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Prodrug research: futile or fertile?

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Dedicated to the memory of Prof. Jacques Gielen, devoted editor, eminent scientist, and gentleman.

Abstract

The objective of this Commentary is to help clarify and illustrate what prodrugs are, what they are not, which benefits they can offer, and what their limits are. To this end, a number of criteria of classification and evaluation are presented. This is followed by a discussion of the pharmaceutical, pharmacokinetic and pharmacodynamic objectives of prodrug research. Recent examples (e.g. oseltamivir, bambuterol, capecitabine, clopidogrel and tirapazamine) are discussed in a biochemical perspective to illustrate these objectives and to demonstrate some of the therapeutic benefits afforded by successful prodrugs. Attention is also called to the fact that the in vitro and in vivo behavior of prodrug candidates may differ from that of the parent drug in ways that go beyond the original pharmaceutical, pharmacokinetic or pharmacodynamic objective being pursued. We conclude that prodrugs offer a viable strategy to disentangle pharmacodynamic and pharmacokinetic optimization.

Keywords: Commentary; Drug metabolism; Prodrug design; Pharmaceutical objectives; Solubility; Pharmacokinetic objectives; Absorption; Tissue-selective delivery; Pharmacodynamic objectives; In situ activation

1. Viewpoints in prodrug design

The examples presented in this Commentary are meant to illustrate the various objectives of a prodrug strategy, as well as the biochemical pathways involved in prodrug activation (i.e., hydrolysis, oxidation or reduction). More detailed information can be found in a number of reviews [1–13]. We begin with an overview of definitions, types of prodrugs, and some of the challenges encountered by prodrug researchers.

1.1. Definitions

What make prodrugs different from other drugs is the fact that they are devoid of intrinsic pharmacological activity. Thus, the simplest and clearest definition, in my view, is that given by Albert in 1958 [14], who coined the term. In modified form, the definition reads:

Prodrugs are chemicals with little or no pharmacological activity, undergoing biotransformation to a therapeutically active metabolite.

The complete opposites of prodrugs are thus drugs that have no active metabolite, e.g. paracetamol. However, prodrugs should not be confused (as is too often the case), with drugs which are intrinsically active, yet are transformed into one or more active metabolites. In this case, two or more active agents will contribute to the observed clinical response in proportions that depend on differences in pharmacological activities, in compartmentalization and in time profiles. Examples include *cis*-platine (which is chemically transformed to the monoaqua and diaqua species), morphine and its 6-*O*-glucuronide, diazepam (which is *N*-demethylated to nordiazepam), and codeine (which is *O*-demethylated to morphine).

It is also important to distinguish prodrugs from soft drugs [15,16] defined as "biologically active compounds (drugs) characterized by a predictable and fast in vivo metabolism to inactive and non-toxic moieties, after they have achieved their therapeutic role". An example is afforded by the short acting (β-blocker esmolol, whose

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half-life of hydrolysis in human blood at pH 7.4 and 37 °C is 23 min [17].

1.2. Types of prodrugs

A number of criteria are available to classify the various types and sub-types of prodrugs. While some of these criteria are quite useful in prodrug research, others are of a more historical and didactic interest.

1.2.1 Research-related criteria

A historical discrimination can be made between *intentional and fortuitous prodrugs*. As the name indicates, the former are designed prodrugs obtained by chemical derivatization or modification of a known active agent. Most prodrugs in clinical use are of this type, and they were developed to improve the pharmaceutical and/or pharmacokinetic properties of an active agent, be it a lead candidate, a lead compound, a clinical candidate, a drug candidate or a marketed drug. A historically interesting example is that of hexamine (Fig. 1), an intentional prodrug of formaldehyde introduced over a century ago and used for decades as a throat desinfectant.

Whereas fortuitous prodrugs are few compared to intentional prodrugs, the discovery of the active agent they generate will contribute significantly to the understanding of the mechanism of action of the (pro)drug and may even lead to the discovery of a new therapeutic class. The antibacterial medicine Prontosil offers a dramatic illustration of this phenomenon, since the discovery in 1935 of sulfanilamide as its active metabolite [18] was the milestone that led to the creation of all antibacterial sulfonamides.

When evaluating prodrugs, it may sometimes be useful to distinguish between "posthoc" and "adhoc" design [4,9]. Post hoc design implies well-accepted drugs endowed with useful qualities but displaying some unwanted property which a prodrug form should ameliorate. In other words, post hoc prodrugs derive from established drugs. The therapeutic gain they afford compared to the drug is usually modest, but may be marked if a better pharmaceutical property or improved targeting is achieved.

In contrast, ad hoc design implies lead candidates suffering from some severe drawback in drug-like properties (e.g. high hydrophilicity restricting bioavailability) which prevents therapeutic use or imposes unwanted routes of administration. Here, a prodrug strategy may prove necessary and should be implemented as early as possible into the iterative process of lead optimization. A significant therapeutic gain is obviously expected.

