Letters

Design and Synthesis of 8-Hydroxy-[1,6]Naphthyridines as Novel Inhibitors of HIV-1 Integrase in Vitro and in Infected Cells

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Abstract: Naphthyridine **7** inhibits the strand transfer of the integration process catalyzed by integrase with an IC_{50} of 10 nM and inhibits 95% of the spread of HIV-1 infection in cell culture at 0.39 μM. It does not exhibit cytotoxicity in cell culture at $\leq$12.5 μM and shows a good pharmacokinetic profile when dosed orally to rats. The antiviral activity of **7** and its effect on integration were confirmed using viruses with specific integrase mutations.

Introduction. Human immunodeficiency virus-type 1 (HIV-1) is the etiological agent of acquired immune deficiency syndrome (AIDS). Current antiretroviral therapies target two viral enzymes, HIV-1 reverse transcriptase and HIV protease, to interrupt the viral replicative cycle.[1,2] The HIV-1 genome encodes a third enzyme, HIV integrase, a target yet to be exploited for antiviral therapy.[3] Integrase catalyzes the integration of double-stranded viral DNA into the host cell's genomic DNA. Integration consists of three biochemical steps: the assembly of integrase on viral DNA, endonucleolytic cleavage of the first two nucleotides from each 3′ terminal of the viral DNA, and strand transfer of the recessed viral DNA to the host cell DNA. Recently, diketoacids such as **1** (Figure 1)[4] were reported by our laboratory to be selective inhibitors of the strand transfer reaction. Although these compounds effectively prevent viral DNA integration and inhibit HIV-1 replication in cell culture,[4a] the presence of the biologically labile 1,3-diketoacid moiety in the molecules is a concern for development of these compounds as chemotherapeutic agents. The 1,3-diketoacid functionality is essential for the enzyme inhibitory activity of these inhibitors;[4b] therefore, efforts were directed toward seeking viable replacements for this critical pharmacophore. In this paper, we describe the design and syntheses of a set of analogues that led to the discovery of 8-hydroxy-[1,6]-naphthyridine as a suitable replacement for the 1,3-diketoacid motif.

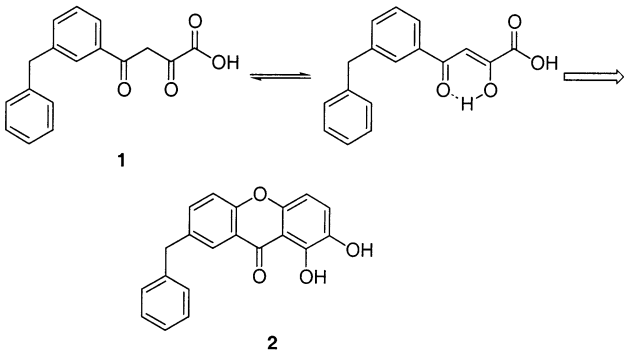

Figure 1. Structures of diketoacid **1** and catechol **2**.

Results and Discussion. On the basis of the SAR from the 1,3-diketoacid HIV integrase inhibitors,[4a] the following assumptions for their biologically active conformation were made. The 1,3-diketoacid moiety enolizes at the α-position, and the resultant conjugated *Z*-4-oxo-2-hydroxy-2-butenoic acid side chain is coplanar with the central benzene ring (Figure 1). It was hypothesized that a similar arrangement of key hydroxyl groups could be achieved by a 1,2-catechol and by incorporating an oxygen bridge to maintain coplanarity of the two phenyl rings (see **2**, Figure 1).[5] Compound **2** inhibits the strand transfer step of the HIV-1 integration process with an IC_{50} value of 0.6 μM (Table 1).[6] This result establishes that the α-enol and carboxylic acid moiety of the diketoacid side chain can be replaced with a 1,2-catechol and provides support for the proposed active conformation of the 4-aryl-1,3-diketoacids.

