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SYNTHESIS AND CHARACTERIZATION OF PRODRUG

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ABSTRACT

The prodrug term involves chemically modified inert compound which upon administration releases the active parent drug to elicit its pharmacological response within the body. For many years, prodrug strategy has been developed enormously to solve many unwanted drug properties. This approach has several advantages over conventional drug administration and it has the potential to be quite effective method for the treatment of diseases in the future. In most cases, prodrugs contain a promoiety (linker) that is removed by enzymatic or chemical reactions, while other prodrugs release their active drugs after molecular modification, such as an oxidation or reduction reactions. In some cases, two biologically active drugs can be linked together in a single molecule called a codrug. In a codrug, each drug acts as a linker for the other. It is important to ensure that the prodrug should be pharmacologically inactive, rapidly converted to its active drug and a non-toxic moiety by metabolic reactions. In this chapter we describe the general terms related to prodrugs, and the ways by which prodrug strategy is used to overcome many pharmaceutical and pharmacokinetic problems such as, low bioavailability by increasing or decreasing lipophilicity of the parent drug, site selectivity for higher absorption and less toxicity, short duration of action to increase patient compliance, rapid metabolism to increase oral bioavailability and masking bitter sensation of commonly used drugs, which is crucial for geriatric and pediatric patient compliance.

KEYWORDS: Prodrugs, Prodrugs history, Physicochemical properties, Chemical reactions, Prodrug metabolism, Permeability, Lipophilicity.

INTRODUCTION

Generally, a drug is characterized by its biological and physicochemical properties. Some of the used drugs have undesirable properties that result in an inefficient delivery and unwanted side effects. The physicochemical, biological and organoleptic properties of these drugs should be improved in order to increase their usefulness and their utilization in clinical practice.[1,2]

During the last few decades, many methods have been developed in order to facilitate the drug design and discovery phases.

Most of these methods were devoted to find new chemical entities that provide the most meaningful interaction with the desired receptors or enzymes with the potential to have minimal unwanted interactions. However, this strategy is time consuming, costly and requires screening of thousands of molecules for biological activity of which only one might enter the drug market. One of the most attractive and promising method is the prodrug approach, in which the active drug molecule is masked by a promoiety to alter its undesired properties.[3,4]

The prodrug (predrug, proagent) term was introduced for the first time by Albert as a pharmacologically inactive moiety which is converted to an active form within the body.[5]

This term has been successfully used to alter the physicochemical, pharmacokinetic properties, (absorption, distribution, excretion and metabolism) of drugs and to decrease their associated toxicity.[6] 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.

A prodrug must undergo chemical and/or enzymatic biotransformation_in a controlled or predictable manner prior to exert its therapeutic activity.[7]

Basically, the use of the term prodrug implies a covalent link between an active drug and a promoiety (Figure 1).[8]

This strategy is designed to overcome barriers through a chemical approach rather than a formulation approach.[9]

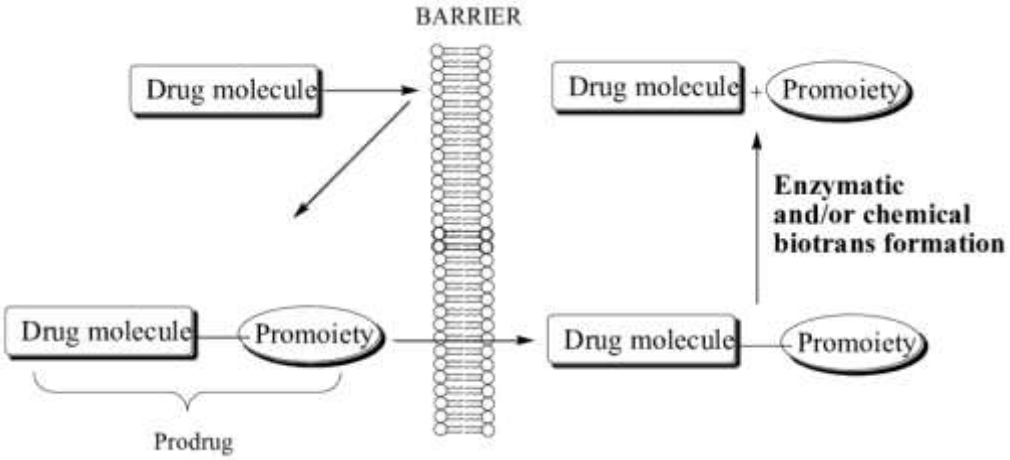


Figure 1: Schematic representation of a prodrug.[8]

In general, the imminent goal behind the use of prodrugs is to develop new entities that possess superior efficacy, selectivity, and reduced toxicity.[3] An ideal prodrug should undergo biotransformation rapidly via chemical or enzymatic process to its active form and a non-toxic moiety within the body.[7,10]

The prodrug must release the active drug and the promoiety prior to, during, or after absorption, or in a specific target tissue or organ, depending upon the purpose of which the prodrug has been designed.[11]

Nowadays, the prodrug approach is considered as one of the most promising site-selective drug delivery strategies that utilize target cell- or tissue-specific endogenous enzymes and transporters.[12]

One of the few examples that were designed to increase the efficiency of a drug by accumulation into a specific tissue or organ is the anti-Parkinson agent L-DOPA. Dopamine is a hydrophilic neurotransmitter, which does not efficiently cross the blood-brain barrier and is rapidly metabolized by oxidative deamination that causes peripheral side effects. However, the prodrug of dopamine, L-DOPA, enables the uptake and accumulation of dopamine into the brain via the L-type amino acid transporter (Figure 2).[6,13]

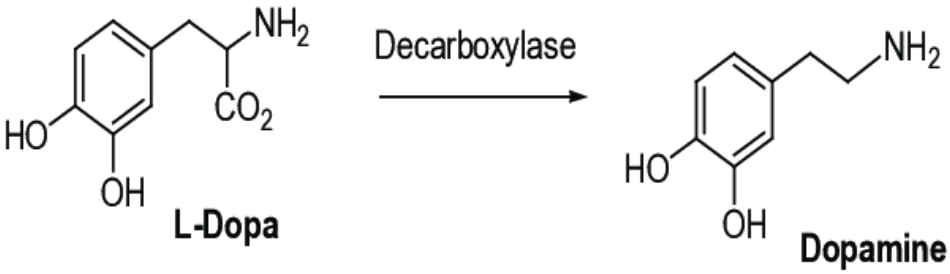


Figure 2: L-DOPA prodrug conversion.

HISTORY

In 1958, Albert introduced the prodrug term for the first time in his book „selective toxicity". This term includes any inert compound that undergoes in vivo biotransformation.[5] Others such as Harper also promoted the concept but used the term drug latentiation which includes the prodrug intentionally designed to undergo biotransformation within the body.[14] Few years later, Albert apologized for having invented an inaccurate term, because "pre-drug" would be a more descriptive term.[15]

The first prodrug was not originally designed as a prodrug,_but its nature was determined later._Earlier examples of compounds fulfill the classical criteria of prodrug were acetanilide and phenacetin, which exhibit_their activities after being metabolized within the body.[16]

Acetanilide is an antipyretic agent that was in use in 1886. It undergoes metabolism (aromatic hydroxylation) to paracetamol. This is similar to phenacetin which produces paracetamol via O-dealkylation (Figure 3).[17]

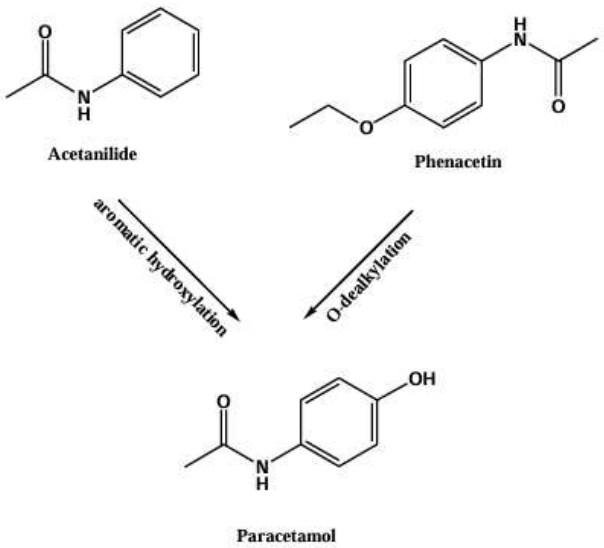


Figure 3: Phenacetin and acetanilide metabolism.

