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Phosphonylmethoxyethyl Purine Derivatives, a New Class of Anti-Human Immunodeficiency Virus Agents

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A study of the structure-activity relationship of a series of newly synthesized phosphonylmethoxyalkyl purine and pyrimidine derivatives revealed that several adenine derivatives substituted at the N^9 position by a 2-phosphonylmethoxyethyl (PME) group inhibited human immunodeficiency virus (HIV)-induced cytopathogenicity and HIV antigen expression in vitro at concentrations significantly below the toxicity threshold for the host cells. In terms of anti-HIV potency in MT-4 cells, the PME 2,6-diaminopurine derivative (50% effective dose [ED_{50}], 1 μM) ranked first, followed by the PME adenine derivative (ED_{50}, 2 μM [MT-4]) and the PME 2-monoaminopurine derivative (ED_{50}, 45 μM). Antiretroviral activity was also demonstrated in ATH8 and H9 cells, which were de novo infected with HIV, and extended to C3H mouse fibroblasts infected with Moloney murine sarcoma virus. Unlike 2′,3′-dideoxyadenosine, these compounds were not found to be degraded by deaminases derived from bovine intestine.

As the number of patients with the acquired immune deficiency syndrome (AIDS) and AIDS-related complex is still rising throughout the world, there is an urgent need for therapeutic agents to counteract and cure this life-threatening disease which is caused by a human retrovirus, now commonly referred to as human immunodeficiency virus (HIV) (5, 13, 22). Of crucial importance in the pathogenesis of the disease is the interaction of HIV with T4 cells; HIV seriously impairs the functioning of T4 cells in host cell defense responses (11).

In recent years several agents have been described which inhibit HIV replication in vitro (for reviews, see references 6 and 19). Among the most potent and selective HIV inhibitors rank the 2′,3′-dideoxynucleoside analogs (7, 18). Clinical trials that have been carried out in patients with AIDS with one of these 2′,3′-dideoxynucleoside analogs, namely, 3′-azido-2′,3′-dideoxythymidine (AZT; also known as AzddThd, Retrovir, Zidovudine), revealed a significant delay in mortality, improvement of immunological and viral parameters, and decreased incidence of opportunistic infections (12, 23). These findings provide a significant impetus for the development of antiviral agents for potential use in the treatment of AIDS and related disorders. As the clinical usefulness of AZT is compounded by severe side effects, i.e., bone marrow toxicity, which may preclude the long-term administration of this drug, therapeutic modalities should be developed that are as equally efficient as or more efficient than AZT and that are less toxic.

Recently, De Clercq et al. (8, 9a) reported that the 9-(3-hydroxy-2-phosphonylmethoxypropyl) (HPMP) derivative of adenine [(*S*)-HPMPA] had a selective inhibitory effect on a broad variety of herpesviruses (herpes simplex virus, varicella-zoster virus, cytomegalovirus, and Epstein-Barr virus) in vitro. Other DNA viruses that were found to be susceptible to the inhibitory effect of (*S*)-HPMPA included adenoviruses (1), iridoviruses (African swine fever virus) (14), and poxviruses (i.e., vaccinia virus) (8). De Clercq et al. (8) also mentioned that (*S*)-HPMPA and its congener 9-(2-phosphonylmethoxyethyl)adenine (PMEA) inhibited transformation of murine fibroblast cells by the retrovirus Moloney murine sarcoma virus (MSV).

PMEA, (*S*)-HPMPA, and various other phosphonylmethoxyalkyl derivatives were the subject of our detailed investigation of their anti-HIV properties in vitro. The assays were carried out in three different T-cell lines (MT-4, ATH8, and H9) and were based on two different parameters of HIV growth (virus-induced cytopathogenicity and antigen expression). In addition, the compounds were examined for their inhibitory effects on the transformation of murine fibroblast cells by MSV. PMEA and the 2,6-diaminopurine derivative of *N*-(2-phosphonylmethoxy)ethyl (PME) (PMEDAP) were evaluated further for their cytostatic properties against a series of human B- and T-cell lines. Finally, their resistance to bovine intestine deaminase was investigated.

