2-Amino-6-arylsulfonylbenzonitriles as Non-nucleoside Reverse Transcriptase Inhibitors of HIV-1

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A series of 2-amino-5-arylthiobenzonitriles (**1**) was found to be active against HIV-1. Structural modifications led to the sulfoxides (**2**) and sulfones (**3**). The sulfoxides generally showed antiviral activity against HIV-1 similar to that of **1**. The sulfones, however, were the most potent series of analogues, a number having activity against HIV-1 in the nanomolar range. Structural−activity relationship (SAR) studies suggested that a meta substituent, particularly a meta methyl substituent, invariably increased antiviral activities. However, optimal antiviral activities were manifested by compounds where both meta groups in the arylsulfonyl moiety were substituted and one of the substituents was a methyl group. Such a disubstitution led to compounds **3v**, **3w**, **3x**, and **3y** having IC_{50} values against HIV-1 in the low nanomolar range. When gauged for their broad-spectrum antiviral activity against key non-nucleoside reverse transcriptase inhibitor (NNRTI) related mutants, all the di-meta-substituted sulfones **3u**−**z** and the 2-naphthyl analogue **3ee** generally showed single-digit nanomolar activity against the V106A and P236L strains and submicromolar to low nanomolar activity against strains E138K, V108I, and Y188C. However, they showed a lack of activity against the K103N and Y181C mutant viruses. The elucidation of the X-ray crystal structure of the complex of **3v** (739W94) in HIV-1 reverse transcriptase showed an overlap in the binding domain when compared with the complex of nevirapine in HIV-1 reverse transcriptase. The X-ray structure allowed for the rationalization of SAR data and potencies of the compounds against the mutants.

Introduction

The first successful combination therapy for the treatment of HIV-1 infections with the non-nucleoside reverse transcriptase inhibitor (NNRTI) nevirapine[1] as a component in the treatment regimen has revived interests in the search for novel and potent NNRTIs. Unlike the nucleosides that act at the catalytic site of HIV reverse transcriptase (RT) by terminating DNA synthesis,[2] NNRTIs bind in a region of the enzyme, which is approximately 10 Å away from the catalytic site. Their binding appears to result in a distortion of the catalytic site because of changes in the position of the key aspartic acid residues, which affects the ability of the enzyme to carry out its catalytic functions.[3] NNRTIs do not require anabolism for activation. Since they are not analogues of natural compounds and do not utilize the biochemical machinery of the host cells, NNRTIs usually manifest different toxicity profiles. Side effects are usually milder than those resulting from treatments with nucleosides.[4] Generally, the most severe and treatment-limiting NNRTI-related adverse event seen in the clinic is rash.[5]

Unfortunately, because of the rapid emergence of resistant strains when used in monotherapy,[6] NNRTIs were initially considered to be of little therapeutic value. Their potential for the treatment of HIV infections was more broadly realized when nevirapine in combination with ddI and AZT was found to lead to a sustained viral load reduction.[1] Thus, the approval by the FDA of nevirapine for combination therapy was rapidly followed by that of delavirdine[7] and efavirenz.[8]

Combination therapy avoids or delays emergence of resistant viral strains, particularly when such pressure comes from potent drugs with different mechanisms of inhibition. Such multiple-drug treatment approach has indeed contributed to the declining morbidity and mortality among HIV-infected patients.[9,10] Yet, because of a high pill burden, coupled with the high cost of treatment and toxicity profile, much still remains to be done to advance HIV chemotherapy. These limitations could potentially be overcome by a treatment regimen containing a potent NNRTI with a favorable side effect profile.

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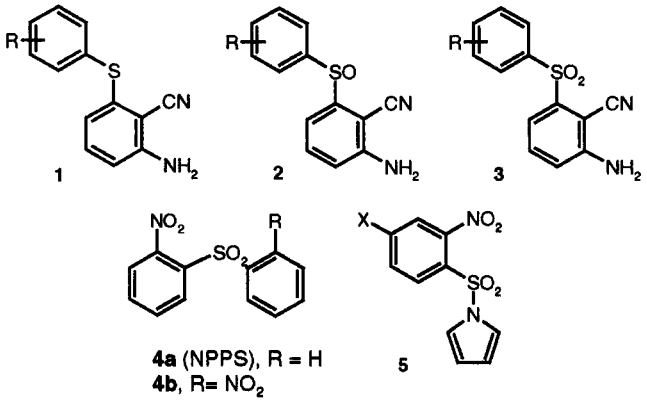
NNRTIs are generally noncompetitive potent inhibitors of HIV-1 RT with drug substance of very low cost and an acceptable toxicity profile. Indeed, an array of structurally diverse compounds including MKC442 (emivirine),[11,12] AG1549,[13] PNU-142721,[14] DPC961,[15] and DPC963[15] are currently in various stages of clinical development.

Our own efforts in this area began with the random screening of compounds from the Glaxo Wellcome database and have resulted in the initial identification of 6-arylthio-2-aminobenzonitriles (**1**) with micromolar activity against HIV-1. Further modification of **1** yielded nanomolar inhibitors in the 6-arylsulfinyl-2-aminobenzonitriles (**2**) and 6-arylsulfonyl-2-aminobenzonitriles (**3**) classes of NNRTIs. While most analogues of **2** showed comparable antiviral activity to those of **1**, analogues in the 6-arylsulfonyl series **3** were generally more potent than that in series **1** or **2**.

Precedence exists in the literature on the antiviral activity against HIV-1 of sulfones.[16−21] For example, the structure−activity relationship (SAR) of sulfones **4**[16,17] and **5**[18] had been previously pursued systematically. Among these sulfones, NPPS (**4a**) was possibly the first to have received extensive evaluation as a potential antiviral agent against HIV-1. One of the findings from the SAR studies of **4** was that two ortho nitro groups as in sulfone **4b**, one on each phenyl ring, resulted in an analogue with the most optimal antiviral activity. However, the mononitrated **4a** was shown to have better in vivo bioavailability and was thus chosen as the compound for further evaluation.[16,17]

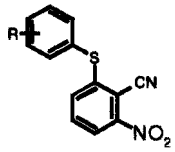


In this paper, we disclose our synthetic efforts and structure−activity relationship (SAR) studies that led to the identification of a group of analogues with the 6-arylsulfonyl-2-aminobenzonitrile (**3**) scaffold having potent antiviral activity against HIV-1 and good therapeutic indices. Additionally, we have determined the crystal structure of compound **3v** (739W94) in complex with HIV-1 RT, allowing some rationalization of both structure−activity data as well as the effects of resistance mutations on the binding potencies of the inhibitor.

