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Review Article

Novel aryl diketo-containing inhibitors of HIV-1 integrase

Godwin C.G. Pais and Terrence R. Burke, Jr.*

*Laboratory of Medicinal Chemistry, Center for Cancer Research, National Cancer Institute, National Institutes of Health, P.O. Box B, Bldg. 376, Boyles St., Frederick, MD 21702-1201, USA. *Correspondence*

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Abstract

HIV-1 integrase is a promising therapeutic target for the development of drugs to treat HIV infection. Aryl diketo-based analogs, disclosed independently by scientists from Merck and Shionogi pharmaceutical companies, are a unique class of compounds that exhibit potent integrase inhibition and display good antiviral effects in HIV-infected cells. The progress of Merck's L-870810 and Shionogi's S-1360 to phase II clinical trials has promised the inclusion of integrase inhibitors in "cocktail" combination therapies in the near future. This review presents a critical overview of research related to this new class of integrase inhibitors.

Introduction

Treatment of patients infected with human immunodeficiency virus (HIV) has improved dramatically with the introduction of highly active antiretroviral therapy (HAART). This has resulted in HIV infection changing from a death sentence to a chronic, manageable disease. The current and most widely used combination treatment for AIDS is the HAART protocol consisting of 2 reverse transcriptase inhibitors plus 1 protease inhibitor (1-3). However, in approximately 30-50% of patients, HAART therapy fails due to the emergence of strains resistant to the currently used drugs (2). This makes it essential to develop drugs targeted at alternative steps in the viral replication cycle. Integrase is essential for HIV replication (3). The integrase-catalyzed insertion of retrotranscribed viral DNA into the host chromosome takes place through a complex process, including processing of the viral cDNA by removal of 2 bases from each 3'-terminus (3'-P) and joining of the recessed ends to the host DNA (strand transfer). Studies have shown that in the absence of a functional integrase enzyme, HIV is not infectious. Therefore, integrase, which has no cellular equivalent, is an attractive target for drug design.

A number of integrase inhibitors have been reported, including nucleotide-based inhibitors, DNA binders, catechols and hydrazides (4-7). Most of these inhibit integrase function in extracellular oligonucleotide assays (6) but often lack inhibitory potency when assayed using fully assembled preintegration complexes (8) or fail to show antiviral effects against HIV-infected cells (5). Recently reported aryl diketo acids (ADKs) such as 5CITEP (**1.1**) (9) and L-731988 (**1.2**) (10) represent a remarkable class of compounds that are both selective HIV-1 integrase inhibitors and antiviral agents (Fig. 1). Members of the ADK family inhibit viral replication by blocking the second step of the integrase reaction which is the strand transfer step (10, 11). Selective inhibition of strand transfer during HIV-1 infection allows the viral DNA to become accessible to metabolism by cellular recombination and repair enzymes. The result is an incompetent and unstable integration leading to the irreversible block of viral replication. ADKs were identified in special assays using

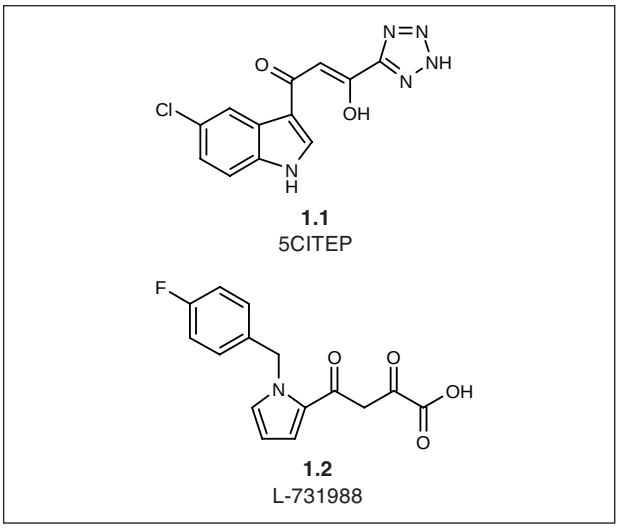


Fig. 1. Structures of 2 ADK integrase inhibitors.

recombinant integrase assembled onto immobilized oligonucleotides as surrogates for authentic HIV-1 preintegration complexes. The relative potencies of these analogs in strand transfer assays are comparable to values obtained in assays using viral preintegration complexes isolated from HIV-1 infected cells (10). There are several excellent reviews on HIV-1 integrase inhibitors (12-23) and this review will concentrate solely on ADK-based integrase inhibitors.

Aryl diketo-containing compounds as integrase inhibitors

Structural features of ADK inhibitors

ADKs are comprised of 3 structural components (**2.1**) each of which lends itself to potential structure-activity relationship (SAR) study (Fig. 2). ADKs have been extensively studied, including replacement of the left aryl portion by various nitrogen-, sulfur- and oxygen-containing heterocycles. The right acid functionality has been replaced with a variety of groups including triazoles, thiazoles, pyridine, naphthyridine, *etc*. One objective of the current paper is to present an overview of these modifications.

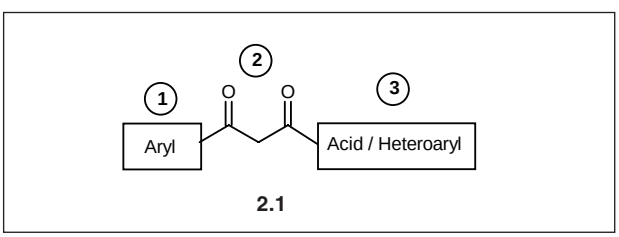


Fig. 2. General aryl diketo-based integrase inhibitor showing 3 conceptual components.

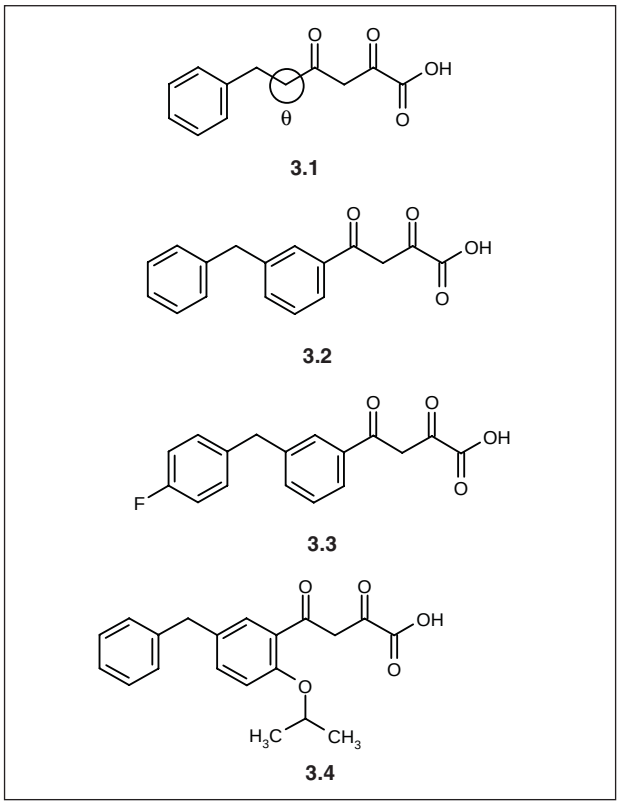


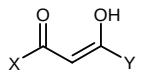
Fig. 3. Examples of ADKs discussed in the study by Wai *et al.* (26).

