Prodrug Approach: An Overview of Recent Cases

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Abstract: In this review we highlight the most modern trends in the prodrug strategy. In drug research and development, the prodrug concept has found a number of useful applications. Selected examples of this approach are provided in this paper and they are classified according to the aim of their design.

Keywords: Prodrug, Carrier-linked prodrug, Bioprecursors

Contents

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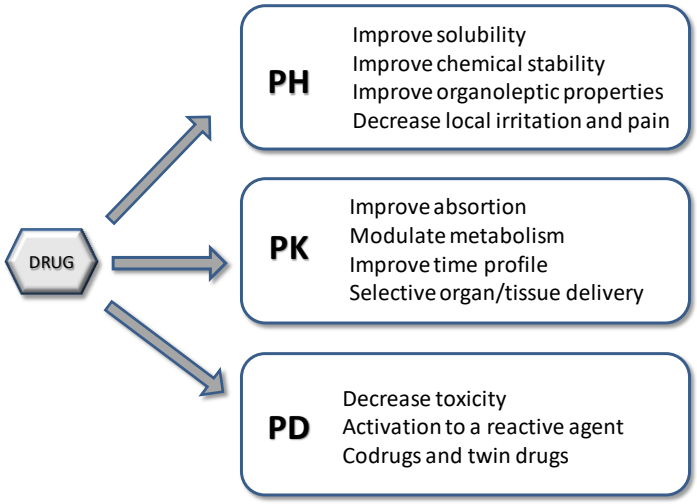
1. Introduction

The concept of prodrug was first introduced in medicinal chemistry by Albert [1] in 1951: “A prodrug is a molecule which does not have any intrinsic biological activity but which is capable during the different phases of its metabolism to generate a biologically active drug”. According to this definition and to that accepted by IUPAC [2], a prodrug is any compound that undergoes biotransformation before exhibiting its pharmacological effects. Prodrugs can thus be viewed as drugs that contain specialized non-toxic protective groups used in a transient manner to alter or to eliminate undesirable properties in the parent molecule.



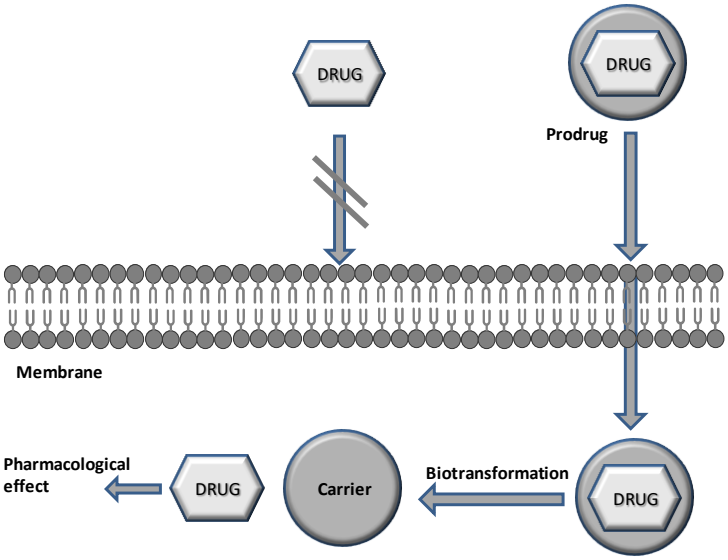
(single-column fitting image) **Fig. 1.** A schematic classification of some objectives in prodrug research, classified by objectives related to pharmaceutical (PH), pharmacokinetic (PK) and pharmacodynamic (PD) phases.

Generally, the metabolic transformation necessary to convert the prodrug into the drug is catalyzed by specific enzymes, mainly hydrolases, and ideally this should selectively occur at the target tissue to prevent undesirable side effects.

In drug research and development, the prodrug concept has found a number of useful applications since it allows several, sometimes contradictory, biological and/or physicochemical objectives to be satisfied. Some examples are shown in Fig. 1, including cellular permeation, solubility, chemical or enzymatic stability, bioavailability, toxicity, or blood brain barrier penetration [3]. One has to bear in mind that many of these objectives are intertwined [4].

A potent suitable prodrug should overcome the crucial paradox: it has to be lipophilic enough to cross a membrane or metabolic barrier (Fig. 2) and simultaneously

it should be hydrophilic enough to fulfill solubility, bioavailability and transport criteria [5,6].



(single-column fitting image) **Fig. 2.** Schematic representation of some prodrugs designed to by-pass a membrane (adapted from [6]).

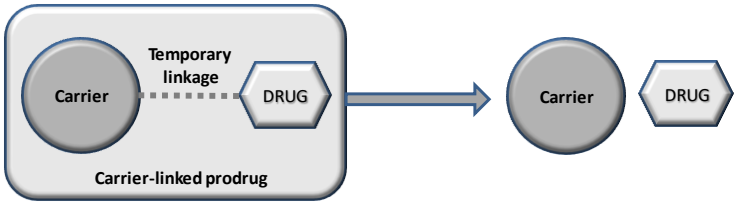
Many therapeutically active agents have low bioavailability after oral administration due to poor absorption or susceptibility to first-pass metabolism, which leads to drug inactivation and/or the production of toxic metabolites. A possible approach to improve the oral absorption is a *formulation solution*, which improves oral bioavailability through the use of suitable excipients that increase intestinal membrane permeability. Such permeation enhancers can be surfactants, fatty acids, glycerides, steroidal detergents and amino acid derivatives amongst others. However, these excipients sometimes cause serious damage to the intestinal epithelium [7]. An attractive alternative is a *chemical solution* that involves a prodrug approach. The prodrug approach has also been widely used to improve delivery of drugs to their site of action by modulation of physico-chemical properties that affect absorption or by targeting specific enzymes or membrane transporters [8]. Thus, a prodrug design is a lead modification approach that is used to correct a flaw in a drug candidate and may be useful in circumventing problems associated with formulation and solubility, absorption and distribution, instability, site specificity of liberation, prolonged release and toxicity, amongst other effects [9].

2. Types of prodrugs

Prodrugs essentially fall into two classes, namely carrier-linked prodrugs and bioprecursor prodrugs. In carrier-linked prodrugs the drug is linked to a carrier moiety by a temporary covalent linkage. Cleavage of a carrier prodrug generates a molecular entity of increased bioactivity (drug) and at least one side product, the carrier, which may be biologically inert [for instance polyethylene glycol (PEG)] or may have targeting properties (for instance antibodies). The bioprecursors do not contain a carrier group and are activated by the metabolic modification of a functional group.

2.1. Carrier-linked prodrugs

According to IUPAC [2], a carrier-linked prodrug "is a prodrug that contains a temporary linkage of a given active substance with a transient carrier group that produces improved physicochemical or pharmacokinetic properties and that can be easily removed *in vivo*, usually by hydrolytic cleavage" (Fig. 3).

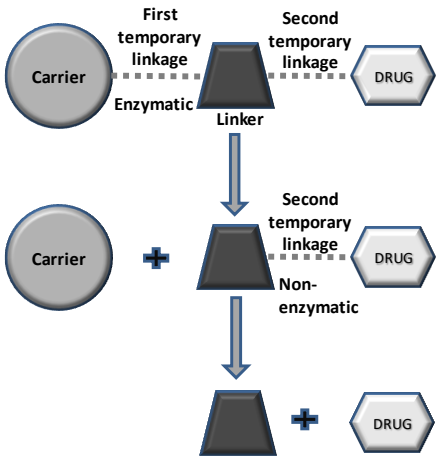


(single-column fitting image) **Fig. 3**. Schematic representation of a carrier-linked prodrug.

A well-designed carried-prodrug may satisfy the following criteria [10]:

- The linkage between the drug substance and the transport moiety is usually a covalent bond.
- As a rule, the prodrug is inactive or less active than the parent compound.
- The linkage between the drug substance and the transport moiety must be broken *in vivo*.
- The prodrug, as well as the *in vivo* released transport moiety, must be non-toxic.
- The generation of the active form must take place with rapid kinetics to ensure effective drug levels at the site of action and to minimize either alternative prodrug metabolism or gradual drug inactivation.

