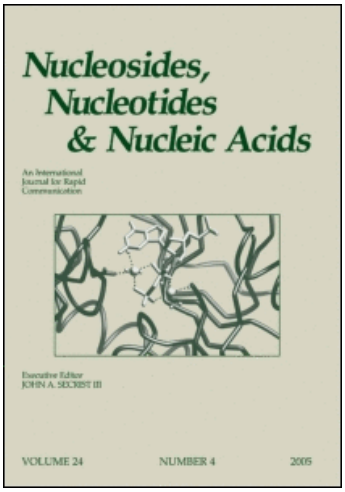

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

6-[2-Phosphonomethoxy)Alkoxy]-2,4-Diaminopyrimidines: A New Class of Acyclic Pyrimidine Nucleoside Phosphonates with Antiviral Activity

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Online publication date: 27 October 2004

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, Nos. 8 & 9, pp. 1321–1327, 2004

6-[2-Phosphonomethoxy)Alkoxy]-2,4-Diaminopyrimidines: A New Class of Acyclic Pyrimidine Nucleoside Phosphonates with Antiviral Activity

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ABSTRACT

Acyclic nucleoside phosphonate derivatives containing a pyrimidine base preferably bearing amino groups at C-2 and C-4 (DAPym), and linked at the C-6 position to (*S*)-[3-hydroxy-2-(phosphonomethoxy)propoxy] (HPMPO), 2-(phosphonomethoxy) ethoxy (PMEO) or (*R*)-[2-(phosphonomethoxy)propoxy] (PMPO), display an antiviral sensitivity spectrum that closely mimic that of the parental (S)-HPMP-, PME- and (*R*)-PMP-purine derivatives. Several PMEO-DAPym derivatives proved as potent as PMEA (adefovir) and (R)-PMPA (tenofovir) in inhibiting Moloney murine sarcoma virus (MSV)-induced tumor formation in newborn NMRI mice. The HPMPO-, PMEO- and PMPO-DAPym derivatives represent a novel well-defined subclass among the acyclic nucleoside phosphonates endowed with potent and selective antiviral activity.

Key Words: Acyclic nucleoside phosphonates; HIV; AIDS; Herpesviruses; Poxviruses; Hepadnaviruses.

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DOI: 10.1081/NCN-200027573
1525-7770 (Print); 1532-2335 (Online)

INTRODUCTION

Acyclic nucleoside phosphonates (ANPs) represent a group of structurally related compounds characterized by the phosphonate entity linked through an uncleavable ether linkage to an aliphatic chain linked at N-1 of the pyrimidine base or N-9 of the purine base. To date, three subclasses of ANPs have been identified, each of which displays a unique antiviral activity spectrum. (*S*)-9-[3-Hydroxy-2-(phosphonomethoxy)-propyl]-adenine (Fig. 1) is the prototype of the first class of ANPs, and is endowed with inhibitory potential against a broad spectrum of DNA viruses, including the herpesviruses [i.e. herpes simplex virus type 1 (HSV-1) and HSV-2, cytomegalovirus (CMV), varicella-zoster virus (VZV)], poxviruses [i.e. vaccinia virus (VV)] and adenoviruses. It is virtually inactive against retroviruses such as the human immunodeficiency virus (HIV). The cytosine analogue of (*S*)-HPMPA [*viz.* (S)-HPMPC, cidofovir] is a potent anti-CMV agent that has been approved for the treatment of CMV retinitis in AIDS patients (Vistide®).

9-[2-(Phosphonomethoxy)ethyl] (PME) purines represent the second class of ANPs (Fig. 1). The prototype adenine derivative PMEA (adefovir) displays activity against DNA viruses (i.e. HSV-1, HSV-2, VV) albeit to a lesser extent than (*S*)-HPMPA, and also shows pronounced activity against retroviruses (including HIV) and hepatitis B virus (HBV). The oral prodrug of PMEA (adefovir dipivoxil, Hepsera®) has been approved for the treatment of HBV infections.

