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Synthesis of 5-(1-H or 1-alkyl-5-oxopyrrolidin-3-yl)-8-hydroxy-[1,6]-naphthyridine-7-carboxamide inhibitors of HIV-1 integrase

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ABSTRACT

HIV-1 integrase catalyzes the insertion of viral DNA into the genome of the host cell. Integrase inhibitor *N*-(4-fluorobenzyl)-8-hydroxy-1,6-naphthyridine-7-carboxamide selectively inhibits the strand transfer process of integration. 4-Substituted pyrrolidinones possessing various groups on the pyrrolidinone nitrogen were introduced at the 5-position of the naphthyridine scaffold. These analogs exhibit excellent activity against viral replication in a cell-based assay. The preparation of these compounds was enabled by a three-step, two-pot reaction sequence from a common butenolide intermediate.

The HIV-1 integrase enzyme is responsible for the insertion of the viral genetic code into the host genome. Integrase assembles onto double-stranded viral DNA in the cytoplasm and processes the 3′ ends to prepare for viral DNA integration. The resultant pre-integration complex then enters the nucleus, where integrase catalyzes viral DNA insertion (strand transfer) into the host DNA.[1] Many laboratories have pursued discovery of integrase inhibitors suitable for dosing in patients. Recently, raltegravir was approved as the first integrase inhibitor to treat HIV-infected patients.[1d–f]

Earlier reports from our laboratories have described a series of 8-hydroxyl-7-carboxamide-naphthyridine inhibitors. Compound **1**[2] (Fig. 1) is a potent inhibitor of the strand transfer process of HIV-1 integration with an IC_{50} of 33 nM in the enzyme inhibition assay.[3] It also inhibits the replication of HIV-1 in cell culture with an IC_{95} of 1250 nM in the presence of 10% fetal bovine serum (FBS).[4] Addition of 50% normal human serum (NHS) to the cell culture results in a fourfold loss in efficacy (IC_{95} = 5000 nM), as a result of the compound's high affinity for serum protein (99.2% protein bound).

A series of 5-dihydrouracil 8-hydroxy-[1,6]-naphthyridine-7-carboxamides **2** (Fig. 1) was reported to more potently inhibit HIV-1 replication in cell culture compared to **1**.[5] The authors suggested that sp^3 hybridized linkages attached at the 5-position of the naphthyridine-based inhibitors were important for HIV-1 inhibition in cells. Among a number of alternative substituents proposed, the 4-substituted pyrrolidinone moiety of naphthyridine **3**

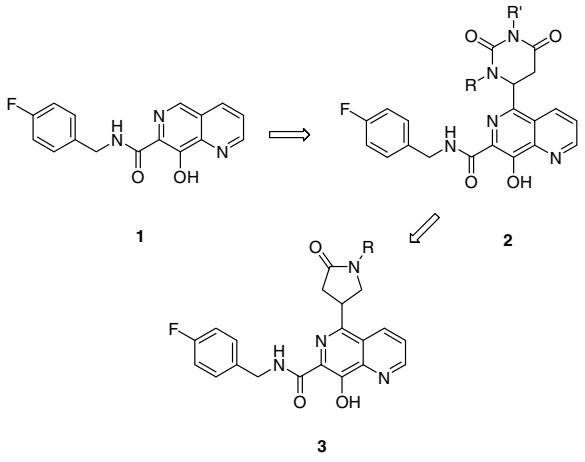

Figure 1. HIV-integrase naphthyridine inhibitors.

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appeared to be an attractive target due to its low molecular weight relative to other polar heterocyclic systems. In this letter, we describe a series of 5-(1-H or 1-alkyl-5-oxopyrrolidin-3-yl)-8-hydroxy-[1,6]-naphthyridine-7-carboxamides, which inhibit HIV-1 viral replication in cell culture.

Depicted in Scheme 1 is our synthetic route developed to allow for efficient introduction of various substituents on the pyrrolidinone nitrogen toward the end of the synthesis, while circumventing protection and deprotection of the 1,6-naphthryridine –OH. Another key consideration in development of the synthesis was that the 1,6-naphthyridine core does not tolerate strongly basic, oxidizing, or reducing conditions.

