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Antiviral evaluation of octadecyloxyethyl esters of (*S*)-3-hydroxy-2-(phosphonomethoxy)propyl nucleosides against herpesviruses and orthopoxviruses☆

Nadejda Valiaeva[a,b], Mark N. Prichard[c], R. Mark Buller[d], James R. Beadle[a,b], Caroll B. Hartline[c], Kathy A. Keith[c], Jill Schriewer[d], Julissa Trahan[a,b], Karl Y. Hostetler[a,b,*]

[a] *Department of Medicine, Division of Infectious Disease, University of California, San Diego, La Jolla, CA 92093-0676, United States*
[b] *Veterans Medical Research Foundation, San Diego, CA 92161, United States*
[c] *Department of Pediatrics, University of Alabama School of Medicine, Birmingham, AL 35233, United States*
[d] *Department of Molecular Microbiology and Immunology, St. Louis University Doisy Research Center, St. Louis, MO 63104, United States*

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ABSTRACT

Our previous studies showed that esterification of 9-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl] adenine (HPMPA) or 1-(*S*)-[3-hydroxy-2-(phosphonomethoxy)-propyl]cytosine (HPMPC) with alkoxyalkyl groups such as hexadecyloxypropyl (HDP) or octadecyloxyethyl (ODE) resulted in large increases in antiviral activity and oral bioavailability. The HDP and ODE esters of HPMPA were shown to be active in cells infected with human immunodeficiency virus, type 1 (HIV-1), while HPMPA itself was virtually inactive. To explore this approach in greater detail, we synthesized four new compounds in this series, the ODE esters of 9-(*S*)-[3-hydroxy-2-(phosphonomethoxy)-propyl]guanine (HPMPG), 1-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]thymine (HPMPT), 9-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]-2,6-diaminopurine (HPMPDAP) and 9-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]-2-amino-6-cyclopropylaminopurine (HPMP-cPrDAP) and evaluated their antiviral activity against herpes simplex virus, type 1 (HSV-1), human cytomegalovirus (HCMV), and vaccinia, cowpox and ectromelia. Against HSV-1, subnanomolar EC_{50} values were observed with ODE–HPMPA and ODE–HPMPC while ODE–HPMPG had intermediate antiviral activity with an EC_{50} of 40 nM. In HFF cells infected with HCMV, the lowest EC_{50} values were observed with ODE–HPMPC, 0.9 nM. ODE–HPMPA was highly active with an EC_{50} of 3 nM, while ODE–HPMPG and ODE–HPMPDAP were also highly active with EC_{50}s of 22 and 77 nM, respectively. Against vaccinia and cowpox viruses, ODE–HPMPG and ODE–HPMPDAP were the most active and selective compounds with EC_{50} values of 20–60 nM and selectivity index values of 600–3500. ODE–HPMPG was also active against ectromelia virus with an EC_{50} value of 410 nM and a selectivity index value of 166. ODE–HPMPG and ODE–HPMPDAP are proposed for further preclinical evaluation as possible candidates for treatment of HSV, HCMV or orthopoxvirus diseases.

1. Introduction

Phosphonate nucleoside analogs are an important class of antiviral agents including cidofovir, adefovir and tenofovir which are approved for treatment of cytomegalovirus, hepatitis B and HIV infections, respectively (De Clercq and Holý, 2005; Holý, 2003). However, their cellular uptake is limited by their double negative charge which leads to lower antiviral activity (Aldern et al., 2003; Magee et al., 2008). For example, HPMPA was previously reported to be inactive in HIV-infected cells (Balzarini et al., 1993; De Clercq, 1991). However, we recently found that esterification of HPMPA with alkoxyalkyl groups such as hexadecyloxypropyl (HDP) or octadecyloxyethyl (ODE) results in a marked increase in cell uptake and antiviral activity (Hostetler et al., 2006). We also synthesized a series of 5-phosphono-pent-2-en-1-yl (PPen) nucleosides and found that the unmodified PPen nucleosides lacked antiviral activity in vitro (Choo et al., 2007). However, the PPen compounds were active antivirals when esterified with HDP groups (Choo et al., 2007). Thus, it appears likely that the antiviral activity of phosphonate nucleoside analogs has

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* Corresponding author at: Department of Medicine (0676), University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0676, United States. Tel.: +1 858 552 8585x2616; fax: +1 858 534 6133.
E-mail address: khostetler@ucsd.edu (K.Y. Hostetler).

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been systematically underestimated because of poor cell penetration.