MATERIALS AND METHODS

Compounds. The phosphonylmethoxyalkyl derivatives were synthesized from the sodium salt of the corresponding heterocyclic base by its condensation with diethyl 2-(4-toluenesulfonyloxyethoxy)methane phosphonate following the procedure described for PMEA (16). The resulting N-substituted 2-(diethoxyphosphonylmethoxy)ethyl derivatives were isolated by silica gel chromatography and treated with bromotrimethylsilane in acetonitrile solution. The PME derivatives obtained were purified by ion-exchange chromatography and stored as water-soluble sodium salts. The compounds were homogeneous by high-pressure liquid chromatography (C_{18} silica in triethylammonium hydrogen carbonate [pH 7.5]). The structure was confirmed by ^1H ^{32}P nuclear magnetic resonance spectra and UV spectra. The 1,N^6-etheno derivative was obtained by chloroacetaldehyde treatment of PMEA in aqueous solution at neutral pH and isolated by octadecylsilica gel chromatography (10). The *N*-(*S*)-(3-hydroxy-2-phosphonylmethoxypropyl) derivatives were prepared from the corresponding *N*-(*S*)-(2,3-dihydroxypropyl) derivatives of the heterocyclic bases by successive treatment with chloromethanephosphonyl dichloride

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and aqueous sodium hydroxide. The isomers were separated by preparative high-pressure liquid chromatography and isolated as sodium salts (17). The formulas of the compounds are presented in Fig. 1. 2',3'-Dideoxycytidine (ddCyd) was purchased from Pharmacia, Inc. (Piscataway, N.J.). All stock solutions were made in phosphate-buffered saline and stored at 4°C until use. Concentrations of the compounds were determined spectrophotometrically.

Viruses. HIV was obtained from the culture supernatant of the persistently HIV-infected H9 cell line H9/HTLV-III_B, which was kindly provided by R. C. Gallo (National Institutes of Health, Bethesda, Md.) (22). The supernatant was clarified by low-speed centrifugation and stored in portions at −70°C until use. Moloney MSV was prepared from tumors that were induced following intramuscular injection of 3-day-old NMRI mice with MSV, as described previously (9).

Anti-HIV assays. The procedure to determine the anti-HIV activity in MT-4 cells was done as described previously (21). Briefly, MT-4 cells were seeded at 5×10^5 cells per ml and infected with a freshly prepared HIV stock dilution at a multiplicity of infection of 100 50% cell culture infective doses per microtiter well. After 90 min of incubation at 37°C, 5×10^4 cells were transferred to the wells of a flat-bottom, 96-well microtiter tray containing 100 μl of various concentrations of the test compounds. Several parameters of the infectious process were evaluated. The inhibitory effects of the compounds on viral antigen expression in HIV-infected MT-4 cells were determined qualitatively at day 4 after HIV infection by indirect immunofluorescence microscopy and then quantitated by laser flow cytofluorometry, using a high-titer polyclonal antibody derived from a patient with AIDS-related complex as a probe. After 5 days of incubation at 37°C, the clustering pattern of the cells, which is characteristic for normal-growing MT-4 cells and completely absent in HIV-infected cells, was examined as described previously (21). The number of viable cells was determined in parallel for both HIV- and mock-infected cells in a blood cell counting chamber following trypan blue staining. The 50% effective dose (ED_{50}) was defined as the concentration of compound that protected HIV-infected cells by 50%, whereas the 50% cytotoxic dose (CD_{50}) corresponded to the concentration of compound that reduced the viability of the mock-infected cells by 50%.

Viral antigen expression in HIV-infected H9 cells was revealed by an indirect immunofluorescence procedure combined with laser flow cytofluorometry, as described above for the MT-4 assays. H9 cells were suspended in 4.5 ml of culture medium at a density of 10^6/ml and infected with 0.5 ml of a virus stock solution, after which 100 μl of the suspension was brought into each well of a microtiter plate; each well contained 100 μl of various dilutions of the test compunds. After 4 days of incubation, half of the culture medium was replaced with fresh medium containing the same concentration of compound. Four days later the cells were examined for HIV antigen expression. Under these experimental conditions, 10 to 20% of the cells proved to be HIV antigen positive.

HIV-induced cytopathogenicity in ATH8 cells was monitored as described above for MT-4 cells, as described previously (20). The ATH8 cells were infected with 3,000 HIV virions per cell for 60 min at 37°C. The number of virus particles of the virus stock was determined by electron microscopy. The number of viable cells was determined after 7 days of incubation at 37°C.