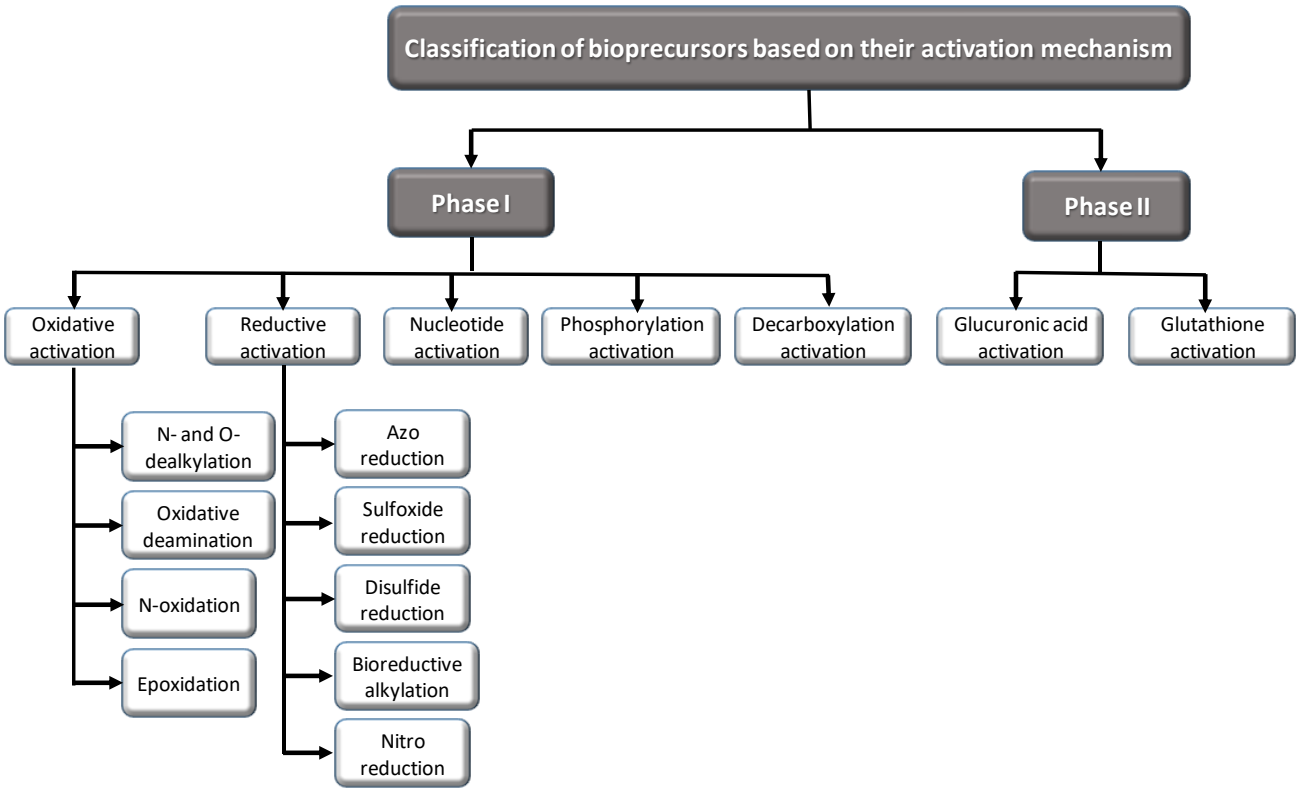
At least one functional group in the drug molecule is employed for attachment of the carrier moiety. Preferred functional groups are hydroxyl or amino groups, but carboxylic acids or carbonyl groups can also be found and, consequently, both the attachment chemistry and hydrolysis conditions can vary markedly between these functionalities. The carrier, which is generally lipophilic in nature, could be a small molecule, e.g., a fatty chain, a polymer or PEG, or a macromolecule, like albumin or an antibody. Carrier-linked prodrug activation may occur by enzymatic or non-enzymatic cleavage of the temporary bond between the carrier and the drug molecule, or by a sequential combination of both, i.e., an enzymatic step followed by a non-enzymatic rearrangement (Fig. 4).



(single-column fitting image) **Fig. 4**. Schematic representation of a carrier-linked prodrug activation by a sequential combination of an enzymatic step followed by a non-enzymatic rearrangement.

As indicated, when designing prodrugs, it is important to ensure that any groups that are cleaved from the prodrug are non-toxic. In this context an ideal prodrug should only provide the drug and natural metabolites in the human body. However, a large number of prodrugs have been designed to liberate aldehydes, such as formaldehyde or acetaldehyde. These species, toxic themselves, are naturally occurring compounds in healthy human individuals. Additionally, they are short-lived compounds and they are converted to the corresponding carboxylic acids, by aldehyde dehydrogenases. For additional information about toxicity of these species when used in prodrug design, see ref. 11 [11].

2.2. Bioprecursor prodrugs



(2-column fitting image) **Fig. 5**. Classification of bioprecursor prodrugs based on their activation mechanisms (adapted from [12]).

Bioprecursor prodrugs result from a molecular modification of the active principle. The modification generates a new compound that is capable of being a substrate of the metabolic enzymes, with the metabolite being the expected active compound [3,10,12]. For example, if the drug contains a carboxylic acid group, the bioprecursor may be an alcohol that is metabolized by oxidation to the aldehyde and then to the carboxylic acid drug. Although pharmacologically active metabolites are generally formed by phase I reactions (oxidation, reduction or phosphorylation), phase II conjugation reactions can also produce biologically active compounds (Fig. 5).

3. Objetives of the prodrug approach

The principal objectives of the prodrug approach can be summarized as follows: (**3.1**) improving drug water-solubility, (**3.2**) improving absorption and membrane permeability, (**3.3**) targeted release, and (**3.4**) reducing metabolism and side effects,

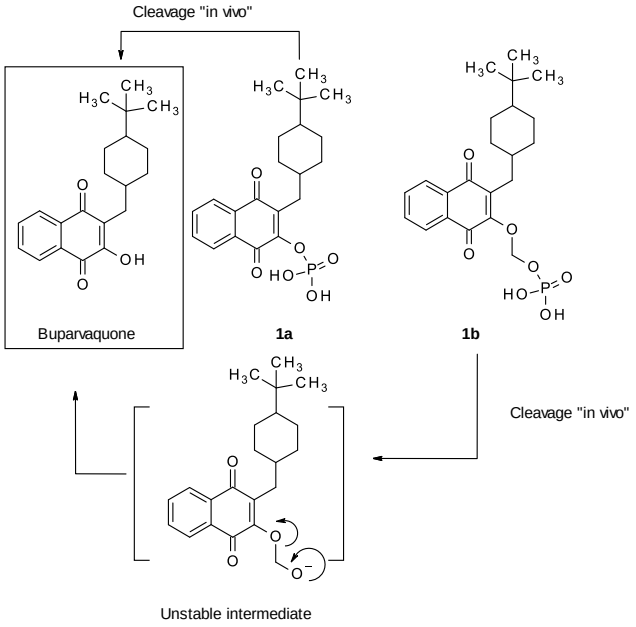
bearing in mind that in some cases two or more objectives are interrelated. This review highlights some modern strategies from the period 2004–2016 in the prodrug approach, including both carrier-linked prodrugs and bioprecursors.

3.1. Improving drug water-solubility

An inadequate water-solubility affects, amongst other factors, the via of drug administration and the pharmaceutical form. A general strategy involves the introduction into this poorly water-soluble drug of some ionizable groups, such as phosphate, hemisuccinate or amino acid esters, or a link to neutral macromolecules such as polyethylene glycol.

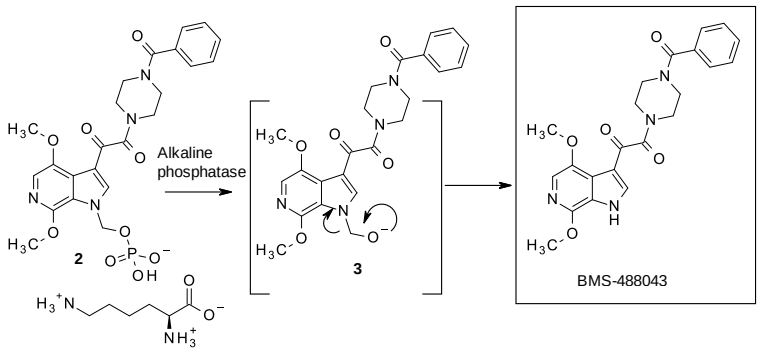
Thus, phosphate or phosphate-derived prodrugs, either directly attached to a molecule or incorporated *via* linkers, have been used to successfully enhance the water solubility of a range of compounds administered by either intravenous or oral routes. Some examples of this strategy are outlined below.

Buparvaquone is a hydroxynaphthoquinone derivative (Scheme 1) that is more active *in vitro* than *in vivo* against *Leishmania donovani* infections after subcutaneous administration. Two factors can account for this lack of *in vivo* activity: poor distribution from the site of injection to the intracellular target when administered subcutaneously, and a combination of low aqueous solubility and high lipophilicity, which lowers the drug bioavailability.



(single-column fitting image) **Scheme 1.** Buparvaquone and its prodrugs.

Mäntylä and co-workers [13] synthesized a series of phosphate prodrugs of Buparvaquone by introducing a phosphate moiety on a hydroxyl group, with the aim of improving both the oral and topical drug delivery properties by increasing the aqueous solubility. The human skin permeation of compounds **1a** and **1b** was studied *in vitro*. While Buparvaquone did not permeate across skin, both **1a** and **1b** permeated readily and **1b** easily released the parent drug in human skin homogenate. This compound is therefore a promising prodrug candidate to deliver Buparvaquone for the treatment of cutaneous leishmaniasis.

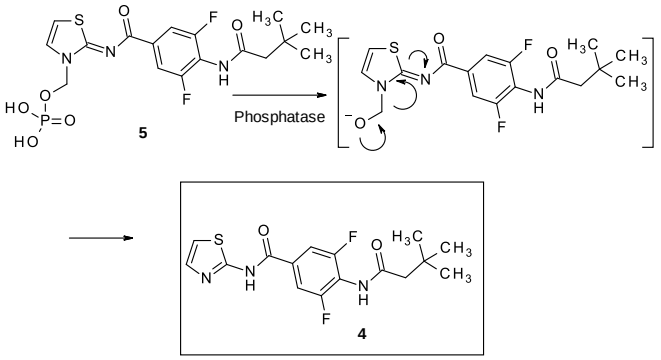


(single-column fitting image) **Scheme 2.** BMS-488043 and its prodrug.

BMS-488043 (Scheme 2) [14] is a member of the class of orally bioavailable HIV-1 attachment inhibitors (AIs). These inhibitors have been shown to bind and induce conformational changes within the HIV-1 viral envelope gp120 protein that interfere with its interaction with the cellular CD4 receptor, the initial point of host cell engagement by the virus. However, the experimental evidence available suggests that absorption of BMS-488043 was limited by its poor water solubility. The lysine salt **2**, a phosphonooxymethyl prodrug of BMS-488043, is significantly more soluble and has sufficient chemical stability in both the solid state and solution to allow oral dosing studies. This prodrug can provide the parent BMS-488043 *via* the intermediacy of a short-lived hydroxymethyl intermediate **3** after phosphatase cleavage [14].

