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A series of 5-aminosubstituted 4-fluorobenzyl-8-hydroxy-[1,6]naphthyridine-7-carboxamide HIV-1 integrase inhibitors

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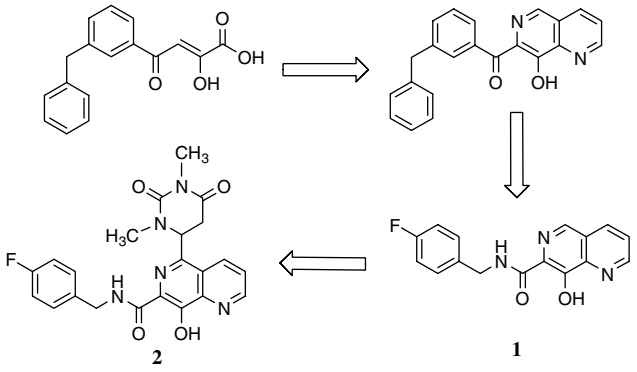
Abstract—A series of 5-amino derivatives of 8-hydroxy[1,6]-naphthyridine-7-carboxamide exhibiting sub-micromolar potency against replication of HIV-1 in cell culture was identified. One of these analogs, compound **12**, displayed excellent pharmacokinetic properties when dosed orally in rats and in monkeys. This compound was demonstrated to be efficacious against replication of simian-human immunodeficiency virus (SHIV) 89.6P in infected rhesus macaques.

Resistance to the current chemotherapeutic agents for the treatment of HIV-1 infections continues to drive research toward developing new and better antiretroviral treatments. Principal therapies currently in use target reverse transcriptase and protease, two of the three virally encoded enzymes that play critical roles in the reproductive cycle of HIV. The third enzyme, integrase, is responsible for inserting the pro-viral DNA into the cellular genome via endonucleolytic processing and strand transfer processes.[1]

The 8-hydroxy-[1,6]naphthyridine scaffold was discovered to be a potent replacement for the diketoacid pharmacophore of HIV-1 integrase inhibitors in our laboratories.[2,3] Further exploration led to the identification of the *N*-(4-fluorobenzyl)-8-hydroxy-[1,6]naphthyridine-7-carboxamide **1** as a potent platform for extensive studies (Fig. 1). Compound **1** inhibits the strand transfer process of integration with an IC_{50} of 33 nM[3,4] and also inhibits the replication of HIV-1 in cell culture with a CIC_{95} of 1250 nM.[5] In the presence of 50% normal human serum (NHS), a 4-fold drop in potency (CIC_{95} = 6000 nM) is observed due to the binding of drug to serum protein (99.2%).[6]



2 1

Figure 1. Evolution of naphthyridine integrase inhibitors.

Keywords: HIV-1; Integrase; Inhibitor; Monkey; Rhesus; Macaque; Naphthyridine.
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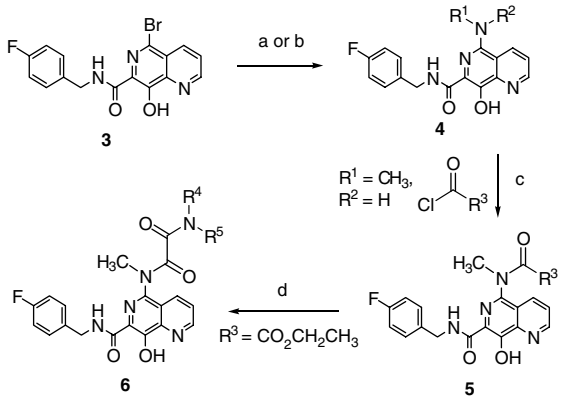
We explored the incorporation of polar heterocycles into compound **1** to improve both intrinsic potency and physical properties. Recently, a series of 5-dihydrouracil 8-hydroxy[1,6]naphthyridine-7-carboxamides **2** was reported to potently inhibit HIV-1 replication in cell culture and to give good pharmacokinetic profiles in rats and dogs.[7] In this communication, we describe a series of 5-amino derivatives of 8-hydroxy[1,6]naphthyridine-7-carboxamide as potent inhibitors of HIV-1 integrase and viral replication in cell culture. One of these analogs, compound **12**, was shown to be efficacious against replication of simian-human immunodeficiency virus (SHIV) 89.6P in infected rhesus macaques.[8]