9-[2-(Phosphonomethoxy)propyl] (PMP) purines are recognized as a third class of antiviral compounds, represented by the prototype adenine derivative (*R*)-PMPA (Fig. 1). Its oral prodrug, tenofovir disoproxil fumarate has been approved for the treatment of AIDS (Viread™). Members of this class of ANPs have completely lost

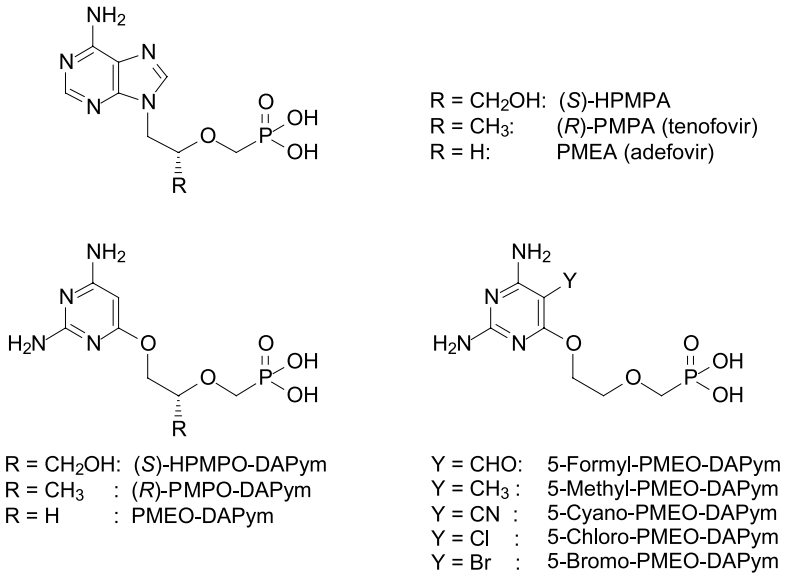

Figure 1. Structural formulae of acyclic nucleoside phosphonate derivatives.

their activity against DNA viruses, except for HBV. They are, however, also markedly active against a variety of retroviruses, including HIV.

With the exception of cidofovir, all other ANPs with biological (antiviral) activity contain a purine base such as adenine, 2,6-diaminopurine, guanine or derivatives thereof. We have recently discovered a new class of ANPs, where the base moiety is a pyrimidine, preferentially 2,4-diaminopyrimidine (DAPym), that is linked *via* an ether linkage to the aliphatic phosphonate side chain through an oxygen atom at the C-6 position of the pyrimidine ring (Fig. 1). The antiviral activity spectrum of these novel pyrimidine ANPs closely resembles that of the [(S)-HPMP-, PME- and (R)-PMP-] purine types of compounds.

MATERIALS AND METHODS

The origin of the viruses and the methods used for measuring in vitro cytostatic activity, in vitro antiviral activity and activity in vivo against (Moloney) murine sarcoma virus (MSV) have been described before.[1,2] The chemical synthesis of the new ANP derivatives have also been described before[1,3] except for the 5-cyano- and 5-formyl DAPym ANPs for which the synthesis is described in Fig. 2.

RESULTS

The broad-spectrum antiviral activity of (*S*)-HPMPO-, PMEO- and (*R*)-PMPO-derivatives of DAPym (Fig. 1) has been investigated against a variety of DNA viruses, retroviruses and RNA viruses (Table 1). (*S*)-HPMPO-DAPym was inhibitory to the DNA viruses to a similar extent as (*S*)-HPMPA and (*S*)-HPMPC, except for cytomegalovirus that was poorly inhibited by (*S*)-HPMPO-DAPym. The compound did not show anti-retrovirus activity, nor did it inhibit the replication of any other RNA