Another example of phosphate or phosphate-derived prodrugs was reported by Sams et al. [15]. The hA2A receptors, a subtype of adenosine receptors that are highly expressed in the striatum, seem to be involved in the modulation of the release of GABA in CNS and, as a consequence, hA2A receptor antagonists may have clinical utility in the treatment of Parkinson's disease. Taking into account the recent literature on the structural elucidation of the hA2A receptor and the structure-based approaches for the identification of new chemotypes of hA_{2A} receptor ligands [16], these authors described the discovery of a novel class of hA_{2A} receptor antagonists with a thiazole

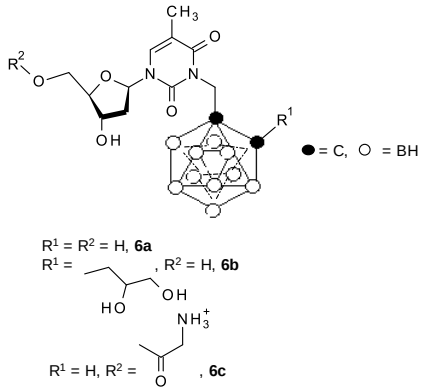
structure. From this series, compound **4** (Scheme 3) was selected as a highly selective compound with acceptable pharmacokinetic properties and with a good effect in an *in vivo* model of Parkinson's disease. The poor water solubility of **4**, however, was a limiting factor in its pharmaceutical development. Consequently, **4** was derivatized as the phosphonooxymethylene derivative **5** and this had much improved aqueous solubility. Compound **5** was fully converted into **4** *in vivo* without detectable traces of prodrugs in the systemic circulation. The proposed mechanism for the release of **4** from **5** is shown in Scheme 3.



(single-column fitting image) **Scheme 3.** Proposed mechanism for the release of **4** from **5**.

As mentioned above, in addition to phosphate groups, other ionizable units can be introduced into the drug to improve the water solubility. Boron Neutron Capture Therapy (BNCT) is a binary cancer treatment modality that relies both on a chemical and a radiation component: When ^{10}B, a stable isotope, is selectively delivered to tumor cells and then irradiated with low energy (thermal) neutrons, a neutron capture reaction occurs to produce lithium and helium nuclei (α-particles). These high linear energy transfer (high LET) particles can produce a variety of cytocidal effects, including DNA double strand breaks [17]. In recent years, 3-carboranyl tymidine analogs (3-CTAs) such as N5 and N5-2OH (compounds **6a** and **6b**, Fig. 6) have attracted considerable attention as compounds developed for BNCT of high-grade brain tumors [17a]. Thus, human thymidine kinase 1 (hTK1) is an enzyme of the DNA salvage pathway synthesis, a metabolic route in which free bases (purine and pyrimidine) or their nucleosides, obtained during DNA-degradation, can be converted back into nucleosides or nucleotides, respectively. hTK1 is predominantly active in proliferative cells and it catalyzes the 5'-monophosphorylation of 3-CTAs and causes, apparently, their selective accumulation in cancer cells. This finding clearly establishes the

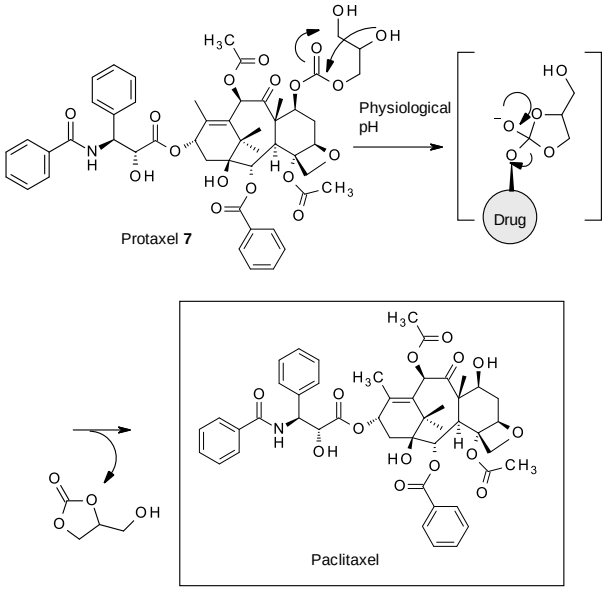
therapeutic potential of **6a** and **6b**. However, these compounds are very lipophilic due to the presence of the carborane cluster and the absence of any functional group that could be ionized under physiological conditions. These characteristics may complicate an adequate parenteral administration.



(single-column fitting image) **Fig. 6.** Chemical structures of N5 and N5-2OH and one of its prodrugs.

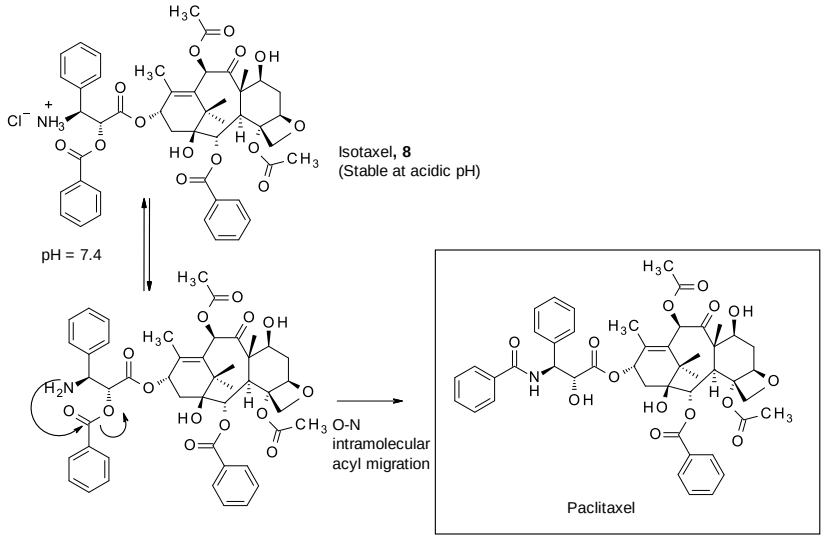
A successful approach to overcome the lack of water solubility of these drugs involved the preparation of carried-linked prodrugs. Tjarks et al. [17] designed and prepared a series of water-soluble amino acid ester prodrugs of **6a** and **6b**. In this respect compound **6c**, the glycine acid ester prodrug (Fig. 6), showed rapid cleavage by chemical hydrolysis to give parent **6a** and this can undergo the crucial 5'-monophosphorylation reaction.

Paclitaxel [18] (Scheme 4) is a taxoid in clinical use for the treatment of a variety of cancers as it promotes, in cancer cells, tubulin polymerization leading to apoptotic death. The water solubility of Paclitaxel is very low and it is therefore co-injected with Cremophor EL, a detergent that causes undesired side effects. Early attempts to prepare more soluble prodrugs, such as succinate, glutarate, sulfonate or amino acid derivatives, were unsuccessful due either to instability in aqueous media or the inability to furnish the parent drug in human plasma.



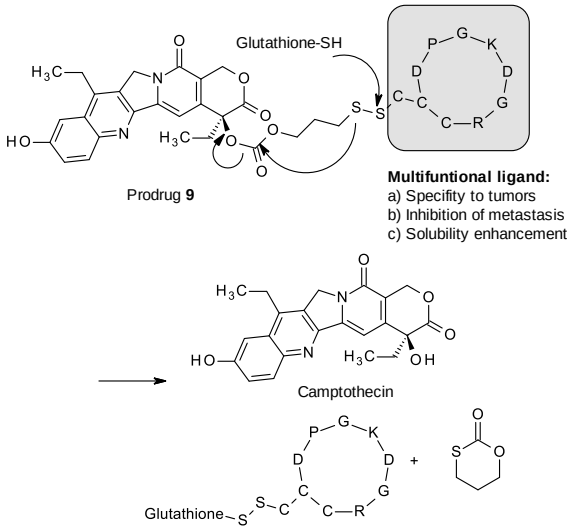
(single-column fitting image) **Scheme 4.** Proposed mechanism for the intramolecular cyclization of Protaxel **7** to release the drug Paclitaxel.

More promising was the prodrug Protaxel **7**, a carbonate conjugate of Paclitaxel that is much more water soluble than Paclitaxel. Compound **7** can release Paclitaxel by a pH-dependent intramolecular cyclization mechanism: A stable stock solution was prepared at acidic pH and the shift to physiological pH resulted in a rapid release of Paclitaxel. This mechanism is outlined in Scheme 4.



(single-column fitting image) **Scheme 5**. *O-N* Intramolecular acyl migration mechanism of Isotaxel **8** to release Paclitaxel.

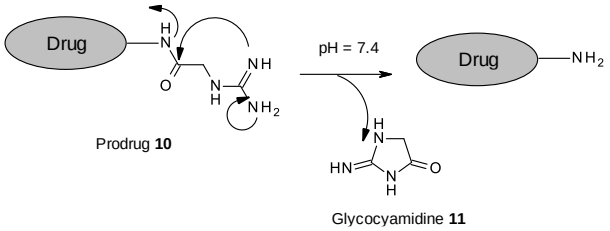
Additionally, Kiso and co-workers [18b] developed Isotaxel **8**, a 2'-*O*-acyl isoform of Paclitaxel. This prodrug is water soluble and can release the parent drug *via* an *O-N* intramolecular acyl migration reaction, without any side reactions, under physiological conditions (Scheme 5).The generation of the parent drug was again pH-dependent: The prodrug was stable in the solid form and under acidic conditions but it furnished the drug at physiological pH.



(single-column fitting image) **Scheme 6**. Cleavage of the disulfide bond to supply the corresponding drug-OH, 7-ethyl-10-hydroxycamptothecin.

