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Prodrug Design and Development for Improved Bioavailability across Biological Barriers

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Dr. M.S Rathore*, Sandeep Kumar

Maharishi Markandeshwar College of Pharmacy, M.M University, Mullana (Ambala), Haryana, India.

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ABSTRACT

The prodrug concept has been used to alter and mask the undesirable properties of the active parent drug. Prodrugs are pharmacologically inactive compounds which are converted into its active product by metabolic transformation like enzymatic and chemical transformations. The rationale behind the development of prodrugs is the delivery of appropriate concentrations of drug to the target cells as compared to administration of parent drug. There are many pharmacokinetic, pharmaceutical and pharmacodynamic barriers encountered by the drug. Prodrug approach has been found to be very helpful to overcome these barriers to a certain extent. This article describes and takes a review on the concept of a prodrug, barriers overcome by prodrug approach, various prodrug designs significant for distribution of drugs which are transporters proteins and bioactive enzymes, different types of transporters i.e SLC transporters & ABC transporters and Enzyme-targeted prodrug therapy. Further, this article focuses on the main chemical moieties and some of the examples of parent drugs using these moieties will be discussed.

INTRODUCTION

A prodrug is pharmacologically inactive compound that is converted into its active drug by a metabolic biotransformation. Prodrugs enhanced the usefulness of various therapeutic agents by altering their physicochemical properties, pharmacokinetics, and biopharmaceutical properties. Prodrug might alter the tissue distribution, efficacy, and toxicity of parent drug. Below are some reasons why prodrug approach should be used in drug design:

· Improved aqueous solubility.
· Improved absorption and distribution
· Site specificity
· Improved stability of drugs
· For prolonged release
· To reduce toxicity
· In poor patient acceptability
· In formulation problems.

It has been reported that about 10% of drugs marketed all over the world can be classified as prodrugs. [1] In 1867, Cahn and Hepp introduced acetanilide as a prodrug for the first time in medical practice. Acetanilide undergoes hydroxylation in the body and gets converted to biologically active compound acetaminophen having both antipyretic and analgesic activities. The main ideology of using prodrug approach is to mask undesirable properties of the parent compound like low solubility in water, irritation or pain after local administration, low target selectivity, chemical stability and enzymatic stability etc.[2, 3] In general prodrug is introduced to optimize absorption, distribution, metabolism, excretion and toxicity of the parent drug.

This review aims to discuss and present the information about the prodrug concept in details, the role of transporters in drug absorption and the different chemical moieties that can be used to design prodrug for improvement of aqueous solubility. Research being conducted in past 10 years in this area is taken into account and attempt has been made to present available information in a lucid manner in this review.

Concept of Prodrug

The fundamental principle of the prodrug is to eradicate undesirable properties of drug-like low aqueous solubility, low lipid solubility, poor target selectivity, undesirable taste, low chemical stability, presystemic metabolism and to mask irritation upon local administration etc.[4] The term prodrug was first introduced by Adrein Albert in 1958, according to Adrein Albert prodrug is biologically inactive derivative of parent drug which undergoes chemical and enzymatic conversions in the body to release biologically active parent drug. The simple illustration of prodrug concept is given in figure 1.

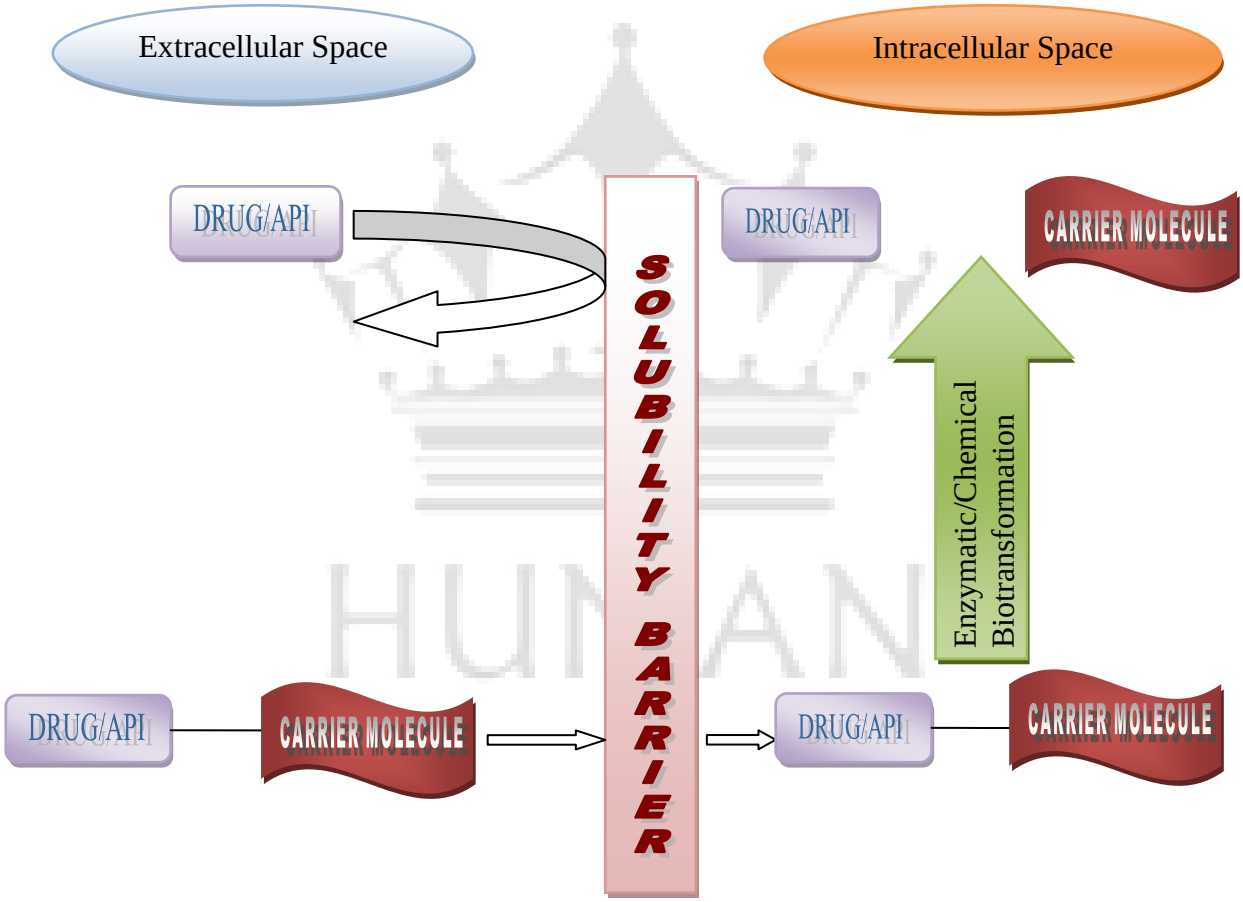


Fig. 1: A simple Illustration of Prodrug Concept

Classification of Prodrugs based on structure of the drug

The prodrugs are classified into various categories as follows:

(A) Carrier-linked Prodrugs

(B) Bioprecursors

(C) Macromolecular Prodrugs

(D) Spacer or Linker Prodrugs

(A) Carrier-Linked Prodrugs:

A carrier-linked prodrug is a complex that comprises an active drug which is temporarily attached to some carrier with covalent linkage. The carrier group can be detached enzymatically. After administration to the body, the prodrug undergoes biotransformations and converted to the active compound. [5] The ideal carrier should have following properties:

· It should save the drug until it reaches the site of therapeutic action.
· Confine the drug at the site of therapeutic action.
· Allow the liberation of the drug by chemical or enzymatic action.
· It should bear biochemical inertness.
· To be easily prepared and inexpensively.
· It should be inert and stable.

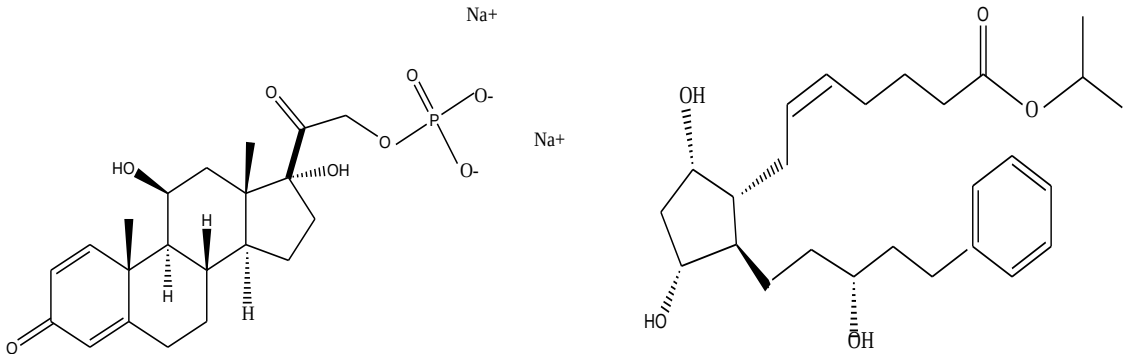
Carrier-linked prodrugs are further classified as:

(i) Bipartite Prodrugs

(ii) Tripartite Prodrugs

(iii) Mutual Prodrugs

(i) Bipartite Prodrug: A bipartite prodrug is a prodrug comprised of one carrier linked to the parent drug. The examples of bipartite prodrugs are Prednisolone sodium phosphate **(1)**, latanoprost **(2)**. (Figure 2)



Prednisolone Sodium Phosphate **(1)** Latanoprost Ester Prodrug **(2)**

Fig. 2: Chemical Structures of Prednisolone prodrug and Latanoprost Prodrug

Tripartite Prodrugs: In tripartite prodrug, a carrier is associated to the linker which is further attached to the drug. For example, Prodrug of ampicillin **(3)** in which the carrier is pivalic acid and linker is $–CH_2-$ as shown in figure 3.

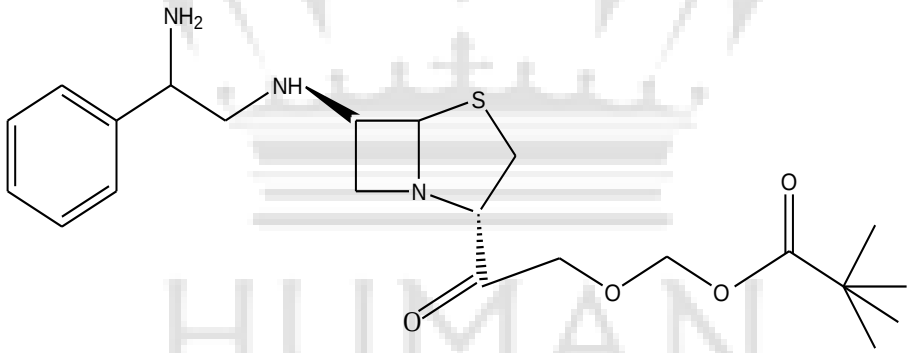


Fig. 3: Chemical Structure of Pivampicillin (Prodrug of Ampicillin)

(ii) Mutual Prodrug or Co-drug: In co-drug approach two pharmacologically active compounds are combined and both acts as promoieties for each other. Mutual prodrugs may be of both types bipartite or tripartite. The carrier in mutual prodrug may have the same therapeutic action as that of parent drug or it may have some different therapeutic action which is not shown by parent drug. For example, a mutual prodrug of sulbactam and ampicillin (Sultamicillin) is shown in figure 4. Sultamicillin **(4)** bears more improved pharmacokinetic properties as compared to alone parent drugs. (Figure 4)

