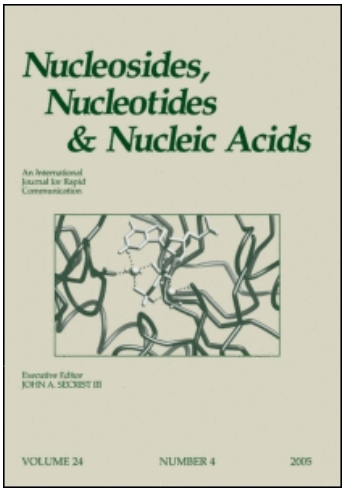

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Effect of Acyclic Nucleoside Phosphonates on the HIV-1 Integrase in Vitro

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EFFECT OF ACYCLIC NUCLEOSIDE PHOSPHONATES ON THE HIV-1 INTEGRASE IN VITRO

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ABSTRACT: Integrase (IN) is an essential enzyme in the human immunodeficiency virus type-1 (HIV-1) replication cycle and, thus, a potential target for chemotherapeutic agents. Because various nucleotide analogues have been reported to inhibit IN in vitro, we investigated the effect of acyclic nucleoside phosphonates. Both unphosphorylated and diphosphorylated derivatives were inhibitory to IN at concentrations ranging between 60 and 800 μM, with diphosphorylated derivatives being 5- to 8-fold more potent than unphosphorylated counterparts.

The 3'-end of the HIV-1 *pol* gene encodes for a 32 kDa protein, the integrase (IN), which catalyzes the insertion of a double-stranded DNA copy of the viral genome into the host cell DNA (1). Being an essential enzyme in the HIV-1 replication cycle and having no counterparts in uninfected cells, IN is an ideal target for the development of selective drugs. In order to obtain more insights into the integration mechanism and to identify IN inhibitors, in vitro assays have been developed which employ recombinant enzyme and double-stranded oligos reproducing the viral LTR termini as substrate. Numerous compounds have been described so far. Among them are nucleotide analogues such as 3'FdTMP, AZTMP, D4TMP, AZTTP, ddATP, L-ddATP, L-ddCMP, L-5FddCMP, L-5FddCTP, which have been shown to inhibit both 3'-processing and strand transfer reactions at concentrations ranging between 50 and 250 μM (2-5). Due to the fact that inhibition seemed to correlate with modifications of the sugar rather than with the type of base or the extent of phosphorylation, interactions between IN and the sugar moieties are taught to play a critical role in the binding of and inhibition by nucleotide analogues (4).

TABLE 1. Anti-HIV-1 activity of acyclic nucleoside phosphonates in cell-based and enzyme assays

Compound	[a]CC_{50} (μM)	[b]EC_{50} (μM)	IC_{50} (μM)	
			[c]RT	[d]IN
PMEA	200	4.8	> 100	840 ± 90
PMEApp	> 200	> 200	5.7 ± 2.5	200 ± 10
PMPA	> 200	2.2	> 100	375 ± 50
PMPApp	> 200	> 200	25 ± 9	65 ± 5
PMEG	1.6	0.14	> 100	640 ± 50
PMEGpp	> 200	> 200	3.7 ± 1.5	82 ± 17
PMPG	> 200	3.0	> 100	300 ± 50
PMPGpp	> 200	> 200	1.9 ± 0.15	63 ± 2
ddAdo	>200	10	> 1000	> 1000
ddATP	-	-	0.6 ± 0.08	150 ± 20
ddGuo	1.4	0.11	> 1000	> 1000
ddGTP	-	-	0.5 ± 0.04	> 1000

[a]Compound concentration required to reduce the viability of mock-infected MT-4 cells by 50%, as determined by the MTT method.
[b]Compound concentration required to achieve 50% protection of infected cultures from the HIV-1-induced cytopathogenicity, as determined by the MTT method.
[c]Compound concentration required to reduce the HIV-1 RT activity by 50%. Data represent average ± standard deviation for three independent determinations. RT activity was measured using activated DNA and [^{3}H]-dGTP 10 μM or [^{3}H]-dATP 10 μM.
[d]Compound concentration required to reduce the HIV-1 IN 3'-processing activity by 50%. Data represent average ± standard deviation for three independent determinations.

We therefore investigated whether acyclic nucleoside phosphonates (ANP) and their diphosphorylated (ANPpp) counterparts, i.e. analogs of phosphorylated nucleosides in which the furanose ring is replaced by an acyclic side chain (6), were also able to inhibit the HIV-1 IN. To this end, recombinant enzyme was incubated with a U5 LTR substrate that was labeled at the dinucleotide which is removed in the 3'-processing reaction. Multiscreen filtration plates and PAGE were used to measure the extent of 3'-processing inhibition with the various test compounds, as previously described (7).

As shown in Table 1, with the sole exception of PMEG, whose CC_{50} was 1.6 μM, ANPs confirmed non cytotoxic for exponentially growing MT-4 cells and fairly active inhibitors of the HIV-1 multiplication in acutely infected cells. While only ANPpp inhibited the HIV-1 reverse transcriptase (RT) in enzyme assays, both ANP and ANPpp inhibited the recombinant HIV-1 IN. PMPGpp, PMPApp and PMEGpp were the most potent acyclic phosphonates (63-82 μM), followed by ddATP (used as reference inhibitor) and PMEApp (150-200 μM), PMPG and PMPA (300-375 μM), PMEG and PMEA (640-840 μM). It is noteworthy that ddGTP confirmed inactive at 1000 μM).

Overall, these results indicate that: i) IN binds and is inhibited by nucleotide analogues which are devoid of a sugar moiety; ii) unlike other nucleoside analogues, the extent of phosphorylation of ANPs is directly related to their anti-IN potency; iii) the type of alkyl side chain is more relevant for the anti-IN activity than the type of base. Furthermore, kinetic studies (data not shown) reveal that the mode of interaction with IN of acyclic nucleoside phosphonates, as well as that of other nucleoside analogues, is non competitive. Therefore, the nucleotide and the substrate binding sites do not overlap.

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