

Prodrugs: design and clinical applications

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Abstract | Prodrugs are bioreversible derivatives of drug molecules that undergo an enzymatic and/or chemical transformation *in vivo* to release the active parent drug, which can then exert the desired pharmacological effect. In both drug discovery and development, prodrugs have become an established tool for improving physicochemical, biopharmaceutical or pharmacokinetic properties of pharmacologically active agents. About 5–7% of drugs approved worldwide can be classified as prodrugs, and the implementation of a prodrug approach in the early stages of drug discovery is a growing trend. To illustrate the applicability of the prodrug strategy, this article describes the most common functional groups that are amenable to prodrug design, and highlights examples of prodrugs that are either launched or are undergoing human trials.

Combinatorial chemistry

The rapid synthesis or the computer simulation of a large number of different but structurally related molecules.

The application of modern discovery technologies such as high-throughput screening and combinatorial chemistry can produce novel lead structures with high pharmacological potency, but the physicochemical and biopharmaceutical aspects of the initial leads have frequently been neglected. This can lead to drug candidates with poor drug-like properties that face significant problems later in drug development¹.

The development of prodrugs — chemically modified versions of the pharmacologically active agent that must undergo transformation *in vivo* to release the active drug — is now well established as a strategy to improve the physicochemical, biopharmaceutical or pharmacokinetic properties of pharmacologically potent compounds, and thereby increase the developability and usefulness of a potential drug^{2–9} (FIG. 1a). For example, prodrugs provide possibilities to overcome various barriers to drug formulation and delivery such as poor aqueous solubility, chemical instability, insufficient oral absorption, rapid pre-systemic metabolism, inadequate brain penetration, toxicity and local irritation. Prodrugs can also improve drug targeting, and the development of a prodrug of an existing drug with improved properties may represent a life-cycle management opportunity.

In most cases, prodrugs are simple chemical derivatives that require only one to two chemical or enzymatic transformation steps to yield the active parent drug. 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 co-drugs^{10,11}. Prodrugs have also been referred to as reversible or bioreversible derivatives, latentiated drugs or biolabile drug-carrier conjugates^{12–14}, but the term prodrug is now standard. A bioprecursor prodrug is a prodrug that does not contain a carrier or promoiety, but results from a molecular modification of the active agent itself. This modification (for example, oxidation or reduction) generates a new compound that can be transformed metabolically or chemically, with the resulting compound being the active agent (it can also be referred to as an active metabolite). Finally, soft drugs, which are often confused with prodrugs, also find applications in tissue targeting. However, in contrast to prodrugs, soft drugs are active drugs that are designed to undergo a predictable and controllable deactivation or metabolism *in vivo* after achieving their therapeutic effect^{15–17}.

Currently, 5–7% of the drugs approved worldwide can be classified as prodrugs, and approximately 15% of all new drugs approved in 2001 and 2002 were prodrugs^{2,6}. However, we have only begun to realize their full potential, and this is mainly due to the only recent understanding of various biological phenomena enabling the design of more sophisticated, safer and better-targeted prodrugs. With the aim of illustrating the full potential of the prodrug approach, this article will provide an overview of functional groups that are amenable to prodrug design, and then highlight the major applications of the prodrug strategy, including the ability to improve oral absorption and aqueous solubility, enhance lipophilicity and active transport, as well as achieve site-selective delivery.

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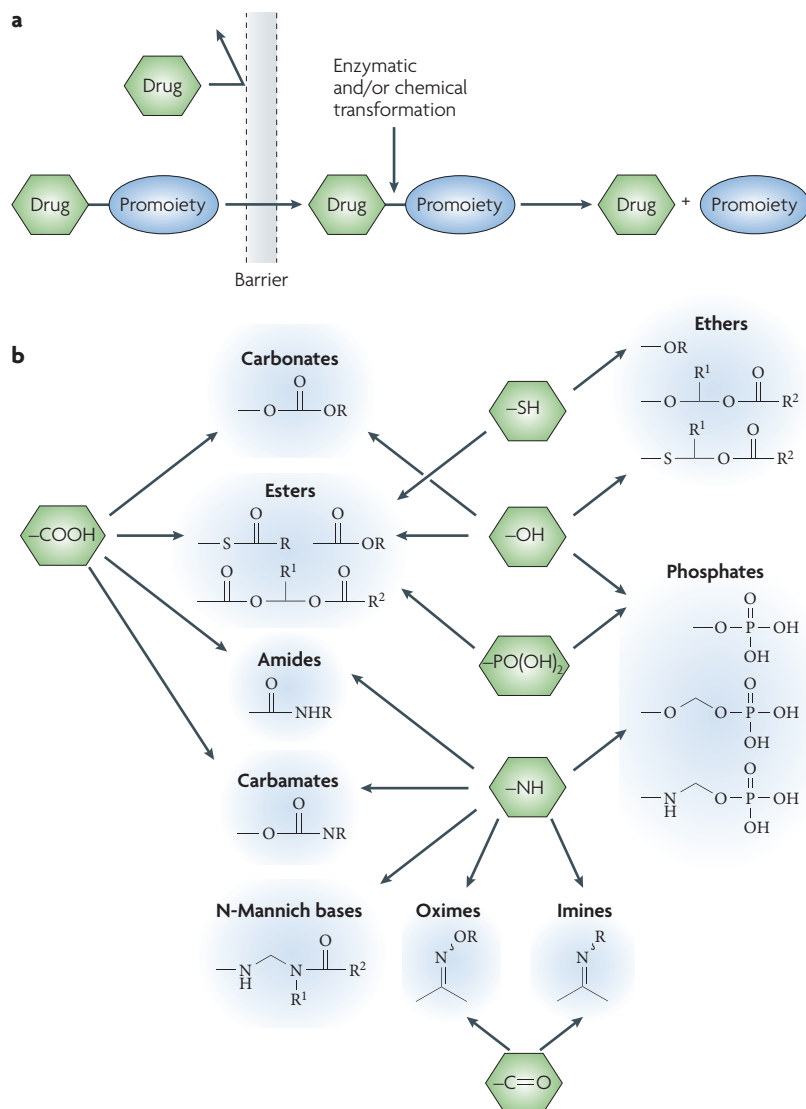


Figure 1 | A simplified representative illustration of the prodrug concept.

a | The drug–promoiety is the prodrug that is typically pharmacologically inactive. In broad terms, the barrier can be thought of as any liability or limitation of a parent drug that prevents optimal (bio)pharmaceutical or pharmacokinetic performance, and which has to be overcome for the development of a marketable drug. The drug and promoiety are covalently linked via bioreversible groups that are chemically or enzymatically labile, such as those shown here. The ‘ideal’ prodrug yields the parent drug with high recovery ratios, with the promoiety being non-toxic. **b** | Common functional groups on parent drugs that are amenable to prodrug design (shown in green). Most prodrug approaches require a ‘synthetic handle’ on the drug, which are typically heteroatomic groups.

Functional groups amenable to prodrug design

Ideally, the design of an appropriate prodrug structure should be considered at the early stages of preclinical development, bearing in mind that prodrugs might alter the tissue distribution, efficacy and the toxicity of the parent drug. Several important factors should be carefully examined when designing a prodrug structure, including:

- Parent drug: which functional groups are amenable to chemical prodrug derivatization?
- Promoiety: this should ideally be safe and rapidly excreted from the body. The choice of promoiety

should be considered with respect to the disease state, dose and the duration of therapy.

- Parent and prodrug: the absorption, distribution, metabolism, excretion (ADME) and pharmacokinetic properties need to be comprehensively understood.
- Degradation by-products: these can affect chemical and physical stability and lead to the formation of new degradation products.