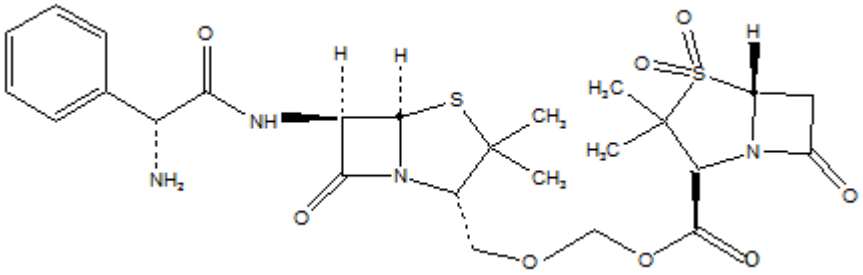


Fig 4: Chemical Structure of Sultamicillin

(B) Bioprecursors :

Bioprecursors results from the molecular modification of the active compound. There is no need of promoiety in bioprecursor prodrugs. Bioprecursor needs oxidation and reduction mechanisms for activation *in vivo*. The bioprecursor prodrugs are metabolized into a new compound that may be active or it may be further metabolized to release the active compound. [6]

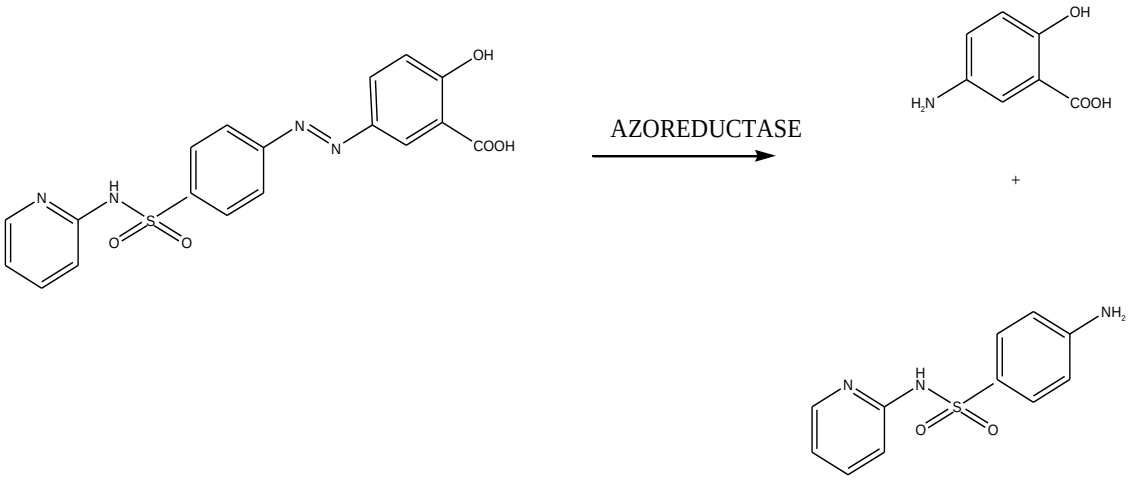
Oxidation: For example Carbamazipine-10,11-oxide **(6)**, a prodrug of Carbamazepine **(5).** (Figure 5)

(5)
Carbamazepine

(6)
Carbamazepine-10,
11-Oxide (Active Form)

Fig 5: Chemical Structures of Carbamazepine and its Prodrug

Reduction: For example, Sulphapyridine **(8)** a prodrug of Sulphasalazine **(7)** .(Figure 6)



(7) Sulphasalazine

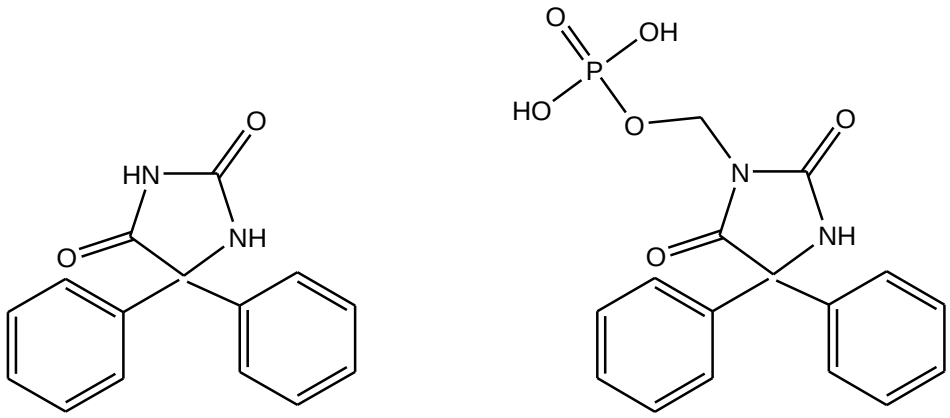
(8) Sulphapyridine

Fig 6: Chemical Structures of Sulphasalazine and its Prodrug

(C) Macromolecule Prodrugs:

In macromolecule prodrugs, the promoiety is a macromolecule like polysaccharides, proteins, dextrans, cyclodextrins, and polymers etc.

(D) Spacer or Linker Prodrugs: The spacer or linker approach can be used in the case where it is difficult to attach the promoiety with parent drug directly due to steric hindrance or any other functional barrier. The attachment of spacer with promoiety increases the distance between parent drug and promoiety. The spacers are cleaved by enzymatic or chemical action on the bond between promoiety and spacer. [7] For example, Fosphenytoin **(10)** is a linked prodrug of phenytoin **(9)** with improved aqueous solubility are shown in figure 7.



(9) **(10)**

Phenytoin Fosphenytoin

Fig 7: Chemical Structures of Phenytoin and Fosphenytoin (Prodrug of Phenytoin)

Classification on the basis of site of conversion:

The studies revealed that there are two main objectives while designing and developing a prodrug that are:

1. Complete and fast conversion of the prodrug into its active drug form.
2. Toxicity profiles of active drug.

The above-said objectives are interrelated to each other. The prodrug is developed to improve the quality of the active drug, its efficacy and its toxicity. Therefore, keeping in view the above points, a prodrug classification system would be developed by Kuei-Meng Wu in 2009, on the basis of site of conversion into the active form of the drug. According to this classification prodrug is classified into two main classes:

(a) Type I Prodrugs:

Type I prodrugs are those which are metabolized intracellularly. Type I class is further classified into two subclasses: Type IA and Type IB. Type IA is the prodrug that is metabolized at target tissues/cells. These include various antimicrobial and chemotherapeutic agents. Type IB is the prodrug which is metabolized in metabolic tissues like liver and GI mucosal cells.

(b) Type II Prodrugs:

These are the prodrugs which are metabolized extracellularly. Further, Type II prodrugs are classified into three subclasses, Type IIA, Type IIB and Type IIC. Type IIA is the prodrugs which are metabolized in GI fluid. Type IIB is metabolized in systemic circulation or other extracellular compartments. Type IIC are the prodrugs which are metabolized at target tissue/cells.[8] Table 1 illustrates the classification of prodrugs with examples.

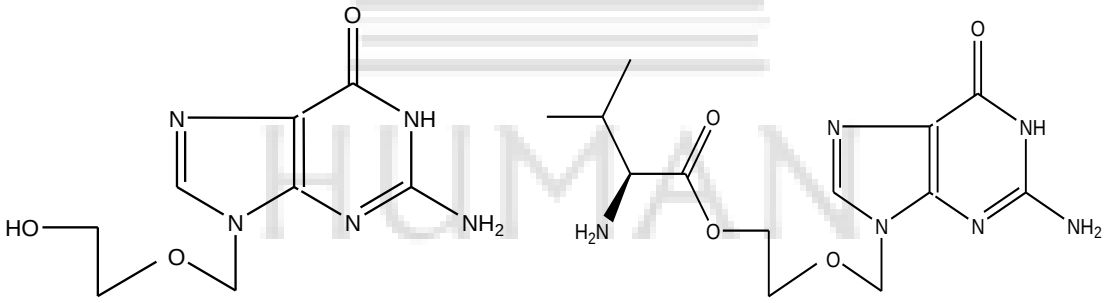
Table 1: Modern Classification of Prodrugs:

Type of Prodrug	Subtypes	Site of conversion	Tissues	Examples
Type I	Type IA	Intracellular	Target tissues/cells	Acyclovir 5-Fluorouracil L-Dopa
	Type IB	Intracellular	Metabolic tissues like liver and GI mucosal cells	Captopril Phenacetin Carbamazepine
Type II	Type IIA	Extracellular	GI Fluids	Loperamide oxide Sulphasalazine
	Type IIB	Extracellular	Systemic circulation or other extracellular compartments	Chloramphenicol succinate
	Type IIC	Extracellular	Target tissue/cells	ADEPT GDEPT VDEPT

Rationale of Prodrug Approach

(1) Prodrugs having improved water solubility:

The poor aqueous solubility of the many active therapeutic agents is of major concern as these active agents possess potential therapeutic activity. In achieving optimum solubility is one of the greatest challenges in drug discovery. The incorporation of prodrug approach helped to overcome the problem of aqueous solubility of many therapeutic agents by improving dissolution rate. This can be done by the utilization of esters and amides of amino acids and phosphoric acid. [9, 10] Among these phosphate esters are widely used to enhance the aqueous solubility of orally and parentally administered drugs. [11] Endogenous phosphatase enzymes release the active parent drug from the phosphate ester prodrug, e.g prednisolone sodium phosphate prodrug, fosphenytoin sodium. The amino acid esters and amide prodrugs are also used to improve the aqueous solubility of active parent drugs e.g valacyclovir **(12)** and valganciclovir which are valine esters of the antiviral drugs acyclovir **(11)** and gancyclovir.[12] The aqueous solubility of acyclovir is found to be 15-30%, while its valine-prodrug exhibits 50% aqueous solubility.[13,14] These prodrugs are good substrates of small peptide transporters (PEPT 1) present in intestinal epithelial cells. (Figure 8)



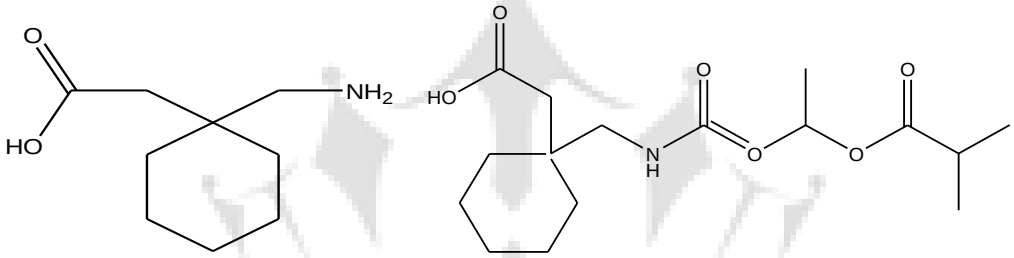
(11) Acyclovir

(12) Valaciclovir

Fig 8: Chemical Structures of Acyclovir and its Valine-Prodrug

(2) Prodrugs as substrates:

The drug has to bypass various pharmacokinetic and pharmaceutical barriers after administration. To overcome this problem, nowadays site-selective drug delivery approach is used i.e prodrug design approach. The prodrugs act as substrates for various endogenous biological transporters e.g Gabapentin enacarbil is a prodrug of gabapentin **(13)** which is substrate for monocarboxylic acid transporter-1 (MCT) and Sodium-dependent multivitamin transporter (SMVT) located all over the intestine. Gabapentin enacarbil **(14)** is having better absorption, bioavailability, and pharmacokinetic properties than parent drug gabapentin. [15] Other examples are ACE inhibitors, antiviral drugs, and anticancer prodrugs act as a substrate for (PEPT 1). (Figure 9).



