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Perspective

Lessons Learned from Marketed and Investigational Prodrugs

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1. Introduction

Prodrugs are an established concept to overcome barriers to a drug's usefulness. In Germany, about 6.9% of all marketed medicines can be classified as prodrugs, an estimate based on a conservative prodrug definition that does not include soft drugs and limited prodrugs. The limited prodrugs, which comprise an estimated 4% of the medicines marketed in Germany, are defined as active agents whose metabolite(s) also contribute(s) to the observed therapeutic activity. Approximately 49% of all marketed prodrugs are activated by hydrolysis, and 23% are bioprecursors (i.e., lacking a promoiety) activated by a biosynthetic reaction.[1] Noteworthy blockbuster prodrugs are, for example, omeprazole, simvastatin, lovastatin, enalapril, and aciclovir (Figure 1).

Regulatory guidelines pay limited attention to issues specific to prodrugs.[2] Thus, active drugs developed from prodrugs are considered as active metabolites. In medicinal chemistry, a prodrug strategy is practically never considered in the early phases of drug design but only when classical analoguing programs fail to provide the required drug profile. What, then, makes prodrug development so special and interesting? Prodrugs provide a rationale and opportunities to reach target physicochemical, pharmacokinetic, and pharmacodynamic properties. They can be designed to overcome pharmaceutical, pharmacokinetic, or pharmacodynamic barriers such as insufficient chemical stability, poor solubility, unacceptable taste or odor, irritation or pain, insufficient oral absorption, inadequate blood−brain barrier permeability, marked presystemic metabolism, and toxicity.[3] A developing field of high importance is that of rationally designed prodrugs for tissue or cell targeting. However, it is worth recalling that many successful prodrugs in current use are in fact accidental prodrugs, namely, agents that were not designed as prodrugs and were recognized as such only late in development or even postmarketing. This article intends to provide some arguments and guidelines for the early

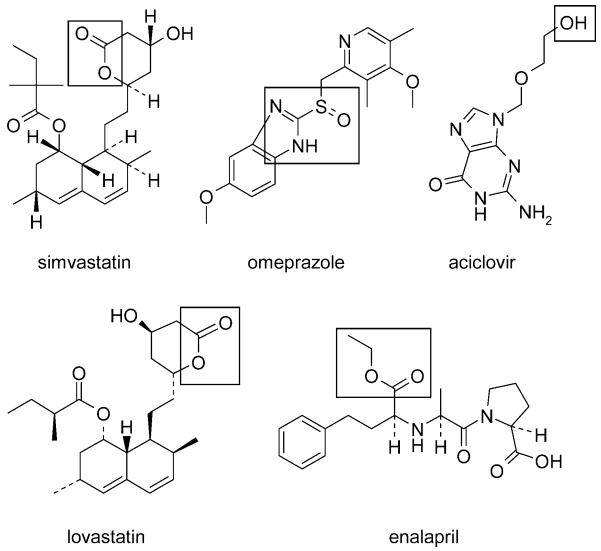

Figure 1. Some blockbuster prodrugs.

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recourse to, and successful application of, a prodrug strategy in drug discovery.

2. Definitions and Classification of Prodrugs

The term prodrug was first introduced in 1958 by Adrien Albert[4] to describe compounds that undergo biotransformation prior to eliciting their pharmacological effects. According to his definition (which we consider the best available), prodrugs are "therapeutic agents which are inactive per se but are transformed into one or more active metabolites." A later definition by Bundgaard[5] states that "By attachment of a pro-moiety to the active moiety, a prodrug is formed which is designed to overcome the barrier that hinders the optimal use of the active principle." Such a definition, however, is restricted to prodrugs carrying a promoiety. This definition is somewhat narrow because it excludes bioprecursors, modular prodrugs (i.e., comprising a trigger, a linker, and the active agent), and metabolites produced by conjugation reactions.

Albert's broader definition posits prodrugs as the opposite of soft drugs. The soft drugs (see section 3.3) are active analogues of a lead compound that are deactivated in a predictable and controllable way after having achieved their therapeutic effect.[6] But since both prodrugs and soft drugs find applications in local tissue targeting, they are sometimes confused in the literature.

Prodrugs can be classified according to two major criteria, namely, (a) chemical classes (carrier-linked prodrugs, bioprecursors (i.e., prodrugs lacking a promoiety), site-specific chemical delivery systems, macromolecular prodrugs, and drug−antibody conjugates) and (b) mechanism of activation (enzymatic versus nonenzymatic, activation by oxidation, reduction or hydrolysis, catabolic versus anabolic reactions).

The enzymatic versus nonenzymatic activation is of particular interest because both routes have advantages and disadvantages. While prodrug activation through bioconversion as a time- and tissue-controlled process has a clear benefit, inter- and intraspecies variability, genetic polymorphism, and the potential for drug−drug interactions (see section 3.1.4) pose significant challenges for such a prodrug strategy. When purely chemical prodrug activation is chosen (e.g., spontaneous chemical cleavage at physiologic pH), interspecies variability, genetic polymorphisms, and drug−drug interaction problems are no concern. However, there might be chemical stability issues (insufficient shelf life) and the site of prodrug activation is undefined. Interestingly, there are only a few reports on prodrugs designed to rely exclusively on a nonenzymatic activation principle[7] maybe because it is very difficulty to ascertain the absence of enzymatic participation.

The complexity of this broad multidisciplinary field makes it difficult to cover in depth all complementary viewpoints. Because some topics have already been covered by reviews and monographs,[8−10] this report will focus mainly on the inherent therapeutic benefit prodrugs can offer in terms of overcoming pharmaceutical, pharmacokinetic, or pharmacodynamic barriers and increased patient convenience.

3. Major Objectives of Prodrug Design

The drugs mentioned in the sections below are meant to illustrate various opportunities medicinal chemists may find in a prodrug strategy. However, this presentation should not be understood as covering all prodrugs in therapeutic use or in clinical development.

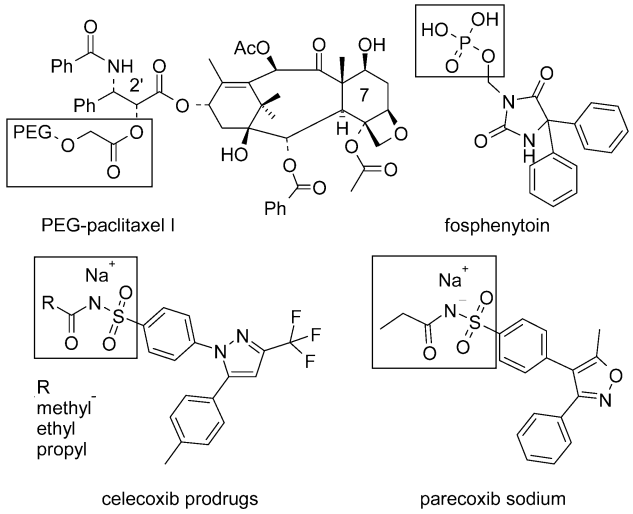


Figure 2. Examples of promoieties for improved aqueous solubility.

3.1. Improved Bioavailability. There are a variety of possibilities for a prodrug to improve bioavailability following oral dosing. We examine in turn aqueous solubility, passive intestinal absorption, targeted active absorption, and metabolic switching as well-established factors limiting bioavailability.

