Part One
Prodrug Design and Intellectual Property

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Prodrug Strategies in Drug Design

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1.1
Prodrug Concept

Prodrugs are bioreversible derivatives of pharmacologically active agents that must undergo an enzymatic and/or chemical transformation *in vivo* to release the active parent drug, which can then elicit its desired pharmacological effect [1–4]. According to this strict definition, active agents whose metabolites contribute to a pharmacological response and salts of active drugs, which have sometimes mistakenly been referred to as prodrugs, are not considered to be prodrugs. In most cases, prodrugs are simple chemical derivatives that are one or two chemical or enzymatic steps away from the active parent drug. Some prodrugs lack an obvious carrier or promoiety, but result from a molecular modification of the active drug itself *in vivo*. Such a modification can be, for example, a metabolic oxidation or reduction that generates a new and active compound. These prodrugs are usually referred to as "bioprecursor prodrugs." In some cases, a prodrug may consist of two pharmacologically active drugs that are coupled together in a single molecule, so that each drug acts as a promoiety for the other. Such derivatives are called "codrugs" [5]. Finally, "soft drugs," which are often confused with prodrugs, also find applications in tissue targeting. In contrast to prodrugs, soft drugs are active drugs as such but are designed to transform into an inactive form *in vivo* after achieving their therapeutic effect [6]. The prodrug concept is illustrated in Figure 1.1.

Prodrugs have been classified according to several criteria; these being, for example, based on therapeutic categories, or based on categories of chemical linkages between the parent drug and the promoiety, or based on mechanism of action of a prodrug. A recently proposed more systematic approach categorizes prodrugs on the basis of their two cellular sites of conversion: intracellular (e.g., antiviral nucleoside analogues and statins) and extracellular be it in digestive fluids or the systemic circulation (e.g., valganciclovir, fosamprenavir, and antibody-, gene-, or virus-directed enzyme prodrugs) [7, 8]. Both types can be further categorized into subtypes depending on whether or not the intracellular converting location is also the site of therapeutic action, or the conversion occurs in the gastrointestinal fluids or

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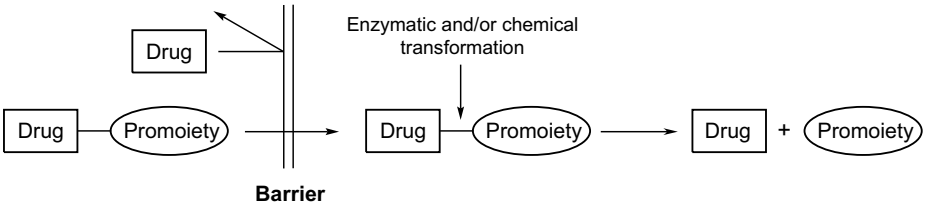


Figure 1.1 Simplified representation of the prodrug concept. The drug–promoiety molecule is the prodrug that is typically inactive pharmacologically. In broad terms, the barrier can be thought of as any biological liability for a parent drug that prevents optimal (bio)pharmaceutical or pharmacokinetic performance. This barrier must be overcome in order to achieve a marketable drug.

systemic circulation. From a regulatory perspective, this new classification system will certainly help in the understanding of a prodrug's pharmacokinetics and safety.

1.2 Basics of Prodrug Design

The design of an appropriate prodrug structure should be considered in the early stages of preclinical development, bearing in mind that prodrugs may alter the tissue distribution, efficacy, and even the toxicity of the parent drug. Although designing a prodrug so as to include all important factors in one molecule is admittedly very challenging, it can still be more feasible than searching for an entirely new therapeutic agent that has the desired properties. Moreover, the prodrug approach can enable the selection of a suitable drug candidate faster. The main factors that should be carefully considered when designing a prodrug structure are as follows:

- Which functional groups on the parent drug are amenable to chemical derivatization?
- Chemical modifications made to the parent drug must be reversible and allow the prodrug to be converted back into the parent drug by an *in vivo* chemical and/or enzymatic reaction.
- The promoiety should be safe and rapidly excreted from the body. The choice of promoiety and relative safety should be considered with respect to the disease state, the dose, and the duration of therapy.
- The absorption, distribution, metabolism, and excretion (ADME) properties of parent drug and prodrug require a comprehensive understanding.
- Possible degradation by-products can affect both chemical and physical stability that lead to the formation of new degradation products.

Arguably, the most common approaches for prodrug design are aimed at prodrugs undergoing metabolic bioconversion to the active parent molecule by functionally prominent and diversity-tolerant hydrolase enzymes such as peptidases, phosphatases, and, especially, carboxylesterases [9]. Because they are distributed throughout

the body, the potential for carboxylesterases to become saturated or the potential for their substrates to become involved in drug–drug interactions is generally insignificant [10], but not unprecedented [11]. Although esterases in general provide a good starting point for prodrug design strategy, premature bioconversion by first-pass metabolism may hinder the success of prodrugs that rely on esterase activation. Moreover, *in vitro* assessments of the hydrolysis rates are not always good predictors of the relative rates of the *in vivo* conversion of a prodrug because of confounding physiological processes that cannot be completely controlled in such studies. Cytochrome P450 (CYP450) enzymes, which are prominent in the liver and are also present in the intestine and lung, have been both intentional [12–14] and unintentional [15] targets for some prodrug strategies; however, these enzymes are not as reliable as esterases in prodrug design due to individual variations in liver functions. Finally, there is a growing interest to design prodrugs that are devoid of a detachable promoiety; in other words, bioprecursor prodrugs that are activated by oxidative or reductive metabolism [16, 17]. Figure 1.2 illustrates prodrug structures for the most common parent drug functionalities. Further discussion of functional group approaches in prodrug design, with representative examples, is given in Chapter 3.

1.3 Rationale for Prodrug Design

Drug discovery is an exceedingly complex and demanding enterprise. During the drug discovery process, new molecular entities (NMEs) are identified by using various techniques that include rational and receptor-based drug design, combinatorial chemistry, and high-throughput screens, or isolating and characterizing active components from living organisms, such as plants, fungi, or bacteria. These technologies can produce novel lead structures with high pharmacological potency. However, until the mid-1990s these technologies frequently ignored important physicochemical and biopharmaceutical aspects of the discovered molecules. These classical drug discovery paradigms often led to drug candidates with poor "drug-like" properties and faced significant problems in later drug development [18, 19]. The term "drug-like" is defined as those compounds that have sufficiently acceptable absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties [19, 20]. Poor outcome resulted in a high attrition rate of potential drug candidates in initial clinical studies when ADMET parameters were not thoroughly studied during preclinical phases. This poor outcome eventually prompted an emphasis to prioritize ADMET-related parameters into the HTS format at earlier decision points, which has enabled the optimization of lead compounds for ADMET properties during the early stages of drug discovery.

