The expanding role of prodrugs in contemporary drug design and development

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Abstract | Prodrugs are molecules with little or no pharmacological activity that are converted to the active parent drug in vivo by enzymatic or chemical reactions or by a combination of the two. Prodrugs have evolved from being serendipitously discovered or used as a salvage effort to being intentionally designed. Such efforts can avoid drug development challenges that limit formulation options or result in unacceptable biopharmaceutical or pharmacokinetic performance, or poor targeting. In the past 10 years, the US Food and Drug Administration has approved at least 30 prodrugs, which accounts for more than 12% of all approved small-molecule new chemical entities. In this Review, we highlight prodrug design strategies for improved formulation and pharmacokinetic and targeting properties, with a focus on the most recently marketed prodrugs. We also discuss preclinical and clinical challenges and considerations in prodrug design and development.

Solid form selection
The selection and characterization of the preferred pharmaceutical solid-state form of a drug.

Formulation
The combination of excipients with the active pharmaceutical ingredient.

Dosage form design
A formulation configured to provide a particular delivery function via a commercializable format.

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doi:10.1038/nrd.2018.46
Published online 27 Apr 2018

The effort to increase the number of druggable targets has coincided with a substantial expansion of the chemical property space in which new drug candidates can reside. As a consequence, contemporary molecules are often associated with undesirable physicochemical properties that create considerable challenges for their delivery to the appropriate biological target. The pharmaceutical industry has responded with major advancements in solid form selection, formulation and dosage form design in an effort to overcome or mitigate those challenges. Structural modifications designed to influence the inherent physicochemical properties of a molecule to enable its delivery and development have increasingly been integrated into the design of these molecules in the discovery phase. Although analogue optimization is generally recognized as the preferred path forward, it has become more and more common to invoke prodrug strategies during the design phase, rather than as a salvage effort once formulation and delivery strategies have been exhausted[1–3]. Implementing an early prodrug strategy may actually result in more rapid clinical development and, ultimately, commercialization of a drug product. Prodrugs can provide a path forward when it becomes challenging, if not impossible, to optimize physicochemical properties that are incompatible with development for the desired delivery routes.

Prodrug strategies are often invoked to overcome deficiencies in the physicochemical properties of a molecule that limit formulation options and result in unacceptable biopharmaceutical performance[4,5]. Similarly, modifications in physicochemical properties can be used to overcome barriers for absorption, distribution, metabolism, excretion and toxicity (collectively referred to as ADMET). Through modification of fundamental properties such as polarizability, electronic factors (which include ionization constants), topological or steric factors, hydrophobicity, hydrogen bonding and chemical reactivity, key biopharmaceutical properties such as solubility, permeability or partitioning, chemical or enzymatic stability and transporter affinities can intentionally be altered.

Prodrug strategies were formally recognized by Adrian Albert in 1958 (REF. [6]) but actually have their roots in the early part of the previous century, as exemplified by methenamine, phenacetin and prontosil[7–9]. Prodrugs are molecules with little or no pharmacological activity in their own right but have a built-in structural lability, whether by chance or by design, that permits bioconversion in vivo. This conversion can occur through a chemical or enzymatic process or a combination of the two. Conversion liberates the active drug from the masking promoiety or drug carrier or triggers a structural modification or rearrangement (such as an intramolecular reaction or oxidation) such that the resulting molecule is an active metabolite. Prodrugs that undergo structural rearrangements are usually referred to as bioprecursor prodrugs[10]. Prodrug strategies for the most common functional groups on parent drugs are described in FIG. 1.

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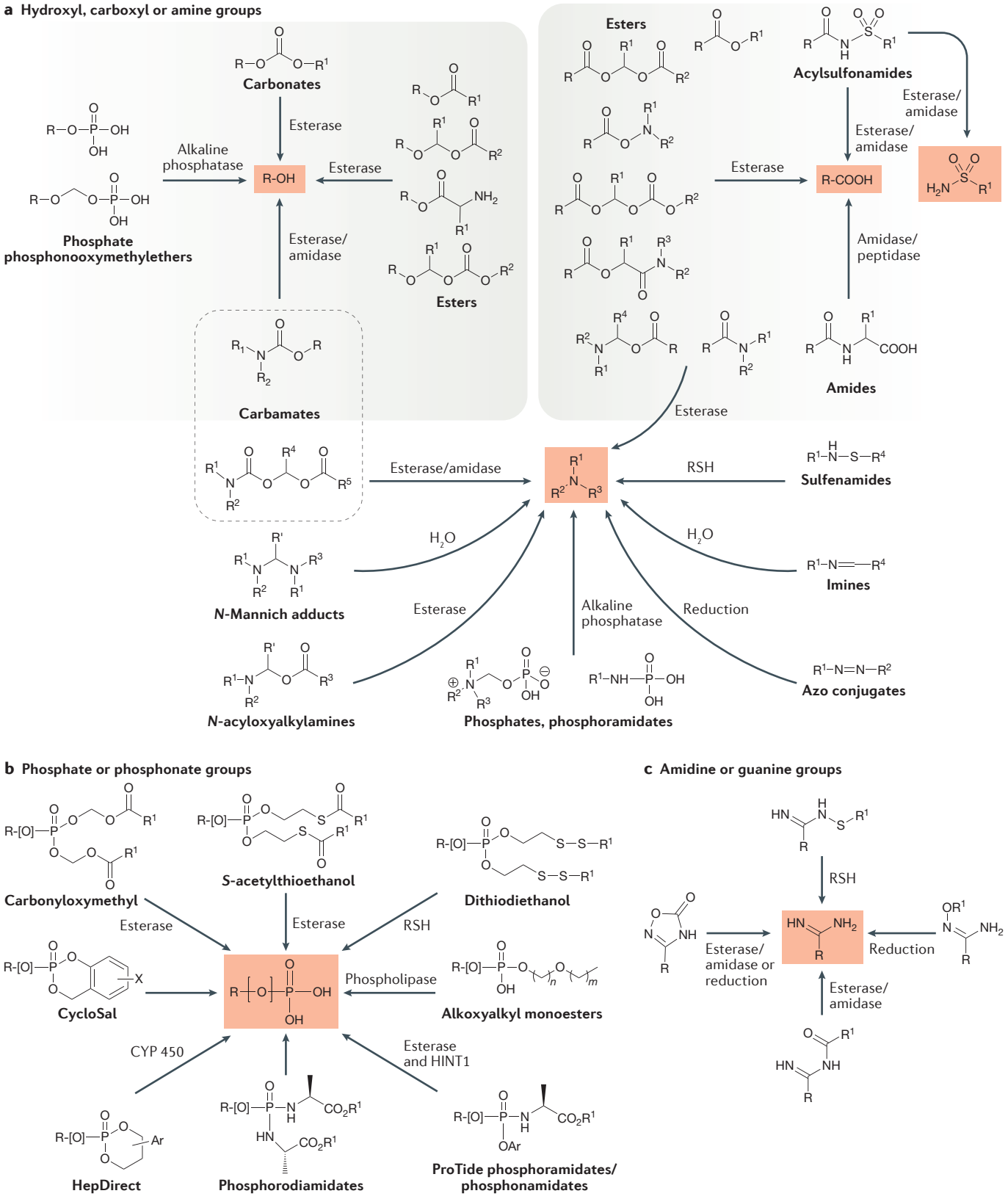

Figure 1 | **Prodrug strategies for the most common functional groups on parent drugs.** Most prodrug strategies require a 'synthetic handle' (functional group) on the drug. The selected prodrug approach is dictated by the liability of the drug that needs to be overcome by the prodrug strategy. Polar and/or ionized functional groups, such as hydrophilic hydroxyl, carboxyl or amine groups (part **a**), phosph(on)ate groups (part **b**) or amino groups (part **c**), can be converted to more lipophilic alkyl or aryl esters or *N*-acyl derivatives using prodrug strategies to improve permeability. These prodrugs can be converted back to the active form by the enzymes or reactions as indicated. The parent drugs are shown in orange. CYP450, cytochrome p450; HINT1, histidine triad nucleotide-binding protein 1.

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Prodrugs, soft drugs (also known as antedrugs) and codrugs (also known as mutual drugs) can often be confused, but each group is designed with different objectives in mind. In contrast to prodrugs, soft drugs are pharmacologically active molecules that, once appropriately delivered, are rapidly converted to a less pharmacologically active or even completely inactive form[11]. Such soft drug approaches are often used for specific tissue targeting (such as to the eye, skin or lung) to minimize overall systemic exposure and undesirable side effects. Clevidipine butyrate (Cleviprex), an ultrashort-acting calcium-channel blocker, is an example of a soft drug that is rapidly hydrolysed into an inactive form after intravenous administration in patients with hypertension, with a half-life of approximately 1 minute[12]. This short half-life enables titration to achieve the desired blood pressure reduction. Codrugs, on the other hand, are similar to prodrugs in that they consist of pharmacologically active drugs, but for codrugs, two compounds are coupled together and each is the promoiety for the other. Upon bioconversion, codrugs liberate both active molecules in the same target tissue[13]. A good example of a marketed codrug is the antibacterial agent sultamicillin, which releases both the β-lactam antibiotic ampicillin and the β-lactamase inhibitor penicillanic acid sulfone upon hydrolysis[14].

Over the past decade (2008–2017), at least 30 prodrugs have been approved by the US Food & Drug Administration (FDA) (TABLE 1), which accounts for over 12% of all small-molecule new chemical entities (NCEs) and almost 10% of all approved drugs (which encompasses both biologics and NCEs). Although this 10-year period resulted in approximately three prodrugs approved per year, it is interesting to note that over the past 3 years, 17% of NCE approvals were prodrugs (in total, 30% of the approvals were biologics, and the remaining 70% were NCEs). Approximately 10% of all marketed drugs worldwide can be considered prodrugs.

Prodrugs have been extensively reviewed from various perspectives[1–3,9,15–18]. A previous prodrug Review was published in this journal 10 years ago, in 2008 (REF. [10]). In the present 'anniversary' Review, we demonstrate various prodrug design opportunities to overcome ADMET hurdles that would have otherwise limited the developability of active molecules. We focus on prodrugs that have been approved by the FDA since 2008, but some older prodrugs, either approved or clinically evaluated but unapproved, are also discussed to demonstrate the versatility and variety of prodrug strategies. We also discuss some of the key considerations and challenges in bringing prodrugs from the bench to the clinic.

Promoiety
A covalently bound, inactive moiety bound to a drug to form a prodrug that provides the desired pharmaceutical properties.

Parenteral
Administered via skin penetration (typically intravenous, subcutaneous or intramuscular).

Benefits of prodrugs

Aqueous solubility: parenteral delivery

Sufficient solubility of a drug is essential for parenteral or injectable drug dosing. Typically, sparingly soluble drugs can be solubilized in a liquid dosage form with various formulation strategies, such as pH adjustment and inclusion of co-solvents, surfactants, solubilizers or cyclodextrins[19]. However, when these formulation strategies prove inadequate or cause irritation or toxicity, prodrug approaches can enable parenteral administration[20,21]. The majority of parenteral prodrugs utilize either a polar or, most often, an ionizable promoiety to increase aqueous solubility. Whereas prodrugs with a non-ionizable but polar promoiety (for example, glycol, polyethylene glycol and sugars) can typically increase solubility by 2–3-fold, ionizable promoieties can improve solubility by several orders of magnitude. Correspondingly, many commercially successful parenteral prodrugs contain an ionized promoiety, such as a succinic acid, amino or phosphate group.

Several early parenteral prodrugs in clinical use are succinate esters of their respective parent drugs. Examples include succinate esters of the adrenal corticosteroids prednisolone (FIG. 2) and methylprednisolone as well as the antibiotic chloramphenicol. However, drawbacks of succinate esters often include limited solubility in the pH range of optimal ester stability, insufficient chemical stability of an ester in solution and incomplete conversion of succinate esters into parent drugs in vivo, properties that have limited the use of this strategy in more recent prodrugs[21–24].

Recently approved prodrugs in parenteral formulations utilize a phosphate group either directly attached to the parent drug or, when direct attachment is not possible, through a short linker such as OCH_2, which is liberated as formaldehyde. Relative to succinate prodrugs, phosphate prodrugs are usually more stable in solution and seem to undergo fast and quantitative bioconversion in vivo by alkaline phosphatases. Fosfluconazole is an example of a phosphate prodrug that results from the direct phosphorylation of a tertiary alcohol (FIG. 2). Because the phosphate promoiety exists in a dianionic form at most physiological pH values, it greatly increases the solubility of the broad-spectrum antifungal drug fluconazole. Although fluconazole itself is available as a 2 mg mL^{-1} dilute solution in saline for infusion, fosfluconazole has an aqueous solubility of over 300 mg mL^{-1} as its disodium salt, which allows lower dosing volumes for bolus infusion as well as higher intravenous doses[25]. Fosfluconazole is readily bioconverted to fluconazole in humans after an intravenous bolus dose of up to 2,000 mg, with less than 4% of the dose excreted intact in the urine[26]. Fosfluconazole has been available in Japan since 2003.

Fospropofol (Lusedra) is a phosphonooxymethyl prodrug of the sedative or hypnotic drug propofol in which the phosphate promoiety is linked to the sterically hindered hydroxyl group of propofol through an OCH_2 spacer[27,28] (TABLE 1, #3). This spacer projects the biolabile phosphate moiety beyond the sterically encumbered phenol, allowing improved access by alkaline phosphatase. Once the phosphate group is enzymatically cleaved, the resulting hydroxymethyl intermediate spontaneously degrades to form formaldehyde and propofol. The hydrolysis occurs rapidly in humans, with a liberated propofol maximum plasma concentration (C_{max}) being achieved by 8 minutes following an intravenous bolus 10 mg kg^{-1} dose of fospropofol[28]. Propofol itself is sparingly water soluble (0.1 mg mL^{-1} at pH 7.4 (REF. [29])) and is formulated in an oil and water emulsion, which can lead to hyperlipidaemia after long-term use,

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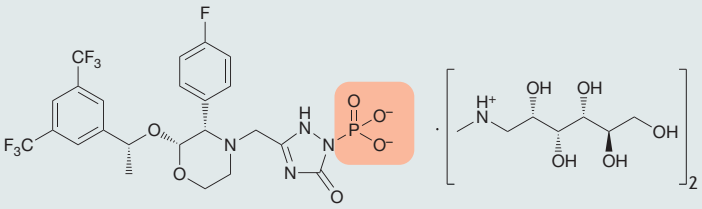
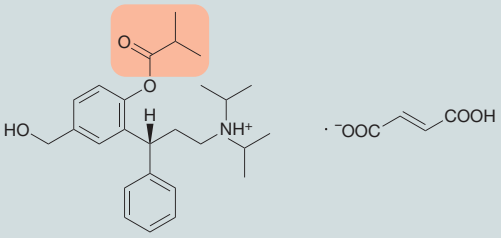
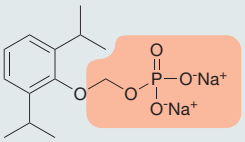
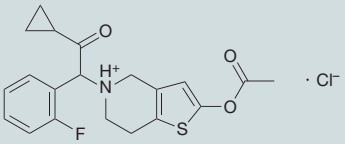
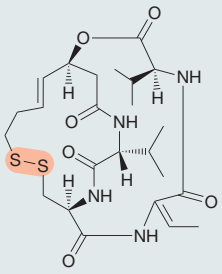
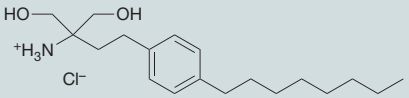
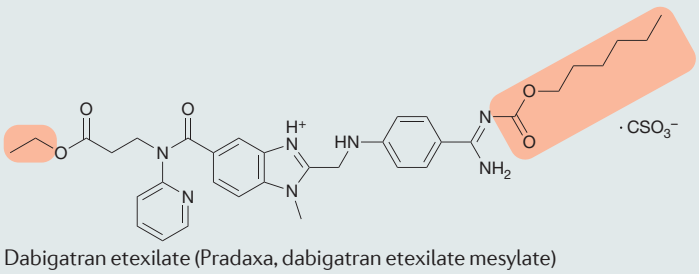
Table 1 | **Prodrugs approved by the FDA in 2008–2017 (in the order of their approval date)**

