Bioprecursor Prodrugs: Molecular Modification of the Active Principle

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Abstract: A large number of therapeutic medications have undesirable properties that may generate pharmacological, pharmaceutical, or pharmacokinetic barriers in clinical drug applications. Metabolism of drugs by Phase-I & Phase-II metabolic pathways for possibility of active metabolites, which could in turn useful for rational designing of bioprecursor prodrugs of the active principle of interest. This review summarizes various approaches & development of drugs, namely bioprecursor prodrugs and active metabolites related to bioprecursor prodrugs.

Keywords: Bioprecursor prodrugs, active metabolites, oxidative activation, glucuronic acid conjugation, bioreductive alkylation.

INTRODUCTION

Numerous prodrugs have been designed and developed to overcome pharmaceutical and pharmacokinetic barriers in clinical drug application, such as low oral absorption, lack of site specificity, chemical instability, toxicity and poor patient acceptance [1]. Prodrugs can be designed to target specific enzymes or carriers by considering enzyme-substrate specificity or carrier-substrate specificity in order to overcome various undesirable drug properties [2].

The definition of the prodrug indicates that the protective group is covalently linked to the drug molecule [3]. The term "prodrugs" or "proagent" was first introduced by Albert in 1958 to signify pharmacologically inactive chemical derivatives that could be used to alter the physicochemical properties of drugs, in a temporary manner, to increase their usefulness or to decrease their toxicity [4]. These compounds have also been called "latentiated drugs", "bioreversible derivatives", and "congeners", but "prodrugs" is now the most commonly accepted terminology [5].

Prodrug design may be useful in circumventing problems associated with: solubility, absorption & distribution, site specificity, instability, prolonged release, toxicity, poor patient acceptability and formulation problems [6, 7].

Classification of Prodrug

Basically prodrugs are classified as carrier linked prodrugs & bioprecursor prodrugs [8]. Carrier linked prodrugs are those which results from a temporary linkage of active molecule with a transport moiety through a bioreversible, covalent linkage (Fig. **1**).

Bioprecursor prodrugs results from a molecular modification of the active principle. The modification generates a new compound, able to be a substrate for the metabolizing enzymes, the metabolite being the expected active principle (Fig. **2**) [9].

A survey of a great number of examples of active metabolites in this review shows that they belong exclusively to the phase - I metabolic products. Taking into the account, the common metabolic pathways, one can imagine the design of a given molecule so that it will be converted *in-vivo* into the desired compound by one or more of the phase - I reactions. In other words, the active metabolite concept can be used in a forward-looking way, by analogy to the retro-synthetic reasoning [10].

Bioprecursor Prodrugs

There is increasing evidence that the metabolites of some drugs are pharmacologically active [11]. Numerous examples of pharmacologically active metabolites have been used as a source of new drug candidates because these metabolites often are subjects to phase II reactions and have better safety profiles. The best known example of bioprecursor is acetaminophen, which is an O-demethylated metabolite of phenacetin. Acetaminophen shows superior analgesic activity when compared with phenacetin. The main advantage of acetaminophen over phenacetin is that, it does not produce methemoglobinemia and hemolytic anemia. Although pharmacologically active metabolites are generally formed by phase I oxidative reactions, phase II conjugation reactions also can produce biologically active metabolites [12]. Classification of bioprecursor prodrugs mainly based on the activation mechanism either phase I or Phase II metabolism (Fig. **3**), on this basic one can design such a molecule, which on Phase I or Phase II metabolism, gives desired activity of interest.

Modulation of drug-metabolizing enzymes can change the plasma concentrations of drugs and result in serious drug interactions in humans. Phase-I and phase-II drug-metabolizing enzymes play important roles in the determination of pharmacological and toxicological effects of drugs [13].

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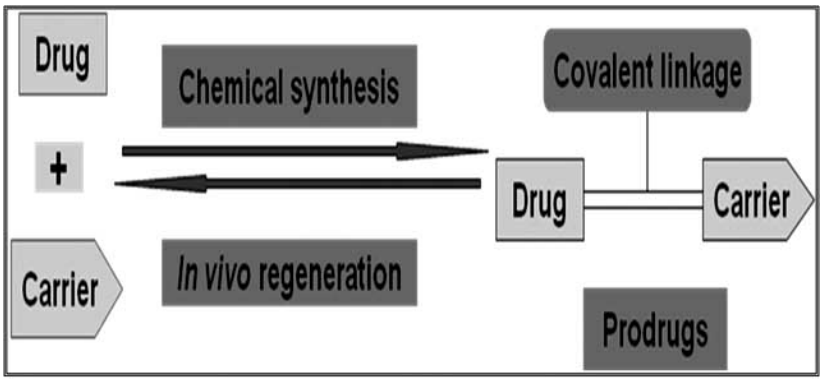

Fig. (1). Activation mechanism of carrier linked prodrug is enzymatic or chemical.

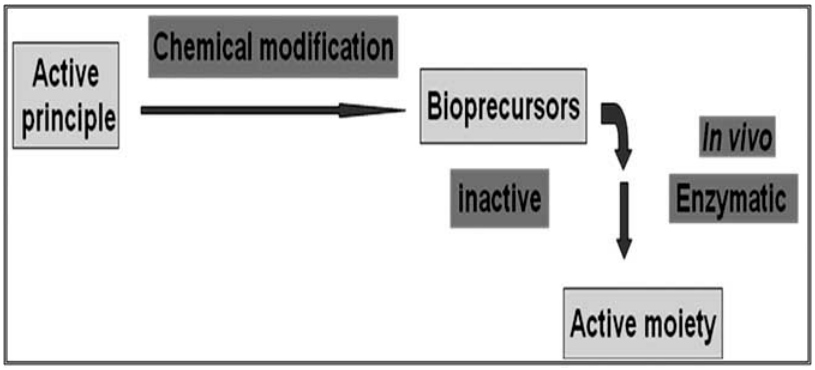

Fig. (2). The activation mechanism of bioprecursor prodrug.

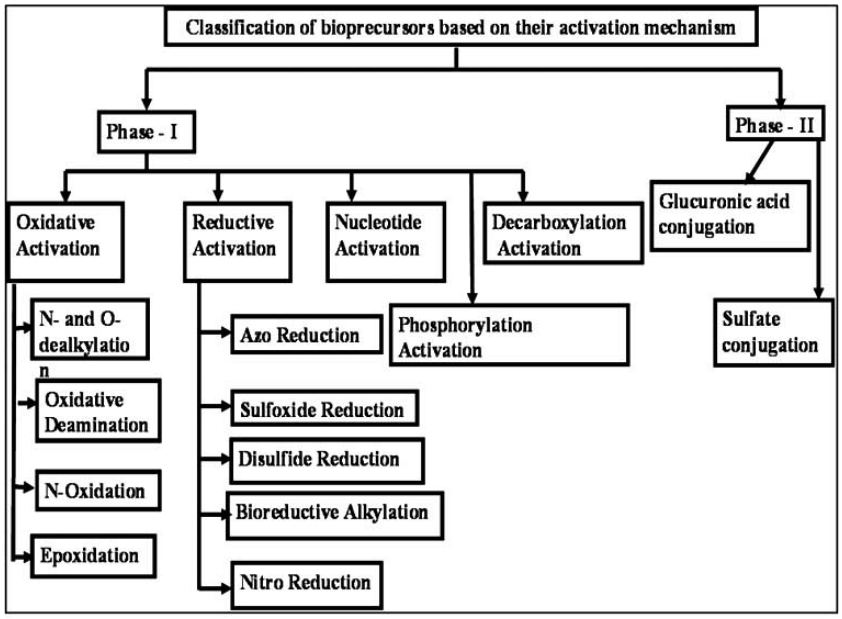

Fig. (3). classification of bioprecursor prodrugs based on their activation mechanisms.

