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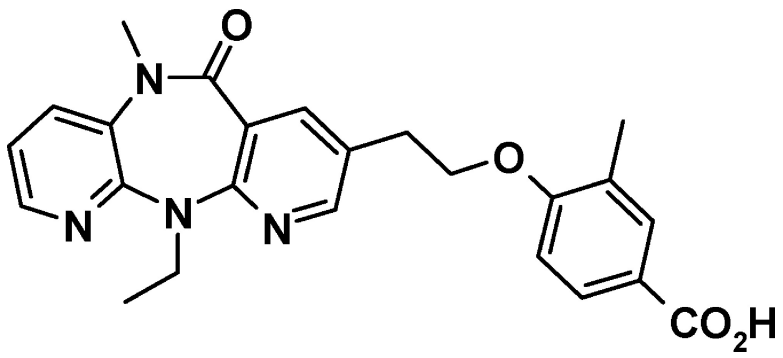

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Novel 8-Substituted Dipyridodiazepinone Inhibitors with a Broad-Spectrum of Activity against HIV-1 Strains Resistant to Non-nucleoside Reverse Transcriptase Inhibitors

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A series of novel 8-substituted dipyridodiazepinone-based inhibitors were investigated for their antiviral activity against wild type human immunodeficiency virus (HIV-1) and the clinically prevalent K103N/Y181C mutant virus. Our efforts have resulted in a series of benzoic acid analogues that are potent inhibitors of HIV-1 replication against a panel of HIV-1 strains resistant to non-nucleoside reverse transcriptase inhibitors (NNRTIs). Furthermore, the combination of good antiviral potency, a broad spectrum of activity, and an excellent pharmacokinetic profile provides strong justification for the further development of compound **7** as a potential treatment for wild type and NNRTI-resistant HIV-1 infection.

Introduction

Acquired immune deficiency syndrome (AIDS) is a disease caused by the human immunodeficiency virus (HIV). Upon entering the cell, the virus relies on three enzymes to establish infection: the reverse transcriptase (RT), integrase, and protease. In the last two decades, progress has been made to understand and control viral replication.[1] In particular, the introduction of highly active antiretroviral therapy (HAART) has been effective in reducing mortality and morbidity. It is well established that RT inhibitors are important drugs in cocktail-based therapy.[2] They are divided into the following two classes: nucleoside reverse transcriptase inhibitors (NRTIs), such as zidovudine (AZT, Retrovir), and non-nucleoside reverse transcriptase inhibitors (NNRTIs), such as nevirapine (Viramune). Although structurally different, all NNRTIs share a common mode of action; they interfere with RT activity via binding to an allosteric site adjacent to the catalytic domain of the protein.[3,4]

As is often observed in any antiviral therapy, the use of NNRTIs in treatment eventually leads to the development of resistance due to drug-specific mutations of the RT enzyme.[5] Furthermore, cross-resistance between presently available NNRTIs results in a loss of further NNRTI options for experienced patients. For these reasons, the development of new NNRT inhibitors is still an active field of pharmaceutical research.[6,7] Thus, new drugs must have a broad spectrum of activity against a variety of clinically relevant mutant RT enzymes combined with a favorable pharmacokinetic profile and low toxicity.

We have recently reported that 8-heteroarylthiomethyldipyridodiazepinone derivatives,[8] exemplified by compound **1** (Figure 1), provide potent inhibitors of HIV-1 viral replication. However, the metabolic instability of this series of inhibitors precluded further development. We have also described a series of inhibitors containing an ethoxy linker,[9,10] as seen in compounds **2** and **3** (Figure 1), which displayed excellent broad antiviral activity against a panel of prevalent RT mutants in addition to good biopharmaceutical properties. We would like to report here our synthetic work as well as structure−activity relationships (SARs) using 8-(2-hydroxyethyl)dipyridodiazepinones (**5a**−**d**) as our starting intermediates. Our effort has resulted in a series of analogues that are potent against wild type (WT) HIV-1 and a panel of NNRTI-resistant HIV-1 strains. Furthermore, this work has led to the identification of a candidate for toxicological evaluation.

Chemistry

The syntheses of the key intermediates 8-(2-hydroxyethyl)dipyridodiazepinones (**5a**−**d**) were realized starting from the corresponding tricyclic bromides (**4a**−**d**).[11] Introduction of the 2-hydroxyethyl substituent was accomplished using a palladium-mediated cross-coupling reaction with allyltributyltin followed by ozonolysis and a reductive workup to give **5a**−**d**, as depicted in Scheme 1. In all cases, assembly of the inhibitors was accomplished via the Mitsunobu reaction. Saponification of the esters resulted in the formation of the desired free acids.

The Mitsunobu[12] reaction represents one of the most convenient routes to the synthesis of aryl ethers. For example, the use of triphenylphosphine and the required phenol **6** in the presence of diisopropyl azodicarboxylate (DIAD) resulted in the conversion of primary alcohol **5a** to the corresponding aryl ether followed by saponification to yield the inhibitor **7** as shown in Scheme 2. The major disadvantage often encountered with the Mitsunobu reaction, particularly in large scale synthesis, is the purification of the desired product from the

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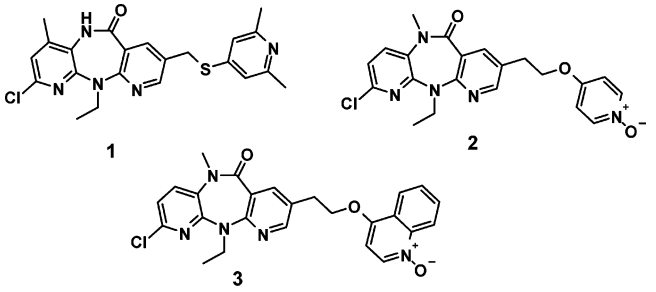
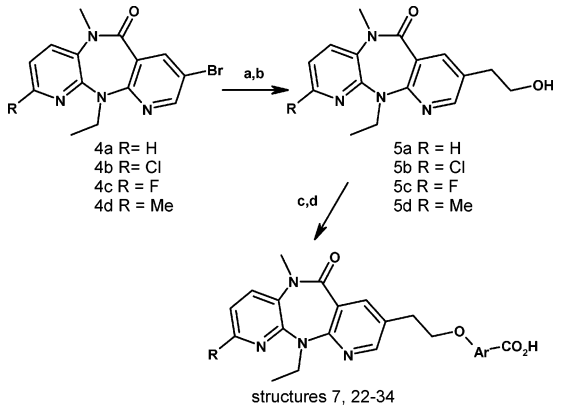


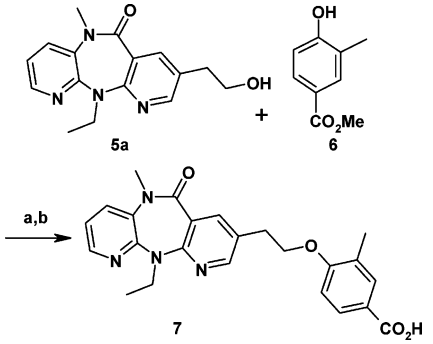
Figure 1. Previously reported NNRTIs in the dipyridodiazepinone class.

Scheme 1



Conditions: (a) $CH_2{=}CHCH_2SnBu_3$, $(Ph_3P)_4Pd$, DMF 80 °C; (b) O_3, CH_2Cl_2/MeOH followed by $NaBH_4$; (c) Ph_3P, DIAD, THF, ArOH; (d) NaOH.

Scheme 2

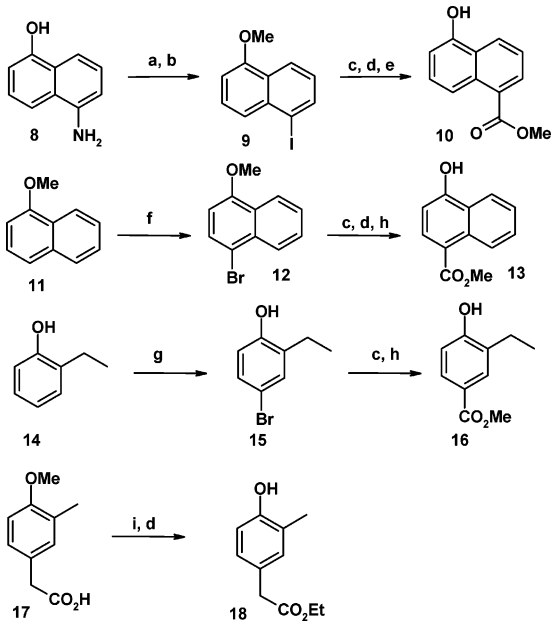


Conditions: (a) Ph_3P, DIAD, THF or DPPBE, DIAD, THF; (b) 1 N LiOH, THF/MeOH.

triphenylphosphine oxide generated as a by-product. To alleviate this problem, we have used 4-diphenylphosphanylbenzoic acid 2-trimethylsilanylethyl ester (DPPBE)[13] as an efficient replacement for triphenylphosphine. The corresponding phosphine oxide was easily removed after cleavage of the trimethylsilanyl ethyl ester and washing with aqueous base. Thus, reaction on a 5 g scale of alcohol **5** in the presence of phenol **6** using DPPBE and DIAD in THF gave the corresponding ether with excellent yield (81%) and purity that did not necessitate further purification.