1.2.2 Chemical criteria

Another way to classify prodrugs is based on chemical arguments. Thus, medicinal chemists find it useful to distinguish between four major classes of prodrugs, namely:

- *Carrier-linked prodrugs*: where the active agent (the drug) is linked to a carrier (also known as a promoiety), and whose activation occurs by hydrolysis (esters, amides, imines. . .), oxidation or reduction (e.g. Prontosil in Fig. 1);
- *Bioprecursors*: which do not contain a promoiety yet are activated by oxidation, reduction or hydrolysis [12];
- *Macromolecular prodrugs*: where the carrier is a macromolecule such as a PEG (polyethyleneglycol) [19];
- *Drug–antibody conjugates*: where the carrier is an antibody raised against tumor cells [1,20].

The present Commentary focuses on carrier-linked prodrugs and bioprecursors, which remain by far the largest groups of prodrugs in use. Indeed, of 1562 different active substances marketed in Germany in 2002, 6.9% are prodrugs, with one-half of these being activated by hydrolytic cleavage of a promoiety, and one-quarter being bioprecursors [1].

1.3. Challenges in prodrug research

A number of challenges await medicinal chemists and biochemists carrying out prodrug research, such as the additional work involved in synthesis, physicochemical profiling, pharmacokinetic profiling and toxicological assessment [1]. Two of these challenges are introduced here, namely biological variability and toxicity potential.

The challenge of *biological variety* results principally but not only from the huge number and evolutionary diversity of enzymes involved in xenobiotic metabolism. Inter- and intra-species differences in the nature of these enzymes, as well as many other differences such as the nature and level of transporters, may render prodrug optimization difficult to predict and achieve. The high levels of carboxylesterases in the plasma of rodents but not of other mammals is but one example of a biological difference that may affect the rate and site of activation of some prodrug esters, as seen for example with the ACE inhibitor benazepril [21]. A chemical strategy developed by medicinal chemists to overcome the problem of biological variety is the development of prodrugs activated by non-enzymatic hydrolysis, e.g. imines, Mannich bases, (2-oxo-l,3-dioxol-4-yl)methyl esters, or oxazolidines [2,4]. A more promising approach appears to be the two-step activation of carrier-linked prodrugs, involving first a relative facile enzymatic hydrolysis to unmask a nucleophilic group, followed by a non-enzymatic, intramolecular nucleophilic substitution and cyclization. Given the ability of chemists to modulate the rate of the second step, this two-step reaction appears particularly promising, as exemplified for peptides (Fig. 2) [6,22–24].

A second challenge is the *toxicity potential* of some prodrugs. Schematically, prodrug designers must be aware of at least two specific sources of toxicity, namely a toxic metabolite formed from the promoiety, or a reactive metabolic intermediate generated during the activation of some

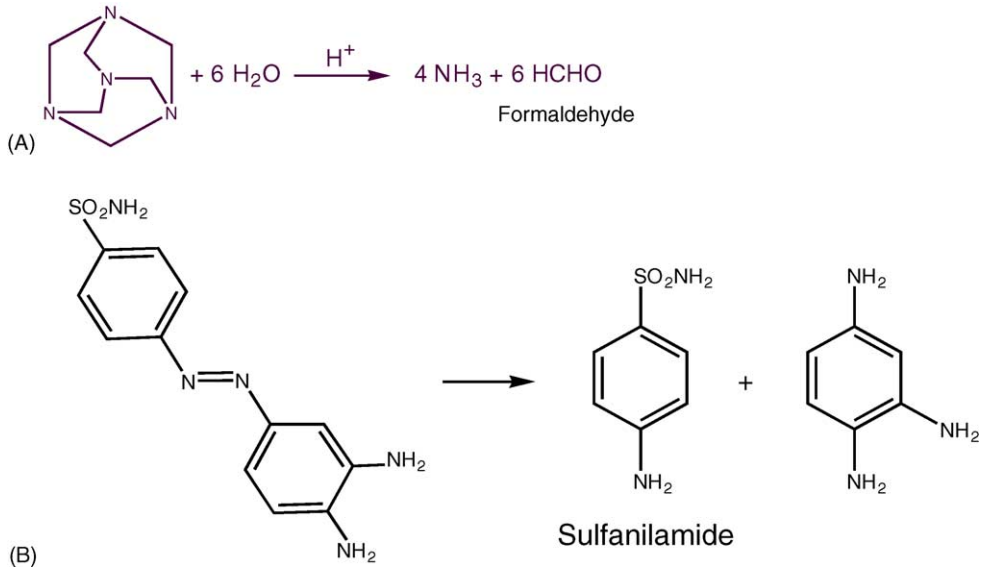


Fig. 1. Two historical examples of prodrugs. (A) Hexamine, an intentional prodrug of formaldehyde. (B) Prontosil, a fortuitous prodrug of sulfanilamide.

bioprecursors. The former case is illustrated by the liberation of formaldehyde, as seen with Mannich bases or some double esters [2,4]. Liberation of formaldehyde, however, is predictable, and most prodrugs of this type were published as academic exercises which never made it to development.

A number of prodrugs have been described as bioprecursors of cytotoxic and antitumor agents, as discussed later in this text. These should not be confused with the very few known examples of failed bioprecursors whose activation involved a reactive and toxic intermediate. Thus, arylacetylenes were examined as potential bioprecursors of non-steroidal antiinflammatory agents [25]. Whereas the nature of the final (and stable) metabolite (an arylacetic acid) was known, researchers at the time were not aware that the metabolic pathway involved an intermediate and highly reactive ketene.

