

**AS PER PCI REGULATIONS  
SECOND YEAR B. PHARM. | SEMESTER-IV**

# **MEDICINAL CHEMISTRY-I**

**Dr. K. G. BOTHARA**



**A Text Book Of**

# **MEDICINAL CHEMISTRY-I**

**As Per PCI Regulations**

**SECOND YEAR B. PHARM.**  
**Semester IV**

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# Preface

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In the last two decades, the phases of ever-growing volume and ever-changing nature of the drug information are witnessed. This is mainly due to an increase in the rate of introduction of new drugs and an increase in the number and depth of published work on both, new as well as existing drugs. Above facts necessitated addition of all recent information wherever it deserves, while presenting the first edition of this book. New chapters on drugs used in neurodegenerative diseases and Anti-migraine agents have been included.

The book was appreciated in all corners of the profession. It has now attained the reputation as a class-room text book for undergraduate and post-graduate students of pharmacy. However, our aim remains the same as to present a review of basic principles of medicinal chemistry and to explain the effects of structural modifications of the lead nucleus on the selectivity of action, duration of action and on the intensity and frequency of adverse-effects.

Each chapter is revised thoroughly to meet the needs of future facts and fantasies. Since this book is written basically for degree students, a backbone understanding in basic disciplines is assumed.

I wish to place on record my sincere thanks to the publisher Mr. D. K. Furia for his kind cooperation. I am greatly indebted to my colleagues for their generous help and criticism. I also wish to acknowledge indebtedness to all who have assisted for the completion of the book.

Suggestions from all corners of the profession are welcome. I am responsible for any deficiencies or errors that might have remained and would be grateful if readers would call them to my attention.



# Syllabus

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## Unit-I

(10 Hours)

### **Introduction to Medicinal Chemistry**

### **History and development of medicinal chemistry**

### **Physicochemical properties in relation to biological action**

Ionization, Solubility, Partition coefficient, Hydrogen bonding, Protein binding, Chelation, Bioisosterism, Optical and Geometrical isomerism.

### **Drug metabolism**

Drug metabolism principles – Phase I and Phase II.

Factors affecting drug metabolism including stereo chemical aspects.

## Unit-II

(10 Hours)

### **Drugs acting on Autonomic Nervous System**

#### **Adrenergic Neurotransmitters :**

Biosynthesis and catabolism of catecholamine.

Adrenergic receptors (Alpha and Beta) and their distribution.

#### **Sympathomimetic agents : SAR of Sympathomimetic agents**

Direct acting : Nor-epinephrine, Epinephrine, Phenylephrine and Dopamine,

Methyldopa, Clonidine, Dobutamine, Isoproterenol, Terbutaline, Salbutamol\*, Bitolterol, Naphazoline, Oxymetazoline and Xylometazoline.

- Indirect acting agents: Hydroxyamphetamine, Pseudoephedrine, Propylhexedrine.
- Agents with mixed mechanism: Ephedrine, Metaraminol.

#### **Adrenergic Antagonists:**

**Alpha adrenergic blockers:** Tolazoline\*, Phentolamine, Phenoxybenzamine, Prazosin, Dihydroergotamine, Methysergide.

**Beta adrenergic blockers:** SAR of beta blockers, Propranolol\*, Metibranolol, Atenolol, Betazolol, Bisoprolol, Esmolol, Metoprolol, Labetolol, Carvedilol.

## Unit-III

(10 Hours)

**Cholinergic neurotransmitters:** Biosynthesis and catabolism of acetylcholine.

Cholinergic receptors (Muscarinic & Nicotinic) and their distribution.

#### **Parasympathomimetic agents: SAR of Parasympathomimetic agents**

**Direct acting agents:** Acetylcholine, Carbachol\*, Bethanechol, Methacholine, Pilocarpine.

#### **Indirect acting/Cholinesterase inhibitors (Reversible & Irreversible):**

Physostigmine, Neostigmine\*, Pyridostigmine, Edrophonium chloride, Tacrine hydrochloride, Ambenonium chloride, Isofluorophate, Echothiophate iodide, Parathione, Malathion.

**Cholinesterase reactivator:** Pralidoxime chloride.

**Cholinergic Blocking agents:** SAR of cholinolytic agents

**Solanaceous alkaloids and analogues:** Atropine sulphate, Hyoscyamine sulphate, Scopolamine hydrobromide, Homatropine hydrobromide, Ipratropium bromide\*.

**Synthetic cholinergic blocking agents:** Tropicamide, Cyclopentolate hydrochloride, Clidinium bromide, Dicyclomine hydrochloride\*, Glycopyrrolate, Methantheline bromide, Propantheline bromide, Benztropine mesylate, Orphenadrine citrate, Biperidine hydrochloride, Procyclidine hydrochloride\*, Tridihexethyl chloride, Isopropamide iodide, Ethopropazine hydrochloride.

## Unit-IV

(08 Hours)

### Drugs Acting on Central Nervous System

#### A. Sedatives and Hypnotics:

**Benzodiazepines:** SAR of Benzodiazepines, Chlordiazepoxide, Diazepam\*, Oxazepam, Chlorazepate, Lorazepam, Alprazolam, Zolpidem

**Barbiturates:** SAR of barbiturates, Barbitol\*, Phenobarbital, Mephobarbital, Amobarbital, Butobarbital, Pentobarbital, Secobarbital

#### Miscellaneous:

Amides & imides: Glutethimide.

Alcohol & their carbamate derivatives: Meprobamate, Ethchlorvynol.

Aldehyde & their derivatives: Triclofos sodium, Paraldehyde.

#### B. Antipsychotics

**Phenothiazines:** SAR of Phenothiazines - Promazine hydrochloride, Chlorpromazine hydrochloride\*, Triflupromazine, Thioridazine hydrochloride, Piperacetazine hydrochloride, Prochlorperazine maleate, Trifluoperazine hydrochloride.

**Ring Analogues of Phenothiazines:** Chlorprothixene, Thiothixene, Loxapine succinate, Clozapine.

**Fluoro buterophenones:** Haloperidol, Droperidol, Risperidone.

**Beta amino ketones:** Molindone hydrochloride.

**Benzamides:** Sulpiride.

**C. Anticonvulsants:** SAR of Anticonvulsants, mechanism of anticonvulsant action

**Barbiturates:** Phenobarbital, Methobarbital.

**Hydantoins:** Phenytoin\*, Mephenytoin, Ethosuximide.

**Oxazolidine diones:** Trimethadione, Paramethadione.

**Succinimides:** Phensuximide, Methsuximide, Ethosuximide\*

**Urea and monoacylureas:** Phenacemide, Carbamazepine\*

**Benzodiazepines:** Clonazepam

**Miscellaneous:** Primidone, Valproic acid, Gabapentin, Felbamate

**Drugs Acting on Central Nervous System****General anesthetics:**

**Inhalation anesthetics:** Halothane\*, Methoxyflurane, Enflurane, Sevoflurane, Isoflurane, Desflurane.

**Ultra short acting barbiturates:** Methohexital sodium\*, Thiamylal sodium, Thiopental sodium.

**Dissociative anesthetics:** Ketamine hydrochloride.\*

**Narcotic and non-narcotic analgesics**

**Morphine and related drugs:** SAR of Morphine analogues, Morphine sulphate, Codeine, Meperidine hydrochloride, Anileridine hydrochloride, Diphenoxylate hydrochloride, Loperamide hydrochloride, Fentanyl citrate\*, Methadone hydrochloride\*, Propoxyphene hydrochloride, Pentazocine, Levorphanol tartarate.

**Narcotic antagonists:** Nalorphine hydrochloride, Levallorphan tartarate, Naloxone hydrochloride.

**Anti-inflammatory agents:** Sodium salicylate, Aspirin, Mefenamic acid\*, Meclofenamate, Indomethacin, Sulindac, Tolmetin, Zomepirac, Diclofenac, Ketorolac, Ibuprofen\*, Naproxen, Piroxicam, Phenacetin, Acetaminophen, Antipyrine, Phenylbutazone.



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*Chapter...1*

# INTRODUCTION TO MEDICINAL CHEMISTRY

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## ♦ SYNOPSIS ♦

**1.1 INTRODUCTION****1.2 PROCESSES OF DRUG ABSORPTION****1.3 DISTRIBUTION OF DRUGS****1.4 STORAGE DEPOTS****1.5 METABOLISM AND EXCRETION****1.6 HISTORY AND DEVELOPMENT OF  
MEDICINAL CHEMISTRY**

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## 1.1 INTRODUCTION

The desired pharmacological response of a drug can only be achieved if it is present at the sites of action in an appropriate concentration for sufficiently long time. This appropriate concentration is generally governed by many factors. The important amongst them are:

- (i) Amount and frequency of drug administered.
- (ii) Route of administration.
- (iii) Factors affecting drug absorption, distribution and elimination.

### **(A) Factors affecting accessibility of drugs to the active sites:**

Once the drug is administered in the body, it undergoes a chain of complex events till it reaches to its site of action. The process by which a drug is released in the body from its dosage form is known as 'absorption'. Since the duration and the intensity of drug action is a function of rate at which the drug is absorbed, an understanding of the factors which can affect the rate of absorption of a drug is necessary. These factors include,

- (i) concentration of the drug administered or dose.
- (ii) route of administration.
- (iii) drug solubility.
- (iv) in case of solid dosage forms, the rate of dissolution may govern the rate of absorption.
- (v) in the local application, the blood circulation to the site of application and area of absorbing surface are the important factors.
- (vi) physico-chemical parameters of the drug play an important role in governing the rate of absorption.

These factors are lipid solubility, dissociation constant, pH-partition theory, dissolution rate, Donnan membrane equilibrium principle, salt forms, effective surface areas, crystal form, complexation, viscosity, surface active agents and drug stability in gastrointestinal tract.

### **(B) Passage of drugs through natural membranes:**

To yield the desired effect in optimum measure, the drug should reach the site of action quickly and remain there in adequate concentration for adequate period of time. To reach the site of action, drug molecules have to cross one or more membranous barriers. This passage is a function of physical and chemical properties and the pharmaceutical make up of the drug.

#### **Biological Membrane:**

The biological membrane, though lipoidal in nature, is intermittently interrupted by small aqueous channels or pores of different sizes. In addition to this, the absorption of the drug molecules may be accelerated by the electrical charges present on these membranes on both sides.

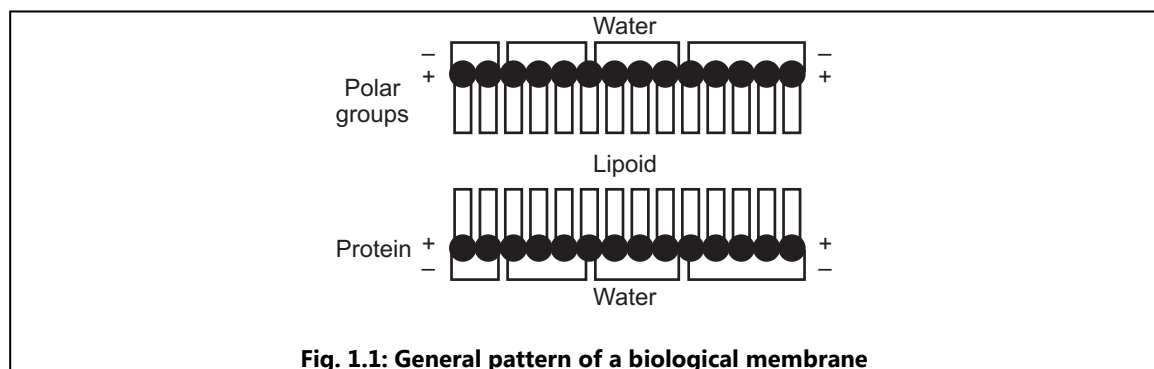
#### **Main Variables:**

Many weak acidic or basic drugs do not present in their ionized form. Similarly, many drugs are lipid soluble. These features characterise slow elimination of such drugs. Hence, metabolism of such drugs usually tends to produce such drug metabolites which are more polar and hence can be excreted readily from the body. If a drug is already polar in nature, it may not even require the bio-transformation and may be excreted unchanged. Though kidney is a major site for elimination of a drug or drug metabolite, other sites like milk, saliva, sweat, tears, bile, intestine, lungs and skin are also recognized as the sites for elimination.

#### **Structure of Membrane:**

A membrane surrounds all living cells. It separates components of vital metabolic processes from the external medium. Thus, the membrane provides an identity to a cell. Since a cell depends upon and communicates with the external environment, its membrane must allow the passage of certain molecules while preventing the passage of others. Thus, cell membranes are sites of a large variety of cellular processes ranging from permeability, transport and excitability to intercellular interaction, morphological differentiation and fusion.

Biological membrane is essentially a two dimensional matrix. It is a membrane of thickness equal to two lipid molecules i.e., some 50 to 60 Å. The three major components of the membrane are proteins, lipids and water. Although the different membranes have the same general pattern, they vary widely in the proportion in which proteins and lipids form their composition.



**Fig. 1.1: General pattern of a biological membrane**

Most biological membranes consist of a lipid bilayer which contains proteins and other molecules that serves as recognition sites, signal transmitters or parts of entrance and exit. The thermodynamic properties of membranes are then described in terms of surface properties such as surface chemical potential and surface tension.

Communication between the inside and outside of a cell includes the exchange of metabolites and electrical signals, the flow of heat and changes in shape. These processes depend on the differences in temperature, pressure and electrochemical potential on both sides of the membrane. Temperature differences cause heat flow, pressure differences cause changes in shape and electrochemical potential differences cause molecular transport and electrical signals. Usually, membranes consist an assortment of lipid molecules with diverse chemical structures, together with proteins and sometimes polysaccharides. The lipids are typically fatty acid esters that differ in the length of fatty acid chain, the degree of unsaturation, the change or polarity of the esterifying group and the number of fatty acids esterified per molecule.

**Table 1.1: Some important functions of biological membranes**

Function	Membrane
Permeability barrier of ions and molecules	Plasma
Ion accumulation or active transport	Plasma, nerve
Conduction of nervous impulse	Nerve axon
Conversion of light into chemical energy	Thylakoid
Conversion of light into electrical energy	Visual receptor
Oxidative and photosynthetic phosphorylation	Mitochondrial chloroplast
Site of immunological reactions	Plasma
Protein synthesis	Cell organelle
Phagocytosis and pinocytosis	Plasma

Proteins may constitute upto 80% of the cell membrane (e.g., red cell membrane) or as little as 18%. (e.g., nerve myelin). Variations are even wider in bacteria. The lipid contents also vary widely from membrane to membrane, constituting upto 80% of myelin, but only 15% of skeletal muscle membrane. Not only the total lipid content varies, but also the chemical nature of the lipids. The hydrocarbon chains vary widely in the nature of polar groups, length and saturation extent.

Although biological membranes are known to be complex and highly variable both in structure and in function, it seems probable that there is a common structural basis to all of them. Under the electron microscope, all the natural membranes thus far examined are in the order of 100 Å in thickness and are generally considered to consist of a lipid bilayer of the Gorter - Grendel type with adsorbed protein or non-lipid layers.

## 1.2 PROCESSES OF DRUG ABSORPTION

The main processes by which a drug molecule crosses the natural barriers are:

- (a) Simple diffusion
- (b) Diffusion of ions across the membrane
- (c) Facilitated diffusion
- (d) Active transport
- (e) Pore transport
- (f) Filtration
- (g) Phagocytosis and pinocytosis.

**(a) Simple diffusion:** The drugs are absorbed from gastrointestinal tract, cross the intestinal endothelium by simple diffusion. This process can be defined as, the flow of drug across a cell membrane from a solution of higher concentration ( $C_A$ ) to the solution of lower concentration ( $C_B$ ) without energy utilization.

The important features of simple diffusion are:

- (i) It depends and proceeds along a concentration gradient.
- (ii) It does not involve energy expenditure.
- (iii) Partition coefficient plays a governing role in the transport of lipophilic drugs by this process.
- (iv) The transport of ionic or polar drugs by this process is influenced by the difference in pH on both the sides of the membrane.
- (v) The process terminates as soon as the concentration of free drug is same on both the sides of the membrane (i.e., at equilibrium).

Simple diffusion can be expressed mathematically using Fick's law, which is as follow:

$$\frac{dm}{dt} \propto \frac{dc}{dx}$$

$$\therefore \frac{dm}{dt} = -DA \frac{dc}{dx} \quad \dots (1.1)$$

where,

$\frac{dm}{dt}$  = Rate of drug diffusion

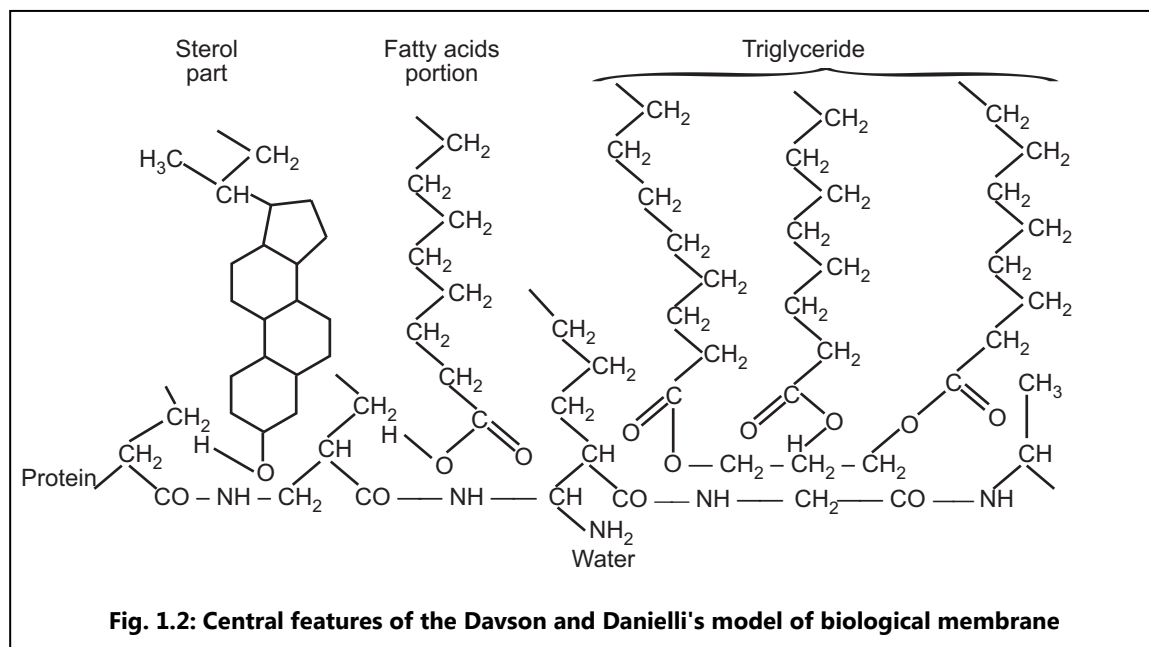
A = Surface area of the absorbing membrane

dc = Difference in solute concentration on both sides of the membrane

dx = Membrane thickness

(i.e.  $\frac{dc}{dx}$  = concentration gradient)

D = Proportionality constant. Here it is distribution coefficient. It includes all other factors that may affect drug, nature and condition of the absorbing membrane



Movement of GIT membrane facilitates the contact of drug molecules with the absorbing surface. This leads to an increased absorption of drug. Food in the stomach interferes in the drug absorption, hence drugs that inhibit gastric emptying (e.g. atropine, amphetamine, morphine etc.) may decrease the rate of absorption.

If we replace  $\frac{D}{dx}$  by another term, P (i.e. diffusion constant), equation (1.1) can be rewritten as,

$$T = -PA \cdot dc \quad \dots (1.2)$$

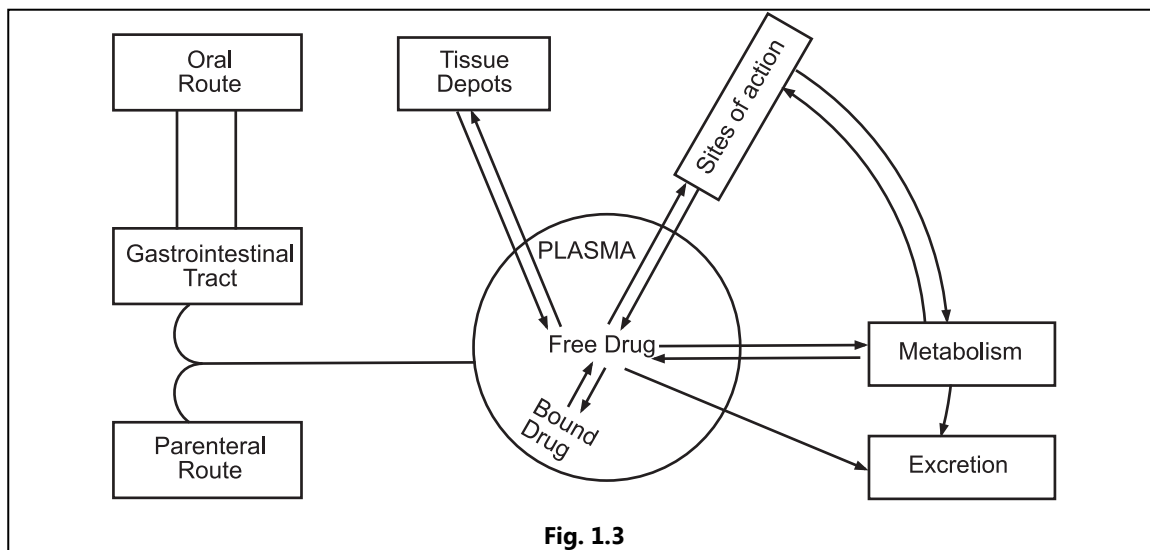


Fig. 1.3

Fick in 1885, derived this equation and now it is known as **Fick's law of diffusion**. The minus sign indicates the passage of a drug from the area of higher concentration to the area of lower concentration. The continuous removal of the drug molecules from the serosal side of the intestinal wall by blood circulation tends to keep the concentration on the other side always negligible. This serves as an additional driving force for the transport of drug molecules.

The rate of diffusion of a drug is a function of an area of absorbing surface. It is much greater in the small intestine due to its folding and refolding into valves of Kerckring and villi. It, thus provides an absorbing area of some 4500 m<sup>2</sup>.

The pH difference across the cell membrane and the dissociation constant (pKa or pKb) of drug also govern the rate of drug absorption. Since, most of the drugs are either weak acids or weak bases, their acidity value (ratio of ionised and unionised forms) is dependent upon pH and pKa. Hence, the dissociation constant plays a vital role in determining the ability of drug to cross cell membrane e.g. barbiturates.

**(b) Diffusion of ions across the membrane:** Sometimes, a potential difference develops which leads to polarisation of biological membrane. One side of the membrane becomes positively charged and other side gets a negative charge. When a positively charged ion (i.e. cation) comes in contact with the positively charged face of the membrane, it will be kicked away from the membrane. Similarly, anions will be driven away by negatively charged face of the membrane in the opposite direction. This process works on the forces of repulsion and naturally does depend upon the electrochemical concentration gradient i.e. on bioelectrical properties of the membrane generated due to polarisation of the membrane.

**(c) Facilitated diffusion:** As the name indicates, it is an accelerated movement or diffusion of molecules that can not be justified by their lipophilicity or molecular size. This diffusion proceeds generally along the concentration gradients. Such diffusion is termed as

downhill diffusion (i.e. from higher concentration to lower concentration). Downhill diffusion requires no net expenditure of energy. A series of acceptor-donor macromolecules carry out this diffusion. But sometimes, facilitated diffusion also transports molecules against concentration gradient. This is known as uphill diffusion where energy is expended. Uphill diffusion is also termed as Active transport.

The accelerated diffusion of drug molecules is brought out by carrier macromolecules which oscillate back and forth across the cell-membrane. The loose complex is formed between carrier and drug molecule. Arriving at another side, loose complex dissociates to relieve the molecule and carrier returns back to lift again a new passenger. The process to certain degree, exhibits substrate specificity. Facilitated diffusion can be well illustrated with the example of transport of antibiotics like, valinomycin or gramicidin A. The eight carbonyl oxygens of the four valine residues in valinomycin skeleton, face inward, forming a cage within which, potassium ions can easily be held by co-ordinate bonds. Thus, potassium ions can get entry through the hydrophobic interior of the membrane when it is in "sound-sleep" within a mosaic of hydrophobic side chain of the antibiotic. Gramicidin A acts as transport antibiotic by forming channels that transverse the membrane.

Limitations of this process include:

- (a) Its saturable nature due to limited number of carrier macromolecules and
- (b) Competitive inhibition of transport of one drug by the presence of another drug bearing similar structural features.

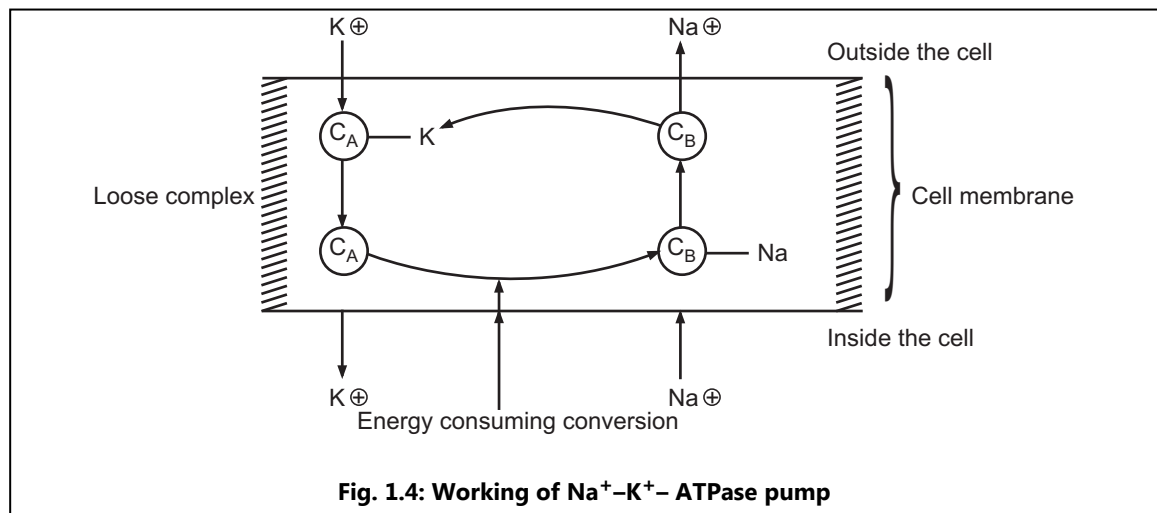
**(d) Active transport:** Some substances diffuse across the biological membrane at much more faster rate that cannot be accounted on the basis of their lipid solubility or molecule size. This transport which proceeds against the concentration gradient and utilizes a series of specialised carrier moieties is termed as 'active transport of drug'.

This carrier aided transport system is characterized by,

- (i) utilization of energy supplied by metabolic activity of membrane,
- (ii) proceeds against the concentration gradient,
- (iii) absorption rate is independent of concentration.

The mechanisms that bring these and similar movements are often termed as pumps e.g.,  $\text{Ca}^{++}$  ATPase pump,  $\text{Na}^+$ ,  $\text{K}^+$  ATPase pump etc. Under the conditions of non-availability of energy, the material thus transported, drifts back again until equilibrium on both sides of the barrier is reached.

Active transport is identical in most of the aspects with facilitated transport. The only difference exists between facilitated transport and active transport is, that the former does not utilize energy (i.e. proceeds along concentration gradient) i.e. downhill diffusion whereas the latter process proceeds against the concentration gradient i.e. uphill diffusion and needs energy consumption. In the case of ionic molecules, transport may occur against an electrochemical potential gradient. The exact mechanism of active transport is still not clear



but it would appear that, on the mucosal surface side of GIT, carrier proteins form a loose complex with the drug molecule. This complex trains away the drug molecule to the serosal side where the complex dissociates to relieve its passenger.

The carrier may then return to the mucosal surface empty handed or may pick up another molecule during its journey back to mucosal side. Before picking up another molecule, it is involved in an energy consuming chemical reaction that converts the carrier protein ( $\text{C}_A$ ) into a new form ( $\text{C}_B$ ). The new form  $\text{C}_B$ , releases that molecule to mucosal side and undergoes a spontaneous change to its original form,  $\text{C}_A$ .

### Limitations:

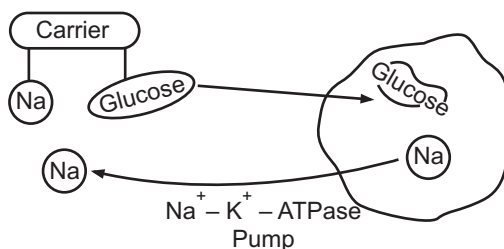
(a) Active transport is site specific as well as a substrate specific process. It means that special carrier channels are appointed to carry particular types of chemical structures. Similarly these substrates are usually absorbed from their corresponding specific sites located in a limited segment of the small intestine. For example, ileum is a site of diffusion for bile acids.

(b) Since carrier channels with a specific carrier molecules are allotted to transport drugs from particular chemical structural class, the carrier system becomes saturated,

- (i) if the drug is present at higher concentration or/and
- (ii) if another substrate of close structural similarity is simultaneously administered.

(c) Substrates that interfere with cell metabolism or in energy generation, may cause non-competitive inhibition of active transport system.

Active transport plays an important role in renal tubule reabsorption, secretion of  $\text{H}^+$  into the stomach, accumulation of iodide ions in the thyroid gland, absorption of glucose, amino acids, some vitamins and metabolites in intestine, absorption processes across placenta and blood brain barrier. The process is of great importance and enables the cell to accumulate metabolites from an external environment where the concentration of the substance may be relatively very low, to excrete unwanted substance, to develop membrane potentials and probably to maintain a normal cell volume.



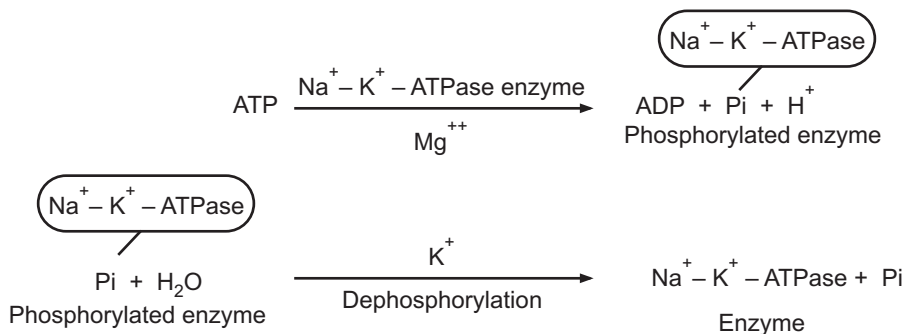
**Fig. 1.5: Co-transport of Glucose**

The active transport of glucose across biological membrane is aided by sodium ions. The glucose molecule and sodium ion both, bind to a specific carrier protein. This complex when enters the cell, the sodium ion is effluxed out through the operation of  $\text{Na}^+ - \text{K}^+$  ATPase pump. Such aided type of transport mechanism is termed as co-transport.

$\text{Na}^+ - \text{K}^+ - \text{ATPase}$  pump is widely distributed in the cell-membranes and especially present in high number in different secretory cells along with excitable tissue such as nerve and muscle cell. It is mainly concerned with the transport of amino acid and glucose during nerve excitability and maintenance of cell-volume. The enzyme,  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  was discovered in 1957 by Jens Skou in the cell-membrane. It hydrolyses ATP molecule to release energy necessary for functioning of this pump. The enzyme and the pump, both are tightly bound with the plasma membrane. The hydrolysis of ATP molecule needs the presence of  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Mg}^{++}$  ions.

The phosphorylation reaction is catalysed by  $\text{Na}^+$  and  $\text{Mg}^{++}$  ions whereas the dephosphorylation needs the presence of  $\text{K}^+$  ions.

The  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  enzyme is the pharmacological receptor for digitalis. Digitalis like drugs bind to the external surface of the enzyme. Cardiac glycosides induce conformational changes and inhibit dephosphorylation reaction of the  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  enzyme.



**Fig. 1.6**

**(e) Pore transport:** The aqueous filled pores or channels are present across the cell-membrane. The diffusion of small sized polar molecules is mainly governed by these channels. The diameter of these pores was estimated to be near about 4°A which serves as a major limitation to the transport process. It is an example of passive diffusion where the rate of transport depends upon the concentration of drug and does not utilize energy. Various electrolytes, urea, low molecular weight sugars, etc. are transported by this mechanism.

**(f) Filtration:** The natural membrane consists of numerous pores of different sizes embedded in it, which generally control the diffusion of small sized molecules of water-soluble or lipid-insoluble substance. If a mechanical pressure (hydrostatic pressure) is imposed on the biological membrane, the drug molecules will ooze out to the other side. Such transport mechanism is termed as filtration. It means,

$$\text{Filtration} = \text{Simple diffusion} + \text{Hydrostatic pressure}$$

The hydrostatic force arises due to the pressure of a drug solution (solvent drag) at one side of the membrane which imposes its pressure at the site of absorption. These pores may have electrical charges that may influence the diffusion of charged bodies, like cations or anions.

In summary, there are three possible routes through which a polar substance can be passively transported across a membrane. These are:

- (i) Diffusion down a concentration gradient (polar transport).
- (ii) Diffusion down a gradient of electric potential (ion transport).
- (iii) Filtration.

In this case of diffusion restricted by a lipid barrier, the penetrating molecule can enter the cell provided it has the appropriate solubility characteristics to dissolve first in the lipid of the membrane and then in the aqueous phase on the other side. This mechanism does not require aqueous pores in the membrane. It has been supported experimentally by the correlation of lipid-water partition coefficients with membrane penetration rates in the case of many non-electrolytes.

**(g) Phagocytosis and pinocytosis:** Droplets of extracellular fluid along with solute molecules are carried into the cell through the formation of vacuoles.

Phagocytosis is described as cell eating process whereas pinocytosis is referred to as cell drinking process. Both these processes are examples of engulfing of extracellular fluid and substances dissolved in it. Phagocytosis can carry macromolecules (such as proteins) into the cell, whereas pinocytosis has limitations of carrying large molecules.

Principle behind these processes has been exploited to develop new drug delivery system. Recently, techniques have been developed to envelope drug molecules by 'liposomes' which can be engulfed by the cells by pinocytosis.

### 1.3 DISTRIBUTION OF DRUGS

If a drug is administered into the body, blood circulation serves as a transport system for it, to reach at its site of action. The most prominent organs like heart, kidney, liver and brain share major portion of the drug, thus characterising the first or initial phase of distribution. The entry of drugs into the cells depends upon many mechanisms. Very small water-soluble molecules and ions (e.g.,  $K^+$ ,  $Cl^-$ ) evidently diffuse through aqueous channels. Lipid-soluble molecules of any size diffuse freely through the cell-membranes. Water-soluble molecules and ions of moderate size including the ionic forms of most drugs, can not enter cells readily except by special transport mechanisms. Drug that is too large to pass through any pore and is also practically insoluble in the membrane, can form a lipid-soluble complex at the membrane surface. The complex then moves by diffusion within the membrane. In a complex biological system like human body, along its way to the site of action, a drug may meet with a number of outward instances which control the distribution of the drug. These instances, however, are dependent upon the physico-chemical properties of a drug and may be one of the following types:

- (1) A considerable amount of drug administered may be retained by reversible storage depots.
- (2) The drug may undergo certain metabolic alteration by biological enzyme systems before it reaches to its site of action, which may result into more or less active form.
- (3) Before a drug gets a chance to act on its normal site, it may be excreted unchanged or in its metabolic form.

### 1.4 STORAGE DEPOTS

Plasma proteins, certain tissues, neutral fat, bone and transcellular fluids (gastrointestinal tract), are found to act as drug reservoirs or storage sites for drug.

The drug stored in these depots is in equilibrium with that in plasma and is released as the plasma concentration of the drug falls below its therapeutic concentration. Thus, the plasma concentration of the drug is maintained which sustains and prolongs the duration of action of the drug.

**Plasma Proteins:** Approximately 6.5% of the blood constitute the proteins, of which 50% is albumin. Most drugs bound to plasma proteins in the albumin fraction; binding to other plasma proteins, generally occur to a much less extent. Albumin has a net negative charge but can interact with anions as well. The binding generally involves ion-ion interaction which is further strengthened by the presence of secondary binding like hydrogen bonding (non-ionic polar portions), hydrophobic and van der Waal's forces (non-polar portions) of the molecule. Ionization is thus not a major factor in the specificity and intensity of protein binding. The protein binding is found to be a reversible process.

Protein binding reduces diffusion of the drug to the sites of action, metabolism and excretion. The size of drug-protein complex is large and hence cannot pass through glomerular filtration which prolongs its duration of action. Protein binding also delays the metabolism of the drug.

Since protein binding is rather a non-selective process, other drugs with similar physico-chemical characteristics, may exert an indirect biological effect by displacing active substances from protein binding which may result into (a) dangerous, adverse effects and (b) misinterpretation about the actions and dose of the drug.

**Tissue Reservoirs:** Depending upon its physico-chemical characteristics, a drug may be stored in various tissues, like liver (antimalarial drugs), thyroid (Iodine), lung, spleen and muscle. Tissue binding of drugs usually occurs with proteins, phospholipids or nucleoproteins and is generally a reversible process.

**Neutral Fat:** Since fat constitutes around 10% (starvation) to 50% of the total body weight, it serves as a main storage site for drugs having a high partition coefficient (lipid/water system) or a high lipid solubility (thiobarbiturates).

Other drugs which get readily deposited into the fat, are adrenergic blocking agents (dibenamine), neuromuscular blocking agents (hexa-fluorenum) etc.

**Bone:** Heavy metals (like lead or radium), divalent metal ion chelating agents and antibiotic (tetracycline group) are the examples of the compounds which, in considerable concentration, are retained by bone.

## 1.5 METABOLISM AND EXCRETION

The termination of drug effect is caused by biotransformation (alteration in the structure of a drug due to the action of enzymes or due to other biochemical processes) and excretion.

The effects of drugs are terminated by: redistribution between the compartments, storage, excretion of the unchanged drug and its metabolites. Compounds having a molecular weight less than about 400 are excreted in urine; larger molecules are cleared by the liver. Bile is excreted into duodenum, where a proportion of drugs (e.g., antibiotics, cardiac glycosides, vitamins) is reabsorbed by the enterohepatic cycle.

### Drugs excreted unchanged in urine

- |                            |                                       |
|----------------------------|---------------------------------------|
| • Digitalis                | • Phenformin/metformin/chlorpropamide |
| • Bretylium                | • Gonadotropin                        |
| • Methotrexate             | • Thiacetazone                        |
| • Sodium stilboglucuronate | • Amino glycoside                     |
| • Acyclovir                | • Neomycin                            |
| • Gallamine                | • Norfloxacin.                        |

## 1.6 HISTORY AND DEVELOPMENT OF MEDICINAL CHEMISTRY

Medicinal chemistry is a discipline or intersection of organic chemistry, biochemistry, anatomy-physiology and pharmacology. There is a long history of plants being used to treat various disease, specially in early civilizations of Egypt, India, China. In the beginning of nineteenth century, the isolation of a number of alkaloids including morphine (1803), quinine (1823) and atropine (1833) led the foundation of medicinal chemistry. Thus, in the very beginning, medicinal chemistry was restricted to ancient folk medicines and application of natural product chemistry.

The use of organic chemistry in attempts to synthesize the semisynthetic/fully synthetic derivatives (structural mimicry) of these plant origin drugs started after 1860s. For example, aspirin (1899) from salicin, benzocaine (1892) from cocaine, etc. Due to the improvisation of biological activity in these semisynthetic/fully synthetic rigid analogs, the scientists started believing about a definite relationship between chemical structure and biological activity (SAR studies). Thus Crum-Brown and Fraser in 1869 proposed that cells can respond to the signals from specific molecule. Ehrlich in 1890s expressed the idea of specific receptors for biologically active compounds – lock and key relationship.

The first phase of modern medicinal chemistry (1890 – 1940) witnessed the development of effective drugs for the treatment of infectious diseases like tuberculosis, typhoid, malaria, infective hepatitis, tetanus, cholera, etc. These drugs were the outcome of mostly the study of microbiology.

Dale (1910) and Ablquist (1946) were amongst the first to propose receptor sub-types for cholinergic and adrenergic receptors respectively. During the same period synthetic antimalarials such as pamaquine (1926), mepacrine (1932) and chloroquine (1943) were invented to replace natural alkaloid, quinine.

Sulphonamides (1936), penicillin antibiotics (1940), streptocine (1944), chloramphenicol (1949) and tetracyclines (1949) emerged as powerful anti-infective agents and used to save life of several thousands soldiers during Second World War.

The second phase of modern medicinal chemistry (1940-1980) had seen the introduction of all modern therapeutic classes. These drugs were the outcome of organic chemistry.

The period (1945 – 1965) may be considered as 'Golden Era' in the history of medicinal chemistry where in many important therapeutic classes of drugs were invented. Examples include corticosteroids (1949), oral contraceptives (1959), antipsychotics (1950), antidepressants (1955), benzodiazepines (1960), and hypoglycemics (1957).

**Table 1.2: Medicinally active alkaloids**

Sr. No.	Active Compound	Herbal Source	Year of Isolation	Activity
01	Morphine	Papaver somniferum	1803	Analgeric
02	Emetine	Ipecacuanha	1816	Vomiting inducer
03	Caffeine	Coffea arabica beans	1819	CNS stimulant
04	Colchicine	Colchicum autumnale	1820	Treatment of rheumatism and gout
05	Quinine	Cinchona bark	1823	Anti-malarial
06	Atropine	Atropa belladonna	1833	Anti-cholinergic
07	Physostigmine	Calabar bean	1864	To treat glaucoma
08	Cocaine	Coca leaves	1884	Local anaesthetics
09	Digoxin	Digitalis purpurea	1930	Cardiotonic
10	Reserpine	Rauwolfia serpentina	1952	Anti-hypertensive

The teratogenic effect (birth of deformed children when mother consumed the drug during pregnancy) came to limelight after thalidomide (sedative) episode in 1960. This made new drug registration and regulations more stringent and tight. The second important event was birth of QSAR (Quantification of SAR) by Hansch in 1964. Both these events have a drastic impact on conventional thinking process of a medicinal chemist and changed further tools and techniques used in medicinal chemistry.

The spectacular advances in medicinal chemistry over the past 03 decades are based on artificial intelligence. These mainly include QSAR, molecular modelling, fragment library, comparative molecular field analysis (CoMFA), pharmacophore mapping, genomic drug, recombinant drugs, proteomics, homology modelling, high throughput screening, virtual screening, etc. All these novel tools helped to reduce huge amount of data leading to faster results.

Inventing a new drug molecule is a complex, costly and time consuming process. It may take around 12-20 years for a single new drug with an estimated cost of about US \$ 1.5 billions to reach from pipeline to patients. However, with the involvement of artificial intelligence, the process of drug discovery has less “failures” due to elimination of human errors.

**Table 1.3: Evolution of Medicinal Chemistry**

Sr. No.	Category	Prototype example	Year of introduction
01	Narcotic analgesics	Morphine	1803
02	General anaesthesia	Diethyl ether	1842
03	CNS depressant	KBr	1850
04	Nonsteroidal anti-inflammatory	Aspirin	1853
05	Sedative/Hypnotics	Phenobarbital	1911
06	Antibiotics	Penicilline	1930
07	Antibacterial	Sulphonamide	1936
08	Anti-allergics	Tripellenamine	1938
09	Antipsychotics	Chlorpromazine	1950
10	Steroid anti-inflammatory	Corticostroid	1952
11	Hypoglycemics	Metformine	1957
12	Benzodiazepine	Diazepam	1960
13	Beta blockers	Propranolol	1965
14	Recombinant drugs	Insulin	1970
15	Antacids	Ranitidine	1976
16	Proton pump inhibitors	Omeprazole	1979
17	COX-2 inhibitors	Celecoxib	1999
18	Gene therapy	Human genom	2000



# PHYSICO-CHEMICAL PARAMETERS AND DRUG ACTION

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### ♦ SYNOPSIS ♦

2.1 INTRODUCTION	2.8 COMPLEXATION
2.2 FERGUSON PRINCIPLE	2.9 SURFACE-ACTIVITY
2.3 IONIZATION	2.10 OXIDATION-REDUCTION POTENTIAL
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2.5 PARTITION COEFFICIENT	2.12 OPTICAL AND GEOMETRICAL ISO-MERISM
2.6 HYDROGEN BONDING	
2.7 PROTEIN BINDING	

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## 2.1 INTRODUCTION

Drug design is an integrated developing discipline which portends an era of 'tailored drug', a drug sans side-effects. It seeks to explain effects of biological compounds on the basis of molecular structures or its physico-chemical properties involved. It studies the processes by which the drugs produce their effects; how they react with the protoplasm to elicit a particular pharmacological effect or response, how they are modified or detoxified, metabolised or eliminated by organism. These concepts are the building stones upon which the edifice of drug design is built.

In an effort to interpret the SAR of a drug, two main approaches have emerged viz. (1) The group and moiety approach and (2) Integral approach.

The former places emphasis on the significance of certain chemical groups in the drug molecule as a whole and particularly concerned with overall physico-chemical properties. Modulating the structure of a drug implies introduction, elimination or substitution of certain groups in the drug. This may lead to the development of a parallel drug with the characteristics similar to the lead compound, like vitamin analogues and hormone analogues. Hence, the activity is maintained, although structure is changed. This can be expressed by an idea of 'bio-isosteric groups' which generally have similar biological activity. The spectrum of action of the existing compound may be changed or side-effects can be changed to main effects.

E. J. Ariens mentioned the following physicochemical parameters affecting drug-activity.

"The chemical properties of a drug are determinant for its biological action and activity. The various physico-chemical properties of bioactive compounds in general, are parameters related to the interaction of the drug with its environment."

The physico-chemical parameters can be divided into three main categories.

**(1) Parameters which are an expression of the hydrophobic aggregation forces at site of action:**

These include partition coefficient, surface activity,  $R_f$  value, and the partial vapour pressure of a solution.

Hydrophobic groups and hydrophobic aggregation forces represented by these parameters give relatively large contribution to binding energy. They allow, by variation in size of the groups, a gradual change in the lipid water balance which rules distribution by passive transport.

**(2) Parameters which are an expression of the charge distribution in the molecule and thus of the electrostatic force at site of action:**

These include redox potential, the base or acid dissociation constants, the electronic polarization, dipole moments, the inductive field effect and the resonance effect, especially in conjugated systems, the capacity of chelate formation, and H-bond formation and finally the characteristics in IR and NMR spectra. Electrostatic forces represented by these parameters give a relatively low contribution to binding energy. They contribute more to the selectivity in drug-receptor interaction and are essentially involved in substrate activation in enzymes and conformational skeletons important for the induction of a stimulus in the macromolecular receptor molecules.

**(3) Parameters which are an expression of spatial arrangement of the molecule:**

These parameters represent spatial arrangement of various groups in the molecule and play a role in the possible steric hindrance on the intramolecular level. The location, size, volume and charge of particular groups play a role here.

The intensity of the pharmacological response elicited by many drugs is probably directly related to the concentration or activity of a drug in the immediate vicinity of the receptor site in the body. Since it is not possible to measure this concentration directly, the study of physico-chemical parameters presents a picture of indirect measurement of the concentration of a drug at receptor site. It follows, therefore, that drug molecules exert their effect by influencing receptor sites in living systems through their physico-chemical properties.

## 2.2 FERGUSON PRINCIPLE

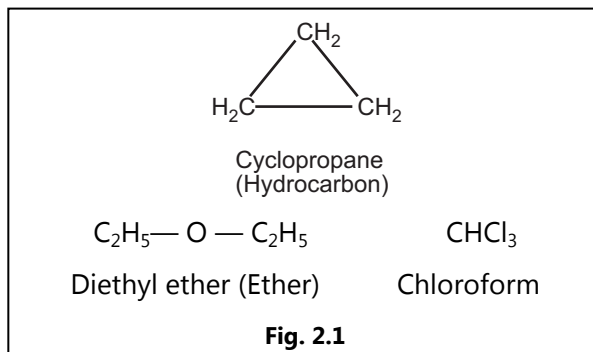
Pharmacologically active compounds can be divided into two major groups:

- (a) the structurally specific and
- (b) structurally non-specific.

The structurally specific drugs bring about their effects by combining with a specific receptor. The SAR of such groups can only be varied within relatively narrow limits.

The structurally non-specific drugs do not act on specific receptor. Instead, they penetrate into the cell or accumulate in cellular membranes, where they interfere by chemical or physical means, with some of the fundamental cellular processes e.g. general anaesthetics, hypnotics, volatile insecticides and certain bactericidal agents. The biological effect of such drugs is more closely correlated with the physical properties of the molecule than with the chemical structure e.g. cyclopropane, diethyl ether and chloroform, though having different structures, are good general anaesthetics.

Ferguson suggested in 1939, that the potency of structurally non-specific drugs was determined by their thermodynamic activity. This quantity is a measure of the proportion of the molecules which are free to react with enzyme systems, nerve membranes and similar biologically important sites. The molecules which are not free to act in this way, are reacting with



one another, with the molecules of the solvent or with molecules of other solutes. It follows, therefore, that the thermodynamic activity of a drug in solution is not determined entirely by its concentration. In the case of volatile anaesthetics administered with air or oxygen, the thermodynamic activity is proportional to the relative saturation of a drug (a). The relative saturation of a drug is defined as  $\frac{P_t}{P_o}$  for volatile drugs and gases.

$$\text{Relative saturation (a)} = \frac{P_t}{P_o} \quad \dots(2.1)$$

where,

$P_t$  = partial pressure of the drug in solution or in the gaseous mixture and

$P_o$  = vapour pressure of the pure drug at the same temperature.

For non-volatile drugs of limited solubility the relative saturation (a), is given by

$$\text{Relative saturation (a)} = \frac{S_t}{S_o} \quad \dots (2.2)$$

where,

$S_t$  = molar concentration required to produce the biological effect and

$S_o$  = molar solubility of the drug.

Ferguson's theory predicts that the anaesthetic agents will show the same degree of biological activity if their concentrations are adjusted so that their thermodynamic activities are equal (or relative saturation value (a) are equal). This theory is also applicable to substances other than anaesthetics and it was originally applied to insecticides and antibacterial substances.

**Table 2.1: Concentrations of gases and vapours producing the same degree of anaesthesia in mice at 37°C**

<b>Anaesthetic agent</b>	<b>Saturation pressure at 37°C (<math>P_s</math>) (mm Hg)</b>	<b>Activity (<math>P_t/P_s</math>)</b>
Nitrous oxide	59,300	0.01
Acetylene	51,700	0.01
Methyl ether	6,100	0.02
Ethylene oxide	5,900	0.01
Ethyl chloride	1,780	0.02
Diethyl ether	830	0.03
Methylal	630	0.03
Ethyl bromide	725	0.02
Dimethylacetal	288	0.05
Diethylformal	110	0.07
Dichlorethylene	450	0.02
Carbon disulphide	560	0.02
Chloroform	324	0.01

**Table 2.2: Bactericidal concentrations of miscellaneous organic compounds toward *Salmonella typhosa***

<b>Compound</b>	<b>Bactericidal Concentration (<math>S_t</math>)</b>	<b>Solubility (<math>S_o</math>)</b>	<b>Relative Saturation (<math>S_t/S_o</math>)</b>
Thymol	0.0022	0.0057	0.38
Propaldehyde	1.08	2.88	0.37
Methyl ethyl ketone	1.25	3.13	0.40
Acetone	3.89	9.7	0.40
Aniline	0.17	0.40	0.44
Cyclohexanol	0.18	0.38	0.47
Butyraldehyde	0.39	0.51	0.76

## 2.3 IONIZATION

Ionized form imparts good water solubility to the drug which is essential for good binding interactions of drug with its receptor. While non-ionized form helps the drug to cross cell membranes. Hence, a good balance of ionized: non-ionized forms is essential for better pharmacokinetic and pharmacodynamic features. Most of the effective drugs are amines having a pKa value in the range 6 - 8. Hence, they are partially ionized at blood pH to create balanced ratio of ionized: non-ionized forms.

The rate of absorption of a drug which is capable of existing both in ionised and unionised forms, is dependent on the concentration of its unionised form rather than on its total concentration. The unionised form is a function of both, the dissociation constant (pKa or negative logarithm of acidic dissociation constant) and the pH of the environment which is represented by Henderson-Hasselbach equation.

$$\text{For Acid, } \text{pKa} - \text{pH} = \log (\text{Cu/Ci}) \quad \dots(2.3)$$

$$\text{For Base, } \text{pKa} - \text{pH} = \log (\text{Ci/Cu}) \quad \dots(2.4)$$

where, Ci and Cu are the concentrations of the ionised and unionised drugs respectively. It can be seen that a solution of weak acid, aspirin (pKa = 3.5) in the stomach, (pH = 1.0) will be more than 99% unionised and since unionised form is lipid soluble, it will get more easily absorbed in the stomach. Quinine, a weak base (pKa = 8.5) in stomach (pH = 1.0) would have only one out of 10,000,000 molecules in unionised state, hence would be most unabsorbable in stomach. In spite of the fact that certain drugs exist in unionised state, they are poorly absorbed due to their low lipid solubility. The distribution or partition coefficient of drug in unionised state between fat-like solvents (such as chloroform) and water or an aqueous buffer mixture nearly at the pH of the site of absorption gives an idea of the lipid solubility of the drug.

**Table 2.3: pKa Values of acids and bases**

	Acids	pKa Scale	Bases	
Strong	Sulphonic acids Benzyl Penicillin	1	Antipyrin	Weak
	Salicylic acid	3		
	Aspirin	3		
	Benzoic acid	4		
	Phenyl butazone	4		
		5	Amidopyrin	
	Sulphadiazine	7	Reserpine	
	Barbital	8	Morphine	
	Sulphapyridine	8	Quinine	
Weak	Diphenyl-hydantoin	9	Procaine Ephedrine	Strong

For weak bases or acids, the pKa value together with the pH of the medium determine which fraction of the drug molecules is undissociated and thus available for penetration through the various lipid barriers. The rate of penetration thus is strongly dependent on the lipophilicity of the drug molecule in its unionised form.

The lipophilic-hydrophilic balance plays a role not only in passive transport but also in active transport and drug metabolism.

**Table 2.4:  $\text{CHCl}_3/\text{H}_2\text{O}$  partition coefficient of unionised barbiturates and % absorption from rat colon**

Barbiturates	Partition Coefficient	% Absorption
Barbital	0.7	12
Amobarbital	4.9	17
Phenobarbital	4.8	20
Cyclobarbital	13.9	24
Pentobarbital	28.0	30
Secobarbital	50.7	40

**Table 2.5: Anaesthesia produced by primary alcohols in tadpoles (Overton and Meyer)**

Alcohol	Anaesthetic concentration in aqueous medium	Partition coefficient (cotton seed oil/water)
$\text{CH}_3\text{OH}$	0.57	0.00966
$\text{C}_2\text{H}_5\text{OH}$	0.29	0.0357
$\text{C}_3\text{H}_7\text{OH}$	0.11	0.156
Iso- $\text{C}_4\text{H}_9\text{OH}$	0.045	0.588

As the length of the hydrophobic chain increases, both the partition coefficient and the anaesthetic potency increases while the aqueous concentration decreases. For weak acids and bases the ionised and non-ionised forms have completely different lipid/water partition coefficients. The ionised groups (usually  $\text{COO}^-$  or  $-\text{N}^+\text{HR}_2$ ) interact strongly with water dipoles and consequently penetrate only poorly or not at all into the lipoidal cell-membranes. Thus, drugs that are partially ionised at body pH enter cells at rates that are strongly pH dependent.

Phenobarbital, a weak acid, caused a drop in the plasma drug level, when the plasma pH was lowered by  $\text{CO}_2$  inhalation in dog. It is because the greater fraction of the total phenobarbital in the blood assumed the non-ionised acid form. The plasma concentration of undissociated diffusible phenobarbital was thus increased and a large amount of the drug

moved across the cell-membranes and into cells where the pH remains relatively stable. Plasma alkalosis produced opposite effect. Hence, to promote just such a shift of the drug out of the tissues, alkalosis is induced therapeutically in the treatment of barbiturate poisoning.

The co-ordinated effect of pKa and lipid solubility of a drug on its absorption led to the development of erythromycin propionate. The pKa value of erythromycin is 8.6 while that of ester is 6.9. Since, the partition coefficient of ester form is about 180 times larger than that of erythromycin, the ester yields 2 to 4 times higher blood levels than does erythromycin. These observations are in accordance with the Handerson-Hasselbach equation.

## 2.4 SOLUBILITY

About 30% of drug candidate molecules are rejected due to pharmacokinetic related failures. As the bioavailability of drugs from liquid orals mainly depends on their solubility in the given solvent system, it is considered as one of the important parameters for assessing the absorption of drugs into the systemic circulation.

Lipinski et al. found that poor absorption or permeability is seen when

- (i) Compound has molecular weight above 500 amu (Atomic mass unit),
- (ii) Compound has  $\log P > 5$ , and
- (iii) Compound has either five H-bond donars or ten H-bond acceptors.

Solubility is the function of ionization, molecular structure, molecular weight, stereochemistry and electronic structure. Solubility is affected by pH. For example, many sparingly soluble compounds have solubilities that depend on pH. By changing the pH of the solution, you can change the charge state of the solute. If the pH of the solution is such that a particular molecule carries no net electric charge, the solute often has minimal solubility and precipitates out of the solution.

Sparingly soluble salts derived from weak acids tend to be more soluble in an acidic solution.

- (i) Acidic drugs: e.g., barbiturates, NSAIDs
- (ii) Basic drugs: e.g., phenothiazines,  $\beta$ -blockers
- (iii) Amphoteric: e.g., tetracyclines, ACE-Is

Any drug to be absorbed must be present in the form of solution at the site of absorption. The solubility of a compound depends on the structure and solution conditions. Structure determines the lipophilicity, H-bonding, molecular volume and ionizability. Insufficient solubility of the compound influence both pharmacokinetic and pharmacodynamic properties of the compound. Hence, good solubility ensures good bioavailability and good ability of the drug to reach its target sites at effective concentrations. The cause of low oral bioavailability is the poor solubility and low permeability. Permeability is a kinetic parameter related to lipophilicity.

Sufficient solubility and membrane permeability are the factors governing oral absorption and distribution of the drug in various body organs. The aqueous solubility of the drug depends upon:

- (1) Buffer and ionic strength
- (2) Polymorphism of the sample
- (3) pH of the solution
- (4) Super saturation, and
- (5) Thermodynamics vs kinetic solubility.

Aqueous solubility decreases as the viscosity, surface area and lipophilicity of the drug increase. If any two of these properties are present, the compound is likely to show poor oral absorption. This is known as 'Rule of 5'. It assumes passive absorption of the drug in the body.

## 2.5 PARTITION COEFFICIENT

The relationship of lipophilicity with narcotic activity dates back to almost a century. Using a series of simple neutral organic compounds, Meyer and Overton, postulated the parallelism between, the values of partition coefficients and narcotic potencies of these agents.

The partition coefficient value ( $\pi$ ) expresses the relative free energy change occurring when a drug molecule moves from one phase to another. It means, a positive value of  $\pi$  suggests that the drug favours organic (lipoidal) layer while a negative value implies that it prefers an aqueous phase. An excellent correlation between partition coefficients determined in  $\text{CCl}_4$  / 0.1 N HCl solvent system and gastric absorption rate for different barbiturates was established.

The partition coefficient determined in the solvent system having pH nearly in the range of pH at the site of absorption gives a better understanding of drug absorption. Hence partition coefficient serves as a good physicochemical guide to estimate % absorption of the drug.

**Table 2.6**

Barbiturate	Partition coefficient	% absorption
1. Barbital	0.7	12
2. Phenobarbital	4.9	17
3. Butethal	11.7	24
4. Cyclobarbitone	13.9	24
5. Pentobarbital	28.0	30
6. Secobarbital	50.7	40

Increase in lipophilicity of a drug, beyond its optimum (i.e.  $\log P_o$ ), leads to its deposition into the first lipoidal biomembrane or macromolecule with which, it comes in contact. A sort of parabolic relationship exists between biological activity and partition coefficient values of drugs whose biological activity is dependent mainly upon lipophilicity (e.g. CNS depressants). This implies that drugs having  $\log P$ , far fluctuating from optimum  $\log P$  value, in either direction, would not be effective CNS depressants, other forces being equal. Similarly the ionization of drug to a certain extent does not affect the narcotic activity if it is compensated with enough lipophilicity. Table 2.7 encloses a survey of different categories of drugs (with special attention to CNS acting drugs) and the relationship of their activity with the  $\log P_o$  value of respective class. From the table, it can be concluded that the  $\log$  values in the range of 1.5 to 3.0, serve as a passport for the easy entry into the brain, where these drugs may produce sedative effects.

This statement serves as an important clue in designing the drugs where CNS depression is an undesirable effect. e.g. antihistamines. New drugs can be developed utilizing the lipophilicity, as minimum as possible and thus avoiding the  $\log P$  values in the range of 1.5 - 3.00.

**Table 2.7**

<b>Class</b>	<b>Log <math>P_o</math></b>	<b>Comment</b>
1. Volatile anaesthetics	2.35	At pH = 7.4
2. Hypnotics - Barbiturates - Carbamates - Alcohols	Near 2.00	At pH = 7.4
3. Nitrous oxide	0.43	At pH = 7.4
4. Ether	0.89	At pH = 7.4
5. Antipsychotic agent	$2.40 \pm 0.8$	At pH = 7.4
6. Tricyclic antidepressants	$2.15 \pm 0.7$	At pH = 7.4
7. Hallucinogens - LSD analogs - Phenylamines - Amphetamines	3.14	At pH = 6
8. Steroids - Testosterone - Progesterone - Deoxycorticosterone	3.29 3.87 3.08	Should possess CNS depressant activity

Lipophilicity governs the CNS penetration of a drug. Certain drugs disturb the brain function and may exhibit lethal effects. Toxicity of such agents was found to mainly governed by their lipophilic character. In summary, log P is a parameter of major importance in drug development.

Log P Calculations :

- (a) can supply guidelines in the development of novel bioactive agents and
- (b) become useful in toxicological estimations.

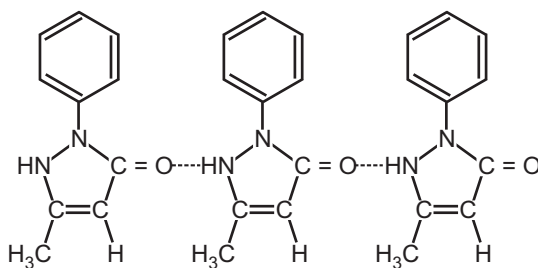
## 2.6 HYDROGEN BONDING

Atoms which are capable of forming H-bonds are electronegative atoms; these include F, Cl, N, O and S.

Though H-bonds are relatively weak bonds their presence may have a profound effect on the biological action of a drug.

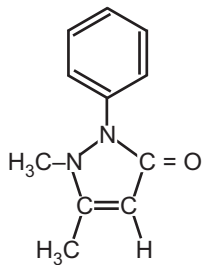
### For Example:

(1) 1-phenyl-3-methyl-5-pyrazolone shows no analgesic properties while 1-phenyl-2,3-dimethyl-5-pyrazolone (antipyrine) is a well known analgesic agent. This effect appears to be best explained by the fact that the first compound through intermolecular H-bonding forms a linear polymer.



Intermolecular hydrogen bonding  
1-Phenyl-3-methyl-5-pyrazolone

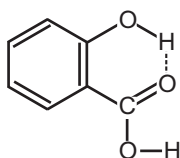
The resulting large attractive force between molecules lowers the solubility, especially in the non-polar solvents which are not capable of breaking the H-bonds



1-Phenyl-2,3-dimethyl-5  
pyrazolone (Antipyrine)

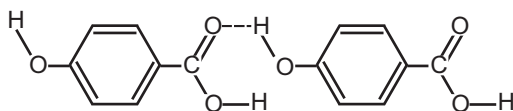
On the other hand, antipyrine cannot form H-bonds and has only comparatively weak attractive forces between its molecules and hence it is freely soluble in non-polar solvents and has the proper partition characteristics to penetrate the CNS.

(2) Salicylic acid (o-hydroxy benzoic acid) has quite an appreciable antibacterial activity, but the para isomer (p-hydroxybenzoic acid) is inactive, because salicylic acid is the ortho isomer that can form intramolecular H-bonds.



Salicylic acid

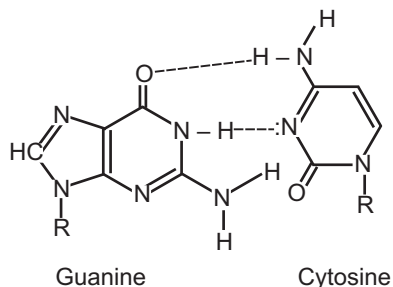
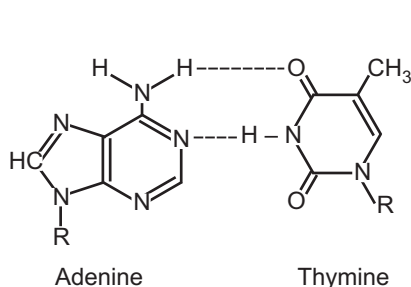
The m- and the p-isomers can form only intermolecular H-bonds.



p-Hydroxybenzoic acid (dimer)

Salicylic acid is less soluble in water than the p-isomer but its partition coefficient (benzene water) is approximately 300 times greater, while p-hydroxy benzoic acid has low partition coefficient and hence low anti-bacterial action. In salicylic acid, intramolecular H-bond has the phenolic hydroxyl group masked but the carboxylic acid group is free and can function as an anti-bacterial agent similar to benzoic acid.

(3) The nucleic acids, fundamental reproductive units of cells, provide an important example of molecules held together by specific hydrogen bonds. The genetic code of the cell, which constitutes the instruction for the synthesis of the cell proteins, is present in the cell nucleus, in the form of DNA. The code consists of sequences of 4 purine and pyrimidine bases-pyrimidine pairs are held together by specific hydrogen bonds.



Thus, H-bonds play a key role in maintaining the structural integrity of the base pairs of DNA.

## 2.7 PROTEIN BINDING

The reversible binding of drug with non-specific and non-functional sites on the body proteins without showing any biological effect is called as *Protein Binding*. Strong drug interactions with serum proteins can influence permeability.

A drug molecule, to less or more extent, has a capacity to enter into specific combination with plasma-proteins. These molecular interactions play an important role in deciding the intimate nature of drug action. For example, using paramecia as test organism, Busck, in 1906, observed the inhibitory effects of serum on the photodynamic and other toxic properties of certain dyes. This inhibition was attributed to the formation of dye-albumin complexes. Moore and Roaf reported that protein binding of volatile anaesthetics, ether and chloroform make them more soluble in plasma than in saline. Rabbit serum has excellent binding properties towards various drugs.

Drug molecules in blood are present in two forms :

**(a) Free form :** This form is pharmacologically active. It is diffusible and available for both, metabolism and excretion.

**(b) Bound form :** It is non-diffusible (being complexed with plasma-proteins) and hence inactive. It acts as reservoir of drug.

The free drug diffuses into various body compartments through biological membranes and barriers. It is consumed at sites of action for physiological effects, for metabolism and excretion.

Numerous alliances of drug molecule with different body proteins are possible. The one which is responsible for pharmacological response, is known as '*Primary interaction*' whereas all other interactions fall under the term, '*Secondary interactions*'. These secondary alliances are responsible for side-effects and storage of drug. It means the key fits many locks but there is only one door.

The body proteins which take part in binding are mainly available in blood and to small extent in tissues. They function as a specially designed transport system for the regulated distribution of drugs throughout the body.

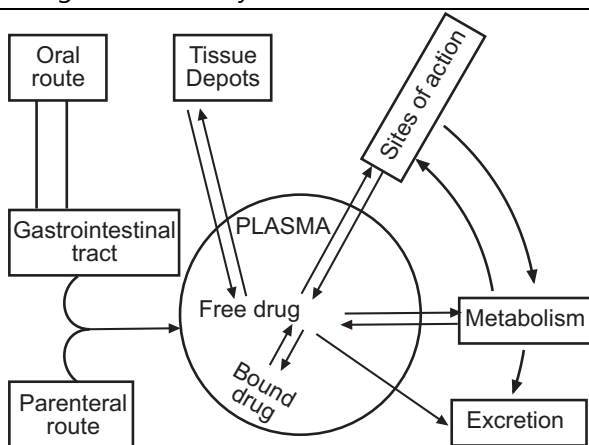
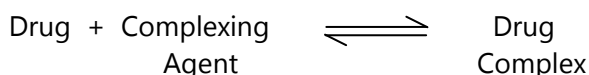


Fig. 2.2

## 2.8 COMPLEXATION

Since complexes of drug molecules cannot cross the natural membranous barriers, they render the drug biologically ineffective. The rate of absorption is therefore, proportional to the concentration of the free drug molecules i.e., the diffusible drug.

Due to the reversibility of the complexation, there always exists an equilibrium between the free drug and the drug complex. Such equilibrium is represented below:



Complexation reduces the rate of absorption of the drug but does not affect the total availability of it, because the absorption of the free drug molecules shifts the equilibrium to the right, causing the free drug molecules to be released from the drug complex.

### Examples of Drug-Complexes:

- (1) Phenobarbital forms a non-absorbable complex with polyethylene glycol-4000. The dissolution rate of phenobarbital tablets containing PEG-4000, is only one-third of that of control tablets.
- (2) Amphetamine carboxymethylcellulose is yet another example of non-absorbable complex.
- (3) Tetracyclines have been known to form complexes with divalent and trivalent cations, which are much less effectively absorbed.
- (4) Calcium is an important constituent of the mucous membrane of GIT. The complexation of this calcium with EDTA, increases the permeability of the membrane, probably due to the widening of space between epithelial cells due to removal of calcium. Therefore, presence of EDTA, increases the absorption of mannitol, quaternary ammonium compounds of sulphanilic acid and heparin, which are very poorly absorbed in ordinary condition.

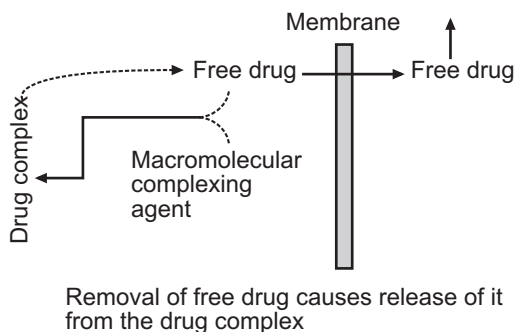


Fig. 2.3

## 2.9 SURFACE-ACTIVITY

As regards to the effect of surfactants or surface active agents on drug absorption through biological membrane, there have been opposing claims both in favour of enhancement and retardation of drug absorption. The main controlling factors in this regard are - the chemical nature of the surfactant, its concentration, its effect on biological membranes and the micelle formation.

It is evident that while in lower concentrations the surfactant enhanced the rate, the same in higher concentrations reduced the absorption rate. In lower concentration, the amphiphiles reduce the surface tension and bring about better absorption through better contact of the molecules with the absorbing membrane. But when the concentration crosses the Critical Micelle Concentration (C.M.C.), the surfactant molecules in the bulk of the solution form colloidal aggregates comprising nearly a few hundreds of themselves, and these molecular aggregates are called micelles, which entrap the drug molecules in their hydrophobic core, resulting in the retardation of the rate of absorption.

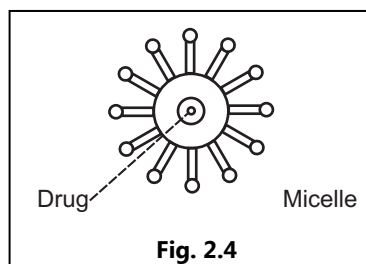


Fig. 2.4

Bile salt solutions of approximately physiological concentration greatly enhance the dissolution rate of poorly water-soluble drugs like griseofulvin and hexestrol by virtue of micellar solubilization effect.

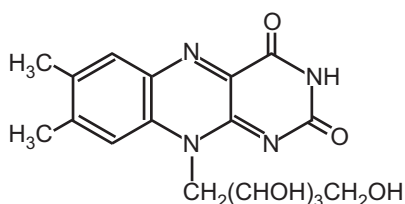
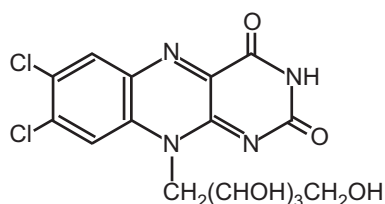
## 2.10 OXIDATION-REDUCTION POTENTIALS

The tendency of a compound to give or to receive electrons, is measured quantitatively by its oxidation-reduction potential or redox potential.

Since the oxidation-reduction potential applies to a single reversible ionic equilibrium which does not exist in a living organism, the correlations between redox potential and biological activity can only be drawn for the compounds of very similar structure and physical properties. Following are the examples:

(1) The optimum bacteriostatic activity in quinones is associated with the redox potential at + 0.03 volt, when tested against *Staphylococcus aureus*.

(2) The biological activity of riboflavin is due to its ability to accept electrons and is reduced to the dihydro form. This reaction has a potential of  $E_0 = -0.185$  volt. By retaining most of the structural features and altering its redox potential, one may develop compounds antagonistic to riboflavin. Kuhn prepared the analogue, in which the two methyl groups of riboflavin were replaced by chlorines and having a potential of  $E_0 = -0.095$  volt. Its antagonistic properties are due to the dichloro-dihydro form being a weaker reducing agent than the dihydro form of riboflavin. It may be absorbed at specific receptor sites but not have a negative enough potential to carry out the biological reduction of riboflavin.

Riboflavin  $E_o = -0.185 \text{ V}$ Riboflavin analogue  $E_o = -0.095 \text{ V}$ 

(3) The optimum anthelmintic activity in a series of substituted phenothiazines is associated with the  $E_m$  potential of 0.583 volt (acetic acid - water) which could lead to maximal formation of semiquinone ion (a radical ion) at physiologic pH. (against mixed infestation of *Syphacia obvelata* and *Aspiculurus tetraptera* in mice). The semiquinone facilitates an essential biological electron transfer reaction, producing a toxic or paralysing effect.

The necessity of a free 3 or 7 position in the phenothiazine nucleus for significant anthelmintic activity and the inactivity of phenothiazine tranquillizing drugs (2-substituted 10-dimethylaminopropyl phenothiazines) is only due to the difficulty of correlating redox potential and activity.

## 2.11 BIO-ISOSTERISM

In SAR studies and drug design, it is always necessary to compare the formal and three dimensional structure with the substituent and functional groups of compounds that show a similar spectrum of biological activities. In most instances, one may find similarities in molecular shape and overall chemical functions and will base one's explanation of biological similarities on these resemblances. This total complex of analogies that comprises steric, electronic and molecular orbital comparison is called bio-isosterism.

Bio-isosteric replacement is the principal guide followed by medicinal chemists in developing analogues of the 'lead' compound, whether as agonists or antagonists of biological effects. The parameters being changed are molecular size, steric shape, bond angles, hybridisation, electron distribution, lipid solubility, water solubility, pKa, the chemical reactivity to cell components and metabolising enzymes and the capacity to undergo H-bonding (receptor interactions).

In order to develop a new drug the structure of the drug is considered to consist of two parts,

**(1) Critical or essential.**

**(2) Non-critical or non-essential:** The non-critical part allows sufficient changes without a considerable change in the biological activity. The various molecular modifications done on this non-critical part are classified as follows.

**(a) Selectophores:** Those modifications which confirm selectivity in action of the drug by regulating drug distribution.

**(b) Contactophores:** The modifications which by increasing penetration, help the drug to reach the receptor site.

**(c) Carrier moieties or conducting moieties:** These moieties increase affinity of a drug.

Thus, non-critical part of a drug molecule is not involved in drug receptor interactions but is involved in passive transport of the drug.

While any change or modification of critical part of the drug molecule will result in the change of its biological activity, only those groups having similar steric, electronic and solubility characteristics can be interchanged. The study of such groups (bio-isosters) and their application in medicinal chemistry is known as Bio-isosterism.

More recently Burger classified and subdivided bio-isosters as:

**(1) Classical bio-isosters:**

- (a) Monovalent atoms and groups, e.g.  $\text{CH}_2$ ,  $\text{NH}_2$ ,  $\text{OH}$  and  $\text{SH}$ .
- (b) Divalent atoms and groups, e.g.  $\text{R}-\text{O}-\text{R}'$ ,  $\text{R}-\text{NH}-\text{R}'$ ,  $\text{R}-\text{CH}_2-\text{R}'$  and  $\text{R}-\text{Si}-\text{R}'$
- (c) Trivalent atoms and groups, e.g.  $\text{R}-\text{N}=\text{R}'$ , and  $\text{R}-\text{CH}=\text{R}'$
- (d) Tetrasubstituted atom, e.g.,  $=\text{C}=$ ,  $=\text{N}^{\oplus}=$ , and  $=\text{P}^{\oplus}=$
- (e) Ring equivalents, e.g.  $-\text{CH}=\text{CH}-$ ,  $-\text{S}-$ ,  $-\text{O}-$ ,  $-\text{NH}$  and  $-\text{CH}_2-$

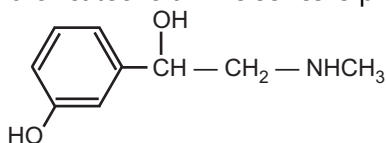
**(2) Non-classical bio-isosters:**

These non-classical bio-isosters do not rigidly fit the steric and electronic rules of the classic bio-isosters. These are further subdivided into,

- (a) Exchangeable groups
- (b) Rings versus non-cyclic structures.

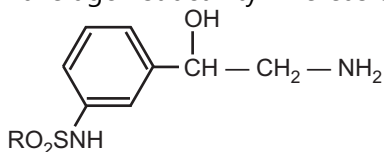
**BIO-ISOSTERIC APPLICATIONS**

- (1) An important compound of catecholamine series is phenylephrine.



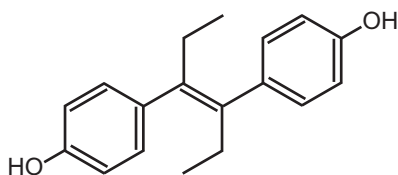
Phenylephrine

An alkylsulphonamido group may be substituted for the phenolic hydroxyl group. Some of the resulting compounds have agonist activity whereas others are antagonist.

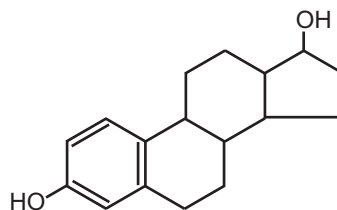


Alkylsulphonamidophenylethanolamine

While a classic example of rings versus non-cyclic structures is diethylstilbestrol and estradiol.



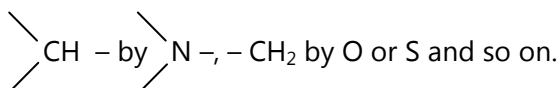
Diethyl stilbestrol



Estradiol

Diethylstilbestrol has about the same potency as the naturally occurring estradiol. The central double bond of diethyl stilbestrol is highly important for the correct orientation of the phenolic and ethyl groups (trans) at the receptor site. Table 2.6 contains a variety of bio-isosters including classical and non-classical examples, incorporating marketed drugs as well as interesting experimental compounds.

Applications of bio-isosterism were also found in the designing of histamine -1- receptor antagonists and anticholinergics (antispasmodics) by replacing benzene by thiophene,



The first application of classical isosterism may be found in ring equivalents. Examples include pyridine and thiazole, benzene and thiophene.

The sulphur atom of the phenothiazine ring system of neuroleptic agents was replaced by  $-\text{CH}=\text{CH}-$  or  $-\text{CH}_2\text{CH}_2-$  leading to the azepine ring analogues that opened up the field of tricyclic antidepressants. In imipramine by isosteric exchange of  $\diagup \text{N} -$  with  $\diagup \text{C} =$ , amitriptyline was obtained.

The purpose of molecular modification is usually to seek subtle change in the compound that should not alter some properties but change others in order to improve potency, selectivity, duration of action and reduce toxicity. Bioisosterism makes it possible to limit some of these changes. All aspects considered, retention of overall molecular shape is the overriding condition for analogy of action.

In the design of bio-isosters an appreciation of the biochemical mode of action may play an important role e.g., aspirin acetylates prostaglandin synthetase and thereby deactivates this enzyme which ordinarily catalyses the biosynthesis of nociceptive prostaglandins. Isosters of aspirin in which the phenolic oxygen atom (X) has been replaced by 'classical' isosteric groups or atoms are inactive because they cannot release the acetyl group at all ( $\text{X} = \text{CH}_2$ ) or at an adequate rate (when  $\text{X} = \text{S}, \text{NH}$ ).

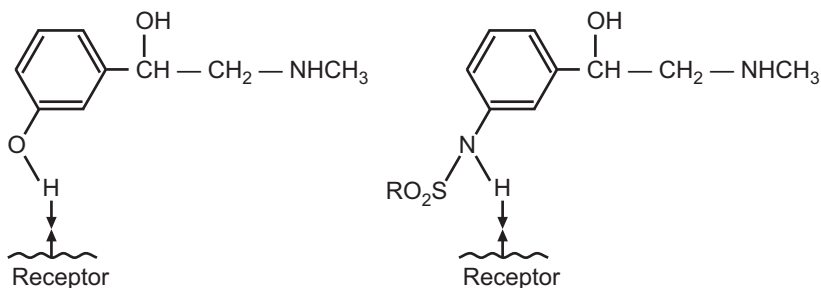
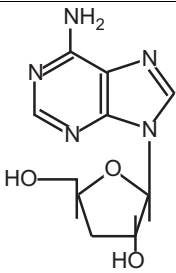
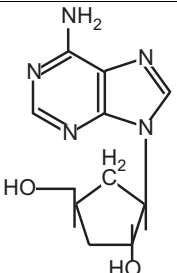
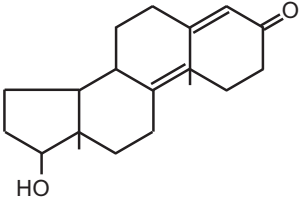
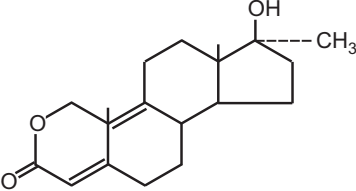
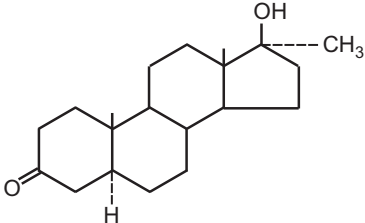
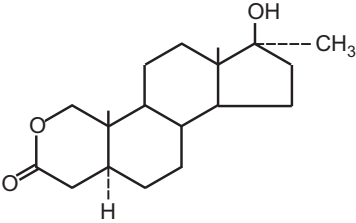
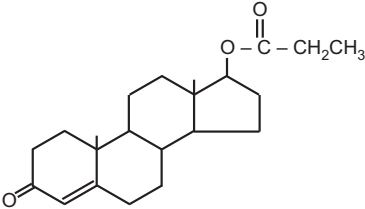
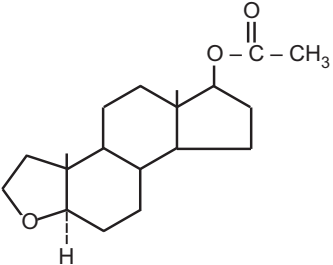
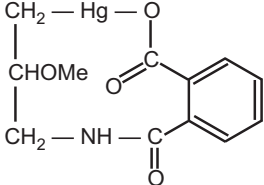
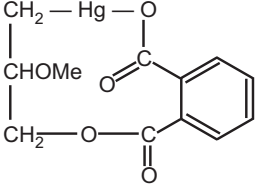
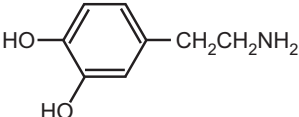
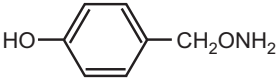


Table 2.8

Parent compound	Bio-isosters	Activity of parent compound
		Adenosine deaminase activity (-)
		Androgenic (+)
		Androgenic (+)
		Androgenic (-)
		Diuretic (+)
		Increases inhibition

(+) → activity of bio-isoster greater than parent compound.

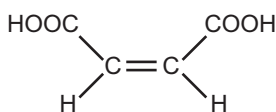
(-) → activity of bio-isoster less than parent compound.

**2.12 OPTICAL AND GEOMETRICAL ISOMERISM**

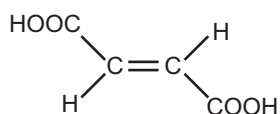
Stereochemistry helps to define the structure of a molecule and orientation of the atoms and functional groups present, in three dimensions. Stereoisomers possess the same molecular and structural formulae and the same functional groups but differ in the three-dimensional spatial orientation of these atoms or groups within the molecule. Due to the difference in orientation of the functional group and geometry of the molecule, stereoisomers differ in their physical, chemical, physicochemical and biochemical properties. Based on symmetry and energy criteria, stereoisomers are divided into three classes.

- (a) Geometrical isomers
- (b) Optical isomers
- (c) Conformational isomers.

**(a) Geometrical isomers (cis-trans isomerism) :** Maleic acid (m.p. 130°C) and fumaric acid (m.p. 287°C) have the same molecular formula but differ in the arrangement of functional groups around double bond. They have different physical and, to some extent, chemical properties. This type of isomerism is known as **geometrical isomerism**.



Maleic acid  
(cis-butenedioic acid)



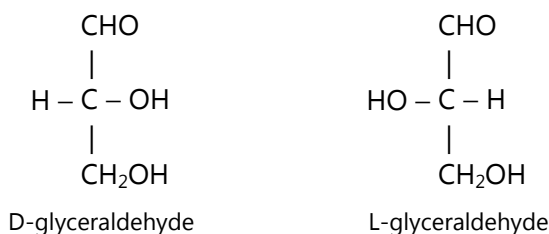
Fumaric acid  
(trans-butenedioic acid)

The presence of a carbon-carbon double bond restricts the freedom of rotation about double bond. The designation cis (Latin word : same side), is used to denote the presence of like atoms or groups on the same side and trans (Latin word, across) is used when they are on opposite sides. Isomerism seen in non-cyclic, open-chain compound due to the presence of a double bond, is called as  **$\pi$  diastereoisomerism** while when it occurs in a cyclic skeleton lacking a double bond, it is termed as  **$\sigma$  diastereoisomerism**.

**(b) Optical Isomerism (enantiomerism) :** In 1815, Biot found that a number of organic and inorganic compounds in the solution form, have the ability to rotate the plane of polarized light in opposite directions but in identical amplitude, passing through them. Optical isomerism is seen in compounds that can rotate plane polarised light. A carbon atom connected to four chemically different functional groups is known as asymmetric or chiral carbon and the presence of at least one asymmetric carbon atom in the structure is the pre-requirement for a molecule to show optical isomerism.

If there is one asymmetric carbon then two optically active isomers are possible. Isomer rotating plane of polarized light to the right is said to be dextrorotatory (Latin, dexter : right) while isomer showing rotation to the left is known as laevorotatory (Latin, laevus : left). Both isomers are mirror images of each other yet are not superimposable. They are called as enantiomers and the pair of enantiomers is called as enantiomorph. An enantiomer does not possess a plane or center of symmetry.

For example,



When the enantiomers are present together in equal concentration, the rotation of plane polarized light caused by laevo isomer will be neutralized by a dextro rotating isomer and the mixture will be optically inactive. Such mixtures are called as racemic mixtures. The conversion of an enantiomer into a racemic form is called as racemization. While the separation of racemic mixture into individual enantiomers is called as resolution. The maximum number of optically active isomers possible for a molecule having more than one asymmetric carbon atoms may be given by the formula

$$N = 2^n$$

where,  $N$  = Number of optically active isomers, and

$n$  = Number of asymmetric carbon atoms.

With the exception of rotation of plane-polarised light, enantiomers have identical physical and chemical properties like boiling point, melting point, solubility. Their chemical properties are same towards achiral reagents, solvents and conditions. Towards chiral reagents, solvents and catalysts, enantiomers react at different rates.

### Importance of Optical Isomerism:

Nearly all naturally occurring substances having asymmetric carbon atoms are in either the *d* or the *l* form rather than as racemic mixtures. In drugs and pharmaceuticals, most of the adverse effects and low potency may be related to the utilisation of the drug in the form of its racemic mixture. Since, enantiomer in its pure form, is more active and selective, there is now an increasing interest to present the drug in the market in its active enantiomeric form instead of its racemic form. Optical isomerism has also been successfully utilized in elucidating the mechanism of many chemical reactions.



## DRUG METABOLISM

### ♦ SYNOPSIS ♦

#### 3.1 INTRODUCTION

#### 3.2 METABOLIC BIOTRANSFORMATION OF DRUGS

#### 3.3 CONJUGATION REACTIONS

#### 3.4 CYTOCHROME OXIDASE ENZYMES

#### 3.5 FACTORS INFLUENCING METABOLIC PATHWAYS OF THE DRUG

#### 3.6 INDUCERS OF DRUG METABOLISM

#### 3.7 INHIBITORS OF DRUG METABOLISM

### 3.1 INTRODUCTION

Drug undergoes metabolism which leads to loss of its physiological activity and an increase in the polarity and water solubility of the drug which results in more rapid elimination of the metabolite.

The metabolism of any drug is generally characterised by two phases of reaction, namely metabolic transformation (biotransformation) and conjugation.

Metabolic transformations or biotransformations are enzyme reactions in which drug may undergo a wide variety of oxidation, reduction and hydrolysis, resulting in the introduction or unmasking of functional groups which increase the polarity and hydrosolubility of the molecule and serve as the centres for the second phase of metabolic reaction i.e., conjugation.

Conjugation reactions are biosynthesis by which the drug or its metabolites are combined with endogenous molecules or groups, such as glucuronic acid, sulphate, amino acids, acetyl group or methyl group, making the molecule more polar, less lipid soluble and therefore it is readily excreted.

Most drugs are metabolised, at least to some extent, by both phases of metabolism e.g., acetyl salicylic acid undergoes hydrolysis to salicylic acid (metabolic transformation), which is then conjugated with glycine to form salicyluric acid (conjugation). The enzymes, oxidases, reductases, and hydroxylases, which carry out the metabolic transformations are located mostly in the liver, blood, intestinal mucosa, kidneys, lungs and the skin.

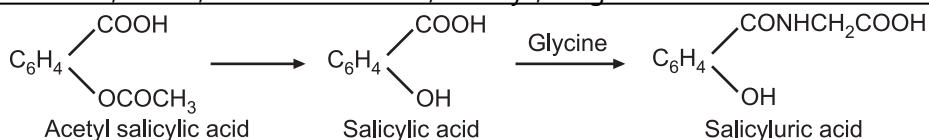


Fig. 3.1: Metabolism of aspirin

Some drugs are considered biochemically inert as they have been excreted unchanged i.e. without any metabolic transformation e.g., barbitone, diethyl ether, only because they cannot easily penetrate the lipoprotein membrane of tissues and were rapidly excreted by active transport mechanisms of kidney.

### 3.2 METABOLIC BIOTRANS-FORMATION OF DRUGS: PHASE I REACTIONS

On chemical basis, it is classified into reactions which are fundamentally oxidation, reduction and hydrolysis.

Most of these reactions occur in the liver while hydrolytic reactions of esters and amides occur in the gut wall, plasma and the lung.

Depending upon the nature and localisation of the enzymes which catalyse these reactions, it is further classified as:

(a) Metabolic transformations which are catalysed by enzymes of endoplasmic reticulum of the liver and the other tissues or the microsomal drug metabolising enzymes.

(b) Reactions catalysed by non-microsomal mammalian enzymes i.e. enzymes present in the mitochondria, lysosomes or cytoplasm of the tissues or in the blood plasma.

(c) Reactions catalysed by intestinal microflora.

#### (a) Microsomal drug metabolising enzymes:

Among the many enzymes associated with the endoplasmic reticulum, are a group of enzymes known as 'drug metabolising enzymes'.

These include:

(1) Mixed function oxidases    (2) Reductases    (3) Esterases.

Very often a drug is subjected to several competing pathways simultaneously and the extent of formation of the various metabolites depends on the relative rates of the various interactions. While simple metabolic reactions are followed by conjugation e.g., an alkyl side chain of a drug may be oxidised to an alcohol which then forms a conjugate with glucuronic acid or an ester may be hydrolysed to its acid form which then, is coupled with glycine.

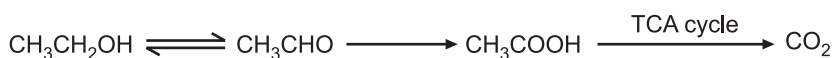
#### Examples:

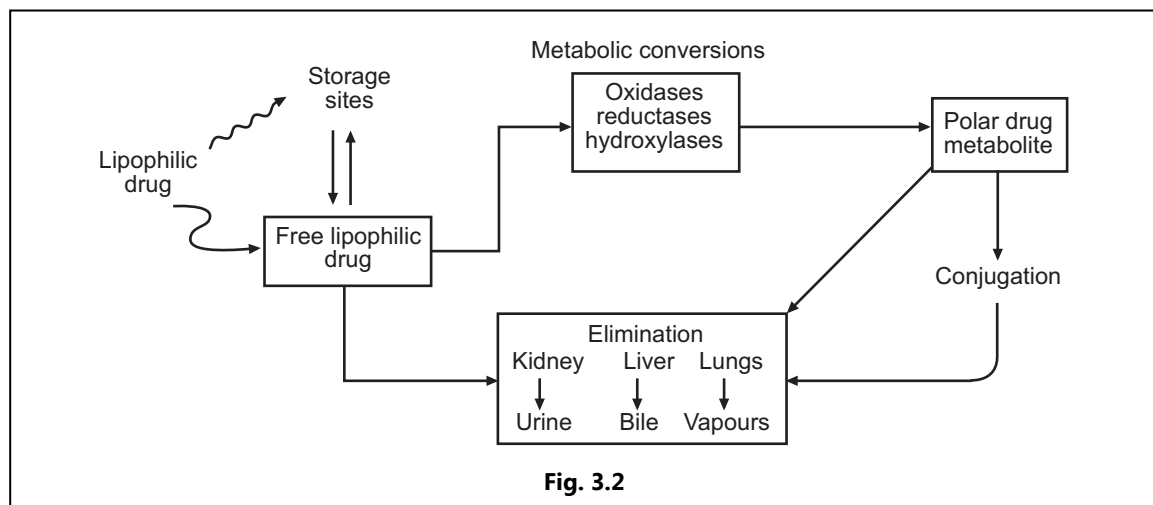
##### [A] Oxidation:

Oxidation mainly occurs at aromatic rings, terminal positions of alkyl chains, N-methyl groups, alcoholic groups and exposed corners of alicyclic rings.

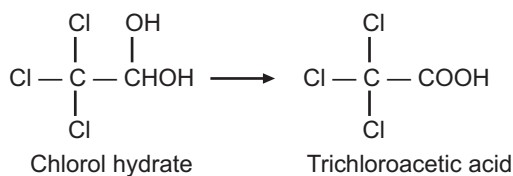
(1) Ethanol, in mammals, is rapidly oxidised by liver alcohol dehydrogenases to a toxic intermediate acetaldehyde. This is a reversible reaction.

The latter is rapidly oxidised to acetic acid by acetaldehyde oxidase and other enzymes. This reaction is irreversible and proceeds faster than the former. Acetic acid, then may enter the tricarboxylic acid cycle and reaches to final stage of oxidation to CO<sub>2</sub>.

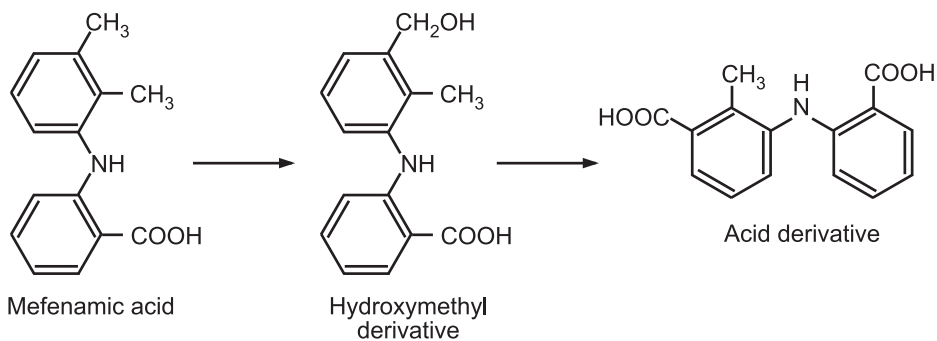




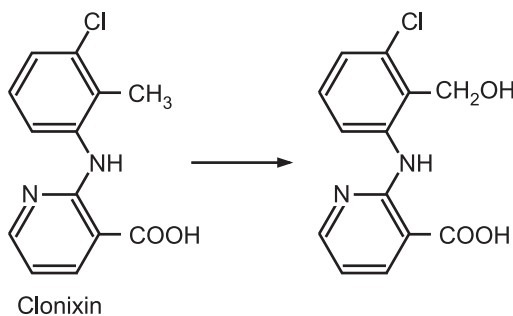
(2) Chloral hydrate is transformed to trichloro-acetic acid by aldehyde dehydrogenase.



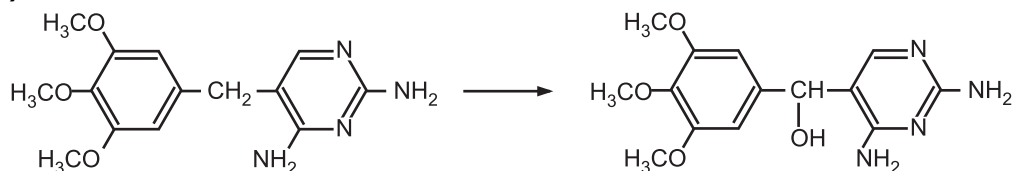
(3)



(4)

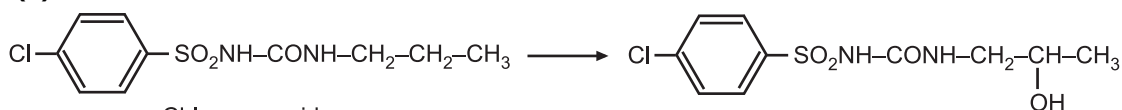


(5)

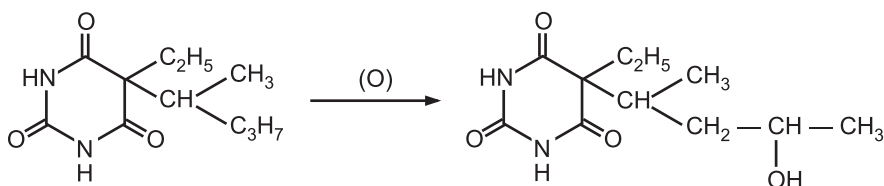


Trimethoprim

(6)



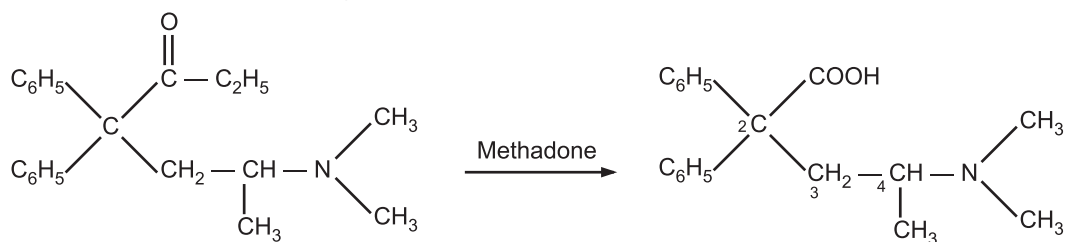
Chlorpropamide



Pentobarbital

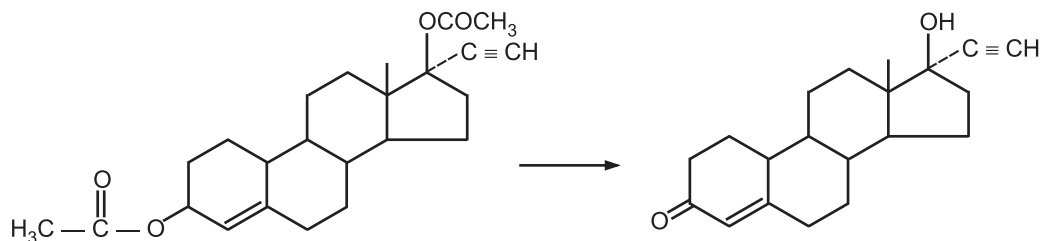
Pentobarbital alcohol

(7) Ketone oxidation: e.g.



