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Review

Prodrugs of phosphates, phosphonates, and phosphinates

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Abstract

The objective of this paper is to review the literature on the use of prodrugs to overcome the drug delivery obstacles associated with phosphate, phosphonate and phosphinate functional group-containing drugs. This is an important area of research because, although we have been successful at identifying numerous phosphate and phosphonate functional group-containing drugs as antiviral and anticancer agents, as well as for other uses, our ability to orally deliver these drugs and to target them to desired sites has led to limited success. Various acyloxymethyl- and aryl-ester prodrugs have shown promise. Alternative and imaginative approaches may be necessary before complete success is realized. It is our hope that this review will stimulate further innovative prodrug research into overcoming the barriers to the delivery of these important drugs.

Keywords: Nucleotide analog; Nucleotide prodrug; Bioavailability; Ester; Permeability

Contents

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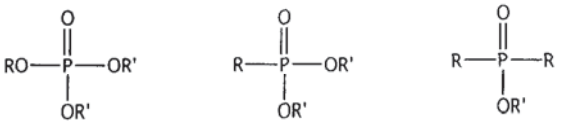


Drug: R = organic residue; R' = H or (-)

Prodrug: R = organic residue; R' = promoeity

Fig. 1. General structure of phosphate, phosphonate, and phosphinate drugs. R represents an organic residue while R′ represents H or anionic charge for the parent drug or the promoiety for the prodrug.

1. Introduction

The intent of this paper is to review the literature on the use of prodrugs to overcome drug delivery obstacles associated with phosphate, phosphonate and phosphinate functional group containing drugs. The general structures of phosphate, phosphonate, and phosphinate-containing drugs are shown in Fig. 1 where R represents an organic residue, and R′ represents either an anion charge or a hydrogen atom in the case of the parent compound, or R′ is a neutral ester in the case of the prodrug.

Initial research on the problems associated with the delivery of phosphate functional group-containing drugs began in the early 1960s with phosphate-containing nucleoside analogs used in cancer and viral chemotherapy. Effort continued to expand and has recently increased in interest with the advent of the variety of drug classes utilizing the phosphate, phosphonate or phosphinate functionalities, especially antiviral agents and various molecules designed to alter cell signaling processes.

2. Why prodrugs of phosphates, phosphonates and phosphinates?

Although the range of drugs containing either a phosphate, phosphonate or phosphinate-containing functional has changed, the underlying problem with the delivery of such drugs has not. The shortcomings in the delivery of these drugs can be broken down into two basic problems:

1. Phosphate, phosphonate and phosphinate groups impart an anionic charge (mono- or di-) at nearly all physiological pH values making them very polar. This high polarity can be the basis for many deficiencies in the efficacy of drug delivery. Specifically, highly ionized species do not readily undergo passive diffusion across cellular membranes.
2. Because of the increased polarity, these agents often exhibit a low volume of distribution and therefore tend to be subject to efficient renal clearance as well as possibly biliary excretion. In addition to renal clearance, phosphates, particularly those of primary alcohols and phenols, are known to be substrates for many phosphorylases present in the body which readily clip the phosphate group from the drug. The rapid dephosphorylation results in a short duration of action. Phosphonates and phosphinates have the advantage of being more chemically stable and showing essentially no enzymatic lability.

3. How can prodrugs overcome these problems?

In an attempt to overcome these shortcomings, the ionizable phosphate, phosphonate and phosphinate groups have been neutralized via chemical derivatization. This generally involves derivatization of the phosphorus-coupled oxygen(s) to form neutral ester(s). If the intention of the ester is for it to breakdown in the body to release the parent drug, then such a derivative would be a prodrug. The neutralization of the charge(s) has been proposed to serve a number of purposes:

1. The first is to decrease the polarity by increasing the lipophilicity of the drug molecule thus allowing access to cells and tissues that might not be available to the non-modified specie and possibly altering the distribution/elimination pattern of the parent drug.
2. Another use of the neutral ester prodrugs is particularly important for phosphates which are substrates for nonspecific serum phosphohydrolases, such as alkaline and acid phos-

phatases. With these drugs, it has been proposed that the neutral ester serves to disguise the phosphate from the enzymes thereby altering the apparent elimination and half-life as illustrated in Scheme 1. A flaw in this thinking is that once deprotected, the intrinsic properties of the drug should not be dramatically altered. Nevertheless, entrapment of the parent drug in tissues in which it is not normally accessible could lead to apparent changes in its pharmacokinetic/pharmacodynamic properties.

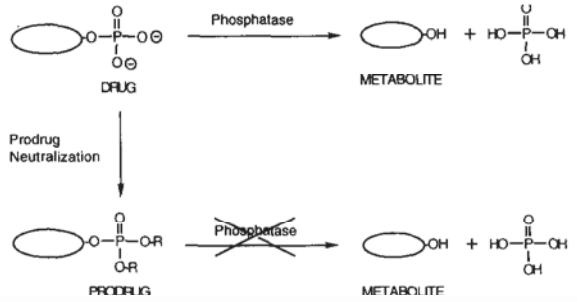
Although alterations in apparent clearance rates may be important, the principal goals of most prodrug modification efforts on phosphate, phosphonate and phosphinate drugs is alteration of membrane permeability to improve oral (GI permeability), brain, tumor and cellular delivery (mainly to virally infected cells) of these agents.

