1.1 Non-Steroidal Anti-Inflammatory Drugs (NSAIDS): These are the drugs which act to relieve inflammation but which are not structurally related to the corticosteroids.

1.1.1 NSAIDs have four major activities:

i. Analgesia - refers to the relief of pain by a mechanism other than the reduction of inflammation (for example, headache). These agents produce a mild degree of analgesia which is much less than the analgesia produced by opioid analgesics such as morphine.

ii. Antipyretic - These drugs lower elevated body temperature by their action on the hypothalamus. However, they will not reduce normal body temperature.

iii. Anti-inflammatory - These drugs are used to treat rheumatoid disorders, and also in other inflammatory diseases and injuries. Their anti-inflammatory activity is due to their ability to inhibit the cyclooxygenase activity of prostaglandin synthase, an enzyme which mediates the production of prostaglandins from arachadonic acid. These drugs were developed as an alternative to the corticosteroids and their analogues, which have many side effects.

iv. Uricosuric - Many of these agents cause the excretion of uric acid and thus are useful in the treatment of gout.

1.1.2 Mechanism of Action:

The puzzle of NSAIDs action was resolved by the elegantly simple experiments of Sir John Vane, who in 1971 demonstrated that aspirin and other NSAIDs could prevent the synthesis of the prostaglandins (Vane et al., 1971). The NSAIDs provide an alternative to steroid therapy and exert their activity through cyclooxygenase (COX) inhibition (Dubios et al., 1998). COX exists in three isoforms, COX-1, COX-2 and COX-3 (Chandrasekharan et al., 2002). The COX-1 is primarily responsible for cytoprotection and COX-2 induces inflammatory response to stimuli and COX-3 has no role in inflammation (Habeeb et al., 2001).
Anti-inflammatory Effects of NSAIDs:

This effect of NSAIDs is due to the inhibition of the enzyme COX, which converts arachidonic acid to prostaglandins, TXA₂ and prostacyclin. Acetylsalicylic acid irreversibly inactivates COX-1 and COX-2 by acetylation of a specific serine residue. Other NSAIDs reversibly inhibit COX-1 and COX-2. Additional anti-inflammatory mechanism may include:

- Interference with the potentiative action of other mediators of inflammation, viz. bradykinin, histamine, serotonin
- Modulation of T-cell function
- Stabilization of lysosomal membranes
- Inhibition of chemotaxis

Analgesic Effects of NSAIDs:

This effect of NSAIDs is thought to be related to the peripheral inhibition of prostaglandin production, but it may also be due to the inhibition of pain stimuli at a
subcortical site. NSAIDs prevent the potentiating action of prostaglandins on endogenous mediators of peripheral nerve stimulation (e.g. bradykinin)

**Antipyretic Effect of NSAIDs:**

This effect is believed to be related to inhibition of the interleukin-1 and interleukin-6 induced production of prostaglandins in the hypothalamus and the resetting of the thermoregulatory system, leading to vasodilation and increased heat loss.

### 1.1.3 Classification of NSAIDs:

NSAIDs are classified on different basis like

- On the basis of COX selectivity
- On the basis of Chemical structure.

**Classification on the basis of COX selectivity**

<table>
<thead>
<tr>
<th>NSAID</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salicylates</strong></td>
<td></td>
</tr>
<tr>
<td>Acetylated</td>
<td>Aspirin</td>
</tr>
<tr>
<td>Nonacetylated</td>
<td>Salsalate, trisalicylate</td>
</tr>
<tr>
<td><strong>Nonsalicylates</strong></td>
<td></td>
</tr>
<tr>
<td>Nonselective NSAIDs</td>
<td>Ibuprofen, naproxen, sulindac, ketoprofen, ketorolac, flurbiprofen, diclofenac, indomethacin, piroxicam</td>
</tr>
<tr>
<td>Partially selective NSAIDs</td>
<td>Etodolac, nabumetone, meloxicam</td>
</tr>
<tr>
<td>Selective COX-2 inhibitors</td>
<td>Celecoxib, rofecoxib, valdecoxib</td>
</tr>
</tbody>
</table>
Fig. 1.2: Relative Specificity Ratio COX-1 to COX-2 of different NSAIDs (Patrignani P et al., 2011)

Classification on the basis of presence of chemical group:

(i) **Aminoarylcarboxylic acid derivatives**: Enfenamic acid, Etofenamate, Flufenamic acid, Isonixin, Meclofenamic Acid, Mefenamic Acid, Niflumic Acid, Tahniflumate, Terofenamate, Tolfenamic Acid.

![Mefenamic acid]

(ii) **Arylacetic acid derivatives**: Aceclofenac, Acemetacin, Alclofenac, Amfenac, Amtolmetin Guacil, Diclofenac, Erodolac, Indomethacin, Lonazolac, Sulindac.

![Diclofenac Sodium]
(iii) **Arylbutyric acid derivatives:** Bumadizon, Butibufen, Fenbufen, Xenbucin.

![Fenbufen](image)

(iv) **Arylcarboxylic acids:** Clidanac, Ketorolac, Tinoridine

![Ketorolac](image)

(v) **Arylpropionic acid derivatives:** Alminoprofen, Benoxaprofen, Bermoprofen, Carprofen, Fenoprofen, Flunoxaprofen, Flurbiprofen, Ibuprofen, Naproxen.

![Ketoprofen](image)

(vi) **Pyrazoles:** Difenamizole, Epirizole

![Epirizole](image)
(vii) **Pyrazolones**: Apazone, Benzipiperylon, Feprazone, Mofebutazone, Morazone, Oxyphenbutazone, Phenylbutazone, Pipebuzone, Propyphenazone, Ramifenazone, Suxibuzone, Thiazolinobutazone.

![Phenylbutazone](image)

Phenylbutazone

(viii) **Salicylic acid derivatives**: Acetaminosalol, Aspirin, Balsalazide, Benorylate, Calcium acetylsalicylate, Diflunisal, Fendosal, Gentisic acid, Glycol salicylate, Imidazole salicylate, Lysine acetylsalicylate, Mesalamine, Morpholine salicylate, 1-Naphthyl salicylate, Olsalazine, Parsalmide, Phenyl acetylsalicylate, Phenyl salicylate, Salacetamide, Salicylamide, Salicylsulfuric acid, Salsalate, Sodium Salicylate, Sulfasalazine.

![Aspirin](image)

Aspirin

(ix) **Thiazinecarboxamides**: Ampiroxicam, Drocicam, Isoxicam, Lornoxicam, Piroxicam, Tenoxicam.

![Piroxicam](image)

Piroxicam
(x) **Others:** γ-Acetamidocaproic acid, S-Adenosylmethionine, 3-Amino-4-hydroxybutyric acid, Bendazac, Benzydamine, α-Bisabolol, Bucolome, Celecoxib, Difenpiramide, Ditazol, Emorfazone, Etanercept, Fepradinol, Guaiazulene, Infliximab, Interleukin-10, Lexipafant, Nabumetone, Nimesulide, Oxaceprol, Paranyline, Perisoxal, Proquazone, Rofecoxib, Superoxide dismutase, Tenidap.

3-amino-4-hydroxybutyric acid

It can be summarized as shown in fig. 1.3.

---

**Fig. 1.3: Classification of NSAIDs on basis of Chemical Structure**

1.2 **Problems Associated With NSAIDs:**

Despite the intensive research that has been aimed at the development of NSAIDs, their clinical usefulness is still restricted by their GI side effects like *gastric irritation, ulceration, bleeding, perforation, nausea, vomiting, dyspepsia,*
diarrhoea and in some cases may develop into life threatening conditions (Robert et al., 1989).

