SECTION I

Physicochemical Factors in Relation to Biological Activity of Drugs

CHAPTER 1

Physicochemical Properties

INTRODUCTION

The biological activity of a targeted drug molecule is solely dependent on its physicochemical characteristics, essentially the nature and type of functional moieties, and the spatial arrangement of such groups in the molecule. 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. Hence, the activity is maintained, although the structure is changed. This can be expressed by an idea of bioisosteric groups that generally have similar biological activity. Physicochemical properties play an important role in modifying the biological activities of many compounds. Thus, pharmacological or therapeutic effects of a drug also relate to its biodistribution or physicochemical parameters of a drug such as:

- Hydrogen bonding
- Chelation
- Oxidation–reduction potential
- Dissociation constant
- Bioisosterism
- Surface activity

The physicochemical parameters that affect the biological activity can be divided into three main groups, as listed below.

1. Parameters that are an expression of the hydrophobic aggregation forces at the site of action. These parameters contribute relatively large to binding energy. Examples for these parameters are listed below.
   - Partition coefficient
   - Rf value
   - Surface activity
   - Partial vapour pressure of a solution
2. Parameters that are an expression of the charge distribution in the molecule and the electrostatic forces at the site of action. They are relatively large or contribute more to drug–receptor interaction. The examples for these parameters are enumerated below.
   - Acid–base dissociation constant (pK_a)
   - Dipole moment

- Redox potential
- Electronic potential
- Resonance effect for conjugated system
- Hydrogen bonding

3. Parameters that are an expression of spatial arrangement of the molecule, which includes the ones listed below.
   - Spatial arrangement of various groups in molecule
   - Steric hindrance

Medicinal chemistry undoubtedly rests its main focus on the broad-based variations embracing the influence of numerous possible manipulations with regard to the chemical structure on the biological activity. In light of the above statement of facts supported by copious volumes of scientific evidence reported in many of the literatures, it is important and necessary for the medicinal chemist to decipher and logically understand not only the 'mechanism of drug action' *in vivo* by which a drug substance exerts its effect, but also the overall physicochemical properties of the molecule. In a rather most recent conceptualized theoretical basis, the terminology *physicochemical characteristics* invariably refers to the cognizable influence of the plethora of organic functional moieties strategically positioned within a drug substance. It is, however, pertinent to mention here that most of the aforesaid properties covertly and overtly exert a significant influence on the various biological phenomena *in vivo*, such as absorption, distribution, metabolism, and excretion of newer targeted drug molecules.

Therefore, a creative medicinal chemist should ponder over the intricacies, complexities, and legitimate presence of each functional moiety to the overall physical and chemical properties of the targeted drug molecule with a view to arrive at or design safer, better, and efficacious medicinal agents. Nevertheless, such critical studies have to be carried out in a rather methodical and systematic manner vis-à-vis their effects on biological activities.

CHAPTER 2

Ferguson Principle

INTRODUCTION

The observation that many compounds containing diverse chemical groups exhibit narcotic or anaesthetic action is indicative of the fact that mainly physical rather than chemical properties are involved. The fact that narcotic action is attained rapidly and remains at the same level as long as reservoir or critical concentration of the drug is maintained but quickly disappears when the supply of drug is removed suggests that equilibrium exists between the external phase and the biophase.

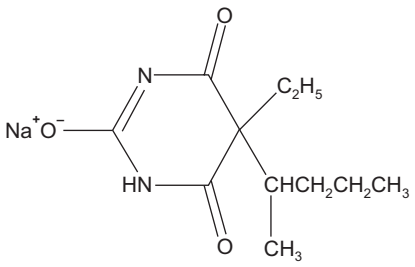
According to Ferguson, it is unnecessary neither to define the nature of the biophase or the receptor nor to measure the concentration of the drug at this site. If equilibrium conditions exist between the drug in molecular biophase and in extracellular fluids, the tendency to escape from each phase is the same, even though the concentrations in the two phases are different. This tendency is called *thermodynamic activity*. It is approximately equivalent to the degree of saturation of each phase. Since the thermodynamic activity is the same in both the biophase and the extracellular phase, measurements made in the extracellular phase, which is measurable, may be directly equated with biophase, which is not possible to be measured.

Pharmacologically active drugs are classified into two main categories (Table 2.1):

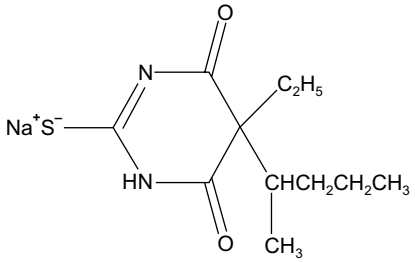
1. Structurally specific drugs
2. Structurally non-specific drugs

Structurally specific drugs: These drugs produce their effect by binding with specific receptors. The physical property of the drug plays an important role on its biological activity, yet the chemical properties do exert their influence on the activity.

Example 1: Pentobarbitone sodium (short-acting hypnotic drug; duration of action less than 3 hrs)



Example 2: Thiopental sodium (ultra short-acting hypnotic drug; duration of action 15 min)



Structurally non-specific drugs: They do not interact with receptors but penetrate to the cell or accumulate in cell membranes where they interfere, e.g. hypnotics, volatile insecticides, bactericidal agents, etc.

The thermodynamic activity of non-volatile drugs may be calculated from the expression S/S_o, where S = molar concentration of the drug and S_o = solubility of the drug.

In the case of volatile drugs, their thermodynamic activity is calculated by using the formula p_t/p_s, where p_t = Partial pressure of the substance in solution.

$p_t = 760 \times (C/100)$

p_s = Saturated vapour pressure

C = Concentration

For example,

Saturated vapour pressure of $CHCl_3$ (p_s) = 324

Narcotic concentration, $C = 0.5$

Partial pressure of $CHCl_3$ (p_t) $= 760 \times (C/100)$

$= 760 \times (0.5/100)$

$= 760 \times 0.005$

$= 3.8 \approx 4$

Approximate thermodynamic activity $= p_t/p_s$

$= 4/324$

$= 0.01$

Table 2.1: Differences between structurally non-specific drugs and structurally specific drugs

S. No.	*Structurally non-specific drugs*	*Structurally specific drugs*
1.	Their biological action is directly related to the thermodynamic activity	Their biological action does not depend on the thermodynamic activity
2.	Thermodynamic activity value varies from 0.01 to 1	Thermodynamic activity value is below 0.01
3.	High doses are needed for biological activities	They are effective in low concentrations
4.	Chemical structures are different, but they produce similar biological responses	They have some structural characteristics in common to produce the biological responses
5.	Slight modifications in their chemical structure do not result in pronounced changes in biological action	Slight modifications in their chemical structures may result in substantial changes in biological activity

The isoanaesthetic concentrations of gases and vapours are given in Table 2.2.

Table 2.2: Isoanaesthetic concentrations of gases and vapours in man at 37°C

Substance	*Vapour pressure, P (mm Hg)*	*Anaesthetic concentration (C)*	*Partial pressure at anaesthetic concentration, p_t 760 × (C/100)*	*Approximate thermodynamic activity (p_t/ p)*
Nitrous oxide	59,300	100	760	0.01
Ethylene	49,500	80	610	0.01
Acetylene	51,700	65	495	0.01
Ethyl chloride	1780	5	38	0.02
Ethyl ether	830	5	38	0.05
Vinyl ether	760	4	30	0.04
Ethyl bromide	725	1.9	14	0.02
1,2-Dichloroethylene	450	0.95	7	0.02
Chloroform	324	0.5	4	0.01

These findings coined the Ferguson's principle, which states that 'substances that are present at the same proportional saturation in a given medium have the same degree of biological action'.

CHAPTER 3

Hydrogen Bonding

INTRODUCTION

Hydrogen bond (H-bond) is a bond in which a hydrogen atom serves to hold two other atoms together. The H-bond usually is formed only between hydrogen and electronegative atoms. In addition, the atoms capable of forming H-bonds have at least one unshared pair of electrons.The most common atoms capable of forming H-bonds are F, O, and N, and to a lesser extent Cl and S.

The compounds that are capable of forming H-bonds are only soluble in water. Proteins are held in a specific configuration by H-bonds, and denaturations of proteins involve the treating of some bonds.

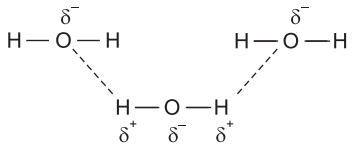
CLASSIFICATION

Generally, hydrogen bonding is classified into two types:

1. Intermolecular hydrogen bonding
2. Intramolecular hydrogen bonding

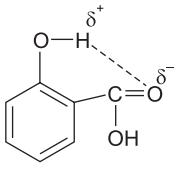
Intermolecular Hydrogen Bonding

Hydrogen bonding occurs between two or more molecules.

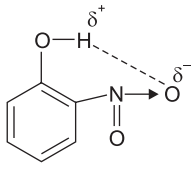


Intramolecular Hydrogen Bonding

Hydrogen bonding occurs within the molecules.



Salicylic acid



o-Nitrophenol

Table 3.1: H-bond and its bond strength

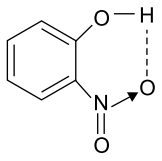
H-bond	*Bond strength (kcal/mol)*
F–H.....F	7
O–H.....O	4.5–7.6
O–H.....N	4–7
C–H.....pi electrons	2–4
C–H.....O	2–3
N–H.....O	2–3
N–H.....N	1.3

There is also some evidence that the hydrogen attached to a triple-bonded carbon (e.g. HCN and $CHCl_3$) forms a H-bond. The strength of H-bonds ranges from 1 to 10 kcal/mol, and usually is about 5 kcal/mol. The distance between the electronegative elements in a H-bond is usually in the range of 2.5–2.7 Å. At distance greater than 3 Å, there is very little interaction (Table 3.1). The stability of H-bonds falls roughly in the order: OHO > OHN >NHN.

H-bonds may occur between molecules (intermolecular H-bond), within one molecule (intramolecular H-bond), or as a combination of these two. Intermolecular bonds are frequently much weaker than the intramolecular bonds. Multiple hydrogen bonding groups in any drug molecule would greatly increase its potential for aqueous solubility. Minimal aqueous solubility is essential for all the drug molecules to transport to the site of action on a receptor.

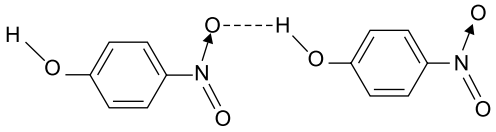
Generally, the more H-bonds that are possible, the greater the water solubility of the molecule. The strength of the H-bond depends on the solvent as well as on the physical state. For example, the H-bond strength of O–H ····· O for CH_3COOH dimer in vapour state is 7.64 kcal/mol, but CH_3COOH dimer in benzene is 4.85 kcal/mol. In water, the H-bond strength is 4.5 kcal/mol; in ice, the bond strength is 6 kcal/mol.

Intramolecular hydrogen bonding



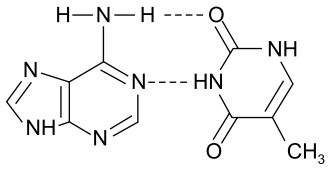
o-Nitrophenol

Intermolecular hydrogen bonding

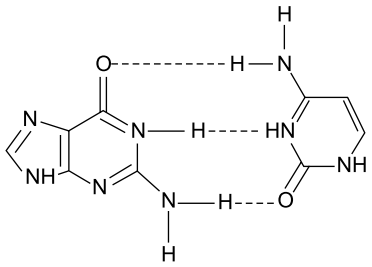


p-Nitrophenol

The description of the drug receptor interaction based on hydrogen bonds is closely related to the importance of these bonds in maintaining the integrity of biological systems and in determining the physicochemical properties of drug molecules. The physical states of substances, such as water, DNA, protein, and various drug molecules, are maintained by hydrogen bonding. The most frequently observed H-bonds in biological systems are between the hydroxyl (OH) and amino (NH) groups. In the DNA helix, hydrogen bonding links the complementary base pairs of adenine–thymine and guanine–cytosine.



Adenine-thymine



Guanine-cytosine

Since the physical and chemical properties of a compound may be greatly altered by hydrogen bonding, it is reasonable to expect that it may also have a significant effect and some correlation with biological properties. In a number of cases, such a correlation is present (Tables 3.2 and 3.3).

Table 3.2: Differences between 1-phenyl-3-methyl-5-pyrazolone and 1-phenyl-2,3-dimethyl-5-pyrazolone (antipyrine)

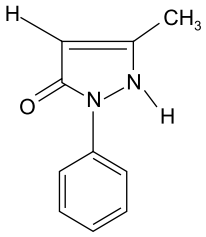
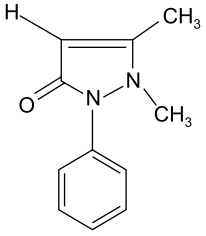
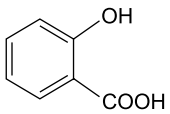
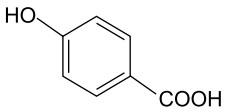
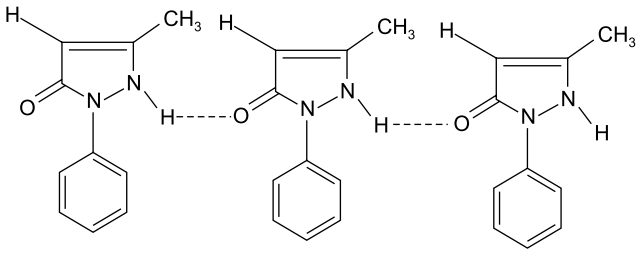
1-Phenyl-3-methyl-5-pyrazolone	***1-Phenyl-2,3-dimethyl-5-pyrazolone (antipyrine)***
	
No analgesic property Melting point 127°C, Insoluble in water Slightly soluble in ether Forms intermolecular hydrogen bonding	Good analgesic agent Melting point 112°C Soluble in water Moderately soluble in ether Does not form intermolecular hydrogen bonding

Table 3.3: Differences between *o*-hydroxybenzoic acid and *p*-hydroxybenzoic acid

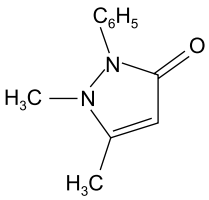
o-Hydroxybenzoic acid (salicylic acid)	***p-Hydroxybenzoic acid***
	
pK_a 3.0 Less soluble in water Low-melting point Good antibacterial action Forms intramolecular hydrogen bonding	pK_a 4.5 More soluble in water High melting point Low antibacterial action Forms intermolecular hydrogen bonding

1-Phenyl-3-methyl-5-pyrazolone forms intermolecular hydrogen bonding.

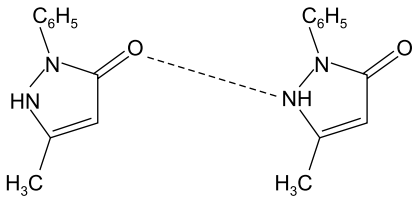


The alkylating agents (nitrogen mustards) are thought to act by replacing the weak and reversible H-bonds between adjacent nucleic acid strands with strong and relatively irreversible covalent bonds. In this way, nucleic acid regeneration and cell division in the rapidly proliferating cancer cells may be inhibited.

Antipyrine, 1-phenyl-2,3-dimethyl-5-pyrazolone has analgesic activity, but 1-phenyl-3-methyl-5-pyrazolone is inactive. This is due to the formation of hydrogen bonding in the 1-phenyl-3-methyl-5-pyrazolone and gives rise to a linear polymer, which cannot pass through biomembranes.



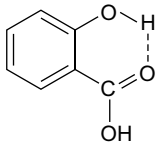
Antipyrine



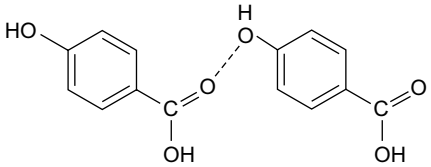
1-Phenyl-3-methyl-5-pyrazolone

Salicylic acid (*o*-hydroxy benzoic acid) has antibacterial activity, but not the *p*-isomer and *m*-isomer; that is, *p*- and *m*-hydroxy benzoic acids are inactive. This is because salicylic acid forms intramolecular hydrogen bonding; therefore, it is less water-soluble and its partition coefficient is also greater.

The *m*- and *p*-isomers can form intermolecular H-bonds, result in dimer, and do not easily pass through the biomembranes. Their partition coefficient is also less, and hence, low antibacterial action.



Salicylic acid
(intramolecular hydrogen bonding)



Dimer of *p*-hydroxy benzoic acid
(intermolecular hydrogen bonding)

CHAPTER 4

Ionization and pK_a Value

INTRODUCTION

If the biological activity of a drug results from ions, the activity intensifies with increase in the degree of ionization. However, if the activity results from undissociated molecules, increase in the degree of ionization of active compounds causes a decrease in activity.

Increase in ionization intensifies a drug's water solubility and decreases its liposolubility or lipophilicity. In general, drugs cross cellular membranes in undissociated forms, as intact molecules, and act in dissociated forms, as ions. This happens because the passage of ions across the cellular membrane is prevented by two factors.

1. The cellular membrane is made up of layers of electrically charged macromolecules (lipids, proteins, and mucopolysaccharides) that attract or repel ions.
2. Hydration of ions increases their volumes rendering their diffusion through pores difficult.

Weakly acidic drugs are predominantly of the unionized form at lower pH of the gastric fluid, and absorbed from the stomach as well as intestine. Some very weak acidic drugs, such as phenytoin and many barbiturates, whose p$K_a$values are greater than 7, are essentially unionized at all pH values. Therefore, for these weak acidic drugs transport is more rapid and independent of pH.

Most weak bases are poorly absorbed in the stomach since they are present largely in the ionized form at low pH. Strong bases, those with pK_a values between 5 and 11, show pH-dependent absorption. Stronger bases, such as guanithidine (pK_a >11), are ionized throughout the gastrointestinal tract and tend to be poorly absorbed.

The compounding or the formulation, pharmacist can adjust the pH to ensure water solubility or maximum solubility in non-polar media. So the understanding of acid–base chemistry becomes important.

Note the following reactions:

HA	+	H_2O	↔	H_3O^+	+	A^- (ionized)
Acid (unionized)		Base	↔	Conjugated acid		Conjugated base
BH^+	+	H_2O	↔	H_3O^+	+	B (unionized)
Acid (ionized)		Base	↔	Conjugated acid		Conjugated base

Acids can be divided into two types: HA and BH^+. HA acids go from unionized (non-polar) acids to ionized conjugated bases, while BH^+ acids go from ionized (polar) acids to unionized (non-polar) conjugated bases.

Pharmaceutically, important HA acids are listed below:

- Inorganic acids: HCl, H_2SO_4
- Carboxylic acids: Salicylic acid, arylanthranilic acid of low molecular weight
- Amides: Sulfonamides
- Imides: Saccharin
- Enols: Hydantoins, barbiturates

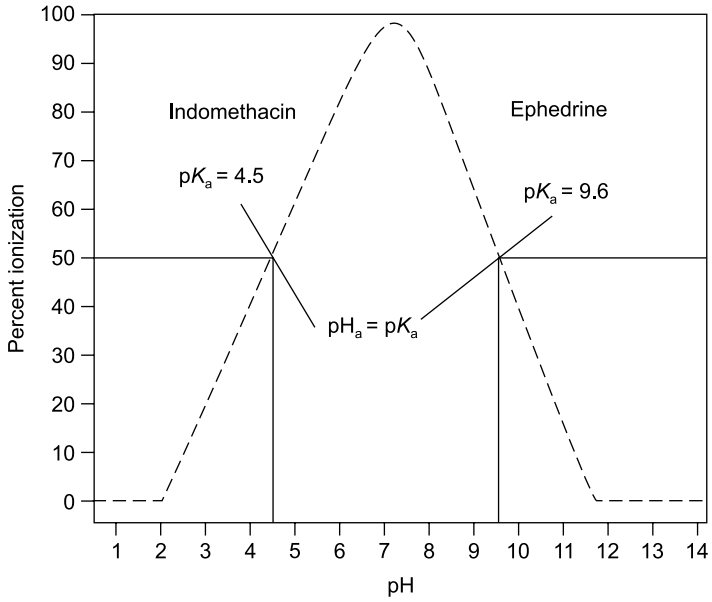


Figure 4.1: Percent ionized versus pH for indomethacin (pK_a 4.5) and ephedrine (pK_a 9.6).

Relationship between Percent Ionization and pH

A plot of percent ionization versus pH shows how the degree of ionization can be influenced by small changes in the pH. For example, the curves for an HA acid (indomethacin) and BH^+ (protonated ephedrine) are shown in Figure 4.1.

When the pK_a= pH, the molar concentration of the acid equals the molar concentration of its conjugated base.

In Henderson–Hasselbalch equation,

$$pK_a = pH\ [(\text{Conc. base})/(\text{Conc. acid})] = 1$$

An increase in 1.0 pH value causes a 90.9 percent ionization of indomethacin, but in ephedrine (HCl) the percent ionization is only about 9.1 percent.

A pH increase of 2 units shifts the HA indomethacin to 99 percent ionization (complete), but BH^+ acid (ephedrine) to non-conjugated form (0.99%).

Therefore, whenever the non-polar form of an acid or a base is used, the rate of percent ionization will be maintained. This ratio depends on the pK_a and pH of the medium.

pK_a VALUE

The partially lipidic nature of cellular membranes, such as the ones that enwrap the stomach, small intestine, mucosa, and nervous tissue, facilitate the passage of drugs with high liposolubiltiy across them. The liposolubility is affected by pH of the environmental medium and by the degree of dissociation pK_a. Usually, drugs are weak acids or weak bases. The degree of dissociation, pK_a, is calculated from the following Henderson–Hasselbalch equation.

In the case of acid,

$$pH = pK_a + \log \frac{\text{Ionized drug concentration}}{\text{Unionized drug concentration}}$$

$$\text{Percentrage of drug ionized} = \frac{10^{pH - pK_a}}{1+10^{pH - pK_a}} \times 100$$

In the case of unionized acid,

$$pH = pK_a + \log \frac{\text{Unionized drug concentration}}{\text{Ionized drug concentration}}$$

$$\text{Percentrage of drug ionized} = \frac{10^{pK_a - pH}}{1+10^{pK_a - pH}} \times 100$$

The biological activity of certain acids and bases is directly related to their degree of ionization. While some (e.g. phenols, carboxylic acids) act in the molecular form, others (quaternary ammonium salts) act in an ionized form. In these cases, the pH plays an important role. For example, acids are more active at lower pH and bases are more active at higher pH.

- Strong acid has low pK_a value.
- Weak acid has high pK_a value.
- Strong base has high pK_a value.
- Weak base has low pK_a value.

Drug Exerting Action as Undissociated Molecules

In a large number of potent medical compounds, the dissociation plays a vital role for their respective biological characteristics. The unusual structural grouping in the tetracycline results in three distinct acidity constants in aqueous solutions of the acid salts. The particular functional groups responsible for each of the thermodynamic pK_avalue have been determined by Lessen *et al.*, as described in Figure 4.2.

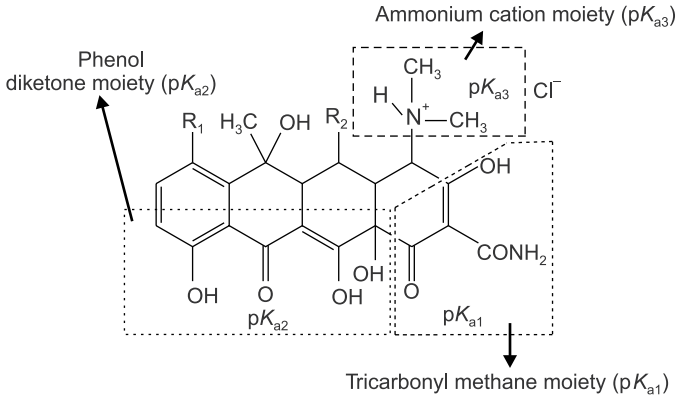


Figure 4.2: Functional groups responsible for each of the thermodynamic pK_a value.

Table 4.1: pK_a values of tetracyclines

S. No.	Name	pK_{a1}	pK_{a2}	pK_{a3}
1.	Tetracycline	3.3	7.7	9.5
2.	Chlorotetracycline	3.3	7.4	9.3
3.	Demeclocycline	3.3	7.2	9.3
4.	Oxytetracycline	3.3	7.3	9.1

The approximate pK_a values for each of these groups in the four commonly used tetracyclines are shown in Table 4.1.

Besides the activities of several local anaesthetics, *d*-tubocurarine and phenol have also been proved to be related to their degree of ionization.

Drug Exerting Action as Ionized Molecules

A plethora of medicinal compounds exerts their pharmacodynamic action exclusively as the ionized molecules, namely acetylcholine, quaternary salts as ganglionic blocking agents, muscle relaxants, and antiseptics.

CHAPTER 5

Redox Potential

INTRODUCTION

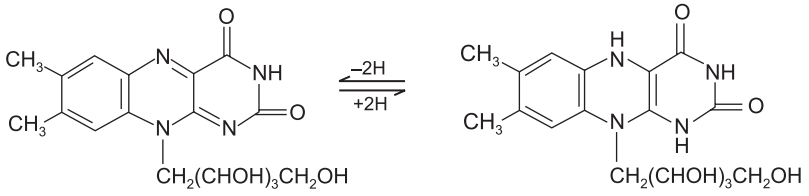
Redox potential may be defined as a quantitative expression of the tendency of a compound that has to give or receive electrons. It may be compared with an acid–base reaction. In the case of acid–base reaction, there is transfer of a proton from an atom in one molecule to the atom in another molecule, while in the case of oxidation–reduction reaction, there is an electron transfer. Since living organisms function at an optimum redox potential range, which varies with the organism, it might be assumed that the redox potential of the compounds of a certain type would correlate with the observed biological effect. This correlation is applicable for all compounds of similar structure and physical properties.

The redox potential of a system may be calculated from the following equation: $E_h = E_1^0 - 0.06/n$ (concentration of reductant/concentration of oxidant), where

E_h = Redox potential of the system being studied
E_1^0 = Standard potential at given pH
n = Number of electrons transferred

However, there are a number of reasons why only a few satisfactory correlations have been observed:

- The redox potential applies to a single reversible ionic equilibrium, which does not exist in a living system.
- A living cell carries on many reactions simultaneously involving oxidation of ionic and non-ionic character, some of which are reversible and others are irreversible.
- The access of a drug to the sites of oxidation–reduction reactions in the intact animal is hindered by the complex competing events occur during absorption, distribution, metabolism, and excretion.

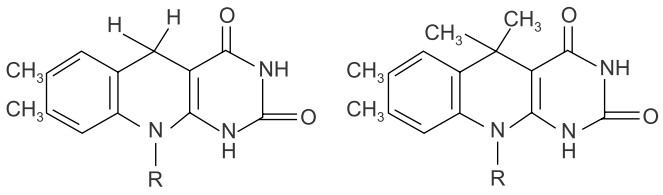


Riboflavin (**I**) Dihydroriboflavin (**II**)

- Therefore, it is to be expected that correlations between redox potential and biological activity, generally, hold only for compounds of very similar structure and physical properties. In such series, variations in the route of distribution and in steric factors, which might modify the redox system interaction, would be minimized.

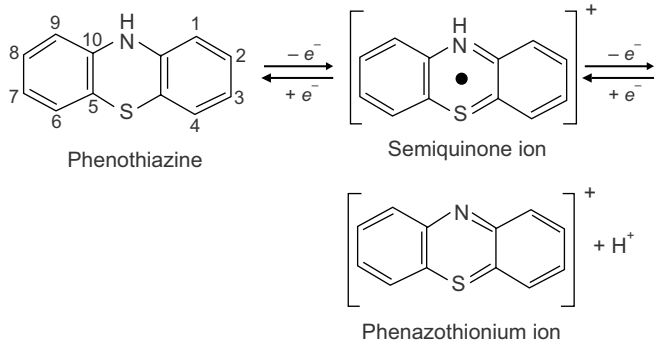
When riboflavin (I) accepts electrons, it is converted into its dihydro (II) form. This reaction has a redox potential E_0 = –0.185 volt. Kuhn (1943) prepared the analogue in which the two methyl groups of riboflavin were replaced by chlorine. The resulting compound had a potential of E_0 = –0.095 volt, and its antagonistic properties were suggested as being due to the dichloro-dihydro form being a weaker reducing agent than the dihydro form of riboflavin. It may be absorbed at the specific receptor site, but may not have a negative potential to carry out the biological reductions of riboflavin.

Reist *et al.* (1960) prepared the non-redox analogues of riboflavin as potential anticancer agents. Replacement of the N_5-nitrogen of dihydroriboflavin (1,5-dihydro-7,8-dimethyl-10-ribitylisoalloxazine) by a methylene group (III) would be expected to have a profound effect on the redox potential as compared to riboflavin. Similarly, replacement of the N_5-nitrogen of dihydroriboflavin by an isopropylidene group (IV) fixes the molecule in the dihydro form, thus eliminating the redox system completely.



Although compound IV is derived from dihydroriboflavin (II) rather than from riboflavin, the redox enzyme system employing riboflavin coenzymes utilizes both the oxidized and reduced forms; thus, analogues of either I or II should be effective antagonists.