4-Dimethylamino-2,2-diphenyl pentanoic acid

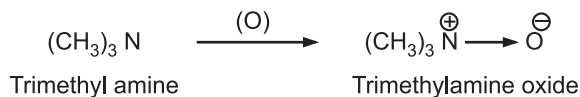
(8)



Ethynodiol diacetate

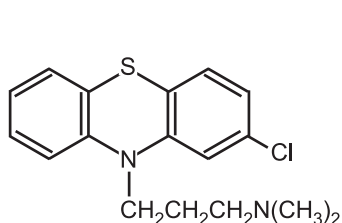
Norethisterone

(9)

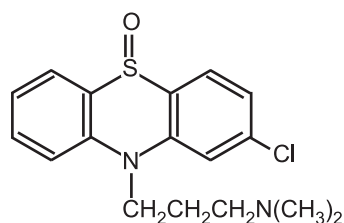


Trimethyl amine

Trimethylamine oxide

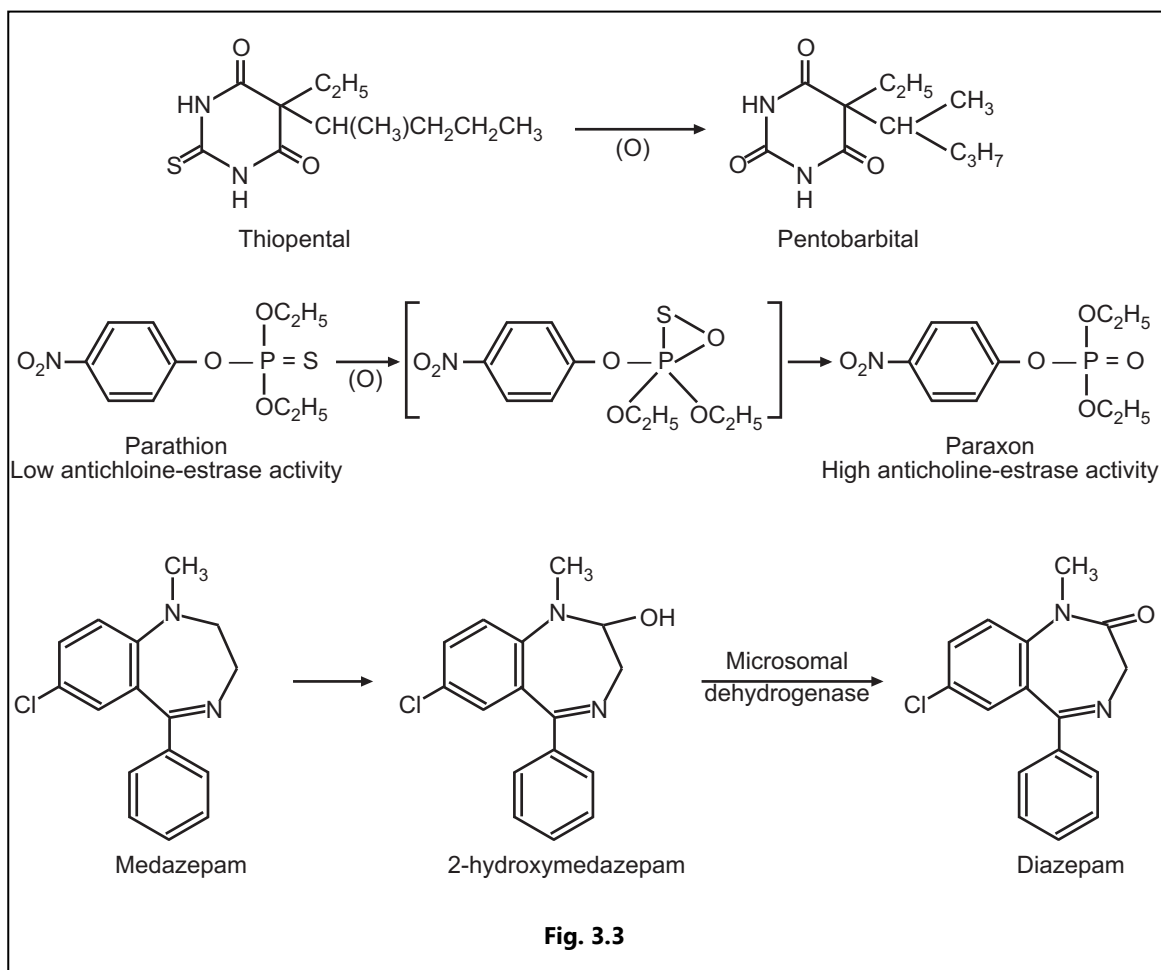
**(10) Sulfoxidation:**

Chlorpromazine



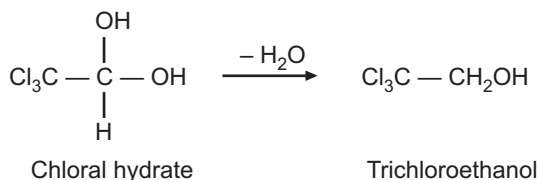
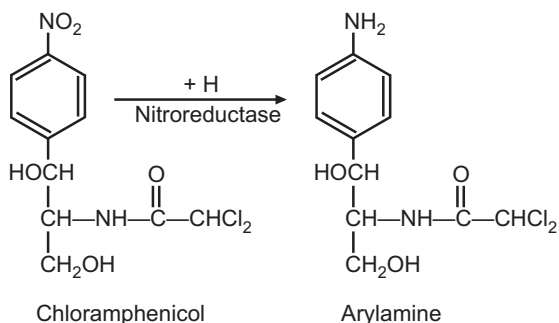
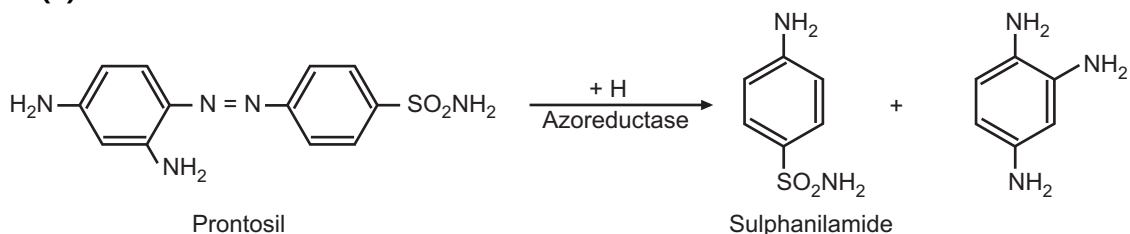
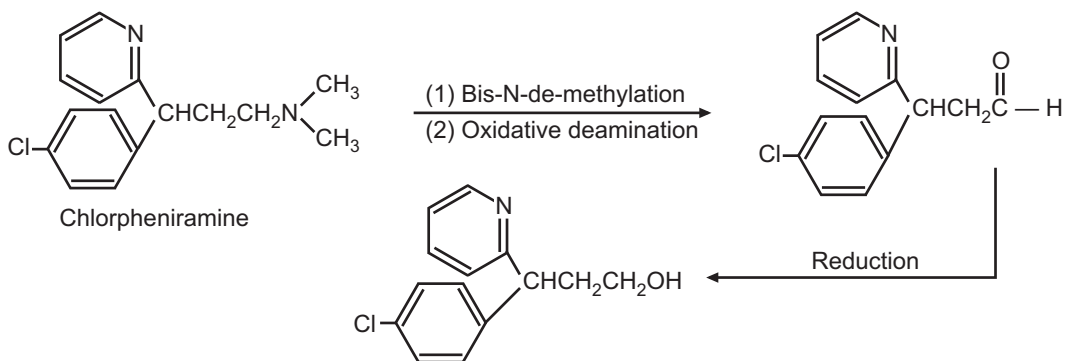
Chlorpromazine sulfoxide

**Other oxidative reactions:** These include sulphur atom replacement reaction and ring formation. Former is an important and significant metabolic reaction for thiobarbiturates and for phosphorothionate insecticides. Parathion through the latter reaction, is transformed to a very toxic compound, paraxon (an active cholinesterase inhibitor in mammals) by the liver microsomes as shown in Fig. 3.3.

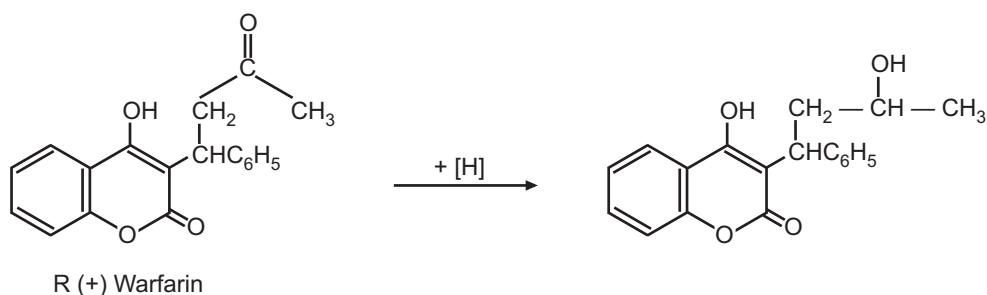
**Fig. 3.3**

**[B] Reduction:**

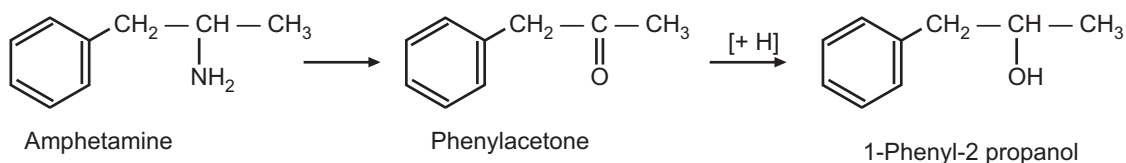
These processes play an important role in the metabolism of many compounds containing carbonyl, nitro and azo groups. Bioreduction of carbonyl compounds generates alcohol derivatives, while nitro and azo reduction leads to amino derivative. Since, the hydroxyl and amino groups are much more susceptible to conjugation than the functional groups of the parent compounds, reductive processes facilitate the drug elimination.

**Examples:****(1)****(2)****(3)****(4)**

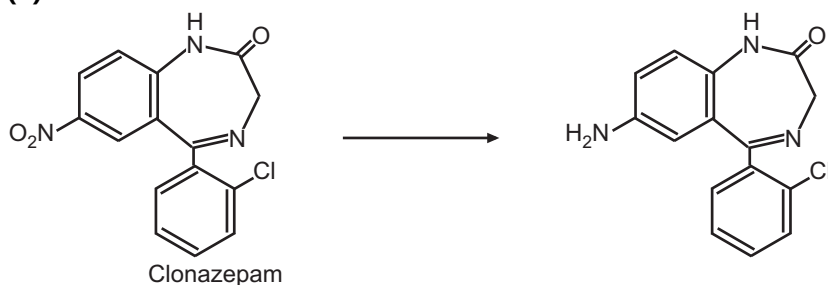
(5)



(6)



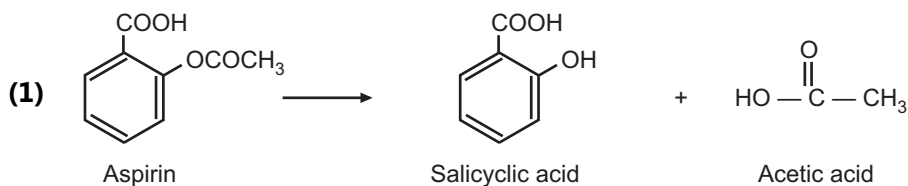
(7)

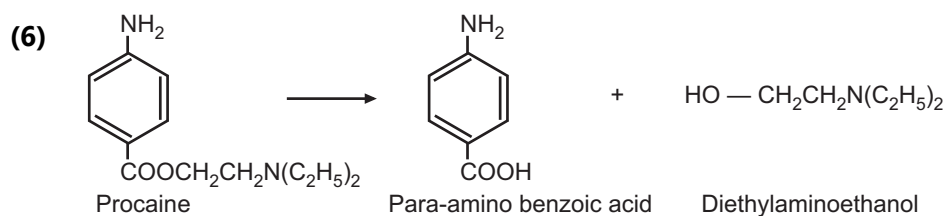
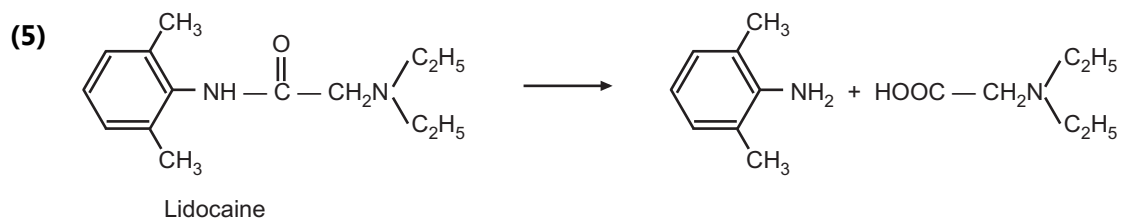
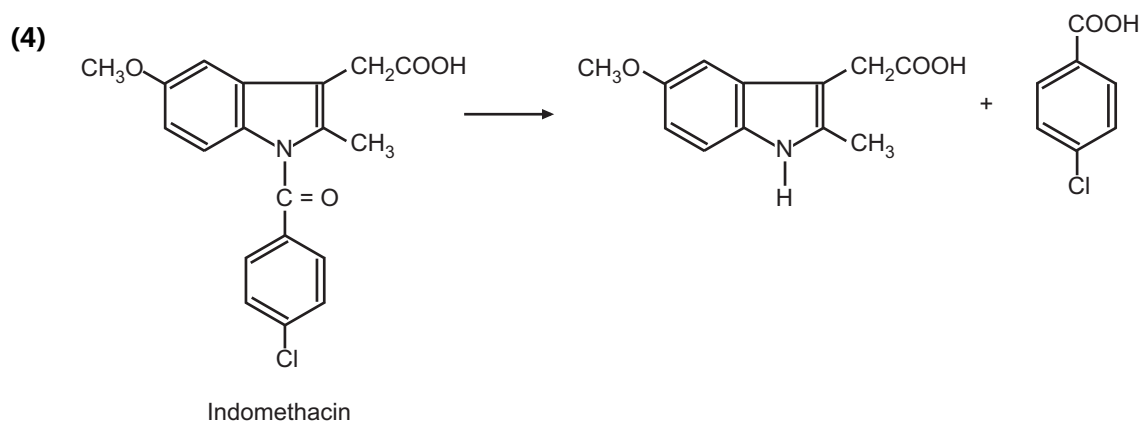
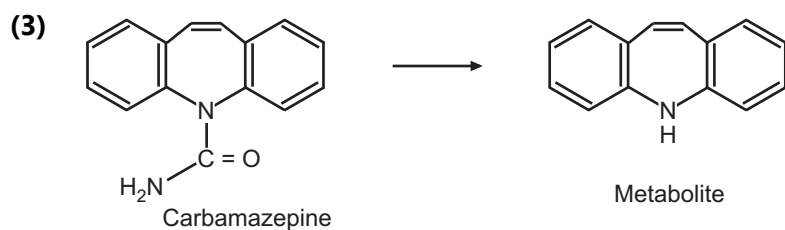
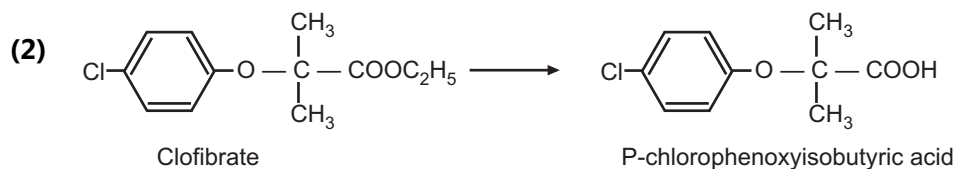


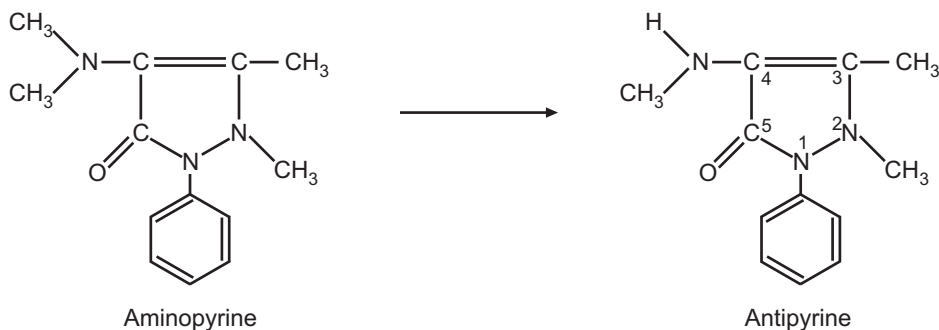
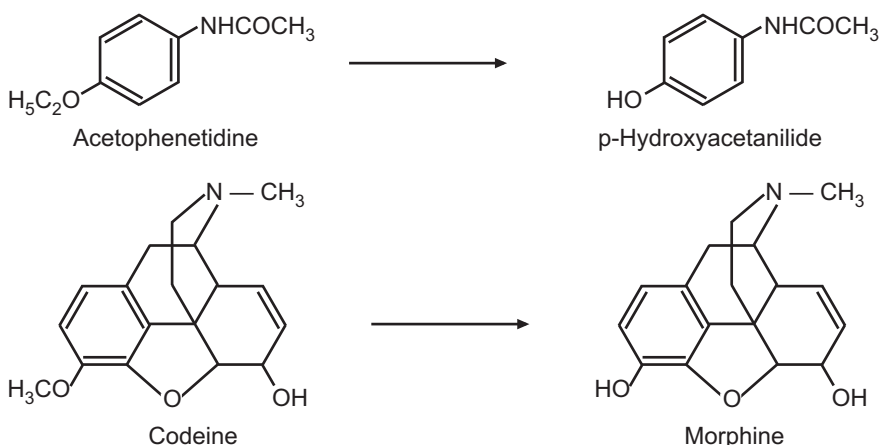
### [C] Hydrolytic Reactions:

The metabolism of ester and amide linkages in many drugs is catalysed by hydrolytic enzymes present in liver, kidney, intestine, blood and other tissues. The metabolic products formed, namely carboxylic acids, alcohols, phenols and amines generally are polar and functionally more susceptible to conjugation and excretion than the parent ester and amides.

Amide hydrolysis appears to be mediated by liver microsomal amidases, esterases and deacylases.





**[D] Other Important Reactions:****(1) N-dealkylation:****(2) O-dealkylation:****(3) Aromatization:****3.3 CONJUGATION REACTIONS OR PHASE II REACTIONS**

Metabolic transformations or phase - I reactions, do not always produce hydrophilic (more polar and water soluble) or pharmacologically inactive metabolites. Drugs exhibiting increased activity or activity different from the parent drug, generally undergo further metabolism through conjugation or phase II reactions, resulting in deactivation and excretion of the inactive, highly polar conjugates. All the phase II reactions do not increase the polarity. Methylation and acetylation, for example, decrease the polarity of drug metabolite.

Phase II reactions are classified mainly into:

- Methylation and acetylation which do not generally increase water solubility but serve mainly to terminate the pharmacological activity.
- Attachment of small, polar and ionisable endogenous molecules such as glucuronic acid, sulphate, glycine and glutamine to the phase I metabolite.
- Of minor importance, are the other conjugative pathways e.g. conjugation with glycosides, phosphate, other amino acids and conversion of cyanide to thiocyanate

**Table 3.1: Phase II or conjugation reactions**

Conjugation	Site	Conjugating agent	Functional groups
Glucuronidation	Microsomes	UDPGA	— OH, — COOH, — NH <sub>2</sub> , — SH
Sulphatation	Cytosol	PAPS	— OH, — NH <sub>2</sub>
Acetylation	Cytosol	Acetyl-CoA	— COOH
Glutathion	Cytosol	Glutathion	Epoxides, arene oxides
Methylation	Cytosol	SAM	— OH, —NH <sub>2</sub>
Amino acid	Cytosol	Glycine	— COOH
<b>UDPGA: Uridine diphosphoglucuronic acid, PAPS: 3'-phosphoadenosine-5'-phosphosulphate</b> <b>SAM: S-adenosylmethionine</b>			

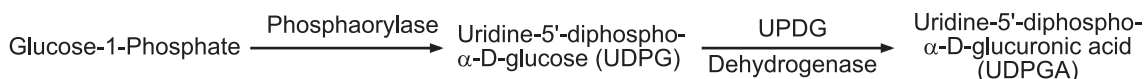
Thus, phase II reactions include -

- Glucuronic acid conjugation.
- Sulphate conjugation.
- Conjugation with glycine, glutamine and other amino acids.
- Glutathione or mercapturic acid conjugation.
- Acetylation.
- Methylation.
- Nucleoside and nucleotide formation.

### (I) Conjugation with Glucuronic Acid:

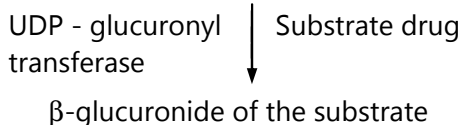
The reaction involves the condensation of the drug or its metabolite with the activated form of a readily available glucuronic acid, [i.e. uridine diphosphate glucuronic acid (UDPGA)], which is synthesised from glucose-1-phosphate. Glucuronic acid conjugation proceeds in two steps.

- Formation of the activated form from glucose 1- phosphate.



- (2) Subsequent transfer of the glucuronic group from UDPGA to an appropriate substrate.

Uridine-5'-diphospho- $\alpha$ -D-glucuronic Acid (UDPGA)



The transfer step is catalysed by microsomal enzymes called UDP-glucuronyltransferases present mostly in the liver but also occur in many other tissues like kidney, intestine, skin, lung and brain.

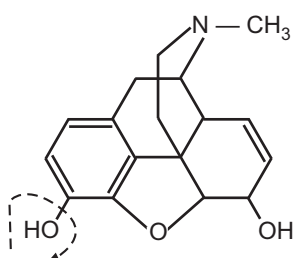
### Types of compounds forming glucuronides:

- (1) Alcohols and phenols form ether type glucuronides.
- (2) Aromatic and some aliphatic carboxylic acids form ester type glucuronides.
- (3) Aromatic amines form N-glucuronides and
- (4) Sulphydryl compounds form S-glucuronides.

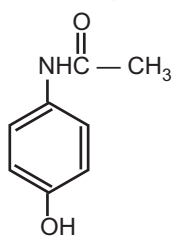
Phenols, alcohols, amines and amides all form O- or N- glucuronides. Many endogenous substances, like steroids, are also excreted in this way. Glucuronides are normally non-toxic, highly water soluble and excreted in the urine or bile.

Glucuronidation of one functional group is usually sufficient to effect excretion, diglucuronide conjugates usually do not occur.

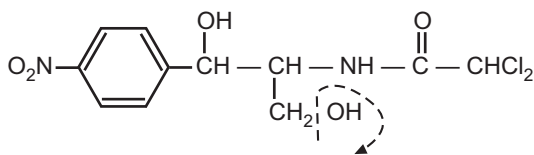
### (a) Compounds containing hydroxyl group:



Morphine

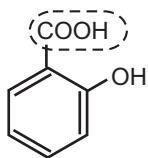


Acetaminophen

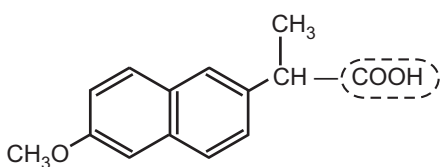


Chloramphenicol

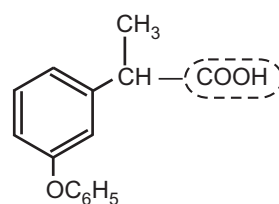
### (b) Compounds containing carboxyl group:



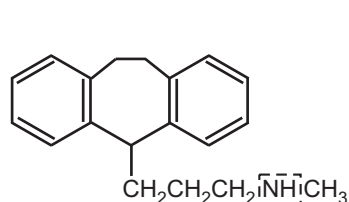
Salicylic acid



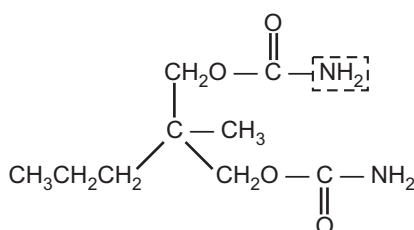
Naproxen



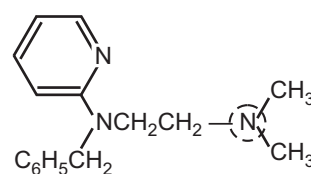
Fenopfen

**(c) Aromatic amines:**

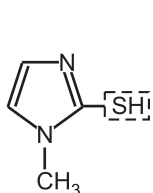
Desipramine



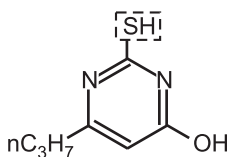
Meprobamate



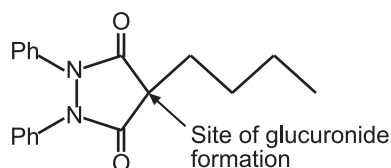
Tripeleennamine

**(d) Sulphydryl compounds:**

Methimazole



Propylthiouracil

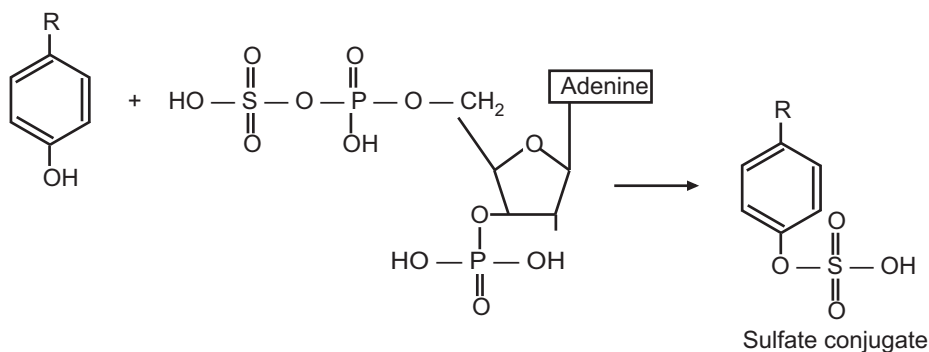
**(e) C - Glucuronide:**

Phenyl butazone

**(II) Sulphate Conjugation:**

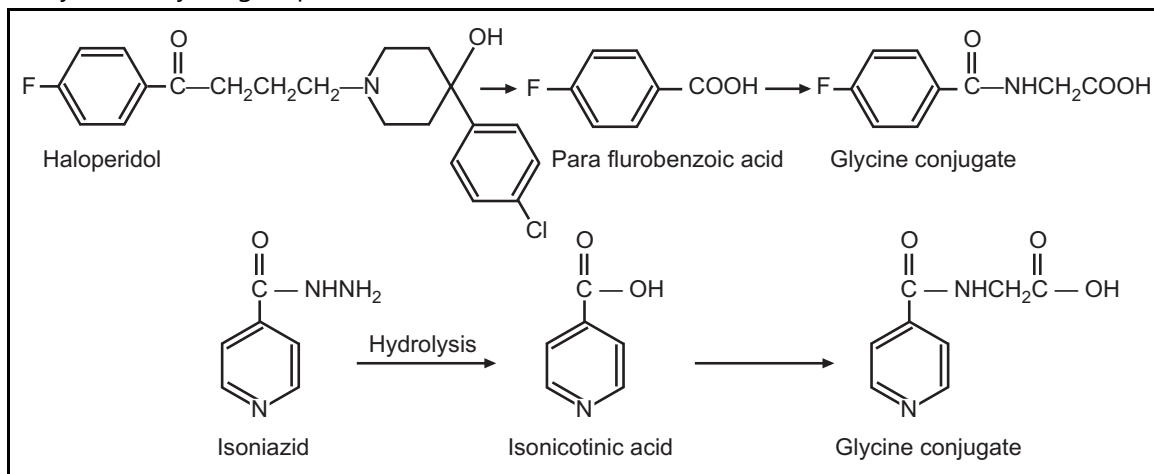
Sulphate conjugates are formed by the reactions of phenolic and aliphatic hydroxyl group and of certain amino groups with an activated form of sulphate through an ether linkage. Hence, they are also termed as "etheral sulphates". Sulphate conjugation generally results into highly polar compounds that are readily excreted in the urine. The soluble fraction of liver contains the enzymes that catalyse the sulphur activation and transfer of sulphate to the substrate.

The sulphate moiety is present in activated state in 3' - phosphoadenosine - 5' - phosphosulphate (PAPS). The sulphotransferase enzyme then catalyses the transfer of sulphate group to the phenolic acceptor. The sulphotransferase enzymes are structure specific. Hence for different substrates, specific sulphotransferase enzymes catalyse the reactions.



### (III) Conjugation with Glycine, Glutamine and other Amino Acids:

The amino acids, glycine and glutamine are utilized by mammalian systems to conjugate carboxylic acids. In contrast to glucuronic acid, glycine and glutamine are not converted to activated form. Instead the carboxylic acid substrate is activated with ATP to acetyl CoA complex. This complex then reacts with glycine and glutamine to form conjugate and free-acetyl Co-enzyme group.

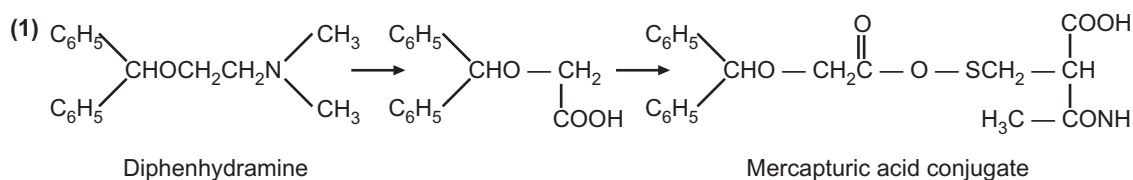


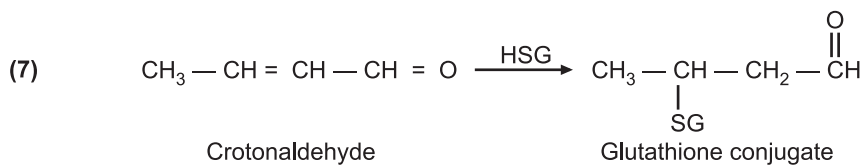
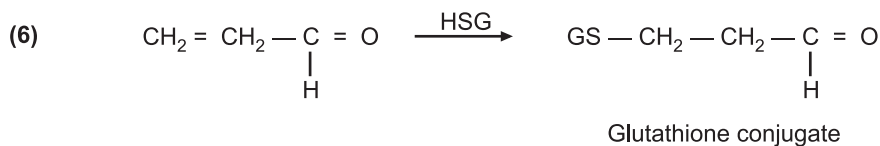
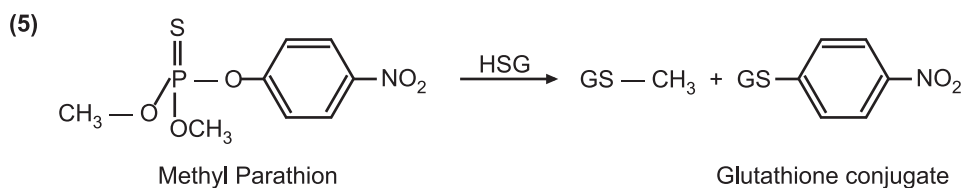
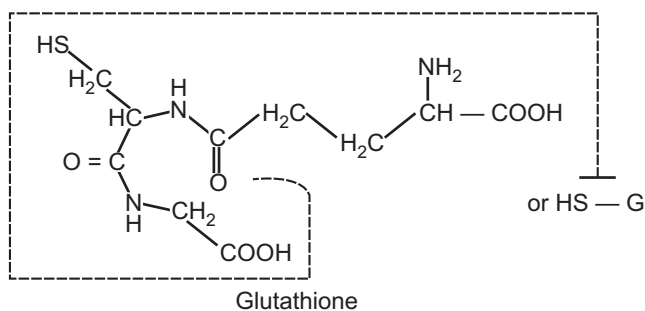
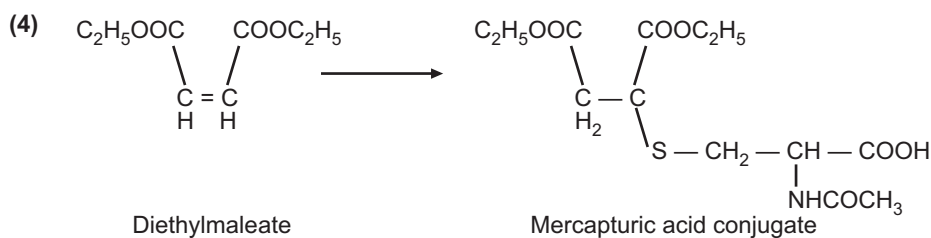
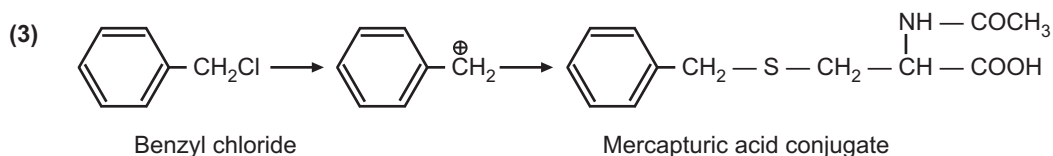
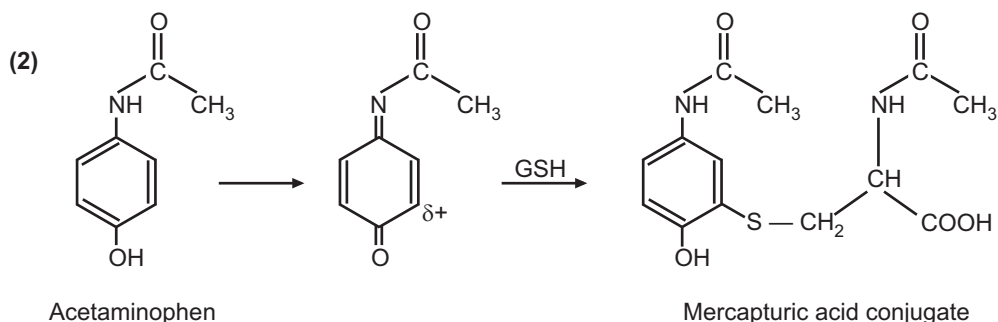
### (IV) Glutathione or Mercapturic Acid Conjugates:

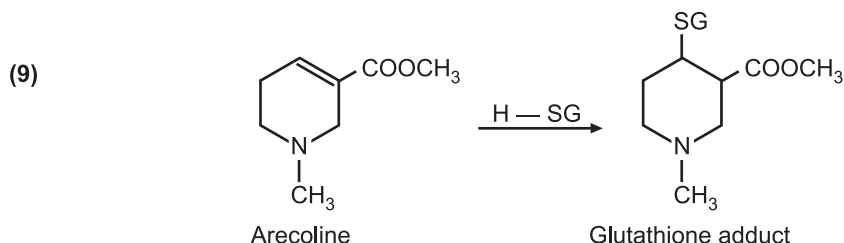
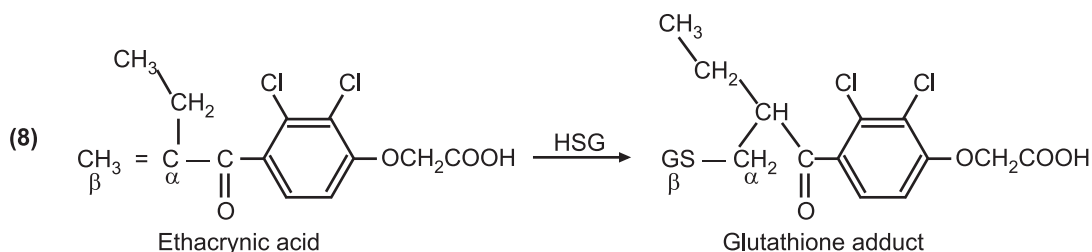
The glutathione conjugation is important in the elimination of polycyclic phenols and halides. The metabolically generated reactive electrophilic species manifest their toxicity (e.g., tissue necrosis, carcinogenicity, mutagenicity, teratogenicity) by combining covalently with nucleophilic groups present in vital cellular proteins and nucleic acids. The tripeptide, glutathione (cysteine glycine-glutamate) may be coupled via its sulfhydryl group to various compounds possessing an electrophilic centre.

The sulfhydryl group (-SH), of glutathione reacts with these electrophilic species to form S-substituted glutathione adducts and thus protects the vital cellular constituents by effective disposal of electrophiles (i.e., reactive epoxides).

Paracetamol, busulphan and azathioprine are other examples of drugs conjugated by this pathway. Glutathione conjugates are polar and of high molecular weight and are eliminated as such in the bile. The glutathione portion of the conjugate may further be metabolised via the peptide bond to mercapturic acid that are the normal urinary products of this conjugation pathway.



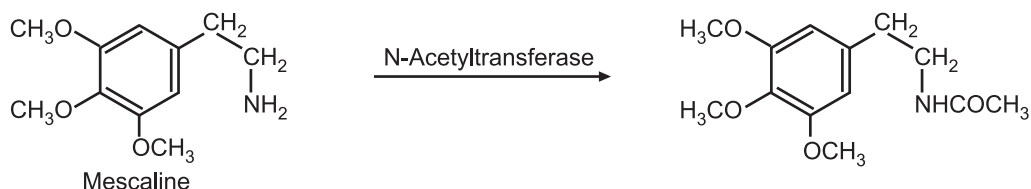
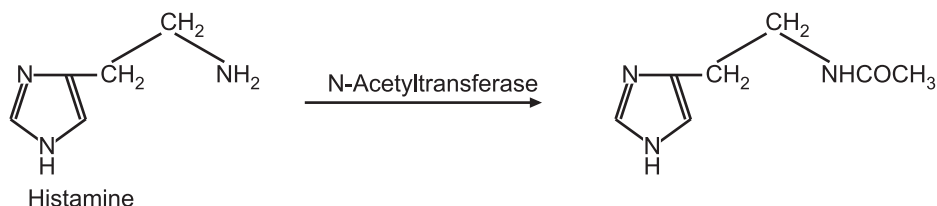


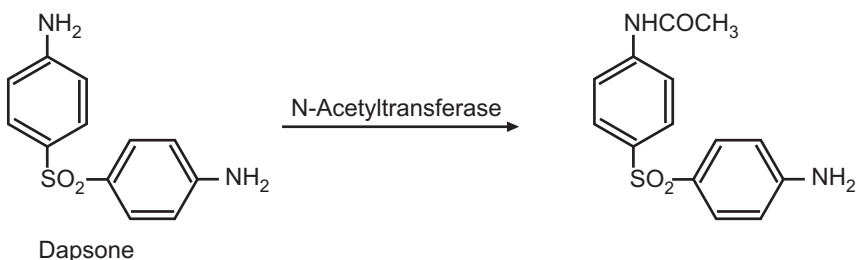
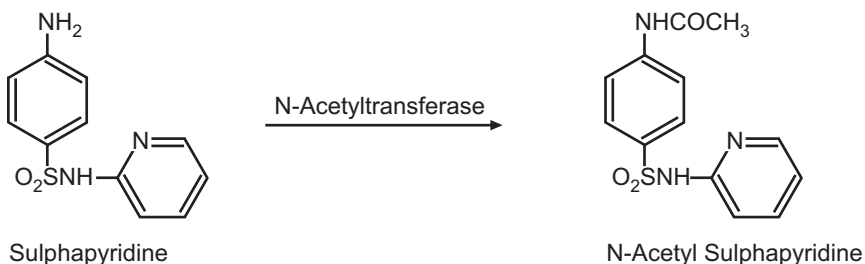


## (V) Acetylation Reactions:

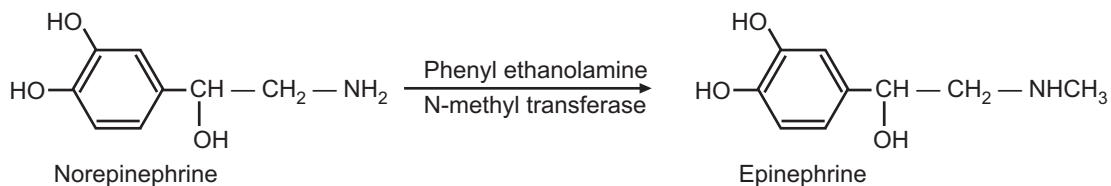
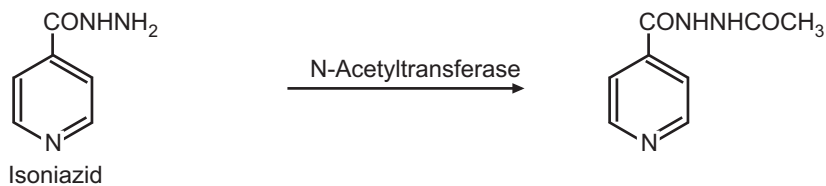
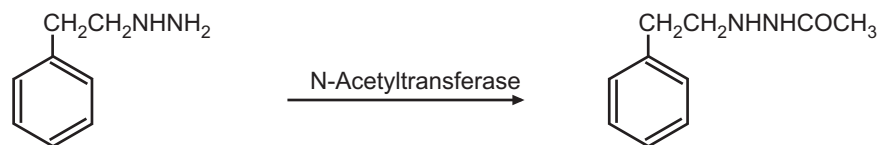
Acetylation reactions serve as an important metabolic route for drugs containing primary amino groups, sulphonamides, hydrazines and hydrazides, which upon conjugation get converted to their corresponding amide derivatives which are generally inactive and non-toxic. The transfer of acetyl group is catalysed by N-acetyltransferase enzymes present mainly in hepatic reticuloendothelial cells, which display broad substrate specificity. Thus, aromatic primary amines (e.g., sulphonamides) and hydrazine derivatives (e.g., isoniazid) are acetylated, utilizing acetyl coenzyme A. The acetyl transferase appears to be located in the soluble fraction of reticuloendothelial cells present in the liver and kidney. Because of a lowered solubility at acid pH, there is the danger of injury to the kidney resulting from precipitation of the conjugated sulphonamide in the renal tubular fluid as the kidney concentrates urine and lowers its pH.

### (1) Aliphatic 1° Amines:



**(2) Aromatic Amines:****(3) Sulphonamides:**

N-Acetyl Sulphapyridine

**(4) Hydrazines and hydrazides:**

Epinephrine

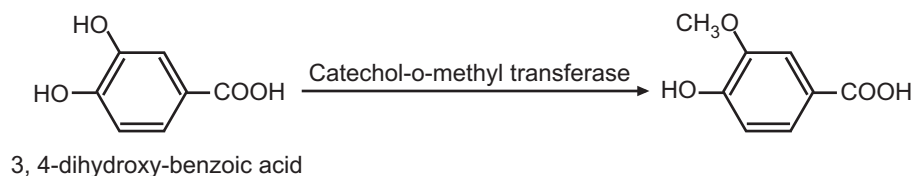
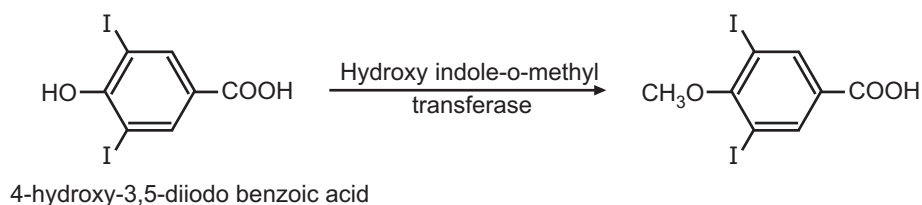
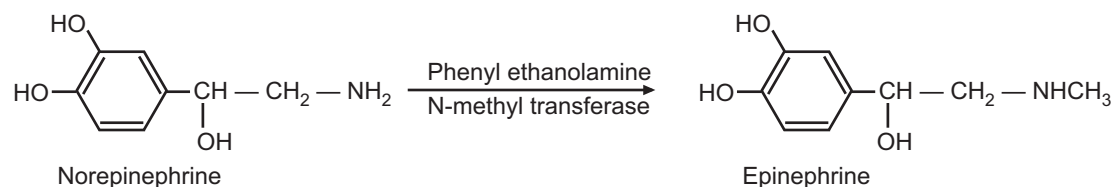
**(VI) Methylation Reactions:**

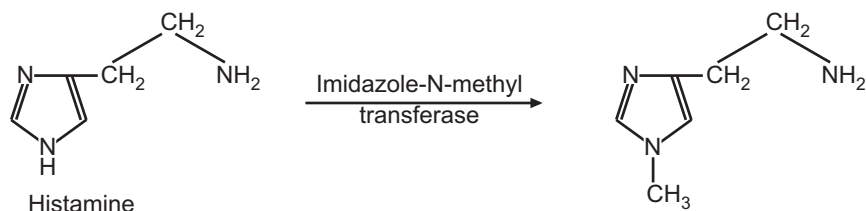
Methylation differs from other conjugation processes in that,

- (1) It is of greater significance in the metabolism of endogenous compounds.
- (2) In some cases, it results in the products having greater pharmacological activity than the parent molecules.

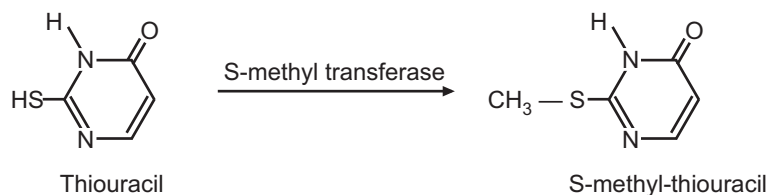
In these reactions, methionine transfers its methyl group via the activated intermediate, S-adenosylmethionine to the substrate under the influence of methyl transferase enzymes. Drugs or their metabolites containing primary aliphatic amine, phenolic or sulfhydryl group may be N-, O or S-methylated respectively. These enzymes are as follows:

- (1) Catechol-O-methyl transferases (COMT) They catalyse O-methylation of catecholamines.
- (2) Hydroxyindole-O-methyl transfer-ases. They catalyse O-methylation of substrate other than catecholamines.
- (3) N-methylation is catalysed by substrate specific enzymes. e.g., Phenyl ethanol amine-N-methyl transferase, Imidazole-N- methyl transferase.
- (4) S-methyl transferases: They catalyse S-methylation.

**(1) O-methylation:****(2) N-methylation:**



### (3) S-methylation:



### (VII) Nucleoside and Nucleotide Formation:

Addition of ribose and phosphate to the drug or its metabolite containing purine or pyrimidine nucleus, results in the formation of nucleoside or nucleotide.

Certain sugars like ribose, through an activated form, react with drugs to form ribonucleosides and ribonucleotides. The drugs that undergo this metabolic pathway should have either purine or pyrimidine nucleus. The activated form in which ribose reacts with the drug molecule is 5-phosphoribosyl-1-pyrophosphate (PRPP).

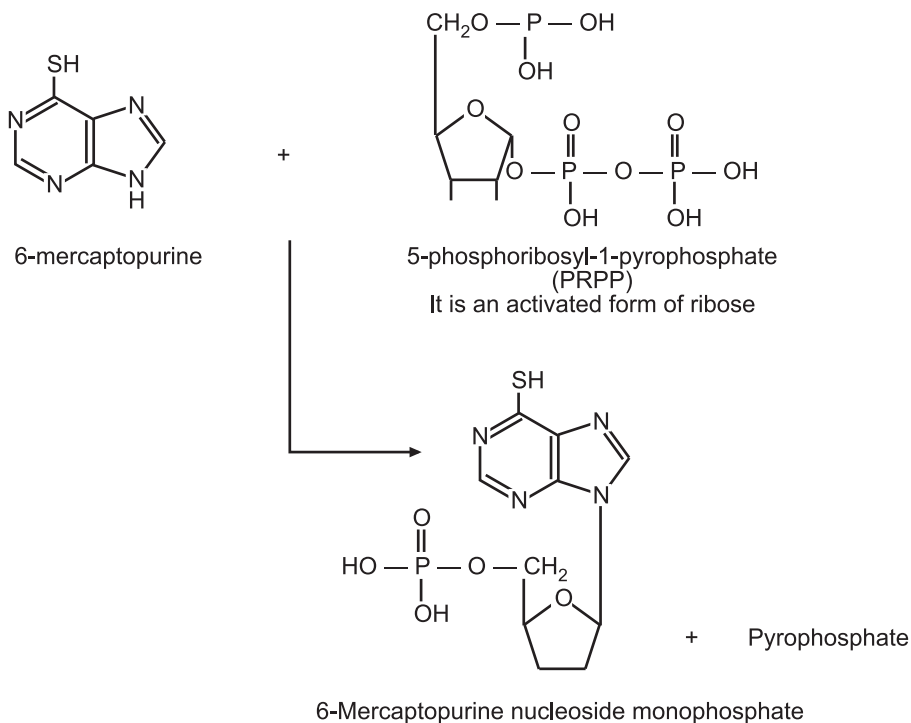
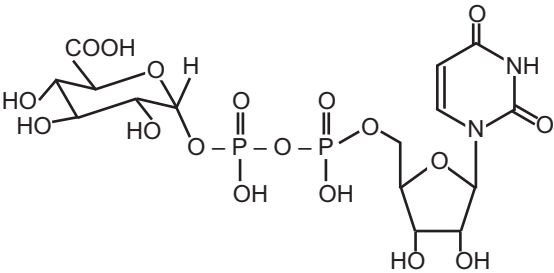
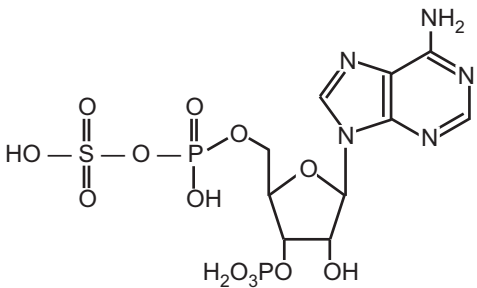
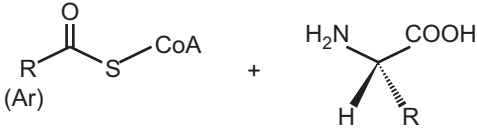
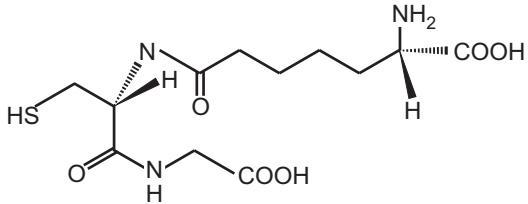
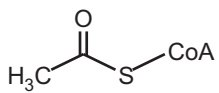
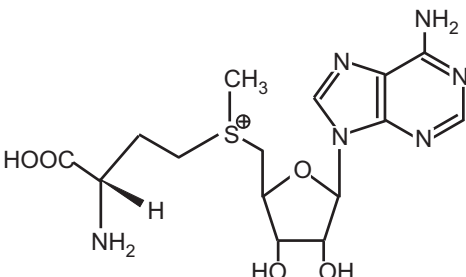


Table 3.2: Mammalian phase II conjugating agents

Type of conjugate	Coenzyme form	Groups conjugated	Transferase Enzyme
Glucuronide	Uridine 5'-diphospho- $\alpha$ -D-glucuronic acid (UDPGA) 	– OH, – COOH, – NH <sub>2</sub> , – NR, – SH, C – H	UDP-Glucuronosyl transferase
Sulfate	3-Phosphoadenosine-5'-phosphosulphate 	– OH, – NH <sub>2</sub>	Sulfotransferase
Glutathione	Activated acyl or aryl coenzyme 	– COOH	Glycine N-acyltransferase
Acetyl	Glutathione (GSH) 	Ar-X, arene oxide, epoxide, carbocation	Glutathione S-transferase
	Acetyl coenzyme A 	– OH, –NH <sub>2</sub>	Acetyl transferase

Methyl	S-Adenosyl methionine (SAM) 	– OH, –NH <sub>2</sub> , – SH, heterocyclic N	Methyl transferase
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### 3.4 CYTOCHROME OXIDASE ENZYMES

The endoplasmic reticulum of mammalian cells plays an important role in drug metabolism. A variety of enzymes that carries out a large number of vital cellular functions and detoxification are located in the endoplasmic reticulum. This intracellular membrane is richly endowed with the mono-oxygenase system which catalyses various oxidation reduction reactions involved in drug metabolism.

Mono-oxygenase reactions take place on or near the outer surface of the membrane. Cytochrome P-450 is deeply embedded in the membrane but a small portion is open to the surface. The exposed region contains the catalytic and substrate binding sites. The endoplasmic reticulum is equipped with a set of oxidation/ reduction enzymes (microsomal enzymes) and conjugation enzymes, whereas the hydrolytic enzymes are mainly present in the plasma.

Cytochrome P-450, molecular oxygen, a reducing agent (NADPH) and  $Mg^{++}$  ions are the basic requirements for mono-oxygenase enzyme system. The system consists of an undetermined number of species of cytochrome P-450 linked with NADPH-cytochrome P-450 reductases. Cytochrome P-450 can be defined as any hemoprotein that has an ability to show a peak absorbance at 450 nm, when it is reduced and reacts with carbon monoxide to form a complex. The term includes either a single molecular species or a group of cytochromes. At least 15 different cytochromes of P-450 type have been identified. They differ in substrate selectivity, molecular weight, catalytic ability, immunologic reactivities, electrophoretic mobility or response to enzyme inducers. The enzyme inducers that cause an increase in the activity of microsomal enzymes include, phenobarbital, steroidal hormones and 3, 4-benzpyrene.

A conclusive evidence about the presence of cytochrome P-450 and its role in drug metabolism was documented in 1962 due to the efforts of G.R. Williams of Johnson Foundation for Medical Physic, University of Pennsylvania. Sato and Omura provisionally named the carbon monoxide binding pigment as cytochrome P-450.

(a) Cytochrome P-450 hemoproteins are present in relatively higher concentration in liver and adrenals. In adrenal cortex, hemoproteins function in mitochondria to hydroxylate steroids. The location of cytochrome P-450 is not restricted to microsomes of liver but they

**Table 3.3: Cytochrome P-450 activity in various organs**

Organ	Relative activity (%)
Liver	100
Lung	20 - 30
Kidney	09
Intestine	07
Placenta	05
Adrenal	02
Skin	01

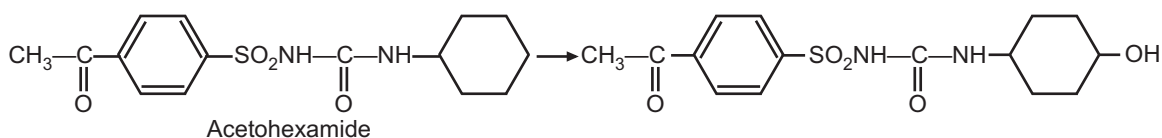
have been reported to be present in microsomes of the kidney, intestinal mucosa, lung, brain, skin, testis, and placenta. Spleen, gonads, eye and leukocytes also exhibit less significant cytochrome P-450 activity. Some higher plants along with yeasts, molds and bacteria also have cytochrome P-450 activity.

(b) NADPH (reduced nicotinamide adenine dinucleotide phosphate) is a source of electrons. NADPH-cytochrome P-450 reductase enzyme catalyses the oxidation of NADPH to release protons. The reductase enzyme is a FAD and FMN containing flavoproteins. The flavoprotein transfers an electron to cytochrome C or other electron acceptor (e.g., menadione, methylene blue) if they are present at the site. Another electron is consumed for the conversion of  $2\text{H}^+$  to  $2\text{H}$ .

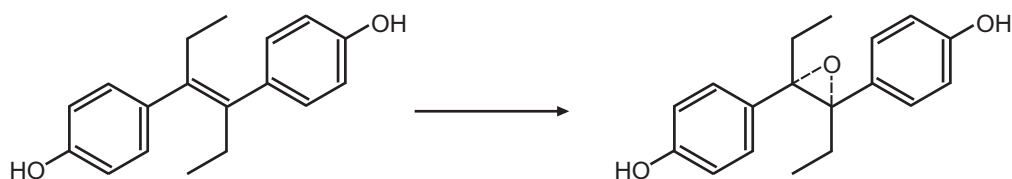
(c) Phosphatidylcholine is necessary for electron transport from NADPH to cytochrome P-450. The mono-oxygenase system is thus a multicomponent membrane bound enzyme. Cytochrome  $\text{b}_5$  is yet another microsomal hemoprotein which sometimes participates in metabolism of drugs.

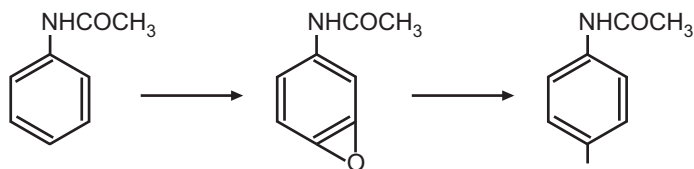
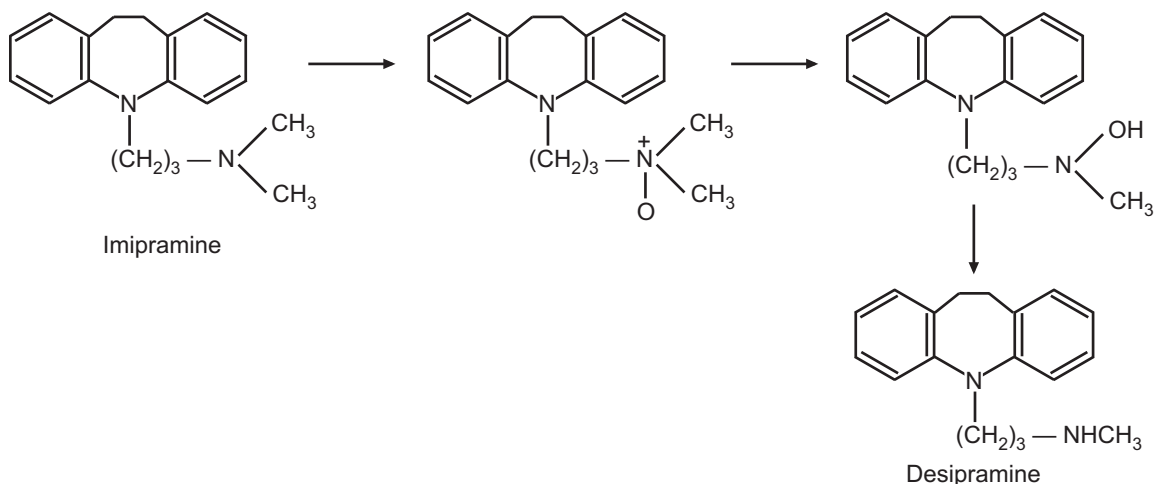
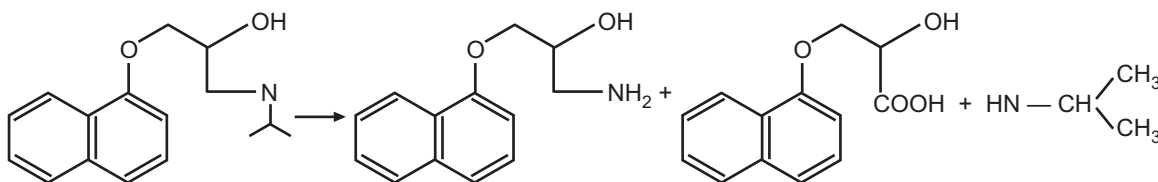
### Metabolic oxidations catalysed by cytochrome P - 450:

#### (a) Ring hydroxylation:



#### (b) Olefin oxidation:



**(c) Aromatic oxidation:****(d) N-Dealkylation:****(e) Deamination:****3.5 FACTORS INFLUENCING METABOLIC PATHWAYS OF THE DRUG**

In most cases, the metabolism of a drug is a first order process which means that a constant fraction of the drug is metabolized in unit time. Saturation of one metabolic pathway may allow for a shift in the metabolic pattern of a drug. Thus after paracetamol overdose, the glucuronide and sulphate conjugation pathways become saturated, making available a greater fraction of the dose for oxidation to a reactive and potentially toxic metabolite where glutathione conjugation plays an important role.

**Important factors that affect metabolic pattern of the drug include:**

- (1) Dose and frequency of administration of drug.
- (2) Species and strain of animal used.
- (3) Diet and nutritional status of animal and the sex and weight of the animal used.

- (4) Route of administration.
- (5) Time of administration.
- (6) Interactions with other drugs and environmental contaminations.
- (7) Pregnancy and psychological abnormalities.
- (8) Inducers of drug metabolism.
- (9) Inhibitors of drug metabolism.

Age of the patient governs the pattern of drug metabolic pathways. For example, the extent and types of drug metabolites observed in very old patient is different from that in very young patient. Besides this, normal adult handles the drug in quite a different fashion.

### 3.6 INDUCERS OF DRUG METABOLISM

Although the liver is the main site for drug metabolism, other sites like placenta, skin, lung, gut or white blood cells also offer platform for metabolism of drugs. The hepatic microsomal P-450 mixed function oxygenases catalyse numerous biotransformations by simply inserting oxygen into drug skeletons. Certain chemically reactive metabolites may produce a range of toxic effects by reacting covalently with essential cellular components. Hence the microsomal P-450 mixed function enzymes, therefore, play a key role in governing drug toxicity.

The rate of elimination of many lipophilic drugs thus depends upon the activity of these hepatic enzymes. Consequently, any alteration in the activity of these enzymes may result in the fluctuation in the drug activity. Enzyme inducing agents can cause an increase in the rate of drug metabolism. Such agents include phenobarbital, steroidal hormones (e.g., estrogen, androgens, progestins, and corticosteroids),  $\beta$ -naphthoflavone, ethanol, 3, 4-benzpyrene, rifampicin, phenytoin, phenyl-butazone, glutethimide, carbamazepine etc. Enzyme induction not only reduces the drug activity but may also decrease toxicity in some cases. However, the effects of enzyme induction and enzyme inhibition are reversible. As soon as the interacting agent is withdrawn, the metabolizing ability of enzymes is brought to normal level.

Stimulation of drug metabolism may result due to

- (i) an increased rate of synthesis of new enzyme proteins or
- (ii) decreased rate of degradation of drug metabolizing enzymes, and
- (iii) an increase in the amount of smooth endoplasmic reticulum that serves as site for drug metabolism.

Phenobarbital treatment increases the amount of microsomal proteins per gm of liver and also causes an increase in the amount of smooth endoplasmic reticulum of the liver.

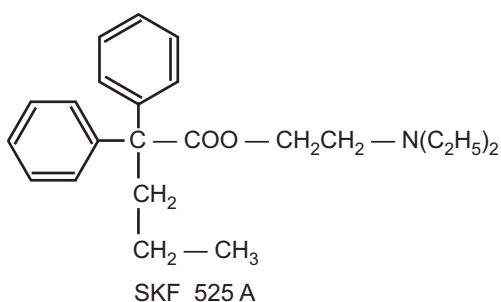
The blockage of enzyme induction by actinomycin or puromycin (protein synthesis inhibitors) suggests the role of inducers at the level of transcription, resulting into an increase in the m-RNA synthesis. Thus, the microsomal enzyme inducers increase the rates of protein and DNA dependent RNA synthesis. Different receptors and genes thus participate in the induction of microsomal enzyme activity, that is reflected in the increased rates of synthesis of cytochrome P-450, cytochrome P-450 reductase and other enzymes.

An enzyme inducer, 3, 4-benzpyrene is present in kidney, adrenal glands, placenta, gastrointestinal tract, pancreas and skin, can also be induced to a limited extent.

### 3.7 INHIBITORS OF DRUG METABOLISM

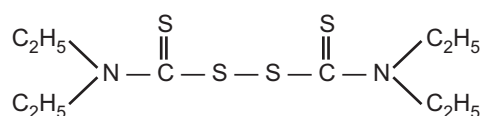
The enzyme inhibitors may display competitive and non-competitive kinetics. For example, cimetidine, MAO-inhibitors (tranylcypromine, iproniazid), disulfiram (aldehyde dehydrogenase) and allopurinol (xanthine oxidase). Novobiocin has been reported to cause jaundice in the new born, arising from its inhibition of bilirubin conjugation with glucuronic acid.

Since microsomal P-450 enzymes exhibit broad substrate specificity, many substances try to occupy the enzymes competitively. Hence any drug may inhibit the metabolism of another drug. Prominent inhibitors of drug metabolism include, SKF 525A, disulfiram, MAO inhibitors, allylbarbiturates, dicumarol, phenylbutazone, piperonyl butoxide, sulfaphenazole etc. Similarly various disease conditions, like hepatitis, obstructive jaundice, advanced cirrhosis, diabetes, or hypothyroidism may lead to the interference or suppression of microsomal enzyme activity. Recently it has been found that the hepatic drug metabolizing activity is inversely proportional to the plasma glucocorticoidal activity.



(i) The drug SKF 525 A is pharmacologically inactive but when administered along with another drug, like hexobarbital, prolongs duration of hypnosis. The prolongation of action of other barbiturates, amphetamine, and analgesic agents has also been reported. It also inhibits the non-microsomal metabolism (e.g., hydrolysis of procaine) and some glucuronidation reactions which require microsomes but are not oxidative in nature.

Since SKF 525 A does not have activity of its own, it can be used as a tool to find out whether the activity is due to the drug or due to its metabolite.

**(ii) Disulfiram:**

Disulfiram is yet another inhibitor of drug metabolism. It is devoid of any pharmacological activity of its own. It specifically inhibits metabolism of ethyl alcohol and hence, is useful in the treatment of chronic alcoholism. The conversion of acetaldehyde intermediate to acetic acid is inhibited by disulfiram.

The accumulation of acetaldehyde in body results into violent unpleasant syndrome including, flushing, dyspnea, nausea, vomiting and hypotension. The drug inhibits an enzyme, acetaldehyde dehydrogenase. Due to the violent syndrome that results from disulfiram in the treatment of chronic alcoholism, the treatment must strictly be given under the medical supervision.

**(iii) MAO Inhibitors:**

Mono amino oxidases (MAO) is a group of enzymes that metabolizes catecholamines, Iproniazide, phenelzine and isocarboxazide are the inhibitors of MAO enzymes.

Since, these agents inhibit the metabolism of catecholamines which act as neurotransmitters in brain, these agents are useful in the treatment of psychotic disorders. They are effective MAO inhibitors, both, *in-vivo* as well as *in-vitro*.

**(iv) Pyrazole:**

Though it is a potent competitive inhibitor of liver alcohol dehydrogenase enzymes, it can not be used clinically due to its severe toxicity.

Several structurally unrelated but lipophilic drugs having an allyl group in their structure, were found to inhibit and destroy cytochrome P-450 enzymes. Allyl barbiturates (e.g., secobarbital, allobarbital) increase the duration of action of other drugs by this mechanism.

Besides starvation and parenchymal liver damage, some drugs on chronic long term use, may depress the microsomal drug-metabolizing system. Such drugs include, oxyphenbutazone, nortriptyline, methyl-phenindate and allopurinol.

**Table 3.4: Therapeutic activity of drug metabolites**

Parent drug	Active metabolite(s)
Acetanilide	N-acetyl-p-aminophenol
Acetohexamide	Hydroxyhexamide
Acetylmethadol	Nor-acetylmethadol and dinoracetylmethadol.
Adriamycin (doxorubicin)	Adriamycinol
Allopurinol	Oxipurinol
Amitriptyline	Nortriptyline
Amphetamine	p-Hydroxyamphetamine
Carbamazepine	Carbamazepine-10, 11- epoxide
Carbimazole	Thiamazole (antithyroid)
Cephaloglycin	Desacetylcephaloglycin
Cephalothin	Desacetylcephalothin
Cephapirin	Desacetylcephapirin
Chloral hydrate	Trichloroethanol
Chlordiazepoxide	N-desmethylchlordiazepoxide, demoxepam
Chlorpromazine	7-Hydroxychlorpromazine
Chloroguanide	Cycloguanil (antimalarial)
Chlorpropamide	2-Hydroxy metabolite, 3-hydroxy metabolite
Clofibrate	Free acid metabolite
Codeine	Morphine, norcodeine
Cortisone	Cortisol
Daunorubicin	Daunorubicinol
Diazepam	N-Desmethyldiazepam, N-methyloxazepam, oxazepam
Digitoxin	Digoxin
Diphenoxylate	Difenoxine (free acid metabolite)
Ethynodiol	Norethisterone
Fenfluramine	Norfenfluramine
Floctafenin	Floctafenic acid
Flurazepam	N-desalkyl metabolite, flurazepam N <sub>3</sub> -ethanol

Parent drug	Active metabolite(s)
Guanethidine	N-oxide metabolite
Imipramine	Desipramine
Lignocaine	N-desmethyl metabolite, N-didesethyl metabolite (glycinexylidide)
$\Delta^9$ -tetrahydrocannabinol	11-hydroxymethyl metabolite
Mephobarbitone	Phenobarbitone
Methsuximide	N-desmethyl metabolite
$\beta$ -Methyl digoxin	Digoxin
Morphine	Nor-morphine
Miracil D	Hycanthone (schistosomicide)
Nalidixic acid	Hydroxynalidixic acid
Naloxone	6- $\beta$ -Hydroxynaloxone
Nor-ethynodrel	Nor-ethisterone
Oxisuran	Oxisuran alcohols
Pethidine	Nor-pethidine
Phenacetin	Paracetamol
Phenylbutazone	Oxyphenbutazone
Prednisone	Prednisolone
Primidone	Phenobarbitone
Probenecid	Side chain hydroxyl metabolites, N-despropyl metabolite
Procainamide	N-acetylated metabolite
Propranolol	4-Hydroxypropranolol, Propranolol glycol, N-Desisopropyl metabolite
Quinidine	3-Hydroxyquinidine
Rifampicin	Desacetylated metabolite
Thioridazine	Mesoridazine
Trimethadione	Dimethadione
Vitamin D	1, 25-dihydroxy metabolite
Warfarin	Warfarin alcohols
Zoxazolamine	Chlorzoxazone

**Table 3.5: Toxic metabolites of drugs**

<b>Drug</b>	<b>Metabolite</b>	<b>Toxicity of metabolite</b>
Chloroform	Phosgene	Renal tubular and hepatic necrosis
Dapsone	N-Hydroxydapsone	Methaemoglobin formation
Diazepam	N-Desmethyl diazepam	Adverse autonomic nervous symptoms
Glutethimide	4-Hydroxy metabolite	Metabolite contributes to the coma produced by glutethimide overdose
	4-Hydroxy metabolite	Metabolite is twice as potent as parent drug in producing drug induced ataxia
Imipramine	2-Hydroxy-imipramine	Cardiotoxic (depressed contractility)
Isoniazid	Acetylhydrazine	Isoniazid hepatitis results from liberation of acetylhydrazine in the body.
Lignocaine (lidocaine)	N-desethyl metabolite (MEGX)	MEGX has convulsant activity.
	Glycinexylidide (GX)	Adversely affected mental performance, caused headaches and
	Glycinexylidide (GX)	Potentiates seizures produced by lignocaine and MEGX.
Methoxy-flurane	Fluoride ion	Nephrotoxicity.
Methsuximide	N-desmethyl metabolite	Metabolite implicated in production of delayed coma after methsuximide overdose and ataxia.
Norethy-nodrel	3 $\beta$ -hydroxy metabolite	Dysmorphogenic
Paracetamol	N-acetyl -p-quinoneimine	Hepatic necrosis
Phenacetin	p-phenetidine	Methaemoglobinaemia
Salicylate	Semiquinone radical	Renal tubular necrosis
Testo-sterone	Etiocholanolone	Pyrogenic and inflammatory reaction.



## Chapter...4

# DRUGS ACTING ON AUTONOMIC NERVOUS SYSTEM

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## ♦ SYNOPSIS ♦

**4.1 INTRODUCTION****4.2 NERVOUS SYSTEM****4.3 NEURO-CHEMICAL TRANSMITTERS****4.4 AUTONOMIC NERVOUS SYSTEM****4.5 DIVISIONS OF AUTONOMIC NERVOUS SYSTEM****4.6 ACH AND NE AS NEUROTRANSMITTERS****4.7 NEUROTRANSMITTERS PRESENT IN CNS**

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### 4.1 INTRODUCTION

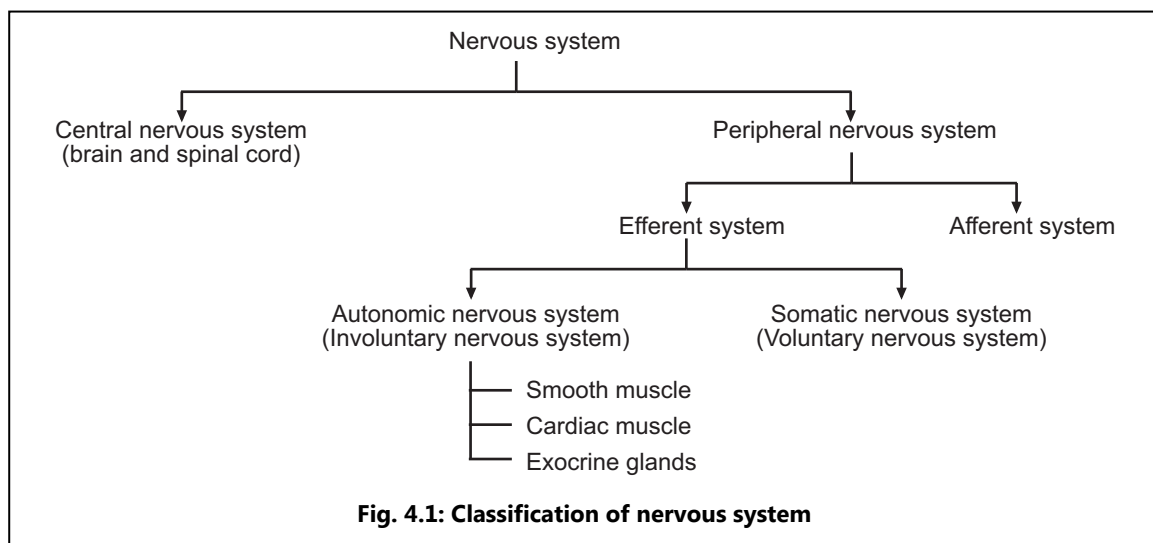
Cellular systems for the transduction of external stimuli into intracellular signals are essential components of the plasma membranes.

According to the theory of neurohumoral transmission, specific chemical agents are responsible for transmission of nerve impulse across most synapses and neuro-effector junctions. These agents are known as *neurohumoral transmitters*. The concept of "chemical neurotransmission" was first proposed by Dale and co-workers, instead of "electrical transmission" hypothesis. The release of transmitter substances occurs when the nerve impulse elicits the responses at smooth, cardiac and skeletal muscles, exocrine glands and postsynaptic neurons. These neurotransmitters cross the synapse or the neuro-effector junction to initiate activity in another neuron or in a muscle or a gland cell by interacting with the postsynaptic receptors. A clear understanding of the impulse transmission therefore, is essential to study the pharmacology of the drugs acting on autonomic nervous system.

### 4.2 NERVOUS SYSTEM

Principally the nervous system may be described as a device of,

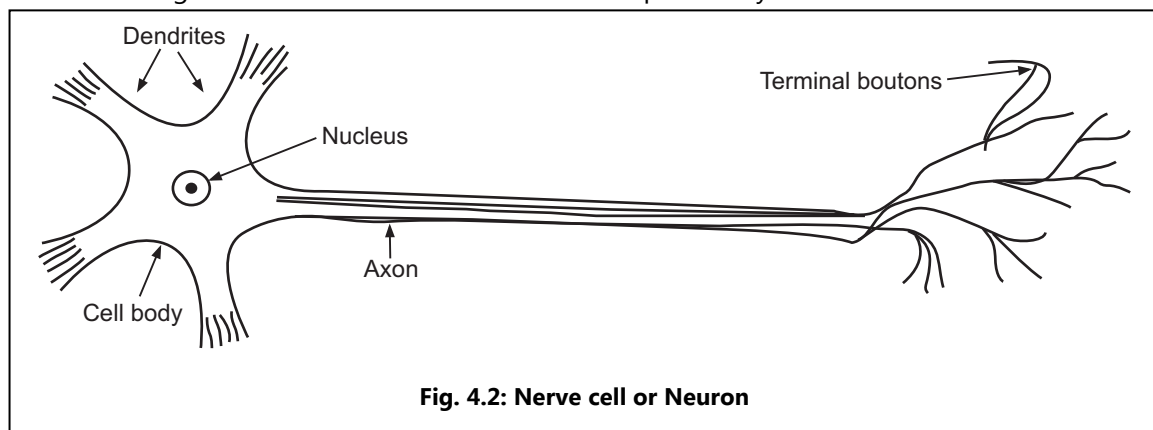
- (1) receiving information (i.e. sensory input),
- (2) processing information (i.e. integration) and
- (3) transmitting information (i.e. motor output).

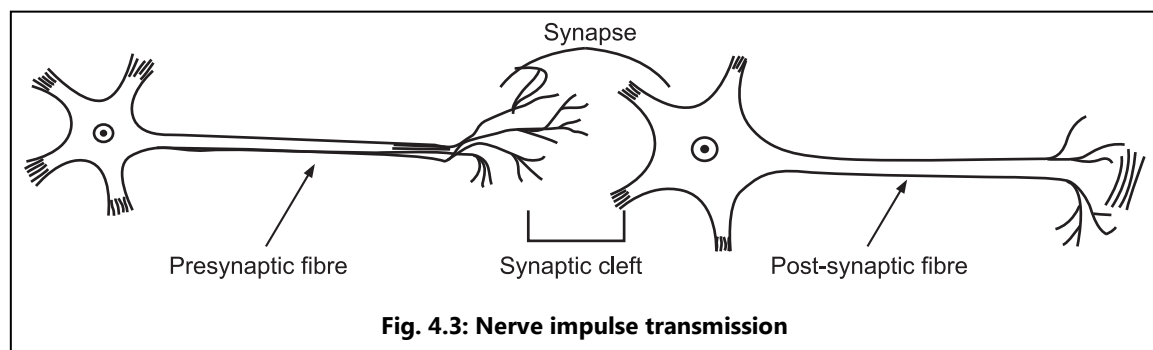


The fundamental unit of a nervous system is the neuron or a nerve cell. Each neuron consists of a nucleus and a cell body i.e., stems (an extensive network of branches), the axon (long process) and the dendrites (short process).

The surface membrane of a neuron consists of a semipermeable layer of lipoproteins. The composition of salt solution inside the membrane is usually different from that on the outside. This is due to differences in the permeability of the membrane to the various ions like  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{++}$ ,  $\text{Cl}^-$ ,  $\text{HCO}_3^-$  etc.

In the resting state, the inside of neuronal membrane is more negative than the outside. This normal situation is known as resting state or polarised state. When any exogenous stimulus is applied; a change in the electrical activity occurs within the neuron. At the point, where an exogenous stimulus occurs, the inside of neuronal membrane becomes positive than the outside. As a result, local action currents are set up, which have the effect of transferring the area of reversed polarisation to an adjoining region of the nerve while normal resting conditions are re-established in the previously stimulated area.





In this way, the path of reversed polarisation is transmitted along with the nerve. The process is continued until the whole length of the nerve has been visited by the impulse. The nerve impulse, in other words, jumps from one patch to other patch. As soon as this impulse reaches the terminal boutons, it activates the influx of extracellular  $\text{Ca}^{++}$  ions. These ions, upon their entry in the cytoplasm lead to the release of intracellular  $\text{Ca}^{++}$  ions from the sacs present on sarcoplasmic reticulum. When the cytoplasmic concentration of  $\text{Ca}^{++}$  ions reaches a threshold value, the storage granules for neurotransmitter get ruptured and a discrete amount of neurotransmitter is discharged from the presynaptic nerve endings into the synaptic cleft. The synaptic cleft or junctional cleft is generally about  $200 - 400 \text{ \AA}$  wide but in some blood vessels, it may be as wide as  $10,000 \text{ \AA}$ .

The transmitter, then diffuses across the synaptic space and binds to the receptor sites present on the cell body of post-synaptic neuron. This binding causes conformational changes in these receptors, which in turn, produce a change in ion-permeability of the axon membrane of post-synaptic neuron. As a result, local action currents are set up into the post-synaptic neuron. The post-synaptic axon branches many times upon entering the effector tissue forming a plexus among the innervated cells. The release of neurotransmitter from post-synaptic nerve terminals into the neuro-effector space then leads to the biological response in a muscle or a gland cell. This synapse between a motor neuron and effector cell is also termed as a neuro-effector junction.

Once the neurotransmitter has interacted with the receptors, it is either removed by active uptake processes back to the terminal boutons of pre-synaptic neuron or by surrounding glial cells where it is destroyed by metabolic deactivation.

### 4.3 NEURO-CHEMICAL TRANSMITTERS

Following are the examples of chemical agents that act as neuro-chemical transmitters in nervous system.

- Aspartic acid, taurine, glycine, gamma amino butyric acid (GABA), and glutamic acid. These can be grouped as amino acids.
- Acetylcholine, dopamine, tyramine, norepinephrine, epinephrine, histamine and serotonin (5-HT). These can be grouped as amines.

- (c) **Miscellaneous:** Peptide substance P, ATP, c-AMP, c-GMP, prostaglandin E, enkephalins, neurotensin, cholecystokinin etc.

Neurotransmitters have an ability to initiate the impulse propagation. Certain substances do not initiate the process of impulse transmission but can modify it.

Such substances are termed as modulators of transmission. For example, most of the autonomic drugs act either by mimicking or modifying the actions of the neurotransmitter released by the autonomic fibres at either synaptic cleft or effector cells. Besides this, the nerve cell is provided with a number of feedback control systems which regulate the biosynthesis, release and metabolism of the neurotransmitter and thus exercise a control over the biological response.

#### 4.4 AUTONOMIC NERVOUS SYSTEM

The ANS consists of central and the peripheral components. It is evident from the investigations that elicitation of autonomic reflexes (e.g. blood pressure changes, vaso-motor responses to alterations of body temperature, sweating, constriction of urinary bladder) can occur at the level of the spinal cord. However, integration of many autonomic functions occurs at supraspinal levels. Thus, regulation of respiration and blood pressure is integrated in medulla. The hypothalamus plays a prominent role in integration of various autonomic functions, e.g. regulation of blood pressure, sleep, emotions, sexual reflexes and carbohydrate-fat metabolism. Posterior and lateral hypothalamic nuclei are connected with the sympathoadrenal system, while anterior and midline nuclei are concerned with parasympathetic functions. The posterio-medial hypothalamus is involved in the modulation of the baro-receptor reflex. The other higher centres involved in the integration of various autonomic functions include the neostriatum, limbic system and cerebral cortex.

The autonomic nervous system controls all involuntary actions aimed to maintain the constancy of the internal environment. It provides a homeostasis for the regulation of all metabolic changes which are essential for life. The ANS is termed as the visceral, vegetative or involuntary nervous system. In the periphery, it functions through nerves, ganglia and plexuses and regulates autonomic functions which are not under the conscious control. These include, breathing, regulation of the cardiovascular system, glandular secretions, digestion, body temperature and metabolism. Except skeletal muscles, all innervated organs of the body, are supplied with efferent nerves of ANS, while skeletal muscles are provided with somatic nerves. Thus, ANS is essentially a motor system. The sensory fibres are numerous than autonomic motor nerves and they pass into the cerebrospinal axis via either somatic nerves or various ramifications of ANS without synaptic interruption.

Hypothalamus is a principle control centre for organisation and co-ordination of the autonomic nervous system. The cells of the adrenal medulla constitute an integral part of the ANS, which upon activation, release epinephrine and norepinephrine into the circulation.

**4.5 DIVISIONS OF AUTONOMIC NERVOUS SYSTEM**

The autonomic nervous system controls tissues, e.g., glands, smooth muscles and cardiac muscles that are not under voluntary control. It consists of two main divisions:

- (a) Sympathetic nervous system and
- (b) Parasympathetic nervous system.

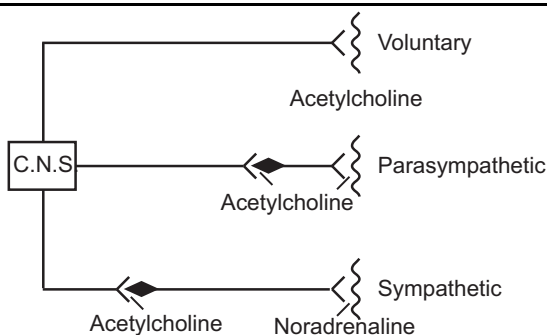
Both these divisions have essentially opposite actions. The sympathetic nervous system is associated with catabolic effects whereas parasympathetic nervous system is characterised by its anabolic effects.

The principle neurotransmitter present in parasympathetic nerves liberate acetylcholine.

- (i) The pre-ganglionic and post-ganglionic fibres of the parasympathetic nerves liberate acetylcholine.
- (ii) The pre-ganglionic fibres and some postganglionic fibres (e.g., salivary glands) of sympathetic nerves liberate acetylcholine.
- (iii) All autonomic ganglia and skeletal muscle end plate region need acetylcholine as a neurotransmitter to evoke biological response. The end plate is a specialised region of the muscle with which the terminal ramifications of the motor nerve fibres are associated. The ganglionic transmission is a highly complex process and several secondary transmitters or modulators either enhance or diminish the sensitivity of the post-ganglionic cell to acetylcholine.

Stimulation of parasympathetic nervous system induces the constriction of the pupils and bronchi, decrease in heart activity and an increase in the activity of the digestive system-salivation and GIT secretions are promoted, the motility of the intestine is increased.

Similarly the principal neurotransmitters present in the sympathetic nervous system include epinephrine (adrenaline), norepinephrine (noradrenaline) and dopamine. The post-ganglionic fibres of the sympathetic nerves with few exceptions, bring about their effects by the liberation of norepinephrine.



**Fig. 4.4**

Stimulation of the sympathetic nervous system causes dilation of the pupils, acceleration of rate of (positive chronotropic) and the force of (positive inotropic) heart contraction, peripheral vasoconstriction, glycogenolysis, inhibition of intestinal motility and of gastrointestinal secretory activity (except salivary gland).

When the transmitter substance reacts with the post-synaptic receptors, it may produce either excitation or inhibition. The action of the transmitter results in selective increase or decrease of ionic permeability of membrane for ions. In inhibition, there is a negligible change in the ion potential and the fibre remains at near to the resting potential, thereby preventing the fibre to get in an excited position.

The transmitters in the neurons are in a state of flux, being continuously biosynthesised, released and metabolised, thus producing profound changes in the activity of the nerves. The nerves in the peripheral nervous system are classified on the basis of their functions into,

- (1) Sensory (afferent) neuron,
- (2) Motor (efferent) neuron and
- (3) Internuncial neuron.

Sensory neurons transmit impulses from CNS to or towards the muscle or tissues.

Internuncial neurons are located in CNS and they transmit impulses from sensory to the motor neurons.

Many neurotransmitters play an important role in the propagation of the nerve impulse in the sensory neurons. These include substance P, somatostatin, vasoactive intestinal polypeptides and cholecystokinin.



Substance P

The efferent (motor) nervous system of ANS can be broadly categorised into,

- (a) Parasympathetic (or craniosacral) division and,
- (b) Sympathetic (or thoracolumbar) division.

This classification is mainly based upon the type of neurotransmitter that predominates in each division.

The cholinergic nervous system consists of pre-ganglionic and post-ganglionic fibres. The pre-ganglionic fibres have their origin in midbrain, medulla oblongata and the sacral part of the spinal cord. Thus, the principal site of control and co-ordination of both sympathetic and parasympathetic nervous system is hypothalamus. The hypothalamus along with cerebral cortex serves as a locus of integration of the entire autonomic nervous system. Hypothalamus also plays an important role in the regulation of gastrointestinal, cardiovascular, sexual, emotional and the functioning of limbic system.

In contrast to other cholinergically innervated organs, the cardiac impulse conduction system (i.e. S-A node, atrium, A-V node and the His-perkinje system) has its own activity where the conduction of impulse can be influenced but not initiated by autonomic nervous system. In cardiac cell, cholinergic influence results into inhibitory response due to hyperpolarization. The hyperpolarization results due to the increased permeability of the axon membrane to potassium ions.

In parasympathetic division, a preganglionic fibre synapses with one or at the most two post-ganglionic neurons. The synapses are located very close to or within the organ innervated. Due to the limited distribution, parasympathetic preganglionic neurons can affect only specific organ and do not influence a wide region of the body. In contrast to this, sympathetic synapses are located in the vertebral and prevertebral ganglia. Hence, a single sympathetic preganglionic fibre may synapse with 60 to 189 post-ganglionic neurons provided to a widely separated regions of the body. Naturally upon activation, sympathetic nervous system can evoke and influence the biological activities of the whole body. The area of functioning of parasympathetic division is thus limited and involves accumulation and preservation of body resources. While sympathetic division regulates body compartments of vital importance and prepares the person in conditions of stress and emergencies. Its stimulation results in a generalised somatic or mass reflex action.

#### **(a) Parasympathetic Nervous System:**

Acetylcholine is the neurotransmitter which propagates impulse transmission in the parasympathetic division. Besides this, acetylcholine also functions as a neurotransmitter in,

- (i) Motor nerves to skeletal muscles and
- (ii) Certain neurons within CNS.

Reid Hunt and Taveau (1906) were first to report the properties of acetylcholine. In 1921, Otto Loewi, a German pharmacologist identified the nervous stimulation of the heart as a chemically mediated event. Loewi then, along with Navratil demonstrated in 1926 that acetylcholine functions as a neurotransmitter in cholinergic nerves. This was later confirmed by Dale and Feldeberg in 1934 on stimulation of vagal fibres to the stomach.

The three important responses mediated by acetylcholine in man or laboratory animals include

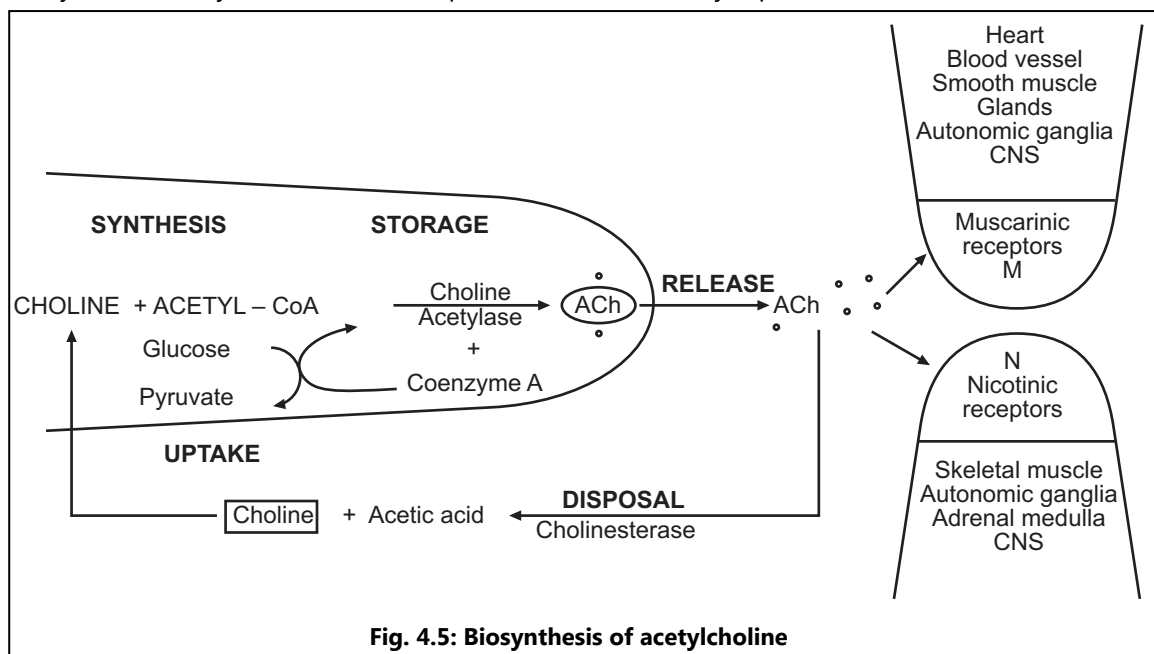
1. Contraction of smooth muscles.
2. Cardiac inhibition and
3. Peripheral vasodilation.

Since upon stimulation, parasympathetic nerve fibre liberates acetylcholine, the parasympathetic division is also termed as cholinergic nervous system. The terms cholinergic and adrenergic were first presented by Dale to denote neurons that release acetylcholine and norepinephrine respectively.

Acetylcholine is biosynthesised by the acetylation of choline molecule. Choline itself has a weak parasympathomimetic activity and upon injection, causes a fall in blood pressure. Acetylcholine is about 10,000 times more active than choline molecule.

Acetylcholine is biosynthesised in the nerve terminals as shown in Fig. 4.5.

Active transport mechanisms are involved in picking up choline molecules from the extrasynaptic fluid into the exoplasm. This transport is dependent upon the intracellular concentration of  $\text{Na}^+$  and  $\text{K}^+$  ions. The choline molecule is acetylated in the cytoplasm by acetyl coenzyme A which is biosynthesised in the mitochondria present in the nerve terminal. The acetylation of choline is catalysed by choline acetyl transferase enzyme. The enzyme is synthesised within the perikaryon and has a molecular weight of about 68,000. In peripheral cholinergic nerves, it is usually present in higher concentrations. As soon as acetylcholine is synthesised, it is sequestered within the synaptic vesicles.



**Site 1:** Acetylcholine synthesis can be blocked by styryl pyridine derivatives such as NVP.

**Site 2:** Acetylcholine transport into vesicles is blocked by vesamicol (AH 5183). ( $\pm$ ) Vesamicol is a potent inhibitor of vesicular ACh storage with L (-)-Vesamicol being more potent than D(+) - Vesamicol.

**Site 3:** Release is promoted by  $\alpha$ -bungarotoxin, black widow spider venom, and  $\text{Ca}^{++}$ . Release is blocked by botulinum toxin, cytochalasin B, collagenase pre-treatment, and  $\text{Mg}^{++}$ .

**Site 4:** Postsynaptic receptors are activated by cholinomimetic drugs and anticholinesterases. Nicotinic receptors, at least in the peripheral nervous system, are blocked by rabies virus, curare hex amethonium, or dihydro- $\beta$ -erythroidine; n-methylcarbamylcholine and dimethylphenyl piperazinium are nicotinic agonists. Muscarinic receptors are blocked by atropine, pirenzepine, and quinuclidinyl benzilate.

- Site 5:** Presynaptic muscarinic receptors may be blocked by AFDX - 116 (an  $M_2$  - antagonist), atropine or quinuclidinyl benzilate. Muscarinic agonists (e.g. oxotremorine) will inhibit the evoked release of acetylcholine by acting on these receptors.
- Site 6:** Acetylcholinesterase is inhibited reversibly by physostigmine (eserine) or irreversibly by DFP.
- Site 7:** Choline uptake competitive blockers include hemicholinium 3, troxypyrrolium tosylate.

The biosynthesised acetylcholine is stored within the synaptic vesicles immediately inside the membrane of the nerve terminal. Each vesicle is expected to contain about 5,000 - 10,000 molecules of acetylcholine. The number of such vesicles present in the nerve terminal varies in different organs. For example, a motor nerve terminal may contain 300,000 or more synaptic vesicles.

When an impulse reaches to nerve terminal depolarisation causes an activation of calcium ionophore. It allows an influx of extracellular calcium ions which is an essential step for the rupturing of storage vesicles of almost all neurotransmitters. The extracellular calcium then leads to the release of acetylcholine from the vesicles. Four calcium ions are taken up for each molecule of acetylcholine released. The ruptured synaptic vesicles again re-shape to store fresh neurotransmitter.

The vesicular release of acetylcholine is reported to be inhibited by excess of magnesium ions. The released acetylcholine along with extracellular calcium ions then mobilise intracellular calcium ions from the sacs present on sarcoplasmic reticulum. The increase in the concentration of free intracellular calcium ions then activates calmodulin dependent myosin light chain kinase and phosphorylation of myosin, in turn, creates the conditions that initiate muscle contraction. In general, minimum concentration of calcium ions needed to evoke muscle contraction is estimated to be  $10^{-6}$  mol/litre.

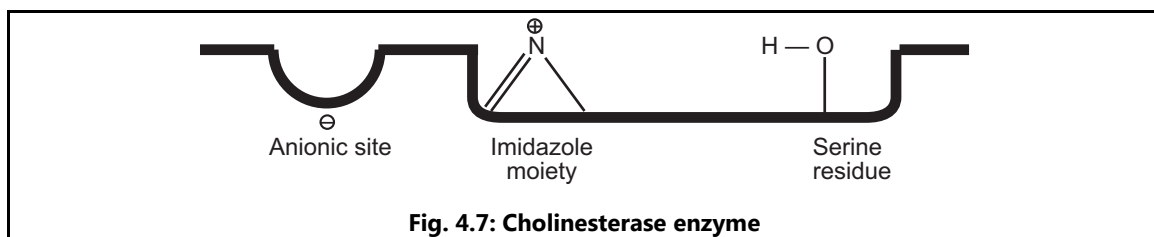
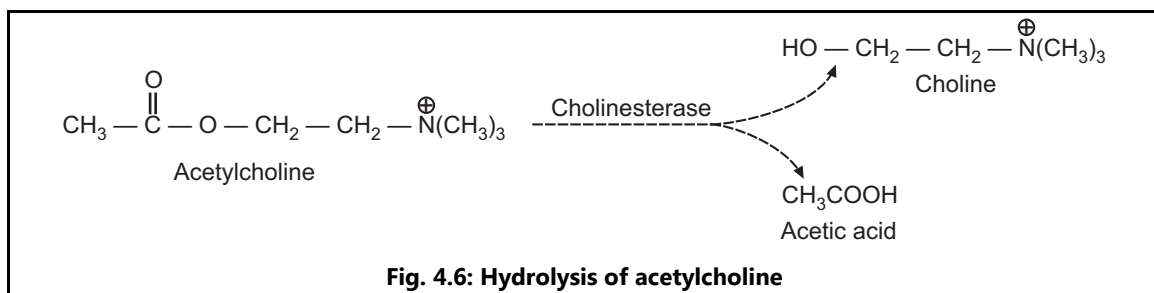
The free acetylcholine present in blood and other tissues, gets quickly hydrolysed by either e-cholinesterase (present in erythrocytes) or s-cholinesterase (present in serum). Upon hydrolysis, acetylcholine is converted into acetic acid and choline molecule.

The cholinesterase enzyme is present in high concentration in the synapses of both, cholinergic and somatic nerves and striated muscle. Hydrolysis occurs in the immediate vicinity of the nerve ending. At the neuromuscular junction, hydrolysis occurs at the end plate region after acetylcholine has initiated the muscle twitch. In the autonomic ganglia, cholinesterase is usually present in the preganglionic fibre. While serum esterase is present in glial cells, plasma, liver and at other sites.

Cholinesterase enzymes are present in two different forms.

- (i) Simple oligomers of a 70,000 dalton catalytic subunit, and
- (ii) Elongated forms of complex structure.

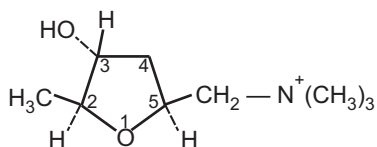
The cholinesterases are not very selective enzymes. Both these types hydrolyse a large number of esters, both of, choline and other carboxylic acids. Cholinesterase is one of the most efficient enzyme present in the body. It can hydrolyse about  $3 \times 10^5$  acetylcholine molecules per mole per minute.



During hydrolysis, acetylcholine binds with the enzyme surface through tetrahedral orientation. The tetrahedral intermediate then gets converted first to choline and then to acetic acid.

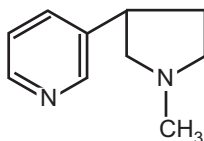
If the enzymatic hydrolysis of acetylcholine is inhibited, it will lead to prolongation and potentiation of neurotransmitter action. This can be done by the inhibition of cholinesterase enzyme.

Muscarine is a naturally occurring plant alkaloid, obtained from the poisonous mushroom, *Amanita muscaria*. Its actions on the smooth muscle, cardiac muscles, exocrine glands and its vascular effects are very much alike to that exhibited by acetylcholine.



**Cis-L-(+) muscarine**

Similarly nicotine, yet another alkaloid, mimics the actions of acetylcholine on autonomic ganglia and the adrenal glands. Hence, it was proposed that acetylcholine exhibits some of its action via muscarinic receptors while remaining actions are propagated through the nicotinic receptors.



**Nicotine**

Muscarinic receptors are further subdivided into  $M_1$  (neuronal type),  $M_2$  (cardiac type) and  $M_3$  (smooth muscles/glandular type) depending upon the selectivity of certain agonists and antagonists. The prototype of  $M_1$ -selective agonist is the quaternary ammonium

compound McN-A343. The corresponding  $M_1$ -selective antagonists are tertiary amine, pirenzepine and the quaternary ammonium compound, o-methoxy-sila-hexocyclium. Pirenzepine is a valuable drug in the treatment of peptic ulcer.

N-Ethyl-guvacine-propargyl ester was found to be a potent partial  $M_2$  agonist in the heart but a competitive antagonist at ileal  $M_3$ -receptors.

$M_2$ -selective antagonists include pirenzepine derivative AF-DX 116, the alkaloid himbacine and polymethylene tetra-amine methoctamine.  $M_2$ -receptor agonists may become useful in lowering heart rate in patients with tachyarrhythmias, whereas  $M_2$ -selective antagonists are regarded as promising drugs in the therapy of bradycardia.  $M_3$ -selective antagonists are expected to serve as antispasmodics with minor side-effects.

The nicotinic cholinergic receptor is a protein of five sub-units (i.e.  $\alpha_2$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ) having a molecular weight of about 280,000 and is embedded into the cell surface membrane.

The actions of acetylcholine thus, are classified into:

### (I) Muscarinic actions:

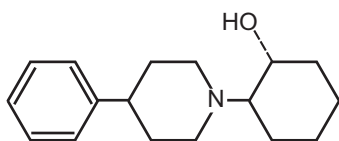
These include,

- (1) Cardiac inhibition
- (2) Peripheral vasodilation
- (3) Constriction of the eye pupils
- (4) Increased salivation and increased flow of secretory glands and
- (5) Contractions and peristaltic action on the GIT and urinary tract.

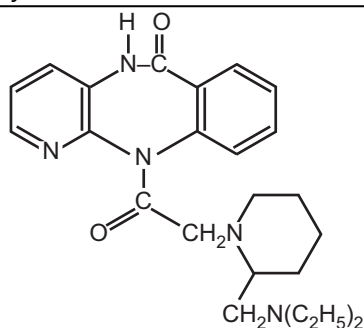
All muscarinic actions are antagonised by atropine.

### (II) Nicotinic actions:

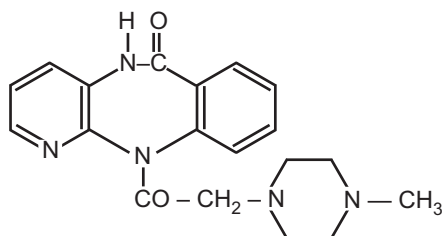
Nicotinic receptors occur at striated muscle and at autonomic ganglia. Nicotine first stimulates and then paralyzes all autonomic ganglia. The nicotinic actions are thereby involved in the stimulation and maintenance of tone of the skeletal muscle. These actions are not antagonised by atropine. Nicotinic receptors ( $N_1$ ) at the neuromuscular junction are activated by phenyl-trimethylammonium and are blocked by decamethonium, succinylcholine and d-tubocurarine. Nicotinic receptors ( $N_2$ ) in autonomic ganglia are activated by tetramethylammonium and are blocked by hexamethonium and trimethaphan.



**Vesamicol (AH5 183)**



**AF - DX 116**



Pirenzepine

Fig. 4.8: Structures of some drugs that affect the cholinergic nervous system

**(b) Sympathetic Division:**

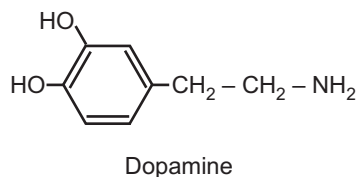
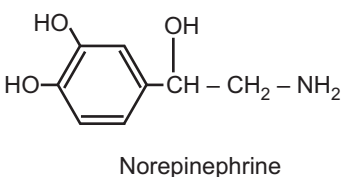
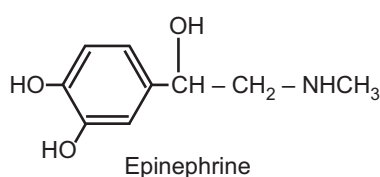
Epinephrine, norepinephrine and dopamine are the principle neurotransmitters present in the sympathetic nervous system. In many cases, synaptic transmission may be mediated by the release of more than one neurotransmitter.

Dopamine is a predominant transmitter in the human extrapyramidal system, mesocortical and mesolimbic neuronal pathways. The first evidence for norepinephrine as a principal neurotransmitter in ANS was given by Euler in 1946. The sympathetic system is distributed to effector cells throughout the body. It is also called as thoracolumbar division because the preganglionic neurons of sympathetic nervous system have their cell bodies in the thoracic and lumbar regions of the spinal cord. The sympathetic ganglia are categorised into,

- (a) Paravertebral,
- (b) Prevertebral and
- (c) Terminal.

This classification is based upon their sites of location. The terminal ganglia are few in number and consist especially of those connected with the urinary bladder and rectum.

The sympathetic system is normally active at all times and by stimulating mental alertness, respiration, energy production and heart activity, it prepares the person for 'fight or flight' situation. Receptors for a number of hormones including norepinephrine and autacoids, function by regulation of the concentration of the intracellular second messenger, cyclic adenosine-3', 5'-monophosphate (cyclic AMP) through the activation of adenylate cyclase. Cyclic AMP was discovered by Suderland and co-workers in 1956.



Epinephrine and norepinephrine catalyse many of the responses of the autonomic nervous system. The biosynthesis of epinephrine occurs in the nerve terminals using tyrosine as a starting material. This scheme of biosynthesis was first proposed by Blaschko in 1939.

Phenylalanine is converted to dopamine in cytoplasm via two intermediates. The hydroxylation of tyrosine to DOPA is generally regarded as the rate limiting step in the biosynthesis of catecholamines. To meet increased demands for norepinephrine, acute regulation mechanisms are available at nerve terminals to activate tyrosine hydroxylase enzyme.

The biosynthesised norepinephrine is stored in the synaptic vesicles or chromaffin granules which are about 0.05 to 0.2  $\mu\text{m}$  in diameter. In these vesicles, catecholamine is present along with ATP in the molecular ratio 4 : 1. The vesicles may contain other substances like ascorbic acid, enzymes, chromogranin proteins and endogenous peptides. The release of stored norepinephrine requires ATP and magnesium ions and is blocked by reserpine like drugs. The release is effected by the process of exocytosis which is influenced by a number of cytoplasmic proteins. These proteins include: calmodulin, tubulin, neurin (actin-like), and stenin (myosin-like). *l*-Norepinephrine is released almost exclusively at the post-ganglionic sympathetic nerve endings. While adrenal medulla releases a mixture of both, i.e., *l*-norepinephrine and *l*-epinephrine.

