Amide Prodrugs of Cyclic 9-(S)-[3-Hydroxy-2-(phosphonomethoxy)propyl]adenine with Potent Anti-Herpesvirus Activity

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Supporting Information

ABSTRACT: A series of amide prodrugs of cyclic 9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine (cHPMPA) featuring different amino acid motifs were synthesized. All phosphonamides derived from (S)-cHPMPA displayed a broad spectrum activity against herpesviruses with EC₅₀ values in the low nanomolar range. A phosphonobisamide prodrug of (S)-HPMPA also exhibited a remarkably potent antiviral activity. In addition, the leucine ester prodrug of (S)-cHPMPA and phosphonobisamide valine ester prodrug of (S)-HPMPA proved stable in human plasma. These data warrant further development of cHPMPA prodrugs, especially against human cytomegalovirus (HCMV), for which there is a high need for treatment in transplant recipients.

KEYWORDS: acyclic nucleoside phosphonates, phosphonamide prodrugs, DNA viruses, antiviral activity, metabolic stability

Virus	EC ₅₀ (nM)
HSV	0.73-20
VZV	0.45-4
HCMV	3.8-65

Human herpesviruses are double-stranded DNA viruses belonging to the Herpesviridae family, which includes nine human viruses causing a wide range of diseases. In particular, the Alphaherpesvirinae subfamily comprises herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) and varicella-zoster virus (VZV), while human cytomegalovirus (HCMV) and human herpes viruses 6A, 6B, and 7 (HHV-6A, HHV-6B, and HHV-7) are classified as Betaherpesvirinae. Epstein–Barr virus and Kaposi’s sarcoma-associated herpesvirus, considered as oncogenic viruses, belong to the Gammaherpesvirinae.^{1,2}

All drugs currently marketed for the treatment of herpesvirus infections, such as acyclovir, ganciclovir, penciclovir, and brivudine, target viral DNA polymerases.^{3–5} For several of these compounds, some prodrugs also received marketing approval (valacyclovir, valganciclovir, and famciclovir). The antiviral activity of these “classical” nucleosides depends upon their intracellular metabolism within virus-infected cells to be sequentially converted to the mono-, di-, and triphosphates. The latest are the pharmacologically active species, as they can be incorporated into a growing viral DNA strand by viral DNA polymerases, resulting in chain termination or fraudulent DNA. Cidofovir [(S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)-cytosine, (S)-HPMPC] is an acyclic nucleoside phosphonate analogue (Figure 1), which is essentially a monophosphate mimic, officially approved as injectable medication for the treatment of cytomegalovirus (CMV) retinitis in AIDS patients.⁶ Its adenine counterpart, namely (S)-HPMPA (Figure 1), was also described to exhibit a similarly potent inhibitory

Figure 1. Structures of (S)-HPMP and (S)-cHPMP nucleoside analogues active against DNA viruses.

activity against a broad spectrum of DNA viruses⁷ while showing greater toxicity in vitro.^{8,9} Owing to the presence of the phosphonate moiety, such nucleotide analogues are metabolically stable and do not depend on viral enzymes for activation but undergo only two phosphorylation steps by cellular kinases.

To reduce the recognized toxic side effects associated with the use of HPMP-type nucleosides, their corresponding cyclic forms (S)-cHPMPC and (S)-cHPMPA (Figure 1) were synthesized and shown to retain the remarkable antiviral potency of the parent compounds while allowing for improved selectivity indexes.^{10,11}

However, all of these HPMP derivatives invariably suffer from a low oral bioavailability due to the presence of negatively

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charged group(s) at physiological pH that restrict their ability to penetrate the lipid-rich cell membrane. Therefore, the development of orally bioavailable, less toxic prodrug forms of cHPMPs is highly desirable. Up to now, no prodrugs of (S)-HPMPA have been approved for clinical use, while the derivatization of cHPMPA has purely focused on the synthesis of phosphoester prodrugs.^{12,13}

One of the most promising prodrug strategies that has been developed over the past few years for nucleoside phosphates and phosphonates is the use of aryloxy monoamides.¹⁴ This approach has not been applied to HPMP-based compounds, owing to the potential chemical instability of the resulting phenoxyphosphonamide with concomitant formation of a cyclic phosphonate analogue. Thus, we decided to exploit this reactivity in order to synthesize a series of phosphono amide prodrugs of cHPMPA bearing different amino acid motifs.

