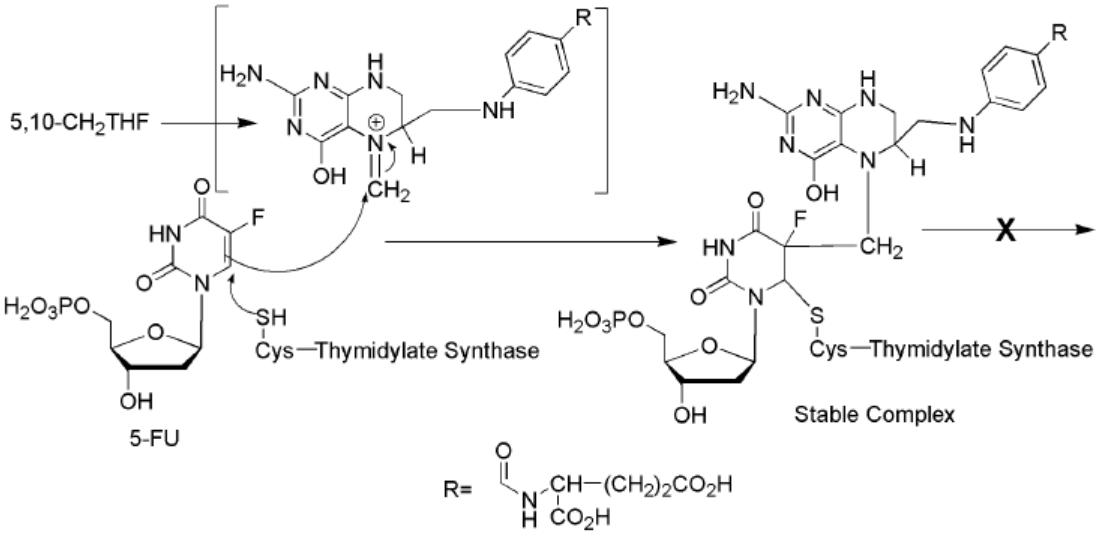
5-Fluorouracil is activated by conversion to the corresponding nucleotide species, 5-fluoro-2-deoxyuridylic acid (see Scheme 10.18). The resulting 5-fluoro-2_-deoxyuridylic acid is a powerful inhibitor of thymidylate synthetase, the enzyme that converts 2_-deoxyuridylic acid to thymidylic acid.



Scheme 10.18 ● Metabolic activation of 5-fluorouracil to 5-FdUMP.

In the inhibiting reaction, the sulfhydryl group of TS adds via conjugate addition to the 6-position of the fluorouracil moiety (Scheme 10.19). The carbon at the 5-position then binds to the methylene group of 5,10- methylenetetrahydrofolate following initial formation of the more electrophilic form of folate the N-5-iminium ion. In the normal process, this step is followed by the elimination

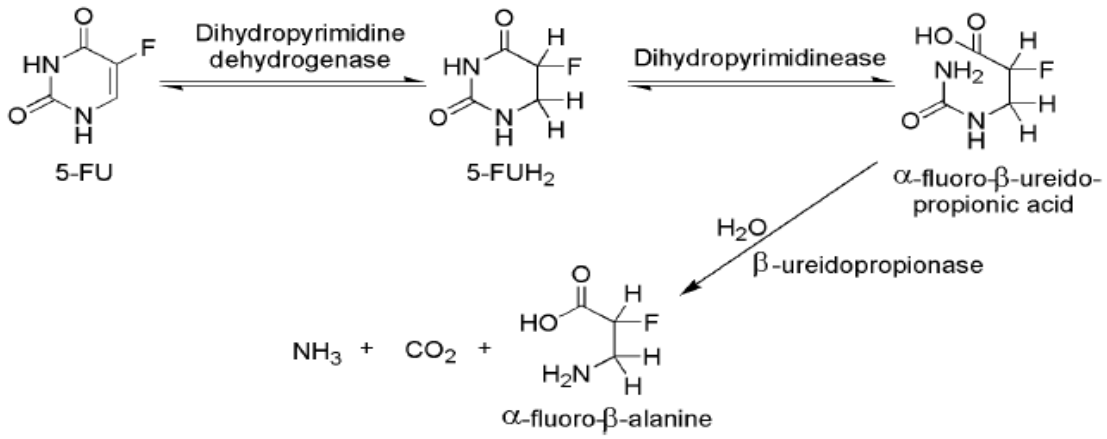
of dihydrofolate from the ternary complex, regeneration of the active enzyme species, and the product thymidine. Central to this process is the loss of the proton at the 5-position of uracil to form the exocyclic methylene uracil species. The 5-fluorine is stable to elimination, and a terminal product results, involving the enzyme, cofactor, and substrate, all covalently bonded (Scheme 10.19).



Scheme 10.19 • Mechanism of inhibition of TS by 5-fluorouracil.

TS is the most obvious and well-documented mechanism of action for 5-FU cytotoxic activity. However, other mechanisms may play a role in the overall value of this drug in the treatment of human cancer. The triphosphate of 5-FU nucleotide is a substrate for RNA polymerases, and 5-FU is incorporated into the RNA of some cell lines. The incorporation of 5-FU into DNA via DNA polymerase occurs in some tissue lines even though uracil is not a common component of human DNA. The 5-FU in DNA likely serves as substrate for the editing and repair enzymes involved in DNA processing for cell division and tissue growth.

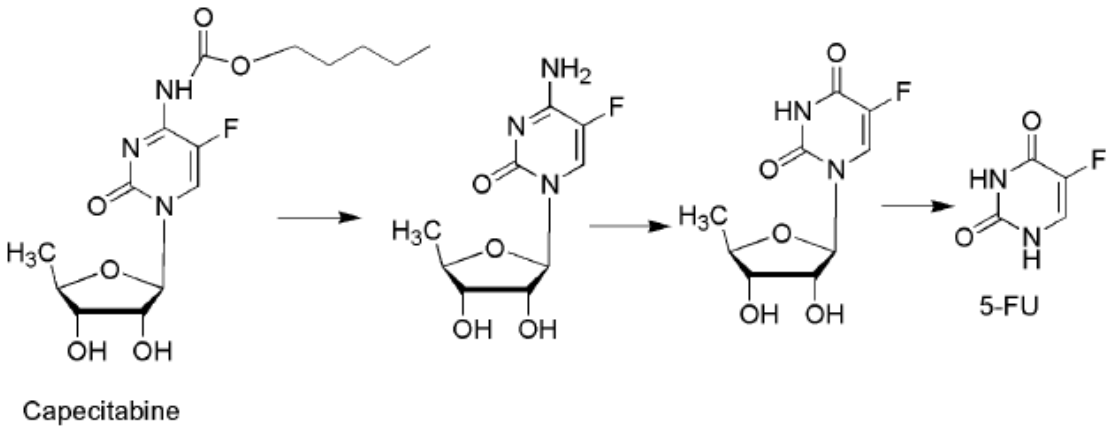
The metabolic activation (anabolism) of 5-FU required to produce the anticancer effects accounts for no more than 20% of the administered amount of drug in most patients. Catabolic inactivation via the normal pathways for uracil consumes the remaining approximate 80% of the dose. The major enzyme of pyrimidine catabolism is dihydropyrimidine dehydrogenase (DPD), and 5-FU is a substrate for this enzyme. The DPD catabolism of 5-FU is shown in Scheme 10.20.



Scheme 10.20 • Catabolic inactivation of 5-FU by dihydropyrimidine dehydrogenase.

The formation of dihydro-5-FU (5-FU-H2) occurs very rapidly and accounts for the majority of the total 5-FU dose in most patients. Thus, *alpha* -fluoro – *beta*- alanine is the major human metabolite of 5-FU. Uracil is a substrate for this enzyme system also and has been dosed with 5-FU and 5-FU prodrugs in an attempt to saturate DPD and conserve active drug species. Variability in the levels of DPD activity among the patient population is a major factor in the bioavailability of 5- FU. Inhibitors of DPD such as uracil or 5-chloro-2,4- dihydroxypyridine (CDHP)53 increase the plasma concentration–time curve of 5-FU by preventing 5-FU catabolism. One mechanism of drug resistance in 5-FU–treated patients may be caused by increased levels of DPD in the target tissue.

The carbamate derivative of 5————————————-deoxy-5-fluorocytidine is known as capecitabine, and it is converted to 5-FU through a series of activation steps. The activation sequence is shown in Scheme 10.21. The initial step is carbamate hydrolysis followed by deamination, then hydrolysis of the sugar moiety to yield 5-FU. Some of these activation steps take place at a higher rate in tumor tissue leading to selective accumulation in those cells.53 The last step in the sequence shown in Scheme 10.21 is catalyzed by phosphorolases, and these enzymes occur in higher levels in colorectal tumors.



Scheme 10.21 • Metabolic activation of capecitabine to 5-FU.

Pyrimidine analogs as antimetabolites for cancer therapy have been developed based on the cytosine structure as well. Modification of the normal ribose or deoxyribose moiety has produced useful drug species such as cytarabine (ara-C) and gemcitabine, see Figure 10.8. Cytosine arabinoside (ara-C or cytarabine) is simply the arabinose sugar instead of ribose, and the only difference in structure is the epimeric hydroxyl group at the 2————————————-position of the pentose sugar. This epimeric sugar is similar enough to the natural ribose to allow ara-C to be incorporated into DNA, and its mechanism of action may include a slowing of the DNA chain elongation reaction via DNA polymerase or cellular inefficiencie in DNA processing or repair after incorporation.

Gemcitabine59 is the result of fluorination of the 2————————————-position of the sugar moiety. Gemcitabine is the 2————————————,2————————————-difluoro deoxycytidine

species and after its anabolism to diphosphate and triphosphate metabolites, it inhibits ribonucleotide reductase and competes with 2-deoxycytidine triphosphate for incorporation into DNA. The mechanism of action for gemcitabine is likely similar to that of ara-C including alteration of the rate of incorporation into DNA as well as the rate of DNA processing and repair.

Modification of the pyrimidine ring has also been explored for the development of potential anticancer drugs based on antimetabolite theory. Several pyrimidine nucleoside analogs have one more or one less nitrogen in the heterocyclic ring. They are known as azapyrimidine or deazapyrimidine nucleosides. 5-Azacytidine is an example of a drug in this category (see Fig. 10.8). The mode of action of this compound is complex involving reversible inhibition of DNA methyltransferase, and this lack of methylated DNA activates tumor suppressor genes.

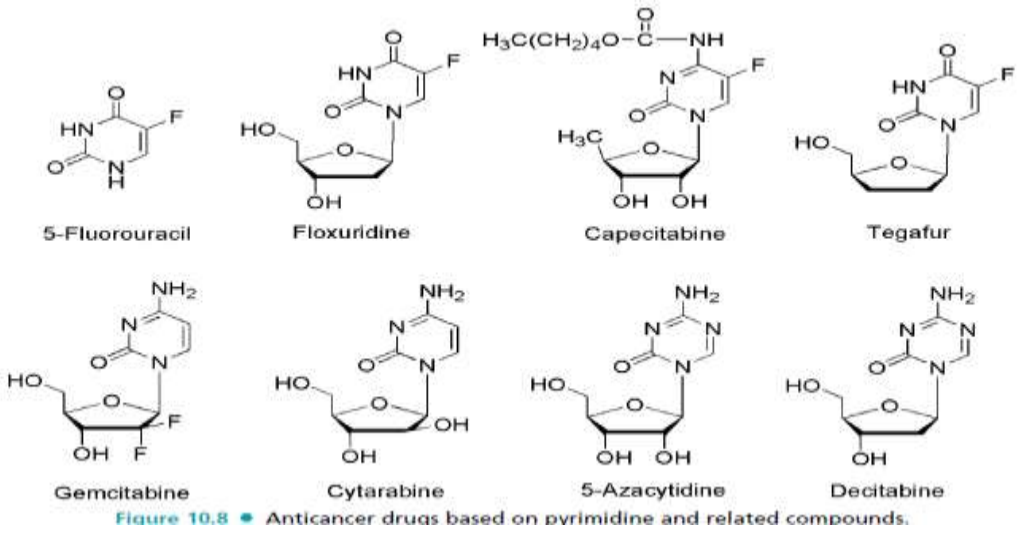
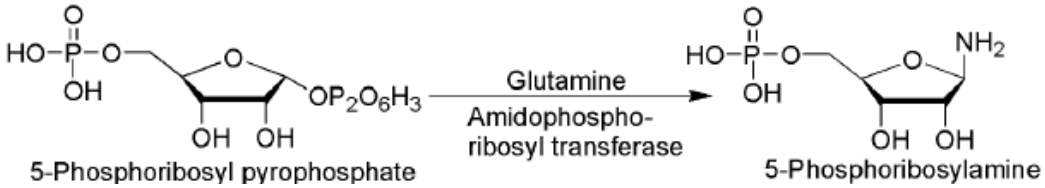
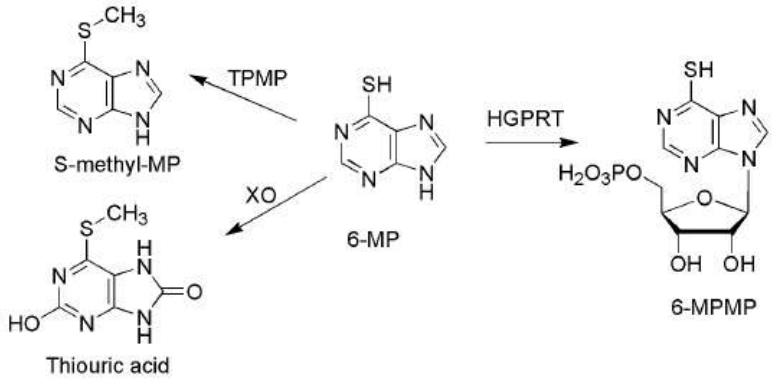


Figure 10.8 • Anticancer drugs based on pyrimidine and related compounds.

Purine Drugs

The anticancer drugs based on purine structure are shown in Figure 10.9. The design of antimetabolites based on purine structure began with isosteric thiol/sulfhydryl group to replace the 6-hydroxyl group of hypoxanthine and guanine. One of the early successes was 6-mercaptopurine (6-MP), the thiol analog of hypoxanthine.This purine requires bioactivation to its ribonucleotide, 6-thioinosinate (6-MPMP), by the enzyme HGPRT. The resulting nucleotide (Scheme 10.22) is a potent inhibitor of an early step in basic purine biosynthesis, the conversion of 5-phosphoribosylpyrophosphate into 5-phosphoribosylamine (see Scheme 10.16). The ribose diphosphate and triphosphates of 6-mercaptopurine are active enzyme inhibitors, and the triphosphate can be incorporated into DNA and RNA to inhibit chain elongation.61 However, the major antineoplastic action of 6-MP appears to be related to the inhibition of purine biosynthesis.





Scheme 10.22 • Conversion of 6-MP to active 6-thioinosine-5-monophosphate (6-MPMP) by HPGRT and inactivation by xanthine oxidase and thiopurine methyl transferase.

Thioguanine (6-TG) is the 6-mercapto analog of guanine, analogous to 6-MP. Thioguanine is converted into its ribonucleotide by the same enzyme that acts on 6-mercaptopurine. It is converted further into the diphosphates and triphosphates. These species inhibit most of the same enzymes that are inhibited by 6-mercaptopurine. Thioguanine is also incorporated into RNA, and its 2^{-}-deoxy metabolite is incorporated into DNA. The incorporation into RNA and DNA and the subsequent disruption of these polymers may account for a greater portion of the antineoplastic activity of thioguanine compared with 6-MP.

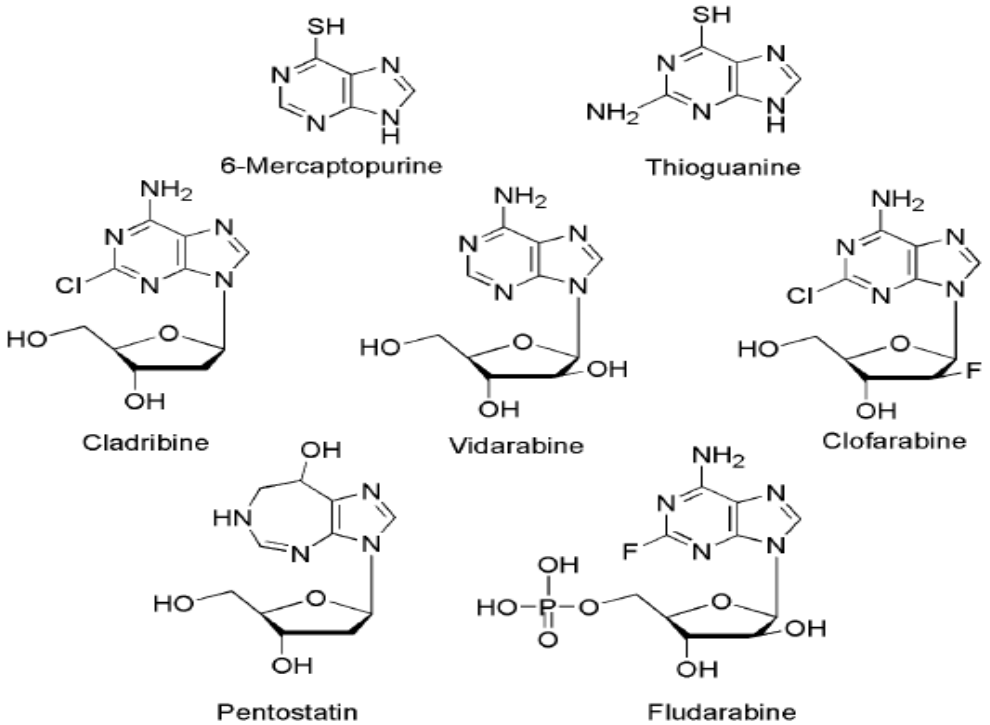


Figure 10.9 • Anticancer drugs based on purines and related compounds.

Drug resistance in certain cell lines may be caused by lower activity of activating enzymes or higher activity of catabolic enzymes. For the classic purine antimetabolites 6-MP major pathways of inactivation (see Scheme 10.22) include *S*-methylation via thiopurine-Smethyl- transferase (TPMT) and oxidation by the enzyme xanthine oxidase (XO). Xanthine oxidase converts the drugs to the inactive thiouric acid, and inhibition of the enzymes responsible for the catabolic breakdown of the purine drugs can potentiate the drug's antineoplastic activity.

Allopurinol is a potent inhibitor of xanthine oxidase and is often used as an adjuvant in purine anticancer drug therapy. Allopurinol increases both the potency and the toxicity of 6- mercaptopurine. Its main importance is that it prevents the uric acid kidney toxicity caused by the release of purines from destroyed cancer cells.

Adenine arabinoside (Vidarabine) contains the sugar, D-arabinose, which is epimeric with D-ribose at the 2^{-}-position. This structural change makes it a competitive inhibitor of DNA polymerase, and this activity accounts for its antineoplastic activity as well as its antiviral action. Adenine arabinoside and some of its derivatives are limited in their antitumor effect by susceptibility to adenosine deaminase. This enzyme converts them into the inactive hypoxanthine arabinoside derivatives. High levels of adenosine deaminase accounts for resistance of certain tumors to the action of adenine arabinoside. The addition of fluorine to the sugar moiety has produced some purine-based drugs with resistance to the catabolic activity of adenosine deaminase. In contrast to the susceptibility of adenosine arabinoside to adenosine deaminase, its 2-fluoro derivative, fludarabine, is stable to this enzyme. The antineoplastic activity of fludarabine depends on its anabolic conversion to the corresponding triphosphate. 2-Chloro-2^{-}-deoxyadenosine (cladribine) also is resistant to adenosine deaminase. It is phosphorylated in cells to the triphosphate by cytidine kinase, and the triphosphate inhibits enzymes required for DNA repair.

Folates

Folic acid and the structures of the major antifolate anticancer drugs are shown in Figure 10.10. Methotrexate is the classic antimetabolite of folic acid structurally derived by *N*methylation of the para-aminobenzoic acid residue (PABA) and replacement of a pteridine hydroxyl by the bioisosteric amino group. The conversion of -OH to $-NH_2$ increases the basicity of N-3 and yields greater enzyme affinity. This drug competitively inhibits the binding of the substrate folic acid to the enzyme DHFR, resulting in reductions in the synthesis of nucleic acid bases, perhaps most importantly, the conversion of uridylate to thymidylate as catalyzed by thymidylate synthetase. In addition, purine synthesis is inhibited because the *N*-10-formyl tetrahydrofolic acid is a formyl donor involved in purine synthesis. The interconversion of the various folate species is shown in Scheme 10.23. Recall that in Scheme 10.16, THFs are cofactors in at least two key steps in the normal biosynthesis of purines.

Methotrexate66 is a broad-spectrum antineoplastic agent commonly used in the treatment of acute lymphoblastic and myeloblastic leukemia and other lymphomas and sarcomas. The major side effects seen are bone marrow suppression, pulmonary fibrosis, and GI

ulceration. Leucovorin is often given 6 to 24 hours after methotrexate to prevent the longterm effects on normal cells by preventing the inhibition of DNA synthesis. Related to this is Pemetrexed, but its scope is greater in that it not only inhibits DHFR but also TS and glycinamide ribonucleotide formyltransferase (GARFT), which is involved in purine biosynthesis (see Fig. 10.16).