Anti-MSV assay. The procedure to determine the anti-MSV activity in murine C3H cells was performed as described previously (4). Briefly, murine fibroblast (C3H) cells were seeded into wells (diameter, 2.3 cm^2) of tissue culture plates (Costar, Cambridge, Mass.) at 5×10^4 cells per ml. The cell cultures were infected 24 h later with 150 focus-forming units of MSV for 90 min at 37°C, after which the medium was replaced with 1 ml of fresh culture medium containing various concentrations of the test compounds. After 6 days, the transformation of the cell cultures was examined microscopically.

Cytostatic assay. The cytostatic effects of the compounds were assessed by measuring inhibition of cell proliferation as described previously (3, 21). Briefly, Raji, Molt/4F, MT-4, CEM, and H9 cells were suspended in growth medium and added to microplate wells at a density of 5×10^4 to 7.5×10^4 cells per well in the presence of various concentrations of the test compounds. The cells were then allowed to proliferate for 72 h at 37°C. At the end of the incubation period, the cells were counted in a Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.). The ID_{50} value was defined as the concentration of compound that reduced the cell increment by 50%.

Enzyme assay. Adenosine deaminase was derived from bovine intestine (Boehringer GmbH, Mannheim, Federal Republic of Germany). The reaction mixture contained 800 μl of potassium phosphate buffer (50 mM, pH 7.4), 100 μl of a solution of the test compound (100 μM), and 100 μl (1 U) of enzyme. The deamination rates of PMEA, PMEDAP, and the 2-monoaminopurine derivative of PME (PMEMAP) were determined at room temperature by measuring the decreases in the A_{265}, A_{280}, and A_{302}, respectively.

RESULTS

Antiviral activity of PME derivatives. The anti-HIV activities of various phosphonylmethoxyalkyl purine and pyrimidine derivatives were determined in MT-4 cells which were infected with HIV at a multiplicity of infection that would completely destroy the cells by day 5 postinfection. PMEA, PMEDAP, PMEMAP, and 9-(2-phosphonylmethoxyethyl) guanine (PMEG) (Fig. 1) proved to be potent inhibitors of HIV replication, with ED_{50} values for inhibition of cytopathogenicity of 2, 1, 45, and 3.8 μM, respectively (Table 1). Substitution of the heterocyclic moiety by hypoxanthine (PMEHx) or any of the pyrimidine bases uracil (PMEU), cytosine (PMEC), or thymine (PMET) annihilated the inhibitory effects on HIV replication. With these compounds no anti-HIV activity could be detected at concentrations up to 125 μM (Fig. 2; data not shown). Complete protection was achieved at concentrations of ≥5 μM with PMEA and PMEDAP and 125 μM with PMEMAP (Fig. 2). At these concentrations, MT-4 cells, which are unable to form clusters if infected with HIV, showed the usual clustering pattern. When ddCyd was evaluated under these conditions, 50% inhibition of cytopathogenicity was seen at about 0.5 μM, whereas the compound proved to be 50% cytotoxic at 80 μM (Table 1).

PMEA and PMEDAP were then assayed further for their anti-HIV properties in ATH8 cells which were infected with 3,000 virions per cell. PMEDAP and PMEA proved effective in protecting the cells against destruction by the virus (Fig. 2), with ED_{50} values of 1.5 and 13 μM, respectively.

In both MT-4 and ATH8 cells, the antiviral concentrations of PMEA, PMEDAP, and PMEMAP required to inhibit HIV replication were clearly lower than the toxicity threshold for the host cells, as demonstrated in Fig. 2 and further shown by the selectivity index in Table 1. PMEG was the most toxic