No.	Prodrug (drug name) and structure (promoiety in orange box)	Indication (mechanism of action)	Prodrug strategy and improved property	Approval date
1	 Fosaprepitant (Emend, fosaprepitant dimeglumine)	Prevention of chemotherapy-induced nausea and vomiting (SPR antagonist)	• *N*-Phosphoryl prodrug • Improved solubility allowing intravenous administration[30] • NCE exclusivity was granted in 2009	25 Jan 2008
2	 Fesoterodine (Toviaz, fesoterodine fumarate)	Overactive bladder (competitive muscarinic receptor antagonist)	• Isobutyryl ester • Reduced interpatient pharmacokinetic variability[258]	31 Oct 2008
3	 Fospropofol (Lusedra, fospropofol disodium)	Anaesthetic (sedative–hypnotic agent)	• Phosphonooxymethyl ester • Improved aqueous solubility of propofol from 150 μg mL^{-1} to ~500 mg mL^{-1} (REFS [27,29])	12 Dec 2008
4	 Prasugrel (Effient, prasugrel hydrochloride)	Reduction of thrombotic and cardiovascular events (inhibitor of the platelet $P2Y_{12}$ receptor that is activated by ADP)	Bioprecursor prodrug that releases a thiolactone by ester hydrolysis and undergoes further metabolic activation to a form that sulfenylates the platelet $P2Y_{12}$ receptor[259]	7 Oct 2009
5	 Romidepsin (Istodax)	Cutaneous T cell lymphoma (zinc-dependent HDAC inhibitor)	Reduction of the disulfide bond by glutathione results in an active monocyclic dithiol[260]	5 Nov 2009
6	 Fingolimod (Gilenya, fingolimod hydrochloride)	Multiple sclerosis (S1P agonist)	• Phosphorylation in vivo at one hydroxyl group forms the active S1P agonist[261] • Hydroxyl form is a more lipophilic prodrug that enables permeation	21 Sept 2010
7	 Dabigatran etexilate (Pradaxa, dabigatran etexilate mesylate)	Thromboembolism (direct inhibitor of thrombin)	• Both an ethyl ester on the carboxylic acid and *N*-hexyloxycarbonylamidine on the amidine • Permeation through increased lipophilicity[64]	19 Oct 2010

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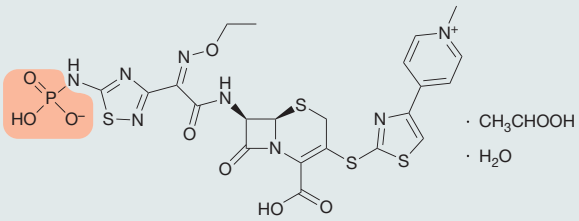
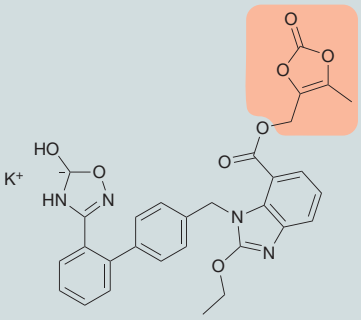
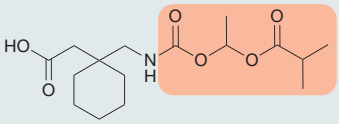
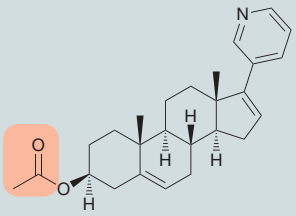
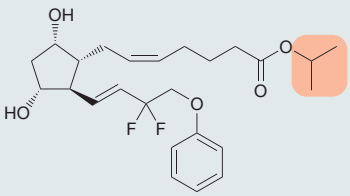
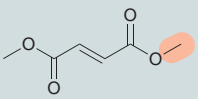
8	 Ceftaroline fosamil (Teflaro, ceftaroline fosamil monoacetate monohydrate)	Pneumonia or skin or skin structure infections (antibacterial; inhibits cell wall synthesis)	• *N*-Phosphono prodrug • Improved solubility (from 2.3 mg mL^{-1} to >100 mg mL^{-1}), thus allowing intravenous administration[31]	29 Oct 2010
9	 Azilsartan medoxomil (Edarbi, azilsartan medoxomil monopotassium)	Hypertension (angiotensin II antagonist)	• Oxodioxolyl methyl ester[262] • The absolute bioavailability of azilsartan following oral administration of the prodrug tablet is ~58%	25 Feb 2011
10	 Gabapentin enacarbil (Horizant)	Restless leg syndrome or postherpetic neuralgia (GABA and calcium-channel modulator)	• *N*-[1-(isobutyryloxy)ethoxy] carbonyl prodrug • Increased permeation via gastrointestinal transporters[97,98]	6 Apr 2011
11	 Abiraterone acetate (Zytiga)	Metastatic castration-resistant prostate cancer (CYP17A1 inhibitor)	• Acetic acid ester • Rapid dissolution and subsequent prodrug hydrolysis may generate intraluminal supersaturated solution of abiraterone, leading to better absorption[263]	28 Apr 2011
12	 Tafluprost (Zioptan)	Intraocular hypertension (prostaglandin analogue)	• Isopropyl ester • Ocular permeation through increased lipophilicity[75,76]	10 Feb 2012; first approval in Germany on 1 Mar 2008
13	 Dimethyl fumarate (Tecfidera)	Multiple sclerosis (activator of the NFE2-related factor 2 pathway)	• Methyl ester • Increased permeation, probably through increased lipophilicity • Marketed in Germany since the mid-1990s for the treatment of psoriasis[264]	27 Mar 2013

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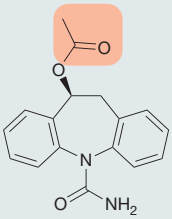
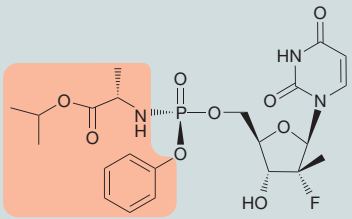
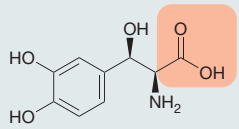
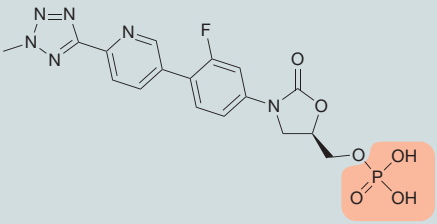
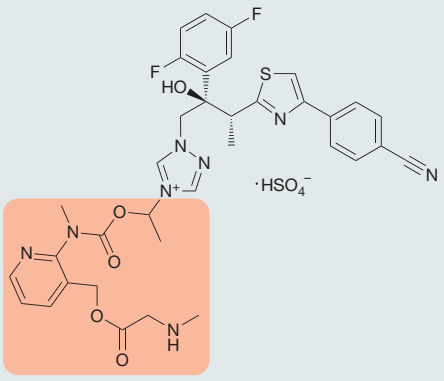
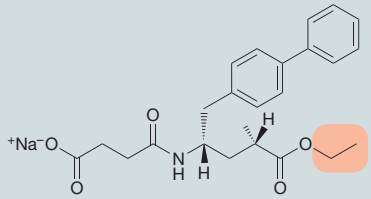
14	 Eslicarbazepine acetate (Aptiom)	Epilepsy (anticonvulsant; voltage-gated sodium-channel blocker)	• Acetate ester • Changes the metabolic profile to reduce potential drug interactions and a have a more favourable safety profile[265,266]	8 Nov 2013
15	 Sofosbuvir (Sovaldi and others)	HCV infection (nucleotide analogue; HCV NS5B polymerase inhibitor)	• Aryloxy phosphoramidate pronucleotide • Improved membrane permeation and targeting to the liver[252]	6 Dec 2013
16	 Droxidopa (Northera)	Neurogenic orthostatic hypotension or Parkinson disease – off-label (dopamine precursor)	• Carboxylated noradrenaline • Improved brain permeability[267] • Marketed in Japan since 1989	18 Feb 2014
17	 Tedizolid phosphate (Sivextro)	Acute bacterial skin or skin structure infections (oxazolidinone-class antibacterial; binds 50S ribosomal subunit)	• Phosphate ester • Improved solubility[268]	20 Jun 2014
18	 Isavuconazonium (Cresemba, isavuconazonium sulfate)	Invasive aspergillosis or invasive mucormycosis (azole antifungal; inhibits synthesis of ergosterol, a cell wall component)	• Sarcosine ester is cleaved to release an alcohol that self-immolates to release isavuconazole • Improved solubility[47]	6 Mar 2015
19	 Sacubitril sodium (part of Entresto, which contains anionic forms of sacubitril and valsartan, sodium cations and water)	Heart failure (neprilysin inhibitor)	• Ethyl ester • Likely improved permeability through increased lipophilicity[58]	7 Jul 2015

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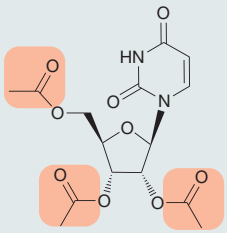
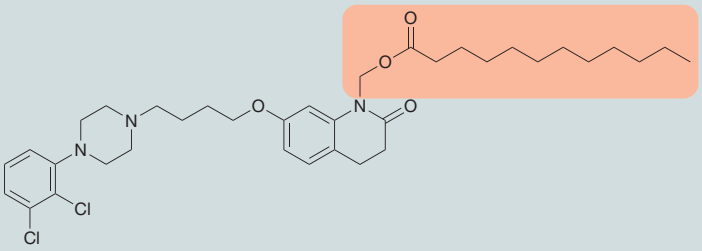
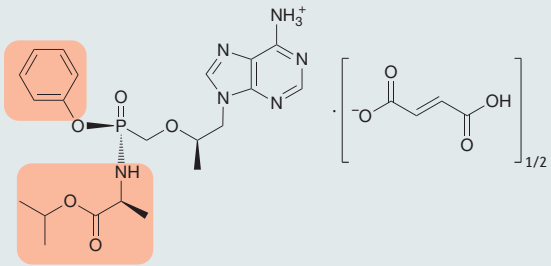
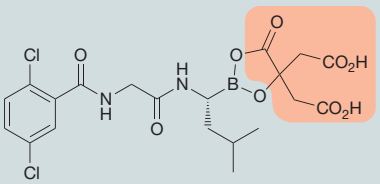
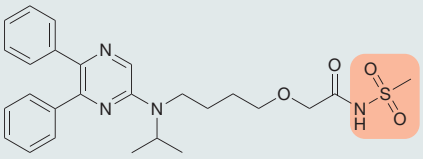
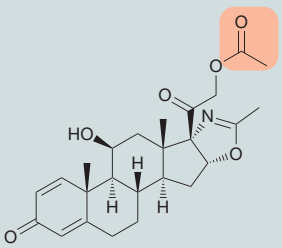
20	 Uridine triacetate (Xuriden)	Hereditary orotic aciduria (pyrimidine analogue for uridine replacement)	• Triacetate ester • Improved membrane permeability through increased lipophilicity[269]	4 Sept 2015
21	 Aripiprazole lauroxil (Aristada)	Schizophrenia (antipsychotic; partially owing to inhibition of the D_2 receptor)	• *N*-Acyloxymethyl prodrug • Sustained release and prolonged duration of action following intramuscular administration[108]	6 Oct 2015
22	 Tenofovir alafenamide (Genvoya and other combinations, tenofovir alafenamide hemifumarate)	HIV-1 infection (HIV-1 nucleoside analogue reverse-transcriptase inhibitor)	• Aryloxy phosphoramidate pronucleotide • Increased permeability through increased lipophilicity and enhanced intracellular targeting[247,248] • Prodrug targets T cells for HIV-1 but is also cleaved in the liver and thus is also used for HBV infection	5 Nov 2015
23	 Ixazomib citrate (Ninlaro)	Multiple myeloma (reversible proteasome inhibitor)	• Citrate ester • Likely increased stability of oxidatively unstable boronic acid[50]	20 Nov 2015
24	 Selexipag (Uptravi)	Pulmonary hypertension (non-prostanoid prostacyclin receptor agonist)	• *N*-Acylsulfonamide prodrug • Prolonged duration of action and reduced side effects because of the reduction in peak–trough fluctuations[113,114]	22 Dec 2015
25	 Deflazacort (Emflaza)	Duchenne muscular dystrophy (corticosteroid)	• Acetate ester • Likely increased lipophilicity[270] • Marketed in Europe since the mid-1980s for various indications	9 Feb 2017

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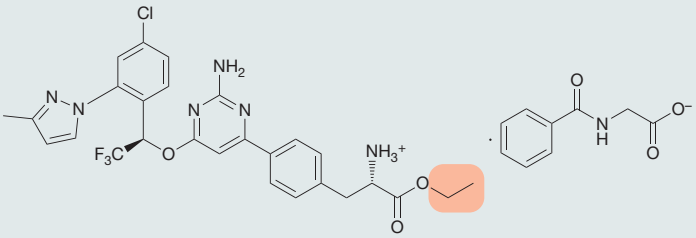
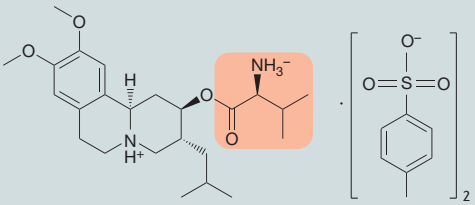
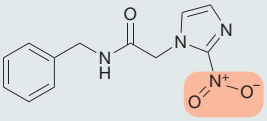
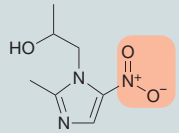
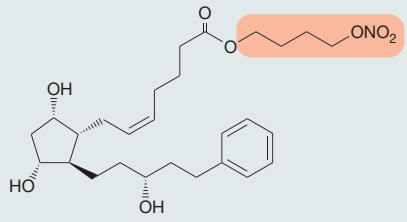
Table 1 (cont.) | **Prodrugs approved by the FDA in 2008–2017 (in the order of their approval date)**

No.	Prodrug (drug name) and structure (promoiety in orange box)	Indication (mechanism of action)	Prodrug strategy and improved property	Approval date
26	 Telotristat etiprate (Xermelo, hippurate salt of telotristat ethyl)	Carcinoid syndrome diarrhoea (tryptophan hydroxylase inhibitor)	• Ethyl ester • Likely improved permeability through increased lipophilicity[59]	28 Feb 2017
27	 Valbenazine (Ingrezza, valbenazine tosylate)	Tardive dyskinesia (VAT2 inhibitor)	• L-Valine ester of NBI-98782 (REF. [271]) • Valbenazine has 50-times less affinity for VAT2 than NBI-98782, a metabolite of tetrabenazine (Xenazine) • Improved pharmacokinetic profile[272] (valbenazine is likely not a PEPT1 substrate)	11 Apr 2017
28	 Benznidazole (Benznidazole)	Chagas disease in children aged 2–12 years (nitroimidazole antimicrobial; inhibits DNA synthesis)	• 2-Nitroimidazole prodrug • Reduced by nitroreductases into active species[149] • Benznidazole has been in medical use since 1971	29 Aug 2017
29	 Secnidazole (Solosec)	Bacterial vaginosis (nitroimidazole antimicrobial)	• 5-Nitroimidazole prodrug • Reduced by bacterial enzymes into active species[151,273]	15 Sept 2017
30	 Latanoprostene bunod (Vyzulta)	High intraocular pressure in patients with open-angle glaucoma or ocular hypertension (prostaglandin analogue)	• NO-donating prodrug or codrug • Hydrolysis by corneal esterases into latanoprost acid and butanediol mononitrate, which is further metabolized to NO and the inactive 1,4-butanediol[273]	2 Nov 2017

CYP17A1, cytochrome p450 17A1; D_2 receptor, dopamine D2 receptor; HBV, hepatitis B virus; HCV, hepatitis C virus; GABA, γ-aminobutyric acid; HDAC, histone deacetylase; NCE, new chemical entity; NFE2, nuclear factor erythroid 2; $P2Y_{12}$ receptor, P2Y purinoceptor 12; PEPT1, peptide transporter 1; S1P, sphingosine-1-phosphate; SPR, substance P receptor; VAT2, vesicular amine transporter 2 (also known as SLC18A2).

cause local pain at the injection site and increase the sensitivity of the formulation to bacterial contamination[20]. Owing to its much improved aqueous solubility (approximately 500 mg mL^{-1} (REF. [27])), fospropofol can be formulated in a more favourable aqueous ready-to-use formulation.