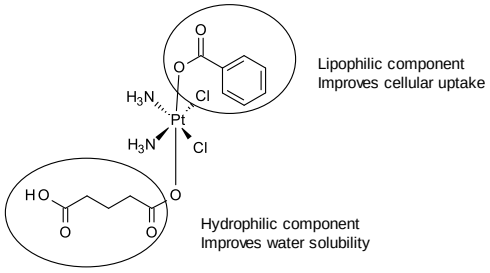
Other water-soluble prodrugs of Paclitaxel include self-immolative disulfide linkers, which can be cleaved with the assistance of glutathione – bearing in mind that glutathione is overexpressed in many tumor cells [19]. Also based on a self-immolative disulfide linker, Wang et al. [20] presented a design concept for multifunctional anticancer prodrugs that simultaneously induce apoptosis and suppress cancer cell metastasis. This class of prodrugs **9** (Scheme 6) includes an antitumor drug, a self-immolative linker and the small cyclic peptidic motif iRDG (Cys-Arg-Gly-Asp-Lys-Gly-Pro-Asp-Cys), which acts as a cell targeting ligand, anti-metastatic agent and highly hydrophilic fragment, thus conferring aqueous solubility on the prodrug.

Based on the decomposition reaction of arginine methyl esters, as reported by Photaki and Yiotakis [21], Hamada [22] designed a novel prodrug strategy especially for drugs that contain amino groups on heterocyclic or aromatic rings and are sparingly water soluble. These prodrugs **10** (Scheme 7) can be converted to the respective parent drugs with acceptable $t_{1/2}$ values. Release of the heterocyclic compound Glycocyamidine **11** is the driving force of the process.



(single-column fitting image) **Scheme 7**. Design of prodrugs **10** based on the decomposition reaction of arginine methyl ester.

In some cases, a drug requires both hydrophilic and lipophilic groups on the same scaffold in order to improve its aqueous solubility and enhance its cellular uptake.



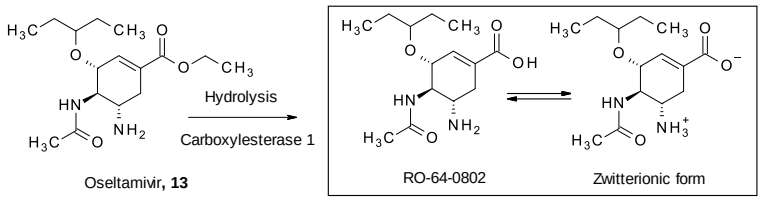
(single-column fitting image) **Fig. 7.** Prodrugs of platinum(IV) bis-carboxylates **12** containing both hydrophilic and lipophilic groups.

This is the case for anticancer prodrug **12** (Fig. 7) [23] based on platinum(IV), which overcomes the limitations of drugs based on platinum(II) by resisting premature “aquation” and binding to essential plasma proteins.

3.2. Improving absorption and membrane permeability

The ability of a drug to cross cell membranes is frequently related to the lipophilicity of that drug. However, many polar groups are important to receptor binding but hinder the drug from crossing membranes. This is the case, for example, of carboxylic acid groups. In these cases, one solution is to protect the acid group in the form of a less polar ester. The ester could cross fatty membranes and once it is in the bloodstream it is hydrolyzed back to the parent acid drug. The variety of ester prodrugs in the literature is immense and has served to mask carboxylate, phosphate and tetrazole groups, as well as alcoholic or phenolic functions. An example of this approach is RO-64-0802 [24], a neuraminidase inhibitor of therapeutic value against type A and B influenza in humans. This compound shows very high *in vitro* inhibitory

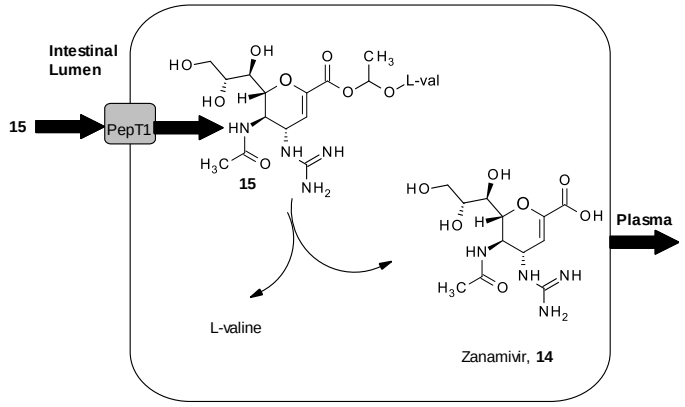
efficacy toward the enzyme but low oral bioavailability as a zwitterion that is too hydrophilic by virtue of basic amino and acidic carboxylate groups. To circumvent this problem, the active agent was derivatized to its ethyl ester, which is known as Oseltamivir **13** (Scheme 8). This prodrug is well absorbed orally and offers an increase in bioavailability from 5% to 80% with respect to the parent drug. Compound **13** undergoes rapid enzymatic hydrolysis by human carboxylesterase 1 to produce high and sustained plasma levels of the active agent.



(single-column fitting image) **Scheme 8.** Hydrolysis of Oseltamivir **13** to RO-64-0802.

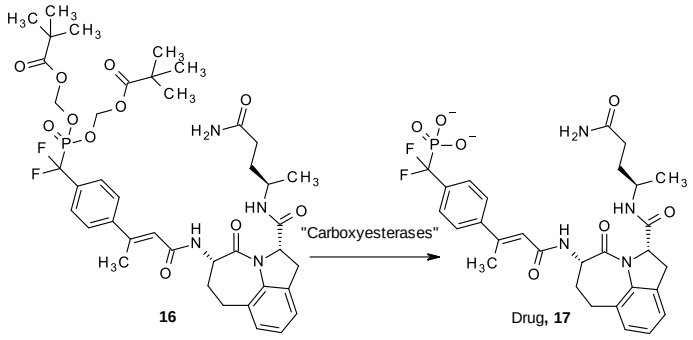
Other recent examples of this approach are collected in reference 25 [25].

Another potent neuraminidase inhibitor is Zanamivir **14** [26], which is active against emerging Oseltamivir-resistant strains. Unfortunately, the oral availability of Zanamivir is very low due its polar structure. A modern prodrug strategy has been developed that targets a carried transporter in the intestinal epithelial cell, such as PepT1. This strategy consists of the incorporation of acyloxy ester groups conjugated with amino acids to supply prodrug **15**, which show improved intestinal absorption by the PepT1 peptide transport system (Scheme 9). Other studies in which this strategy is used are shown in reference 27 [27].



(single-column fitting image**) Scheme 9.** A prodrug **15** of Zanamavir **14** with improved oral absorption (adapted from [26]).

A signal transducer and activator of transcription 3 (Stat3) is a transcription activator factor [28] that communicates signals from extracellular receptors directly to the nucleus, where it mediates the expression of some genes and plays a significant role in cellular processes such as apoptosis. Stat3 contains an SH2 domain, a protein fragment commonly found in signal-transduction proteins, which confers on the protein the ability to bind to phosphotyrosine residues on other proteins and, as a result, Stat3 becomes activated. This activation process is the key event in the proliferation of several cancer types, and some small molecules that target Stat3 through its SH2 domain would be potential anticancer agents.



(single-column fitting image) **Scheme 10.** Structure of phosphopeptide mimetic prodrug **16** and the drug **17**.

McMurray et al. [28] targeted this SH2 domain with inhibitors based on the peptide Ac-pTyr-Leu-Pro-Gln-Thr-Val-NH_2 and reported its conversion into a conformationally constrained version to supply a cell-permeable, phosphatase-stable peptidomimetic prodrug **16** (Scheme 10). In order to facilitate cell penetration, the negatively charged oxygen atoms of the difluoromethylphosphonate group in **17** were masked with pivaloyloxymethyl groups, which are *in vivo* labile to carboxyesterases.

Micellar systems of polyethylene glycol-poly(aspartic acid) copolymer have been used by Silva and co-workers [29] as carriers for the hydrophilic tuberculostatic Pyrazinamide. Pyrazinamide is covalently linked to a polymer to form a micelle, which has a hydrophobic core consisting of the drug-ligand group and a hydrophilic outer coat (PEG-PASP-PZA). The advantage of this micellar carrier lies in its structural stability and good water solubility. In addition, this structure allows a controlled rate of drug release, reduced toxicity and selective action on the chosen target. Furthermore, the PEG-PASP-PZA derivative, when assayed for its anti-Mycobacterium activity, exhibited stronger activity than the simple drug.

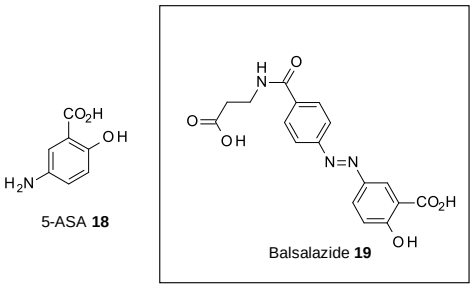
Nanostructured delivery systems such as liposomes, micelles, micro- and nanoemulsions, polymeric nanoparticles (PNPs) and solid lipid nanoparticles (SLNs)

have frequently been used as ideal choices for drug delivery due to the improved bioavailability, solubility, stability and biocompatibility [30].

3.3. Targeted release

A method that unifies the prodrug approach with target specificity, where the drug is inactive during transport and is activated only when released to specific target tissue without any toxic effects, would be very effective in the field of drug delivery. This concept comes from the scientist Paul Ehrlich's principle known as the "magic bullet": he considered the chemical as a bullet that could search out and destroy the invading microorganism without adversely affecting the host. Several strategies have been pursued in an effort to improve the selectivity of low molecular weight drugs and thus to increase the concentration of the active agent in the desired tissue, while its concentration is reduced in healthy tissues in order to reduce side effects. Some examples of this strategy are described below.

