Advances in Prodrug Design

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Abstract: The background of prodrug design is presented herein as the basis for introducing new and advanced latent systems, taking into account mainly the versatility of polymers and other macromolecules as carriers. ***PDEPT*** *(**P**olymer-**D**irected **E**nzyme **P**rodrug **T**herapy);* ***PELT*** *(**P**olymer-**E**nzyme **L**iposome **T**herapy);* ***CDS*** *(**C**hemical **D**elivery **S**ystem);* ***ADEPT****(**A**ntibody-**D**irected **E**nzyme **P**rodrug **T**herapy);* ***GDEPT/VDEPT*** *(**G**ene-**D**irected **E**nzyme **P**rodrug **T**herapy/**V**irus-**D**irected **E**nzyme **P**rodrug **T**herapy);* ***ODDS*** *(**O**steotropic **D**rug **D**elivery **S**ystem) and* ***LEAPT*** *(**L**ectin-directed **e**nzyme-**a**ctivated **p**rodrug **t**herapy)* are briefly described and some examples are given.

Keywords: Prodrug design, Latent advanced systems, Macromolecules carriers, Selective delivery systems.

INTRODUCTION

Although there are more than 7000 drugs available for the treatment of most diseases, many physico-chemical, pharmacokinetic, pharmacological, and toxicological properties can be barriers for their clinical use, in addition to organoleptic unwanted properties [1-3].

With the aim of optimizing mainly the physico-chemical properties of a drug, which is reflected in its poor pharmacokinetic characteristics, the latentiation process, currently known as prodrug design, is a good alternative [3-8].

The interest in prodrug design as an alternative for improving drug effectiveness has increased. For example, among the blockbuster drugs in the pharmaceutical market, five – lovastatin, sinvastatin, omeprazole, acyclovir, and enalapril – are prodrugs [1].

Prodrug design is a molecular modification process, first proposed by Harper in 1959, although Albert, a year previously, introduced the term prodrug to signify any compound that needs biotransformation prior to exhibiting its pharmacological effects. This term comprehends the transformation of a drug in an inactive transport form that *in vivo*, through chemical or enzymatic reactions, releases the drug either at or near the site of action (Fig. **1**). Despite being introduced in the 60's, this process was only defined in parallel with the advances in the knowledge of drug targets and in their pharmacokinetics in the middle of the 70's [2]. Advanced prodrug design has been one of the most useful tools for the development of new chemotherapeutic agents against cancer and AIDS [7].

Hans Bundgaard decisively contributed to the development of prodrug design. In 1978, he started the well-recognized Prodrug Research Group, which he was in charge of until his death in October 1992. He was a pioneer, introducing the importance of drug delivery in industrial drug development. He published more than 300 papers, besides many book chapters and patent applications. Several marketed prodrugs are based on his initial prodrug research[a].

In terms of the industrial aspects of drug introduction, the advantage of prodrug design is the relative facility of obtaining the new derivatives, which are not considered to be "me too" drugs, thus, allowing patent recognition [9].

Prodrugs and analogs, although having similar structures, present some differences, as depicted in (Fig. **2**). Currently, the main difference is related to the bioreversibility of the former. The principal reversible linkages used in prodrug design are described by Friis and Bundgaard [5].

There are many reasons that justify the need for drug molecular modification through prodrug design; these include [10] *pharmaceutical problems*, such as low solubility, low chemical stability, undesirable taste, odor, high irritation and pain; *pharmacokinetic problems*, such as low oral absorption, high rate of presystemic metabolism, low absorption by non-oral routes, low time profile, and low selectivity in organ/tissue active agent delivery, and *pharmacodynamic problems*, such as low therapeutic index and lack of selectivity at the action site [5, 7, 11-14].

A strategic approach for choosing a prodrug design as the solution for a definite drug problem deserves consideration. This strategy begins when the structure-activity relationship of a compound indicates an incompatibility between pharmacokinetics and pharmacodynamics [1]. Figure **3** shows two situations that can de found in a research project. In case A, a favorable situation is represented, where PD – Pharmacodynamic – and PK – Pharmacokinetic chemical spaces have a partial overlap, signifying that the candidates

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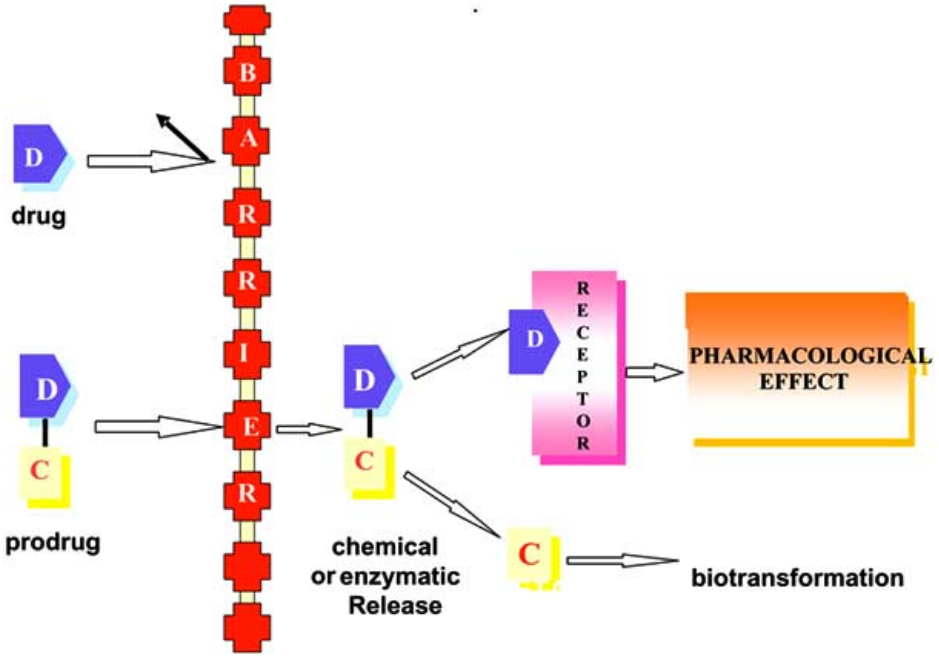

Fig. (1). Prodrug concept (adapted from [4]). D – drug. C- carrier.

have good PD and PK features. In case B, an unfavorable situation has to be faced, since there is incompatibility concerning structural characteristics, avoiding good pharmacodynamic and pharmacokinetic profiles. In this circumstance the prodrug approach may be the only alternative for solving this problem. The success of this strategy depends on how soon the diagnostic of the situation – case A or case B -- is made.

Some researchers [9] believe that the prodrug approach should be available as soon as a drug candidate arises from the discovery step in the general fluxogram of drug/medicine development [15]. In this sense, they [9] have distinguished two types of prodrug design, namely *posthoc* and *adhoc* design. The former corresponds to a modification of a well-known drug that presents some of the problems described above and the later means the modification of a lead candidate that shows some undesirable properties that can restrict its further approval as a drug.

The main pharmacokinetic problems for drug use are: low oral bioavailability, due to polarity and/or solubility, to

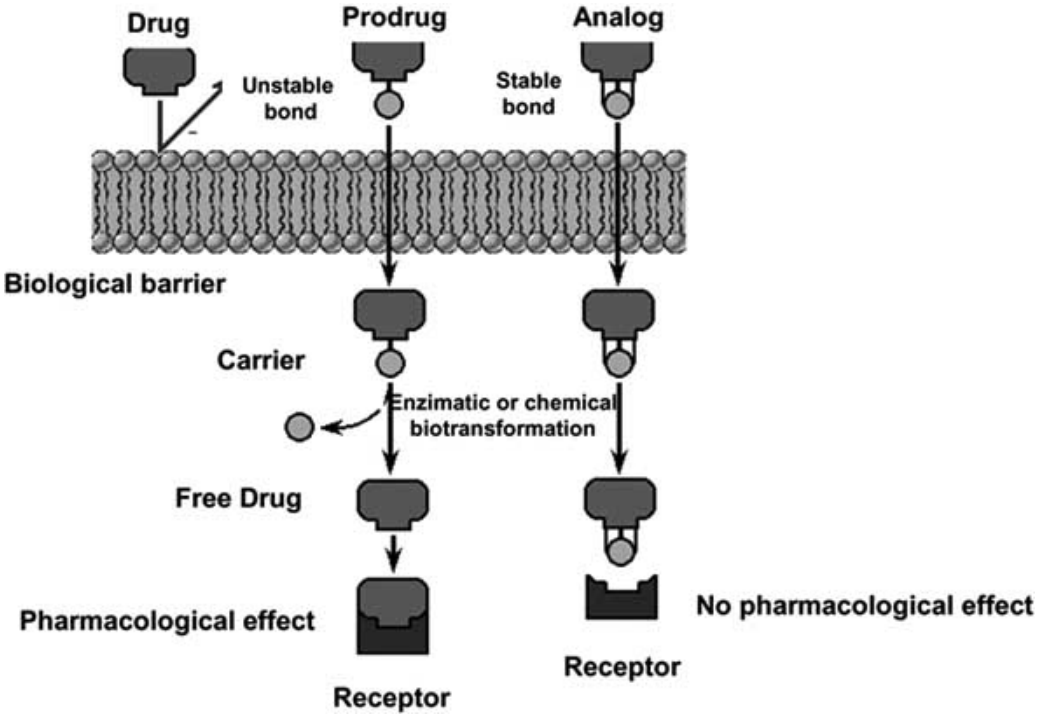

Fig. (2). Differences between prodrug and analog.

gastric instability or to a high level of first-pass metabolism; insufficient distribution to the local site and inability to cross different kinds of biological barriers (gastric mucous, skin, cornea and blood brain barrier) [5].

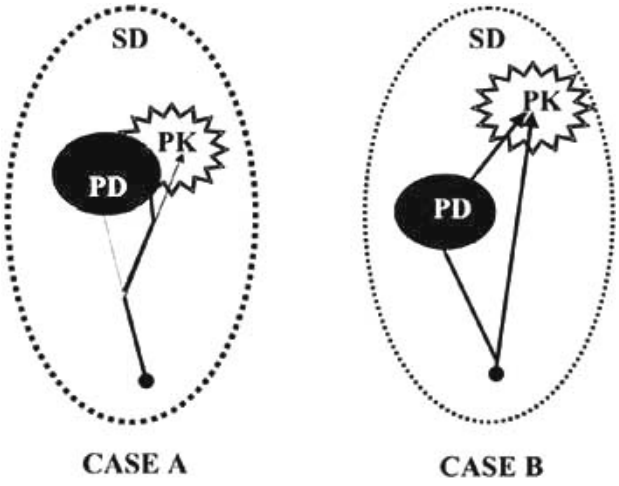

Fig. (3). Schematic situations found in a reserch project (adapted from [1]). SD represents de structural diversity space; PD is the pharmacodinamic of the molecules considered; PK is the pharmacokinetics of those molecules.

In order to improve drug properties, prodrugs must have some important characteristics such as, biological inactivity (or very low activity, in comparison with the prototype); bioreversible linkage with the carrier that provides the minimal drug efficient concentration at the local site of action; lack of toxicity in the carrier. In addition, the synthesis should be less complex than that of the drug.

Based on the discussed points, the main objectives of prodrug design are [4, 8]:

1. Modification of drug pharmacokinetics;
2. Prolongation of action;
3. Diminution of toxicity and side-effects;
4. Increase in selectivity;
5. Resolution of formulation problems, such as stability, solubility and organoleptic properties.

"LATENT DRUGS" CLASSIFICATION

Wermuth, in 1984 [16], classified "latent drugs" as *prodrugs*, subdivided into bioprecursors and classic prodrugs, and *targeted drugs*. The carrier nature of the drug is one of the characteristics that differentiate these two categories of latent drugs, with special emphasis on the specificity used for targeted drugs. In addition to these classes, *mixed* and *mutual prodrugs* exist.

Bioprecursors

Bioprecursors are latent drugs that do not have a carrier, but must be biotransformed *in vivo*, although through generally non-hydrolytic enzyme systems [8].

Lovastatin (Fig. **4**), for example, a HMG CoA (3-hydroxy-3-methylglutaryl-coenzime A) reductase inhibitor that has introduced a new perspective in the treatment of hypercholesterolemias [8], is active due to its biotransformation to the open chain nonlactone metabolite. The lactone derivative provides a more suitable partition coefficient to the drug, although the carboxyl group must be free to provide similarity with the enzyme substrate.

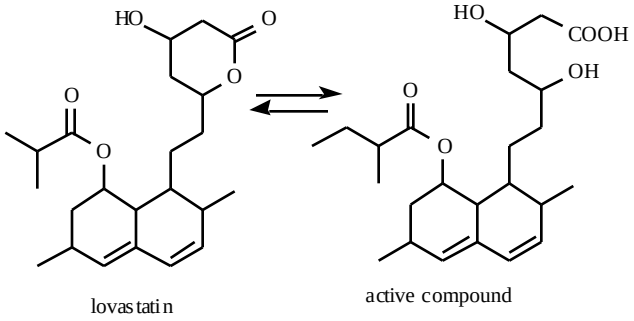

Fig. (4). Lovastatin and its non-lactone active derivative.

Photosensitizer prodrugs, used in photodynamic therapy, have been considered as bioprecursors [17], and represent a good alternative for tumor treatment although still in experimental studies.

Classic Prodrugs

Classic prodrugs follow the classic definition of latentiation, being inactive or much less active than the drug and must be hydrolyzed by chemical or enzymatic means, releasing the active molecule [8]. The drugs are linked to a suitable carrier in order to improve some therapeutic properties, such as bioavailability, selectivity, reduced toxicity and prolonged action. In addition, some prodrugs are designed with the aim of making the drug formulation possible.

In spite of being used for all the goals above mentioned, the main objective pursued by classic prodrugs has been the bioavailability improvement [5]. In general, an undesirable partition coefficient causes low bioavailability of a drug and, by choosing a suitable carrier, either the liposolubility or the hydrosolubility can be increased. Classical examples are the ester prodrugs of ampicillin. This beta-lactam antibiotic has an absorption of about 40% due to high polarity and, hence, hydrosolubility. Through the latentiation process, lipophilic acyloxyalkyl esters were designed (Fig. **5**) and increased the absorption to about 90%, thus improving the bioavailability of the antibiotic. Once absorbed, ampicillin, responsible for the antibacterial activity, is released in approximately 15 minutes.

Hydrosolubility can also be increased by means of decreasing either intra or intermolecular hydrogen bonds [4], since those interactions lead to more organized structures, being less soluble in aqueous medium. Hydroxymethyl derivatives of acidic drugs, such as amides, allow aqueous solubility to be increased, and are also intermediate derivatives for ester drugs, with modulation of the partition coefficient.

