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# Rational design of 2-pyrrolinones as inhibitors of HIV-1 integrase

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ABSTRACT

HIV-1 integrase is an essential enzyme for viral replication and a validated target for the development of drugs against AIDS. With an aim to discover new potent inhibitors of HIV-1 integrase, we developed a pharmacophore model based on reported inhibitors embodying structural diversity. Eight compounds of 2-pyrrolinones fitting all the features of the pharmacophore query were found through the screening of an in-house database. These candidates were successfully synthesized, and three of them showed strand transfer inhibitory activity, in which, one compound showed antiviral activity. Further mapping analysis and docking studies affirmed these results.



HIV-1 integrase (IN) is an important enzyme involved in mediating the full integration of proviral DNA into the human genome, which is essential for retroviral replication.[1] This step consists of two reactions with DNA. (1) The first step is referred to as 3′-processing which includes the endonucleolytic cleavage of the 3′-ends of the viral DNA. (2) The second step, called strand transfer, results in the ligation of the 3′-processed proviral DNA into host chromosome. IN is an attractive target because no known human enzyme exists with similar activity.[2] Therefore, the addition of an IN inhibitor to existing components of antiretroviral therapy is expected to improve the outcome of therapy by potential synergism without exacerbating toxicity.[3]

The search for HIV-1 IN inhibitors has spanned more than a decade.[3–8] The β-diketo acids have been identified as strand transfer specific IN inhibitors and have been shown to be 'druggable'.[9] Many bioisosteres of β-diketo acids have been developed for years. Some of them, such as **12** (S1360),[2] **2** (L870810),[10] **9** (GS-9137),[11] **6** (GSK-364735)[12] shown in Figure 1 have already entered into clinical phase. Moreover, Raltegravir, **4** (MK-0518)[13] has been recently approved by FDA as the first IN inhibitor. However, the inevitable emergence of drug resistant substitutions in IN will require a constant effort toward the discovery of novel inhibitors to maintain a therapeutic advantage over the virus. Our laboratories focus on finding new potent inhibitors of 3′-processing and strand transfer especially the new selective strand transfer inhibitor.

Pharmacophore modeling is one of the widely used analog-based drug design methods.[14,15] Some IN inhibitors with structural diversity have been successfully discovered by this approach.[11,16–19] In this study, we focus on the development of the pharmacophore model, which was constructed based on the chemical features of fifteen reported potent IN inhibitors. In fact, several HIV IN pharmacophores have already been published.[3,16,17] However, to the best of our knowledge, no attempts to build the model on these potent β-diketo acid (DKA)-like strand-transfer-selective inhibitors have been reported. The resulting model can generate the common features of these effective inhibitors, and it is further utilized to predict novel bioactive molecules through the virtual screening of an in-house database. Eight representatives selected from a group of hits have been successfully synthesized for a biological assay, and three of them exhibit strand transfer inhibition in vitro.

Fifteen compounds (Fig. 1) for the training set were selected from literatures,[19–22] which was based on the principles of structural diversity and certain coverage of activity range. These compounds were provided to the HipHop module to generate the common features of the pharmacophore model. The chemical structures of these drugs/candidates were sketched using ISIS/Draw (MDL Informations Systems, Inc., San Leandro, CA, USA) as shown in Table 1, and their 3D structures with hydrogens were converted by CORINA (a fast generation of high-quality 3D molecular models, Molecular Networks, http://www.molecular-networks.com/products/corina). Atomic types and bond types of these compounds were inspected and modified manually, and Gasteiger charges were assigned to them. Furthermore, the structures were optimized by means of molecular mechanics, using a Tripos force field encoded in SYBYL v6.9 (Tripos Associates, St. Louis, MO, USA). A set of energetically reasonable conformations were built within the CATALYST (Version 4.0; Accelrys, Inc., 2003) CatConf module using the Poling Algorithm.[23] The 'Best conformer generation' option was used with

