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Review

Prodrugs and soft drugs

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Abstract:
This review focuses on a new approach to the development of drugs, namely on prodrugs and soft drugs. Nowadays, we try to design drugs that heal sick people having the best acceptance of patients. They must be efficient and selective on their site of action and must be metabolized to non-toxic derivatives. Both, prodrugs and soft drugs should have good distributive properties to enhance their quality. They are designed to maximize the amount of an active drug that reaches its target, through changing the physicochemical, biopharmaceutical or pharmacokinetic properties of drugs. Prodrugs are changed into the active drug within the body through enzymatic or non-enzymatic reactions. Soft drugs are novel and active analogues of already known therapeutic agents.
It is expected that continued studies will improve drug properties so as to achieve the best drug delivery system.

Key words:
prodrugs, soft drugs, bioprecursors, drug delivery system, GDEPT, ADEPT

Abbreviations: ADAPT – antibody-directed "abzyme" prodrug therapy, ADEPT – antibody-directed enzyme prodrug therapy, BBB – blood-brain barrier, BZD – 1,4-benzodiazepine, CNS – central nervous system, CPA – cyclophosphamide, DA – dopamine, DNA – deoxyribonucleic acid, GDEPT – gene-directed enzyme-prodrug therapy, HepG2 – hepatocellular carcinoma HSV herpes simplex virus, HSV-Tk – herpes simplex type-1 thymidine kinase enzyme, IUPAC – The International Union of Pure and Applied Chemistry, LEAPT – lectin-directed enzyme-activated prodrug therapy, NTX – naltrexone, RhaDox – prodrug rhamnose-bound doxorubicin, TPZ – tirapazamine, VZV – varicella-zoster virus

Introduction

A large number of therapeutic medications have undesirable properties that may generate pharmacological, pharmaceutical, or pharmacokinetic barriers in clinical drug application. The chemical approach using reversible derivatives such as prodrugs and soft drugs, can be useful in the optimization of the clinical application of a drug. Obviously, this approach can offer the highest flexibility and improve the drug efficacy.

According to The International Union of Pure and Applied Chemistry (IUPAC), the term of prodrug is defined as "any compound that undergoes biotransformation before exhibiting its pharmacological effects. Prodrugs can thus be viewed as drugs containing specialized non-toxic protective groups used in a transient manner to alter or to eliminate undesirable properties in the parent molecule" [15].

Numerous prodrugs have been designed and developed to overcome pharmaceutical and pharmacokinetic barriers in clinical drug application, such as low oral absorption, lack of site specificity, chemical instability, toxicity, and poor patient acceptance (bad taste, odor, pain at the site of application, etc.) [13]. Prodrugs can be designed to target specific enzymes or carriers by considering enzyme-substrate specific-

ity or carrier-substrate specificity in order to overcome various undesirable drug properties. The definition of the prodrug indicates that the protective group is covalently linked to the drug molecule.

The term "prodrugs" or "proagent" was first introduced by Albert in 1950 to signify pharmacologically inactive chemical derivatives that could be used to alter the physicochemical properties of drugs, in a temporary manner, to increase their usefulness or to decrease their toxicity [2]. During the next years, these compounds have also been called "latentiated drugs", "bioreversible derivatives", and "congeners", but "prodrugs" is now the most commonly accepted term. Prodrugs can be considered in different aspects and divided into several categories.

Classification of prodrugs

Drug delivery

The prodrug's design may be useful in avoiding problems associated with the following issues.

Absorption and distribution

Orally administrated drugs must cross the cell membrane barrier twice. When the drug is taken, it must initially cross the cell membrane barrier to get into the blood stream for transportation. After reaching the affected part of the body, it must cross the cell membrane again, this time to enter the affected cell [16].

Physicochemical constants (e.g. the dissociation constant, K, and the partition coefficient, log P) are useful for enabling us to understand the behavior of drug molecules, the lipophilicity and degree of ionization, which can affect absorption, excretion, and the penetration into the central nervous system (CNS). If the aim is to improve oral drug absorption, gastrointestinal enzymes may be the main targets for prodrug design, and the use of a nutrient moiety as a substituent group permits more specific targeting the gastrointestinal enzymes to improve oral drug absorption.

The membrane of the gastrointestinal epithelial cells is composed of tightly packed phospholipids interspersed with proteins. According to that, the transcellular transport of drugs depends on their permeability characteristics and ability to penetrate the lipid bilayer of the epithelial cell membrane, which is dependent on the lipophilicity of the drugs [27].

The effect of lipophilicity on oral absorption is best exemplified by classical study with barbiturates [33], which are the group of drugs responsible for profound sedative-hypnotic effect. They are weakly acidic in nature and are converted to the corresponding sodium salt in aqueous sodium hydroxide. The sodium salt is extensively employed for its intravenous anesthetic properties.

Barbituric acid is the parent member of this group of compounds. Various barbiturates differ in the time required for the onset of sleep and in the duration of their effect. 1,5,5-Trisubstitiuted barbiturates are more acidic and lipophilic than 5,5-disubstituted barbiturates [23]. Barbituric acid was found to be an effective drug but its membrane permeability was observed to be low. However, hexobarbitone a simple derivative of the parent drug was found to have better permeability characteristic. The effectiveness of substituting the hydrogen atom by the methyl group at N-atom decreases polarity of amine and increases penetration through the cell membrane. After intake, the N-methyl group is cleaved in the liver to release the physiologically active drug.

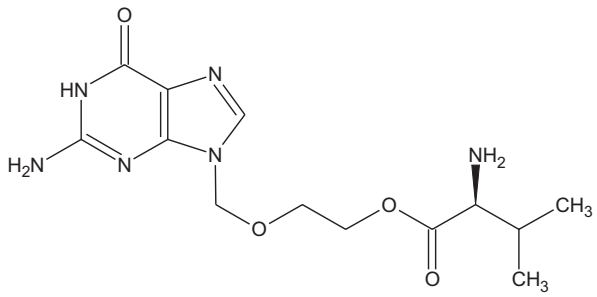


Fig. 1. Valaciclovir

Valaciclovir (Fig. 1) is an interesting example of a prodrug with 3–5 times better bioavailability than aciclovir. It is the L-valyl ester of the parent drug, and is rapidly converted to acyclovir after oral administration.