Chung, in 1996 [18], synthesized the nitrofurazone hydroxymethyl derivative (NFOH) (Fig. **6**) as a synthesis intermediate of the mutual prodrugs of primaquine and nitrofurazone, which are potentially active in Chagas'

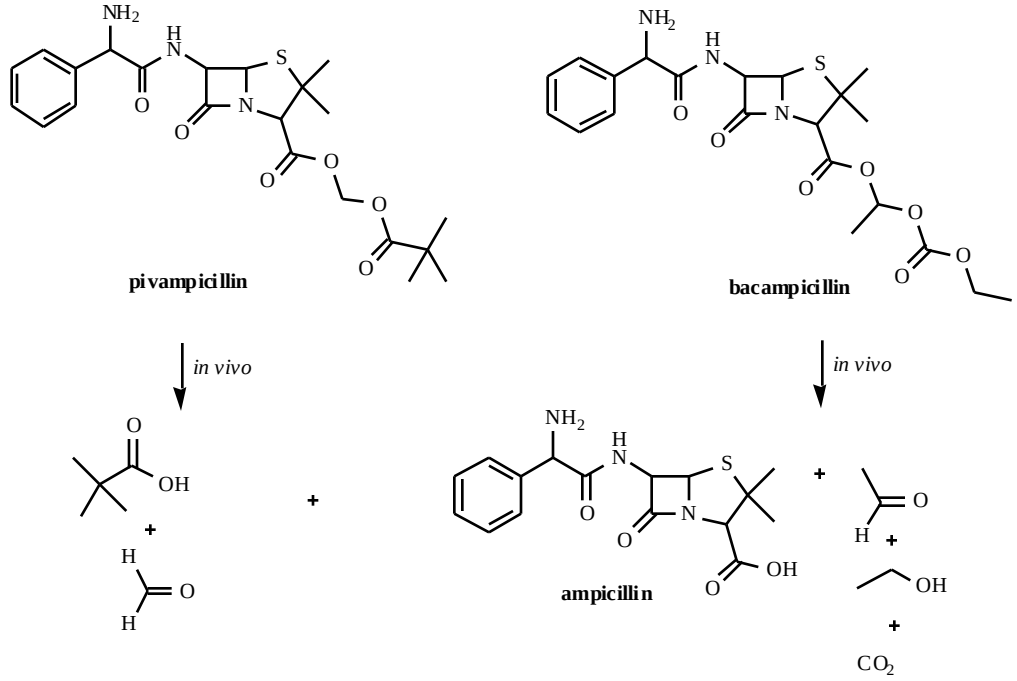

Fig. (5). Lipophilic acyloxyalkyl ester prodrugs of ampicillin.

disease. This compound was shown to be highly active in cell cultures infected with *Trypanosoma cruzi* and much less toxic than the drug, when submitted to the Ames'mutagenicity test. [19, 20].

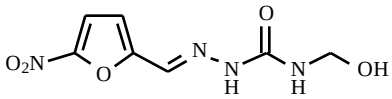

Fig. (6). Nitrofurazone hydroxymehtyl prodrug (NFOH) with higher hydrosolubility than the drug.

Mixed Prodrugs

Mixed prodrugs accumulate bioprecursors and classic prodrugs characteristics, requiring biotransformation by chemical or enzyme reactions, and increasing the concentration of the drug in a specific site of action. In some instances, the carrier needs biotransformation prior to absorption, as in the Chemical Selivey System (CDS). This **CDS**, idealized by Bodor and Abdelalim, in 1985 [21], has been used for prodrugs directed to the central nervous system (Fig. **7**). In this system, the carrier – the reduced form of methylnicotinic acid - needs biotransformation through an oxidative enzyme process prior to crossing the blood brain barrier. Once positively charged, the molecule has difficulty in crossing back across the blood brain barrier, thus the prodrug is concentrated in the brain. Although this biotransformation also occurs peripherally, the concentration into the brain leads to higher efficacy and lower toxicity of the drug [22].

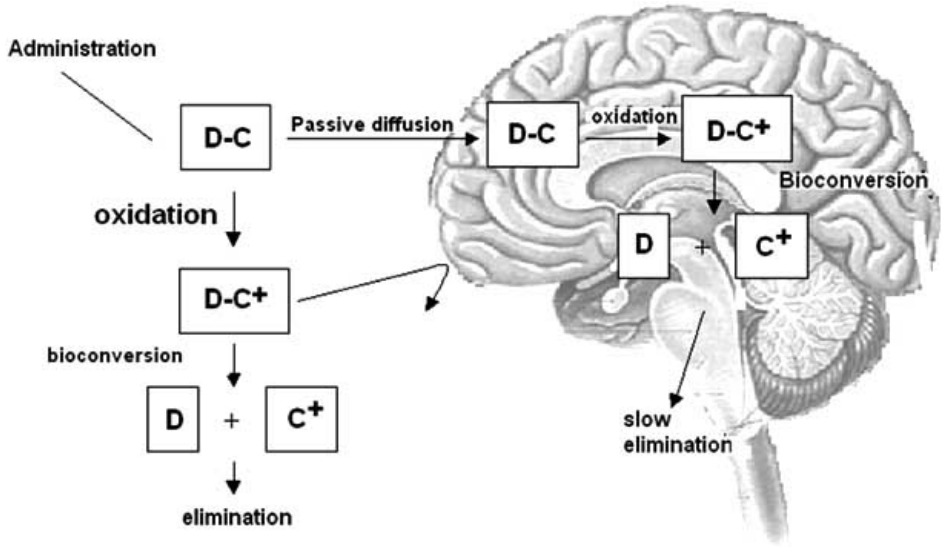

Fig. (7). CDS(*Chemical Delivery System)* in SNC. D – drug; C – carrier; C+ - charged carrier.

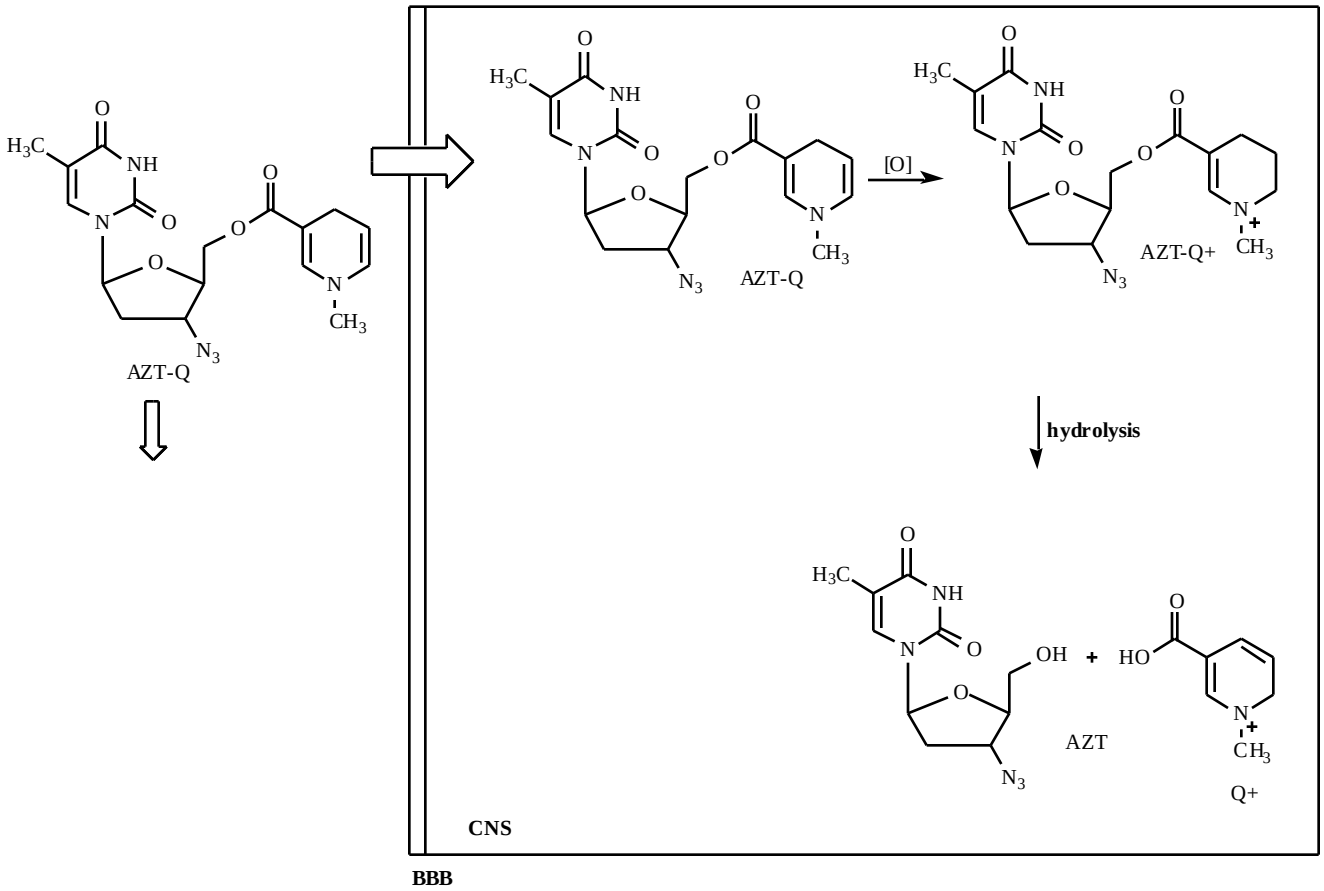

Fig. (8). AZT-CDS system. Zidovudine has been coupled with 1,4-dihydrotrigoneline carrier (AZT-Q), which enters into CNS. The carrier is oxidized to trigoneline (Q+), and in this form it can accumulate in the brain tissue. The linkage between the carrier and the drug is then hydrolyzed, releasing the drug [24].

This CDS has been used for many drugs that need CNS concentration. As an example, it has been used in the design of many antiviral agents, particularly those employed in AIDS therapeutics. Zidovudine (AZT) was included in a CDS [23, 24](Fig. **8**) with the purpose of being accumulated in CNS in neurological AIDS.

With the same objective, Somogyi and coworkers, in 2002 [25], used another carrier, an acyloxyalkyl phosphonate, called anionic CDS. The release follows the mechanism as depicted in (Fig. **9**).

Antiinflammatory drugs such as diclophenac, ibuprofen, ketoprofen, tiaprofen acid and tolmetin have been linked to trigoneline in order to concentrate in CNS [26]. The purpose was to obtain prodrugs against Alzheimer disease.

Glaucoma has also been a target for CDS. Prodrugs of *ter*-butalone [27], and testosterone derivatives [25, 28], for example, have been synthesized as potential antiglaucoma agents.

In addition to CNS and glaucoma, cardiac tissue has been the target for antiarrhythmic drugs with high affinity. Bodor and coworkers, in 2001 [29], synthesized a prodrug of tryptamine, using trigoneline as the carrier (Fig. **10**). The compound showed to be selectively bound to the heart muscles, and the concentrations achieved were different depending on the heart tissues. Its activity in the cardiovascular system allowed predicting its effectiveness and safeness as antiarrhythmic.

Mutual Prodrugs

These "latent drugs", in contrast to classic prodrugs where the carriers do not show any biological activity, are comprised of two or more drugs where one is the carrier of the other. Their advantage is the possibility of adding different or similar therapeutic activities, achieving synergistic effect or higher efficacy, respectively. Singh and Sharma, in 1994 [30], published a review with many examples of mutual prodrugs. Interestingly, this class of prodrugs was introduced prior to the concept of the prodrug itself. Sulfasalazine, for example, was used in 1942 in rheumatoid artritis and has now been used for ulcerative colitis. However, this drug, formerly considered a mutual prodrug, showed to be a classical prodrug, since the activity was only due to the anti-inflammatory effect of aminosalicyclic acid (Fig. **11**).

As a recent example, Vlieghe and colleagues [31] developed a κ-carragenan-3'-azido-3'-desoxytimidine, a polymer mutual prodrug of zidovudine, in which the carrier α-carragenine is responsible for intrinsic anti-HIV activity (Fig. **12**).

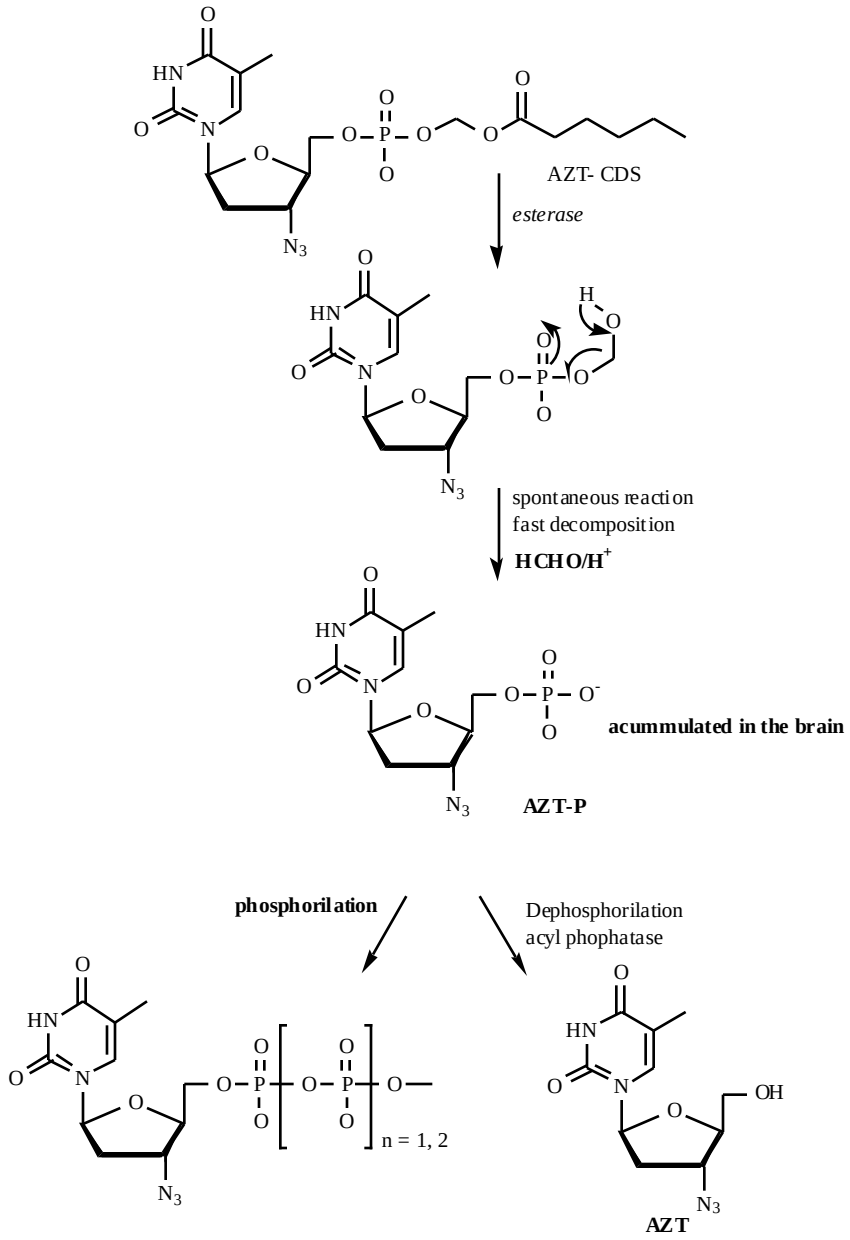

Fig. (9). Mechanism of accumulation and releasing of AZT in CNS, using anionic CDS [28].

