81

3-Dimensional structure studies of Thiadiazole-derivatives for anti-HIV-1 RT activity

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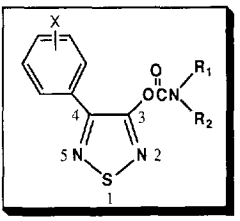

1,2,5-Thiadiazole derivatives (TDAs) were found to be novel and potent non-nucleoside reverse transcriptase (RT) inhibitors of human immunodeficiency virus type 1 (HIV-1). The 4-Phenyl and 3-(*N,N*-dialkylcarbamyl) groups were important moieties which affected to the anti-HIV-1 activity. Namely,4-(4'-halogenophenyl)-TDAs disappeared the anti-HIV-1 activity, and the order of RT inhibition activity of 3-(*N,N*-dialkylcarbamyl) derivatives was *N,N*-dimethyl ≥ *N*-methyl-*N*-ethyl > *N*-methyl-*N*-propyl > *N*-methyl-*N*-butyl > *N*-methyl-*N*-hexyl. To explain the inhibition property of these compounds, the relationship between 3-dimensional structures and RT inhibition activity of the TDAs was studied.

82

Characterization of a cellular line expressing a defect of thymidine kinase activity and displaying resistance to the antiviral activity of AZT.

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In a previous work we have shown that multidrug-resistant cells, expressing the multidrug transporter P-glycoprotein, are less sensitive to the antiviral activity of AZT. Subsequently, we addressed the question whether AZT itself is able to induce cellular resistance to the drug. In order to study this phenomenon, CEM cells were treated continously with low, and gradually increasing, concentrations of AZT. After 4 months of treatment we could obtain the growth of CEM cells in the presence of 2mM AZT. These cells, called CEMazt, are much less sensitive to the antigrowth activity of AZT and the TC50 value is much higher (about 50 fold) than that of the parental cell line. The same cell line is also less sensitive to the antiviral activity of AZT and the ID50 value for HIV-1 is 20,000 fold higher in CEMazt than in the parental cell line. However, although resistant to AZT, CEMazt cells do not express detectable level of P-glycoprotein. Sensitivity of these cells to other compounds, such as vinblastine, vincristine, remained unchanged indicating that they do not display a multi-drug resistant phenotype. Interestingly, in CEMazt cells the intracellular accumulation of AZT is significantly reduced when compared with the parental cell line. The same results were obtained when intracellular accumulation of thymidine (dT) was evaluated. Aimed experiments have shown that the inability of CEMazt to accumulate AZT and dT may be due to a defect of the thymidine kinase 1 (TK1) activity (TK activity: CEM = 13.7 U/ml; CEMazt = 0.6 U/ml). Further experiments have shown that after 9 days of culture in HAT medium, the growth rate of CEMazt was drammatically diminished when compared to the parental cell line, thus confirming that CEMazt cells are not able to phosphorylate the dT present in the medium. Interestingly, CEMazt cells displayed resistance only to AZT and dT, while they showed complete sensitivity to other drugs, such as ddI, ddC, and AraT, which are activated by other cellular kinases or by TK2, suggesting that these phosphorylation pathways are unaltered in AZT resistant cells. Taken toghether these findings suggest that the failure of the antiviral activity of AZT may be also due to a defect of TK1 activity. Experiments are being carried out to explore whether these findings may have clinical relevance.