

Review

Prodrugs for Amines

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Received: 14 December 2007; in revised form: 25 February 2008 / Accepted: 25 February 2008 /

Published: 3 March 2008

Abstract: The purpose of this work is to review the published strategies for the production of prodrugs of amines. The review is divided in two main groups of approaches: those that rely on enzymatic activation and those that take advantage of physiological chemical conditions for release of the drugs. A compilation of the most important approaches is presented in the form of a table, where the main advantages and disadvantages of each strategy are also referred.

Keywords: Prodrugs, amines, enzymatic activation, chemical activation

Introduction

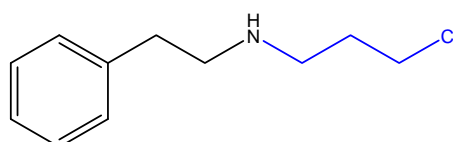
The prodrug strategy may be defined as the temporary derivatisation of a functional group of a drug in order to improve its pharmaceutical utility. Sometimes the functional group is merely a handle for the introduction of a moiety that confers on the new entity some desirable characteristic; more frequently, the group is intimately connected with the pharmaceutical deficiency and its masking directly addresses the deficiency. Of the commonly occurring drug functional groups, perhaps greatest effort has been directed at temporarily masking the amino group. The most easily identified liability of candidate amino drugs is their tendency to ionise under physiological conditions, leading to poor

membrane penetration by passive diffusion. The impact of this is amplified for the large number of amino drugs that are required to penetrate the blood brain barrier in order to reach their pharmacological targets. A second issue that can affect the development of amino drugs is instability. An example of this is the tendency of primary amines to undergo first-pass metabolism due to *N*-acetylation and oxidation by monoaminoxidase (MAO) [1]. The same applies to peptides [2] containing basic amino acid side-chains. Low water solubility, poor stability and low permeability through biological membranes often hinder the clinical development of biologically active peptides [3]. The major problem in designing amine prodrugs is the general robustness of amine derivatives particularly those, such as amides, in which the capacity to ionize has been obviated. On the other hand, the very robustness of amino derivatives means that subtle drug targeting effects can be achieved if an appropriate local vector can be identified and accommodated in the design process. A number of prodrugs of cytotoxic agents fit this description. In structuring the review we have in the first instance sought to classify prodrugs according to whether in vivo activation is enzymatic or pH/redox dependent. Within this major division the various types are identified by the nature of the derivative functional group. The approach is admittedly somewhat arbitrary as prodrug systems rarely undergo activation exclusively by one route. Also, a derivative type that undergoes unmasking enzymatically in one prodrug, may be removed primarily chemically in another because of overall structural differences. Finally, although peptides can be derivatised on other functionalities, we have included only those approaches that involve the amino group alone or conjointly with other functional groups. Table 1 presents a summary of the most important amine prodrug designs along with their dominant activation mechanism and the advantages/disadvantages of each approach. The table should be consulted where difficulties arise in identifying the chemical structure of a group from its name.

Prodrugs that rely (mostly) on enzymatic activation

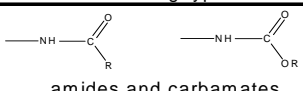
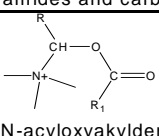
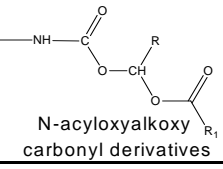
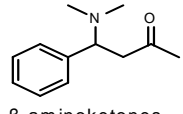
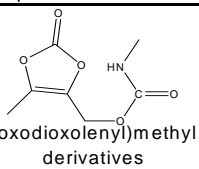
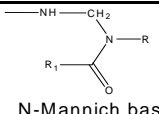
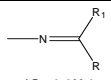
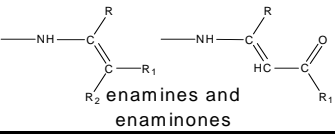
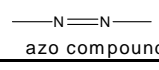
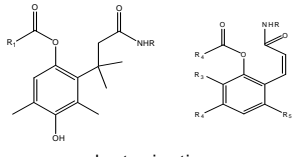
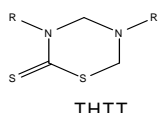
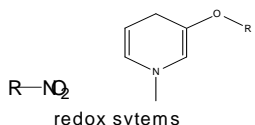
N-Alkylation

Secondary and tertiary alkyl amines are reported to undergo dealkylation mediated by MAO-B to an amine and the corresponding aldehyde or ketone [4]. This has been investigated as a prodrug approach for the CNS active agent 2-phenylethylamine (PEA). In comparison with the free drug, *N,N*-dipropargyl-2-phenylethylamine and *N*-propargyl-2-phenylethylamine produced increased levels of PEA in the brain of rats [5,6]. *N*-(2-cyanoethyl)-2-phenylethylamine [7] and *N*-(3-chloropropyl)-2-phenylethylamine (**1**) [8] also caused sustained elevations of PEA in rat brain.



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Table 1. Compilation of a series of published prodrug approaches to amine drugs.

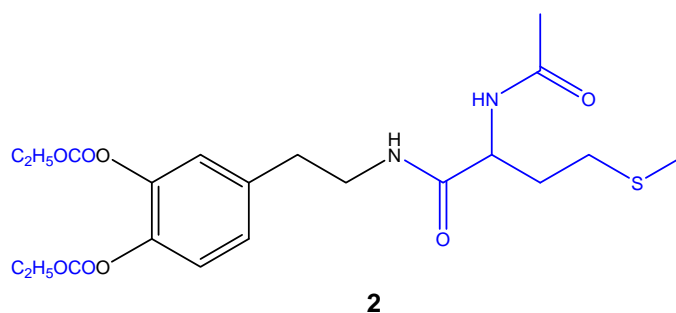
Prodrug type	mechanism of transformation	advantages	disadvantages
 <p>amides and carbamates</p>	enzymatic, pH activated	lipid solubility may be improved slow release	Needs to be activated by an electron withdrawing substitute
 <p>N-acyloxyalkyl derivatives</p>	enzymatic followed by spontaneous	improved lipophilicity	only applicable to tertiary amines
 <p>N-acyloxyalkoxy carbonyl derivatives</p>	enzymatic followed by spontaneous	produces neutral compounds	usually not suitable for primary amines
 <p>β-aminoketones</p>	pH activated	lowers pKa increases lipophilicity	easily hydrolysed in aqueous solution
 <p>(oxodioxolonyl)methyl derivatives</p>	base catalysis and/or enzymatic	also applicable to primary amines	
 <p>N-Mannich bases</p>	base catalysis	lowers pKa up to 3 units	formation of formaldehyde low stability
 <p>imines (Schiff bases)</p>	pH activated	lowers pKa	easily hydrolysed in aqueous solution
 <p>enamines and enamionones</p>	chemical	lowers pKa improved lipophilicity	not stable enough at low pH
 <p>azo compounds</p>	azo-reductases	possibility of targeting	only applicable to aromatic amines
 <p>lactonization systems</p>	enzymatic followed by spontaneous	possible to manipulate phys/chem characteristics	poor aqueous solubility in most cases
 <p>THTT</p>	enzymatic and chemical	improved lipophilicity	only applicable to primary amines
 <p>redox systems</p>	chemical or enzymatic activation	possibility of targeting	oxidation in solid state
PEG	chemical and enzymatic activation	improved solubility	need association with other systems

N-Acylation: amides and simple carbamates

Bioreversible masking of the hydroxyl group with an ester functionality is a practical prodrug approach as ester compounds are in general readily hydrolysed by the rich variety of hydrolase enzymes present in the human body. Acylation of amines is ostensibly less promising because of the chemical and enzymatic stability of amides and carbamates: in this context, mammalian amidases appear to be less promiscuous than esterases. Indeed amides also undergo hydrolysis by esterases *in vitro* [9], but the rates are usually too slow and insufficiently competitive to be used for amine release *in vivo*.

Nevertheless, several successful *N*-acyl prodrugs are in clinical use or in development. In some cases, activation relies on specific substrate-peptidase relationships while in others, slow prodrug hydrolysis by non-specific enzymes is not undesirable. A well-known example of the former is the exploitation of renal γ -glutamyl transpeptidase as a vector for amino drug release from γ -glutamic acid conjugates [10]. Dopamine has been a candidate for this and numerous other prodrug approaches because it is inactivated by sulfotransferase, MAO and COMT in the intestinal wall and liver [11] following oral administration [12]. The dopamine double prodrug, γ -glutamyl-L-dopa (gludopa), achieves kidney dopamine levels about five-fold higher than those obtained with an equimolar quantity of the single prodrug L-dopa [13]. Gludopa itself however, suffers from poor oral bioavailability [14].