The process of developing a prodrug is now more focused also on optimizing the ADMET properties of potential pharmacological compounds, which consequently increases the eventual utility of potential drug candidates [1, 21–26]. Some of the main barriers, which are not limited to a drug's ADMET properties yet may be

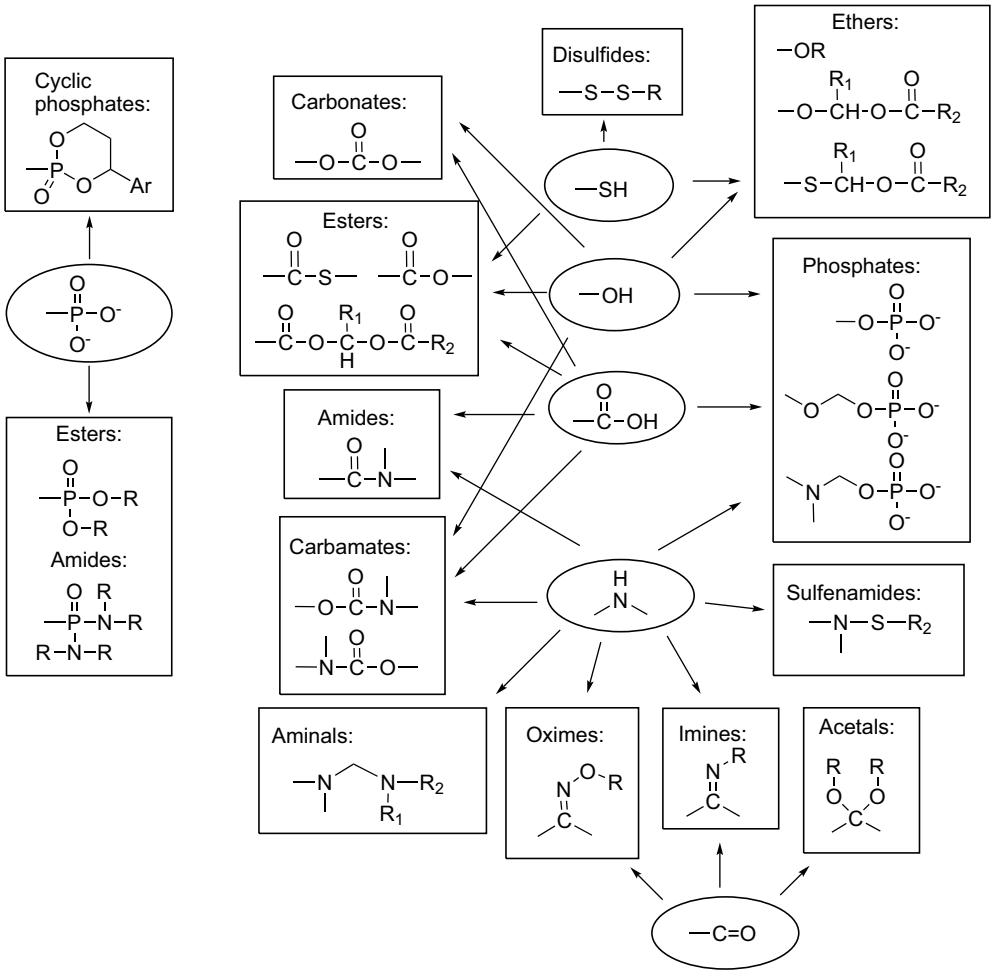


Figure 1.2 Common functional groups amenable to prodrug design. Most prodrugs require "synthetic handles," which are typically heteroatomic combinations.

overcome by a prodrug modification, are listed in Table 1.1 [4, 24]. It should be understood that these obstacles are often intertwined. Several of these issues are also briefly discussed in the following sections.

1.3.1 Overcoming Formulation and Administration Problems

Sufficient aqueous solubility of a drug is a prerequisite for the preparation of aqueous-based solutions for parenteral or injectable drug dosing. When conventional formulation techniques are not always successful or even possible, such as salt

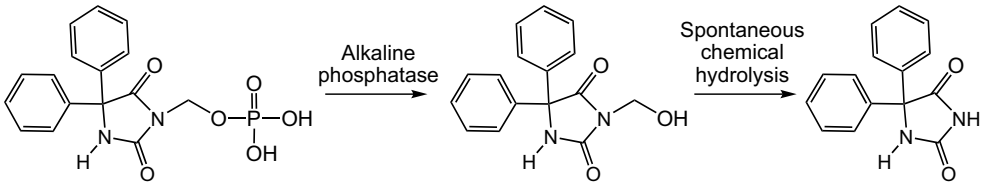
Table 1.1 Prodrugs can be used to address the following barriers to a drug's usefulness.

Formulation and administration
• Inadequate aqueous solubility for liquid dosage forms
• Inadequate shelf life for solid or liquid dosage forms
• Pain or irritation after local administration*
• Unpleasant taste or odor
Absorption
• Inadequate dissolution rate due to low aqueous solubility
• Poor membrane permeation and low oral or topical (e.g., dermal and ocular) bioavailability due to poor lipophilicity
• Inadequate stability in acidic gastric juices or during first-pass metabolism
• Inadequate availability due to efflux mechanisms
Distribution
• Lack of site specificity (e.g., poor brain or tumor targeting)*
• Need to decrease plasma protein binding or deposition in lipophilic compartments
Metabolism and excretion
• Lack or need of site-specific bioactivation*
• Short duration of action
Toxicity
• See entries above marked with asterisk* (typically associated with lack of site specificity)
• Need to temporarily mask a reactive, inherently active, functional group
Life cycle management
• Development of a prodrug from an existing drug, to achieve improved properties that may represent a life cycle management opportunity.

formation, a prodrug strategy becomes an extremely valuable option. From a technical and a commercial point of view, there are several successful prodrugs with improved aqueous solubility properties that serve as solved examples of formulation and administration problems with parenteral administration [27, 28]. The most common approach has been to increase water solubility by introducing an ionizable/polar promoiety to the parent drug. A number of phosphoric acid esters have been developed as potential water-soluble prodrugs, especially for parenteral administration, as the increase in solubility imparted by the dianionic phosphate group is often several orders of magnitude [27].

An excellent example of this is fosphenytoin (**1**), a phosphate ester prodrug of the poorly water-soluble anticonvulsant phenytoin (**3**), which can be used in both intravenous and intramuscular administration [29, 30]. Although the simple sodium salt of this weakly acidic ($pK_a = 8.3$) drug also exhibits good water solubility at high pH values, it can cause local irritation at the injection site, which is due to drug precipitation as the pH adjusts to the physiological range. With fosphenytoin, a phosphate ester is attached to an acidic amine functionality of phenytoin via an oxymethylene spacer. Therefore, this prodrug example demonstrates the use of "spacer" or "linker" group in prodrug design, which can be a viable option to form prodrug if there are no functional groups on the parent drug amenable to direct chemical derivatization. With fosphenytoin this modification leads to a remarkable

increase in aqueous solubility (from 20–25 μg/ml for phenytoin to 140 mg/ml for fosphenytoin) [31]. While being stable at neutral pH values, fosphenytoin is completely converted back to phenytoin *in vivo* by alkaline phosphatases through a chemically unstable aminol intermediate (**2**), with half-lives ranging from 7 to 15 min in humans [30, 31].



