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Acyclic phosphonomethylether nucleoside inhibitors of respiratory viruses

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Summary

A series of acyclic phosphonomethylether nucleosides were synthesized and then evaluated for inhibitory activity against respiratory viruses of clinical significance using CPE inhibition, neutral red uptake and virus yield reduction assays. Of the 20 compounds synthesized, none significantly inhibited influenza A or B viruses or respiratory syncytial virus strains A2, Long or 18537; the selective indices (SI) were less than 10. A new compound, GS-2128 (2R, 5R-9-[2,5-dihydro-5-(phosphonomethoxy)-2-furanyl]adenine; D4API), selectively inhibited adenovirus 5 (SI>10) as did GS-0577 (9-(3-hydroxy-2-phosphonylmethoxypropyl)-adenine; HPMPA) and GS-0504 [(*S*)-1-[3-hydroxy-2-(phosphonylmethoxypropyl)]-cytosine; HPMPC]. The 50% effective concentrations (EC_{50}) ranged from 8–100 μg mL^{-1} and 50% cell inhibitory concentrations (CC_{50}) from 40–1000 μg mL^{-1}. All three compounds were also found to be active against laboratory strains and clinical isolates of adenovirus types 1, 2, 8 and 41 with EC_{50} values ranging from 0.2 to 10 μg mL^{-1}. Two compounds, GS-438 (9-(2-phosphonylmethoxyethyl)guanine, PMEG) and GS-2542 (9-[3-phosphonomethoxy)methoxymethyl]guanine) inhibited parainfluenza virus 3 strain C243, with SI of 52 and >333, respectively. PMEG also inhibited measles virus strains CC, Halonen and Chicago with EC_{50} values ranging from 0.03–9 μg mL^{-1}. These data suggest that these compounds should be considered for possible development as therapeutic agents for respiratory virus infections.

Keywords: Acyclic; phosphonomethylether; nucleoside; adenovirus; measles virus; parainfluenza virus 3.

Introduction

Acyclic phosphonomethyl ether nucleosides, first described by De Clercq *et al.* (1986), are a novel class of compounds with interesting biological activities. Two members of this group in the most advanced stages of clinical development are (*S*)-1-[3-hydroxy-2-(phosphonylmethoxypropyl)]-cytosine (HPMPC) and 9-(2-phosphonylmethoxyethyl)-adenine (PMEA). HPMPC (cidofovir), which has broad spectrum antiherpesvirus activity (De Clercq, 1993), has recently been approved for treatment of human cytomegalovirus retinitis in the US and is currently in clinical trials as a topical treatment for herpes simplex virus (HSV). PMEA (**1**) inhibits herpesviruses and retroviruses in animal models (Tsai *et al.*, 1994) and is being evaluated against clinical human immunodeficiency virus and Epstein–Barr virus infections. This class of compounds also inhibits parasites (Kaminsky *et al.*, 1994) and has anti-proliferative (Otova *et al.*, 1993a,b; Rose *et al.*, 1990) and immunomodulatory (Otova *et al.*, 1994; Calio *et al.*, 1994; Del Gobbo *et al.*, 1991) activity. Inhibitory activity against respiratory viruses has received only scant attention.

In this study, we report on the *in vitro* inhibitory activity of 20 phosphonates against influenza A and B viruses, adenovirus (Ad), respiratory syncytial virus (RSV), measles virus (MV) and parainfluenza virus 3 (PIV). Some were very specific and potent inhibitors of virus replication.

Materials and Experimental Procedures: Chemistry

Reactions were done under a dry argon atmosphere unless otherwise noted. Solvents were anhydrous grade (Aldrich). A 230–400 mesh silica gel was used for column chromatography. ^{1}H and ^{31}P NMR spectra were recorded

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at 300 MHz and 121.7 MHz, respectively; *J* values are reported in Hz. Solutions after extractive isolation were dried over $MgSO_4$ unless otherwise indicated.