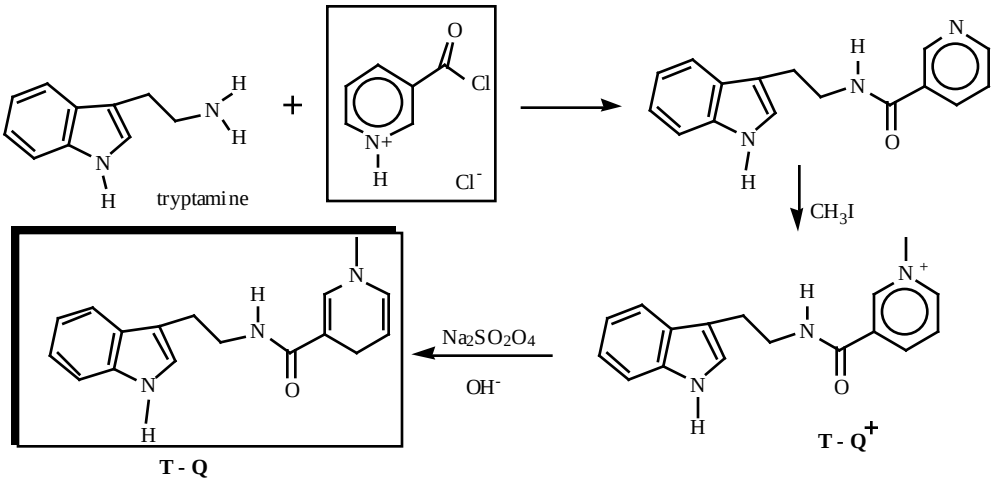

Fig. (10). Synthesis of tryptamine-CDS (T-Q) with high affinity for cardiac tissue [31].

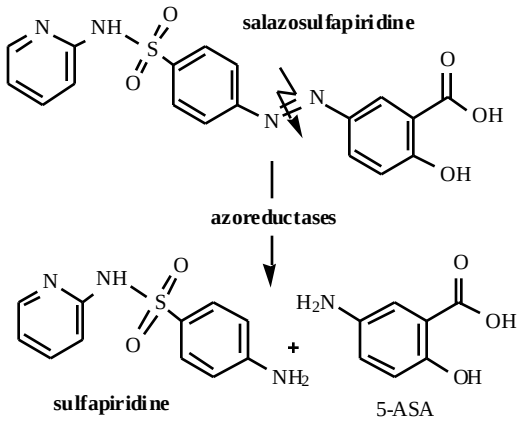

Fig. (11). Salazosulfapiridine, formerly considered as mutual prodrug of sulfapyridine and aminosalycilic acid [32].

this approach was the mechanism of the drugs: while primaquine increases the oxidative stress in the parasite, nitrofurazone (a trypanothione reductase inhibitor) does not allow the protective action of the enzyme, provoking the increase in the intracellular oxidative stress. The compound in which Lys-Arg was the spacer group has been the most active (Fig. **13**). Dipeptide prodrugs of primaquine, as synthesis intermediates, also showed to be active in cell cultures infected with *T. cruzi* [32].

Targeted Drugs

Cell selective drug delivery through the latentiation approach has received increasing interest recently [2, 32]. In targeted drugs, the carriers assume a fundamental importance due to the selectivity they must have, since they should interact with receptors or enzymes, generally located at cell

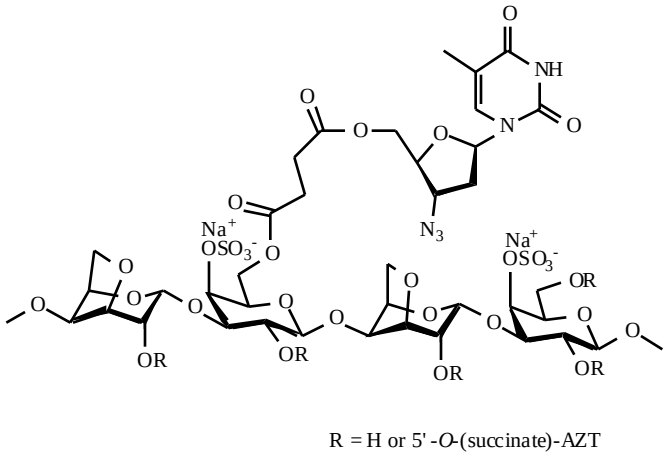

Fig. (12). Zidovudine mutual prodrug [33].

Chung, in 1996 [18], synthesized mutual prodrugs of Primaquine and nitrofurazone using dipeptides as spacer groups. These peptides are selectively cleaved by cruzipain, a cysteinyl-protease found only in *T. cruzi*. The rational for membranes, decreasing the unwanted side-effects on either organs or tissues not related to the biological action.

Targeted drugs can be constituted of polymers working either as supports for director groups or as their own specific

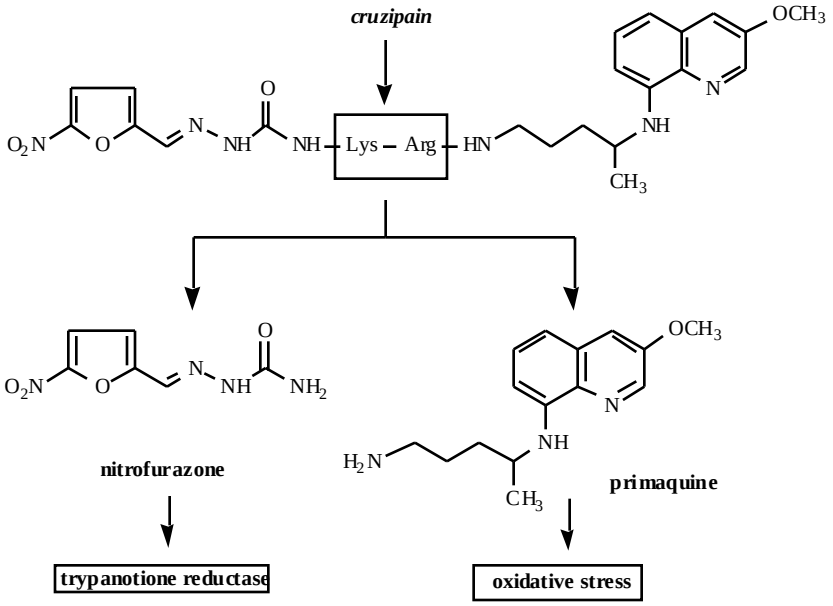

Fig. (13). Mutual prodrug of primaquine and furazone with Lys-Arg as spacer group [19].

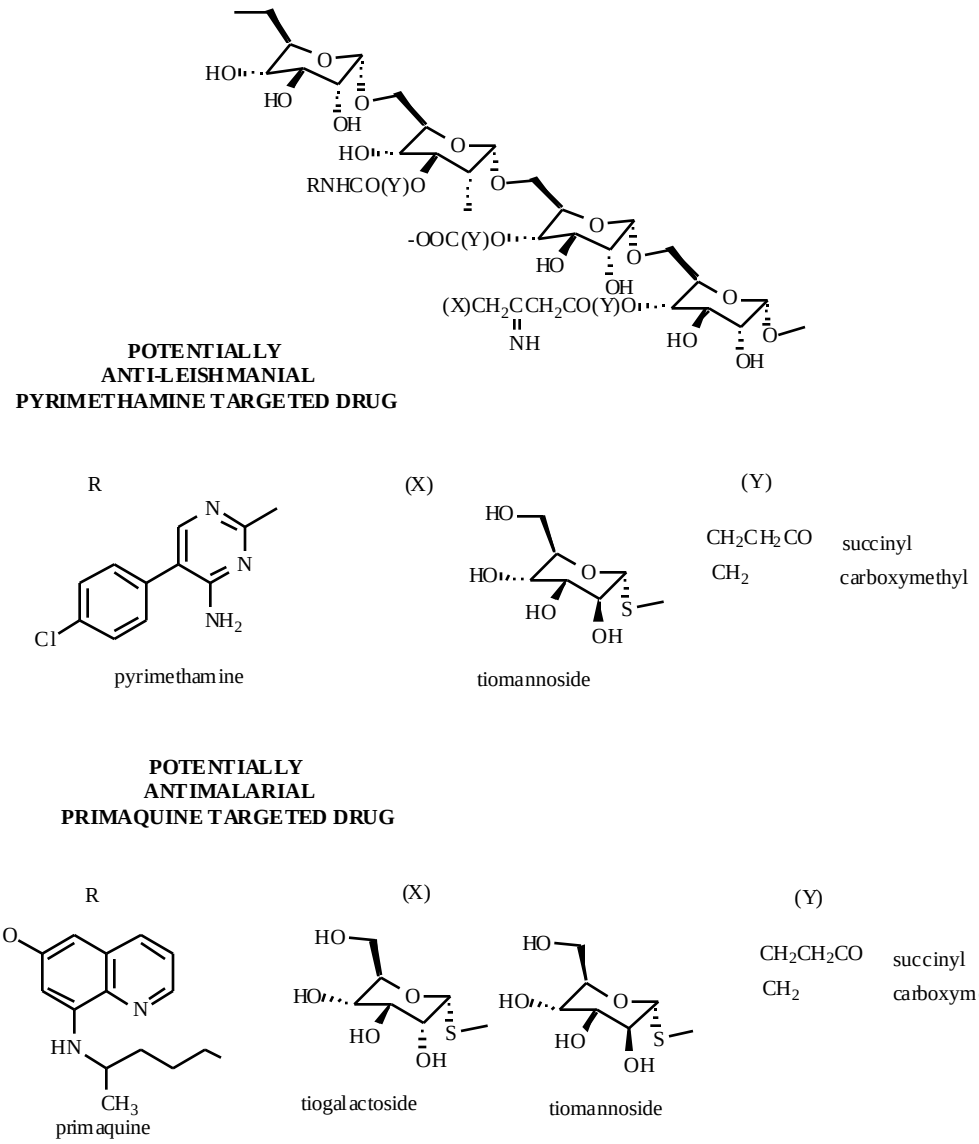

Fig. (14). Potentially anti-leishmanial drug targeted from pyrimethamine [37] and antimalarial drug targeted from primaquine.

groups. The latter are found among specific macromolecules as antibodies [1, 33]. Nishikawa and coworkers [35] have used carboxymethyl and succinyldextrans as carriers in which the director groups were linked with the main objective of obtaining selective liver delivery. Applied to mitomycin, the carriers were demonstrated to be promising for the drug targeting of antineoplastic agents.

Based on this approach and with the purpose of obtaining drugs targeted to mannoside receptors found in macrophages, Carvalho and coworkers [36] designed potential anti-leishmanial drugs (Fig. **14**). The targeted drugs, carboxymethyldextran-thiomannopyranoside- and succinyldextran-thiomannopyranoside-pyrimethamine demonstrated an inhibition of approximately 46.4% at a concentration of 200 μg/mL, when submitted to *in vitro* tests with macrophage culture infected with *Leishmania*.

Using the same approach, Scarlato, Alves, Cardoso, Andrade Junior and Ferreira[b] synthesized potentially-antimalarial targeted drugs of primaquine with selective hepatic delivery (Fig. **14**). The synthesis followed Nishikawa and coworkers [36], in which tiogalactopyranoside and succinyl/carboxymethyl dextrans are firstly and separately synthesized. The director group is linked through ethylenediamine spacer group. The compounds were dialised and liofilised and then identified by IR, ^{1}H-RMN and ^{13}C-RMN, and showed about 30% of drug substitution in the carboxyl groups of the carriers. Succinyldextran-galactopyranoside-primaquine showed to be promising in tests with mice infected with *P. berghei*, increasing the efficacy and decreasing the toxicity of the drug. This potential targeted drug has significantly decreased the parasitemia in the ninth/eleventh day of parasite inoculation and drug administration to mice. While primaquine lead to 25% of mice mortality, its targeted drug did not cause any death in the tested animals.

Mannan is a mannose polymer with linear and branched chains, extracted from *Saccharomyces cerevisae*. Since macrophages have receptors that specifically interact with

[b]Congress of the American Association of Pharmaceutical Scientists, 1999.

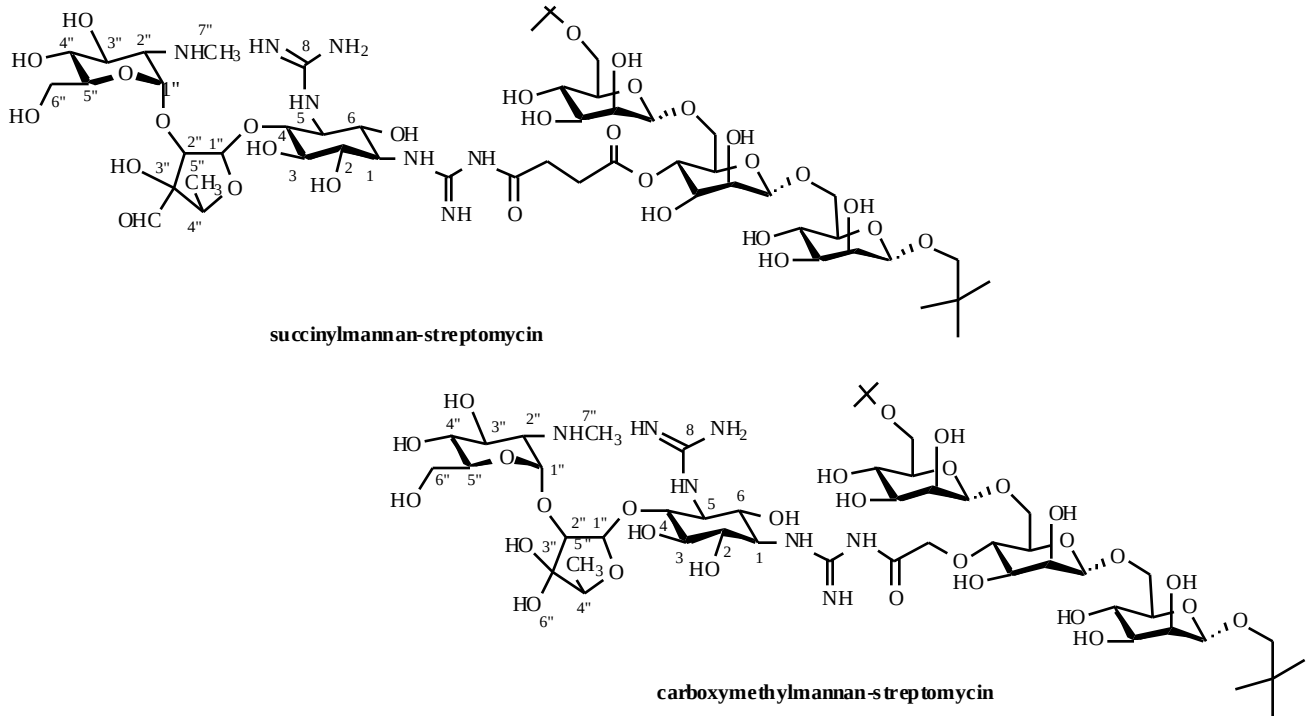

Fig. (15). Targeted drug from streptomycin using derivatized mannan as selective carrier.

mannose, Ricceli [37] synthesized a potentially tuberculostatic targeted drug using carboxymethyl and succinylmannan as specific carriers for streptomycin (Fig. **15**). The compounds are synthesized using dicyclohexylcarbodiimide previously dissolved in dymethylformamide with carboxymethyl or succinylmannan and streptomycin is added in the last step. The prodrugs were dialised and liofilised and characterized by IR, ^{1}H-RMN and ^{13}C-RMN. The calculated mean amount of streptomycin coupled to the functionalized polysaccharides was 0.6 mmol/g of the product. These derivatives showed to be promising in *in vitro* MIC and intracell tests.

Worthy of particular mention is the type of carrier built up with di or tri- modified peptides used, due to their affinity, by the intestine transporter system for di or tripeptides Pep T1 [38]. These researchers have used these carriers for the design of purine and pyrimidine analogs targeted drugs.