(13) Gabapentin

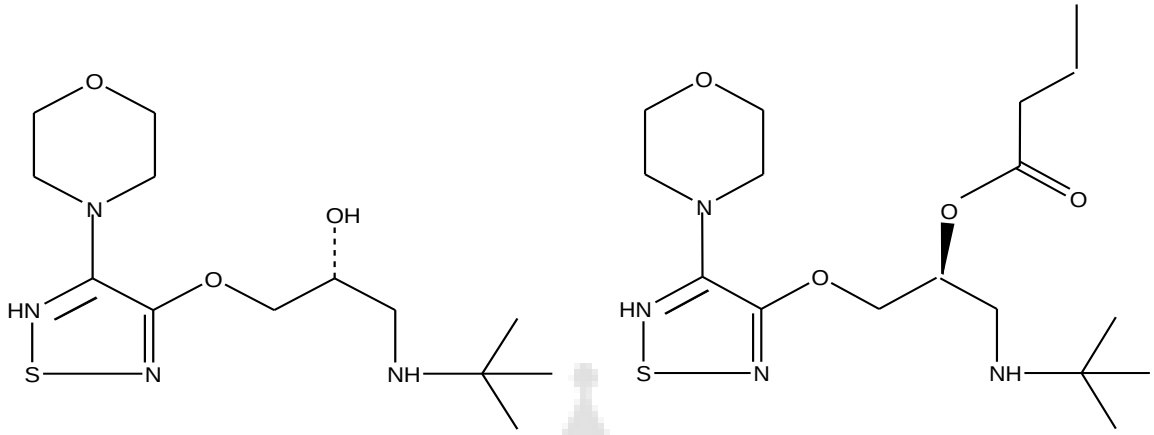
(14) Gabapentin enacarbil

Fig 9: Chemical Structures of Gabapentin and Gabapentin enacarbil

(3) Prodrugs with improved lipophilicity:

The biological membranes consist of phospholipids, therefore, lipophilicity is required to transport through biological membranes. The lipophilicity of polar and ionized drugs can be improved by converting them into esters. [16] The hydrophilic groups present in parent drugs like hydroxyl, thiol, carboxyl, phosphates and amines can be converted to more lipophilic aryl and alkyl esters. These esters can be converted to their active parent drug by the enzymatic action of esterases. [17] For example, Dabigatran, which is polar and permanently charged molecule and therefore has very low bioavailability due to high polarity. The bioavailability of dabigatran was enhanced by the introduction of dabigatran etexilate prodrug which acts by masking the polar

functionalities with carbamic acid ester and carboxylic acid ester groups. Another example includes o-butyryl timolol **(16)**, a prodrug of timolol **(15)** having logP/D value of 2.08 while that of timolol logP/D is -0.04.[18] (Figure 10)



(15) Timolol

(16) o-butyryl Timolol

Fig 10: Chemical Structures of Timolol and its prodrug

(4) Chemotherapeutic prodrugs for improved targetability and efficacy:

Chemotherapy is used to treat many disorders. One of the main disease treated by chemotherapeutic agents is cancer. The majority of anticancer agents exerts their oncostatic action by inhibition of proliferation and arresting cell cycle at a certain stage. But these oncostatic drugs have poor selectivity in selecting tumor cells, so they affect not only tumor cells but also normal cells. Therefore, this problem decreases the effectiveness of these agents for long-term use. This problem is overcome by prodrug approach. An anticancer prodrug should be transported to neoplastic cells and there it will go through a conversion to cytotoxic parent drug by local or recombinant enzymes.[19] Anticancer drugs can be intended to target specific molecules that are highly expressed in tumor cells as compared to normal cells. The new chemotherapeutic prodrugs include Enzyme-activated prodrug therapy, which is further divided into Antibody-directed enzyme prodrug therapy (ADEPT) and Gene-directed enzyme prodrug therapy (GDEPT), Target ligand conjugated prodrugs, enzyme-cleavable prodrugs, membrane transporter associated prodrugs and polymeric prodrugs.

(5) Effect of prodrugs on Presystemic metabolism and excretion:

The availability of the drug in systemic circulation is affected by presystemic metabolism in the gastrointestinal tract and in liver. This presystemic metabolism results in the presence of an inadequate quantity of drug at the site of action or target. This problem has been overcome by various methods of altering the route of administration and by formulation development e.g sublingual route and by controlled release formulations. Presystemic metabolism can also be inhibited by the prodrug approach by masking the metabolically labile functional groups, e.g terbutaline undergoes rapid presystemic metabolism, therefore, it has been prevented by converting its phenolic groups to bis-dimethylcarbamate.[20] Another problem of extensive excretion is associated with a more aqueous solubility of the parent drug. This can be controlled by incorporating lipophilic promoieties.

(6) The role of prodrugs for CNS delivery:

The development of drugs acting on CNS faces one major problem that is the inability of many therapeutic compounds to cross the blood-brain barrier (BBB). The composition of BBB involves the endothelial cells of brain microvessels connected by very tight junctions. Therefore, the passage of the compounds through BBB can be achieved by the aid of carrier involving intrinsic transporter proteins localized on the luminal and abluminal sides of epithelial cells. [21] So there are basically three mechanisms for a compound to enter the brain.

(a) Increasing the passive diffusion by masking polar groups.

(b) Increasing the carrier-mediated or receptor-mediated transport through BBB.

(c) Decreasing the efflux of drug from the brain into the blood.[22]

The various endogenous transporters present at the brain capillary endothelial which forms BBB are:

LAT1 (Large neutral amino acid transporters)

MCT (Monocarboxylic acid transporters)

GLUT1 (Glucose transporters**)**

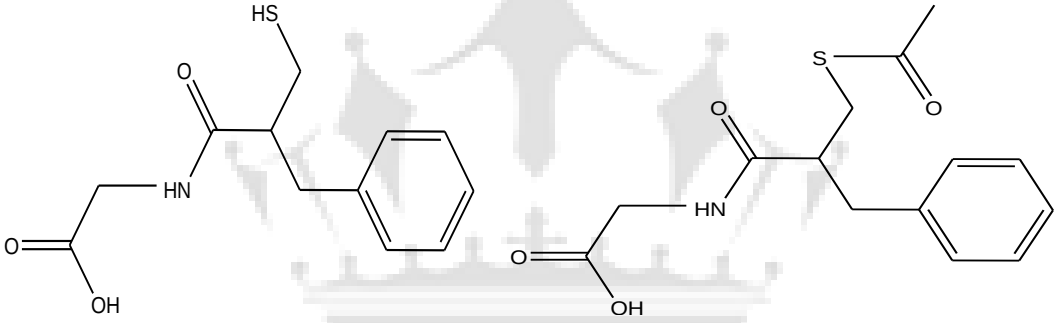
PEPT1 (Peptide transporters)

OCT (Organic cation transporters)

OAT (Organic anion transporters)

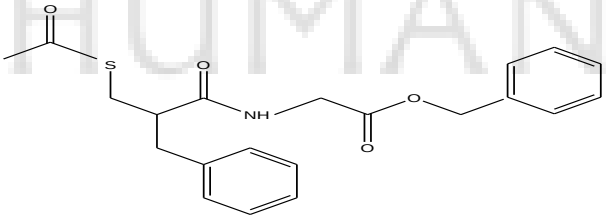
CNT (Concentrative nucleoside and nucleotide transporters)

To improve the bioavailability and permeation of drug through BBB, the targeted prodrug approach has been suggested as a potential alternative. The targeted prodrugs synthesis depends on the types of cells and tissues, types of enzymes and transporters present at the target site. In-depth knowledge about transporters, enzymes at the target site and their interaction with parent drug or ligand to get them recognized at the target site are required before synthesis of the prodrug. [23, 24] For example, Thiorphan (17) has limited BBB permeability but thiol derivatives of thiorphan have been proven with remarkable BBB permeation and therefore high analgesic effect. These derivatives are the monoacylated product of thiorphan (S-acetylthiorphan, 18) and benzyl ester of S-acetylthiorphan (Acetorphan, 19).[25,26] (Figure 11)



(17) **(18)**

Thiorphan S-acetylthiorphan



(19)

Acetorphan

Fig 11: Chemical structures of Thiorphan, S-acetylthiorphan, and Acetorphan

Approaches to Prodrug Design For Desired Distribution At Target Site

Prodrugs can be designed to target specific carriers or enzymes to overcome the undesirable properties of the parent drug. This type of targeted prodrug design requires a thorough knowledge of carrier systems and enzymes which can be used in targeting. Therefore, the two main categories in which the targeted prodrugs will be divided are Targeting specific membrane transporters and Targeting specific enzymes.

(A) Prodrug Design Targeting Membrane Transporters

There are numerous therapeutic agents which have achieved a remarkable improvement in their absorption across the biological membranes with the help of transporters. These transporters play a vital role in absorption, distribution, and elimination of drugs. It is therefore very important to study the nature and functions of these transporters as they have a major role in altering the pharmacokinetic properties of the drug. It has been suggested that a given drug will interact with these membrane transporters during disposition in the body.[27] The transporters are widely distributed in the body especially in the organs like intestine, liver, and kidney as these organs play a remarkable role in absorption, distribution and elimination processes. The drug transporters are widely categorized into two groups i.e SLC (Solute carriers) and ABC (ATP-binding cassette). The transporters move the substrate in either direction across the cell membrane so depending on the direction of movement these can be classified as efflux transporters and influx transporters. The ABC transporters are efflux transporters because they utilize the energy from the hydrolysis of ATP for the active export of substrate from intracellular to the extracellular milieu. The SLC transporters are influx transporters because they make possible the cellular uptake or influx of substrate by facilitated diffusion. There are few SLC transporters which act as both efflux and influx transporters or bidirectional depending on the concentration gradient of the substrate and the ions coupled across the membrane. At this point, it is important to understand the interplay between the transporters present in the apical and basolateral membranes of epithelial cells. This study of efflux and influx transporters is mandatory to determine the degree and direction of drug movements in the organs like intestine, liver, and kidney. The transporters located in intestine, liver and kidney are shown below in fig.12, fig.13 and fig.14 [28]:

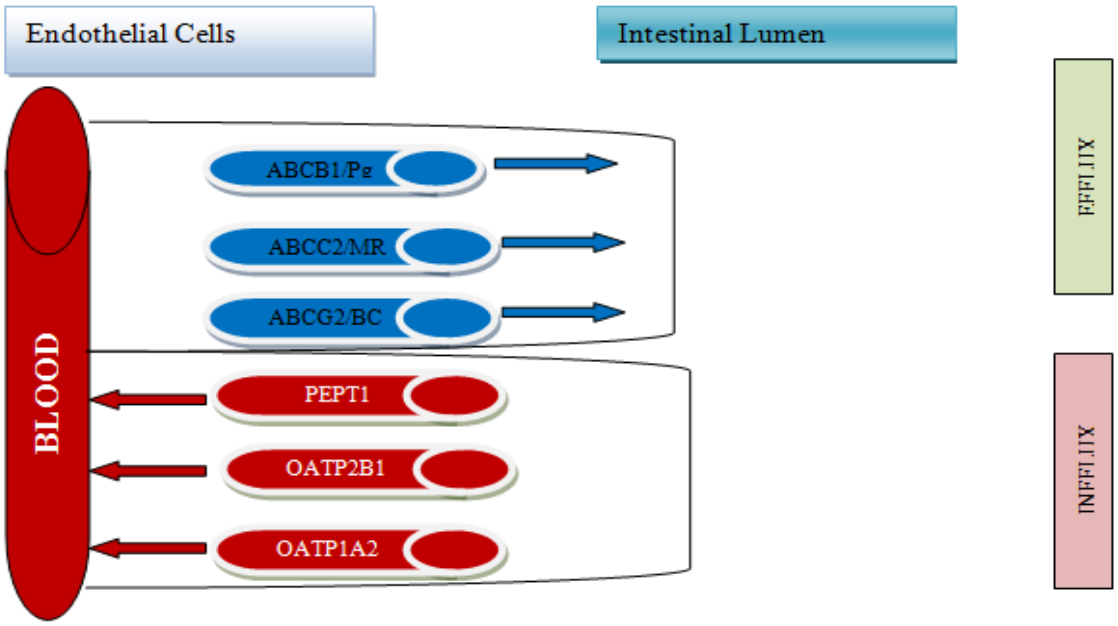


Fig.12: Transporters expressed in the GI tract. Efflux transporters are in red color which effluxes back the substrate into the intestinal lumen while influx transporters are in blue color which influx the substrate from intestinal lumen into the blood