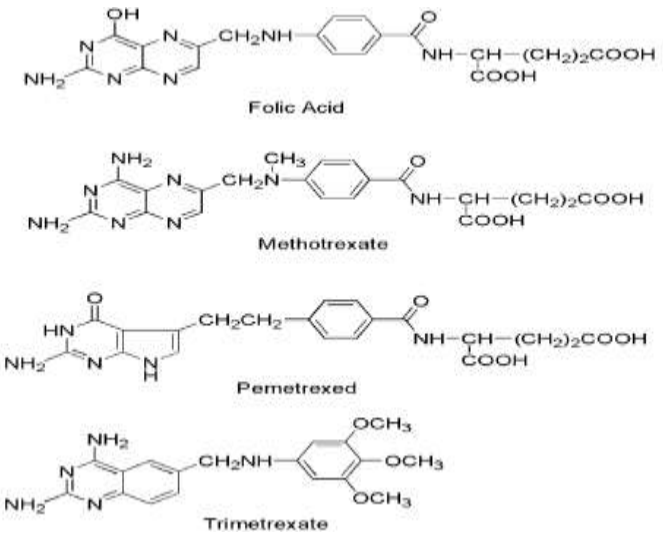
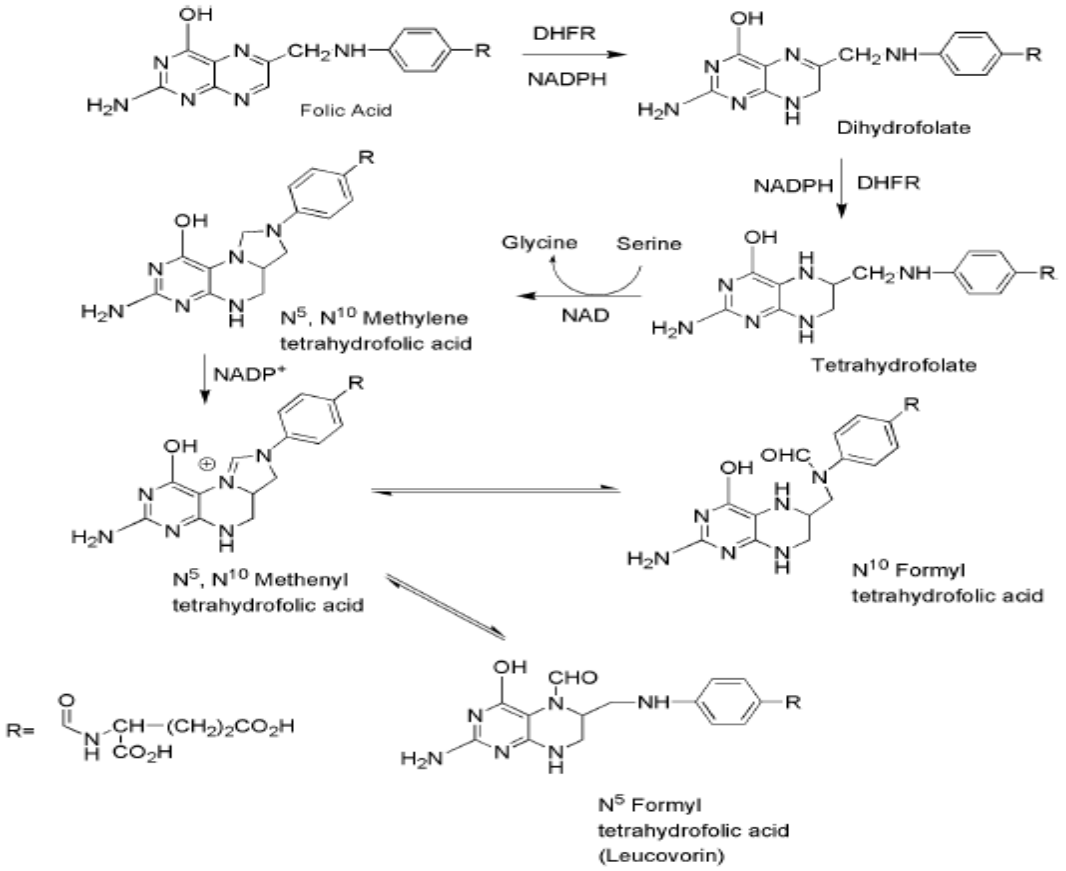
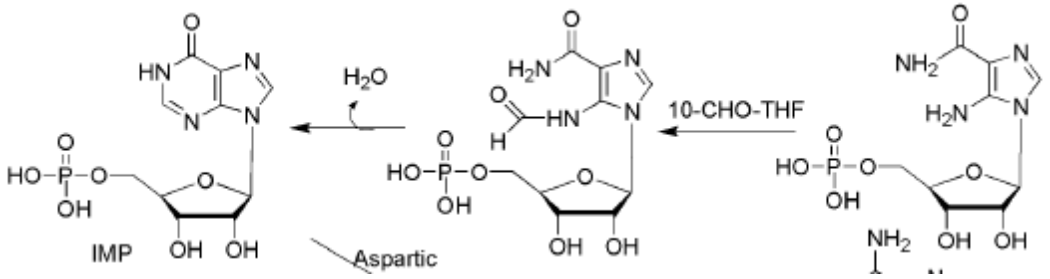


Figure 10.10 • Structures of folic acid and antifolate anticancer drugs.



Scheme 10.23 • Interconversion of various folate species. (DHFR, dihydrofolate reductase.)



ANTIBIOTICS AND NATURAL PRODUCTS

A variety of the anticancer agents available today are derived from natural sources with several of these being obtained from microbial sources (antibiotics). Many of the antineoplastic antibiotics are produced by the soil fungus *Streptomyces*. Both the antibiotic and natural product classes have multiple inhibitory effects on cell growth; however, they primarily act to disrupt DNA function and cell division. There are several mechanisms by which these agents target DNA, including intercalation, alkylation, and strand breakage either directly or as a result of enzyme inhibition. Intercalation is a process by which a planar molecule of the appropriate size inserts itself between adjacent base pairs of DNA and in so doing, it causes a local unwinding that may disrupt the normal template function of DNA. Intercalation requires that the drug induce a cavity between base pairs so that insertion may occur. The interaction of the intercalator and the adjacent base pairs occurs by the overlap of p-orbitals of the intercalator and the base pairs. The p-orbitals of the intercalation species are provided by a combination of aromatic and conjugated systems that impart the planarity required for intercalation. The drug–DNA interaction is further stabilized by side chains attached to the intercalation species. The side chains often include a cationic moiety, which may form ionic bonds with the anionic phosphate backbone. Alternative modes of stabilization may occur through a combination of van der Waals interaction or hydrogen bonds. The overall result of these interactions is to cause a local bend or kink in DNA resulting in a local shap distortion. This may produce several effects but is often associated with inhibition of normal DNA function.

Intercalators may also result in inhibition of topoisomerase enzymes Topoisomerase enzymes are responsible for the unwinding and relaxation of DNA so that transcription may occur.67 There are two major types of topoisomerase enzymes, which are important sites of action for antineoplastics. Topoisomerase I makes a single-strand break in DNA and subsequently allows the other strand to spin, relieving any tension associated with the packing process and subsequently reseals the broken strand. Topoisomerase II makes double-strand breaks in DNA allowing an intact chain to pass through and then subsequently reseals the double-strand break. There are several natural products that are capable of disrupting the formation and function of the mitotic spindle. These include the epipodophyllotoxins, the taxanes, and the vinca alkaloids. The mitotic spindle forms during the M phase of the cell cycle and is responsible for moving the replicated DNA to opposite ends of the cell in preparation for cell division.

Actinomycins

The actinomycins are a group of compounds that are isolated from various species of *Streptomyces*, all of which contain the same phenoxazone chromophore but differ in the attached peptide portion. Originally, these materials were investigated for use as antibiotics, but they proved to be too toxic. From this group emerged actinomycin D, which is known as *dactinomycin* and contains identical pentapeptides bound through an amide linkage utilizing the amino group of L-threonine with carbonyls at positions 1 and 9.The pentapeptides namely L-threonine, D-valine, L-proline, sarcosine, and L-methylvaline form a lactone via the side chain hydroxyl of L-threonine and the carboxyl group of L-methylvaline (Fig. 10.11).

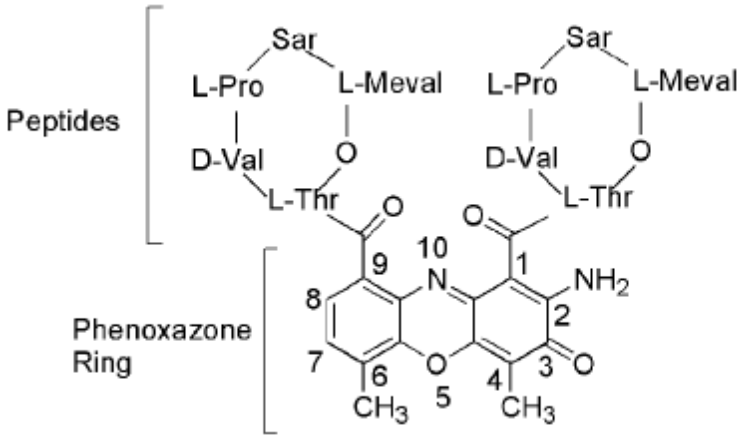


Figure 10.11 • Structure of dactinomycin.

Dactinomycin binds noncovalently to double-stranded DNA by partial intercalation between adjacent guaninecytosine bases resulting in inhibition of DNA function. The structural feature of dactinomycin important for its mechanism of cytotoxicity is the planar phenoxazone ring, which facilitates intercalation between DNA base pairs. The peptide loops are located within the minor groove and provide for additional interactions . The preference for GpC base pairs is thought to be partly related to the formation of a hydrogen bond between the 2-amino groups of guanine and the carbonyls of the L-threonine residues. Additional hydrophobic interactions and hydrogen bonds are proposed to form between the peptide loops and the sugars and base pairs within the minor groove. The primary effect of this interaction is the inhibition of DNA-directed RNA synthesis and specifically RNA polymerase. DNA synthesis may also be inhibited, and the agent is considered cell cycle specific for the G1 and S phases. The drug has been found to bind to single-stranded DNA and double-stranded DNA without adjacent GpC sequences. Inhibition of topoismerase II also occurs such that the enzyme-DNA complex is stabilized and strand breakage may be seen. Resistance is caused by a decreased ability of tumor cells to take up the drug and P-glycoprotein (Pgp)-mediated efflux.

Anthracyclines

The anthracycline antibiotics (Fig. 10.13) are characterized by a planar oxidized anthracene nucleus fused to a cyclohexane ring that is subsequently connected via a glycosidic linkage to an amino sugar. Initially discovered in the early 1960s when they were isolated from *Streptomyces peucetius*, hundreds of compounds belonging to this class have subsequently been discovered of which five are used clinically in the United States (doxorubicin, daunorubicin, idarubicin, epirubicin, and valrubicin).

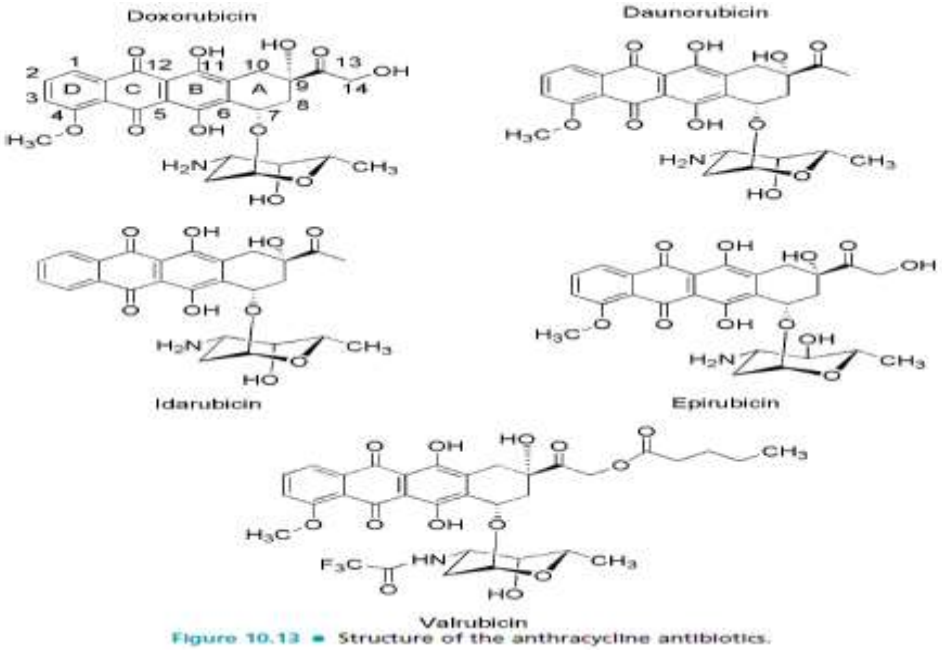
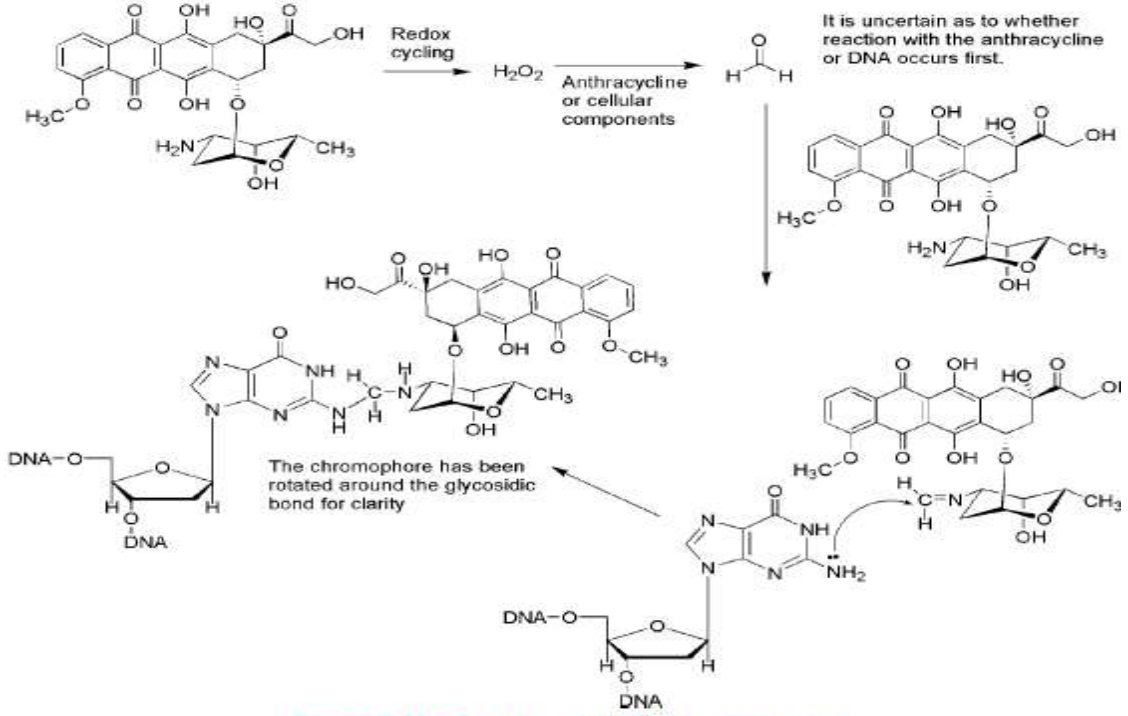


Figure 10.13 ■ Structure of the anthracycline antibiotics.

Studies of the mechanism by which the anthracyclines exhibit their cytotoxic effects initially focused on the ability of the compounds to associate with DNA resulting from intercalation of their planar ring system reinforced by auxiliary binding of the amino sugar. Subsequent work focused on the ability of these compounds to generate free radicals. Currently, the accepted mechanism involves intercalation followed by inhibition of topoisomerase II resulting in strand breakage leading to apoptosis. The anthracyclines are considered specific for the S phase of the cell cycle. There is data to support increased binding of p53 with DNA when anthracyclines are administered, which would stimulate the apoptotic process, The general features show rings B and C intercalating between CpG base pairs and ring D protruding into the major groove. The sugar moiety lies within the minor groove and along with ring A is important for interaction and inhibition of topoisomerase IIby stabilization of the cleavable complex. The amino sugarseems to play an especially important role and compounds lacking this functionality failed to inhibit the enzyme. In the case of doxorubicin and daunorubicin, specificity is provided by the hydrogen bonding between O-9 of the anthracycline and N-2 and N-3 of guanine.

The formation of covalent bonds between anthracyclines and DNA also is supported by several studies in which formaldehyde is produced by oxidation of cellular components or other anthracycline molecules. This oxidation results from the production of ROS such as H_2O_2, which are generated during redox cycling of anthracyclines (Scheme 10.24).

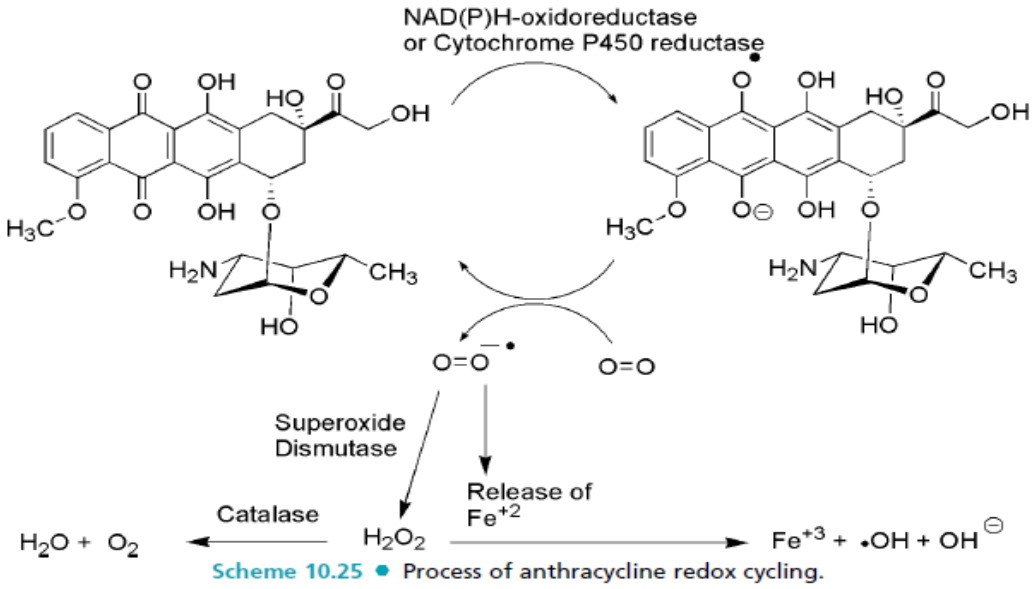
The generated formaldehyde may then form a methylene bridge between the 4-amino group of the anthracycline and the 2-amino group of guanine in DNA.



Scheme 10.24 • Formation of anthracycline-DNA adduct.

Although the anthracyclines produce several adverse effects that are typical for antineoplastics, cardiotoxicity is a special concern with this class of agents. The associated cardiomyopathies and congestive heart failure (CHF) have been related to the ability of these compounds to undergo redox cycling. The C ring of the anthracyclines is subject to a one or two electron reduction by several different enzymes including NAD(P)H-oxidoreductases and CYP reductases to give the semiquinone or hydroquinone. The case of the one electron reduction is shown in Scheme 10.25. This may undergo reversion to the starting quinone in futile redox cycling accompanied by the production of superoxide radical. The radical is normally converted to H_2O_2 by superoxide dismutase and H_2O_2 is then converted to H_2O and O_2 by catalase. However, the production of superoxide (O_2^-) is also associated with the release of iron from intracellular stores, which may be chelated by the anthracycline. The iron then diverts the normal detoxification pathway by catalase so that more potent radicals such as hydroxyl radical (^-OH) are produced. Myocardial cells possess lower levels of catalase and therefore are less able to detoxify the H_2O_2 that results from redox cycling.

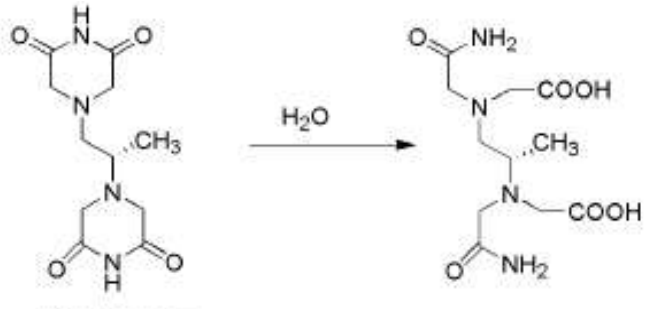
Materials such as $O2^-$, ^-OH, and H_2O_2 are known as *reactive oxygen species* or ROS and in sufficiently high concentration produce cellular damage. Hydroxyl radicals cause single-strand breaks in DNA, which would activate p53 and enhance apoptosis in cardiac cells. Studies have also shown that doxorubicin administration stimulates both the intrinsic and extrinsic pathways of apoptosis in numerous ways, and this has been linked to the increased production of H_2O_2 and hydroxyl radical.



Scheme 10.25 • Process of anthracycline redox cycling.

Additional mechanisms of cardiotoxicity have been proposed, which involve the metabolic reduction of the anthracycline C-13 ketone to the alcohol.79 Several pharmacological effects have been associated with the resulting alcohols including loss of Ca+2 homeostasis and inhibition of Na^+/K^+ -ATPase in cardiac cells.

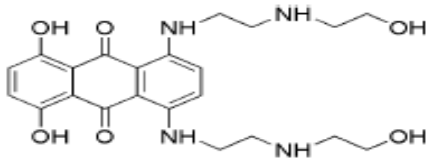
Several strategies have been developed to reduce the cardiotoxicity associated with the anthracyclines. Slow infusion over 48 to 96 hours versus administration of a bolus given over 15 minutes was implemented in the 1980s and has proven to be effective in reducing the toxicity without adversely affecting the antineoplastic effects. An alternative strategy is to chelate the iron required for the activation of H_2O_2, and for this, dexrazoxane (Totect) is used.81 Radiolabeling experiment have shown that dexrazoxane is rapidly taken up into myocardial cells, where it is subsequently hydrolyzed by a two-step process to yield the diamide acid (Scheme 10.26), which chelates free iron and iron bound to the anthracycline. Dexrazoxane is also an inhibitor of topoisomerase II that, unlike the anthracyclines, does not result in strand breaks.82 This additional property is important in its utility as antidote in cases of anthracycline.



Dexrazoxane

Scheme 10.26 • Aqueous hydrolysis of dexrazoxane to yield the iron-chelating metabolite.

Related to the anthracyclines is **mitoxantrone**, which is shown in Figure 10.14. Although mitoxantrone is a synthetic agent, it is included with the natural products because it is mechanistically similar to the anthracyclines. Produced in the late 1970s, it is a derivative of a synthetic dye and is classified as an anthracenedione. The mechanism involves intercalation by the chromophore, which is stabilized by the 2-[(2-aminoethyl)amino]ethanol side chain presumably as a result of an ionic interaction of the protonated amines with the phosphate backbone of DNA. The formation of covalent adducts with DNA have also been demonstrated to occur in a manner similar to the anthracyclines. Topoisomerase II is inhibited, and strand breakage occurs similar to that seen with the anthracyclines. In contrast to the anthracyclines, mitoxantrone is not a substrate for the reductase enzymes responsible for the conversion to the semiquinone so that ROS are not generated by this process. This lack of activation has been attributed to the presence of the side chains. This has the effect of reducing the cardiotoxicity but not completely eliminating it.

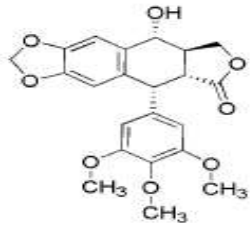


Mitoxantrone

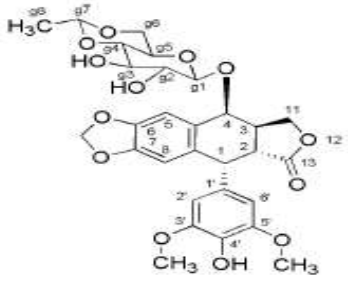
Figure 10.14 • Structure of mitoxantrone.