Chemistry

2-Amino-6-arylthiobenzonitriles **1a**−**d**, **1f**, **1n**, **1r**−**s**, **1u**−**v** (Table 2) were obtained from the stannous chloride reduction of the corresponding 2-arylthio-6-nitrobenzonitriles **8a**−**j** (Table 1),[22,23] as depicted in Scheme 1. The other analogues in Table 2, **1e**, **1g**−**m**, **1o**−**q**, **1t**, and **1w**−**y**, were obtained from the reaction of the corresponding 2-arylthio-6-fluorobenzonitriles **10** in Table 3 with concentrated ammonium hydroxide or ethanolic ammonia at 130−140 °C in a glass-lined Parr bomb (Scheme 2).[23,24] The details of this chemistry had been previously described in connection with the synthesis of a series of 2,4-diaminoquinazolines, and compounds **1b**−**d**, **1g**−**j**, **1m**, and **1r** were reported as chemical intermediates.[23]

Table 1. Physical Constants for 6-Arylthio-2-nitrobenzonitriles (**8a**−**j**)[a]



compd	R	% yield	mp (°C)	empirical formula	elemental analysis
8a	H	89	106−107	$C_{13}H_8N_2O_2S$	C,H,N,S
8b	2-OCH_3	70[b]	145−147	$C_{14}H_{10}N_2O_3S$	C,H,N,S
8c	3-OCH_3	51[c]	144−147	$C_{14}H_{10}N_2O_3S$	C,H,N,S
8d	4-OCH_3	93[c]	139−140	$C_{14}H_{10}N_2O_3S$	C,H,N,S
8e	3-CH_3	80[c]	129−130	$C_{14}H_{10}N_2O_2S$	C,H,N,S
8f	3-F	86[d]	128−129	$C_{13}H_7N_2O_2FS$	C,H,N,S
8g	3-CF_3	76[d]	102−104	$C_{14}H_7N_2O_2F_3S$	C,H,N,S
8h	3-NH_2	88[d]	178−179	$C_{13}H_9N_3O_2S$	C,H,N,S
8i	3,5-Cl_2	84[d]	157−158	$C_{13}H_6N_2O_2Cl_2S$	C,H,N,Cl,S
8j	3,5-$(CH_3)_2$	80[d]	155−156	$C_{15}H_{12}N_2O_2S$	C,H,N,S

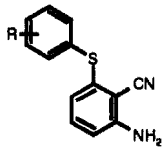
[a] Compounds were prepared according to method A described in the Experimental Section. See also ref 23 for compounds **8a**−**d**. [b] Solvent used for chromatography: EtOAc/hexane (2:3). [c] Solvent used for chromatography: CH_2Cl_2. [d] Solvent used for chromatography: EtOAc/hexane (1:1).

Intermediates **8a**−**j** were obtained from the reaction at 0 °C of 2,6-dinitrobenzonitrile **6** with an appropriately substituted arylthiol (**7**) and *t*-BuOK in DMSO or K_2CO_3 in DMF as the base (Scheme 1). Intermediates **10a**−**o**, **10s**−**v**, and **10z**−**bb** were obtained by displacing a fluoro group in 2,6-difluorobenzonitrile (**9**) by an appropriately substituted arylthiol **7** under conditions similar to those described above for the synthesis of the nitro analogues **8**. Compounds **10p**−**r** were obtained from the displacement of the bromo group in **10k**−**m**, respectively, by copper cyanide in DMF.[25] Details are described in the Experimental Section.

The synthesis of compounds **10w**−**y** is depicted in Scheme 3 and started with the bromination of anilines **13a**−**c** to their respective bromo analogues **14a**−**c**. Deamination through diazonium salts formation resulted in intermediates **15a**−**c**.[26] Subsequently, a one-pot reaction of these intermediates utilizing a bromolithium exchange with *sec*-butyllithium and then reacting the resultant aryllithium with sulfur resulted in their respective thiolates that were then reacted in situ with **9** to give **10w**−**y**. Table 8 summarizes the physical constants of the intermediates **14a**−**c** and **15a**−**c**.

Oxidation of a select group of 6-arylthio-2-fluorobenzonitriles (**10b**−**g**, **10k**−**m**, **10p**−**t**, **10v**, **10x**, and **10z**) from Table 3 with 1 equiv of *m*-chloroperoxybenzoic acid or OXONE[27] led to 2-arylsulfinyl-6-fluorobenzonitriles (**11a**−**q**) (Scheme 2, Table 4). Oxidation of **10a**−**bb** using 2 equiv of the same oxidants gave 2-arylsulfonyl-6-fluorobenzonitrile **12a**−**z** and **12ee**−**ff** (Scheme 2, Table 6). Compound **12aa** was obtained from the demethylation of **12y** using boron tribromide in methylene

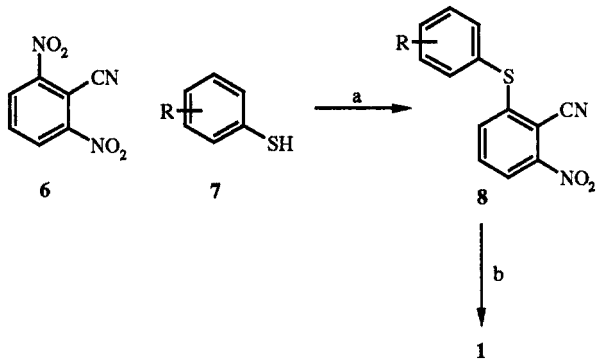
Table 2. Physical Constants for 2-Amino-6-arylthiobenzonitriles (**1a**−**y**)[a]



compd	R	% yield	mp (°C)	empirical formula	elemental analysis
1a	H	94[b]	73−74	$C_{13}H_{10}N_2S$	C,H,N,S
1b	2-OCH_3	70[c]	145−147	$C_{14}H_{12}N_2OS$	C,H,N,S
1c	3-OCH_3	51[b]	98−100	$C_{14}H_{12}N_2OS$	C,H,N,S
1d	4-OCH_3	55[d]	95−98	$C_{14}H_{12}N_2OS{\cdot}0.2H_2O$	C,H,N,S
1e	2-CH_3	42[e]	83−85	$C_{14}H_{12}N_2S$	C,H,N,S
1f	3-CH_3	57[f]	114−115	$C_{14}H_{12}N_2S$	C,H,N,S
1g	4-CH_3	21[b]	114−115	$C_{14}H_{12}N_2S$	C,H,N,S
1h	2-Cl	83[b]	113−115	$C_{13}H_9N_2ClS$	C,H,N,Cl,S
1i	3-Cl	58[b]	88−90	$C_{13}H_9N_2ClS$	C,H,N,Cl,S
1j	4-Cl	77[b]	118−120	$C_{13}H_9N_2ClS$	C,H,N,Cl,S
1k	2-Br	62[f]	93−95	$C_{13}H_9N_2BrS$	C,H,N,Br,S
1l	3-Br	81[f]	84−86	$C_{13}H_9N_2BrS$	C,H,N,Br,S
1m	4-Br	88[b]	119−120	$C_{13}H_9N_2BrS$	C,H,N,Br,S
1n	3-F	65[f]	63−65	$C_{13}H_9N_2FS$	C,H,N,S
1o	2-CN	50[b]	112−114	$C_{14}H_9N_3S$	C,H,N,S
1p	3-CN	24[b]	110−111	$C_{14}H_9N_3S$	C,H,N,S
1q	4-CN	44[b]	169−171	$C_{14}H_9N_3S{\cdot}0.2CH_3OH$	C,H,N,S
1r	3-CF_3	67[f]	66−68	$C_{14}H_9N_2F_3S$	C,H,N,S
1s	3-NH_2	49[f]	99−100	$C_{13}H_{11}N_3S{\cdot}0.1H_2O$	C,H,N,S
1t	2,5-Cl_2	42[f]	100−103	$C_{13}H_8N_2Cl_2S$	C,H,N,Cl,S
1u	3,5-Cl_2	76[f]	132−134	$C_{13}H_8N_2Cl_2S$	C,H,N,Cl,S
1v	3,5-$(CH_3)_2$	64[f]	124−125	$C_{15}H_{14}N_2S$	C,H,N,S
1w	3-Cl, 5-CH_3	64[g]	125−127	$C_{14}H_{11}N_2ClS$	C,H,N,Cl,S
1x	3-OCH_3, 5-CH_3	30[b]	108−110	$C_{15}H_{14}N_2OS$	C,H,N,S
1y	3-OCH_3, 5-CF_3	79[g]	85−86	$C_{15}H_{11}N_2OF_3S$	C,H,N,S