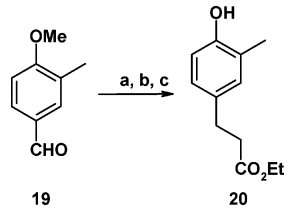
Most phenols used in this study were commercially available or accessed using published procedures or are exemplified in published patents.[14,15] For instance, phenol **6** was obtained from commercially available 4-hydroxy-3-methylbenzoic acid by treatment with refluxing methanol in the presence of a catalytic amount of HCl. A few phenols required a slightly more elaborate

Scheme 3



Conditions: (a) $NaNO_2$, HCl, followed by NaI; (b) NaH, MeI, DMF; (c) *n*-BuLi, THF, from −78 °C to 0 °C, then CO_2(g); (d) BBr_3/CH_2Cl_2; (e) MeOH, H^+; (f) NBS, CH_3CN; (g) Br_2, $CHCl_3$, HCl; (h) CH_2N_2, Et_2O; (i) oxalyl chloride, CH_2Cl_2, DMF(cat), EtOH.

Scheme 4



Conditions: (a) $(EtO)_2P(O)CH_2CO_2Et$, *n*-BuLi, THF; (b) H_2, Pd/C (10%); (c) BBr_3, CH_2Cl_2, EtOH.

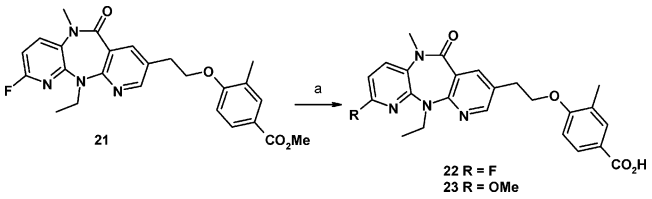
synthesis, as shown in Schemes 3 and 4. Diazotization of 5-amino-1-naphthol (**8**) followed by in situ conversion to the iodo compound and methyl ether formation gave compound **9**. Lithium−halogen exchange and trapping with CO_2 followed by cleavage of the methyl ether and esterification afforded the desired phenol **10**.

Commercially available 1-methoxynaphthalene (**11**) was brominated using *N*-bromosuccinimide. Lithium−halogen exchange and trapping with CO_2 gave the corresponding acid. Deprotection of the methyl ether and diazomethane treatment of the acid gave ester **13**. Alternatively, regioselective bromination of commercially available phenol **14** gave ester **16** in excellent yield using a similar synthetic sequence. Finally, phenol **18** was obtained by esterification of the acid followed by cleavage of the methyl ether using boron tribromide.

Homologated carboxylic acid was obtained from commercially available aldehyde **19** using Horner−Emmons conditions to yield the corresponding unsaturated ester (Scheme 4). Hydrogenation of the double bond followed by deprotection gave the needed phenol **20**.

Interestingly, as highlighted in Scheme 5, saponification of ester **21** using aqueous LiOH in methanol allowed us to isolate not only the corresponding carboxylic acid **22** but also the product of the methanolysis at the 2 position, giving the analogous 2-methoxy derivative **23**. 2-Fluoropyridine is known to be more

Scheme 5



Conditions: (a) 1 N LiOH, MeOH.

reactive toward methoxide in methanol than the corresponding 2-chloro analogue.[16] Indeed, this side reaction was not observed when compound **33** was prepared.

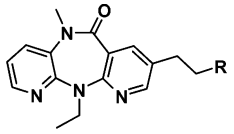
Results and Discussion

In the early stages of our work, we relied on the enzymatic activity (IC_{50})[17] against the WT RT as well as the key mutant K103N/Y181C for determination of structure−activity relationships. The choice of the K103N/Y181C mutant stems from the necessity for a next generation NNRTI to display potent antiviral activity against this genotype, which is the most prevalent double mutant observed in the clinic.[5] Furthermore, good activity against K103N/Y181C was an indicator of broad activity against other clinically relevant mutants. For potent compounds, the enzymatic profiling was followed by the evaluation of the antiviral activity (EC_{50})[17] in the WT virus as well as the double mutant virus. Broad antiviral profiling was done on the most promising inhibitors.

On the basis of a model structure of HIV-1 RT in the presence of our inhibitors,[18] the C-8 heterocycle (for example, the pyridine-*N*-oxide moiety present in compound **2** of Figure 1) appears to be mostly solvent exposed. On the basis of this observation, we hypothesized that a well positioned carboxylic acid should provide new derivatives with equivalent potency but a different physicochemical profile. In particular, the presence of an acid functionality should improve solubility and cellular permeability, thus having a positive impact on pharmacokinetic profile.

Our initial search for carboxylic acid-containing inhibitors was directed to the evaluation of a family of naphthoic acid derivatives, as outlined in Table 1. In the case of naphthoic acid derivatives, going from the 4-ethoxy (**24**) to the 5-ethoxy (**25**) substitution caused a loss of potency of over 2-fold against both the WT and mutant RT. Interestingly, moving the ether to the 6 position of the naphthyl ring did not affect the activity against the WT enzyme (cf. compounds **26** and **25**); however, the activity against the K103N/Y181C mutant enzyme dropped by 7.2-fold. Modifying the position of the carboxyl group (e.g., **27**) led to excellent activity against the WT enzyme (IC_{50} = 14 nM); however, activity against the mutant was inadequate. Inhibitor **24**, having the best enzymatic profile, was evaluated for antiviral potency. The 10-fold difference in potency between the WT and mutant enzymatic activities was maintained in the cellular assay, with $EC_{50,WT}$ = 4.8 nM and $EC_{50,K103N/Y181C}$ = 40 nM. Compound **24** was then selected for in vivo pharmacokinetic evaluation in rats. After oral gavage[19] of the naphthoic acid **24** at 5 mg/kg, we observed an area under the curve (AUC_{po}) of 6.9 μM·h and a maximum plasma concentration (C_{max}) of 0.64 μM which corresponds to ~130-fold the EC_{50}

Table 1. Activities of Naphthoic Acid Derivatives



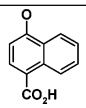
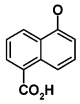
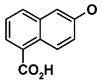
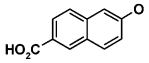
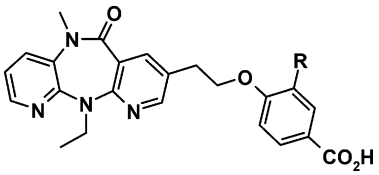
cpd	R	IC_{50} (nM) WT	IC_{50} (nM) K103N/Y181C
24		27	194
25		60	550
26		63	4000
27		14	1100

Table 2. Activities of Benzoic Acid Derivatives

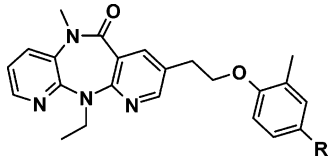


cpd	R	IC_{50} (nM) WT	IC_{50} (nM) K103N/Y181C	EC_{50} (nM) WT	EC_{50} (nM) K103N/Y181C	stability HLM $t_{1/2}$ (min)
28	H	95	4480	3.3	162	76
7	Me	17	221	1.6	15	134
29	Et	19	430	2.2	24	140
30	Br	18	300	3.2	35	55

value against wild type HIV but only 16-fold for the double mutant. Following intravenous injection[19] (1 mg/kg), inhibitor **24** exhibited an excellent half-life[20] ($t_{1/2}$ = 8.7 h). The apparent bioavailability was 75%. In an attempt to capitalize on the excellent biopharmaceutical profile, we looked at the SAR around the tricyclic core, but it proved to be challenging to further improve the antiviral potency of the naphthoic acid derivatives. Thus, we decided to evaluate substituted benzoic acid analogues as mimics of the naphthoic acid.