After the exocytosis, the neurotransmitter is released into the synaptic cleft. It crosses the synaptic cleft and releases at the adrenergic receptors present at post-synaptic neuron. The action of epinephrine at the myoneural junction is blocked by ergotamine. The transmitter action is terminated by a number of processes. These include,

(a) Re-uptake mechanisms carry the neurotransmitter into the nerve terminals or glial cells.

(b) Part of the neurotransmitter diffuses out into the surrounding tissue fluid and blood circulation where it is metabolised by catechol-o-methyl transferase (COMT) enzymes.

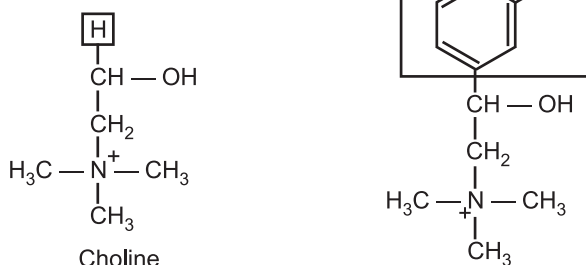
(c) And part of the neurotransmitter is attacked by mono amino oxidase (MAO) enzymes present in the mitochondria of the nerve terminal and it results into the metabolic deactivation of neurotransmitter.

MAO and COMT enzymes are present in almost every vital organ of the body. The highest concentration of these enzymes is reported in brain, liver and kidney.

## 4.6 ACETYLCHOLINE AND EPINEPHRINE AS NEUROTRANSMITTERS

Acetylcholine and epinephrine are the neurotransmitters of major importance which lead the list of parasympathetic and sympathetic functions respectively. The Fig. 4.9 shows an element of structural similarity between these neurotransmitters.

The replacement of H-atom present on  $\beta$ -carbon of choline molecule by a catechol nucleus results into formation of dimethyl derivative of epinephrine. Thus, by an introduction of catechol nucleus in a choline molecules, the parasympathetic activity is shifted into sympathomimetic activity. Table 4.1 illustrates some points of differences between the sympathetic and parasympathetic divisions of ANS.



**Fig. 4.9: Structural similarities between epinephrine and choline part of acetylcholine**

In most instances, the sympathetic and parasympathetic divisions act as physiological antagonists. Exception is male sexual organ where both divisions act to promote sexual function.

The sympathetic fibres to sweat glands and to certain blood vessels provided to skeletal muscles, release acetylcholine and hence the effect of stimulation of both the divisions at these target sites is similar.

For example, in salivary glands, both divisions upon activation leads to an increase in saliva production.

Some organs are innervated only by sympathetic nervous system. These include, most blood vessels, spleen, sweat glands, etc.

**Table 4.1**

Sr. No.	Parasympathetic division	Sympathetic division
1.	Craniosacral division.	Thoracolumbar division.
2.	Acetylcholine is a principal neurotransmitter.	Epinephrine and norepinephrine are principal neurotransmitters.
3.	Emerges at segmental level S <sub>2</sub> to S <sub>4</sub> of spinal cord.	Emerges at segmental level T <sub>1</sub> to L <sub>2</sub> or L <sub>3</sub> of spinal cord.
4.	Ganglia are small and suited very close to the organs innervated.	Ganglion contains neurons that are distributed to a number of organs.
5.	Effects are localised and limited and affects only a small region of the body.	Influences a wide region of the body.
4.	It is involved mainly in storage and preservation of body resources.	Prepares the person to face emergency cases.
7.	In rare cases, c-GMP is used as second messenger.	Operates through c-AMP which acts as second messenger.
8.	In the organs, innervated by both these divisions, effects upon stimulation of parasympathetic system is exactly opposite to that obtained after the stimulation of sympathetic system.	

**4.7 NEUROTRANSMITTERS PRESENT IN CENTRAL NERVOUS SYSTEM**

Along with acetylcholine and norepinephrine, other neurotransmitters which function in the CNS include, dopamine, epinephrine, serotonin, glycine, gamma amino butyric acid, aspartic acid, taurine, histamine, substance P, enkephalins and ATP.

It is usually difficult to establish an identity of any other substance as a candidate to function as neurotransmitter in CNS. Many drugs affect CNS functioning by influencing the release, action or metabolism of the neurotransmitter. In such cases, it can not be judged easily whether the altered CNS pattern is due to involvement of new neurotransmitters or due to modulating effect of drug given. It can be decided by developing such specific agents that will block the specific form of the nervous activity in CNS that was supposed to be operated by the drug which was claimed to be a new neurotransmitter.

Acetylcholine has been searched out in the brain cortex, limbic system, extrapyramidal nuclei and reticular formation. It regulates the sensor functions, short term memory, the classical phase of sleep and elimination of hormones, especially vasopressin.

Norepinephrine acts as a neurotransmitter in limbic system, reticular formation, locus coeruleus, hypothalamus and medulla oblongata. It is involved in thermoregulation, memory, motor activity and vegetative functions.

Dopamine is present at higher concentration in the extrapyramidal nuclei and limbic structures. It regulates motor activity, emotional tonus, memory and release of hormones.

Serotonin is a mediator which influences thermoregulation, learning, classical phase of sleep, analgesia and sensory functions. It is present in limbic system, hypothalamus, spinal cord and Raphe nuclei. Lysergic acid diethylamide (LSD) interferes in and reduces serotonin turnover in the brain. This explains the basis of hallucinogenic action of LSD. The depression of serotonin activity results in the inhibition of visual and other sensory inputs.

CNS excitation occurs as a result of the release of excitatory neurotransmitters like acetylcholine, catecholamines, dopamine, glutamic acid etc. Similarly CNS depression arises either due to,

- (a) Inhibition of the release of CNS excitatory neurotransmitter, or
- (b) Release of inhibitor, neurotransmitter which then stimulates inhibitory responses.

The inhibitory neurotransmitters include, serotonin, glycine, taurine and gamma-amino butyric acid (GABA).

**Glycine:**

It acts as an inhibitory neurotransmitter predominantly in the reticular formation. Strychnine appears to antagonise selectively the glycine responses but fails to antagonise the effects mediated by GABA.

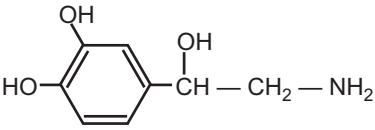
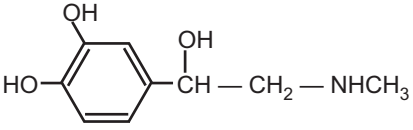
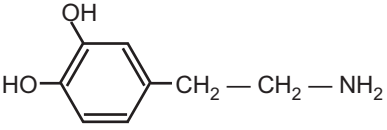
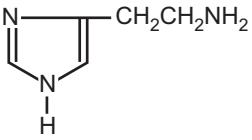
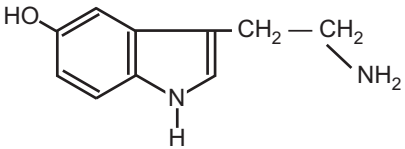
**Taurine:**

It uniformly depresses the functioning of CNS except the cortex where it has very weak depressant action.

**GABA:**

Though it has widespread depressant action on the various regions of CNS, its main sites of action involve local interneurons in the brain and presynaptic sites within the spinal cord. Its presence in brain was first reported in 1950.

**Table 4.2: Some of the neurotransmitters present in CNS**

$\text{CH}_3 - \overset{\text{O}}{\parallel} \text{C} - \text{O} - \text{CH}_2 - \text{CH}_2 - \overset{\oplus}{\text{N}} - (\text{CH}_3)_3$ <p>Acetylcholine</p>	 <p>Norepinephrine</p>
 <p>Epinephrine</p>	 <p>Dopamine</p>
 <p>Histamine</p>	 <p>5-Hydroxytryptamine (Serotonin)</p>
$\text{NH}_2 - \text{CH}_2 - \text{COOH}$ <p>Glycine</p>	$\begin{array}{c} \text{H}_2\text{N} - \text{CH} - \text{CH}_2 - \text{COOH} \\   \\ \text{COOH} \end{array}$ <p>Aspartic acid</p>
$\begin{array}{c} \text{H}_2\text{N} - \text{CH} - \text{CH}_2 - \text{CH}_2 - \text{COOH} \\   \\ \text{COOH} \end{array}$ <p>Glutamic acid</p>	$\text{H}_2\text{N} - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 - \text{COOH}$ <p><math>\gamma</math> - Amino butyric acid</p>
$\text{H} - \text{Tyr} - \text{Gly} - \text{Gly} - \text{Phe} - \text{Met} - \text{OH}$ <p>Methionine enkephaline</p>	$\text{H} - \text{Tyr} - \text{Gly} - \text{Gly} - \text{Phe} - \text{Leu} - \text{OH}$ <p>Leucine enkephaline</p>
$\text{H} - \text{Arg} - \text{Lys} - \text{Pro} - \text{Pro} - \text{Gln} - \text{Gln} - \text{Phe} - \text{Phe} - \text{Gly} - \text{Leu} - \text{Met} - \text{NH}_2$ <p>Substance P</p>	

Many drugs lead to excessive CNS stimulation (convulsant action) mainly due to the blockade of inhibitory nerve-channels mediated by GABA. For example, the action of benzodiazepines is linked with the potentiation of the functions of receptor-chloride ionophore systems that are regulated by GABA. The activation of chloride ionophore causes influx of chloride ions which causes the hyperpolarisation of the nerve tract.



## SYMPATHOMIMETIC AGENTS

### ♦ SYNOPSIS ♦

#### 5.1 INTRODUCTION

#### 5.2 BIOSYNTHESIS OF NEUROTRANSMITTER

#### 5.3 SYNAPTIC INTERACTIONS

#### 5.4 PHARMACOLOGICAL ACTIONS OF CATECHOLAMINES

#### 5.5 METABOLISM

#### 5.6 ADRENOCEPTORS

#### 5.7 CLASSIFICATION

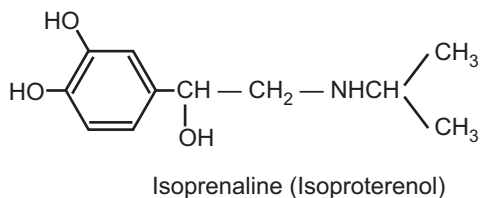
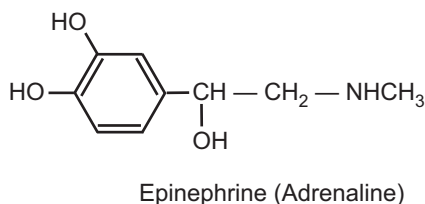
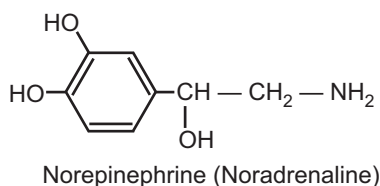
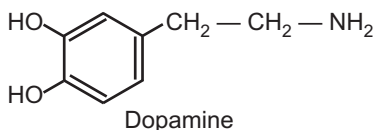
#### 5.8 DESIGN OF DRUGS AFFECTING THE ADRENERGIC NERVOUS SYSTEM

### 5.1 INTRODUCTION

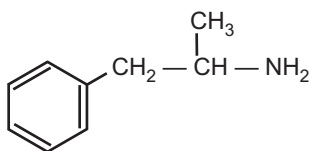
The sympathetic nervous system controls various important systems including cardiovascular, bronchial airway tone, muscular, metabolic etc. It prepares the organism against the conditions of stress, either of physical or physiological origin. In addition to epinephrine, a large number of agents can mimic the responses obtained as a result of stimulation of adrenergic nerves. They bear structural resemblance with the neurotransmitter, epinephrine. Hence, they can be used to mimic or alter the functioning of sympathetic nervous system in several clinical disorders like hypertension, asthma, arrhythmia and various allergic conditions. Majority of these substances contain an intact or a partially substituted amino group and hence, also called as sympathomimetic amines.

These drugs are divided into two broad categories according to their structures.

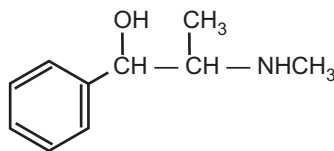
**(a) Compounds with 3, 4-dihydroxy -phenyl nucleus or a catechol nucleus:** They are termed as catecholamines.



**(b) Compounds those lack hydroxy groups on phenyl ring:** They are termed as non-catecholamines.



Amphetamine



Ephedrine

### Catecholamines:

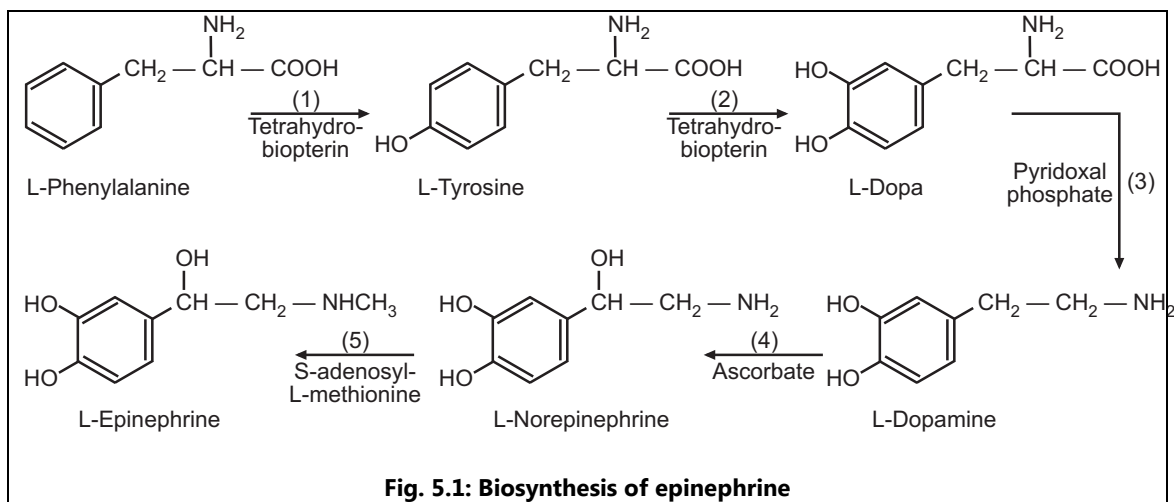
With few exceptions, drugs which act on the adrenergic nervous system, all possess some chemical elements of the endogenous agonist, epinephrine. Epinephrine, norepinephrine and dopamine are the naturally occurring catecholamines. They control most of the familiar responses of the "flight or fight" system.

Norepinephrine is a neurotransmitter present in the sympathetic nerves and in brain. It also serves as a precursor for the synthesis of adrenaline in the adrenal gland. Adrenaline is the hormone of adrenal medulla. A pressor effect exerted by adrenal extracts was observed in 1895. It was first separated from the adrenal medulla by Abel and Crawford (1897) in the form of its polybenzoyl derivative. The active principle was called epinephrine by Abel. One of the laboratories (Japanese chemist, Takamine) engaged in isolation and purification of this pressor constituent, named it as adrenaline. It was first synthesised by Stoltz in 1904.

Catecholamines are prone to easy oxidation to produce ortho quinone like compounds. The development of a pink to brown colour is indicative of oxidative breakdown. Hence, antioxidants such as ascorbic acid or sodium bisulfite may be used to stabilize the solution of catecholamines.

A large number of synthetic amines, structurally related to epinephrine were prepared and evaluated for the activity by Barger and Dale in 1910. They described the activity of these compounds as sympathomimetic. The racemic mixture thus formed, was resolved by Tullar in 1948. Upon resolution, dextro-epinephrine was found to be about one twelfth potent as laevo-epinephrine. Its role as a neurotransmitter in the sympathetic nervous system, was proposed by Elliott in 1904.

Dopamine, a third naturally occurring catecholamine, acts as a neurotransmitter in the basal ganglia of CNS. Dopamine-β-hydroxylase enzyme converts dopamine into norepinephrine. This enzyme is not present in the dopaminergic neuron. Hence, dopamine remains in its original form to carry out the function of neurotransmitter. For example, dopamine acts through dopaminergic neuronal mechanisms to dilate mesenteric and renal vascular beds.



## 5.2 BIOSYNTHESIS OF NEUROTRANSMITTER

The Fig. 5.1 represents the biosynthetic pathway for norepinephrine and epinephrine in the nerve terminals.

The enzymes which catalyse the intermediate steps of biosynthesis of sympathetic neurotransmitter are denoted by the number present on the arrow. They are listed as below:

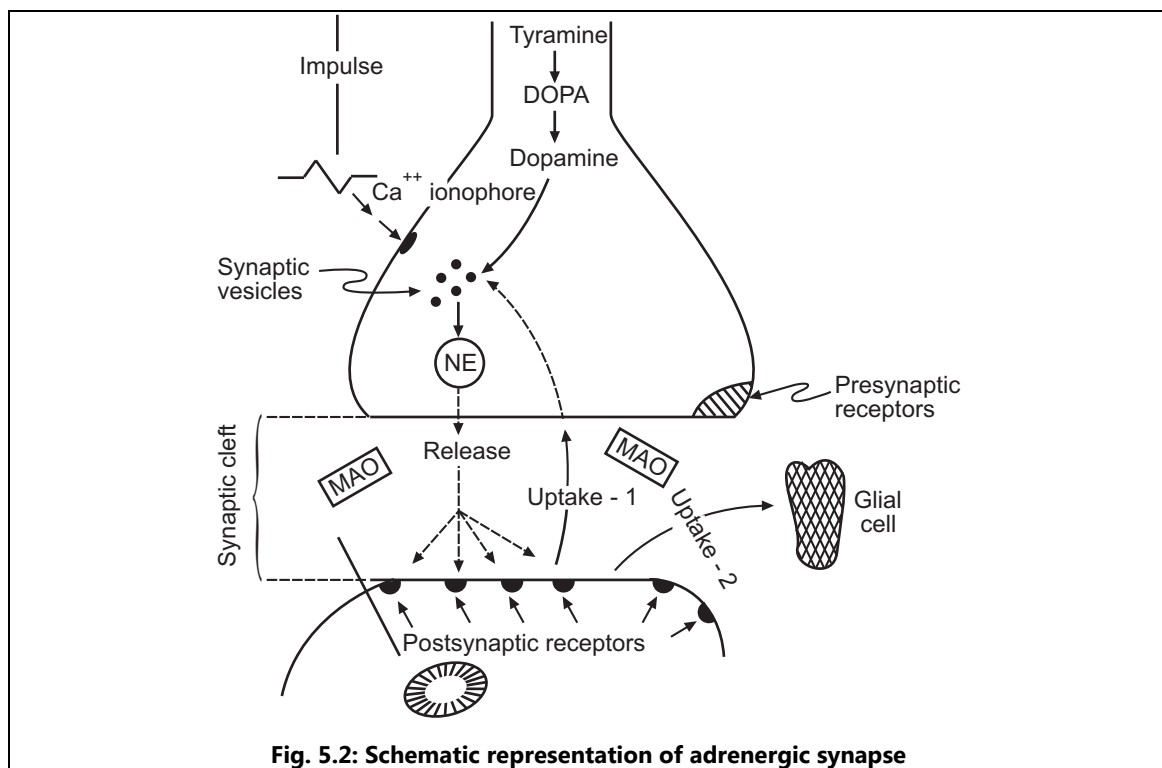
### Enzymes Participating:

1. Phenylalanine hydroxylase
2. Tyrosine hydroxylase
3. DOPA decarboxylase
4. Dopamine- $\beta$ -oxidase
5. Phenylethanolamine-N-methyl-transferase.

These enzymes are synthesised within the cell bodies of the adrenergic neurons and are then transported along the axons to their nerve terminals. The activity of tyrosine hydroxylase is low and conversion of tyrosine to DOPA is a rate - limiting step in neurotransmitter synthesis. The remaining enzymes are of generally low specificity. Steps 1 to 3, takes place in cytoplasm. Dopamine, the end product of step 3, then enters into the synaptic vesicles where it is converted into norepinephrine. Norepinephrine thus synthesized is then stored inside the nerve endings within the synaptic vesicles.

## 5.3 SYNAPTIC INTERACTIONS

(1) When the impulse reaches to the nerve terminals, the membrane becomes more permeable for the influx of  $\text{Ca}^{++}$  ions. This causes the release of neurotransmitter from the synaptic vesicles through exocytosis. The transmitter migrates across the synapse and binds to its receptor sites upon the target organ.



**Fig. 5.2: Schematic representation of adrenergic synapse**

The neuro-transmitters being highly flexible molecules, can switch on different receptors to give different biological responses. Hence, body takes care to release them close to their target receptors, then quickly inactivating them to avoid their journey to other receptors.

(2) After its interaction with receptor, norepinephrine may be removed by the following routes:

**(a)** Norepinephrine is rapidly and efficiently 'reabsorbed' into the neuron i.e. nerve terminals and then into its storage sites. The maximum quantity of norepinephrine is removed in this way. This type of uptake (uptake-1) has strict ionic requirements, being completely dependent on the presence of Na<sup>+</sup> ions (and low concentration of K<sup>+</sup> ions) in the external surrounding medium. Similarly this uptake exhibits high stereochemical selectivity and operates against concentration gradient. Certain drugs like cocaine and imipramine selectively block this neuronal uptake and avail high concentration of norepinephrine in the synaptic cleft.

**(b) Extraneuronal Uptake:** In addition to neuronal uptake, there exists a second uptake process of norepinephrine from synaptic cleft to supporting tissues (glial cells). This process is not stereo-selective and is not inhibited by usual inhibitors of neuronal uptake. This extraneuronal uptake is reported to be inhibited by metanephine and corticosteroids. This extraneuronal uptake can be regarded as transport and metabolism while neuronal uptake is transport and retention of neurotransmitter.

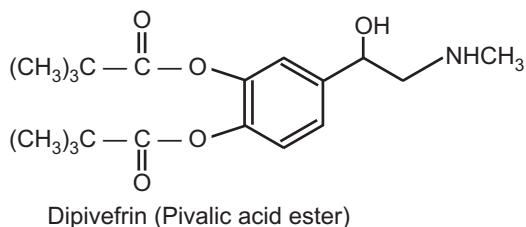
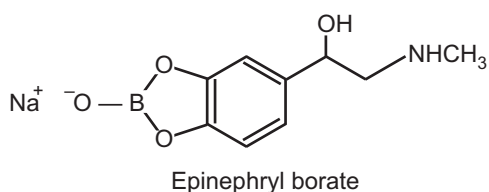
**(c) Part of the neurotransmitter may also be lost due to its diffusion across the synaptic cleft:** In the adrenergic synapses, diffusion mechanism is a route of minor importance in removing norepinephrine. The blood vessels, probably stand as an exception where the immediate disposition of released norepinephrine is accompanied largely by a combination of extra-neuronal uptake, diffusion and enzymatic breakdown of neurotransmitter.

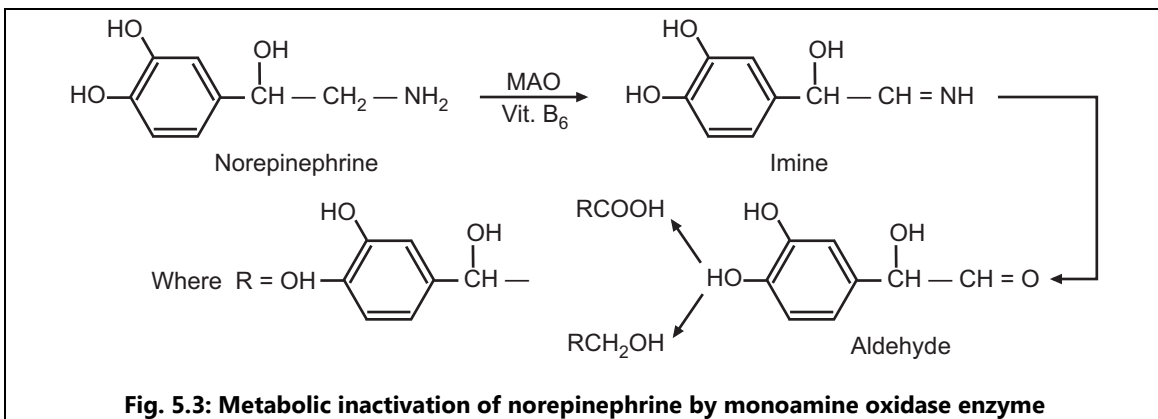
The other routes of removing norepinephrine are metabolic in nature.

For example, norepinephrine through its interaction with cytoplasmic monoamino oxidase (MAO) enzymes, is converted to the corresponding aldehyde which then non-enzymatically get further oxidised. Similarly, catechol-o-methyl transferase (COMT) enzyme methylates the m-hydroxy group of the phenyl ring of the catecholamines, rendering them less active.

#### 5.4 PHARMACOLOGICAL ACTIONS OF CATECHOLAMINES

- (1) They exert excitatory effects on smooth muscles present in blood vessels and on salivary as well as sweat glands.
- (2) They initiate inhibitory responses on smooth muscles of GIT, bronchial tract and of blood vessels provided to skeletal muscles. Thus, the blood vessels get dilated to supply the skeletal muscles with more blood.
- (3) Depending upon the drug employed, the secretion of various endocrine glands either increases or decreases.
- (4) They exert excitatory effects on cardiac cells resulting into an increase in force of contraction (i.e. positive inotropic effect) and an increase in the rate of contraction (i.e. positive chronotropic effect).
- (5) The increased level of catecholamines in the CNS leads to respiratory stimulation, alertness, an increase in psychomotor activity and a reduction in appetite.
- (6) Catecholamines promote glyco-genolysis both in liver and skeletal muscles and cause a reduction in the production of free fatty acids from adipose tissue.
- (7) Epinephrine reduces intraocular pressure. Epinephrine bitartrate, dipivefrin and epinephryl borate (complex between boric acid and epinephrine) are preferred preparations used in the treatment of open-angle glaucoma. In the lacrimal fluid, epinephryl borate readily dissociates to release free drug.





**Fig. 5.3: Metabolic inactivation of norepinephrine by monoamine oxidase enzyme**

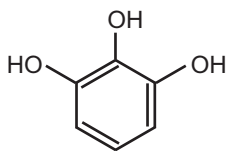
## 5.5 METABOLISM

Monoamine oxidase (MAO) and catechol-o-methyl transferase are the main enzymes which metabolise the sympathomimetic drugs. About 60% of the administered dose of epinephrine or norepinephrine in man remains untouched by these main enzymes which then is excreted in its original form or in its conjugated form with sulphuric or glucuronic acid. Conjugation usually occurs at phenolic hydroxyl groups.

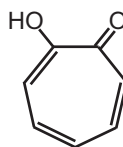
The major fraction of natural catecholamines is attacked by MAO and / or COMT. At periphery, they are preferentially oxidised to the acid and in the CNS, they are reduced to glycol.

Thus, the principal metabolites of norepinephrine (MPOGAL, MOMA and MOPEG) are excreted through urine alongwith a free or conjugated form of unaltered norepinephrine. Of these, 3- methoxy-4-hydroxymandelic acid is the principal metabolite and the estimation of its content in urine can be taken as an index of catecholamine metabolism. On an average, about 70% of metabolised dose of epinephrine or norepinephrine follow metabolism by COMT enzymes while only 20% favour the attack by MAO enzymes.

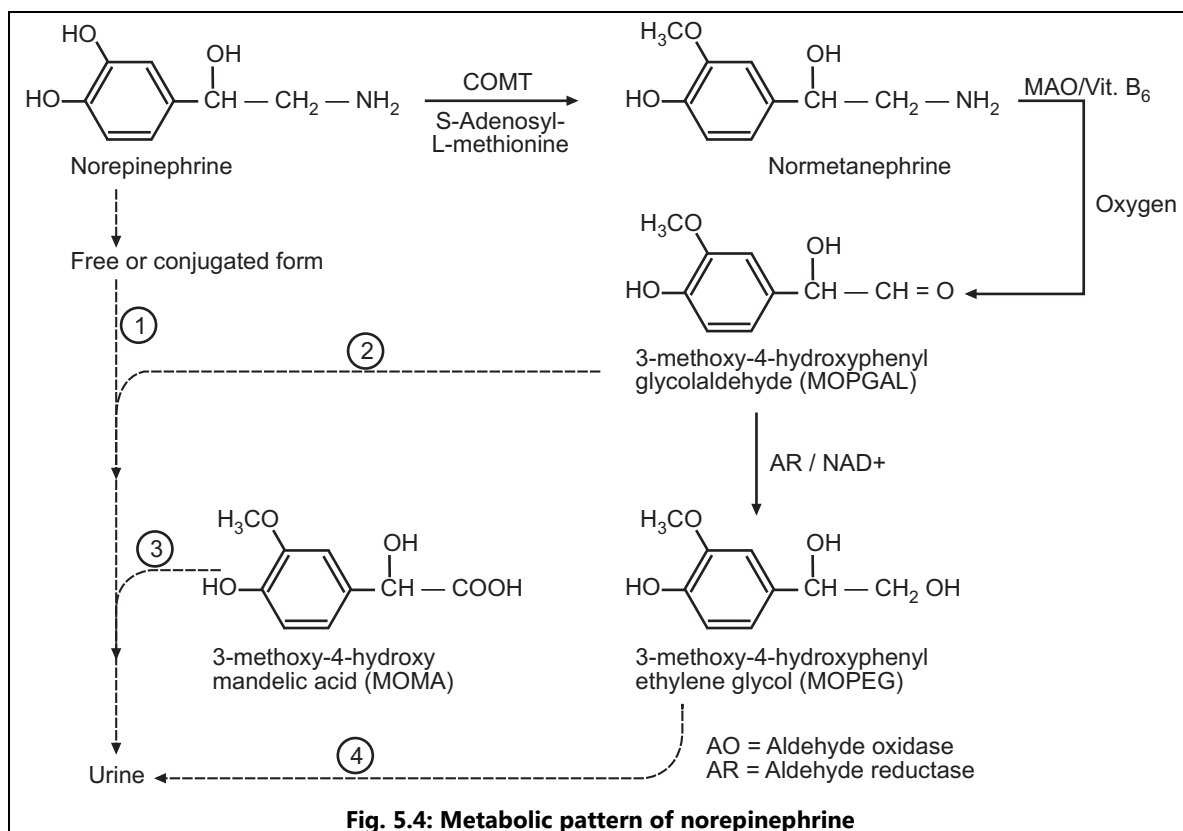
By using drugs which inhibit these metabolising enzymes, the duration and intensity, of effects can be raised. For example, some agents can specifically block the MAO enzymes and are in clinical use under the name of MAO inhibitors, while only few agents can block the activity of COMT enzymes on circulating catecholamines and did not find clinical applicability. These include pyrogallol and tropolone derivatives.



Pyrogallol



Tropolone



## 5.6 ADRENOCEPTORS

Upon discharge from nerve terminals, norepinephrine reacts with post-synaptic receptor sites to evoke its pharmacological response. In 1948, Ahlquist observed that, the tissues he examined, carried two kinds of adrenergic responses, i.e. alpha and beta responses, as shown in the table 5.1.

From the table 5.1, it can be easily seen that (with the last response as an exception)  $\alpha$ -responses are mainly excitatory in nature, while  $\beta$ -responses are inhibitory in nature. In general, inhibitory  $\beta$ -receptors can be activated at quite low concentration of catecholamines than that is needed to activate excitatory  $\alpha$ -receptors.

**Table 5.1: Results of Ahlquist experiment**

Group 1 responses ( $\alpha$ -responses)	Group 2 responses ( $\beta$ -responses)
Vasoconstriction	Vasodilation
Contraction of Uterus	Relaxation of Uterus
Contraction of Ureters	Increased rate and force of heart beats
Constriction of Pupil	
Relaxation of Intestine	

**Table 5.2: Distribution of receptor sub-types**

<b><math>\beta</math>-receptor predominance</b>	<b><math>\alpha</math>-receptor predominance</b>	<b><math>\alpha</math> and <math>\beta</math></b>
Cardiac cells ( $\beta_1$ ) Metabolic effects – lipolysis ( $\beta_1$ ) – glycogenolysis ( $\beta_2$ )  Bronchial muscles ( $\beta_2$ ) Ciliary muscles ( $\beta_2$ ) Bladder muscles	Blood vessels to – skin – visceral region – brain region  – renal region Intestinal sphincter Sweat gland Bladder sphincter Dilated pupils	Coronary blood vessels Skeletal muscle Blood vessel Mucous membrane of alimentary tract

Lands and co-workers in 1967, based on the differences in the cardiac and bronchial responses of the sympathomimetic agents, proposed a further subdivision of the  $\beta$ -receptors into:

- (i)  $\beta_1$ -receptors whose activation accounts for cardiac stimulation, lipolysis and intestinal relaxation effects of sympathomimetic drugs, and
- (ii)  $\beta_2$ -receptors whose activation accounts for relaxation in vascular bed, bronchial tree, uterus and ureter alongwith metabolic effects of sympathomimetic agents.

With an exception of  $\beta_2$ -receptors present in pancreas (which have excitatory response), the activation of most of the  $\beta_2$ -receptors is linked with the inhibitory responses. While the activation of  $\beta_1$ -receptors leads to the excitatory responses in general. The type of response is mainly governed by  $\text{Ca}^{++}$  ion fluxes at the nerve endings.

On the same line,  $\alpha$ -receptors can be categorised into  $\alpha_1$ - and  $\alpha_2$ -receptors.  $\alpha_1$ -receptors are present on post-synaptic receptor sites of smooth muscles of blood vessels and gland cells. While  $\alpha_2$ -receptors are present on pre- and post-synaptic sites on the nerve terminals and are also present in the CNS.

An increased prominence of  $\alpha_2$ -receptor responses accompanies hypertension and may contribute to the elevated blood pressure. The post-synaptic sites of  $\alpha_2$ -receptor include the tissues like brain, uterus, parotid glands and extra-synaptic region at some blood vessels. The pre-synaptic  $\alpha_2$ -receptors are present on the nerve terminal. Their activation leads to inhibition of neurotransmitter release (norepinephrine or acetylcholine) through negative feedback inhibitory mechanism. Their function is:

- (i) to govern the release of neurotransmitter, and
- (ii) as per need, to alter the rate of synthesis of neurotransmitter.

Thus, the activation of  $\alpha_2$ -receptors on the cholinergic nerve terminals within the intestinal wall leads to the inhibition of release of acetylcholine.

Table 5.3

Effector organ	Receptor responses		
	$\alpha$	$\beta_1$	$\beta_2$
Vascular system	constriction	–	dilation
Uterus	constriction	dilation	dilation
Intestine	decreased motility	decreased motility	decreased motility
Heartbeat	–	increased	–
Bronchial muscle	constriction	–	relaxation

Clonidine, yohimbin and  $\alpha$ -methyl-norepinephrine are more effective agonists on  $\alpha_2$ -receptors than  $\alpha_1$ -receptors. While phenylephrine, prazosin and methoxamine act prominently on  $\alpha_1$ -receptors.

Thus in tissues, the overall effect of the adrenergic nerve stimulation depends upon the population of  $\alpha$  and  $\beta$ -receptors present in that organ.

For example, in cardiac cells, positive inotropic and positive chronotropic actions are due to the activation of  $\beta$ -receptors whereas  $\alpha$ -receptor activation leads to ectopic excitation induced by sympathetic stimulation.

Further subclassification suggests the presence of four  $\alpha_1$  ( $\alpha_{1A}$  to  $\alpha_{1D}$ ) and four  $\alpha_2$  ( $\alpha_{2A}$  to  $\alpha_{2D}$ ) receptors. In case of  $\beta$ -receptors, the presence of third ( $\beta_3$ ) type is postulated. All four  $\alpha_1$ -receptors produce vasoconstrictor responses through different biochemical mechanisms for increasing cytosolic free  $\text{Ca}^{++}$  ions.

Tachyphylaxis or reduced response is a common problem encountered in the prolonged treatment of adrenergic drugs. Upon continuous exposure, the receptors lose their efficiency resulting into decrease in the magnitude of biological response. This is known as desensitisation, refractoriness, down regulation or tachyphylaxis. Various mechanisms are proposed to account for this event.

Thus, tachyphylaxis may be due to the following processes:

- Feedback regulatory mechanisms governed by cyclic-AMP.
- Some receptors may undergo degeneration causing decrease in total number of receptors.
- Receptors may be inactivated or blocked due to irreversible phosphorylation, or
- The correlation between the receptor and adenylate cyclase may get paralyzed.

Norepinephrine is the most active agent at  $\alpha$ -receptor and the latter is least responsive to isoproterenol. The responses mediated through  $\alpha$ -receptors are blocked by antagonists like phenoxybenzamine or phentolamine. The excitatory nature of  $\alpha$ -receptors and inhibitory nature of  $\beta$ -receptors can easily be seen from the table 5.4.

**Table 5.4: Adrenergic responses**

<b><math>\alpha</math>-receptor mediated responses</b>	<b><math>\beta</math>-receptor mediated responses</b>
Vasoconstriction	Vasodilation
Mydriasis	Bronchial smooth muscle relaxation
Release of ACTH	
Uterine myometrial contraction	Uterine myometrial relaxation
Retractor penis contraction	Intestinal smooth muscle relaxation
Seminal vesicle contraction	Positive inotropic effect on the heart
Pilomotor muscle contraction	
Orbital contraction	Positive chronotropic effect on the heart
Nictitating membrane contraction	
Intestinal smooth muscle relaxation	Hepatic glycogenolysis and Lipolysis

The  $\beta$ -receptor is most responsive to isoproterenol while the least responsive agent is norepinephrine. The  $\beta$ -receptor mediated responses remain unaffected by the usual  $\alpha$ -adrenergic blockers and are blocked by agents like nadolol or timolol.

## 5.7 CLASSIFICATION

Norepinephrine, epinephrine or isoproterenol-like drugs mimic the responses of adrenergic stimulation by acting directly on the receptor sites. While some agents when administered, do not act on the adrenergic receptors. They enter the adrenergic nerve terminals and cause stoichiometric displacement of norepinephrine from the synaptic vesicles. Their pharmacological responses are thus due to this displaced neurotransmitter. The adrenergic agonists can be conveniently divided into:

### (a) Direct-acting drugs:

These amines produce their pharmacological responses by their direct action on adrenoceptors. The actions produced are of rapid onset and short-lived. Most of the agents can influence both  $\alpha$ - and  $\beta$ -receptors, thus ranging from pure  $\alpha$ -agonist (phenylephrine) to pure  $\beta$ -agonist (isoproterenol). The intensity of their effects remains unaffected by the use of reserpine, cocaine or imipramine.

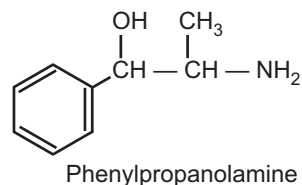
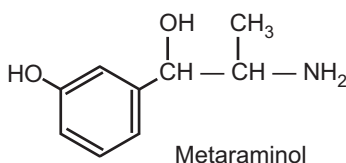
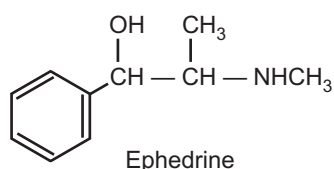
### (b) Indirect-acting drugs:

Tyramine does not act directly on the adrenoceptors. The fact that reserpine depletes tissues of norepinephrine (Bertler et al, 1956) indicated that tyramine acts by releasing endogenous norepinephrine. Many sympathomimetic agents exert a large fraction of their effects by releasing (through displacement) norepinephrine from storage sites. The responses of this released norepinephrine are prominently  $\alpha$ -receptor mediated, slower in onset and generally long lasting.

Examples of indirect acting drugs include tyramine, amphetamine, etc. These drugs usually lack catechol nucleus. Indirect acting agents have little or no action in reserpinized animals. Cocaine or imipramine also lower the intensity of activity by inhibiting the drug-induced displacement and release of norepinephrine. Since, these drugs lack the phenolic hydroxyl groups, the increased lipophilicity imparts pronounced central effects to these drugs. If given repeatedly, tachyphylaxis is likely to occur due to the depletion of norepinephrine stores.

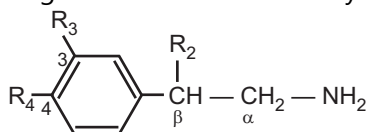
### (c) Mixed action drugs:

Many sympathomimetic drugs exert their actions partly by acting directly on the receptor sites and partly by their effect on the norepinephrine release. They are termed as mixed action drugs. Examples include:



They share structural features of both classes. The presence of cocaine, reserpine or imipramine only reduce (and not abolish) the intensity of their effects, where higher doses of these drugs will be needed to produce comparable effects.

Thus if we assume the following skeleton essential for sympathomimetic activity,



then,

- (i) Direct-acting drugs will have  $R_3 = R_4 = \text{OH}$  or  $\text{OCH}_3$  and  $R_2 = \text{OH}$ .
- (ii) Indirect acting drugs will have only a hydroxyl group at  $\beta$ -carbon atom or no substitution at  $R_2$ ,  $R_3$  and  $R_4$ . They retain the phenylethylamine framework, and
- (iii) Mixed action drugs share the structural features of both the above classes.

Thus, examples of direct-acting drugs include:

- norepinephrine (predominantly on  $\alpha$ -receptor)
- epinephrine (on  $\alpha$ ,  $\beta_1$  and  $\beta_2$  receptors)
- isoprenaline (on  $\beta_1$  and  $\beta_2$  receptors)
- Tazolol (predominantly on  $\beta_1$ - receptor)
- Salbutamol (predominantly on  $\beta_2$  receptor)
- Phenylephrine (predominantly on  $\alpha$ -receptor)

Prototype of indirect-acting drugs is amphetamine. Prototype of mixed action drugs is ephedrine.

The commonly used alpha blocking agent is dibozane or 1, 4-(bis-1, 4-benzodioxan-2-yl-methyl) piperazine while the commonly used beta blocking agent is propranolol.

## 5.8 DESIGN OF DRUGS AFFECTING ADRENERGIC NERVOUS SYSTEM

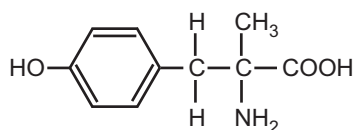
Development of a new drug can be done in the following areas:

- (A) Drugs that affect the biosynthesis of norepinephrine.
- (B) Drugs that affect the storage of norepinephrine.
- (C) Drugs that affect the metabolism and/or removal of norepinephrine from the area surrounding the receptor.
- (D) Drugs that mimic the effects of norepinephrine at the receptor sites (agonists).
- (E) Drugs that block the interaction of norepinephrine with the receptor (antagonists).
- (F) Drugs that affect post-synaptic regulation of hormone action.

### (A) Drugs Affecting Biosynthesis of Nor-epinephrine:

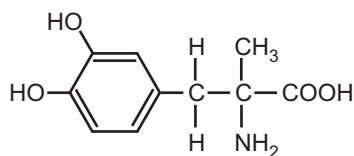
The key enzyme is tyrosine hydroxylase, which requires a tetrahydrofolate coenzyme,  $O_2$  and  $Fe^{++}$  and is quite specific. DOPA-decarboxylase acts on all aromatic amino acids and requires vitamin  $B_6$  as cofactor. Dopamine- $\beta$ -hydroxylase, located in the membranes of storage vesicles, is a copper containing protein, a mixed function oxygenase that uses  $O_2$  and ascorbic acid. Finally phenyl-ethanolamine-N-methyl transferase located in the adrenal medulla (the main site of adrenaline synthesis) and in the brain, uses S-adenosyl-methionine as a  $CH_3$ -donor.

(i)  $\alpha$ -Methyl tyrosine effectively inhibits the action of tyrosine hydroxylase in the first step of biosynthesis, which is rate determining step. Unfortunately, the compound is not clinically useful.



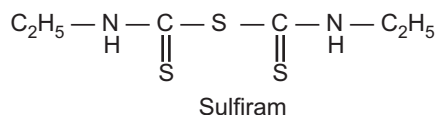
$\alpha$ -Methyl tyrosine

(ii) DOPA-decarboxylase may be inhibited by  $\alpha$ -methyl DOPA. Since, the rate of decarboxylation of  $\alpha$ -methyl DOPA is considerably slower than that of DOPA, it ties up the enzyme for a longer period of time and acts as an effective inhibitor.



$\alpha$ -Methyl DOPA

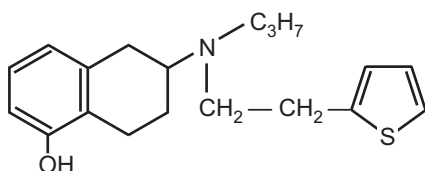
(iii) Dopamine hydroxylase may be inhibited by a variety of compounds including disulfiram. Disulfiram inhibits dopamine hydroxylase probably by breaking into two molecules diethyldithiocarbamate which forms a chelate with  $Cu^{++}$ -ion of enzyme's prosthetic group.



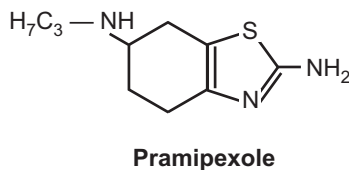
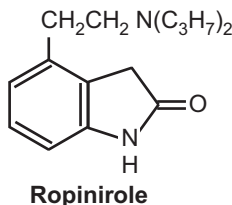
**(B) Drugs Affecting the Release of Stored Norepinephrine (Indirect acting agonists):**

**(a) False neurotransmitters:** Tyramine, produced by decarboxylation of tyrosine (and especially the  $\beta$ -OH derivative of tyramine, octopamine), can be taken up through the pre-synaptic membrane by the not very selective uptake-1 mechanism. Tyramine then enters the storage granules to a certain extent and displaces neurotransmitter norepinephrine which, when released causes post-synaptic effects. Octopamine, is taken up even more readily into storage vesicles and is, in turn, released when the neuron fires. As an agonist, it is only about 1/10 active as norepinephrine. Compounds that behave like neurotransmitters of low potency are called as false neurotransmitters.

**Rotigotine:** It is a non-ergoline dopamine agonist indicated for treatment of Parkinson's disease and restless legs syndrome.

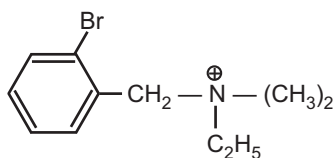


**Ropinirole and Pramipexole** are non-ergoline dopamine agonists used in the treatment of Parkinson's disease and restless leg syndrome.



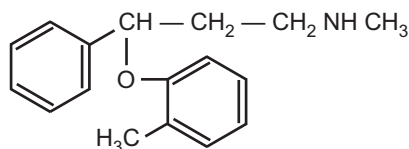
**(b) True uptake inhibitors:** They block the amine pump of the reuptake-1 mechanism in central adrenergic, dopaminergic and serotonergic neurons. e.g., tricyclic antidepressant agents.

- (i) Cocaine interferes with norepinephrine uptake at the neuron and thus increases the concentration of norepinephrine at the receptor. Reserpine depletes the neuronal storage sites. Released norepinephrine is then metabolised by MAO.
- (ii) A number of antihypertensive agents exert their activity by affecting the storage and release of norepinephrine e.g. Bretylium and Guanethidine.

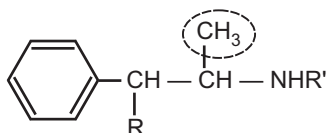


Bretylium

**Atomoxetine:** It is selective NE reuptake inhibitor. It is used in the treatment of attention deficit hyperactivity disorder.



(iii) The following structure is representative of indirect acting drugs.

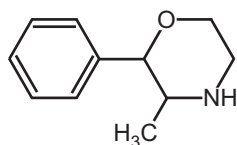


R = H or OH

R' = H, CH<sub>3</sub> or heterocyclic ring

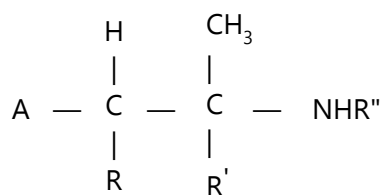
- Indirect acting adrenergic agents do not contain phenolic hydroxyl groups at the 3, 4-positions. The lack of these ionic groups increases oral absorption and penetration into CNS.
- The encircled methyl group is not present in norepinephrine or its direct acting analogues. It provides increased oral activity, probably by preventing interaction with enzyme that metabolise the direct acting analogues.
- The benzylic hydroxyl group may or may not be present in indirect acting compounds. Those without this alcoholic hydroxyl group, are less polar, pass more readily through Blood-Brain-Barrier and demonstrate greater CNS stimulation.
- The amino nitrogen may be primary or secondary amine or a part of a heterocyclic ring.
- The phenyl group may be replaced by other aromatic group, cycloalkyl group or alkyl group.

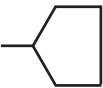
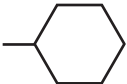
In addition to the actions that mimic adrenergic responses, a number of indirect acting adrenergic drugs have an anorexic or appetite depressing action. In attempts to modify the phenylethylamine structure to provide anorexic activity without pronounced CNS stimulation, compounds were prepared in which the amino nitrogen is a part of heterocyclic system, but they failed to separate totally the two effects.



Phenmetrazine

It represents an attempt to produce an appetite suppressant lacking the CNS-stimulant properties of ephedrine or amphetamine.

**Table 5.5: Indirect-acting agonists of norepinephrine**

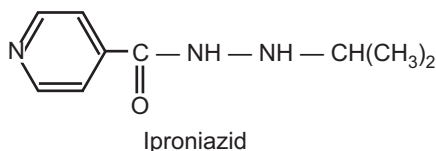
Sr. No.	Name	A	R	R'	R''
1.	Phenylpropanolamine	C <sub>6</sub> H <sub>5</sub>	OH	H	H
2.	Amphetamine	C <sub>6</sub> H <sub>5</sub>	H	H	H
3.	Methamphetamine	C <sub>6</sub> H <sub>5</sub>	H	H	CH <sub>3</sub>
4.	Phentermine	C <sub>6</sub> H <sub>5</sub>	H	CH <sub>3</sub>	H
5.	Chlorphentermine	P - Cl C <sub>6</sub> H <sub>4</sub>	H	CH <sub>3</sub>	H
6.	Methoxyphentermine	P - CH <sub>3</sub> O C <sub>6</sub> H <sub>4</sub>	H	CH <sub>3</sub>	H
7.	Cyclopentamine		H	H	CH <sub>3</sub>
8.	Propylhexedrine		H	H	CH <sub>3</sub>

**(C) Drugs Inhibiting the Metabolism of Norepinephrine:**

MAO oxidises norepinephrine through the removal of two hydrogens to give an imine, using pyridoxal phosphate (vitamin B<sub>6</sub>), and then the non-enzymatic hydrolysis of this imine results into an aldehyde.

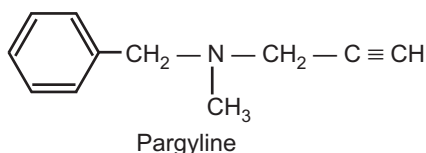
Inhibition of these enzymes will increase the concentration of norepinephrine at the receptor. Thus, MAO-inhibitors will be useful in the therapy of depression. They are divided into three types.

(a) Hydrazines and hydrazides.

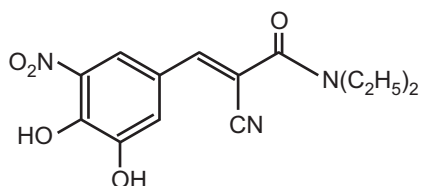


(b) The rigid analogues of phenylethylamine e.g. tranylcypromine.

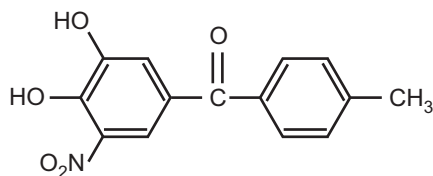
(c) Structures not directly related to norepinephrine.



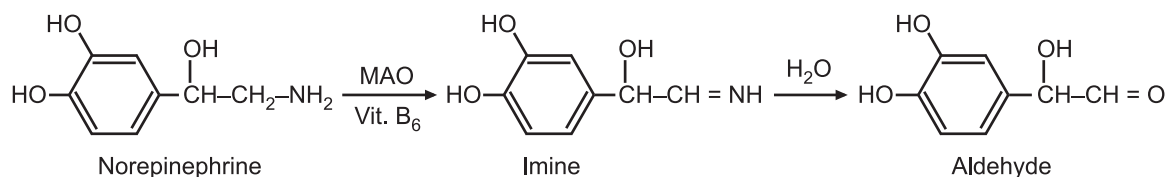
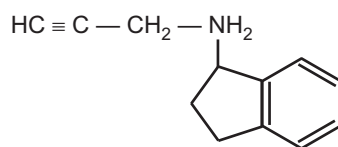
**Entacapone:** It is a COMT inhibitor, used in the treatment of Parkinson's disease.



**Tolcapone:** It inhibits COMT enzyme. It is used in the treatment of Parkinson's disease as an adjunct to levodopa/carbidopa medication.



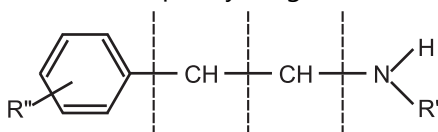
**Rasagiline:** It is an irreversible inhibitor of MAO used in the treatment of Parkinson's disease.



**(D) Drugs that Mimic the Effects of Norepinephrine at Receptor Sites (Direct acting agonists):**

The structural requirements for agonist activity at adrenergic receptors are:

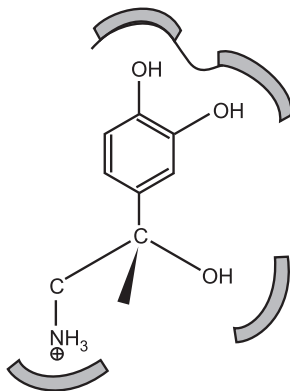
- (I) A phenylethylamine parent structure.
- (II) 3, 4-dihydroxy substitution on the phenyl ring:



Phenylethylamine structure

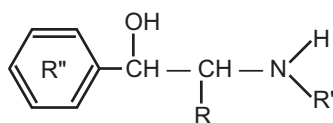
Although the catechol group is of major importance for agonist activity, the phenolic groups can be successfully replaced by alkyl or arylsulphonamide functions.

- (a) The amino group should be separated from the aromatic ring by two carbon atoms for optimal activity.
- (b) Direct acting agonist activity is enhanced by the presence of a hydroxyl group of the correct stereochemical configuration (i.e. laevo rotatory) on the  $\beta$ -carbon but is reduced by the presence of a methyl group on  $\alpha$ -carbon. The presence of  $\alpha$ -methyl group increases the duration of action by making the compound more resistant to metabolic deamination by MAO.
- (c) Small substituents ( $H$ ,  $CH_3$ ,  $C_2H_5$ ) may be placed on the carbon without affecting agonist activity significantly.
- (d) Small substituents ( $H$  or  $CH_3$ ) may be placed on the nitrogen atom, without affecting agonist activity.
- (e) The nitrogen atom must have at least one H-atom.
- (f) The highly critical factor in the interaction of adrenergic agonists with their receptor is that of stereoselectivity. Stedman and Easson proposed a three point interaction of the catechol,  $\beta$ -hydroxyl and amino group as shown for norepinephrine.



**Fig. 5.5: Representation of the stereoselective three-point binding of norepinephrine**

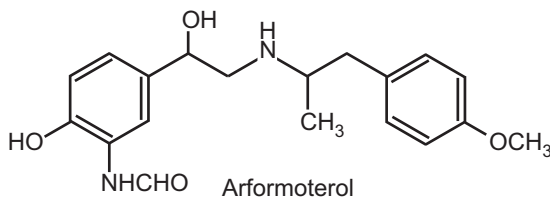
Table 5.6: Direct-acting adrenergic agonists



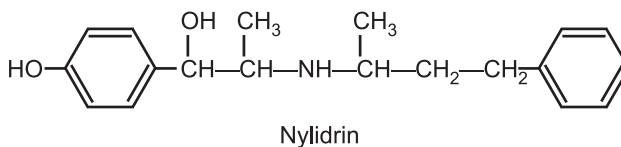
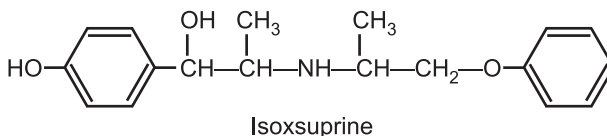
Compound	R''	R	R'	Primary receptor site
1. Norepinephrine	3, 4, - di OH	H	H	$\alpha$
2. Epinephrine	3, 4, - di OH	H	CH <sub>3</sub>	$\beta$
3. Phenylephrine	3 - OH	H	CH <sub>3</sub>	$\alpha$
4. Isoproterenol	3, 4, - di OH	H	- CH (CH <sub>3</sub> ) <sub>2</sub>	$\beta$
5. Isoetharine	3, 4, - di OH	- C <sub>2</sub> H <sub>5</sub>	- CH (CH <sub>3</sub> ) <sub>2</sub>	$\beta$
6. Metaproterenol	3, 5, - di OH	H	- CH (CH <sub>3</sub> ) <sub>2</sub>	$\beta$
7. Metaraminol	3 - OH	CH <sub>3</sub>	H	$\alpha$

For epinephrine, norepinephrine and related compounds, the more potent enantiomer has the R (-) configuration. None of these compounds can be considered totally specific for either receptor and that they interact to some extent with both of the receptors listed in Table 5.6. Phenylephrine is the most specific drug acting chiefly at the  $\alpha$ -receptor, whereas isoproterenol acts most specifically at the  $\beta$ -receptor.

**Arformoterol:** It is a long acting  $\beta$ -adrenoceptor agonist effective in the treatment of chronic obstructive pulmonary disease.

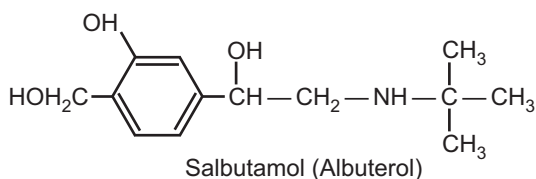


Two clinically useful compounds of this category are isoxsuprine and nylidrin.

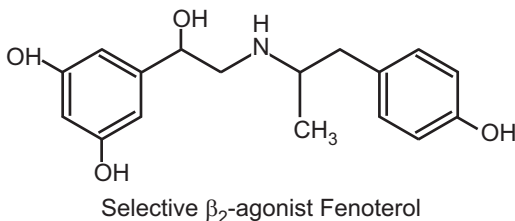
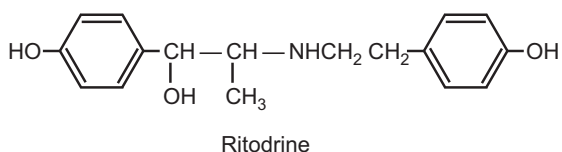
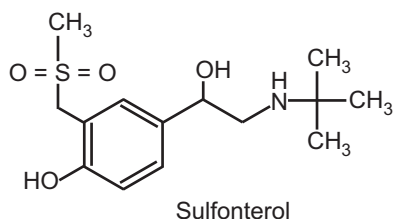
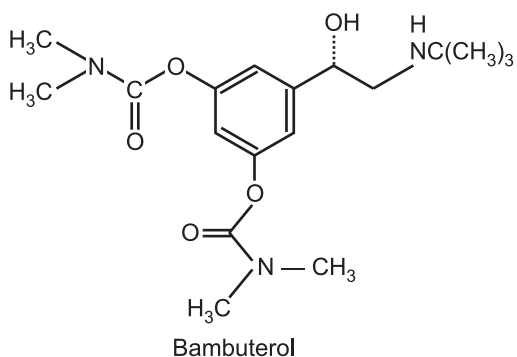
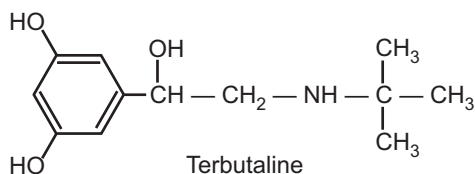


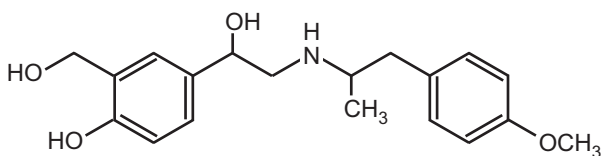
If the  $\alpha$ -CH<sub>3</sub> group is dropped, more selectivity for bronchial  $\beta_2$ -receptor is obtained. The phenolic groups in nonpinephrine are involved in H-bonding to receptor. The COMT enzymes cause metabolic methylation of one of these phenolic groups which accounts for its shorter duration of action.

Replacing one of these phenolic groups by a hydroxyethyl group saves the hydroxyl group from COMT enzymes without affecting its ability to activate receptor through H-bonding. e.g. Salbutamol.



**Bambuterol:** It is a long acting  $\beta$ -adrenoceptor agonist used in the treatment of asthma. It is also a prodrug of terbutaline.

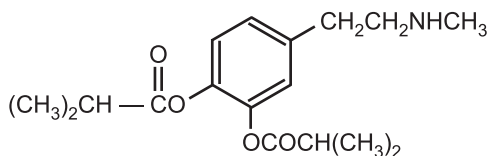




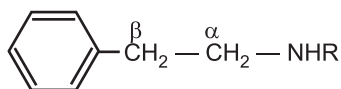
Salmefamol

Salmefamol is 1.5 times more active than salbutamol. It has a longer duration of action.

**Ibopamine:** It is a sympathomimetic used in ophthalmology to induce mydriasis.

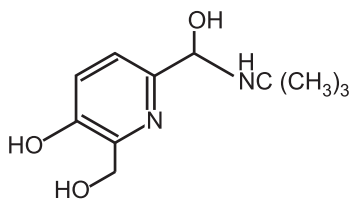


### Selective $\beta_2$ Agonists:

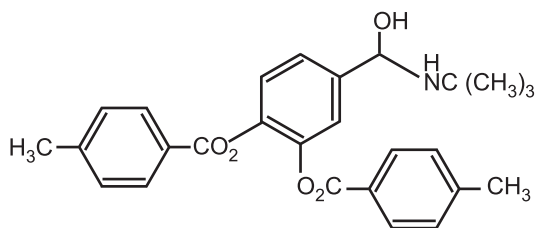


General formula

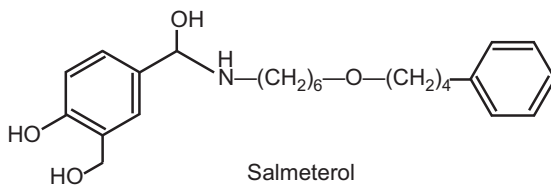
- (i) Only one aromatic hydroxyl group is necessary (usually at the para, but sometimes at meta position).
- (ii) An  $\alpha$ -methyl or ethyl group is preferred for vascular effects.



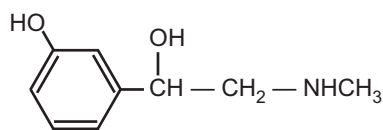
Pirbuterol



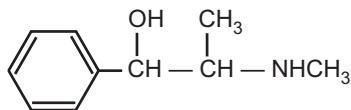
Bitolterol



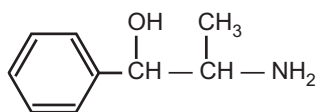
Salmeterol

**Nasal Decongestants (Selective  $\alpha_1$ -agonists)**

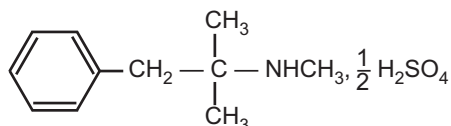
Phenylephrine



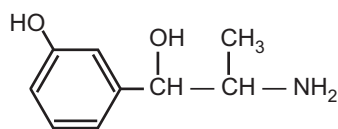
Ephedrine



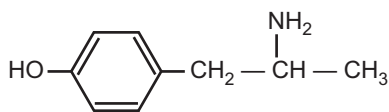
Phenylpropanolamine



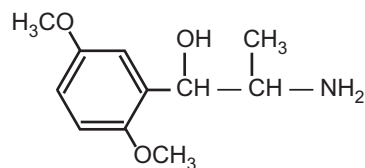
Mephentermine sulphate



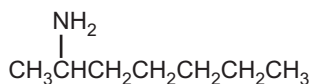
Metaraminol



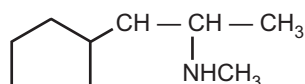
Hydroxyamphetamine



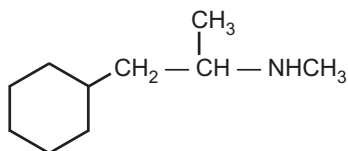
Methoxamine



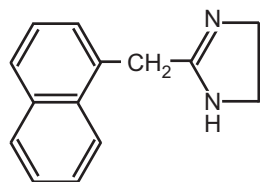
Tuaminoheptane



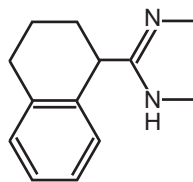
Cyclopentamine



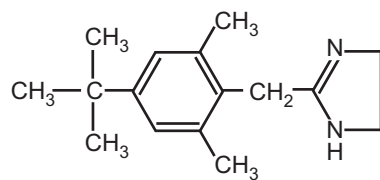
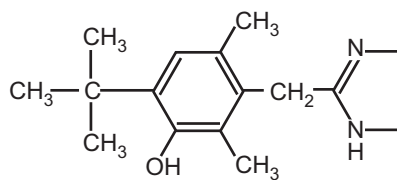
Propylhexedrine

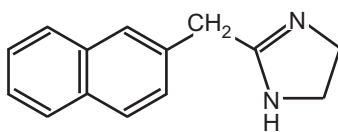


Naphazoline



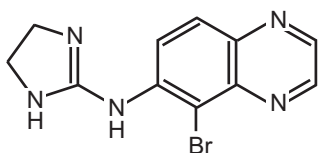
Tetrahydrozoline

Xylometazoline  
(Otrivin, Ciba)Oxymetazoline  
(Nasivion, Merck)

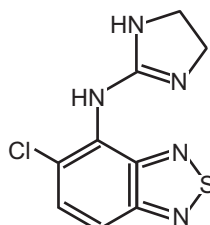
**(E) Drugs that act as  $\alpha_2$ -adrenoceptor agonists:**

Naphazoline

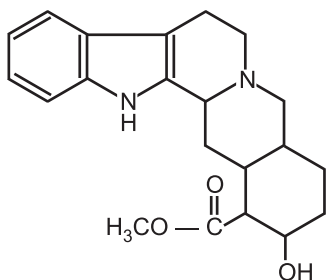
**Brimonidine:** It is peripheral  $\alpha_2$ -receptor agonist used to treat open angle glaucoma or ocular hypertension. It is a centrally acting adrenergic  $\alpha_2$ -receptor agonist used to treat chronic muscle spasticity conditions, such as multiple sclerosis.



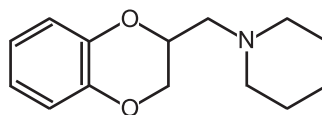
Brimonidine



Tizanidine



Yohimbine

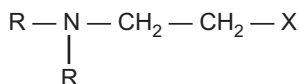


Piperoxan

Clonidine is yet another  $\alpha_2$ -adrenoreceptor agonist which is used as a central antihypertensive. It may perhaps act on the baroreceptor (blood pressure) reflex pathway, on cardiovascular centers in the medulla and also peripherally. It abolishes most symptoms of opiate withdrawal and stimulates histamine  $H_2$  receptors. It also acts as antianxiety agent that stimulates  $\alpha_2$ -adrenoreceptors and therefore decreases norepinephrine levels.

**(F) Drugs that block the interaction of norepinephrine with receptor (antagonists)****Alpha Adrenergic Blockers:**

**(a)  $\beta$ -Haloalkylamines:** These compounds resemble the nitrogen mustard antineoplastic agents and may be represented by the following general formula:

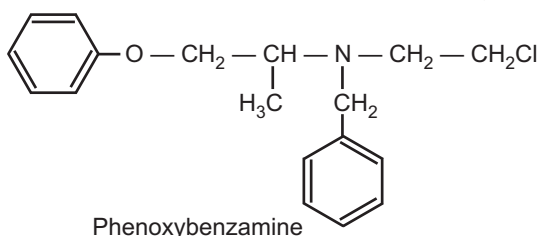


where, R = arylalkyl group

X = halogen

The effectiveness is dependent upon the nature of R. e.g., phenoxybenzamine.

Dibenzamine [N, N, dibenzyl-N-( $\beta$ -chloroethyl) amine] was investigated by Nickerson and Goodman as an anti-leukemic nitrogen mustard. Phenoxybenzamine was the outcome of molecular modification of dibenzamine to remove the toxicity.



The groups attached to the nitrogen are important for transport of the drug to the receptor area and binding to the receptor surface.

The  $\beta$ -haloalkylamines through the formation of an ammonium ion react with a nucleophilic group, present in the alpha receptor, forming a stable and perhaps only slowly reversible covalent bond, as shown in the Fig. 5.6.

#### Mechanism of Action:

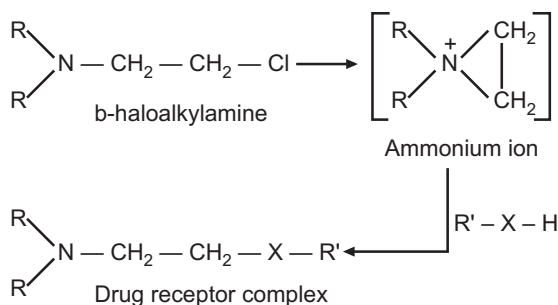
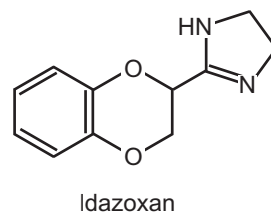
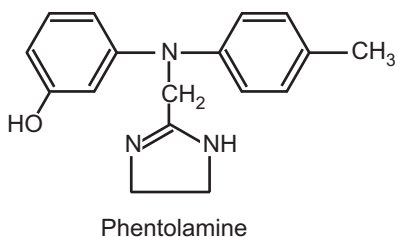
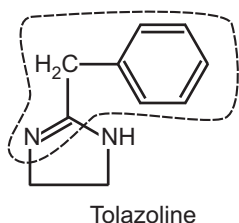


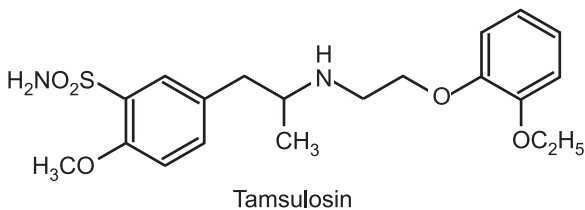
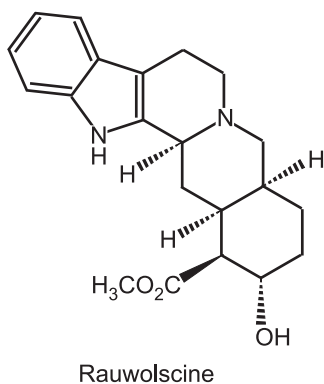
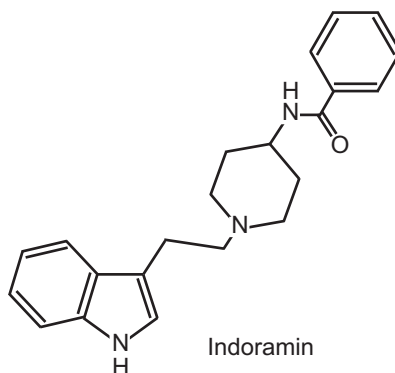
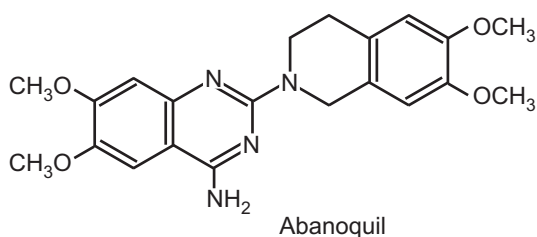
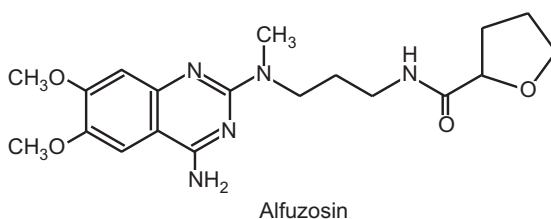
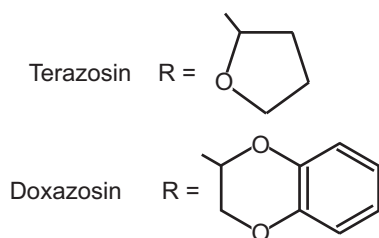
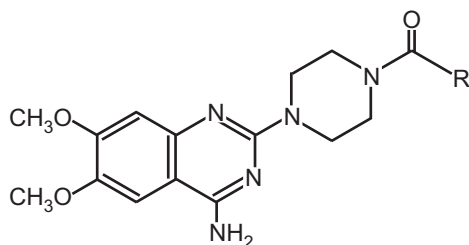
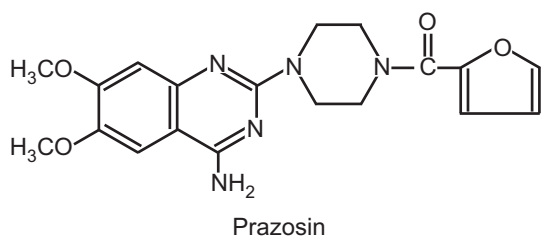
Fig. 5.6

**(b) Ergot alkaloids:** Ergot was recognised as  $\alpha$ -adrenergic agent in 1906. The parent compound is Lysergic acid. Ergocristine, ergocryptine, ergocornine and ergonovine which are the derivatives of lysergic acid are found to possess adrenergic blocking action. A number of other amides of lysergic acid have been prepared of which methylergonovine and methylsergide are employed clinically.

**(c) Imidazolines:** Following are the examples of clinically useful agents used in the management of hypertension, i.e. Tolazoline. The encircled portion resembles structurally with norepinephrine



(d) Other alpha receptor blocking agents include, some benzodioxanes and dibenzazepines. Prazosin, a quinazoline derivative is one of the newer clinically available  $\alpha$ -blocking agents.



**Tamsulosin:** Selective  $\alpha_1$ -blocker. It is used in symptomatic treatment of benign prostate hyperplasia.

**$\beta$ -Adrenergic Blockers:**

Unlike  $\alpha$ -adrenergic blockers, the structural requirements for  $\beta$ -adrenergic blockers have been fairly well defined.

**SAR:** (1) Phenolic OH groups are important for adrenergic agonist activity. Removal of the 4-OH leaves intact only  $\alpha$ -agonist activity, (e.g., phenylephrine, methoxamine - both vasoconstrictors used in treating hypotension and nasal congestion), whereas removal of the 3-OH group abolishes both  $\alpha$ - and  $\beta$ -agonist activity. The 3-OH group can, however, be replaced by a  $\text{SO}_2\text{NH}_2$  (soterenol) or a  $\text{OHCH}_3$ -(salbutamol) group. 3-amino compounds can be extremely potent. Replacement of 4-OH group by any such groups leads to an almost total loss of activity and compound may become an antagonist.

(2) The two-carbon side-chain is essential for activity. The benzylic carbon (next to the ring) must have (R) absolute configuration.

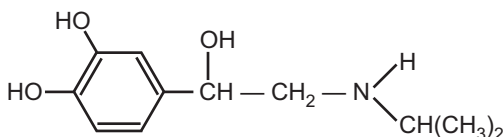
(3) The alcoholic OH can be replaced only by an amino or  $-\text{CH}_2\text{OH}$  group.

(4) Small ( $-\text{H}$ ,  $-\text{CH}_3$ ) N-substituents produce  $\alpha$ -activity; larger ones [ $-\text{CH}(\text{CH}_3)_2$ , aryl] produce  $\beta$ -activity.

There are two main classes of these agents:

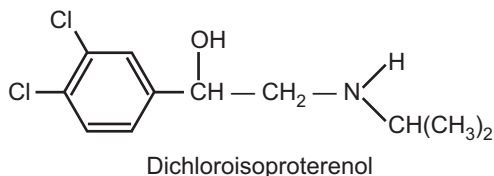
(a) Arylethanolamines, (b) Aryloxypropanolamines

**(a) Arylethanolamines:** Isoproterenol is a basic structure to yield good  $\beta$ -adrenergic blocking compounds.

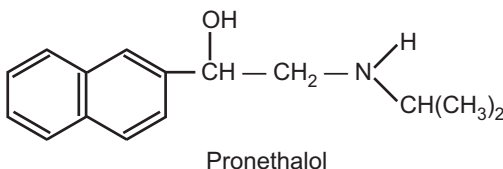


Certain modifications can be made on this basic structure, like,

(i) Replacement of catechol hydroxyl groups with chlorine to give dichloroisoproterenol (DCI), a classic  $\beta$ -blocking agent. It is the first useful  $\beta$ -blocker discovered in 1948. Since DCI is also a partial  $\beta$ -agonist and it cannot be used as a hypotensive agent. DCI turned out to be carcinogenic.

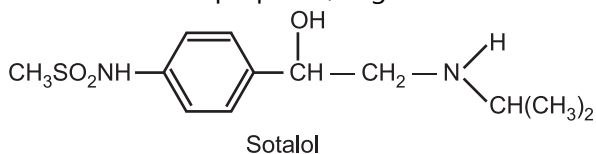


(ii) Replacement of the electron rich hydroxyl groups with an electron rich phenyl at 3, 4 positions gives pronethalol, which is even better  $\beta$ -blocker than dichloroisoproterenol.



(iii) **N-Substitution:**

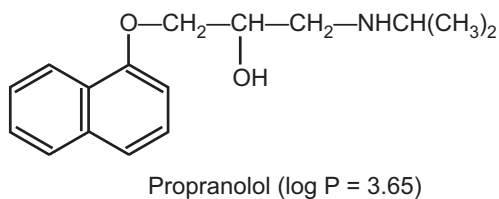
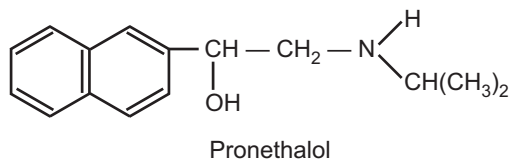
- (a) N, N-disubstitute, compounds are inactive.
- (b) Alpha-methyl group decreases activity.
- (c) Activity is maintained when phenylethyl, hydroxyphenylethyl or methoxyphenylethyl groups are added to amine part of the molecule.
- (d) Cyclic alkyl substituents are better than corresponding open chain substituents at nitrogen atom of amine.
- (e) Chain length may extend to a total of 4-atoms with or without a terminal phenyl carbon.
- (iv) Reduction of one ring to give either of two tetraline analogues did not affect activity.
- (v) Converting the aromatic portion to phenanthrene or anthracene was disadvantageous.
- (vi) Other derivatives, in which the parahydroxyl group on phenyl ring is replaced by methylsulphonamide were also prepared, e.g. Sotalol.



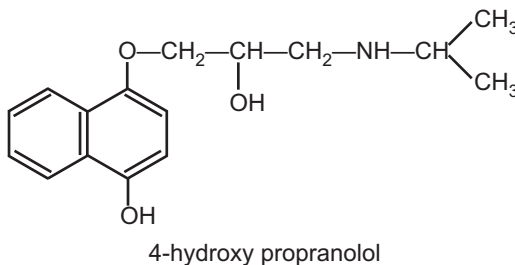
In this series, if the methylsulphonamide group is replaced by nitro, appreciable activity is maintained.

(b) **Aryloxypropanolamines:**

(1) Pronethalol, an aryloxypropanolamine, was withdrawn from clinical testing because of reports that it caused thymic tumours in mice. However, within two years of this report, Black and co-workers discovered, a potent  $\beta$ -blocker propranolol, a close structural relative of pronethalol.



Propranolol is the prototype of the group of  $\beta$ -blocking agents known as aryloxypropanolamines. A propranolol metabolite of particular interest is 4-hydroxypropranolol, which is a potent  $\beta$ -antagonist.

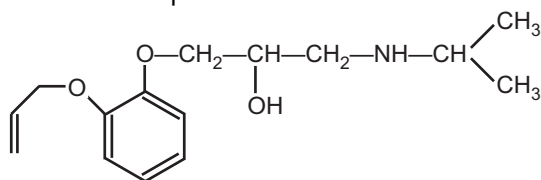


(2) (a) Most derivatives of this series possess various substituted phenyl rings rather than the naphthyl ring. The catechol ring system can be replaced by a great variety of other ring systems varying from phenylether (oxprenolol) and sulphonamides (sotalol) to amides (labetolol), indoles (pindolol, benzpindolol) and naphthalene (propranolol). N-substituents must be bulky to ensure affinity to  $\beta$ -receptors; isopropyl is the smallest effective substituent.

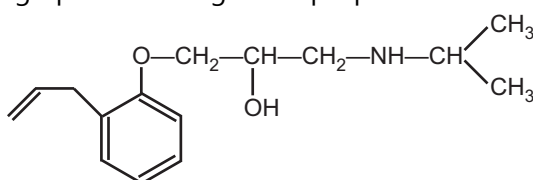
(b) Substitution of  $\text{CH}_3$ ,  $\text{Cl}$ ,  $\text{OCH}_3$  or  $\text{NO}_2$  groups on the phenyl ring was most favoured at 2 and 3 positions and least favoured at 4 - position.

(c) Alkenyl and alkenyloxy groups in the ortho positions on phenyl ring, provided good activity. e.g. oxprenolol, alprenolol.

These compounds could be considered as ring opened analogues of propranolol.



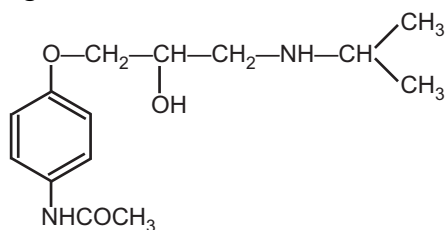
Oxprenolol



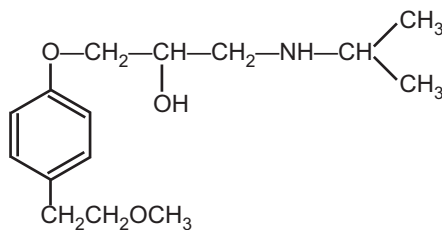
Alprenolol

(d) Longer alkyl chains are less effective but isopropyl or t-butyl, which gives an optimal basicity or nucleophilicity to the amino group for receptor affinity are most preferred.

(3) A major clinical problem with propranolol was its high lipid solubility, which allowed it to penetrate nerve tissue and exert an undesirable cardiodepressant effect in addition to its  $\beta$ -blocking. To avoid this problem, use of polar group (such as methanesulphonamide- $\text{NHSO}_2\text{CH}_3$ ) was considered. The prototype of this series of compounds was practolol, which is devoid of the depressant effect of propranolol. It was the first cardioselective  $\beta_1$ -antagonist.

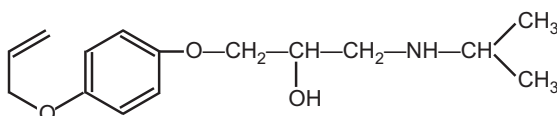
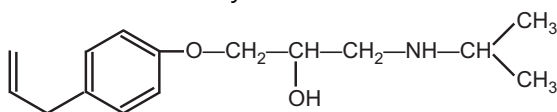


Practolol (log P = 0.79)



Metoprolol

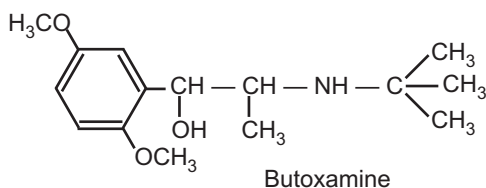
Other selective  $\beta_1$ -blockers under study are:



These compounds are characterised chiefly by p-substitution rather than o-substitution.

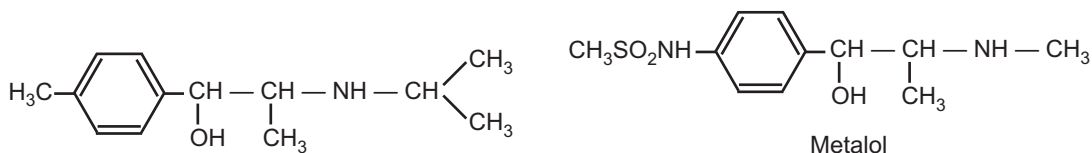
**Selective  $\beta_2$ -Antagonists:**

Butoxamine has a selective  $\beta_2$ -antagonistic action. It blocks  $\beta_2$ -receptors present in smooth muscle and in skeletal muscle.

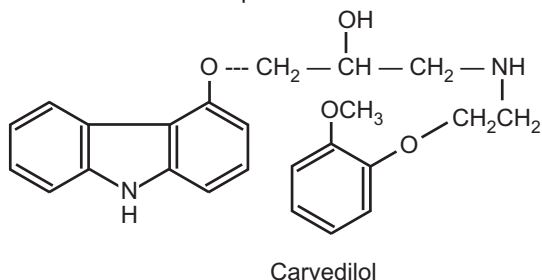
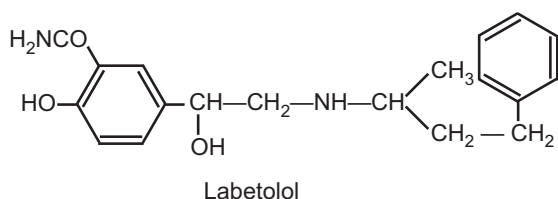
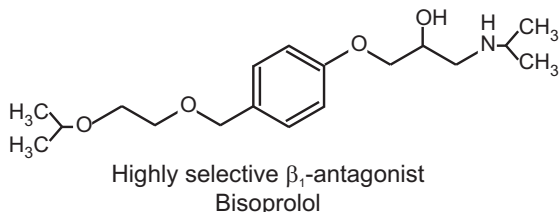
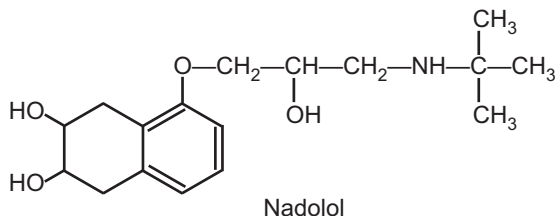


It is an useful research tool but it does not, at present, have clinical use.

Other compounds which have selective  $\beta_2$ -blocking action, are

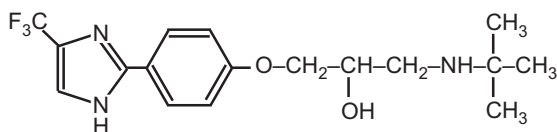
**Non-selective  $\beta$ -Blockers:**

Alongwith alprenolol, propranolol, oxprenolol, other new compounds such as nadolol, timolol and labetalol, also exhibit non-selective  $\beta$ -receptor blocker activity.



Labetolol and carvedilol are the examples of non-selective  $\beta$ -blockers with  $\alpha_1$ -receptor antagonistic activity.

Another compound having direct vasodilating effects in addition to  $\beta$ -blockage which offers an additional advantage of a reduced peripheral resistance with the original antihypertensive action is:



**Clinical Significance of  $\beta_1$ -Blockers as Antihypertensive Agents:**

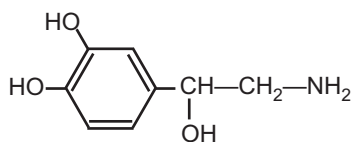
It is found that  $\beta_1$ -receptors are predominant in heart (alongwith few  $\beta_2$ -receptors). Stimulation of  $\beta_1$ -receptors therefore, results in an increase in heart rate and increased force of contraction of heart muscles. Therefore, selective  $\beta_1$ -blockers gained a high clinical importance as antihypertensive agents.

Similarly,  $\beta_2$ -receptors are predominant in lungs particularly bronchial muscles (alongwith few  $\beta_1$ -receptors). Hence, selective  $\beta_2$ -blockers will cause bronchial muscles constriction, a case contraindicated in patients suffering from bronchial asthma and hence clinically useless, but can serve as a research tool for a medicinal chemist to develop new, potent, selective  $\beta_1$ -blockers or antihypertensive agents. Cardioselective  $\beta$ -antagonists are drugs that have much greater affinity for the  $\beta_1$ -receptors of the heart than for  $\beta_2$  receptors in other tissues. Such agents should have two important features: (a) The lack of an antagonist effect on the  $\beta_2$ -receptor in bronchi. This would make  $\beta_1$ -blockers, safe for use in patients who have bronchial asthma. (b) The absence of blockage of the vascular  $\beta_2$ -receptors (which mediate vasodilation), which otherwise leads to vasoconstriction resulting in increased peripheral resistance, an undesirable effect in antihypertensive activity of non-selective  $\beta_2$ -antagonist. Theoretically, one cannot obtain complete cardioselectivity because of the presence of both  $\beta_1$ - and  $\beta_2$ -receptors in cardiac and lung tissues. Hence, on strict pharmacological ground, antihypertensive agents of this category could be expected to raise rather than lower the arterial pressure by blocking the vasodilation-mediated by vascular  $\beta_2$ -receptor. They are antihypertensive and act through the following postulated mechanisms:

- (1) Inhibition of renin release.
- (2) Inhibition of cardiac output ( $\beta_1$ -blockage).
- (3) Inhibition of sympathetic output by central action.
- (4) Restoration of vascular relaxation response.
- (5) Inhibition of the synaptic norepinephrine release.

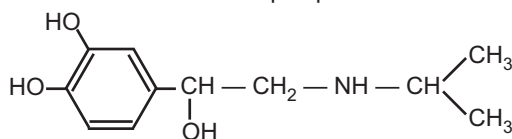
**Receptor Structure:**

The most effective compound, acting on alpha receptor is norepinephrine.

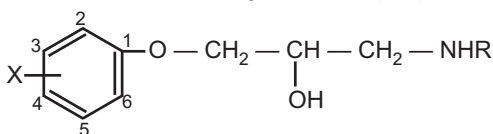


Norepinephrine

The more bulkier the substituents on nitrogen, alpha receptor activity decreases e.g. Isoprenaline.

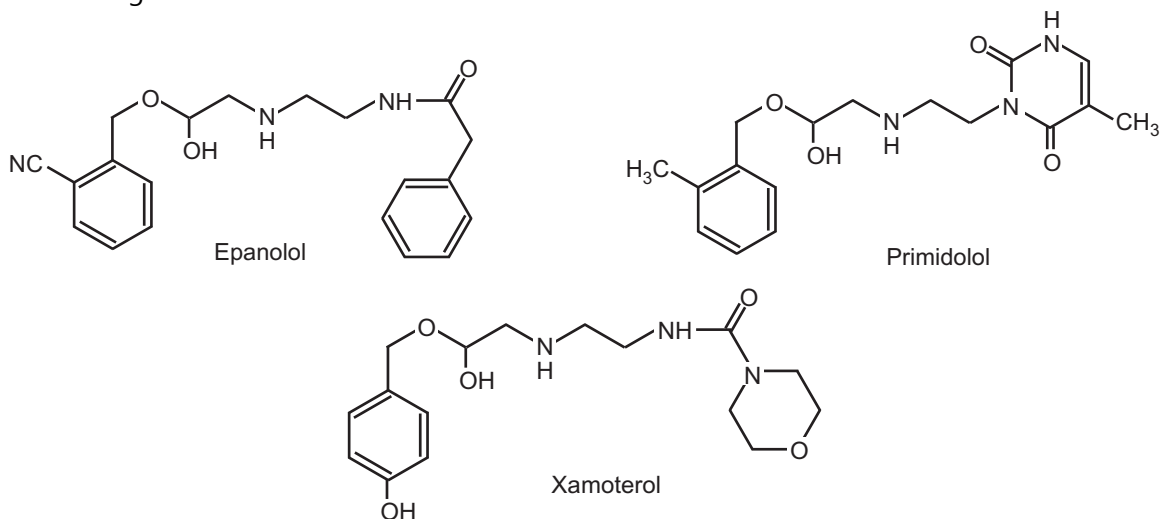


Isoprenaline

**Table 5.7: Useful  $\beta$ -blocking agents**

Sr. No.	Name	X	R	Selectivity on receptor
1.	Pindolol	2, 3-	isopropyl	non-selective
2.	Bunitrolol	2 - CN	t - butyl	non-selective
3.	Nadolol		t-butyl	non-selective
3.	Metoprolol	4-CH <sub>2</sub> CH <sub>2</sub> OCH <sub>3</sub>	isopropyl	$\beta_1$ - receptor
4.	Atenolol	4-CH <sub>2</sub> CONH <sub>2</sub>	isopropyl	$\beta_1$ - receptor
5.	Acebutolol	4-NHCOC <sub>3</sub> H <sub>7</sub>	isopropyl	$\beta_1$ - receptor
6.	Timolol			

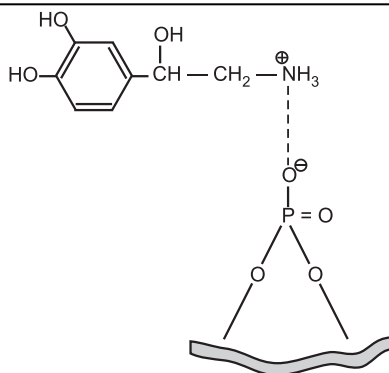
A new series of selective  $\beta_1$ -blockers replacing N-alkyl groups (e.g. isopropyl, t-butyl) by aralkyl groups was developed which bind to the  $\beta_1$ -receptor through an additional H-bonding interaction.



**$\alpha$ -Receptor:**

The  $\alpha$ -receptor carries a negatively charged group (probably a phosphate) which will then react with the positively charged ammonium nitrogen.

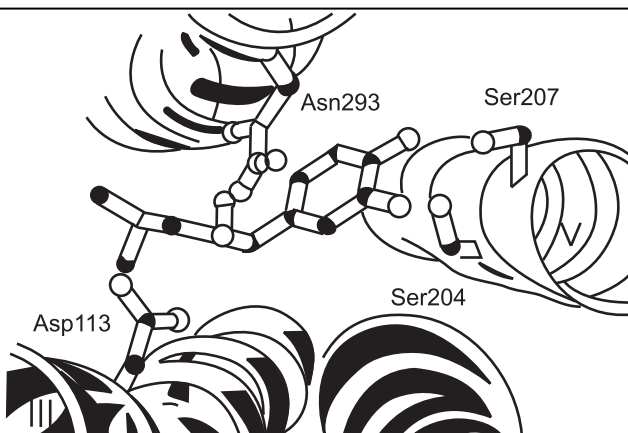
Bulky substituents present on the nitrogen (as in the case of isoprenaline), would hinder the attack of positively charged cation on phosphate anion. Hence, isoprenaline has less affinity for  $\alpha$ -receptors.



**Fig. 5.7:  $\alpha$ -receptor activation**

 **$\beta$ -Receptor:**

The seven  $\alpha$ -helices proposed for the  $\beta$ -adrenoceptor are radically arranged around a central "pore" in which the receptor ligands bind. It has been postulated that the phenyl ring of Phe 290 in the sixth transmembrane helix, participate in binding of the aromatic ring of agonist ligands. The m- and p-hydroxyl groups of the catecholamine agonists are postulated to form H-bonds to Ser 204 and Ser 207. The  $\beta$ -hydroxyl group in the side chain may interact with either Ser 165 of transmembrane helix 4 or ASn 293 of transmembrane helix 6. An aspartic acid residue ASp 113, located in third transmembrane spanning helix is required for both agonist and antagonist binding to  $\beta$ -adrenoceptor. The free carboxyl group of this amino acid interacts with the protonated amino group of adrenergic ligand.



**Fig. 5.8**

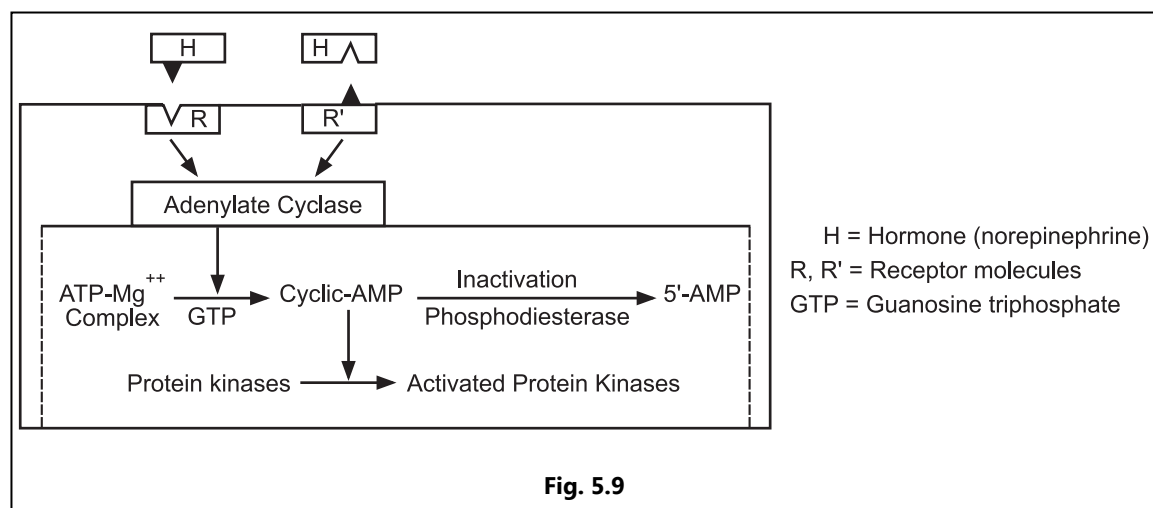
**Indications of  $\beta$  Blockers:**

- Angina pectoris Myocardial infraction
- Thyrotoxicosis Anxiety
- Glaucoma Hypertension
- Cardiac arrhythmias Pheochromocytoma
- Migraine Essential tremors
- Hypertrophic subaortic stenosis

**Post Receptor Binding Events:**

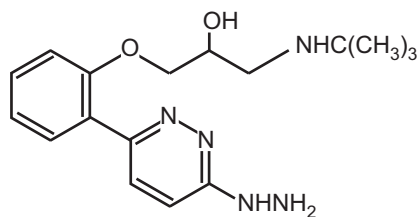
Receptors for a number of hormones and autacoids function by regulating the concentration of the intracellular second messenger cyclic adenosine 3', 5'-monophosphate (cyclic AMP) through the stimulation or inhibition of the membrane - bound enzyme, adenylate cyclase. Adenylate cyclase and  $\beta$ -adrenergic receptors are considered to be proteins. They are embedded in lipid matrix of the cellular membrane in such a way that adenylate cyclase faces the intracellular fluid, whereas the receptor faces the extracellular fluid. The activation of adenylate cyclase enzyme leads to the formation of cyclic AMP from  $Mg^{++}$ -ATP complex. An additional protein appears to mediate this conversion in guanosine triphosphate (GTP). Similarly, phosphodiesterase enzymes metabolise cyclic AMP to inactive 5' - AMP.

The function of cyclic AMP is to regulate the activity of a class of enzymes, the protein kinases, which activate a wide variety of reactions which are characteristic of  $\beta$ -adrenergic responses e.g. increased cardiac contractility, smooth muscle relaxation, glycogenolysis etc.



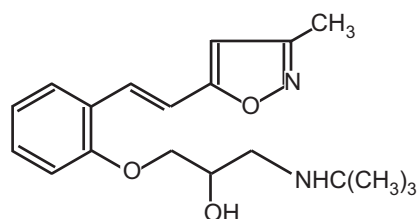
**Dual-acting Antihypertensive Agents:**

Introduction of a known vasodilator moiety into the o-position of a  $\beta$ -blocker, which is known to be sterically undemanding, has given the  $\beta$ -blocker vasodilator prizidolol.



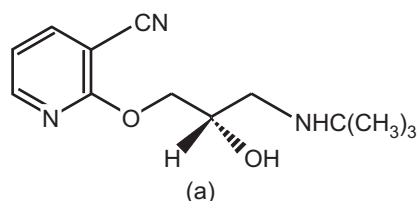
Prizidolol

Isoxaprolol was 16 times more potent than labetalol as a  $\beta$ -blocker and 4-times more potent as an  $\alpha$ -blocker.



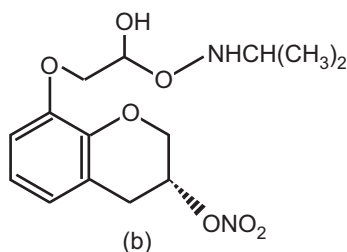
Isoxaprolol

MK-761 (a) was equipotent with timolol as a  $\beta$ -blocker and 3.8-times more potent than hydralazine as a vasodilator, and was not a  $\beta_2$ -agonist.



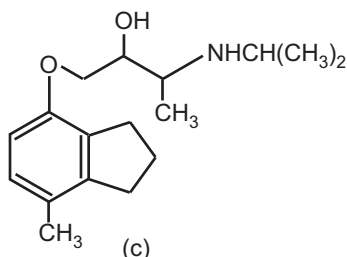
(a)

Although it is not formally described as a combination,  $\beta$ -blocker-nitrate molecule, K 351 (b) clearly owes its vasodilator activity to the presence of the nitrate ester.



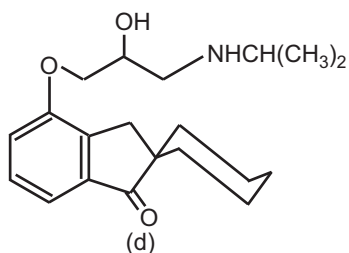
(b)

An extension of the work led to the potent  $\beta_2$ -selective blocker, ICI 118551 (c). A  $\beta_2$ -selectivity ratio of 123 : 1 has been reported from *in-vitro* studies and greater than 250 : 1 *in-vivo*. ICI 118551 has no partial agonist activity; it has the same degree of membrane stabilizing activity as propranolol and is currently undergoing clinical evaluation for potential use in migraine and essential tremor.

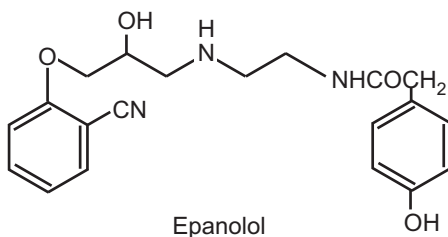


(c)

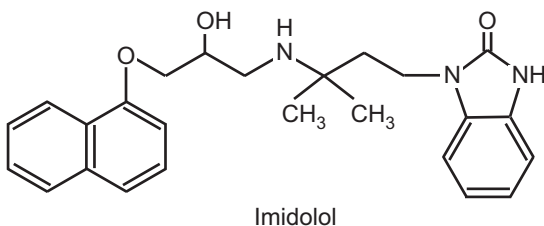
Spirendolol (LI 32468) (d) is another  $\beta_2$ -selective blocker in clinical trial. It is effective in controlling essential tremor at doses which have no effect on heart rate.



It was hypothesized that the cardioselectivity was the result of an interaction between the oxygen or sulphur atom and a complementary site on the  $\beta$ -receptor.



Epanolol (ICI 141292), a cardioselective  $\beta$ -blocker with partial agonist activity, is undergoing clinical evaluation.



Imidolol is reported to be  $\beta$ -blocker with  $\alpha$ -blocking activity.



## Chapter...6

## CHOLINERGIC NEUROTRANSMITTERS

## ♦ SYNOPSIS ♦

- |  |  |
|--|--|
| 6.1 ACETYLCHOLINE                                | 6.8 CLASSIFICATION OF ANTICHOLINESTERASES                |
| 6.2 CYCLIC ANALOGUES OF ACH                      | 6.9 THERAPEUTIC USES                                     |
| 6.3 CHOLINERGIC RECEPTORS                        | 6.10 ANTIDOTES FOR NERVE GASES                           |
| 6.4 METABOLISM OF ACH                            | 6.11 URINARY ANTISPASMODIC (M <sub>3</sub> -ANTAGONISTS) |
| 6.5 CHOLINERGIC AGONISTS                         |  |
| 6.6 ANTICHOLINESTERASES                          |  |
| 6.7 STRUCTURAL FEATURES OF CHOLINESTERASE ENZYME |  |

## 6.1 ACETYLCHOLINE

Acetylcholine was first synthesised by Baeyer in 1867. In general, stimulation of parasympathetic nervous system induces constriction of pupil and bronchi, decrease in heart activity and an increase in the activity of digestive system i.e. salivation and other GIT secretions are promoted. Motility of the intestine is also increased.