Craig *et al.* (1960) studied a series of substituted phenothiazine with regard to potentiometric titration, electrode potentials, and their correlation with anthelmintic activity. They measured them in the biological assay using mixed infestation of *Syphacia obvelata* and *Aspicularis tetraptera* in mice. From these studies, it appeared that two factors were necessary for their activity, namely, the ability to form a high proportion of a stable semiquinone radical (as measured by the index potential in aqueous CH_3COOH) and the presence of free 3 or 7 position.



In addition to the two factors mentioned above, Craig *et al.* (1960) also noted that only these compounds with electrode potential in the range of 550–850 mV in aqueous CH_3COOH had significant activity. If the toxic or paralyzing effect of the phenothiazines was due to an inhibition by the semiquinone of the oxidation–reduction system in the parasite, it would seem reasonable that active phenothiazines would

have reduction potentials corresponding to those of oxidation–reduction enzyme system or the system which they inhibit. At similar potentials, the semiquinone concentration would be maximal, and thus facilitates or competes with the electron transfers in the enzyme system involved.

For example, it has been suggested that the semiquinone of chlorpromazine is responsible for the inhibition of certain oxidoreductase *in vitro* and some of the biological activities of phenothiazines correlate with the formation of their semiquinones *in vivo*.

CHAPTER 6

Surface Tension

INTRODUCTION

A surfactant is defined as a material that can reduce the surface tension of water at lower concentrations. This molecule is made up of water-soluble and water-insoluble components. Surface agent may enhance or retard the drug absorption, which depends upon the chemical nature of surfactant, its concentration, its effect on biological membrane, and micelle formation.

At lower concentrations, the surfactant enhances the absorption rate; the same in higher concentrations reduces the absorption rate. In lower concentrations, they reduce the surface tension and bring about better absorption through better contact of the molecules with absorbing membrane, but when the concentration crosses the critical micelle concentration, the surfactant aligns them at the surface so that the hydrophilic end is towards the water and hydrophobic end is squeezed away from the water. These molecular aggregates are called *micelle*, which entrap the drug molecule in their hydrophobic core, and result in the retardation of the rate of absorption (Figure 6.1).

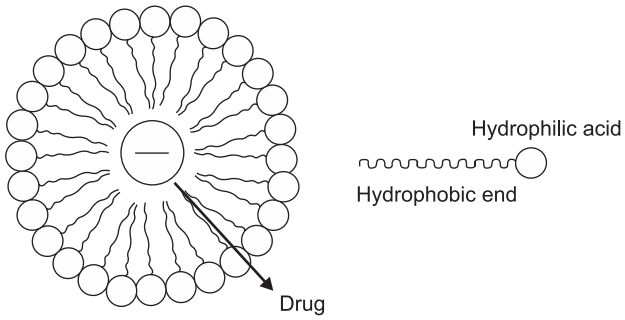


Figure 6.1: Micelle formation

The orientation of surface-active molecules at the surface of water or at the interface of polar and non-polar liquids takes place with the non-polar (hydrocarbon) portion of the molecule oriented towards the non-polar liquid or vapour phase and polar groups (e.g. –COOH, –OH, $–NH_2$, $–NO_2$, etc.) towards the polar liquid. Three forces are involved in the orientations of this type, namely, Van der Waals forces, hydrogen bonds, and ion dipoles.

A surfactant molecule exhibits two distinct regions of lipophilic and hydrophilic character, and such compounds are commonly categorized as *amphiphilic* or as *amphiphils*. Molecules of this type may

vary markedly from predominantly hydrophilic to predominantly lipophilic, depending on the relative ratio of polar to non-polar groups present.

CLASSIFICATION

Surface-active agents are classified as follows:

- Anionic surfactants: Ordinary soaps, salts of bile acids, salts of the sulphate or phosphate esters of alcohols, salts of sulphonic acids
- Cationic surfactants: High molecular weight aliphatic amines, quaternary ammonium derivatives
- Non-ionic surfactants: Polyethylene ethers, glycol esters of fatty acids
- Amphoteric surfactants

Applications

The bactericidal activity of cationic quaternary ammonium compounds, such as benzalkonium chloride, cetrimide, cetyl pyridinium chloride, etc., is explained through their surface-active property. Many compounds such as detergents, disinfectants, and antibiotics act through the surface phenomenon.

The anthelmintic activity of hexylresorcinols is reported to be increased by low concentrations of soap and decreased by high concentrations of soap. If the soap concentration is kept below critical micelle concentration (CMC), a 1:1 association of phenol and soap occurs, which facilitate the penetration of phenol through the surface of the worm. If the CMC is exceeded, the micelle competes favourably with the worms for phenol, and there is decreased activity.

Compounds showing pronounced surface activity usually are unsuited for use in the animal body. Such compounds are lost through their adsorption by proteins, and they also have an undesirable feature of disorganizing the cell membrane and producing haemolysis of red blood cells. In general, highly surface-active agents are not used internally, but only topically, as skin disinfectants or sterilizers for sterilization of instruments. This is the case for ionic surfactants. Non-ionic surfactants are largely employed in pharmaceutical preparations for oral (sometimes even parenteral) use as solubilizing agents of water-insoluble or slightly soluble drugs.

Surface-active agents can be expected to have a pronounced effect on the permeability of a cell. Mildly surface-active agents may be adsorbed by cell membranes, and thereby interfere with the absorption of other compounds through this membrane or may alter membrane structure and function. Many central nervous system depressant drugs, such as sedative–hypnotic, anticonvulsant, and central relaxant agents possess the general structure of non-ionic surface-active compounds.

The most commonly used surfactants are anionic and non-ionic surfactants. Since the process of solubilization occurs due to the presence of micelles, generally, high concentrations of surfactants are needed to improve drug solubility significantly. One example of a surfactant-based solution is Taxol (paclitaxel, Sigma- Aldrich, USA), an anticancer drug that is solubilized in 50% solution of Cremophor. Other examples include Valrubicin in 50% Cremophor and Cyclosporin in 65% Cremophor.

Surfactant preparations are used as replacement therapy for the treatment of premature infants suffering from neonatal respiratory distress syndrome (also known as hyaline membrane disease). A substantial deficiency in the endogenous lung surfactant is the principal factor contributing to the pathology of respiratory distress syndrome. The lung surfactant preparations are used in combination with supplemental oxygen and mechanical ventilation to facilitate gas exchange. The exogenous surfactants are either derived from animals or synthesized, for example Beractant (modified bovine extract), Calfactant (extracted from the lungs of calves), and Poractant alfa (extract of porcine lung).

CHAPTER 7

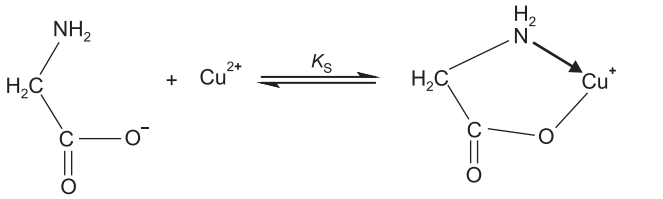
Complexation

INTRODUCTION

Complexes or coordination results from a donor–acceptor mechanism (donating/accepting electron or rather an electron pair) or Lewis acid–base reaction (donating accepting protons). Since complex drugs cannot cross the natural membranous barriers, they reduce the rate of absorption of the drug. The compounds that are obtained by donating electrons to metal ions with the formation of ring structures are called chelates. The compounds that are capable of forming a ring structure with metal atoms are termed as ligands.

Both biological molecules and medicinal agents may develop chelate structures by forming a ring structure with a metal through coordinate bonds (i.e. bonds in which electron pairs are from the same atom).

Example: Glycine forms complex with Cu^{2+}.



Glycine anion Copper Glycine–copper complex

The stability constant (K_S) reflects the strength of the interaction, and larger the constant the greater the stability.

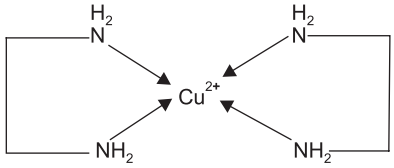
$$K_s = \frac{[\text{Complex}]}{[\text{Glycine}]\,[Cu^{2+}]}$$

Two coordinate covalent bonds are formed between glycine and copper in the complex. The nitrogen and oxygen atoms of glycine serve as the electron-donating groups, each supplying an electron pair, whereas the cupric ion is the electron acceptor.

Electron-donating groups are almost always limited to O, N, and S atoms; electron acceptors include various bivalent and trivalent metals, particularly those of the transition group. Stability of the metal ions in the complex:

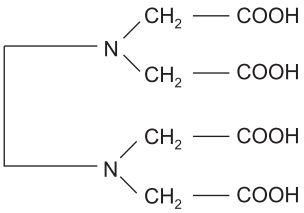
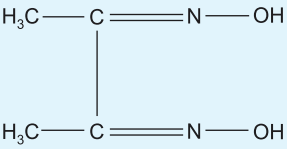
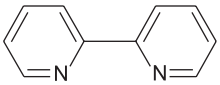
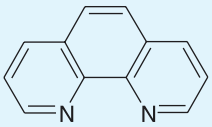
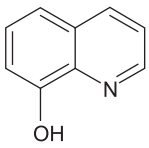
$Fe^{3+}, Hg^{2+} > Cu^{2+}, Al^{3+} > Ni^{2+}, Pb^{2+} > Co^{2+}, Zn^{2+} > Fe^{2+}, Cd^{2+} > Mn^{2+} > Mg^{2+} > Ca^{2+}$

A ligand, such as ammonia, that has a single basic group capable of bonding to the metal ion is a unidentate ligand. A ligand having more than one accessible basic binding site is multidentate, for example ethylenediamine, $NH_2CH_2CH_2NH_2$, is a bidentate ligand form and chelates with Cu(II).



Some important multidentates are listed in Table 7.1.

Table 7.1: Multidentates

Ethylenediamine	$NH_2\ CH_2\ CH_2\ NH_2$
Ethylenediamine tetra acetic acid	
Dimethylglyoxime	
2,2-Bipyridine	
1,10-Phenanthroline	
8-Hydroxyquinoline	

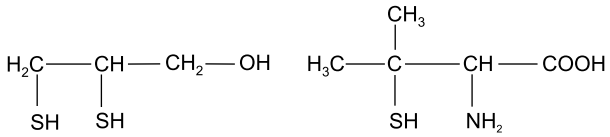
The number of rings formed in the chelate depends on the electron-donating groups. S can form the stable four-member rings, O and N can form five- and six-member rings, but five-member rings are generally more stable. Heavy metals are required for the following enzymes and biomacromolecular components (Table 7.2).

Important metal-binding hormones are thyroxine, insulin, histamine, epinephrine, and norepinephrine. In clinical practice, chelating agents have been used primarily as antidotes in heavy metal poisoning. In principle, any chelating agent with a sufficiently high K_S value *in vitro* could be used as an antidote, but some limitations are there. For example, ethylene diamine tetra acetic acid (EDTA) because of its powerful chelating effect can displace toxic heavy metals, such as lead and mercury from cellular layer, but EDTA is not highly selective in its action; it tightly binds some essential metals, including calcium.

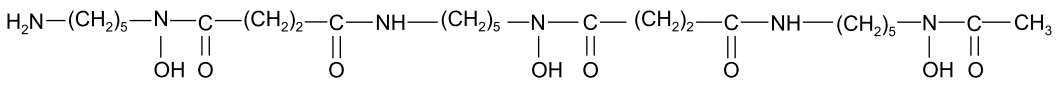
Table 7.2: Metals required for enzymes and biomacromolecular components

S. No.	Metal	Cellular components
1.	Cobalt	Vitamin B_{12}, carboxypeptidase
2.	Copper	Oxidase enzymes
3.	Iron	Porphyrin enzymes, haemoglobulin,iron-storage molecules (ferritin, haemosiderin)
4.	Magnesium	Chlorophyll
5.	Manganese	Chloroplasts
6.	Molybdenum	Xanthine oxidase, aldehyde oxidase
7.	Nickel	Urease
8.	Zinc	Carbonic oxidase, alcohol dehydrogenase

To prevent excessive loss of calcium from the body during EDTA therapy, it is necessary to administer this antidote as disodium calcium salt. Iron deficiency anaemia may be treated using the iron complex of EDTA in place of ferrous sulphate or ferrous gluconate. Pernicious anaemia is treated effectively using Vitamin B_{12}, a naturally occurring cobalt complex. Deferoxamine is a highly selective antidote, which strongly chelates iron in iron poisoning. Dimercaprol (BAL) is used in the treatment of lead poisoning. L-Penicillamine is used in the treatment of copper and Wilson's disease.



Dimercaprol L-Penicillamine



Deferoxamine

Numerous antimicrobial and antineoplastic agents are believed to exert their action by means of complex formations with DNA base pairs. These drug molecules are large planar aromatic compounds, and they can be inserted between the planar base pair assemblies on the DNA double helix. This type of inserted molecular interaction is called *intercalation*. Intercalating drugs include ethidium, quinacrine, proflavin, daunorubicin, adriamycin, and actinomycin D.

Undesirable side effects are caused by drugs that chelate with metals. A side effect of hydralazine, an antihypertensive agent, is the formation of anaemia, and this is due to chelation of the drug with iron. 8-Hydroxy quinoline and its analogues act as antibacterial and antifungal agents by complexing with iron or copper.

CHAPTER 8

Solubility

Solubility is the ability of a substance to dissolve in a solvent. Solubility of a substance in a solvent depends on the nature of the solute and solvent, and the interactions involved. It is expressed by molarity, molality and percentage. In general, polar solvents dissolve polar solutes and non-polar solvents dissolve non-polar solutes, and semipolar solvents act as intermediate. Electrolytes have appreciable solubility and their solubility depends largely on the electrostatic forces of attraction and repulsion.

All biochemical reactions are based on the solubility of a particular drug molecule in an aqueous phase or on macromolecules, in this phase usually body. A highly important physical property of all physiologically and pharmacologically important drug molecules is their solubility in body's aqueous and non-aqueous environment, because the solution form of the drug can interact with cellular and subcellular structures that carry drug receptors, triggering pharmacological reactions. The degree of solubility differs in each compartment.

The proportion of concentrations at equilibrium is called *partition coefficient*. Most of the drugs exhibit solubility to some extent in both water and lipid environments.

Solubility is a function of many molecular parameters, as mentioned below:

- Ionization
- Size and molecular structure
- Stereochemistry
- Electronic structure

All the above parameters are involved in the interaction between the solvent and the solute. For example, H_2O forms hydrogen bonds with ions or non-polar compounds through –OH, –SH, –NH and –C=O groups or with lone pair of electrons of O and N atoms.

The interaction of non-polar drugs with lipids is based on hydrophobic interaction, e.g. the solubility with pharmacological activity.

1. Local anaesthetic activity of *para*-amino benzoic acid (PABA) esters partially depends on their lipid solubility.
2. In the homogeneous series beginning with *n*-butanol and ending with *n*-octanol, the bactericidal activity changes with molecular weight. *n*-Butanol and *n*-pentanol are active against *Staphylococcus aureus*. Higher members of this series fail to kill the bacteria since the necessary concentration cannot be reached due to the lack of solubility in water.

Solubility of Weak Electrolytes

Most of the drugs are either weak acids or weak bases, which are less soluble in water. Their solubility depends on the pH. They are reacted with acids or bases to produce respective salts to make it water soluble. Here, we consider the two factors:

1. Increase the solubility of the weak electrolyte by adjusting the polarity of the solvent.
2. Decrease the dissociation of weak electrolyte (pK_a increases) to decrease the solubility.

The solubility of weakly acidic and basic drugs may be enhanced by micellar solubilization.

The solubility of weakly acidic or weakly basic drugs depends on the pH.

Consider the following equation:

$$NaD \longrightarrow Na^+ + D^-$$

(Salt of weakly acidic drug)

$$D^- + H_2O \rightleftharpoons HD + OH^-$$

NaD is dissolved in water. HD = Conjugate acid

Then,

$$pH_{(p)} = pK_a + \log (C_s - S_o/S_o)$$

where, $pH_{(P)}$ = pH below which precipitation occurs

K_a = Ionization constant for conjugate acid (HD)

C_s = The total amount of drug in solution (ionized + unionized)

S_o = Aqueous solubility of unionized drug (HD)

For weak base

$$pH_{(p)} = pK_a + \log (S_o/C_s - S_o)$$

where pK_a = Ionization constant for conjugate acid

According to *Indian Pharmacopoeia* (IP), the solubility is indicated by the following table:

Descriptive term	***Parts of solvent required for part of solute***
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10,000
Insoluble	More than 10,000

Factors Affecting Solubility of Drugs

The factors affecting solubility of drugs are classified as follows:

1. *Solute related (nature of solute)*: Size, shape, melting point, pK_a, etc.
2. *Solvent related (nature of solvent)*: Polarity, pH, etc.
3. *Environmental related*: Temperature and pressure
4. *Formulation related*: Surfactants, common ion effect and other electrolytes effect

Applications of Solubility

1. The solubility of drug in gastrointestinal fluids (dissolution) is important for better absorption of drugs. If the aqueous solubility of drug is more than 1 percent, good absorption of drug will take place.
2. The action of drug also depends on the poor aqueous solubility.
3. The release and absorption of drug from an ointment or intramuscular injection depends on the degree of saturation of the drug in the particular solvent.
4. Solubility of the drug provides information about intermolecular forces of interaction, which is useful for finding out the drug–receptor interaction.
5. Solubility is used for purification purpose of drugs, e.g. extraction and recrystallization.

Drug Solubility and Absorption

An important prerequisite for the absorption of drug by all mechanisms (except endocytosis) is that the drug must be present in aqueous solution.

After oral administration, absorption of the particular drug depends mainly on two factors (Figure 8.1):

1. Drug solubility and rate of dissolution
2. Permeation rate of the drug through membrane

The desired solubility of a particular drug for good oral absorption depends on permeability of the drug, and the required doses are mentioned in Table 8.1.

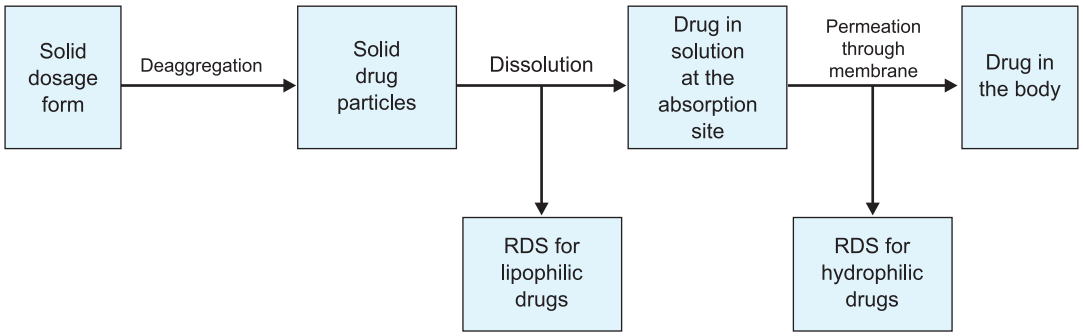


Figure 8.1: Two rate-determining steps (RDSs) in the absorption of drugs from oral administration.

Table 8.1: Desired solubility related to therapeutic doses

Dose (mg/kg)	*Desired solubility values (mg/ml) for drug with*		
	High permeability	*Medium permeability*	*Low permeability*
0.1	1	5	21
1	10	52	207
10	100	520	2100

The maximum absorbable dose (MAD) of a particular drug also depends on its solubility.

$$MAD = K_a S_{GI} V_{GI} t_r$$

where,

K_a = Intrinsic absorption rate constant
S_{GI} = The solubility of the drug in GIT fluid
V_{GI} = The volume of GIT fluid
t_r = Residence time of drug in GIT

From the above equation, the two drugs have same K_a value, but one with greater or higher solubility will have a high MAD value.

Based on the solubility and permeability of drugs, Amidon *et al.* developed biopharmaceutics classification system of drugs, which is shown in Table 8.2.

Table 8.2: Biopharmaceutics classification system for drugs

Class	*Solubility*	*Permeability*	*Absorption*	*Example*	*RDS in absorption*
I	High	High	Well absorbed	Diltiazem	Gastric emptying
II	Low	High	Variable	Nifedipine	Dissolution
III	High	Low	Variable	Insulin	Permeability
IV	Low	Low	Poor absorption	Taxol	Variable by each one (case by case)

Drug Design Related to Solubility and Membrane Permeability

We shall now look how drug design principle can be used to solve some pharmacokinetic problems faced by drugs. In this aspect, first of all we consider about how the solubility and membrane permeation of a drug can be improved. In this respect, the ionization and drug's polarity are two important considerable factors.

Drugs with high polarity do not cross cell membranes and are easily excreted, whereas non-polar drugs are poorly soluble in hydrophilic (aqueous) solution and absorbed by fatty tissues. In common, the polarity and ionization of compounds can be altered by changing the substituents. The following approaches are adopted to perform this operation:

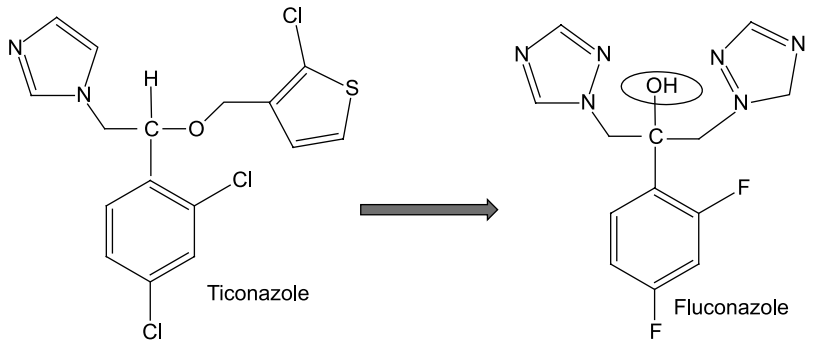
1. Varying polar functional group to alter polarity
2. Variation of N-alkyl substituents to vary pK_a
3. Variation of aromatic substituents to vary pK_a
4. Variation of acyl or alkyl substituents to alter polarity

Let us discuss each of them one by one.

1. Varying polar functional groups to alter polarity

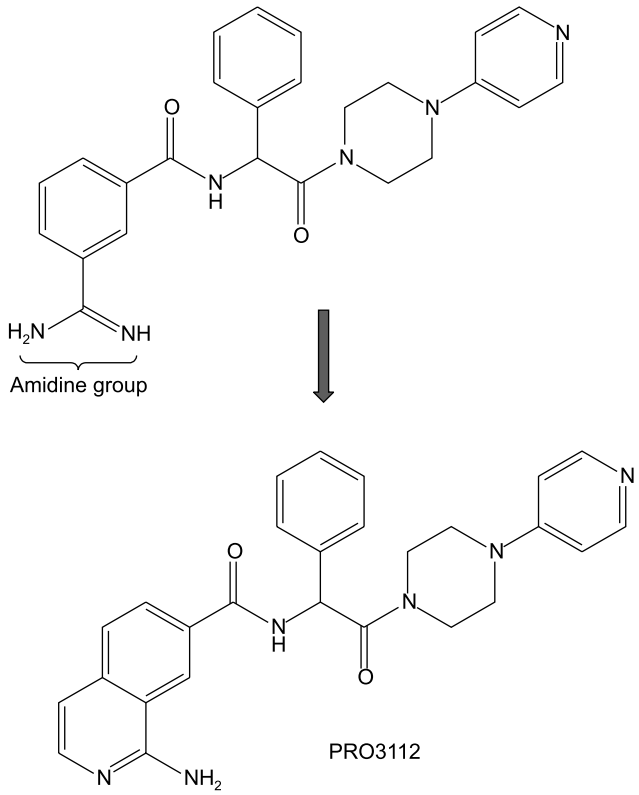
A polar functional group is added to a drug to increase its polarity, e.g. the antifungal agent ticonazole. Ticonazole is used only for skin infection because of its poor solubility in blood and non-polar nature.

The oral activity of the compound is increased by adding one polar hydroxyl group, and polar heterocyclic ring leads to the formation of an antifungal agent, fluconazole, with high solubility and enhanced (improved) activity against systemic infection.



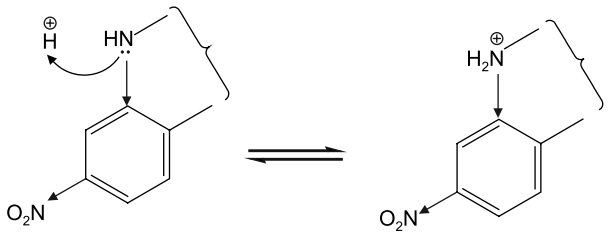
2. Variation of N-alkyl substituents to vary pK_a

Drugs having pK_a more than 6–8 are easily ionized and poorly absorbed through the membranes. However, the pK_a can be altered within the preferred range. For example, the benzamidine structure of the following antithrombic drug is too basic due to the presence of amidine group. The incorporation of isoquinoline ring reduces basicity and increases permeability.



3. Variation of aromatic substituents to vary pK_a

The pK_a value of a carboxylic acid or amine can be altered by adding electron-withdrawing or electron-donating group. For example, the antischistosomiasis drug oxaminaquine pK_a is increased by high electronegative substituent NO_2 group because a strongly electron-deficient aromatic ring pulls the lone pair of electrons present in the nitrogen atom into the ring that reduces its basicity. This, in turn, increases the pK_a, ionization and solubility of the drug totally, which increases the permeation of the drug.

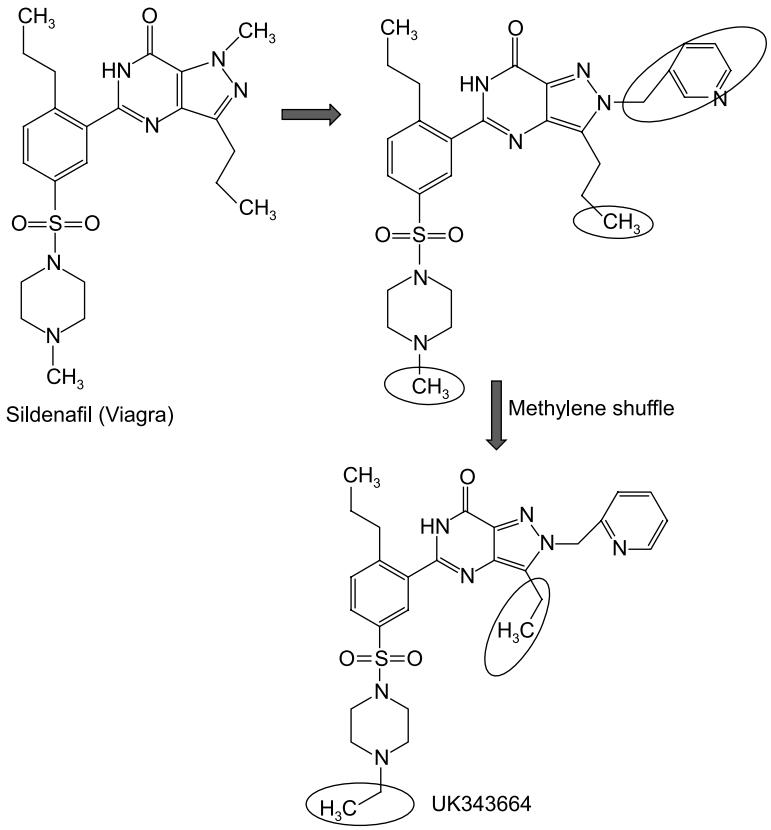


4. Variation of acyl or alkyl substituents to alter polarity

Molecules with high polarity can be converted into those with low polarity using the following ways:

- Protecting or masking a polar functional group with acyl or alkyl group
- Adding an extra large hydrophobic group

Molecules with low polarity can be converted into those with high polarity by using the opposite strategy, i.e. entirely removing the alkyl group or replacing the bulky alkyl group with smaller alkyl groups or increasing the size of another one. This strategy is known as *methylene shuffle*, which is used to increase the hydrophobicity of a compound, e.g. antiimpotence drug sildenafil (Viagra).



In the above compound, methylene shuffle is carried out in a propyl, and a methyl group results in two ethyl groups, which reduces its lipid solubility and increases the *in vivo* activity.

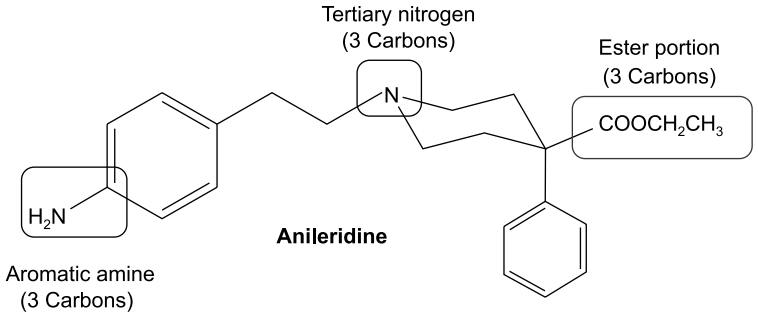
Predicting Water Solubility by Empirical Approach

The water solubility of molecules is based on carbon-solubilizing potential of the organic functional groups (Table 8.3). If the solubilizing potential of the drug or functional group exceeds the total number of carbon atoms present, then the drug is considered water soluble.

