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

Vasu Nair,*,§ Guochen Chi,§ Roger Ptak,† and Nouri Neamati‡

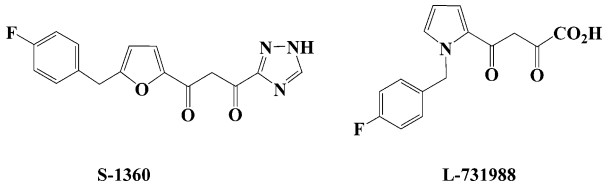
The Center for Drug Discovery and the Department of Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, Georgia 30602, Southern Research Institute, Infectious Disease Research Department, Frederick, Maryland 21701, and Department of Pharmaceutical Sciences, University of Southern California, Los Angeles, California 90089

Received September 8, 2005

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.



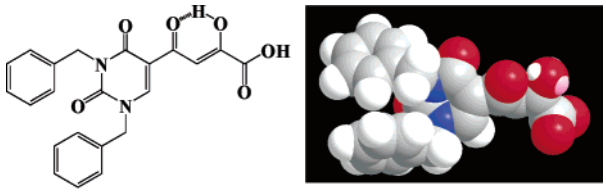
S-1360 L-731988

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

Table 1. Anti-HIV Data for Two of the Most Active, Known β-Diketo Compounds (see Figure 1 for structures)

identification no.	S-1360	L-731988
lit. ref no.	25	19
IC_{50} (μM)	0.14	1.0
CC_{50} (μM)	110	*b*
cell line	PBMC	H-9
TI[a]	786	*b*

[a] Therapeutic index. [b] Not specified.



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.

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 (Figure 2). This compound, 4-(1,3-dibenzyl-1,2,3,4-tetrahydro-2,4-dioxopyrimidin-5-yl)-2-hydroxy-4-oxo-but-2-enoic acid (**1**), was synthesized in our laboratory from readily available ethyl β-ketobutyrate.

* To whom correspondence should be addressed. Tel.: (706) 542-6293; Fax: (706) 583-8283, E-mail: vnair@rx.uga.edu.
§ The University of Georgia.
† Southern Research Institute.
‡ University of Southern California.

10.1021/jm0508890 CCC: $33.50
Published on Web 12/21/2005

Table 2. IC_{50} Data for the Inhibition of Wild-Type HIV-1 Integrase by Compound **1** and Its Closely Related Analogues

inhibitors	3′-processing (μM)	strand transfer (μM)
compound **1** (Figure 1)	3.7 (±1.0)	0.2 (±0.1)
2 (bis-(*o*-F−Bn) analogue of **1**)	4.1 (±0.4)	< 0.6
3 (bis-(*p*-F−Bn) analogue of **1**)	3.9 (±1.1)	< 0.7
4 (**1** minus N^3-benzyl)	10 (±2.0)	0.5 (±0.2)

