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Biaryl Ethers as Novel Non-nucleoside Reverse Transcriptase Inhibitors with Improved Potency against Key Mutant Viruses

Dai-Shi Su,*[,†] John J. Lim,[†] Elizabeth Tinney,[†] Bang-Lin Wan,[†] Mary Beth Young,[†] Kenneth D. Anderson,[†] Deanne Rudd,[†] Vandna Munshi,[‡] Carolyn Bahnck,[‡] Peter J. Felock,[‡] Meiquing Lu,[‡] Ming-Tain Lai,[‡] Sinoeun Touch,[§] Gregory Moyer,[§] Daniel J DiStefano,[§] Jessica A. Flynn,[§] Yuexia Liang,[∥] Rosa Sanchez,[∥] Rebecca Perlow-Poehnelt,[†] Mike Miller,[‡] Joe P. Vacca,[†] Theresa M. Williams,[†] and Neville J. Anthony[†]

[†]*Department of Medicinal Chemistry and Structural Biology,* [‡]*Antiviral Research,* [§]*Vaccines and Biologics Research and* [∥]*Drug Metabolism, Merck Research Laboratory, P.O. Box 4, West Point, Pennsylvania 19486*

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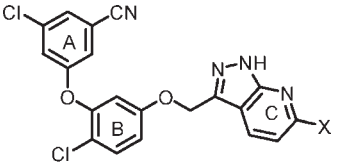
Biaryl ethers were recently reported as potent NNRTIs. Herein we disclose a detailed SAR study that led to the biaryl ether **6**. This compound possessed excellent potency against WT RT and key clinically observed RT mutants and had an excellent pharmacokinetic profile in rats, dogs, and rhesus macaques. The compound also exhibited a clean safety profile in preclinical safety studies.

Introduction

Non-nucleoside reverse transcriptase inhibitors (NNRTIs)[a] are key components of the most current HIV therapy, highly active antiretroviral therapy (HAART), a combination of nucleoside and non-nucleoside reverse transcriptase inhibitors (NRTIs, NNRTIs) and protease inhibitors (PIs). HAART can be very effective at delaying disease progression. However, due to the propensity of HIV to rapidly mutate, the efficacy and durability of HAART can be compromised. The most frequent HIV RT mutations observed in patients failing therapy with first generation NNRTIs are K103N and Y181C. Therefore, new agents with better activity profiles against mutant HIV-1 RT are needed. Recently, biaryl ethers were reported to be potent NNRTIs (Figure 1).[1−3] In this manuscript, we report the detailed structural−activity relationship (SAR) study of this series of compounds that led to a compound which exhibited excellent potency against wild type (WT) reverse transcriptase (RT) and key clinically observed RT mutants and had a favorable pharmacokinetic profile.

Chemistry

Compounds described in this paper were prepared according to the route depicted in Scheme 1. Treatment of the commercially available 1-fluoro-3,5-dimethyoxybenzene with BBr_3 followed by chlorination of the resulting intermediate with NCS provided a mixture of two regioisomers **1a** and **1b** in a ratio of about 2:1. The S_NAr reaction of **1a** with 3-chloro-5-fluorobenzonitrile and demethylation by BBr_3 yielded the key intermediate **1c**. Alkylation of phenol (**1c**) with the previously reported 1-Boc-3-bromomethyl-6-fluoro-pyrazolopyridine[2] delivered **1d**. The final compound (**6**) was prepared in a two-step sequence via displacement of the fluorine atom with 4-methoxybenzylamine and simultaneous removal of the 4-methoxybenzyl and Boc protecting groups with TFA. The other compounds in Table 1 were synthesized in an analogous fashion.



compound **1**: X = H
compound **2**: X = NH_2

Figure 1. Lead compounds **1** and **2**.

The isoxazolopyridines **12** to **20** in Table 2 were synthesized according to the route depicted in Scheme 2. Deprotonation of 2,6-difluoropyridine by the action of LDA followed by {[*t*-butyl(dimethyl)silyl]oxy}acetaldehyde addition afforded the desired alcohol **1e**. Swern oxidation and treatment of the resulting ketone with hydroxyl amine and $Ti(iOPr)_4$ formed the oxime derivative **1f**. Thermal cyclization yielded a mixture of desired cyclized product (**1g**), the desilylated cyclized product (**1h**), and the desilylated starting material. Conversion of the resulting alcohol (**1h**) to mesylate provided the compound **1i**. Coupling 3-chloro-5-(2-chloro-5-hydroxyphenoxy)benzonitrile[2] with mesylate **1i** under basic conditions afforded the mesylate **1m** in 71% yield, presumably via an in situ displacement of fluorine by the newly generated anionic mesylate group. The compounds (**13**−**20**) in Table 2 were then prepared by the displacement of mesylate with a variety of amines.

The synthesis of the intermediates for the preparation of compound **21** is shown in Scheme 3. Treatment of fluoride **1g** with sodium cyanide afforded the cyano intermediate **1n**.

*To whom correspondence should be addressed. Phone: 610-917-5879. Fax: 610-917-6151. E-mail: Dai-Shi.X.Su@gsk.com. Present address: GlaxoSmithKline, 1250 South Collegeville Road, UP1205, Collegeville, PA 19426.

[a] Abbreviations: NNRTI, non-nucleoside reverse transcriptase inhibitors; HAART, highly active antiretroviral therapy; PI, protease inhibitors; SAR, structural−activity relationship; WT, wild type, RT, reverse transcriptase; FBS, fetal bovine serum; NHS, normal human serum; PK, pharmacokinetic; ECG, electrocardiogram.

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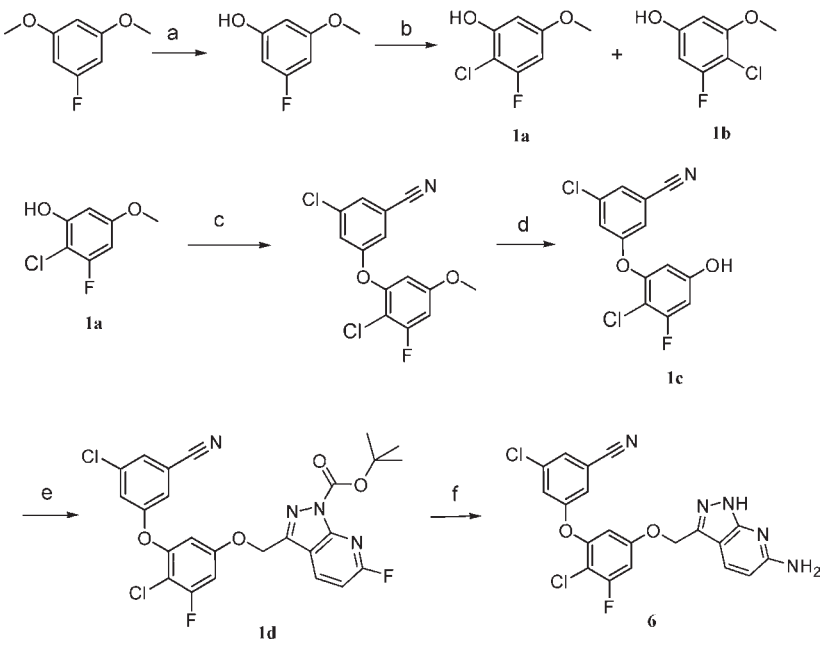
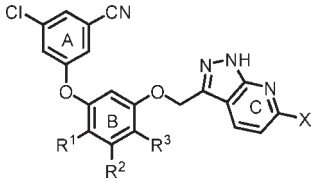
Scheme 1. Synthesis of Compound **6**[a]



