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Phosphonoacid Prodrugs with Greatly Increased Antiviral Activity in HCMV-Infected Cells, *in vitro*. K.Y. Hostetler, K.A. Aldern, G.D. Kini, C.J. Wheeler and C.N. Sridhar, University of California, San Diego, La Jolla, CA 92093-0676; Veterans Affairs Medical Center, San Diego, CA. 92161; and Vical Inc., San Diego, CA., 92121.

Phosphonoformate (PFA) and phosphonoacetate (PAA) were first synthesized in 1924 by Nylén and were later shown to inhibit a variety of viral DNA polymerases. PFA is currently approved for treating human cytomegalovirus (HCMV) infection but has significant toxicity. It would be useful to discover more effective forms of PFA. We synthesized several lipid prodrugs to see if activity could be increased. Batyl alcohol (1-0-octadecyl-sn-glycerol) and dimyristoylglycerol (DMG) were conjugated to the phosphate of PFA and PAA and antiviral activity was assessed in MRC5 human lung fibroblasts infected with the AD169 strain of HCMV. Viral replication was assessed with and without graded levels of PFA and its analogs using a DNA probe method. Cytotoxicity was assessed in subconfluent MRC5 cells by a visual grading method. DMG-PFA, batyl-PFA (B-PFA) and batyl-PAA (B-PAA) were applied to HCMV-infected MRC5 human fibroblasts and HCMV-specific DNA levels were determined. DMG-PFA was less effective than PFA, IC_{50} 141 vs 46 μM. However, the batyl alcohol conjugates (1-0-octadecyl-sn-glycero-3-PFA and -PAA) exhibited greatly increased antiviral activity; IC_{50} values for B-PFA and B-PAA were 0.43 and 0.22 μM representing a 108- and 210-fold increase versus PFA. The antiviral activity of ganciclovir in the same assay was 1.5 μM and a batyl alcohol control had no antiviral activity. Cytotoxicity was modest with TC_{50} values of 32-100 μM for B-PAA and >1000 μM for B-PFA. B-PFA and B-PAA are potent and selective antiviral prodrugs which may provide an improved approach to therapy of HCMV and other susceptible viruses.

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Synthesis and Antiviral Activity of Novel Phosphonomethyl Ether Nucleosides.
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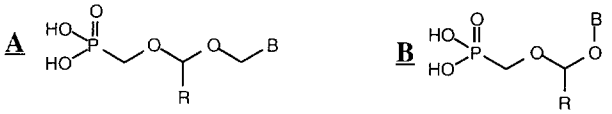

The synthesis and antiviral activity of some compounds of type A and B have previously been described, (Tetrahedron Lett. 33, 25-28 (1992), J. Med. Chem. 34, 2286-94 (1991), Eur. Pat. Appl. 0494370A1, (1992), Eur. Pat. Appl. 0398231A2, (1990)). We report the synthesis and biological evaluation of a number of compounds in both series, some of which are novel. Series A: 1 B=G, R=H; 2 B=A, R=H; 3 B=DAP, R=H; 4 B=A, R=CH_2OH; 5 B=G, R=CH_2OH; 6 B=C, R=CH_2OH; 7 B=G, R=CH_3; 8 B=A, R=CH_3. Series B: 9 B=G, R=H; 10 B=A, R=H; 11 B=A, R=CH_2OH. All compounds were evaluated for their *in vitro* antiviral activity against HSV-2, HCMV, HIV and HBV. Compounds 5 and 9 were potent inhibitors of HBV: 5: IC_{50} = 0.18μM, CC_{50} = >100μM; 9: IC_{50} = 0.08μM, CC_{50} = 15μM. Compounds 1 and 5 were potent inhibitors of HCMV: 1: EC_{50} = 2μM; 5: EC_{50} = 0.3μM and MCMV: 1 EC_{50} = 0.1μM; 5 EC_{50} = 0.01μM. Compound 1 was further evaluated *in vivo* against MCMV infection in mice. Compound 1 was administered to mice i.p. twice daily for 5 days. When treatment was initiated 24 hours post infection significant protections was observed at concentrations as low as 2mg/kg. When therapy was delayed until +72 hours, 10mg/kg significantly altered mortality. In these experiments, compound 1 was comparable to Ganciclovir.