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Mechanism of Anti-HIV Action of Masked Alaninyl 2',3'-Dideoxy-2',3'-Didehydrothymidine 5'-Monophosphate Derivatives

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So324 is a prodrug of 2',3'-dideoxy-2',3'-didehydrothymidine 5'-monophosphate (d4T-MP). Its phosphate moiety is linked to a phenyl group and, through a phosphoramidate linkage, also to the methyl ester of L-alanine. The anti-HIV activity of So324 in CEM and MT-4 cells is slightly superior to that of d4T. In contrast to d4T, So324 also inhibits HIV replication in thymidine kinase-deficient CEM cells, and suppresses HIV-1 infection in natural peripheral blood lymphocytes and freshly isolated monocyte/macrophages. So324 derivative is equally active against HIV-1 and HIV-2 strains, and has also proved inhibitory to other retroviruses including simian immunodeficiency virus (SIV) and feline immunodeficiency virus (FIV), Visna virus and Moloney murine sarcoma virus in cell culture. So324 is metabolized to d4T-MP, d4T-DP and d4T-TP, and also to the novel compound alaninyl d4T-MP that markedly accumulates in So324-exposed cells. Virtually equal levels of d4T-TP and alaninyl d4T-MP were formed in the wild-type CEM and thymidine kinase-deficient CEM cells. Alaninyl d4T-MP has a relatively short intracellular half-life, but may be considered as a depot form for d4T-MP and d4T. Therefore, the masked alaninyl d4T-MP derivative represents an interesting phosphoramidate prodrug of d4T.

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2-glycineamide-5-chlorophenyl(2-pyril) ketone is a novel Tat inhibitor which have no benzodiazepine formula

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In the search for effective anti-Tat agents using a screening assay system that has recently been developed(Kira et al., 1995), 2-glycineamide-5-chlorophenyl (2-pyril) ketone (GCPK) has proved to be a potent and selective inhibitor of human immuno defi ciency virus type 1 (HIV-1) replication in vitro. This compound was inhibitory to HIV-1 replication in both acutely and chronically infected cells. The 50% effective concentrations (EC50s) of GCPK in acutely infected MOLT-4 and CEM cells were 0.62 and 0.13 μg/ml, respectively. These values were similar to those of the known Tat inhibitors Ro5-3335 and Ro24-7429. Like these inhibitors, GCPK could inhibit HIV-1 replication in MOLT-4/IIIB (MOLT-4 cells chronically infected with HIV-1) and TNF-α-induced viral activation in OM10.1 cells (a HL-60 clone latently infected with HIV-1). Although GCPK is related to Ro5-3335 and Ro24-7429 in chemical structure, it is a novel Tat inhibitor that has no benzodiazepin ring.

Kira et al., (1995) AIDS Res. Hum. Retroviruses, 11, 1359-1366.

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Pentafuside (T20), A Novel Inhibitor of HIV-1 Fusion: Pharmacokinetics and In Vivo Efficacy. D. M. LAMBERT, T. VENETTA, B. DIMASSIMO, S. HOPKINS, P. L. BLACK[1], O. L. WOOD[1], D. D. BROUD[1], M. BACHO[1], S. C. KUNDER[1], S. PAPERMASTER[1], S. BARNEY, AND M. A. USSERY[1]. Trimeris, Inc., Research Triangle Park, NC. and [1]U.S.F.D.A., Rockville, MD

Pentafuside, a 36-mer synthetic peptide derived from the HIV-1 gp41 transmembrane protein, is a selective and potent inhibitor of HIV-1 fusion (IC_{50}=1ng/ml) and infection (IC_{50}=80 ng/ml) *in vitro*. To evaluate T-20 as a potential lead compound for clinical investigation, the *in vivo* stability of T-20 in rodents was assessed. Pharmacokinetic studies revealed a surprisingly long half-life in circulation ($t_{1/2}$=2.4 hr), with sustained levels above the IC_{50} for at least 6 hr. We tested the ability of T-20 to inhibit HIV-1 replication *in vivo* in the HuPBMC-SCID mouse model of infection. Two weeks after reconstitution, mice were infected I.P. on day 0 with 10^3 $TCID_{50}$ HIV-1 9320 passed in PBMC (AZT-sensitive isolate A018, D. Richman). Treatment with T-20 was I.P., bid, for total daily doses of 2, 20, or 200 mg/kg, beginning on day -1. The extent of infection in blood cells, splenocytes, lymph nodes (LN), and peritoneal cells (PC) was assayed by quantitative coculture with human PBMC blasts 1 week later. Infectious HIV-1 was recovered from none of the tissues from the animals treated with the 200 mg/kg/day dose. Furthermore, the two lower doses of T-20 reduced the recovery of HIV-1 from cells in PC, LN, and blood, but not from spleen. Additionally, viral load was quantitated by the NASBA method, and the results were generally consistent with those of cocultures. Antiviral effects also correlated with the protection of human CD4 lymphocytes (assayed by flow cytometry) from the cytotoxic effects of HIV-1 infection, with a dose-dependent restoration fo the CD4/CD8 ratio by T-20. These results support the potential therapeutic efficacy of this novel class of antiviral agents.

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Antiviral Activity of the Protease Inhibitor KNI-272 *In Vivo* in the HIV-Infected HuPBMC-SCID Mouse Model. MICHAEL A. USSERY, OWEN L. WOOD, DENNIS D. BROUD, MATTHEW BACHO, STEVEN C. KUNDER, SUSAN PAPERMASTER, YOSHIAKI KISO[1], and PAUL L. BLACK. U.S.F.D.A., Rockville, MD, USA, and [1]Kyoto Pharmaceutical University, Kyoto, Japan.

In order to validate a murine model of HIV infection for use in AIDS drug development, we have examined compounds from a variety of chemical classes in the HuPBMC-SCID mouse model. Female SCID mice (5-7 wk old) received 5-10X10^7 adult human PBMC I.P. Two weeks after reconstitution, mice were infected I.P. on day 0 with 10^3 $TCID_{50}$ HIV-1 9320 (AZT-sensitive isolate A018, D. Richman). Treatment with KNI-272 was I.P., bid, for total daily doses of 20, 50, or 100 mg/kg, beginning on day -1. The extent of infection in blood cells, splenocytes, lymph nodes (LN), and peritoneal cells (PC) was assayed by quantitative coculture with human PBMC blasts 1 wk later. A significant proportion of samples from animals treated with the 50 and 100 mg/kg/day doses did not have detectable levels of HIV in PC and LN sites. The average HIV titer (TCID/10^6 cells) decreased in PC from 390 to 29 and 23 at the 50 and 100 mg/kg/day doses, respectively, and in LN from 850 to 6.6 and not detectable at the 50 and 100 mg/kg/day doses, respectively. Blood cells showed a decrease from 440 TCID/ml to 18 at the highest dose tested. Spleen cells were the only tissue in which KNI-272 did not reduce virus recovery. Additionally, viral load was quantitated by the NASBA assay, and the results were generally consistent with those of cocultures. Antiviral effects also correlated with the protection of human CD4 lymphocytes (assayed by flow cytometry) from the cytotoxic effects of HIV infection, with a dose-dependent restoration of CD4/CD8 ratio by KNI-272. In a subsequent experiment, levels of HIV recovery from tissues were much higher, but the results followed those described above. The antiviral effect of KNI-272 at 100 mg/kg/day was again observed in PC, LN, and blood cells (but not splenocytes), but the magnitude of the antiviral effects was more modest than in the previous experiment. Further experiments are underway to confirm and expand these results.