Journal of Medicinal Chemistry

© Copyright 2000 by the American Chemical Society

Volume 43, Number 26 December 28, 2000

Communications to the Editor

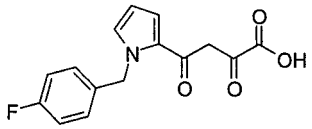
4-Aryl-2,4-dioxobutanoic Acid Inhibitors of HIV-1 Integrase and Viral Replication in Cells

John S. Wai,*,† Melissa S. Egbertson,† Linda S. Payne,† Thorsten E. Fisher,† Mark W. Embrey,† Lekhanh O. Tran,† Jeffrey Y. Melamed,† H. Marie Langford,† James P. Guare, Jr.,† Linghang Zhuang,† Vanessa E. Grey,† Joseph P. Vacca,† M. Katharine Holloway,‡ Adel M. Naylor-Olsen,‡ Daria J. Hazuda,§ Peter J. Felock,§ Abigail L. Wolfe,§ Kara A. Stillmock,§ William A. Schleif,§ Lori J. Gabryelski,§ and Steven D. Young†

Departments of Medicinal Chemistry, Molecular Systems, and Antiviral Research, Merck Research Laboratories, West Point, Pennsylvania 19486

Received April 19, 2000

Introduction. Human immunodeficiency virus type 1 (HIV-1) is the etiological agent of acquired immunedeficiency syndrome (AIDS). The unique nature of the replicative cycle of HIV-1 provides many potential targets for chemotherapeutic intervention. One of these, the viral integrase, catalyzes the insertion of the proviral DNA into the genome of the host cell. Integration is a multistep process which includes three different biochemical processes: assembly of proviral DNA on integrase, endonucleolytic processing of the proviral DNA, and strand transfer of the proviral DNA to host cell DNA.[1] Recently, diketo acid derivative **1** was reported to be a selective inhibitor of the strand-transfer process. This compound effectively prevents proviral DNA integration and inhibits HIV-1 replication in cell culture.[2] In this Communication, we describe the chemistry and structure−activity relationships (SAR) of a series of diketo acids derived from **1**.



1

Strand Transfer IC_{50} 0.17 μM (n = 37)[1,3]

Antiviral Activity CIC_{95} 9.6 μM (n = 166)[4]

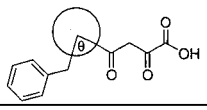
Results and Discussion. Replacing the central pyrrole ring of **1** with a series of aromatic systems having various substitution patterns provided a quick survey of the optimum relative orientation of the benzyl and diketo acid side chains. This variable is a function of the angle between the two lines extended from the benzyl and diketo acid side chains into the aromatic systems (Table 1). In the preliminary survey, the set of compounds prepared did not have a fluoro substituent on the distal benzene ring as in lead compound **1**. The intrinsic potency of these inhibitors increases as the angle of bisection increases from 60° to 118° (Table 1, compounds **2**−**5**). Compound **5** has a 1,3-disubstituted central benzene ring, and its activity appears to exceed the sensitivity limits of the strand-transfer enzyme assay at 0.1 μM.[3] This increase in potency against HIV integrase translates into improvement in inhibitory activity against replication of HIV-1 in cell culture.[4] No cytotoxicity was observed with these inhibitors at concentrations up to 50 μM as determined by cell viability.[5] As the angle of bisection increases further, a gradual loss in both inhibitory activities against the enzyme and HIV-1 replication in cell culture was observed (Table 1, compounds **6**−**8**).

The significant difference in cell potency of **1** versus the corresponding desfluoro analogue **3** (CIC_{95} 9.6 μM vs 42.0 μM, respectively) prompted us to substitute different positions of the distal benzene ring of compound **5**. Table 2 summarizes the results. Introduction of a chloro substituent at the 2′-position of the distal

* To whom correspondence should be addressed at: Department of Medicinal Chemistry, WP14-3, Merck Research Laboratories, P.O. Box 4, West Point, PA 19486. Tel: 215-652-3020. Fax: 215-652-3971. E-mail: john_wai@merck.com.
† Department of Medicinal Chemistry.
‡ Department of Molecular Systems.
§ Department of Antiviral Research.

