97

Anti-orthopoxviral activity of the 2-Cycloalkylimino-5-(4-Nitrophenyl)-1,3,4-Thiadiazine Derivatives

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During this study, we synthesized a series of the 2-cycloalkylimino-5-(4-nitrophenyl)-1,3,4-thiadiazinesof the general formula (1) wherein the group (N) represents: piperidino-, pyrrolidino-, methylpiperazino-, hexamethyleneimino-group. These derivatives were tested for cytotoxicity and antiviral activity against the orthopoxviruses: vaccinia, cowpox and mousepox in cell cultures. Some of the derivatives show antiviral activity which depended from type of viruses and from the structural features of the compounds. Thus, we find a new class of heterocyclic compounds with antiviral activity against the orthopoxviruses.

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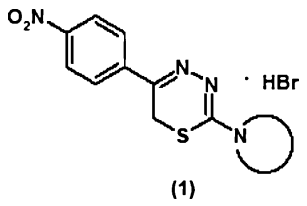

(1)

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98

Resistance of Human Cytomegalovirus to Cyclopropavir Involves a Novel Mutation in UL97

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We have previously described a second-generation methylenecyclopropane with two hydroxymethyl groups on the cyclopropane ring. This guanine nucleoside analog termed cyclopropavir (CPV) was active in vitro against HCMV and MCMV with IC_{50}'s of 0.27–0.49 μM and no cytotoxicity at 100 μM [*J. Med. Chem.* **47** (2004) 566]. It also was active when administered orally to MCMV-infected mice [*Antimicrob. Agents Chemother*. **48** (2004) 4745]. Last year we reported the isolation of HCMV resistant to CPV by passage of Towne strain HCMV in selected concentrations of the drug. Dose–response experiments with plaque-purified virus (termed 2696^r) gave IC_{50}'s of 22 and 42 μM for CPV and ganciclovir (GCV) compared to IC_{50}'s of 0.9 and 1.5 μM, respectively, for wt virus [*Antiviral Res.* **74** (2007) A83]. The virus also was resistant to two first generation analogs. Data such as these and the fact that both CPV and GCV are guanine nucleoside analogs led us to hypothesize that like GCV, CPV is phosphorylated to its active form by the CMV kinase pUL97. Consequently we sequenced UL97 from twice plaque-purified 2696^r and found a deletion of base pair 498. This deletion produced a frame shift resulting in the acquisition of a stop codon at base pairs 502-4 that normally occurs at base pairs 2122-4. The resulting putative protein would be 168 amino acids in length (normally 708) and would contain neither the ATP-binding region (codons 460–520) nor the substrate-recognition site (codons 590–607) found in wild-type UL97. Nonetheless virus 2696^r grew at nearly the same rate and to a titer only one $\log_{10}$ lower than wt virus. In contrast HCMV with UL97 deleted (kindly provided by Mark Prichard) grew much more slowly and to a titer several $\log_{10}$'s lower. These data demonstrate virus 2696^r is resistant to both GCV and CPV plus imply that it can replicate with a severely truncated pUL97.

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99

Synthesis and Activity of Vidarabine D-Amino Acid Prodrugs as Potential Pox Virus Agents

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Vidarabine (ara-A) was originally developed as an anti-tumor agent and later was found to be active against HSV types 1 and 2. It was the first FDA-approved drug for treatment of systemic herpes infections but its use is limited due to metabolism by adenosine deaminase (ADA) to ara-H, low lipophilicity, and low intestinal membrane permeability. Its low aqueous solubility also limits formulation options. We recently reported that vidarabine was three- to fivefold more active in plaque reduction assays against vaccinia and cowpox viruses than was cidofovir [Antiviral Res. 70 (2006) A14]. Furthermore, its activity against these viruses was enhanced approximately 10-fold by combination with 1 μM 2′-deoxycoformycin, a potent inhibitor of ADA. We also reported that minimizing the conversion of vidarabine to ara-H by synthesizing 5′-L-amino acid substituted prodrugs gave more potent anti-pox activity. We showed that the prodrugs are resistant to inactivation by deamination and that delivery of the L-amino acid prodrugs to the small intestine resulted in a 10-fold increase in the vidarabine plasma levels when compared with vidarabine. We now report the synthesis and antiviral activity of D-amino acid prodrugs of vidarabine as well as the 5′-valerate analog. The 5′-valerate and the D-valyl, -leucyl, -isoleucyl, phenylalanyl, -alpha and beta aspartyl prodrugs all were active against vaccinia and cowpox viruses at non-cytotoxic concentrations. Time-of-addition studies indicated the activity of vidarabine against vaccinia