In the late nineteenth century a chemist, Felix Hoffman in Bayar Company, synthesized the antipyretic agent Aspirin (acetylsalicylic acid), which was introduced for the first time in clinical practice in 1899; it can be considered a less corrosive prodrug form of salicylic acid to minimize the gastric irritation and ulcerogenicity associated with salicylic acid (Figure 4)._However, it remains a matter of debate whether aspirin is a true prodrug or not.[18]

Another example of an accidental prodrug and how serendipity aided in prodrug development is methenamine and the first sulfa prodrug, prontosil.[6]

Methenamine was discovered in 1899 by Schering as inactive prodrug that delivers the antibacterial formaldehyde. It is useful in the treatment of urinary tract infection, when transported to urinary bladder it becomes acidified to provide a medium in which formaldehyde is generated (Figure 5).[19]

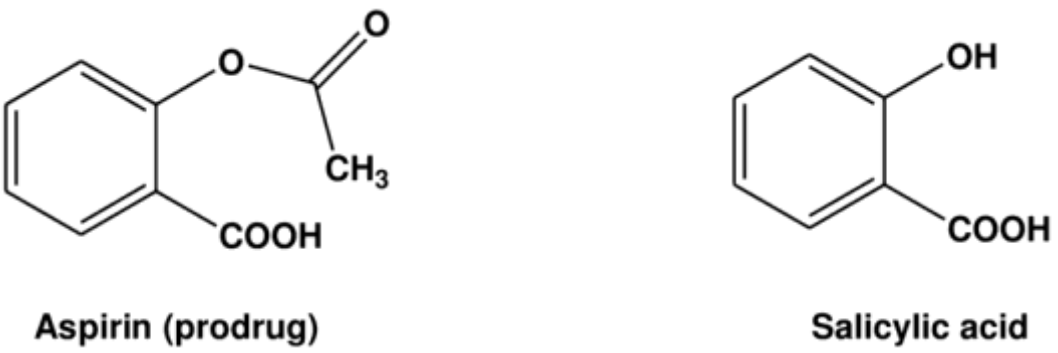


Figure 4: Chemical structure of salicylic acid and its prodrug aspirin.

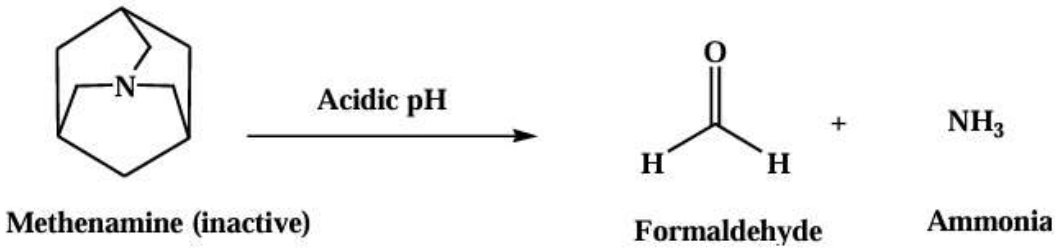


Figure 5: Methenamine prodrug activation at acidic pH.

Prontosil was found to be effective against microorganisms only in vivo, and not in vitro. When administered in the body it was metabolized by the enzyme azo reductase to sulfanilamide, the first sulfonamide to be discovered (Figure 6).[16]

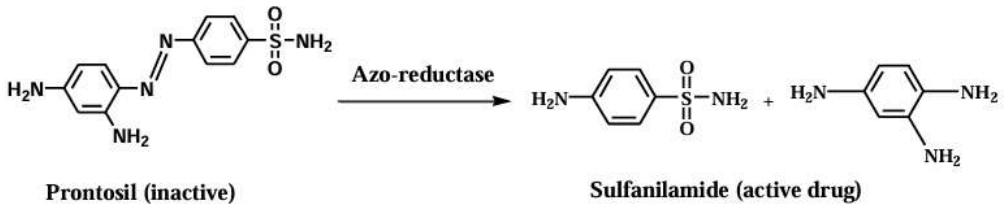


Figure 6: Prontosil prodrug activation by azo reductase.

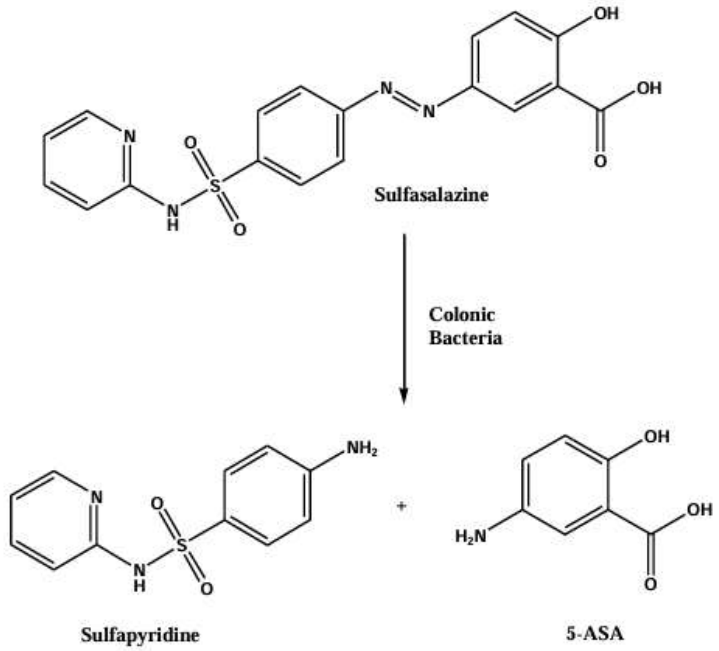


Figure 7: Chloramphenicol prodrugs and their conversion to chloramphenicol.

In the mid twentieth century the prodrug concept was intentionally used for the first time when Parke-Davis Company modified the structure of chloramphenicol in order to improve its bitter sensation and poor water solubility. Two prodrugs of chloramphenicol were synthesized, chloramphenicol sodium succinate with a good water solubility for IV, IM, and ophthalmic administration, and chloramphenicol palmitate used in the form of suspension for children (Figure 7).[6,20]

The prodrug approach has been successfully applied to a wide variety of drugs. It is estimated that currently about 10% of world-wide marketed drugs can be classified as prodrugs,_20% of all small molecular medications approved between 2000-2008 were prodrugs, and in the year 2008, one third of approved drugs were prodrugs.[1]

PRODRUGS CLASSIFICATION

The conventional method used to classify prodrugs is based on derivatization and the type of carriers attached to the drug.

This method classifies prodrugs into two sub-major classes

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, in which the promoiety is covalently linked to the active drug but it can be easily cleaved by enzymes (such as an ester or labile amide) or non-enzymatically to provide the parent drug. Ideally, the group removed is pharmacologically inactive, nontoxic, and non-immunogenic, while the promoiety must be labile for in vivo efficient activation.[3,21]

A WELL-DESIGNED CARRIER-LINKED PRODRUG SHOULD SATISFY CERTAIN CRITERIA

- The linkage between the drug and the carrier should usually be a covalent bond.
- As a rule, the prodrug itself should be inactive or less active than the parent drug.
- The linkage should be bio reversible.
- The prodrug and the carrier released after in vivo enzymatic or non-enzymatic attack should be nontoxic.
- The generation of the active form must take place with rapid kinetics to ensure effective drug levels at the site of action.

The bioavailability of carrier-linked prodrug is modulated by using a transient moiety. The lipophilicity is generally the subject of profound alteration of the parent molecule. The bio activation process is exclusively hydrolytic and sometimes a redox system.

IDEAL CRITERIA FOR CARRIERS

- An ideal carrier should be without intrinsic toxicity.
- It should be non-immunogenic and non-antigenic and should not accumulate in the body.

- It should possess a suitable number of functional groups for drug attachment and adequate loading capacity.
- It should be stable to chemical manipulation and autoclaving.
- It should be easy to characterize and should mask the liganded drug activity until the release of the active agent at the desired site of action.

CARRIER-LINKED PRODRUGS CAN BE FURTHER SUBDIVIDED INTO

(a) bipartite which is composed of one carrier (promoiety) attached directly to the drug,

(b) tripartite which utilizing a spacer or connect a group between the drug and a promoiety. In some cases bipartite prodrug may be unstable due to inherent nature of the drug-promoiety linkage. This can be solved by designing a tripartite prodrug and

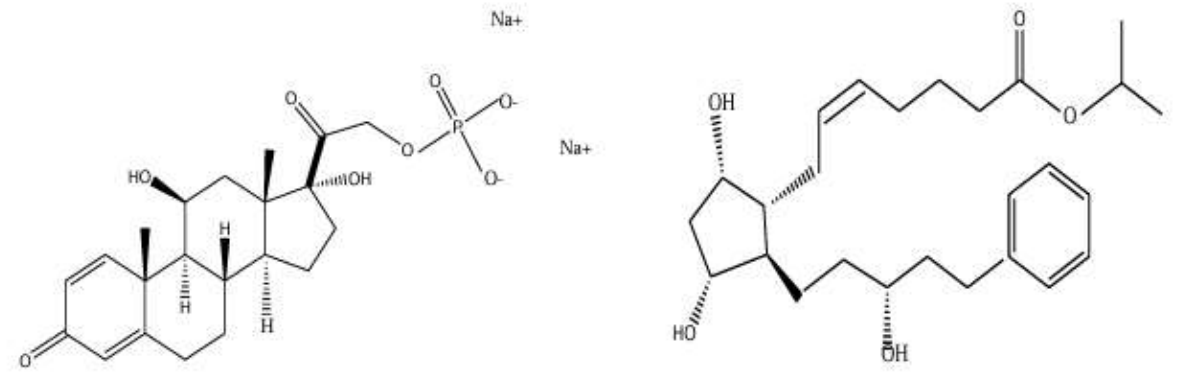
(c) mutual prodrugs, which are consisting of two drugs linked together.

