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

Jack M. Hinkley [1,*], Katherine Z. Borysko [2], Julie M. Breitenbach [2], Keenan Bora [1], Shigetada Kosai [1], John C. Drach [1,2], Leroy B. Townsend [1]

[1] Department of Medicinal Chemistry, College of Pharmacy, USA; [2] Department of Biologic & Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI 48109, USA

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

Kathy Keith [1,*], Earl Kern [1], Shalisa Sanders [1], Robin Conley [1], Robert Jordan [2], Dennis Hruby [2], George Painter [3], Mark Prichard [1]

[1] University of Alabama School of Medicine, Department of Pediatrics, Birmingham AL, USA; [2] SIGA Technologies, Inc., Corvallis, OR, USA; [3] Chimerix, Inc., Research Triangle Park, NC, USA

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