Novel HIV-1 Integrase Inhibitors Derived from Quinolone Antibiotics

Motohide Sato,† Takahisa Motomura,† Hisateru Aramaki,† Takashi Matsuda,† Masaki Yamashita,† Yoshiharu Ito,† Hiroshi Kawakami,† Yuji Matsuzaki,† Wataru Watanabe,† Kazunobu Yamataka,† Satoru Ikeda,† Eiichi Kodama,‡ Masao Matsuoka,‡ and Hisashi Shinkai*,†

Central Pharmaceutical Research Institute, JT Inc., 1-1 Murasaki-cho, Takatsuki, Osaka, 569-1125, Japan, and Institute for Virus Research, Kyoto University, 53 Shogoin Kawaramachi, Sakyo-ku, Kyoto, 606-8507, Japan

Received January 6, 2006

Abstract: The viral enzyme integrase is essential for the replication of human immunodeficiency virus type 1 (HIV-1) and represents a remaining target for antiretroviral drugs. Here, we describe the modification of a quinolone antibiotic to produce the novel integrase inhibitor JTK-303 (GS 9137) that blocks strand transfer by the viral enzyme. It shares the core structure of quinolone antibiotics, exhibits an IC_{50} of 7.2 nM in the strand transfer assay, and shows an EC_{50} of 0.9 nM in an acute HIV-1 infection assay.

Human immunodeficiency virus type 1 (HIV-1) integrase, along with HIV-1 reverse transcriptase and HIV-1 protease, is an essential enzyme for retroviral replication and represents an important target for interrupting the viral replication cycle.[1] HIV-1 integrase first catalyzes removal of the terminal dinucleotide from each 3′-end of viral DNA (3′-processing) and subsequently mediates joining of the 3′-end of the viral DNA to host DNA (strand transfer).[2] Reverse transcriptase inhibitors and protease inhibitors have already made significant advances in antiretroviral therapy but cannot achieve complete suppression and risk producing resistant HIV-1.[3,4] On the other hand, despite numerous attempts to develop integrase inhibitors, only the diketo acid class of compounds is at an advanced stage of development and no integrase inhibitors have yet been approved for therapeutic use.[1,5−7] Here, we report that the core structure of quinolone antibiotics can be used as an alternative to the diketo acid class of HIV-1 integrase inhibitors and how this finding led to a novel quinolone integrase inhibitor, JTK-303 (GS 9137).

The diketo acid moiety (γ-ketone, enolizable α-ketone, and carboxylic acid) was believed to be essential for the inhibitory activity of this series of integrase inhibitors,[8] and the structures of diketotriazole **2**,[6] diketotetrazole **3**,[9] diketopyridine **4**,[10] and 7-carbonyl-8-hydroxy-(1,6)-naphthyridine **5** [7,11] were reported to be bioisosters of the diketo acid pharmacophore (Figure 1). The carboxylic acid could be replaced with not only acidic bioisosters, such as tetrazole and triazole, but also by a basic heterocycle bearing a lone pair donor atom, such as a pyridine ring. It has been reported that the heteroaromatic nitrogen in the pyridine ring mimics the corresponding carboxyl oxygen in the diketo acid as a Lewis base equivalent.[11] The enolizable ketone at the α-position of diketo acids can be replaced with a phenolic hydroxyl group, indicating that the α-enol form of each diketo acid is its biologically active coplanar conformation.[11]