Given the well-established beneficial effect of a L-aspartic acid amyl ester moiety instead of the classical L-alanine ester on the antiviral activity of different phosphonamide prodrugs,^{15–17} our study started with the synthesis of the L-aspartate prodrugs of (R)- and (S)-cHPMPA. Both enantiomers of MMT-protected HPMPA **6a/b** could be readily obtained from commercially available (R)-(+)- and (S)-(-)-glycidol **2a/b**, as illustrated in Scheme 1. Interestingly, when **2a/b** were subjected to a tritylation reaction in the presence of pyridine as base, ring-opened compounds **3a/b** were formed as the sole products instead of the anticipated MMT-glycidols.¹⁸ The following alkylation of 3-chloro-1-O-monomethoxytrityl-1,2-propanediols **3a/b** with adenine in the presence of 0.9 equiv of

sodium hydride afforded moderate yields (40–50%) of compounds **4a/b** in a regioselective and stereospecific manner. Subsequently, the phosphonate group was introduced using diethyl tosyloxymethylphosphonate and NaH, furnishing **5a/b** in 35–40% yield. Standard hydrolysis of the diester group with TMSBr in dry acetonitrile was carried out overnight to form **6a/b** in good yield. The free phosphonic acid moiety of **6a/b** was then converted into the corresponding key intermediate aryloxyphosphonamides **7a/b** by treatment with L-aspartic acid diamyl ester (Asp-diamyl)¹⁷ and phenol using 2,2'-dithiodipyridine and triphenylphosphine as activating agents.¹⁹ Under acid conditions, the phenoxy group served as a good leaving group, efficiently generating cyclic (S)/(R)-phosphonamides **8a/b**.

Additionally, guided by the preliminary data obtained for **8a/b** (Table 1) and in order to determine the influence of the amino acid side chain on the antiviral activity, a restricted library of (S)-cHPMPA phosphonoamide prodrugs **10–16** was prepared by introducing a range of structural variations. To this aim, phosphonoacid **6a** was coupled with a variety of L-amino acid (phenylalanine, methionine, alanine, glutamic acid, valine, leucine, and isoleucine) esters yielding the desired compounds in moderate yields over two steps (Scheme 2).

Phosphonobisamide **17** was also prepared using a slightly modified protocol from the parent nucleoside phosphonate **6a** and featured L-valine as amino acid motifs (Scheme 3).

Compounds **8a/b** and **10–16** were evaluated for their antiviral activity against HSV-1, HSV-2, VZV [strains (TK⁺) Oka and thymidine kinase deficient (TK⁻) 07-1], and HCMV (strains AD-169 and Davis) in human embryonic lung (HEL) cells (Table 1). In parallel, the potential toxic effects were assessed on the same cell line. The (S)-enantiomer of cHPMPA diamyl aspartate phosphonoamide prodrug **8a** showed good activity against HSV, VZV, and HCMV with EC₅₀ values in the 0.0009–0.0032, 0.0014–0.0015, and 0.009–0.027 μ M ranges, respectively. On the other hand, the (R)-counterpart **8b** displayed 24- to 180-fold decreased potency. Notably, all other tested analogues **10–16** displayed very potent anti-HSV, VZV, and HCMV activity. In particular, (S)-Phe-cHPMPA (**10**), (S)-Glu-cHPMPA (**13**), (S)-Val-cHPMPA (**14**), (S)-Leu-cHPMPA (**15**), and (S)-Ile-cHPMPA (**16**) emerged as the most potent compounds against HSV with EC₅₀ values consistently in the 0.0012–0.0042 μ M range. Good selectivity indexes (SI: CC₅₀/EC₅₀ > 4400) were obtained, in particular, for (S)-Phe-cHPMPA (**10**) and (S)-Ile-cHPMPA (**16**). Compounds **10–16** proved also very potent against all VZV and HCMV strains with EC₅₀ values in the 0.00045–0.0043 and 0.0038–0.064 μ M range, respectively. Among these compounds, (S)-Ile-cHPMPA (**16**) emerged as the most selective one with SI's of 20000 and 1800 in the anti-VZV and anti-HCMV assays, respectively.

