Synthesis and Antiviral Activity of 7-Benzyl-4-hydroxy-1,5-naphthyridin-2(1*H*)-one HIV Integrase Inhibitors

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The medicinal chemistry and structure−activity relationships for a novel series of 7-benzyl-4-hydroxy-1,5-naphthyridin-2(1*H*)-one HIV-integrase inhibitors are disclosed. Substituent effects were evaluated at the N-1, C-3, and 7-benzyl positions of the naphthyridinone ring system. Low nanomolar IC_{50} values were achieved in an HIV-integrase strand transfer assay with both carboxylic ester and carboxamide groups at C-3. More importantly, several carboxamide congeners showed potent antiviral activity in cellular assays. A 7-benzyl substituent was found to be critical for potent enzyme inhibition, and an *N*-(2-methoxyethyl)-carboxamide moiety at C-3 significantly reduced plasma protein binding effects in vitro. Pharmacokinetic data in rats for one carboxamide analogue demonstrated oral bioavailability and reasonable in vivo clearance.

Introduction

Current drug treatments for HIV and AIDS target primarily the inhibition of two viral enzymes, HIV reverse transcriptase[1] and HIV protease.[2] The recent introduction of entry inhibitors, such as enfuvirtide[3] and maraviroc,[4] has further improved treatment options. These regimens are highly successful at reducing viral load and the incidence of AIDS, but drug resistance and side effects still warrant a need for new drug targets in HIV therapy.

The third viral enzyme involved in HIV replication, namely, HIV integrase,[5] is an especially attractive target for drug therapy because it is critical for viral infectivity. In its initial state, HIV integrase is thought to chelate one divalent metal ion by two aspartic acid residues (D64, D116) in its catalytic triad (D64, D116, E152). The carboxyl group of the glutamic acid (E152) may chelate a second divalent metal ion upon binding of the viral DNA substrate. Integrase catalyzes the integration of reverse-transcribed viral DNA into the host chromosomal DNA through a two-step, metal-dependent process. In the first catalytic step (3′ processing), the enzyme promotes cleavage of a dinucleotide from the two 3′ ends of double stranded viral DNA; in the subsequent strand transfer (ST[a]) step, the viral DNA is inserted into the host cell's DNA. The gaps that remain in the product proviral DNA are subsequently repaired by cellular enzymes.

The global research effort to identify drugs that inhibit HIV integrase has spanned approximately 1.5 decades and recently led to human trials with several candidates, including S-1360 (**1**),[6] L-870,810 (**2**),[7] GS-9137 (**3**),[8] and MK-0518 (**4**) (Figure 1).[9] Compound **4** (raltegravir) was recently launched in the U.S. and is the first FDA-approved HIV integrase inhibitor for the treatment of HIV/AIDS. All of these molecules inhibit the ST step of HIV-1 integrase and possess submicromolar antiviral activity.

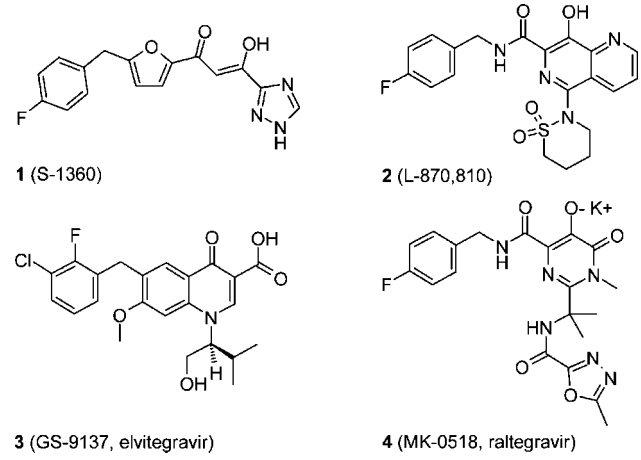

Figure 1. Clinical HIV-1 integrase strand transfer inhibitors.

Crystallographic analysis of inhibitors bound to the HIV integrase/viral DNA complex continues to be a significant challenge; consequently, the exact nature of their binding modes remains unclear. Nevertheless, the structural requirements for potent inhibition are relatively well-understood and both one-metal[10] and two-metal[11,12] binding models exist for the design of new inhibitor scaffolds. In general, these pharmacophore models require a diketoacid (or diketoacid-like bioisostere) attached to an aromatic ring by a flexible tether. The three-heteroatom metal binding region of the inhibitor, which typically includes a central acidic hydroxyl group, is believed to bind a magnesium ion (or ions) within the catalytic domain of the HIV integrase/viral DNA complex (following the 3′-processing step). In addition, because the tethered hydrophobic region (typically a benzyl substituent) is important for potent inhibition, it is plausible that this group makes an energetically favorable hydrophobic interaction within the catalytic domain of the

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[a] Abbreviations: ST, strand transfer assay; PHIV, pseudotype HIV assay; HSA, human serum albumin; MT-4, HIV replication assay in MT-4 cell lines; MT-4-$CCIC_{50}$, compound-induced cytotoxicity in MT-4 cells.

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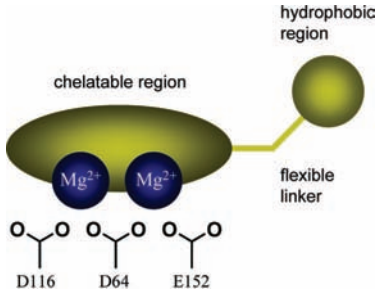


Figure 2. Generalized two-metal binding pharmacophore model for HIV-1 integrase strand transfer inhibitors.

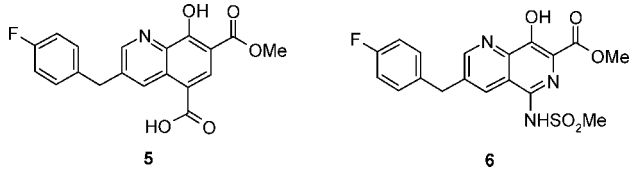


Figure 3. 3-Benzyl-8-hydroxyquinoline (**5**) and 3-benzyl-8-hydroxynaphthyridine (**6**) HIV-1 integrase strand transfer inhibitors.

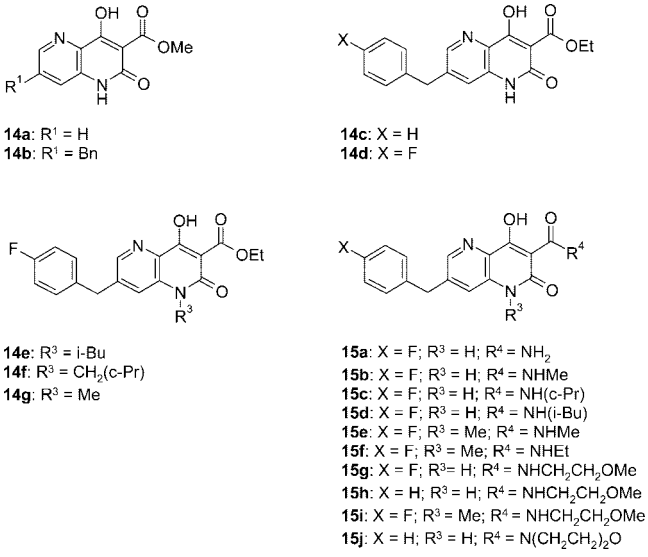


Figure 4. Structures of 4-hydroxy-1,5-naphthyridin-2(1*H*)-ones **14a**−**g** and **15a**−**j**.

enzyme.[10,12] The clinical inhibitors shown in Figure 1 all possess structural features in accordance with these models.

Scientists at Shionogi Research Laboratories have utilized the two-metal binding model (Figure 2) in the development of novel inhibitors containing a benzyl group directly attached to the heterocycle (or heterocyclic ring system) bearing the metal binding moiety. These include (in addition to compound **1**) the 3-benzyl-8-hydroxyquinolines[13,14] (e.g., **5**) and 3-benzyl-8-hydroxynaphthyridines[13,15] (e.g., **6**) (Figure 3). Interestingly, placement of the benzyl substituent in **5** and **6** (relative to inhibitor **2**) appears to reverse the binding orientation of the metal-chelating pyridyl nitrogen and carbonyl oxygen lone pairs while maintaining a centralized acidic hydroxyl group. Consequently, inhibitors **5** and **6** have been described as reversed[13] metal-binding scaffolds.