[a] (a) BBr_3, CH_2Cl_2, −15 to 23 °C, 67%; (b) NCS, DCE, 84 °C, ~2:1 ratio, 36% for **1a**; (c) 3-chloro-5-fluorobenzonitrile, Cs_2CO_3, NMP, 120 °C, 88%; (d) BBr_3, CH_2Cl_2, 0−23 °C, 35%; (e) 1-Boc-3-bromomethyl-6-fluoro-pyrazolopyridine, Cs_2CO_3, NMP, 23 °C, 85%; (f) (1) PMB-amine, NMP, 95 °C, (2) TFA, 65 °C, 4% for two steps.

Table 1. B-Ring SAR Results



					RT-Pol[a]	Spread (nM)[b]			
compd	R^1	R^2	R^3	X	WT/K103N/Y181C(nM)	10% FBS/50%NHS (WT)	K103N[c]	Y181C[c]	K103N/Y181C[c]
1	Cl	H	H	H	0.1/0.2/0.2	10/52	15	22	53
2	Cl	H	H	NH_2	0.2/0.4/0.4	5/26	11	27	101
3	Cl	Cl	H	H	2.7/4.6/3.2	18/103	34	31	277
4	Cl	CH_3	H	H	0.4/1.9/0.9	11/103	103	31	278
5	Cl	F	H	H	0.5/0.9/0.6	16/59	11	31	93
6	Cl	F	H	NH_2	0.1/0.4/0.3	8/69	18	14	62
7	H	F	Cl	H	5.2/0.8/13	11/103	11	31	93
8	H	F	Cl	NH_2	120/ > 910/270	2778/ > 8333	8333		
9	H	F	H	NH_2	02/3.3/1.3	< 3.8/103	309		
10	H	H	F	H	47/350/120	3767/ > 8300	> 8300		
11	H	H	Cl	H	17/140/100	> 8300/ > 8300	> 8300		

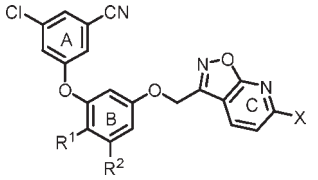
[a] K_i, compounds were evaluated in a standard ECL assay. The values are the geometric mean of at least two determinations. All individual values are within 25% of the mean. [b] CIC95 (cell culture inhibitory concentration) is defined as the concentration at which the spread of virus is inhibited by > 95% in MT-4 human T-lymphoid cells maintained in RPMI 1640 medium containing either 10% fetal bovine serum (FBS) or 50% normal human serum (NHS). The antiviral activity of compound against wild-type ABI R8 virus was measured. The values are the geometric mean of at least two determinations. All individual values are within 25% of the mean. [c] In the presence of 10%FBS.

The cyano group in **1n** was then converted to imidazoline by a two-step sequence: formation of the ethyl imidate and cyclization with ethylene diamine. Treatment of the resulting alcohol with thionyl chloride afforded the intermediate **1p**. Compound **21** was prepared in a manner analogous to the route described in Scheme 2.

Biological Results and Discussion

The previously reported compounds **1** and **2** showed excellent potencies and had good oral bioavailability in preclinical species (Figure 1). The SAR study results suggested that the chlorine atom at the R^1 position of B-ring is critical for the potency and mutation profile of compound (Table 1).[1] Therefore, multiple substitutions on the B-ring with chloride anchoring the R^1 position were investigated. Several potent analogues were produced. At the R^2 position, a chlorine atom, a methyl group, and a fluorine atom were well tolerated (**3**, **4**, and **5**). The chlorine and fluorine combination was the most promising (**5**). Compound **5** maintained high antiviral activity against WT RT and its key mutants in both enzyme (RT_Pol)[4] and cell-based

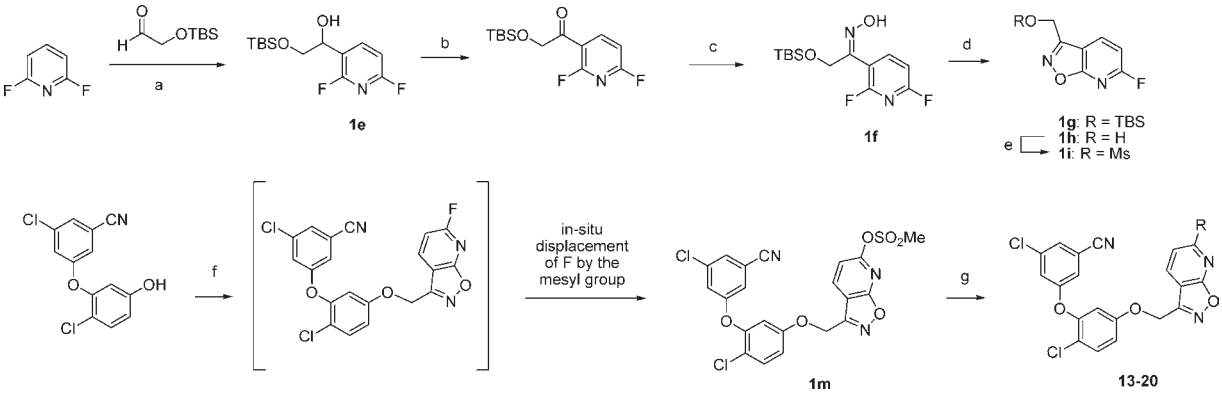
Table 2. C-Ring SAR Results



Compound	R^1	R^2	X	RT-Pol [a] WT/K103N/Y181C (nM)	Spread (nM)[b] 10% FBS / 50%NHS (WT)	K103N[c]	Y181C[c]	K103N/Y181C[c]
12	Cl	H	H	1.6/2.1/9.4	19/103	103	93	278
13	Cl	H	NH2	0.3/0.4/0.8	11/34	34	93	278
14	Cl	F	NH2	0.2/0.8/0.1	11/103	103	31	833
15	Cl	H	NHMe	0.3/0.9/1.6	103/103	309	-	-
16	Cl	H	NMe_2	0.3/0.9/3.5	103/309	309	-	-
17	Cl	H		2.2/7.2/24	>8333	>8333	>8333	-
18	Cl	H		1.8/8.6/6.6	103/956	926	-	-
19	Cl	H		1.7/3/8.7	33/309	103	833	833
20	Cl	H		0.2/0.8/0.8	33/309	103	278	833
21	Cl	H		2.3/6.3/12	309/309	309	-	-

