Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 1920–1923

β-Diketo acids with purine nucleobase scaffolds: Novel, selective inhibitors of the strand transfer step of HIV integrase

Vasu Nair,[a,*] Vinod Uchil[a] and Nouri Neamati[b]

[a]*The Center for Drug Discovery and the Department of Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, GA 30602, USA*
[b]*Department of Pharmaceutical Sciences, University of Southern California, Los Angeles, CA 90089, USA*

Received 4 November 2005; revised 22 December 2005; accepted 22 December 2005
Available online 24 January 2006

Abstract—The HIV *pol* gene encodes three viral enzymes that are required for its replication. While drug discovery involving the viral targets, reverse transcriptase and protease, has resulted in useful therapeutic agents, such efforts on HIV integrase have not produced a single FDA-approved drug. In the work focused on the discovery of inhibitors of HIV integrase, we have synthesized new β-diketo acids with purine nucleobase scaffolds that are potent inhibitors of the strand transfer steps of wild-type HIV-1 integrase.

Enzymes of the *pol* gene of the human immunodeficiency virus (HIV) are required for HIV replication and therefore represent significant viral targets for the discovery of anti-HIV therapeutic agents.[1–5] Drug discovery directed at inhibitors of two of these enzymes, HIV reverse transcriptase (RT) and HIV protease (PR), has produced clinically useful compounds for the treatment of acquired immunodeficiency syndrome (AIDS).[4–8] However, while 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 whose mechanism of action is inhibition of HIV integrase.[9–11] Nevertheless, because integrase is essential for HIV replication and has no human counterpart, it remains a significant 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 and is responsible for the integration of HIV DNA into host chromosomal DNA.[12–16] Prior to the initiation of integration, there is assembly of viral DNA, previously produced by reverse transcription, on HIV integrase. Following this assembly, endonucleatic cleavage of two nucleotides from each 3′-end of double-stranded viral DNA (3′-processing) produces tailored viral DNA recessed by two nucleotides. In the next step, which occurs in the nucleus and is 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. 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.

While a number of structurally diverse compounds have been reported to be inhibitors of HIV integrase,[8–10,17–26] only a few compounds of one group, the β-diketo acids, represent the most convincing, biologically validated inhibitors[23,24] of this viral enzyme (Fig. 1).

In this report, we disclose the synthesis and HIV-1 integrase data of new β-diketo acids with purine nucleobase scaffolds (Fig. 2) that are inhibitors of the strand transfer steps of HIV-1 integrase. We have discovered that the nucleobase scaffold, the substituents, and the specific spatial relationship of substituents in the scaffold are critical for integrase inhibitory activity involving either strand transfer inhibition selectivity (as in this case) or inhibition of both the 3′-processing and strand transfer steps (as in other cases).

Keywords: HIV integrase inhibitors; Purine diketo acids; Mechanism of inhibition.
* Corresponding author. Tel.: +1 706 542 6293; fax: +1 706 583 8283; e-mail: vnair@rx.uga.edu

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.12.093

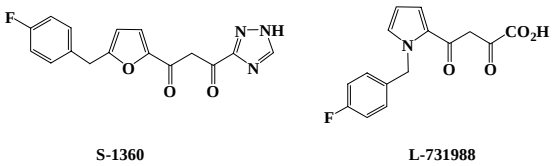


Figure 1. Structures of two β-diketo compounds that are inhibitors of HIV-1 integrase.