[a] Compounds **1a**−**d**, **1f**, **1n**, **1r**−**s**, and **1u,v** were prepared according to method B and compounds **1e**, **1g**−**m**, **1o**−**q**, **1t**, and **1w**−**y** according to method G described in the Experimental Section. [b] Solvent used for chromatography: CH_2Cl_2. [c] Solvent used for chromatography: EtOAc/hexane (2:3). [d] Recrystallized from MeOH/H_2O. [e] Recrystallized from EtOAc/hexane. [f] Solvent used for chromatography: EtOAc/hexane (1:1). [g] Solvent used for chromatography: CH_2Cl_2/hexane/EtOAc (4:5.5:0.5).

Scheme 1[a]



[a] Conditions: (a) K_2CO_3, DMF; (b) $SnCl_2{\cdot}2H_2O$, HCl, diglyme.

chloride, and **12bb**−**dd** were obtained from the reaction of the sodium salt of **12aa** with alkyl bromide having the appropriate chain lengths (Scheme 4). Amination of **11a**−**q** and **12a**−**ff** using either concentrated ammonium hydroxide or ethanolic ammonia in a Parr bomb at 130−140 °C resulted in 2-amino-6-arylsulfinylbenzonitriles **2a**−**q** (Table 5) and 2-amino-6-arylsulfonylbenzonitriles **3a**−**z** and **3bb**−**ff** (Table 7), respectively. Compound **3aa** was obtained from the demethylation of **3y**, a procedure similar to that used for the synthesis of **12aa**.

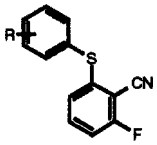
The synthesis of the diarylsulfones **4a** (NPPS) and **4b** made use of a procedure slightly modified from that reported.[16] NPPS was synthesized by reacting 1-bromo-2-nitrobenzene (**16**) with the anion of thiophenol (**7a**), resulting in 2-nitrophenyl phenylsulfide (**17**), as depicted in Scheme 5. Oxidation of the arylsulfide with potassium permanganate gave NPPS. The synthesis of the dinitro analogue **4b** was accomplished using the same procedure, starting with 2-nitrophenylthiolate. Selective oxidation of the sulfide **18** (Scheme 5), however, was accomplished using OXONE[27] to give sulfoxide (**19**), which was further oxidized with hydrogen peroxide to give **4b**.

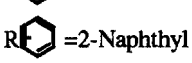
As depicted in Scheme 6, the *n*-butylamino and cyclohexylamino analogues **20a** and **20b** were obtained from the displacement of **12v** by *n*-butylamine and cyclohexylamine, respectively, using potassium carbonate in DMF as the base. The 3-amino analogue **21** was purchased from Sigma.

Biological Results, X-ray Crystallography, and Discussion

Compounds listed in Tables 2, 5, and 7 as well as compounds **12u**−**v**, **12y**, **20a**−**b**, and **21** were evaluated in an assay to measure their antiviral activity in HIV-1-infected MT4 cells. The assay assesses the reduction of the cytopathic effect of HIV-1 on MT4 cells in the presence of compounds, and the results are reported as IC_{50} values, which are the concentrations of compounds that would produce a 50% decrease in the cytopathic effect. As a gauge of the therapeutic index of the compounds assayed, compound-induced cytotoxicity of MT4 cells was also measured in parallel with the antiviral activity, and the results are reported as the cell culture cytotoxicity IC_{50} ($CCIC_{50}$) values. Addition-

Table 3. Physical Constants for 2-Arylthio-6-fluorobenzonitriles (**10a**−**bb**)[a]



compd	R	% yield	mp (°C)	empirical formula	elemental analysis
10a	H	35[b]	104−106	$C_{13}H_8NFS$	C,H,N,S
10b	2-OCH_3	40[c]	*d*	$C_{14}H_{10}NOFS$	*d*
10c	3-OCH_3	13[c]	132−134	$C_{14}H_{10}NOFS$	C,H,N,S
10d	4-OCH_3	71[e]	83−85	$C_{14}H_{10}NOFS$	C,H,N,S
10e	2-CH_3	47[f]	58−60	$C_{14}H_{10}NFS$	C,H,N,S
10f	3-CH_3	72[c]	96−98	$C_{14}H_{10}NFS$	C,H,N,S
10g	4-CH_3	70[f]	93−95	$C_{14}H_{10}NFS$	C,H,N,S
10h	2-Cl	91[g]	85−87	$C_{13}H_7NClFS$	C,H,N,Cl,S
10i	3-Cl	51[g]	80−81	$C_{13}H_7NClFS$	C,H,N,Cl,S
10j	4-Cl	85[g]	98−100	$C_{13}H_7NClFS$	C,H,N,Cl,S
10k	2-Br	57[g]	88−90	$C_{13}H_7NBrFS$	C,H,N,Br,S
10l	3-Br	51[h]	104−107	$C_{13}H_7NBrFS$	C,H,N,Br,S
10m	4-Br	84[i]	80−82	$C_{13}H_7NBrFS$	C,H,N,Br,S
10n	2-F	44[e]	83−85	$C_{13}H_7NF_2S{\cdot}0.2H_2O$	C,H,N,S
10o	3-F	47[f]	95−97	$C_{13}H_7NF_2S$	C,H,N,S
10p	2-CN	85[g]	118−120	$C_{14}H_7N_2FS$	C,H,N,S
10q	3-CN	58[j]	110−112	$C_{14}H_7N_2FS{\cdot}0.2H_2O$	C,H,N,S
10r	4-CN	25[g]	92−94	$C_{14}H_7N_2FS{\cdot}0.1H_2O$	C,H,N,S
10s	3-CF_3	72[g]	*d*	$C_{14}H_9N_2F_3S$	*d*
10t	2,5-Cl_2	76[b]	*d*	$C_{13}H_6NCl_2FS$	C,H,N,Cl,S
10u	3,5-Cl_2	34[b]	74−77	$C_{13}H_6NCl_2FS$	C,H,N,Cl,S
10v	3,5-$(CH_3)_2$	68[k]	98−99	$C_{15}H_{12}NSF$	C,H,N,S
10w	3-Br, 5-CH_3	35[l]	87−90	$C_{14}H_9NBrFS$	C,H,N,Br,S
10x	3-Cl, 5-CH_3	26[k]	*d*	$C_{14}H_9NClFS$	C,H,N,Cl,S
10y	3-OCH_3, 5-CH_3	46[k]	94−95	$C_{15}H_{12}NOFS$	C,H,N,S
10z	3-OCH_3, 5-CF_3	77[m]	78−80	$C_{15}H_9NOF_4S$	C,H,N,S
10aa	 =1-Naphthyl	79[e]	124−126	$C_{17}H_{10}NSF$	C,H,N,S
10bb	=2-Naphthyl	44[e]		$C_{17}H_{10}NSF$	C,H,N,S