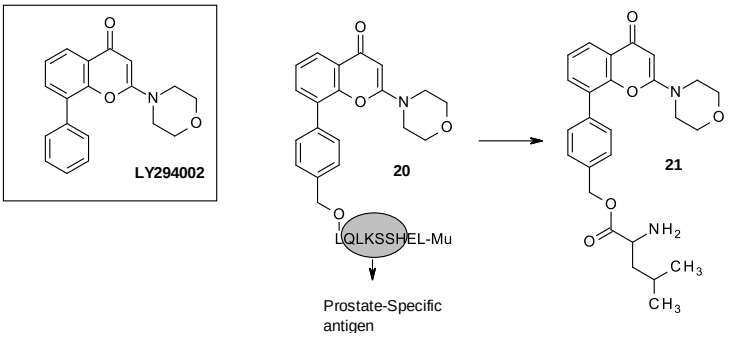
5-Aminosalicylic acid (5-ASA **18**, Fig. 8) is a drug used to attenuate the inflammatory response in idiopathic inflammatory bowel diseases, such as ulcerative colitis, although its mechanism of action is not fully understood. However, 5-ASA usually fails to reach the colon and this leads to significant adverse effects, such as ulcerogenic potential. Therefore, a prodrug approach for colon delivery of 5-ASA has become a rational system of drug delivery for the topical treatment of such diseases. Balsalazide (**19**, Fig. 8), a prodrug in which 5-ASA is linked to the carrier 4-aminobenzoyl β-alanine by an azo bond, is specifically converted to 5-ASA by azo-reducing bacteria present in the colon [31]. Thus, the prodrugs based on non-sulfapyridine azo-conjugates of 5-ASA, such as Balsalazide **19**, protect against proximal absorption and have the potential to separate dose-ranging benefits from side effects related to the presence of the sulfapyridine moiety.



(single-column fitting image) **Fig. 8**. Molecular structures of 5-ASA **18** and Balsalazide **19**.

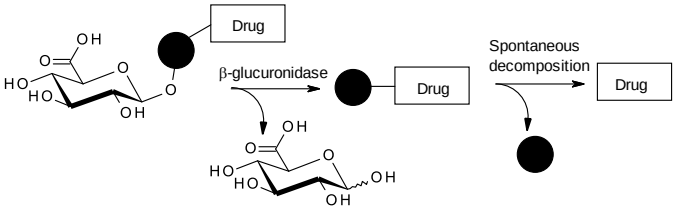
However, more progress has been made with the above approach for anticancer drugs. PI3K inhibitors such as LY294002, an analog of quercetin, have been

considered as an adjuvant therapy for advanced prostate cancer. However, experiments in clinical models did not afford the expected results. One possible approach to improve the efficacy of PI3K inhibitors against prostate cancer is to convert the inhibitor into an inactive prodrug by attaching a specific prostate-specific-antigen (PSA) cleavable peptide (Scheme 11). Prostate-specific-antigen (PSA) in systemic circulation is inactive and its protease activity is confined to the prostate or prostate-derived cancer cells. The cleavable peptide has the total sequence Mu-LEHSSKLQL (*N*-(4-morpholinylcarbonyl)-Leu-Glu-His-Ser-Ser-Lys-Leu-Gln-Leu)- where the internal sequence HSSKLQ is the substrate for PSA. Thus, the activation of water-soluble prodrug **20** yields **21**, a PI3K inhibitor LY294002-analog, and this increases the delivery to the tumor site while minimizing systemic toxicity [32].



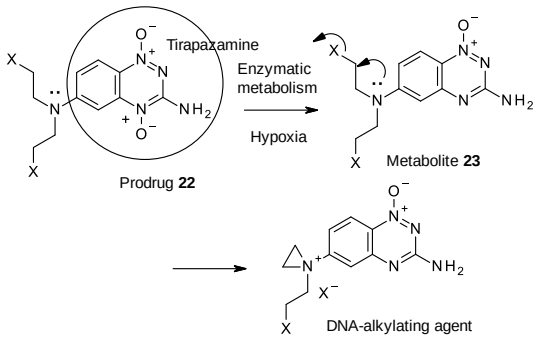
(single-column fitting image) **Scheme 11.** PSA-dependent activation of **20** to **21**.

β-Glucuronidases are enzymes that catalyze hydrolysis of β-D-glucuronic acid residues, mainly in mucopolysaccarides [33]. However, this enzyme is overexpressed in a wide range of cancer tumors, including lung, breast and gut cancer. A possible strategy to delivery anticancer drugs in the neighborhood of malignant cells is the denominated β-glucuronidase-responsive prodrug [34]. These prodrugs include, in general, a self-immolative linker between the β-D-glucuronic acid moiety and the corresponding drug, and the release of the drug occurs by an initial step of enzymatic hydrolysis by β-glucuronidase and a second step of spontaneous decomposition to furnish the active drug (Scheme 12) [34].



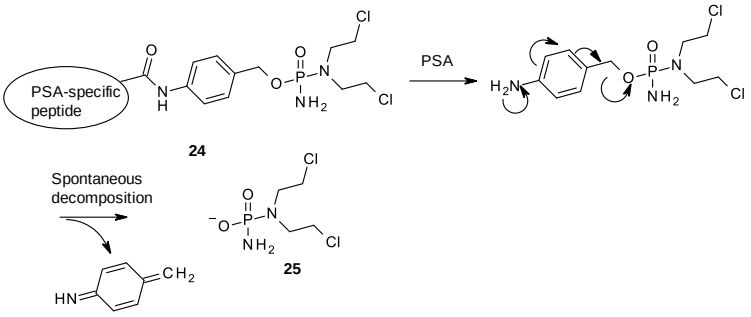
(single-column fitting image) **Scheme 12.** General scheme for β-glucuronidase-responsive prodrugs (adapted from [34]).

Nitrogen mustards were the first anticancer drugs and they operate through the generation of aziridinium ions that are capable of alkylating DNA, mainly in purine rings. However, nitrogen mustards have very significant side effects that arise from indiscriminate alkylation of biomolecules in nonmalignant tissues and cells. On the other hand, it is an axiom in medicinal chemistry that aziridinium ion formation is suppressed in *N*-aryl nitrogen mustards affected by the presence of electron-withdrawing groups and, in contrast, favored by activating groups on the aryl moiety. Taking this into consideration and bearing in mind the predominantly reductive metabolism in (hypoxic) cancer cells, Gates et al. [35] reported the preparation of a new prodrug of nitrogen mustards **22**, in which the mustard is coupled to Tirapazamine, a heterocyclic di-*N*-dioxide that undergoes enzymatic deoxygenation in the hypoxic cells found in solid tumors to generate a mono-*N*-oxide metabolite **23** (Scheme 13). This process causes a substantial increase in reactivity of the mustard unit and unmasks the bioactive agent inside hypoxic cells. A similar strategy in the construction of prodrugs of *N*-aryl nitrogen mustards has been described by Peng et al. [36].



(single-column fitting image) **Scheme 13.** Activation of prodrug **22** to a DNA-alkylating agent in cancer cells.

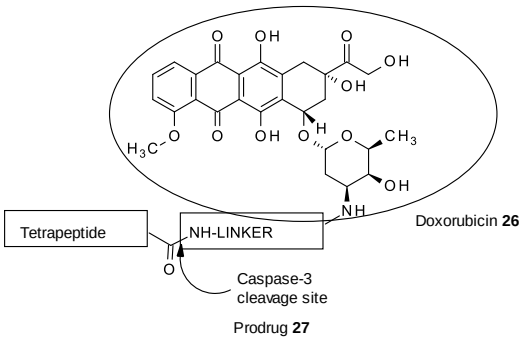
As mentioned above (see Scheme 11 and reference 32) prostate-specific drug design is an attractive approach to localize the cytotoxicity at the prostate tumor site.



(single-column fitting image) **Scheme 14.** Activation of prodrug **24** by PSA proteolysis.

Based on a nitrogen mustard and a PSA-specific peptide, Hu et al. [37] described a prodrug approach to a specifically released phosphoramide mustard in prostate tumor cells by the proteolytic action of PSA. The prodrug **24** (Scheme 14) contains a self-immolative *para*-aminobenzyl linker between a PSA-specific peptide and a phosphoramide mustard. The proteolytic cleavage of the PSA-specific peptide supplies an intermediate that suffers spontaneous decomposition to provide the cytotoxic alkylating agent **25** inside the prostate tumor tissues.

Doxorubicin **26** is an anthracycline antitumor agent that acts as a DNA intercalator. This compound is used in the treatment of a wide range of cancer processes, including hematological cancers and many types of solid tumors, either alone or in combination with other therapies. Common adverse effects of Doxorubicin **26** include, amongst others, cumulative cardiotoxicity and myelosuppression – side effects related to the nonselective distribution of drug. Thus, considerable effort has been made to liberate Doxorubicin selectively within tumor cells.



(single-column fitting image) **Fig. 9.** Schematic representation of prodrug **27**.

Kim et al. [38] reported the preparation of prodrugs **27** (Fig. 9) based on Doxorubicin **26**, including an example in which the anthracycline is conjugated with a tetrapeptide *via* a functionalized linker. This peptide is specifically recognized by

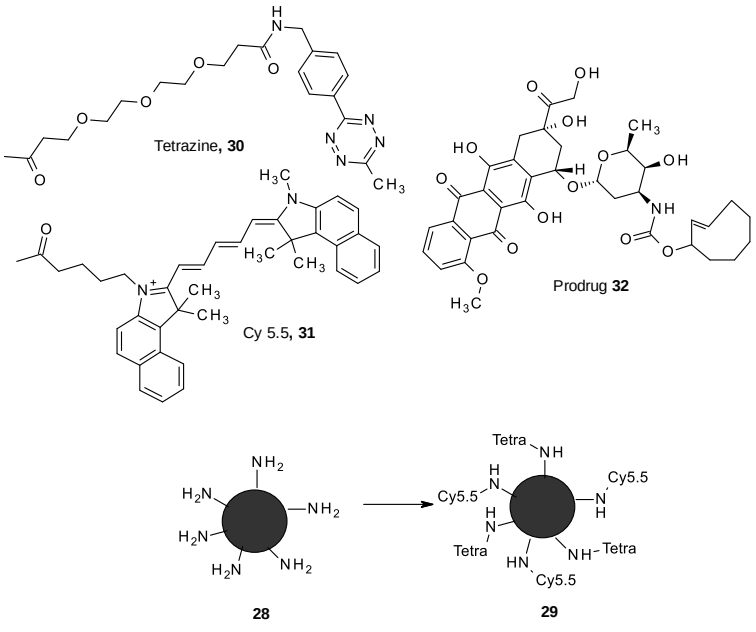
caspase-3, a cystein protease overexpressed in apoptotic cells, which selectively hydrolyzes the amide bond. Thus, these prodrugs can be activated in the presence of caspase-3, the expression of which can be exogenously regulated by inducing apoptosis with radiation therapy at the specific site of interest.