Fig. 1.4: Peptic Ulcer caused by NSAIDs

It is a well-accepted fact that the GI side effect of acidic NSAIDs is a result of two different mechanisms:

a) Local Effect on GI Tract

The first mechanism involves a local action comprising of a direct contact effect and an indirect effect on the GI mucosa. The direct effect can be attributed to the local inhibition of prostaglandin (PG) synthesis in the GI tract. The indirect effect can be attributed to a combination of anion-trapping mechanism of NSAIDs in mucosal cells and back diffusion of H⁺ ions from the lumen into the mucosa. Topical irritation by the free carboxylic group of the NSAIDs is considered an important factor in establishing superficial stomach erosion, particularly in the corpus region of the stomach.

b) Systemic Effects:

The second mechanism is based on the generalized systemic action occurring after absorption and can be manifested even after intravenous dosing (Cioli et al., 1979). The
systemic effects are manifested due to inhibition of synthesis of gastric PGs like PGI$_2$ and PGE$_2$. NSAIDs are also responsible for altered membrane signaling proteins, which is shown in Fig.1.5.

Fig. 1.5: Effect of NSAID on Membrane proteins

1.3 **Approaches Used To Counter The Problems:**

As NSAIDs cause several serious side effects, so to counter these problems there are different approaches which are in practice. Attempts to develop non-steroidal anti-inflammatory remedies devoid of these shortcomings—especially gastrointestinal toxicity—have followed several strategies. These strategies are:

1. **Association of NSAIDs with gastroprotective agents (Combination therapy):** Non-steroidal anti-inflammatory drugs have, therefore, been associated with gastroprotective agents that counteract the damaging effects of prostaglandin synthesis suppression; however, this combination therapy introduces other problems of pharmacokinetic, toxicity, and patient's compliance.

2. **Incorporation of a nitric oxide (NO)-generating moiety into the molecule of several NSAIDs:** This approach was shown to greatly attenuate their ulcerogenic activity; however, several findings suggest a possible involvement of NO in the pathogenesis of arthritis and subsequent tissue destruction.
3. Preparation of novel NSAIDs targeted at the inducible isoform of prostaglandin synthase (COX-2):- They appear to be devoid of gastrointestinal toxicity, in that they spare mucosal prostaglandin synthesis. However, a number of recent studies have raised serious questions about the two central tenets that support this approach, namely that the prostaglandins that mediate inflammation and pain are produced solely via COX-2 and that the prostaglandins that are important in gastrointestinal and renal function are produced solely via COX-1. So, a growing body of evidence shows that COX-2 (not only COX-1) also plays a physiological role in several body functions and that, conversely, COX-1 (not only COX-2) may also be induced at sites of inflammation. More recent and puzzling data shows that COX-2 is induced during the resolution of an inflammatory response, and at this point it produces anti-inflammatory (PGD$_2$ and PGF$_{2\alpha}$), but not proinflammatory (PGE$_2$) prostaglandins; inhibition of COX-2 at this point thus results in persistence of the inflammation. Moreover, COX-2 selective NSAIDs have lost the cardiovascular protective effects of non-selective NSAIDs which are mediated through COX-1 inhibition (in addition, COX-2 has a role in sustaining vascular prostacyclin production).

4. Use of dual acting anti-inflammatory drugs:– These drugs inhibit both 5-lipoxygenase and cyclooxygenase. Such compounds retain the activity of classical NSAIDs, while avoiding their main drawbacks, in that curtailed production of gastroprotective prostaglandins is associated with a concurrent curtailed production of the gastro-damaging and bronchoconstrictive leukotrienes. Moreover, thanks to their mechanism of action, dual acting anti-inflammatory drugs could not merely alleviate symptoms of rheumatic diseases, but might also satisfy, at least in part, the criteria of a more definitive treatment.

5. Use of adequate pharmaceutical formulation:– Sometimes, an adequate pharmaceutical formulation like enteric coated tablets of some NSAIDs, use of proper buffers etc. can overcome these drawbacks, but often the galenic formulation is inoperant and a chemical modification of active molecule is necessary to correct its pharmacokinetic insufficiencies.
6. **Change in route of administration:** Most NSAIDs are given as oral tablets or capsules; others are given by injection to avoid gastric irritation (e.g. ultragin 0.5 g/ml inj. in 2 ml amp and 30 ml amp). Some NSAIDs are also given by topical route in the form of gels (e.g. diclofenac 1% gel, ibuprofen 10% gel, naproxen 10% gel etc.)

7. **Prodrug approach:** This chemical formulation process whose objective is to convert an interesting active molecule into a clinically acceptable drug, often involves the so called “Prodrug design”.

**1.4 PRODRUG APPROACH:**

1.4.1 **Introduction:**

Initially, the term prodrug was introduced by Albert to describe any compound that undergoes biotransformation prior to exhibiting its pharmacological effects (Albert *et al.*, 1958). Harper referred to this process as drug latentiation, that is, chemical modification of a biologically active compound to form a new compound that, upon *in vivo* enzymatic attack, will liberate the parent compound (Harper *et al.*, 1959).

1.4.2 **Classification of Prodrug:**

Wermuth, after surveying the literature, has classified the prodrugs into two broad categories: the *carrier-linked prodrugs* and *bioprecursors* (Wermuth *et al.*, 1998).

1.4.2.1 **Carrier linked Prodrugs:**

These are drugs that have been attached through a metabolically labile linkage to another molecule, the so called promoiety, which is not necessary for activity but may impart some desirable property to the drug such as decreased toxicity. The promoiety should be easily and completely removed after it has served its function and should be non-toxic. E.g. Chloramphenicol succinate (Scheme 1) has increased water solubility and facilitated parenteral administration as compared to poorly water soluble chloramphenicol. Carrier – linked prodrugs can be subdivided even further into bipartate, tripartate and mutual prodrugs (Bhosle *et al.*, 2006).
(1) **Bipartate prodrug**: A bipartate prodrug is a prodrug comprised of one carrier attached to the drug. Example:

![Diagram of Bipartate Prodrug](image)

**Functional group link**

![Functional group link](image)

**Tolmetin-glycine prodrug**
Depending upon functional group these are sub classified as:

1. **Carboxylic acids and Alcohols:** Prodrugs of agents that contain carboxylic acid or alcohol functionalities can often be prepared by conversion to an ester. eg. Chloramphenicol palmitate. Alcohol containing drugs can be acylated with aliphatic or aromatic carboxylic acids to decrease water solubility (increase lipophilicity) or with carboxylic acids containing amino or additional carboxylate groups to increase water solubility. Conversion to phosphate or sulphate esters also increases water solubility. By using these approaches a wide range of solubilities could be achieved which in turn affect the absorption and distribution properties of the drug.

2. **Amines:** Derivatization of amines to give amides has not been widely used as a prodrug strategy due to high chemical stability of the amide linkage and the lack of amidase enzymes necessary for hydrolysis. However certain activated amides are sufficiently chemically labile for example the L–iso-leucyl derivative of dopamine, an orally absorbable prodrug of dopamine. The utility of carbamates as prodrug derivatives for amines (R – NH – (C = O) –OR) is limited due to the general resistance of carbamates to
undergo enzymatic cleavage, in vivo. By introduction of an enzymatically labile ester
function in the carbamate structure it is however, possible to circumvent this problem.

