Synthesis and Bioactivity of Novel Bis(heteroaryl)piperazine (BHAP) Reverse Transcriptase Inhibitors: Structure−Activity Relationships and Increased Metabolic Stability of Novel Substituted Pyridine Analogs

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The major route of metabolism of the bis(heteroaryl)piperazine (BHAP) class of reverse transcriptase inhibitors (RTIs), atevirdine and delavirdine, is via oxidative N-dealkylation of the 3-ethyl- or 3-isopropylamino substituent on the pyridine ring. This metabolic pathway is also the predominant mode of metabolism of (alkylamino)piperidine BHAP analogs (AAP−BHAPs), compounds wherein a 4-(alkylamino)piperidine replaces the piperazine ring of the BHAPs. The novel AAP−BHAPs possess the ability to inhibit non-nucleoside reverse transcriptase inhibitor (NNRTI) resistant recombinant HIV-1 RT and NNRTI resistant variants of HIV-1. This report describes an approach to preventing this degradation which involves the replacement of the 3-ethyl- or 3-isopropylamino substituent with either a 3-*tert*-butylamino substituent or a 3-alkoxy substituent. The synthesis, bioactivity and metabolic stability of these analogs is described. The majority of analogs retain inhibitory activities in enzyme and cell culture assays. In general, a 3-ethoxy or 3-isopropoxy substituent on the pyridine ring, as in compounds **10**, **20**, or **21**, resulted in enhanced stabilities. The 3-*tert*-butylamino substituent was somewhat beneficial in the AAP−BHAP series of analogs, but did not exert a significant effect in the BHAP series. Lastly, the nature of the indole substitution sometimes plays a significant role in metabolic stability, particularly in the BHAP series of analogs.

Introduction

The development of non-nucleoside inhibitors of HIV-1 reverse transcriptase (NNRTIs) as potential candidates for the treatment of HIV infection has been the focus of extensive research efforts in many laboratories.[1−6] Such research at Upjohn led to the discovery of the bis(heteroaryl)piperazine (BHAP) class of HIV-1 RT inhibitors.[7,8] This discovery served as the impetus for an extensive structure−activity relationship (SAR) program[9] which led to the development of the clinical candidates atevirdine mesylate (**1**)[10] and delavirdine mesylate (**2**).[11,12] Both of these compounds are potent inhibitors of HIV-1 RT *in vitro* and viral replication in cell culture.

The major route of metabolism *in vivo* for both **1** and **2** is via oxidative N-dealkylation of the ethylamino or isopropylamino substituent on the pyridine ring (Scheme 1). The ratio of drug to metabolite circulating *in vivo* has been determined to be approximately 1:1.[13] Unfortunately the amino metabolites, **3** and **4**, are both inactive in the *in vitro* assay for RT inhibition (Table 1). Therefore, we have been interested in replacing the ethylamino and isopropylamino groups of **1** and **2** with appropriate surrogates in order to slow metabolic degradation while at the same time retaining potency. More recently, we have shown that BHAP analogs containing an (alkylamino)piperidine (AAP−BHAPs) in place of the piperazine, as in compound **14** (Table 2), possess remarkable broad spectrum activity against a panel of recombinant RTs containing mutations known to confer resistance to other NNRTIs.[14,15] Unfortunately, N-dealkylation of these compounds occurs even more rapidly than in their piperazine forerunners. Thus, developing metabolically stable 3-pyridine substituents became critically important to the discovery of pharmaceutically acceptable AAP−BHAP clinical candidates.

Since cytochrome P450 mediated N-dealkylation proceeds through decomposition of a carbinolamine which is formed via either a one-electron oxidation on nitrogen or direct proton abstraction α to the nitrogen,[16,17] replacing the ethyl or isopropyl group with a group containing a quaternary carbon such as *tert*-butyl should preclude metabolism via the carbinolamine. Consideration of other potential substituents that would fit the structural criteria required for bioactivity led to the possibility of employing ethers. Even though metabolism of ethers is reported to occur via a pathway similar to N-dealkylation (i.e. via abstraction of an α-hydrogen and formation of an unstable hemiacetal which decomposes to the alcohol),[18] the relative rate of such a process compared to N-dealkylation was unknown for the BHAP chemical class. Literature reports indicating that the rate of O-dealkylation is decreased as the *n*-alkyl chain of an aromatic ether is lengthened[18,19] supported our decision to pursue the synthesis of the targeted ether analogs. Consequently, the piperazine-containing BHAP analogs **6**−**13** (Table 1) and the piperidine-containing AAP−BHAP analogs **17**−**23** (Table 2) were synthesized and tested for HIV-1 RT inhibitory activity. The active analogs which resulted from this effort were further evaluated to determine their metabolic stability *in vitro*.

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Synthesis

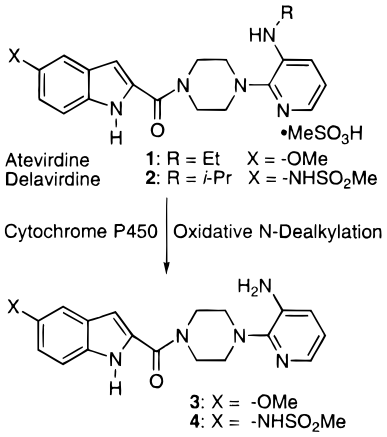
The ethylamine analog of delavirdine (**5**) was obtained by treating the precursor 5-aminoindole BHAP with methanesulfonyl chloride in a manner similar to the methods described by us previously.[8,9] Coupling of 1-[3-[(1,1-dimethylethyl)amino]-2-pyridyl]piperazine[20] with 5-methanesulfonamidoindole-2-carboxylic acid afforded *tert*-butylamine congener **6**. The synthesis of the alkoxy-BHAP analogs **7**−**13** began with the preparation of the 2-bromo-3-alkoxypyridines **25**−**28** (Scheme 2). The isopropyl, ethyl, and benzyl ethers were prepared in high yields by heating the commercially available 2-bromo-3-pyridinol (**24**) with the appropriate alkyl iodide in DMF. Standard methods for *tert*-butylation with isobutylene and acid failed under various conditions due to very poor solubility of the starting 2-bromo-3-pyridinol. Thus, the *tert*-butyl ether of 2-bromo-3-pyridinol was synthesized via a modification of the procedure developed by Jackson *et al.*[21] Alcohol **24** was treated with an excess of *tert*-butyl trichloroacetimidate and catalytic boron trifluoride etherate in a THF/cyclohexane mixture to afford the *tert*-butyl ether. These ethers were heated with excess piperazine in a stainless steel autoclave to afford the (pyridyl)piperazines **29**−**32** as oils in yields of 59−81%. Subsequent coupling with an indole carboxylic acid derivative provided the desired products **7**−**13**.

The synthesis of the AAP−BHAPs **14**−**16** was straightforward and began with the condensation of 1-benzyl-4-(*N*-methylamino)piperidine with 2-chloro-3-nitropyridine.[15] The remaining steps were conducted as described for the piperazine containing BHAPs: nitro group reduction, reductive alkylation, debenzylation, and coupling to indole-2-carboxylic acids. *tert*-Butylation of 1-benzyl-4-[*N*-methyl-*N*-(3-amino-2-pyridyl)amino]piperidine[20,22] followed by deprotection and coupling to 5-methanesulfonamidoindole-2-carboxylic acid afforded analog **17**. Alkoxypiperidine analogs **18**−**21** were prepared in a manner similar to that of the corresponding piperazine congeners (Scheme 3). Condensation of the piperidine moiety with 2-bromo-3-isopropoxypyridine was more difficult than experienced in the piperazine series. Although the same conditions were used, lower yields were usually obtained. Subsequent debenzylation and coupling with the appropriate indole-2-carboxylic acid proceeded uneventfully. The deuterated analogs **22** and **23** were prepared as depicted in Scheme 4 via reductive alkylation of (3-aminopyridyl)piperidine **37** with acetone and sodium borodeuteride for **38** or acetone-d_6 and sodium cyanoborohydride for **39**. Debenzylation and coupling of **38** and **39** with 5-methanesulfonamidoindole-2-carboxylic acid led to the deuterated AAP−BHAPs **22** and **23**.

