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Dihydroxypyridopyrazine-1,6-dione HIV-1 integrase inhibitors

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Abstract—A series of potent novel dihydroxypyridopyrazine-1,6-dione HIV-1 integrase inhibitors was identified. These compounds inhibited the strand transfer process of HIV-1 integrase and viral replication in cells. Compound **6** is active against replication of HIV with a CIC_{95} of 0.31 μM and exhibits no shift in potency in the presence of 50% normal human serum. It displays a good pharmacokinetic profile when dosed in rats and no covalent binding with microsomal proteins in both in vitro and in vivo models.

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: assembly of the proviral DNA on integrase, endonucleolytic processing of the proviral DNA, and strand transfer of the proviral DNA to host cell DNA.[1]

1,3-Diketoacids **1** were reported to be effective integrase inhibitors and prevent HIV-1 replication in cell culture.[2] Recently, novel naphthyridines **2** and **3** were identified to be suitable replacements for the diketoacid pharmacophore.[3] Furthermore, naphthyridine carboxamide **3** was found to be efficacious in rhesus macaques infected with the simian-human immunodeficiency virus (SHIV) 89.6P.[4] In this communication, we describe the discovery, SAR, computer modeling, pharmacokinetic profile, and synthesis of a novel series of dihydroxypyridopyrazine-1,6-diones HIV-1 integrase inhibitors.

Incorporation of the dihydroxycarbonyl pharmacophore (in red) into a pyridinone scaffold led to the dihydroxypyridinone[5] moiety on the right-hand portion of the bicyclic system **4**. Addition of the benzyl amide functionality (in blue) and imposing restrictions on the rotation of the amide side chain via a cyclic constraint (in magenta) led to the novel dihydroxypyridinone-carboxylic derivatives depicted as **4**. Literature searches revealed no precedent for the preparation of such structure (Fig. 1).

Among the three different ring constrained compounds prepared, compounds **6** and **7** were found to be significantly more active against HIV integrase than compound **5** (Table 1). Molecular modeling (MMFF)[7] of the bicyclic core suggests that the amide carbonyl group in the constrained seven-membered ring compound **5** is not coplanar relative to the remaining pharmacophore (Fig. 2), while in both 6-membered ring constrained analogs **6** and **7**, the amide groups are coplanar with respect to the dihydroxypyridinone core.

Keywords: HIV; Integrase; Strand transfer inhibitors; Dihydroxypyridopyrazine-1, 6-dione.
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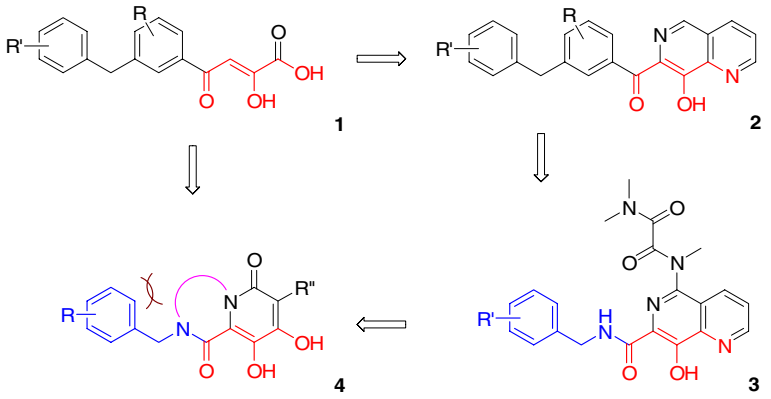
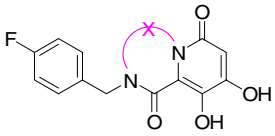


Figure 1. Conception of dihydroxypyridopyrazine-1,6-diones inhibitors.

Table 1. Effect of different constraints



Compound	X	Inhibition of strand transfer IC_{50}[a] (μM)
5	$CH_2CH_2CH_2$	1.04 (±0.05)
6	CH_2CH_2	0.10 (±0.06)
7	CH=CH	0.04 (±0.02)

[a] Assays were performed with recombinant HIV-integrase (0.1 μM) preassembled on immobilized oligonucleotides.[6] Values are means of three experiments, standard deviation is given in parentheses.