*Mannich bases.* The pKa of amines can be lowered by approximately three units by
conversion to their N–Mannich bases. Mannich bases result from the reactions of two
amines with an aldehyde or ketone, eg. Rolitetracycline. They are generally formed
by reacting an NH–acidic compound with formaldehyde or in very rare cases, other
aldehydes and a 10 and 20 aliphatic or aromatic amine.

\[
\text{R-CONH}_2 + \text{CH}_2\text{O} + \text{R}_1\text{R}_2\text{NH} \rightarrow \text{RCONH-CH}_2\text{-NR}_1\text{R}_2 + \text{H}_2\text{O}
\]

N–Mannich bases lower the basicity of the amine so that at physiological pH few of
the prodrug molecules are protonated, thereby increasing its lipophilicity. For
example, the partition coefficient between octanol and phosphate buffer at pH 7.4 for
the N–Mannich base derived from benzamide and the decongestant phenylpropanolamine hydrochloride is almost 100 times greater than for the parent
amine. By benzamidomethylation the pKa of phenylpropanolamine decreases from
9.4 to 6.2, revealing that the derivative is predominantly unprotonated at pH 7.4, a
major contributing factor to the enhanced P (lipophilicity) value of the latter
compound.

\[
\text{R=H.HCl Phenylpropanolamine Hydrochloride}
\]

\[
\text{R= CH}_2\text{NHCOC}_6\text{H}_5, \text{N- Mannich base derived from Benzamide}
\]

*Schiff bases:* Another approach for lowering the pKa of the amines, and, thereby making
them more lipophilic, is to convert them to imines (Schiff bases); however imines are too
labile in aqueous solution. The anticonvulsant agent Progabide is a prodrug form of γ-
amino butyric acid, an important inhibitory neurotransmitter. Once inside the brain it is
hydrolyzed to γ-amino butyric acid.
Azo linkage: Amines have occasionally been incorporated into an azo linkage to produce a prodrug. This type of linkage appears in sulfasalazine; which is used in the treatment of ulcerative colitis. The azo linkage is broken in the gut by the action of azo reductases produced by microflora. This releases the active agent, aminosalicyclic acid, which has an anti-inflammatory effect on the colon, and sulfapyridine. Generation of aminosalicyclic acid prior to the absorption prevents the systemic absorption of the agent and helps to concentrate the active agent at the site of action.

3. Carbonyl Compounds: A number of different functionalities have been evaluated as prodrug derivatives of carbonyls eg. aldehydes, ketones, oximes, imines and enol esters, although this approach has not found wide clinical utility. So, under hydrolysis conditions, these functionalities are reconverted to the carbonyl compounds. eg. methenamine. The most important prodrug forms of aldehydes and ketones are Schiff bases, oximes, acetal (ketals), enol esters, oxazolidines and thiazolidines. Thiazolidines have been applied as prodrug derivatives for various steroids containing a 3-carbonyl group to improve their topical anti-inflammatory activity. Thiazolidines (spirothiazolidines) of hydrocortisone and hydrocortisone 21-acetate prepared with cysteine esters (structure-1) or related β- aminothiols have been shown to be readily converted to the parent corticosteroids at conditions similar to those prevailing in the skin, thus meeting the requirement for a prodrug. Thiazolidine ring opening proceeds...
by a spontaneous $S_N^1$ cleavage of the carbon-sulphur bond to give a Schiff base intermediate which is hydrolyzed.

In particular, cysteine derivatives may be attractive as pro-moieties due to release of cysteine as a by-product. Also the carboxyl group of cysteine is easily esterifiable thus providing a convenient method for changing the lipophilicity/hydrophilicity of the spirothiazolidine prodrugs.

![Structure-1](image)

Prodrug forms of various functional groups (Table 1.1) can be summarized as follows:

**Table 1.1: Prodrug forms of various functional groups**

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Prodrug Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>C=O</td>
<td>Ketals</td>
</tr>
<tr>
<td>C=O</td>
<td>Imines</td>
</tr>
<tr>
<td>C=O</td>
<td>Enol esters</td>
</tr>
<tr>
<td></td>
<td>Oxazolidines</td>
</tr>
<tr>
<td></td>
<td>Thiazolidines</td>
</tr>
</tbody>
</table>

![Table 1.1](image)
### Chapter-1

**Introduction**

<table>
<thead>
<tr>
<th>Ester</th>
<th>$\text{HO}_2\text{C}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$-acycloxyalkyl ester</td>
<td>$\text{HO}_2\text{CCH}_2\text{OOR}_1$</td>
</tr>
<tr>
<td>Amides</td>
<td>$\text{NH}_2\text{CONHR}$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Esters</th>
<th>$\text{O}_2\text{C}-\text{R}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbonate ester</td>
<td>$\text{O}_2\text{C}-\text{O}-\text{R}$</td>
</tr>
<tr>
<td>Phosphate esters</td>
<td>$\text{PO}_2\text{OH}$</td>
</tr>
<tr>
<td>Ethers</td>
<td>$\text{OR}$</td>
</tr>
<tr>
<td>$\alpha$-acyloxyalkyl ethers</td>
<td>$\text{HC}_2\text{O}_2\text{C}-\text{R}_2\text{OR}_1$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Thioester</th>
<th>$\text{S}_2\text{C}-\text{R}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$-acyloxyalkyl ethers</td>
<td>$\text{S}_2\text{HC}_2\text{O}_2\text{C}-\text{R}_2\text{OR}_1$</td>
</tr>
<tr>
<td>Disulphides</td>
<td>$\text{S}_2\text{S}-\text{R}$</td>
</tr>
</tbody>
</table>

---

**Diagram:**

- Ester: $\text{HO}_2\text{C}$
- $\alpha$-acycloxyalkyl ester: $\text{HO}_2\text{CCH}_2\text{OOR}_1$
- Amides: $\text{NH}_2\text{CONHR}$
- Esters: $\text{O}_2\text{C}-\text{R}$
- Carbonate ester: $\text{O}_2\text{C}-\text{O}-\text{R}$
- Phosphate esters: $\text{PO}_2\text{OH}$
- Ethers: $\text{OR}$
- $\alpha$-acyloxyalkyl ethers: $\text{HC}_2\text{O}_2\text{C}-\text{R}_2\text{OR}_1$
- Thioester: $\text{S}_2\text{C}-\text{R}$
- $\alpha$-acyloxyalkyl ethers: $\text{S}_2\text{HC}_2\text{O}_2\text{C}-\text{R}_2\text{OR}_1$
- Disulphides: $\text{S}_2\text{S}-\text{R}$
<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-\text{NH}_2)</td>
<td>Amides</td>
</tr>
<tr>
<td>(-\text{NH}_2)</td>
<td>Carbamates</td>
</tr>
<tr>
<td>(-\text{R}_1\text{C}=\text{R}_2)</td>
<td>Imines</td>
</tr>
<tr>
<td>(-\text{R}_1\text{C}=\text{R}_2)</td>
<td>Enamines</td>
</tr>
<tr>
<td>(-\text{R}_1\text{N}^+\text{C}=\text{R}_2)</td>
<td>N-Mannich bases</td>
</tr>
<tr>
<td>(-\text{R}_1\text{N}^+\text{C}=\text{R}_2)</td>
<td>N-acyloxy alkoxy carbonyl derivatives</td>
</tr>
<tr>
<td>(-\text{SO}_2\text{NH}_2)</td>
<td>N-sulfonyl imidates</td>
</tr>
<tr>
<td>(-\text{SO}_2\text{NH}–\text{CH}_2\text{O}–\text{R})</td>
<td>N-sulfonyl imidates</td>
</tr>
<tr>
<td>NH-Acidic group</td>
<td>N-mannich bases</td>
</tr>
<tr>
<td>Heterocyclic amine</td>
<td>N-acyloxyalkyl derivatives</td>
</tr>
</tbody>
</table>
2) **Tripartate Prodrug**: When a carrier is connected to a linker that is to the drug, it is called a tripartate prodrug.

![Diagram of a tripartate prodrug structure](image)

1.4.2.2 **Bioprecursor Prodrugs**

It contain no promoiety but rather rely upon metabolism to introduce the necessary functionality to create an active species. Eg. Sulindac is inactive as the sulphoxide and must be reduced metabolically to the active sulfide (Scheme 2)

![Scheme 2](image)

Depending upon type of metabolic activation, these are sub-classified as:

a) **Oxidative activation**: Isozymes of cytochrome P-450 are capable of oxidizing a wide variety of functionalities, generally to produce more polar compounds. Eg. Nabumetone requires oxidative activation.

b) **Reductive activation**: It is less common than oxidative activation due to a lower number of reducing enzymes. Eg. Mitomycin C requires reductive activation.

c) **Phosphorylation**: The type of activation achieved is dependent upon the molecule phosphorylated. It is commonly required for the bioactivation of antiviral agents. These agents are commonly nucleosides, which must be converted to the nucleotides to have activity like disruption of synthesis or function of DNA or RNA. Eg. Iodoxuridine.
An ideal prodrug should possess following properties:

1. It should not have intrinsic pharmacologic activity.
2. It should rapidly transform, chemically or enzymatically, into the Active form where desired.
3. The metabolic fragments, apart from the active drug, should be non-toxic.