PHASE-I

A. OXIDATION

1. Oxidation by Using Cytochrome P_{450} Mono-Oxygenase System

Cytochrome P_{450} (CYP) dependent mono-oxygenase, is the main phase-I enzyme that catalyzes oxidative metabolism of drugs. CYP-catalyzed oxidations require electron transfer through NADPH-CYP reductase. For some CYP enzymes, cytochrome b_5 is essential for the optimal catalytic activity [14]. A series of evidence showed that alteration of the CYP pool could affect the biological effects of xenobiotics possibly due to the broad substrate specificities of CYP enzymes [15, 16].

2. Flavin-Containing Monooxygenase System (FMOs)

Phase-I oxidation reactions namely the flavin-containing mono-oxygenases (FMOs) system involved in various oxido-reductase reactions of phase-I. The FMO family of enzymes converts lipophilic compounds into more polar metabolites and decreases activity of the compounds, a similar activity to that of the cytochrome P_{450} [17].

3. Monoamine Oxidase (MAO)

Monoamine oxidase is an integral protein of outer mitochondrial membranes and occurs in neuronal and non-neuronal cells in the brain and in peripheral organs. It oxidizes amines from both endogenous and exogenous sources thereby influencing the concentration of neurotransmitter amines as well as many xenobiotics [18, 19].

It occurs as two subtypes, MAO-A and MAO-B which have different inhibitor and substrate specificities. MAO-A preferentially oxidizes nor-ephinephrine and serotonin and is selectively inhibited by clorgyline, while MAO-B preferentially breaks down the trace amine phenethykunine and is selectively inhibited by L-deprenyl [20, 21]. Both forms oxidize dopamine, tyramine and octopamine. Oxidation is accompanied stoichiometrically by the reduction of oxygen to hydrogen peroxide. The relative ratios of MAO A and B are organ and species specific [22, 23].

Alcohol dehydrogenase and aldehyde dehydrogenase are also involved in phase-I oxidation metabolism [24].

B. REDUCTION

1. Reduction by NADPH-Cytochrome P_{450} Reductase

NADPH-cytochrome P_{450} reductase (CPR) is a membrane bound flavoprotein that interacts with the membrane *via* its N-terminal hydrophobic sequence (residues 1-56). CPR is the main electron transfer component of hydroxylation reactions catalyzed by microsomal cytochrome P_{450}. The membrane bound hydrophobic domain of NADPH-cytochrome P_{450} reductase is easily removed during limited proteolysis and is the subject of spontaneous digestion of membrane binding fragment at the site Lys56–Ile57 by intracellular trypsin like proteases that makes the flavoprotein very unstable during purification or expression in *E. coli*. The removal of the N-terminal hydrophobic sequence of NADPH cytochrome P_{450} reductase results in loss of the ability of the flavoprotein to interact with and transfer electrons to cytochrome P_{450} [25].

2. Reduction by Using Reduced (Ferrous) Cytochrome P_{450}

The mechanism for the reduction of ferric cytochrome P_{450cam} by reduced putidaredoxin, the physiological electron donor for the cytochrome. The monooxygenation reaction requires, in addition to *d*-camphor and molecular oxygen, two reducing equivalents, which are transferred from NADH to P_{450cam} through a specific electron transfer system, composed of NADH-putidaredoxin reductase (PdR), a flavoprotein, and putidaredoxin (Pd), an iron-sulfur (Fe_2S_2) protein. In the reaction, Pd receives electrons from PdR and transfers them to P450cam; Pd serves as the direct electron donor for P_{450cam} [26].

When the mechanism for the electron transfer reaction between Pd and P_{450cam} was examined, reduced Pd and ferric P_{450cam} molecules were found to associate rapidly to form a bimolecular complex, followed by an intracomplex electron transfer giving ferrous P450cam and oxidized Pd [27, 28].

Examples of Bioprecursor Prodrugs

Phase – I Activation

a. Oxidative Activation

Oxidative activation of alcohols (**-OH**) and ethers (**R-O-R**) into its corresponding carboxylic acids is useful for designing a better prodrug with maximum efficacy, stability and less toxicity e.g. Nabumetone, Dexpathenol, 3-pyridine methanol.

Nabumetone is a bioprecursor prodrug, which exhibits reduced gastric irritation as compared to other NSAIDs. The modifications of the carboxyl group in NSAIDs into ether will results in reduced gastric irritation [29] (Fig. **4**), so one can use this approach to reduce the undesired effect of NSAIDs due to free carboxylic acid group.

Bioprecursor pro-vitamins provide improved stability towards racemization as compared to the respective vitamins (Fig. **5**).

Dexpathenol is the alcoholic analogue of d-pantothenic acid is a prodrug that is converted *in-vivo* to pantothenic acid, a B-complex vitamin. The vitamin is a precursor for coenzyme-A, the cofactor for enzyme-catalyzed reactions involving

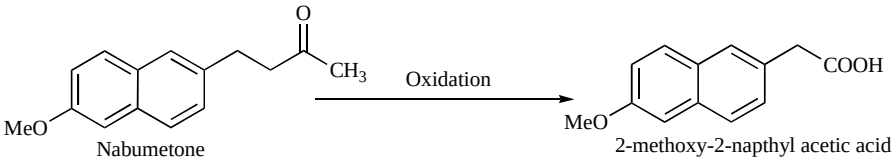

Fig. (4). Activation of Nebumetone by oxidative metabolism.

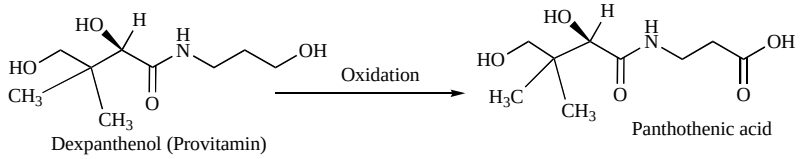

Fig. (5). Activation of pro-vitamin dexpanthenol to pantothenic acid.

the transfer of acetyl group [30]. **3-pyridine methanol** is a bioprecursor prodrug for nicotinic acid, which undergo oxidation reaction *in-vivo* to results in nicotinic acid [31].

b. Reductive Bio-Activation

Reductive bio-activation is effectively utilized for the various drugs to improve their lipophilicity, duration of pharmacological action, potency and specificity e.g. Sulindac, mitomycin, nitrogen mustard, omeprazole.

Sulindac (sulfoxide) is a prodrug in reversible metabolic equilibrium with its pharmacologically active metabolite, the corresponding sulfide. It is having various advantages over indomethacin like improved lipophilicity and duration of pharmacological effects and low ulcerogenic potential on oral administration [32] (Fig. **6**).

Reductive bioactivation of mitomycin results in the formation of an analog, which is activated only in hypoxic condition, such as those found in the slow growing tumors that are poorly vascularized. In these tissues with a low oxygen content, it was thought that reductive metabolism might be more prevalent than in normal tissues & the agents would be selectively activated & therefore selectively toxic [33] (Fig. **7**).

Reductive bio-activation of nitrogen mustard results in formation of hypoxia – selective alkylating active metabolite [34] (Fig. **8**).

Omeprazole effectively inhibits gastric secretion by inhibiting the gastric H^+, K^+ - ATPase & thus gives its anti-ulcerative effect. This enzyme is responsible for gastric acid production & is located in the secretory membrane of parietal cells [35] (Fig. **9**).

c. Mixed Bio-Activation Mechanisms

Mixed bio-activation which involve both oxidation and reduction simultaneously and the use of this mixed activation can be apply for rational designing of drug molecules for its specificity, efficacy and less toxicity.