2. Pharmaceutical objectives

Pharmaceutical scientists are often faced with serious formulation problems resulting from *poor solubility*, *insufficient chemical stability* or *poor organoleptic properties* (bad smell or bitterness endangering patients' compliance). While pharmaceutical technology can solve such problems in favorable cases (e.g. by improving the solubility of cyclosporine), success may be uncertain and time-consuming to achieve. Rather than to wait for an uncertain and

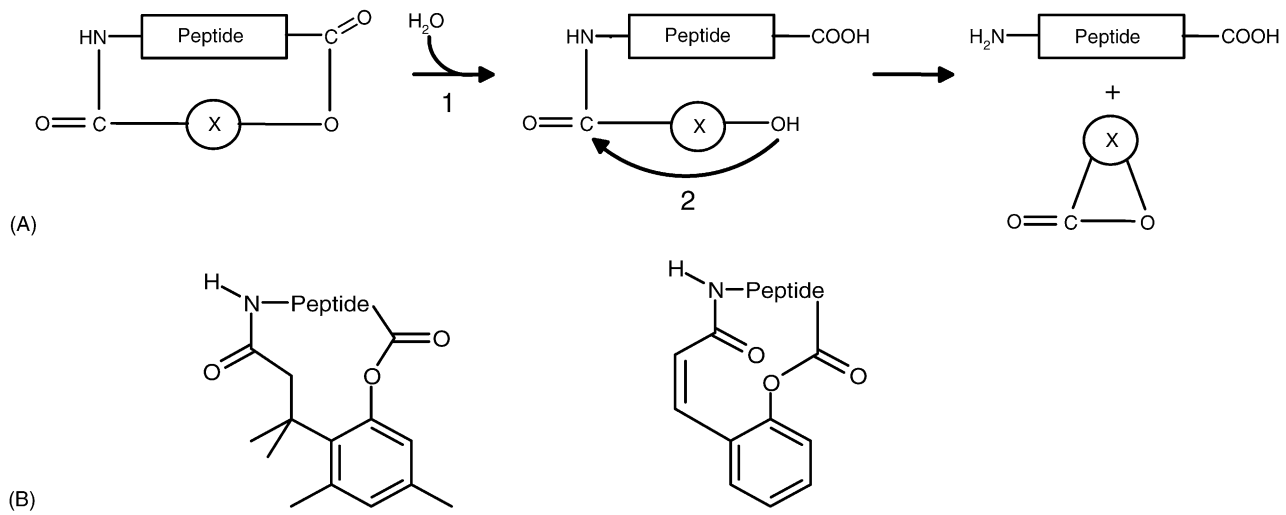
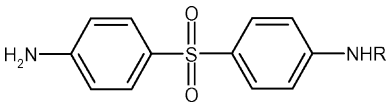
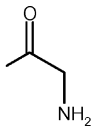
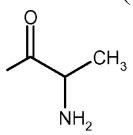
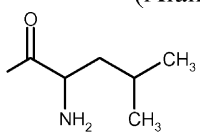
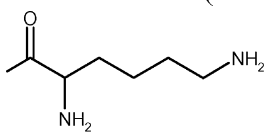
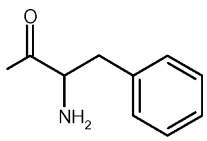


Fig. 2. The two-step activation of peptide prodrugs. (A) Principle of the reaction, whereby an enzymatic reaction cleaves the ester group (step 1), thus unmasking a nucleophilic group (e.g. a phenol) which then attacks non-enzymatically the amide bond (step 2) and cleaves it in an intramolecular reaction of cyclization-elimination. (B) Two examples of pro-moieties that have proved quite promising in this two-step approach [6,23,24].

Table 1
Solubility and enzymatic lability of amino acid derivatives of dapsone as water-soluble prodrugs [26]

	Solubility at 25 °C (in mg dapsone equivalent/mL)		$t_{1/2}$ at 37 °C in human blood (in min ± S.D.)
	In water as HC1 salt	In pH 7.4 phosphate buffer	
Dapsone (R=H)	0.16	0.14	
R =  (Glycyl)	>15	0.87	14.6 ± 0.9
R =  (Alanyl)	>30	6.6	20.5 ± 0.7
R =  (Leucyl)	>25	0.31	1.7 ± 0.2
R =  (Lysyl)	>65	>65	10.9 ± 0.7
R =  (Phenylalanyl)	1.3	0.002	8.8 ± 0.7

delayed pharmaceutical solution to a problem of solubility or stability, project leaders may prefer to take advantage of a prodrug strategy and hope on an early solution.

A representative example of a prodrug solution to a solubility problem is afforded by dapsone. In a project aimed at improving the water solubility of this drug, a number of prodrugs were prepared by creating an amido bridge between dapsone and an amino acid [26]. Good water solubilities were indeed contributed by the more polar pro-moieties (i.e., glycyl, alanyl and lysyl). The half-lives of hydrolysis in human blood and plasma were very promising, as shown in Table 1.

