readily available for routine drug screening. Our laboratory has utilized a cell proliferation assay in Human Foreskin Fibroblast (HFF) cells that is predictive for toxicity in bone marrow. This labor intensive method uses a Coulter counter to enumerate live cells and is the current method of choice in our laboratory. We have compared this assay with three other assay systems in an attempt to automate the assay. Cellular replication was measured in HFF cells using a neutral red uptake assay, a crystal violet/formalin stained assay, a luminescent assay (CellTiter-Glo, Promega), and our standard cell counting assay. These experiments were conducted in both HFF and human embryonic lung (HEL-299) cells to see if results were cell line dependent. The neutral red uptake assay is based upon the uptake of a vital dye by living cells, whereas crystal violet stains all adherent cells. CellTiter-Glo generates a signal proportional to the amount of ATP present, which is proportional to the number of cells present. We compared a panel of drugs with well-characterized toxicities in each of the assay systems. Comparison of the IC_{50} values from the various assays demonstrated that the cell counting method using a Coulter counter was the most sensitive and predictive of toxicity in bone marrow. The neutral red uptake and the luminescent assay were less sensitive, while the crystal violet staining was relatively insensitive. There was no significant difference in the HFF and HEL-299 cells regardless of which method was utilized.

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Synthesis and Structure Activity Relationships among Non-nucleoside Analogs of Toyocamycin Active against Herpesviruses

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Toyocamycin (4-amino-5-cyano-7-β-D-ribofuranosylpyrrolo [2,3-*d*]pyrimidine) is a cytotoxic nucleoside. In contrast, certain of its deoxyribosyl, arabinosyl and acyclic analogs are less or non-cytotoxic. Some of these analogs have potent activity against HSV and HCMV (Renau et al., 1996. J. Med. Chem. 39, 873). The compounds act by a unique mechanism (Jacobson et al., 1999. Antimicrob. Agnts. Chemother. 43, 1888) early in the viral replication cycle (Evers et al., 2004. Antimicrob. Agnts. Chemother. 48, 3918). The simplest of these, the 7-methyl toyocamycin analog, was neither active against herpesviruses nor cytotoxic. The compounds that had antiviral activity, however, also had some cytotoxicity. Consequently we extended the original research and now report the synthesis and antiviral activity of other 7-alkyl analogs that also are substituted in the 6-position. These compounds were synthesized in a manner similar to that described by Renau et al. Using this and an analogous procedure, a series of 4-amino-5-cyano-pyrrolo[2,3-*d*]pyrimidines was synthesized with either H, Br, or NH2 in the 6-position and alkyl groups from methyl to octyl in the 7-position. These changes affected activity against HSV-1, HCMV, and cytotoxicity. In contrast to the 6-unsubstituted- and 6-Br-7-methyl analogs that were inactive, the 6-NH2 compound was active against both HSV-1 and HCMV (IC50's = 25 and 2 μM, respectively). Increasing the length of the 7-alkyl group increased activity against both viruses with propyl or butyl being optimal. Although the 6-Br-7-methyl analog was inactive at 100 μM against both viruses, 6-Br-7-ethyl and longer 7-alkyl analogs were active in the low to sub-micromolar range. The 6-NH2 analogs exhibited some cytotoxicity at 20–70 μM whereas the 6-Br analogs were not cytotoxic at 100 μM. Consequently, we conclude that compounds such as the 6-Br-7-butyl analog have specific antiviral activity against HSV-1 and HCMV.

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Combinations of CMX-001 and ST-246 Synergistically Inhibit Orthopoxvirus Replication In Vitro

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The necessity for the development of compounds for use in the treatment of orthopoxvirus infections originating from either a bioterror release or a natural endemic infection has yielded several drug candidates. A few of these are under active development for the treatment of orthopoxvirus infections including ST-246 and CMX-001 (HDP cidofovir), that are highly active both in vitro and in vivo. Our experiments were designed to determine if combinations of these two drugs would result in enhanced efficacy since: (1) they are the most advanced candidates under development, (2) they have different mechanisms of action and might be expected to act synergistically, and (3) they potentially could be used together in the clinic to avoid certain issues such as drug resistance. Combination assays were initially performed in human foreskin fibroblast (HFF) cells using the Copenhagen strain of vaccinia virus. Results from these studies revealed a robust synergistic interaction against viral replication suggesting that this drug combination might be particularly effective. Simultaneous cytotoxicity controls did not reveal any increased toxicity and suggested that it was a true antiviral effect. Treating viral infections with combinations of drugs with different mechanisms of action is advantageous because the combinations can offer improved efficacy at lower

dosages and minimize the development of drug resistance. The results of these experiments indicate that combinations of CMX-001 and ST-246 are particularly effective in the treatment of orthopoxvirus infections in vitro and suggest that this combination may also be optimal in treating these infections in animals and humans.