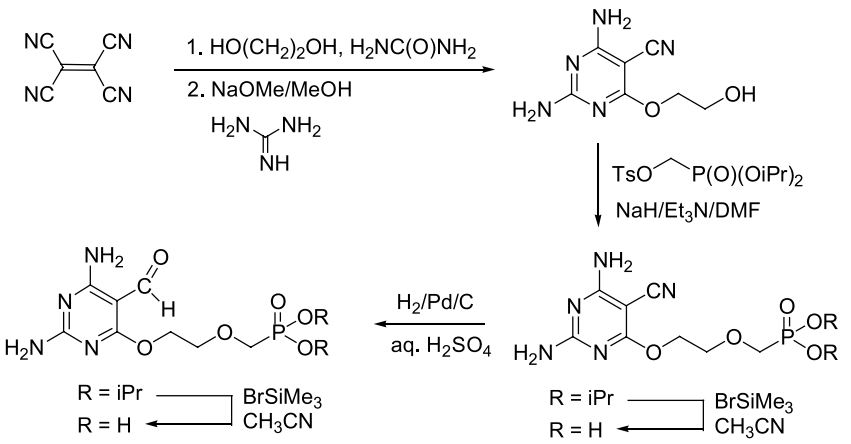

Figure 2. Synthesis of 5-cyano- and 5-formyl-2,4-diamino-6-[2-(phosphonomethoxy)ethoxy]-pyrimidines.

Table 1. Antiviral activity of (*S*)-HPMPO-, PMEO- and (*R*)-PMPO-DAPym and comparison with their corresponding adenine counterparts.

	50% Effective concentration (μM)[a]					
	(*S*)-HPMPA	(*S*)-HPMPO-DAPym	PMEA	PMEO-DAPym	(*R*)-PMPA	(*R*)-PMPO-DAPym
Herpesviruses						
HSV-1 (KOS)	6.6	14	26	25	>350	58
HSV-2 (G)	13	51	26	91	≥350	17
VZV (OKA, YS)	0.07	0.51	38	2.7	≥350	17
CMV (AD-169, Davis)	0.63	139	95	>190	>350	>180
Poxviruses						
VV	2.3	19	555	570	>350	>720
Hepadnaviruses						
HBV[b]	1.1	–	0.2	–	2.5	–
Retroviruses						
HIV-1	>50	>15	11	3.0	3.8	6.8
HIV-2	>50	>15	21	1.6	4.9	4.7
MSV	>50	>15	1.5	0.57	15	0.18
RNA viruses[c]	>50	>75	>185	>190	>525	>180

[a]Compound concentration required to inhibit virus-induced cytopathicity in cell culture by 50%.

[b]Data taken from Ref. [4].

[c]The following viruses were included in the antiviral assays: vesicular stomatitis virus, Coxsackie B4 virus, reovirus-1, Semliki forest virus, parainfluenza-3 virus, Sindbis virus, Punta Toro virus and respiratory syncytial virus.

Table 2. Antiretroviral activity of 5-substituted PMEO/(*R*)-PMPO DAPym derivatives.

		EC_{50} (μM)[a]					
Virus	Cells	PMEO-DAPym	5-Cl-PMEO-DAPym	5-Br-PMEO-DAPym	5-Methyl-PMEO-DAPym	5-Cyano-PMEO-DAPym	5-Formyl-PMEO DAPym
HIV-1(III_B)	CEM	3.4	3.1	7.1	0.32	11	4.5
HIV-2(ROD)	CEM	2.5	4.2	9.1	0.32	4.5	2.9
MSV	C3H	0.57	2.8	6.8	0.18	9.4	21

[a]50% effective concentration, or compound concentration required to inhibit HIV-induced giant cell formation in CEM cells or MSV-induced transformation of C3H cells by 50%.