Docarpamine, [*N*-(*N*-acetyl-L-methionyl)-*O,O*-bis(ethoxycarbonyl)dopamine], **2**, a pseudopeptide prodrug of dopamine, is administered orally in the treatment of renal and cardiovascular pathologies [15,16].

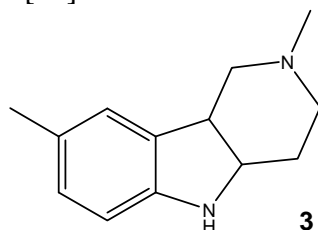


Docarpamine is well absorbed from the oral route, has weak intrinsic vasodilatory effects [17], has no effect in the CNS even at high doses [11], and is easily converted to dopamine *in vivo* [15]. Non-peptide *N*-acyl derivatives of dopamine have also been evaluated [18]. Several other amine conjugate with amino acids via amide bonds have been investigated with significant improvement in solubility, for example, dapsone [19,20].

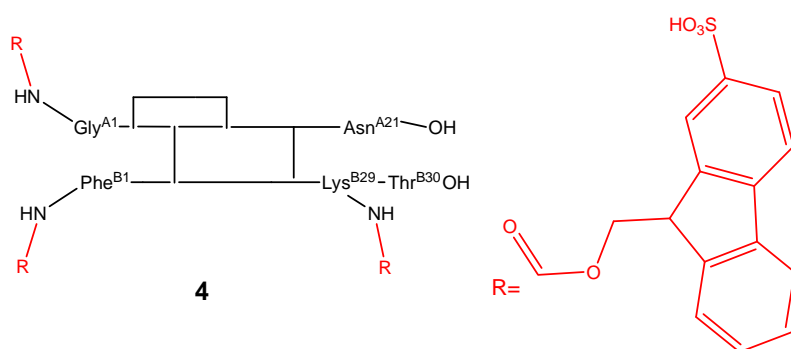
More recently, amino acids have been linked to anti-tumour amines to produce water-soluble amide prodrugs that release, *in vivo*, the parent amine [21]. Glycine and valine amides of monoaminoxidase A (MAO-A) inhibitors were also prepared as an attempt to deliver the active compounds to the brain before inhibiting MAO-A in the intestinal mucosa which leads to the potentiation of tyramine induced hypertension [22].

Derivatization with amino acids has also been used to target intestinal transporters such as PEPT1. One such case is the vasoconstrictor midodrine, which releases the active form 1-(2',5'-dimethoxyphenyl)-2-aminoethanol following cleavage of the glycine residue [23].

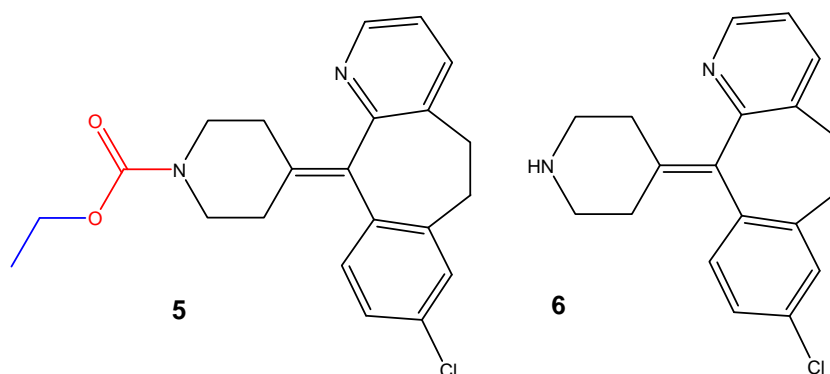
One synthetic amide prodrug type system that has been extensively studied are the *N*-acyl derivatives of allopurinol, which are more lipophilic than the parent drug, while at the same time being in some cases more water soluble [24]. The acetyl, veleroyl and nicotinoyl amides of stobadine (**3**) are highly lipophilic and penetrate the BBB [25].



Simple *N*-acyl prodrugs in which slow cleavage is desirable are the [(2-sulfo)-9-fluorenylmethoxycarbonyl]-3 derivatives of glucose lowering drugs like insulin [26] (**4**, R=H) and exendin-4 [27]. These prodrugs have the advantage of delivering the drugs slowly to the systemic circulation reducing the risk of hypoglycaemia.



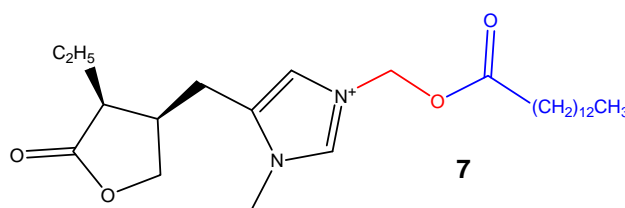
Another system that involves carbamate and urea prodrugs has been developed for antibody-directed enzyme prodrug therapy [ADEPT]. In this system, the carbamate and the urea groups are substrates for the enzyme tyrosinase present in melanomas, where it triggers the release of the drug [28]. New cephalosporins have also been subjected to acyl derivatisation in order to increase solubility [29]. The prototypical non-sedating antihistamine loratadine (**5**) is an ethyl carbamate, which undergoes a CP450-mediated conversion *in vivo* to the active desloratadine (**6**) [30]. It is interesting to note that loratadine was not designed in response to any apparent pharmaceutical liability in desloratadine, and it was certainly not clear at the outset that one acted as a precursor for the other [31].



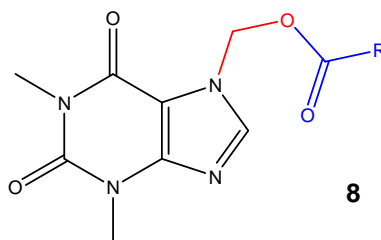
The anti-cancer drug capecitabine is a cascade-type prodrug that produces 5-fluorouracil (5-FU) in the liver and in response to enzymes overexpressed in cancer cells. The first step in the cascade is hydrolysis of a pentyl carbamate mediated by liver carboxylesterase (CES-1). Significant species differences were observed when a panel of homologous carbamates was subjected to hydrolysis by human, monkey and mouse carboxylesterases and the discovery scientists point out that the correct analogue would not have been selected for development without the availability of human intestinal and liver carboxylesterases [32]. Capecitabine has lower gastrointestinal toxicity than 5FU, better tumour targeting and is widely used now clinically [33]. Pentyl PABC-Doxaz (PPD) is a doxaz carbamate prodrug that contains a pentylcarbamate of an anilide, though in this case hydrolysis is mainly mediated by human intestinal carboxylesterase (CES-2) [34]. Neither of these pentyl carbamates is an amine prodrug in the truest sense, since in neither case is the pharmacologically active moiety an amine. However they illustrate that carbamates of anilides may be processed by intestinal or liver carboxylases, predominantly CES-2 or CES-1 respectively.

N-Acyloxyalkylation, *N*-hydroxyalkylation and *N*-(phosphoryloxy)alkylation

N-Acyloxyalkyl derivatives of primary and secondary amines are not usually suitable as prodrugs due to their high lability in aqueous solution. However, with tertiary or *N*-heterocyclic amines it is possible to produce stable quaternary ammonium salts that are nonetheless susceptible to enzymatic hydrolysis by esterases and subsequent spontaneous decomposition. An example of this type is the tetradecyloxymethyl quaternary salt of pilocarpine (**7**) [35].



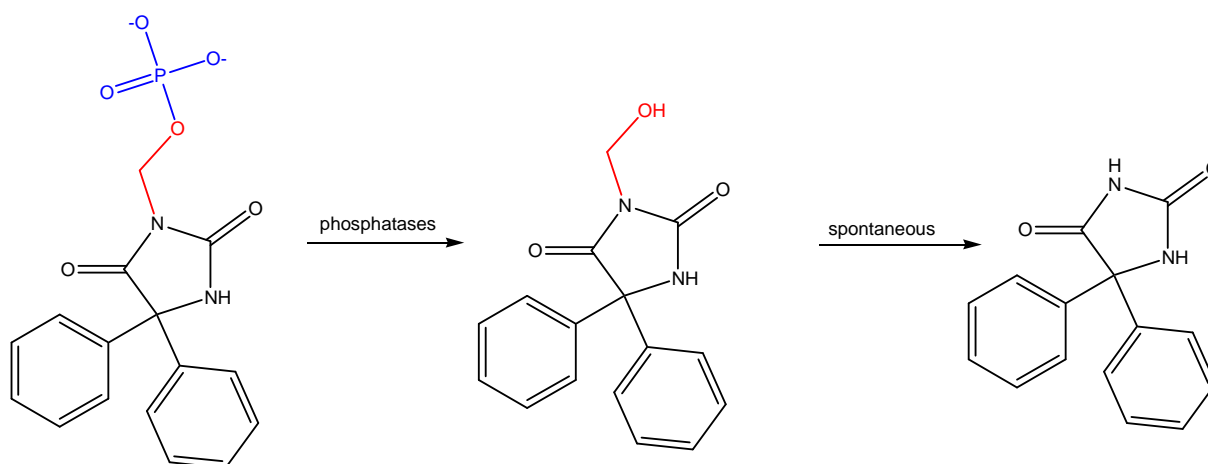
N-acyloxyalkyl derivatives **8** of the topical antiproliferative drug theophylline have been evaluated. No increase in skin permeability was apparent with alkyl chains of up to five carbons; however, the intermediate 7-hydroxymethyltheophylline, possesses twice the permeability of the drug itself [36].