Some of the most common functional groups that are amenable to prodrug design include carboxylic, hydroxyl, amine, phosphate/phosphonate and carbonyl groups. Prodrugs typically produced via the modification of these groups include esters, carbonates, carbamates, amides, phosphates and oximes. However, other uncommon functional groups have also been investigated as potentially useful structures in prodrug design. For example, thiols react in a similar manner to alcohols and can be derivatized to thioethers¹⁸ and thioesters¹⁹. Amines may be derivatized into imines^{20,21} and N-Mannich bases²². The prodrug structures for the most common functionalities are illustrated in FIG. 1b and discussed below.

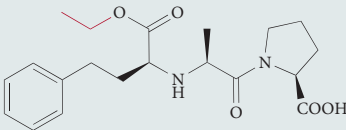
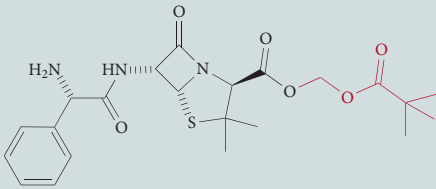
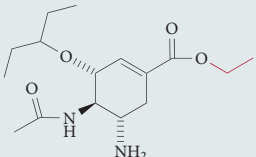
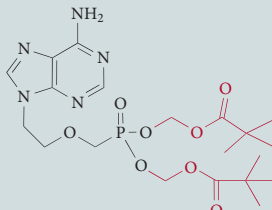
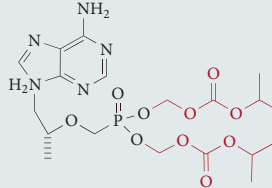
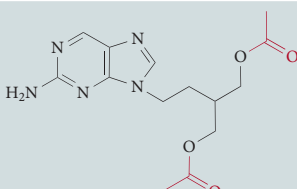
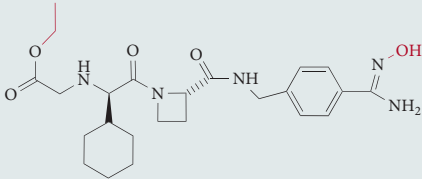
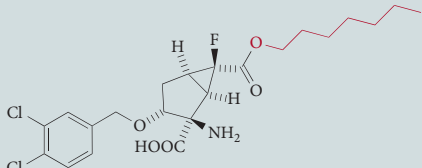
Esters as prodrugs of carboxyl, hydroxyl and thiol functionalities. Esters are the most common prodrugs used, and it is estimated that approximately 49% of all marketed prodrugs are activated by enzymatic hydrolysis⁴. Ester prodrugs are most often used to enhance the lipophilicity, and thus the passive membrane permeability, of water-soluble drugs by masking charged groups such as carboxylic acids and phosphates^{3,23}. The synthesis of an ester prodrug is often straightforward. Once in the body, the ester bond is readily hydrolysed by ubiquitous esterases found in the blood, liver and other organs and tissues²⁴, including carboxylesterases, acetylcholinesterases, butyrylcholinesterases, paraoxonases and arylesterases. However, one significant challenge with ester prodrugs is the accurate prediction of pharmacokinetic disposition in humans, owing to significant differences in specific carboxylesterase activities in preclinical species²⁵, as reported for the exploratory intravenous diester prodrug of nalbuphine²⁶. A comprehensive review on ester prodrugs that enhance oral absorption of predominantly poorly permeable and polar parent drugs was recently published by Beaumont *et al.*³

Several alkyl and aryl ester prodrugs are in clinical use³, of which angiotensin-converting enzyme (ACE) inhibitors are some of the most successful²⁴, with a representative sample shown in TABLE 1. However, the relatively slow and incomplete bioconversion of some simple alkyl esters in human blood can sometimes result in lower than predicted bioavailability. For example, the oral bioavailability of enalaprilate in humans is 36–44%, despite 53–74% of the administered dose being absorbed³. In some cases, faster bioactivation has been achieved by the use of a double prodrug (pro-prodrug), which requires an enzymatic breakdown after which a spontaneous chemical reaction releases the parent drug. The double prodrug approach has been the preferred choice when preparing oral acyloxyalkyl ester prodrugs of β -lactam antibiotics (TABLE 1).

Promoiety

A functional group used to modify the structure of pharmacologically active agents to improve physicochemical, biopharmaceutical or pharmacokinetic properties.

Table 1 | Prodrugs for improved lipophilicity or permeability

Prodrug name (therapeutic area)	Functional group	Structure	Prodrug strategy
Enalapril (angiotensin-converting enzyme inhibitor)	Monoethyl ester of enalaprilat		- Bioconversion by esterases - The oral bioavailability of enalaprilat in humans is 36–44% 53–74% of the administered dose is absorbed^{3,172}
Pivampicillin (β-lactam antibiotic)	Pivaloylmethyl ester of ampicillin		- Bioconversion by esterases - The oral bioavailability of 32–55% for ampicillin increased to 87–94% for pivampicillin^{173,174}
Oseltamivir (anti-influenza)	Ethyl ester of oseltamivir carboxylate		- Bioconversion by esterases - The oral bioavailability of less than 5% in rat and marmoset for oseltamivir carboxylate increased to 80% for oseltamivir in humans^{80–82}
Adefovir dipivoxil (antiviral)	Bis-(pivaloyloxy-methyl) ester of adefovir		- Bioconversion by esterases and phosphodiesterases - The oral bioavailability of ~10% for adefovir increased to 30–45% for adefovir dipivoxil^{178,79}
Tenofovir disoproxil (antiviral)	Bis-(isopropoxy-carbonyloxymethyl) ester of tenofovir		- Bioconversion by esterases and phosphodiesterases - The oral bioavailability of tenofovir from tenofovir disoproxil is 39% in the fed state^{74,76,77}
Famciclovir (antiviral)	Dimethyl ester of penciclovir		- Bioconversion by esterases and oxidation from purine to guanine - The oral bioavailability of 4% for penciclovir increased to 75% for famciclovir^{175–177}
Ximelagatran (anticoagulant)	Hydroxyamidine and ethyl ester of melagatran		- Bioconversion by esterases and reductive enzymes - The oral bioavailability of 3–7% for melagatran increased to 20% for ximelagatran^{84,86}
MGS0210 (glutamate receptor (MGLUR2) antagonist)	n-Heptyl ester of MGS0039		- Bioconversion by esterases - The oral bioavailability of less than 13% for MGS0039 in monkeys increased to 44% for MGS0210 in monkeys^{41,50}

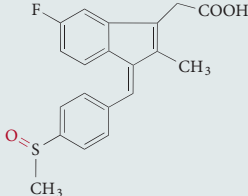
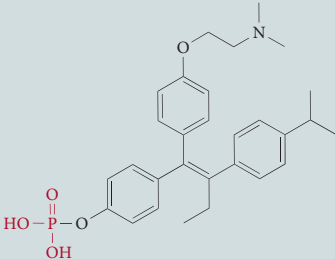
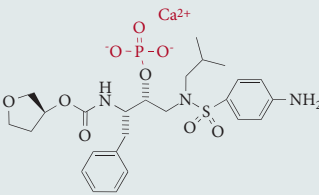
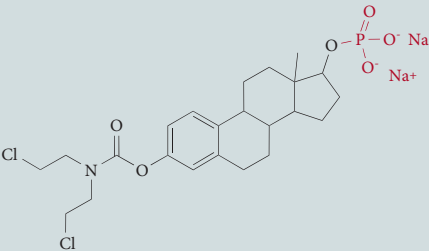
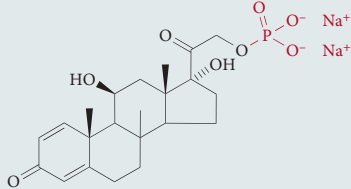
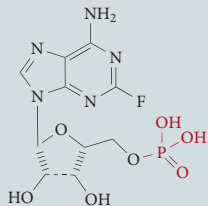
Co-drug

A chemical structure that undergoes conversion to two or more active drugs within a biological system, such conversion usually involves the metabolism of the co-drug.