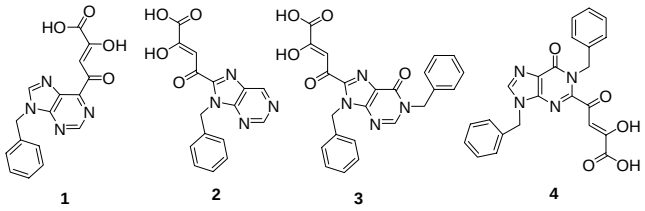
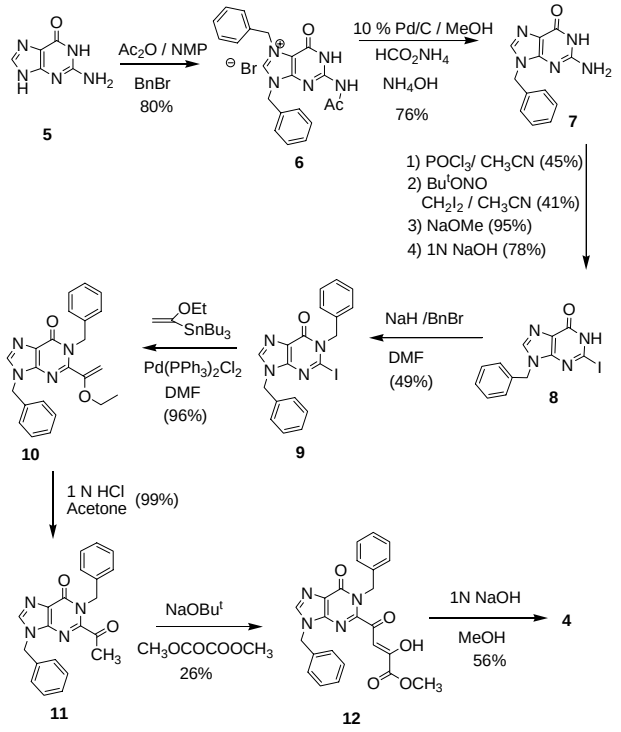


Figure 2. Compounds investigated for HIV integrase activity showing the regiochemical arrangement of functional and hydrophobic groups.

The synthesis of these compounds using both known and new steps is illustrated with one example in which the β-diketo acid is at the C-2 position (see Scheme 1). Guanine (**5**) served as the starting material and was selectively benzylated at the 9-position (to compound **7**) via the intermediate **6**.[27] Conversion of **7** to intermediate **8** required initial protection of the lactam group,[28] a radical deamination-halogenation reaction at the 2-position,[29] and then a two-step hydrolytic dehalogenation at the 6-position. Intermediate **8** was benzylated at N-1 and converted to the 2-acetyl intermediate **11** by a palladium-catalyzed cross-coupling reaction[28,30] followed by hydrolysis. Conversion of **11** to **4** involved a cross-Claisen condensation[31] followed by base-catalyzed ester hydrolysis.[32] The overall yield of the target compound **4** from guanine involving 11 steps was 0.56%. The characterization data for compounds **1**–**4** are summarized.[33]



Scheme 1. Representative example of the synthesis of an integrase inhibitor with a purine nucleobase scaffold.