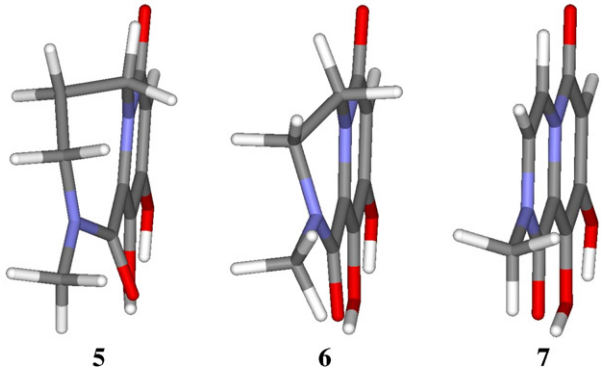


Figure 2. Molecular modeling of bicyclic core of inhibitors **5**–**7**.

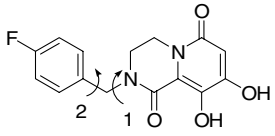


Figure 3. Torsional search on compound **6**.

It was anticipated that the cyclic constraint would also bias the orientation of the benzyl side chain through steric interaction. Torsional search on compound **6** (varying the two bonds identified as 1 and 2 in Fig. 3) revealed four low energy regions. Two of the minima correspond to rotations about the 4-fluorophenyl ring

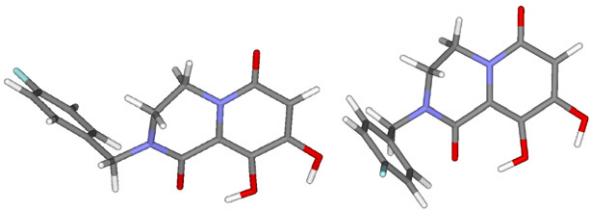
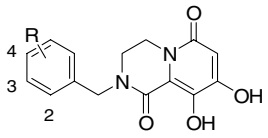


Figure 4. Two low energy conformers of compound **6**.

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	0.10 (±0.06)	0.31 (±0.08)
8	H	0.23 (±0.06)	2.50 (±0.12)
9	2-Cl	0.37 (±0.10)	>1.00
10	3-Cl	0.04 (±0.01)	0.50 (±0.11)
11	4-Cl	0.38 (±0.07)	1.00 (±0.18)
12	4-F, 3-Cl	0.04 (±0.02)	0.25 (±0.03)

[a] Assays were performed with recombinant HIV-integrase (0.1 μM) preassembled on immobilized oligonucleotides.[6] Values are means of three experiments, standard deviation is given in parentheses.

[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.[8] Cytotoxicity is not observed in cell culture at concentrations up to 20 μM.

(bond 2). The two lower energy conformers differ only by rotation about bond 1 and are depicted in Figure. 4. This suggests that the bicyclic dihydroxypyrido-pyrazine-1,6-dione significantly limited conformation flexibility of the 4-fluorobenzyl side chain in compound **6**.

Compound **6** inhibits 95% of the replication of HIV-1 in cell culture at 0.31 μM (Table 2). There is no shift in potency when the compound is assayed in the presence of 50% normal human serum.[8] Removal of the 4-fluoro substitution in compound **6** leads to a significant drop in

potency (cf. compound **8**). A chloro substitution walk around the benzyl group shows that a 3-chloro substituent improves integrase inhibition relative to the 2- and 4-position isomers (compounds **10** vs **9** and **11**). However, combining both the 4-fluoro and 3-chloro substituents only leads to a modest improvement in inhibitory potency in the cell based assay (compound **12**). These compounds do not exhibit cytotoxicity in cell culture at concentrations up to 20 μM.

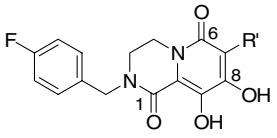
In our attempt to attenuate the acidity of the 8-hydroxyl group to mimic diketoacid **1**, electron-withdrawing substituents were incorporated adjacent to the 8-hydroxyl group on the bicyclic system. This led to a significant improvement in their intrinsic potency (Table 3, compounds **13**, **14**, **15** vs **6**). However, this did not translate into improvements in antiviral activity. One possible explanation is that the equilibrium between neutral and ionized inhibitor has shifted to an extent which adversely affects the cell permeability of these analogs.