Table 8.3: Water solubilizing potential of organic functional groups

Functional group	*Monofunctional molecule*	*Polyfunctional molecule*
Alcohol	5–6 carbons	3–4 carbons
Phenol	6–7 carbons	3–4 carbons
Ether	4–5 carbons	2 carbons
Aldehyde	4–5 carbons	2 carbons
Ketone	5–6 carbons	2 carbons
Amine	6–7 carbons	3 carbons
Carboxylic acid	5–6 carbons	3 carbons
Ester	6 carbons	3 carbons
Amides	6 carbons	2–3 carbons
Urea, carbonate, carbamate		2 carbons

Let us discuss here an example of anileridine, a narcotic analgesic that contains three organic functional groups, which determine water solubility.



The three organic functional groups are (1) an aromatic amine (very weak base), (2) a tertiary alkylamine (weak base) and (3) an ester (neutral).

The compound contains 21 carbon atoms with a solubilizing potential of 9 carbon atoms. Since the solubilizing potential is less than that of the total number of carbon atoms present, the prediction would be that the drug is insoluble in water, but according to the *United States Pharmacopeia* (USP), it is soluble in water (1/10000 ml), which indicates that not only the three functional groups and solubilizing potential of 9 carbon atoms, but it also depends upon the positive charge of alkyl ammonium group.

The charge of the solubilizing potential for this group (anileridine HCl) is 29–39 carbons, which is more than that of the total number of carbon present. Hence, this compound is soluble in water.

CHAPTER 9

Partition Coefficient

The hydrophobicity of a drug can be determined experimentally by the relative distribution of the drug in an octanol–water mixture. Hydrophobic molecules prefer to dissolve in the octanol layer of this two-phase system. Hydrophilic molecules prefer the water layer. The relative distribution of drug between the two-phase systems is called *partition coefficient* (*P*).

$$P = \frac{\text{Concentration of drug in octanol}}{\text{Concentration of drug in water}}$$

Hydrophobic compounds will have a high *P* value and hydrophilic compounds will have a low *P* value.

Partition coefficient is difficult to measure *in vivo*. They are usually determined *in vitro* only by using n-octanol as a lipid phase and water or aqueous phosphate buffer (pH 7.4) as an aqueous phase. This gives the standardized measurement of *P*, since it is the ratio. *P* is an additive property and is dimensionless, so it is used to determine the polarity, and hydrophilic and lipophilic characters of the molecule.

Partition Coefficient Versus Biological Activity

Partition coefficient fully influences the drug transport characteristics during pharmacokinetic phase.

It affects the way a drug reaches the site of action. Extremely water-soluble drugs cannot penetrate the lipid barriers (blood–brain barriers), and hence cannot exhibit the CNS activity. The biological activity of anticonvulsants and general anaesthetics depend on the partition coefficients of these drugs only.

Lead compounds with different substituents yield a series of analogues of varying hydrophobicities and different *P* values. Any relationship between partition coefficient and biological activity of these drugs is determined by plotting these *P* values against the biological activity.

The biological activity is expressed as $1/C$.

Here, *C* is concentration of drug needed to produce a defined level of biological (pharmacological) activity.

Generally, the reciprocal of concentration ($1/C$) is used, since more active drugs will produce particular biological activity at low concentration.

The graph is drawn by plotting $1/C$ versus log *P*.

For the log *P* values between 1 and 4, a straight line graph is obtained (Figure 9.1).

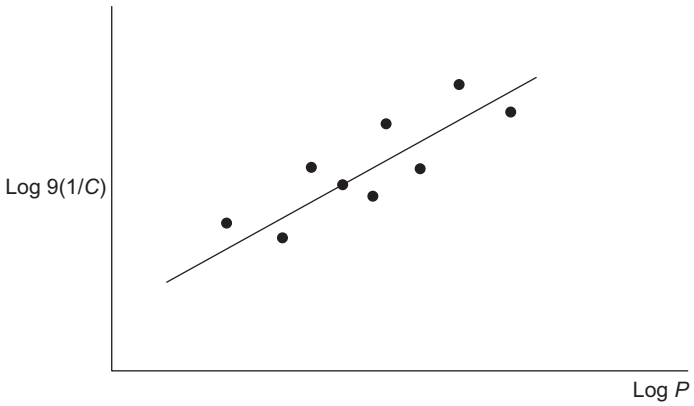


Figure 9.1: Biological activity versus partition coefficient.

It indicates the relationship between hydrophobicity and biological activity, e.g. binding of serum albumin.

The binding of serum albumin is determined by their hydrophobicity. A study of 40 compounds results in the following equation:

$$\text{Log } (1/C) = 0.75 \log P + 2.30$$

The above equation indicates that increase of log *P* increases serum albumin-binding capacity.

Compounds containing the log *P* values from 0.78 to 3.82 have restricted serum albumin binding.

When we plot the values, a parabolic graph is obtained (Figure 9.2). In the graph shown below, the biological activity increases as log *P* increases until a maximum value is obtained.

In the graph shown below, log P° = Optimum partition coefficient for biological activity. Beyond this point, an increase in log *P* results in decrease in biological activity.

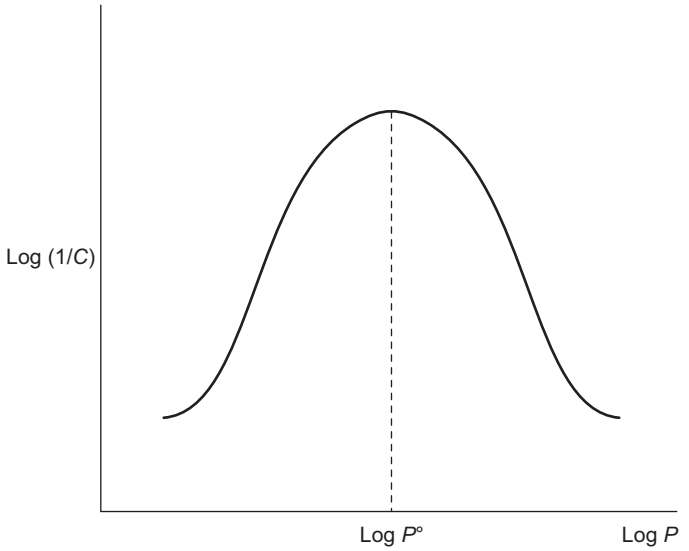
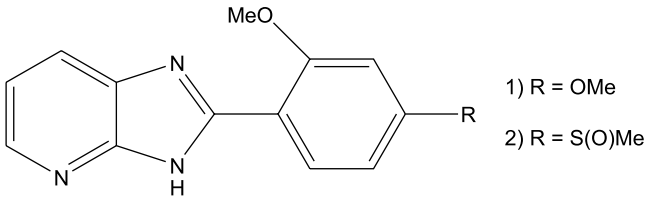


Figure 9.2: Parabolic log (1/C) versus log *P*.

Examples for Drugs in which Biological Activity is Related to the Log *P* Factor Alone

1. **General anaesthetics:** The main mechanism of general anaesthetics is entering the CNS and dissolving into cell membranes where it affects membrane structure and nerve function. In this condition, the action of drug is purely controlled by its hydrophobicity. From the series of QSAR studies, the optimum hydrophobicity (log P°) value for anaesthetic activity is found to be around 2.3.
2. Compounds having a log *P* value close to 2 should be capable of entering into CNS efficiently. For example, the log *P* value of potent barbiturates for sedative and hypnotic activity is close to 2.

The cardiotonic agent sulmazole, which enters the CNS due to log *P* value of 2.59, was found to produce 'bright visions' in some patients.



Sulmazole [R = S(O)Me]

To prevent the entry of drug into CNS, the 4-methoxy group was replaced by 4-S(O)Me group with a low log *P* value of 1.17. Now, the drug is too hydrophilic, hence unable to enter the CNS and was free of CNS side effects.

From the above examples, we conclude that the partition coefficient is a measure of the drug's overall hydrophobicity, and it is an important measure of how efficiently a drug is transported to its target's site.

Determination of Partition Coefficient

The determination of partition coefficient is a time-consuming procedure and depends on the stability and purity of the particular drug. One of the most popular method or approach has been HPLC or TLC.

In this case, the mobile phase usually contains some water-miscible organic solvent. The stationary phase is non-polar by using octadecylsilane, mineral oils or coating of octanol.

Determination of Partition Coefficient through Regression Analysis by Using the Following Equation

Log $P = a$ (log retention) + C

a = Regression coefficient or slope of the straight line

C = The intercept terms on the y-axis (physical and chemical properties equal to 0.)

This method or model has the following two limitations:

1. Partition coefficient of related compounds is same.
2. The retention in chromatographical system.

Determination of Partition Coefficient on the Basis of Atomic Components of the Molecule

According to this model, each atom type is assumed to contribute a fixed amount to the chemical partition coefficients, e.g. cyclohexane. The major disadvantage of this model is that it needs several of correction factors.

There are several methods or commercial drug design software packages that contain modules determining chemical partition coefficient.

CHAPTER 10

Steric Features of Drugs

INTRODUCTION

The potential biological activity of a targeted drug molecule solely depends on its physicochemical characteristics and essentially comprises the nature and type of functional moieties and also the spatial arrangement of such groups in the molecules. Interestingly, the human body itself represents an asymmetric environment, wherein drug molecules interact with proteins and biological macromolecules (receptors). Hence, it is virtually important and necessary that the decisive functional moieties must be strategically located with respect to exact spatial region circling the targeted drug molecule, so as to enable the crucial and productive bonding interactions, particularly with the receptor, thereby potentially accomplishing the desired pharmacological effect. It is, however, pertinent to state here that the right fitment of correct 3D-orientation of the functional moieties in a drug substance may ultimately result in the formation of an extremely viable and reasonably strong interaction with its receptor.

Steric factors determined by the stereochemistry of the receptor site surface and that of the drug molecules are, therefore, of primary importance in determining the nature and the efficiency of the drug–receptor interaction. The drug must approach and fit closely into the receptor surface to evoke the pharmacological action. Hence, the drug must possess a high degree of structural specificity or stereoselectivity. Many drugs show stereoselectivity because mostly receptor binds are optically active biological macromolecules, such as proteins, polynucleotides, or glycolipids.

GEOMETRICAL ISOMERISM

Isomerism caused by the difference in spatial arrangement of groups about the double bonded carbon atoms is known as geometrical isomerism. Due to the hindered rotation, the positions of different groups attached to the two carbon atoms are fixed in space. Two different compounds are distinguished by the terms *cis* and *trans*.

Cis Isomer

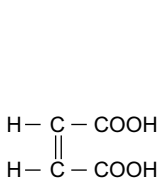
In this isomer, similar groups lie on the same side and it is known as *cis* isomer (in Latin term *cis* means—same side).

Trans Isomer

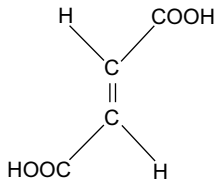
In this isomer, similar groups lie on opposite side and is known as *trans* isomer (in Latin term *trans* means—across).

Due to *cis* and *trans* isomers, geometrical isomerism is otherwise known as *cis-trans* isomerism.

Example: Maleic acid and fumaric acid possess same molecular formula of $C_4H_4O_4$, but they differ each other in most of their physical properties and some of their chemical properties.



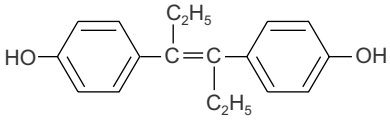
Cis form (maleic acid)



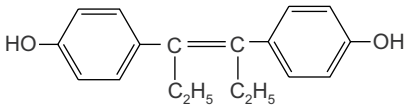
Trans form (fumaric acid)

The two isomers exhibit different pharmacological response due to their orientation in the body enzymes or receptors. Some of the examples are described below.

Diethylstilbestrol exists in two fixed stereoisomeric forms—*trans*-diethylstilbestrol is oestrogenic, whereas *cis* isomer is only 7% active. In *trans*-diethylstilbestrol, resonance interaction and minimal stearic interference tend to hold the two aromatic rings and connecting ethylene carbon atom in the same plane.



Trans-diethylstilbestrol

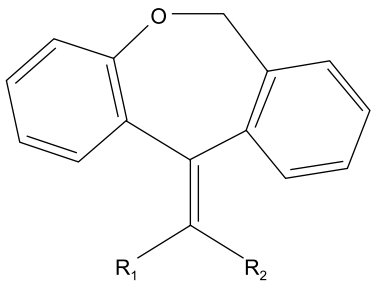


Cis-diethylstilbestrol

In geometric isomers, *cis* and *trans* isomers differ in their physical and chemical properties. Therefore, distribution in the biological medium is different.

The geometric isomers (E–Z forms) of some drugs are found to be pharmacologically active and are approved for various therapeutic uses as shown below.

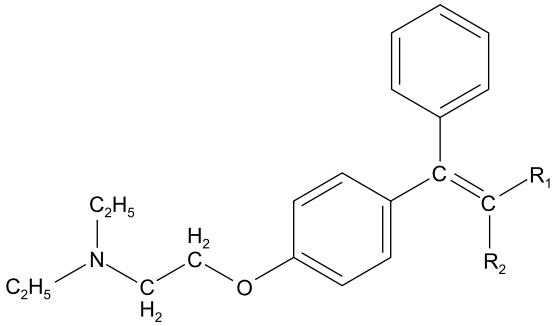
Doxepin



Z-Doxepin: $R_1 = CH_2CH_2N(CH_3)_2$, R_2 = H; ***E*-Doxepin:** R_1 = H, $R_2 = CH_2CH_2N(CH_3)_2$

Used as a mixture of E–Z isomers in the ratio of 85:15 (approximately) for treatment of insomnia.

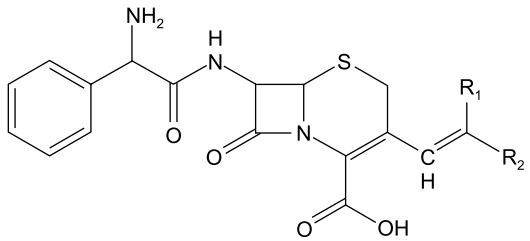
Clomiphene



***Z*-Clomiphene:** R_1= C_6H_5, R_2 = Cl; ***E*-Clomiphene:** R_1 = Cl, R_2 = C_6H_5

It contains the mixture of E–Z isomers and used as an orally active non-steroidal agent.

Cefprozil



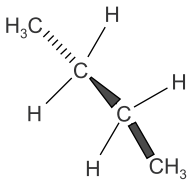
***Z*-Cefprozil:** R_1= H, R_2 = CH_3; ***E*-Cefprozil:** R_1 = CH_3, R_2 = H

It is a cephalosporin antibiotic exhibits E–Z isomerism, used to treat bronchitis, skin and ENT infections.

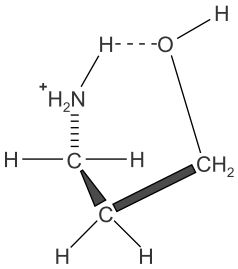
Conformational Isomers

Different arrangements in the space for atoms or groups in single bonds are called *conformations*. Rotations about the bonds allow interconversion of conformers (conformational isomers). The energy barrier between isomers is often high enough for their independent existence and reaction. Differences in the reactivity of functional groups or interaction with biological receptors may be due to differences in steric requirements of the receptors.

Open chains of atoms form an important part of many drug molecules. Energy barrier to the free rotations of the chains is present because of the interactions of non-bonded atoms; for example, the atoms tend to position themselves in space so that they occupy staggered positions with no two atoms directly facing each other (eclipsed). Non-bonded interactions in polymethylene chains tend to favour the most extended anticonformations, although some partially extended gauche conformations also exist. The conformational isomers show significant differences in biological activities.

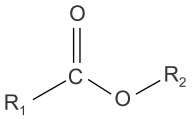


n-Butane anticonformation

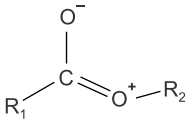


3-Amino-*n*-propanol eclipsed conformation

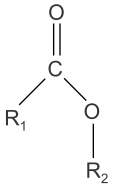
Stabilized planar structure of esters



Antistablized

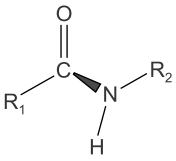


Resonance stabilized

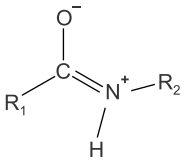


Gauche

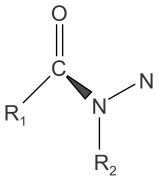
Stabilized planar structure of amides



Antistabilized

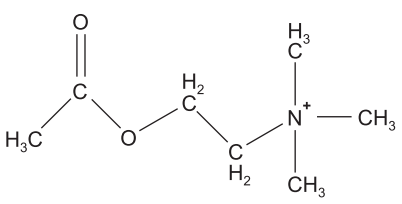


Resonance stabilized

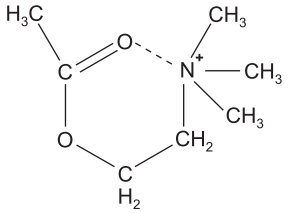


Gauche

The potential interaction energy of trimethyl ammonium ion and acetoxy group is lowest in the staggered (also called, though erroneously, *trans* or *transoid*) conformation, and highest when the two groups are eclipsed (*cis* or *cisoid* conformation). It has been suggested that acetylcholine interacts with the muscarinic receptor in fully extended staggered conformation and interacts with nicotinic receptor in folded (gauche) conformation. To study the relationship between the possible conformations of rigid analogues of acetylcholine and their biological effects conformationally rigid analogues of acetylcholine have been used. The *cis* and *trans* isomers of 2-acetoxy cyclopropyl trimethyl ammonium iodide are two such compounds. The (+) – *trans* isomer in which the quaternary nitrogen atom and acetoxy groups are held apart in a shape approximating that of extended conformation of acetylcholine was found to be almost equipped with acetylcholine at the muscarnic receptor, but shows little nicotinic activity. It is easily hydrolyzed by acetyl cholinesterase. In contrast (+) – *cis* isomer showed practically no activity at the nicotinic or muscarinic receptor. The results indicate that acetylcholine assumes staggered conformation at the muscarinic receptors.

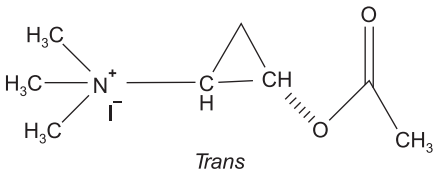


Extended

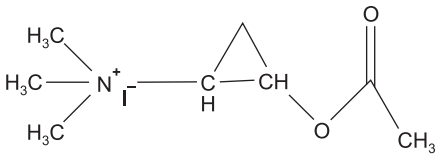


Quasi-ring

Acetylcholine



Trans



Cis

2-Acetoxy cyclopropyl trimethylammonium iodide

Acetylcholine conformation

OPTICAL ISOMERISM

When a plane polarized light passes through a solution of chiral compound, plane of polarization changes because the molecules are asymmetric. Hence, a chiral compound rotates plane polarized light either clockwise or anticlockwise direction. A compound which rotates a plane polarized light is said to be optically active. In other words chiral compounds are optically active and achiral compounds are optically inactive.

Dextrorotatory: If an optically active compound or chiral compound rotates the plane polarized light in a clockwise direction, it is known as dextrorotatory and indicated by the symbol (+).

Levorotatory: If an optically active compound or chiral compound rotates the plane polarized light in anticlockwise direction, it is known as levorotatory and indicated by the symbol (–). 'Dextro and levo' are Latin prefixes for 'to the right' and 'to the left', respectively.

While naming stereochemical compounds, the D, L, R, S indicates stereochemical relation (structural features) and the (+) or (–) indicates actual direction of rotation of plane polarized light.

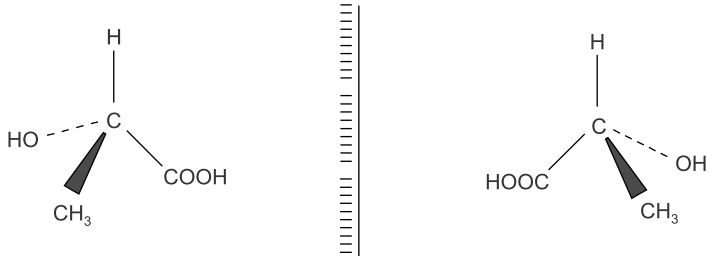
Note: Do not get confuse (+) and (–) with R and S or D and L. The (+) or (–) symbol indicates the direction in which a chiral compound rotates a plane polarized light. But R and S or D and L indicate the arrangement of the groups around a chiral centre.

Enantiomers

If four different atoms or groups are attached at the four corners of a regular tetrahedron, then the molecule is asymmetric and can exist in two forms. The three-dimensional structures cannot be superimposed on each other, and hence, are different, even though they represent the same structural arrangement of

atoms. They bear a relationship to each other corresponding to what exists between an object and its mirror image. Mirror image molecules are not superimposable and are called *enantiomers*.

A tetrahedral carbon atom carrying four groups that are all different, therefore, must invariably constitute a centre of asymmetry and permits two arrangements of the groups in space. This asymmetry calls for the existence of two isomers identical in all respects except optical properties, for example lactic acid and 2-hydroxy propanoic acid.



(+)-Lactic acid Mirror (–)-Lactic acid

A chiral compound containing one asymmetric centre has two enantiomers. Although each enantiomer has identical chemical and physical properties, they may have different physiological activities such as the interaction with receptor, metabolism, and protein binding.

Examples of enantiomers possessing varying biological activity are the following:

(S)(+)-Naproxen sodium
Active as analgesic, antipyretic and anti-inflammatory

(R)(–)-Naproxen sodium
(inactive)

Many optical isomers exhibit variation in the intensity of their biological properties. For example,

(–)-Hyoscyamine is 15–20 times more active as a mydriatic than (+)-hyoscyamine.

(–)-Hyoscine is 16–18 times as active as (+)-hyoscine.

(–)-Epinephrine is 12–15 times more active as vasoconstrictor than (+)-epinephrine.

(–)-Isoprenaline is 800 times more active bronchodilator than (+)-isoprenaline.

(+)-Nor homoepinephrine is 160 times more active as a pressor than (–)-nor homoepinephrine.

(+)-Amino acids are sweet, whereas (–)-amino acids are either sweetless or bitter.

(+)-Ascorbic acid has good antiscorbutic properties, whereas (–)-ascorbic acid has none.

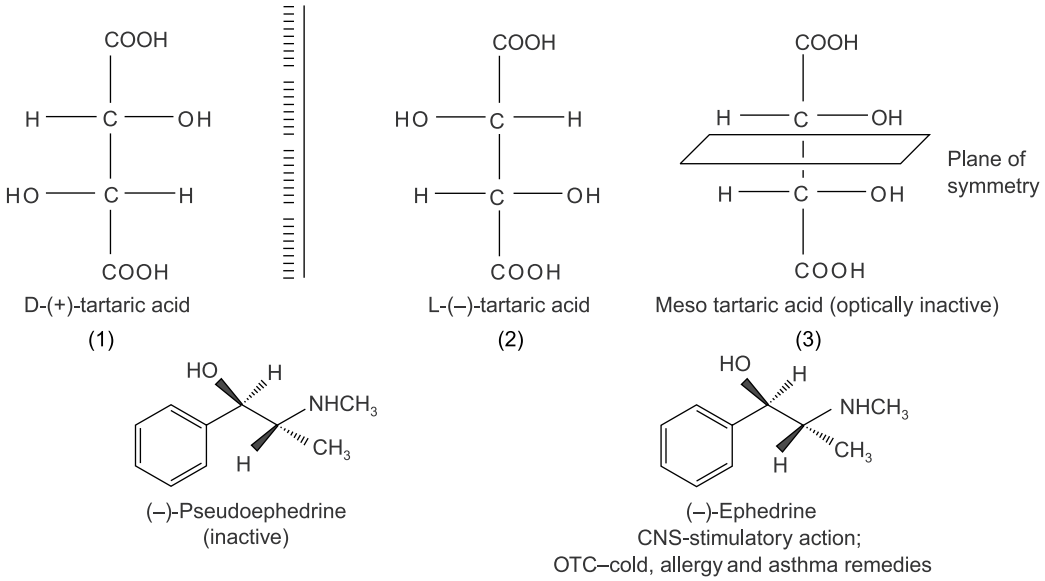
(S)-Thalidomide is more teratogenic than (R)-thalidomide.

Diastereomers

Tartaric acid is an example of a compound having two similar asymmetric carbon atoms since each carbon atom has attached hydrogen atom, a hydroxyl group, a carboxyl group (CH OH COOH group).

If we represent the configurations of two mirror images about each asymmetric carbon atom, then the structures are represented as enantiomers of D- and L-tartaric acid.

A third structure is possible, which possesses a plane of symmetry (achiral) and is, therefore, optically inactive. Such molecules are designated as *mesoforms*. Stereoisomers (1) and (2) are enantiomers, but (1) and (3) are not enantiomers, they are called *diastereomers*. These stereoisomers are not mirror images, unlike enantiomers, but diastereomers possess different physical and chemical properties. Examples of diastereomers possessing varying activity are the following.



Reasons Behind Varying Activity of Optical Isomers

The difference is due to the interaction of asymmetric carbon atom of the molecule with stereospecific receptors. According to Easson-Stedman hypothesis, if binding ions are specific for one enantiomer, then a three-point attachment must occur between the enantiomer and the asymmetric surface of the receptor, since only one of the entantiomers will fit, and the other one is only capable of a two-point attachment, as shown in Figure 10.1.

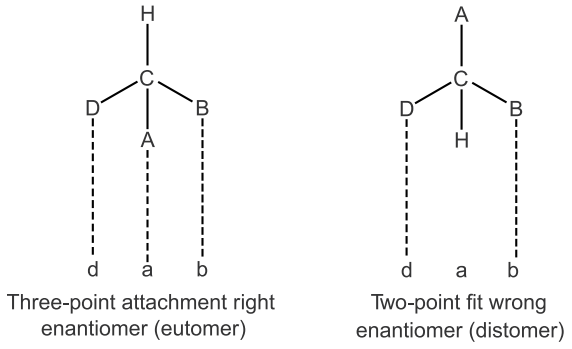


Figure 10.1: Varying activity of optical isomers.

Binding of Enantiomers to Receptors

The enantiomer (Figure 10.2) that has a high affinity for receptor is called *eutomer*, whereas the one with a lower affinity is called *distomer*. The ratio of activity of the eutomer and distomer is called *eudismic ratio*.

Examples of Easson-Stedman principle: For epinephrine, the benzene ring, benzylic hydroxyl, and protonated amine bind with the hydrophobic or aromatic region, anionic site, and hydrogen bonding at the centre of the receptor.

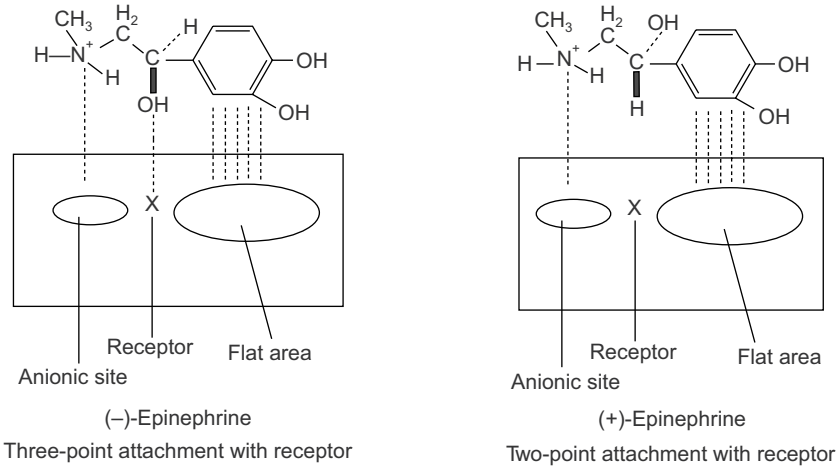


Figure 10.2: Binding of epinephrine with receptor.

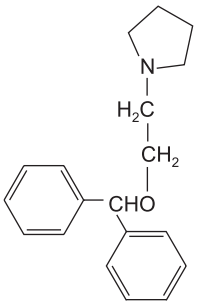
CHAPTER 11

Bioisosterism

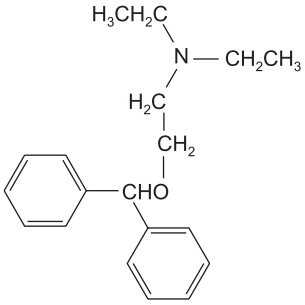
INTRODUCTION

Isosterism is of vital importance to the medicinal chemists because the biological characteristics of isosteres appear to be similar, more frequently than physical or chemical characteristics. Keeping in view the numerous advantageous application of isosterism in resolving biological problems effectively, Friedman proposed the following definition of bioisosterism—the phenomenon by which compounds usually fit the broadest definition of isosteres and possess the same type of biological activity.

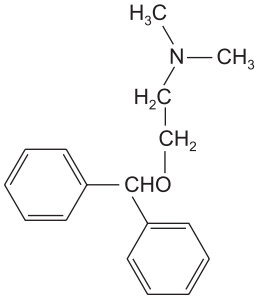
For instance, among antihistamines it is always preferable to have small compact substituents on the terminal nitrogen.



