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Development of the next generation of HIV-1 integrase inhibitors: Pyrazolone as a novel inhibitor scaffold

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ABSTRACT

HIV-1 integrase (IN), one of the essential enzymes in HIV infection, has been validated as a target for HIV treatment. While more than 20 drugs have been approved by the FDA to treat HIV/AIDS, only one drug, Raltegravir (**1**), was approved as an IN inhibitor. The rapid mutation of the virus, which leads to multidrug resistant HIV strains, presents an urgent need to find potent compounds that can serve as second-generation IN inhibitors. The pyrazolone scaffold, predicted by a computational modeling study using GS-9137(**2**) as a pharmacophoric model, has shown to inhibit the IN catalytic activities in low micromolar range. We have synthesized various analogs based on the pyrazolone scaffold and performed SAR studies. This paper will showcase the up-to-date result of this scaffold as a promising HIV-1 IN inhibitor.

Acquired immunodeficiency syndrome (AIDS) is a debilitating disease affecting more than 39 million people worldwide. Since the disease was first detected in 1981, more than 25 million people have died of AIDS.[1] Consequently, the World Health Organization recognizes HIV as the fourth largest cause of death globally.[1] As the most recent advancement toward eradication of this pandemic, a highly successful combination therapy of three HIV drugs known as HAART (highly active antiretroviral therapy) is considered as the best treatment against AIDS so far. However, increasing drug resistance due to rapid mutation of this retrovirus has prompted the faster development of many reserved drugs against resistant viral strains.

HIV-1 integrase (IN) has been validated to be a crucial target for shutting down the HIV reproductive cycle. This viral enzyme catalyzes the insertion of proviral DNA into the host genome in two-step processes: (1) 3′-processing step that removes two nucleotides from the 3′-hydroxyl end of its viral DNA and (2) strand transfer step, which joins 3′-end of the viral DNA onto the host DNA by nucleophilic addition. Additionally, the presences of divalent metals such as Mg^{2+} or Mn^{2+} are required for the catalysis of these two steps.[2]

Two compounds demonstrate effective inhibition of IN (Fig. 1). Presently the only FDA approved drug is Raltegravir (MK-0518, **1**), which selectively inhibits the strand transfer step and has shown an excellent efficacy in HIV-1 patients.[3] Another IN inhibitor, GS-9137 (Elvitegravir, **2**), a quinolone 3-carboxylic acid, is currently in late-stage clinical trials.[4] GS-9137 showed highly potent antiretroviral activity in both treatment-naive and experienced patients in the Phase II clinical trials. Like other IN inhibitors, resistant mutants against this drug have been identified in the catalytic core domain of the IN.[5]

Although the recent success in the discovery and the clinical development of IN inhibitors as novel antiretroviral agents provided us a new choice in the treatment of HIV/AIDS, multidrug resistant HIV-1 strains have emerged as a result of the dynamic nature of the HIV genome coupled with the requirement of a sustained antiretroviral treatment regimen in chronic HIV-1 patients. Once the first generation of IN inhibitors becomes commonly used in the clinic, the emergence of resistant HIV-1 virus strains containing mutations in the IN is inevitable. Therefore, alternative second generation agents with improved resistance profiles are urgently needed.

It has been our goal to design and discover novel and structurally diverse second-generation IN inhibitors that show favorable inhibition profiles against known resistant mutant strains. We quickly became interested in the work of Dayam et al.[6] who

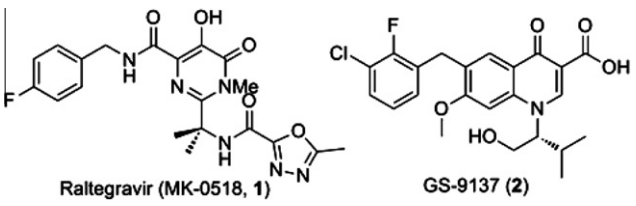

Figure 1. Examples of clinically used HIV-1 integrase inhibitors.