The unsubstituted benzoic acid derivative (compound **28**, Table 2) had a disappointing activity profile. However, addition of a single methyl (compound **7**) not only improved the enzymatic activity but also translated into an 11-fold improvement in EC_{50} against the mutant. Ethyl and bromo substitution (compounds **29** and **30**) were also well tolerated with only a slight loss of antiviral potency. Although not shown, we have looked at a large number of other groups, such as isopropyl, benzyl, F, CF_3, OMe, NO_2, and NH_2, with less success. As shown in Table 2, compounds were also evaluated for first-pass metabolism[21] using male human liver microsomes.[22] The nonsubstituted compound **28** and the

Table 3. Impact on Activity of Selected Carboxylates



cpd	R	IC_{50} (nM) WT	IC_{50} (nM) K103N/Y181C	EC_{50} (nM) WT	EC_{50} (nM) K103N/Y181C	stability HLM $t_{1/2}$ (min)	Caco-2 A−B (10^{-6} cm/s)
7	CO_2H	17	221	1.6	15	134	27.5
31	CH_2CO_2H	23	370	12	139	216	18.4
32	$(CH_2)_2CO_2H$	22	200	5.5	101	206	46.9

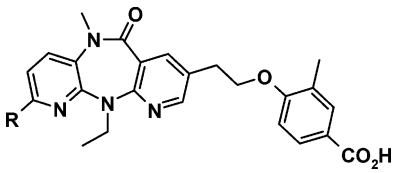
bromo derivative **30** were rapidly metabolized with a half-life shorter than 100 min.

We then turned our attention to homologues by inserting one or two methylene spacers between the phenyl and the acid group, as shown in Table 3. It was interesting to find that the acetic acid **31** and the propanoic acid **32** gave IC_{50} values similar to that of the carboxylic acid **7** in both IC_{50} assays. However, significant differences were observed in the cellular assay (EC_{50}), in particular, against the K103N/Y181C virus. This discrepancy could not be explained by poor cell penetration. Indeed, measuring the permeability across the Caco-2 cell monolayer gave good permeability for the three compounds. Inhibitors **31** and **32** have more ionizable carboxyl groups, which could in part explain these differences in cell culture media. Furthermore, regardless of the structural class they belong to, NNRTIs usually display more potent activity in cell culture than in enzymatic reverse transcriptase assays. This is true for the first generation NNRTIs efavirenz, nevirapine, and delavirdine, and it extends as well to new chemical series such as capravirine, etravirine, and our own 8-substituted dypyridodiazepinone compounds (Tables 2−4).

NNRTIs provoke pausing in the transcription, with RT often falling off the primer/template (P/T). In most enzymatic RT assays, the readout is based on incorporation of radiolabeled nucleotides to a P/T. Inhibition is detected as reduced overall incorporation compared to control. Whether RT pauses or falls off, transcription can always resume (on the same or another P/T) without any consequences other than reduced incorporation. However, in cell culture, these effects are believed to have a more dramatic impact. Repeated RT pausing or falling off not only prevents full transcription of the viral RNA in a timely fashion but also slows down the process considerably, which makes the reaction product more susceptible to degradation. Therefore, pausing viral transcription leads to a greater negative impact on the virus than on the incorporation of radiolabeled nucleotides in an in vitro assay.

Having established that the best aryl group was the 3-methylbenzoic acid derivative, we decided, based on previous knowledge,[8,9] to further optimize at the C-2 position of the dipyridodiazepinone ring. As outlined in Table 4, these derivatives were equipotent against WT virus in the enzymatic assay, which was also reflected in the antiviral potency. In the K103N/Y181C mutant assay, the 2-chloro derivative (**33**) was found to be the most potent with an excellent EC_{50} value, which for the first time in this series of inhibitors was below 10 nM.

Table 4. SAR at C-2



cpd	R	IC_{50} (nM) WT	IC_{50} (nM) K103N/Y181C	EC_{50} (nM) WT	EC_{50} (nM) K103N/Y181C	stability HLM $t_{1/2}$ (min)
7	H	17	221	1.6	15	134
22	F	15	150	1.5	14	79
23	OMe	15	300	1.9	15	105
33	Cl	10	64	1.6	8.1	35
34	Me	19	280	1.3	33	106

Table 5. Antiviral Activity against Various Mutants (nM)

cpd	V106A	K103N/P225H	K103N/V108I	K103N/L100I	Y188L
7	36	5	5	4	172
22	27	2	4	3	27

Unfortunately compound **33** was the least stable in the human liver microsome preparation, which precluded further evaluation. The 2-fluoro and 2-methoxy analogues (**22** and **23**) gave compounds which were equipotent to inhibitor **7**. Inhibitor **34**, containing the 2-methyl substituent, was only 2-fold less potent against the double mutant virus. However, all four analogues were found to be less stable than compound **7** when evaluated in human liver microsome preparations.

The two most interesting compounds were evaluated for their antiviral activities against a panel of mutant viruses, as shown in Table 5. Compounds **7** and **22** have similar overall potencies, with the notable exception observed against the virus containing the Y188L mutation, where the fluoro analogue **22** was 6-fold more potent. Furthermore, we evaluated the antiviral activity in the presence of 50% human serum as an assessment of activity lost upon protein binding. Protein binding is an important factor in the prediction of in vivo potency of any drug. Both compounds **7** and **22** exhibited similar profiles with moderate 3- and 4-fold shifts, respectively.

Additional predevelopment studies revealed that compounds **7** and **22** were not potent inhibitors of any of the five major human Cyp450 isoforms. As shown in Table 6, both inhibitors had IC_{50} values significantly greater than their respective antiviral activity. This finding is important in the context of HIV therapy, where drug−drug interaction is of particular concern.

Table 6. Cyp450 Inhibition Profile

	IC_{50} (μM)					
cpd	1A2	2C9	2C19	2D6	3A4-BFC[a]	3A4-BQ[a]
7	>30	10	2	>30	24	>30
22	19	6	2	>30	10	>30

[a] 7-Benzyloxy-4-(trifluoromethyl)coumarin (BFC) and 7-benzyloxy-quinoline (BQ) are fluorogenic substrates for Cyp3A4.

Table 7. DMPK Profile for Inhibitor **7**

	intravenous (1 mg/kg)			oral (5 mg/kg)			
species	Vss (L/kg)	$t_{1/2}$ (h)	Cl (mL/(kg min))	C_{max} (μM)	AUC (μM h)	%*F*	stability[a] LM $t_{1/2}$ (min)
rat	4.6	11	7.1	6.5	12	46	291
dog	2.5	5.9	10	16	20	115	253
monkey	1.0	1.3	24	1.5	3.4	43	39

[a] In vitro stability in liver microsome.

Among the large number of compounds selected for in vivo pharmacokinetic profiling in rats, inhibitor **7** was found to have the best overall profile. As shown in Table 7, compound **7** had an excellent half-life after intravenous administration and was very well absorbed after oral gavage[19] at 5 mg/kg. Furthermore, we evaluated the potential of this benzoic acid derivative for development by determining the pharmacokinetic characteristics in dogs and monkeys. As outlined in Table 7, the compound was well absorbed in both species. The half-life was shortest in monkeys (1.3 h), and high clearance (24 mL/(kg min)) was observed after intravenous injection. In addition, after oral administration, we observed low C_{max} and AUC values (1.5 μM and 3.4 μM h, respectively), which is consistent with the lower stability in monkey liver microsome preparations. In dogs, however, compound **7** showed an interesting profile with a half-life intermediate between those in rats and monkeys ($t_{1/2}$ = 5.9 h) and an excellent bioavailability. The oral bioavailability (*F*) ranged between 43 and 100% across the different species tested.

In summary, we have identified novel 8-substituted dipyridodiazepinone inhibitors with a broad spectrum of activity against wild type as well as clinically relevant NNRTI-resistant mutants. In particular, we have shown good potency against a panel of double mutants containing K103N, which account for a significant portion of failures in the clinic. The combination of good antiviral potency, a broad spectrum of activity, and an excellent pharmacokinetic profile provides strong justification for the further development of compound **7** as a potential treatment for wild type and NNRTI-resistant HIV-1 infection.

Experimental Section

Chemistry. Unless otherwise noted, materials were obtained from commercial sources and used without further purification. The purity of each inhibitor was determined by high-performance liquid chromatography (HPLC) and was >95% unless otherwise noted. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker AMX 400 spectrometer. ^{1}H assignment abbreviations are the following: singlet (s), doublet (d), triplet (t), quartet (q), pentet (p), broad singlet (bs), broad multiplet (bm), broad quartet (bq), doublet of doublets (dd), doublet of triplets (dt), and multiplet (m). Electrospray mass spectrum (ES-MS) were recorded on a Micromass-Quattro-II mass spectrometer. Purification was performed using flash column chromatography (FCC) on silica gel (10−40 μm or 230−400 mesh ASTM, E. Merck) or by preparative HPLC using a Partisil 10 ODS-3, C18 preparative column (50 cm × 22 mm). Analytical HPLC was carried out on the following systems. System A: ODS-AQ, Combiscreen, 5 μm analytical column (50 mm × 4.6 mm); mobile phase, acetonitrile/0.06% trifluoroacetic acid (TFA) in water/0.06% TFA. System B: Symmetry C18, 5 μm analytical column (2.1 mm × 150 mm); mobile phase, acetonitrile/50 mM NaH_2PO_4, pH = 4.4.