(B) Bioprecursors which are chemical entities that are metabolized into new compounds that may be active or further are metabolized to active metabolites (such as amine to aldehyde to carboxylic acid). In this prodrug type there is no carrier but the compound should be readily metabolized to induce the necessary functional groups.[6,9, 22]

In addition, prodrugs can be classified based on cellular site of interconversion into the active drug form. This classification strategy includes two types: Type I: includes prodrugs that are converted to active drug intracellularly (e.g. anti-viral nucleoside analogs, and lipid-lowering statins). Type II: involves prodrugs that are converted extracellularly, especially in digestive fluids or in the systemic circulation (e.g. etoposide phosphate, valganciclovir, fosamprenavir, antibody-directed/gene-directed enzyme prodrugs (ADEP/GDEP) for chemotherapy). Both types are further subdivided into subtypes (Type IA, IB and Type IIA, IIB, and IIC) based on whether or not the intracellular converting location is the site of therapeutic action, or whether the conversion occurs in the gastrointestinal (GI) fluids or systemic circulation (Table 1).[23]

(a) 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 8)



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

Fig. 8: Chemical Structures of Prednisolone prodrug and Latanoprost Prodrug.

(b) 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 –CH2- as shown in figure 9.

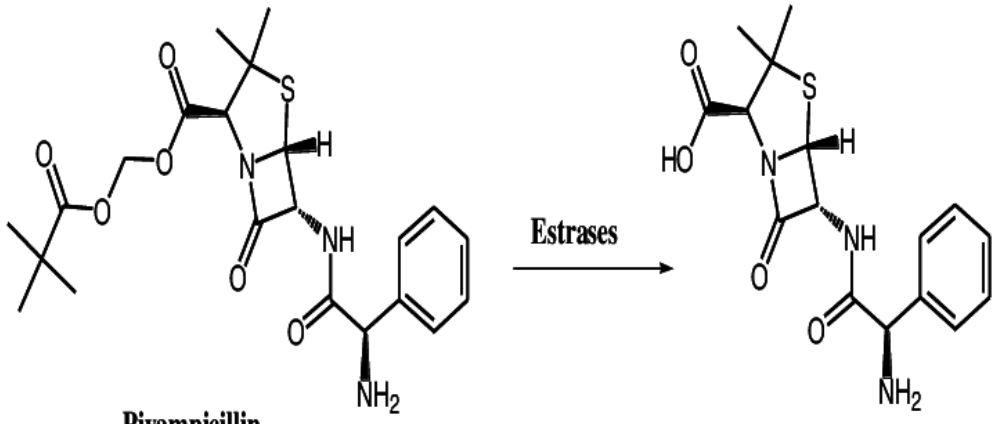


Fig. 9: Chemical Structure of Pivampicillin (Prodrug of Ampicillin)

(c) MUTUAL PRODRUG OR CO-DRUG

Mutual prodrugs are a type of carrier linked prodrugs by which two pharmacologically active agents linked together to form a single molecule. Each of these drugs acts as a carrier for the other. Mutual prodrugs approach offers an efficient tool for improving the clinical and therapeutic effectiveness of a drug that is suffering from some undesirable properties hindering its clinical usefulness.

A common example of this approach is benorylate (Figure 8), by which aspirin linked covalently to paracetamol through an ester linkage, which claims to have decreased gastric irritation with synergistic analgesic effect.[24]

OBJECTIVES BEHIND DESIGNING OF MUTUAL PRODRUGS

- To bring both active drugs to their respective active sites.
- To provide the desired pharmacological effects while minimizing adverse metabolic and/or toxicological events.
- To improve the clinical and therapeutic effectiveness of those drugs which suffer from some undesirable properties that otherwise hinder their clinical usefulness
- To avoid the practice of clinically co-administering two drugs to enhance pharmacological activity or prevent clinical side effects. Simultaneous administration does not guarantee equivalent absorption or transportation to the site of action. So, the mutual prodrug concept is useful when two synergistic drugs need to be administered at the same site at the same time. Mutual prodrugs are synthesized toward a pharmacological objective of improving each drug's efficacy, optimizing delivery, and lowering toxicities.

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.

Table 1 illustrates the classification of prodrugs with examples.

Table 1: Prodrug classification.[23]

Prodrug types	Site of conversion	Subtypes	Tissue/location of conversion	Examples
Type I	Intracellular	**A**	Therapeutic Target Tissues/Cells	Acyclovir 5-Flurouracil L-Dopa
		B	Metabolic Tissues (Liver, GI mucosal cell, lung, etc…)	Cabamazepine Captopril Suldinac
Type II	Extracellular	**A**	GI Fluids	Loperamide oxide Oxyphenisatin Sulfasalazine
		B	Systemic circulation and other_extracellular fluid _compartments	Acetylsalicylate Bacampicillin Fosphenytoin
		C	Therapeutic Target Tissues/Cells	ADEPs

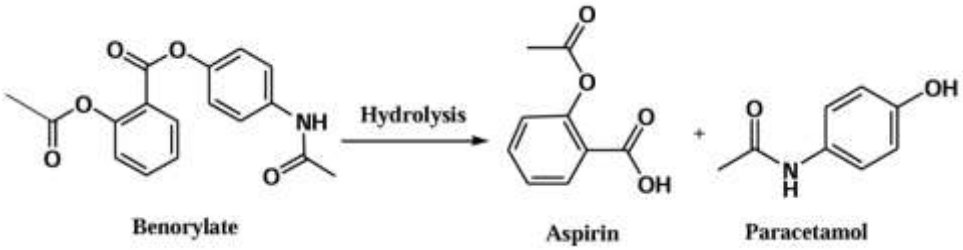


Figure 8. Mutual prodrug of acetylsalicylic acid and paracetamol.

Another example of mutual prodrug is sulfasalazine. It's a colon selective mutual prodrug, of 5-aminosalicylic acid (5-ASA) and sulfapyridine used in the treatment of ulcerative colitis.[25]

Sulfasalazine was the first sulfa drug to be used in inflammatory bowel disease and was developed in 1950. It is composed of 5-aminosalicylic acid (5-ASA) linked to sulfapyridine via a diazo bond (Figure 9). This bond is readily cleaved by bacterial azo-reductases in the colon. Where 5-ASA has been found to be the therapeutically active component, while sulfapyridine is assumed to function solely as a carrier molecule and serves as a delivery system that transports 5-ASA to the affected region of the lower GI.[26,27]

The advantage of this approach is that the cleavage of the azo linkage and generation of 5-ASA prior to the absorption prevents its systemic absorption and helps to concentrate the active drug at the active site. Even though sulfapyridine proved to be a good carrier for targeting 5-ASA to colon, it gave rise to many side effects resulting from its systemic toxicity.

Due to disadvantages related to the use of sulfasalazine, another interesting mutual prodrug of 5-ASA, olsalazine, has been developed. This mutual prodrug is actually a dimer of 5-ASA, where 5-ASA is linked through azo linkage to another molecule of 5-ASA. When it reaches the large intestine, it is cleaved, releasing two molecules of 5-ASA for every molecule of olsalazine administered. This design eliminates the drawbacks of sulfasalazine, targets 5-ASA to the colon, and fulfills all requirements of mutual prodrug as well. Improvement of the bioavailability of 5-ASA was also achieved using this design.

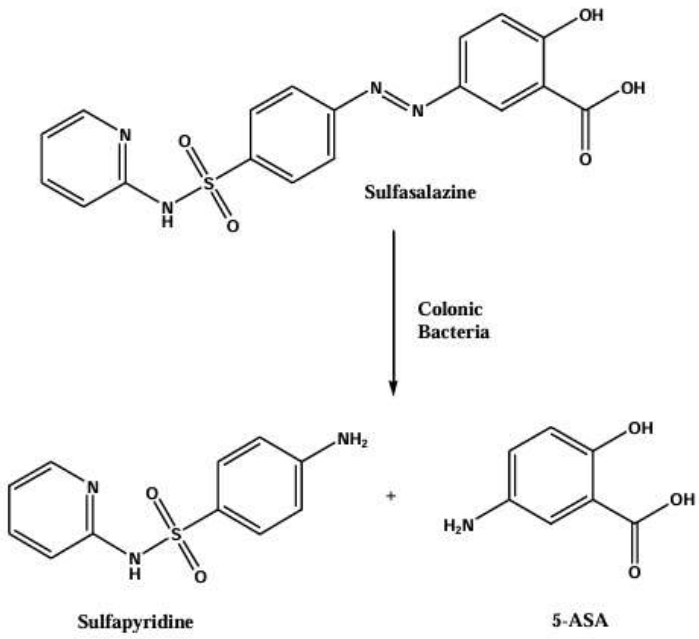


Figure 9: Conversion of the mutual prodrug, sulfasalazine.