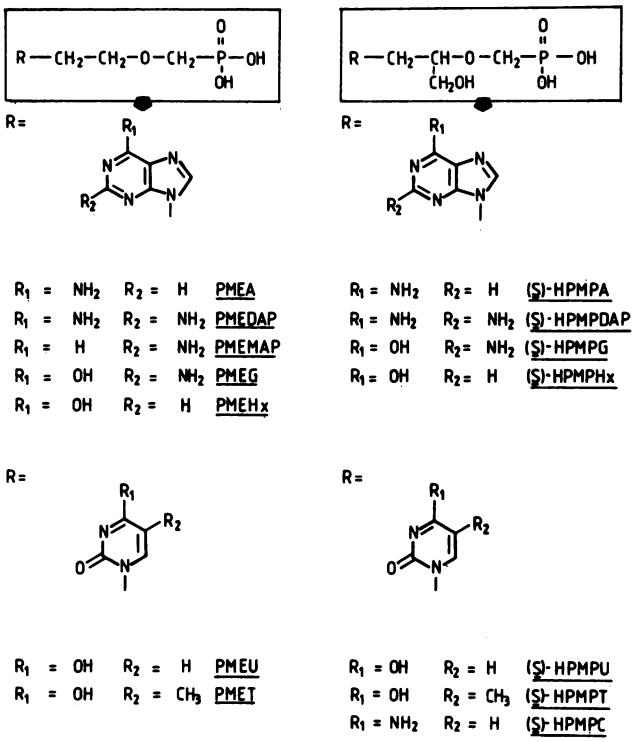

FIG. 1. Structural formulas of PME and HPMP purine and pyrimidine derivatives.

TABLE 1. Anti-HIV activity and cytotoxicity of phosphonylmethoxyalkyl purine derivatives in MT-4 or H9 cells

Compound	Cell line	CD_{50} (μM)[a]	ED_{50} (μM) by[b]:		Selectivity index[c]
			CPE[d]	IF[e]	
PMEA	MT-4	67 ± 4	2.0 (± 0.4)	1.6	33
PMEA	H9	40 ± 1.3	ND[f]	0.4	
PMEDAP	MT-4	18 ± 9	1.0 (± 0.1)	0.9	18
PMEDAP	H9	10.5 ± 2.7	ND	<0.2[g]	
PMEMAP	MT-4	>1,250	45.0 (± 37)	52	>28
PMEG	MT-4	11 ± 4	3.8 (± 1.6)	2.1	3
PMEHx	MT-4	>625	>125	ND	
(*S*)-HPMPA	MT-4	52	>125	>125	<1
(*S*)-HPMPDAP	MT-4	90	>125	ND	<1
(*RS*)-HPMPG	MT-4	25	>25	ND	<1
(*S*)-HPMPHx	MT-4	>125	>125	ND	
ddCyd	MT-4	80 ± 56	0.47 (± 0.05)	ND	170

[a] Dose required to reduce the viability of mock-infected host cells by 50% after 5 days of incubation in the presence of the compound. Data represent average values ± standard deviation from two to five separate experiments.

[b] The 50% antiviral effective dose, or the dose required to achieve 50% protection of the cells against HIV infection, as monitored by either cytopathogenicity or immunofluorescence. Data represent average values ± standard deviation from two to five separate experiments.

[c] Ratio of CD_{50} to ED_{50} (based on the cytopathic effect assay).

[d] CPE, Cytopathic effect; cytopathogenicity assay based on cell viability determined 5 days after HIV infection.

[e] Immunofluorescence (IF) assay based on HIV antigen expression determined by quantitative laser flow cytofluorometry 4 days after HIV infection.

[f] ND, Not determined.

[g] Protection of 67.5 ± 6.4% was seen at 0.2 μM.