9-[[2-Iodo-1-[(diethylphosphinyl)methoxy]-ethoxy]methyl]adenine **24**

To a suspension of **21** (956 mg, 5.0 mmol) and diethyl (hydroxymethyl)phosphonate (5.04 g, 30.0 mmol) in CH_2Cl_2 (20 mL) at 0°C was added an I_2 solution (2.54 g, 10.0 mmol, in 70 mL CH_2Cl_2). The mixture was stirred for 30 min at 0°C. The organic phase was washed with saturated $NaHCO_3$, 10% NaS_2O_3 and brine, and dried (Na_2SO_4), concentrated and chromatographed (5% MeOH in CH_2Cl_2) to afford **24** (602 mg, 25%) as a white foam: ^{1}H NMR ($CDCl_3$) δ 8.39 (s, 1H), 8.05 (s, 1H), 5.85 (d, 1H, *J* 10.8), 5.70 (d, 1H, *J* 10.8), 5.60 (br s, 2H), 4.96 (t, 1H, *J* 5.4), 4.21 (m, 4H), 3.92 (m, 2H), 3.20 (d, 2H, *J* 5.1), 1.37 (t, 6H, *J* 6.9); ^{31}P NMR ($CDCl_3$) δ 20.04.

9-[[2-Iodo-1-[(diethylphosphinyl)methoxy]-ethoxy]methyl]cytosine **25**

This compound was prepared from **22** as described for compound **24** and the mixture was stirred for 1.5 h at room temperature. After preparing the organic phase as described above for **24**, the residue was chromatographed (10% MeOH in CH_2Cl_2) to yield **25** (539 mg, 54%) as a white foam: ^{1}H NMR ($CDCl_3$) δ 8.03 (br s, 1H), 7.40 (d, 1H, *J* 7.3), 6.97 (br s, 1H), 6.03 (d, 1H, *J* 7.3), 5.50 (d, 1H, *J* 10.3), 5.14 (d, 1H, *J* 10.3), 4.96 (t, 1H, *J* 5.1), 4.20 (m, 4H), 3.98 (m, 2H), 3.24 (d, 2H, *J* 5.1), 1.36 (t, 6H, *J* 7.0).

2-(Acetylamino)-6-[(diphenylcarbamoyl)oxy]-9-[[2-iodo-1-[(diisopropylphosphinyl)methoxy]-ethoxy]-methyl]purine **26**

This compound was prepared from **23** as described for compound **24** and the mixture was stirred for 1 h. The organic phase was washed with saturated $NaHCO_3$, 10% NaS_2O_3 and brine, dried and the residue chromatographed (gradient 2–4% 2-propanol in CH_2Cl_2) to yield **26** (5.6 g, 41%) as a white foam: ^{1}H NMR ($CDCl_3$) δ 8.61 (br s, 1H), 8.45 (s, 1H), 7.52–7.20 (m, 10H), 5.85 (d, 1H, *J* 10.5), 5.70 (d, 1H, *J* 10.5), 4.97 (t, 1H, *J* 5.0), 4.90–4.75 (m, 2H), 3.96–3.80 (m, 2H), 3.20 (m, 2H), 2.51 (s, 3H), 1.35 (m, 12H); ^{31}P NMR ($CDCl_3$) δ 18.1.

9-[[2-Acetoxy-1-[(diethylphosphinyl)methoxy]-ethoxy]methyl]adenine **27**

To a solution of **24** (401 mg, 0.83 mmol) in DMF (8.3 mL) was added tetraethylammonium acetate (2.16 mg, 8.3 mmol). The reaction mixture was heated at 60°C for 5 h and the solvent was evaporated. The residue was diluted with $CHCl_3$, washed with water and brine, and dried (Na_2SO_4) and evaporated. The residue was chromatographed (10% MeOH in CH_2Cl_2) to afford **27** (184 mg, 53%) as a white foam: ^{1}H NMR ($CDCl_3$) δ 8.37 (s, 1H), 8.05 (s, 1H), 6.06 (br s, 2H), 5.87 (d, 1H, *J* 10.5), 5.71 (d, 1H, *J* 10.5), 5.08 (t, 1H, *J* 5.2), 4.21 (m, 4H), 4.11 (m, 2H), 3.92 (m, 2H), 1.96 (s, 3H), 1.37 (t, 6H, *J* 6.9).