Early reports of ADK integrase inhibitors

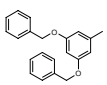
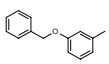
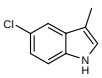
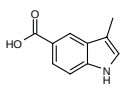
Members of the ADK family have been used previously as therapeutics for a variety of diseases. For example, 2,4-dioxo-4-substituted-1-butanoic acid derivatives are known to be useful in treating urinary tract oxalate lithiasis (24) and 4-substituted 2,4-dioxobutanoic acid-containing compounds have been shown to be active against influenza virus endonuclease (25). Scientists from Shionogi and Merck Pharmaceutical Companies independently discovered that certain diketo acids behave as integrase inhibitors. Subsequently, there has been some structural overlap in patent claims by these groups.

SAR studies by Wai *et al.* (26) on compounds typified by **3.1-3.4**, which are similar to L-731988 (**1.2**), have highlighted the importance of relative orientations of the aryl substituents and the diketo acid side chain of ADK inhibitors (Fig. 3). Cell culture inhibitory concentrations (CIC_{95}) of several analogues presented were in the range of 0.10-0.62 μM. Important conclusions from this study include the finding that inhibitory potency is maximal when the angle between the aryl substituent and the diketo acid side chain is approximately 118°. Electronic effects, size and position of substituents on the distal aryl ring also have profound effects on inhibitory activity. Methoxy, ethoxy or isopropoxy groups at the 2-position on

Table I: Inhibitory potencies as measured in an extracellular HIV-1 integrase assay (27).



General structure

No.	X	Y	IC_{50} (μM) 3'-P	IC_{50} (μM) ST
1a		$-CO_2H$	>100	1.43 ± 0.15
1b		$-CO_2Et$	>100	6.9
1c		$-CO_2H$	48, 80	0.52 ± 0.10
L-708906 **1d**		$-CO_2H$	>1000	0.48 ± 0.08
1e		$-CO_2H$	65, 100	0.35 ± 0.13
1f		$-CO_2H$	>100	24.2, 25.0
1g			>100	1.9, 2.0
1h			35	0.65 ± 0.19
1i			40	40

the central benzene ring result in significant enhancement of antiviral potency whereas a methoxy group at the 3 or 4 position decreases potency. It is not clear whether this behavior is due to increased affinity or to changes in physical properties such as pKa that improve cell membrane penetration.

Follow-up studies on ADK integrase inhibitors

Comparative studies on aryl and acidic portions

Our own interest in ADK integrase inhibitors was predicated on the disclosure of an X-ray crystal structure of 5CITEP bound to the catalytic core of integrase (9). Since at this time there was little structure-activity or synthetic work on this class of compounds (26), we undertook a limited study of ADKs (27). Our results are summarized in Table I. As shown in the table, a halogen on the indole ring has little effect on strand transfer processes, whereas 3'-P inhibitory potency is slightly affected. Introduction of a carboxyl group at the indolyl 5-position significantly decreases inhibitory potency against strand transfer, while approximately doubling potency against 3'-P as compared to 5CITEP. This leads to a complete loss of 3'-P/strand transfer selectivity. When the tetrazole group was replaced with carboxyl group, as in compounds **1a** and **1c** there was little change in either 3'-P or strand transfer potencies. Compound **1b** maintained significant strand transfer inhibitory potency and selectivity over 3'-P, indicating that an acidic proton may not be required for strand transfer inhibitory activity. Consistent with literature reports (10), L-708906 (**1d**) exhibited potent inhibition of strand transfer with no effect on 3'-P up to 1000 μM. Removal of one benzyloxy group resulted in a slight increase in strand transfer inhibitory potency accompanied by some loss of selectivity for 3'-P, while providing an *in vivo* IC_{50} of 0.6 μM. Compound **1f** showed significant loss in strand transfer inhibitory potency, suggesting that a substituent at the aryl 3-position potentially is crucial for effective strand transfer inhibition.

Our studies also found that tetrazole analogues **1g** and **1h** are not antiviral, which is in accordance with a study from Grobler *et al.* (11) that attributed this to differences in metal dependency of carboxylic acid *versus* tetrazole functionalities of ADK inhibitors. Binding affinity and inhibitory potencies of carboxylate derivatives are not metal-dependent, while tetrazoles exhibit reduced binding and inhibition in Mg^{2+} relative to Mn^{2+}. Hence, **1.2** is antiviral but **4.1** is not (11). The weak activity of the tetrazole analogue of **1.2** in Mg^{2+} may be one reason for the latter's poor antiviral activity. These ADK inhibitors disrupt viral infectivity in a manner consistent with inhibition of integration.

Compounds that contain a diketo acid moiety selectively inhibit integrase strand transfer *in vitro* and in infected cells and are effective as inhibitors of HIV-1 replication. Reports indicate that an acidic moiety such as carboxylate or isosteric heterocyle is not required for

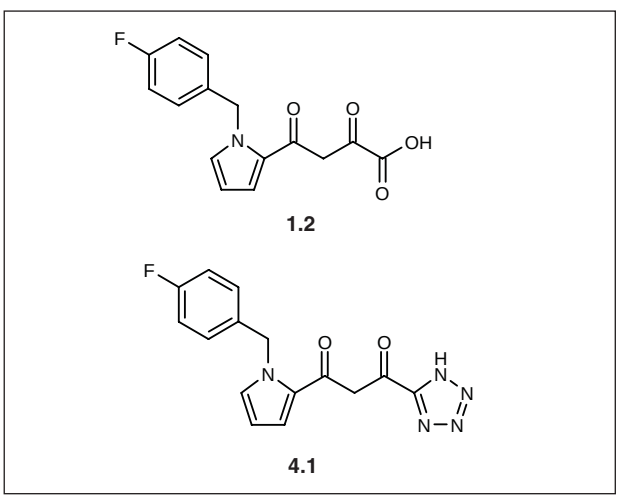
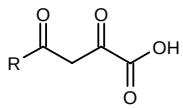
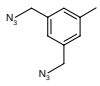
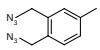
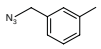
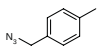
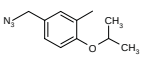
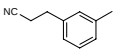


Fig. 4. ADKs discussed in the study by Grobler *et al.* (11).

Table II: Inhibitory potencies of azido-containing β-diketo acids (28).



General structure

No.	R	Integrase assay IC_{50} (μM) 3'-P	ST	Antiviral potency (IC_{50} μM)
2a		>100	2.0, 2.8	5 ± 2.5
2b		>100	0.26, 0.15	14.3 ± 4.7
2c		>100	1.53 ± 0.27	5.9 ± 1.1
2d		>100	6.1, 7.1	>25
2e		>100	25, 23	5.9 ± 1.1
2f		>100	1.5, 1.8	>25

binding to the enzyme complex but it is essential for inhibition and confers distinct metal-dependent properties on the inhibitor. The mechanism of action of ADK inhibitors depends on the interaction between the acid moiety and metal ions in the integrase active site resulting in the sequestration of the critical metal cofactors (11).

Azide-containing ADK inhibitors

In further studies it was found that when the aryl substitution on L-708906 (**1d**) was replaced by azido functionality (compound **2a**), it was as potent as L-708906 (**1d**) (Table II) in extracellular integrase assays, as well as being antiviral (28). The isomeric 3,4-di-(azidomethyl) analogue **2b** is approximately 10-fold more potent than the original 3,5-substituted compound. This enhanced potency could potentially be attributable to preferential orientation of the azido functionality induced by *O*-neighboring group crowding. Among the monosubstituted analogues of **2a**, the 3-substituted isomer **2c** was approximately equivalent to the diazide-containing **2a** in both integrase and antiviral assays, suggesting that the 3-substitution is sufficient for high potency. Introduction of methoxy or isopropoxy groups into **2c** resulted in a reduction of integrase inhibitory potency. A nonazide containing equivalent of **2c** had nearly equivalent integrase inhibitory potency, however antiviral potency was reduced significantly for the nitrilo analogue **2f**. Both azido and nitilomethano groups were found to be sterically and electronically similar and to exhibit similar metal dependencies. Therefore, the reasons for differences in antiviral potencies were not clear (Table II).

