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Bioorganic & Medicinal Chemistry Letters 17 (2007) 6511–6515
Bioorganic & Medicinal Chemistry Letters

8-Hydroxy-3,4-dihydropyrrolo[1,2-*a*]pyrazine-1(2*H*)-one HIV-1 integrase inhibitors

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Received 10 September 2007; accepted 25 September 2007
Available online 29 September 2007

Abstract—A series of potent novel 8-hydroxy-3,4-dihydropyrrolo[1,2-*a*]pyrazine-1(2*H*)-one HIV-1 integrase inhibitors was identified. These compounds inhibited the strand transfer process of HIV-1 integrase and viral replication in cells. Compound **12** is active against replication of HIV-1 in cell culture with a CIC_{95} of 0.31 μM. Further SAR exploration led to the preparation of pseudosymmetrical tricyclic pyrrolopyrazine inhibitors **23** and **24** with further improvement in antiviral activity.

Human immunodeficiency virus-type 1 (HIV-1) is the etiological agent of acquired immunodeficiency syndrome (AIDS). The unique nature of the replicative cycle of HIV-1 provides many potential targets for chemotherapeutic intervention. One of these, the viral enzyme integrase, catalyzes the insertion of the proviral DNA into the genome of host cells. Integration is a multistep process which includes three different biochemical steps: the assembly of proviral DNA on integrase, endonucleolytic processing of the proviral DNA, and strand transfer of the proviral DNA to host cell DNA.[1]

We have previously reported from our laboratories that 1,3-diketoacids **1** are potent integrase inhibitors and prevent HIV-1 replication in cell culture.[2] In addition, we have shown that the diketoacid pharmacophore is effectively replaced with a novel series of napthyridines **2**,[3] and were found to be efficacious in rhesus macaques infected with the simian-human immunodeficiency virus (SHIV) 89.6P.[4] Recently, we described the discovery of dihydroxypyridopyrazine-1,6-diones **3** as an alternative scaffold for the diketoacid pharmacophore.[5a] In this communication we describe the discovery, structure–activity relationships (SAR),and synthesis of a series of novel 8-hydroxy-3,4-dihydropyrrolo[1,2-*a*]pyrazine-1(2*H*)-one HIV-1 integrase inhibitors.

Contraction of the six membered pyridinone ring in dihydroxypyridopyrazine-1,6-dione **3** to a five membered pyrrole ring led to a 8-hydroxy-3,4-dihydropyrrolo[1,2-*a*]pyrazine-1(2*H*)-one system **4**. The addition of an exocyclic carboxyl group, indicated in the overlay in Figure 1, provided a new template that mimics the diketoacid pharmacophore. Although electron-rich 3-hydroxypyrroles were known to be unstable towards air oxidation, they are rendered air stable with the addition of electron withdrawing groups such as carboxylic esters.[6] These structural requirements for chemical stability coincide with those required for activities against HIV-1 integrase. No precedent for the preparation of these structures is evident in a survey of the literature.

The effect of varying ring size was first investigated and found to be consistent with those observed in the dihydroxypyridopyrazine-1,6-dione integrase inhibitors.[5a] Analog **5**, with a 7-membered ring constraint, is significantly less active against HIV integrase than the

Keywords: HIV; Integrase inhibitor; Antiviral.
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doi:10.1016/j.bmcl.2007.09.086