Famciclovir (Fig. 2) is an oral prodrug of penciclovir and has a similar spectrum as aciclovir. The advantage of valaciclovir and famciclovir over aciclovir is their better oral bioavailability, which permits less frequent dosing when treating *herpes simplex virus* (HSV) or *varicella-zoster virus* (VZV) infections [28].

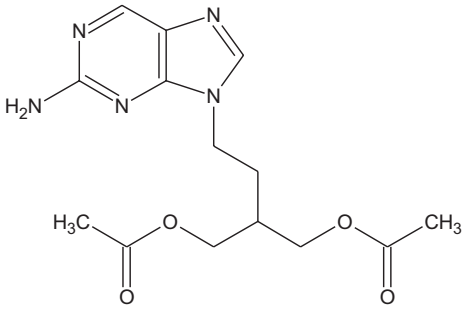
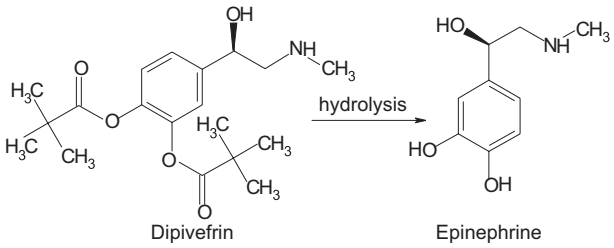


Fig. 2. Famciclovir

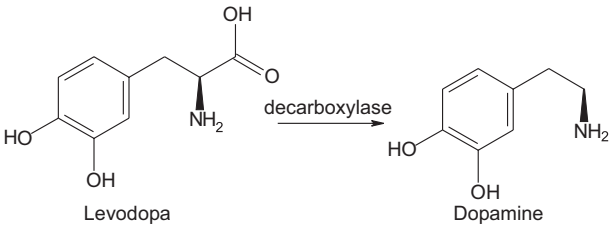
Dipivefrin is a prodrug of epinephrine formed by the diesterification of epinephrine and pivalic acid. The addition of pivaloyl groups to the epinephrine molecule enhances its lipophilic character thereby improving its penetration into the anterior chamber of the eye. Dipivefrin is converted to epinephrine inside the human eye by enzymatic hydrolysis. The liberated epinephrine, an adrenergic agonist, appears to exert its action on intraocular pressure by decreasing aqueous humor production and by enhancing outflow facility. The dipivefrin prodrug delivery system is a more efficient way of delivering the therapeutic effects of epinephrine, with fewer side effects than are usually associated with conventional epinephrine therapy [22], (Scheme 1).



Scheme 1

Treating CNS diseases is particularly challenging because a variety of formidable obstacles often limit drug delivery to the brain and spinal cord. By targeting drugs to their desired site of action one can reduce toxicity and increase treatment efficiency. In response to the insufficiency of conventional delivery mechanisms, research efforts have recently focused on the development of new strategies to deliver drug molecules to the CNS more effectively. The major problem in drug delivery to the brain is the presence of the blood-brain barrier (BBB). Drugs that are effective against diseases of the CNS and reach the brain through the blood compartment must pass the BBB [30].

The "trojan horse" approach for carrier proteins is the most significant solution to delivery of drugs to the brain. Design of a prodrug, which can take advantage of carrier proteins in the cell membrane, such as ones responsible for carrying amino acids into cells is often the most advisable approach. Levodopa is a prodrug of the neurotransmitter dopamine and has been used for the treatment of Parkinson disease, which is the condition primarily due to a dopamine deficiency. Dopamine itself cannot be used because it is too polar and cannot cross the blood-brain barrier. Levodopa (L-3,4-dihydroxy-phenylalanine) is an amino acid and is recognized by the amino acid carrier proteins and is carried across the cell membrane. When it is inside the cell, the enzyme decarboxylase removes the acid group to produce dopamine [7], (Scheme 2).



Scheme 2

Solubility

Solubility is another important determinant of drug permeability because the drug must be reasonably soluble in the aqueous environment to be absorbed properly. Prodrugs used to increase solubility in water have been proved useful in preventing pain associated with some injections.

Injections of the antibacterial drug clindamycin (Fig. 3) are painful, but using a prodrug, which is its phosphate ester, improves solubility and prevents pain.

Solubility is particularly important for drugs that are given intravenously, because higher concentrations and smaller doses can be used.

The steroid estrone, which as most steroid hormones has low water solubility, is dissolved in fat

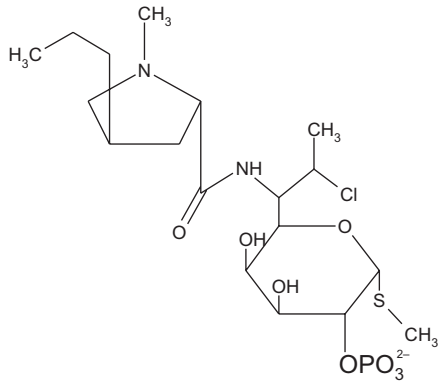
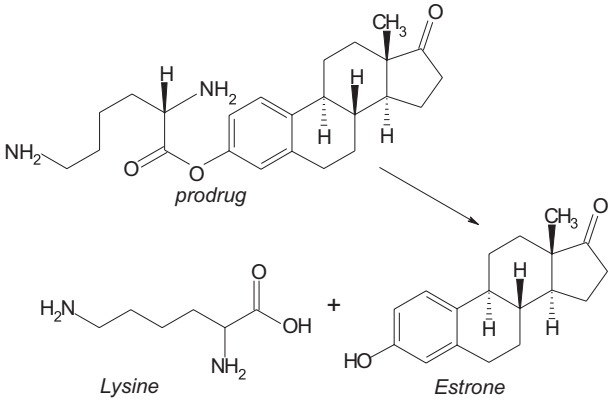


Fig. 3. Phosphate clindamycin

globules and fails to interact effectively with the gut wall and does not get easily absorbed. Its lysine ester prodrug improves water solubility and increases absorption [23], (Scheme 3).



Scheme 3

Site specificity

The most important feature of efficient drug is right site of action. It is necessary to deliver the drug precisely to the affected part of the body, where it is supposed to attack.

It has been suggested that at least three following factors should be optimized to obtain a prodrug acting at the specific site [11]:

– the prodrug must be directly transported to the site of action, and uptake at the site must be rapid and essentially perfusion rate must be limited;

– once the prodrug reaches the site of its action, it must be selectively cleaved yielding the active drug, relative to its conversion at other sites;

– once selectively generated at the site of action, the active drug must be retained by the tissue.