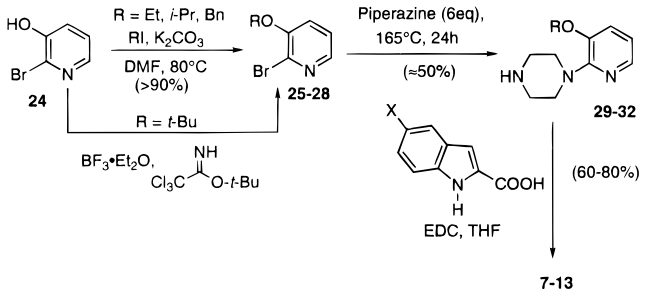
Results and Discussion

The analogs **5**−**23** were initially evaluated for their ability to inhibit recombinant HIV-1 RT in an *in vitro* assay.[8,9] The compounds were tested at 100 μM, and IC_{50} values were determined for most analogs. These analogs were further tested in a cell culture assay to determine their ability to inhibit HIV1 replication in human lymphocytes (PBMC) as previously described.[8] These results are summarized in Tables 1 and 2. From these data, it is clear that several analogs of both the

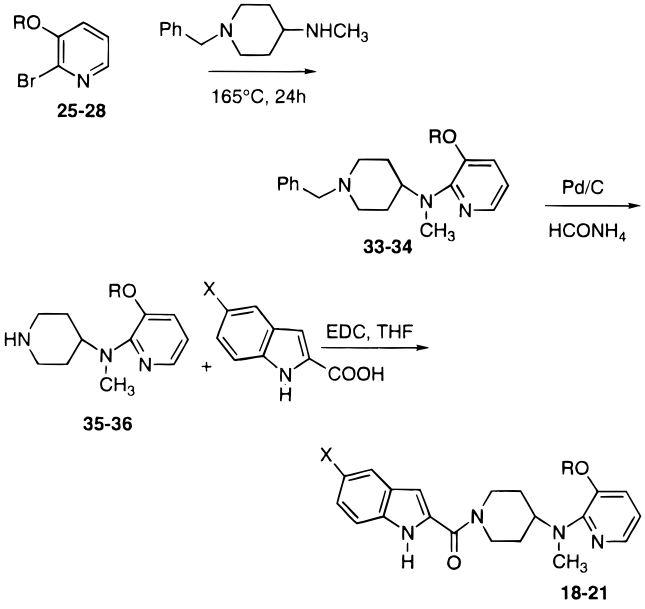
Scheme 1



Scheme 2



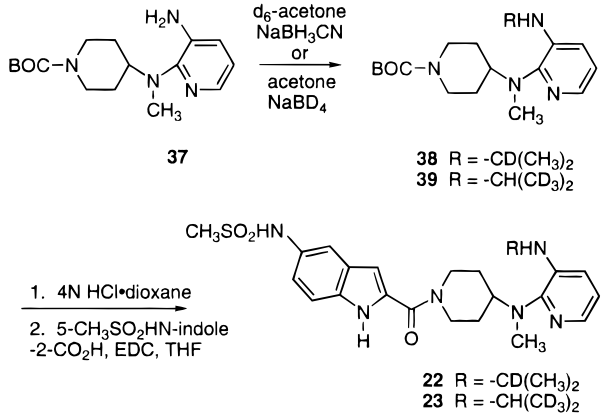
Scheme 3



piperazine and piperidine types are potent inhibitors of HIV-1 RT *in vitro* and HIV-1 replication in cell culture.[23]

Specifically, the isopropyl and ethyl ethers (**7**−**10**) retain activity comparable to the parent drugs **1** and **2**, demonstrating that the alkoxy group is a good surrogate for the alkylamine moiety. The *tert*-butylamine and *tert*-butyl ether analogs **6** and **13** were also quite active. In fact the *tert*-butylamino compound **6** possessed potency similar to the parent drug, delavirdine (**2**), in the tissue culture assay measuring inhibition of viral replication. Finally, both benzyl ethers **11** and **12** showed no activity in the initial assay. This result was

Scheme 4



not surprising since the corresponding benzylamines were previously shown to be inactive.[8]

The AAP−BHAPs containing a 3-alkylamino substituent such as compounds **14**−**17** exhibited very good potency in the *in vitro* antiviral assay. Replacing the 3-ethylamino group with a 3-ethoxy group resulted in a loss of anti-HIV-1 potency in the cell-based assay (e.g. **18** vs **14**). However within the alkoxy series, the piperazine-linked compounds appeared to possess activities comparable to the piperidine-linked compounds (**9** vs **18**, **10** vs **20**). Even though preliminary results indicated that substitution with the alkoxy group diminished antiviral potency in the AAP−BHAP series, several compounds containing the 3-alkoxypyridine possessed sufficient antiviral potency to warrant further evaluation. Therefore, selected potent analogs were evaluated for metabolic stability ($t_{1/2}$) in the presence of hepatic microsomal cytochrome P450 *in vitro*. The results of these experiments are also summarized in Tables 1 and 2.

In the piperazine-linked series reference compound 3-(ethylamino)pyridine **5** was more metabolically stable than either of the closely related parent drugs **1** or **2** with a half-life 3 times that of the corresponding 3-(isopropylamino)pyridine, delavirdine (**2**). This result parallels the trend in metabolic stabilities previously observed in other 5-substituted indole series such as atevirdine (**1**) and its 3-(isopropylamino)pyridine congener.[24] Compound **5** differs from atevirdine (**1**) solely in the substitution at the 5-position of the indole. Whereas atevirdine contains a 5-methoxy-substituted indole, **5** contains a 5-methanesulfonamido-substituted indole. This particular substitution resulted in a more than 2-fold increase in half-life.

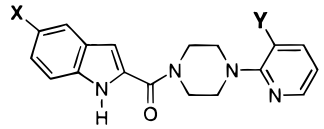
Unfortunately, no enhancement in metabolic stability was obtained for the (*tert*-butylamino)pyridine **6** relative to delavirdine. As detailed above, it was anticipated that the absence of an abstractable hydrogen α to the heteroatom would significantly decrease the dealkylative metabolism of **6**, since carbinolamine formation is precluded. Literature precedent indicates that N-dealkylation of *tert*-butylamines can occur via a cytochrome P450 mediated oxidation of one of the methyl groups.[25] The resulting alcohol is converted to the carboxylic acid via two consecutive oxidations by alcohol dehydrogenase and aldehyde dehydrogenase. Finally, decarboxylation of the acid to the isopropylamine, subsequent oxidation by cytochrome P450 to the carbinolamine, and decomposition provide the dealkylated amine.[16] Even though *tert*-butylamines can still be dealkylated, any diminution in the efficiency of the initial hydroxylation reaction relative to N-dealkylation of the isopropylamine would result in increased metabolic stability for *tert*-butylamine **6**. Preliminary results with a closely related compound that lacked indole substitution indicated that the analog containing the *tert*-butylamino substituent reduced the amount of *N*-desalkyl metabolite observed relative to compound containing the isopropylamino substituent.[8] Similarly, the dealkylated material was not observed as the major metabolite in the case of **6**. The fact that the presence of the *tert*-butylamine did not increase the half-life in this assay, which measured disappearance of starting material, suggests that other equally efficient metabolic processes such as the side-chain hydroxylation or pyridine ring hydroxylation were competitive.

Alkoxy-BHAPS containing 5-unsubstituted indoles such as **7** and **9** were much less metabolically stable in comparison to their 5-methanesulfonamido-substituted counterparts **8** and **10**. This was also observed in the case of the (ethylamino)- and (isopropylamino)-BHAPs with unsubstituted versus substituted indoles and is likely due to hydroxylative metabolism at the 5-position of the indole.[26] A dramatic increase in metabolic stability was observed in the case of compound **10** which contains the requisite 5-substituted indole and a 3-ethoxypyridine. This compound possessed a half-life 3−4 times longer than that of either atevirdine or delavirdine, and approximately 4 times longer than that of the corresponding isopropyl ether **8**. Moreover, direct comparison of the half-lives of ethyl ether **10** to its ethylamino counterpart **5** demonstrated that the ether is superior in its metabolic stability. Unfortunately, the isopropyl ether **8** and *tert*-butyl ether **13** do not possess similar enhanced stabilities compared to the isopropylamine **2** or *tert*-butylamine **6**. In summary, the alkoxy BHAPs follow the same general trends in metabolic stability as the (alkylamino)-BHAPs, i.e. isopropyl ethers and *tert*-butyl ethers have similar half-lives, both of which are shorter than the ethyl ethers. The longer half-life of ether **10** suggests that dealkylation was slowed or that metabolism was shunted to a less efficient pathway such as pyridine ring hydroxylation and/or hydroxylation of the ether side chain.[18]

The exciting discovery that certain AAP−BHAPs had the ability to inhibit various mutant RT enzymes, particularly the RT containing the P236L mutation linked to BHAP resistance,[14] led to the investigation of their pharmacokinetic properties. This in turn led to the disappointing discovery that these compounds were unsuitable from a pharmacokinetic standpoint due to their rapid metabolism.[15] The piperidine AAP−BHAPs possessed half-lives much shorter than the corresponding piperazine BHAPs (e.g. **16** vs **2**, and **17** vs **6**). Once again the 3-*tert*-butylamino substituent as found in compound **17** provided only modest protection from metabolic degradation, although, relatively, it contributed more to metabolic stability in the piperidine series than in the piperazine series. In any case, we were anxious to apply the alkoxy-substituted pyridine solution to this series of compounds to see if benefits similar to those obtained in the piperazine series (Table 1) could be realized.