When compared to cidofovir, all compounds displayed a dramatically improved antiviral activity. This can be explained by an improved cellular permeability, due to an increased lipophilicity, as evidenced by the clogP values. Importantly, HPMPA prodrugs proved more potent than the reference drugs, i.e., acyclovir (HSV and VZV) and ganciclovir (HCMV). Furthermore, the newly synthesized compounds were equally active against TK⁺ and TK⁻ HSV and VZV strains.

The main drawback of these amide prodrugs of (S)-cHPMPA is that diastereomeric mixtures are formed due to the chirality of the phosphorus atom. To avoid the tedious separation of both diastereomers, the symmetrical phosphonodiamide prodrug **17** was also evaluated. This compound was

Scheme 1. Synthesis of (S)- and (R)-cHPMPA L-Asp-diamylphosphonamides **8a and **8b**^a**

^aReagents and conditions: (a) MMTCl, Pyr, 50 °C, 20 h, 80%; (b) NaH, DMF, 110 °C, 6 h, 40–50%; (c) diethyl tosyloxymethylphosphonate, NaH, THF, 0 °C to rt, 5 h, 35–40%; (d) TMSBr, 2,6-lutidine, CH₃CN, 0 °C to rt, 12 h, 70%; (f) (i) L-aspartic acid amyl ester HCl salt, PhOH, 2,2'-dithiodipyridine, PPh₃, Et₃N, Pyr, 60 °C, 12 h; (g) TCA (6% in DCM), rt, 2 h, 37–42% over 2 steps.

Table 1. Antiviral Activity and Cytotoxicity of Compounds 8a and 8b and 10–17 against HSV, VZV, and HCMV in HEL Cells