3.1.1. Improved Aqueous Solubility. Inadequate aqueous solubility is an important factor limiting parenteral, percutaneous, and oral bioavailability. In such cases, a prodrug strategy may bring great pharmaceutical and pharmacokinetic benefit. Charged promoieties (e.g., esters such as phosphates, hemisuccinates, aminoacyl conjugates, dimethylamino acetates) and neutral promoieties (e.g., poly(ethylene glycol)s, PEG) can be used. The latter promoieties, however, require PEG of high molecular weight to avoid a rapid clearance characteristic of low MW PEG conjugates. Improved aqueous solubility for better iv administration has been demonstrated for PEG−paclitaxel[11] (Figure 2), but we note here that 2′-PEG esters, but not 7′-PEG esters, are hydrolyzed enzymatically. Representative water solubilities of PEG−paclitaxel prodrugs are as follows: 2′-PEG^{5000}, 666 mg mL^{-1}; PEG^{20000}, 200 mg mL^{-1}; PEG^{40000}, 125 mg mL^{-1}; compared to 25 μg mL^{-1} for paclitaxel itself.[12]

Fosphenytoin is another example showing how parenteral delivery problems associated with a sparingly water-soluble drug can be overcome by a prodrug. Fosphenytoin[13] (Figure 2) is a hydrophilic phosphate prodrug of the anticonvulsant phenytoin and is hydrolyzed rapidly by phosphatases. Another example can be found in the parental administration of COX-2 inhibitors of the diarylheterocyclic class (e.g., celecoxib[14] and valdecoxib[15]). Intravenous treatment of acute pain and inflammation is hampered by their modest aqueous solubility (<50 μg mL^{-1}). A solution to the problem was afforded by the sodium salt of the acetylated sulfonamide prodrug (parecoxib sodium[15]) (Figure 2), which exhibits a significantly improved aqueous solubility (∼15 mg mL^{-1}). The acyl residue is rapidly cleaved in hepatic and intestinal preparations and in vivo to

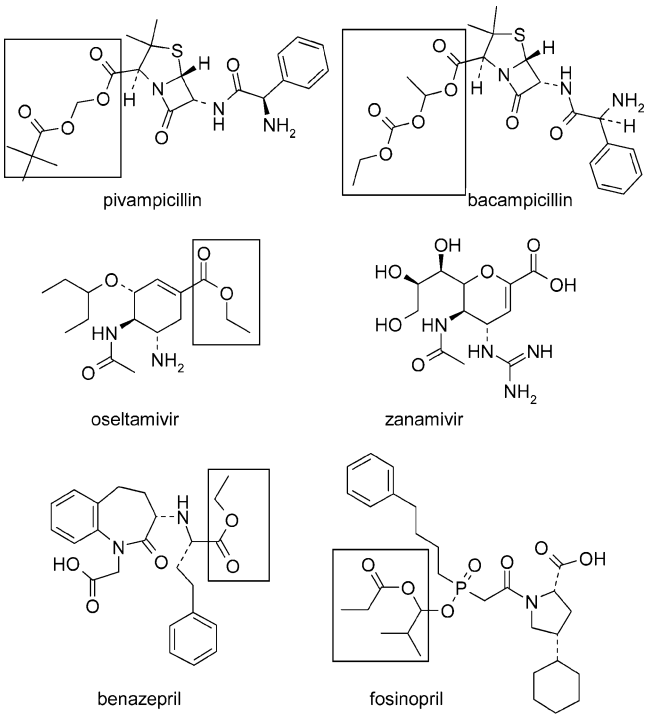


Figure 3. Examples of ester prodrugs showing improved passive intestinal absorption.

liberate the active drug. Parecoxib sodium is currently in the preregistration phase for parental administration. Improved oral bioavailability and dose linearity in pharmacokinetics have also been reported for acylated celecoxib prodrugs[16] whose sodium salt showed improved oral bioavailability with dose linearity over a wider dose range compared to celecoxib itself.

One of the potential problems in this approach is that solubilizing groups may sometimes generate toxic effects. Thus, the phosphate prodrug fosphenytoin was designed to enhance the aqueous solubility of phenytoin for intravenous administration, but the high phosphate concentrations at the site of injection produced some hypocalcemic effects leading to mild pruritus and paresthesia.[17] Interestingly, such side effects were moderate compared to those of the standard injectable sodium phenytoin.

3.1.2. Improved Passive Intestinal Absorption. Providing enhanced lipophilicity for increased passive intestinal absorption is the most frequent rationale when adopting a prodrug strategy (approximately 49% of all marked prodrugs are activated by hydrolysis[1]). Various esters of carboxylic acids (a frequently encountered group of carrier-linked prodrugs) are cleaved by hydrolysis (enzymatic and/or chemical) to liberate the active carboxylic acid. There are many examples such as ACE inhibitors[18] benazepril and fosinopril (Figure 3), statins such as simvastatin and lovastatin (Figure 1), and some antibiotics such as pivampicillin and bacampicillin (Figure 3). While this article was in preparation, Kevin Beaumont et al.[8] comprehensively reviewed the design of ester prodrugs to enhance oral absorption. We refer the reader to this in-depth analysis of ester prodrugs and the related challenges for the discovery scientists.

A nice example of the competitive advantage provided by a prodrug strategy is found in the class of neuraminidase inhibitors used against type A and type B influenza viruses. The prototypal drug is zanamivir (Figure 3), which shows that a very high polarity is incompatible with intestinal absorption and has to be administered via inhaler. Another neuramidase inhibitor is RO-64-0802, which like zanamivir is not absorbed orally because of its high hydrophilicity. However, RO-64-0802 is not marketed as such but as the ethyl ester known as oseltamivir (Figure 3). This prodrug is well absorbed orally, and its rapid in vivo enzymatic hydrolysis provides high and sustained plasma levels of the active parent drug.[19]

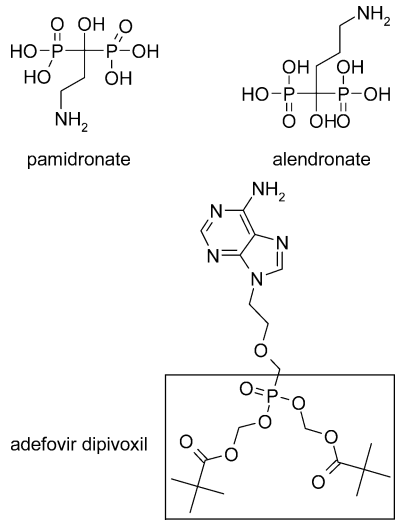


Figure 4. Examples of drugs incorporating phosphonate moieties and prodrugs thereof.

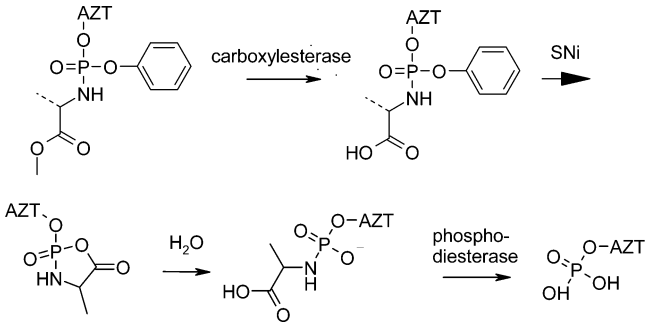


Figure 5. Proposed conversion of AZT-phosphoramidate to AZT-monophosphate.

