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Substituted 2-pyrrolinone inhibitors of HIV-1 integrase

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Abstract—The β-diketoacid class of HIV-1 integrase (IN) inhibitors represent the first potent class of compounds specific for the strand transfer catalytic activity of the viral enzyme. Previously, utilizing a β-diketoacid pharmacophore as a search query, we identified a substituted 2-pyrrolinone with modest IN inhibitory activity from a database of small-molecules [Dayam, R.; Sanchez, T.; Neamati, N. *J. Med. Chem.* **2005**, *48*, 8009]. In efforts to optimize this class of IN inhibitors, we carried out a structure–activity relationship analysis around the 2-pyrrolinone core. Here, we present a new class of 2-pyrrolinone IN inhibitors.

The *pol* gene of HIV-1 encodes for three essential enzymes for viral replication, reverse transcriptase (RT), protease (PR), and integrase (IN). Currently, there are a number of US FDA-approved drugs targeting RT and PR, but none targeting IN. Highly active anti-retroviral therapy, or HAART, is the current treatment strategy for HIV-1 infected patients, and consists of a cocktail of drugs largely targeting RT and PR. HAART does result in reducing viral loads below detectable limits, but sustained treatment is associated with a range of toxicity issues and the emergence of drug resistant viral strains. Drug resistant viral strains often display cross-resistance to other HAART components targeting the same viral enzyme. Drugs targeting a different stage of the HIV-1 life cycle, such as integration, are expected to complement HAART and be effective against current drug resistant viral strains.

Integration of the proviral DNA transcript into the host cell genome is a prerequisite for proviral gene transcription and translation, and ultimately virion assembly, budding, and release. IN catalyzes this essential step via two successive DNA reaction events, termed 3′-processing and strand transfer. 3′-Processing occurs in the cytosol following reverse transcription of the viral RNA genome. Here, IN cleaves off a dinucleotide from each 3′-end of the proviral DNA transcript resulting in two 3′-recessed hydroxyl groups utilized for a nucleophilic attack in the subsequent strand transfer step. Next, IN bound to the processed proviral DNA, in the context of a large nucleo-protein complex termed the preintegration complex, translocates to the nucleus. In the nuclear environment, IN catalyzes the concerted integration of this DNA product into the host cell genome.[1] The early development of a convenient in vitro IN enzymatic assay using isolated systems that contain purified IN, a DNA oligonucleotide substrate with ends corresponding to the U3 or U5 viral DNA termini, and Mg^{2+} or Mn^{2+} as a cofactor[2] has enabled the identification of numerous IN inhibitors over the past decade.[3–5]

The β-diketoacid class of compounds represent the first strand transfer specific small-molecule IN inhibitors and helped validate IN as a viable anti-retroviral drug target for HIV/AIDS therapy.[6] The β-diketoacid bioisostere S-1360 (**1**), discovered by Shionogi & Co., was the first IN inhibitor to enter human clinical trials.[7,8] Unfortunately, S-1360 failed in efficacy studies due to its metabolism into an inactive metabolite followed by a rapid body clearance. It was later shown that the aldo–keto reductase family of enzymes, which are cytosolic, NADPH-dependent, carbonyl reducing enzymes in the human liver, were responsible for S-1360 metabolism.[9] Currently, two strand transfer specific IN inhibitors, MK-0518[10,11] (**2**) and GS-9137[12] (**3**), are in advanced stages of clinical trials (Fig. 1).