9-[[1-[(Diethylphosphinyl)methoxy]ethoxy]-methyl]adenine **28**

Compound **24** (258 mg, 0.53 mmol) in MeOH (5.3 mL) was treated with triethylamine (538 mg, 5.3 mmol) and stirred with 10% Pd/C (258 mg) under H_2 for 1 h. Celite (1 g) was added, the mix stirred for 5 min and filtered. The filtrate was evaporated and the residue chromatographed (5% MeOH in CH_2Cl_2) to give **28** (157 mg, 82%), a white foam: ^{1}H NMR ($CDCl_3$) δ 8.39 (s, 1H), 8.04 (s, 1H), 5.78 (d, 1H, *J* 10.8), 5.67 (d, 1H, *J* 10.8), 5.60 (br s, 2H), 5.03 (t, 1H, *J* 5.3), 4.21 (m, 4H), 3.82 (d, 2H, *J* 10.2), 1.36 (t, 6H, *J* 7.1), 1.32 (t, 3H, *J* 5.3); ^{31}P NMR ($CDCl_3$) δ 21.49.

9-[[2-Acetoxy-1-[(diethylphosphinyl)methoxy]-ethoxy]methyl]cytosine **29**

This compound was prepared from **25** as described for compound **27** and the solution was heated at 60 °C for 6 h. The solvent was evaporated and the residue was diluted with $CHCl_3$ and washed with water and brine, and dried (Na_2SO_4). The solvent was evaporated and the residue was chromatographed (10% MeOH in CH_2Cl_2) to afford **29** (259 mg, 66%) as a white foam: ^{1}H NMR ($CDCl_3$) δ 7.87 (br s, 1H), 7.40 (d, 1H, *J* 7.4), 6.94 (br s, 1H), 6.05 (d, 1H, *J* 7.4), 5.47 (d, 1H, *J* 9.8), 5.21 (d, 1H, *J* 9.8), 5.11 (t, 1H, *J* 4.9), 4.71 (m, 4H), 4.07 (m, 2H), 3.97 (m 2H), 2.11 (s, 3H),1.37 (t, 6H, *J* 6.7).

2-(Acetylamino)-6-[((diphenylcarbamoyl)oxy)-9-[(2-acetoxy)-1-[(diisopropylphosphinyl)methoxy]-ethoxy]-methyl]purine **30**

This compound was prepared from **26** as described for compound **27** and the solution was heated at 50°C for 3 h. The solvent was evaporated and the residue was diluted with ethyl acetate and was washed with water and brine, and dried. The residue was purified by chromatography (2.5% MeOH in CH_2Cl_2) to afford **30** (2.2g, 44%) as a white foam: ^{1}H NMR ($CDCl_3$) 8.63 (br s, 1H), 8.15 (s, 1H), 7.45–7.21 (m, 10H), 5.84 (d, 1H, *J* 10.8), 5.69 (d, 1H, *J* 10.8), 5.07 (t, 1H, *J* 5.2), 4.86–4.73 (m, 2H), 4.16–4.04 (m, 2H), 3.89 (d, 2H, *J* 9.9), 2.52 (s, 3H), 1.97 (s, 3H), 1.35 (m, 12H); ^{31}P NMR ($CDCl_3$) δ 18.3.

2-(Acetylamino)-6-[((diphenylcarbamoyl)oxy)-9-[1-[(diisopropylphosphinyl)methoxy]ethoxy]-methyl]purine **31**

Iodide **26** (0.1 g, 0.32 mmol) was dissolved in MeOH (5 mL). Triethylamine (0.2 mL, 1.4 mmol) was added followed by 5% Pd/C (10% w/w). The suspension was stirred under H_2 (1 atmosphere) for 48 h. The reaction mixture

was filtered through celite and the filtrate was evaporated. The residue was resuspended in CH_2Cl_2 (10 mL), washed with water and brine, dried and evaporated. The crude product was chromatographed (3% MeOH in CH_2Cl_2) to give **31** (40.6 mg, 47%) as a white foam: ^{1}H NMR ($CDCl_3$) δ 8.55 (br s, 1H), 8.16 (s, 1H), 7.5–7.2 (m, 10H), 5.82 (d, 1H, *J* 11.6), 5.62 (d, 1H, *J* 11.6), 5.05–4.98 (m, 1H), 4.86–4.70 (m, 2H), 3.85–3.6 (m, 2H), 2.53 (s, 3H), 1.40–1.24 (m, 15H); ^{31}P NMR ($CDCl_3$) δ 16.2.