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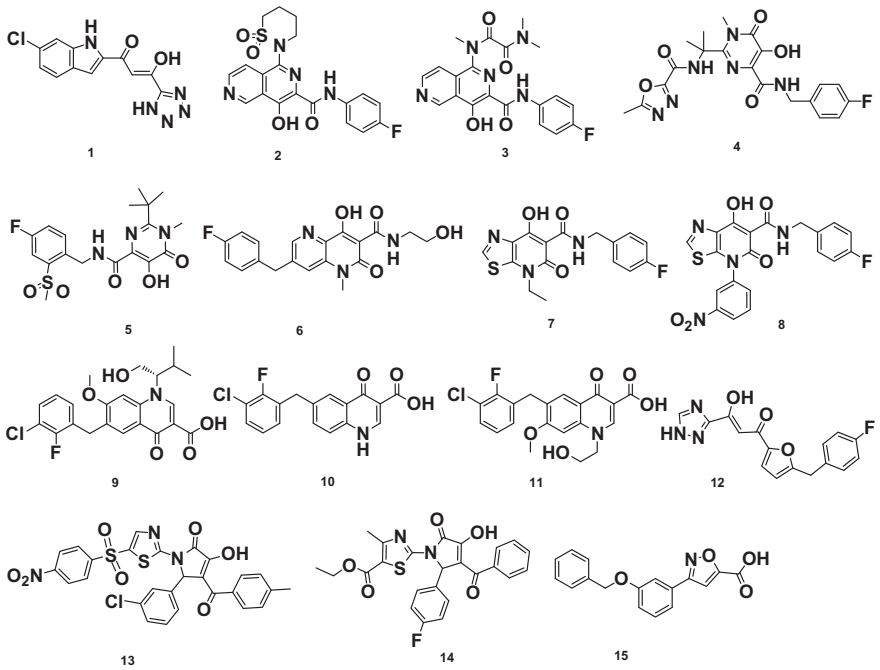
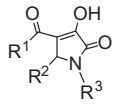


**Figure 1.** Chemical structures of the 15 training set compounds.

**Table 1**
The IN inhibitory activities and the pharmacophore fit values of the 2-pyrrolinone derivatives



| Compound | $R^1$ | $R^2$ | $R^3$ | Inhibition of HIV-1 integrase ($IC_{50}$)[a] | | Pharmacophore fit value[b] |
|---|---|---|---|---|---|---|
| | | | | 3′-Processing (μM) | Strand transfer (μM) | |
| **20** | Ph | 4-$NO_2$-Ph– | Ph | 89 | 44 | 2.47 |
| **21** | Ph | Ph | –$(CH_2)_2$-Ph-4-OMe | 80 | 45 | 2.98 |
| **22** | 2-Py | 3-Cl-Ph | –$(CH_2)_2$-Ph-4-OMe | 77 | 40 | 2.97 |
| **23** | 2-Py | 3-Cl-Ph | –Me | >100 | >100 | 2.59 |
| **24** | Ph | 4-OH-Ph | –$(CH_2)_2$-Ph-4-OMe | >100 | 100 | 2.97 |
| **25** | Ph | 3-Cl-Ph | –Me | >100 | >100 | 2.23 |
| **26** | Ph | 4-$NO_2$-Ph– | –Me | >100 | >100 | 2.76 |
| **27** | Ph | Ph | –$CH_2CH(CH_2)_2$ | >100 | >100 | 2.26 |

[a] HIV-1 IN inhibitory activity was measured according to the procedure described in Experimental. Values are means of three determinations and deviation from the mean is <10% of the mean value.
[b] The overall fit value is 4.

a 20 kcal/mol energy cut off. Default settings were used for the other parameters.

The top ranked pharmacophore model (Hypo1) had the best predictive power and statistical significance and was characterized by the lowest total cost value (82.4009), the highest cost difference (100.0801), the lowest RMSD (0.5003), and the best correlation coefficient (0.9785). The hypothesis (Hypo1) (shown in Fig. 2A) has four features, namely a hydrophobic aromatic feature (HRA) and three H-bond acceptors (HBA). Three excluded volumes were used to define the entrance of the active site. Compound **6** (GSK-364735) was mapped onto Hypo1 with high pharmacophore fit values (3.99) between the key chemical features of the compound and the pharmacophore features of Hypo1 (Fig. 2B).

The Hypo1 model was applied to an in-house database as a query to find the compounds that fitted all the above features. Our in-house database is a chemical database with searchable 3D structure multiconformers, most of which are based on an available compound library.

Virtual screening was carried out in CATALYST after the library compounds were minimized to the closest local minimum. A total of 89 2-pyrrolinone compounds as primary hits were obtained by use of a 'best fit'. Among them, compounds **20–27** show pharmacophore fit values ranging from 2.23 to 2.98 when one feature of the Hypo1 is allowed to be missing in the mapping process. In order to find more active compounds based on the corrected pharmacophore model, several representative hits selected on the basis of the pharmacophore fit value were synthesized and tested for their HIV-1 IN inhibitory activities.