Erion and coworkers [39] have synthesized some hepatospecific carriers, named HelpDirect® targeted drugs, derived from phosphates and phosphonates. The resultant compounds are released in the liver, after an oxidative reaction by CYP-450 enzymes (Fig. **16**). These derivatives did not lead to toxic byproducts in the liver, when assayed in animals. They are considered promising for use in therapies against some liver diseases, such as hepatitis B, C and hepatocelullar carcinoma.

Hypoxia-selective drug delivery systems are also an interesting approach; these systems are based on bioreductive drug activation [40]. These bioreductive targeting drugs have always been used with nitrobenzyl quaternary ammonium mustards and further studies have led to other nitroheterocycles that have been employed in ADEPT and GDEPT systems that will be described elsewhere in this review, and to indolequinone, which also undergoes a reductive elimination process.

POLYMERS AS DRUG CARRIERS

The use of macromolecules in prodrug design has many objectives, among them the prolongation of action paralleled with decreasing toxicity [41, 42]. The increasing importance of these compounds in drug design allows the interface between polymer chemistry and biomedical sciences, originating the 21st century "polymer therapeutics" [43].

Many natural and synthetic biological macromolecules have been employed as carriers of some chemotherapeutic agents, such as antineoplastic drugs, for example. This use is based on the difference in anatomic and physiological characteristics between tumour and normal cells. The anatomical structure of tumoral vessels has an essential role in the drug distribution through the interstitial space, allowing: 1. the increase in microvascular permeability related to the normal vessel, thus leading to better macromolecules penetration; 2. high interstitial pressure, which can conduct to a delay in macromolecules efflux, and 3. lack of a lymphatic drainage system, accumulating macromolecules into the tumor tissues [44-48].

To be used as carriers in the latentiation approach, polymers should have the following features [49]: 1. biodegradability; 2. lack of either toxicity or intrinsic antigenicity; 3. incapacity of accumulating in the body; 4. functional groups for chemical bioreversible linkage, and 5. stability of drug linkage until the polymer prodrug reaches the site of action. Box **1** shows some examples of these carriers and (Fig. **17**) depicts the chemical structures of the polymers most used as carriers with the purpose of

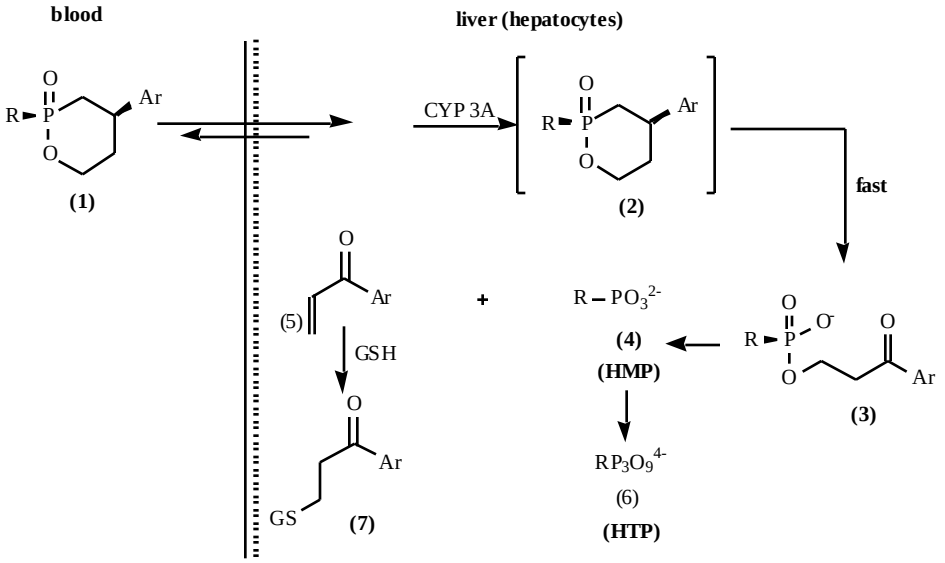

Fig. (16). HelpDirect® targeted drugs (1) and their mechanism of cleavage [42]. (1). enters into hepatic cells and is oxidized by CYP-3A, leading to C4-hydroxyl derivative (2). The ring is fastly and irreversibly opened, leading to a monoacidic intermediary (3). This compound produces the corresponding phosphate or phosphonate (4) and a vinyl aryl ketone (5), which suffers either a •-elimination or an enzymatic hydrolysis. Compound 4 is biologically converted in a triphosphate nucleoside analog (NTP, 6) by celular nucelotide kinases, when RPO3 2-, is a monophosphate nuceloside (MPN) and by PRPP synthase, when MPN analog is a PMEA (9-[2-phosphonylmethoxyethyl]adenine or adefovir).

prolonging action and decreasing toxicity, besides being used for director group support in targeted drugs.

Box 1. Classification of Macromolecules Used as Nonspecific Carriers

Natural macromolecules
· Proteins (albumin, globulin)
· Polysaccharides (dextran, chitin, chitosan, inuline)
· Nucleic acids (DNA)
Synthetic macromolecules
· Polyamine acids (polylysine, polyaspartic acid, polyglutamic acid)
Mixed macromolecules
· Styrene anhydride of maleic acid co-polymer (SMA)
· Divynil maleic ether anhydride co-polymer (DIVEMA)
· *N*-(2-hydroxypropyl)metacrylamide co-polymer (HPMA)
· Polyethyleneglycol (PEG)
· Polyvinyl alcohol (PVA)

Source: Adapted from [36].

Another type of polymer carrier that has received interest in prodrug design is the one obtained by Yokoyama and collaborators [50, 51]. These researchers synthesized a micell-forming polymer to be used as a doxorrubicine carrier. The antineoplastic was linked through its amino group to the free carboxyl group of the aspartic acid of the poly(ethyleneglycol)-(polyaspartic acid), which resulted in an amphiphilic drug conjugate.

The micell obtained by Yokoyama and collaborators [50, 51] presented a hydrophobic core with an external hydrophylic layer. The doxorubicin micell conjugate showed to be more potent than the free drug, when tested either in leukemia or solid tumors in mice. Notwithstanding, it is believed that the cytotoxic activity can be attributed to the micell, without the need for drug release, considering the high stability of the drug linkage to the polymer [50]. These authors have advanced the hypothesis that targeted drugs may be obtained for specific cells by means of adjusting the micell size.

Silva and coworkers, in 2001 [52], synthesized the micelle-forming polymer prodrug of isoniazid (Fig. **18**), using the same approach as Yokoyama and collaborators [51, 52]. The prodrug showed to be active *in vitro* in *M. tuberculosis* culture. Other similar derivatives with rifampin/isoniazid combination and pyrazinamide showed to be promising [53].

Peptides and aminoacids have been used as drug carriers with the aim of either decreasing the toxicity or improving the bioavailability through the increase in hydrosolubility [54-56]. The employment of peptides as drug carriers was first used by Carl and coworkers [57], who researched antineoplastics agents with lower toxicity. With the same purpose, Chakravarty and colleagues, in 1983 [58], synthesized peptide prodrugs of doxorubicine, rationally designed based on plasmin selectivity. These prodrugs could be locally activated in tumors with high levels of plasmin produced from associated plasminogen activators. The prodrugs showed to be more selective than the parent drug in *in vitro* tests. However, these derivatives proved to be ineffective in *in vivo* tests, probably because of the poor release of the drug from their latent forms.

More recently, Trouet and colleagues [59], using the same peptide carrier as Chakravarty and collaborators [58] synthesized several aminoacid and peptide prodrugs of

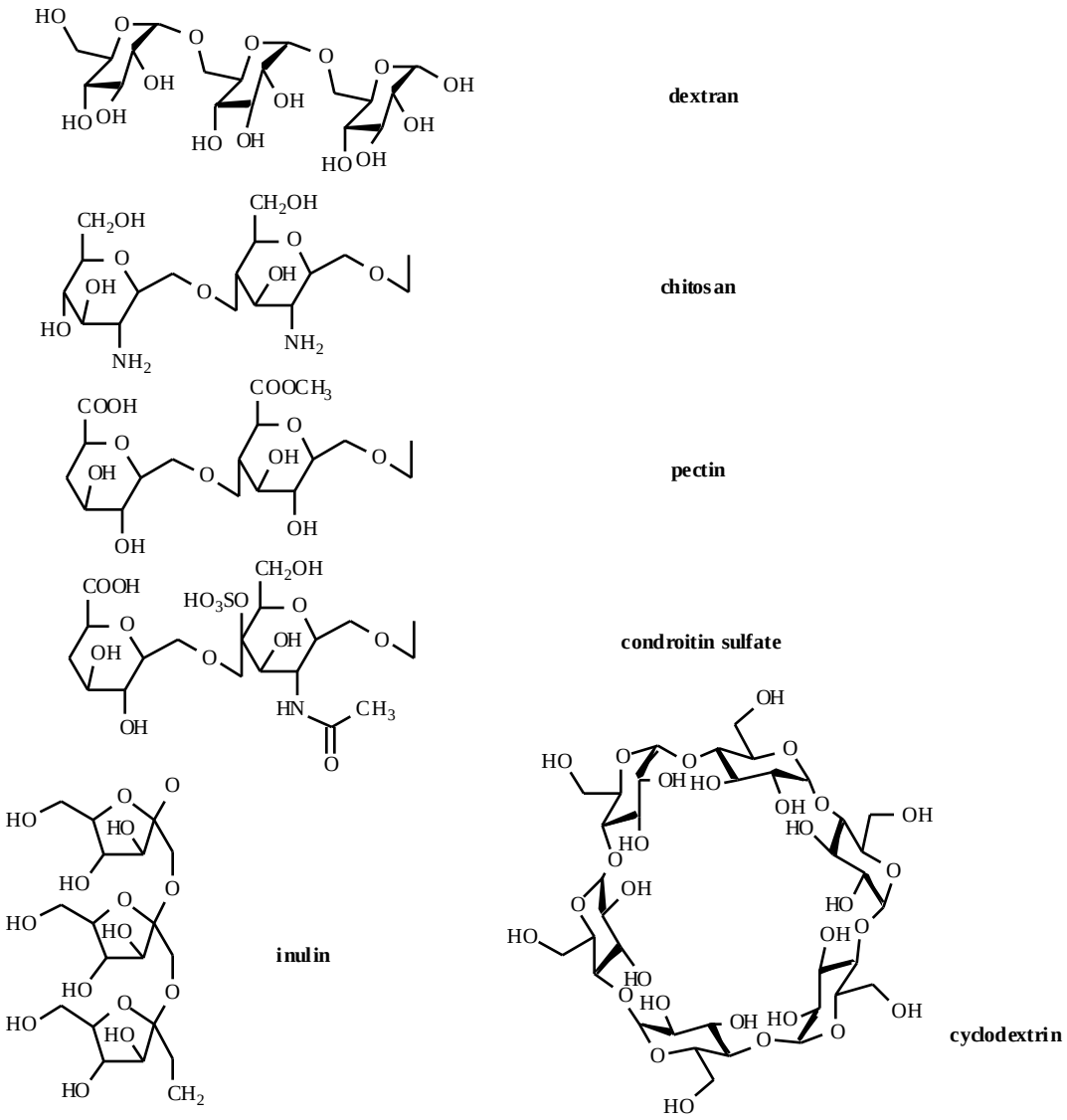

Fig. (17). Some polysaccharides used as drug carriers.

primaquine with potential antimalarial activity. The compounds showed to be less toxic than the parent drug.

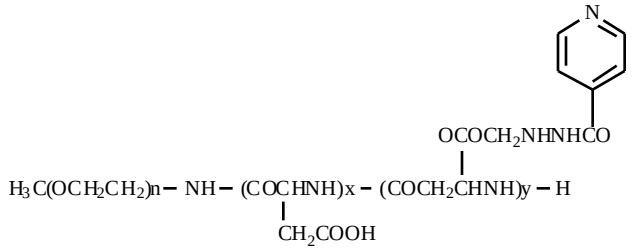

Fig. (18). Proposed structure of micelle-forming polymer prodrug of isoniazid [54].

As previously mentioned, polymers can be used as support for director groups besides being drug carriers. They can highly decrease the toxicity due to selectivity achieved. As an example, the drug targeted PK-2, HPMA-doxorubicin-galactosamine (Fig. **19**), was designed to be selectively delivered to the liver, since it contains the director group, galactosamine, which interacts with specific receptors in hepatic cells. This targeted drug, originally produced for use in hepatocellular carcinoma therapeutics as well as in secondary hepatic diseases, was in Clinical Phase II [60].

Another derivative, the antitumoral prodrug PK1 (HPMA-Gly-Phe-Ley-Gly-doxorubicin) has been shown to be neither toxic nor immunogenic [62]. In addition, this prodrug has been found to be more concentrated in tumours than doxorubicin in *in vivo* tests. This behavior could be explained based on the different anatomical and physiological features of tumoral tissue in comparison to normal cells, as has been already mentioned [41].

The prodrug FK 506-dextran (Fig. **20**), was synthesized by Yura and collaborators, in 1999 [63]. FK 506 is tacrolimus, an extremely potent (about 100 times more potent than cyclosporin) immunosuppresor agent that has been used in the USA, Europe and Japan for the prevention of liver and kidney transplant rejection. However, it requires administration by either frequent injections or slow infusions, leading to serious side-effects, mainly renal toxicity. The prodrug, FK 506-dextran, has been shown to be more advantageous than the drug itself, since it allows prolonged action and reduced unwanted effects.

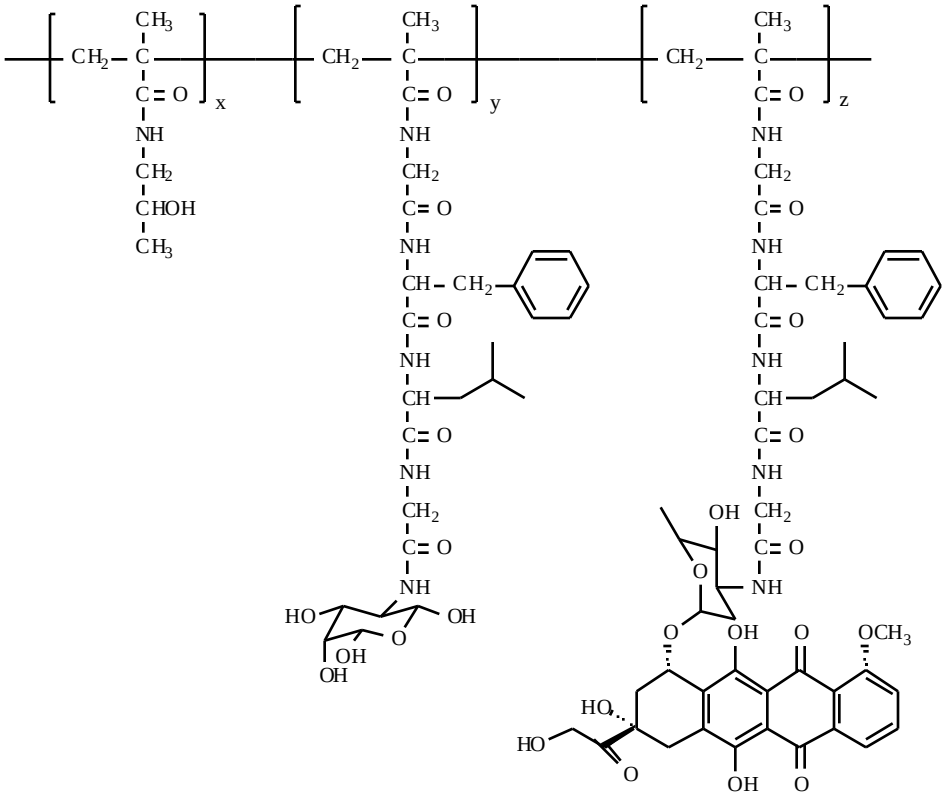

Fig. (19). Drug targeted PK-2 (HMPA-doxorubicine-galactosamine) [62, 63].