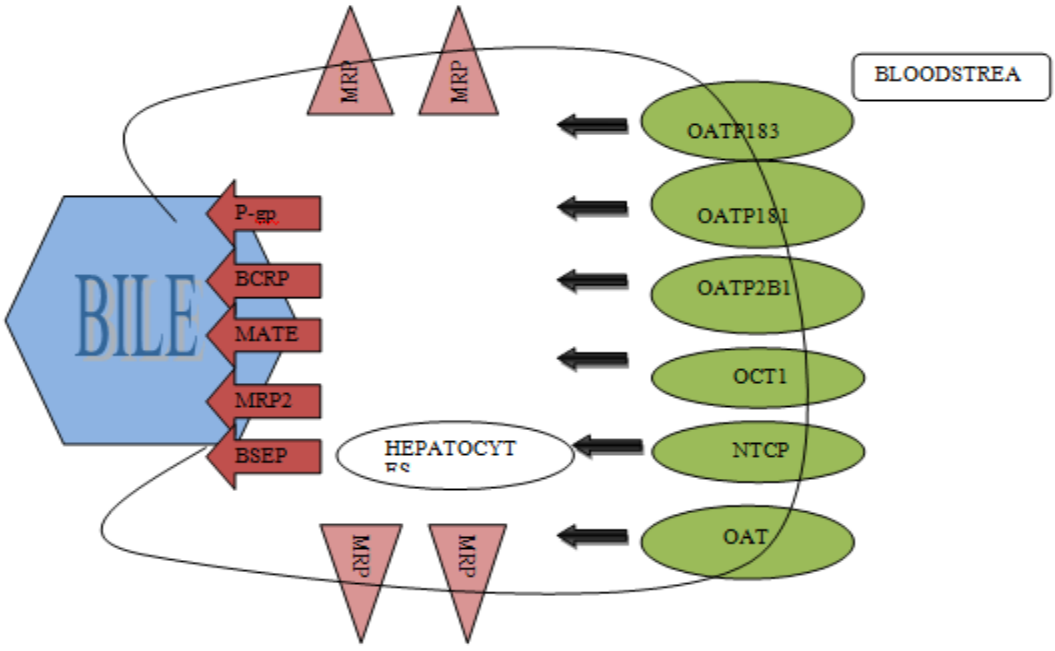


Fig.13: Transporters expressed on hepatocytes. Influx transporters are in blue color which transports drugs from the blood into hepatocytes where they are metabolized, efflux transporters are in red color which effluxes drugs and their metabolites into bile or back into the blood.

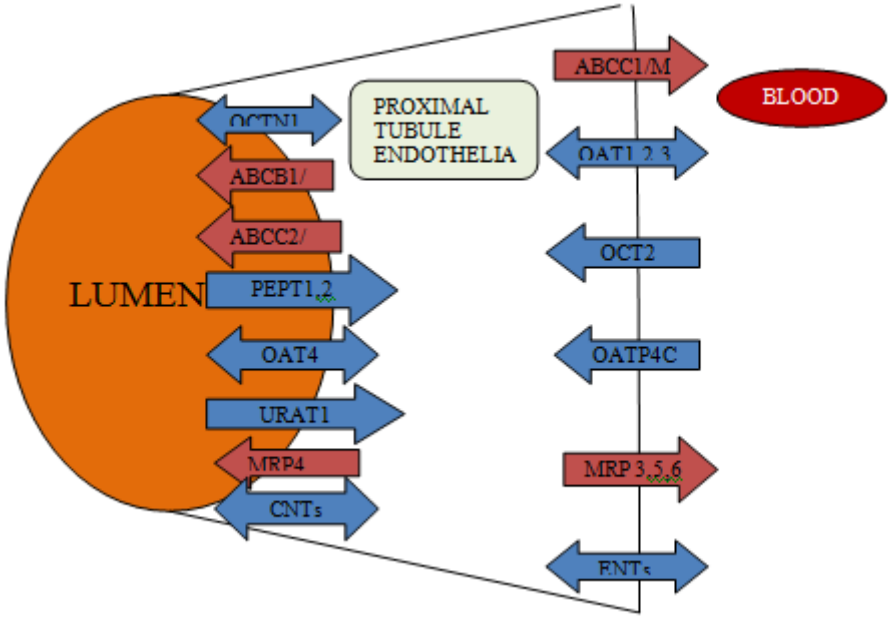


Fig.14: Transporters expressed in the renal proximal tubule. Influx transporters in blue color on Basolateral membrane (BLM) efflux substrates back into the renal epithelial cells from blood. Efflux transporters are in red color transport substrates back to blood.

SLC Transporters

The SLC drug transporters play a significant role in improving the pharmacokinetic properties of the drug and in the uptake of the drug in intestine, liver, and kidney. There are many SLC transporters which belong to different SLC subfamilies. The main transporters at this point under consideration are oligopeptide transporters(PEPT1/2), Organic anion transporters (OATs), Organic cation transporters (OCTs) and Organic anion transporting polypeptides (OATPs).

(i) Oligopeptide Transporters: The two main oligopeptide transporters are PEPT1 and PEPT2. The oligopeptide transporters are capable of transporting peptidomimetic and other drug substrates. PEPT1 transporters are widely expressed in the intestine and also in low levels in kidney. These transporters are of low affinity and high capacity. PEPT2 transporters are widely distributed in kidneys. These are renal transporters with high-affinity and low-capacity.[29, 30] The amino and carboxylic acid groups present in the substrates of PEPT1 transporters allows the prodrug design and development. This prodrug design is responsible for the improved oral

availability of drug by increasing the intestinal absorption. PEPT2 transporters are involved in reabsorption from proximal tubules.

(ii) Organic Anion Transporters (OATs): Organic anion transporters are widely expressed in kidney and in some other organs like liver, placenta, and brain. They perform their function by pairing the cellular influx of an organic anion to the replace with carboxylates and other organic anions in the cell. The active uptake of the anionic drugs takes place with the help of driving force occurs due to the concentration gradient of these anions.

(iii) Organic Cation Transporters (OCTs): Organic cation transporter is more commonly referred to as OCT1. OCT1 is a hepatic transporter which is widely distributed in hepatocytes and plays major role in drug disposition and hepatic clearance. OCT1 transporters are also present in enterocytes of the small intestine, renal proximal tubules and at low levels in neurons.[31] OCT1 allows and mediates Na^+-independent transport of protonated molecules and bulkier molecules. OCT2 transporter is mainly present in kidney and almost absent in liver. This has been reported that with the help of QSAR studies hydrophobicity, molecular size and shape are important determinants for binding of OCT2 transporter to substrate drugs and to predict unwanted drug interactions.[32]

(iv) Organic Anion Transporter Polypeptides (OATPs): Organic Anion Transporter Polypeptides are capable of transportation and cellular uptake of more bulky (MW>450 Da) and hydrophobic organic anionic groups. There is a wide range of compounds which acts as a substrate for OATPs like amphipathic organic compounds, bile salts, steroid conjugates thyroid hormones and various drugs. The distribution of OATPs is very organ specific. Different types of OATPs are distributed in their specific organ types e.g OATP1A2 is expressed in kidney, small intestine and bile duct. OATP1B1 and OATP1B3 are expressed only in the liver.[33]

ABC TRANSPORTERS

ABC transporters are mainly responsible for the efflux mechanisms. ABC transporters are widely distributed in the intestine, liver, and kidney. These transporters play an important role in absorption, distribution, and removal of drugs. These are well known as ATP-binding cassette (ABC), therefore, binds and hydrolyze ATP to drive the efflux processes. There are many

subfamilies of ABC transporters but there are few types of ABC transporters which are important to discuss at this point of discussions like P-gp, Multidrug resistance-associated Proteins (MRPs), Bile salt export pump (BSEP) and Breast cancer resistance protein (BCRP).

(i) P-gp Transporters: P-glycoprotein transporter is located mainly in the apical membrane of the intestine, liver, and kidney. P-gps exhibit broad range substrate specificity, therefore, many compounds having a size of 350 Da (small molecules) to 4000 Da (polypeptides) acts as a substrate for P-gps. A large number of substrates for P-gps falls in the class of polyvalent, large, organic cations.[34] It has been reported that multispecificity of P-gps is due to overlap drug-binding sites to some extent in the internal cavity of the protein.[35]

(ii) Bile Salt Export Pump (BSEP): BSEP is expressed mainly in the apical membrane of liver.[36] The substrate specificity of BSEP is narrow and specific for bile acids. The main function of BSEP is excretion of bile acids into bile and also acts as a dynamic force for the flow of bile.[37]

(iii) Multidrug Resistance-Associated Proteins (MRPs): There are about nine MRPs which have been identified in the subfamily of ABC transporters. Among these nine MRPs, MRP2, MRP3, and MRP4 are the most vital transporters. The MRPs plays a major role in transporting organic anionic compounds and uncharged amphipathic compounds.[38] The apical membrane of polarized cells mainly comprise MRP2 and therefore its important function is terminal excretion of the anionic drugs.[39] MRP3 are localized in basolateral membrane, therefore, mediating the cellular efflux of glucuronidated drugs from intestine and liver into the blood.[40] MRP4 are localized in both apical and basolateral membranes. In hepatocytes, they are expressed in basolateral membrane whereas in renal proximal tubule cells they are expressed in the apical membrane.[41]

Amino Acid Transporters

The amino acid transporters are responsible for the transport of amino acids in the body. Based on their ionic nature and substrate specificity, the amino acid transporters are of following types:

(i) Anionic Amino acid Transporters: The major anionic amino acid transporters Glutamate transporters. The glutamate transporters are mainly responsible for transport of glutamate through plasma membrane in neurons. The glutamate transport also transports aspartate and known as a glutamate-aspartate transporter. The main members of glutamate transporter are excitatory amino-acid transporters (EAATs) and vesicular glutamate transporters (VGLUT). The EAATs are classified into five subclasses i.e $EAAT_{1-5}$. [42] The main difference between EAATs and VGLUT is that EAATs are dependent on the electrochemical gradient of Na^+ ions while VGULT is independent of the electrochemical gradient of Na^+.[43] The subfamilies of EAATs are located at different sites. $EAAT_{1-2}$ are located glial membranes. $EAAT_2$ performs the main functions of 90% glutamate reuptake in the brain.[44] $EAAT_{3-4}$ are expressed in neurons. $EAAT_5$ are located in bipolar neurons of retina and photoreceptors.[45]

VGLUT is classified into three subfamilies-$VGLUT_{1-3}$. The main function of these transporters is the uptake of neurotransmitter by synaptic vesicles so that they can be released into the synapse. The proton gradient of secretary system regulates the functioning of VGLUT. [46]

(ii) Cationic Amino Acid Transporters: The transport of cationic amino acids is facilitated by transport systems. The two major transport systems which are involved in the transport of cationic amino acids are Na+-independent y^+ system and N^+ dependent system. The system y^+ is responsible for transport of amino acids like arginine, lysine, and ornithine while Na^+ dependent system transports glutamine and homoserine. The other transport systems which facilitate the transport of cationic amino acids are y^{+L}, $b^{0,+}$ and $B^{0,+}$. The transport systems have different substrate specificity. The main difference is that some transport systems are specific for the transport of cationic amino acids while others have interactions with both cationic and neutral amino acids. The specificity of transport systems has been showed earlier by Shennan *et al*. It has been reported that in mammary tissues of rat Na^+-independent system which is involved in the transport of cationic amino acids also have interaction with neutral amino acids (L-leucine and L-glutamine).[47] Further, it was concluded by Sharma and Kansal that transport system exhibits broad specificity i.e it interacts with both cationic and neutral amino acids is y^{+L} type of transporter and the specific uptake of cationic amino acids is due to the y^+ system.[48]

(B) Prodrug Design: Targeting Specific Enzymes

The enzyme-targeted prodrug design approach can be widely used to improve oral absorption of drugs and also site-specific drug delivery. Enzymes can be an important target for improving oral drug absorption of the drugs.[49] Secondly, a major reason for using enzyme-targeted prodrug approach is site-specificity which is a very important aspect for precise and direct effects at the site of action with minimal effect on rest of the body.[50] In this section improvement in site-specificity of prodrugs with the aid of enzyme targeted prodrug design will be discussed.