The chemistry used to prepare these analogs is illustrated in Scheme 1. Reaction of the accessible bromonaphthyridine **3**[9] with primary or secondary amines provided a series of substituted aminonaphthyridines **4**. The products derived from reactions with primary amines were further functionalized to compounds **5** and **6** for optimization of biological and physical properties.

Substitution at the 5 position of 8-hydroxy[1,6]naphthyridine-7-carboxamide with secondary amino groups, such as *N*-acetylpiperazine and *N*-methylpiperazine, provided inhibitors **7** and **8**, respectively (Table 1). Compounds **7** and **8** exhibited comparable potency to compound **1** against HIV-1 integrase in the enzyme assay. Both analogs were significantly more active against replication of HIV-1 in cell culture in the presence of 10% fetal bovine serum (FBS). Presumably the polar heterocyclic substitution improved cell penetration of these analogs. However, when the antiviral replication assay was repeated with these compounds in the presence of 50% normal human serum (NHS), there was a 10-fold decrease in potency with compound **7** and a nearly 3-fold decrease with compound **8**. These shifts in potency appear to correlate with the high affinities of the compounds for human serum proteins as determined in vitro (Table 1; 98–99% for compounds **1**, **7**, and **8**). Two amino functionalities, $MeNCH_2CH_2NMe)Ac$ and $MeNCH_2CONMe_2$, were recently reported to exhibit low affinity for the serum proteins fibrinogen and lysozyme.[10] These two functional groups were incorporated into the naphthyridine inhibitor scaffold to provide compounds **9** and **10** (Table 1). Compound **10** displayed significantly less affinity toward human serum proteins (83.3% compared to 98–99% for compounds **7** and **8**). This reduction in protein binding with compound **10** translated to a less than 2-fold shift in potency against HIV-1 replication in the presence of 50% NHS (Table 1). Little decrease in affinity for serum protein was observed with inhibitor **9**.



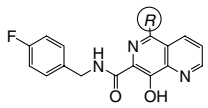
Scheme 1. Reagents and conditions: (a) R^1R^2NH, DIEA, DMSO, THF, 140 °C, 72 h; (b) R^1R^2NH, *i*-Pr_2NEt, DMSO, THF, microwave at 170 °C, 1 h; (c) *i*-Pr_2NEt, THF, 0–25 °C; (d) R^4R^5NH, MeOH, 0 °C.

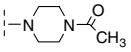
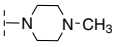
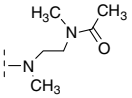
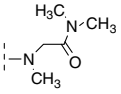
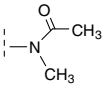
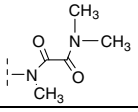
Intravenous dosing of DMSO solutions of analogs **7**, **8**, and **10** in rats at 2 mg/kg resulted in rapid clearance of the compounds from plasma (Table 1; clearance 42–150 mL/min/kg). Further studies indicated that these analogs were primarily cleared via glucuronidation. It has been reported that acetylation of the methylamino group *para* to the hydroxyl group of 4-*N*-methylaminophenol reduces its rate of glucuronidation by 5-fold relative to the parent compound.[11] This feature was incorporated into the 5-amino-8-hydroxy[1,6]naphthyridine-7-carboxamide template to afford the acetyl aminonaphthyridine **11** (Table 1). Compound **11** exhibited very low clearance (1.8 mL/min/kg) following intravenous dosing in rats. It also had antiviral activity comparable to those of inhibitors **7**–**10** when assayed in the presence of 10% FBS. However, there was a 5-fold shift in its antiviral activity in the presence of 50% NHS. Compound **11** was found to have higher affinity for human serum protein than **10** (95.6% vs. 83.3%), although its affinity was lower than those observed for compounds **7**–**9**.

