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Peptide-based Inhibitors of HIV-1 RT Dimerization and Pol-polyprotein Maturation

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Reverse transcriptase plays an essential role in HIV replication and even if it is a major target for current AIDS therapy it remains an option for future anti-HIV treatments. Therefore, identification of new molecules targeting new sites on RT remains an important task, particularly against resistant virus strains. The active form of HIV-RT is the p51/p66 heterodimer, and its formation is a two-step mechanism, including a rapid protein/protein interaction, the dimerization step followed by conformational changes, the maturation step. Herein, we have elaborated a new strategy based on short interfacial peptides that target protein/protein interfaces. We have designed a short 9-mer peptide (Pep-71) derived from the Trp-rich cluster of the connection subdomain, which blocks RT dimerization. Pep-71 interacts preferentially with the p51 subunit within heterodimeric RT, and destabilizes the dimeric conformation, thereby triggering dissociation. The binding site of Pep-71 has been located to a cleft between the connection and fingers domains of the small p51 subunit and found to involve contacts between the highly conserved residues Trp24 and Phe61 on the p51 subunits. When delivered into cells using the cell penetrating peptide MPG, Pep-71 abolishes HIV-1-LAI replication (IC50: 0.5 nM). These sub-nanomolar concentrations of Pep-71 block the replication of either multi-drug resistant strains or primary HIV isolates from subtypes A to G; it is at least as efficient as AZT. Similarly, Pep-71 presents the same efficiency than AZT in monocyte-derived macrophages infected with HIV-1/Ba-L (IC50: 3.8 nM). Preliminary evaluation of Pep-71 in an HIV susceptible transgenic rat model has confirmed the potency of the MPG-Pep-71 nanoparticles in vivo. From a mechanistic point of view, the high efficiency of Pep-71 in cellulo is not only associated to inhibition of RT dimerization, but also to an alteration of the maturation of the Pol-polyprotein. The combination of interfacial peptide inhibitors with currently used treatments could have a major impact on AIDS therapy.

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Low *In Vitro* Potential for Class-specific Toxicity of GS-9148, A Novel Nucleotide Reverse Transcriptase Inhibitor

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GS-9148 is a novel ribose-modified nucleotide with a favorable in vitro activity profile against HIV-1 strains resistant to multiple NRTIs. Its oral prodrug, GS-9131, is currently under clinical evaluation. Since adverse symptoms caused by mitochondrial toxicity limit the therapeutic benefit of some antiretroviral nucleosides and a various degree of renal dysfunction has been associated with the clinical use of nucleotides, we conducted in vitro studies to characterize the potential of GS-9148 for these class-specific effects. As determined by both DNA hybridization and quantitative PCR analysis, treatment with up to 300 μM GS-9148 or tenofovir for 10–21 days did not reduce the mitochondrial DNA content in HepG2 cells. In contrast, treatment with 30–100 μM ddI, d4T, or ddC depleted the levels of mitochondrial DNA by 70–90%. Unlike ddI, neither GS-9148 nor tenofovir affected the lactic acid production in treated cells. In accordance with these observations, diphosphates of GS-9148 and tenofovir did not inhibit DNA polymerase gamma ($IC_{50} > 300$ μM) whereas the active metabolite of ddI was a potent inhibitor of the enzyme ($IC_{50} = 1.0$ μM). Potential for nephrotoxicity was assessed in primary human renal proximal tubule cells, in which GS-9148 and tenofovir exhibited reduced cytotoxicity ($CC_{50} > 2000$ μM) compared to cidofovir and adefovir ($CC_{50} = 260$ and 495 μM, respectively). GS-9148 also showed 60–100-fold lower efficiency of transport by human renal organic anion transporter type 1 (hOAT1) and was 20–300-fold less cytotoxic in cells overexpressing hOAT1 than cidofovir, adefovir, and tenofovir. Finally, approximately fivefold lower accumulation of GS-9148 compared to tenofovir was observed in fresh human renal tissue incubated in vitro; a similar difference was found in the renal accumulation of radiolabeled material following oral administration of GS-9131and tenofovir DF to dogs. In summary, the lack of effects on mitochondrial functions, low cytotoxicity in renal cells, and limited efficiency of renal transport suggest a low potential for GS-9148 to be associated with class-specific toxicities observed with some antiviral nucleosides or nucleotides.

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