Other commercially available phosphate prodrugs for parenteral use are fosaprepitant[30] (Emend, TABLE 1, #1), in which the phosphate promoiety is directly attached to an amide group of the anti-emetic drug aprepitant, and ceftaroline fosamil[31] (Teflaro, TABLE 1, #8), which is an *N*-phosphonoamino prodrug of the cephalosporin antibacterial ceftaroline. Fosaprepitant has substantially improved aqueous solubility over aprepitant (12 mg mL^{-1} versus 0.2 μg mL^{-1} in isotonic saline)[30]. In vitro bioconversion studies have demonstrated that the conversion of fosaprepitant to aprepitant can occur in multiple extrahepatic tissues, in addition to the liver[30] (see FDA label in Related links). Rapid conversion was later confirmed in humans, with plasma concentrations of fosaprepitant falling below quantifiable levels (10 ng mL^{-1}) within 30 minutes after intravenous infusion. Ceftaroline fosamil also exhibits superior aqueous solubility compared with its active form ceftaroline (>100 mg mL^{-1} versus 2.3 mg mL^{-1})[31]. It is converted into bioactive ceftaroline by plasma phosphatases at a rate that allows detection of intact prodrug in plasma during intravenous infusion (see Related links).

Anti-emetic
A drug effective against vomiting and nausea.

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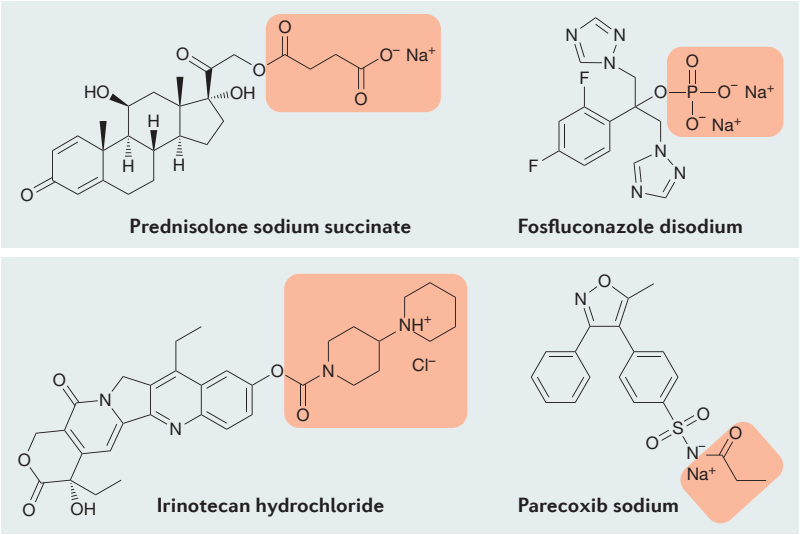

Figure 2 | **Water-soluble prodrugs with various prodrug strategies to increase aqueous solubility.** Utilization of a succinate ester (as in prednisolone sodium succinate), a phosphate group (as in fosfluconazole disodium), an alternative ionizable promoiety (irinotecan hydrochloride) and attachment of a neutral promoiety to create an ionizable element (parecoxib sodium) are applicable prodrug strategies that have been successfully used to increase aqueous solubility. Promoieties are shown in orange boxes.

Irinotecan is a parenteral water-soluble prodrug of the anticancer agent SN-38, an analogue of the highly potent plant alkaloid camptothecin[32] (FIG. 2). It has an alternative ionizable promoiety for better aqueous solubility. In the irinotecan molecule, a 1,4′-dipiperidinyl promoiety results in a solubility of >20 mg mL^{-1} at pH 3–4 owing to protonation of the tertiary amino group of the piperidine ring[33]. The bioconversion of the carbamate bond between SN-38 and the promoiety occurs mainly in the liver and, to a lesser extent, in the intestine, plasma and tumours[34]. However, in vivo, both irinotecan and the released SN-38 exist in a pH-dependent equilibrium between the active lactone and inactive carboxylate forms, resulting in a very complex pharmacokinetic profile.

In contrast to the above-mentioned ionizable prodrugs, parecoxib uses a different approach in which a water-soluble prodrug was created through the attachment of a neutral promoiety to create an ionizable element[35,36] (FIG. 2). Acetylation of the sulfonamide group of the highly selective parent drug, the cyclooxygenase 2 (COX2; also known as PTGS2) inhibitor valdecoxib, produces an acidic moiety, in which the pK_a value of the sulfonamide is reduced from 9.8 to 4.9 in the acyl sulfonamide. The more acidic sulfonamide group enables improved water solubility suitable for parenteral formulation in salt form in a physiologically acceptable pH range. Parecoxib is rapidly and almost completely hydrolysed to valdecoxib and propionic acid, primarily in the liver. Following single intravenous and intramuscular 20 mg doses of parecoxib, the C_{max} values of valdecoxib are achieved in approximately 30 minutes and 1 hour, respectively. Although parecoxib is not approved by the FDA, it is in clinical use in Europe for short-term perioperative pain control.

Oral bioavailability
The fraction of orally dosed drug that reaches the systemic circulation.

Excipients
Substances added to formulations to improve delivery or efficacy.

Aqueous solubility: oral delivery

Aqueous solubility is one of the most important properties, regardless of the administration route of a drug[19,37]. For oral drug delivery, a drug with low solubility may face low and variable oral bioavailability, which leads to an unpredictable clinical response. Prodrug strategies to improve solubility for oral administration are similar to those used for solubilizing drugs in aqueous formulations for parenteral administration[20]. In addition to attaching an ionized or a polar promoiety to a drug, improved solubility at the absorption site in the gastrointestinal tract — and consequently better oral bioavailability — may also be achieved by using a promoiety that disrupts the crystal packing of the drug[20,38].

Similar to parenteral water-soluble prodrugs, the most successful oral prodrug strategy introduces an ionizable promoiety to a sparingly soluble parent drug. Once the prodrug is dissolved in the intestinal lumen, it is typically designed to undergo conversion in proximity to the site of absorption to release the parent drug into solution. Because ionized prodrug molecules are expected to have inherently poor passive permeability, this ensures that minimal intact prodrug reaches the systemic circulation, leading to simplified analytical and toxicological profiling[39]. From this point of view, phosphate prodrugs again offer great promise because of their typically good chemical stability and ready conversion at the intestinal brush border by membrane-bound alkaline phosphatase[20,40].

A prototype of an orally administered phosphate prodrug is fosamprenavir, which is a phosphate monoester of the HIV-1 protease inhibitor amprenavir[40–42]. Amprenavir itself was marketed before fosamprenavir, but owing to its low solubility in water (approximately 0.04 mg mL^{-1} at 25 °C), it required a solubilizing formulation that contained a large amount of excipients, such as propylene glycol, polyethylene glycol 400 and D-α-tocopherol polyethylene glycol succinate (vitamin E TPGS), all in soft gelatin capsules. The required dose was 1,200 mg to be taken in eight large 150 mg capsules twice daily. By contrast, the calcium salt of fosamprenavir has approximately tenfold higher aqueous solubility, which allowed fosamprenavir to be formulated in a solid tablet that contains the molar equivalent of 600 mg of amprenavir[43–45]. This substantially greater drug loading permits a dosage regimen of only four tablets per day. Orally administered fosamprenavir is rapidly and extensively hydrolysed by alkaline phosphatase in the brush border of the intestinal epithelium to liberate amprenavir and inorganic phosphate before absorption, with only minimal amounts of intact fosamprenavir (<0.17% of the amprenavir concentration) reaching the systemic circulation[43]. Although amprenavir and fosamprenavir possess comparable therapeutic efficacy and safety profiles, fosamprenavir provides a simplified and more patient-compliant dosage regimen for patients with HIV-1 infection.

Other clinically approved oral phosphate ester prodrugs include estramustine phosphate, fludarabine phosphate, prednisolone phosphate and tedizolid phosphate[40] (Sivextro, TABLE 1, #17). The specific challenges

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encountered in the development of fostemsavir, the phosphonooxymethyl prodrug of the HIV-1 entry inhibitor temsavir, which is in late clinical testing, are outlined in BOX 1.

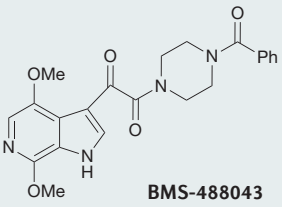
The recently approved isavuconazonium sulfate (Cresemba, TABLE 1, #18) introduced a very novel prodrug strategy for improved aqueous solubility. Isavuconazonium sulfate is an acyloxyalkyl triazolium salt-based prodrug of the broad-spectrum antifungal agent isavuconazole[46]. Isavuconazonium exhibits an excellent water solubility of >100 mg mL^{-1} as a hydrochloride salt[47] owing to a positively charged triazolium ring and the sarcosine element of the promoiety. Although the initial prodrug effort was designed to improve the solubility of isavuconazole for parenteral administration[47], the greatly enhanced solubility enabled both parenteral and oral administration of the prodrug. Following intravenous administration, isavuconazonium is rapidly and completely hydrolysed by plasma esterases, mainly by butyrylcholinesterase (see FDA label in Related links),

Box 1 | **Temsavir, an inhibitor of HIV-1 attachment, and its phosphonooxymethyl prodrug fostemsavir**

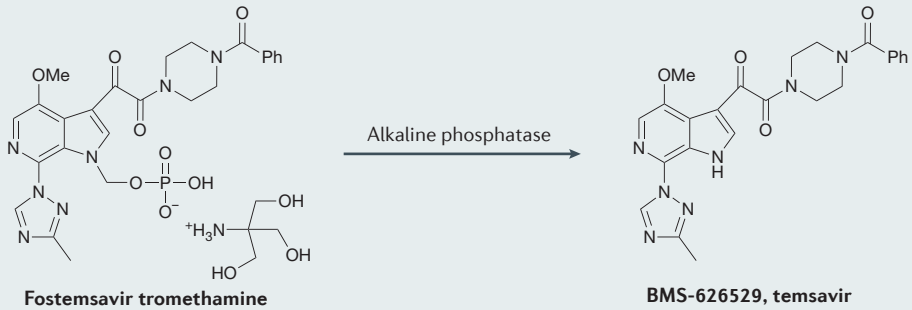
The prodrug fostemsavir was advanced into clinical trials following experience with the parent HIV-1 attachment inhibitor temsavir and its predecessor BMS-488043, which identified dissolution-limited and/or solubility-limited absorption as a major barrier to achieving optimal plasma exposure of this chemotype[240,241]. In the proof-of-concept efficacy study conducted in patients with HIV-1 infection and dosed with BMS-488043, concomitant administration of a high-fat meal was required in order to achieve targeted plasma levels.

The design of phosphonooxymethyl prodrugs for this unique class of anti-HIV-1 agents was explored as a potential solution to the delivery problem but required a delicate balance between release kinetics of the prodrug and absorption of the parent molecule, which was categorized as a biopharmaceutics classification system (BCS) class II molecule (low solubility and high membrane permeability)[209]. The rapid release of parent drug would require facile absorption in the gut in order to avoid precipitation, but a slowly releasing prodrug may be too inefficient as a delivery system[242]. Because these factors are challenging to address with de novo design, phosphonooxymethyl prodrugs of BMS-488043 and temsavir were evaluated in vivo and found to effectively address the formulation challenge, with preclinical studies indicating rapid release of the parent drug when exposed to alkaline phosphatase either in vitro or in vivo[240]. Importantly, the phosphonooxymethyl prodrugs demonstrated vastly improved plasma exposure of the parent molecule compared with a suspension of the parent compound. Furthermore, there was no major food effect in dogs, and plasma exposure was more responsive to dose escalation. However, dosing of the prodrugs of BMS-488043 and temsavir to humans revealed a short intrinsic plasma half-life for both compounds, which differed from observations in preclinical species[243]. The true pharmacokinetic profiles of these molecules were masked by the prolonged absorption of these poorly soluble compounds from the gastrointestinal (GI) tract in animal models, a phenomenon referred to as flip-flop kinetics.

In order to prolong the plasma exposure of the parent drug to allow twice-daily dosing, an extended-release form of fostemsavir was considered[244,245]. An absorption site study was first undertaken; the prevalence of alkaline phosphatase in the lower GI tract was not well understood at the outset of the experiment. Drug release of fostemsavir in the proximal small intestine, the distal small intestine and the ascending colon afforded good plasma exposure of temsavir, but release in the ascending colon provided the targeted profile of a minimized C_{max}:C_{min} (maximum:minimum plasma concentration) ratio. This result informed the design of the extended-release formulation that was used to prepare tablets for the phase IIb clinical trials of the drug. In the phase IIb study, fostemsavir was administered in combination with the integrase inhibitor raltegravir and the nucleoside-based reverse-transcriptase inhibitor tenofovir, and this regimen was compared with one that combined these two marketed drugs with the protease inhibitor atazanavir. Fostemsavir was assessed in both twice-daily and daily dosing schedules, with doses of 400 and 800 mg twice daily and 1,200 mg daily performing comparably to the atazanavir arm. A dose of 600 mg twice daily was selected for the pivotal phase III studies, which have been completed (see Related links).



BMS-488043



Fostemsavir tromethamine **BMS-626529, temsavir**

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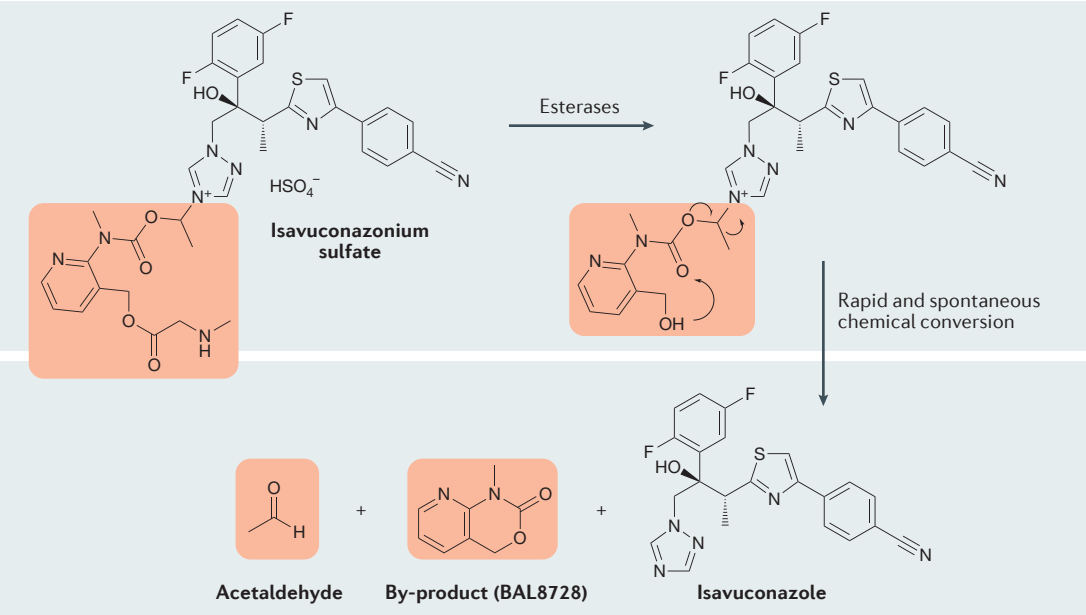

Figure 3 | **Bioconversion of isavuconazonium to isavuconazole.** Isavuconazonium sulfate is a salt-based prodrug of the broad-spectrum antifungal agent isavuconazole. In isavuconazonium, the positively charged triazolium ring and the sarcosine element of the promoiety improve aqueous solubility such that it can be administered orally or intravenously. In vivo, isavuconazonium is hydrolysed by plasma esterases to a pyridin-3-ylmethanol intermediate, which subsequently undergoes an intramolecular cyclization that triggers *N*-dealkylation to release isavuconazole, an inactive and innocuous cyclic by-product (BAL8728) and acetaldehyde. The promoiety and its derivatives are shown in orange boxes.

to a benzyl alcohol, which subsequently undergoes an intramolecular cyclization that triggers *N*-dealkylation to release isavuconazole, an inactive and innocuous cyclic by-product, and acetaldehyde[47] (FIG. 3). After oral administration, no substantial concentrations of the prodrug were detectable in plasma, indicating a rapid presystemic conversion of isavuconazonium in the intestinal tract and/or liver as well as the plasma. The bioavailability of isavuconazole after oral administration of single and multiple doses of the prodrug in humans was determined to be almost complete (98%)[48,49]. In addition to having excellent bioavailability, isavuconazole shows low interindividual variability in serum concentrations. Isavuconazonium sulfate was granted approval by the FDA in 2015 for the treatment of invasive aspergillosis and invasive mucormycosis in adults[46]. It is available in both oral capsules and lyophilized powder for reconstitution with water for intravenous administration for once-a-day dosing.