The synthesis of inhibitor **9** starts with treatment of 5-bromonaphthyridine **3**[6] (Scheme 1) with 4-tributylstannylfuran-2(5H)-one[7] **4** and $PdCl_2(PPh_3)_2$, to give **5** in excellent yield. It was anticipated that treatment of **5** with methylamine would provide compound **6**, which could be cyclized to the desired unsaturated pyrrolidinone **7**. However, after treating **5** with methylamine, hydroxy amide **6** was present in only minute quantities. Detailed NMR analysis revealed that the major products were *trans* and *cis* aminopyrrolidinones **8a** and **8b** (8a:8b, 2:1).[8] The *cis* isomer **8b** converted on standing in d_6-DMSO at room temperature to the *trans* diastereomer **8a** following re-running the NMR after 4 days. The *trans* diastereomer **8a** is active against replication of HIV with an IC_{95} of 94 nM (NHS). Another minor component isolated from the reaction mixture was identified to be **8c**.[9] Compound **8c** is potent against integrase in the strand transfer enzyme assay (IC_{50} = 40 nM), but shows ~20-fold less potency in cell culture (IC_{95} = 1000 nM NHS).

Diastereomers **8a** and **8b** were taken up in TFA and heated for 2 h to effect elimination of methylamine, providing a 4:1 inseparable mixture of alkene isomers **7a** and **7b**.[10] The mixture exhibits potent antiviral activity in cells with an IC_{95} of 78 nM, but shows a >10-fold decrease in potency in the presence of NHS. The reduction in potency is consistent with the high protein binding of **7a** and **7b** (99.7% protein bound). Treatment of the crude mixture of **8a** and **8b** with triethylsilane in the presence of TFA at 75 °C for 1.5 h provides the desired racemic naphthyridine **9** in a two-step, one-pot sequence.[11] Further exploratory work on a model system showed that the reaction works well with other aryl systems.[12]

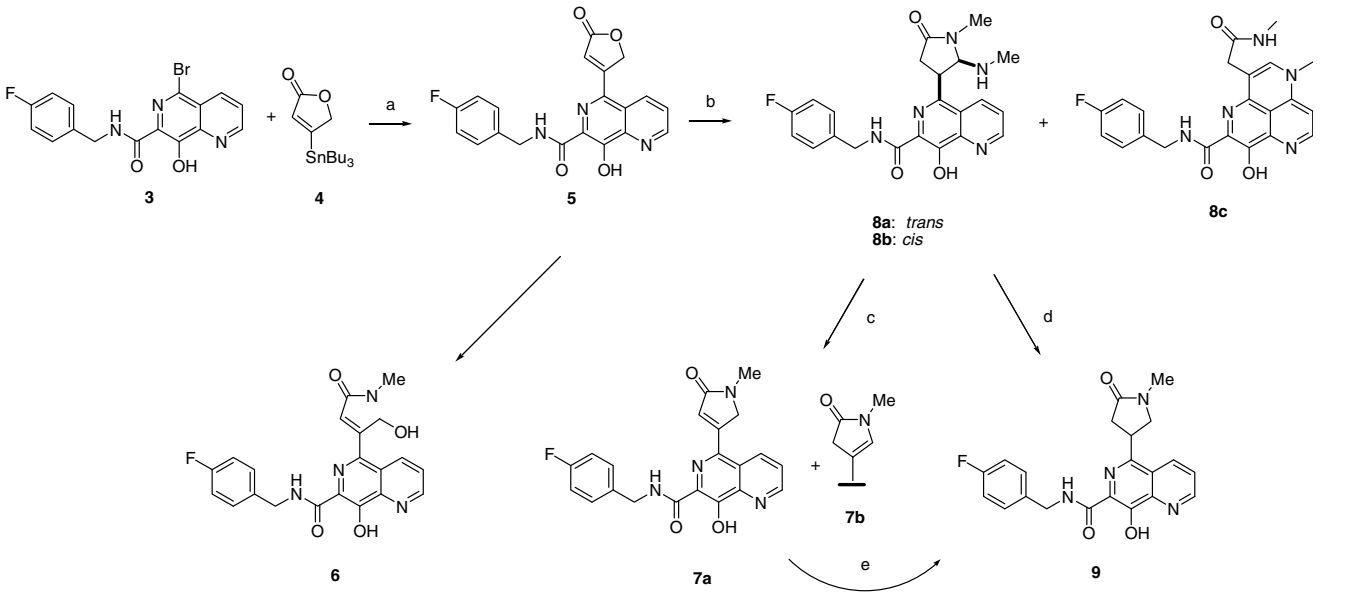
Pyrrolidinone **9** (Table 1) is a potent inhibitor of strand transfer with an IC_{50} of 20 nM in the enzyme inhibition assay. It inhibits the replication of HIV-1 in cell culture with an IC_{95} of 31 nM in the presence of 10% fetal bovine serum (FBS). Addition of 50% normal human serum (NHS) to the cell culture results in a fourfold loss in potency (IC_{95} = 125 nM).[13]