Bifunctional diketo acids

Other studies demonstrated that replacement of the benzyloxy group on **1e** by 2,4-diketobutyric acid (**5.2**) had little effect on the strand transfer inhibitory potency but it resulted in enhanced 3'-P inhibitory potency. When the side chain was attached to the 4-position, as in **5.1**, the inhibitory potency was similar to **5.2**. This suggests that mode of action of bis-diketo acids is different from that of the monofunctional diketo acids. Based on these findings, a new binding mechanism has been proposed for beta-diketo acids (29). Because viral integrase catalyzes the insertion of a donor DNA substrate into an acceptor DNA template, both DNA duplexes are required to bind two adjacent sites. In this model (29) it was suggested that monofunctional DKAs such as L-708906 (**1d**) bind only to the acceptor site. Consequently, such inhibitors do not inhibit 3'-processing and cannot compete for binding with

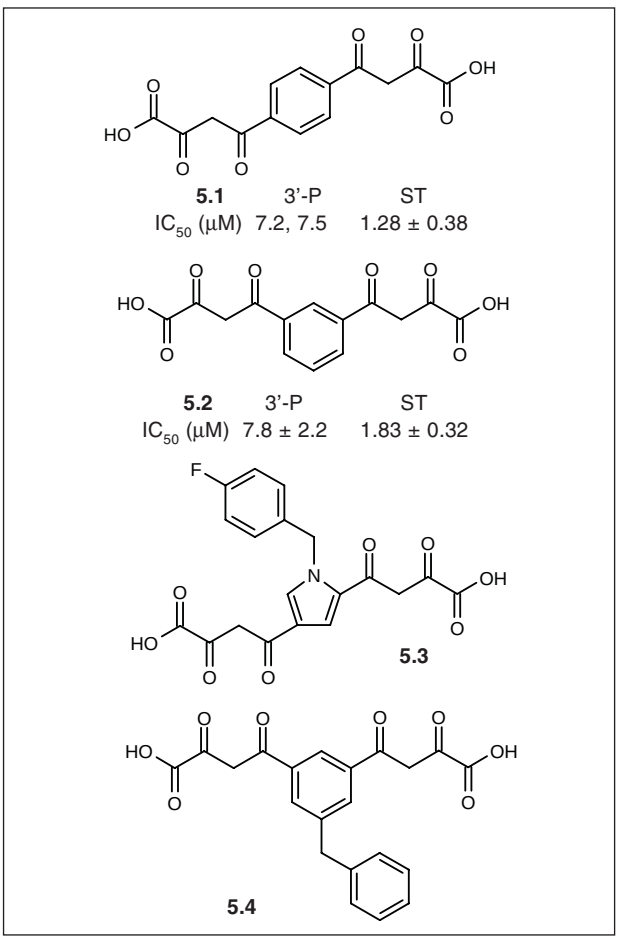


Fig. 5. Representative bifunctional diketo acids.

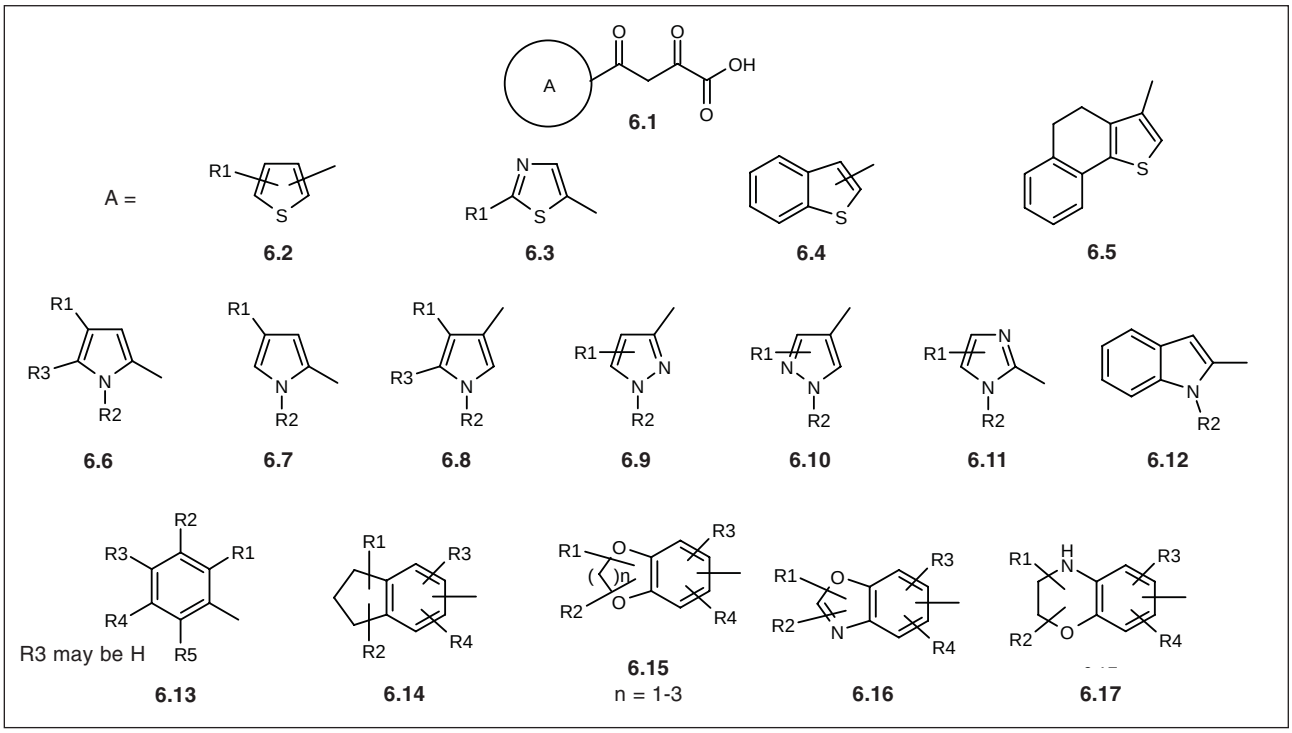


Fig. 6. General structures of ADKs from Merck (31-33).

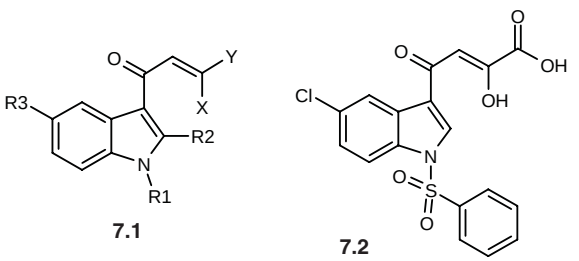
the donor (30). In contrast, bifunctional diketo acids are capable of interacting with both donor and acceptor sites. At low concentrations they could preferentially bind to the acceptor site while at higher concentrations they could bind to the donor site. Similar bis-diketo acids have also been reported by Merck (**5.3, 5.4**) (31).