(a) Pyrrolidone analogue



(b) Diethyl analogue



(c) Dimethyl analogue

In the above-mentioned three-structural analogues, it has been observed that 'A' possesses twice the activity of 'C', whereas it showed an activity that is many times greater than that of the open-chain diethylamino analogue.

It has been duly observed that it is more or less difficult to correlate the biological properties vis-a-vis physicochemical properties inherited by specific individual atoms, functional groups, or entire molecule by virtue of the glaring and established fact that a host of physical and chemical parameters are involved simultaneously, and therefore, it is extremely difficult to quantify them justifiably.

Besides simpler relationships, for example, isosterism invariably does not delay across the several varieties of biological systems that are often encountered with medicinal agents. In other words, a specific isosteric replacement in one particular biological system may either work or fail in response to the other. Thus, bioisosteres were further explained as follows: Bioisosteres are (functional) groups or molecules that have chemical and physical similarities producing broadly similar biological properties.

CLASSIFICATION

Bioisosteres are classified into the following two types:

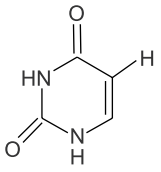
1. Classical bioisosteres
2. Non-classical bioisosteres

Classical Bioisosteres

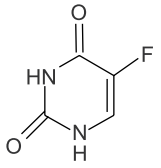
Classical bioisosteres have similarities in shape and electronic configuration of atoms, groups, and molecules, that they replace. Actual applications of bioisosteres in the successful design of a specific given molecule interacting with particular receptor is one glaring example, and very often either fails or negates the biological characteristics in another environment. Therefore, it is pertinent to state at this juncture that the logical use of biological replacement (classical or non-classical) in the design of a new target drug molecule is solely and significantly depend on the specific biological system under critical investigation. Hence, there are no predetermined, well-established, predictable hard and fast guidelines, or laid generalized rules that may be useful to a medicinal chemist to affect biosteric replacement gainfully towards improved biological activity. Various classical bioisosteres with their appropriate examples are listed as follows:

1. Monovalent atoms and groups

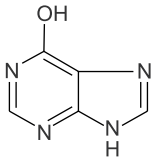
- F, H
- OH, NH
- F, OH, NH, or CH_3 for H
- SH, OH
- Cl, Br, and CF_3



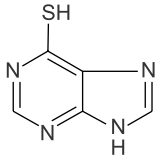
Uracil



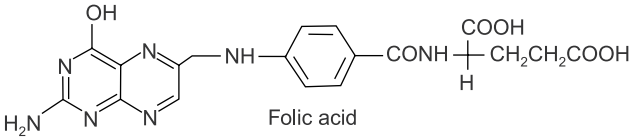
5-Fluorouracil



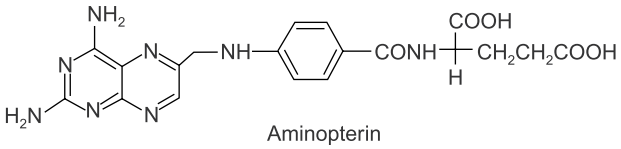
Hypoxanthine



6-Mercaptopurine



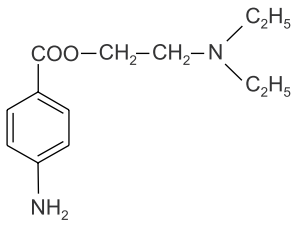
Folic acid



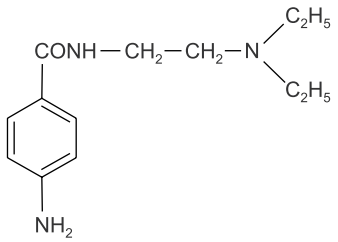
Aminopterin

2. Divalent atoms and groups

–C=S, –C=O, –C=NH, –C=C–



Procaine



Procainamide

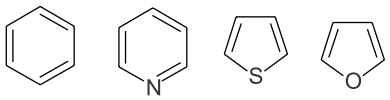
3. Trivalent atoms and groups

–CH=, –N=, –P=, –As=

4. Tetravalent atoms and groups

$=N^{+}=$, =C=, $=P^{+}=$, $=As^{+}=$

5. Ring equivalents

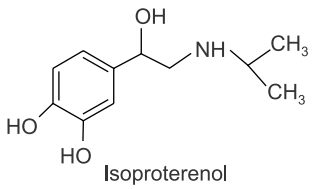


Non-classical Bioisosteres

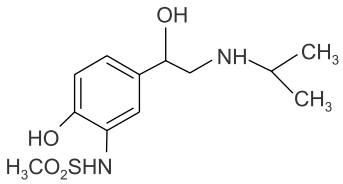
They do not obey the steric and electronic definition of classical isosteres. Also, they do not have the same number of atoms as replacement. Although, many of these functional moieties practically just behave as one, they have one of the following characteristic features, such as

- Electronic properties
- Physicochemical properties
- Spatial arrangements
- Functional moiety critical for biological activity

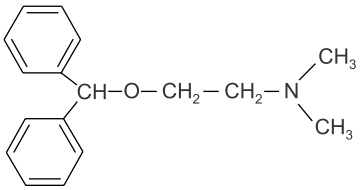
1. Exchangeable groups



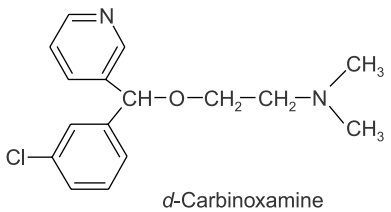
Isoproterenol



Soterenol

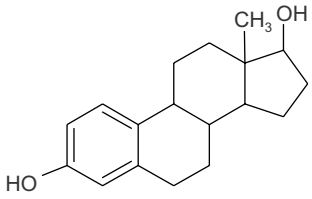


Diphenhydramine

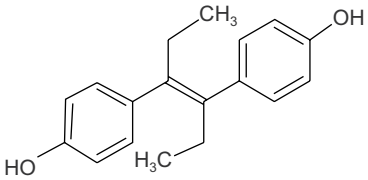


d-Carbinoxamine

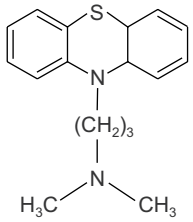
2. Cyclic versus non-cyclic structure



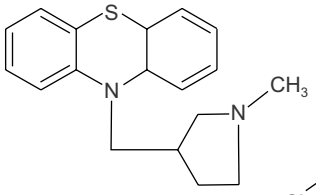
Estradiol



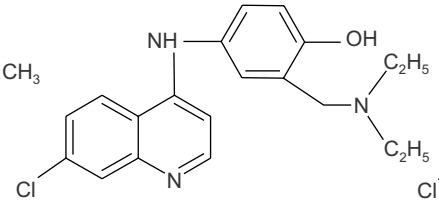
Diethylstilbestrol (*trans*)



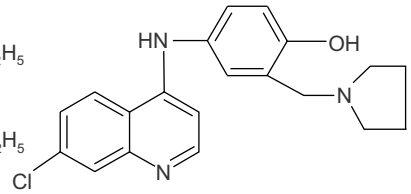
Promazine



Methdilazine



Amodiaquine



Amopyroquine

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CHAPTER 12

Protein Binding of Drugs

Once the drug enters into the systemic circulation, it undergoes various binding events. It may be present in solution, but majority of drugs bind with various proteins such as albumin, etc. The drug can interact with various tissue components such as blood and extravascular tissues. The interacting molecules are DNA or adipose tissues.

"The phenomenon or process of formation of complex with proteins is known as protein binding of drugs."

CLASSIFICATION OF PROTEIN BINDING

1. Intracellular binding
2. Extracellular binding

Intracellular Binding: In this process, the drug is bound to the cell proteins such as drug receptors which produces the pharmacological response. This kind of receptors are called primary receptors.

Extracellular Binding: In this process, the drug is bound to the extracellular proteins but does not elicit any pharmacological response. This kind of receptors are called secondary receptors or silent receptors. The secondary receptors are plasma proteins.

MECHANISM OF PROTEIN–DRUG BINDING

Binding of drugs to the proteins is a reversible reaction and involves weak chemical bonding. The different types of bond occur between the drugs and the proteins are as follows.

1. Hydrogen bonds
2. Hydrophobic bonds
3. Ionic bonds
4. Van der Waals forces

Irreversible binding of drugs leads to the carcinogenicity or tissue toxicity. For example, irreversible covalent binding of drug paracetamol to the liver tissue leads to hepatotoxicity.

Binding of drugs is categorized into two classes:

I. Binding of drugs into blood components such as
   i. Plasma proteins
   ii. Blood cells
II. Binding of drugs into extravascular tissues.

Let us discuss in detail.

I. Binding of Drugs into Blood Compartments

Once the drug reaches the systemic circulation, first it can interact with plasma proteins, blood cells and haemoglobin. Due to the abundant or huge amount of plasma proteins, the drug mainly interacts with these proteins. The process of drug binding to the plasma proteins is reversible one. The order of binding of drug to the plasma proteins is as follows:

Albumin > α_1-Acid glycoprotein > Lipoproteins > Globulins

The process of protein binding involves the following steps:

1. Binding of drugs to human serum albumin (HSA)
2. Binding of drugs to α_1-acid glycoprotein (AAG)/binding of drugs to orosomucoid
3. Binding of drugs to lipoproteins
4. Binding of drugs to globulins
5. Binding of drugs to blood cells

1. Binding of Drugs to HSA

It has the largest amount of plasma proteins and has the capacity to bind with maximum number of drugs. Most of the drugs as well as the endogenous substances such as tryptophan, bilirubin, etc. can also bind with HSA. HSA contains four different drug-binding sites as mentioned below.

- **Site-I or Warfarin and azapropazone binding site:** In this site, large number of drugs interact and bound. Example for drugs binding to these sites are various kind of NSAIDs (indomethacin, naproxen, etc.) sulphonamides, phenytoin and bilirubin.
- **Site-II or Diazepam binding site:** Example for drugs binding to this region are ibuprofen, probenecid, cloxacillin, benzodiazepines, etc.
- **Site-III or Digitoxin binding site and site -IV or tamoxifen binding site:** Site-I and II are the most important drug binding sites for maximum number of drugs. Very few number of drugs are binding to the site-III and IV. If the drug is bound to more than one site, then majority binding site of the drug is called primary binding site or the other site is known as secondary binding site.

2. Binding of Drugs to α_1-Acid Glycoprotein (AAG)/Binding of Drugs to Orosomucoid

The plasma concentration range of this protein is 0.04 to 0.1g. The examples for drug binding to this protein are imipramine, propranolol, lidocaine (basic drugs), etc.

3. Binding of Drugs to Lipoproteins

Lipoproteins are classified into four types according to their density. They are:

1. Chylomicrons (least dense and largest in size)
2. Very low-density lipoproteins (VLDL)
3. Low-density lipoproteins (LDL) (mostly present in humans)
4. High-density lipoproteins (HDL) (denser and smallest in size)

The molecular weight of lipoproteins ranges from 2 lakhs to 34 lakhs depending upon their chemical compositions. The main role of lipoproteins is the transport of lipids from the circulation to the tissues. They also transport or circulate the drugs to the tissues. Binding of drugs to the lipoproteins is a non-competitive process, i.e. there is no specific or absence of specific binding site. Binding of drugs do not depend upon the concentration. Examples of drug bound to the lipoproteins are acidic drug—diclofenac, basic drug—chlorpromazine and the neutral drug such as cyclosporine A.

4. Binding of Drugs to Globulins

The various types of globulins are α_1, α_2, β_1, β_2, and γ-globulins.

1. **α_1-globulins or transcortin or corticosteroid binding globulin:** Steroidal drugs like cortisone, prednisone and thyroxin, cyanocobalamine are the examples of the drugs which bind to the globulins.
2. **α_2-globulin or ceruloplasmin:** Fat soluble vitamins A, D, E, K and cupric ions bind to this site.
3. **β_1-globulin or transferring:** Ferrous ions bind to this site.
4. **β_2-globulins:** Carotenoids bind to this site.
5. **γ-globulins:** Antigens bind to this site.

5. Binding of Drugs to Blood Cells

The major cellular component in the blood is RBC cell. Hence, the drugs majorly bind with RBC cells only. The three major components of RBC cells to which drugs bind are:

1. **Haemoglobin:** Drugs like phenytoin, phenobarbital and phenothiazine bind to this.
2. **Carbonic anhydrase:** Carbonic anhydrase inhibitors such as acetazolamide and chlorthalidone are drugs which bind to this.
3. **RBC cell membrane:** Drugs like chlorpromazine and imipramine bind to the RBC cell membrane.

Note: Highly lipophilic drugs bind to RBCs than hydrophilic drugs such as ampicillin, etc.

II. Binding of Drugs to Extravascular Tissues

Some kind of drugs possess special affinity towards certain tissue components. Hence, it increases the duration of action of the drug and decreases the elimination of drug.

Importance of Tissue–Drug Binding

1. It increases the apparent volume of distribution of drugs.
2. It leads to the localization and accumulation of drug into specific site of the body.

Factors Influencing the Tissue–Drug Binding

1. Structural features of the drug
2. Lipophilicity or hydrophobicity of the drug
3. pH difference
4. Perfusion rate

The extensive binding of drugs to the tissues leads to storage of that drugs in that site.

The order of binding of drugs into tissue is as follows.

Liver > kidney > lungs > muscles

Some examples of drugs which bind to the various tissues are as follows (Table 12.1).

Table 12.1: Drugs which bind to the various tissues

S. No.	*Tissues in which drugs to be bind*	*Drugs*	*Responses*
1.	Liver	Paracetamol, halogenated hydrocarbons	Covalent binding leads to hepatotoxicity
2.	Lungs	Antihistamines, imipramine and chlorpromazine	—
3.	Kidney	Lead, mercury and cadmium bind with the metallothionin	Toxicity
4.	Skin	Phenothiazine and chloroquine bind with the tissue by interaction with melanin	—
5.	Eyes	Phenothiazine and chloroquine bind with the tissue by interaction with melanin	Retinopathy
6.	Hairs	Arsenicals, chloroquine	—
7.	Bones	Tetracyclines	Discoloration of teeth
8.	Fats	Thiopental and DDT	—
9.	Nucleic acids	Chloroquine and quinacrine	Distortion of double helical structure

Factors Influencing Drug–Protein Binding

Various factors affect the binding of drugs to the proteins. They are described as follows:

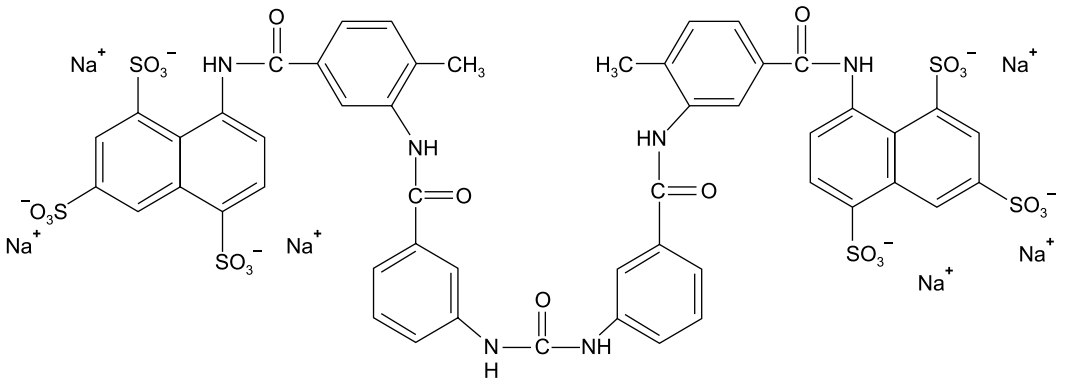
1. **Drug-related factors**
   i. Physicochemical properties of the drug
   ii. Drug concentration
   iii. Drug affinity to the particular binding site
2. **Protein- or tissue-related factors**
   i. Physicochemical properties of the protein
   ii. Protein or drug binding component concentration
   iii. Number of binding sites on the protein or binding agent
3. **Patient-related factors**
   i. Age
   ii. Disease condition
   iii. Intersubject variations
4. **Drug interactions**
   i. Competition between drugs for the binding sites or proteins
   ii. Competition between drugs and the normal body constituents
   iii. Allosteric variations or changes in the binding proteins

Applications of Protein Binding

1. The albumin–drug complex acts as a reservoir to provide the required concentration of drug to elicit pharmacological response.

2. It may also limit the access to body compartments. The placenta prevents the entry of drugs from maternal to fetal circulation. Protein binding prevents the normal passage through placental barrier and produce toxicity to that area in the maternal circulation and bound to the mother's serum protein.
3. The protein binding of drugs prolongs or increases the duration of action of drugs. The drug–protein complex is too long and big, large to pass via the renal globular membranes that prevents the rapid excretion of drugs.
4. It also prevents the metabolism of drugs and the interaction of drugs with receptor site.

   For example, the drug suramin is used to treat sleeping sickness remains in the body in the form of protein bound as long as 3 months. The maintenance dose of the drug is administered once in a week. It produces advantages to the patient and at the same time leads to several side effects.



Suramin sodium

5. The protein binding of drugs phenomenon leads to some clinically important drug–drug interactions. Hence, one drug displaces another one from its binding site on albumin. For example, anticoagulant drug warfarin is displaced from its binding site albumin by various kind of drugs. This increases the concentration of warfarin at receptors, leading to increase in prothrombin time and severe haemorrhage.

CHAPTER 13

Biotransformation or Metabolism of Drugs

INTRODUCTION

The biochemical process of conversion of drugs or xenobiotics in the body is called biotransformation or metabolism. During this process, the drugs are converted into water soluble conjugates and eliminated through kidney. Body treats drugs as a xenobiotics. Hence, metabolism plays a peculiar role in the elimination of drugs and xenobiotics. A well-known understanding of metabolic pathway of drugs is an important tool for the pharmacist in selection and monitoring appropriate drugs in the treatment for their patients.

Importance of Metabolism

Most of the organic drugs are highly lipophilic in nature, when they are administered via oral route, they cross the lipoprotein membranes of GIT and reach the bloodstream. Once the drugs enter into various target organs, elicit their pharmacological response. Due to reabsorption in the renal tubule, the lipophilic drugs are not excreted from the body via urine. Hence, they undergo various changes by metabolic enzymes and render them to be more water soluble one, and then eliminated.

If the lipophilic xenobiotic is not metabolised to polar, readily excreted hydrophilic products, then they would remain in the body which leads to the development of toxic effects. It indicates that the conversion of lipid soluble xenobiotics into highly hydrophilic compounds not only increases the drug metabolism but it also leads to the compounds that are pharmacologically inactive metabolites and non-toxic compounds. Hence, the drug metabolism is also known as **detoxification.**

During the metabolism not all drugs are converted into the inactive form. The consequences of drug metabolism are as enumerated below.

1. Conversion of drugs into pharmacologically active metabolites, e.g. morphine to codeine
2. Conversion of inactive xenobiotics into active metabolite (prodrug), e.g. levodopa, bacampicillin, prednisone, etc.
3. Conversion of active drug into inactive metabolite, e.g. ibuprofen, lignocaine, etc.
4. Conversion of active drug into toxic metabolites, e.g. paracetamol, cyclophosphamide, etc.

General Pathways of Drug Metabolism

Drug metabolism reactions are broadly classified into two types:

1. Phase-I reactions or functionalisation reactions
2. Phase-II reactions or conjugation reactions

Phase-I or Functionalisation Reactions

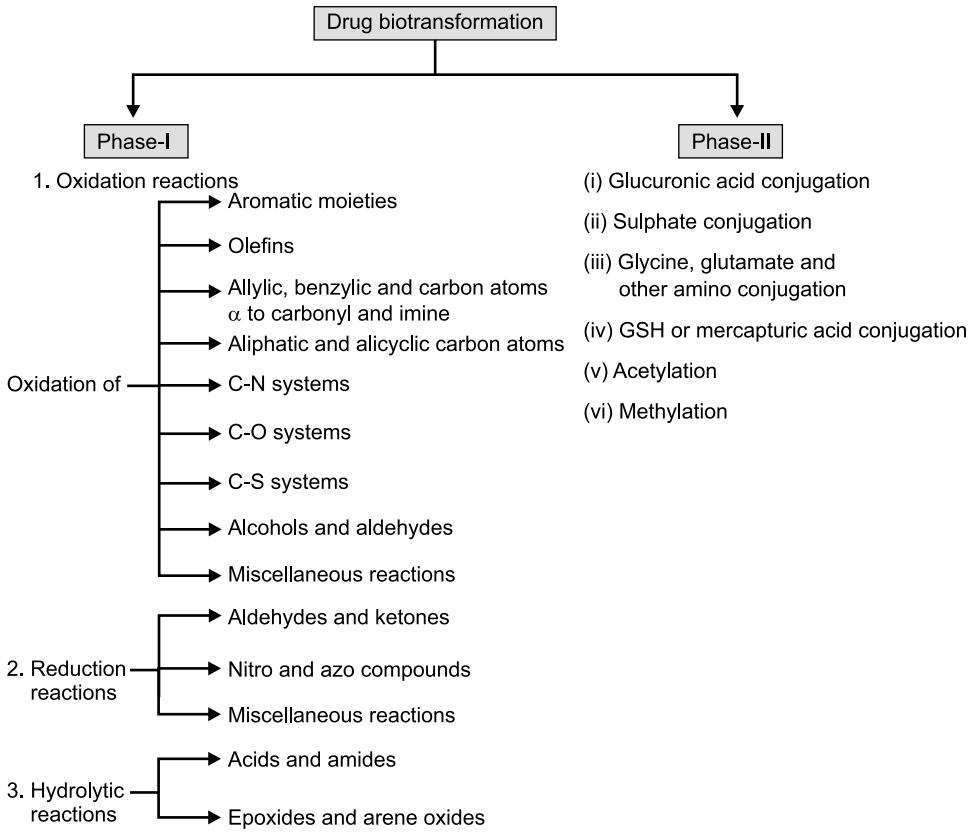
The main principle of this reaction is to introduce or attach polar functional groups such as –OH, –COOH, $–NH_2$ to the xenobiotic and convert them into a highly water-soluble compounds. This can be performed or achieved by two ways:

1. Direct introduction of the functional group by aromatic or aliphatic hydroxylation.
2. Modifying or masking the already existing functional groups by the reactions like oxidation of alcohols to acids, hydrolysis of ester and amides, reduction of aldehydes and ketones to alcohols, etc.

The phase-I reactions involve hydroxylation, oxidation, reduction and hydrolytic transformation reactions.

Phase-II Reactions or Conjugation Reactions

These reactions help to attach a small, polar and easily ionizable endogenous substances such as glucuronic acid, sulphates, glycine and other amino acids into the functional group of phase-I metabolites or parent compounds that already possess suitable functional moieties to form highly water-soluble conjugate products. These conjugates generally does not possess any pharmacological activity and are non-toxic to human being. These are readily excreted by urine. The other phase-II reactions involve acetylation and methylation which does not increases the water solubility but attenuate or terminate the pharmacological activity, whereas glutathione conjugation protects the body from the effect of chemical reactive conjugates or metabolites.



Phase-I and phase-II reactions are competent to one another, by this way detoxifying and facilitating the elimination of drugs and xenobiotics from the body take places.

Sites of Drug Metabolism

Drug metabolism occurs in various tissues of our body. Liver is a major organ for the biotransformation of major number of drugs, i.e. endogenous or exogenous compounds. The second organ or site especially for the metabolism of orally administered drugs is intestinal mucosa. The intestinal mucosa contains the enzyme cytochrome P-450 and P-glycoprotein that can capture the drugs and secrete it back into the intestinal tract. But the liver secretion contains lot of drug metabolising enzymes.

Once the drug is administered via oral route, it gets absorbed by bloodstream and enter into the liver before distributing into the various compartments of body. Hence, they easily undergo hepatic metabolism called first pass effect, before entering into the systemic circulation, hence oral availability of the drug gets reduced. For example, nitroglycerine, pentazocine, propranolol, lidocaine, etc.

Many of the aromatic nitro drugs and azo drugs are metabolised in the bacterial flora present in the colon. The intestinal β-glucuronide conjugate takes place and it is excreted in the bile, thereby producing the free drug or its metabolite for reabsorption known as enterohepatic cycle.

The other organs involved in biotransformation are lungs, kidney, placenta, brain, adrenal glands and skin.

Cytochrome P-450 or Monooxygenase Enzymes and their Role in Oxidative Biotransformations

Among the various phase-I reactions, the oxidative biotransformation processes are the most common and most important one in drug biotransformation process. The general oxidation reactions of many xenobiotics substrates (RH) into their respective oxidized metabolites (ROH) involve the following chemical equation:

$$R\text{–}H + NADPH^{+} + O_2 \longrightarrow ROH + NADP^{+} + H_2O$$

Enzymes which participate or carry out these reactions are collectively called monooxygenases or mixed function oxidases.

Nomenclature of CYP-450 Enzymes

In the nomenclature, the CYP refers cytochrome system. This is followed by the Arabic numbers specifies the cytochrome family (CYP1, CYP2). Next the capital letter indicates the subfamily (CYP1A, CYP1B etc). Finally, the last Arabic number next to the capital letter indicates the specific enzyme involved in the particular reaction (CYP1A2, CYP12C9).

Reaction Process

1. The reaction needs both molecular oxygen and the reduced form of NADPH.
2. During this reaction, one oxygen atom is added into the substrate RH to give ROH and the other oxygen atom is attached into the water.
3. The CYP-450 enzyme is responsible for the transfer of oxygen atom to the substrate molecule.
4. The other two enzymes NADPH-dependent cytochrome P-450 reductase and NADPH-linked cytochrome b_5 which is responsible for the supply of reducing electrons needed for the overall reactions. The overall reaction is depicted as follows.

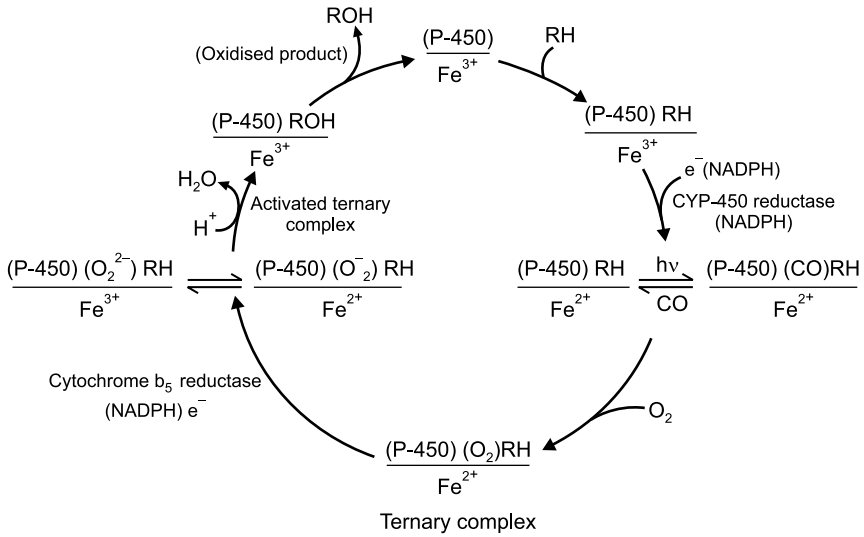


Figure 13.1: Catalytic reaction cycle involving cytochrome P-450.

The reaction sequence is as follows:

1. The first step of the reaction involves the attachment of the substrate to the (oxidised form of Fe^{3+}) resting state of CYP-450 to yield a P-450 (Fe^{3+}) substrate complex.
2. The second step is the transfer of one electron from the NADPH dependent CYP P-450 reductase to the CYP-450 (Fe^{3+}) substrate complex, that leads to the formation of P-450 (Fe^{2+})(RH). During this reaction, the oxidised form of Fe^{3+} is reduced to the Fe^{2+} which is capable of binding with molecular oxygen.
3. The complex obtained from the above step further reduced to yield a peroxide dianions P-450 (Fe^{3+})–substrate complex.
4. Water is eliminated from the intermediate to yield activated oxygen–P-450 substrate complex.
5. The activated oxygen $(FeO)^{3+}$ in this complex is highly electron deficient and a potent oxidising agent.
6. The activated O_2 is transferred to the substrate (RH) and the oxidised product (ROH) is released from the complex to regenerate the oxidised form of cytochrome P-450.

PHASE-I REACTIONS OR ASYNTHETIC REACTIONS OR FUNCTIONALIZATION REACTIONS

Characteristic Properties of Phase-I Reactions

1. **Improves the hydrophilicity:** In phase-I reactions, polar functional groups like –OH, $–NH_2$, –COOH, etc. are introduced into the lipid soluble substrate or unmasked the protecting groups which are already existing in the substrate. These are also called asynthetic reactions which are opposite to the phase-II reactions.
2. **Facilitation or improving conjugation:** During phase-I reactions, the substrate is made susceptible to phase-II reactions which convert the non-polar substrate into highly polar compounds which are easily excreted through urine.

3. **Reduction in stability:** These phase-I reactions reduce the stability of drug and enable the drugs to react with cellular components.

Phase-I Reactions

These are classified into three types.