Camptotecins (CTP), such as itinotecan (CPT-11), T-2513, SN-38 and topotecan, for example, are clinically available and represent a very promising antineoplastic class. However, they present a high level of toxicity including marrow suppression, gastrointestinal effects and diarhrea. Okuno and coworkers, in 2000 [64], with the purpose of modifying the pharmacokinetics of one of those CTP, compound T-2513, synthesized its polymer prodrug, using carboxymethyldextran as the carrier and a tripeptide Gly-Gly-Gly as the spacer group (Fig. **21**). This prodrug has been responsible for a solid tumour regression, and demonstrates a higher tissue concentration compared to the drug itself.

New Strategies for the Use of Polymer Carriers

When a drug is administered intravenously, the following events can be observed:

1. distribution inside the vascular space;
2. penetration through the microvascular wall;
3. movement through the intersticial space;
4. interaction with cell surface.

Based on these effects, new strategies have been proposed toward second generation therapeutic polymers. Among them are lysosomotropic and intracytoplasmic delivery

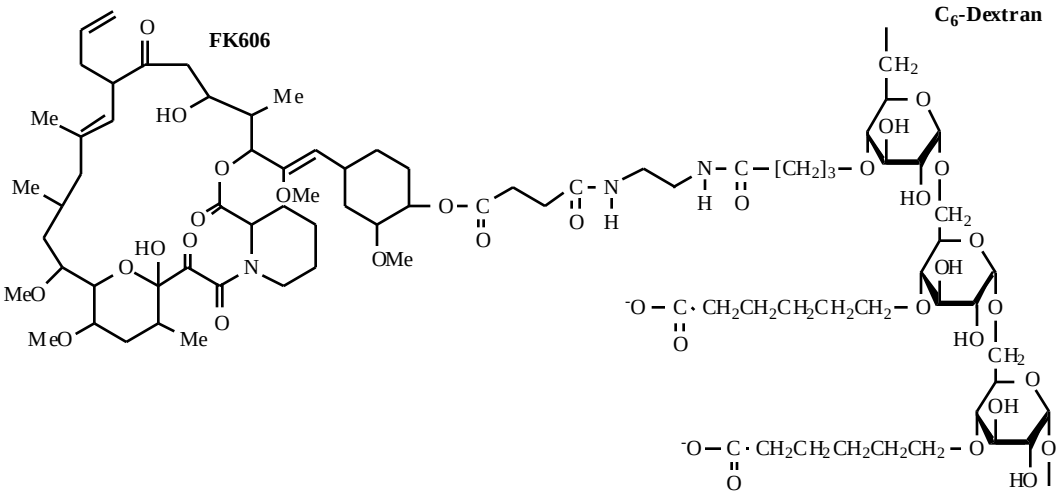

Fig. (20). Polymer prodrug tacrolimus(FK506)-dextran [64].

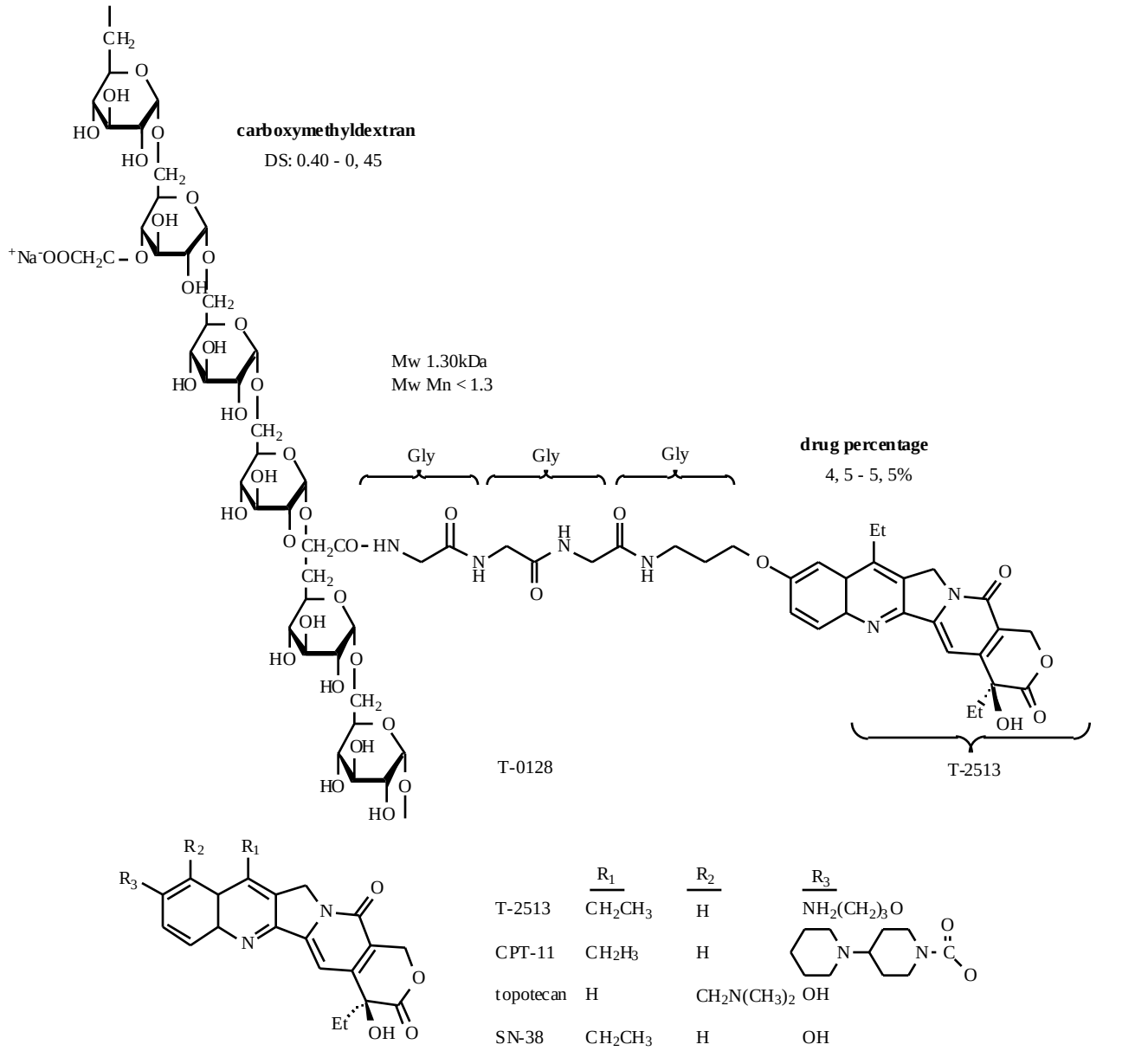

Fig. (21). Prodrugs of T-2513, CPT-11, topotecan and SN-38. DS=degree of substitution of carboxymethyl groups [65].

systems: **PDEPT** (*Polymer-Directed Enzyme Prodrug Therapy*) and **PELT** (*Polymer–Enzyme Liposome Therapy*) [60]. These systems can be schematically viewed in (Fig. **22**). Intracellular release can be by means of lysosomotropic(1) or endosomotropic(2) hydrolysis, depending on the carrier. PDEPT and PELT are classified as two-steps system(3) and the enzyme mediated hydrolysis occur extracellularly. The enzymes are attached to polymers and specifically cleave the linkage between polymer or liposome carrier. The drug released from these carriers is then absorbed by the cells. Neverthless, drugs from polymer prodrugs (4) are released before entering the cell.

A system similar to that of PDEPT was recently developed by Robinson and coworkers [65]. This system, named **LEAPT (***Lectin-Directed Enzyme-Activated Prodrug Therapy*), is based on the interaction of endogenous carbohydrate to lectin in order to place a glycosylated-enzyme conjugate in specific cells. In a second step, the prodrug, specifically activated by the enzyme in the conjugate, is released at the site of action (Fig. **23**). This system was studied in liver cells to obtain targeted drugs for this organ. Polymer prodrugs are also used in association with pharmaceutical techniques to improve their activity. Prodrugs using poly-(dl-lactic-co-glycolic) acid (PLGA) as a carrier in conjugation with microspheres of controlled delivery [66] are schematically shown in (Fig. **24**).

MODERN SELECTIVE LATENTIATION SYSTEMS

The advance in cloning methods and gene controlled expression in mammalian cells has allowed the elucidation of enzymes and membrane transporters tridimensional structure, making possible the rational design of highly selective targeted drugs [2]. These advanced latent forms comprehend the following systems: **CSDDS** – ***C****olon-****S****pecific* ***D****rug* ***D****elivery* ***S****ystem*; **ADEPT** – ***A****ntibody-*

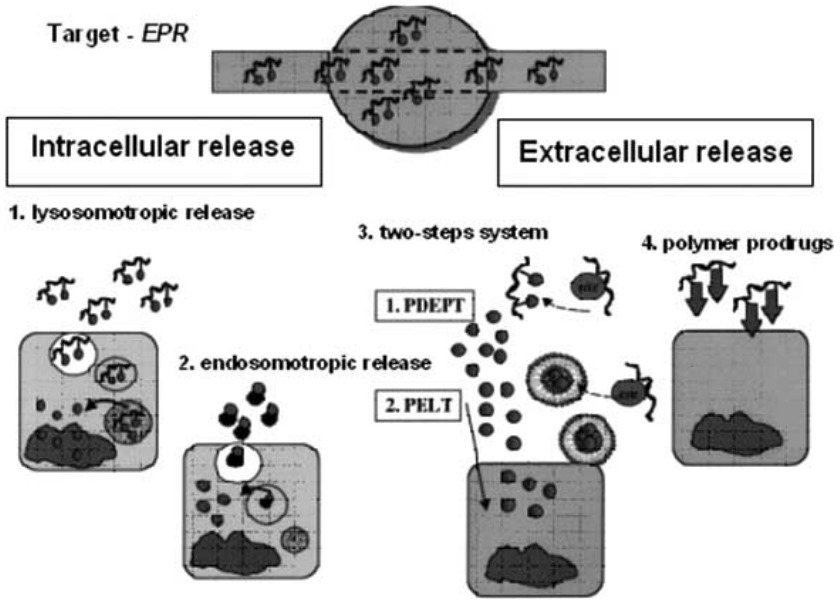

Fig. (22). PDEPT and PELT systems [61].

Target - EPR = Target - Enzyme Polymer Release.

***D**irected **E**nzyme **P**rodrug **T**herapy*; **GDEPT/VDEPT** – ***G**ene-**D**irected Enzyme Prodrug Therapy/**Virus** **D**irected **E**nzyme **P**rodrug **T**herapy*, and **ODDS** – ***O**steotropic **D**rug **D**elivery **S**ystem.*

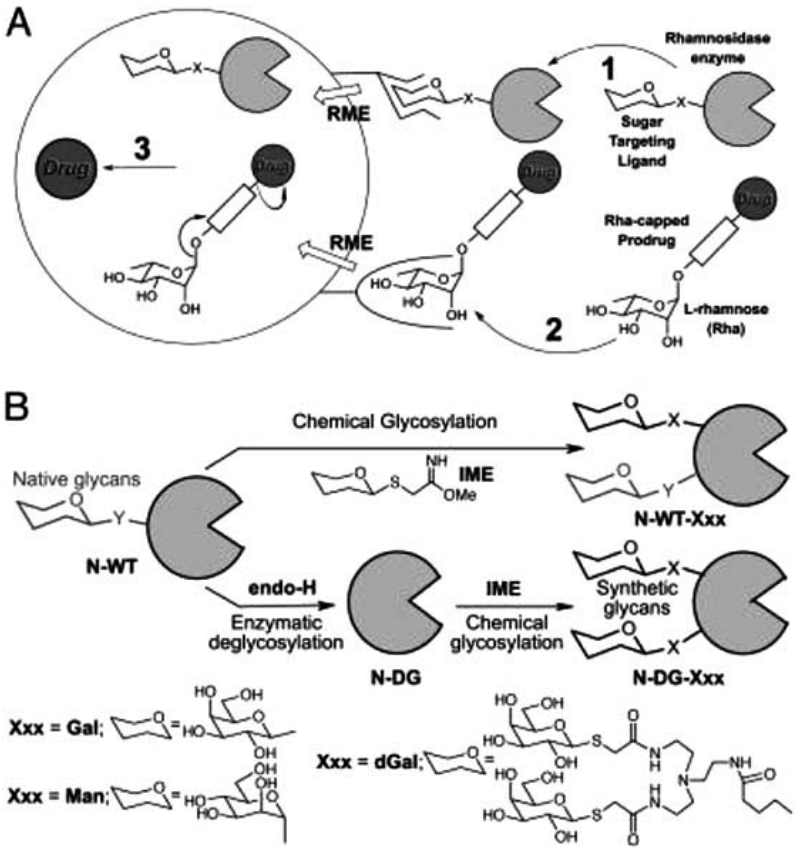

Fig. (23). The LEAPT strategy[68]. (*A)* (Step 1) Site-selective delivery of a glycosylated rhamnosidase (Rha-cleaving) enzyme by sugar-mediated RME (Receptor-mediated endocytosis). (Step 2) Delivery of a Rha-capped prodrug that can be cleaved only by the delivered glycosylated rhamnosidase. (Step 3) Activation of the prodrug which results in site-selective release of the parent drug. (*B)* Glycosylated enzyme construction. RHa- lrhamnopyranose; DG – deglycosylated; Gal – •-D-galactose; Man – D-mannose; IME – 2-imino-2-methoxyethyl 1-thioglycoside.

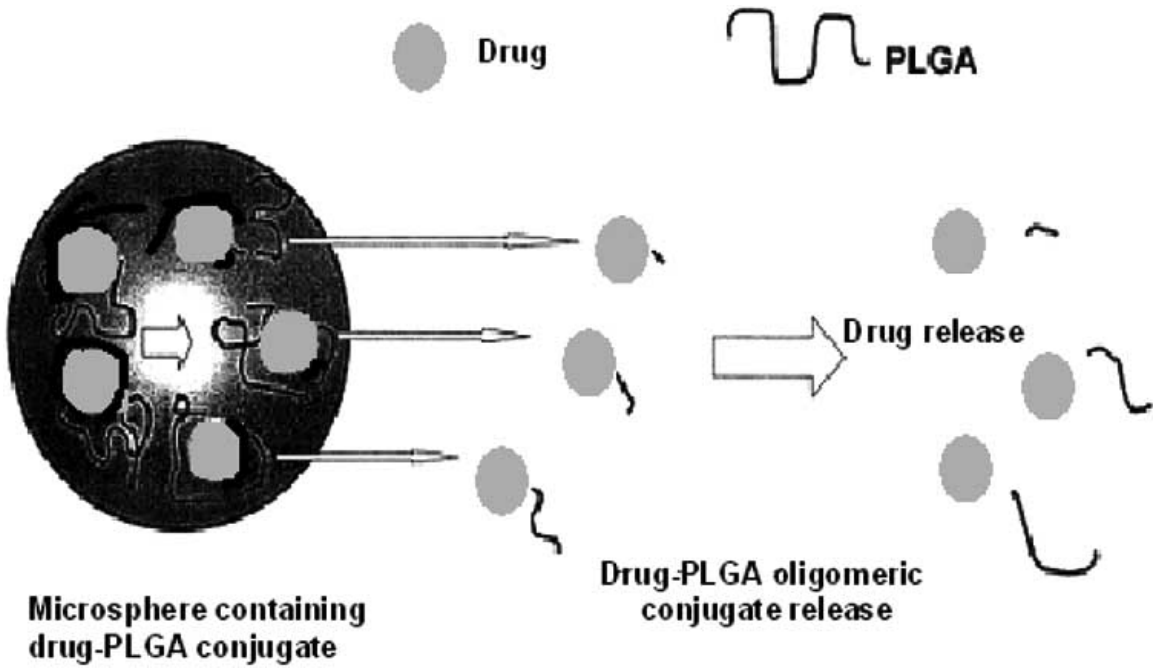

Fig. (24). Microspheres with PLGA-drug conjugates [69].