Steroid glycosides and the unique glycosidase activity of the colonic microflora create an opportunity to develop a new colon-targeted drug delivery system. Dexamethasone and prednisolone are corticosteroids used for anti-inflammatory properties. They are steroid drugs and are hydrophobic in nature. They are absorbed efficiently from the small intestine and as such do not reach colon area for treatment. However, when prodrugs dexamethasone-21-β-glucoside and prednisolone-21-β-glucoside were used, they were absorbed in the colon more efficiently compared to their parent drugs. The prodrugs are more hydrophilic than their parent drugs and, therefore, are absorbed poorly in the intestine. The glucosidase enzymes present in the bacteria located in the colon release the parent hydrophobic drugs for absorption in the area [33].

Hexamine (Fig. 4) is a stable inactive compound at pH greater than 5. However, in more acidic pH, the compound disintegrates spontaneously to form formaldehyde, which has antibacterial properties. This is useful for treatment of urinary tract infections. The normal pH of the blood is slightly alkaline and so the

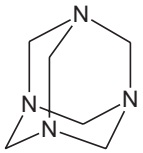
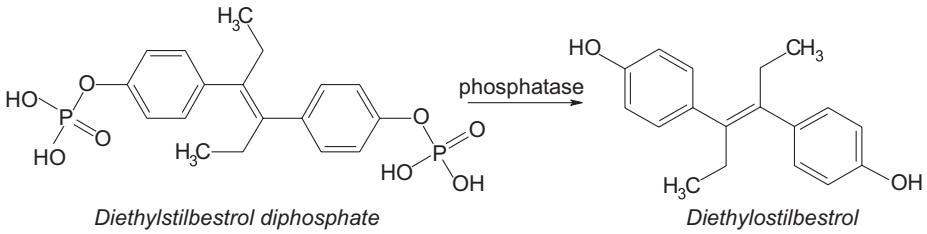


Fig. 4. Hexamine

drug circulates around the body unchanged. However, once it is excreted into the urinary tract, it encounters urine, which is acidic as a result of the bacterial infection. Consequently, formaldehyde is generated just where it is needed [19].

Most of drugs do not specifically attack the affected parts of the body. Orally or intravenously administered drugs need to travel through bloodstream to the site where they are required. In this process, they may cause toxic side effects.

Anticancer chemotherapeutic agents are cytotoxic, because they attack growing normal cells. An example of site-specific prodrug is diethylstilbestrol diphosphate, which is designed for the treatment of breast cancer. The site-specific drug delivery can be obtained by the tissue-specific activation of the



Diethylstilbestrol diphosphate *Diethylostilbestrol*

Scheme 4.

prodrug, which is a result of metabolism by an enzyme that is either unique for the tissue or present at the higher concentration if we compare with other tissues. The tumor cells possess higher concentrations of phosphatases and amidases than normal cells (Scheme 4).

Bodor and co-workers discovered an interesting prodrug approach. They developed a reversible redox drug delivery system for delivering drugs to the CNS. They designed a dihydropyridine derivative with di-O-pivaloyl-DA allowing the compound to cross the blood-brain barrier. Upon oxidation of the dihydropyridine ring, a pyridinium ion is formed that cannot leave the brain, because ions cannot naturally migrate over the blood-brain barrier. The ionic dopamine prodrug is locked inside the CNS where upon hydrolysis it yields DA. Oxidation of dihydropyridine unfortunately does not exclusively occur in the brain but also throughout the periphery [4].

The example of a prodrug, whose design is based on site-specific conditions such as lack of oxygen in hypoxic cells, is tirapazamine. Tirapazamine (TPZ) (Fig. 5) is a bioreductive drug that exhibits greatly enhanced cytotoxicity in hypoxic tumor cells, which are frequently radiation-resistant and chemoresistant. It has two N-oxide moieties, and on one electron reduction twice gets converted to highly reactive diradicals. The diradicals are responsible for deoxyribonucleic acid (DNA) cleavage. Even though such diradicals are generated in normal cells, they get reconverted to N-oxides due to the presence of oxygen, whereas in hypoxic cells the diradicals have longer lifetime to interact with DNA molecules and further cleave them. Such a cleavage of single- or double-strand of DNA leads to the destruction of cells. Thus, the N-oxide prodrugs were found to be highly effective in hypoxic cells.

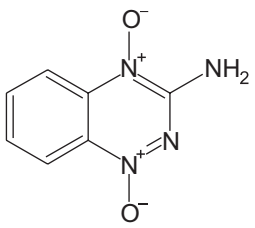


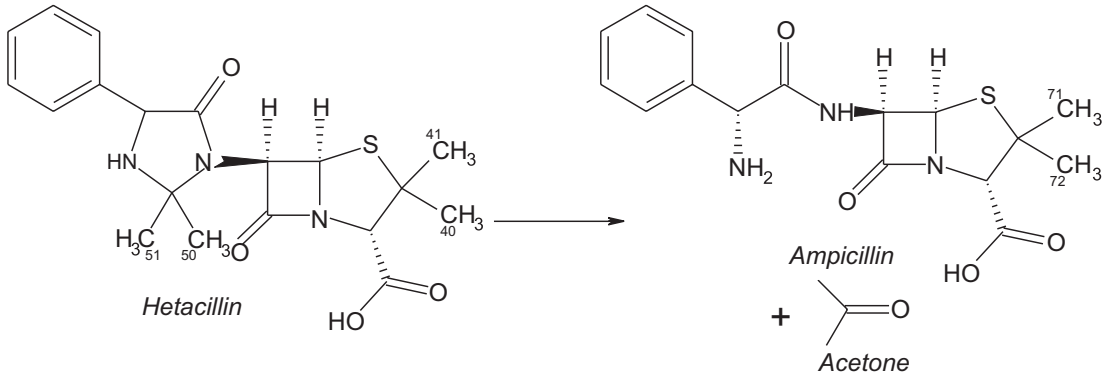
Fig. 5. Tirapazamine

TPZ exhibits particularly good activity when combined with alkylating agents such as cyclophosphamide (CPA). Some studies examined the potential of combining TPZ with CPA in a cytochrome P450-based prodrug activation gene therapy strategy. Recombinant retroviruses were used to transduce 9L gliosarcoma cells with the genes encoding P450 2B6 and NADPH-P450 reductase [17]. Although these tumors showed little response to TPZ treatment alone, tumor growth was significantly delayed, by up to approximately four doubling times, when TPZ was combined with CPA. Some toxicity from the drug combination was apparent, however, as determined by body weight profiles. These findings suggest the potential benefit of incorporating TPZ, and perhaps other bioreductive drugs, into a P450/P450 reductase-based gene therapy strategy for cancer treatment.

