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The ProTide Prodrug Technology: Where Next?

Ashwag S. Alanazi,[†] Edward James,[†] Youcef Mehellou^{†,*}

Cardiff School of Pharmacy and Pharmaceutical Sciences, Redwood Building, King Edward VII Avenue, Cardiff University, Cardiff CF10 3NB, U.K.

The ProTide prodrug technology has proved very useful in the discovery of nucleotide therapeutics and has successfully led to two FDA-approved drugs. However, with the extensive application of this prodrug approach to nucleotides for nearly three decades, the intellectual property (IP) landscape is becoming congested and, to overcome this, new inventive applications of the ProTide prodrug technology are emerging.

EARLY DAYS AND SUCCESS OF THE PROTIDES

Since the approval of the first synthetic nucleoside analogue, iodouridine in 1963, nucleoside analogues have since been a constant area of interest in drug development.¹ It was understood back then that these compounds require phosphorylation to the active triphosphate species. Later, the inefficient phosphorylation of some of these nucleoside analogues, particularly the first phosphorylation step, emerged as the rate limiting-step in their activation.¹ Hence, prodrugs of nucleoside analogue monophosphates were developed to bypass the first prerequisite phosphorylation step of therapeutic nucleoside analogues. Among the most widely used and successful monophosphate and monophosphonate prodrug approaches is the aryloxy triester phosphoramidate prodrug technology, commonly referred to as the ProTide technology, which was pioneered by Chris McGuigan (Cardiff University, UK).² In this approach, the monophosphate and monophosphonate groups are masked by an aryl motif and an amino acid ester (**Figure 1A**).² These masking groups are cleaved off inside cells by esterases and carboxypeptidase-type enzymes, *e.g.* hint-1, to release the unmasked monophosphate or monophosphonate species (**Figure 1B**).²

To date, the ProTide prodrug technology has been extensively exploited by the pharmaceutical industry and medicinal chemistry academic researchers in the discovery of nucleoside analogue monophosphate and monophosphonate therapeutics.² Such interest is reflected in the increasing number of research publications and granted patents on the ProTide technology since its inception in the early 1990s (**Figure 1C**).² This has so far translated into two FDA-approved antiviral ProTides, sofosbuvir and tenofovir alafenamide (**Figure 1D**), and many more ProTide clinical candidates, *e.g.* GS-5734, Acelarin and NUC-3373 (**Figure 1E**).²