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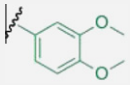
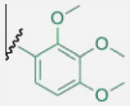
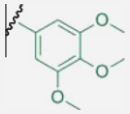
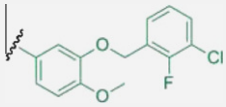
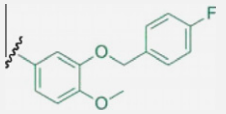
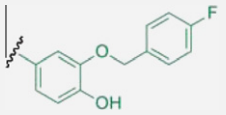
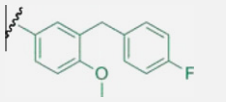
reported a series of potential lead compounds for IN inhibitor by using structure-based pharmacophore model derived from quinolone 3-carboxylic acids (such as in GS-9137). Among the compounds, the pyrazolone **3** is the most potent in inhibiting IN and its IC_{50} is in the low micromolar range.

The preliminary study of this pyrazolone scaffold prompted us to develop a more diversified library that includes phenyl-substituted pyrazolone compounds. This pyrazolone can be modified on three possible sites (R_1, R_2, and R_3) as shown in Figure 2. The R_1 modification involves the benzylidene ring and its benzyloxy substituent of compound **3**, which is believed to bind to the hydrophobic region in the active site of IN. The R_2 modification will change the functional group in the proximity of the pyrazolone's carbonyl group, which is crucial for metal chelation inside the enzyme active site. Lastly, R_3 modification could tune the electronic factors on the pyrazolone core ring.

For modification on the benzylidene ring of the pyrazolone, the following **4a**–**4g** analogs were synthesized as depicted in Scheme 1. This modification will determine the factors needed for increasing binding affinity to the metal ion inside the IN active site. They are as follows: the position of the methoxy group, the position of the benzyloxy group, and the linker space joining two aromatic rings. Starting with 3-bromoaniline (**7**), the corresponding hydrazine was synthesized quantitatively via a diazotiation reaction followed by tin (II) chloride reduction. The resulting hydrazine was reacted with trifluoroacetoacetate in boiling ethanol to yield the pyrazolone **8**, which was then reacted with various aromatic aldehydes (R_1CHO)[7a] in boiling water to yield the desired analogs bearing different benzylidene tethers.

These compounds were subjected to in vitro testing, and the results (Table 1) showed that compound **4g** was the most potent compound in this R_1 modified library with an IC_{50} of 11 ± 1 μM for inhibition on strand transfer activity.

Table 1
HIV-1 integrase inhibitory activity of compound **4** analogs

Compds	R_1	3′-Processing IC_{50} (μM)	Strand transfer IC_{50} (μM)
4a		89 ± 10	34 ± 2
4b		>100	>100
4c		61 ± 7	15 ± 3
4d		50 ± 8	30 ± 7
4e		83 ± 5	40 ± 9
4f		>100	22 ± 3
4g		21 ± 1	11 ± 1

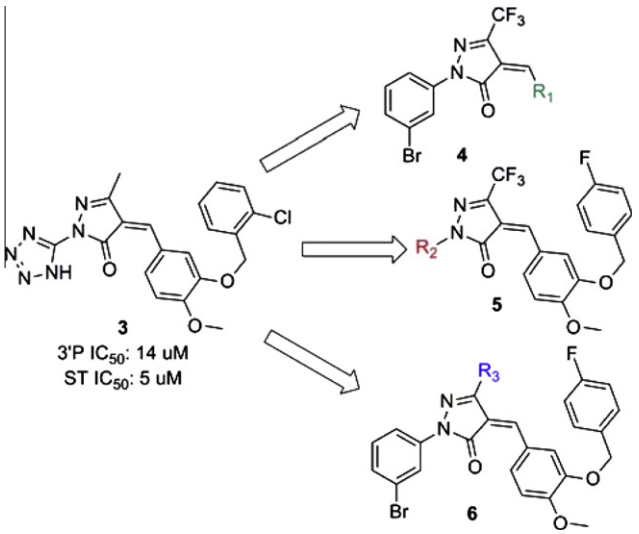
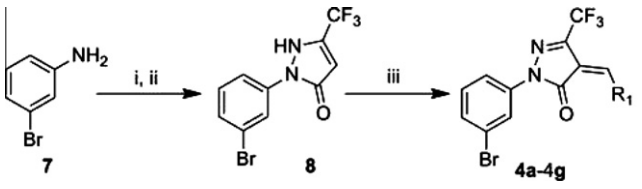


