96-well plates containing monolayers of primary human fibroblasts were infected at an MOI of 0.01 PFU/cell and the infection was allowed to proceed for 7 days. The level of luciferase activity expressed by the virus was assayed and used as a surrogate marker for viral replication. The assay yielded EC_{50} values that were comparable to those generated in standard assays for several compounds that are currently licensed for use against HCMV, but appears to offer significant advantages. Chief among them were reduced processing time, reduced incubation time (7 days instead of 14 days) and reduced sensitivity to colored compounds. This assay, paired with the CellTiter Glo® toxicity assay, promises to provide a rapid means to assess cytotoxicity as well as antiviral activity against HCMV.

doi:10.1016/j.antiviral.2008.01.121

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Carbocyclic L-Nucleoside Analogues as Potential Antiviral Agents

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The application of nucleosides in antiviral therapy has grown to a common process over the last three decades. There exists a huge variety of nucleosides, which show several modifications on the nucleobase or the sugar moiety. Due to these changes in nucleoside structure new antiviral activities were found. Beside the commonly used D-configurated natural nucleosides there is the important class of L-nucleosides which are the mirror-images of the natural ones. L-Nucleosides are known for their significant bioactivity, especially their antiviral activity towards Hepatitis B. There are several FDA-approved L-nucleoside analogues, e.g. lamivudinde (3TC), telbivudine (L-thymidine) and clevudine (L-FMAU). With regard to these derivatives we decided to connected the concept of carbocyclic and L-nucleosides within this work to obtain similar carbocyclic L-nucleoside analogues as potential antitumor and antiviral agents. As starting material, we chose a chiral cyclopentenol, which can be prepared from cyclopentadiene by alkylation and a subsequent asymmetric hydroboration. After protection of the formed hydroxy group, the remaining double bond can easily be hydroxylated by different methods, yielding a chiral cyclopentanol. Using a modified Mitsunobu protocol, heterocycles were condensed to this precursor leading to L-configurated pyrimidine und purine carbocyclic nucleosides, e.g.: L-*carba*-dT, L-*carba*-dA, L-*carba*-BVDU or L-*carba*-d4T. The obtained enantiomerically pure carbocyclic nucleosides can simply be converted into the corresponding *cyclo*Sal-pronucleotides or their monophosphate esters (nucleotides) with the aim to improve their activity.

doi:10.1016/j.antiviral.2008.01.122

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Immunoprophylaxis of Phleboviral Infection in Hamsters with Recombinant Eimeria Protozoan Surface Antigen

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Recombinant *Eimeria* antigen (rEA) has been shown to have potent anticancer and antiviral activity in respective mouse disease models, presumably through robust immune stimulation that occurs via TLR11, a pattern recognition receptor that recognizes profilin-like proteins expressed on apicomplexan protozoans. Comparable immunostimulatory activity in other species has yet to be demonstrated. Since rEA is known to be highly effective in treating Punta Toro virus (PTV) infection in mice, its ability to elicit protective immunity in the hamster PTV infection model was investigated. rEA was given alone, or in combination with IL-18 or IL-2, and virally challenged hamsters were observed for mortality. A dose of 100 μg of rEA, given once 4 h prior to viral challenge, and a second time on day 3 of the infection, was found to be the most effective prophylactic therapy protecting 60% of treated hamsters from mortality, compared to only 5–10% observed in animals receiving placebo. In addition, splenic cytokine transcript profiles for IL-12, IL-21, IFN-γ and TNF-α were assessed at various times after a single 100-μg dose treatment of rEA. Only IFN-γ and IL-12 were found to have remarkably increased expression following exposure. The data suggest that rEA does induce host antiviral responses in hamsters that result in significant protection from death, although determining the most appropriate dose for intervention in other species, including humans, will likely be very challenging.

Acknowledgement: Supported in part by contract grant NO1-AI-15435 from the Virology Branch, NIAID, NIH.

doi:10.1016/j.antiviral.2008.01.123

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Novel Inhibitors of Orthopoxvirus Replication Target Vaccinia Virus P37 Envelope Protein