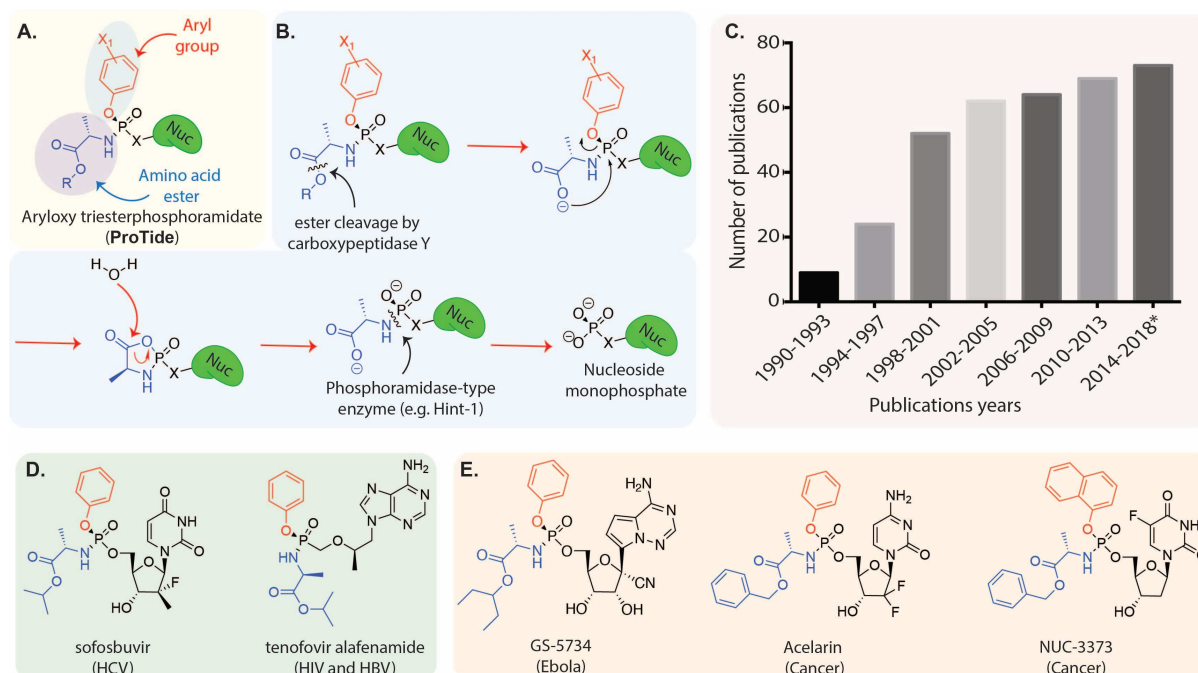


Figure 1. A. The general chemical structure of the aryloxy triester phosphoramidate (ProTide) prodrug technology. The key masking groups, the aryl motif and the amino acid ester, are labelled and highlighted. X= O (phosphate) or CH₂ (phosphonate). X1 = any aromatic substitution. R = any group. **B.** The postulated mechanism of the ProTides' metabolism. **C.** The number of ProTide publications from 1990 to 2018 at the indicated time periods. This was gathered by searching PubMed using the terms “phosphoramidate and nucleoside” for the time periods shown. *Indicates November 25th, 2018 when the search was last performed. **D.** Chemical structures of the two FDA-approved drugs, sofosbuvir and tenofovir alafenamide along with diseases they are being used to treat. **E.** Examples of ProTide clinical candidates along with diseases they are being pursued to treat.

A SHRINKING IP LANDSCAPE?

Notably, almost all of the applications of the ProTide technology in drug discovery to date have been on nucleoside monophosphates and monophosphonates and this, as mentioned above, has led to a series of nucleotide ProTide clinical candidates. Thus, it is of no surprise to see that a large number of biotech and pharmaceutical companies, such as GSK, Roche, Gilead, Novartis and Merck,³ have patents that cover the application of this prodrug technology to the discovery of nucleotide therapeutics. However, this has come at the expense of a shrinking IP landscape and an overexploited “inventive step” for the application of the ProTide technology to nucleotide therapeutics. As a result, it is becoming more difficult to have patents on the application of the ProTide technology to nucleoside monophosphates and monophosphonates granted successfully. Therefore, new avenues for applying this ever useful prodrug technology to non-traditional molecules, *e.g.* non-nucleotides, with more freedom to operate have emerged. The move towards non-nucleotide applications of the ProTide technology may have also been influenced by the various ongoing legal battles between pharmaceutical companies over proprietary issues concerning certain nucleoside monophosphate and monophosphonate ProTides, *e.g.* Gilead Sciences vs. Merck & Co.⁴

SO, WHAT NEXT?

Throughout the period in which the ProTide technology has been applied to the discovery of nucleotide therapeutics, what has been consistent is how effective this technology is in delivering

monophosphorylated compounds into cells and the superior drug-like properties they have when compared to the parent nucleoside monophosphates. Despite the ProTide technology's burgeoning potential, the previously highlighted issues surrounding intellectual property and the "inventive step" are limiting further applications of this technology to nucleosides.

Thus, it seems that the future of this prodrug technology lies in the non-nucleoside monophosphate and monophosphonate arena where there may be more freedom to operate. The medicinal chemistry field, with interest in the ProTide technology, has been trying to break away from nucleotide ProTides. Indeed, there have been an increasing number of applications of the ProTide technology to non-nucleoside drug molecules. As shown in **Figure 2**, this prodrug technology has been used with success on many non-nucleotide substrates such as glucosamine monophosphate (**1-3**), small molecule phosphoantigens (**4-6**), phosphopantothenate (**7**), sphingosine-1 phosphate (S1P) receptor modulators (**8**), 6-phosphogluconate dehydrogenase (6-PGDH) inhibitors (**9**) and phosphotyrosine-containing molecules (**10**).

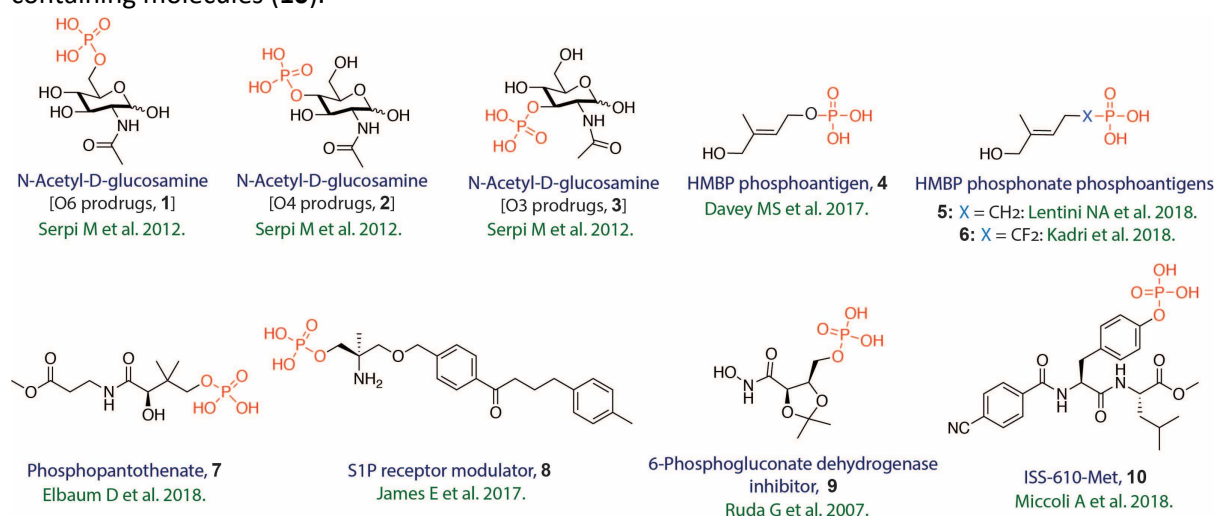


Figure 2. Examples of non-nucleoside monophosphate and monophosphonate-containing molecules to which the ProTide technology has been applied as a means of improving their drug-like properties.