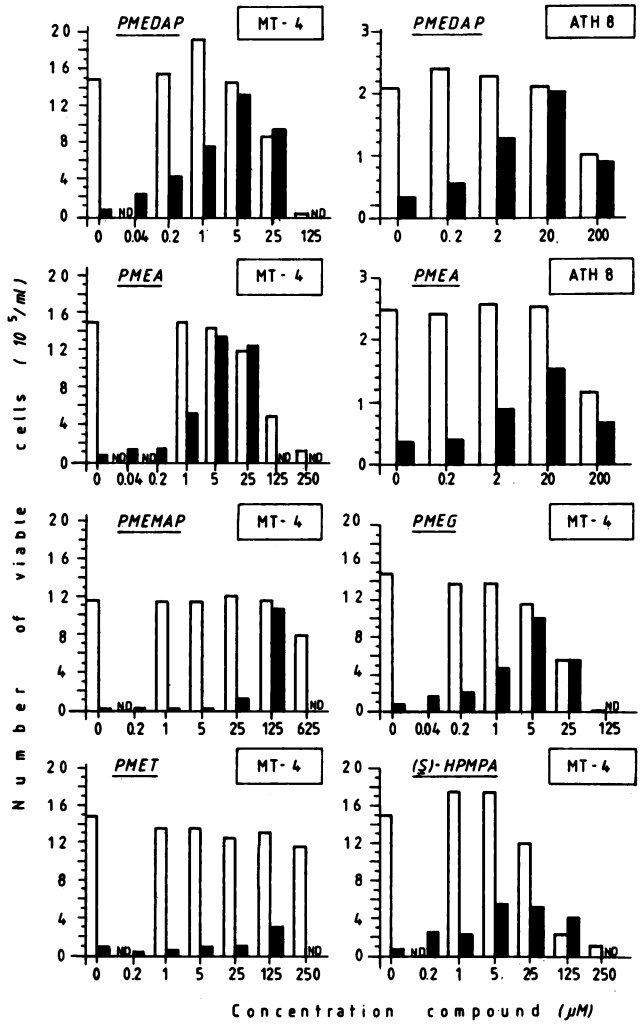

FIG. 2. Inhibition of HIV cytopathogenicity in MT-4 or ATH8 cells by various PME purine derivatives. Viability of the cells was measured by the trypan blue exclusion method on days 5 and 7 postinfection (MT-4 and ATH8 cells, respectively). The viabilities of HIV-infected cells (■) and mock-infected cells (□) are shown. ND, Not determined.

of the whole group, with a selectivity index not greater than 3. PMEA and PMEDAP were less cytotoxic in ATH8 cells (CD_{50}s, ≥200 μM) than MT-4 cells (CD_{50}s, 67 and 18 μM, resepectively). The lowest cytotoxicity was observed with PMEMAP, which decreased MT-4 cell viability by only 30% at a concentration of 1,250 μM.

Next, we examined the effects of the test compounds on HIV antigen expression in MT-4 and H9 cells. The viral proteins were detected by an indirect immunofluorescence technique, using a polyclonal antibody as a probe, after which the cells were analyzed by laser flow cytofluorometry. The histograms shown in Fig. 3 readily visualize the inhibitory effects of various concentrations of PMEDAP and PMEA on viral antigen expression in MT-4 cells. The dose-response effects were quantitated by computer analysis based on the accumulative data for 10^4 cells per sample and are depicted in Fig. 4. PMEDAP completely inhibited the expression of HIV proteins at a concentration of 5 μM, whereas PMEA and PMEG did so at 25 μM and PMEMAP did so at 125 μM. At 125 μM, PMET and (*S*)-HPMPA