More recently, a joint research effort of scientists from Shionogi and GlaxoSmithKline exploited a new series of reversed scaffolds, namely, the 7-benzyl-4-hydroxy-1,5-naphthyridin-2(1*H*)-ones exemplified in Figure 4. This manuscript is the first in a series of planned reports on this joint research effort. As described below, the evolution from carboxylic ester analogues of the title series to carboxamide congeners was accompanied by a marked increase in antiviral activity. In addition, acceptable oral bioavailabity and in vivo clearance results in rats are presented for one 7-benzylnaphthyridinone congener.

Chemistry

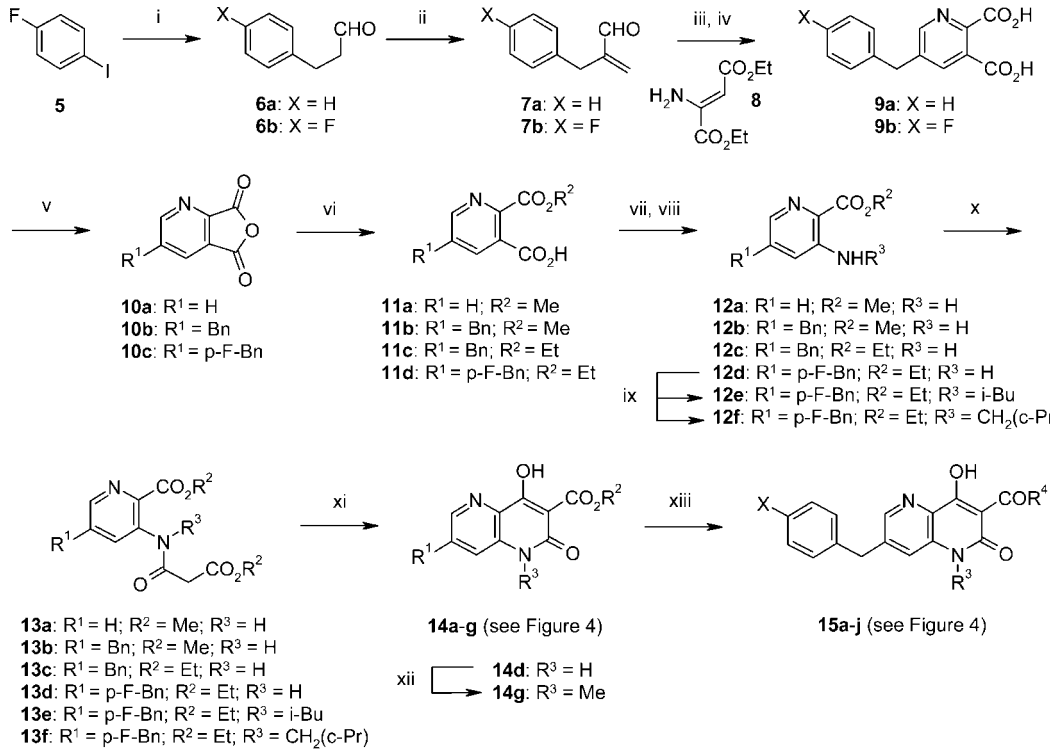
The synthetic pathways employed to prepare the title compounds are illustrated in Scheme 1. Heck coupling of 1-fluoro-4-iodobenzene (**5**) with allyl alcohol[16] afforded 4-fluorophenylpropanal (**6b**). Mannich methylenation[17] of propanals **6a**,**b** with formalin in the presence of diethylamine hydrochloride afforded 2-(phenylmethyl)-2-propenals **7a**,**b**. Reaction of propenals **7a**,**b** with diethyl (2*Z*)-2-amino-2-butenedioate (**8**)[18] gave crude pyridine dicarboxylic ester intermediates which were hydrolyzed to provide the corresponding diacids **9a**,**b**. Treatment of **9a**,**b** with acetic anhydride afforded the corresponding cyclic anhydrides **10b**,**c**. Ring-opening of cyclic anhydrides **10a**−**c** with MeOH or EtOH gave predominantly the 2-alkoxycarbonyl-3-pyridinecarboxylic acids **11a**−**d**[19,20] along with lesser amounts of the unwanted 3-alkoxycarbonyl-2-pyridinecarboxylic acid isomers. Treatment of **11a**−**d** with DPPA in *t*-BuOH provided *N*-BOC protected alkoxycarbonylaminopyridines by Curtius rearrangement. The resulting products were N-deprotected with TFA and purified by chromatography to afford the requisite 3-amino-2-pyridinecarboxylic esters **12a**−**d**. An alternative synthesis of the corresponding methyl ester of **12d** was subsequently developed for scale-up purposes and is described elsewhere.[21] The aminopyridine **12d** was converted to its *N*-isobutyl (**12e**) and *N*-cyclopropylmethyl (**12f**) derivatives by reductive amination with isobutyraldehyde or cyclopropanecarboxaldehyde in the presence of $NaBH(OAc)_3$. Treatment of **12a**−**f** with methyl- or ethylmalonyl chloride produced the malonylamide intermediates **13a**−**f** which were cyclized to naphthyridinone esters **14a**−**f** by addition of sodium methoxide or ethoxide. Compound **14d** was converted to its N1-methyl derivative **14g** by reaction with MeI in DMF in the presence of LiHMDS. Conversion of selected naphthyridinone esters (**14c**, **14d**, and **14g**) to carboxamides **15a**−**j** was performed by reaction with the requisite amines under thermal or microwave conditions.

Results and Discussion

In Vitro SAR. Structural formulas for the title compounds are provided in Figure 4. Results for ST inhibition are shown in Table 1 along with pseudotype HIV (PHIV) antiviral data, both with and without added human serum albumin (HSA). Antiviral data in MT-4 cell lines (with and without added HSA) and compound-induced cytotoxicity ($CCIC_{50}$) results are shown in Table 2. The MT-4 assay generally produced IC_{50} values higher than those seen in the PHIV screen, especially for compounds with high protein binding, and we attributed this difference to the higher protein concentrations in the MT-4 method. Integrase inhibitors **1** (S-1360) and **2** (L-870,810) were used as comparator compounds for these studies (see footnotes in Tables 1 and 2).

As shown in Table 1, a number of 7-benzylnaphthyridinone analogues were potent ST inhibitors of HIV integrase. Indeed, 7-benzyl substitution in the title series was found to be crucial for potent enzyme inhibition. Comparing the ST IC_{50} values of the des-benzyl methyl ester **14a** and its 7-benzyl congener **14b** showed a >60000-fold increase in inhibition potency as a result of this substitution, supporting the belief that this substituent

Scheme 1. Synthesis of 4-Hydroxy-1,5-naphthyridin-2(1*H*)-ones **14a**−**g** and **15a**−**j**[a]



[a] Reagents and conditions: (i) allyl alcohol, $Pd(OAc)_2$, $NaHCO_3$, BTEAC, DMF, 50 °C; (ii) CH_2O, $Et_2NH(HCl)$, 110 °C; (iii) $TsOH(H_2O)$, *n*-BuOH, 120 °C; (iv) NaOH, EtOH; (v) Ac_2O, 120 °C; (vi) R^2OH, reflux; (vii) DPPA, *t*-BuOH, TEA, 80 °C; (viii) TFA, DCM; (ix) R^3CHO, $Na(OAc)_3BH$, HOAc, DCE, room temp; (x) methyl- or ethylmalonyl chloride, DCE, reflux; (xi) $NaOR^2$, R^2OH; (xii) LiHMDS, MeI, DMF, room temp; (xiii) amine (R^4H), DMF, DMA or EtOH, 120−150 °C (sealed tube).

