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Affinity Purification OF HIV and Visna Virus Reverse Transcriptases Using a Novel Inhibitor. C. Flexner, P.R. Hubbs, Y-J. Chan and H. Prochaska. Departments of Medicine and Pharmacology and Molecular Sciences, Johns Hopkins University, Baltimore, Maryland, USA.

The reverse transcriptase (RT) of lentiviruses is essential for replication and can be inhibited by several reactive sulfonated dyes. We identified a novel sulfonated monochlorotriazine dye which was a potent and selective inhibitor of the RTs of both HIV-1 and a closely related ovine lentivirus, visna virus. This dye had an IC_{50} for crude RTs of 1 to 5 μg/ml, and prevented visna virus cytopathic effect in sheep choroid plexus cells with an IC_{99} of approximately 25 μg/ml. Covalent linkage of this compound to agarose resulted in a complex which retained high affinity for RT in crude extracts of cells infected with live recombinant vaccinia virus expression vectors. Elution of inhibitor-affinity columns with potassium chloride yielded peak fractions with up to a 2000-fold increase in RT specific activity. Recombinant visna virus RT produced in cells infected with a vaccinia virus expression vector was purified in this manner. This enzyme, which has a 50% amino acid sequence homology with the HIV-1 RT, had nearly identical biochemical characteristics and was equally sensitive to inhibition by dideoxynucleoside triphosphates. Inhibitor-affinity purification using an antiviral compound is a simple and efficient way to characterize viral enzymes of laboratory and clinical interest.

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5'-HYDROGENPHOSPHONATES OF ANTI-HIV NUCLEOSIDE ANALOGUES: CONTROVERSIAL MODE OF ACTION

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The 5'-hydrogenphosphonates of AZT and ddU were synthesized and their anti-HIV activity as well as their stability in various media were studied.

From the obtained results, we concluded that whether they may penetrate cell membranes or not, such derivatives of anti-HIV dideoxynucleosides most likely exert their action through a release of their parent nucleoside.

Thus, these compounds cannot be used in order to overcome the often observed resistance to nucleoside analogues, when the resistance is due to a loss or to a lack of activity of the enzymes required for anabolic phosphorylations.