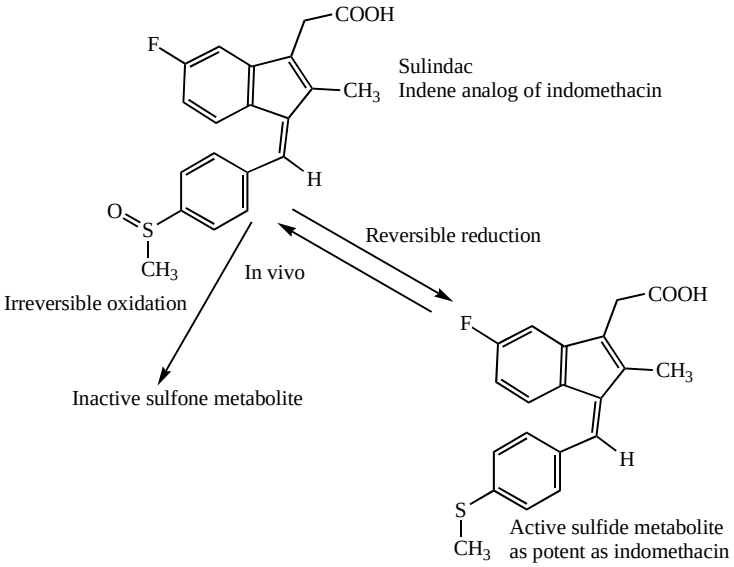

Fig. (6). *In-vivo* activation of sulindac.

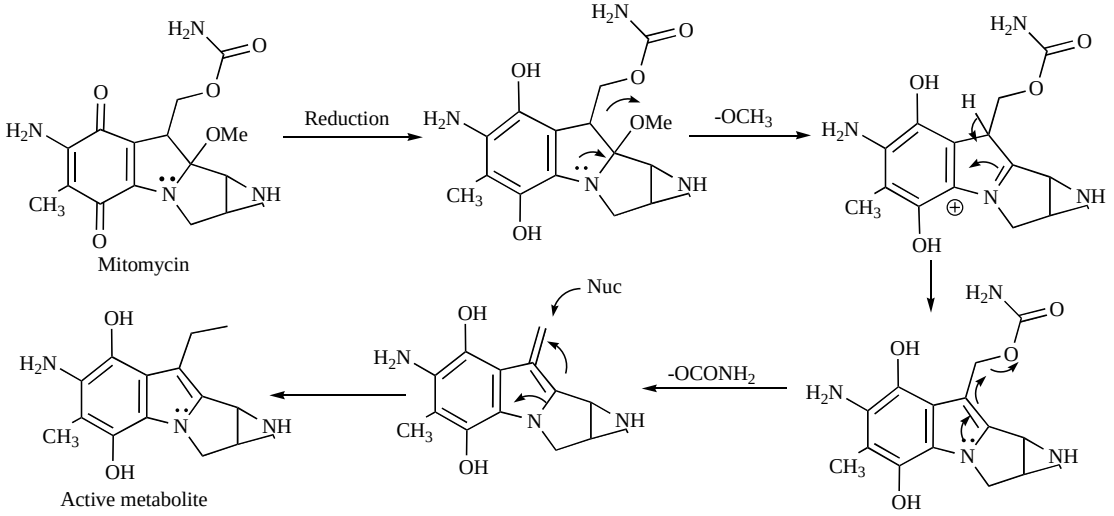

Fig. (7). Activation of mitomycin.

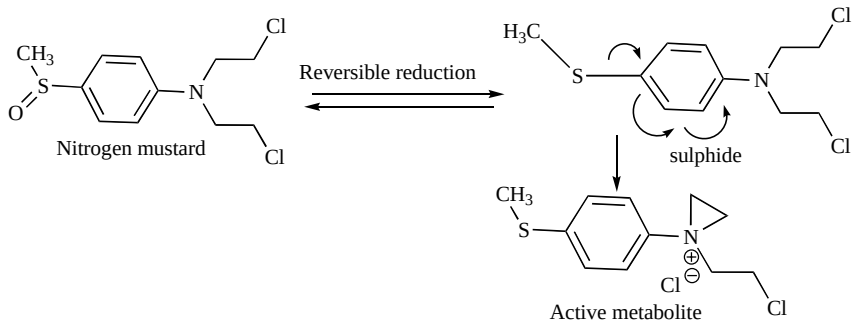

Fig. (8). Activation of Nitrogen mustard.

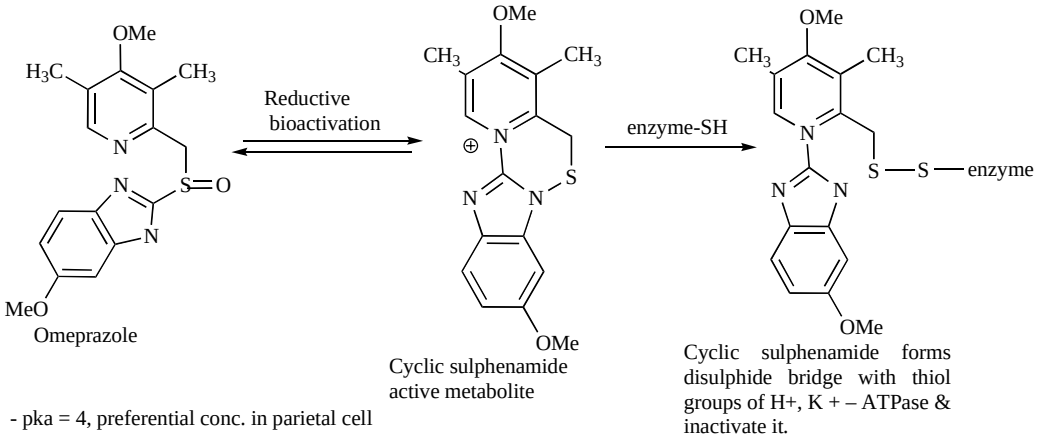

Fig. (9). Activation of omeprazole.

SAH 51-641 is a potent hypoglycemic agent that inhibits gluconeogenesis by inhibition of fatty acid oxidation. It is activated by sequential oxidation/reduction into hydroxy acid and this active metabolite inhibits fatty acyl CoA ligase involved in fatty acid oxidation, thus inhibits hepatic gluconeogenesis [36] (Fig. **10**).

Biotransformation of nicotine & haloperidol involves formation of **aroylpropionic acids** which subsequently undergo progressive degradation of aryl acetic acid. In this the progressive metabolic degradation of β- aroylpropionic acid into arylacetic acids takes place through a multi step process implying reductive, oxidative & hydration-dehydration sequences [37] (Fig. **11**).

The arylacetic acid obtained was used to design bucloxic acid, fenbufen & furobufene which are the bioprecursor forms of anti-inflammatory arylacetic acid [38] (Fig. **12**).

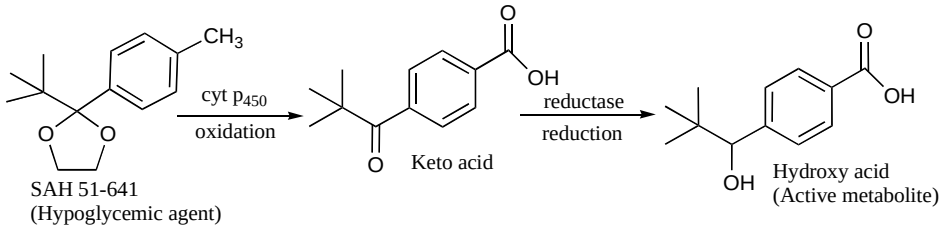

Fig. (10). Activation of SAH 51-641 as potent hypoglycemic agents.

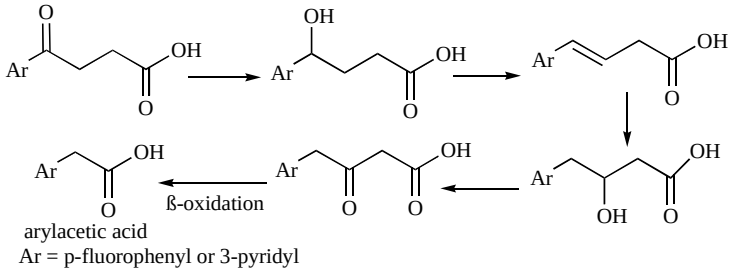

Fig. (11). Activation of nicotine and haloperidol.