Foscan is a chlorine photosensitizing agent that is under clinical trial for a treatment of a number of cancers. By itself this agent has little effect. Given intravenously, it accumulates within cells and has some selectivity for cancer cells. If these cancer cells are then treated with light, the porphyrin is converted into the excited state and is induced to produce highly toxic singlet oxygen that can attack proteins and unsaturated lipids in the cell membrane resulting in the formation of damaging hydroxyl radicals thereby leading to cell destruction [26].

Stability. Resistance

The drug must be resistant to degradation in different body parts and fluids. L-DOPA is the most important



Scheme 5

prodrug that is used in the treatment of Parkinson's disease. It is the biological precursor of DA and may be considered to be a prodrug. The increased oral bioavalibility of the catecholamine, while retaining the catecholamine itself as an active component, is achieved by protection of the hydroxyl and amino groups. The protecting groups are designed to be less susceptible to metabolism and to gradual dissociation from the catecholamine molecule *in vivo*. There are also other prodrugs of DA and of analogues of catecholamines, designed to cross the blood-brain barrier and to centrally undergo slow hydrolysis to give the active species. The hydroxyl groups on the catechol ring are usually protected by formation of di-O-pivaloyl or di-O-benzoyl esters.

The antibacterial agent ampicillin is decomposed because of the intramolecular attack of the side chain amino group on the lactam ring. The prodrug hetacillin locks up the offending amino group in a ring and prevents the decomposition reaction by bacterial β-lactamases. Once the drug has been administered, the drug undergoes hydrolysis on its own to release ampicillin and acetone [36], (Scheme 5).

Prodrugs may protect a drug from first-pass effects. Propranolol is a β-blocker and antihypertensive drug, which suffers from the first-pass elimination resulting in decreased bioavalibility of oral doses compared to intravenous injections. One of the major metabolites is the O-glucuronide. The hemisuccinate ester (Fig. 6) was designed to block glucuronide formulation resulting in 8-fold increase in plasma levels of propranolol [9].

Naltrexone (NTX) (Fig. 7) is an opioid antagonist used for treatment of narcotic dependence and alcoholism. Transdermal NTX delivery is desirable to help to improve patient compliance. In order to increase the delivery rate of NTX across human skin, lipophilic alkyl ester prodrugs are used. Straight-chain naltrexone-3-alkyl ester prodrugs of 2–7 carbons in chain length were synthesized and evaluated [32]. The prodrugs are almost completely hydrolyzed upon passing through the skin and appear as NTX in the receiver compartment. The amount of drug detected in the skin is significantly greater after treatment with

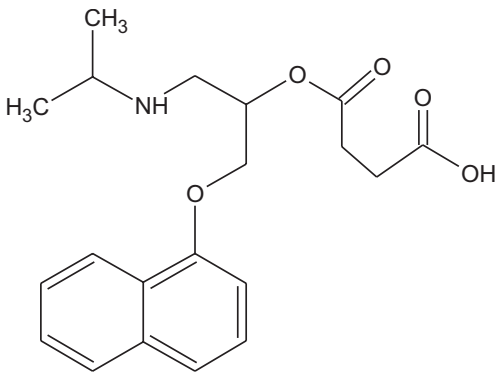


Fig. 6. Propranolol hemisuccinate

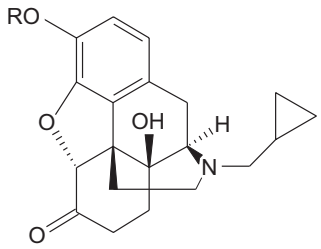


Fig. 7. Naltrexone

the prodrug solutions compared with treatment with NTX base. The extent of the parent drug (NTX) regeneration in the intact skin ranged from 28 to 91%. Higher NTX regeneration percentages in the skin appeared to correlate with the increased drug delivery rates. Definitively, the highly oil-soluble prodrugs provide a higher NTX flux across human skin *in vitro* and undergo significant metabolic conversion in the skin. NTX is used as a treatment for opioid addiction, is non-addicting and is readily absorbed from the gut and as a result undergoes extensive first-pass metabolism. Ester prodrugs, such as the anthranilate (o-aminobenzoate) and the acetylsalicylate increased bioavalibility 45- and 28-fold, respectively, (Fig. 8).

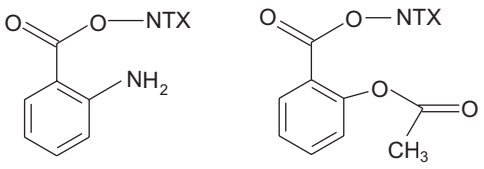


Fig. 8. Prodrugs of naltrexone

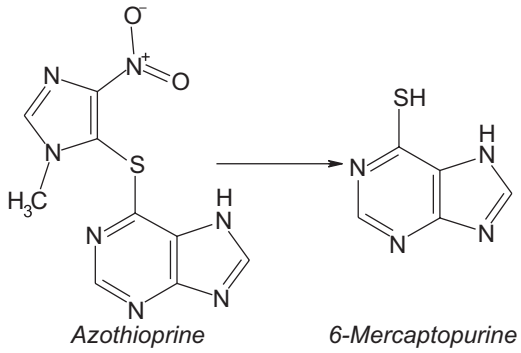
Sustained release

It is also desirable to retain the drug molecule in specific parts of the body for a longer duration, so that its effective activity profile could be completely realized. A common strategy for assuring the slow release is to introduce a long-chain aliphatic ester to slow the hydrolysis. It is particularly useful for the treatment of psychoses where patients require medication for extended periods.

Haloperidol is a potent orally active CNS depressant, sedative and tranquillizer. Its main peak plasma level appears between 2–6 h after administration. However, when haloperidol decanoate is injected intramuscularly as a solution in sesame oil, its antipsychotic activity lasts 1 month [3].

Some prodrugs are designed to be slowly converted to the active drug, in order to prolong the drug activity.