Table 1. HIV Integrase Strand Transfer (ST) Inhibition and Pseudo-HIV (PHIV) Antiviral Activity of 4-Hydroxy-1, 5-naphthyridin-2(1*H*)-ones[a]

	IC_{50}, nM [*N*]		
compd	ST	PHIV	PHIV (with 40 mg/mL HSA)
14a	>500000 [1]	>14000 [2]	NT
14b	8 ± 2 [2]	~14000 [2]	NT
14c	3 [1]	3700 ± 400 [2]	NT
14d	13 [1]	1200 [1]	NT
14e	22 ± 4 [2]	155 [1]	NT
14f	15 ± 6 [2]	358 [1]	NT
14g	6 [1]	77 ± 11 [2]	~12000 [1]
15a	45 [1]	66 ± 2 [2]	656 ± 92 [2]
15b	6 [1]	3.4 ± 0.4 [3]	119 [1]
15c	10 [1]	8 ± 1 [2]	>14000 [2]
15d	6 [1]	4 [1]	2500 ± 400 [2]
15e	4.1 ± 0.6 [2]	1.4 ± 0.3 [2]	57 ± 17 [2]
15f	4 ± 1 [2]	1.6 ± 0.2 [3]	661 ± 67 [4]
15g	5 ± 1 [2]	4.2 ± 0.2 [2]	36 ± 6 [2]
15h	7 ± 4 [2]	14 ± 2 [2]	59 ± 12 [4]
15i	4 ± 2 [2]	2 ± 1 [2]	50 ± 9 [4]
15j	427 [1]	2821 [1]	NT

[a] Data are expressed as mean values ± standard error ($N > 1$). Compound **1**: ST IC_{50} = 160 nM; PHIV IC_{50} = 110 nM (10 μM with HSA). Compound **2**: ST IC_{50} = 5 nM; PHIV IC_{50} = 2 nM (49 nM w/ HSA). *N* = number of experiments. NT = not tested.

Table 2. Anti-HIV Activity of 4-Hydroxy-7-benzyl-1, 5-naphthyridinones in MT-4 Cells[a]

	IC_{50}, nM [*N*]		
compd	MT-4	MT-4 (with 40 mg/mL HSA)	$CCIC_{50}$, μM [*N*], MT-4
14a	>125000 [1]	NT	>125 [1]
14b	>125000 [1]	NT	>125 [1]
14c	>39500 [1]	NT	103 [1]
14d	1200 [1]	NT	NT
14g	1300 [1]	NT	6 [1]
15b	30 [1]	NT	>8 [1]
15c	78 [1]	NT	>8 [1]
15d	68 [1]	NT	7 [1]
15e	11 ± 1 [2]	NT	20 [1]
15f	3.5 ± 0.6 [2]	4000 ±400 [3]	>50 [1]
15g	36 [1]	NT	>8 [1]
15h	91 ± 16 [2]	NT	>10 [2]
15i	5 ± 2 [3]	48 [1]	>20 [2]
15j	108000 [1]	NT	>125 [1]

[a] Data are expressed as mean values ± standard error ($N > 1$). Compound **1**: MT-4 $IC_{50} \approx 2$ μM; $CCIC_{50} = 42$ μM. Compound **2**: MT-4 $IC_{50} = 11$ nM; $CCIC_{50} = 3$ μM. *N* = number of experiments. NT = not tested.

makes a key hydrophobic interaction with the integrase/viral DNA complex. The corresponding 7-benzyl substituted ethyl ester (**14c**) also showed potent inhibition in the ST assay.

Despite the excellent enzyme inhibition effects of **14b** and **14c**, both compounds showed relatively low antiviral activity compared to our positive controls (**1** and **2**). Nonetheless, **14b** and **14c** served as good leads for SAR exploration at the N-1, C-3, and 7-benzyl positions.

Fluorine substitution on the benzyl group of the title series was important for optimizing antiviral activity. For example, 4-fluorobenzyl analogue **14d**, although slightly less potent than **14c** in the enzyme assay, showed improved antiviral activity in the cellular assays, particularly in the MT-4 ($IC_{50} \simeq 1.2$ μM) (Table 2). Similar results were observed in the amide series described below.

Following these observations, structural modifications at N-1 and C-3 positions of the naphthyridinone pharmacophore were investigated. Replacing the N-1 hydrogen substituent in the ester series with *N*-isobutyl (**14e**) and *N*-cyclopropylmethyl (**14f**) improved the antiviral activity in the PHIV assay. Methyl

substitution at N-1 (**14g**) improved both enzyme inhibition and PHIV antiviral activity compared to **14e** and **14f**. However, when **14g** was evaluated in the PHIV assay in the presence of serum protein (40 mg/mL HSA), a significant drop in potency was observed (>150-fold negative shift). Although the antiviral activity of **14g** in the MT-4 screen was disappointing (MT-4 $IC_{50} = 1.3$ μM), the compound did show a small separation between antiviral activity and cytotoxicity (MT-4 $CCIC_{50} \approx 6$ μM).

Marked improvements in antiviral activity were realized by converting ester derivatives **14c**, **14d**, and **14g** to carboxamide congeners **15a**−**i**. Primary carboxamide analogue **15a** showed two-digit nanomolar IC_{50} values in both the ST and PHIV assays and only a 10-fold negative protein shift in the PHIV. The corresponding *N*-methylcarboxamide analogue **15b** was a single digit nanomolar inhibitor in both the ST and PHIV assays but showed a more significant protein shift compared to **15a**. Similarly, the *N*-cyclopropyl (**15c**) and *N*-isobutyl (**15d**) amide analogues, although potent inhibitors, lost considerable antiviral activity on addition of serum protein. Compounds **15b**, **15c**, and **15d** showed two-digit nanomolar IC_{50} values in the MT-4 assay and >100-fold separation between antiviral activity and cytotoxicity. Comparing the inhibition potency and anti-PHIV activities of **15a**−**d** suggested that simple *N*-alkyl carboxamide groups, although well-tolerated for enzyme inhibition purposes, posed significant protein binding issues.

Interestingly, the presence of an N-1 methyl substituent in the *N*-methylcarboxamide series (e.g., **15e**) appeared to increase enzyme inhibition and antiviral potency (relative to **15b**) without a significant increase in protein binding. The corresponding *N*-ethylcarboxamide analogue (**15f**) was also a potent ST inhibitor, but its antiviral activity was again hampered by added HSA. In the MT-4 assay, compounds **15b**−**f** showed one- to two-digit nanomolar antiviral activity and produced no cytotoxicity up to concentrations of ≥ 7 μM. On the other hand, it was apparent from these results that lipophilic *N*-alkylcarboxamide substituents (larger than methyl) were detrimental to the druglike properties of the target molecules.

The negative influence of HSA on the antiviral potencies of **15c**, **15d**, and **15f** prompted us to incorporate a more polar, hydrophilic substituent on the amide nitrogen. We were pleased to discover that *N*-(2-methoxy)ethylcarboxamide analogue **15g** was quite potent in the PHIV assay and displayed a relatively low protein shift (<9-fold negative). Desfluoro analogue **15h** was also synthesized and found to be nearly equipotent to its 4-fluorobenzyl congener (**15g**) in the ST assay but less potent in both the PHIV and MT-4. This result further demonstrated the importance of a fluorobenzyl substituent for enhanced antiviral effects. Both compounds **15g** and **15h** showed two-digit nanomolar antiviral activity in the MT-4 assay and no cytotoxicity up to 8−10 μM.

By combining optimal substituents at all three positions (i.e., methyl group at N-1, *N*-(2-methoxy)ethylcarboxamide at C-3, and 4-fluorobenzyl at C-7), we obtained a new inhibitor (**15i**) that was single-digit nanomolar in all three assays (ST, PHIV, and MT-4) with a <10-fold negative protein shift in the MT-4 assay. In addition, compound **15i** showed a large separation between antiviral activity and cytotoxicity in the MT-4 relative to comparator compounds **1** and **2**.