10.1021/jm000176b CCC: $19.00 © 2000 American Chemical Society
Published on Web 11/28/2000

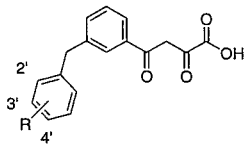
Table 1. Inhibition of HIV-1 Integrase Catalytic Activities and HIV-1 Replication in Cells by a Series of Diketo Acids



Compound	Central Ring	Angle θ[a]	Inhibition of Strand Transfer[b] IC_{50} (μM)	Antiviral Activity[c] CIC_{95} (μM)
2		60.1°	5.67 ± 1.89 (n = 3)	>50, >50 (n = 2)
3		69.3°	0.22 ± 0.11 (n = 3)	41.6 ± 11.8 (n = 12)
4		74.6°	0.18 ± 0.08 (n = 4)	25.0, 25.0 (n = 2)
5		118.2°	<0.10 (n = 4)	1.11 ± 0.61 (n = 16)
6		138.6°	0.16, 0.10 (n = 2)	2.50 ± 0.70 (n = 3)
7		141.3°	0.5, 0.60 (n = 2)	3.0 (n = 1)
8		148.9°	0.50 ± 0.22 (n = 5)	12.5 ± 0.10 (n = 3)

[a] Each angle of bisection is an average of 10−26 determinations based on X-ray coordinates of similarly substituted heterocyclic/aromatic compounds. [b] Assays were performed with recombinant HIV-1 integrase (0.1 μM) preassembled on immobilized oligonucleotides. Inhibitors were added after assembly and washings. For details see ref 3. [c] 95% Cell culture inhibitory concentrations (CIC_{95}) are defined as those which inhibited by 95% the spread of HIV-1 infection in cell culture, using the HIV-1IIIb variant and MT-4 T-lymphoid cells. For details see ref 4.

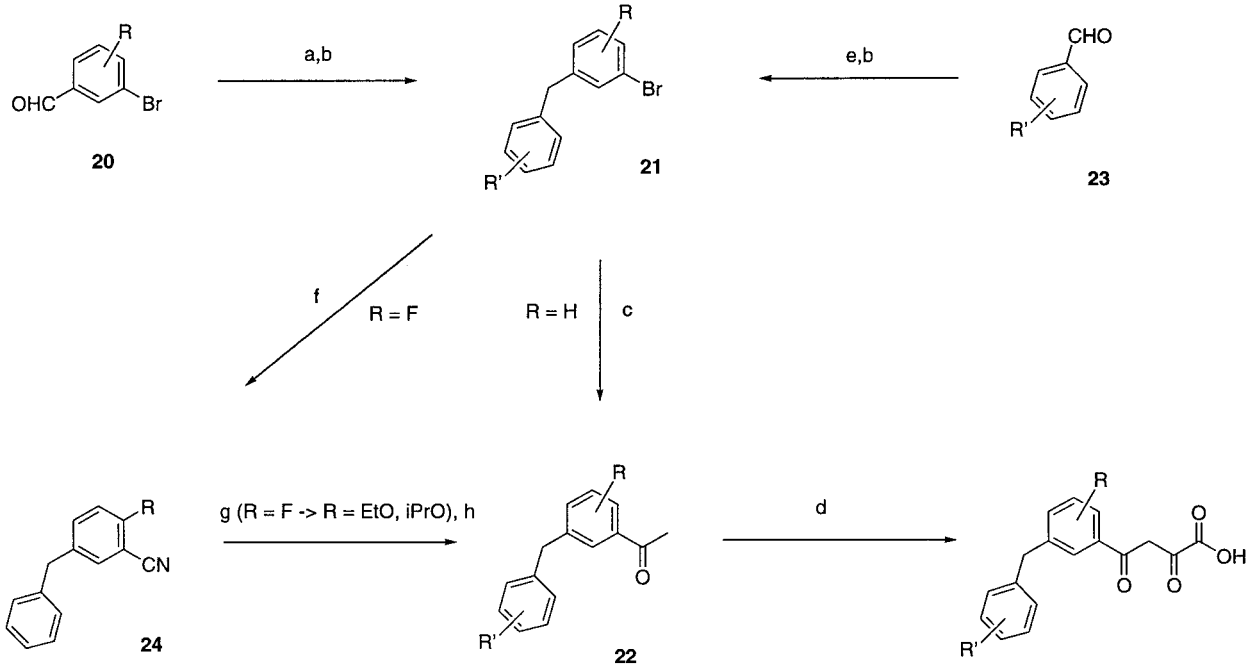
Table 2. Inhibition of HIV-1 Integrase Catalytic Activities and HIV-1 Replication in Cells by a Series of Diketo Acids