Epipodophyllotoxins

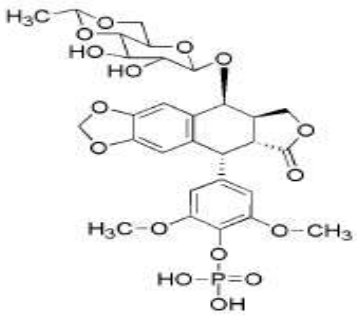
The epipodophyllotoxins (Fig. 10.15) are semisynthetic derivatives of podophyllotoxin, which is isolated from the mayapple (mandrake) root and functions as an inhibitor of microtubule function. Chemical modification has led to compounds with a different mechanism of action, which involves inhibition of topoisomerase enzymes.



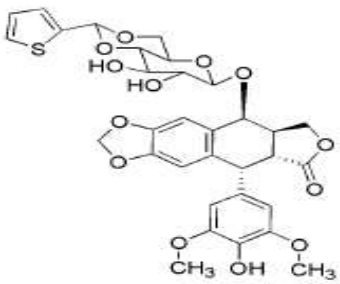
Podophyllotoxin



Etoposide



Etoposide Phosphate

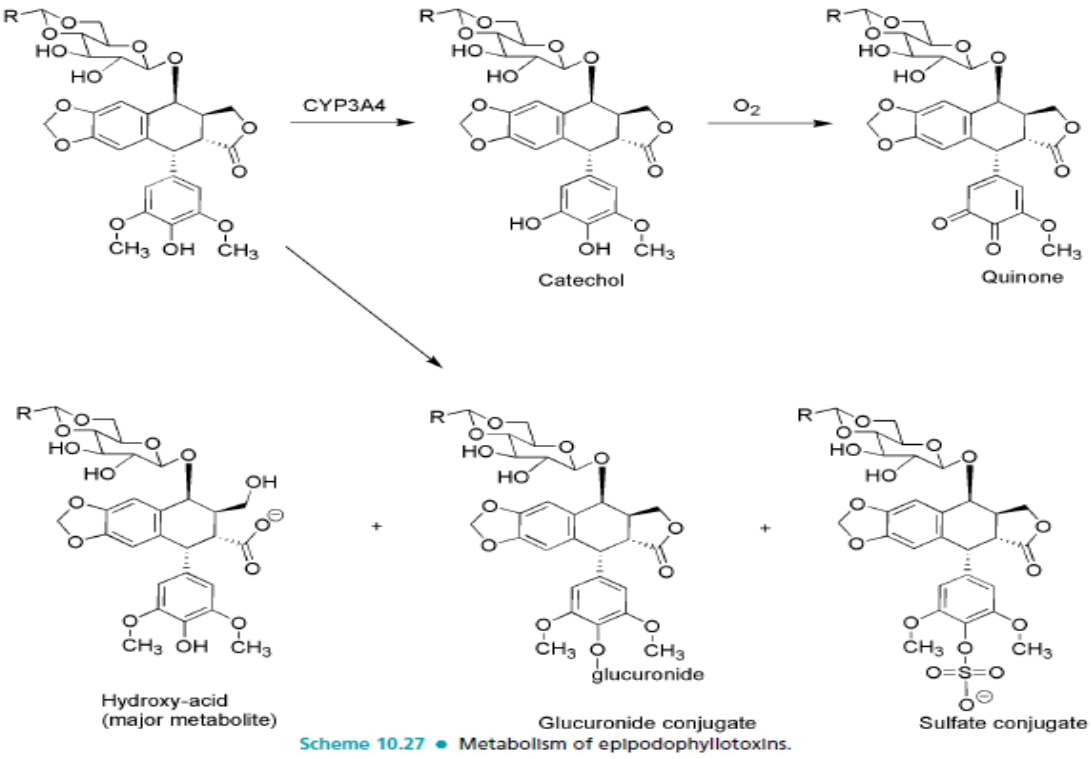


Teniposide

Figure 10.15 • Structures of the epipodophyllotoxins.

Etoposide acts on topoisomerase II stabilizing the cleavable complex leading to single- and double-strand breaks. If enough breaks are initiated, apoptosis is activated. The etoposide-topoisomerase II complex then binds DNA, and strand cleavage occurs, One etoposide molecule stabilizes the cleavable complex of one chain, and therefore two etoposide molecules are necessary to mediate double-strand breaks. The agents are considered cell cycle specific and act in the late S and G2 phases of the cell cycle.

The glycosidic moiety of the epipodophyllotoxins, which is lacking in podophyllotoxin, is associated with converting these compounds from tublin binders to topoisomerase inhibitors. Replacement of the glycosidic 8-methyl group with thiophene gives tenoposide, which is 10-fold more potent than etoposide. The glycosidic moiety is not an absolute requirement for activity, and other more active compounds are known in which it has been replaced. The 4'-OH group is important for the activity of the compounds, and loss of this functionality results in greatly reduced levels of strand breaks. Removal of one of the adjacent methoxy groups by CYP3A4 mediated oxidative-*O*-dealkylation gives the catechol analogs, which are more potent than the parent molecules. The catechol analogs may be further oxidized to give the quinones, which are also more active than the parent (Scheme 10.27).



Scheme 10.27 • Metabolism of epipodophyllotoxins.

There are several mechanisms by which cells become resistant to the epipodophyllotoxins including increased efflux by Pgp as seen for many of the other natural products. Additionally, topoisomerase II levels may decrease or develop altered binding sites with lower affinity for these agents. Increased DNA repair mechanisms may also decrease the effectiveness of these agents. There are mechanisms by which double-strand breaks in DNA can be repaired. If for example a strand break occurs in the late portion of the S phase or in the G2 phase after DNA has been replicated, the sister

chromatid can be utilized as a template to make the repair in a process known as *homologous recombination* without the loss of genetic material. However, if a sister chromatid is not available, then a process known as *nonhomologus end joining* may be utilized. In this process, after a double-strand break has occurred, exonucleases remove additional base pairs from each strand creating overhangs.

Camptothecins

The camptothecins (Fig. 10.16) are inhibitors of topoisomerase I and are used clinically for the treatment of various cancers.

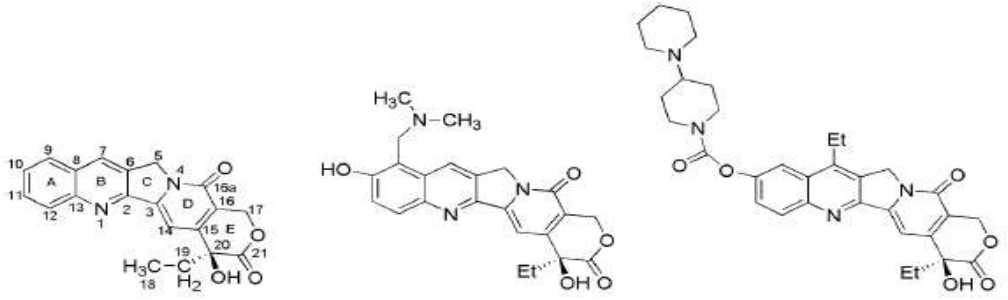
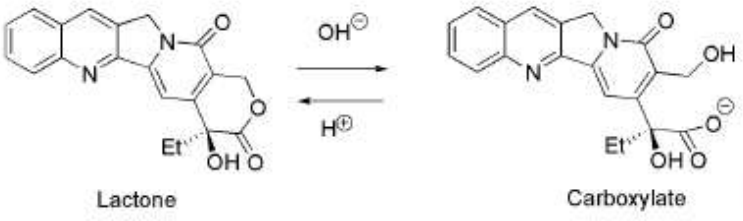


Figure 10.16 • Structures of topoisomerase I inhibitors.

The lead drug for this class of agents was camptothecin, which was discovered in the 1960s by Wani and Wall who isolated the material from *Camptotheca acuminata*, an ornamental tree found in China. Initial testing of the isolated material revealed promising antitumor activity, but testing in phase II trials gave disappointing results. The reason for this outcome was that camptothecin had low water solubility, and to overcome this, the sodium salt had been prepared and used during the trials. This was accomplished by hydrolysis of the E-ring lactone to give the carboxylate salt (Scheme 10.28).



Scheme 10.28 • Chemical hydrolysis of camptothecin lactone.

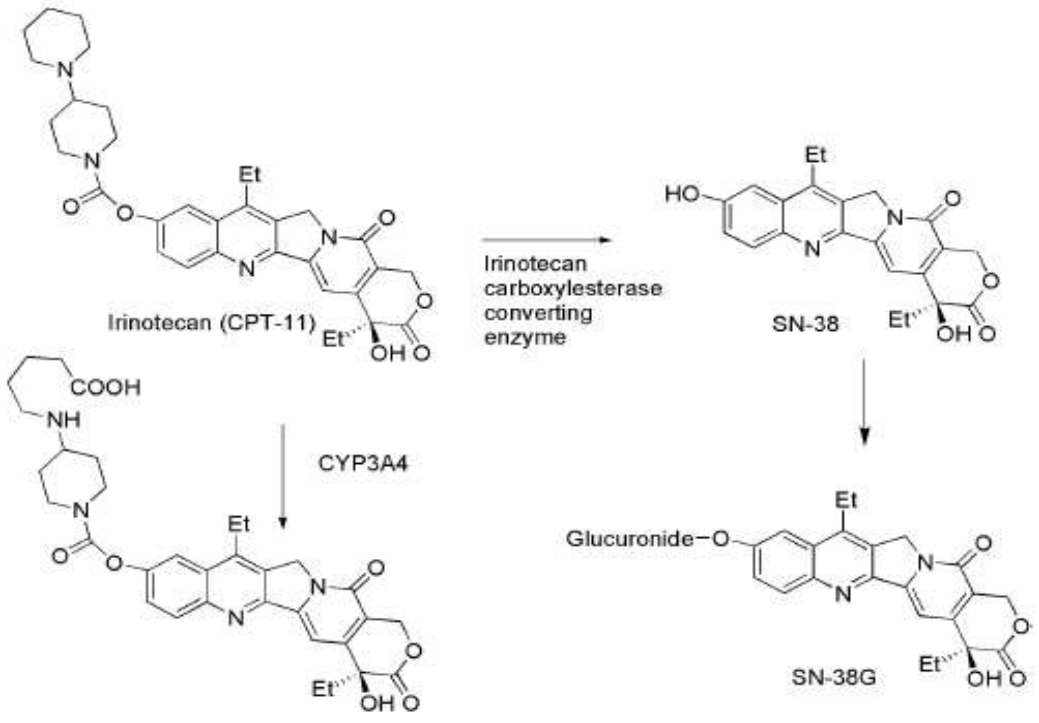
The resulting ring-opened material was 10 times less active and more toxic producing inflammation of the small intestines, blood in the urine, and myelosuppression.

the incorporation of side chains containing basic amines led to the more water-soluble derivatives, topotecan and irinotecan (Fig. 10.16). These agents could be administered as the lactone giving better clinical results. Topoisomerase I produces single-strand breaks in DNA, utilizing a similar mechanism to topoisomerase II. The enzyme binds to supercoiled DNA and cleaves a single strand resulting in the formation of a cleavable complex with formation of a transient phosphodiester bond between DNA and a tyrosine residue of topoisomerase I. The camptothecin analogs bind to the enzyme DNA complex after strand cleavage has occurred, such that the planar structure of the drug can intercalate between DNA base pairs and then stabilize the cleavable complex. The

binding site for this intercalation is only formed after the enzyme is bound to DNA, and once this site is occupied by the drug, it prevents the realignment necessary for resealing of the initial strand break.

Several mechanisms of resistance to the camptothecin analogs are known. Different neoplasms express different levels of topoisomerase I. Increased DNA-repair enzymes may limit the damage to DNA, and these agents are susceptible to Pgpmediated efflux. The agents have also been shown to activate nuclear factor-$_k$ B (NF-$_k$B), which has an antiapoptotic effect so that strand breaks may not initiate apoptosis.

Irinotecan undergoes hydrolysis of its carbamate moiety by irinotecan-converting enzyme to give SN-38, which is 1,000 times more potent than the parent compound (Scheme 10.29).There is wide interpatient variability in the extent of this transformation, which may explain differential responses to the agent. Further metabolism involves the glucuronidation (isozyme UGT1A1) of the resulting phenolic function of SN-38 to give SN-38G, which is inactive. An additional metabolite forms as a result of CYP3A4-mediated conversion to [4-*N*-(5-aminopentanoic acid)-1- piperidino] carbonyl camptothecin (APC), which is 100 times less active than SN-38.



Scheme 10.29 • Metabolism of Irinotecan.

Bleomycin

Bleomycin is a glycopeptide antibiotic complex isolated from *Streptomyces verticillus* initially by Umezawa. At least 13 different fractions of bleomycin have been isolated with the clinically used product (Blenoxane) being a mixture of predominantly A2 (55%–70%) and B2 (25%–32%) fractions (Fig. 10.18). Of these fractions, A2 appears to possess the greatest antineoplastic activity. Copper is found in the naturally occurring material, and its removal is important for the material used clinically because it significantly reduces activity.

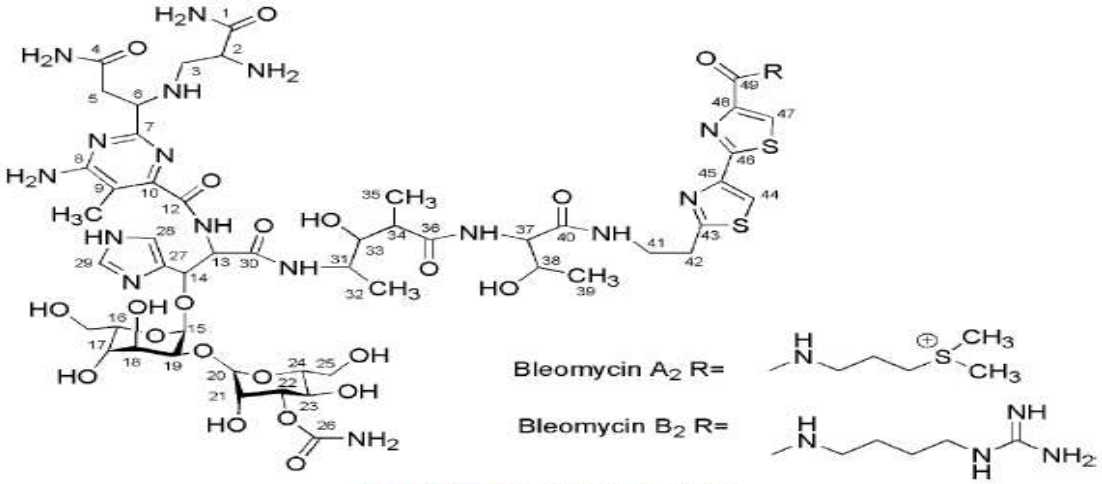


Figure 10.18 • Structure of bleomycins.

Bleomycin binds Fe+2 through multiple interactions with the amino terminal end of the peptide chain (Fig. 10.19). Bleomycin may itself initiate the release of iron necessary for this complexation. Interaction with DNA subsequently occurs through the bithiazole portion of the molecule, which intercalates between G-C base pairs with a preference for genes undergoing transcription. Held in proximity to DNA by this interaction, in an aerobic environment, Fe+2 is oxidized to Fe+3 in a one-electron process with the electron being transferred to molecular oxygen. This gives the activated form of bleomycin, which has been formulated as HOO^{-}Fe(III)-bleomycin. This then results in the production of ROS in the form of superoxide and hydroxide radical, which initiate single-strand breakage of the phosphodiester backbone and release of DNA bases by oxidative cleavage of the 3^{-} - 4^{-} bond of the deoxyribose moiety. This activity may be enhanced in the presence of mercaptans such as glutathione, which can facilitate the action of reductase enzymes in reducing the Fe+3 that is generated back to Fe+2 so that the process may continue. The agent is most active in the G_2 and M phases of the cell cycle.

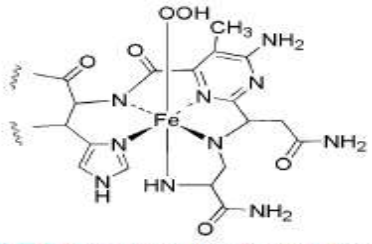


Figure 10.19 • Structure of the bleomycin-Fe-O_2 complex.

Bleomycin is notable for its lack of myelotoxicity, and this allows it to be combined with other myelosuppressants without a resulting additive effect. The acute toxicities seen with bleomycin are erythema (reddening of the skin), hyperpigmentation (skin darkening) found predominately on the extremities, and pulmonary toxicity. The pulmonary toxicity may first occur as pneumonitis (inflammation of lung tissue), which normally responds to glucocorticosteroid therapy. Chronic pulmonary toxicity is expressed as pulmonary fibrosis, which is irreversible and limits utility of the agent. The toxicity profile of bleomycin is explained by its route of inactivation. Hydrolysis of the N-terminal amide to the carboxylic acid increase the pKa of the amine at C-2 from 7.3 to 9.4, resulting in a greater degree of ionization and decreased binding to DNA.119 The enzyme responsible for this conversion is known as *bleomycin hydrolase*, and it is present in most tissue but found in low concentration in skin and lung tissue. Tumor cells that are resistant to bleomycin may contain high levels of this enzyme.

Vinca Alkaloids

The vinca alkaloids (Fig. 10.21) are extracted from the leaves of *Catharanthus roseus* (periwinkle), and were originally investigated for their hypoglycemic properties but latter found to possess antineoplastic actions. The alkaloids are composed of a catharanthine moiety containing the indole subunit and the vindoline moiety containing the dihydroindole subunit joined by a carbon–carbon bond. Vincristine and vinblastine differ only in the group attached to the dihydroindole nitrogen, which is a methyl group in vinblastine and a formyl group in vincristine. Vinorelbine is a semisynthetic material resulting from loss of water across the 3^{-} - 4^{-} bond.

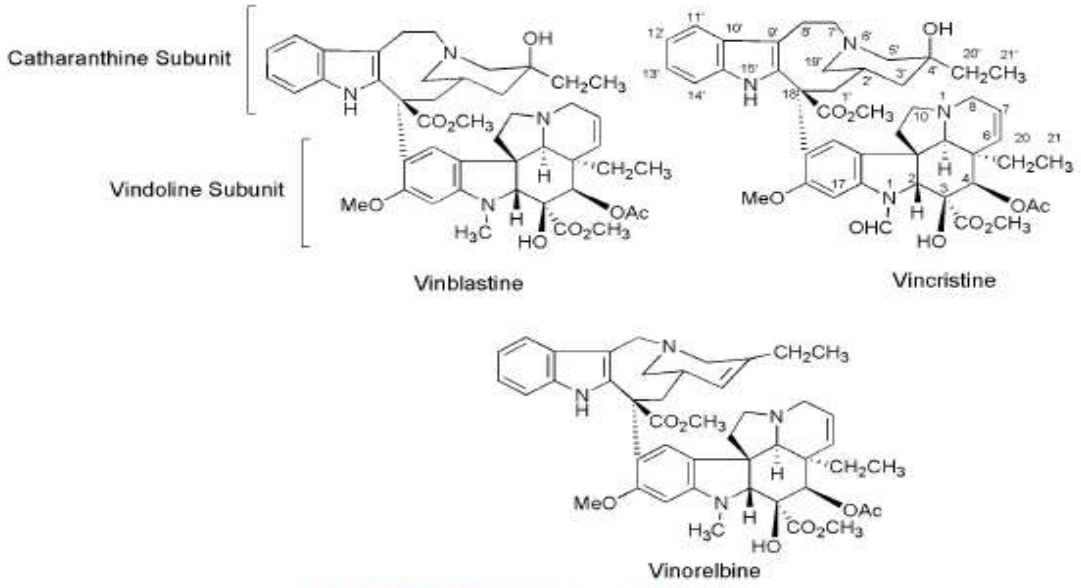


Figure 10.21 • Structures of vinca alkaloids.

The vinca alkaloids were initially believed to gain entry

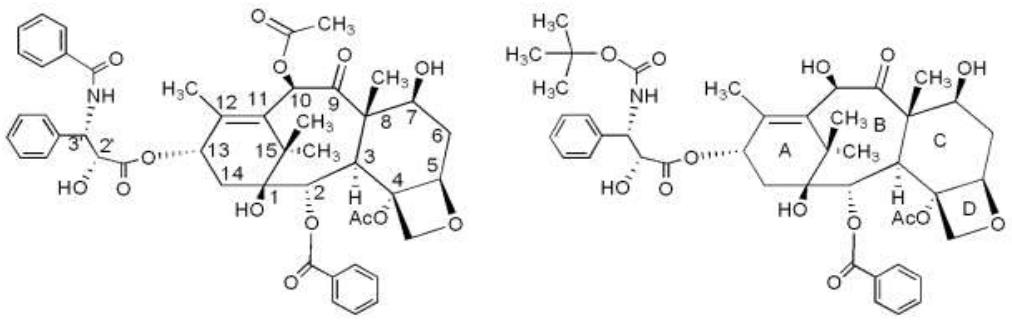
into the cell by an energy-dependent process, but more recent work suggests that entry occurs by an energy- and temperature- independent mechanism similar to passive diffusion. The agents then begin to accumulate in cells with intracellular concentrations 5- to 500-fold higher than extracellular concentrations. Once in the cell, the vincas bind to tubulin disrupting formation and function of the mitotic spindle. The mitotic spindle is composed of the microtubules, which function as part of the cell's cytoskeleton and are important in maintaining cellular shape. They are also involved in transport within the cell and cell signaling as well as playing a pivotal role in the movement of chromosomes during mitosis. The microtubules are composed of heterodimers of γ -tubulin and β-tubulin, which may arrange as alternating heterodimers around a hollow axis to form the protofilaments of the microtubule.

The vincas bind to tubulin in a reversible manner at sites different from those at which other inhibitors of spindle function bind including the podophyllotoxins and the taxanes. Combinations of these agents may give synergistic effects because of their unique binding sites. X-ray studies indicate that vinblastine binds between the γ - and β-tubulin heterodimers and other studies have shown that there are both high-affinity binding sites located at the end of the spindle and low-affinity sites located along the intact spindle. Binding at the high-affinity sites prevents both lengthening and shortening of the spindle and thereby disrupts its function. Both dynamic instability and treadmilling are inhibited by the vinca alkaloids. Binding at low-affinity sites, which occurs at higher drug concentration, leads to breakdown of the spindle as tubulin depolymerizes. As a result of these actions, the mitotic spindle fails to form properly, chromosomes do not move to the metaphase plate, anaphase fails to occur, and the cell undergoes apoptosis. The agents are considered specific for the M phase of the cell cycle. Other activities have been observed including antimetabolite activity, inhibition of protein synthesis, and altered lipid metabolism, but these are only seen at very high concentrations of the drugs. Inhibition of angiogenesis has also been associated with the vinca alkaloids.

Resistance to the vinca alkaloids occurs by several different mechanisms that are also associated with resistance to several high–molecular-weight molecules with diverse mechanisms of action also used in treating cancer.This multidrug resistance (MDR) is also seen with the taxanes, epipodophyllotoxins, and the anthracyclines although the resistance is usually greatest to the principal agent to which the patient was exposed. The MDR has been associated with several proteins including permeability glycoprotein (Pgp) and multidrug resistance protein (MRP1), which function to actively secrete the molecules from the cell. There are several inhibitors of Pgp such as calcium channel blockers and cyclosporine.