6-Mercaptopurine suppresses the immune response and is useful for protecting donor grafts. However, it is eliminated from the body too quickly. The prodrug, azothioprine is slowly converted to 6-mercaptopurine allowing for more sustained activity, (Scheme 6). This conversion is spontaneous and no enzymes are involved. The rate can be controlled depending on the electron withdrawing ability of the heterocyclic group [41].



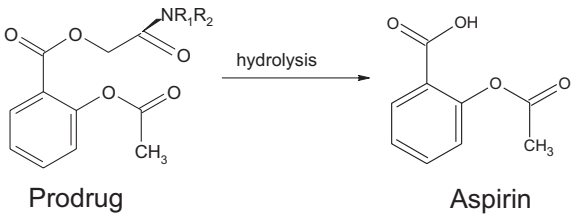
Scheme 6.

Ethyl loflazepate (Victan) is original 1,4-benzodiazepine (BZD) being used as a potent, non-sedative, minor tranquillizer. Ethyl loflazepate was designed to be a prodrug that would gradually release an active 1,4-BZD within the organism. It was hypothesized that this type of pharmacokinetic profile would dissociate anxiolytic from sedative activities. Previously published pharmacokinetic studies demonstrated that ethyl loflazepate gradually releases an active 1,4-BZD, descarboxyloflazepate in plasma. The activities of ethyl loflazepate and its metabolites were compared to those of reference BZDs: diazepam, bromazepam, nitrazepam, flunitrazepam, lorazepam, clorazepate. Ethyl loflazepate and its metabolites revealed a typical profile of activity of minor tranquillizers. The activity of ethyl loflazepate was long-lasting. The overall anxiolytic potency of ethyl loflazepate was similar to that of diazepam but ethyl loflazepate appeared to be less sedative than diazepam [8].

Another tactic is to deliberately link a very lipophilic group to the drug. Then, the majority of the drugs would be stored in the fat tissue and if the lipophilic group is only slowly removed over time, the drug is steadily released into the bloodstream. The antipsychotic drug fluphenazine is used as a lipophilic ester, and given by intramuscular injection, slowly diffuses into blood where it is rapidly hydrolyzed [20].

Toxicity

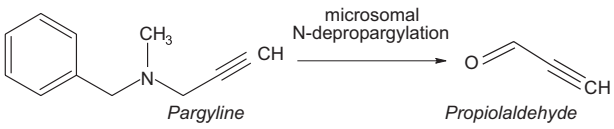
Derivatives of salicylic acid are one of the oldest examples that are characterized by lesser toxicity than their parent drugs. Salicylic acid is a good pain-killer but causes gastric irritation and bleeding because of



Scheme 7.

the carboxy group. It accumulates in the gastric mucosal cells. Aspirin esters suppress gastric irritation. N,N-disubstituted 2-hydroxyacetamide esters are chemically stable and quickly hydrolyzed by plasma esterases [33], (Scheme 7).

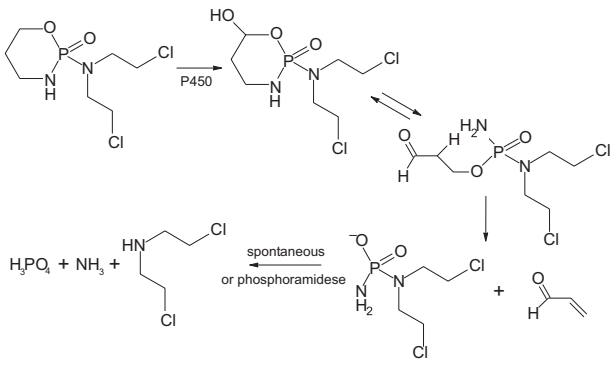
Prodrugs can be used to afford drugs, that would be too toxic to be given directly, a feature of the slow release. Propiolaldehyde is useful for aversion therapy in patients addicted to alcohol. However, it is a highly



Scheme 8.

irritating chemical and causes allergic reactions. As an alternative, a closely related compound, pargyline is used. The prodrug can be converted to propiolaldehyde by enzymes only in the liver (Scheme 8).

A prodrug that is converted to the active drug at the target site itself greatly reduces side-effects of highly



Scheme 9.

toxic drugs. Cyclophosphamide is a successful anticancer drug for the treatment for a broad range of malignant tumors, which is not toxic itself, but becomes a toxic alkylating agent after being metabolized [31]. It can, therefore, be taken orally without causing damage to the gut wall. It was hoped that the high level of phosphoramidase enzyme present in some tumor cells would lead to a greater concentration of the alkylating agent in these cells and would result in selectivity of action (Scheme 9).

Poor patient acceptability

Some drugs have a revolting taste. One way to reduce this problem is to decrease their solubility in water so that they do not dissolve on the tongue. For example the bitter taste of the antibiotic chloramphenicol can be avoided by using the palmitate ester (Fig. 9), which is quickly hydrolyzed once swallowed [40].

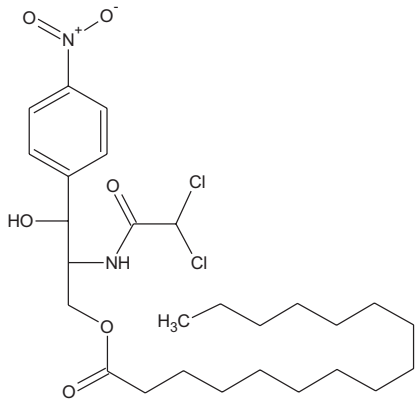


Fig. 9. Palmitate ester of chloramphenicol

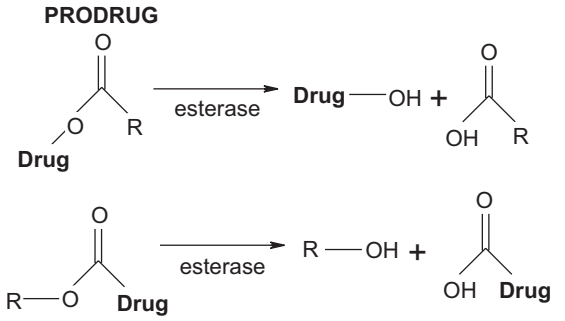
Chemical structure

Esters

Esters as prodrugs can be readily synthesized from alcohol-containing parent drug and an alcohol-containing carrier (R).

Prodrugs can be also synthesized from carboxylic acids-containing parent drug and an alcohol-containing carrier (R), (Scheme 10).