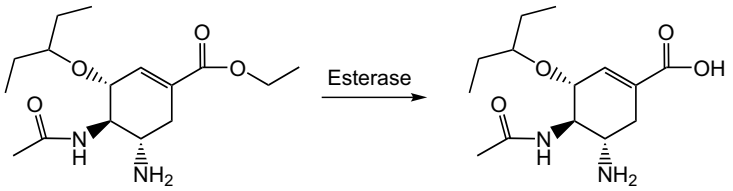
1 **2** **3**

1.3.2
Overcoming Absorption Barriers

The most successful area of prodrug research has so far been the improvement of passive drug permeation across various epithelial cell membranes, and a majority of these prodrugs have been developed to improve absorption from the GI tract. Similarly, the prodrug approach has been used to improve topical absorption through transdermal and ocular administration. These two approaches are discussed in Chapters 7 and 8.

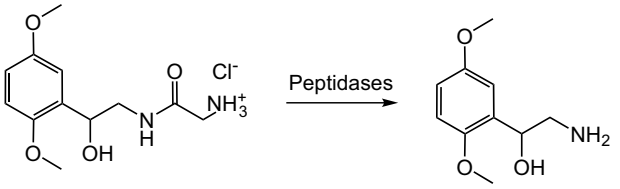
The oral bioavailability of a drug may be limited by its aqueous solubility, low permeability, propensity to be an efflux substrate, and both rapid and extensive hepatic metabolism followed by biliary excretion. Since unfacilitated and largely nonspecific passive transport mechanisms are the most common absorption routes in oral drug delivery, better diffusion across the biologic phospholipid bilayers, and thus better oral bioavailability, can be achieved by increasing the lipophilicity of an active agent by masking polar ionized or nonionized functional groups.

Several lipophilic alkyl and aryl ester prodrugs are in clinical use [22], of which oseltamivir (Tamiflu®) represents a very recent and successful example. Oseltamivir (**4**) is an orally active prodrug of oseltamivir carboxylate (**5**, GS4071, Ro 64-0802), which is a selective inhibitor of viral neuraminidase glycoprotein in both influenza A and B [32–34] and has some antiviral activity against the H1N1 influenza virus ("swine" flu). As an ethyl ester, oseltamivir is both rapidly and well absorbed, and this modification also increases the oral bioavailability of oseltamivir carboxylate from 5 to 79% [34, 35]. Oseltamivir undergoes fast bioconversion to oseltamivir carboxylate, mostly by human carboxylesterase 1, and maximum plasma levels of oseltamivir carboxylate are reached within 3–4 h after oral dosing [32, 35]. It is interesting to mention another neuraminidase inhibitor in this context: zanamivir (Relenza®), which was the first of the two to be marketed, but was available only in an inhaled form due to its hydrophilicity, and was soon outsold by oseltamivir as an oral tablet formulation.



4 **5**

An alternative means of increasing oral absorption of a drug is carrier-mediated transport, which is particularly important where a drug is either polar or charged and where passive transcellular absorption is negligible. While surprisingly many drugs already take advantage of gastrointestinal tract transporters [36], a number of prodrugs have been designed to have structural features that would allow them to be recognized and taken up by one of these transporters. A good example that exploits carrier-mediated transport is midodrine (**6**), which is an oral prodrug of desglymidodrine (**7**, DMAE), a selective α1-receptor agonist for the treatment of orthostatic hypotension [37, 38]. Midodrine contains a glycine promoiety that is attached to the amine functionality of DMAE, and it is converted into its parent active drug primarily in the liver and in the systemic circulation by unknown peptidases [37]. Midodrine is a substrate for the di- and tripeptide transporter (hPEPT1), and this carrier-mediated transport raises the bioavailability of midodrine to 93% from 50% for that of DMAE [38].

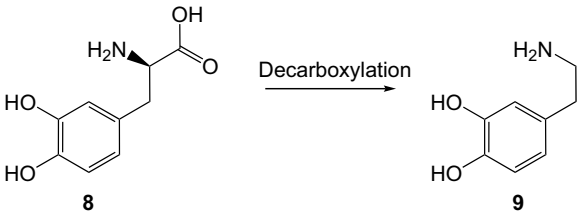


6 **7**

1.3.3
Overcoming Distribution Problems

There are several prodrugs that have been able to successfully alter the distribution of an active parent drug by achieving site-selective delivery. While most of these examples also address side effects and toxicity issues associated with a parent drug, and are discussed in more detail in Section 1.3.5, a classic yet still interesting example of directing distribution by a prodrug is given by levodopa (**8**, L-dopa). The neurotransmitter dopamine (**9**) is not able to cross the blood–brain barrier and is poorly distributed in the brain because of its hydrophilic nature. However, the conversion of dopamine into its α-amino acid, levodopa, enables the uptake of dopamine into the brain via the large neutral amino acid transporter (LAT1) [39, 40]. After entering the brain tissue, levodopa is rapidly converted back to dopamine by dopa decarboxylase, and being a very hydrophilic molecule, it is trapped close to the active site, thus

enabling its pharmacodynamic effects. Because decarboxylation also takes place prematurely in the peripheral tissues, a polar dopa decarboxylase inhibitor, carbidopa, is consequently coadministered with levodopa.



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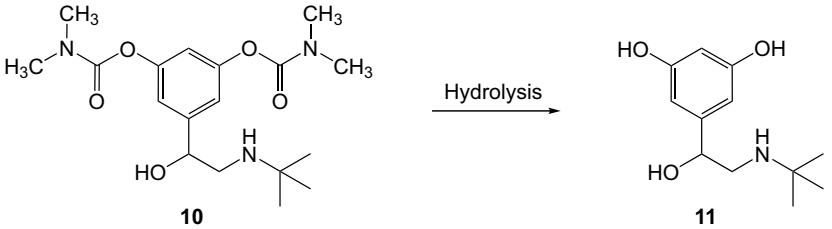
1.3.4
Overcoming Metabolism and Excretion Problems

The use of prodrugs to attenuate the rapid elimination of a drug caused by metabolism and/or excretion is not as frequently used strategy as various controlled release formulations or making drug analogues, but a few examples exist that use prodrugs. The rationale for making a prodrug derivative is often to mask that metabolically labile but pharmacologically essential functional group(s) in order to avoid rapid metabolism. In the case of the bronchodilator and β_2-agonist terbutaline (**11**), a sustained drug action is achieved with its bis-dimethylcarbamate derivative, bambuterol (**10**). Protection of the phenolic moieties, which are susceptible to rapid and extensive presystemic metabolism, results in some avoidance of first-pass intestinal and hepatic metabolism. After oral administration, bambuterol is slowly bioconverted to terbutaline, predominantly by nonspecific butyrylcholinesterase, and also by lung tissue that is capable of the same metabolism [41–43]. As a result of slower release and prolonged action, a once-daily bambuterol treatment provides relief from asthma with a lower incidence of side effects than terbutaline taken three times a day [44].