As will be discussed later, increasing solubility is a pharmacokinetic as well as a pharmaceutical objective. Indeed, and as made explicit in the biopharmaceutics classification scheme (BCS) [27], solubility is one of the main factors influencing oral absorption.

3. Pharmacokinetic objectives

Pharmacokinetic objectives are currently the most important ones in prodrug research. Foremost among these is a need to improve oral bioavailability, be it by improving the oral absorption of the drug, and/or by decreasing its presystemic metabolism. Other objectives are to improve absorption by parenteral (non-enteral, e.g. dermal, ocular) routes, to lengthen the duration of action of the drug by slow metabolic release, and finally achieve the organ/tissue-selective delivery of an active agent. Some of these objectives are exemplified below with clinically successful prodrugs.

Achieving *improved oral absorption* by a prodrug strategy is a frequent rationale in marketed prodrugs [28]. An apt illustration is found within the neuraminidase inhibitors of therapeutic value against type A and B influenza in humans [29]. Here, target-oriented rational design has led to highly hydrophilic agents which are not absorbed orally. One of the two drugs in current clinical use is zanamivir, a highly hydrophilic drug administered in aerosol form (Fig. 3). The other active agent is Ro-64-0802, which also shows very high in vitro inhibitory efficacy toward the enzyme but low oral bioavailability due to its high polarity [30]. To circumvent this problem, Ro-64-0802 is marketed as oseltamivir, its ethyl ester prodrug (Fig. 3). Following intestinal absorption, the prodrug undergoes rapid enzymatic hydrolysis and produces high and sustained plasma levels of the active agent. As demonstrated by this example, the prodrug concept may thus be a valuable alternative to disentangle pharmacokinetic and pharmacodynamic optimization (see Section 5).

Pharmaceutical formulation is the most frequent method used to achieve slow release and *prolong the duration of*

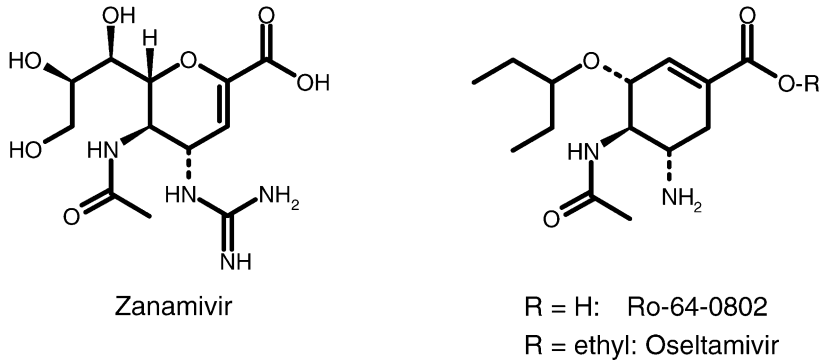


Fig. 3. Structure of the two neuraminidase inhibitors in current clinical use against types A and B influenza in humans, namely the drug zanamivir and the prodrug oseltamivir whose active agent is Ro-64-0802 [29,30].

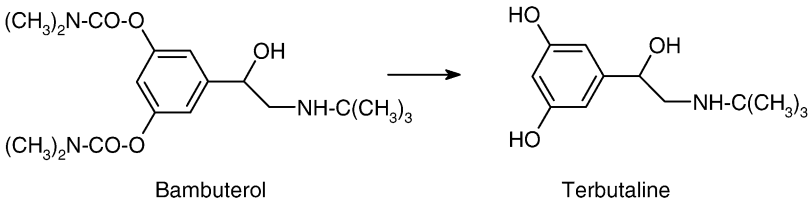


Fig. 4. Structure of terbutaline and its prodrug bambuterol.

action of a given drug. However, a prodrug strategy can also be useful, as exemplified by depot formulation of esters of steroid hormones. A conceptually different and particularly elegant approach to slow metabolic release has been achieved with bambuterol, a prodrug of the β_2-adrenoreceptor agonist terbutaline [31,32] (Fig. 4). Compared to terbutaline 5 mg taken three times daily, bambuterol 20 mg taken once daily provides smooth and sustained plasma levels of terbutaline, and a greater symptomatic relief of asthma with a lower incidence of side-effects [33]. Similar results were obtained when comparing a slow-release formulation of terbutaline 10 mg with bambuterol 0.085 mg kg^{-1} [34]. As shown in Fig. 5, the two preparations elicited a comparable bronchodilating effect, but that caused by bambuterol appeared to be more sustained. The plasma levels of terbutaline were markedly more constant after bambuterol than terbutaline, but they were also significantly lower. This higher effect-to-plasma concentration ratio is another valuable feature of this prodrug, and it results from a marked uptake of bambuterol by the lung, where activation occurs in part [32].