Integrase inhibition studies were conducted with recombinant wild-type HIV-1 integrase and a 21-mer oligonucleotide substrate, following a previously described procedure.[34] Percent inhibition was calculated using the following equation: inhibition = $100 \times [1 - (D - C)/(N - C)]$, where C, N, and D are the fractions of 21-mer substrate converted to 19-mer (3′-processing product) or strand transfer products for DNA alone, DNA plus integrase, and integrase plus drug, respectively. The 50% inhibitory concentrations (IC_{50}) were graphically determined from the plot of the log of drug concentration versus % inhibition. Integrase inhibition data are shown in Table 1. Compounds **3** and **4** with hydrophobic benzyl groups, and the β-keto acid functionality at the 8- and 2-positions, respectively, were strong inhibitors of the strand transfer step of HIV-1 integrase. However, both compounds showed much lower inhibition of the 3′-processing step of HIV integrase action. In sharp contrast, β-diketo acids with pyrimidine nucleobase scaffolds are potent inhibitors of both the 3′-processing and strand transfer steps.[35] For example, 4-(1,3-dibenzyl-1,2,3,4-tetra-hydro-2,4-dioxo-pyrimidin-5-yl)-2-hydroxy- 4-oxo-but-2-enoic acid, a β-diketo acid with a pyrimidine nucleobase scaffold synthesized in our laboratory, exhibits strong inhibition of both 3′-processing and strand transfer steps (IC_{50} 3.7 and 0.2 μM, respectively).[35] Closely related analogs of this pyrimidine-based inhibitor also exhibit potent anti-HIV integrase inhibitory activity for both key steps of integrase enzymology. The reason for this difference is not entirely clear. However, our molecular modeling data reveal that the regiochemical arrangement and preferred conformation of the β-diketo acids with pyrimidine nucleobase scaffolds allow for more effective overlap of these diketo acids with both the 3′-processing and strand transfer pockets within the catalytic site.[35,36] Figure 3 shows the docking of HIV integrase inhibitor, (**4**), with the 'two magnesium model' of HIV-1 integrase core structure. The catalytic triad is colored in blue-green. The Mg^{2+} ion present in the crystal structure is shown in green and is coordinated by Asp64 and Asp116. The modeled Mg^{2+} ion (red sphere) is located in the strand transfer pocket of the active site and is coordinated by Asp64 and the side-chain carbonyl of Asn155 (shown in orange) and also interacts with the β-diketo acid moiety.

Table 1. IC_{50} data (μM) for the inhibition of wild-type HIV-1 integrase by the compounds of Figure 2

Inhibitors	3′-Processing (μM)	Strand transfer (μM)
1	1000	790
2	100	10
3	31.5	4.1
4	30	2.7

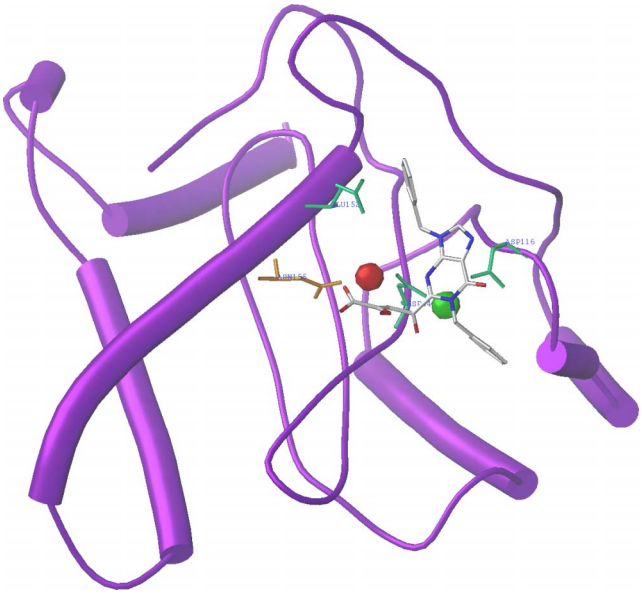


Figure 3. Docking of HIV integrase inhibitor, (**4**), with HIV-1 integrase core structure (PDB code 1BL3).

In summary, new β-diketo acids, assembled on purine nucleobase scaffolds, have been synthesized and found to exhibit moderate selectivity of inhibition of the strand transfer step of HIV-1 integrase. Further work with other novel purine-based inhibitors of HIV integrase is in progress.

Acknowledgments

This project was supported by Grant No. RO1 AI 43181 from the National Institutes of Health (NIAID). The contents of this paper are solely the responsibility of the authors and do not necessarily represent the official views of the NIH. We thank Dr. Arthur Cox of our research group for the molecular modeling data.

