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HIV Integrase Inhibitors with Nucleobase Scaffolds: Discovery of a Highly Potent anti-HIV Agent

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Abstract

HIV integrase is essential for HIV replication. However, there are currently no integrase inhibitors in clinical use for AIDS. We have discovered a conceptually new β -diketo acid that is a powerful inhibitor of both the 3'-processing and strand transfer steps of HIV-1 integrase. The *in vitro* anti-HIV data of this inhibitor were remarkable as exemplified by its highly potent antiviral therapeutic efficacy against HIV_{TEKI} and HIV-1_{NL4-3} replication in PBMC (TI >4,000 and >10,000, respectively).

The enzymes of the *pol* gene of the human immunodeficiency virus (HIV) have been identified as important viral targets for the discovery and development of anti-HIV therapeutic agents.¹⁻⁵ Drug discovery involving two of the enzymes, HIV reverse transcriptase (RT) and HIV protease (PR), and subsequent clinical utilization of some of these compounds for the treatment of acquired immunodeficiency syndrome (AIDS), have suggested that the methodology of targeting key viral enzymes for inhibition represents a viable approach in antiviral chemotherapy.⁴⁻⁸ However, while the viral targets, HIV RT and HIV PR, have been successfully investigated for the development of clinically-useful therapeutic agents, research efforts on drug discovery pertaining to the third enzyme of the *pol* gene, HIV integrase, have not resulted in a single FDA-approved drug where the mechanism of action is inhibition of HIV integrase.⁹⁻¹¹ Nevertheless, as integrase has no human counterpart and is essential for HIV replication, it remains a superbly attractive target for the discovery of new anti-HIV agents.

HIV-1 integrase is a 32 kDa protein encoded at the 3'-end of the HIV *pol* gene.¹²⁻¹⁴ Incorporation of HIV DNA into host chromosomal DNA, which is catalyzed by HIV integrase, occurs by a specifically defined sequence of 3'-processing and strand transfer reactions.^{12, 13,15,16} Prior to the initiation of integration in the cytoplasm, there is assembly of viral DNA, previously produced by reverse transcription, on HIV integrase. Following assembly, site-specific endonucleatic cleavage of two nucleotides from each 3'-end of double-stranded viral DNA (3'-processing) produces tailored viral DNA recessed by two nucleotides and with terminal CAOH-3'. In the next step, identified as strand transfer, there is staggered nicking of chromosomal DNA and joining of each 3'-end of the recessed viral DNA to the 5'-ends of the host DNA. Interestingly, the strand transfer step, occurring in the nucleus, is partitioned from 3'-processing and is carried out after transport of the processed, preintegration complex from the cytoplasm into the nucleus.

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While a number of structurally diverse compounds have been reported to be inhibitors of HIV integrase,^{8-10,17-27} only a few compounds of one group, the β -diketo acids, represent the most convincing, biologically-validated inhibitors^{23,24} of this viral enzyme. However, many of the reported β -diketo acids are inhibitors of only the strand transfer step. The *in vitro* anti-HIV data for perhaps the most active β -diketo compound,²⁵ **S-1360** (Figure 1, Table 1), reveal a therapeutic index (CC_{50}/IC_{50}) of 786. Another well-known β -diketo compound, **L-731988**, appears to be somewhat less active than **S-1360** (Table 1).

In this report, we disclose for the first time, conceptually new β -diketo acids with nucleobase scaffolds that are potent inhibitors of *both* the *3'-processing* and *strand transfer* steps of HIV integrase. We have discovered that the nucleobase scaffold, the substituents, and the specific spatial relationship of substituents in the scaffold, are critical for potent integrase inhibitory activity. The discovery makes these compounds unique among diketo acids, not only in terms of integrase activity, but also because of their *remarkably potent anti-HIV activity* (discussed below). Our work involved examples of *both pyrimidine and purine* scaffolds. The discovery is best illustrated with one notable example, in which the structural features are a pyrimidine nucleobase scaffold that bears three specific substituents in a defined spatial relationship: hydrophobic benzyl groups at N-1 and N-3 and a diketo acid (enolic form predominant) at C-5 (Fig. 2). This compound, 4-(1,3-dibenzyl-1,2,3,4-tetrahydro-2,4-dioxypyrimidin-5-yl)-2-hydroxy-4-oxo-but-2-enoic acid (**1**), was synthesized in our laboratory from readily available ethyl β -ketobutyrate.