Phosphate esters as prodrugs of hydroxyl or amine functionalities. Phosphate ester prodrugs are typically designed for hydroxyl and amine functionalities of poorly water-soluble drugs with an aim to enhance their aqueous solubility to allow a more favourable oral or parenteral administration (see examples in TABLES 2,3). The synthesis

of phosphate prodrugs is fairly straightforward, and the presence of the dianionic phosphate promoiety usually raises the aqueous solubility^{27,28}. Phosphate prodrugs typically display excellent or adequate chemical stability and rapid bioconversion back to the parent drug by phosphatases present at the intestinal brush border or

Table 2 | **Prodrugs for improved aqueous solubility**

Prodrug name (therapeutic area)	Functional group	Structure	Prodrug strategy
Sulindac (non-steroidal anti-inflammatory)	Oxide prodrug of sulindac sulphide		• Bioprecursor prodrug that is reduced to the active sulphide form after oral absorption • ~ 100-fold increase in aqueous solubility^{62,65}
Miprosifene phosphate, TAT-59 (anticancer)	Phosphate ester of miprosifene/DP-TAT-59		• Bioconversion by alkaline phosphatases • Aqueous solubility at pH 7.4 increased by ~1,000-fold⁶⁹ • Enhanced bioavailability to 28.8% in rats and 23.8% in the dog⁶⁶ • Dose-linear pharmacokinetics in humans⁶⁹
Fosamprenavir (antiviral)	Phosphate ester of amprenavir		• Bioconversion by alkaline phosphatases • 10-fold increased aqueous solubility • More simplified and patient compliant dosage regimen • Prolonged exclusive patent⁷⁰⁻⁷²
Estramustine phosphate (anticancer)	Phosphate ester of estramustine		• Bioconversion by alkaline phosphatases • Marketed both as injectable and oral formulations for the treatment of prostate carcinoma since the mid-1970s^{178,179}
Prednisolone phosphate (glucocorticoid)	Phosphate ester of prednisolone		• Bioconversion by alkaline phosphatases • The prodrug enabled the development of a liquid formulation, and thus, improved childrens' compliance to prednisolone treatment^{28,180}
Fludarabine phosphate (antiviral)	Phosphate ester of fludarabine		• Bioconversion by alkaline phosphatases • Until recently, fludarabine phosphate was marketed only for parenteral use¹⁸¹ • Based on a modest advantage over the parent drug, development of an oral prodrug of fludarabine may have only been as a consequence of the prior existence of a commercial parenteral prodrug^{28,181}

Bioprecursor prodrug

A prodrug that does not contain a carrier or promoiet, but is metabolically or chemically transformed into an active drug.

Soft drug

Soft drugs are the opposite of prodrugs. They are active drugs that are designed to undergo a predictable and controllable deactivation or metabolism *in vivo* after achieving their therapeutic effect.

Bioconversion

A process in which the pharmacologically active drug is released or formed.

in the liver^{27,28}. Unlike carboxylic acid esters, phosphate esters are typically hydrolysed at similar rates in different preclinical species by alkaline phosphatases²⁹, and there are no published reports on species differences resulting in poor human pharmacokinetic performance.

There are many successful examples of parenteral phosphate prodrugs, but because of the challenges that they may encounter in the development phases, only a few phosphate prodrugs for oral administration have reached the market²⁷. A highly ionic phosphate prodrug may, for example, exhibit suboptimal enzymatic bioconversion by phosphatases (usually the aqueous solubility of the phosphate prodrug is enhanced to such an extent that passive diffusion through the intestinal membrane is prevented); lead to precipitation of the parent drug following cleavage in the intestinal lumen — this eventually leads to poor absorption or drug flux of the parent drug, as the parent drug is not in a soluble form²⁷; or show reduced bioavailability in the presence of calcium, for example, milk products or antacids⁸.

Carbonates and carbamates as prodrugs of carboxyl, hydroxyl or amine functionalities. Carbonates and carbamates differ from esters by the presence of an oxygen or nitrogen on both sides of the carbonyl carbon. They

are often enzymatically more stable than the corresponding esters but are more susceptible to hydrolysis than amides. Carbonates are derivatives of carboxylic acids and alcohols, and carbamates are carboxylic acid and amine derivatives. The bioconversion of many carbonate and carbamate prodrugs requires esterases for the formation of the parent drug³⁰ (see examples in TABLES 1,3,4).

Amides as prodrugs of carboxylic acids and amines.

Amides are derivatives of amine and carboxyl functionalities of a molecule. In prodrug design, amides have been used only to a limited extent owing to their relatively high enzymatic stability *in vivo*. An amide bond is usually hydrolysed by ubiquitous carboxylesterases³⁰, peptidases or proteases³¹. Amides are often designed for enhanced oral absorption by synthesizing substrates of specific intestinal uptake transporters^{31,32} (TABLE 4).

Oximes as derivatives of ketones, amidines and guanidines.

Oximes (for example, ketoximes, amidoximes and guanidoximes) are derivatives of ketones, amidines and guanidines, thus providing an opportunity to modify molecules that lack hydroxyl, amine or carboxyl functionalities. Oximes are hydrolysed by the versatile microsomal cytochrome P450 (CYP450) enzymes^{33,34}, better known as xenobiotic metabolizing enzymes^{35,36}. Oximes, especially strongly basic amidines and guanidoximes, can be used to enhance the membrane permeability and absorption of a parent drug³⁷ (see an example in TABLE 1).

Summary. Using the functional groups described above, the prodrug strategy has been successfully applied to a wide range of drug molecules. The major applications of prodrugs are described below, with examples that have been approved for marketing, or that are currently or have been in clinical trials, shown in TABLES 1–6.

Improved oral absorption

The oral bioavailability of a potential drug may be limited by its aqueous solubility, low permeability, propensity to be an efflux substrate, and rapid and extensive hepatic metabolism and biliary excretion. It is imperative to understand the physicochemical and biological factors that are limiting the oral bioavailability of a compound before embarking on a prodrug strategy (BOX 1). For most drugs, the bioavailability is controlled by the fraction absorbed (F_a) and by the clearance of the drug. F_a is largely controlled by the physicochemical parameters of the parent or prodrug, that is, its gastrointestinal permeability, solubility, dissolution rate and dose number³⁸.

Most marketed oral prodrugs are derived from parent drugs with a low F_a or minimal first-pass metabolism. This Review also assumes negligible gut-wall and pre-systemic metabolism, although there are several prodrugs — for example, bambuterol (Bambec/Oxeol; AstraZeneca) (TABLE 6) — that have been designed to protect against rapid metabolic breakdown³⁹, with varying degrees of success. F_a can be improved by enhancing the dissolution rate, aqueous solubility or permeability, assuming that the gut-wall metabolism is insignificant. Some prodrug strategies overcome poor absorption by

Box 1 | Factors affecting oral bioavailability

The maximum achievable oral bioavailability, F_{max} , of a parent drug is a function of three factors:

$$F_{max} = F_a * F_g * F_h \quad (1)$$

where F_a is the fraction of the dose that is absorbed after oral administration, F_g is the fraction of the dose that escapes intestinal metabolism and F_h is the fraction of the dose that escapes hepatic metabolism. Since $F_h = 1 - E_h$, where E_h is the hepatic extraction ratio (which is a measure of the liver's ability to extract drug from the systemic circulation), and $E_h = CL_h / Q_h$, one obtains:

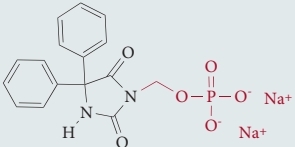
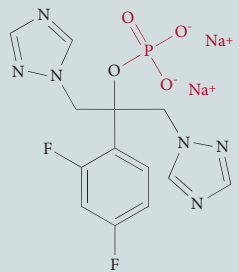
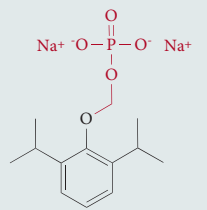
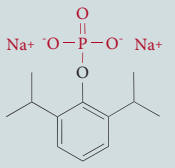
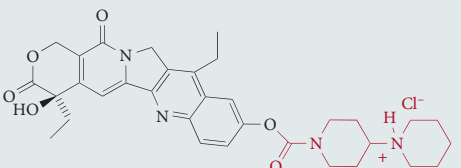
$$F_{max} = F_a * F_g * (1 - \frac{CL_h}{Q_h}) \quad (2)$$

where CL_h is the hepatic drug blood clearance and Q_h is the hepatic blood flow. When hepatic metabolism is the primary clearance mechanism and the fraction of drug excreted renally, f_e , is small, and with the assumption that drug is equally distributed between blood and plasma (blood/plasma ratio = 1), the total blood clearance (CL) is equal to CL_h since $CL_h = (1 - f_e) CL$ and $f_e \sim 0$ (REFS 168, 169). For the purposes of this Review it will be assumed that gut-wall metabolism is negligible, that is, F_g is near unity, which leads to a simplified equation:

$$F_{max} = F_a * (1 - \frac{CL}{Q_h}) \quad (3)$$

Equation 3 states that for most drugs the bioavailability is controlled by the fraction absorbed and by the clearance of the drug. The F_a is largely controlled by the physicochemical parameters of the parent or prodrug, that is, its gastrointestinal permeability, solubility, dissolution rate and dose number³⁸. F_a can be calculated in a preclinical species once CL and absolute bioavailability have been determined¹⁷⁰. Q_h is a known constant in preclinical species and humans¹⁷¹; if the clearance is half of the liver blood flow in a given species¹⁷¹ the theoretically maximum achievable bioavailability is 50%. For example, a compound with a CL of 35 ml per min per kg in the rat will have a calculated maximum bioavailability of only 50%, given the rat liver blood flow of ~70 ml per min per kg¹⁷¹, even if absorption is complete and gut-wall metabolism is negligible, that is, F_a is near unity. Similarly, if a parent drug series has a rat blood clearance >50 ml per min per kg, then $E_h > 0.7$ and its maximum oral bioavailability is limited to ~30%.

Table 3 | Prodrugs for improved parenteral administration

Prodrug name (therapeutic area)	Functional group	Structure	Prodrug strategy
Fosphenytoin (anticonvulsant)	Phosphonooxymethyl amine of phenytoin		• Rapidly converted to phenytoin by alkaline phosphatases (half-lives 7–15 min)^{101,102} • Increased aqueous solubility from 20–25 µg per ml of phenytoin to 140 mg per ml of fosphenytoin
Fosfluconazole (antifungal)	Phosphate ester of fluconazole		• Bioconversion by alkaline phosphatases¹⁰⁶ • Allows a low-volume bolus and higher dose product for intravenous administration • Increased aqueous solubility of fosfluconazole (over 300 mg per ml)
Phosphonooxymethyl propofol (anaesthetic)	Phosphonooxymethyl ether of propofol		• Is rapidly converted to propofol after intravenous administration by alkaline phosphatases^{106,185} • Significantly increased the aqueous solubility of propofol from 150 µg per ml to ~500 mg per ml
Propofol phosphate (anaesthetic)	Phosphate ester of propofol		• Significantly increased the aqueous solubility of propofol (150 µg per ml) • But bioconversion to propofol after intravenous administration is significantly slower when compared with phosphonooxymethyl propofol¹⁰⁷
Irinotecan (anticancer)	Dipiperidino carbamate of camptothecin		• Increased aqueous solubility from 2–3 µg per ml (in water) of camptothecin to 20 mg per ml (at pH 3–4) of irinotecan • Undergoes rapid, pH-dependent equilibrium with closed and open forms of the lactone ring • Only the lactone form is active^{112,115}

enhancing permeability, which is achieved by masking polar or charged moieties of a poorly permeable parent drug. These prodrugs are often carboxylic acid esters^{3,40,41}, such as GS-4104, the ethylester of GS-4071 (oseltamivir; marketed as Tamiflu by Gilead/Roche) (TABLE 1), or phosphonic acid esters^{42,43} of poorly permeable but aqueous soluble parent drugs. Thus, these are prodrugs of Class III parent drugs in the [Biopharmaceutical Classification System](#) (BCS)³⁸, with parent drugs of low permeability, but high solubility (FIG. 2). As these prodrugs are designed to enhance permeability, and as they typically do not alter the metabolic clearance or half-life of parent drugs, ‘ideal’ parent drug candidates will have low to moderate clearance in preclinical species with an hepatic extraction ratio (E_h) that is less than 0.5.

For parent drugs that are not significantly metabolized by the liver in preclinical species^{44,45}, it can be seen from equation 2 in BOX 1 that the bioavailability

is determined by the fraction absorbed, that is, $F_{max} \sim F_a$. For example, the bioavailability of adefovir (9-[2-(phosphonomethoxy)ethyl] adenine; PMEA) in rats is low (near 8%)⁴⁶, which is in agreement with adefovir’s low F_a (reported to be close to 12% using *in silico* methods⁴⁷). The low F_a resulting in low bioavailability is likely to be caused by the low cellular permeability of the charged phosphonic acid⁴⁸, and not by poor aqueous solubility or high E_h . The bioavailability of adefovir in the rat was greatly enhanced with the bis-(pivaloyloxy-methyl) prodrug to almost 40%⁴⁹. The bis-(pivaloyloxy-methyl) prodrug, also known as adefovir dipivoxil, is now marketed as Hepsera by Gilead (TABLE 1).

MGS0039 (TABLE 1) is a recent example of a successful prodrug discovery strategy to enhance the bioavailability by increasing the F_a of ester prodrugs of the metabotropic glutamate receptor 2 (MGLUR2; also known as GRM2) antagonist^{52,53}. *In vitro* screening experiments included

parent-drug release from the prodrug in human liver S9 fractions as well as *in vivo* testing in rat and monkey. The parent drug is a poorly permeable carboxylic acid, with a low permeability of less than 1×10^{-6} cm per s in the intestinal Caco-2 cell line, and no evidence of intestinal efflux. The clearance of MGS0039 is low in the rat (2.9 ml per min per kg)⁵⁰ and in the monkey (5.8 ml per min per kg)⁴¹ with $E_h \leq 0.1$. Using equation 3 in BOX 1, an F_a of less than 0.15 can be calculated from rat and monkey *in vivo* data, indicating incomplete absorption. Combined, the data suggest that the poor bioavailability of less than 13% in rats and monkeys is caused by poor permeability, not by high hepatic extraction⁴¹. However, it should be noted that theoretically, intestinal metabolism of the parent drug may also be saturated with the prodrug (more accurately increased intestinal availability, F_g), so that increased bioavailability of a prodrug may be the result of an increased $F_a \cdot F_g$, or absorption. For MGS0210, alkyl prodrugs did increase the bioavailability to 40–70% in the rat and up to 44% in the monkey. MGS0210 (TABLE 1), the *n*-heptyl prodrug, was chosen as the most promising candidate for further development on the basis of its high bioavailability in the monkey (39%), combined with its high parent-drug-release ratio in human S9 incubations (77%) and little or no formation of additional unknown metabolites⁴¹.

Intestinal drug absorption may also be limited by efflux transporters, which secrete drugs and intracellularly formed metabolites back into the intestinal lumen. Well-known examples of efflux pumps are P-glycoprotein (P-gp)^{51–53} and breast cancer resistant protein (BCRP)^{54,55}, which are expressed on the apical surfaces of the gut wall epithelium among various other tissues. Although intestinal efflux proteins may be reasons for incomplete oral absorption and variable bioavailability of drugs, particularly for parent drugs with low intestinal permeability^{52,53,56}, these transporters are also important to consider as a liability in terms of clinical drug–drug interactions and inter-individual variability. Various prodrugs intended to avoid efflux-protein-mediated transport of drugs from the intestinal lumen and thereby enhance the oral absorption and bioavailability of the parent drug are under preclinical investigation^{57–59}.

In the following sections, some of the more common prodrug approaches for improving oral drug delivery are discussed, including improved aqueous solubility, improved lipophilicity and carrier-mediated absorption.

