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New prodrugs of two pyrimidine acyclic nucleoside phosphonates: Synthesis and antiviral activity

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ABSTRACT

New 2,4-diamino-6-[2-(phosphonomethoxy)ethoxy]pyrimidine (PMEO-DAPy) and 1-[2-(phosphonomethoxy)ethyl]-5-azacytosine (PME-5-azaC) prodrugs were prepared with a pro-moiety consisting of carbonyloxymethyl esters (POM, POC), alkoxyalkyl esters, amino acid phosphoramidates and/or tyrosine. The activity of the prodrugs was evaluated *in vitro* against different virus families. None of the synthesized prodrugs demonstrated activity against RNA viruses but some of them proved active against herpesviruses [including herpes simplex virus (HSV), varicella-zoster virus (VZV), and human cytomegalovirus (HCMV)]. The bis(POC) and the bis(amino acid) phosphoramidate prodrugs of PMEO-DAPy inhibited herpesvirus replication at lower doses than the parent compound although the selectivity against HSV and VZV was only slightly improved compared to PMEO-DAPy. The mono-octadecyl ester of PME-5-azaC emerged as the most potent and selective PME-5-azaC prodrug against HSV, VZV and HCMV with EC_{50}'s of 0.15–1.12 μM while PME-5-azaC only had marginal anti-herpesvirus activity. Although the bis (hexadecylamido-L-tyrosyl) and the bis(POM) esters of PME-5-azaC were also very potent anti-herpesvirus drugs, these were less selective than the mono-octadecyl ester prodrug.

1. Introduction

Acyclic nucleoside phosphonates (ANPs) are compounds of great importance due to the broad spectrum of biological activities, especially antiviral but also cytostatic, immunomodulatory and antiparasitic.[1–4] Some of them have become already clinically available drugs: cidofovir for the treatment of human cytomegalovirus (CMV) retinitis in AIDS patients, adefovir in a prodrug form as adefovir dipivoxil for the treatment of hepatitis B (HBV) and tenofovir, either as tenofovir disoproxil fumarate, or newly (since 2015) also as a new prodrug form tenofovir alafenamide (TAF) for the treatment of HIV and HBV infections. On the other hand, it should be noted that over the last thirty years of systematic investigation of ANPs, there are dozens of other therapeutically attractive structures synthesized but never advanced to the stage of preclinical/clinical investigations.[5] These structures are namely a) antiretroviral purine 3-fluoro-2-[(phosphonomethoxy)propyl] derivatives,[6] b) acyclic nucleoside phosphonates with 5-azacytosine base moiety,[7] c) 6-[2-(phosphonomethoxy)alkoxy]-2,4-diaminopyrimidines ("open-ring" derivatives)[8–10] and d) aza/deaza analogues of purine [(phosphonomethoxy)ethyl] derivatives.[11]

The common structural attribute of all ANPs is their highly polar character caused by the presence of the phosphonic acid residue which is responsible for their unfavourable pharmacological properties: low cell permeability and low oral bioavailability. To overcome this problem, transformation of free acyclic nucleoside phosphonates to appropriate prodrugs is often a solution.

In our work, we focus on two pharmacologically interesting ANP structures: 2,4-diamino-6-[2-(phosphonomethoxy)ethoxy] pyrimidine – an example of the so-called "open-ring" ANPs and on the group of 5-azacytosine derivatives, namely 1-[2-(phosphonomethoxy)ethyl]-5-azacytosine, and their antiviral potential in diverse prodrug forms.

Open-ring ANPs are characterized by the phosphonomethoxy group containing an aliphatic part linked to the position 6 of 2,4-diaminopyrimidine *via* the oxygen atom. They are evidently mimics of the appropriate 2,6-diaminopurine derivatives with an open imidazole ring. Their antiviral activity is essentially identical to that of their parent compounds, including the enantiomeric specificity. Compounds as PMEO-DAPy, (*R*)-PMPO-DAPy and 5-substituted PMEO-DAPy (Fig. 1) are very efficient inhibitors of

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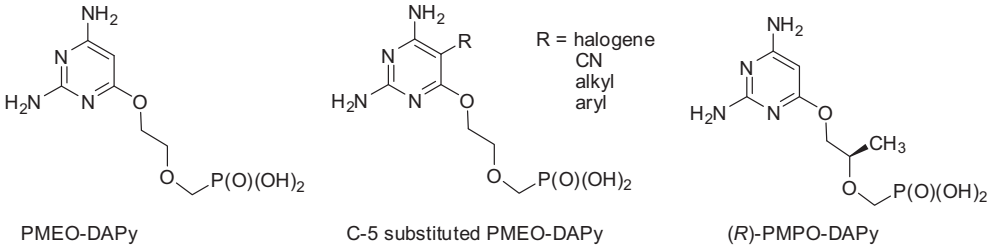

Fig. 1. Structures of "open-ring" acyclic nucleoside phosphonates.

retroviruses[8–10] and HBV. Despite the number of antiretroviral drugs currently available on the market, further investigation of new structures is advisable for several reasons: 1) the risk of emergence of resistance, 2) none of the known drugs is able to eradicate HIV infection completely, 3) no vaccination are yet available, 4) early development of new drug candidates is necessary due to the longtime process lasting from *in vitro* laboratory testing to clinical phases and final approval. Moreover, there is an additional reason to focus on open-ring analogs of the PMEO-DAPy type: they are incorporated more efficiently than (*R*)-PMPA (tenofovir) by the K65R HIV-1 reverse transcriptase (RT) mutant and they are not as efficiently excised as (*R*)-PMPA by the HIV-1 RT containing thymidine analog mutations.[12] Additionally, PMEO-DAPy is active not only against retroviruses but also against DNA viruses, especially herpesviruses, which often affect immunocompromised patients, including those with HIV/AIDS.