A drug may have a carboxylic group that plays an important role in its activity by binding to an active



Scheme 10

site *via* ionic or hydrogen bonding. However, it is ionic so it may not be able to cross the membrane. Protecting this group by its estrification makes it less polar, thereby enabling it to cross the membrane. Once in the blood stream, it is hydrolyzed back to the free acid by esterases.

Esters are easily hydrolyzed by various and ubiquitous esterases. An ester as a prodrug alters lipophilicity of the drug and consequently, adsorption and distribution may also be affected as desirable.

Bacampicillin and pivampicillin are penicillin prodrugs, that are the examples of prodrugs, which fluently cross the cell membranes. Ampicillin is poorly absorbed from oral administration (about 40%

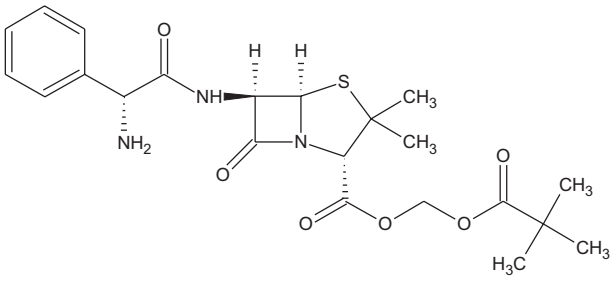


Fig. 10. Becampicillin

is absorbed) and unabsorbed drugs may destroy important intestinal bacteria and cause dysbacteriosis. Bacampicilin (Fig. 10) and pivampicilin (Fig. 11) possess double ester substitution in order to improve absorption. Acyloxyl methyl ester extends the terminal ester away from thiazolidine ring and decreases steric hindrance for enzyme activation by hydrolases. Bacampicillin is absorbed in 98–99% and liberates the free drug into the blood within 15 min [24].

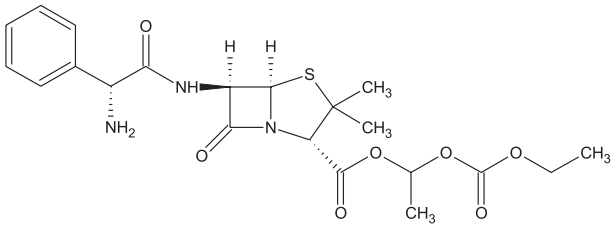


Fig. 11. Pivampicillin

Similar example of the esters is the enalapril prodrug, which reduces blood pressure [1].

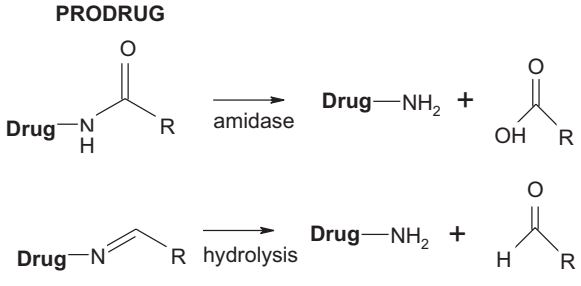
Unfortunately, not all such prodrugs are ideal substrates for the activating enzymes. It is sometimes necessary to consider modifying the carrier with electron withdrawing or donating groups to facilitate the hydrolysis. Not all esters are hydrolyzed equally efficiently. Hydrolysis can be improved by introducing electron-withdrawing groups into the alcohol part (in order to make the alcohol a better leaving group), for example OCH_2CF_3, or CH_2COOR, or OAr. However, making the ester too reactive may result in its hydrolysis before it crosses the membrane.

Amides and amines

Amides are synthesized from an amine-containing parent drug and a carboxylic acid. Amides are more resistant than esters to hydrolysis. However, activated amides or amides of amino acids are more susceptible to enzyme-mediated cleavage.

Amines may be converted to imines (Schiff's bases). As a result, such compounds are more lipophilic (allowing access across blood-brain barrier) although labile in aqueous solutions, (Scheme 11).

Prodrugs can be designed to target specific enzymes or carriers by utilizing knowledge of enzyme-substrate



Scheme 11.

specificity or carrier-substrate specificity in order to overcome various undesirable drug properties.

Bioprecursors

They are prodrugs that are metabolized into a new compound that may be active or further metabolized to an active metabolite.

We can consider the process of biotransformation to be the way of producing new active compounds. There is increasing evidence that the metabolites of some drugs are pharmacologically active. Numerous examples of pharmacologically active metabolites have been used as a source of new drug candidates because these metabolites often are subjects to phase II reactions and have better safety profiles.

The best known example of bioprecursor is acetaminophen, which is an *O*-deethylated metabolite of phenacetin. Acetaminophen shows superior analgesic activity when compared with phenacetin. The main advantage of acetaminophen over phenacetin is that it does not produce methemoglobinemia and hemolytic anemia [38].

Although pharmacologically active metabolites are generally formed by phase I oxidative reactions, phase II conjugation reactions also can produce biologically active metabolites. Morphine 6-glucuronide is a more potent μ-opioid receptor agonist than morphine itself. Recent clinical studies in cancer patients given morphine 6-glucuronide indicated that appreciable analgesic effects are achieved without the side effects of nausea and vomiting that are often associated with morphine therapy.

Sulfation also produces biologically active metabolites. Minoxidil (Fig. 12), a potent vasodilator, is a good example. Studies concerning the action of minoxidil revealed that the therapeutic activities were mediated by its sulfate conjugate. In addition to the advantages that active metabolites may have in terms of efficiency with fewer unwanted side effects, active metabolites can also be preferred over the parent drugs for kinetic reasons.

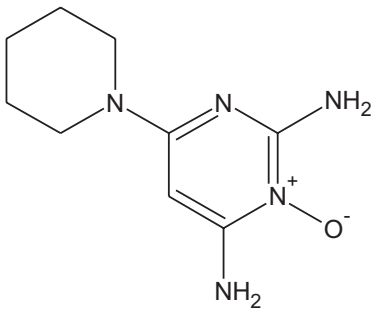


Fig. 12. Minoxidil

The newest therapies

Recently, new therapies have been proposed to overcome this limitation of prodrug therapy. These new approaches are referred to as an antibody-directed enzyme prodrug therapy (ADEPT) and gene-directed enzyme-prodrug therapy (GDEPT). They attempt to localize prodrug-activating enzymes in specific cancer cells prior to prodrug administration. Appropriately designed drugs have been found to be effective in the treatment of human tumors possessing high levels of an activating enzyme.