PRODRUG ACTIVATION

Conversion of prodrugs into the active form occurs enzymatically or chemically. Numerous and most commonly carrier linkage prodrugs are designed to be activated via esterases. A wide range of esterases distributed throughout the body, differ in their substrate specificity. Examples of esterases found in the body are acetylcholinesterases, butyrylcholinesterases, carboxylesterases and arylesterases.[15]

Therefore, prodrug approach can be employed to control the release of the active compound at_a specific site. One of the most important enzymes involved in ester bioactivation are carboxylesterases (CESs). These enzymes are a multi-gene family whose genes are localized in the endoplasmic reticulum (ER) of several tissues. These enzymes efficiently catalyze the hydrolysis of a variety of ester- and amide-containing prodrugs to the corresponding free acids. CESs show ubiquitous tissue expression profiles with the highest levels of CESs activity present in the liver microsomal site.[28] Hence the potential for their substrates to become involved in drug-drug interactions is generally considered to be negligible.[29] Examples of prodrug hydrolyzed by this type of esterases are enalapril (Figure 10) and pivampicillin (Figure 11).

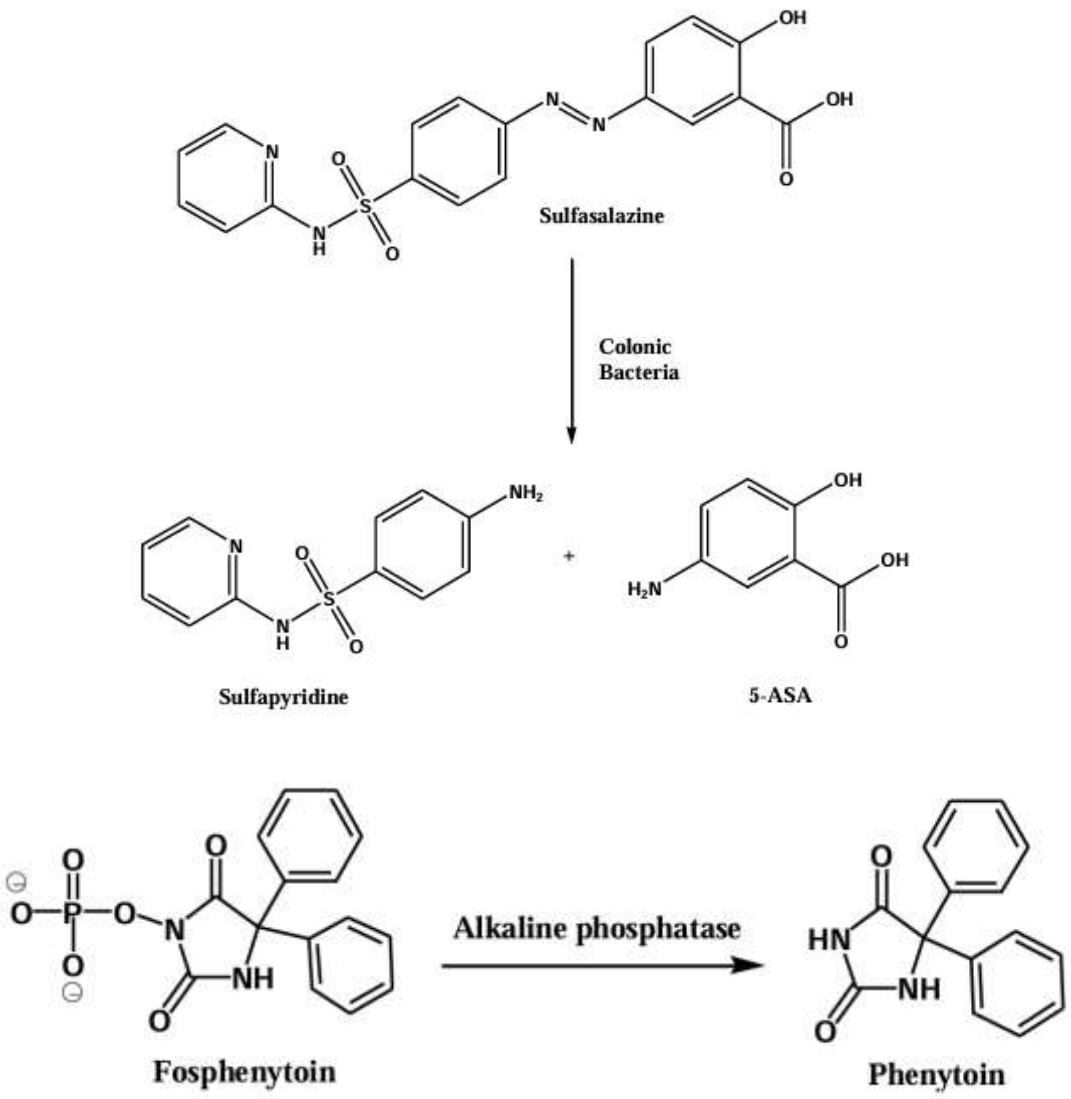


Figure 10: Enalapril conversion via carboxylestrase.

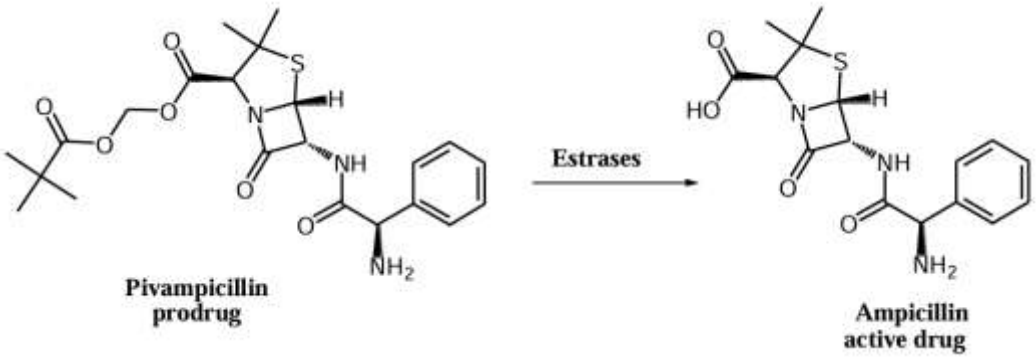


Figure 11: Pivampicillin conversion via carboxylestrase.

Another enzymes involved in the activation of prodrugs are phosphatases.[30] An example of such activation is shown in Figure 12.

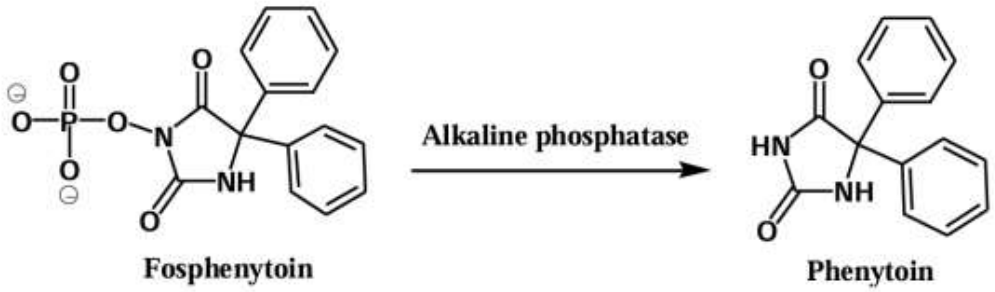


Figure 12: Fosphenytoin activation via alkaline phosphatase.

BIOPRECURSOR ACTIVATION

Upon administration many compounds are metabolized by molecular modification into new compounds that are active in principle or can be metabolized further into the active drugs.

Five types of reactions can be involved in bioprecursor activation:

1. Oxidative reaction, _catalyzed by CYP450 such as

(i) O and N dealkylation; _e.g (bioprecursor prodrug for alprazolam),

(ii) oxidative deamination; e.g. (cyclophosphamide activation) and

(iii) N-oxidation; e. g (procarbazine activation).

2. Reductive activation such as

(i) disulfide reaction; e. g (activation of thiamine prodrug) and

(ii) bioreductive alkylation; e.g. (activation of anticancer antibiotic, mitomycin c).