Synthetic approaches for preparation of the hit compounds are depicted in Scheme 1. This strategy is concise with just a two-step process. Firstly, condensation of acetophenone (**16**) or 1-(pyridin-2-yl) ethanone (**17**) with diethyl oxalate in the presence of sodium

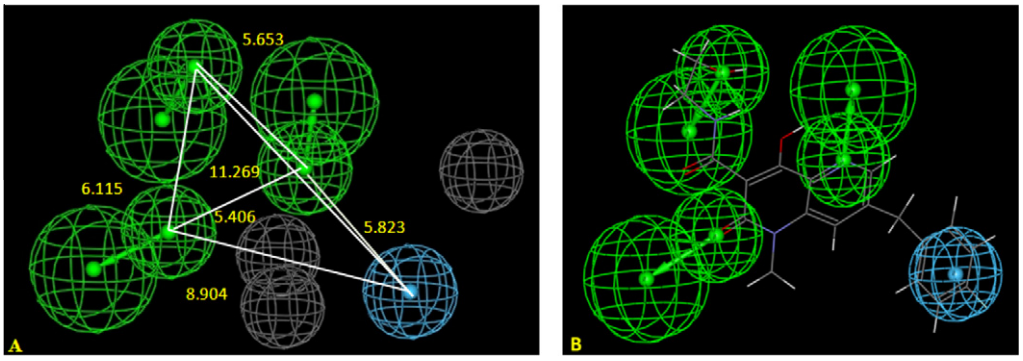
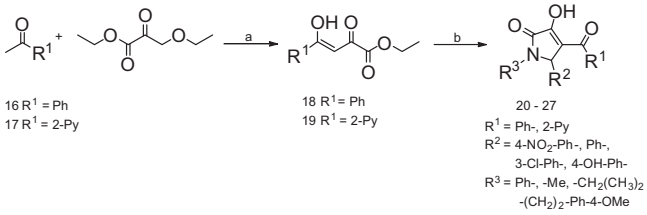



**Figure 2.** Pharmacophore models. (A) The best-ranked HipHop pharmacophore model (Hypo1). The pharmacophore with four features are color coded as follows: H-bond acceptors (HBA) as green and hydrophobic aromatic (HRA) features as blue. Interfeature distances are given in Angstroms. (B) The mapping plot of HIV-1 integrase inhibitor **6** from the training set onto best HipHop pharmacophore model (Hypo1).




**Scheme 1.** General route for the synthesis of hits of 2-pyrrolinones. Reagent and conditions: (a) NaH, toluene, rt, 90%; (b) $R^3$-$NH_2$, $R^2$-CHO, THF, rt, 90%.

hydride furnish 2,4-diketo esters (**18, 19**). At the second step, the objects of 2-pyrrolinones (**20**–**27**) are successfully prepared via Mannich reaction following intramolecular ring closure in one pot just by treatment of such resulting esters with various amines and aldehydes.

We examined the ability of the eight hits to inhibit IN catalytic activities using in vitro assays. The chemical structures, IN inhibitory activities, and the pharmacophore fit values of these hits are shown in Table 1.

Three compounds (**20**, **21**, and **22**) show partial selectivity toward the IN strand transfer. This result agreed with the general belief that the character of acyl-diketo can function as a selective strand transfer inhibitor. The presence of a bulky hydrophobic group at the nitrogen atom seems to be important due to the lack of activity of derivatives with short and linear substituents in this position (**20** vs **26**, **21** vs **27**, and **22** vs **23**). The activity of the compound substituted with phenyl at the nitrogen atom (**20**) is superior to that of compounds substituted with methyl or isobutyl (**23**, **27**). The $R^2$ group seems also an impact factor for the activity (**21** vs **24**), an observation that warrants further study. For inhibition of the 3′-processing reaction, the compound with pyridinyl as $R^1$ group (**22**) is more potent than the compound with a phenyl substitution (**20**).