10 **11**

1.3.5
Overcoming Toxicity Problems

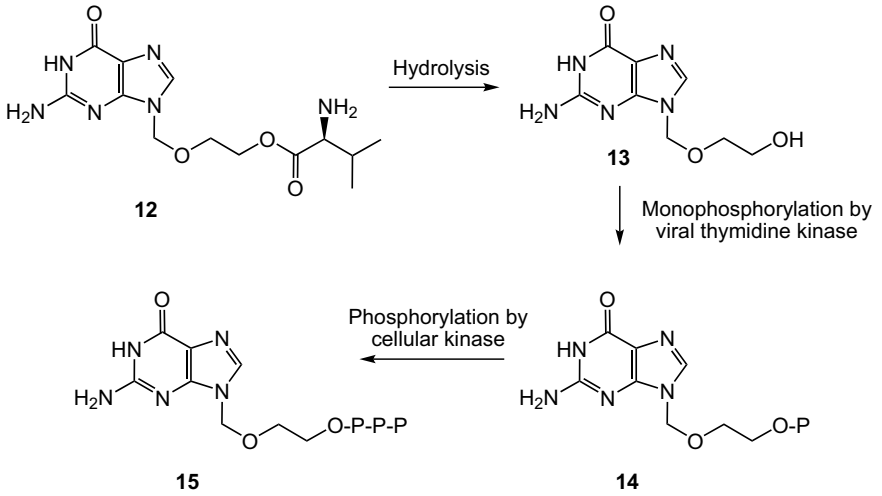
Overcoming a parent drug's toxicity can sometimes be accomplished through prodrug strategies that alter one or more of the ADME barriers discussed earlier

(see also Table 1.1). Of particular interest is the exclusive, and often elusive, site-selective drug delivery to a particular target, also known as the "magic bullet," to minimize toxicity associated with "freely" distributed drug. In a prodrug approach, such site selectivity can generally be achieved either by site-specific drug bioactivation or site-directed drug delivery. While site-directed drug delivery consists of the selective or primary transport of the intact prodrug to the site of drug action, as in the case of ocular and dermal administration, in site-specific bioactivation the prodrug releases the active drug predominantly at the desired site by a selective metabolic activation reaction.

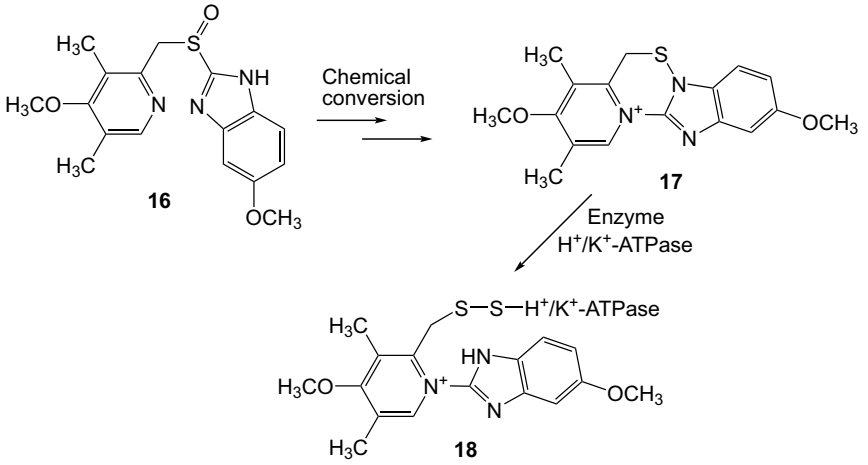
There are several excellent prodrugs that undergo site-specific activation by exploiting endogenous enzymes of the target tissue. A recent and clinically significant example is capecitabine, an orally active prodrug of the anticancer drug 5-fluorouracil, which requires a cascade of three enzymes for its bioconversion. Capecitabine and several other interesting site-selective prodrugs are discussed in more detail in Chapter 11.

The ubiquitous distribution of most endogenous enzymes that are responsible for bioactivating a prodrug diminishes the opportunities for selective activation and, consequently, targeting. Therefore, prodrugs that rely on bioactivation by exogenous enzymes selectively delivered via monoclonal antibodies (i.e., antibody-directed enzyme prodrug therapy, ADEPT) or generated from genes encoding an exogenous enzyme (i.e., gene-directed enzyme prodrug therapy, GDEPT) have received considerable attention over the past decade, especially in cancer therapy. These approaches are described in more detail in Chapter 12. The rest of this chapter will focus on other examples of site-activated prodrugs.

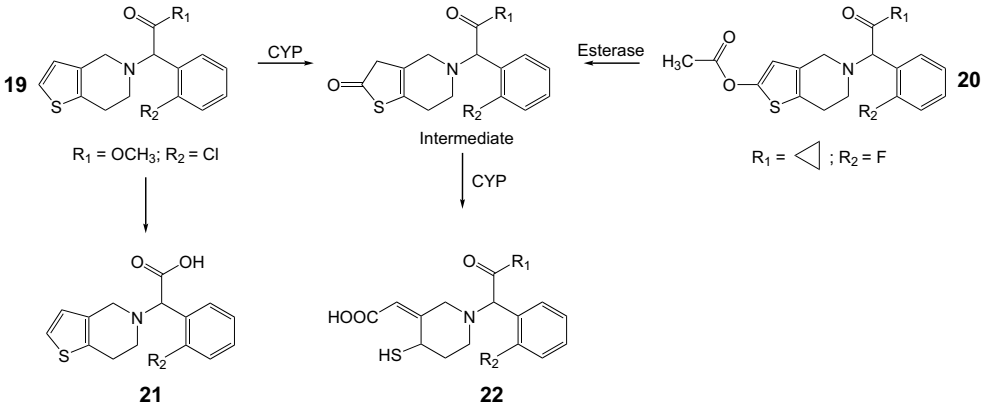
Nucleoside analogues and nucleoside phosphonates are widely used for the treatment of antiviral infections, and some also have antineoplastic properties. A representative example of selectivity for viral infected cells is acyclovir (**13**) [45, 46]. This prodrug requires intracellular phosphorylation by kinases to produce the active triphosphate nucleotide form (**15**), which cannot be administered as such due to its high polarity and consequently its poor absorption. Desirable site activation is obtained by viral thymidine kinase, which is far more effective (3000 times) in the monophosphorylation of acyclovir than thymidine kinase in uninfected cells. Subsequently, the monophosphate form (**14**) must be further phosphorylated to the triphosphate by cellular kinases in order to become active. This triphosphorylated form has demonstrated a very potent inhibition of viral DNA polymerase and shown approximately 100 times greater affinity for viral than cellular polymerases. However, due to the low oral bioavailability of acyclovir itself (only 10–30%), a valine derivative of acyclovir (**12**) with 3–5 times higher oral bioavailability was also eventually developed for clinical use [47, 48]. Typically, it has been more common to think of these derivatives of nucleoside analogues, such as valacyclovir, which get metabolized into their respective nucleosides to become nucleosidic prodrugs. However, to be more precise, these are prodrugs of prodrugs (or, pro-prodrugs).