Table 3. Anti-MSV activity of 5-methyl-PMEO-DAPym and PMEA in newborn NMRI mice.[a]

Drug	Daily dose (mg/kg)	Mean day of tumor initiation (% tumor free, day 12)	Mean day of animal death (% survivors, day 12)
5-Methyl-PMEO-DAPym	50	>12 (100%)	10.1
	20	>12 (100%)	>12 (100%)
	5	8 (90%)	>12 (100%)
PMEA (adefovir)	50	>12 (100%)	8.8
	20	>12 (100%)	9.0 (90%)
	5	9.3 (70%)	>12 (100%)
Control	0	5.0	10.9

[a]Compound was administered intraperitoneally (i.p.) at days 1, 2, 3, 4 and 5 post MSV infection. MSV was inoculated intramuscularly (i.m.).

virus. PMEO-DAPym also had a comparable antiviral activity spectrum as PMEA (moderately active against herpesviruses, but virtually inactive against vaccinia virus), and was markedly inhibitory to retroviruses in cell culture (Table 1). (*R*)-PMPO-DAPym proved not active against CMV or VV, but showed pronounced activity against retroviruses (Table 1).

The anti-retrovirus activity of several 5-substituted PMEO-DAPym derivatives (Fig. 1) was also determined (Table 2). Interestingly, 5-methyl-PMEO-DAPym was at least 7- to 10-fold more active against HIV replication in CEM cells and 3-fold more active against MSV in C3H cells, than its parental unsubstituted PMEO-DAPym derivative. Also, 5-bromo-, 5-chloro-, 5-cyano- and 5-formyl-substituted PMEO-DAPym derivatives were highly active against these viruses (Table 2).

The anti-MSV activity of 5-methyl-PMEO-DAPym was studied in newborn NMRI mice that had been inoculated with (Moloney) murine sarcoma virus (Table 3). The mean day of tumor initiation in the untreated mice was 5 days and the mean day of animal death was 10.9 days. At day 12, the experimental results for the drug treated MSV-inoculated mice were evaluated. Interestingly, 5-methyl-PMEO-DAPym proved remarkably efficient in preventing tumor formation at compound doses of 5, 20 and 50 mg/kg/day. At day 12 of the experiment, all mice were alive in the 20 and 5 mg/kg-treatment group and did not bear any visible tumor, whereas at 50 mg/kg, none of the mice survived due to toxic effects of the drug. In comparison, PMEA was slightly more antivirally active, but also more toxic than 5-methyl-PMEO-DAPym (Table 3).

DISCUSSION

A novel subclass of acyclic pyrimidine nucleoside phosphonates has been discovered. A virtually similar antiviral activity spectrum has been observed as that of the prototype acyclic purine nucleoside phosphonates. These are the first pyrimidine ANP derivatives in the PME(O) and (*R*)-PMP(O) series that show antiviral activity. Due to the fact that the aliphatic phosphonate chain is linked to C-6 (and not N-1) of the pyrimidine ring, this novel group of pyrimidine ANPs may mimic an incomplete

purine ring system in which the ether oxygen of the pyrimidine PMEO derivatives replaces the N-9 of the intact purine ring. Moreover, when the 2,4-diaminopyrimidine in the PMEO derivative is substituted at C-5 by a methyl, cyano or formyl entity, the imidazole ring of the purine base is even more closely imitated.

The identification of the cellular enzymes converting these compounds to their diphosphate analogues (i.e. CMP-UMP kinase, dTMP kinase, AMP kinase, NDP kinase) is currently under investigation in our laboratories. Also, it will be of interest to decipher whether HIV-1 reverse transcriptase recognize these compounds as purine or pyrimidine nucleotide analogues, and whether/how these compounds are incorporated in the growing (viral) DNA chain.

ACKNOWLEDGMENTS

This work was supported by grants from the Fonds voor Geneeskundig Wetenschappelijk Onderzoek-Vlaanderen (G.0104.98) and the Geconcerteerde Onderzoeksacties (GOA) Krediet no. 00/12. It was performed as a part of the research project # 4055905 of the Institute of Organic Chemistry and Biochemistry. It was supported by the Programme of Targeted Projects of the Academy of Sciences of the Czech Republic (# S4055109), by the René Descartes Prize-2001 of the European Commission and by Gilead Sciences (Foster City, CA).

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