Some quaternary derivatives of tertiary amines have been mentioned as potential prodrugs since these compounds degrade at physiological pH releasing the parent amine [10] in a similar fashion to the acyloxyalkyl derivatives. Another approach involves a salt form of an *N*-phosphoryloxymethyl prodrug, from which the parent drug is released by a first step enzyme-catalysed rate-determining dephosphorylation, followed by spontaneous chemical breakdown of the *N*-hydroxymethyl intermediate [37]. This approach which has been applied to loxapine, improves the aqueous solubility

and stability of the drug [38] and *in vivo* tests suggest that there is quantitative reversion of the prodrug to the parent drug [39]. The phosphoryloxymethyl spacer approach has also been successfully employed in fosphenytoin (Scheme 1), a prodrug of the anti-epileptic phenytoin [40].

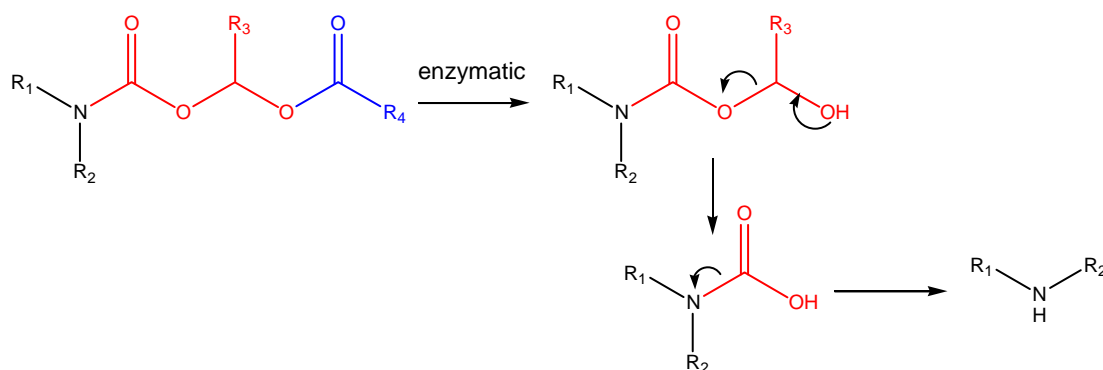
Scheme 1. Tripartite prodrug system for tertiary amines (illustrated for fosphenytoin).



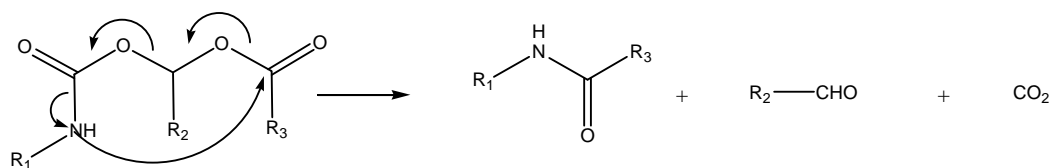
(Acyloxy)alkyl and (phosphoryloxy)alkyl carbamates

N-Acyloxyalkoxycarbonyl derivatives or (acyloxy)alkyl carbamates (R_4 = alkyl or aryl), have received attention as possible prodrug types for amines [41-44]. The compounds possess an esterase sensitive terminal group, whose hydrolysis triggers a spontaneous decomposition of the intermediate (hydroxyalkoxy)carbonyl derivative liberating the parent amine (Scheme 2). This approach neatly addresses the relative enzymatic stability of simple *N*-acyl groups.

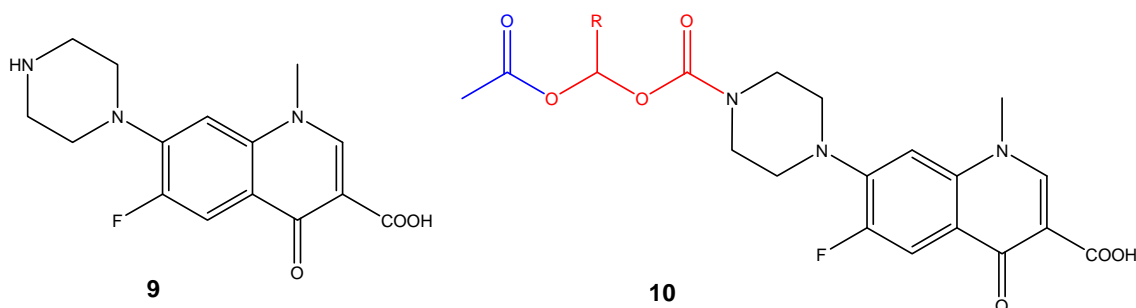
Scheme 2. Hydrolysis of (acyloxy)alkyl carbamate and amine prodrugs.



The system has limited applicability to primary amines whose *N*-acyloxyalkoxycarbonyl adducts can undergo intramolecular acyl transfer, leading to the formation of stable *N*-acyl compounds (Scheme 3) [45].

Scheme 3. Intramolecular acyl transfer in *N*-acyloxyalkoxycarbonyl derivatives of primary amines.

(Acyloxy)alkyl carbamates derivatives of hydrophilic beta-blockers exhibited several fold increase in rat skin and rabbit cornea permeation in comparison with the original drugs [46]. Other examples of (acyloxy)alkyl carbamates are the taste-masking prodrugs **9** of the bitter anti-bacterial norfloxacin (**10**) [47] and XP13512, a prodrug of gabapentin recognized by MCT1 and the sodium-dependent multivitamin transporter (SMVT) [48]. XP13512 was efficiently absorbed and rapidly converted to gabapentin after oral dosing. In monkeys, the oral bioavailability of gabapentin from XP13512 was about 84% compared to 25% after similar oral administration of gabapentin [49].



The use of (acyloxy)methyl esters as bridging groups (Scheme 2, $R_2=H$) is generally a topic of controversy due to the generation of formaldehyde during breakdown. For this reason, (acyloxy)ethyl esters (Scheme 2, $R_2=CH_3$) are usually preferred. On the other hand, (acyloxy)ethyl derivatives introduce a chiral centre into the system; if the drug already has a chiral centre, diastereomers are formed, which can display very different hydrolytic rates [50].

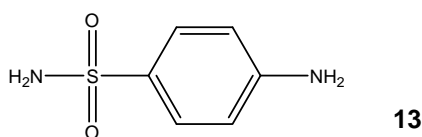
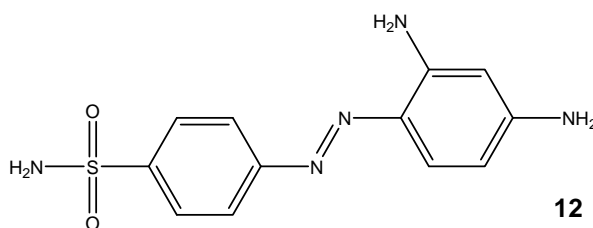
(Alkoxy-carbonyloxy)methyl carbamates have also been prepared (Scheme 2, $R_4=$ alkoxy or aryloxy) [43], as well as (phosphoryloxy)methyl carbamates **11** which would, *in vivo*, be cleaved by alkaline phosphatases. *In vitro* tests with the phosphate esters showed that following the initial enzymatic triggering, a spontaneous cascade leads to the release of the amine [51].