We were gratified to find that comparison of the

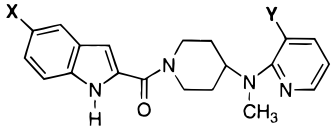
Table 1. Biological Activities and Metabolic Stability ($t_{1/2}$) of BHAP Analogs



compound	X	Y	RT inhibition[a] in vitro % at 100 μM	RT inhibition[a] in vitro IC_{50} (μM)	PBMC[b] D34 ED_{50} (μM)	$t_{1/2}$[c] (min)
atevirdine (**1**)	OMe	NHEt	92	5.2	0.001	14.7
delavirdine (**2**)	$NHSO_2Me$	NH−*i*−Pr	98	1.1	0.0001	10.8
3	OMe	NH_2	0	ND	ND	ND
4	$NHSO_2Me$	NH_2	0	ND	ND	ND
5	$NHSO_2Me$	NHEt	96	2.7	0.01−0.1	36.5
6	$NHSO_2Me$	NH-*tert*-Bu	97	4.8	0.0001	10.1
7	H	O-*i*-Pr	98	1.8	0.001−0.01	3.1
8	$NHSO_2Me$	O-*i*-Pr	99	1.1	0.001−0.01	11.7
9	H	OEt	98	2.1	0.1	6.1
10	$NHSO_2Me$	OEt	98	1.9	0.01	46.8
11	H	OBn	0	ND	ND	ND
12	$NHSO_2Me$	OBn	0	ND	ND	ND
13	$NHSO_2Me$	O-*tert*-Bu	92	ND	0.1	10.6

[a] The HIV-1 RT *in vitro* assay was carried out with recombinant enzyme[32] using the template:primer poly(rA):$(dT)_{10}$ and dTTP as the mononucleotide substrate as described.[8,33] IC_{50} values were determined by assaying at four drug concentrations. [b] See footnote 34 for a description of the assay. [c] Half-life of parent compound upon microsomal incubation. ND = not determined.

Table 2. Biological Activities and Metabolic Stability ($t_{1/2}$) of AAP−BHAP Analogs



compound	X	Y	RT inhibition[a] *in vitro* % at 100 μM	RT inhibition[a] *in vitro* IC_{50} (μM)	PBMC[b] D34 ED_{50} (μM)	$t_{1/2}$ (min)
14	H	NHEt	96	2.2	0.0001	0.9
15[c]	H	NH-*i*-Pr	96	2.0	0.0001	1.03
16	$NHSO_2Me$	NH-*i*-Pr	98	2.2	<0.0001	1.4
17	$NHSO_2Me$	NH-*tert*-Bu	97	3.1	<0.0001	3.6
18	H	OEt	94	3.2	0.01−0.1	2.8
19	H	O-*i*-Pr	96	2.0	ND	ND
20	$NHSO_2Me$	OEt	96	3.4	0.01−0.1	22.0
21	$NHSO_2Me$	O-*i*-Pr	91	1.4	ND	10.0
22	$NHSO_2Me$	$NHCD(CH_3)_2$	98	ND	ND	1.88
23	$NHSO_2Me$	$NHCH(CD_3)_2$	98	ND	ND	1.25

[a] The HIV-1 RT *in vitro* assay was carried out with recombinant enzyme[32] using the template:primer poly(rA):$(dT)_{10}$ and dTTP as the mononucleotide substrate as described.[8,33] IC_{50} values were determined by assaying at four drug concentrations. [b] See footnote 34 for a description of the assay. A less than (<) symbol indicates that the ED_{50} was below the lowest concentration tested. [c] Compound **15** was tested as the mesylate salt. ND = not determined.

3-ethoxy-substituted AAP−BHAP, **18**, with the corresponding 3-ethylamino congener, **14**, demonstrated a 3-fold improvement in the half-life. Moreover, an even greater benefit was derived from the analog which possessed an indole 5-methanesulfonamido group in concert with a 3-isopropoxy substituent (**21**), relative to its 3-isopropylamino-substituted counterpart (**16**) wherein a 7-fold enhancement in half-life was achieved. The trend wherein a 3-ethoxy substituent, as in compound **20**, proved to be more robust than the 3-isopropoxy substituent (**21**) continued. In short, a half-life similar to that of clinical candidate delavirdine was realized with AAP−BHAP **21** and a half-life 2-fold longer was realized with AAP−BHAP **20**. The enhanced stability of the alkoxypyridines carries over into the AAP−BHAP series of analogs.

Another approach to enhancing the metabolic stability of the AAP−BHAP template involved the synthesis of compound **22** wherein the α-hydrogen of the isopropylamine is replaced by a deuterium atom. If the mechanism of metabolic degradation involves a direct proton abstraction, then a significant primary deuterium isotope effect ($k_H/k_D = 10$) should be observed. Since production of deuterated **22** involves a simple modification of the chemical procedure utilized to produce **16**, such an effect might easily be used to our advantage to afford a compound with increased metabolic stability. The hexadeuterated compound **23** was also synthesized for comparison. Only a small increase in the half-life of **22** was observed, indicative of a secondary isotope effect ($k_H/k_D = 2$). Thus it appears likely that cytochrome P450 is acting via a one-electron oxidation of the nitrogen.

The effect of alkoxy versus (alkylamino)pyridine substituents on the broad spectrum activity of the AAP−BHAPs **15**−**21** was assessed in enzyme assays employing recombinant RTs containing a mutation at amino acid 236 (proline to leucine)[27] or 181 (tyrosine to cysteine)[28] (Table 3). The alkoxy-AAP−BHAPs **18**−**21** demonstrated slightly lower IC_{50} values against WT RT

Table 3. Activities of Selected AAP−BHAP Analogs against Wild-Type and Mutant RT Enzymes

	IC_{50} (μM)[a]			fold resistant[b]	
compound	WT	P236L	Y181C	P236L/WT	Y181C/WT
15[c]	0.23	0.74	0.80	3.2	3.5
16	0.50	1.5	1.1	3.0	2.2
17	0.39	1.5	0.51	3.8	1.3
18	0.18	1.5	4.8	5.6	26.7
19	0.09	1.27	3.7	14.1	41.1
20	0.06	1.5	2.0	16.7	33.3
21	0.10	1.2	2.0	12.0	20.0

[a] RNA-dependent DNA polymerase activity of mutant RTs was assayed as described in the Experimental Section. IC_{50} values were determined by nonlinear least-squares fit of data from duplicate points at six drug concentrations. Differences in the IC_{50} values against WT RT in Table 3 and those in Table 2 are presumed to reflect the fact that two sets of experiments were performed in different laboratories using somewhat different assay conditions (cf. Experimental Section). [b] Expressed as the ratio of IC_{50} mutant RT/IC_{50} WT RT. [c] Compound **15** was tested as the mesylate salt.

Table 4. Comparison of the Antiviral Activity of an Alkoxy-Substituted AAP−BHAP to an Alkylamino AAP−BHAP against NNRTI Resistant Viruses

	IC_{90} (μM)	
compound	U-90152^R HIV-1_{MF} (P236L)	L-697,661^R HIV-1_{IIIB} (Y181C)
16	5.3	1.0
21	4.0	5.1

than those of the (alkylamino)-AAP−BHAPs **15**−**17**, while both types of analogs possessed comparable activities against the P236L enzyme. However, when activities against the Y181C mutant enzyme were compared, the alkoxy analogs (**18**−**21**) were less potent (i.e. higher IC_{50} values) than their alkylamino counterparts (**15**−**17**). Moreover, comparison of the P236L RT or Y181C RT IC_{50} values to WT RT IC_{50} values to generate fold resistance ratios for each compound revealed that the mutant RTs were more resistant to the alkoxy-AAP−BHAPs (Table 3). For example, the fold resistance of the Y181C RT to alkoxy compounds **18**−**21** ranged from 20 to 41.1, whereas the fold resistance to alkylamino compounds **15**−**17** ranged from 1.3 to 3.5. This effect is due to the increased activity of the alkoxy compounds against WT RT in concert with their decreased activity against Y181C RT. Differences in P236L RT fold resistance ratios were observed between the two sets of analogs but were of lesser magnitude. The ratios also indicated that the Y181C mutation conferred a higher level of resistance to the alkoxy compounds **18**−**21** than did the substitution at residue 236. This trend was not observed for the alkylamino compounds **15**−**17** as indicated by the fold resistance ratios for both mutant enzymes which were comparable.