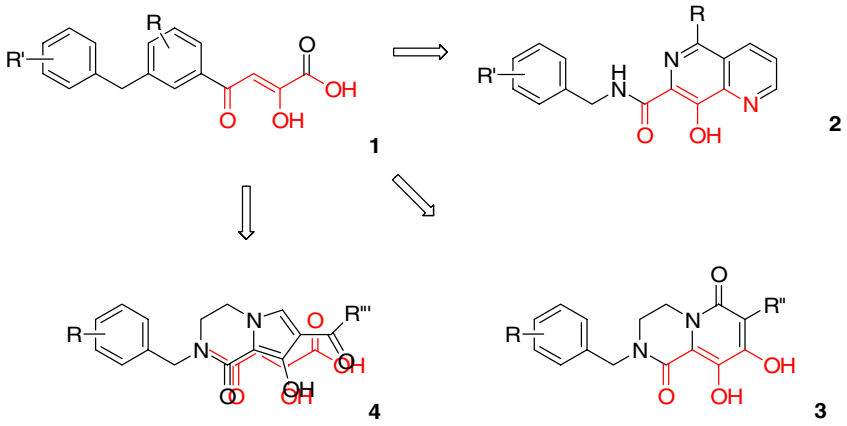
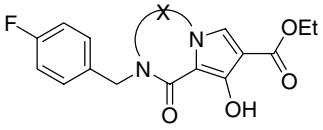


Figure 1. Evolution of hydroxy-3,4-dihydropyrrolo[1,2-*a*]pyrazine-1(2*H*)-one inhibitors.

6-membered ring compound**6** (Table 1). Molecular modeling suggested that only the latter compound **6** presents the key pharmacophore in a coplanar manner.[5a] It was also anticipated that the cyclic constraint would bias the orientation of the benzyl side chain through steric interaction in a manner similar to those observed for **3**.[5a]

Halogen substitution on the benzyl group showed significant potency effects on the inhibitory activity against HIV-1 integrase and viral replication in cell culture. Compound **6** inhibits 95% of the replication of HIV-1 in cell culture at 3.23 μM (Table 2). When the 4-fluoro substituent is replaced by a 4-chloro group, intrinsic integrase inhibition potency is maintained and the antiviral activity improves by 3 fold (compound **8** versus **6**). While migration of the fluoro substituent from the 4- to the 3-position results in a slight loss in antiviral potency (compare compounds **6** and **9**), the 4-chloro and 3-chloro analogs are equipotent with CIC_{95} values ~ 1 μM (compounds **8** and **10**). All of these mono-halogenated analogs are significantly more potent than the des- halo inhibitor **7**. Combination of chloro and fluoro substituents at both the 3- and 4-positions leads to further improvements in potency in the cell based assay with sub-micromolar CIC_{95} (compounds **11** and **12**). These compounds do not exhibit cytotoxicity in cell culture at concentrations up to 20 μM.

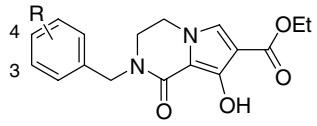
Table 1. Effect of different constraints



Compound	X	Inhibition of strand transfer IC_{50}[a] (μM)
5	$CH_2CH_2CH_2$	>5.00
6	CH_2CH_2	0.04 (±0.02)

[a] Assays were performed with recombinant HIV-integrase (0.1 μM) preassembled on immobilized oligonucleotides.[12] Values are means of three experiments, standard deviation is given in parentheses.

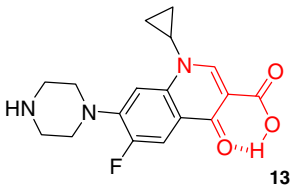
Table 2. Effect of substitutions on benzyl group



Compound	R	Inhibition of strand transfer IC_{50}[a] (μM)	Antiviral activity in cell culture, CIC_{95}[b] (μM)
6	4-F	0.04 (±0.02)	3.23 (±1.72)
7	H	0.22 (±0.03)	20.00 (±5.55)
8	4-Cl	0.04 (±0.02)	1.02 (±0.33)
9	3-F	0.11 (±0.03)	9.38 (±4.42)
10	3-Cl	0.03 (±0.02)	1.25 (±0.40)
11	4-F, 3-Cl	0.02 (±0.01)	0.94 (±0.40)
12	3,4-di-Cl	0.01 (±0.01)	0.31 (±0.10)

[a] See footnotes under Table 1.