Cmpd	HSV-1			HSV-2		antiviral activity EC ₅₀ ^a (μM)			HCMV		cytotoxicity (μM)		logP ^d
	KOS strain	KOS ACV ^c strain	G strain	VZV			AD-169 strain	Davis strain	MCC ^b		HEL		
				TK ⁺ strain (OKA)	TK ⁺ strain (07-1)	HEL			CC ₅₀ ^c				
8a	0.0032 ± 0.0043	0.0073 ± 0.00043	0.0020 ± 0.0015	0.0014 ± 0.0011	0.0015 ± 0.00080	0.027 ± 0.027	0.0090 ± 0.00051	13.6 ± 8.54	2.73 ± 0.50	2.22			
8b	0.21 ± 0.14	0.076 ± 0.063	0.11 ± 0.068	0.033 ± 0.020	0.062 ± 0.042	4.39 ± 3.61	1.62 ± 1.21	>18.5	11.6 ± 3.86	2.22			
10	0.0035 ± 0.0027	0.0015 ± 0.00037	0.0023 ± 0.00044	0.00052 ± 0.00074	0.0032 ± 0.0026	0.031 ± 0.023	0.017 ± 0.0061	9.29 ± 9.19	30.6 ± 24.4	3.34			
11	0.0089 ± 0.0075	0.0057 ± 0.0049	0.011 ± 0.0050	0.0026 ± 0.0024	0.0040 ± 0.0021	0.032 ± 0.014	0.029 ± 0.011	4.11 ± 8.54	39.6 ± 25.8	2.07			
12	0.020 ± 0.015	0.010 ± 0.010	0.017 ± 0.011	0.0020 ± 0.0015	0.0043 ± 0.00067	0.064 ± 0.015	0.065 ± 0.017	23.5	3.40 ± 0.40	1.93			
13	0.0012 ± 0.00015	0.0012 ± 0.00084	0.0025 ± 0.0029	0.00058 ± 0.00067	0.00095 ± 0.00029	0.014 ± 0.012	0.0076 ± 0.00072	13.2 ± 8.33	0.52 ± 0.051	3.53			
14	0.0030 ± 0.0023	0.0018 ± 0.0015	0.0042 ± 0.0025	0.00057 ± 0.00035	0.0015 ± 0.00029	0.0059 ± 0.0014	0.0048 ± 0.00033	4.4	7.76 ± 4.03	2.85			
15	0.0013 ± 0.00079	0.0013 ± 0.00072	0.0019 ± 0.0010	0.00047 ± 0.00035	0.00059 ± 0.0011	0.0057 ± 0.0030	0.0038 ± 0.00025	4.27	1.62 ± 0.91	2.38			
16	0.0020 ± 0.0012	0.0019 ± 0.0019	0.0027 ± 0.0012	0.00045 ± 0.00045	0.00054 ± 0.00026	0.0070 ± 0.0028	0.0065 ± 0.0042	9.96 ± 9.86	12.0 ± 0.41	3.38			
17	0.0023 ± 0.0014	0.00090 ± 0.00084	0.0028 ± 0.0029	0.0015 ± 0.00093	0.00095 ± 0.00037	0.10 ± 0.097	0.072 ± 0.063	8.57 ± 9.92	0.60 ± 0.28	4.11			
acyclovir	0.47 ± 0.21	>88.8	0.34 ± 0.090	2.84	54.8 ± 17.08	ND ^e	ND ^e	>88.8	>444				
peniclovir	0.48 ± 0.41	>79.0	0.84 ± 0.51	ND ^e	ND ^e	ND ^e	ND ^e	>79.0	>395				
brivudine	0.043 ± 0.027	>30.0	>30.0	0.053 ± 0.052	22.1 ± 25.5	ND ^e	ND ^e	>30.0	>300				
ancidovir	0.057 ± 0.053	>29.5	0.051 ± 0.048	ND ^e	ND ^e	7.41 ± 4.94	3.31 ± 1.01	>39.2	>319				
cidofovir	4.18 ± 3.22	1.95 ± 0.82	2.73 ± 1.10	ND ^e	ND ^e	1.60 ± 0.87	0.84 ± 0.21	>71.6	>358	-2.39			

^aEffective concentration required to reduce virus-induced cytopathicity (HSV and HCMV) or plaque formation (VZV) by 50%. ^bMinimum concentration required to cause a microscopically detectable alteration of cell morphology. ^cCytotoxic concentration required to reduce cell viability by 50%. ^dLogP values were calculated using ChemBioDraw Ultra version 14.0 from CambridgeSoft. ^eNot determined.

Scheme 2. Synthesis of (S)-cHPMPA Phosphoramidates 10–16^a

^aReagents and conditions: (a) (i) L-amino acid (di)amyl ester HCl salts **9a–g** (see the [Supporting Information](#)), PhOH, 2,2'-dithiodipyridine, PPh₃, Et₃N, Pyr, 60 °C, 12 h; (ii) TCA (6% in DCM), rt, 2 h, 28–35% over two steps.

Scheme 3. Synthesis of (S)-cHPMPA Phosphonobisamide 17^a

^aReagents and conditions: (a) (i) L-valine amyl ester HCl salt **9e**, 2,2'-dithiodipyridine, PPh₃, Et₃N, Pyr, 60 °C, 12 h; (ii) TCA (6% in DCM), rt, 2 h, 32% over two steps.

endowed with potent anti-HSV and VZV activity, displaying EC₅₀ values in the range of 0.0009–0.0015 μM, along with decreased CC₅₀ values. With regard to the anti-HCMV activity, compound **17** was less potent than the (S)-cHPMPA prodrugs, with EC₅₀ values in the range of 0.072–0.10 μM.