As mentioned previously, the three-heteroatom metal-binding regions of the title compounds and *N*-benzylhydroxynaphthyridinecarboxamides (e.g., **2**)[7] are similar, but the relative placement of their tethered aromatic rings are inverted. Because the benzyl substituent is of paramount importance for potent inhibition, the most favored three-dimensional binding space occupied by the tethered aromatic ring should be similar for both pharmacophores. Consequently, it is reasonable to assume that the binding modes of their chelatable regions are reversed (Figure 5).[22]

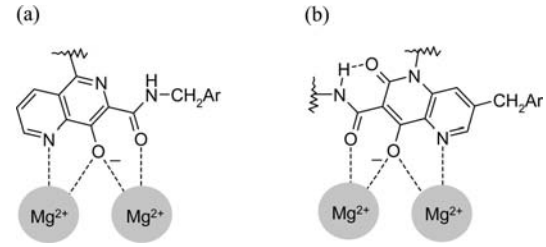


Figure 5. Proposed reversed-binding orientations of *N*-benzylnapthyridinecarboxamide (a) and 7-benzylnaphthyridinone (b) pharmacophores. It is assumed that the most favored binding space occupied by the tethered aromatic ring (Ar) is similar for each pharmacophore.

The data for compounds in Figure 4 indicate that potent enzyme inhibition may be achieved by incorporating a 7-benzyl substituent on the metal binding hydroxynaphthyridinone template and that druglike properties (i.e., cellular antiviral activity and plasma protein binding) may be modulated by varying the substitution at both the N-1 and the C-3 positions. This is consistent with the minimal pharmacophore requirements of the two-metal binding model shown in Figure 2.

This model also appears to favor a planar orientation of the three-heteroatom chelatable region on the inhibitor. The metal binding motif of ester congeners **14b**−**d** could readily adopt a planar configuration. In the case of carboxamide analogues **15a**−**i**, the amide N−H can form an intramolecular hydrogen bond (via a six-membered ring) with the naphthyridinone carbonyl oxygen at C-2 (Figure 5b) which should further stabilize a planar conformation of the metal binding motif. Consistent with this hypothesis, morpholinocarboxamide **15j** was significantly less potent than its closest congener (**15h**) in the ST assay (Table 1). The relatively weak inhibition activity of **15j** may be explained by intramolecular steric repulsion between the ring carbonyl oxygen at C-2 and the morpholino group which forces the exocyclic amide carbonyl out of plane with the naphthyridinone ring.

In Vivo Pharmacokinetics. The pharmacokinetic properties of **15i** were subsequently evaluated in male rats, and the results are illustrated in Figure 6. Following iv administration of **15i** in male CD rats (1 mg/kg), systemic clearance averaged 11.8 (mL/min)/kg, approximately 20% of rat hepatic blood flow;[23] steady-state volume of distribution was 0.6 L/kg, similar to total body water volume in the rat.[23] Oral administration of **15i** (5 mg/kg) resulted in a bioavailability of 25% in fasted rats and 20% in nonfasted rats. Furthermore, fasted rats had a second peak in their oral concentration−time profile, suggestive of enterohepatic recirculation, which may have contributed to increased bioavailability compared with nonfasted rats. In both groups, oral absorption was rapid, with peak plasma concentrations occurring at 15−30 min following dose administration.

Conclusion

In summary, the 7-benzyl-4-hydroxynaphthyridinone template proved to be a viable starting point for developing druglike HIV integrase inhibitors for the potential treatment of HIV/AIDS. By taking advantage of the observed substituent effects at the N-1, C-3, and 7-benzyl positions of the core template, improvements in enzyme inhibition and antiviral activity were achieved coupled with reduced protein binding. A 7-(4-fluorobenzyl)

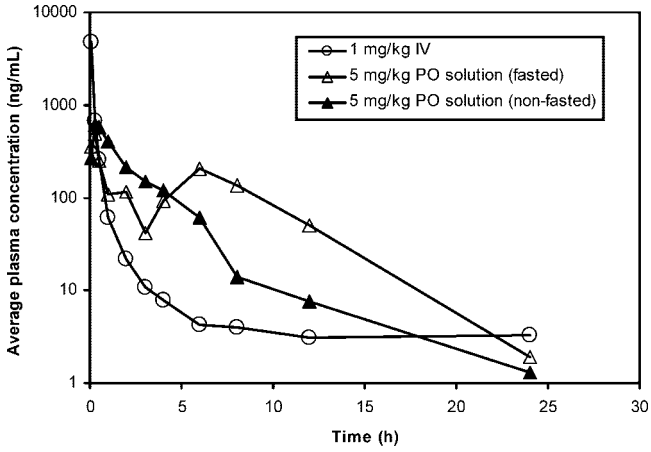


Figure 6. Plasma concentration−time profiles for compound **15i** following iv (1 mg/kg) and po (5 mg/kg) administration in fasted and nonfasted male CD rats.

substituent in combination with an N-1 methyl group and C-3 carboxamide moiety produced optimum cellular antiviral activity; moreover, by incorporation of an *N*-(2-methoxy)ethyl carboxamide moiety at C-3, a significant reduction in plasma protein binding was accomplished. Pharmacokinetic data for compound **15i** in rats demonstrated reasonable oral bioavailability (20−25%) and acceptable in vivo clearance (11.8 (mL/min)/kg). Additional SAR studies in the title series leading to the discovery and development of clinical candidate GSK364735[24] will be reported in future publications.

Experimental Section

Chemistry (General). ^{1}H NMR spectra were recorded at 400 MHz; melting points are uncorrected. Compounds **5**, **6a**, and **10a** were obtained commercially. Compound **12e** was prepared from **12d** and isobutyraldehyde, employing a method similar to that described for the preparation of **12f**. Intermediates **13a**, **13b**, **13c**, **13e**, and **13f** were prepared from **10a**−**c** by methods similar to those described for preparation of **13d** from **10c**. Purities of test compounds were established by analytical HPLC (C-18 column, 5.0 μm, 0% → 100% CH_3CN (or MeOH)/water with 0.05−0.1% HCOOH (or TFA)) and UV detection with or without evaporative light scattering detection (ELSD). All test compounds showed >95% purity (AUCs by UV detection). Preparative HPLC conditions were as follows: C-18 column, 5 μm, 21.2 mm × 150 mm; flow rate = 4 mL/min; mobile phase, 10% → 100% CH_3CN/H_2O/0.1% HCOOH (10 min run).

3-(4-Fluorophenyl)propanal (6b). To a mixture of **5** (300 g, 1.35 mol), benzyltriethylammonium chloride (300 g, 1.35 mol), $NaHCO_3$ (283 g, 3.4 mol), and allyl alcohol (138 mL, 2.0 mol) in DMF (300 mL) was added palladium acetate (3.0 g, 13.5 mmol). The mixture was heated at 50 °C for 5 h with stirring. Water (1 L) and Et_2O (1 L) were added at room temperature. After filtration through Celite, the filtrate was extracted with Et_2O. The extracts were washed with H_2O and brine, then dried and concentrated to yield **6b** (205 g, 100% crude yield): ^{1}H NMR ($CDCl_3$) δ 9.81 (1H, s), 7.16 (2H, m), 6.97 (2H, m), 2.93 (2H, t, J = 7.5 Hz), 2.77 (2H, t, J = 7.5 Hz).

5-(Phenylmethyl)-2,3-pyridinedicarboxylic Acid (9a). A stirred mixture of **6a** (49 mL, 0.37 mol), diethylamine hydrochloride (41 g, 0.37 mol), and 37% formalin (33 mL, 0.45 mol) was heated at 110 °C for 1 h. The mixture was cooled to room temperature, diluted with water (150 mL), and extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated to afford **7a** as a pale-amber oil. Neat **7a** (44 g, 0.3 mol) was added dropwise over 2.5 h to a stirred solution of **8**[18] (47 g, 0.25 mol) and $TsOH\cdot H_2O$ (0.5 g, 2.6 mmol) in 1-butanol (100 mL) at 120 °C. The reaction mixture was stirred an additional 10 h at this temperature and then concentrated at reduced pressure. The crude product was dissolved in EtOH (125 mL), and a solution of NaOH (30 g, 0.75 mol) in water (100 mL) was added. The reaction mixture was stirred for 2 h at room temperature, diluted with water (100 mL), and concentrated by rotovap. The remaining material was acidified with 6 N HCl (125 mL) and stirred at room temperature for 1.5 h. The supernatant was decanted, and the remaining solid was washed with water and dried under high vacuum to afford **9a** as an off-white solid (73 g, 76% overall crude yield): ^{1}H NMR (DMSO-d_6) δ 13.5 (2H, br), 8.62 (1H, s), 8.08 (1H, d, J = 1.2 Hz), 7.35−7.02 (m, 5H), 4.05 (2H, s); MS m/z 255 $(M - H)^-$.