Enzyme-Targeted Prodrug Approach for Site-Specificity: The enzyme-targeted prodrug approach can be obtained by the process of tissue-specific activation of a prodrug which is further processed by the metabolic process by an enzyme present in the tissue. The enzyme present in the tissue can be tissue specific or can be present in higher concentrations. The enzyme-targeted site-specificity now days have been suggested to play a vital role in chemotherapy of cancer. This has been found that high concentrations of activating enzymes provide site-specificity to the prodrugs and responsible for the effective treatment of animal tumors.[51] It was found that human tumors containing high concentrations of activating enzymes were rare and also, on the other hand, the activating enzymes present in high concentrations were not linked with any specific type of tumor.[52] So, it was a major problem of using the enzyme-targeted approach in the treatment of human tumors. It has been suggested that this problem is resolved with the introduction of newer techniques which helps in the localization of prodrug activation enzymes in the specific tumor cells prior to the administration of prodrug. These techniques are referred as:

ADEPT (Antibody Directed Enzyme Prodrug Therapy)
GDEPT (Gene Directed Enzyme Prodrug Therapy).

General Concept of ADEPT and GDEPT for Site-Specificity of Prodrugs

In ADEPT strategy, the drug-activating enzyme is localized onto the tumor cell surface by forming conjugate with a monoclonal antibody which targets only tumor cells. The non-toxic prodrug is administered systemically which is converted to a toxic drug by the pre-localized drug-activating enzyme resulting is cytotoxic effects in tumor cells. [53, 54] It has been shown that

various classes of human tumor xenografts are sensitive to ADEPT by using combinations of different antibodies, enzymes, and prodrug. [55] In GDEPT strategy, it consists of a prodrug which is an inactive form of the active drug which is delivered to the body systemically and a gene which is decoded at target cells to form the enzyme. The vectors are used to transport prodrug activated enzyme gene to tumor cells and normal cells. The main challenge in GDEPT is vector delivery. It has been suggested that there are main two types of strategies i.e 1) Search & destroy approach and 2) Induction approach.

In search & destroy strategy, vector identifies the tumor cells selectively and kill the tumor cells whereas in induction strategy vector is delivered locally to stimulate the immune system and therefore killing the tumor cells. The selection of vectors for the delivery of gene is a very crucial step in terms of efficacy in this strategy. The vectors may be synthetic in origin or more commonly used that are derived from microbes like viruses and bacteria. The second major concern in both ADEPT and GDEPT is the selection of enzyme. The general considerations while selecting enzyme for ADEPT and GDEPT are as follows:

1. The enzyme would be monomeric and of low molecular weight so that it would be easy to handle and protein modification would be possible.[56]
2. The enzymes from the non-human or non-mammalian origin are preferred targets.
3. The enzymes from the microbiological origin are of significant importance in terms of specificity.[57] (Table 2)

Table 2: Endogenous Enzymes Responsible For Prodrug Activation:

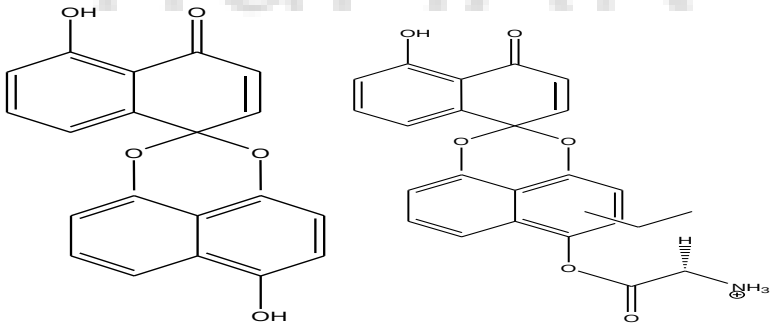
CLASS	ENZYME	DRUG	PRODRUG	P'COLOGY
Oxidoreductase	Aldehyde Oxidase	5-ehynyluracil	5-ethynyl-2(1H)-pyrimidinone	Mechanism-based inhibitor of dihydropyrimidine dehydrogenase (DPD)[58]
	Amino acid oxidase	Hydrogen peroxide	d-alanine	Oxidative stress[59]
	Cytochrome P450 reductase	Nitroxide radical	Tirapazamine	DNA alkylation and oxidative stress[60]
	DT- diaphorase	Semiquinone radical	Diaziquone	DNA alkylation and oxidative stress[61]
	Cytochrome P450	AQ4	AQ4N	Topoisomerase II inhibitor[62]
	Tyrosinase	Phenol mustard	Phenyl mustard	DNA alkylation[63]
Transferases	Glutathione S-transferase	6-MP	PTA	Antimetabolite[64]
	Thymidine phosphorylase	5-FU	5'-deoxy-5-flurouridine	Upregulation of pyrimidine nucleoside phosphorylases by the cytokine interferon[65]
Hydrolases	Carboxylesterase	5-FU	Capecitabine	Thymidylate synthase inhibitor[66]
	Alkaline phosphatase	3-AP	3-AP phosphate(67)	--
	β-glucuronidase	paclitaxel	Paclitaxel glucuronide	Microtubule binding[68]
Lyases	Cysteine conjugate-β-lyase	Selenol	SeCys conjugate	Apoptosis[69]

Major Chemical Moieties For Prodrug Design

As per the information about prodrug design, molecular modification in the parent drug is the main strategy adopted to alter the physicochemical and pharmacological profile of the parent drug. There are many functional groups which act as carriers are available for molecular modification but their choice depends on the chemical groups or moieties already present on parent drug. These chemical groups or carriers which can be incorporated in the structure of parent drug are mainly Esters, Amides, Carbamates, Phosphates.[70] In this section of the article, we will discuss them one by one in details.

(i) Prodrugs with Esters: Esters are the promising bond linking groups due to their capability of undergoing hydrolysis easily. From the literature search, it has been found that there are many examples of esters prodrugs which have attained more aqueous solubility than the parent drug. For example, palmarumycin, etoposide, NSAIDS and many antiviral drugs. We will hereby discuss few examples to understand the intensity of the effect of prodrug design on aqueous solubility of the parent drug.

(a) Palmarumycin **(20)** is a lipophilic drug with poor aqueous solubility, therefore it was suggested that this drug has shown poor anticancer activity *in vivo*. So, the chemists synthesized the amino-ester prodrugs of palmarumycin. They designed glycyl ester **(21)** derivative which was found to be about seven times increased aqueous solubility than the parent drug.[71] (Figure 15)

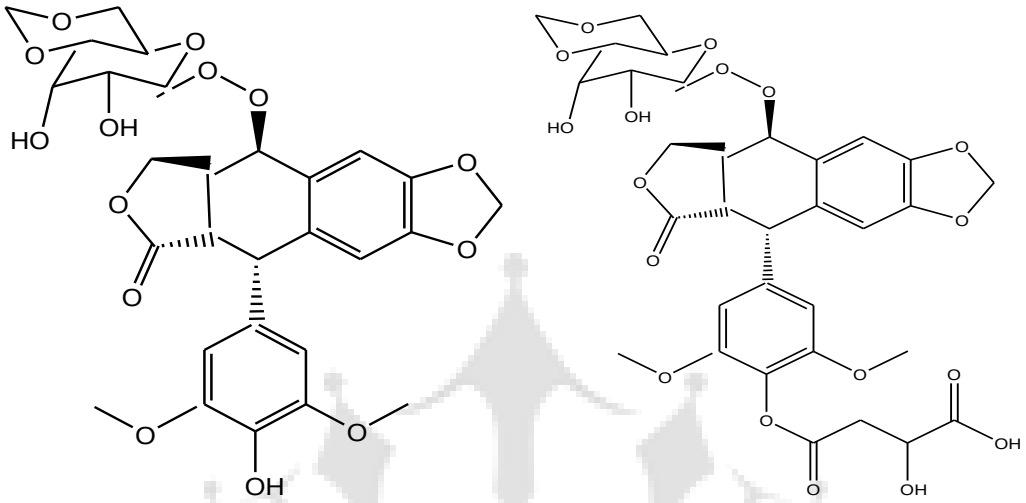


(20) Palmarumycin

(21) Glycyl-ester of Palmarumycin

Fig. 15 : Chemical Structures of Palmarumycin and its ester prodrug

(b) Etoposide **(22)** which is a topoisomerase inhibitor having low aqueous solubility. The prodrug design was adopted and prodrugs were synthesized whose aqueous solubility was demonstrated as about 120 folds higher than the parent drug. The prodrug of etoposide shown below **(23)** attains water solubility of about 9.0 mg/ml as compared to etoposide which has 0.1 mg/ml of water solubility.[72] (Figure 16)

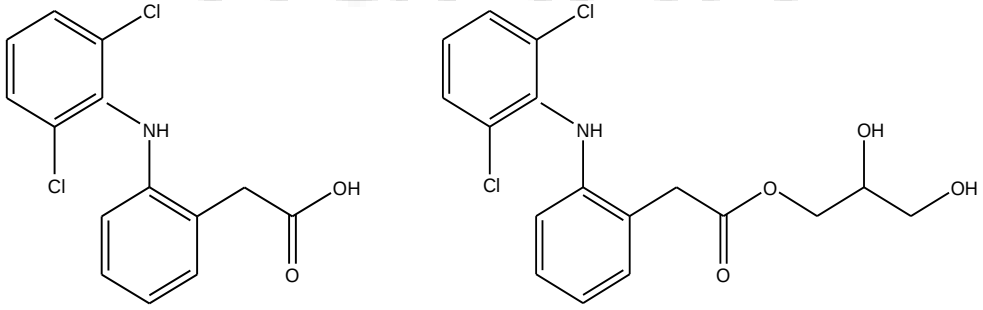


(22) Etoposide

(23) Malic acid ester of etoposide

Fig 16: Chemical Structures of Etoposide and its malic acid prodrug

(c) Diclofenac ester **(25)** prodrugs of Diclofenac **(24)** were synthesized by chemists and it was found that glycerol ester has been shown better water solubility of about 0.551 µmol/ml as compared to parent drug having 0.0034 µmol/mL of water solubility.[73] (Figure 17)