11-Ethyl-8-(2-hydroxyethyl)-5-methyl-5,11-dihydro-6*H*-dipyrido[2,3-*e*:3′,2′-*b*][1,4]diazepin-6-one (5a). Allyltributyltin (30.7 mL, 99.0 mmol) and $Pd(Ph_3P)_4$ (5.20 g, 4.50 mmol) were added to a degassed (N_2 through solution for 30 min) solution of **4a** (30.0 g, 90.0 mmol) in dimethylformamide (DMF, 450 mL) at room temperature. The mixture was stirred at 90 °C for 1.5 h and, then, was cooled to room temperature and concentrated under reduced pressure. The residue was purified (FCC; hexane/EtOAc, 8:2 to 7:3) to give 11-ethyl-5-methyl-8-(2-propenyl)-5,11-dihydro-6*H*-dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one (22.2 g, 84% yield). This compound (22.2 g, 75.4 mmol) was dissolved in CH_2Cl_2 (150 mL) and MeOH (150 mL) and cooled to −78 °C. Ozonized oxygen was bubbled through the resulting solution for 15 min; then, solid $NaBH_4$ (4.99 g, 132 mmol) was added to the solution. The reaction mixture was allowed to warm to room temperature. After 1 h, aqueous saturated NH_4Cl (200 mL) was added, and the mixture was stirred at room temperature for 2 h. The organic solvents were removed under reduced pressure. Water (300 mL) and $CHCl_3$ (300 mL) were added to the residue. The phases were separated, and the aqueous layer was extracted with $CHCl_3$. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified (FCC; EtOAc/$CHCl_3$, 4:1) to give **5a** (16.1 g, 72% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ 8.28 (d, J = 2.2 Hz, 1H), 8.20 (d, J = 4.4 Hz, 1H), 7.88 (d, J = 2.2 Hz, 1H), 7.83 (d, J = 7.8 Hz, 1H), 7.25 (dd, J = 7.8, 4.4 Hz, 1H), 4.63 (t, J = 5.1 Hz, 1H), 4.06 (bm, 2H), 3.55 (dt, J = 6.1, 5.6 Hz, 2H), 3.42 (s, 3H), 2.69 (t, J = 6.4 Hz, 2H), 1.17 (t, J = 7.1 Hz, 3H); MS (*m*/*z*) 299.2 (MH^+).

2-Chloro-11-ethyl-8-(2-hydroxyethyl)-5-methyl-5,11-dihydro-6*H*-dipyrido[2,3-*e*:3′,2′-*b*][1,4]diazepin-6-one (5b). 77% yield: ^{1}H NMR (DMSO-d_6) δ 8.31 (d, J = 2.4 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.88 (d, J = 8.4 Hz, 1H), 7.35 (d, J = 8.4 Hz, 1H), 4.63 (t, J = 5.4 Hz, 1H), 4.00 (bq, J = 7.1 Hz, 2H), 3.57 (td, J = 6.4, 5.4 Hz, 2H), 3.41 (s, 3H), 2.70 (t, J = 6.4 Hz, 2H), 1.18 (t, J = 7.1 Hz, 3H); MS (*m*/*z*) 333.0, 335.0 (MH^+).

11-Ethyl-2-fluoro-8-(2-hydroxyethyl)-5-methyl-5,11-dihydro-6*H*-dipyrido[2,3-*e*:3′,2′-*b*][1,4]diazepin-6-one (5c). 72% yield: ^{1}H NMR (DMSO-d_6) δ 8.29 (d, J = 2.4 Hz, 1H), 8.02 (dd, J = 8.5, 7.5 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.02 (dd, J = 8.5, 3.8 Hz, 1H), 4.63 (t, J = 5.1 Hz, 1H), 3.98 (bq, J = 7.1 Hz, 2H), 3.57 (dt, J = 5.1, 6.4 Hz, 2H), 3.42 (s, 3H), 2.70 (t, J = 6.4 Hz, 2H), 1.18 (t, J = 7.1 Hz, 3H); MS (*m*/*z*) 317.0 (MH^+).

11-Ethyl-8-(2-hydroxyethyl)-2,5-dimethyl-5,11-dihydro-6*H*-dipyrido[2,3-*e*:3′,2′-*b*][1,4]diazepin-6-one (5d). 81% yield: ^{1}H NMR (DMSO-d_6) δ 8.27 (d, J = 2.6 Hz, 1H), 7.89 (d, J = 2.6 Hz, 1H), 7.71 (d, J = 8.5 Hz, 1H), 7.12 (d, J = 8.5 Hz, 1H), 4.61 (t, J = 5.1 Hz, 1H), 4.03 (bq, J = 7.0 Hz, 2H), 3.57 (dt, J = 6.4, 5.1 Hz, 2H), 3.41 (s, 3H), 2.68 (t, J = 6.4 Hz, 2H), 2.48 (s, 3H), 1.18 (t, J = 7.0 Hz, 3H); MS (*m*/*z*) 313.1 (MH^+).

General Mitsunobu/Hydrolysis Procedure. 4-[2-(11-Ethyl-5-methyl-6-oxo-6,11-dihydro-5*H*-dipyrido[2,3-*e*:3′,2′-*b*][1,4]diazepin-8-yl)ethoxy]-3-methylbenzoic Acid (7). A solution of DIAD (99 μL, 0.51 mmol) in THF (2 mL) was added dropwise via syringe pump over 2 h to a THF (5 mL) solution of **5a** (100 mg, 0.34 mmol), **6** (56 mg, 0.34 mmol), and PPh_3 (132 mg, 0.50 mmol). The resulting solution was stirred for 1 h and then concentrated *in vacuo*. The residue was purified (FCC; hexane/EtOAc, 7:3 to 1:1) to yield methyl 4-[2-(11-ethyl-5-methyl-6-oxo-6,11-dihydro-5*H*-dipyrido[2,3-*e*:3′,2′-*b*][1,4]-diazepin-8-yl)ethoxy]-3-methylbenzoate (127 mg, 85% yield) as a white solid. The intermediate ester (100 mg, 0.224 mmol) was dissolved in THF (6 mL) and MeOH (2 mL), and 1.0 N LiOH (1.8 mL, 1.8 mmol) was added. The resulting solution

was stirred at room temperature for 24 h and then concentrated under reduced pressure. The residue was diluted with H_2O (20 mL) and washed with Et_2O (20 mL). The aqueous phase was acidified with 1.0 N HCl (3 mL), and the material was extracted with EtOAc. The organic phases were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting material was triturated from Et_2O using sonication. The solid was collected via suction filtration, washed with cold Et_2O, and dried under high vacuum to give **7** (90 mg, 93% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ 12.49 (bs, 1H), 8.40 (d, J = 2.1 Hz, 1H), 8.19 (dd, J = 4.7, 1.4 Hz, 1H), 8.04 (d, J = 2.1 Hz, 1H), 7.83 (d, J = 7.8 Hz, 1H), 7.73 (dd, J = 8.6, 1.4 Hz, 1H), 7.69 (s, 1H), 7.25 (dd, J = 7.8, 4.7 Hz, 1H), 6.99 (d, J = 8.6 Hz, 1H), 4.21 (t, J = 6.1 Hz, 2H), 4.05 (bm, 2H), 3.42 (s, 3H), 3.06 (t, J = 6.1 Hz, 2H), 2.11 (s, 3H), 1.16 (t, J = 7.0 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 167.0, 166.3, 159.9, 157.6, 154.0, 151.2, 144.0, 141.2, 131.7, 131.5, 131.1, 129.2, 129.1, 125.7, 122.4, 120.3, 120.2, 110.6, 67.9, 40.4, 36.8, 30.9, 15.9, 13.5; MS (*m/z*) 433.2 (MH^+); Anal. ($C_{26}H_{29}N_4O_4$) C, H, N.

Alternatively the Mitsunobu reaction can be carried out using a DPPBE[12] reagent: A solution of DIAD (4.1 mL, 20.9 mmol) in THF (1.9 mL) was added dropwise via syringe pump over 1 h to a THF (43.5 mL) solution of **5a** (5.0 g, 16.7 mmol), **6** (3.1 g, 18.4 mmol), and DPPBE (40 mL of a 0.5 M solution in THF, 20 mmol). The resulting yellow solution was stirred for 18 h at room temperature. Tetrabutylammonium fluoride (42 mL of a 1.0 M solution in THF, 42 mmol) was added, and the reaction was stirred for 3 h. The reaction mixture was concentrated *in vacuo* and diluted with EtOAc. The organic phase was washed with H_2O, 1 N NaOH, and brine, dried over $MgSO_4$, and concentrated *in vacuo* to give a pale yellow gum. The residue was purified by trituration from 3:2 hexane/MeO*t*Bu to give 7.5 g of a pale yellow solid. The solid was triturated a second time from Et_2O, collected by suction filtration, washed with Et_2O, and dried under high vacuum to give methyl 4-[2-(11-ethyl-5-methyl-6-oxo-6,11-dihydro-5*H*-dipyrido[2,3-*e*:3′,2′-*b*][1,4]diazepin-8-yl)ethoxy]-3-methylbenzoate (6.1 g, 81% yield) as a white amorphous solid. This intermediate ester could be hydrolyzed with LiOH to give **7** as described above.