An assessment of the effects of the alkylamino and alkoxy substituents on antiviral activity is shown in Table 4. The antiviral activities of **16** and **21** were compared in cell culture assays that employed drug resistant virus stocks derived by serial passage of virus in the presence of the NNRTIs U-90152 (BHAP) or Merck's L-697,661 (pyridinone). From genotypic analyses of these two HIV-1 variants, the BHAP and L-drug resistant viruses contained the P236L and Y181C RT substitutions, respectively. Comparison of the IC_{90} values revealed **16** and **21** to be comparable in inhibiting the replication of BHAP resistant virus; however, the IC_{90} value for the alkoxy analog **21** versus L-drug resistant virus was 5-fold higher than the IC_{90} for the alkylamino analog **16**. The relative susceptibilities of these two viruses to **16** and **21** are in accordance with the *in vitro* inhibitory activities observed for these two compounds against the mutant RTs (Table 3). In short, the 3-alkoxy substitution preserved the *in vitro* inhibitory activities against both the wild-type and P236L RT enzymes. However, the alkoxy substituents conferred a reduction in potency against the Y181C RT *in vitro* and L-drug resistant virus expressing the Y181C RT. Nevertheless, we evaluated the pharmacokinetic parameters of isopropoxy AAP−BHAP **21** upon intravenous and oral administration to rats at 15 mg/kg. The enhanced metabolic stability observed in liver microsomes correlated with the *in vivo* results obtained in rats. For example, alkoxy analog **21** had an iv clearance rate 0.8-fold that of its isopropylamino counterpart **16**, and an absolute oral bioavailability 3-fold greater than that of **16**.[15] These results indicate substantial improvement in the metabolic stability and/or absorption efficiency of the AAP−BHAP template as a result of the alkoxy substitution. The oral bioavailability of **21** was 40% that of delavirdine[15] at comparable doses. Thus even with the improvements offered by the alkoxy substituent, compounds containing the AAP−BHAP template possess lower oral bioavailabilities than the corresponding compounds containing the piperazine template.

Conclusion

The synthesis of *tert*-butylamine and various alkyl ether BHAP and AAP−BHAP analogs designed to possess enhanced metabolic stability relative to the parent drugs **1** and **2** was accomplished. The majority of the analogs synthesized retained potent RT inhibitory activities. Compounds which contained indoles unsubstituted in the 5-position in either the alkylamine or alkyl ether series possessed metabolic stabilities less than the parent compounds, as evidenced by their relative half-lives in the *in vitro* microsomal assay. In addition, the nature of the 5-indole substituent was found to be an important contributor to the metabolic stability of the BHAPs. The 5-methanesulfonamido substituent found in delavirdine decreased the metabolism of the piperazine BHAP analogs relative to the 5-methoxy group found in atevirdine. The relative order of metabolic stability among the 3-pyridine substituents in both the alkylamine or alkyl ether series in the BHAP compounds which contained the 5-methanesulfonamidoindole was ethyl > isopropyl = *tert*-butyl. As a result, the piperazine-linked analogs which incorporated a 5-substituted indole in concert with an 3-(ethylamino)-pyridine or 3-ethoxypyridine, such as compounds **5** and **10**, possessed the longest half-lives, 3−4 times those of the parent drugs.

In general, the AAP−BHAPs were metabolically degraded in this assay more rapidly than their piperazine counterparts. The presence or absence of a 5-indole substituent did not appreciably alter the metabolic stability. On the other hand, a 3-(*tert*-butylamino)-pyridine substituent resulted in relatively greater stability in this series than it did in the piperazine series. Incorporation of a 3-alkoxy substituent exerted a very beneficial effect on metabolic stability providing analogs with half-lives similar to or greater than delavirdine.

Once again the ethoxy-substituted analog was more stable than the isopropoxy analog. The compounds embodying these changes, **20** and **21**, possessed good activities in cell culture and against the WT and P236L RT enzymes. Activities against the Y181C RT *in vitro* and virus harboring a Y181C containing a Y181C mutation were diminished. Therefore we are continuing to investigate alternate solutions to this problem.

Experimental Section

Flash chromatography utilized E. Merck silica gel (230−400 mesh). Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. High-resolution mass spectra and combustion analysis were obtained from the Structural, Analytical and Medicinal Chemistry Department at Pharmacia & Upjohn. Proton NMR spectra were recorded on a Bruker Aspect 3000 300-MHz spectrometer. Carbon NMR spectra were performed on the same instrument at 75 MHz.

General Procedure for Preparation of 3-Alkoxypyridines 25−27. 2-Bromo-3-isopropoxypyridine (25). A mixture of 2-bromo-3-pyridinol (1.74 g, 10 mmol), 2-iodopropane (2 mL, 20 mmol), and K_2CO_3 (2.5 g, 18 mmol) in dry DMF (20 mL) was heated to 80 °C for 2 h. The solvent was removed *in vacuo*, and the residue was partitioned between EtOAc and H_2O. The aqueous layer was extracted with EtOAc and the combined organic layers were washed with H_2O and brine and dried (Na_2SO_4). Removal of solvent *in vacuo* gave a tan liquid which was homogeneous by TLC and used without further purification: yield 2.15 g (99%); ^{1}H NMR ($CDCl_3$) δ 7.80 (dd, J = 2.4, 3.8 Hz, 1 H), 7.03−7.10 (m, 2 H), 4.39−4.51 (m, 1 H), 1.24 (d, J = 6.1 Hz, 6 H); ^{13}C NMR ($CDCl_3$) δ 151.24, 140.87, 133.96, 123.08, 121.48, 72.15, 21.56; HRMS calcd for $C_8H_{10}N_{10}$-OBr 214.9946, found 214.9945.

2-Bromo-3-ethoxypyridine (26): yield 92%; ^{1}H NMR ($CDCl_3$) δ 7.31 (dd, J = 1.8, 4.4 Hz, 1 H), 6.53−6.62 (m, 2 H), 3.48 (q, J = 7.0 Hz, 2 H), 0.84 (t, J = 7.0 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 152.30, 140.95, 132.89, 123.43, 119.56, 64.94, 14.52; HRMS calcd for $C_7H_8N_{10}$OBr 200.9790, found 200.9801.

2-Bromo-3-(benzyloxy)pyridine (27): yield 66%; ^{1}H NMR ($CDCl_3$) δ 7.86−7.91 (m, 1 H), 7.37−7.41 (m, 2 H), 7.22−7.34 (m, 3 H), 7.05−7.13 (m, 2 H), 5.03 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 151.69, 141.19, 135.33, 132.83, 128.47, 128.01, 126.83, 123.35, 120.17, 70.48; HRMS calcd for $C_{12}H_{10}$NOBr 262.9946, found 262.9944. Anal. ($C_{12}H_{10}$NOBr) C, H, N.

2-Bromo-3-*tert*-butoxypyridine (28). 2-Bromo-3-pyridinol (0.5 g, 2.9 mmol) was dissolved in a minimum amount of dry THF, and cyclohexane was added until the solution became turbid. Then *tert*-butyl trichloroacetimidate (2.1 mL) was added followed quickly by BF_3·Et_2O (0.06 mL). The reaction mixture became homogeneous and was stirred at room temperature for 1 h after which time additional *tert*-butyl trichloroacetimidate (1 mL) was added. The reaction mixture was stirred overnight, and solid $NaHCO_3$ was added. The mixture was filtered through a plug of silica gel, and the solvent was removed *in vacuo*. The white solid thus obtained was triturated in hexane, filtered, and washed with copious amounts of hexane. The filtrate was concentrated *in vacuo* to a colorless liquid which was chromatographed on a 2 × 40 cm column with EtOAc/hexane (1:3). The product was isolated as a colorless liquid in a yield of 0.42 g (64%): ^{1}H NMR ($CDCl_3$) δ 7.96 (dd, J = 4.6, 1.6 Hz, 1 H), 7.29 (dd, J = 8.01, 1.68 Hz, 1 H), 7.09 (dd, J = 8.05, 4.48 Hz, 1 H), 1.36 (s, 9 H).

General Procedure for the Preparation of 29−34 via Condensation of Piperazine or 1-Benzyl-4-(methylamino)piperidine with 3-Alkoxypyridines 25−28. 1-(3-Isopropoxy-2-pyridyl)piperazine (29). A neat mixture of piperazine (2.69 g, 31.25 mmol) and **25** (1.35 g, 6.25 mmol) was heated in a sealed stainless steel autoclave at 165 °C for 24 h. The resulting mixture was partitioned between $CHCl_3$ and 1 M $NaHCO_3$ solution. The organic layer was washed with H_2O and dried (Na_2SO_4). Removal of solvent *in vacuo* gave a brown oil which was chromatographed on a 2 × 30 cm column with MeOH/$CHCl_3$ (1:9) as the eluting solvent. The product, 1.12 g (81%), was isolated as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.69 (dd, J = 1.5, 4.9 Hz, 1 H), 6.86 (dd, J = 1.4, 7.8 Hz, 1 H), 6.60 (dd, J = 4.8, 7.8 Hz, 1 H), 4.32−4.40 (m, 1 H), 3.20−3.24 (m, 4 H), 2.85−2.88 (m, 4 H), 2.50 (s, 1 H), 1.16 (d, J = 6.1 Hz, 6 H); ^{13}C NMR ($CDCl_3$) δ 153.32, 144.61, 139.24, 121.80, 116.58, 70.44, 49.25, 45.94, 22.02; HRMS calcd for $C_{12}H_{19}N_3O_1$ 221.1521, found 221.1528. Anal. ($C_{12}H_{19}N_3O$·0.25H_2O) C, H, N.