*9-[[2-Hydroxy-1-(phosphonomethoxy)ethoxy]-methyl]adenine disodium salt **12***

A solution of **27** (146 mg, 0.37 mmol) in CH_3CN (3.8 mL) at 0°C was treated with 2,6-lutidine (0.891 mL) and trimethylsilyl bromide (586 mg, 3.8 mmol). The reaction mixture was stirred at room temperature for 18 h. The volatiles were evaporated and the residue was dissolved in ammonium hydroxide (2 mL), stirred for an additional 30 min and evaporated. The ammonium salt was converted to the sodium salt by ion exchange (Ag 50W-X8) and precipitated with water/acetone to afford **12** (102 mg, 80%) as a white powder: ^{1}H NMR (D_2O) δ 8.30 (s, 1H), 8.22 (s, 1H), 5.82 (ABq, 2H, *J* 12.0), 4.86 (t, 1H, *J* 4.8), 3.49–3.71 (m, 4H).

*9-[[2-Hydroxy-1-(phosphonomethoxy)ethoxy]-methyl]guanine ammonium salt **13***

To a solution of **30** (980 mg, 1.38 mmol) in MeOH (22 mL) was added ammonium hydroxide (11 mL) and the solution was refluxed for 1.5 h. The solvents were evaporated and the residue was evaporated twice from MeOH. The residue was chromatographed (10% MeOH in CH_2Cl_2) to afford a white solid (500 mg) which was suspended in CH_3CN (10 mL), cooled to 0°C and treated sequentially with 2,6-lutidine (2.8 mL, 23.8 mmol) and trimethylsilyl bromide (1.6 mL, 11.9 mmol). After stirring for several min at 0°C the reaction was warmed to room temperature and stirred for 18 h. To the resulting suspension was added 1.4 M ammonium hydroxide (25 mL) and the solution was evaporated. The residue was dissolved again in 1.4 M ammonium hydroxide (25 mL), evaporated and the solid obtained was slurried in 1:1 acetone:water (30 mL), filtered, and washed with 1:1 acetone:water and acetone, and dried to afford **13** (382 mg, 78%) as a white solid: ^{1}H NMR (D_2O) δ 7.96 (s, 1H), 5.65 (ABq, 2H, *J* 11.4), 4.86 (t, 1H, *J* 4.9), 3.71 (dd, 1H, *J* 9.4, 12.6), 3.61 (dd, 1H, *J* 5.1, 12.3), 3.56–3.49 (m, 2H); ^{31}P NMR (D_2O) δ 14.3.

*9-[[1-(phosphonomethoxy)ethoxy]methyl]adenine ammonium salt **14***

To a solution of **29** (146 mg, 0.37 mmol) in DMF (3.7 mL) was added trimethylsilyl bromide (569 mg, 3.7 mmol) at 0°C. The reaction mixture was stirred for 3 h at room temperature, ammonium hydroxide (3.8 mL) was added and the solution was evaporated. Purification of the residue by C_{18} reverse phase HPLC (CH_3CN/water) afforded **14** (82 mg, 83%) as a white solid: ^{1}H NMR (D_2O) δ 7.82 (d, 1H, *J* 6.6), 6.10 (d, 1H, *J* 6.6), 5.39 (ABq, 2H, *J* 10.0), 4.88 (t, 1H, *J* 4.8), 3.74 (m, 2H), 3.63 (m, 2H); ^{31}P NMR (D_2O) δ 15.22.