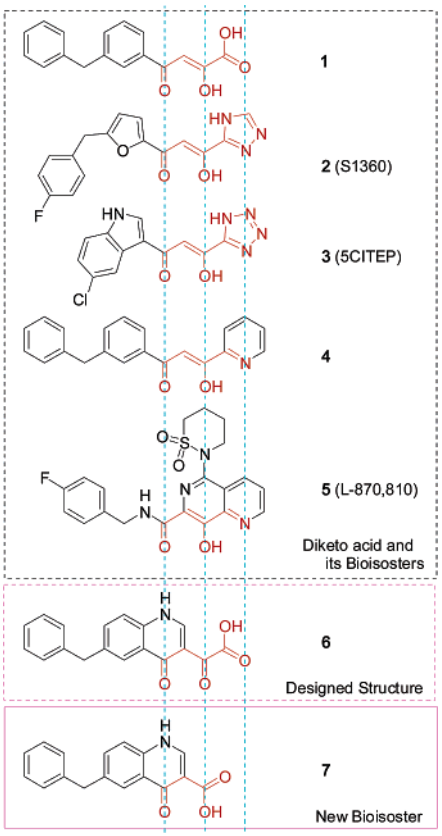


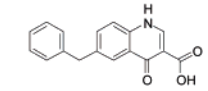
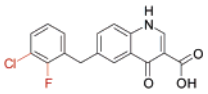
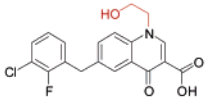
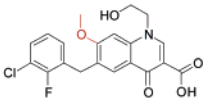
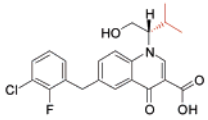
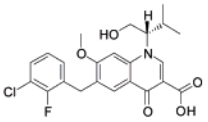
Figure 1. Structures of the diketo acid family and its new bioisoster.

All bioisosters of the diketo acid motif have the three functional groups that mimic a ketone, enolizable ketone, and carboxyl oxygen and can have a coplanar conformation (Figure 1). Therefore, we designed the structure of 4-quinolone-3-glyoxylic acid **6** as a new scaffold that maintained the coplanarity of diketo acid functional groups (Figure 1). Interestingly, not the 4-quinolone-3-glyoxlic acid **6** but its precursor 4-quinolone-3-carboxylic acid **7** showed integrase inhibitory activity. The 4-quinolone-3-carboxylic acid **7** only had two functional groups, a β-ketone and a carboxylic acid, which were coplanar. This result showed that the coplanar monoketo acid motif in 4-quinolone-3-carboxylic acid **7** could be an alternative to the diketo acid motif and provided novel insight into the structural requirements and the binding mode of this type of inhibitor. Quinolone **7** had an IC_{50} of 1.6 μM in the strand transfer assay, and structural modification of **7** led to a far more potent integrase inhibitor **12** with stronger antiviral activity (Table 1). Introduction of 2-fluoro and 3-chloro substituents into the distal benzene ring of **7** (**8**) led to a significant improvement of its inhibition of strand transfer (IC_{50} = 44 nM) and to the appearance of antiviral activity (EC_{50} = 0.81 μM). Compound **9**, bearing a hydroxyethyl group at the 1-position of the quinolone ring, was 1.8-fold more potent at inhibiting strand transfer (IC_{50} = 24 nM) and displayed about 11-fold stronger antiviral activity (EC_{50} = 76 nM) than **8**. Introduction of a methoxy group at the 7-position of the quinolone ring of **9** (**10**) led to a significant improvement of its inhibition of strand transfer (IC_{50} = 9.1 nM) and of antiviral activity (EC_{50} = 17.1 nM). Compound **11**, bearing an isopropyl group at the 1*S*-position of the hydroxyethyl moiety, was about 3-fold more potent at inhibiting strand transfer (IC_{50} = 8.2 nM) and about 10-fold stronger at inhibiting HIV

* To whom correspondence should be addressed. Phone: +81 72 681 9700. Fax: +81 72 681 9725. E-mail: hisashi.shinkai@ims.jti.co.jp.
† JT Inc.
‡ Kyoto University.

10.1021/jm0600139 CCC: $33.50
Published on Web 02/15/2006

Table 1. Summary of the Structural Optimization Process for Quinolone Integrase Inhibitors[e]