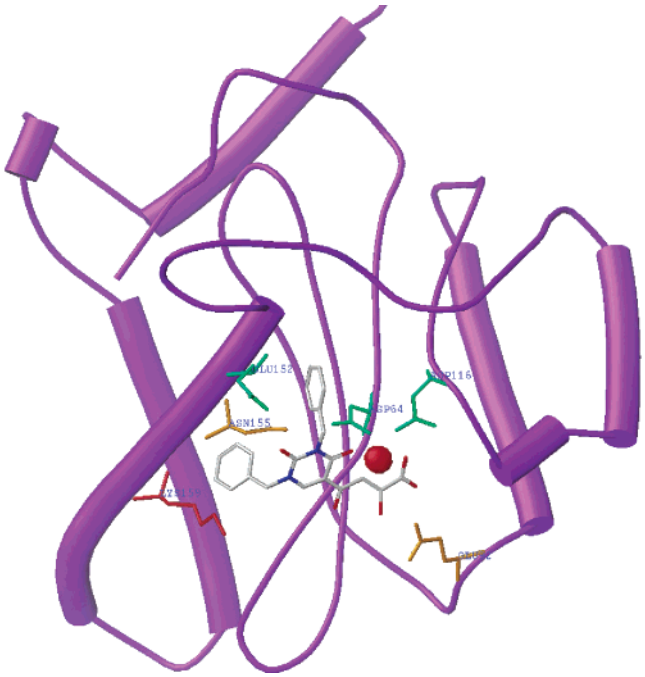


Figure 3. Docking of HIV integrase inhibitor (**1**) with HIV-1 integrase core structure (PDB code 1BL3). The DDE catalytic triad (Asp64, Asp116, and Glu152) is shown in blue-green, the Mg^{2+} as a red sphere, other H-bonding residues (Asn155 and Glu92) in orange, the lysine residue with NH_3^+ (electrostatic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

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 analogues, **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 (Figure 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 μM (3′-processing) and 0.48 μM ± 0.08 μM (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 1/2log10 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.

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 concn (μM)	HIV-1 isolate	IC_{50} (nM)	CC_{50} (μM)	TI
1[a]	200	TEKI	50	>200	>4000
1[a]	200	NL4-3	<20	>200	>10000
AZT	1	TEKI	0.14	>1	>7143
AZT	1	NL4-3	0.18	>1	>5556

[a] For compound **1**: IC_{90} 3.91 μM (HIV-1_{TEKI}), CC_{90} > 200 μM. IC_{90} 1.54 μM (HIV-1_{NL4-3}), CC_{90} > 200 μM.

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 μM) was extremely active with IC_{50} values in the nM range and with antiviral efficacy data (therapeutic indices, TI = CC_{50}/IC_{50}) of > 4000 (HIV-1_{TEKI}) and >10000 (HIV-1_{NL4-3}). The IC_{90} data for **1**, which were in the low μM range, were also compelling (Table 3). Cell viability data showed only mild cellular cytotoxicity at higher test concentrations; however, a CC_{50} (>200 μM) was not reached. The control compound, AZT (highest test concentration = 1 μM), gave therapeutic indices of > 7143 (HIV-1_{TEKI}) and >5556 (HIV-1_{NL4-3}). Also, in comparison, the antiviral efficacy data of **1** in PBMC (HIV-1_{NL4-3}) were well over 1 order of magnitude greater than those for the most active known β-diketo integrase inhibitor, S-1360 in PBMC (see Table 1).[25]

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 μM) 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.

Acknowledgment. This project was supported by Grant No. RO1 AI 43181 (to V.N.) from the National Institutes of Health (NIAID). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

One of us (V.N.) also thanks the Terry Endowment and the Georgia Research Alliance for their support. The in vitro anti-HIV data were determined at the Southern Research Institute, Frederick, MD, under NIAID Contract NO1-AI-25478 (to R.P.). We thank Dr. Steven Turk, Program Officer, NIAID, Division of AIDS, for his support and facilitation of the anti-HIV studies. We also acknowledge the contribution of Dr. Arthur Cox with the molecular modeling representation.