3. Nucleotide activation

4. Phosphorylation activation; e. g (antiviral drug, acyclovir activation).

5. Decarboxylation activation; e. g (Nabumetone).

APPLICATIONS OF PRODRUGS

Prodrugs are used to overcome pharmacokinetic and pharmaceutical barriers to increase drug biological bioavailability. After overcoming those barriers the prodrug must be converted to the active form in the targeted site of action.

Pharmacokinetic applications

1. Improvement of bioavailability by alteration of drug‟s solubility.
2. Prodrugs for site selective drug delivery.
3. Prolongation of action.
4. Minimizing toxicity.
5. Protection from presystemic metabolism.

(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.

RATIONALE OF PRODRUG APPROACH

I. IMPROVEMENT OF BIOAVAILABILITY

Chemical modification of drugs is used to improve physicochemical properties solubility, stability, and lipophilicity.[32]

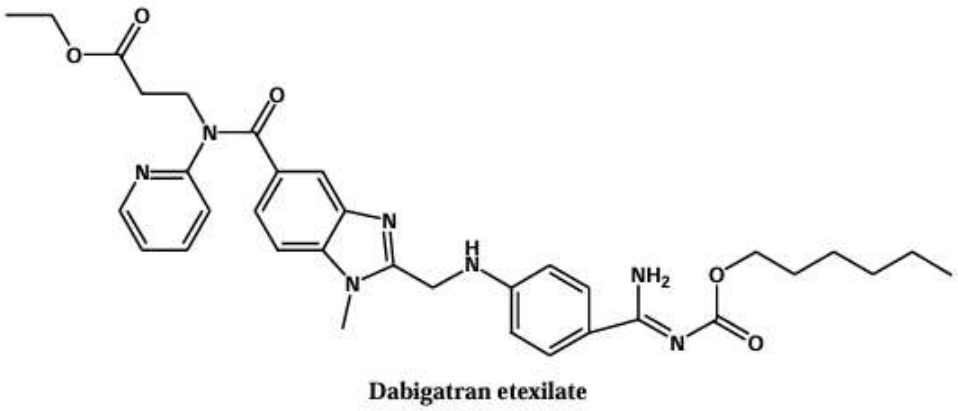
Oral drug bioavailability is critical for the development of new drugs, because low oral absorption leads to inter- and intra-patient variability. One of the strategies developed to improve oral bioavailability is prodrugs.[33]

Oral bioavailability of lipophilic drugs depends on the dissolution in the gastrointestinal fluids, and polar drug's bioavailability depends on the transport across gastrointestinal mucosa.[11] Therefore, prodrugs are designed to increase or decrease lipophilicity.

II. PRODRUGS TO INCREASE LIPOPHILICITY

Prodrugs are used to increase lipophilicity so that the drugs are available for oral administration, ocular or topical drug delivery.

The main reason for designing prodrugs is to increase oral bioavailability, and the intestinal absorption, which are enhanced by masking the polar moiety of the drug.[34] For example, dabigatran, a potent inhibitor of the active site of thrombin is very polar molecule with a logP of −2.4 (n-octanol/buffer pH 7.4), therefore its oral bioavailability is negligible.[35] Dabigatran etexilate, the first oral alternative to warfarin, was developed as a prodrug of dabigatran (Figure 13). After oral administration, dabigatran etexilate is converted to the active drug dabigatran by esterases. The oral bioavailability of dabigatran etexilate is 6.5%.[36]



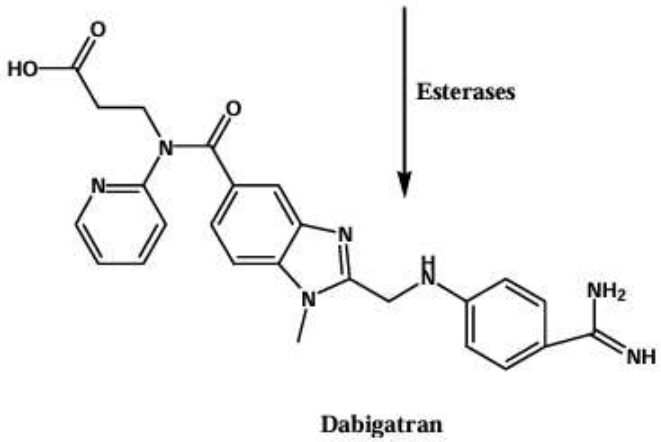


Figure 13: Chemical structures of dabigatran prodrug and its active drug.

III. PRODRUG DESIGN: TARGETING SPECIFIC ENZYMES

The enzyme-targeted prodrug design approach can be widely used to improve oral absorption ofdrugs 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]

OPHTHALMIC DRUG DELIVERY

Lipophilic prodrugs are also used to enhance ocular absorption. For example latanoprost and travoprost are isopropyl esters of the parent latanoprost and travoprost carboxylic acids (Figure 14). These ester prodrugs have an increased lipophilicity, which enables them to penetrate the cornium epithelium.[37]

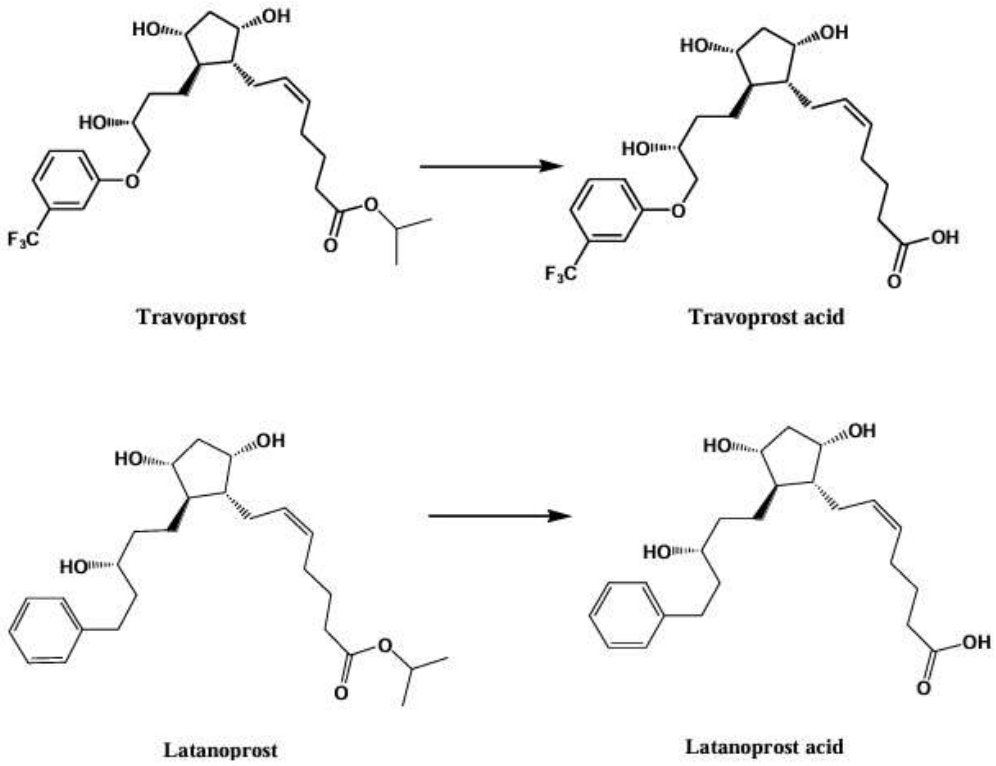


Figure 14: Chemical structures of travoprost and latanoprost acids, and their corresponding ester prodrugs.

TOPICAL DRUG DELIVERY

Another use of lipophilic prodrugs is to increase transdermal absorption for certain drugs. For example, ester prodrugs with increased lipophilicity allow them to accumulate in the skin leading to higher efficacy and lower side effects.[37]

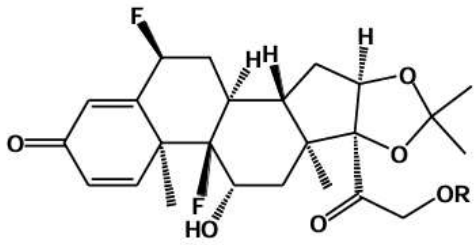


Figure 15: Chemical structures of fluocinolone and its prodrug, fluocinolone acetonide.