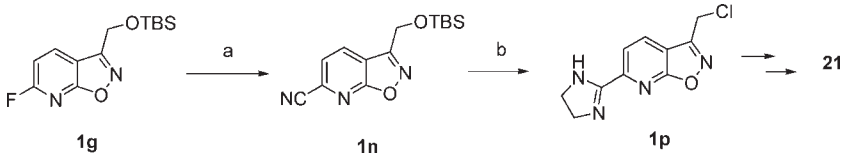
[a] K_i, compounds were evaluated in a standard ECL assay. The values are the geometric mean of at least two determinations. All individual values are within 25% of the mean. [b] CIC95 (cell culture inhibitory concentration) is defined as the concentration at which the spread of the virus is inhibited by >95% in MT-4 human T-lymphoid cells maintained in RPMI 1640 medium containing either 10% fetal bovine serum (FBS) or 50% normal human serum (NHS). The antiviral activity of compound against wild-type ABI R8 virus was measured. The values are the geometric mean of at least two determinations. All individual values are within 25% of the mean. [c] In the presence of 10%FBS.

Scheme 2. Synthesis of Compound **13**−**20**[a]



[a] Reagents and conditions: (a) LDA, then aldehyde, 77%; (b) $(COCl)_2$, DMSO, TEA, DCM, 94%; (c) NH_2OH, $Ti(iPrO)_4$, 66%; (d) ethylene glycol, 150 °C, 5 h, 17% (**1h**), 42−60% (**1g**); (e) MsCl, TEA, 73%; (f) tBuOK, **1i**, THF, 78%; (g) amine, Cs_2CO_3, DMF, 60 °C.

Scheme 3. Synthesis of the Intermediates for Compound **21**[a]



[a] Reagents and conditions: (a) NaCN, DMSO, 88%; (b) (i) HCl (g), EtOH, (ii) ethylene diamine, EtOH, 88%, (iii) $SOCl_2$.

assays (Spread assay).[5] Introduction of an amino group on the pyrazolo pyridine C-ring was reported to affect both the physiochemical properties and the potency of compounds **1** and **2**.[2] Incorporating the amino group in **5** led to compound **6**. Gratifyingly, compound **6** exhibited excellent intrinsic enzyme inhibitory potency versus WT and mutant RTs and also had

excellent antiviral activity in cell-based assays against the same panel of mutants. Relocation of the chlorine atom from R^1 to R^3 position (**7**) was also well tolerated. We were encouraged that **7** had equivalent potency compared with **5** in our assays. However, introduction of the amino group in the C-ring unexpectedly caused a significant loss of activity (**8**). Monohalogenation at the R2 and R3 positions of the B-ring led to a loss of potency in the Spread assay (**9**−**11**).

Co-crystallization of compound **2** in the allosteric WT RT binding site suggested that the NH group and the imido nitrogen atom in the pyrazole motif make key interactions with the carbonyl oxygen and NH of the backbone K103 residue, respectively.[2] To our surprise, the isoxazolopyridine analogue **12**, in which the NH of pyrazolopyridine was replaced with an oxygen atom, exhibited nanomolar activities in RT_Pol assay against WT and K103N and Y181C mutants and potency decreased only 2-fold in the WT Spread assay compared with that of **1** (Table 2). The amino analogues (**13** and **14**) showed similar intrinsic potency to that of **2** and **6**, respectively, and were about 2-fold less potent in the cell-based assay. Although the substituted amino analogues (**15** and **16**) showed nanomolar antiviral activities in the enzyme assay, they generally were less potent than the corresponding pyrazolopyridines in the Spread assay. The 6-amino group in the C-ring of pyrazolopyridine (**2**) is accommodated within the solvent channel lying under Pro-236.[2] We speculated that introduction of a polar and basic group at the 6-position of the isoxazolopyridine C-ring would decrease the lipophilicity of compounds and hence reduce the fold-shift in the Spread assay. Therefore a structurally diverse array of basic heterocycles were installed at the 6-position of the C-ring. Nearly all the compounds prepared (**17**−**21**) exhibited potency less than 5 nM against the WT and the key mutant RTs in the enzyme assay. However, these compounds proved less potent in the cell-based assay compared to the unsubstituted amino-compounds (**13** and **14**). A higher shift between sera was also observed for the most of analogues, indicating the influence of protein binding. Of the heterocycles, **20**, containing imidazole at the 6-position, had the best overall profile. While compound **20** displayed subnanomolar potency against the WT RT and the key mutants in the enzyme assay, it was significantly less potent in the cell-based assay.

Compounds **3** and **6** showed promising pharmacokinetic profiles in rats following iv administration (**3**: Cl, 5.5 mL/min/kg; V_d, 1.8 L/kg; AUC_{iv}, 3.5 μM·h; $t_{1/2}$, 9.4 h; **6** (vide infra): Cl, 10.1 mL/min/kg; V_d: 3.5 L/kg; AUC_{iv}, 7.4 μM·h, $t_{1/2}$, 4.6 h). However, other compounds in this series generally exhibited high clearance in rats following iv administration.[6] In a related in vitro study in hepatocytes, an analogue structurally similar to **6** underwent predominantly *O*-dealkylation with loss of the heterocyclic C-ring to form a phenolic metabolite (M1) along with glucuronidation of parent molecule and M1. The major metabolic pathways in microsomes were *O*-dealkylation to form M1 and subsequent further oxidation of M1.

On the basis of its overall profile (potency against WT and mutants and PK), compound **6** was selected for further evaluation. Examination of compound **6** against a broader panel of clinically relevant mutant HIV-1viruses showed that the compound possessed excellent antiviral activity against these mutants (Figure 2).[7] In terms of pharmacokinetics, compound **6** had excellent bioavailability, low clearance, good volume distribution, and half-life in three preclinical species (rat, dog, and monkey), showing a similar profile to that of compound **2** but with higher V_d, leading to longer $t_{1/2}$ across species. (Table 3).

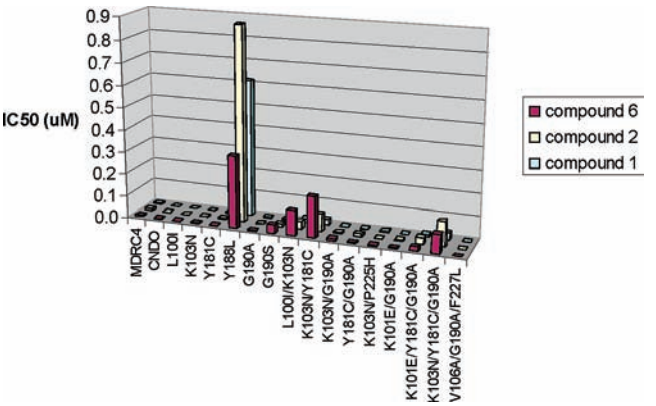


Figure 2. Antiviral potency vs clinically isolated RT mutants of compounds **1**, **2**, and **6**.