Compound	R	Inhibition of Strand Transfer[a] IC_{50} (μM)	Antiviral Activity[b] CIC_{95} (μM)
5	H	<0.10 (n = 4)	1.11 ± 0.61 (n = 16)
9	2'-Cl	<0.10 (n = 4)	0.62 ± 0.00 (n = 3)
10	3'-Cl	<0.10 (n = 3)	0.94 ± 0.51 (n = 6)
11	4'-Cl	1.00, 0.50 (n = 2)	25.00 (n = 1)
12	2'-F	<0.10 (n = 4)	0.52 ± 0.15 (n = 3)
13	3'-F	0.25 ± 0.12 (n = 4)	2.08 ± 0.59 (n = 3)
14	4'-F	<0.10 (n = 2)	0.69 ± 0.36 (n = 5)

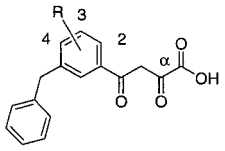
[a] See ref 3. [b] See ref 4.

Scheme 1[a]



[a] Reagents: (a) PhMgBr, THF; (b) Et_3SiH, $BF_3{\cdot}Et_2O$, CH_2C1_2; (c) *n*-BuLi, $CH_3CON(OCH_3)CH_3$, THF; (d) $(CO_2CH_3)_2$, $NaOCH_3$, THF, then NaOH, H_2O; (e) 1,3-dibromobenzene, *n*-BuLi, THF; (f) $Zn(CN)_2$, $(Ph_3P)_4Pd$, DMF; (g) EtOH or *i*-PrOH, KHMDS, THF; (h) CH_3MgI, benzene.

Table 3. Inhibition of HIV-1 Integrase Catalytic Activities and HIV-1 Replication in Cells by a Series of Diketo Acids



Compound	R	Inhibition of Strand Transfer[a] IC_{50} (μM)	Antiviral Activity[b] CIC_{95} (μM)
5	H	<0.10 (n = 4)	1.11 ± 0.61 (n = 16)
15	4-OCH_3	0.15 ± 0.06 (n = 4)	1.83 ± 1.00 (n = 4)
16	3-OCH_3	0.14 ± 0.01 (n = 2)	2.08 ± 0.59 (n = 3)
17	2-OCH_3	<0.10 (n = 6)	0.62 ± 0.38 (n = 4)
18	2-OCH_2CH_3	<0.10 (n = 6)	0.15, 0.25 (n = 2)
19	2-$OCH(CH_3)_2$	<0.10 (n = 6)	0.10 ± 0.05 (n = 5)
Indinavir[4] (Crixivan®)	-	-	0.055 ± 0.019 (n = 30)

[a] See ref 3. [b] See ref 4.

benzene ring leads to a slight improvement in inhibition of HIV replication. Chloro substitution at the 3′-position has no effect, while at the 4′-position it is not well-tolerated (Table 2, compounds **5** vs **9**−**11**). Similarly, only a slight improvement in inhibition of HIV replication was observed with the introduction of a 2′- or 4′-fluoro substituent, and a moderate loss in potency was observed when it was introduced at the 3′-position (Table 2, compounds **5** vs **12**−**14**). In these compounds (**5**, **9**−**14**), the benzyl group and diketo acid side chains are spread further apart than in pyrrole analogues (**1**, **3**), and the benzyl group may have already extended into the region responsible for the potency enhancement observed with a fluorine substitution in the pyrrole series.