1. Oxidative reactions
2. Reductive reactions
3. Hydrolytic reactions.

OXIDATIVE REACTIONS

These are the most common and most important metabolic reactions. Maximum all type of drugs or xenobiotics undergo these reactions.

Objectives

1. Oxidative reactions increase the water solubility of the compounds by introducing polar functional groups. Hence, these compounds easily undergo phase-II reactions and excreted through kidney.
2. Oxidation of xenobiotics is catalyzed by various kind of enzymes present in microsomes which needs molecular oxygen and reducing agent NADPH which affects the reaction. These enzymes are called mixed function oxidases.

Steps involved in oxidation reactions

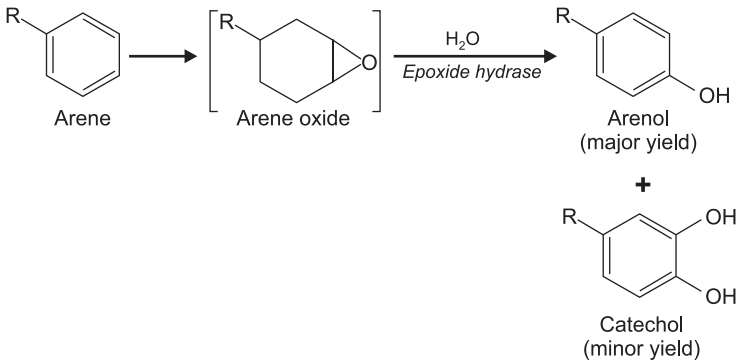
1. Reaction or binding of the xenobiotics or substrates or drugs (RH) to the oxidized cytochrome P-450 reductase, leads to the formation of complex.
2. Transfer of one electron from NADPH to the complex by the enzyme cytochrome P-450 reductase, leads to the formation of Fe^{2+}–P-450-substrate complex (reduced form of substrate enzyme complex).
3. Combination of molecular oxygen with the reduced form of substrate–enzyme complex to yield ternary complex.
4. The second electron transfer from NADPH to ternary complex in the presence of the enzyme cytochrome b_5 reductase to form a ternary activated oxygen–P-450–substrate complex.
5. The final step of the reaction is combination of one oxygen atom from the activated oxygen complex to the substrate to give the oxidized form of the product. The other atoms react together to form water.
6. Now, the free form of cytochrome P-450 is readily available to attach another molecule of xenobiotics.

Reactions

The various oxidation reactions of phase-I reactions can be explained as follows.

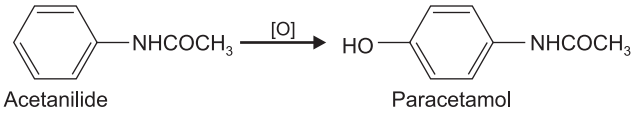
1. **Oxidation of aromatic carbons or aromatic hydroxylation:**

   The basic principle of the reaction involves the formation of highly effective arene oxide or epoxide of the substrate or drugs which undergo rearrangement to yield arenols.



Note: Monosubstituted arenes are oxidized at *ortho*, *para* or *meta* positions but *para* product is formed normally.

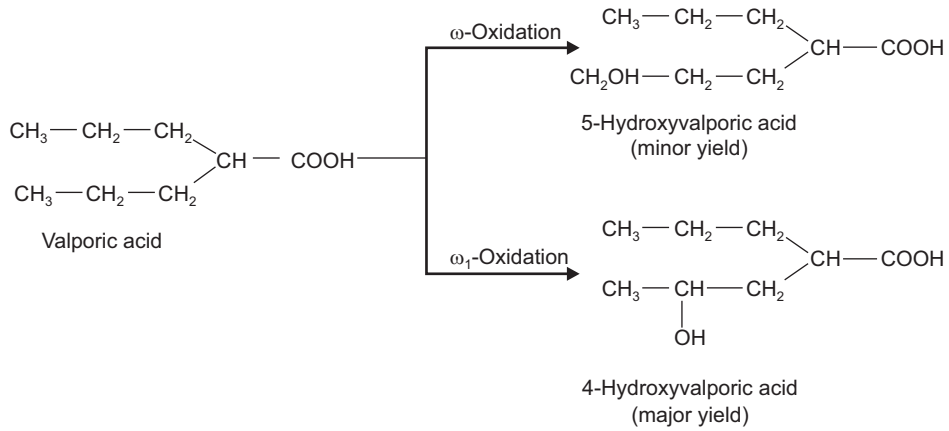
Example: Oxidation of acetanilide to paracetamol.



2. Oxidation of aliphatic carbons or aliphatic hydroxylation

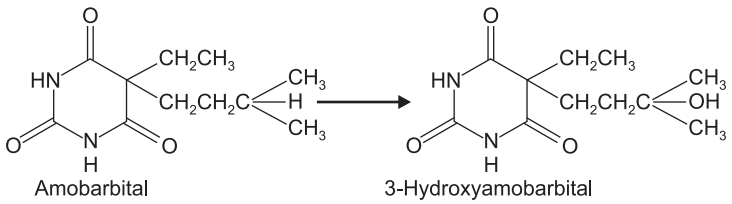
The aliphatic or alkyl carbon atoms are oxidized at two positions such as terminal methyl group (ω-oxidation) and the penultimate atom (ω_1-oxidation), but the later one predominates.

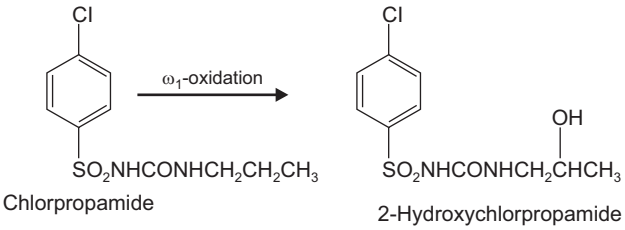
Example 1: Oxidation of the NSAIDs drug valproic acid.



This type of oxidation is called mixed function oxidation.

Example 2: Maximum number of barbiturates and some oral hypoglycemic sulphonylureas are metabolized by ω- and ω_1-oxidation method.



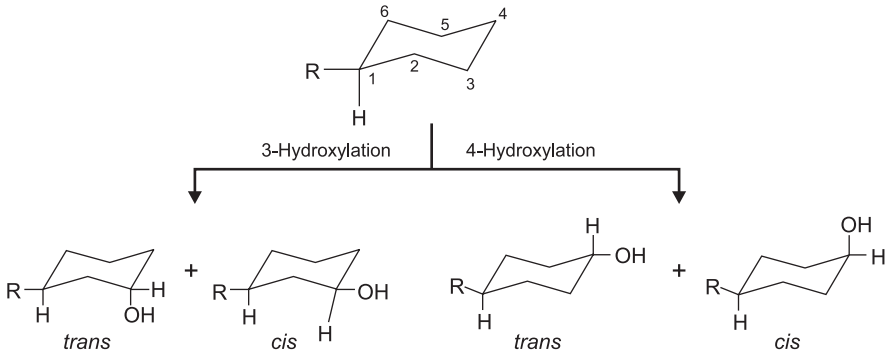


The other examples of drugs which undergo aliphatic hydroxylation are phenylbutazone, glutethimide, meprobamate etc.

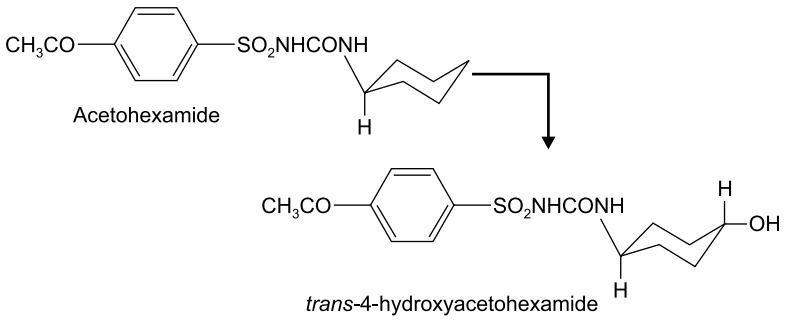
Note: The initial products obtained undergo oxidation to give aldehydes, ketones or carboxylic acids. These may also undergo glucuronide conjugation. This type ω- and ω_1-oxidation commonly occurs in drugs containing straight or branched chains.

3. Oxidation of alicyclic carbons or alicyclic hydroxylation

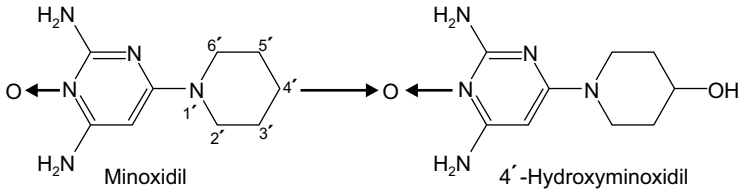
The cyclohexyl group present in many of the drugs undergoes mixed-function oxidation. The metabolism of these drugs mainly takes place in C-3 or C-4 position and gives *cis* and *trans* isomers. In human beings, the *trans* isomers are obtained as the major metabolite.



Example 1:



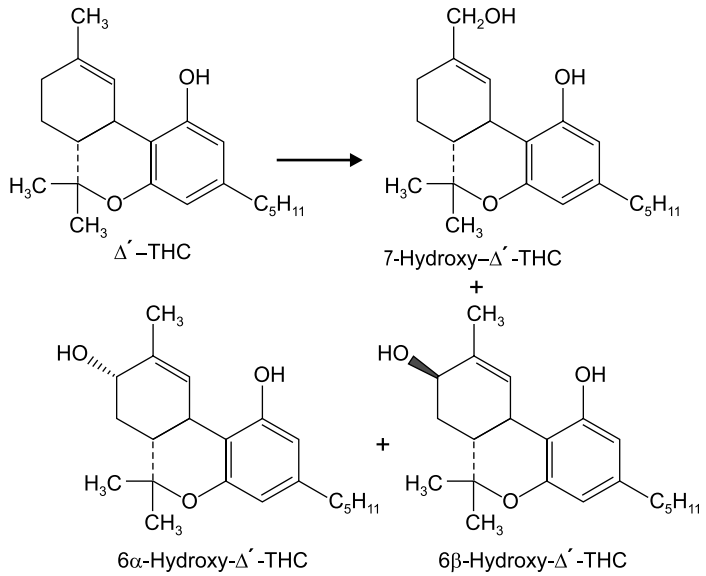
Example 2: Metabolism of alicyclic six-membered piperidyl moiety in minoxidil also follow the above hydroxylation.



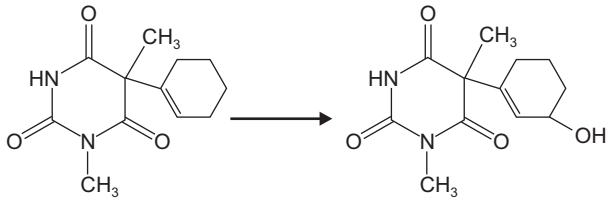
4. Oxidation of allylic carbon atoms:

Allylic carbon atoms also metabolized by hydroxylation.

Example: Metabolism of psychoactive drug Δ′-tetra hydrocannabinol-(Δ′-THC). This drug contains 3-allylic carbon centres, i.e. C-3, C-6 and C-3′. But the allylic hydroxylation chiefly takes place in C-7 carbon centre. The 7-hydroxy metabolite obtained is pharmacologically more active than the parent drug.



The S (+)-isomer of hexobarbital is easily metabolized by hydroxylation than *R* (–)-isomer.

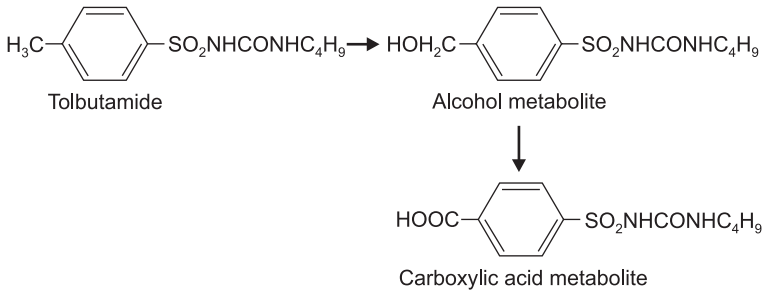


Note: Generally, allylic hydroxylation does not form any reactive intermediates.

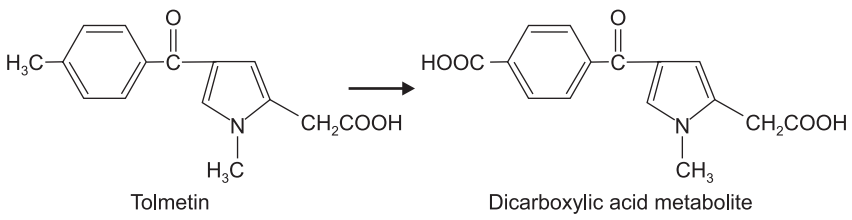
5. Oxidation of benzylic carbons or benzylic hydroxylation

Carbon atoms attached to the aromatic ring undergo oxidation and form respective carbinol or alcohol. The primary alcoholic metabolites obtained are further oxidized to yield aldehydes and carboxylic acids and the 2° alcoholic metabolites are converted into ketones with the aid of soluble alcohol and aldehyde dehydrogenase enzymes. Sometimes, the alcohol may be conjugated with glucuronic acid directly.

Example 1:



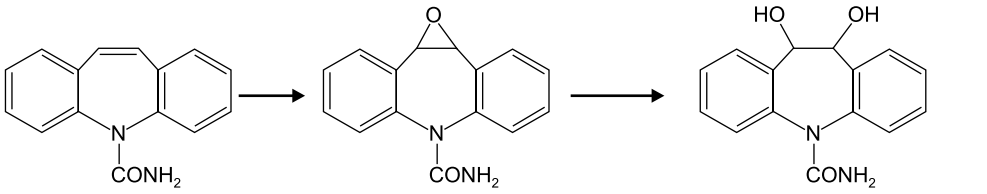
Example 2:

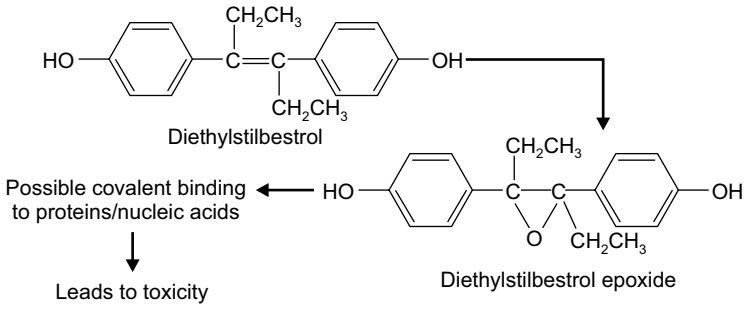


6. Oxidation of olefins

Olefins undergo metabolism and lead to the formation of corresponding epoxides or oxiranes which are stable than arene oxides. The epoxide metabolites further undergo enzymatic hydration by epoxide hydrase to form *trans*-1,2-dihydrodiols or 1,2-diols or 1,2-dihydroxy compounds.

Example:

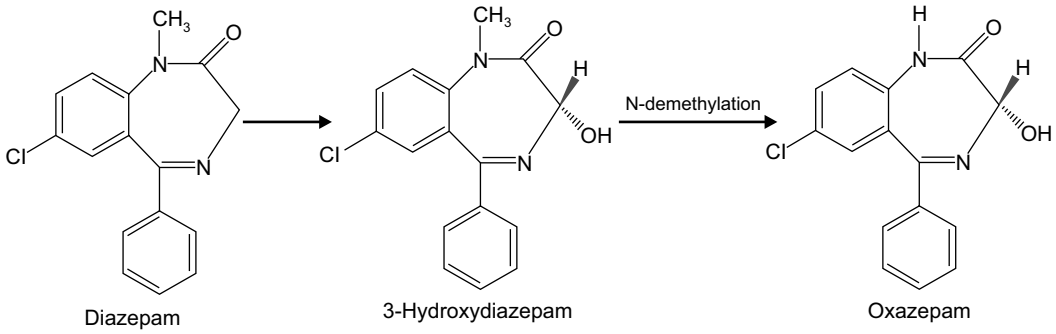




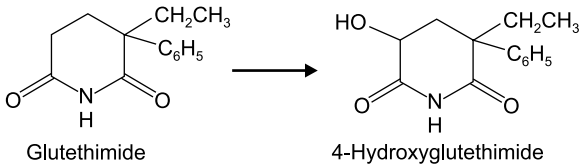
7. Oxidation of carbon atoms α to carbonyls and imines

The mixed functional oxidase also metabolizes the carbon atoms adjacent to carbonyl and imino groups.

Example 1:



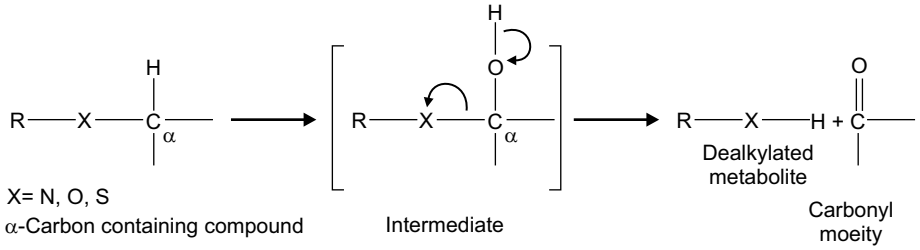
Example 2:



8. Oxidation involving carbon-heteroatom

Maximum number of drugs contains nitrogen and oxygen atoms. Sulphur atom is present occasionally. Metabolic oxidation of C-N, C-O, C-S involves two basic types.

1. Hydroxylation of the α-carbon which is directly attached to the N (nitrogen), O (oxygen) and S (sulphur). The intermediate obtained is unstable and decomposes with the cleavage of C-hetero-atom bond.



The reactions of oxidative N-dealkylation, O-dealkylation and S-dealkylation belong to this category.

2. Hydroxylation or oxidation of the heteroatoms (N, S-only undergoes this type of reaction), depending upon various factors one of the above pathways is preferred among this.

a. Oxidation involving Carbon–Nitrogen Systems

Biotransformation of nitrogen containing compounds are important one because of their occurrence in many natural products and in vast number of important drugs such as alkaloids, antihistamines, benzodiazepines, etc.

Nitrogen containing compounds are classified as follows:

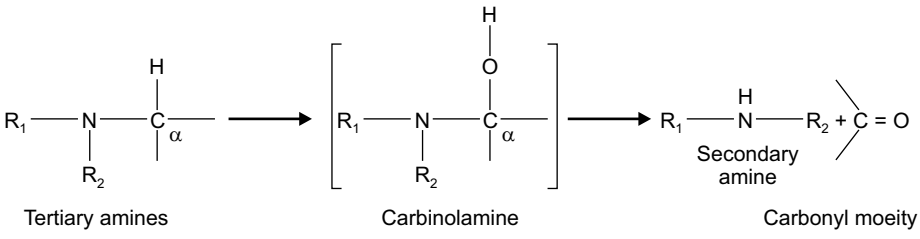
1. Aliphatic (1°, 2°, 3°) and alicyclic (2°, 3°) amines
2. Aromatic amines
3. Amides

The main enzymes responsible for these reactions are cytochrome P-450 mixed function oxidases and amine oxidases or N-oxidases. The activation of later one needs NADPH and molecular oxygen. Biotransformation of C-N system takes places by following reactions.

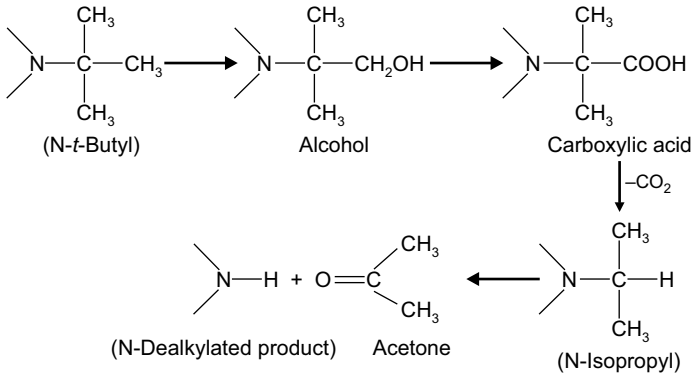
i. N-Dealkylation
ii. Oxidative deamination
iii. N-oxide formation
iv. N-Hydroxylation.

i. N-Dealkylation

3° aliphatic and alicyclic amines: The oxidative removal of alkyl groups from 3° aliphatic and alicyclic amines is called oxidative N-dealkylation. These reactions are carried out by cytochrome P-450 mixed function oxidase enzyme. This reaction takes place via the formation of unstable carbinolamine intermediate which upon the hydrolytic cleavage of C–N gives the 2° amines and carbonyl moiety (aldehyde or ketones).

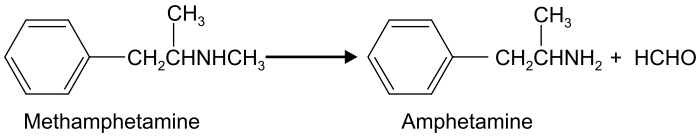


When compared with 2° nitrogen, 3° nitrogen easily undergoes dealkylation because of its higher solubility. Thus, the first alkyl group is removed fast and the second one is removed slowly. N-dealkylation of 3° butyl group is not possible because the α-carbon cannot be hydroxylated.

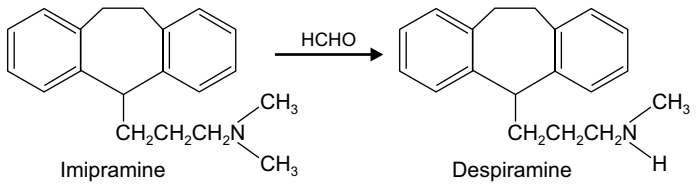


If the tertiary nitrogen atom is attached to different alkyl groups, then the smaller sized alkyl group is dealkylated first.

Secondary aliphatic amines:

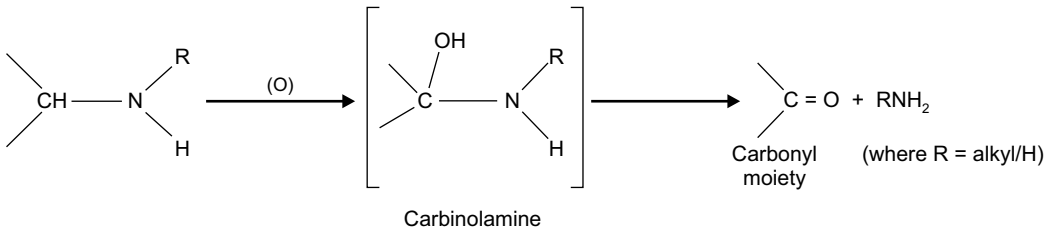


Tertiary aliphatic amines:

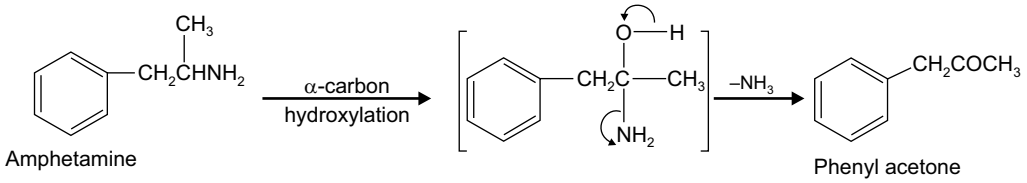


ii. Oxidative deamination:

This reaction also takes place via the formation of carbinolamine intermediate. Here, the C-N bond breaking takes place where the bond links the amino group to the larger portion of the molecule gets cleaved.



The oxidative deamination is carried out by the enzyme mixed-function oxidases. Endogenous neurotransmitters are metabolized by a specialized family of enzymes, known as monoamine oxidases (MAOs).

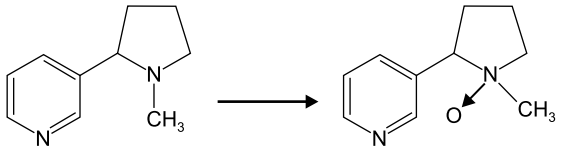


Amphetamine Phenyl acetone

iii. N-Oxide formation:

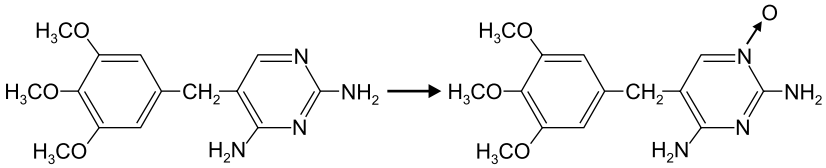
Tertiary amines with basic properties undergo N-oxide formation. The various types of tertiary amines are:

1. Aliphatic amines
2. Alicyclic amines



Nicotine Nicotine-N-oxide

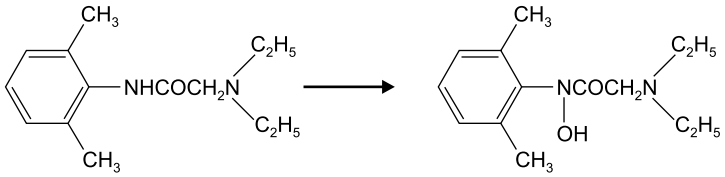
3. Aromatic heterocycles containing nitrogen



Trimethoprim Trimethoprim-N-oxide

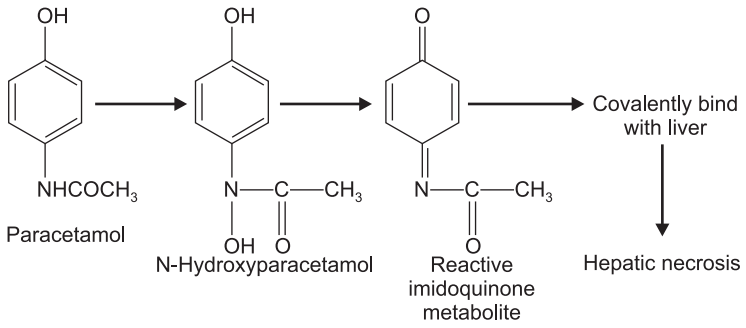
iv. N-Hydroxylation:

i. *Amide nitrogen containing compounds*

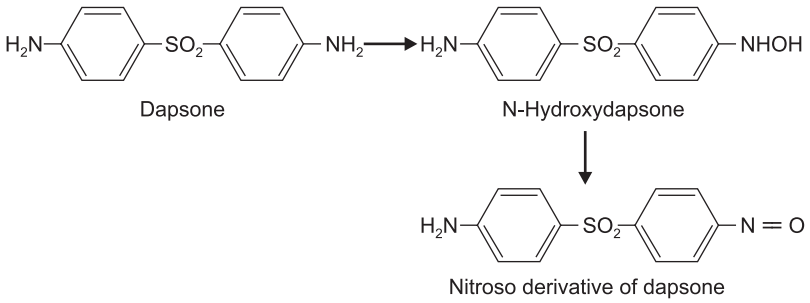


Lidocaine N-Hydoxylidocaine

N-hydroxylation of amide sometimes gives chemically reactive intermediate which covalently binds with the receptors. The best example for this reaction is paracetamol Therapeutic doses of paracetamol are safe because it produces the active metabolite of paracetamol imidoquinone and it is neutralized by glutathione. But in higher doses, the paracetamol metabolite covalently binds with liver tissue and produces hepatic necrosis.

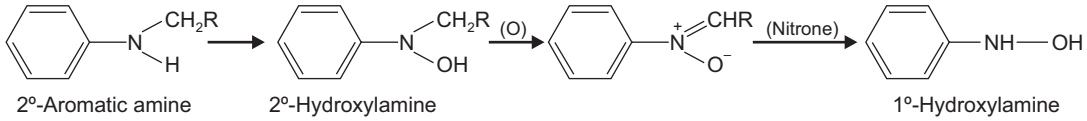


ii. *N-Hydroxylation of 1° amines:* Primary amines are hydroxylated and give nitroso derivatives.

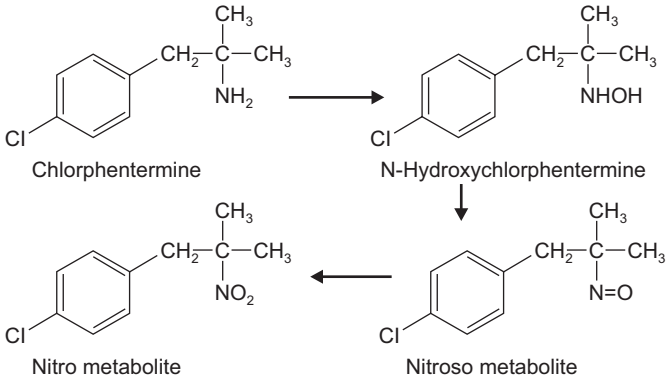


Note: N-Hydroxydapsone oxidizes the ferrous form of hemoglobin into ferric form which leads to the toxicity of methemoglobinemia.

iii. *N-hydroxylation of 2º-amines:* 2º-aromatic amines undergo N-hydroxylation to yield 2º-hydroxylamine which upon oxidation leads to nitrone. Hydration of later provides 1º-hydroxylamine.