[b] Cell culture inhibitory concentrations (CIC_{95}) are defined as those which inhibited by >95% the spread of HIV-1 infection in MT-4 human T-lymphoid cells maintained in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum.[13] Cytotoxicity is not observed in cell culture at concentrations up to 20 μM.



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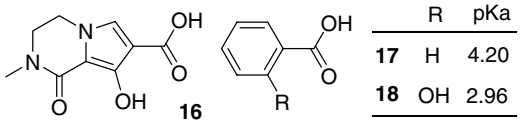
The pharmacokinetic profile of compound **12** was examined in rat. When dosed intravenously as a solution in DMSO (2 mg/Kg), a relatively high clearance of 30 ± 6 mL/min/Kg and a short half life of 0.5 h were observed. The ethyl ester was found to be rapidly hydrolyzed in vivo. It was surmised that, unlike other carboxylic acids which are ionized at physiological pH and are apparently cell impermeable, the corresponding acids from these esters would have pK_a values similar to that of the quinoline antibiotic ciprofloxacin **13** (pK_a 6.0).[7] This relatively high pK_a is attributed to the electron donating effect of the aromatic nitrogen to the carbonyl carbon and the hydrogen bonding interaction of

the acidic proton with the neighboring hydroxy group in both systems. In silico pK_a estimation using ACD Labs software suggests that these pyrrolocarboxylic acids should have pK_a values *ca.* 5.9.[8] This result was encouraging in that, similar to ciprofloxacin **13**, these pyrrolocarboxylic acid inhibitors might be cell permeable.

Analogs **7** and **9** (Table 2) were hydrolyzed to the corresponding acids **14** and **15** (Table 3). They were found to be significantly more active against HIV integrase in the enzyme assay than their ester precursors. However, this did not translate into improvements in antiviral activity. In fact, no antiviral activities were observed with these inhibitors up to 20 μM.



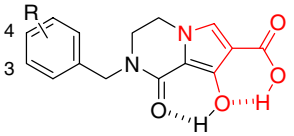
16

	R	pKa
17	H	4.20
18	OH	2.96

Compound **16**, a water soluble *N*-methylated analog of these carboxylic acids, was determined to have a pK_a value of 3.76.[9] Examination of pK_a values reported for benzoic acid (**17**) and *o*-salicyclic acid (**18**) indicates that an *ortho*-hydroxy substitution in benzoic acid greatly enhances its acidity.[10] The *ortho*-hydroxy substituent seems to have a more dominant effect in influencing the acidity observed with our hydroxy-pyrrolocarboxylic acids. The algorithm employed by the ACD Lab pK_a prediction software appears to have underestimated the effect of this substitution. At physiological *p*H, it is very likely that inhibitors **14** and **15**, with pK_a's of approximately 3.8 (based on compound **16**) exist primarily as the negatively charged carboxylate. This would likely compromise their ability to penetrate cells.

It was reasoned that the more metabolically stable and cell permeable amide analogs might resolve the liabilities encountered with the ester and acid inhibitors. Furthermore, the amide inhibitors, with higher electron density at the exocyclic carbonyl oxygen, were expected to be more potent than the ester inhibitors. However, the methyl amide inhibitors **19**, **20,** & **21** (Table 4) were found to be slightly less active than the corresponding esters (Table 2, compounds **6**, **8**, & **12**) in both the enzyme and HIV replication inhibition assays. Molecular modeling (PM3 semi-empirical calculation) suggests that there may be a bias for orientation of the exocyclic

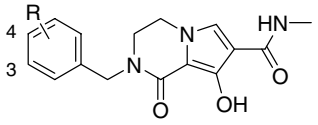
Table 3. Integrase and HIV replication inhibition activities of acids



Compound	R	Inhibition of strand transfer IC_{50}[a] (μM)	Antiviral activity in cell culture, CIC_{95}[a] (μM)
14	H	0.08 (±0.01)	>20.00
15	3-Cl	0.01 (±0.01)	>20.00

[a] See footnotes under Table 2.