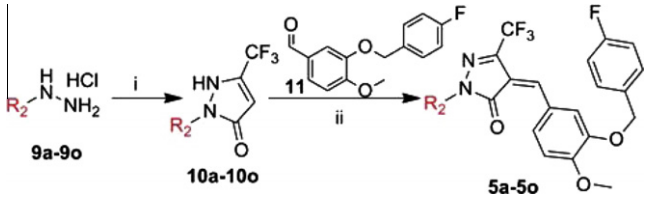
Figure 2. Three possible sites of modification in the pyrazolone scaffold.

Since the second type of alteration is crucial for the metal chelation ability of pyrazolone compounds, the choice of starting hydrazines should be important. Cyclization of various hydrazines **9a**–**9o** with ethyl trifluoroacetoacetate in boiling ethanol yielded the corresponding pyrazolone intermediates **10a**–**10o**, which were then treated with substituted benzaldehyde **11** to generate the R_2 modified pyrazolone analogs (Scheme 2). Unfortunately, several attempts to make pyrazolones with an *ortho*-halophenyl R_2 substituent (Cl and Br) were not successful due to decomposition of the intermediates when refluxing in boiling water. Also, the syntheses of pyrazolones with heterocyclic R_2 groups (such as pyridine, pyrimidine, and tetrazole) were not as straightforward as those of their phenyl-substituted pyrazolone counterparts. The last condensation step to synthesize these heterocyclic pyrazolones failed to generate the desired products even after prolonged reaction time.

The in vitro results of R_2 modified pyrazolones are summarized in Table 2. The presence of larger substituents (–Ph and –I) was more favored than the smaller substituents (–F and $–CF_3$). *Meta*



Scheme 1. Synthesis of R_1 modified pyrazolone compounds. Reagents and conditions: i) $NaNO_2$, HCl, 0 °C, then $SnCl_2.2H_2O$; ii) Ethyl trifluoroacetoacetate, EtOH, reflux; iii) R_1CHO, H_2O, reflux.



Scheme 2. Synthesis of R_2 modified pyrazolone compounds. Reagents and conditions: i) ethyl trifluroacetoacetate, EtOH, reflux; ii) 3-(4-fluorobenzyloxy)-4-methoxybenzaldehyde (**11**), H_2O, reflux.

Table 2
HIV-1 integrase inhibitory activity of compound **5** analogs

Compds	R_2	3′-Processing IC_{50} (μM)	Strand transfer IC_{50} (μM)
5a	Phenyl	>100	>100
5b	*m*-Tolyl	>100	>100
5c	*m*-Trifluoromethylphenyl	90 ± 13	24 ± 4
5d	*m*-Phenylphenyl	>100	16 ± 5
5e	*m*-Nitrophenyl	52 ± 13	19 ± 2
5f	*m*-Fluorophenyl	>100	60 ± 0.1
5g	*m*-Chlorophenyl	88 ± 24	30 ± 6
5h (=**4e**)	*m*-Bromophenyl	83 ± 5	40 ± 9
5i	*m*-Iodophenyl	>100	35 ± 3
5j	3,5-Dichlorophenyl	>100	12 ± 1
5k	*p*-Bromophenyl	82 ± 1	25 ± 6
5l	*p*-Iodophenyl	>100	69 ± 9
5m	*p*-Carboxylphenyl	>100	41 ± 12
5n	*p*-Sulfonyamidophenyl	>100	>100
5o	Benzothiazolyl	>100	27 ± 6

substitution was slightly more preferred to the *para* substitution. The best compound in this group was **5j**, which has IC_{50} value of 12 ± 1 μM for the strand transfer reaction.