A comparable situation is found in the field of bisphosphonates, where drugs with improved oral bioavailability are badly needed.[20] Indeed, pamidronate, alendronate (Figure 4), and analogues have a poor oral bioavailability (<1%).[21] While the methyl and ethyl phosphate esters are chemically and enzymatically too stable, the pivaloyloxymethyl and *S*-acetylthioethylphosphonate esters are converted enzymatically to the free phosphonate.[22] Similarly, adefovir dipivoxil, a bis(pivaloyloxymethyl) ester of the antiviral adenine nucleotide analogue adefovir,[23] was recently launched in the U.S. for the treatment of hepatitis B virus infection (Figure 4). Other conceptually successful phosphate prodrugs are the aryl phosphoramidates, which deliver the nucleoside monophosphates to cells. This has allowed the efficacy of AZT to be increased by several orders of magnitude (Figure 5).[24] Interestingly, this promising prodrug concept has not yet been applied to

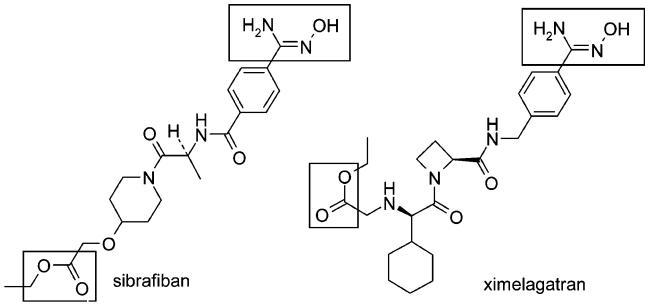


Figure 6. Amidoxime prodrugs on the market or in development.

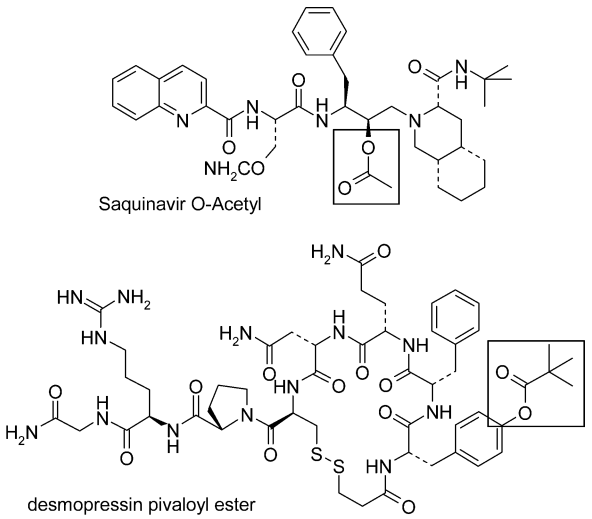


Figure 7. Examples of prodrugs of peptidomimetics.

phosphotyrosines. It should be mentioned in this context that one should be cautious with prodrugs of highly charged drugs, since their intracellular release might lead to entrapment and thus to toxicity.[25]

Besides carrier-linked prodrugs, there exist other approaches to reach optimal lipophilicity. Amidoximes can be used as bioprecursors for amidines (Figure 6). Amidoximes are less basic and thus unprotonated under physiological conditions, thereby enhancing intestinal absorption.[26] Reductases in the kidneys, liver, brain, lungs, and gastrointestinal tract are responsible for the rapid conversion of the inactive amidoximes to amidines. A GP IIb/IIIa-receptor-antagonist (sibrafiban) and a thrombin inhibitor ximelagatran (the "double" prodrug of melagatran) are based on the amidoxime strategy and are in clinical development. Ximelagatran is expected to become the first orally acting, direct thrombin inhibitor in medical use.[27]

Increasing evidence is being published on prodrugs of peptides, a class of pharmacologically important compounds that almost always suffer from mucosal permeation problems and rapid enzymatic degradation.[8,28] The same is true for some peptidomimetics. Thus, the pivaloyl ester of desmopressin (Figure 7), a synthetic analogue of vasopressin, showed improved transport across Caco-2 monolayers.[29] The perbutanoylated prodrug glycovir showed improved bioavailability in rat, dog, and monkey compared to the parent compound SC-48334. Transient cyclization of linear peptides using an esterase-sensitive linker yielded peptide prodrugs with improved Caco-2 permeability and increased stability toward peptidases. The *O*-acetyl

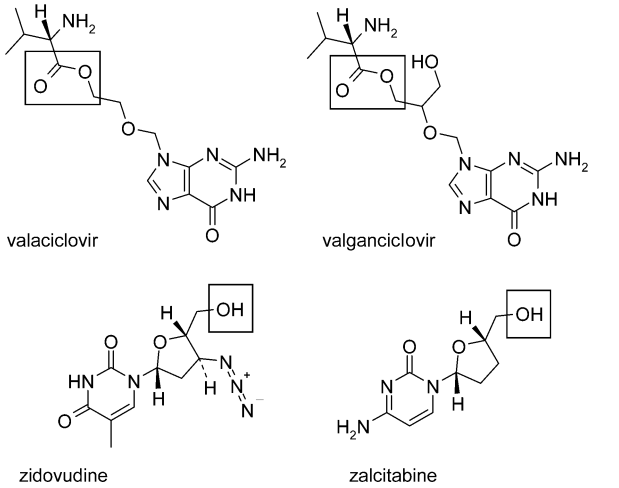


Figure 8. Example of (pro)drugs utilizing oligopeptide and nucleoside transporters.

prodrug of the HIV-protease inhibitor saquinavir showed improved oral bioavailability (Figure 7).[30]

Designing carrier-linked prodrugs, one should keep in mind to balance the increased lipophilicity necessary for transcellular absorption with sufficient aqueous solubility, otherwise oral bioavailability will become dissolution limited. Such cases have been reported for the bifunctional prodrugs of cephalosporines[31] and for the pivaloyloxymethyl ester of ceftizoxime.[32] In addition the toxicity potential of the promoiety should be evaluated or promoieties used that are already accepted by registration authorities and are known to be nontoxic. Recently the commonly used pivalic acid (trimethylacetic acid) was associated with some toxicity.[33] Also, the formaldehyde released upon hydrolysis of methylene-bridged double ester prodrugs can be a toxicological concern. The toxicologically more acceptable acetaldehyde, on the other hand, introduces a chiral center that increases complexity, since the two enantiomers might have distinct activation profiles.

3.1.3. Improved Transporter-Mediated Intestinal Absorption. Utilizing carrier-mediated transport is another successful prodrug strategy to actively enhance intestinal absorption.[34] For example, oligopeptide transporters are responsible for the active uptake of β-lactam antibiotics, the ACE inhibitor enalapril[35] (Figure 1), valaciclovir, and valganciclovir, to name a few (Figure 8). Valaciclovir, the valine ester of aciclovir, showed an oral bioavailability 5-fold higher than that of aciclovir itself.[36] The increased oral absorption is due to transport by the intestinal dipeptide transporter PEPT1.[37] Nucleoside transporters are involved in the uptake of nucleoside analogues such as zidovudine, zalcitabine (Figure 8), cladribine, ara-C, ara-A, and fludarabine. Bile acid transporters are used for the uptake of thyroxine, chlorambucil, and crilvastatin. Further classes of transporters include vitamin transporters (whose substrates include methotrexate, nicotinic acid, thiamine, and vitamin B12), organic cation transporters (e.g., choline, spermine, (+)-tubocurarine, and dopamine), organic anion transporters (e.g., methotrexate, cefodizime, ceftriaxone, and pravastatin), and glucose transporters (e.g., glucopyranosides and galactopyranosides). Successful targeting of active transport depends on the specificity and capacity of the transporters.

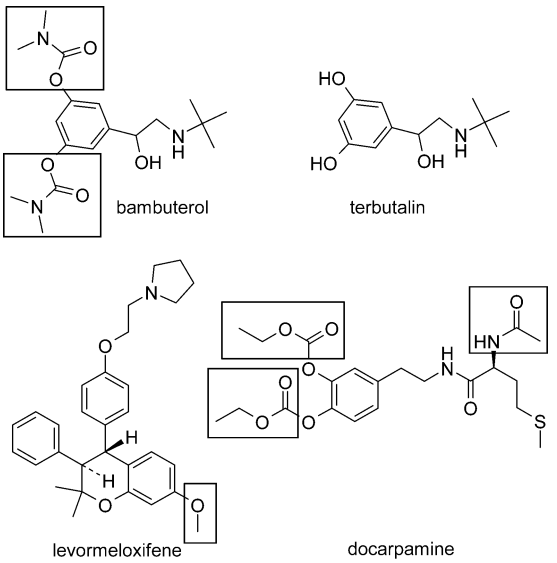


Figure 9. Example of prodrugs of metabolically labile drugs.