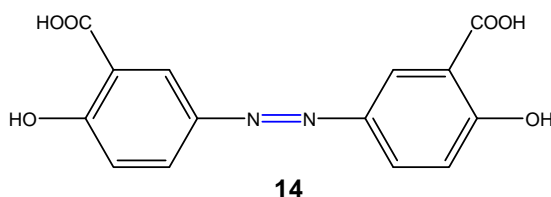
Azo compounds

Numerous azo compounds have been investigated as site-specific prodrugs that exploit the facile reduction of the azo linkage by azo-reductase enzymes. A remarkable story of an early amine azo prodrug is that of Prontosil (**12**), which was used for the treatment of streptococcal infections. Prontosil is a prodrug of sulphanilamide (**13**) and it was developed by Gerhard Domagk in 1932,

probably for commercial reasons rather than pharmacokinetic ones, as the active compound was not patentable. A group at the Pasteur Institute later speculated that the azo link might not be necessary for therapeutic efficacy, and that the active principal might be formed by reduction of the azo bond. They later proved that the antibacterial activity resided in the sulfanilamide portion of the molecule [52].



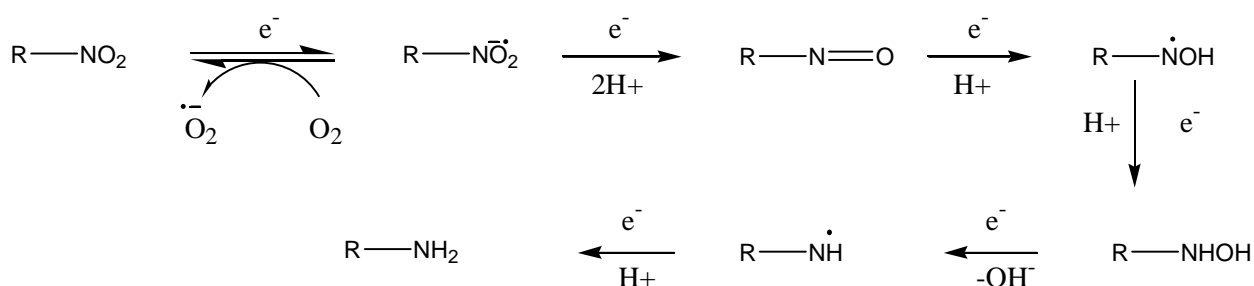
A well-known azo-drug class that exploits azoreductases for site-specific drug release are the 5-aminosalicylic acid prodrugs (e.g. olsalazine, **14**). These pass unaffected through the intestine where they are poorly absorbed [10, 45], but are reduced by azoreductases associated with the high levels of colonic bacteria.



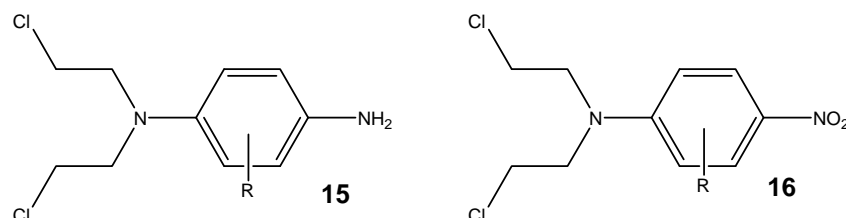
Other clinically used prodrugs in this class include balsalazide and ipsalazide, in which the 5-aminosalicylic acid moiety is conjugated to 4-aminobenzoyl- β -alanine and 4-aminobenzoylglycine, respectively [53]. It should be noted that the azo-approach is generally restricted to primary aromatic amines, azo derivatives of aliphatic amines being unstable. More recently, conventional *N*-acyl type conjugates of 5-aminosalicylic acid with various amino acid derivatives were evaluated for colon specific delivery. 5-Aminosalicylic acid-L-aspartic acid was effectively delivered to the large intestine, releasing about half of the administered dose of 5-aminosalicylic acid [54].

Redox systems

The design of prodrug systems poised to undergo redox reaction receives growing attention. The principal merit of this type of drug-release triggering mechanism is its potential to achieve site-specific delivery. For example, several reductive systems which are selectively activated in hypoxic conditions have been developed and applied in cancer therapy. The systems involve amines latent as nitro groups which, after conversion, form adducts with DNA. The reduction pathway involves several radical intermediates as well as nitroso, hydroxylamine and amine intermediates (Scheme 4).

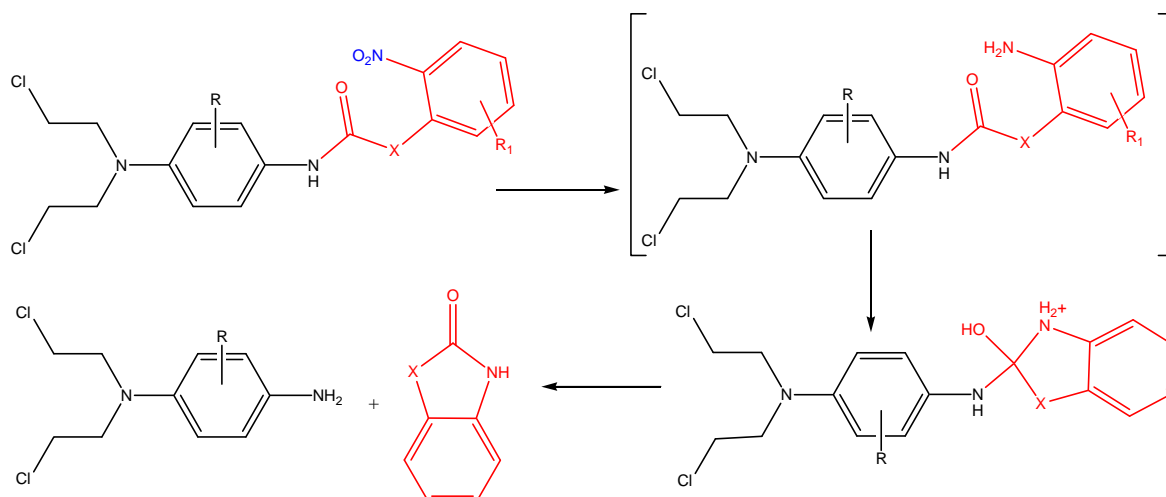
Scheme 4. Generalised reduction pathway for nitro-heterocycles [55].

This type of drug delivery system has been evaluated for the 2-nitroimidazoles, mitomycin C, tirapazamine [55] and indolequinones [56], amongst others. The reduction can be facilitated by the action of endogenous or specially delivered enzymes [ADEPT or GDEPT]. A similar approach has been applied to aromatic mustards **15** to produce prodrugs to target hypoxic tumours. Although the direct use of nitroaromatic mustards **16** could be envisaged as a hypoxic activated system, its application is limited because both the nitro and the alkylating groups are attached to the same aromatic rings and have opposing electronic requirements. This means that the prodrug has a very low reduction potential with consequent low hypoxic selectivity. Substitution on the aromatic ring with electron-withdrawing groups greatly reduced the cytotoxicity of the drug [57]. Nevertheless a water-soluble phosphate ester of a 3,5-dinitrobenzamide-2-nitrogen mustard is currently under clinical trials. The prodrug is converted in vivo to the corresponding alcohol and afterwards activated by reduction to the corresponding 5-hydroxylamine and 5-amine [58].

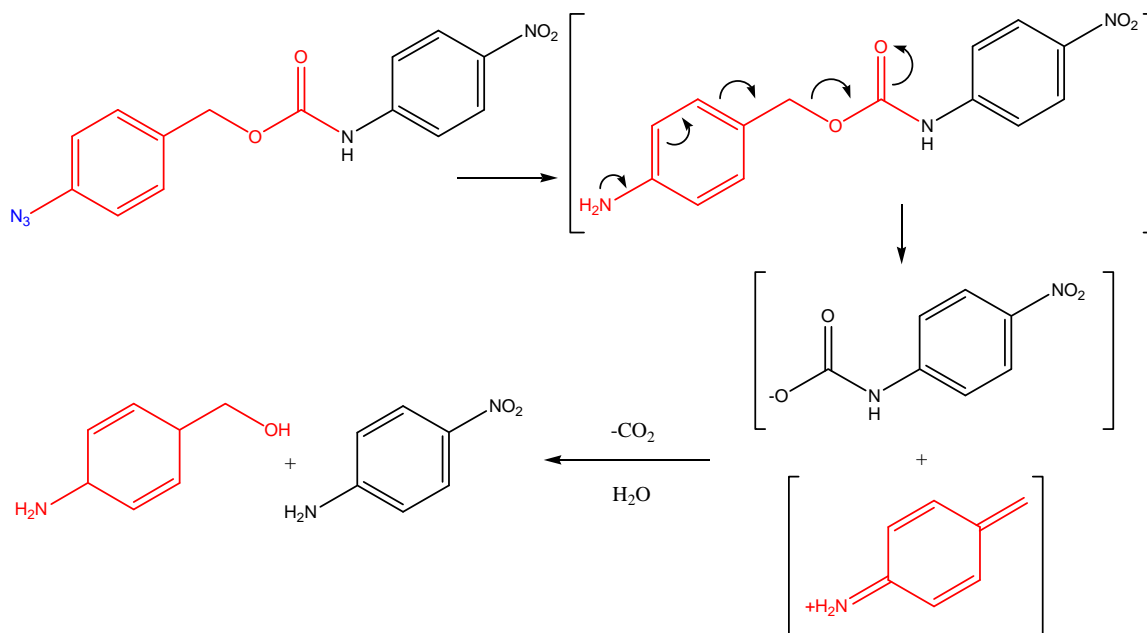


However, the design of a reductively activated system where the reduction potential can be manipulated independently (R_1) allows the production of prodrugs of the more cytotoxic non-substituted mustards. Moreover, the selection of the linker group X (Scheme 5) allows some control over the rates of cyclization [57].