*9-[[2-Hydroxy-1-(phosphonomethoxy)ethoxy]-methyl]cytosine ammonium salt **15***

To a solution of **28** (72 mg, 0.20 mmol), 2,6-lutidine(0.48 mL) and CH_3CN (2.0 mL) was added trimethylsilyl bromide (308 mg, 2.0 mmol) at 0°C. The reaction mixture was warmed to room temperature and stirred for 18 h. The reaction was evaporated and ammonium hydroxide (2 mL) was added and stirred for additional 5 min. The solvent was evaporated, converted to the sodium salt by ion exchange (Ag 50W-X8) and precipitated with water/acetone to afford **15** (62 mg, 89%) as a white powder: ^{1}H NMR (D_2O) δ 8.33 (s, 1H), 8.24 (s, 1H), 5.79 (ABq, 2H, *J* 10.3), 5.02 (t, 1H, *J* 5.4), 3.54 (m, 2H), 1.29 (d, 1H, *J* 5.4); ^{31}P NMR (D_2O) δ 14.64.

*9-[[1-(Phosphonomethoxy)ethoxy]methyl]-guanine disodium salt **16***

To a solution of **31** (0.25 g, 0.06 mmol) in MeOH (1 mL) was added ammonium hydroxide (0.5 mL), the solution was heated to 60°C for 3.5 h and the volatiles evaporated. The residue was chromatographed (10% MeOH in CH_2Cl_2) to yield the diisopropyl ester (24.9 mg) which was suspended in CH_3CN, cooled to 0°C and treated with 2,6-lutidine (0.23 mL, 2 mmol) followed by trimethylsilyl bromide (0.132 mL, 1 mmol). The reaction was stirred for 5 min at 0°C, warmed to room temperature and stirred for 15 h. Volatiles were evaporated, ammonium hydroxide (2 mL) was added and evaporated twice. The residue was purified by C_{18} reverse phase HPLC (gradient: 0–30% B; A, 5% CH_3CN in 0.1 M triethylammonium acetate; B, 75% CH_3CN in 0.1 M triethylammonium acetate) and subsequently desalted by C_{18} reverse phase HPLC (gradient: 0–100% CH_3CN in water). The ammonium salt was converted to the sodium form by ion exchange (Ag 50W-X8) and evaporated to give **16** (22 mg, 98%), a white solid: ^{1}H NMR (D_2O) δ 7.95 (s, 1H), 5.61–5.59 (m, 2H), 5.03–4.99 (m, 1H), 3.66–3.55 (m, 2H), 1.30 (d, 3H, *J* 5.4); ^{31}P NMR (D_2O) δ 17.4.

Materials and Experimental Procedures: Virology

Cells

Embryonic African green monkey kidney cells (MA-104) were obtained from BioWhittaker (Walkersville, Md., USA) and were grown in MEM (Gibco BRL) sup-

cytotoxicity. The 50% cytotoxic doses (CC_{50}) were calculated by regression analysis and a selectivity index (SI) was calculated using the formula $SI=(CC_{50})/(EC_{50})$.

Neutral red (NR) uptake assay

A modified method of Cavenaugh *et al.* (1990) was used. Medium was removed from each well on a plate used for the CPE inhibition assay and 0.2 mL of NR (0.034% in physiological saline) was added to the wells of that plate and incubated for 2 h at 37°C in the dark. The NR solution was then removed from the wells and the wells were rinsed twice with PBS pH 7.4. Equal volumes (0.1 mL) of absolute ethanol and Sorenson citrate buffer (0.1 M sodium citrate, 0.1 M HCl pH 4.2) were mixed together and added to the wells. Plates were incubated in the dark for 30 min at room temperature to solubilize the dye. The plates were then gently mixed on a 96-well plate-adapted vortexer for 1 min. Absorbance at 540 nm and 450 nm was read with a microplate reader (Bio-Tek EL 1309). All compound concentrations were assayed in quadruplicate. Absorbance values were expressed as a percentage of untreated controls and EC_{50} and CC_{50} values were calculated by regression analysis.

Virus yield assay

As a follow-up to the CPE inhibition and NR uptake assays, virus yields from a second CPE inhibition assay were determined for each active compound (SI>10). After CPE was scored as described above, each plate was frozen at −80°C and thawed. Samples wells at each compound concentration tested were pooled and titrated for infectious virus. Titration was accomplished by using a 10-fold dilution series in medium without serum. Each dilution was plated in triplicate on monolayers of susceptible cells. After absorption (1 h) the sample was removed, maintenance medium added and the plates incubated at 37°C until the virus control wells showed 4+ CPE.