Heteroaryl-containing ADK inhibitors

The patent literature contains a plethora of aryl diketo acid derivatives in which the aryl portion is a variety of heterocycles. Merck has conducted an exhaustive study on these compounds (31-33). Although the HIV-1 integrase inhibitory potencies of most compounds are not provided, representative compounds showed IC_{50} values less than 1 μM in integrase assays (34). In preintegration complex assays (35), IC_{95} values for the inhibition of acute HIV infection of T-lymphoid cells were often less than 10 μM. Although the above patents cover a diverse range of aryl diketo acids, they do very little to aid in the understanding of the SAR requirements for high-affinity interaction with HIV-1 integrase.

Indole diketo acids

In addition to patents by Merck covering various heterocycles, patents from Shionogi also included heterocycles, including several indolyl compounds. One patent



Where X = OH, and Y = H or tetrazole or triazole; R_1, R_2 may be hydrogen, phenyl sulfonate or substituted phenyl sulfonate or benzyl; R_3 may be halogen.

Fig. 7. Indole-3-diketo derivatives from Shionogi (36).

entitled, “Indole derivatives with antiviral activity”, reports a number of indole-3-(1,3-diketo) derivatives (Fig. 7) (36). Exemplary of these compounds, **7.2** is reported to exhibit an IC_{50} value of 0.13 μg/ml.

Inclusion of a spacer between aryl and diketo moieties

Patents from Bristol-Myers Squibb (37, 38) describe a new class of diketo acids as shown in Figure 8, which are either amides (**8.1**) or contain a keto group β to the usual diketo acid side chain (**8.2**). At a concentration of 70 μM, some of these compounds inhibit integrase by up to 100%.

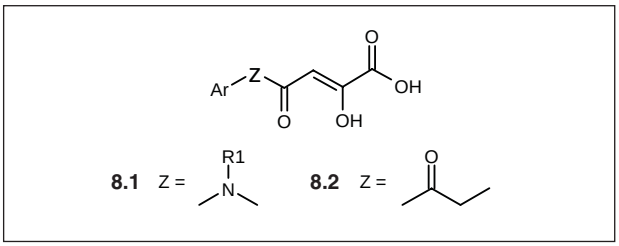
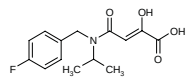
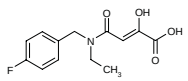
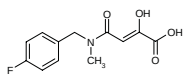
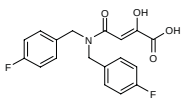
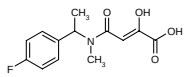
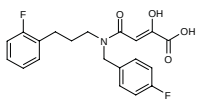
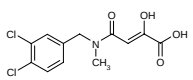
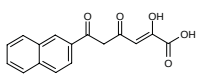
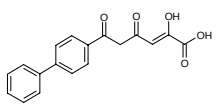
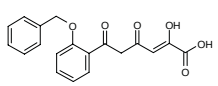
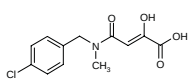


Fig. 8. General structures of modified ADKs.

Table III: Percent integrase inhibition at a concentration of 70 μM of inhibitor (37, 38).

No.	Compound	% Inhibition
3a		38
3b		93
3c		97
3d		97
3e		99
3f		100
3g		100
3h		95
3i		99
3j		97
3k		99

From Table III it is evident that a compound with only one 4-fluorobenzyl substituent on nitrogen (**3c**) is as effective as the one with two 4-fluorobenzyl groups (**3d**). When the second substituent is an alkyl group, steric factors have profound effects on inhibitory activity. The position of the fluoro substituent on the aromatic ring also has a minor influence on the activity, while a second chloro-substituent on the aromatic ring slightly enhances the inhibitory activity.

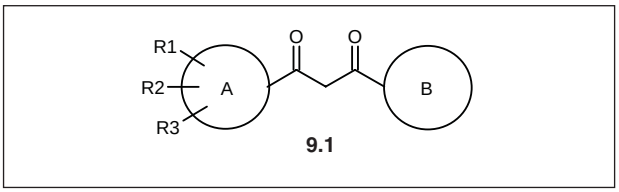


Fig. 9. General structure of 1,3-propanedione integrase inhibitors from Merck (39).

1,3-Propanedione derivatives

Carboxylic acid replacements

A further step in ADK inhibitor development has been replacement of the acid carboxyl group with Lewis base equivalents. A number of 1-(aromatic- or heteroaromatic-substituted)-3-(heteroaromatic-substituted)-1,3-propanediones of the general structure as shown in Figure 9 have been disclosed by Merck (39). Some examples of this class are shown in Figure 10. Compound S-1360 (**10.9**), which was disclosed by Shionogi as exhibiting an EC_{50} of 57 ng/ml, is in phase II clinical trials against HIV infection (40, 41).

In further work from Shionogi (42), compounds of the general structure **11.1** have been described as integrase inhibitors (Fig. 11). In **11.1,** X is hydroxyl, protected hydroxyl or substituted amino; Y is COOR (wherein R is hydrogen or an ester residue, $CONR^1R^2$ (wherein R^1 and R^2 may be hydrogen or an amide residue), or substituted aryl or substituted heteroaryl; and A is substituted heteroaryl. Some of the examples reported in this patent are shown in Figure 12.

Modified 1,3-propanedione derivatives

Further work by Shionogi discloses heterocyclic compounds such as **13.1-13.3** (43). In these compounds one keto functionality is replaced by the nitrogen of a heterocyclic ring (Fig. 13).

An interesting patent from Shionogi reports variants of propanediones where one of the ketones is constrained in the ring or is replaced by ring nitrogen (Fig. 14) (44). These compounds are capable of chelating 2 divalent metal ions at the same time. Some selected compounds from this patent are shown in Figure 15. Some inferences from this patent are that the position of carboxylic acid is crucial, change of ring size from 6 to 5 results in loss of activity (pyridine to pyrrole), and position of nitrogen on the ring is also important (2-pyridyl to 3-pyridyl >100).

In spite of their high integrase inhibitory potencies, 1,3-propanediones are limited as candidates for clinical development. As electrophilic compounds, they bind

