106

Benzimidazole Ribonucleosides: Design, Synthesis and Antiviral Evaluation of some TCRB Analogs. Rodrigo V. Devivar, Etsuko Kawashima, Edward D. Kreske, John C. Drach, and Leroy B. Townsend*. Department of Medicinal Chemistry, College of Pharmacy, and the Department of Biologic and Materials Sciences, School of Dentistry. The University of Michigan, Ann Arbor, MI 48109-1065.

Our laboratories have long been involved in the synthesis of nucleosides as potential antiviral agents. As part of our comprehensive structure activity relationship study related to 2,5,6-trichloro-1-(β-D-ribofuranosyl)benzimidazole (TCRB) and its 2-bromo analog (BDCRB) we have prepared a number of 2-substituted-5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazoles. Our synthetic efforts have revealed that the 2-position of the lead compounds is very amenable toward synthetic modification and the antiviral activity seems to be very dependent on the type of functional group residing at the 2-position. We have now incorporated the known broad biological activity of 2-mercaptobenzimidazoles into the central structure of our lead compound (TCRB) and have synthesized several 5,6-dichloro-2-substitutedmercaptobenzimidazole ribonucleosides. These compounds have been evaluated for their ability to inhibit the replication of human cytomegalovirus (HCMV) and for their cytotoxicity in uninfected cells. 5,6-Dichloro-2-benzylthio-1-(β-D-ribofuranosyl)benzimidazole was active against HCMV in plaque assays (IC_{50} = 22 μM) and in yield reduction experiments (IC_{90} = 7 μM). Its cytotoxicity to uninfected human diploid fibroblasts (HFF cells) and a neoplastic cell line (KB cells) was found to be low (IC_{50} = 100 μM). Other analogs substituted on the 2-benzyl moiety were found to have IC_{50}s in the range 20-40 μM. This research was supported by a University of Michigan Minority Merit Fellowship and federal funds from the Department of Health and Human Services research contracts NO1-AI-72641, NO1-AI-42554 and grant UO1-AI-25739 from NIAID.

107

Benzimidazole Ribonucleosides: Cytotoxicity of TCRB and BDCRB to Human Cells. M.R. Nassiri, S.R. Turk, M.P. Petrillo, J.P. Robinson, R.V. Devivar, L.B. Townsend, and J.C. Drach. University of Michigan, Ann Arbor, Michigan 48109, and Purdue University, West Lafayette, Indiana 47907, U.S.A.

With the high incidence of human cytomegalovirus (HCMV) infections in AIDS and other immunosuppressed individuals plus the emergence of strains resistant to existing drugs, there is a critical need for new and safe treatments of this viral infection. We have reported elsewhere at this conference that benzimidazole ribonucleosides are potent and selective inhibitors of HCMV replication *in vitro*. In order to explore the potential cytotoxicity of two of these compounds – 2,5,6-trichloro-1-(ß-D-ribofuranosyl)benzimidazole and its 2-bromo analog (TCRB and BDCRB, respectively) – we examined the effects of these compounds in a neoplastic and a normal human diploid cell line (KB and WI-38 cells, respectively). In cell growth studies, a slight prolongation of population doubling time occurred from 21 hours (untreated cultures) to 24 hours in KB cells treated with 100 μM TCRB, however this growth inhibitory effect was reversible. Similar results were obtained with WI-38 cells. BDCRB also did not inhibit the growth of KB cells at concentrations up to 100 μM. In contrast, a 100 μM concentration of the 2-unsubstituted analog of TCRB (termed DRB by Tamm *et. al, J. Exp. Med.* **99**:227, 1954) completely inhibited the growth of KB and WI-38 cells and was not reversible upon the removal of drug following 24 hours of treatment. In plating efficiency experiments, KB cells produced colonies in 100 μM TCRB. In experiments which measured incorporation of radiolabeled precursors into DNA, RNA, and protein, IC_{50}'s of >100 μM were noted for DNA and protein and >50 μM for RNA. DNA flow cytometry studies revealed that concentrations of TCRB up to 100 μM did not perturb the progression of the KB cell cycle. Only a slight reduction in the number of cells in G_2/M phase was noted when the cultures were treated with 320 μM TCRB, showing that it did not affect DNA synthesis. These data establish that both compounds produce little cytotoxicity in their antiviral dose range. Consequently TCRB or BDCRB may have the potential to be a drug of choice for treatment of HCMV infections. This work was supported by contracts N01-AI42554 and N01-AI72641 and grant U01-AI31718 from N.I.A.I.D.