## Chemical Features of Acetylcholine:

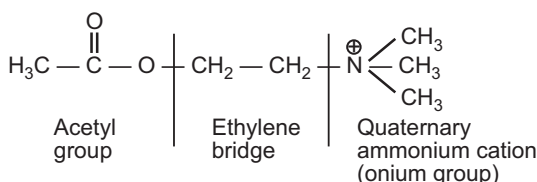


Fig. 6.1: Acetylcholine

Following are some of the important chemical features of acetylcholine molecule:

- (i) Chemically it is an ester of acetic acid and choline, an amino alcohol.
- (ii) On the structural basis, it offers three sites for molecular modifications:
  - (a) acetyl group,
  - (b) ethylene bridge and
  - (c) quaternary ammonium group.

- (iii) The quaternary ammonium group (i.e. onium group) is linked by an ethylene bridge to an ester group.
- (iv) Acetylcholine is stable in acidic solutions but it is very unstable in alkaline media.
- (v) Free acetylcholine present in the tissue fluids and circulation, is rapidly hydrolysed to acetic acid and choline molecule by cholinesterase enzyme.
- (vi) Acetylcholine exhibits some of its actions via G-protein coupled muscarinic receptors while remaining actions are propagated through nicotinic receptors.

Muscarinic receptors are also reported to be present at cortical and subcortical site within the CNS and in autonomic ganglia. Nicotinic actions at autonomic ganglia are antagonised by hexamethonium and related drugs whereas at neuromuscular junction of skeletal muscle, they are antagonised by tubocurarine. At nicotinic receptor sites, acetylcholine produces stimulant effects in small doses whereas large doses of acetylcholine lead to receptor inhibition.

### **Pharmacological Actions:**

#### **(a) Cardiovascular System:**

If acetylcholine is injected intravenously, the muscarinic effects of acetylcholine are predominantly seen on cardiovascular system. These effects include:

- (i) Vasodilation.
- (ii) Decrease in the force of heart contraction  
(negative chronotropic effect)
- (iii) Decrease in the force of heart contraction (negative inotropic effect).

Low doses acetylcholine are sufficient to produce vasodilation including the pulmonary and coronary vasculature. This is brought about mainly by the stimulation of muscarinic receptors present in the endothelial cells of vasculature. The vasodilatory effect of acetylcholine on peripheral vasculature is very marked but is quickly terminated. The latter two effects of acetylcholine can be observed only in higher doses. In heart, cholinergic innervation is provided mainly to the sinoarterial node, atrioventricular node and the atrial muscles. The activation of this innervation leads to an increase in the permeability of cardiac fibres to potassium resulting into a decrease in the activity of S-A node.

The effects of acetylcholine on cardiovascular system just described, sometimes may be reversed by the release of catecholamines from the adrenal medulla and sympathetic ganglia. This release may be due to the stimulation of nicotinic cholinergic receptor sites present in these organs.

#### **(b) Smooth Muscle:**

At moderate doses, acetylcholine stimulates the muscarinic receptors present on the smooth muscles of GIT, urinogenital and respiratory tract, and eye resulting into contraction of these muscles. The increased muscle tone of GIT may lead to nausea and vomiting. The lacrimal, salivary, gastric, pancreatic, and sweat glands are also stimulated. The motility of gall bladder and bile ducts is also increased.

The stimulatory effects of acetylcholine can be explained on the basis of an increase in the permeability of the muscle cell to  $\text{Na}^+$  and  $\text{Ca}^{++}$  ions which results into a depolarisation of the cell membrane. When acetylcholine reacts with the post-synaptic receptors, it may produce either excitation or inhibition. The action of the transmitter results in selective increase or decrease of ionic permeability of membrane. The ratio of permeability for  $\text{K}^+$  to that of  $\text{Na}^+$ , if increased may lead to hyperpolarisation (as in cardiac cells) and if decreases, may cause depolarisation (as in smooth muscle cells). For example, high concentration of acetylcholine may lead to the complete heart block due to hyperpolarisation.

Thus, enhanced permeability to monovalent cations, an increase in the concentration of intracellular calcium ions, an increase in the concentration of guanosine - 3', 5'-monophosphate (cyclic GMP) or the inhibition of adenylate cyclase enzyme are some of the mechanisms associated with muscarinic receptor stimulation. Investigations in the role of c-GMP started from 1960. It is widely distributed in the tissues. It is present at higher concentration in cerebellum and particularly in retina.

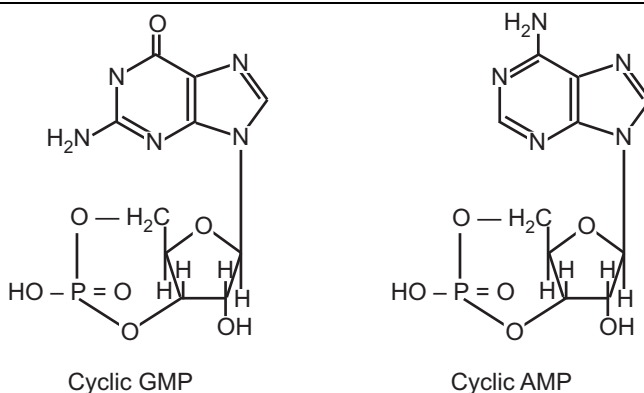
Due to the quaternary cationic nature, acetylcholine, at low doses, can not readily reach the skeletal muscles embedded by the fatty layers. Hence at low doses, the nicotinic actions of acetylcholine are not prominent. At large doses, acetylcholine can stimulate nicotinic receptors present in both, sympathetic and parasympathetic ganglia.

Acetylcholine is poor therapeutic agent since it gets easily hydrolysed by cholinesterase enzyme. Clinically, its use as acetylcholine chloride, is restricted in ophthalmic surgery to obtain rapid and complete miosis during cataract removal. For this purpose, 0.5 to 2.0 ml of 10 mg/ml solution of acetylcholine chloride can be applied locally.

### Structure-Activity Relationship:

(i) Any change in the ethylene bridge may affect the chemical stability of acetylcholine molecule.

(ii) A cationic ammonium group is essential for the manifestation of both muscarinic and nicotinic receptor activities. If one or more of the methyl groups on nitrogen atom are replaced by hydrogen or ethyl group, both activities are reduced.



**Fig. 6.2: Important cyclic nucleotides**

(iii) The quaternary nitrogen atom itself may be replaced by arsenic, antimony, phosphorus or sulphur atom without the loss of all acetylcholine-like activities.

(iv) Ing in 1949 proposed that for maximal muscarinic activity, there should be not more than four atoms between the nitrogen and terminal C-atom.

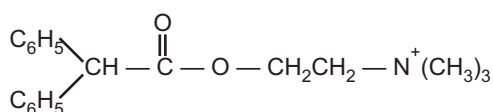
(v) If bulky substituents are placed on the terminal C-atom of acetyl group, through a firm binding and 'Umbrella effect,' these substituents block the access of acetylcholine to the receptor. This results in the antimuscarinic activity. Examples include, benzilylcholine, tropylcholine etc.

(vi) Carbachol and acetyl- $\beta$ -methylcholine are the cholinergic agonists acting chiefly at muscarinic receptors while propionylcholine and acetyl  $\alpha$ -methylcholine act chiefly at nicotinic cholinergic receptors.

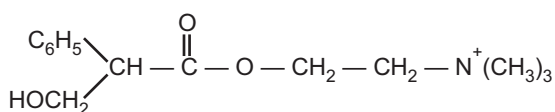
## 6.2 CYCLIC ANALOGUES OF ACETYLCHOLINE

Muscarine is a cyclic analogue of acetylcholine, devoid of nicotinic receptor activity. It has a quaternary ammonium group but does not possess an ester function. Hence, it is not enzymatically metabolised by cholinesterase enzyme. This explains its long duration of action. If administered in sufficient concentration, it reaches the CNS by crossing blood-brain barrier and evokes cortical arousal.

The following structures, along with the rigid size requirements for the esters of choline, also indicate steric and conformational requirement for optimal fit on the muscarinic receptors.

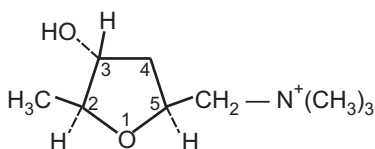


Benzilylcholine

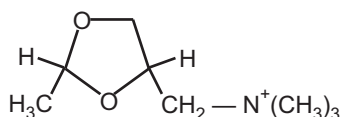


Tropyllcholine

### Cyclic analogues of acetylcholine:

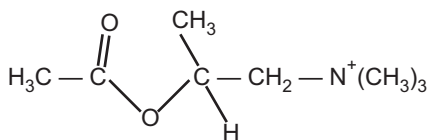


Cis-L-(+) muscarine



2-methyl-4-trimethyl ammonium  
- methyl-1,3-dioxolane

For example, Acetyl- $\beta$ -methyl choline adopts following conformation.



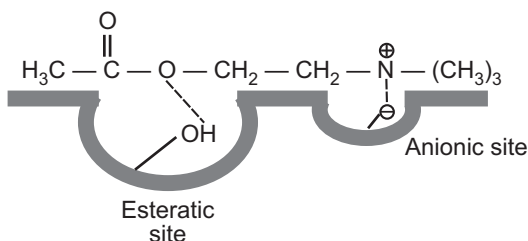
Acetyl- $\beta$ -methylcholine

Fig. 6.3

### 6.3 CHOLINERGIC RECEPTORS

From the SAR studies, the structure of a cholinergic receptor is predicted as shown in the Fig. 6.4.

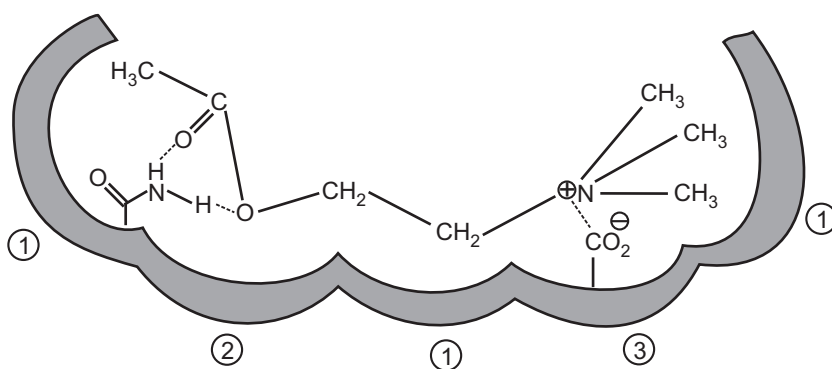
The negative charge at the anionic site of the receptor may result from the ionisation of a dicarboxylic amino group (i.e., aspartic or glutamic acid) present in the receptor. The quaternary ammonium group forms an electrostatic bond with this anionic site. The ester or other group capable of forming H-bond interacts at the esteratic site through H-bonding.



**Fig. 6.4: Cholinergic receptor**

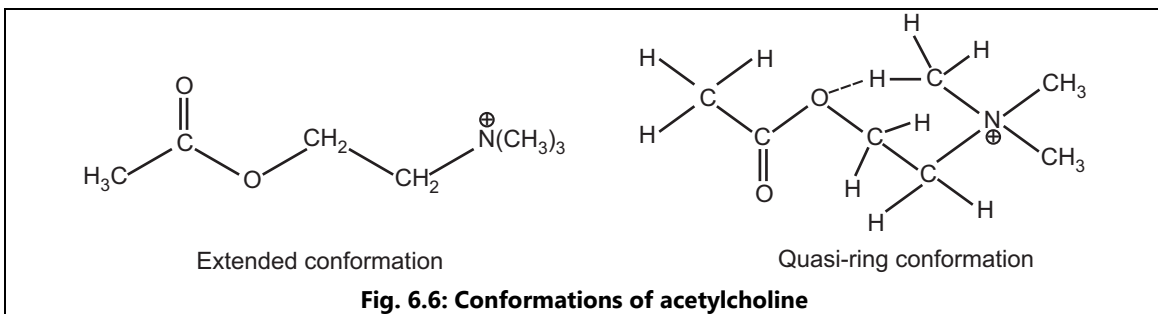
Since the tissues containing muscarinic receptors are extremely complex, binding studies between acetylcholine analogues and cholinesterases were made. They indicate that the methyl groups present on N-atom along with the terminal methyl group are bound to the receptor by both hydrophobic and van der Waal's forces. This binding assures a close fit of the molecule to the receptor as shown in Fig. 6.5.

But, due to the free rotation around most of its covalent bonds, acetylcholine can exist in a large number of conformations. The following two structures represent extremes in all such possible conformations (Fig. 6.6).



- (1) : Region of hydrophobic binding (van der Waal's forces)
- (2) : Region of H – bonding
- (3) : Region of ionic bonding

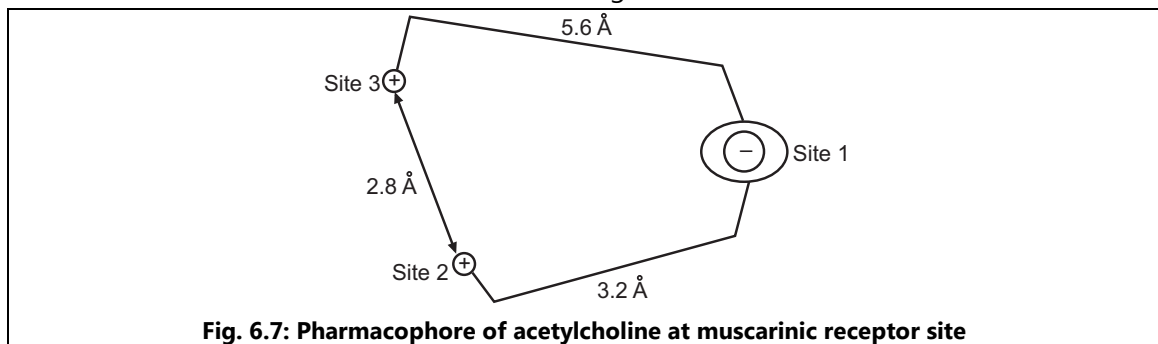
**Fig. 6.5: Acetyl choline - receptor interactions**



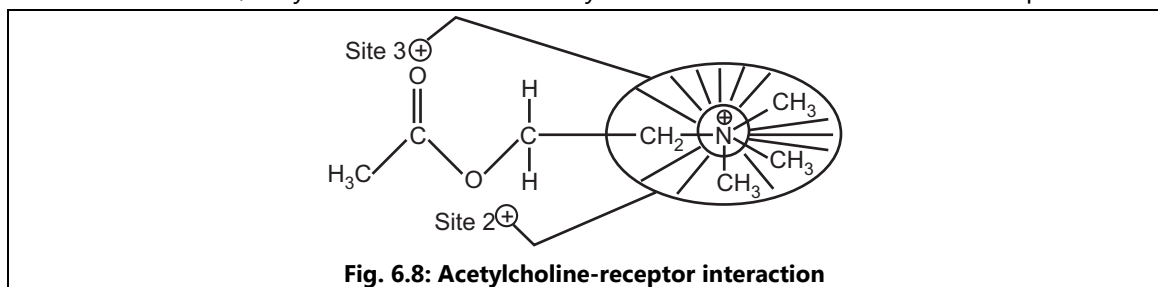
The structure of any drug can be categorised into an essential part (necessary for better intrinsic activity) and supporting part (necessary for better affinity and pharmacokinetic properties). The supporting structure also helps to bring the essential structure in the correct three-dimensional arrangement with respect to the receptor surface.

The essential as well as supporting structures, both differ in their conformations as regard to the action of acetylcholine on muscarinic and nicotinic receptors. For example, acetylcholine is present in an extended conformational form when it fits on muscarinic receptor while it adopts quasi ring conformation when it acts on nicotinic cholinergic receptors.

While in another hypothesis, Beckett proposed that, the muscarinic receptors contain one anionic and two cationic sites as shown in Fig. 6.7.



The anionic binding site (site 1) accommodates the quaternary nitrogen of muscarine. The site 2 or a H-bonding site is for ether oxygen of muscarine or acetylcholine while the site 3 can interact with the carbonyl group of acetylcholine or the ether oxygen of dioxolone or with the alcohol group of muscarine as shown in Fig. 6.8. Although acetylcholine has three reactive sites, only two sites are necessary for the various actions of the compound.



## 6.4 METABOLISM OF ACETYLCHOLINE

The free acetylcholine present in the blood and other tissues, gets quickly hydrolysed by either e-cholinesterase (present in erythrocytes) and s-cholinesterase (present in the serum). Dale (1914) first proposed the concept of enzymatic destruction of acetylcholine in the blood and other tissues. Serum cholinesterase is also known as butyrylcholinesterase while e-cholinesterase is also termed as acetylcholinesterase. The cholinesterases are not very selective enzymes. A number of other s-cholinesterases share some of the properties of e-cholinesterases. Both these types hydrolyse a large number of esters, both, of choline and of other carboxylic acids.

The basic unit of the enzyme is a tetramer with a molecular weight of 320,000; each of the protomers contains an active site. Normally, three such tetrameric units are linked through disulphide bonds to a  $50 \times 2$  nm stem. Analysis of the amino acid composition of the enzyme shows that it bears a close similarity to the acetylcholine receptor in its high proportion of acidic amino acids.

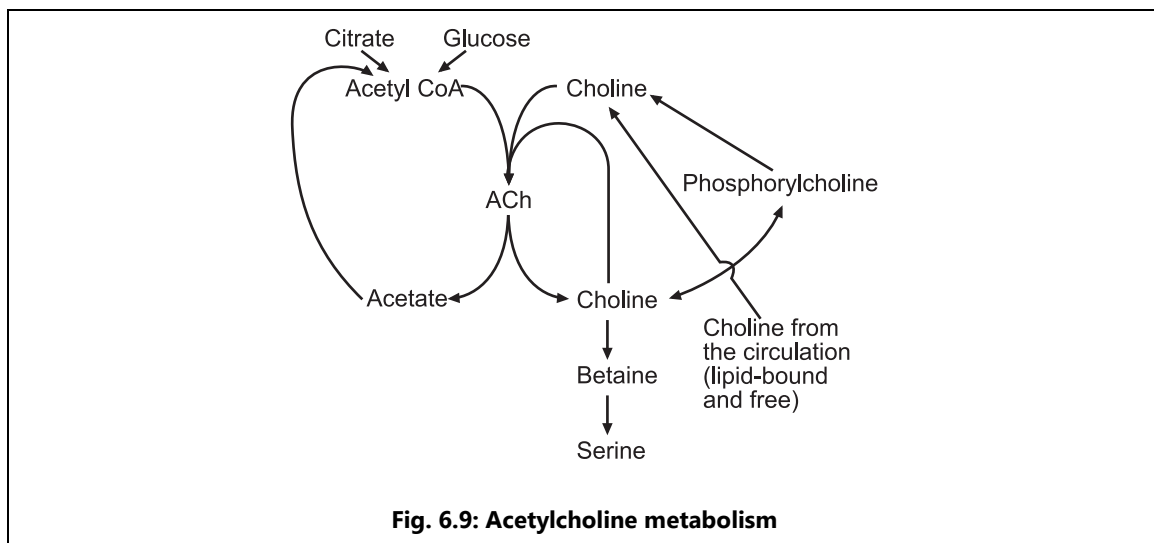


Fig. 6.9: Acetylcholine metabolism

## 6.5 CHOLINOMIMETICS OR CHOLINERGIC AGONISTS

The cholinomimetics have as their primary action the excitation or inhibition of autonomic effector cells that are innervated by post-ganglionic parasympathetic nerves. They differ from acetylcholine in,

- (1) their selectivity on muscarinic and nicotinic receptors.
- (2) their chemical stability.
- (3) their resistance to hydrolysis by cholinesterases and
- (4) their duration of action.

On the structural basis, the cholinomimetic agents can be divided into,

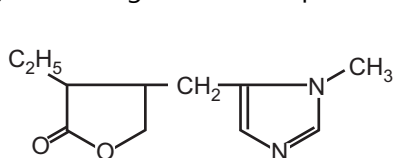
- (a) Acetylcholine and several synthetic choline esters.
- (b) Naturally occurring and synthetic alkaloids.
- (c) Cholinesterase inhibitors or anticholinesterases and
- (d) Ganglionic stimulants.

The last two categories do not act at post-ganglionic cholinergic effector sites and produce their effects by acting in an indirect way.

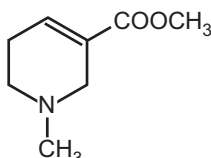
### Structure-Activity Relationship

#### (a) Modifications of the Onium (Quaternary ammonium) Group:

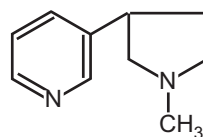
(i) The trimethylammonium group is the optimal functional requirement for activity, although following are the exceptions.



**Pilocarpine**



**Arecoline**



**Nicotine**

(ii) Phosphonium, sulphonium, arsenonium or substances larger than methyl on the nitrogen, had less activity and not used clinically.

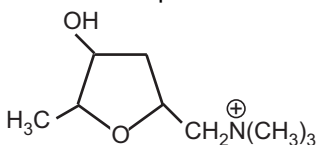
#### (b) Modifications of the Ethylenic Bridge:

(i) In studying a series of n-alkyl tri-methyl ammonium salts, Ing noted that for maximal muscarinic activity, there should be not more than four atoms between the nitrogen and terminal carbon atom. This rule was referred to as five atom rule.

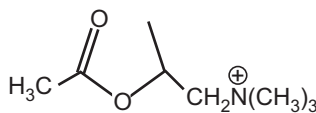
(ii) Replacement of the hydrogen atoms of the ethylenic bridge by alkyl groups produces far less active compounds except when a single, methyl group is placed either at  $\alpha$  or  $\beta$  to the quaternary nitrogen atom.

(iii) The presence of a methyl group  $\beta$  to the quaternary nitrogen atom increases the muscarinic activity, e.g. Methacholine.

The high selective muscarinic action is due to orientation of methyl group of methacholine in the same position as a methylene group in muscarine.



**Muscarine**



**(S) - Methacholine**

Moreover the added methyl group hinders the attack of esterase enzyme, thus slows down enzymatic hydrolysis.

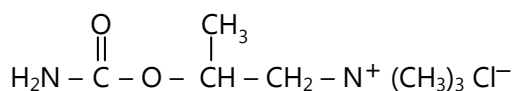
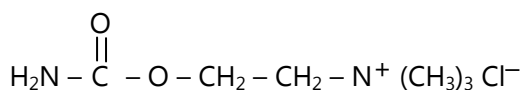
(iv) A methyl group alpha to the nitrogen increases nicotinic activity e.g. Acetyl Methylcholine.

**(c) Modifications of the Acyl Group:**

(i) When the acyl group is substituted by its higher homologues (i.e. the propionyl, butyryl etc.), less active compounds are formed.

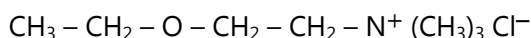
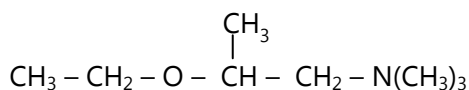
(ii) Choline esters of aromatic or higher molecular weight acids are cholinergic antagonists rather than agonists.

(iii) When the terminal methyl group is replaced by – NH<sub>2</sub> group, the resulting compound, (the carbamic acid ester), however, is a potent cholinergic agent with both muscarinic and nicotinic activities.

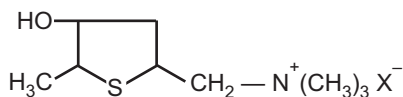
**Bethanechol chloride****Carbachol chloride**

Carbachol is certainly stable to hydrolysis and has the right size to fit the cholinergic receptor. The carbamic acid ester of β-methylcholine is also a stable therapeutic agent. The measured interprosthetic distances in acetylcholine are 7.0 Å ketone oxygen to methyl and 5.3 Å ether oxygen to methyl. Obviously, the interprosthetic distances for acetylcholine, methacholine, carbaminoylcholine, and urecholine are the same. Apparently, if the interprosthetic distances are optimal, the receptors on the cell do not differentiate between ether, ketone, ester, or acetyl oxygen atoms.

(iv) In carbachol, the terminal methyl group of acetylcholine is replaced by -NH<sub>2</sub> group, while size of the molecule remains the same as that of acetylcholine. So it becomes apparent that the size of the molecule may be more important to its activity than the acyl group present. Similarly, the ether oxygen appears to be of primary importance for high muscarinic activity. As a result of such reasoning, ethers of choline and alkylaminoketones were examined for activity.

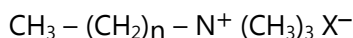
**Choline ethyl ether (High muscarinic activity)****β-Methylcholine ethyl ether (High muscarinic activity)**

(v) The reduced biological activity of compounds in which oxygen is replaced by sulphur (e.g. thiomuscarine) suggests the presence of H-bonding or dipole-dipole interaction between the drug and the receptor because sulphur atom has a less ability to form H-bonds with the receptors.



**Thiomuscarine**

(vi) The concept that the ester i.e. carbonyl or other group is not essential for activity but may enhance it by increasing the affinity of the molecule for the receptor was confirmed by a study of the muscarinic properties of N-alkyltrimethyl ammonium salts.



**N-alkyl trimethyl ammonium salts**

Compounds in this series showed muscarinic activity when  $n = 1, 2, 3$  or  $4$ . Compounds with groups larger than pentyl were partial agonists and those with groups larger than heptyl were antagonists. This appears to believe the hypothesis that size rather than functional groups is necessary for the intrinsic activity.

### Adverse Reactions:

All synthetic choline esters should never be administered by intravenous route. They are usually administered preferably by oral or by subcutaneous route. The usual side-effects include, salivation, vomiting and severe gastrointestinal cramps.

### Contraindications:

These synthetic derivatives of acetylcholine are contraindicated in patients suffering from peptic ulcer, bronchial asthma, hypotension, presence of organic urinary tract or gastrointestinal obstruction.

## 6.6 ANTICHOLINESTERASE

The cholinesterase enzyme terminates the biological activity of acetylcholine by hydrolyzing acetylcholine into acetic acid and a choline molecule, thus limiting the turnover time of acetylcholine to 150 microseconds. The hydrolysis of acetylcholine occurs through deacetylation reaction which is catalysed by cholinesterase enzyme.

The cholinesterases present in the human body can be broadly categorised into,

- (a) Acetylcholinesterase or e-cholines-terase or true cholinesterase or specific cholinesterase and
- (b) Butyrylcholinesterase or s-cholines-terase or pseudo cholinesterase or non-specific cholinesterase.

The specific or acetylcholinesterase is found in R.B.C., in the brain and other nerve tissues. It is present in high concentration on presynaptic sites, post-synaptic membrane sites and at motor nerve end plate regions of cholinergic nervous system. At presynaptic sites, its role is to regulate the acetylcholine levels in cholinergic nerve terminals. It is also located in autonomic ganglia and certain cholinergic synapses in the CNS. The non-specific or butyrylcholinesterase is present in plasma, glial cells, intestine and other organs. The cholinesterases present in different species or organs sometimes bear basic differences and need not be identical.

These enzymes are mainly located in the outer basement membrane of the synapses and in the neuromuscular junctional cleft. They are also reported to be present in the cisternae of the endoplasmic reticulum.

Sometimes cholinesterase enzymes have been located in such regions where they can not claim the role of 'acetylcholine-killer'. In such cases, they are supposed to be tied up with some independent activities like,

- (a) to control the membrane permeability and
- (b) to control the blood level of fatty substances.

Cholinesterase inhibitors, as the name indicates, increase the concentration of the acetylcholine at the receptor sites by inhibiting its metabolism by cholinesterases, resulting into prolongation and potentiation of acetylcholine activity at both, muscarinic and nicotinic receptors. They do so mainly through competitive antagonism and hence often resemble with acetylcholine in structure.

The unhydrolysed acetylcholine accumulates and exerts its actions. Hence, cholinesterase inhibitors are also termed as indirectly acting cholinomimetic agents.

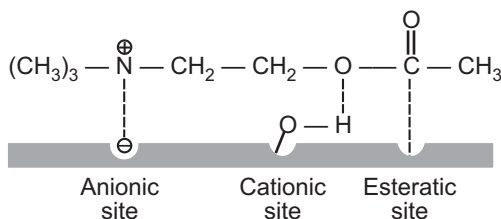
The activation of muscarinic receptors results into various muscarinic effects which include miosis, contractions of smooth muscles, diarrhoea, vasodilation, bradycardia, nausea, vomiting, salivation, perspiration, lacrimation etc. All these effects can be blocked by administration of muscarine blocker like, atropine.

The activation of nicotinic receptors by high levels of accumulated acetylcholine results into generalised muscle twitching followed by the muscle weakness and ganglionic stimulation. The activation of nicotinic receptors present in the autonomic ganglia and adrenal medulla leads to the release of catecholamines which may further alter the cardiovascular function.

Some anticholinesterases have an independent direct cholinomimetic action of their own while some may cause neuromuscular blockade. The toxic effects of some drugs may be due to their *in-vivo* metabolism to toxic metabolites.

## 6.7 STRUCTURAL FEATURES OF CHOLINESTERASE ENZYME

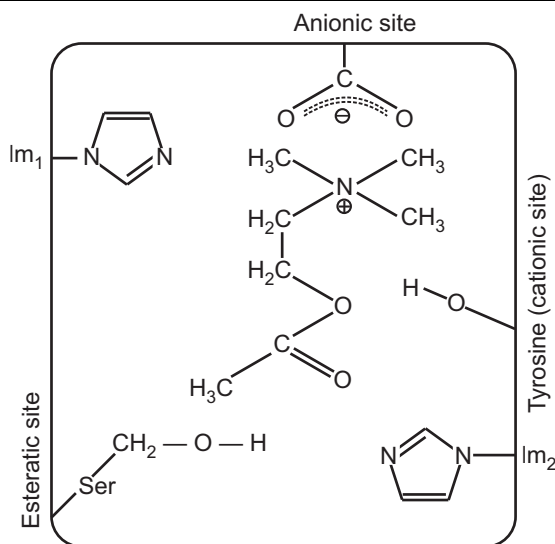
Cholinesterase constitutes an example of one of the most effective enzyme systems present in the body. It is a tetramer having a molecular weight of about 3,20,000. The cholinesterase molecule consists of three important sites namely, anionic, cationic and esteratic site. The anionic site is formed by an ionised gamma-carboxylate group of a glutamic acid residue and is stereospecific. The cationic site possesses hydroxyl group probably that of tyrosine residue. While the esteratic site consists of two imidazole groups ( $\text{Im}_1$  and  $\text{Im}_2$ ) from histidine moieties and a serine residue.



**Fig. 6.10: Possible interactions between acetylcholine and cholinesterase enzyme**

The process of hydrolysis of acetylcholine, thus occurs in the following steps:

(i) The imidazole group  $\text{Im}_2$ , of histidine, accepts a proton from a serine hydroxyl group at the esteratic site, creating a strong nucleophile while  $\text{OH}^-$  from tyrosine just serves as binding site to ether oxygen of the acetoxy group of acetylcholine.



**Fig. 6.11: Cholinesterase molecule and acetylcholine**

(ii) The anionic site of the enzyme binds with the quaternary nitrogen of the acetylcholine through both ionic and hydrophobic forces. The latter binding force is provided by the presence of three methyl groups which are present on the nitrogen. The

activated serine, being a strong nucleophile, then attacks on the C-atom of carbonyl group of acetylcholine resulting into a tetrahedral intermediate. This intermediate is very short-lived and its collapse results into the release of choline molecule, leaving the acetylated serine residue on the enzyme.

(iii) The choline molecule readily dissociate from the anionic site, since it is bound only by van der Waal's forces and hydrophobic forces. The acetyl group, however, forms a covalent bond with the nucleophilic group (activated serine residue) of the enzyme. The acetylated enzyme then undergoes a conformational change which brings the acetylated serine in close proximity to the second imidazole ( $\text{Im}_1$ ) residue. In presence of a water molecule, the second imidazole residue catalyzes hydrolysis of acetylated serine to give acetic acid and serine residue. This step is rate limiting step which occurs at a very rapid rate and the enzyme is thereby efficiently regenerated back.

The cholinesterase enzyme from a purified sample of OX red blood cell is found to hydrolyse  $3 \times 10^5$  molecules of acetylcholine per minute.

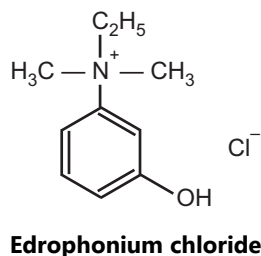
## 6.8 CLASSIFICATION OF ANTICHOLINE-STERASES

The anticholinesterases are classified into:

- I. reversible anticholinesterases
- II. irreversible anticholinesterases.

### (I) Reversible Anticholinesterases:

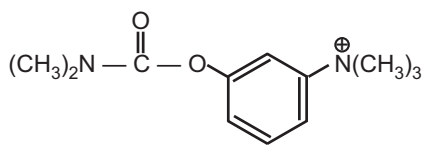
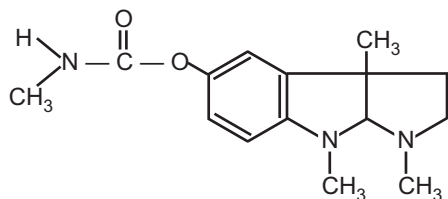
They bear a structural resemblance to acetylcholine, hence capable of combining with the anionic and esteratic sites of cholinesterases and receptors as well. They have a great affinity for active sites but no intrinsic activity. This produces the temporary inhibition of the enzyme. In contrast to other reversible cholinesterases, edrophonium forms reversible complex only with the anionic site and hence has a shorter duration of action.



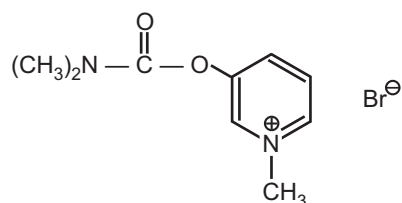
These can further be divided into:

(a) **Naturally occurring:** e.g. physostigmine

(b) **Synthetic:** e.g. Neostigmine, pyridostigmine, ambenonium, miotine, demacarium, edrophonium and benzpyrinium.



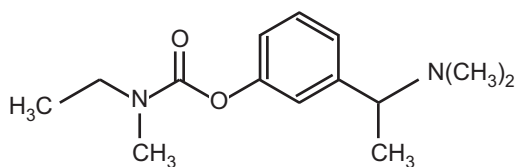
In neostigmine, increased stability to hydrolysis is achieved by using a dimethyl carbamate in place of methyl carbamate group. Because of charged nitrogen, neostigmine can not cross the blood-brain barrier and cause CNS side-effects.



**Pyridostigmine bromide**

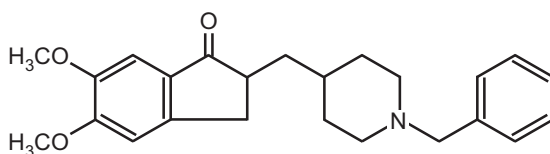
Patients with Alzheimer's disease present with progressive impairment of memory and cognitive functions such as a lack of attention, disturbed language function and an inability to complete common tasks. Although the exact defect in the central nervous system has not been elucidated, evidence suggests that a reduction in cholinergic nerve function is largely responsible for the symptoms.

**Rivastigmine (1997):** It is a cholinesterase inhibitor used for the treatment of mild to moderate dementia associated with Parkinson's disease.



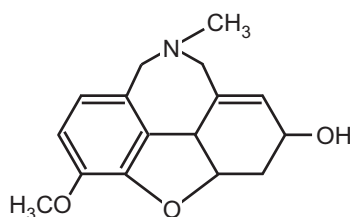
**Rivastigmine**

**Donepezil:** It is centrally acting reversible cholinesterase inhibitor used in the palliative treatment of mild to moderate Alzheimer's disease.



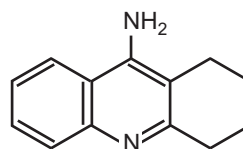
**Donepezil**

**Galantamine:** It is an alkaloid obtained synthetically or from the bulbs and flowers of *Galanthus caucasicus*. It is a reversible cholinesterase inhibitor and also is nicotinic receptor modulator. It is used in the treatment of mild to moderate vascular dementia and Alzheimer disease.

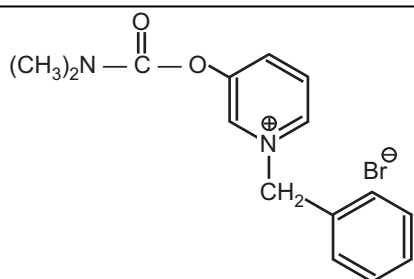
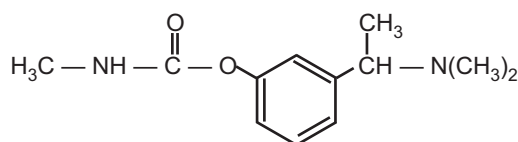
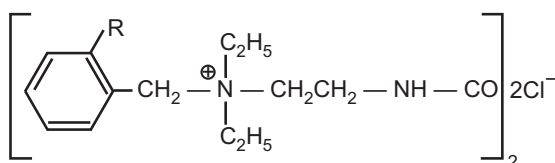


**Galantamine**

**Tacrine:** It is the first centrally acting reversible cholinesterase inhibitor approved for the treatment of Alzheimer's disease.



**Tacrine**

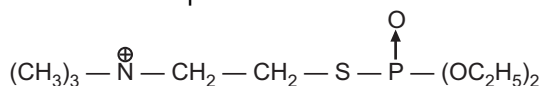
**Benzpyrinium bromide****Miotine****R = - Cl; Ambenonium chloride****R = - OCH<sub>3</sub>; Methoxyambenonium chloride****Structure-Activity Relationship:**

(i) The distance across the ether oxygen and nitrogen atom is approximately same as that between the ether oxygen and nitrogen atom in acetylcholine.

(ii) The two heterocyclic rings of physostigmine are not essential for anticholinesterase activity. During hydrolysis, the phenolic fragment of this drug is eliminated, leaving the carbamoyl group attached to the enzyme. The rate of hydrolysis of carbamoyl group is about 60 times less than the rate of hydrolysis of acyl group of acetylcholine.

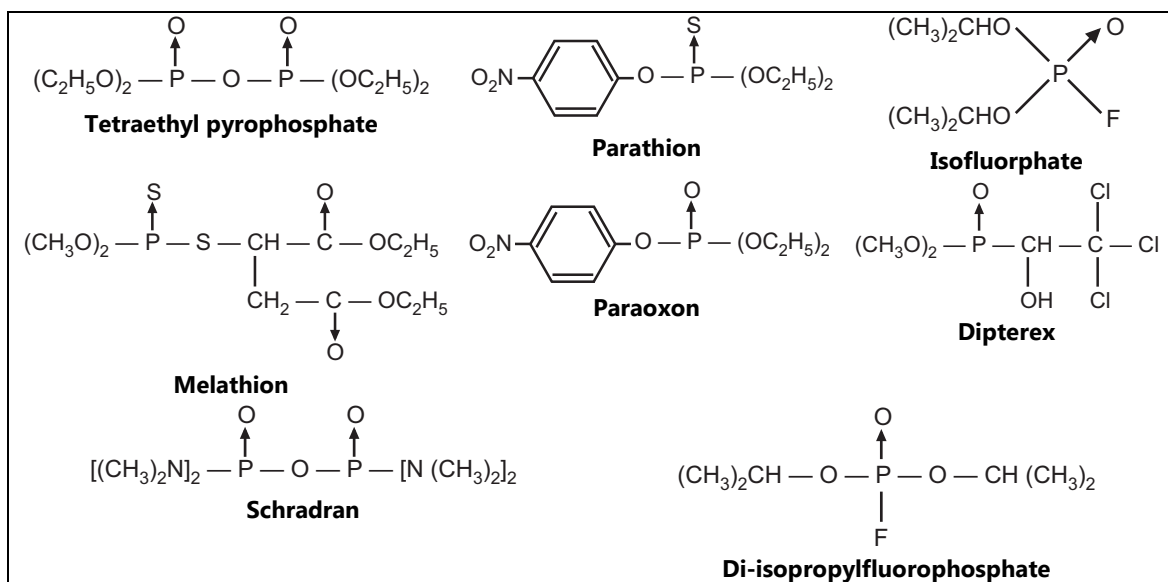
**(II) Irreversible Anticholinesterases:**

Organophosphorus compounds combine only with esteratic site of cholinesterases and the esteratic site is phosphorylated. The hydrolysis of this phosphorylated site, however, is extremely slow which produces a long term inhibition of cholinesterases. In contrast to other organophosphorus compounds, echothiophate forms complex with both anionic and esteratic sites and hence is much more potent.

**Echothiophate**

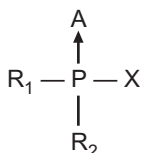
A number of phosphate, pyrophosphate and phosphonate esters apparently react irreversibly with cholinesterase by forming phosphate ester with the esteratic site. Because the rate of hydrolysis of the phosphorylated enzyme is measured in hours, these compounds have long duration of action. These compounds esterify the serine residue in the cholinesterase enzyme. The hydrolysis rate of the phosphorylated serine is extremely slow and hydrolysis to the free enzyme and phosphoric acid derivative is so limited that the inhibition is considered irreversible.

### Organophosphorus compounds : Irreversible anticholinesterases



### Structure-Activity Relationship:

A general formula for these compounds is as follows:



where,  $\text{R}_1$  = alkoxyl

$\text{R}_2$  = alkoxyl, alkyl or tertiary amine.

$\text{X}$  = A good leaving group,

e.g. F, CN, thiomalate, p-nitrophenoxy.

- (i) A is usually oxygen or sulphur, but may also be selenium. When A is other than oxygen, biological activation is required before compound becomes effective.
- (ii) X is good leaving group when the molecule reacts with the enzyme.
- (iii) The R moiety imparts lipophilicity to the molecule and contributes its absorption through skin.
- (iv) In alkoxy series, compounds which contain fluorine are more active than those containing iodine or other radical.

These organophosphorus compounds are nerve poisons and used as:

- (1) Nerve gases in warfare.
- (2) As agricultural insecticides in which the death of insects can be attributed to the inactivation of their acetylcholinesterases.
- (3) In the treatment of glaucoma.

These compounds are very toxic to humans and must be handled with extreme caution. Toxic symptoms are nausea, vomiting, excessive sweating, salivation, miosis, bradycardia, low blood pressure and respiratory difficulty that is usually the cause of death.

## 6.9 THERAPEUTIC USES

The reversible cholinesterase inhibitors find their clinical uses mainly due to their the following basic properties:

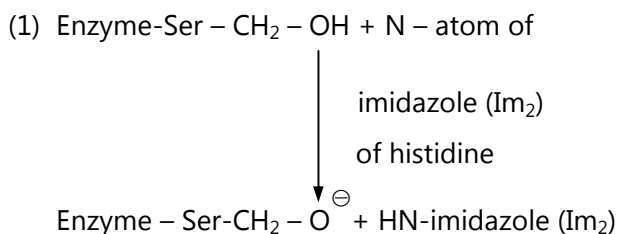
- (1) They activate muscarinic receptors by accumulating acetylcholine at the receptor sites.
- (2) Activation of nicotinic receptor sites leads to stimulation, followed by depression or paralysis of skeletal muscles.
- (3) Activation of muscarinic receptors in the CNS leads to stimulation followed by depression of the centrally governed cholinergic effects.

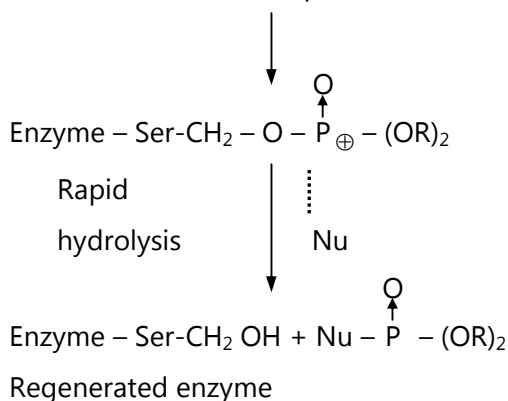
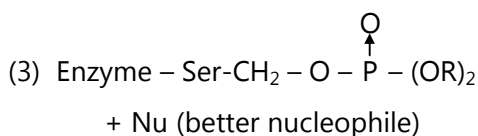
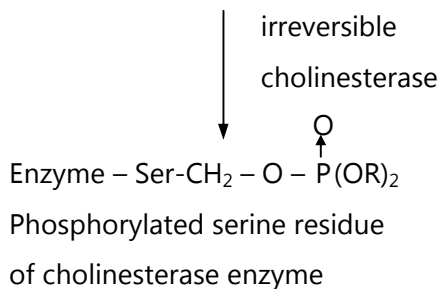
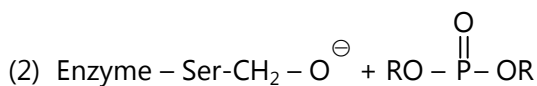
The cholinesterase inhibitors are recommended under the following condition,

- (i) in glaucoma, in which high intraocular pressure can lead to permanent damage to the optic disk, resulting in blindness,
- (ii) in myasthenia gravis: They increase the acetylcholine concentration and excitation of the neuromuscular junction. Disease is characterised by muscular weakness and abnormal fatigue that patients can not even keep their eyes open. These drugs increase the strength and endurance, and
- (iii) as curare antidotes because the increased acetylcholine levels displace the blocker more readily.

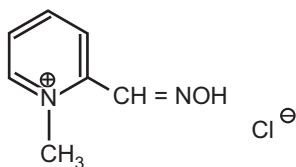
## 6.10 ANTIDOTES FOR NERVE GASES

When an irreversible cholinesterase is used, it combines with cholinesterase and the OH group of the serine gets phosphorylated. It was usually considered that water in body fluids attacks the phosphorylated serine residue and causes its hydrolysis. But the rate of hydrolysis is very slow and that a more effective agent was required for rapid hydrolysis i.e. it involves the administration of a better nucleophile than water to attack the phosphorus atom and thereby eliminate or liberate the enzyme back to its original form, as shown below:

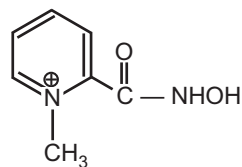




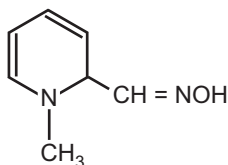
The effective antidotes for irreversible anticholinesterases developed are as follows:



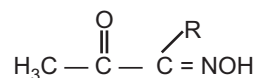
**2-pyridine aldoxime methchloride (Pralidoxime chloride)**



**2-Pyridine hydroxamic acid methiodide**



**Dihydro 2-Pyridine aldoxime methiodide**



**R = -CH<sub>3</sub>; Diacetylmonoxime**

**R = -H; Pyruvaldoxime**

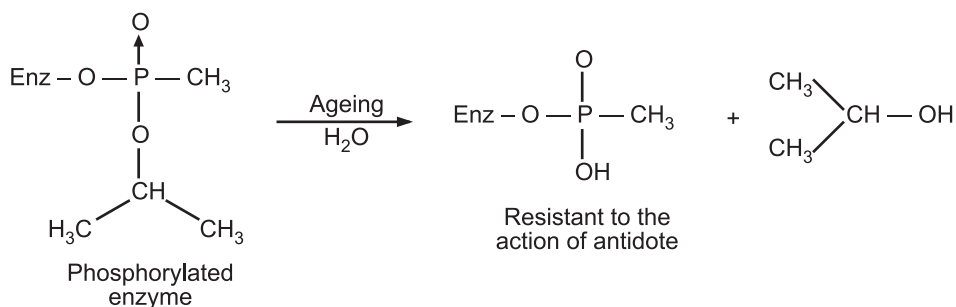
Hydroxamic acid or aldoxamine part of the structure is known for zinc (metalloenzymes) binding properties.

To potentiate the action of 2-PAM, atropine is also used along with it. Atropine acts as competitive antagonist for the accumulated acetylcholine at muscarinic sites.

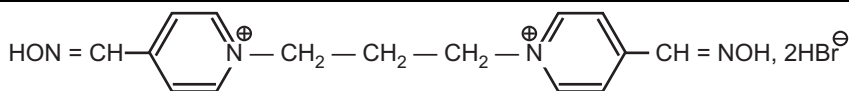
The effectiveness of the oximes as antidotes is not entirely attributed to their reactivating action on cholinesterases. They may also alter the distribution of the inhibitor, diverting it to the liver as well as exerting a curare like action sufficient to effect a partial relief to the neuromuscular blockade.

### Limitations of Oximes as Antidotes:

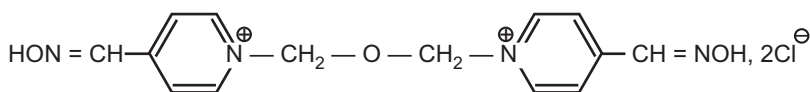
- (i) These reactivators (antidotes) are only effective if they are given immediately before or soon after the exposure to the inhibitor. The phosphorylated enzyme undergoes a fairly rapid process, called as 'ageing' (which probably involves the loss of an alkyl part of alkoxy group) as a result of which it becomes resistant to the action of the antidote.



- (ii) The reactivators are not very successful in restoring cholinesterase activity in CNS.
- (iii) They are not effective against all organophosphorus compounds, the toxicity of few of which is actually increased by the oximes.



**1, 3 bis (pyridinium - 4 - aldoxime) propane dibromide**



**Obidoxime chloride (Toxogenin)**

**Parasympatholytics or Antispasmodics or Cholinergic Blocking Agents:**

They are anticholinergic drugs that inhibit the effects of acetylcholine released from postganglionic parasympathetic nerve endings. They block muscarinic actions of acetylcholine including smooth muscle contractions and exocrine gland secretion. Atropine is the prototype of this class.

Two obvious approaches are there, to treat the conditions characterised by overstimulation of cholinergic nerves.

- (i) Use of agents that inhibit the synthesis or release of acetylcholine.
- (ii) Use of agents that block the acetylcholine from reacting with the receptors.

Compounds that inhibit acetylcholine synthesis have been discovered but have not proved to be clinically useful while drugs that block the interaction of acetylcholine with the receptor, however, are widely employed in medicine. These drugs are of three types:

- (a) Those who block the transmission at parasympathetic post-ganglionic nerve terminals e.g. Atropine.
- (b) Those who block transmission, at sympathetic and parasympathetic ganglia e.g. hexamethonium.
- (c) Those who block neuromuscular junctions in skeletal muscles e.g. d-tubocurarine. Such compounds should have the opposite effects of cholinergic agonists and their administration should be characterised by decreased secretion of saliva and gastric juices, decreased mobility of GIT and urinary tract (antispasmodic) and dilation of pupil.

Because of their ability to relax smooth muscle, they are referred to as antispasmodic. Such compound should have an affinity for cholinergic receptor but should lack intrinsic activity.

The interaction of atropine with muscarinic receptors was found to be loose and reversible.

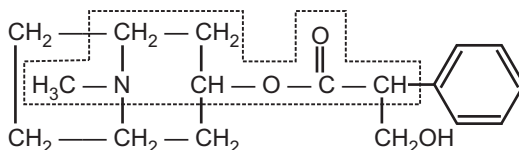
**Classification:**

Chemically, antispasmodic drugs are classified as:

1. Atropine and its synthetic analogues
2. Synthetic aminoalcohol esters
3. Aminoalcohol ethers
4. Aminoalcohols
5. Aminoamides
6. Papaverine and its synthetic analogues
7. Miscellaneous agents.

**Structure-Activity Relationship:**

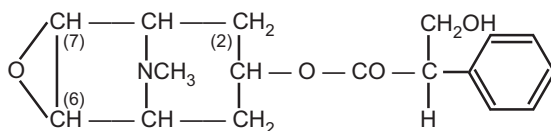
**(A)** Anticholinergic compounds may be considered as chemicals that have some structural similarity to acetylcholine but contain additional substituents which enhance their binding to the cholinergic receptors.



**Fig. 6.12: Atropine**

The circled portion of atropine molecule reveals the segment resembling with acetylcholine.

*Atropa belladonna* was named by Linnaeus in 1753, after Atropos, the eldest of the three Fates of Greek mythology and the one whose duty was to cut the thread of life. "Belladonna" does refer to an Italian name ("handsome woman") for the plant, which was used by Venetian ladies to give them "sparkling eyes". The greater molar potency of atropine helps it to block several moles of acetylcholine. The umbrella-like atropine molecule may mechanically or electrostatically inactivate adjacent receptors on the cell surface so that these receptors are also unavailable for acetylcholine or other parasympathomimetic stimulants. Atropine and scopolamine are esters of tropic acid with the complex organic bases *tropanol* (*tropane*) and *scopine*, respectively. Scopine differs from tropanol only by the oxygen bridge between C-6 and C-7. The alkaloid atropine was first isolated by Mein and also by Geiger and Hesse in 1832. Ladenburg, who, in 1880, produced the semisynthetic derivative homatropine, which is still widely used.

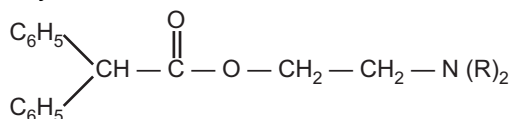


**Scopolamine (Hyoscine)**

The action of scopolamine differs from that of atropine in one important respect: when given by injection, in ordinary doses, scopolamine, in addition to its cholinergic blocking effect, exerts a powerful sedative or hypnotic action.

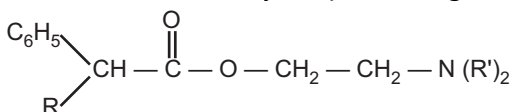
To reduce CNS side-effects, quaternary salts of atropine like, ipratropium (bronchodilator) and atropine methonitrate (to lower GI tract motility) are used clinically.

**(B)** Since acetylcholine and atropine both are acetic acid ester of aminoalcohol, many substituted acetic acid esters of amino alcohols were prepared and evaluated for biological activity. Such esters of phenylacetic acid had little activity. Similar esters of diphenyl acetic acid are found therapeutically useful.



R = alkyl group

Therefore, the minimum structure necessary for pure antagonistic activity is:



where

R = hydroxyalkyl, alkyl, cycloalkyl or heterocyclic

R' = alkyl

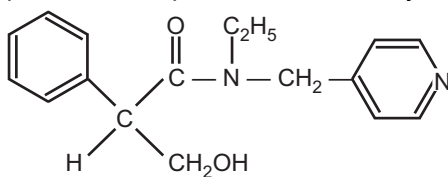
(i) In the above general formula, the antagonist may contain larger groups than methyl on the nitrogen atom. In general, these groups should not be larger than butyl, if the compound is to be an effective antagonist.

(ii) The nitrogen atom in an antagonist need not be always quaternised. Since the pH of the receptor is acidic, this amino group gets protonated and carries a positive charge that interacts with the anionic site of the receptor.

(iii) The acyl group in an antagonist, is always larger than the acyl group in acetylcholine. The larger acyl group ensures that the compound is not a partial agonist.

**(C)** The acetylcholine molecule does not cover all the area of receptor. The area of a receptor, which is not covered by a acetylcholine molecule appears to be chiefly hydrophobic in nature. Hence, hydrophobic substituents increase the affinity of the antagonist by binding to this area. However, this area is not uniform in its hydrophobic nature. The fact that esters of triphenyl acetic acid have low potency can be justified only if the hydrophobic area does not accommodate binding by a third phenyl ring.

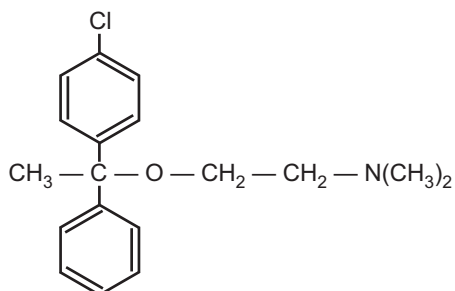
**(D)** The high potency of esters and amides of tropic acid result from their ability to H-bond with a suitable group on the receptor, surrounded by the hydrophobic area.



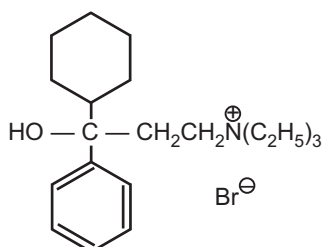
**Tropicamide**

It is used to produce mydriasis and cycloplegia.

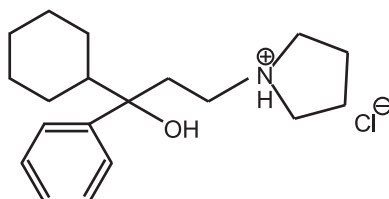
(E) Since, a number of alcohols, esters and ethers resembling choline are less potent than acetylcholine, but still demonstrate appreciable agonist properties, it might be expected that the addition of two large groups to these molecules would produce cholinergic blocking agents. The reasoning was correct and yielded therapeutically useful compounds.



**Chlorphenoxamine**

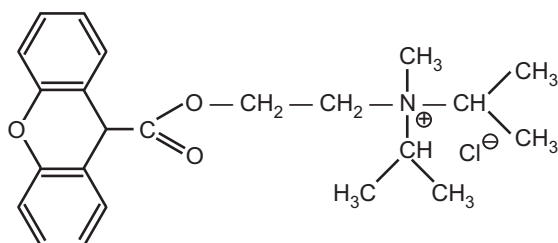


**Tridihexethyl bromide**

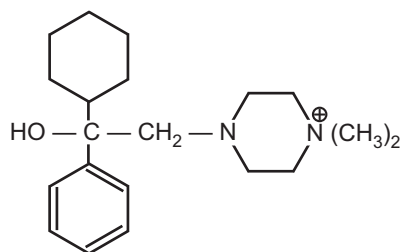


**Procyclidine hydrochloride**

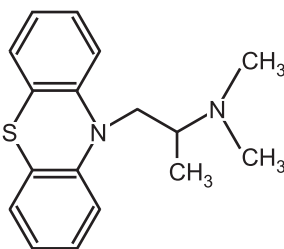
(To treat Parkinsonism and akathisia)



**Propantheline chloride**



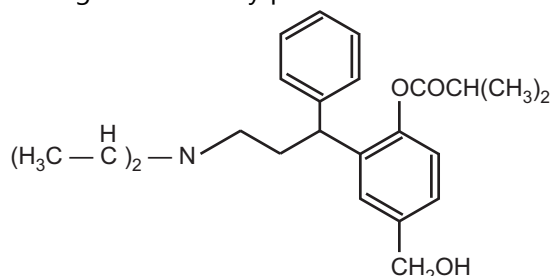
**Hexocyclium**



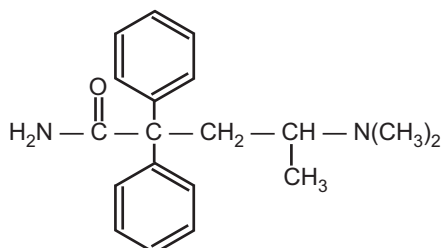
**Ethopropazine HCl (Profenazine HCl)**

**Phenothiazine derivative used to treat Parkinsonism**

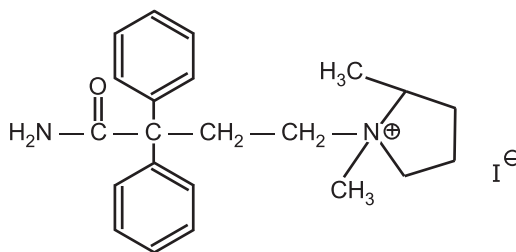
**Fesoterodine:** It is an antimuscarinic agent used to treat over active bowel. It is a prodrug converted by plasma esterases to active metabolite, 5-hydroxymethyl toterodine.



Fesoterodine



Aminopentamide



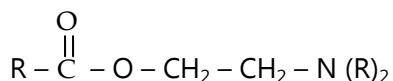
Isopropamide iodide

This is an indication for the need of at least one portion of the molecule to have the space occupying, umbrella like shape which leads to firm binding at the receptor.

**(F)** Size alone, is not the sole criteria for potent blocking agents. The special arrangement or the stereochemical features of the molecule are also important presumably because of a good fit of its prosthetic group with the receptor site.

### Mode of Action:

The main difference in cholinergic and anticholinergic agents appears to be the size of the acyl group.



In cholinergic compounds R = small group.

In anticholinergic compound R = large group.

The large (alkyl or aryl) group may not only increase the affinity of the blocking agent but through an 'Umbrella effect' may also block the approach of acetylcholine to the receptor.

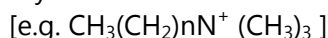
**Adverse Effects:** Adverse effect of the antispasmodic drugs are dose dependent and include dry mouth and skin, flushing, tachycardia, pupillary dilatation with blurred vision, cerebral excitement and delirium. The quaternary ammonium compounds may also cause postural hypotension and impotence because of their ganglionic blocking effects.

**Uses:** The anticholinergic drugs have been widely used in the treatment of peptic ulcer disease and irritable bowel and functional disorders, including diarrhoea. The main contraindications to anticholinergic drug use are narrow angle glaucoma, pyloric outlet obstruction and reflux oesophagitis.

**Bellaue's Concept of Enzyme Perturbation:**

This concept views that the receptor alters its conformation to fit the acetylcholine or its agonists. Since, it is bound to the membrane, it sufficiently changes membrane structure to alter the transport of ions through the membrane to generate muscle contraction.

The applicability of this concept is further enhanced by the fact that as the size increases in the series of compounds with cholinergic activity, there is not an abrupt change from cholinergic to anticholinergic activity.



when (1)  $n = 1$  to 4, potency increases

(2)  $n = 5$  to 7, partial agonists

(3)  $n = \text{more than } 7$ , compound is antagonist.

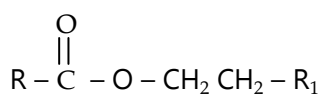
Bellaue's concept can be represented as shown in Table 6.1.

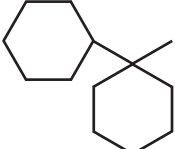
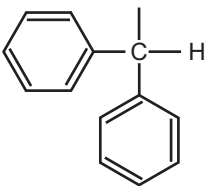
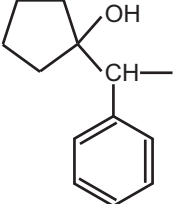
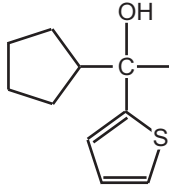
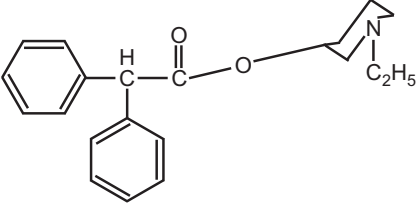
In this table, compound 1 (ACh) can exactly fit on the receptor to provide orientation favourable for agonist activity. Some molecules like compound 2 and 3, when in an unfavourable orientation [i.e. 2 (a) and 3 (a)] exhibit antagonistic activity, while remaining molecules can assume a conformation i.e. 2 (b) and 3 (b), that will fit on the receptor to give an orientation favourable for agonist activity. Therefore, the combined effect is being partial agonist. Molecule 4 is too large to fit the receptor in such a way as to provide a favourable conformation. It acts totally as an antagonist.

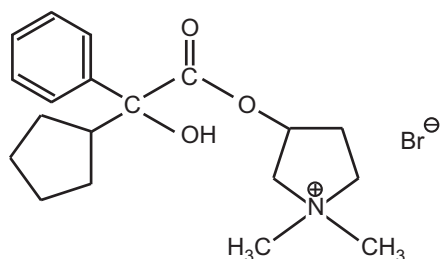
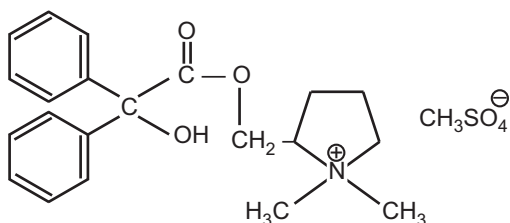
**Table 6.1: Belleau's concept of enzyme perturbation**

Favourable Orientation		Unfavourable orientation
1	ACh	$(\text{CH}_3)_3\text{N}^+ - \text{CH}_2 - \text{CH}_2 - \text{O} - \text{C}(=\text{O}) - \text{CH}_3$
2	(a) 5	$(\text{CH}_3)_3\text{N}^+ - \text{CH}_2 - \text{CH}_2 - \text{O} - \text{C}(=\text{O}) - \text{CH}_2 - \text{CH}_3$
	(b) 5	$(\text{CH}_3)_3\text{N}^+ - \text{CH}_2 - \text{CH}_2 - \text{O} - \text{C}(=\text{O}) - \text{CH}_2 - \text{CH}_3$
3	(a) 6	$(\text{CH}_3)_3\text{N}^+ - \text{CH}_2 - \text{CH}_2 - \text{O} - \text{C}(=\text{O}) - \text{CH}_2 - \text{CH}_2 - \text{CH}_3$
	(b) 6	$(\text{CH}_3)_3\text{N}^+ - \text{CH}_2 - \text{CH}_2 - \text{O} - \text{C}(=\text{O}) - \text{CH}_2 - \text{CH}_2 - \text{CH}_3$
4	9	$(\text{CH}_3)_3\text{N}^+ - \text{CH}_2 - \text{CH}_2 - \text{O} - \text{C}(=\text{O}) - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 - \text{CH}_3$

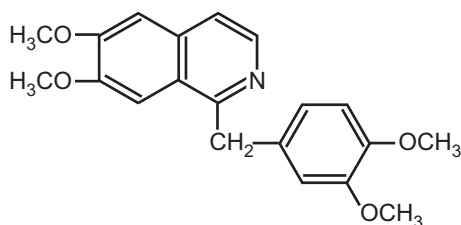
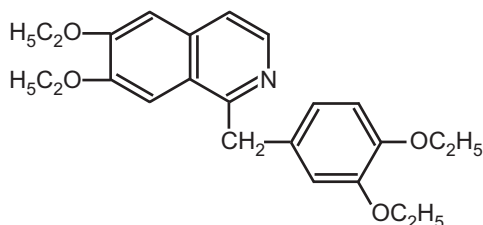
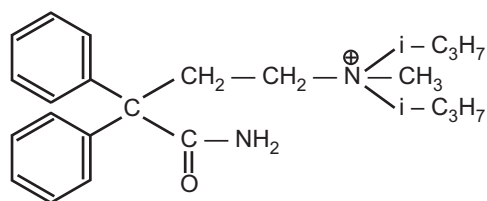
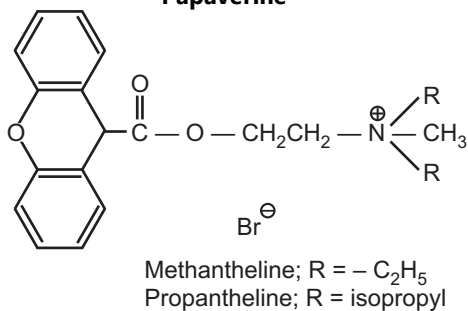
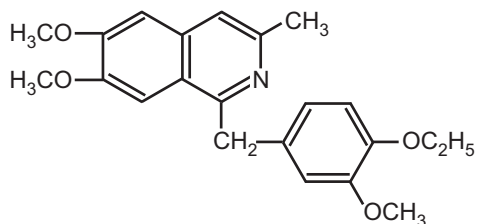
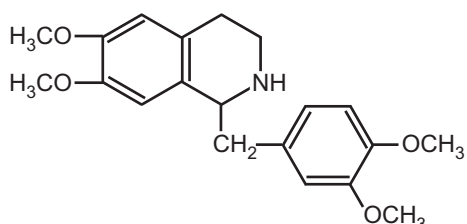
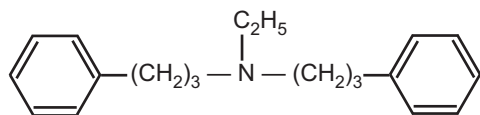
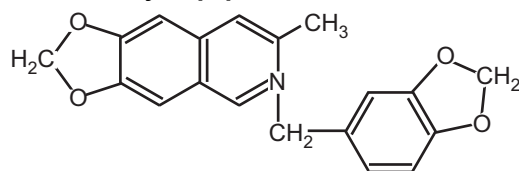
Table 6.2: Compounds with cholinergic blocking action

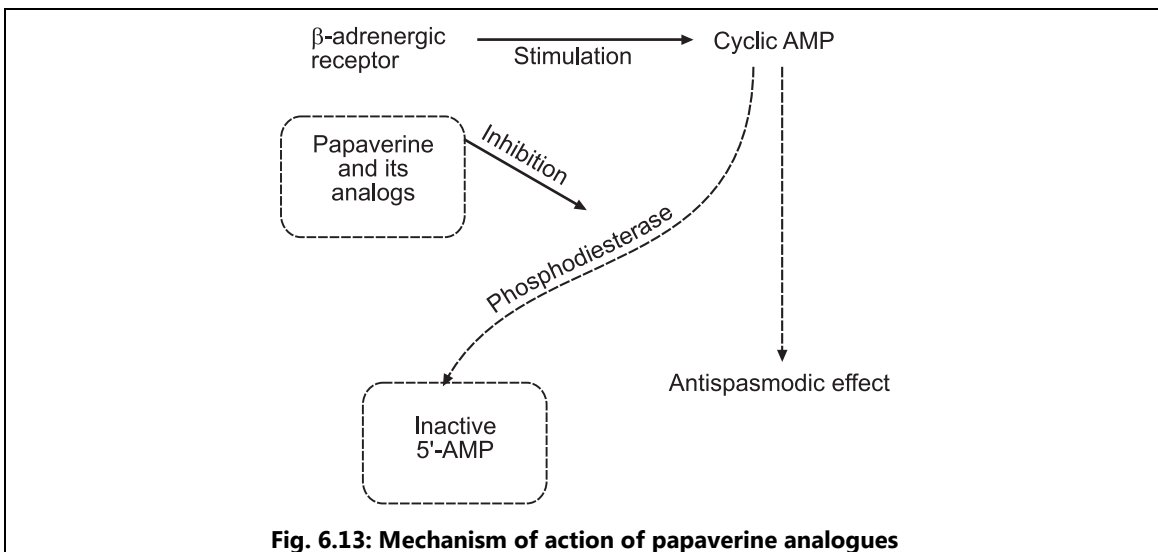


	R	R <sub>1</sub>	Names
(1)		$-\text{N}(\text{C}_2\text{H}_5)_2$	Dicyclomine
(2)		$-\text{N}(\text{C}_2\text{H}_5)_2$	Trasentine
(3)		$-\text{N}(\text{CH}_3)_2$	Cyclopentolate
(4)		$\begin{array}{c} \text{CH}_3 \\   \\ -\text{N} - (\text{C}_2\text{H}_5)_2 \\ \oplus \end{array}$	Penthienate
(5)			Piperidolate
(6)	$\begin{array}{c} \text{n-CH}_3\text{CH}_2\text{CH}_2\text{CH}_2 \\ \quad \quad \quad \diagdown \\ \quad \quad \quad \text{N} - \\ \quad \quad \quad \diagup \\ \text{n-CH}_3 - \text{CH}_2\text{CH}_2\text{CH}_2 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\   \\ -\text{N} - (\text{CH}_3)_2 \\ \oplus \end{array}$	Dibutoline

**Clinically Useful Antispasmodic Agents****Glycopyrrrolate****Poldine Methylsulphate****Papaverine and its Synthetic Analogues:**

This is a group of antispasmodic agents that do not act by interfering with cholinergic nerve transmission.

**Papaverine****Ethaverine****Isopropamide****Dioxyline****Tetrahydropapaverine****Alverine****Neupaverine**

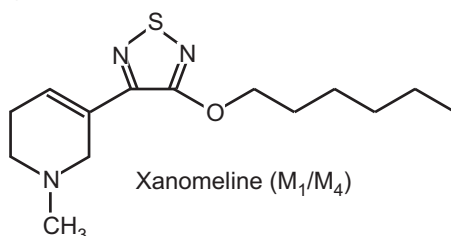


**Fig. 6.13: Mechanism of action of papaverine analogues**

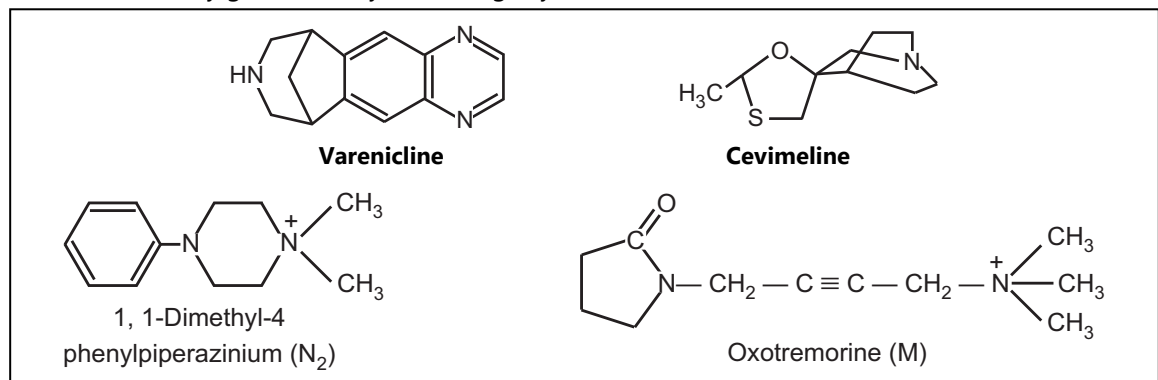
### Mechanism of Action:

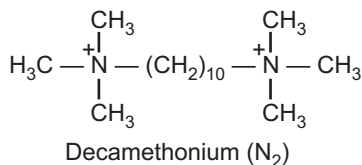
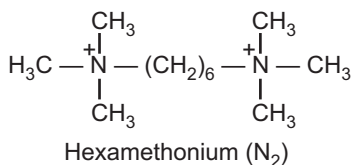
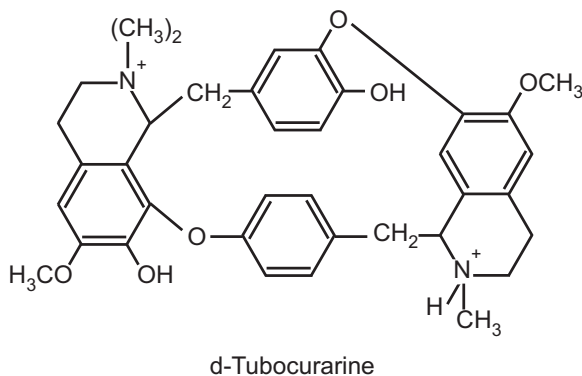
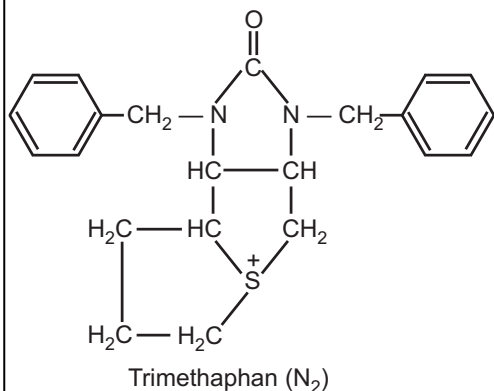
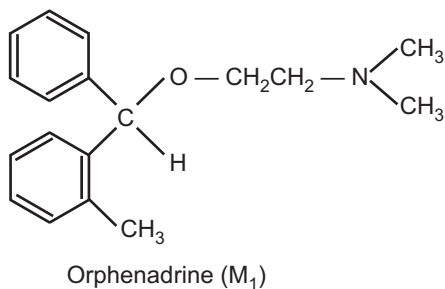
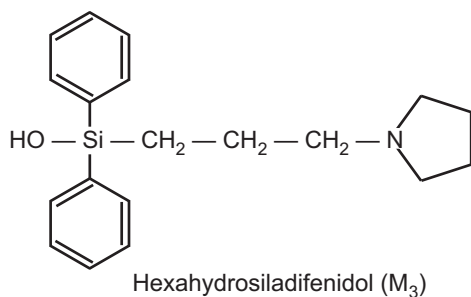
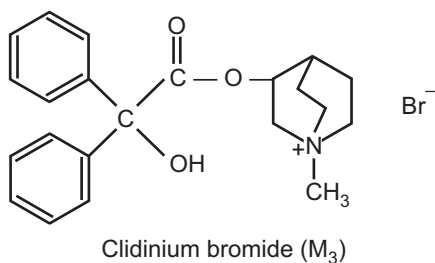
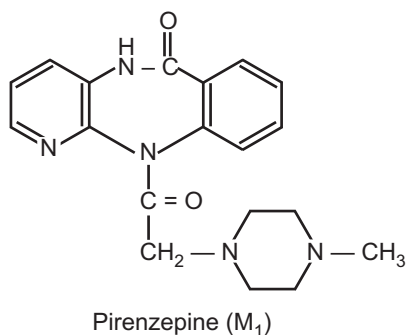
Since, cholinergic nerve stimulation increases peristaltic movements of GIT (spasmodic), adrenergic nerve stimulation will produce antispasmodic effect through the stimulation of β-adrenergic receptors. Cyclic-AMP (cyclic-3', 5'-adenosine monophosphate) is the active factor which is a product of the response of β-adrenergic receptors. Papaverine and its analogues are inhibitors of phosphodiesterase, an enzyme that destroys cyclic-AMP.

### Nicotinic and Muscarinic Agonists:



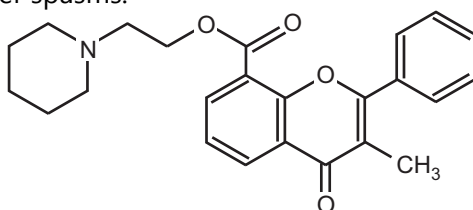
**Varenicline (2006):** It is nicotinic receptor partial agonist. It may be used to treat smoking addiction. **Cevimeline** is a parasympathomimetic and  $M_3$  – muscarinic agonist. It stimulates salivary gland, thereby alleviating dry mouth.



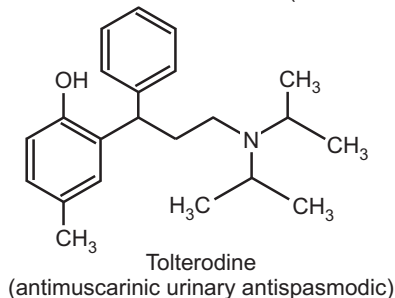
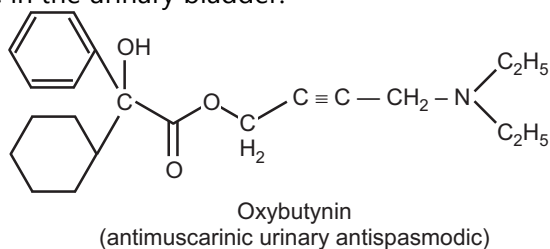
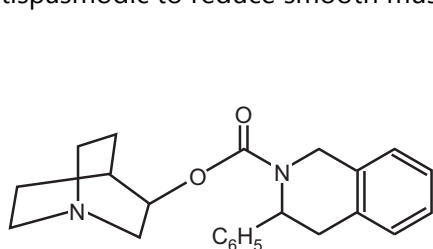
**Nicotinic antagonists:****Muscarinic antagonists:**

### 6.11 URINARY ANTISPASMODIC (M<sub>3</sub>-ANTAGONISITS)

**Flavoxate:** It has antimuscarinic activity in addition to direct muscle relaxant action. It is used to treat urinary bladder spasms.



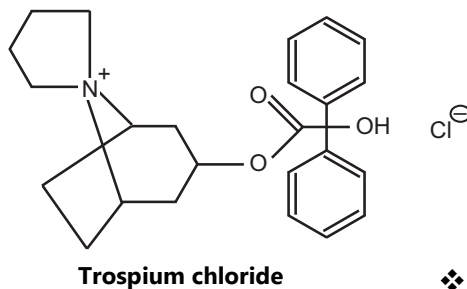
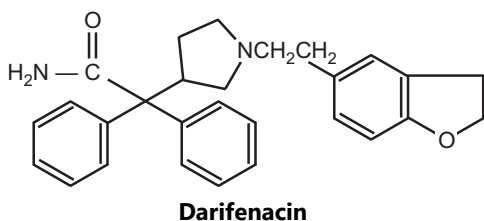
**Solifenacin:** It is a competitive muscarinic (M<sub>3</sub>) receptor antagonist. It is used as urinary antispasmodic to reduce smooth muscle tone in the urinary bladder.



Tolterodine is a selective M<sub>3</sub> antagonist and is considered to be the drug of choice for hyperactive bladder. This condition is characterized by frequent and involuntary urination. The drug is better tolerated than oxybutynin. **Fesoterodine** is a related drug with similar activity.

**Darifenacin:** It is M<sub>3</sub> – muscarinic receptor blocker, used to decrease the urgency to urinate in overactive bladder.

**Tropium chloride:** It is a urinary antispasmodic used for the treatment of overactive bladder.



## Chapter...7

# SEDATIVES AND HYPNOTICS

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### ♦ SYNOPSIS ♦

#### 7.1 INTRODUCTION

#### 7.2 CLASSIFICATION

##### 7.2.1 Barbiturates

##### 7.2.2 Benzodiazepines

##### 7.2.3 Acyclic Hypnotics Containing Nitrogen

##### 7.2.4 Cyclic hypnotics Containing Nitrogen

##### 7.2.5 Alcohols And Aldehydes

##### 7.2.6 Acetylene Derivatives

##### 7.2.7 Miscellaneous Agents

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## 7.1 INTRODUCTION

Ideally, a sedative hypnotic should induce sleep that is similar in sleep pattern to the natural sleep.

A sedative drug decreases activity and excitement of the patient and calms the anxiety by producing mild depression of CNS without causing drowsiness or sleep.

A hypnotic drug produces drowsiness, compelling the patient to sleep by depressing the CNS, particularly the reticular activity which characterises wakefulness.

Pharmacologically, sedative-hypnotic and general anaesthetic agents are usually regarded as agents causing only increasing depths of the CNS depression.

Since there is a lack of structural specificity in both these classes, these agents appear to be non-specific in their action.

The various sedative-hypnotic agents are employed as,

- (1) Antianxiety agents in the emotional strain and chronic tension state
- (2) Anticonvulsant agents
- (3) Muscle relaxants
- (4) General anaesthetics
- (5) Potentiation of analgesic drugs
- (6) Adjuvants to anaesthesia
- (7) In hypertension

The prolonged administration of these agents is not recommended because it may result into -

- (1) Alteration in the pattern of naturally occurring sleep.
- (2) Increasing tolerance and physical dependence to the drug.
- (3) Many hypnotics produce a hangover effect.
- (4) Since death can be caused by respiratory collapse if used in higher concentration, sedative-hypnotics are frequently used agents in attempting suicides.

## 7.2 CLASSIFICATION

Though, it is said that, the sedative-hypnotic drugs are not characterized by common structural features i.e. non-specific drugs, two important properties compel them to share certain common structural features.

(1) The presence of hydrophilic groups is necessary for their transport from gastrointestinal tract to aqueous body fluids while their penetration into CNS necessitates the drug to be sufficiently lipophilic. These objectives can be fulfilled by the non-ionic surfactant characteristics.

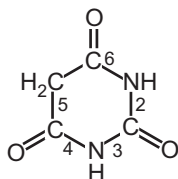
(2) They should possess such structural features which would normally resist their rapid metabolism. In these agents, generally polar groups like,  $-\text{CONHCONHCO}$  (barbiturates),  $-\text{CONH}_2$  (amides),  $-\text{OH}$  (alcohol),  $\text{OCONH}_2$  (carbamates), are attached to a non-polar moiety, usually alkyl, aryl or a haloalkyl groups.

Arbitrarily, the sedative-hypnotics may be classified as,

- (1) Barbiturates
- (2) Benzodiazepines
- (3) Acyclic hypnotic containing nitrogen
- (4) Cyclic nitrogen containing hypnotics e.g., piperidinediones and quinazolinones.
- (5) Alcohols and aldehydes
- (6) Acetylene derivatives
- (7) Miscellaneous agents
  - (a) Bromides
  - (b) Acids and esters
  - (c) Antihistamines and anticholinergics
  - (d) Sulfones
  - (e) Plant extracts
  - (f) Endogenous substances

## 7.2.1 Barbiturates

The first agent from this class, i.e. barbital (5, 5-diethylbarbituric acid) was introduced in 1903 by Fischer and Von Mebring followed by the introduction of phenobarbital in 1912. Since both these agents turned out to be powerful hypnotic drugs, over 2500 barbiturates were synthesised and evaluated, of which very few were proved of clinical utility. The parent compound in this series is Barbituric acid or 2, 4, 6 trioxohexahydropyrimidine which is devoid of CNS depressant activity but the presence of alkyl or aryl groups at position 5 confers sedative-hypnotic and sometimes other activities.



### Structure-Activity Relationship:

In 1951, Sandberg postulated that, a good hypnotic barbituric acid derivative must have -

(a) The acidity value within certain limits to give a proper ratio of ionised (dissociated) and unionised (undissociated) forms which is important to cross blood-brain-barrier.

It takes approximately 40-60% dissociation to enable a barbiturate to cross BBB and exerts an effect on CNS. A determination of the pKa can thus be predictive of CNS activity.

(b) A lipid-water solubility (partition coefficient) between certain limits.

### (a) Acidity:

On the basis of acidity values, barbiturates are divided into two classes.

### Hypnotic Class:

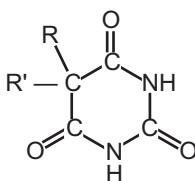
- (i) 5, 5-disubstituted barbituric acids.
- (ii) 5, 5-disubstituted thiobarbituric acids.
- (iii) 1, 5, 5-trisubstituted barbituric acids.

### Inactive Class:

- (i) 1-substituted barbituric acids.
- (ii) 5-substituted barbituric acids.
- (iii) 1, 3-disubstituted barbituric acids.
- (iv) 1, 5-disubstituted barbituric acids.
- (v) 1, 3, 5, 5-tetrasubstituted barbituric acids are inactive since they are not acidic. They upon metabolism, produce 1, 5, 5-trisubstituted barbituric acids, which are acidic.

**(b) Lipid-Water Solubility:**

Once the acidity value criteria is satisfied, the lipid-water solubility or partition coefficient is calculated to find out whether the compound is active or not. The following structural skeleton is essential for hypnotic activity.

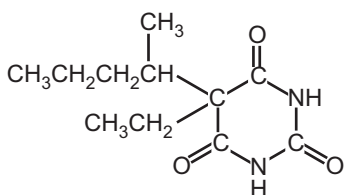


- (1) The sum of the carbon atoms of both substituents at carbon 5 should be between 6 and 10 in order to attain optimal hypnotic activity. This sum is also an index of duration of action.
- (2) Within the same series, the branched chain isomer has greater lipid solubility and hypnotic activity but has shorter duration action.

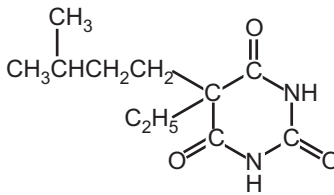
Sum value	Duration of action
(1) 7 – 9	Rapid onset and shortest duration
(2) 5 – 7	Intermediate duration of action
(3) 4	Slowest onset and longest duration (Two ethyl groups or an ethyl and a phenyl)

Branched, cyclic or unsaturated chains at 5 position generally reduce the duration of action due to increased ease of metabolic conversion to a more polar, inactive metabolite.

The greater the branching, more potent will be the drug e.g. pentobarbital is more potent than amobarbital.



Pentobarbital



Amobarbital

- (3) However, the stereoisomers possess approximately same potencies.
- (4) Within the same series the unsaturation (i.e. allyl, alkenyl, cycloalkenyl analogues) may result in greater potency than the saturated analogues with the same number of carbon atoms.
- (5) Alicyclic or aromatic substituted analogues are more potent than analogues with aliphatic substituents with the same number of carbon atoms.
- (6) Introduction of a halogen atom into the 5-alkyl substituent increases potency.
- (7) Introduction of a polar substituent (OH, NH<sub>2</sub>, COOH, CO, RNH, SO<sub>3</sub>H) into the aromatic group at C-5 results in decreased lipid solubility and potency.
- (8) Alkylation at 1 or 3 position may result in compounds having shorter onset and duration of action since N-methyl group reduces acidity value.
- (9) Replacement of oxygen by sulphur at 2-carbon, shortens the onset and duration of action due to increased lipid solubility.
- (10) Introduction of more sulphur atoms (at C-4 and C-6) decreases the hypnotic activity.

### Classification:

Depending upon the duration of action barbiturates are divided into four classes like -

- (1) Long acting barbiturates (6 hours or more).
- (2) Intermediate acting (3-6 hours).
- (3) Short acting (less than 3 hours).
- (4) Ultra-short acting (I.V.).

### General Scheme for Synthesis of Barbiturate:

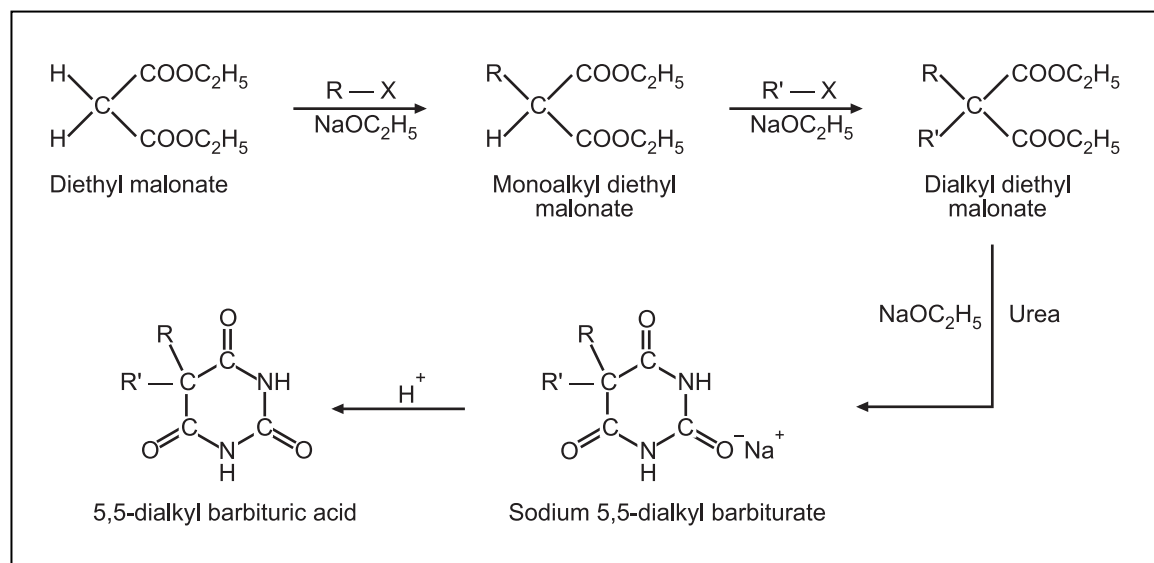
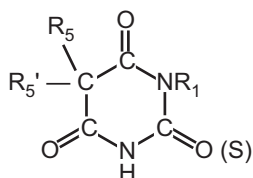


Table 7.2: Barbiturate Classification



Name	R <sub>1</sub>	Substituents R <sub>5</sub>	R <sub>5</sub> '	Sedative dose (mg)	Hypnotic dose (mg)
<b>1. Long acting Barbiturates:</b>					
Barbital	H	C <sub>2</sub> H <sub>5</sub>	– C <sub>2</sub> H <sub>5</sub>	–	300
Phenobarbital	H	C <sub>2</sub> H <sub>5</sub>	– C <sub>6</sub> H <sub>5</sub>	15 - 30	100
Mephobarbital	CH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	– C <sub>6</sub> H <sub>5</sub>	30 - 100	100
<b>2. Intermediate acting Barbiturates:</b>					
Amobarbital	H	C <sub>2</sub> H <sub>5</sub>	– CH <sub>2</sub> CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	20 - 40	100
Butobarbital	H	C <sub>2</sub> H <sub>5</sub>	– CH – CH <sub>2</sub> CH <sub>3</sub>   CH <sub>3</sub>	15 - 30	100
Probarbital	H	C <sub>2</sub> H <sub>5</sub>	– CH – (CH <sub>3</sub> ) <sub>2</sub>	50	150 - 400
<b>3. Short acting Barbiturates:</b>					
Cyclobarbital	H	C <sub>2</sub> H <sub>5</sub>		–	100 - 300
Heptabarbital	H	C <sub>2</sub> H <sub>5</sub>		100	200 - 400
Pentobarbital	H	C <sub>2</sub> H <sub>5</sub>	– CH – CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>   CH <sub>3</sub>	30	100
Secobarbital	H	– CH <sub>2</sub> CH = CH <sub>2</sub>	– CH – CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>   CH <sub>3</sub>	30	100
<b>4. Ultra-short acting Barbiturates:</b>					
Hexobarbitone	CH <sub>3</sub>	CH <sub>3</sub>		–	400 - 500
Thiopentone	H	C <sub>2</sub> H <sub>5</sub>	– CH – CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>   CH <sub>3</sub>	–	C <sub>2</sub> oxygen is replaced by sulphur

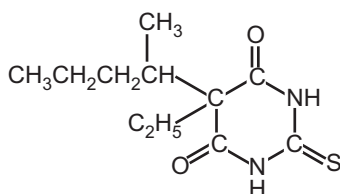
**Metabolism:**

A few barbiturates (which already possess enough polar groups) with low lipid water partition coefficients are largely excreted unchanged in urine. While barbiturates with non-polar structure (lipophilic character) are metabolised resulting into the introduction of more polar groups in the structure which may be excreted in the urine in free form or as conjugates of glucuronic acid.

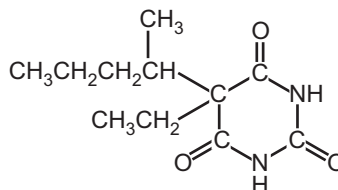
Liver remains as the principal site of metabolism. The metabolic changes that may occur with non-polar barbiturate include -

- (1) Oxidation of radicals present at C<sub>5</sub> to yield hydroxy, keto or carboxy derivatives.
- (2) Opening of the barbiturate ring by hydrolytic cleavage.
- (3) N-dealkylation (N-demethylation) in N-substituted barbiturates.
- (4) De-sulfurisation of 2-thiobarbiturates is a common metabolic process e.g. thiopental when metabolised, results into a pentobarbital molecule.

All these metabolic changes result in an increase in polar characteristics of the barbiturate molecule.



Thiopental



Pentobarbital

**Therapeutic Uses:**

Barbiturates, depending upon their potency and duration of action, may be clinically employed as -

- (1) Non-analgesic sedative - hypnotic drug.
- (2) Anticonvulsant agent.
- (3) General anaesthetic (basal anaesthetics).
- (4) In psychiatric treatments, as diagnostic and therapeutic aids.

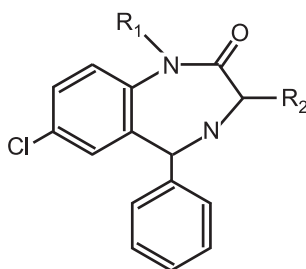
**7.2.2 Benzodiazepines**

An extensive research had been carried out on the benzodiazepoxide displayed psychotropic activity, in experimental animals. Over 2000 compounds belonging to this series have been synthesised and pharmacological screening of these compounds has been carried out in the search of better tranquilizer, muscle relaxant, anticonvulsant or a sedative hypnotic drug.

All benzodiazepines exhibit hypnotic action to more or less extent with varying degree of metabolism in liver. Hence only those benzodiazepines which are quickly metabolised and excreted, can be used as hypnotics in clinical practices.

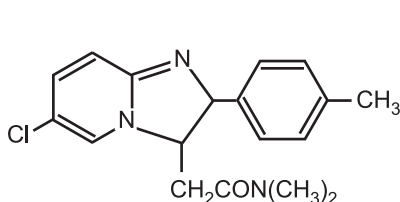
**Structure-Activity Relationship:**

- (1) All CNS - depressant benzodiazepines are usually substituted with a 5-aryl or 5-cyclohexenyl group.
- (2) The chemical nature of substituents at positions 1 to 3 does not affect the activity.
- (3) N(4) should usually be substituted with a group with low electron density.
- (4) Position 7, if substituted with electron withdrawing groups, results into an enhancement of activity, while substitution elsewhere in this aromatic ring results in decrease in activity. Same holds true, for  $R'_2$  substitution in phenyl ring on  $C_5$ . In addition to their antianxiety action, the benzodiazepines which are exclusively used as hypnotics are summarised in the Table 7.2.

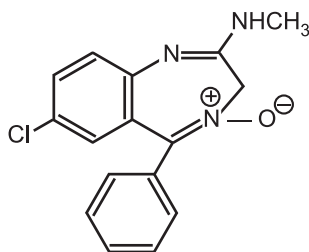


Diazepam : ( $R_1 = -CH_3$ ;  $R_2 = -H$ )

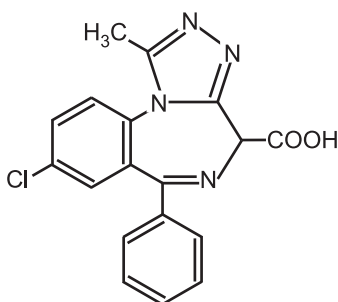
Oxazepam : ( $R_1 = -H$ ;  $R_2 = -OH$ )



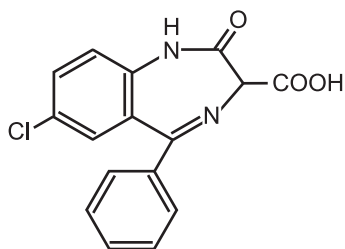
Zolpidem



Chlordiazepoxide

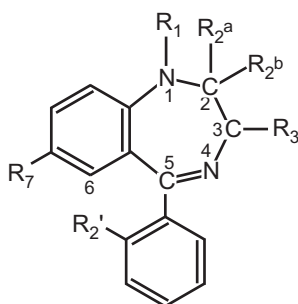


Alprazolam



Chlorazepate

Table 7.2: Clinically used benzodiazepines



5-aryl-1, 4-benzodiazepine

Name	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>7</sub>	R' <sub>2</sub>
Chlordesmethyl diazepam	H	= O	H	Cl	Cl
Fosazepam	$\begin{array}{c} \text{O} \\ \uparrow \\ (\text{CH}_2)_2 \text{P} (\text{CH}_3)_2 \end{array}$	= O	H	Cl	H
Nitrazepam	H	= O	H	NO <sub>2</sub>	H
Nordiazepam	H	= O	H	Cl	H
Nimetazepam	CH <sub>3</sub>	= O	H	NO <sub>2</sub>	H
Flunitrazepam	CH <sub>3</sub>	= O	H	NO <sub>2</sub>	F
Flurazepam	(CH <sub>2</sub> ) <sub>2</sub> N(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub>	= O	H	Cl	F
SAS 643	(CH <sub>2</sub> ) <sub>2</sub> OH	= O	OH	Cl	F
Quazepam	CH <sub>2</sub> CF <sub>3</sub>	= S	H	Cl	F
Lorazepam	H	= O	OH	Cl	Cl
Temazepam	CH <sub>3</sub>	= O	OH	Cl	H
Potassium chlorazepam	H	2 a OH 2 b OK	COOK	Cl	H
Estazolam	$\begin{array}{c} \text{R}_2(\text{a}) = \text{N} \diagdown \\ \quad \quad \quad \text{N} \\ \text{R}_1 - \text{C} = \text{N} \end{array}$	—	H	Cl	H
Triazolam	$\begin{array}{c} \text{R}_2(\text{a}) = \text{C} \diagdown \\ \quad \quad \quad \text{N} \\ \text{R}_1 - \text{C} = \text{N} \\ \quad \quad \quad \text{CH}_3 \end{array}$	—	H	Cl	Cl
Clonazepam	H	= O	H	NO <sub>2</sub>	Cl

**Table 7.3: Benzodiazepines versus Barbiturates**

<b>Benzodiazepines</b>	<b>Barbiturates</b>
1. Act on limbic system, thus reduce alertness and wakefulness.	1. Act by depressing the cortical response.
2. They do not cause true anaesthesia.	2. They can cause true anaesthesia.
3. Sleep induced by benzodiazepines resembles much with natural sleep.	3. Likely to produce hangover and psychomotor impairment.
4. In overdoses, less chances to cause unconsciousness and respiratory depression and hence relatively more safe.	4. Overdoses may result into a death.
5. They get slowly eliminated from the body.	5. They get eliminated more readily than benzodiazepines.
6. Long-term use is rarely indicated.	6. Long-term use is rarely indicated.

**Mode of Action of Benzodiazepines:**

(1) In central nervous system, benzodiazepines do not equally affect the activity at all levels and hence cannot be classified as general depressant of CNS.

(2) The midbrain reticular activating system, which is responsible for the maintenance of a wakefulness, is depressed by benzodiazepines.

(3) The limbic system incorporates a balanced complex of excitatory and inhibitory components. The antianxiety activity of benzodiazepines may be attributed to the depressant action of these drugs on the mechanisms which evoke anxiety and aggression.

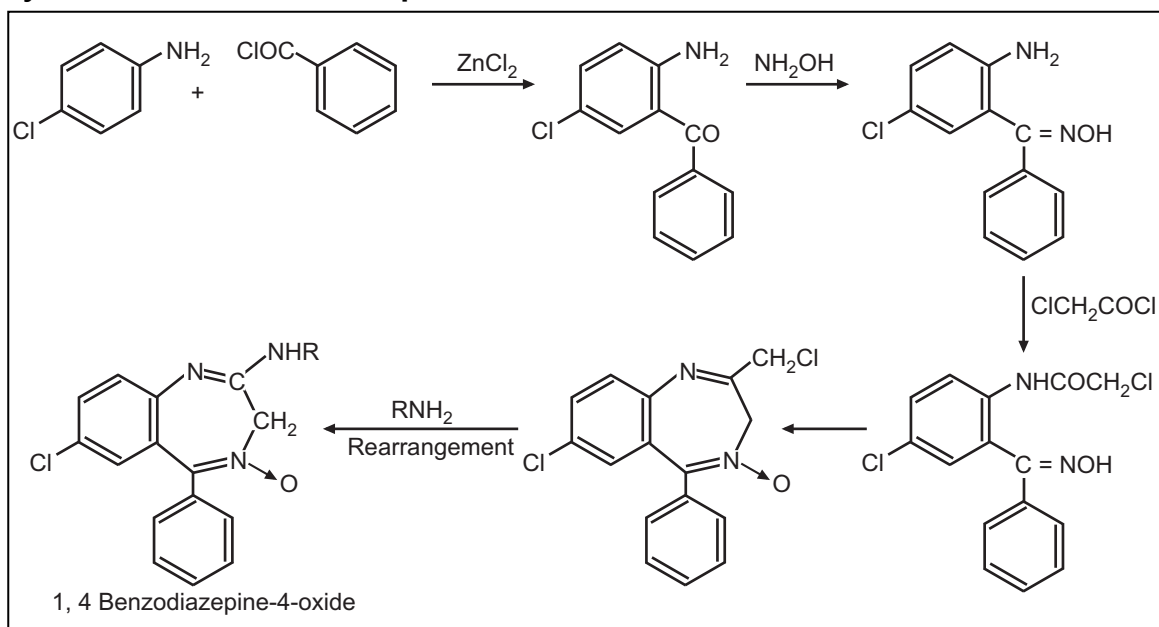
(4) In convulsions, these drugs neither elevate the threshold for convulsions nor suppress the repeated stimulation. They just prevent the spread of the seizures.

(5) Just like the action of gamma amino butyric acid, benzodiazepines cause either presynaptic or (rarely) postsynaptic inhibition in polysynaptic neuronal pathways in CNS, affecting the turnover of various neurotransmitters in the brain. This may result in the interference in the transmission processes. Since, benzodiazepines are found to act indirectly, they are not GABA-mimetic in true sense.

**Clinical Uses:**

- (1) As antianxiety agents.
- (2) As sedative or to potentiate the action of a hypnotic drug.
- (3) Muscle relaxant and anticonvulsant agent.
- (4) Psychostimulant agent.
- (5) Preanaesthetic medication.
- (6) During withdrawal of alcohol in chronic alcoholics.