A series of *N*-dinitrophenylamino acid amides that also release primary amines via nitroreduction and intramolecular cyclization has been studied. This system does not seem to be efficiently activated by nitroreductases but the reduction can be radiation-induced, which is a possible approach in cancer therapy [59].

Scheme 5. Mechanism of activation of 2-aminoaryl derivatives.

Another system that can be activated by reduction is the 4-azidobenzoyloxycarbonyl (Scheme 6). In this system the drug is linked through a carbamate to an aromatic azide that is converted by reduction to an amine: a cascade reaction eliminates the carbamic acid, which is readily converted to the amine drug [60].

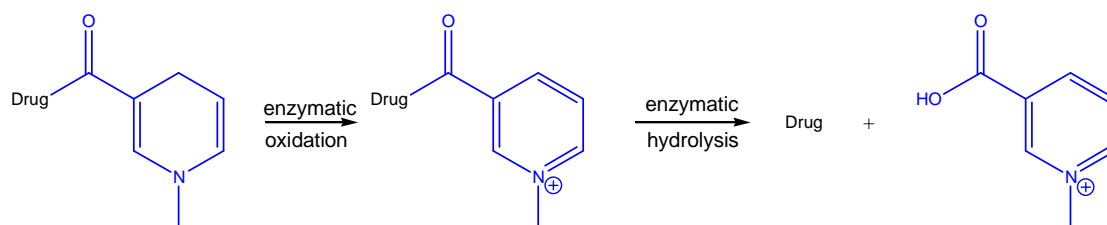
Scheme 6. Activation of the 4-azidobenzoyloxycarbonyl prodrug system.

N-oxides have been suggested as bioreductive prodrugs for tertiary amines. *N*-oxidation masks the cationic charge of the amine reducing their DNA binding affinity and toxicity. The prodrugs are activated by metabolic reduction under hypoxic conditions [61].

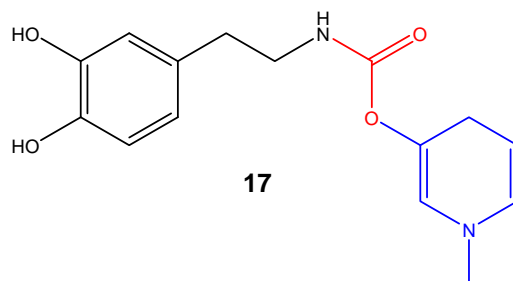
The dihydropyridinepyridinium salt system [62,63] (Scheme 7) is an example of a site-specific prodrug, developed for brain penetration of amines (but that is also applicable to alcohols and carboxylic acids), which employs an oxidative pathway for prodrug localisation. Application of this system to amines was illustrated for desipramine, amongst others. In this case, although there was no

evidence of a more efficient delivery, there was a prolonged presence of the drug in the rat brain at a constant level [64].

Scheme 7. Redox carrier system to the brain.



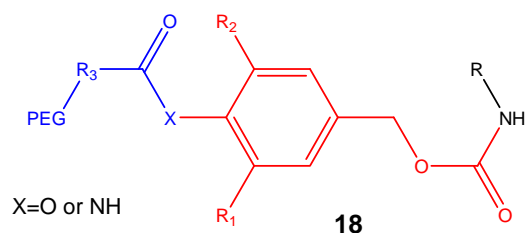
The system has also been applied to dopamine [65,66]. A dimeric form of this prodrug has also been used [17]. The system has also been modified to include an activated carbamate ester **17**.



The relatively lipophilic prodrug penetrates the BBB and the salt obtained after oxidation is trapped in the brain, where it slowly releases the drug. Any oxidation in periphery results in rapid elimination of the intermediate due to its high polarity. This way, the drug can be preferentially accumulated in the brain. The approach has wide applicability to other functional groups such as alcohols and carboxylic acids [67,68]. One significant limitation to the design is the facile oxidation of the dihydropyridine function, which makes the development of a stable formulation difficult [13]. The application of this method to several different drugs has been reviewed [69]. An analogous thiazolium system [70] and carrier groups that involve alkoxycarbonyl methyl derivatives of 7,4-dihydropyridine-3,5-dicarboxylate [71] have been proposed to overcome the stability problems of the dihydropyridine type prodrugs.

PEG and other macromolecular systems

Double prodrug systems that consist of poly(ethylene glycol) (PEG) linked through a spacer to the amine drug (**18**) have been explored for drug solubilization and for extending the plasma half-life of the drug. In one approach, ester, carbonate, carbamate or amide bonds are introduced as spacers and triggers for enzymatic activation and release of the PEG group. After that, the drug, latentiated in the form of a carbamate or an ester, is released by a spontaneous 1,4- or 1,6-benzyl elimination [72,73].



PEG can also be used in conjunction with the "trimethyl lock" system to produce prodrugs for amines with improved solubility and potentially capable of targeting specific tumors. In this system, which has been applied to Daunorubicin, PEG is connected through a spacer to the phenol group of the open lactone. Manipulation of the spacer or the substituents in the aromatic ring allows for tuning of release rates [73-76]. Analogous conjugates have been prepared for Doxorubicin with different results from the ones obtained for Daunorubicin [77], revealing that individual compounds should be evaluated with different linkers in order to determine the most effective combination.

Low molecular weight proteins (LMWP) have also been used to prepare systems for kidney targeting that can be cleaved by aminopeptidases or lysosomal lysates. The LMWP can also be linked to the drug through an acid sensitive spacer. Using β -naphthylamine (β -naph) as a model compound, it was found that the Leu- β -naph and the Gly-Phen- β -naph conjugates were stable in buffer solution, but released the amine completely in cortex homogenates and lysosomal lysates solutions. However the results were not as promising with adriamycin, triametrene and sulfamethoxazole as model drugs [78].

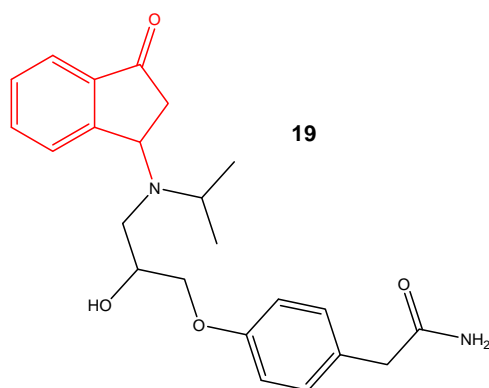
Dextran conjugates have also been prepared using aminocarboxylic acids as spacers [79]. The concept has been applied for slow release of Mitomycin C; different release rates were observed for different linkers.

Prodrugs that rely mostly on chemical activation

β -aminoketones

A recently reported design involves the preparation of β -aminoketones, which are usually stable in acidic conditions but cleave into the parent amine and an α,β -unsaturated ketone at neutral to basic pH [80,81]. Elimination from β -aminoketones by retro-Michael reaction is well known, but hadn't been used previously in prodrug systems for amines. Placement of the amine in a benzylic position seems to have a positive effect on the rate of elimination in comparison with aliphatic systems. This is probably due to the extended conjugation of the double bond upon elimination of the amine.