1-(3-Ethoxy-2-pyridyl)piperazine (30): yield 59%; ^{1}H NMR ($CDCl_3$) δ 7.86 (dd, J = 1.5, 4.9 Hz, 1 H), 7.01 (dd, J = 1.4, 7.9 Hz, 1 H), 6.80 (dd, J = 4.9, 7.9 Hz, 1 H), 4.03 (q, J = 7.0 Hz, 2 H), 3.38−3.41 (m, 4 H), 3.04−3.07 (m, 4 H), 2.75 (s, 1 H), 1.46 (t, J = 7.0 Hz, 6 H); ^{13}C NMR ($CDCl_3$) δ 151.88, 145.71, 138.41, 118.15, 116.44, 63.29, 49.04, 45.58, 14.38; HRMS calcd for $C_{11}H_{17}N_3O_1$ 207.1372, found 207.1528. Anal. ($C_{11}H_{17}N_3O$·H_2O) C, N; H: calcd, 8.50; found, 7.78.

1-[3-(Benzyloxy)-2-pyridyl]piperazine (31): yield 49%; ^{1}H NMR ($CDCl_3$) δ 7.89 (dd, J = 1.4, 4.9 Hz, 1 H), 7.28−7.41 (m, 5 H), 7.10 (dd, J = 1.4, 7.9 Hz, 1 H), 6.82 (dd, J = 4.9, 7.8 Hz, 1 H), 5.09 (s, 2 H), 4.52 (br s, 1 H), 3.53 (br s, 4 H), 3.11 (br s, 4 H); ^{13}C NMR ($CDCl_3$) δ 151.86, 145.70, 139.29, 136.23, 128.53, 128.00, 127.00, 119.65, 116.92, 70.30, 48.13, 45.08; HRMS calcd for $C_{16}H_{19}N_3O$ 269.1528, found 269.1522.

1-(3-*tert*-Butoxy-2-pyridyl)piperazine (32): yield 78%; ^{1}H NMR ($CDCl_3$) δ 7.95 (dd, J = 4.77, 1.54 Hz, 1 H), 7.18 (dd, J = 7.78, 1.71 Hz, 1 H), 6.77 (dd, J = 7.84, 4.79 Hz, 1 H), 3.61−3.58 (m, 4 H), 1.33 (s, 9 H). Anal. ($C_{13}H_{21}N_3O$·0.6H_2O) C, H, N.

1-Benzyl-4-[*N*-methyl-*N*-(3-ethoxy-2-pyridyl)amino]-piperidine (33). A neat mixture of 1-benzyl-4-(methylamino)-piperidine[15] (2.92 g, 14.3 mmol) and **26** (1.44 g, 7.15 mmol) was treated as described above for **29**: yield 63%; ^{1}H NMR ($CDCl_3$) δ 7.82 (dd, J = 1.5, 4.8 Hz, 1H), 7.32−7.22 (m, 5H), 6.97 (dd, J = 1.5, 7.8 Hz, 1H), 6.70 (dd, J = 4.8, 7.8 Hz, 1H), 4.01 (q, J = 7.0 Hz, 2H), 3.78 (m, 1H), 3.50 (s, 2H), 2.97 (m, 2H), 2.88 (s, 3H), 2.05−1.85 (m, 4H), 1.69 (m, 2H), 1.45 (t, J = 7.0 Hz, 3H). Anal. ($C_{20}H_{27}N_3O$) C, H, N.

1-Benzyl-4-[*N*-methyl-*N*-[3-(1-methylethoxy)-2-pyridyl]-amino]piperidine (34): yield 51%; ^{1}H NMR ($CDCl_3$) δ 7.82 (dd, J = 1.5, 4.8 Hz, 1H), 7.32−7.23 (m, 5H), 6.98 (dd, J = 1.4, 9.3 Hz, 1H), 6.70 (dd, J = 4.9, 7.9 Hz, 1H), 4.53 (sept, J = 6.0 Hz, 2H), 3.82 (m, 1H), 3.49 (s, 2H), 2.97 (m, 2H), 2.86 (s, 3H), 1.97 (m, 4H), 1.69 (m, 2H), 1.34 (d, J = 6.1 Hz, 6H). Anal. ($C_{21}H_{29}N_3O$) C, H, N.

General Procedure for the Debenzylation of Benzylpiperidines 33 and 34 To Afford 35 and 36. 4-[*N*-Methyl-*N*-(3-ethoxy-2-pyridyl)amino]piperidine (35). A mixture of **33** (1.40 g, 4.30 mmol), 10% Pd/C (1.4 g), and ammonium formate (813 mg, 12.9 mmol) in methanol (25 mL) was degassed, stirred at 65 °C for 45 min, cooled to RT, and filtered through Celite to remove catalyst. The filtrate was concentrated *in vacuo* to provide 832 mg (83%) of the title compound as a colorless film: ^{1}H NMR ($CDCl_3$) δ 7.83 (d, J = 4.8 Hz, 1H), 6.97 (d, J = 7.7 Hz, 1H), 6.71 (dd, J = 4.9, 7.5 Hz, 1H), 4.02 (q, J = 6.9 Hz, 2H), 3.88 (m, 1H), 3.15 (bd, J = 11.9 Hz, 2H), 2.88 (s, 3H), 2.63 (m, 2H), 1.75 (m, 4H), 1.46 (t, J = 6.9 Hz, 3H); HRMS (EI) calcd for $C_{13}H_{21}N_3O$: 235.1685, found 235.1688.

4-[*N*-Methyl-*N*-[3-(1-methylethoxy)-2-pyridyl]amino]-piperidine (36): yield 80%; ^{1}H NMR ($CDCl_3$) δ 7.82 (m, 1H), 6.98 (d, J = 7.5 Hz, 1H), 6.71 (m, 1H), 4.54 (m, 1H), 3.92 (m, 1H), 3.15 (bd, J = 11.5 Hz, 2H), 2.87 (s, 3H), 2.62 (m, 2H), 1.74 (m, 5H), 1.35 (d, J = 5.8 Hz, 6H); HRMS (EI) calcd for $C_{14}H_{23}N_3O$ 249.1841, found 249.1843.

1-[(1,1-Dimethylethoxy)carbonyl]-4-[*N*-methyl-*N*-[3-[(1-deuterio-1-methylethyl)amino]-2-pyridyl]amino]-piperidine (38). 1-[(1,1-Dimethylethoxy)carbonyl]-4-[*N*-methyl-*N*-(3-amino-2-pyridyl)amino]piperidine (0.61 g, 2.0 mmol) was dissolved in 2.5 mL of acetone and 0.72 mL of acetic acid-*d*. The solution was cooled to 0 °C, and sodium borodeuteride (0.146 g, 3.5 mmol) was added. The ice bath was removed, and the reaction mixture was stirred at room temperature for 30 min. Then another 0.07 g of sodium borodeuteride was added, and the reaction mixture was stirred another 30 min. The reaction mixture was quenched with D_2O, and the mixture was partitioned between 1 N NaOH and $CHCl_3$. The organic

layer was dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (20% ethyl acetate/hexane) afforded 0.51 g of the title compound (73%) as an oil: ^{1}H NMR ($CDCl_3$) δ 7.58 (dd, J = 1.6, 4.7 Hz, 1H), 6.80 (dd, J = 4.7, 8.0 Hz, 1H), 6.71 (dd, J = 1.6, 8.0 Hz, 1H), 4.36 (br, 1H), 3.98 (br, 2H), 3.11 (tt, J = 3.7, 10.8 Hz, 1H), 2.65 (br t, 2H), 2.51 (s, 3H), 1.63 (m, 2H), 1.42 (m, 2H), 1.35 (s, 9H), 1.11 (s, 6H); MS (EI) *m/e* (rel int): 350 (26), 349 (41), 166 (41), 165 (59), 164 (40), 57 (100); HRMS calcd for $C_{19}H_{31}DN_4O_2$ 349.2588, found 349.2588.

