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A series of 5-(5,6)-dihydrouracil substituted 8-hydroxy-[1,6]naphthyridine-7-carboxylic acid 4-fluorobenzylamide inhibitors of HIV-1 integrase and viral replication in cells

Mark W. Embrey,[a,*] John S. Wai,[a] Timothy W. Funk,[a] Carl F. Homnick,[a] Debbie S. Perlow,[a] Steven D. Young,[a] Joseph P. Vacca,[a] Daria J. Hazuda,[b] Peter J. Felock,[b] Kara A. Stillmock,[b] Marc V. Witmer,[b] Gregory Moyer,[c] William A. Schleif,[c] Lori J. Gabryelski,[c] Lixia Jin,[d] I-Wu Chen,[d] Joan D. Ellis,[d] Bradley K. Wong,[d] Jiunn H. Lin,[d] Yvonne M. Leonard,[a] Nancy N. Tsou[e] and Linghang Zhuang[a]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, West Point, PA 19486, USA*
[b]*Department of Biological Chemistry, Merck Research Laboratories, West Point, PA 19486, USA*
[c]*Department of Vaccine and Biologics Research, Merck Research Laboratories, West Point, PA 19486, USA*
[d]*Department of Drug Metabolism and Pharmacology, Merck Research Laboratories, West Point, PA 19486, USA*
[e]*Department of Medicinal Chemistry, Merck Research Laboratories, Rahway, NJ 07065, USA*

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Abstract—Introduction of a 5,6-dihydrouracil functionality in the 5-position of *N*-(4-fluorobenzyl)-8-hydroxy-[1,6]naphthyridine-7-carboxamide **1** led to a series of highly active HIV-1 integrase inhibitors. These compounds displayed low nanomolar activity in inhibiting both the strand transfer process of HIV-1 integrase and viral replication in cells. Compound **11** is a 150-fold more potent antiviral agent than **1**, with a CIC_{95} of 40 nM in the presence of human serum. It displays good pharmacokinetics when dosed in rats and dogs.

The etiological agent of acquired immune-deficiency syndrome (AIDS) is the human immunodeficiency virus type 1 (HIV-1). One of the possible targets for chemotherapeutic intervention is a virally encoded enzyme, HIV integrase, which catalyzes the insertion of double-stranded viral DNA into the host cell's DNA. Integration is a three-step process consisting of the assembly of the viral DNA onto the integrase enzyme, endonucleolytic processing of the viral DNA, and strand transfer of the processed viral DNA into the host cell's DNA.[1]

Recently our laboratory reported the discovery of 8-hydroxy-[1,6]naphthyridine as a viable replacement to the diketoacid pharmacophore in our early integrase inhibitors.[2,3] Further research led to the discovery of the *N*-(4-fluorobenzyl)-8-hydroxy-[1,6]naphthyridine-7-carboxamide **1** as a potent structural backbone for integrase inhibition (Fig. 1). Compound **1** inhibits the strand transfer process of HIV-1 integration with an IC_{50} of 33.4 nM.[3,4] It inhibits the replication of HIV-1 in cell culture with a CIC_{95} of 1250 nM (assay run with 10% fetal bovine serum (FBS)).[5] In the presence of 50% normal human serum (NHS) a 4-fold drop in potency (CIC_{95} = 5000 nM) is observed due to the binding of drug to serum protein (99.2%).[6]

We attempted to incorporate polar heterocycles into compound **1** for improvement in both intrinsic potency and physical properties. The bromonaphthyridine **2** was readily accessible in quantities of several grams (Scheme 1).[7] Reacting **2** with methyl acrylate, under Fu's Heck

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* Corresponding author. Tel.: +1 215 652 0881; fax: +1 215 652 3971; e-mail: mark_embrey@merck.com