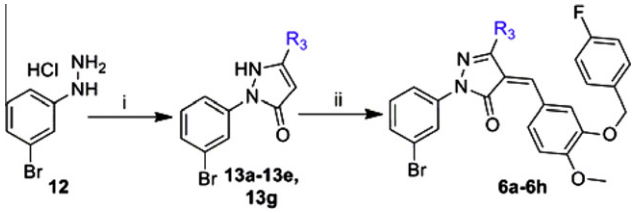
The role of the last modification is to investigate the effect of the 5-substituent of the pyrazolone ring on IN inhibition. Table 3 lists many analogs bearing different R_3 substituents on the ring. We predicted that variation of the electronic nature of the pyrazolone through electron donating (NHC(=O)CF_3), electron withdrawing (CF_3, CO_2H, CO_2Et), or weakly interacting substituents (Me and Et) may assist in determination of the electronic factor necessary for binding toward the metal inside the IN active site. In general, synthesis of this analog was accomplished by cyclization of **12** with a variety of ethyl acetate derivatives to generate intermediates **13**, followed by sequential condensation to form pyrazolones **6**. The compounds **6a**–**6c** which contain different aliphatic carbon R_3 groups (e.g., Me, Et, and CF_3) were synthesized from the reaction of *m*-bromophenyl hydrazine with methyl acetoacetate, methyl propionoacetate, and methyl trifluoroaceto-acetate, respectively, followed by subsequent condensation with **11**. For the synthesis of **6d**, **12** was condensed with ethyl cyanoacetate[7b], acylated with trifluoroacetic anhydride to generate **13d**, and then condensed further with **11** to give **6d**. The similar two-step protocol was carried out for the synthesis of **6e**–**6h** utilizing but-2-ynedioic acid diethyl ester and dimethylacetonedicarboxylate[7c] as depicted in Scheme 3.

The presence of electron donating or electron neutral group did not increase the inhibitory activity against the IN. However, the presence of electron withdrawing group such as CF_3 and CO_2H increased the inhibitory activity. Compound **6h** exhibited the most potent analog with ST IC_{50} of 19 ± 3 μM.

To conclude the optimization study, we combined the best features obtained from the prior optimization studies. The combination of these features for each modification effort yielded a second prototype compound **14a**, which after bioassay did not give

Table 3
HIV-1 integrase inhibitory activity of compound **6** analogs

Compds	R_3	3′-Processing IC_{50} (μM)	Strand transfer IC_{50} (μM)
6a	Methyl	>100	>100
6b	Ethyl	>100	>100
6c (=**5h**)	Trifluoromethyl	83 ± 5	40 ± 9
6d	Trifluoroacetamide	>100	>100
6e	Methyl homoacetate	>100	100
6f	Homoacetate	>100	57
6g	Ethyl carboxylate	>100	88 ± 11
6h	Carboxylate	55 ± 5	19 ± 3



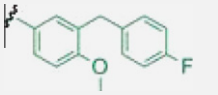
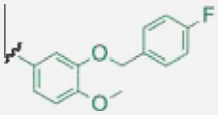
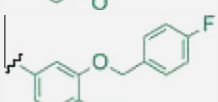
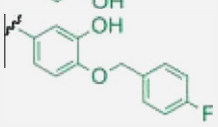
Scheme 3. Synthesis of R_3 modified pyrazolone compounds. Reagents and conditions: i) (for **12a**-**12c**), methyl acetoacetate, methyl propionacetate, or methyl trifluoroacetoacetate in boiling EtOH; (for **12d**), methyl cyanoacetoacetate in EtOH, then trifluoroacetic anhydride; (for **12e**), dimethylacetone-1,3-dicarboxylate in AcOH, 100 °C; (for **12g**), diethyl acetylenedicarboxylate, K_2CO_3 in EtOH; ii) 3-(4-fluorobenzyloxy)-4-methoxybenzaldehyde (**11**), H_2O, reflux; (for **4f** and **4h**) LiOH, MeOH/THF/H_2O.

a better inhibitory activity (ST IC_{50} of 11 μM). Puzzled with this result, we elongated the tether of the second benzyl group as in compound **14b**. Finally, switching the position of the fluorobenzyl group provided compound **14d** which has the lowest ST IC_{50} so far (3 ± 0.4 μM).