The improvement in the pharmaceutical utility of formulations — including improved dissolution rates in an aqueous environment — of oxidatively unstable boronic acids may have been one of the reasons behind the discovery of ixazomib citrate[50] (Ninlaro, TABLE 1, #23). In this prodrug, ixazomib exists in a citrate ester form (ixazomib citrate), which, when exposed to aqueous solutions or plasma, is hydrolysed rapidly to release the biologically active boronic acid form, ixazomib.

Examples in this section and BOX 1 provide various prodrug strategies designed to modulate solubility properties and improve the oral drug delivery of poorly soluble drugs. In particular, a phosphate group that is either directly attached to a functional group (such as an alcohol or amine) of a drug or attached via a CH_2O spacer group can improve solubility by several orders of magnitude. Generating phosphate-based prodrugs is particularly useful for drugs that are administered in high doses and that exhibit dissolution-limited or solubility-limited absorption[40]. The innovative solubilizing strategy presented by isavuconazonium sulfate may offer an alternative way to improve solubility for compounds not suitable for direct phosphorylation.

Passive permeability

Permeability across biological membranes remains one of the main obstacles for polar and charged drugs. Poor membrane permeability often leads to low and variable oral absorption and, consequently, to low oral bioavailability, and oral absorption of polar and charged drugs is often associated with substantial interspecies variability. Poorly permeable drugs also have low exposure levels in specific target organs even after topical administration. Improving membrane permeability has been one of the most fruitful areas of prodrug research to date.

Most frequently, the lipophilicity of a parent drug has been improved by masking its polar and ionized functionalities by short-chain hydrocarbon promoieties. Hydrophilic hydroxyl, carboxyl, phosphate or amine groups have been converted to more lipophilic alkyl or aryl esters or *N*-acyl derivatives, which are rapidly hydrolysed back to the parent drugs in the body by ubiquitous esterases or peptidases[51–53] (FIG. 1). A very good example is oseltamivir, which is an oral prodrug of oseltamivir

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carboxylate, an inhibitor of influenza neuraminidase, that was FDA-approved in 1999 (REF. [54,55]). Although oseltamivir carboxylate shows low bioavailability in preclinical species (<5%)[56], its more lipophilic ethyl ester is readily absorbed in humans after oral administration. Oseltamivir undergoes extensive bioconversion, predominantly by human carboxylesterase 1 (CES1) in the liver, resulting in maximum plasma levels of oseltamivir carboxylate within 3–4 hours and approximately 80% oral bioavailability in humans[55,57]. More recently approved ethyl ester prodrugs in clinical use are sacubitril[58] (Entresto, TABLE 1, #19) and telotristat etiprate[59] (Xermelo, TABLE 1, #26), which are lipophilic prodrugs of the endopeptidase inhibitor sacubitrilat and the serotonin synthesis (via tryptophan hydroxylase) inhibitor telotristat, respectively.

Dabigatran is a direct thrombin inhibitor with very limited oral bioavailability due to its hydrophilic and zwitterionic nature[60,61]. Dabigatran etexilate (Pradaxa, TABLE 1, #7) is a double prodrug in which the carboxylic acid is derivatized to an ethyl ester and an *N*-hexyloxycarbonyl moiety is added on the amidine to mask the ionizable groups[62]. Therefore, the formation of dabigatran requires two metabolic reactions: cleavage of the *N*-hexyloxycarbonyl by intestinal carboxylesterase 2 (CES2) to the amidine-containing ethyl ester intermediate, followed by the removal of the ethyl promoiety by CES1 in the liver[63]. Following oral administration of dabigatran etexilate in humans, the absolute bioavailability of dabigatran is 3–7%, which can be improved to 5–12% by formulation efforts[64].

Nucleoside analogues have demonstrated their effectiveness in the treatment of cancer and various infections. To exert their therapeutic effect, nucleoside analogues must undergo the stepwise addition of phosphate groups mediated by cellular kinases to form the corresponding active nucleoside triphosphates. In this activation process, the first phosphorylation step is often limiting. To circumvent this shortcoming, nucleoside analogues are frequently administered as their monophosphorylated forms or are configured to include a phosphonate moiety, both of which have further been transformed into more lipophilic ester prodrugs. These prodrugs are designed to efficiently cross biological membranes and, once in the target cell, to release the monophosphorylated form, which becomes active after intracellular conversion to the corresponding nucleoside diphosphates or triphosphates[65–67].

To date, four nucleoside monophosphate and monophosphonate prodrugs have been approved by the FDA. Adefovir dipivoxil is the bis(pivaloyloxymethyl) prodrug of adefovir[68,69] and is used in the treatment of hepatitis B virus (HBV) infection. Adefovir dipivoxil has almost fivefold higher (an increase from 12% to 59%) oral bioavailability than does adefovir itself[70]. Tenofovir disoproxil is a bis(isopropyloxymethyl) carbonate prodrug of tenofovir used for the treatment of HIV-1 and HBV infections (BOX 2). It provides dose-proportional pharmacokinetic exposure, and the oral bioavailability of tenofovir is approximately 25% in the fasted state (see Related links). The discovery of the ProTide-based prodrugs tenofovir alafenamide and sofosbuvir paved the way for the development of a novel class of nucleoside monophosphate and monophosphonate prodrugs, which are referred to as aryloxy phosphoramidate pronucleotides[71,72] (BOX 2).

The ProTide phosphoramidate drug delivery technology has also been used in NUC-1031 (Acelarin), a prodrug of gemcitabine that is in phase II clinical trials[73], and the C-nucleoside analogue GS-5734, which is being developed as a treatment for infections with Ebola and other emerging viruses and is also undergoing phase II clinical trials[74] (FIG. 4). The ester moiety in GS-5734 is different from the isopropyl ester found in tenofovir alafenamide and sofosbuvir and was identified on the basis of the antiviral potency in vitro and physicochemical properties appropriate for intravenous administration[65].

Whereas the majority of lipophilic prodrugs have been developed to improve membrane permeability and oral absorption, the same prodrug strategy has also been employed to improve topical administration of parent drugs that are absorbed through the skin or eye. Tafluprost (Zioptan, TABLE 1, #12) is a prodrug of tafluprost acid, an analogue of prostaglandin $F_{2\alpha}$ and an agonist of the human prostaglandin F receptor[75,76]. It is approved for the treatment of high intraocular pressure in glaucoma. As a lipophilic isopropyl ester, tafluprost readily penetrates the cornea after ocular instillation. Tafluprost is hydrolysed predominately by ocular carboxylesterases to tafluprost acid, which starts lowering the intraocular pressure 2–4 hours following the initial instillation of tafluprost, with a maximum effect reached after approximately 12 hours.

Several other prostaglandin $F_{2\alpha}$ analogue prodrugs with potent and long-lasting intraocular-pressure-lowering properties are widely used as first-line therapies for glaucoma or ocular hypertension. These agents include the isopropyl esters latanoprost[77] and travoprost[78], as well as the ethanolamine amide-based prodrug bimatoprost[79].

Exploiting carrier-mediated transport

Transporters are membrane proteins that play an important role in controlling the intake and efflux of crucial polar endogenous nutrients[80,81]. Their specificity is, however, not limited to endogenous substrates, and other molecules, including drugs that bear a close structural resemblance to endogenous substrates, can also be carried across cell membranes by transporters. Carrier-mediated transport is particularly important for polar and charged drugs, as they have negligible passive diffusion across biological membranes.

Prodrugs that are able to take advantage of carried-mediated transport mechanisms offer intriguing targets in drug design[82]. A long-standing but still interesting example is afforded by levodopa, which delivers dopamine to the brain for the treatment of Parkinsonism[83]. The hydrophilic dopamine cannot cross the blood–brain barrier, but levodopa is carried into the brain by the L-type amino acid transporter 1 (LAT1; also known as SLC7A5)[84,85]. Although the role of LAT1 in brain uptake

ProTide-based prodrugs
The ProTide technology is most commonly used to deliver nucleotide analogues to cells. The hydroxyls of the monophosphate or monophosphonate are masked by an aromatic group and an amino acid ester.

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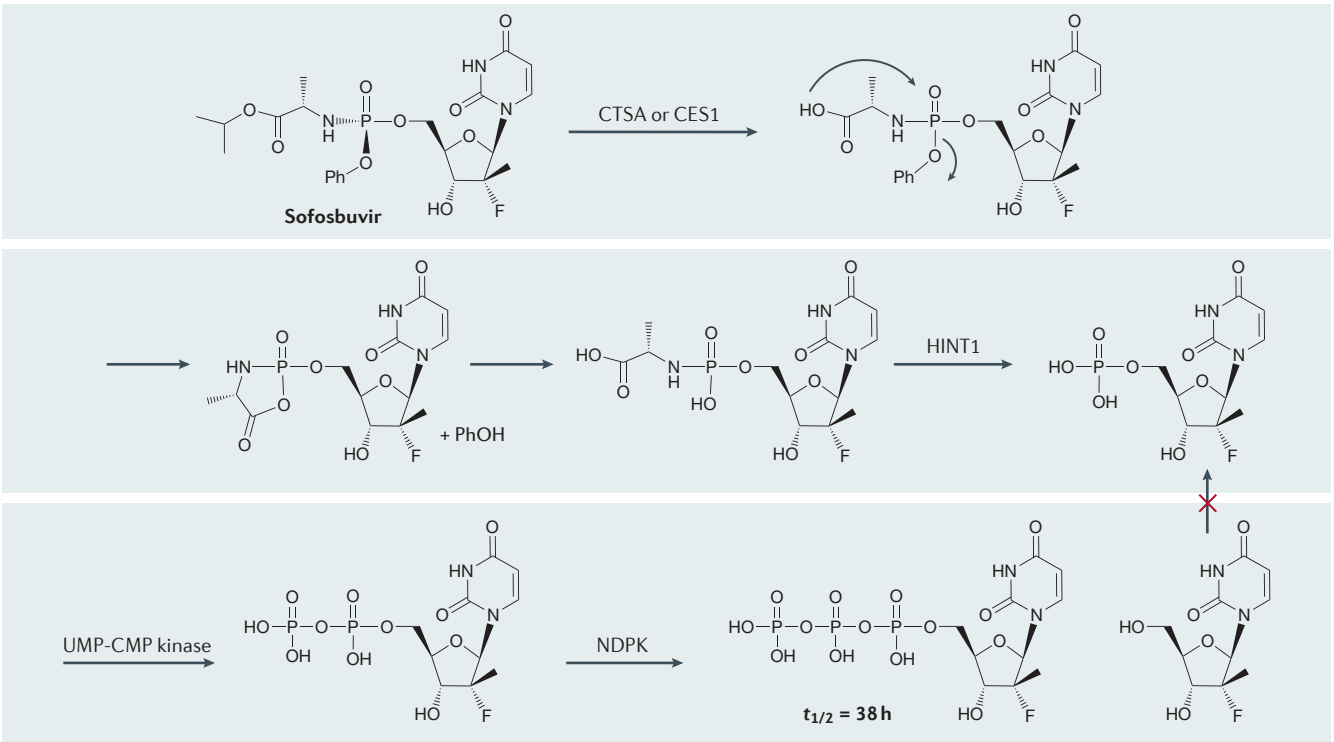
Box 2 | **Nucleoside analogue prodrugs: tenofovir, tenofovir alafenamide and sofosbuvir**

The design of nucleoside analogue drugs presents a sizeable challenge because of the complexity of their metabolism, which utilizes three consecutive phosphorylation steps to generate a triphosphate derivative, which then must be recognized by the viral polymerase. Phosphonate-based nucleoside analogues require only two consecutive phosphorylation steps but require the polarity of the phosphonic acid to be masked in order to facilitate oral absorption. The HIV-1 nucleoside reverse-transcriptase inhibitor tenofovir is configured as tenofovir disoproxil, a bis(isopropyloxymethyl) carbonate ester, which is cleaved both intracellularly and extracellularly to release tenofovir. Although tenofovir disoproxil has been a successful therapeutic agent for HIV-1 infection, the development of phosphoramidate ProTide phosphate or phosphonate prodrug technology by Christopher McGuigan[71,72,246] provided an opportunity to refine oral delivery, and the resulting compound, tenofovir alafenamide[247,248] (TABLE 1, #22), was approved by the US Food and Drug Administration in 2015 as a component of Genvoya, a fixed-dose combination tablet containing elvitegravir, cobicistat and emtricitabine. This fixed-dose combination includes 10 mg of tenofovir alafenamide rather than the 300 mg dose of tenofovir disoproxil prodrug that is formulated in the fixed-dose combination Stribild. The prodrug moiety on tenofovir alafenamide is predominantly cleaved intracellularly (as discussed below) in contrast to tenofovir disoproxil, for which prodrug cleavage occurs more broadly in plasma and tissues, providing a level of tissue targeting that allows for the use of a lower dose, which is associated with fewer side effects (including bone marrow depletion and kidney toxicity)[65,249–251]. In 2016, tenofovir alafenamide (25 mg) was approved for the treatment of hepatitis B virus (HBV) infection on the basis of the outcome of two phase III clinical trials in which the compound was non-inferior in efficacy to tenofovir disoproxil in treatment-naive and treatment-experienced adults with hepatitis B e antigen (HBeAg)-negative and HBeAg-positive chronic HBV. Tenofovir alafenamide was the first nucleoside analogue to demonstrate the clinical efficacy of phosphoramidate prodrug technology.

Sofosbuvir (TABLE 1, #15) is a phosphoramidate prodrug of β-D-2′-deoxy-2′-α-fluoro-2′-β-C-methyluridine monophosphate that efficiently delivers this compound to the cytosol of hepatocytes where, after cleavage, it is readily phosphorylated twice to generate the triphosphate, a potent inhibitor of hepatitis C virus (HCV) NS5B polymerase (see Figure)[252]. The uridine nucleoside was not an appropriate drug, as it is not an efficient substrate for 5′-phosphorylation and has poor potency in vitro. This problem was solved by the phosphoramidate prodrug, which effectively delivered the monophosphate to the cytosol. The process of unmasking the phosphoramidate to reveal the monophosphate has been studied in some detail and primarily occurs intracellularly, as the isopropyl ester is stable in plasma[253,254]. Cathepsin A (CTSA, also known as lysosomal protective protein) and carboxylesterase 1 (CES1) cleave the ester moiety, and the released carboxylate is believed to react intramolecularly at the phosphate phosphorus atom to expel phenol. The inherent chemical reactivity of the resulting acyl phosphoramidate intermediate is such that it rapidly hydrolyses to give a phosphoryl alanine derivative. This derivative is a substrate for histidine triad nucleotide-binding protein 1 (HINT1), which can remove the alanine to form the monophosphate. However, knockdown experiments indicate that HINT1 is only partially responsible for the production of the monophosphate. Conversion to the triphosphate occurs through the sequential action of uridine monophosphate–cytidine monophosphate kinase (UMP–CMP kinase) and nucleoside diphosphate kinase (NDPK). The triphosphate is recognized by the HCV NS5B polymerase and incorporated into viral RNA, where it leads to termination of chain growth, although the presence of the 3′-hydroxyl means that it is not an obligate chain terminator[255].

Sofosbuvir is rapidly absorbed from the gastrointestinal (GI) tract of animals and humans and is subject to high first-pass hepatic extraction. The uridine nucleoside is the major circulating metabolite, produced by hydrolase activity, but animal studies have indicated that the triphosphate is formed in the liver and has a terminal half-life ($t_{1/2}$) of 18 hours. In primary human hepatocytes, the $t_{1/2}$ of the triphosphate was 12 hours, consistent with exposure in human liver explants, which was estimated at ~55 μM, much higher than the inhibition constant (K_i) value of 0.42 μM for inhibition of the HCV NS5B polymerase[256,257]. Sofosbuvir is a component of the HCV combination therapies Harvoni, Epclusa and Vosevi, in which it is paired with ledipasvir, velpatasvir and the combination of velpatasvir and voxilaprevir, respectively.