The amide prodrugs of (S)-cHPMPA showed promising in vitro antiviral activity against HSV, VZV, and HCMV. However, to achieve antiviral efficacy in vivo, such prodrugs must be stable during the absorption and distribution processes, since their partial or full hydrolysis before reaching the target organs may reduce their antiviral effect. To exert such effect, the phosphonoamidate prodrugs must penetrate the target cells efficiently, and undergo intracellular metabolism, yielding HPMPA, which is further phosphorylated to the pharmacologically active diphosphophosphonate analogue. The intracellular activation pathway of ProTides has been extensively studied.^{20–23} McGuigan et al. proposed an activation route for phosphorodiamidates.²⁴ It has been reported that cHPMPC is intracellularly converted to cidofovir by a cyclic CMP phosphodiesterase.²⁵

The in vitro stability of three representative prodrugs (**8a**, **15**, and **17**) in human plasma and human liver microsomes was investigated (Table 2). (S)-Leu-cHPMPA (**15**) was found to be

Table 2. Metabolic Stability of Prodrugs **8a**, **15**, and **17** in Human Plasma and Human Liver Microsomes

compd	human plasma $t_{1/2}$ ^a (min)	human liver microsomes $t_{1/2}$ ^a (min)
8a	22	4
15	84	16
17	>120	4

^aResults are the mean of two independent experiments.

stable in human plasma with a $t_{1/2}$ value of almost 1.5 h, whereas the half-life of prodrug **8a** (bearing an aspartic acid as amino acid motif) was only 22 min. The phosphonobisamide prodrug of HPMPA (**17**) displayed high stability in human plasma ($t_{1/2}$ > 2 h). On the other hand, very short half-lives of prodrugs **8a** and **17** ($t_{1/2}$ = 4 min) were observed in human liver microsomes, suggesting that they are quickly metabolized in liver cells. The microsomal stability of prodrug **15** was slightly better with a $t_{1/2}$ value of 16 min, indicating that the structural variation of the amino acid moiety offers the possibility to improve the microsomal stability.

In summary, the synthesis of amide prodrugs of cHPMPA was performed using an aryloxyphosphoramidate as key intermediate. In addition, one phosphonobisamide prodrug of (S)-HPMPA was synthesized. All prodrugs based on (S)-HPMPA exhibited potent antiviral activity against HSV, VZV, and HCMV with EC₅₀ values in the low nanomolar range, whereas a prodrug derived from (R)-HPMPA resulted much less active. Three representative prodrugs were tested for metabolic stability in the presence of human plasma and human liver microsomes. (S)-Leu-cHPMPA **15** and the phosphonobisamide prodrug of HPMPA **17** were found to be stable in human plasma with $t_{1/2}$ values exceeding 1 h. On the other hand, all prodrugs underwent fast metabolism in the presence of human liver microsomes. Further chemistry could focus on structural variations of the amino acid side chain and ester moieties in order to further optimize the prodrug structure in function of antiviral activity and metabolic stability in plasma and microsomes.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the [ACS Publications website](#) at DOI: [10.1021/acsmedchemlett.8b00079](https://doi.org/10.1021/acsmedchemlett.8b00079).

Experimental details and characterization data for the reported compounds; NMR spectra; and biological assays ([PDF](#))

Reaction scheme for compounds **9a–g** ([PDF](#))

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

HSV, herpes simplex virus; VZV, varicella zoster virus; HCMV, human cytomegalovirus; HHV, human herpesvirus; cHPMPC, cyclic cidofovir; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil; HPMPA, 1-(3-hydroxy-2-phosphonylmethoxypropyl)adenine; HPMP, 1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine; TMSBr, bromotrimethylsilane; NaH, sodium hydride; HEL, human embryonic lung; TK, thymidine kinase; Phe, phenylalanine; Glu, glutamic acid; Val, valine; Leu, leucine; Ile, isoleucine

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