Furthermore, one has to be aware of potential drug−drug interactions due to saturation/inhibition of intestinal transporters.

3.1.4. Protection against Fast Metabolism (Slow-Release Prodrugs). The modification of metabolic pathways (metabolic switching) to alter the biotransformation pattern and protect against fast metabolic breakdown is another validated concept in prodrug design. While drugs with decreased metabolic stability (soft drugs) will be considered in section 3.3, we focus here on prodrugs offering increased metabolic or chemical stability and hence intensified and prolonged activity. In other words, the prodrug here is a protected form of the drug. Cases where the prodrug protects the drug against fast metabolism are presented first. At the end of the section, we discuss prodrugs that release a highly reactive and unstable active agent.

Metabolically labile but important pharmacophoric elements can be masked or capped to avoid rapid metabolism. In bambuterol (Figure 9), a prodrug of terbutaline, the phenolic groups are masked as carbamates.[38] This designed, long-acting bronchodilator is hydrolyzed by nonspecific cholinesterases. Its competitive advantage is demonstrated by once-daily dosage compared to 3 times daily for terbutaline.

A wealth of other examples can be briefly mentioned. Thus, docarpamine (Figure 9) is a cardiotonic, orally bioavailable double prodrug of dopamine whose bis-ethylcarbonate groups are cleaved in the intestine, followed by amide hydrolysis and conjugation in the liver.[39] Yet another example is levormeloxifene (Figure 9), an O-methylated prodrug of a selective estrogen receptor modulator (SERM).[40] Oxidative demethylation in vivo leads to enhanced oral bioavailability of the parent drug. Estrogen deficiency is also the target of SCH-57050, a bis-pivaloyl derivative of a 4-hydroxyphenylchromen-7-ol compound. Despite such success stories, one should not forget that in oxidative activation catalyzed by the cytochrome P450 (CYP) enzymes, special attention should be paid to potential drug−drug interactions. Concomitantly administered drugs utilizing the same CYPs for metabolism can produce drug−drug interactions potentially causing unacceptable systemic exposure and hence toxicity.

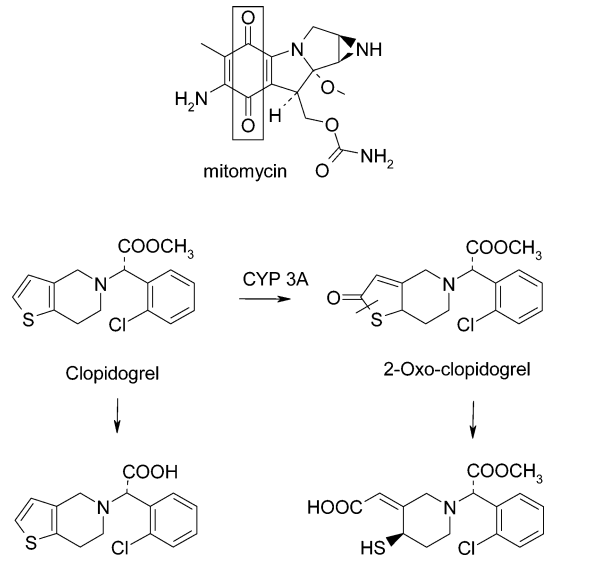


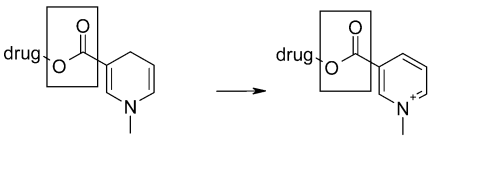
Figure 10. Prodrugs activated to a highly unstable active agent. The antiaggregating agent clopidogrel undergoes extensive hydrolysis in humans (ca. 85% of a dose) to the inactive acid. A smaller proportion of the dose is activated by cytochrome P450 3A to 2-oxoclopidogrel, which irreversibly antagonizes platelet ADP receptors via a covalent S−S bridge.[42]

Groups of particular interest are prodrugs activated to a highly unstable active agent. Thus, a number of antitumor drugs are in fact reduced within tumor cells to a reactive metabolite. This is exemplified by mitomycin, which is reduced by NADPH-cytochrome *c* reductase to provide the active semiquinone, which would not be stable enough to be effective in the cerebrospinal fluid (Figure 10).[41] While NADPH-cytochrome *c* reductase is not specific for tumor cells, its activity is favored in such cells because of their higher reductive capacity.

A comparable situation exists for the polyol nitrates marketed as coronary vasodilators (e.g., glycerol trinitrate, isosorbide dinitrate, isosorbide mononitrate), which can be seen as clinical release forms of nitric oxide.

A recent and quite revealing example is that of clopidogrel, whose mechanism of activation and action has been uncovered only recently[42] (Figure 10). Clopidogrel is an antiaggregating medicine that undergoes extensive hydrolysis in humans (ca. 85% of a dose) to the inactive acid. A smaller proportion of the dose is activated by cytochromes P450 3A to 2-oxoclopidogrel, which spontaneously and rapidly hydrolyzes to a highly unstable thiol.[43] The latter is the active agent, whose mechanism of action involves irreversible binding to platelet ADP receptors via a covalent S−S bridge.

3.2. Tissue-Selective Delivery. Selective delivery to the target cells or tissues is known to the public as the "magic bullet" metaphor.[44] While some drugs have a built-in capacity to accumulate in target tissues or cells, such a favorable pharmacokinetic feature is usually the objective of a prodrug strategy. And indeed, rationally designed tissue targeting is probably the most exciting objective of a prodrug strategy. This can be achieved by (a) passive enrichment in the target tissue, (b) targeting specific transporters, (c) targeting tissue- or cell-specific enzymes, and (d) targeting surface antigens. These strategies are presented and exemplified in turn below.



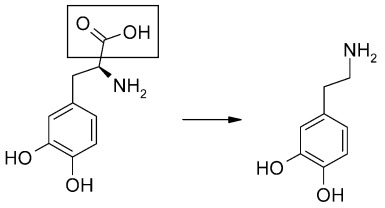


Figure 11. Example of prodrugs targeting the brain.

3.2.1. Passive Enrichment in the Target Tissue. Cytostatic and cytotoxic agents conjugated to PEG with MW > 50 000 (e.g., PEG−paclitaxel, Figure 2) are reported to exhibit an improved therapeutic efficacy due to their longer half-lives and selective accumulation in tumor cells. This passive tumor targeting is called the "enhanced permeability and retention" ("EPR") effect, and its mechanism is not fully understood.[45] Clinical phase II trials are ongoing using a PEG−Ala-camptothecin conjugate (prothecan[46]) for the treatment of stomach tumors. The low drug load achievable by such macromolecular conjugates requires more potently cytotoxic agents than traditional anticancer drugs.