CSDDS – Colon-Specific Drug Delivery System

This system is based on the existence of enzymes from normal intestinal microbiota that can be used to promote colon drug delivery [2]. Since the normal gastrointestinal microbiota and azoreductase presence in this medium was known, the use of prodrugs with azo linkage was considered as an attractive form of targeting drugs to the intestine.

Among the derivatives synthesized with this objective [34], aminosalicylic acid prodrugs targeted to the colon through aminoacids as spacer groups, were able to slowly deliver the drug (Fig. **25**). Dextran, **PHEA** (poly[*N*-(2-hydroxyethyl)-dl-aspartamide]) and **PVP-MA** (poly(1-vinyl-2-pirrolidone-co-maleic anhydride) were used as carriers. The azo linkage between the spacer group and the carrier was selectively biotransformed by azoreductases in the colon. Another example of CSDDS with an azoic group is balsalazine, recently approved by the FDA[67] (Fig. **26**).

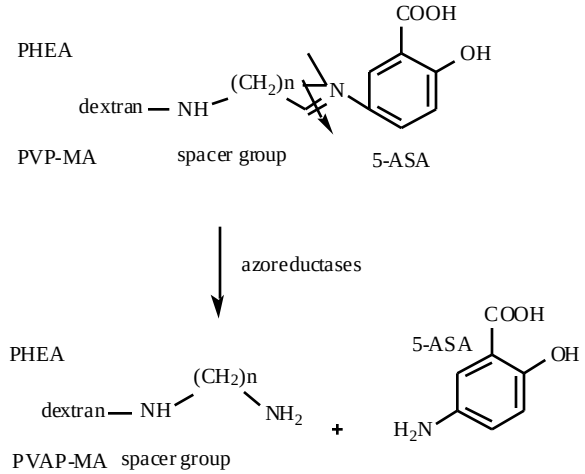

Fig. (25). Aminosalycilic acid prodrugs colon-directed [36].

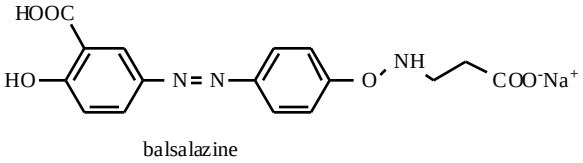

Fig. (26). Balsalazine. Available at www.fda.gov [70].

ADEPT – Antibody-Directed Enzyme Prodrug Therapy

It is well known that cancer chemotherapy is highly limited by serious side-effects of drugs, due to their lack of selectivity to neoplastic cells [68, 69]. Thus, most of the studies developed in this area are related to selective antineoplastic chemotherapeutic agents. However, this approach can also be applied to parasites, bacteria and other infectious agents.

The ADEPT approach, by definition, involves a non-existent enzyme in the body, coupled to a monoclonal antibody, in order to activate the prodrug, which is selectively cleaved by this enzyme [1] (Fig. **27**). The monoclonal antibody-enzyme conjugate is administered at first, and the antigen-antibody interaction occurs. The prodrug is then administered and the enzyme from the enzyme-antibody-antigen complex selectively cleaves the carrier-drug linkage, releasing the drug, responsible for the action against either the pathogenic organism (bacteria, helminth or protozoa) or the tumour cell [1, 2, 69-71].

Interestingly, a 100% interaction between the cell surface antigen-conjugate and enzyme-antibody is not necessary. Only about 20% of the conjugate enzyme-monoclonal antibody is needed to bind the antigen to the pathogenic cell surface to impart effectiveness to the system.

Many human tumours have been shown to be sensitive to different antibodies, enzymes and prodrugs in ADEPT systems. Recent clinical experiments have shown that this

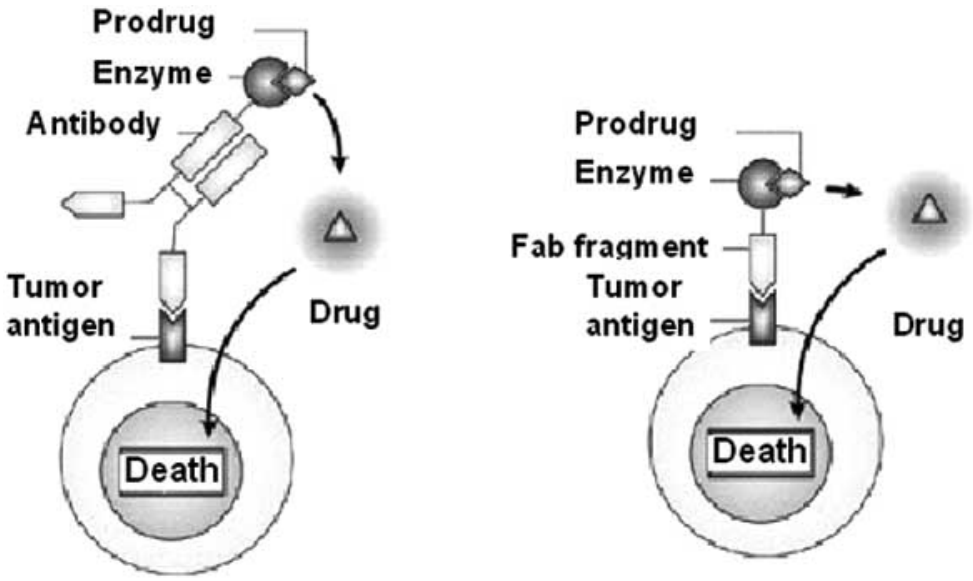

Fig. (27). ADEPT with full antibody and with Fab fragment. Available in (www.nature.com/nrc/journal/v2/n2/slideshow/nrc723_F4.html). [Acess in 27/02/2003].

approach can be an efficient tool for solid cancer treatment, since the specific cell surface antibodies are known [2]. Since one of the major problems in cancer chemotherapy is the poor tumour tissue vascularity and its physiological barrier, Fab and scFv antibody fragments can be used instead of full antibody [71] (Fig. **27**). ADEPT prodrugs must have a suitable partition coefficient to penetrate the tumour membrane barrier as well.

In addition, ADEPT prodrugs must be less cytotoxic than the drugs, and their use requires a good knowledge of structure-activity relationships [71].

In both the ADEPT and the GDEPT system, non-mammal and non-human enzymes should be used to avoid the biotransformation of prodrugs prior to reaching the enzyme-antibody-antigen complex on the cell (protozoa, helminth, and bacteria) surface. Enzymes from bacteria, which can have their immunogenicity easily controlled, are advantageous, since they impart a high selectivity for drug release from the prodrug, in the second phase of the process [71].

Enzymes for ADEPT system can be classified according to their origin [71] into:

Mammalian enzymes

- alkaline phosphatase;
- α-galactosidase (α-g).

Non mammalian-with mammalian homology enzymes:

- carboxypeptidase A;
- β-glucuronidase (β-g) from *E. coli*;
- Nitroreductase (NR) from *E. coli.*

Non mammalian-without mammal homology:

- β-lactamase (β-L);
- carboxypeptidase G2 (CPG2);
- cytosine deaminase (CD);
- benzylpenicilin amidase (PGA);
- phenoxymethylpenicilin amidase (PVA).

Many combinations between enzymes and prodrugs have been proposed for ADEPT systems, and also for GDEPT, as described later on. Box **2** shows some of these combinations. It is worth noting that the combinations useful for ADEPT can sometimes be different from those recommended for GDEPT, since in the former the activation is carried out in extracellular medium, while in the latter, activation occurs in the intracellular environment [2].

The ADEPT system has some advantages and disadvantages, as described below [71]:

Advantages

- Possibility of clinical use;
- Increase in the selectivity of malignant cells;
- Release of drug that has low molecular weight and easily penetrates in tumor cell;
- The drug reaches a higher concentration in the tumor cell when it is administered as a prodrug;
- There is no need for internalizing the complex enzyme-antibody;
- Effect of amplification, since one molecule of enzyme can act in many prodrugs.

Disadvantages

- Immunogenicity of enzyme-antibody complex. This problem can be overcome using mammalian enzymes;
- Potential to kill normal cell due to drug release for dead tumor cell. This inconvenience can be solved using low half-life drugs.

Box 2. Enzymes, Prodrugs and Respective Drugs Proposed for Cancer Therapy in ADEPT/GDEPT Approach

ENZYME	PRODRUG	DRUG
DT diaforase	5-(aziridin-1-yl)-2,4-nitrobenzamide (CB 1954)	5-(aziridin-1-yl)-4-hydroxyl-amine-2-nitrobenzamide
Plasmin	peptidyl-*p*-phenylethyleneamine mustard	phenylethyleneamine mustard
Carboxypeptidase G2	benzoic acid glutamates mustard	benzoic acid mustard (many)
Timidine quinase (viral)	gancyclovir 6-methoxypurine arabinonucleoside (araM)	gancyclovir triphosphate adenine arabinonucleoside (araATP)
Cytosine deaminase	5-fluorcytosine	5-fluoruracil
Glucose oxidase	glucose	hydrogen peroxide
Xantine oxidase	hypoxantine	superoxide, hydrogen peroxide
Carboxypeptidase A	methothrexate-alanin	methothrexate
α-Galactosidase	*N*-[4-(α-d-galactopiranosyl)benzyloxycarbonyl]-daunorubicin	daunorubicin
β-Glucosidase	amidalin	cianide
Azoreductase	azobenzene mustard	phenylethyleneamine mustard (many)
γ-Glutamyl transferase	γ-glutamyl-*p*-phenylethylenediamine mustard	phenylethyleneamine mustard
β-Glucuronidase	phenol-glucuronide mustard epirubicin-glucuronide	phenol mustard epirubicin
β-Lactamase	vinka-cephalosporin phenylethyleneamine mustard-cephalosporin nitrogen mustard-cephalosporin	4-desacetylvinylblastin-3-carboxyhydrazine phenylethyleneamine mustard nitrogen mustard (many)
Alkaline phosphatase	phenol mustard phosphate doxorubicin phosphate mitomycin phosphate etoposide phosphate	phenol mustard doxorubicin alcoholic mitomycin etoposide
Penicilin amidase	palitoxin-4-hydroxyphenyl-acetamide doxorubicin-phenoxyacetamide melphalan-fenoxyacetamide	palitoxin doxorubicin melphalan
Cytocromo P-450	cyclophosphamide iphosphamide	phosphamide mustard (+acrolein)
Nitroreductase	CB 1954 4-nitrobenzylcarbonyl derivatives	5-(aziridin-1-yl)-4-hydroxyl-amine-2-nitrobenzamide dactinomycin, mitomycin C

Source: [2].

Examples of Alkylant Agent Prodrugs Used for ADEPT

ZD2767 (Fig. **28**) is the prodrug phenol *bis*-iodide mustard, which is in the preclinical phase. This is a substrate for CPG2, coupled with an antibody, which has been shown to be able to reduce colo-rectal tumors [71].

Niculesco-Duvaz and Springer [71] report other examples (Fig. **29**), such as prodrug 5-FC (5-fluorcytosine), an antifungal agent that is converted into cytosine by CD in 5-FU (5-fluorouracil), and floxuridine prodrug, that releases the nucleoside by β-g action.

The ADEPT strategy has also been applied for other antitumor agents and many enzyme systems [73, 74]. Paclitaxel derivatives [75], antracyclines [76], and dinitrobenzamides [77] are some examples of drugs that have been submitted to this approach.

Fluordeoxyuridine 5'-dipeptidyl derivatives (FdU) (1a-d) (Fig. **30**) were synthesized by Wei and Pei, in 2001 [78], in order to be biotransformed by peptidyldeformylase (PDF), which removes the dipeptide formyl *N*-terminal group, releasing the drug (FdU). This enzyme is solely found in

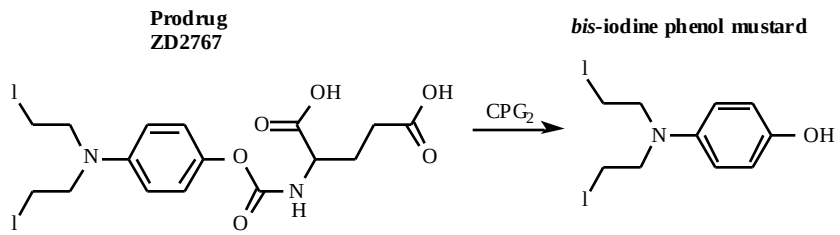

Fig. (28). ZD2767 and *bis*-iodine phenol mustard release [74].

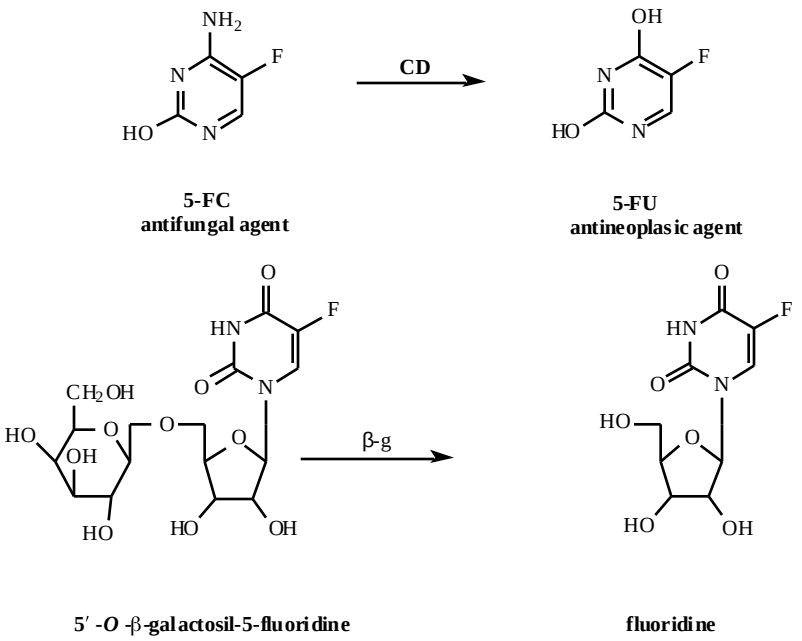

Fig. (29). Fluoruracil and floxuridine release from the respective prodrugs [72].

bacteria and is able to selectively generate potent antibacterial agents.

In 2001, Wang and coworkers [70] synthesized a prodrug constituted by a cephalosporin and a CC-1065 analog (Fig. **31**) for releasing by β-L coupled to an antibody. This release is due to the enamine resonance effect of groups in position 3 of the cephalosporan ring, which are released as living groups. The prodrug obtained demonstrated to be 10 times less toxic than the free drug in *in vitro* testing. In addition, it was effective against rat tumors.

Based on these studies, these authors have synthesized analogs of CC-1065 to be used in a β-g sensitive ADEPT system [72]. The mechanism of release is presented in (Fig. **32**).