Indeed, bambuterol is activated to terbutaline by hydrolysis in blood serum, and by monooxygenase-catalyzed oxidation in the liver, lung and other tissues. The reaction

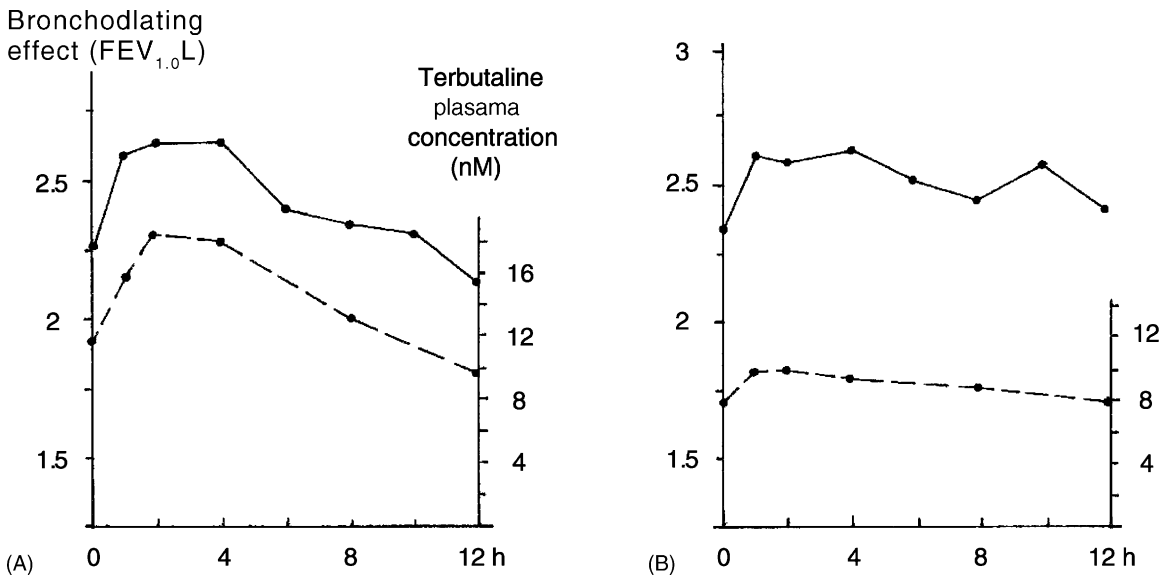


Fig. 5. Bronchodilating effects ((—) S.D. ~0.25 $FEV_{1.0}$L) and terbutaline plasma concentrations ((— — —) S.D. <1 nM) in 18 patients during the forth day after treatment twice daily with terbutaline 10 mg in a slow-release formulation (A), or with bambuterol 0.085 mg kg^{-1} (B). Modified from [34].

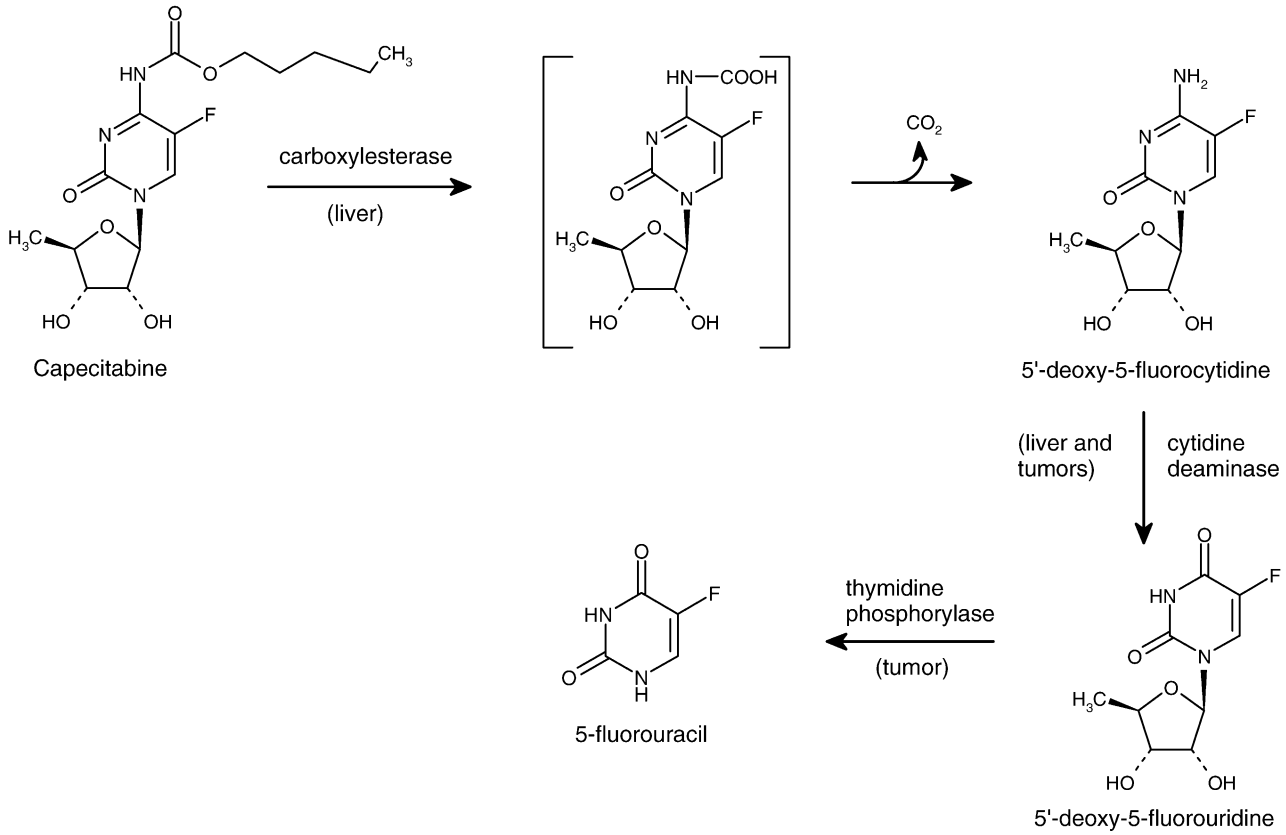


Fig. 6. The stepwise activation of capecitabine to the antitumor drug 5-fluorouracil. Note the tissue-selectivity of the last step.