For further modification of **2**, three factors were taken into consideration (Figure 2). First, 1,2-catechols are biologically labile,[7] so it was necessary to replace one of the hydroxyl groups in **2** with other suitable functionality while maintaining activity against integrase. On the basis of our earlier SAR,[8a] the 2-hydroxyl group in **2** was replaced with a heteroaromatic nitrogen (Figure 2). This aromatic nitrogen is a Lewis base equivalent of the corresponding carboxylate oxygen in diketoacid **1**. Second, coplanar polyaromatic ring systems in general exhibit poor physical properties as therapeutic agents. As such, the oxygen bridge in **2** was removed. Third, to allow for adoption of coplanar conformations between the central benzene ring and the heteroarylpyridine, a heteroatom was incorporated at an appropriate position of the heteroaryl ring. This avoids the unfavorable conformational bias presented in their carbon counterparts (see **5**, Figure 2).[5] These

* To whom correspondence should be addressed. Address: Department of Medicinal Chemistry, WP14-3, Merck Research Laboratories, P.O. Box 4, West Point, PA 19486. Phone: 215-652-3584. Fax: 215-652-3971. E-mail: linghang_zhuang@merck.com.
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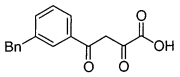
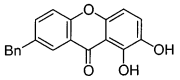
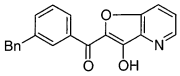
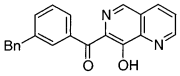
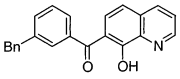
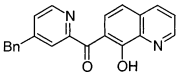
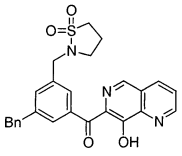
Table 1. Inhibition of HIV-1 Integrase Catalytic Activities and HIV-1 Replication in Cells by Compounds **1**−**7** (Bn = Benzyl)

Compound	Structure	Inhibition of Strand Transfer[a] IC_{50} (μM)	Antiviral Activity[b] CIC_{95} (μM)	Cytotoxicity[c] (μM)
1		0.01 (n = 4)	1.11 ± 0.61 (n = 16)	> 50.00
2		0.60 (n = 2)	6.00 (n =1)	3.00
3		1.00 (n = 2)	> 50.00 (n = 1)	12.50
4		0.04 ± 0.02 (n = 4)	6.20 ± 1.8 (n = 3)	12.50
5		0.37 ± 0.06 (n = 2)	5.00 (n = 1)	1.25
6		0.05 ± 0.01 (n = 2)	2.50 (n = 1)	2.50
7		0.01 (n = 1)	0.39 ± 0.16 (n = 4)	> 12.50

[a] Assays were performed with recombinant HIV-1 integrase (0.1 μM) preassembled on immobilized oligonucleotides. Inhibitors were added after assembly and washings. For details, see ref 6. [b] Cell culture inhibitory concentrations (CIC_{95}) are defined as those that inhibited by ≥95% the spread of HIV-1 infection in susceptible cell culture, using the HIV-1 IIIb variant and MT-4 T-lymphoid cells. For details, see ref 9. [c] See ref 10.

considerations led to the design and synthesis of furanopyridine **3** and [1,6]naphthyridine **4**.[5]

Furanopyridine **3** (IC_{50} = 1000 nM) is found to be 100-fold less potent than the diketoacid **1** (IC_{50} = 10 nM) in the strand transfer assay (Table 1).[6] On the other hand, naphthyridine **4** (IC_{50} = 40 nM) exhibits good potency in the same assay and is only 4-fold less potent than **1** (Table 1). The corresponding quinoline **5** exhibits an IC_{50} of 370 nM in this assay and is thus 9-fold less potent than naphthyridine **4**. Shifting the aromatic nitrogen

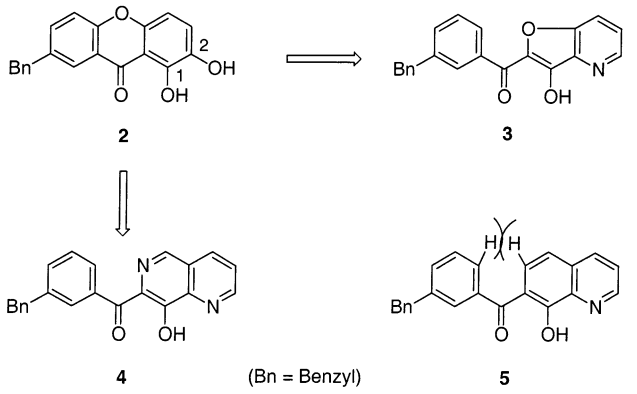
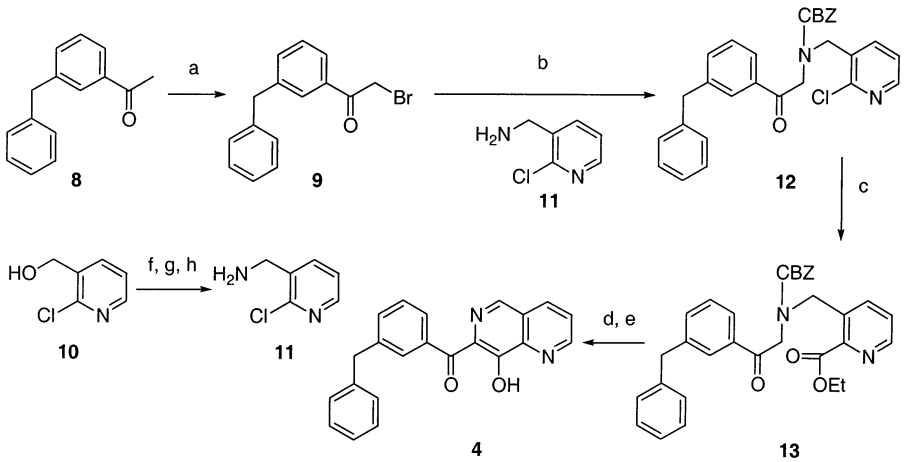