Utilizing Autodock Vina[8] to validate this experimental finding (Table 4), we began checking the lowest energy conformations of compounds **14a**–**14d**. Compound **14d** was the only compound with the anticipated conformation, where the carbonyl in the pyrazolone core was aligned closely to metal cofactor, so it would disrupt the IN activity toward cleaving DNA strand and/or transferring its viral DNA by sequestering the active site of IN (Fig. 3a). On the other hand, the rest of optimized compounds (**14a**–**14c**) interacted with metal ion through their carboxylate moieties. We postulated that the absence of the methyl on the benzylidene ring gave rise to extra stabilization possibly through hydrogen bonding donor interaction with His67, while the presence of the carboxylate functional group also serves as a hydrogen bond acceptor from Asn155 as shown in Figure 3b. The nitro substituent gave an extra stabilization through hydrogen bond acceptor with Gln148.

In conclusion, we have exploited SAR studies of pyrazolone scaffold for IN which was discovered through pharmacophore models. The compound **14d** exhibited single-digit micromolar activity against IN strand transfer process. This work is expected to provide helpful information for the discovery of novel IN inhibitor.

Table 4
HIV-1 integrase inhibitory activity of second round optimized compounds

Compds	R_1	3′-Processing IC_{50} (μM)	Strand transfer IC_{50} (μM)
14a		15	11
14b		60 ± 14	14 ± 4
14c		95 ± 7	10 ± 1
14d		21 ± 8	3 ± 0.4

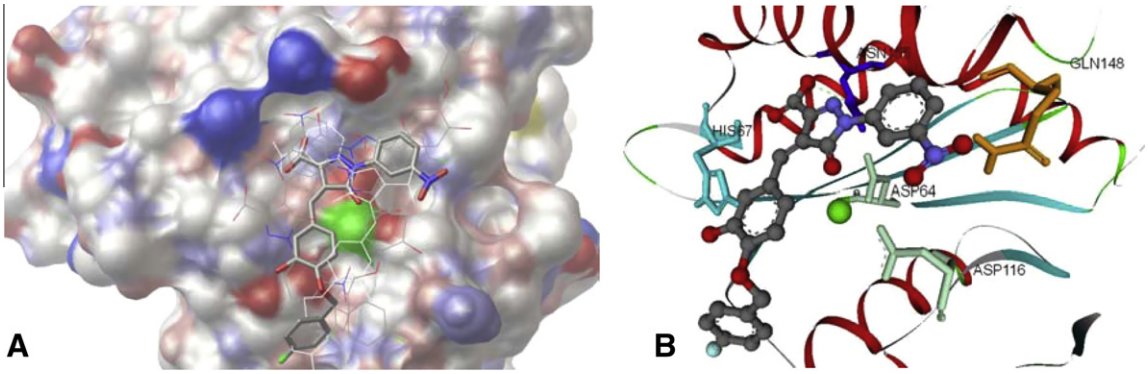


Figure 3. (A) Predicted binding conformations of compound **14d** inside the HIV-1 integrase active site (modified PDB1QS4). The lowest energy conformation is represented as a solid stick representation, while the rests are the other possible conformation. The docking analyses were performed around the green sphere which represents the metal active site. (B) Predicted protein–ligand interaction between compound **14d** and HIV-integrase. Three residues (H67, N155, and Q148) show plausible interactions with this docked compound.

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