It is worth noting that the reaction of **5** with ammonia and other alkyl amines, including the more sterically hindered isopropylamine, provided pyrrolidinones **10–12** in good yields. All of the compounds are intrinsically potent against the Integrase enzyme ($IC_{50} \leqslant 40$ nM). Compound **10** is the most active compound against replication of HIV-1 in cell culture with an IC_{95} of 16 nM (FBS). Due to the relatively high plasma protein binding (98.9%), this compound shifts eightfold to 125 nM in the presence of 50% NHS. The unsubstituted analog **12** also exhibits good cell potency (FBS) and shows only a twofold loss in potency in the presence of NHS. We were unable to determine the protein binding of **12** due to low solubility.

The improved cell activity of **12** relative to **9–11** prompted us to introduce polar substituents to reduce plasma protein binding. The synthetic route that was developed was found to tolerate more functionalized amines as exemplified by glycine dimethylamide, thus enabling the synthesis of **13.** Compound **13** exhibited significantly lower protein binding (94.8%), and proved to be among the most potent inhibitors in this class of compounds in the presence of NHS with an IC_{95} of 63 nM (twofold loss of potency when assayed in 10% FBS) compared to compound **9** (fourfold loss in potency from FBS).

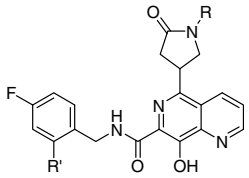
We recently reported that substitution at the 2-position of the benzyl moiety with polar substituents led to integrase inhibitors with improved physical properties and increased antiviral potency in the presence of NHS.[2] Application of this finding led to pyrrolidinones **14** and **15**. Addition of a methyl sulfone[16] to **9** provides **14**, which shows very good antiviral activity in the presence of NHS with an IC_{95} of 63 nM (twofold loss of potency from FBS). Similar activity was observed with the ethyl analog **15**. These results correlate well with decreased protein binding and log P (see Table 1).

The rat pharmacokinetic (PK) profiles of representative compounds are shown in Table 2. Compound **9** exhibits low clearance and good oral bioavailability. The more potent analogs **13** and **14** displayed higher clearance and low oral bioavailability.



Scheme 1. Reagents: (a) $PdCl_2(PPh_3)_2$, toluene, reflux (92%); (b) $MeNH_2$, MeOH, 70 °C, sealed tube (48% of a 2.1/1 mixture of 8a:8b); (c) TFA, sealed tube, 105 °C (76%); (d) TFA 105 °C, sealed tube, 2 h followed by TFA, Et_3SiH, 75 °C, sealed tube (48%, one pot, 2 steps); (e) TFA, Et_3SiH, sealed tube, 75 °C.

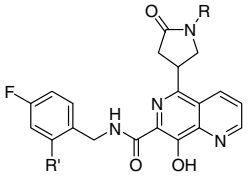
Table 1
Inhibition of HIV-1 integrase catalytic activities and HIV-1 replication in cells by a series of 5-(1-H or 1-alkyl-5-oxopyrrolidin-3-yl)-8-hydroxy-[1,6]-naphthyridine-7-carboxamides



Compound	R	R′	Inhibition of strand transfer IC_{50} (nM)[a]	Antiviral activity IC_{95} (nM) (10% FBS)[b]	Antiviral activity IC_{95} (nM) (50% NHS)[b]	%Prot.Bnd[c]	Log P[d]
9	Me	H	20	31 ($n = 3$)	125 ($n = 3$)	98.8	2.3
10	Et	H	14	16 ($n = 2$)	125 ($n = 3$)	98.9	2.7
11	*i*-Pr	H	40	31 ($n = 2$)	125 ($n = 3$)	97.8	2.7
12	H	H	24	42 ($n = 3$)	83 ($n = 3$)	insol.	n.d.
13	$CH_2C(O)NMe_2$	H	33	31 ($n = 3$)	63 ($n = 3$)	94.8	1.8
14	Me	SO_2Me	123	31 ($n = 3$)	63 ($n = 3$)	97.0	1.6
15	Et	SO_2Me	26	38 ($n = 5$)	81 ($n = 3$)	96.0	1.9

[a] The strand transfer assay was performed with recombinant HIV-integrase (100 nM) preassembled on immobilized oligonucleotides using 0.5 nM DNA.[2] Values are means of at least two experiments with a lower limit of accuracy ~5 nM, standard deviation is approximately twofold.
[b] Antiviral activity was assessed by measuring the decrease in HIV-1 p24 core antigen in MT-4 human T-lymphoid cells/HIV-1 IIIb cultured in the presence of increasing concentrations of inhibitor. Antiviral activity in cell culture (IC_{95}) is the drug concentration, which inhibits 95% viral growth relative to control. FBS: run with 10% fetal bovine serum, NHS: run with 50% normal human serum, see Ref. 4
[c] Measure of the percentage of test compound bound to human serum proteins, see Ref. 14.
[d] Log P measurement (partition coefficient), see Ref. 15.

