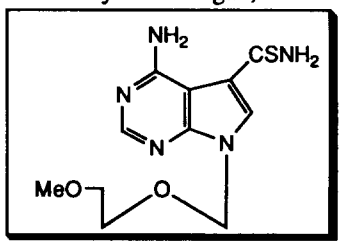
104

Activity Against Human Cytomegalovirus, Cytotoxicity and Mode of Action of a Non-Nucleoside Pyrrolo[2,3-*d*]pyrimidine. T. E. Renau, L. B. Townsend and J. C. Drach. University of Michigan, Ann Arbor, Michigan 48109, USA.

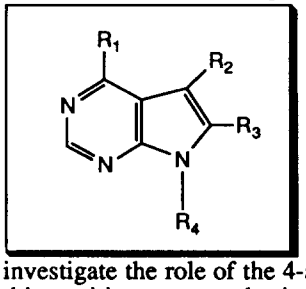


From an extensive study evaluating the potential of non-nucleoside pyrrolo[2,3-*d*]pyrimidines as inhibitors of HCMV, we have chosen to investigate further one active analog in this series: 4-amino-7-[(2-methoxyethoxy)methyl]pyrrolo-[2,3-*d*]pyrimidine-5-thiocarboxamide (**1**). In an HCMV plaque reduction assay, **1** had an IC_{50} of 6 μM. Similar results were obtained in an HCMV ELISA assay (2 μM). Cytotoxicity in HFF, L1210 and MRC-5 cells was >100 μM. The IC_{50} for effect on the growth of KB cells was 165 μM. The compound was not stable in Fischer's cell culture medium ($t_{1/2}$= 51 hr). It was converted to the corresponding 5-nitrile derivative, which was inactive at concentrations below 100 μM in both HCMV plaque reduction and ELISA assays. In yield reduction assays, compound **1** produced multiple $\log_{10}$ reductions in virus titer which was comparable to the activity of DHPG (IC_{99}= 2.2 μM and 2.1 μM, respectively). In contrast to DHPG, the reduction of viral titers by **1** decreased with increasing multiplicity of infection (MOI). Time of addition studies at an MOI of 0.005 PFU/cell demonstrated that compound **1** was active only when added at times up to 24 hr post-infection whereas DHPG produced multiple $\log_{10}$ reductions in virus titers up to 3 days. These results establish that **1** acts prior to viral DNA replication. Initial combination studies with **1** and DHPG in a plaque reduction assay demonstrated a one log decrease in the IC_{50} compared with each compound alone. Further studies using a three-dimensional model to analyze drug-drug interactions showed that the effect of the two compounds on HCMV was not synergistic but additive. We conclude that a number of non-nucleoside pyrrolo[2,3-*d*]pyrimidines may have usefulness as antiviral agents because they have activity against HCMV and act by a unique mechanism. This study was supported by Dept. of Health and Human Services contract N01-AI72641 from N.I.A.I.D.

105

Structure-Activity Relationships of Non-Nucleoside Pyrrolopyrimidine Analogs Active Against Human Cytomegalovirus. T. E. Renau, J. C. Drach and L. B. Townsend. University of Michigan, Ann Arbor, Michigan 48109, USA.

The activity against HCMV of a number of non-nucleoside derivatives related to the pyrrolopyrimidine nucleosides toyocamycin, sangivamycin and thiosangivamycin has recently been described by us (*Biorg. Med. Chem. Lett.*, 2, 1755, 1992). We now have expanded our studies in this area and report herein the SAR of this class of compounds. Initially, the effect of



changes at N-7 (R_4) was examined. A series of 4-aminopyrrolo[2,3-*d*]pyrimidine analogs substituted at R_4 with various alkyl, benzyl or ether groups was synthesized from the known 5-amino-2-bromo-3,4-dicyanopyrrole. The biological results demonstrated that like our initial report, the major factor required for activity against HCMV was not the substituent at R_4 but a thioamide moiety ($CSNH_2$) at R_2. The substituent at R_4 did act, however, to modulate the selectivity of the compounds so that the best separation between HCMV activity and toxicity with the thioamide analogs was observed with N-7 ether derivatives. To investigate the role of the 4-amino (R_1) group on activity, a series of compounds substituted at this position was synthesized from the 4-chloro derivatives. The results demonstrated that modifications at R_1 were not well tolerated and in almost all cases no activity against HCMV was observed. In a series of compounds substituted with an amino group at both R_1 and R_3, activity with either a nitrile or a thioamide substituent at R_2 was observed. In this series, the antiviral activity was well separated from the cytotoxicity. Overall, our results show that many non-nucleoside derivatives of 4-aminopyrrolo[2,3-*d*]pyrimidines have potent activity against HCMV. We are investigating further the biological activity of one active compound, 4-amino-7-[(2-methoxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamide. This work was supported by Department of Health and Human Services research contract N01-AI-72641 from N.I.A.I.D.