### Synthesis of 1, 4 - Benzodiazepine -4-oxides



Both the amide and urea moieties seen in barbiturates are reproduced in numerous acyclic CNS sedatives (e.g., carbamates, amides, ureides, etc.). They may contain saturated, unsaturated, halogenated, epoxide or alicyclic carbon skeletons.

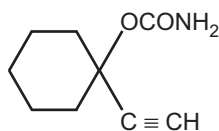
#### Non-barbiturate Sedative-Hypnotics:

There are many drugs, though structurally not related to barbiturates, can produce sedation and hypnosis. The time of onset, duration of action and hypnosis, and untoward effects may be the probable points of difference.

#### 7.2.3 Acyclic Hypnotics Containing Nitrogen

Besides, therapeutic efficacy and relative safety of some agents, this class does not offer any advantage over other hypnotic classes. The clinically used agents are listed below:

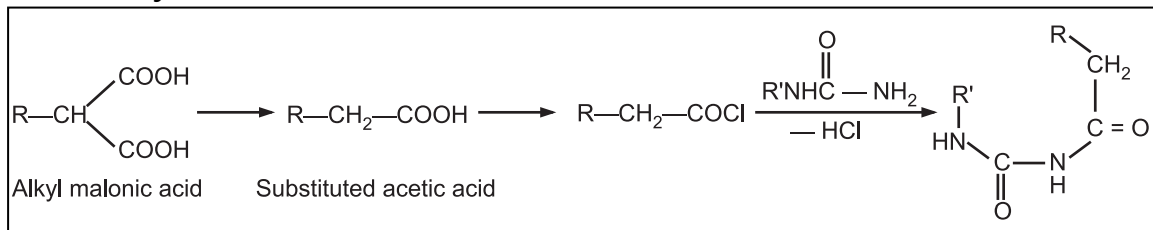
##### (a) Urethanes:



It is generally employed for the prompt treatment of simple insomnia. Prolonged use may result into tolerance and physical dependence and hence is not advisable.

##### (b) Ureides:

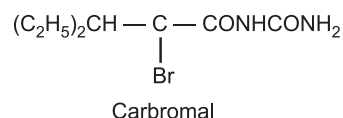
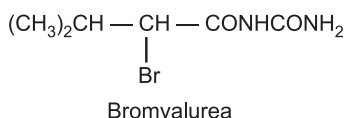
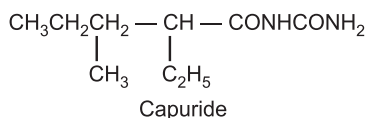
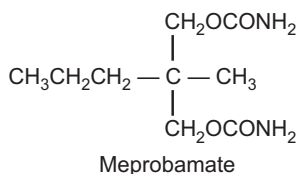
Acylation of urea yields ureides. Many of the ureides are found to be useful hypnotics.

**Route for Synthesis:**

Bromvalurea and carbromal are in clinical use from long time due to their short acting, mild hypnotic action. They are considered to be relatively safe drugs.

Since both of these agents can release bromide ion *in-vivo*, their prolonged use may lead to acute bromide toxication and hence is not recommended.

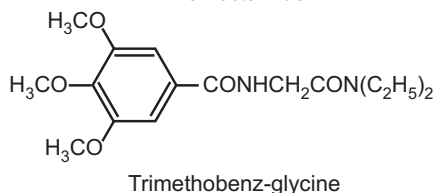
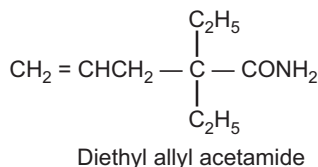
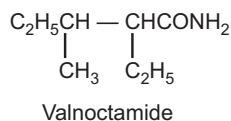
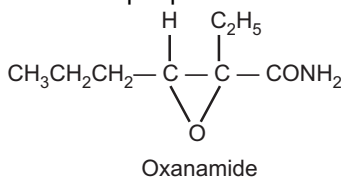
Examples of this class are:

**(c) Carbamates:**

It is used primarily as centrally acting muscle relaxant and antianxiety drug. In larger doses (800 mg), it is sometimes used as a hypnotic. It is less toxic but exhibits some degree of addiction as that of barbiturates.

**(d) Amides:**

The following agents from this class are marketed as tranquilizers and muscle relaxants, having good sedative properties.



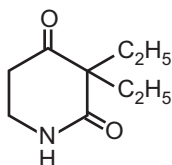
Diethyl allyl acetamide is a more potent hypnotic than the corresponding saturated analogue.

### 7.2.4 Cyclic Hypnotics Containing Nitrogen

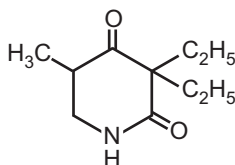
After the success of barbiturates in sedative- hypnotic therapy, many heterocyclic ring structures bearing a close structural relationship with barbiturates were synthesised and screened for CNS depressant activity. The clinically introduced agents from this class are,

#### (a) Piperidinediones:

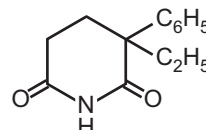
When compared with barbiturates, agents from this class are less active but better tolerated sedative-hypnotics.



Dihydropyrrone

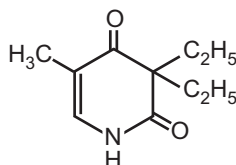


Methypyrrone



Glutethimide

Methypyrrone undergoes metabolic alterations in the body and changes to Ethypicone, which also possesses hypnotic activity.



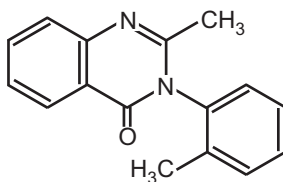
Ethypicone

Methypyrrone does not exhibit analgesic, tranquilizing or muscle relaxant activities. Similar to barbiturates, it causes dependence.

Glutethimide does not offer any advantage over barbiturates. More potent than any other non-barbiturate hypnotics, other properties remain same as that of methypyrrone.

#### (b) Quinazolinones:

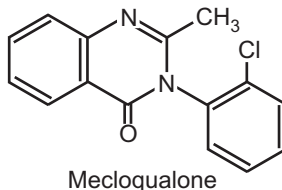
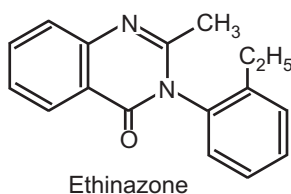
2 - Methyl - 3 - aryl - 4 - (3H)-quinazolinones preserve oxypyrimidine structure of barbiturates. The following agents from quinazoline class are in clinical practices.



Methaqualone

Methaqualone lacks analgesic, local anaesthetic and spasmolytic activity. Though similar to barbiturates in its hypnotic effects, it is also a potent antitussive agent. The undesirable effects include delusions, hallucinations, disorientation with confusion and convulsions.

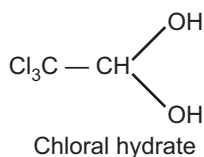
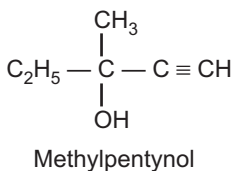
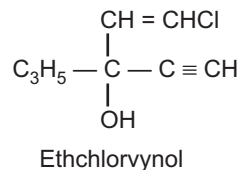
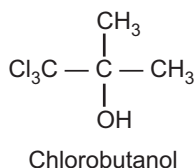
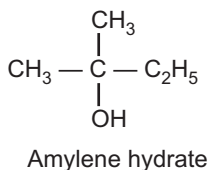
Ethinazone and mecloqualone are other quinazoline type drugs which exert a potent analgesic and antitussive activity in addition to hypnotic activity.



### 7.2.5 Alcohols and Aldehydes

(a) Since branching of the alkyl chain in alcohols, affords a greater resistance to metabolic inactivation, resulting in increased activity, all clinically useful alcohols are tertiary alcohols. The presence of an electron-withdrawing group or unsaturation characterized by electron density near the alcohol group seems to potentiate the activity further.

Clinically useful agents from this class are,

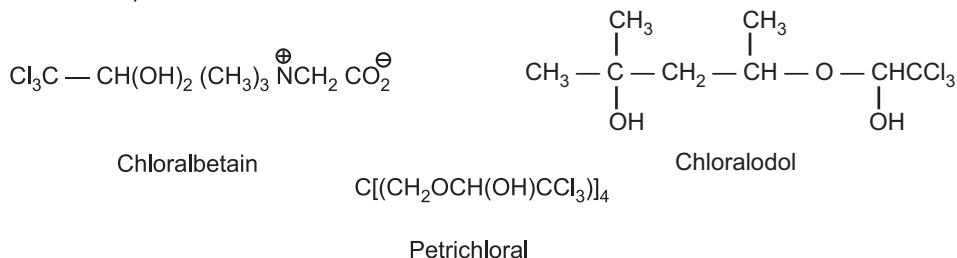


#### Chloral Hydrate Derivatives:

Despite a safe and reliable hypnotic agent, chloral hydrate possesses the following disadvantages:

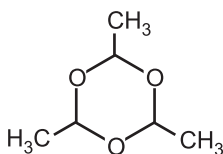
- (i) Poor analgesic property.
- (ii) Quite irritating to the mucous membrane and skin, and may cause nausea, vomiting and diarrhoea.
- (iii) In high dose, may cause marked respiratory depression.
- (iv) Has an unpleasant taste and odour.
- (v) Causes physical dependence.

To overcome these problems, a number of derivatives were prepared; the important amongst them are,

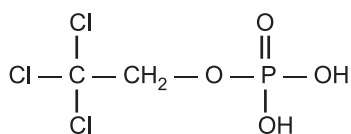


### (b) Aldehydes:

Paraldehyde, introduced in 1882, is one of the oldest and safest hypnotic drugs. Its unpleasant taste, pungent odour and mucous membrane irritating properties limited its widespread use.



Paraldehyde

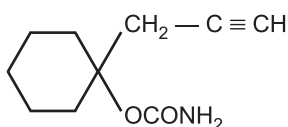


Triclofos

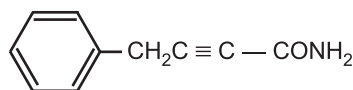
Triclofos is a sedative drug. It is a prodrug which is metabolized in the liver into the active drug, trichloroethanol.

### 7.2.6 Acetylene Derivatives

A few analogues ethinamate, hexapropymate and carfimate can be grouped under this class.



Hexapropymate

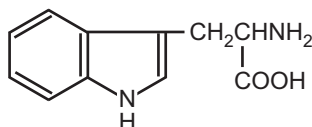


Carfimate

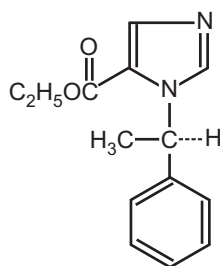
### 7.2.7 Miscellaneous Agents

**(a) Bromides:** Though they were used as anticonvulsants and sedatives, due to their extremely low rate of excretion, they tend to accumulate resulting into intoxication e.g. Potassium bromide.

**(b) Acids and esters:** Examples,



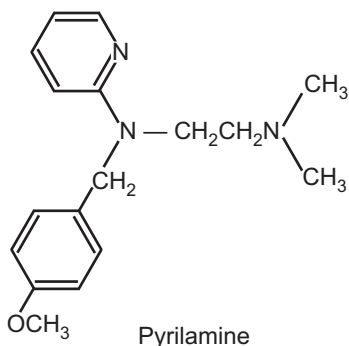
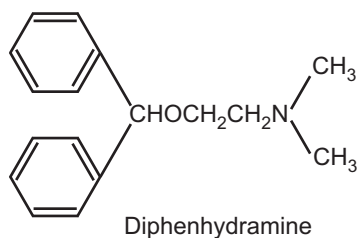
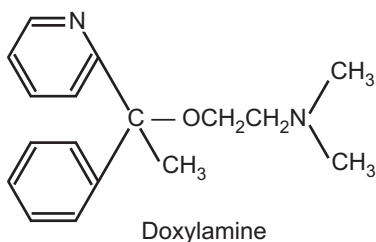
L-Tryptophan



Etomidate

**(c) Antihistamine and Anticholinergics:**

Examples,



**(d) Sulfones:** The agents from this class induce toxic effects at therapeutic doses and hence no longer are used as sedative-hypnotic drugs.

**(e) Plant extracts:** A number of plant extracts are reported to possess sedative-hypnotic activity. Examples: *Radix valerianae*, *Rauwolfia serpentina*, *Avana sativa* and *Glandulae lupuli*.

**(f) Endogenous substances:** It has been postulated that, the sleep inducing substances are present in the cerebrospinal fluids, which are responsible for occurrence and nature of the sleep. Although the characterisation of these substances has not yet been completed, they are thought to possess peptide-like structure, more precisely a non-peptide structure.

Such sleep inducing factors were isolated from the cerebrospinal fluids of goat, dogs and rat brains and efforts to identify and characterise such factors are still on the way.



## ANTIPSYCHOTICS

### ♦ SYNOPSIS ♦

#### 8.1 INTRODUCTION

#### 8.3 MECHANISM OF ACTION

#### 8.2 NEUROLEPTICS

#### 8.4 THERAPEUTIC APPLICATIONS

### 8.1 INTRODUCTION

The antipsychotic agents, now more commonly called as the "neuroleptics" are one of the most important and widely used classes of drugs which developed after the second world war.

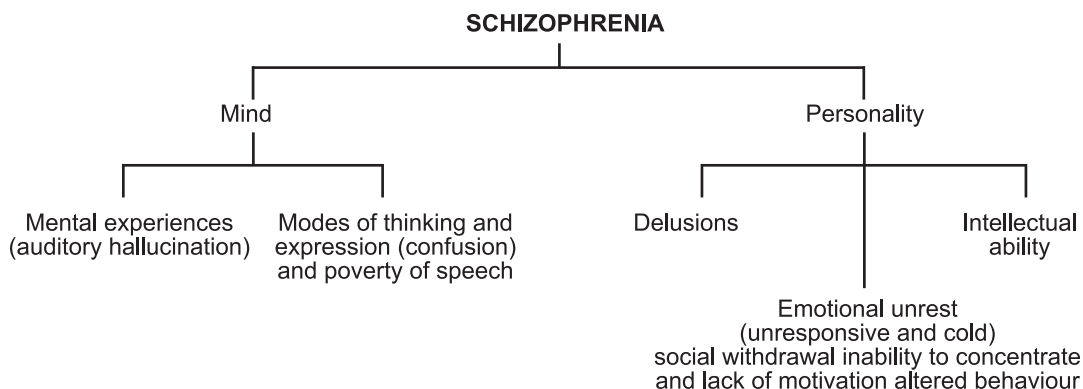
A psychotropic, psychoactive or phenotropic drug is one that inhibits, sharpens or alters behavioural, mood and emotional responses. Psychiatric illnesses can be divided into the neuroses and the psychoses. A neurotic patient usually retains sufficient insight to realise that he is ill while the psychotic patient lives in a world of his own, believes that only his own actions are completely rational and is a victim of his hallucinations and delusions.

The antipsychotic agents have the capacity to sedate or tranquilize the blunt emotional expressions, aggressive and impulsive behaviour, leaving the higher intellectual functions relatively unaffected. Hence, they are also known as major tranquilizers.

Various psychiatric illnesses include:

#### (I) Neurotic Disorders:

**(a) Schizophrenia:** The full panoply of symptoms was first described by Professor Emil Kraepelin at Heidelberg University in 1899 Schizophrenia is a neurological as well as psychological disorder. It is known in general by fundamental and characteristic distortions of thinking and perception. Consciousness and intellectual capacity are usually maintained. The most common symptoms include thought disorders, delusional perception, hallucinatory voices, delusion of control, absent or inappropriate emotional response, poverty of speech, social withdrawal, inability to experience pleasure, inability to concentrate and lack of motivation. Besides this, intense thirst and excess salivation may be seen. The course of schizophrenia may be either continuous or episodic. There can be one or more episodes with complete or incomplete remission.



The patient may feel that his thoughts are disrupted by some outside agency and that his thoughts are withdrawn from his mind or other thoughts are inserted into it. Delusions constitute false beliefs about bodily control. The patient behaves without a will of its own. He seems to be hypnotised by some outside force or power. He feels being forced to make a particular movement and speaks with a special voice.

**(b) Phobias:** These are the fearful reactions to leaving home, entering shops, crowds and public places.

**(c) Obsessive compulsive disorder:** It is characterized by recurrent obsessional thoughts or compulsive acts. Obsessional thoughts are ideas, images or impulses that enter the patient's mind again and again in a stereotyped form. They are almost invariably distressing and the patient often tries, unsuccessfully to resist them. Compulsive acts or rituals are stereotyped behaviours that are repeated again and again. Anxiety is almost invariably present. If compulsive acts are restricted, anxiety gets worse.

**(d) Dissociative (conversion) disorders:** A partial or complete loss of normal integration between memories of past, awareness of identity and immediate sensation and control of bodily movements are the characteristic features. They tend to remit after a few weeks or months, particularly if their onset is associated with the traumatic life event.

**(e) Mental retardation:** It is composed of arrested or incomplete development of the mind, which is especially characterized by impairment of skills manifested during the developmental period, that contribute to the overall level of intelligence, i.e., cognitive, language, motor and social abilities. Retardation can occur with or without other mental or physical condition.

The psychological dysfunction mediating the schizophrenic process is to be found in the information processing sequence - the sensory, attentional, perceptual, cognitive processes - and is probably linked to impairment in arousal mechanisms. Many schizophrenic patients, both hyperactive or inactive-retarded types, are in a state of overarousal.

The neural organizations usually considered to be involved in regulating the level of arousal are the brain stem and thalamic systems that make up the "classical" ascending reticular activating or arousal system. Behavioural and electrophysiological observations suggest that the antipsychotic agents, by an action on this system, decrease sensory input or

responsiveness to stimuli, lower arousal level, and thus bring about clinical improvement. However, some of the electrophysiological effects of the neuroleptics may merely reflect the "sedative" effects and not the antipsychotic action of these drugs. The arousal system includes reticular activating system, limbic structures and basal ganglia.

There may be more than one arousal system or a network of systems. Therefore, there may be many varieties of "arousal," some leading to schizophrenia and some leading to other forms of psychopathology or to none at all. Possibly the quality of arousal in affective disorders, e.g. manic-depression, is different from that in schizophrenia.

## **(II) Mood (Affective) Disorders:**

They include disorders in which the fundamental disturbance is a change in affect (or mood) to depression (with or without associated anxiety) or to elation. The change in mood is usually associated with a change in the overall level of activity; most of other symptoms are either secondary to the change in mood and activity. Most of these disorders tend to be recurrent and onset can often be related to stressful events. Depressive illnesses vary greatly in symptoms and severity. The terms mania and severe depression denote opposite ends of affective spectrum.

The affective disorders include:

- manic episode
- bipolar affective disorder
- depressive episodes-mild, moderate and severe
- recurrent depressive disorder
- persistent mood (affective) disorder
- other mood (affective) disorders.

A large proportion of population is affected by depressive disorders at some stage of their lives. Mood disorders are very often chronic and recurrent. Depressed persons often become of suicidal nature. They may attempt suicide even using overdoses of their prescribed anti-depressant drugs.

(1) Major depression is a unipolar (one-phase) affective (emotional) disorder. The completely different manic depressive disorder is called bipolar affective disorder.

(2) The depressed patient has feeling of helplessness, hopelessness and worthlessness, and often thinks of suicide. Thus, depression can be fatal. The depressed person may be socially withdrawn with retarded activity, speech and thinking or there may be restlessness and anxiety. Patients may come to their doctors because of insomnia, appetite and weight loss and impaired sexual performance.

(3) The same symptoms appear in the depressive phase that usually begins the onset of manic depressive disorder. If a doctor prescribes an anti-depressant in such a case, it could trigger a manic episode.

(4) Not so rarely, depression may occur with delusions. Hallucinatory voices may accuse them, or they may have morbid visions. Such delusions and hallucinations may suggest schizophrenia. But prescription of an antipsychotic drug exposes such patients to risks of incurable movement disorders as side-effects. Currently available anti-depressant agents take 2-3 weeks to become effective. This is because neurotransmitter receptors take that long time to adapt the drug and increase their sensitivity.

### **(III) Anxiety Disorders:**

Each of us has experienced anxiety and fear. Apprehension, fright, nervousness, panic etc. are some of the few words which are used to describe anxiety. The terms, anxiety and fear are often used interchangeably. Thus, anxiety describes an unpleasant state of mental or psychological tension often accompanied by physical or physiological symptoms in which one feels helpless and exhausted.

Panic disorder can be low-level and constant. Symptoms are anxiety, nervousness, fatigue, headaches and insomnia. Physical symptoms can include heart pounding, abnormal heart rhythms, chest pains, trembling, sweating, nausea, diarrhoea, dizziness, faintness and rapid breathing. The symptoms resemble the body's normal flight or fight reaction to arousal by danger. Chronic anxiety and panic attacks thus may be caused by abnormal adrenergic activity in the central and peripheral nervous systems.

Many psychiatrists have linked phobic disorders to panic disorders. Examples are agoraphobia (fear of open spaces), claustrophobia (fear of cramped spaces) and social phobia (feeling of embarrassment when among people). Doctors treat panic disorder with heterocyclic antidepressants or MAO-I plus alprazolam. In addition, benzodiazepine minor tranquilizers can help patients to face phobic situation with less anxiety.

The diagnostic and statistical manual of mental disorders divides anxiety disorders into eight sub-types:

- (i) panic disorder with or without agoraphobia,
- (ii) agoraphobia without history of panic disorder,
- (iii) social phobia,
- (iv) simple phobia,
- (v) obsessive compulsive disorder,
- (vi) generalized anxiety disorder,
- (vii) post traumatic stress disorder,
- (viii) other anxiety disorder.

The two anxiety disorders most common in prevalence are panic and generalised anxiety. In generalized anxiety disorders, the anxiety is persistent and the patient complains of feeling on edge whole time. Typical symptoms of generalized anxiety disorder include motor tension (trembling), palpitations, tachycardia, nausea, and emotional overresponsiveness (e.g., irritability, difficulty in concentrating, insomnia). While the panic

disorders consist of recurrent spontaneous sudden episodes of fear and apprehension which are not restricted to any particular situation or set of circumstances. Hence, these attacks are unpredictable. Symptoms include sudden onset of palpitations, chest pain, choking sensations, dizziness and depersonalization. There is often a secondary fear of dying, losing control or becoming insane.

Virtually all depressed patients suffer from anxiety, although the reverse may not be true. Anxiety has an arousal function, whereas depression inhibits. In most depressions, these two states exist together, albeit with contradictory functions and in opposite directions.

Cardiovascular, respiratory, neurological, gastrointestinal, urinary etc. are some of the systems which are affected by anxiety. A number of diseases can also generate anxiety.

Anxiety symptoms may be caused by many medicines. Similarly discontinuation of a variety of drugs (e.g. anxiolytics) may cause prominent anxiety.

The psychotropic drugs can be divided into three major categories depending on their clinical usefulness, namely

- (1) The neuroleptics,
- (2) The anti-depressants and
- (3) The anxiolytics.

### **(1) The neuroleptics (Antipsychotics or major tranquilizers):**

These drugs are used in the treatment of psychoses. Clinically, these agents counteract hallucinations and delusions, alleviate psychomotor excitement and facilitate the social adjustment of the patient, by reducing dopaminergic activity in the CNS.

### **(2) The anti-depressants (Thymoleptics):**

These include

- (a) Monoamine Oxidase Inhibitors
- (b) Tricyclic Anti-depressants
- (c) Lithium Salts.

They increase the availability of catecholamines and indoleamines at the appropriate receptor sites of the brain.

### **(3) The anxiolytics (Antianxiety agents or minor tranquilizers):**

These mainly include:

- (a) Benzodiazepines and
- (b) Propanediol carbamates e.g. meprobamate

These agents reduce the turnover of norepinephrine and serotonin and facilitate  $\gamma$ -aminobutyric acid activity.

## 8.2 NEUROLEPTICS

The drugs from this category, are used primarily for the treatment of schizophrenia and mania though many of them have anxiolytic actions too.

### Classification:

The antipsychotic agents are best classified in terms of their chemical structures:

[I] Phenothiazines (Chlorpromazine)

[II] Rauwolfia alkaloids (Reserpine)

[III] Butyrophenones (Haloperidol)

[IV] Miscellaneous drugs

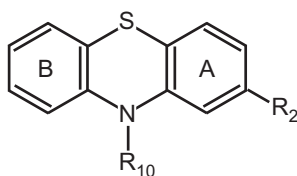
### [I] Phenothiazines:

During second world war, a number of phenothiazine derivatives were prepared in the laboratories of the French pharmaceutical manufacturer, 'RHONE POULENC' in Paris. Among these, were series of 10-(2 - dimethyl aminoalkyl) phenothiazines which on pharmacological screening were found to possess strong anti-histaminic properties. The 10 - (2-dimethyl amino) propylphenothiazine (Promethazine) was studied extensively. Among its diverse pharmacologic properties are sedative, anti-histaminic, antiemetic and potentiating effect on the sedative action of barbiturates.

With the synthesis of chlorpromazine, by Charpentier in 1950, a very large number of phenothiazine derivatives possessing diversified pharmacological activities like skeletal muscle relaxant, tranquilizers, sedative-hypnotic, potent antihistaminic, antiserotonin, antiemetic, analgesic, anthelmintic, urinary antiseptic have been developed. Further departure from the basic phenothiazine ring structure resulted into the development of new tricyclic ring compounds.

Promethazine was prepared as a potent antihistaminic agent. It caused pronounced sedation. Similar CNS-depression was encountered in yet another anti-histaminic anti-cholinergic drug, diphenhydramine, in which the etheral oxygen isosterically takes the place of one  $\text{CH}_2$  group. An attempt to separate CNS-depressant activity from anti-histaminic activity, chain length increase was suggested in promethazine. The resulting compound promazine was further modified by traditional chlorine (e.g., chlorpromazine) to stabilize aromatic phenothiazine system against premature oxidative detoxification. Thus, an antipsychotic drug, chlorpromazine was born.

Chlorpromazine HCl is the first major tranquilizer of the phenothiazine group of compounds. It is used in the treatment of anxiety, tension, agitation and in lessening motor activity in both psychoneurotics and psychotic patients. The antiemetic effects of chlorpromazine make it valuable in the treatment of nausea and vomiting associated with carcinomatosis, uraemia, acute infections, nitrogen mustard therapy, radiation sickness and vomiting during pregnancy.

**Structure-Activity Relationship:**

Phenothiazines

These substances are chemically constituted by a lipophilic linearly fused tricyclic system having a hydrophilic basic amino alkyl side chain.

The sites of modifications thus include:

[A] Tricyclic system

[B] The basic aminoalkyl side chain.

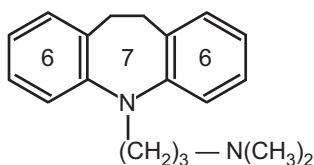
Relative to ring A, ring B plays a less specific role in binding of tricyclic neuroleptic to receptor sites. The apparent necessity of a tricyclic ring for neuroleptic activity perhaps stems from a requirement that ring B be fixed in such a way that it can not assume certain conformations that may prohibit effective binding to the receptors. The ring substituents like chloro or trifluoromethyl increase the lipophilicity of the molecule. Moving the substituents to other positions of the ring may create steric factors unfavourable for drug-receptor interactions.

**[A] Tricyclic System :**

- (a) Most of these compounds have either a six-membered central ring. e.g., Phenothiazine class (6-6-6)

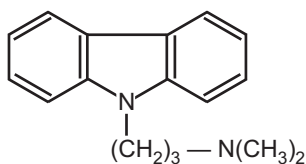
OR

- (b) A seven-membered central ring. e.g., Imipramine class (6-7-6).



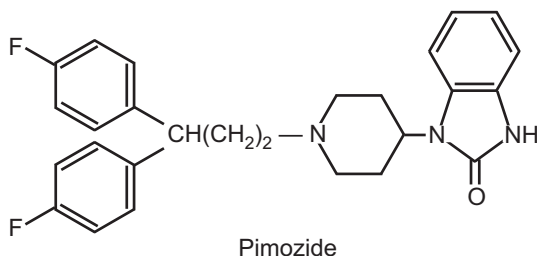
Imipramine

- (c) Compounds having larger central ring are usually devoid of significant antipsychotic activity.
- (d) Compounds with a five-membered central ring e.g., Carbazole, also lack antipsychotic activity and produce only anti-depressant effects.



Carbazole

- (e) Analogs of tricyclic compounds that lack a central ring, are (with some exceptions) generally devoid of antipsychotic activity.



It is clearly derived from benperidol.

**(a) Phenothiazine derivatives:**

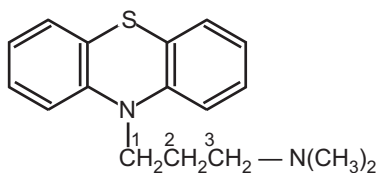
The activity of these compounds is determined by:

- (i) The nature of side chain at C-10.
- (ii) The amino group of the side chain.
- (iii) The substituents on the aromatic nucleus.

**(i) Modifications of the alkyl side chain:**

(1) Maximum potency is retained when there is a three carbon spacing between the basic amino group and the nitrogen of the phenothiazine nucleus because it permits the maximum resemblance of phenothiazine conformation with that of most preferred conformational form of dopamine. The homologue of chlorpromazine in which the ring and amino nitrogen is separated by four methylene units does not produce desired effects.

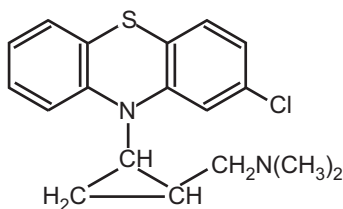
(2) Substitution on the propylene chain of 10-(3-aminopropyl) phenothiazines may also influence antipsychotic potency.



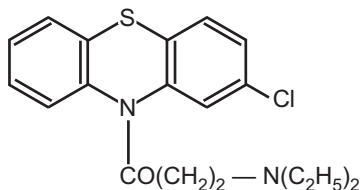
**For example:**

(a) Introduction of methyl group at position 1, decreases antipsychotic activity and may result in imipramine-like activity.

(b) If position 1 of the side chain is incorporated into a cyclopropane ring, potent imipramine-like actions are noted in pharmacological screening.



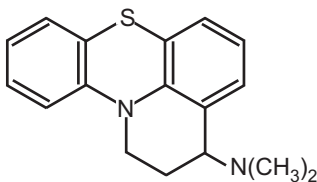
(c) If oxygen is introduced into the position 1 of the 3-aminopropyl side chain, it results into potent anti-depressant like actions e.g.,



Chloracizine

(d) Introduction of a  $\text{CH}_3$  substituent at position 2 or position 3 of the side chain apparently has little influence on activity.

(e) Bridging of position 3 of the side chain to position 1 of the phenothiazine nucleus, reduces the neuroleptic activity, e.g.,



## (ii) Modifications of the basic amino group:

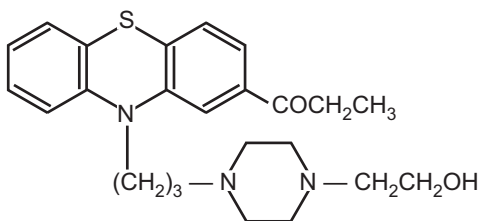
(a) Maximum neuroleptic potency is retained in compounds having a  $3^\circ$  amino group. In compounds with  $2^\circ$  or  $1^\circ$  amino group, activity is either reduced or abolished.

(b) In general, alkylation of the basic amino group with groups larger than methyl, decreases neuroleptic activity.

(c) In pharmacological screening, potency is found to be decreased by replacement of dimethylamino group of chlorpromazine with pyrrolidinyl, morpholinyl or thiomorpholinyl groups.

(d) Activity is retained or increased if the amino group is incorporated in cyclic systems like piperidyl or piperazine, e.g., Mesoridazine, and Carphenazine.

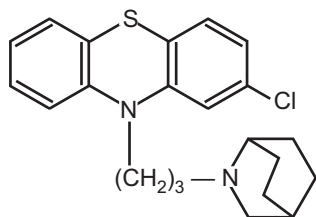
The piperazine compounds are more potent than aliphatic compounds and have relatively less adrenoceptor blocking activity while piperidine group is intermediate in potency and possesses more antimuscarinic activity than chlorpromazine.



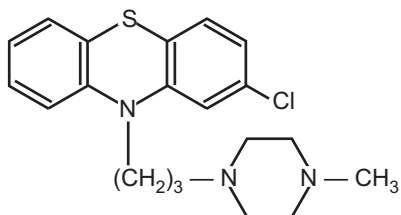
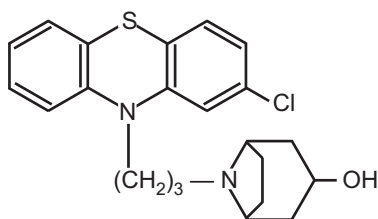
Carphenazine

(e) Bridged piperidine derivatives though are bulky, still retain a high degree of neuroleptic activity.

(f) Introduction of hydroxyl, methyl, hydroxyethyl groups at position 4 of piperidine and piperazine moieties results in increase in potency e.g.,

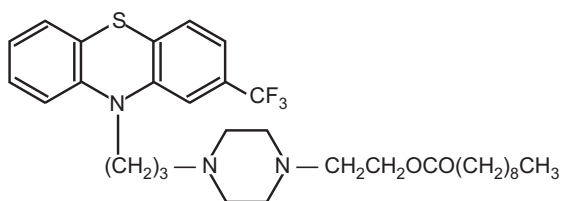


Bridged piperidine derivative



Prochlorperazine

(g) Piperazine phenothiazines may be esterified with long chain fatty acids to produce slowly absorbed, long acting, lipophilic prodrugs. e.g.,

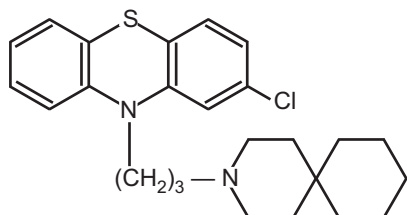


Fluphenazine decanoate

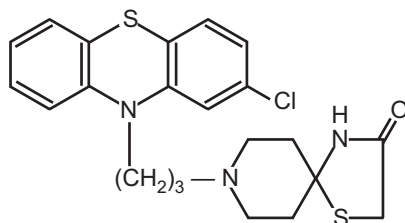
Prolonged duration is due to its slow release from an oily depot.

(h) Significant activity is retained when N-4 piperazine substituents are as large as phenylethyl or p-aminophenylethyl.

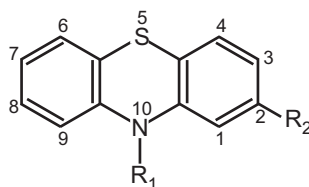
(i) In the series of 4, 4-disubstituted piperidiny propylphenothiazines, azaspirane and chlorspirane are clinically effective antipsychotics.



Azaspirane

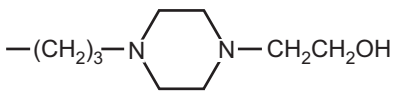
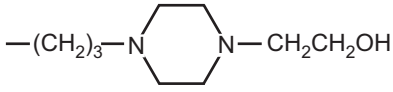
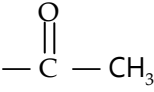
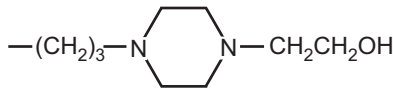
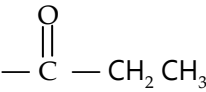
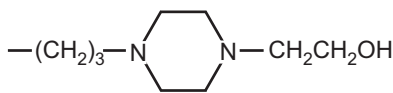


Chlorspirane

**Table 8.1: Phenothiazines**Phenothiazine ( $R_1 = R_2 = H$ )

Generic Name	$R_2$	$R_1$
<b>(A) Propyl dialkylamino side chain:</b>		
(i) Promazine hydrochloride	H	$-(CH_2)_3 N(CH_3)_2 \cdot HCl$
(ii) Chlorpromazine	Cl	$-(CH_2)_3 N(CH_3)_2$
(iii) Triflupromazine	$CF_3$	$-(CH_2)_3 N(CH_3)_2$
<b>(B) Alkyl piperidyl side chain:</b>		
(i) Thioridazine	$SCH_3$	
(ii) Mesoridazine	$O \leftarrow SCH_3$	
<b>(C) Propyl piperazine side chain:</b>		
(i) Prochlorperazine	Cl	$-(CH_2)_3-N \text{ (piperazine ring) } -CH_3$
(ii) Trifluoperazine	$CF_3$	$-(CH_2)_3-N \text{ (piperazine ring) } -CH_3$
(iii) Thioethylperazine	$-SCH_2CH_3$	$-(CH_2)_3-N \text{ (piperazine ring) } -CH_3$
(iv) Butaperazine	$-CO(CH_2)_3CH_3$	$-(CH_2)_3-N \text{ (piperazine ring) } -CH_3$

Contd...

(v) Perphenazine	Cl	
(vi) Fluphenazine	CF <sub>3</sub>	
(vii) Acetophenazine		
(viii) Carphenazine		

The most widely used two-substituents are Cl (chlorpromazine, prochlorperazine, perphenazine); CF<sub>3</sub> (triflupromazine, trifluperazine, fluphenazine); SCH<sub>3</sub> (thioridazine).

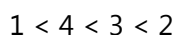
Replacement of a terminal N, N-diethylamino group by piperidino exploits the decreasing valency angle at the 3°-nitrogen of the latter so that access of the basic group to anionic sites might be improved.

### (iii) Phenothiazine ring substitution:

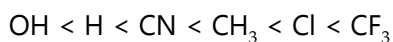
The potency of antipsychotic phenothiazines is mainly influenced by both, the location and the nature of substitution on the tricyclic nucleus.

(a) With some exceptions, substitution at position 2 is optimal for neuroleptic potency.

In general, potency increases in the following order of position of ring substitution:



(b) 2-Substitution of the phenothiazine nucleus increases the neuroleptic potency in the following order:



(c) In general, disubstitution and trisubstitution of neuroleptically active 2-substituted phenothiazines have little effect or are harmful to potency.

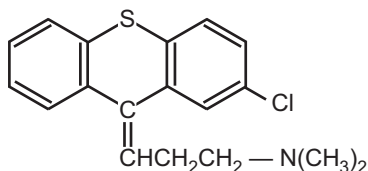
(d) Oxidation of the sulphur at 5-position in antipsychotic phenothiazines decreases neuroleptic activity.

**Ring Analogs of Phenothiazines:**

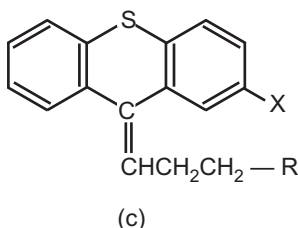
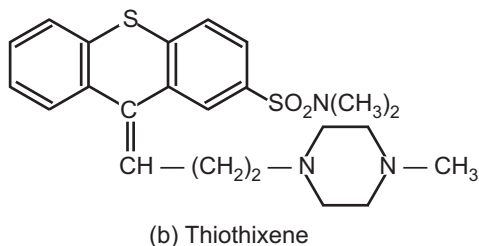
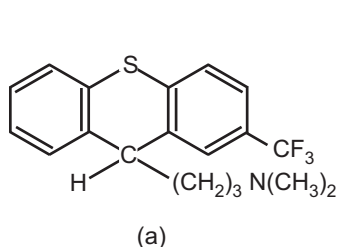
These agents are derived by isosteric replacement of one or more atoms/groups in the structure of the effective antipsychotic phenothiazines.

**(1) Chlorprothixene:**

It is an isostere of chlorpromazine in which the nitrogen is replaced by a methylene group. Released in 1961, it is effective in the treatment of schizophrenia and in psychotic and several neurotic conditions.

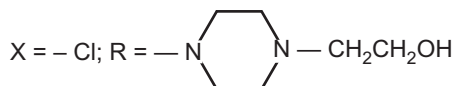


**(2) Other drugs having considerable antipsychotic activity are:**

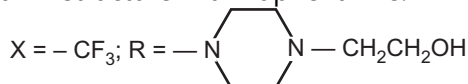


where:

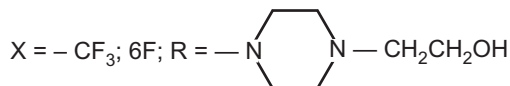
(i) Clopenthixol:



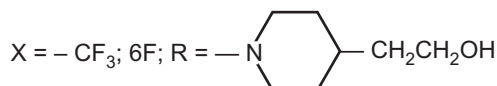
(ii) Flupenthixol is similar in structure with fluphenazine:



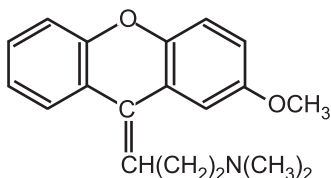
(iii) Tefluthixol:



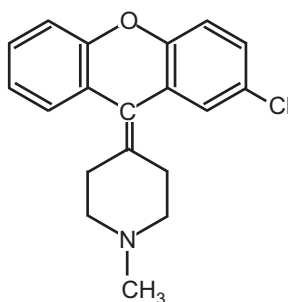
(iv) Piflutixol:



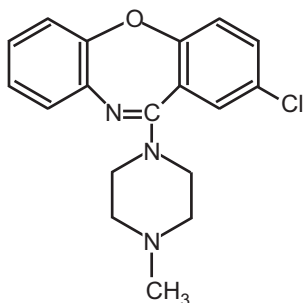
(d)



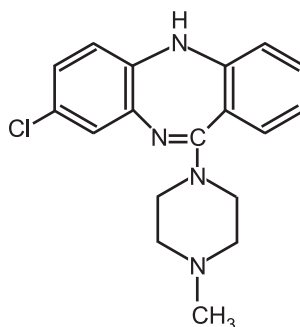
Dimeprozan



Clopipazan



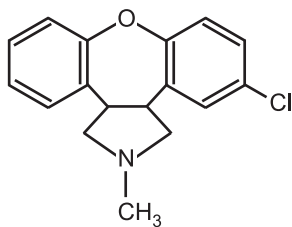
Loxapine



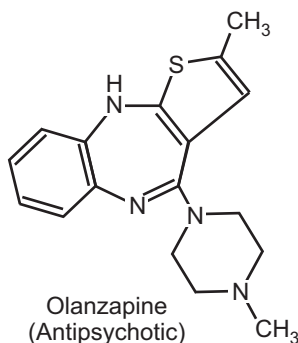
Clozapine

The central ring was expanded to 7-membered heterocyclic structure motivated by possibility that antipsychotic and anti-depressant will be present in one drug. Loxapine has a central ring having the unique oxazepine structure. It is used in the treatment of acute and chronic schizophrenia.

**Asenapine:** It is a new atypical antipsychotic developed for treatment of schizophrenia and acute mania associated with bipolar disorder.



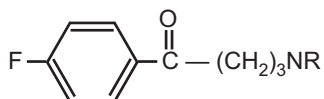
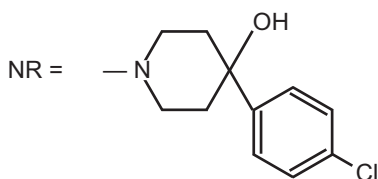
Asenapine

Olanzapine  
(Antipsychotic)

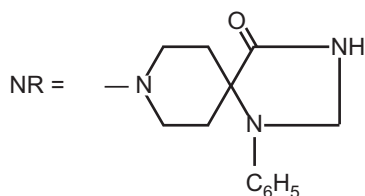
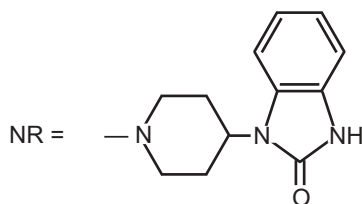
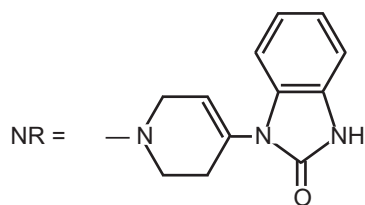
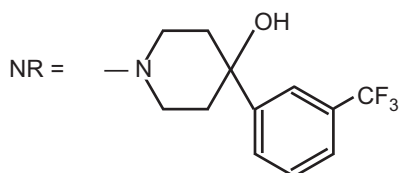
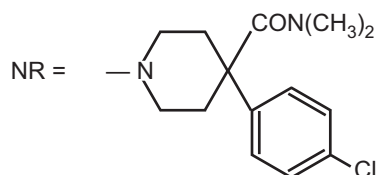
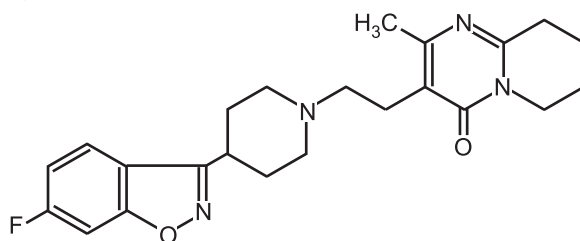
**[II] Butyrophenones:**

Butyrophenones were developed by P. A. Jansen, a Belgian scientist from pethidine fentanyl type analgesics.

A number of compounds from a series of fluorobutyrophenones was found to be effective in the treatment of major psychoses. Haloperidol stands as a prototype of this series.

**(a) Haloperidol:**

Other clinically used agents from this category are:

**(b) Spiroperidol:****(c) Benperidol:****(d) Droperidol:****(e) Trifluoperidol:****(f) Paraperidide:****(g) Risperidone:**

The most potent neuroleptics in this series are 4-aminobutyrophenones in which

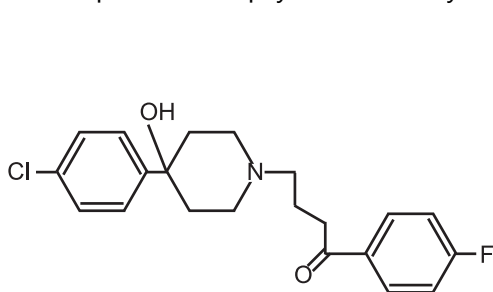
- (1) The aryl group is optimally a 4-fluorophenyl,
- (2) The bridge between the benzoyl and amino group is an unbranched propylene and
- (3) NR is a 4-substituted piperidiny or tetrahydropyridyl.

Greatest potency is noted in tertiary amines, but a few secondary amines are also active.

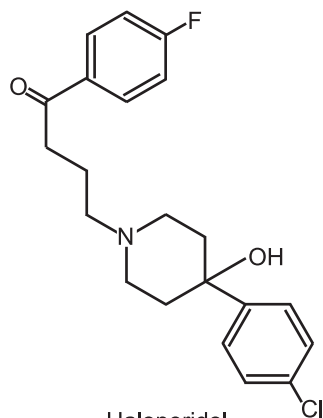
The SAR of this category is determined by:

- (1) The nature and position of aryl substituents.
- (2) Variation of carbonyl functionality.
- (3) Alteration of the propylene bridge and
- (4) Changes involving the basic amino group.

In all types of antipsychotic compounds (e.g., phenothiazines, reserpine, butyrophenones) the aromatic center should be separated from neuroleptic nitrogen by four atoms for optimum antipsychotic activity.



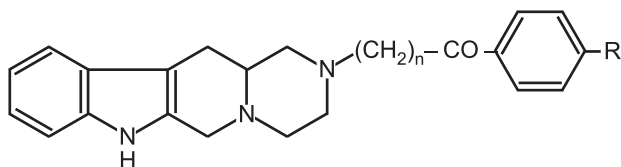
Haloperidol



Haloperidol

It binds with  $D_2$  dopaminergic receptor due to structure similarity with antipsychotic phenothiazine.

**Centbutinole:** Its a new neuroleptic and anti-hypertensive. It is a hybrid skeleton comprising of reserpine and butyrophenone leads. It has conformation having structural similarity with reserpine alkaloids.

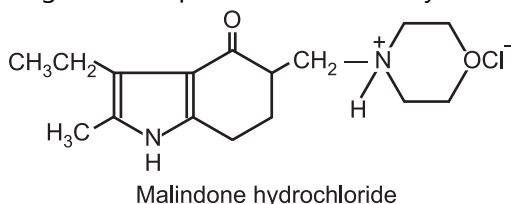


Centbutinole;  $n = 3$ ;  $R = -F$

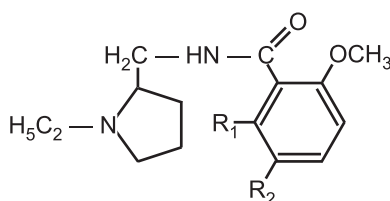
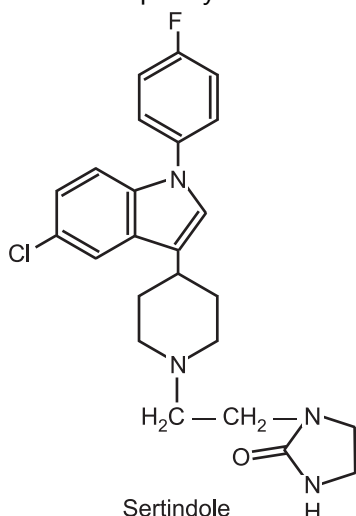
Other butyrophenones like terfenadine and oxatamide have anti-histaminic activity without sedative actions. They are used in the treatment of seasonal and perennial allergic rhinitis.

### Miscellaneous Antipsychotic Drugs:

Various compounds which are distinctly different from the phenothiazines and their ring analogs and from the fluorobutyrophenones, have been successfully used in the treatment of major psychoses. Following are examples of such clinically used agents.

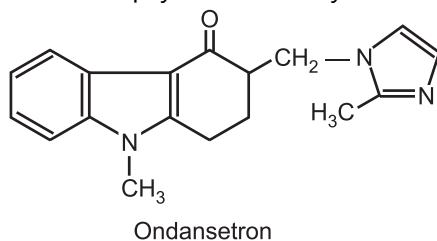


**Sertindole:** It is a phenylindole derivative used in the treatment of schizophrenia.

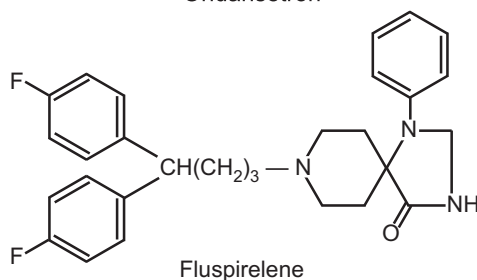
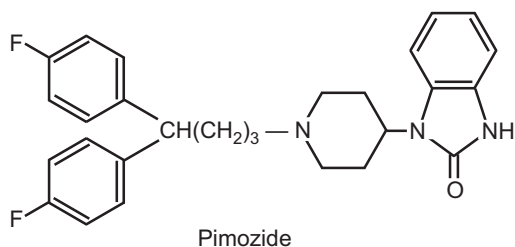


Benzamide derivatives (e.g., sulpiride) were developed through the modification of the structure of procainamide. This led to metoclopramide, a drug having antispasmodic and antidopaminic effect in the periphery as well as showing some antipsychotic activity.

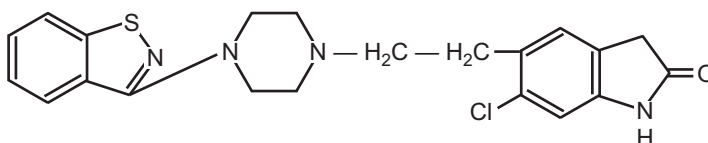
Ondansetron has anxiolytic - antipsychotic activity, besides its use to control nausea and vomiting of cancer chemotherapy.



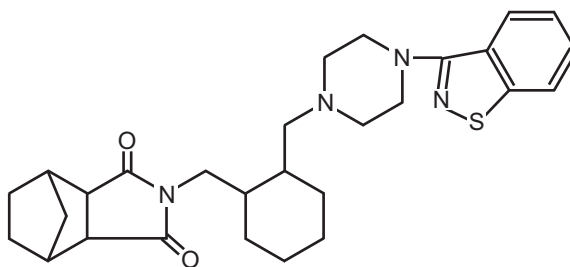
Pimozide, fluspiroline and penfluridol are the outcome of structural modifications on butyrophenones.



(7)

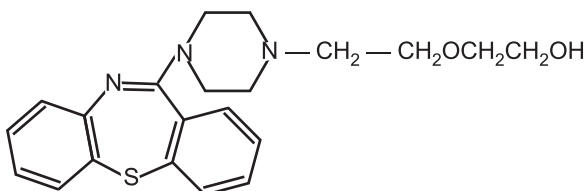
**Ziprasidone**

Ziprasidone is an atypical antipsychotic used in the treatment of schizophrenia, mania and bipolar disorder.

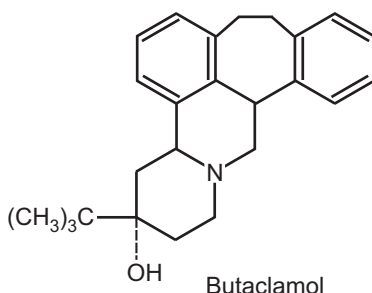
**Lurasidone**

Lurasidone is an atypical antipsychotic used in the treatment of schizophrenia and bipolar disorder.

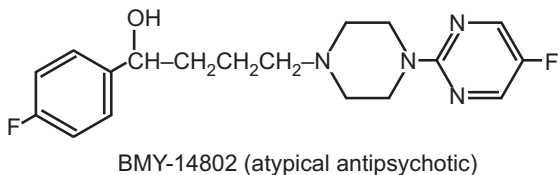
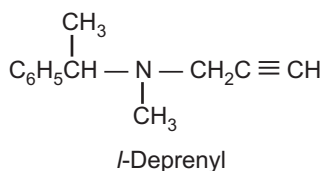
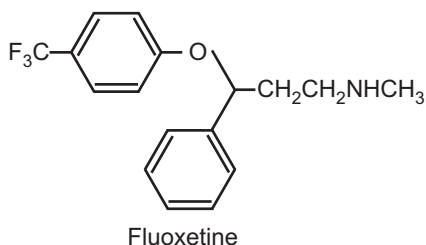
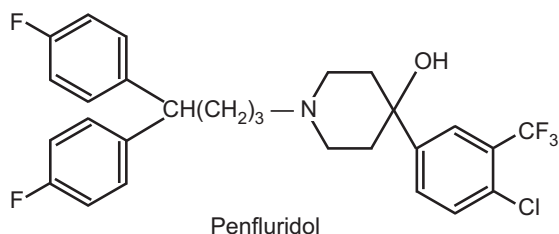
**(8) Quetiapine:** It is an atypical antipsychotic approved in the treatment of schizophrenia, bipolar disorder and depression. Atypical antipsychotics (2<sup>nd</sup> generation antipsychotics) are less likely to cause extrapyramidal side effects (like abnormal gait, body rigidity and involuntary tremors) clozapine is the first atypical antipsychotic.

**Quetiapine**

Butaclamol is a potent dopaminergic blocking agent and is an effective antipsychotic agent.

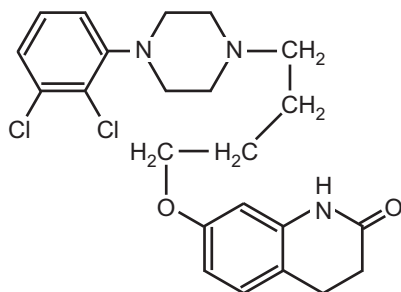
**Butaclamol**

Similarly the neuropeptide, neurotensin (NT), a co-transmitter in dopaminergic neurons, may have an antipsychotic effect through modulation of dopamine release.



Fluoxetine exhibits relatively selective inhibition of serotonin reuptake. It has its own side-effects of occasional nausea, nervousness and insomnia. But its selectivity leaves it without the side-effect of dry mouth, constipation, blurred vision, orthostatic hypotension or abnormal heart rhythms. Indeed patient may not be able to commit suicide with an overdose.

**Aripiprazole:** It is an atypical antipsychotic and anti-depressant used in the treatment of schizophrenia, bipolar disorder and clinical depression.

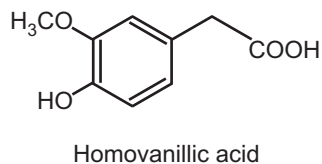


### 8.3 MECHANISM OF ACTION OF ANTI-PSYCHOTIC OR NEUROLEPTIC DRUGS

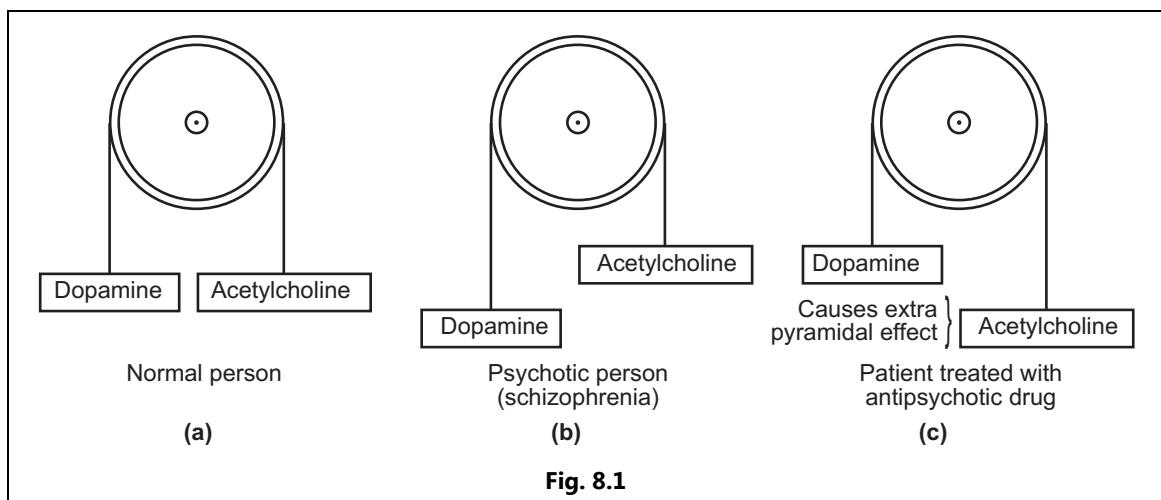
(1) In 1958, Carlsson suggested that dopamine, besides a precursor of noradrenaline and adrenaline, might also function independently as a neurotransmitter.

(2) The highest concentrations of dopamine have been found in the thalamus, the hypothalamus and some of the basal ganglia.

Dopamine not utilized in receptor reactions, is degraded to homovanillic acid in the brain,



(3) Dopamine usually has a depressant action, particularly on cells that have been excited by glutamate.



(4) In the control of emotional responses, the hypothalamus is closely associated with the reticular and the limbic systems, the latter, incorporates a balanced complex of excitatory (ACh) and inhibitory (dopamine) components.

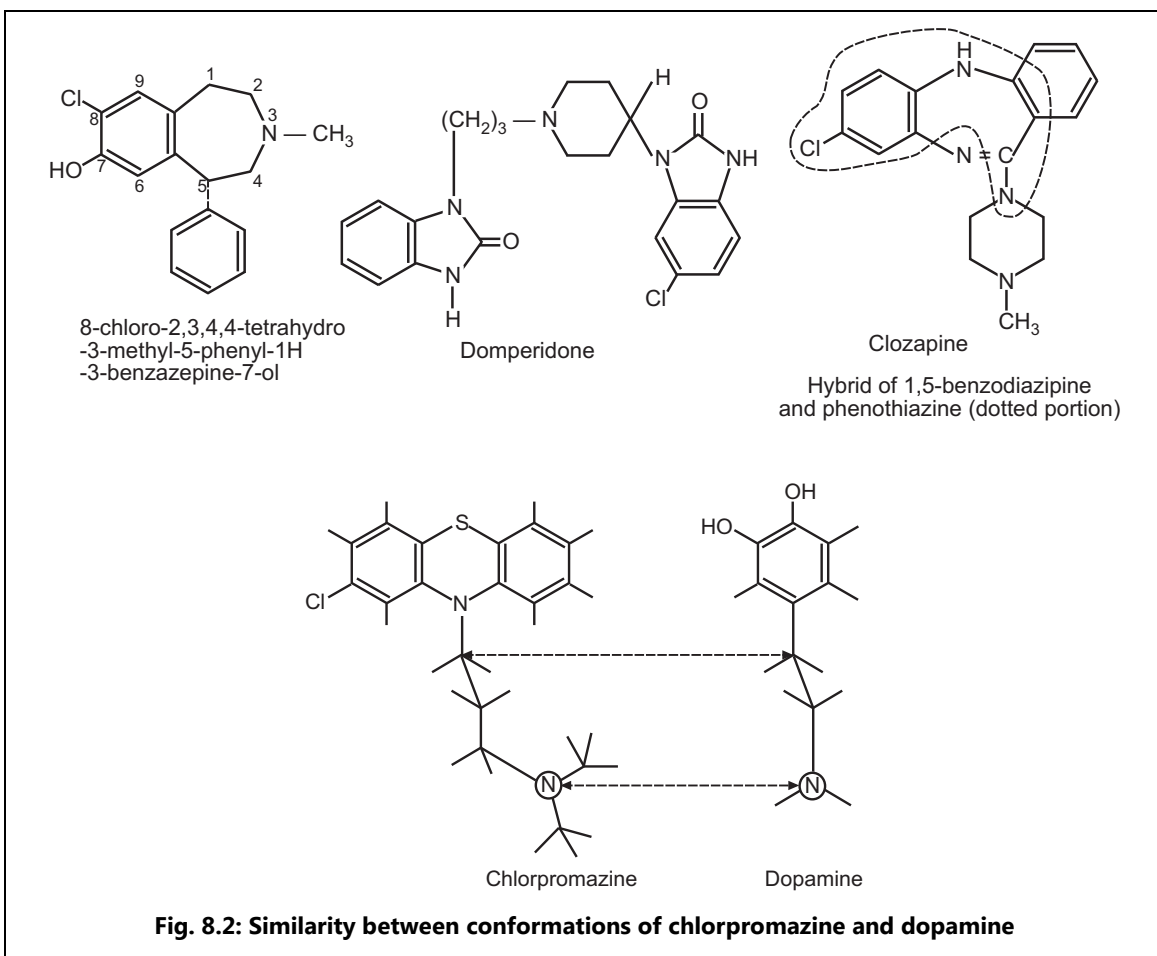
(5) There is an evidence which suggests that schizophrenia is associated with the presence of greater than normal amounts of dopamine (inhibitory component) at central synapses in the striatum and other brain regions where it results in overfiring of neurons. Since Parkinson's disease is associated with a deficiency of dopamine, neuroleptic therapy may evoke Parkinsonism like symptoms.

There are five identified dopamine pathways in the brain, each accounting for an effect of neuroleptics. The mesolimbic pathway is most probably involved in the antipsychotic efficacy of neuroleptics. The nigrostriatal pathway is implicated in extrapyramidal side-effects. The mesocortical pathway is probably involved in sedative action and hypothalamic-hypophyseal and incertohypothalamic pathways are responsible for the neuroendocrine side-effects caused by neuroleptics.

(6) The antipsychotic drugs e.g., chlorpromazine acts by: (a) Increasing metabolic rate of dopamine. (b) Blocking dopamine (D<sub>2</sub>) receptors and reserpine like drugs which cause depletion of dopaminergic neurons. This results in an increase in concentration of excitatory component (ACh) and decreasing the concentration of inhibitory component (dopamine). There is an evidence of a direct quantitative correlation between the antipsychotic activity of various neuroleptic drugs and their ability to block H-dopamine in rat neostriatal slices. All the antipsychotic drugs increase the turnover of dopamine in the brain.

Some of these drugs also have a sedative effect, because of their  $\alpha$ -adrenolytic and anti-histaminergic properties.

(7) Antipsychotic drugs shift the balance (a), in favour of ACh (c), this difference between the concentration of dopamine and ACh, leads to an increase in extrapyramidal effects (posture and the involuntary aspects of movement). Hence, all antipsychotic drugs are always associated with a varying degree of extrapyramidal effects.



Extrapyramidal symptoms take their name from cone-shaped structures, called pyramids, that extend from the medulla oblongata to the spinal cord. The pyramids lie along the course of impulse transmissions that govern voluntary movement. Involuntary movements are controlled along another pathway involving the corpus striatum. Because movement disorders arise in a pathway outside that of the pyramids, they are termed as extrapyramidal. Clozapine appears to work in mesolimbic and mesocortical tissues, but not in striatal tissues, thus avoiding production of extrapyramidal symptoms. Unfortunately, clinical use of clozapine was subsequently curtailed by incidence of blood dyscrasias, and especially agranulocytosis, a potentially lethal non-neurological disorder. The agent most similar to clozapine is fluperlapine.

(8) The superimposability of the conformations of dopamine and chlorpromazine blocks the dopamine receptors. It further explains why an effective antipsychotic phenothiazine derivative should possess three carbons in the side chain, separating the two nitrogen atoms.

## 8.4 THERAPEUTIC APPLICATIONS

- (1) Antiemetic agents
- (2) Potentiation of actions of, analgesic and sedative agents.
- (3) In treatment of moderate and severe mental and emotional disturbances.

Relapse occurs in about 30-40% of patients within a year of commencing treatment. Illnesses in patients on maintenance drug therapy are often precipitated by acute stress (e.g., traumatic life events) or chronic stress associated with patient's life-style. Life events and factors related to excessive levels of stress at home, particularly of an emotional and interpersonal type is responsible. In actual practice of medicine, therefore, besides drug therapy, social and psychological treatments are also employed for better results.



# ANTICONVULSANT DRUGS

### ♦ SYNOPSIS ♦

<b>9.1 INTRODUCTION</b>	<b>9.3.4 Succinimides</b>
<b>9.2 TYPES OF EPILEPSY</b>	<b>9.3.5 Phenacemide</b>
<b>9.3 CLASSIFICATION OF ANTICONVULSANT DRUGS</b>	<b>9.3.6 Benzodiazepines</b>
<b>9.3.1 Anticonvulsant Barbiturates</b>	<b>9.3.7 Sodium Valproate</b>
<b>9.3.2 Hydantoins</b>	<b>9.3.8 Iminostilbenes</b>
<b>9.3.3 Oxazolidinediones</b>	<b>9.3.9 Carbonic anhydrase inhibitors</b>
	<b>9.3.10 GABA-nergic Agnoist</b>

## 9.1 INTRODUCTION

Epilepsy is one of such diseases where selectively acting drugs are still lacking. The prevalence of epilepsy is between 3 to 6 per 1000 population.

The term epilepsy is derived from the Greek word epilambenein which means 'to seize' or convulsions. A convulsion is a violent involuntary spasmodic contraction of the skeletal musculature.

Epilepsy is a collective designation for a group of chronic CNS disorders having in common the occurrence of brief and self limited, sudden and transitory seizures of abnormal motor, sensory, autonomic or psychic origin resulting into a repeated neuronal discharge.

All forms of epilepsy have their origin in the brain. Epilepsy results when many neurons in union, under a high excited stage, deliver massive discharges abolishing a finely organised pattern of the integrative activity of the brain.

John Jackson proposed that these seizures are caused by occasional, sudden, excessive, rapid and local discharges of grey matter and once initiated by the abnormal focus, the seizures attack the neighbouring normal brain resulting into generalised convulsions.

This abnormal focus may originate as a result of local biochemical changes, ischemia or the loss of vulnerable cell inhibitory systems.

The normal inhibitory mechanisms generally restrict the spread of convulsive activity to the neighbouring normal cells. Hence, a seizure focus in man may remain normal over long period of time and may not cause signs and symptoms of epilepsy. However, certain physiological changes may trigger the focus and thus facilitate the spread of abnormal electrical activity to normal tissue.

Such factors include:

- (1) Changes in blood glucose concentration.
- (2) Blood gas tension.
- (3) Plasma pH.
- (4) Total osmotic pressure and electrolyte composition of extracellular fluids.
- (5) Fatigue.
- (6) Emotional stress.
- (7) Nutritional deficiency.
- (8) Trauma, infection, meningitis, brain tumours, cerebrovascular disease, or metabolic abnormalities. Epileptic seizures of unidentified cause are known as primary or idiopathic epilepsy while epileptic attacks of known causes are called as secondary or symptomatic epilepsy.

Seizures, in fact, are nothing but electrical explosions of the brain. Once initiated, a seizure is maintained by re-entry of excitatory impulses in a closed feed-back fashion which may not include the original seizure focus. Complete depletion of neurotransmitter, metabolic factors (like accumulation of  $\text{CO}_2$  and adenosine, depletion of  $\text{O}_2$  and high energy phosphate intermediates) may contribute to self control of intensity and duration of seizures.

In summary, epilepsy is a CNS malfunctioning, which leads either to generalised hyperactivity (involving essentially all parts of the brain) or to hyperactivity of only a portion of the brain.

## 9.2 TYPES OF EPILEPSY

Vitamin  $\text{B}_6$  is the precursor in the formation of coenzyme pyridoxal - 5 - phosphate which is responsible for the decarboxylation of glutamic acid to form GABA and since hydrazine derivatives can inactivate the coenzyme, pyridoxal - 5 - phosphate via hydrazone formation, these facts confirm the fundamental role of GABA in the arrest of convulsions.

### [A] Generalized epilepsy:

Once initiated, it spreads quickly into the entire or at least the greater part of the brain.

**(i) Tonic-clonic seizures (grand mal):** It has a close resemblance with electrically induced convulsions where the mass stimulation of cortical neurons occurs. As the name indicates, initially there is a generalized tonic activity followed by a clonic phase. It results due to a potent cerebral excitation and is also known as major seizures. Its onset is pre-intimated to the patient by a warming sensation that is known as aura. Patient may become cyanotic. Heart rate and blood pressure increase and dilation of pupils also occur. These signs are characteristics of sympathetic nervous system stimulation. The total attack lasts for several minutes. After the attack, sleeps prevails due to neuronal store-exhaustion.

**(ii) Absence or minor seizures (petit mal):** It is reported to occur mainly in young children between the age of 6 to 14. Seizures frequently disappear spontaneously after adolescence. Seizures are usually of brief durations (in seconds) accompanied by a momentary loss of consciousness and originate due to synchronization of both, excitatory and inhibitory neurons within the brain stem and mesial reticular activating system.

**(iii) Myoclonic seizures:** The attack is characterized by the jerky muscular movements of head, limbs or body as such. The duration of attack remains near about 1 second and it reappears at about 5 seconds intervals for a period of a minute. The etiology of attack is not clear and is supposed to be due to brain damage.

**(iv) Infantile spasms:** The attack, sometimes begins with a cry and is often associated with momentary unconsciousness. The structural or functional brain abnormalities or pyridoxine deficiency are some recognized causes responsible for infantile spasms.

### [B] Partial or Focal Epilepsy:

In this type, the initial neuronal discharge originates from a specific, limited cortical area e.g.

**(i) Complex partial seizures (psychomotor or temporal lobe seizures):** It usually originates in the mesial anterior temporal lobe and is characterized by hallucinations, fear, hate or other emotional and behavioural abnormalities. Symptoms are extremely complex and varied and may sometimes be confused with psychotic disorder.

**(ii) Motor epilepsy:** Only one entire side of the body is affected. Consciousness is usually not lost. In severe cases, motor epilepsy is transformed into grand mal followed by paralysis of the hyperactive side of the body. Motor epilepsy is mainly witnessed in the childhood and is due to more limited cortical abnormalities.

**(iii) Sensory epilepsy:** This is similar to motor epilepsy except the fact that it arises in the sensory cortex. Simultaneous attack of both, motor and sensory epilepsy in the patients, is also reported.

**(iv) Akinetic seizures:** Superficially no convulsions are seen. Patient may suddenly fall down on the ground without loss of consciousness.

**Status epilepticus (acute repetitive seizures):** It is the condition in which one attack follows another without patients regaining consciousness. The attack may be of grand mal, petit mal or partial seizures. If it remains untreated, it may be fatal. Status epilepticus originates due to failure of the patient to follow therapeutic regimen prescribed for him. Diazepam, clonazepam, thiopentone or lignocaine may be administered intravenously to control this condition. If the treatment fails, general anaesthesia may be required.

### Mechanism of Action of Anticonvulsant Drugs:

(1) Many carbonic anhydrase inhibitors (Acetazolamide) are found to possess anticonvulsant or anti-epileptic activity. Carbonic anhydrase plays a role in promoting the elimination of excess carbon dioxide from the brain and blood circulation. Since, excess carbon dioxide depresses nerve functioning, these drugs are thought to exert their anticonvulsant action by decreasing the cerebral respiration.

(2) It has been postulated that excessive discharge of neurotransmitter is the cause of generation of seizures. Various anticonvulsant drugs (like phenobarbital, diphenylhydantoin), increase the levels of serotonin in brain which causes non-specific depression of CNS functions and thus controls the release of neurotransmitters.

(3) The anticonvulsant activity of barbiturates is attributed to their ability to exert conformational rearrangements of oxidative enzymes essential for brain respiration.

(4) Gamma-amino butyric acid level in brain is also important to prevent the spread of the seizures. Many anticonvulsant drugs are reported to operate by increasing GABA levels in brain.

Since, GABA hypofunction is thought to be one of the major aetiological factors in epilepsy during the last two decades, most of the attention has been focussed on the development of GABA-ergic anticonvulsants. The discovery and clinical usefulness of sodium valproate gave the necessary impetus. The future strategy would be to develop anticonvulsants with GABA mimetic and antiglutamate (e.g., dizocilpine) action without serious side-effects.

### 9.3 CLASSIFICATION OF ANTICONVULSANT DRUGS

The first serious attempt to treat epilepsy was by Sir Charles Locock who, in 1857, gave large doses of bromides under the erroneous idea that the disease was due to masturbation, in order to produce an aphrodisiac effect. In virtue of their effects on the motor cortex, bromides were used in the treatment of epilepsy. Sedation is induced by decreasing the central reflex responses. The irritability of the motor cortex is also diminished. Mental efficiency is retarded. Bromism comprises a chronic syndrome showing mental depression, deficient memory, general stupidity, muscular weakness, rash, psychosis, conjunctivitis, ataxia etc. and is symptomatic of a chronic intoxication.

From 1857 to 1912, no much advance was made in the treatment of epilepsy. With the introduction of barbiturates, a number of other chemical substances have been synthesised to combat this disease. The various structurally diversified antiepileptic agents can be broadly categorised as:

- (a) *Drugs which were introduced before 1960:* These are closely related in structure to phenobarbital. Examples include hydantoins, deoxybarbiturates, oxazolidinediones and succinimides.
- (b) *Drugs which were introduced from 1960 to 1981:* Examples include benzodiazepines, iminostilbenes and valproic acid, and
- (c) *Drugs which were introduced after 1981:* These agents are either GABA-nergic agonists (e.g. progabide, vigabatrin) or antiglutamates (e.g. Iomotrigine).

Since epilepsy primarily results through excessive stimulation of the brain neurons, anticonvulsant drug is principally, a CNS depressant drug. Many anticonvulsants have sedative-hypnotic effects and some may cause mental disturbances and hence search for a more ideal, newer structural types of potential antiepileptic agent, which should possess a broad spectrum of efficacy and minimal side-effects, is still going on.

**Therapeutic Aspects:**

The various structurally diversified antiepileptic drugs can be broadly divided into

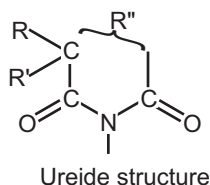
- (1) Barbiturates
- (2) Hydantoins
- (3) Oxazolidinediones
- (4) Succinimides
- (5) Phenacemide
- (6) Benzodiazepines, and
- (7) Sodium valproate.

If the seizures are not controlled by a single drug treatment, multiple drug therapy is generally used, since more than one type of seizures may occur in the patient.

The additional drug is usually chosen from different chemical class.

Though plasma concentration of anticonvulsant drug is usually related to its anticonvulsant activity, it may sometimes lead to wrong interpretation, since many antiepileptic drugs are highly bound to plasma proteins and the concentration of free drug is only a small fraction of the total plasma concentration of a drug.

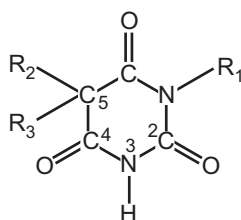
**Table 9.1: Common structural features of anticonvulsant agents**



R''	Class	R''	Class
	Barbiturates		Succinimides
	Hydantoins		Phenacemide
	Oxazolidinedione		Glutarimides

### 9.3.1 Anticonvulsant Barbiturates

The clinically effective anticonvulsant barbiturates are:



Barbiturates

**(1) Phenobarbitone:**  $R_1 = \text{H}$ ;  $R_2 = \text{C}_2\text{H}_5$ ;  $R_3 = \text{C}_6\text{H}_5$

**(2) Mephobarbitone:**  $R_1 = \text{CH}_3$ ;  $R_2 = \text{C}_2\text{H}_5$ ;  $R_3 = \text{C}_6\text{H}_5$

**(3) Metharbital:**  $R_1 = \text{CH}_3$ ;  $R_2 = \text{C}_2\text{H}_5$ ;  $R_3 = \text{C}_2\text{H}_5$

Amongst barbiturates, phenobarbitone is the first introduced and most widely used anti-epileptic drug. Though the above mentioned barbiturates (long acting class) exhibit a high degree of effectiveness in grand mal seizures, phenobarbitone is relatively non-selective. Other barbiturates like methyl phenobarbitone also possesses slight anticonvulsant action.

#### Structure-Activity Relationship:

- (1) Optimum activity is observed when one of the substituents at  $\text{C}_5$  is phenyl.
- (2) The 5, 5 - diphenyl derivative has less activity than phenobarbitone.
- (3)  $\text{N}_2$  and  $\text{N}_3$  substitutions, in some cases also resulted in an increased activity.
- (4) 5, 5 - dibenzyl barbituric acid, causes convulsions.

#### Metabolic Studies:

(1) About 40-60% of the total phenobarbital administered is bound to plasma proteins. The major metabolite parahydroxyphenyl derivative is obtained through oxidative hydroxylation by hepatic microsomal enzymes.

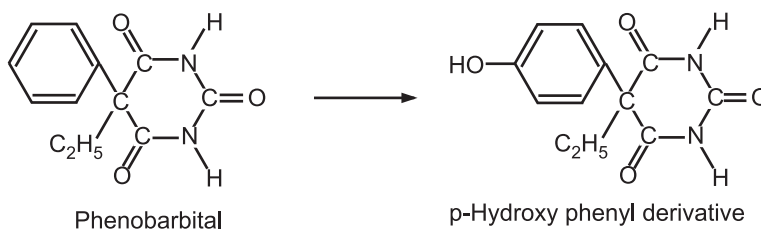
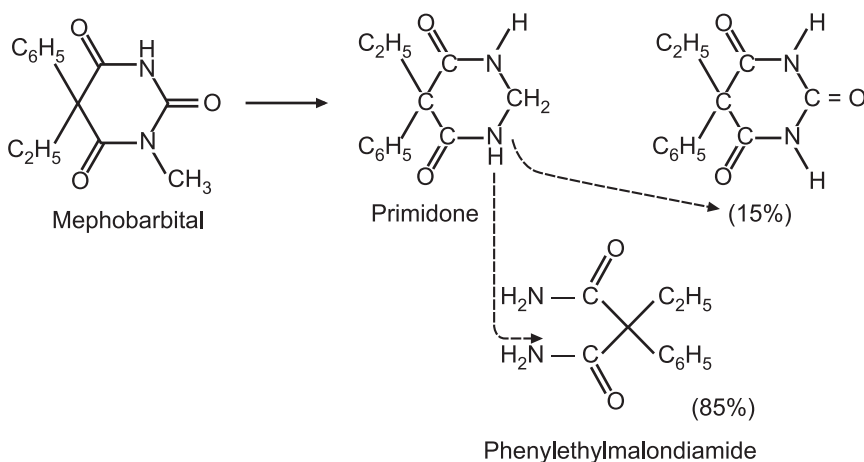


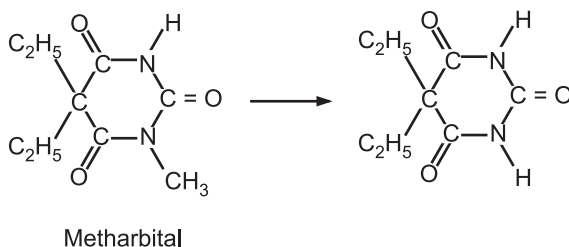
Table 9.2: Drugs used clinically in the control of epilepsy

	Type of epilepsy	Drugs of choice	Less effective drugs
<b>(A) Generalized epilepsy:</b>	1. Grand Mal	Phenobarbitone, Phenytoin Carbamazepin	Primidone, Methoin Acetazolamide
	2. Petit Mal (Absences)	Nitrazepam, Clonazepam, Sodium Valproate	Primidone
	3. Myoclonic Seizures	Phenobarbitone, Nitrazepam Clonazepam, Sodium Valproate	
	4. Infantile Spasms	Nitrazepam Clonazepam, Vitamin B <sub>6</sub>	
	<b>(B) Partial epilepsy:</b>		
	1. Psychomotor	Phenytoin, Carbamazepine, Methsuximide	Primidone, Phenacemide, Methoin

(2) Mephobarbital and primidone (Deoxybarbiturate), both get metabolised *in-vivo*, to phenobarbital and are active antiepileptic drugs only due to the fact that they serve simply as a source of *in-vivo* phenobarbital.



While metharbital undergoes N - demethylation to give



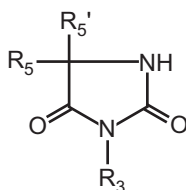
**Toxicity:**

- (1) Sedation and drowsiness are the most common effects with the beginning of therapy while tolerance develops during chronic treatment.
- (2) Irritation and hyperactivity in children and confusion in older aged people are observed with phenobarbitone.
- (3) Folate deficiency, hypocalcaemia and coagulation defects in the new-born are other toxic reactions.

**9.3.2 Hydantoins**

As a chemical species, the hydantoins have been known since the 1860s. Phenytoin had been first synthesized by Blitz in 1908, through a condensation of urea with benzyl which exploited a pinacolone rearrangement.

The concept that 'antiepileptics need not impair consciousness' is emerged with the discovery of the most extensively used antiepileptic agent phenytoin in 1938 by T. Putnam at ParkeDavis Company. It is a non-sedative structural relative of phenobarbital. Since then, many hydantoins were synthesised and were evaluated for their antiepileptic activity. The hydantoins are most effective against grand mal while they remain ineffective against petit mal. The clinically used antiepileptic hydantoins are,



Hydantoin

- (1) Phenyethylhydantoin:  $R_3 = -H$ ;  $R_5 = -C_2H_5$ ;  $R'_5 = -C_6H_5$

(not used clinically now)

- (2) Phenytoin:  $R_3 = -H$ ;  $R_5 = -C_6H_5$ ;  $R'_5 = -C_6H_5$

- (3) Mephentyoin:  $R_3 = -CH_3$ ;  $R_5 = -C_2H_5$ ;  $R'_5 = -C_6H_5$

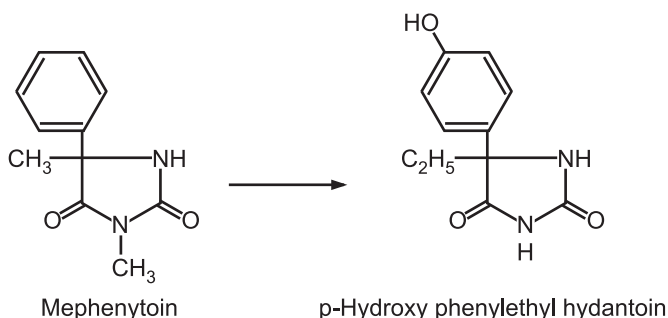
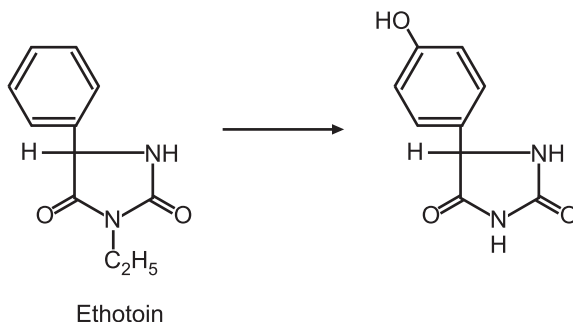
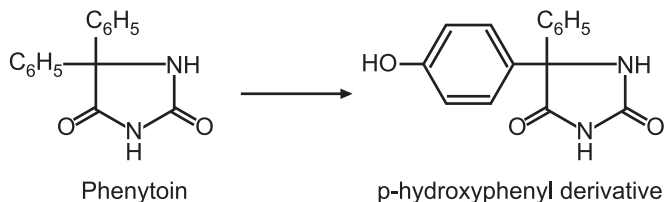
- (4) Ethotoin:  $R_3 = -C_2H_5$ ;  $R_5 = -H$ ;  $R'_5 = -C_6H_5$

**Structure-Activity Relationship:**

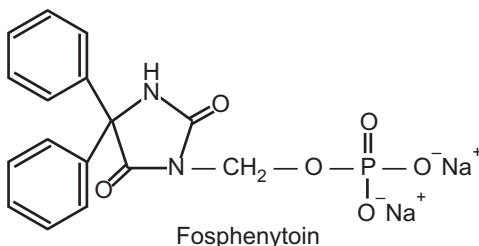
- (1) A 5-phenyl or other aromatic substituent is essential for the activity.
- (2) Alkyl substituents at position 5 may contribute to sedation, a property absent in phenytoin.
- (3) Among other hydantoins, like spirohydantoins, thiohydantoins, dithiohydantoins and 1, 3-disubstituted hydantoins, some exhibit activity against chemically induced convulsions while remaining are ineffective against electroshock induced convulsions.

**Metabolic Studies:**

Metabolism is catalysed by hepatic microsomal enzymes.

**Mode of Action:**

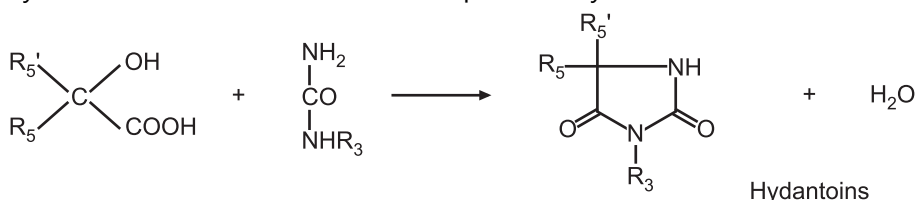
Epileptic seizures cause an accumulation of  $\text{Na}^+$  ions within the cerebral neurons, which initiates enhanced synaptic transmission following rapid, repetitive presynaptic stimulation. Phenytoin decreases the intracellular  $\text{Na}^+$  ion concentration by activating the biochemical process that normally extrudes  $\text{Na}^+$  ions from neurons.



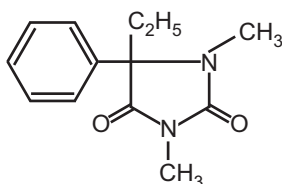
Fosphenytoin is a phosphonoxy ester attached to the C<sub>3</sub> position of the five-membered ring.

**Synthesis:**

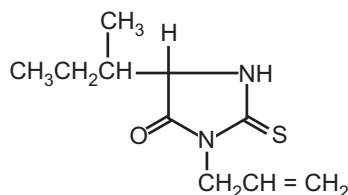
Hydroxy acids when condensed with urea, produce hydantoins.

**Other hydantoins:**

Other less important antiepileptic hydantoins include:



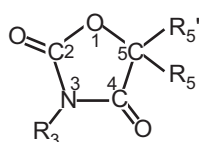
1-methyl mephentyoin



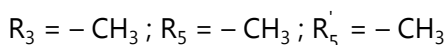
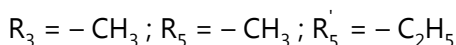
3-Allyl-5-isobutyl-2 thio-hydantoin

**9.3.3. Oxazolidinediones**

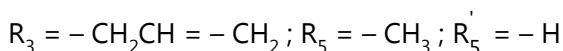
Like other antiepileptic drugs, the oxazolidine -2, 4-diones were originally developed as hypnotics or analgesics but were introduced into anticonvulsant therapy between 1946 to 1948. These compounds are isosterically related to the hydantoin, differing only in that an oxygen atom is replaced by NH group. Trimethadione, paramethadione, and allylmethyloxazolidinedione are the clinically used drugs from this class. The oxazolidinediones are effective in the treatment of petit mal seizures but if used alone, are ineffective against other types of epilepsy.



Oxazolidinediones

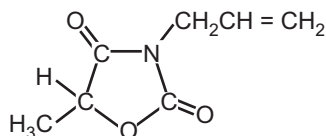
**(1) Trimethadione****(2) Paramethadione****(3) Allylmethyloxazolidinedione**

(Malidione)

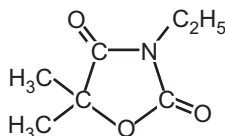


**Structure-Activity Relationship:**

(1) The nature of the substituents on C<sub>5</sub> is important e.g., lower alkyl substituents tend towards anti-petit mal activity while aryl substituents towards anti-grand mal activity e.g.,

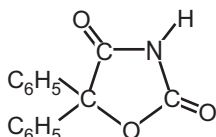


Malidione



Dimidione

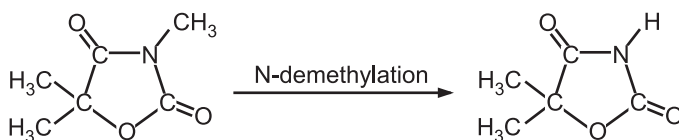
Both are active against petit mal epilepsy. While,



5, 5-diphenyloxazolidine-2, 4-dione

It is active against grand mal epilepsy.

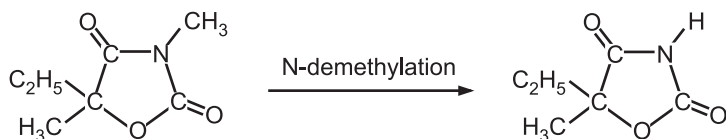
(2) The N-alkyl substituent does not affect the activity since all clinically used agents from this class, undergo N-dealkylation in metabolism. e.g. The anticonvulsant activity of trimethadione is due to mainly its N-demethylated metabolite, dimethadione.



Trimethadione

Dimethadione

Similarly paramethadione also undergoes N-demethylation *in-vivo* to yield 5-ethyl-5-methyl oxazolidine -2, 4-dione, which is responsible for observed anticonvulsant action of paramethadione.



Paramethadione

5-ethyl-5-methyl oxazolidine-2,4-dione

Paramethadione is similar to trimethadione but less effective and less toxic.

**Mode of Action:**

The petit mal epilepsy involves low frequency discharges in the thalamus and cerebral cortex which are induced through reticular activating system.

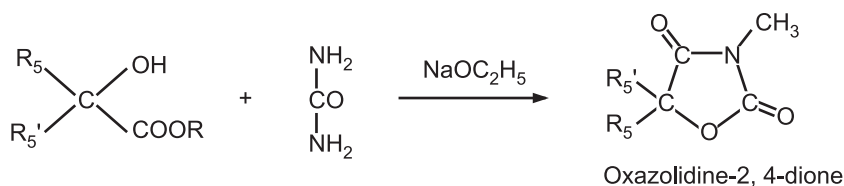
Oxazolidinediones are effective only against petit mal condition.

This effectiveness is due to two fold action of these agents.

- (1) they increase the threshold (of excitability) for production of petit mal seizures of the thalamic centres and thus prevent the spread of electrical activity to the thalamus;
- (2) they decrease synaptic transmission by increasing the duration of the refractory period in the neurons through which repetitive discharges occur;
- (3) a slight inhibitory action on the resting respiration of the brain cells is an additional effect.

### Synthesis:

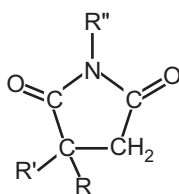
A glycolic ester when condensed with urea in the presence of a base, yields oxazolidine-2, 4-dione molecule.



### 9.3.4 Succinimides

Though less potent, succinimides have enjoyed more success over oxazolidinediones. Since, they possess less significant side-effects. These drugs are moderately effective against petit mal seizures but remain ineffective against grand mal. The first drug from this series, Phensuximide, introduced in 1953 is the weakest and now rarely used. It is followed by Methsuximide (1958) and ethosuximide (1960).

### Structure-Activity Relationship:



Succinimides

- (1) Phensuximide:  $R = -C_6H_5$ ;  $R' = -H$ ;  $R'' = -CH_3$
  - (2) Methsuximide:  $R = -C_6H_5$ ;  $R' = -CH_3$ ;  $R'' = -CH_3$
  - (3) Ethosuximide:  $R = -C_2H_5$ ;  $R' = -CH_3$ ;  $R'' = -H$
- (a) Methsuximide and phensuximide have phenyl substituents which make them active against electrically induced convulsion.
  - (b) N-methylation decreases activity against electroshock seizures and impart more activity against chemically induced convulsions.
  - (c)  $\alpha$ -Methylalkoxyphenyl succinimides and alkoxybenzylsuccinimides were active anticonvulsants. The length of the alkoxy group here determines the activity.

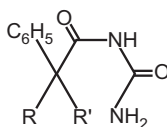
**Toxicity:**

The most commonly occurring side-effects with these drugs are:

- (1) Gastrointestinal complaints like nausea, vomiting and anorexia.
- (2) CNS effects include drowsiness, euphoria, lethargy and headache.
- (3) Others include restlessness, agitation, anxiety and skin reactions.
- (4) Certain degree of tolerance to above effects may develop in some patients.

**9.3.5 Phenacemide**

Acetylureas is a group of compounds structurally related with barbiturates and hydantoin. Phenacemide is an open chain analogue of the hydantoin. Introduced in 1951, it possesses a high anticonvulsant activity, associated with serious toxicity which limits its use.



Acetylurea

- (1) Phenacemide:  $R = -H$ ;  $R' = -H$
- (2) Phenylethylacetylurea:  $R = -H$ ;  $R' = -C_2H_5$

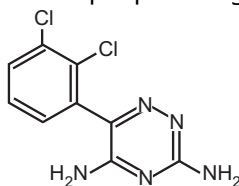
Not only these compounds possess a good anticonvulsant action but also possess serious toxic effects.

**Structure-Activity Relationship:**

- (1) Among aliphatic acetylureas, the highest anticonvulsant activity is found in those derived from branched chain acids of about seven carbon atoms.
- (2) With a further increase in molecular weight, the anticonvulsant activity gradually terminates and hypnotic effect predominates.
- (3) Phenacemide is most active agent amongst the aromatic acetylurea.
- (4) Any substitution on the nitrogen of phenacemide does not increase further the anticonvulsant activity.
- (5) Activity decreases with aromatic substitution of phenacemide with a gradual increase in hypnotic activity.
- (6) Diphenylacetylurea is inactive.

**Mode of Action:**

The acetylureas inhibit the metabolism of other antiepileptic drugs and hence effective when given in combination with other antiepileptic drugs.



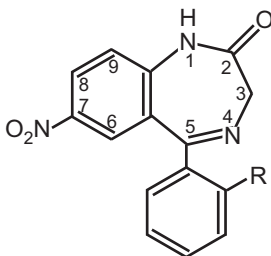
Lamotrigine

Chronic treatment with phenytoin, phenobarbitone and primidone has been known for some time to disturb folate metabolism, resulting in megaloblastic anemia in some patients. A search for compounds not interfering with folate metabolism by Wellcome laboratories (UK) resulted in the phenyltriazine series from which lamotrigine was developed. It stabilizes presynaptic membranes by blocking voltage-dependent  $\text{Na}^+$  channels, thereby preventing the release of excitatory neurotransmitters, particularly glutamate and aspartate.

### 9.3.6 Benzodiazepines

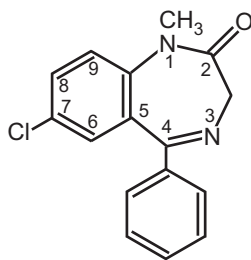
Benzodiazepines were discovered by Leo Sternbach in 1956 at Roche laboratories. The benzodiazepines started their career primarily as sedative-anxiety drugs but established themselves also as effective antiepileptic drugs in recent years.

Chlordiazepoxide was the first clinically used (1960) antiepileptic agent from this class, followed by oxazepam, nitrazepam, diazepam and clonazepam.



(1) Nitrazepam:  $\text{R} = -\text{H}$

(2) Clonazepam:  $\text{R} = -\text{Cl}$



Diazepam

#### Structure-Activity Relationship:

- (1) The electron withdrawing atom or group at position 7 increases the anti-epileptic activity while electron donating substituents at 7, 8 or 9 positions decrease it.
- (2) A phenyl group at position 5 is necessary for activity. But only halogen substituents are allowed in the ortho position.
- (3) The electron withdrawing groups at ortho or diortho positions at 5-phenyl increase the activity while any substituent on meta or para position at 5-phenyl decreases the activity.
- (4) Methyl substitution at position 1 confirms high activity.

**Mode of Action:**

Benzodiazepines somehow increase the effectiveness of GABA, an inhibitory neurotransmitter by,

- (1) Making easy the functioning of variety of GABA mediated synaptic systems.
- (2) Increasing the affinity of GABA for receptor sites in brain membranes.

**Metabolic Studies:**

- (1) Clonazepam is principally metabolised to inactive 7-amino derivative.
- (2) Diazepam metabolises to the N-dimethyl analogue and oxazepam, both are biologically active.

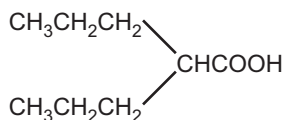
**Toxicity:**

- (1) Drowsiness and fatigue are among the most common symptoms.
- (2) Muscular inco-ordination, behavioural disturbances and increased salivary and bronchial secretions constitute less frequent side-effects.

**9.3.7 Sodium Valproate (Valproic Acid)**

Valproate was first synthesized by B. S. Baron in 1882 and was initially used as an organic solvent.

It is the latest antiepileptic drug. Chemically, it is n-dipropylacetic acid.



Valproic acid

Among other relatives of valproic acid, 3, 3, 4-trimethylpentanoic acid is also as active as valproic acid. In this series, [i.e. dialkylalkanoic acid having less than 14 carbon atoms]

- (1) The anticonvulsant activity increases with increased chain length.
- (2) Introduction of a double bond decreases the activity.
- (3) Introduction of a secondary or tertiary hydroxyl group or replacement of carboxyl by hydroxyl group has no effect.

Divalproex consists of a compound of sodium valproate and valproic acid in a 1 : 1 molar relationship in an enteric coated form.

**Mode of Action:**

*Valproic acid inhibits:*

- (1) GABA deactivating enzymes.

It blocks succinic semialdehyde dehydrogenase, the enzyme oxidising the semialdehyde metabolite. As this metabolite accumulates GABA-T activity is decreased by end-product inhibition and the GABA concentration increases.

- (2) Re-uptake by glial cells and nerve endings and thus increases the synaptic concentrations of GABA.

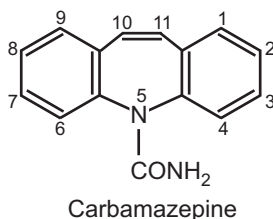
**Toxicity:**

It is one of the potent antiepileptic drugs having minimal sedation and other CNS side effects. GIT disturbances are most commonly observed.

Tolerance to its anticonvulsant effects is yet not reported.

**9.3.8 Iminostilbenes**

Carbamazepine is introduced (1960s) in clinical practices. It is structurally related to tricyclic antidepressants. It is known to increase available adenosine which is a natural anticonvulsant or convulsion modulator.

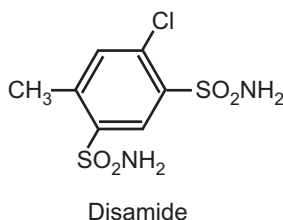
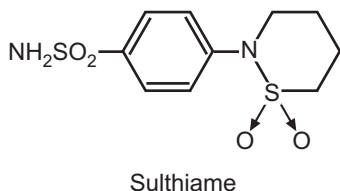
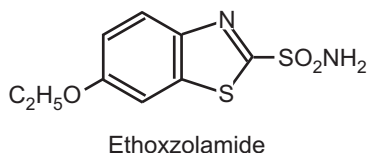
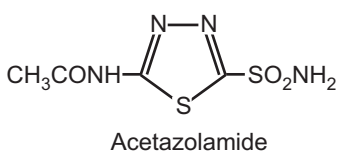


In majority of the cases, toxicity is relatively minor and may include mild drowsiness, skin rash, and gastric irritation.

Carbamazepine is metabolised to 10, 11-epoxide which also has anticonvulsant activity.

**9.3.9 Carbonic Anhydrase Inhibitors (Sulphonamides)**

Acetazolamide, ethoxzolamide, sulthiame and disamide were shown to possess anti-convulsant property.



Their anticonvulsant action is due to their direct inhibition of brain carbonic anhydrase enzymes.

In addition, sulthiame inhibits oxygen consumption by the brain.

### 9.3.10 GABA-nergic Agonists

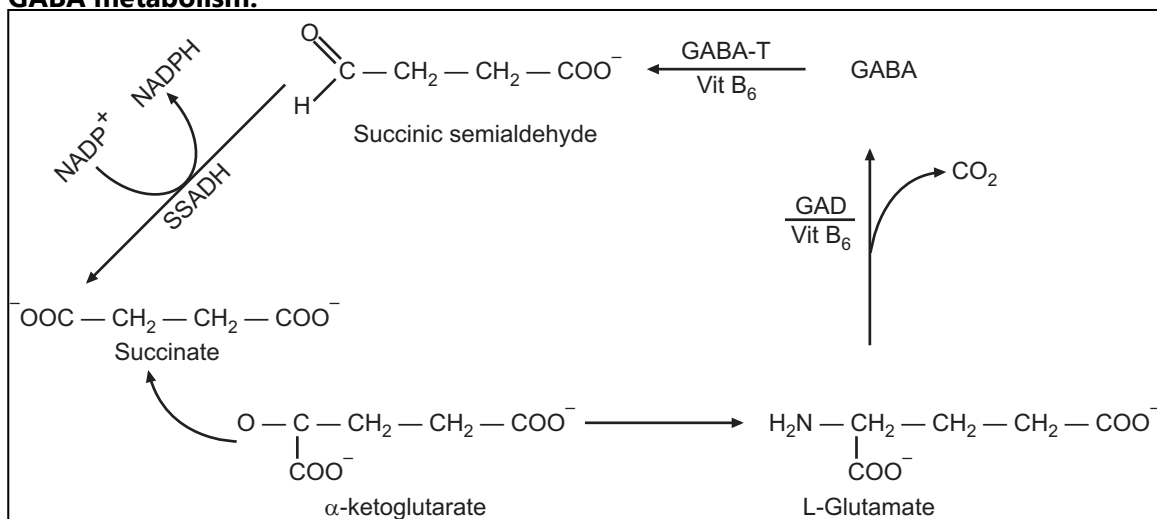
There seem to be numerous GABA-nergic pathways in the CNS. GABA is found in the highest concentration in the substantia nigra. It is also found in hypothalamus and occurs in low concentration in practically all brain structures as well as in the spinal cord.

**GABA-receptor** was thoroughly investigated in the early 1980s. The GABA-A receptor is a high affinity binding site with fairly constant density. The GABA-B receptor has a low binding affinity and shows great variation in receptor density in various brain areas. GABA-receptor is regulated by a thermostable protein called GABA-modulin, having a molecular weight of 150,000. This protein is an inhibitor of the  $\text{Ca}^{++}$  dependent and both C-AMP dependent and C-AMP-independent protein kinases. Its removal increases protein phosphorylation in the synapses by 20 fold and the number of receptors (or their transmitter recognition ability) increases. The receptor assembly seems to be composed of GABA-receptor, the protein kinase with attached GABA-modulin, the ionophore mediated  $\text{Cl}^-$  ion transport. The GABA-modulin binding site is apparently the same as benzodiazepine binding site.

The existence of peripheral GABA-receptors has been shown. Since, they are located at outer mitochondrial membrane, they may be involved in the modulation of intermediary metabolism.

GABA is involved in feeding, sleep, hormonal secretion, CVS functions and in analgesia that is not mediated by opiate-sensitive neurons. The GABA mimetic THIP (tetrahydroisoxazolo-pyridinol), a non-addictive pain-relieving agent, is reported to be equivalent to morphine. The correlation may be enkephalinergic neurons, involved in pain pathways, seem to be regulated by GABA-ergic neurons.