Methyl 5-Hydroxy-1-naphthoate (10). Naphthol **8** (1.5 g, 9.43 mmol) was dissolved in 10% HCl (50 mL) and cooled to 0 °C; $NaNO_2$ (700 mg, 10.1 mmol) was dissolved in H_2O (5 mL) and added rapidly to the above solution. The reaction mixture was stirred for 20 min with rapid stirring. NaI (2.83 g, 18.9 mmol) was dissolved in H_2O (10 mL) and cooled to 0 °C and then added to the above solution all at once. The resulting reaction mixture was stirred for 3 h at 0 °C. The reaction was poured into Et_2O/H_2O (250:100 mL), and the entire mixture was filtered through a cotton plug. The filtrate was extracted with Et_2O, washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified (FCC; 20% EtOAc/hexane) to give 5-iodonaphthol (1.65 g, 65% yield). This compound (300 mg, 1.11 mmol) was dissolved in THF (15 mL), NaH (60 mg of a 60% dispersion in mineral oil, 1.44 mmol) was added, and the resulting solution was stirred for 15 min, during which time hydrogen gas evolved. MeI (315 mg, 2.2 mmol) was added, the flask was sealed, and the reaction mixture was stirred for 16 h. The reaction mixture was quenched with H_2O and extracted with EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, filtered, and concentrated to give **9** (310 mg, 98%) which was suitably pure for the next step. Compound **9** (300 mg, 1.1 mmol) was dissolved in THF (15 mL) and cooled to −78 °C. *n*-BuLi (2.0 M solution in hexane, 0.74 mL, 1.48 mmol) was added slowly, and then, the solution was stirred for 15 min at −78 °C. CO_2(g) was bubbled through the reaction mixture for 5 min, the cold bath was removed, and the reaction was warmed to room temperature. The reaction mixture was diluted with H_2O and Et_2O and extracted with 1.0 N NaOH, the aqueous phase was acidified with 1 N HCl and extracted with EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 5-methoxynaphthoic acid (168 mg, 79% yield). This acid (150 mg, 0.75 mmol) was dissolved in CH_2Cl_2 (25 mL), and BBr_3 (4.5 mL of a 1.0 M solution, 4.5 mmol) was added. The reaction was stirred for 16 h at room temperature, quenched with H_2O, and extracted with EtOAc. The combined organics were washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting material was dissolved in EtOAc and filtered through a short silica plug to give 5-hydroxynaphthoic acid (130 mg, 93% yield), which was dissolved in CH_2Cl_2 (10 mL), and CH_2N_2 in Et_2O was added until no more starting material was present, as proven by thin-layer chromatography (TLC). Silica gel was added to destroy the excess CH_2N_2, and the resulting mixture was concentrated *in vacuo* and purified (FCC; 20−30% EtOAc/hexane) to give **10** (93 mg, 66% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 8.46 (d, J = 8.6 Hz, 2H), 8.18 (d, J = 7.2 Hz, 1H), 7.50 (t, J = 7.4 Hz, 1H), 7.43 (t, J = 8.6 Hz, 1H), 6.87 (d, J = 7.4 Hz, 1H), 5.32 (s, 1H), 4.00 (s, 3H); MS (*m/z*) 202.8 (MH^+).

Methyl 4-Hydroxy-1-naphthoate (13). Compound **11** (1.5 g, 9.5 mmol) was dissolved in CH_3CN (50 mL), *N*-bromosuccinimide (1.83 g, 10.2 mmol) was added, and the reaction was stirred for 90 min at room temperature. The solution was concentrated *in vacuo* and diluted with Et_2O and H_2O. The organic phase was separated and washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified (FCC; 5−10% EtOAc/hexane) to give **12** (1.71 g, 76% yield). Compound **12** (1.0 g, 4.2 mmol) was dissolved in THF (40 mL) and cooled to −78 °C. *n*-BuLi (2.5 mL of a 2.2 M solution in hexane, 5.5 mmol) was added dropwise. After the addition was complete, the mixture was stirred for 20 min at −78 °C. CO_2 (g) was bubbled through the reaction mixture for 20 min, and the reaction was allowed to warm to room temperature. The reaction mixture was concentrated *in vacuo*, diluted with Et_2O, and extracted twice with 1.0 N NaOH. The combined basic extracts were acidified with 1.0 N HCl, extracted with EtOAc, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 4-methoxynaphthoic acid (887 mg, 100% yield) as a white solid. This compound (300 mg, 1.5 mmol) was dissolved in CH_2Cl_2 (20 mL), BBr_3 (5.2 mL of a 1.0 M solution in CH_2Cl_2, 5.2 mmol) was added, and the reaction was stirred for 16 h at room temperature. The reaction was quenched carefully by the addition of H_2O, and the resulting mixture was diluted with EtOAc and H_2O. The mixture was extracted with EtOAc, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material (230 mg, 82% yield, 1.21 mmol) was dissolved in CH_2Cl_2, CH_2N_2 (10 mL of a 1.6 M solution in Et_2O) was added, and the mixture was stirred for 15 min. Silica gel (3 g) was added, and the mixture was concentrated *in vacuo*. The product was purified (FCC; 20−50% EtOAc/hexane) to give compound **13** (138 mg, 56% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ 9.04 (d, J = 8.8 Hz, 1H), 8.36 (d, J = 7.8 Hz, 1H), 8.26 (d, J = 8.2 Hz, 1H), 7.65 (dt, J = 7.2, 1.3 Hz, 1H), 7.54 (dt, J = 7.2, 1.0 Hz, 1H), 6.81 (d, J = 8.2 Hz, 1H), 5.72 (s, 1H), 3.97 (s, 3H); MS (*m/z*) 202.8 (MH^+).

Methyl 3-Ethyl-4-hydroxybenzoate (16). Phenol **14** (2.0 g, 16.4 mmol) was dissolved in $CHCl_3$ (15 mL), and bromine (2.9 g, 16.4 mmol) was dissolved in $CHCl_3$ (5 mL) and added dropwise to the above solution over 20 min. The resulting mixture was stirred at room temperature for 1 h, then, diluted with EtOAc, washed with H_2O, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The resulting gum was purified (FCC; hexane/EtOAc 9:1) to give **15** (2.32 g, 71% yield) as a red oil. A solution of 1.6 M *n*-BuLi in hexane (6.2 mL, 9.9 mmol) was added rapidly to a cold solution (−78 °C) of **15** (870 mg, 4.33 mmol) in THF (20 mL). The mixture was stirred at −78 °C for 10 min, then, allowed to warm to 0 °C, and maintained at 0 °C for 1 h. A stream of CO_2 (g) was introduced into the reaction mixture for 10 min, and the solution was rendered acidic by the addition of aqueous 1.0 N HCl solution. The mixture was extracted with EtOAc. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was taken up in Et_2O (20 mL) and treated with an excess of ethereal CH_2N_2. The mixture was concentrated under reduced pressure, and the residue was purified (FCC; hexane/EtOAc, 4:1 to 7:3) to give compound **16**

(130 mg, 17% yield) as a colorless gum. ^{1}H NMR (DMSO-d_6) δ 10.25 (s, 1H), 7.70 (d, J = 2.1 Hz, 1H), 7.66 (dd, J = 8.4, 2.1 Hz, 1H), 6.86 (d, J = 8.4 Hz, 1H), 3.77 (s, 3H), 2.56 (q, J = 7.5 Hz, 2H), 1.13 (t, J = 7.5 Hz, 3H); MS (*m/z*) 178.9 (M − H)$^-$.

Ethyl (4-Hydroxy-3-methylphenyl)acetate (18). Acid **17** (1.0 g, 5.6 mmol) was dissolved in CH_2Cl_2 (50 mL), oxalyl chloride (0.73 mL, 8.3 mmol) was added followed by DMF (0.1 mL), and the reaction mixture was stirred for 90 min. EtOH (15 mL) was added, and the mixture was stirred for 1 h at room temperature. The reaction was concentrated *in vacuo*, and the resulting residue was dissolved in EtOAc, washed with H_2O, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give ethyl (4-methoxy-3-methylphenyl)acetate. This ester was dissolved in CH_2Cl_2 (50 mL), BBr_3 (7.2 mL of a 1.0 M solution in CH_2Cl_2, 7.2 mmol) was added dropwise over 5 min, and then, the mixture was stirred for 3 h at room temperature. EtOH (5 mL) was added, and the reaction mixture was stirred for an additional 30 min. The reaction mixture was concentrated *in vacuo*, taken into EtOAc, washed with H_2O, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The product was purified (FCC; 30% EtOAc/hexane) to give compound **18** (802 mg, 74% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ 9.19 (s, 1H), 6.92 (s, 1H), 6.84 (dd, J = 8.2, 2.0 Hz, 1H), 6.69 (d, J = 8.2 Hz, 1H), 4.04 (q, J = 7.0 Hz, 2H), 3.46 (s, 2H), 2.08 (s, 3H), 1.16 (t, J = 7.0 Hz, 3H); MS (*m/z*) 192.9 (M − H)$^-$.

