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Synthesis and Anti-HIV-1 Activity of Pyrrolobenzothiadiazepines.

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RS654 (11-oxo-10,11-dihydro-pyrrolo[1,2-b][1,2,5]benzothiadiazepine 5,5-dioxide) is the representative of a new group of compounds that we have recently synthesized by reacting 2-nitrobenzenesulphonyl-chloride with 2-ethoxycarbonylpyrrole, and by cyclizing the resulting sulphonylpyrrole.

RS654 selectively inhibited the multiplication of several HIV-1 strains ($H9III_B$, NM and RF) and of an AZT^r mutant in a variety of T4 lymphocyte cell lines, namely MT4, C8166, MOLT4, in the monocytic cell line U937 and in HeLa T4 cells. Effective doses were in the range of 1.0 - 5 μM, whereas doses up to 400 μM were non-cytotoxic. No activity against HIV-2 (ROD and CBL20 strains) could be demonstrated.

In enzyme assays, RS654 inhibited the HIV-1 rRT at doses comparable to those inhibiting the HIV-1 multiplication in cell cultures; these results, together with time of addition studies indicated that the reverse transcriptase is the target of RS654.

Combinations of RS654 with AZT (0.01 μM) or ddI (40 μM) gave additive effects on the HIV-1 multiplication. Moreover, when RS654 + ddI was added to HIV-1-infected MT4 cells, virus breakthrough was noted after 6 subcultivations, whereas no viral breakthrough occurred after 16 subcultivations in the presence of RS654 + AZT. It is worth noting that virus breakthrough occurred after 3 subcultivations when each drug was used alone, and after 11 subcultivations when AZT and ddI were combined.

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Comparison of Antiretroviral Drug Combinations in a Murine Leukemia Virus (MuLV) Model. L. B. Allen, D. C. Quenelle, L. Westbrook, W. M. Shannon, A. D. Brazier, M. G. Hollingshead, Southern Research Institute, Birmingham, AL.

Each compound (AZT, ddI, ribavirin) was screened for activity in the Rauscher MuLV UV-XC plaque reduction assay and active levels were determined for each. From these data, combinations were planned using a checkerboard design with concentrations of compounds that were active and non-toxic. In addition to the plaque reduction assay, supernatants from infected, untreated and treated cells were frozen at -70°C and subsequently thawed for titration by plaque assay. For these studies, we used the Prichard and Shipman 3-Dimensional technique for analyzing the drug-drug interactions (Antiviral Res. 14:181-206, 1990) to evaluate the experimental data. When ddI and ribavirin were evaluated, the combination was significantly synergistic with the volume under the curve being +324.95 $\mu g^2\%$. When the supernatants from these cultures were titrated for virus, titers were not reduced by either drug individually. In contrast, several of the drug combinations reduced the titer of virus by 2.3 to 0.2 $\log_{10}$. When AZT and ribavirin were combined, the combinations were found to be significantly antagonistic with a volume under the curve of -212.71 $\mu g^2\%$. While AZT did not reduce virus titers at the concentrations tested, ribavirin reduced the titer by 1 $\log_{10}$ at the highest dose. Titration of the supernatants from drug combinations revealed variations of the ribavirin activity. Since AZT was not active in this assay, no antagonism was seen. In anticipation of performing a 3 drug combination with ddI, ribavirin and AZT, we evaluated combinations of ddI and AZT. In this Rauscher MuLV, ddI and AZT were found to be additive. Moderately synergistic doses of ddI and ribavirin were selected for combination with AZT. In the three drug combination experiment, there was evidence of minimal antagonism; however, the drugs in combination were generally additive. These combination experiments are good examples of synergism and antagonism against which other drug combinations can be compared. This work was supported by NIAID, Contract No. NO1-AI-05086.