References and notes

1. Fauci, A. S. *Science* **1988**, *239*, 617.
2. Katz, R. A.; Skalka, A. M. *Annu. Rev. Biochem.* **1994**, *63*, 133.
3. Frankel, A. D.; Young, J. A. T. *Annu. Rev. Biochem.* **1998**, *67*, 1.
4. De Clercq, E. *Nat. Rev. Drug Disc.* **2002**, *1*, 13.
5. De Clercq, E. *Clin. Microbiol. Rev.* **1997**, *10*, 674.
6. Johnson, S. C.; Gerber, J. G. 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. *Antimicrob. Agents Chemother.* **1995**, *39*, 1993.
8. De Clercq, E. *J. Med. Chem.* **2005**, *48*, 1297.
9. Pommier, Y.; Johnson, A. A.; Marchand, C. *Nat. Rev. Drug Disc.* **2005**, *4*, 236.
10. Nair, V. *Front. Med. Chem.* **2005**, *2*, 3.
11. Dayam, R.; Neamati, N. *Curr. Pharm. Des.* **2003**, *9*, 1789.
12. Asante-Appiah, E.; Skalka, A. M. *Adv. Virus Res.* **1999**, *52*, 351.
13. Esposito, D.; Craigie, R. *Adv. Virus Res.* **1999**, *52*, 319.
14. Dyda, F.; Hickman, A. B.; Jenkins, T. M.; Engelman, A.; Craigie, R.; Davies, D. R. *Science* **1994**, *266*, 1981.
15. Wu, Y.; Marsh, J. W. *Science* **2001**, *293*, 1503.
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. *Proc. Natl. Acad Sci. U.S.A.* **2000**, *97*, 11244.
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. *Mol. Pharmacol.* **1997**, *51*, 567.
18. Taktakishvili, M.; Neamati, N.; Pommier, Y.; Pal, S.; Nair, V. *J. Am. Chem. Soc.* **2000**, *122*, 5671.
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. *Science* **2000**, *287*, 646.
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., Jr.; 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. *J. Med. Chem.* **2000**, *43*, 4923.
21. Marchand, C.; Zhang, X.; Pais, G. C. G.; Cowansage, K.; Neamati, N.; Burke, T. R., Jr.; Pommier, Y. *J. Biol. Chem.* **2002**, *277*, 12596.
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. *Science* **2004**, *305*, 528.
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. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 6661.
24. Goldgur, Y.; Craigie, R.; Cohen, G. H.; Fujiwara, T.; Yoshinaga, T.; Fujishita, T.; Sugimoto, H.; Endo, T.; Murai, H.; Davies, D. R. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 13040.
25. Yoshinaga, T.; Sato, A.; Fujishita, T.; Fujiwara, T. 9th Conference on Retroviruses and Opportunistic Infections, Seattle, WA, February 24–28, 2002; Abstract 8, p 55.
26. Pannecouque, C.; Pluymers, W.; Van Maele, B.; Tetz, V.; Cherepanov, P.; De Clercq, E.; Witvrouw, M.; Debyser, Z. *Curr. Biol.* **2002**, *12*, 1169.
27. Kalayanov, G.; Jaksa, S.; Scarcia, T.; Kobe, J. *Synthesis* **2004**, 2026.
28. Nair, V.; Turner, G. A.; Buenger, G. S.; Chamberlain, S. D. *J. Org. Chem.* **1988**, *53*, 3051.
29. Nair, V.; Young, D. A.; De Silvia, R. *J. Org. Chem.* **1987**, *52*, 1344.
30. Gundersen, L.-L.; Bakkestuen, A. K.; Aasen, J. A.; Øverås, H.; Rise, F. *Tetrahedron* **1994**, *50*, 9743.
31. Jiang, X.-H.; Song, L.-D.; Long, Y.-Q. *J. Org. Chem.* **2003**, *68*, 7555.
32. Selnick, H. G.; Hazuda, D. J.; Egbertson, M.; Guare, J. P.; Wai, J. S.; Young, S. D.; Clark, D. L.; Medina, J. C. PCT, WO 62513, 1999.
33. Data for 4-(9-benzyl-9*H*-purin-6-yl)-2-hydroxy-4-oxo-but-2- enoic acid (**1**): mp 152–153 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.49 (s, 2H, CH_2), 6.72 (s, 1H, olefinic CH), 7.29–7.41 (m, 5H, Ar-H), 8.07 (s, 1H, purine C_8-H), 9.10 (s, 1H, purine C_2-H). ^{13}C NMR ($CDCl_3$,125 MHz): δ 47.6, 102.2, 128.0, 128.1, 128.1,