Taxanes

The taxanes, specifically, taxol (or paclitaxel) was discovered in the 1960s. Taxol (Fig. 10.22), isolated from the bark of the pacific yew tree, proved to be active against various cancer models. The formulation problems seen in the early development of paclitaxel were caused by poor water solubility.



Paclitaxel Docetaxel

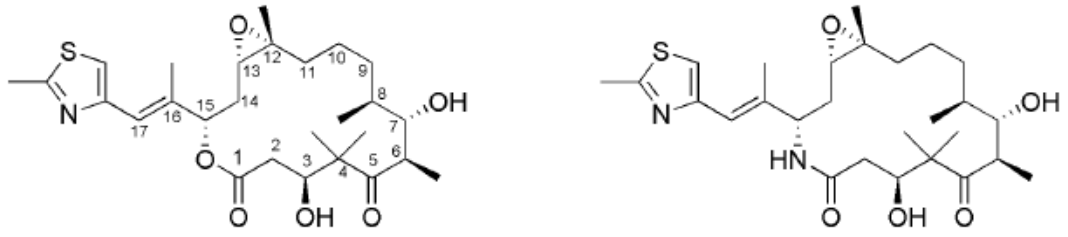
Figure 10.22 ● Structures of the taxanes.

The taxanes bind to tubulin at a site distinct from the vinca alkaloids. In the absence of x-ray crystal structures of bound drug, photoaffinity probes of paclitaxel have identified two sites at which binding may occur to *β*-tubulin. The first site was located at the N-terminus and involved residues 1 to 31. The second site involved residues 217 to 231 of *β*-tubulin. The binding site is located on the luminal side of the microtubule and located in the middle of the *β*-tubulin subunit. Docetaxel binds to the same site with greater affinity. Binding of taxanes at low concentration results in stabilization of the microtubule and prevents depolymerization. At higher concentrations, polymerization is enhanced. The taxanes inhibit both treadmilling and dynamic instability, and cells are most affected in the M phase when microtubule dynamics are undergoing the greatest change. Mitosis is blocked at the metaphase anaphase boundary, and cells undergo apoptosis. Paclitaxel has also been shown to enhance phosphorylation of a serine residue of Bcl-2, an antiapoptotic protein resulting in inhibition of Bcl-2^{-} s ability to block apoptosis. The proapoptotic proteins Bad and Bax are stimulated and in a similar manner, docetaxel has been shown to induce apoptosis by activation of caspase enzymes.

Resistance to the taxanes is like that seen for the vinca alkaloids and other agents and involves Pgp-mediated efflux. Alterations in the structure of *β*-tubulin may also occur and result in decreased binding of taxanes to the microtubule and therefore reduced cytotoxicity.

IXABEPILONE (AZAEPOTHILONE B, IXEMPRA)

The epothilones are macrocyclic lactones that have a mechanism of action similar to that of the taxanes but offer several advantages (Fig. 10.23). Ixabepilone is the semisynthetic amide analog of epothilone B



Epothilone B Ixabepilone

Figure 10.23 ● Structures of epithilones.

The epothilones showed potent in vitro activity but greatly decreased activity in vivo caused by metabolic instability via hydrolysis of the macrocyclic lactone. Conversion to the lactam increased stability and maintained in vivo activity. Ixabepilone has been recently (2007) approved for the treatment of metastatic breast cancer that is resistant to the taxanes. The agent is believed to bind to the same site occupied by the taxanes. Like the taxanes, ixabepilone binds to β-tubulin and stabilizes microtubules resulting in cell death. The agent is useful in cancers that have become resistant to the taxanes, because it is not removed by Pgp and is still capable of binding to altered beta tubulin to which the taxanes no longer bind. Increased water solubility also allows the agent to be administered without the need for Cremophor EL, reducing the chance of hypersensitivity reactions. The current indications for the agent are in metastatic breast cancer in combination with capecitabine after the failure of an anthracycline and a taxane and as monotherapy in metastatic breast cancer after failure of an anthracycline, a taxane, and capecitabine.

MITOMYCIN C

Mitomycin C (Fig. 10.24) was isolated from *Streptomyces caespitosus* in 1958 by Japanese workers and is considered the prototype of the bioreductive alkylating agents.

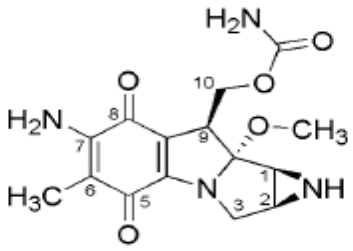
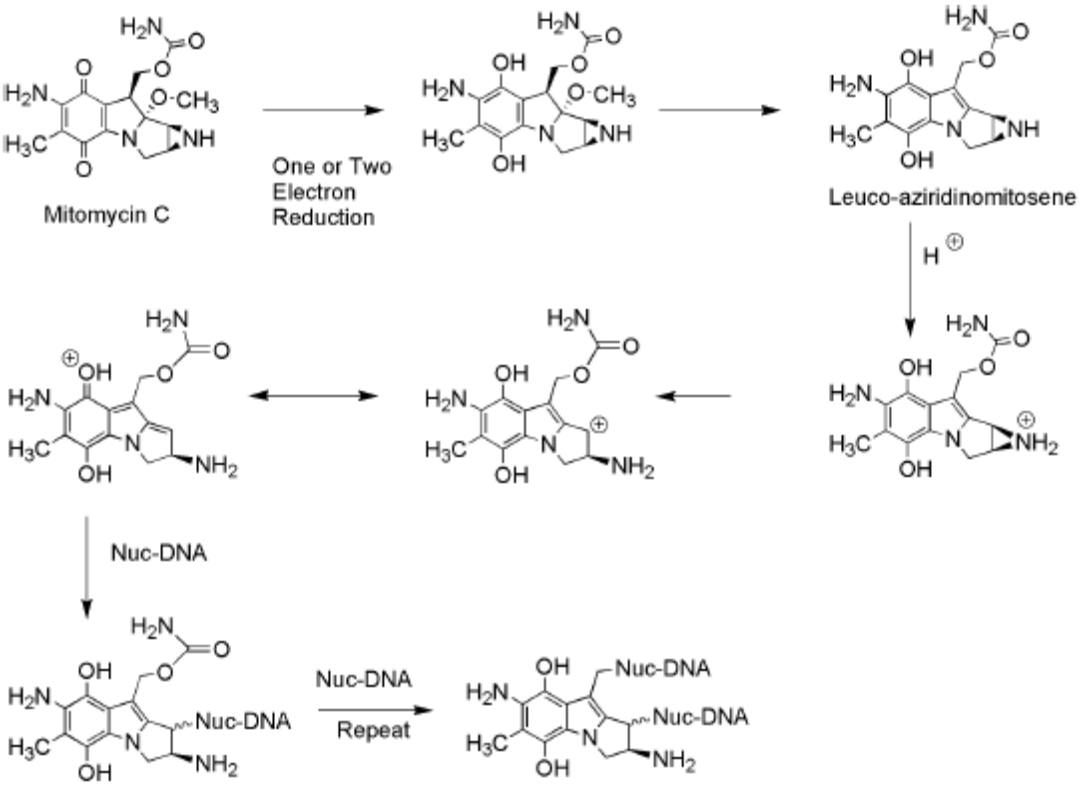


Figure 10.24 • Structure of mitomycin C.

Mitomycin is sometimes included as an alkylating agent but is included here because it is a naturally occurring material. The drug contains what would appear to be reactive functionalities, including the quinone and aziridine functionalities, both of which would be thought to be susceptible to nucleophilic attack; however, the reactivity of these functionalities is reduced because of steric and electronic effects in the parent molecule.

Mitomycin C is capable of being activated and alkylating DNA in an anaerobic environment, but there is actually little selectivity for hypoxic cells. Activation can occur enzymatically by both one- and twoelectron processes. Reductive enzymes such as NADPH CYP reductase and DT-diaphorase have been implicated in these processes. Involvement of one-electron processes such as those seen for the anthracylines result in redox cycling and the production of ROS that may result in DNA damage, but the cytotoxicity of mitomycin C is primarily associated with its ability to alkylate DNA. The hydroquinone can result from a single two-electron process or a one-electron process followed by disproportionation to give the hydroquinone (Scheme 10.30). The electrons of the amine are no longer withdrawn by the quinone system and are now free to expel methoxide followed by loss of a proton to give the leuco-aziridinomitosene. This reduction renders positions C-1 and C-10 reactive to nucleophilic attack because of the fact that there are leaving groups attached, and they are now benzylic positions so that the

intermediate carbocations can be stabilized. Protonation of the aziridine is followed by ring opening to give the carbocation, which can be stabilized by resonance involving the hydroquinone system. Nucleophiles on DNA may attack at C-1 with concomitant electron movement. This can be repeated in a similar manner at C-10, which allows for cross-linking. There are several other possibilities under different conditions. DNA is alkylated by mitomycin C at both the N-2 and N-7 positions of guanine.

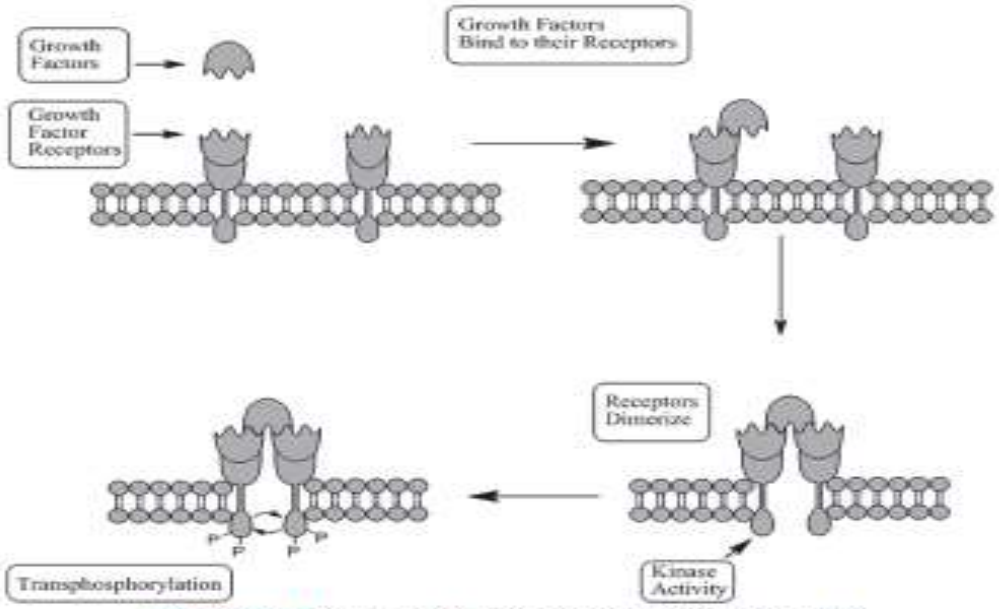


Scheme 10.30 • Alkylation of DNA by mitomycin C.

PROTEIN KINASE INHIBITORS

In the last several years, several new treatment options have become available based on increased knowledge of growth factors and cell signaling. It was realized early on that the growth and proliferation of many tissues was under the control of the endocrine system, in which endocrine glands secreted hormones and that hormones or their antagonists were useful in controlling the overgrowth of these tissues. It was found later that other growth factors, many of which were secreted by nearby cells, were also involved in controlling the growth and proliferation of the target tissue and this became known as paracrine control. Further work identified the pathways by which interaction with these cell surface receptors resulted in growth and proliferation of cells. There are various mechanisms by which these processes occur, but a generalized pathway involves interaction of a growth factor with a growth factor receptor occurring as a monomer present in the phospholipid bilayer of the cell membrane, which results in dimerization of the receptors (Scheme 10.31). The receptors themselves possess an extracellular-binding

domain for interaction with the growth factors, an intracellular domain, and a connecting trans membrane region. The intracellular domain of these receptor proteins often function as kinases, many of which are TKs that phosphorylate residues on the mother monomer receptor. The resulting phosphorylated residues are recognized by the Src homology 2 domainm(SH2) of cytoplasmic proteins such as Shc, Grb-2, and Sos, which activate Ras. These cytoplasmic proteins then serve to transmit the growth signals from the growth factor receptors to Ras (Fig. 10.25). **Ras** is a G protein tethered to the cytoplasmic membrane, which may bind GTP resulting in activation of Ras or bind GDP resulting in Ras inactivation. Ras also normally has the ability to hydrolyze GTP to inactivate itself. Many cancers are found to have altered forms of Ras, which is present in a hyperactive state. Ras, in its activated form activates a series of kinases, many of which are TKs with various effects on cells. Notable is the **Ras- Raf-MEK-ERK** pathway also known as the ***mitogen activated protein kinase*** (MAPK) pathway.



Schemo 10.31 ● Schematic of the activation of growth factor receptors.

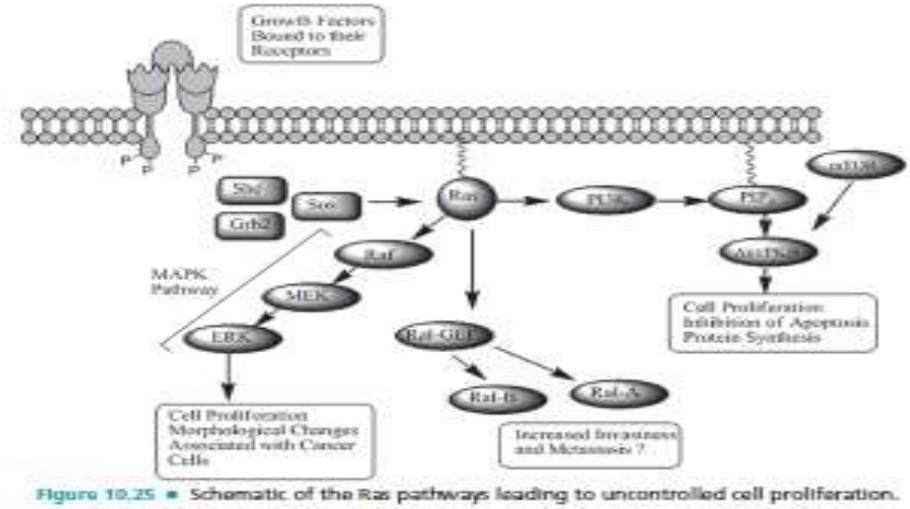


Figure 10.25 ● Schematic of the Ras pathways leading to uncontrolled cell proliferation.

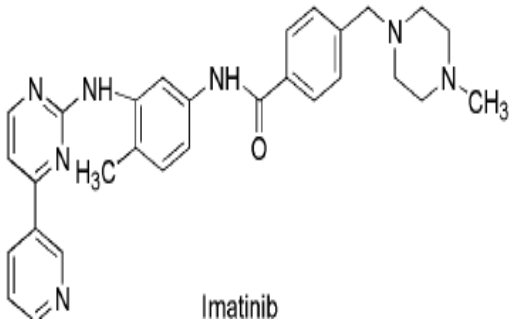
This is a cascade effect, whereby one kinase phosphorylates and activates more of the next kinase in the pathway amplifying the signal in the process. Stimulation of this pathway results in the activation of several transcription factors involved in the expression of growth factors, cyclin D1 (involved in controlling cell division, see Fig. 10.1), and transcription factors Fos and Jun, which were first discovered as viral oncogenes and are known to be elevated in several human cancers. In addition, overactivation of this pathway may result in loss of contact inhibition, loss of anchorage dependence, and changes in cell shape, all of which are characteristic of cancer cells.

The second major pathway activated by Ras is **PI3K PIP3-Akt/PKB** whose activation increases cell proliferation but perhaps most importantly, inhibits apoptosis.

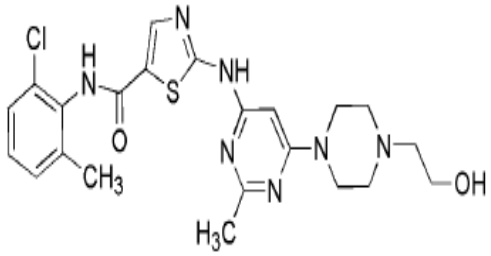
The third pathway involves Ras-mediated activation of **Ral-GEF** (Guanine nucleotide exchange factor), which acts on Ral-A and Ral-B to stimulate their exchange of GDP for GTP and hence their activation. The activation of this pathway, although less well understood, seems to allow cancer cells to metastasize and invade.

Imatinib was developed to specifically inhibit this unique TK and does so rather selectively by binding to the ATP-binding pocket and stabilizing an inactive form of the enzyme. Protein kinases have similar structures and consist of C- and N-terminal lobes with the active site formed by a cleft between the two lobes. The activity of the enzyme is regulated by an activation loop that is extended in the active form of the enzyme and provides for substrate binding. X-ray crystal studies of imatinib binding to Abl show that the drug binds in the cleft formed by the two lobes of the enzyme through the formation of several hydrogen bonds and hydrophobic interactions with the pyridine ring assuming the position occupied by ATP in the active enzyme. Imatinib binding locks the enzyme in a conformation in which the activation loop is oriented so as to block substrate binding.

dasatinib can bind to either an active or inactive form of the enzyme and is 100 times more potent compared with imatinib. It has the ability to overcome resistance associated with amino acid alterations that leads to decreased affinity for imatinib.

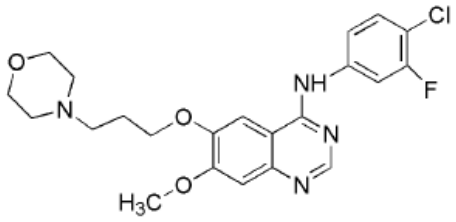


Imatinib

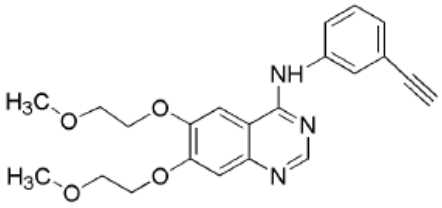


Dasatinib

Currently, there are two structurally similar agents in use, **gefitinib** and **erlotinib**, which bind to the ATP-binding site and thereby inhibit the resulting cascade that would normally occur from activation of the EGF receptor.The benefit of the agents was hoped to be caused by inhibition to the Ras pathway so that ultimately, all three arms could be affected (MAPK, Akt/PKB, and Ral-GEF). The agents are modestly selective for this kinase. Currently both compounds have indications for NSCLC generally as alternatives after the failure of more traditional therapy, and gefitinib has an additional indication against pancreatic cancer.

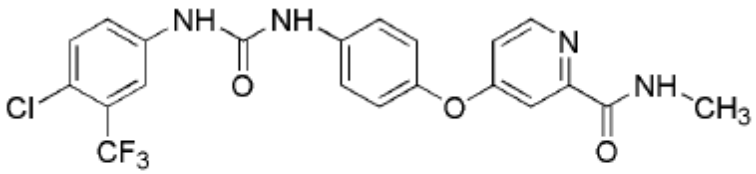


Gefitinib



Erlotinib

Sorafenib was originally developed as an inhibitor of the serine-threonine kinase Raf . It proved to be a potent inhibitor of this enzyme with an IC50 6 nM and acted by stabilizing an inactive form of the enzyme similar to imatinib. Subsequent x-ray analysis of the drug receptor complex showed that the pyridine ring occupied the ATP-binding site of Raf, whereas the trifluoromethyl-chloro–substituted phenyl ring occupied a hydrophobic pocket in the cleft formed by the two lobes of the protein.



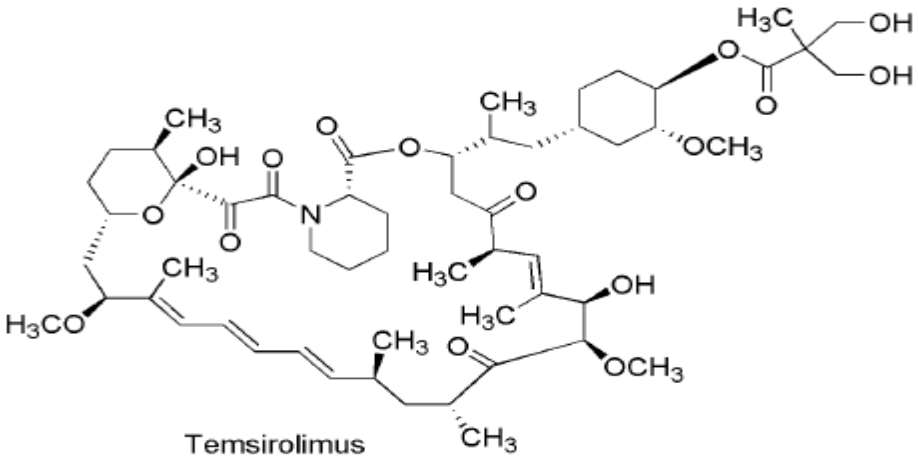
Sorafenib

As a group of agents, the protein kinase inhibitors have the advantage of oral administration and better patient tolerability. The major adverse effects include a skin rash that normally appears early in therapy on the upper torso and is generally mild but may become more serious in some cases. Mild diarrhea and nausea are also commonly seen but are generally controlled with the administration of antiemetics and antidiarrheals. Mild myelosuppression is also seen with several of these agents. There has been some evidence for cardiotoxicity in this class.

MISCELLANEOUS COMPOUNDS

TEMSIROLIMUS

Temsirolimus (Fig. 10.27) is an esterified derivative of rapamycin and in a similar manner binds initially to the protein FKBP-12(FK506-binding protein). This complex then acts to inhibit the mammalian target of rapamycin (mTOR), a serine-threonine kinase that plays a crucial role in cell division. It is somewhat unique in its method of kinase inhibition, because it actually binds to an allosteric modulator of the kinase rather than just binding to the ATP-binding site like most other kinase inhibitors. Binding of temsirolimus inhibits the phosphorylating activity of mTOR. mTOR regulates the Akt/PKB pathway, and therefore, one way in which the agent acts is to inhibit this pathway. An additional mechanism involves the ability of mTOR to initiate protein synthesis independent of the Akt/PKB pathway. Blockade of mTOR results in inhibition of protein synthesis and prevents the cell from moving past the G1 phase into the S phase. More specifically, mTOR is prevented from phosphorylating 4E-binding protein-1(4E-BP1) an initiating factor and 40S ribosomal protein S6 kinase (p70S6 kinase), both of which are involved in initiating protein synthesis necessary for the cell cycle. In addition, mTOR is involved in the control of several growth factors such VEGF, PDGF, and TGF, which are involved in cell growth and angiogenesis. This agent, like rapamycin, possesses immunosuppressant properties and there is an increased risk of infection. The most serious side effects are interstitial lung disease, perforation of the bowel, and acute renal failure although these occur only rarely.