You et al. [39] employed a related methodology to produce a new prodrug of Paclitaxel (see Scheme 5) that is activated by far-red light *via* a singlet oxygen-cleavable aminoacrylate linker.

The accumulation and cellular uptake of non-toxic magnetic nanoparticles (MNPs, **28**) (Fig. 10) of iron oxide in cancer cells [40] and the possibility of modifying their amine *termini* on the surface were used by Royzen et al. [41] in a general strategy for imaging-guided prodrug activation by a “click and release” process. The methodology involves two steps and two different classes of reagents:

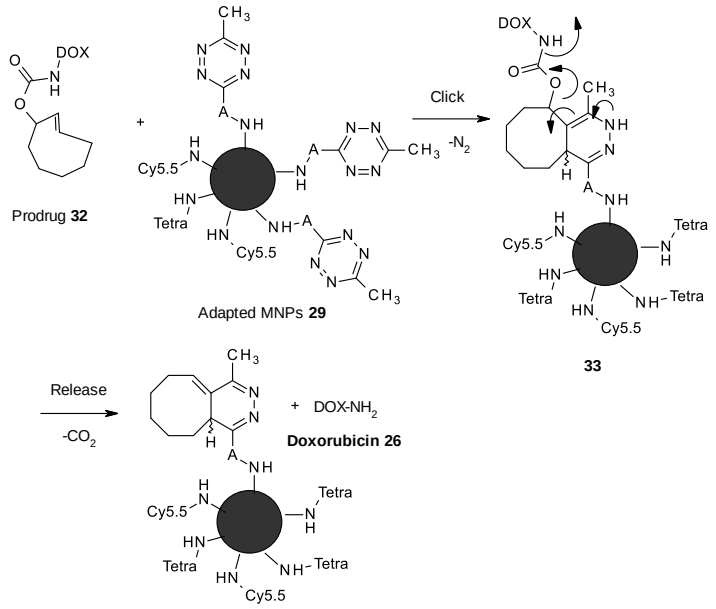
- Adapted MNPs **29**, concomitantly modified with tetrazine moieties **30** and a near infrared fluorescent dye, such as cy5.5 (NIRFcy 5.5, **31**).

- A prodrug **32** of Doxorubicin, obtained by modification of its amine group with a carbamate derived from *trans*-cyclooctene, where the cytotoxicity of Doxorubicin **26** is appreciably attenuated by the chemical alteration.



(single-column fitting image) **Fig. 10.** Chemical structures of **28**–**32**.

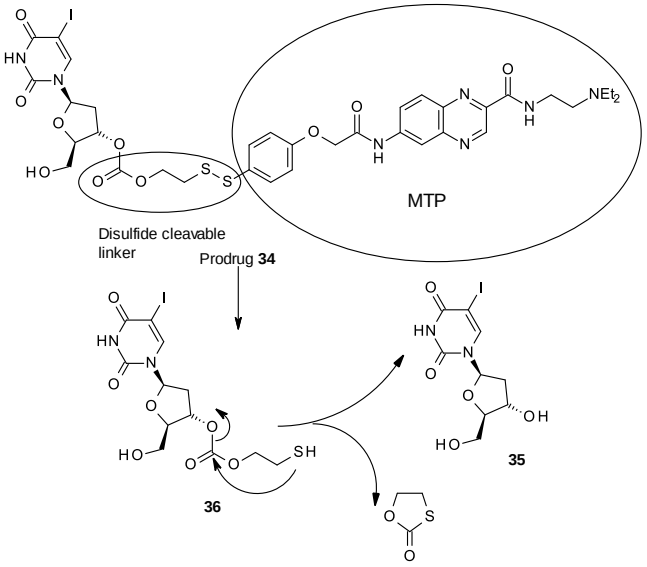
Systemic administration of non-toxic adapted MNPs **29** can be monitored by Magnetic Resonance Imaging (MRI) techniques because MNPs are MRI contrast agents. As soon as localization of adapted MNPs **29** within cancer cells is confirmed, **32** is systemically administered at the concentration corresponding with its IC_{20} value: The activation of the prodrug and its metabolic transformation occurs when it reaches the cells containing adapted MNPs **29**, by the "click and release" mechanism shown in Scheme 15. The inverse demand Diels–Alder reaction between the *trans*-cyclooctene (in the prodrug **32**) and the tetrazine moieties (in the adapted MNPs **29**, about 60 tetrazines per nanoparticle) can supply intermediate **33**, which spontaneously releases Doxorubicin **26**.



(single-column fitting image) **Scheme 15.** Activation of prodrug **32** by "click and release" chemistry.

Melanoma is the most dangerous type of skin cancer and it develops from melanocytes. The primary cause of melanoma is exposure to ultraviolet light (UV). Melanin-Targeting Probes (MTPs) comprise a group of arylcarboxamide families with high affinity for melanins, the molecular target detected in more than 90% of primary melanoma cases and in 30–50% of metastatic lesions. Moreau et al. [42] explored the use of such MTPs to transport an anticancer drug into the melanoma tumor site. The authors reported the synthesis and evaluation of a prodrug **34** (Scheme 16), which was developed for pigmented melanoma therapy and contains an MTP conjugated to the antimetabolite 5-iodo-2'-deoxyuridine **35** as a model anticancer agent. The moieties are connected by a linker that contains a disulfide bond, which is stable in the plasma but

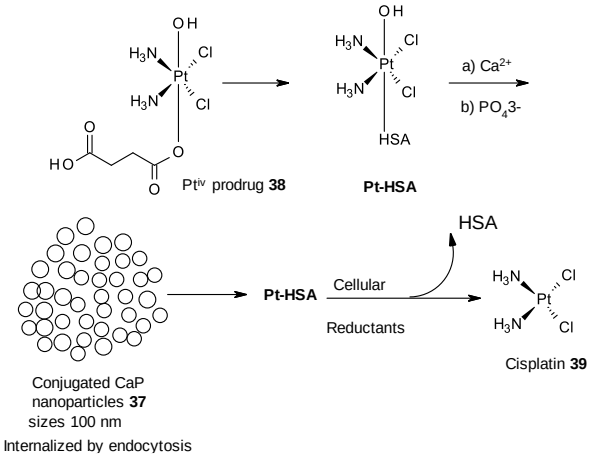
efficiently cleaved by intracellular reducing systems present in hypoxic tumor cells. The resulting thiol **36** would undergo cyclization to supply the free anticancer agent **35**.



(single-column fitting image) **Scheme 16.** Prodrug **34** containing a cleavable disulfide linker.

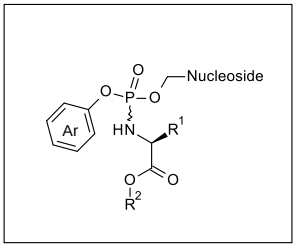
Also based on a reducible disulfide bond, Chmielewski et al. [43] reported some prodrugs of Abacavir, an HIV reverse transcriptase inhibitor developed to eradicate HIV reservoirs in the brain. Prodrugs of Abacavir have two functions: They inhibit the drug efflux of P-glycoproteins to facilitate brain drug penetration and they revert to the antiviral agent Abacavir in cellular reducing environments.

Human serum albumin (HSA) is the most abundant plasma protein. It is a natural carrier of endogenous hydrophobic molecules (such as vitamins, hormones, and other water-insoluble plasma substances) that are bound in a reversible non-covalent manner [44]. Moreover, albumin seems to help endothelial endocytosis of protein-bound and unbound plasma constituents principally by binding to a cell-surface glycoprotein (gp60) receptor. Some characteristics make HSA an attractive drug vehicle in oncology: HSA accumulates in cancer cells to cover their enhanced need for amino acids and energy. This accumulation is favored by the deficient lymphatic drainage and the enhanced permeability of the vascular system inside the tumors. Taking into account these premises, Liu et al. [45] reported HSA-conjugated nanoparticles (conjugated CaP nanoparticles **37**, Scheme 17) of a prodrug of Cisplatin (Pt^{iv} prodrug, **38**), with sizes of about 100 nm, that can be internalized into cells by endocytosis. Nanoparticles were decomposed in the acidic and hypoxic environment and, after reduction in cancer cells, furnished Cisplatin **39**.



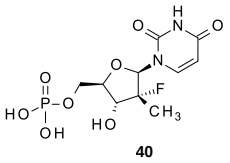
(single-column fitting image) **Scheme 17.** Schematic representation of the preparation of **37** and their transformation to **39** in the acidic and hypoxic cancer cells.

Perhaps the most important development in the prodrug field is the methodology ProTide, discovered by McGuigan [46], especially for nucleoside phosphates. The ProTide technology is a phosphoramidate prodrug that contains an aryl phosphate and an amino acid ester linked by the *N–P* bond to the active nucleoside (Fig. 11). This was designed to deliver nucleoside monophosphate into the cells, mainly hepatocytes, by increasing intracellular concentrations of the active nucleoside and taking advantage of first pass metabolism, where the liver enzymes are capable of hydrolyzing the carboxylic ester to convert the prodrug into the 5′-monophosphate nucleoside.