#### GABA metabolism:

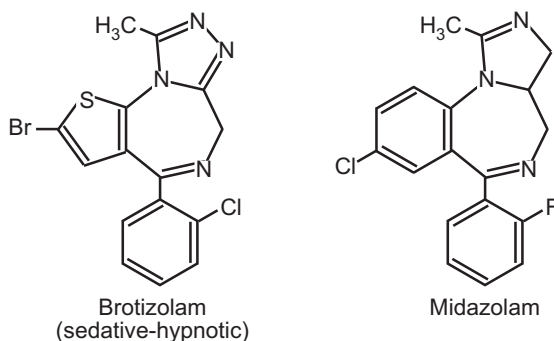


**GAD:** Glutamic acid decarboxylase. Cofactor is vitamin B<sub>6</sub>. GAD is found only in mammalian CNS and in the retina.

**GABA-T:** GABA-transaminase. **SSADH:** Succinic semialdehyde dehydrogenase.

**GABA** can be deactivated and recycled by transamination reaction with  $\alpha$ -ketoglutarate to yield glutamate.

Huntington's chorea, a neuromotor disorder involves defective central GABA-metabolism. It is hereditary disease that manifests in involuntary movements which disappear only during sleep and leads to mental deterioration in adults. It arises due to deficiency of glutamic acid decarboxylase (GAD).



### New and Potential Anticonvulsants:

(a) Vigabatrin (gamma-vinyl GABA) is an irreversible inhibitor of the enzyme aminotransferase and interferes with the catabolism of GABA.

(b) Lamotrigine is believed to exert antiepileptic action by inhibiting the release of excitatory neurotransmitters like glutamic and aspartic acids.

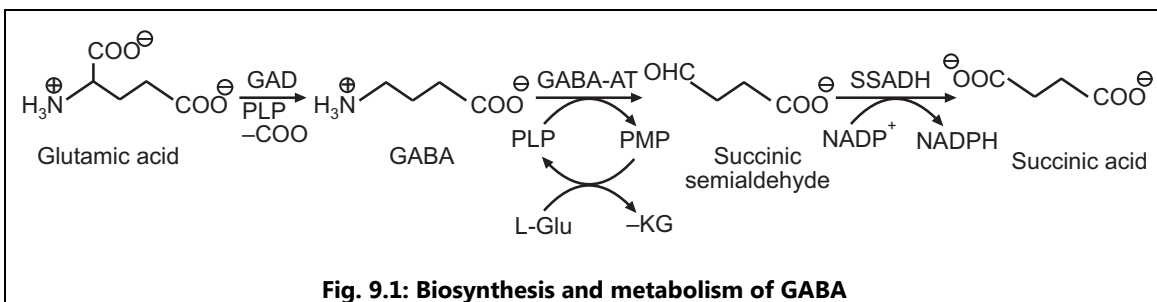
(c) Denzimol is an antiepileptic agent. An involvement of purinergic and benzodiazepine mechanisms is suggested.

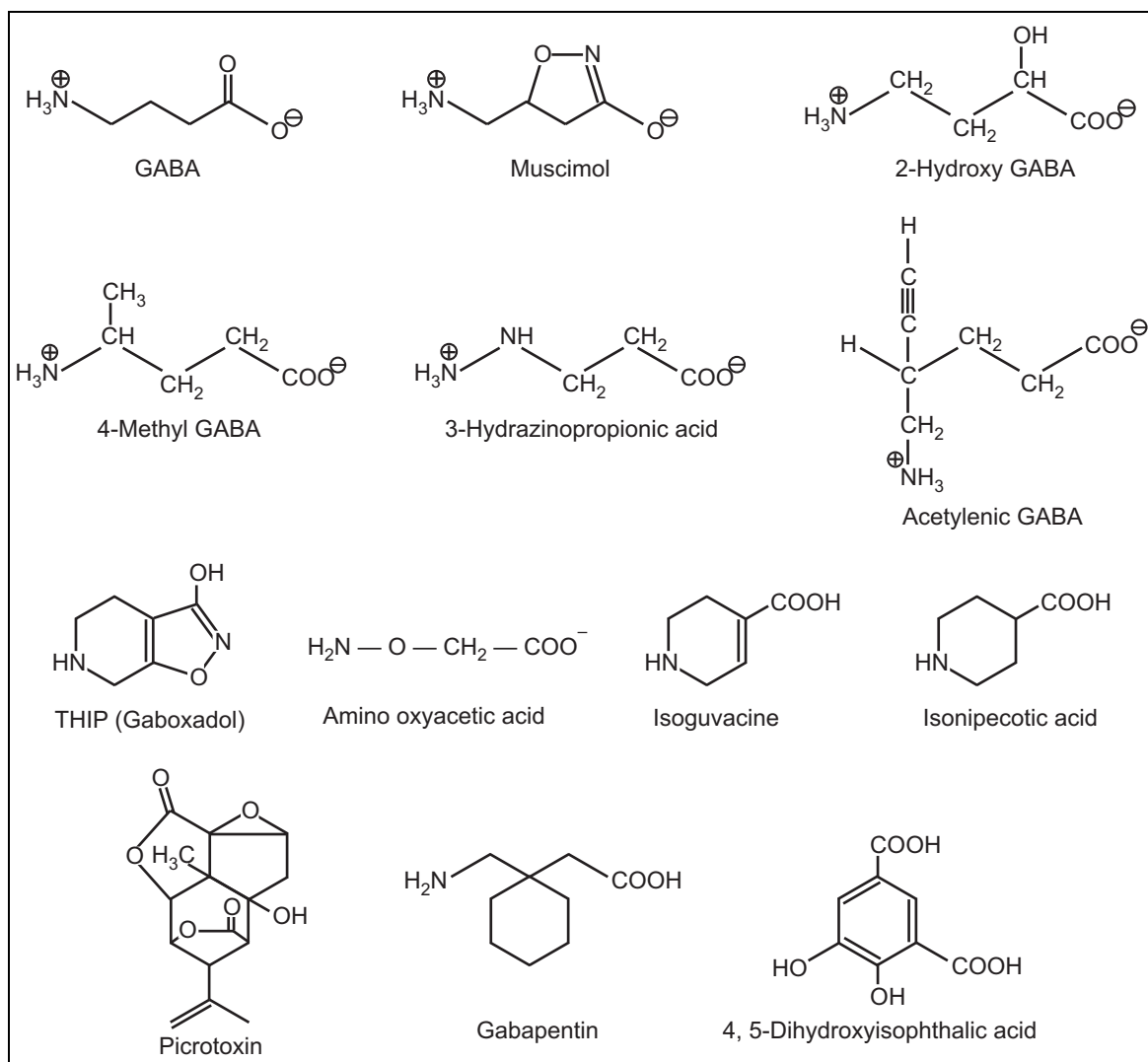
(d) Stripentol is a ethylene alcohol derivative. It acts by inhibition of synaptosomal GABA uptake and inhibition of metabolic transformation of GABA.

(e) Eterobarb has anticonvulsant action probably due to its metabolic conversion into phenobarbitone.

Other examples include zonisamide, topiramate, iodaxaprine and oxacarbazepine. Unlike carbamazepine, oxacarbazepine does not undergo oxidative metabolism to toxic carbamazepine-10, 11-epoxide metabolite.

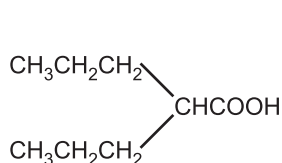
Valdice behaves as a prodrug like valpromide, while di-isopropylacetamide differs from valpromide in being more stable. The amide linkage in the former is protected from hydrolysis by two methyl groups at  $\beta$ -position. The methyl groups prevent attack of amidases through steric hindrance.



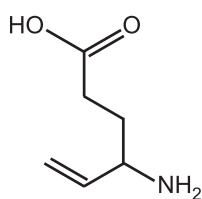
**Table 9.3: Structure of compounds that act at GABA - nergic synapses****Vigabatrin:**

Symptomatic epilepsy can result from specific physiological phenomenon such as brain tumours, syphilis, cerebral arteriosclerosis, multiple sclerosis, Buerger's disease, Pick's disease, Alzheimer's disease, sunstroke or headstroke, acute intoxication, lead poisoning, head trauma, vitamin  $\beta_6$  deficiency, hypoglycaemia and labour.

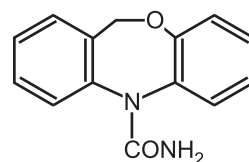
The concentration of GABA is regulated by two pyridoxal -5'-phosphate (PLP) dependent enzymes, L-glutamic acid decarboxylase (GAD) which converts glutamate to GABA and GABA-aminotransferase (GABA-AT) which degrades GABA to succinic semialdehyde (SS). Although succinic semialdehyde is toxic to cells there is no build up of this metabolite because it is efficiently oxidised to succinic acid by the enzyme succinic semialdehyde dehydrogenase (SSADH).



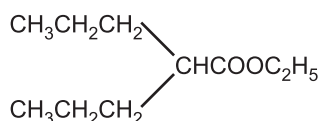
Valproic acid



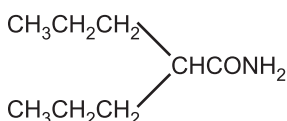
Vigabatrin



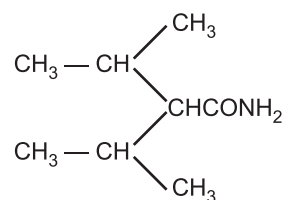
Oxcarbazepine



Valdice



Valpromide



Di-isopropylacetamide

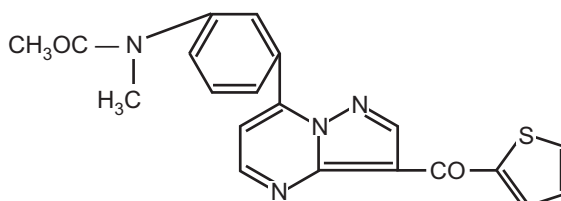
Vigabatrin is an unreactive compound that is converted by the normal catalytic mechanism of the target enzyme (GABA-AT) to a reactive compound which attaches to same enzyme.

Because of vinyl substituent in vigabatrin (1) lipophilicity increases and (2) vinyl, being an electron withdrawing substituent, has the effect of lowering the pKa of the amino group. This would increase the concentration of non-zwitterion form which is more lipophilic than zwitterion.

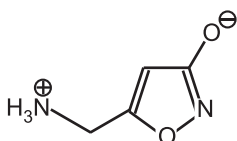
**Table 9.4: Drugs that work by either facilitating (e.g. benzodiazepine agonists, barbiturates), or inhibiting GABA (e.g. picrotoxin, bicuculline, PTZ) ergic transmission**

### (I) GABA AGONISTS / ANTAGONISTS

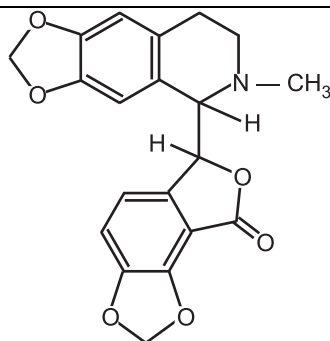
**Indiplon:** It is a non-benzodiazepine hypnotic sedative that acts as GABA-A receptor agonist.



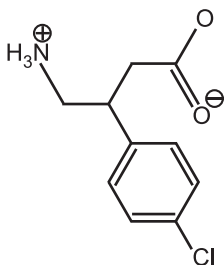
Indiplon



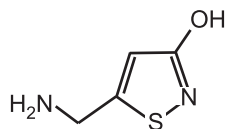
Muscimol  
(GABA-A receptor agonist)



Bicuculline  
(GABA-A receptor antagonist)



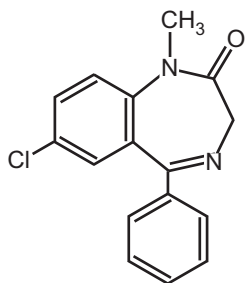
Baclofen  
(GABA-B receptor agonist)



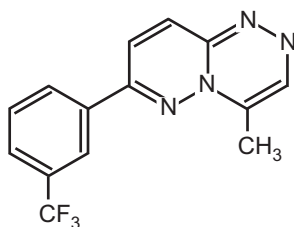
Thiomuscimol  
(GABA-A receptor agonist)

## (II) BENZODIAZEPINE LIGANDS

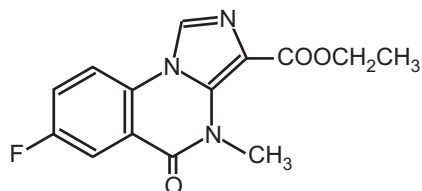
## (III) BENZODIAZEPINE ANTAGONISTS



Diazepam

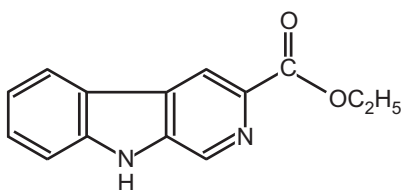


CL 218872  
GABA-modulin inhibitor

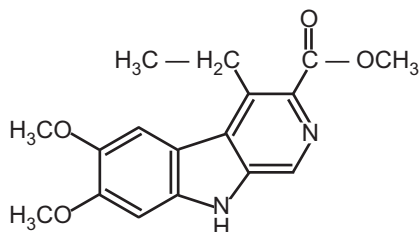


RO15-1788

## (IV) $\beta$ - CARBOLINES

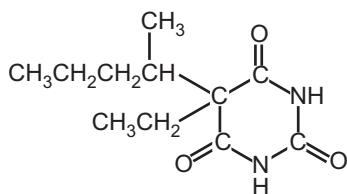


Ethyl- $\beta$ -carboline-3-carboxylate

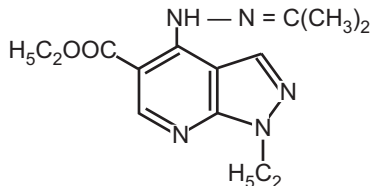


DMCM

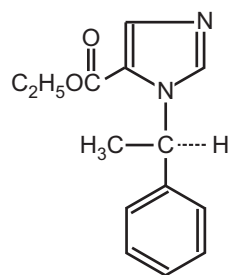
## (V) OTHER MODULATOR

**(a) Depressants:**

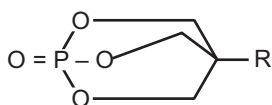
Pentobarbital



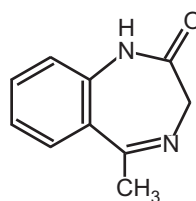
Etazolate



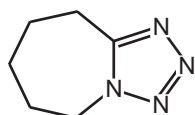
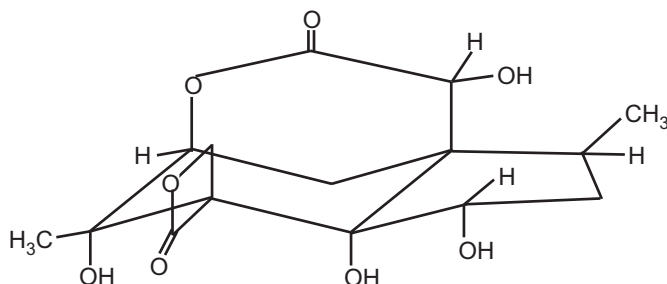
Etomidate

**(b) Convulsants:**

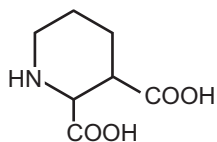
Bicyclophosphate esters



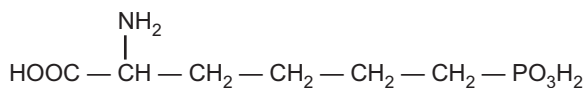
RO5-3663

Pentylene tetrazole  
(PTZ)

Anisatin

**Table 9.5: Structures of analogues of glutamate****(a) Antagonists of glutamic acid:**

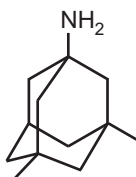
Cis-2, 3-piperidine dicarboxylic acid



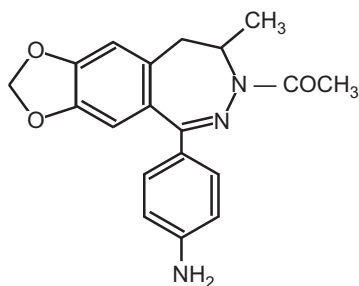
D-2-amino-5-phosphonovalerate

**Memantine** helps to reduce abnormal activity in the brain by binding to NMDA receptors on brain cells and blocking the activity of neuro-transmitter, glutamate. It is used

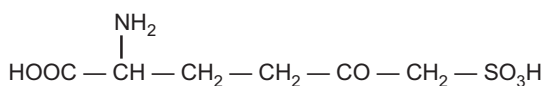
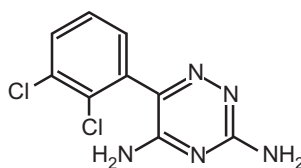
in the treatment of moderate to severe Alzheimer's disease. **Talampanel** may be used in the treatment of epilepsy. It is a non-competitive antagonist of glutamate receptors in brain.



Memantine

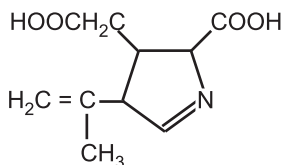


Talampanel

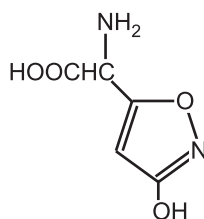
 $\gamma$ -D-glutamylaminomethylsulphonate

Lamotrigine

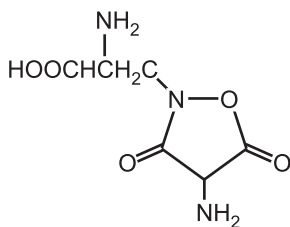
### (b) Agonists of glutamic acid:



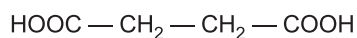
Kainic Acid



Ibotenic Acid



Quisqualic Acid



N-Methyl-D-Aspartate

**Table 9.6: Comparison of oral anticonvulsant drugs**

<b>Anticonvulsant drug</b>	<b>Optimum serum concentrations <math>\mu\text{mol/l}</math> (<math>\mu\text{g/ml}</math>)</b>	<b>Half-life (hours)</b>	<b>Common or important adverse effects</b>
Carbamazepine (Tegretol)	25 - 50 (6 -12)	25 - 50 initially 10 - 30 long term	Drowsiness, dizziness, ataxia, diplopia, rashes, leucopenia, heart block, hyponatremia.
Clobazam (Frisium)	–	18	Drowsiness, dizziness, confusion, ataxia.
Clonazepam (Rivotril)	–	20 - 60	Drowsiness, ataxia, behavioural disturbance, bronchial hyper-secretion in infants.
Ethosuximide (Zarontin)	300 - 700 (40-100)	30 - 70	Nausea, vomiting, tiredness, dizziness, mood disturbances, leucopenia, rashes.
Phenytoin (Epanutin)	40 - 80 (10- 20)	10 - 60	Drowsiness, impaired memory and attention, ataxia, blurred vision, diplopia, gum hyperplasia, hirsutism, acne, facial coarsening, rashes, sensory neuropathy, liver damage, osteomalacia.
Phenobarbitone	80 - 180 (15 - 40)	70- 120	Drowsiness, memory impairment, behavioural disorders, hyperactivity.
Primidone (Mysoline)	–	4 - 11	As for phenobarbitone, occasionally pronounced at start.
Sodium valproate (Epilim)	350 - 700 (50 - 100)	6 - 15	Drowsiness, confusion, tremor, insomnia, nausea, weight gain, alopecia, bleeding tendency, hepatotoxicity, pancreatitis.
Vigabatrin (Sabril)	–	5 - 7	Somnolence, fatigue, confusion, dizziness, weight gain.

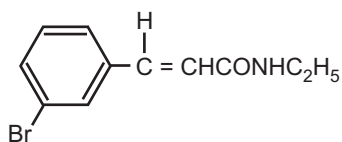
**Antiepileptic drugs marketed in the USA:**

Year introduced	Drug	Trade name (US)	Company
1912	phenobarbital	Luminal	Winthrop
1935	mephobarbital	Mebaral	Winthrop
1938	phenytoin	Dilantin	Parke-Davis
1946	trimethadione	Tridione	Abbott
1947	mephenytoin	Mesantoin	Sandoz
1949	paramethadione	Paradione	Abbott
1951	phenacemide	Phenurone	Abbott
1952	metharbital	Gemonil	Abbott
1953	phensuximide	Milontin	Parke-Davis
1954	primidone	Mysoline	Ayerst
1957	methsuximide	Celontin	Parke-Davis
1957	ethotoin	Peganone	Abbott
1960	ethosuximide	Zarontin	Parke-Davis
1968	diazepam	Valium	Roche
1974	carbamazepine	Tegretol	Geigy
1975	clonazepam	Clonopin	Roche
1978	valproate	Depakene	Abbott
1981	clorazepate	Tranxene	Abbott
1993	felbamate	Felbatol	Carter-Wallace
1994	gabapentin	Neurontin	Parke-Davis

**(11) Miscellaneous agents:**

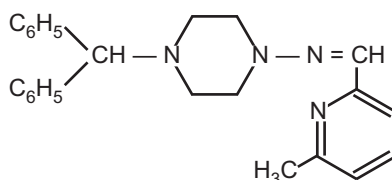
This class includes:

- (1) Amphetamine and related compounds
- (2) Steroids: Corticotropin
- (3) Local anaesthetics: Lidocaine i.v.
- (4) Antimalarials: e.g. quinacrine.
- (5) Vitamins; e.g. vitamin B<sub>6</sub>
- (6) Prostaglandin: e.g., PGE<sub>1</sub> and PGE<sub>2</sub>
- (7) Cinromide
- (8) Tiagabine



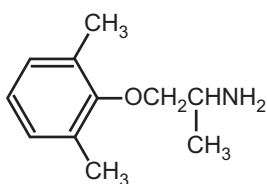
Cinromide

(9) Ropizine:



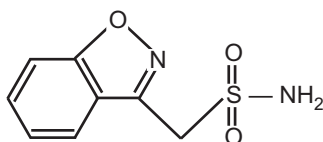
Ropizine

(10) Antiarrhythmic:



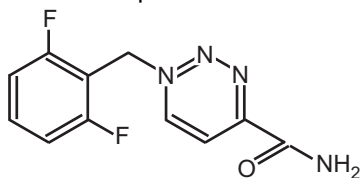
Mexiletine

(11) 2-Amino-7-phosphonoheptanoic acid: It is a derivative of convulsive dicarboxylic acid like aspartic or glutamic acid.

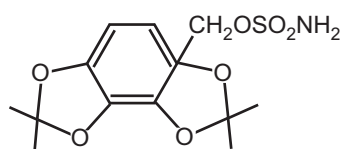


Zonisamide

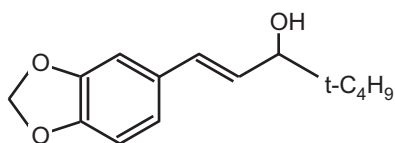
It is sulphonamide drug used for generalized seizures, partial seizures, Lennox-Gestaut syndrome and infantile spasm. It is  $\text{Na}^+$ -channel blocker.



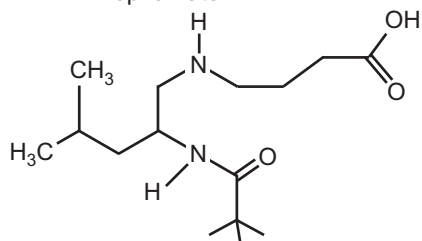
Rufinamide



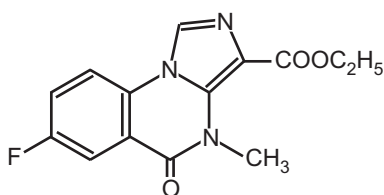
Topiramate



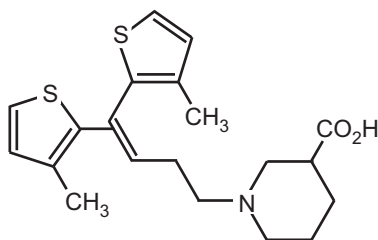
Stiripentol



Progabide



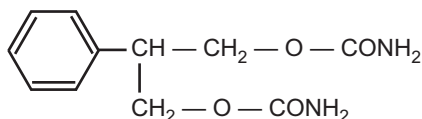
Flumazenil  
(Benzodiazepine antagonist)



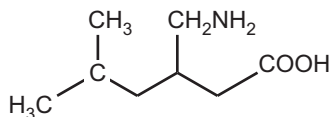
Tigabine

Tigabine is GABA agonist. Chemically it is R (-) N - [4, 4-di - (3-methylthien-2-yl) but - 3-enyl] nipecotic acid HCl. It is a potent inhibitor of GABA uptake into synaptosomal membranes, neurons and glial cells. It thus augments and prolongs the synaptic inhibitory effect of GABA.

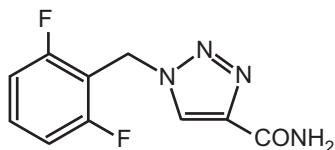
**Felbamate:** It is an anticonvulsant drug used in the treatment of epilepsy. It is used to treat partial seizures.



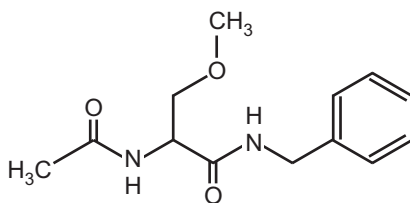
**Pregabalin:** It is an anticonvulsant used to treat neuropathic pain, partial seizures and generalized anxiety disorder.



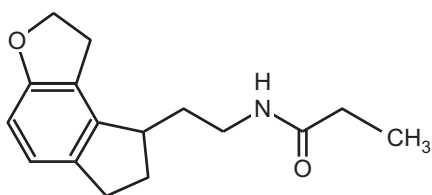
**Rufinamide:** It is a triazole derivative used as an anticonvulsant drug.



**Lacosamide (erlosamide):** It is used as adjunctive treatment of partial onset seizures and diabetic neuropathic pain.



**Ramelteon:** It is a melatonin ( $MT_1$  and  $MT_2$ ) receptor agonist used to treat insomnia, particularly delayed sleep onset. It is a new class of sleep inducing agents that do not bind to GABA receptors. Hence, it is devoid of addiction and withdrawal symptoms.



*Chapter...10***GENERAL ANESTHETICS****♦ SYNOPSIS ♦****10.1 INTRODUCTION****10.2 CHARACTERISTICS OF GENERAL ANAESTHETICS****10.3 STAGES OF ANAESTHESIA****10.4 INHALATION ANESTHETICS****10.5 ULTRASHORT ACTING BARBITURATES****10.6 MISCELLANEOUS AGENTS****10.1 INTRODUCTION**

As the name indicates the principal pharmacological action of this class is to depress the central nervous system. These include,

- (a) General anaesthetics
- (b) Hypnotic-Sedatives
- (c) Tranquilizing agents
- (d) Anticonvulsants
- (e) Central nervous system depressants with skeletal muscle relaxant properties.

**General Anaesthetics**

General anaesthetic is a class of CNS depressant drugs which produce partial or total loss of the sense of pain with a controlled and reversible depression of the functional activity of CNS.

In order to perform more complicated surgical operations, the surgeon needs time and needs a patient whose muscles are relaxed. General anaesthetic serves both these objectives.

**10.2 CHARACTERISTICS OF GENERAL ANAESTHETIC**

- (1) The agents in this class enjoy wide structural variation and hence strict structure-activity relationship cannot be framed out.
- (2) These agents are non-specific in action i.e., they do not interact with specific receptors. Hence, they are thought to be simple general cellular poisons.
- (3) They are used at high concentration and have access to all areas of the body.

**10.3 STAGES OF ANAESTHESIA**

The concept of Blood - Brain - Barrier has been put forward to explain the unique and specialised mechanism by which, brain excludes many substances presented to it by circulation. Important physiological properties governing entry of compounds in brain include: (i) the degree of ionisation at physiological pH (non-ionised compounds penetrate

more readily) (ii) molecular size: above molecular mass 400, there is noticeable increase in penetration and also when cross-section of molecule, (second smallest Van der Waal's diameter) exceeds 10 Å or 1.05 nm and (iii) lipophilicity.

$\log P_o = 2.0 \pm 0.3$  is an ideal lipophilic character to design a neutral molecule for passive penetration into CNS. Under physiologically healthy conditions, polar molecules only enter CNS via specific uptake mechanisms. Hence to target polar compounds in brain by passive diffusion, one should avoid polar H-bonding groups and keep molecular size to a minimum.

### Overton-Meyer Hypothesis of Anaesthetic Activity:

Anaesthesia refers to the complete lack of somatic sensation. Overton, at the turn of the century, attempted to explain drug-induced anaesthesia. He, and later H. H. Meyer, stated that:

- (1) All neutral lipid-soluble substances have depressant properties on neurons.
- (2) This activity is most pronounced in lipid-rich cells.
- (3) The effect increases with increasing partition coefficient, regardless of the structure of the substance.

Although the absolute drug concentration necessary to achieve anaesthesia varies greatly, the drug concentration in the lipid phase - that is, in the cell membrane is within one order of magnitude, or 20-50 nm, for all anaesthetic agents.

In 1954, Mullins, in a modification to the Overton-Meyer hypothesis, proposed that besides the membrane concentration of the anaesthetic, its volume, expressed as its volume fraction (mole fraction  $\times$  partial molar volume), is important. This reasoning implies that the anaesthetic expands the cell membrane, and that anaesthesia occurs when a critical expansion value is reached at about 0.3-0.5 % of the original volume. The surface area of the membrane will also expand by several percentage points.

In general, lipophilic and unionised molecules pass most readily into the central nervous system. In case of general anaesthetic agents, as the concentration is increased, penetration into the CNS increases, resulting into increased depth of anaesthesia.

For convenience, Guedel divided anaesthesia into four separate stages in 1937:

**Stage I: Analgesia:** Consciousness is maintained, analgesia is produced. Since higher cortical centres are depressed, this stage is also called as Cortical stage.

**Stage II: Delirium or stage of excitement:** Consciousness is lost. The further removal of cortical inhibition leads to excitement and the patient may shout and struggle violently. He may salivate, vomit or develop cough.

The first two stages are combinedly termed as induction period.

**Stage III: Surgical anaesthesia:** Skeletal muscles are relaxed and hence, most of the operative procedures are performed at this stage. It is further subdivided into four planes representing progressive increase in depth of anaesthesia and decreased respiration. Respiration ceases altogether as stage IV is entered.

**Stage IV: Respiratory Paralysis or Medullary Paralysis:** This is a toxic or overdose stage in which there is a respiratory and cardiovascular collapse and the tissues rapidly become anoxic.

In modern practice, anaesthetics either by intravenous or rectal routes (basal anaesthetics or fixed anaesthetics) are generally given to cause loss of consciousness before a volatile anaesthetic is given and hence transition from complete consciousness (Stage I) to surgical anaesthesia (Stage III) is so rapid that none of the earlier stages of anaesthesia can be seen.

#### Preanaesthetic Medications:

- (1) Generally a hypnotic is given on the night before, to assure a good night sleep.
- (2) One or two hours before surgery, atropine (or hyoscine) is usually administered to prevent excess secretion of saliva or mucous which might impede the work of anaesthetist.
- (3) Morphine (or Pethidine) is also given to minimize fear and apprehension. Although anaesthesia is often induced with a basal anaesthetic agent, it is often maintained with a gaseous or volatile anaesthetic which is administered by various techniques. The simplest one consists of dropping the liquid anaesthetic on a gauze or other absorbent material supported over the patient's nose and mounted by a framework, forming a mask. But the vapours, often explosive (ether) may spread into the surrounding area. The new techniques (anaesthetic machine) allow controlled supply of oxygen, carbon dioxide and anaesthetic concentrations by means of flowmeters.

#### Classification:

Besides on chemical basis, general anaesthetic may also be classified on the basis of their physical state, like.

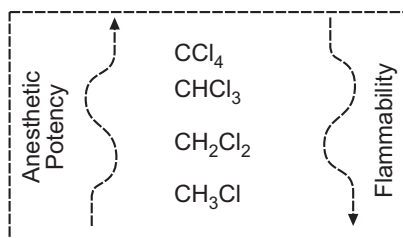
- (a) **Volatile anaesthetics:** e.g., Ether, chloroform, trichloroethylene, halothane, fluroxene, methoxyflurane, vinyl ether and ethylchloride.
- (b) **Gaseous anaesthetics:** Nitrous oxide and cyclopropane.
- (c) **Non-volatile anaesthetics:** Ultra short acting barbiturates, ketamine and propanidid.

**Table 10.1: Classification of volatile anaesthetic agents**

Class	Examples
1. Hydrocarbon	Cyclopropane, Ethylene
2. Halogenated hydrocarbon	Halothane, Desflurane
3. Ether	Diethyl ether, Vinyl ether
4. Alcohol	Trichloroethanol
5. Ultrashort acting barbiturate	Thiopental sodium, Methohexital sodium
6. Miscellaneous agents	Nitrous oxide, Ketamine hydrochloride, Propanidid

## 10.4 INHALATION ANESTHETICS

The replacement of hydrogen of low molecular weight ethers and hydrocarbons by halogen results in an increase in its anaesthetic potency with the proportional decrease in its flammability, e.g. –

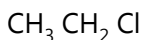


But this halogen substitution is also accompanied by an increase in toxicity which has limited their use as anaesthetics.

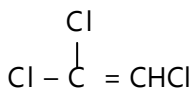
(1) Among the halogens, some of the chlorinated analogues are used clinically to some extent. e.g. chloroform, ethyl chloride and trichloroethylene.



**Chloroform**



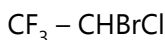
**Ethyl chloride**



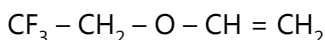
**Trichloroethylene**

(2) Bromination of hydrocarbons is also tried but none of the compounds is found clinically applicable.

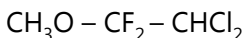
(3) Additional qualifications (like decreased toxicity, decreased flammability, decreased boiling point with an increase in the anaesthetic potency) are associated with the fluorinated hydrocarbons and ethers e.g. Halothane, Fluroxene, Methoxyflurane, Enflurane, Isoflurane and Sevoflurane.



**Halothane (1956)**



**Fluroxene**



**Methoxyflurane**



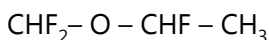
**Enflurane (1973)**



**Isoflurane (1981)**



**Sevoflurane**



**Desflurane (1992)**

Halothane, a volatile and non-flammable liquid, is one of the most commonly employed anaesthetic agents (2 - 2.5%). Since, it is light sensitive, it is stored in brown bottles and stabilised by 0.01% thymol.

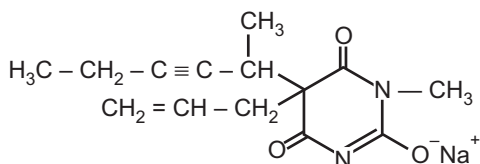
## 10.5 ULTRASHORT ACTING BARBITURATES

For rapid induction of anaesthesia, the sodium salts of ultrashort acting barbiturates are usually administered intravenously or by retention enema. The advantages associated with these agents are:

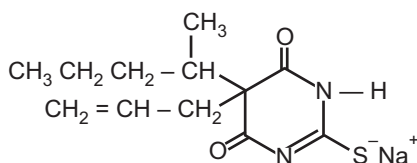
- Smooth induction
- Fair muscular relaxation
- Absence of salivary secretions
- Non-explosive nature
- Short and uncomplicated recovery.

The potent respiratory depression is the risk generally associated with their use and hence they are used to produce rapid and pleasant anaesthesia, which is then maintained with the volatile anaesthetics. Their high lipid solubility and rapid destruction of these drugs by liver, contribute to their short-duration of action.

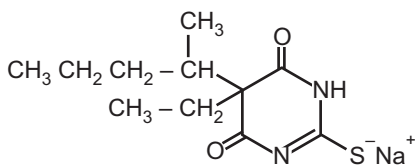
### Examples:



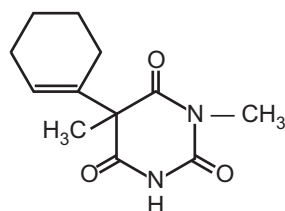
Methohexital sodium



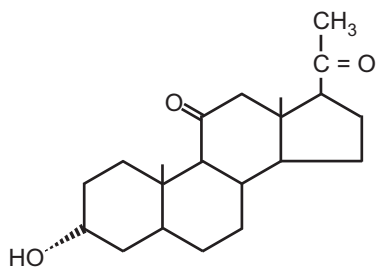
Thiampylal sodium



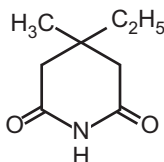
Thiopental sodium



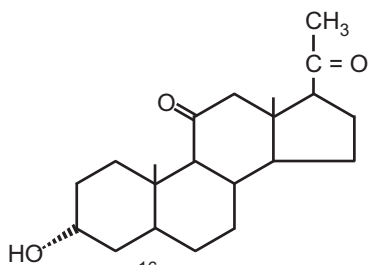
Hexobarbital



Alfaxalone



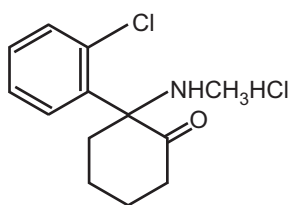
Bemegride

 $\Delta^{16}$  - Alfaxalone

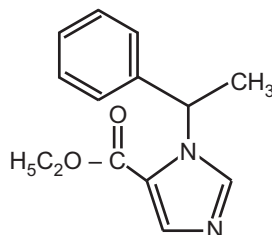
**10.6 MISCELLANEOUS AGENTS**

**(a) Ketamine hydrochloride:** Generally used as an induction anaesthetic prior to the use of other anaesthetic due to its rapid onset and short duration of action on parenteral administration and may be of value in short surgical procedures which do not require skeletal muscle relaxation.

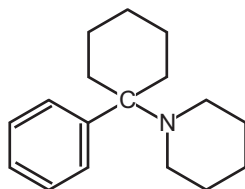
The side-effects include an increase in blood pressure, delirium, hallucinations and unpleasant dreams. Diazepam, promethazine, etc. are the drugs which reduce or abolish these untoward effects.



Ketamine hydrochloride



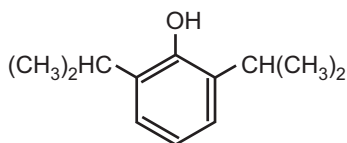
Etomidate



Phencyclidine

Ketamine was developed in 1970 as a structural analogue of phencyclidine, a parenteral anaesthetic agent.

**(b)**

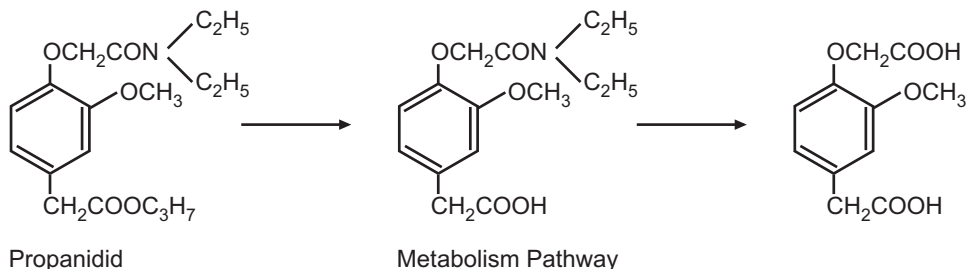


Propofol

It acts via GABA receptor

**(c) Nitrous oxide (laughing gas):** Nitrous oxide was introduced in 1844. (one of the oldest of anaesthetics). Nitrous oxide is a non-flammable, non-irritating and powerful analgesic with least potent anaesthetic properties. If used alone, a concentration of 80 - 85 % of nitrous oxide is required to produce surgical anaesthesia, which is associated with the risk of hypoxia and hence it is currently used as an adjunct to ether or halothane in most of the procedures. But since it is a good analgesic in subtherapeutic concentration (20 - 30%), it is used for minor dental operations, painful procedure e.g. dressing of burns etc. Very prolonged administration of nitrous oxide may result in the interference with the production of both leukocytes and red blood cells. It is one of the safest anaesthetic agents. It does not exert any toxic effect on liver, kidney, the gastrointestinal tract and the CNS. It is rapidly excreted in unchanged form, mainly through lungs.

**(d) Propanidid:** It is a eugenol derivative (Eugenol is a principal constituent of oil of cloves). It is an oily liquid having anaesthetic action of very short duration when given intravenously. Unlike other anaesthetics it has a stimulant action on respiration while depressant action on myocardium, resulting into hypotension. The nausea and vomiting is more frequent with propanidid than any other I.V. anaesthetic. The cardiovascular collapse is frequent in (an inborn or acquired) hypersensitive patients when propanidid is given intravenously.



Propanidid is attacked at its ester linkage by the serum cholinesterase in plasma and liver. The resulting acid further undergoes metabolism with the loss of diethylamino group.

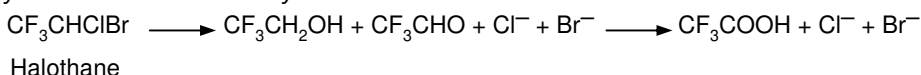
### Mechanism of action:

Due to wide structural variations, general anaesthetics are thought to be of non-specific in their action i.e. they do not act on specific receptor sites. The most recent theories include the lipid solubility hypothesis proposed by Overton and Meyer, which correlates the potency of the anaesthetic agent with its lipid solubility. The hydrocarbon core of the lipid bilayer region of nerve membranes accommodates the anaesthetic molecules which expand the membrane by fluidizing or disordering the phospholipid bilayer and thus inhibits the essential conformational changes of membrane protein involved in ionic conductance. The membrane protein itself, may also be the site of action. These proteins comprise the apolar amino acid residues embedded in the lipid bilayer of the nerve membrane. The anaesthetic agents modify the properties of the lipid bilayer in which these proteins function and thus inactivate these proteins which are essential for proper functioning of CNS.

### Metabolism of Volatile Anaesthetics:

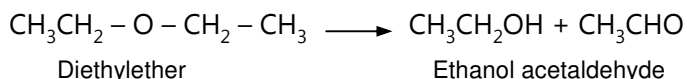
A small amount of the anaesthetic undergoes the metabolism.

(a) Hydrocarbons are mainly converted to



(b) Halogenated hydrocarbons undergo dehalogenation by microsomal enzymes.

(c) Ether metabolism occurs in two phases. In the first phase, ether is converted to an alcohol



and aldehyde which are further metabolised to  $\text{CO}_2$  in second phase.



## OPIOID ANALGETICS

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### ♦ SYNOPSIS ♦

11.1 INTRODUCTION

11.2 OPIUM ALKALOIDS

11.3 OPIOID RECEPTORS

11.4 CHEMISTRY OF OPIOIDS

11.5 NARCOTIC ANTAGONISTS

11.6 ENDOGENOUS OPIOIDS

11.7 THERAPEUTIC USES OF OPIOID  
ANTAGONISTS

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### 1.1 INTRODUCTION

Analgesia may be defined 'as a state of relative insensitivity to pain, where the capacity to tolerate pain is increased without the loss of consciousness'. The term "analgesic" is generally applied to the agents or actions required to produce analgesia.

#### Classification:

Analgesics are divided into two main classes:

- (1) Narcotic analgesics (Centrally acting drugs)
- (2) Non-narcotic analgesics (Peripherally acting drugs)

#### (1) Narcotic analgesics:

Serturmer, in 1805, isolated and discovered the potent analgesic activity of Morphine, an alkaloid isolated from the juice of unripened seed capsules of the poppy plant, *Papaver somniferum*. The word opium is derived from Greek word "opos" means juice.

The term opioid is used generally to designate collectively the drugs (natural or synthetic) which bind specifically to any of subspecies of receptors of morphine and produce, to varying degrees, morphine like actions. They are often known as the narcotic analgesics due to their ability to produce drug dependence. With the development of many analgesics which are morphine derivatives with little tendency to produce physical dependence, the term narcotic is no longer useful.

Other actions which are associated with narcotic analgesics are sedation, and constipation (useful in the control of diarrhoea). In therapeutic doses, morphine sometimes produces nausea or vomiting. The related compound, apomorphine is a powerful emetic agent.

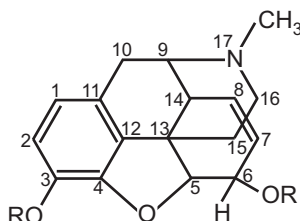
## 11.2 OPIUM ALKALOIDS

Opium contains 25% by weight alkaloidal compounds. The opium alkaloids can be divided chemically into two distinct classes:

- (a) Phenanthrenes: e.g., morphine, codeine and thebaine.
- (b) Benzyloisoquinolines: e.g. papaverine and noscapine.

### Opium alkaloids:

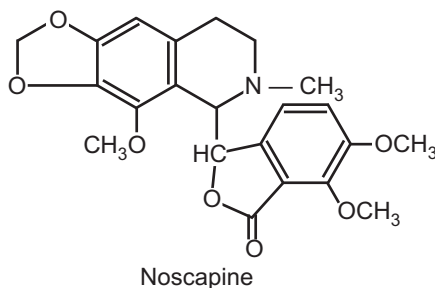
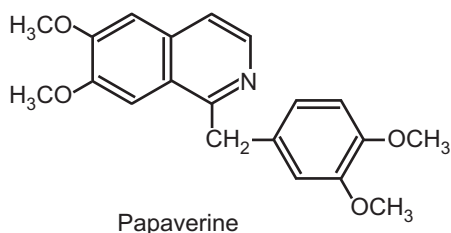
#### (a) Phenanthrenes:



- (1) Morphine,  $R = -H$ ;  $R' = -H$
- (2) Codeine;  $R = -CH_3$ ;  $R' = -H$
- (3) Thebaine;  $R = -CH_3$ ;  $R' = -CH_3$

A double bond between  $C_5$  and  $C_6$ .

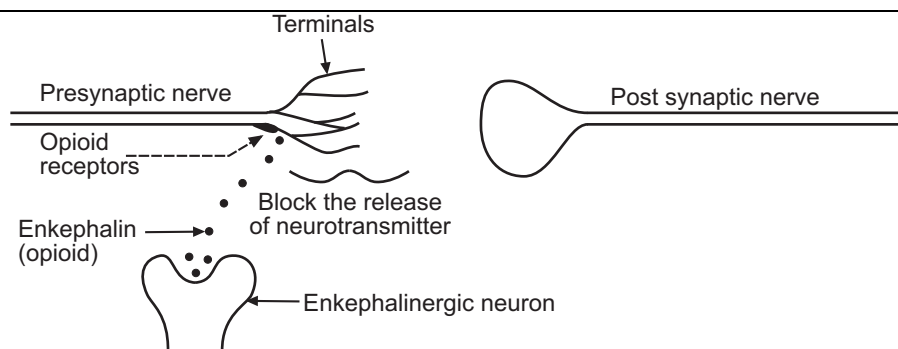
#### (b) Benzyloisoquinolines:



Opioids act as agonists of endogenous substances known as endorphins (a group of morphine like peptides), interacting with stereospecific binding sites or receptors in the brain and other tissues. Enkephalins represent the simplest members of endorphins. They are located in short interneurons predominantly in the areas of the CNS which are related to the perception of pain, to movement, mood, and behaviour and to the regulation of neuroendocrinological functions.

Opioid mediated inhibition of transmitter release in various mammalian cells has been reported to involve either a reduction in the influx of  $Ca^{++}$  through activation of  $k$  – receptors or an increased outward  $K^+$  conductance through  $Ca^{++}$  activated  $K^+$  channels following activation of either  $\mu$  or  $\sigma$  receptors. The inflow of potassium ions hyperpolarizes the membrane potential. This results in decrease in neurone excitability.

Opioids have been shown to inhibit either basal or neurotransmitter-stimulated increases in adenylate cyclase activity in several areas of the mammalian CNS. The mechanism for opioid inhibition of adenylate cyclase appears to involve stimulation of a high affinity membrane associated GIPase, reflecting an activation of the guanine nucleotide regulatory binding protein, G.



**Fig. 11.1: Role of enkephalin in pain impulse perception**

Thus, under the influence of enkephalin, pre-synaptic terminals fail to release neurotransmitter in the synaptic cleft and pain impulse is not received by post-synaptic neuron. The opioid mediated fall in cyclic AMP levels also contributes to produce analgesia.

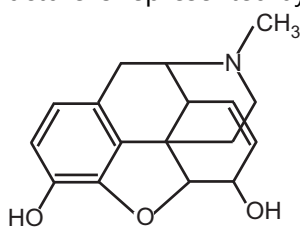
It is assumed that all opioids (morphine like drugs) produce their effects by mimicking the actions of endogenous enkephalins.

### 11.3 OPIOID RECEPTORS

Morphine causes analgesia by selectively acting on receptors situated both in the higher centers and the spinal cord. The existence of an opioid receptor is supported by:

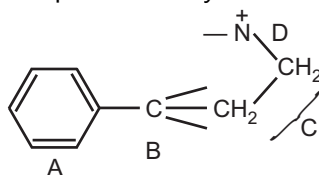
- (1) SAR of morphine like compounds.
- (2) A close structural similarities between opioid agonists and antagonists.
- (3) Competitive inhibition of actions of morphine agonists by narcotic antagonists.

The ideal narcotic analgesic structure is represented by Morphine.

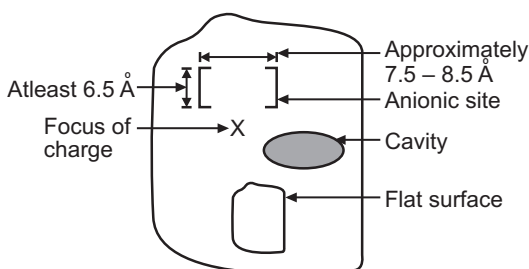


Morphine

The structural features which are recognised to be essential for the perfect fit of a narcotic analgesic on receptors are represented by A, B, C and D.



- where, A = Phenyl or aromatic portion  
 B = Quaternary carbon  
 C = Ethylene bridge  
 D = Tertiary nitrogen



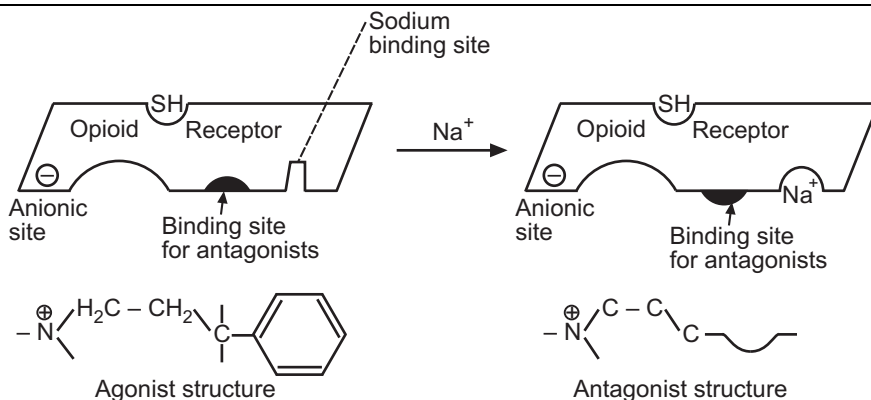
**Fig. 11.2: Beckett and Casy's model of the analgesic receptor site**

Beckett and Casy (1954) proposed that an opioid receptor is composed of three prominent parts.

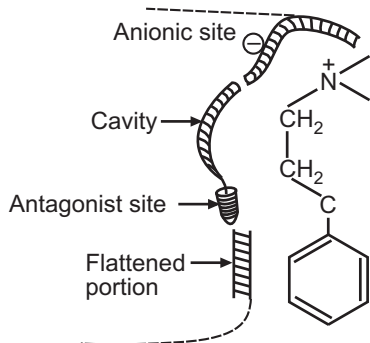
- (1) A flattened part which holds the aromatic portion of an analgesic molecule through van der Waal's forces.
- (2) A cavity or a hollow portion which entraps the ethylene bridge.
- (3) An anionic site which holds the tertiary nitrogen which is assumed to be ionised at physiological pH. The pKa values of most of analgesics fall in the range of 7.8 - 8.9 so that tertiary nitrogen is equally present in ionized and un-ionized forms at physiological pH. The drug crosses the blood-brain barrier as the free base while interacts with the receptor in ionic form.

The fact that these sites do not bind other substances and are saturated by even very low concentrations of opioids explains the highly stereospecific orientation of these three components of opioid receptors.

Similarly GTP, GDP and the non-hydrolyzable analogue Gpp (NH)p, reduce agonist affinity while divalent ions such as magnesium increase agonist affinity. Sodium ions and Gpp (NH)p were found to decrease the binding of agonists to  $\mu$  sites more effectively than to  $\delta$  sites with binding to  $\kappa$  sites least affected.



**Fig. 11.3: Sodium ion effect on opioid receptor as proposed by Snyder and co-workers**



**Fig. 11.4: Receptor–Drug interaction**

The narcotic antagonists competitively inhibit the access and binding of morphine agonists to the opioid receptors but lack the intrinsic activity (ability to initiate the biological response).

The irregular distribution of these opioid receptors in the various regions of central nervous system explains the untoward effects associated with the opioids; like, euphoria, sedative and emetic actions.

Sodium ions are reported to reduce the affinity of opioid receptors for agonists and to increase the affinity for narcotic antagonists. It is suggested that Na<sup>+</sup> protects the sulfhydryl group of receptors from the alkylating agents by changing the conformation at opioid receptors. And while doing so, Na<sup>+</sup> ions modify the opioid binding sites, which are now more suitable for binding of antagonist molecules than that of the agonists.

No other ion showed this selectivity. Na<sup>+</sup> ion effect is used to distinguish pure agonist, antagonist and mixed agonist-antagonist.

Sodium index -  $IC_{50}^{Na}/IC_{50}$  - It is the ratio of concentration of test drug required to inhibit by 50% the stereospecific binding of standard tagged, pure antagonist in presence of sodium ion.

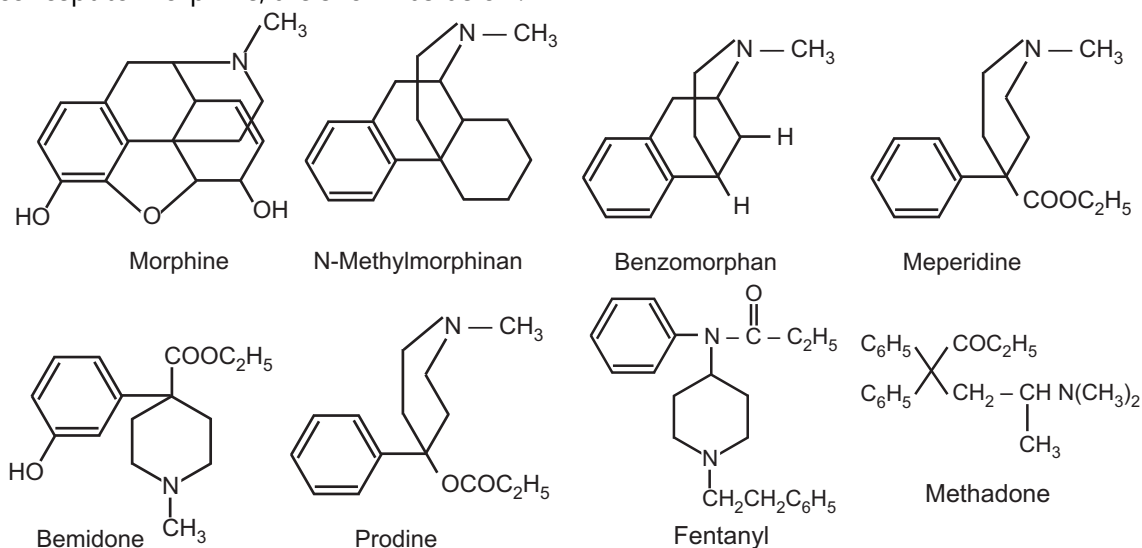
For pure antagonist index will be in range of 1-2 (ideally 1).

For pure agonist it is 10-60 and for mixed agonist - antagonist the ratio is 3-7.

### **Molecular dissection of morphine:**

Morphine is a complex pentacyclic skeleton which was considered to be responsible for a number of adverse effects associated with morphine skeleton. Hence, attempts were made to identify the pharmacodynamically essential part through the application of molecular dissection concept. In this attempt, various components of the skeleton are gradually removed one by one. Since the resulting new skeletons were found to retain the activity, the part removed, was considered non-essential for the analgesic activity.

Various series which can be obtained through the application of molecular dissection concept to morphine, are shown as below.



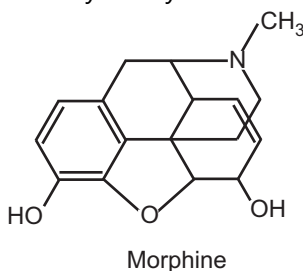
## 11.4 CHEMISTRY OF OPIOIDS

Though morphine itself is a potent analgesic agent, the serious side-effects (like, euphoria, sedation, respiratory depression, addiction and tolerance) associated with morphine, initiated the attempts for modification of this structure, in order to increase the therapeutic usefulness and to widen the difference between desirable action and toxicity syndromes.

The various modifications of the morphine molecule are categorised as:

- (1) Early changes on morphine nucleus.
- (2) Modifications carried out in 1929 by Small, Eddy and co-workers.
- (3) Modifications carried out in 1938 by Eisleb and Schaumann.
- (4) Modifications carried out by Grewe in 1946.

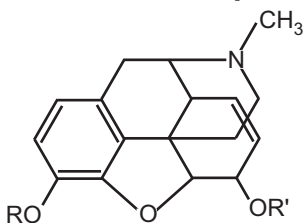
Like other simple semisynthetic analogues of morphine (like, codeine, heroin, hydromorphone, hydrocodone) many other classes of chemically distinct opioids have been prepared, some of which have been employed clinically. These include the morphinans, benzomorphan, methadones, phenylpiperidines, propionanilides, and thiambutene and benzimidazole derivatives. All these classes share certain common characteristics with the prototype, morphine, which are shown by heavy lines in the structure of the morphine.



**(1) Early changes on morphine prior to the study of Small, Eddy and co-workers:**

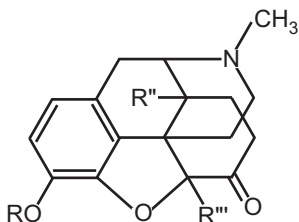
The analgesic properties of morphine are found in the (–) enantiomer which has the absolute configuration 5(R), 6(S), 9(R), 13(S), 14(R).

Prior to 1929, many analogues of morphine had been prepared by attempting simpler molecular modifications. Except hydromorphone and hydrocodone, which remain in clinical use, none were found to be superior to morphine.

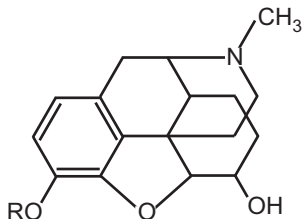
**Simpler Derivatives of Morphine Prior to 1929:****(A)**

- (1) Codeine; R = – CH<sub>3</sub>; R' = – H
- (2) Ethylmorphine; R = – C<sub>2</sub>H<sub>5</sub>; R' = – H
- (3) Heroin; R = – CH<sub>3</sub>CO; R' = – CH<sub>3</sub>CO

Codeine, is only one tenth as analgesic as morphine. This indicates the need of a free phenolic hydroxyl for greater potency. While heroin is more potent despite having a significantly weaker opioid receptor binding affinity. Reasons – (1) Heroin is more lipophilic than morphine. (2) It gets rapidly converted *in-vivo* to the active metabolite,  $\sigma$ -acetylmorphine and morphine.

**(B)**

- (1) Hydromorphone; R = – H; R'' = – H; R''' = – H.
- (2) Hydrocodone; R = – CH<sub>3</sub>; R'' = – H; R''' = – H.
- (3) Oxycodone; R = – CH<sub>3</sub>; R'' = – OH; R''' = – H.
- (4) Methylhydromorphone R = – H; R'' = – H; R''' = – CH<sub>3</sub>.

**(C)**

- (1) Dihydromorphine; R = H
- (2) Dihydrocodeine; R = CH<sub>3</sub>

All the above compounds were prepared by modifying only the easily changeable peripheral groups and not according to the principles of structure–activity relationship.

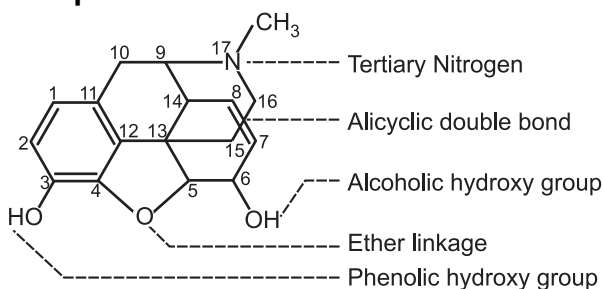
## (2) Modifications carried out after 1929 by Small, Eddy and co-workers:

Prior to 1929, all morphine analogues had been prepared through non-rational, random search for new drugs.

The first systematic effort had been made by Small, Eddy and co-workers to investigate the structure–activity relationship in morphine molecule during their 10 year synthesis and testing programme, initiated by the National Research Council of United States. Though their studies were far more comprehensive, the principal targets, chosen for modifications in morphine structure were,

- (1) The peripheral groups and simple skeletal modifications on alicyclic ring.
- (2) The peripheral group and simple skeletal modifications on aromatic ring.
- (3) The tertiary nitrogen.

### Peripheral Groups on Morphine:



### (1) Modifications on alicyclic ring:

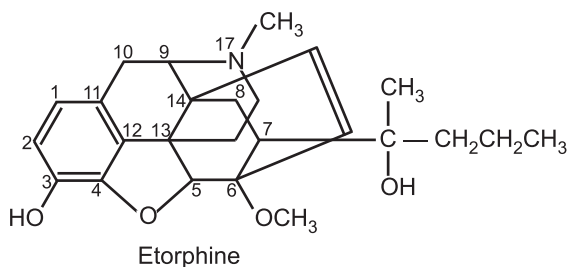
**(a)** The C-6  $\alpha$ -hydroxyl group is methylated, esterified, oxidised, removed or replaced by halogen in order to get more potent analgesics. e.g., codeine, heroin, chloromorphide. But there is also a parallel increase in toxicity.

#### **(b) The C-8 presents the next site for modification:**

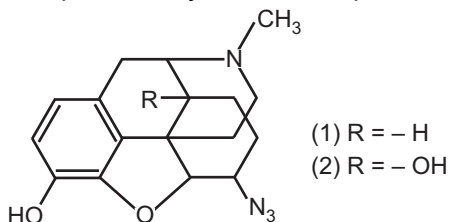
It has got a hydrogen atom and a double bond. The outcome of catalytic hydrogenation is the compounds dihydrocodeine and dihydromorphine which are the precursors of more potent ketones, dihydrocodeinone and dihydromorphinone. Similarly C-8  $\beta$ -halo derivatives are found to be more potent analgesics than morphine.

#### **(c) C-14:**

Introduction of 14-OH group in dihydroforms yielded the still more potent 14-hydroxy-dihydrocodeinone and 14-hydroxydihydromorphinone. Bridging of C<sub>6</sub> and C<sub>14</sub> through a ethylene linkage is also tried e.g., etorphine. It is about 200 times more potent than morphine in man.



(d) Introduction of any new substituent further, does not enhance the activity. 5-Methyl dihydromorphine and azidomor-phinines may be the exceptions.



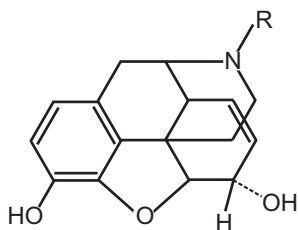
Azidomorphines

## (2) Modifications on phenyl ring:

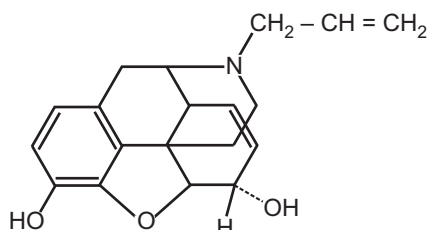
- (a) An intact benzene ring is, in general, essential for analgesic activity.
- (b) Modification of C<sub>3</sub> – phenolic hydroxyl group causes a decrease in analgesic activity.
- (c) Any further substitution in phenyl ring generally diminishes activity. The only exception is 1-fluoro codeine which possesses the same analgesic activity as that of codeine.

## (3) The tertiary nitrogen:

- (a) When R is methyl, n-pentyl or n-hexyl chain, it results into opioid agonists.
- (b) The N-phenylethyl group enhances the analgesic activity in desmorphine, codeine and heterocodeine.
- (c) N-allyl and N-cycloalkylmethyl functions impart narcotic antagonistic properties to the molecule. e.g.,

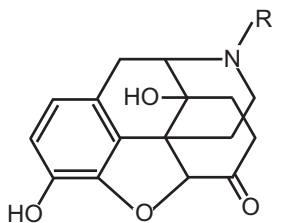


Morphine; R = - CH<sub>3</sub>



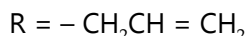
N-allylmorphine (Nalorphine)

Nalorphine was the first clinically useful narcotic antagonist. Its unpleasant psychomimetic and hallucinogenic properties preclude its use as analgesic, though it is a potentially valuable non-addict drug with partial agonistic features.

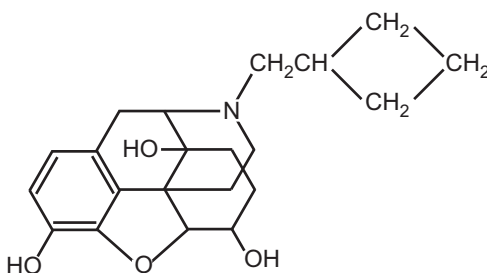
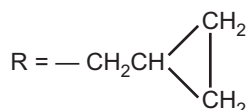


Oxymorphone; R = - CH<sub>3</sub>

**(1) Naloxone:** It is a hydrazone of naltrexone. It is a long acting antagonist with a comparable duration of action.



**(2) Naltrexone:**

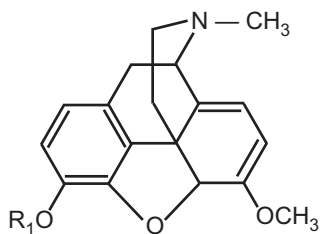


Nalbuphine

### Derivatives of Thebaine:

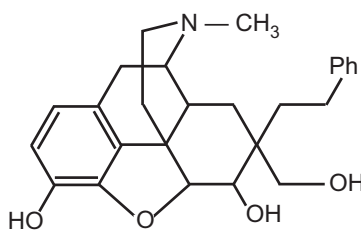
Since, most of the opioids discovered in this period (1929 – 38) of morphine prototype, though more potent than morphine, are also associated with the undesirable psychotomimetic effects. So Bentley and Hardy postulated that it might be a more rigid molecular structure which is important to act with a single pain relieving receptor and not with other side-effects evoking centres. This led to the synthesis of thebaine derivatives.

Diel-Alder adducts of the diene system in thebaine are known collectively as oripavines.

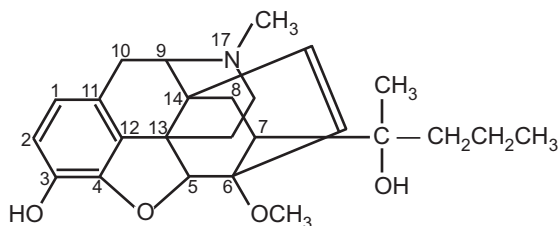


Thebaine;  $R_1 = -CH_3$

Oripavine;  $R_1 = -H$



(1)

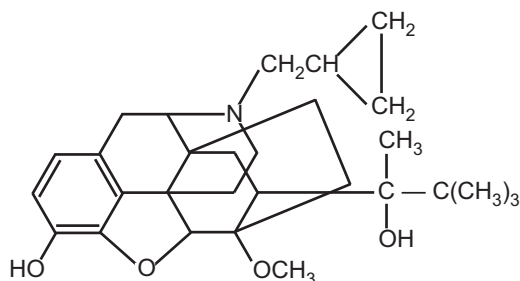


Etorphine (Pure agonist)

Above compound (1) is about 700 times more potent than morphine.

(1) Etorphine is a pure agonist (1000 times more potent than morphine). Because of its side-effect profile, its use is restricted to veterinary medicine as a sedative for large animals.

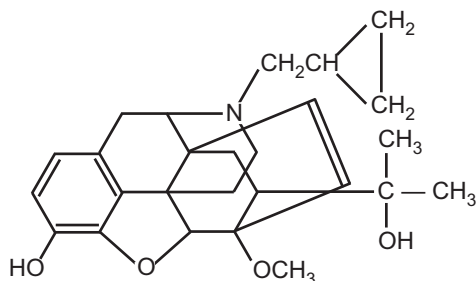
(2)



Buprenorphine (Partial agonist)

Buprenorphine is about 100 times active as morphine as agonist and four times as active as nalorphine as antagonist and is therefore non-addicting and without psychotomimetic effects.

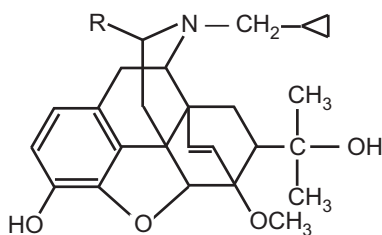
(3)



Diprenorphine (Antagonist)

Diprenorphine is a potent narcotic antagonist (100 times more potent than nalorphine). The additional alkyl substitution at C-7 is able to produce agonist, partial agonist and antagonist which suggests an evidence for an additional lipophilic binding site at the opioid receptor.

The oripavine derivative, 16 – methyl cyprenorphine is the most selective nonpeptide delta antagonist.



Cyprenorphine (R = H)

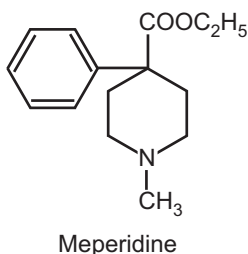
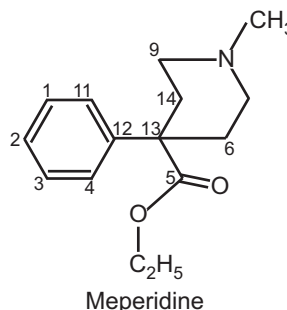
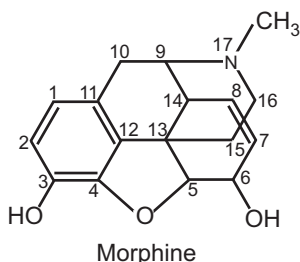
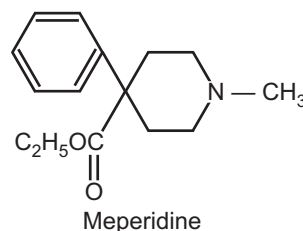
In general, in morphine series, replacement of N-methyl group by larger alkyl groups not only lowers analgesic activity but potentiates narcotic antagonistic properties of the molecule. The only exception to this generalisation, is the N- phenethyl series.

### (3) Modifications carried out by Eisleb and Schaumann:

The 1930s saw many new antispasmodics of general formula  $\text{ArCO}_2(\text{CH}_2)_2\text{NR}_2$ ,  $\text{Ar}_2\text{CHCO}_2 - (\text{CH}_2)\text{NR}_2$  and similar structures. The rules of isosterism emerging at that time emphasized that reversed esters could be a good variation to improve anticholinergic activity. This led to synthesis of meperidine by Eisleb in 1930.

It had lived upto its expectations and had moderate antispasmodic as well as sedative properties. When Schaumann tested it in the cat, he was surprised by an exhibition of Straub's tail, a phenomenon (test for analgesic activity) associated with morphine. Further studies indicated that meperidine had 10–12% of overall activity of morphine. Schaumann succeeded to spot the segment in morphine structure similar to meperidine as a result of molecular dissection.

The discovery of analgesic properties of meperidine opened new avenues for the search of simpler, relatively small, structurally uncomplicated analgesics.



Meperidine was not designed by molecular dissection. It was of "reversed" antispasmodic structure and its analgesic properties were observed during pharmacological workup.

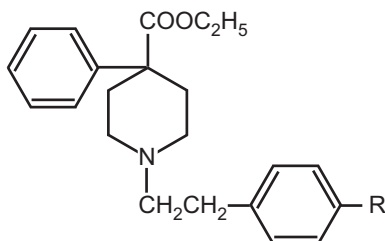
The various modifications of meperidine and related compounds are vaguely divided into four major categories:

- |                 |                     |
|-----------------|---------------------|
| (a) meperidines | (b) bemidones       |
| (c) prodines    | (d) fentanyl series |

**Structure-Activity Relationship:**

(1) Replacement of 4-phenyl group by hydrogen, alkyl, aroalkyl or heterocyclic group results in reduced activity.

(2) Many N-substituted analogues of meperidine have been prepared. Anileridine is employed clinically.

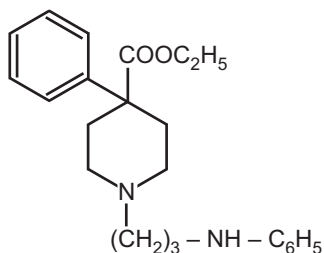


Anileridine : R =  $\text{-NH}_2$

Pheneridine : R =  $\text{-H}$

(3) Replacement of carbethoxyl group ( $\text{-COOC}_2\text{H}_5$ ) by acyloxy group ( $\text{OCOC}_2\text{H}_5$ ) results in better analgesic activity.

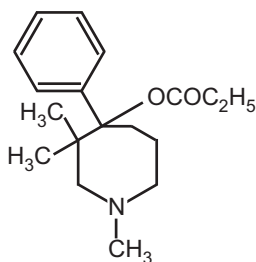
(4) The replacement of N-methyl group by various aralkyl groups can increase the analgesic property markedly.



Piminodine

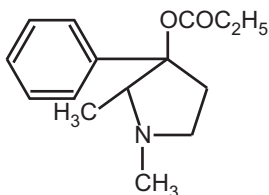
(5) Series of compounds were prepared where piperidine is enlarged to 7- membered azepine ring.

Proheptazine is among the more active analgesic agents in higher ring homologue of meperidine.



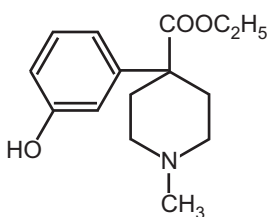
3, 3-Dimethyl-4-phenyl-4-propionoxy hexahydroazepine (proheptazine)

(6) Substitution of the piperidine ring with 5-membered pyrrolidine ring is also successful.

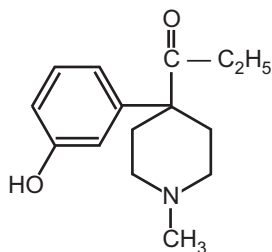


Prodilidene

(7) The presence of m-hydroxyl group in the phenyl ring resembles that of  $C_3$  phenolic hydroxyl group in the morphine. Bemidone represents this class.



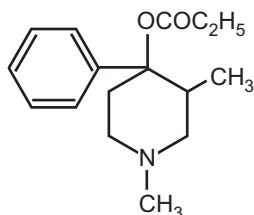
Bemidone



Ketobemidone

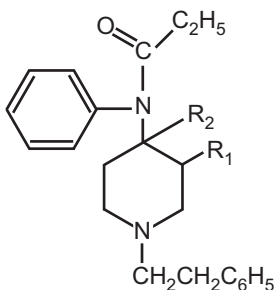
Replacement of the ester moiety by a ketone function in the bemidone, yielded a new series of compounds, ketobemidone.

(8) Prodines are the reversed esters of meperidines. Here the ester of meperidine ( $COOC_2H_5$ ) is replaced by ( $OCOC_2H_5$ ) propionoxy function.



Prodine (1940)

(9) In all the above structures, phenyl ring and acyl group are directly attached to the piperidine ring. In fentanyl series, phenyl ring and acyl group are separated from the ring by a nitrogen.

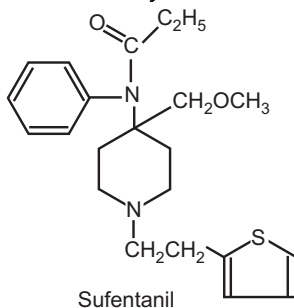


Fentanyl:  $R_1 = -H$ ;  $R_2 = -H$

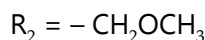
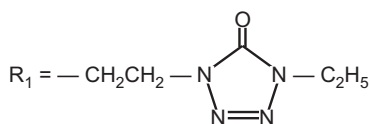
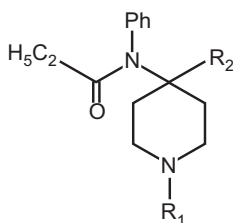
Lofentanyl:  $R_1 = -CH_3$ ;  $R_2 = -COOCH_3$

Carfentanyl:  $R_1 = -H$ ;  $R_2 = -COOCH_3$

Sufentanil is a recent example from fentanyl series.



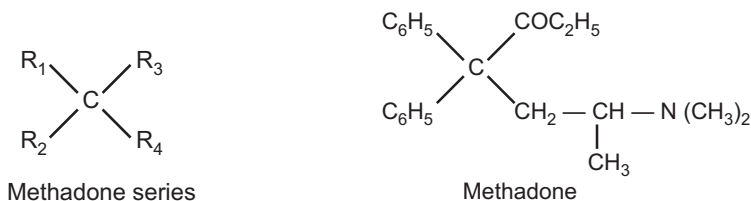
(a) Alfentanil;



Fentanyl is about 500 times as potent as pethidine. Some of the 4, 4-disubstituted piperidines, alfentanil, sufentanil and carfentanil are even more potent. The latter two have a much longer duration of analgesia and respiratory depression and indicate a different therapeutic use e.g. anaesthesia.

### Methadone Series:

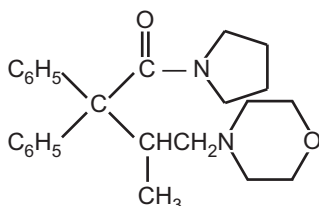
The further simplification of morphine nucleus by opening of the nitrogen ring, resulted into methadone series of compounds. Methadone, itself possesses analgesic as well as spasmolytic properties.



### Structure-Activity Relationship:

- (1) Unlike meperidine or bemidone series, the insertion of m-hydroxyl group in one of the phenyl rings of methadone causes a marked decrease in analgesic activity.
- (2) The methadone derivatives are generally more potent analgesics (and also more toxic) than the isomethadone analogues.
- (3) The replacement of propionyl ( $\text{COC}_2\text{H}_5$ ) group by hydrogen, hydroxyl or acetyloxy, led to decrease in activity.

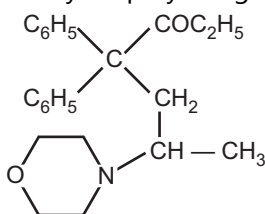
Similarly attempts were also made to replace the propionyl group by amide functions. e.g. Racemoramide; it is more active than methadone.



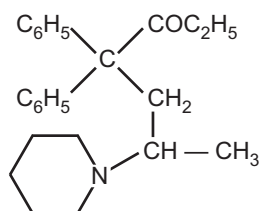
Racemoramide

(4) Removal of any of the two phenyl rings results into decreased activity.

(5) The dimethylamino group is replaced by heterocyclic rings like morpholine and piperidine. The clinically employed agents from this class are:

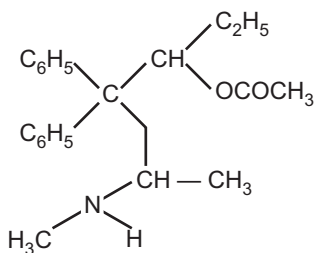


Phenadoxone

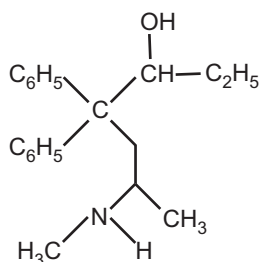


Dipanone

(6) The following are N-demethylated derivatives which are metabolites of methadone analogues in man and are found to retain the analgesic activity.

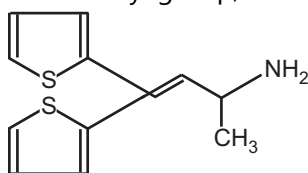


Metabolite of alphacetylmethadol

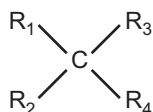


Metabolite of methadone

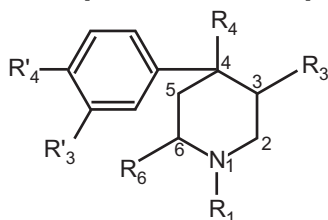
Molecular modifications of Methadone include: homologation and cyclization of dimethylamino group, reduction of CO to -CHOH, removal and relocation of CH<sub>3</sub> branching, isosteric replacement of one or both phenyls by thienyl etc. Examples: Replacement of the keto group by an amide group results into dextromoramide. Insertion of an ester oxygen between blocking groups and carbonyl (as well as a benzyl instead of one phenyl) gave dextropropoxyphene. In thiambutene, the blocking thiophene rings converge on an amine chain and instead of an electron rich carbonyl group, a double bond is introduced.



Thiambutene

**Table 11.1: Compounds from methadone series**

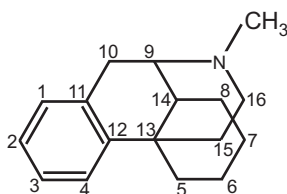
Name	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>
1. Methadone	- C <sub>6</sub> H <sub>5</sub>	- C <sub>6</sub> H <sub>5</sub>	- COC <sub>2</sub> H <sub>5</sub>	- CH <sub>2</sub> CH(CH <sub>3</sub> )N(CH <sub>3</sub> ) <sub>2</sub>
2. Isomethadone	- C <sub>6</sub> H <sub>5</sub>	- C <sub>6</sub> H <sub>5</sub>	- COC <sub>2</sub> H <sub>5</sub>	- CH(CH <sub>3</sub> ) CH <sub>2</sub> N (CH <sub>3</sub> ) <sub>2</sub>
3. Normethadone	- C <sub>6</sub> H <sub>5</sub>	- C <sub>6</sub> H <sub>5</sub>	- COC <sub>2</sub> H <sub>5</sub>	- CH <sub>2</sub> CH <sub>2</sub> N (CH <sub>3</sub> ) <sub>2</sub>
4. Alphacetylmethadol	- C <sub>6</sub> H <sub>5</sub>	- C <sub>6</sub> H <sub>5</sub>	$  \begin{array}{c}  -CHC_2H_5 \\    \\  O - COCH_3  \end{array}  $	- CH <sub>2</sub> CH - (CH <sub>3</sub> )N(CH <sub>3</sub> ) <sub>2</sub>
5. Dextromoramide	- C <sub>6</sub> H <sub>5</sub>	- C <sub>6</sub> H <sub>5</sub>	$  \begin{array}{c}  -C- \\     \\  O  \end{array}  -N \begin{array}{c} \diagup \quad \diagdown \\ \diagdown \quad \diagup \end{array}  $	$  \begin{array}{c}  -CHCH_2- \\    \\  CH_3  \end{array}  -N \begin{array}{c} \diagup \quad \diagdown \\ \diagdown \quad \diagup \end{array} O  $
6. Propoxyphene	- C <sub>6</sub> H <sub>5</sub>	- CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	$  \begin{array}{c}  O - C - C_2H_5 \\     \\  O  \end{array}  $	$  \begin{array}{c}  -CHCH_2 N (CH_3)_2 \\    \\  CH_3  \end{array}  $

**Table 11.2: Compounds from meperidine series**(R<sub>6</sub> = H, except trimeperidine)

Name	R <sub>1</sub>	R <sub>3</sub>	R <sub>4</sub>	R' <sub>3</sub>	R' <sub>4</sub>
1. Meperidine	- CH <sub>3</sub>	H	- COOC <sub>2</sub> H <sub>5</sub>	H	H
2. Bemidone	- CH <sub>3</sub>	H	- COOC <sub>2</sub> H <sub>5</sub>	- OH	H
3. Prodine	- CH <sub>3</sub>	CH <sub>3</sub>	- OCOC <sub>2</sub> H <sub>5</sub>	H	H
4. Trimeperidine	- CH <sub>3</sub> (R <sub>6</sub> = - CH <sub>3</sub> )	CH <sub>3</sub>	- OCOC <sub>2</sub> H <sub>5</sub>	H	H
5. Diphenoxylate	- CH <sub>2</sub> CH <sub>2</sub> C - (C <sub>6</sub> H <sub>5</sub> ) <sub>2</sub>   CN	H	- COOC <sub>2</sub> H <sub>5</sub>	H	H
6. Loperamide	- CH <sub>2</sub> CH <sub>2</sub> C - (C <sub>6</sub> H <sub>5</sub> ) <sub>2</sub>   C - N(CH <sub>3</sub> ) <sub>2</sub>    O	H	- OH	H	- Cl

**(4) Modifications carried out by Grewe in 1946:**

Various compounds belonging to morphinan and benzomorphan series have been synthesized and tested clinically.

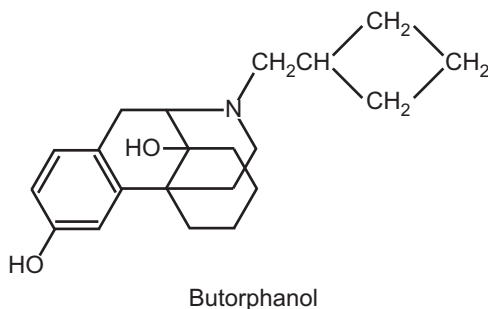
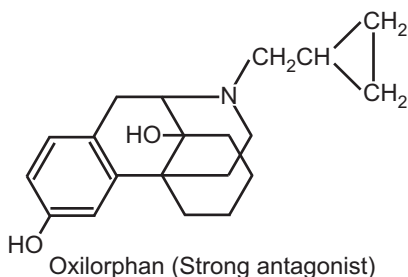


N-methylmorphinan

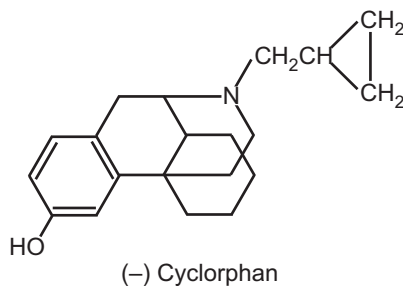
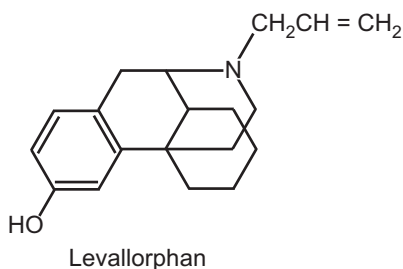
These compounds lack the ether bridge between the carbon atoms 4 and 5.

**Structure-Activity Relationship:**

- (i) Introduction of a hydroxyl group at C - 3 enhances the analgesic activity.
- (ii) The ethers and acylated derivatives of the 3-hydroxyl form also have considerable analgesic activity.
- (iii) The 14-hydroxylation results in potent derivatives with both agonist and antagonist properties e.g.

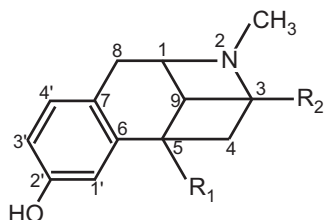


(iv) N-substitution may result into either agonist or antagonist depending upon the nature of substituent e.g., the N-phenylethyl or N-p-amino phenylethyl derivative of levorphanol are potent analgetics. The N-furylethyl and N-acetophenone analogues are also potent analgetics, while N-allyl derivative (cyclorphan) possesses antagonistic properties.

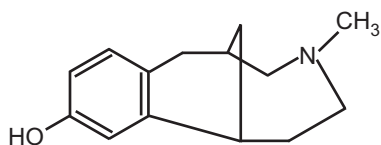


**(5) Benzomorphans:**

The fact that, the removal of ether bridge and all the peripheral groups in the alicyclic ring of the morphine did not destroy its analgesic activity, encouraged May and Murphy to synthesize a new series of compounds known as benzomorphans (in which the alicyclic ring was replaced by one or two methyl groups).

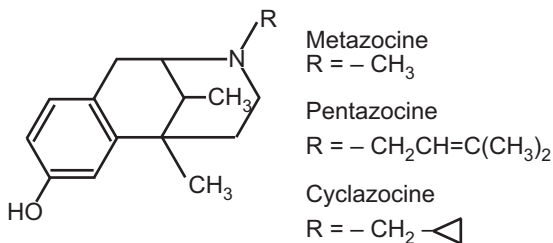
Benzomorphan ( $R_1 = R_2 = H$ )**Structure-Activity Relationship:**

- (1) Amongst the various substitutions at position 2', following SAR is observed  
 $OH \geq H \geq NH_2, NO_2, F$  and  $Cl$
- (2) The trimethyl compound ( $R_1 = R_2 = CH_3$ ) is about 3 times more potent than the dimethyl analogue ( $R = H; R_2 = CH_3$ ).
- (3) Insertion of methyl group at C-9 increases analgesic activity.
- (4) Insertion of hydroxyl group at C-9 (which is equivalent to 14-hydroxylation in morphine series) decreases the activity.
- (5) The N-phenylethyl analogues always possess greater potency over N-methyl analogues.
- (6) Eptazocine is a mixed agonist-antagonist introduced by Morishita in 1987 in Japan.

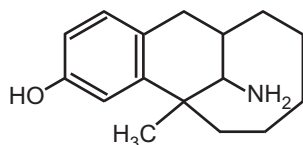


Eptazocine (1987)

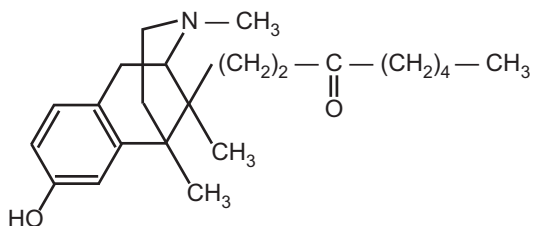
- (7) Pentazocine and cyclazocine are the mixed agonist-antagonists from benzomorphan series.



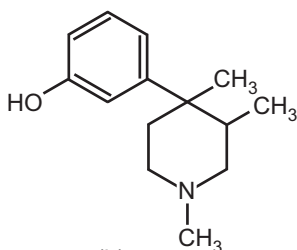
Dezocine is a mixed agonist-antagonist of the benzomorphan series.



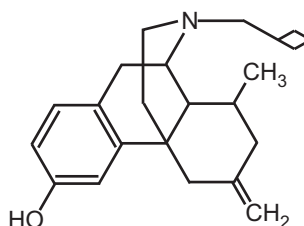
Dezocine (1986)



(a) Tonazocine



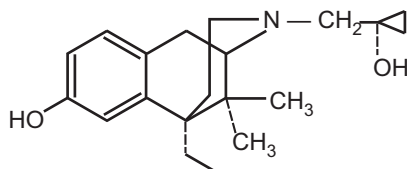
(b)



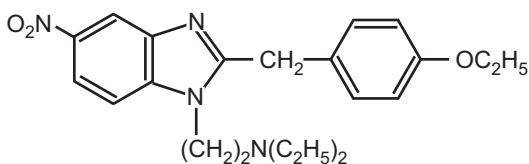
(c) Xorphanol

Xorphanol is found to be a partial agonist at the  $\kappa$ -receptor and antagonist at the  $\mu$ -receptor.

(8) Introduction of N-furfuryl group into the benzomorphans have provided a new series of potent agonists and antagonists that are now undergoing clinical evaluation.



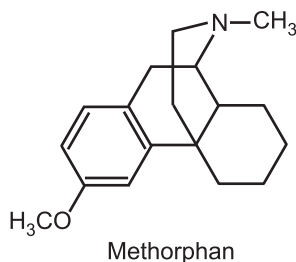
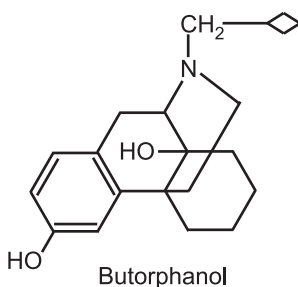
A new benzomorphan, bremazocine is a powerful  $\kappa$  – agonist of long duration of addictive properties and respiratory depressant activity. It is about 200 times more active than morphine.



Etonitazene

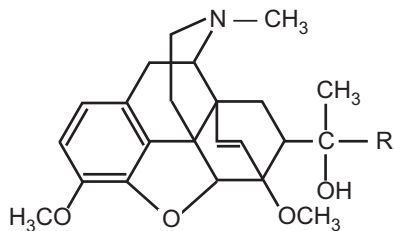
Etonitazene can be related to morphine. It is a benzimidazole derivative and is highly potent, having about 1000 times activity of morphine.

**(6) Antitussive-opioidal agents:** A compound synthesized as, and found to be, a narcotic antagonist turned out to be a potent and long lasting antitussive, is (+) butorphanol.



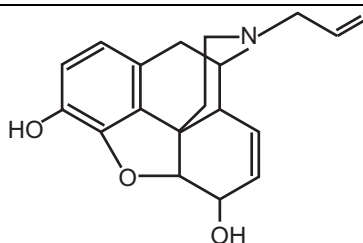
The (+) isomer of methorphan (dextromethorphan) is essentially devoid of analgesic and additive properties but is effective antitussive agent.

Other examples of antitussive skeletons include –

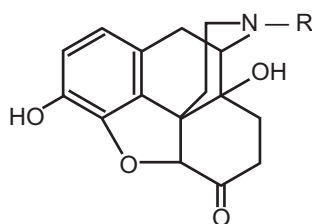


6,14-endo-Ethenotetrahydro-thebaine  
(7-substituted-16 methyl)

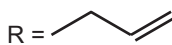
## 11.5 NARCOTIC ANTAGONISTS



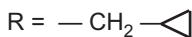
Nalorphine (1940)



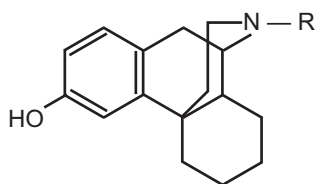
Oxymorphone



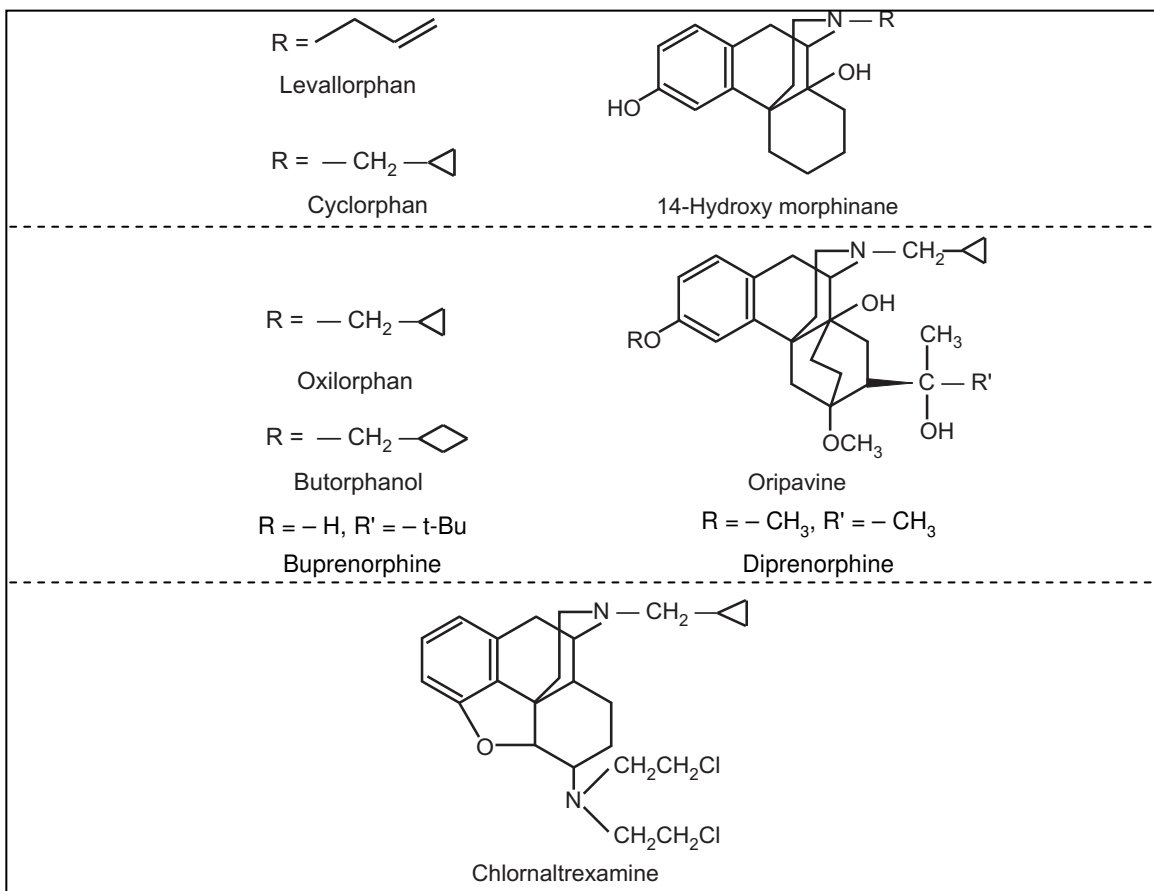
Naloxone (1961)



Naltrexone



Morphinane

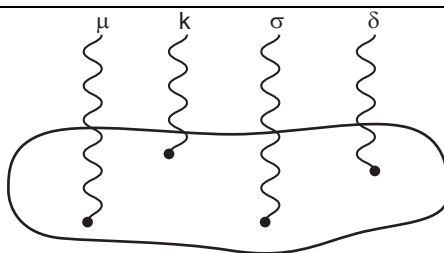


The recently described chlornaltrexamine, which acts as an irreversible alkylating affinity label on the opiate receptor, can maintain its antagonistic effect for the astonishing period of 3 days. Other examples of narcotic antagonists include cyclazocine and pentazocine (benzomorphan series).

Narcotic antagonists competitively antagonise the effects of opioid analgetics by binding at several subspecies of opioid receptor.

The original concept of opioid receptor was first postulated from the stereo-selective studies of Beckett and Casy (1954). They proposed a model of opioid receptor. In the early 1970s biochemical binding experiments with radio labelled naloxone, which antagonises the pharmacological effects of morphine, led to the identification of stereo-specific opioid receptors in mammalian brain tissue. In 1976, Martin classified opioid receptors into three subtypes:  $\mu$  receptors (morphine-like),  $\kappa$  receptors (ketozocine-like) Ethylketozocine, an analgesic that is chemically unrelated to morphine and  $\sigma$  receptors (N-allylnormetazocine-like) on the basis of effects of opioids on respiration, heart rate and locomotor activity.

A steric theory of opiate agonists and antagonists was proposed in 1983 by Martin. This theory helps to understand both agonistic and antagonistic features of opioid. The antagonistic activity of N-allyl or N-cyclopropylmethyl substituent can be explained on the basis of van der Waal's interaction of these substituents with the receptor. This may result in moving the nitrogen away from the position required for agonism.



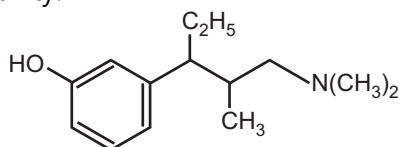
**Fig. 11.5: Opioid receptor**

Opioid receptors are a group of G-protein coupled receptors with opioids as ligands. The opioid receptors are ~40% identical to somatostatin receptors.

Martin and Gilbert in 1977 have postulated the existence of three subspecies of opioid receptors, designated as  $\mu$ ,  $\kappa$  and  $\sigma$ . While Lord et al., in 1977 designated  $\delta$ . It is thought that

- (i)  $\mu$  subspecies are involved in producing: analgesia, respiratory depression, euphoria, addiction, miosis and reduced GI motility.

**Tapentadol:** It is an opioid analgesic ( $\mu$ -receptor agonist) with norepinephrine reuptake inhibitory activity.



- (ii)  $\kappa$  receptor subspecies are involved in producing: spinal analgesia, sedation and miosis.
- (iii)  $\sigma$  subspecies are involved in producing: dysphoria, hallucinations, respiratory stimulation and
- (iv)  $\delta$  subspecies are involved in producing analgesia, antidepressant effects and addiction.

An agonistic or antagonistic activity of the drugs depends only on their relative affinity for these receptor subspecies.

Opiates may cause respiratory depression which is attributed to the stimulation of both  $\mu$  and  $\delta$  receptors present in areas of the brain stem associated with control of respiration. Thus,  $\mu$  and  $\delta$  agonist appear to alter respiratory function by reducing the responsiveness of both central chemoreceptors in the brain stem and peripheral chemoreceptors in the carotid body to carbon dioxide.

### Classification:

Depending upon the activity, drugs can be classified as:

- (a) Pure antagonists: Naloxone
- (b) Partial antagonist: Nalorphine, Levallorphan and Cyclazocine
- (c) Partial agonists of morphine: Propiram and Profadol.

In particular, the pure agonist molecule can be converted into a partial agonist or a pure antagonist by relatively minor changes in the structure. The most common substitution is that of a larger moiety (like an allyl or methylcyclopropyl group) for the N-methyl group of an opioid.

## 11.6 ENDOGENOUS OPIOIDS

In 1975, Hughes and Kosterlitz isolated extracts from pig brain which had opioid activity similar to that of morphine. This activity was shown to be due to mixture of two pentapeptides which they characterized and named Leu enkephalin and Met enkephalin.

The term 'endorphin' (endogenous morphine) is used to describe any endogenous opioid substance including the two enkephalins, e.g.  $\alpha$ -,  $\beta$ -, or  $\gamma$ - endorphin and dynorphin.

The term 'opioid' is applied to any substance which produces its biological effects through an interaction with any of the three major types of opioid receptor ( $\mu$ ,  $\kappa$  or  $\delta$ ) and whose actions are reversed by naloxone. An opiate is an opioid whose chemical structure and biological properties are similar to morphine.

The three families of peptides that have been isolated and identified are, the Enkephalins, the Endorphins and the Dynorphins. Met-enkephalin has a sequence of amino acids identical with that of residues 61– 65 of the pituitary hormone  $\beta$ -lipotropin. This fragment itself has a potent-opioid activity.

Elevated levels of immunoreactive  $\beta$ -endorphin and enkephalin have been reported in human plasma after exercise and after surgical stress.

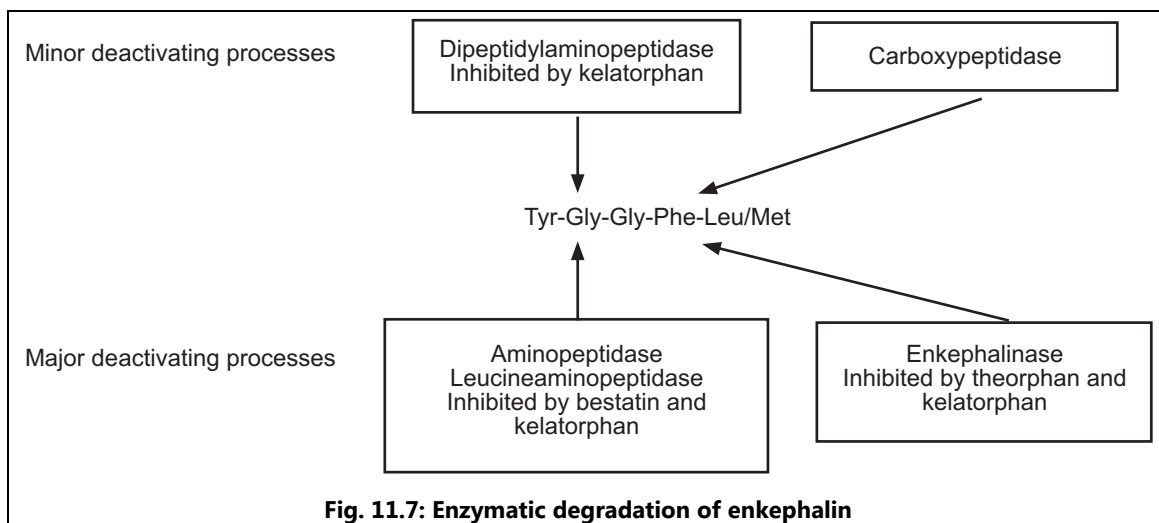
### Metabolism:

The enkephalins and dynorphin have a much shorter half-life than  $\beta$ -endorphin *in-vivo* because of faster hydrolysis by a variety of non-specific metallopeptidases. Consequently,  $\beta$ -endorphin is the only endogenous opioid which causes sustained analgesia after i. v. administration to mice. The two major metabolic processes for the enkephalins are the cleavage of Tyr<sup>1</sup> – Gly<sup>2</sup> by membrane bound aminopeptidases, which are inhibited by bestatin or kelatorphan, and the hydrolysis of Gly<sup>3</sup> – Phe<sup>4</sup> by a variety of metalloendopeptidases including 'enkephalinase', which is inhibited by thiorphan or kelatorphan.