An interesting prodrug strategy for brain targeting is that of site-specific chemical delivery systems (CDS).[47] After brain penetration, a lipophilic prodrug is converted there into a more hydrophilic molecule that remains "locked in". The same conversion taking place in the rest of the body results in increased peripheral elimination. The most frequently reported CDS is the 1,4-dihydrotrigonelline−trigonelline targetor system attached to the drug via an esterase-sensitive bond (Figure 11).[47] 1,4-Dihydrotrigonelline is rapidly oxidized by oxidoreductases, resulting in the formation of the inactive quaternary ammonium metabolite in the periphery and the brain. When formed in the periphery, this polar metabolite is rapidly excreted. In contrast, its formation in the brain leads to entrapment followed by enzymatic hydrolysis. This liberates the drug in situ, whereas the polar quaternary pyridinium ion moiety is rapidly eliminated from the brain, probably by a transporter. Dexamethasone-CDS and estradiol-CDS entered clinical development (phase I/II), but trials were stopped in 2001.

Prodrug activation in osteoclasts and osteoblasts can be utilized for bone targeting. The osteotropic drug delivery system (ODDS) relies on the bone-homing properties of bisphosphonates for the targeted delivery of drugs to the bone and bone marrow.[48] A few examples of ODDS delivery systems of estrogen and diclofenac are in exploratory stages.

3.2.2. Targeting Specific Transporters. The number of well-documented examples of prodrugs targeting tissue-specific transporters is relatively limited. Levodopa affords an apt example of enrichment resulting from active import of the prodrug into the brain followed by tissue entrapment of the active metabolite dopamine (Figure 11).[49] Levodopa is a substrate for the neutral amino acid transporters present at the blood−brain barrier (BBB). After brain entry, levodopa is decarboxylated to dopamine, which can act locally, being no longer a substrate for the neutral amino acid transporter. To increase the systemic half-life of levodopa, it has become customary to coadminister peripherally acting inhibitors of DOPA decarboxylase.

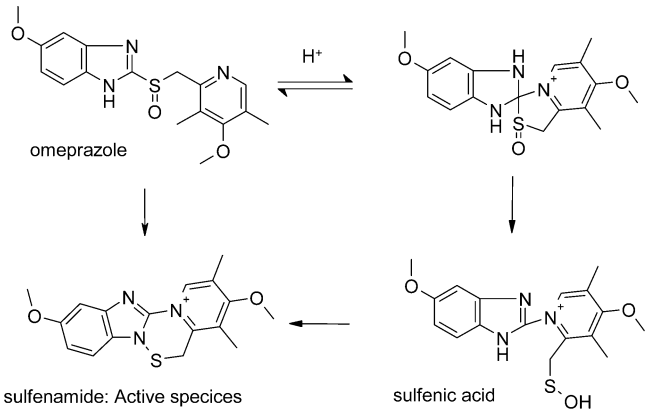


Figure 12. Activation of omeprazole.

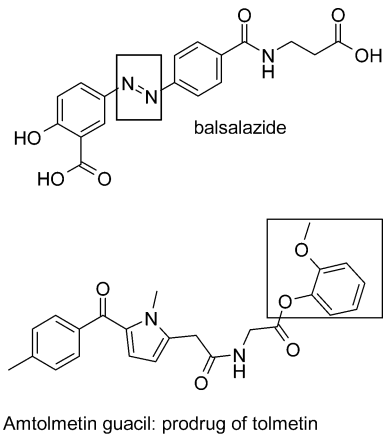


Figure 13. Examples of prodrugs for colon targeting or to avoid gastrointestinal side effects.

Prodrugs that target the liver utilize transporters predominately expressed in this organ. There are publications on bile acid conjugates of various cytostatic compounds, but no clinical reports appear to have been published.[50]

3.2.3. Targeting Tissue- or Cell-Specific Enzymes. The acidic environment of the stomach is utilized by the proton pump inhibitor omeprazole (Figure 12).[51,52] This potent antiulcer agent is a prodrug of a sulfenamide that exerts its effects by covalently modifying cysteine residues on the luminal side of the proton pump, i.e., the H^+/K^+-ATPase of the parietal cell in the oxyntic mucosa of the stomach. The prodrug omeprazole is only activated in the acidic enviroment of the oxyntic mucosa of the stomach where the drug exercises its antisecretory effect.

The reductive environment or the bacterial flora can be utilized for colon targeting. Balsalzide, a prodrug of 5-aminosalicylic acid (5-ASA) and an analogue of sulfasalazine, is specifically converted to 5-aminosalicilic acid by azo-reducing bacteria present in the colon (Figure 13).[53] The prodrug remains intact in the gastrointestinal tract until it reaches the colon, where it releases 5-ASA

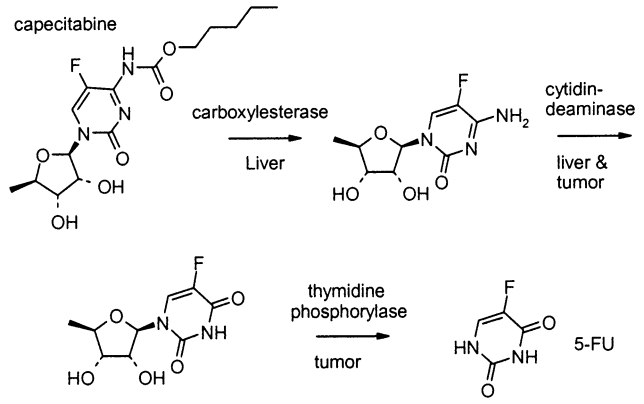


Figure 14. Activation of capecitabine.

to exert a local anti-inflammatory effect directly on the inflamed lining of the intestinal wall.

Alternatively to colon targeting, many prodrug projects are directed toward avoiding gastrointestinal side effects. A nice example of prodrug design is amtolmetin guacil, a lipophilic prodrug of the anti-inflammatory drug tolmetin created to reduce gastrointestinal side effects. In addition to beneficial lipophilicity, its hydrolysis in the gastrointestinal (GI) tract releases guajacol, which in turn activates inducible gastric nitric oxide synthase,[54] thereby reducing GI irritation (Figure 13). It is interesting in this context to mention that the extensively investigated esters of nonselective COX inhibitors did not show the desired GI protection, since GI side effects are a consequence of COX-1 inhibition and not of local irritation.

Specific targeting of virus-infected cell is elegantly realized by the antiviral prodrug aciclovir (Figure 1) and all other selective antimetabolites (a subgroup of bioprecursor prodrugs) used as antiviral agents. The activity of such antimetabolites implies their intracellular phosphorylation by kinases to form a nucleotide analogue that inhibits DNA synthesis. The older nonselective antiviral drugs were phosphorylated by native and by virus-induced thymidine kinases. In contrast, the selective antimetabolites are phosphorylated only by kinases coded by the viral genome and expressed in infected cells. As a result, aciclovir and all other selective antimetabolites are active only in infected cells.[55]

The selective delivery of cytotoxic drugs to tumor cells, without concomitant damage to normal tissues, is a major challenge in cancer chemotherapy. Prodrugs for active tumor targeting are therefore of high interest. Tumor specificity can be achieved by targeting transporter systems, enzymes having a higher activity in tumor cells, as discussed here, or surface markers (see section 3.2.4) or by gene-directed enzyme prodrug strategy (see section 3.2.5).