1.5 **Applications of Prodrug Design:**

The various applications of prodrug design are:

1. **Pharmaceutical Applications:** The undesirable organoleptic properties and the physicochemical problems associated with drug formulations can be resolved.
   a) Improvement of taste
   b) Improvement of odour
   c) Change of physical form for preparation of solid dosage forms
   d) Reduction of GI irritation
   e) Reduction of pain on injection
   f) Enhancement of drug solubility and dissolution rate
   g) Enhancement of chemical stability of drug.

2. **Pharmacokinetic Applications:**
   a) Enhancement of bioavailability (lipophilicity)
   b) Prevention of presystemic metabolism
   c) Prolongation of duration of action
   d) Reduction of toxicity
   e) Site-specific drug delivery (Drug targeting).
Introduction

a) Improvement of Taste:
One of the reasons for poor patient compliance, particularly in case of children, is the bitterness, acidity or causticity of the drug. Two approaches can be utilized to overcome the bad taste of the drug. The first in reduction of the drug solubility in saliva and the other is to lower the affinity of drug toward taste receptors, thus making the bitterness or causticity imperceptible. Examples of such drugs are:

<table>
<thead>
<tr>
<th>Parent Drug</th>
<th>Prodrug with improved taste</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloramphenicol</td>
<td>Palmitate ester</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>Palmitate ester</td>
</tr>
<tr>
<td>Sulfisoxazole</td>
<td>Acetyl ester</td>
</tr>
<tr>
<td>Triamcinolone</td>
<td>Diacetate ester</td>
</tr>
</tbody>
</table>

b) Improvement of odour:
The odour of a compound depends upon its vapour pressure (and hence boiling point); a liquid with high vapour pressure (and low boiling point) will have a strong odour. Ethyl mercaptan is one such drug which is a foul smelling liquid of b.p 35 degree. The drug useful in the treatment of leprosy is converted into its phthalate ester, diethyldithio- isophthalate which has higher boiling point and is odourless. The prodrug is administered by rubbing on the skin. After absorption, the ester is metabolized to parent drug by thioesterases.

\[
\text{Phthalate ester of Ethyl mercaptan}
\]

c) Change of physical form of the drug:
Some drugs which are in liquid form are unsuitable for formulation, a tablet especially if their dose is high. The method of converting such liquid drugs into solid prodrugs involves formation of symmetrical molecules having tendency to crystallize; for example esters of ethyl mercaptan and trichloroethanol.
d) **Reduction of GI irritation:**
Several drug causes irritation and damage to the gastric mucosa through direct contact, increased stimulation of acid secretion or through interference with the protective mucosal layer. The NSAIDs, especially the salicylates, have such tendency. They are designed to overcome the problem of gastric pH and induce or aggravate ulceration. Examples of prodrugs designed to overcome such problems of gastric distress are:

<table>
<thead>
<tr>
<th>Parent drug</th>
<th>Prodrugs that causes no gastric distress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salicylic acid</td>
<td>Salsalate, Asprin</td>
</tr>
<tr>
<td>Diethyl stilbestrol</td>
<td>Fosfestrol</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>Kanamycin pamoate</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>Nicotinic acid hydrazide</td>
</tr>
</tbody>
</table>

![Nicotinic acid hydrazide](image)

e) **Reduction of pain on injection:**
Intramuscular injections are particularly painful when the drug precipitates or penetrates into the surroundings cells or when the solution is strongly acidic, alkaline or alcoholic; for example, the low aqueous solubility of clindamycin hydrochloride and the alkaline solution of phenytoin are responsible for pain on injection. This can be overcome by the use of more water soluble prodrugs of such agents, for example, the 2-phosphate ester of clindamycin.
f) **Enhancement of solubility and dissolution rate (Hydrophilicity) of drug:**

Hydrophilic or water soluble drugs are desired where dissolution is the rate limiting step in the absorption of poorly aqueous soluble agents or when parenteral or ophthalmic formulation of such agents are desired. Drugs with hydroxyl function can be converted into their hydrophilic forms by use of half esters such as hemisuccinates, hemiglutarates or hemiphthalates; the other half of these acidic carriers can form sodium, potassium or amine salts or render the moiety water soluble. For phenolic drugs and some alcohols as in the case of steroidal drugs such as cortisol, predinosolone, betamethasone and dexamethasone, the sodium succinates salts have poor chemical stability and hence phosphate esters are preferred. Glycosidic prodrugs of some agents and L-lysine esters of benzodiazepines are also water soluble. Such hydrophilic promoieties when meant for parenteral use are advantageous over their propylene glycol solutions which are toxic or painful. Many water soluble prodrugs for oral use acts as substrates for enzymes in the brush border region of microvilli where they are biotransformed into free active drug having lipophilic property facilitating permeation across the biomembrane and rapid absorption. Examples of hydrophilized prodrugs are:

<table>
<thead>
<tr>
<th>Parent Drug</th>
<th>Prodrugs with Enhanced Hydrophilicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tocopherol</td>
<td>Sodium succinate ester</td>
</tr>
<tr>
<td>Testosterone</td>
<td>Phosphate ester</td>
</tr>
<tr>
<td>Menthol</td>
<td>Beta-glycoside</td>
</tr>
<tr>
<td>Diazepam</td>
<td>L-lysine ester</td>
</tr>
</tbody>
</table>
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Introduction

Testosterone phosphate ester

g) Enhancement of chemical stability:
A drug may destabilize either during its shelf-life or in the GIT when used orally. Shelf-life stability is particularly important in case of drugs for intravenous use. The conventional approach is to lyophilize such solutions into powder which can be reconstituted before use. An example of this is the antineoplastic drug, Azacytidine. The aqueous solution of this drug is readily hydrolyzed but the bisulfite prodrug is stable to such degradation at acidic pH and is more water soluble than the parent drug. The prodrug converts to active drug at the physiological pH of 7.4.

Azacytidine                                          Azacytidine bisulfite

h) Enhancement of bioavailability (Lipophilicity):
Most drugs are absorbed by passive diffusion for which lipophilicity is an important prerequisite. Two reasons can be attributed to the enhanced oral bioavailability of lipophilic compounds:

i. The lipophilic form of a drug has enhanced membrane or water partition coefficient as compared to the hydrophilic form thus favouring passive diffusion; for example, the pivampicillin, bacampicillin and talampicillin prodrugs of ampicillin are more lipophilic, better absorbed (above 98%) and rapidly hydrolyzed to the parent drug in blood.

ii. The lipophilic prodrugs, for example, the esters of erythromycin, have poor solubility in gastric fluids and thus greater stability and better absorption.
A big advantage of increased bioavailability through increased lipophilicity is reduction in drug dosage. For example, Bacampicillin is as effective as ampicillin in just one-third the dose of latter. The bioavailability of topically administered drug also depends upon lipid solubility. Skin penetration of polar drugs can be improved by esterification to form lipid soluble compounds. For example glyceryl ester of naproxen.

\[
\begin{align*}
\text{Glyceryl ester of naproxen} \\
\text{Propranolol succinate}
\end{align*}
\]

i) **Prevention of presystemic metabolism:**
Several corticosteroids undergo extensive first pass hepatic metabolism which can be prevented by the use of their ester or ester prodrugs – for example, triamcinolone acetonide. Propranolol is another drug with high first-pass hepatic metabolism: its hemisuccinate prodrug is resistant to esterases of liver. The first pass hepatic metabolism of opiate narcotic agonist or antagonist can be reduced by protecting the enzyme labile phenolic hydroxyl group (that undergo rapid glucuronidation) by forming a prodrug with aspirin. The parent drug is regenerated during first pass transit of the prodrug through liver.

j) **Reduction of Toxicity:**
An important objective of drug design is to develop one with high activity and low toxicity. Example of prodrug design is the bioprecursor sulindac. As a sulfoxide, it
does not cause any gastric irritation and is absorbed better. In blood, it is converted to its active sulfide form.

\[
\text{Sulfide (Active)} \quad \text{Sulindac (Inactive)} \quad \text{Sulfoxide (Inactive)}
\]

k) **Prolongation of duration of action:**

Frequent dosing is required for drugs having short biological half lives; this can be overcome by use of both controlled release and prodrug approaches. The two rate-controlling steps in the enhancement of duration of drug action are:

1. The rate of release of prodrug form the site of application or administration into the systemic circulation, and
2. The rate of conversion of prodrug into active drug in blood.

