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Efficient Synthesis and Biological Properties of Base Substituted 2,4-Diamino-6-(*R*)-[3-hydroxy-2-(phosphonomethoxy) propoxy]pyrimidine (HPMPO-DAPy)

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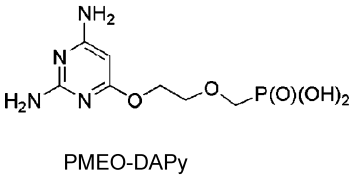
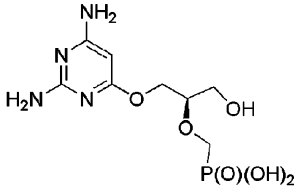
New generation of acyclic nucleoside phosphonates (ANPs) is derived from 2,4-diamino-6-hydroxypyrimidine (Holý et al., 2002; Balzarini et al., 2002). 2,4-Diamino-6-[(phosphonomethoxy)ethoxy]pyrimidine (PMEO-DAPy), its 4-hydroxy congener and 5-substituted derivatives possess pronounced antiviral activity against retroviruses (Hocková et al., 2003, 2004). 2,4-Diamino-6-(*R*)-[3-hydroxy-2-(phosphonomethoxy)propoxy]pyrimidine (HPMPO-DAPy) exhibited antiviral activity against various DNA viruses (adenovirus (Naesens et al., 2005), polyomavirus (Lebeau et al., 2007) and orf virus (Dal Pozzo et al., 2005)). To further study structure–activity relationship we decided to prepare base substituted derivatives bearing the 3-hydroxy-2-(phosphonomethoxy)propoxy (HPMPO) side chain. Various synthetic methods were developed for preparation of HPMPO-DAPy derivatives substituted at positions 2-, 4- and 5- of the pyrimidine base. Details of synthesis and biological activities will be discussed.



PMEO-DAPy



HPMPO-DAPy

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A Single Intranasal Administration of DEF201 Protects Against Lethal Respiratory Challenge with Western Equine Encephalitis Viruses

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Objective: The aim of this study is to evaluate the prophylactic and treatment efficacy of DEF201 against lethal challenge of WEEV strains in a mouse model. DEF201 is a proprietary replication deficient adenovirus type 5 expressing mouse IFNα under development as a broad spectrum antiviral.

Method: Groups ($n = 10$) of female Balb/c mice were treated with a single dose intranasal administration of 1×10^7 pfu of DEF201 to each group up to 21 days prior to challenge. Groups of untreated mice were included as controls. On Day 0 all mice were challenged intranasally with a lethal dose of either WEEV California strain or WEEV CBA87 strain. A group of mice daily treated with a single intraperitoneal injection of 2×10^7 IU/kg IFNα recombinant protein served as a positive control group. Mice were monitored daily for clinical signs of disease during the 14 day post challenge period.

Results: Treatment of mice with DEF201 provided complete protection against otherwise lethal challenge with either WEEV California or CBA87 strains when given at Days 21, 14, 7 or 1 prior to challenge. In addition, when treatment was given 4 h post-challenge 100% protection was still conferred against WEEV California strain whereas 70% of mice survived the challenge with WEEV CBA87 strain. Protected mice demonstrated no drastic change in body weight with little to no clinical signs of WEEV infection which typically include ruffled hair, hunched posture, lethargy and ataxia.

Conclusion: This study demonstrated that DEF201 induced rapid and long-lasting protection against lethal WEEV infection of mice and could potentially be used as a prophylactic and possibly a therapeutic against biothreat agents and emerging pathogens. This work was funded by NIAID-DMID contract N01-AI-30063.

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Synthesis and Solution Structure of DNA Duplexes Containing the Potent Anti-poxvirus Agent Cidofovir

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Cidofovir (1-(S)-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine, CDV) is a potent inhibitor of poxvirus replication. Prior studies showed that the inhibitory mechanism involves (at least) two effects of the active intracellular metabolite of CDV, CDV diphosphate (CDVpp), on reactions catalyzed by vaccinia virus DNA polymerase: (1) after CDV and one more deoxynucleoside monophosphate are incorporated into a growing DNA strand, addition of the next base by the polymerase is greatly slowed, and the 3′-to-5′ exonuclease activity is completely blocked; and, (2) templates containing a CDV residue cannot be extended beyond the CDV base by the vaccinia DNA polymerase, effectively blocking further rounds of replication. As part of our studies to further characterize the mechanism of inhibition of vaccinia virus DNA