Effect of substitution on the central benzene ring was investigated. Introduction of a methoxy group at the 3- or 4-position of the central benzene ring leads to a drop in antiviral activity (Table 3, compounds **15** and **16**). However, introduction of a 2-methoxy group leads to a significant improvement in potency against replication of HIV-1 in cell culture (Table 3, compound **17**, CIC_{95} 0.62 μM, vs compound **5**, CIC_{95} 1.11 μM). Further improvement is observed with ethoxy and isopropoxy substitutions (Table 3, compounds **18** and **19**). Since the activity of inhibitors **5** and **17**−**19** exceeds the limit of detection of the enzyme assay, it is difficult to ascertain whether the improvement in antiviral activity is due to increased intrinsic potency or to a change in physical properties which improves cell penetration.

Scheme 1 depicts the chemistry employed in the preparation of this series of 3-benzylphenyl diketo acids. For the preparation of compounds **15**−**17**, treatment of the appropriately substituted 3-bromobenzaldehyde **20** with phenylmagnesium bromide, followed by exposure of the resulting crude adduct to triethylsilane in the presence of boron trifluoride etherate,[6] provided the corresponding 3-benzylphenyl bromide **21**. Bromide **21** was then lithiated, and the resulting solution was treated with *N*-methoxy-*N*-methylacetamide to provide ketone **22**.[7] Treatment of **22** with dialkyl oxalate and sodium alkoxide provided the intermediate ester adducts,[8] which were hydrolyzed in situ to provide the target diketo acids. For the preparation of compounds **5** and **9**−**14**, the required bromide **21** was prepared by treatment of benzaldehyde or a suitably substituted halobenzaldehyde **23** with 3-bromo-l-lithiobenzene, followed by exposure of the resulting crude adduct to triethylsilane in the presence of boron trifluoride etherate. Further elaboration as described above provided the target compounds. For the synthesis of the 2-ethoxy and 2-isoproxy analogues **18** and **19**, the required acetophenone was prepared in three steps (Scheme 1, steps f−h). Treatment of 3-benzyl-6-fluoro-l-bromobenzene with zinc cyanide in the presence of tetrakis(triphenylphosphine)palladium(0) provided nitrile **24**.[9] Compound **24** was exposed to a mixture of an alcohol and KHMDS[10] and then to methylmagnesium iodide to provide the appropriately substituted ketone **22**.[11]

Conclusion. In summary, modification of a screening lead **1** provided a series of potent 3-benzylphenyl diketo acid based HIV-1 integrase inhibitors. The most active compounds from this series inhibit replication of HIV-1 in cell culture at CIC_{95} 0.10−0.62 μM. This result represents a 100-fold improvement in potency versus the lead **1**. Furthermore, compound **19** is only 2-fold less potent than the protease inhibitor indinavir (CIC_{95} 0.05 μM) in the same assay (Table 3). Cytotoxicity was not observed in cell culture at concentrations up to 50 μM. Further work on this approach to new antiviral agents to treat HIV infection will be reported in due course.

Supporting Information Available: Experimental procedures and elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) For recent reviews on biology of HIV-1 integrase, see: Esposito, D.; Craigie, R. HIV Integrase Structure and Function. *Adv. Virus Res.* **1999**, *52*, 319−333. Asante-Appiah, E.; Skalka, A. M. HIV-1 Integrase: Structural organization, conformational changes, and catalysis. *Adv. Virus Res.* ***1999***, *52*, 351−369. For a recent review on HIV-1 integrase inhibitors, see: Pommier, Y.; Neamati, N. Inhibitors of Human Immunodeficiency Virus Integrase. *Adv. Virus Res.* **1999**, *52*, 427−458.

(2) (a) Hazuda, D. J.; Felock, P.; Witmar, 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. (b) Selnick, H. G.; Hazuda, D. J.; Egbertson, M.; Guare, J. P.; Wai, J. S.; Young, S. D.; Clark, D. L.; Medina, J. C. HIV integrase Inhibitors. Patent W09962513 A (Merck & Co. Inc.). (c) Toshio, F.; Tomokazu, Y. Preparation of indole derivatives with antiviral activity. Patent written in Japanese. Patent W099-JP1547 (Shionogi & Co. Ltd.).