The easier approach that of controlling the release rate of prodrug is useful when in vivo conversion of the latter into active drug is rapid. Examples include the IM depot injections of lipophilic ester prodrug of steroids (testosterone cypionate and propionate, estradiol propionate) and antipsychotics (fluphenazine enanthate and decanoate). Since testosterone and estradiol are natural soft drugs, their lipophilic prodrugs are sometimes called prodrug-soft drugs.

The second approach of controlled conversion of prodrug to active drug, although difficult, was successfully utilized to deliver pilocarpine to eyes in the treatment of glaucoma. The diesters of the drug when applied as ophthalmic solutions showed better intraocular penetration due to improved lipophilicity, and slow conversion of the ester prodrug to active pilocarpine prolonged the therapeutic effect. The rate of conversion, however, is greatly dependent upon the ester group.

\[
\text{Estradiol propionate}
\]
l) **Site – specific drug delivery (Drug targeting):**

After its absorption into the systemic circulation, the drug is distributed to the various parts of the body including the target site as well as the nontarget tissues. Such a distribution pattern has several disadvantages.

1. The drug may lead to undesirable toxic effects in the nontarget tissues (if therapeutic index is low).
2. If the target site has a long distribution time, the drug may get eliminated without reaching such a site.
3. Even if the drug reaches the target cells in sufficient amounts, it may not be able to penetrate into them.
4. A smaller fraction of the drug will reach its target site because of dilution due to distribution which may be insufficient to evoke the therapeutic response.

These problems can be overcome by targeting the drug to its site of action by altering its disposition characteristics. It includes the following:

   1. Selective Uptake System
   2. Redox System for Drug Delivery to Brain
   3. Site - Specific Drug Delivery in Cancer
MAJOR APPLICATIONS OF PRODRUGS

↑ Corneal absorption
eg. Dipivefrin HCl

↑ Oral absorption
eg. Hetacillin

↓ Side effects and toxicity;
1) Alleviation of pain at site of injection. eg. Chloramphenicol succinate;
2) ↓GI toxicity eg. Sulindac and NSAIDs esters

↓ Bioavailability

Major applications of prodrugs

Prevention of first pass metabolism.
eg. Cefpodoxime Proxetil

Synergistic action of Mutual prodrugs
eg. Estramustine

Prolonged duration of action eg. Dipivefrin

Site specific drug delivery

Site directed drug delivery

Localized delivery eg. Dipivefrin in cornea

Site specific bioactivation
eg. Sulfasalazine

Systemic delivery eg. L-Dopa

Fig. 1.8: The diagrammatic representation of applications of prodrugs
1.6 **Various Approaches To Prodrug Design:**

1. **Mutual Prodrug approach:**

   In this approach, carrier used is another biologically active drug instead of some inert molecule. A mutual prodrug consists of two pharmacologically active agents coupled together so that each acts as a promoiety for the other agent and vice versa. The carrier selected may have the same biological action as that of the parent drug and thus might give synergistic action, or the carrier may have some additional biological action that is lacking in the parent drug, thus ensuring some additional benefit. The carrier may also be a drug that might help to target the parent drug to a specific site or organ or cells or may improve site specificity of a drug. The carrier drug may be used to overcome some side effects of the parent drugs as well.

2. **Macromolecular prodrug approach:**

   In this approach, macromolecules like polysaccharides, dextrans, cyclodextrins, proteins, peptides and polymers are used as carriers.

3. **Double prodrug or cascade-latentiated prodrug approach:**

   In this, a prodrug is further derivatized in a fashion such that only enzymatic conversion to prodrug is possible before the latter can cleave to release the active drug.

4. **Site-specific prodrug approach:**

   This approach involves a carrier which acts as a transporter of the active drug to a specific targeted site.

1.7 **Mutual Prodrug Approach:**

   One of the recent trends emerging in prodrug research is the increased interest in the application of mutual prodrug concept. Mutual prodrug is a type of carrier-linked prodrug, where the carrier used is another biologically active drug instead of some inert molecule (Bhosle et al., 2006). Mutual prodrugs are synthesized toward a pharmacological objective of improving each drug’s efficacy, optimizing delivery and lowering toxicities. In mutual prodrug, each component drug functions as the “pro” portion with respect to the other. Like a prodrug, a mutual prodrug is converted into the component active drugs within the body through enzymatic and/or non-enzymatic reactions. Mutual prodrugs can be classified as, for example, carrier- linked prodrugs, bio-precursor prodrugs, or chemical activation prodrugs, depending upon their constituents and composition (Rao et al., 2003). Mutual prodrugs are
typically similar to single active agent prodrugs in regard to pharmaceutical and pharmacological activities, such as absorption, disposition, metabolism and excretion.

The concept of Mutual prodrug of Nonsteroidal anti-inflammatory drugs (NSAIDs) got attention in 1942 with the synthesis of sulfasalazine, a mutual prodrug of 5-amino salicylic acid and sulfapyridine to be used in ulcerative colitis. Carrier selected for mutual prodrug of NSAIDs either may have the same biological action as that of the parent drug for synergistic action or the carrier may have some additional action that is lacking in the parent drug for additional benefit. Mutual prodrug research of NSAIDs has undergone considerable expansion during the past two decades and an overview of the research work done in design and development of mutual prodrugs can be found in many books and reviews published on this subject (Singh et al., 1994).

Till now, mutual prodrug approach was gaining popularity for reducing gastrointestinal side effects and ulcerogenicity of NSAIDs. Recently this approach is also used in site-specific delivery of NSAIDs.

1.7.1 **Classification of mutual prodrug:**

Depending upon their constituents and composition, Mutual prodrugs can be classified as,

- Carrier- linked mutual prodrug. It can be further subdivided into two types:
  - Bipartate carrier- linked mutual prodrug
  - Tripartate carrier- linked mutual prodrug

- Bio-precursor mutual prodrugs or Chemical activation prodrugs.

*Carrier- linked mutual prodrug* is bipartate or tripartate where a synergistic drug acts as the carrier. Bipartate mutual prodrug is having a pharmacologically active carrier drug which is directly attached to parent drug where as in case of tripartate mutual prodrugs; the carrier drug is not linked directly to the parent drug but instead through a linker so it allows for decreased steric hindrance during enzymatic cleavage that may occur with bipartate prodrugs. Here Carrier drug is enzymatically cleaved from linker in first step then linker is spontaneously cleaved from parent Drug.

*Bio-precursor mutual prodrugs* produce their effects after in vivo chemical modification of their inactive form. Bioprecursor prodrugs rely on oxidative or
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reductive activation reactions unlike the hydrolytic activation of carrier-linked prodrugs.

1.7.2 **Objective/ Reasons of Mutual Prodrug:**

Mutual prodrug design is really no different from the general drug discovery process, in which a unique substance is observed to have desirable pharmacological effects, and studies of its properties lead to the design of better drugs. The main objectives of a mutual prodrug designing are:

- To bring both active drugs to their respective active sites. To provide the desired pharmacological effects while minimizing adverse metabolic and/or toxicological events.

- To improve the clinical and therapeutic effectiveness of those drugs which suffer from some undesirable properties that otherwise hinder their clinical usefulness.

- To avoid the practice of clinically co-administering two drugs in order to enhance pharmacological activity or prevent clinical side effects. Simultaneous administration does not guarantee equivalent absorption or transportation to site of action. So, mutual prodrug concept is useful when two synergistic drugs need to be administered at the same site at the same time. Mutual prodrugs are synthesized toward a pharmacological objective of improving each drug's efficacy, optimizing delivery, and lowering toxicities.