Ethyl 3-(4-Hydroxy-3-methylphenyl)propanoate (20). Triethyl phosphonoacetate (2.6 mL, 13.3 mmol) was dissolved in THF (50 mL) and cooled to −78 °C. *n*-BuLi (9.6 mL of a 1.4 M solution in hexane, 13.4 mmol) was added dropwise over 5 min, and the resulting solution was stirred for 30 min at −78 °C. Aldehyde **19** (2.0 g, 13.3 mmol) was dissolved in THF (10 mL) and added dropwise via syringe pump over 1 h. After the addition was complete, the reaction mixture was stirred at −78 °C for 45 min and then warmed to 0 °C for 90 min. The reaction mixture was carefully concentrated *in vacuo* and diluted with Et_2O and H_2O. The aqueous phase was extracted twice with Et_2O; the combined organics were washed twice with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified (FCC; 10% EtOAc/hexane) to give ethyl (2*E*)-3-(4-methoxy-3-methylphenyl)acrylate (2.6 g, 89%) as a yellow oil. The α,β-unsaturated ester (1.0 g, 4.5 mmol) was dissolved in EtOH (20 mL) and degassed under vacuum for 10 min. 10% Pd/C (0.1 g) was added, and the resulting mixture was stirred under an H_2 atmosphere for 18 h. The mixture was filtered through Celite and concentrated *in vacuo* to give the reduced compound (0.96 g, 95%) as a pale yellow oil. This compound (350 mg, 1.58 mmol) was dissolved in CH_2Cl_2 (15 mL) and cooled to 0 °C. BBr_3 (4 mL of a 1.0 M solution in CH_2Cl_2, 4 mmol) was added dropwise over 5 min. The mixture was stirred at 0 °C for 4 h. The reaction was quenched by the addition of EtOH (0.5 mL) and stirred for 30 min. The resulting solution was diluted with EtOAc, washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give compound **20** (175 mg, 54%) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 9.03 (s, 1H), 6.88 (s, 1H), 6.80 (dd, J = 8.0, 1.7 Hz, 1H), 6.65 (d, J = 8.0 Hz, 1H), 4.02 (q, J = 7.0 Hz, 2H), 2.69 (t, J = 7.7 Hz, 2H), 2.55 (m, 2H), 2.06 (s, 3H), 1.15 (t, J = 7.0 Hz, 3H); MS (*m/z*) 207.0 (M − H)$^-$.

4-[2-(11-Ethyl-2-fluoro-5-methyl-6-oxo-6,11-dihydro-5*H*-dipyrido[2,3-*e*:3′,2′-*b*][1,4]diazepin-8-yl)ethoxy]-3-methylbenzoic Acid (22) and 4-[2-(11-Ethyl-2-methoxy-5-methyl-6-oxo-6,11-dihydro-5*H*-dipyrido[2,3-*e*:3′,2′-*b*]-[1,4]diazepin-8-yl)ethoxy]-3-methylbenzoic Acid (23). Compound **21** (100 mg, 0.215 mmol) was dissolved in 25% MeOH in THF (6 mL), 1.0 M LiOH (1.0 mL, 1.0 mmol) was added, and the reaction mixture was stirred for 24 h at room temperature. The reaction mixture was diluted with H_2O and washed with Et_2O. The aqueous phase was acidified with 1.0 N HCl and extracted with EtOAc. The combined organics were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified (FCC; 50% EtOAc/hexane containing 1% AcOH). Compound **23** (25 mg, 25%) elutes from the column first as a white solid upon concentration of the appropriate fractions. ^{1}H NMR (DMSO-d_6) δ 8.26 (d, J = 2.4 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.66 (d, J = 8.0 Hz, 1H), 7.50−7.46 (m, 2H), 6.65 (d, J = 8.2 Hz, 1H), 6.53 (d, J = 8.0 Hz, 1H), 4.03−3.90 (bm, 4H), 3.72 (s, 3H), 3.21 (bs, 3H), 2.92 (t, J = 6.2 Hz, 2H), 1.95 (s, 3H), 1.11 (t, J = 7.2 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 174.1, 169.9, 166.3, 159.2, 158.0, 157.0, 151.7, 150.7, 141.2, 135.4, 131.6, 129.5, 128.1, 124.0, 123.6, 120.4, 109.5, 106.0, 67.5, 53.3, 40.1, 36.7, 31.1, 16.1, 13.5; MS (*m/z*) 463.2 (MH$^+$); HRMS calcd for $C_{25}H_{26}N_4O_5$ (MH$^+$) 462.1903, found 462.1894. **Compound 22** (48 mg, 50% yield) elutes second and as a white solid. ^{1}H NMR (DMSO-d_6) δ 8.47 (d, J = 2.3 Hz, 1H), 8.12 (d, J = 2.3 Hz, 1H), 8.11 (t, J = 5.3 Hz, 1H), 7.66−7.64 (m, 2H), 7.08 (dd, J = 8.6, 3.5 Hz, 1H), 6.80 (d, J = 9.0 Hz, 1H), 4.18 (t, J = 6.3 Hz, 2H), 4.04 (bm, 2H), 3.47 (s, 3H), 3.11 (t, J = 6.3 Hz, 2H), 2.11 (s, 3H), 1.24 (t, J = 7.02 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 175.1, 170.1, 166.4, 158.9, 157.4, 152.4, 151.4, 141.6, 137.8, 132.1, 131.9, 130.4, 128.9, 128.5, 124.0, 109.8, 105.4, 67.7, 40.7, 37.3, 31.3, 16.3, 13.5; MS (*m/z*) 451.2 (MH$^+$); HRMS calcd for $C_{24}H_{24}N_4O_4F$, (MH$^+$) 451.1782, found 451.1800.

4-[2-(11-Ethyl-5-methyl-6-oxo-6,11-dihydro-5*H*-dipyrido-[2,3-*e*:3′,2′-*b*][1,4]diazepin-8-yl)ethoxy]-1-naphthoic Acid (24). ^{1}H NMR (DMSO-d_6) δ 12.58 (bs, 1H), 8.92 (d, J = 8.5 Hz, 1H), 8.43 (d, J = 2.3 Hz, 1H), 8.12−8.09 (m, 3H), 8.05 (d, J = 2.3 Hz, 1H), 7.75 (dd, J = 8.0, 1.6 Hz, 1H), 7.54 (t, J = 8.0 Hz, 1H), 7.42 (t, J = 8.0 Hz, 1H), 7.17 (dd, J = 8.0, 4.7 Hz, 1H), 6.97 (d, J = 8.5 Hz, 1H), 4.35 (t, J = 6.3 Hz, 2H), 3.99 (bm, 2H), 3.36 (s, 3H), 3.15 (t, J = 6.3 Hz, 2H), 1.10 (t, J = 7.0 Hz, 3H); MS (*m/z*) 469.2 (MH$^+$); HRMS calcd for $C_{27}H_{25}N_4O_4$ (MH$^+$) 469.1876, found 469.1886.

5-[2-(11-Ethyl-5-methyl-6-oxo-6,11-dihydro-5*H*-dipyrido-[2,3-*e*:3′,2′-*b*][1,4]diazepin-8-yl)ethoxy]-1-naphthoic Acid (25). ^{1}H NMR (DMSO-d_6) δ 13.25 (bs, 1H), 8.67 (d, J = 2.2 Hz, 1H), 8.52 (d, J = 8.8 Hz, 1H), 8.50 (d, J = 8.6 Hz, 1H), 8.37 (dd, J = 4.7, 1.3 Hz, 1H), 8.29 (d, J = 2.2 Hz, 1H), 8.27 (d, J = 7.4 Hz, 1H), 8.02 (dd, J = 8.0, 1.3 Hz, 1H), 7.69−7.65 (m, 2H), 7.43 (dd, J = 8.0, 4.7 Hz, 1H), 7.22 (dd, J = 7.7 Hz, 1H), 4.53 (t, J = 6.1 Hz, 2H), 4.25−4.12 (bm, 2H), 3.61 (s, 3H), 3.37 (t, J = 6.1 Hz, 2H), 1.35 (t, J = 7.0 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 168.9, 166.4, 157.7, 154.1, 153.9, 151.2, 144.2, 141.2, 131.8, 131.6, 131.2, 130.0, 129.4, 127.8, 127.7, 126.1, 125.3, 124.2, 120.3, 117.7, 105.7, 68.2, 40.44, 36.81, 31.0, 13.5; MS (*m/z*) 469.2 (MH$^+$); HRMS calcd for $C_{27}H_{25}N_4O_4$ (MH$^+$) 469.1876, found 469.1887.