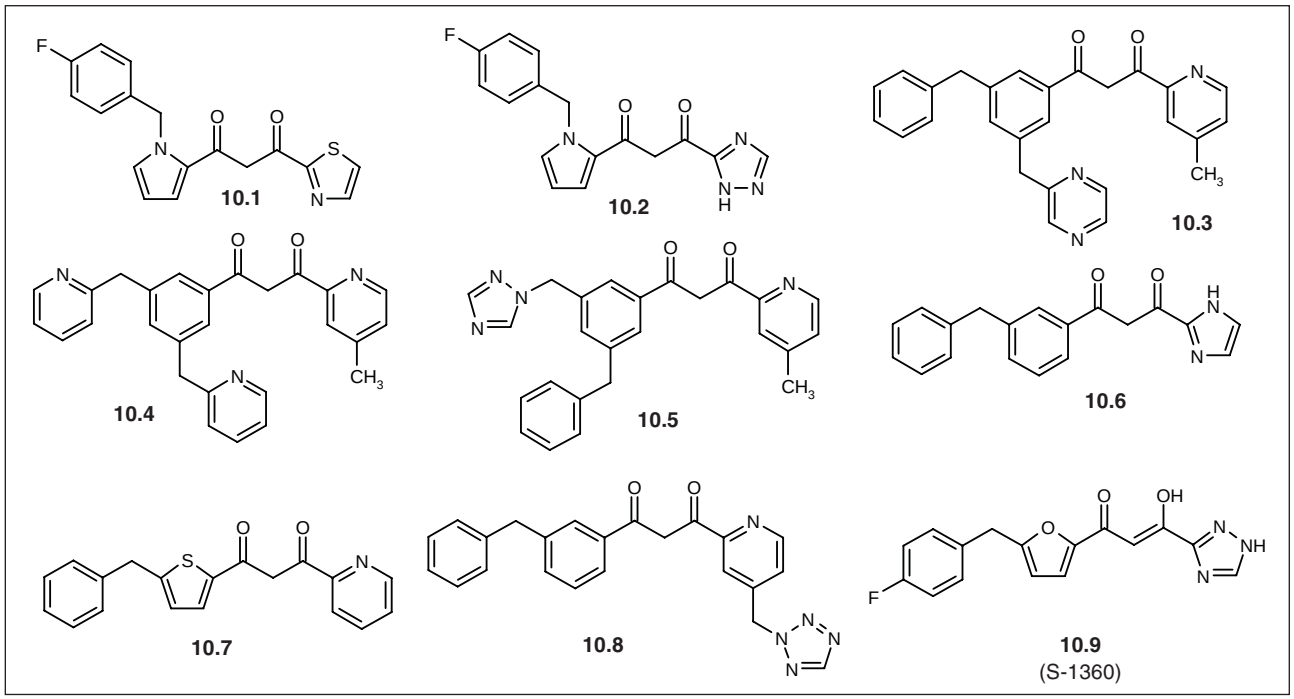


Fig. 10. Representative 1,3-propanedione HIV-1 integrase inhibitors.

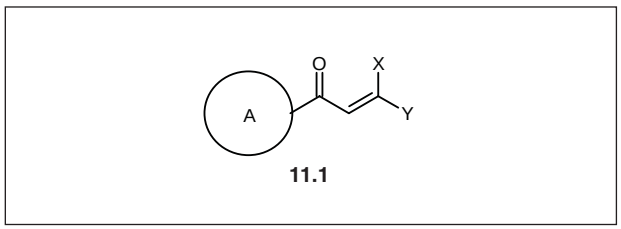


Fig. 11. General structure of ADKs from Shionogi (42).

covalently to human cellular proteins. It has been reported by Merck (45) that these types of compounds have high affinity towards serum albumin. This has led to the evaluation of a new class of heterocyclic metabolically stable compounds, which are disclosed in a patent (46). In these compounds, the second keto functionality is on the aromatic ring (Fig. 16). The naphthyridine analogue L-870810 (**16.3**) is potent inhibitor of integrase and has excellent selectivity for strand transfer. It has an IC_{95}

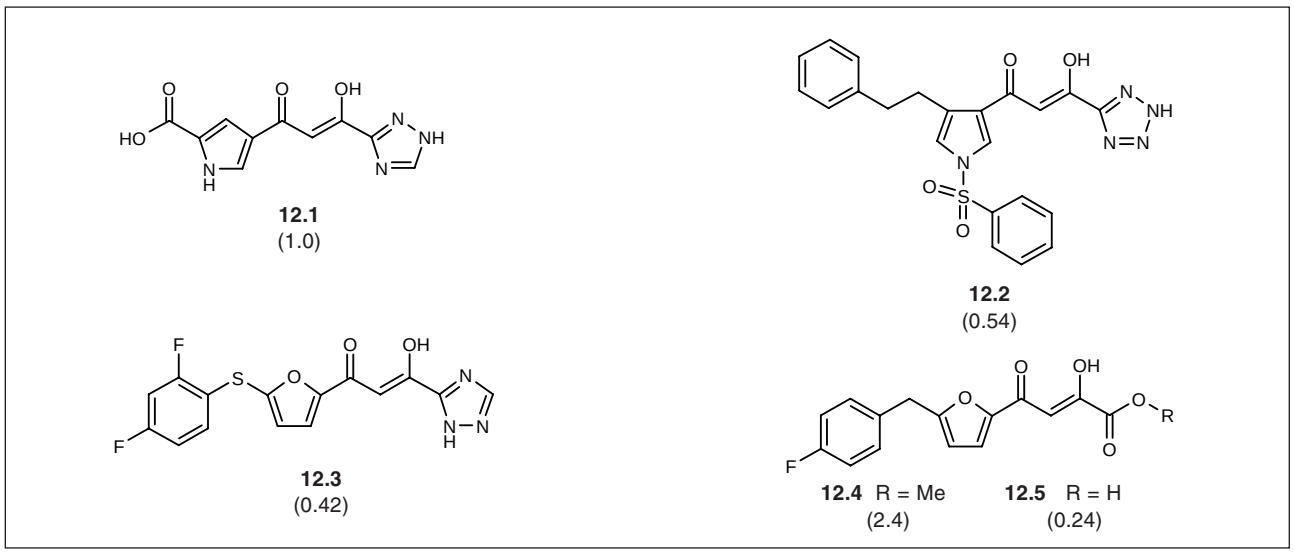


Fig. 12. Examples of 1,3-propanedione integrase inhibitors from Shionogi (42). IC_{50} values (μg/ml) are in parentheses.

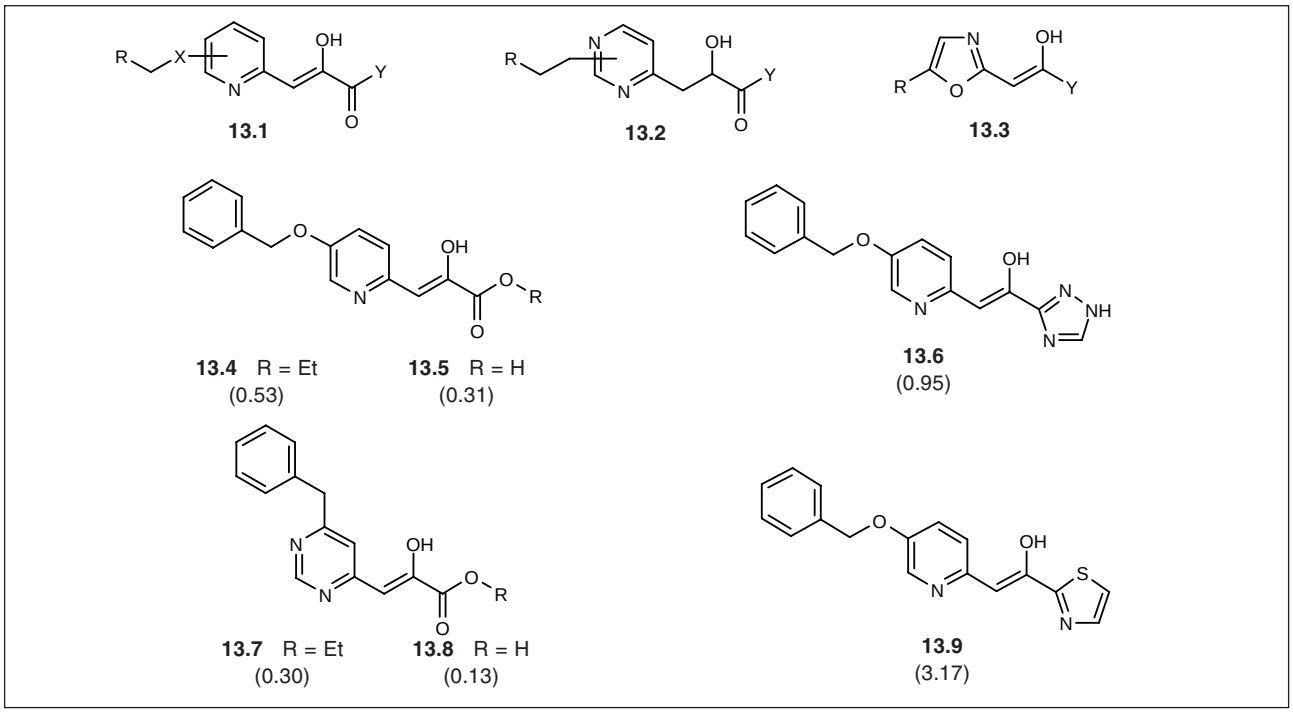


Fig. 13. Examples of modified 1,3-propanediones from Shionogi (43). IC_{50} values (μg/ml) are in parentheses.