[a] Compounds **10a**−**o**, **10s**−**v**, and **10z**−**bb** were prepared according to method C, **10p**−**r** according to method D, and **10w**−**y** according to method J described in the Experimental Section. [b] Solvents used for chromatography: CH_2Cl_2/hexane (1:1). [c] Recrystallized from acetone/water. [d] Data not obtained. [e] Solvents used for chromatography: CH_2Cl_2/hexane (3:7). [f] Recrystallized from EtOAc/hexane. [g] Solvents used for chromatography: CH_2Cl_2/hexane (7:3). [h] Solvents used for chromatography: CH_2Cl_2/hexane (1:4). [i] Solvents used for chromatography: CH_2Cl_2/hexane (2:3). [j] Solvents used for chromatography: CH_2Cl_2/hexane (1:1). [k] Solvents used for chromatography: EtOAc/hexane (1:4). [l] Solvents used for chromatography: EtOAc/hexane (1:9). [m] Solvents used for chromatography: EtOAc/hexane (1.5:8.5).

ally, these compounds were evaluated for their inhibitory activity against HIV-1 RT. The results from the cell-based and RT assays are summarized in Tables 9−12. As mentioned earlier, certain diarylsulfones have previously been reported to have potent activity against HIV-1. We have synthesized two such analogues, NPPS (**4a**) and its dinitro derivative **4b**. These two analogues were reported to be the most potent analogues in the diarylsulfone series.[18] Compounds **4a** and **4b**, the precursors bisarylsulfide **18**, and the bisarylsulfoxide **19** have been included in Tables 9−11 for comparison purposes.

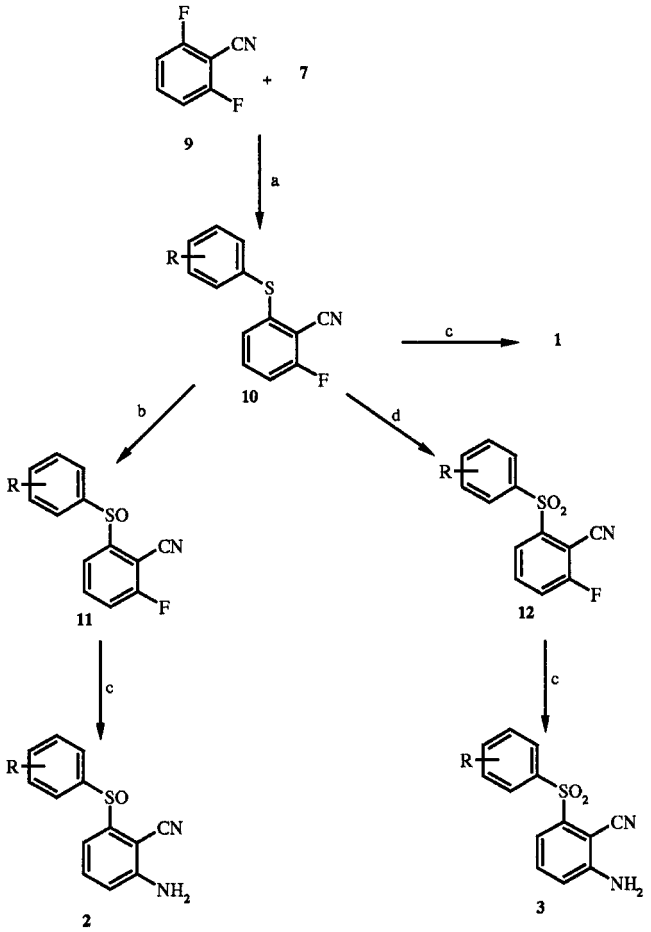
A select group of the more potent 6-arylsulfonyl-2-aminobenzonitriles **3** were additionally evaluated against a panel of mutant viruses genetically engineered to contain RT mutations considered to be relevant to NNRTIs. The results are included in Table 13. The details of the procedures for all assays mentioned above are described in the Experimental Section.

The initial SAR studies centered on substituent variations on the arylthio moiety in **1**. Subsequently, the SAR studies were expanded to include sulfoxides **2**, sulfones **3**, and modifications of the benzonitrile ring system.

As shown in Table 9 for the sulfide series, the moderate activity against HIV-1 of the parent compound **1a** was in general enhanced by the presence of a substituent in the C-3 position (compounds **1c**, **1f**, **1i**, **1l**, **1m,** and **1o**) of the arylthio ring. The 3-trifluoromethyl-substituted **1q**, however, was almost equipotent to **1a**, and the 3-amino analogue **1r** was 2-fold less active against the virus than **1a**. The reduced activity against HIV-1 for **1r** might suggest the detrimental effect of having a polar substituent in the C-3 position of the arylthio group.

The antiviral activity of the C-2 substituted analogues appeared to vary considerably with the substituents. The 2-methoxy analogue **1b** and the 2-chloro analogue **1h** were the most active in the series, and the 2-methyl analogue **1e** retained activity comparable to that of **1a**. On the other hand, the 2-bromo analogue **1k** was much less active against HIV-1 than that of **1a**. Such a disparity of activity could not simply be due to the electronic properties of the substituents alone. The fact that the 2-bromo analogue **1k** was approximately 7-fold less active than the 2-chloro **1h** might suggest that the size of the 2-substituent is important for maximizing antiviral activity.

Scheme 2[a]



[a] Conditions: (a) *t*-BuOK, DMSO or K_2CO_3, DMF; (b) 1.2 equiv of mCPBA, CH_2Cl_2, or 1.1 equiv of OXONE[27] H_2O; (c) concentrated NH_4OH or NH_3, MeOH; (d) 2.2 equiv of mCPBA, CH_2Cl_2, or 2.2 equiv of OXONE, MeOH.

The least optimal position to place a substituent was C-4 (compounds **1d**, **1g**, **1j**, and **1p**). The much reduced antiviral activity of these C-4 substituted analogues compared to that of analogues having C-2 and C-3 substituents might suggest unfavorable interactions in the region of HIV-1 RT into which the substituents projected.