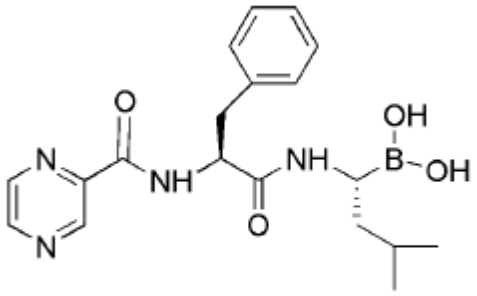


Temsirolimus

BORTEZOMIB

Proteasomes normally function to degrade proteins that are no longer needed by the cell. Such proteins are normally marked by the addition of ubiquitin, a 76 amino acid protein that is added to the ε-amino group of lysine residues on the target proteins. The marked proteins are then hydrolyzed by the large barrel-shaped proteasomes to give peptides of 7 to 8 residues that may be further hydrolyzed and reutilized by the cell. This process serves to regulate protein levels within the cell, remove defective proteins, and becomes important in maintaining normal signal transduction. Inhibition of the proteasomes results in the buildup of ubiquitylated proteins, which disrupts cell-signaling processes and cell growth (Fig. 10.28). The signaling by transcription factor NF-kB (nuclear factor kB) appears to be especially sensitive to bortezomib. NF-kB is associated with the transcription of antiapoptotic and proliferative genes but is under the control of IkB (inhibitor of NF-kB). IkB can itself be phosphorylated by IKK (IkB kinase), which marks IkB for ubiquitylation and destruction allowing NF-kB to mediate its antiapoptotic and proliferative effects The agent is used primarily in treating multiple myeloma, The agent contains the unique boronic acid group, which serves as a bioisosteric replacement for an aldehyde functionality and forms a tetrahedral complex with a threonine hydroxyl group present on the proteasome.

The major toxicities seen with the agent are generalized weakness, nausea, vomiting, diarrhea, peripheral neuropathy, fever, and orthostatic hypotension. Myelosuppression also occurs normally as thrombocytopenia and neutropenia.



Bortezomib

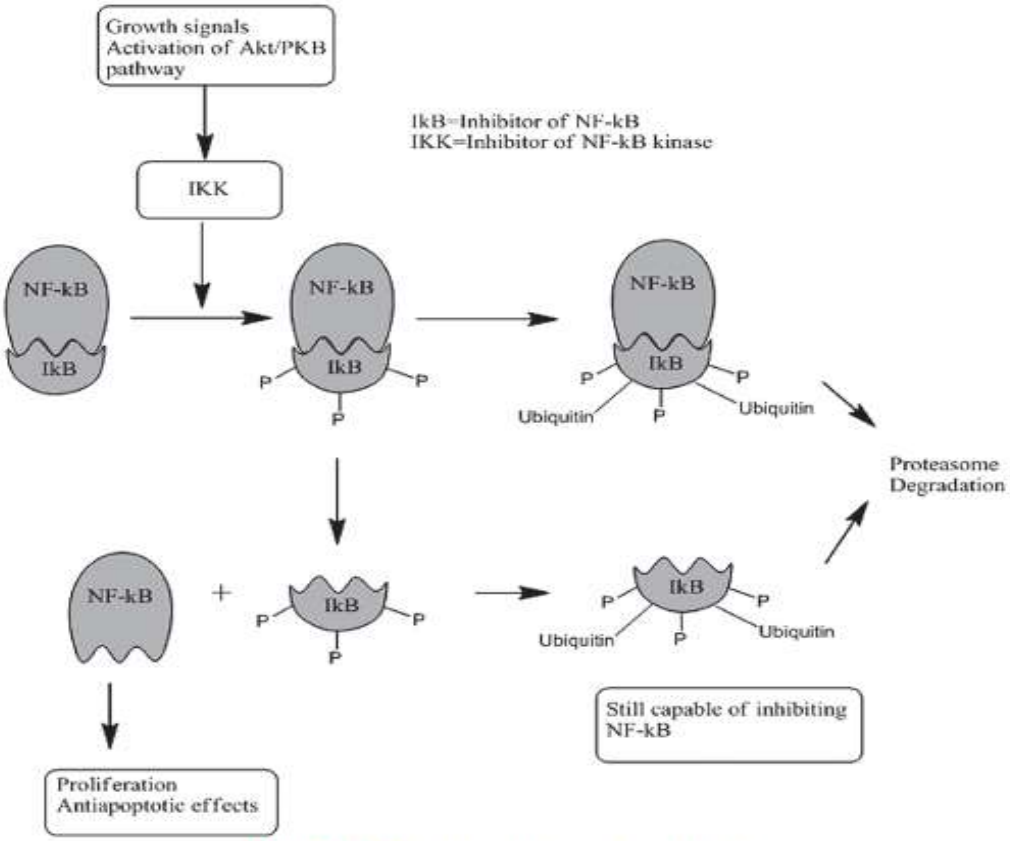
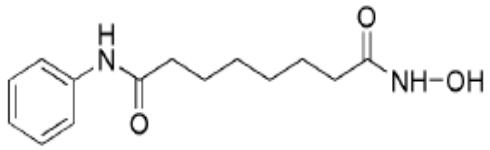


Figure 10.28 • Actions of proteasomes on NF-κB.

VORINOSTAT



Vorinostat

Histones are proteins around which DNA is wound in the process of packing DNA into the nucleus. They also have a role in regulating the transcription of genes, and this is controlled by the covalent modifications acetylation, phosphorylation, and methylation to which they are subject. Acetylation occurs at the ε-amino group of lysine and is accomplished by histone acetyltransferase enzymes, whereas deacetylation is accomplished by histone deacetylase enzymes. The result of inhibition of histone deacetylase is hyperacetylation of lysine residues of the histone proteins. The positively charged ε-amino groups of the lysine residues are believed to interact with the negatively charged phosphate backbone of DNA. Once acetylation has occurred, this interaction is prevented, and the binding of transcription factors is favored. Therefore, the inhibition of

deacetylation by vorinostat, a histone deacetylase inhibitor (HDACis), results in the increased transcription of certain genes. Specifically, this has been associated with upregulation of a regulatory protein known as p21, which serves to inhibit progression past the G1 phase of the cell cycle. Other genes and their proteins are also effected by vorinostat such as Hsp90 (heat shock protein 90) and BCL6.

Vorinostat fits the basic pharmacophore for the HDACis (Fig. 10.29), which consists of a hydrophobic cap region connected to a zinc coordinating functionality by a hydrophobic linker. The hydroxamic acid functionality is capable of bidendate binding to zinc present in the enzyme and is a major factor in the overall binding of the compound.

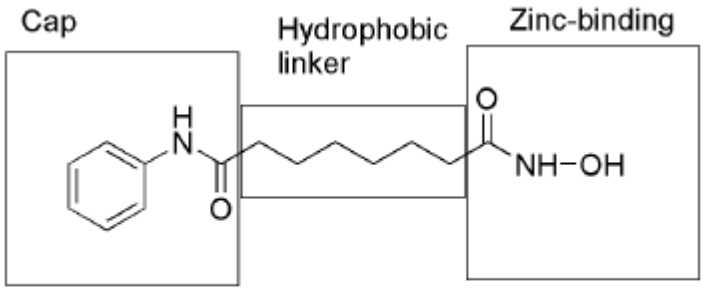


Figure 10.29 ● Pharmacophore for histone deacetylase inhibitors.

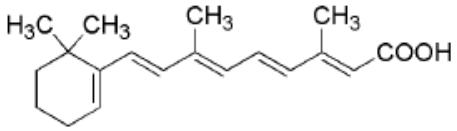
The most commonly reported adverse effects are fatigue, diarrhea, and nausea. Elevations in glucose and triglyceride levels are commonly seen with the agent. The agent has been associated with thrombocytopenia, an increased risk of clotting resulting in pulmonary embolism.

ARSENIC TRIOXIDE (AS2O3)

Arsenic trioxide is used as second-line therapy in the treatment of acute promyelocytic leukemia (APL). The mechanism of the agent has not been well characterized; however, work has indicated that the agent may cause the degradation of a protein that blocks myeloid differentiation.

Arsenic trioxide is capable of degrading this protein and allowing the cells to differentiate. Additional effects have included stimulation of apoptosis by decreasing Bcl-2 activity and stimulation of caspase enzymes and p53. Angiogenesis is inhibited by the inhibition of VEGF at the protein level.

Tretinoin



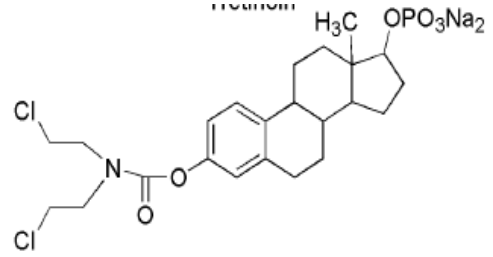
Tretinoin

It is used in the treatment of APL. The mechanism of action involves passive diffusion through the cell membrane and then movement to the nucleus where it interacts with the retinoic acid receptor (RAR) portion of the PML-RARα fusion protein. Binding of tretinoin allows the cell to differentiate and has also been shown to result in the destruction of the PML-RARα fusion protein. Resistance to tretinoin is problematic and associated with an increase in cellular retinoic acid–binding proteins (CRABPs) located in the cytosol. The complexation with tretinoin prevents movement into the nucleus and may present the drug to metabolizing enzymes that inactivate it. Amino acid mutation of the PML-RARα protein has also been established as a mechanism of resistance.
Vitamin A toxicity is seen in nearly all patients and presents as headache, fever, dryness of the skin, skin rash, mucositis, and peripheral edema.

ASPARAGINASE

Asparaginase is used in the treatment of acute lymphocytic leukemia. Tumor cells are unable to synthesize asparagine, and therefore must utilize what is available in the extracellular environment. The agent acts by hydrolyzing extracellular asparagine to aspartate and ammonia. The tumor cells are then deprived of a necessary nutrient, and protein synthesis is inhibited leading to cell death. The agent is specific for the G1 phase of the cell cycle. Resistance occurs because of the development of the tumor cells ability to produce asparagine synthetase that allows them to synthesize the required amino acid. Antibody production directed at asparaginase may be stimulated by the agent as well.
Adverse effects include hypersensitivity reactions, fever, chills, nausea, lethargy, confusion, hallucinations, and possibly coma. Myelosuppression is not generally seen. An increased risk of bleeding and clotting is seen in half of the patients taking the agent.

Estramustine



Estramustine

Estramustine as the phosphate is used for the treatment of prostate cancer. Although originally designed as an alkylating agent, it has been shown to be devoid of alkylating activity and functions as an inhibitor of microtubule function by binding to microtubule associate proteins (MAPs) and also binds to tubulin at a site that is distinct from that of the vinca alkaloids but thought to partially overlap with that of paclitaxel. The major mechanism by which cells become resistant to the agent involves increased efflux, although this is not mediated by Pgp as is the case with other microtubule inhibitors such as the taxanes and vinca alkaloids. Therefore, the agent does not show cross-resistance with these agents. As an inhibitor of microtubules, it is cell cycle specific acting in the M phase.

Metabolism involves the formation of the active estromustine, which arises from oxidation of the C17 alcohol to give the ketone. Additional inactive metabolites result from carbamate hydrolysis to give estradiol and estrone.

The adverse effects of the agent are nausea and vomiting, which is generally mild but the severity may increase upon prolonged administration. Gynecomastia also commonly occurs, and diarrhea may also be seen. Less commonly seen effects include myelosuppression, skin rash, and cardiovascular abnormalities including CHF.

Hsp90 (heat shock protein 90) is a chaperone protein that assists other proteins to fold properly, stablizes proteins against heat stress, and aids in protein degradation. It also stabilizes a number of proteins required for tumor growth, which is why Hsp90 inhibitors are investigated as anti-cancer drugs.

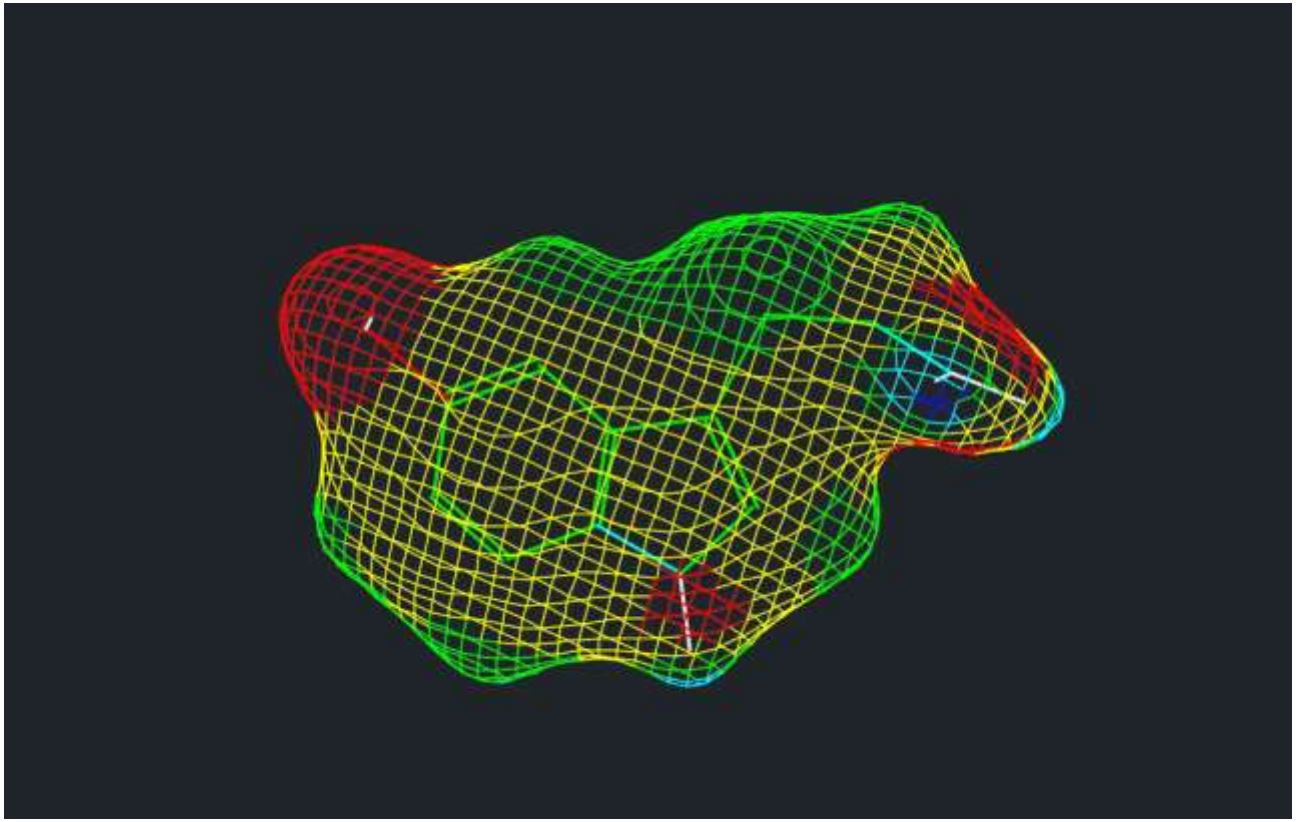
ORGANIC MEDICINAL AND PHARMACEUTICAL CHEMISTRY

INTRODUCTION

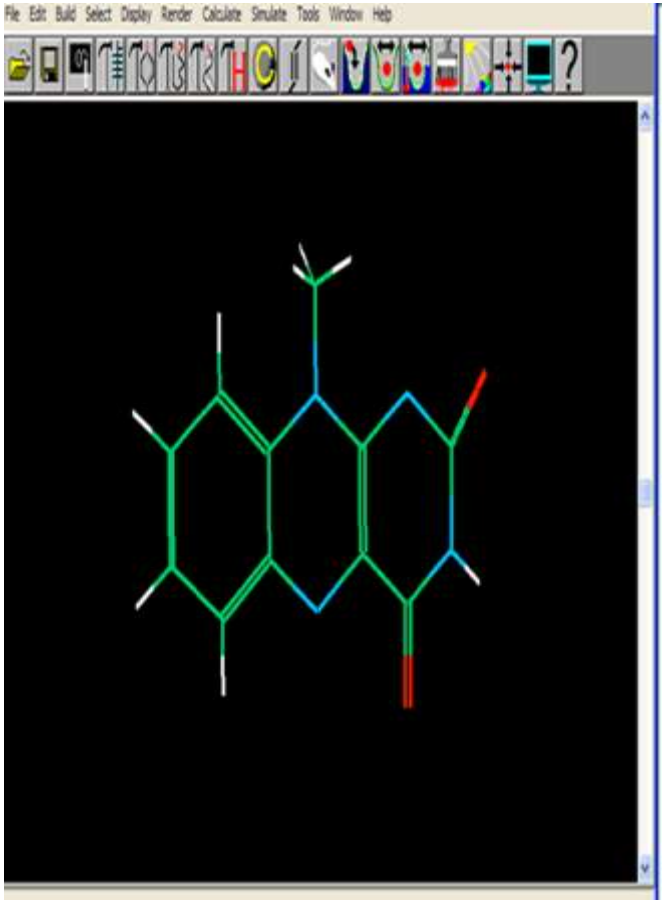
INTRODUCTION

- ##Medicinal chemistry is devoted to discovery and development of mostly new natural or synthetic organic agents for treating diseases. Paralleling the development of medicinal agents has come a better understanding of receptor chemistry which has been greatly facilitated by low-cost computers running software that calculates molecular properties and structure and pictures it.
--Development of organic compounds has grown beyond traditional synthetic methods. It now includes biotechnology using cell's biochemistry to synthesize new compounds . Techniques like recombinant DNA and site-directed mutagenesis and fusion of cell lines have greatly broadened the possibilities for new entities that treat disease. Now , the pharmacist dispenses modified human insulins that provide more convenient dosing , cell-stimulating factors that have changed the dosing regimens for chemotherapy , humanized monoclonal antibodies that target specific tissues and fused receptors that intercept immune cell-generated cytokines.

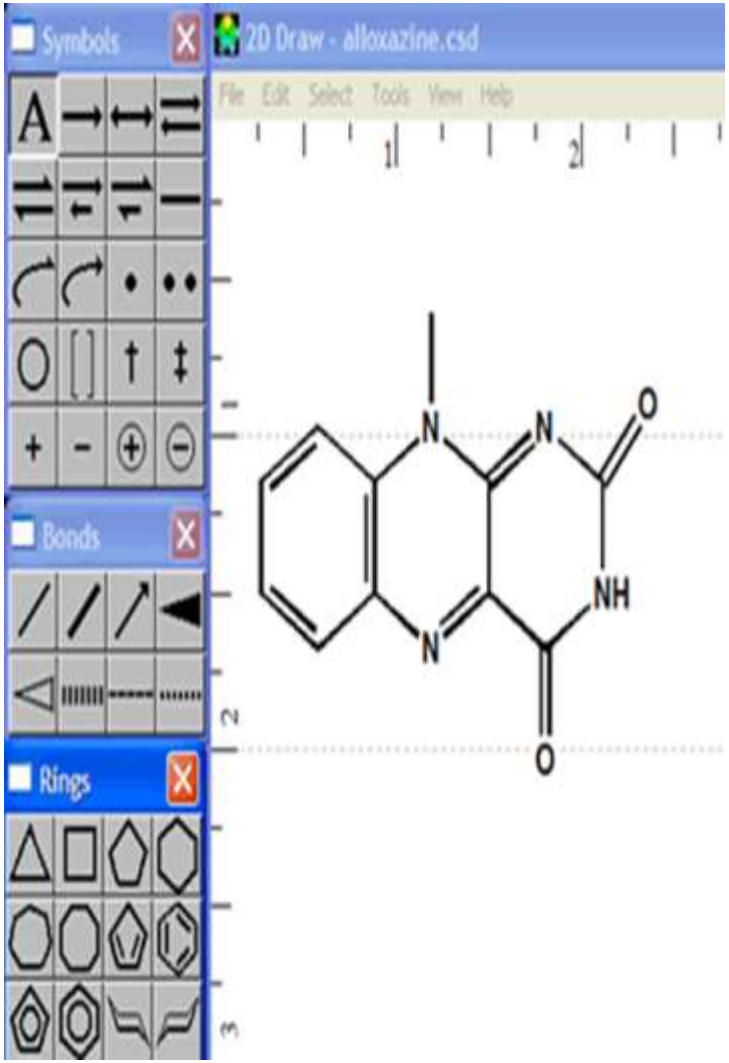
INTRODUCTION(electrostatic surface map of serotonin)

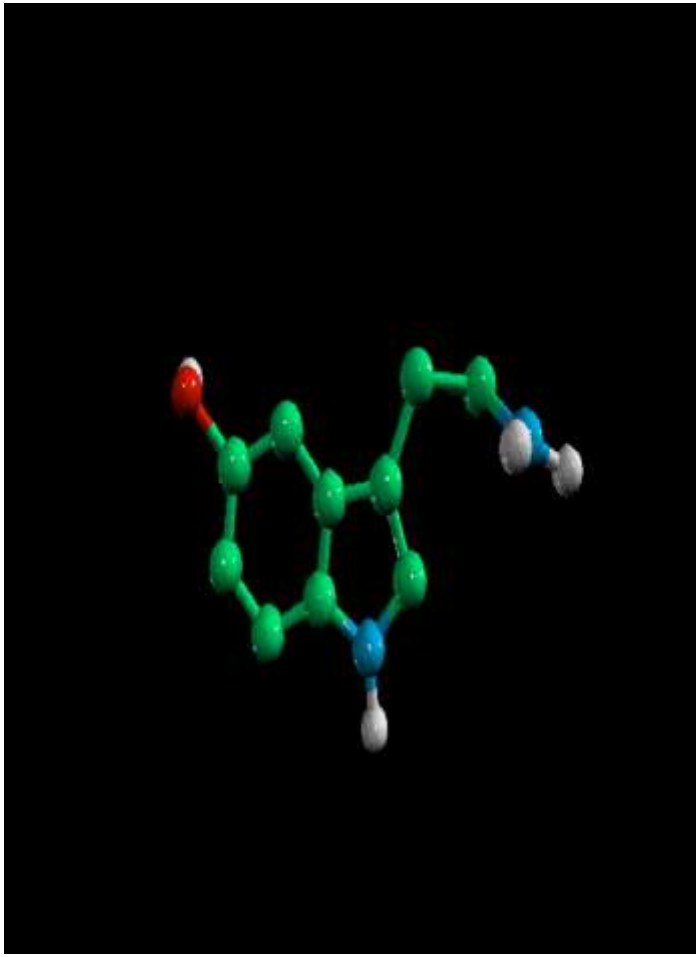


INTRODUCTION(CHEMSITE program)



INTRODUCTION(draw in 2D & 3D)





INTRODUCTION

##Organic medicinal and pharmaceutical chemistry presents the scientific basis of medicinal chemistry describing how organic medicinals are discovered , how they act and how they developed into clinical agents.
--Establishing a new pharmaceutical is exceedingly complex and involves talents of chemistry , biochemistry, molecular biology, physiology, pharmacology and workers in pharmaceutics and medicine.
--Medicinal chemistry is concerned mainly with the organic , analytical and biochemical aspects of such establishing . Also the chemist must interact productively with others . Medicinal chemistry occupies a strategic position at the interface of chemistry and biology.
--All of the principles discussed in Wilson textbook are based on organic chemistry , physical chemistry and biochemistry . To understand such principles of medicinal chemistry , we have to consider the physicochemical properties used to develop new pharmacologically active compounds and their mechanisms of action , the drug's metabolism , including possible biological activities of metabolites,

INTRODUCTION

- importance of stereochemistry in drug design and the methods used to determine what “space” a drug occupies.