1.7.3 **Selection criteria for mutual prodrug synthesis**

a) The candidate drugs selected for mutual prodrug synthesis can be from one therapeutic category or from different therapeutic categories. Similarly, the constituent drugs of a mutual prodrug can act on the same biological target with similar mechanism of action or act on different biological targets with different mechanisms of action.

b) The candidates for making mutual prodrugs can be the pairs of drugs that are currently used in combination therapy (including those combination studies at investigational stage) in various therapeutic areas provided each of those drugs possesses the requisite functional group(s). There are a number of therapeutic areas where such combination therapy is applied routinely and successfully.
c) The linkage between the first and second component should be a cleavable linkage. For example, the linkage may be hydrolyzable and/or may be enzymatically cleavable. Preferably, the linkage should be cleavable under physiological conditions, such as those present in a mammalian body, particularly a human body.

1.7.4 Mechanism of activation:

Like a prodrug, a mutual prodrug is converted into the component active drugs within the body through enzymatic and/or non-enzymatic reactions

1. In vivo metabolic activations of bioprecursor mutual prodrugs
   a) Oxidative Activation
      • N- and O-Dealkylation
      • Oxidative Deamination
      • N-Oxidation
      • Epoxidation
   b) Reductive Activation
      • Azo Reduction
      • Sulfoxide Reduction
      • Disulfide Reduction
      • Bioreductive Alkylation
      • Nitro Reduction
   c) Nucleotide Activation
   d) Phosphorylation Activation
   e) Decarboxylation Activation

2. Intramolecular activation:

Active Drug as the cyclic product of intramolecular activation is one of the important approach proposed to explain the activation of some mutual prodrugs. This approach found application in explaining the release the parent drugs from carbamate mutual prodrugs in aqueous buffer (pH 6-11) and plasma (pH 7.4) through intramolecular reactions due to a hydroxyl nucleophile (Vigroux et al., 1995).
1.7.5 Methodologies Used for Mutual prodrug synthesis:

Synthesis of Mutual prodrug is basically a concept of designing drug through conjunction of two different pharmacophores having similar or different pharmacological activities. Before synthesizing mutual prodrugs, following queries arises regarding the linkage between two pharmacologically active drugs:

- What types of groups are the easiest to link to a carrier drug?
- What types of groups are the easiest to cleave from a carrier drug?

These are suitably answered by study of nature of functional groups forming a suitable linkage or bond between two drugs which get easily hydrolyzed by suitable enzymes. Mutual Prodrug forms of various functional groups are shown in Table 1.

There are so many methodologies followed to synthesize mutual prodrug depending upon the functional group attached to parent drug or carrier drug. Among them some are given below:

- Esterification
- Amidation
- Using spacer technique
- Azo linkage for example Sulfasalazine
- Enzymatic Regioselective methodology
- Elaborate protection/deprotection and separation strategies
- Multi-step chemical reaction synthesis

1.7.6 Methods of Evaluation of Mutual Prodrug:

1. Solubility Measurement:

The solubility measurement of mutual prodrug is carried out by placing an excess amount of mutual prodrug in separate vials containing different solvents like 10 ml deionized water, n-hexane, phosphate buffer of different pH etc and then stirring at 37 °C for 24 hours. The solutions are centrifuged for 5 min at 9000 rev/min and the supernatant is filtered with cellulose acetate membrane filters. The mutual prodrug concentration in each filtrate is determined by suitable analytical technique like HPAE-PAD / UV spectroscopy/ HPLC after the appropriate dilution.
2. **Determination of Partition Coefficients:**

   By shaking flask method partition coefficient of mutual prodrugs can easily be determined.

3. **In vitro Hydrolytic study in Different Buffers:**

   Hydrolysis studies are carried out in aqueous buffer so as to study whether the mutual prodrug hydrolyzes in an aqueous medium and to what extent or not, suggesting the fate of mutual prodrug in the system. Mutual prodrugs may also be subjected to *in vitro* hydrolysis in simulated gastric fluid (SGF) at pH 1.2, simulated intestinal fluid (SIF) at pH 7.4 and SIF + 80% human plasma at pH 7.4. The kinetics of hydrolysis is monitored by the increase of free drug concentration with time and the order of the reaction and half-life \(t_{1/2}\) are calculated. The rate of hydrolysis is calculated using the equation:

   \[
   k_H = \frac{(2.303)}{t} \log \left(\frac{a}{a-x}\right)
   \]

   Where \(k_H\) represents the hydrolysis constant, \(t\) is the time in min, \(a\) is the initial conjugate concentration, \(x\) is the amount of Mutual prodrug hydrolyzed and \((a-x)\) is the amount of the remaining prodrug.

   The graph between % cumulative amounts of drug release after hydrolysis versus time is also plotted to study the *in vitro* hydrolysis of mutual prodrug in SGF, SIF and 80% plasma. For better absorption, mutual prodrug should not hydrolyze appreciably in these fluids. Jyoti Rawat, have studied *in vitro* hydrolysis of mutual prodrugs of isoniazid, *p*-amino salicylic acid and ethambutol *(Rawat et al., 2007)*.

4. **In vivo enzymatic hydrolysis:** *An in vivo* study are conducted to determine the plasma concentration drug time profile using suitable analytical technique like UV spectrophotometric determination, HPLC etc. In this study all mutual prodrugs and individual drugs are administered to animals and after particular intervals drug concentrations can be determined in serum.

   The reactions are initiated by adding stock solution of mutual prodrug in suitable solvent to preincubated 80% human plasma with isotonic phosphate buffer (pH 7.4) and incubated at 37°C. At appropriate time intervals the plasma reaction are withdrawn and deproteinized by mixing with acetonitrile. After centrifugation for 10
min at 104 rpm, clear supernatant is analyzed by suitable analytical technique like HPLC.

5) **In vitro biological activity:** In vitro transport (diffusion) is evaluated by Franz cell diffusion experiments using shed snakeskin. Shed snakeskin is widely recognized as a sufficient model membrane to human skin for preliminary permeability studies due to the similarity in its composition to the human stratum corneum. Benorylate (4-acetamido phenyl-O-acetylsalicylate) hydrolysis in vitro by human plasma and by human liver microsomes and cytosol has been investigated by F M Williams (Williams et al., 1989).

6) **Chemical stability (kinetics of chemical hydrolysis):** A Mutual prodrug should be chemically stable so that it can be formulated in an appropriate pharmaceutical dosage form with optimum half life. At the same time, it should be biolabile to regenerate the parent drug molecules to exhibit therapeutic activity. For this purpose the kinetics of chemical hydrolysis is studied at 37 °C using buffer solutions of different pH. Different pharmacokinetic parameters obtained/ calculated during this study like rate constant, order of reaction and half-life helps in determining the chemical stability of mutual prodrug. The chemical stability of mutual prodrug is also studied at various temperatures.

7) **Protein binding studies:** Protein binding of mutual prodrug is studied by preparing a solution of the mutual prodrug in phosphate buffered saline (PBS, pH 7.4). 100 mL of this solution is placed in a beaker. The cellophane membrane firstly washed with distilled water and then with buffer solution (pH 7.4) is tied at the opening end of a dialysis tube; the dialysis tube containing (6 %) egg albumin is dipped into the drug solution and covered. The whole assembly is placed on a magnetic stirrer and set at low revolutions per minute. The temperature must be maintained at 37 ± 0.5 °C. After each 1 h, 1 mL of the PBS containing drug solution is replaced with fresh 1 mL of PBS. The withdrawn sample is further diluted with 1 mL phosphate buffer and the concentration of the mutual prodrug is estimated using a suitable analytical technique.