Although the proton pump inhibitor omeprazole (**16**) was initially not designed to behave as a prodrug, it offers another excellent example of a site-activated prodrug. Omeprazole has a basic pK_a of 3.97, which causes it to accumulate in the acidic secretory parietal cells due to pyridine protonation [49]. In the acidic conditions of parietal cells, omeprazole is converted to its active sulfenamide (**17**), followed by irreversible binding with a cysteine group in H^+/K^+ ATPase (**18**), thereby inhibiting the ability of the parietal cells to produce gastric acid [50]. The excellent safety profile of omeprazole can be attributed to the fact that nongastric H^+/K^+ ATPases lack the highly acidic compartment seen in the parietal cells and are thus unable to convert omeprazole to its active form. Therefore, omeprazole is converted to its active form by molecular rearrangement under acidic conditions that are only close to the acid-producing enzyme it inhibits. Other proton pump inhibitors such as lansoprazole, pantoprazole, rabeprazole, esomeprazole, and tenatoprazole share the same activation mechanism as omeprazole.



More examples of prodrugs with decreasing systemic toxicities include the antiplatelet drugs clopidogrel (**19**) and prasugrel (**20**), which are converted to their active species at or close to their site of action. Clopidogrel must be metabolized by CYP450 enzymes to produce the active metabolite (**22**), which selectively inhibits platelet aggregation by forming an irreversible disulfide bond with the platelet adenosine diphosphate (ADP) receptor, and thus prevents ADP binding [51]. This *in situ* biological effect takes place primarily at the bioconversion site, in the liver, and thus accounts for the absence of an antiaggregating action in the plasma. Interestingly, only a small proportion of administered clopidogrel is metabolized by CYP450 enzymes to the active species, whereas about 85% of the clopidogrel dose is hydrolyzed by esterases to an inactive carboxylic acid derivative (**21**) [17, 52]. This drawback has led to the design of prasugrel that is primarily bioconverted to the active thiol in a cascade of events that involve enzymatic hydrolysis by carboxylesterases 1 and 2, and oxidation reactions by CYP450 enzymes [16, 53]. It is also worth noting that genetic polymorphism in CYP450 enzymes is known to significantly affect the disposition and response of many drugs. For example, variability in the response to clopidogrel is well documented (see Chapter 16).



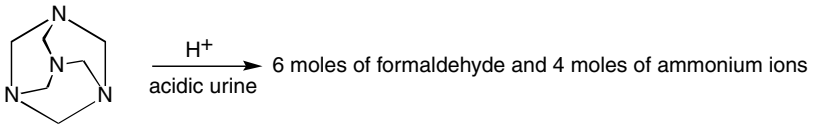
1.3.6
Life Cycle Management

As new chemical entities (NCEs), prodrugs are likely to have the added benefit of being considered as intellectual property (IP). Therefore, the development of a prodrug from an existing drug, with improved properties, represents an opportunity for life cycle management. For example, fosphenytoin is a prodrug of the older off-patent phenytoin and has recaptured a market position that was essentially lost when injectable sodium phenytoin became generic [54]. Similarly, as the protection for the fosamprenavir patent will continue up to at least 2017, the protection by the composition of matter patent for its parent drug, amprenavir, will expire in 2013 (*FDA Orange Book*). Therefore, the additional costs to develop fosamprenavir will probably be leveraged by revenues created by its extended patent life.

1.4 History of Prodrug Design

After giving a modern rationale for prodrug design, a glimpse into the history of and very early examples of prodrugs is worthwhile, and it may be surprising to learn that many are still in clinical use. The term "prodrug" itself was first introduced by Albert in 1958 to describe compounds that undergo biotransformation prior to exhibiting their pharmacological effects [55]. For such compounds, the term "drug latentiation" was also introduced at nearly the same time by Harper [56, 57], which further promoted the concept. Prodrugs have also been called reversible or bioreversible derivatives and biolabile drug–carrier conjugates [58], but at present the term prodrug is both standard and simple.

Several examples of prodrugs precede the formal introduction of the concept. While most of these examples were intentionally designed to function as prodrugs, the actual mechanisms of many were discovered later. Methenamine (**23**), one of the first prodrugs, was introduced in 1899 and intentionally relied on the formation of antibacterial formaldehyde in urine. Because of the relatively selective conversion of methenamine at urinary acidic pH and not in plasma or other tissues, it also serves as an early example of site-activated prodrugs.



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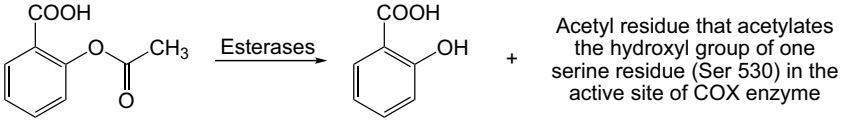
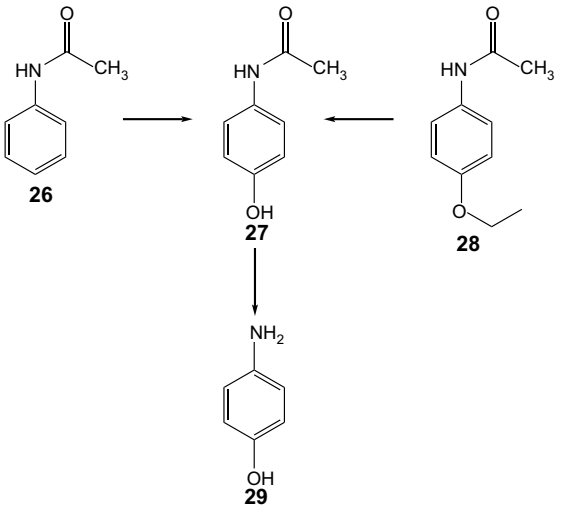
In the very same year, another prodrug was introduced that certainly falls into the category of the best-known and the most widely used prodrug ever, namely, aspirin (or acetylsalicylic acid, **24**), which was intentionally designed to be a less-irritating replacement for the common salicylate medicines of that time. Aspirin has a relatively short half-life in circulation and it hydrolyzes to both salicylic acid (**25**) and an acetate. Much of aspirin's mechanism of action is due to its ability to suppress the production of prostaglandins and thromboxanes by an irreversible inactivation of cyclooxygenase (COX). While released salicylic acid is a weak COX inhibitor, the released acetyl group is mainly responsible for aspirin's pharmacological effects by covalently and irreversibly attaching to a serine residue on an active site of the COX enzyme [59].



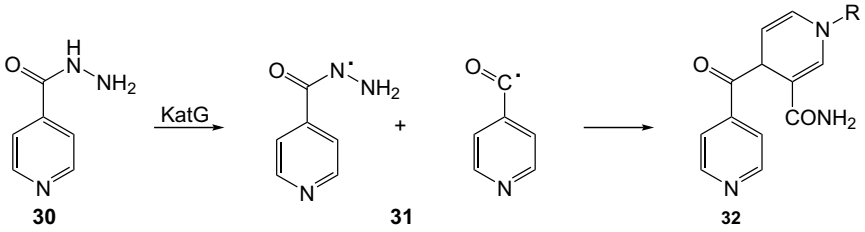
24 **25**

Another very popular analgesic and antipyretic drug with a weak anti-inflammatory action, paracetamol (**27** or acetaminophen in the United States), may also

function via the metabolite *para*-aminophenol (**29**), which makes it a prodrug (the exact mechanism by which paracetamol reduces fever and pain is still debated). *para*-Aminophenol reacts further with arachidonic acid to form *N*-arachidonoylphenolamine, which is responsible for most of the paracetamol's analgesic action [60]. The predecessors of paracetamol, acetanilide (**26**) (introduced in 1886) and phenacetin (**28**) (introduced in 1887), were the first aniline derivatives found to provide both analgesic and antipyretic properties, but were later discovered to provide their therapeutic efficacy via their common major metabolite, paracetamol. In this way, both acetanilide and phenacetin may be considered to be unintentional prodrugs of paracetamol.