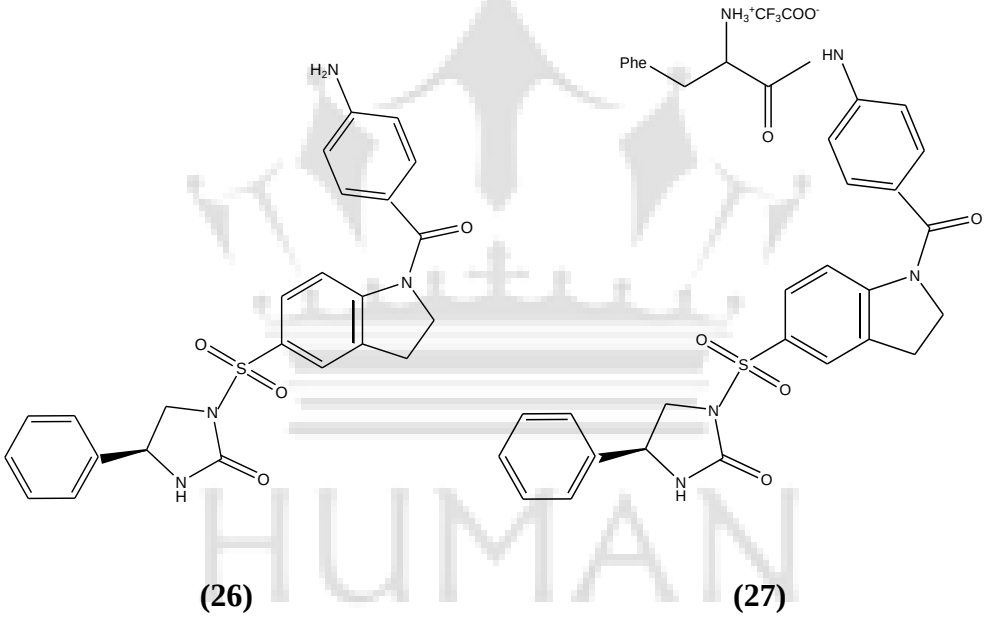
(24) Diclofenac

(25) Diclofenac Prodrug

Fig 17: Chemical Structures of Diclofenac and Its glycerol Prodrug

(b) Prodrugs with Amides: The amide prodrugs are also promising compounds for increasing aqueous solubility of parent drug and its bioavailability. As compared to esters, amide bonds are more stable to enzymatic hydrolysis. There are numerous examples of amide prodrugs, some are discussed below:

(a) DW2282 **(26)** is chemically (S)-1-[1-(4-aminobenzoyl)-2,3-dihydro-1H-indol-6-sulphonyl]-4-phenyl-imidazolidin-2-one, which is an anticancer drug with low water solubility (0.024 mg/mL) and higher gastrointestinal toxic effects.[74] Many amino acid prodrugs were synthesized almost all of them attained higher water solubility as compared to the parent drug. One of the compound **(27)** have shown very good aqueous solubility (0.865 mg/mL) and bioavailability by oral route is given below[75]: (Figure 18)



(26) DW2282

(27) Prodrug of DW2282

Fig 18: DW2282 and its prodrug

Acyclovir **(28)** is another example with poor aqueous solubility. It is an antiviral drug having a low bioavailability of about 10% to 20 %.[76] To overcome these problems of low bioavailability and poor aqueous solubility, an amide prodrug **(29)** and ester prodrug **(30)** were synthesized. Both the prodrugs showed a remarkable increase in water solubility i.e amide prodrug showed about 17-fold higher aqueous solubility than parent drug and ester prodrug showed about 9-fold higher water solubility than the parent drug.[77] (Figure 19)

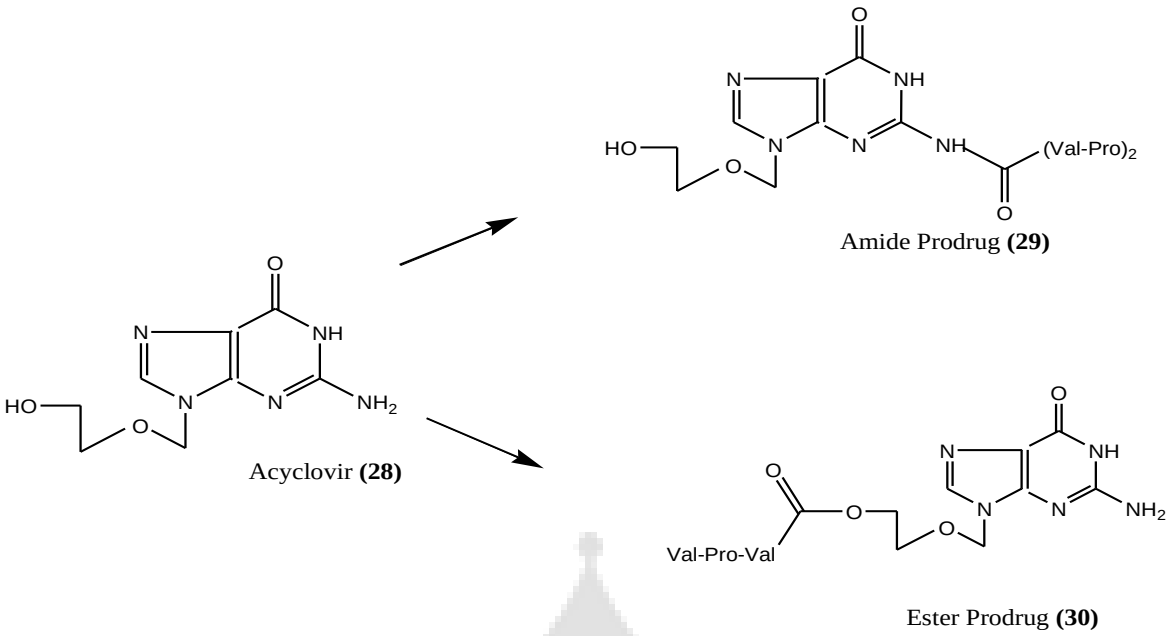
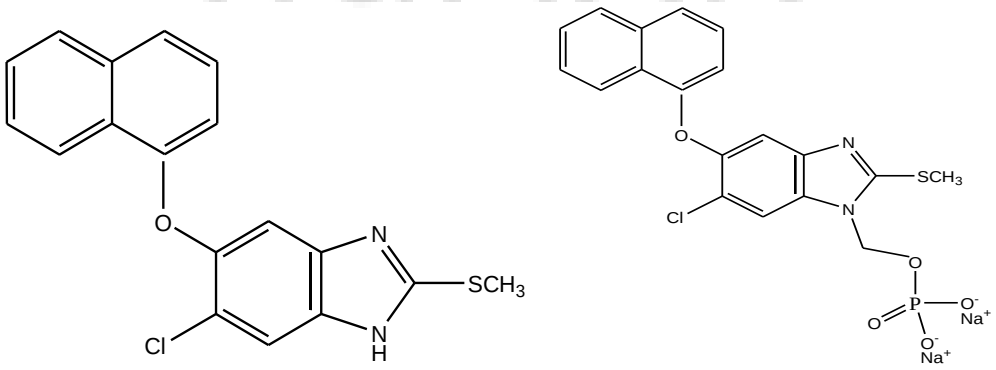


Fig. 19: Amide and Ester Prodrugs of Acyclovir

(c) Prodrugs with Phosphates: The phosphate prodrugs have been proven good candidates to increase the aqueous solubility and bioavailability of the parent drug. The phosphate prodrugs get converted to its parent drug by the action of intestinal alkaline phosphatase enzyme.[78]

(a) Flores-ramos *et al.* synthesized a prodrug of benzimidazole derivative i.e α-6-chloro-2-(methylthio)-5-(napthalen-1-yloxy)-1H-benzo[*d*] imidazole. **(31)**. The prodrug **(32)** synthesized by linking disodium phosphate to the structure and found be 50,000-folds higher water soluble than the parent drug.[79]

(Figure 20)



(31) **(32)**

Fig. 20: Benzimidazole derivative and its Prodrug

(b) Another lipophilic drug which encounters difficulty in aqueous solubility is propofol **(33)**. The phosphate prodrugs were synthesized to overcome this problem either by directly attaching phosphate group to hydroxyl group of parent drug **(34)** or by using spacers such as oxymethyl and ethylenedioxy moieties **(35)**.[80] (Figure 21)

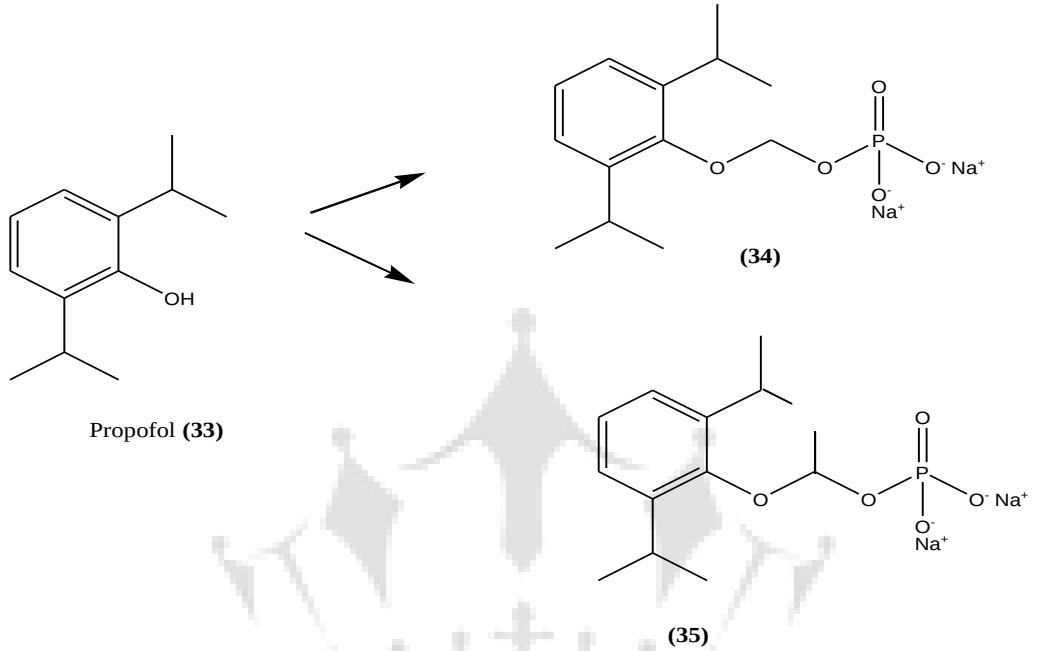
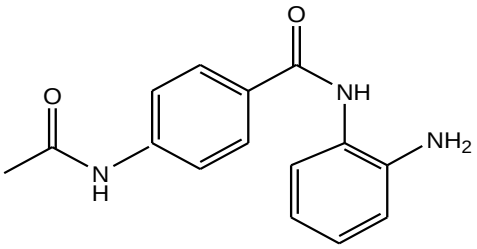


Fig. 21: Propofol and its Prodrugs

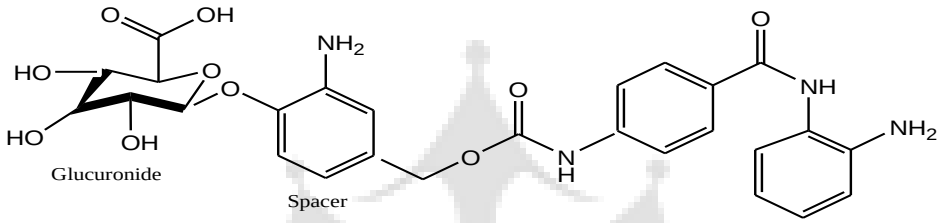
(d) Prodrugs with Carbamates: Carbamates are derivatives of carbamic acid. The carbamates are generally exhibited very good chemical and proteolytic stability. Carbamates are able to permeate through cell membranes and also they have the capability to alter intermolecular and intramolecular interactions with the target receptor or enzyme. Due to their contribution in drug designing carbamates have received special attention since past few years.[81]