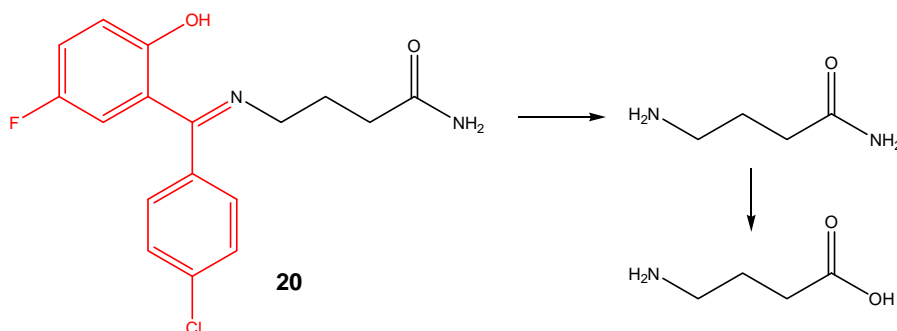
Half-lives at pH=7.4 range from less than one minute to several hours depending on the amine and on the progroup. The indanone prodrug of atenolol **19** has a half-life of 1.3 minutes in pH=7.4 buffer and 2.3 minutes in plasma.



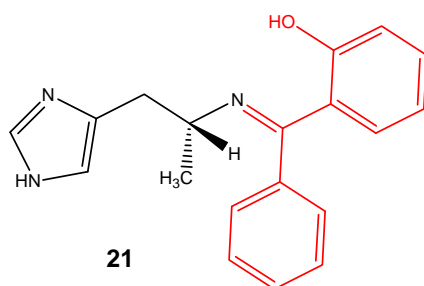
Schiff bases

Imines (Schiff bases), are usually too easily hydrolysed to be useful as amine prodrugs. Nevertheless, in some particular cases they may be surprisingly stable and useful as they impart increased lipophilicity to the parent amine and depress the pKa values. The anticonvulsant progabide under the trade name Gabrene[®] (**20**) was prepared as a prodrug form of γ -aminobutyric acid (GABA) since it crosses the BBB, while the free drug doesn't. The prodrug is converted to γ -aminobutyramide and GABA (Scheme 8), which are then trapped if produced in the brain. However, it might not be considered a true prodrug as it possesses intrinsic pharmacological activity [10].

Scheme 8. Metabolism of progabide.



Chemically activated azomethine prodrugs **21** [82,83] of the reference histamine H3 receptor agonist *R*- α -methylhistamine have been evaluated, as well as enzymatically [84] activated prodrugs (amide, esters and carbamates).

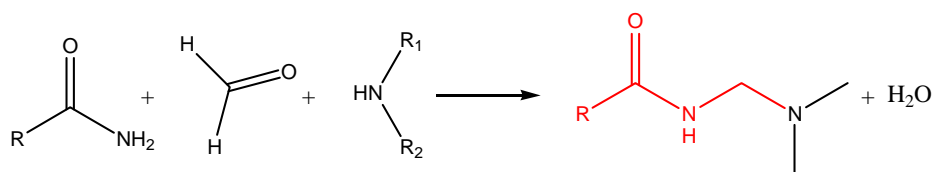


The new compounds, being more lipophilic, have improved oral absorption and better BBB penetration than the original drug.

S and N-Mannich bases

N-Mannich bases are synthesised through the Mannich reaction, which involves a NH-acidic compound, an aldehyde (usually formaldehyde) and an amine in ethanol (Scheme 9).

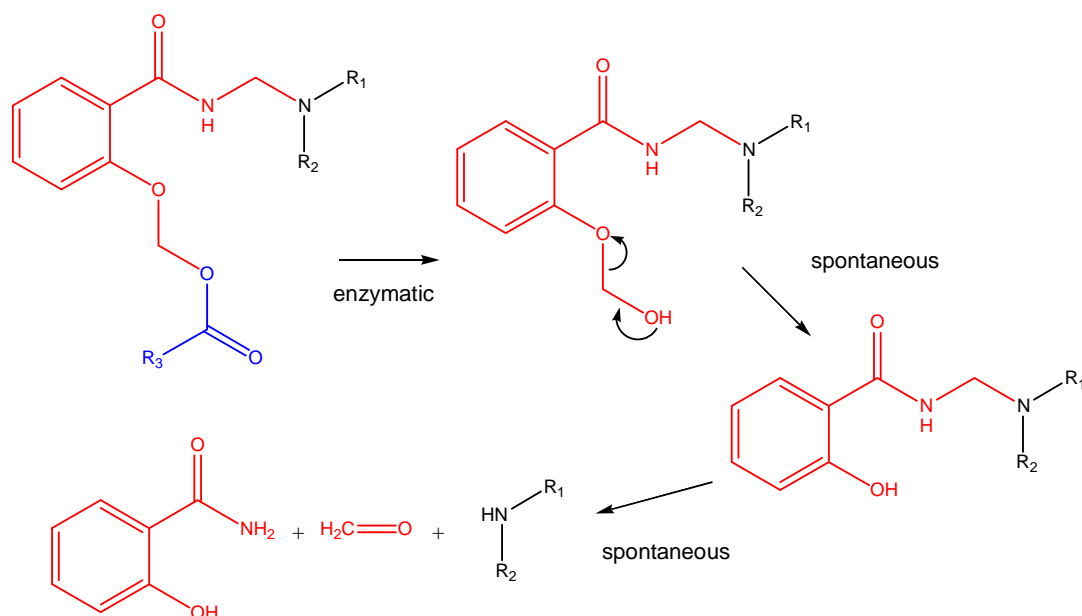
Scheme 9. Synthesis of *N*-Mannich bases.



This system can be used to produce prodrugs that are more soluble than the parent [85], not only for amines but also for amides, as in the case of rolitetracycline [45]. *N*-Mannich bases are useful when an increase in the lipophilicity of amines is desirable. *N*-Mannich base formation also suppresses the pKa (a difference of up to 4 units) with respect to the amine, which means that an important proportion remains unionized at the pH of the intestine [1]. However, the range of biologically acceptable amide type transport groups affording an appropriate cleavage rate is limited [86].

Cleavage of the prodrug in this case is strictly pH dependent: it has been found that *N*-Mannich bases of salicylamide and different aliphatic amines and amino acids show a bell shaped pH/rate profile with a high breakdown rate at pH 7.4. In the case of salicylamide, the hydroxyl group is thought to be responsible for the high reactivity, possibly by intramolecular catalysis, when the compound is in the neutral or zwitterionic forms. At high pH, when the compound is in the anionic form, the reactivity decreases markedly [87]. However, derivatisation of this group by acyloxymethylation, provides new possibilities of controlling *in vivo* cleavage as well as improved *in vitro* stability (Scheme 10) [88].

Scheme 10. Esterase sensitive *N*-Mannich bases of salicylamide as prodrugs for amines.



It was established that the acidity of the parent amide and steric effects within the amine component correlate with reactivity [85,89]. For amines with similar steric properties, a decreasing basicity is associated with decreasing reactivity [85]. For example, the rate of breakdown of *N*-Mannich bases of aromatic amines with succinamide increases markedly with increasing amine basicity [1]. For amines with similar pK_as, some correlation was found between reaction rates and the difference in pK_a between the amine and the corresponding Mannich base (for the same amide) [90].

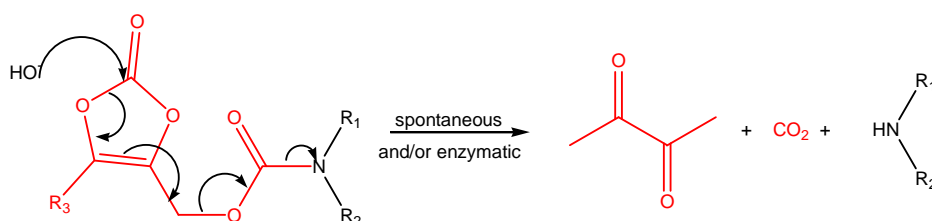
A drawback of the *N*-Mannich bases is their limited *in vitro* stability, raising some stability/formulation problems [45]. The unavoidable release of formaldehyde during decomposition is another factor that has to be taken into consideration due to its toxicity [85].

An example of an *S*-Mannich base is dipyrone (metamizole) the methanesulfonic acid of the analgesic 4-(methylamino)antipyrine, which is highly water soluble and therefore more suitable for parenteral administration. When administered orally, dipyrone appears to undergo fast hydrolysis in the stomach followed by intestinal absorption of the active form [91].

(Oxodioxolenyl)methyl carbamates and other selfimmolative systems

(Oxodioxolenyl)methyl carbamates were prepared as an attempt to avoid the drawbacks of (acyloxy)ethyl and (acyloxy)methyl esters. The prodrugs break by base catalysis according to Scheme 11, but the rate of hydrolysis in plasma solutions is higher than in pH 7.4 buffer solutions [43,50].

Scheme 11. (Oxodioxolenyl)methyl carbamate prodrugs of amines: mechanism of base-catalysed cleavage.