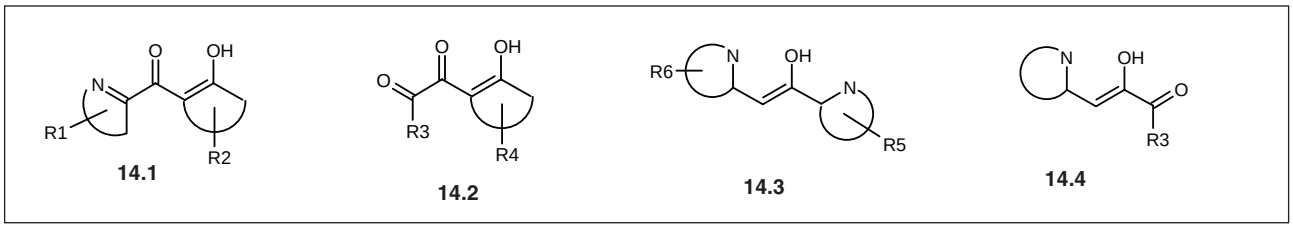


Fig. 14. General structures of modified 1,3-propanediones from Shionogi (44).

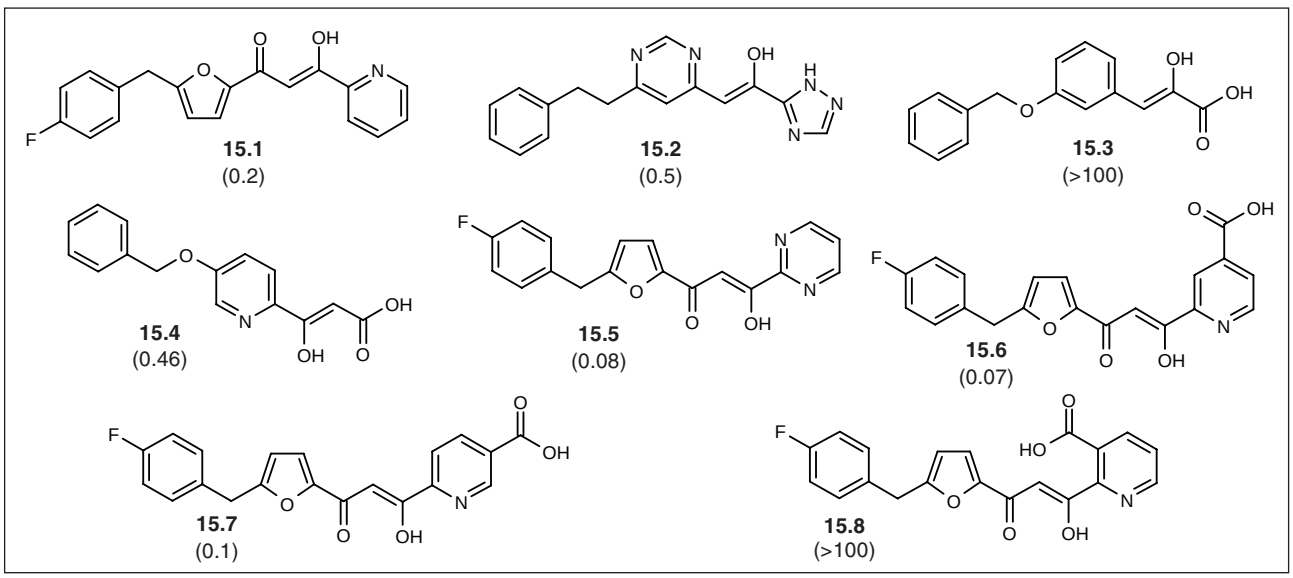


Fig. 15. Modified 1,3-propanedione integrase inhibitors from Shionogi (44). IC_{50} values (ng/ml) are in parentheses.

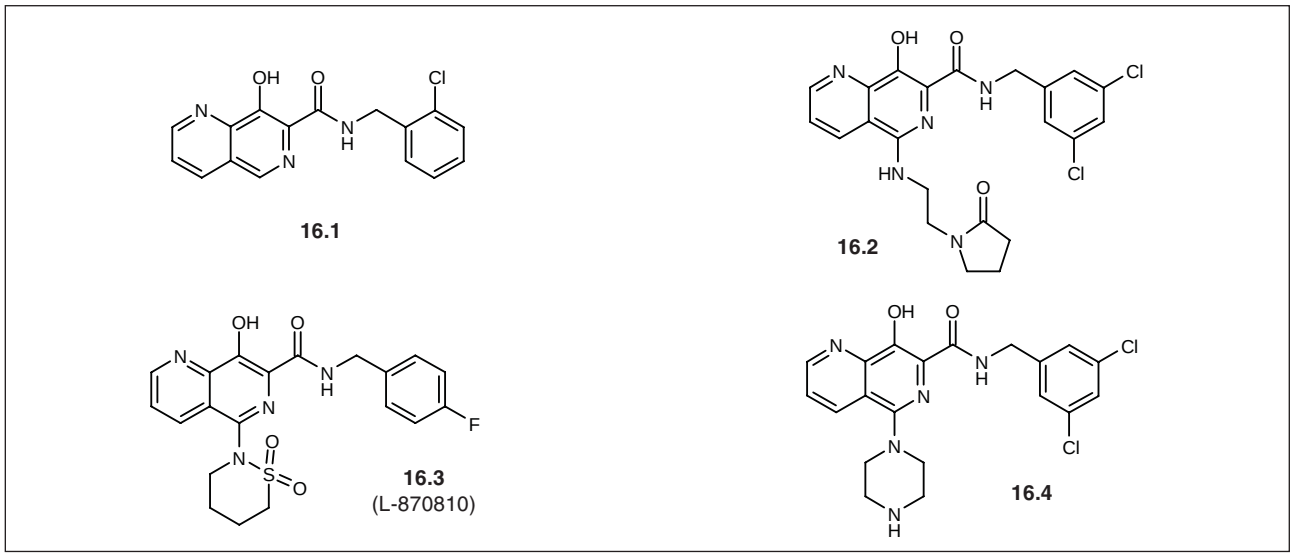


Fig. 16. Modified 1,3-propanedione integrase inhibitors from Merck (46).