Table 4. Integrase and HIV replication inhibition activities of amides



Compound	R	Inhibition of strand transfer IC_{50}[a] (μM)	Antiviral activity in cell culture, CIC_{95}[a] (μM)
19	4-F	0.08 (±0.02)	10.00 (±2.11)
20	4-Cl	0.06 (±0.02)	2.50 (±0.55)
21	3,4-di-Cl	0.04 (±0.01)	1.25 (±0.20)

[a] See footnotes under Table 2.

carboxamide at 180 degrees from the desired conformation for binding (Fig. 2),[11] which would negatively impact the inhibitory activities of compounds **19**–**21** against HIV-1 integrase.

To circumvent this unfavorable conformational bias, an additional cyclic constraint was introduced between the central pyrrole ring and the exocyclic carboxamide group. The pseudosymmetry of the scaffold permits the pharmacophore to be examined in both a forward and reverse spatial configuration or mode (Table 5). Analogs were prepared in both modes and tricyclic inhibitors **22** and **23** exhibited enzyme inhibition below the lower limit of the assay (0.01 μM) and enhancement in their antiviral activities. Furthermore, preparation of the highly pseudo-symmetrical bis-4-fluorobenzyl inhibitor **24** led to even greater activity against HIV replication in cell culture.

The pseudosymmetrical nature of compounds **22**, **23,** and **24**, discussed above, showcases the characteristic 1,3-diketoacid pharmacophore of HIV-1 integrase

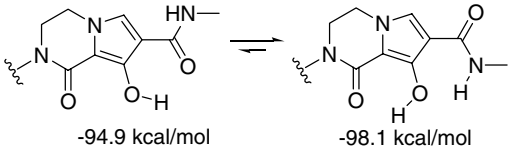
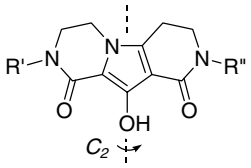


Figure 2. Conformational bias of pyrrolopyrazine carboxamide.

Table 5. Integrase and HIV replication inhibition activities

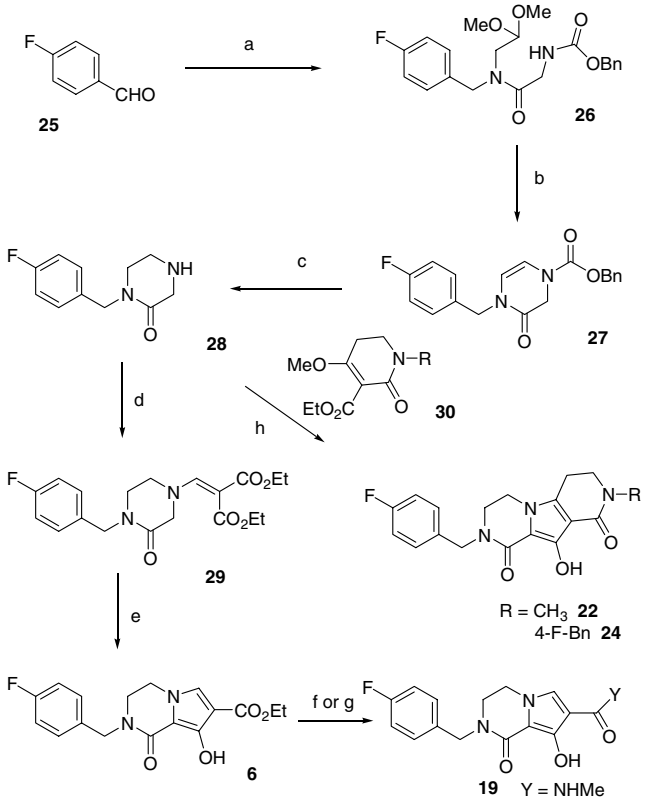


Compound	R′	R″	Inhibition of strand transfer IC_{50}[a] (μM)	Antiviral activity in cell culture, CIC_{95}[a] (μM)
22	4-F-Bn	CH_3	<0.01	0.39 (±0.12)
23	CH_3	4-F-Bn	<0.01	0.16 (±0.11)
24	4-F-Bn	4-F-Bn	<0.01	0.07 (±0.02)

