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In Vitro Evaluation Of Selected New Compounds As Inhibitors Of HIV-1 Replication. M. A. Chirigos[3], J. S. Driscoll[2], J. B. Kahlon[1], M. Tucker[1], L. E. White[1], A. D. Brazier[1], and W. M. Shannon[1]. Southern Research Institute, Birmingham, AL 35255 USA1, Laboratory of Medicinal Chemistry, Division of Therapeutics Program, Division of Cancer Treatment, NCI, NIH, Bethesda, MD[2], U.S. Army Medical Research, Institute of Infectious Diseases, Fort Detrick, Frederick, MD[3].

Four compounds coded as DDF-92, DDG-1, DDG-39 and DDG-48 were tested for antiviral activity against the human immunodeficiency virus (HIV-1) *in vitro*. AZT and ddC, two well-known compounds with antiviral activity against HIV-1 were tested in parallel. *In vitro* antiviral evaluations were conducted using MT-2 cells (Harada *et al.* Science *229*:563-566) and CEM-SS cells (P.Nara, NIH). Inhibition of viral cytopathic effects (CPE) in the presence of the test compounds was determined by measuring either cell viability (MTT staining; Tada *et al.* J Immunol Methods, *93*:157-165) or cellular DNA (to determine cell proliferation). Cytotoxic effects of the compounds were measured at the same concentrations. Three compounds (DDF-92, DDG-1 and DDG-39) were significantly active against HIV-1 [selectivity index (SI): >312.5, >16.74 and >12.01, respectively; SI AZT=312.50 and SI ddC=67.30] with no cytotoxicity demonstrated at the tested concentrations. The compounds were tested for direct inhibition of HIV-1 reverse transcriptase (RT) activity and RT was also employed as a measure of progeny virus in infected treated samples. RT results correlated well with results obtained from cell proliferation/cpe-inhibition data. Two of the most active compounds (determined by their SI) were followed up in experiments designed to target HIV-1 markers, i.e., HIV-1 RNA (measured by hybridization) and HIV-1 P24, the gag gene product, quantitatively measured by an indirect immune-fluorescence assay. Data indicate a good correlation between the various assay methods used and provide a basis for evaluation of the *in vitro* and *in vivo* antiviral properties of these compounds.

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5-Chloropyrimidine 2',3'-Dideoxyribosides: Synthesis and Anti-HIV Evaluation
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In view of the selective anti-HIV activity of 2',3'-dideoxy-3'-fluoro-5-chlorouridine (J. Balzarini *et al.*, Mol. Pharmacol. 35: 571, 1989; A. Van Aerschot *et al.*, J. Med. Chem. 32: 1743, 1989), a series of eight 2',3'-dideoxyribo-5-chloropyrimidines were synthesized and evaluated for their inhibitory activity against human immunodeficiency virus type 1 (HIV-1) replication in MT-4 cells. The 5-chloro substituent was introduced through reaction of the 5'-O-protected 2',3'-dideoxyuridine derivatives with N-chlorosuccinimide. The 2',3'-dideoxycytidine analogues were derived from the 2',3'-dideoxyuridine analogues. A marked increase in selectivity was noted for the 5-chlorouracil derivatives of 2,3-dideoxyribofuranose, 3-azido-2,3-dideoxyribofuranose and 3-fluoro-2,3-dideoxyribofuranose, as compared to their non-chlorinated counterparts. This was mainly due to a decreased toxicity of the chlorinated compounds for the host cells. While introduction of a chlorine at the C-5 position of 2',3'-dideoxycytidine annihilated its anti-HIV activity in MT-4 cells, chlorination of 3'-fluoro-2',3'-dideoxycytidine, 3'-azido-2',3'-dideoxycytidine and 2',3'-didehydro-2',3'-dideoxycytidine led to a reduction in cytotoxicity without concomitant reduction in anti-HIV activity.