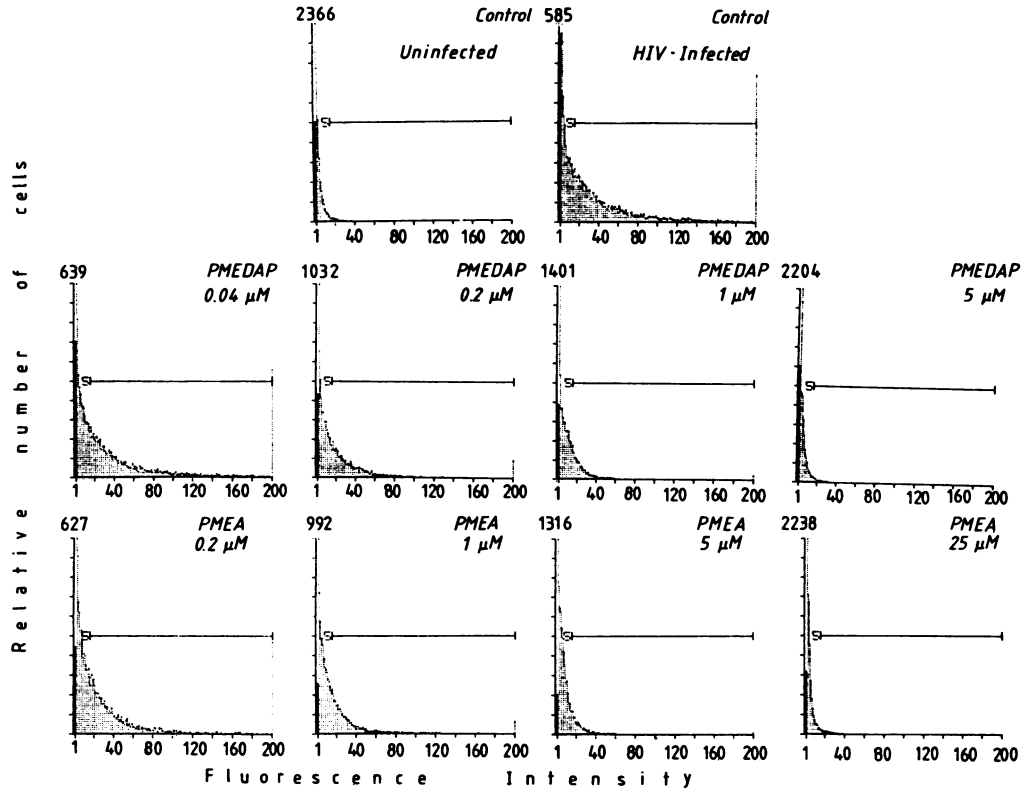

FIG. 3. Laser flow cytofluorometric histograms of HIV-infected MT-4 cells treated with various concentrations of PMEA and PMEDAP. Duplicate samples were processed by an indirect immunofluorescence method, using polyclonal antibodies as probes. The values indicated on top of the histograms represent the relative numbers of cells scored.

inhibited HIV antigen expression by only 30 or 42%, respectively. When H9 cells were infected with HIV and treated with various concentrations of PMEA and PMEDAP, 50% inhibition of viral antigen expression was demonstrated at 0.4 and <0.2 μM, respectively (Table 1). At the latter concentration, PMEDAP inhibited HIV antigen expression by about 70%. H9 cell viability was inhibited by 50% at 40 and 10 μM, respectively.

We also investigated whether the antiretrovirus activity of the phosphonylmethoxyalkyl purine and pyrimidine derivatives extended to retroviruses other than HIV and, therefore, determined the effects of the compounds on the transformation of murine C3H cells by MSV. In this assay system, PMEDAP, PMEA, and PMEMAP achieved 50% inhibition of MSV-induced focus formation at concentrations of 0.6, 1.0, and 1.2 μM, respectively (Table 2), whereas cytotoxicity (microscopic alteration of normal cell morphology) was seen at 40 μM (PMEDAP), 200 μM (PMEA), and 1.6 μM (PMEMAP). The PMEG derivative was active at an ED_{50} of 0.12 μM, but was cytotoxic at 0.3 μM, which

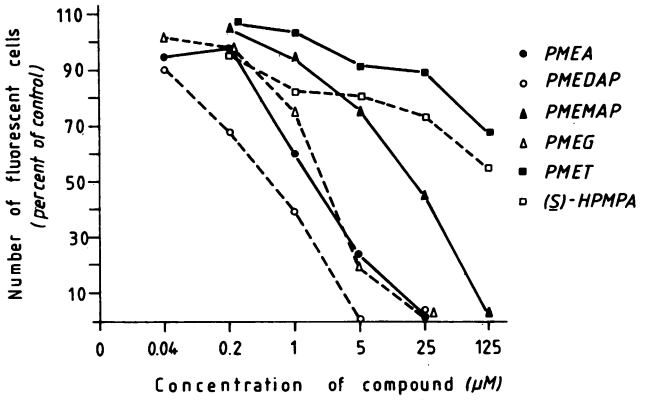

FIG. 4. Dose-response curves for inhibition of viral antigen expression in MT-4 cells by various PME and HPMP derivatives of purines and pyrimidines.

TABLE 2. Effects of phosphonylmethoxyalkyl purine derivatives on MSV-induced transformation of C3H cells[a]

Compound	Minimum cytotoxic concn (μM)[b]	ED_{50} (μM)[c]
PMEA	200	1.01 ± 0.54
PMEDAP	40	0.60 ± 0.33
PMEMAP	1.6	1.21 ± 0.33
PMEG	0.3	0.12 ± 0.08
PMEHx	>200	>200
(*S*)-HPMPA	40	>8
(*RS*)-HPMPDAP	200	6 ± 0.8
(*RS*)-HPMPG	40	6 ± 1.9
(*S*)-HPMPHx	>200	>200
ddCyd	>200	29 ± 8.8

[a] All data represent average values for at least two separate experiments.
[b] Dose required to cause a microscopically detectable alteration of normal cell morphology.
[c] The 50% antiviral effective dose, or dose required to reduce the number of MSV-induced foci by 50%.