[a] See footnotes under Table 2.

inhibitors. Together with the observation that both 4-fluorobenzyl groups on **24** contributes to binding, it is very likely that the mono-benzyl inhibitors (compounds **6**–**23**) may bind to HIV integrase in more than one mode. This observation represents the first structural evidence for the hypothesis presented in a recent communication from our laboratories that there is potentially more than one mode of binding for HIV-1 integrase inhibitors.[3b] It is our contention that these analogs provide a unique scaffold to be optimized for binding in multiple modes. Potentially if one binding mode is rendered less effective by mutation of the integrase enzyme, the inhibitors may be able to maintain inhibitory activity against replication of the mutant viruses by binding in a different mode. Further progress towards optimization of this series of tricyclic hydroxypyrrole integrase inhibitors with a higher genetic barrier to mutation has been recently reported.[5b]

The synthesis of compound **6** is depicted in Scheme 1. Reductive alkylation of dimethoxyethylamine with 4-fluorobenzaldehyde **25** in the presence of sodium borohydride provided the corresponding benzylamine, which was treated with N-CBz-glycine under a standard coupling protocol (EDC and HOBt in DMF) to provide the amide **26**. Acid catalyzed cyclization[14] of **26**, followed by a one pot stepwise saturation of the resultant product **27** in the presence of 5% Pt on charcoal and cleavage of the CBz group with 5% Pd on charcoal provided the piperazinone **28**. Compound **28** and diethyl ethoxymethylenemalonate were heated in toluene at 80 °C overnight to provide the adduct **29** and its subsequent addition to a refluxing solution of lithium hexamethyldisilylamide in THF afforded the cyclization product **6**. Ester **6** was then hydrolyzed to the corresponding acid with aqueous sodium hydroxide in ethanol in a sealed tube at 100 °C overnight. Alternatively, treatment of the ester **6** with a suspension of $AlCl_3$ in anhydrous chloroform purged with methylamine gas at 70 °C overnight provided the corresponding methyl amide **19**.



Scheme 1. Synthesis of Compounds **6**, **19**, **22**, and **24**. Reagents and conditions: (a) i—$H_2NCH_2CH(OMe)_2$, $NaBH_4$, MeOH, rt (82%); ii—N-Cbz-Gly, EDC, HOBt, *i*-Pr_2NEt, DMF, rt, overnight (95%); (b) TsOH, toluene, 80 °C, 5 days (62%); (c) H_2, 10% Pt/C, EtOH; H_2, 20% $Pd(OH)_2$/C, EtOH (95%); (d) diethyl ethoxymethylenemalonate, toluene, 100 °C, 4 h; (e) LiHMDS, THF, 80 °C (78%); (f) for Y = OH, NaOH, EtOH, 100 °C, overnight; (g) $AlCl_3$, $MeNH_2$, $CHCl_3$, 70 °C (85%); (h) Ethylene glycol, microwave at 250 °C for 20 min (25%).

Tricyclic inhibitors such as **22** and **24** were prepared by heating a mixture of the piperazinone **28** and an appropriate lactam **30**[15] in ethylene glycol or 1,2-dichlorobenzene in a sealed tube at 250 °C in a microwave reactor for 20 minutes. The crude product mixture was purified by C-18 reverse phase HPLC eluting with a water-acetonitrile gradient.

In summary, a series of potent 8-hydroxy-3,4-dihydropyrrolo[1,2-*a*]pyrazine-1(2*H*)-one HIV-1 integrase inhibitors which inhibit replication of HIV-1 in cell culture has been established. Further exploration revealed the pseudosymmetrical nature of the integrase inhibitor pharmacophore. Efforts are ongoing to identify the potential of the tricyclic pyrrolopyrazine template and analogous bicyclic systems.

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