Table 3. Pharmacokinetics of Compounds **2** and **6**[a]

	2			**6**		
	rat[b]	dog[c]	rhesus[d]	rat[b]	dog[c]	rhesus[d]
CL	9	5.8	16	10	4	15
V_d	2	1.7	1.9	3.5	2.3	3.6
AUC_{po}	23	31	0.3	18	6	1.7
$t_{1/2}$	3.5	3.9	2.1	4.6	7	4.2
F	52	47	1	49	63	13

[a] CL: mL/min/kg; V_d: L/kg; AUC_{po}: μM·h; $t_{1/2}$: hours; *F*: % oral bioavailability. [b] Sprague−Dawley rats (n = 3); po: 10 mg/kg, iv: 2 mg/kg. [c] Beagle dogs (n = 3) for dog singles; po: 1 mg/kg, iv: 0.5 mg/kg. [d] Rhesus macaques (n = 3); po: 5 mg/kg; iv: 1 mg/kg. Interanimal variability was less than 20%.

Compound **6** is not a potent competitive inhibitor of the major cytochrome P450 isoforms (CYP3A4 IC50: 4.4 μM; CYP2C9 10.3 μM; CYP2D6 47.4 μM) nor did it show time-dependent inhibition of CYP3A4 in human liver microsomes. The binding affinity of **6** in a human PXR-dependent reporter gene assay showed an inductive response value of 51% of that of the positive control (10 μM rifampcin) at a concentration of 3.3 μM.

Further preclinical evaluation of compound **6** showed that it is devoid of ancillary activities, including effects on mean arterial pressure, heart rate, or electrocardiogram (ECG) in a conscious cardiovascular dog study with peak plasma exposure of 25.9 μM at 10 mg/kg (cumulative dosing).[8] Compound **6** was negative in preliminary genotoxicity determinations.[9] There are no significant findings for **6** in a seven-day rat study in which high levels of exposure were observed (21.78 μM·h and 180.3 μM·h over a period of 24 h at 10 and 100 mg/kg, respectively).[10]

Conclusion

In summary, compound **6** was identified in an effort to modify the previously reported compound **2**. Compound **6** possessed excellent antiviral activity against WT RT and key clinically observed RT mutants with a favorable pharmacokinetic profiles. The compound also exhibited a clean safety profile in preclinical safety studies. While compound **6** had an excellent overall profile for a second generation NNRTI, overall, it was very similar to **2**. Further study is required to determine if compound **6** has developmental potential.

Experimental Procedures

General Procedures. ^{1}H and ^{13}C (300, 400, or 600 MHz) NMR spectra were recorded on a Varian VXR 300, Unity Inova 400, or Unity Inova 600 spectrometer. The chemical shifts are reported in δ (ppm) using the δ 0.00 signal of Me_4Si as an internal standard. LC/MS data were obtained on a Waters 2690 separations module and Micromass ZMD. High resolution MS (HRMS) data were obtained on a Bruker 3T or 7T FTICR MS with either electrospray ionization or APCI. HPLC spectra were recorded on a Hewlett-Packard 1100 with a YMC-Pack Pro C-18 column or Atlantis dC_{18} column with a 5−95% CH_3CN/H_2O (with 0.05% TFA) gradient at 215 nm. The purity of compound is ≥95%.

2-Chloro-3-fluoro-5-methoxyphenol (1a). Under nitrogen atmosphere, 1-fluoro-3,5-dimethoxybenzene (25 g, 160 mmol) was diluted in CH_2Cl_2 (200 mL, 0.8M) and then cooled to −15 °C. BBr_3 (176 mL, 176 mmol, 1 M in CH_2Cl_2) was slowly added to the reaction mixture. The reaction mixture was stirred at −15 °C for one and a half hours at room temperature for 10 min. The reaction mixture was then cooled to 0 °C and slowly quenched with water (150 mL). The aqueous layer was then extracted with methylene chloride (3 × 100 mL). The organic extracts were dried over sodium sulfate, filtered, and concentrated in vacuo. Silica gel chromatography (1%-30% EtOAc/hexanes) of the crude residue gave 3-fluoro-5-methoxyphenol (15 g, 67%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 3.76 (s, 3H), 4.93 (s, 1H), 6.19 (m, 3H).

Under a nitrogen atmosphere, 3-fluoro-5-methoxyphenol (15 g, 106 mmol) was diluted in DCE (150 mL, 0.7 M). To this solution NCS (15.5 g, 116 mmol) was added, and the reaction mixture was heated to reflux for 4 h. The reaction was then cooled to room temperature and quenched with water (100 mL). The aqueous layer was extracted with methylene chloride (3 × 50 mL). The organic extracts were dried over sodium sulfate, filtered, and concentrated in vacuo. Silica gel chromatography (1−30% EtOAc/hexanes) of the crude material gave 2-chloro-3-fluoro-5-methoxyphenol (**1a**) (6.7 g, 36%), ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 3.76 (s, 3H), 5.59 (s, 1H), 6.33 (d, 1H), 6.36 (d, 1H), 6.40 (dd, 1H). ESMS, M + H$^+$ found 177.2, and 4-chloro-3-fluoro-5-methoxyphenol (**1b**) (2.8 g, 15%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 3.87 (s, 3H), 4.93 (s, 1H), 6.28 (dd, 1H), 6.29 (d, 1H), 6.31 (d, 1H). ESMS, M + H$^+$ found 177.2. LCMS (ES) m/z 177.2 (M)$^+$, 179.2 (M + 1)$^+$. The starting material, 3-fluoro-5-methoxyphenol was also recovered (4.3 g).

3-Chloro-5-(2-chloro-3-fluoro-5-hydroxyphenoxy)benzonitrile (1c). Under a nitrogen atmosphere, 2-chloro-3-fluoro-5-methoxyphenol (**1a**) (6.7 g, 37.9 mmol) was diluted in NMP (40 mL, 0.95 M). To this solution Cs_2CO_3 (24.73 g, 76 mmol) was added, and the reaction was allowed to stir at room temperature for 5 min. Then 3-chloro-5-fluorobenzonitrile (11.81 g, 76 mmol) was added to the reaction and it was then heated to 120 °C. After 2 h, the reaction was cooled to room temperature and then diluted with EtOAc (40 mL). It was partitioned with water (20 mL) and then extracted with EtOAc (3 × 30 mL). The organic extracts were then washed with water (3 × 20 mL) and brine (1 × 20 mL), dried over sodium sulfate, filtered, and concentrated in vacuo. Silica gel chromatography (1−15% EtOAc/hexanes) of the crude residue gave 3-chloro-5-(2-chloro-3-fluoro-5-methoxyphenoxy)benzonitrile (10.4 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 3.80 (s, 3H), 6.47 (t, 1H), 6.69 (dd, 1H), 7.07 (t, 1H), 7.19 (t, 1H), 7.36 (s, 1H).