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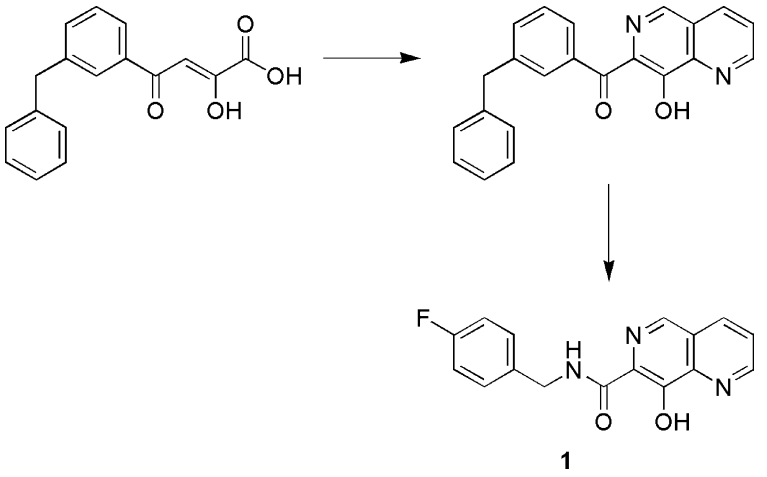
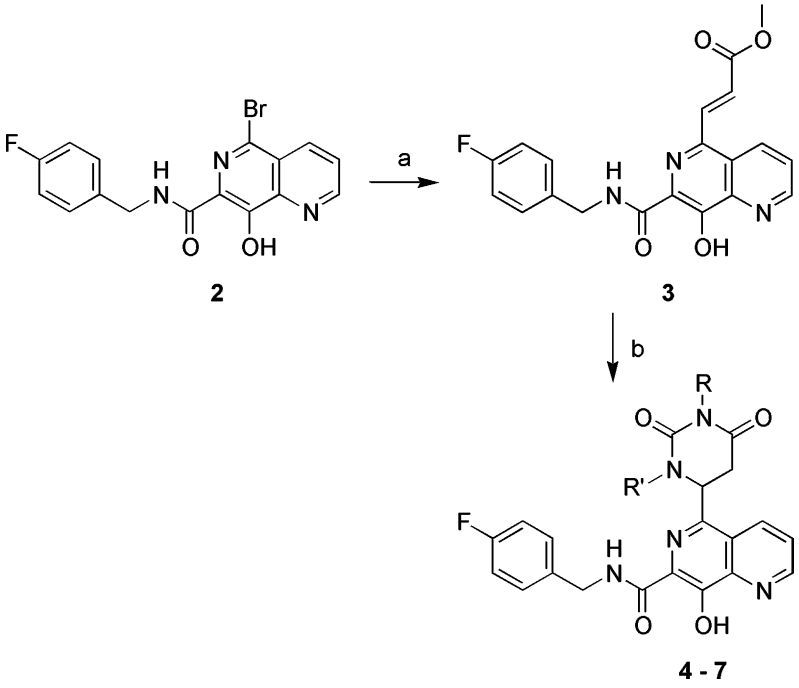


Figure 1. Evolution of **1**.



Scheme 1. Reagents and conditions: (a) Pd[P(*t*-Bu)$_3$]$_2$, Cy_2NMe, P(*t*-Bu)$_3$, $Pd_2(dba)_3$, methyl acrylate, 1,4-dioxane, sealed tube, 120 °C; (b) R,R′-urea (50–260 eq), melt 165 °C.

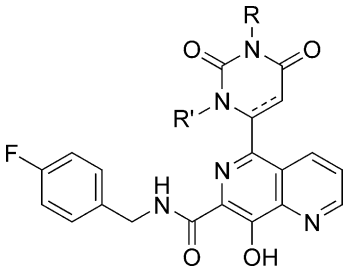
reaction conditions,[8] provided the α,β-unsaturated methyl ester **3**. The latter served as the common intermediate toward various heterocycles.

For example, melting **3** with urea at 165 °C in a sealed tube provided dihydrouracil **4**.[9] Compound **4** inhibits the strand transfer reaction of HIV-1 integrase with an IC_{50} of 10 nM and inhibits the replication of virus in cell culture with a CIC_{95} of 55 nM (Table 1). In the presence of 50% NHS, no drop in potency is observed, due to the lower protein binding nature of **4** (92.1%). Compound **4** is not cytotoxic at concentrations up to 10 μM.[10] Resolution of the enantiomers by chiral HPLC separation provided the (+) and (−) enantiomers, **8** and **9**, respectively. Both enantiomers exhibit similar potency in the in vitro assay. In the cell-based assay, neither compound shifts in the presence of human serum, but the (+) enantiomer displays slightly better activity against HIV-1 replication in cells versus the (−) enantiomer (CIC_{95} of 93.8 nM vs. 125 nM) (Table 1).

Treating **3** with methylurea and subsequent separation of the reaction products by preparative HPLC provided the regio-isomers **5** and **6** (as determined by NOE experiments) in poor yield. Although potency is similar in the in vitro assay, in the cellular anti-viral assay, **5** is fourfold more potent than **6** with a CIC_{95} of 15.6 nM in the presence of 50% NHS (Table 1).