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Inhibition of Cellular Entry and Spread of Lymphocytic Choriomeningitis Virus by Amphipathic DNA Polymers

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Arenaviruses merit significant attention as powerful experimental models and the causative agents of several severe human hemorrhagic fevers with high mortality. Lymphocytic choriomeningitis virus (LCMV) serves as the prototype of the Old World arenavirus family and represents a powerful experimental model system for the study of arenavirus pathogenesis and treatments. In the present study, we tested the activity of novel sequence-independent amphipathic DNA polymers for activity against LCMV. Our findings indicate that REP 9, a 40mer degenerate PS-ON is a potent antiviral drug against LCMV infection, and revealed a novel antiviral mechanism for this class of drugs against this family of viruses. We determined that REP 9 targets the viral glycoprotein and blocks the initial steps of infection and cell-cell propagation of the virus. REP 9 blocks the binding of LCMV to its cellular receptor, a-dystroglycan, and can dissociate the stable virus-receptor complex. There is no apparent effect of REP 9 on the association between GP1 and GP2 or on the conformation of neutralizing antibody epitopes. Structure-function studies revealed that the action of REP 9 is sequence independent but has a critical dependence on size and hydrophobicity as seen for antiviral activity against type 1 enveloped viruses targeted by this class of compounds. The results herein suggest REP 9 has potential for treatment and prevention of arenavirus infection, and highlight our efforts towards the development of a unique class of anti-arenaviral drugs with a novel mechanism of action.

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Benzimidazole with Broad Spectrum of Antiviral Action

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Benzimidazoles show large-scale biological activity. The series of benzimidazoles and their derivatives were synthesized. An antiviral action of 2-methyl-4-dimethyl-aminomethyl-5-oxybenzimidazole was studied. Antiviral efficacy of this compound was predicted using developed by us hierarchical QSAR technology for molecular design of the promising antiviral compounds. Anti-HSV action was tested using cytomorphological method. Hep-2 cells were infected with HSV-1 strain US in dose 5 IFU/cell. The cells were incubated in Eagle's Medium that contained compound in experimental samples or without them in control samples. Then cells were fixed with 96% ethanol and stained with 0.01% acridine orange solution. The amount of infected cells with DNA-containing virus inclusion bodies was counted by fluorescent microscopy. Anti-HSV activity of compound was calculated as the difference between of the percentage of infected cells in treated cell cultures to the percentage of infected cells in untreated cell cultures.

Anti-influenza activity of the compound was studied on the model of replication of human A/Hong Kong/1/68 (H3N2) and avian H5N3 and H7N3 strains in tissue culture of chorioallantoic membranes of chicken embryos.

Anti-NDV activity was tested on the same model. 2-Methyl-4-dimethyl-aminomethyl-5-oxybenzimidazole in dose 20 mg/ml decreased amount of cells infected with HSV-1 by 60%. This compound in concentration 100 mg/ml inhibited of human influenza virus A/Hong Kong/1/68 (H3N2) replication on 1.0 $\log_{10}$ TID_{50}. In dose 100 mg/ml it decreased of avian influenza virus H5N3 reproduction on 1.0 $\log_{10}$ TID_{50}. 2-Methyl-4-dimethyl-aminomethyl-5-oxybenzimidazole did not show antiviral action in dose 200 mg/ml toward avian influenza virus H7N3, but this compound was active toward NDV. In concentration 100 mg/ml it decreased virus amount on 0.8 $\log_{10}$ TID_{50}.

The results of this research show that benzimidazoles are the perspective class of compounds for search of new agents with broad spectrum of antiviral activity.

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