The opioid peptides are formed in the brain, the pituitary gland and in the adrenal medulla by the proteolytic cleavage of three protein precursors; these are preproopiomelanocortin (POMC) [also known as corticotropin- $\beta$ -lipotropin precursor (ACTH- $\beta$ -LPH precursor)]; preproenkephalin A (also known as preproenkephalin) and preproenkephalin B (also known as preprodynorphin).

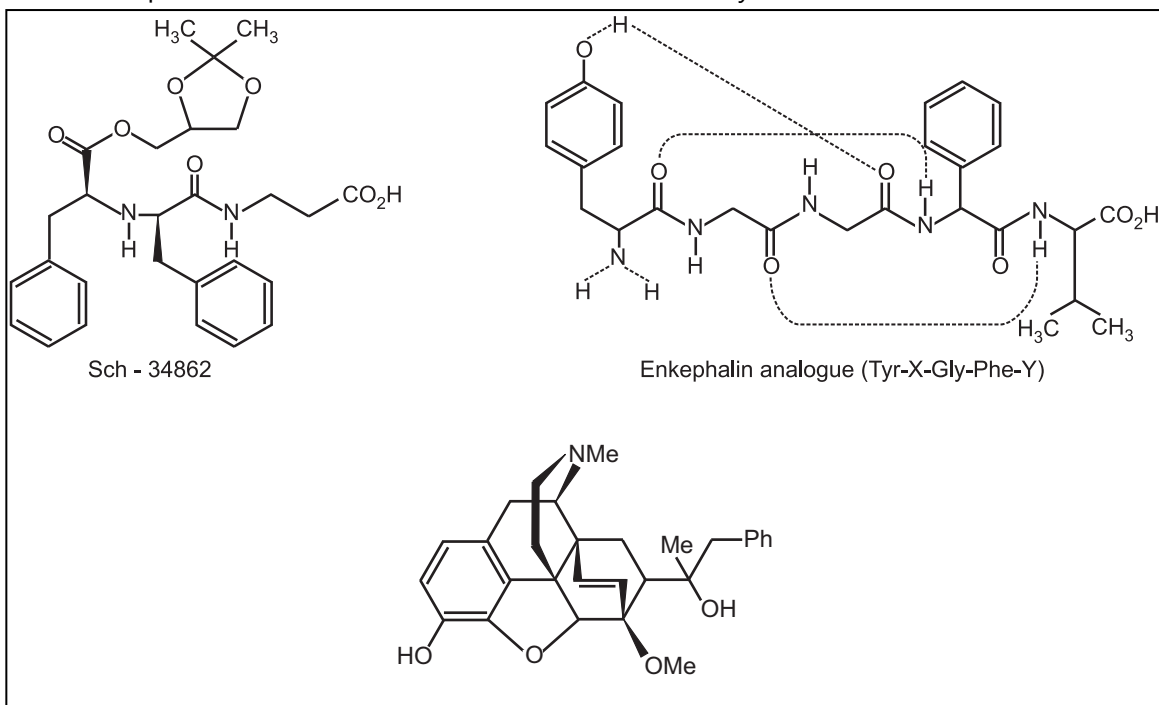
Tyr – Gly – Gly – Phe – Leu	Tyr – Gly – Gly – Phe – Met
<b>Leu-enkephalin</b>	<b>Met-enkephalin</b>
Tyr – Gly – Gly – Phe – Leu – Arg – Arg – Ile – Arg – Pro – Lys – Trp – Asp – Asn – Gln	
<b>Dynorphin</b>	
Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Gly	
<b><math>\beta</math>- Endorphin</b>	
Tyr-Pro-Trp-Glu-NH <sub>2</sub> $\alpha$ - Endorphin: 1– 16 sequence	Tyr-Pro-Glu-Glu-NH <sub>2</sub>
Endomorphin-1 $\gamma$ - Endorphin: 1– 17 sequence	Endomorphin-2
$\delta$ - Endorphin: 1– 27 sequence	

**Fig. 11.6: Structures of endogenous opioid peptides**



Over 1000 analogues of enkephalins have been synthesized. Greater stability towards metabolizing enzymes can be attained by conversion of the terminal carboxyl to  $-\text{CONH}_2$ , or by inserting a D-amino acid at this position. The tyrosyl group which provides a link between the enkephalins and thebaine derivatives, is an essential feature. A  $10.0 \pm 1.1 \text{ \AA}$  distance between the aromatic rings of Tyr<sup>1</sup> and Phe<sup>4</sup> may be important in the design of enkephalins with other aromatic moieties.

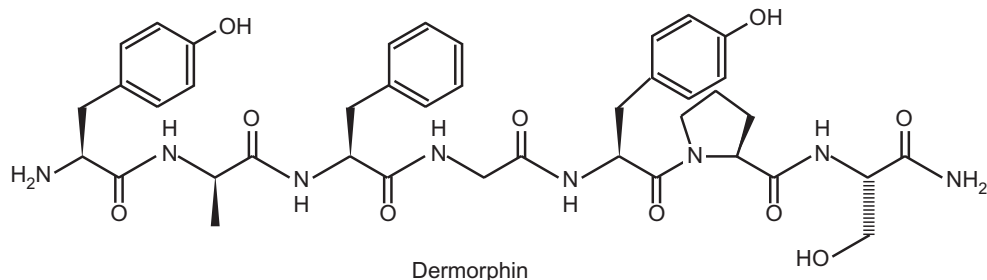
Enkephalinase inhibitors' might be of use as analgesic drugs, but to date there is little clinical data to support this. Schering and Plough are reported to have developed an orally active enkephalinase inhibitor, Sch-34862, which is currently in clinical trials.



**Table 11.3: Effect of the position of thiomethylene linkage on the half-life of synthetic Leu-enkephalin derivatives**

Structure	Amide bond replaced	Half-life (min) in human serum at pH 7.4 (HPLC assay)	Opioid binding (versus etorphine) (nM) affinity
Tyr-Gly-Gly-Phe-Leu	–	12.5	256
Tyr-ψ (CH <sub>2</sub> S) Gly-Gly-Phe-Leu	1 - 2	11.8	1060
Tyr-Gly-ψ (CH <sub>2</sub> S) Gly-Phe-Leu	2 - 3	85.5	–
Tyr-Gly-Gly-ψ (CH <sub>2</sub> S) Phe-Leu	3 - 4	134	–
Tyr-Gly-Gly-Phe-ψ (CH <sub>2</sub> S) Leu	4 - 5	318	480

Other physico-chemical techniques that have been used to study the conformations of the enkephalins lead to the conclusion that the enkephalins are flexible molecules whose conformation depends upon their molecular environment. In aqueous solution at room temperature they may adopt several different conformations.



A 70 mg parenteral dose of metkephamid (Tyr – Ala – Gly – Phe – NMeGly – NH<sub>2</sub>) was equivalent to 100 mg of meperidine in treating post-operative pain. Side-effects included a sensation of heavy limbs, dry mouth, redness of eyes and nasal stuffiness which are different from μ-selective opiate drugs and may be due to the relatively high affinity of metkephamid for δ-receptors.

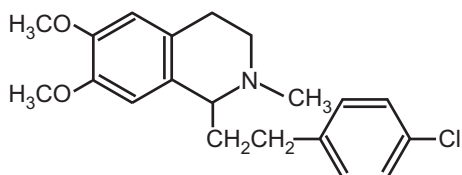
The δ-selective peptide (Tyr–D-Ala–Gly– Phe–D-Leu) has been found to produce effective analgesia after intrathecal administration to cancer patients who had become tolerant to the analgesic effects of morphine.

The heptapeptide dermorphin was isolated from the skin of the frog *Phyllomedusa bicolor*.

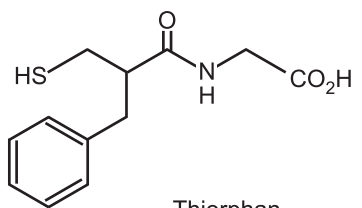
## 11.7 THERAPEUTIC USES OF OPIOID ANTAGONISTS

- (1) In the treatment of opioid induced respiratory depression.
- (2) Chronic administration of nalorphine along with morphine prevents or minimizes the development of dependence on morphine.
- (3) Therapeutic agents in the treatment of compulsive users of opioids.
- (4) Reduce the intensity of various untoward effects of opioids, e.g., euphoria, drowsiness, vomiting and muscular inco-ordination.
- (5) An abstinence syndrome characterized by abnormal pain, irritability, cold sweats, diarrhoea, nausea and vomiting. These effects usually last 4–10 weeks. Nalorphine precipitates the withdrawal symptoms in patients addicted to heroin and methadone.
- (6) In acute poisoning due to morphine and related compounds.

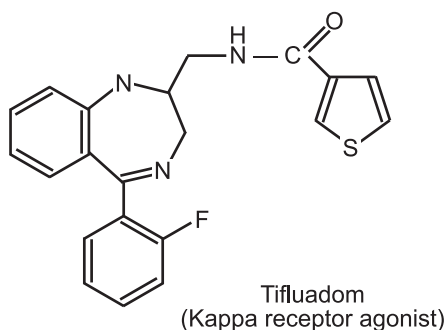
### Other Narcotic Analgesic Leads:



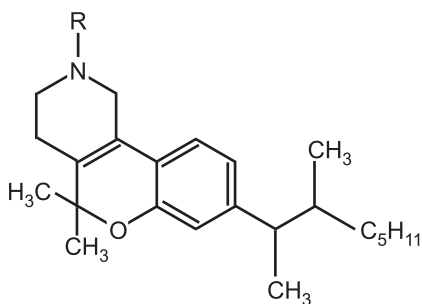
A chloro homolog of laudanoline, an alkaloid that occurs in opium.



Thiorphan  
(Enkephalinase Inhibitor)

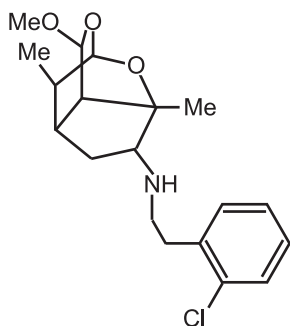


Tifluadom  
(Kappa receptor agonist)

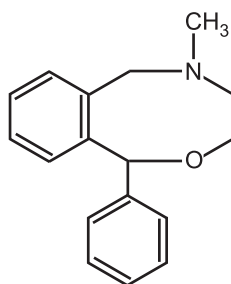


Aza-cannabinoids : R =  $-\text{CH}_3$  ;  $-\text{CH}_2\text{C}\equiv\text{CH}$   
(Cannabis sativa)

$\mu$  receptors = supraspinal analgesia, respiratory depression, miosis, reduced GI-motility and euphoria.



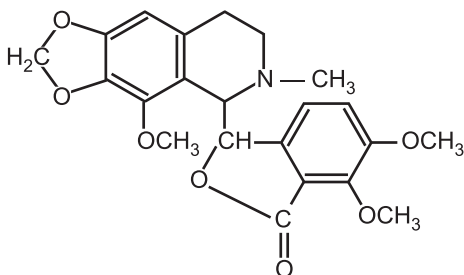
Valorphin ( $\mu$ -selective agonists)



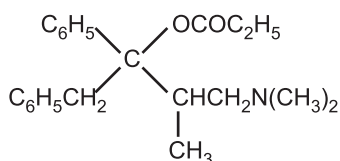
Nephopam

Nephopam is a novel analgesic agent having very rapid onset of action. It possesses minimum side-effects.

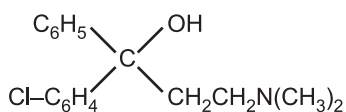
**Table 11.4: Non-narcotic antitussive agents**



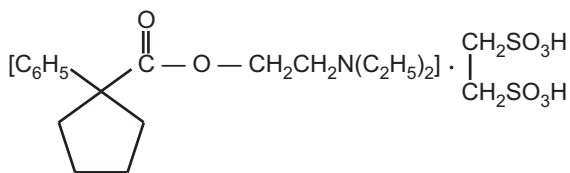
Noscapine



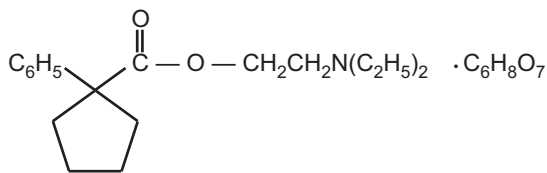
Levopropoxyphene



Chlophedianol



Caramiphen Edisylate



Carbetapentane Citrate



## ANTI-INFLAMMATORY ANALGESICS

### ♦ SYNOPSIS ♦

12.1 INTRODUCTION	12.7 PHENYLACETIC ACID AND PROPIONIC ACID DERIVATIVES
12.2 CLASSIFICATION	12.8 FENAMATES
12.3 SALICYLIC ACID DERIVATIVES	12.9 MISCELLANEOUS AGENTS
12.4 PARA-AMINO PHENOL DERIVATIVES	12.10 MECHANISM OF ACTION OF NON-STEROIDAL ANTI-INFLAMMATORY AGENTS
12.5 PYRAZOLONE DERIVATIVES	12.11 TREATMENT OF GOUT
12.6 INDOMETHACIN AND OTHER ARYLACETIC ACID DERIVATIVES	

### 12.1 INTRODUCTION

Inflammation can be defined as 'a defensive but exaggerated local tissue reaction in response to exogenous or endogenous insult'. It is a complex phenomenon, comprising of biochemical as well as immunological factors. It is recognised by the following symptoms:

- (1) Calor (Heat)
- (2) Rubor (Redness)
- (3) Tumour (Swelling), and
- (4) Dolor (Pain).

Tissue damage initiates or activates the local release of various chemotactic factors that provoke directly or indirectly the appearance of the mediators of pain and inflammation. These factors include:

**(a) Amines:** Histamine, serotonin.

**(b) Proteases:** Kallikrein, plasmin. Release of lysosomal enzymes usually occurs from mast cells, macrophages, polymorphonuclear leucocytes and platelets.

**(c) Prostaglandins.**

**(d) Hageman factor:** It was discovered in 1955 in a preoperative blood of 37 years old patient, John Hageman. This factor is activated when it comes in contact with a foreign surface.

Once activated, Hageman factor is known to act upon a number of macromolecular substrates present in the plasma.

Hageman factor is a serum globular protein ( $\beta$ -globulin) of high molecular weight (110 000). The three main functions performed by Hageman factor in the inflammatory reaction can be summarised as:

- (a) Generation of thromboplastin activity in the pathways leading to coagulation. It is also termed as coagulation factor XII.
- (b) Conversion of plasminogen pre-activator to plasminogen activator in the pathway leading to fibrinolysis.
- (c) Conversion of pre-kallikrein in the pathway leading to kinin production.

**Kinins** are polypeptides formed in blood from inactive precursors called kininogens, induce vasodilation and increase permeability and serve as chemotactic agents for phagocytes.

Bradykinin is the major final biologically active product of the kallikrein-kinin pathway. Bradykinin has been cited as mediating vasodilatation, increasing vascular permeability and producing pain. Bradykinin also increases local lymph flow, another characteristic of local inflammation.

**(e) Other factors:** These include leucotoxin, leucocytosis promoting factor and lymph node permeability factor.

**Blood and interstitial fluids contain three main types of antimicrobial proteins:**

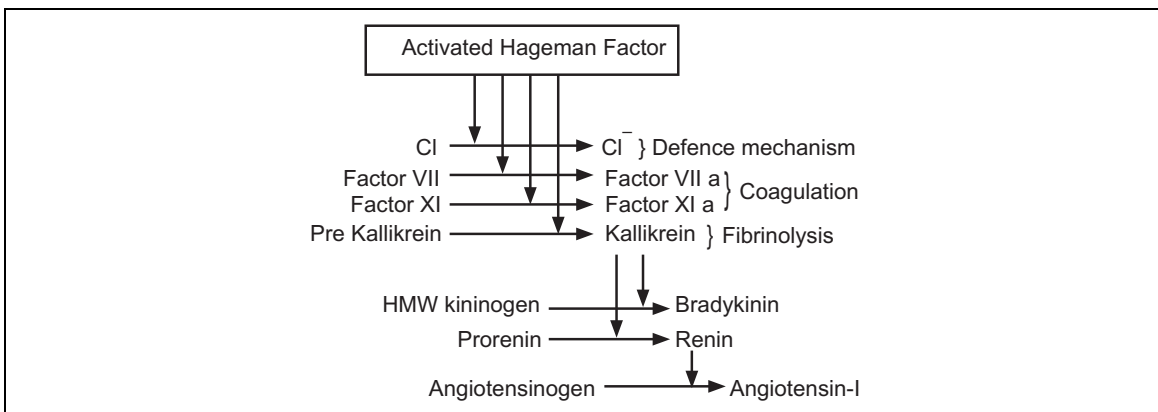
**(i) Interferons:** Lymphocytes, macrophages and fibroblasts infected with virus, produce antiviral proteins called interferons.

**(ii) Complement system:** A group of normally inactive proteins in blood plasma and on plasma membrane when get activated, causes cytolysis (bursting) of microbes, promotes phagocytosis and contributes to inflammation.

**(iii) Transferrins:** Iron binding proteins that inhibit growth of certain bacteria by reducing the amount of available iron.

Complement is a complex cascade system comprising about 20 plasma proteins, many of which are enzymes. It helps the ability of antibodies and phagocytes to clear pathogens. This cascade acts as:

- opsonizing pathogens,
- inducing inflammatory responses (release of small peptide mediator which invite increased flow of phagocytes),
- enhancing antibody responses and
- attacking some pathogens directly.



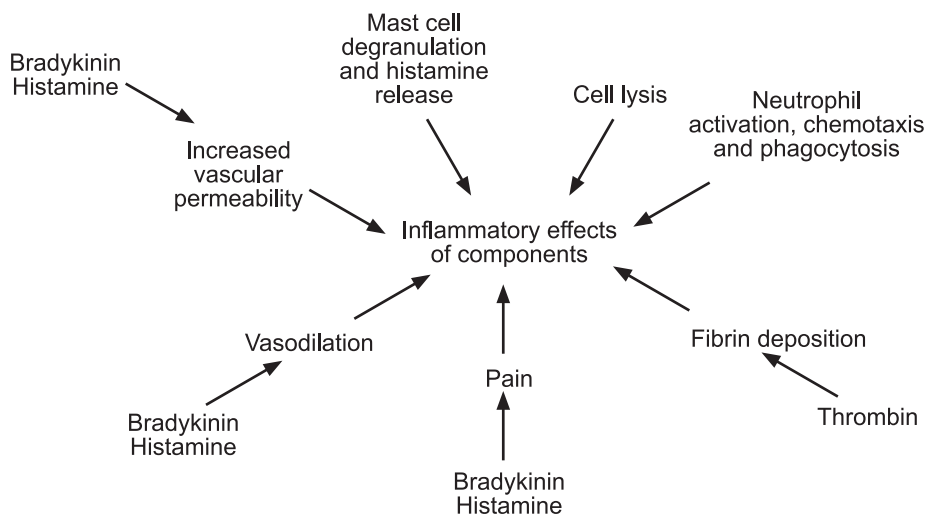
Different components of complement system stimulate histamine release, attract neutrophils by chemotaxis and promote phagocytosis. Some components can also destroy bacteria. A number of complement components mediate various inflammatory effects, in particular C<sub>3</sub>a and C<sub>5</sub>a. C<sub>5</sub>a in particular is chemotactic for neutrophils and increases vascular permeability. Conditions where complement is involved include glomerulonephritis, rheumatoid arthritis, rheumatic fever and drug allergies.

Complement peptides trigger cell function, aid in the recognition of invading pathogens and regulate the phagocytic process via interactions with specific cell surface receptors.

There are five types of WBC (Neutrophils + Lymphocytes + Monocytes + Eosinophils + Basophils). WBC combat pathogens by phagocytosis or immune responses. Several different chemicals released by microbes and inflamed tissues attract phagocyte, a phenomenon called chemotaxis. At site of inflammation, basophils leave capillaries, enter tissues and release granules that contain heparin, histamine and serotonin. Mast cells are fixed and found particularly in connective tissues of skin and mucous membranes of respiratory and GI tracts.

Hydrolytic enzymes are released by cells from intracellular vacuoles (known as lysosomes) during phagocytosis and also during cell death. There are two classes of these enzymes. Those in the first group act at acid pH (3 - 5) and are normally contained within lysosomes that fuse with vacuoles to form secondary phagosomes. The activity of these acid hydrolases is normally intracellular, but on cell death they may well be liberated at the site of inflammation and cause considerable damage.

Lysosomal enzymes are secreted from human neutrophils by cyclic nucleotides, autonomic neurohormones, prostaglandins, glucocorticoids and calcium. Discharge of lysosome granule contents from neutrophils results in the provocation of acute inflammation and connective tissue degradation. Agents that enhance lysosomal enzyme secretion include c-GMP, immune reactants, Ach, PGF<sub>2</sub>. and Ca<sup>++</sup> ions. While inhibiting occurs due to accumulation of intracellular c-AMP, epinephrine and several prostaglandins (PGE, PGA).

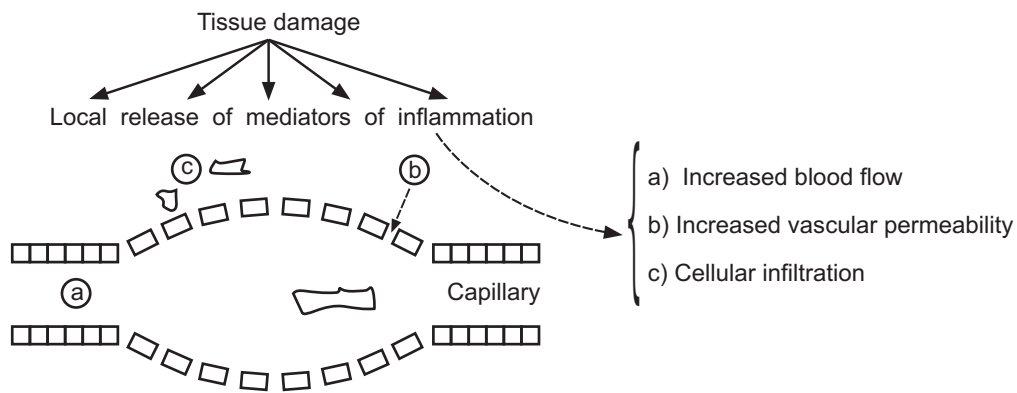


**Fig. 12.1: Inflammatory effects of components**

The second group of hydrolytic enzymes consists of those that act at neutral pH and are contained in cell organelles other than lysosomes. These are probably more important in the early stages of tissue damage during inflammation. Normal phagocytosis seems to involve these neutral pH enzymes; they include collagenase and elastase.

All these mediators cause local vascular response, which is characterized by:

- (1) Increased blood flow to the affected area.
- (2) Increased vascular permeability which may cause oedema.
- (3) Cellular infiltration of platelets and macrophages from the capillaries into the tissue spaces.



**Fig. 12.2: Acute inflammation**

So in brief, the sequence of early events in inflammation may be summarised as:

- (a) Initial injury which causes the release of inflammatory mediators.
- (b) Vasodilation.
- (c) A glycoprotein E-selectin appears on the inner surface of vascular endothelium during inflammation. It induces the adhesion of WBCs by attracting the tetrasaccharide sialyl Lewis X which is displayed on the surfaces of WBCs. After further adhesion the white blood cells are then able to squeeze through gaps between endothelial cells and enter the adjacent tissues to help repair injury. Thus, this increased vascular permeability, results into cellular infiltration.
- (d) Migration of phagocytic cells to the inflamed area, resulting into release of lytic enzymes due to rupturing of cellular lysosomal membranes.

An inflammation may be either a primary or a secondary response to the tissue damage. A primary inflammation involves direct and generally acute defence reaction while in the secondary inflammation, it is an indirect consequence of the exaggerated cell physiology, arising due to pathological condition, e.g., rheumatoid arthritis.

The anti-inflammatory analgesic agents, also popularly known as non-steroidal anti-inflammatory drugs are associated with analgesic and antipyretic activities. The peripheral nerve fibres which conduct pain impulses may be categorised as:

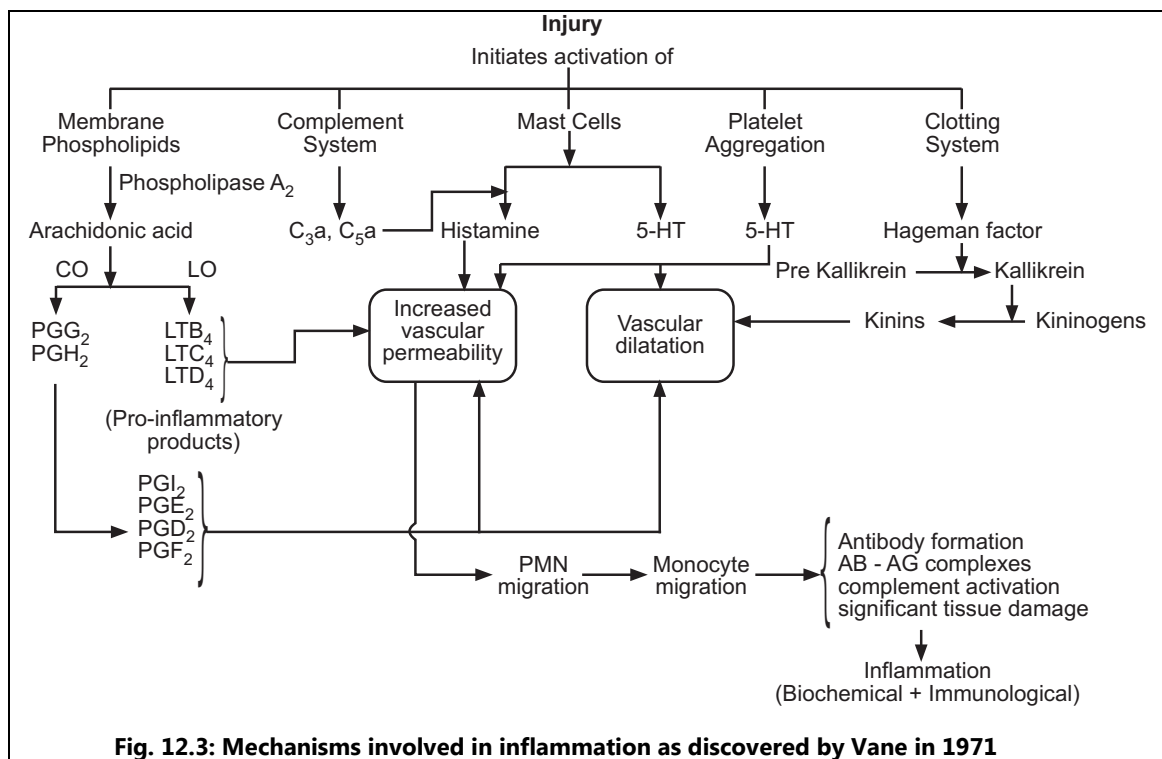
- (a) The large myelinated A fibres that conduct fast, more intense and precise pain.
- (b) The myelinated B fibres that conduct pain impulses of medium intensity, and
- (c) Unmyelinated C fibres that conduct slow and diffused pain.

The drugs covered in this chapter, have an ability to inhibit the synthesis of thromboxane and prostaglandins. This fact was first discovered by Vane in 1971, who assigned the therapeutic as well as adverse effects of aspirin-like drugs to their ability to prevent prostaglandin biosynthesis.

Considerable evidence has supported the concept that non-steroidal anti-inflammatory analgesic drugs act by inhibiting the biosynthesis of prostaglandins which are the basic cause behind pain, fever and inflammatory conditions. They have the ability to sensitise the pain receptors to mechanical and chemical stimulation. The biosynthesis of prostaglandins is catalysed by microsomal enzymes present in almost every mammalian cell type, except erythrocytes.

Prostaglandins are a group of cyclopentane derivatives formed from poly-unsaturated fatty acids by most mammalian tissues. The basic structure of all prostaglandins contains about 20 carbon atoms having a cyclopentane ring with two adjacent side-chains.

Arachidonic acid serves as a precursor for biosynthesis of prostaglandins in humans. Arachidonic acid is probably stored in the phospholipid fraction of the cell. The biosynthetic route for the formation of various prostaglandins is shown in Fig. 12.3.



Prostaglandin release has been demonstrated under a variety of conditions, for example, at the sites of inflammation (skin, joints, eye, white cells); during anaphylactic reactions; in platelets during aggregation; in the cerebral ventricles during fever, in subcutaneous fat during lipolysis and in the uterus during labour or during menstruation.

Prostaglandins potentiate the early inflammatory response, causing vasodilation, increased permeability, facilitating cellular infiltration and sensitising the pain receptors. The non-steroidal anti-inflammatory analgesics do not act centrally to intervene in the perception of the pain. They act peripherally to inhibit both, the synthesis and release of prostaglandins. Thus, they have minimum CNS side-effects. They neither induce mood alterations nor have a tendency to cause drug dependence. Thus, morphine like drugs act on CNS while these drugs act mainly peripherally at the site of origin of pain.

An elevation of body temperature is usually seen in many infectious diseases. It is also often associated with the inflammatory process. The centre for control of body temperature is located in the hypothalamus. An elevation of the body temperature occurs due to the attack of pyrogenic substances on this regulatory centre. Pyrogens are the metabolic products of bacteria and leucocytes. They induce changes in the normal regulatory process of body temperature resulting into reduced heat loss by peripheral vasoconstriction associated with an increase in the heat production. The net result is a rise in body temperature.

Prostaglandin E<sub>1</sub> is known to be a potent pyrogen (fever inducing) and PGE<sub>2</sub> causes pain, oedema, fever and reddening of the skin.

In an inflammatory disorder, the endogenous pyrogen apparently passes into the CNS and stimulates the release of prostaglandin-like substances from some specific sites within the brain. The non-steroidal anti-inflammatory analgesic agents have antipyretic activity. They block the synthesis and release of these substances, followed by peripheral vasodilation and increased sweating, resulting into considerable heat loss from the body. This brings down the body temperature to normal.

Anti-inflammatory agents are believed to act by disrupting the arachidonic acid cascade. These drugs are widely used for the treatment of minor pain and also for the management of oedema and the tissue damage resulting from arthritis.

They also provide relief to the patient from the emotional trauma of fever, pain and insomnia. Besides inhibiting the cyclooxygenase enzymes involved in prostaglandin biosynthesis, they also interfere with a variety of other enzymes. The inhibition of cyclooxygenase enzyme is probably only one of several mechanisms for the anti-inflammatory activity of these drugs, since indomethacin does not block this enzyme. The adverse effects, in part, can be accounted on this basis. The major adverse-effects common to different classes of these drugs are as follows:

(a) Except para amino phenol derivatives, these drugs produce gastrointestinal side-effects. In the untreated normal person, the GIT-membrane is protected from mucosal damage by prostaglandins like  $\text{PGI}_2$  and  $\text{PGE}_2$ . These drugs inhibit the synthesis of gastric prostaglandins and expose the mucosal membrane to increased gastric acid attack resulting into gastric or intestinal ulceration.

(b) Their ability to inhibit the biosynthesis of prostaglandins enables them to prevent the formation of thromboxane  $\text{A}_2$ , a potent aggregating agent. Thus, treatment with non-steroidal anti-inflammatory drugs leads to increase in the bleeding time.

It revealed that medium dose of aspirin (75-325 mg daily) produced reductions of about a quarter in heart attack, stroke or other arterial diseases such as angina or peripheral vascular diseases. A long term therapy is beneficial in almost all patients with suspected heart attack or unstable angina or with any history of heart attack, stroke, angina, arterial bypass surgery or angioplasty, or other occlusive diseases of the blood vessels, irrespective of age, hypertension or diabetes. Higher doses do not increase the antiplatelet effect but onset of action is quicker. Because side-effects are possible, long-term aspirin intake may even do more harm than good in low-risk individual. Therefore, treatment of normal people with prophylactic aspirin is not recommended. However, it is rather suggested that advice on a healthy life-style would be of greater benefits to the patient.

(c) The gestation or spontaneous labour is found to be prolonged.

(d) Due to their higher affinity for plasma proteins, these agents cause easy displacement of other plasma protein-bound drugs. This may lead to a sudden, unexpected rise in the plasma concentration of co-administered drug resulting in potentially dangerous effects.

(e) In some individuals, hypersensitivity reaction may be seen during therapy which is characterized by oedema, generalised urticaria and sometimes bronchial asthma. Epinephrine is usually used to control such hypersensitivity reaction.

The anti-inflammatory analgesics popularly known as non-narcotic analgesic agents are also associated with antipyretic property. The prototype of this class is aspirin, and hence, though this class comprises the chemically unrelated heterogenous group of compounds, these compounds are often referred to as 'aspirin-like' drugs. They are valuable for the non-specific relief of pain of mild to moderate intensities, like headache, arthritis, neuralgia, dysmenorrhea etc. Due to their ability to inhibit the synthesis and release of thromboxane  $A_2$  (platelet aggregating factor), some of these agents are also useful in the treatment of diseases characterized by platelet hyperaggregation such as, coronary artery disease, myocardial infarction etc.

Their use in rheumatism is, however, symptomatic only. The remission of rheumatoid or osteoarthritis requires corticosteroid treatment, often combined with the use of penicillamine, antimalarials, gold compounds (e.g., auranofin) or immunosuppressive agents.

Their effectiveness in various inflammatory conditions is due to their ability to inhibit the biosynthesis of prostaglandins. Aspirin itself inactivates the cyclooxygenase enzyme by acetylating serine group at its active site. With the exception of indomethacin, the aspirin-like drugs irreversibly inhibit the cyclooxygenase enzymes.

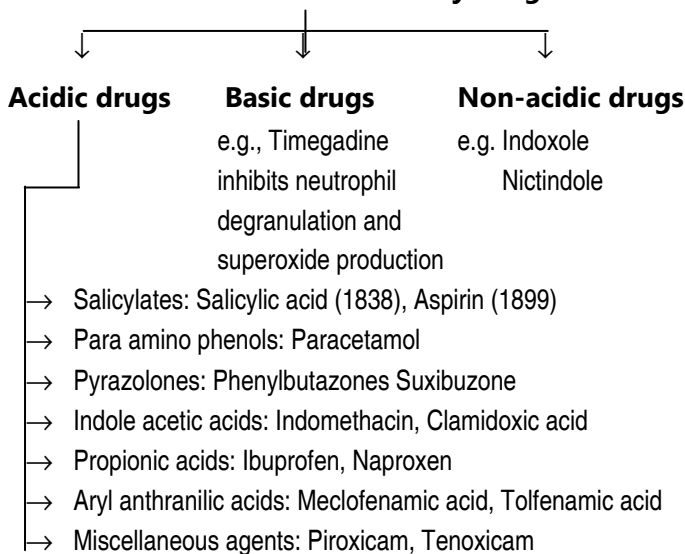
Besides this, some of these agents have an ability to speed up the breakdown of mucopolysaccharides, in addition to inhibiting its synthesis. They also stabilize the lysosomes and cool down other mediators of inflammation.

Some of 'aspirin-like' drugs have uricosuric effect (i.e., promote excretion of uric acid) and hence may be useful in the treatment of gout.

## 12.2 CLASSIFICATION

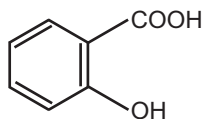
The various analgesic-antipyretic anti-inflammatory agents can be classified as:

### Non-Steroidal Anti-inflammatory Drugs



## 12.3 SALICYLIC ACID DERIVATIVES

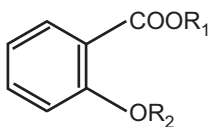
Salicylic acid was first used in rheumatic fever by Mac Lagan in 1877. The salicylic acid derivatives are most widely employed to treat arthritis.



Salicylic acid

The aspirin like drugs are mild analgesics and are effective against pain of low to moderate intensity.

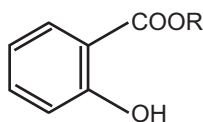
**Table 12.1: Salicylic acid derivatives**



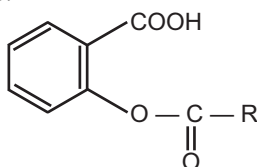
<i>Name</i>	<i>R<sub>1</sub></i>	<i>R<sub>2</sub></i>	<i>Year of introduction</i>
1. Salicylic acid	H	H	1838
2. Methyl salicylate	CH <sub>3</sub>	H	1844
3. Sodium salicylate	Na <sup>+</sup>	H	1875
4. Phenyl salicylate	C <sub>6</sub> H <sub>5</sub>	H	1886
5. Acetyl salicylic acid (Aspirin)	H	COCH <sub>3</sub>	1899

Since, salicylic acid is so irritating, (that it can only be used externally) various derivatives have been synthesized for systemic use. These derivatives can broadly be divided into:

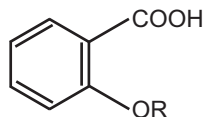
(i) Esters of salicylic acid:



(ii) Salicylate esters of organic acids:



(iii) Phenoxy derivatives:

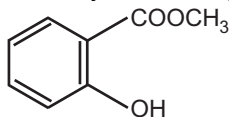


**Important landmarks of Aspirin:**

The antipyretic (fever reducing) property of the bark of the Willow tree (*Salix alba*) was known to the ancient Greeks.

Sr. No.	Year	Landmarks
1.	1763	Edward Stone noticed that chewing the bark of the willow tree helped to relieve the symptoms of malaria – chills and fever.
2.	1827	The active ingredient in willow bark, <b>salicin</b> , was isolated.
3.	1838	Raffaele Piva, an Italian Chemist, hydrolyzes <b>salicin</b> to produce glucose and <b>salicyl alcohol</b> . He further oxidizes salicyl alcohol to salicylic acid, establishing a connection between that substance and the active ingredient in willow bark.
4.	1843	A related compound, <b>methyl salicylate</b> was found by the French chemist, Auguste Cahours and the American chemist, William Proctor, to be a major constituent of oil of wintergreen, which was extracted from the leaves of the wintergreen plant.
5.	1853	Charles Gerhardt of Strasbourg replaced the OH of salicylic acid with an acetyl group using acetic anhydride, the first synthesis of <b>acetyl salicylic acid</b> , which was later called aspirin.
6.	1859-1993	During this period, salicylic acid which is moderately strong acid ( $pK_a = 3$ ) was widely used as a medicine. The acid burned the mouth. Efforts to moderate the effects of its acidity resulted in the administration of the sodium salt of salicylic acid, sodium salicylate. The salt, however has an unpleasant taste.
7.	<b>1893</b>	In an effort to find a less unpleasant way to administer salicylic acid. Felix Haffman, a chemist working for the Bayer pharmaceutical company in Germany, reinvestigated the acetylation reaction first conducted by Gerhardt in 1853. Hoffman's father was rheumatic, which added a personal motivation for finding such a substitute. The synthetic material called <b>aspirin</b> lacked the strong acidity of the salicylic acid and the unpleasant taste of its sodium salt.

**(i) Esters of salicylic acid:** The alkyl and aryl esters are used externally, mainly as counter irritants. These compounds have very little analgesic value e.g.



Methyl salicylate

Salicylic acid is moderately strong acid ( $pK_a = 3.0$ ). It burns the mouth. Efforts to dilute the effects of its acidity resulted in sodium salicylate. It has however an unpleasant taste.

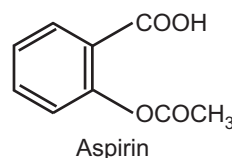
A few inorganic salicylates are used internally as analgesics. These compounds vary in their stomach irritation property.

These include the following salts of salicylic acid:

- (a) Sodium salicylate
- (b) Sodium thiosalicylate
- (c) Magnesium salicylate
- (d) Choline salicylate
- (e) Less commonly used
  - (i) Ammonium salicylate
  - (ii) Lithium salicylate
  - (iii) Strontium salicylate.

**(ii) Salicylate esters of organic acids:** Examples from this class are:

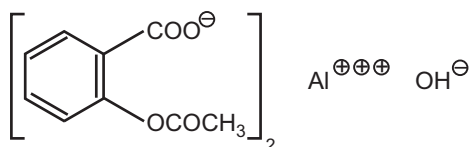
Aspirin lacks the strong acidity of salicylic acid and unpleasant taste of its sodium salt. It is still the most extensively employed analgesic-antipyretic and anti-inflammatory agent associated with few untoward effects like allergic reactions (asthma and urticaria) and gastric irritation (due to its hydrolysis to salicylic acid).



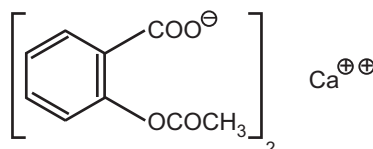
The name was coined by adding an "a" for acetyl to spirin for Spiraea, the plant species from which salicylic acid was once prepared.

The following salts of aspirin appear to have fewer undesirable side-effects and to induce analgesia faster than aspirin.

(a) Aluminium aspirin

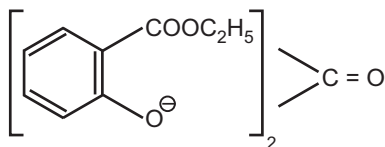


(b) Calcium aspirin

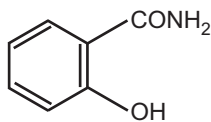


Other compounds of interest are:

**(1) Carbethyl salicylate:** It is an ester of ethyl salicylate and carbonic acid and thus is a combination of a type I and type II derivatives of salicylic acid.



**(2) Salicylamide:** It gets excreted more rapidly than other salicylates and exerts a moderately quicker and deeper analgesic effect than does aspirin.



Salicylamide

### Structure-Activity Relationship:

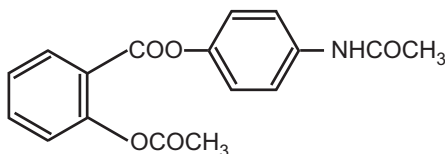
- (a) Various substitutions on the carboxyl or hydroxyl group result into change in potency as well as toxicity.
- (b) The ortho position of the OH group is an important feature for the action of the salicylates.
- (c) Benzoic acid, though much weaker, shares many of the actions of salicylic acid.
- (d) Various approaches have been made towards the design of a superior analogue.

### For Example:

**(1) Trilisate (Choline Magnesium Trisalicylate):** It is a complex salt of salicylic acid with choline and magnesium which possesses longer duration of action and lesser gastrointestinal irritation than aspirin.

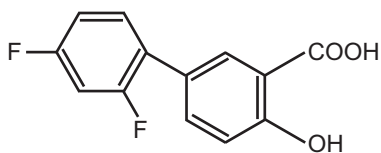
**(2) Trolamine salicylate:** It is a topical analgesic used in sunscreen. The salicylic acid possesses both the sun protection effect (by absorbing UV radiation) and analgesic effect. Triethanolamine neutralizes the acidity of salicylic acid.

**(3) Benorylate:** It is the N-acetylaminophenol ester of aspirin.



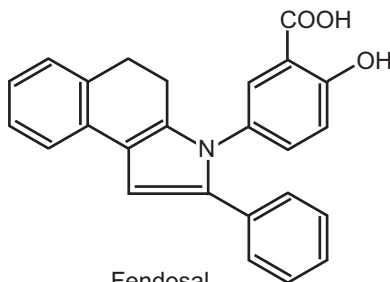
Benorylate

**(4) Diflunisal (1971):** It is recently introduced for clinical use in some parts of the world. It has a long duration of action.



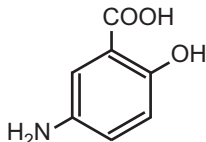
5-(2,4-difluorophenyl) salicylic acid

**(5) Fendosal:** It possesses an analgesic activity comparable to that of diflunisal.

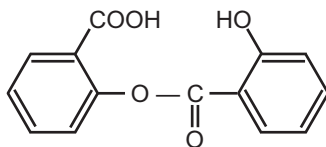


Fendosal

**(6) Mesalazine (5-aminosalicylic acid):** It is a bowel specific anti-inflammatory drug used to treat inflammation of the digestive tract ulcerative colitis. It is considered as active metabolite of sulfasalazine.

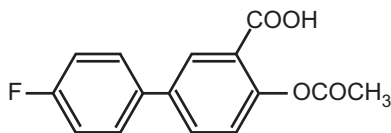


**(7) Salsalate:** It is the ester formed between two salicylic acid molecules. Since, it is relatively insoluble in the stomach and is not absorbed until it reaches the small intestine, it is said to cause less gastric irritation.



Salsalate

**(8) Flufenisal:** With the introduction of a hydrophobic group (F) at 5' position, the compound became more potent, longer acting and with less gastric irritation.



Flufenisal

**Local Actions:**

Salicylic acid and methyl salicylate, since, both are too irritant to the gastric mucosa internally; these compounds are used for topical applications due to their keratolytic, antiseptic and fungistatic actions.

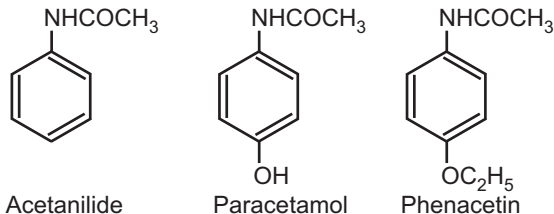
**Salol Principle:**

Salol, (phenyl salicylate) was introduced by Nencki in 1886. It is an ester of two toxic substances like phenol and salicylic acid.

When both the components (i.e., alcohol and acid) of an ester are active compounds, the ester is called as True salol or Full salol e.g. phenyl salicylate (salol) and  $\beta$ -naphthol benzoate (betol). When only one component of the ester (either alcoholic or acidic part) is active, toxic or corrosive compound, the ester is referred to as a partial salol e.g. methyl salicylate and thymol carbonate.

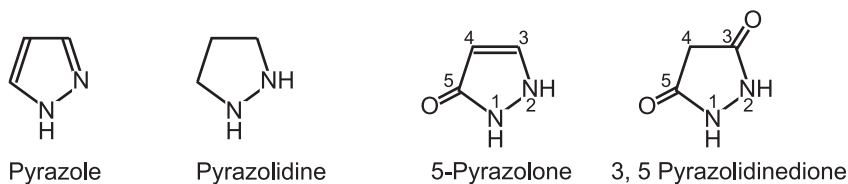
**12.4 PARA-AMINO PHENOL DERIVATIVES**

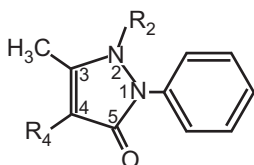
Since p-amino phenol is the metabolite of aniline (aniline also possesses antipyretic activity) these analgesics are also being called as "coal tar analgesics". The only agents of interest, from this class are:



Acetanilide is metabolised into paracetamol and aniline. The toxicity of the latter compound discouraged its use in therapeutics.

The p-amino phenol derivatives are analgesics and antipyretics. They do not have anti-inflammatory activity. They are safe in children and patients with ulcers. However, they are hepatotoxic.

**12.5 PYRAZOLONE DERIVATIVES**

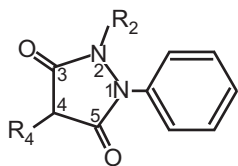
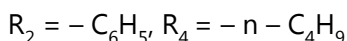
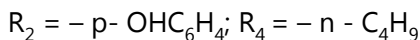
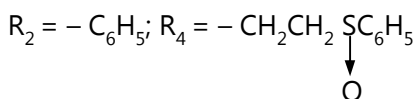
**(a) 5-Pyrazolone derivatives:**

Compound	R <sub>2</sub>	R <sub>4</sub>
Antipyrine	-CH <sub>3</sub>	H
Aminopyrine	-CH <sub>3</sub>	-N (CH <sub>3</sub> ) <sub>2</sub>
Dipyrone	-CH <sub>3</sub>	$\begin{array}{c} \text{--- N --- CH}_2 \text{ SO}_3 \text{ Na} \\   \\ \text{CH}_3 \end{array}$

Antipyrine is the parent drug from this category. Its modification further resulted into the introduction of aminopyrine and dipyrone in clinical use.

Antipyrine and aminopyrine have analgesic, antipyretic and antirheumatic activities. Fatal agranulocytosis caused by dipyrone and aminopyrine has limited their usefulness. The patient is at high risk of infection due to low count of granulocytes (i.e. neutrophils + basophils + eosinophils).

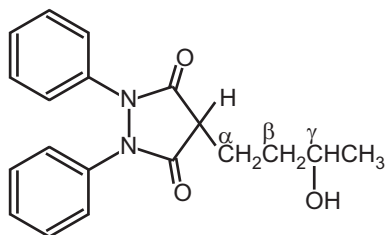
Aminopyrine is no longer an official drug. Chemically aminopyrine is N-phenyl-N-alkyl-substituted pyrazoline-3-one. Being considered as structural analogue of quinine, the antipyretic action of aminopyrine was discovered. Due to haematological toxicity search for improved analogue of aminopyrine lead to phenylbutazone.

**(b) 3, 5-Pyrazolidinedione derivatives:****(1) Phenylbutazone****(2) Oxyphenbutazone****(3) Sulfipyrazone**

To eliminate GI-disorders and occasional agranulocytosis, the n-butyl group of phenylbutazone may be replaced by (CH<sub>2</sub>)<sub>2</sub>SOC<sub>6</sub>H<sub>5</sub> which increases analgesic activity and uricosuric activity in sulfipyrazone.

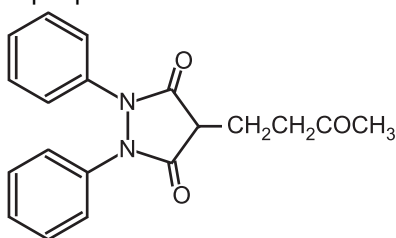
Phenylbutazone, although analgesic itself, was originally developed as a solubilizer, for the insoluble aminopyrine. Compared with phenyl butazone, oxyphenbutazone is an equally potent anti-inflammatory analgesic but is slightly less toxic.

Phenylbutazone completely undergoes metabolism by liver microsomal enzymes to (a) oxyphenbutazone and (b)  $\gamma$ -hydroxy-phenylbutazone. Other compounds of interest from this series are:



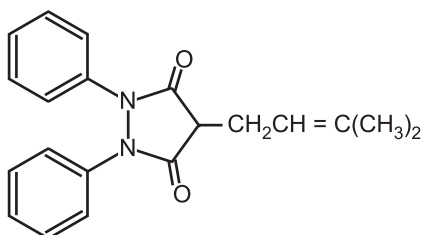
$\gamma$ -Hydroxyphenylbutazone

**(a) Kebuzone:** It has similar properties and actions as that of phenylbutazone.

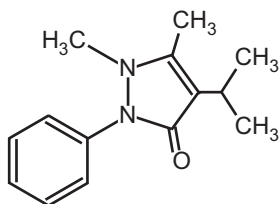


Kebuzone

**(b) Propyphenozone**

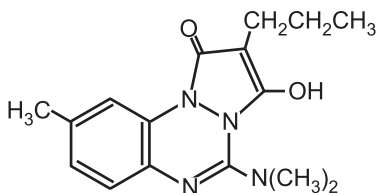


**(c) Phenazone:**



Propyphenazone

**(d) Azapropazone:** It is a pyrazolo benzotriazinedione derivative, having similar activity as that of phenylbutazone.



Azapropazone

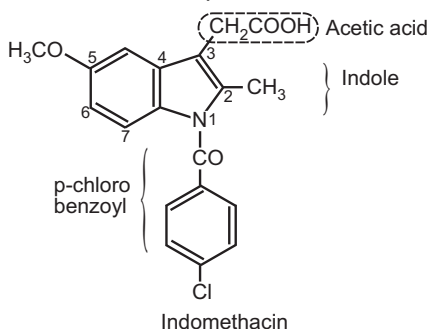
**SAR for pyrazolidine diones:**

- (i) Activity decreases if n-butyl group at C<sub>4</sub> is replaced by propyl or allyl group.
- (ii) Substitution (e.g. CH<sub>3</sub>, Cl, NO<sub>2</sub>, OH) only at para position in phenyl ring retains the activity.
- (iii) Replacement of nitrogen in pyrazolidines with oxygen yields equipment isoxazole analog.
- (iv) Analogs with lower pK<sub>a</sub> value passes shower plasma half life.
- (v) Substitution at C<sub>4</sub> by methyl group destroys anti-inflammatory activity.
- (vi) The most active analog has log P value of 0.7.

**12.6 INDOMETHACIN AND OTHER ARYLACETIC ACID DERIVATIVES**

The possibility that 5-hydroxytryptamine might be an important mediator of inflammation led to the discovery of indomethacin, after the laboratory evaluation of 350 indole derivatives.

Introduced in 1964, indomethacin is a powerful anti-inflammatory analgesic agent.



In man, it is largely metabolised by O-demethylation and N-deacylation. Excretion is facilitated by conjugation with glucuronic acid.

The most frequent side-effects include peptic ulceration, blood disorders, severe frontal headache and GIT disturbances.

**Structure-Activity Relationship:**

(1) The following substituents generally give expected activities:

(a) Indole substituents:

5-Methoxy, F, (CH<sub>3</sub>)<sub>2</sub>N;

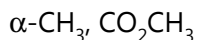
5-Methoxy-6-F; 2-Methyl

(b) Benzoyl substituents:

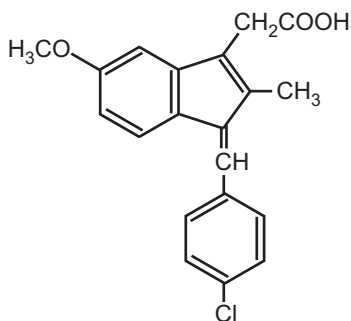
CF<sub>3</sub> or SCH<sub>3</sub> at para position provides greatest anti-inflammatory activity:

p - Cl, F or CH<sub>3</sub>S

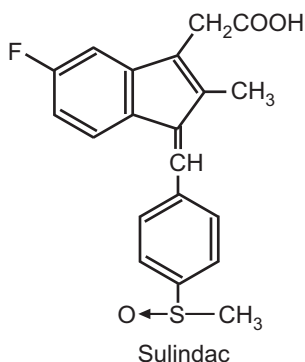
(c) Acetic acid substituents:



- (2) The carboxyl group is necessary for antiinflammatory activity. The more acidic the carboxyl group, the greater the antirheumatic activity.
- (3) N-substitution of indole derivative increases anti-inflammatory activity in the order benzoyl > alkyl > H.
- (4) The 1-indene isostere has activity similar to that of indomethacin.



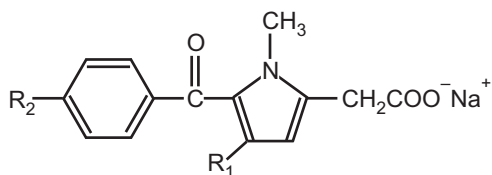
The study of SAR of this class resulted in the development of following clinically used agents.



Sulindac is a pro-drug, the active form being its reduced sulfide ( $-\text{S CH}_3$ ) derivative. It is also an indene isostere, substituted by F in the indene and by methylsulphoxide in phenyl group.



### Pyrroleacetic Acid Derivatives:

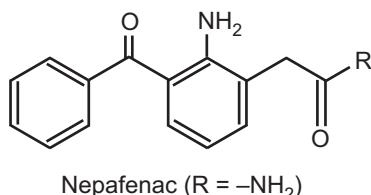
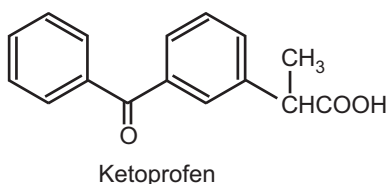
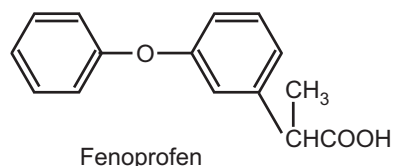
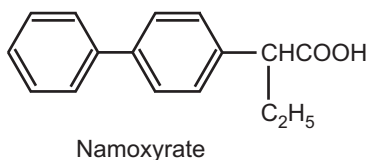
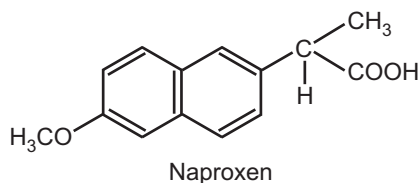
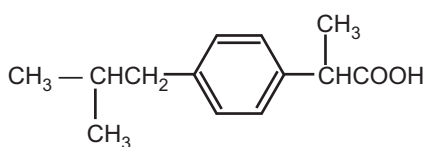


Tolmetin:  $R_1 = -\text{H}$ ;  $R_2 = -\text{CH}_3$

Zomepirac:  $R_1 = -\text{CH}_3$ ;  $R_2 = -\text{Cl}$

## 12.7 PHENYLACETIC ACID AND PROPIONIC ACID DERIVATIVES

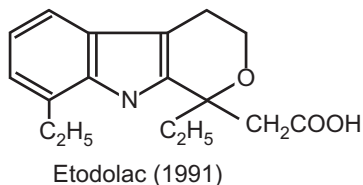
Numerous phenylacetic acid and propionic acid derivatives have been synthesized and found to possess anti-inflammatory activity. The most commonly employed agents from this class are:



Amfenac (R = -OH)

Nepafenac is a prodrug. After topical ocular dosing, it penetrates the cornea and is converted by ocular tissue hydrolases to amfenac, a non-steroidal anti-inflammatory drug.

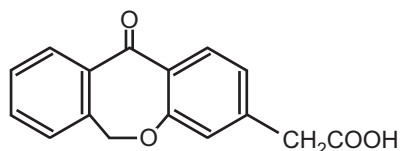
Indomethacin, piroxicam and ibuprofen inhibit PG - synthesis by macrophages (present in inflammatory exudates as well as by syncoviocytes and chondrocytes which contribute to inflammation of joint) almost to the same extent.



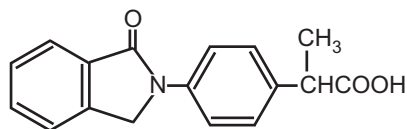
Etodolac is also suggested for the treatment of osteoporosis. It is a potent anti-inflammatory drug with a high gastric tolerance. The apparent elimination half-life is 7 hours.

It extensively binds to plasma-proteins. Etodolac is found to possess potent anti-inflammatory, anti-arthritic and analgesic activity. It is superior to other NSAIDs in having less faecal blood loss. Chemically, it is 1, 8 - diethyl - 1, 3, 4, 9 - tetrahydro pyrano [3, 4-b] indole -1- acetic acid. In man, a dose of 200 mg per day is suggested as minimum effective dose for the relief of active rheumatoid arthritis.

Several other arylacetic acid derivatives are under clinical trials. These include alcofenac, fenclofenac, pirprofen, prodolic acid, ketoprofen and oxepinac.

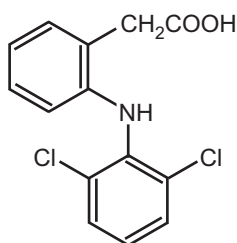


Oxepinac



Indoprofen

A hybrid of fenamate and phenylacetic acid is diclofenac.



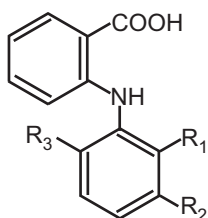
Diclofenac

Replacement of carboxyl group by an ester, alcohol, amide, hydroxamic acid (NHOH) or tetrazole ( $\text{CHN}_4$ ) generally produces less active compound. Among enantiomers, activity usually resides in the S(+) isomer.

The metabolism of substituted phenyl acetic acids involves mainly aromatic or aliphatic hydroxylation followed by glucuronide conjugation at the hydroxyl and/or carboxyl group.

## 12.8 FENAMATES OR DERIVATIVES OF N-ARYLANTHRANILIC ACID

Replacing phenolic OH of salicylic acid by an aryl substituted amino group which results in isosters of aryl ethers of salicylic acid. Fenamates are a family of N-arylanthranilic acids, which are nitrogen analogues of salicylic acid.

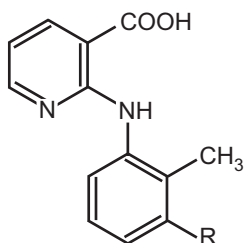


N-arylanthranilic acid

- (1) Mefenamic acid:  $R_1 = R_2 = -\text{CH}_3$
- (2) Flufenamic acid:  $R_1 = -\text{H}$ ,  $R_2 = -\text{CF}_3$
- (3) Meclofenamic acid:  $R_1 = -\text{Cl}$ ,  $R_2 = -\text{CH}_3$ ,  $R_3 = -\text{Cl}$   
( $R_3 = \text{H}$ ; except in meclofenamic acid)

Mefenamic acid, flufenamic acid and meclofenamic acid are the clinically useful fenamates. Mefenamic acid has moderate anti-inflammatory activity and mainly used as a short term analgesic. Diarrhoea, drowsiness and headache are among the principal side-effects. It is also used in the management of primary dysmenorrhea, which is thought to be caused by excessive concentrations of prostaglandins.

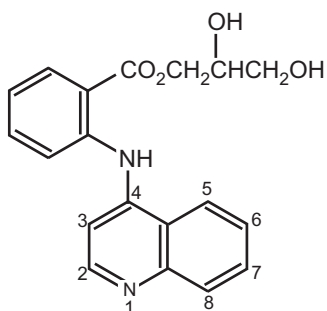
The following heterocyclic isosters of fenamates are under clinical trials.



(i) Clonixin: R = - Cl

(ii) Flunixin : R = - CF<sub>3</sub>

### Substituted aza analogues:



(i) Glafenine: 7-Cl

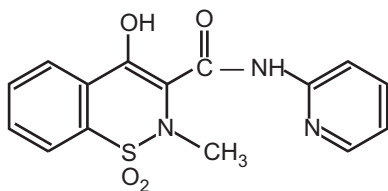
(ii) Floctafenine: 8-CF<sub>3</sub>

Glaphenine is a combination of 7-chloroquinoline and anthranilic acid. These compounds possess only weak anti-inflammatory activities.

High incidences of anaphylactic reactions and acute renal failure have led to the withdrawal of glafenine in most of the countries.

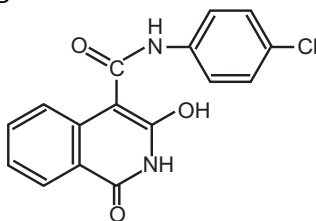
## 12.9 MISCELLANEOUS AGENTS

(a) Many o-hydroxy aromatic carboxylates have been tried (salicylic acid) in which benzene ring of salicylic acid is replaced by other aromatic or quasiaromatic nuclei. The group of oxicams represents N-aryl-carboxamides of 4-hydroxy-1, 2-benzothiazine 1, 1-dioxides. e.g., piroxicam.



Piroxicam  
Pfizer (Feldene)  
Log P = 0.26, pKa = 4.6

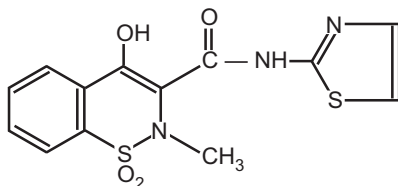
1. The initial compounds, isoquinoline carboxanilides showed AI potency similar to phenylbutazone. The enhanced acid properties of these cyclic  $\beta$  - diketones were responsible in part for their biological activities.



Tescicam

Metabolic studies in animal suggested that chlorine substituted at 4-position of the phenyl ring extended the half-life and duration of action.

2. 2H - 1, 2 - benzothiazine - 3 (4H) - one - 1, 1-dioxides as bioesters of tescicam was synthesized. This series did not prove fruitful. Consequently, the isomeric carboxamides of the 4 - hydroxy - 2H - 1, 2 - benzothiazine - 1, 1 - dioxide were prepared.

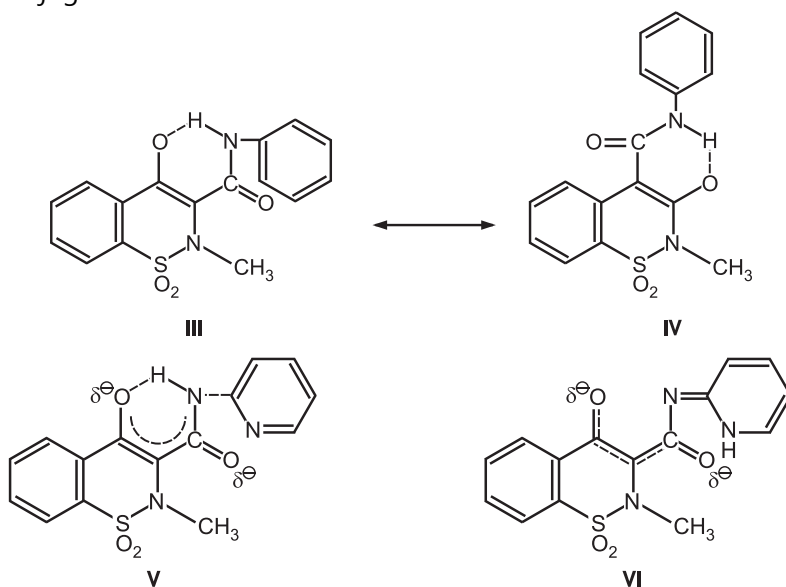


Sudoxicam

Sudoxicam is more potent than tescicam and has a longer duration of action.

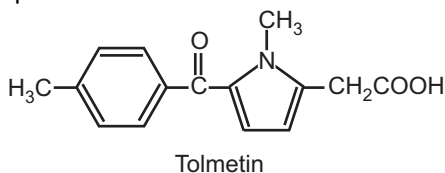
3. Further research led to piroxicam which has a half-life of 45 hours.

4. The tautomeric structures impart further stability to the enolate anion. Such stabilization of the enolate anion would thereby contribute to a further increase in the acidity of the conjugated acid.



5. It is assumed that first step of the reaction involves a reduction of the enzymatic  $\text{Fe}^{+++}$  to  $\text{Fe}^{++}$  by abstracting H-atom at  $\text{C}_9$  of arachidonic acid to give a delocalised radical which reacts with molecular oxygen. This results into formation of superoxide anion which is metabolised to reactive oxygen species including  $\text{H}_2\text{O}_2$ , hydroxyl radical and singlet oxygen. These reactive oxygen species are thought to contribute to the inflammatory process and tissue destruction.

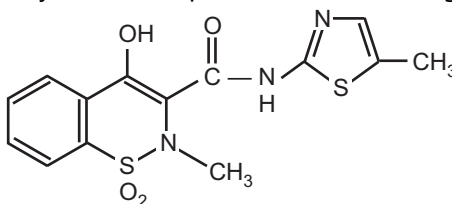
Piroxicam and tolmetin are recently developed promising agents having good analgesic-antipyretic and anti-inflammatory activities. Both these agents have an ability to inhibit cyclo-oxygenase enzyme. They are rapidly and completely absorbed from GIT, and get extensively bound to plasma-proteins.



Piroxicam is metabolised mainly by hydroxylation in the pyridyl ring followed by glucuronide conjugation, while tolmetin metabolism occurs by the oxidation of para methyl group to COOH. The metabolites are excreted in urine in both, free and conjugated forms. Piroxicam is better tolerated agent, having long half-life.

Both these drugs cause gastric erosions and increase in the bleeding time. The most frequent adverse effects include nausea, vomiting, epigastric pain, anxiety, skin rash, gastric and peptic ulceration.

**Meloxicam:** It is structurally related to piroxicam and belongs to oxicam family.



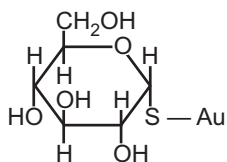
### SAR for Oxicams:

- (i) The nitrogen of benzothiazine having  $\text{CH}_3$  substituent and electron withdrawing groups ( $\text{C}_1$ ,  $\text{CF}_3$ ) on anilide phenyl ring increase anti-inflammatory activity.
- (ii) The introduction of a heterocyclic ring in the amide chain significantly increases the activity. Sudoxicam is more potent than indomethacin.
- (iii) The benzothiazines having pKa range of 6 to 8 have more activity.

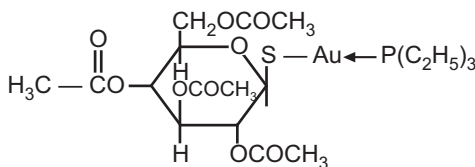
**(b) Gold compounds:** The clinically used agents from this category include aurothioglucose, auranofin and gold sodium thiomalate. In all these agents, the gold is directly attached to sulphur. Hence, these compounds are supposed to act by the inhibition of vital sulfhydryl systems in the body. Gold gets accumulated in the lysosomes where it inhibits the activity of acid phosphatase,  $\beta$ -glucuronidase and cathepsin enzymes which have a catalytic role in various inflammatory disorders.

In addition, gold compounds inhibit the synthesis of connective tissues. Hence, they can be used in the treatment of rheumatoid arthritis in patients who do not respond well to the therapy with aspirin-like drugs.

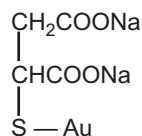
They are usually administered by intramuscular route, since the absorption from oral route is erratic and incomplete. They are extensively bound to plasma-proteins. Their onset of action is slow and signs of inflammation are reduced in intensity gradually. The slow rate of excretion of gold compounds can be enhanced by concomitant administration of sulfhydryl agents like, penicillamine and dimercaprol. They are primarily excreted in urine and faeces.



Aurothioglucose



Auranofin



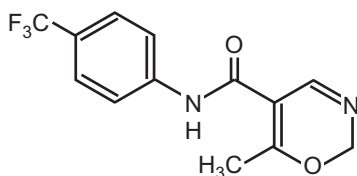
Gold sodium thiomalate

The adverse effects associated with gold therapy include cutaneous reactions, aplastic anaemia, leucopenia, agranulocytosis, thrombocytopenia, nephrosis, hepatitis and peripheral neuritis. They are contraindicated in patients with anaemia, renal disease, hepatic dysfunction and in pregnancy.

**(c) D-penicillamine:** Only D-isomer is clinically used in the treatment of rheumatoid arthritis because L-penicillamine is reported to cause optic neuritis due to its anti-pyridoxine activity. Being a metabolite of penicillin, it has a structural resemblance with cysteine. In certain cases, combination of D-penicillamine with aspirin-like drugs may give better results. It, alongwith a disulphide metabolite is excreted in urine and faeces. Due to its high toxicity, it should not be used frequently or for a long-term treatment.

**(d) Abatacept:** It is a fusion protein composed of an immunoglobulin fused to the extra cellular domain of CTLA-4, a molecule capable of binding B7. It is used in delaying the progressing of structural damage and reducing symptoms of rheumatoid arthritis. It is also beneficial in the treatment of psoriasis and in organ transplantation.

**(e) Leflunomide:** It is a pyrimidine synthesis inhibitor used to treat rheumatoid arthritis and psoriatic arthritis.



**(f) Other agents which have beneficial effects in the treatment of inflammatory disorders include:**

- (i) Antimalarial agents: Chloroquine and hydroxy chloroquine.
- (ii) Glucocorticoids
- (iii) Immunosuppressive agents: Azathioprine, cyclophosphamide.
- (iv) Sulphonamides: Diflumidone.

## 12.10 MECHANISM OF ACTION OF NON-STEROIDAL ANTI-INFLAMMATORY AGENTS

**(a) Biosynthesis of prostaglandins:** The biochemical effects of NSAIDs include inhibition of lysosomal membrane stabilization, inhibition of the biosynthesis of mucopolysaccharides, uncoupling oxidative phosphorylation, fibrinolytic activity, sulfhydryl disulfide stabilization, collagenase production and at times suppression of lymphocytic functions.

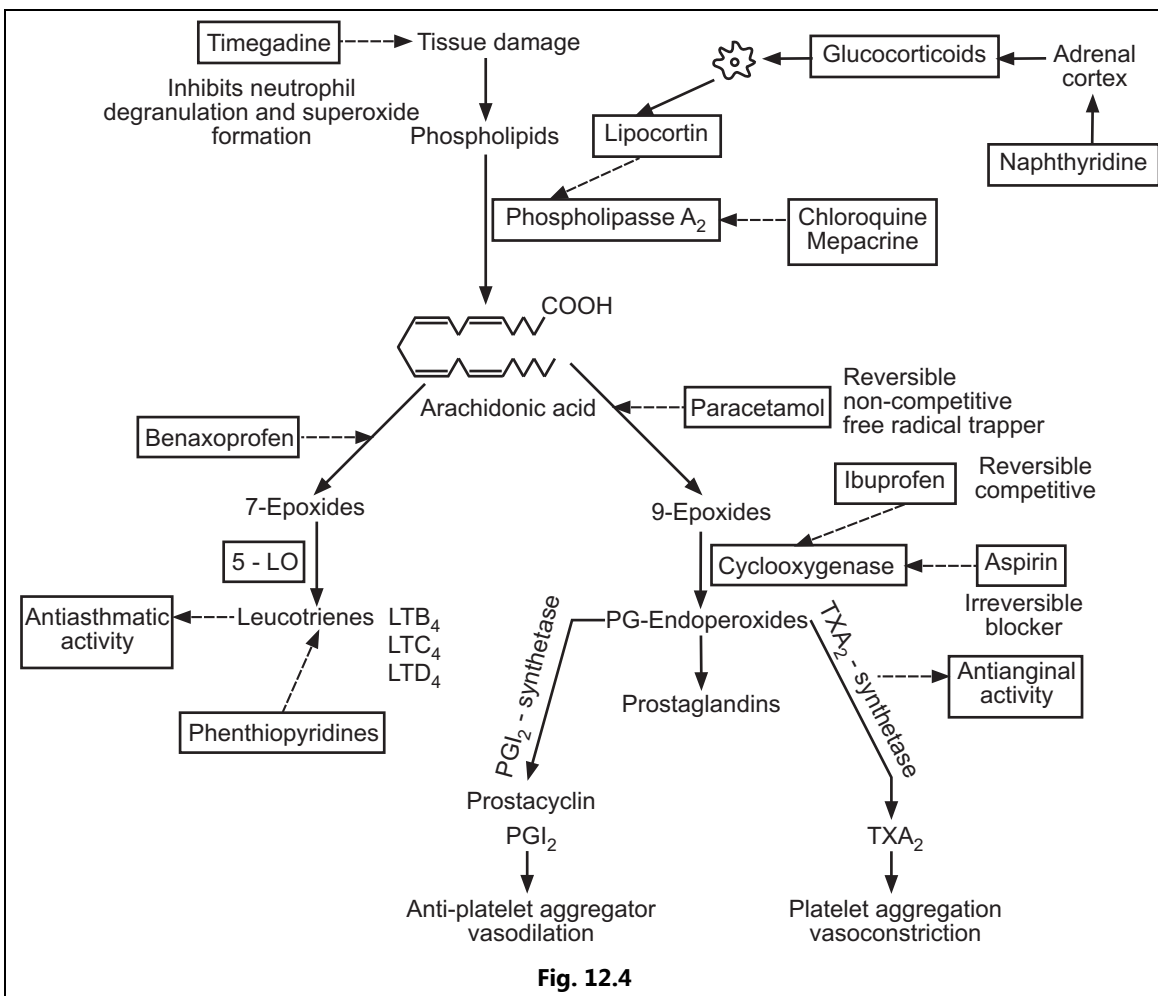


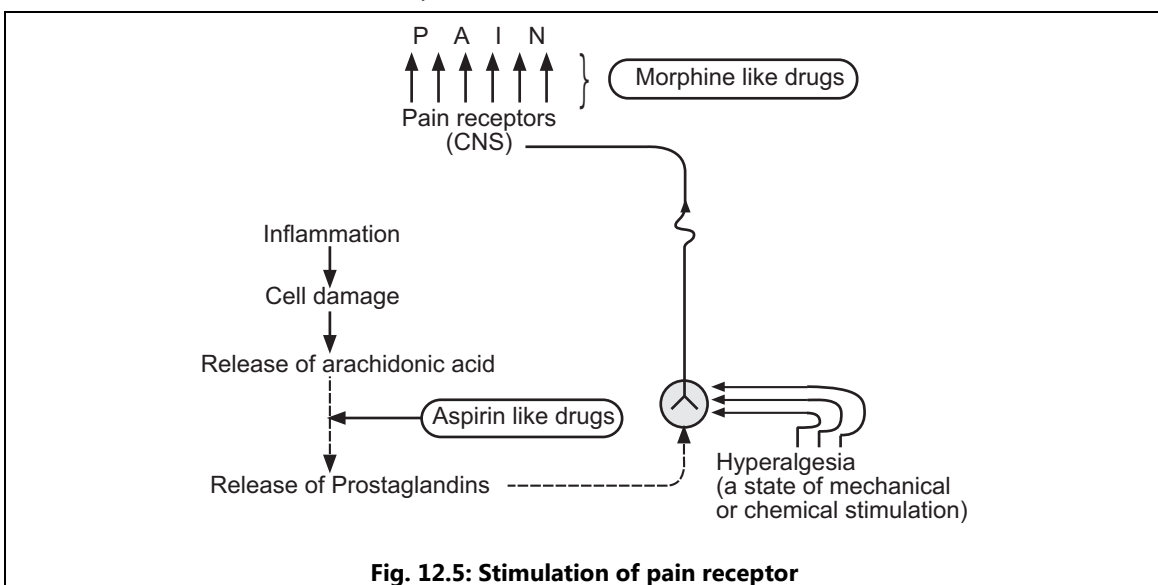
Fig. 12.4

Cyclooxygenase catalyses two enzymatic processes (1) The incorporation of oxygen in a dioxygenase step to form  $\text{PGG}_2$  and (2) the subsequent peroxidation to  $\text{PGH}_2$ . The reaction is initiated by the stereospecific abstraction of hydrogen at  $\text{C}_{13}$  followed by oxygen attack at  $\text{C}_{11}$  and  $\text{C}_{15}$  and ring closure between  $\text{C}_8$  and  $\text{C}_{12}$  in next reaction. The presence of hemein and molecular oxygen is required. Most of NSAIDs act by inhibiting cyclooxygenase by preventing the abstraction of hydrogen from  $\text{C}_{13}$  and therefore blocking peroxidation at  $\text{C}_{11}$  and  $\text{C}_{15}$ . This action is highly specific, for similar abstraction and peroxidation reactions at other points in the fatty acid molecule are not inhibited.

On the basis of mechanism of action, these chemically diversified NSAIDs can be broadly divided into:

**(a) Reversible competitive inhibitors:** Examples of this class are fatty acids, closely related to the substrate which have a comparable affinity for the cyclooxygenase (CO) and lipooxygenase (LO) enzymes but are not converted to oxygenated inflammatory products of CO pathway (i.e., prostaglandins) and LO pathway (leucotrienes). Ibuprofen has a binding affinity for cyclooxygenase similar to that of arachidonic acid. The carboxyl function of aryl acidic NSAIDs is said to resemble the terminal carboxyl of arachidonic acid while the planar hydrophobic groups bind to the enzyme to prevent hydrogen abstraction at  $\text{C}_{13}$ . The presence of aryl halogen is supposed to enhance this activity due to its lipophilicity.

**(b) Reversible non-competitive inhibitors:** The reversible non-competitive inhibitors have anti-oxidant or radical trapping properties. During inflammation, a continual presence of lipid peroxides induces a free radical chain reaction that sustains cyclooxygenase activity. This can be blocked by an addition of radical scavengers or anti-oxidants (e.g. paracetamol) which acts as reversible non-competitive inhibitors.



**Fig. 12.5: Stimulation of pain receptor**

**(c) Irreversible inhibitors:** The irreversible inactivation of cyclooxygenase is done by aspirin through the transacetylation of the lysyl amino group in the enzyme which is important for its activity. As salicylic acid is chemically incapable of acylating the enzyme, the anti-inflammatory action of salicylic acid may depend more on other mechanisms such as inhibition of leukocyte emigration and lysosomal stabilization.

Aspirin like drugs block this release of prostaglandins in the brain, followed by peripheral vasodilation and increased sweating resulting into considerable heat loss from the body. This brings down the body temperature to its normal.

### 12.11 TREATMENT OF GOUT

Gout is a term representing a heterogeneous group of genetic and acquired diseases manifested by hyperuricemia and a characteristic acute inflammatory arthritis induced by crystals of monosodium urate monohydrate. Some patients develop aggregated deposits of these crystals (tophi) in and around the joints of the extremities that can lead to severe crippling. Many patients develop a chronic interstitial nephropathy. In addition, uric acid urolithiasis is common in gout. Primarily gout is chiefly a disease of adult men. The frequency of gout is increased in patients taking diuretics, especially of the thiazide group. Gout in all of its forms makes up about 5% of arthritis cases. Humans lack uricase, therefore, uric acid is the end product of purine metabolism. In normal subjects, approximately one third of uric acid disposed of each day is degraded by bacteria in the gut and two third is excreted unchanged by kidney. Both increased purine biosynthesis and decreased renal excretion of uric acid play important roles in the pathogenesis of primary hyperuricemia.

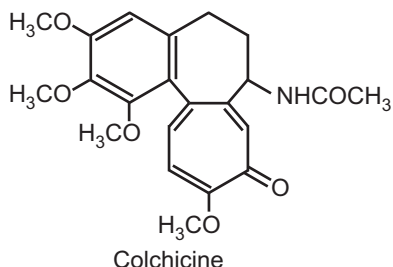
Once the uric acid is filtered by renal glomeruli, it is almost completely reabsorbed into circulation from proximal tubules. In normal circumstances, some of the reabsorbed uric acid is again driven back into the urine by distal tubules. Due to low solubility of undissociated form of urates, the crystals of sodium urate (the end product of purine metabolism) get deposited in the joint cavities and on articular cartilages. Their deposition initiates inflammatory reactions which involve local infiltration of phagocytes that, after ingestion of urate also release chemotactic substances and probably lactic acid. Lactic acid further lowers down the pH of the surrounding medium, resulting into further deposition of uric acid. The phagocytosis of urate crystals releases a glycoprotein which is responsible to produce acute gouty arthritis. An acute attack of gout occurs as a result of an inflammatory reaction. Usually small joints are affected before larger ones.

Uricosuric agents enhance the rate of excretion of uric acid by reducing the rate of its tubular reabsorption. They thus relieve the signs and symptoms of acute attack of gout and offer symptomatic relief in this condition. Phenylbutazone is such a uricosuric agent.

**(a) Colchicine:**

It is an alkaloid obtained from *colchicum autumnale*. It was clinically introduced for the treatment of acute attacks of gout in 1763 by Von Storck.

It does not possess analgesic activity. In inflammatory disorders, it is effective only against acute gouty arthritis. It neither effectively inhibits the prostaglandin biosynthesis nor it influences the tubular reabsorption of uric acid. It probably acts to inhibit the release of lactic acid during phagocytosis of the urate crystals. Its central effects include, depression of the respiratory centers, central vasomotor stimulation and antipyretic action.



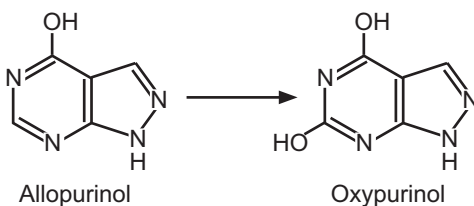
The alkaloid is readily absorbed from GIT and by intravenous route. The drug along with its metabolites is mainly excreted through urine and faeces.

The adverse effects are mild and include nausea, vomiting, diarrhoea, abdominal pain and leucopenia. These effects appear in the dose-dependent fashion and are reversed, if treatment is discontinued. In severe toxicity, death usually results due to respiratory arrest.

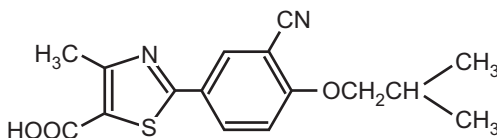
**(b) Allopurinol:**

Chemically, it resembles in structure with hypoxanthine. It inhibits the formation of uric acid by competitively antagonising xanthine oxidase enzyme which catalyses the conversion of hypoxanthine to xanthine the precursor for uric acid synthesis. Due to the structural similarity, it competes with hypoxanthine which is the substrate for xanthine oxidase enzyme. At higher concentration, due to the non-specific nature of the enzyme, it acts as non-competitive inhibitor. Instead of hypoxanthine, allopurinol is attacked by xanthine oxidase and is converted primarily to oxypurinol which is also effective enzyme inhibitor. Thus by inhibiting the uric acid formation, it lowers down hyperuricemia and prevents the formation of uric acid stones. In order to enhance therapeutic effectiveness, allopurinol may sometimes be combined with uricosuric agent.

The adverse reactions include, nausea, vomiting, diarrhoea, gastric irritation, headache, fever, drowsiness, and cutaneous reactions. In some patients, hypersensitivity reactions may also be seen.



**Febuxostat:** It is a xanthine oxidase inhibitor used in the treatment of hyperuricemia and gout.



The pK<sub>a</sub> of uric acid is 5.6. The solubility of undissociated urates is usually low. Hence, their solubility can be increased by inducing their ionisation.

Hence alkalization of urine is one of the effective ways to minimise the intra-renal urate deposition.

Probenecid and sulfinpyrazone also mobilise the uric acid. They are also useful agents in the treatment of chronic gout disorders though they lack analgesic and anti-inflammatory activities.

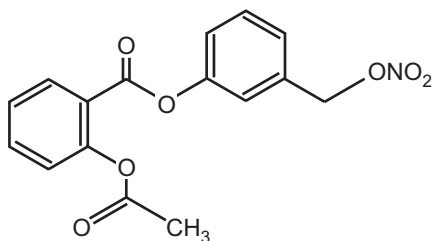
Recent strategies adopted to minimize the side effects of NSAIDs include the use of the dual LOX/COX inhibitors, the use of selective COX-2 inhibitors and the use of hybrid molecules made up of non-selective or selective COX inhibitors together with a nitric oxide releasing function. Recent data revealed serious cardiovascular side effects to selective COX-2 inhibitors. In addition, such drugs only minimize the development of new gastric ulcers but do not affect the existing ones. The strategy involving the use of hybrid molecules made up of non-selective COX inhibitors together with a nitric oxide donor, constitute one of the most promising approaches, because nitric oxide supports several endogenous GIT defence mechanisms, including increase in mucus, bicarbonate secretions, increase in mucosal blood flow and inhibition of activation of proinflammatory cells. Moreover because of the beneficial cardiovascular effects of NO, such drugs are expected to be devoid of the potential adverse CVS effects associated with the use of selective COX-2 inhibitors. Among those NO-NSAIDs that came into clinical trials are nitroaspirin, nitronaproxene, nitroketoprofen, nitroibuprofen etc. Among the nitric oxide donors adopted to prove the validity of this principle are furoxans, oximes, hydrazides and organic nitrates.

This may be due to a reduction in the level of the desirable platelet aggregation inhibitor and vasodilatory prostacyclin (PGI<sub>2</sub>) in conjunction with an increased level of the undesirable potent platelet activator and aggregator thromboxane A<sub>2</sub> (TxA<sub>2</sub>).

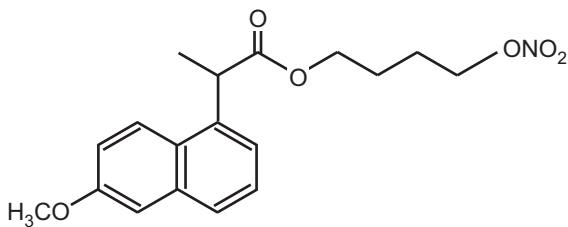
At nanomolar concentrations NO reversibly activates soluble guanylate cyclase by 400 fold, catalyzing the conversion of GTP to c-GMP. Elevation of c-GMP relaxes vascular smooth muscles, inhibits platelet aggregation and adhesion and blocks the adhesion of white cells to blood vessel walls. Similarly, NO acts as a critical mediator of gastrointestinal mucosal defense, exerting many of the same actions as prostaglandins in the GIT.

Inhibition of 5-lipo-oxygenase results in the decrease of autocoids which are involved in the pathophysiological production of gastrointestinal ulceration and also promote inflammation due to chemotactic effects. Hence the discovery of novel dual and selective inhibitors of COX-2 and 5-LO has led to a new generation of NSAIDs.

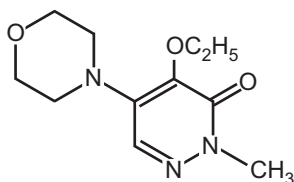
### Recently developed NSAIDs



NO-aspirin



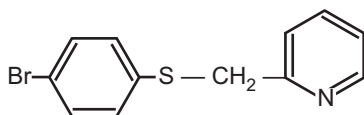
NO-naproxen



**Emarfazole**

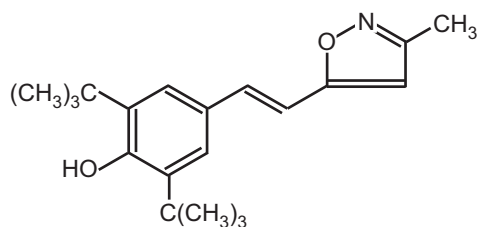
(Japan, Nandran)

(inhibits vascular permeability and release of bradykinin)



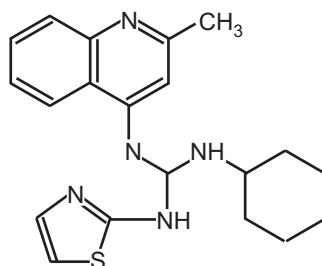
**Phenthiopyridine** (1987)

(inhibits immune complex induced inflammation)



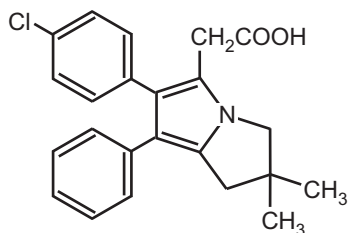
**Isoxazole** (1993)

(dual inhibitor of CO & 5-LO)



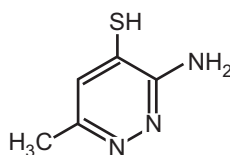
**Timegadine** (1983)

(inhibits neutrophil degranulation and superoxide production)



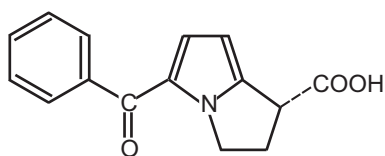
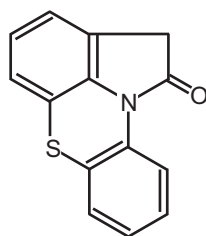
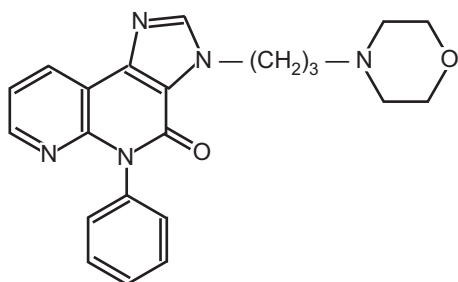
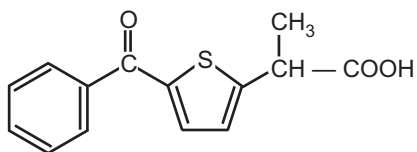
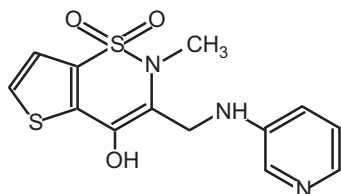
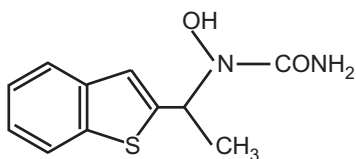
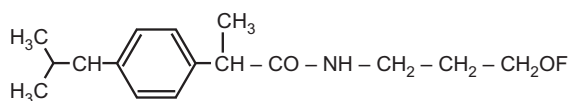
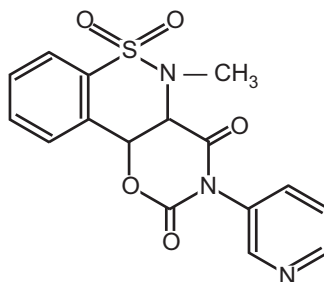
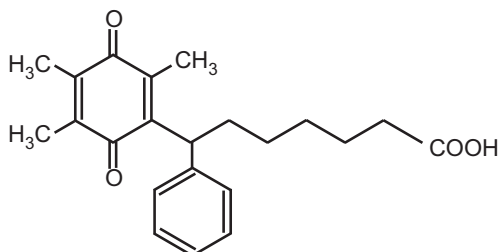
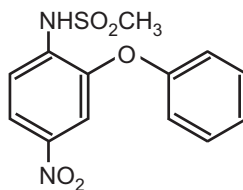
**Licofelone**

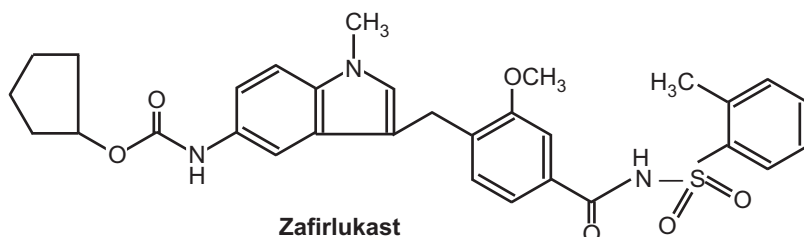
(dual COX/LOX inhibitor)



**ES-1007** (Germany)

(central mode of action)

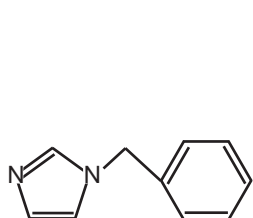
**Ketorolac (1990)****Oxindole carboxamide (1989)**  
(dual inhibitor of CO and 5-LO)**Naphthyridine (1994)**  
(induces release of endogenous Glucocorticoids)**Tiaprofenic acid****Tenoxicam (Hoffmann-La-Roche, 1987)****Zileuton**  
(It blocks leukotriene synthesis)**Aminoprofen (Aldounion, 1990)****Droxicam (Abbott, 1990)****Seratrodast**  
(It blocks thromboxane receptors)**Nimesulide**  
(Preferential COX-2 inhibitor)



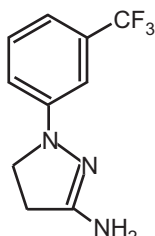
Zafirlukast

(It blocks leukotriene receptor)

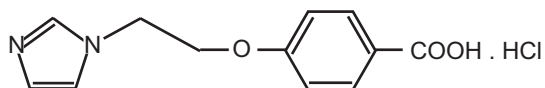
**Thromboxane synthase inhibitors:** Low doses of aspirin impair platelet  $\text{TXA}_2$  synthesis with no effect on formation of prostacyclin by the vascular endothelium. Aspirin acetylates the CO and therefore the inhibitory effect remains effective until new enzyme is produced. Whereas the vascular endothelium is capable of generating new enzyme, the platelets do not have the biochemical machinery necessary for protein synthesis. Therefore, the ability of platelets to form  $\text{TXA}_2$  is blocked while prostacyclin synthesis recontinue after exposure to aspirin. This explains the anti-thrombotic activity of aspirin. Other specific thromboxane synthase inhibitors include -



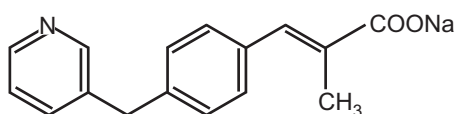
Benzyl imidazole



BW 755 C

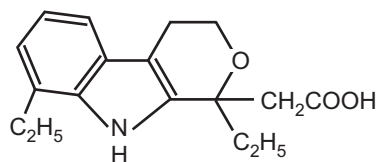
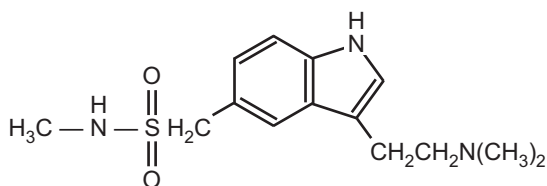


Dazoxiben

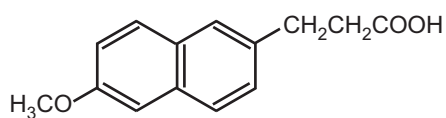


OKY 1518

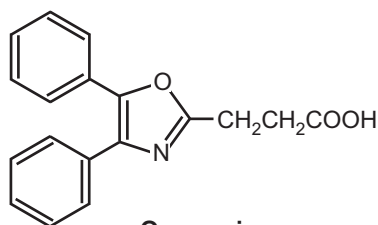
**Sumatriptan:** It is a selective  $5\text{HT}_1$ -receptor agonist used in the treatment of migraine attack. It belongs to triptan class. It affects a natural chemical (serotonin) that constricts blood vessels in the brain. It may also block other pain pathways in the brain. Other examples include zolmitriptan, naratriptan, rizatriptan, eletriptan, almotriptan and frovatriptan.



Etodolac (1991)

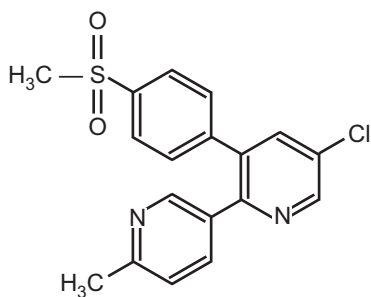


**Nabumetone**  
(Naproxen derivative)

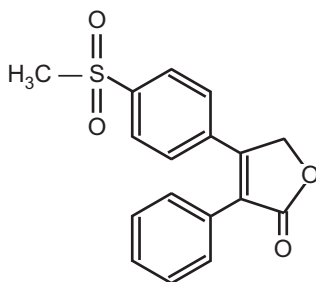


**Oxaprozin**

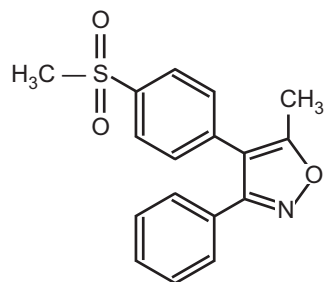
### COX-2 selective inhibitors:



**Etoricoxib**

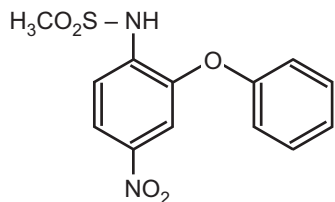


**Rofecoxib (Merck)**  
withdrawn in 2004

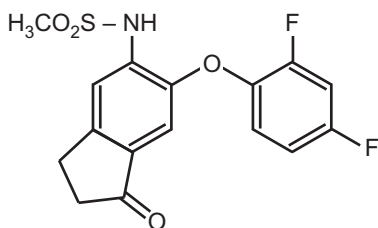


**Valdecoxib (Searle)**  
withdrawn in 2005

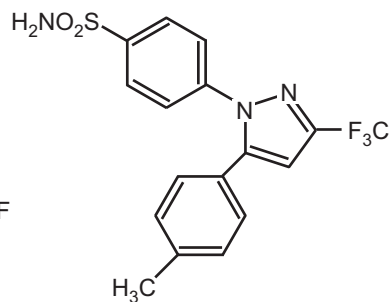
### Application of bio-isosterism in selective COX<sub>2</sub>-inhibitors.



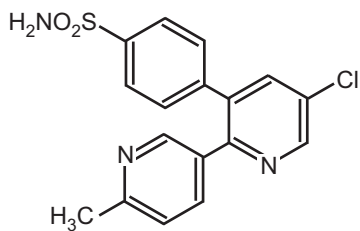
**Nimesulide**



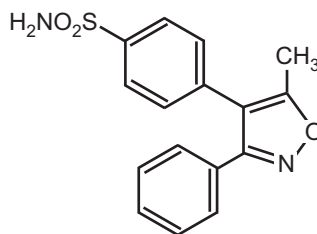
**Flosulide**



**Celecoxib**



**Etoricoxib**



**Valdecoxib**



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# SAMPLE QUESTIONS

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- Discuss in detail the chemistry of acetyl choline.
  - Relate the structural features of bethanechol, carbachol and methacholine with acetyl choline.
- What are reversible and irreversible anticholinestrases? Give mechanism of action of irreversible anticholinestrases.
- Give a detailed account of SAR of parasympathomimetic drugs.
- Give an enlightened account of the cholinergic receptors.
- Write short notes on :
  - Cholinergic agonists
  - Reactivators of cholinestrases
  - Antispasmodic agents
- Give the classification of antispasmodic agents. Review the SAR of each class.
- Give a brief account of 'Belleau's concept of enzyme perturbation'.
  - Discuss the significance of Ing's 'Rule of five', in SAR of parasympathomimetic drugs.
- Classify the sympathomimetic drugs. Give the chemical features of each class. Discuss the SAR in general.
- Write notes on :
  - Adrenergic receptors
  - $\alpha$ -adrenergic receptor blockers
  - $\beta$ -adrenergic receptor blockers
  - $\beta$ -adrenergic agonists
  - Clinical significance of  $\beta$ -blockers
- " $\beta$ -adrenergic blockers will serve to continue as a source of new promising anti-hypertensive agents." Comment on the above statement with suitable examples.
- Give a detailed account of biosynthesis and metabolism of neurotransmitter in a sympathetic nerve.
- Discuss the drug-receptor interactions in sympathetic nervous system.
  - Outline the synthesis of epinephrine from catechol.
- (+) Acetyl-  $\beta$ -methyl choline has about 200 times higher muscarinic activity than its epimer. Explain.
  - (l-) Epinephrine is more active than (d-) epinephrine. Explain.

14. Discuss the SAR in dialkylaminoalkyl esters of aromatic acids for local anaesthetic activity. Give the synthesis and specific uses of :
- (i) Lignocaine hydrochloride
  - (ii) Cyclomethycaine
  - (iii) Procaine hydrochloride
15. Classify the anticonvulsant drugs on the basis of chemical structure. Show the common structural features shared by them. Give the mode of action of hydantoins. Outline the synthesis of any two drugs (each one from different chemical class).

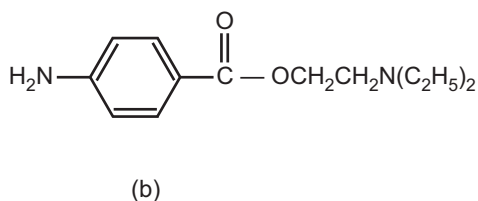
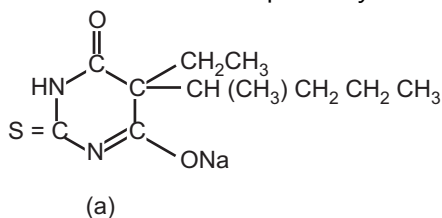
16. The structure  $R_1 - \overset{\overset{\text{O}}{\parallel}}{\text{C}} - \text{X} - (\text{CH}_2)_n - \text{Y} \begin{cases} \text{R}_2 \\ \text{R}_3 \end{cases}$  is common to many local anaesthetic

agents. Comment on the possible variations with respect to  $R_1$ ,  $R_2$ ,  $R_3$ , X and Y and their effects on local anaesthetic activity. Give the synthesis of (i) Cyclomethacaine (ii) Lignocaine.