When these prodrugs are used for improving oral bioavailability, various issues dealing with GI absorption of drugs must be considered. The ability to address these issues will ultimately determine the proper selection of the prodrug system and its likely success. The optimal scenario for enhanced systemic delivery of prodrugs after oral dosing is as follows:

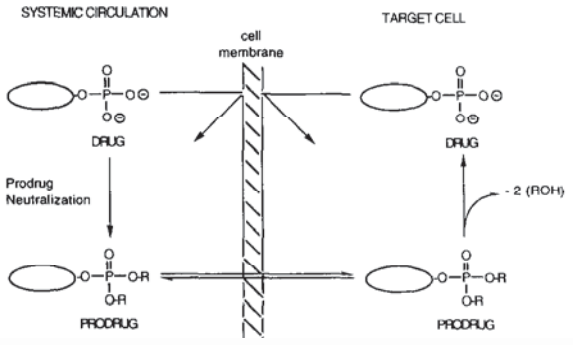
1. The prodrug must display adequate chemical stability for formulation purposes as well as stability in the variable pH environment of the GI tract.
2. The prodrug should have adequate solubility in the GI tract environment to allow for dissolution.
3. Once dissolved, the prodrug should also display enzymatic stability to lumenal contents as well as the enzymes found in the brush border membrane.
4. The prodrug should have properties that allow for good permeability (generally associated with an adequate log P value).
5. After permeation of the lumenal membrane, the prodrug could revert to the parent drug either in the enterocyte or once absorbed into systemic circulation. Post-enterocyte reversion is desired because conversion in the enterocyte would also allow for back diffusion into the GI lumen, a problem which is not generally recognized.

When the prodrug is formulated to increase cellular permeability into viral-infected cells, tumor cells or across barriers like the blood brain barrier, the desired characteristics might change. Replacing the desire for complete and rapid post absorption reversion, is a need for balance in lability. The most optimal scenario, however unrealistic, would be for the prodrug to have complete enzymatic and chemical stability during the absorption process and in blood but readily revert to the parent compound once it has permeated the targeted cell, thereby 'trapping' the drug in the cell (Scheme 2).

Considering both of these scenarios, prodrugs for improved oral delivery and prodrugs for improved cell targeted delivery, the rate of bioreversion is a very important process that



Scheme 1. Scheme showing the possible advantages of prodrugs in altering the phosphatase cleavage of phosphate-containing drugs.



Scheme 2. Proposed scheme illustrates the potential advantages of prodrugs over the parent molecule for intracellular targeting.

must be considered in detail when designing prodrug systems. For example, if bioreversion is very fast and non-specific, prodrug reversion may take place before the limiting barrier is overcome. On the other hand, if reversion is slow and inefficient at all sites, the prodrug may readily reach the site of action but never release enough parent drug to elicit a pharmacological response. With these factors in mind, choosing a suitable bioreversible protective group for phosphates, phosphonates and phosphinates presents a major challenge.

4. Specific examples

4.1. Nucleotide analog prodrugs

Purine and pyrimidine nucleoside analogs have found great utility in treatment of neoplastic and viral diseases [1–8]. Please note, the word nucleoside will be equated with nucleoside analog for the remainder of this manuscript for simplicity purposes. These nucleosidic drugs mostly rely on viral or kinase-mediated (i.e., thymidine kinase) activation step(s) to produce the phosphorylated nucleotide, necessary to display biological activity i.e., the nucleosides are themselves prodrugs. Unfortunately, dependency on kinase mediated synthesis can lead to the development of resistance [9–11]. The first step in the phosphorylation of a nucleoside to the monophosphate is known to be highly specific and often causes the development of resistance [12–15]. Therefore, it can be argued that an approach to circumventing the resistance development problem is to administer the monophosphate-containing nucleoside drug. This strategy has two flaws:

1. The highly polar monophosphate has limited passive absorption properties and therefore, transcellular transport is very restricted [16–19]. This flaw is supported by the work of Leibman et al. [18] who demonstrated the lack of passive as well as the absence of an active transport mechanism for ara-CMP (the monophosphate derivative of ara C; Fig. 2).
2. Rapid in vivo dephosphorylation of the monophosphate is observed with this class of drugs [20–22].

Could prodrugs be used to overcome these problems? The aim of this portion of the review is to focus on phosphorus-coupled oxygen prodrug esters of nucleotides, namely the monophosphates and their phosphonate analogs. An effort has been made to group the various prodrug strategies according to the type of neutral ester chosen. The interested reader may wish to refer to other reviews on this subject that take a little different approach from those taken here [23–25].

4.1.1. Simple and substituted alkyl and aryl ester prodrugs of phosphates and phosphonates

Rosowsky et al. [26] have examined several mono-5′-(alkyl phosphate) esters of ara-C (Fig.