Improved aqueous solubility. Approximately 40% of the drug candidates produced from combinatorial screening programmes have poor aqueous solubility; that is, they have an aqueous solubility of less than $10 \mu\text{M}$ ^{60,61}. Sometimes conventional formulation technologies, such as salt formation, particle size reduction, solubilizing excipients and complexation agents, can not provide adequate solubility. In these cases, prodrugs offer an alternative tool to overcome the solubility limitations of poorly soluble drugs when first-pass metabolism is low to moderate and not the main cause of systemic drug availability. Parent drug properties for which a solubility-enhancing prodrug strategy could be appropriate are listed in BOX 2.

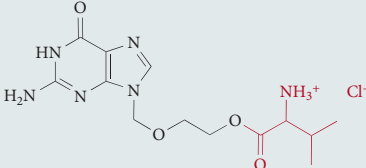
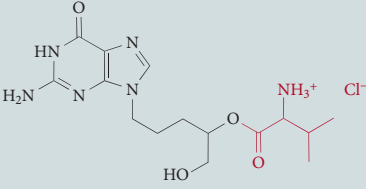
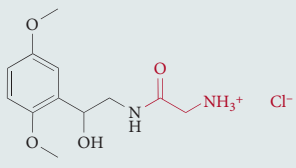
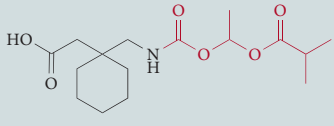
Although there are many examples of successful water-soluble prodrugs for parenteral administration, only a few water-soluble prodrugs have been developed exclusively for oral administration. Many of the water-soluble prodrugs for enhanced oral drug delivery include the addition of an ionizable progroup to the parent compound (such as a phosphate group). However, enhanced water solubility, and thus, better oral bioavailability may also be achieved by decreasing the crystal packing or by affecting the melting point of the parent drug⁹.

A good example of a water-soluble oral prodrug is the non-steroidal anti-inflammatory indene derivative, sulindac (TABLE 2). This is a bioprecursor prodrug that does not contain a promoiety, but instead, its inactive sulphoxide form is reduced to the active sulphide form after oral absorption^{62,63}. Sulindac sulphoxide, the prodrug, is about 100-times more water-soluble than the pharmacologically active sulphide. Greater solubility incorporated with the prodrug's optimal lipophilicity (logP 1.52 at pH 7.4) provides more efficient oral absorption^{64,65}.

Phosphate esters can increase the oral bioavailability of many poorly water-soluble drugs. They are especially useful for drug candidates that require a high dose and exhibit a dissolution-rate limited absorption²⁸. Nearly all oral phosphate ester prodrugs are rapidly hydrolysed to the parent drug by endogenous alkaline phosphatases at the intestinal cell surface during absorption, leading to low prodrug concentrations in the systemic circulation. An example is a water-soluble phosphate ester miproxifene phosphate (TAT-59; TABLE 2), the prodrug of DP-TAT-59 (REFS 28,66). The parent drug, DP-TAT-59, has low to moderate hepatic clearance (CL_h) compared with the hepatic blood flow (Q_h) in preclinical species⁶⁶ and in humans⁶⁷, and a low E_h in the rat (0.4) and dog (0.2). DP-TAT-59 is apparently not bioavailable in preclinical species owing to the 'brickdust' nature of this parent drug, with a solubility of less than $1 \mu\text{g}$ per ml⁶⁸. After TAT-59 prodrug dosing, DP-TAT-59 bioavailability was greatly enhanced to 28.8% in rats and 23.8% in the dog⁶⁶, and in human trials DP-TAT-59 showed dose-linear pharmacokinetics after TAT-59 dosing⁶⁹.

Another example, fosamprenavir (Lexiva/Telzir; GlaxoSmithKline) (TABLE 2), is a phosphate ester of the HIV protease inhibitor amprenavir (Agenerase; GlaxoSmithKline), which shows improved water solubility and an oral bioavailability that is equivalent or higher to that of amprenavir⁷⁰. While the marginally water-soluble amprenavir (0.04 mg per ml) requires a high dose (1,200 mg twice a day, or 8 capsules), fosamprenavir as a calcium salt has a 10-fold higher water solubility, permitting a more simplified and patient-compliant dosage regimen (4 tablets once a day)^{71,72}. Fosamprenavir is rapidly hydrolysed by gut epithelial alkaline phosphatases to amprenavir during absorption, with only minimal concentrations of fosamprenavir reaching the circulation^{70,71}. Fosamprenavir is also a good example of prodrugs having improved life-cycle management over the parent drug. The additional costs to develop fosamprenavir will probably be covered by its extended patent life of 6 years over the parent amprenavir⁷³.

Table 4 | Prodrugs to exploit carrier-mediated absorption

Prodrug name (therapeutic area)	Functional group	Structure	Prodrug strategy
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)^{90–92,182}
Valganciclovir (antiviral)	L-Valyl ester of ganciclovir		- Bioconversion by intestinal and hepatic esterases - Transported predominantly by hPEPT1 - Oral bioavailability improved from 6% (ganciclovir) to 61% (valganciclovir)^{183,184}
Midodrine (vasopressor)	Glycyl amide of desglymidodrine		- Bioconversion by unknown peptidase - Transported by hPEPT1 - Oral bioavailability improved from 50% (desglymidodrine) to 93% (midodrine)⁹⁴
XP13512 (restless leg syndrome, neuropathic pain)	Isobutanoyloxy-ethoxy carbamate of gabapentin		- Bioconversion by esterases - Transported by both MCT1 and SMVT - Oral bioavailability improved from 25% (gabapentin) to 84% (XP13512) in monkeys^{98,99}

hPEPT1, human peptide transporter 1 (also known as SLC15A1); MCT1, monocarboxylic acid transporter 1 (also known as SLC16A1); SMVT, sodium-dependent vitamin transporter (also known as SLC5A6).

Other orally administered water-soluble phosphate ester prodrugs include estramustine phosphate, prednisolone phosphate, and fludarabine phosphate (Fludara; Bayer) (TABLE 2).

Improved lipophilicity. Prodrugs are most frequently applied to mask polar and ionizable groups within a drug molecule with the aim of improving oral drug delivery. Increasing drug lipophilicity promotes membrane permeation and oral absorption. BOX 2 lists some parent drug properties for which a permeability-enhancing prodrug strategy could be appropriate.

Some of the best examples of prodrugs in this category include ACE inhibitors and ampicillin prodrugs, which have already been described. Most nucleoside antivirals are polar, and thus poorly absorbed after oral administration. In the case of tenofovir and adefovir, high hydrophilicity of the phosphonic acid moieties have been postulated to account for their poor oral bioavailability (<5%). From a series of bis-carbonate esters of tenofovir⁷⁴, tenofovir disoproxil (Viread; Gilead) (TABLE 1) was selected for further development. In clinical studies, tenofovir disoproxil has been well tolerated, with an oral bioavailability of approximately 39%^{75,76}, and is now approved for the treatment of HIV^{76,77}. Similarly, the lipophilic adefovir dipivoxil (TABLE 1) was developed and approved as a bis-pivalate ester prodrug for the treatment of hepatitis B after

an initial trial for the treatment of HIV^{78,79}. Both tenofovir disoproxil and adefovir dipivoxil are rapidly converted back to their parent drugs by esterases^{74,78,79}.

Oseltamivir (TABLE 1) is an oral prodrug of oseltamivir carboxylate (GS4071, Ro 64-0802), a selective inhibitor of viral neuramidase glycoprotein in influenza A and B^{80–82}. As an ethyl ester, oseltamivir is rapidly and well absorbed, and increases the oral bioavailability from 5% to 79%^{82,87}. Oseltamivir undergoes fast bioconversion to oseltamivir carboxylate mostly by human carboxylesterase 1 (CES1), resulting in the production of small quantities of ethanol as a by-product^{80,83}.