Similarly, treating **3** with *N*,*N*′-dimethyl urea provided **7**. This compound maintains inhibitory activity against HIV-1 integrase strand transfer with an IC_{50} of

Table 1. Inhibition of HIV-1 integrase catalytic activities and HIV-1 replication in cells by a series of 5-(dihydrouracil or uracil) substituted 8-hydroxy-[1,6]naphthyridine-7-carboxylic acid 4-fluorobenzylamides



Compound	R	R′	Optical purity	Inhibition of strand transfer IC_{50} (nM)[a,b]	Antiviral activity CIC_{95}(nM) (with 10% FBS)[c]	Antiviral activity CIC_{95} (nM) (with 50% NHS)[c]	% Binding to human plasma protein[d]
1	—	—	—	32.8 ± 7.8 (n = 6)	1250 (n = 3)	5000 (n = 2)	99.2
Dihydrouracil							
4	H	H	Racemic	10(n = 8)	53.7 ± 25.1 (n = 8)	55.7 ± 22.6 (n = 8)	92.1
5	H	Me	Racemic	12.5 ± 4.3 (n = 4)	15.6 (n = 1)	15.6 (n = 1)	
6	Me	H	Racemic	12.8 ± 3.0 (n = 4)	31.3 (n = 2)	62.5 (n = 2)	
7	Me	Me	Racemic	8.7 ± 1.9(n = 6)	18.6 ± 6.1 (n = 16)	60.7 ± 7.4 (n = 17)	94.3
8	H	H	(+)97.2%ee	11 ± 1.7 (n = 4)	93.8 ± 31.3 (n = 2)	93.8 ± 31.3 (n = 2)	
9	H	H	(−)92.8%ee	13.3 ± 3.7 (n = 4)	125 (n = 2)	125 (n = 2)	
10	Me	Me	(+) 100%ee	11.3 ± 1.9 (n = 3)	41.7 ± 14.7 (n = 3)	166.7 ± 58.9 (n = 3)	96.0
11	Me	Me	(−)93.1%ee	35 ± 5(n = 2)	20.1 ± 7.1 (n = 7)	40.2 ± 14.1 (n = 7)	95.0
Uracil							
14	Me	Me		27.5 ± 13.0 (n = 4)	250 (n = 1)	1000 (n = 1)	97.8

[a] See Refs. 3,4.
[b] The lower limit of activity of this assay is ~10 nM.
[c] See Ref. 5.
[d] See Ref. 6.

8.7 nM (Table 1). Compound **7** has a CIC_{95} of 18.6 nM in 10% FBS, which increases to 60.7 nM in the presence of 50% NHS, due to its higher protein binding of 94.3%. Chiral HPLC separation of the racemic **7** provided the (+) and (−) enantiomers, **10** and **11** respectively. The (−) enantiomer **11** displays better cellular activity (CIC_{95} of 20.1 nM with 10% FBS and 40.2 nM with 50% NHS) as compared to the (+) enantiomer **10** (CIC_{95} of 41.7 nM with 10% FBS and 166.7 nM with 50% NHS) even though **11** is less active in the enzyme assay (IC_{50} = 35 nM vs. IC_{50} = 11.3 nM for **10**) (Table 1). When **11** is dosed to rats as the sodium salt, it displays good pharmacokinetics with a clearance of 9.76 mL/min/kg, a C_{max} of 4.81 μM, and an absolute oral bioavailability of 28.0%. Similarly when dosed to dogs, **11** displays good pharmacokinetics with a clearance of 3.47 mL/min/kg, a C_{max} of 1.58 μM, and a bioavailability of 41.5%.[11]

Additional features of **7** were revealed by X-ray crystallographic structure determination (Fig. 2).[12] The naphthyridine ring is positioned in a pseudo-axial orientation relative to the dihydrouracil ring. The naphthyridine and the exocyclic amide are in an extended coplanar conformation. The dihydrouracil ring is almost planar, orientates perpendicular to the naphthyridine ring, and points toward the 4-fluorobenzyl amide.