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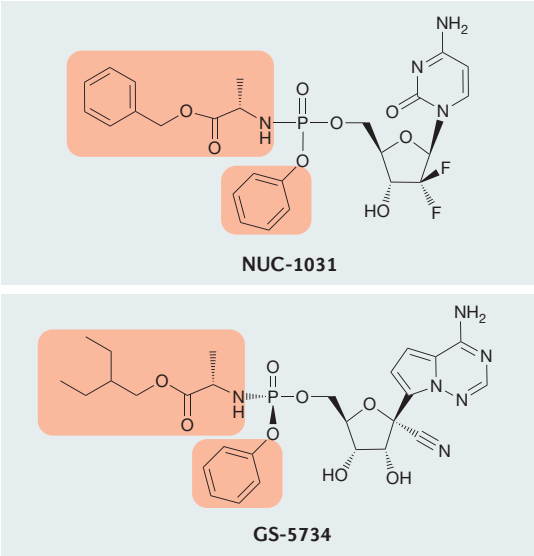

Figure 4 | **ProTide prodrugs of nucleoside monophosphate derivatives undergoing clinical trials.** NUC-1031 (Acelarin) is the phosphoramidate prodrug of the antitumour agent gemcitabine, and GS-5734 is a *C*-nucleoside analogue undergoing clinical evaluation for treatment of infections including Ebola and other emerging viruses. The ester moieties in both prodrugs are different from the isopropyl ester found in tenofovir alafenamide and sofosbuvir. The promoieties are shown in orange boxes.

and distribution of levodopa was likely discovered in hindsight, more recent carrier-mediated prodrugs have been deliberately designed for specific transporters, especially those that could enhance intestinal absorption after oral administration.

Improved oral absorption of several amino acid prodrugs has been attributed to their carrier-mediated transport via intestinal peptide transporter 1 (PEPT1; also known as SLC15A1)[86]. PEPT1 recognizes both dipeptides and tripeptides, and its expression increases from the duodenum to the ileum[87]. Valacyclovir and valganciclovir are the L-valine amino acid esters of the herpesvirus inhibitor acyclovir and the human cytomegalovirus inhibitor ganciclovir, respectively, and are the pioneering examples of marketed prodrugs that utilize PEPT1 to overcome the limited and variable oral bioavailability of the polar parent nucleoside-based drugs. The first of these drugs marketed, valacyclovir, achieved 3–5-fold higher oral bioavailability (~60%)[88] than dosing of the parent drug acyclovir (10–20%)[89]. For valganciclovir, the oral bioavailability was approximately 60%, which is almost tenfold higher than that of ganciclovir itself (6–8%)[90]. The intestinal carrier-mediated absorption of prodrugs occurs mainly by PEPT1 (REFS [91,92]) and, to a lesser extent, by other transporters such as the Na^+-dependent neutral amino acid transporter (ATB0+; also known as SLC6A14)[93]. After absorption, both prodrugs are hydrolysed to their hydroxyl forms, predominantly by valacyclovir hydrolase[94]. Furthermore, both acyclovir and ganciclovir require conversion to their active phosphorylated forms by viral kinases, thus reflecting complex, dual prodrug species.

First-pass effect
An effect in which the concentration of a drug is greatly reduced via presystemic metabolism before it reaches the systemic circulation.

Valacyclovir and valganciclovir are both valine esters, whereas midodrine is a glycine-based prodrug of desglymidodrine (DMAE) in which the glycine promoiety is attached to an amine group of DMAE via an amide bond. This prodrug is also absorbed mainly via PEPT1, which leads to an increase in the oral bioavailability of DMAE from 50% to 93%[95]. Midodrine is bioconverted by as yet unknown peptidases in the liver and systemic circulation[96]. The released DMAE acts as a selective peripheral α_1-receptor agonist and can be used in the treatment of neurogenic orthostatic hypotension.

Gabapentin is structurally related to γ-aminobutyric acid (GABA) and has been used in the treatment of epilepsy and neuropathic pain. However, gabapentin demonstrates suboptimal pharmacokinetic properties, including saturable absorption, lack of a linear dose–response relationship, high interpatient variability and a short half-life (5–7 hours), which have prompted the development of treatment options with prolonged exposures. Gabapentin enacarbil (Horizant, TABLE 1, #10) takes advantage of both monocarboxylate transporter 1 (MCT1), which is expressed with increasing abundance along the gastrointestinal tract with predominant expression in the colon, and sodium-dependent, multivitamin transporter (SMVT; also known as SLC5A6)[97,98]. Following oral administration to humans, gabapentin enacarbil produces a dose-proportional increase in gabapentin exposure, with consistently high (~75%) bioavailability in the fed state over the dose range of 300–6,000 mg[99,100]. The carbamate bond in gabapentin enacarbil is efficiently hydrolysed by nonspecific carboxylesterases, primarily in enterocytes and, to a lesser extent, in the liver, to form gabapentin, carbon dioxide, acetaldehyde and isobutyric acid (see Related links). Gabapentin enacarbil is currently available for the treatment of restless leg syndrome and post-herpetic neuralgia in adults.

Improved metabolic stability

Metabolic instability is typically attributed to hepatic metabolism, although the role of intestinal metabolism is becoming increasingly recognized. This instability can greatly reduce the total amount of a drug that reaches the systemic circulation and, ultimately, its target. Prodrugs can be used to protect active drugs from this first-pass effect by masking a metabolically labile but pharmacologically essential functional group, such as a phenol, to avoid rapid metabolism. This strategy is effectively illustrated by bambuterol, which is a long-lasting prodrug of the bronchodilator and β_2-adrenergic receptor agonist terbutaline. In bambuterol, two metabolically susceptible phenol moieties on terbutaline are protected by dimethylcarbamate promoieties designed to avoid rapid and extensive first-pass metabolism in the gut and liver[101,102]. After oral administration, bambuterol is slowly converted to terbutaline via its monocarbamate metabolite, mainly outside of the lungs by a nonspecific butyrylcholinesterase[101–104]. Other hydrolytic and oxidizing enzymes may also be involved in the release of active drug. Interestingly, because bioactivation of bambuterol leads to transfer of

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the carbamoyl moiety to the active-site serine of butyrylcholinesterase and formation of a slowly hydrolysed covalent bond, bambuterol release slows its own bioconversion. This leads to a sustained release in vivo, and once-daily bambuterol treatment provides relief from asthma symptoms with a lower incidence of side effects than terbutaline taken three times a day[105]. Bambuterol is marketed in several countries, although it is not available in the United States.

Prolonged duration of action

Sustained plasma levels and, consequently, a prolonged duration of drug action, are typically enabled by controlled-release formulations, such as suspensions and polymeric matrices. Advantages of such formulations are multifold, including reduced dosing frequency, improved patient compliance, elimination of variability in exposure and the blunting of high peaks of drug concentration in plasma. Prodrugs can be used to achieve controlled release of an active drug by modifying its aqueous solubility and dissolution properties in a way that affects the release rate of the active drug, the rate of absorption or its tissue distribution. In this regard, prodrugs have been especially useful in the development of several subcutaneous or intramuscular sustained-release depot injections, which maintain therapeutic plasma levels of a parent drug for weeks to months[106]. These prodrugs are typically fatty acid esters, such as decanoates, palmitates, enanthates, cypionates or valerates, that are formulated in an oil-based vehicle, which results in the slow release of the prodrug and, hence, modulates the disposition of the parent drug. The high lipophilicity of these prodrugs also results in binding to blood and tissue proteins, which slows enzymatic conversion and consequently results in the slow appearance of an active drug in the systemic circulation. This approach has resulted in many marketed sustained-release injectables of, for example, oestrogens (for example, oestradiol), neuroleptics (for example, fluphenazine, flupentixol, haloperidol, pipotiazine and zuclopenthixol), contraceptives (for example, hydroxyprogesterone and norethisterone) and anabolic steroids (for example, nandrolone and testosterone)[107].

Aripiprazole lauroxil (Aristada, TABLE 1, #21) is the most recently approved intramuscularly administered prodrug that provides a prolonged duration of action and is used in the treatment of adults with schizophrenia[108]. It is an *N*-acyloxymethyl prodrug of aripiprazole in which the *N*-hydroxymethyl group has been acylated with the very lipophilic dodecanoic acid. The solubilizing ingredients in the aripiprazole lauroxil suspension for intramuscular injection include sorbitan monolaurate and polysorbate 20. Once injected into the body, aripiprazole lauroxil likely undergoes enzyme-mediated hydrolysis to form the *N*-hydroxymethyl intermediate, which is susceptible to non-enzymatic chemical degradation to release aripiprazole and formaldehyde[109]. Systemic appearance of aripiprazole following a single intramuscular injection of the prodrug occurs within 5–6 days, with elevated plasma levels persisting for an additional 36 days (see Related links).

Disposition
The distribution of a drug throughout the body following administration.

Prolonged duration of action by prodrugs can also be the result of sustained bioconversion after oral administration. Because of the consistent rate of bioconversion in the body, these prodrugs can be less vulnerable to absorption-related changes than prodrugs with formulation-based delivery systems are. Prodrug strategies for sustained release typically consist of prodrug bonds such as amides, which are reasonably resistant to bioconversion. An example is lisdexamfetamine (Vyvanse), which is the L-lysine amino acid amide prodrug of dextroamphetamine and has been marketed since 2007. Lisdexamfetamine is used as a psychostimulant for the treatment of attention-deficit hyperactivity disorder in children aged 6–12 years as well as in adults[110,111]. The major advantage of lisdexamfetamine is that, being a sustained-release prodrug, it has less abuse potential than other amphetamines do[110]. Following oral administration in humans, conversion of lisdexamfetamine to dextroamphetamine occurs by enzymatic hydrolysis mainly in red blood cells[112] in approximately 1.5 hours, with a duration extending from 1.5–13 hours in children and 2–14 hours in adults[110]. The therapeutic duration of action of lisdexamfetamine is considerably longer than that of an equivalent dose of immediately released dextroamphetamine, allowing once-daily dosing of lisdexamfetamine.

Selexipag (Uptravi, TABLE 1, #24) is a recently approved prodrug of a non-prostanoid prostacyclin mimetic that exhibits a prolonged duration of action owing to a slow bioconversion process. Selexipag is an *N*-acylsulfonamide prodrug that is slowly hydrolysed back to the pharmacologically active carboxylic acid form (ACT-333679) by hepatic CES1 (REFS [113,114]). In preclinical studies in monkeys, selexipag administration resulted in a threefold lower C_{max} and almost a twofold increase in the apparent terminal half-life ($t_{1/2}$) compared with the active acid form, thereby resulting in sustained formation and a prolonged duration of action of the parent acid[114]. Selexipag is rapidly absorbed after oral administration in humans, showing dose-proportional pharmacokinetic exposure, with maximum plasma concentrations of selexipag and its active form ACT-333679 attained within 1–3 and 3–4 hours, respectively[115] (see Related links). Selexipag is approved for the treatment of pulmonary arterial hypertension[116].

Better targeting, fewer side effects

Targeted drug action can be achieved in general by either site-directed delivery or site-specific bioactivation of a prodrug. In site-directed drug delivery, the intact prodrug is selectively delivered to its site of action using drug conjugation strategies, wherein the drug is typically linked to a macromolecule carrier that recognizes target-specific markers, such as antigens or receptors. In site-specific bioactivation, a prodrug can be widely distributed throughout the body but is activated only or predominantly at the desired site. This site-specific bioactivation can be achieved, for example, by exploiting physiological conditions or endogenous enzymes in the target tissue. Alternatively, prodrug-activating enzymes can be directed to the target site by various strategies before prodrug administration. Several targeting strategies are discussed below.

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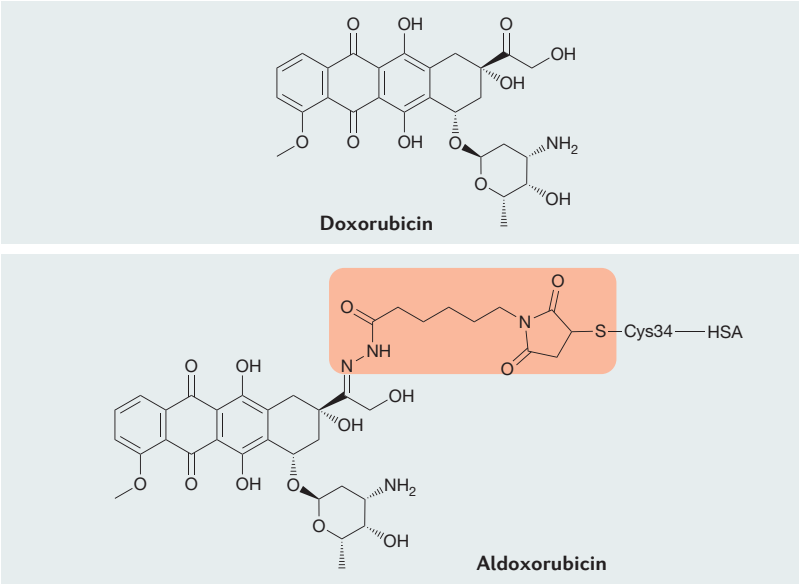

Figure 5 | **Doxorubicin and its prodrug, aldoxorubicin.** The anticancer drug doxorubicin can be attached to a thiol-reactive maleimide moiety through an acid-labile hydrazone bond to form aldoxorubicin. In vivo, the maleimide moiety reacts with cysteine 34 (Cys34) of human serum albumin (HSA) and is released upon cleavage of the hydrazone bond, which occurs selectively in the acidic environment of the tumour. The promoiety is shown in an orange box.

Targeting physiological conditions. Site-selective prodrug activation and release of the active drug can be achieved by exploiting the physiological conditions of the target site if they differ from conditions in the rest of the body. One such condition is the acidic environment found in tumour tissue, endosomes and lysosomes, which can be used for site-selective conversion of acid-sensitive bonds, such as an imine and hydrazone, in anticancer prodrugs[117,118]. Aldoxorubicin (INNO-206; FIG. 5) is a prodrug wherein the anticancer drug doxorubicin is bound to a thiol-reactive maleimide moiety through an acid-labile hydrazone bond[119]. Following intravenous administration, the maleimide moiety reacts selectively with a cysteine residue of human serum albumin. Doxorubicin is released from the albumin carrier by the cleavage of the hydrazone bond in the acidic environment of the tumour. Aldoxorubicin has reached phase III clinical trials for the treatment of soft tissue sarcoma.

Another prodrug example that utilizes unusual pH conditions in its activation process is provided by the marketed drug omeprazole. Omeprazole is an inhibitor of the enzyme H^+/K^+-ATPase, also known as the proton pump, which is responsible for acid secretion from parietal cells[120]. Omeprazole incorporates a weakly basic pyridine group ($pK_a = 3.97$), which is not protonated at physiological pH, allowing omeprazole to be absorbed and distributed into the secretory canaliculus of the parietal cells. Because the pH of parietal cells is approximately 1, omeprazole becomes protonated and accumulates inside the canaliculus of the cell. Protonation also initiates the chemical transformation of omeprazole into its active sulfenamide metabolite, which binds covalently to the thiol of a cysteine residue of H^+/K^+-ATPase to form a disulfide derivative[121,122]. This binding inhibits the ability of parietal cells to secrete gastric acid. Other proton pump inhibitors (PPIs) include structurally related analogues of omeprazole, such as lansoprazole, pantoprazole and rabeprazole, as well as single isomers of those molecules, including esomeprazole (the (*S*)-isomer of omeprazole) and dexlansoprazole (the (*R*)-isomer of lansoprazole). Although these PPIs were not originally developed as prodrugs, they provide good examples of targeted prodrugs with site-selective and use-dependent bioconversion into active species. Because the PPIs are effective only on H^+/K^+-ATPases that are located in highly acidic compartments, non-gastric H^+/K^+-ATPases are not affected; thus, these compounds have excellent safety profiles.

Targeting site-specific enzymes. Another strategy for targeted drug delivery is to design a prodrug that undergoes bioconversion by an enzyme expressed predominantly at the desired site of action. Alternatively, enzymes expressed in multiple locations can be used for prodrug activation if the prodrug can be preferentially directed to the target site using, for example, specific receptors or transporters. Less ideal prodrugs are those that undergo bioconversion in close proximity to the target site and then have activity through a bystander effect. Enzymes that provide site-selective prodrug conversion and, consequently, targeting include the cytochrome P450 (CYP) enzyme CYP3A4 in the liver[123]; β-glucuronidase[124,125], glutathione *S*-transferase[126–128], thymidine phosphorylase[129,130], tyrosinase[131,132], NADPH–cytochrome P450 reductase[133,134], DT-diaphorase (also known as NQO1)[135] or tumour-associated proteases[136,137] in cancer cells and bacterial reductases in the colon[138].