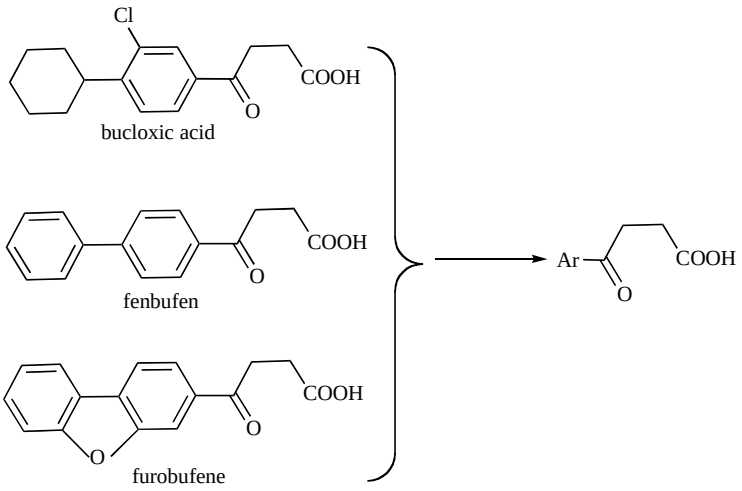

Fig. (12). Designing of arylacetic acid as anti-inflammatory agents.

More recently, arylhexenoic acids were shown to undergo same metabolic degradation as arylacetic acids. e.g. hexanoic analogue of indomethacin acts as a bioprecursor prodrug of indomethacin [39] (Fig. **13**).

d. Phosphorylation Reactions

Bioactivation through phosphorylation reactions results in the formation of drug which is activated to a greater extent in the virally infected cells and achieves selective toxicity. It also offers increased cell penetration because nucleosides can easily enter the cell *via* active transport mechanism. E.g. **Idoxuridine** a nucleoside, better substrate for viral enzymes than corresponding mammalian enzymes gives a triphosphate nucleotide, inhibits viral DNA polymerase & incorporation into DNA, resulting in incorrect base pairing & inhibition of DNA synthesis by Phosphorylation reaction in presence of Viral thymidine kinase [40] (Fig. **14**).

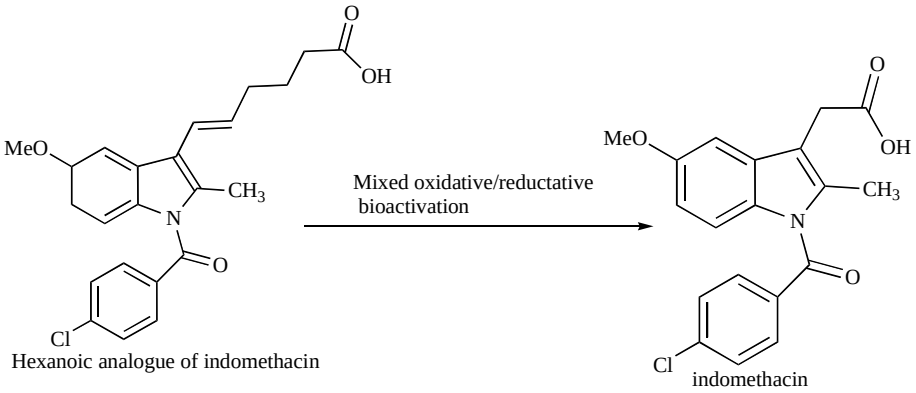

Fig. (13). Hexanoic analogue of indomethacin.

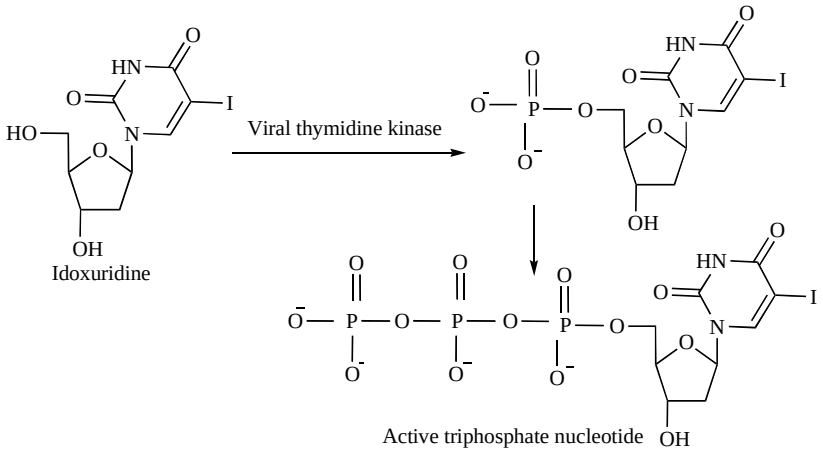

Fig. (14). Activation of idoxuridine.

Another example of phosphorylation is of **Acyclovir** a potent antiherpes drug – poor/erratic absorption due to high polarity. Phosphorylation of acyclovir essential for antiviral activity. In normal mammalian cells phosphorylation is extremely low but in cells infected with virus, rate is very high due to virus coded thymidine kinase. **Desoxyacyclovir:** bioprecursor of acyclovir which is activated by xanthine oxidase into acyclovir. The ultimate target enzyme of acyclovir is the viral DNA polymerase, which is inhibited by the triphosphate metabolite of acyclovir [41] (Fig. **15**).

e. Reactions without Change in the State of Oxidation

Glutathione is a tripeptide of glycine, glutamic acid & L-cysteine. L-2-Oxothiazolidine-4-carboxylate, a cyclic thiocarbamate may serve as an intracellular delivery system for cysteine & has a potential to act as a therapeutic agent for the hepatic glutathione depletion [42] (Fig. **16**).

PHASE – II

1. METHYLATION

A. Methyltransferase

Methylation is a relatively minor component to drug or xenobiotic metabolism but is rather important in the biosynthesis of endogenous compounds such as epinephrine and melatonin. Usually the cofactor SAM (S-adenosyl methionine) serves as a methyl donator. Methylation (like acetylation) differs from most conjugation reactions in that it produces products with lower hydrophilicity. The exception is methylation of tertiary or pyridine-type nitrogens resulting in a charged, quaternary ammonium salts. Methylation is a 2-step process whereby the cofactor S-adenosylmethionine (SAM) which transfers a methyl group is first biosynthesized from methionine. Once available, SAM is utilized by a methyltransferase to transfer an activated methyl group to an acceptor molecule (nucleophile = alcohol, amine, thiol). A variety of transferases are used depending on the nature of the acceptor [43].

2. SULPHATION

A. Glutathione S-Transferases

Glutathione-*S*-transferase (GST) catalyzes the nucleophilic conjugation of glutathione (GSH) with many diverse electrophilic substrates. Glutathione conjugation is a major mechanism of detoxification in mammals and detoxification of at least six major families of herbicides in plants. Although the role of GST in detoxification-degradation of xenobiotics by terrestrial microorganisms has been postulated, there is need to elucidate the role of this enzyme in biodegradation of xenobiotic compounds [44, 45].