Topical corticosteroids are widely used as anti-inflammatory and immune-suppressants agents for skin problems. However, they may be absorbed systemically and cause side effects.[38] For example, fluocinolone acetonide ester prodrugs (Figure 15) have high membrane retention (in epidermis) and low permeation which is preferred for local application of corticosteroids.[39] The high lipophilicity of the fluocinolone acetonide prodrug makes it more potent than its less lipophilic parent drug, fluocinolone.[40]

IV. PRODRUGS TO INCREASE POLARITY

Prodrugs are designed to increase aqueous solubility by esterification with amino acids or phosphate group.[34]

PHOSPHATE PRODRUG FOR ORAL DELIVERY

For example, fosamprenavir (Figure 16), a protease inhibitor used as antiviral, is converted to amprenavir by alkaline phosphatase in the gut epithelium.[41] The phosphate promoiety is linked to a free hydroxyl group which makes fosamprenavir 10-fold more water soluble than amprinavir. An enhanced patient compliance is achieved by producing this antiviral prodrug, instead of administering the drug 8 times daily, dosage regimen is reduced into 2 times per day.[42]

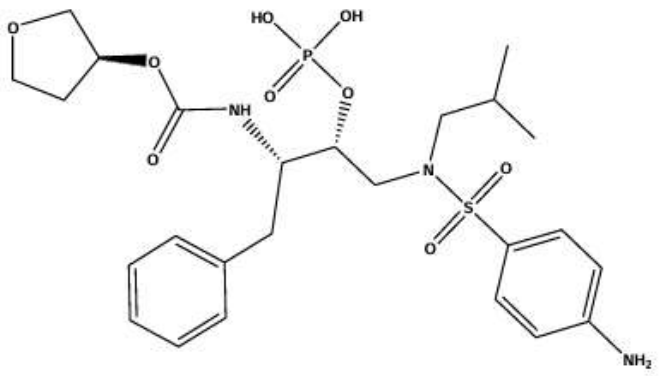


Figure 16: Chemical structure of the oral prodrug fosamprenavir.

Another example is 2-fluoroadenosine (F-ara-A), which has a clinical use as anti-neoplastic agent. However, it is difficult to be formulated because of its lipophilicity. Therefore fludarabine phosphate (2F-ara-AMP), which is a prodrug that is rapidly dephosphorylated to give fludarabine (F-ara-A), was synthesized (Figure 17).[43]

Fludarabine phosphate is available as an oral dosage form and its systemic bioavailability is 85%.[44] Fludarabine enters the cells by a carrier mediated transport and it undergoes phosphorylation_to_furnish 2-fluoroadenosine (F-ara-ATP, the cytotoxic form of the drug).[43]

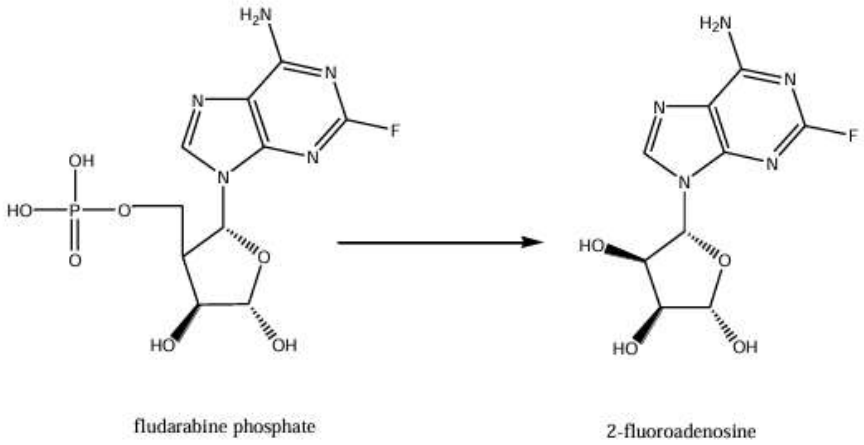


Figure 17: Chemical structures of 2-fluoroadenosine and its prodrug, fludarabine phosphate.

V. PRODRUGS FOR SITE SELECTIVE DRUG DELIVERY

Prodrugs are applied for targeting drugs to a specific organ or tissue; they are widely used in chemotherapy. Targeted prodrugs are used to increase absorption and decrease toxicity, they are targeted to an enzyme or membrane transporter.[32]

TUMOR TARGETED DRUG DELIVERY

Cancer chemotherapeutics are toxic and nonselective which limits their use for cancer therapy.[45] Their selectivity depends on the rapidly dividing cells that are more prone to toxic effects.[46] Hence, they are toxic for rapidly proliferating normal tissue such as hair follicles, gut epithelia, bone marrow, and red blood cells. Therefore, in order to improve toxicity and efficacy chemotherapy prodrugs were designed to target tumor cells; this targeting is achieved by binding drugs to ligands having high affinity to specific antigens, receptors, or transporters that are over expressed in tumor cells.[47]

One of the targeting methods is enzyme activated prodrug therapy where the nontoxic prodrug is converted to the active drug in the tumor tissue.[37] The enzyme should be specifically expressed or over expressed in tumor. Plasmin, prostate specific antigen, matrix metalloproteaes, cathepsin B, D, H and L are examples of tumor associated enzymes that are used for prodrug activation in malignant cells.[48]

Monoclonal antibodies (mAbs) have a high affinity, hence they are the first ligands used for tumor targeting.[47] MAbs are designed as drug-antibody conjugate or antibody enzyme conjugate.[48]

Drug-Antibody Conjugate

Tumor specific mAbs bind to receptors on tumor cells and the cytotoxic drug is selectively delivered to the tumor. For example, mylotarge consists of anti-CD33 mAbs conjugated to the cytotoxic ozogamicin, which was approved by the FDA for treatment of AML, acute myeloid leukemia.[46]

VI. 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.[70] 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.[71]

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:

Antibody Enzyme Conjugates

- Antibody-directed enzyme prodrug therapy (ADEPT)

In this approach tumor specific antibody is delivered into tumor cells. Then the prodrug is administered systemically, and converted to the active toxic drug inside the tumor.[48]

- Gene-directed enzyme prodrug therapy (GDEPT)

In this method, a gene encoding the activating enzyme is delivered to tumor cells as a first step. The second step is an administration of the inactive prodrug which is converted to the toxic drug by the tumor enzyme.[47] Viral vectors are the most popular vectors used for gene delivery.[48]

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)
	Amino acid oxidase	Hydrogen peroxide	d-alanine	Oxidative stres
	Cytochrome P450 reductase	Nitroxide radical	Tirapazamine	DNA alkylation and oxidative stress
	DT- diaphorase	Semiquinone radical	Diaziquone	DNA alkylation and oxidative stress
	Cytochrome P450	AQ4	AQ4N	Topoisomerase II inhibitor
	Tyrosinase	Phenol mustard	Phenol mustard	DNA alkylatio
Transferases	Glutathione S transferase	6-MP	PTA	Antimetabolite
	Thymidine phosphorylase	5-FU	5'-deoxy-5 flurouridine	Upregulation of pyrimidine nucleoside phosphorylases by the cytokine interferon
Hydrolases	Carboxylesterase	5-FU	Capecitabine	Thymidylate synthase inhibitor
	Alkaline phosphatase	3-AP	3-AP phosphate	--
	β-glucuronidase	paclitaxel	Paclitaxel glucuronide	Microtubule binding
Lyases	Cysteine conjugate-β-lyase	Selenol	SeCys conjugate	Apoptosis

Membrane Transporter Prodrug Targeting

Membrane transporters selectively transport peptides, amino acids, phosphates, ascorbic acid, bile acids and others. For example, dipeptides and tripeptides are transported in the intestinal epithelial cells by peptide transporters (PepT1).[32]

Targeting specific transporters, which have an important role in drug absorption, distribution, and elimination, via a prodrug is efficient and selective strategy[32], in which a prodrug is selectively attached to a molecule that targets a specific membrane transporters; PepT1 is the most promising transporter due to its selectivity and high capacity.[34]

For example, the antiviral drug acyclovir, used to treat herpes simplex virus, by acting as a competitive substrate for DNA polymerase[49], has low oral bioavailability, because of its hydrophilic nature and poor permeability which limited its efficacy.[50]

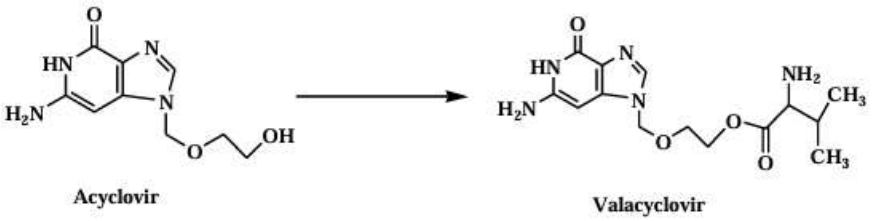


Figure 18: Chemical structures of acyclovir and its valine prodrug, valacyclovir.