polymerase, we analyzed by nuclear magnetic resonance spectroscopy the structure and dynamics of a DNA duplex containing cidofovir and an isosequential control DNA. The oligonucleotide was synthesized from the 5′-to-3′ end by adapting the solid-phase procedure described by Birkus et al. for (S)-HPMPA oligonucleotides [Birkus et al., 2004, Antivir. Chem. Chemother. 15, 23–33]. Reversed 5′ phosphoramidites were introduced into the oligonucleotide chain using phosphoramidite chemistry. The appropriately protected CDV monomer was prepared by a multistep synthesis starting from N^4-benzoylcytosine and (S)-glycidyl trityl ether and was added to the oligo using the phosphotriester condensation method. From the NMR it is clear that the cidofovir containing DNA is distorted in structure. No imino–imino contacts were found in the cidofovir containing DNA, implying that the base pairing dynamics are much higher than that of the control DNA. Further, the imino protons of the neighboring nucleobases near the cidofovir moiety exchange more easily than those in control DNA. These experiments suggest there is a bulge near to the location of cidofovir in the DNA core. These results should provide further details about the mechanisms by which CDV inhibits vaccinia polymerase activity.

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Inhibition of Calicivirus Replication in Mammalian Cells by RNAI

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Caliciviruses are non-enveloped viruses with a single stranded positive orientated RNA genome. They include human pathogenic (norovirus and sapovirus) and non-human pathogenic genera (vesivirus and lagovirus). Because of the increasing number of human infections and the absence of possibilities for vaccination as well as an antiviral therapy, it is of interest to develop antiviral strategies against human pathogenic caliciviruses. The objective of our study is to use the feline calicivirus in order to proof the concept of siRNA application as a new approach to control calicivirus infections. In contrast to the vast majority of caliciviruses that so far remain non-cultivable, the feline calicivirus (FCV, vesivirus) can be passed in CRFK-monolayers and is therefore used as a cell model for antiviral research. Our investigations include two different FCV strains (FCV-DD06 [GB Acc. No. DQ424892] and FCV-2024 [GB Acc. No. AF479590]). We have observed antiviral effects on several aspects of viral replication like reduction of infectious particles, the level of viral genomic RNA and inhibition of viral translation. siRNA-molecules targeting conserved genomic sequences lead either to a complete reduction of the viral titer or to a decrease of virions by two log scales (1.95E + 05 PfU/ml vs. a positive control of 4.08E + 07 PfU/ml). Similarly, viral genomic RNA level was decreased by about 30–50% in comparison to a positive control. Analysis by western blot reveals no detectable signal for structural and non-structural viral proteins after siRNA application. Quantification of the antiviral activity was performed with a cell proliferation assay resulting in an EC50 value of 0.69 μM. Our data underlines the potential of the siRNA approach as a novel strategy to control calicivirus infection.

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Developing Capsid Inhibitor V-073 for Poliovirus

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V-073, an enterovirus capsid inhibitor, has potent broad spectrum of anti-poliovirus activity *in vitro*. The compound has exhibited desirable attributes in nonclinical pharmacologic and toxicologic studies, which support the continued advancement of V-073 to first-in-human clinical studies. These attributes will be reviewed, as will the rationale for additional poliovirus antiviral development.

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Addition Antiflogistic to Viral Inhibitor

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Earlier we have been shown new viral inhibitors may be found among compounds with reliable anti-inflammatory properties. It may be supposed inflammatory and virus inhibitors influence at two independent parts of viral reproduction and therefore combine action of antiflogistic and antiviral remedies may be more potent in comparison with activity level of antiflogistic or virus inhibitor per se. These results present in table. Previous result seems to support this conclusion. Acyclovir is more potent than indometacin but a mixture is active as (I) ever in diminished dose. I + II overestimated as I and II if virus is added after I + II.

Comp	HSV-1, initial titer 4 IgID50, compounds are added in 24 h after virus infection			HSV-1, initial titer 5 IgID50, compounds are added in 24 h before virus infection		
	Acyclovir (I)	Indometacin (II)	I + II (mol:mol)	I	II	I + II (mol:mol)
Dose mkg/m	50	50	25	50	50	37.5
Titer $IgID_{50}$	1	2	1	1	3	<1.0

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Unsymmetrical CADA Analogs as Novel Down-modulators of the CD4 Receptor

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Cyclotriazadisulfonamide (CADA) inhibits the entry of HIV into $CD4^+$ target cells. This effect is due to the compound's ability to down-modulate the expression of the primary cellular receptor for HIV, CD4. Structural modifications of CADA have been made to increase potency, reduce toxicity, and improve physical properties. For 19 CADA analogs, a strict correlation between the CD4 down-modulating and antiviral activities has been observed. The interest