B. Sulfotransferases System

Sulfonate conjugation was first reported by Baumann in 1876 and has since been shown to be an important pathway in the biotransformation of numerous xeno- and endobiotics such as drugs, chemical carcinogens, hormones, bile acids, neurotransmitters, peptides and lipids. The universal sulfonate donor for these reactions is 3'-phosphoadenosine 5'-phosphosulfate (PAPS) and the transfer of sulfonate (SO_3-) to a hydroxyl or amino- group is catalysed by a super gene family of enzymes called sulfotransferases (SULTs). In the case of most xenobiotics and small endogenous substrates, sulfonation has generally been considered a detoxification

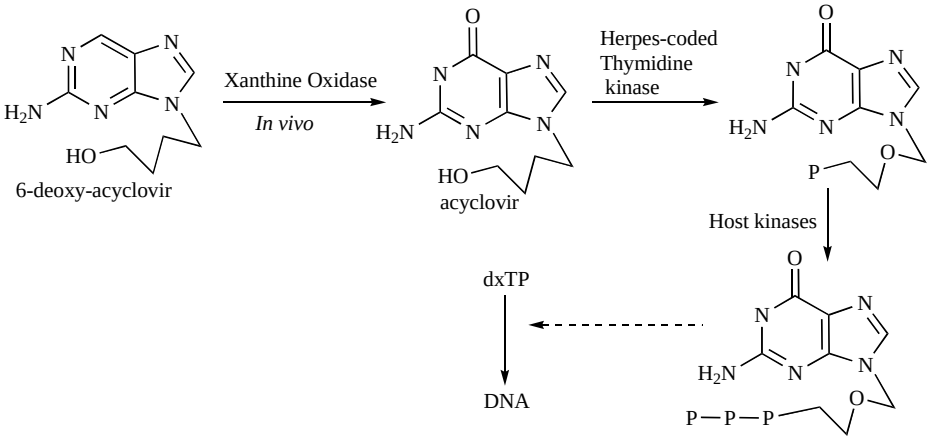

Fig. (15). Activation of acyclovir.

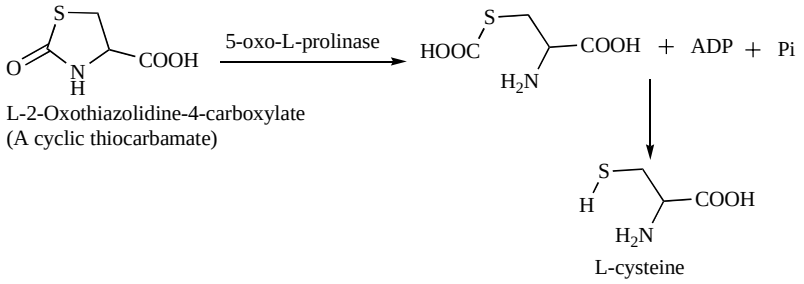

Fig. (16). L-2-Oxothiazolidine-4-carboxylate for the hepatic glutathione dep.

pathway leading to more water-soluble products and thereby aiding their excretion *via* the kidneys or bile [46].

3. ACETYLATION

A. N-Acetyltransferases System

Phase II enzymes, such as the arylamine N-acetyltransferases (NAT) catalyze activation or detoxification reactions for endogenous and exogenous arylamines and hydrazines. The proteins designated NAT catalyze three types of acetylation reactions: N-acetylation of arylamines or hydrazines, O-acetylation of N-hydroxylamines, and the intramolecular acyl transfer with hydroxyarylacetamides. There are two members in this enzyme family, NAT1 and NAT2. The importance of these isoforms in the biotransformation of environmental chemicals and therapeutic drugs is well recognized [47, 48].

B. Amino Acid N-Acyl Transferases

In this again the acetylation occurs by N-acyl transferase enzyme but in presence of amino acids as a cofactor [49].

4. GLUCURONIDATION

A. UDP-Glucuronosyltransferases

Glucuronidation, a typical reaction of phase II metabolism, is probably the main pathway of conjugation with anabolic androgenic steroid metabolites, but detailed information about the factors that determine and affect this biotransformation is still very limited. More is known, however, on glucuronidation of clinically important endogenous steroids, which have been taken as an estimate for the total androgenic pool in men, or for the production of dihydrotestosterone in extrahepatic tissues [50]. UDP-glucuronosyl transferases are a family of membrane-bound enzymes of the endoplasmic reticulum. They catalyze the glucuronidation of various endogenous and exogenous compounds, including steroids, thereby converting the substrate molecules (the aglycones) into a less toxic and more polar D-glucuronides [51]. The human genome encodes at least 16 different UGTs, and they are divided into families (1 and 2) and subfamilies (2A and 2B) according to the degree of sequence identities and genomic organization. Most of the UGTs are expressed in the liver, the organ that is considered to be the major site of glucuronidation. However, some UGTs are extrahepatic enzymes, and many of the liver UGTs are also found in other tissues [52].

Examples of Bioprecursor Prodrugs

Phase – II Activation

Although pharmacologically active metabolites are generally formed by phase - I oxidative reactions, phase - II conjugation reactions also can produce biologically active metabolites. For example morphine- 6-glucuronide is more potent as a μ-opioid receptor agonist than morphine itself. Recent clinical studies in cancer patients given morphine- 6-glucuronide indicated that useful analgesic effects are achieved without the side effects of nausea & vomiting that are often associated with morphine. These findings have led to the commercial marketing of morphine-6-glucoronide [53].

Sulfation also produces biologically active metabolites. For example the action of minoxidil (Fig. **17**), a potent vasodialator, is mediated through its sulfate conjugation [54].

Paclitaxel (Fig. **18**) is prodrug designed to be activated into the drug by human's β-glucuronidase. In order to enhance the liberation of rate of paclitaxel, an elongated spacer system including a nitro-aromatic derivative & N,N-methylethylenediamine was incorporated between the sugar moiety & the drug [55].

Bioprecursors of Nature

There are some bioprecursors which originated by nature and plays a vital role in day today's life. These are mostly vitamins: like vitamin D and vitamin A.

Activation of 7-dehydrocholestrol into vitamin D by UV-rays (sunlight) is a very well known example of bioprecursor prodrug [56] (Fig. **19**).

Beta– carotene i.e. Vitamin A, in the intestinal mucosa transformed to retinal, by β-carotene-15, 15'-dioxygenase [57] (Fig. **20**).

Applications of Bioprecursor Prodrugs:

1) Improved Bioavailability

Oxidative bioactivation of *losartan* which is a nonpeptide angiotensin II receptor antagonist used as an antihypertensive medication [58, 59] (Fig. **21**).

Bisthiazolium precursors are used as potent antimalarials with improved bioavailability. 1, 6-hexadecamethylenebis (N-methylpyrrolidinium) dibromide has charged cationic group essential for activity but detrimental to oral absorption. Its bisthiazolium precursors like TE4a, TE4gt & TE3 mask ionisable groups & are quantitatively converted to active metabolites T4 & T3 respectively & shows impressive antimalarial activity against *Plasmodium cynomolgi* [60] (Fig. **22**).

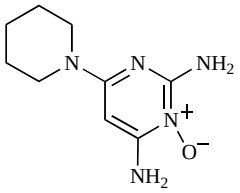

Fig. (17). Structure of Minoxidil.

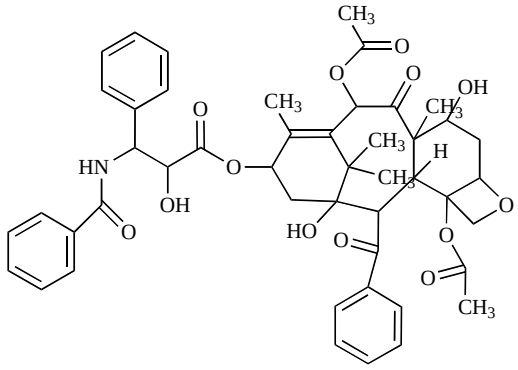

Fig. (18). Structure of paclitaxel.