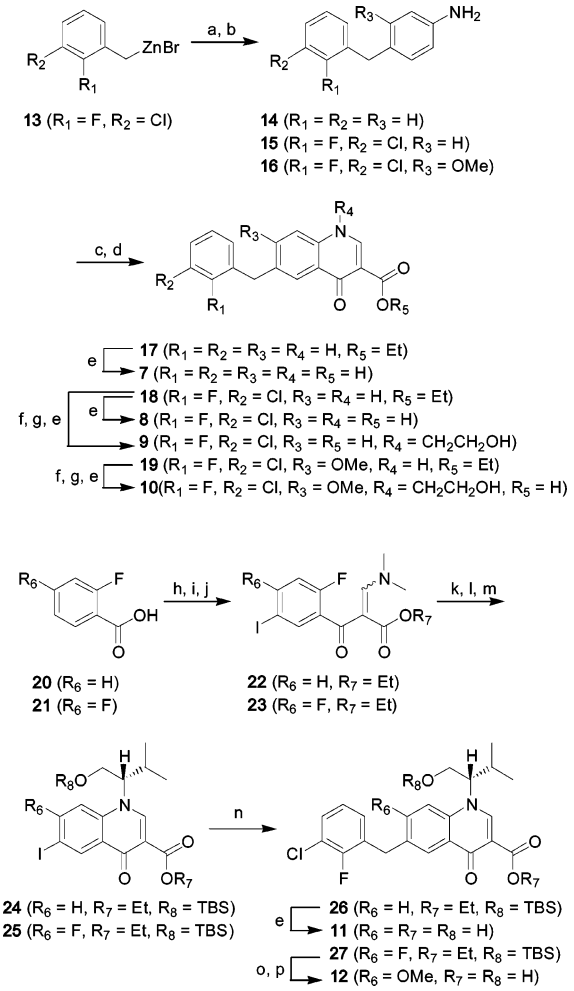
	compd	Inhibition of Strand Transfer[a] IC_{50} (nM)	Antiviral Activity[b] EC_{50} (nM)	Cytotoxicity (μM)
7		1600 ± 300	> 30000	> 30
8		43.5 ± 8.8	805.2 ± 225.0	> 12
9		24.2 ± 11.6	76.3 ± 3.7	> 15
10		9.1 ± 2.1	17.1 ± 2.9	5.3 ± 1.1
11		8.2 ± 1.7	7.5 ± 0.8	14.0 ± 2.0
12		7.2 ± 2.2	0.9 ± 0.4	4.0 ± 0.8
5 (L-870,810)[c]		22.8 ± 4.3	3.6 ± 0.4	0.7 ± 0.06
Ciprofloxacin[d]		> 100000	–	–

[a] The strand transfer assay was performed according to the method of Hazuda[14] with some modifications.[15] [b] Antiviral activity was measured by the acute HIV-1 infection assay[16] with some modifications.[17] [c] Prepared according to the reported method.[18] [d] Available from Wako Pure Chemical [e] Data are given as the mean ± SD ($n = 3$).

replication (EC_{50} = 7.5 nM) than **9**, although introduction of an isopropyl group at the 1*R*-position of the hydroxyethyl moiety could not enhance inhibitory acitivity. Introduction of both a methoxy group at the 7-position of the quinolone ring and an isopropyl group at the 1*S*-position of the hydroxyethyl moiety of **9** (**12**) led to a synergistic improvement of antiviral acitivity (EC_{50} = 0.9 nM), but there was no additive or synergistic improvement in the inhibition of HIV-1 integrase (IC_{50} = 7.2 nM). This may be due to the condition of the strand transfer assay using 5 nM of target DNA that influences potencies of inhibitors.

Preparation of the quinolone analogues (**7**−**12**) is shown in Scheme 1. Palladium-catalyzed coupling of 3-chloro-2-fluorobenzylzinc bromide **13**, which was derived from the corresponding benzylbromide, with 1-iodo-4-nitrobenzene or 1-iodo-2-methoxy-4-nitrobenzene (Negishi coupling) and subsequent reduction of the nitro group gave the aniline **15** or **16**. Condensation of **15**, **16**, or commercially available **14** with diethyl ethoxymethylenemalonate and subsequent thermal cyclization of the aminoacrylate products in diphenyl ether led to the quinolone esters **17**, **18** and **19**.[19] Hydrolysis of **17** and **18** gave **7** and **8**, respectively. N-Alkylation of **18** or **19** with the *tert*-butyldimethylsilyl (TBS) ether of 2-hydroxyethylbromide and subsequent hydrolysis of the ethyl ester and TBS ether resulted in **9** or **10**. After 5-iodonation of 2-fluorobenzoic acids **20** and **21**, the acid chlorides of **20** and **21** were coupled with ethyl 3-(dimethylamino)acrylate to produce the acrylates **22** and

Scheme 1[a]



[a] Reagents and conditions: (a) 1-iodo-4-nitrobenzene, $PdCl_2(Ph_3P)_2$, THF, reflux; (b) Zn, AcOH; (c) diethyl ethoxymethylenemalonate, toluene, reflux; (d) Ph_2O, 250 °C; (e) NaOH, EtOH/H_2O, reflux; (f) $TBSOCH_2CH_2Br$, K_2CO_3, DMF, 80 °C; (g) TBAF, THF; (h) NIS, H_2SO_4; (i) $SOCl_2$, DMF, toluene, reflux; (j) ethyl 3-(dimethylamino)acrylate, THF, 50 °C; (k) (*S*)-valinol, THF; (l) K_2CO_3, DMF, 70 °C; (m) TBSCl, imidazole, DMF; (n) **13**, $Pd(dba)_2$, trifurylphosphine, THF, reflux; (o) NaOH, *i*-PrOH/H_2O, reflux; (p) NaOMe, MeOH, reflux.