The bioavailability of compound **6** was 69% when dosed orally in rats at 10 mg/Kg as a solution in 1% aq methylcellulose (Chart 1). Plasma concentrations of compound **6** were maintained between 0.64 and 0.50 μM from the second to the twenty-fourth hour. This is well above the concentration (0.31 μM) required for >95% inhibition of HIV-1 replication in cell culture.

There was a general concern about the biological liability of a dihydroxypyridinone core and its potential metabolites. ^{14}C Labeled compound **6** was prepared[9] to assess whether this series of compounds would irreversibly interact with liver microsomal proteins. Tables 4 and 5 depict the findings. There is only a low level of covalent interaction for radiolabeled **6** with liver microsomes derived from rat and human, well within the <50 pmol equiv/mg/60 min criteria (Table 4).[10] Furthermore, there is no difference in the results obtained with or without addition of NADPH. Results obtained in vivo in rat are also consistent with the observation made in vitro (Table 5).

The synthesis of compound **6** is depicted in Scheme 1. Reductive alkylation of dimethoxyethylamine with 4-fluorobenzaldehyde **16** in the presence of sodium borohydride provided the corresponding benzylamine, which was treated with a mixture of *N*-acetylglycine, EDC, and HOBt in DMF to provide the bis amide **17**. Acid catalyzed cyclization[11] of **17**, followed by hydrogenation of the resultant product **18** in the presence of 5% Pd/C in ethanol, provided the acetyl piperazinone **19**. Treatment of **19** in anhydrous DMF with LiHMDS, followed by addition of diethyl oxalate, led to three oxalation products, one major and two minor ones, as indicated by LC-MS analysis. Upon addition of more base, product **6** was generated from one of the minor oxalation products. The other two resisted cyclization and decomposed upon further exposure to base. The crude product mixture was purified by C-18 reverse phase HPLC eluting with a water–acetonitrile gradient.

Table 3. Effect of substitutions on pyridopyrazine-1,6-dione core



Compound	R′	Inhibition of strand transfer IC_{50}[a] (μM)	Antiviral activity in cell culture, CIC_{95}[b] (μM)
6	H	0.10 (±0.06)	0.31 (±0.08)
13	CN	0.02 (±0.06)	>1.00
14	Br	0.03 (±0.01)	1.00 (±0.16)
15	I	0.02 (±0.02)	>1.00

[a] Assays were performed with recombinant HIV-integrase (0.1 μM) preassembled on immobilized oligonucleotides.[6] Values are means of three experiments, standard deviation is given in parentheses.

[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.[8] Cytotoxicity is not observed in cell culture at concentrations up to 20 μM.

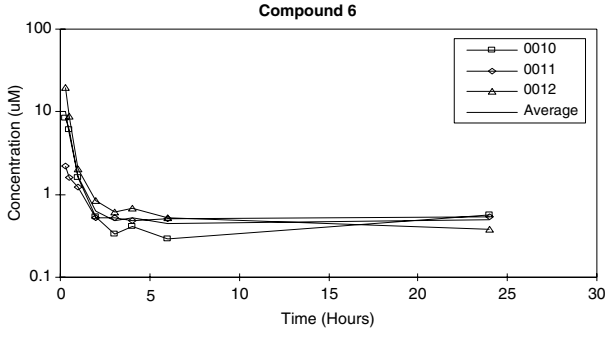
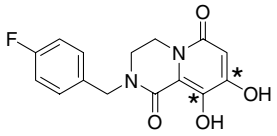


Chart 1. Oral pharmacokinetic profile of compound **6** in rat.