1-[(1,1-Dimethylethoxy)carbonyl]-4-[*N*-methyl-*N*-[3-[[1-(1,1,1-trideuteriomethyl)-2,2,2-trideuterioethyl]amino]-2-pyridyl]amino]piperidine (39). 1-[(1,1-Dimethylethoxy)-carbonyl]-4-[*N*-methyl-*N*-(3-amino-2-pyridyl)amino]-piperidine (0.5 g, 1.63 mmol) was dissolved in 3.2 mL of CH_3-OH and cooled to 0 °C. Then acetone-d_6 (0.18 mL, 2.4 mmol) and acetic acid (0.92 mL, 16 mmol) were added. $NaCNBH_3$ (0.15 g, 2.4 mmol) was added in one portion, and the reaction was warmed to room temperature. Since the reaction proceeded only modestly, additional acetone-d_6 (0.5 mL), acetic acid (0.5 mL), and $NaCNBH_3$ (0.1 g) were added in batches four times, at which point the reaction mixture was complete as detected by TLC. Then the reaction mixture was partitioned between 1 N NaOH and $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Flash column chromatography (20% ethyl acetate/hexane) afforded 0.57 g (99%) of the title compound: ^{1}H NMR ($CDCl_3$) δ 7.54 (dd, J = 1.6, 4.7 Hz, 1H), 6.77 (dd, J = 4.7, 8.0 Hz, 1H), 6.67 (dd, J = 1.6, 8.0 Hz, 1H), 3.84 (br, 2H), 3.33 (s, 1H), 3.02 (tt, J = 3.7, 10.8 Hz, 1H), 2.50 (br t, 2H), 2.44 (s, 3H), 1.51 (m, 2H), 1.33 (m, 2H), 1.26 (s, 9H); MS (EI) *m/e* (rel int): 355 (22), 354 (100), 211 (21), 171 (29), 170 (89); HRMS calcd for $C_{19}H_{26}D_6N_4O_2$ 354.2902, found 354.2897.

1-[(5-Methanesulfonamidoindol-2-yl)carbonyl]-4-[3-(ethylamino)-2-pyridyl]piperazine (5). 1-[(5-Aminoindol-2-yl)carbonyl]-4-[3-(ethylamino)-2-pyridyl]piperazine[15] (0.16 g, 0.43 mmol) was dissolved in 4 mL of CH_2Cl_2 and cooled to 0 °C. Then pyridine (0.037 mL, 0.45 mmol) was added followed by methanesulfonyl chloride (0.035 mL, 0.45 mmol), and the reaction mixture was slowly warmed to room temperature and stirred for 24 h. The reaction mixture was diluted with $CHCl_3$, washed with aqueous $NaHCO_3$, and dried over Na_2SO_4. Purification by flash column chromatography (90% EtOAc/hexane) provided 0.11 g (58%) of the title compound: mp 215−216 °C (EtOAc/hexane); ^{1}H NMR (CD_3OD) δ 7.48 (m, 2H), 7.34 (d, J = 8.8 Hz, 1H), 7.07 (dd, J = 2.1, 8.8 Hz, 1H), 6.90 (m, 2H), 6.75 (s, 1H), 3.95 (br, 4H), 3.11 (q, J = 7.0 Hz, 2H), 3.02 (m, 4H), 2.80 (s, 3H), 1.19 (t, J = 7.0 Hz, 3H). Anal. ($C_{21}H_{26}N_6O_3S$) C, H, N, S.

General Procedure for Synthesis of 6−13 via EDC Coupling of Indole-2-carboxylic Acids with Pyridylpiperazines or 4-(*N*-Methyl-*N*-(3-alkoxy-2-pyridyl)amino]-piperidines. 1-[(5-Methanesulfonamidoindol-2-yl)carbonyl]-4-[3-[(1,1-dimethylethyl)amino]-2-pyridyl]-piperazine (6). 5-Methanesulfonamidoindole-2-carboxylic acid[29] (0.46 g, 1.80 mmol), 1-[3-[(1,1-dimethylethyl)amino]-2-pyridyl]piperazine[8] (0.42 g, 1.80 mmol), and EDC (0.35 g, 2.2 mmol) were coupled in 3 mL of THF and 3 mL of DMF as described for **7**. Workup, chromatography (60% EtOAc/hexane to 10% CH_3OH/EtOAc), and crystallization (DMF) afforded 0.41 g (48%) of the title compound: mp 259−260 °C; ^{1}H NMR (DMSO) δ 7.53 (m, 1H), 7.44 (m, 1H), 7.34 (d, J = 8.7 Hz, 1H), 7.07 (m, 2H), 6.88 (dd, J = 4.7, 7.9 Hz, 1H), 6.80 (s, 1H), 4.56 (s, 1H), 3.87 (br, 4H), 2.93 (br, 4H), 2.83 (s, 3H), 1.32 (s, 9H). Anal. ($C_{23}H_{30}N_6O_3S\cdot0.5H_2O$) C, H, N, S.

1-(Indol-2-ylcarbonyl)-4-(3-isopropoxy-2-pyridyl)piperazine (7): yield 71%; mp 169−170 °C; ^{1}H NMR ($CDCl_3$) δ 9.43 (s, 1 H), 7.88 (dd, J = 1.5, 4.9 Hz, 1 H), 7.66 (d, J = 7.9 Hz, 1 H), 7.44 (d, J = 8.2 Hz, 1 H), 7.26−7.31 (m, 1 H), 7.07−7.17 (m, 2 H), 6.83−6.87 (m, 2 H), 4.54−4.62 (m, 1 H), 4.09 (br s, 4 H), 3.55−3.58 (m, 4 H), 1.39 (d, J = 6.1 Hz, 6 H); ^{13}C NMR ($CDCl_3$) δ 162.27, 152.0, 144.49, 138.50, 135.42, 129.11, 127.26, 124.15, 121.64, 121.19, 120.32, 116.90, 111.52, 105.11, 70.52, 48.13, 21.86; HRMS calcd for $C_{21}H_{24}N_4O_2$ 364.1899, found 364.1903. Anal. ($C_{21}H_{24}N_4O_2$) C, H, N.

1-[(5-Methanesulfonamidoindol-2-yl)carbonyl]-4-(3-isopropoxy-2-pyridyl)piperazine (8): yield 83%; mp 210−211 °C; ^{1}H NMR ($CDCl_3$) δ 9.65 (s, 1 H), 7.88 (dd, J = 1.5, 4.9 Hz, 1 H), 7.60 (s, 1 H), 7.42, (d, J = 8.7 Hz, 1 H), 7.69 (dd, J = 2.1, 8.7 Hz, 1 H), 7.09 (d, J = 6.6 Hz, 1 H), 6.86, (dd, J = 4.9, 7.9 Hz, 1 H), 6.83 (s, 1 H), 6.78 (s, 1 H), 4.52−4.64 (m, 1 H), 4.07 (br s, 4 H), 3.55−3.58 (m, 4 H), 2.97 (s, 3 H), 1.40 (d, J = 6.1 Hz, 6 H); ^{13}C NMR ($CDCl_3$) δ 162.02, 144.72, 138.84, 134.13, 130.74, 129.36, 127.87, 121.33, 120.99, 117.26, 116.42, 112.84, 105.30, 70.71, 48.29, 38.79, 22.09; HRMS calcd for $C_{22}H_{27}N_5O_4S$: 457.1784, found 457.1809. Anal. ($C_{22}H_{27}N_5O_4S$) C, H, N.

1-(Indol-2-ylcarbonyl)-4-(3-ethoxy-2-pyridyl)piperazine (9): yield 78%; mp 160−162 °C; ^{1}H NMR ($CDCl_3$) δ 9.50 (s, 1 H), 7.88 (dd, J = 1.4, 4.9 Hz, 1 H), 7.66 (d, J = 8.0 Hz, 1 H), 7.44 (d, J = 8.2 Hz, 1 H), 7.26−7.31 (m, 1 H), 7.06−7.16 (m, 2 H), 6.84−6.89 (m, 2 H), 4.10 (t, J = 7.0 Hz, 2 H), 4.05−4.12 (m, 4 H), 3.55−3.58 (m, 4 H), 1.50 (t, J = 7.0 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 162.42, 151.06, 146.01, 138.33, 135.58, 129.19, 127.36, 124.27, 121.76, 120.43, 118.68, 117.21, 111.67, 105.23, 63.82, 48.34, 14.69; HRMS calcd for $C_{20}H_{22}N_4O_2$ 350.1743, found 350.1740. Anal. ($C_{20}H_{22}N_4O_2$) C, H, N.