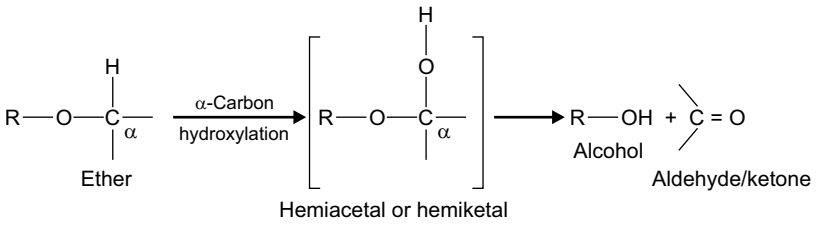


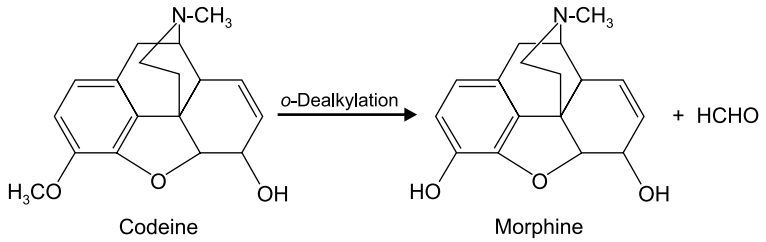
iv. *N-hydroxylation of α-substituted 1° amines:*

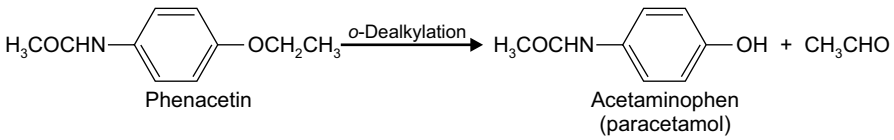


b. Oxidation of Carbon–Oxygen Systems

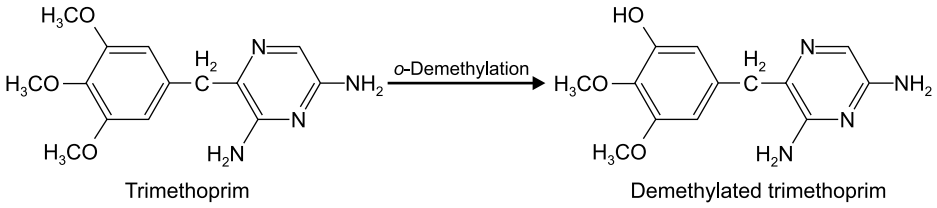
The metabolism of C–O system involves the formation of hemiacetal and hemiketal by the hydroxylation of α-carbon which further undergoes spontaneous C–O bond cleavage to yield phenol or alcohol and aldehyde or ketone. Small alkyl groups like methyl, ethyl, propyl, etc. attached to the oxygen atoms gets dealkylated rapidly.







The other drugs which follow this reaction are indomethacin, metoprolol, etc. some of the drugs contains non-equivalent $-OCH_3$ groups, in that case one particular methoxy group preferentially undergoes *o*-demethylation as shown in the following example:



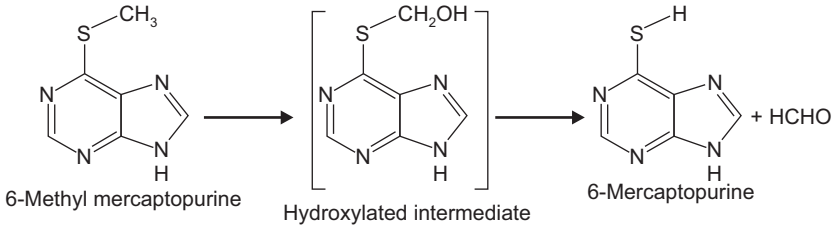
Oxidation of Carbon–Sulphur Systems

Oxidation of carbon–sulphur systems involves the following reactions.

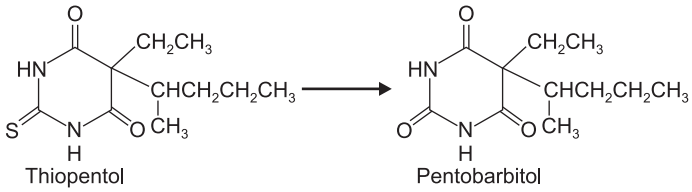
1. S-dealkylation
2. S-oxidation
3. Desulphuration

The S-dealkylation and desulphuration needed oxidative C-S bond cleavage.

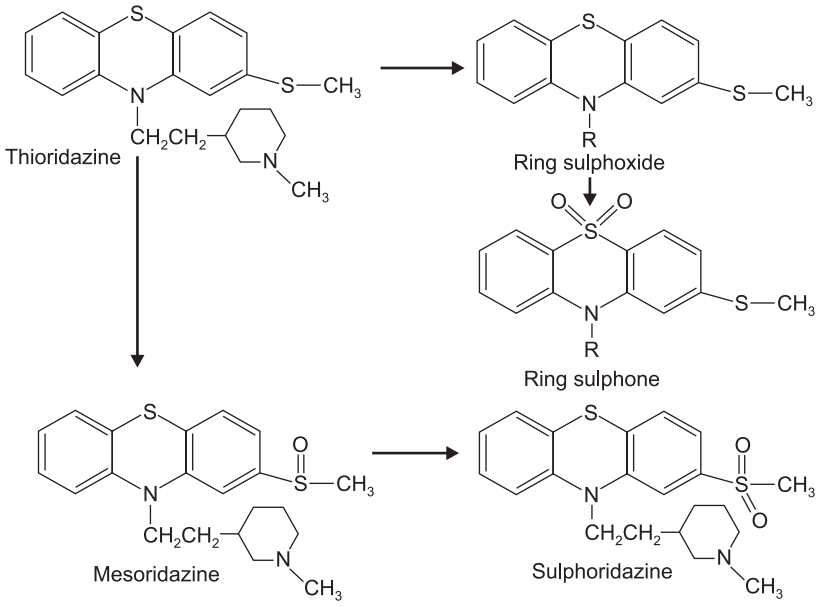
1. **S-dealkylation:** The metabolism by S-dealkylation in human is less.



2. **Desulphuration:** Oxidative conversion of carbon–sulphur double bond into carbon–oxygen double bond is called desulphuration.



3. **S-oxidation:** Many phenothiazine derivatives and organosulphur containing xenobiotics are metabolized by S-oxidation.

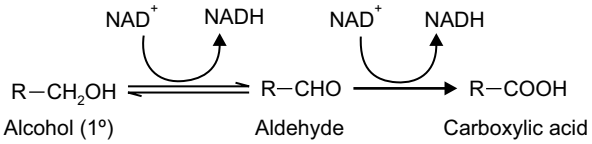


Note:

1. S-oxidation is an important way of metabolism of H_2-antihistamines like cimetidine.
2. Sulphoxide drugs and metabolites further undergo oxidation leads to sulphones.

9. Oxidation of alcohols and aldehydes

Many oxidation reactions leads to the formation of alcohols, as these reactions lead to the formation of alcohols as reactive intermediates. If they do not undergo phase-II reactions, they are further oxidized and converted into aldehydes and ketones. The aldehyde metabolic product is also obtained from oxidative deamination of 1° aliphatic amines which are further oxidized to carboxylic acids and its derivatives.



The bioconversion of alcohols to aldehydes and ketones is catalyzed by the enzyme soluble *alcohol dehydrogenase* present in liver and other tissues. NAD^+ and $NADP^+$ are needed as coenzyme. The further conversion of aldehydes to their respective carboxylic acids is effected by *aldehyde oxidase* and *xanthine oxidase*.

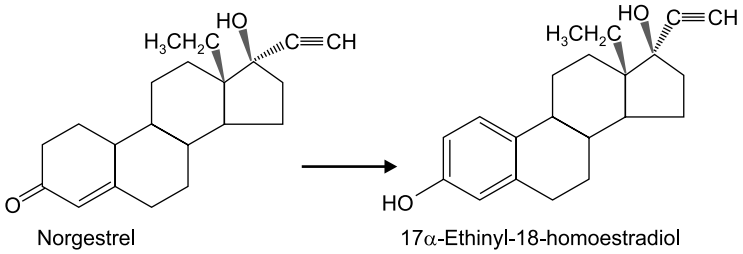
10. Miscellaneous oxidation reactions

It includes the following two reactions.

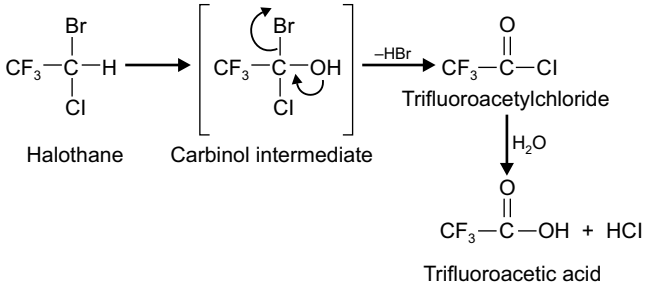
a. Oxidative aromatization/dehydrogenation
b. Oxidative dehalogenations

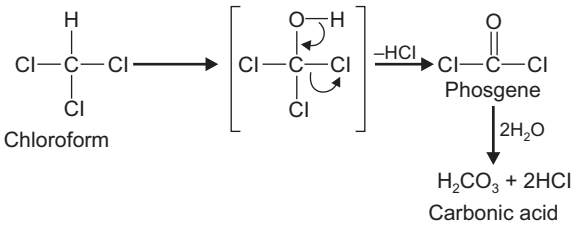
Let us discuss these reactions.

a. Oxidative aromatization results in the formation of aromatic ring as shown in norgestrel steroidal drug.



b. Oxidative dehalogenation: Many of the halogen containing compounds are metabolized by oxidative dehalogenation. For example, halothane and chloroform.





Note: Oxidative dehalogenation leads to the formation of toxic acylhalide intermediates.

REDUCTIVE REACTIONS

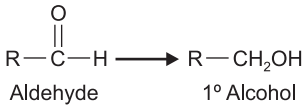
Reductive reactions are important one for compounds containing carbonyl, nitro and azo groups. The bioreduction of carbonyl compounds yields alcohol derivatives whereas the nitro and azo compounds give amino derivatives.

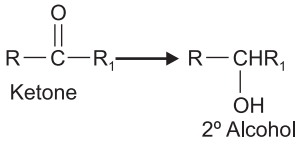
1. Reduction of Aldehydes and Ketones

According to the reactivity towards bioreduction carbonyl compounds are classified into three categories:

1. Aliphatic aldehydes and ketones
2. Aromatic aldehyde and ketones
3. Ester, acids and amides.

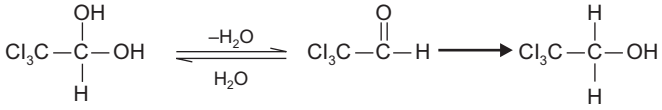
Majority of the drugs have ketone groups. The aldehyde group present in the substrate is reduced to primary alcohols whereas the ketones are reduced to 2° alcohols. The metabolites formed in this further undergoes conjugation with glucuronides.



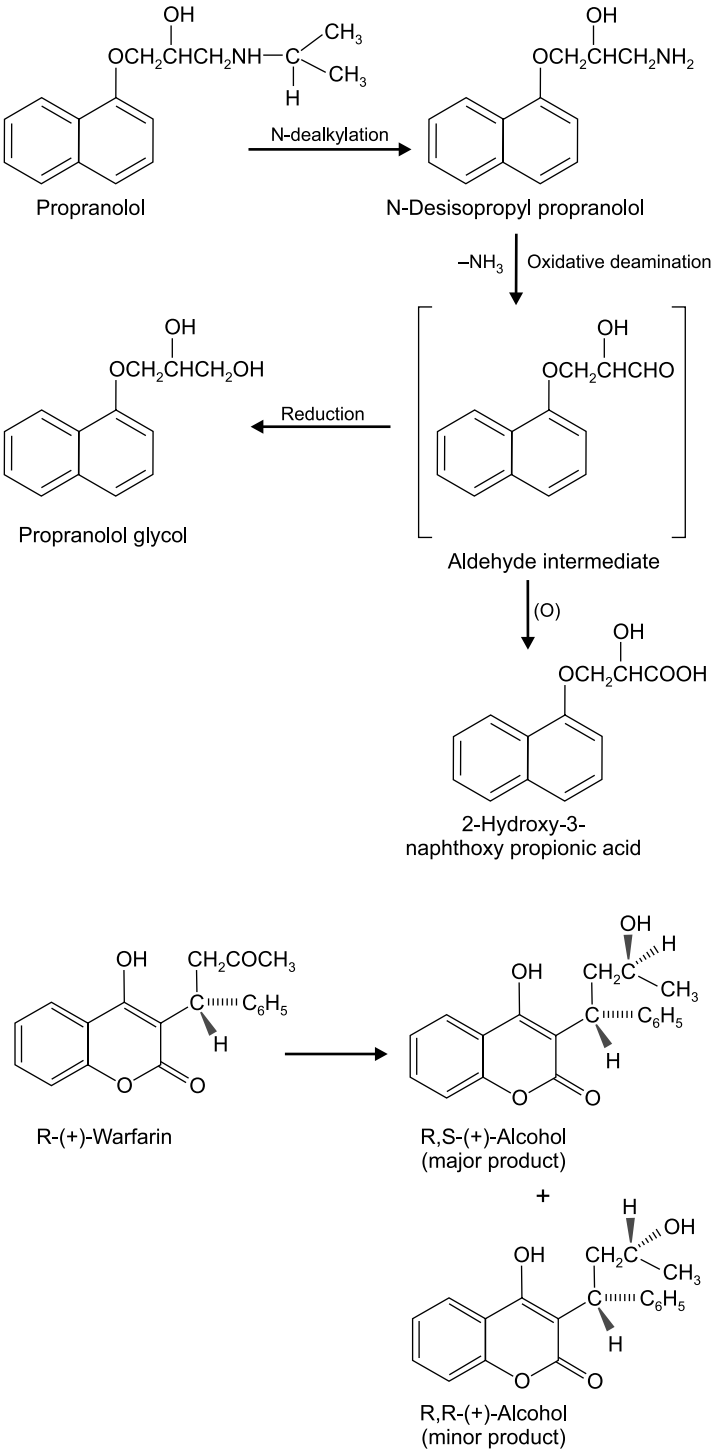


The enzyme aldo-keto reductase is responsible for this reaction. Sometimes, other *oxidoreductase* enzymes are also involved in these reactions. Various examples for this reaction are as follows.

a. Aliphatic aldehydes

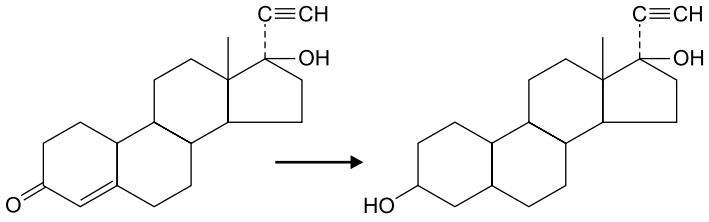


Sometimes, the metabolites obtained from oxidative deamination also undergo reduction to a minor extent.


OH
OCH2CHCH2NH—C
CH3
CH3
H
N-dealkylation
OH
OCH2CHCH2NH2
Propranolol
N-Desisopropyl propranolol
–NH3
Oxidative deamination
OH
OCH2CHCHO
OH
OCH2CHCH2OH
Reduction
Propranolol glycol
Aldehyde intermediate
(O)
OH
OCH2CHCOOH
2-Hydroxy-3-
naphthoxy propionic acid
OH
CH2COCH3
C6H5
H
O
O
R-(+)-Warfarin
OH
H
CH2C
CH3
C6H5
R,S-(+)-Alcohol
(major product)
+
R,R-(+)-Alcohol
(minor product)

2. Reduction of α,β-unsaturated Ketones

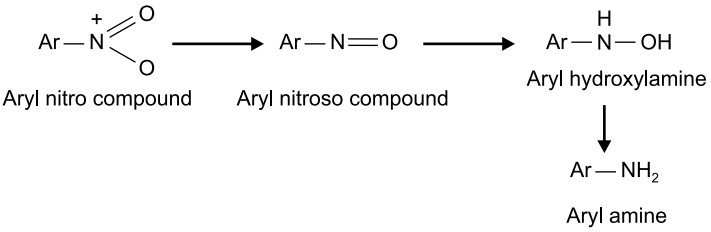
These compounds are not only reduced at ketone group but also reduced at carbon-carbon double bond.



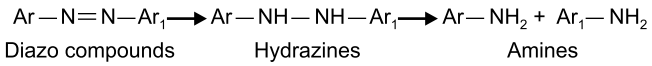
Norethindrone 3β,5β-Tetrahydronorethindrone

3. Reduction of Nitro and Azo Compounds

The reduction of nitro and azo compounds leads to the formation of aromatic primary amine metabolites, the sequence of reductions is as follows.

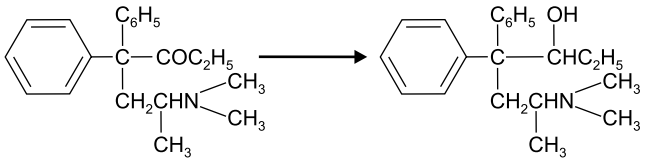


Aryl nitro compound → Aryl nitroso compound → Aryl hydroxylamine → Aryl amine



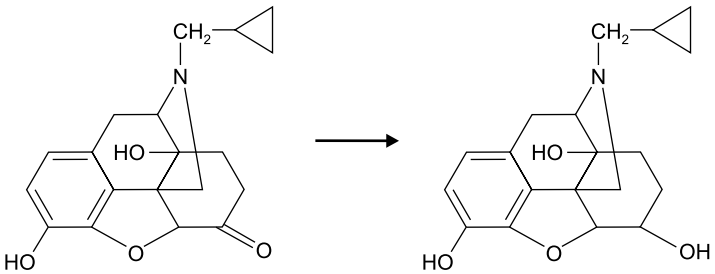
Diazo compounds → Hydrazines → Amines

Aliphatic ketones



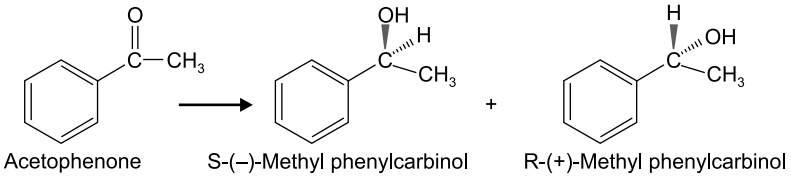
Methadone Methadol

Alicyclic ketones



Naltrexone Naltrexol

Aromatic ketones

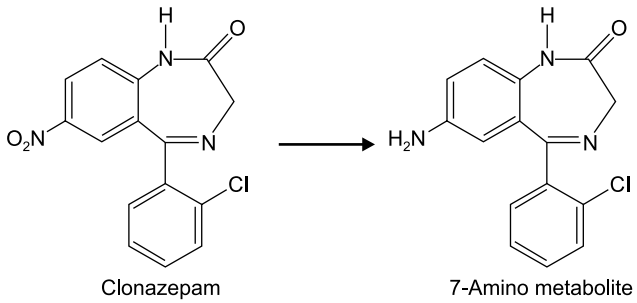


Acetophenone S-(–)-Methyl phenylcarbinol R-(+)-Methyl phenylcarbinol

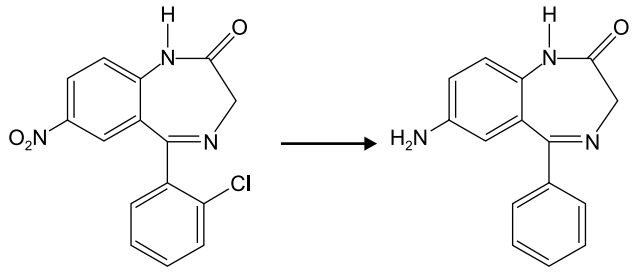
Ketones with chiral carbons also reduced to two epimeric or diastereomeric alcohols. For example, warfarin is reduced to R, S-(+)-alcohol and R, R-(+)-alcohol.

Bioreduction of nitro compounds is carried out by NADPH-dependent nitro reductase enzymes. The reduction of azo compounds is carried out by the enzyme bacterial hepatic microsomal reductase. The enzyme bacterial hepatic microsomal reductase also reduces nitro and azo compounds.

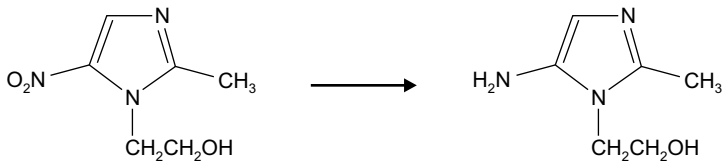
Example:



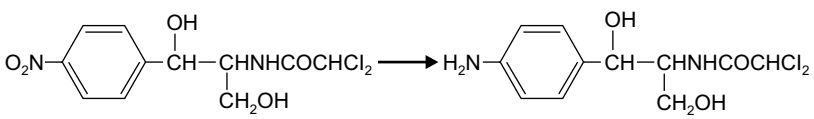
Clonazepam 7-Amino metabolite



Nitrazepam Amino metabolite

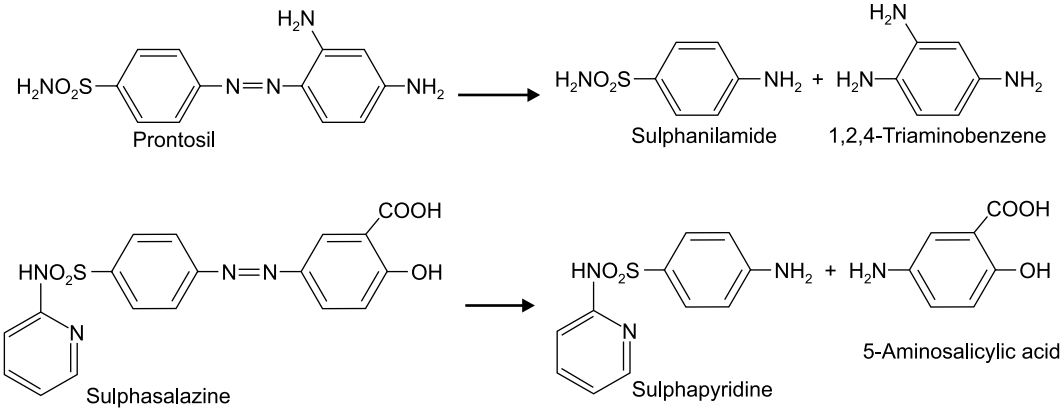


Metronidazole Amino metabolite



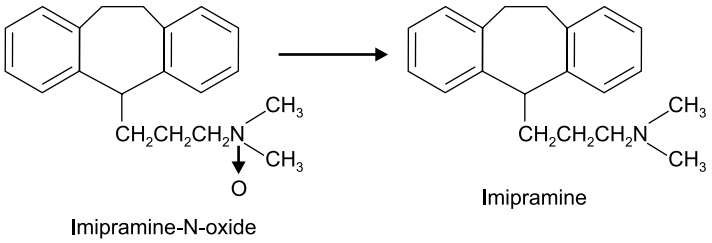
Chloramphenicol Amino metabolite

Reduction of azo compounds is an historical example for the discovery of sulphanilamide antibiotic from prontosil. Bacterial reductase in the intestine is responsible for the reduction of azo compounds.

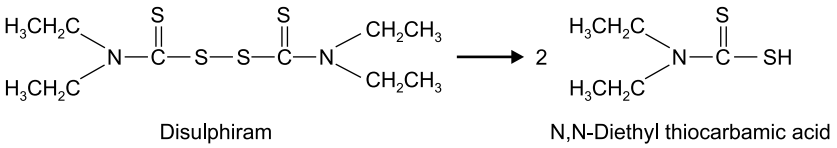


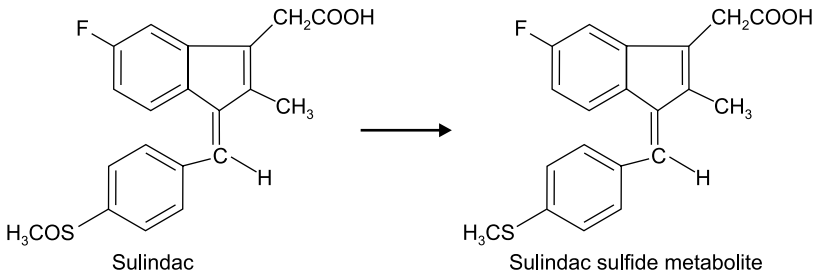
4. Miscellaneous Reductions

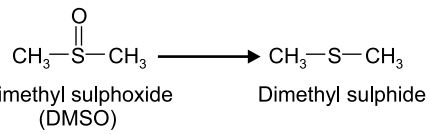
1. **Reduction of N-oxides:** N-oxides are reduced to respective tertiary amines.



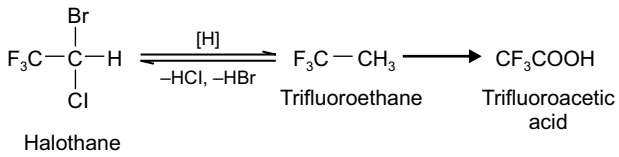
2. **Reduction of sulphur containing compounds:** It is a minor pathway of metabolic reductions.







3. **Reductive dehalogenation:** This reaction follows the displacement or removal of halogen atom from the attached carbon atom.

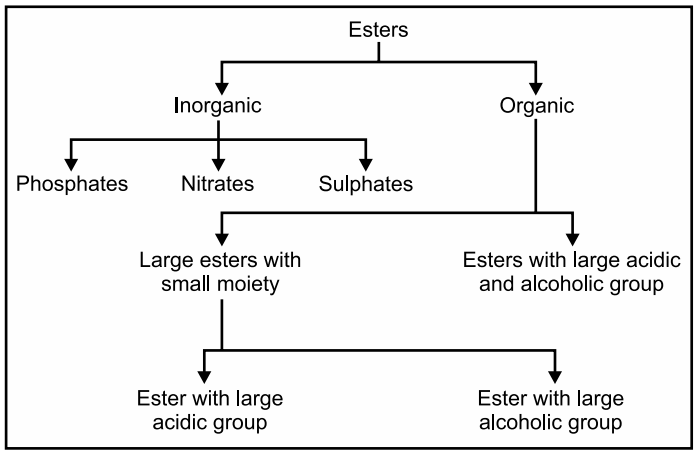


HYDROLYTIC REACTIONS

Hydrolysis plays an important role in the metabolism of amide linkage and ester linkage containing drugs. In most of the drugs, it is catalyzed by the hydrolytic enzymes. The metabolic products obtained are polar compounds and are excreted easily from body.

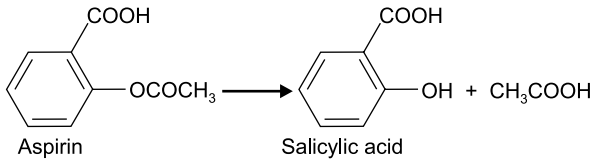
Hydrolysis of Esters

CLASSIFICATION OF ESTERS

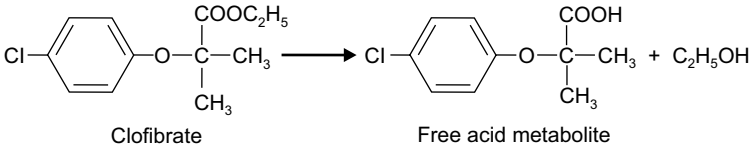


Organic acid esters

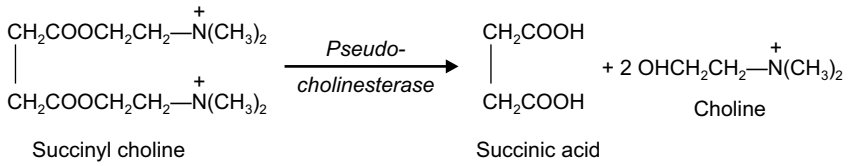
1. **Esters with large alcoholic group**



2. **Esters with large acidic group and small alcoholic group**



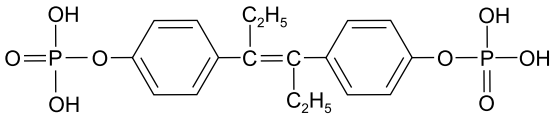
3. **Esters with large acidic and alcoholic group**



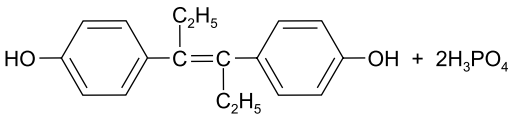
Succinyl choline → Succinic acid

Inorganic Acid Esters

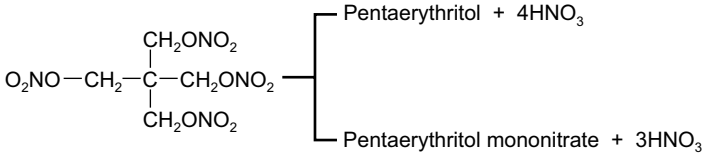
Example: Sulphate esters



Stilbestrol diphosphate



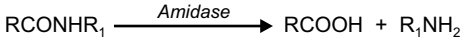
Stilbestrol



Pentaerythritol tetranitrate

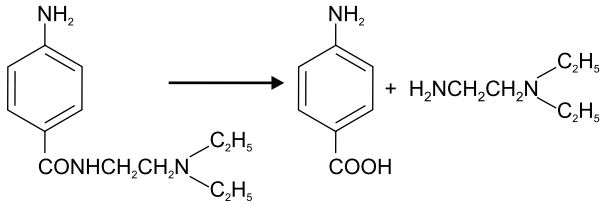
Hydrolysis of Amides

The rate of hydrolysis of amides is lower than esters. Amides are hydrolyzed by the enzyme amidase which involves the cleavage of C-N linkage to yield corresponding carboxylic acids.