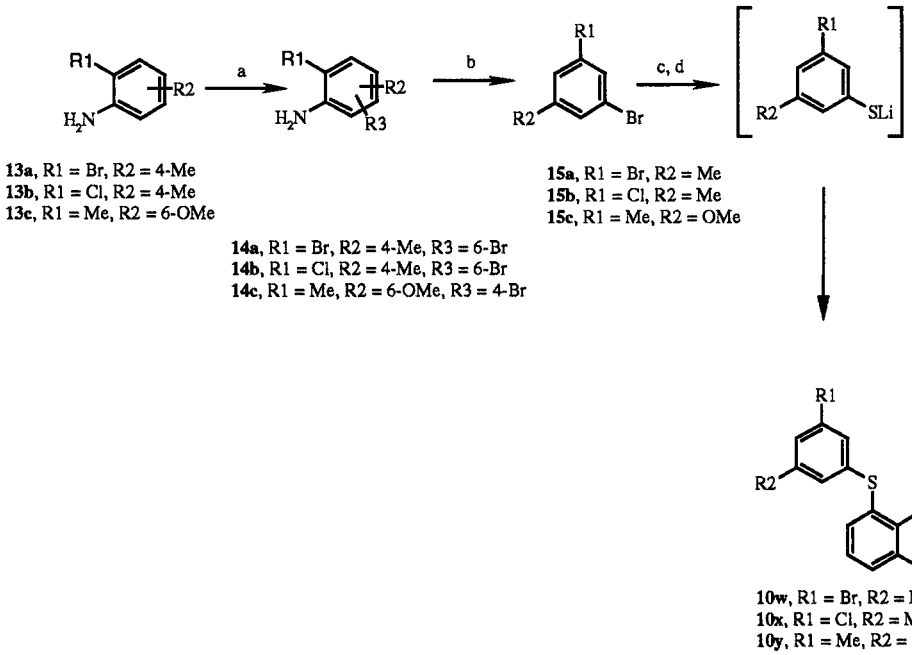
Disubstution on the arylthio group, each having a meta methyl substituent, resulted in a marked increase in antiviral activity. Thus, compounds **1t**, **1v**, and **1w** were about 14-, 4-, and 3-fold more active against HIV-1 than the corresponding monosubstituted **1f**, **1i**, and **1c**. On the other hand, if the additional meta substituent were electron-withdrawing, such as the chloro and trifluoromethyl groups in **1u** and **1x**, respectively, the resultant compounds at best retained equipotency to the corresponding monosubstituted analogues.

A number of the sulfides showed relatively low cytotoxic IC_{50} values, resulting in marginal therapeutic indices. These values were in direct contrast to those found for the sulfoxide and sulfone series, which generally showed therapeutically acceptable indices (vide infra). That the antiviral activity was due to the inhibition of RT was well established by the correlation of activity against HIV-1 RT of the sulfide analogues. Compared to the bisnitrophenylsulfide **18**, the 6-arylthio-2-aminobenzonitriles were generally more potent against HIV-1.

Utilizing the sulfides in Table 9 as the starting point in an ensuing SAR study, we next synthesized a select group of the sulfoxide analogues and an expanded group of the sulfone analogues. The antiviral activities of these compounds are summarized in Tables 10 and 11, respectively.

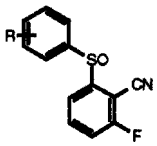
Interestingly, in general, for a comparably substituted compound, the antiviral activity of the sulfide and sulfoxide analogues was quite similar. However, the SAR for antiviral activity of the sulfoxides was not as clear-cut as in the sulfide series. In the methoxy-substituted analogues, the trend of activity was C-2 analogue **2a** > C-3 analogue **2b** > C-4 analogue **2c**. This

Scheme 3[a]



[a] Conditions: (a) Br_2, AcOH, MeOH; (b) $NaNO_2$, concentrated HCl, AcOH, H_2O, H_3PO_2; (c) *sec*-BuLi, S_8; (d) 2,6-difluorobenzonitrile, THF, DMSO.

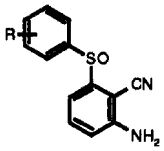
Table 4. Physical Constants for 2-Arylsulfinyl-6-fluorobenzonitriles (**11a**−**q**)[a]



compd	R	% yield	mp (°C)	empirical formula	elemental analysis
11a	2-OCH_3	40[b]	138−140	$C_{14}H_{10}NO_2FS$	C,H,N
11b	3-OCH_3	13[b]	110−111	$C_{14}H_{10}NO_2FS$	C,H,N,S
11c	4-OCH_3	50[c]	73−75	$C_{14}H_{10}NO_2FS$	C,H,N,S
11d	2-CH_3	58[d]	87−88	$C_{14}H_{10}NOFS$	C,H,N,S
11e	3-CH_3	72[e]	96−98	$C_{14}H_{10}NOFS$	*h*
11f	4-CH_3	67[e]	200−202	$C_{14}H_{10}NOFS$	C,H,N,S
11g	2-Br	83[e]	105−107	$C_{13}H_7NOBrFS$	C,H,N,Br,S
11h	3-Br	51[e]	104−107	$C_{13}H_7NOBrFS$	C,H,N,Br,S
11i	4-Br	67[d]	121−124	$C_{13}H_7NOBrFS$	C,H,N,Br,
11j	2-CN	50[f]	152−160	$C_{14}H_7N_2OFS{\cdot}0.3H_2O$	C,H,N,S
11k	3-CN	89[d]	153−155	$C_{14}H_7N_2OFS{\cdot}0.2H_2O$	C,H,N,S
11l	4-CN	40[d]	160−162	$C_{14}H_7N_2OFS$	C,H,N,S
11m	3-CF_3	60[d]	85−87	$C_{14}H_7NOF_4S$	C,H,N,S
11n	2,5-Cl_2	83[g]	*h*	$C_{13}H_6NO_2ClFS$	*h*
11o	3,5-$(CH_3)_2$	68[i]	98−99	$C_{15}H_{12}NOFS$	C,H,N,S
11p	3-Cl, 5-CH_3	62[j]	131−133	$C_{14}H_9NOClFS$	C,H,N
11q	3-OCH_3, 5-CF_3	77[k]	127−128	$C_{15}H_9NO_2FS$	C,H,N

[a] All compounds were prepared according to method E described in the Experimental Section. [b] Recrystallized from acetone/water. [c] Solvent used for chromatography: CH_2Cl_2/MeOH (99:1). [d] Solvent used for chromatography: CH_2Cl_2. [e] Solvent used for chromatography: CH_2Cl_2/hexane/EtOAc (4:5:1). [f] Isolated analytically pure from the reaction medium. [g] Yield is based on crude material isolated. [h]Data not obtained. [i] Solvents used for chromatography: EtOAc/hexane (1.:4). [j] Solvents used for chromatography: EtOAc/hexane (1:9). [k] Solvents used for chromatography: CH_2Cl_2/hexane (1:9).