17. Give an account of non-barbiturates used as sedative and hypnotics.
18. What is an isosteric replacement of atoms or groups ? Explain with reference to phenothiazine.
19. Define the term "Neuroleptics".  
"Structural changes in molecule produces not only quantitative changes but also qualitative changes in biological activity."  
Explain this statement giving examples of phenothiazine derivatives. Outline the synthesis of promethazine.
20. What do you mean by tranquilizer ? Discuss the chemistry in details. Give the synthesis of any two drugs in this category.
21. Write notes on :  
(a) Mechanism of action of neuroleptics  
(b) Mechanism of action of local anaesthetics  
(c) Mechanism of action of benzodiazepine.
22. (a) Define Sedative and hypnotic agents. What do you mean by Hansch Quantitative SAR ?  
(b) Classify sedative and hypnotic drugs. Write SAR of barbiturates and give synthesis of any two barbiturates.  
(c) Write the synthesis and SAR of phenothiazine.
23. Draw the structures, give IUPAC name and medicinal uses of :  
(a) Cyclobarbitone (b) Trimethadione  
(c) Iodipamide (d) Diazepam  
(e) Diphenhydramine

24. (a) Give a brief account of applications of 'Ferguson Principle'.  
(b) Outline the metabolic pathways of :  
(i) Procaine (iii) Phenobarbitone  
(ii) Meperidine (iv) Chlorpromazine
25. (a) Give the structure activity relationship of hydantoins and oxazolidine -2, 4 - diones as anticonvulsants.
26. (a) How do adrenergic blocking agents act as antihypertensive ?  
(b) 'In hypertension therapy, diuretic agent gives better results'. Explain with suitable examples.
27. Draw structure of morphine and show important functional groups in it due to which it exhibits biological activity. Give the structure and synthesis of any two synthetic agents which are potent analgesics as morphine. Write brief note on analgesic receptors and drug action.
28. What are narcotic analgesics? Discuss the sites of modification on Morphinan prototype.
29. Explain the term 'drug dependence' and give SAR of Morphine.
30. Write a note on 'morphine and its analogs.'
31. (a) Describe morphine modifications initiated by Grewe.  
(b) Outline the synthesis of methadone and pethidine.
32. What do you mean by non-narcotic analgesics ? Give their classification and mechanism of action. Give the structural features of salicylates.
33. Classify the non-narcotic analgesics. Give the synthesis of one drug from each class.
34. Give the chemical classification of non-steroidal anti-inflammatory agents. Give the structures of at least two drugs from each class.
35. (A) Give structure of :  
(a) Oxyphenbutazone (b) Indomethacine  
(c) Aspirin (d) Antipyrine  
(e) Mefenamic acid (f) Tolmetin  
(g) Ibuprofen (h) Naproxen.  
(B) Give the mechanism of action of non-steroidal anti-inflammatory agents.  
(C) Outline the synthesis of Ibuprofen.
36. Give an illustrated account of your understanding of drug metabolism studies. How such information can be helpful in drug design ? Give examples.
37. Give an enlightened account of the forces involved in drug-receptor interactions.
38. "Biological response is not the function of purity of the drug but is function of its physiochemical parameters". Illustrate above statement with suitable examples.
39. Discuss in detail the factors affecting accessibility of a drug to its active sites.

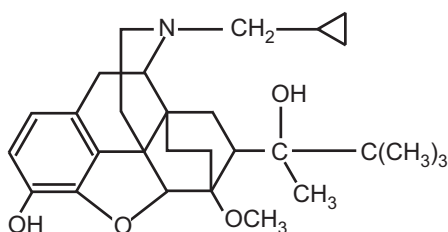
40. Write notes on :
- Receptor site theories
  - Protein binding of drugs
  - Steric factors affecting pharmacological activity
  - Isosterism and pharmacological activity
  - Bioisosterism
  - Drug metabolism
41. What do you mean by the term 'Bioisosterism' ? Discuss in detail the recent bioisosteric applications.
42. "The pharmacological activity of a drug in many ways, is the consequence of its metabolism." Justify the above statement with suitable examples.
43. Write short notes on :
- Drug interactions
  - Fergusson principle
  - Drug conjugation pathway
  - Hansch quantitative SAR
  - Stereochemical aspects of drug metabolism
  - Drug elimination process
44. 'Ionisation of a drug modifies biological activity '. Explain the statement giving suitable examples.
45. Outline the metabolic pathways of



46. Give chemical classification of hypnotics and sedatives with structure of atleast one drug from each class. Outline the general scheme of synthesis for barbiturates. Discuss the SAR of barbiturate class.
47. Discuss the molecular modifications in  $\beta$ -phenylethylamine for adrenergic (pressor) activity.
48. Write short notes on (any three) :
- Therapeutic applications of anticholinergic drugs.
  - Narcotic antagonists
49. "Gone are the days when the importance of Physico-chemical parameters in drug design was looked upon with scepticism". Justify above statement with suitable examples.

50. (a) Discuss the significance of Belleau's concept of enzyme perturbation with reference to parasympathomimetic drugs.  
(b) Define the term receptor. Give an illustrated account of occupation theory.  
(c) Write a note on : "Recent bio-isosteric applications in drug design. "
51. "A lot has been talked about Benzodiazepines as antiepileptics and anxiolytics, yet there exists a very fine shade of difference between the personalities of above two categories of benzodiazepines". Illustrate this difference with suitable examples.
52. Discuss in detail the SAR of barbiturates, used as sedative-hypnotics.
53. Searchlight the various corridors of activities exhibited by barbiturates with the examples of front runner drugs from each category.
54. "The benzodiazepines started their carrier primarily as sedative-antianxiety drugs but also established themselves as effective antiepileptic drugs in past years". Comment.
55. "Many drugs designed purely on the excellent SAR calculations die in the course of preclinical trials if their physico-chemical status is not interrogated properly". Explain the statements with the help of suitable examples.
56. (i) Give the classification of adrenergic drugs.  
(ii) Give the general formula of direct-acting adrenergic agonists.  
(iii) Give the structure of prototype of  $\beta$ -adrenergic blocking agents of anti-hypertensive category.  
(iv) Give the name of 'second messenger' involved in adrenergic system.  
(v) What is Ing's rule of five ?  
(vi) Give the name along with structure of one irreversible anticholinesterase agent.
57. Draw the structure of cholinesterase enzyme along with its active functional groups.
58. (i) Draw the conformations of acetylcholine necessary to exhibit  
(a) muscarinic and (b) nicotinic activity respectively.  
(ii) Illustrate with figure, three areas of acetylcholine molecule which offer sites for molecular modifications.  
(iii) Illustrate with figure, mechanism of action of cardiac glycosides.
59. (i) Give two examples alongwith structures of para amino phenol category of non-narcotic analgetics.  
(ii) Sketch out the basic skeleton or structural features required to develop an ideal narcotic analgetic.  
(iii) Give the name along with structure of prototype of third generation of norcotic analgetics.

60.



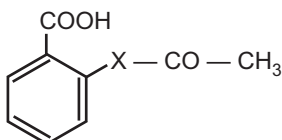
Buprenorphine

Buprenorphine is a very lipophilic drug and as such would be expected to easily cross the blood brain-barrier for entry into central nervous system. In fact, such drugs normally should have very rapid onset of action. But buprenorphine has slow onset and long duration of action. Justify.

61. (a) "Where observation is concerned, success favours only the prepared mind."

Justify above statement with reference to nuclear modifications of morphine to evolve methadone series.

62. (i)



(a)  $X = -O \rightarrow$  Acetylsalicylic acid

(b)  $X = -NH \rightarrow$  Bioisoster of (a)

(b)  $X = -S \rightarrow$  Bioisoster of (a)

Forecast the activities of (b) and (c), if any. Which one of above three, will be a better analgetic anti-inflammatory agent?

- (ii) Salicylic acid has quite appreciable antibacterial activity but the para isomer is inactive. Why?
- (iii) Figure out the part of morphine nucleus at which antagonistic activity appears to reside.
- (iv) Explain the terms: 'tolerance and drug dependance' in the light of neuronal changes.

63. Write short notes on (any three) :

(a) Narcotic antagonists (b) Non-steroidal oestrogens

64. (a) "The acetylcholine molecule offers three area for the required molecular modifications". Define these areas and show how structural changes in each of these areas affect the activity?
- (b) Discuss in brief the SAR of phenylethylamine derivatives having agonist property. List out the therapeutic uses.

65. Answer the following questions in brief:
- (a) Benzodiazepines do not produce hangover effect. Why?
  - (b) The more bulky the substituents on nitrogen,  $\alpha$ -receptor activity decreases and  $\beta$ -receptor activity increases in adrenergic series. Explain.
  - (c) Hypocalcemia increases the sensitivity of post-synaptic membrane towards seizure generation. Explain.
  - (d) N-alkylation does not affect the antiepileptic activity. Why?
  - (e) Replacement of oxygen by sulfur at C-2 in barbiturates, shortens the onset and duration of action. Why?
  - (f) Draw the structural features common to most of the antiepileptic classes.
  - (g) Show the isosteric relationship in hydantoin, oxazolidine-2, 4-dione, succinimide and acetylureas anticonvulsant agents.
  - (h) Give the examples of inhibitory neurotransmitters.
  - (i) Give two examples along with structure of mind expanding drugs.
66. Write notes on:
- (a) Sympathomimetics with CNS-stimulant activity.
  - (b) Ultra-short acting barbiturates
  - (c)  $\beta$ -blockers
67. Give an account of any two of the following:
- (a) Complex of events between drug administration and its action.
  - (b) Pro-drugs in drug metabolism.
  - (c) Oxidation-reduction potential and drug action
  - (d) Bioisosterism and drug action.
68. Outline the synthesis of 5, 5-dialkylbarbituric acid and SAR of barbiturates.
69. What is the meaning of antipyretic analgesics? Classify the various classes of antipyretic analgesics with suitable examples. How they reduce pain and fever simultaneously?
70. Classify the various psychotherapeutic agents. Explain their clinical utility in psychosis.
71. "The story of psychotropic drugs may go on for centuries. The more we ask, the little we seem to know". Illustrate your answer with reference to SAR and mechanism of action of tricyclic neuroleptics.
72. "The term drug design is used to impress the people rather than to express it". Discuss in detail, how drug metabolism studies help in discovery of new drug?
73. Give an account of any two of the followings:
- (a) Role of cytochrome P-450 in oxidative biotransformation.
  - (b) Bioisosterism.
  - (c) Partition coefficient and biological activity of drug molecules.

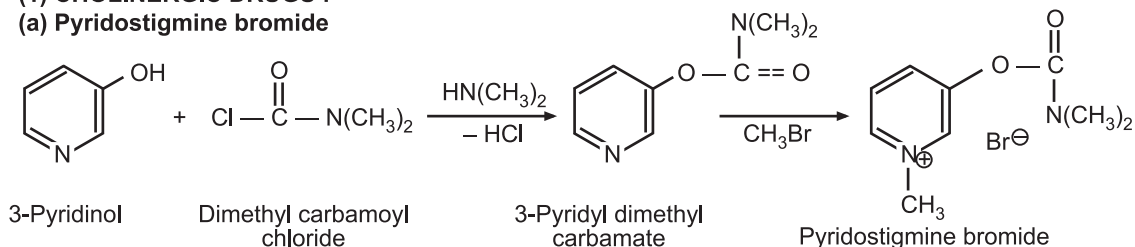
74. Discuss the SAR of morphine. Outline the synthesis of a morphine derivative which is devoid of addictive properties but having analgesic property.
75. (a) Classify the non-steroidal anti-inflammatory agents with one example from each class.  
(b) Describe the general mode of action of non-steroidal anti-inflammatory agents.  
(c) Give the scheme of synthesis for the following :  
(i) Ibuprofen,  
(ii) Indomethacine
76. (a) Discuss the properties of  $\alpha$  and  $\beta$ -adrenergic receptors.  
(b) Classify  $\alpha$  and  $\beta$ -adrenergic blocking agents with one example from each category.  
(c) Write steps involved in the synthesis of  
(i) Propranolol,  
(ii) Tolazoline
77. Give an account of any two of the following :  
(a) Forces involved in drug-receptor interaction.  
(b) Oxidation-reduction potential and biological action.  
(c) Suggest the metabolic pathways of propranolol and diazepam.



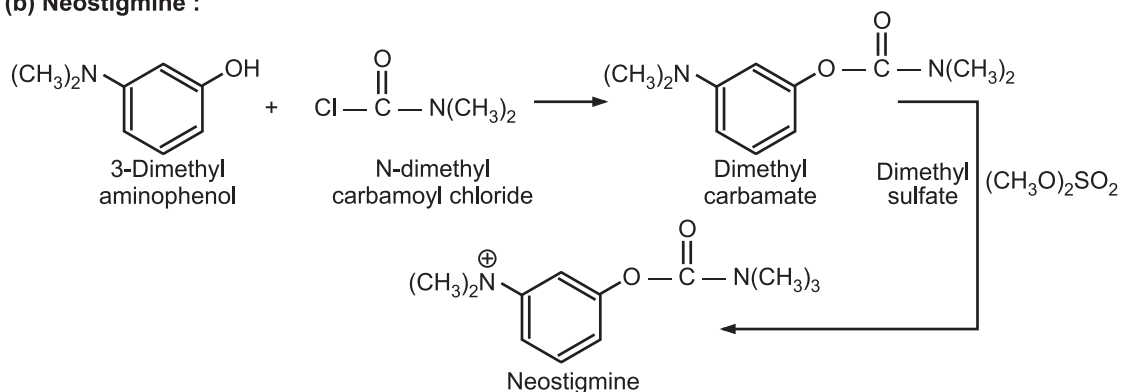
# SYNTHESIS

## (1) CHOLINERGIC DRUGS :

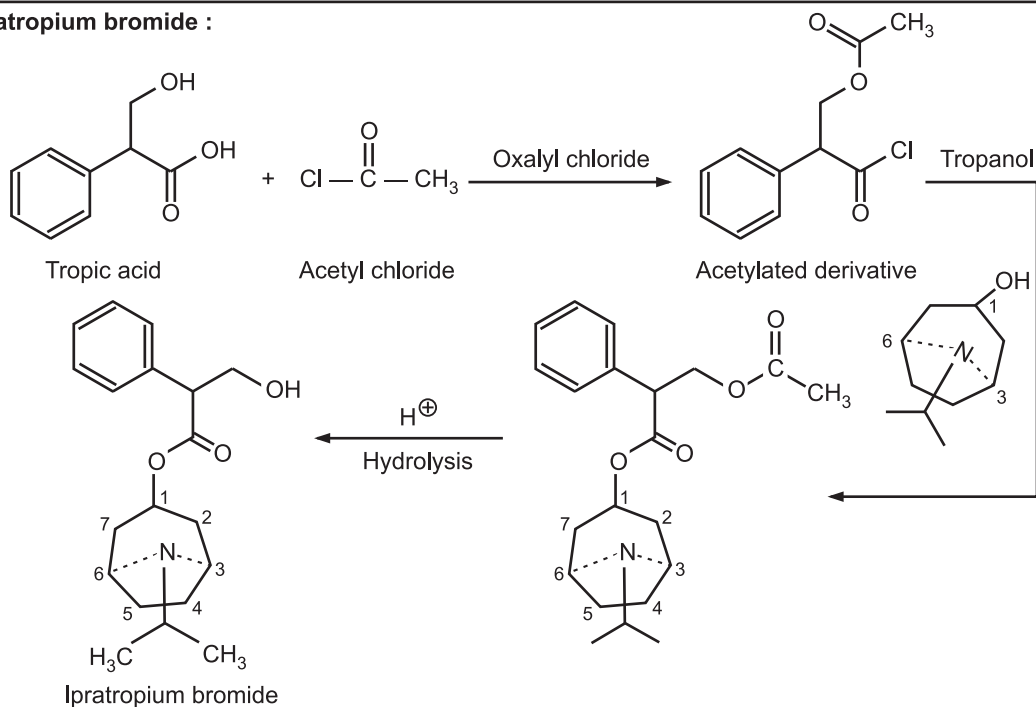
### (a) Pyridostigmine bromide

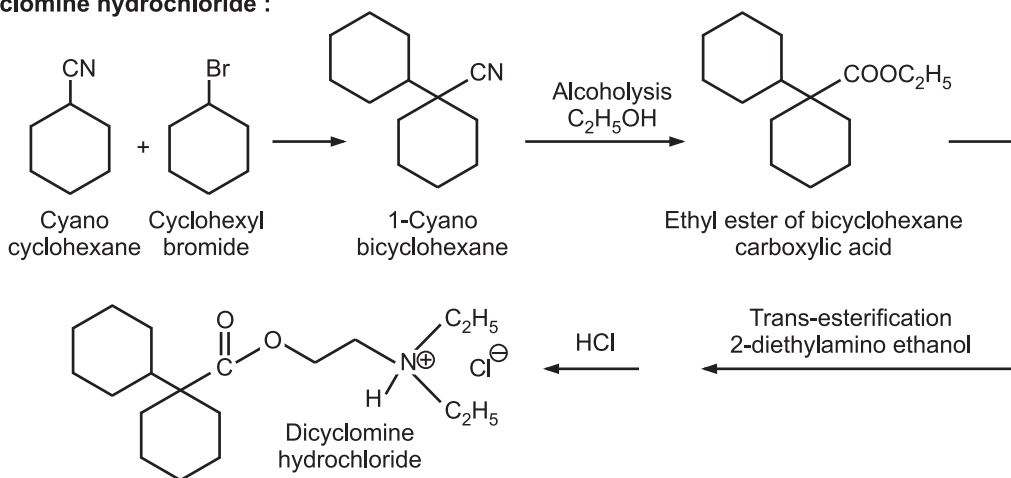
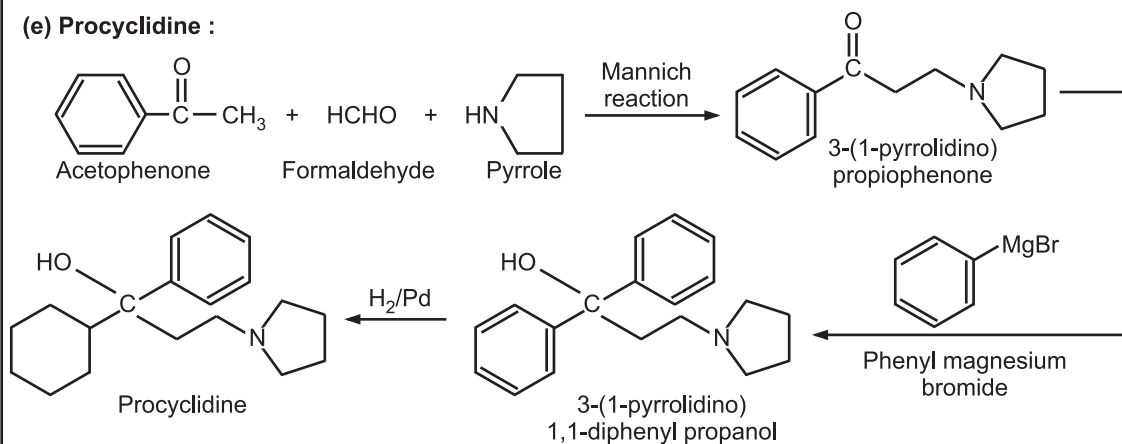
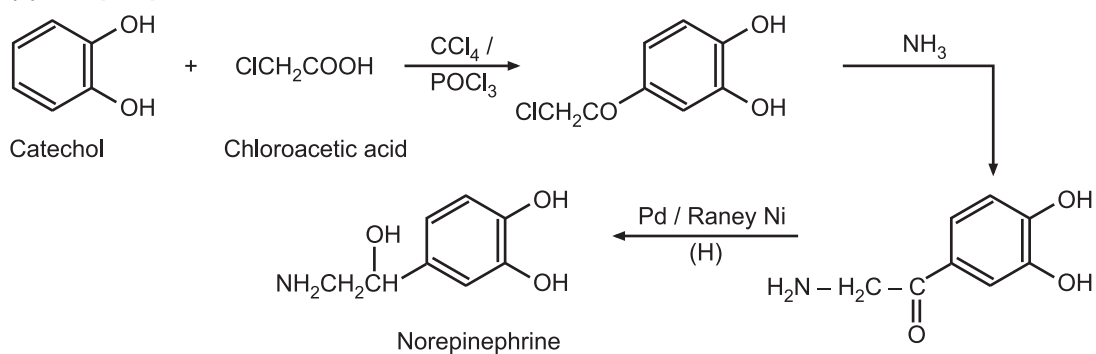


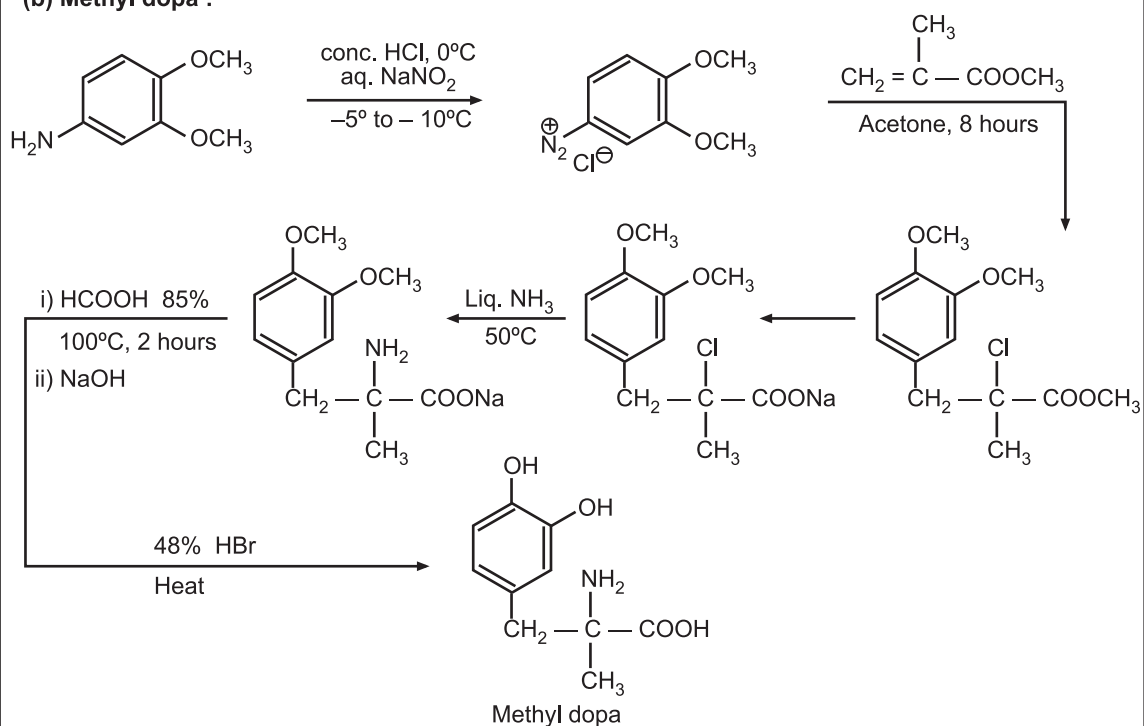
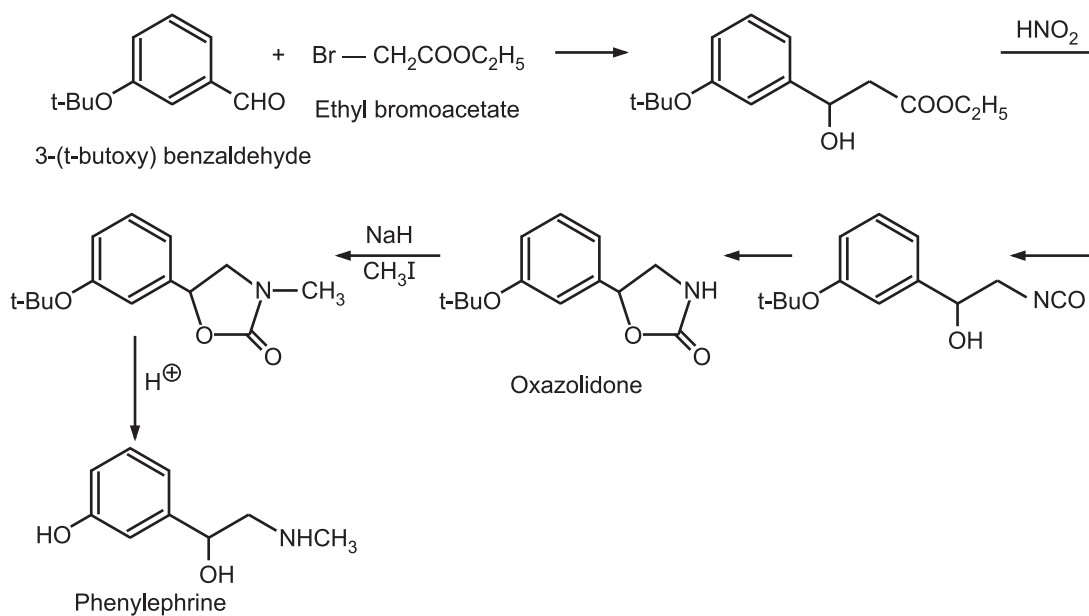
### (b) Neostigmine :

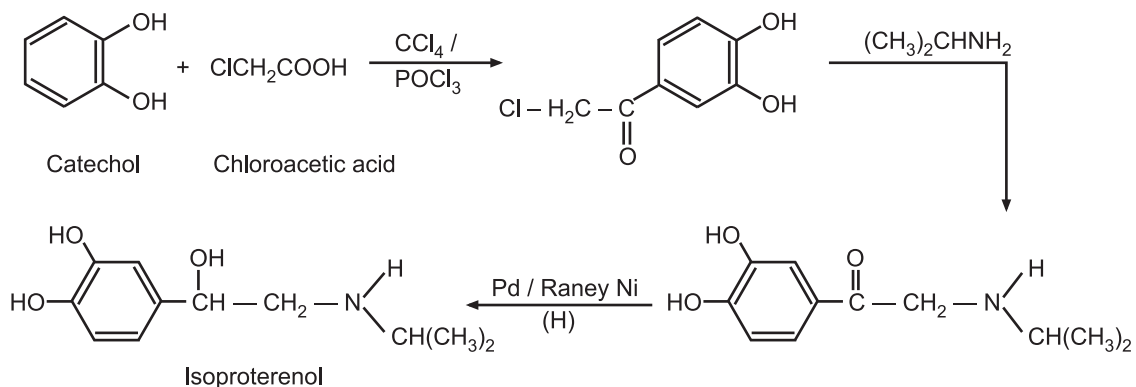
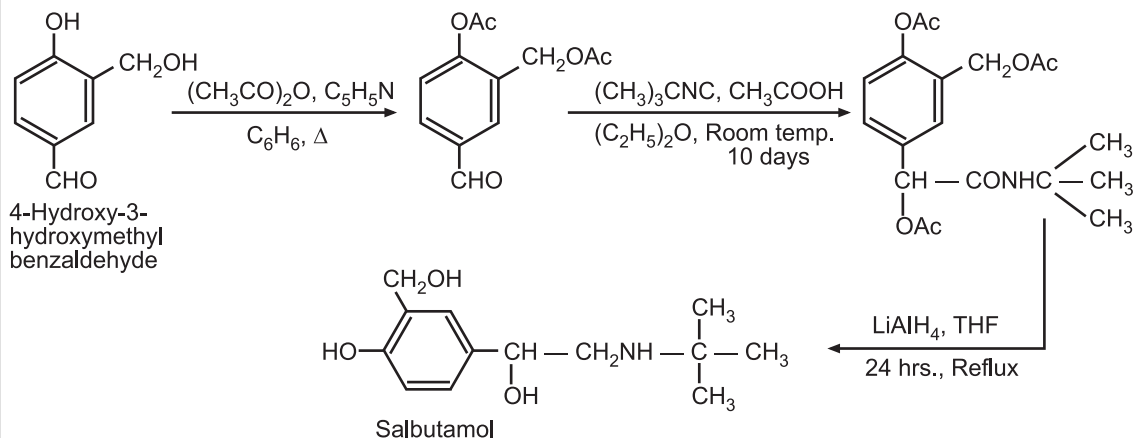
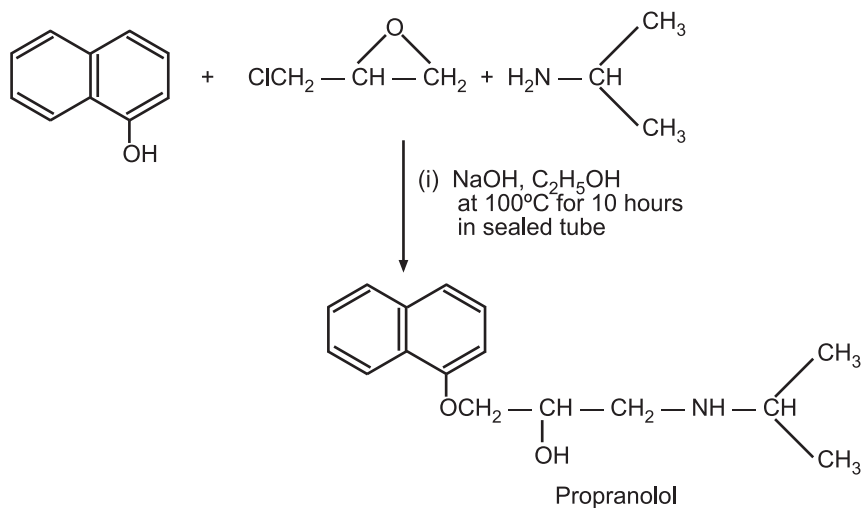


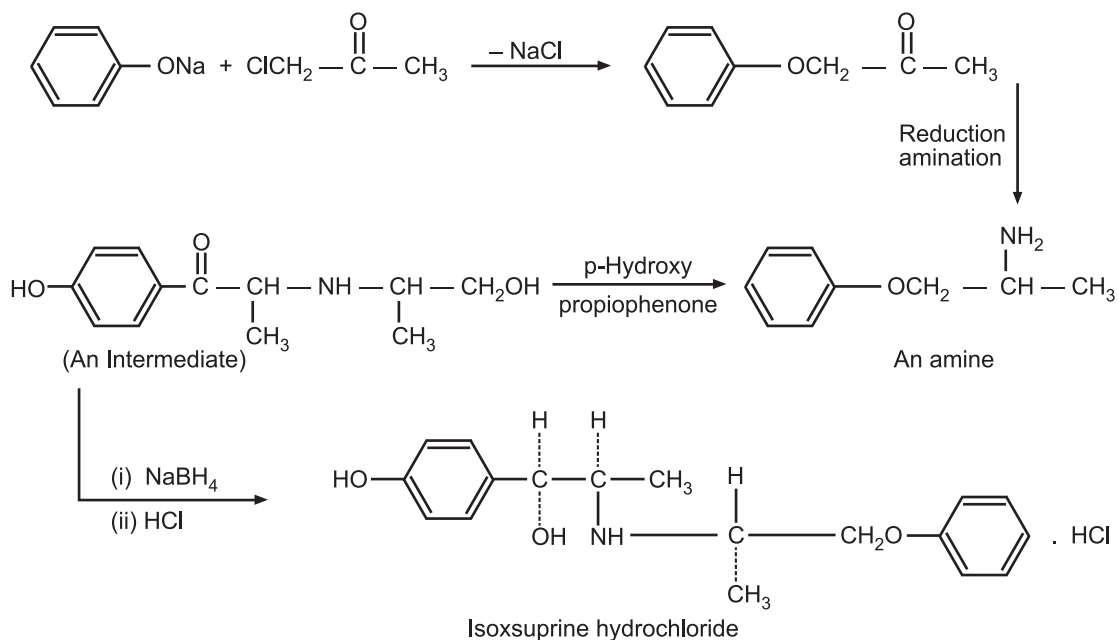
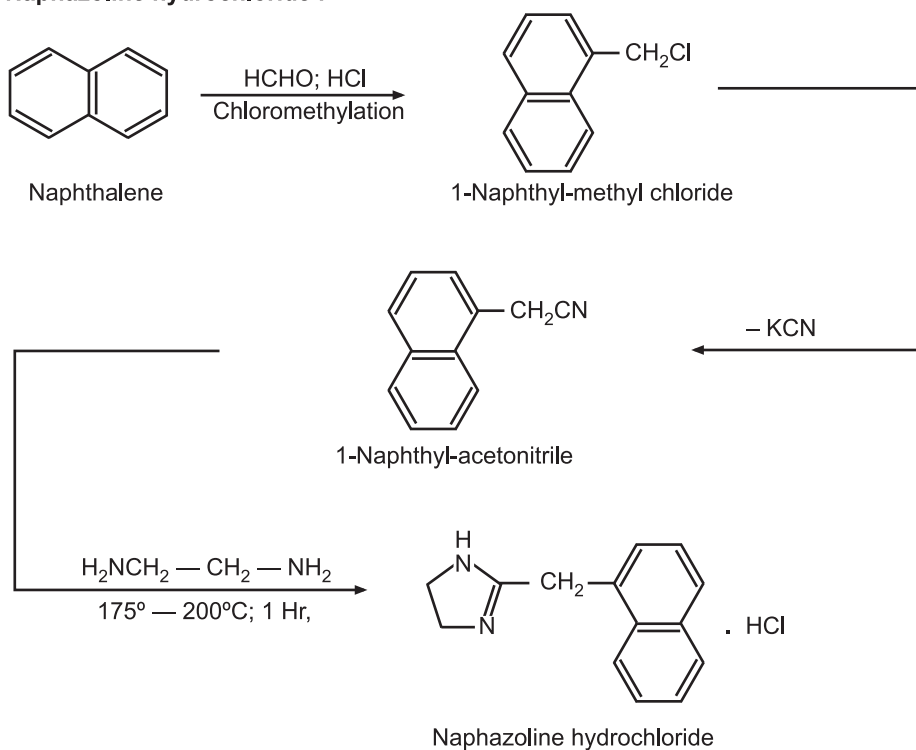
### (c) Ipratropium bromide :

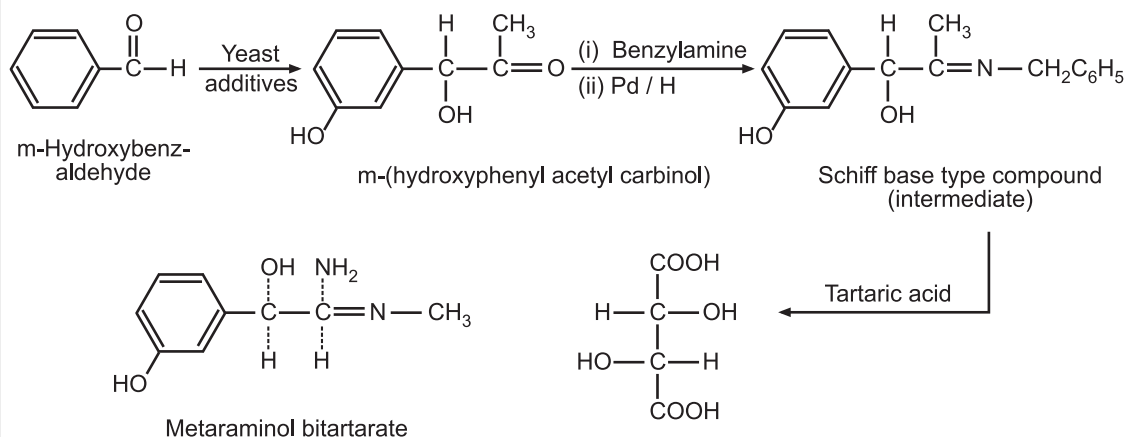
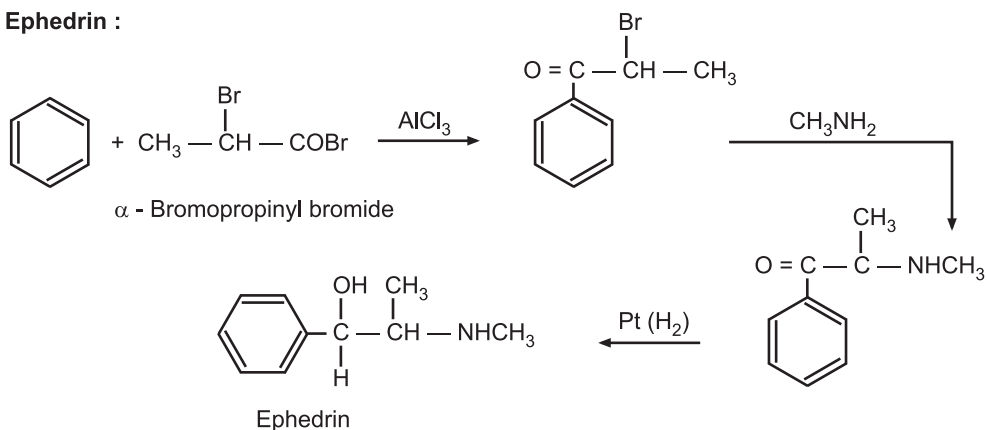
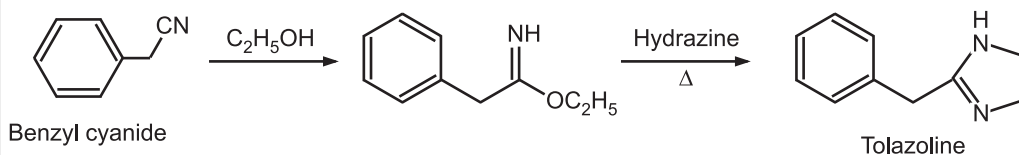
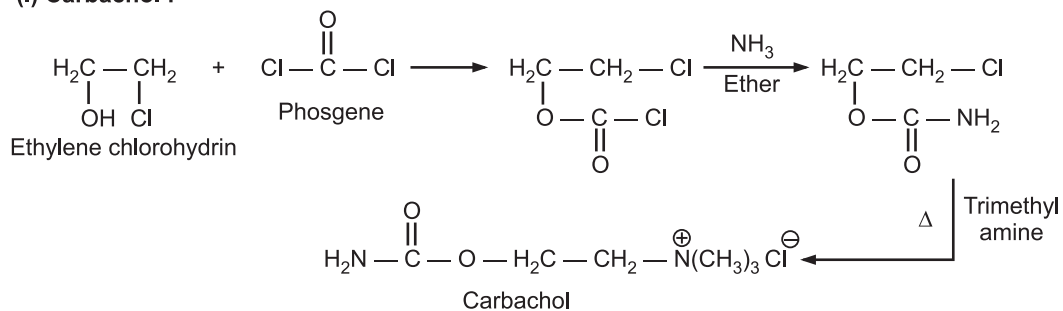


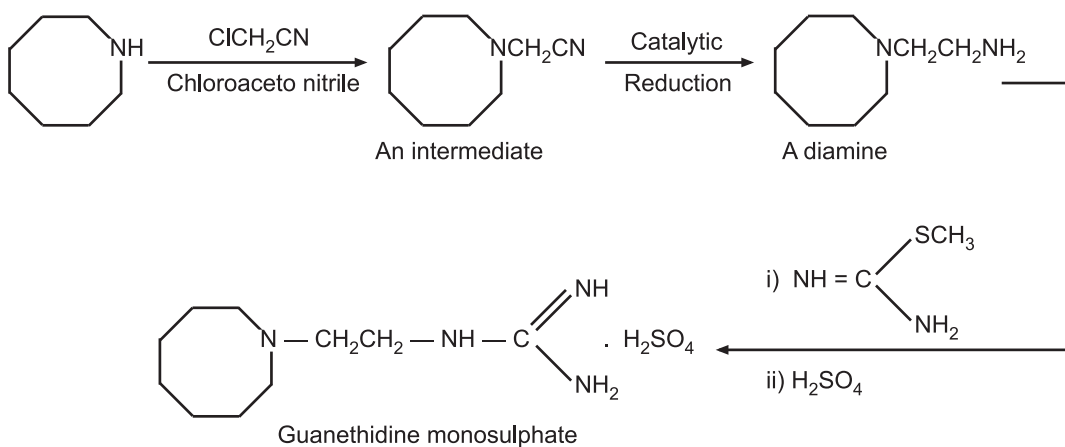
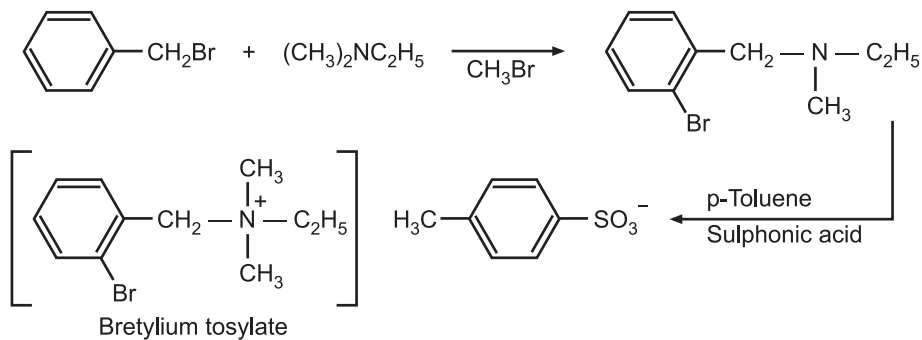
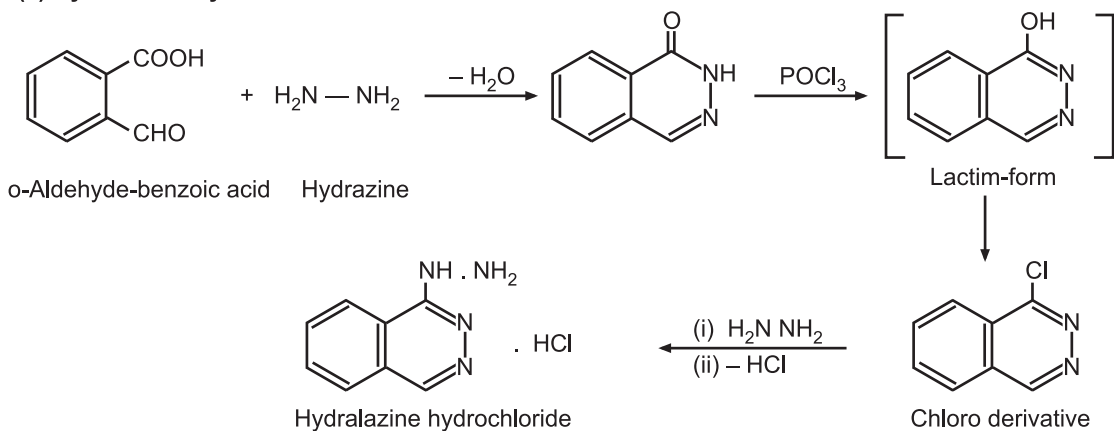
**(d) Dicyclomine hydrochloride :****(e) Procyclidine :****(2) ADRENERGIC DRUGS :****(a) Norepinephrine :**

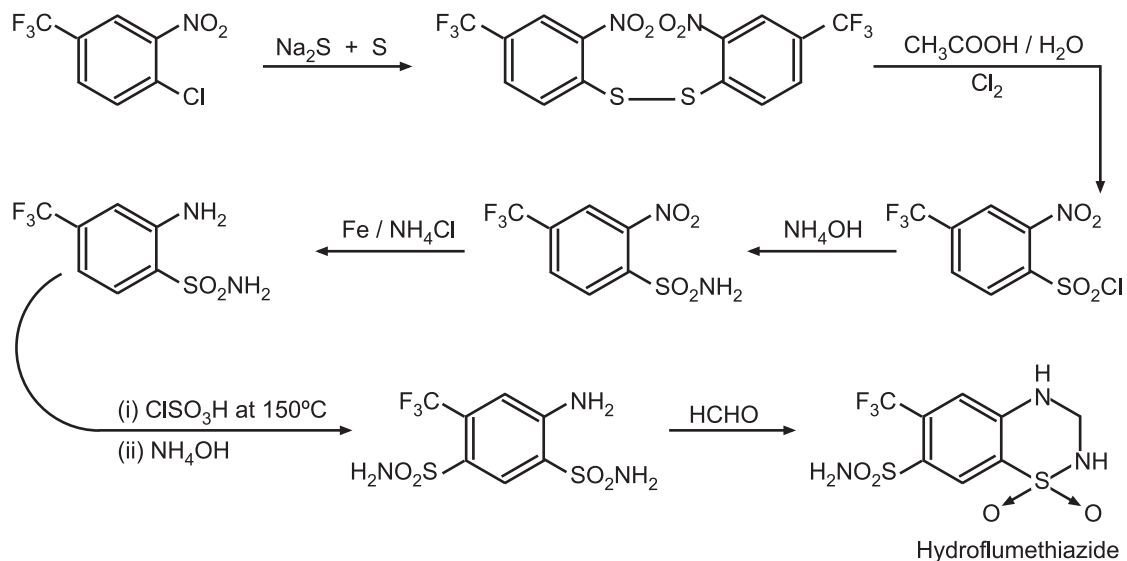
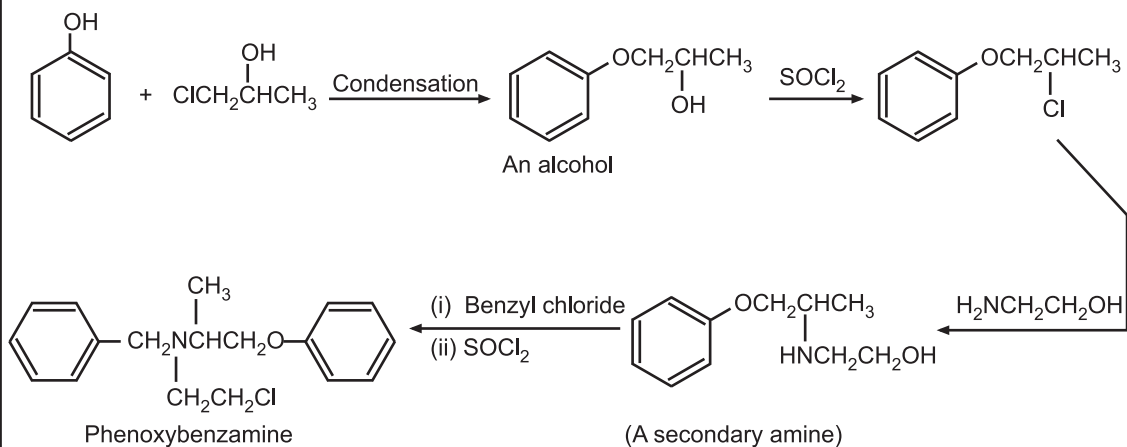
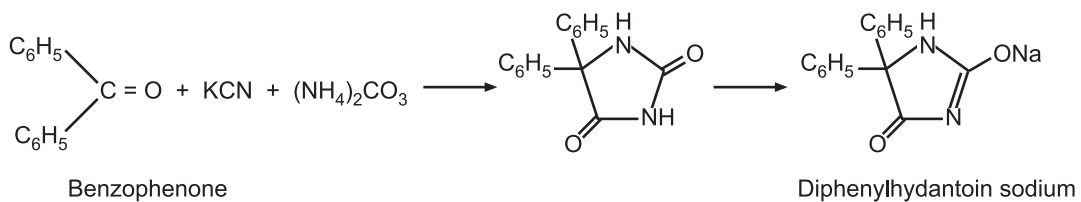
**(b) Methyl dopa :****(c) Phenylephrine :**

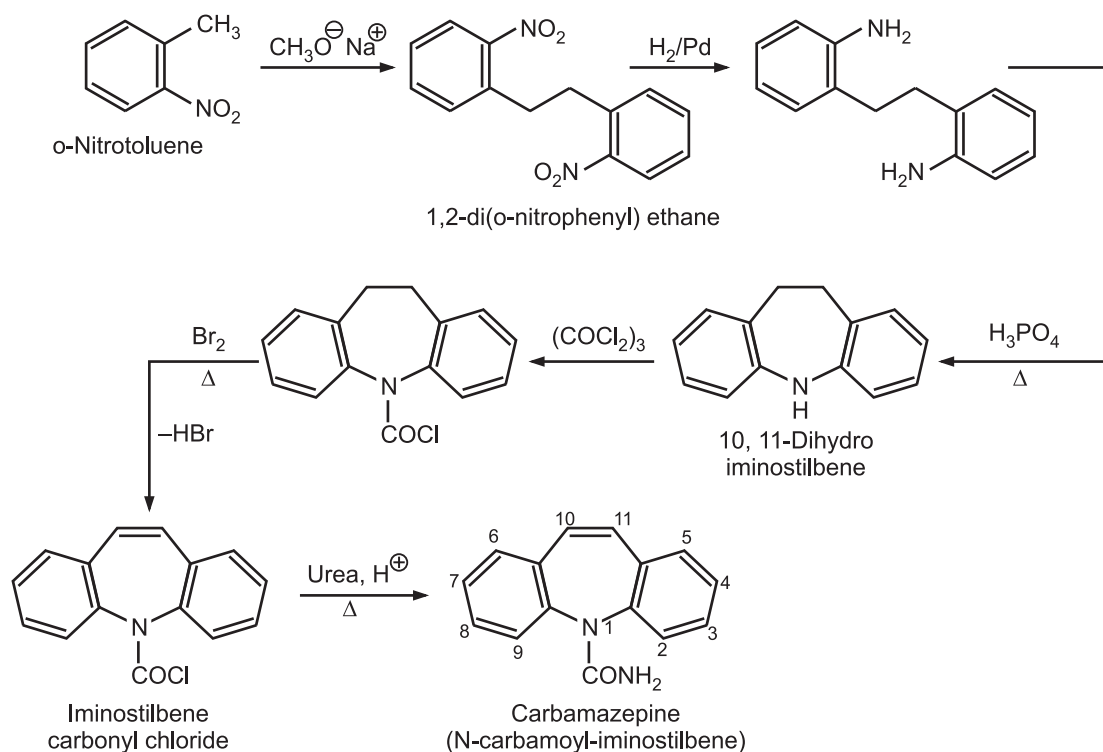
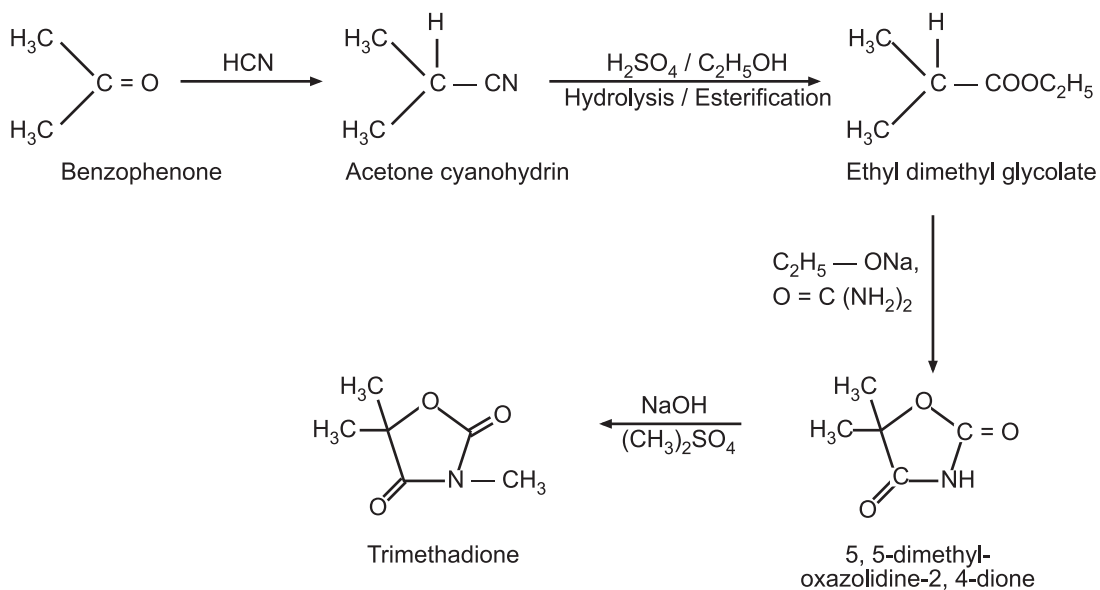
**(d) Isoproterenol :****(e) Salbutamol :****(f) Propranolol :**

**(g) Isoxsuprine hydrochloride :****(h) Naphazoline hydrochloride :**

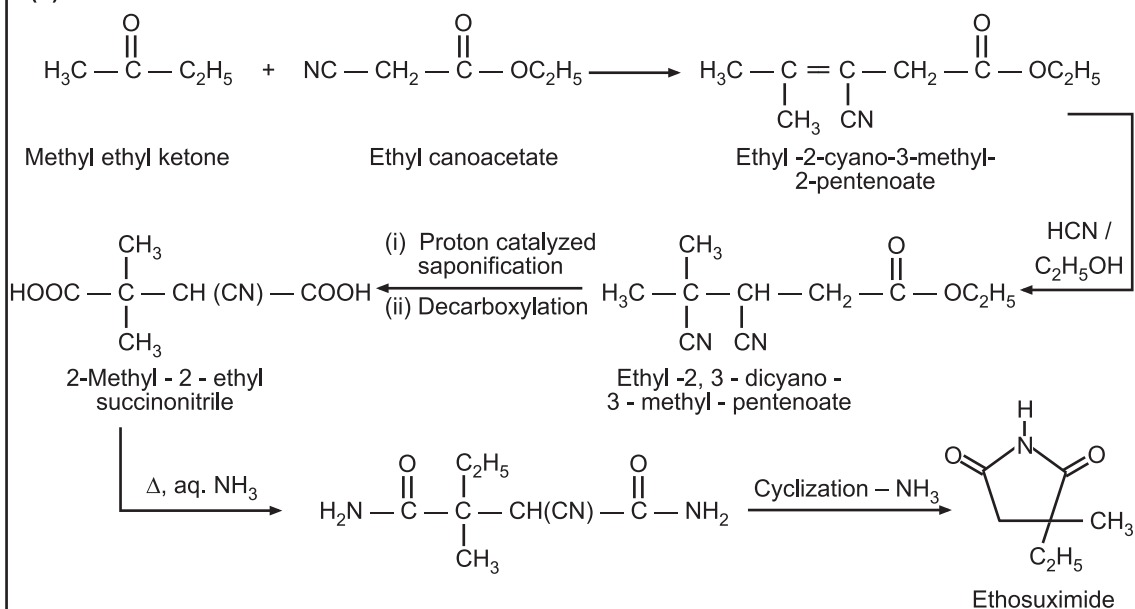
**(i) Metaraminol bitartrate :****(j) Ephedrin :****(k) Tolazoline :****(l) Carbachol :**

**(3) ANTIHYPERTENSIVE AGENTS :****(a) Guanethidine monosulphate :****(b) Bretylium tosylate :****(c) Hydralazine hydrochloride :**

**(d) Hydroflumethiazide :****(e) Phenoxybenzamine :****(4) ANTICONVULSANT AGENTS :****(a) Diphenylhydantoin sodium :**

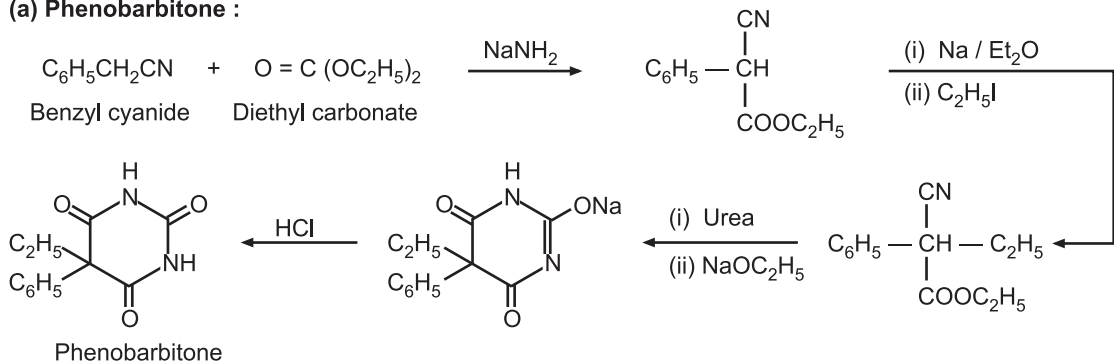
**(b) Carbamazepine :****(c) Trimethadione :**

**(d) Ethosuximide :**

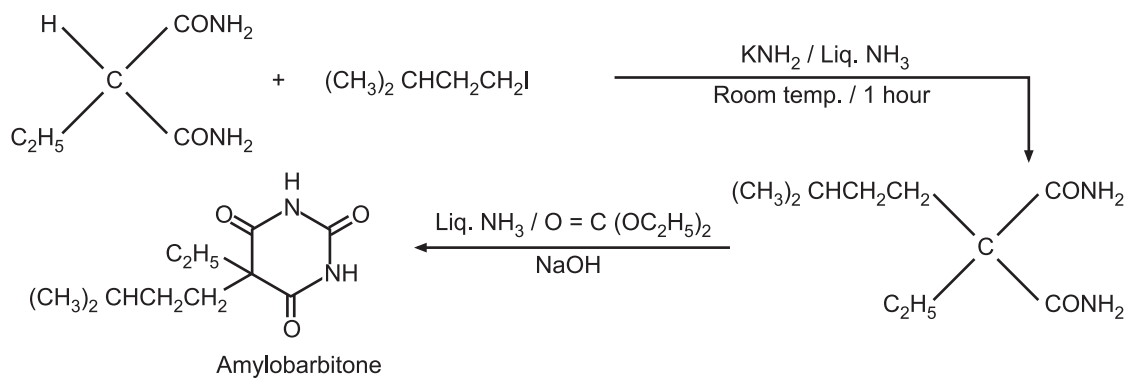


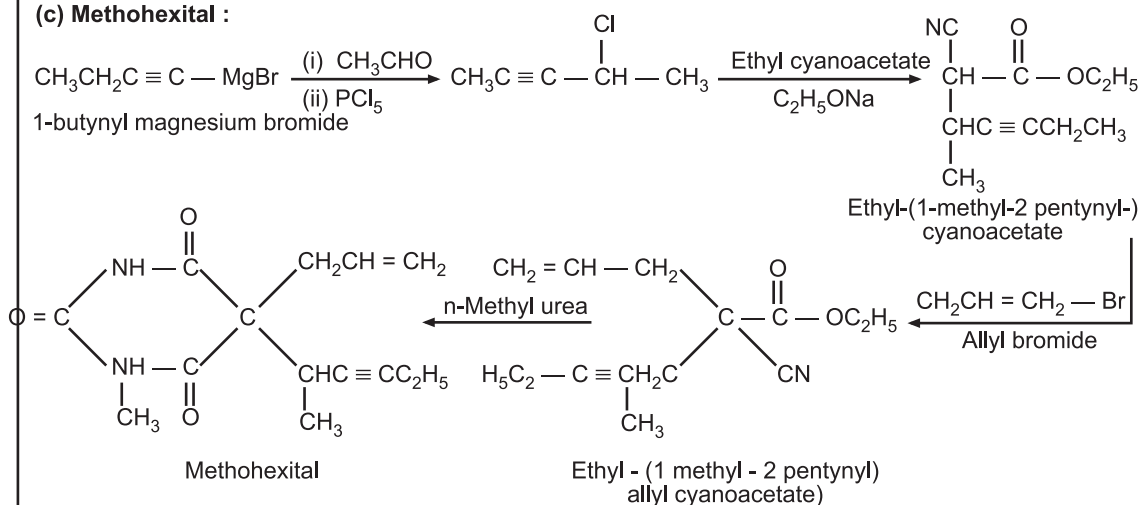
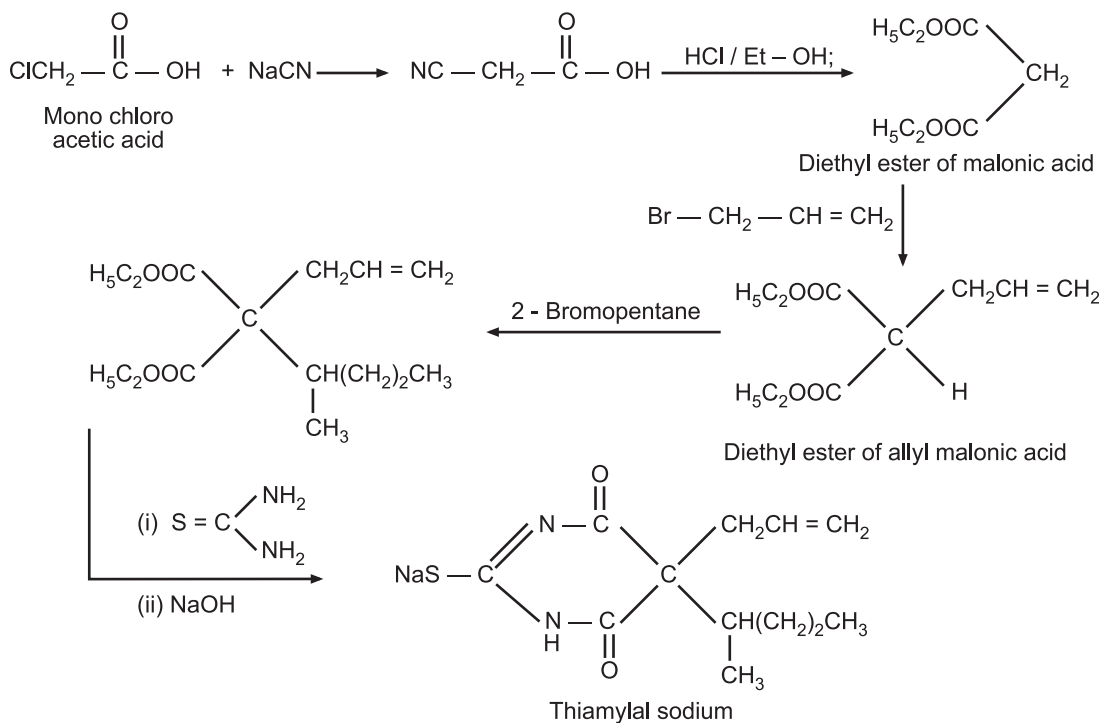
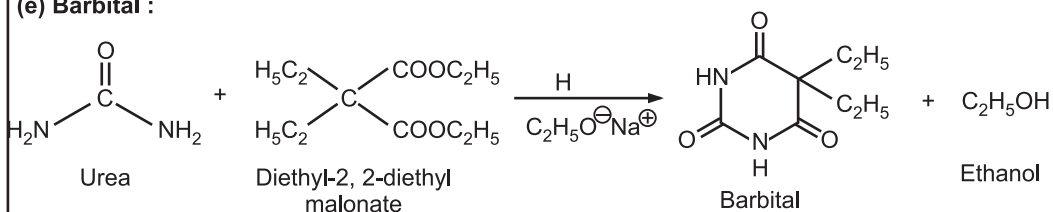
**(5) SEDATIVE-HYPNOTICS :**

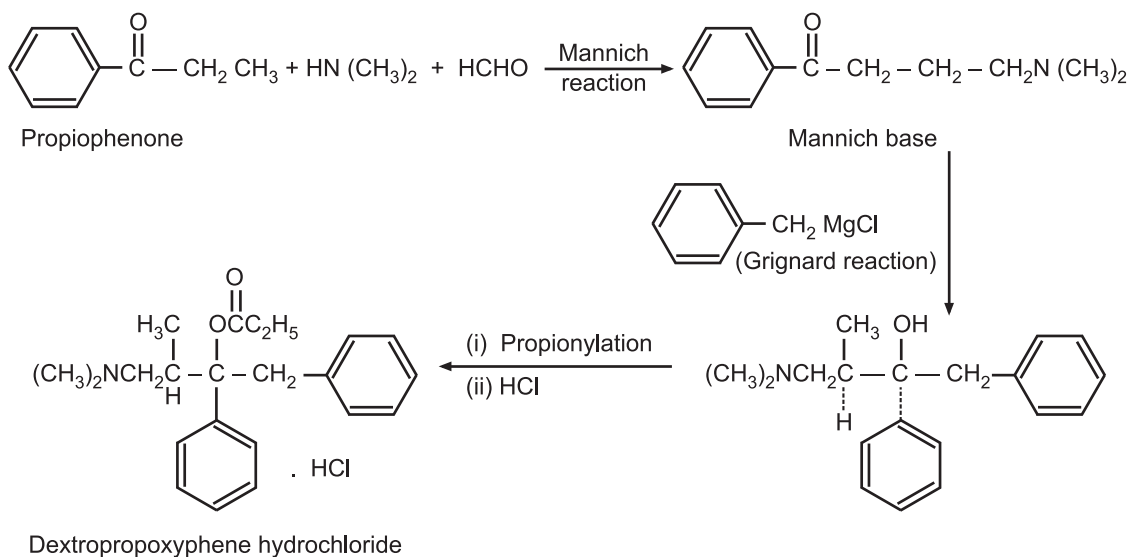
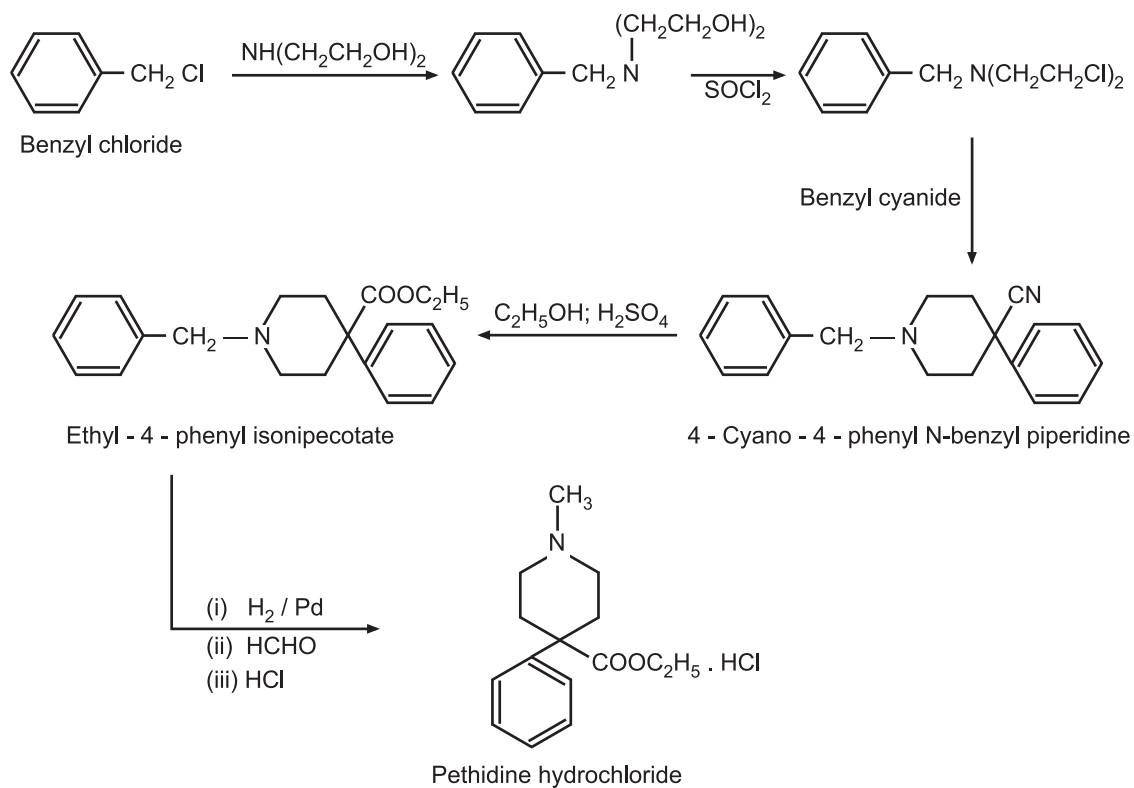
(a) **Phenobarbitone :**

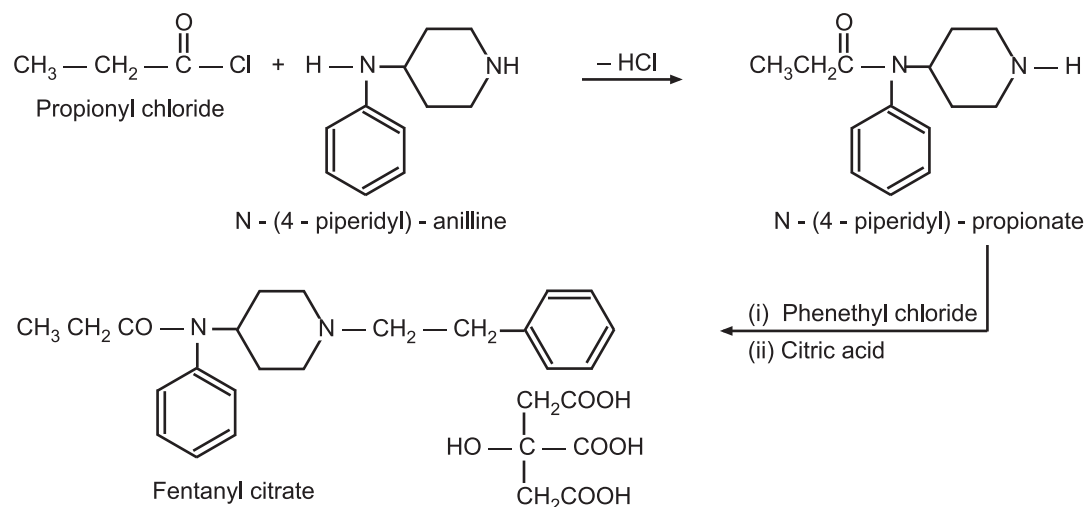
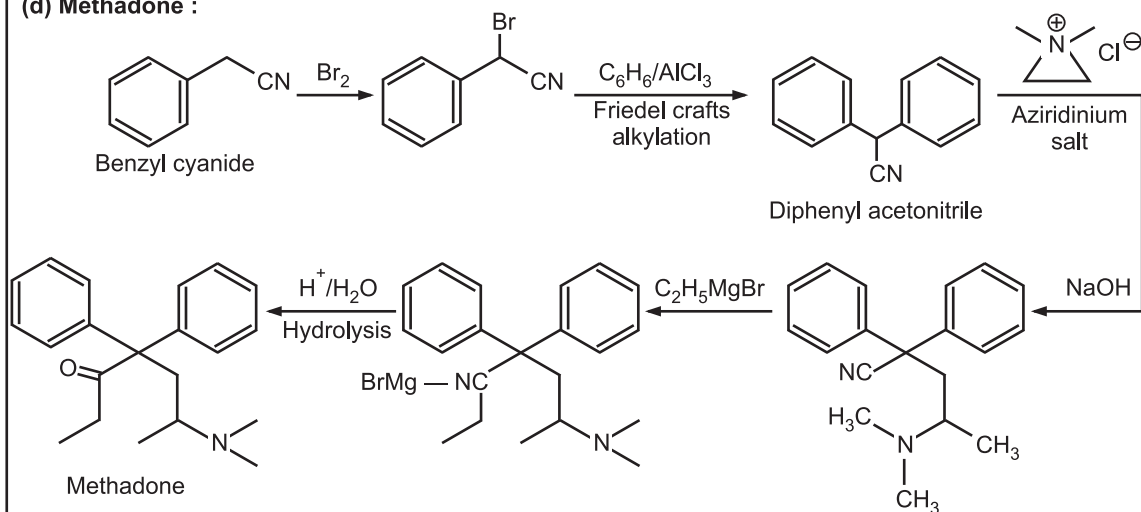
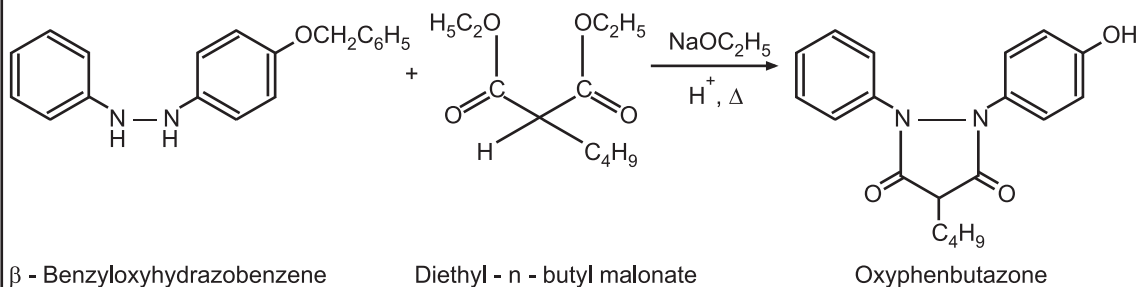


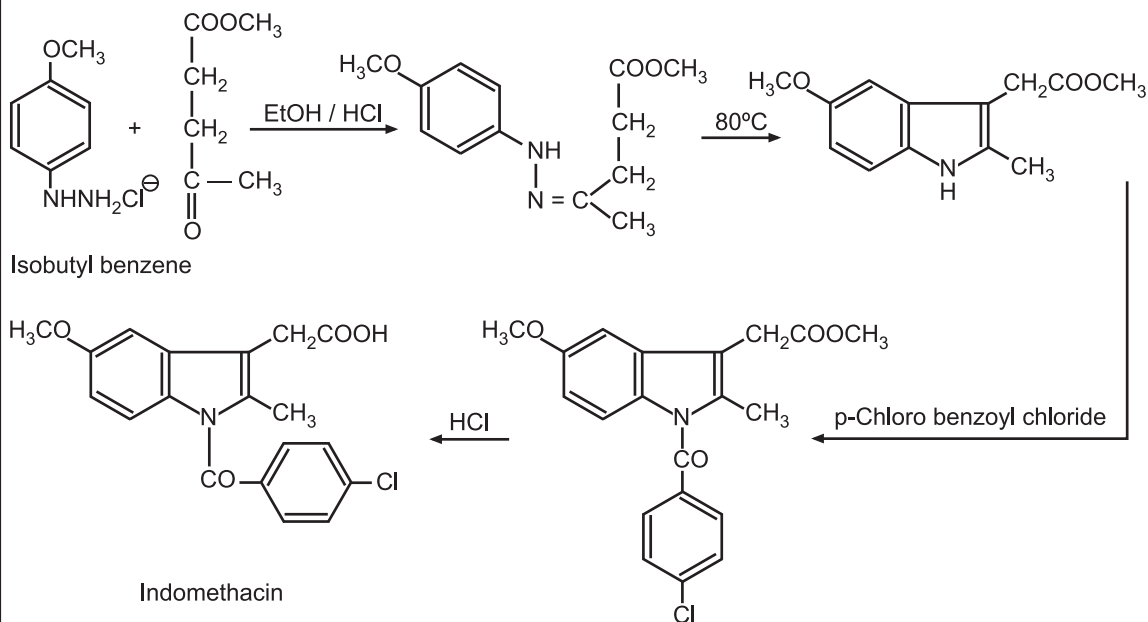
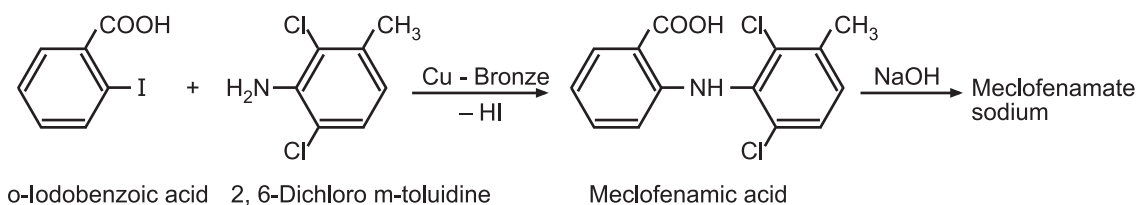
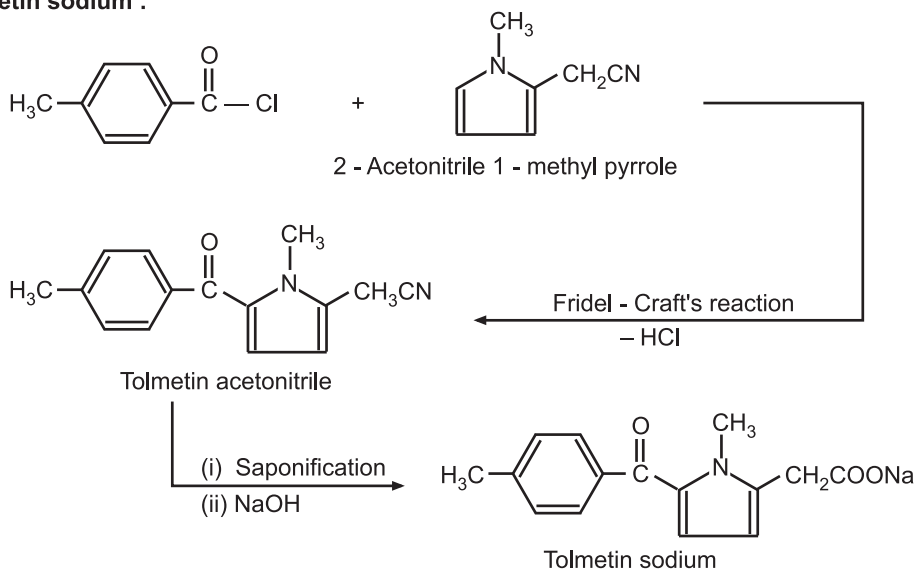
**(b) Amylobarbitone :**

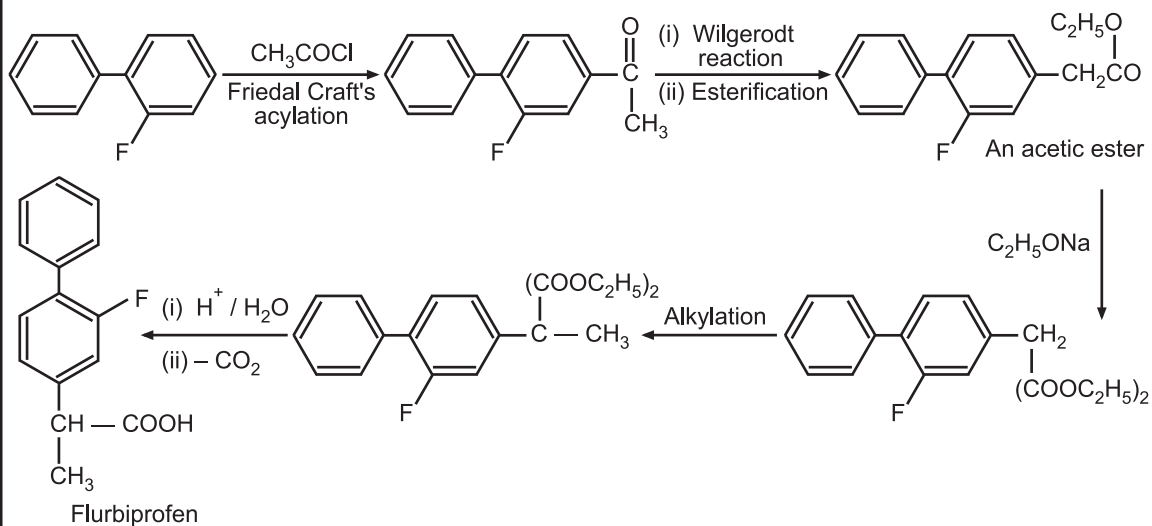
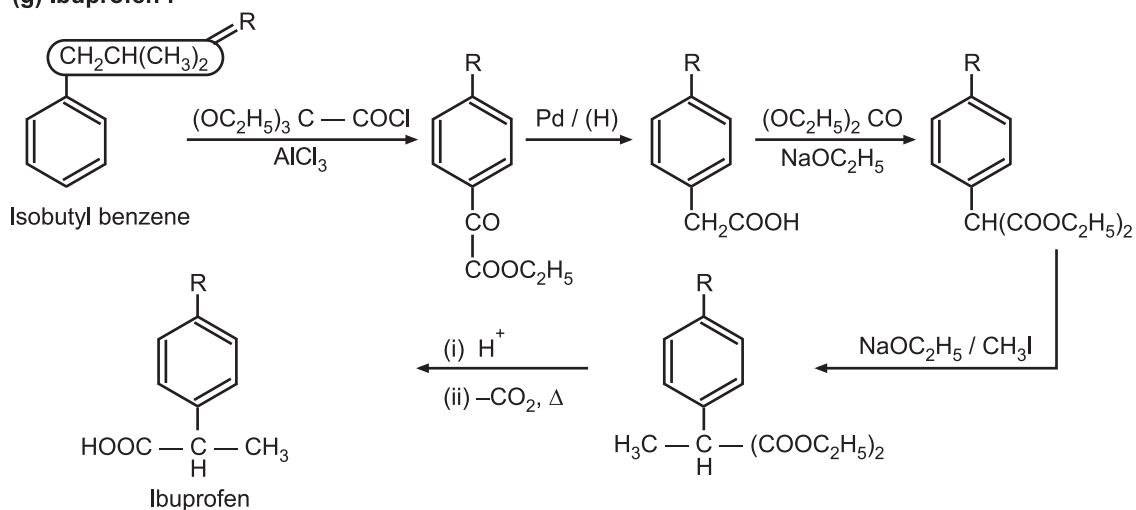
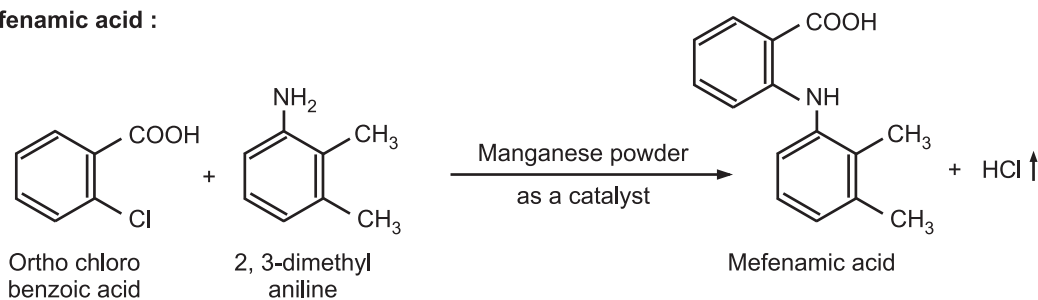


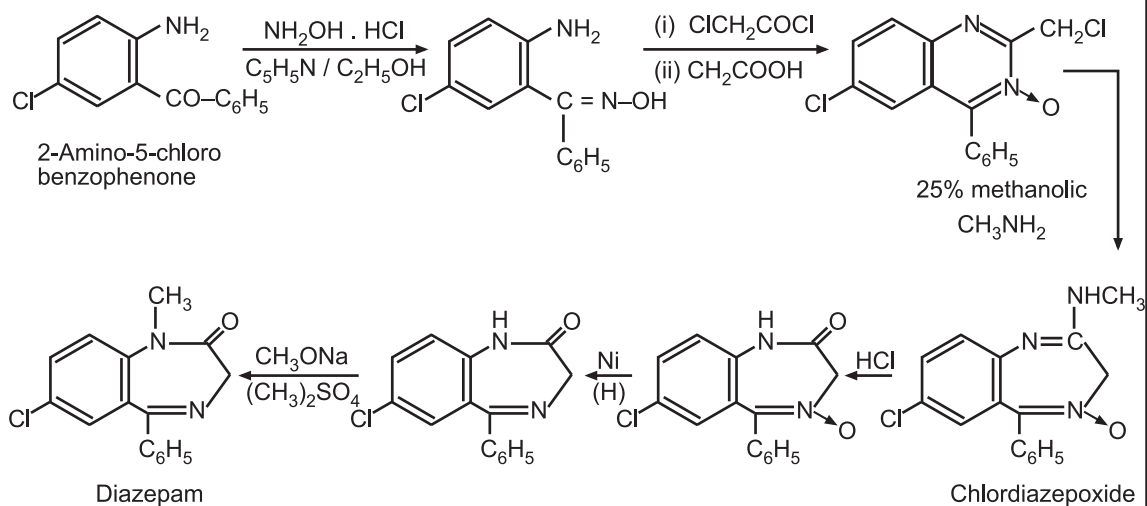
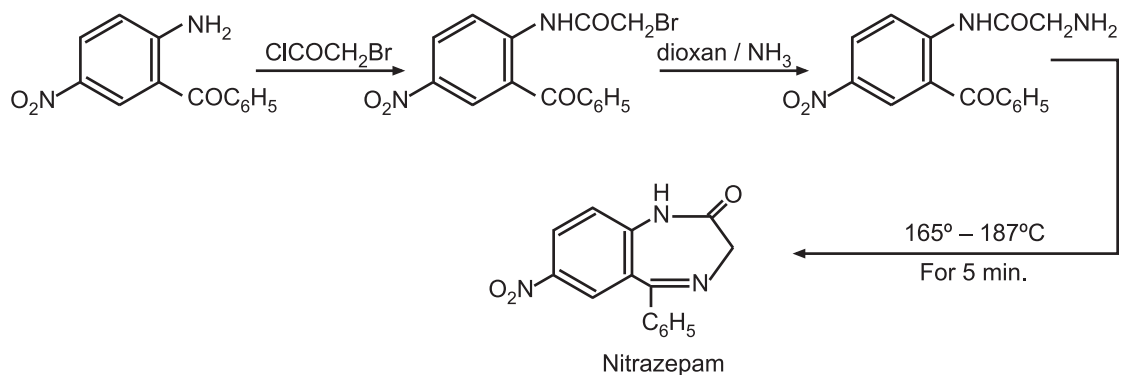
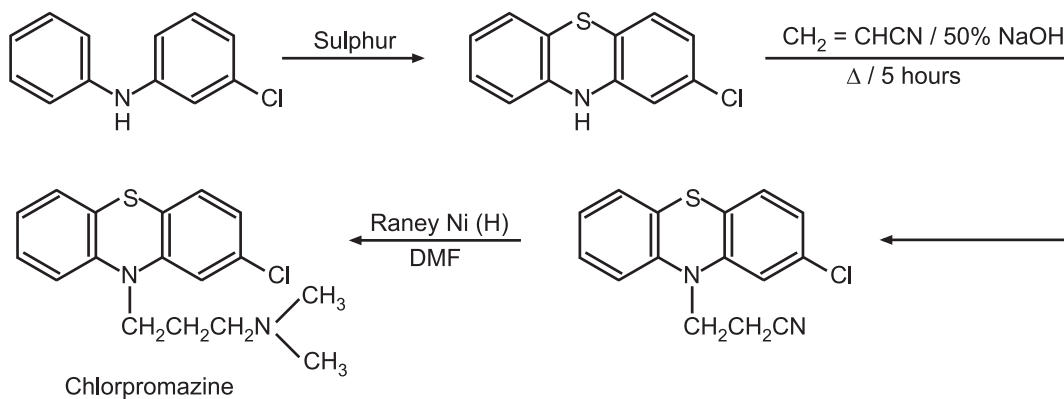
**(c) Methohexital :****(d) Thiamylal sodium :****(e) Barbital :**

**(6) NARCOTIC ANALGESIC AGENTS :****(a) Dextropropoxyphene hydrochloride :****(b) Pethidine hydrochloride :**

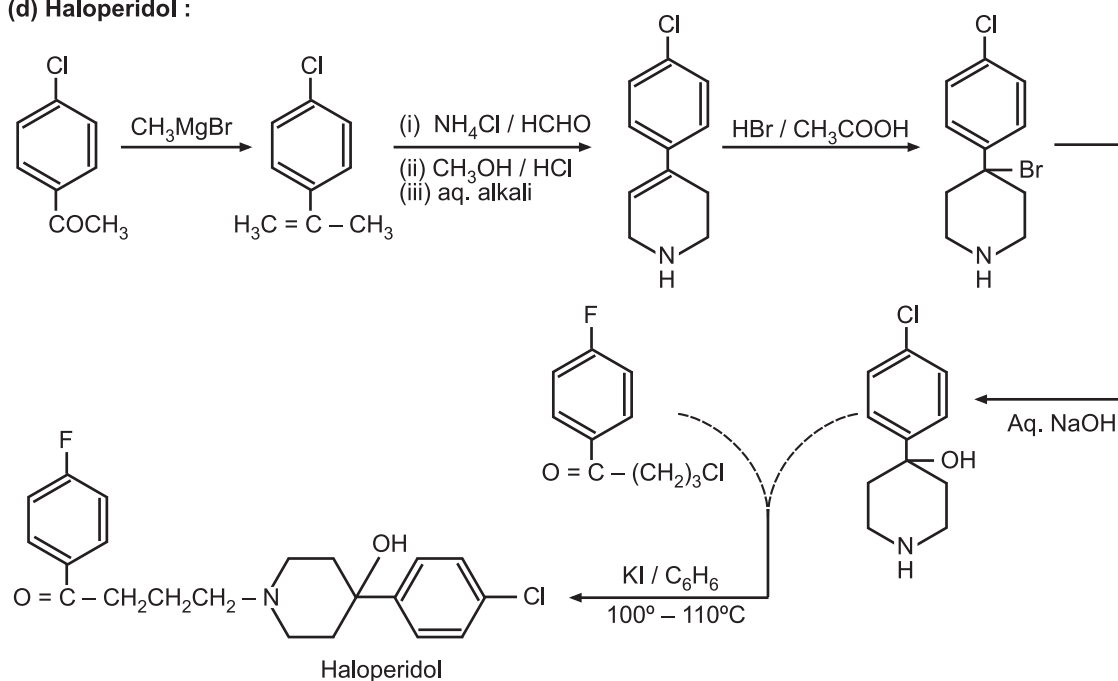
**(c) Fentanyl citrate :****(d) Methadone :****(7) NON-STEROIDAL ANTI-INFLAMMATORY AGENTS :****(a) Oxyphenbutazone :**

**(c) Indomethacin :****(d) Meclofenamate sodium :****(e) Tolmetin sodium :**

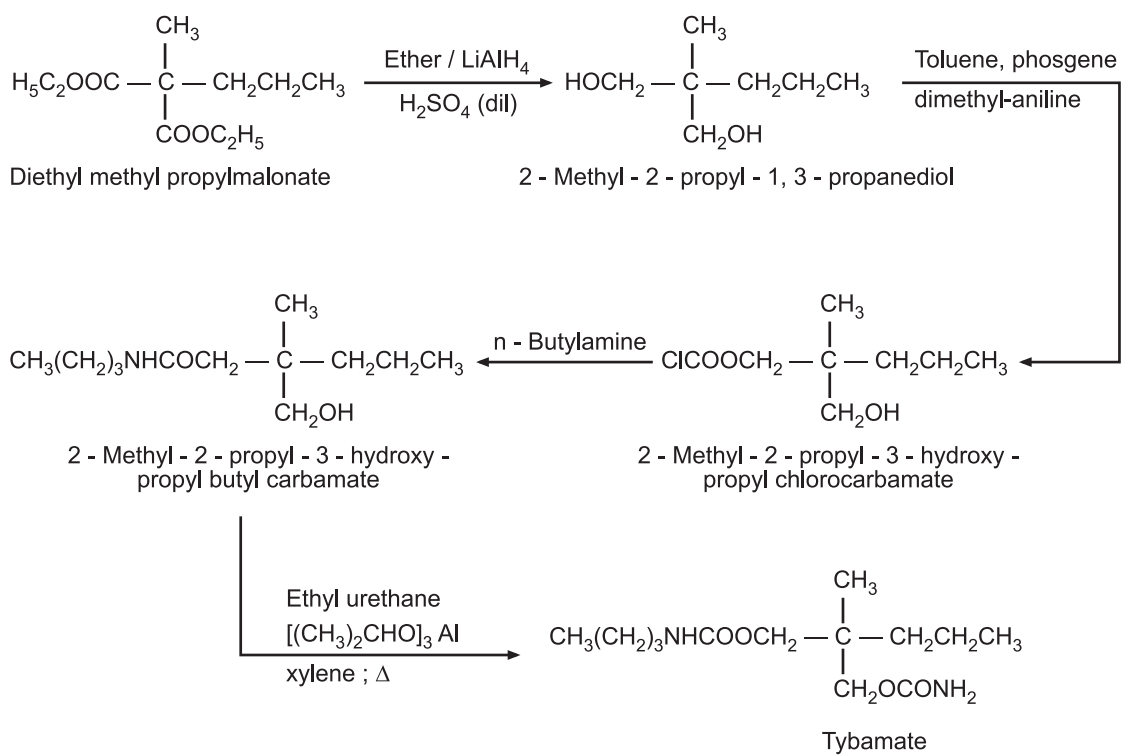
**(f) Flurbiprofen :****(g) Ibuprofen :****(h) Mefenamic acid :**

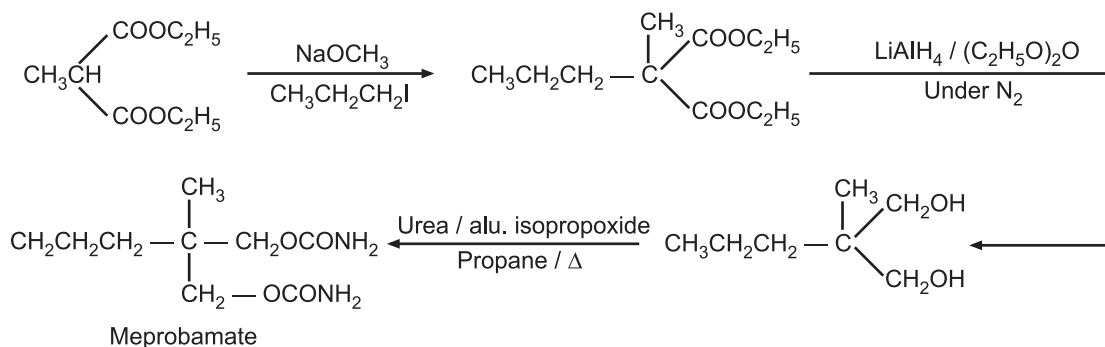
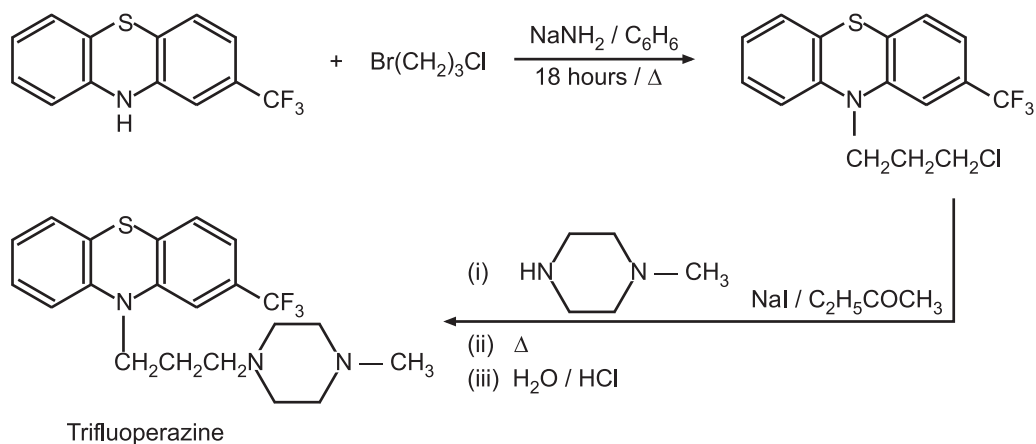
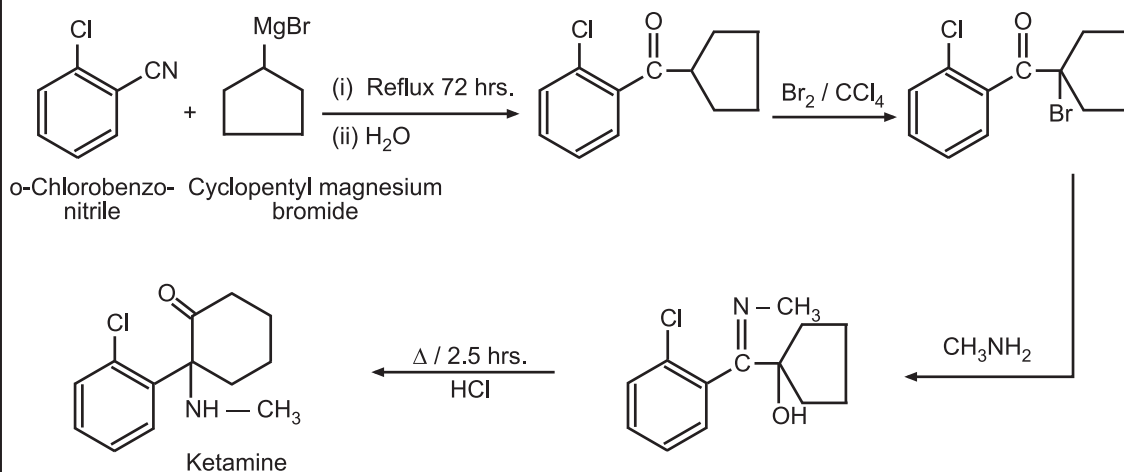
**(8) TRANQUILLIZERS :****(a) Chlordiazepoxide and Diazepam :****(b) Nitrazepam :****(c) Chlorpromazine :**

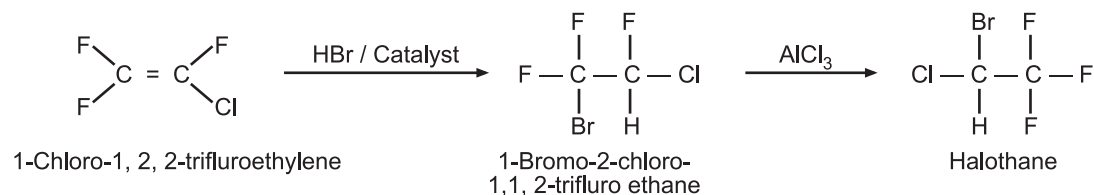
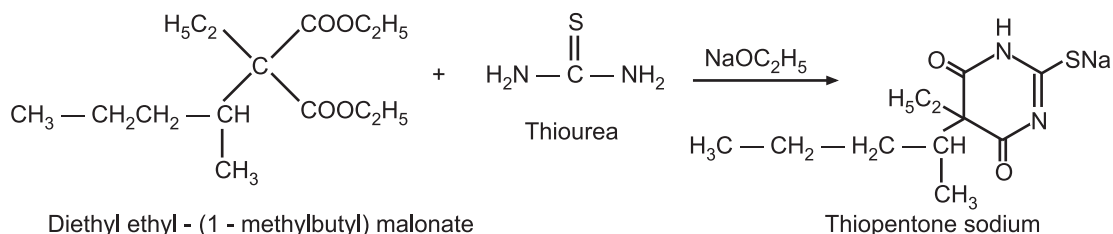
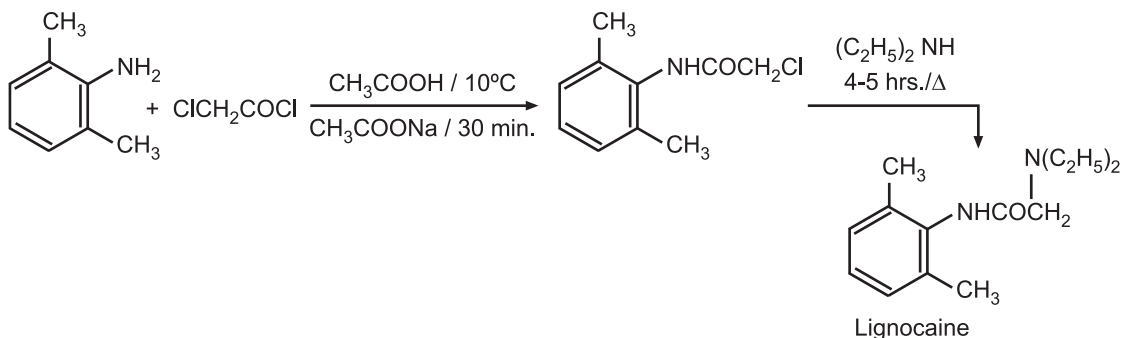
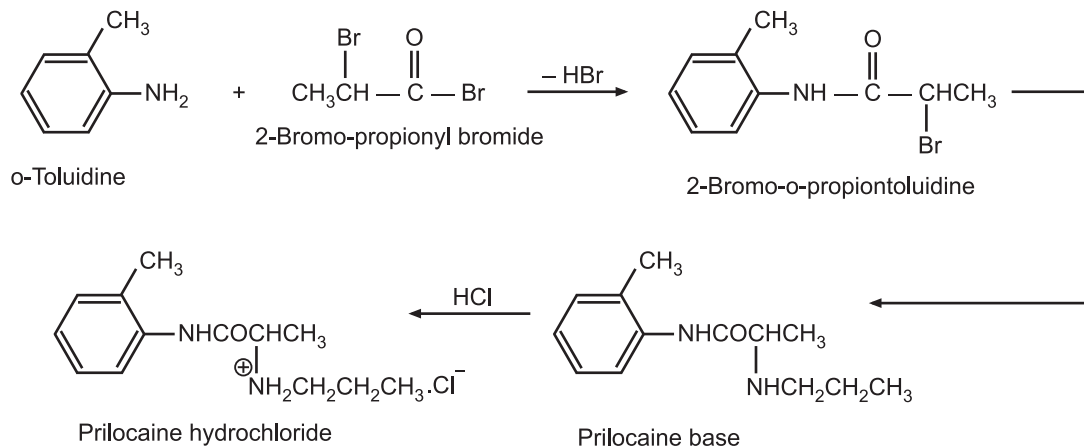
## (d) Haloperidol :

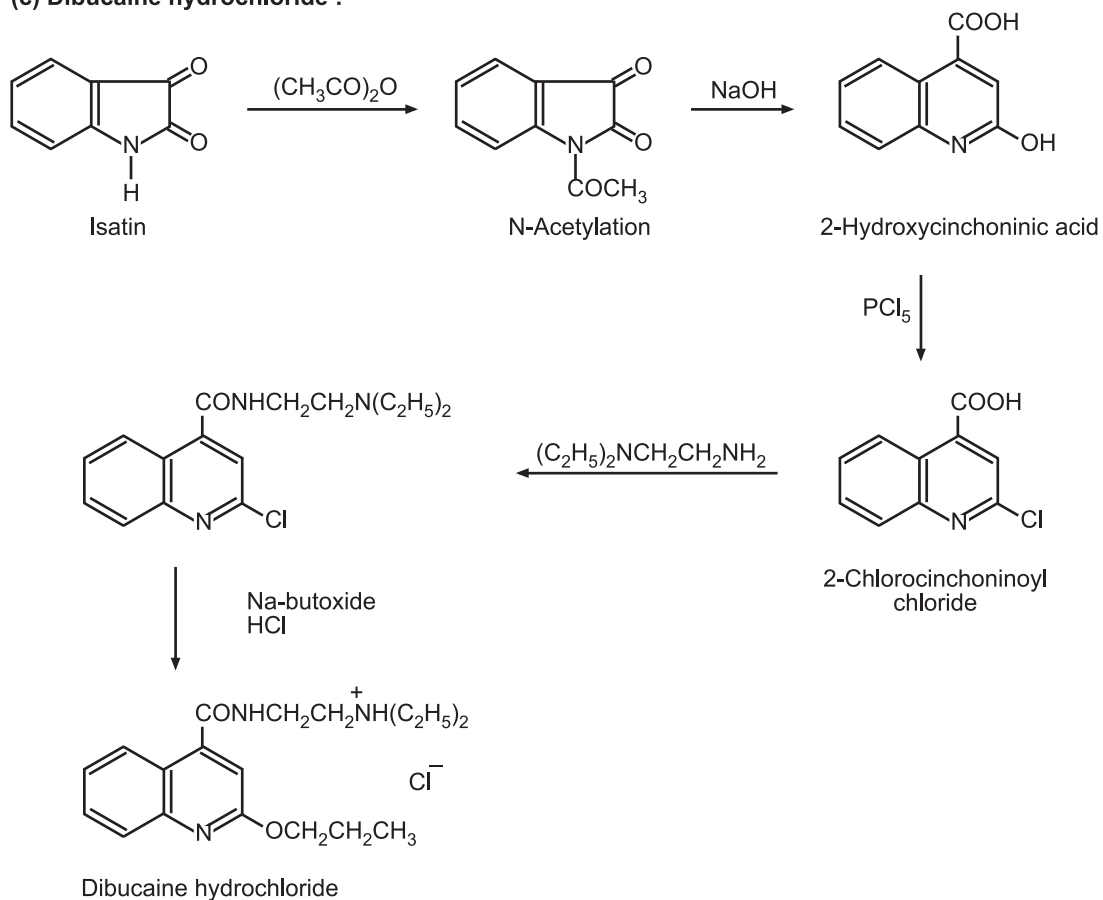


## (e) Tybamate :



**(f) Meprobamate :****(g) Trifluoperazine :****(9) ANAESTHETIC AGENTS :****'(a) Ketamine :**

**(b) Halothane :****(c) Thiopentone sodium :****(10) LOCAL ANAESTHETIC AGENTS :****(a) Lignocaine :****(b) Prilocaine hydrochloride :**

**(c) Dibucaine hydrochloride :****(c) Procaine :**