##The earliest drug discoveries were made by random sampling of higher plants . Some of this sampling(based on anecdotal (stories)evidence)led to use of such crude plant drugs as opium , belladonna and ephedrine that have been important for centuries . With the accidental discovery of penicillin came the screening of microorganisms and the large number of antibiotics from bacterial and fungal sources . Many of these antibiotics provided the prototypical structure that the medicinal chemist could modify to obtain antibacterial drugs with better therapeutic profiles.

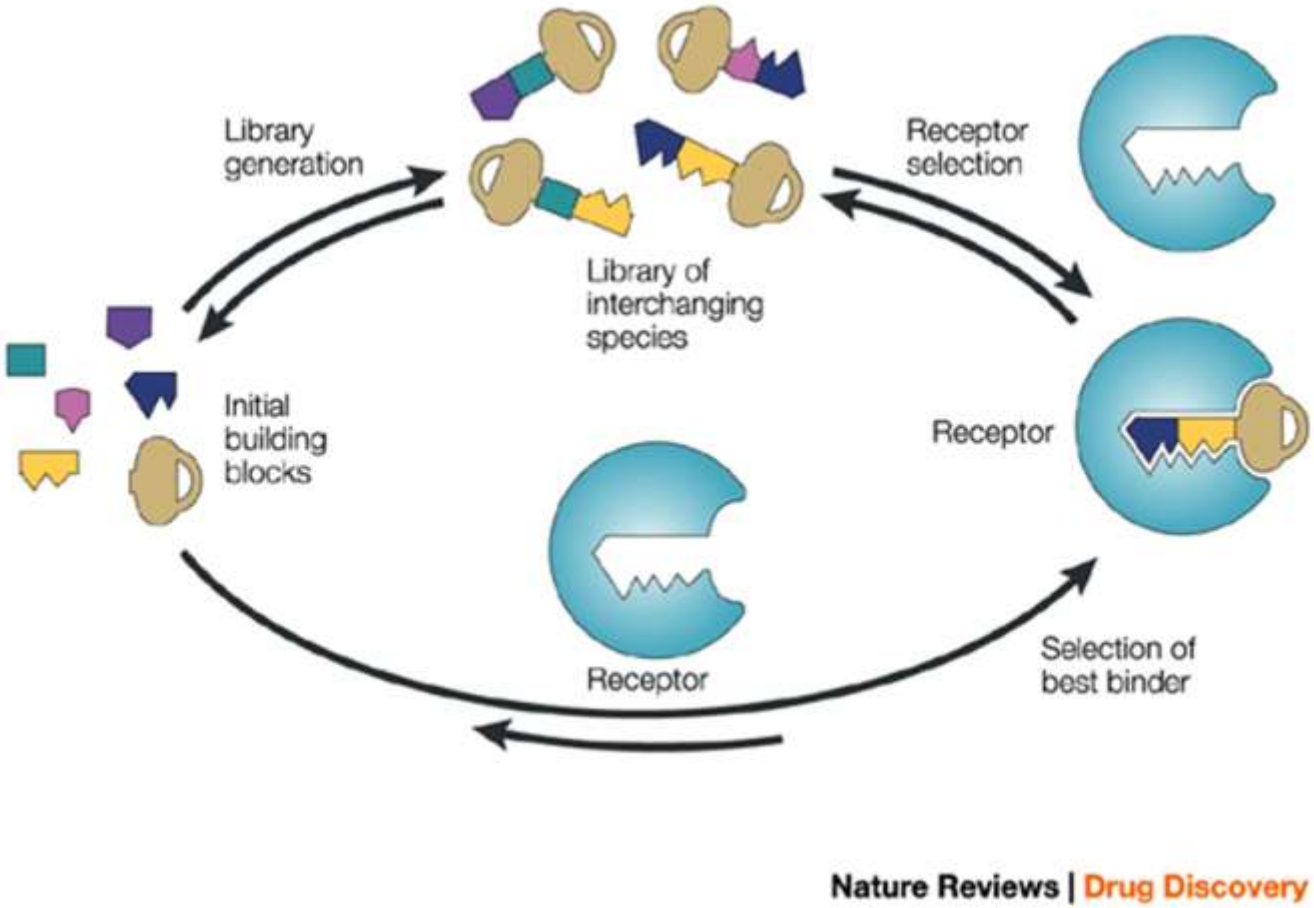
INTRODUCTION

- ##Hundreds of thousands of new organic chemicals are prepared annually throughout the world , and many of them are entered into pharmacologic-al screens to determine whether they have useful biological activity. This random screening(inefficient) has resulted in identification of new lead compounds with optimized structures giving clinical agents.
--Sometimes , a lead develops by careful observation of the pharmacological behavior of an existing drug . This discovery that amantadine protects and treats early influenza A came from a general screen for antiviral agents . The use of amantadine in long-term care facilities showed that it also could be used to treat Parkinsonism.
--More recently , automated high-throughput screening systems utilizing cell culture systems with linked enzyme assays and receptor molecules derived from gene cloning have greatly increased the efficiency of random screening.

INTRODUCTION

- --It is now practical to screen enormous libraries of peptides and nucleic acids obtained from combinatorial chemistry procedures.
##Rational design, the opposite approach to high-volume screening, is also flourishing. Statistical methods based on correlation of physicochemical properties with biological potency are used to explain and optimize biological activity .
--Significant advances in X-ray crystallography and nuclear magnetic resonance have made it possible to obtain detailed representations of enzymes and other drug receptors.
--The techniques of molecular graphics and computational chemistry have provided novel chemical structures that have led to new drugs with potent medicinal activities. Development of HIV protease inhibitors and ACE inhibitors came from understanding of the geometry and chemical character of the respective enzyme’s active site.

INTRODUCTION



INTRODUCTION

--Even if the receptor structure is not known in detail, rational approaches based on the physicochemical properties of lead compounds can provide new drugs. For example , development of cimetidine involved a careful study of the changes in antagonism of H2-receptors induced by varying the physical properties of structures based on histamine.

-

DRUG DESIGN STRATEGIES

-Modern drug design continues to evolve rapidly as an approach to solving a drug design problem.

-***Classical approach was about making a change on an existing compound or synthesizing a new structure and seeing what happens***.

-Combination of increasing power and decreasing cost of desktop computing has had a major impact on solving drug design problems . Drug design is based on modern computational chemical techniques . Drug design uses the knowledge of disease mechanisms and receptor properties . Also knowledge(about the drug transport into body , distribution throughout body compartments , metabolism by liver and other organs excretion from patient along with the structural characteristics of receptor)is required.

-Acid-base chemistry is used to aid in formulation and biodistribution

.

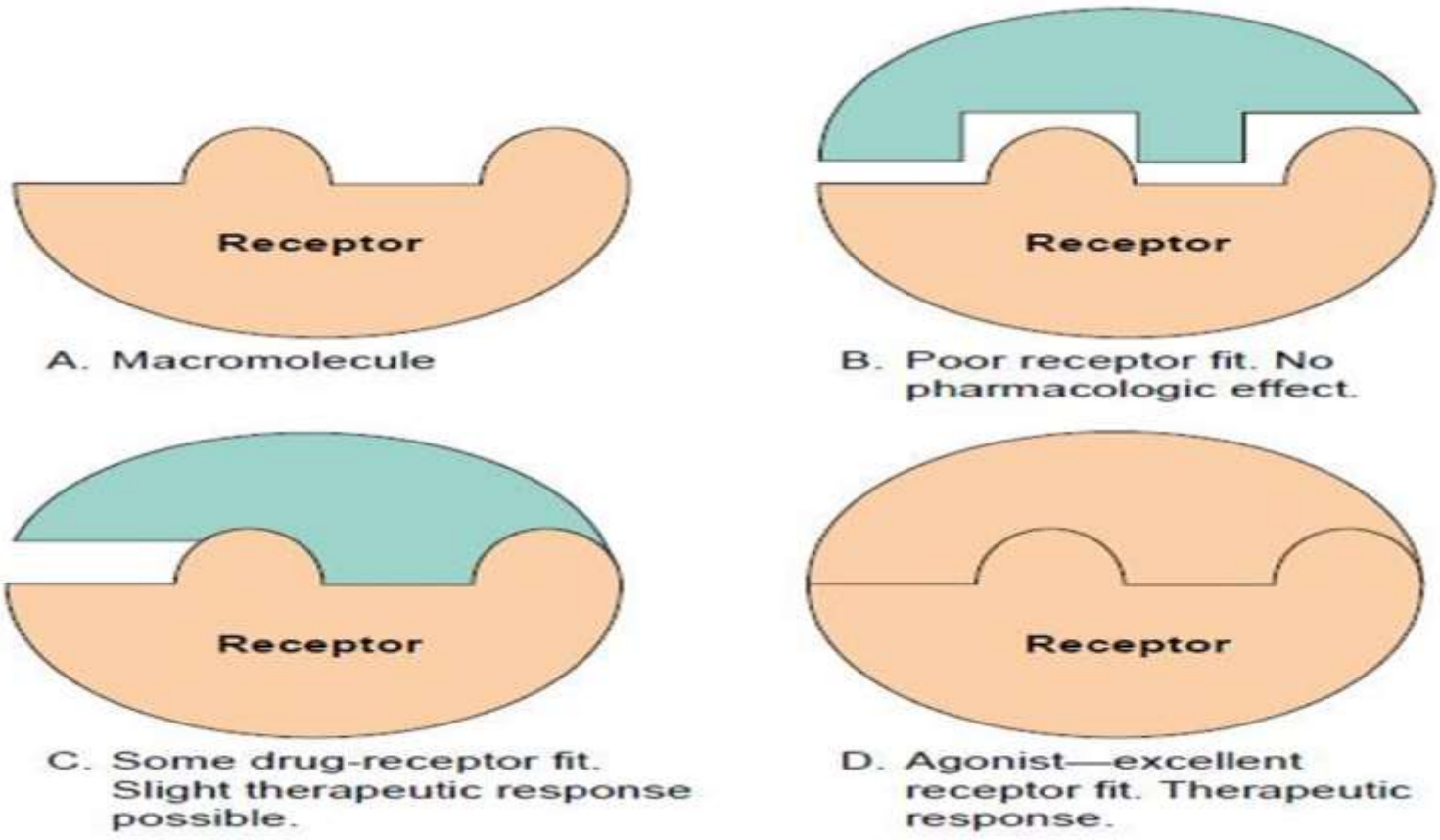
-

DRUG DESIGN STRATEGIES

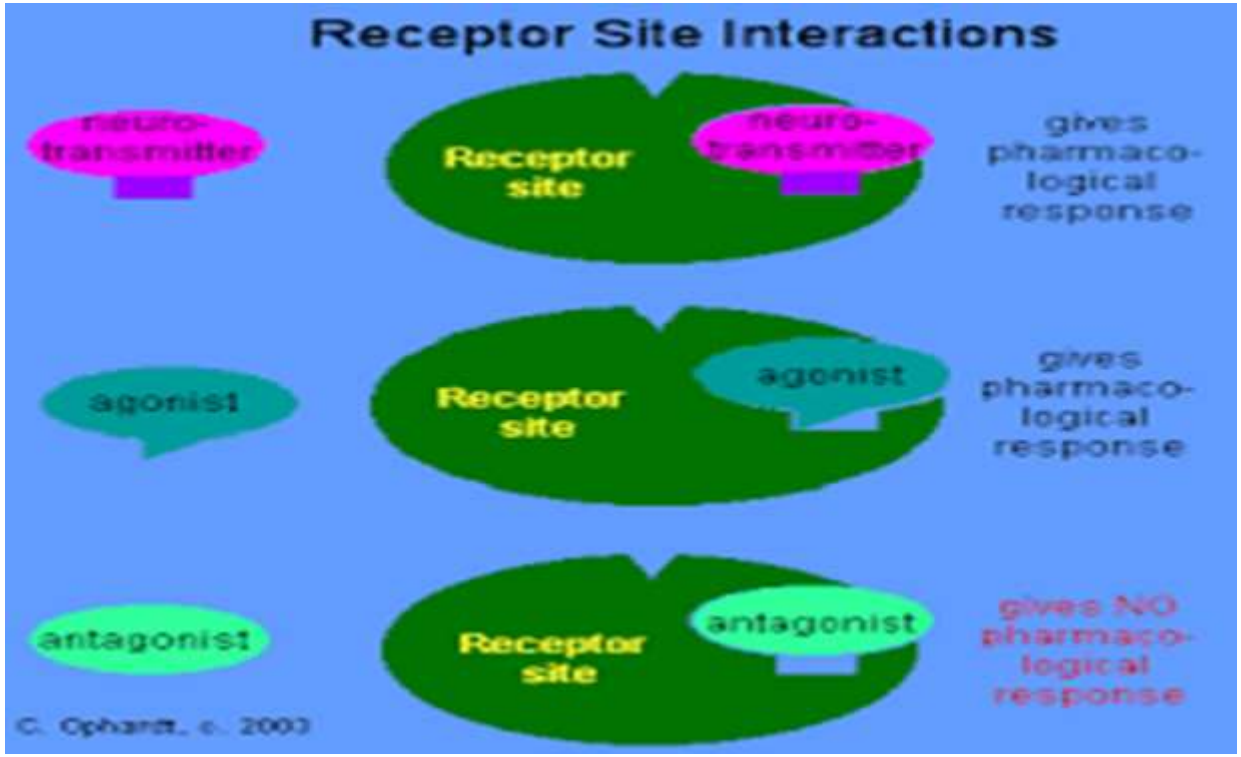
-Structural attributes and substituent patterns responsible for optimum pharmacological activity can often be predicted by statistical techniques such as regression analysis.

-Computerized conformational analysis permits the medicinal chemist to predict drug's three-dimensional(3D)shape that is seen by the receptor . With isolation and structural determination of specific receptors ***and*** availability of computer software that can estimate the 3D shape of receptor, it is now possible to design molecules that will show an optimum fit to the receptor.

DRUG DESIGN STRATEGIES



DRUG DESIGN STRATEGIES



DRUG DISTRIBUTION

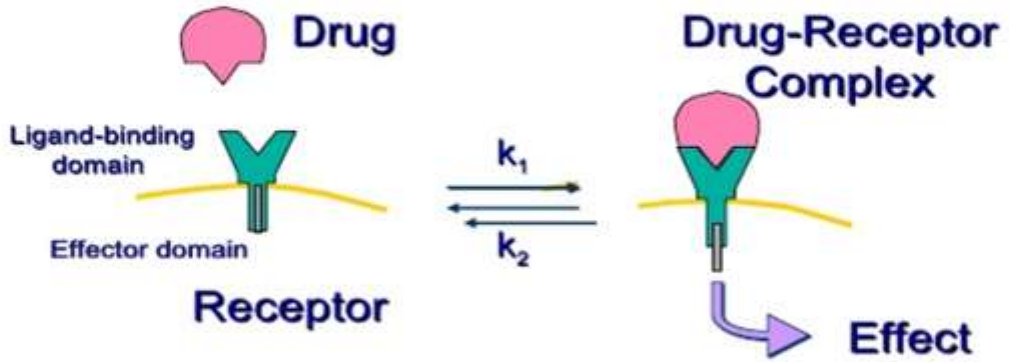
-A drug is a chemical molecule . Following introduction into body, a drug must pass through many barriers , survive alternate sites of attachment and storage and avoid significant metabolic destruction before it reaches site of action, ususlly a receptor on or in a cell . At receptor , the following equilibrium usually holds:

-Ideal drug molecule will show favorable binding characteristics to the receptor and the equilibrium will lie to right.At the same time , drug will be expected to dissociate from the receptor and re-enter the systemic circulation for excretion.

DRUG DISTRIBUTION

Drug(Ligand) ↔ Receptor interaction

Langley (1878)



$$D + R \underset{k_2}{\overset{k_1}{\rightleftharpoons}} DR \longrightarrow \text{Effect}$$

DRUG DISTRIBUTION

-Exceptions include alkylating agents used in cancer chemotherapy , a few inhibitors of acetylcholinesterase , suicide inhibitors of monoamine oxidase...etc. These agents form covalent bonds with receptor(usually an enzyme's active site. In these cases , cell must destroy the receptor or enzyme. But in alkylating agents , cell would be replaced ,ideally with a normal cell. i.e. , usual use of drugs in medical treatment calls for drug's effect to last for a finite period of time. Then if it is to be repeated , drug will be administered again. I f the patient does not tolerate drug well, the agent dissociate from the receptor and be excreted from body and the drug can be replaced with alternatives.

DRUG DISTRIBUTION

1-Oral Administration

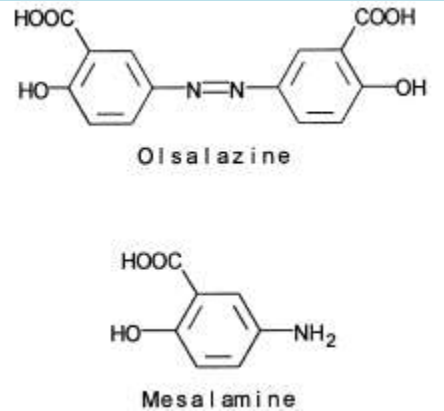
-Examination of obstacle course(following fig) faced by drug.

Oral Administration

- This examination will give an understanding of what is involved in developing a commercially feasible product.
-If drug administered orally, it must go into solution to pass through GIT mucosa. Drugs as true solutions may not remain in solution as they enter acidic stomach and then pass into alkaline intestinal tract(acid-base chemistry).
-Ability of drug to dissolve is governed with several factors, including its chemical structure , variation in particle size and particle surface area ,nature of crystal form ,type of tablet coating and type of tablet matrix.
-Varying dosage form and physical characteristics of drug make it possible to have a drug dissolve quickly or slowly, with the latter being situation for many of sustained-action products . Example orally administered sodium phenytoin , with which variation of both crystal form and tablet adjuvents can significantly alter bioavailability of this drug widely used in epilepsy.

Oral Administration

-Also , chemical modification is used to a limited extent to facilitate a drug reaching its desired target. An example is olsalazine , used in treatment of ulcerative colitis .This compound is a dimer of the pharmacologically active mesalamine(5-aminosalicylic acid).The latter is not effective orally because it is metabolized to inactive forms before reaching colon . The dimeric form passes through a significant portion of the intestinal tract before being cleaved by intestinal bacteria to two equivalents of mesalamine.

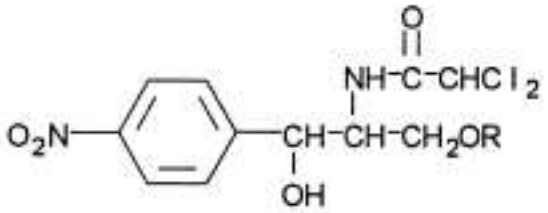


Oral Administration

Therefore any compound passing through GIT will encounter a large number and variety of digestive and bacterial enzymes which ,***in theory*** ,can degrade the drug molecule .***But in practice*** , a new drug entity under investigation will likely be dropped from further consideration if it cannot survive in intestinal tract or its oral bioavailability is low ,necessitating parenteral dosage forms only . Exception is a drug which we do not have its effective alternative or which is more effective than existing products and can be administered by an alternate route, including parenteral, buccal or transdermal.

-These same digestive enzymes can be useful. Chloramphenicol is water-soluble enough(2.5mg/ml)to come in contact with the taste receptors on the tongue, producing an unpalatable bitterness.To mask this bitter taste,the palmitic acid moiety is added as an ester of chloramphenicol's primary alcohol.This reduces the parent drug's water solubility(1.05mg/ml) enough so that it can be formulated as a suspensionn that passes over the bitter taste receptors on the tongue.Once in intestinal tract , ester linkage is hydrolyzed by digestive esterases to the active chloramphenicol.

Oral Administration



Chloramphenicol: R = H

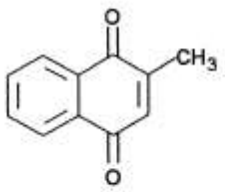
Chloramphenicol Palmitate: R = $CO(CH_2)_{14}CH_3$

Oral Administration

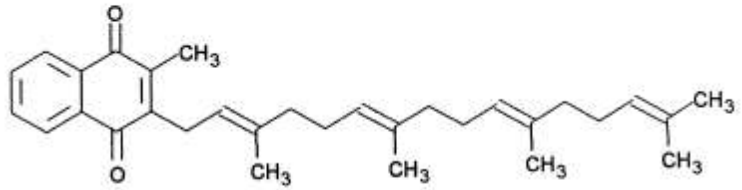
-Olsalazine and chloramphenicol palmitate are prodrugs , cleaved to smaller compounds , one is active .

- Most prodrugs are compounds that are inactive as native form but are easily metabolized to the active agent.

-Others are metabolic precursors to the active form, an example on such type of prodrug is menadione, a simple naphthoquinone that is converted in the liver to phytonadione(vitamine K2).



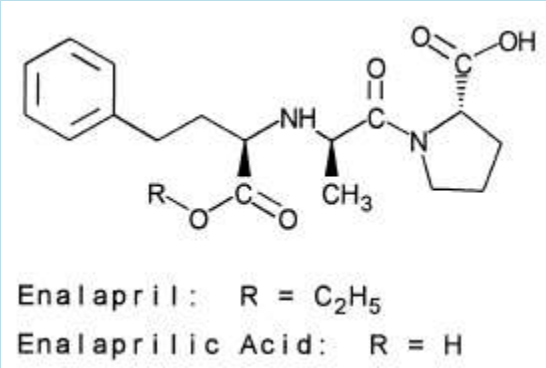
Menadione



Phytonadione (Vitamin $K_{2(20)}$)

Oral Administration

-Occasionally,prodrug approach ususallly is used to enhanced absorption of a drug poorly absorbed from GIT.Enalapril is ethyl ester of enalaprilic acid,an active inhibitor of ACE.The ester prodrug is much more readily absorbed orally than the pharmacologically active carboxylic acid .