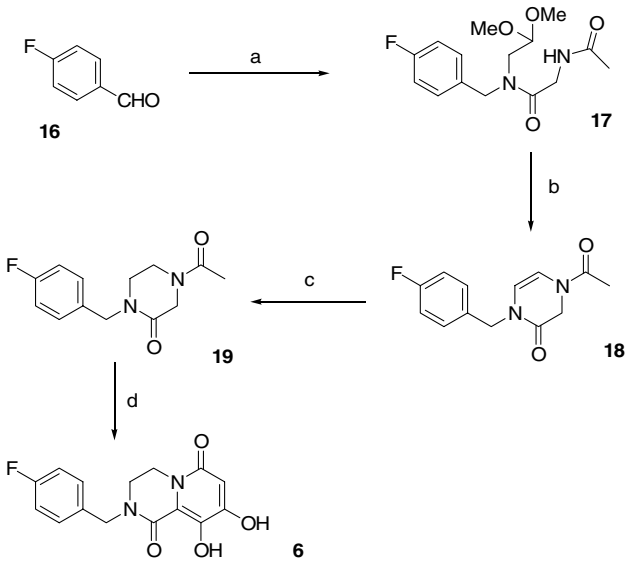
Table 4. In vitro covalent binding of [^{14}C] compound **6** with liver microsomes



Species	Irreversibly bound radioactivity (pmol equiv/mg/60 min)	
	−NADPH	+NADPH
Rat	24.7	24.2
Human	15.2	19.6

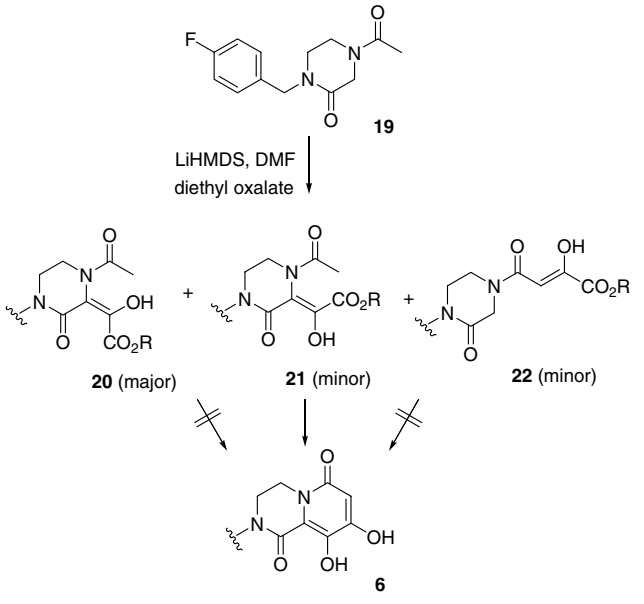
Table 5. In vivo covalent binding of [14C] Compound **6** in rats

Time (h)	Irreversibly bound radioactivity (pmol equiv/mg protein)	
	Plasma	Liver
2	<5	<5
6	<5	<5
24	<5	<5



Scheme 1. Synthesis of compound **6**. Reagents: (a) i—$H_2NCH_2CH(OMe)_2$, $NaBH_4$, MeOH (82%); ii—*N*-Acetyl-Gly, EDC, HOBt, *i*-Pr_2NEt, DMF (95%); (b) MsOH, CH_2Cl_2 (62%); (c) H_2, 5% Pd/C, EtOH (95%); (d) i—LiHMDS, diethyl oxalate, DMF; ii—excess LiHMDS (15–20%).

NMR studies of the purified oxalation products led to the identification of the major component as **20**, and one of the minor oxalation products as **22**. Treatment of each one of them independently with LiHMDS in DMF did not lead to cyclization product **6**. The third oxalation product readily cyclized to compound **6** upon workup. However, **20** was readily converted to the third oxalation product when exposed to a mixture of acetonitrile, water, and trifluoroacetic acid. NMR data of this material are consistent with those of structure **21**. Treatment of **21** in DMF with LiHMDS led to the cyclization product **6**.



In summary, a series of potent dihydroxypyrido-pyrazine-1,6-dione HIV-1 integrase inhibitors which inhibited replication of HIV-1 in cell culture has been identified. The lead compound **6** inhibits replication of HIV-1 in cell culture at $CIC_{95} = 0.31$ μM, and exhibits no significant shift in potency when assayed with addition of 50% normal human serum. No cytotoxicity is observed in cell culture at concentrations up to 20 μM. When compound **6** was dosed orally in rat at 10 mg/kg as a solution in 1% methylcellulose, it displayed good pharmacokinetic profile and maintained excellent exposure through 24 h of the study. No covalent binding of compound **6** with microsomal proteins was observed in both vitro and in vivo models. Further exploration of this dihydroxypyridopyrazine-1,6-dione template and analogous bicyclic systems is in progress.

References and notes

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