of hydrolysis is catalyzed by cholinesterase (butyrylcholinesterase, EC 3.1.1.8). Following a first burst of terbutaline release, the enzyme is inhibited by covalent attachment of the dimethylcarbamate moiety (Me_2N–CO–). Indeed, bambuterol is also characterized by its high inhibitory capacity toward cholinesterase (IC_{50} = 17 nM), and reactivation of the enzyme occurs very slowly. As a result, the inhibition of cholinesterase by bambuterol is of long duration but fully reversible, 2 days or more being necessary for complete recovery. Another important feature of the inhibition is its selectivity toward unspecific cholinesterase, since inhibition of acetylcholinesterase (EC 3.1.1.7) would increase the levels of acetylcholine and exacerbate asthmatic symptoms. As it turns out, bambuterol is about 2000 times less potent in inhibiting acetylcholinesterase (IC_{50} = 41 μM) than cholinesterase.

The last pharmacokinetic objective discussed here is the *organ- or tissue-selective delivery* of a given drug, also known as the search for the ''magic bullet''. A recent and clinically very significant example is that of capecitabine, a multistep, orally active prodrug of 5-fluorouracil [35,36] (Fig. 6).

Capecitabine is well absorbed orally and undergoes three activation steps resulting in high tumor concentrations of the active drug. It is first hydrolyzed by liver carboxylesterase, the resulting metabolite being a carbamic acid which spontaneously decarboxylates to 5′-deoxy-5-fluorocytidine. The enzyme cytidine deaminase, which is present in the liver and tumors, then transforms 5′-deoxy-5-fluorocytidine into 5′-deoxy-5-fluorouridine. Transformation into 5-FU is catalyzed by thymidine phosphorylase and occurs selectively in tumor cells.

Table 2
Tissue distribution of 5-fluorouracil (5-FU) following administration of 5-FU or its triple prodrug capecitabine[a]

	Reported clinical dose range (μmol kg^{-1})	AUC of 5-FU (nmol min mL^{-1})[b]			AUC ratios[b]	
		GI tract	Blood	Tumor	Tumor/GI	Tumor/blood
Capecitabine p.o.	59–92	220–480	100–200	970–3,100	4.4–6.5	9.7–15.5
5-FU continuous i.v.	6–210	30–1,300	300–10,000	8–400	0.3–0.3	0.03–0.04
5-FU p.o.	19–41	4,400–13,000	25–100	0.9–3.4	0.002–0.003	0.03–0.03

[a] The reported clinical ranges, AUCs and ratios are rounded off values taken from graphical data calculated by Tsukamoto et al. [35] using a physiologically based pharmacokinetic analysis.

[b] The first and second values correspond respectively to the lower and upper limit of the reported clinical range.

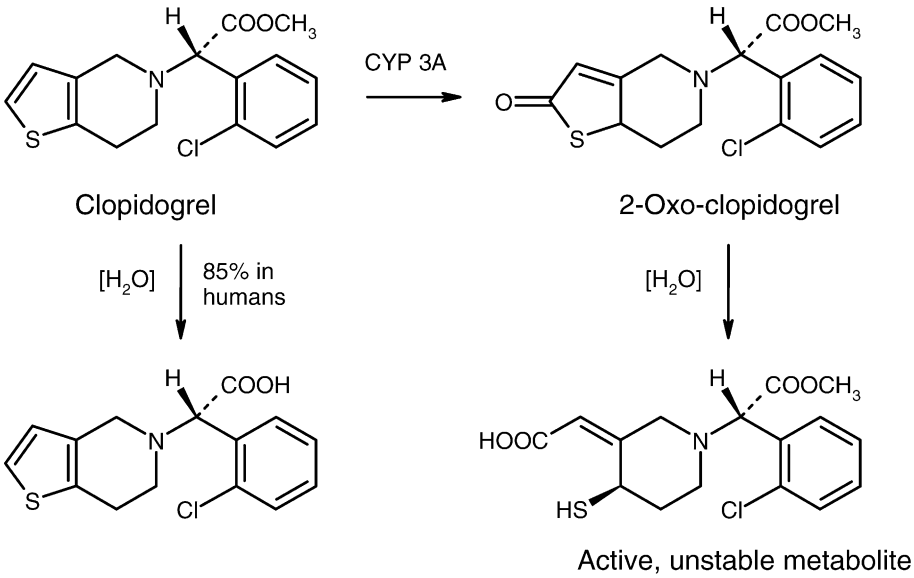


Fig. 7. Major metabolic reactions of clopidogrel in humans. Most of a dose is inactivated by hydrolysis, whereas a smaller part is activated by CYP3A to 2-oxo-clopidogrel, followed by spontaneous hydrolytic ring opening to the active agent, a highly reactive thiol metabolite which irreversibly antagonizes platelet ADP receptors via a covalent S–S bridge [37,38].