CYPs are potentially attractive prodrug activators for liver-targeted drug delivery because these enzymes are present at the highest concentrations in the liver and act on a vast array of structurally diverse substrates[15]. CYP-catalysed bioconversion of the anticancer prodrugs cyclophosphamide and ifosfamide paved the way for the discovery of liver-specific cyclic phosphate and phosphonate derivatives, termed HepDirect prodrugs[123,139–141] (FIG. 1). In this strategy, oxidative hydroxylation at the benzylic position of an aryl-substituted cyclic phosphodiester is mediated by CYP3A4. The hydroxylated intermediate undergoes rapid and irreversible ring opening to generate a negatively charged form that is largely retained within the hepatocyte as a consequence of its anionic nature, which reduces membrane permeability. The HepDirect prodrug strategy was explored as an approach to target adefovir to the liver for the treatment of HBV infection. When the HepDirect derivative of adefovir, pradefovir, was explored, the approved form of adefovir was the bispivalyoloxymethylene prodrug adefovir dipivoxil, which causes renal toxicity at high doses. By contrast, pradefovir resulted in a 12-fold and an 84-fold increase in the liver-to-kidney and liver-to-intestine ratio, respectively, in preclinical studies in rats relative to adefovir dipivoxil[141]. In clinical trials, the ability of HepDirect prodrugs to undergo prodrug activation

Parietal cells
Epithelial cells lining the stomach that secrete hydrogen chloride.

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in humans was demonstrated with evidence of liver targeting in patients infected with HBV[139,142]. The success of the phosphoramidate strategy (BOX 2) may have halted the development of HepDirect prodrugs.

It is worth noting that although cyclophosphamide and ifosfamide undergo a relatively site-selective bioconversion by CYPs in the liver, they fail to provide site-selective drug action. The relatively long-lived intermediates that are formed during the bioconversion process are sufficiently lipophilic to escape from hepatocytes and readily diffuse into the systemic circulation before being converted into active drugs. Therefore, these anticancer drugs are used for the treatment of extrahepatic tumours but are also associated with extrahepatic side effects[123]. Drug targeting can therefore be achieved by site-selective prodrug activation only if the active drug is formed and largely retained in the target cells.

Thymidine phosphorylase is a pentosyltransferase that catalyses the phosphorolytic cleavage of uridine and its derivatives. This enzyme is present in many human tissues, but its levels are increased by at least 10% in many types of tumour[143,144]. Doxifluridine (5′-deoxy-5′-fluorouridine or 5′-DFUR) is an orally administered prodrug for the treatment of various cancers that was designed to undergo site-selective conversion into the active antimetabolite drug 5-fluorouracil (5-FU)[130,143]. However, thymidine phosphorylase is also expressed in the gastrointestinal tract, and as a consequence, the conversion of 5′-DFUR to 5-FU during the absorption process causes dose-related diarrhoea[130]. An approach to overcome the unwanted gastrointestinal side effects of doxifluridine led to the rational discovery of capecitabine, which is now an approved therapy for certain types of cancer. Capecitabine is an oral prodrug that requires three enzymatic steps to release 5-FU[130,144] (FIG. 6). After administration, capecitabine is first hydrolysed to 5′-deoxy-5′-fluorocytidine (5′-DFCR) by carboxylesterase activity in the liver, which is followed by the conversion of 5′-DFCR into 5′-DFUR by cytidine deaminase, either in the liver or in tumour tissue. Finally, the release of active 5-FU takes place relatively site selectively in cancer cells by the action of thymidine phosphorylase, which has 3–10-times higher activity in various cancers than in healthy tissue[145]. The oral bioavailability of 5-FU after capecitabine administration is almost complete, with maximal concentrations of 5-FU reached within 1.5–2 hours. The concentration of 5-FU in tumour tissue was 2.5-fold higher than in healthy tissue and 14-fold higher than in plasma after capecitabine administration in patients with advanced breast cancer[146].

Sulfasalazine is activated by reduction of its azo bond by anaerobic bacteria in the lower bowel to mesalazine (also known as 5-aminosalicylic acid) and sulfapyridine (FIG. 6). After an oral dose, only a limited amount of the prodrug (10–30%) is absorbed from the small intestine (in some populations, however, the amount absorbed is <10%), so most of the dose reaches the colon. Sulfasalazine is approved for the treatment of inflammatory bowel disease and rheumatoid arthritis[147]. Mesalazine is responsible for therapeutic activity, and sulfapyridine produces adverse effects. Similar azo-based prodrugs, such as olsalazine and balsalazide, which rely upon less toxic promoieties, have been developed for clinical use[148].

Both benznidazole (TABLE 1, #28) and secnidazole (Solosec, TABLE 1, #29) are nitroimidazole derivatives that have been designed to undergo reduction of the nitro moiety to generate a reactive radical species that damages DNA in anaerobic protozoa and bacteria. Benznidazole has been used since the 1970s to inhibit the synthesis of DNA, RNA and proteins of the *Trypanosoma cruzi* parasite that causes Chagas disease but was only recently approved by the FDA. The trypanocidal activity of benznidazole depends on a parasite type I nitroreductase that has been shown to form several potentially cytotoxic metabolites from benznidazole[149,150]. Because this enzyme is absent from mammalian cells, prodrug activation occurs site selectively. Secnidazole is also active against many anaerobic bacteria that are associated with bacterial vaginosis. Bacterial reductases reduce secnidazole to radical anions, which interfere with bacterial DNA synthesis[151].

Directed enzyme prodrug therapies. One of the greatest challenges facing efforts to design prodrugs for targeted drug delivery is the lack of enzymes that are both site-specific and capable of catalysing the activation of prodrugs. Many enzymes that are relatively site-specific are also highly substrate-specific and unable to tolerate large structural changes; as such, they often fail to catalyse the bioconversion of prodrugs. To expand the selection of enzymes capable of site-selectively activating prodrugs, efforts have been undertaken to develop directed enzyme prodrug therapies (DEPTs) with the particular aim of improving targeted cancer chemotherapy. DEPTs can be further divided by the strategy used to direct the prodrug-activating enzyme to the tumour cells. The enzyme can be conjugated with a vehicle that is capable of targeting tumour cells (such as an antibody in antibody-directed enzyme prodrug therapy (ADEPT)[152,153] or a polymer in polymer-directed enzyme prodrug therapy (PDEPT)[154]), be produced in cancer cells by expression of the gene encoding the enzyme (physical gene delivery in gene-directed enzyme prodrug therapy (GDEPT) or delivered by a viral vector in virus-directed enzyme prodrug therapy (VDEPT))[155,156] or be expressed inside bacteria that accumulate in cancer cells (bacterial-directed enzyme prodrug therapy (BDEPT))[157,158]. In the next step, a non-toxic prodrug is administered and activated to a cytotoxic drug by the targeted enzyme.

The two most heavily explored therapies are ADEPT and GDEPT. In ADEPT, the prodrug-activating enzymes are linked to an antibody, which binds to the target cell surface, whereas in GDEPT, the delivered genes express the enzymes inside the target cells. ADEPT has been evaluated in clinical trials using, for example, a bacterial carboxypeptidase G2 conjugated with murine monoclonal antibodies to activate a prodrug of a benzoic acid mustard glutamate. The treatment, in a phase I trial, was well tolerated, but the antibody–enzyme conjugate did not target the tumour exclusively[159]. The most extensively

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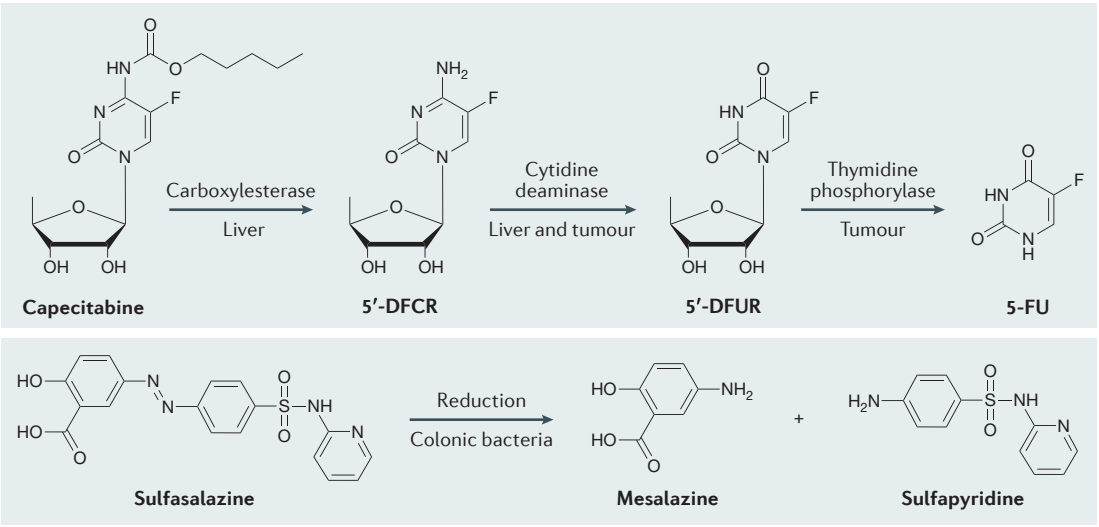

Figure 6 | **Bioconversion mechanisms of capecitabine and sulfasalazine.** Capecitabine, which is used for the treatment of numerous cancers, is first hydrolysed to 5′-deoxy-5′-fluorocytidine (5′-DFCR) by carboxylesterase activity in the liver and then converted to 5′-deoxy-5′-fluorouridine (5′-DFUR) by cytidine deaminase in either the liver or the tumour (top panel). 5′-DFUR is then converted to 5-fluorouracil (5-FU) by thymidine phosphorylase in a site-selective manner in the tumour. Sulfasalazine is activated by reduction of its azo bond by anaerobic bacteria in the lower bowel to an anti-inflammatory agent mesalazine (5-aminosalicylic acid) and the antibacterial agent sulfapyridine (bottom panel).

evaluated enzyme–prodrug pairs in GDEPT are herpes simplex virus thymidine kinase (HSV-TK) with ganciclovir, cytosine deaminase of *Escherichia coli* with 5-fluorocytosine, CYP with cyclophosphamide or ifosfamide and nitroreductase with CB1954. The HSV-TK–ganciclovir treatment has reached phase III clinical trials[155,160]. Other clinical trials are under way or are recruiting patients.

Macromolecule-based prodrugs for drug targeting. In a macromolecular prodrug delivery system, the drug is covalently bound to a macromolecule, such as a synthetic polymer, glycoprotein, lipoprotein, lectin, hormone, albumin, a liposome, DNA or a cell[161–164]. In typical cases, the aims of macromolecule–drug conjugation are to achieve improved drug targeting to a tumour and, as a consequence, to reduce drug toxicity and overcome the mechanisms of drug resistance. A potential advantage of this strategy is that the distribution of the drug depends on the macromolecular carrier, not the drug. Macromolecules typically cannot penetrate healthy membranes, which minimizes the delivery of conjugated drugs to non-targeted tissues and organs, but endothelial membranes of cancerous tissue are leaky, so macromolecules enter the tumour microenvironment from the bloodstream. Furthermore, inefficient lymphatic drainage from tumours leads to prolonged localization of the macromolecules. These two phenomena are collectively referred to as the 'enhanced permeation and retention' (EPR) effect, which is currently the subject of extensive investigation. Intracellular access of macromolecules into tumour cells can occur by pinocytosis or ligand–receptor docking. Depending on the linkers used, the drug can usually be released intracellularly upon exposure to lysosomal enzymes, such as cathepsin B, or the lower pH in endosomes and lysosomes.

In the 1990s, the synthetic non-biodegradable polymer *N*-(2-hydroxypropyl)methacrylamide (HPMA) conjugated with doxorubicin (PK1; FCE 28068) became the first macromolecular prodrug to enter phase I/II clinical trials[165]. It has a molecular mass of approximately 28 kDa, ~8.5% of which is drug (8.5 wt%). The anticancer drug doxorubicin was attached to the polymer through a peptidyl Gly-Phe-Leu-Gly linker that was demonstrated to be stable in the circulation but cleaved by lysosomal proteases after internalization by endocytosis[166]. Despite the prolonged plasma circulation half-life for redistribution to tissues ($t_{1/2\alpha}$ = 1.8 hours), an absence of liver accumulation and considerable renal elimination in a phase I evaluation[165,167], broad antitumour efficacy was not observed in phase II trials[168]. HPMA has also been conjugated to platinates, including carboplatin[169] and diaminocyclohexane (DACH) platinates[170], with reduced platinum-related toxicity observed in clinical trials. The DACH platinate also demonstrates antitumour efficacy in humans.

Polyethylene glycol (PEG) is another non-biodegradable polymer that has been used in macromolecular prodrugs to exploit EPR[171,172]. PEG has a limited drug-carrying capacity because it contains only two terminal groups suitable for conjugation. A PEG–camptothecin conjugate (Prothecan), which contains the anticancer agent camptothecin (~1.7 wt%) attached to PEG at the 20-OH position through an alanine linker, has been tested clinically[173]. This macromolecular prodrug demonstrated a prolonged plasma half-life ($t_{1/2}$ > 72 hours) and improved efficacy, with neutropenia and thrombocytopenia as dose-limiting toxicities. Another PEG conjugate, EZN-2208, consists of a 40 kDa, 4-pronged multiarm PEG with 3.7 wt% drug loading of the camptothecin derivative SN-38. EZN-2208 has been explored in a phase II clinical

Pinocytosis
The transport of fluid into a cell via vesicles created by local infoldings by the cell membrane.

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investigation of patients with advanced colorectal cancer[174] and metastatic breast cancer. Positive results in patients with previously treated metastatic breast cancer warranted further clinical study.

Concern for the potential intracellular accumulation of non-biodegradable polymer elements has prompted the use of biodegradable polymers, both natural and synthetic, to create drug conjugates. Polymers explored clinically include polyglutamic acid (PGA, degraded by lysosomal cathepsin B), dextrin (degraded by α-amylase), hyaluronic acid (degraded by hyaluronidase), hydroxyethyl starch (HES), polyoxymethylene and polysialic acid[161,164,175]. An example of a biodegradable polymer–drug conjugate that has been evaluated in clinical trials is the conjugate of PGA and the anticancer agent paclitaxel (Opaxio, formerly Xyotax)[176,177]. Paclitaxel is conjugated via an ester bond to the γ-carboxylic acid side chains of PGA to give a high drug loading of 37 wt% and a molecular mass of ~49 kDa. A small amount of paclitaxel is released from the conjugate by slow hydrolysis (up to 14% over 24 hours), but the release mainly occurs by lysosomal cathepsin B degradation of the polymer backbone after endocytic uptake[178]. The PGA–paclitaxel macromolecular prodrug showed greatly reduced severe side effects when compared with either gemcitabine or vinorelbine in a randomized phase III clinical trial in patients with non-small-cell lung cancer[179]. Although the conjugate failed to demonstrate improved survival of patients with non-small-cell lung cancer over both compounds used as single agents, it showed a large 40% improvement in survival over vinorelbine.

Several innovative anticancer treatments in which PEG is covalently attached to a bioactive substance other than a small molecule have progressed into clinical development[175]. The first anticancer product in clinical use was pegaspargase, in which L-asparaginase is covalently conjugated to PEG. This pegylated enzyme has a substantially increased half-life (357 hours) compared with the native enzyme (20 hours) and produces fewer hypersensitivity reactions[180]. Pegaspargase is used for the treatment of acute lymphoblastic leukaemia because L-asparaginase depletes the amino acid asparagine, which is essential for tumour growth.