Integrase inhibition studies were conducted with recombinant wild-type HIV-1 integrase and a 21-mer oligonucleotide substrate, following a previously described procedure.²⁸ Compound **1** and its analogs, **2** and **3**, showed strong inhibition of both 3'-processing and strand transfer steps of HIV-1 integrase (Table 2). The observed integrase inhibition is unusual, as the reported β -diketo compounds are commonly inhibitors of only the strand transfer step (e.g., **S-1360**). Although the same active site residues appear to be involved in 3'-processing and strand transfer, it is not clear whether the mechanism of inhibition of HIV integrase by **1** is the same for 3'-processing in the cytoplasm and strand transfer in the nucleus, i.e., interaction with the DDE motif (Asp64, Asp116 and Glu152) and other proximal amino acid residues and possible sequestration of critical metal cofactors in the catalytic site (Fig. 3). Participation of the uracil amide carbonyl (4-position) in the binding of this inhibitor to the active site is suggested by our docking experiments. Some support for the contribution of the uracil ring in the inhibition of both steps of HIV integrase comes from the inhibition data for **L-708906** that lacks the nucleobase scaffold [IC_{50} for **L-708906** >1000 μ M (3'-processing) and 0.48 μ M $\pm$ 0.08 μ M (strand transfer), TI = 16, MT-4 cells].²⁷

Compound **1** and the positive control compound, AZT, were tested in a PBMC cell-based, microtiter anti-HIV assay against the clinical isolate, HIV-1_{TEKI} (NSI phenotype) and HIV-1_{NL4-3} (SI phenotype), and in a MAGI-X4 assay against HIV-1_{NL4-3} performed with HeLa-CD4-LTR- β -gal cells. All antiviral determinations were performed in triplicate with serial $\frac{1}{2}$ log₁₀ dilution of the test materials (six to nine concentrations total). The overall performance of both assays was validated by the MOI-sensitive positive control compound, AZT, exhibiting the expected level of antiviral activity.

As summarized in Table 3 (see also, "Supporting Information"), *in vitro* anti-HIV studies against HIV-1 isolates in PBMC showed that compound **1** (highest test concentration = 200 μ M) was extremely active with IC_{50} values in the nM range and with antiviral efficacy data (*therapeutic indices*, $TI = CC_{50}/IC_{50}$) of >4,000 (HIV-1_{TEKI}) and >10,000 (HIV-1_{NL4-3}). The IC_{90} data for **1**, which were in the low μ M range, were also compelling (Table 3). Cell viability data showed only mild cellular cytotoxicity at higher test concentrations; however, a CC_{50} (>200 μ M) was not reached. The control compound, AZT (highest test concentration = 1 μ M),

gave therapeutic indices of $>7,143$ (HIV-1_{TEKI}) and $>5,556$ (HIV-1_{NL4-3}). Also, in comparison, the antiviral efficacy data of **1** in PBMC (HIV-1_{NL4-3}) were *well over an order of magnitude greater* than those for the most active known β -diketo integrase inhibitor, **S-1360** in PBMC (see Table 1).²⁵

In order to obtain further supporting information for the aforementioned PBMC data and the anti-HIV-1 integrase data, evaluations were performed for compound **1** against HIV-1_{NL4-3} in a MAGI-X4 assay. This assay is designed to detect compounds that block HIV-1 replication *via* targets in the viral life cycle up to and including Tat transactivation (e.g., virus attachment/fusion/entry, uncoating, reverse transcription, nuclear import, *integration*, LTR transactivation). Inhibitors of viral targets after LTR transactivation (e.g., Rev, virus egress/packaging/release, protease) do not score well in this assay.