5-[(4-Fluorophenyl)methyl]-2,3-pyridinedicarboxylic Acid (9b). This compound was prepared from crude **6b** by methods similar to those described above for the preparation of **9a**. The product (**9b**) was obtained as a yellow solid (12% overall yield): ^{1}H NMR (DMSO-d_6) δ 13.5 (2H, br), 8.64 (1H, d, J = 2 Hz), 8.01 (1H, d, J = 2 Hz), 7.32 (2H, dd, J = 9, 6 Hz), 7.12 (2H, t, J = 9 Hz), 4.05 (2H, s); MS m/z 276 $(M + H)^+$.

2-[(Ethoxy)carbonyl]-5-(phenylmethyl)-3-pyridinecarboxylic Acid (11c). A stirred mixture of **9a** (73 g, 0.25 mol crude) and Ac_2O (300 mL, 3.2 mol) was heated at 120 °C for 3 h. The mixture was concentrated by rotovap chasing with toluene. The resulting material was dissolved in EtOH (300 mL), and the stirred solution was heated at reflux overnight. The solution was concentrated by rotovap chasing with toluene to afford crude **11c** (78 g, >100% crude yield). This material contained ~40% of the corresponding 3-[(ethoxy)carbonyl]-2-pyridinecarboxylic acid isomer. Major isomer (**11c**): ^{1}H NMR (DMSO-d_6) 8.53 (1H, s), 7.97 (1H, s), 7.32−6.99 (5H, m), 4.23 (2H, q, J = 7 Hz), 4.03 (2H, s), 1.22 (3H, t, J = 7 Hz); MS m/z 286 $(M + H)^+$.

2-[(Ethoxy)carbonyl]-5-[(4-fluorophenyl)methyl]-3-pyridinecarboxylic Acid (11d). This compound was prepared from **9b** by a method similar to that described above for the synthesis of **11c**. The crude product (**11d**) contained ~33% of the corresponding 3-[(ethoxy)carbonyl]-2-pyridinecarboxylic acid isomer). Major isomer (**11d**): ^{1}H NMR (DMSO-d_6) δ 13.5 (1H, br), 8.67 (1H, d, J = 2 Hz), 8.06 (1H, d, J = 2 Hz), 7.31 (2H, dd, J = 9, 6 Hz), 7.11 (2H, m, J = 9 Hz), 4.25 (2H, q, J = 7 Hz), 4.06 (2H, s), 1.24 (3H, t, J = 7 Hz); MS m/z 304 $(M + H)^+$.

Ethyl 3-Amino-5-(phenylmethyl)-2-pyridinecarboxylate (12c). A mixture of **11c** (78 g, 0.25 mol crude), *t*-BuOH (600 mL), TEA (80 mL, 0.58 mol), and DPPA (86 mL, 0.4 mol) was heated at reflux for 5 h. The mixture was concentrated by rotovap, dissolved in EtOAc, and washed with saturated NH_4Cl solution, $NaHCO_3$ solution, and brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by flash chromatography on silica gel, eluting with 33% EtOAc/hexanes to afford a mixture of *N*-BOC protected ethyl aminopyridinecarboxylate isomers (42 g). This material was dissolved in CH_2Cl_2 (300 mL). TFA (100 mL) was added, and the solution was stirred at room temperature for 3 h. The mixture was concentrated by rotovap, reconstituted in EtOAc, and washed with $NaHCO_3$ solution. The organic phase was dried over Na_2SO_4, concentrated, and purified by flash chromatography on silica gel, eluting with 20−60% EtOAc/hexanes to afford **12c** (8.7 g, 13% yield) as a solid: mp 119−122 °C; ^{1}H NMR (DMSO-d_6) δ 7.77 (1H, d, J = 2 Hz), 7.32−7.27 (2H, m), 7.22−7.18 (3H, m), 6.94 (1H, d, J = 2 Hz), 6.62 (2H, br s), 4.23 (2H, q, J = 7 Hz), 3.87 (2H, s), 1.26 (3H, t, J = 7 Hz); HRMS m/z calcd for $C_{15}H_{17}N_2O_2$, 257.1290 $(M + H)^+$; found, 257.1286.

Ethyl 3-Amino-5-[(4-fluorophenyl)methyl]-2-pyridinecarboxylate (12d). This compound was prepared from **11d** by methods similar to those described above for the preparation of **12c**. The product (**12d**) was obtained as a light-yellow solid (50% yield): mp 137−138 °C; ^{1}H NMR (DMSO-d_6) δ 7.76 (1H, d, J = 1.7 Hz), 7.25 (2H, m), 7.15 (2H, m, J = 9 Hz), 6.92 (1H, d, J = 1.7 Hz), 6.62 (2H, br s), 4.23 (2H, q, J = 7 Hz), 3.87 (2H, s), 1.26 (3H, t, J = 7 Hz); HRMS m/z calcd for $C_{15}H_{16}FN_2O_2$, 275.1196 $(M + H)^+$; found, 275.1206.

Ethyl 3-[(Cyclopropylmethyl)amino]-5-[(4-fluorophenyl)methyl]-2-pyridinecarboxylate (12f). A stirred solution of **12d** (416 mg, 1.52 mmol) in 1,2-dichloroethane (10 mL) was cooled to 0 °C. Cyclopropanecarboxaldehyde (0.17 mL, 2.27 mmol) and acetic acid (0.45 g, 7.55 mmol) were added followed by $Na(OAc)_3BH$ (640 mg, 3.02 mmol). The mixture was allowed to warm to room temperature and stirred for 2 days. The mixture was quenched with saturated $NaHCO_3$ solution and extracted with CH_2Cl_2. The organic layer was washed with brine, dried, and concentrated. The crude product was purified by flash chromatography on silica gel, eluting with 67% petroleum ether/EtOAc to afford **12f** as an oil (450 mg, 91% yield): ^{1}H NMR ($CDCl_3$) δ 7.85 (1H, d, $J = 2$ Hz), 7.78 (1H, br), 7.15−7.06 (2H, m), 6.96 (2H, m), 6.75 (1H, s), 4.42 (2H, q, $J = 7$ Hz), 3.89 (2H, s), 2.96 (2H, m), 1.42 (3H, t, $J = 7$ Hz), 1.07 (1H, m), 0.57 (2H, m), 0.23 (2H, m).

Ethyl 3-{[3-(Ethoxy)-3-oxopropanoyl]amino}-5-[(4-fluorophenyl)methyl]-2-pyridinecarboxylate (13d). A solution of **12d** (1.2 g, 4.2 mmol) and ethyl malonyl chloride (1.08 mL, 8.4 mmol) in 1,2-dichloroethane (65 mL) was heated at reflux for 2.5 h. The reaction mixture was cooled to room temperature, diluted with CH_2Cl_2, and washed with saturated $NaHCO_3$ solution. The organic phase was dried and concentrated. The crude material was purified by flash chromatography on silica gel, eluting with 0−5% MeOH/CH_2Cl_2 to afford **13d** as an oil (1.5 g, 94% yield): ^{1}H NMR ($CDCl_3$) δ 11.52 (1H, br s), 8.96 (1H, d, $J = 2$ Hz), 8.32 (1H, d, $J = 2$ Hz), 7.19−7.12 (2H, m), 7.10 (2H, m), 4.53 (2H, q, $J = 7$ Hz), 4.29 (2H, q, $J = 7$ Hz), 4.02 (2H, s), 3.54 (2H, s), 1.48 (3H, t, $J = 7$ Hz), 1.33 (3H, t, $J = 7$ Hz); MS *m*/*z* 387 $(M - H)^-$.