Figure 2. Structures of furanopyridine **3**, naphthyridine **4**, and quinoline **5**. A CH−CH interaction in **5** prevents the central phenyl ring and the quinoline ring from a adopting coplanar conformation.

to the central benzene ring led to quinoline **6** (Table 1),[5] which exhibits an IC_{50} of 50 nM in the strand transfer assay and is comparable to that of naphthyridine **4** (40 nM). These results are in accordance with the earlier assumption that the ability of these inhibitors to adopt a coplanar arrangement of the central phenyl ring (or pyridine ring) and the naphthyridine ring (or quinoline ring) is important for the inhibitory activity of these inhibitors. It is also noteworthy to point out that 8-hydroxy-[1,6]naphthyridine **4** is significantly less cytotoxic than the corresponding 8-hydroxyquinolines **5** and **6** (12.50 μM vs 1.25 and 2.50 μM, respectively) in the cell assay.[9,10] This may be related to the observation that unsubstituted 8-hydroxy-[1,6]naphthyridine exhibits significantly weaker affinity for metal ions versus unsubstituted 8-hydroxyquinoline.[11]

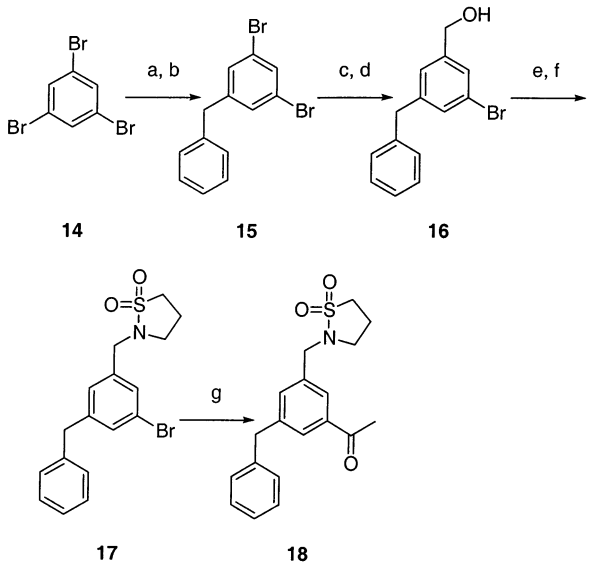
Further elaboration of **4** led to naphthyridine **7**, bearing a sultam substitution at the 3 position of the central phenyl ring. Naphthyridine **7** exhibits an IC_{50} of 10 nM in the strand transfer assay; it is essentially as potent as diketoacid **1**. In a single-cycle HIV-1 infectivity assay,[4c] **7** exhibits significant loss in potency against HIV-1-containing integrase mutations previously shown to confer resistance to the 1,3-diketoacid inhibitors (EC_{50} > 0.8 μM; T66I, S153Y) versus the wild-type virus (EC_{50} = 0.14 μM; NL4-3). This result dem-

Scheme 1[a]



[a] Reagents: (a) Br_2, $AlCl_3$ (cat.), 1,4-dioxane; (b) *i*-Pr_2NEt, CH_3CN, CBZCl, 76% for steps a and b; (c) Pd(II)$(PPh_3)_2Cl_2$, Et_3N, CO (250 psi), EtOH, 50%; (d) NaHMDS, THF; (e) 48% HBr, CH_3CN, air oxidation, 42% for steps d and e; (f) $SOCl_2$, toluene, 98%; (g) LiN_3, DMSO; (h) 5% Pt/C, H_2, EtOH, 51% for steps g and h.