GDEPT/VDEPT – Gene-Directed Enzyme Prodrug Therapy/Vírus-Directed Enzyme Prodrug Therapy

This process is based on a gene that expresses enzymes able to activate prodrugs. The genes can be transported by liposomes, cationic lipids or virus (retrovirus or adenovirus). These transporters reach tumor and normal cells. In genes of virus origin, the approach is named VDEPT. Gene expression can be performed by linking them in downstream sequence extremity of tumor transcriptional unities (Fig. **33**). This approach has shown to be promising in experimental tests [2, [79] and has been particularly investigated for cancer therapies, with the purpose of obtaining highly selective antineoplastic drugs [80].

Examples of GDEPT Prodrugs

Some examples are given below and can be also applied to ADEPT systems.

In 1999, Hay and coworkers [81] synthesized a 2-nitroimidazol-5-ylmethyl carbamate prodrug, which was activated by nitroreductase (NR) expressed by specific genes (Fig. **34**). This prodrug was 10 to 24 times more cytotoxic against human ovarian carcinoma (SKOV3) in GDEPT than in the normal system of prodrug administration. This activity was increased to 15 to 40 times under hypoxy.

Also using nitroreductase (NR) activation, Sagnou and coworkers, in 2000 [82], synthesized three N^{10}-(4-nitrobenzyl)carbamate prodrugs to be evaluated for its use in ADEPT and GDEPT systems. The DC-81_9a prodrug (Fig.

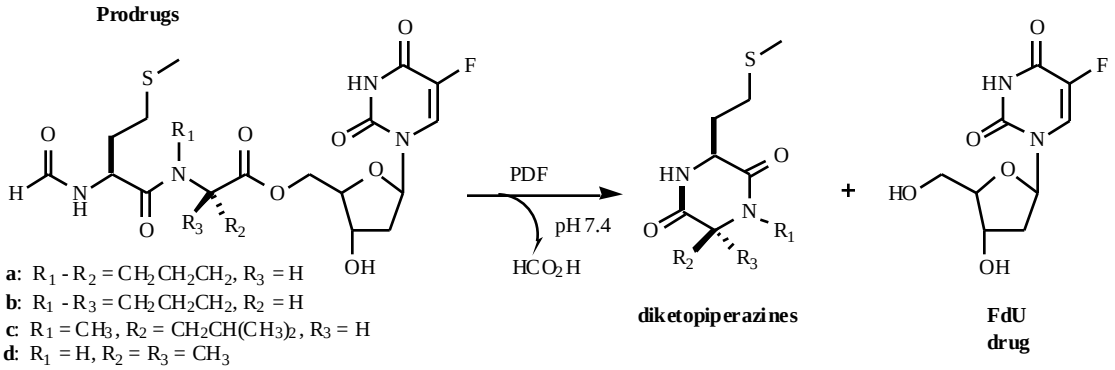

Fig. (30). Fluordeoxyuridine 5'-dipeptidyl derivatives(FdU) and their release by peptidyldeformilase (PDF) [79].

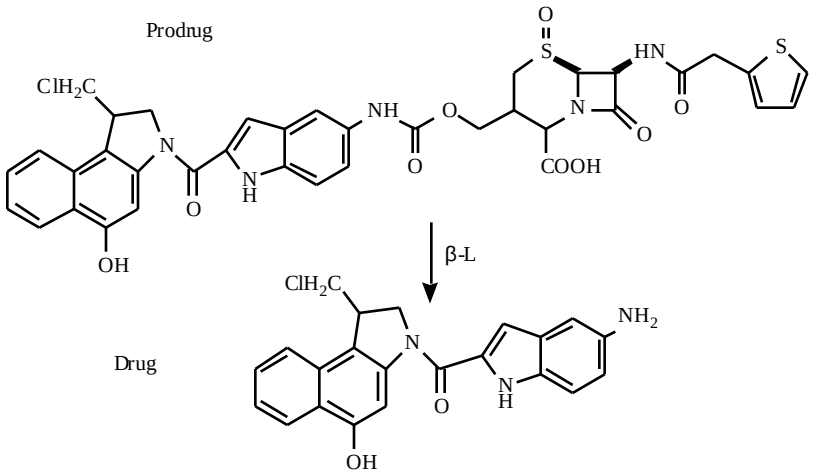

Fig. (31). Cephalosporin prodrug [71].

35) was 100 times more active against human adenocarcinoma. In addition, Helsby and coworkers [83] have developed a SAR (Structure Activity Relationship) study with aziridinylnitrobenzamides that are activated by nitroreductase in GDEPT systems.

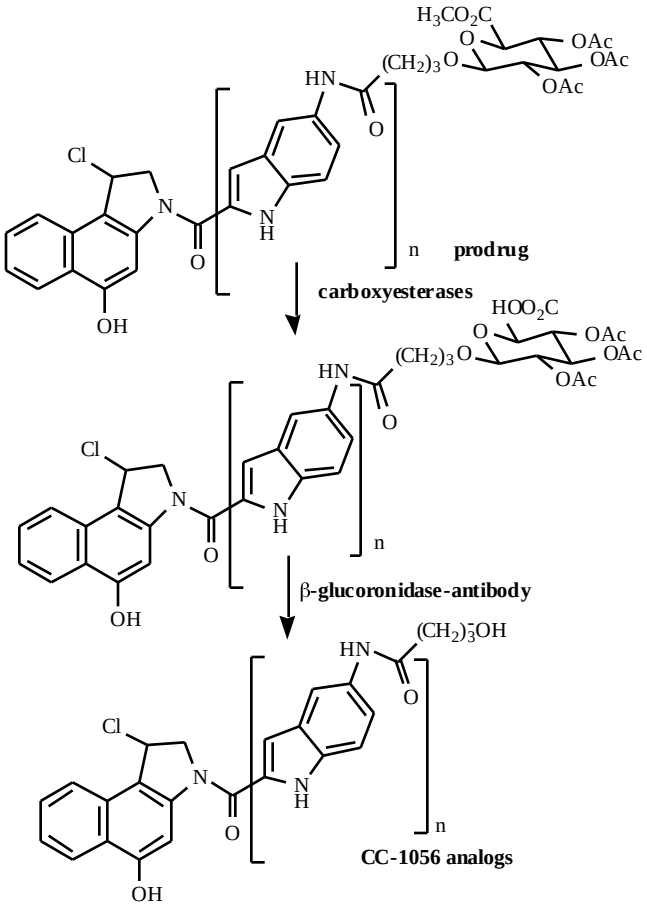

Fig. (32). Proposed mechanism for CC-1056 analogs release [73].

Prodrugs, designed using the GDEPT approach, are in pre-clinical phase as antineoplastic against prostatic tumours [84]. Although extremely promising, with respect to the selectivity that they provide, GDEPT/VDEPT systems have one important feature related to what kind of gene carrier is more suitable. In addition, the selective transport of the genes to the tumor rather than to normal cells has been a great challenge.

ODDS – Osteotropic Drug Delivery System

Despite many attempts to develop prodrugs that could be useful for bone diseases, bone tissue has continued to be a limited target. Its biological properties and the lack of a circulatory system like those of other tissues in the body are some of the reasons for this limitation. A new and promising prodrug system was recently proposed for the delivery of drugs to bone tissue. This system is comprised of biphosphonate molecules linked to specific drugs for bone diseases (Fig. **36**) and is named the osteotropic drug delivery system (**ODDS**) [85, 86].

Biphosphonates comprehend a new class of synthetic compounds, structurally related to pirophosphate, which is an endogenous calcium homeostasis modulator [85, 87]. These derivatives are clinically useful in many bone metabolic diseases, such as Paget's disease, malignum hypercalcemia, bone metastasis and osteoporosis [86]. These compounds have a high affinity for hydroxyapatite and the calcified tissues are the main targets for their accumulation after their administration. Based on biphosphonate tropism, the ODDS system makes bone structures or bone marrow drug release possible.

CONCLUSION AND PERSPECTIVES

The prodrug approach has been useful for the treatment of many diseases, either infectious or provoked by normal physiological disturbances. This approach has been an important, rational and possible alternative to introduce better drugs in therapy, due to the rapid advance in the biotechnological field and in organic compound identification.

Despite the use of, and also due to, generally simple synthetic routes, the modern systems that have been used for prodrug design deserve more and more interest, mainly because they allow us to obtain highly selective compounds that are potentially useful for therapeutic purposes. The studies regarding advanced systems of prodrug design have

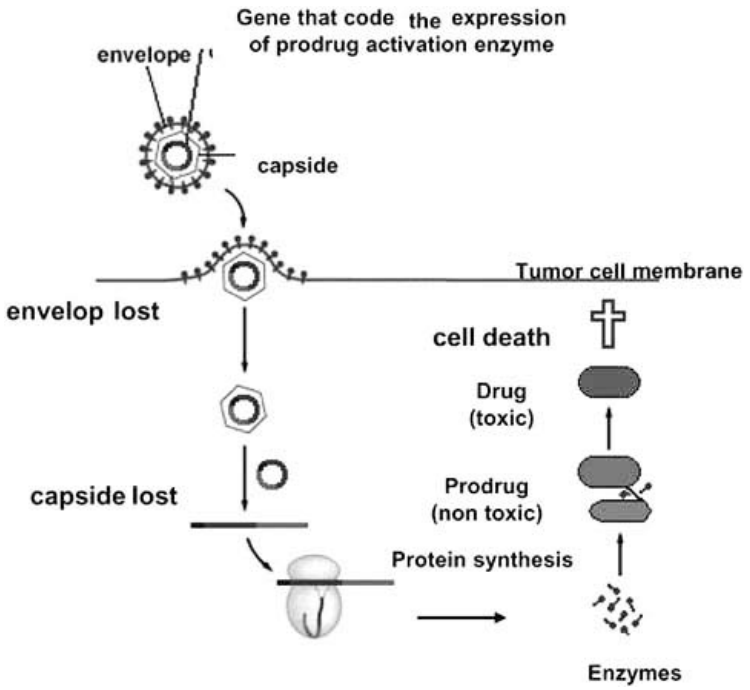

Fig. (33). VDEPT system.

concentrated on the cancer field, due to the urgent need for selective antineoplastic drugs. Nevertheless, it is of utmost importance to extend these modern approaches to infectious diseases, such as tropical endemics and tuberculosis, for instance. These diseases affect mainly poor people in undeveloped countries and new and better drugs need to be found. We have been working on prodrug design with some antimalarial, antileishmanial, anti-Chagas' disease and tuberculostatic agents with the objective of improving their activity and obtaining derived selective systems.

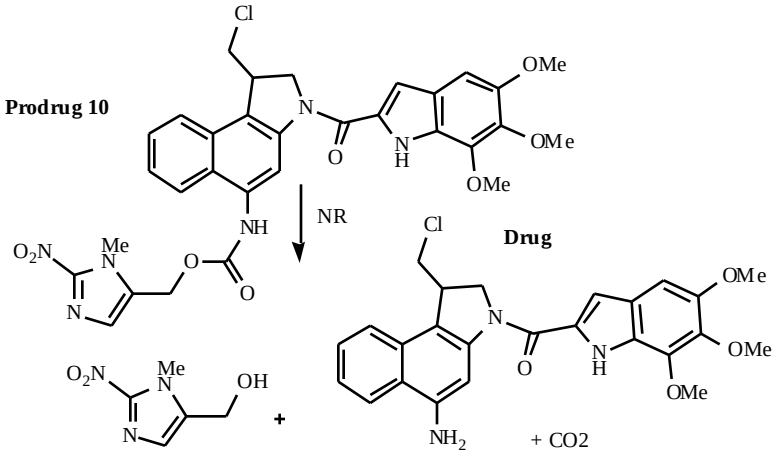

Fig. (34). Nitroreductase(NR) activation of the prodrug [82].

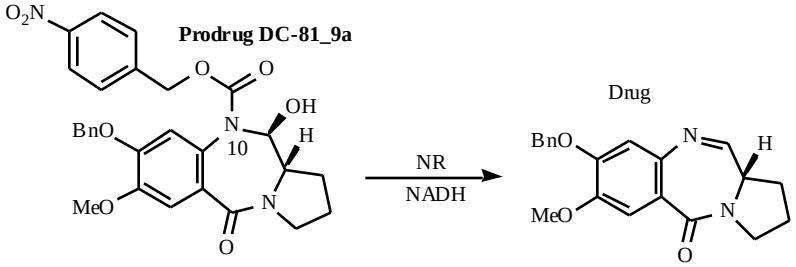

Fig. (35). DC-81_9a prodrug activation by nitroreductase (NR) [83].

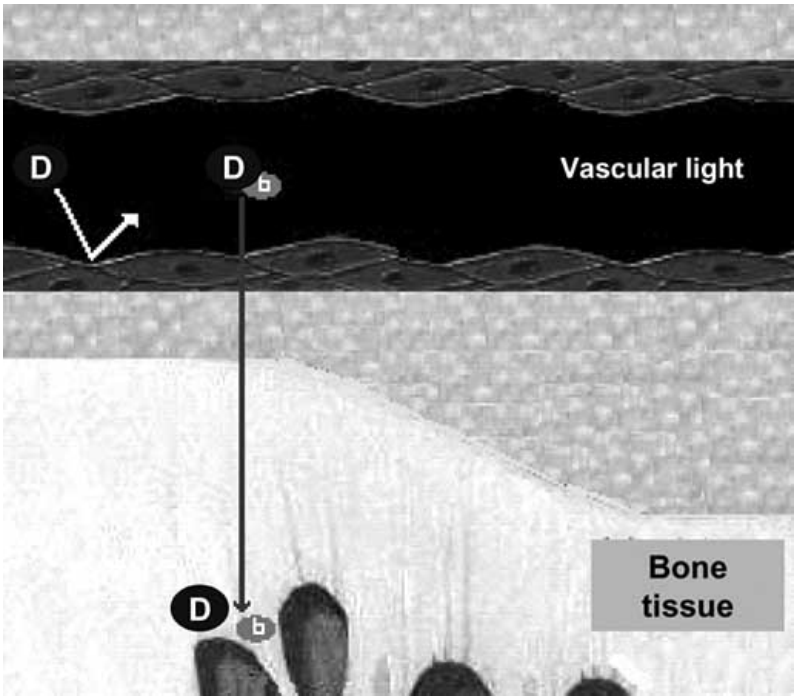

Fig. (36). ODDS representation. D–drug; b-biphosphonate carrier [87].

Non-steroid antiinflammatory drugs (NSAID) were tested in ODDS against induced arthritis in rats [86]. Diclofenac showed to be highly potent and less toxic when used in ODDS system (Fig. **37**).

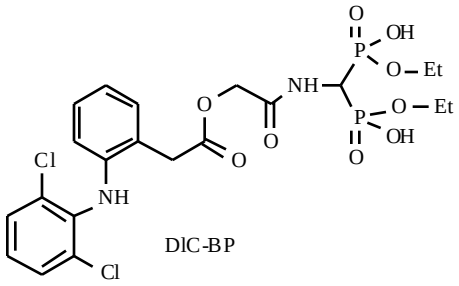

Fig. (37). Diclofenac ODDS [87].

Prodrug design advances have been briefly presented herein and the involvement of Immunology, Enzymology, Hystology, Molecular biology professionals is essential. In addition, Pharmacology and Organic Chemistry expertise, among others from science areas currently comprised by Medicinal Chemistry are needed to make possible the clinical use of so promising selective systems as ADEPT, GDEPT/VDEPT, ODDS and other that are coming up in the near future.