The unsaturated *N*,*N*′-dimethyluracil analog **14** was also synthesized. In this case, the plane of the uracil ring would be expected to orient perpendicular to the plane of the naphthyridine ring and as such, the uracil ring would not be pointed toward the 4-flourobenzyl amide. Attempts to oxidize the dihydrouracil to the uracil were unsuccessful. The compound **14** was synthesized by coupling the iodide **12** with the dimethyl uracil zinc iodide **13** using the method of Knochel (Scheme 2).[13]

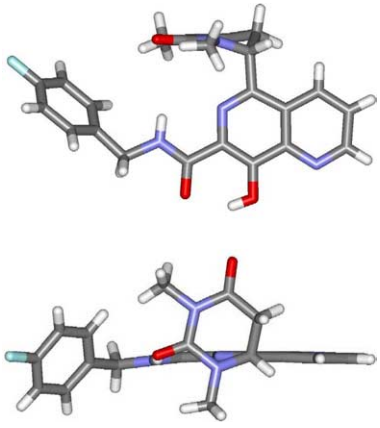
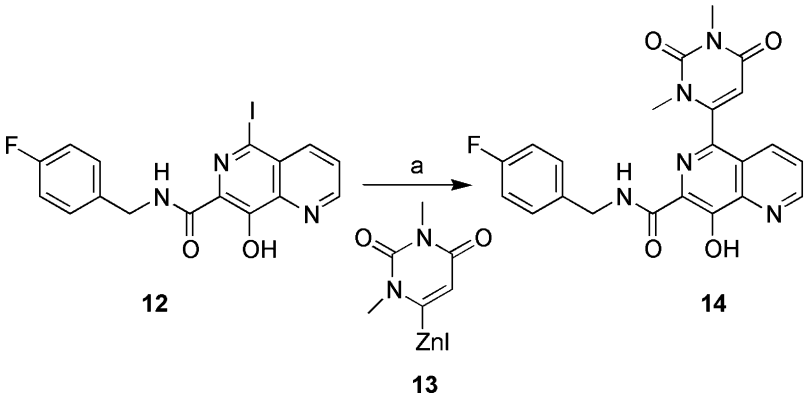


Figure 2. X-ray crystal structure of **7**.

Compared to the saturated analogs, the unsaturated uracil analog **14** is less active in the enzyme assay (IC_{50} = 27.5 nM). This decrease in activity is also more significantly reflected in the cell-based assay with a



Scheme 2. Reagents and conditions: (a) $Pd_2(dba)_3$, (2-furyl)$_3$P, DMA, 80 °C.

CIC_{95} of 250 nM (with 10% FBS) and a CIC_{95} of 1000 nM with 50% NHS (PB = 97.8%) (Table 1).

This observation suggests that sp^3 hybridized linkages off of the 5-position of naphthyridine-based inhibitors will provide more potent analogs against viral replication in cells. Further research was done in this area by our group, which will be reported at a later date.

In summary, a potent series of dihydrouracil heterocycles were synthesized that displays nanomolar inhibition of HIV-1 replication in a cell-based assay with little shift in efficacy in the presence of human serum. This represents a 100-fold improvement in potency over the unsubstituted analog **1**. In addition, the (−) enantiomer of the dimethylated dihydrouracil **11** displays good pharmacokinetic properties when dosed in rats and in dogs.

Acknowledgments

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was dissolved in water and the solution was acidified to pH 5–6. The resultant precipitate was filtered, dissolved in DMSO, and subjected to HPLC purification on C-18 stationary phase eluted with water/acetonitrile/TFA mobile phase. Lyophilization of appropriate fractions provided compound **4** in 44% yield. ^{1}H NMR (400 MHz, ~5:1 $CDCl_3$-DMSO-d_6) δ 13.35 (s, 1H), 9.19 (br d, 1H), 8.68–8.59 (m, 2H), 8.14 (br s, 1H), 7.71 (dd, J = 8.6, 4.2 Hz, 1H), 7.42 (dd, J = 8.4, 5.5 Hz, 2H), 7.06 (t, J = 8.6 Hz, 2H), 5.36 (br d, 1H), 4.65 (br s, 2H), 3.03 (br s, 2H), 2.59 (br s, 1H)

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.
11. Compound was dosed intravenously in dimethyl sulfoxide (DMSO) at a concentration of 2 mg/kg in rats and at 1 mg/kg in dogs. Compound was dosed orally in aqueous solution (with 0.5% methylcellulose) at a concentration of 10 mg/kg in rats and at 1 mg/kg in dogs.
12. Crystallographic data (excluding structure factors) for **7** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 264140. Copies of the data can be obtained free of charge, on application to CCDC, 12 Union Rd, Cambridge CB2 1EZ, UK [fax: +44(00)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].
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