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Molecular Properties of HIV-1 Resistant to (+)-Enantiomers and Racemates of Oxathiolane Cytosine Nucleosides and their Potential for the Treatment of HIV and HBV Infections. Schinazi, R.F.,[1]* McMillan, A.,[1] Lloyd, R.L., jr.,[1] Schlueter-Wirtz, S.,[1] Liotta, D.C.,[1] and Chu, C.K.[2] Emory University/VA Medical Center, Decatur, GA,[1] and Univ. of Georgia, Athens, GA, USA.[2]

The development of resistant viruses to (-)-β-L-oxathiolane cytosine nucleosides produced variants that are highly (> 1,000-fold) resistant to 3TC [(-)-BCH-189] and (-)-FTC, but that remained susceptible other nucleosides, NNRTI, and protease inhibitors. The combination of AZT and 3TC is one of the most successful drug combination for treating HIV infected individuals, especially when combined with protease inhibitors. In cell culture the (+)-β-D-enantiomers and racemates of both FTC and BCH-189 are effective inhibitors of HIV and hepatitis B virus (HBV). However, (+)-BCH-189 was found to be toxic in certain cells such as CEM and human bone marrow cells, whereas (+)-FTC or racemic FTC are essentially non-toxic. In this study, we demonstrate that in HIV-1 infected human PBM cells, (+)-BCH-189 readily selected for a virus that was > 8-fold less susceptible to (+)-BCH-189 or racemic BCH-189. DNA sequence analysis of the RT gene amplified from (+)-BCH-189-resistant viruses consistently identified a single mutation at codon 215 from T (ACC) to Y (TAC). Clinically this change has been associated with AZT-resistance. It was markedly more difficult to select for resistant virus with the 215 mutation with (+)-FTC or racemic BCH-189, although mutations at codon 184 (M to V) were observed with racemic BCH-189, but not with either (+)-enantiomers. Racemic FTC appeared to prevent the development of a mutation at codon 215, but not at 184. These studies demonstrate that it may not always be rational to clinically develop only pure enantiomers of nucleoside antivirals. Unfortunately, one cannot use (+)-BCH-189 or racemic BCH-189 clinically or as part of a combination due to the high toxicity *in vitro*. However, the potential use of (+)-FTC or racemic FTC should be considered for the therapy of HIV and HBV. (Supported by the Dept. of Veterans Affairs and NIH).

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FROM CARBOVIR TO 1592.

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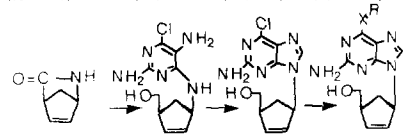
Carbocyclic-2',3'-didehydro-2',3'-dideoxyguanosine is the parent compound in a series of 6-substituted-2-amino purine carbocyclic nucleosides (called carbovir, CVR) that are potent and selective inhibitors of human immunodeficiency virus [Vince et al., BBRC 156: 1046, 1988; Vince and Hua, US patents 4,916,224, 4,931,559, 4,950,758, (1990)].



We have identified the active CVR enantiomer, characterized its metabolism, identified its active form (CVR-triphosphate) as an inhibitor of reverse transcriptase, and demonstrated its potent synergy with AZT. The 6-alkylamino and 6-alkoxy derivatives were also active against against HIV. The 6-substituted CVR's are metabolized directly to the monophosphates and are subsequently converted to CVR-triphosphate via of CVR-monophosphate. The adenosine deaminase inhibitor, EHNA, had no effect on anti-HIV activity of these compounds, while the adenylic acid deaminase inhibitor, 2-deoxycoformycin, inhibited antiviral activity. The 6-substituted CVR, 1592, a prodrug of CVR-monophosphate, has recently been reported as a clinically active candidate for the treatment of AIDS.

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Identification of the Antiviral Nucleoside Drug Binding Site of HIV-1 Integrase by Proteolytic Peptide Mapping. R. Drake, N. Neamati, P. Sunthankar, A. Mazumder, Y. Pommier. Dept. of Biochemistry, Univ. of Arkansas for Med. Sci., Little Rock, AR 72205 and Laboratory of Mol. Pharm., NCI, NIH, Bethesda, MD, 20892.

The recent successes of combination therapies targeted to HIV-1 reverse transcriptase and protease provide an increased impetus for identifying HIV-1 integrase (HIV-IN) inhibitors. Phosphorylated metabolites of antiviral nucleoside drugs, typified by azidothymidine monophosphate (AZTMP), are capable of inhibiting the 3'-processing and strand transfer reactions of HIV-IN via a specific low affinity nucleotide site located within the catalytic core residues 50-212. A photoaffinity analog of AZTMP, 5-azido-3'-azido-2',3'-dideoxyuridine monophosphate (5N$_3$AZddUMP), was used to identify this nucleotide site in recombinant WT and mutant HIV-IN. Photoincorporation of [^{32}P]5N$_3$AZddUMP into HIV-1 integrase was half-maximal at 90 μM, and it was competitively inhibited by AZTMP. Also, 5N$_3$AZddUMP was an inhibitor of the 3'-processing and strand transfer reactions with 50-70 μM IC$_{50}$ values. Non-nucleotide, high affinity inhibitors thought to bind in the D,D-35-E catalytic region had little inhibitory effect on photolabeling. A new proteolytic mapping procedure was used to identify photocrosslinked peptides, and by comparison with different protease digestions, a peptide region corresponding to amino acids 158-170 was determined as the site of crosslinking. Structurally, this peptide corresponds to a surface site on the α4 helix domain adjacent to the catalytic core D,D-35-E region. Site-specific mutants in this region result in loss of DNA binding and catalytic activity. Because most potent HIV-IN inhibitors bind at the catalytic site and are predominantly lipophilic, identification of this 158-170 site will allow the design and testing of new hydrophilic drugs for HIV-IN.

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Piperazinyloxoquinoline derivatives, potent and selective inhibitors of HIV-1 transcription

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We have found novel piperazinyloxoquinoline derivatives to be potent and selective inhibitors of HIV-1 replication in both acutely and chronically infected cells. K-12, the most potent congener of the series, completely inhibited HIV-1 replication in MOLT-4 cells (acutely infected with IIIB strain) at a concentrations of 0.10-0.8 μM without showing any cytotoxicity. Its 50% effective concentration (EC$_{50}$) and 50% cytotoxic concentration (CC$_{50}$) were 0.061 and 6.6 μM, respectively. K-12 was also inhibitory to HIV-1 in MT-4 and peripheral blood mononuclear cells. It was active against an HIV-1 clinical isolate (HE) and HIV-2 (EHO). The compound completely suppressed tumor necrosis factor (TNF)-α-induced HIV-1 expression in latently infected cells (OM-10.1) and constitutive viral production in chronically infected cells (MOLT-4/IIIB) at a concentration of 0.8 μM, as determined by p24 antigen production in culture supernatants. The HIV-1 protease inhibitor VX-478 was also effective, however, the Tat inhibitor Ro24-7429 showed marginal effect in OM-10.1 cells. The reverse transcriptase inhibitor zidovudine was totally inactive. Furthermore, K-12 could inhibit HIV-1 antigen expression in OM-10.1 and MOLT-4/IIIB cells, whereas VX-478 did not affect the antigen expression at non-toxic concentrations. Northern blot analysis revealed that K-12 selectively prevented the accumulation of HIV-1 mRNA in MOLT-4/IIIB and TNF-α-treated OM-10.1 cells in a dose-dependent fashion. These results indicate that the piperazinyloxoquinoline derivatives represent a group of HIV-1 inhibitors with a unique mechanism of action.