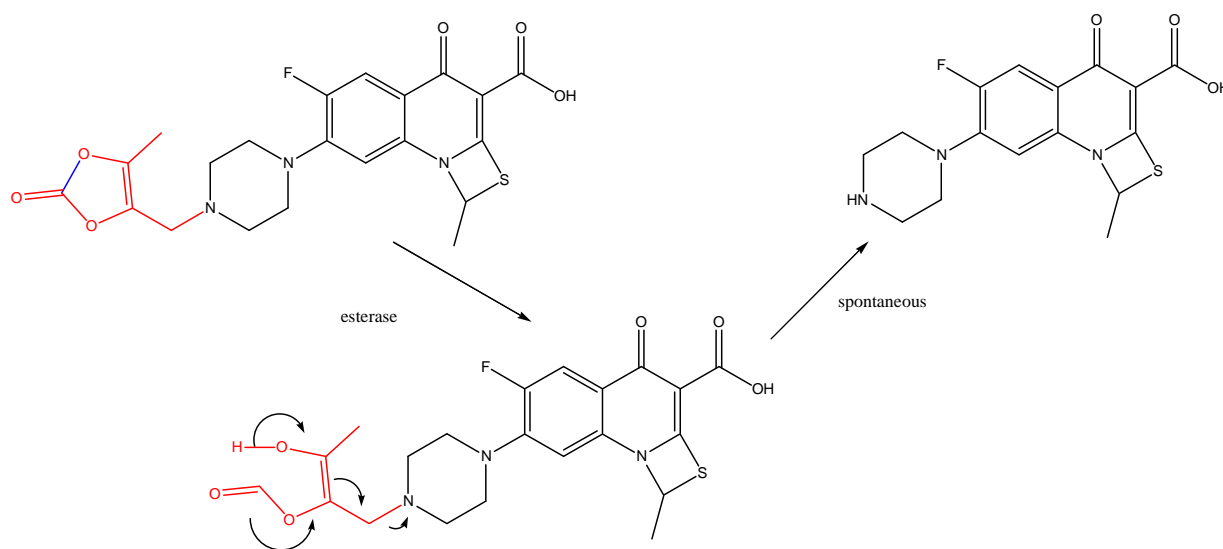
In these systems, the cleavage of the dioxolenone ring by the amino group attack on the reactive vinylene carbonate function is precluded, which makes this approach potentially applicable to primary amines. Aryl R₃ substituents generally have a destabilising influence, reducing the half-life of the prodrugs [50]. The system has been applied to pseudomycins and some of the prodrugs exhibited comparable *in vivo* efficacy to that achieved by the parent compounds, with reduced side effects [92].

In a similar approach a selfimmolative linker was used to attach tryptophan to a bisphosphonate component through a carbonate-labile linker, 4-hydroxy-3,5-dimethoxybenzyl alcohol, which was further attached through a stable carbamate linkage to the amine group of tryptophan [93]. The carbonate linkage hydrolysed with a half-life of 90 h, but it can be modulated through the nature of the substituent on the aromatic ring of the self-immolative linker.

Another example using oxodioxolenyl group is that of Prulifloxacin (Scheme 12) which is a fluoroquinolone antibacterial agent with a broad spectrum of activity against Gram-positive and -negative bacteria [94] currently under clinical phase 3 trials. The prodrug uses the (5-methyl-2-oxo-1,3-dioxolyl)methyl promoiety linked to the secondary amine group of the active form by *N*-alkylation. Amine release is triggered by esterase attack on a distal oxodioxolenyl group, but formally

speaking, the connection to the amine group being masked is an alkyl group. Prulifloxacin is available for oral use, and after absorption is metabolized to the active form ulifloxacin by esterases, mainly paraoxonase [95].

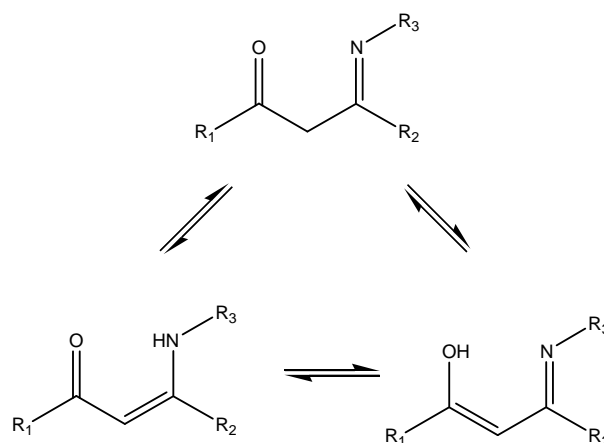
Scheme 12. Enzymatic hydrolysis of Prulifloxacin [95].



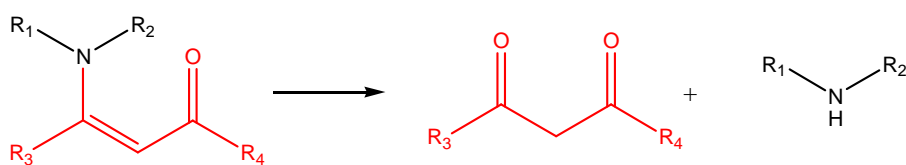
Enamines and enaminones

Enamines [96], (α,β -unsaturated amines), just like imines, are generally unstable particularly at low pH, which make them unsuitable for the preparation of prodrugs for oral delivery. Nevertheless, an enamine prodrug of ampicillin was found to promote the rectal absorption of the drug [97]. Enaminones, which are enamines of β -dicarbonyl compounds are more stable, probably due to keto-enol and imine-enamine tautomeric equilibria (Scheme 13) [91], and were thought to have potential use as prodrugs [10].

Scheme 13. Stabilisation of enaminones.



The hydrolysis of enaminones derived from some amino acids and antibiotics is rapid, releasing the amine and a diketone (Scheme 14).

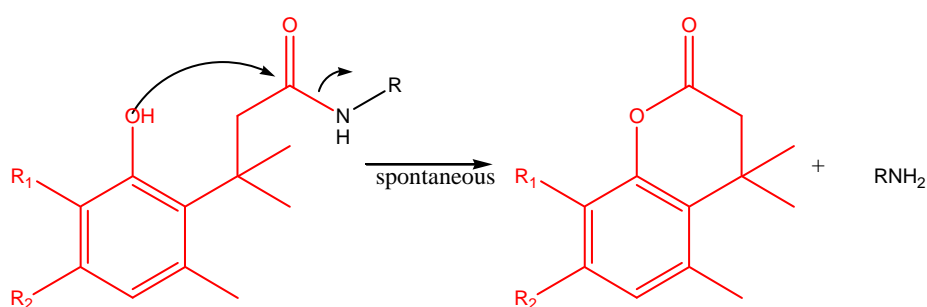
Scheme 14. Hydrolysis of enamines.

The prodrugs are more lipophilic than the corresponding drugs, which usually results in improved absorption [10]. The system seems to be relatively insensitive to the type of amine used, but very sensitive to minor changes in the structure of the 1,3-dicarbonyl compound used to produce the prodrug. Closed structures like the compounds derived from cyclohexane-1,3-dione show considerably lower rates of hydrolysis. This is probably due to their rigid geometry and the inherent stability of this system. The maximum rate of hydrolysis occurs in the pH range 2-5 [98].

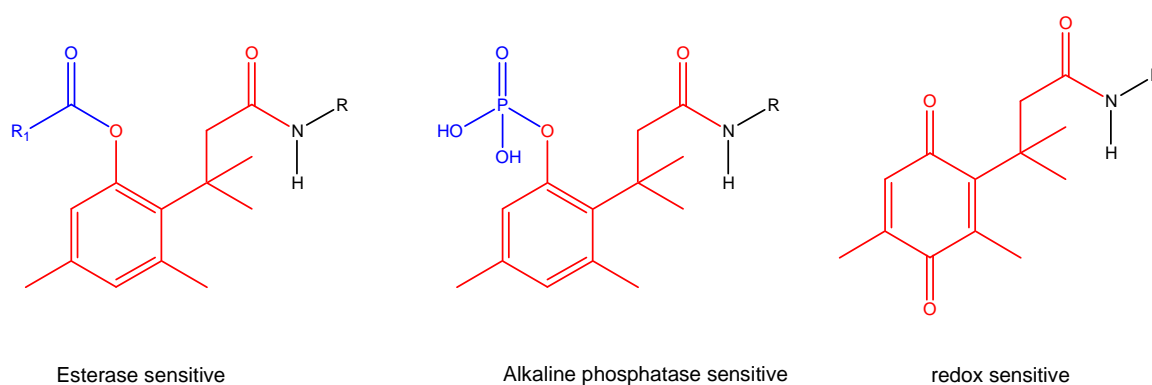
Based on chemical stability considerations, enamines do not seem promising as prodrugs. However it has been speculated that enamines obtained from ketoesters and lactones may be better candidates as they may be subjected to enzyme-catalysed degradation [99].