(single-column fitting image) **Fig. 11.** Schematic representation of a ProTide prodrug.

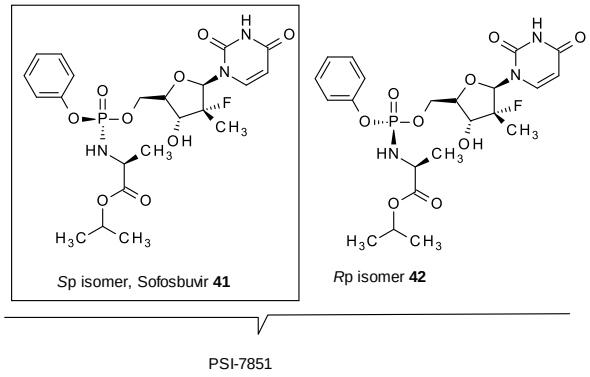
In this context, hepatitis C is a severe liver disease caused by the hepatitis C virus (HCV), a single stranded RNA virus of the Flaviviridae family. NS5B polymerase [HCV NS5B (RdRp)] is an enzyme responsible for the replication of viral RNA and it could be inhibited in the presence of nucleoside analogs that function as defective substrates.



40

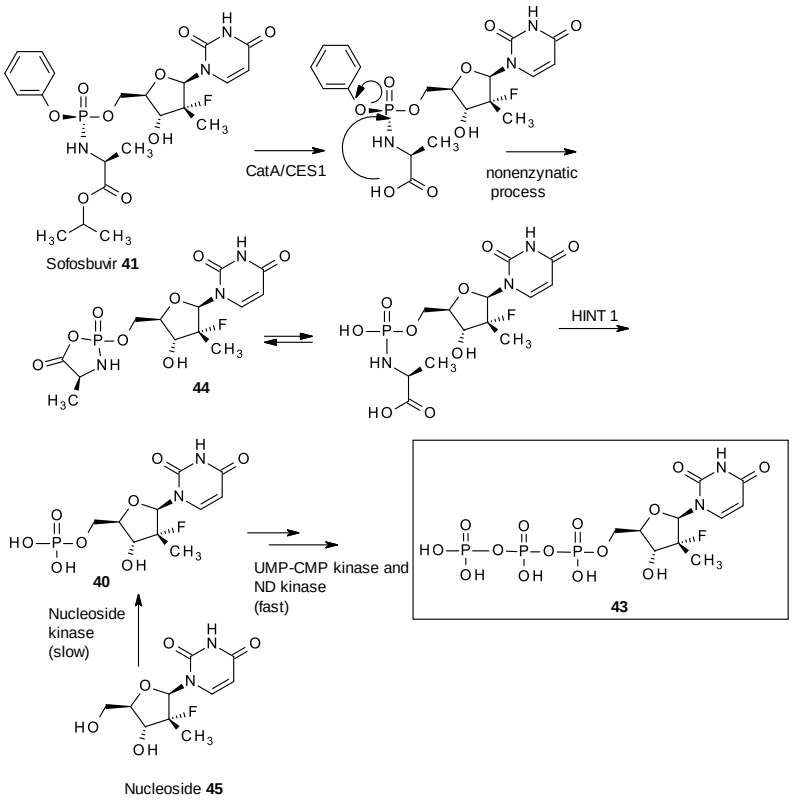
(single-column fitting image) **Fig. 12.** Chemical structure of **40**, an inhibitor of HCV NS5B polymerase.

Thus 2'-deoxy-2'-α-fluoro-2'-β-5-methyluridine-5'-mono-phosphate **40** (Fig. 12) is a nucleoside phosphate inhibitor of the NS5B polymerase and Sofosbuvir **41** (Fig. 13), launched on the marked by Gilead in 2014, is its ProTide prodrug. Sofosbuvir **41** is the Sp isomer of the diastereomeric mixture PSI-7851 and it is approximately18-fold more active than the corresponding Rp isomer **42**, where Sp and Rp refer to the stereochemistry of asymmetric phosphorus.



(single-column fitting image) **Figure 13.** Sofosbuvir **41** and its diastereomer **42**.

Sofosbuvir **41** is metabolized to the drug **40** and then to active antiviral agent 2'-deoxy-2'-α-fluoro-2'-β-5-methyluridine-5'-triphosphate **43** by liver enzymes and it is efficiently incorporated by HCV polymerase, causing a complete chain termination in the process of RNA replication. The metabolic pathway for conversion of Sofosbuvir into the triphosphate **43** is outlined in Scheme 18 and involves hydrolysis of the ester moiety by cathepsin A (cat A) and/or carboxyesterase 1 (CES1) to supply the carboxylic acid **44**. The spontaneous intramolecular displacement of the phenolic group by nucleophilic attack of the free carboxylic acid on the phosphate group and removal of the alanine fragment by histidine triad nucleotide-binding protein 1 (HINT1), produce the monophosphate nucleoside **40**. Phosphorylation reactions catalyzed by cellular kinases supply the active triphosphate agent **43**. This structural design avoids the slow step of the first phosphorylation on nucleoside **45** [47].



(single-column fitting image) **Scheme 18.** Metabolic pathway for Sofosbuvir **41**.

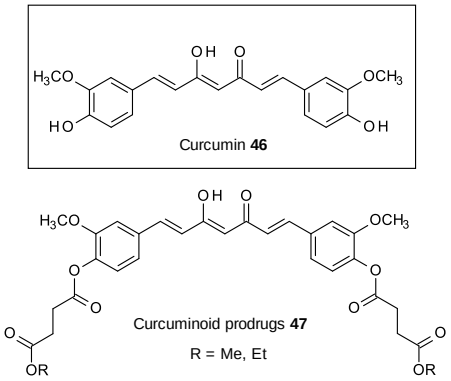
Based on the same methodology Chang et al. [48] reported a prodrug of 5-fluoro-2'-deoxyuridine that is orally active and indicated in the treatment of hepatocellular carcinoma.

3.4. Reducing metabolism and side effects

Many drugs suffer from extensive metabolism leading to drug inactivation, frequently due to the presence of metabolically labile chemical groups. In some cases drugs undergo metabolic activation into toxic chemical species and sometimes toxicity is associated with high initial plasma levels. In all cases, these drugs are attractive targets for a prodrug strategy.

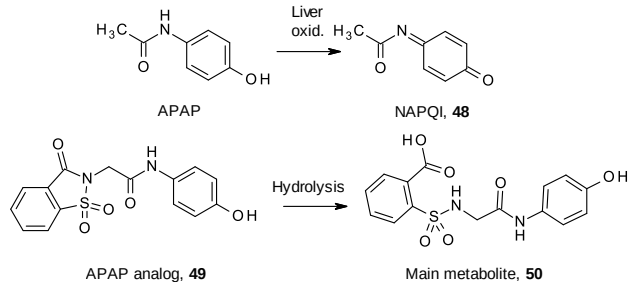
Curcumin **46** [49] (Fig. 14) is a polyphenolic compound. It is the main bioactive ingredient of turmeric extract and has been widely investigated as an antioxidant, anti-inflammatory and antimicrobial agent. The most attractive feature of curcuminoids is the lack of significant toxicity, as shown in animal and human studies. The major obstacle in clinical trials has been the low bioavailability of Curcumin **46** due to its

instability in a biological environment, inadequate absorption and fast metabolism resulting in rapid systemic elimination. Phenolic groups and double bonds are the main sites of *in vitro* and *in vivo* degradation by pathways that include conjugations and reduction process. Numerous strategies have been used to enhance the bioavailability of Curcumin and these include the conjugation of small endogenous molecules such as amino acids and glucose at the phenolic groups [50]. Rojsitthisak and co-workers [51] reported the synthesis of a series of curcuminoid prodrugs **47** (Fig. 14) carrying succinyl ester moieties and these compounds showed enhanced stability and anticolon cancer activity.



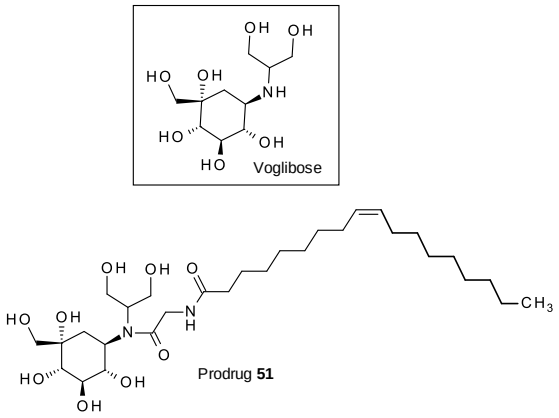
(single-column fitting image) **Fig. 14.** Structure of Curcumin **46** and its succinate prodrugs **47**.

Acetaminophen (APAP), the drug of choice for mild to moderate pain and fever, is a compound with a mechanism of action not yet clearly understood. The activity is mainly related with the antagonist activity on COX-2, but also associated to other non-COX related mechanisms. When the drug is in the liver tissue the oxidation produces NAPQI **48**, a very electrophilic compound as the main metabolite, to which the toxicity on hepatocyte of APAP is due. Bazan and co-workers [52] described a series of acetaminophen analogs, such as compound **49**, eventually more easily transported to CNS. As a result, 2-(1,1-dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl) derivatives showed an unexpected lack of liver toxicity due to an alternative metabolic pathway, yielding the hydrolysis compound **50**, much more hydrophilic, as the main metabolite (Scheme 19).