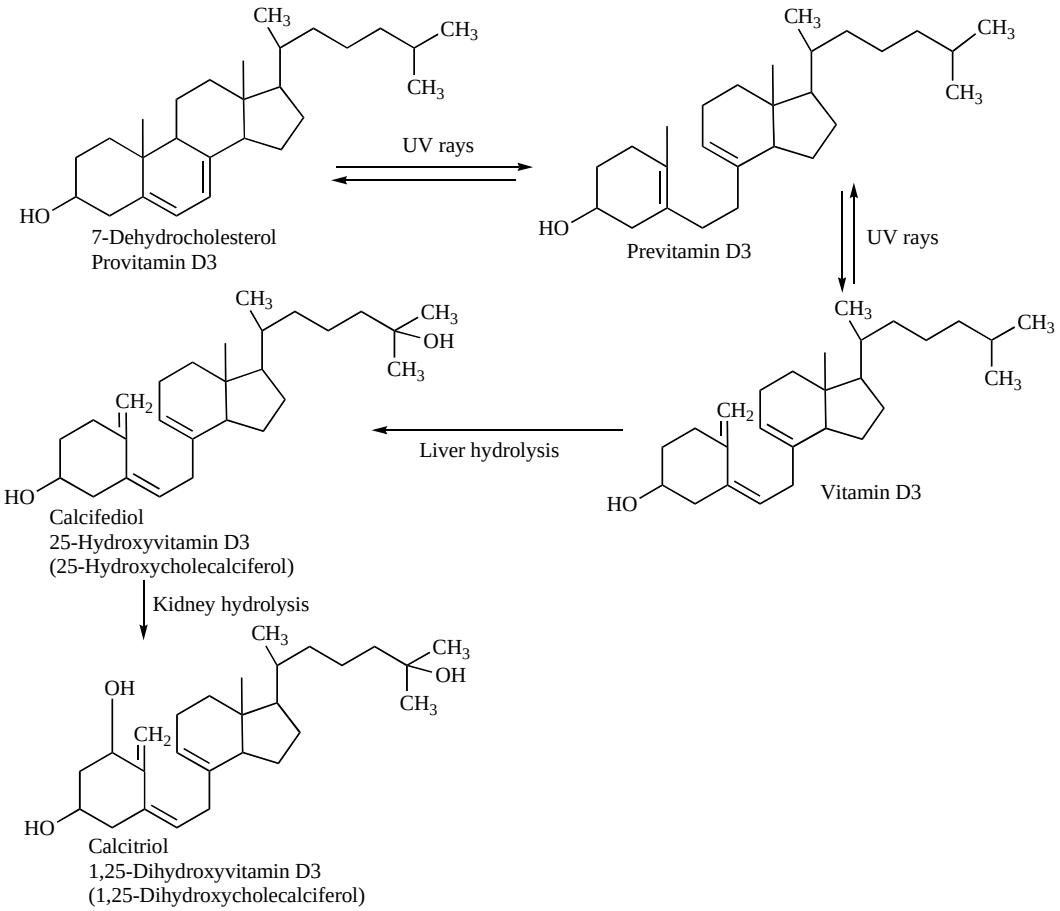

Fig. (19). Activation of 7-dehydrocholestrol into vitamin D by UV-rays.

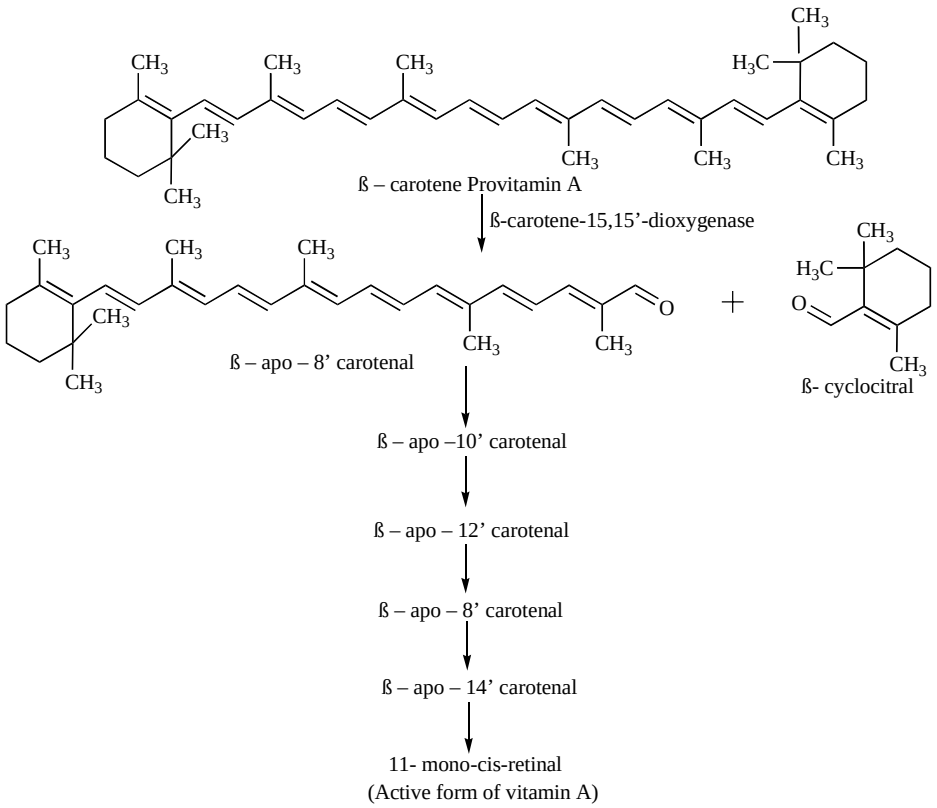

Fig. (20). Activation of Vitamin A to retinal.

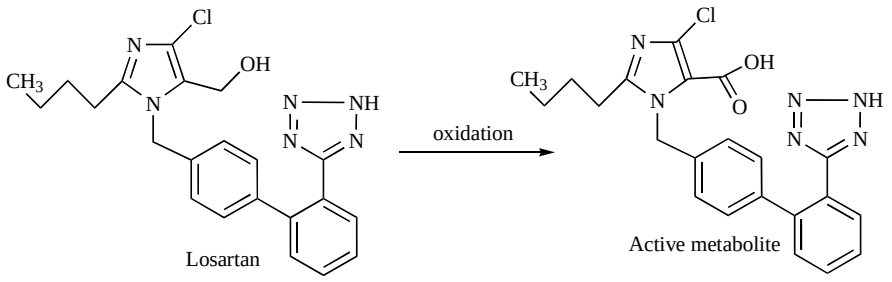

Fig. (21). Activation of losartan.

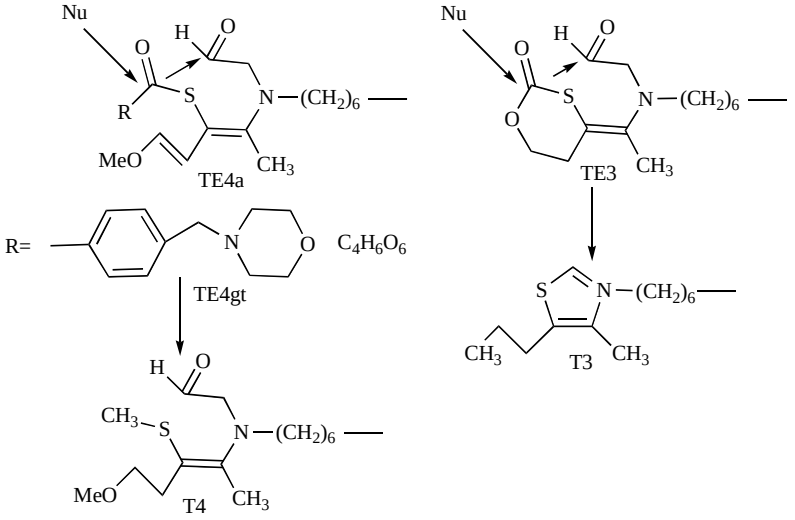

Fig. (22). Activation of bisthiazolium precursor as potent antimalerial.

2) Prevention of First Pass Metabolism

Methylenedioxy derivatives are used as bioprecursors of catechols. Catechols are very succeptible to first metabolism in liver. Their methyledioxy bioprecursors are resistant to first pass metabolism as & shows longer duration of action [61] (Fig. **23**).