To increase the oral bioavailability of acyclovir, L-valine (valacyclovir) prodrug was developed to target PepT transporters in the G.I (Figure 18). This prodrug has a high affinity for PepT transporter, therefore, it is highly absorbed through small intestine and is converted to acyclovir in the gut lumen.[51] Oral bioavailability of acyclovir is 21.5%, whereas the bioavailability of valacyclovir after oral administration of the prodrug is 70.1%, in addition to the three-fold increase in bioavailability the inter-individual variations are less in the case of valacyclovir.[52]

Several valine-valine dipeptide prodrugs of acyclovir were synthesized, in order to protect the prodrug against enzymatic hydrolysis before it reaches the PepT transporter. For example, D-isomers of valine were incorporated, since the hydrolytic enzymes have low affinity for the D-isomer. It was found that dipeptide prodrug with one D-valine isomer keeps its affinity for the PepT transporter.[50]

Another example, is zanamivir, which has poor oral bioavailability because of its polar nature, which limits its use in influenza infection.[53] L-Valyl zanamivir a prodrug of zanamivir was developed (Figure19). The prodrug has a higher uptake through the PepT transporters, which improved its absorption after oral administration.[34]

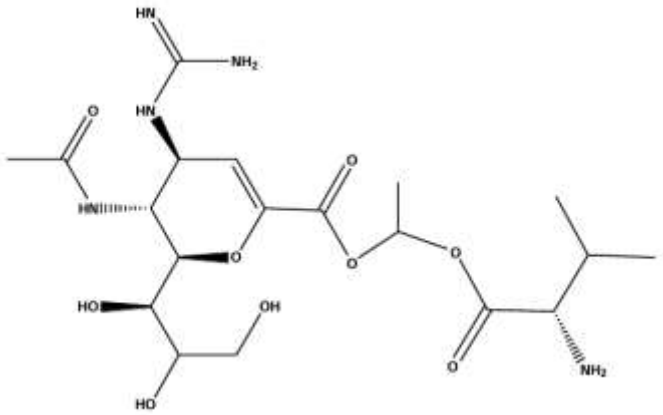


Figure 19: Chemical structure of L-valyl zanamivir.

PRODRUGS FOR LONGER DURATION OF ACTION

Drugs with short half-life require frequent dosing, to maintain blood concentration, which leads to poor patient compliance and fluctuation in the drug concentration. The development of prodrugs with long duration of action can be used to overcome these problems.[11]

Long acting antipsychotic therapy is important to control symptoms and prevent relapse. These long acting agents also improve patient compliance and increase efficacy. For example, fluphenazine decanoate, an ester prodrug of fluphenazine (Figure 20), is used as long acting intramuscular depot injection for the treatment of schizophrenia; this prodrug is administered once every 2 weeks.[54]

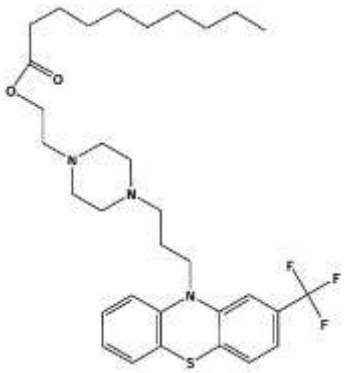


Figure 20: Chemical structure of fluphenazine decanoate prodrug.

Buprenorphine ester prodrugs are another example of prodrugs used for longer duration of action. Patients with moderate to severe pain like postoperative pain and burn pain may need analgesics for 3 days after a trauma. Therefore, there is a need for depot analgesics. Buprenorphine decanoate, enanthate, and propionate were synthesized and formulated in sesame oil for I.M injection. Pharmacokinetic studies on buprenorphine decanoate (Figure 21) showed that it produced 4.1 day duration of action, which is 14-fold longer than the traditional buprenorphine.[55]

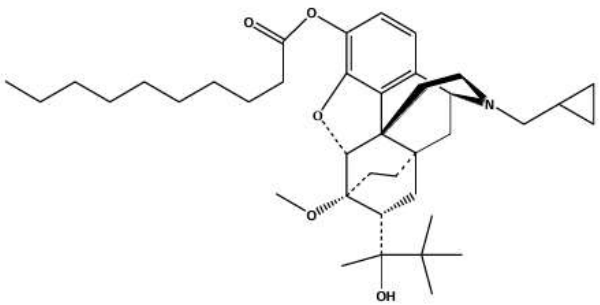


Figure 21: Chemical structure of buprenorphine decanoate prodrug.

REDUCTION OF TOXICITY

For therapeutically active drugs it is preferred to have minimum or no toxicity, therefore, prodrugs can be used to minimize toxicity of many drugs.[11] For example, doxorubicin, _an anthracycline antibiotic, which is highly used as anticancer drug, but its use, is limited by its cardiotoxicity. Hence, there was a crucial need to design drug targeting system to increase doxorubicin availability in tumor tissue and decrease its accumulation in cardiac tissue.[56] A galactoside prodrug that is linked to doxorubicin via a carbamate spacer was developed (Figure 22). This prodrug is solely activated by β-galactosidase that is highly expressed in tumor tissue; additionally, the hydrophilic nature of galactoside moiety prevents its distribution to other tissues. This prodrug is more effective and less toxic than its parent drug due to low concentration in cardiac tissue.[57]

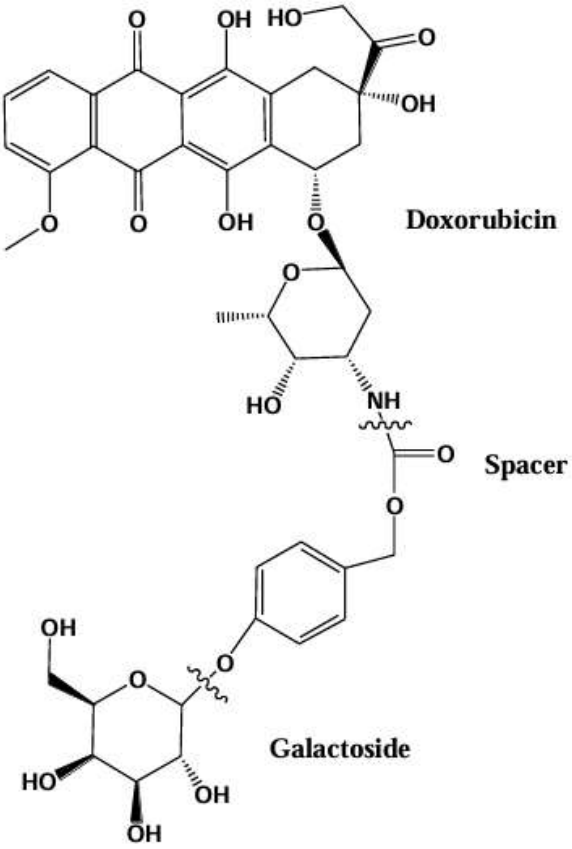


Figure 22: Chemical structure of doxorubicin galactoside prodrug.

PROTECTING FROM RAPID METABOLISM AND EXCRETION

Presystemic metabolism causes low oral bioavailability of drugs; certain sites or groups in the molecule are subjected to presystemic metabolism therefore prodrugs can be used to block these sites and increase oral bioavailability.[45]

Nalbuphine is a potent analgesic used in moderate to severe pain; it has a low oral bioavailability of only 17% due to presystemic metabolism at the 3-hydroxyl position. Nalbuphine acetylsalicylate (Figure 23) an ester prodrug of nalbuphine was synthesized and it has shown an increased oral bioavailability in dogs by 5-folds.[58]

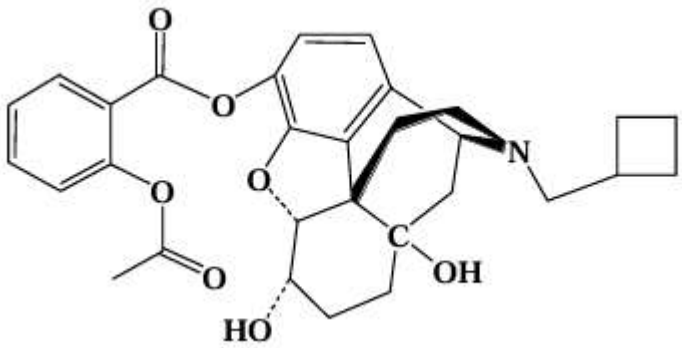


Figure 23: Chemical structure of nalbuphine ester prodrug.

Estrogens such as estradiol and ethinyl estradiol have low oral bioavailability due to the conjugation at the phenolic hydroxyl position.[45] Estrogen sulfamate prodrug (Figure_24) was synthesized by replacing the phenolic hydroxyl group with sulfamate; this sulfamate prodrug protects estrogens from the liver first pass effect which leads to higher systemic activity of oral estrogens.[59]

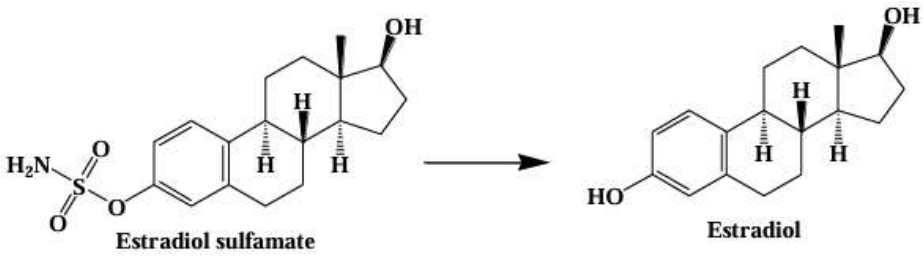


Figure 24: Chemical structures of estrogen sulfamate prodrug and the active drug estradiol.