In order to rationalize the obtained results, we mapped the common feature hypothesis 1 (Hypo1) onto compound **21**. The mapping plot showed a good agreement between chemical features of the compound and pharmacophoric features of Hypo1 (Fig. 3A). The benzene ring linked to the nitrogen atom and two carbonyl groups were well mapped onto the HRA, HBA1, and HBA3, respectively. Furthermore, we docked compound **21** into the IN active site abstracted from the IN crystal structures with the PDB entry code 3L2V (Fig. 3B)[24] to build the binding model. Docking was performed with GOLD 4.1.2 (The Cambridge Crystallographic Data Centre: Cambridge, UK). The carbonyl groups of the ligand formed hydrogen bonds with the SER184 and HIS213. The binding mode of compound **21** in the active site was very close to that observed in the crystal structure of Raltegravir with IN.[24] Consequently, these observations provided an excellent explanation for the in vitro inhibitory activity of compounds **20–22**.

Although the IN inhibitory activity was not as potent as we expected, compound **22** exert potent inhibition on the stimulated luciferase enzymatic activity of HIV in TZM-bl cells with an $EC_{50}$ value of 0.317 μM (Table 2). More importantly, this compound possessed low cytotoxicities with a SI value of 83.

A pharmacophore-based virtual screening of the in-house library was performed to identify HIV-1 IN inhibitors with novel

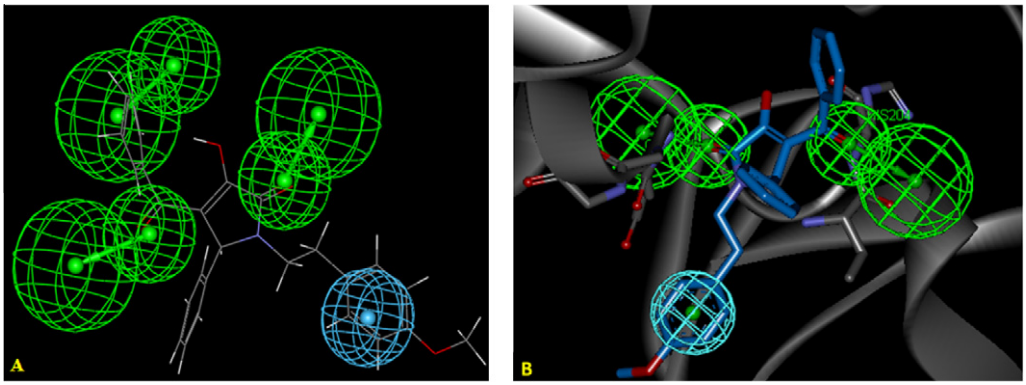



**Figure 3.** The mapping plot of the pharmacophore model and the plot of the docking model. (A) Mapping of common feature hypothesis 1 (Hypo1) onto compounds **21**, which explained HIV-1IN inhibitory activity of **21**. (B) Predicted binding orientation of compound **21** inside the HIV-1 IN active site.

**Table 2**
The antiviral effect of the 2-pyrrolinone derivatives

| Compound | Anti-HIV-1 activity | | SI[c] |
|---|---|---|---|
| | $EC_{50}$[a] (μM) | $CC_{50}$[b] (μM) | |
| **20** | N/A | 790 | |
| **21** | N/A | 830 | |
| **22** | 0.317 | 26.6 | 83.7 |
| **24** | N/A[d] | 459 | |

[a] Effective concentration required to protect TZM-bl cells against the stimulated luciferase enzymatic activity of HIV by 50%.
[b] Cytostatic concentration required to kill TZM-bl cells by 50%.
[c] Selectivity index (SI) is a ratio of $CC_{50}$ value/$EC_{50}$ value.
[d] N/A: no activity.

scaffolds. Firstly, the common feature of six clinical candidates was visualized by generating a pharmacophore model. Secondly, based on the application of resulting pharmacophore model, eight compounds with a common 2-pyrrolinones core were selected from 89 primary hits to be synthesized in a concise strategy. Their catalytic IN inhibitory activities were tested as well. The compounds **20**, **21**, and **22** exhibit strand transfer inhibitory activity with $IC_{50}$ values of 44, 45, and 40 μM, respectively. Furthermore, the best antiviral effect was exhibited by compound **22** with an $EC_{50}$ value of 0.317 μM. The mapping analysis and the docking study showed that the *p*-methoxylphenyl moiety was well docked in the vicinity of the aromatic pocket, forming hydrophobic interactions. The analysis is well supported by the biological activities. These results provide useful information for the design of new potent antiviral agents. Further structural optimization based on this pharmacophore model and the potent inhibitor structure is in progress.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.09.054.

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