Capecitabine, an orally available triple prodrug of 5-fluorouracil (5-FU), offers a good example for a prodrug activated by tumor-specific enzymes (Figure 14). Following oral absorption, capecitabine undergoes three activation steps resulting in high tumor concentrations of the active drug, namely, (a) carboxylesterase-mediated hydrolysis in the liver, (b) cytidine deaminase mediated deamination in the liver and tumor cells, and (c) specific liberation of 5-FU by thymidine phosphorylase in tumor cells.[56]

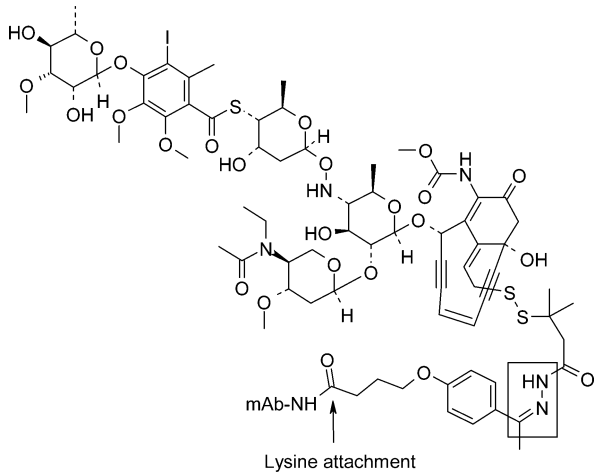


Figure 15. *N*-Acetyl-γ-calicheamicin linked via a hydrolytically labile hydrazone linker to a lysin N-atom of recombinant humanized anti-CD33 antibody.

3.2.4. Targeting Surface Antigens. Targeting surface markers appears as a selective and promising strategy in developing tumor-selective prodrugs. In 2000, the FDA approved the use of CMA-676 (gemtuzumab ozogamicin, mylotarg) for the treatment of acute myelotic leukemia (AML).[57] CMA-676 is an antibody-targeted chemotherapy agent consisting of a recombinant humanized anti-CD33 antibody linked via the lysin N-atom to *N*-acetyl-γ-calicheamicin, a potent cytotoxic agent (Figure 15). The linker incorporates a hydrazone moiety, a site for hydrolytic release,[58] previously shown to be required for activity.[59] Since this antibody recognizes AML blast cells but not hematopoietic stem cells, it allows selective delivery of the cytotoxic agent to leukemia cells. It was demonstrated that after CMA-676 infusion, the CD33 sites are quickly saturated and internalization of the conjugate occurs. However, elimination of leukemia correlates with the low capacity of these cells to extrude the drug conjugate. This finding is in good agreement with the fact that CMA-676 was ineffective in multidrug-resistant (MDR) sublines expressing P-glycoprotein. However, by combination of CMA-676 and MDR modifiers, an increased effect in MDR AML may be possible. While there are numerous preclinical reports on such carrier-linked prodrugs, successful clinical developments are slow to come.

3.2.5. Enzyme−Prodrug Cancer Therapy. Selective activation of prodrugs in tumor tissues can also be achieved by exogenous enzymes in a two-step approach. Prodrug-activating enzyme gene or functional protein is delivered selectively to tumor tissues, followed by systemic administration of a nontoxic prodrug that is activated by the exogenous enzyme. The net gain is a high local concentration of an active anticancer drug in tumors. Delivery of the enzyme gene is accomplished via viral vectors (VDEPT, virus-directed enzyme prodrug therapy) and nonviral vectors composed of chemical gene delivery agents (GDEPT, gene-directed enzyme prodrug therapy). Functional prodrug-activating enzymes are targeted to tumor tissue using enzyme−antibody conjugates (ADEPT, antibody-directed enzyme prodrug therapy). An exogenous enzyme is coupled to a monoclonal antibody (mAb) targeted to tumor cells. This enzyme−mAb conjugate is administered and allowed sufficient time to localize on tumor cells and clear from

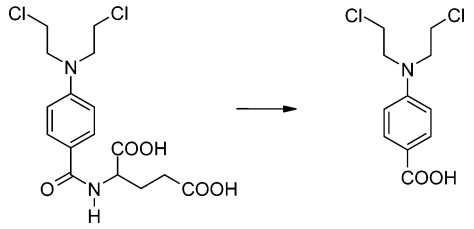


Figure 16. Structure of 4-[bis(2-chloroethyl)amino]benzoyl-L-glutamic acid used in ADEPT as a prodrug of the antitumor alkylating agent 4-[bis(2-chloroethyl)amino]benzoic acid.[62,63]

circulation. In a second step, a prodrug is administered that, being a selective substrate of the exogenous enzyme, will be selectively activated at the tumor site. ADEPT is in fact a more elaborate form of antigen-targeted therapy where the active drug is coupled to an antibody raised against tumor cells[60] (see also section 3.2.4).

A more recent example of the GDEPT strategy is the activation of methotrexate-Phe and other methotrexate-α-peptides in the vicinity of tumor cells by activated carboxypeptidase A (CPA).[61] CPA is normally synthesized as a zymogen that requires activation by proteolytic removal of its propeptide end by trypsin. To adapt this system to GDEPT, a mutant form of the enzyme (CPA_{ST3}) was engineered. This mutant did not require trypsin-dependent zymogen cleavage but was activated by ubiquitously expressed intracellular propeptidases. All evidence indicated that mature CPA_{ST3} was structurally and functionally similar to the trypsin-activated, wild-type enzyme. Furthermore, tumor cells expressing CPA_{ST3} were sensitized to the methotrexate prodrugs in a dose- and time-dependent manner.

To limit diffusion of CPA_{ST3}, a cell surface localized form was generated by constructing a fusion protein between CPA_{ST3} and the phosphatidylinositol linkage domain from decay accelerating factor (DAF). After retroviral transduction, both CPA_{ST3} and $CPA_{ST3-DAF}$ exhibited a potent bystander effect, even when <10% of the cells were transduced, because extracellular production of MTX sensitized both transduced and nontransduced cells.[60]

A relevant example of ADEPT using carboxypeptidases to release the anticancer agent 4-[bis(2-chloroethyl)amino]benzoic acid is presented here. Carboxypeptidase G2 [CPG2; EC 3.4.17.11] is a bacterial enzyme unknown in mammalian cells. It has been used with promising success to target cytotoxic alkylating agents to tumor cells. In a series of investigations, a CPG2−monoclonal antibody conjugate was targeted to a human choriocarcinoma cell line and a human colorectal cell line.[62] The prodrugs investigated, 4-[bis(2-chloroethyl)-amino]benzoyl-L-glutamic acid (Figure 16) and two analogues, were designed as selective substrates of CPG2. And indeed, the two tumor cell lines labeled with the CPG2−mAb conjugate effectively activated the prodrug to the anticancer agent 4-[bis(2-chloroethyl)-amino]benzoic acid (Figure 16), eliciting a strong cytotoxic response. Promising results were obtained in athymic mice with transplanted choriocarcinoma or colorectal xenografts. In a clinical study, an antibody-directed enzyme prodrug therapy using the prodrug in Figure 16 met conditions for effective antitumor therapy and gave evidence of tumor response in colorectal cancer.[63]

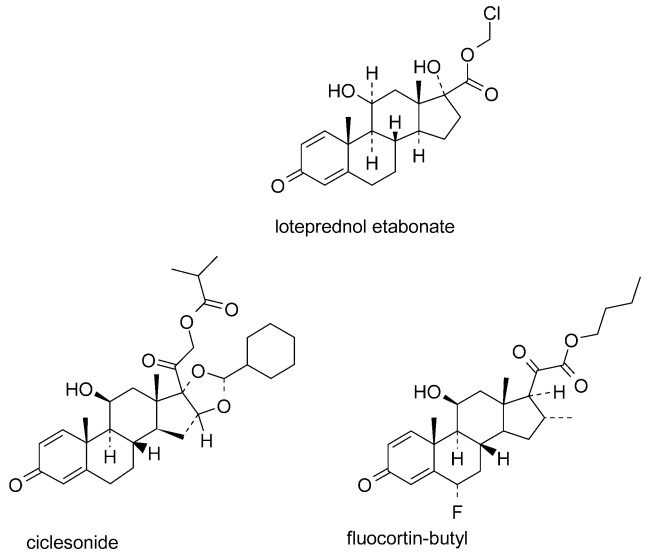


Figure 17. Examples of anti-inflammatory soft drugs.