(3) Hazuda, D. J.; Felock, P.; Hastings, J. C.; Pramanik, B.; Wolfe, A. Differential Divalent Cation requirements uncouple the Assembly and Catalytic Reactions of Human Immunodeficiency Virus Type 1 Integrase. *J. Virol.* **1997**, *71*, 7005−7011. Assays

were performed with recombinant HIV-1 integrase (0.1 μM) preassembled on immobilized oligonucleotides. Inhibitors were either added during assembly without washing or after assembly and washings. Inhibition was determined in relation to the integrase control reaction (without inhibitor) performed in quadruplicate and averaged. All samples were background subtracted. In each case, there was no difference between either assay format as anticipated for strand-transfer inhibitors (see ref 2a).

(4) Vacca, J. P.; Dorsey, B. D.; Schleif, W. A.; Levin, R. B.; McDaniel, S. L.; Darke, P. L.; Zugay, J.; Quintero, J. C.; Blahy, O. M.; Roth, E.; Sardana, V. V.; Schlabac, A. J.;Graham, P. I.; Condra, J. H.; Gotlib, L.; Holloway, M. K.; Lin, J.; Chen, I.-W.; Vastag, K.; Ostovic, D.; Anderson, P. S.; Emini, E. E.; Huff, J. R. L-735,524: An orally bioavailable human immunodeficiency virus type 1 protease inhibitor. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4096−4100. 95% Cell culture inhibitory concentrations (CIC_{95}) are defined as those which inhibited by >95% the spread of HIV-1 infection in susceptible cell culture. MT-4 human T-lymphoid cells were maintained in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum. Cells were infected en masse at low multiplicity (0.01) using HIV-1 strain IIIb and were incubated for 24 h. At this time, cells were washed and distributed into 96-well microtiter dishes. Serial 2-fold dilutions of inhibitor were added to the wells, and the cultures were maintained for 3 additional days. Virus spread was assessed by HIV-1 p24 core antigen ELISA. Control cultures in the absence of inhibitor were fully infected at 4 days.

(5) Cytotoxicity is evaluated by visual inspection of the culture for cytopathic effects distinguished as gross morphological changes, growth pattern change, and metabolic change as indicated by lack of change in the medium pH indicator.

(6) Smonou, I. One step reduction of diaryl ketones to hydrocarbons by etherated boron trifluoride-triethylsilane system. *Synth. Commun.* **1994,** *24*, 1999−2002.

(7) Nahm, S.; Weinreb, S. M. *N*-Methoxy-*N*-methylamides as effective acylating agents. *Tetrahedron Lett.* **1981**, *22*, 3815−3818.

(8) Bachmann, W. E.; Cole, W.; Wilds, A. L. The total synthesis of the sex hormone Equilenin and its stereoisomers. *J. Am. Chem. Soc.* **1940**, *62*, 824−839.

(9) Tschaen, D. M.; Desmond, R.; King, A. O.; Fortin, M. C.; Pipik, B.; King, S.; Verhoeven, T. R. An improved procedure for aromatic cyanation. *Synth. Commun.* **1994**, *24*, 887−890. Tschaen, D. M.; Abramson, L.; Cai, D.; Desmon, R.; Dolling, U.-H.; Frey, L.; Karady, S.; Shi, Y.-J.; Verhoeven, T. R. Asymmetric Synthesis of MK-0499. *J. Org. Chem.* **1995**, *60*, 4324−4330.

(10) Woiwode, T. F.; Rose, C.; Wandless, T. J. A simple and efficient method for the preparation of hindered Alkyl−Aryl ethers. *J. Org. Chem.* **1998**, *63*, 9594−9596.

(11) Burden, P. M.; Capper, H. R.; Allan, R. D.; Johnston, G. A. R. The synthesis of 1,8-disubstituted 10,11-dihydrodibenz[*b*,*f*]-oxepin-10-ones. Analogues of anaesthetic steroids. *J. Chem. Soc., Perkin Trans. 1* **1991**, 3291−3294.

JM000176B