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REFERENCES

[1] Ettmayer, P.; Amidon, G. L.; Clement, B.; Testa, B. *J. Med. Chem*., **2004**, *47*, 2393.
[2] Han, H. K.; Amidon, G. L. *AAPS Pharm. Sci*., **2000**, *2*, E6.
[3] Zheng, A.; Wang, W.; Zhang, H.; Wang, B. *Tetrahedron*, **1999**, *55*, 4237.
[4] Bundgaard, H., Ed. *Prodrug design*, Elsevier: Amsterdam, **1985**.
[5] Friis, G.J.; Bundgaard, H. In *A textbook of drug design and development*; Krogsgaard-Larsen, P.; Liljefors, T.; Madsen, U., Eds.; Harwood Academic: Amsterdam, **1996**; pp. 351-385.
[6] Stella, V. J.; Charman, W. N. A. *Drugs*, **1985**, *29*, 455.
[7] Chung, M.-C.; Ferreira, E. I. *Quim. Nova*, **1999**, *22*, 75.
[8] Wermuth, C. G. In *The practice of Medicinal Chemistry*; Wermuth, C. G., Ed.; Academic Press: London, **2003**; pp. 697-716.
[9] Testa, B.; Caldwell, J. *Med. Res. Rev*., **1996**, *16*, 233.
[10] Testa, B. *Biochem. Pharmacol*., **2004**, *68*, 2097.
[11] Bundgaard, H.; Krosgaard-Larsen, P., Eds. *A Textbook of Drug Design and Development*, Harwood Academic Publishers: Academic, **1991**.
[12] Choi, J. S.; Jo, B. W. *Int. J. Pharm*., **2004**, *6*, 221.
[13] Majumdar, S.; Duvvuri, S.; Mitra, A. K. *Adv. Drug Delivery Rev*., **2004**, *56*, 1437.
[14] Skoblov, Y.; Karpenko, I.; Shirokova, E.; Popov, K.; Andronova, V.; Galegov G.; Kukhanova, M. *Antiviral Res*. **2004**, *63*, 107.
[15] Yevich, J. P. In *A textbook of drug design and development*; Krogsgaard-Larsen, P.; Liljefors, T.; Madsen, U., Eds.; Harwood: Amsterdam, **1996**; pp. 508.
[16] Wermuth, C.G. In *Drug design: Fact or fantasy*? Jolles, G.; Wooldridge, K.R.H., Eds.; Academic: London, **1984**; pp. 47-72.
[17] Brown, S.B.; Brown, E. A.; Walker, I. *Lancet Oncol*., **2004**, *5*, 497.
[18] Chung, M.-C. Planejamento e síntese de pró-fármacos recíprocos de nitrofural e primaquina potencialmente antichagásicos. Faculdade de Ciências Farmacêuticas, USP, **1996** [PhD Thesis].
[19] Chung, M. C.; Güido, R. .V.; Martinelli, T. F.; Goncalves, M. F.; Polli, M. C.; Botelho, K. C. A.; Varanda, E. A.; Colli, W.; Miranda, M. T.; Ferreira, E. I. *Bioorg. Med. Chem.*, **2003**, *3*, 4779.
[20] Güido, R. V. C.; Ferreira, E. I.; Nassute, J. C.; Varanda, E. A.; Chung, M. C. *Rev. Ciên. Farm*., **2001**, *22*, 319.
[21] Bodor, N.; Abdelalim, A. M. *J. Pharm. Sci*., **1985**, *74*, 241.
[22] Prokai, L.; Prokai-Tatrai, K.; Bodor N. *Med. Res. Rev*., **2000**, *20*, 367.
[23] Little, R.; Bailey, D.; Brewster, M.; Estes, K.; Clemmons, R.; Saab, A.; Bodor, N. *J. Biopharm. Sci.*, **1990**, *1*, 1.
[24] Brewster, M. E.; Anderson, W.R.; Webb, A.I.; Pablo, L.M.; Meinsma, D.; Moreno, D.; Derendorf, H.; Bodor, N.; Pop, E. *Antimicrob. Agents Chemother*., **1997**, *41*, 122.

[25] Somogyi, G.; Buchwald, P.; Bodor, N. *Pharmazie*, **2002**, *57*, 135.
[26] Perioli, L.; Ambrogi, V.; Bernardini, C.; Grandolini, G.; Ricci, M.; Giovagnoli, S.; Rossi, C. *Eur. J. Med. Chem*., **2004**, *39*, 715.
[27] Reddy, I. K.; Vaithiyalingam, S. R.; Khan, M. A.; Bodor, N. S. *J. Pharm. Sci*., **2001**, *90*, 1026.
[28] Somogyi, G.; Buchwald, P.; Bodor, N. *Pharmazie*, **2004**, *59*, 378.
[29] Bodor, N.; Farag, H. H.; Polgar, P. *J. Pharm. Pharmacol*., **2001**, *53*, 889.
[30] Singh, G.; Sharma, P.D. *Indian J. Pharm. Sci*., **1994**, *56*, 69.
[31] Vlieghe, P.; Clerc, T.; Pannecouque, C.; Witvrouw, M.; De Clercq, E.; Salles, J. P.; Kraus, J. L. *J. Med. Chem*., **2002**, *45*, 1275.
[32] Chung, M.-C.; Gonçalves, M. F.; Colli, W.; Ferreira, E. I.; Miranda, M. T. *J. Pharm. Sci*., **1997**, *86*, 1127.
[33] Hirabayashi, H.; Takahashi, T.; Fujisaki, J.; Masunaga, T.; Sato, S.; Hiroi, J.; Tokunaga, Y.; Kimura, S.; Hata, T. *J. Controlled Release*, **2001**, *70*, 183.
[34] Schacht, E.; Gevaert, A.; Kenawy, E. R.; Molly, K.; Verstraete, W.; Adriaensens, P.; Carleer, R.; Gelan, J. *J. Controled Release*, **2000**, *39*, 327.
[35] Nishikawa, M.; Kamijo, A.; Fujita, T.; Takakura, Y.; Sezaki, H.; Hashida, M. *Pharm. Res*., **1993**, *10*, 1253.
[36] Carvalho, P.B.; Ramos, D. C.; Cotrim, P.C.; Ferreira, E.I. *J. Pharm. Sci*., **2003**, *92*, 2109.
[37] Ricceli, N. L. *Tuberculostáticos potenciais: síntese e ensaios preliminares de fármacos dirigidos de estreptomicina e amicacina.* Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, **2003** [MSc Dissertation].
[38] Steffansen, B.; Nielsen, C. U.; Brodin, B.; Eriksson, A. H.; Andersen, R.; Frokjaer, S. *Eur. J. Pharm. Sci*., **2004**, *21*, 3.
[39] Erion, M. D.; Van Poelje, P. D.; Mackenna, D. A.; Colby, T. J.; Montag, A.; Fujitaki, J. M.; Linemeyer, D. L.; Bullough, D. A. *J. Pharmacol. Exp. Ther*, **2005**, *312*, 554.
[40] Naylor, M. A.; Thomson, P. *Mini Rev. Med. Chem*., **2004** [www.bentham.org/mrmc1].
[41] Takakura, Y.; Hashida, M. *Crit. Rev. Oncol. Hematol*., **1995**, *18*, 207.
[42] Hoste, K.; De Winne, K.; Schacht, E. *Int. J. Pharm*., **2004**, *277*, 119.
[43] Duncan, R. *Nat. Rev. Drug Discov*., **2003**, *2*, 347.
[44] Takakura, Y.; Takagi, A.; Hashida, M.; Sezaki, H. *Pharm. Res*., **1987**, *4*, 293.
[45] Takakura, Y.; Fujita, T.; Hashida, M. E.; Sezaki, H. *Pharm. Res*., **1990**, *7*, 339.
[46] Jain, R. K. *Cancer Metastasis Rev*., **1987**, *6*, 559.
[47] Matsumura, Y. E.; Maeda, H. *Cancer Res*., **1986**, *46*, 6387.
[48] O'Connor, S. W. E.; Bale, W. F. *Cancer Res*., **1984**, *44*, 3719.
[49] Sezaki, H.; Hashida, M. *Crit. Rev. Ther Drug Carrier Syst*., **1984**, *1*, 1.
[50] Yokoyama, M.; Miyauchi, M.; Yamada, N.; Okano, T.; Sakurai, Y.; Kataoka, K.; Inoue, S. *Cancer Res.*, **1990**, *50*, 1693.
[51] Yokoyama, M.; Okano, T.; Sakurai, Y.; Ekimoto, H.; Shibazaki, C.; Kataoka, K. *Cancer Res.*, **1991**, *51*, 3229.
[52] Silva, M.; Lara, A. S.; Leite, C. Q. F.; Ferreira, E. I. *Arch. Pharm*.**, 2001,** *334*, 189.
[53] Silva, M. Tuberculostáticos potenciais: pró-fármacos formadores de micelas derivados de isoniazida, rifampicina e pirazinamida.Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, SP, **2001** [PhD Thesis].
[54] Clerici, C.; Gentili, G.; Boschetti, E.; Santucci, C.; Aburbeh, A. G.; Natalini, B.; Pellicciari, R.; Morelli, A. *Dig. Dis. Sci*., **1994**, *39*, 2601.
[55] Nishida, K.; Kido, M.; Sasaki, H.; Nakamura, J. *J. Pharm. Res*., **1994**, *11*, 160.
[56] Vitols, K. S.; Haag-Zeino, B.; Baer, T.; Montegano, Y. D.; Huennekens, F. M. *Cancer Res*., **1995**, *55*, 478.
[57] Carl, P. L.; Chakravarty, P. K.; Katzenellenbogen, J. A. E.; Weber, M. J. *Proc. Natl. Acad. Sci. USA*, **1980**, *77*, 2224.
[58] Chakravarty, P. K.; Carl, P. L.; Weber, M. J.; Katzenellenbogen, J. A. *J. Med. Chem*., **1983**, *26*, 633.
[59] Trouet, A.; Jolles, G. *Semin. Oncol.*, **1984**, *11*, 64.
[60] Julyan, P. J.; Seymour, L. W.; Ferry, D. R.; Daryani, S.; Boivin, C .M.; Doran, J.; David, M.; Anderson, D.; Christodoulou, C.; Young, A. M.; Hesslewood, S.; Kerr, D.J. *J. Control Release*, **1999**, *57*, 281.
[61] Duncan, R.; Gac-Breton, S.; Keane, R.; Musila, R.; Sat, Y. N.; Satchi, R.; Searle, F. *J. Controlled Release*, **2001**, *74*, 135.
[62] Loadman, P. M.; Bibby, M. C.; Double, J. A.; Al-Shakhaa, W. M.; Duncan, R. *Clin. Cancer Res*., **1999**, *5*, 3682.
[63] Yura, H.; Yoshimura, N.; Hamashima, T.; Akamatsu, K.; Nishikawa, M.; Takakura, Y.; Hashida, M. *J. Controlled Release*, **1999**, *57*, 87.
[64] Okuno, S.; Harada, M.; Yano, T.; Yano, S.; Kiuchi, S.; Tsuda, N.; Sakamura, Y.; Imai, J.; Kawaguchi, T.; Tsujihara, K. *Cancer Res*., **2000**, *60*, 2988.
[65] Robinson, M. A.; Charlton, S. T.; Garnier, P.; Wang, X. T.; Davis, S. S.; Perkins, A. C.; Frier, M.; Duncan, R.; Savage, T. J.; Wyatt, D. A.; Watson, S. A.; Davis, B. G. *Proc. Natl. Acad. Sci*.USA, **2004**, *101*, 14527.
[66] Oh, J. E.; Nam, Y. S.; Lee, K. H.; Park, T. G. *J. Control. Release*, **1999**, *57*, 269.
[67] FDA, **2002**, [www.fda.gov].
[68] Xu, G.; Mcleod, H. L. *Clin. Cancer Res*., **2001**, *7*, 3314.
[69] Houba, P. H. J.; Leenders, R. G. G.; Boven, E.; Scheeren, J. W.; Pinedo, H. M.; Haisma, H. J. *Biochem. Pharmacol*., **1996**, *52*, 455.
[70] Wang, Y.; Yuan, H.; Wright, S. C.; Wang, H.; Larrick, J. W. *BMC Chem. Biol*., **2001**, *1*, 4.
[71] Niculescu-Duvaz, I. I.; Springer, C. J. *Adv. Drug Delivery Rev*., **1997**, *26*, 151.
[72] Wang,Y.; Yuan, H.; Wright, S. C.; Wang, H.; Larrick, J. W. *Bioorg. Med. Chem*., **2003**, *11*, 1569.
[73] Chen, X.; Wu, B.; Wang, P. G. *Curr. Med. Chem. Anti-Cancer Agents*, **2003**, *3*,139.
[74] Heinis, C.; Alessi, P.; Neri, D. *Biochemistry*, **2004**, *43*, 6293.
[75] Bouvier, E.; Thirot, S.; Schmidt, F.; Monneret, C. *Org Biomol Chem.*, **2003**, *7*, 3343.
[76] Harikrishna, D.; Rao, A. R.; Krishna, D. R. *Drug News Perspect*., **2003**, *16*, 309.
[77] Johansson, E.; Parkinson, G. N.; Denny, W. A.; Neidle, S. *J. Med. Chem*., **2003**, *46*, 4009.
[78] Wei, Y.; Pei, D. *Bioorg. Med. Chem. Lett.*, **2000**, *10*, 1073.
[79] Grove, J. I.; Searle, P. F.; Weedon, S. J.; Green, N. K.; Mcneish, I. A.; Kerr, D. J. *Anticancer Drug Des*., **1999**, *14*, 461.
[80] Kerr, D. J.; Young, L. S.; Searle, P. F.; Mcneish, I. A. *Adv. Drug Deliv. Rev*., **1997**, *26*, 173.
[81] Hay, M. P.; Sykes, B. M.; Denny, W. A.; Wilson, W. R. *Bioorg. Med.Chem*., **1999**, *9*, 2237.
[82] Sagnou, M. J.; Howard, P. W.; Gregson, S. J.; Eno-Amooquaye, E.; Burke, P. J.; Thurston, D. E. *Bioorg. Med. Chem. Lett.*, **2000**, *10*, 2083.
[83] Helsby, N. A.; Atwell, G. J.; Yang, S.; Palmer, B. D.; Anderson, R. F.; Pullen, S. M.; Ferry, D. M.; Hogg, A.; Wilson, W. R.; Denny, W. A. *J. Med. Chem*., **2004**, *47*, 3295.
[84] Wang, Y.; Martiniello-Wilks, R.; Shaw, J. M.; Ho, T.; Coulston, N.; Cooke-Yarborough, C.; Molloy, P. L.; Cameron, F.; Moghaddam, M.; Lockett, T. J.; Webster, L. K.; Smith, I. K.; Both, G. W.; Russell, P. J. *Gene Ther*., **2004**, *11*, 1559.
[85] Castro, L. F.; Silva, A.T. A.; Ferreira, A. G.; Ferreira, E. I.; Chung, M. C. *Quim. Nova*, **2004**, *27*, 456.
[86] Hirabayashi, H.; Takahashi, T.; Fujisaki, J.; Masunaga, T.; Sato, S.; Hiroi, J.; Tokunaga, Y.; Kimura, S.; Hata, T. *J. Controlled Release*, **2001**, *70*, 183.
[87] Hirabayashi, H.; Fujisaki, J. *Clin. Pharmacokinet*., **2003**, *42*, 1319.