1-[(5-Methanesulfonamidoindol-2-yl)carbonyl]-4-(3-ethoxy-2-pyridyl)piperazine (10): yield 79%; mp 195−197 °C; ^{1}H NMR ($CDCl_3$) δ 9.43 (s, 1 H), 7.88 (dd, J = 1.4, 4.9 Hz, 1 H), 7.60 (s, 1 H), 7.43, (d, J = 8.7 Hz, 1 H), 7.17 (dd, J = 2.1, 8.7 Hz, 1 H), 7.08 (d, J = 7.9 Hz, 1 H), 6.87 (dd, J = 4.9, 7.9 Hz, 1 H), 6.80 (s, 1 H), 6.55 (s, 1 H), 4.09 (q, J = 7.0 Hz, 2 H), 4.10 (br s, 4 H), 3.55−3.58 (m, 4 H), 2.97 (s, 3 H), 1.50 (t, J = 7.0 Hz, 3 H); ^{13}C NMR (DMSO-d_6) δ 161.78, 145.36, 138.13, 133.60, 130.77, 130.37, 126.89, 119.43, 119.11, 117.02, 114.28, 112.45, 104.01, 63.38, 47.87, 38.22, 14.43; HRMS calcd for $C_{21}H_{25}N_5O_4S$ 443.1627, found 443.1627. Anal. ($C_{21}H_{25}N_5O_4S$) C, H, N, S.

1-(Indol-2-ylcarbonyl]-4-[3-(benzyloxy)-2-pyridyl]piperazine (11): yield 46%; mp 247−249 °C; ^{1}H NMR ($CDCl_3$) δ 11.59 (s, 1 H), 7.82−7.85 (m, 1 H), 7.61 (d, J = 8.0 Hz, 1 H), 7.30−7.51 (m, 7 H), 7.16−7.20 (m, 1 H), 7.01−7.07 (m, 1 H), 6.92 (dd, J = 4.8, 7.9 Hz, 1 H), 6.83 (s, 1 H), 5.17 (s, 2 H), 3.88 (br s, 4 H), 3.40−3.50 (m, 4 H); ^{13}C NMR ($CDCl_3$) δ 162.15, 151.26, 145.41, 138.72, 136.80, 135.97, 128.61, 127.96, 127.45, 126.87, 123.26, 121.41, 120.05, 119.78, 117.25, 112.11, 104.18, 69.74, 48.16; HRMS calcd for $C_{25}H_{24}N_4O_2$ 412.1899, found 412.1892. Anal. ($C_{25}H_{24}N_4O_2\cdot H_2O$) C, H, N.

1-[(5-Methanesulfonamidoindol-2-yl)carbonyl]-4-[3-(benzyloxy)-2-pyridyl]piperazine (12): yield 79%; mp 260−262 °C; ^{1}H NMR (DMSO-d_6) δ 11.65 (s, 1 H), 9.37 (s, 1 H), 7.85 (d, J = 3.5 Hz, 1 H), 7.30−7.52 (m, 8 H), 7.12, (d, J = 8.7 Hz, 1 H), 6.94 (dd, J = 4.8, 7.8 Hz, 1 H), 6.85, (s, 1 H), 5.19 (s, 2 H), 3.88 (br s, 4 H), 3.44 (br s, 4 H), 2.88 (s, 3 H); ^{13}C NMR (DMSO-d_6) δ 162.06, 151.34, 145.48, 138.83, 136.88, 133.85, 130.99, 130.67, 128.69, 128.05, 127.53, 127.15, 120.12, 119.71, 117.35, 114.53, 112.70, 104.27, 69.82, 48.25, 38.49; HRMS calcd for $C_{26}H_{27}N_5O_4S$ 505.1784, found 505.1786. Anal. ($C_{26}H_{27}N_5O_4S\cdot0.75H_2O$) C, H, N, S.

1-[(5-Methanesulfonamidoindol-2-yl)carbonyl]-4-(3-*tert*-butoxy-2-pyridyl)piperazine (13): yield 86%; mp 238−239 °C; ^{1}H NMR (DMSO-d_6) δ 11.45 (s, 1 H), 9.18 (s, 1 H), 7.76 (dd, J = 4.7, 1.7 Hz, 1 H), 7.31 (d, J = 1.88 Hz, 1 H), 7.21 (d, J = 8.72 Hz, 1 H), 7.14 (dd, J = 7.77, 1.67 Hz, 1 H), 6.93 (dd, J = 8.75, 2.10 Hz, 1 H), 6.67−6.71 (m, 2 H), 3.71 (br s, 4 H), 3.26 (br s, 4 H), 2.70 (s, 3 H), 1.16 (s, 9 H); ^{13}C NMR (DMSO-d_6) δ 161.86. 155.94, 141.74, 141.38, 133.66, 130.94, 130.81, 130.46, 126.96, 119.49, 116.62, 114.32, 112.49, 104.09, 80.46, 47.94, 38.30, 28.37; HRMS calcd for $C_{23}H_{29}N_5O_4S$ 471.1940, found 471.1933. Anal. ($C_{23}H_{29}N_5O_4S$) C, H, N.

General Procedure for Synthesis of 18−19 via CDI Coupling of Indole-2-carboxylic Acids with Pyridylpiperazines or 4-[*N*-methyl-*N*-(3-alkoxy-2-pyridyl)amino]-piperidines. 1-(Indol-2-ylcarbonyl)-4-[*N*-methyl-*N*-(3-ethoxy-2-pyridyl)amino]piperidine (18). Indole-2-carboxylic acid (86 mg, 0.53 mmol) and CDI (0.085 g, 0.533 mmol) were dissolved in 2.7 mL of THF and stirred at room temperature for 1.5 h. A solution of **35** (0.114 g, 0.484 mmol) in 7 mL of THF was added, and the mixture was stirred for 40 h and then concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2, washed with saturated $NaHCO_3$, water, and brine, dried over

Na_2SO_4, and concentrated *in vacuo*. Purification by flash column chromatography (10−50% ethyl acetate/hexane) provided the product as a white solid (0.123 g, 67%): ^{1}H NMR ($CDCl_3$) δ 9.47 (bs, 1H), 7.85 (dd, J = 1.5, 4.8 Hz, 1H), 7.66 (d, J = 8.0 Hz, 1H), 7.01 (dd, J = 1.4, 7.8 Hz, 1H), 6.81 (d, J = 1.3 Hz, 1H), 6.76 (dd, J = 5.0, 7.8 Hz, 1H), 4.86 (bd, J = 12.8 Hz, 2H), 4.15 (m, 1H), 4.05 (q, J = 7.0 Hz, 2H), 3.05 (bs, 2H), 2.88 (s, 3H), 1.94 (m, 4H), 1.48 (t, J = 7.0 Hz, 3H). Anal. ($C_{22}H_{26}N_4O_2$) C, H, N.

1-(Indol-2-ylcarbonyl)-4-[*N*-methyl-*N*-(3-(1-methylethoxy)-2-pyridyl)amino]piperidine (19): yield 94%; ^{1}H NMR ($CDCl_3$) δ 9.68 (bs, 1H), 7.85 (dd, J = 1.5, 4.8 Hz, 1H), 7.65 (d, J = 7.9 Hz, 1H), 7.44 (d, J = 8.2 Hz, 1H), 7.27 (m, 1H), 7.13 (td, J = 0.9, 8.0 Hz, 1H), 7.03 (dd, J = 1.4, 7.9 Hz, 1H), 6.81 (d, J = 1.3 Hz, 1H), 6.75 (dd, J = 4.8, 7.9 Hz, 1H), 4.86 (bd, J = 12.8 Hz, 2H), 4.56 (sept, J = 6.1 Hz, 1H), 4.18 (m, 1H), 2.87 (s, 3H), 1.92 (m, 4H), 1.37 (d, J = 6.1 Hz, 6H). Anal. ($C_{23}H_{28}N_4O_2$) C, H, N.

1-[(5-Methanesulfonamidoindol-2-yl)carbonyl]-4-[*N*-methyl-*N*-(3-ethoxy-2-pyridyl)amino]piperidine (20). 5-Nitroindole-2-carboxylic acid and **35** were treated as described above for **18**: yield 79%; ^{1}H NMR ($CDCl_3$) δ 10.84 (bs, 1H), 8.63 (d, J = 2.2 Hz, 1H), 8.15 (dd, J = 2.2, 9.0 Hz, 1H), 7.86 (dd, J = 1.4, 4.8 Hz, 1H), 7.49 (d, J = 9.1 Hz, 1H), 7.04 (dd, J = 1.4, 7.9 Hz, 1H), 6.97 (d, J = 1.5 Hz, 1H), 6.78 (dd, J = 4.9, 7.8 Hz, 1H), 4.86 (bs, 2H), 4.20 (m, 1H), 4.06 (q, J = 7.0 Hz, 2H), 3.4−2.8 (bm, 2H), 2.90 (s, 3H), 1.96 (m, 4H), 1.49 (t, J = 6.9 Hz, 3H); HRMS (EI) calcd for $C_{22}H_{25}N_5O_4$ 423.1906, found 423.1906.