Table 5. Physical Constants for 2-Amino-6-arylsulfinylbenzonitriles (**2a**−**q**)[a]



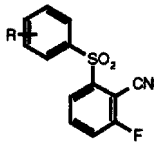
compd	R	% yield	mp (°C)	empirical formula	elemental analysis
2a	2-OCH_3	40[b]	159−162	$C_{14}H_{12}N_2O_2S$	C,H,N
2b	3-OCH_3	13[b]	110−114	$C_{14}H_{12}N_2O_2S$	C,H,N,S
2c	4-OCH_3	67[c]	155−157	$C_{14}H_{12}N_2O_2S{\cdot}0.1CH_3OH$	C,H,N,S
2d	2-CH_3	32[d]	153−155	$C_{14}H_{12}N_2OS{\cdot}0.2EtOAc$	C,H,N,S
2e	3-CH_3	72[b]	96−98	$C_{14}H_{12}N_2OS$	C,H,N,S
2f	4-CH_3	52[c]	200−202	$C_{14}H_{12}N_2OS{\cdot}0.1CH_3OH$	C,H,N,S
2g	2-Br	55[d]	140−142	$C_{13}H_9N_2OBrS$	C,H,N,Br,S
2h	3-Br	51[e]	104−107	$C_{13}H_9N_2OBrS$	C,H,N,Br,S
2i	4-Br	42[d]	155−157	$C_{13}H_9N_2OBrS$	C,H,N,Br,S
2j	2-CN	66[d]	200−202	$C_{14}H_9N_3OS{\cdot}0.1H_2O$	C,H,N,S
2k	3-CN	78[d]	148−149	$C_{14}H_9N_3OS{\cdot}0.8H_2O$	C,H,N,S
2l	4-CN	44[c]	169−171	$C_{14}H_9N_3OS$	C,H,N,S
2m	3-CF_3	18[c]	148−150	$C_{14}H_9N_2OF_3S{\cdot}0.3H_2O$	C,H,N,S
2n	3,5-$(CH_3)_2$	68[f]	98−99	$C_{15}H_{14}N_2OS$	C,H,N,S
2o	2,5-Cl_2	59[c]	180−182	$C_{13}H_8N_2OCl_2S$	C,H,N,S,Cl
2p	3-Cl, 5-CH_3	26[g]	192−193	$C_{14}H_{11}N_2OClS$	C,H,N,Cl,S
2q	3-OCH_3, 5-CF_3	77[g]	78−80	$C_{15}H_{11}N_2O_2F_3S$	C,H,N,S

[a] All compounds were prepared according to method G described in the Experimental Section. [b] Recrystallized from acetone/water. [c] Solvents used for chromatography: CH_2Cl_2/MeOH (99:1). [d] Recrystallized from EtOH/H_2O. [e] Solvents used for chromatography: CH_2Cl_2/hexane (1:4). [f] Solvents used for chromatography: EtOAc/hexane (1:4). [g] Solvents used for chromatography: EtOAc/hexane (1:9).

trend was similar for the cyano analogues (**2j**−**l**). However, this trend was not observed for the methyl (**2d**−**f**) or bromo (**2g**−**i**) analogues. As was observed previously in the sulfide series, the addition of another meta substituent also resulted in an increase in antiviral activity (compounds **2n**, **2o**, **2p**, and **2q**). Again, the increase in antiviral activity was more pronounced when a methyl group was the additional meta substituent (**2n** and **2p**). The sulfoxides in Table 10 were synthesized as the racemates, and no attempt was made to resolve them. Nonetheless, judging from the range of antiviral activity, resolution of the racemates might not result in a significant increase in antiviral activity of the resultant optical isomers. The sulfoxides, in contrast to the sulfides, showed relatively high cell cytotoxic IC_{50} values, resulting in acceptable therapeutic indices. Additionally, the correlation between antiviral activity and RT activity was much more pronounced than that in the sulfide series (Table 10). The bisnitrophenylsulfoxide **19** did not show any appreciable activity against HIV-1 at concentrations up to 200 μM.

Previous work on the bisarylsulfones demonstrated that for a similar substitution, the bisarylsulfones were generally more potent than the corresponding bisaryl-

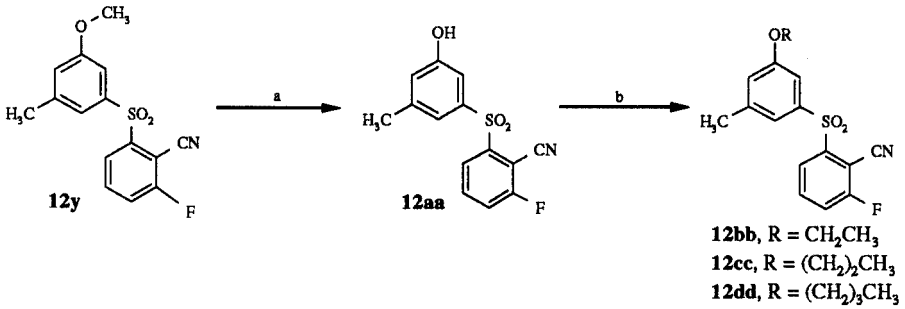
Table 6. Physical Constants for 6-Arylsulfonyl-2-fluorobenzonitriles (**12a**−**ff**)[a]