Methyl 4-Hydroxy-2-oxo-1,2-dihydro-1,5-naphthyridine-3-carboxylate (14a). A solution of **13a** (1 g, 4 mmol) in MeOH was treated with 4.36 M NaOMe/MeOH (1.73 mL, 8 mmol) and stirred at room temperature for 1 h. The reaction mixture was neutralized with 1 N HCl (8 mL, 8 mmol), and the product was collected by filtration. The solid was triturated with MeOH and dried under vacuum to afford **14a** as a white solid (0.55 g, 63% yield): mp 282−285 °C; ^{1}H NMR (DMSO-d_6) δ 11.71 (1H, s), 8.51 (1H, m), 7.69−7.61 (2H, m), 3.75 (3H, s); MS *m*/*z* 221 $(M + H)^+$; HRMS *m*/*z* calcd for $C_{10}H_9N_2O_4$, 221.0562 $(M + H)^+$; found, 221.0571.

Methyl 4-Hydroxy-2-oxo-7-(phenylmethyl)-1,2-dihydro-1,5-naphthyridine-3-carboxylate (14b). This compound was prepared from **13b** employing methods similar to those described above for synthesis of **14a**. Compound **14b** was obtained as a tan solid (65% yield): mp 210−211 °C (dec); ^{1}H NMR (DMSO-d_6) δ 11.59 (1H, s), 8.46 (1H, d, $J = 2$ Hz), 7.43 (1H, d, $J = 2$ Hz), 7.33−7.30 (2H, m), 7.26−7.20 (3H, m), 4.11 (2H, s), 3.74 (3H, s); MS *m*/*z* 311 $(M + H)^+$; HRMS *m*/*z* calcd for $C_{17}H_{15}N_2O_4$, 311.1032 $(M + H)^+$; found, 311.1025.

Ethyl 4-Hydroxy-2-oxo-7-(phenylmethyl)-1,2-dihydro-1,5-naphthyridine-3-carboxylate (14c). A solution of **13c** (1 g, 2.7 mmol) in EtOH (10 mL) was treated with 2 M NaOEt/EtOH (2.7 mL, 5.4 mmol), and the solution was stirred 30 min at room temperature. The reaction mixture was neutralized with concentrated HCl. The product was collected by filtration washing with 1:1 brine/water and dried to afford a white solid (879 mg, 100% yield): mp >275 °C (dec); ^{1}H NMR (DMSO-d_6) δ 10.72 (1H, br s), 8.24 (1H, br s), 7.36−7.23 (6H, m), 4.14 (2H, q, $J = 7$ Hz), 4.06 (2H, s), 1.22 (3H, t, $J = 7$ Hz); MS *m*/*z* 325 $(M + H)^+$. Anal. ($C_{18}H_{16}N_2O_4 \cdot 1.38NaCl$) C, H, N.

Ethyl 7-[(4-Fluorophenyl)methyl]-4-hydroxy-2-oxo-1,2-dihydro-1,5-naphthyridine-3-carboxylate (14d). This compound was prepared from **13d** employing methods similar to those described above for synthesis of **14c**. Compound **14d** was obtained as a beige solid (86% yield): mp 235−236 °C; ^{1}H NMR (DMSO-d_6) δ 11.54 (1H, br s), 8.54 (1H, d, $J = 1.4$ Hz), 7.44 (1H, s), 7.32 (2H, dd, $J = 8, 6$ Hz), 7.17 (2H, t, $J = 9$ Hz), 4.23 (2H, q, $J = 7$ Hz), 4.12 (2H, s), 1.26 (3H, t, $J = 7$ Hz); MS *m*/*z* 343 $(M + H)^+$; HRMS *m*/*z* calcd for $C_{18}H_{16}FN_2O_4$, 343.1094 $(M + H)^+$; found, 343.1088.

Ethyl 7-[(4-Fluorophenyl)methyl]-4-hydroxy-1-(2-methylpropyl)-2-oxo-1,2-dihydro-1,5-naphthyridine-3-carboxylate (14e). An ice-cooled solution of **13e** (307 mg, 0.69 mmol) in EtOH (3 mL) was treated with solid NaOEt (104 mg, 1.53 mmol), and the reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated by rotovap, reconstituted in water, and extracted with Et_2O. The aqueous phase was acidified to pH 5 with 1 N HCl and extracted with EtOAc. The organic phase was dried and concentrated to afford **14e** as a rigid foam (279 mg, 99% yield): ^{1}H NMR ($CDCl_3$) δ 13.75 (1H, br), 8.49 (1H, s), 7.26−7.21 (1H, m), 7.19−7.11 (2H, m), 7.04 (2H, m), 4.50 (2H, q, $J = 7$ Hz), 4.11 (2H, s), 3.95 (2H, br), 1.95 (1H, m), 1.45 (3H, t, $J = 7$ Hz), 0.87 (6H, d, $J = 7$ Hz); MS *m*/*z* 399 $(M + H)^+$; HRMS *m*/*z* calcd for $C_{22}H_{24}FN_2O_4$, 399.1720 $(M + H)^+$; found, 399.1720.

Ethyl 1-(Cyclopropylmethyl)-7-[(4-fluorophenyl)methyl]-4-hydroxy-2-oxo-1,2-dihydro-1,5-naphthyridine-3-carboxylate (14f). This compound was prepared from **13f** by methods similar to those described above for synthesis of **14e**. Compound **14f** was obtained as a rigid foam (99% yield): ^{1}H NMR ($CDCl_3$) δ 13.80 (1H, br), 8.50 (1H, s), 7.41 (1H, s), 7.22−7.09 (2H, m), 7.03 (2H, m), 4.51 (2H, q, $J = 7$ Hz), 4.12 (2H, s), 4.06 (2H, d, $J = 8$ Hz), 1.45 (3H, t, $J = 7$ Hz), 0.97 (1H, m), 0.54−0.31 (4H, m); MS *m*/*z* 397 $(M + H)^+$; HRMS *m*/*z* calcd for $C_{22}H_{22}FN_2O_4$, 397.1564 $(M + H)^+$; found, 397.1563.

Ethyl 7-[(4-Fluorophenyl)methyl]-4-hydroxy-1-methyl-2-oxo-1,2-dihydro-1,5-naphthyridine-3-carboxylate (14g). Solid LiHMDS (610 mg, 3.65 mmol) was added to a stirred mixture of **14d** (500 mg, 1.46 mmol) in DMF (24 mL). After the mixture was stirred for a few minutes, MeI (545 μL, 8.76 mmol) was added and the reaction mixture was stirred for 1 h. The DMF was removed by rotovap under high vacuum, and the crude material was diluted with water and extracted with CH_2Cl_2. The organic phase was dried and concentrated to afford **14g** as an amber glass (520 mg, 100% yield): ^{1}H NMR ($CDCl_3$) δ 13.9 (1H, br), 8.51 (1H, d, $J = 1$ Hz), 7.34 (1H, s), 7.15 (2H, m), 7.02 (2H, m), 4.50 (2H, q, $J = 7$ Hz), 4.12 (2H, s), 3.55 (3H, s), 1.46 (3H, t, $J = 7$ Hz); MS *m*/*z* 357 $(M + H)^+$; HRMS *m*/*z* calcd for $C_{19}H_{18}FN_2O_4$, 357.1250 $(M + H)^+$; found, 357.1244.

7-[(4-Fluorophenyl)methyl]-4-hydroxy-2-oxo-1,2-dihydro-1,5-naphthyridine-3-carboxamide (15a). A mixture of **14d** (35 mg, 0.10 mmol), 28−30% NH_3/H_2O (0.5 mL), and EtOH (1.5 mL) was heated in a sealed tube in a microwave at 120 °C for 40 min. Additional 28−30% NH_3/H_2O (0.1 mL) was added, and heating was continued for 20 min. The mixture was cooled to room temperature, and the product was collected by filtration. The filter cake was washed with EtOH and dried to afford **15a** as a light-yellow powder (22 mg, 69% yield): mp 314−315 °C (dec); ^{1}H NMR (*d*-TFA) δ 8.77 (1H, s), 8.51 (1H, s), 7.32−7.25 (2H, m), 7.13 (2H, t, $J = 9$ Hz), 4.43 (2H, s); MS *m*/*z* 314 $(M + H)^+$; HRMS *m*/*z* calcd for $C_{16}H_{13}FN_3O_3$, 314.0941 $(M + H)^+$; found, 314.0947.