23, respectively. Substitution with (*S*)-valinol and subsequent cyclization with potassium carbonate and protection of the alcohol with TBS ether gave the quinolones **24** and **25**, respectively.[20] Negishi coupling of **24** and **25** with **13** led to the quinolone esters **26** and **27**, respectively. Hydrolysis of **26** gave **11**. Hydrolysis of **27** and subsequent methoxylation with sodium methoxide produced **12**.

In summary, modification of quinolone antibiotics, which did not show HIV-1 integrase inhibitory activity (Table 1), led to discovery of the coplanar monoketo acid motif in their scaffold, 4-quinolone-3-carboxylic acid, as an alternative to the diketo acid motif. These novel quinolone integrase inhibitors were structurally optimized in the highly potent **12**, which had little antibacterial activity although it still retained the core structure of quinolone antibiotics. Compound **12** was much more potent at inhibiting integrase-catalyzed strand transfer processes than 3′-processing reactions, as previously reported for compounds of the diketo acid class.[12,13] This indicates that it probably inhibits HIV-1 integrase via a mechanism similar to that of diketo acids, although there is no direct evidence (such as cocrystal data) that the coplanar monoketo acid motif shows the same mode of binding to the enzyme as the diketo acid

motif. Clinical studies of the novel quinolone integrase inhibitor **12** (GS 9137) are currently being conducted by Gilead Sciences.

Acknowledgment. We thank H. Isoshima and K. Kondo for sample preparation; S. Kato for comments on the manuscript; and S. Ishiguro, J. Haruta, and M. Kano for support.