Supporting Information Available: Synthetic methods and analytical data for **1** and its precursors, and for compounds **2**−**4**; procedures for HIV integrase assays and HIV antiviral assays, representative graphical anti-HIV data and standard deviation data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Fauci, A. S. The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. *Science* **1988**, *239*, 617−622.
(2) Katz, R. A.; Skalka, A. M. The retroviral enzymes. *Annu. Rev. Biochem.* **1994**, *63*, 133−173.
(3) Frankel, A. D.; Young, J. A. T. HIV-1: fifteen proteins and an RNA. *Annu. Rev. Biochem.* **1998**, *67*, 1−25.
(4) De Clercq, E. Strategies in the design of antiviral drugs. *Nature Rev. Drug Discovery* **2002**, *1*, 13−25.
(5) De Clercq, E. In search of a selective antiviral chemotherapy. *Clin. Microbiol. Rev.* **1997**, *10*, 674−693.
(6) Johnson, S. C.; Gerber, J. G. Advances in HIV/AIDS therapy. In *Advances in Internal Medicine*; Schrier, R. W.; Baxter, J. D.; Dzau, V. J.; Fauci, A. S., Eds,; Mosby: St. Louis, 2000; Vol. 45, pp 1−40.
(7) Nair, V.; St. Clair, M. H.; Reardon, J. E.; Krasny, H. C.; Hazen, R. J.; Paff, M. T.; Boone, L. R.; Tisdale, M.; Najera, I.; Dornsife, R. E.; Averett, D. R.; Borroto-Esoda, K.; Yale, J. L.; Zimmerman, T. P.; Rideout, J. L. Antiviral, metabolic, and pharmacokinetic properties of the isomeric dideoxynucleoside 4(S)−(6-amino-9H-purin-9-yl)-tetra-hydro-2(S)-furanmethanol. *Antimicrob Agents Chemother.* **1995**, *39*, 1993−1999.
(8) De Clercq, E. New approaches toward anti-HIV chemotherapy. *J. Med. Chem.* **2005**, *48*, 1297−1313.
(9) Pommier, Y.; Johnson A. A.; Marchand, C. Integrase inhibitors to treat HIV/AIDS. *Nature Rev. Drug Discovery* **2005**, *4*, 236−248.
(10) Nair, V. Novel inhibitors of HIV integrase: the discovery of potential anti-HIV therapeutic agents. *Frontiers Med. Chem.* **2005**, *2*, 3−20.
(11) Dayam, R.; Neamati, N. Small-molecule HIV-1 integrase inhibitors: the 2001−2002 update. *Curr. Pharm. Design* **2003**, *9*, 1789−1802.
(12) Asante-Appiah, E.; Skalka, A. M. HIV-1 integrase: structural organization, conformational changes, and catalysis. *Adv. Virus Res.* **1999**, *52*, 351−369.
(13) Esposito, D.; Craigie, R. HIV integrase structure and function. *Adv. Virus Res.* **1999**, *52*, 319−333.
(14) Dyda, F.; Hickman, A. B.; Jenkins, T. M.; Engelman, A.; Craigie, R.; Davies, D. R. Crystal structure of the catalytic domain of HIV-1 integrase: similarity to other polynucleotidyl transferases. *Science* **1994**, *266*, 1981−1986.
(15) Wu, Y.; Marsh, J. W. Selective transcription and modulation of resting T cell activity by preintegrated HIV DNA. *Science* **2001**, *293*, 1503−1506.
(16) Espeseth, A. S.; Felock, P.; Wolfe, A. Witmer, M.; Grobler, J.; Anthony, N.; Egbertson, M.; Melamed, J. Y.; Young, S.; Hamill, T.; Cole, J. L.; Hazuda D. J. HIV-1 integrase inhibitors that compete with the target DNA substrate define a unique strand transfer conformation for integrase. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 11244−11249.
(17) Mazumder, A.; Uchida, H.; Neamati, N.; Sunder, S.; Jaworska-Maslanka, M.; Wickstrom, E.; Zeng, F.; Jones, R. A.; Mandes, R. F.; Chenault, H. K.; Pommier, Y. Probing interactions between viral DNA and human immunodeficiency virus type 1 integrase using dinucleotides. *Mol. Pharmacol.* **1997**, *51*, 567−575.