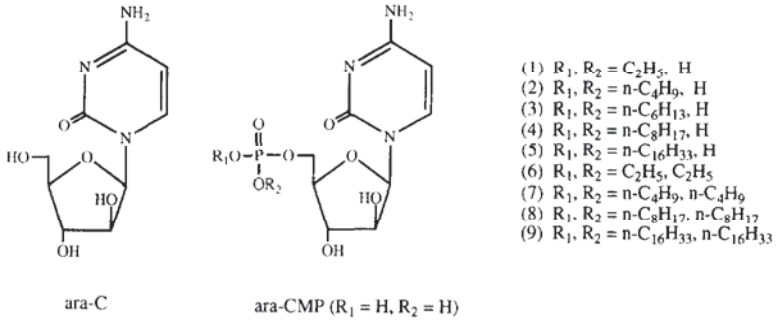


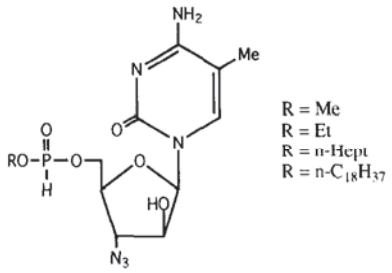
Fig. 2. Structures of ara-C, ara-CMP, and araCMP alkyl prodrugs. Structures (**1**)–(**4**) are mono-alkyl prodrugs [26] while (**5**)–(**9**) are dialkyl prodrugs [30].

2, **1–5**) in an effort to deliver ara-CMP to cancer cells. The cytotoxicity of the proposed prodrugs toward cultured L1210 leukemia and B16 melanoma cells appeared to obey an inverse structure-activity relationship with respect to alkyl chain length. The *n*-butyl and *n*-hexyl prodrugs were approximately half as active as the ethyl prodrug. The structure-activity relationship seemed to plateau as chain length became longer as exemplified by *n*-octyl and *n*-C_{16} H_{33} esters having nearly the same $ID_{50\ values}$. Similarly, Mullah and co-workers [27] produced a 5′-*O* methyl and 5′-*O* phenyl esters of 2′,3′-didehydro-2′,3′-dideoxyadenosine and 2′,3′-didehydro-2′,3′-dideoxycytosine which displayed similar in vitro results to the parent nucleosides. When incubated in serum containing medium, the phenyl prodrug produced parent nucleoside along with nucleoside monophosphate; the methyl prodrug was not evaluated in this manner. In an in vivo experiment, assessing activity against adenocarcinoma 755 in mice, Montgomery et al. [28] has evaluated the mono- and diethyl, butyl, and phenyl esters of 6-mercaptopurine ribonucleotide. The monoester prodrugs were of the same order of effectiveness as 6-mercaptopurine ribonucleotide, whilst the diesters were markedly less effective.

In general, the mono-alkyl/aryl ester analogs of phosphates failed to act as efficient prodrugs for the delivery of nucleoside-monophosphate analogs intracellularly. The poor activity of the monoalkyl/aryl esters can be attributed to:

1. High degree of polarity. Due to presence of the mono-anionic charge, limited passive transport across cells can be expected.
2. The relative ease of in vivo conversion of the monoalkyl esters in vivo back to the parent nucleoside before the cell barrier has been overcome. This could be a possible explanation for the monoalkyl prodrugs and parent nucleoside sharing similar activities.

McGuigan et al. [29] recently synthesized and evaluated, in vitro, a series of alkyl prodrugs of a hydrogen-phosphonate derivative of AZT (Fig. 3) in an attempt to increase its antiviral activity. They showed that the short chain (C1–C7) alkyl



AZT H-phosphonate analog (R = H)

Fig. 3. Structures of an AZT H-phosphonate analog and its prodrugs [29].

hydrogen phosphonates to be more active than the long-chain (C18) ester prodrugs in HIV-1-infected C8166 cells. The short chain phosphonate prodrugs were 5–10 times more potent than the parent phosphonate; however, all prodrugs were poorly active in an infected JM cell line, which is thought to lack the kinase mediated phosphorylation to produce the active nucleotide analog. This demonstrated lack of activity in the JM cell line suggested that these prodrugs were acting as depot forms of the nucleoside rather than the nucleotide.

To cause a further reduction in polarity, investigators have also synthesized diesters of phosphates/phosphonates and evaluated their effectiveness as prodrugs in in vitro and in in vivo tests. The results were not consistent with the observation with the monoesters. For example, Colin et al. [30] showed a clear relationship between inhibition of thymidine incorporation by mammalian epithelial cells and lipophilicity (increased alkyl chain length) of diester prodrugs of ara-CMP utilizing a similar series of protecting groups to those studied by Rosowsky et al. (Fig. 2, **6–9**). The in vitro activity was lowest for the ethyl ester and highest for the hexyl ester. Similar work performed on ara-AMP gave a correlation between an in vitro inhibition of DNA synthesis and lipophilicity; however, no anti-viral activity at concentrations up to 100 μg/ml was detected against a range of viruses [31,32]. AZTMP alkyl esters were also employed to improve membrane permeability [33,34]. In vitro, the triesters showed a complete lack of

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