A more recent example of an ethyl ester prodrug is ximelagatran (Exanta; AstraZeneca) (TABLE 1), a prodrug of melagatran, which was the first member of orally administered direct thrombin inhibitors⁸⁴. As a zwitterion, melagatran has an oral bioavailability of only 3–7%. Ximelagatran is a double prodrug, as, in addition to an ethyl ester group in the carboxylic acid end, it contains an *N*-hydroxyamidine group in the amidine end of melagatran. Therefore, the formation of melagatran requires two metabolic reactions. The *N*-hydroxy group is reduced to an amidine in the liver, and to some extent also in the intestine, by CYP450 enzymes. The ethyl ester is then hydrolysed to free carboxylic acid in the liver by carboxyl esterases^{84,85}. The oral bioavailability of melagatran is improved up to 20% by ximelagatran⁸⁶. Ximelagatran was

Table 5 | Prodrugs for improved ophthalmic and dermal delivery

Prodrug name (therapeutic area)	Functional group	Structure	Prodrug strategy
Dipivefrin (glaucoma)	Dipivalic acid diester of adrenaline		- Bioconversion by esterases - More lipophilic (600-fold) dipivefrin is able to permeate the human cornea 17-times faster than adrenaline^{119,120}
Latanoprost (glaucoma)	Isopropyl ester of latanoprost acid		- Bioconversion by esterases - Improved lipophilicity achieves better ocular absorption and safety^{124,186}
Tazarotene (topical skin disorders, psoriasis, acne)	Ethyl ester of tazarotenic acid		- Bioconversion by esterases - Is both a prodrug and soft drug (undergoes oxidative deactivation) - Improved lipophilicity and maintained adequate aqueous solubility; resulted in better skin permeation^{131,132}

approved for marketing in Europe in 2004, but was withdrawn in February 2006 after an extended clinical trial confirmed the initial concerns of severe liver toxicity, possibly caused by the chemical template and not the promoiety⁸⁷.

Carrier-mediated absorption. Recent progress in molecular biology has allowed the identification and cloning of nutrient transporters and the elucidation of the functional and structural characteristics and regulation of these proteins⁸⁸. Prodrugs targeted towards specific membrane transporters are designed to have structural features that would allow them to be taken up by one of the endogenous transporters present at the intestinal epithelium⁸⁹. Targeting specific transporters is particularly important for polar or charged drugs that have negligible passive absorption.

Peptide transporters appear to be attractive targets for prodrug design, as they are widely distributed throughout the small intestine and show sufficiently high transport capacity and broad substrate specificity^{32,88,89}. Good examples of prodrugs that exploit carrier-mediated transport are valacyclovir (Valtrex; GlaxoSmithKline) (TABLE 4) and valganciclovir (Valcyte; Roche) (TABLE 4). They are L-valyl esters (for example, amino-acid valine as the promoiety) of acyclovir and ganciclovir, which both have limited and variable oral bioavailability owing to their high polarity. These amino-acid prodrugs increased the intestinal permeation of their parent drugs by 3–10-fold, and their membrane transport was not passive but mediated predominantly by the di- and tripeptide transporter (hPEPT1)^{90–93} expressed in the intestinal epithelial cells⁹⁴. Following their membrane transport, both prodrugs are readily bioconverted back to their parent drugs by intracellular hydrolysis^{93,95,96}.

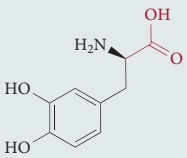
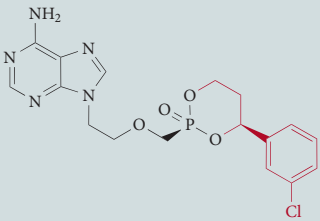
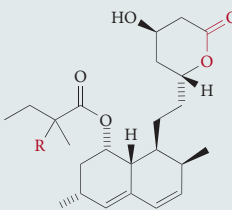
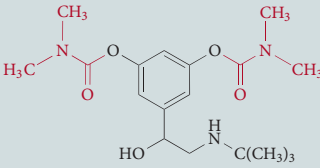
An amino-acid prodrug approach was also used in the development of an oral prodrug of desglymidodrine (DMAE), a selective α_1 -receptor agonist for the treatment of orthostatic hypotension^{94,97}. The prodrug midodrine (TABLE 4) contains a glycine promoiety that is attached to the amine functionality of DMAE and it is converted into its active drug mainly in the liver and in the systemic circulation by unknown peptidases⁹⁷. Midodrine is a substrate for hPEPT1, and this carrier-mediated transport raises the bioavailability of midodrine to 93%, compared with 50% for DMAE⁹⁴.

Gabapentin (Neurontin; Pfizer) is a structural analogue of GABA (γ -aminobutyric acid) that is marketed for the treatment of epilepsy and post-herpetic neuralgia. Gabapentin has suboptimal pharmacokinetic properties including saturable absorption, high inter-patient variability, lack of dose proportionality and a short half-life. XP13512 (TABLE 4) is a carbamate prodrug of gabapentin developed by Xenoport. The prodrug takes advantage of both a monocarboxylate transporter type 1 (MCT1), which is highly expressed in all segments of the colon and upper gastrointestinal tract, and a sodium-dependent multivitamin transporter (SMVT), responsible for absorption of multiple essential nutrients^{98,99}. The oral bioavailability of gabapentin was increased from 25% to 84% by use of the prodrug (XP13512) in monkeys, and showed dose-proportional gabapentin exposure in humans⁹⁸. XP13512 is currently in Phase III development for restless legs syndrome and in Phase II development for neuropathic pain.

Improved parenteral administration

There are numerous successful prodrugs with improved aqueous solubility properties for parenteral administration. The most commonly used prodrug-based approach

Table 6 | Prodrugs for other purposes

Prodrug name (therapeutic area)	Functional group	Structure	Prodrug strategy
Levodopa (Parkinson's disease)	Carboxylic acid of dopamine		• Crosses the blood–brain barrier and enters the brain by using LAT1 • Is decarboxylated to dopamine by aromatic amino-acid decarboxylase^{136,137}
Pradefovir mesylate (antiviral)	2-(3-chlorophenyl)-[1,3,2]dioxaphosphinane of adefovir		• Undergoes cytochrome P450-catalyzed oxidation to adefovir predominantly in the liver^{154,155,187}
Simvastatin, R=CH ₃ ; lovastatin, R=H (hypercholesterolaemia)	Inactive lactone forms		• Bioprecursor prodrugs that are converted into the active hydroxyl acid forms in the liver^{156,157,188}
Bambuterol (asthma)	Bisdimethylcarbamate of terbutaline		• Prolongs duration of drug action • Undergoes cascade of hydrolysis and oxidation reactions to terbutaline^{163,165}

LAT1, type 1 L-type amino-acid transporter.

to increase water solubility is to introduce an ionizable/polar moiety to the parent drug. As the increase in solubility imparted by the dianionic phosphate group is often of several orders of magnitude, several phosphoric acid esters have been developed as potential water-soluble prodrugs for parenteral administration and, less commonly, for oral administration.

Fosphenytoin (Cerebyx; Pfizer/Eisai) (TABLE 3) is a phosphate ester prodrug of poorly water-soluble phenytoin for the acute treatment of seizures, and can be used for both intravenous and intramuscular administration^{100,101}. In fosphenytoin, a phosphate ester is attached to a weakly acidic ($pK_a = 8.3$) amine functionality of phenytoin via an oxymethyl spacer group, leading to a remarkable increase in aqueous solubility (from 20–25 μg per ml to 140 mg per ml)¹⁰². Following intravenous administration in patients with epilepsy, endogenous alkaline phosphatases completely convert fosphenytoin back to phenytoin, with a half-life ranging from 7–15 minutes^{101,102}. As an oxymethyl-linked prodrug, the bioconversion of fosphenytoin leads to the liberation of formaldehyde within the body¹⁰³. The recovery of phenytoin is almost quantitative, with only 1–5% of the fosphenytoin dose being recovered in urine¹⁰⁰.