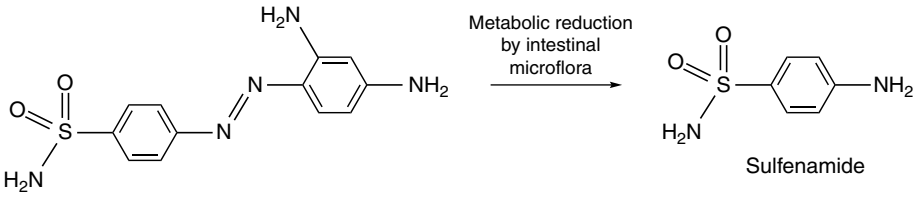


In a similar way, the prodrug activity of the antituberculosis drug isoniazid (**30**) was recently discovered in hindsight. For decades, after its introduction in the early 1950s, the mechanisms of action of isoniazid remained unclear. Developments in late 1990s have shown that isoniazid is activated by the mycobacterial catalase–peroxidase enzyme called KatG. This activation generates the reactive species (**31**) that form adducts with NAD^+ and $NADP^+$ (**32**), which are potent inhibitors of lipid and nucleic acid biosynthetic enzymes [61].



More examples of such hindsight recognition are given by the antibacterial prontosil (**33**) and prodrugs of morphine (**35**), namely, heroin (**34**) and codeine (**36**). The discovery in 1935 of sulfanilamide as the active metabolite of prontosil ushered in the era of sulfonamide antibiotics [62].

Diacetylmorphine was synthesized in 1874 and subsequently marketed as an over-the-counter drug in 1895 under the name heroin and used as a morphine substitute for treating coughs and as a cure for both cocaine and morphine addictions. Interestingly, it is still available by prescription in the United Kingdom and other European countries. However, the discovery of rapid metabolism of heroin into morphine became eventually a historic blunder for Bayer.



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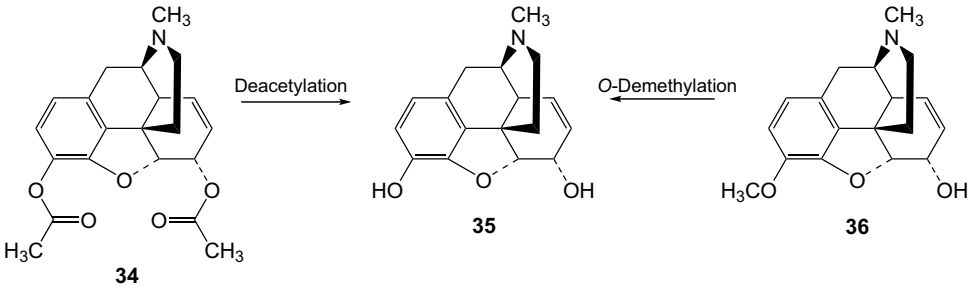
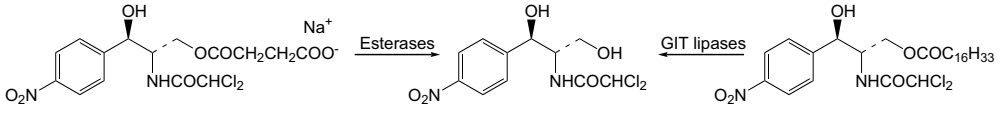


34 **35** **36**

The pioneering research on chloramphenicol derivatives in the 1950s by Glazko and coworkers at Parke-Davis is also worth recognizing. Both a sparingly water-soluble palmitate ester (**39**), to mask the bitter taste of chloramphenicol (**38**) in pediatric use [63], and a more water-soluble chloramphenicol sodium hemisuccinate (**37**) [64], for aqueous-based formulations, are early examples of rationally developed prodrugs by a pharmaceutical company.



37 **38** **39**

1.5
Recently Marketed Prodrugs

1.5.1
Prodrug Prevalence

A review of pharmacologically active, new chemical entities approved by the US FDA between 2004 and 2008 shows 6 prodrugs (Table 1.2) out of the total 84 NCEs entering the market. Therefore, 7% of the recently approved drugs can be classified as prodrugs, which is very similar to a value of 5–7% estimated by Stella in 2007 for all drugs approved worldwide [54]. However, these numbers do not include the previously discussed nucleoside analogues, which require intracellular phosphorylation by kinases to produce the active nucleotide analogues capable of inhibiting RNA or DNA polymerases. When drugs such as azacitidine (introduced in 2004), clofarabine (2005), entecavir (2005), decitabine (2006), nelarabine (2006), telbivudine (2006), and clevudine (2007) are included, as well as those nucleoside analogues brought to market earlier, a prodrug prevalence of about 15% is seen between 2004 and 2008, which raises the prodrug market share closer to 10%.

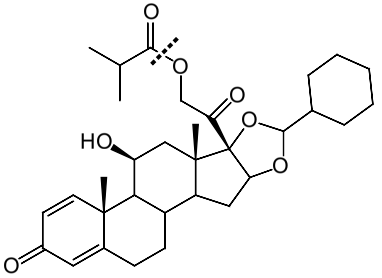
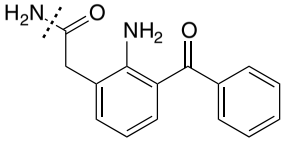
The prevalence of prodrugs among world's 100 top selling pharmaceuticals is also substantial (data from 2008; http://www.drugs.com/top200.html). Out of the 100 blockbuster drugs, 9 are biologics and 91 small molecules. Of the small molecules, 14 can be classified as prodrugs (Table 1.3), which gives a prodrug prevalence of 15.4% among the 100 best selling small molecular weight drugs. These 14 prodrugs include only one nucleosidal analogue (emtricitabine), therefore further demonstrating the extent of the successful implementation of the prodrug approach.

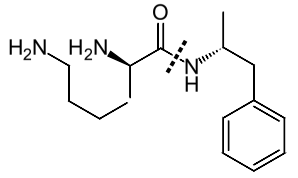
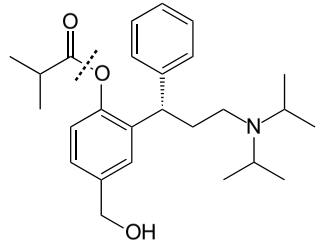
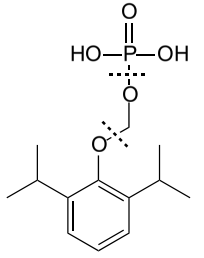
1.5.2
Recent Prodrug Approvals

Several prodrugs were launched worldwide during 2004–2008, and these are summarized in Table 1.2 (FDA approved) and Table 1.4 (approved elsewhere, excluding prodrugs in Table 1.2). These products demonstrate the versatility of the prodrug approach. They include four different routes of administration (oral, inhalation, ocular, and intravenous), many types of activity (anticoagulant, anti-inflammatory, stimulant, antimuscarinic, sedative, and reduction of ocular hypertension), and other indications (thrombin inhibition, asthma, ocular pain, attention deficit hyperactivity disorder (ADHD), overactive bladder, sedation, and glaucoma). The common feature for all these recently approved prodrugs is their bioconversion mechanisms, as they all rely on hydrolase activation. This is not a total surprise; by estimation half of all marketed prodrugs are hydrolytically bioconverted to their parent drugs [23].