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Three compounds with antiviral activity against orthopoxviruses were identified through routine in vitro screening of over 1800 compounds from a chemical library utilizing cytopathic effect assays. Plaque reduction assays in human foreskin fibroblast cells confirmed the activity of three compounds against both vaccinia (VV) and cowpox (CV)

viruses. Compounds 05-103227, 05-102300 and 05-102691 yielded effective concentration (EC_{50}) values of 1–9 μM for VV and 6–58 μM for CV. Neutral red uptake and CellTiter-Glo cell viability assays were used to measure cellular cytotoxicity and it was determined that all of the compounds were relatively non-cytotoxic. Thus, these compounds are highly selective agents with 05-103227 and 05-102300 yielding selective indices of >167 and >346, respectively. The chemical structures of these small molecules shared characteristics with the potent antipoxvirus drug ST-246. We hypothesized that the compounds might act by a similar mechanism and tested them against an ST-246 resistant strain of VV. This mutant proved to be highly resistant to both 05-102300 and 05-102691, suggesting that these compounds also inhibited the F13L gene product, p37, which is the target for ST-246. The most effective compound of the three, 05-103227, retained activity against the F13L mutant suggesting that it does not target the same binding site on p37, or that it inhibits a different viral function. Additional experiments are underway to identify the molecular target of this compound and to determine the activity of each of these compounds in experimental animal infections.

doi:10.1016/j.antiviral.2008.01.124

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Synthesis and Antiviral Activity of Various N^4-Acyl Derivatives of Cidofovir and its 5-Azacytosine Counterpart, 1-(*S*)-[3-Hydroxy-2-(Phosphonomethoxy)Propyl]-5-Azacytosine

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Investigation of new types of acyclic nucleoside phosphonates (ANPs) as antiviral agents resulted among others in discovery of 1-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine (HPMP-5-azaC), its cyclic form and several types of ester prodrugs, compounds active against DNA viruses with activity data similar or better compared to cidofovir and higher index of selectivity *in vitro*. In contrast to cidofovir, HPMP-5-azaC has more complicated metabolic profile due to its chemical and enzymatic instability. In aqueous solutions ring opening between C-6 and N-1 of the triazine moiety occurs and HPMP-5-azaC is successively degraded to 2-{[(2*S*)-3-hydroxy-2-(phosphonomethoxy)propyl]carbamoylguanidine via the intermediary *N*-formyl derivative. The final decomposition product has no cytotoxicity *in vitro* but it is antivirally inactive. Besides chemical decomposition, HPMP-5-azaC undergoes also extensive enzymatic deamination in cell cultures. To improve the stability towards deamination process we tried to transform HPMP-5azaC to diverse N^4-acyl prodrugs on the level of free phosphonic acids as well as on the level of some earlier already prepared ester prodrugs, e.g. hexadecyloxyethyl ester of cyclic HPMP-5-azaC. As acyl groups we selected even number fatty acid residues (e.g. behenoyl, stearoyl). Similar N^4-acyl compounds were prepared also from HPMPC (cidofovir) and some of its esters. Different reactivity of both systems towards acylation reactions and influence of introduction of N^4-acyl groups to stability and antiviral activity of compounds will be discussed.

doi:10.1016/j.antiviral.2008.01.125

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Antiviral Effects of Sulfated Exopolysaccharide from the Marine Microalga *Gyrodinium impudicum* Strain KG03

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The sulfated exopolysaccharide p-KG03, which is produced by the marine microalga *Gyrodinium impudicum* strain KG03, exhibited impressive antiviral activity in vitro (EC_{50} = 26.9 mg/ml) against the encephalomyocarditis virus (EMCV). Depending on the p-KG03 concentration, the development of cytopathic effects in EMCV-infected HeLa cells was either inhibited completely or slowed. Moreover, p-KG03 did not show any cytotoxic effects on HeLa cells, even at concentrations up to 1000 mg/ml. The polysaccharide was purified by repeated precipitation in ethanol, followed by gel filtration. The p-KG03 polysaccharide had a molecular weight of 1.87×10^6, and was characterized as a homopolysaccharide of galactose with uronic acid (2.96%, w/w) and sulfate groups (10.32%, w/w). Antiviral activities of p-KG03 against various viruses – various picornaviruses, herpesviruses, influenza viruses and feline coronaviruses and HIV – will be reported. The biological activities of p-KG03 suggest that sulfated metabolites from marine organisms are a rich source of antiviral agents. The p-KG03 polysaccharide may be useful for the development of marine bioactive exopolysaccharides for use in biotechnological and pharmaceutical products.

doi:10.1016/j.antiviral.2008.01.126

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Developing a Novel High-throughput Screening Assay against Bluetongue Virus

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Arthropod borne viruses (arboviruses) are important human/animal pathogens that cause acute virus infections with severe diseases and/or death. Several recent human/animal epidemics are caused by arboviruses, including Dengue virus (DNV) in Asia, West Nile virus (WNV) in North America and Bluetongue virus (BTV) in Europe. There are no antiviral drugs available against these diseases. We have designed,