Table 2
Rat pharmacokinetics



Compound	R	R'	Cl (mL/min/kg)[b]	$T_{1/2}$ (h)[b]	% *F*[c]
9[a]	Me	H	2.5	1.3	65
13[a]	$CH_2C(O)NMe_2$	H	23.4	3.4	5
14	Me	SO_2Me	17.2	0.67	4

[a] Compound dosed as Na salt.
[b] Dosed iv in DMSO at 2 mg/kg.
[c] Dosed po in 1% methylcellulose at 10 mg/kg.

In summary, we have developed an efficient route for the preparation of 4-[5-(1,6-naphthyridyl)]pyrrolidinones. The synthetic sequence permits late-stage variation of the lactam nitrogen substituent without tedious protection/deprotection steps. This methodology can also be used to prepare various 4-aryl substituted pyrrolidinones. Pyrrolidinone **9** is a potent inhibitor of HIV-1 integrase in cell culture. The compound exhibited low clearance and good oral bioavailability in rat. Installation of polar substituents on the pyrrolidinone nitrogen or the benzylamide provides compounds with lower protein binding and reduced shift in antiviral activity.

Acknowledgments

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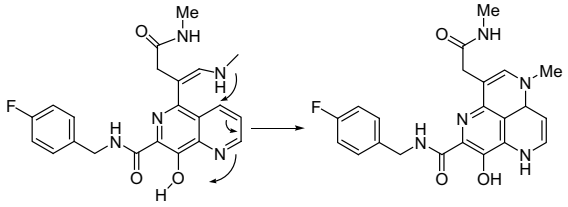
7. Compound **4** was prepared by the procedure described by Hollingworth, G. J.; Perkins, G.; Sweeney, J. *J. Chem. Soc., Perkin Trans. 1*, **1996**, 1913. Compound **5** was prepared as follows: To a stirring solution of *N*-(4-fluorobenzyl)-8-hydroxy-5-bromo-1,6-naphthyridine-7-carboxamide **3** (2.0 g, 5.32 mmol) in anhydrous toluene (35 mL) under nitrogen were added dichloro[bis(triphenylphosphine)]palladium (II) (0.374 g, 0.53 mmol) and 4-(tributylstannyl)furan-2(5H)-one **4** (1.98 g, 5.32 mmol). The solution was heated to reflux for 4.5 h, cooled, and the solids that precipitated from the solution were collected by vacuum filtration (1.85 g, 92%). The compound was taken onto the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.77 (1H, t, J = 6.4 Hz), 9.23 (1H, d, J = 3.4 Hz), 8.92 (1H, d, J = 8.0 Hz), 7.91–7.88 (1H, dd, J = 8.6, 4.2 Hz), 7.46–7.42 (2H, dd, J = 8.6, 5.6 Hz), 7.18 (2H, t, J = 8.9 Hz), 6.99 (1H, s), 5.69 (2H, s), 4.60 (2H, d, J = 6.4 Hz). ES HRMS calcd for $C_{20}H_{14}N_3O_4F$ (M+H): 380.1041, Found: 380.1050.
8. To a heavy wall glass pressure vessel was added **5** (0.83 g, 1.93 mmol) dissolved in a saturated solution of methylamine in MeOH (15.0 mL). The solution was heated to 55 °C for 2 h, cooled, and concentrated under reduced pressure. The crude material was dissolved in DMF (2 mL) and injected on the

Gilson autoprep using a Waters 6 μM Nova Pak reverse phase column with an 18 min gradient of 95:5 to 5:95, H_2O/CH_3CN/0.1% TFA. The desired fractions were combined and concentrated to afford 0.45 g (48%) of a mixture of diastereomers **8a** and **8b**, which were taken onto the next step. (NMR of analytically pure *trans* diastereomer): ^{1}H NMR (400 MHz, DMSO-d_6), δ 13.45 (1H, br s), 9.28 (1H, br s), 9.45–9.25 (1H, br s), 9.21 (1H, dd, J = 4.2, 1.4 Hz), 8.78–8.75 (1H, dd, J = 8.7, 1.3 Hz), 7.93–7.90 (1H, dd, J = 8.6, 4.2 Hz), 7.44–7.41 (2H, dd, J = 8.6, 5.6 Hz), 7.20 (2H, t, J = 8.9), 5.95 (1H, br s), 4.71–4.57 (3H, br s), 3.29–3.22 (1H, dd, J = 17.2, 9.6 Hz), 2.92 (3H, s), 2.64 (3H, s), 2.38–2.34 (1H, d, J = 18.5 Hz). ES HRMS calcd for $C_{22}H_{22}N_5O_3F$ (M+H): 424.1786, Found: 424.1781.