Supporting Information Available: Analytical data for **5** and **7**−**12**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Pommier, Y.; Johnson, A. A.; Marchand, C. Integrase inhibitors to treat HIV/AIDS. *Nat. Rev. Drug Discovery* **2005**, *4*, 236−248.
(2) Craigie, R. HIV integrase, a brief overview from chemistry to therapeutics. *J. Biol. Chem.* **2001**, *276*, 23213−23215.
(3) Richman, D. D. HIV chemotherapy. *Nature* **2001**, *410*, 995−1001.
(4) Weiss, R. A. HIV and AIDS: looking ahead. *Nat. Med.* **2003**, *9*, 887−891.
(5) Johnson, A. A.; Marchand, C.; Pommier, Y. HIV-1 integrase inhibitors: a decade of research and two drugs in clinical trial. *Curr. Top. Med. Chem.* **2004**, *4*, 1059−1077.
(6) Barreca, M. L.; Ferro, S.; Rao, A.; Luca, L. D.; Zappala, M.; Monforte, A. M.; Debyser, Z.; Witvrouw, M.; Chimirri, A. Pharmacophore-based design of HIV-1 integrase strand-transfer inhibitors. *J. Med. Chem.* **2005**, *48*, 7084−7088.
(7) 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.
(8) Grobler, J. A.; Stillmock, K.; Binghua, H.; 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.
(9) Goldgur, Y.; Craigie, R.; Cohen, G. H.; Fujiwara, T.; Yoshinage, 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.
(10) Hazuda, D. J.; Anthony, N. J.; Gomez, R. P.; Jolly, S. M.; Wai, J. S.; Zhuang, L.; Fisher, T. E.; Embrey, M.; Guare, J. P., Jr.; Egbertson, M. S.; Vacca, J. P.; Huff, J. R.; Felock, P. J.; Witmer, M. V.; Stillmock, K. A.; Danovich, R.; Grobler, J.; Miller, M. D.; Espeseth, A. S.; Jin, L.; Chen, I. W.; Lin, J. H.; Kassahun, K.; Ellis, J. D.; Wong, B. K.; Xu, W.; Pearson, P. G.; Schleif, W. A.; Cortese, R.; Emini, E.; Summa, V.; Holloway, M. K.; Young, S. D. A naphthyridine carboxamide provides evidence for discordant resistance between mechanistically identical inhibitors of HIV-1 integrase. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 11233−11238.
(11) Zhuang, L.; Wai, J. S.; Embrey, M. W.; Fisher, T. E.; Egbertson, M. S.; Payne, L. S.; Guare, J. P., Jr.; Vacca, J. P.; Hazuda, D. J.; Felock, P. J.; Wolfe, A. L.; Stillmock, K. A.; Witmer, M. V.; Moyer, G.; Schleif, W. A.; Gabryelski, L. J.; Leonard, Y. M.; Lynch, J. J., Jr.; Michelson, S. R.; Young, S. D. Design and synthesis of 8-hydroxy-[1,6]naphthyridines as novel inhibitors of HIV-1 integrase in vitro and in infected cells. *J. Med. Chem.* **2003**, *46*, 453−456.
(12) 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.
(13) Sechi, M.; Derudas, M.; Dallocchio, R.; Dessi, A.; Bacchi, A.; Sannia, L.; Carta, F.; Palomba, M.; Ragab, O.; Chan, C.; Shoemaker, R.; Sei, S.; Dayam, R.; Neamati, N. Design and synthesis of novel indole β-diketo acid derivatives as HIV-integrase inhibitors, *J. Med. Chem.* **2004**, *47*, 5298−5310.
(14) Hazuda, D. J.; Hastings, J. C.; Wolfe, A. L.; Emini, E. A. A novel assay for the DNA strand-transfer reaction of HIV-1 integrase. *Nucleic Acids Res.* **1994**, *22*, 1121−1122.
(15) Donor DNA (which was processed at the 3′ end of the strand and biotinylated at the 5′ end) was immobilized on streptavidin-coated microtiter plates. Recombinant integrase (300 nM) was assembled on the immobilized donor DNA (0.5 pmol per well) in 100 μL of reaction buffer (30 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS), 5 mM $MgCl_2$, 3 mM dithiothreitol (DTT), 0.1 mg/mL bovine serum albumin (BSA), 5% glycerol, 10% DMSO, 0.01% Tween-20) by incubation for 60 min at 37 °C. Then excess enzyme was removed and a test compound was added. The strand transfer reaction was initiated by addition of target DNA (5 nM), which was labeled at the 3′ end with digoxigenin. After incubation at 37 °C for 10 min, the plates were washed with phosphate-buffered saline (PBS) containing 0.1% Tween-20. The digoxigenin-labeled products were detected using anti-digoxigenin-peroxidase (POD) Fab fragments (Roche Diagnostics) and a POD substrate, tetramethylbenzidine (TMB). Then 100 μL of anti-digoxigenin-POD Fab fragment solution was added to each well, and the plates were incubated at 37 °C for 60 min. After the mixture was washed with PBS containing 0.1% Tween-20, 100 μL of the POD substrate (TMB) was added to each well, and the plates were incubated at room temperature. The colorimetric reaction was stopped by addition of 100 μL of 0.5 M H_2SO_4, and the absorbance was measured at 450 nm by a microplate reader (SPECTRA max 340, Molecular Devices).
(16) Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds. *J. Virol. Methods* **1988**, *20*, 309−321.
(17) MT-4 human T lymphoid cells (1×10^5 cells/mL) in RPMI 1640 medium containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin were infected with HIV-1 strain IIIb at a multiplicity of 0.01 and were distributed into 96-well microtiter plates. Test compounds were added to the wells, and cultures were incubated at 37 °C for 5 days. Cell viability was determined by the MTT assay, which measures living cells on the basis of mitochondrial dehydrogenase activity. The cells were incubated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (7.5 mg/mL). The MTT formazan crystals were dissolved in acidic 2-propanol containing 4% Triton-X, and the absorbance was measured at 595 nm.
(18) Anthony, N. J.; Gomez, R. P.; Young, S. D.; Egbertson, M.; Wai, J. S.; Zhuang, L.; Embrey, M.; Tran, L.; Melamed, J. Y.; Langford, H. M.; Guare, J. P.; Fisher, T. E.; Jolly, S. M.; Kuo, M. S.; Perlow, D. S.; Bennett, J. J.; Funk, T. W. Aza- and polyaza-naphthalenyl carboxamides useful as HIV integrase inhibitors. PCT Int. Appl. WO02/30930 A2, 2002 (Merck & Co., Inc.).
(19) Gould, R. G., Jr.; Jacobs, W. A. The synthesis of certain substituted quinolines and 5,6-benzoquinolines. *J. Am. Chem. Soc.* **1939**, *61*, 2890−2895.
(20) Cecchetti, V.; Fravolini, A.; Lorenzini, M. C.; Tabarrini, O.; Terni, P.; Xin, T. Studies on 6-aminoquinolones: synthesis and antibacterial evaluation of 6-amino-8-methylquinolones. *J. Med. Chem.* **1996**, *39*, 436−445.

JM0600139