compd	R	% yield	mp (°C)	empirical formula	elemental analysis
12a	H	88[b]	97−98	$C_{13}H_8NO_2FS$	C,H,N,S
12b	2-OCH_3	4[c]	174−177	$C_{14}H_{10}NO_3FS$	C,H,N,S
12c	3-OCH_3	23[c]	113−115	$C_{14}H_{10}NO_3FS$	C,H,N,S
12d	4-OCH_3	86[b]	210−212	$C_{14}H_{10}NO_3FS$	C,H,N,S
12e	2-CH_3	50[d]	118−123	$C_{14}H_{10}NO_2FS$	*e*
12f	3-CH_3	28[d]	119−121	$C_{14}H_{10}NO_2FS$	C,H,N,S
12g	4-CH_3	83[d]	200−202	$C_{14}H_{10}NO_2FS$	C,H,N,S
12h	2-Cl	89[f]	135−137	$C_{13}H_7NO_2ClFS$	C,H,N,Cl,S
12i	3-Cl	44[f]	123−125	$C_{13}H_7NO_2ClFS$	C,H,N,Cl,S
12j	4-Cl	76[f]	133−135	$C_{13}H_7NO_2ClFS$	C,H,N,Cl,S
12k	2-Br	37[f]	113−115	$C_{13}H_7NO_2BrFS$	C,H,N,Br,S
12l	3-Br	82[d]	162−166	$C_{13}H_7NO_2BrFS$	C,H,N,Br,S
12m	4-Br	73[f]	130−132	$C_{13}H_7NO_2BrFS$	C,H,N,Br,S
12n	2-F	78[b]	134−135	$C_{13}H_7NO_2F_2S$	C,H,N,S
12o	3-F	64[b]	122−124	$C_{13}H_7NO_2F_2S$	C,H,N,S
12p	2-CN	59[c]	208−210	$C_{14}H_7N_2O_2FS{\cdot}0.2H_2O$	C,H,N,S
12q	3-CN	63[c]	*e*	$C_{14}H_7N_2O_2FS{\cdot}0.4H_2O$	C,H,N,S
12r	4-CN	76[c]	192−195	$C_{14}H_7N_2O_2FS{\cdot}0.4H_2O$	C,H,N,S
12s	3-CF_3	94[g]	184−185	$C_{14}H_7NO_2F_4S$	C,H,N,S
12t	2,5-Cl_2	16[h]	183−184	$C_{13}H_6NO_2Cl_2FS{\cdot}0.05CH_2Cl_2$	C,H,N,Cl,S
12u	3,5-Cl_2	75[g]	84−86	$C_{13}H_6NO_2Cl_2FS$	C,H,N,Cl,S
12v	3,5-$(CH_3)_2$	65[i]	167−168	$C_{15}H_{12}NO_2FS$	C,H,N,S
12w	3-Br, 5-CH_3	78[f]	*e*	$C_{14}H_9NO_2BrFS$	C,H,N,Br,S
12x	3-Cl, 5-CH_3	85[f]	164−166	$C_{14}H_9NO_2SClF$	C,H,N,Cl,S
12y	3-OCH_3, 5-CH_3	83[b]	160−162	$C_{15}H_{12}NO_3FS$	C,H,N,S
12z	3-OCH_3, 5-CF_3	78[d]	111−113	$C_{15}H_9NO_3F_4S$	C,H,N,S
12aa	3-OH, 5-CH_3	50[i]	178−180	$C_{14}H_{10}NO_3FS$	*e*
12bb	3-OCH_2CH_3, 5-CH_3	92[j]	*e*	$C_{16}H_{14}NO_3FS$	*e*
12cc	3-$O(CH_2)_2CH_3$, 5-CH_3	49[j]	*e*	$C_{17}H_{16}NO_3FS$	*e*
12dd	3-$O(CH_2)_3CH_3$, 5-CH_3	55[j]	*e*	$C_{18}H_{18}NO_3FS$	*e*
12ee	R = 1-Naphthyl	95[c]	155−158	$C_{17}H_{10}NO_2FS$	*e*
12ff	R = 2-Naphthyl	90[c]	110−112	$C_{17}H_{10}NO_2FS$	*e*

[a] Compounds **12a**−**z** and **12e**−**ff** were prepared according to method F, **12aa** according to method K, and **12bb-dd** according to method L described in the Experimental Section. [b] Isolated analytically pure from the reaction medium. [c] Solvent used for chromatography: CH_2Cl_2. [d] Solvent used for chromatography: CH_2Cl_2/hexane/EtOAc (4:5:1). [e] Data not taken. [f] Solvent used for chromatography: CH_2Cl_2/hexane (7:3). [g] Solvent used for chromatography: CH_2Cl_2/hexane (1:1). [h] Solvent used for chromatography: CH_2Cl_2/hexane (3:7). [i] Solvent used for chromatography: EtOAc/hexane (1:1). [j] Purified by silica preparative plate with CH_2Cl_2 as the eluent.

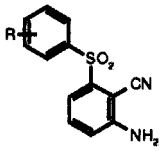
Scheme 4[a]



[a] Conditions: (a) BBr_3, CH_2Cl_2; (b) NaH, RBr, DMF.

sulfide analogues.[16] For example NPPS (**4a**) was found to be more active than nitrophenyl phenylsulfide **18** (antiviral IC_{50} values against HIV-1 of 2.2 and 93.5 μM, respectively, in our assays). In addition, the NNRTI pocket in the HIV-1 RT is very hydrophobic.[3,28,29] Utilizing these two pieces of information in a continuing effort to expand the SAR studies, we selected the dimethyl analogue **1t** and synthesized its sulfone counterpart. The resultant compound **3v** (739W94) was approximately 43-fold more active against HIV-1 than **1t**. Encouraged by such a potent level of antiviral activity, we undertook an extensive SAR work around the sulfone scaffold, and the antiviral activity of the resultant compounds is listed in Table 11. In general, the sulfones **3** showed much enhanced antiviral activity versus both the sulfides **1** and the sulfoxides **2**. The pattern of activity generally mirrored that of the sulfides. Potent antiviral activity required at least a

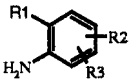
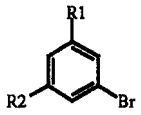
Table 7. Physical Constants for 2-Amino-6-arylsulfonylbenzonitriles (**3a**−**ee**)[a]



compd	R	% yield	mp (°C)	empirical formula	elemental analysis
3a	H	67[b]	185−187	$C_{13}H_{10}N_2O_2S·0.2H_2O$	C,H,N,S
3b	2-OCH_3	35[c]	191−193	$C_{14}H_{12}N_2O_3S$	C,H,N,S
3c	3-OCH_3	23[c]	186−191	$C_{14}H_{12}N_2O_3S$	C,H,N,S
3d	4-OCH_3	61[c]	220−222	$C_{14}H_{12}N_2O_3S$	C,H,N,S
3e	2-CH_3	85[b]	213−215	$C_{14}H_{12}N_2O_2S$	C,H,N,S
3f	3-CH_3	80[b]	191−193	$C_{14}H_{12}N_2O_2S$	C,H,N,S
3g	4-CH_3	75[b]	200−202	$C_{14}H_{12}N_2O_2S·0.2H_2O$	C,H,N,S
3h	2-Cl	80[b]	135−137	$C_{13}H_9N_2O_2ClS$	C,H,N,Cl,S
3i	3-Cl	91[b]	185−187	$C_{13}H_9N_2O_2ClS$	C,H,N,Cl,S
3j	4-Cl	68[b]	212−215	$C_{13}H_9N_2O_2ClS$	C,H,N,Cl,S
3k	2-Br	62[b]	235−238	$C_{13}H_9N_2O_2BrS$	C,H,N,Br,S
3l	3-Br	19[b]	209−210	$C_{13}H_9N_2O_2BrS$	C,H,N,Br,S
3m	4-Br	100[d]	226−228	$C_{13}H_9N_2O_2BrS$	C,H,N,Br,S
3n	2-F	85[b]	224−226	$C_{13}H_9N_2O_2FS$	C,H,N,S
3o	3-F	92[b]	162.5−164	$C_{13}H_9N_2O_2FS$	C,H,N,S
3p	2-CN	73[e]	267−268	$C_{14}H_9N_3O_2S·0.1H_2O$	C,H,N,S
3q	3-CN	78[e]	211−213	$C_{14}H_9N_3O_2S·0.2H_2O$	C,H,N,S
3r	4-CN	77[e]	224−226	$C_{14}H_9N_3O_2S$	C,H,N,S
3s	3-CF_3	80[c]	184−185	$C_{14}H_9N_2O_2F_3S$	C,H,N,S
3t	2,5-Cl_2	58[b]	>200	$C_{13}H_8N_2O_2Cl_2S$	C,H,N,Cl,S
3u	3,5-Cl_2	86[b]	227−228	$C_{13}H_8N_2O_2Cl_2S$	C,H,N,Cl,S
3v	3,5-$(CH_3)_2$	56[e]	208−209	$C_{15}H_{14}N_2O_2S$	C,H,N,S
3w	3-Br, 5-CH_3	59[c]	201−203	$C_{14}H_{11}N_2O_2BrS$	C,H,N,Br,S
3x	3-Cl, 5-CH_3	76[c]	203−205	$C_{14}H_{11}N_2O_2ClS$	C,H,N,Cl,S
3y	3-OCH_3, 5-CH_3	56[h]	165−166	$C_{15}H_{14}N_2O_3S$	C,H,N,S
3z	3-OCH_3, 5-CF_3	92[c]	144−146	$C_{15}H_{11}N_2O_3F_3S$	C,H,N,S
3aa	3-OH, 5-CH_3	50[f]	178−180	$C_{14}H_{12}N_2O_3S$	C,H,N,S
3bb	3-OCH_2CH_3, 5-CH_3	60[g]	112−114	$C_{16}H_{16}N_2O_3S·0.2H_2O$	C,H,N,S
3cc	3-$O(CH_2)_2CH_3$, 5-CH_3	68[g]	120−122	$C_{17}H_{18}N_2O_3S$	C,H,N,S
3dd	3-$O(CH_2)_3CH_3$, 5-CH_3	56[g]	140−142	$C_{18}H_{20}N_2O_3S·0.4H_2O$	C,H,N,S
3ee	 =1-Naphthyl	72[c]	228−232	$C_{17}H_{12}N_2O_2S$	C,H,N,S
3ff	 =2-Naphthyl	75[c]	205−206	$C_{17}H_{12}N_2O_2S$	C,H,N,S