virus is due to inhibition of viral DNA synthesis. Interestingly, both vidarabine and prodrugs were more active against vaccinia (IC_{50}'s = 2.5–6 μM) than cowpox (IC_{50}'s = 8–54 μM). Both the D- and L-val prodrugs were stable at three pH's but D-val was more stable in intestinal and liver homogenates and in plasma. These properties make the D-amino acid prodrugs good candidates for further study as orally bioavailable anti-pox virus agents.

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100

Antiviral Activity of (−)-D-Carbocyclic Cytosine (Carbodine) Against Avian Influenza Virus (H5N1)

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Natural and synthetic carbocyclic nucleosides are well known for their interesting biological properties, including antitumor as well as antiviral activities against a wide variety of RNA and DNA viruses. The carbocyclic analogue of cytosine (carbodine) was previously prepared as a racemic mixture and has been shown to possess inhibitory activity against human influenza type-A virus, measles, vesicular stomatitis virus and herpes simplex viruses. These interesting biological properties of carbodine, prompted us to develop an efficient synthetic method for the enantiomerically pure (−)-D-carbodine for further antiviral evaluations. Herein, we report the antiviral activity of carbodine against various strains of avian influenza virus (H5N1). Anti-influenza activity of (−)-D-carbodine was evaluated in comparison to its (+)-L- as well as (+)-DL analog against Duck, Gull, Hong Kong/2003 and Vietnam/2004 (H5N1) strains in vitro, and the results indicate that (−)-D-carbodine demonstrated potent antiviral activity with EC50 values of 0.57, 0.27, 0.18 and 0.18 μM, respectively, while the (+)-L-analog was inactive. Further biological and biochemical studies of (−)-D-carbodine as a potential antiviral agent for H5N1 virus are warranted.

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101

Application of the Phosphoramidate Protide Approach to the Antiviral Drug Ribavirin

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Ribavirin is a nucleoside analogue with antiviral activity against a number of DNA and RNA viruses in vitro and in vivo (Sidwell et al., 1972). Ribavirin has been approved for the treatment of respiratory syncytial virus and for the treatment of hepatitis C virus in association with interferon. The bioactivation of ribavirin involves intracellular phosphorylation to its 5′-monophosphate mediated by adenosine kinase followed by further phosphorylation to the di- and tri-phosphate. Its broad spectrum of antiviral activity prompted us to design and synthesise a new series of ribavirin phosphoramidates in order to investigate its activity against different viruses. The synthesis and the biological evaluation for these compounds will be reported.

Reference

Sidwell, R.W., Huffman, J.H., Khare, G.P., Allen, L.B., Witkowski, J.T., Robins, R.K., 1972. Broad-spectrum antiviral activity of virazole: 1-beta-D-ribofuranosyl-1,2,4-triazole-3-carboxamide. Science 177, 705–706.

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102

Design, Synthesis and Biological Evaluation of Novel Acyclovir ProTides

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Acyclovir and its prodrug valacyclovir are currently the treatments of choice for herpes simplex virus (HSV) and varicella-zoster virus (VZV) infections. Intracellular conversion of acyclovir to its active triphosphate form is severely limited by the first phosphorylation step, which is carried out by a herpes virus encoded thymidine kinase (Elion et al., 1977). Further conversions to the di- and triphosphate are mediated by cellular guanosine monophosphate kinase and nucleoside diphosphate kinase respectively. Importantly, the activation of the compound by the viral nucleoside kinase is a target for drug resistance in both HSV and VZV strains (Larder et al., 1983). Our phosphoramidate ProTide approach was applied to acyclovir as a means to bypass the limiting step of its activation. However, no signifi-