7-[(4-Fluorophenyl)methyl]-4-hydroxy-*N*-methyl-2-oxo-1,2-dihydro-1,5-naphthyridine-3-carboxamide (15b). A mixture of **14d** (35 mg, 0.10 mmol), 2 M $MeNH_2$/MeOH (0.5 mL, 1 mmol), and EtOH (0.75 mL) was heated in a sealed tube in a microwave at 140 °C for 45 min. The mixture was cooled to room temperature, and the crude product was collected by filtration. The filter cake was triturated with EtOH/water and dried to afford **15b** as a white solid (18 mg, 52% yield): mp >380 °C (dec); ^{1}H NMR (DMSO-d_6) δ 11.62 (1H, br), 10.41 (1H, br), 8.22 (1H, br), 7.27−7.20 (3H, m), 7.12 (2H, m), 4.01 (2H, s), 2.78 (3H, br s); MS *m*/*z* 328 $(M + H)^+$; HRMS *m*/*z* calcd for $C_{17}H_{15}FN_3O_3$, 328.1097 $(M + H)^+$; found, 328.1084.

***N*-Cyclopropyl-7-[(4-fluorophenyl)methyl]-4-hydroxy-2-oxo-1,2-dihydro-1,5-naphthyridine-3-carboxamide (15c).** A neat mixture of **14d** (35 mg, 0.10 mmol) and cyclopropylamine (292 mg, 5.11 mmol) was heated in a sealed tube at 120 °C for 24 h. The mixture was cooled to room temperature and diluted with EtOH, and the crude product was collected by filtration. The filter cake was triturated with hot EtOH and dried to afford **15c** as a white solid (21 mg, 57% yield): mp 315−316 °C (dec); ^{1}H NMR (DMSO-d_6) δ 11.80 (1H, br), 10.35 (1H, br), 8.35 (1H, br), 7.38 (1H, s), 7.30 (2H, m), 7.15 (2H, t, $J = 8.7$ Hz), 4.06 (2H, br s), 2.85 (1H, m), 0.74 (2H, m), 0.51 (2H, m); MS *m*/*z* 354 $(M + H)^+$; HRMS *m*/*z* calcd for $C_{19}H_{17}FN_3O_3$, 354.1254 $(M + H)^+$; found, 354.1255.

7-[(4-Fluorophenyl)methyl]-4-hydroxy-*N*-(2-methylpropyl)-2-oxo-1,2-dihydro-1,5-naphthyridine-3-carboxamide (15d). This compound was prepared from **14d** and isobutylamine employing methods similar to those described above for synthesis of **15c**. Compound **15d** was obtained as a white solid (59% yield): mp 320−321 °C (dec); ^{1}H NMR (DMSO-d_6) δ 8.60−8.10 (2H, br), 7.41 (1H, br s), 7.48−7.31 (1H, m), 7.29 (2H, m), 7.13 (2H, m), 4.13−3.96 (2H, br), 3.15 (2H, br m), 1.79 (1H, br m), 0.90 (6H, d, J = 7 Hz); MS m/z 370 (M + H)$^+$; HRMS m/z calcd for $C_{20}H_{21}FN_3O_3$, 370.1567 (M + H)$^+$; found, 370.1559.

7-[(4-Fluorophenyl)methyl]-4-hydroxy-*N*,1-dimethyl-2-oxo-1,2-dihydro-1,5-naphthyridine-3-carboxamide (15e). This compound was prepared by treatment of **14g** with 2 M $MeNH_2$/MeOH using methods similar to those described above for synthesis of **15b**. Compound **15e** was purified by preparative HPLC followed by recrystallization from CH_3CN and was obtained as a white solid (32% yield): mp 189−190 °C; ^{1}H NMR ($CDCl_3$) δ 10.09 (1H, br), 8.57 (1H, d, J = 2 Hz), 7.39 (1H, s), 7.19−7.13 (2H, m), 7.02 (2H, t, J = 9 Hz), 4.14 (2H, s), 3.58 (3H, s), 3.02 (3H, d, J = 5 Hz); MS m/z 342 (M + H)$^+$; HRMS m/z calcd for $C_{18}H_{17}FN_3O_3$, 342.1254 (M + H)$^+$; found, 342.1254.

***N*-Ethyl-7-[(4-fluorophenyl)methyl]-4-hydroxy-1-methyl-2-oxo-1,2-dihydro-1,5-naphthyridine-3-carboxamide (15f).** This compound was prepared by treatment of **14g** with 2 M $EtNH_2$/MeOH employing methods similar to those described above for synthesis **15b**. Compound **15f** was purified by preparative HPLC and was obtained as a pale-yellow solid (15 mg, 38% yield): mp 184−185 °C; ^{1}H NMR ($CDCl_3$) δ 10.12 (1H, br), 8.56 (1H, s), 7.39 (1H, s), 7.18−7.12 (2H, m), 7.02 (2H, t, J = 9 Hz), 4.13 (2H, s), 3.57 (3H, s), 3.48 (2H, m), 1.27 (3H, t, J = 7 Hz); MS m/z 356 (M + H)$^+$; HRMS m/z calcd for $C_{19}H_{19}FN_3O_3$, 356.1405 (M + H)$^+$; found, 356.1410.

7-[(4-Fluorophenyl)methyl]-4-hydroxy-*N*-[2-(methoxy)ethyl]-2-oxo-1,2-dihydro-1,5-naphthyridine-3-carboxamide (15g). This compound was prepared from **14d** and 2-(methoxy)ethylamine employing methods similar to those described above for synthesis of **15c**. Compound **15g** was obtained as a white solid (66% yield): mp 302−303 °C (dec); ^{1}H NMR (DMSO-d_6) δ 11.80 (1H, br), 10.40 (1H, br), 8.37 (1H, br), 7.39 (1H, br), 7.31 (2H, m), 7.15 (2H, br t, J = 9 Hz), 4.07 (2H, br s), 3.47 (4H, br), 3.33 (3H, s); MS m/z 372 (M + H)$^+$; HRMS m/z calcd for $C_{19}H_{19}FN_3O_4$, 372.1360 (M + H)$^+$; found, 372.1372.

4-Hydroxy-*N*-[2-(methoxy)ethyl]-2-oxo-7-(phenylmethyl)-1,2-dihydro-1,5-naphthyridine-3-carboxamide (15h). This compound was prepared from **14c** and 2-(methoxy)ethylamine employing methods similar to those described above for synthesis of **15b**. The crude product was triturated with CH_2Cl_2/MeOH and dried to afford **15h** as a white solid (51% yield): mp 301−302 °C (dec); ^{1}H NMR (DMSO-d_6) δ 11.85 (1H, br), 10.75 (1H, br), 8.23 (1H, br s), 7.35−7.22 (6H, m), 4.03 (2H, s), 3.45 (4H, br), 3.28 (3H, s); MS m/z 354 (M + H)$^+$; Anal. ($C_{19}H_{19}N_3O_4 \cdot 0.25CH_2Cl_2$) C, H, N.

7-[(4-Fluorophenyl)methyl]-4-hydroxy-1-methyl-*N*-[2-(methoxy)ethyl]-2-oxo-1,2-dihydro-1,5-naphthyridine-3-carboxamide (15i). This compound was prepared from **14g** and 2-(methoxy)-ethylamine employing methods similar to those described above for synthesis of **15b**. Compound **15i** was purified by preparative reverse phase HPLC and was obtained as a white solid (50% yield): mp 188−190 °C; ^{1}H NMR ($CDCl_3$) δ 10.34 (1H, br), 8.59 (1H, d, J = 1 Hz), 7.41 (1H, s), 7.16 (2H, m), 7.03 (2H, m), 4.14 (2H, s), 3.65 (2H, m), 3.60 (2H, m), 3.59 (3H, s), 3.42 (3H, s); MS m/z 386 (M + H)$^+$; HRMS m/z calcd for $C_{20}H_{21}FN_3O_4$, 386.1516 (M + H)$^+$; found, 386.1531.