Under nitrogen atmosphere, 3-chloro-5-(2-chloro-3-fluoro-5-methoxyphenoxy)benzonitrile (9.6 g, 30.8 mmol) was diluted in CH_2Cl_2 (60 mL, 0.5M) and then cooled to 0 °C. To this solution BBr_3 (61.5 mL, 61.5 mmol, 1 M in CH_2Cl_2) was slowly added to the reaction. The reaction mixture was allowed to slowly warm to room temperature and stir for 12 h. It was then cooled to 0 °C and slowly quenched with water (60 mL). The aqueous layer was then extracted with methylene chloride (3 × 30 mL). The organic extracts were dried over sodium sulfate, filtered, and concentrated in vacuo. Silica gel chromatography (1−20% EtOAc/hexanes) of the crude material gave 3-chloro-5-(2-chloro-3-fluoro-5-hydroxyphenoxy)benzonitrile (**1c**) (3.2 g, 35%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 6.40 (dd, 1H), 6.63 (dd, 1H), 7.09 (dd, 1H), 7.19 (t, 1H), 7.38 (t, 1H). LCMS (ES) m/z 298.2 (M)$^+$, 300.2 (M + 1)$^+$.

3-{5-[(6-Amino-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)methoxy]-2-chloro-3-fluorophenoxy}-5-chlorobenzonitrile (6). Under a nitrogen atmosphere, 3-chloro-5-(2-chloro-3-fluoro-5-hydroxyphenoxy)benzonitrile (**1c**) (150 mg, 0.503 mmol) was dissolved in NMP (4 mL, 0.1 M). Cs_2CO_3 (164 mg, 0.503 mmol) was added, and the reaction was allowed to stir at room temperature for 15 min. Then, 1-Boc-3-bromomethyl-6-fluoro-pyrazolopyridine (166 mg, 0.503 mmol, prepared by the method described in ref 2) was added to the reaction. The reaction was stirred at room temperature for 2 h. It was diluted with EtOAc (4 mL), partitioned with water (3 mL), and then extracted with EtOAc (3 × 4 mL). The organic extracts were then washed with water (3 × 4 mL) and brine (1 × 4 mL), dried over sodium sulfate, filtered, and concentrated in vacuo. Silica gel chromatography (1−20% EtOAc/hexanes) of the crude gave intermediate **1d** (230 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 1.73 (s, 9H), 5.39 (s, 2H), 6.59 (m, 1H), 6.84 (dd, 1H), 6.98 (dd, 1H), 7.06 (m, 1H), 7.14 (t, 1H), 7.38 (m, 1H), 8.24 (t, 1H). ESMS, M + H$^+$ found 446.9 (M-100, loss of Boc).

Under a nitrogen atmosphere, intermediate **1d** (230 mg, 0.420 mmol) was dissolved in NMP (4 mL, 0.1 M). 4-Methoxybenzylamine (288 mg, 2.101 mmol) was added and the reaction mixture was heated to 95 °C for 2 h. It was then diluted with EtOAc (4 mL), partitioned with water (3 mL), and then extracted with EtOAc (3 × 4 mL). The organic extracts were then washed with water (3 × 4 mL) and brine (1 × 4 mL), dried over sodium sulfate, filtered, and concentrated in vacuo. The crude reaction material was then diluted with TFA (3 mL) and heated to 65 °C for 2 h. The reaction mixture was concentrated and purified directly on a reverse phase HPLC system (5−95% AcCN/H_2O with 0.05% TFA) to give **6** (8 mg, 4%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 4.72 (b, 2H), 5.30 (s, 2H), 6.41 (d, 1H), 6.61 (s, 1H), 6.81 (dd, 1H), 7.04 (s, 1H), 7.17 (m, 1H), 7.38 (s, 1H), 7.81 (d, 1H), 9.90 (b, 1H). ESMS, M + H$^+$ found 444.0.

3-Chloro-5-[2,3-dichloro-5-(1*H*-pyrazolo[3,4-*b*]pyridin-3-ylmethoxy)phenoxy] Benzonitrile (3). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 5.43 (s, 2H), 6.76 (d, 1H), 7.04 (s, 1H), 7.15 (m, 2H), 7.22 (dd, 1H), 7.38 (s, 1H), 8.19 (d, 1H), 8.61 (s, 1H), 10.6 (b, 1H). High resolution MS: m/z found 445.0014 (M + 1), calculated 445.0021 (M + 1).

3-Chloro-5-[2-chloro-3-methyl-5-(1*H*-pyrazolo[3,4-*b*]pyridin-3-ylmethoxy)phenoxy] Benzonitrile (4). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 2.39 (s, 3H), 5.43 (s, 2H), 6.69 (d, 1H), 6.89 (d, 1H), 6.99 (m, 1H), 7.13 (t, 1H), 7.22 (dd, 1H), 7.32 (t, 1H), 8.21 (d, 1H), 8.60 (s, 1H). High resolution MS: m/z found 425.0565 (M + 1), calculated 425.0567 (M + 1).

3-Chloro-5-[2-chloro-3-fluoro-5-(1*H*-pyrazolo[3,4-*b*]pyridin-3-ylmethoxy) henoxy] Benzonitrile (5). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 5.49 (s, 2H), 6.62 (t, 1H), 6.83 (dd, 1H), 7.03 (s, 1H), 7.16 (t, 1H), 7.39 (s, 1H), 7.44 (dd, 1H), 8.56 (m, 2H). ESMS, M + H$^+$ found 429.6.

3-Chloro-5-[4-chloro-3-fluoro-5-(1*H*-pyrazolo[3,4-*b*]pyridin-3-ylmethoxy)phenoxy] Benzonitrile (7). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 5.43 (s, 2H), 6.63 (t, 1H), 6.84 (dd, 1H), 7.07 (t, 1H), 7.17 (t, 1H), 7.23 (dd, 1H), 7.38 (t, 1H), 8.20 (dd, 1H). 8.60 (d, 1H). ESMS, M + H$^+$ found 429.0.

3-{3-[(6-Amino-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)methoxy]-4-chloro-5-fluorophenoxy}-5-chlorobenzonitrile (8). ^{1}H NMR (400 MHz, CD_3OD, ppm): δ 5.41 (s, 2H), 6.47 (d, 1H), 6.65 (dd, 1H), 6.87 (t, 1H), 7.34 (m, 2H), 7.61 (t, 1H), 7.92 (d, 1H). ESMS, M + H$^+$ found 444.1.

3-{3-[(6-Amino-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)methoxy]-5-fluorophenoxy}-5-chlorobenzonitrile (9). ^{1}H NMR (400 MHz, CD_3OD, ppm): δ 5.44 (s, 2H), 6.55 (dt, 1H), 6.63 (s, 1H), 6.66 (d, 1H), 6.78 (dt, 1H), 7.32 (s, 1H), 7.35 (t, 1H), 7.59 (s, 1H), 8.22 (d, 1H). ESMS, M + H$^+$ found 410.0.