IMPROVEMENT OF TASTE

Taste is an important factor in the development of dosage forms, and masking bitter taste of oral drugs is crucial for patient compliance especially in pediatric and geriatric patients.

Drugs interact with taste buds on the tongue to give bitter taste. Many technologies were developed to prevent this interaction, including use of physical barrier, chemical or solubility modification and solid dispersion.[60]

Chemical modification to eliminate interaction with taste receptors can be achieved by using prodrug approach. For example, paracetamol, an antipyretic and pain killer drug, has a bitter taste, it is believed that the phenolic hydroxyl group of paracetamol interacts by hydrogen bonding with bitter taste receptors. Therefore, blocking the hydroxyl group with a suitable linker could inhibit the interaction and mask the bitter taste of paracetamol. Karaman's group synthesized some paracetamol prodrugs that were found to lack the bitter taste sensation of paracetamol (Figure 25).[61]

Another example is cefuroxime antibiotic, which has extremely bitter taste. This bitter taste is mostly due to the interaction between the amido group at position 3 and the active site of bitter taste receptors. Some prodrugs with an amide linker were proposed (Figure 26).[62]

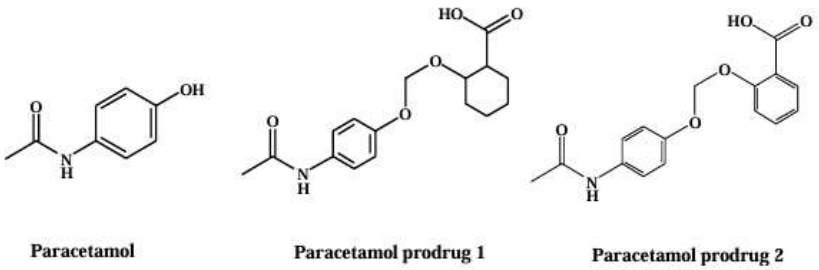


Figure 25: Chemical structures of _paracetamol and its bitterless taste prodrugs.

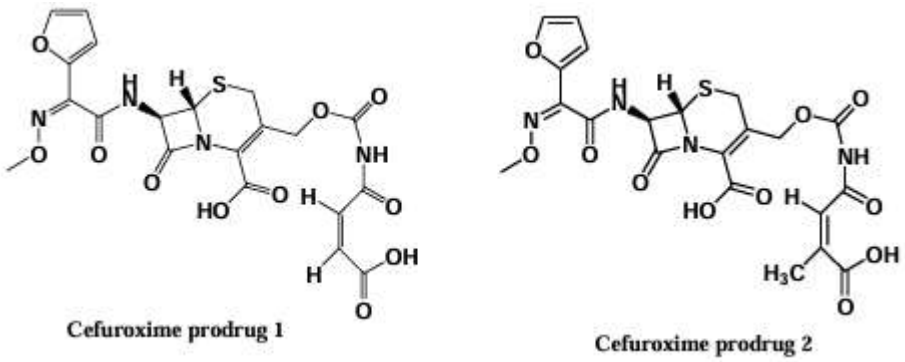


Figure 26: Chemical structures of cefuroxime prodrugs.

IMPROVEMENT OF ODOR

Odor is an aesthetic concern for drugs with high vapor pressure or low boiling point, which makes them difficult to be formulated. For example, ethyl mercaptan a tuberculostatic agent used for the treatment of leprosy has unpleasant smell because of low boiling point 25°C. The most attractive derivative prodrugs were its ethyl thiol esters[63]; diethyl dithiolisophthalate

prodrug of ethyl mercaptan was developed (Figure 27); this prodrug was found to be highly active and odorless.[64]

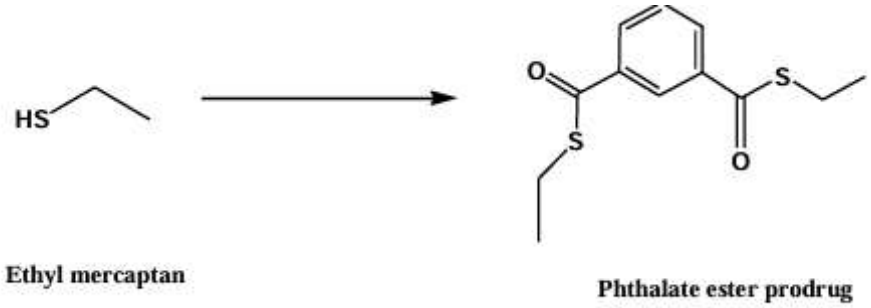


Figure 27: Chemical structures of ethyl mercaptan and its phthalate prodrug.

MINIMIZING PAIN AT INJECTION SITE

Pain at the injection site is caused by precipitation of drug that causes cell lyses and tissue injury. This problem may be related to the vehicle composition or vehicle pH needed for formulation purposes.

For example, phenytoin injection, which is approved for the treatment of status epilepticus has poor aqueous solubility, therefore, the pH in the vehicle for injection is adjusted to 12, which leads to soft tissue injury and pain in the site of administration, due to _phenytoin precipitation.[65]

Fosphenytoin, a phosphate ester prodrug of phenytoin was approved by the FDA in 1996 (Figure 12). This prodrug has high aqueous solubility, no apparent pain was observed upon its use and its intramuscular bioavailability was 100%.[66]

Another example is propofol injectable formulations, which are difficult to be developed because propofol is highly lipid soluble. The available formulations are oil in water emulsions that are associated with pain on the site of the injection.[67] Fospropofol, a phosphoester prodrug of propofol was synthesized (Figure 28)._The synthesized prodrug is a water soluble form of propofol and upon its use no pain on the site of the injection was detected.[68]

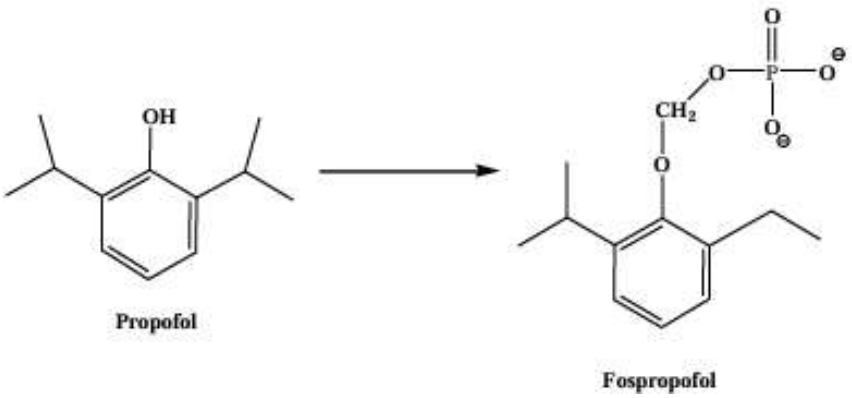


Figure 28: Chemical structures of propofol and its prodrug fospropofol.

SUMMARY AND CONCLUSION

Over the past 50 years, considerable attention has been focused on the development of bioreversible derivatives, such as prodrugs, to alter the physicochemical, pharmacokinetic and biopharmaceutical properties of drugs. Prodrugs are pharmacologically inactive form of their active agents, which undergo chemical and/or enzymatic biotransformation to release the corresponding active drug. The conversion product (i.e. parent drug) subsequently elicits the desired pharmacological response. Since the synthesis of new compounds is a time consuming and too costly, designing derivatives of existing clinically used drugs is definitely an interesting and promising area of research.

Pharmacokinetic and pharmaceutical problems are the most important causes of high attrition rates in drug development. Prodrug design is an efficient approach used to overcome these problems. The lipophilicity of poorly permeable drugs can be increased by linking the drug to a lipophilic linker such that it can be used for oral, ocular or local drug delivery.[69] Prodrugs can be also used to increase aqueous solubility by linking the drug to a polar or ionizable groups.[69] Site selectivity can be achieved by targeting a specific enzyme or receptor, such as targeting an enzyme that is over expressed in tumor cells. Additionally, mAbs are also used as ligands to transport prodrugs to tumor cells. They are designed as drug-antibody conjugate or antibody enzyme conjugate[48], targeting membrane transporters_ are used to increase absorption such as in the case of valacyclovir prodrug.

Prodrugs are also have been used for prolongation of action, such as buprenorphine decanoate and fluphenazine decanoate ester prodrugs.[55] Prodrugs also are applied to decrease pain at injection site by making drugs more water soluble. Masking bitter taste and improvement of odor are important applications of prodrugs to increase patient compliance. Taste masking is

achieved by blocking chemical groups that are involved in the drug interaction with bitter taste receptors.

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