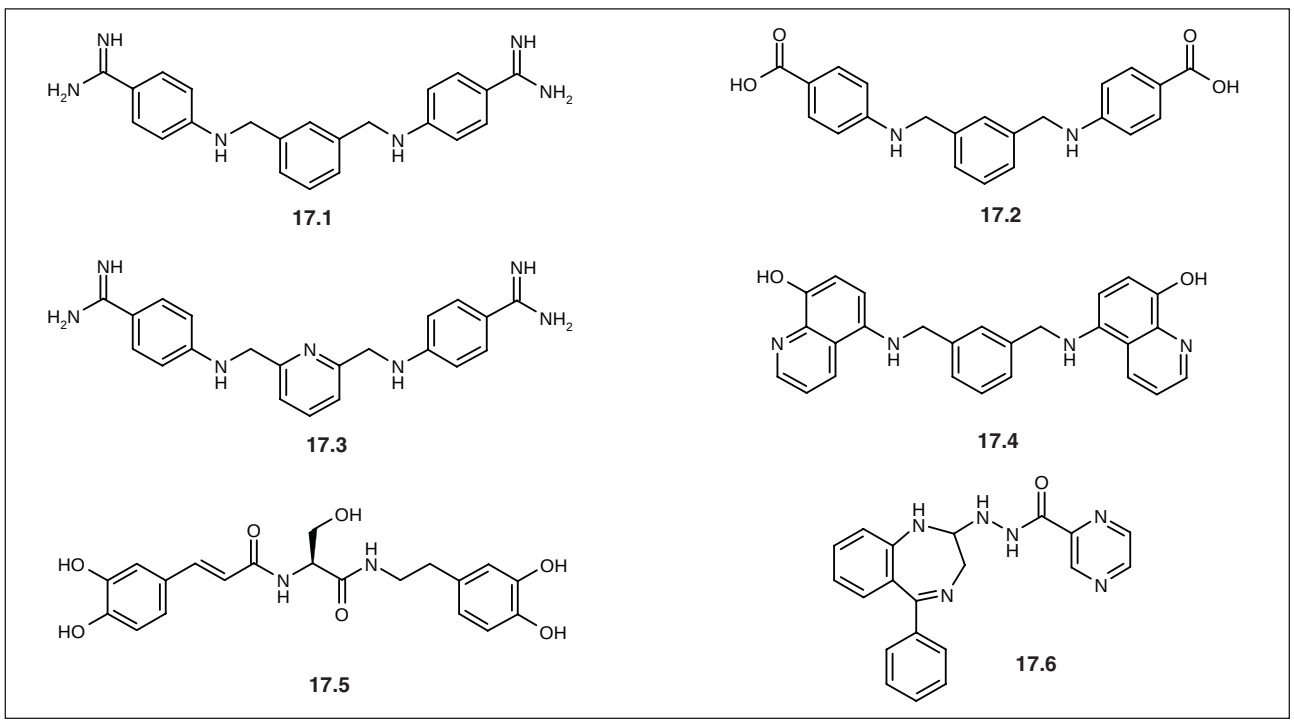


Fig. 17. Examples of miscellaneous ADK-related inhibitors.

value of 102 nM with human serum. It showed promising results in rats, dogs and rhesus macaques and has now moved to phase I clinical studies (45).

Miscellaneous ADK-related inhibitors

A patent from Harrison and Skalka reports symmetric compounds as inhibitors of HIV integrase, some of which are active in cell culture and inhibit the replication of ASLV and HIV at concentrations below cell toxicity (47). Some of the selected examples are shown in Figure 17 (**17.1-17.4**). The IC_{50} values in the HIV integrase assay ranged from 0.01-5 μM while the IC_{95} values in the cellular assay ranged from 0.01-5 μM for selected compounds of this category. Patents from Pharmacor, Inc. (48, 49) include hydroxyphenyl compounds of the type **17.5** as integrase inhibitors. Merck & Co. published benzodiazapine

hydrazide derivatives such as **17.6** as HIV integrase inhibitors (50).

Conclusions

Ten years of research on HIV-1 integrase has resulted in establishing ADK derivatives as bona fide inhibitors of integrase. As exemplified by S-1360 (**10.9**), a diketo analogue which has progressed into phase II clinical trials, and L-870810 (**16.3**), a modified 1,3-propandione which is in phase I clinical trials, integrase inhibitors may find a place in HAART protocols in the near future. Although integrase does not have a cellular equivalent, the potential for nonspecific inhibition of certain cellular enzymes by integrase inhibitors should not be ignored. RAG1/2 proteins essential for the development of mammalian immune system have similar modes of action as retroviral integrases. In a recent report, it was clearly shown that the 2 diketo acids tested interfered with DNA cleavage and disintegration activities of RAG1/2 (51). This implies that certain integrase inhibitors may have the potential to interfere with aspects of B- and T-cell development.

References

1. De Clercq, E. *Toward improved anti-HIV chemotherapy: Therapeutic strategies for intervention with HIV infections.* J Med Chem 1995, 38: 2491-517.

2. Richman, D.D. *HIV chemotherapy*. Nature 2001, 410: 995-1001.

3. LaFemina, R.L., Schneider, C.L., Robbins, H.L. et al. *Requirement of active human immunodeficiency virus type 1 integrase for productive infection of human T-lymphoid cells.* J Virol 1992, 66: 7414-9.

4. Neamati, N., Sunder, S., Pommier, Y. *Design and discovery of HIV-1 integrase inhibitors.* Drug Discov Today 1997, 2: 487-98.

5. Pommier, Y., Marchand, C., Neamati, N. *Retroviral integrase inhibitors year 2000: Update and perspectives*. Antiviral Res 2000, 4: 139-48.

6. Pommier, Y., Neamati, N. *Inhibitors of human immunodeficiency virus integrase*. Adv Virus Res 1999, 52: 427-58.

7. Neamati, N., Marchand, C., Pommier, Y. *HIV-1 integrase inhibitors: Past, present and future.* Adv Pharmacol 2000, 49: 147-65.

8. Farnet, C.M., Wang, B.B., Lipford, J.R., Bushman, F.D. *Differential inhibition of HIV-1 preintegration complexes and purified integrase protein by small molecules*. Proc Natl Acad Sci USA 1996, 93: 9742-7.

9. Goldgur, Y., Craigie, R., Cohen, G.H. et al. *Structure of the HIV-1 integrase catalytic domain complexed with an inhibitor: A platform for antiviral drug design*. Proc Natl Acad Sci USA 1999, 96: 13040-3.

10. Hazuda, D.J., Felock, P., Witmer, M. et al. *Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells*. Science 2000, 287: 646-50.

11. Grobler, J.A., Stillmock, K., Hu, B. et al. *Diketo acid inhibitor mechanism and HIV-1 integrase implications for metal binding in the active site of phosphotransferase enzymes*. Proc Natl Acad Sci USA 2002, 99: 6661-6.

12. Farnet C.M., Bushman, F.D. *HIV cDNA integration: Molecular biology and inhibitor development*. AIDS 1996, 10: S3-11.

13. Asante-Appiah, E., Skalka, A-M. *Molecular mechanism in retrovirus DNA integration*. Antiviral Res 1997, 36: 139-56.

14. Pommier, Y., Pilon, A., Bajaj, K., Mazumdar, A., Neamati, N. *HIV-1 integrase as a target for antiviral drugs*. Antiviral Chem Chemother 1997, 8: 463-83.

15. Hansen, M.S., Carteau, S., Hoffmann, C., Li, L., Bushman, F.D. *Retroviral cDNA integration: Mechanism, applications and inhibition*. Genet Eng 1998, 20: 41-61.

16. Robinson, W.E. Jr. *HIV integrase: The next target?* Infect Med 1998, 15: 129-37.

17. Young, S.D. *Inhibition of HIV-1 integrase by small molecules: The potential for a new class of AIDS chemotherapeutics*. Curr Opin Drug Discov Dev 2001, 4: 402-10.