Combination of the unique structural features of compounds **10** and **11** led to the preparation of naphthyridine oxalylamide **12** (Table 1). Compound **12** exhibited balanced biological and physical properties, with an antiviral CIC_{95} of 103 nM and a moderate affinity for human serum protein (93.2%) that led to a modest 2.5-fold shift of the CIC_{95} to 250 nM in the presence of 50% NHS. Furthermore, analog **12** exhibited low clearance of 5.6 mL/min/kg in rats when dosed intravenously at 2 mg/kg as a solution in DMSO. Compound **12** was found to be well absorbed with an oral bioavailability of 71% and excellent exposure (AUC of 52 μM h) when dosed at 10 mg/kg as a suspension in 0.5% methylcellulose (Fig. 2). Mean plasma concentration of compound **12** at the 24th hour was determined to be ~600 nM, which was significantly higher than the 250 nM required for inhibition of 95% of viral growth in the presence of 50% human serum.

Replacement of the dimethylamino moiety of the oxalylamide **12** with methylamino and amino groups led to the preparation of compounds **13** and **14**. Improvements in their intrinsic activities against integrase were observed (Table 2); however, both compounds were less active against replication of HIV-1 in cell assays. Presumably, the introduction of additional amido hydrogen(s) adversely affects cell penetration and increases binding to human serum proteins[10] (Table 2). Substitution with piperidine and pyrrolidine led to the preparation of compounds **15** and **16**. The pyrrolidine analog **16**

Table 1. SAR of 5-substituted amino 1,6-naphthyridines



Compound	R =	IC_{50}[a] (nM)	CIC_{95} (nM) (10% FBS)[b]	CIC_{95} (nM) (50% NHS)[c]	PB (%)[d]	Cl[e]
1	H	33	1250	6000	99.2	na
7		25	128	1344	99.0	77.3
8		40	234	677	97.9	42.3
9		30	156	625	98.3	na
10		58	156	234	83.3	150.0
11		25	125	612	95.6	1.8
12		40	103	250	93.2	5.6

[a] Assays were performed with recombinant HIV-integrase (0.1 μM) preassembled on immobilized oligonucleotides.[4]
[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.[5a] Cytotoxicity was not observed in cell culture at concentrations up to 20 μM.[5b]
[c] Cell culture assayed in the presence of 50% normal human serum.[5a]
[d] Percentage of compound bound to human serum proteins.[6]
[e] Rat clearance values; i.v. dosing as a solution in DMSO (2 mg/kg).

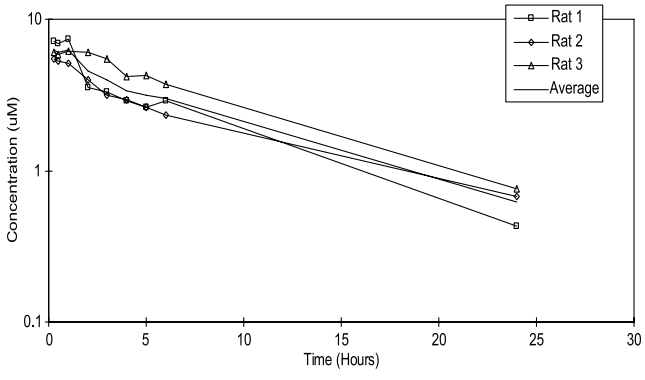


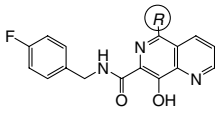
Figure 2. Pharmacokinetic profile of compound **12** in rats dosed orally at 10 mg/kg as a suspension in 0.5% aqueous methylcellulose.