3-Chloro-5-[4-fluoro-3-(1*H*-pyrazolo[3,4-*b*]pyridin-3-ylmethoxy)phenoxy] Benzonitrile (10). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 5.53 (s, 2H), 6.60 (m, 1H), 6.93 (m, 1H), 7.06 (s, 1H), 7.11 (m, 2H), 7.24 (t, 1H), 7.31 (s, 1H), 8.32 (d, 1H), 8.61 (s, 1H), 11.20 (b, 1H). ESMS, M + H$^+$ found 395.1.

3-Chloro-5-[4-chloro-3-(1*H*-pyrazolo[3,4-*b*]pyridin-3-ylmethoxy)phenoxy] Benzonitrile (11). ^{1}H NMR (400 MHz, CD_3OD, ppm): δ 5.54 (s, 2H), 6.66 (dd, 1H), 7.06 (d, 1H), 7.24 (m, 3H), 7.42 (d, 1H), 7.53 (t, 1H), 8.40 (dd, 1H), 8.52 (dd, 1H). ESMS, M + H$^+$ found 411.5.

2-{[*t*-Butyl(dimethyl)silyl]oxy}-1-(2,6-difluoropyridin-3-yl)-ethanol (1e). 2,6-Difluoro pyridine (3.3 g, 28.7 mmol) was dissolved in 20 mL of THF and cooled to −78 °C. To this solution was added LDA (mono THF complex in hexanes, 1.5M, 28.7 mmol) dropwise, and the resulting mixture was stirred for 2 h at −78 °C. In a separate flask {[*t*-butyl-(dimethyl)silyl]oxy}acetaldehyde (5 g, 28.7 mmol) was dissolved in 20 mL of THF and cooled to −78 °C. The lithiated pyridine solution was then transferred into the aldehdyde solution via cannula over 15 min. The reaction mixture was stirred an additional 15 min at −78 °C and then warmed to room temperature for 1 h. The reaction mixture was quenched with 50 mL of water and diluted with EtOAc. The combined organic layer was washed once each with water and brine, dried over sodium sulfate, filtered, and concentrated in vacuo. Flash chromatography of the crude residue (0−20% EtOAc/hexanes) gave 6.4 g (77%) of **1e** as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 0.07 (m, 6H), 0.942 (s, 9H), 3.05 (t, 1H), 3.53 (t, 1H), 3.92 (dd, 1H), 5.01 (d, 1H), 6.88 (d, 1H), 8.11 (q, 1H). ESMS, M + H$^+$ found 290.2.

2-{[*t*-Butyl(dimethyl)silyl]oxy}-1-(2,6-difluoropyridin-3-yl)-*N*-hydroxyethanimine (1f). DMSO (0.123 mL, 1.73 mmol) was added dropwise to a solution of oxalyl chloride (0.302 mL, 3.46 mmol) in 10 mL of dichloromethane at −78 °C and then stirred for 30 min. 2-{[*t*-Butyl(dimethyl)silyl]oxy}-1-(2,6-difluoropyridin-3-yl)ethanol (500 mg, 1.728 mmol) was added to the cooled solution, and the mixture was stirred for 15 min. TEA (1.2 mL, 8.64 mmol) was added and then warmed to 0 °C for 30 min. The reaction mixture was diluted with EtOAc. The organic layer was washed once each with water and brine, dried over sodium sulfate, filtered, and concentrated in vacuo. Flash chromatography of the crude residue (5−15% EtOAc/hexanes) gave 470 mg (94%) of 2-{[*t*-butyl(dimethyl)silyl]oxy}-1-(2,6-difluoropyridin-3-yl)ethanone as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 0.126 (s, 6H), 0.930 (s, 9H), 4.82 (s, 2H), 6.97 (d, 1H), 8.52 (q, 1H). ESMS, M + H$^+$ found 287.2.

To a solution of 2-{[*t*-butyl(dimethyl)silyl]oxy}-1-(2,6-difluoropyridin-3-yl)ethanone (6.8 g, 23..66 mmol) in 200 mL of dichloromethane was added titanium isopropoxide (13.87 mL, 47.3 mmol) followed by hydroxyl amine hydrochloride (3.13 g, 47.3 mmol). The reaction mixture was stirred at room temperature for 1 h and then quenched with 10 mL of water. The reaction was allowed to stir overnight and then filtered and concentrated in vacuo. Flash chromatography of the crude residue (5−20% EtOAc/hexanes) gave 4.7 g (66%) of **1f**. ESMS, M + H$^+$ found 303.2.

(6-Fluoroisoxazolo[5,4-*b*]pyridine-3-yl)methanol (1h) and 3-({[*t*-Butyl(dimethyl)silyl]oxy}methyl)-6-fluoroisoxazolo[5,4-*b*]-pyridine (1g). 2-{[*t*-Butyl(dimethyl)silyl]oxy}-1-(2,6-difluoropyridin-3-yl)-*N*-hydroxyethanimine (3 g, 9.92 mmol) was dissolved in 100 mL of ethylene glycol and heated to 150 °C for 5 h. The mixture was cooled to room temperature and diluted with EtOAc. The organic layer was washed once each with water and brine, dried over sodium sulfate, filtered, and concentrated in vacuo. Flash chromatography of the crude residue (1−4% MeOH/CH_2Cl_2) afforded 480 mg (17%) of **1h**, 1 g (60%) of **1g**, and 550 mg (30%) of desilylated starting material. For **1h**, ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 5.1 (s, 2H), 7.00 (d, 1H), 8.34 (t, 1H). ESMS, M + H$^+$ found 169.2.

(6-Fluoroisoxazolo[5,4-*b*]pyridine-3-yl)methanesulfonate (1i). To a solution of (6-fluoroisoxazolo[5,4-*b*]pyridine-3-yl)methanol (500 mg, 2.97 mmol) in 10 mL of dichloromethane was added TEA (0.415 mL, 2.97 mmol) and cooled to 0 °C, and then methane sulfonyl chloride was added (0.232 mL, 2.97 mmol) and stirred for 15 min at 0 °C. The mixture was quenched with 5 mL of water and diluted with dichloromethane. The organic layer was washed once each with water and brine, dried over sodium sulfate, filtered, and concentrated in vacuo. Flash chromatography of the crude residue (10−60% EtOAc/hexanes) afforded 533 g (73%) **1i** as a yellow oil. ESMS, M + H$^+$ found 247.2.