18. Nair, V. *HIV integrase as a target for antiviral chemotherapy*. Rev Med Virol 2002, 12: 179-93.

19. Condra, J.H., Miller, M.D., Hazuda, D.J., Emini, E.A. *Potential new therapies for the treatment of HIV-1 infection*. Annu Rev Med 2002, 53: 541-55.

20. Pluymers, W.D., De Clercq, E., Debyser, Z. *HIV-1 integration as a target for antiretroviral therapy: A review*. Curr Drug Targets-Infect Disord 2001, 1: 133-49.

21. Pani, A., Marongiu, M.E. *Anti-HIV-1 integrase drugs: How far from shelf?* Curr Pharm Des 2000, 6: 569-84.

22. Neamati, N. *Structure based HIV-1 integrase inhibitor design: A future perspective*. Expert Opin Invest Drugs 2001, 10: 281-96.

23. Neamati, N. *Patented small molecule inhibitors of HIV-1 integrase: A 10-year saga*. Expert Opin Ther Patents 2002, 12: 709-24.

24. Williams, H.W.R., Eichler, E., Randall, W.C. et al. *Inhibitors of glycolic acid oxidase. 4-Substituted 2,4-dioxobutanoic acid derivatives.* J Med Chem 1983, 26: 1196-200.

25. Tomassini, J., Selnick, H., Davies, M.E. et al. *Inhibition of CAP (M(7)GPPPXM)-dependent endonuclease of influenzavirus by 4-substituted 2,4-dioxobutanoic acid compounds*. Antimicrob Agents Chemother 1994, 38: 2827-37.

26. Wai, J.S., Egbertson, M.S., Payne, L.S. et al. *4-Aryl-2,4-dioxobutanoic acid inhibitors of HIV-1 integrase and viral replication in cells.* J Med Chem 2000, 43: 4923-6.

27. Pais, G.C.G., Zhang, X., Marchand, C. et al. *Structure activity of 3-aryl-1,3-diketo-containing compounds as HIV-1 integrase inhibitors.1*. J Med Chem 2002, 45: 3184-94.

28. Zhang, X., Pais, G.C.G., Svarovskaia, E.S. et al. *Azido-containing aryl β-diketo acid HIV-1 integrase inhibitors*. Bioorg Med Chem Lett, in press.

29. Marchand, C., Zhang, X., Pais, G.C.G. et al. *Structural determinants for HIV-1 integrase inhibition by β-diketo acids*. J Biol Chem 2002, 277: 12596-603.

30. Espeseth, A.S., Felock, P., Wolfe, A. et al. *HIV-1 integrase inhibitors that compete with the target DNA substrate define a unique strand transfer conformation for integrase.* Proc Natl Acad Sci USA 2000, 97: 11244-9.

31. Young, S.D., Egbertson, M., Payne, L.S. et al. (Merck & Co., Inc.; Tularik, Inc.). *HIV integrase inhibitors*. PCT Int Appl 1999, WO-99/62520.

32. Young, S.D., Wai, J.S., Embrey, M.W., Fisher, T.E. (Merck & Co., Inc.). *HIV integrase inhibitors*. PCT Int Appl 1999, WO-99/62897.

33. Selnik, H.G., Hazuda, D.J., Egbertson, M. et al. (Merck & Co., Inc.; Tularik, Inc.). *HIV integrase inhibitors*. PCT Int Appl 1999, WO-99/62513.

34. Wolfe, A.A., Felock, P.J., Hastings, J.C., Blau, C.U., Hazuda, D.J. *The role of manganese in promoting multimerization and assembly of human immunodeficiency virus type 1 integrase as a catalytically active complex on immobilized long terminal repeat substrates*. J Virol 1996, 70: 1424-32.

35. Farnet C.M., Bushman, F.D. *HIV-1 cDNA integration: Requirement of HMG I(Y) protein for function of preintegration complexes in vitro*. Cell 1997, 88: 483-92.

36. Fujishita, T., Yoshinaga, T., Yamaguchi, H. (Shionogi & Co., Ltd.). *Indole derivatives with antiviral activity*. PCT Int Appl 1999, WO-99/50245.

37. Walker, M.A., Johnson, T.D., Meanwell, N.A., Banville, J. (Bristol-Myers Squibb Co.). *HIV integrase inhibitors*. PCT Int Appl 2001, WO-01/96283.

38. Walker, M.A., Johnson, T.D., Kim, O.A. (Bristol-Myers Squibb Co.). *HIV integrase inhibitors.* PCT Int Appl 2001, WO-01/98248.

39. Payne, L.S., Tran, L.O., Zhuang, L.H. et al. (Merck & Co., Inc.; Tularik, Inc.). *1-(Aromatic- or heteroaromatic-substituted)-3-(heteroaromatic substituted)-1,3-propanediones and uses thereof*. PCT Int Appl 2001, WO-01/00578.

40. Sato, A., Yamaguchi, H. et al. (Shionogi & Co., Ltd.). *Medicinal compositions containing propenone derivatives*. PCT Int Appl 2001, WO-96329.

41. Yoshinaga, T., Sato, A., Fujishita, T., Fujiwara, T. *In vitro activity of a new HIV-1 integrase inhibitor in clinical development*. 9th Conf Retroviruses Opportunistic Infect (Feb 24-28, Seattle) 2002, Abst 8.

42. Fujishita, T., Yoshinaga, T., Satao, A. (Shionogi & Co., Ltd.). *Aromatic heterocycle compounds having HIV integrase inhibiting activities*. PCT Int Appl 2000, WO-00/39086.

43. Kawasuji, T., Yoshinaga, T. (Shionogi & Co., Ltd.). *Integrase inhibitors containing aromatic heterocyclic derivatives*. PCT Int Appl 2001, WO-01/17968.

44. Kiyama, R., Kawasuji, T. (Shionogi & Co., Ltd.). *Inhibitor for enzyme having two divalent metal ions as active centers*. PCT Int Appl 2001, WO-01/95905.

45. Young, S.D., Anthony, N., Wai, J., Fisher, T. et al. *Discovery of a potent HIV integrase inhibitor with potential clinical utility.* 5th Int Workshop HIV Drug Resistance Treatment Strategies (Seville) 2002.

46. Anthony, N.J., Gomez, R.P., Young, S.D. et al. (Merck & Co., Inc.). *Aza- and polyaza-naphthalenyl carboxamides useful as HIV integrase inhibitors*. PCT Int Appl 2002, WO-02/30930, WO-02/30931.

47. Harrison, R.W., Skalka, A.M. (Thomas Jefferson Univ.). *Symmetric inhibitors of HIV integrase, mammalian topoisomerase and serine protease*. PCT Int Appl 2002, WO-02/02516.

48. Sauvé, G., Yelle, J. (Pharmacor Inc.). *Hydroxyphenyl derivatives with HIV integrase inhibitory properties*. US6362165; 2002, PCT Int Appl WO-00/59867, 2000.

49. N'Zemba, B.M., Sauvé, G., Sevigny, G., Yelle, J. (Pharmacor Inc.). *Aromatic derivatives with HIV integrase inhibitory properties*. PCT Int Appl 20002, WO-02/26697.

50. Bell, I.M., Guare, J.P. Jr., Hazuda, D.J., Munson, P.M., Thompson, W.J., Vacca, J.P. (Merck & Co., Inc.). *Benzodiazepine hydrazide derivatives as inhibitors of HIV integrase*. PCT Int Appl 1998, WO-98/18473.

51. Melek, M., Jones, J.M., O'Dea, M.H. et al. *Effect of HIV integrase inhibitors on the RAG1/2 recombinase*. Proc Natl Acad Sci USA 2002, 99: 134-7.