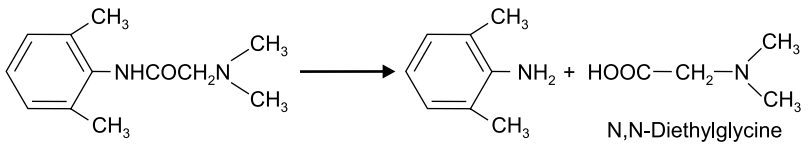
Amide → Acid + Amine

1. **Secondary amides with aliphatic substitution at N-atom**



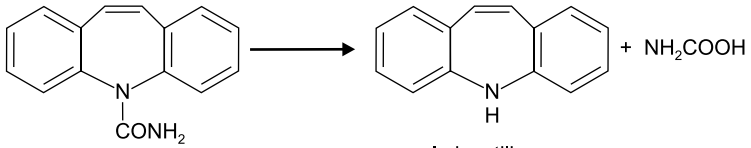
Procainamide → *Para* amino benzoic acid

2. **Secondary amides with aromatic substitutes at N-atom**



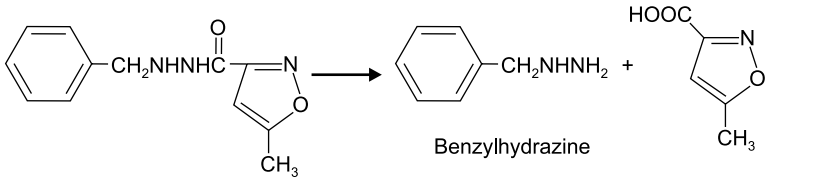
Lidocaine — 2,6-Xylidine — N,N-Diethylglycine

3. **Tertiary amides**



Carbamazepine — Iminostilbene

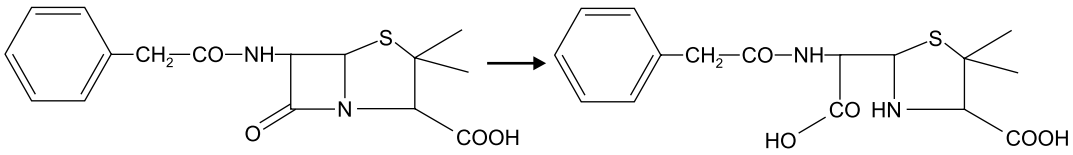
4. **Hydrazides**



Isocarboxazide — Benzylhydrazine — 5-Methylisoxazole-3-carboxylic acid

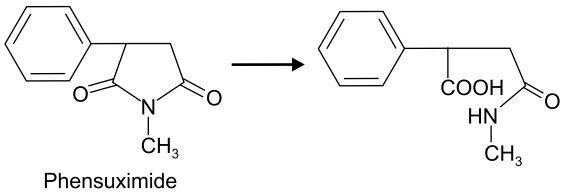
Hydrolysis of Non-aromatic Heterocycles

1. **Four-membered lactam rings**



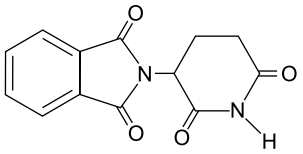
Benzyl pencillin — Pencillonic acid

2. **Five-membered lactam rings**



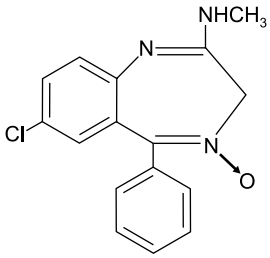
Phensuximide

3. **Six-membered lactam rings**



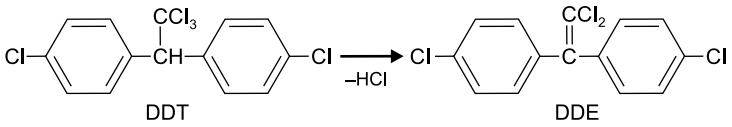
Phthalidomide

4. **Seven-membered lactam rings:**



Chlordiazepoxide

5. **Hydrolytic dehalogenation:** Example for this reaction is the pesticide DDT.



DDT DDE

Miscellaneous Hydrolytic Reactions

Recombinant human peptide drugs and hormones at the N- or C- terminal amino acids undergo phase-I metabolism by carboxy and amino peptidases. Example for this category is growth hormone, human insulin, parathyroid hormone, etc.

PHASE-II REACTIONS OR TRUE DRUG DETOXIFICATION PROCESS OR CONJUGATION REACTIONS

The phase-I reactions do not yield fully hydrophilic or pharmacologically inactive metabolites. But the phase-II reactions convert the metabolites formed from the phase-I reactions into highly polar and water-soluble products. The phase-II conjugation reactions required many number of conjugative enzymes. The main role of this enzyme is attaching a small, polar and ionizable endogenous molecules such as, glucuronic acid, glycine sulphate and glutamine to the phase-I metabolites or products or parent xenobiotics. The resulting conjugates formed are relatively water soluble and excreted rapidly from the body. The conjugates formed are non-toxic and pharmacologically inactive in nature. The other phase-II reactions such as acetylation and methylation do not increase the hydrophilicity of the drugs but attenuate or terminate the pharmacological activity of the drug molecules. The role of glutathione (GSH) is to combine with chemically reactive compounds to inhibit damage to the important biomacromolecules such as DNA, RNA and proteins. Hence, the phase-II reactions are called true detoxification process or reaction pathways in drug biotransformation.

Distinguishing Features of Phase-II Reactions

1. Glucuronic acid, sulphate, acetyl and methyl conjugation takes place and the conjugative groups are initially activated in the form of a co-enzyme before attaching to accepting substrate in the presence of corresponding transferase enzyme.
2. In glycine and glutamine conjugation, the substrate is activated initially.
3. Apart from this, many endogenous compounds such as catecholamines, steroids, bilirubin and histamine also undergo conjugation by using the same coenzymes or specific transferase enzymes.
4. Other than the above said conjugation reactions, conjugation with glycosides, phosphate and other amino acids, cyanide to thiocyanate also involved, but they do not play a role in drug metabolism.

Glucuronic Acid Conjugation/Glucuronidation

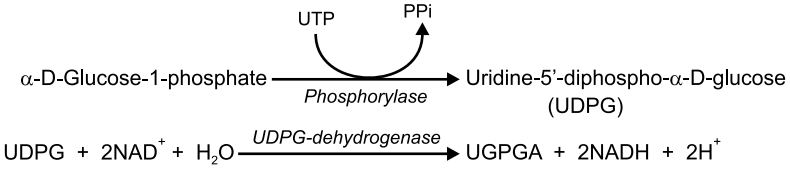
It is the most common pathway in phase-II drug metabolism. The various reasons behind this reaction are as follows:

a. The D-glucuronic acid required for this reaction is easily derived from D-glucose.
b. Large number of functional groups are easily attached to the glucuronic acid.
c. Combination of glucuronic acid with various functional groups increases the hydrophilicity of parent xenobiotics or conjugated products.

Formulation of β-glucuronide conjugates: It involves two steps:

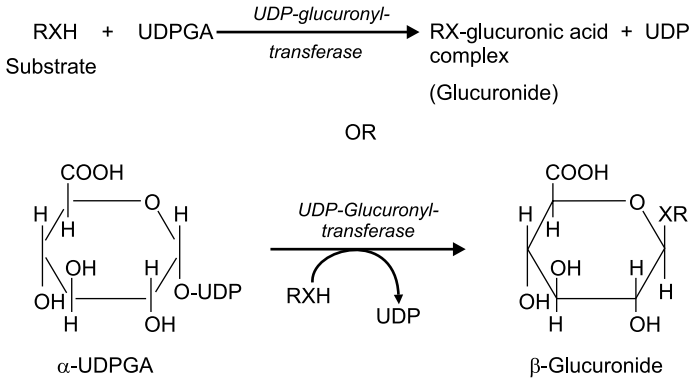
Step 1 [Synthesis of UDPGA (uridine-5'-diphospho-α-D-glucuronic acid), an active co-enzyme]:

The active co-enzyme UDPGA is synthesized from UDP-glucose (UDPG). Here, the co-enzyme UDPGA acts as a donor of glucuronic acid. The UDPG needed for this reaction is synthesized by the reaction between α-D-glucose-1-phosphate and UTP (uridine triphosphate).



Step 2: Transfer of glucuronyl moiety from UDPGA to the substrate or the product of phase-I metabolism:

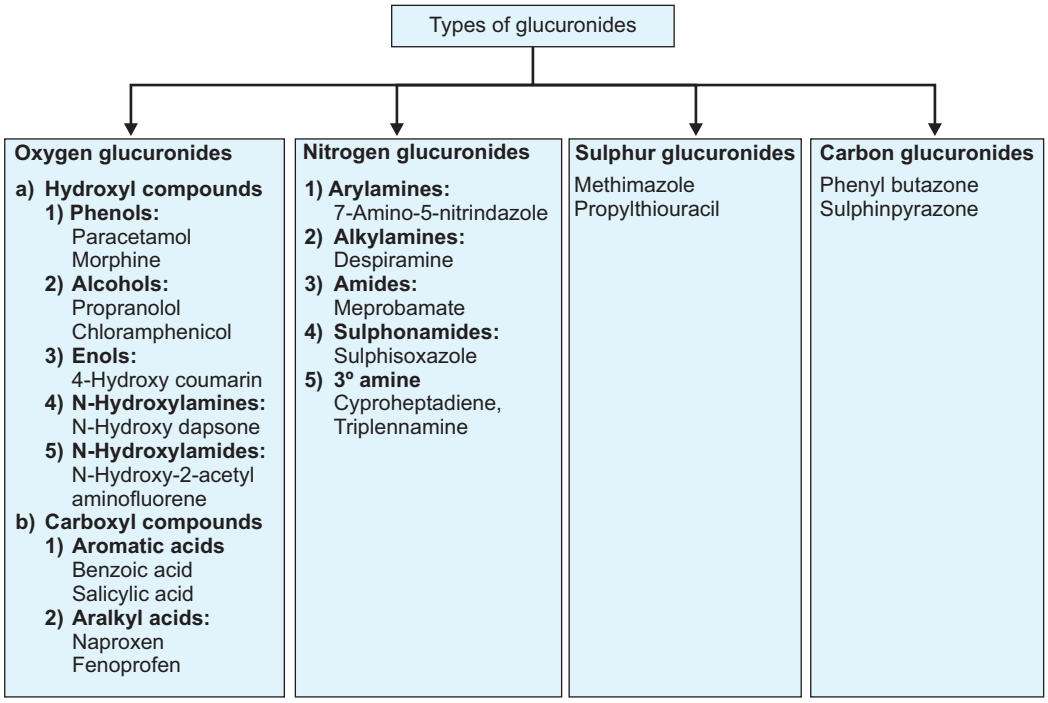
The glucuronyl moiety present in the UDPGA is transferred into the substrate by UDP-glucuronyl transferase to give the substrate glucuronic acid conjugate. The glucuronyl transferase enzyme is mainly present in the liver.



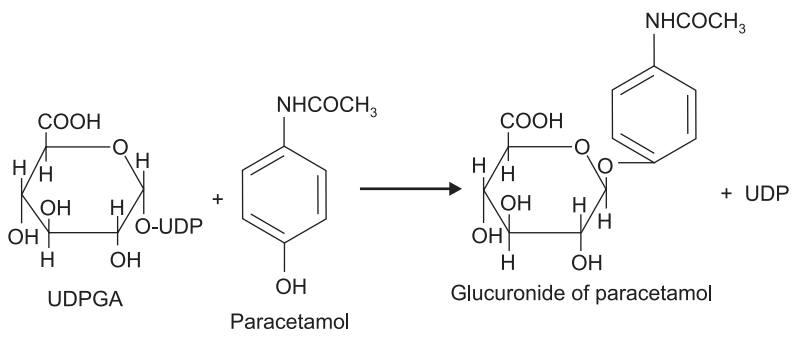
Note:

1. All the glucuronide conjugates possess β-linkage or β-configuration at C-1, but the co-enzyme UDPGA has α-linkage.
2. In the enzymatic transfer, replacement of α-linked UDP-moiety from UDPGA to the substrate molecule RXH takes place via the complete inversion of configuration, at C-1 position to yield β-glucuronide.
3. Glucuronidation of one functional group is an effective way of elimination of metabolites.

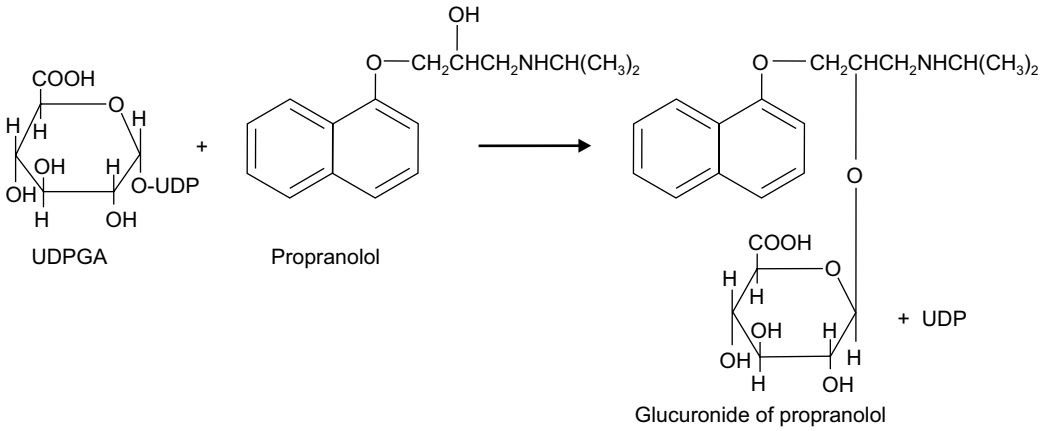
The metabolic products obtained are classified as oxygen, sulphur, nitrogen or carbon-glucuronides depending upon the type of heteroatom attached to the C-1 atom of glucuronyl moiety.



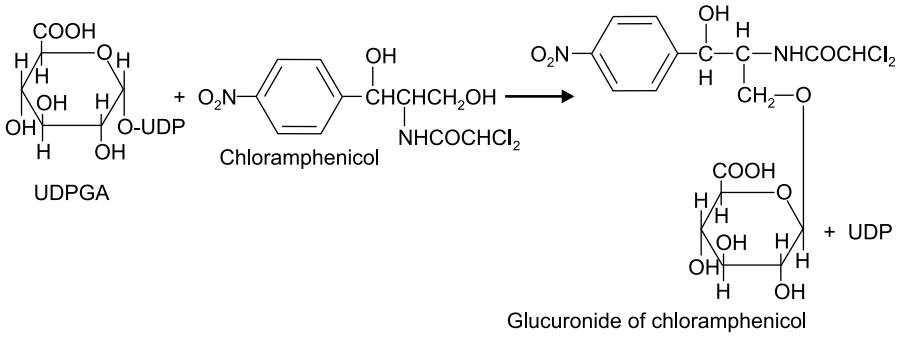
- **Oxygen glucuronides:** Phase-I products or xenobiotics containing hydroxyl (–OH) and carboxyl (–COOH) group easily undergo O-glucuronidation. In that, phenol and hydroxyl groups are the important functional groups which easily get glucuronised. For example:
  a. *Phenol derivatives:* Phase-II reaction of paracetamol.



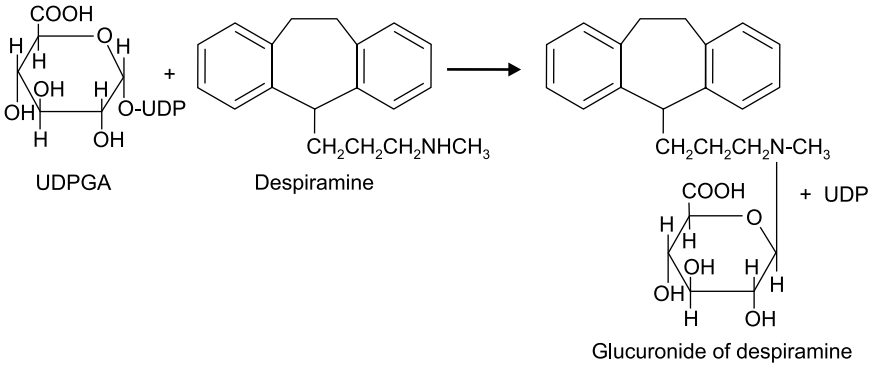
b. *Alcohols:* Phase-II reaction of the antihypertensive drug propranolol.



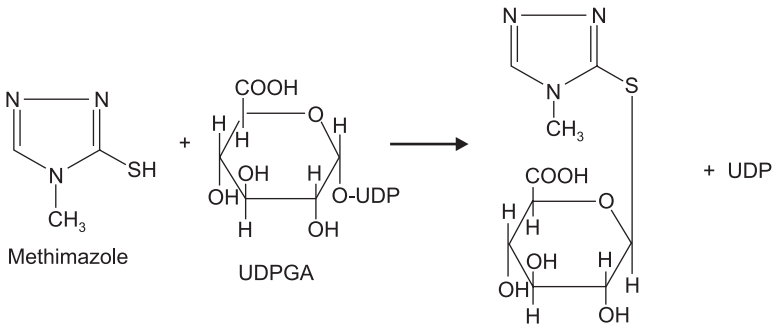
c. *Hydroxyl compounds:* Phase-II reaction of chloramphenicol.

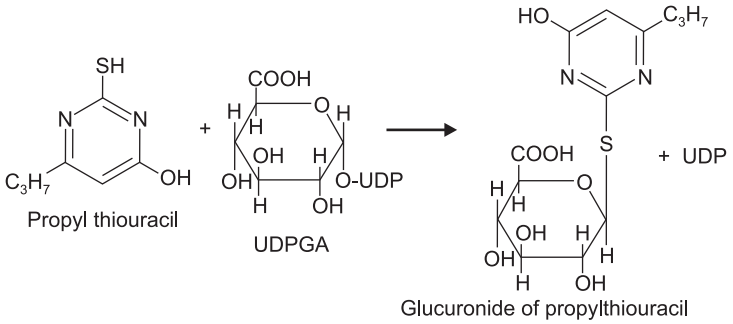


d. *Nitrogen glucuronides:* N-Glucuronides are also obtained with aliphatic amines, arylamines, amides, sulphonamides, etc. It is a minor pathway when compared with N-acetylation and oxidative deamination. Example for this reaction is the metabolism of desipramine mentioned below.

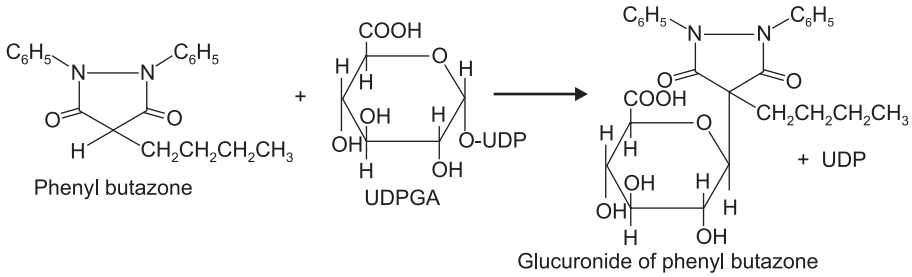


- **Sulphur glucuronides:** Only few numbers of drugs undergo this reaction due to the minimal usage of sulphur containing drugs. Some of the examples are mentioned below.





- **Carbon glucuronides or C-glucuronides:** The formation of C-glucuronides is an important pathway in drug metabolism as shown below.



Note:

1. Apart from the above mentioned heteroatoms, some of the xenobiotics also undergo glucuronidation such as steroids and bilirubin, these conjugates are primarily excreted from urine easily.
2. If the molecular weight of the glucuronides is more than 300 Dalton, then they are chiefly excreted from bile, which may undergo hydrolysis by β-glucuronidase present in the intestine and get reabsorbed, thus lead to enterohepatic recycling. In neonates and children the glucuronidation process is not completely developed, hence the drugs and hormones are accumulated and lead to severe toxicity. For example, the inability of neonates and infants to conjugate bilirubin and chloramphenicol that leads to neonatal hyperbilirubinemia and Grey baby syndrome, respectively.

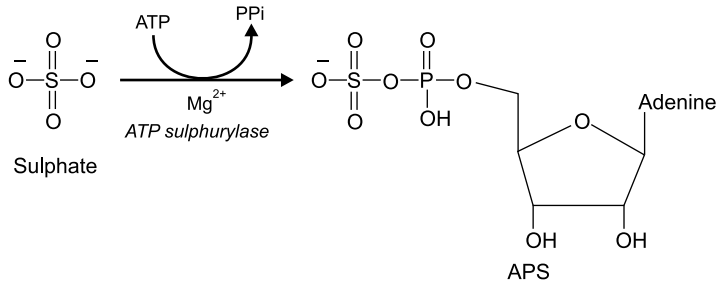
SULPHATE CONJUGATION

Conjugation of xenobiotics or substrate with sulphate primarily takes place with phenols and occasionally occurs in alcohols, aromatic amines, etc. The limited amount of sulphate moiety is available in our body; hence it utilizes significant amount of sulphate pool to make conjugate with numerous endogenous substances such as steroids, catecholamines, heparin, thyroxin, etc.

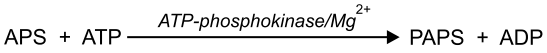
Principle: The main principle involved in this reaction is the synthesis of activated co-enzyme 3′-phosphoadenosine-5′-phosphosulphate which takes place in two steps.

Step 1:

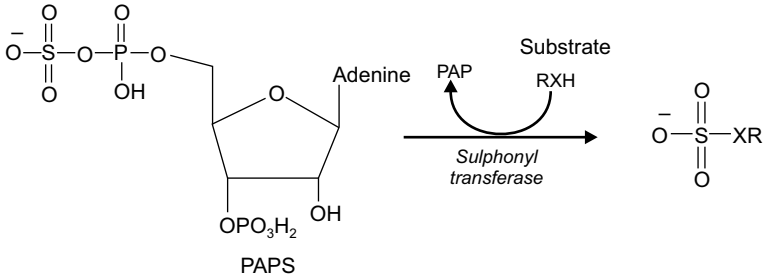
a. **Synthesis of adenosine-5′-phosphosulphate (APS):** It is prepared by the reaction between sulphate and ATP in the presence of ATP sulphurylase to yield APS.



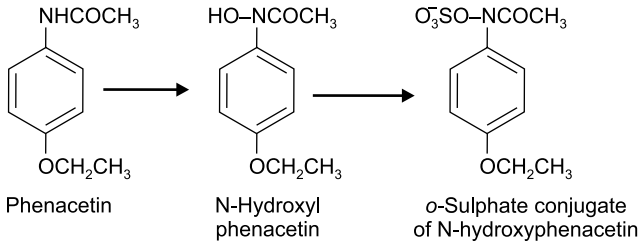
b. **Activation of APS to PAPS (3-phosphoadenosine-5-phosphate):**



Step 2: Transfer of sulphate group from PAPS to the substrate RXH in the presence of soluble sulphotransferase enzyme or sulphokinase, followed by the liberation of PAP (phosphoadenosine phosphate).



Phenolic compounds are the important group of compounds which undergo sulphate conjugation. But in phenolic compounds, glucuronidation predominates. Alcohols and aromatic amines also undergo sulphate conjugation but have minor metabolic pathways. For example, the sulphate conjugation reaction of phenacetin is as follows:

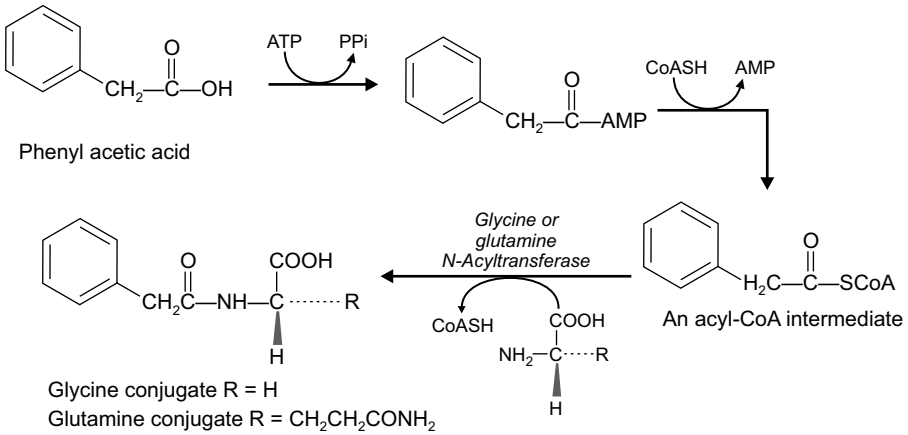


Conjugation with Glycine, Glutamine and Other Amino Acids

Carboxylic acids especially aromatic acids and aryl alkyl acids utilize this type of metabolism. Glycine conjugation is common to most mammals, whereas glutamine conjugation is common to mammals and other primates. The extent of amino acid conjugation formed by xenobiotics is less because of the limited availability of amino acids in the body. The main difference between the glucuronic acid, sulphate and the glycine, glutamine is the later one is not converted into activated co-enzymes.

The important conjugation reaction occurs in this reaction is as follows:

1. Activation of carboxylic acid drug substrate with ATP and the co-enzyme A (CoA) gives an acyl-CoA complex.
2. The intermediate obtained in the above step, acetylase glutamine or glycine catalyzed by the enzyme glutamine or glycine N-acyl transferase enzymes.
3. The activation and acylation steps occur in the mitochondria and kidney. The sequence of the reactions are explained as follows.

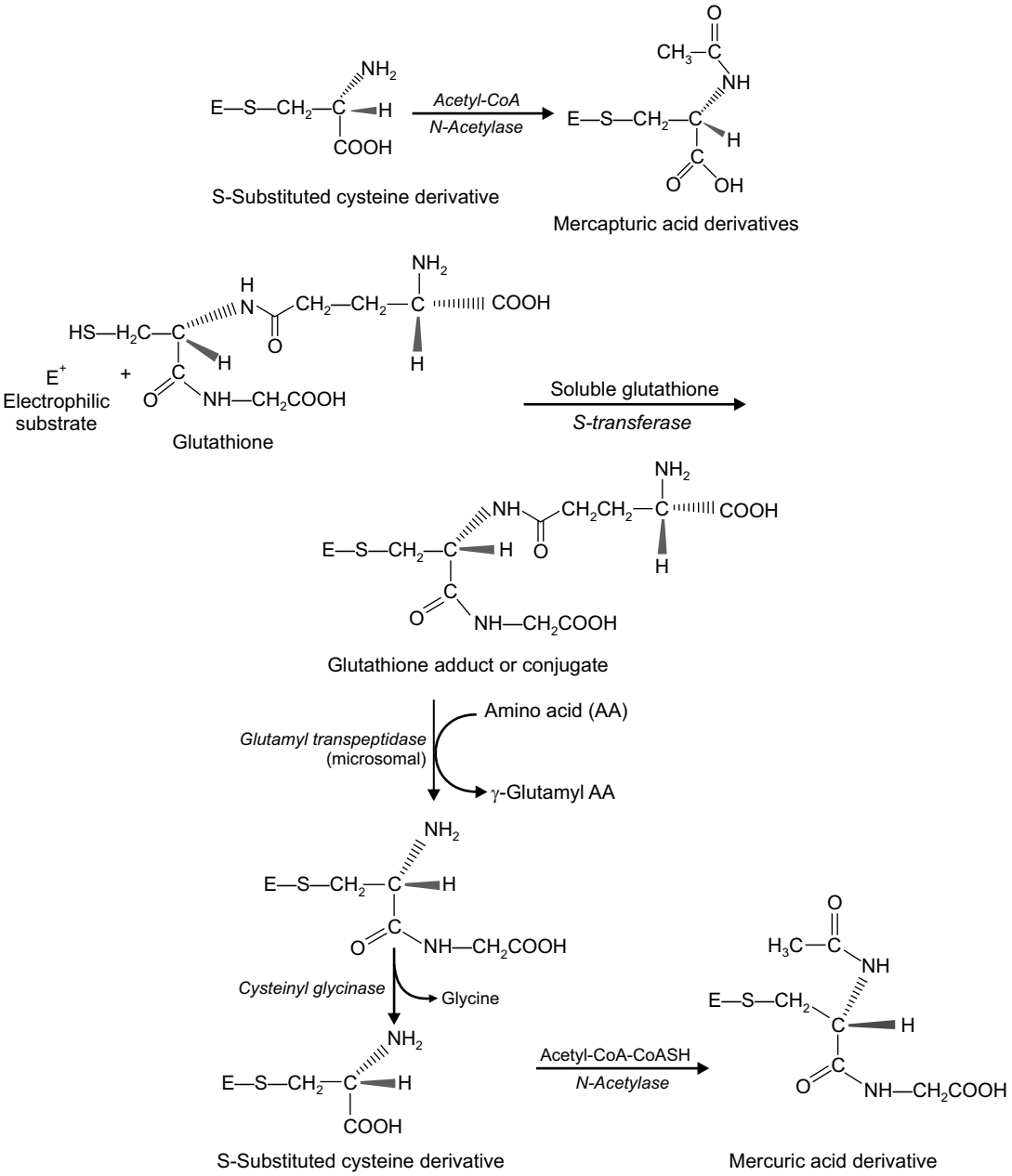


Other examples of drugs or compounds metabolized by this pathway are benzoic acid and salicylic acid. Aromatic acids and aralkyl acids are the major kind of substrate undergo glycine conjugation. Glutamine conjugation mainly takes place in aryl acetic acid such as phenylacetic acid and 3-indolyl acetic acid. Some other types of amino acids also involve in biotransformation such as aspartic acid and serine (in rats), ornithine (in birds), alanine (in mouse and hamster), etc.