8) **In vivo pharmacokinetic study:** Tissue homogenates are used for in vivo pharmacokinetic study.
9) **Biochemical studies:** Suitable markers are used to determine biological activity of mutual prodrug. For example for evaluating anti-inflammatory activity of mutual prodrug TNF- alpha, IL-1 β and IL-6 etc are used. Biochemical evaluation was carried by M. Madhukar et al (2010) by using various peripheral markers of oxidative stress including lipid peroxidation (MDA levels), myeloperoxidase activity (MPO levels), superoxide dismutase activity (SOD) and catalase activity to study the effect of mutual prodrug of 4-biphenylacetic acid and quercetin tetramethyl ether (BPAeQTME) (Madhukar et al., 2010).

1.7.7 **Mutual prodrugs of different therapeutic categories:**

There are so many mutual prodrugs which are basically designed for specific purpose like to decrease the dose of parent drug if carrier moiety is also of same pharmacological action, to show additional effect than parent drug which is required for specific diseased condition, to improve pharmacokinetic profile, to decrease side effects, to shorten as well as prolong action and to alter bioactivation etc. Mutual prodrugs belonging to different therapeutic area are listed in table 1.2.

Table 1.2: Examples of Mutual prodrugs belonging to therapeutic areas

<table>
<thead>
<tr>
<th>Therapeutic Area</th>
<th>Mutual Prodrug</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antitubercular Drugs</td>
<td>Mutual prodrugs of isoniazid, p-amino salicylic acid and ethambutol (Rawat et al., 2007)</td>
<td>To eliminate the problem of fast metabolism, toxicity and local irritation and reduction of therapeutic doses.</td>
</tr>
<tr>
<td>Antipsychotic</td>
<td>Mutual prodrug ester of GABA and perphenazine (Nudelman et al., 2008).</td>
<td>To minimize the extrapyramidal effects</td>
</tr>
<tr>
<td>Anlgesic in Neuropathic pain</td>
<td>Gabapentin-Pregabalin Mutual Prodrugs (Shi et al., 2005).</td>
<td>To show better effectiveness in reversing tactile allodynia in CCI rats</td>
</tr>
</tbody>
</table>
### Anticancer

<table>
<thead>
<tr>
<th>Prodrug Type</th>
<th>Description</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutual prodrugs All-trans-Retinoic Acid and Histone Deacetylase Inhibitors</td>
<td>To show differential antiproliferative potencies in both MDA-MB-231 and PC-3 cell lines.</td>
<td></td>
</tr>
<tr>
<td>5-Fluorouracil / Cytarabine Mutual Prodrugs</td>
<td>To show synergistic effect therefore help in reduction of dose as well as toxicity</td>
<td></td>
</tr>
</tbody>
</table>

### Anti-viral Agents

<table>
<thead>
<tr>
<th>Prodrug Type</th>
<th>Description</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutually prodrugs of 2′,3′-dideoxyinosine with 3-octadecyloxy-propane-1,2-diol</td>
<td>To show synergistic effect with different mechanisms and to release the parent drugs at desired site of action.</td>
<td></td>
</tr>
</tbody>
</table>

### Pulmonary Inflammation and Bronchoconstriction

<table>
<thead>
<tr>
<th>Prodrug Type</th>
<th>Description</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutual Prodrugs of Anti-Inflammatory Signal Transduction Modulators (AISTM’s) and Beta-Agonists</td>
<td>For producing synergistic effects with different mechanism of action in the treatment of Pulmonary inflammation and Bronchoconstriction</td>
<td></td>
</tr>
</tbody>
</table>

### Cardiovascular Drugs

<table>
<thead>
<tr>
<th>Prodrug Type</th>
<th>Description</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutual prodrugs of Amlodipine and Atorvastatin</td>
<td>For the treatment of arthrosclerosis, angina pectoris, combined hypertension and hyperlipidaemia and the management of cardiac risk</td>
<td></td>
</tr>
</tbody>
</table>

### Non-steroidal Anti-inflammatory Drugs (NSAIDS)

<table>
<thead>
<tr>
<th>Prodrug Type</th>
<th>Description</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benorylate (Mutual prodrug of paracetamol and aspirin)</td>
<td>For Reduction of Gastro-intestinal side effects and ulcerogenicity of NSAIDs</td>
<td></td>
</tr>
<tr>
<td>4-biphenylacetic acid and quercetin tetramethyl ether (BPA-QTME)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**Introduction**
Chlorzoxazone esters of acidic NSAIDs (Ahmed et al., 2009).

Indomethacin–flavonoid Mutual prodrug (Sawraj et al., 2010).

Aminoalcohol ester analogues of Indomethacin (Parmeshwari et al., 2007).

Coupling with amino acids (Mishra et al., 2008).

Paracetamol (acetaminophen) esters of some acidic NSAIDs (Bhosale et al., 2004).

Naproxen propyphenazone mutual prodrugs (Sheha et al., 2002).

Mutual prodrugs of NSAIDs and natural antioxidants (Manon et al., 2009).

Conjugation of NSAIDs with H$_2$ antagonist (Ueda et al., 1991).

Chemically coupling a nitric oxide (NO) releasing moiety to the parent NSAID (Burgaud et al., 2002).
Glucosamine conjugate prodrug of NSAIDs (Capomacchia et al., 2008).

Colon-specific mutual prodrug of 5-aminosalicylic acid (5-ASA) and sulfapyridine (Nagpal et al., 2006).

Aceclofenac colon specific mutual amide prodrug (Rasheed et al., 2009).

### 1.7.8 Advantages of making mutual prodrug:

1. Help in reduction of Side-effects of Parent Drugs
2. Produces synergistic effect
3. Give additional biological action as that of parent drug.
4. Reduction in dose due to synergistic effect
5. Improve pharmacokinetics of Parent drug

### 1.7.9 Limitations of mutual prodrug design:

Even if mutual prodrug design has proven highly beneficial in overcoming various undesirable properties of drugs, it can also give rise to a large number of newer difficulties, especially in the assessment of pharmacological, pharmacokinetic, toxicological and clinical properties (Bhosle et al., 2006).

i. **Problems at the pharmacological level:** These compounds cannot be submitted to preliminary in vitro screening tests like binding studies, reuptake of neurotransmitter and enzyme inhibition measurement because bioactivation to their active species is necessary.

ii. **Problems at the toxicological level:** Even though mutual prodrugs are derived from well-known active principles, they have to be regarded as new entities. In a review by Gored, he has cited certain toxicity mechanisms like formation of...
toxic metabolite of total prodrug which is not produced by the parent drugs, consumption of vital constituent during prodrug activation process, generation of a toxic derivative, release of a pharmacokinetic modifier which may cause enzyme induction or alter drug excretion.

iii.  
Problems at the pharmacokinetic Studies: The mutual prodrug may not be an ideal substrate for the activating enzymes. So, it is necessary to consider modifying the carrier with electron withdrawing or donating groups to facilitate the hydrolysis. Pharmacokinetic studies may lead to numerous misinterpretations. When mutual prodrug and parent molecules are being compared, one must take into account the differences in their respective time courses of action. The maximum activity may appear later for mutual prodrug than for parent compounds, so area under the curve should be compared as it presents a better criterion for comparison.

iv.  
Problems at the clinical stage: The predictive value of animal experiments is also questionable. The active doses of two mutual prodrugs of the same parent drugs may appear to be same in rats but may be quite different in clinical investigations.