While all three enzyme−prodrug strategies (GDEPT, VDEPT, and ADEPT) have been tested in clinical trials, there is still room for future improvements. GDEPT faces its major challenges in the selective delivery of the gene to the target tissue,[64] anti-DNA antibody formation, and insertional mutagenesis.[65] The drawback in VDEPT is that retroviral vectors only target dividing cells.[66] Even in a rapidly growing tumor only 6−20% of the cells are proliferating.[67] In ADEPT the immunogenicity of the enzyme−antibody complex can diminish efficiency in prolonged therapies. Furthermore, enzymes delivered by ADEPT might need to enter the cell for best prodrug activation. This requirement is challenged by the poor penetration rates of the large antibody−enzyme complexes.

3.3. A Glance at Soft Drugs. Drugs for local delivery (e.g., to the skin, the eyes, or the lungs) belong predominantly to the class of soft drugs. While being locally active, these drugs are systemically and readily inactivated. Although soft drugs are in essence different from prodrugs, their indisputable role in local delivery justifies a brief glance at this interesting subclass.

As stated above, soft drugs are active analogues of a lead compound that are deactivated in a predictable and controllable way after achieving their therapeutic role.[6] The majority of soft drugs are based on the inactive metabolite design. A known (or hypothetical) inactive metabolite of an existing drug is converted into an active structural analogue. This approach is complemented by the soft analogue design. These close structural analogues of known active drugs have a metabolically labile moiety to allow facile, one-step controllable deactivation and detoxification. Soft drugs play an important role as short-acting systemic drugs (e.g., esmolol in surgery, remifentanil in anesthesia, and ATI-2001 in arrhythmia).[68,69]

Soft drugs have also been examined as local delivery agents but with limited success. Fluocortin butyl, an ester soft drug of the inactive metabolite of fluocortolone, is on the market as a topical anti-inflammatory agent (Figure 17).[70] However, the gain in therapeutic benefit is limited because of low intrinsic activity.

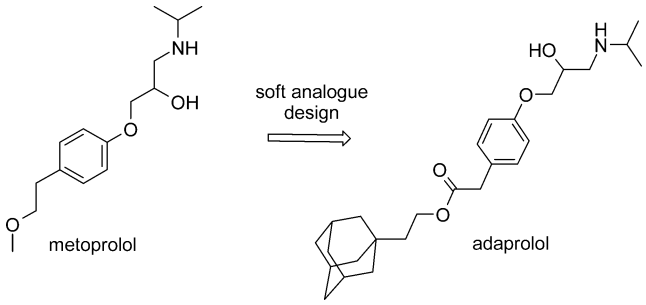


Figure 18. Soft analogue design of adaprolol.

Loteprednol etabonate, a soft drug analogue of hydrocortisone, is marketed for the local treatment of eye inflammation.[71] It was designed as an ester of the inactive metabolite cortienic acid, yielding a 20-fold improved therapeutic index compared to hydrocortisone itself. Loteprednol etabonate is currently under investigation in allergic rhinitis (Figure 17).

In the context of unsuccessful attempts to make soft derivatives of ascomycin, rapamycin, and FK-506,[72] it should be mentioned that soft drugs of already metabolically labile drugs are problematic. On the basis of the major inactive metabolite of the β-blocker metoprolol, the soft drug adaprolol (Figure 18) was originally designed for the local treatment of glaucoma, but the phase II results were disappointing and development stopped in 1995.[73]

The most promising pro-soft-drug for topical treatment of lung diseases (asthma and chronic obstructive pulmonary disease) is ciclesonide (Figure 17). This inhaled corticosteroid pro-soft-drug is activated by intracellular esterases yielding the active metabolite CIC-AP. The prodrug and its active metabolite have a very short plasma half-life owing to their high sensitivity to hepatic oxidases.[74]

4. Strategic Considerations

4.1. When Should a Prodrug Strategy Be Considered? Kevin Beaumont et al. in his recent review on the design of ester prodrugs conclude with the recommendations that the prodrug strategy should only be considered as a last resort to improve the oral bioavailability of important therapeutic agents.[8] While we certainly agree that a prodrug approach should not be taken lightly because of the additional complexity, we think that it may be considered in parallel with classical analoguing as soon as a problem becomes apparent. As stated several times, the prodrug strategy is especially promising to achieve pharmaceutical and/or pharmacokinetic properties that would be incompatible with a given pharmacophore. A prodrug might be considered when the structure−activity relationship (SAR) of a compound class for the drug target and the pharmacokinetic properties appear chemically incompatible, as schematized in Figure 19, e.g., when pharmacokinetically problematic moieties are essential requisites for biological activity (e.g., amidines, guanidines, phenolic groups, phosphate groups).[75,76] Alternatively, a prodrug can be designed for tissue (e.g., tumor) targeting to reduce systemic toxicity.

In summary, a prodrug approach should be explored when development of an innovative and very promising agent is precluded by a major pharmacokinetic or pharmaceutical defect. In contrast, a prodrug approach to overcome a defect should not be considered when several drugs already exist that lack this defect.

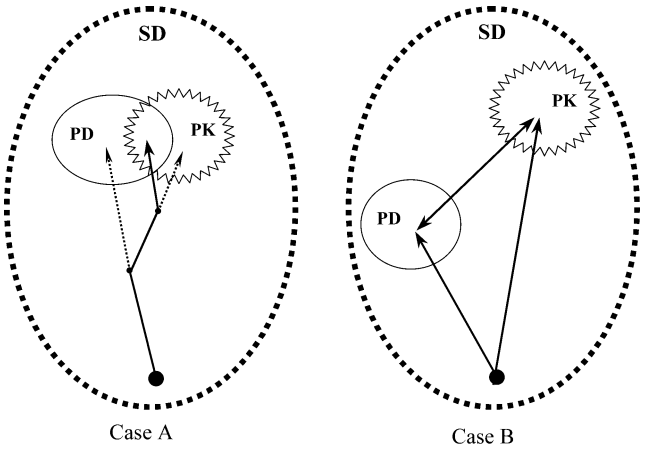


Figure 19. Two schematic situations encountered in a research project. SD symbolizes the undefined structural diversity space theoretically accessible in a given research project. PD symbolizes the ensemble within SD of all molecules having good pharmacodynamic properties. PK symbolizes the ensemble within SD of all molecules having good pharmacokinetic properties. Case A represents the favorable situation where there is partial overlap of the PD and PK chemical spaces. Case B represents the difficult situation where there is incompatibility between the structural conditions for good pharmacodynamic and good pharmacokinetic properties. Case A is seen to evolve toward candidates that combine good PD and PK properties. This objective is out of reach in case B, where a prodrug strategy may be the only alternative to save the project.[76] The sooner a given research project is found to belong to case B, the more successful a prodrug strategy may be.

At the beginning of each prodrug program, there should be a clear definition of the problem to solve and defect to improve. If, for example, poor oral bioavailability is the problem, one should try to identify its cause, be it poor aqueous solubility, mucosal absorption, or metabolic lability. The prodrug approach will then aim at improving the identified defect(s). However, the prodrug approach should not be misunderstood as a universal solution to all barriers to a drug's usfulness.