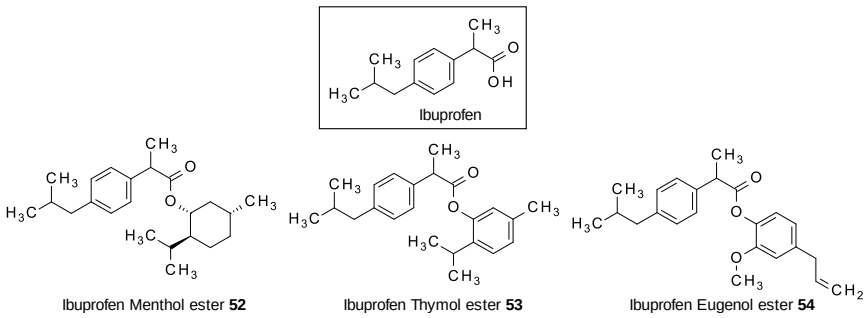
(single-column fitting image) **Scheme 19.** Metabolic pathway for APAP and for **49**.

Highly soluble drugs bearing primary or secondary amines have been converted to prodrugs by creating an amide bond between the parent drug and a lipophilic carrier. In this way, gastro-intestinal side effects are reduced and membrane permeability is increased. This is the case of prodrugs of Voglibose (Fig. 15), which is a carbasugar α-glycosidase inhibitor used to lower post-prandial blood glucose levels in patients with diabetes mellitus. Voglibose is very soluble and this causes gastro-intestinal discomfort, such as flatulence, constipation and diarrhoea. By using the lipid conjugate derivative **51** (Fig. 15), the molecule becomes less water soluble and more lipophilic, which reduces the gastro-intestinal side effects [53].



(single-column fitting image) **Fig. 15.** Chemical structure of Voglibose and its prodrug **51**.

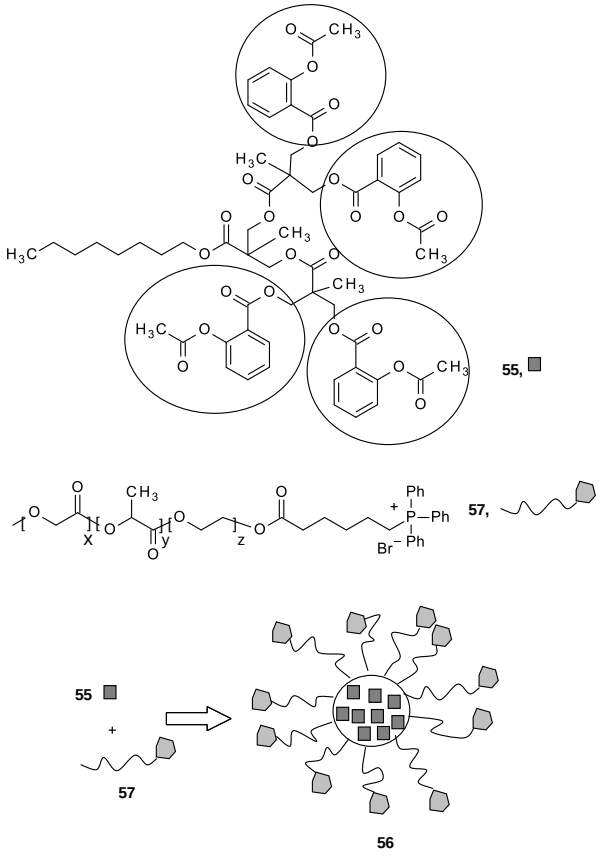
Redasani et al. described an ester prodrug designed to improve oral therapeutic efficacy by retarding gastrointestinal side effects [54]. These authors reported some ester derivatives of Ibuprofen (**52**–**54**, Fig. 16) as mutual prodrugs with natural phenolic and alcoholic compounds such as Menthol, Thymol and Eugenol. In this context, mutual prodrugs is a very important class of prodrugs: A mutual prodrug is a product containing two or more synergistic drugs with improved potency, and frequently, with potential to overcome unwanted side effects [3]. Thus, prodrugs **52**–**54** show increased anti-inflammatory activity, which was attributed to a synergistic effect as Ibuprofen conjugates to natural analgesics and minimizes gastrointestinal toxicity.



(single-column fitting image) **Fig. 16.** Structures of Ibuprofen and prodrug esters **52**–**54**.

Other remarkable examples of the mutual prodrug approach are collected in reference 55 [55].

Acetylsalicylic acid and other NSAIDs (nonsteroidal anti-inflammatory drugs) seem to have some neuroprotective roles in neurodegenerative diseases. Unfortunately, the physicochemical properties and gastric toxicity limits the effectiveness of this compound.

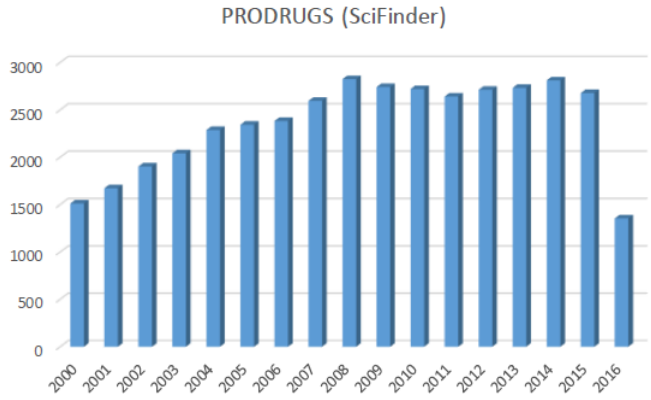


(single-column fitting image) **Fig. 17.** Chemical structures of **55** and **57**, and schematic representation of **56**.

In this context, Dhar et al. [56] developed a hydrophobic prodrug **55** of acetylsalicylic acid encapsulated in polymeric nanoparticles **56** (Fig. 17). This approach can specifically deliver the drug in the neighbourhood of the brain mitochondrias, the drug target for a possible application in neurodegenerative diseases. The prodrug **55** contains four acetylsalicylic acid molecules incorporated into a hydrophobic structure. Encapsulation of prodrug **55** in the presence of biodegradable and mitochondria-specific polymer **57** [poly(lactic-*co*-glycolic acid)-*block*-polyethylene glycol], functionalized with a terminal triphenylphosphonium cation (PLGA-*b*_PEG-TPP) furnished nanoparticles **56**. These nanoparticles overcome the disadvantages arising from the strong hydrophilic properties and the gastric toxicity of the drug while retaining its anti-inflammatory properties.

4. Conclusion

In conclusion, in drug research the prodrug concept has found many useful applications, especially in the target releasing field, but other problems such as a poor water solubility, incorrect absorption rate, early metabolic destruction or unwanted side effects continue to be topics to consider for the successful development of a prodrug. The area has been extensively exploited in the last 15 years, as can be seen in Fig. 18, with a continuous growth in the references appearing in the literature.



(single-column fitting image) **Fig. 18**. Publications referenced in SciFinder during the period 2000–2016 in relation to the prodrug concept.

This review is not intended to be exhaustive but it aims to provide an idea of the most modern trends in the chemical development of prodrugs. This review is intended for chemists and an extensive knowledge of pharmacology or physiology is not required.

Acknowledgements

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Fig. 1. A schematic classification of some objectives in prodrug research, classified by objectives related to pharmaceutical (PH), pharmacokinetic (PK) and pharmacodynamic (PD) phases.

Fig. 2. Schematic representation of some prodrugs designed to by-pass a membrane (adapted from [6]).

Fig. 3. Schematic representation of a carrier-linked prodrug.

Fig. 4. Schematic representation of a carrier-linked prodrug activation by a sequential combination of an enzymatic step followed by a non-enzymatic rearrangement.

Fig. 5. Classification of bioprecursor prodrugs based on their activation mechanisms (adapted from [12]).

Scheme 1. Buparvaquone and its prodrugs.

Scheme 2. BMS-488043 and its prodrug.

Scheme 3. Proposed mechanism for the release of **4** from **5**.

Fig. 6. Chemical structures of N5 and N5-2OH and one of its prodrugs.

Scheme 4. Proposed mechanism for the intramolecular cyclization of Protaxel **7** to release the drug Paclitaxel.

Scheme 5. *O-N* Intramolecular acyl migration mechanism of Isotaxel **8** to release Paclitaxel.

Scheme 6. Cleavage of the disulfide bond to supply the corresponding drug-OH, 7-ethyl-10-hydroxycamptothecin.

Scheme 7. Design of prodrugs **10** based on the decomposition reaction of arginine methyl ester.

Fig. 7. Prodrugs of platinum(IV) bis-carboxylates **12** containing both hydrophilic and lipophilic groups.

Scheme 8. Hydrolysis of Oseltamivir **13** to RO-64-0802.

Scheme 9. A prodrug **15** of Zanamavir **14** with improved oral absorption (adapted from [26]).

Scheme 10. Structure of phosphopeptide mimetic prodrug **16** and the drug **17**.

Fig. 8. Molecular structures of 5-ASA **18** and Balsalazide **19**.

Scheme 11. PSA-dependent activation of **20** to **21**.

Scheme 12. General scheme for β-glucuronidase-responsive prodrugs (adapted from [34]).

Scheme 13. Activation of prodrug **22** to a DNA-alkylating agent in cancer cells.

Scheme 14. Activation of prodrug **24** by PSA proteolysis.

Fig. 9. Schematic representation of prodrug **27**.

Fig. 10. Chemical structures of **28**–**32**.

Scheme 15. Activation of prodrug **32** by “click and release” chemistry.

Scheme 16. Prodrug **34** containing a cleavable disulfide linker.

Scheme 17. Schematic representation of the preparation of **37** and their transformation to **39** in the acidic and hypoxic cancer cells.

Fig. 11. Schematic representation of a ProTide prodrug.

Fig. 12. Chemical structure of **40**, an inhibitor of HCV NS5B polymerase.