ADEPT

ADEPT is an antibody-directed enzyme prodrug therapy in which a conjugate of an enzyme and a monoclonal antibody bind to tumor-associated antigens, and is used to convert a non-toxic prodrug into a toxic species selectively at the surface of malignant cells. Analogously, antibody-directed "abzyme" prodrug therapy ADAPT [37], where a catalytic antibody replaces the enzyme used in ADEPT, has also been investigated. In both strategies, the biocatalyst component is localized at an antigen-presenting site, typically resulting in a serum-exposed biocatalyst source. After administration of the appropriate enzyme, the active drug molecule is released by the localized enzyme, resulting in a potential reduction in selectivity due to the diffusion and uptake of active drug into non-targeted bystander cells.

Paclitaxel (Fig. 13) is a new prodrug intended for use in ADEPT. This prodrug was originally designed to be activated into the drug by human β-glucuronidase. In order to enhance the liberation rate of paclitaxel, an elongated spacer system including a nitroaromatic derivative and *N, N*-methylethylenediamine was incorporated between the sugar moiety and the drug. Indeed, this new prodrug proved to be activated significantly faster than the former prodrug paclitaxel containing a conventional spacer [6].

The cytotoxic activity of antitumor drugs such as doxorubicin is enhanced and restricted to tumor-affected tissues by preparing their peptide derivatives.

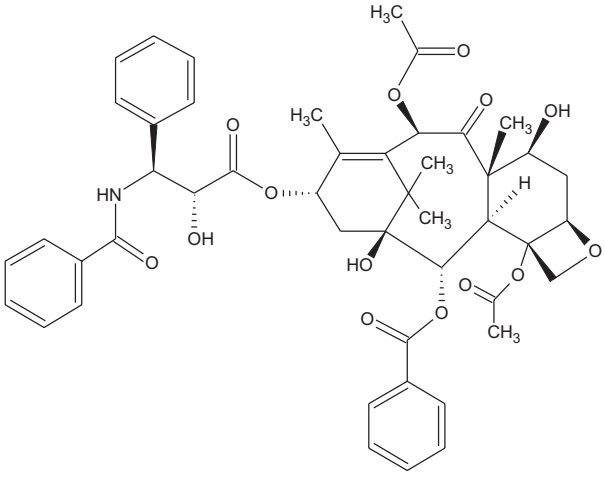


Fig. 13. Paclitaxel

The active site of doxorubicin (Fig. 14) is blocked by strategically attaching a suitable polypeptide to the drug separated by spacer. The spacer is used to expose the polypeptide chain to plasmin activity. The prodrug was found to be inactive and stable under biological

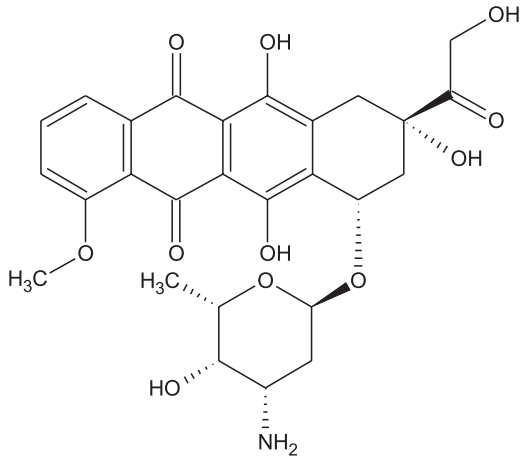


Fig. 14. Doxorubicin

pH conditions but it was readily cleaved with the release of the parent drug in the presence of cytoplasmic enzymes present in tumor cells. The prodrug was synthesized by blocking an important functional group in the molecule with a polypeptide-capping agent to make it inactive. The spacer group was designed to self-eliminate after hydrolysis of the polypeptide chain by the enzyme [18].

GDEPT

GDEPT or suicide gene therapy is composed of three components: the prodrug to be activated, the enzyme used for activation, and the delivery system of the corresponding gene [21]. Prodrugs can be considered as comprised of two major domains; a "trigger" unit that is the substrate for the activating enzyme, and an "effector" unit that is activated or released by the metabolic process. Besides being an efficient and selective substrate for the activating enzyme, the prodrug itself needs to be a systemically active agent, metabolically stable and able to diffuse efficiently by paracellular and transcellular ways to the areas in the tumor where the activating enzyme is being generated. The effector that is achieved or released from the prodrug must be an effective cytotoxin. It is preferably required to kill cells at all stages of the cell cycle, but must also have good bystander effect (an ability to diffuse and kill neighboring tumor cells) [39].

The following agents are examples of prodrugs and their activating enzymes used in GDEPT therapy.

The most prominent GDEPT therapy has been the use of the herpes simplex type-1 thymidine kinase enzyme (HSV-Tk) in co-function with a variety of guanosine-based prodrugs, compounds originally developed as antiviral (anti-herpes) agents. The enzyme converts these prodrugs very efficiently to the monophosphates, which are then converted by cellular enzymes to the toxic triphosphates. They cause cell death by inhibition of dGTP incorporation into DNA, and also by prevention of chain elongation.

Ganciclovir, penciclovir, acyclovir and zidovudine are the most widely known prodrugs, which have been used for HSV-Tk therapy. Beside HSV-Tk, the cytosine deaminase gene is the next most broadly studied for GDEPT. The enzyme catalyzes the conversion of cytosine to uracil and is an important member of the pyrimidine salvage pathway in prokaryotes and fungi, but is absent in multicellular eukaryotes. The therapy focuses only on one drug, which is clinically used as anti-fungal agent-5-fluorocytosine.

Cytochrome P450 is composed of a large number of different isozymes. Many of these enzymes are expressed to greater extent in the liver than in tumor cells [14]. Because of that the main target is to selectively increase tumor cell exposure to cytotoxic drug metabolites by targeting expression of the enzymes to the tumor by gene vectors. This area is dominated only by two prodrugs: cyclophosphamide and ifosfamide [25].

An additional GDEPT strategy involves the delivery of a gene encoding a foreign enzyme to a chosen site, and, after subsequent enzyme expression, a dose of prodrug results in the selective release of the parent drug. GDEPT provides a source of enzyme localized within a cell, thereby reducing the problem of the reduced selectivity associated with the bystander effect, but it requires the development of gene-delivery vehicles appropriate to the particular cell type. Suicide gene therapy is a promising but very complex technology, and to be broadly clinically useful will require maximization of the therapeutic properties of all components.