(18) Taktakishvili, M.; Neamati, N.; Pommier, Y.; Pal, S.; Nair, V. Recognition and inhibition of HIV integrase by novel dinucleotides. *J. Am. Chem. Soc.* **2000**, *122*, 5671−5677.
(19) Hazuda, D. J.; Felock, P.; Witmer, M.; Wolfe, A.; Stillmock, K.; Grobler, J. A.; Espeseth, A.; Gabryelski, L.; Schleif, W.; Blau, C.; Miller, M. D. Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. *Science* **2000**, *287*, 646−650.
(20) Wai, J. S.; Egbertson, M. S.; Payne, L. S.; Fisher, T. E.; Embrey, M. W.; Tran, L. O.; Melamed, J. Y.; Langford, H. M.; Guare, J. P., Jr.; Zhuang, L.; Grey, V. E.; Vacca, J. P.; Holloway, M. K.; Naylor-Olsen, A. M.; Hazuda, D. J.; Felock, P. J.; Wolfe, A. L.; Stillmock, K. A.; Schleif, W. A.; Gabryelski, L. J.; Young, S. D. 4-Aryl-2,4-dioxobutanoic acid inhibitors of HIV-1 integrase and viral replication in cells. *J. Med. Chem.* **2000**, *43*, 4923−4926.
(21) Marchand, C.; Zhang, X.; Pais, G. C. G.; Cowansage, K.; Neamati, N.; Burke, T. R., Jr.; Pommier, Y. Structural determinants for HIV-1 integrase inhibition by β-diketo acids. *J. Biol. Chem.* **2002**, *277*, 12596−12603.
(22) Hazuda, D. J.; Young, S. D.; Guare, J. P.; Anthony, N. J.; Gomez, R. P.; Wai, J. S.; Vacca, J. P.; Handt, L.; Motzel, S. L.; Klein, H. J.; Dornadula, G.; Danovich, R. M.; Witmer, M. V.; Wilson, K. A. A.; Tussey, L.; Schleif, W. A.; Gabryelski, L. S.; Jin, L.; Miller, M. D.; Casimiro, D. R.; Emini, E. A.; Shiver, J. W. Integrase inhibitors and cellular immunity suppress retroviral replication in rhesus macaques. *Science* **2004**, *305*, 528−532.
(23) Grobler, J. A.; Stillmock, K.; Hu, B.; Witmer, M.; Felock, P.; Espeseth, A. S.; Wolfe, A.; Egbertson, M.; Bourgeois, M.; Melamed, J.; Wai, J. S.; Young, S.; Vacca, J.; Hazuda, D. J. Diketo acid inhibitor mechanism and HIV-1 integrase: Implications for metal binding in the active site of phosphotransferase enzymes. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 6661−6666.
(24) Goldgur, Y.; Craigie, R.; Cohen, G. H.; Fujiwara, T.; Yoshinaga, T.; Fujishita, T.; Sugimoto, H.; Endo, T.; Murai, H.; Davies, D. R. Structure of the HIV-1 integrase catalytic domain complexed with an inhibitor: a platform for antiviral drug design. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 13040−13043.
(25) Yoshinaga, T.; Sato, A.; Fujishita, T.; Fujiwara, T. S-1360: in vitro activity of a new HIV-1 integrase inhibitor in clinical development. Presented at the 9th Conference on Retroviruses and Opportunistic Infections, Seattle, WA; Feb 24−28, 2002; Abstract 8, p 55. Data given in Tables 1 for compound S-1360 are those cited by Billich, A. S-1360 Shionogi-GlaxoSmithKline. *Curr. Opin. Invest. Drugs* **2003**, *4*, 206−209.
(26) Pannecouque, C.; Pluymers, W.; Van Maele, B.; Tetz, V.; Cherepanov, P.; De Clercq, E.; Witvrouw, M.; Debyser, Z. New class of HIV integrase inhibitors that block viral replication in cell culture. *Curr. Biol.* **2002**, *12*, 1169−1177.
(27) Pais, G. C. G.; Zhang, X.; Marchand, C.; Neamati, N.; Cowansage, K.; Svarovskaia, E. S.; Pathak, V. K.; Tang, Y.; Nicklaus, M.; Pommier, Y.; Burke, T. R., Jr. Structure activity of 3-aryl-1,3-diketo-containing compounds as HIV-1 integrase inhibitors. *J. Med. Chem.* **2002**, *45*, 3184−3194.
(28) Mazumder, A.; Neamati, N.; Sundar, S.; Owen, J.; Pommier, Y. Retroviral integrase: a novel target in antiviral drug development and basic in vitro assays with purified enzyme. In *Antiviral Methods and Protocols*; Kinchington, D.; Schinazi, R. Eds.; Humana: Totowa, 1999; Vol. 24, pp 327−338.

JM0508890