Conjugation with GSH or Mercapturic Acid Conjugates

1. It is an important pathway of metabolism for detoxifying chemically reactive electrophilic compounds. Many serious kinds of toxicities are produced by the covalent binding of these reactive electrophilic compounds to the cellular nucleophiles of nucleic acids and vital cellular proteins.
2. GSH protects the nucleic acid and vital cellular proteins due to its nucleophilic sulfhydryl (SH) group.

3. The –SH group reacts with electron deficient compounds to form S-substituted GSH adducts. The reaction process is explained as follows.

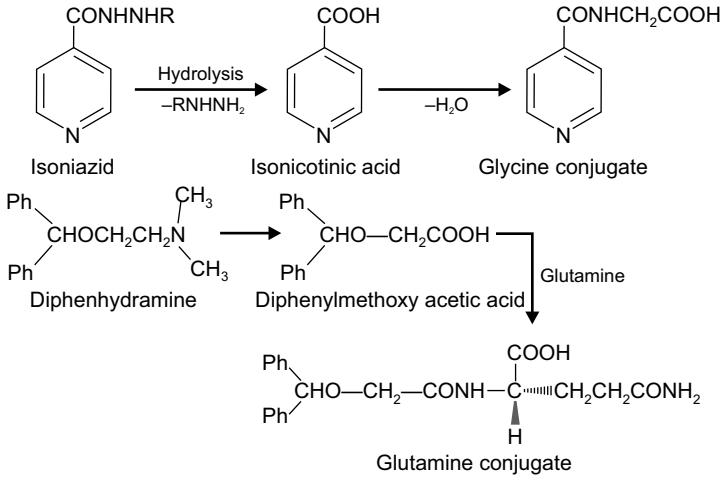


4. Actually, GSH is a tripeptide present in most tissues. Xenobiotics conjugated with GSH usually not excreted as such, but undergo further metabolism to yield S-substituted N-acetyl cysteine products known as mercapturic acids by the enzymatic cleavage of two amino acids from the initially formed GSH adduct and subsequent acetylation of the remaining S-substituted cysteine residue. This reaction is carried out by a family of cytoplasmic enzymes called glutathione-S-transferases. This GSH conjugation do not require the initial formation of an activated substrate

or co-enzyme. The energy needed for the reaction is provided by the reactivity of the nucleophilic GSH towards an electrophilic substrate. The main mechanism of the reaction is as follows.

a. Nucleophilic displacement at an electron deficient carbon or heteroatom.
b. Nucleophilic addition to an electron deficient double bond.

Examples of compounds undergoing mercapturic acid conjugation:



Many of alkyl, aryl, benzylic, allylic substrate, arene oxides, oxiranes also undergo GSH conjugates metabolism.

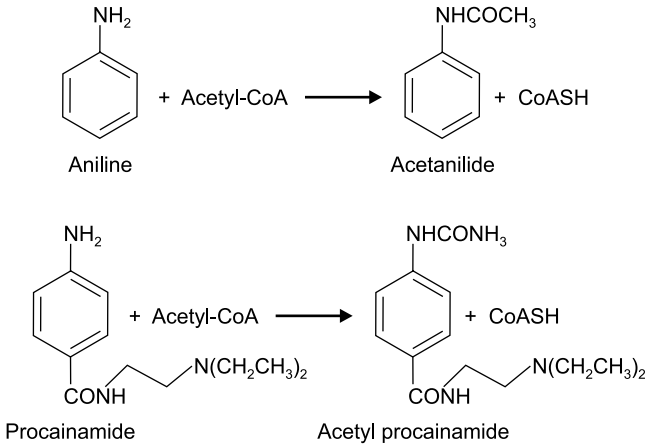
ACETYLATION

The drugs containing primary amino group easily get acetylated and hence it is the major metabolic route for them. During this reaction, the amine group is converted into amide functionalities and are inactive and non-toxic and the water solubility of the metabolites are not increased. Examples of amines undergoing this reaction are $ArNH_2$, $–CONHNH_2$, RNH_2 and sulphonamides.

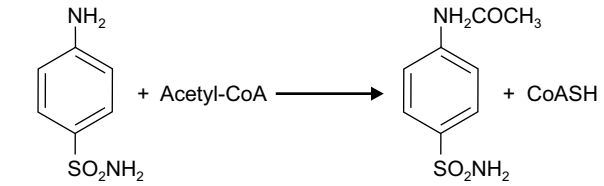
The acetyl group needed for this reaction is obtained from acetyl-CoA. The transfer of acetyl group to the xenobiotics is catalyzed by the enzyme N-acetyl transferase.

Example:

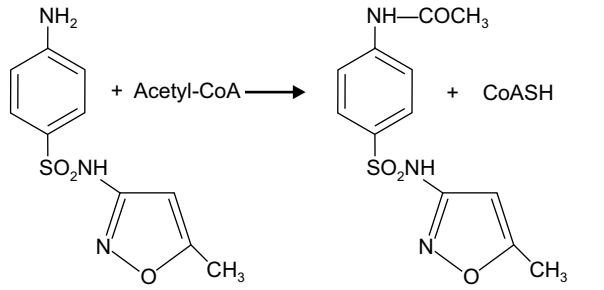
1. Aromatic amines



2. Sulphonamides

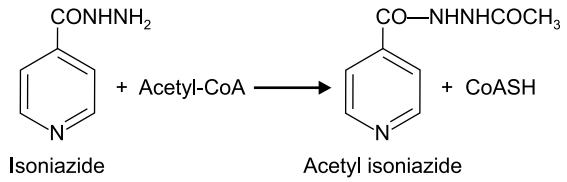


Sulphanilamide Acetyl sulphanilamide



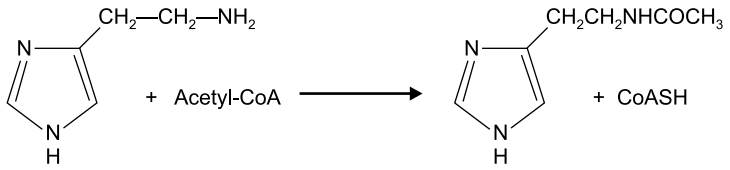
Sulphamethoxazole Acetyl sulphamethoxazole

3. Hydrazines



Isoniazide Acetyl isoniazide

4. Aliphatic amines



Histamine Acetyl histamine

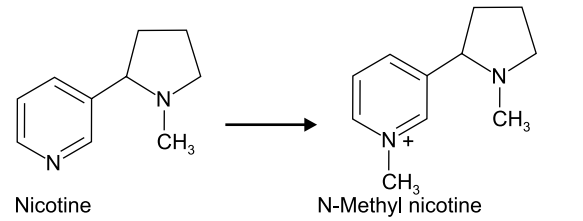
METHYLATION

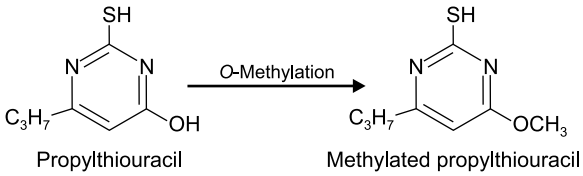
It is a minor pathway of metabolism of drugs. It plays a role for the synthesis of many endogenous compounds and inactivation of various biogenic amines. Methylation of xenobiotics leading to the formation of quaternary ammonium compounds that is in contrast to the reactions.

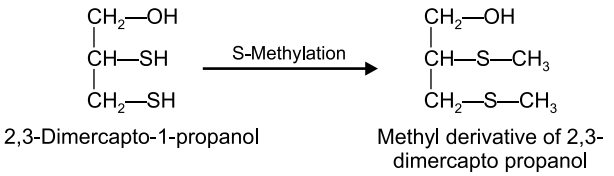
The co-enzyme required to carry out this reaction is S-adenosyl methionine (SAM). The transfer of methyl group from the SAM to the substrate or xenobiotic is catalyzed by different kind of cytoplasmic and microsomal methyltransferases, which mainly includes COMT (catechol-*O*-methyltransferase), phenol-*o*-methyltransferase, S-methyltransferase and N-methyltransferases. Out of these COMT is important one because it catalyses the *O*-methylation of important biotransmitters such as dopamine and norepinephrine. Transferases which are used to methylate histamine, epinephrine and serotonine does not participate in the metabolism of other xenobiotics.

Phenols, catechols, amines, thiols and N-heterocyclic compounds undergo methylation. Catechol and catecholamines are metabolized by methylation and converted into inactive monomethylated catechol products by COMT.

Examples:







Factors Affecting Biotransformation or Metabolism of Drugs

All the drugs and xenobiotics are metabolised by various kind of phase-I and phase-II reactions by various enzymes which form different metabolic products. Depending upon the concentration and activity of the metabolic enzymes, the relative amount of any kind of metabolites is determined. The rate of drug metabolism is important one for the pharmaceutical activity as well as the toxicity of the drugs. For example, when the metabolic rate of the drug increases the intensity and duration of biological action is decreased and if the metabolic rate of drug is decreased, the intensity and duration of action increases and toxicity of the drug also increases. The rate of metabolism is affected by various factors and are listed below.

1. Physicochemical factors/properties of the drugs
2. Chemical factors
    i. Enzyme induction
    ii. Enzyme inhibition
    iii. Environmental chemicals
3. Biological factors
    i. Age
    ii. Sex
    iii. Species variances
    iv. Strain variances
    v. Diet

vi. Stereochemical factors
vii. Altered physiological factors
  a. Disease conditions
  b. Hormonal imbalance/imbalanced hormone conditions
  c. Pregnancy
viii. Temporal factors
  a. Circannual rhythm
  b. Circadian rhythm

1. Physicochemical Properties of the Drug

Generally, absorption and distribution of any kind of drug is majorly depend upon the various types of physicochemical properties of that drug. Similarly, the drug metabolism also affected by the some factors such as molecular weight, size, shape, pK_a, acidity, basicity, solubility, steric and electronic characteristics. All the factors influence the interaction of the drug with its particular enzyme interaction sites and metabolism. But the mechanism of this phenomenon is not yet clearly explained.

2. Chemical Factors

i. Enzyme Induction

The exposure of various drugs, pesticides, environmental xenobiotics, etc. alters the cytochrome P-450 mixed function oxidase system. The process by which the activities of the drug metabolism increased is called enzyme induction. The activity is due to the increased amount of newly synthesized enzymes. Enzyme induction increases the drug metabolism rate and decreases the duration of action. Generally, enzyme inducers increase the rate of their own metabolism as well as those of other related/unrelated drugs or other xenobiotics. Hence, the concomitant administration of two or more drugs may have serious effect or severe drug interaction due to enzyme induction. For example:

a. **Phenobarbital and warfarin:** Phenobarbital is an enzyme inducer, hence, the effect of enzyme induction by phenobarbital increases the metabolism of warfarin and reduces the anticoagulant activity of warfarin. Hence, for the patients receiving anticoagulant therapy careful attention is required to administer phenobarbital by phenobarbital dose adjustment.
b. **Oral contraceptives and phenobarbital or rifampicin:** Concurrent administration of oral contraceptives and phenobarbital/rifampicin to the therapy of oral contraceptives receiving patients leads to decreased pharmacological activity of oral contraceptives due to the enzyme induction activity of the phenobarbital (increased the metabolism of 17-α-ethinyl estradiol) or induced metabolism by rifampicin.

Sometimes, the factors or the microsomal inducers also increases the metabolism of endogenous substances. For example, phenobarbital enhances the metabolism of vitamin D_3, bilirubin, cortisol, etc. in human beings. The increased metabolism of vitamin D_3 by phenobarbital leads to the development of osteomalacia in long-term therapy.

Enzyme induction by chemicals

Along with drugs, sometimes the other chemicals like polycyclic aromatic hydrocompounds and pesticides may also induce some kind of oxidative metabolic reactions.

Cigarette smoking contains minute quantity of polycyclic aromatic hydrocarbons, i.e. benzo[α] pyrene, which is a potent microsomal enzyme inducer. For example, theophylline gets metabolized faster in smokers than in non-smokers. The other drugs like pentazocine, propoxyphene also undergo more rapid metabolism in smokers.

The accidental and occasional exposure of chlorinated insecticides also increases the metabolic rate of particular drugs such as antipyrine, etc.

Some of the drugs like rifampicin, carbamazepine, meprobamate, etc. themselves increase their own metabolism rate, known as self-induction or autoinduction.

The enzyme induction effect leads to stimulation of toxic metabolites in the body.

Table 13.1: Enzyme inducers and their effect on drug metabolism

Enzyme	*Drugs*	*Inducers*
CYP1A2	Imipramine Haloperidol Cloimipramine	Cigarette smokers Ritanovir Ciprofloxacin
CYP2C19	Phenytoin Thioridazine	Carbamazepine Phenytoin
CYP3A4	Alprazolam Atorvastatin	Efavirenz Garlic supplements

ii. Enzyme Inhibition

The activity of metabolic enzymes is sometimes inhibited by drugs or other xenobiotics. A decrease in the metabolic activity of mixed function oxidase enzymes or metabolizing enzymes is called enzyme inhibition. When the metabolism of drugs are decreased means, the concentration or accumulation of drug in the body is increased, which leads to toxicity. There are two types of enzyme inhibition as mentioned below.

1. Direct inhibition
2. Indirect inhibition

1. **Direct inhibition:** It is due to the direct interaction of drugs or xenobiotics at the enzyme site which leads to alteration in the activity of enzyme. It is divided into three types:
   a. Competitive inhibition
   b. Non-competitive inhibition
   c. Product inhibition

   a. **Competitive inhibition:** It takes place due to the binding competition of structurally related analogues to the same active site of enzyme. This is reversible one and easily tackled by the addition of high concentration of any one of the substrates. Metabolism of acetylcholine is inhibited by methacholine by competing for choline esterase.

   b. **Non-competitive inhibition:** It is resulted due to the structurally unrelated agents interacting with the active site of the enzyme and inhibiting the metabolism. This type of interaction is irreversible one. Example, phenytoin metabolism is inhibited by INH.

   c. **Product inhibition:** It is resulted due to the competition between the metabolic products and substrate for the same enzyme. It is otherwise called autoinhibition.

2. **Indirect inhibition:** It takes place by two mechanisms:
   a. Repression
   b. Altered physiology

   a. **Repression or decrease in enzyme content:** In this process, the amount of metabolising enzymes is decreased due to the decreased rate of enzyme synthesis or increased rate of enzyme degradation.

Drugs like actinomycin D, methionine decreases the synthesis of enzymes. Similarly, drugs like disulphiram, carbontetrachloride, etc. increase the degradation of enzymes.

b. Altered physiology: The enzyme inhibition is also affected by hormonal imbalance or nutritional deficiency in the body.

The main example for enzyme inhibition is the effect of phenyl butazone on warfarin plasma concentration. The S-isomer of warfarin is five times more active than R-isomer. Stereoselective inhibition of the metabolism of S-isomer increases the anticoagulant property which leads to haemorrhage. Concomitant administration of isoniazid and phenytoin leads to the prevention of metabolism of phenytoin by former produces toxicity mainly in slow acetylators. Sometimes, the enzyme inhibition also occurs by grape juice. Grape fruit juice is an enzyme inhibitor of CYP_3A_4, if the drugs are administered with grape fruit juice, it alters the metabolic rate of the drug by enzyme inhibition. Some of the examples are given below.

Amiodarone + Grape juice ⟶ Increased bioavailability

Carbamazepine + Grape juice ⟶ Increased plasma level

Diazepam + Grape juice ⟶ Increased bioavailability

Biological Factors

Species Difference

The metabolism of many drugs and foreign substances is species dependent. Same drug or xenobiotic is metabolised in different pathway in different kind of animal species. This type of reactions creates problem in new drug development.

Example 1: Metabolism of phenytoin takes place in human beings by aromatic oxidation leads to (S) (–)-*p*-hydroxyphenytoin. But in dog, the main metabolite formed by oxidation is (R)-(+)-*m*-hydroxy-phenytoin.

Example 2: Metabolism of amphetamine occurs by two ways. They are aromatic hydroxylation and oxidative deamination. Oxidative deamination is predominant in rabbits, humans and guinea pigs, but in rats, amphetamine is predominantly metabolised by aromatic hydroxylation.

Species difference is also present in conjugation reactions, this occurs due to the presence or absence of transferase enzyme involved in the conjugation reaction. For example, in cats, phenolic xenobiotics are metabolised by sulphonation due to the absence of glucuronyltransferase but in case of pigs the phenolic xenobiotics are metabolised by glucuronidation.

The conjugation of aromatic acids with amino acids such as glutamine and glycine depends upon animal species as well as on substrate. For example, in many animals benzoic acid is metabolised by glycine conjugation but in certain birds such as ghoose, turkey and duck, benzoic acid is metabolised by ornithine conjugation.

Strain differences/pharmacogenetics

The altered mechanism of drug also seen in various strain differences especially in rabbits and mice. This is mainly due to the genetic variations in the amount of metabolising enzymes found among the different strains.

For example, the *in vitro* metabolic studies show that the cottontail rabbit metabolises hexobarbital about 10 times faster than New Zealand rabbits. Interindividual differences in metabolism also shown

by human beings. The influence of metabolic enzymes in substrate is under genetic control. Hence, the different metabolic ability is present in the different strains of same species.

Pharmacogenetics: The study of intersubject difference in drug response is known as pharmacogenetics. It is either monogenetically or polygenetically controlled. In identical twins, there is no difference in the metabolism of phenylbutazone, but the same process in non-identical twins showed peculiar differences.

Ethnic variations: Differences occur in the drugs among different races are called ethnic variations. This is may be monomorphic or polymorphic. The appearance of unimodal frequency of distribution present in total population is called monomorphic. For example, the small extent of metabolism of PABA and PAS in total human beings by acetylation. But the metabolism of INH is different in slow acetylator and fast acetylator. In slow acetylator (Japanese and Eskimose) the INH is metabolized slow and in fast acetylators (white and black populations) INH is metabolized fast. Hence, dose adjustment of INH is needed for slow acetylators, otherwise it leads to peripheral neuritis. The other examples of drugs shown strain differences are dapsone, phenytoin, sulphadimidine, etc.

Sex differences: The rate of metabolism of drugs also observed in different sex of population due to the variation in secretion of sexual hormones during puberty. For example, benzodiazepines are metabolized slowly in women than in men.

Age differences: The variations in the metabolic rate of drugs are observed in different age group such as variations in the enzyme secretion, amount of enzyme and haemodynamics. These are explained as follows.

Neonates: In neonates the microsomal enzymes are not fully developed, hence the metabolism occurs in slower rate. For example, absence of glucuronide enzyme in neonates leads to slow metabolism of chloramphenicol and produces Grey baby syndrome. Similarly, paracetamol and sulphonamides lead to hepatotoxicity and nephrotoxicity, respectively.

Infants: Infants also shows similar activity like neonates.

Children: In children and older infants the rapid metabolic rate is observed than adults. Hence, they need large dose than adults. For example, $t_{1/2}$ of theophylline in children is two-thirds of adults.

Elder patients: The decrease in rate of metabolism is observed in elder patients due to decreased hepatic flow and reduced cardiac output.

Diet: The dietary components also alters the metabolism of drugs and xenobiotics.

a. Low protein diet decreases and high protein diet increases the drug metabolism.
b. The high protein–carbohydrate ratio increases the CYP-450 mixed function oxidase activity.
c. Fat free diet decreases the metabolism.
d. Vitamin A, B_2, B_3, C and E deficiency and minerals like Ca, Mg, Cu, etc. retarded the metabolism.
e. Grape juice inhibits the metabolism of various drugs.
f. Malnutrition in females increases the metabolism of sex hormones.
g. Alcohol intake also alters the metabolism of drugs.

Stereochemical Factors

In market, many drugs are available in the form of racemic mixtures and they are also administered in the same form to the humans. The two enantiomers present in the racemic mixtures may differ in their

pharmacological activity. Normally, one enantiomer is more active than another. For example, the (S)-(–) isomer of warfarin is five times more potent than (R)-(+) isomer. In some case, the two enantiomers show totally different pharmacological activities. For example (+)-α-propoxyphene is an analgesic, whereas (–)-α-propoxyphene is an antitussive.

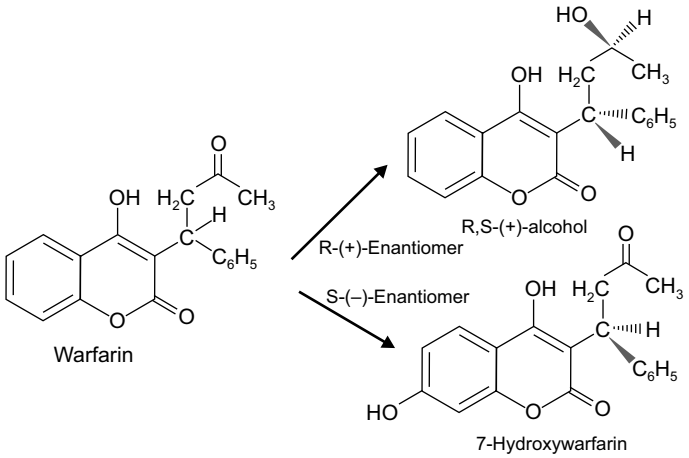
Alterations or differences in metabolism is observed among enantiomers, i.e. one isomer gets metabolized faster than another enantiomer. For example, the less active (+)-enantiomer of propranolol is more rapidly metabolised than corresponding (–) isomer. Another example is allylic hydroxylation of hexobarbital. The (R)-isomer is metabolized faster in humans than (S)-isomer.

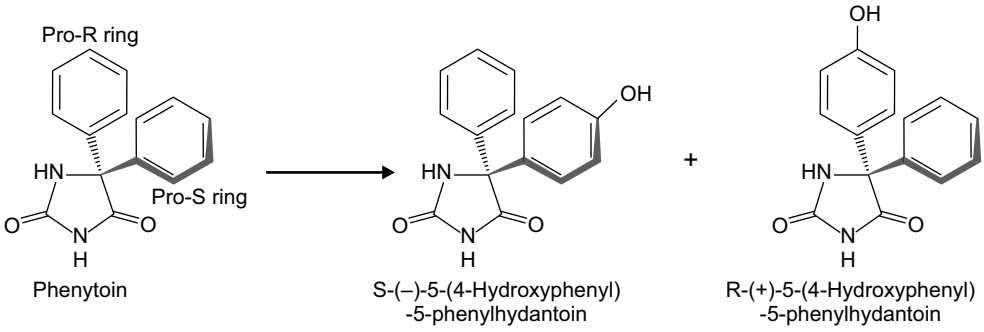
Sometimes, the individual enantiomers of racemic mixtures also metabolised by different pathways. This is called substrate stereoselectivity or enantioselectivity in drug biotransformation.

The more active (S)-(–) isomer of warfarin is metabolized by 7-hydroxylation. But in the less active (R,S) warfarin, alcohol is the major metabolic product.

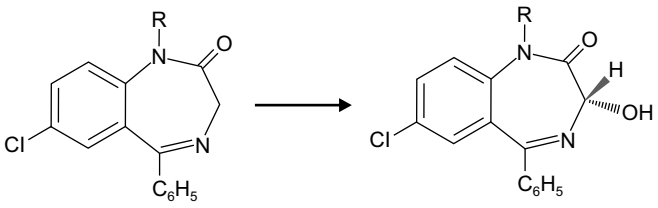
Drug metabolism also leads to the formation of new asymmetric centre in the metabolites or metabolic products. They are known as stereoisomeric or enantiomeric formation of products and are called product stereoselectivity.

Example 1: In humans, the metabolism of phenytoin occurs by aromatic hydroxylation. In which *p*-hydroxylation occurs preferentially (90%) at the pro-(S)-phenyl ring to yield (S)-(–)-5(4-hydroxy-phenyl)-5-phenyl hydantoin. But the hydroxylation of another phenyl ring occurs only in 10%.





Example 2: Microsomal hydroxylation of diazepam yields optically active metabolites. Both of the metabolites are active and one is marketed as a drug in the name of oxazepam.



Diazepam, R = CH_3
Desmethyldiazepam, R = H

(3S)-N-Methyloxazepam, R = CH_3
S-(+)-Oxazepam, R = H

Example 3: Pentazocine, the analgesic agent is metabolized by allylic hydroxylation or by the N-acetylation at the N-butyl side chain to yield *cis–trans* alcohols. In humans, monkey and mouse the *trans*-alcohol is the major metabolic product, whereas in rats *cis*-alcohol is major metabolic product.

Regioselectivity in drug metabolism also observed in some type of drug molecules.

In some drugs, the metabolic studies that denotes the selective metabolism of two or more similar functional groups such as –OH, $–NO_2$, etc. or two or more similar atoms that are positioned in different regions of molecule. For example, the four methoxy groups ($–OCH_3$) present in papavarine, are regioselectively *o*-demethylated in rats, dogs, rabbits, etc. Similarly, two sp^2-hybridised nitrogen atoms are present in trimethoprim (N_1 and N_3). In dogs, the regioselective oxidation of N_3 of trimethoprim yields respective 3-N-oxide.

Altered Physiological Factors

Pregnancy: The metabolic rate of many drugs is reduced during lateral stage of pregnancy, due to the huge amount of steroidal hormones secreted into circulation. For example, the anticonvulsant drugs are metabolized rapidly during pregnancy due to the induction of metabolic enzymes by the circulating progesterone.

Hormonal imbalance: Higher levels of one hormone may interfere the activity of an enzyme and may interfere the activity of another metabolic enzyme. Thyroidectomy and alloxan-induced diabetes in animals showed altered metabolic enzyme activity which leads to decreased metabolism of drugs. Stress-related changes in ACTH levels also effect the drug biotransformation.

Disease state: Liver is the major organ for drug metabolism. Any diseased condition of liver alters the secretion of microsomal enzymes as well as alters the biotransformation of drugs. For example, hepatic carcinoma, obstructive jaundice, etc. of liver reduces the rate of biotransformation. Any impairment of kidney or renal parts alters the biotransformation of salicylates, procaine, etc. In the diabetic patients, glucuronidation rate is decreased due to the decreased availability of UDPGA.

Temporal Factors

Circadian rhythms: Variations in the microsomal enzyme activity with light cycle is known as circadian rhythm in drug metabolism process. The enzyme activity is maximum during early morning (6 am to 9 am) and minimum in late afternoon (2 pm to 5 pm) which is related to the high and low serum levels of corticosterone (because the level of corticosterone depends upon the light–dark sequence in the day).

Chronopharmacology: The study of differences in drug response is influenced by time. The time-dependent change in kinetics is known as chronokinetics. For example, the $t½$ of metyrapone is 2.5 times longer during the night time than in the day in rats.

PROBABLE QUESTIONS

1. Describe the importance of physicochemical properties on biological activity of drug molecules.
2. Enumerate the different physicochemical properties of a drug molecule that influences the biological activity, and describe in detail about the (a) hydrogen bonding and (b) ionization.
3. Write in brief about the following physicochemical properties and their influence on biological activity of drugs (a) redox potential and (b) surface activity.
4. What is bioisosterism? Write its applications in the design of a new and potent drug molecule.
5. Write a brief note on the steric features of drugs and their effects on the biological activity.
6. Describe in detail about the effect of following physicochemical properties on biological activity:
   (a) Solubility
   (b) Partition coefficient
7. Describe in detail about the various methods of determination of partition coefficient.
8. Write a brief note on complexation and its importance on biological activity.
9. Write a note on role of steric features of drugs with suitable examples.
10. Define the following terms with suitable example of drugs.
    (a) Diastereomers (b) Enantiomers (c) conformational isomers
11. What is bio-isosterism and write the significance on biological activity of drugs?
12. Write in detail about classical bioisoteres with examples.
13. Write a short note on non-classical bioisoteres with examples.
14. Describe the importance of protein binding of drugs.
15. Write a note on mechanism of protein drug binding.
16. What is meant by metabolism or biotransformation of drugs and classify with examples.
17. Describe in detail about phase-I reactions.
18. What is meant by phase-II metabolic reactions and explain it with appropriate examples.
19. Enumerate the various factors influencing metabolism.
20. Explain in detail about the oxidation reactions of phase-I metabolism.