Because a 50% reduction of virus titre represents relatively weak antiviral activity, a 90% reduction in virus yield was calculated by regression analysis. This represented a 1 $\log_{10}$ inhibition in titre when compared to untreated virus controls. An SI was calculated by the formula $SI=CC_{50}/EC_{90}$. The CC_{50} was from a cell yield assay of log phase cells exposed to compound.

Cell yield assay

Cytotoxicity in rapidly dividing cells was evaluated by determining the total number of cells after a 3 day exposure to several concentrations of compound. Twelve-well tissue culture plates were seeded with 5×10^4 cells suspended in growth medium. After 4 h incubation at 37°C, the cells were approximately 20% confluent; the medium was replaced with growth medium containing the same concentrations of test compound as in the antiviral experiments. The cells were then incubated at 37°C for 72 h, at which time the medium was removed and the cells washed twice. Trypsin (0.25%) was added to each well and the cells incubated until they rounded and began to come off the plates. The medium containing trypsinized cells from each well was then subjected to vigorous trituration by pipette to provide a uniform cell suspension. From each suspension, 0.2 mL were added to 9.8 mL of Isoton III (Coulter), an

Table 1. Antiviral activity of phosphonomethylether nucleosides against clinically important respiratory viruses.

	CPE inhibition assay			NR uptake assay			Virus yield reduction assay		
Compound	EC_{50} (μg mL^{-1})	CC_{50} (μg mL^{-1})	SI	EC_{50} (μg mL^{-1})	CC_{50} (μg mL^{-1})	SI	EC_{90} (μg mL^{-1})	CC_{50} (μg mL^{-1})[a]	SI
Ad5 (adenoid 75)									
4[b]	10	40	4	3	26	9	0.1	6	60
5	45	140	3	5	50	10	2	>100[c]	>50
17	100	>1000[c]	>10	15	440	36	15	>100[c]	>7
HPMPA	8	120	15	5	>120[c]	>24	1	20	20
MV (CC)									
3	9	120	13	10	300	30	12	>100[c]	>8
Ribavirin	10	560	56	5	450	90	15	180	16
PIV-3 (C-243)									
3	32	350	11	5	260	52	17	>100[c]	>6
11	14	427	31	3	>1000[c]	>333	>100[d]	30	0
Ribavirin	10	560	56	5	450	90	4	180	45

[a]Determined in rapidly growing cells.

[b]HPMPA synthesized by Gilead Sciences (Foster City, Calif., USA) and received coded.

[c]Highest concentration tested.

[d]Complete cytotoxicity was observed at concentrations greater than shown.

Table 2. Inhibitory activity of compound **3** against different PIV-3 strains and clinical isolates.

		CPE inhibition assay			NR uptake assay			Virus yield reduction assay
Compound	Virus strain/type	EC_{50} (μg mL^{-1})	CC_{50} (μg mL^{-1})	SI	EC_{50} (μg mL^{-1})	CC_{50} (μg mL^{-1})	SI	EC_{90} (μg mL^{-1})
3	14798	32	29	1	32	11	0.5	7
	14702	32	29	1	10	11	1	10
	SF	32	29	1	56	11	0	3
11	14798	100	>100[a]	>1	100	75	1	40
	14702	100	>100[a]	>1	100	75	1	11
	SF	100	>100[a]	>1	>100[b]	75	0	31
Ribavirin	14798	4	180	13	4	110	28	5
	14702	10	55	5	8	100	13	15
	SF	10	670	67	16	>1000[a]	63	13

[a]Concentrations greater than those shown were not done.

[b]Complete cytotoxicity was observed at concentrations greater than shown.

isotonic saline solution. Three samples from each cell suspension were then counted using a Coulter cell counter (Model Zb-1). CC_{50} was then determined by regression analysis using percentages of control for each drug dilution lying within the linear portion of the regression line.