Although S-1360 failed in clinical studies, the use of the diketoacid pharmacophore as a search query

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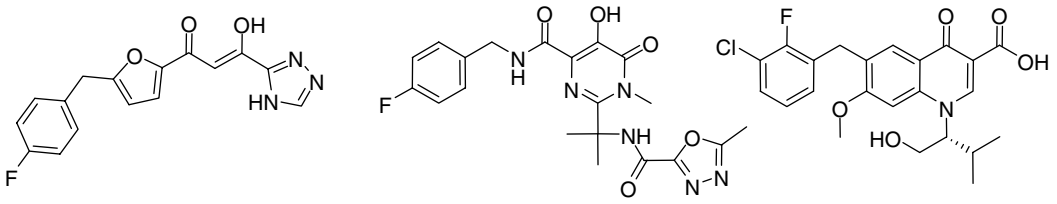


S-1360 (**1**) MK-0518 (**2**) GS-9137(**3**)

Figure 1. Clinically studied HIV-1 integrase inhibitors. Currently, MK-0518 and GS-9137 are in advanced stage clinical trials.

remains an effective approach to identify potent IN inhibitors with diverse chemical scaffolds that may be subject to dissimilar in vivo metabolic fates. We previously employed this approach to discover a series of structurally diverse IN inhibitors including the substituted 2-pyrrolinone **4** (Table 1).[13] Compound **4** displayed modest IN inhibitory activity, but contained a diketoacid pharmacophore on a constrained 2-pyrrolinone ring presenting a different keto-enolic arrangement as compared to previously disclosed open chain diketoacids and bioisosteres (Fig. 2a).

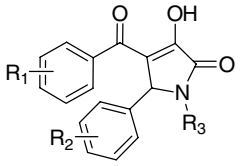
In efforts to optimize the substituted 2-pyrrolinone **4** we used the β-diketoacid pharmacophore-containing 2-pyrrolinone substructure as a search query (Fig. 2b). The search retrieved a series of substituted 2-pyrrolinones (**5**–**21**) from a commercial database of small-molecules (Asinex Corp.) (Table 1). IC_{50} values of each compound against IN were obtained as described previously.[14] The substituted 2-pyrrolinones (**5**–**21**) displayed an interesting IN inhibitory profile. The variation in IN inhibitory activity of the substituted 2-pyrrolinones demonstrates an apparent tolerance to the size and chemical nature of the substitutions (R_3) at the N1 position of the 2-pyrrolinone core (compounds **4**–**6**, Table 1). Compounds bearing optimally substituted aromatic or heterocyclic groups at the R_3 position displayed an improved IN inhibitory activity as compared to compound **4**. For example, compounds **8**–**11** containing an aromatic (**8**) or heterocyclic groups (**9**–**11**) displayed significantly improved IN inhibitory potency. Similarly, compounds **16**–**19** bearing a substituted thiazole moiety at R_3 are the most potent members of this novel series of IN inhibitors. It appears that the substitution pattern at the R_1 and R_2 positions also has a secondary influence on the IN inhibitory potency of this series of compounds (Table 1). In general, compounds bearing a halogen and/or smaller alkyl groups such as a methyl substitution at either the R_1 or R_2 positions in combination with an optimum substitution at the R_3 position display an improved IN inhibitory profile. Compounds **16**–**18** bearing a halogen (*para*-fluoro in **16** and **17**, *meta*-chloro in **18**) at the R_2 position and a hydrogen (**16**) or a methyl (**17** and **18**) at the R_1 position in combination with substituted thiazole groups at position R_3 demonstrate an enhanced IN inhibitory potency. Compound **18** is the most potent substituted 2-pyrrolinone analogue. It inhibited both the 3′-processing and strand transfer activity of IN with IC_{50} values of 27 ± 10 and 8 ± 2 μM, respectively.