(a) Histone deacetylases are responsible for gene expression and several inhibitors of histone deacetylases exhibit anti-tumor activity. One of the histone deacetylases inhibitor is a benzamide compound CI-994 **(36)**[82, 83] The poor aqueous solubility of this compound limits its therapeutic activity. To overcome this problem, Thomas M. *et al* have synthesized two glucuronide prodrugs. In one compound they have linked glucuronide moiety with the aid of spacer **(37)** and in another compound they have directly linked the glucuronide moiety with the carbamate group

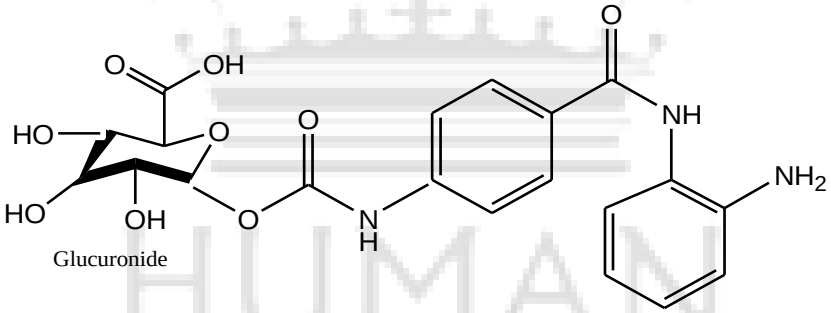
of parent drug **(38)**. The aqueous solubility of parent compound CI-994 was found to be 0.08 mg/mL and both the prodrugs showed aqueous solubility more than 1 mg/mL.[84] (Figure 22)



(36)



(37)



(38)

Fig. 22: Glucuronide Prodrugs of CI-994 Benzamide compound

CONCLUSION

The process of drug discovery and development is time-consuming and also includes cost-related factors. Thus, there are various automated attempts which have been used to rationalize the process such as computer-aided drug design (CADD), combinatorial synthesis and high-throughput screening. During the process of drug discovery and development, the major focus is on various pharmacokinetic, pharmacodynamic and pharmaceutical properties of the drug. The

prodrug design approach is one of the promising approaches to enhance the therapeutic efficacy of the parent drug by various mechanisms and therefore, reduce toxicity. The prodrugs are now powerful alternative approach to improve aqueous solubility, lipophilicity and also to improve target-selectivity. The prodrugs have been found more effective and exhibit target selectivity by various mechanisms such as by transporter proteins (SLC & ABC transporters) and by enzyme-activated prodrug therapy (ADEPT & GDEPT). Despite the commercial success of the prodrugs, the concept is still quite unknown. It is not always easier and faster to develop prodrug. As we know it is responsible to overcome unwanted barriers such as pharmacokinetic, pharmacodynamic, pharmaceutical and economical barriers of the parent drug, but sometimes prodrugs may generate new barriers for the delivery and targeting. For the future research scope and successful prodrug approach, more studies are needed to identify more novel prodrug structures (promoieties) to target desirable tissues and to achieve desired pharmacological profiles.

REFERENCES

[1] K M Huttunen, H. Raunio, J.Rautio. Prodrugs from serendipity to rational design. Pharmacol Rev. 2011; 63: 750-771.
[2] V.J Stella. Prodrugs: some thoughts and current issues. J Pharm Sci. 2010;99: 4755-4765.
[3] B. Testa. Prodrugs bridging pharmacodynamic/pharmacokinetic gaps. Curr Opin Chem Biol. 2009;13: 338-344.
[4] J. Rautio, H. Kumpulainen, T. Heimbach, R. Oliyai, D. Oh, T. Jarvinen, J. Savolainen. Prodrugs: Design and clinical applications. Nat Rev Drug Discov.2008; 7: 255-270.
[5] J.B Zawilska, J. Wojcieszak, A.B Olejniczak. Prodrugs: A challenge for the drug development. Pharmacological Reports. 2013; 65: 1-14.
[6] A. Abu-Jaish, S. Jumma, R. Karaman. Prodrugs Overview in prodrug design- A new era. Nova, USA; 2014.
[7] S. Papot, I. Tranoy, F. Tillequin, J.C Floren, J.P Gesson. Design of selectivity activated anticancer prodrugs: elimination and cyclization strategies.Curr. Med. Chem. Anticancer agents. 2002; 22: 155-185.
[8] Kuei-Meng Wu. A new classification of prodrugs: Regulatory Perspectives. Pharmaceuticals. 2009; 2: 77-81.
[9] P. Fasinu, V. Pillay, V.M Ndesendo, L.C du Toit, Y.E Choonara. Diverse approaches for enhancement of oral drug bioavailability. Biopharm Drug Dispos. 2011; 32: 185-209.
[10] C.E Miller. Prodrug administration for enhancing the bioavailability of drugs with low molecular solubility. Chem Biodivers. 2009; 6: 2071-2083.
[11] V.J Stella, K.W Nti-Addae. Prodrug strategies to overcome poor water solubility. Adv Drug Deliv Rev. 2007; 59: 677-694.
[12] D. Vytla, R.E Combs-Bachmann, A.M Hussey, S.T McCarron, D.S McCarthy, J.J Chambers. Prodrug approaches to reduce hyperexcitation in the CNS. Adv Drug Deliv Rev. 2012; 64: 666-685.
[13] G.E Granero, G.L Amidon. Stability of valacyclovir: implications for its oral bioavailability. Int J Pharm. 2006; 317: 14-18.
[14] S. Jana, S. Mandlekar, P. Marathe. Prodrug design to improve pharmacokinetics and drug delivery properties: Challenge to the discovery scientists. Current Med Chem. 2010; 17: 3874-3908.

[15] B.M Liederer, R.T Borchardt. Enzymes involved in the bioconversion of ester-based prodrugs. J Pharm Sci. 2006; 95: 1177-1195.
[16] W.G Eisert, N. Hauel, J. Stangier, W, Wienen, A. Clemens, J. Van Ryn. Dabigatran: an oral novel potent reversible nonpeptide inhibitor of thrombin. Arterioscler Thromb Vasc Biol. 2010; 30: 1885-1889.
[17] S. Blech, T. Ebner, E. Ludwig-Schwellinger, J. Stangier, W. Roth. The metabolism and disposition of the oral direct thrombin inhibitor, dabigatran, in humans. Drug Metab Dispos. 2008; 36: 368-399.
[18] K.M Huttunen, J. Rautio. Prodrugs an efficient way to breach delivery and targeting barriers. Current topics in Medicinal Chem. 2011; 11: 2265-2287.
[19] R. Mahato, W. Tai, K. Cheng. Prodrugs for improving tumor targetability and efficiency. Adv Drug Deliv Rev. 2011; 63:659-670.
[20] L.A Svensson, A, Tunek. The design and bioactivation of presystemically stable prodrugs. Drug Metab Rev. 1988; 19: 165-194.
[21] N.J Abbott, A.A Patabendige, D.E Dolman, S.R Yusof, D.J Begley. Structure and function of blood- brain barrier. Neurobiol Dis. 2010; 37:13-25.
[22] M.M Patel, B.R Goyal, S.V Bhadada, J.S Bhatt, A.F Amin. Getting into the brain: approaches to enhance brain drug delivery. CNS Drugs. 2009; 23:35-58.
[23] A. Dahan, M. Khamis, R. Agbaria, R. Karaman. Targeted Prodrugs in oral drug delivery:the modern molecular biopharmaceutical approach. Expert Opin Drug Deliv. 2012; 9: 1001-1013.
[24] R. Karaman. Prodrugs design based on inter and intramolecular processes. Chem Biol Drug Des. 2013; 82: 643-668.
[25] J.M Lacomte, J. Costentin, A. Vlaiculescu, P. Chaillet, H. Marcais-Collado, C. Llorens-Cortes, M. Leboyer, J.C Schwartz. Pharmacological properties of acetorphan, a parentrally active “enkephalinase” inhibitor. J Pharmacol Exp Ther. 1986; 237: 937-944.
[26] S.M Lambert, F. Mergen, J.H Poupaert, P. Dumont. Analgesic Potency of S-Acetylthiorphan after intravenous administration to mice. Eur J Pharmacol. 1993; 243: 129-134.
[27] P.D Dobson, D.B Kell. Carrier mediated cellular uptake of pharmaceutical drugs: an exception or the rule? Nat Rev Drug Discov. 2008; 7: 205-220.
[28] T.M Sissung, S.M Troutman, T.J Campbell, H.M Pressler, H. Sung, S.E Bates, W.D Figg. Transporter Pharmacogenetics: Transporter polymorphisms affect normal physiology, diseases, and pharmacotherapy. Discovery Medicine. 2012; 13: 19-34.
[29] M. Brandsch, I. Knutter, E. Bosse-Doenecke. Pharmaceutical and Pharmacological importance of peptide transporters. J Pharm Pharmacol. 2008; 60: 543-585.
[30] M.A Kamal, R,F Keep, D.E Smith. Role and relevance of PEPT1 in drug disposition, dynamics and toxicity. Drug Metab Pharmacokinet. 2008; 23:236-242.
[31] W.J Jonker, A.H Schinkel. Pharmacological and physiological functions of the polyspecific organic cation transporters: OCT1,2 and 3 (SLC 22A1-3). J Pharmacol Exp Ther. 2004;308: 2-9.
[32] W.M Suhre, S. Ekins. C.Chang, P.W Swaan, S.H Wright. Molecular determinants of substrate/inhibitor binding to the human and rabbit renal organic cation transporters hOCT2 and rbOCT2. Mol Pharmacol. 2005; 67: 1067-1077.
[33] B. Hagenbuch, C. Gui. Xenobiotic transporters of the human organic anion transporting polypeptides (OATP) family. Xenobiotics. 2008; 38: 778-801.
[34] S.F Zhou. Structure, function and regulation of p-glycoprotein and its clinical relevance in drug disposition. Xenobiotica. 2008; 38: 802-832.
[35] S.G Aller, J. Yu, A. Ward. Y. Weng, S. Chittaboina, R. Zhou, P.M Harell, Y.T Trinh, Q. Zhang, I.L Urbatch, G.Chang. Structure of p-glycoproteins reveals a molecular basis for poly-specific drug binding. Science. 2009;323: 1718-1722.
[36] B. Stieger, Y. Meier, P.J Meier. The bile salt export pump. Pflugers Arch. 2007;453: 611-620.