A phosphate prodrug approach was also applied to fluconazole (Diflucan; Pfizer), which is a broad-spectrum antifungal drug marketed in both oral and intravenous formulations¹⁰⁴. The phosphate prodrug fosfluconazole (Procif; TABLE 3) has been marketed in Japan since 2003. It exhibits an aqueous solubility of over 300 mg per ml as its disodium salt, which allows lower dosing volumes for bolus administration as well as higher intravenous doses. Fosfluconazole is almost completely converted to fluconazole in humans following an intravenous bolus dose of up to 2,000 mg, with less than 4% of the given dose excreted intact in the urine¹⁰⁵.

Two different phosphate prodrugs of propofol, a highly lipophilic but potent anaesthetic agent, have been developed and tested in clinical trials. Phosphonoxyethyl propofol (TABLE 3) is an oxymethyl-linked phosphate derivative of propofol¹⁰⁶, and propofol phosphate (TABLE 3) is a simple phosphate derivative of propofol¹⁰⁷. Phosphonoxyethyl propofol forms propofol and gives a maximum plasma concentration (T_{max}) more rapidly than propofol phosphate. This may be due to steric hindrance from two large isopropyl groups on either side of the hydroxyl group of the parent drug. The oxymethyl group of phosphonoxyethyl propofol reduces this

steric hindrance around the hydroxyl group, and the rate of bioconversion is enhanced and T_{max} is achieved earlier¹⁰⁸.

Irinotecan (CPT-11, Camptosar; Pfizer) (TABLE 3) is a parenteral water-soluble carbamate prodrug of the lipophilic antineoplastic topoisomerase I inhibitor, camptothecin (SN-38). It represents an alternative ionizable promoiety for improved aqueous solubility^{109–112}. In the irinotecan molecule, a dipiperidino promoiety is attached to the phenol moiety of camptothecin by a carbamate bond. The bioconversion back to camptothecin occurs primarily in the liver, and to a minor extent in tumours¹¹³, by human carboxylesterases¹¹⁰. Both irinotecan and camptothecin exist in a pH-dependent equilibrium between lactone and carboxylate forms, of which the lactone is the pharmacologically active form¹¹⁴. The T_{max} of camptothecin was reached in 2.3 hours after intravenous administration of irinotecan¹¹⁴. It should be noted that the dose-limiting toxicities of the parent drug camptothecin are not altered with the prodrug¹¹⁵.

Improved topical administration

The topical administration of drugs encompasses all external membranes, but here we consider only ocular and dermal prodrug applications.

Ophthalmic drug delivery. The corneal barrier limits the permeation of topically administered ophthalmic drugs into the intraocular tissues. As a result, only a small percentage of the applied dose is absorbed, most (50–99%) of which escapes into the systemic circulation¹¹⁶. Prodrugs were introduced to ophthalmology about 30 years ago when ocular absorption of adrenaline was substantially improved by the use of its prodrug, dipivalyl adrenaline (dipivefrin)^{117,118}. Dipivefrin (TABLE 5), a dipivalic acid diester of adrenaline, penetrates the human cornea 17-times more rapidly than adrenaline¹¹⁹, owing to its 600-fold increase in lipophilicity (at pH 7.2) compared with that of adrenaline¹²⁰. Consequently, a 0.1% dipivefrine eyedrop is only slightly less effective at lowering intraocular pressure than a 2% adrenaline hydrochloride eyedrop¹²¹. In addition, systemic side effects are greatly reduced¹²².

The prostaglandin analogues latanoprost (Xalatan; Pfizer) (TABLE 5), bimatoprost (Lumigan; Allergan), travoprost (Travatan; Alcon) and unoprostone isopropyl (Rescula; Santen/Novartis) represent a new class of active ocular hypotensive agents for the treatment of glaucoma. They are lipophilic isopropyl ester (latanoprost, travoprost, unoprostone) or ethanolamine amide (bimatoprost) prodrugs that are rapidly hydrolysed inside ocular tissue to biologically active prostaglandins^{123–126}. In their carboxylic acid forms, these agents are poorly permeable and cause irritation, whereas the lipophilic prodrugs achieve improved ocular absorption and safety.

Dermal drug delivery. The unfavourable physicochemical properties of many drug molecules lead to poor permeation across the skin, in particular through the most superficial layer, the stratum corneum, which provides high resistance for topical drug delivery. Numerous

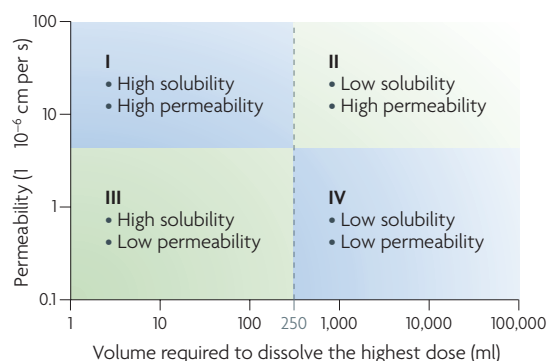


Figure 2 | Biopharmaceutical Classification System (BCS) characterization of drugs based on solubility and permeability measures. Many successful prodrugs are those of parent drugs from BCS Classes II and III (shown in green). Prodrugs of BCS Class II parent drugs enhance solubility, whereas prodrugs of BCS Class III parent drugs are designed to enhance permeability³⁸. The x-axis shows the volume (ml) required to dissolve the highest dose strength of the parent drug at the lowest solubility over the pH 1–7.5. A parent drug is considered ‘highly soluble’ when the highest dose strength is soluble in <250 ml water over a pH range of 1–7.5, in which 250 ml reflects the so-called FDA glass of water. Permeability is defined by various *in vivo* or *in vitro* assays, and a permeable drug is one associated with ≥90% oral bioavailability or ≥90% absorption as assessed by urinary excretion data.

studies have demonstrated that both water and lipid solubilities, or a balance of the two, are important in the optimization of drug permeation^{127–130}. These optimal features can often be achieved by prodrugs. In the case of tazarotene (Tazorac; Allergan) (TABLE 5), its active carboxylic acid form is esterified to a more lipophilic ethyl ester, which still maintains adequate water solubility^{131,132}. Tazarotene was effectively and reliably absorbed percutaneously, exerted less skin irritation and was rapidly converted to tazarotenic acid^{133,134}. The less lipophilic tazarotenic acid subsequently released, showed no accumulation in fat and other tissues in part due to the reduced systemic half-life of this parent drug¹³⁵, achieved by the introduction of a metabolically labile sulphur group that undergoes rapid oxidative deactivation and thus prevents accumulation in tissues. Thus, tazarotene is not only a carboxylic acid prodrug with enhanced skin permeability, but it is also a soft drug with enhanced systemic metabolism. Both can be important features for drugs aimed at topical treatment.

Site-selective drug delivery

An ultimate goal in drug delivery is that it is site selective, and this may be the most exciting possibility that prodrugs offer. Site selectivity may be achieved in four different ways: by passive drug enrichment in the organ; through transporter-mediated delivery; by selective metabolic activation through enzymes; and by antigen targeting⁴. Examples of the most frequently studied applications are listed in sub-sections below. It is to be noted, however, that possible prodrug applications are not restricted to those listed.

Box 2 | Parent-drug properties and prodrug strategies

Solubility-enhancing strategy

A solubility-enhancing strategy can be applied when low intestinal solubility or dissolution rate of the parent drug is a barrier to bioavailability, but not hepatic metabolism:

- Parent is a Biopharmaceutical Classification System (BCS) Class II drug (low solubility, high permeability).
- High dose/solubility ratio, that is, the highest targeted parent drug dose will only dissolve in volumes much larger than 250 ml over a pH range of 1–7.5.
- Low or moderate hepatic clearance, CL_h , in rats or preclinical species.
- Hepatic extraction ratio, E_h , is less than 0.5.
- High permeability in *in vitro* assays such as Caco-2.
- Fraction of dose absorbed after oral administration, F_a , is calculated or measured to be low (less than ~25%).