To evaluate this in more detail, we compared the ODE esters of HPMPA (Beadle et al., 2006) and HPMPC (Beadle et al., 2002) with four newly synthesized compounds, the ODE esters of HPMPG, HPMPT, HPMPDAP and HPMP-cPrDAP. The antiviral activity of these compounds was determined in cells infected with HSV-1, human cytomegalovirus (HCMV), murine cytomegalovirus (MCMV), vaccinia virus, cowpox virus and ectromelia virus. The ODE esters were substantially more active against HSV-1 than published data for the unmodified acyclic nucleoside phosphonates. The ODE esters were also highly active against HCMV and MCMV and were several orders of magnitude more active than ganciclovir. The most active compound against orthopoxviruses was ODE–HPMPA, but it was also the most cytotoxic of the series. The most active and selective compounds against orthopoxviruses were ODE–HPMPG and ODE–HPMPDAP.

2. Materials and methods

2.1. General chemistry methods

^{1}H and ^{31}P nuclear magnetic resonance (NMR) spectra were recorded on a Varian HG-300 spectrometer at 300 MHz for ^{1}H NMR and are reported in units of ppm relative to internal tetramethylsilane at 0.00 ppm. Mass spectra were recorded on either a Finnigan LCQDECA mass spectrometer or ThermoFinnigan MAT900XL high-resolution mass spectrometer at the small molecule facility in Department of Chemistry at University of California, San Diego. In addition to the spectral data provided, purity, of the target compounds (>98%) was confirmed by analytical thin layer chromatography (TLC) using Analtech Uniplate™ silica gel-GF (250 μm) plates. The plates were developed using the solvent system $CHCl_3$/MeOH/con NH_4OH/H_2O (70:30:3:3, v/v) and visualized with UV light, phospray (Supelco; Bellefonte, PA, USA) and charring at 400 °C. Flash chromatography was performed with silica gel (Merck silica gel 60, 230–400 mesh).

2.2. General method for synthesis of 3-trityloxy-2-hydroxypropyl nucleosides (**5**–**8**) (Scheme 1)

A suspension of the corresponding heterocyclic base **1**–**4** (1 mmol) and cesium carbonate (0.1 mmol) or sodium hydride (0.1 mmol) in dry DMF (25 ml) was stirred at room temperature for 1 h. (*S*)-Trityl glycidyl ether (DAISO Co., Ltd.) (0.9 mmol) was added in one portion. The mixture was stirred at 80 °C overnight. DMF was evaporated, the residue was purified by column chromatography on silica gel (eluent: dichloromethane–methanol, 0–20%) to give product.

2.2.1. 1-(S)-(3-Trityloxy-2-hydroxypropyl)-4-methoxy-5-methyl-2-pyrimidone (**5**)

It was synthesized from 4-methoxy-5-methyl-2-pyrimidone and S-trityl glycidyl ether (cesium carbonate base) as described by Wong and Fuchs (1970). Yield 87%. ^{1}H NMR ($CDCl_3$): δ 8.00 (s, 1H), 7.21–7.47 (m, 15H), 4.04–4.14 (m, 2H), 3.65–3.74 (m, 1H), 3.30 (s, 3H), 3.06–3.22 (m, 2H), 1.85 (s, 3H). MS-ESI (*m*/*z*) 479.18 $(M+Na)^+$.

2.2.2. 9-(S)-(3-Trityloxy-2-hydroxypropyl)-6-O-benzylguanine (**6**)

It was synthesized from 6-O-benzylguanine and S-trityl glycidyl ether (sodium hydride base) as reported by Liu et al. (2003). Yield 57%. ^{1}H NMR ($CDCl_3$): δ 8.00 (s, 1H), 7.19–7.50 (m, 20H), 5.49 (s, 2H), 5.20 (br.s, 1H), 4.20–4.30 (m, 1H), 4.10–4.20 (m, 2H), 2.98–3.22 (m, 2H). MS-ESI (*m*/*z*) 558.24 $(M+H)^+$.

2.2.3. 9-(S)-(3-Trityloxy-2-hydroxypropyl)-2,6-diaminopurine (**7**)

It was synthesized from 2,6-diaminopurine activated by sodium hydride and S-trityl glycidyl ether. Yield 75%. ^{1}H NMR ($CDCl_3$): δ 7.35–7.50 (m, 7H), 7.15–7.35 (m, 9H), 5.83 (br.s, 2H), 4.91 (br.s, 2H), 4.05–4.25 (m, 3H), 3.22–3.33 (m, 1H), 2.93–3.05 (m, 1H). MS-ESI (*m*/*z*) 467.21 $(M+H)^+$.