6-[2-(11-Ethyl-5-methyl-6-oxo-6,11-dihydro-5*H*-dipyrido-[2,3-*e*:3′,2′-*b*][1,4]diazepin-8-yl)ethoxy]-1-naphthoic Acid (26). ^{1}H NMR (DMSO-d_6) δ 13.05 (bs, 1H), 8.78 (d, J = 9.4 Hz, 1H), 8.47 (d, J = 2.2 Hz, 1H), 8.23 (dd, J = 4.7, 1.6 Hz, 1H), 8.09 (d, J = 2.2 Hz, 1 H), 8.02 (d, J = 8.2 Hz, 1H), 7.99 (d, J = 5 Hz, 1H), 7.87 (dd, J = 8.0, 1.6 Hz, 1H), 7.54 (t, J = 7.6 Hz, 1H), 7.46 (d, J = 2.5 Hz, 1H), 7.30−7.27 (m, 2H), 4.33 (t, J = 6.4 Hz, 2H), 4.11−4.05 (bm, 2H), 3.46 (s, 3 H), 3.15 (t, J = 6.4 Hz, 2H), 1.21 (t, J = 7.0 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 168.7, 166.4, 157.7, 156.0, 154.1, 151.2, 144.1, 141.1, 135.1, 131.8, 131.2, 129.1, 127.6, 127.5, 127.1, 126.1, 125.4, 120.0, 107.6, 67.6, 40.44, 36.8, 30.9, 13.5; MS (*m/z*) 469.2 (MH$^+$); HRMS calcd for $C_{27}H_{25}N_4O_4$ (MH$^+$) 469.1876, found 469.1875.

6-[2-(11-Ethyl-5-methyl-6-oxo-6,11-dihydro-5*H*-dipyrido-[2,3-*e*:3′,2′-*b*][1,4]diazepin-8-yl)ethoxy]-2-naphthoic Acid (27). ^{1}H NMR (DMSO-d_6) δ 12.84 (bs, 1H), 8.56 (s, 1H), 8.51 (d, J = 2.3 Hz, 1H), 8.27 (dd, J = 4.5, 1.4 Hz, 1H), 8.13 (d, J = 2.3 Hz, 1H), 8.05 (d, J = 9.0 Hz, 1H), 7.97 (dd, J = 6.0, 1.4 Hz, 1H), 7.92−7.88 (m, 2H), 7.48 (d, J = 2.1 Hz, 1H), 7.33 (dd, J = 13.9, 9.2 Hz, 1H), 7.27 (dd, J = 9.0, 2.3 Hz, 1H), 4.38 (t, J = 6.6 Hz, 2H), 4.15−4.05 (bm, 2H), 3.49 (s, 3H), 3.18 (t, J = 6.6 Hz, 2H), 1.24 (t, J = 7.1 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 167.5, 166.3, 158.0, 157.7, 154.0, 151.2, 144.1, 141.1, 136.7, 131.8, 131.2, 130.9, 130.3, 129.1, 127.5, 126.9, 125.7, 120.3, 119.5, 106.8, 67.8, 40.4, 36.8, 30.8, 13.5; MS (*m/z*) 469.2 (MH$^+$); HRMS calcd for $C_{27}H_{24}N_4O_4$ (M) 468.1799, found 468.1797.

4-[2-(11-Ethyl-5-methyl-6-oxo-6,11-dihydro-5*H*-dipyrido-[2,3-*e*:3′,2′-*b*][1,4]diazepin-8-yl)ethoxy]benzoic Acid (28). ^{1}H NMR (DMSO-d_6) δ 8.32 (d, J = 2.3 Hz, 1H), 8.13 (dd, J = 4.7, 1.6 Hz, 1H), 8.12 (d, J = 1.4 Hz, 1H), 7.76 (dd, J = 8.0, 1.4 Hz, 1H), 7.65 (d, J = 8.7 Hz, 2H), 7.19 (dd, J = 8.0, 4.7 Hz,

1H), 6.68 (d, J = 8.7 Hz, 2H), 4.06 (t, J = 6.5 Hz, 2H), 4.00 (bm, 2H), 3.36 (s, 3H), 2.95 (t, J = 6.5 Hz, 2H), 1.10 (t, J = 7.0 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 167.0, 166.4, 161.9, 157.7, 154.1, 151.2, 144.1, 141.1, 131.8, 131.3, 131.2, 129.0, 123.1, 120.4, 114.3, 67.8, 40.5, 40.0, 36.8, 30.8, 13.5; MS (*m/z*) 419.2 (MH^+); HRMS calcd for $C_{23}H_{22}N_4O_4$ (MH^+) 418.1641, found 418.1637.

3-Ethyl-4-[2-(11-ethyl-5-methyl-6-oxo-6,11-dihydro-5*H*-dipyrido[2,3-*e*:3′,2′-*b*][1,4]diazepin-8-yl)ethoxy]benzoic Acid (29). ^{1}H NMR (DMSO-d_6) δ 8.37 (d, J = 2.3 Hz, 1H), 8.18 (dd, J = 4.5, 1.4 Hz, 1H), 8.00 (d, J = 2.3 Hz, 1H), 7.81 (dd, J = 8.0, 1.6 Hz, 1H), 7.68−7.55 (m, 2H), 7.24 (dd, J = 8.0, 4.5 Hz, 1H), 6.73 (d, J = 8.2 Hz, 1H), 4.11 (t, J = 6.0 Hz, 2H), 4.05 (bm, 2H), 3.41 (s, 3H), 3.03 (t, J = 6.0 Hz, 2H), 2.36 (q, J = 7.4 Hz, 2H), 1.16 (t, J = 7.0 Hz, 3H), 0.84 (t, J = 7.4 Hz, 3H); MS (*m/z*) 447.2 (MH^+); HRMS calcd for $C_{25}H_{27}N_4O_4$ (MH^+) 447.2032, found 447.2032.

3-Bromo-4-[2-(11-ethyl-5-methyl-6-oxo-6,11-dihydro-5*H*-dipyrido[2,3-*e*:3′,2′-*b*][1,4]diazepin-8-yl)ethoxy]benzoic Acid (30). ^{1}H NMR (DMSO-d_6) δ 8.43 (d, J = 2.4 Hz, 1H), 8.20 (dd, J = 4.5, 1.6 Hz, 1H), 8.08 (d, J = 2.2 Hz, 1H), 8.01 (d, J = 2.4 Hz, 1H), 7.88−7.82 (m, 2H), 7.25 (dd, J = 8.0, 4.5 Hz, 1H), 7.19 (d, J = 8.0 Hz, 1H), 4.29 (t, J = 6.2 Hz, 2H), 4.02 (bm, 2H), 3.43 (s, 3H), 3.09 (t, J = 6.2 Hz, 2H), 1.17 (t, J = 7.0 Hz, 3H); MS (*m/z*) 497.1, 499.1 (MH^+); HRMS calcd for $C_{23}H_{22}N_4O_4Br$ (MH^+) 497.0824, found 497.0819.

{4-[2-(11-Ethyl-5-methyl-6-oxo-6,11-dihydro-5*H*-dipyrido[2,3-*e*:3′,2′-*b*][1,4]diazepin-8-yl)ethoxy]-3-methylphenyl}acetic Acid (31). ^{1}H NMR (DMSO-d_6) δ 8.31 (d, J = 2.2 Hz, 1H), 8.12 (dd, J = 4.4, 1.2 Hz, 1H), 7.95 (d, J = 2.2 Hz, 1H), 7.76 (dd, J = 8.0, 1.2 Hz, 1H), 7.18 (dd, J = 8.0, 4.7 Hz, 1H), 6.85 (s, 1H), 6.81 (d, J = 8.2 Hz, 1H), 6.63 (d, J = 8.2 Hz, 1H), 3.99 (t, J = 6.2 Hz, 1H), 3.35 (s, 3H), 2.95−2.93 (m, 4H), 1.93 (s, 3H), 1.10 (t, J = 4.9 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 174.7, 166.4, 157.6, 154.1, 151.2, 144.1, 141.1, 131.9, 131.8, 131.4, 131.2, 129.7, 127.2, 124.4, 120.3, 120.3, 110.6, 67.6, 45.3, 40.4, 36.8, 31.2, 16.0, 13.5; MS (*m/z*) 447.2 (MH^+); HRMS calcd for $C_{25}H_{27}N_4O_4$ (MH^+) 447.2032, found 447.2042.