Permeability-enhancing strategy

A permeability-enhancing strategy can be applied when low intestinal permeability of the parent drug is a major barrier to bioavailability, but not hepatic metabolism:

- Parent is a BCS Class III drug (high solubility, low permeability).
- Low or moderate CL_h in the rat or other preclinical species.
- E_h less than 0.5.
- Low *in vitro* permeability in screening assays such as Caco-2.
- F_a is calculated or measured to be low (less than ~25%).

Central nervous system (CNS) drug delivery. Clinically, the CNS is one of the most challenging organs to target, mainly due to the blood–brain barrier (BBB). However, by understanding the transport mechanisms and enzymatic activity at the BBB, it is often possible to achieve substantially enhanced CNS delivery relative to other sites of the body. For example, the prodrug of dopamine, Levodopa (TABLE 6), is a substrate for the neutral amino-acid transporter (LAT1) expressed at the BBB^{136,137}. Having entered the brain tissue, Levodopa is rapidly converted back to dopamine, and being a very hydrophilic molecule, it is trapped there, enabling its pharmacodynamic effects.

A traditional approach to increase CNS drug concentration has been to increase the lipophilicity of the parent drug. For this approach to be successful the prodrug must have easy access to the brain tissue, bioconversion back to the parent drug should be highly site-selective, and the parent drug should exhibit prolonged retention within the brain tissue¹³⁸. Once the lipophilicity of the drug is increased by the development of a prodrug, it has improved access to the CNS. However, increased lipophilicity alone does not ensure a higher concentration of the parent drug in the target tissue. Bioconversion in the target tissue needs to be rapid and selective enough to compete with elimination, and also to ensure that any premature bioconversion of the prodrug is kept to a minimum.

Tumour targeting. The aim in cancer therapy is to target an inactive prodrug selectively to tumour cells, where the active drug may then be released without causing toxicity to normal, healthy tissue^{139,140}. Owing to the high proliferation rates of tumour cells, in addition to bio-reductive activity, the levels of certain enzymes are often

elevated in these cells and have been exploited in targeted prodrug-tumour delivery¹⁴¹. A need for reduced normal tissue exposure of the cytotoxic drug, 5-fluorouracil (5-FU), has led to the development of a prodrug that is activated by tumour-selective enzymes^{142,143}. Capecitabine (Xeloda; Roche) (FIG. 3) is an orally administered carbamate prodrug of 5-FU that requires a cascade of three enzymes for the bioconversion to the active drug¹⁴⁴ (FIG. 3). Intact capecitabine is absorbed from the intestine and undergoes bioconversion in tumours, thus, avoiding any systemic toxicity^{144,145}. The bioavailability of 5-FU after oral administration of capecitabine is almost 100% and the T_{max} of 5-FU is reached within 1.5–2 hours¹⁴³.

To expand the range of tumours susceptible to enzyme-prodrug cancer therapy, prodrug-activating exogenous enzymes can be delivered to tumour cells by using antibodies or genes. The most common approaches are antibody-directed enzyme prodrug therapy (ADEPT) and gene-directed enzyme prodrug therapy (GDEPT). ADEPT is a two-step therapy, in which the enzyme–antibody conjugate first binds to a tumour-specific antigen on the malignant cell membrane^{139,140,146,147}. The inactive prodrug is then administered and activated to a cytotoxic drug by the localized enzyme. The principle of GDEPT is similar, but the enzyme is localized to tumour cells using a targeting vector to deliver the gene encoding the enzyme^{148,149}. Some ADEPT systems have progressed to clinical Phase I studies^{150–152}, and one GDEPT has reached Phase III clinical trials¹⁴⁹.

Liver-targeted delivery. Of all organs, the liver may hold the greatest potential for organ-specific targeted drug delivery, because, as the metabolizing organ, it possesses a wide variety of liver-specific metabolizing enzymes¹⁵³ that are capable of prodrug activation. Pradefovir mesylate (TABLE 6) is a cyclic 1,3-propanyl ester prodrug of a nucleoside monophosphate (NMP), adefovir, that is under development for the treatment of hepatitis B^{154,155}. Pradefovir undergoes a CYP450-catalysed oxidation reaction predominantly in hepatocytes in the liver¹⁵⁵. In Phase II clinical trials in patients with hepatitis B, pradefovir has demonstrated good efficacy with low systemic adefovir levels, which is indirect evidence for liver targeting¹⁵⁴.

Simvastatin (Zocor; Merck) and lovastatin (for example, Mevacor; Merck) are bioprecursor prodrugs of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors used in the treatment of hypercholesterolaemia (TABLE 6). Simvastatin and lovastatin are administered in their inactive hydrophobic lactone forms, which are then transformed into the active hydroxy acid forms in the liver^{156,157}. Moreover, the lactone ring present in cyclic lactone undergoes relatively rapid metabolism by CYP450 enzymes. Lipophilic simvastatin and lovastatin are well absorbed from the gastrointestinal tract and are taken up by hepatocytes by a transport mechanism^{157–159}. Both prodrugs are highly extracted by the liver, and their bioavailability is only 5% or less, in part due to CYP3A4/P-gp-mediated clearance and metabolism of prodrugs in the intestine and in the liver¹⁵⁷.

Capecitabine (Xeloda) is a prodrug that has reduced gastrointestinal toxicity and high tumour selectivity. The enzymatic bioconversion pathway initiates in the liver, where human carboxylesterases 1 and 2 (CES1 and CES2) cleave the ester bond of the carbamate¹⁴². This is followed by a fast, spontaneous decarboxylation reaction resulting in 5'-deoxy-5-fluorocytidine (5'-dFCyd)¹⁴⁴. Generation of the parent drug continues in the liver, and to some extent in tumours, by cytidine deaminase (CDA), which converts 5'-dFCyd to 5'-deoxyuridine (5'-dFurd). Finally, thymidine phosphorylase (dThdPase; also known as ECGF1) liberates the active drug 5'-fluorouracil in tumours^{7,144}.

Although various pharmaceutical formulations are frequently used to prolong the duration of drug action, a few examples that use prodrugs exist. Highly lipophilic prodrugs of several steroids (for example, testosterone nandrolone) and neuroleptics (for example, fluphenazine, flupenthixol, haloperidol) are slowly released in the circulation from the site of intramuscular injection and result in a prolonged duration of action^{160,161}. Once released from the injection site, prodrugs are usually rapidly bioconverted, with no attenuation of their therapeutic action in most cases. As an example, the onset of action of fluphenazine is generally between 24–72 hours after injection of its lipophilic decanoate ester prodrug, which continues for 1–8 weeks with an average duration of 3–4 weeks¹⁶².

In general, while the fundamental process of safety assessment for a prodrug is no different to that of any other drug molecule, the extra facets provided by prodrugs are likely to require additional consideration and, therefore, effort and cost. However, considering the high cost of drug development and the low rate of drug success, carefully designed prodrugs will warrant the increased costs of additional complexity. The safety assessment of prodrugs was recently comprehensively reviewed¹⁶⁶, and is briefly discussed below.

However, there are significant needs that have not yet been adequately addressed by prodrugs. It is surprising how few marketed prodrug examples exist for cancer therapy, such as those designed to increase site-selective drug delivery, despite the prominent side effects of anticancer agents. In addition prevention of pre-systemic drug metabolism and the circumvention of efflux-limited drug absorption and distribution have not received enough attention in prodrug research, despite great possibilities.

In summary, prodrugs have become an integral part of the drug design and delivery process, as illustrated by the increasing number of approved prodrugs and patents. We anticipate that an increased application of rational prodrug approaches at early stages of the drug

discovery process by multidisciplinary teams including medicinal chemists, pharmaceutical chemists, and drug metabolism and pharmacokinetic scientists will lead to the development of compounds with better drug-like properties.

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