Scheme 2[a]



[a] Reagents: (a) *n*-BuLi, PhCHO, Et_2O, −78 °C; (b) Et_3SiH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0 °C, 82% for steps a and b; (c) *n*-BuLi, DMF, Et_2O, −78 °C; (d) $NaBH_4$, MeOH, 77% for steps c and d; (e) PPh_3, CBr_4, CH_2Cl_2, 75%; (f) γ-sultam, K_2CO_3, CH_3CN, reflux, 99%; (g) butyl vinyl ether, Pd(II)$(OAc)_2$, Tl(I)OAc, DPPP, Et_3N, DMF, 100 °C, 1 N HCl, THF, 90%.

onstrates that the antiviral activity of compound **7** is due to its effect on integrase and suggests that it inhibits HIV-1 integrase in a mechanism similar to that of the diketoacids reported earlier. Naphthyridine **7** exhibits a CIC_{95} of 0.39 μM in the cell assay, 1-fold more potent than **1**. Furthermore, it does not show cytotoxicity at concentrations up to 12.5 μM.[10] When naphthyridine **7** is administered intravenously to rats at 2 mg/kg,[12] it exhibits a half-life ($T_{1/2}$) of 9.7 h with a moderate clearance rate (Cl_p) of 2.98 mL min^{-1} kg^{-1}. At 10 mg/kg dose given orally to rats,[12] **7** reaches the peak plasma level (C_{max}) of 1.17 μM at 60 min and maintains a plasma concentration greater than 0.8 μM throughout the first 6 h.

The synthesis of [1,6]naphthyridine **4** is shown in Scheme 1. The known methyl ketone **8**[4a] was monobrominated to give bromoketone **9**, which was treated subsequently with amine **11** and benzyl chloroformate (CBZCl) to afford **12** in 76% yield overall. Amine **11** was prepared from alcohol **10**[13] via chlorination, azide displacement, and platinum-catalyzed hydrogenation in 50% yield. Palladium-catalyzed carbonylation of **12** under 250 psi of carbon monoxide in ethanol provided ester **13** (50%).[14] Treatment of **13** with sodium bis(trimethylsilyl)amide provided the Dieckmann cyclization intermediate. Subsequent CBZ protecting group removal with 48% hydrobromic acid and in situ air oxidation furnished [1,6]naphthyridine **4** in 42% yield. The naphthyridine **7** was prepared in a similar manner starting from sultam-substituted methyl ketone **18**. Preparation of ketone **18** is shown in Scheme 2. Monolithiation of 1,3,5-tribromobenzene **14** followed by treatment with benzaldehyde provided the corresponding benzylic alcohol. This alcohol was reduced with a mixture of triethylsilane and boron trifluoride diethyl etherate to give **15** in overall 82% yield.[15] Dibromobenzene **15** was monolithiated and treated with *N*,*N*-dimethylformamide. The resultant aldehyde was reduced with sodium borohydride to afford benzyl alcohol **16** in 77% yield. Alcohol **16** was converted to its corresponding bromide, which was then reacted with γ-sultam[16] to give **17** in 74% yield. Compound **17** was subjected to Heck reaction conditions with butyl vinyl ether followed by acid hydrolysis to provide methyl ketone **18** in 90% yield.[17]

In summary, starting with diketoacid **1**, novel bioisosteric diketoacid equivalents were designed and prepared. This led to the discovery of 8-hydroxy-[1,6]-naphthyridine as a suitable replacement for the 1,3-diketoacid moiety in our earlier HIV-1 integrase inhibitors. Among them, naphthyridine **7** exhibits excellent potency and a good pharmacokinetic profile. Further work on 8-hydroxy-[1,6]naphthyridine-derived HIV-1 integrase inhibitors is in progress.

Supporting Information Available: Experimental procedures for preparation of compounds **2**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(5) Preparations of compounds **2**−**7** are in Supporting Information.

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(Sapphire II, Tropix). The assay was performed in a final concentration of 10% DMSO. To specifically evaluate inhibition of strand transfer, compounds were added *after* assembly, just prior to the addition of the target DNA.

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(10) Cytotoxicity is evaluated by visual inspection of the culture for cytopathic effects distinguished as gross morphological changes, growth pattern change, and metabolic change as indicated by lack of change in the medium pH indicator. With inhibitor **7**, all cells remain viable after 4 days. During the 4-day incubation period, MT-4 cells will typically double at least 4 times. No effect on cell numbers was observed with **7** at up to 12.5 μM.

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