9. Tricyclic compound **8c** is the major product obtained upon prolonged reactions times and/or higher reaction temperatures. A proposed mechanism for formation of **8c:**

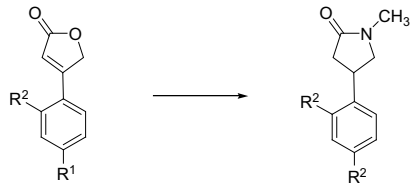


^{1}H NMR of **8c**: (400 MHz, DMSO-d_6), δ 13.25 (1H, br s), 9.68 (1H, t, J = 6.4 Hz), 8.44 (1H, d, J = 7.3 Hz), 8.10 (1H, m), 7.95 (1H, s), 7.43 (2H, m), 7.19 (3H, m), 4.64 (2H, d, J = 6.5 Hz), 3.87 (3H, s), 3.68 (2H, s), 2.48 (3H, d, J = 4.5 Hz).

10. A mixture of **8a** and **8b** (0.1 g, 0.24 mmol) in TFA (3 mL) in a glass pressure tube was heated to 105 °C for 2 h. The reaction mixture was cooled and concentrated, then redissolved in DMF (1 mL). The solution was injected on the Gilson autoprep using a Waters 6 μM Nova Pak reverse phase column with an 18 min gradient of 95:5 to 5:95, H_2O/CH_3CN/0.1% TFA. The desired fractions were combined and concentrated to afford 0.076 g (76%) of an inseparable 4:1 mixture of diastereomers **7a** and **7b**. Major component of mixture (**7a**): ^{1}H NMR (400 MHz, DMSO-d_6), δ 13.84 (1H, br s), 9.75 (1H, br s), 9.20 (1H, d, J = 4.2 Hz), 8.85 (1H, d, J = 8.7 Hz), 7.85 (1H, dd, J = 8.6, 4.2 Hz), 7.44 (2H, m), 7.19 (2H, m), 6.76 (1H, s), 4.80 (2H, s), 4.60 (2H, d, J = 6.4 Hz), 3.05 (3H, s). ES HRMS calcd for $C_{21}H_{17}N_4O_3F$ (M+H): 393.1357, Found: 393.1357.

11. A mixture of **8a** and **8b** (0.45 g, 1.06 mmol) in TFA (3 mL) in a glass pressure tube was heated to 105 °C for 2 h. The reaction mixture was then cooled to room temperature (once it was determined by LC–MS that elimination of methylamine had occurred). After cooling, triethylsilane (0.62 g, 5.31 mmol) was added to the solution, and the reaction mixture was heated to 75 °C for 1.5 h. The mixture was cooled and concentrated under reduced pressure. The crude mixture was re-dissolved in DMF (2 mL) and injected on the Gilson auto prep using a Waters 6 μM Nova Pak reverse phase column with an 18 min gradient of 95:5 to 5:95, water/CH_3CN/0.1% TFA. The desired fractions were combined and concentrated to afford 0.20 g, (48%) of **9** as an off-white solid.^{1}H NMR (400 MHz, DMSO-d_6), δ 13.55.

12. Examples of the synthesis of various substituted 4-aryl-pyrrolidinones from the corresponding butenolides:



R^1	R^2	Yield[a]
CH_3	H	55%
$N(CH_3)_2$	H	60%
NO_2	H	20%
F	H	49%
H	CH_3	98%

a) Isolated yield for two step process, unoptimized.
b) For conditions see Scheme 1, reactions b and d.

13. Resolution of **9** by chiral HPLC provides optically pure (+) and (−) isomers which are equipotent in the enzyme and cellular assays.
14. Analytical HPLC/UV detection-based assay that measures the ability of a compound to bind with human plasma (primarily Albumin) in pH 7.4 buffer at room temperature. Each value is the result of the average of three determinations.
15. UV detection method determining the relative concentration of a methanol solution of a compound partitioned between an octanol and pH 7.4 (KH_2PO_4/NaOH) water layer. Log P = log (Octanol HPLC area)(Octanol dilution)/(Buffer HPLC area)(Buffer dilution).
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