3-{4-[2-(11-Ethyl-5-methyl-6-oxo-6,11-dihydro-5*H*-dipyrido[2,3-*e*:3′,2′-*b*][1,4]diazepin-8-yl)ethoxy]-3-methylphenyl}propanoic Acid (32). ^{1}H NMR (DMSO-d_6) δ 8.31 (d, J = 2.3 Hz, 1H), 8.12 (dd, J = 4.5, 1.4 Hz, 1H), 7.95 (d, J = 2.3 Hz, 1H), 7.75 (dd, J = 8.0, 1.4 Hz, 1H), 7.18 (dd, J = 7.9, 4.5 Hz, 1H), 6.82−6.80 (m, 2H), 6.65 (d, J = 8.6 Hz, 1H), 4.01−3.98 (m, 4H), 3.35 (s, 3H), 2.94 (t, J = 6.1 Hz, 2H), 2.52 (t, J = 7.6 Hz, 2H), 1.98−1.94 (m, 5H), 1.09 (t, J = 7.1 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 175.9, 166.4, 157.6, 154.1, 151.2, 144.1, 135.3, 131.8, 131.2, 130.4, 129.6, 126.2, 125.0, 120.3, 120.2, 110.9, 67.5, 40.7, 40.4, 36.8, 31.9, 31.1, 16.0, 13.5; MS (*m/z*) 461.3 (MH^+); HRMS calcd for $C_{26}H_{29}N_4O_4$ (MH^+) 461.2189, found 461.2195.

4-[2-(2-Chloro-11-ethyl-5-methyl-6-oxo-6,11-dihydro-5*H*-dipyrido[2,3-*e*:3′,2′-*b*][1,4]diazepin-8-yl)ethoxy]-3-methylbenzoic Acid (33). ^{1}H NMR (DMSO-d_6) δ 8.56 (d, J = 2.2 Hz, 1H), 8.19 (d, J = 2.2 Hz, 1H), 8.02 (d, J = 8.4 Hz, 1H), 7.73−7.71 (m, 2H), 7.49 (d, J = 8.4 Hz, 1H), 6.88 (d, J = 8.8 Hz, 1H), 4.26 (t, J = 6.0 Hz, 2H), 4.14 (bm, 2H), 3.54 (s, 3H), 3.18 (t, J = 6.0 Hz, 2H), 2.18 (s, 3H), 1.31 (t, J = 7.0 Hz, 3H); MS (*m/z*) 467.2, 469.2 (MH^+); HRMS calcd for $C_{24}H_{23}N_4O_4Cl$ (MH^+) 466.1408, found 466.1420.

4-[2-(11-Ethyl-2,5-dimethyl-6-oxo-6,11-dihydro-5*H*-dipyrido[2,3-*e*:3′,2′-*b*][1,4]diazepin-8-yl)ethoxy]-3-methylbenzoic Acid (34). ^{1}H NMR (DMSO-d_6) δ 8.39 (d, J = 2.4 Hz, 1H), 8.08 (d, J = 2.4 Hz, 1H), 7.72 (d, J = 8.0 Hz, 1H), 7.60−7.56 (m, 2H), 7.11 (d, J = 8.0 Hz, 1H), 6.79 (d, J = 8.4 Hz, 1H), 4.19−4.00 (bm, 4H), 3.39 (s, 3H), 3.06 (t, J = 6.2 Hz, 2H), 2.41 (s, 3H), 2.08 (s, 3H), 1.19 (t, J = 7.2 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 169.6, 166.4, 157.7, 156.7, 153.3, 152.9, 151.0, 144.1, 133.1, 132.1, 131.6, 130.4, 129.6, 128.5, 128.0, 123.4, 120.4, 119.5, 109.4, 67.5, 40.7, 36.7, 31.1, 23.3, 16.1, 13.5; MS (*m/z*) 447.2 (MH^+); HRMS calcd for $C_{25}H_{27}N_4O_4$ (MH^+) 447.2032, found 447.2042.

Enzymatic Assay: Radiometric 96-Well Microtiter Plate Assay with Streptividin Scintillation Proximity Beads. HIV-1 RT (WT or mutants) was diluted into Tris/HCl mM (pH 7.8) containing NaCl 60 mM, $MgCl_2$ hexahydrate 2 mM, DTT 6 mM, GSH 2 mM, and 0.02% w/v Chaps to give ≈3 nM enzyme. To 30 μL of this enzyme solution was added 10 μL of inhibitor solution (50 μM to 2.5 nM inhibitor in the same assay buffer as above containing 15% v/v DMSO). The plate was preincubated for 15 min at room temperature before proceeding to the next step (in this preincubation step, the highest and lowest inhibitor concentrations were 12.5 μM and 0.62 nM, respectively, and the concentration of DMSO was 3.75% v/v). The enzymatic reaction was initiated by addition of 10 μL of substrate solution. The final reaction mixture contained Tris/HCl 50 mM (pH 7.8), NaCl 60 mM, $MgCl_2 \cdot 6H_2O$ 2 mM, DTT 6 mM, GSH 2 mM, Chaps 0.02% w/v, DMSO 3% v/v, Poly rC 179 nM, Biotin dG_{15} 18 nM, dGTP 288 nM, ^{3}H-dGTP 71 nM, and 1−2 nM enzyme (in this incubation step, the highest and lowest inhibitor concentrations were 10 μM and 0.5 nM, respectively). After the addition of substrate, the plate was covered with a plastic seal and incubated for 1 h at 37 °C in a dry incubator. The reaction was quenched by addition of 75 μL of EDTA 0.5 M containing 5 mg/mL of streptavidin scintillation proximity beads. The plate was shaken for 2 min at medium speed and incubated for 1 h at room temperature. Then, 75 μL of a cesium chloride 7 M solution was added, and the plate was shaken for 2 min at medium speed and incubated again for 1 h at room temperature. The plate was then covered with a plastic seal and counted using a TopCount-NXT Microplate scintillation and luminescence counter (Packard). Each well was counted for 60 s. Each row contained at its extremities a blank and a control well.

Antiviral Assay. Inhibition of HIV replication was performed in cell culture in a 96-well plate assay. Complete RPMI 1640, consisting of RPMI 1640 + 10% fetal bovine serum, 10 μg/mL gentamycin, and 10 μM β-mercaptoethanol, was used for dilution of the compound as well as cell growth medium. The T-lymphocyte cell line C8166 was infected at a multiplicity of infection of 0.001 with viruses coding for wild type and mutant reverse transcriptase. Cells were then incubated for 3 days in the presence of serial dilutions of the compounds. The supernatant was pooled from eight replica wells, and the concentration of extracellular p24 was determined using a commercially available HIV-1 p24 antigen assay kit (Beckman-Coulter).

Liver Microsomes Stability Assay. $t_{1/2}$ values for phase I oxidative metabolic stability were determined using male human liver microsomes, or rat, dog, or monkey liver microsomes, at a starting concentration of 10 μM compounds and 2.5 mM NaDPH as cofactor.

Cyp450 Inhibition. c-DNA-expressed human cytochrome P450 isozymes (supersomes) were obtained from Gentest Corporation. Reaction progress was monitored using fluorescent substrates. Each test compound was dissolved in acetonitrile/methanol at 1.5 mM and serially diluted to determine IC_{50} values. A positive control was included for each isozyme.

Caco-2 Permeability Assay. Cells were cultured in 12-well Transwell membrane plates. Wells were incubated for 180 min at 37 °C with a test solution. Samples from the receiving side (basolateral for A to B direction) were analyzed by HPLC. Apparent permeability was obtained from permeability (apparent) = $(\Delta Q/\Delta t)/(AC_0)$, where $(\Delta Q/\Delta t)$ is the transport rate across the monolayer, A is the area of the monolayer surface, and C_0 is the initial concentration at the donor side.

Pharmacokinetic (PK) Studies. Oral and intravenous (i.v.) PK experiments were carried out in male Sprague−Dawley rats, beagle dogs, and rhesus monkeys. All animals fasted overnight prior to the experiments and were restrained during the sampling period. Plasma extracts were analyzed by HPLC. All PK parameters were determined using the noncompartmental analysis methods provided by TopFit version 2.0.

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(17) Each value represents the mean of at least three determinations. The reproducibility of the assay was gauged using an internal standard.

(18) Data not shown and will be published elsewhere.

(19) For oral gavage, the aqueous vehicle contains 0.5% methocel and 0.3% Tween-80. For intravenous bolus injection, the compound was dissolved in a vehicle containing *N*,*N*-dimethylacetamide and 0.9% aqueous NaCl (30:70).

(20) Half-life ($t_{1/2}$) is calculated using the equation $t_{1/2} = 0.693(V_d/\text{clearance})$ where V_d is the volume of distribution at the pseudoterminal phase ($V_d = 7.3 \pm 0.1$ L/kg).

(21) Thummel, K. E.; Kunze, K. L.; Shen, D. D. Enzyme-catalyzed processes of first-pass hepatic and intestinal drug extraction. *Adv. Drug Delivery Rev.* **1997**, *27*, 99.

(22) For the liver microsome stability assay, $t_{1/2}$ values for phase I oxidative metabolic stability were determined using male human or rat liver microsomes at a starting concentration of 10 μM for the compounds.

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