The two most recently approved prodrugs are dexlansoprazole and prasugrel, which were both launched in 2009. Dexlansoprazole is the slower metabolized *R*-enantiomer of the proton pump inhibitor lansoprazole. The main advantage of this prodrug is not based on chemistry but rather on a dual-release formulation tech-

Table 1.2 Prodrugs approved by the US FDA during 2004–2008.

Prodrug, approval year, therapeutic area	Functional group	Prodrug structure (dashed lines indicate site of bioconversion)	Prodrug strategy
Ciclesonide, 2005, anti-inflammatory corticosteroid	Isobutyryl ester of des-isobutyryl ciclesonide		Bioconversion by lung esterases [65] Reduced systemic exposure and consequently reduced risk of adverse effects [66]
Nepafenac, 2005, anti-inflammatory	Amide of amfenac		Bioconversion by intraocular hydrolases [67] Improved ocular absorption and prolonged duration of activity [68]

Lisdexamfetamine, 2007, psychostimulant (ADHD)	L-Lysyl amide of D-amphetamine		Bioconversion by intestinal or hepatic hydrolases [69] Reduced potential for abuse due to prolonged release of active drug [70]
Fesoterodine, 2008, antimuscarinic (overactive bladder)	Isobutyryl ester of 5-hydroxymethyl tolterodine		Bioconversion by esterases Reduced interpatient pharmacokinetic variability [71, 72]
Fospropofol, 2008, anesthetic	Phosphonooxymethyl ether of propofol		Is rapidly converted to propofol after intravenous administration by alkaline phosphatases [73, 74] Significantly increases the aqueous solubility of propofol from 150 μg/ml to ~500 mg/ml

(*Continued*)

Table 1.2 (*Continued*)

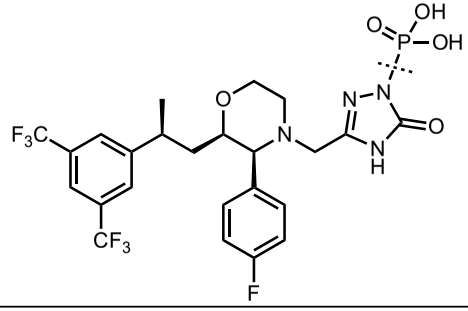
Prodrug, approval year, therapeutic area	Functional group	Prodrug structure (dashed lines indicate site of bioconversion)	Prodrug strategy
Fosaprepitant, 2008, antiemetic	*N*-Phosphono aprepitant		Reduced injection site pain [75] Is rapidly converted to aprepitant after intravenous administration by alkaline phosphate The prodrug enabled the development of a liquid formulation

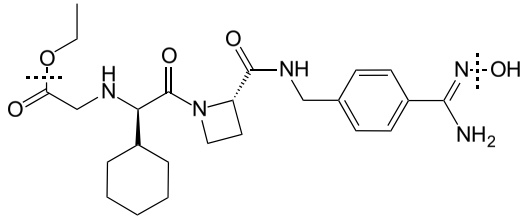
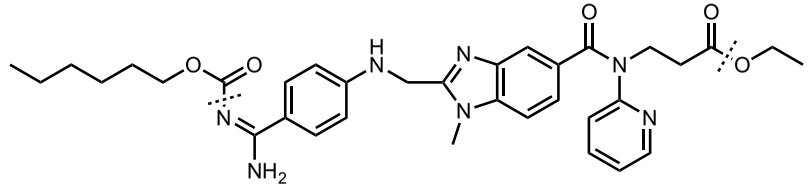
Table 1.3 The occurrence of prodrugs among the world's 100 top selling pharmaceuticals in 2008.

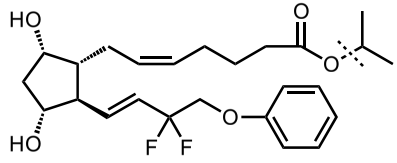
Prodrug name (therapeutic area)	Functional group	Prodrug strategy
Esomeprazole, lansoprazole, pantoprazole, rabeprazole (proton pump inhibitors)	Formation of active sulfenamide form	Bioprecursor prodrugs that are converted into their respective active sulfenamide forms site selectively under acidic conditions of stomach [50]
Clopidogrel (antiplatelet)	Formation of the active thiol	Bioprecursor prodrug that selectively inhibits platelet aggregation [51]
Simvastatin (hypercholesterolemia)	Inactive lactones	Bioprecursor prodrug that is converted into the active hydroxyl acids in the liver [76]
Fenofibrate (hypercholesterolemia)	Isopropyl ester of fenofibric acid	Lipophilic ester of fenofibric acid [77]
Olmesartan medoxomil (hypertension)	Cyclic carbonate ester of olmesartan	Improved bioavailability compared to olmesartan, allowing oral administration [78]
Mycophenolate mofetil (immunosuppressant)	Morpholinyl ethyl ester of mycophenolic acid	Improved oral bioavailability with less variability [79]
Valacyclovir (antiviral)	L-Valyl ester of acyclovir	Bioconversion by valacyclovir hydrolase (valacyclovirase). Transported predominantly by hPEPT1. Oral bioavailability improved from 12–20% (acyclovir) to 54% (valacyclovir) [80–83]
Latanoprost (glaucoma)	Isopropyl ester of latanoprost acid	Bioconversion by esterases. Improved lipophilicity to achieve better ocular absorption and safety [84, 85]
Tenofovir disoproxil (antiviral)	Bis(isopropyloxycarbonyloxymethyl) ester of tenofovir	Bioconversion by esterases and phosphodiesterases. The oral bioavailability of tenofovir from tenofovir disoproxil is 39% after food [86–88]

nology that produces two peaks in plasma drug concentration, which subsequently prolongs exposure to the proton pumps [93]. Prasugrel is a novel platelet inhibitor that was described earlier in this chapter.

Both ciclesonide and fesoterodine are isobutyryl ester prodrugs of their parent active drugs, des-isobutyryl ciclesonide and 5-hydroxymethyl tolterodine, respectively. While ciclesonide is an inhaled corticosteroid used in the preventative treatment of asthma, fesoterodine is a new orally administered antimuscarinic agent for the treatment of overactive bladder. The bioconversion of ciclesonide to its active species takes place in the target organ, that is, the lung, by endogenous esterases located both on airway epithelial cells and on lung fibroblasts [65]. The minimal oral bioavailability

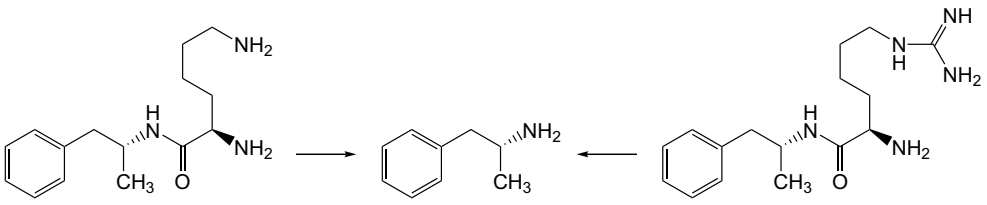
Table 1.4 Prodrugs approved outside the United States during 2004–2008 (excluding the FDA approvals).