Results

Chemistry

The synthesis of compounds (Fig. 1) **1**, **2**, **3**, **4** and **5** have been summarized in Holy (1993), Holy *et al.* (1989) and Bronson *et al.* (1989). Compounds **6**, **7** and **8** were synthesized by the method of Holy *et al.* (A Holy, H Dvorakova, E De Clercq, A Desire, J Balzarini & R Marie, 1994; Anti-retroviral enantiomeric nucleotide analogs. Patent No. WO 94/03467). The preparation of **9**, **10**, **11**, **13** and **16** was described by Kim *et al.* (1990, 1991a), except to derive **10** the diaminopurine sodium salt was used to alkylate the diethyl chloromethoxy-methoxymethyl phosphonate. The synthesis of phosphonates **12**, **13**, **14**, **15** and **16** is shown in Fig. 2. Vinyl ethers **21**, **22** [CU Kim, JC Martin, BY Luh & PF Misco, 1991; antiviral (phosphonomethoxy) methoxy purine/pyrimidine derivatives. Patent No. EP 0494370A1] and **23** (Kim *et al.*, 1991b) were reacted with diethyl or diisopropyl(hydroxymethyl) phosphonate in the presence of I_2 affording iodides **24**, **25**, and **26**, respectively, which were treated with tetraethylammonium acetate in DMF to yield the corresponding acetates. Direct catalytic hydrogenation of iodides **24** and **26** with Pd/C and triethyl-amine gave methyl derivatives **28** and **31**, respectively. Final deprotection of phosphonate esters **27**, **28** and **29** was done using trimethylsilyl bromide (TMSB) and excess 2,6-lutidine. Phosphonate **14** was isolated as the ammonium salt and **12** and **15** as the sodium salts after ion exchange. Deprotection of **30** and **31** was a two step sequence. Cleavage of the diphenyl-carbamoyl and acetate-protecting groups was accomplished with refluxing methanolic NH_4OH. Subsequent removal of the phosphonate esters was done with TMSB and 2,6-lutidine followed by treatment with aqueous NH_4OH and gave **13** as the ammonium salt. Phosphonate **16** was isolated as the disodium salt after HPLC purification and ion exchange. The synthesis of **17** has been described by Kim *et al.* [CU Kim, JC Martin, BY Luh & PF Misco, 1991; antiviral (phosphonomethoxy) methoxy purine/pyrimidine derivatives. Patent No. EP 0494370A1], **18** and **19** by Kim *et al.* (1991b) and **20** by Kim *et al.* (1992).

Activity against influenza virus and RSV

Compound **13** was slightly inhibitory to two influenza A viruses (H1N1; EC_{50} 190, CC_{50} >200, H1N3; EC_{50} 24, CC_{50} >100) and influenza B virus (EC_{50} 22, CC_{50} >100). The EC_{50} values for the other compounds were generally >500 μg mL^{-1} (data not shown). Compound **19** did not inhibit RSV strains A2, Long, 18537 or bovine RSV by CPE inhibition and NR assays, although it did inhibit strain Utah 89 by CPE inhibition and virus yield reduction assays.

Activity against PIV-3

Two compounds, **3** and **11** had activity (SI>10) against PIV-3 with EC_{50} values of 5 and 3 μg mL^{-1} and SI of 52 and >333 in the NR assay (Table 1). However, when **11** was further evaluated in a virus yield reduction assay the EC_{90} was >100 and CC_{50} was 30 (determined in actively growing cells). The anti-PIV activity of **3** was verified by a virus yield reduction assay, giving an EC_{90} of 17 μg mL^{-1} (ribavirin 4 μg mL^{-1}). Compounds **3** and **11** were only slightly inhibitory for two clinical isolates and the bovine

Anti-Ad activity could involve inhibition of viral DNA polymerases, as has been shown with HSV-1 (Holy *et al.*, 1990), inhibition of the processivity of the Ad-mediated DNA polymerization reaction enhanced by Ad DNA binding protein, as has been postulated with HPMPA (Mul *et al.*, 1989), or inhibition of key cellular target proteins necessary for viral replication.

In summary, we have identified promising lead compounds with inhibitory activity *in vitro* against PIV-3, MV and Ad. The true potential of these compounds will be further addressed by more i*n vitro* testing, evaluation in animal models and by exploring their mechanism of action.

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