Enalapril: R = C_2H_5

Enalaprilic Acid: R = H

Oral Administration

-Unless drug is intended to act locally in GIT, it will have to pass through gastrointestinal mucosal barrier into venous circulation to reach site of receptor. The drug's route involves distribution or partitioning between the aqueous environment of GIT, lipid bilayer cell membrane of the mucosal cells ,possibly the aqueous interior of the mucosal cells, the lipid bilayer membranes on venous side of GIT and the aqueous environment of venous circulation. Some very lipid-soluble drugs may follow route of dietary lipids by becoming part of mixed micelles , incorporating into chylomicrons in mucosal cells into lymph ducts , servicing intestines and finally entering venous circulation via thoracic duct.

-Drug's passage through mucosal cells can be passive or active. Lipid membranes are very complex with a highly ordered structure . Part of this membrane is a series of channels or tunnels that form , disappear and reform . There are receptors that move compounds into cell by "pinocytosis".

Oral Administration

-Drugs that resemble a normal metabolic precursor or intermediate may be actively transported into cell by same system that transports the endogenous compound .

-On other hand, most drug molecules are too large to enter the cell by an active transport mechanism through the passages . The latter , ***many times*** , pass into patient's circulatory system by passive diffusion.

2-Parenteral Administration

-Many times , there will be therapeutic advantages in bypassing intestinal barrier by using parenteral(injectable)dosage forms.
*This is common in patients who, because of illness, cannot tolerate or are incapable of accepting drugs orally .
*Some drugs are so rapidly and completely metabolized to inactive products in the liver (first-pass effect)that oral administration is precluded. ***But*** this does not mean drug administered by injection is not confronted by obstacles. ***IV administration*** places drug directly into circulatory system, where it will be rapidly distributed throughout body, including tissue depots and liver, where most biotransformations occur, in addition to receptors , **whereas *Sc and im injections*** slow distribution of drug, because it must diffuse from site of injection into systemic circulation.

Parenteral Administration

-It is possible to inject the drug directly into specific organs or areas of the body. Intraspinal and intracerebral routes will place the drug directly into the spinal fluid or brain, respectively. This bypasses a specialized epithelial tissue, the blood-brain barrier, which protects the brain from exposure to a large number of metabolites and chemicals.

-The blood-brain barrier is composed of membranes of tightly joined epithelial cells lining the cerebral capillaries.The net result is that the brain is not exposed to the same variety of compounds that other organs are. Local anesthetics are examples of administration of a drug directly onto the desired nerve. A spinal block is a form of anesthesia performed by injecting a local anesthetic directly into the spinal cord at a specific location to block transmission along specific neurons.

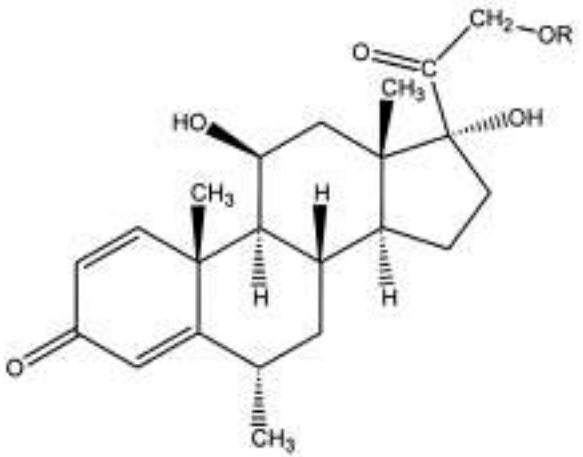
Parenteral Administration

-Most of the injections a patient will experience in a lifetime will be subcutaneous or intramuscular. These parenteral routes produce a depot in the tissues (Fig. 2.1), from which the drug must reach the blood or lymph. Once in systemic circulation, the drug will undergo the same distributive phenomena as orally and intravenously administered agents before reaching the target receptor. In general, the same factors that control the drug's passage through the gastrointestinal mucosa will also determine the rate of movement out of the tissue depot.

Parenteral Administration

-The prodrug approach described previously can also be used to alter the solubility characteristics, which, in turn, can increase the flexibility in formulating dosage forms. The solubility of methylprednisolone can be altered from essentially water-insoluble methylprednisolone acetate to slightly water-insoluble methylprednisolone to water-soluble methylprednisolone sodium succinate. The water-soluble sodium hemisuccinate salt is used in oral, intravenous and intramuscular dosage forms. Methylprednisolone itself is normally found in tablets. The acetate ester is found in topical ointments and sterile aqueous suspensions for intramuscular injection. Both the succinate and acetate esters are hydrolyzed to the active methylprednisolone by the patient's own systemic hydrolytic enzymes (esterases).

Parenteral Administration



Methylprednisolone: R = H

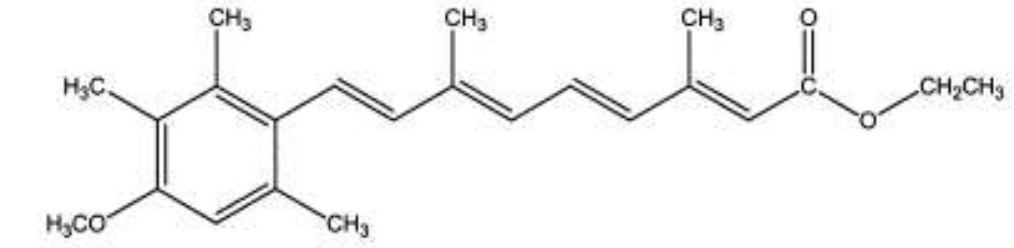
Methylprednisolone Acetate: R = C(=O)CH_3

Methylprednisolone Sodium Succinate: R = C(=O)$CH_2CH_2COO^-$ Na^+

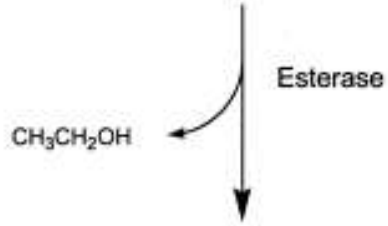
Parenteral Administration

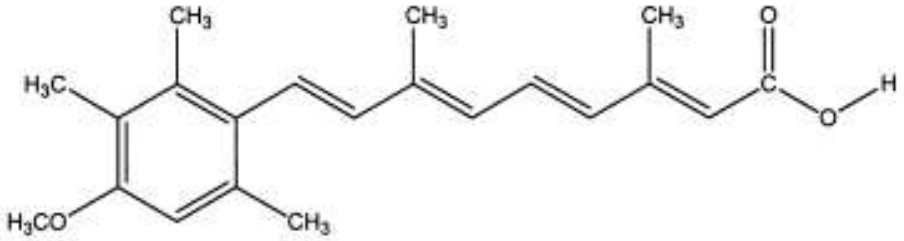
-Another example of how prodrug design can significantly alter biodistribution and biological half-life is illustrated by two drugs based on the retinoic acid structure used systemically to treat psoriasis, a nonmalignant hyperplasia. Etretinate has a 120-day terminal half-life after 6 months of therapy. In contrast, the active metabolite, acitretin, has a 33- to 96-hour terminal half-life. Both drugs are potentially teratogenic. Women of childbearing age must sign statements that they are aware of the risks and usually are administered a pregnancy test before a prescription is issued. Acitretin, with its shorter half-life, is recommended for a woman who would like to become pregnant, because it can clear her body within a reasonable time frame. When effective, etretinate can keep a patient clear of psoriasis lesions for several months.

Parenteral Administration



Etretinate





Acitretin

3-Protein Binding

-Once the drug enters the systemic circulation (Fig. 2.1), it can undergo several events. It may stay in solution, but many drugs will be bound to the serum proteins, usually albumin (Rx. 2.2). Thus, a new equilibrium must be considered. Depending on the equilibrium constant, the drug can remain in systemic circulation bound to albumin for a considerable period and not be available to the sites of biotransformation, the pharmacological receptors, and excretion.

$$\text{Drug} + \text{Albumin} \rightleftharpoons \text{Drug-Albumin Complex}$$

Protein Binding

-Protein binding can have a profound effect on the drug's effective solubility, biodistribution, half-life in the body and interaction with other drugs. A drug with such poor water solubility that therapeutic concentrations of the unbound (active) drug normally cannot be maintained still can be a very effective agent. The albumin–drug complex acts as a reservoir by providing large enough concentrations of free drug to cause a pharmacological response at the receptor.

-

Protein Binding

-Protein binding may also limit access to certain body compartments. The placenta is able to block passage of proteins from maternal to fetal circulation. Thus, drugs that normally would be expected to cross the placental barrier and possibly harm the fetus are retained in the maternal circulation, bound to the mother's serum proteins.

-Protein binding also can prolong the drug's duration of action.

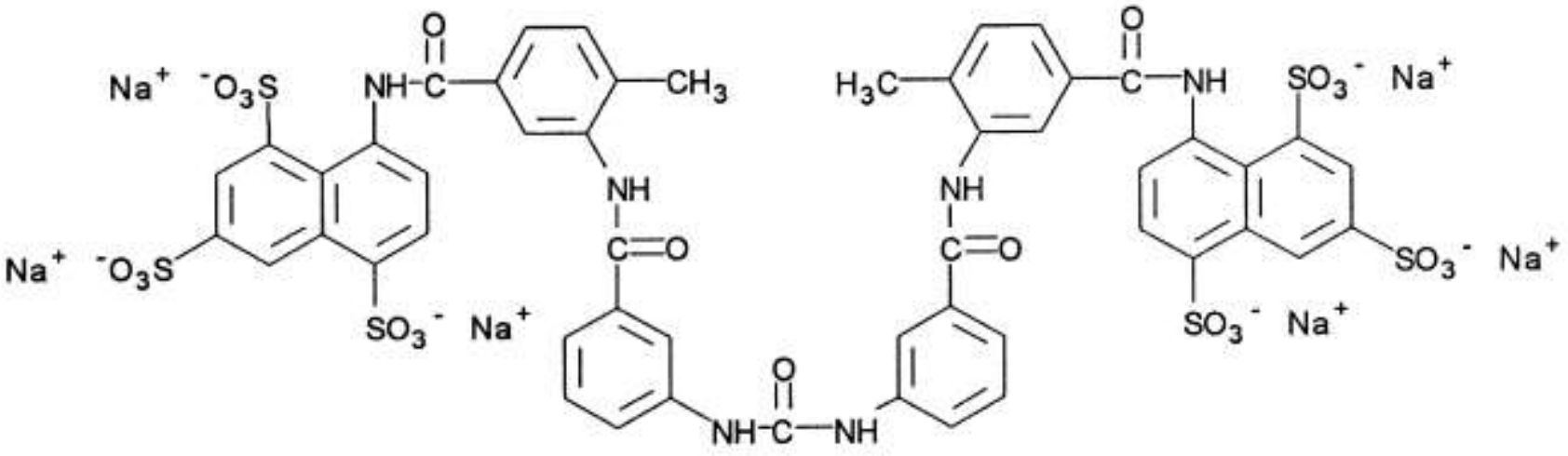
The drug–protein complex is too large to pass through the renal glomerular membranes, preventing rapid excretion of the drug. Protein binding limits the amount of drug available for biotransformation and for interaction with specific receptor sites….

Protein Binding

For example, the large, polar trypanocide suramin remains in the body in the protein-bound form for as long as 3 months (t1/2=50 days). The maintenance dose for this drug is based on weekly administration. At first, this might seem to be an advantage to the patient. It can be, but it also means that, should

the patient have serious adverse reactions, a significant length of time will be required before the concentration of drug falls below toxic levels.

Protein Binding



Suramin Sodium

Protein Binding

-The drug–protein binding phenomenon can lead to some clinically significant drug–drug interactions that result when one drug displaces another from the binding site on albumin. A large number of drugs can displace the anticoagulant warfarin from its albumin-binding sites. This increases the effective concentration of warfarin at the receptor, leading to an increased prothrombin time (increased time for clot formation) and potential hemorrhage.

4-Tissue Depots

-The drug can also be stored in tissue depots. Neutral fat constitutes some 20% to 50% of body weight and constitutes a depot of considerable importance. The more lipophilic the drug, the more likely it will concentrate in these pharmacologically inert depots. The ultrashort-acting, lipophilic barbiturate thiopental's concentration rapidly decreases below its effective concentration following administration. It disappears into tissue protein, redistributes into body fat, and then slowly diffuses back out of the tissue depots but in concentration too low for a pharmacological response. Thus, only the initially administered thiopental is present in high enough concentrations to combine with its receptors. The remaining thiopental diffuses out of the tissue depots into systemic circulation in concentrations too small to be effective (Fig. 2.1), is metabolized in the liver, and is excreted...

Tissue Depots

In general, structural changes in the barbiturate series that favor partitioning into the lipid tissue stores decrease duration of action and induce a rapid central nervous system (CNS) depression. Conversely, the barbiturates with the slowest onset of action and longest duration of action contain the more polar side chains. This latter group of barbiturates both enters and leaves the CNS more slowly than the more lipophilic thiopental.

5-Drug Metabolism

-All substances in the circulatory system, including drugs, metabolites, and nutrients, will pass through the liver .

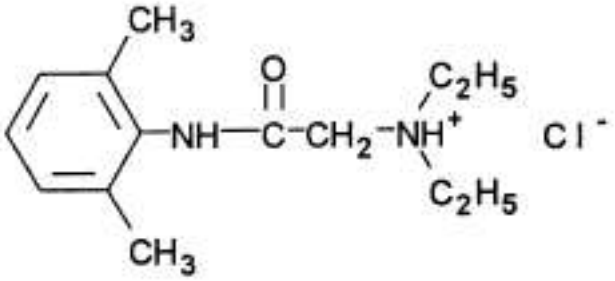
*Most molecules absorbed from the gastrointestinal tract enter the portal vein and are initially transported to the liver.

*A significant proportion of a drug will partition or be transported into the hepatocyte, where it may be metabolized by hepatic enzymes to inactive chemicals during the initial trip through the liver, by what is known as the first -pass effect .

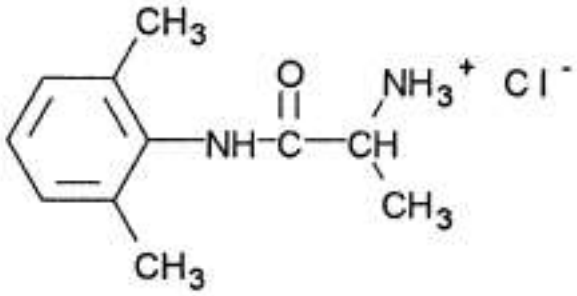
Drug Metabolism

-Lidocaine is a classic example of the significance of the first-pass effect. Over 60% of this local anesthetic antiarrhythmic agent is metabolized during its initial passage through the liver, resulting in it being impractical to administer orally. When used for cardiac arrhythmias, it is administered intravenously. This rapid metabolism of lidocaine is used to advantage when stabilizing a patient with cardiac arrhythmias. Should too much lidocaine be administered intravenously, toxic responses will tend to decrease because of rapid biotransformation to inactive metabolites. An understanding of the metabolic labile site on lidocaine led to the development of the primary amine analog tocainide. In contrast to lidocaine's half-life of less than 2 hours, tocainide's half-life is approximately 15 hours, with 40% of the drug excreted unchanged.

Drug Metabolism



Lidocaine



Tocainide

Drug Metabolism

-A study of the metabolic fate of a drug is required for all new drug products.
*Often it is found that the metabolites are also active.
*Sometimes the metabolite is the pharmacologically active molecule.
*These drug metabolites can provide leads for additional investigations of potentially new products.
*Examples of an inactive parent drug that is converted to an active metabolite include the nonsteroidal antiinflammatory agent sulindac being reduced to the active sulfide metabolite, the immunosuppressant azathioprine being cleaved to the purine antimetabolite 6-mercaptopurine and purine and pyrimidine antimetabolites and antiviral …

Drug Metabolism

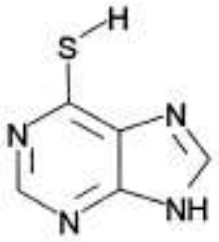
agents being conjugated to their nucleotide form (acyclovir phosphorylated to acyclovir triphosphate).
*Often both the parent drug and its metabolite are active, which has led to additional commercial products, instead of just one being marketed. About 75% to 80% of phenacetin (now withdrawn from the market) is converted to acetaminophen. In the tricyclic antidepressant series , imipramine and amitriptyline are N-demethylated to desipramine and nortriptyline, respectively.

Drug Metabolism

Sulindac: R = $CH_3S(=O)$

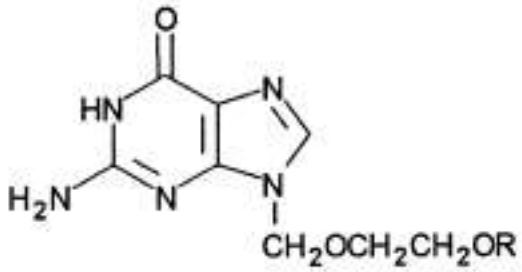
Active Sulfide Metabolite: R = CH_3S

Azathioprine



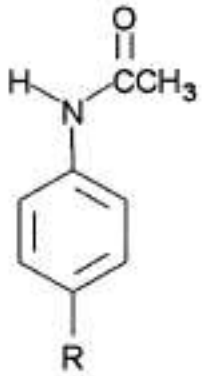
6-Mercaptopurine

Drug Metabolism



Acyclovir: R = H

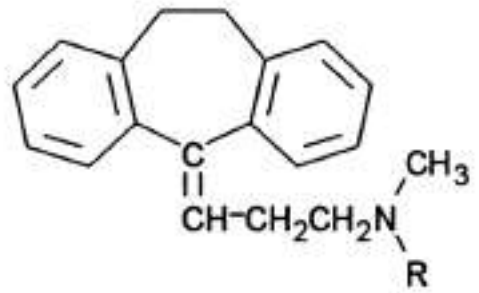
Acyclovir triphosphate: R = O-P-O-P-O-P



Phenacetin: R = OC_2H_5

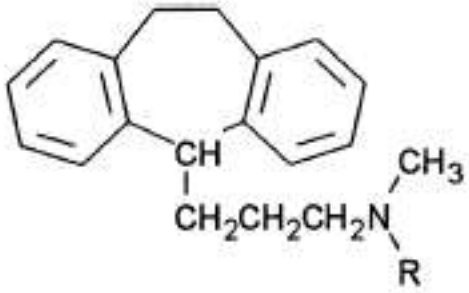
Acetaminophen: R = OH

Drug Metabolism



Amitriptyline: R = CH_3

Nortriptyline: R = H



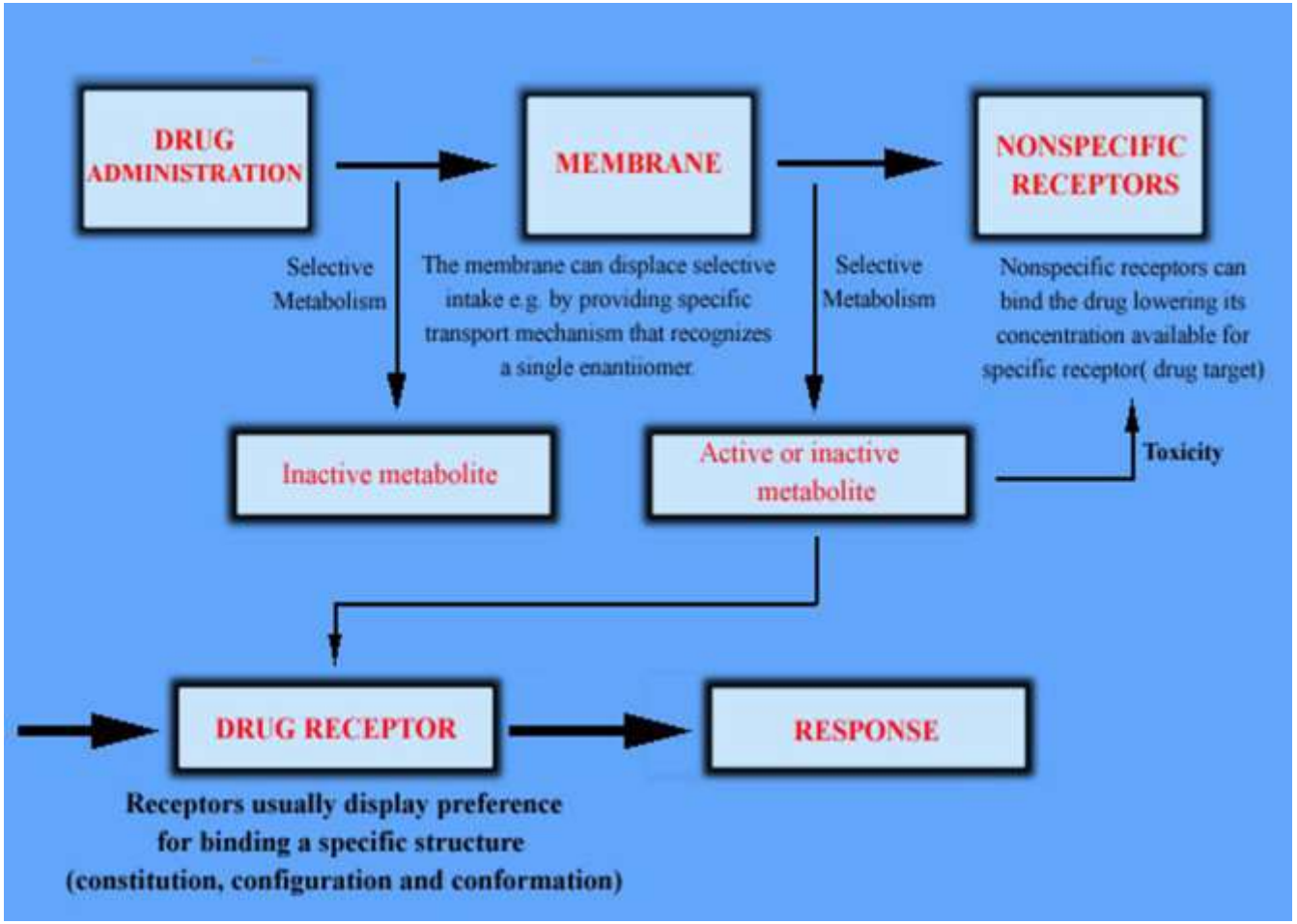
Imipramine: R = CH_3

Desipramine: R = H

Drug Metabolism

Although a drug's metabolism can lead to inconvenience and compliance problems with the patient, it is fortunate that the body has the ability to metabolize foreign molecules (xenobiotics). Otherwise, many of these substances could remain in the body for years. This has been the complaint against certain lipophilic chemical pollutants, including the once very popular insecticide dichlorodiphenyltrichloroethane (DDT). After entering the body, these chemicals reside in body tissues, slowly diffusing out of the depots and potentially harming the individual on a chronic basis for several years. They can also reside in tissues of commercial food animals that have been slaughtered before the drug has washed out of the body.

Drug Metabolism



6-Excretion

-The main ***excretion route*** of a drug and its metabolites is through the kidney.
-For some drugs, enterohepatic circulation (Fig. 2.1), in which the drug reenters the intestinal tract from the liver through the bile duct, can be an important part of the ***agent's distribution*** in the body and ***route of excretion.***

Either the drug or drug metabolite can reenter systemic circulation by passing once again through the intestinal mucosa. A portion of either also may be excreted in the feces.