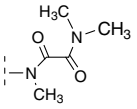
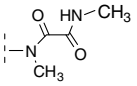
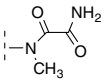
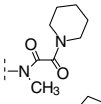
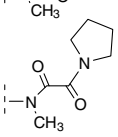
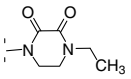
was the most potent compound in this series of naphthyridine oxalylamides in the antiviral assay and was nearly 2-fold more active than compound **12** when assayed in the presence of fetal bovine serum. However, binding to human serum protein also crept up to 96.7%, which rendered compound **16** 2-fold less active than compound **12** in the presence of human serum. Compound **17**, which contains a 2,3-piperazine dione substituent as a cyclic version of the oxalylamide in **12**, maintained potency against integrase, but was 10-fold less active against replication of the virus.

Compound **12**, possessing balanced biological and physical properties, was chosen for further characterization. It was found to exhibit an excellent pharmacokinetic profile in rhesus monkey with 60% bioavailability and a half life of 5 h when dosed orally at 10 mg/kg as a suspension in 0.5% methylcellulose. Compound **12** is active against both HIV-1 and the simian lentivirus, SIV, with CIC_{95} values of 250 and 350 nM, respectively, in the presence of 50% human and rhesus serum. These favorable features made it an excellent tool for evaluation of the antiviral efficacy of an integrase inhibitor against SHIV in an animal model. More than 400 g of compound **12** was prepared for biological studies.[12]

In the early intervention cohort of the study, 10 days after rhesus monkeys were infected intravenously with 50 monkey infectious doses of cell-free SHIV 89.6P,

Table 2. SAR of 1,6-naphthyridine oxalylamides



Compound	R =	IC_{50}[a] (nM)	CIC_{95} (nM) (10% FBS)[a]	CIC_{95} (nM) (50% NHS)[a]	PB (%)[a]
12		40	103	250	93.2
13		15	500	>1000	98.3
14		20	563	750	98.2
15		7	125	500	97.8
16		40	63	500	96.7
17		10	1000	>1000	98.8

[a] See footnotes under Table 1.

dosing of the animals with integrase inhibitor **12** as a suspension in 0.5% aqueous methylcellulose was initiated at 10 mg/kg, twice daily. This resulted in a plasma concentration of ~700 nM of **12** at 12 h, well above the CIC_{95} of 350 nM.[8] Sustained suppression of viral replication in vivo was achieved in less than 4 weeks. Continued inhibition of viral growth was maintained subsequently with a once-daily dose of 20 mg/kg of **12** through 87 days post-infection.

In summary, a potent series of 5-amino derivatives of 8-hydroxy[1,6]naphthyridine-7-carboxamide exhibiting sub-micromolar potency against replication of HIV-1 in cell-based assay was identified. The most potent inhibitor, analog **16**, inhibited viral growth with a CIC_{95} of 63 nM in the presence of 10% FBS. Compound **12** displayed balanced biological and physical properties, and excellent pharmacokinetic profiles in rats and monkey. Oxalylamide **12** was demonstrated to be efficacious against replication of simian-human immunodeficiency virus (SHIV) 89.6P in infected rhesus macaques.