TABLE 3. Inhibitory effects of PME purine derivatives on cell proliferation in human B- and T-cell lines

Compound	ID_{50} (μM) for the following cell lines[a]:				
	Raji	Molt/4F	MT-4	CEM	H9
PMEA	31.8 ± 2.3	55.5 ± 2.4	144 ± 3	69.0 ± 4.2	81.4 ± 13.6
PMEDAP	16.5 ± 0.5	14.9 ± 3.7	46.9 ± 1.6	13.9 ± 0.3	29.9 ± 11.7
2′,3′-Dideoxyadenosine	>1,000	>1,000	>1,000	>1,000	>1,000

[a] Cell number was determined on day 3 by using a Coulter Counter. All data represent average values for at least two separate experiments. ID_{50} is the concentration of compound that inhibited cell proliferation by 50%.

confirms the low selectivity of PMEG observed in the anti-HIV assays. Neither PMEHx nor the pyrimidine derivatives PMEU or PMET were found to be inhibitory toward MSV focus formation at a concentration of 200 μM. ddCyd achieved 50% protection against MSV-induced focus formation at 29 μM, whereas no toxicity was seen at 200 μM (Table 2). PMEA and PMEDAP were further assessed for their susceptibility to deamination. Whereas 2′,3′-dideoxyadenosine was readily deaminated under these experimental conditions of the assays, no deamination was observed for the two PME derivatives (data not shown).

The cytostatic effects of PMEA and PMEDAP compared with that of 2′,3′-dideoxyadenosine against a series of human B- and T-cell lines are summarized in Table 3. The 50% cytostatic dose was determined by enumerating the cells (which were cultured in the absence or presence of the test compounds) at day 3 with a cell Coulter Counter. PMEDAP was found to be consistently more cytostatic (ID_{50}, 14 to 47 μM) than PMEA (ID_{50}, 32 to 144 μM).

Structure-activity relationship study. We also explored the antiviral activities of a number of compounds that were related to the parent compound (*S*)-HPMPA (Tables 1 and 2) but that differed from the PME series by the presence of a (*S*)-hydroxymethyl group linked to the β-carbon atom of the alkyl side chain (Fig. 1). (*S*)-HPMPA and 9-(3-hydroxy-2-phosphonylmethoxypropyl)-2,6-diaminopurine [(*RS*)-HPMPDAP] were nearly 10 times less inhibitory to MSV transformation (ED_{50}, >8 and 6 μM, respectively) than their PME counterparts. Whereas 9-(3-hydroxy-2-phosphonylmethoxypropyl)guanine [(*RS*)-HPMPG] was about as potent and selective in inhibiting MSV focus formation as (*S*)-HPMPA, neither the corresponding hypoxanthine (*S*)-HPMPHx nor the pyrimidine (thymine, uracil) derivatives (*S*)-HPMPT or (*S*)-HPMPU showed any anti-MSV activity at concentrations up to 200 μM (Table 2; data not shown). Nor was any inhibitory effect observed with the hypoxanthine or pyrimidine derivatives on HIV replication (ED_{50}, >125 μM). (*S*)-HPMPA (Fig. 2 and 4) and (*S*)-HPMPDAP (data not shown) exhibited some inhibitory effect on HIV-induced cytopathogenicity and antigen expression, albeit to a much lower extent than their PME counterparts and at concentrations which approached those that caused host cell toxicity.

