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Synthesis, Enzymology, and Anti-HIV Studies of Analogues of Isodideoxyadenosine and its Phosphorylated Derivatives

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4(*S*)-(6-Amino-9H-purin-9-yl)tetrahydro-2(*S*)-furanmethanol [(*S,S*)-isodideoxyadenosine, (*S,S*)-IsoddA] synthesized in our laboratory has anti-HIV activity against HIV-1, HIV-2, and HIV resistant strains. Its triphosphate is a potent inhibitor of HIV reverse transcriptase (K_i 16 nM). This compound, an L-related nucleoside triphosphate, is apparently incorporated in the structure of viral DNA and behaves as a chain terminator of this HIV DNA. We have investigated the synthesis, enzymology and antiviral studies of analogues of IsoddA and its phosphorylated derivatives, and this is the emphasis of the presentation. The analogues will focus on unusual modifications of the purine base of IsoddA. The phosphorylated derivatives will include dinucleotides and DNA models incorporating isomeric nucleosides. Structural data presented and explained will include those obtained from multinuclear 1D and 2D NMR spectra, hypochromicity studies, and circular dichroism. Investigation of the stability of the IsoddA analogues and the derived nucleotides towards various enzymes will be presented and the stabilities will be compared with those of the corresponding natural compounds. Antiviral data of relevance will be discussed.

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SAR and molecular modeling studies for the anti-HIV-1 activity optimization of pirryl-aryl-sulphones (PASs). Identification of derivatives with improved potency. A.G. Loi, °R. Silvestri, °M. Artico, °S. Massa, *E. Novellino, *G. Greco, §A. Ettorre, A. DeMontis, P. La Colla. *Dipartimento di Biologia Sperimentale, Università di Cagliari. °Dipartimento di Studi Farmaceutici, Università di Roma "La Sapienza". *Facoltà di Farmacia, Università di Salerno. §Menarini Ricerca SpA, Roma.*

The high potency and selectivity of non-nucleoside reverse transcriptase inhibitors (NNRTIs) and their clinical efficacy in combination with nucleoside analogues still gives high priority to the search for new NNRTIs with both improved pharmacokinetic profile and, hopefully, increased potency against clinically relevant resistant mutants. Among NNRTIs are pirryl-aryl-sulphones (PASs), whose lead compound, a 2-nitrophenyl-1-pyrryl-sulphone bearing a carbethoxy group at position 2 in the pyrrole ring, has been subsequently improved in potency by reducing the 2-nitro group to amino and by introducing a Cl atom at position 5 in the phenyl moiety (RS-980). In addition to studies aimed at defining the biological properties of RS-980, we started a synthetic project based on molecular modeling-aided design. In order to identify the active conformation of RS-980, we used the published HIV-1 RT-bound structures of nevirapine and of the α-APA analogue R95845 as templates. The superimposition model was used as starting point for docking RS-980 into the NNRTI binding pocket of the HIV-1 enzyme. The investigation of the RT-RS-980 complex and mechanic calculations allowed the design of new PAS derivatives potentially characterized by improved steric and electronic complementarity with the NNRTI site. Derivatives RS-1226, RS-1267 and RS-1281 were thus designed and synthesized. When tested against HIV-1, the latter showed EC_{50}s of 0.04, 0.07 and 0.09 μM, respectively, and no cytotoxicity at concentrations up to 200 μM. At the present time we are in the process of evaluating whether these new derivatives are also able to suppress the HIV-1 multiplication in MT-4 cells infected at high multiplicity of infection, as found with the parent compound RS-980.

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Synthesis and Antiviral Evaluation of Benzyl-Substituted Thiopurine and Tiazofurin Derivatives

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We have recently reported that some imidazo[1,5-*a*]-1,3,5-triazine derivatives containing the benzyl and thio structural units, e.g. 8-(4-methylbenzyl)-2-[(4-metylbenzyl)thio]imidazo [1,5-*a*]-1,3,5-triazine-4-one **1**, are specifically inhibitory to influenza A and respiratory syncytial viruses at concentrations below 5 μM [1]. Two series of analogs being benzyl-substituted thiopurine and tiazofurin derivatives were now synthesized and examined for their inhibitory effects. None of these compounds was inhibitory to influenza A or B virus. Lack of anti-influenza A virus activity of the purine ring counterpart of **1** at a concentration of 750 μM demonstrates that the position of nitrogen atoms within the heterocyclic skeleton is of critical importance. Similarly to benzyl substituted imidazo[1,5-*a*]-1,3,5-triazine derivatives [1], none of the new compounds was inhibitory to HIV-1 or HIV-2 in CEM cell cultures at subtoxic concentrations.

[1] B. Golankiewicz, P. Januszczyk, S. Ikeda, J. Balzarini and E. De Clercq, J. Med. Chem. **1995**, *38*, 3558-3565.

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Targeting the HIV-1 Nucleocapsid Protein by Azodicarbonamide (ADA), Cystamine, and Dithiane Compounds

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Sequence conservation and the essential roles of the retroviral nucleocapsid (NC) protein Cys-X_2-Cys-X_4-His-X_4-Cys (CCHC) zinc finger domains make these structures prime antiviral targets. The HIV-1 NC protein contains two copies of the invariant CCHC zinc finger domain that are required for packaging of genomic RNA during virus assembly and during the early phase of infection. We discovered that these CCHC domains are chemically reactive with certain electrophilic compounds, provided the compounds demonstrate spatial properties that allow access to the reactive sulfur atoms of the domains (*Nature* 361, 473-475, 1993). Both C-nitroso and 2,2'-dithiobisbenzamides (DIBAs) were identified as compounds that exert anti-HIV-1 activity by targeting the NC zinc finger domains (*Science* 270, 1194-1197, 1995). Many of these NC inhibitors are directly virucidal and could be utilized for intravaginal virucidal agents. Their virucidal action is mediated by interruption of the role of the NC protein during initiation and ongoing reverse transcription, thereby mimicking the action of reverse transcriptase inhibitors. These compounds also inhibit the production of infectious virions from cells containing integrated proviral DNA. They modify the zinc fingers of Gag precursor polyproteins, thereby preventing processing of precursors to mature viral proteins and mimicking the action of HIV-1 protease inhibitors (*J. Virol.* 70, 6180-6189, 1996). HIV-1 isolates resistant to NC inhibitors have not been detected. We now report NC zinc ejection as the rationale for the known anti-HIV-1 activity of ADA and cystamine. In addition, cyclic dithiane molecules having enhanced stability properties over other classes of NC inhibitors have been identified. Assignment of these compounds as NC inhibitors is predicated on the antiviral properties of the compounds with acutely and chronically infected cells, as well as evaluations of compounds against various molecular targets. Rational drug design efforts through molecular modeling and medicinal chemistry will be presented.