-Nursing mothers must be concerned, because drugs and their metabolites can be excreted in human milk and be ingested by the nursing infant.

Excretion

-Drug metabolism can be conceptualized as occurring in two stages or phase:

-Intermediate metabolites that are pharmacologically active usually are produced by ***phase I reactions***.

-The products from the phase I chemistry are converted into inactive, usually water-soluble end products by phase II reactions.

-The latter, commonly called conjugation reactions, can be thought of as synthetic reactions that involve addition of water-soluble substitue-nts .In human drug metabolism, the main conjugation reactions add glucuronic acid, sulfate, or glutathione.

Excretion

-Obviously, drugs that are bound to serum protein or show favorable partitioning into tissue depots are going to be metabolized and excreted more slowly for the reasons discussed previously.

Excretion

This does not mean that drugs that remain in the body for longer periods of time can be administered in lower doses or be taken fewer times per day by the patient. Several variables determine dosing regimens, of which the affinity of the drug for the receptor is crucial. If the equilibrium does not favor formation of the drug–receptor complex, higher and usually more frequent doses must be administered. Further, if partitioning into tissue stores or metabolic degradation and/or excretion is favored, it will take more of the drug and usually more frequent administration to maintain therapeutic concentrations at the receptor.

7-Receptor

With the possible exception of general anesthetics , the working model for a pharmacological response consists of a drug binding to a specific receptor. Many drug receptors are the same as those used by endogenously produced ligands. Cholinergic agents interact with the same receptors as the neurotransmitter acetylcholine. Synthetic corticosteroids bind to the same receptors as cortisone and hydrocortisone. Often, receptors for the same ligand are found in various tissues throughout the body. The nonsteroidal anti-inflammatory agents inhibit the prostaglandin-forming enzyme cyclooxygenase, which is found in nearly every tissue. This class of drugs has a long list of side effects with many patient complaints.

Receptor

Note in Figure 2.1 that, depending on which receptors contain bound drug, there may be desired or undesired effects. This is because various receptors with similar structural requirements are found in several organs and tissues. Thus, the nonsteroidal anti-inflammatory drugs combine with the desired cyclooxygenase receptors at the site of the inflammation and the undesired cyclooxygenase receptors in the gastrointestinal mucosa, causing severe discomfort and sometimes ulceration.

Receptor

One of the second-generation antihistamines, fexofenadine, is claimed to cause less sedation because it does not readily penetrate the blood-brain barrier. The rationale is that less of this antihistamine is available for

the receptors in the CNS, which are responsible for the sedation response characteristic of antihistamines. In contrast, some antihistamines are used for their CNS depressant activity because a significant proportion of the administered dose is crossing the blood-brain barrier relative to binding to

the histamine H1 receptors in the periphery.

Receptor

Although it is normal to think of side effects as undesirable, they sometimes can be beneficial and lead to new products. The successful development of oral hypoglycemic agents used in the treatment of diabetes began when it was found that certain sulfonamides had a hypoglycemic effect. Nevertheless, a real problem in drug therapy is patient compliance in taking the drug as directed. Drugs that cause serious problems and discomfort tend to be avoided by patients.

Receptor

At this point, let us assume that the drug has entered the systemic circulation (Fig. 2.1), passed through the lipid barriers, and is now going to make contact with the receptor. As illustrated in Reaction 2.1, this is an equilibrium process. A good ability to fit the receptor favors binding and the desired pharmacological response. In contrast, a poor fit favors the reverse reaction. With only a small amount of drug bound to the receptor, there will be a much smaller pharmacological effect. If the amount of drug bound to the receptor is too small, there may be no discernible response. Many variables contribute to a drug's binding to the receptor. These include the structural class, the 3D shape of the molecule, and the types of chemical bonding involved in the binding of the drug to the receptor.

Receptor

Most drugs that belong to the same pharmacological class have certain structural features in common. The barbiturates act on specific CNS receptors, causing depressant effects; hydantoins act on CNS receptors, producing an anticonvulsant response; benzodiazepines combine with the gama-aminobutyric acid (GABA) receptors, with resulting anxiolytic activity; steroids can be divided into such classes as corticosteroids, anabolic steroids, progestogens, and estrogens, each acting on specific receptors; nonsteroidal antiinflammatory agents inhibit enzymes required for the prostaglandin cascade; penicillins and cephalosporins inhibit enzymes required to construct the bacterial cell wall; and tetracyclines act on bacterial ribosomes.

Receptor

-Before isolation and characterization of receptors has occurred , the concept of receptors began as postulate . Molecules with certain structural features would elucidate a specific biological response. Very slight changes in structure could cause significant changes in biological activity . These structural variations could increase or decrease activity or change an agonist into an antagonist. This early and fundamentally correct interpretation called for the drug (ligand) to fit onto some surface (the receptor) that had fairly strict structural requirements for proper binding of the drug. The initial receptor model was based on a rigid lock-and-key concept, with the drug (key) fitting into a receptor (lock).

Receptor

It has been used to explain why certain structural attributes produce a predictable pharmacological action. This model still is useful, although one must realize that both the drug and the receptor can have considerable flexibility. Molecular graphics, using programs that calculate the preferred conformations of drug and receptor, show that the receptor can undergo an adjustment in 3D structure when the drug makes contact. Using space-age language, the drug docks with the receptor.

Receptor

More complex receptors now are being isolated, characterized, and cloned. The first receptors to be isolated and characterized were the reactive and regulatory sites on enzymes. Acetylcholinesterase, dihydrofolate reductase, angiotensin, and human immunodeficiency virus (HIV) protease-converting enzyme are examples of enzymes whose active sites (the receptors) have been modeled. Most drug receptors probably are receptors for natural ligands used to regulate cellular biochemistry and function and to communicate between cells. Receptors include a relatively small region of a macromolecule, which may be an isolatable enzyme, structural and functional component of a cell membrane, or a specific intracellular substance such as a protein or nucleic acid.

Receptor

Specific regions of these macromolecules are visualized as being oriented in space in a manner that permits their functional groups to interact with the complementary functional groups of the drug. This interaction initiates changes in structure and function of the macromolecule, which lead ultimately to the observable biological response. The concept of spatially oriented functional areas forming a receptor leads directly to specific structural requirements for functional groups of a drug, which must complement the receptor.

Receptor

It now is possible to isolate membrane-bound receptors, although it still is difficult to elucidate their structural chemistry, because once separated from the cell membrane , these receptors may lose their native shape. This is because the membrane is required to hold the receptor in its correct tertiary structure. One method of receptor isolation is affinity chromatography. In this technique, a ligand, often an altered drug molecule known to combine with the receptor, is attached to a chromatographic support phase. A solution containing the desired receptor is passed over this column . The receptor will combine with the ligand. It is common to add a chemically reactive grouping to the drug, resulting in the receptor and drug covalently binding with each other. The drug–receptor complex is washed from the column and then characterized further.

Receptor

A more recent technique uses recombinant DNA. The gene for the receptor is located and cloned. It is transferred into a bacterium, yeast, or animal, which then produces the receptor in large enough quantities to permit further study. Sometimes it is possible to determine the DNA sequence of the cloned gene. By using the genetic code for amino acids, the amino acid sequence of the protein component of the receptor can be determined, and the receptor then modeled , producing an estimated 3D shape. The model for the receptor becomes the template for designing new ligands . Genome mapping has greatly increased the information on receptors. Besides the human genome, the genetic composition of viruses, bacteria, fungi, and parasites has increased the possible sites for drugs to act. The new field (proteomics) studies the proteins produced by structural genes.

Receptor

-The receptor components of the membranes appear to be mainly protein. They constitute a highly organized region of the cell membrane. The same type of molecular specificity seen in such proteins as enzymes and antibodies is also a property of drug receptors. The nature of the amide link in proteins provides a unique opportunity for the formation of multiple internal hydrogen bonds, as well as internal formation of hydrophobic, van der Waals, and ionic bonds by side chain groups, leading to such organized structures as the α-helix, which contains about four amino acid residues for each turn of the helix. An organized protein structure would hold the amino acid side chains at relatively fixed positions in space and available for specific interactions with a small molecule.

Receptor

Proteins can potentially adopt many different conformations in space without breaking their covalent amide linkages. They may shift from highly coiled structures to partially disorganized structures, with parts of the molecule existing in random chain or folded sheet structures, dependent on the environment. In the monolayer of a cell membrane, the interaction of a small foreign molecule with an organized protein may lead to a significant change in the structural and physical properties of the membrane. Such changes could well be the initiating events in the tissue or organ response to a drug, such as the ion-translocation effects produced by interaction of acetylcholine and the cholinergic receptor.

Receptors

The information now available on relationships between chemical structure and biological activity strongly supports the concept of flexible receptors. The fit of drugs onto or into macromolecules is ***rarely*** an all-or-none process as pictured by the earlier lock-and-key concept of a receptor. Rather, the binding or partial insertion of groups of moderate size onto or into a macromolecular pouch appears to be a continuous process, at least over a limited range, as indicated by the frequently occurring regular increase and decrease in biological activity as one ascends a homologous series of drugs.

Receptor

A ***range*** of productive associations between drug and receptor may be pictured, which leads to agonist responses, such as those produced by cholinergic and adrenergic drugs. Similarly, strong associations may lead to unproductive changes in the configuration of the macromolecule, leading to an antagonistic or blocking response, such as that produced by anticholinergic agents and HIV protease inhibitors. Although the fundamental structural unit of the drug receptor is generally considered to be protein, it may be supplemented by its associations with other units, such as mucopolysaccharides and nucleic acids.

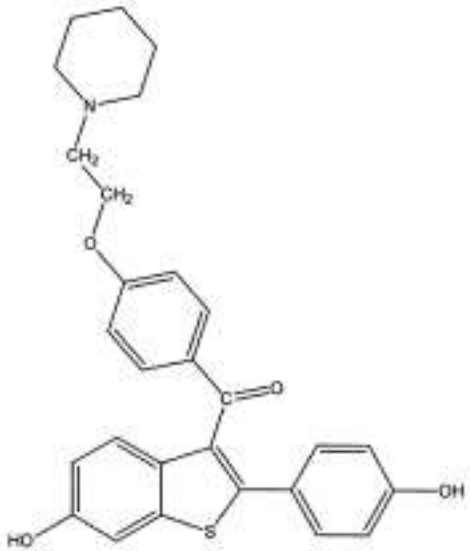
Receptor

Humans (and mammals in general) are very complex organisms that have developed specialized organ systems. The receptors are not distributed equally throughout the body. It now is realized that, depending on the organ in which it is located, the same receptor class may behave differently. This can be advantageous by focusing drug therapy on a specific organ system, but it can also cause adverse drug responses because the drug is exerting two different responses based on the location of the receptors. An example is the ***selective*** estrogen receptor modulators (SERMs). They cannot be classified simply as agonists or antagonists. Rather, they can be considered variable agonists and antagonists. Their ***selectivity*** is very complex because it depends on the organ in which the receptor is located.

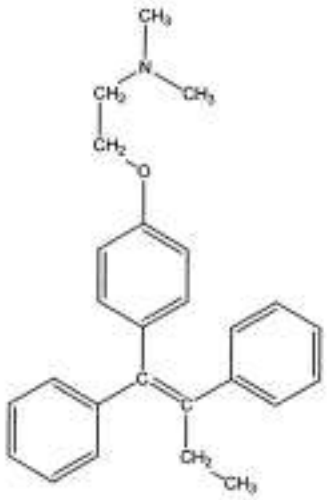
Receptor

This complexity can be illustrated with tamoxifen and raloxifene (Fig. 2.2). Tamoxifen is used for estrogen-sensitive breast cancer and for reducing bone loss from osteoporosis. Unfortunately, prolonged treatment increases the risk of endometrial cancer because of the ***response*** from the uterine estrogen receptors. Thus, tamoxifen is an estrogen antagonist(***no response***) in the mammary gland and an agonist in the uterus and bone. In contrast, raloxifene does not appear to have much agonist property in the uterus but, like tamoxifen, is an antagonist in the breast and agonist in the bone.

Receptor



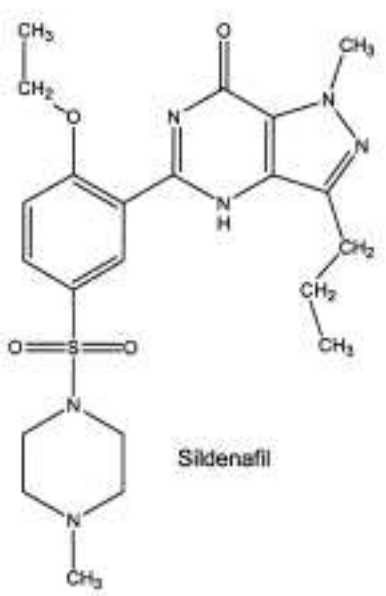
Raloxifene

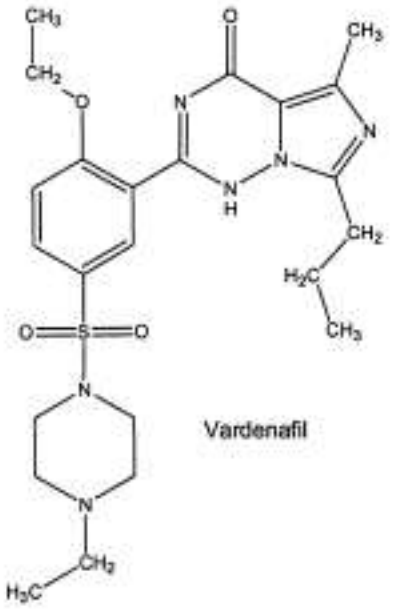


Tamoxifen

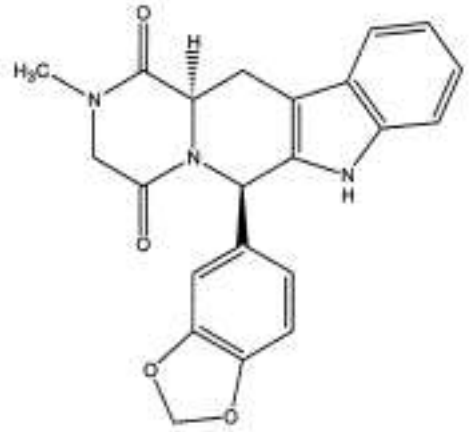
Figure 2.2 • Selective SERMs.

Receptor





Receptor



Tadalafil

Figure 2.3 • Examples of phosphodiesterase type 5 inhibitors.

receptor

There are a wide variety of phosphodiesterases throughout the body. These enzymes hydrolyze the cyclic phosphate esters of adenosine monophosphate (cAMP) and guanosine monophosphate (cGMP). Although the substrates for this family of enzymes are cAMP and cGMP, there are differences in the active sites. Figure 2.3 illustrates three drugs used to treat erectile dysfunction (sildenafil, tadalafil, and vardenafil). These three take advantage of the differences in active site structural requirements between phosphodiesterase type 5 and the other phosphodiesterases. They have an important role in maintaining a desired lifestyle: treatment of erectile dysfunction caused by various medical conditions. The drugs approved for this indication were discovered by accident. The goal was to develop a newer treatment of angina. The approach was to develop phosphodiesterase inhibitors that would prolong the activity of cGMP. The end result was drugs that were not effective inhibitors of the phosphodiesterase that would treat angina, but were effective inhibitors of the one found in the corpus cavernosum. The vasodilation in this organ results in penile erection.

Summary

One of the goals is to design drugs that will interact with receptors at specific tissues. There are several ways to do this, including (a) altering the molecule, which, in turn, can change the biodistribution; (b) searching for structures that show increased specificity for the target receptor that will produce the desired pharmacological response while decreasing the affinity for undesired receptors that produce adverse responses; and (c) the still experimental approach of attaching the drug to a monoclonal antibody that will bind to a specific tissue antigenic for the antibody. Biodistribution can be altered by changing the drug's solubility, enhancing its ability to resist being metabolized (usually in the liver), altering the formulation or physical characteristics of the drug, and changing the route of administration.

Summary

If a drug molecule can be designed so that its binding to the desired receptor is enhanced relative to the undesired receptor and biodistribution remains favorable, smaller doses of the drug can be administered. This, in turn, reduces the amount of drug available for binding to those receptors responsible for its adverse effects.

Summary

The medicinal chemist is confronted with several challenges in designing a bioactive molecule. A good fit to a specific receptor is desirable, but the drug would normally be expected to dissociate from the receptor eventually. The specificity for the receptor would minimize side effects. The drug would be expected to clear the body within a reasonable time. Its rate of metabolic degradation should allow reasonable dosing schedules and, ideally, oral administration. Many times, the drug chosen for commercial sales has been selected from hundreds of compounds that have been screened. It usually is a compromise product that meets a medical need while demonstrating good patient acceptance.

Preparation and Standardization of 1 *N* NaOH Solution

- **Sodium hydroxide is a strong base that is usually used to prepare standard alkaline solutions useful for volumetric analysis of acidic compounds.**
- **Sodium hydroxide is hygroscopic and can react with atmospheric carbon dioxide.**

$$2NaOH + CO_2 \longrightarrow Na_2CO_3 + H_2O$$

contaminant
(water soluble)

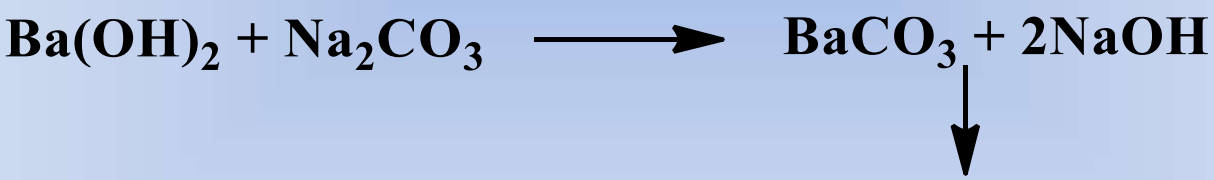
Preparation and Standardization of 1 *N* NaOH Solution

- **preparation of 100 mL of 1 *N* NaOH solution**

  Dissolve 4.5 g of sodium hydroxide in 100 mL distilled water, allow to cool, and then add saturated barium hydroxide solution drop wise with stirring until a precipitate is formed. Leave aside allowing for complete precipitation, filter, and collect the filtrate to be standardized against 1 *N* HCl solution.

Preparation and Standardization of 1 *N* NaOH Solution

contaminant
(water soluble)



water insoluble

Preparation and Standardization of 1 *N* NaOH Solution

- **standardization**

$$NaOH + HCl \longrightarrow NaCl + H_2O$$

- 1 *N* HCl solution is used as a secondary standard

- phenolphthalein is used as the indicator

colourless $\longrightarrow$	***pink***
***p*H: 8.3**	**10**

Preparation and Standardization of 1 *N* NaOH Solution

❑ procedure

- **wash the burette with the D. W. and the titrant (NaOH)**
- **fill the burette with NaOH to a level (adjust it)**
- **wash a 20 mL – bulb pipette with D. W. then by a little of HCl solution; fill it to the mark with the acid**
- **transfer the acid into a clean conical fask; add D.W. (50 mL)**
- **add 2 drops of phenolphthalein indicator**
- **start titration by adding NaOH solution drop wise with continuous stirring until the solution changes from colourless to pink**
- **record the volume of NaOH solution used and calculate the normality**

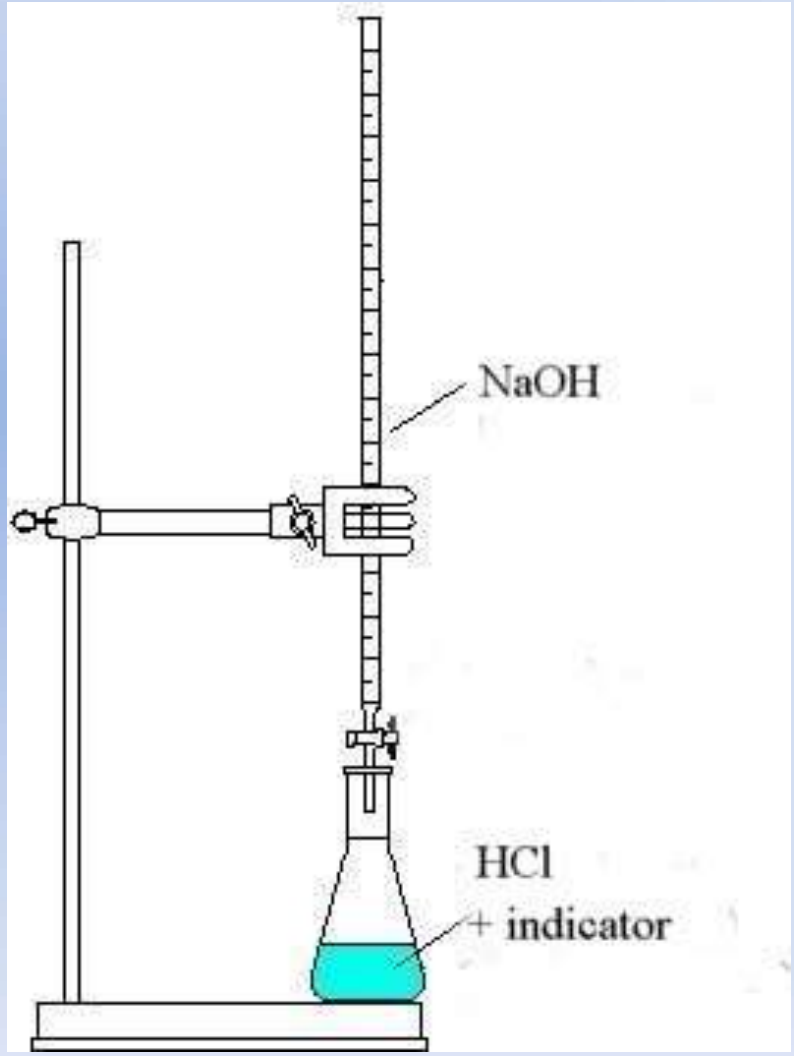
Note ***wash the burette with water thoroughly***

Preparation and Standardization of 1 *N* NaOH Solution

- **procedure**

Preparation and Standardization of 1 *N* NaOH Solution

- **titration apparutus**



Preparation and Standardization of 1 *N* NaOH Solution

end point
(pink)

Preparation and Standardization of 1 *N* NaOH Solution

- **calculations**

$$\underbrace{N_1 \times V_1}_{\text{NaOH}} = \underbrace{N_2 \times V_2}_{\text{HCl}}$$

N_1 :the normality of NaOH solution
V_1 :the volume of NaOH solution used
N_2 :the normality of HCl
V_2 : volume of HCl solution used (20mL in our experiment)

Preparation and Standardization of 1 *N* NaOH Solution

- **Home work**

Why have you used 4.5 g of NaOH to prepare 100 mL of 1 *N* NaOH solution?