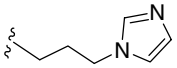
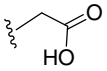
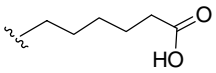
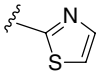
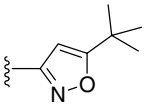
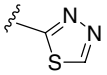
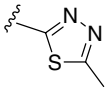
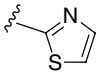
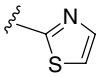
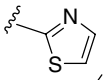
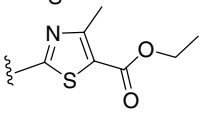
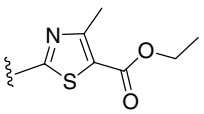
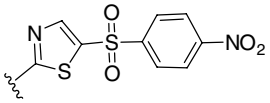
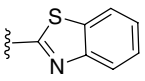
Compound **19** bearing a 2-benzothiazole group at R_3 showed ~3-fold selectivity toward the strand transfer reaction of IN. It inhibited both the 3′-processing and strand transfer activities of IN with IC_{50} values of 57 ± 10 and 21 ± 10 μM, respectively. Interestingly, compounds **20** and **21**, also bearing a 2-benzothiazole group at R_3, and a 3-pyridine (**20**) or a 2-pyridine (**21**) at the 5th position on the pyrrolinone core as opposed to a phenyl group (present in all other substituted 2-pyrrolinones), display a weak inhibitory potency against the IN strand transfer reaction. Compounds **20** and **21** failed to inhibit the 3′-processing activity of IN at a maximum tested concentration of 100 μM. This indicates that a substituted phenyl ring is favored as compared to a pyridine substitution at the 5th position of the pyrrolinone core.

Of note, compounds **6** and **18** exhibited an interesting inhibitory profile in the presence of Mg^{2+} (Fig. 3). Compound **6** bearing an *n*-hexanoic acid group at the N1 position (R_3) of the pyrrolinone core displayed more than a 10-fold selectivity toward the strand transfer reaction of IN. It inhibited the strand transfer activity of IN with an IC_{50} value of 10 μM. Similarly, compound **18** also selectively inhibited the IN strand transfer reaction with an IC_{50} value of 6.1 μM. Compounds **6** and **18** failed to inhibit the 3′-processing reaction of IN at a maximum tested concentration of 100 μM in the presence of Mg^{2+} as a co-factor. Based on the excellent anti-retroviral profile observed with previously reported strand transfer selective IN inhibitors and the biological relevance of using the Mg^{2+} ion as the IN metal co-factor, compounds **6** and **18** are promising leads with suitable characteristics for further therapeutic development.

In conclusion, we have identified a series of novel substituted 2-pyrrolinone IN inhibitors through substructure database search methods. A limited SAR around the substituted 2-pyrrolinone core resulted in the discovery of a series of compounds with improved IN inhibitory potency. Further chemical optimization of the substituents on the constrained 2-pyrrolinone diketoacid pharmacophore-containing core would lead to potent IN inhibitors that may have different in vivo pharmacoki-

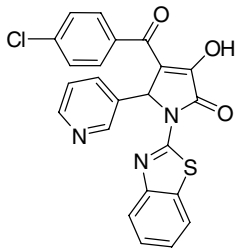
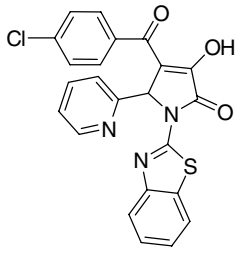
Table 1. Inhibition of HIV-1 integrase catalytic activities by a series of substituted 2-pyrrolinones



Compound	Structure			IN inhibition activity (IC_{50}, μM)	
	R_1	R_2	R_3	3′-Processing	Strand transfer
4	4-CH_3	4-Cl		93 ± 12	67 ± 28
5	4-Cl	4-CH_3		84 ± 23	69 ± 36
6[a]	2, 5-$(OCH_3)_2$	4-Cl		88 (>100)	40 ± 5 (10)
7	3, 4-$(OCH_3)_2$	4-Br	–CH_2CH_2Ph	60 ± 3	42 ± 10
8	4-Br	3, 4-$(OCH_3)_2$	–Ph-3-CF_3	47 ± 7	26 ± 9
9	4-OEt	3-OCH_3,4-OH		57 ± 27	30 ± 7
10	4-Br	3, 4-$(OCH_3)_2$		45 ± 8	43 ± 26
11	4-F	4-*tert*-Butyl		64 ± 28	43 ± 8
12	4-OEt	4-F		>100	>100
13	4-CH_3	4-Cl		56 ± 10	39 ± 3
14	4-CH_3	4-F		>100	78 ± 10
15	4-OEt	4-OCH_3		>100	90 ± 17
16	H	4-F		44 ± 4	21 ± 4
17	4-CH_3	4-F		>100	33 ± 25
18[a]	4-CH_3	3-Cl		27 ± 10 (>100)	8 ± 2 (6.1)
19	H	3,4-OCH_2O–		57 ± 10	21 ± 10