[37] W.A Alrefai, R.K Gill. Bile acid transporters: Structure, function, regulation and pathophysiological implications. Pharm. Res. 2007;24: 1803-1823.
[38] R.G Deelay, C. Westlake, S.P Cole. Transmembrane transport of endo and xenobiotics by mammalian ATP-binding cassette multidrug resistance proteins. Physiol Rev. 2006; 86: 849-899.
[39] A.T Neis, D. Keppler. The apical conjugate efflux pump ABCC2 (MRP2). Pflugers Arch. 2007; 453: 643-659.
[40] P. Brost, C. de Wolf, K. van de Wetering. Multidrug resistance-associated proteins 3,4 and 5. Pflugers Arch. 2007; 453: 661-673.
[41] R.A Van Aubel, P.H Smeets, J.G Peters, R.J Bindels, F.G Russels. The MRP4/ABCC4 gene encodes a novel apical organic anion transporter in human kidney proximal tubules: Putative efflux pump for urinary cAMP and cGMP. J AmSoc Nephrol. 2002; 13: 595-603.
[42] R. Ganel, J.D.Rothstein. Ionotropic Glutamate Receptors in the CNS.Springer Berlin Heidelberg;1999.
[43] N.C.Danbolt. Glutamate Uptake. Prog Neurobiol. 2001; 65: 1-105.
[44] S. Holmseth, H.A. Scott, K. Real, K.P. Lehre, T.B. Leergaard, J.G.Bjaalie, N.C.Danbolt, The concentrations and distributions of three C-terminal variants of the GLT1 (EAAT2:slc1a2) glutamate transporter protein in rat brain tissue suggest differential regulation. Neuroscience. 2009; 162: 1055-71.
[45] D.V. Pow, N.L. Barnett. Developmental expression of excitatory amino acid transporter 5: a photoreceptor and bipolar cell glutamate transporter in rat retina. Neurosci Lett. 2000; 280:21-4.
[46] T. Miyaji, N. Echigo, M. Haisa, S. Senoh, H. Omote, Y. Moriyama. Identification of vesicular aspartate transporter. Proc Natl Acad Sci USA. 2008; 105: 11720-24.
[47] D.B. Shennan, I.D Miller, D.T Calvert. Mammary-tissue amino acid transport system. Proc Nutr Soc. 1997; 56: 117-191.
[48] V.K. Kansal, R.Sharma. Mechanism and regulation of amino acid transport in mammary glands: Review. Asian-Aust J anim Sci. 2001; 14: 718-19.
[49] G.L. Amidon, G.D. Leesman, R.L. Elliot. Improving intestinal absorption of water-insoluble compounds: a membrane metabolism strategy. J Pharm Sci. 1980;69: 1363-1368.
[50] V.J. Stella, K.J. Himmelstein. Prodrugs and site-specific drug delivery. J Med Chem. 1980; 23: 1275-1282.
[51] T.A. Conners. Prodrugs in cancer chemotherapy. Xenobiotica. 1986; 16: 975-988.
[52] K.D.Bhagshave. Antibody-directed enzyme prodrug therapy (ADEPT). Adv Pharmacol. 1993; 24: 99-121.
[53] K.N. Syrigos, A.A. Epenetos. Antibody-directed enzyme prodrug therapy (ADEPT): a review of the experimental and clinical considerations. Anticancer Res. 1999; 19:605-614.
[54] X.U. Guang, L. Howard McLeod. Strategies for enzyme/prodrug cancer therapy. Clinical cancer research. 2001;7: 3314-3324.
[55] H. Hyu-Kyung, G.L. Amidon. Targeted prodrug design to optimize drug delivery. AAPS Pharm Sci 2000; 2: 48-58.
[56] G.M. Anlezark, R.G. Melton, R.F. Sherwood, B. Coles, F. Friedlos, R.J Knox. The bioactivation of 5-(aziridin-1-yl)-2,4-dinitrobenzamide(CB1954)- - I. Purification and properties of a nitroreductase enzyme from Escherichia coli-a potent enzyme for antibody-directed enzyme prodrug therapy (ADEPT). Biochem Pharmacol. 1992; 44: 2289-2295.
[57] K.D. Bhagshave. Antibody-directed enzymes revive anticancer prodrug concept. Br J Cancer, 1987; 56: 531-532.
[58] D.J. Portal, J.A. Harrington, M.R Almond, G.T. Lowen, T.P. Zinnerman, T. Spector. 5-ethymyl-2-(1H)-pyrimidinone: Aldehyde oxidase-activation to 5-ethymyl uracil, a mechanism based inactivator of dihydropyrimidine dehydrogenase. Biochem Pharmacol. 1994; 47: 1165-1171.
[59] L.D. Stegman, H. Zheng, E.R Neal, O. Ben-Yoseph, L. Pollegioni, M.S. Pilone, B.D Ross. Induction of cytotoxic oxidative stressby D-alanine in brain tumor cells expressing Rhodotorula gracilis D-amino acid oxidase: a cancer gene therapy strategy. Hum Gene Ther. 1998; 9: 185-193.
[60] M.I. Walton, C.R. Wolf, P.Workman. Molecular enzymology of the reductive bioactivation of hypoxic cell cytotoxins. Int J Radiat Oncol Biol Phys.1989;16: 983-986.

[61] D.Ross, D. Siegel, N.W. Gibson, D. Pacheco, D.J. Thomas, M.Reasor, D.Wierda. Activation and deactivation of quinines catalyzed by DT-diaphorase. Evidence for bioreductive activation of diaziquone (AZQ) in human tumor cells and detoxification of benzene metabolites in bone marrow stroma. Free Radic Res Commun. 1990;8:373-381.
[62] S.M. Raleigh, E. Wanogho, M.D. Burke, S.R. McKeown, L.H. Patterson. Involvement of human cytochromes P450 (CYP) in the reductive metabolism of AQ4N, a hypoxia activated anthraquinone di-N-oxide prodrug. Int J Radiat Oncol Biol Phys. 1998;42: 763-767.
[63] A.M. Jordan, T.H.Khan, H.M. Osborn, A. Photiou, P.A Riley. Melanocyte-directed enzyme prodrug therapy: development of a targeted treatment for malignant melanoma. Biorg Med Chem. 1999; 7: 1775-1780.
[64] J.N.M. Commandeur, I. Andreadou, M. Rooseboom, M.Out, L.J. De Leur, E. Groot, P.E. Vermeulen. Bioactivation of selenocysteine Se-conjugates by a highly purified rat renal cysteine conjugate β-lyase/glutamine transaminase K^1. J Pharmacol Exp Ther. 2000; 294: 753-761.
[65] S. Gunnarsdottir, A.A. Elfarra. Glutathione-dependent metabolism of cis-3-(9H-purin-6-ylthio) acrylic acid to yield the chemotherapeutic drug 6-mercaptopurine: evidence for two distinct mechanisms in rats. J Pharmacol Exp Ther. 1999; 290:950-957.
[66] H.Eda, K. Fujimoto, S. Watanabe, M. Ura, A. Hino, Y. Tanaka, K. Wada, H. Ishitsuka. Cytokines induce thymidine phosphorylase expression in tumor cells and make them more susceptible to 5'-deoxy-5-fluorouridine. Cancer Chemother pharmacol.1993; 32:333-338.
[67] M. Miwa, M. Ura, M. Nishida, N. Sawada, T. Ishikawa, K. Mori, N. Shimma, I. Umeda, H. Ishitsuka. Design of novel oral fluoropyrimide carbamate, capecitabine, which generates 5-fluorouracil selectively in tumors by enzymes concentrated in human liver and cancer tissue. Eur J Cancer. 1998; 34: 1274-1281.
[68] J. Li, X. Luo, Q. Wang, L.M. Zheng, I. King, T.W Doyle, S.H. Chen, Synthesis and Biological evaluation of a water soluble phosphate prodrug of 3-aminopyridine-2-carboxylaldehyde thiosemicarbazone (3-AP). Bioorg Med Chem Lett. 1998; 8: 3159-3164.
[69] J.L. Guerquin-Kern, A. Volk, E. Chenu, R. Lougerstay-Madec, C. Monneret, J.C Florent, D. Carrez, A. Croizy. Direct in-vivo observation of 5-fluorouracil release from a prodrug in human tumors hetero transplanted in nude mice: a magnetic resonance study, NMR Biomed. 2000; 13: 306-310.
[70] D.H. Jornada, G.F. dos Santos Fernandes, D.E. Cheba, T.R. Ferreira de Melo, J.L. dos Santos, M.C. Chung. The prodrug approach: A successful tool for improving drug solubility, Molecules. 2016; 21: 1-31.
[71] P. Wipf, S.M. Lynch, G. Powis, A. Birmingham, E.E. Englund. Synthesis and Biological activity of prodrug inhibitors of the thioredoxin-thioredoxin reductase system. Org Biomol Chem. 2005; 3: 3880-3882.
[72] J. Chen, W. Du. Synthesis and evaluation of water-soluble etoposide esters of malic acid as prodrugs. Med Chem. 2013; 3: 740-747.
[73] S. Lobo, H. Li, N. Farhan, G. Yan. Evaluation of diclofenac prodrugs for enhancing transdermal delivery. Drug Dev Ind Pharm. 2014; 40: 425-432.
[74] C.W. Lee, D.H. Hong, S.B. Han, S.H. Jung, H.C. Kim, R.L. Fine, S.H. Lee, H.M. Kim. A novel stereo-selective sulfonylurea, 1-[1-(4-aminobenzoyl)-2,3-dihydro-1H-indol-6-sulfonyl]-4-phenyl-imidazolidin-2-one, has antitumor efficacy in *in vitro* and *in vivo* tumor models. Biochem Pharmacol. 2002; 64: 473-480.
[75] K.C. Lee, E. Ventakeswararao, V.K. Sharma, S.H. Jung. Investigation of amino acid conjugates of (S)1-[1-(4-aminobenzoyl)-2,3-dihydro-1H-indol-6-sulfonyl]-4-phenyl-imidazolidin-2-one (DW2282) as water soluble anticancer prodrugs. Eur J Med Chem. 2014; 80: 439-446.
[76] P. De Miranda, M.R. Blum, Pharmacokinetics of acyclovir after intravenous and oral administration. J Antimicrob Chemother. 1983; 12: 29-37.
[77] A. Diez-Torrubia, S. Cabrera, S. de Castro, C. Gracia-Aparicio, G. Mulder, I. De Meester, M.J. Camarasa, J. Balzarini, S. Velazquez. Novel water-soluble prodrugs of acyclovir cleavable by the dipeptidyl-peptidase IV (DPP IV/CD26) enzyme. Eur J Med Chem. 2013; 70: 456-468.
[78] K. Ghosh, D. Majumdar Tagore, R. Anumula, B. Lakshmaiah, P.P. Kumar, S. Singaram, T. Matan, S. Kallipatti, S. Selvam, P. Krishnamurthy, M. Ramaro. Crystal structure of rat intestinal alkaline phosphatase- role of crown domain in mammalian alkaline phosphatases. J Struct Biol. 2013; 184: 182-192.

[79] M. Flores-ramos, F. Ibarra-Velarde, A. Hernandez-campos, Y. Vera-Montenegro, H. Jung-cook, G.J. Canto-Alarcon, L.M. del Rivero, R. Castillo. A highly water soluble benzimidazole derivative useful for the treatment of fasciolosis. Bioorg Med Chem Lett. 2014; 24: 5814-5817.
[80] H. Kumpulainen, T. Jarvinen, A. Mannila, J. Leppanen, T. Nevalainen, A. Mantyla. J. Vepsalainen, J. Rautio. Synthesis, in vitro and in vivocharacterization of novel ethyl dioxy phosphate prodrug of propofol. Eur J Pharm Sci. 2008; 34: 110-117.
[81] A.K. Ghosh, M. Brindisi, Organic carbamates in Drug Design and Medicinal chemistry, J Med Chem. 2015; 58: 2895-2940.
[82] C. Monneret. Histone deacetylase inhibitors. Eur J Med Chem. 2005;40: 1-13.
[83] M. Dokmanovic, C. Clarke, P.A Marks. Histone deacetylase inhibitors: Overview and perspectives. Mol Cancer Res. 2007; 5: 981-989.
[84] M. Thomas, J. Clarhaut, I. Tranoy-Opalinski, J.P Geeson, J. Roche, S. Papot. Synthesis and biological evaluation of glucoronide prodrugs of the histone deacytylase inhibitor CI-994 for application in selective cancer chemotherapy. Biorg Med Chem. 2008; 16: 8109-8116.