The development of 5-azacytosine ANPs was initiated in our laboratory in order to search for new demethylating (epigenetic) drugs similar to 5-azacytosine nucleosides.[13] Although none of the new compounds fulfilled this criterion, we managed to find a new class of antiviral agents, 5-azacytosine analogue of cidofovir 1-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine (HPMP-5-azaC) and various ester prodrugs derived from its cyclic form (Fig. 2).[7,14] Compared to cidofovir, HPMP-5-azaC has improved selectivity. The prodrug hexadecyloxyethyl ester of its cyclic form (HDE-cHPMP-5-azaC) revealed the most potent anti-DNA virus activities and also the highest selectivity indices (ratio activity *vs* toxicity) in the order of thousands, e.g. 1160 for herpes simplex virus (HSV) $\geq$ 5800 for varicella zoster virus (VZV) and $\geq$24,600 for HCMV.[14] The only disadvantage of HPMP-5-azaC is its complicated metabolic profile bound to instability of the 5-azacytosine ring in alkaline conditions including physiological pH. Studying the stability of various 5-azacytosine ANPs, we found much better stability for another 5-azacytosine derivative, i.e. 1-[2-(phosphonomethoxy)ethyl]-5-azacytosine (PME-5-azaC).[15] Despite the fact that its antiviral activity was only marginal in the free phosphonic acid form [7], we selected the compound for syntheses and further studies of its prodrug forms. We considered the fact that the activities of many ANPs were increased after transformation to appropriate prodrugs. Moreover, in some cases not only the activity was enhanced but also the spectrum of activity could be broaden by transformation to prodrugs. Typical examples are the anti-DNA viral agents 1-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine (HPMPA) whose octadecyloxyethyl (ODE) ester is a potent and selective inhibitor of hepatitis C virus replication[16] or cidofovir transformed to its hexadecyloxyethyl ester (brincidofovir) whose efficacy is also enlarged to some RNA viral infections including Ebola virus. In fact, the lipid moiety of brincidofovir was found to be required for *in vitro* antiviral activity against Ebola virus.[17]

2. Chemistry

The starting compound 2,4-diamino-6-[2-(phosphonomethoxy) ethoxy]pyrimidine (**1**) was synthesized according to a procedure described in the literature.[8] Briefly: base-catalysed alkylation of 2,4-diamino-6-hydroxypyrimidine with diisopropyl 2-(chloroethoxy)methylphosphonate gave a mixture of appropriate *O*- and *N*-diisopropyl (phosphonoethoxy)methyl derivatives where the *N*-isomer was separated and diisopropyl ester groups deprotected with bromotrimethylsilane. The synthesis of the prodrugs was rather complicated by a low solubility of the starting PMEO-DAPy; finally we managed to synthesize two biodegradable ester prodrugs – pivaloyloxymethyl (POM) and (isopropoxycarbonyl) oxymethyl (POC) esters and the amino acid phosphoramidate prodrug (Scheme 1). Pivaloyloxymethylation was performed by reaction of the starting phosphonic acid with chloromethyl pivalate using *N,N′*-dicyclohexyl-4-morpholinecarboxamidine as a base. The reaction proceeded very slowly to give a mixture of bis (POM) and mono(POM) esters **2** and **3**. After chromatographic separation, both compounds were isolated in acceptable yields. Alternative reaction conditions (e.g. reaction in dioxane with DBU) completely failed. Also for the introduction of POC groups, different attempts have been examined (various solvents, DBU or diisopropylethylamine as bases). The only way that led to the bis (POC) derivative **4** consisted in transformation of free PMEO-DAPy to its tetrabutylammonium salt, followed by heating with POC-chloride in dioxane. The bis(amino acid) phosphoramidate prodrug **5** was prepared by the direct coupling of PMEO-DAPy with ethyl L-alaninate in pyridine and treatment with a premixed solution of triphenylphosphine and 2,2′-dipyridyl disulfide (Aldrithiol).

Synthetic efforts to obtain 1-[2-(phosphonomethoxy)ethyl]-5-azacytosine prodrugs were targeted to carbonyloxymethyl esters (POM, POC), alkoxyalkyl esters, amino acid phosphoramidates

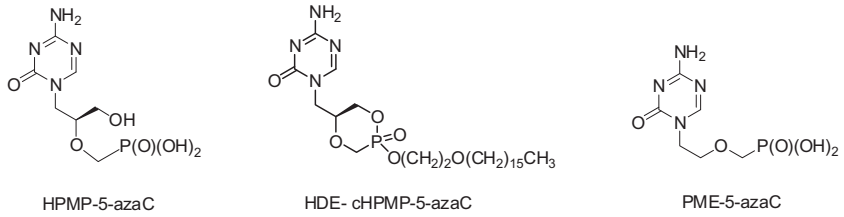

Fig. 2. Examples of acyclic nucleoside phosphonates with 5-azacytosine base.