Antibody–drug conjugates. Antibody–drug conjugates (ADCs) link an active drug to a monoclonal antibody, which specifically recognizes a cellular surface antigen and delivers the drug directly to the target cell[181,182]. The chemical conjugation of the antibody to the cytotoxic drug has a major influence on the pharmacokinetics, selectivity and therapeutic index of the therapy. Because the conjugation is formed through a cleavable bond in most of the clinically used ADCs, these conjugates can be regarded as macromolecular prodrugs. This targeting strategy has been especially successful in the treatment of various cancers. For example, the enediyne anticancer agent calicheamicin is too toxic to be used as a chemotherapeutic. However, a slightly modified calicheamicin, linked to a humanized antibody through a spacer, was developed as gemtuzumab ozogamicin (Mylotarg, FIG. 7a), which exhibited reduced toxicity compared with the parent drug[183]. The antibody is specific for the CD33 antigen that is commonly expressed in myeloid leukaemic cells, and gemtuzumab ozogamicin is approved for the treatment of $CD33^+$ acute myeloid leukaemia[184]. This ADC contains an acid-sensitive hydrazone linker that typically has a plasma half-life of several days (7–8 d) at pH 7 and only a few hours (4–5 hours) at pH 5 (REF. [185]). Gemtuzumab ozogamicin was withdrawn from the market in 2010 owing to toxicities that were attributed, in part, to poor plasma stability of the hydrazone bond, but this drug was reapproved in 2017 following a meta-analysis of prior trial results and additional results from a phase III randomized, open-label study.

One of the new ADCs in clinical use is brentuximab vedotin (Adcetris, FIG. 7b), in which the antibody is targeted to CD30, a defining marker of Hodgkin lymphoma and systemic anaplastic large-cell lymphoma[186]. The drug attached to the monoclonal antibody brentuximab is monomethyl auristatin E (known as vedotin), an antimitotic agent that inhibits cell division by blocking polymerization of tubulin. The linker used for conjugation is the plasma-stable dipeptide valine–citrulline[187], which is designed to be site-selectively cleaved by the proteolytic enzyme cathepsin B once the ADC has been internalized by tumour cells through endocytosis, thus releasing vedotin[188].

Small-molecule–drug conjugates. Low-molecular-weight ligands and peptides can also be utilized as alternatives to antibodies for targeting tumour-associated antigens[189,190]. Some of the most promising ligands include folate derivatives, which bind to the folate receptor, a protein that is highly overexpressed in many epithelial cancers, including cancers of the breast, colon, kidney, lung and ovary[191]. The most advanced conjugate in development is vintafolide, which contains folic acid as a targeting ligand, connected to a cytotoxic desacetyl vinblastine through a linker comprising a charged peptide moiety and a disulfide bond with a self-immolative spacer for drug release[190]. After binding to the folate receptor, vintafolide is translocated through endocytosis, and the cytotoxic drug is released intracellularly. Vintafolide has been clinically tested for several indications, of which a combination therapy with liposomal doxorubicin (Doxil) reached a phase III trial for the treatment of platinum-resistant ovarian cancer[192]. However, this trial was suspended following the recommendation of the data safety monitoring board because vintafolide failed to improve progression-free survival.

Another antigen that is selectively targeted by low-molecular-weight ligands is prostate-specific membrane antigen (PSMA)[189,190]. PSMA is not usually expressed in adult tissues, with the exception of normal prostate tissue and the duodenum. It is also highly expressed in the majority of localized and metastatic prostate cancer cells and in the neovasculature of many malignancies. PSMA can be efficiently targeted using urea-based derivatives of glutamic acid. The PSMA targeting ligand is chemically attached to the radioactive lutetium-177 (^{177}Lu), a cytotoxic β-particle emitter,

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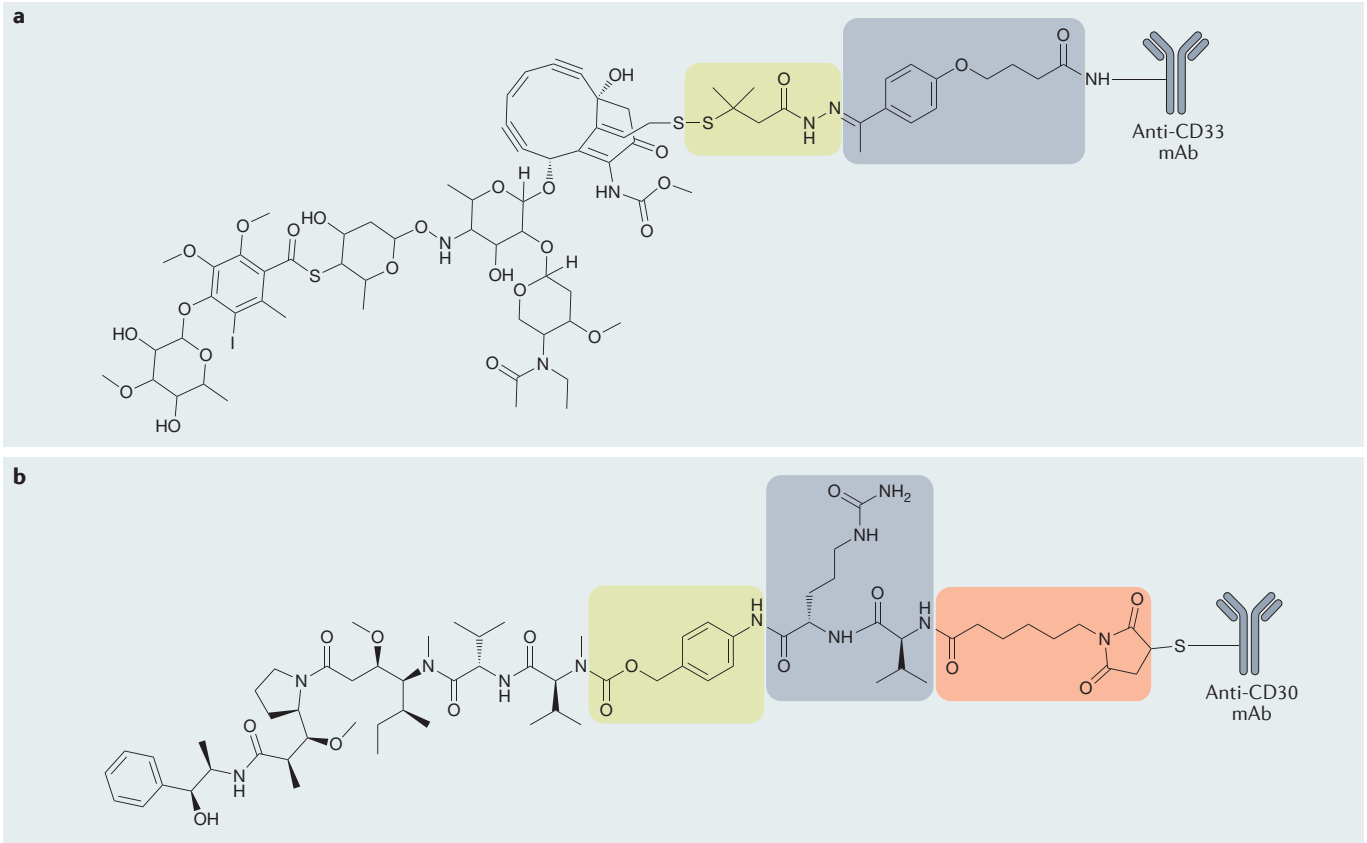

Figure 7 | **Antibody–drug conjugates. a** | Gemtuzumab ozogamicin (Mylotarg). An acid-sensitive hydrazone bond is hydrolysed between the 4-(4-acetylphenoxy)butanoic acid (blue) and the disulfide spacer (green). Next, the disulfide bond undergoes reduction by glutathione, allowing the sulfhydryl intermediate to cyclize onto the enediyne core structure to form a reactive species. **b** | Brentuximab vedotin (Adcetris). A peptide bond between the dipeptide valine–citrulline (blue) and *para*-amino benzyloxycarbonyl (PABC, green) moieties is cleaved by cathepsin B. The PABC-substituted vedotin spontaneously undergoes 1,6-elimination with a loss of *p*-iminoquinone methide, carbon dioxide and vedotin. The purpose of the maleimidocaproyl spacer (orange) is to provide space for cathepsin B access and cleavage. mAb, monoclonal antibody.

in ^{177}Lu-PSMA-617. This drug has shown promising outcomes in clinical trials examining the treatment of men with progressive prostate cancer that has spread beyond the prostate and is no longer responding to hormonal therapy[193].

Advantages of using small molecule–drug conjugates over ADCs are their small size, which enables faster and better penetration into solid tumours, their non-immunogenic nature and their more manageable synthesis. However, virtually all small ligands characterized to date have demonstrated some level of kidney uptake and undesired accumulation in normal organs.

Prodrug challenges and considerations

The ideal properties of most prodrugs include the following: adequate aqueous solubility and membrane permeability to achieve sufficient oral absorption; acceptable stability to reach the desirable site(s) of conversion; an optimal conversion rate that liberates the active parent in an efficient and/or controlled manner while avoiding precipitation if absorption of the parent drug is solubility-limited; nearly complete prodrug conversion, with minimal non-productive pathways and low levels of systemically circulating active drug; a good safety profile, which does not inhibit or induce drug-metabolizing enzymes or transporters; and a safe promoiety, with no undesirable pharmacological effects and rapid excretion from the body[10,51,194]. It is challenging for a prodrug to have all these desirable characteristics, but practical criteria can be set to rank order prodrugs with the most promising in vivo profiles. Both in vitro and in vivo assays have been developed to identify prodrugs with the highest potential to succeed in the clinic[16,51] (Supplementary Figure 1). Though great progress has been made to characterize prodrugs, the limitations of the different approaches should be carefully considered with respect to the uncertainty in translation to humans. The development of prodrugs is, in general, much more complex and less predictable in the clinic than that of other drugs (BOX 3).

Studies to evaluate prodrugs

Solubility. Many prodrugs have been developed to increase aqueous solubility[20]. Even for prodrugs that are intended to improve membrane permeability, stability or other pharmaceutical properties, it is still important to maintain adequate aqueous solubility to facilitate oral absorption or for parental formulation. An example of a prodrug approach to improve membrane permeability

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Box 3 | **Challenges associated with the discovery and development of prodrugs**

- The synthesis can be complex.
- Challenges exist in controlling the site and rate of bioconversion and metabolism.
- Interpretation of the preclinical results is complicated by species differences in prodrug bioconversion.
- Complex analytical profiling is needed and requires analysis of the prodrug, the parent drug and each of their respective metabolites.
- Physiologically based pharmacokinetic modelling can be challenging.
- The toxicity of not only the prodrug and drug but also the released promoieties or by-products needs to be considered.
- Although adding prodrug moieties can decrease chemical stability, the stability must be adequate to allow drug synthesis or isolation at scale as well as formulation.
- The regulatory environment surrounding prodrugs can be difficult to navigate, particularly for prodrugs of marketed drugs.

is found in the prodrugs of cephalosporins, in which the polar carboxylic acid of the parent drugs often contributes to low oral absorption[195]. A methyl ester prodrug increases oral absorption (~twofold increase in C_{max}), but the absorption is slow owing to poor solubility (solubility-limited oral absorption). It is, therefore, important to strike a balance between solubility and permeability in order to achieve optimal absorption when designing prodrugs to overcome specific molecular deficiencies. This is nicely demonstrated by mycophenolate mofetil, which is the 4-(2-hydroxyethyl)morpholine ester prodrug of the immunosuppressive drug mycophenolic acid[196]. Mycophenolate mofetil is slightly basic ($pK_a = 5.6$) and, therefore, exists as a partly ionized species that exhibits good solubility in the acidic regions of the gastrointestinal (GI) tract but also good lipophilicity, resulting in >90% oral bioavailability in humans.

The solubility of prodrugs can be assessed in physiological fluids such as buffers within a pH range of 1–9, fasted-state simulated intestinal fluid (FaSSIF), fed-state simulated intestinal fluid (FeSSIF) and plasma and in formulations to evaluate dissolution compared with parent drugs. The most relevant solubility values of a prodrug are those in the physiological fluids before the site of conversion. After conversion, the solubility of the parent will determine the outcome. Typically, thermodynamic solubility is measured using a shake-flask method with liquid chromatography–ultraviolet light–mass spectrometry (LC–UV–MS) detection. Kinetic solubility can also be used in the early stages of prodrug selection as long as its limitations are understood[37,197]. Dissolution rate measurements are conducted on the final dosage form to determine the dissolution profile. For intravenous formulations, the potential for precipitation after intravenous injection can be evaluated by mixing the dosage form with plasma or blood. Solubility and dissolution data, along with permeability measurements, can be used to estimate the fraction absorbed (F_a) in humans by using various models (for example, physiologically based pharmacokinetic (PBPK) modelling)[198,199].

Permeability. Prodrugs have been explored in numerous efforts to overcome permeability and oral absorption issues[51,52,200]. In vitro cell-based assays (such as Caco-2 or MDCK monolayer transwell assays) or artificial membrane permeability assays (such as the parallel artificial membrane permeability assay (PAMPA)) are frequently used to evaluate the permeation potential of prodrugs. Caco-2 and MDCK cells typically contain drug-metabolizing enzymes, including hydrolases, and transporters of clinical interest. Caco-2 cells express numerous transporters that are present on the apical (luminal) and basolateral (portal circulation) sides of intestinal epithelial cells, such as PEPT1 (apical) and multidrug resistance protein 1 (MDR1, also known as P-glycoprotein; apical), and several multidrug resistance-associated protein (MRP) transporters are expressed on both the apical and basolateral membranes. Both Caco-2 and MDCK cell lines as well as stably transfected cell lines expressing various transporters can be used to streamline prodrug screening and selection and to understand the potential efflux liabilities. Alternatively, physiochemical approaches (for example, PAMPA and shake-flask Log *D*) or computational methods (for example, cLog *D* and topological polar surface area (TPSA) calculations) can be used to estimate permeability. These in vitro permeability data, along with solubility and dissolution profiles, can be modelled using methods, such as PBPK modelling, that incorporate in vivo GI physiology to predict F_a.

Enzymes present in cell systems, such as Caco-2 or MDCK cells, can hydrolyse certain prodrugs, leading to low recovery and unreliable permeability data. In these instances, hydrolase inhibitors (for example, 1 μM bis-*para*-nitrophenylphosphate or 1 μM paraoxon)[201] can be added to the assay to increase the accuracy of the measurement by improving the stability of prodrugs and, hence, assay recovery. It is important to note that although Caco-2 cells are derived from human colon carcinoma cells, they do not express CES2, the esterase present in human intestinal cells, but rather CES1, the esterase present in human hepatocytes[202]. Therefore, the Caco-2 cell assay is not a good model to evaluate intestinal conversion of prodrugs that are susceptible to CES-mediated bioconversion, such as esters, or the fraction escaped from gut wall metabolism (F_g). On the other hand, Caco-2 cells provide a good predictive tool for absorption and bioconversion studies of phosphate prodrugs because of their high alkaline phosphatase activity[203]. Ex vivo studies using a Ussing chamber are often conducted at the later stages of drug development to answer specific questions. In vivo animal studies, such as portal vein-cannulated (PVC) animals, can also be used to evaluate the oral absorption of prodrugs[204,205]. For example, after oral administration of LY544344, a prodrug of the group II metabotropic glutamate receptor agonist LY354740, its bioconversion was extensive and rapid, with >97% of prodrug hydrolysis occurring before it reached the portal circulation in dogs[206]. These data were consistent with the extensive in vitro hydrolysis of the prodrug in jejunal homogenate[207]. It is also important to understand the impact of species differences on human translation with respect to GI physiology (such as length, surface area, pH, bile salt, gastric emptying time and intestine transit time) and levels of enzyme and transporter expression and activity.

Ussing chamber
A perfusion device used to measure transport across epithelial membranes.

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For example, the GI tracts of rodents tend to have much higher esterase activity than those of humans, and they may, therefore, have much lower F_g values than humans. In vitro intestinal S9 data in both species will need to be used in conjunction in order to deconvolute $F_a \times F_g$ data and to better understand the oral absorption potential in humans from animal data.

Stability and bioconversion. The stability of prodrugs in various matrices that mimic physiological conditions is often evaluated to identify potential liabilities and bioconversion rates. A balance of stability at the activation site compared with other sites in the body is critical in order to maximize target exposure of the parent. Oral prodrugs designed to increase intestinal absorption need to have sufficient stability in both the intestinal milieu and enterocytes in order to escape intestinal conversion and enter the blood and liver, where they will be bioconverted to generate the active parent. For example, the oral cephalosporin prodrugs cefuroxime axetil and cefpodoxime proxetil underwent degradation faster in human duodenal secretions than in pH 7.4 phosphate buffer. The oral bioavailability of these esters is generally around 40–50%[208]. Oral prodrugs intended to improve solubility, such as phosphates, need to be stable at various GI pH values and cleaved at the intestinal lumen by alkaline phosphatases[209]. Intravenous phosphate prodrugs designed to increase solubility need to be stable in the formulation vehicle but then be hydrolysed in the blood and tissues by alkaline phosphatases to release the active parent[21].