3-{[4-Chloro-3-(3-chloro-5-cyanophenoxy)phenoxy]methyl}-isoxazolo[5,4-*b*]pyridine-6-yl Methanesulfonate (1m). To a solution of 3-chloro-5-(2-chloro-5-hydroxyphenoxy)benzonitrile (600 mg, 2.14 mmol, prepared by the method described in ref 2) in 10 mL of THF was added *t*-BuOK (1 M solution in THF, 2.14 mmol), stirred for 10 min at room temperature, **1i** (527 mg, 2.14 mmol) dissolved in 5 mL of THF was added and stirred at room temperature for 90 min. The reaction mixture was then quenched with 5 mL of water and diluted with EtOAc. The organic layer was washed once each with water and brine, dried over sodium sulfate, filtered, and concentrated in vacuo. Flash chromatography of the crude residue (100% $CHCl_3$) gave 850 mg (78%) of **1m** as a white solid. (ESMS, M + H$^+$ found 506.0).

3-Chloro-5-(2-chloro-5-{[6-(methylamino)isoxazolo[5,4-*b*]-pyridine-3-yl)methoxy}phenoxy) Benzonitrile (15). To a solution of 3-{[4-chloro-3-(3-chloro-5-cyanophenoxy)phenoxy]methyl}-isoxazolo[5,4-*b*]pyridine-6-yl methanesulfonate (95 mg, 0.221 mmol) in 1 mL of DMF was added Cs_2CO_3 (71.9 mg, 0.221 mmol) and methyl amine (0.22 L mL, 1.0 M solution in THF). The resulting reaction mixture was heated for 12 h at 60 °C, cooled to room temperature, and diluted with EtOAc. The organic layer was washed with water (3 × 5 mL) and brine (1 × 5 mL), dried over sodium sulfate, filtered, and concentrated in vacuo. The crude residue was purified via reverse phase chromatography (5−95% AcCN/water/0.05% TFA) to afford 17 mg (15%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 3.05 (d, 3H), 5.05 (b, 1H), 5.33 (s, 2H), 6.37 (d, 1H), 6.77 (d, 1H), 6.92 (dd, 1H), 6.96 (t, 1H), 7.13 (t, 1H), 7.34 (t, 1H), 7.40 (d, 1H), 7.71 (d, 1H). High resolution MS: *m*/*z* found 441.0497 (M + 1), calculated 441.0516 (M + 1).

3-Chloro-5-[2-chloro-5-(isoxazolo[5,4-*b*]pyridine-3-yl)methoxy)-phenoxy] Benzonitrile (12). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 5.47 (s, 2H), 6.80 (d, 1H), 6.94 (dd, 1H), 7.00 (t, 1H), 7.13 (t, 1H), 7.35 (m, 2H), 7.43 (d, 1H), 8.22 (d, 1H), 8.67 (d, 1H). High resolution MS: *m*/*z* found 412.0234 (M + 1), calculated 412.0250 (M + 1).

3-{5-[(6-Aminoisoxazolo[5,4-*b*]pyridin-3-yl]-2-chlorophenoxy}-5-chlorobenzonitrile (13). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 5.1 (b, 2H), 5.35 (s, 2H), 6.48 (d, 1H), 6.78 (d, 1H), 6.93 (dd, 1H), 6.96 (t, 1H), 7.14 (t, 1H), 7.35 (s, 1H), 7.40 (d, 1H), 7.81 (d, 1H). High resolution MS: *m*/*z* found 427.0341 (M + 1), calculated 427.0346 (M + 1).

3-{5-[(6-Aminoisoxazolo[5,4-*b*]pyridin-3-yl]-2-chloro-3-fluoro-phenoxy}-5-chlorobenzonitrile (14). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 5.34 (s, 2H), 6.50 (d, 1H), 6.58 (t, 1H), 6.81 (dd, 1H), 7.01 (s, 1H), 7.17 (t, 1H), 7.39 (t, 1H), 7.82 (d, 1H). High resolution MS: *m*/*z* found 445.0271 (M + 1), calculated 445.0265 (M + 1).

3-Chloro-5-(2-chloro-5-{[6-(dimethylamino)isoxazolo[5,4-*b*]-pyridine-3-yl)methoxy}phenoxy) Benzonitrile (16). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 3.20 (s, 6H), 5.33 (s, 2H), 6.52 (d, 1H), 6.78 (d, 1H), 6.92 (dd, 1H), 6.98 (t, 1H), 7.12 (t, 1H), 7.35 (t, 1H), 7.39 (d, 1H), 7.76 (d, 1H). High resolution MS: *m*/*z* found 455.0660 (M + 1), calculated 455.0672 (M + 1).

3-Chloro-5-{2-chloro-5-[(6-piperdin-1-yl-isoxazolo[5,4-*b*]pyridine-3-yl)methoxy]phenoxy} Benzonitrile (17). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 1.66 (m, 4H), 1.71 (m, 2H), 3.71 (t, 4H), 5.32 (s, 2H), 6.62 (d, 1H), 6.77 (d, 1H), 6.92 (dd, 1H), 6.99 (t, 1H), 7.12 (t, 1H), 7.34 (t, 1H), 7.39 (d, 1H), 7.73 (d, 1H). High resolution MS: *m*/*z* found 495.0967 (M + 1), calculated 495.0985 (M + 1).

3-Chloro-5-{2-chloro-5-[(6-morpholin-4-yl-isoxazolo[5,4-*b*]-pyridine-3-yl)methoxy]phenoxy} Benzonitrile (18). ^{1}H NMR

(400 MHz, $CDCl_3$, ppm): δ 3.71 (t, 4H), 3.81 (t, 4H), 5.34 (s, 2H), 6.63 (d, 1H), 6.77 (d, 1H), 6.92 (dd, 1H), 6.97 (t, 1H), 7.13 (t, 1H), 7.34 (t, 1H), 7.40 (d, 1H), 7.81 (d, 1H). High resolution MS: m/z found 497.0748 (M + 1), calculated 497.0778 (M + 1).

3-Chloro-5-{2-chloro-5-[(6-piperazin-1-yl-isoxazolo[5,4-*b*]pyridine-3-yl)methoxy]phenoxy} Benzonitrile (19). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 2.98 (t, 4H), 3.71 (t, 4H), 5.33 (s, 2H), 6.63 (d, 1H), 6.77 (d, 1H), 6.91 (dd, 1H), 6.99 (t, 1H), 7.13 (t, 1H), 7.34 (t, 1H), 7.39 (d, 1H), 7.78 (d, 1H). High resolution MS: m/z found 496.0946 (M + 1), calculated 496.0938 (M + 1).

3-Chloro-5-(2-chloro-5-{[6-(1*H*-imidazol-1-yl)isoxazolo[5,4-*b*]-pyridine-3-yl)methoxy]phenoxy} Benzonitrile (20). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 5.47 (s, 2H), 6.80 (d, 1H), 6.93 (dd, 1H), 7.00 (t, 1H), 7.14 (t, 1H), 7.25 (s, 1H), 7.36 (t, 1H), 7.45 (dd, 1H), 7.76 (s, 1H), 8.31 (d, 1H), 8.47 (s, 1H). High resolution MS: m/z found 478.0456 (M + 1), calculated 478.0468 (M + 1).