4-Hydroxy-3-(4-morpholinylcarbonyl)-7-(phenylmethyl)-1,5-naphthyridin-2(1*H*)-one (15j). A mixture of **14c** (50 mg, 0.15 mmol), morpholine (403 mg, 4.62 mmol), and DMA (0.5 mL) was heated in a sealed tube in a microwave at 150 °C for 45 min. The reaction mixture was cooled to room temperature, and the crude product was collected by filtration washing with EtOAc and hexanes. The filter cake was triturated with hot MeOH and collected by filtration as an off-white solid (17 mg, 30% yield): mp >335 °C; ^{1}H NMR (DMSO-d_6) δ 9.93 (1H, br s), 8.04 (1H, s), 7.35−7.16 (6H, m), 3.98 (2H, s), 3.61−3.43 (6H, m), 3.31 (2H, m); MS m/z 366 (M + H)$^+$; HRMS m/z calcd for $C_{20}H_{20}N_3O_4$, 366.1455 (M + H)$^+$; found, 366.1452.

Biological Methods: ST Assay. Compounds were tested as inhibitors of recombinant HIV integrase in the following in vitro ST assay. A complex of integrase and biotinylated donor DNA-streptavidin-coated SPA beads was formed by incubating 2 μM recombinant integrase with 0.66 μM biotinylated donor DNA−4 mg/mL streptavidin-coated SPA beads in 25 mM sodium MOPS, pH 7.2, 23 mM NaCl, 10 mM $MgCl_2$, 10 mM dithiothreitol, and 10% DMSO for 5 min at 37 °C. Beads were pelleted by centrifugation, and supernatant was removed. The beads were resuspended in 25 mM sodium MOPS, pH 7.2, 23 mM NaCl, 10 mM $MgCl_2$. Beads were again spun down, supernatant was removed, and the beads were resuspended in a volume of 25 mM sodium MOPS pH 7.2, 23 mM NaCl, 10 mM $MgCl_2$ that would give 570 nM integrase (assuming all integrase bound the DNA−beads). Test compounds dissolved and diluted in DMSO were added to the integrase−DNA complex to give 6.7% DMSO (typically 1 μL of compound added to 14 μL of integrase complex) and preincubated for 60 min at 37 °C. Then [^{3}H] target DNA substrate was added to give a final concentration of 7 nM substrate, and the ST mixture was incubated at 37 °C typically for 25−45 min, which allowed for a linear increase in covalent attachment of the donor DNA to the radiolabeled target DNA. A 20 μL reaction was quenched by adding 60 μL of the following: 50 mM sodium EDTA, pH 8, 25 mM sodium MOPS, pH 7.2, 0.1 mg/mL salmon testes DNA, 500 mM NaCl. Streptavidin-coated SPA were from GE Healthcare, oligos to make the donor DNA were from Oligos Etc, and [^{3}H] target DNA was a custom synthesis from Perkin-Elmer. Sequences of donor and target DNA were previously described (see the following: Hazuda, D. J.; Hastings, J. C.; Wolfe, A. L.; Emini, E. A. *Nucleic Acids Res.* **1994**, *22*, 1121−1122) with the addition of seven terminal As on each end of the target DNA that allowed for the incorporation of 14 tritiated Ts (specific activity of target DNA of approximately 1300 Ci/mmol).

PHIV Assay. VSV-G pseudotyped HIV vector expressing luciferase was generated by co-transfecting pGJ3-Luci (see the following: Jarmy, G.; Heinkelein, M.; Weissbrich, B.; Jassoy, C.; Rethwilm, A. *J. Med. Virol.* **2001**, *64*, 223−231) and pVSV-G (Clontech) into 293T cells by the calcium phosphate method. Approximately 5 h following transfection, the medium was exchanged. Sodium butyrate was added at 10 mM, and the cultures were incubated for approximately 40 h when the cell supernatants were harvested, filtered through a 0.45 or 1.0 μm filter, and stored at −80 °C. Compounds were dissolved in DMSO, diluted in medium (DMEM + 10% FCS), and plated at 100 μL per well in 96-well black, clear bottom tissue culture plates (Costar). 293T cells were harvested by trypsinization, counted, and mixed with PHIV viral vector in medium (DMEM + 10% FCS). The amount of PHIV was adjusted to give approximately 100K CPM in the assay. One hundred (100) microliters of cell-virus mixture was then plated on top of the compounds to give 2×10^4 cells per well. The plates were incubated at 37 °C and 5% CO_2 for 2 days. The medium was aspirated from the plates, and 100 μL of prepared Steady Glo reagent (Promega) mixed 1:1 with medium was added per well. The plates were then read in a Topcount instrument (Perkin-Elmer) for 1 s per well. The effect of serum proteins on antiviral potency was evaluated with selected test compounds; in these instances, the standard assay was performed with the addition of 40 mg/mL HSA (catalog no. A1653; Sigma, St. Louis, MO).

MT-4 Cell HIV Replication Assay. Antiviral HIV activity and $CCIC_{50}$ values were measured in parallel by means of an methanethiosulfonate tetrazolium (MTS) based procedure in the HTLV-1 transformed cell line MT-4 as previously described (see the following: Hazen, R.; Harvey, R.; Ferris, R.; Craig, C.; Yates, P.; Griffin, P.; Miller, J.; Kaldor, I.; Ray, J.; Samano, V.; Furfine, E.; Spaltenstein, A.; Hale, M.; Tung, R.; St. Clair, M.; Hanlon, M.; Boone, L. *Antimicrob. Agents Chemother.* **2007**, *51*, 3147−3154). Cells were prepared in medium (RPMI 1640, 20% [vol/vol] FBS, 20% [vol/vol] T-cell growth factor [catalog no. 801017, Zeptom-

etrix], and 10 μg/mL gentamicin) and were infected by the addition of HIV-1 strain IIIB, with infection allowed to proceed for 1 h at 37 °C in a tissue culture incubator with humidified 5% CO_2 atmosphere. Then aliquots of the cell suspension were added to each well of the plate containing compounds prediluted in RPMI medium containing 10% [vol/vol] FBS and 10 μg/mL gentamicin. The resulting RPMI assay medium contained 15% [vol/vol] FBS, 10% [vol/vol] T-cell growth factor, and 10 μg/mL gentamicin. Plates were then placed in a tissue culture incubator at 37 °C with humidified 5% CO_2 for 5 days. HIV-induced cytopathic effects were assessed by a CellTiter96 MTS staining method (catalog no. G3581; Promega, Madison, WI). The optical density at 492 nm was measured by using a microplate absorbance reader (catalog no. 20-300; Tecan, Research Triangle Park, NC). The effect of serum proteins on antiviral potency was evaluated with selected test compounds. In these instances, the standard assay was performed with the addition of 40 mg/mL HSA (catalog no. A1653; Sigma, St. Louis, MO).

In Vivo Pharmacokinetic Methods. Male CD rats ($n = 2$ per study arm) received **15i** at doses of 1 mg/kg iv (1 mL/kg, fasted), 5 mg/kg po (5 mL/kg, fasted), or 5 mg/kg po (5 mL/kg, nonfasted), formulated in a 10% DMSO/15% Solutol/75% 0.05 M *N*-methylglutamine dosing vehicle. For fasted animals, the food supply was removed the evening before dosing and replaced at 4 h following dose administration; for all animals, water was provided ad libitum. Blood samples were withdrawn from a surgically implanted venous cannula at timed intervals for 24 h after dose administration, treated with EDTA, and centrifuged to harvest plasma for LC/MS/MS analysis. Plasma concentration−time data for individual rats were analyzed using noncompartmental analysis (WinNonlin, version 3.0A; Pharsight, Mountain View, CA) to generate pharmacokinetic parameter estimates.

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Supporting Information Available: Analytical HPLC chromatograms for compounds **14a**−**g** and **15a**−**j**. This material is available free of charge via the Internet at http://pubs.acs.org.

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