3) Site Specific Drug Delivery

a. Ocular Delivery Through Bioprecursor Prodrugs

Several oxime or methoxime analogs of known β-adrenergic blockers have been synthesized as their bioprecursors using general retrometabolic drug design principles as antiglaucoma agents. In these compounds, a β-amino oxime or alkyloxime function replaces the corresponding β-amino alcohol pharmacophore part of the original molecules. These oxime or alkyloxime derivatives exist in alternatives Z or E configurations. These derivatives ensure ocular delivery of β-blockers. Their intravenous administration does not produce the active β-blocker metabolically, thus void of any cardiovascular activity that is a major drawback of classical antiglaucoma agents. The oxime-type Chemical Delivery System (CDS) approach proposed here provides site specific or site-enhanced delivery through sequential, multi-step enzymatic and/or chemical transformations. The original oximes or methoximes and the intermediate ketones are inactive; they are enzymatically converted into active S-(-) β-adrenergic blocks alcohols in a site and stereospecific manner. In the case of eye-targeting CDS, this is achieved through a targetor (T) moiety that is converted into a biologically active function by enzymatic

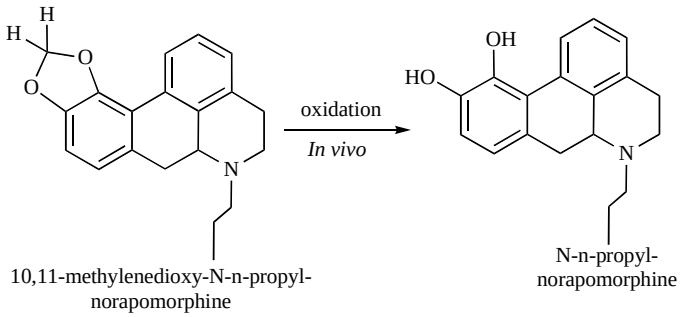

Fig. (23). Bioprecursors of catechols and its active metabolites.

reactions that take place primarily, exclusively, or at higher activity at the site of action as a result of differential distributions of certain enzymes found at the site of action. [62] (Fig. **24**).

b. Liver Targeted Drug Delivery Through Bioprecursors

In liver targeted drug delivery system, series of phosphate & phosphonate bioprecursor prodrugs are used, called as HepDirect prodrugs. This HepDirect prodrugs represent a potential strategy for targeting drugs to the liver & achieving more effective therapies against chronic liver diseases such as hepatitis B, hepatitis C & hepatocellular carcinoma. One conjugated-based strategy capable of delivering drugs to an extravascular site uses glycolipid-containing drug carriers that recognize the asialoglycoprotein receptor expressed on hepatocytes. HepDirect prodrugs are cyclic 1,3-propanyl esters containing a ring substitute that renders them sensitive to an oxidative cleavage reaction catalysed by a cytochrome P450 [63] (Fig. **25**).

Prodrug cleavage mechanism: A HepDirect prodrug (1) diffuses into hepatocytes and undergoes a CYP3A-catalyzed oxidation of the C4 methine hydrogen to produce the C4-hydroxylated product (2). Rapid and irreversible ring-opening leads to the intermediate monoacid (3), which generates the corresponding phosphate or phosphonate (4) following a β -elimination reaction or possibly, in the case of the phosphate, a phosphodiesterase-catalyzed hydrolysis reaction. (4) is converted to the biologically active nucleoside triphosphate analog (NTP) (6) by intracellular nucleotide kinases when $R\text{-}PO_3^{2-}$ is an NMP analog and by PRPP synthase when the NMP analog is PMEA. Aryl vinyl ketone 5 is trapped by intracellular glutathione (GSH) to form conjugate (7) [64].

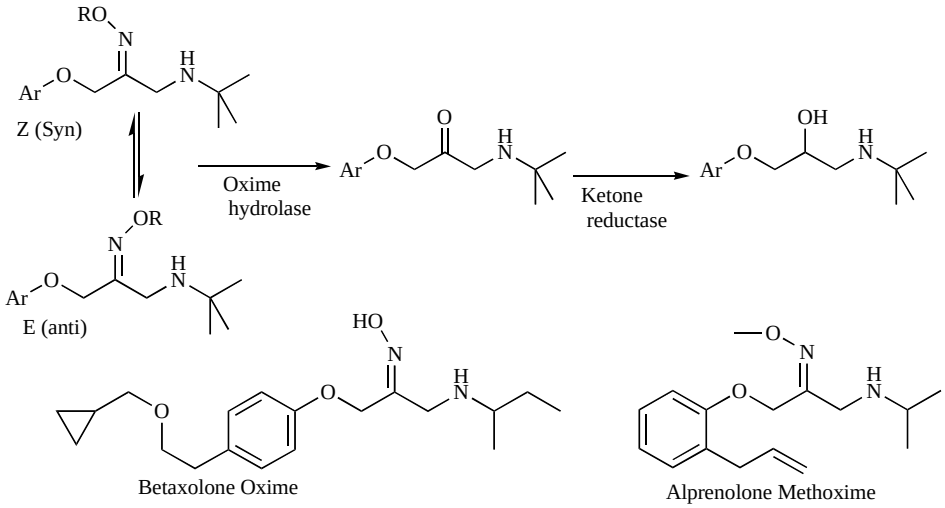

Fig. (24). Activations of oximes for target specific drug delivery.

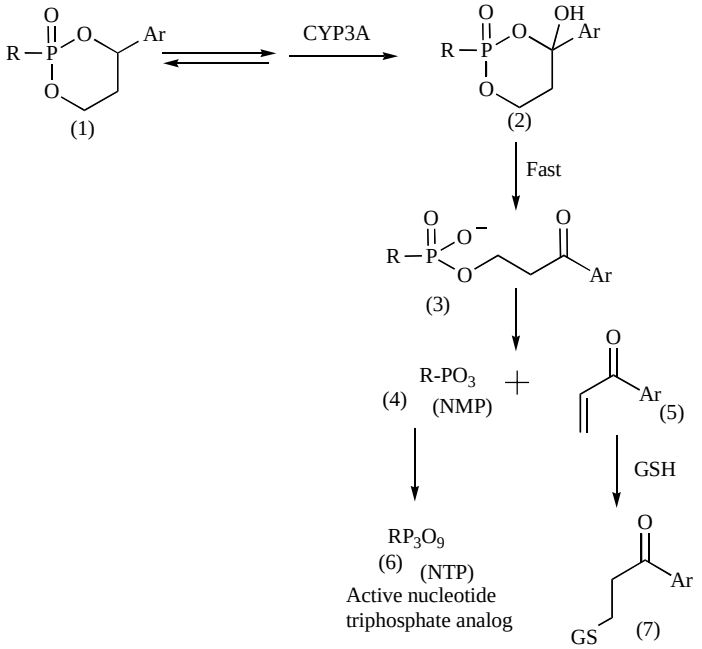

Fig. (25). Liver targeted drug delivery.

c. Delivery of 2- PAM to Brain

N- Methylpyridinium-2-carbaldoxime (2-PAM) is a potent reactivator of acetylcholine-esterase. Due to quaternary nitrogen in 2-PAM its bioavailability is poor due to less penetration through blood brain barrier (BBB). Dihydropyridine-pyrimidine redox system developed for its brain delivery, in this the active drug (a) is administered as 5,6-dihydropyridine derivative (Pro-2-PAM), (b). It exists as a stable immonium salt (c). The lipoidal (b), (pKa = 6.32) easily penetrates the blood – brain barrier where it is oxidised to active (a) [65] (Fig. **26**).