The nutritional supplement *D*-glucosamine is one of the most commonly used agents to treat osteoarthritis. As this compound is phosphorylated *in vivo*, the aryloxy phosphoramidate prodrugs of *N*-acetyl-*D*-glucosamine and its regioisomers (**1-3**, **Figure 2**) were reported by the McGuigan lab⁵ with the aim of improving their oral bioavailability. The results showed that several *N*-acetyl-*D*-glucosamine aryloxy triester phosphoramidate prodrugs were able to significantly reduce the loss of glycosaminoglycan at a noncytotoxic concentration compared to the original compound *D*-glucosamine.⁵

The application of the aryloxy triester phosphoramidate technology to the phosphoantigen (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBP, **4**, **Figure 2**) was reported by Davey MS *et al.*⁶ Although these prodrugs, termed ProPAgens, exhibited potent activation of Vγ9/Vδ2 T-cells, which subsequently led to the lysis of cancer cells, their stability was low.⁶ To address this stability issue, the synthesis of HMBP phosphonates (**5** and **6**, **Figure 2**) and their aryloxy triester phosphoramidate prodrugs was reported by Kadri *et al.*⁷ and Lentini *et al.*⁸ Both studies showed that HMBP phosphonate prodrugs exhibited superior stability and subnanomolar activation of Vγ9/Vδ2 T-cells, which led to efficient eradication of cancer cells.

As means of treating pantothenate kinase-associated neurodegeneration (PKAN), the aryloxy triester phosphoramidate prodrug of phosphopantothenate (PPA, **7**, **Figure 2**), known as Fosmetpantotenate,

was synthesised.⁹ The application of the aryloxy triester phosphoramidate prodrug approach to PPA enhanced both the cell and blood-brain barrier permeability as the parent compound is not membrane permeable due to its anionic character. Clinical trials are currently underway for the use of Fosmetpantotenate as a PPA replacement therapy to treat PKAN disease.⁹

The FDA-approved drug fingolimod, which is used to treat relapsing-remitting multiple sclerosis, is phosphorylated *in vivo* by sphingosine kinase to the pharmacologically active monophosphorylated derivative. In order to improve the efficacy of fingolimod benzyl ether derivatives and bypass the essential phosphorylation of these compounds, James E *et al*¹⁰ synthesised a series of their aryloxy triester phosphoramidate prodrugs. Whilst the biological activity of these prodrugs is yet to be reported, *in vitro* studies indicated that these prodrugs are metabolised to release the monophosphate species **8** (Figure 2).

In the search of new antimicrobials, Ruda *et al*.¹¹ applied the aryloxy triester phosphoramidate prodrug technology to a 6-PGDH inhibitor, **9**. This compound is a potent and selective inhibitor of *T. brucei* parasites, and hence could be an effective treatment for the human African *trypanosomiasis*. However, the poor uptake of this compound into the parasites was thought to limit their activity *in vitro*. Therefore, the aryloxy triester phosphoramidate prodrug technology was applied to this inhibitor and these prodrugs led to a 48-fold increase in efficacy.¹¹

Recently, Miccoli *et al*¹² reported the application of the aryloxy triester phosphoramidate prodrug technology to a phosphotyrosine-containing small molecule, ISS-610-Met (**10**). This compound and its analogue ISS-610 are peptidomimetics that contain a phosphotyrosine motif and inhibit the dimerization of the signal transducer and activator of transcription 3 (STAT3). However, these compounds are weak inhibitors of STAT3 dimerization because of their poor cellular uptake and possible dephosphorylation of the phosphotyrosine moiety *in vivo*. The aryloxy triester phosphoramidate prodrugs of ISS-610-Met exhibited a significant reduction in the expression of anti-apoptotic proteins, whose expression is regulated by STAT3, as compared to ISS-610 and ISS-610-Met. Collectively, these examples further showcase the ability of the aryloxy triester phosphoramidate prodrug technology to enhance the drug-like properties of non-nucleotide monophosphate and monophosphonate compounds by facilitating their cellular uptake and improving their stability. Furthermore, these examples hint at a huge untapped potential of the aryloxy triester phosphoramidate prodrug technology, beyond the intracellular delivery of nucleotides, and may offer more attractive IP opportunities for future drug discovery.

AUTHOR INFORMATION

Corresponding Author

*Tel: +44 (0) 2920875821; E-mail: MehellouY1@cardiff.ac.uk.

Notes

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