Prodrug, approval year, therapeutic area	Functional group	Prodrug structure (dashed lines indicate site of bioconversion)	Prodrug strategy
Ximelagatran, 2004, anticoagulant	Hydroxyamidine and the ethyl ester of melagatran		Bioconversion by esterases and reductive enzymes Increased oral bioavailability of 3–7% for melagatran to 20% for ximelagatran [89, 90]
Dabigatran etexilate, 2008, anticoagulant	Hexyloxycarbonylamidine and ethyl ester of dabigatran		Bioconversion by esterases Oral bioavailability of 7%, measured for dabigatran [91]

Tafluprost, 2008, glaucoma	Isopropyl ester of tafluprost acid		Bioconversion by esterases Improved lipophilicity achieves better ocular absorption and safety [92]

of ciclesonide and des-isobutyryl ciclesonide, which is normally less than 1% for both drugs, combined with a pulmonary bioavailability of about 50%, supports the favorable risk–benefit profile observed in clinical trials for ciclesonide [66]. Fesoterodine is also rapidly and extensively hydrolyzed by nonspecific esterases after oral administration, and no intact prodrug can be detected in plasma. As the oral bioavailability of fesoterodine is 52% [94], a value commonly seen with esterified drugs [22], at least part of the bioconversion probably occurs prematurely during absorption.

Stimulants such as amphetamine have remained the most effective treatment of ADHD for decades. The recently approved lisdexamfetamine dimesylate (**40**) is a prodrug of dextroamphetamine (**41**) covalently attached to the essential amino acid L-lysine, via an amide bond [95]. It was designed to have less abuse potential than other amphetamines, as it is not rapidly converted to the active form if inhaled or injected, compared to amphetamine, and thus reduces the potential for euphoric effects. The bioconversion of lisdexamfetamine occurs through first-pass intestinal and/or hepatic metabolism after oral administration to provide a time-released effect dose [96]. Of course, lisdexamfetamine can still be used illicitly when taken orally, yet the rewarding effects are substantially attenuated when compared to amphetamine. As a consequence, another amino acid prodrug has been introduced: homoarginine dextroamphetamine (**42**), which has maintained similar blood levels of amphetamine in preclinical species compared to lisdexamfetamine, but with a delayed onset and a longer duration of action, which contribute to a lower abuse potential [97].



40 **41** **42**

Nepafenac is an amide prodrug of the NSAID amfenac, which is indicated for the treatment of ocular pain and inflammation [67, 68]. It efficiently permeates the external ocular barriers, cornea, and scleral tissues and has a penetration coefficient that is 4–28 times greater than that achieved with conventional NSAIDs such as diclofenac and ketorolac. This efficient ocular penetration is followed by rapid hydrolysis to amfenac by ocular tissue hydrolases, specifically in the iris, ciliary body, retina, and choroid.

There are several successful phosphate prodrugs in clinical use with improved aqueous solubility properties for parenteral administration. The most recent entrants are phosphonooxymethyl prodrug fospropofol and the *N*-phosphono prodrug fosaprepitant, which were both approved in 2008. The widely used, and unfortunately

abused, anesthetic propofol is formulated as an oil-in-water emulsion because of its high lipophilicity and referred to as "milk of amnesia" in some circles due to its physical appearance and recreational effects. However, pain on injection, inherent emulsion instability, and hyperlipidemia after prolonged administration are major drawbacks, which eventually encouraged the development of fospropofol. Being significantly more water soluble, fospropofol is formulated as a purely aqueous solution, thus avoiding some of the problems associated with propofol. Fospropofol releases propofol rapidly after intravenous administration by alkaline phosphatases [73, 74]. This highly water-soluble phosphate prodrug strategy was also applied to the antiemetic aprepitant. Plasma concentrations of fosaprepitant are below the limits of quantification (10 ng/ml) within 30 min after infusion, which indicates rapid bioconversion by alkaline phosphatases. This is also demonstrated by the similarity in aprepitant plasma concentrations at 24 h postdose for 125 mg of oral aprepitant and 115 mg of intravenous fosaprepitant.

Ximelagatran is an ethoxyl prodrug of melagatran and represents the first example of an orally administered direct thrombin inhibitor [89]. It was approved for the European market in 2004 but was withdrawn in early 2006 after an extended clinical trial confirmed initial concerns of severe liver toxicity. Another anticoagulant, dabigatran etexilate, was approved in some European countries and Canada in 2008. Both ximelagatran and dabigatran etexilate are double prodrugs with an ethyl ester on the carboxylic acid and *N*-hydroxyamidine or *N*-hexyloxycarbonylamidine groups, respectively, on the amidine function. The subsequent formation of the respective parent drugs, melagatran and dabigatran, requires two metabolic reactions, where the *N*-hydroxy group in ximelagatran is reduced to an amidine, mainly in the liver by CYP450 enzymes [89, 98], and *N*-hexyloxycarbonyl is primarily metabolized by esterases [99]. The ethyl ester in both prodrugs is hydrolyzed to provide the free carboxylic acid in the liver. The masking of two ionizable groups in the parent drugs results in more lipophilic prodrugs that have oral bioavailability of 20% for melagatran from ximelagatran [90] and 7% for dabigatran from dabigatran etexilate [99].

1.6 Concluding Remarks

The prodrug strategy is a versatile and powerful method that can be applied to a wide variety of pharmaceuticals whose pharmacologic limitations compromise their clinical use. The development of prodrugs is now well established as a strategy to improve ADMET properties of pharmacologically potent compounds already in the early phases of drug discovery. Also, the economic importance of extending patent protection for an existing pharmaceutical can be significant. These reasons have already led to an increasing number of approved prodrugs that are on the market. About 10% of all approved drugs worldwide can be classified as prodrugs. In addition, the percentage of prodrugs among the most successful drugs is particularly high.

The majority of clinical prodrugs are based on enhanced drug permeation and delivery to the active site via increased drug lipophilicity and, more recently, improved water solubility. However, there are also unmet needs that have not yet been adequately addressed by prodrugs. It is surprising how few prodrugs exist for cancer therapy, even though the side effects of antineoplastic agents are considerable and potentially lethal. Perhaps, this means that the easier problems have been resolved while more complex issues remain unaddressed. More creative prodrug strategies will be required to address drug delivery issues that are related to site-selective drug delivery with reduced side effects, prevention of presystemic drug metabolism, and circumvention of efflux-limited drug absorption.

I hope that this chapter has provided useful examples of potential applications for using prodrugs and will encourage creative prodrug research to solve some of the unmet drug delivery needs. Prodrugs will certainly be an exciting area of research in the future.

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