Acknowledgments

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12. Preparation of compound **12**. *N-(4-Fluorobenzyl)-8-hydroxy-5-(methylamino)-1,6-naphthyridine-7-carboxamide* (**4**, $R^1 = H, R^2 = CH_3$): A mixture of bromonaphthyridine **3** (35 g, 93 mmol)[9], methylamine (107 mL, 214 mmol, 2 M in THF), diisopropylethylamine (12 g, 93 mmol), and 50 mL DMSO in a 400 mL reaction vessel was heated in a microwave reactor at 170 °C for 1 h. The product mixture was cooled to room temperature and poured into ice/water (3 L). The yellow solid which precipitated was collected by filtration and air-dried to yield 20 g (66%) of **4** ($R^1 = H$, $R^2 = CH_3$). This procedure was repeated 21 times to accumulate 415 g of **4**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.11 (m, 1H), 8.30 (m, 1H), 8.10 (d, 8.5 Hz), 7.53 (dd, J = 8.4, 4.3 Hz, 1H), 7.37 (m, 2H), 7.06 (t, J = 8.6 Hz, 2H), 5.00 (m, 1H), 4.67 (d, J = 6.3 Hz, 2H), 3.06 (d, J = 4.8 Hz, 3H). HRMS *m/z* calcd for $C_{17}H_{15}FN_4O_2$ (M+1) 327.1252, found 327.1264. *N-(7-{[(4-fluorobenzyl)amino]carbonyl}-8-hydroxy-1,6-naphthyridin-5-yl)-N,N′,N′-trimethylethanediamide* (**12**): To a cold (0 °C) solution of compound **4** (415 g, 1.27 mol; $R^1 = H$, $R_2 = CH_3$) and diisopropylethylamine (1.3 kg, 10.2 mol) in THF (20 L), ethyl chlorooxoacetate (690 g, 5.1 mol) was added dropwise over a period of 20 min. The resultant mixture was stirred at the same temperature for 2 h, allowed to warm to 25 °C, and stirred for an additional 18 h. The product mixture was filtered and the filtrate was concentrated under vacuum. The residual oil was dissolved in ethyl acetate and filtered again. The ethyl acetate solution was washed successively with 1 M aqueous HCl, water, and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum to provide the bis-oxalation product as a brown oil (670 g). This material was used in the following step without further purification. To a cold (0 °C) solution of the above crude bis-oxalate (670 g) in methanol (8 L), anhydrous dimethylamine gas (400 g, 8.8 mol) was bubbled into the mixture with its temperature maintained below 5 °C. After the reaction mixture was stirred at 0 °C for 18 h, another 400 g of methylamine was added. The resultant mixture was stirred at 0 °C for 4 h, warmed to 25 °C, and stirred for 18 h. The mixture was then purged with nitrogen gas for 15 min. The product mixture was concentrated under vacuum and the residue was dissolved in ethyl acetate (8 L). The organic solution was washed successively with 0.5 M aqueous HCl, water, and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residual oil was dissolved in boiling ethyl acetate (4 L), decolorized with activated charcoal, and filtered. The precipitate that formed upon cooling was filtered to provide compound **12** as a white crystalline solid (400 g, 74% yield from **4**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.21 (m, 1H), 8.35 (dd, J = 8.5, 1.6 Hz, 0.84H, rotamer A), 8.26 (dd, J = 8.5, 1.6 Hz, 0.16H, rotamer B), 8.09 (m, 0.16H), 7.92 (m, 0.84H), 7.72 (dd, J = 8.5, 4.2 Hz, 0.84H), 7.67 (dd, J = 8.5, 4.2 Hz, 0.16H), 7.36 (m, 2H), 7.08 (m, 2H), 4.69 (d, J = 6.1 Hz, 0.16H), 4.66 (d, J = 6.3 Hz, 0.84H), 3.47 (s, 0.16H), 3.41 (s, 0.84H), 3.16 (s, 0.16H), 3.11 (s, 0.16H), 2.88 (s, 0.84H), 2.54 (s, 0.84H). In experiments run at elevated temperatures, peaks coalesced and sharpened, indicating that the rate of equilibration of the two rotamers is temperature-dependent: ^{1}H NMR (600 MHz, DMSO-d_6, 135 °C): δ 9.16 (dd, J = 4.3, 0.9 Hz, 1H), 8.85 (br s, 1H), 8.36 (br s, 1H), 7.81 (dd, J = 4.3, 8.7 Hz, 1H), 7.43 (t, J = 5.9 Hz, 2H), 7.11 (t, J = 8.7 Hz, 2H), 4.61 (d, J = 6.7 Hz, 2H), 3.39 (s, 3H), 2.97 (br s, 3H), 2.51 (br s, 3H). HRMS *m/z* calcd for $C_{21}H_{20}FN_5O_4$ (M+1) 426.1572, found 426.1590. Anal. Calcd for $C_{21}H_{20}FN_5O_4$: C, 59.29; H, 4.74; N, 16.46. Found: C, 59.40; H, 4.65; N, 16.34. Mp: 195–196 °C.