Capecitabine is of great interest in the context of this Commentary. Clinically, it was first approved for the cotreatment of refractory metastatic breast cancer. Its therapeutic spectrum now includes metastatic colorectal cancer, and there are hopes that it might broaden further as positive results of new clinical trials become available. Capecitabine thus affords an impressive gain in therapeutic benefit compared to 5-FU due to its oral bioavailability and a relatively selective activation in and delivery to tumors. This is illustrated with the data presented in Table 2, which show the tissue distribution of 5-FU following the oral administration of capecitabine or 5-FU, or the continuous i.v. administration of 5-FU. The levels of 5-FU in tumors are clearly much higher after capecitabine administration. But even more impressive appear the tumor-to-blood and tumor-to-GI tract ratios achieved after capecitabine administration.

4. Pharmacodynamic objectives

Pharmacodynamic objectives can be understood as being synonymous with decreasing systemic toxicity. Two major cases are illustrated here, namely the masking of a reactive agent to improve its therapeutic index, and the in situ activation of a cytotoxic agent.

The *masking of a reactive agent* to improve its therapeutic index is aptly exemplified by the successful anti-aggregating agent clopidogrel. This compound, whose molecular mechanism of action was poorly understood for years, is now known to be a prodrug. However, it is of interest among prodrugs in that its major metabolic route in humans (ca. 85% of a dose) is indeed one of hydrolysis, but this reaction leads to the inactive acid (Fig. 7). In contrast, clopidogrel is activated by cytochromes P450 3A in a two-step sequence. First, the CYP-catalyzed reaction oxidizes clopidogrel to 2-oxo-clopidogrel. This is followed by a spontaneous and rapid hydrolysis of the cyclic thioester to a highly reactive thiol metabolite which irreversibly antagonizes platelet ADP receptors via a covalent S–S bridge [37,38]. Interestingly, the same activation mechanism appears to account for the potent and irreversible inhibition of human CYP2B6 by clopidogrel [39], again demonstrating the high reactivity of the thiol metabolite.

In situ *activation to a cytotoxic agent* is part of the well-known mechanism of action of the antibacterial and antiparasitic nitroarenes such as metronidazol. Here, we examine this concept as currently intensively applied in the search for more selective antitumor agents [40]. Given that tumor cells have a greater reductive capacity that normal cells, various chemical strategies are being explored to design bioprecursors activated by reductive enzymes to cytotoxic agents, e.g. platinum(IV) complexes [41] or *N*-oxides.

The latter case is aptly illustrated by the hypoxia-activated, bioreductive antitumor agent tirapazamine [42], seemingly the best-studied drug candidate in this class [40]. As shown in Fig. 8, tirapazamine is inactivated by two-electron reduction steps catalyzed by quinone reductase, and activated to a cytotoxic nitroxide by a one-electron reduction catalyzed by NADPH-cytochrome P450 reductase. The nitroxide itself can be inactivated by spontaneous dismutation. In other words, both inactivation and activation involve reactions of reduction, but cytotoxicity will depend on the relative levels of quinone reductase and CYP-reductase in hypoxic cells.