1-[(5-Nitroindol-2-yl)carbonyl]-4-[*N*-methyl-*N*-(3-ethoxy-2-pyridyl)amino]piperidine (from above, 350 mg, 0.83 mmol) was dissolved in 20 mL of DMF, and 20 mL of methanol and 10% Pd/C (88 mg) were added. The mixture was degassed, put under a hydrogen atmosphere for 2.5 h, filtered through Celite, and concentrated *in vacuo*. The residue obtained was chromatographed (5% methanol/CH_2Cl_2) to provide 103 mg of the amine (28%). The amine product was dissolved in CH_2Cl_2 (5 mL), and pyridine (41 μL, 0.51 mmol) was added. Then the reaction mixture was treated with methanesulfonyl chloride (21 μL, 0.267 mmol) and stirred for 16 h. The reaction mixture was diluted with CH_2Cl_2, washed with water, and brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification on three silica gel plates, eluting with 5% $CH_3OH/CHCl_3$ (R_f = 0.27−0.40) afforded 96 mg (80%) of the product as a light brown, amorphous solid: ^{1}H NMR ($CDCl_3$) δ 9.81 (bs, 1H), 7.85 (m, 1H), 7.61 (s, 1H), 7.42 (d, J = 8.7 Hz, 1H), 7.16 (dd, J = 2.0, 8.7 Hz, 1H), 7.02 (d, J = 7.8 Hz, 1H), 6.98 (s, 1H), 6.75 (m, 2H), 4.83 (bd, J = 12.6 Hz, 2H), 4.16 (m, 1H), 4.05 (q, J = 7.0 Hz, 2H), 3.20−2.85 (bs, 2H), 2.96 (s, 3H), 1.95 (m, 4H), 1.48 (t, J = 6.9 Hz, 3H); HRMS (EI) calcd for $C_{23}H_{29}N_5O_4S$ 471.1940, found 471.1931.

1-[(5-Methanesulfonamidoindol-2-yl)carbonyl]-4-[*N*-methyl-*N*-[3-(1-methylethoxy)-2-pyridyl]amino]piperidine (21). Prepared as described for **20** using 5-nitroindole-2-carboxylic acid (600 mg, 2.91 mmol) and **36**: yield 74%; ^{1}H NMR ($CDCl_3$) δ 9.98 (bs, 1H), 7.84 (dd, J = 1.5, 4.8 Hz, 1H), 7.60 (d, J = 1.9 Hz, 1H), 7.41 (d, J = 8.7 Hz, 1H), 7.20 (s, 1H), 7.16 (dd, J = 2.1, 8.7 Hz, 1H), 7.03 (dd, J = 1.4, 8.0 Hz, 1H), 6.75 (m, 2H), 4.83 (bd, J = 12.9 Hz, 2H), 4.56 (sept, J = 6.1 Hz, 1H), 4.18 (m, 1H), 3.10 (bm, 2H), 2.96 (s, 3H), 2.86 (s, 3H), 1.90 (m, 4H), 1.37 (d, J = 6.0 Hz, 6H); HRMS (EI) calcd for $C_{24}H_{31}N_5O_4S$ 485.2097, found 485.2092.

1-[(5-Methanesulfonamidoindol-2-yl)carbonyl]-4-[*N*-methyl-*N*-[3-[(1-deuterio-1-methylethyl)amino]-2-pyridyl]amino]piperidine (22). 38 (0.5 g, 1.43 mmol) was dissolved in 15 mL of 4 N HCl in dioxane for 15 min at room temperature. The reaction mixture was concentrated *in vacuo*, dissolved in $CHCl_3$, and concentrated again. The residue was dried on a vacuum pump for 1 h, dissolved in 3 mL of THF, and 5-methanesulfonylindole-2-carboxylic acid (0.43 g, 1.7 mmol), triethylamine (0.237 mL, 1.7 mmol), and EDC (0.33 g, 1.7 mmol) were added. The reaction mixture was stirred for 18 h at room temperature, concentrated *in vacuo*, and partitioned between $CHCl_3$ and 1 N NaOH. The organic layer was separated, diluted with methanol, dried over Na_2SO_4, and concentrated *in vacuo*. Flash column chromatography (75% ethyl acetate/hexane) afforded the product which was recrystallized from $CH_3OH/CHCl_3$/ether to provide 0.6 g of the title compound (88%): ^{1}H NMR (DMSO-d_6): δ 7.58 (dd, J = 1.6, 4.7 Hz, 1H), 7.44 (m, 1H), 7.35 (d, J = 8.7 Hz, 1H), 7.04 (dd, J = 2.0, 8.7 Hz, 1H), 6.89 (m, 2H), 6.74 (br, 1H), 4.97 (br s, 1H), 4.38 (m, 2H), 3.32 (m, 1H), 2.85 (s, 3H), 2.53 (s, 3H), 1.78 (m, 2H), 1.56 (m, 2H), 1.14 (s, 6H); MS (EI) *m/e* (rel int): 486 (42), 485 (59), 166 (52), 165 (81), 164 (63), 130 (100); HRMS calcd for $C_{24}H_{31}DN_6O_3S$ 485.2319, found 485.2314. Anal. ($C_{24}H_{31}DN_6O_3S\cdot0.6H_2O$) C, H, N.

1-[(5-Methanesulfonamidoindol-2-yl)carbonyl]-4-[*N*-methyl-*N*-[3-[[1-(1,1,1-trideuteriomethyl)-2,2,2-trideuterioethyl]amino]-2-pyridyl]amino]piperidine (23). Prepared as described for **22** from **39**: yield 81%; ^{1}H NMR ($CDCl_3$) δ 9.89 (s, 1H), 7.53 (dd, J = 1.6, 4.7 Hz, 1H), 7.38 (d, J = 2.0 Hz, 1H), 7.20 (m, 2H), 6.95 (dd, J = 2.0, 8.7 Hz, 1H), 6.75 (dd, J = 4.7, 8.0 Hz, 1H), 6.65 (dd, J = 1.6, 8.0 Hz, 1H), 6.51 (d, J = 1.5 Hz, 1H), 4.44 (br, 2H), 3.46 (br s, 1H), 3.32 (m, 1H), 3.40−2.82 (br, 2H), 2.76 (s, 3H), 2.44 (s, 3H), 1.75 (m, 2H), 1.46 (m, 2H). MS (EI) *m/e* (rel int) 491 (8), 490 (28), 237 (43), 222 (36), 130 (100); HRMS calcd for $C_{24}H_{26}D_6N_6O_3S$ 490.2633, found 490.2641.

HIV-RT Assay Protocol. The RT assays described in Table 3 were carried out at 28 °C for 10 min in a 100 μL reaction mixture consisting of 2 mM dithiothreitol, 60 mM NaCl, 10 mM $MgCl_2$, 50 mM Tris-HCl (pH 8.3), 0.05% Nonidet P-40, 50 μM thymidine triphosphate with 12 μCi/mL [*methyl*-^{3}H]thymidine 5′-triphosphate (Amersham), 200 nM template-primer (poly rA_{600}:oligo dT_{10} from Pharmacia), and 4 μg/mL of purified recombinant HIV RT. The RT enzymes (WT, P236L, and Y181C) were used as the homodimers and were obtained as previously described.[30,31] The reaction mixture was stopped with 100 μL of 10% (v/v) trichloroacetic acid. The acid precipitated materials, recovered on glass fiber filters, were analyzed for radioactivity. Non-nucleoside drugs were added from DMSO stocks. In all cases the final DMSO concentration was 1% (v/v).

Assay for Metabolic Stability. Analogs were screened for metabolic stability in the presence of hepatic microsomal cytochrome P450 by measuring loss of the parent drug in the following manner. Hepatic microsomes (0.4 mg), obtained from untreated rats, were diluted into 1 mL (final volume) of 50 mM potassium phosphate buffer, pH 7.4, 0.1 mM EDTA. Drug was added from 1 mM stock in MeOH to a final concentration of 5 μM. Following a preincubation at 37 °C, reaction mixture was started by addition of an NADPH generating system consisting of (final concentration) 0.5 mM NADPH, 5 mM isocitrate, 5 mM $MgCl_2$, and 0.4 unit of isocitrate dehydrogenase. At 2 min intervals between 0 and 12 min from start of reaction, 100 μL aliquots of incubation were removed and quenched with 100 μL acetonitrile followed by addition of an appropriate internal standard contained in 10 μL of acetonitrile. Samples were vortex mixed and centrifuged to pellet protein. The supernatant was diluted 1:1 with HPLC buffer, and a 100 μL sample was assayed by reverse phase HPLC using a Zorbax Rx-C8 column (15 × 0.49 cm). The mobile phase consisted of a 12 min gradient of 20−60% acetonitrile balanced by 100 mM ammonium acetate, pH 4.0, flowing at 1 mL/min. Detection was by UV absorbance at 295 nm. Drug concentration was estimated by peak height ratio to the internal standard. Concentration data were fitted to a monoexponential equation, yielding the rate constant (k) for loss of drug, which was converted to half-life by $t_{1/2} = \ln 2/k$. For purposes of comparison, half-lives of comparator compounds were normalized to delavirdine mesylate or atevirdine mesylate which were run as controls in each assay.

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