2.2.4. 9-(S)-(3-Trityloxy-2-hydroxypropyl)-2-amino-6-cyclopropylaminopurine (**8**)

It was synthesized from 2-amino-6-cyclopropylaminopurine activated by sodium hydride and S-trityl glycidyl ether. Yield 12%. ^{1}H NMR ($CDCl_3$): δ 8.00 (s, 1H), 7.21–7.45 (m, 15H), 5.91 (br.s, 2H), 4.05–4.30 (m, 2H), 3.55–3.75 (m, 1H), 3.15–3.35 (m, 2H), 0.45–0.70 (m, 2H), 0.70–95 (m, 2H). MS-ESI (*m*/*z*) 507.21 $(M+H)^+$.

2.3. General method for synthesis of 2-(octadecyloxy)ethyl (S)-3-trityloxy-2-(phosphonomethoxy)propyl nucleosides **9**–**12**

2-(Octadecyloxy)ethyl p-toluene-sulfonyloxymethyl-phosphonate (1.5 mmol) prepared as described by Beadle et al. (2006) was added to a mixture of the corresponding (*S*)-(3-trityloxy-2-hydroxypropyl)-nucleoside **5**–**8** (1.0 mmol) and sodium tert-butoxide (2.0 mmol) in dry triethylamine (50 ml). The mixture was stirred at 70 °C for 24 h. The solvent was evaporated; the residue was purified by column chromatography to give the product.

2.3.1. 2-(Octadecyloxy)ethyl 1-(S)-[3-trityloxy-2-(phosphonomethoxy)propyl] 4-methoxy-5-methyl-2-pyrimidone (**9**)

Yield 23%. ^{1}H NMR ($CDCl_3$ + methanol-d_4): δ 7.43–7.7.48 (m, 8H), 7.24–7.33 (m, 7H), 7.12 (s, 1H), 3.91–4.09 (m, 4H), 3.80–3.91 (m, 1H), 3.50–3.61 (m, 4H), 3.39–3.43 (m, 2H), 3.36 (s, 3H), 3.25–3.32 (m, 2H), 1.74 (s, 3H), 1.48–1.60 (m, 2H), 1.18–1.38 (m, 30H); 0.88 (t, J = 7 Hz, 3H). MS-ESI (*m*/*z*) 845.49 $(M-H)^-$.

2.3.2. 2-(Octadecyloxy)ethyl 9-(S)-[3-trityloxy-2-(phosphonomethoxy)propyl] 6-O-benzylguanine (**10**)

Yield 23%. ^{1}H NMR ($CDCl_3$ + methanol-d_4): δ 7.90 (s, 1H), 7.18–7.33 (m, 20H), 5.50 (s, 2H), 3.88–4.03 (m, 2H), 3.60–3.80 (m, 2H), 3.39–3.50 (m, 3H), 3.22–3.39 (m, 4H), 3.08–3.20 (m, 2H), 1.45–1.62 (m, 2H), 1.00–1.40 (m, 30H); 0.88 (t, J = 7 Hz, 3H). MS-ESI (*m*/*z*) 948.15 $(M+H)^+$.

2.3.3. 2-(Octadecyloxy)ethyl 9-(S)-[3-trityloxy-2-(phosphonomethoxy)propyl] 2,6-diaminopurine (**11**)

Yield 31%. ^{1}H NMR ($CDCl_3$ + methanol-d_4): δ 7.56 (s, 1H), 7.20–7.48 (m, 15H), 4.00–4.15 (m, 2H), 3.55–3.68 (m, 4H), 3.40–3.55 (m, 3H), 3.20–3.40 (m, 4H), 1.42–1.62 (m, 2H), 1.15–1.40 (m, 30H); 0.89 (t, J = 7 Hz, 3H). MS-ESI (*m*/*z*) 615.41 $(M+H)^+$, 637.31 $(M+Na)^+$, 653.32 $(M+K)^+$, 613.36 $(M-H)^-$.

2.3.4. 2-(Octadecyloxy)ethyl 9-(S)-[3-trityloxy-2-(phosphonomethoxy)propyl] 2-amino-6-cyclopropylaminopurine (**12**)

Yield 49%. ^{1}H NMR ($CDCl_3$ + methanol-d_4): δ 7.96 (s, 1H), 7.26–7.62 (m, 15H), 4.15–4.40 (m, 2H), 3.60–3.72 (m, 4H), 3.40–3.58 (m, 3H), 3.25–3.48 (m, 4H), 1.42–1.62 (m, 2H), 1.15–1.40 (m, 31H); 0.89 (t, J = 7 Hz, 3H), 0.50–0.78 (m, 4H). MS-ESI (*m*/*z*) 895.53 $(M-H)^-$.