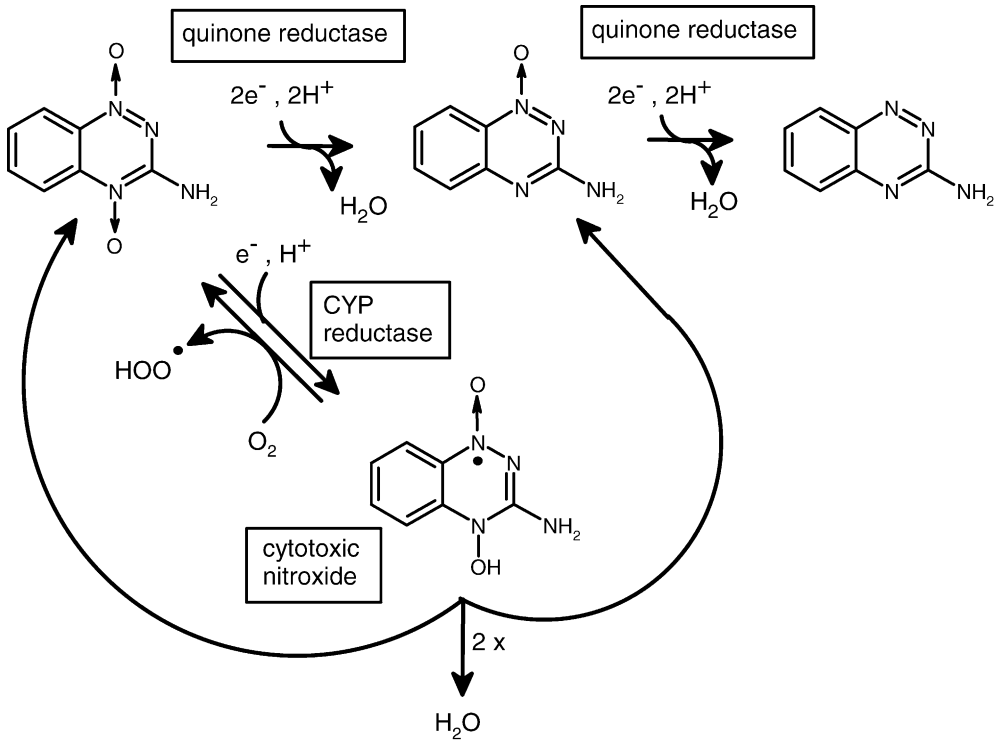


Fig. 8. Pathways of reductive inactivation (two-electron steps catalyzed by quinone reductase) and reduction activation (one-electron step catalyzed by cytochrome P450 reductase) of the hypoxia-selective antitumor agent tirapazamine [40,42].

5. Conclusion: prodrugs are a chemical strategy to bridge gaps

As reviewed above, the prodrug concept has found a number of useful applications in drug research and development. These are summarized in Fig. 9, which repeats the objectives discussed above. However, Fig. 9 goes further in that it illustrates how the various objectives of prodrugs are intertwined. Thus, an improved solubility can greatly facilitate oral absorption, while improving the chemical stability of an active agent can allow tissue-selective delivery and even lead to its in situ activation.

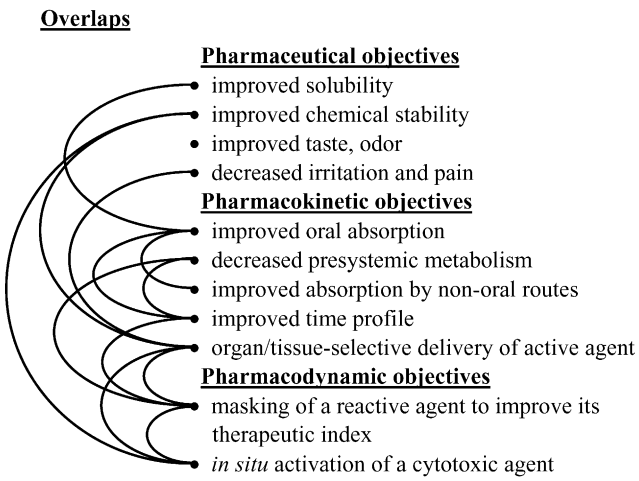


Fig. 9. List of objectives in prodrug research and their overlaps.

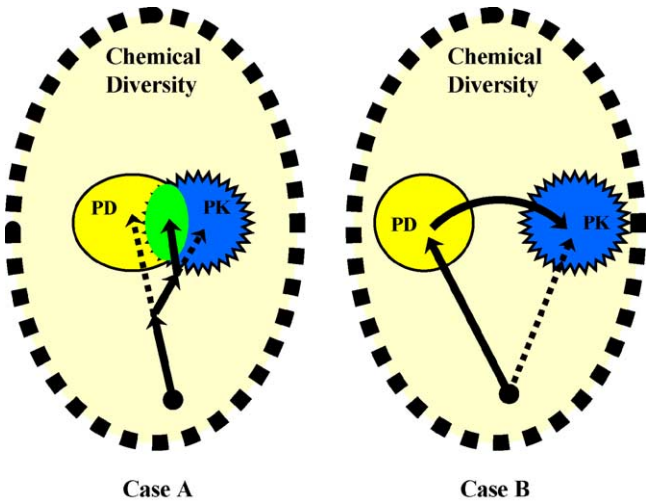


Fig. 10. Two schematic situations in a research project. S.D. symbolizes the undefined structural diversity space theoretically accessible in a given research project. PD symbolizes the ensemble within S.D. of all molecules having the required activity. PK symbolizes the ensemble within S.D. of all molecules having the required drug-like properties (e.g., a good pharmacokinetic behavior). Case A represents the favorable situation where there is partial overlap of the PD and PK chemical spaces, and where structural optimization can evolve toward candidates which combine good PD and PK properties. Case B represents the difficult situation where there is incompatibility between the structural conditions for good activity and good drug-like properties, and where a traditional optimization strategy must fail. Here, a prodrug strategy may be the only alternative to bridge the gap and save the project. The sooner a given research project is found to belong to Case B, the more successful a prodrug strategy may be. Modified from [1].

In practical terms, medicinal chemists and biochemists in prodrug research should be aware that the in vitro and in vivo behavior of their prodrug candidates may differ from that of the parent drug in ways that go well beyond the original pharmaceutical, pharmacokinetic or pharmacodynamic objective being pursued.

A large number of prodrug examples published in the literature are clear cases of post hoc research that never advanced to development. In contrast, the examples presented above illustrate how well-designed or even fortuitous prodrugs allow to achieve medicinal objectives that remain out of reach of the active drug. Indeed, a prodrug approach is most fruitful when a traditional lead candidate optimization fails because the structural conditions for activity (i.e., the pharmacophore) are incompatible with the preset pharmaceutical, pharmacokinetic or pharmacodynamic properties. In other words, the gap between activity and other drug-like properties may be of such a nature that only a prodrug strategy can bridge it, as depicted in Fig. 10.

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