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Ingenol Derivatives, Ingredient of 'Kansui', are Highly Potent Inhibitor of HIV

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We have examined Ingenol derivatives, the extracts from 'kansui' (dried roots of *Euphorbia Kansui* Liou) for their inhibitory effects on HIV replication *in vitro* and found that they are highly potent inhibitors of the virus. In the anti-HIV assays, MT-4 cells were infected with HIV and incubated in the presence of varying concentrations of the test compounds. After a 4- or 5-day incubation period, the anti-HIV activities were determined by the inhibition of virus-induced cytopathic effect in infected cells or the reduction of p24 antigen in culture supernatants. The 50% effective concentration (EC_{50}) and the 50% cytotoxic concentration (CC_{50}) were calculated by the standard method. We have also examined the inhibitory effects of the Ingenol derivatives on syncytium (multinucleated giant cell) formation induced by cocultivation of Molt-4 cells with Molt-4/IIIB (a cell line persistently infected HIV-1) cells. The most potent compound of this series inhibited the replication of HIV-1_{IIIB} at a concentration of 0.1nM with a selectivity index greater than 100,000. The Ingenol derivatives were also inhibitory to the replication of several strains of HIV-1, including clinical isolates, AZT-resistant mutants, and nonnucleoside reverse transcriptase (NNRTI)-resistant mutants, and HIV-2. Syncytium formation was suppressed by the compounds. Studies on their mechanism of action suggest that the Ingenol derivatives act on the stage of viral entry into the host cells.

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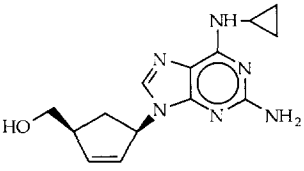
1592U89 Succinate – A Potent, Selective Anti-HIV Carbocyclic Nucleoside. S.M. DALUGE, S.S. GOOD, M.B. FALETTO, M.T. MARTIN, W.H. MILLER, D.R. AVERETT, M.H. ST. CLAIR, L.R. BOONE, M. TISDALE[1], and J.E. REARDON. Wellcome Research Laboratories, Research Triangle Park, NC 27709, USA, and [1]Beckenham, UK.

(1S,4R)-4-[2-Amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol (1592U89) is a carbocyclic nucleoside with potent, selective anti-HIV activity. Clinical evaluation of the succinate salt of 1592U89 was recently initiated. 1592U89 was selected for development after synthesis and evaluation of a wide variety of nucleosides containing the cyclopentenyl sugar mimic of (-)-carbovir (CBV), maximizing anti-HIV potency and improving oral bioavailability. Importantly, 1592U89 penetrated rat brain and monkey CSF as well as AZT, the only approved anti-HIV therapy with demonstrable positive effects on the CNS manifestations of the disease. 1592U89 is activated intracellularly to CBV-TP by a novel phosphorylation pathway involving the recently characterized enzyme adenosine phosphotransferase (Garvey and Krenitsky. 1992. *Arch. Biochem. Biophys.* 296:161-169). 1592U89 is not a substrate for adenosine deaminase and is not converted (<2%) to CBV in animals and cells. Using identical conditions with activated calf thymus DNA as the nucleic acid substrate, the selectivity of CBV-TP for HIV-1 reverse transcriptase (K_i 0.21 μM) over human DNA polymerases α, β, γ, and ε (K_i values 330-, 16,000-, 670-, and 19,000-fold higher, respectively) was confirmed. 1592U89 was equivalent in potency to AZT when tested *in vitro* in human peripheral blood lymphocytes against fresh clinical isolates of HIV-1 from AZT-naive patients (average IC_{50} value 0.26 μM for 1592U89 and 0.23 μM for AZT). 1592U89 was not cross-resistant with AZT. *In vitro* passage in MT4 cells of HIV-1 (IIIB) molecular clones HXB2 and RTMC in the presence of 1592U89 did not rapidly select for resistant virus. Multiple mutations were required to confer up to 4- to 10-fold increases in IC_{50}, and the L74V and M184V mutations are each able to partially revert AZT resistance. The cross-resistance profile and relatively slow emergence of resistance *in vitro*, together with synergy with AZT and other anti-HIV agents, make 1592U89 an important anti-HIV drug candidate.



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