LEAPT

Lectin-directed enzyme-activated prodrug therapy (LEAPT) is a bipartite drug-delivery system that exploits endogenous carbohydrate-to-lectin binding to the localized glycosilated enzyme conjugates to specific, predetermined cell types followed by administration of a prodrug activated by that pre-delivered enzyme at the desired site. Macromolecular glycoconjugates, such as synthetic glycopolymers and glycoproteins, have been used as carriers of covalently conjugated drugs, bearing carbohydrate ligands that provide delivery specificity. To exploit natural carbohydrate binding mechanisms, the LEAPT approach uses two components: the cell-specific delivery of a synthetically glycosylated enzyme, here an α-rhamnosidase, and an appropriately capped, here rhamnoside-capped, prodrug. The glycosylated α-rhamnosidase selected here, non-mammalian glycosidase enzyme, is delivered to specific cell types within the body which is mediated by pre-selected carbohydrate-receptor interactions as determined by appropriate choice of the carbohydrate. Once uptake is completed, a prodrug composed of a drug molecule bearing α-L-rhamnosidase cap can be dosed.

The therapeutic efficacy of lectin-directed enzyme-activated prodrug therapy was demonstrated by doxorubicin, Rha-Dox, and its application to reduce tumor burden in a hepatocellular carcinoma (HepG2) disease model [29].

Soft drugs

According to IUPAC definition, soft drug "is a compound that is degraded *in vivo* to predictable nontoxic and inactive metabolites, after having achieved its therapeutic role". It should be an isosteric/isoelectronic analogue of the parent drug. They are strategically designed to undergo singular metabolic deactivation after they achieve their therapeutic roles. It is important to control and predict the process of metabolism. Metabolism of most drugs is mediated by the cytochrome P450 system. These kinds of compounds are ideal for producing specific action at the site of application without affecting the rest of the body. The idea of designing the soft drugs is based on the concept that we should build a fragment into the molecule that turns the new molecule into a compound that can function as a substrate for the metabolizing enzymes, but still has a sustained activity at its original target. There are a lot of important local sites where application of a drug can be achieved very easily, for example, eye, skin, major parts and compartments of the gastrointestinal tract, and lungs. Local application of a drug to these sites can easily be achieved, and soft drugs then can produce their desired pharmacological activity at the site of application.

Nicholas Bodor was the first one who introduced the idea of soft drugs in 1977 [11]. According to this concept, soft drugs have been divided into four categories as follows:

- soft analogues,
- inactive metabolite-based soft drugs,
- active metabolite-based soft drugs and pro-soft drugs.

Soft analogues

They are close to the structure of known active drugs that have a specific metabolically sensitive moiety built into their structure to allow for a facile, one-step controllable deactivation and detoxification after the desired therapeutic role has been achieved.

Quaternary ammonium compounds, such as benzalkonium chloride, are strong antibacterial agents. Their toxicity limits their usage in humans and animals, and their chemical stability limits their usage for general environmental sanitation. A series of soft analogues of the quaternary ammonium preservatives such as cetyl pyridinium chloride and benzalkonium chloride, currently available strong antibacterial agents, are less toxic. These soft analogues consist of long alkyl chain connected to a polar head group *via* chemically labile spacer. They are characterized by facile non-enzymatic and enzymatic degradation to

form their original non-toxic building blocks. Cetyl pyridinium chloride is used for mouthwash. Unlike the cetyl pyridinium chloride, which needs to undergo oxidative metabolic deactivation, the soft analogue will be metabolized easily by esterases, which ultimately destroy in one step both the quaternary head and the long chain that are together responsible for the surface-active properties and antimicrobial activity of cetyl pyridinium chloride [34].

Soft anticholinergics were intended for topical application, for example, to be used as antiperspirants or as mydriatic agents. By incorporating an adequate metabolically labile moiety into their structure, they can be potent and locally active as anticholinergic agents, but only with minimal systemic anticholinergic effects due to their rapid metabolism in the systemic circulation. Thus, the overall therapeutic index is greatly improved [14].

Inactive metabolites

Soft drugs are active compounds designed starting from a known (or hypothetical) inactive metabolite of an existing drug by converting this metabolite into an isosteric/isoelectronic analogue of the original drug in such way as to allow a facile, one-step controllable metabolic conversion after the desired therapeutic role has been achieved back to the very inactive metabolite the design started from.

Loteprenolol etabonate is structurally similar to other corticosteroids. However, there is no ketone group at the position 20. It is highly lipid soluble which alters its penetration into cells. Loteprenolol etabonate (Fig. 15) is synthesized through structural modification of prednisolone-related compounds so that it will undergo a predictable transformation to an inactive metabolite. It is known that loteprendolol etabonate undergoes extensive metabolism to inactive carboxylic acid metabolites [27].

Active metabolites

They are soft drugs, which refer to compounds or metabolic products of a drug that retain the same affinity as the parent drug. Reasonable selection of an active metabolite can lead to a potent drug that will undergo a one-step deactivation process, since it is already at the highest oxidation state.

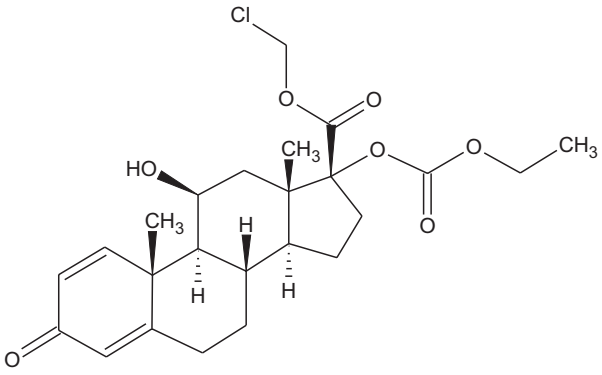


Fig. 15. Loteprenolol etabonate

Pro-soft drugs

They are metabolized through enzymatic transformation into the active soft drug.

The first two approaches are the most popular concepts of soft drugs.

Conclusion

Both, the prodrugs and the soft drugs are used to overcome several undesirable properties in order to achieve the best clinical drug application. The newest discoveries of molecular biology provide the essential information about enzymes and carrier proteins. It is clear from the foregoing that the design of drugs cannot be based just on chemical synthesis. Drug discovery and prodrug and soft drugs development appear to be complementary for the generation of target-specific medicines now and in the future.

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