128.3, 128.8, 132.7, 132.8, 135.9, 146.8, 151.6, 152.6, 155.2, 161.9, 186.2; HRMS: $(M+H)^+$ calcd for $C_{16}H_{13}N_4O_4$ 325.0937, found 325.0930. Data for 4-(9-benzyl-9*H*-purin-8-yl)-2-hydroxy-4-oxobut-2-enoic acid (**2**): mp 162–163 °C. ^{1}H NMR (DMSO, 500 MHz): 5.91 (s, 2H, CH_2), , 7.26–7.34 (m, 6H, Ar-H and olefinic CH), 9.15 (s,1H, purine C_6-H), 9.48 (s, 1H, purine C_2-H). ^{13} C NMR ($CDCl_3$/CD_3OD, 125 MHz): δ 47.6, 101.4, 127.9, 127.9, 128.9, 129.3, 129.3, 131.7, 134.5, 147.4, 147.5, 152.2, 154.3, 162.0, 172.8, 185.7; HRMS: $(M+H)^+$ calcd for $C_{16}H_{13}N_4O_4$ 325.0937, found 325.0926. Data for 4-(1,9-benzyl-6,9-dihydro-6-oxo-1*H*-purin-8-yl)-2-hydroxy-4-oxobut-2-enoic acid (**3**): mp 167 °C, decomposes. ^{1}H NMR (DMSO-d_6, 500 MHz): 5.27 (s, 2H, CH_2), 5.80 (s, 2H, CH_2), 7.25 (s, 1H, olefinic CH), 7.27–7.38 (m, 10H, Ar-H), 8.78 (s,1H, purine C_2-H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 47.9, 49.3, 101.3, 123.9, 127.4, 127.4, 127.5, 127.5, 128.1, 128.2, 128.2, 128.6, 128.9, 129.1, 129.1, 137.1, 137.2, 150.4, 151.5, 156.4, 163.8, 175.9, 179.5. HRMS $(M+H)^+$ calcd for $C_{23}H_{19}N_4O_5$ 431.1355, found 431.1374. Data for 4-(1,9-dibenzyl-6,9-dihydro-6-oxo-1*H*-purin-2-yl)-2-hydroxy-4-oxobut-2-enoic acid (**4**): mp 138–139 °C; ^{1}H NMR ($CDCl_3$/CD_3OD, 500 MHz) δ 5.33 (s, 2H, CH_2), 5.73 (s, 2H, CH_2), 7.11–7.37 (m, 11H, Ar-H and olefinic CH), 7.92 (s,1H, purine C_8-H); ^{13}C NMR ($CDCl_3$/CD_3OD,125 MHz) δ 45.9, 47.9, 124.5, 127.4, 127.5, 127.5, 127.6, 127.7, 128.0, 128.0, 128.1, 128.2, 128.5, 128.6, 128.8, 129.0, 129.1, 134.5, 136.6, 141.9, 145.4, 156.8, 162.8; HRMS $(M+H)^+$ calcd for $C_{23}H_{19}N_4O_5$ 431.1355, found 431.1372.

34. Mazumder, A.; Neamati, N.; Sundar, S.; Owen, J.; Pommier, Y.. In *Antiviral Methods and Protocols*; Kinchington, D., Schinazi, R., Eds.; Humana: Totowa, 1999; Vol. 24, pp 327–338.
35. Nair, V.; Chi, G.; Ptak, R.; Neamati, N. *J. Med. Chem.* **2006**, *49*, in press.
36. Dayam, R.; Neamati, N. *Bioorg. Med. Chem.* **2004**, *12*, 6371.