Integrase inhibitor, **1**, and the known HIV replication inhibitors, AZT (reverse transcriptase inhibitor), T-20 (fusion inhibitor) and dextran sulfate (entry inhibitor) all exhibited anti-HIV activity in the MAGI-X4 assay against HIV-1_{NL4-3} in HeLa-CD4-LTR- β -gal cells. The protease inhibitor, saquinavir was inactive. In addition, compound **1** did not display any cellular cytotoxicity up to the highest concentrations (200 μ M) tested in this assay.

In summary, a conceptually new β -diketo acid discovered by us is a powerful inhibitor of *both* steps of wild-type HIV-1 integrase and exhibits remarkably potent *in vitro* anti-HIV activity, making it the most active inhibitor of HIV replication of this class of biologically-validated integrase inhibitors. The discovery of this remarkably active molecule, representative of a unique set of diketo acids bearing nucleobase scaffolds, has uncovered a whole new direction in the chemistry and biology of integrase inhibitors and their potential therapeutic applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Structures of two of the most active, known β -diketo compounds.

Compound 1

Figure 2.

Our novel integrase inhibitor, **1**, shown in its intramolecularly hydrogen-bonded form (left), and the preferred conformation of its physiologically-relevant anion (right), depicting the relationship of the two benzyl groups and of the enolic hydrogen with the keto carbonyl.

Figure 3. Docking of HIV integrase inhibitor, (**1**), with HIV-1 integrase core structure (PDB code 1BL3). The DDE catalytic triad (Asp64, Asp116 and Glu152) is shown in blue-green, the Mg^{2+} as a red sphere, other H-bonding residues (Asn155 and Glu92) in orange, the lysine residue with NH_3^+ (electrostatic interaction) in red. Involvement of the uracil ring of inhibitor in active site binding is clearly apparent.

Table 1

Anti-HIV data for two of the most active, known β -diketo compounds (see Fig. 1 for structures).

Identification No.	S-1360	L-731988
Lit. Ref. No.	25	19
IC ₅₀ (μ M)	0.14	1.0
CC ₅₀ (μ M)	110	**
Cell Line	PBMC	H-9
TI*	786	**

* Therapeutic Index.

** Not specified

Table 2

IC₅₀ data for the inhibition of wild-type HIV-1 integrase by compound **1**, its closely related analogs and compounds of Table 1.

Inhibitors	3'-Processing (μM)	Strand Transfer (μM)
Compound 1 (Fig. 1)	3.7 (±1.0)	0.2 (±0.1)
2 (bis-(<i>o</i> -F-Bn) analog of 1)	4.1 (±0.4)	< 0.6
3 (bis-(<i>p</i> -F-Bn) analog of 1)	3.9 (±1.1)	< 0.7
4 (1 minus N ³ -benzyl)	10 (±2.0)	0.5 (±0.2)

Table 3

Antiviral efficacy of compound **1** and the positive control compound, AZT, in the inhibition of HIV-1_{TEKI} and HIV-1_{NL4-3} replication in PBMC.

Compd	High Conc. (μM)	HIV-1 Isolate	IC ₅₀ (nM)	CC ₅₀ (μM)	TI
1 [*]	200	TEKI	50	> 200	> 4,000
1 [*]	200	NL4-3	< 20	> 200	> 10,000
AZT	1	TEKI	0.14	> 1	> 7,143
AZT	1	NL4-3	0.18	> 1	> 5,556

* For compound **1**: IC₉₀ 3.91 μM (HIV-1_{TEKI}), CC₉₀ >200 •M. IC₉₀ 1.54 μM (HIV-1_{NL4-3}), CC₉₀ >200 •M.