4.2. Prodrug Design and Characterization. Prodrug design will always start with the investigation of the potential of the parent drug for a prodrug strategy. The potential pharmacophoric groups and sites of metabolism in the candidate have to be assessed. When the attachment of a promoiety is considered, one has to keep in mind that the chemical stability of the linker and hence the prodrug must be sufficient to enable synthesis, purification, and formulation. Especially methylene-bridged double esters have a low intrinsic chemical stability[77] and are justified only when simple alkyl or aryl esters are not hydrolyzed in vivo. Alkyl esters, substituted with basic moieties (e.g., tertiary amines) have not only a very low half-life in human plasma[78] but also a decreased chemical stability in buffered solutions at physiological pH.[79] The toxicity potential of the promoiety should also be evaluated. If possible, promoieties already accepted by registration authorities or that are known to be nontoxic should be used. However, even the commonly used pivalic acid (trimethylacetic acid) was recently associated with some toxicity.[33] When the design of carrier-linked prodrugs

is aimed at improved passive intestinal absorption, one should keep in mind that the increased lipophilicity might give rise to counterproductive physicochemical properties such as insufficient aqueous solubility and a pronounced first-pass effect. Such cases have been reported for the bifunctional prodrugs of cephalosporines[31] and for the pivaloyloxymethyl ester of ceftizoxime.[32] One should be generally cautious with prodrugs of highly charged drugs. Intracellular release of drug might lead to entrapment, nonproductive metabolism, and toxicity.[25]

The majority of all prodrug approaches face the challenge of identifying the optimal prodrug plus its activation system to enhance or prolong the concentration of the active principle at the site of action. Because of the complex situation of prodrug transport and processing, we recommend, especially for novel prodrug principles, that the first step should be to design and investigate different prodrug prototypes of high diversity (different attachment sites, linkers, promoieties, hydrolytic, oxidative, reductive activation, chemical vs enzymatic activation). The feasibility of these prototypes should subsequently be evaluated with appropriate in vivo pharmacokinetic experiments. Unfortunately, the literature is full of sophisticated prodrug design that often lack proper characterization. Therefore, we want to stress the fact that both intravenous and oral pharmacokinetic experiments with both the active principle and the prodrug prototypes have to be performed early on. If superior drug levels can be detected in the blood, plasma and/or target tissue after administration of the prodrugs compared to the drug, one should try to narrow down or, preferentially, to identify the enzyme(s) and potential transporters involved in prodrug transport and activation. Once these enzyme(s) are identified, the prodrug might be optimized with regard to target objectives using in vitro profiling, using enzymes from several species including human.[9] This verification of prodrug processing enzymes is important because the frequently used cleavage rates in plasma may be the wrong parameter to monitor and to optimize.[9] In addition, tissue fractions from metabolically competent organs (liver, intestine) like liver hepatocytes and gut wall microsomes have to be considered.[9]

One has to pay special attention to the metabolic differences among animal species. For example, rats show significant higher rates of esterase-mediated hydrolysis than man.[80] Therefore, pharmacokinetic studies in rats with esterase-activated prodrugs might overestimate the release of the active principle in man. For targeting tissue- or cell-specific enzymes for prodrug activation, an enzyme family should be preferred over a single enzyme because of potential saturation problems. In oxidative activation catalyzed by the cytochrome P450 (CYP) enzymes, special attention should be paid to potential drug−drug interactions. Concomitantly administered drugs utilizing the same CYPs for metabolism can cause unacceptable systemic exposure and hence toxicity. Besides species specificity and potential drug−drug interactions, genetic polymorphism might be an issue for the targeted biotransformation and should be checked as early as possible. There are specific transporters in nearly all tissues that could be utilized for tissue-specific prodrug targeting.[34] Transporters are expected to be functionally equivalent across species consuming the same food.

The full development of both the prodrug and the drug is usually not necessary. However, both compounds should be available for preclinical profiling, e.g., to elucidate metabolic pathways and to asses toxicity. Tissue distribution studies using radiolabeled material should be envisaged at an early stage. Ideally, a double-labeling strategy (drug and promoiety) should be considered so that both the prodrug and the drug can be traced.

5. Conclusions

The aim of this perspective article has been to present and discuss the various advantages a prodrug strategy might bring to a drug discovery program including carrier-linked prodrugs, bioprecursors, site-specific chemical delivery systems, macromolecular prodrugs, and drug−antibody conjugates. The main focus on marketed prodrugs has illustrated that prodrug strategies have been successful for a number of important therapeutic agents! However, the focus on victorious prodrugs should not be misunderstood as neglecting the inherent difficulties and additional layers of complexity a prodrug strategy might face. We hope that this article will further stimulate medicinal chemists to be creative when they face situations where the SARs for the drug target are incompatible with pharmacokinetic objectives or to consider a prodrug approach for rationally designed tissue/cell targeting early in a drug discovery program.

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Biographies

Peter Ettmayer graduated from the Vienna University of Technology, Austria, specializing in synthetic organic chemistry and received his doctoral degree there in 1990 (*sub auspiciis praesidentis*). After a postdoctoral stay at the Christian Doppler Laboratories for Chiral Compounds & Chemical Synthesis, he joined the Novartis Research Institute in Vienna as Laboratory Head in 1991. In 1996 Peter worked 1 year at the Novartis research facility in New Jersey and concomitantly received training in solid-phase chemistry at Pharmacopeia, Princeton. His main areas of interest are virology, oncology, and immunopathology covering all fields of medicinal chemistry. Since 1999 he is Chairman of the Medicinal Chemistry Section of the Austrian Chemical Society.

Gordon L. Amidon is Professor of Pharmaceutical Sciences and the Charles R. Walgreen, Jr. Professor of Pharmacy at the University of Michigan. He is internationally known for his research in the fields of drug absorption, transport phenomena, solubility, dissolution, prodrugs, and molecular drug targeting. He has published extensively in journals, with over 200 published papers and 250 abstracts and 16 U.S. patents, and has contributed chapters to over 30 books. He has developed a Biopharmaceutics Classification System (BCS), with the FDA, which had an impact on bioequivalence standards worldwide. Professor Amidon is the Editor of the new American Chemical Society journal *Molecular Pharmaceutics*.

Bernd Clement graduated with a specialty in pharmacy and chemistry and received his Ph.D. in Pharmaceutical/Medicinal Chemistry from the University of Marburg in 1978 and his habilitation from the University of Freiburg in 1985. He was a Postdoctoral Fellow with Prof. A. H. Beckett at Chelsea College, University of London (1978−1979). Since 1990 he is Full Professor of Pharmaceutical/Medicinal Chemistry and Director of the Pharmaceutical Institute, University of Kiel. His main research interests are all aspects of the biotransformation of nitrogen-containing functional groups including the development of prodrugs. He developed a prodrug principle for amidines, which has been applied to several drug candidates. He is the Chairman of the Pharmaceutical/Medicinal Chemistry Section of the German Pharmaceutical Society.

Bernard Testa graduated with a specialty in pharmacy and obtained his Ph.D. on the physicochemistry of drug−macromolecule interactions. After 2 years as a Postdoctoral Fellow with Prof. Arnold H. Beckett, he became Full Professor and Head of Medicinal Chemistry in 1978. He served as Dean of the Faculty of Sciences (1984−1986), Director of the Geneva-Lausanne School of Pharmacy (1994−1996 and 1999−2001), and President of the University Senate (1998−2000). He has written 4 books, edited 28 others, and (co)authored 450 research and review articles in the fields of drug design and drug metabolism. His recently granted Emeritus status has freed him from administrative duties and gives him more time for writing, editing, and collaborating in research projects.

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(1) *Rote Liste*; Editio Cantor Verlag: Aulendorf, Germany, 2002. This is a list of nearly all marketed drugs in Germany containing 1562 different active substances. Of these, 108 could definitely be classified as prodrugs (6.9%), 53 of which are activated by hydrolysis (49%) and 25 (23%) are bioprecursors (i.e., lacking a promoiety) activated by a biosynthetic reaction. In addition, 62 (4%) can be considered as limited prodrugs (i.e., active substances forming one or more active metabolites).

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