(*continued on next page*)

Table 1 (*continued*)

Compound	Structure			IN inhibition activity (IC_{50}, μM)	
	R_1	R_2	R_3	3′-Processing	Strand transfer
20				>100	90 ± 10
21				>100	83 ± 10

[a] The values in parenthesis are obtained using Mg^{2+} as a metal co-factor.

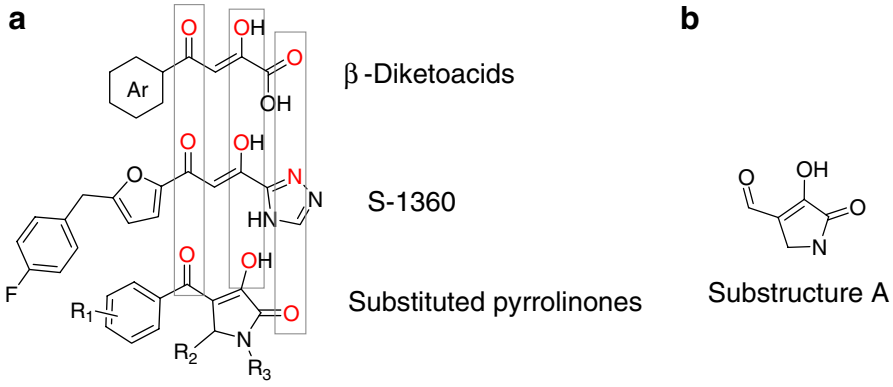


Figure 2. (a) The β-diketoacid pharmacophore is shared by diverse chemical scaffolds. (b) The substructure A was used to retrieve analogues of compound **4** bearing a diketoacid pharmacophore-containing 2-pyrrolinone moiety.

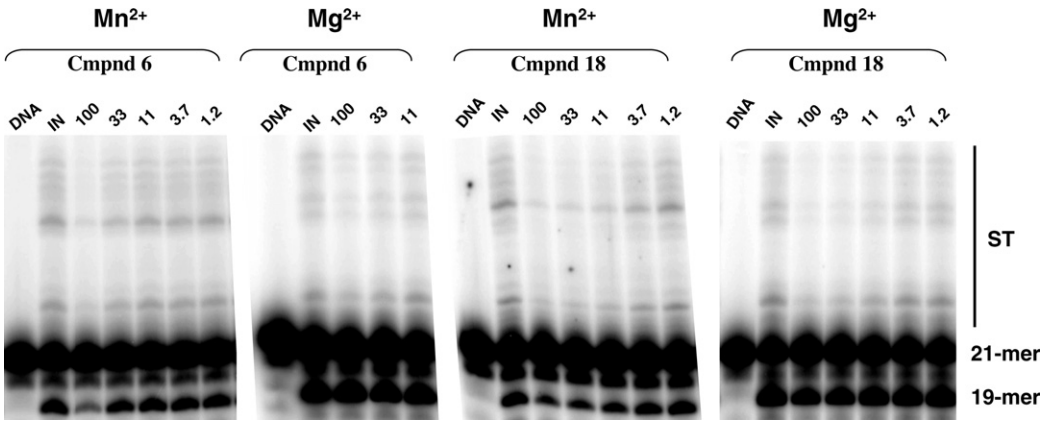


Figure 3. Compounds **6** and **18** both exhibit >10-fold selectivity for strand transfer inhibition when tested using Mg^{2+} as an IN cofactor.

netic/pharmacodynamic properties as compared with the previously clinically studied open-chained β-diketo bioisostere S-1360.

Acknowledgments

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