3-Chloro-5-(2-chloro-5-{[6-(4,5-dihydro-1*H*-imidazol-2-yl)isoxazolo[5,4-*b*]pyridine-3-yl)methoxy}phenoxy] Benzonitrile (21). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 3.70−4.10 (b, 4H), 5.48 (s, 2H), 6.80 (d, 1H), 6.92 (dd, 1H), 7.00 (t, 1H), 7.13 (t, 1H), 7.35 (t, 1H), 7.45 (dd, 1H), 8.30 (s, 2H). High resolution MS: m/z found 480.0623 (M + 1), calculated 480.0625 (M + 1).

3-({[*t*-Butyl(dimethyl)silyl]oxy}methyl)isoxazolo[5,4-*b*]pyridine-6-carbonitrile (1n). To a solution of 3-({[*t*-butyl(dimethyl)silyl]-oxy}methyl)-6-fluoroisoxazolo[5,4-*b*]pyridine (470 mg, 1.66 mmol) in 2 mL of DMSO was added NaCN (82 mg, 1.664 mmol) and the reaction mixture was allowed to stir for 12 h at 90 °C. The reaction was then cooled to room temperature and diluted with EtOAc. The organic layer was washed with water (3 × 10 mL) and brine (1 × 10 mL), dried over sodium sulfate, filtered, and concentrated in vacuo. Flash chromatography of the crude residue (10−85% EtOAc/hexanes) gave 425 mg (88%) of **1n**. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 0.15 (s, 6H), 0.92 (s, 9H), 5.11 (s, 2H), 7.72 (d, 1H), 8.40 (d, 1H). ESMS, M + H^+ found 290.2.

3-(Chloromethyl)-6-(4,5-dihydro-1*H*-imidazol-2-yl)isoxazolo-[5,4-*b*]pyridine (1p). To a solution of 3-({[*t*-butyl(dimethyl)-silyl]oxy}methyl)isoxazolo[5,4-*b*]pyridine-6-carbonitrile (270 mg, 0.933 mmol) in 5 mL of EtOH at 0 °C was bubble HCl (g) for 2 min. The ice water bath was removed, and the reaction mixture was allowed to warm to room temperature for 4 h. The residual HCl gas was purged with N_2, and the reaction mixture was concentrated in vacuo. The crude residue was redissolved in 5 mL of EtOH to which was added ethylene diamine (0.077 mL, 0.993 mmol). The reaction was allowed to stir overnight at room temperature and concentrated in vacuo. Flash chromatography of the crude residue (0−8% MeOH/dichloromethane/0.1% TEA) gave 425 mg (88%) of [6-(4,5-dihydro-1*H*-imidazol-2-yl)isoxazolo[5,4-*b*]pyridin-3-yl]methanol as a clear oil. ^{1}H NMR (400 MHz, DMSO, ppm): δ 3.78 (s, 4H), 4.91 (d, 2H), 5.92 (t, 1H), 8.21 (d, 1H), 8.58 (d, 1H). ESMS, M + H^+ found 219.3.

To a solution of [6-(4,5-dihydro-1*H*-imidazol-2-yl)isoxazolo-[5,4-*b*]pyridin-3-yl]methanol (56 mg, 0.257 mmol) in 2 mL of dichloromethane was added thionyl chloride (37.5 uL, 0.513 mmol) and the reaction was allowed to stir at room temperature for 30 min. The reaction was then concentrated in vacuo to afford 61 mg of crude material as the HCl salt, which was used for the next reaction without further purification. ^{1}H NMR (400 MHz, DMSO, ppm): δ 4.09 (s, 4H), 5.31 (s, 2H), 8.37 (d, 1H), 8.92 (d, 1H), 8.58 (d, 1H), 9.5 (b, 1H), 11.15 (s, 2H) ESMS, M + H^+ found 237.2.

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Supporting Information Available: PhenoScreen assay data from Monogram Bioscience and rat PK of selected compounds (iv administration). This material is available free of charge via the Internet at http://pubs.acs.org.

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(4) The polymerase activity of reverse transcriptase was measured using a 500nt heteropolymeric RNA template and a biotinylated DNA primer. BrdU incorporation by purified recombinant RT (wild-type, K103N, or Y181C) was detected using a ruthenylated anti-BrdU antibody and a Bioveris M384 chemiluminescence detection instrument. Data represent mean and standard deviations of ≥2 experiments. Efavirenz was tested in this assay as a control (K_i (nM): 0.4/8.5/0.3 (WT/K103N/Y181C)).

(5) The antiviral potency of compounds against wild-type (H9IIIB) virus or H9IIIB with K103N or Y181C mutations was measured in a multiple-cycle replication assay in MT2 cells. Cells were infected overnight (moi ≈ 0.01) in the absence of compound, washed, and cultured for 3 days in varying compound concentrations. Viral replication was assessed by measuring as p24 in culture supernatants, and the CIC_{95} is the lowest concentration of compound inhibiting replication by ≥95%. Mutants K103N, Y181C, and K103N/Y181C are prepared by the Advanced Biotechnology, Inc. Also see Vacca, J. P.; Dorsey, B. D.; Schleif, W. A.; Levin, R. B.; McDaniel, S. L.; Darke, P. L.; Zygay, J.; Quintero, J. C.; Blahy, O. M.; Roth, E.; Sardana, V. V.; Schlabach, A. J.; Graham, P. I.; Condra, J. H.; Gotlib, L.; Holloway, M. K.; Lin, J.; Chen, I.; Vastag, K.; Ostovic, D.; Anderson, P. S.; Emini, E. A.; Huff, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4096. Efavirenz was tested in this assay as a control (CIC95 (nM): 4.3/38 (WT, 10% FBS/50% NHS), 232 (K103N, 10% FBS), 8.0 (Y181C, 10% FBS), and 272 (K103N/Y181C, 10% FBS)).

(6) See Supporting Information for detailed rat PK of selected compounds (iv administration).

(7) Assays performed by Monogram Bioscience, San Francisco, CA. Values are the average of two determinations. Detailed assay protocols are available at: http://www.monogramhiv.com/assays/hcp/phenolHIVTechnology.aspx.

(8) iv dose, 1, 3, and 10 mg/kg (cumulative); vehicle: 100% DMSO, 5 mL/30 min; 3 anesthetized, vagotomized, and ventilated dogs.

(9) Compound **6** was tested over a concentration range of 7.11−4000.00 μg/mL with and without S-9, in a forward mutation assay in *Salmonella typhimurium* strain FU100. Compound **6** did not produce any significant increases in mutants compared with the mean historical control levels in either the presence or absence of S-9 metabolic activation and is thus considered negative in the Exploratory 5-Fluorouracil Microbial Forward Mutation Assay developed at Merck.

(10) Exploratory 7-day tolerability study, po dose, vehicle: 10% polysorbate and 80/90% water.