1.7.10 Patents filed in the area of Mutual prodrug

- Monophosphates as Mutual Prodrugs of Anti-Inflammatory Signal Transduction Modulators (AISTM's) and Beta-Agonists for the Treatment of Pulmonary Inflammation and Bronchoconstriction (Swaminathan et al., 2010).
- Mutual prodrugs and methods to treat cancer (Njar et al., 2010).
- Glucosamine and glucosamine/anti-inflammatory mutual prodrugs, compositions, and methods (Capomacchia et al., 2008).
- Mutual prodrug of amlodipine and atorvastatin (Crook et al., 2004).
- Prodrugs Containing Bio- Cleavable Linkers (Satyam et al., 2007).
- Monophosphates as mutual prodrugs of muscarinic receptor antagonists and β-agonists for the treatment of COPD and chronic bronchitis (Baker et al., 2008).
- Hepatoprotectant acetaminophen mutual prodrugs (Muhammad et al., 2009).
1.7.11 Docking:
Docking of the mutual prodrug into active site of receptor is conducted in order to predict the affinity and orientation of prodrug at the enzyme active site. It is very much helpful in predicting the ligand-enzyme interactions at the active sites. Suitable software can be used for this purpose.
A.Z. Abdal Azeem et al. (2009) performed Docking studies of non-steroidal anti-inflammatory drugs and their mutual prodrug esters with chlorzoxazone by MOE (Molecular Operating Environment) using murine COX-2 co-crystallized with SC-558 (PDB ID: 1CX2) as a template. The recent determination of the three-dimensional co-crystal structure of murine COX-2 complexed with SC-558 has led to the development of a model for the topography of NSAIDs binding site in human COX-2. This might enable the prediction of the orientation and interaction of parent drugs and their ester prodrugs into COX-2 active site. They performed 100 docking iterations for each ligand and the top scoring configuration of each of the ligand-enzyme complexes was selected on energetic ground. The output of docking simulation was the scoring function which reflects the binding free energy \( \delta G \) in kcal/mol (S), value proportional to the sum of Gaussian \( R1R2\exp(-0.5d^2) \), where \( R1 \) and \( R2 \) are the radii of atoms in Angstrom Å and \( d \) is the distance between the pair in Å (ASE) a linear combination of \( (S, ASE, Econf) \) where \( Econf \) is an estimated self-energy of the ligand in kcal/mol (E) (Williams et al., 1989).

1.7.12 Applications of Mutual Prodrug approach in Improving therapeutically index of NSAIDs (Bhosle et al., 2006).
1) Reduction of GI side effects & ulcerogenicity of NSAIDs: NSAIDs are amongst the most successful group of drugs ever marketed, which demonstrates their overall efficacy. Despite the excessive use of NSAIDs in various inflammatory conditions and the intensive research going on in the development of NSAIDs, their clinical value is still limited due to gastrointestinal side effects like gastric irritation, ulceration, bleeding and perforation and in some cases may develop into life threatening conditions. These GI side effects are due to direct local action on the gastric mucosa, particularly of acidic NSAIDs and/or generalized systemic action that takes
place after absorption of these agents. This problem has been solved by derivatization of carboxylic function of NSAIDs into ester and amide mutual prodrugs using amino acid like L-tryptophan, L-glycine, and L-histidine as carriers that have marketed anti-inflammatory activity of their own. Paracetamol (acetaminophen) esters of some acidic NSAIDs were synthesized and evaluated as mutual prodrug forms with the aim of improving the therapeutic index through prevention of the gastrointestinal toxicity. These synthesized ester mutual prodrugs were found to have better therapeutic index than the parent drugs. Researchers such as Bhosale and co-workers have also made attempts to produce mutual prodrugs of ibuprofen/paracetamol and ibuprofen/salicylamide. The goal of this research work was to produce prodrugs of NSAIDs to reduce the associated side effects.

2) **Mutual prodrug of NSAIDs with additional antiarthritic activity**: Mutual prodrugs of ketoprofen, ibuprofen, diclofenac and flurbiprofen with an antiarthritic, nutraceutical D-glucosamine have been reported with reduced gastrointestinal ulcerogenicity, better analgesic/anti-inflammatory effects and additional antiarthritic activity. Glucosamine is used as an antiarthritic drug and nutritional supplement in conditions like joint ache, stiffness, severely restricted movements and serious pain. It acts as an essential substrate for the biosynthesis of glucosaminoglycans and the hyaluronic acid backbone needed for formation of proteoglycans found in the structural joints.

3) **Site-specific Drug delivery**: A drug, after its absorption into systemic circulation, gets distributed to target site as well as non-targeted tissues. The distribution of drug to non-targeted tissues may lead to undesirable toxic effects in those tissues and insufficient concentration in the target site to evoke any therapeutic response. If the target site has a longer distribution time, the drug may get eliminated without reaching such a site; and even if the drug reaches the targeted area in sufficient concentrations, it may have such a low penetration power that it may not penetrate the target cells at all. Targeting the drug to its site of action through prodrug concept has been utilized to overcome these problems. While designing the prodrug, utilization of the
enzymes that are specifically present in that organ or tissue or specific constant pH of that area which is different from body pH should be made so that the prodrug releases the drug only in the targeted organ.

Sulfasalazine is the classic example of colon-specific mutual prodrug of 5-aminosalicylic acid (5-ASA) and sulfapyridine, used in the treatment of ulcerative colitis. 5-ASA and sulfapyridine are linked together by azo linkage, which is reduced only in the colon by azo reductases secreted by colonic microflora. This releases the active agent 5-ASA in the colon, having anti-inflammatory effect on the colon along with sulfapyridine. The advantage of this approach is that the cleavage of azo linkage and generation of 5-ASA prior to the absorption prevents its systemic absorption and helps it to concentrate at the active site. Sulfapyridine was selected as a carrier in this mutual prodrug design by taking into account its antibacterial activity, but even though sulfapyridine proved to be a good carrier for targeting 5-ASA to colon, it gave rise to many side effects resulting from its systemic toxicity. Therefore, even if according to definition, sulfasalazine is a mutual prodrug, due to disadvantages of its carrier, it cannot be referred to as a true mutual prodrug. This led to the development of interesting mutual prodrug of 5-ASA called olsalazine which is actually a diamet of 5-ASA, where 5ASA is linked through azo linkage to one more molecule of 5-ASA. When it reaches the large intestine, it is cleaved, releasing two molecules of 5-ASA for every molecule of olsalazine administered. This design overcomes the drawbacks of sulfasalazine, targets 5-ASA to colon, and fulfils all requirements of mutual prodrug too. Improvement in the bioavailability of 5-ASA is also achieved by this design.

4) **Synergistic action with or without some additional benefit:**

Chlorzoxazone [5-chloro-2 (3H)-benzoxazolone] is a centrally active muscle relaxant, while acetaminophen (N-acetyl-p-aminophenol) exhibits analgesic properties. Owing to their synergistic effects, these two drugs can be prescribed together. Using this rationale, a mutual prodrug of chlorzoxazone and acetaminophen has been designed, and its synthesis and kinetics have been reported.
Mutual Prodrug of NSAID-Propyphenazone:

Propyphenazone is a non-acidic pyrazole drug with good analgesic, antipyretic and weak anti-inflammatory property. It is available in Europe for the treatment of mild to moderate pain and fever. It replaced aminopyrine in many combination analgesic products in 1970s, after aminopyrine was discovered to be myelotoxic and carcinogenic. The metabolism of propyphenazone has been reported to proceed via the formation of 3-hydroxymethyl-propyphenazone (HMP), which is pharmacologically as active as the parent drug. Naproxen-propyphenazone hybrid drug esters and/or amides have been already synthesized as mutual prodrugs which revealed improvement in the therapeutic index of the parent drugs.

The rational of present work is to couple carboxylic acid containing NSAIDs and propyphenazone to achieve many advantages related to synergistic analgesic effects with reduced GI irritation. Ester prodrugs should exhibit decreased toxicity since they neither possess a free carboxylic acid group nor do they inhibit prostaglandin synthesis.

Mutual prodrug of NSAID- Allopurinol:

Allopurinol is a widely used agent for the treatment and prevention of hyperuricemic states such as gout. Allopurinol and its main metabolite oxipurinol lower the level of uric acid in plasma and urine by inhibiting xanthine oxidase, the enzyme catalyzing the oxidation of hypoxanthine to xanthine and xanthine to uric acid. In addition to its use as prophylaxis against and treatment of gout and other chronic hyperuricemic states allopurinol is commonly used to prevent the development of hyperuricosuric that often results from the rapid lysis of cells in Patients with malignancies who are undergoing treatment with cytotoxic drugs or radiations.
Fig. 1.9: Mechanism of Action of Allopurinol (Pession A et al., 2008)