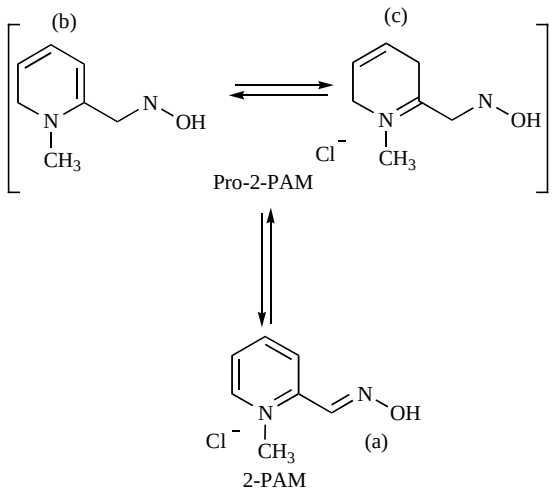

Fig. (26). Brain targeted drug delivery.

d. Delivery to Cancerous Cells

Cyclophosphamide is a cytoxic (cyctostatic), cell - cycle nonspecific, antiproliferative agent, developed by Arnold *et al.* as bioprecursor of potent alkylating nitrogen mustard. Activation by enzymes specifically present in tumors through initial oxidative dealkylation followed by hydrolysis. Used in diverse medical problems as neoplasia, tissue transplantation & inflammatory diseases [66, 67] (Fig. **27**).

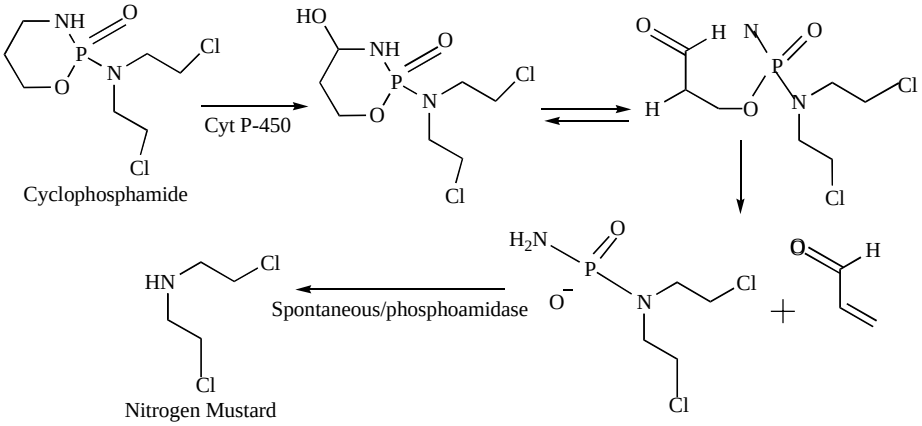

Fig. (27). Cancer cell targeting using bioprecursor prodrug approach.

e. Kidney Targeted Drug Delivery Through Bioprecursors

Dopamine, a neurotransmitter, produces vasodilation of renal tissue by binding to specific receptors in kidney & can be used in the treatment of renal hypertension. But due to it's interaction with α-adrenergic receptors, it precipitates high blood pressure. This can be overcome by targeting dopamine to kidneys in the form of it's bioprecursor γ-glutamyl DOPA that undergoes selective & sequential activation by specific renal enzymes i.e. γ- glutamyl transpeptidase & L-aromatic amino acid decarboxylase to release the active drug dopamine locally. The increase in dopamine levels produces a marked increase in renal blood flow & thus treats renal hypertension [68] (Fig. **28**).

Various examples of bioprecursor prodrugs and their active metabolites used as therapeutic agents are listed in Table **1**.

CONCLUSION

Metabolic activation of the various drugs can be a key to open the locks of undesirable pharmacological, pharmaceutical, or pharmacokinetic barriers. Bioprecursor prodrug approach is an excellent route to overcome these barriers.

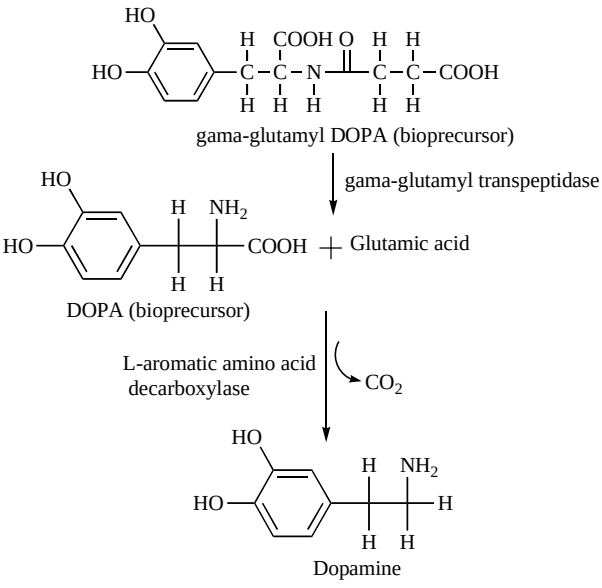

Fig. (28). Kidney targeting drug delivery.

Table 1. Various Examples of Bioprecursor Prodrugs Used as a Therapeutic Agents Various Examples of Bioprecursor Prodrugs

Bioprecursors	Active Metabolite	Category
Citalopram	Des-methyl-citalopram	Antidepressant agent [69]
Amitriptyline	Nortriptyline	Antidepressant agent [70]
Imipramine	Desipramine	Antidepressant agent [71]
Tamoxifen	4-hydroxy tamoxifen	Anticancer agent [72]
Valganciclovir	Ganciclovir	Antiviral agent [73]
Spirodiketopiperazine	β-substituted-(2R,3R)-2-amino-3-hydroxy propionic acid	Antiviral agent [74]
Carbamazepin	10,11-dihydroxy carbamazepine	Anticonvulsant [75]
Enrofloxacin	Ciprofloxacin	Antimicrobial agent [76]
Venlafaxine	O-desmethyl venlafaxine	Antidepressant agent [77]
Amifostine	Aminothiol	Anticancer agent [78]
Prulifoxacin	Ulifloxacin	Antimicrobial agent [79]
Ceftiofur	Desfuroylceftiofur	Antimicrobial agent [80]
Zotepine	Nor-zotepine	Antipsycotic agent [81]
Oxycodone	Oxymorphone	Anti-inflammatory [82]
Glimepiride	Trans-hydroxy-glimepride	Antidiabetic agent [83]
Oxymatrin	Matrine	Antihepatic agent [84]
Ximeleyatran	Melagatran	Antithrombin agent [85]
Ciclesonide	Desisobutyryl-ciclesonide	Antiasthamatic agent [86]
Ramipril	Ramiprilat	Antihypertensive agent [87]
Tramodol	O-desmethyl tramadol	Antiarthritic agent [88]
Rabeprazole	5-methyl-2- { 4-(3-methoxy-propoxy)-3-methyl pyridin-2-yl)methyl sulfinyl }-1H benzimidazole	Proton pump inhibitor [89]

The various activation mechanisms listed in this review are useful for medicinal chemist to design the new molecules namely bioprecursor prodrugs, which in turns minimizes the side effects / undesired effects / toxic effects and maximizes site specificity / improved bioavailability / stability / potency / to prevent first pass metabolism.

ABBREVIATIONS

CYP = Cytochrome P_{450}

NADP = Nicotinamide Adenine Dinucleotide Phosphate

FMOs = Flavin-containing monooxygenase system

MAO = Monoamine Oxidase

CPR = NADPH-Cytochrome P_{450} Reductase

PdR = NADH-Putidaredoxin Reductase

SAM = S-Adenosyl Methionine

GST = Glutathione-*S*-Transferase

PAPS = 3'-Phosphoadenosine 5'-Phosphosulfate

SULTs = Sulfotransferases

NAT = N-Acetyltransferases

UGT = UDP-Glucuronosyl Transferases

CDS = Chemical Delivery System

NTP = Nucleoside Triphosphate

2-PAM = N- Methylpyridinium-2-carbaLdoxime

BBB = Blood Brain Barrier

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