"Trimethyl lock" and coumarin systems

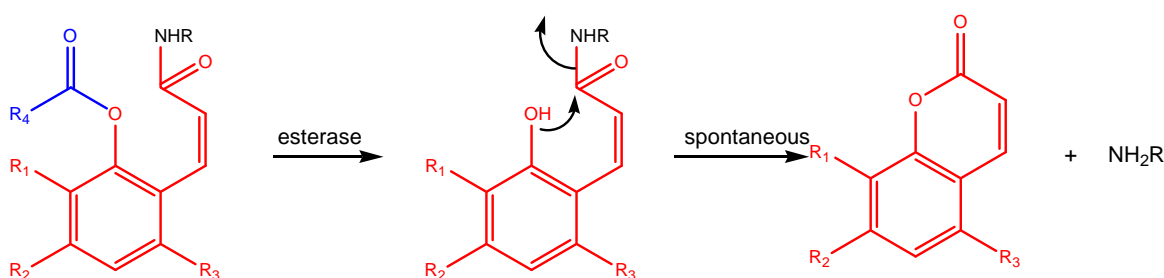
Phenolic amides derived from lactones can be used as amine prodrug systems as they release the amine and a lactone at physiological pH [100] (Scheme 15). In this system, referred to generally as a "trimethyl lock", the side chain is folded back to bring the amide carbonyl group into proximity with the nucleophilic phenolic oxygen. This conformation may account for the facile cyclisation that occurs independently of the drug attached to the side chain. However the half-lives of these systems are usually less than 1 min, which is too short for useful application [101].

Scheme 15. "Trimethyl lock" prodrug system for amino drugs.

The design has been modified to produce compounds that are esterase [102] or redox [103] sensitive (Figure 1). These derivatisations involve the protection of the nucleophilic hydroxyl in a bioreversible manner. The rate-limiting step then becomes the enzymatic exposure of the phenolic group. A further variation to this system involved the introduction of phosphate esters as the phenolic masking group [104].

Figure 1. Tripartite "trimethyl lock" systems.

A conceptually similar system exploits the facile cyclization of coumarinic acid and its derivatives [105] (Scheme 16). The presence of the phenolic hydroxyl group and the *cis*-geometry of the double bond allows lactonization at rates comparable to those of the "trimethyl lock" system. The phenol group is protected by an ester or a phosphate group that serves as an esterase or phosphatase sensitive biological triggering mechanism.

Scheme 16. Coumarin-based esterase sensitive system for amino drugs.

The lactonization rate is higher for primary amines than for secondary amines; it also depends on steric features of the amine to be released [106]. For secondary amines with higher pK_as, the system is sometimes undesirably slow. A further refinement to the design is the use of ring mesomeric effects to tune drug release rates: increases of up to 16-fold can be achieved by placing electron releasing groups on the aromatic ring [107,108].

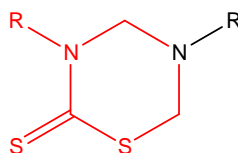
More recently, an attempt was made to prepare a tripartite prodrug (double prodrug) that uses the coumarin system as a spacer between the drug (linked to the side chain) and a carrier group, a peptide or an amino acid, connected to the hydroxyl group of the coumarin. The advantage of this system would be the possibility of targeting drug specific proteases for the cleavage that would release the carrier, which would be followed by spontaneous lactonization, releasing the drug. Poor aqueous solubility has, however, limited the exploitation of this system [109].

THTT

A tetrahydrothiadiazine-2-thione (THTT, Figure 2) was proposed as a prodrug system for primary amines [110], amino acids [111] and peptide drugs [112]. In this system the nitrogen atom from the drug is included in a six membered ring, which is more lipophilic than the original drug. The prodrugs

are enzyme and chemically sensitive at physiological pH, but are stable under acidic conditions. Despite the apparent promise of this system, it does not seem to have been subjected to further development.

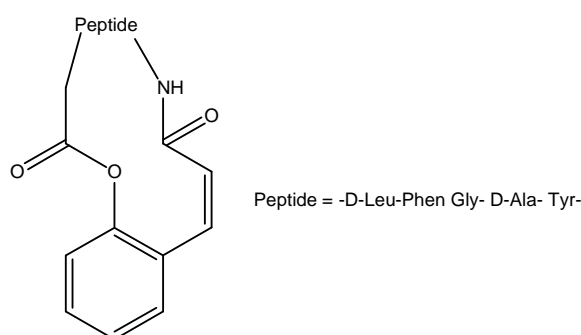
Figure 2. THTT prodrug system for amines and amino acids.



Cyclic derivatives of polyfunctional drugs containing the amino group

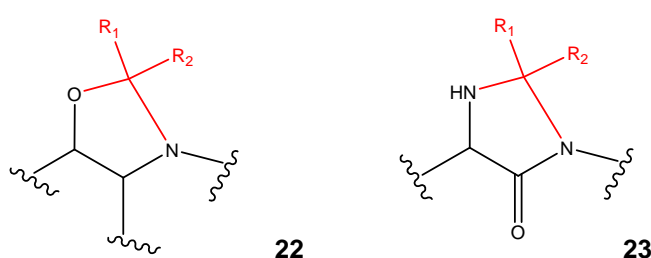
Making prodrugs of compounds with multiple functional groups may be a challenge, particularly in the case of peptides, where the amine group poses a problem of its own due to the lack of suitable biologically reversible masking groups. The "trimethyl lock" and coumarin systems mentioned before as prodrug systems for amines, have also been tested as prodrugs for peptides (linked to the progroup through an amide and an ester link) with promising results (Figure 3) [113-116].

Figure 3. Coumarin system applied to peptides.



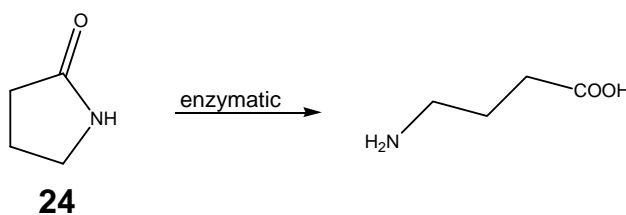
The concept has been applied to peptides such as DADLE, an opioid peptide. The C and N terminal ends of the linear peptide are masked by forming an ester and an amide bond with the phenol hydroxyl and side chain carboxyl groups, respectively, of the linker [107]

Oxazolidines **22**, which are cyclic condensation products of β -aminoalcohols and aldehydes or ketones, are a possible means of formation of peptide prodrugs [86]. These compounds are less basic and more lipophilic than the corresponding β -aminoalcohols and hydrolyse completely in aqueous solution [117]. Thiazolidines can be used for β -aminothiols.



4-Imidazolidinones **23** [118-120] have been proposed for the α -aminoamide moiety, in particular as prodrugs of Leu-enkephalin and prilocaine. The derivatives of Leu-enkephalin afford protection against aminopeptidase-N and angiotensin converting enzyme (ACE) and are cleaved slowly in buffered solutions at pH=7.4, with half-lives of some hundred minutes [120]. The hydrolysis of some prilocaine derivatives, at basic pHs, proceeds to an equilibrium due to reversible kinetics [119]. Lactams and pyrrolines have been shown to revert to the corresponding amino acids by enzymatic action. Moreover, they pass the BBB while the opened structures don't. One example is the cyclic derivative (**24**) of GABA depicted in Scheme 17.

Scheme 17. Conversion of 2-pyrrolidinone to GABA.



Conclusions

Three major themes emerge in amine prodrug chemistry: (i) suppression of ionization in order to promote passive diffusion; (ii) increasing the metabolic stability especially of primary amines and peptides; (iii) tissue targeting, particularly tumor tissue targeting. Other objectives include increasing the water solubility of the amine. Overall then, amino drugs may benefit significantly from prodrug design, but designing appropriate prodrugs for amines has been challenging. This challenge has provoked a markedly disparate variety of responses from pharmaceutical researchers. We considered it timely to gather these into one review article as a sort of catalogue that developers might find useful to consult during the development of new amino drugs or improvement of existing ones. Predicting the suitability of any single approach to a new situation, however, is still problematic. It seems prudent to investigate a number of approaches in parallel with appraisal in a panel of the most relevant human biological matrices, for example, intestinal and liver microsome preparations as well as plasma.

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