Decisions regarding which type of prodrug to develop depend upon which enzyme or enzymes will be involved in the bioconversion. Characterizing enzymatic pathways should preferentially be considered early and deliberately in prodrug design[201]. In practice, however, the selection of bioconversion processes for prodrugs is somewhat empirical, and the activating enzymes involved might not be fully identified or characterized during prodrug design and discovery. For example, the activating enzymes for valacyclovir were identified 8 years after drug approval[94,210] and those for dabigatran etexilate were identified almost 12 years after its discovery[211,212]. Multiple enzymes are often involved in the bioactivation of a prodrug, and the activation pathways can be complex. For example, both CES1 and CES2 are required for the formation of dabigatran from dabigatran etexilate, as it is both an ethyl ester and a hexyloxycarbonyl prodrug[63]. With advancements in new technologies (for example, activity-based protein profiling[210]), the ability to effectively identify prodrug-activating enzymes has greatly improved. This will, in turn, help to increase our knowledge and understanding of these enzymes in the areas of substrate specificity, tissue distribution, species differences, individual variability and genetic polymorphisms, thus enabling prodrug design to optimize the desired site and rate of release.

The most common enzymes involved in the bioactivation of prodrugs are hydrolases (for example, esterases, amidases and phosphatases[213]; TABLE 2), which, owing to their simplicity, activate about half of the prodrugs on the market[51]. The advantages of using this class of enzymes for bioactivation include the following: high catalytic capacity, for which enzyme saturation and dose nonlinearity do not usually occur; low drug–drug interaction potential, as most drugs are not potent inhibitors of these enzymes; and the relatively low impact of genetic polymorphism because of the low minor allele frequency and functional redundancy of multiple enzymes (each of which metabolizes a small fraction of the drug)[214]. The drawback is that many of these enzymes are not fully characterized, making it challenging to rationally design prodrugs and to accurately translate in vitro and animal data to humans. A few hydrolases are relatively well studied, such as CES1, which is known to activate a number of prodrugs (such as trandolapril and benazepril[215]). In humans, CES1 is mainly expressed in the liver and is distributed throughout many tissues (including the lung, muscle and pancreas) but is not found in the intestine[216,217]. However, in certain preclinical species (for example, monkeys), both CES1 and CES2 are expressed in the intestine[217]. On the other hand, there is minimal CES activity in the intestine of dogs[218]. Rodents have high CES activity in blood, whereas humans express no CES in the blood owing to the presence of the His-X-Glu-Leu (HXEL) sequence at the carboxyl terminal of the human versions, which binds to the Lys-Asp-Glu-Leu (KDEL) sequence of the endoplasmic reticulum protein-retaining receptor 1 (KDELR1)[219,220]. These differences in CES expression and localization can have important implications for which species are appropriate to evaluate ester prodrugs. Oseltamivir ethyl ester has the following oral bioavailability: mouse 30%, rat 35%, dog 73% and human 80%. This indicates that degradation before or during intestinal absorption or liver metabolism increases the first-pass effects in species with high CES activity and/or CES1 activity in the intestine or liver[56]. CES1 substrates tend to comprise large acids and small alcohols (such as oseltamivir), whereas CES2 substrates usually contain small acids and large alcohols (for example, irinotecan)[217]. Prodrugs that utilize CES1 for activation are usually designed to improve permeability and oral absorption. Although in vitro to in vivo extrapolation (IVIVE) of hepatic clearance has shown some promise for CES1 substrates by using human hepatocytes or liver S9 fraction[221], further research in this area will help to improve the accuracy in the human pharmacokinetic prediction of CES1 prodrugs.

CYPs are another class of enzymes that can be employed for prodrug bioactivation, although they are less commonly used than hydrolases[15,194,222]. CYPs tend to be more versatile and adaptable for prodrugs designed to target the liver, tumours or hypoxic tissues (for example, regions of solid tumours that are poorly vascularized). CYPs are predominantly expressed in the liver, but some are also found in the intestine and other tissues. Higher CYP3A4 expression in the liver has been utilized in the design of liver-specific cyclic phosphate and phosphonate HepDirect prodrugs[139–141]. Certain CYPs, such as CYP1B1, CYP2S1 and CYP2W1, are preferentially expressed in tumours and/or hypoxic tissues, making them amenable to tissue targeting[223–225].

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Table 2 | **Prodrug-activating enzymes**[213,221]

Enzyme	Gene name	Tissues with high expression in humans	Cellular location	Prodrug example
Carboxylesterase 1	*CES1*	Liver, lung and gallbladder	Endoplasmic reticulum	Oseltamivir
Carboxylesterase 2	*CES2*	Intestine, liver and kidney	Cytosol and Golgi apparatus	Irinotecan
Acetylcholinesterase	*ACHE*	Skeletal muscle, brain, erythrocytes and lymphocytes	Vesicles and Golgi apparatus	Butyryl acyclovir
Butyrylcholinesterase	*BCHE*	Predominantly in plasma, liver, brain, muscle, kidney, intestine, retina and placenta	Intracellular, secreted	Bambuterol
Paraoxonase 1	*PON1*	Synthesized in the liver, secreted into the blood	Secreted	Prulifloxacin
Carboxymethylenebutenolidase	*CMBL*	Kidney, intestine, liver and skeletal muscle	Intracellular	Olmesartan medoxomil
Alkaline phosphatase, intestinal	*ALPI*	Small intestine	Plasma membrane	Fosamprenavir
Alkaline phosphatase, liver/bone/kidney	*ALPL*	Endocrine tissues, brain, lung, liver, bone and kidney	Cytosol	Fosphenytoin
Biphenyl hydrolase-like	*BPHL*	Liver, intestine and kidney	Nucleoplasm and mitochondria	Valacyclovir

See The Human Protein Atlas website for more information

Two experimental cancer prodrugs, 5F-203 [2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole] and GW-610 [5-fluoro-2-(3,4-dimethoxyphenyl)benzothiazole], require CYPs (notably CYP2W1) to produce the intermediates that form adducts with DNA and underlie their antitumour activities[194,226–228]. One advantage of using CYPs for prodrug activation is that they are well characterized. IVIVE has been established through experiments measuring the conversion and disposition of the active parent in model systems but still has high uncertainty in human prediction. The limitations of using CYPs for prodrug activation should also be noted[194,222]: first, conversion of prodrugs by CYPs can be relatively slow, and it takes time for complete conversion, as only portions of the cardiac blood flow (~25%) transit through the liver; second, CYPs are usually high-affinity, low-capacity enzymes and can be saturated at high doses, leading to nonlinear pharmacokinetics; third, there is a high risk of drug–drug interactions due to inhibition or induction of CYP enzymes by other coadministered drugs; and fourth, the impact of genetic polymorphisms can be considerable for certain CYP enzymes (for example, CYP2D6, CYP2C9 and CYP2C19), leading to high interindividual pharmacokinetic variability. An example of alterations in CYP activity due to genetic polymorphism for prodrug activation is provided by tamoxifen. Tamoxifen is bioconverted to the biologically more potent metabolite, endoxifen, by CYP3A and CYP2D6. It has been shown that CYP2D6 poor metabolizers have lower levels of endoxifen and worse clinical outcomes than extensive metabolizers[229].

Advances in PBPK modelling

The prediction of the human pharmacokinetic profile of the prodrug and active parent from in vitro and in vivo data can be challenging because multiple factors can influence the outcome. Properties of both the parent and prodrug, as well as the conversion rates from prodrug to active parent, the site of conversion and the clearance mechanisms involved (for example, metabolic, renal and/or biliary), need to be well understood and characterized. It is therefore important to integrate all the data in order to gain a holistic view of the profiles of the prodrug and active parent drug rather than focusing solely on the property that needs to be improved and/or evaluating individual properties in isolation. Sometimes improving one property can lead to a deterioration of others. PBPK models provide a valuable platform with which to integrate all the information associated with prodrugs and the active parent drug and to evaluate them simultaneously under physiological conditions. This helps to improve the quality of the decision-making process and identify prodrug candidates with a higher likelihood of success. In the early stages of prodrug discovery, the input parameters are mostly derived from in silico, in vitro and in vivo studies conducted in preclinical species. Bottom-up PBPK models are usually developed at this stage to identify prodrugs with an optimal profile. As there are no clinical data to verify the accuracy of these models, the uncertainties of the PBPK models tend to be high at the early stages of drug discovery. However, the models can still be very informative to rank-order a large number of prodrug candidates on a relative scale of properties, including their relative conversion rate, pharmacokinetic profile, half-life, bioavailability and F_a. The accuracy of the models at this point is of less importance because the inaccuracy will likely be comparable across a set of structurally similar prodrugs. An example has been reported using the bottom-up approach to develop PBPK models for three prodrugs: mycophenolate mofetil, midodrine and bambuterol[230]. The pharmacokinetic profiles

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of mycophenolate mofetil and midodrine prodrugs and the active parent drugs were adequately predicted from the model, whereas the model developed for bambuterol was less successful owing to overprediction of oral bioavailability. Most importantly, at this early stage, sensitivity analysis can also be conducted to identify parameters that have the greatest effect on the pharmacokinetic properties so that they can be optimized through structural modification. This example illustrates the potential of PBPK models to prioritize prodrugs with a high probability of success early in drug discovery.

As clinical data become available, models will be refined using top-down or mid-up approaches to best describe the human pharmacokinetic profile. The accuracy of the models is greatly improved at the later stages of drug development. These models are especially useful in predicting the pharmacokinetic profile in special populations (for example, patients with hepatic or renal impairment, paediatric or neonatal patients, and patients who are pregnant), the drug–drug interaction potential and the effect of genetic polymorphisms on prodrug activation. The models can also serve as a starting point for the design of prodrugs that rely on similar structures and activation mechanisms. An example of PBPK modelling of prodrugs is a PBPK model that was developed for oseltamivir and its prodrug, verified using clinical data and then used to understand the impact of the CES1 genetic polymorphism[231]. In another example, a semi-PBPK model for rats and humans was constructed for the prodrug pafuramidine and its active parent furamidine[232]. The models described pafuramidine and furamidine disposition in plasma and predicted furamidine liver and kidney exposure, as well as renal and biliary excretory profiles. The models also predicted substantial first-pass conversion from prodrug to the active drug in the gut. A dosing regimen was derived based on analysis of the dose–plasma–tissue relationship and the therapeutic index. A similar approach could be used to guide human dose selections for future compounds. A PBPK model for ganciclovir and its prodrug, valganciclovir, has also been reported[233]. The model was initially developed using a bottom-up approach based on physiochemical properties, in vitro data and animal data, and was later verified with clinical pharmacokinetic data in adults, children and neonates. The PBPK model could be used to predict pharmacokinetics in infants and neonates and thus aid drug development in patient populations in whom clinical data are challenging to obtain. A full PBPK model of clopidogrel and its active metabolite was developed and validated with CYP2C19-phenotyped data and drug–drug interaction studies[234]. The model was able to accurately describe the pharmacokinetics of the prodrug and the active metabolite.

Safety evaluation and regulatory aspects

Standard safety and toxicity studies are typically conducted for prodrugs regardless of the extent of toxicological characterization of the parent drug in preclinical species. For example, a full toxicology programme was conducted for fosamprenavir even though only very low concentrations of the intact prodrug were detected in the circulation (less than 0.17% of the amprenavir concentration)[45]. Without question, though, a clean safety profile with the parent drug reduces the risk that the prodrug is problematic. Promoieties released from prodrugs need to be safe, which should be a consideration early in prodrug design, but controversy regarding the safety profiles of certain promoieties can still be prohibitive. For example, there are reservations in developing formaldehyde-releasing prodrugs, despite sound scientific arguments regarding its safety[235]. Released pivalic acid from several prodrugs, such as adefovir dipivoxil and pivampicillin, is also of concern because pivalic acid has been shown to interrupt carnitine homeostasis, which can lead to depletion of carnitine and adverse cardiac effects[236]. Exposure to low doses of pivalic acid in most cases has no or only minor toxicological effects. The toxicity associated with long-term exposure to high doses of pivalic acid can be overcome by administration of supplemental carnitine. Therefore, for situations in which prodrugs raise a toxicity concern, daily dosing and duration of treatment should be taken into careful consideration in the overall risk evaluation process.

Conclusions and outlook

Prodrugs have become a well-accepted path to a viable commercialized product and account for more than 10% of the approved NCEs per year over the past decade. Indeed, consideration of prodrug strategies has become an integral part of the discovery process, whereas they had previously been considered only as a means to salvage a molecule after a medicinal chemistry shortcoming. The increased recognition that drug-like properties can be designed into a molecule at early stages has opened the door to the use of prodrug modifications to obtain those properties and thus obtain clinical proof of concept more quickly. Prodrugs, very much like analogues and homologues, require the physicochemical and ADMET properties to be balanced in order to obtain the optimal combination necessary to progress the molecule, and prodrug modifications should be considered as part of the preclinical optimization process.

The decision to pursue a prodrug strategy can be influenced by the nature of the biological target and its physiological location, as well as the nature of the early hit-to-lead molecules and their perceived potential for building in appropriate drug-like properties. With the pressure to generate proof-of-concept data and validate a target in the clinic as soon as possible, employing a prodrug strategy may be an expedient approach. This changing paradigm requires an alteration in thinking about the configuration of a screening funnel during candidate optimization. The presence of a viable prodrug handle on the lead chemical template, coupled with feasible promoieties, could feed into the screening funnel if the final molecule is expected to be a prodrug. In the best-case scenario, the prodrug will also be optimized for a particular drug formulation strategy to effect its delivery. In essence, the design of the prodrug cohesively interlinks the properties of the promoiety and the parent, thus optimizing the final compound through conscious selection rather than by necessity at a late stage.

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Much of the translation from the discovery setting into the clinic is still complicated by the factors involved in bioconversion of the prodrug. Unfortunately, there are many intrinsic metabolic, as well as extrinsic, factors that complicate interspecies relationships and influence bioconversion mechanisms. Rapid advances in the ADMET field and increased patient profiling may be a great advantage in this arena.

In the past 10 years, at least 30 prodrugs have received approval from the FDA. One-third of these newer prodrugs are built to contain a small alkyl group to improve bioavailability through permeability enhancement. This is not surprising because improving membrane permeability has certainly been the most widely pursued and most successful area of prodrug research to date. Four of the recently approved prodrugs utilize either a solubilizing phosphate or phosphonate group. Interestingly, in all four prodrugs, the solubilizing promoiety is linked to a different functional group of the parent drug, demonstrating the importance and versatility of this prodrug strategy to increase the aqueous solubility of poorly soluble compounds.

Isavuconazonium sulfate introduced an unprecedented prodrug strategy for improved aqueous solubility, as discussed above. The future will show if this innovative strategy is applicable to compounds not suitable for direct phosphorylation. Another novel prodrug strategy is demonstrated by selexipag, which has an *N*-acylsulfonamide promoiety that provides sustained formation of the parent carboxylic acid in a clinical setting. Finally, the discovery of the ProTide strategy has resolved the long-term question of how to deliver nucleoside analogue drugs efficiently to intracellular sites after oral administration. The approvals of tenofovir alafenamide and sofosbuvir have already paved the way for other ProTide phosphoramidate prodrugs, some of which are advancing towards approval.

There remains a considerable unmet need for improving drug targeting and the therapeutic index of drugs. Major advances may be made as we continue to understand the factors that influence diseased tissue, organs and cells as well as their healthy counterparts. Our increased understanding of prodrugs and clinically relevant permeability improvements, through both passive and active transport mechanisms exploited by the prodrug, has increased our ability to access intracellular targets, which will hopefully lead to the next wave of progress. As more challenging drug targets that demand physicochemical properties extending beyond that of the traditional drug space are brought into focus[237–239], prodrugs will likely play a prominent role in their delivery. Consequently, prodrugs are not only here to stay, but their utility will probably also expand as they become both more important and more prevalent.

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Acknowledgements
The authors' research on prodrugs was funded by the Academy of Finland (grant number 308329 to J.R.). The authors also want to recognize C. McGuigan (1958–2016) for his outstanding achievements in prodrug research, which paved the way for the development of phosphoramidate ProTide phosphate and phosphonate prodrug technology.

Competing interests
The authors declare no competing interests

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Supplementary information
Supplementary information is available for this paper at https://doi.org/10.1038/nrd.2018.46.

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