The following modifications in the purine or acyclic moieties of the parent compounds PMEA and PMEDAP failed to result in any significant anti-HIV (ED_{50}, >125 μM) or anti-MSV (ED_{50}, >200 μM) activity (data not shown): substitution of the purine moiety by either 6-hydrazinopurine [9-(2-phosphonylmethoxyethyl)-6-hydrazinopurine] or 1,6-ethenoadenine [9-(2-phosphonylmethoxyethyl)-1,6-ethenoadenine]; linkage of the PME side chain to the 2,6-diaminopurine moiety at either N^7 [7-(2-phosphonylmethoxyethyl)-2,6-diaminopurine] (ED_{50} for MSV, 178 μM) or N^3 [3-(2-phosphonylmethoxyethyl)-2,6-diaminopurine]; the esterification of the phosphonyl group in PMEA (PMEA ethyl ester) (ED_{50} for MSV, 101 μM) or PMEDAP (PMEDAP ethyl ester); and, finally, replacement of the ethyl group of the alkyl side chain by methyl [9-(phosphonylmethoxymethyl)adenine].

DISCUSSION

As a rule, nucleoside analogs which are endowed with antiviral properties must be phosphorylated intracellularly to exert their inhibitory effects on viral replication. Phosphorylated nucleosides would seem of little, if any, use, unless their premature hydrolysis by phosphomonoesterases could be prevented. Based on this premise, nucleotide analogs were designed in which the phosphorus atom was attached to the nucleoside via a P-C bond. This has led recently to the discovery of (*S*)-HPMPA, a phosphonyl derivative with potent and selective broad-spectrum anti-DNA virus properties (8, 9a).

Several new acyclic phosphonate nucleoside derivatives have been developed, and some of these compounds have shown marked antiretroviral properties. In terms of anti-HIV activity, PMEDAP ranked first, followed by PMEA and PMEMAP, when evaluated for their inhibitory effects on HIV-induced cytopathogenicity and viral antigen expression in MT-4 cells. The antiviral concentrations were well below the toxicity thresholds for the host cells, as was apparent from the selectivity indexes that were attained in MT-4 cells and the cytostatic properties of PMEDAP and PMEA in four human T-cell lines and one human B-cell line. A variety of related compounds were synthesized and evaluated for their inhibitory effects on HIV replication. The salient feature of this structure-function relationship was that all the active congeners fell in the class of the PME purines. Furthermore, the highest selectivity was obtained with the purine derivatives which were substituted with amino group(s) at the C-2 or C-6 position of the heterocyclic base. Equally important for anti-HIV activity was the presence of a phosphonylmethoxy group linked via an ether linkage to position 2 of the side chain. Substitutions by the (*S*)-hydroxymethyl group (HPMPA) at C-2 of the side chain or esterification of the phosphonyl residue drastically reduced their inhibitory actions against HIV.

PMEA and (*S*)-HPMPA and their 2,6-diaminopurine, 2-monoaminopurine, and guanine counterparts show marked differences in their antiviral activity spectra. HPMP derivatives were much more active than the PME compounds against vaccinia, adeno-, cytomegalo-, and varicella-zoster viruses (8, 9a). The PME compounds were about equally as active as their HPMP counterparts against herpes simplex virus types 1 and 2 and thymidine kinase-negative herpes simplex virus type 1 (8, 9a); and as shown here, the PME derivatives were clearly more active than the HPMP derivatives against HIV. The anti-HIV activities of the phosphonylmethoxyalkyl derivatives correlated with their anti-MSV activities, in that the PME derivatives were about 10-fold more

inhibitory toward C3H cell transformation by MSV than their HPMP counterparts. Thus, the MSV-C3H system is predictive for anti-HIV activity of this class of compounds.

The mechanism of anti-HIV action of PMEDAP, PMEA, and PMEMAP remains to be elucidated. Whether they need to be converted to their mono- and diphosphoryl derivatives, as shown previously for (*S*)-HPMPA (24), also remains a subject of further study.

Reports from several laboratories, including our own (7, 18), have indicated the potent and selective anti-HIV activities of several 2',3'-dideoxynucleosides. When evaluated under the experimental conditions of this study, the 2',3'-dideoxynucleoside analogs of adenine, guanine, and 2,6-diaminopurine achieved 50% protection against HIV-induced cytopathogenicity at concentrations ranging from 1 to 6 μM (2, 4, 15). Thus, the potencies of the PME derivatives reported in this study are comparable to those that have been obtained previously for the purine 2',3'-dideoxyribonucleoside derivatives. Unlike 2',3'-dideoxyadenosine, the PME derivatives were not found to be degraded by deaminase derived from bovine intestine. It seems important, therefore, to further pursue PMEDAP and PMEA for their therapeutic potentials in retrovirus models in vivo.

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