[a] All compounds were prepared according to method G except for compound **3aa**, which was synthesized according to method K. Details are described in the Experimental Section. [b] Solvent used for chromatography: CH_2Cl_2. [c] Solvent used for chromatography: CH_2Cl_2/MeOH (49:1). [d] Isolated analytically pure from the reaction medium. [e] Recrystallized from EtOH/H_2O. [f] Solvents used for chromatography: hexane/EtOAc (1:1). [g] Purified on a silica gel preparative plate with CH_2Cl_2 as the eluent.

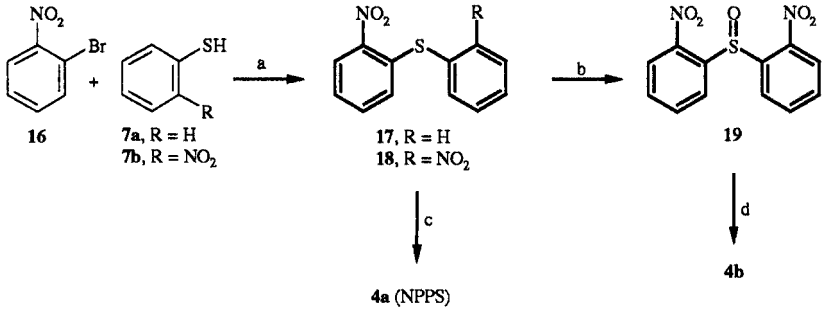
Table 8. Physical Constants for Anilines (**14a**−**c**) and Arylbromide (**15a**−**c**)[a]

compd	R1	R2	R3	% yield	mp (°C)	empirical formula	elemental analysis
							
14a	Br	4-CH_3	6-Br	73[b]	71−73	C_7H_7NBr	C,H,N,Br
14b	Cl	4-CH_3	6-Br	74[b]	*c*	C_7H_7NBrCl	C,H,N,Br,Cl
14c	OCH_3	6-CH_3	4- Br	80[d]	55−56	$C_8H_{10}NOBr$	C,H,N,Br
							
15a	Br	CH_3		68[e]	37−40	$C_7H_6Br_2$	C,H,Br
15b	Cl	CH_3		70[e]	oil	C_7H_6 BrCl	*c*
15c	CH_3	OCH_3		80[f]	oil	C_8H_9OBr	C,H,Br

[a] Compounds **14a**−**c** and **15a**−**c** were prepared according to methods H and I, respectively. Details are described in the Experimental Section. [b] Recrystallized from hexane. [c] Data not obtained. [d] Solvents used for chromatography: CH_2Cl_2/hexane (19:1). [e] Isolated analytically pure from the reaction medium. [f] Solvent used for chromatography: hexane.

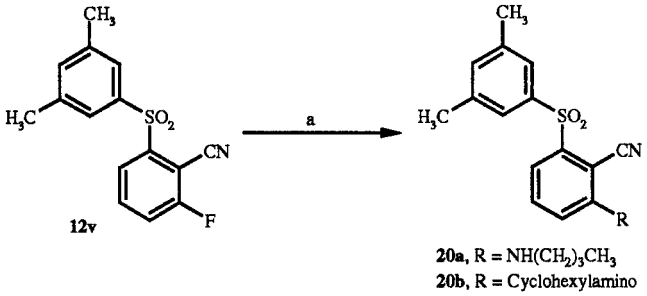
lipophilic C-3 substituent in the phenylsulfonyl moiety (**3c**, **3f**, **3i**, and **3l**). Analogues with C-2 substituents (**3e**, **3h**, **3k**, and **3p**), although still showing reasonable antiviral activity, were in general not as active against HIV-1 as the C-3 substituted compounds. Two exceptions to this observation were the 2-methoxy analogue **3b** and the 2-fluoro analogues **3n**, both of which were of equal potency to their 3-substituted counterparts. The similarity in antiviral activity of the 2- and 3-methoxy analogues **3b** and **3c** could possibly be explained by the

Scheme 5[a]



[a] Conditions: (a) NaH, DMF; (b) OXONE,[27] MeOH, H_2O; (c) $KMnO_4$, H_2O, AcOH; (d) H_2O_2, trifluoroacetic acid.

Scheme 6[a]



[a] Conditions: (a) RNH_2, DMF.

Table 9. Activity against HIV-1 and RT of 2-Amino-6-arylthiobenzonitriles (**1a**−**x**)[a]

compd	R	anti-HIV-1 IC_{50} (μM)	$CCIC_{50}$ (μM)	HIV-1 RT IC_{50} (μM)
1a	H	14.6	168	8.7
1b	2-OCH_3	4.3	>200	2.7
1c	3-OCH_3	6.0	>200	1.5
1d	4-OCH_3	*b*	*c*	>17
1e	2-CH_3	16	>40	*c*
1f	3-CH_3	6.1	79	0.96
1g	4-CH_3	115	>200	5.7
1h	2-Cl	4.1	33	7.2
1i	3-Cl	7.4	53	16
1j	4-Cl	>32	113	12
1k	2-Br	30.0	41	>21
1l	3-Br	5.1	53	15
1m	3-F	9.8	162	12
1n	2-CN	>6.4	42	9.1
1o	3-CN	1.73	80	1.1
1p	4-CN	43.8	>200	>19
1q	3-CF_3	12.8	34	7.1
1r	3-NH_2	31.5	168	>23
1s	2,5-Cl_2	>0.8	4.3	3.5
1t	3,5-$(CH_3)_2$	0.43	>2	1.1
1u	3,5-Cl_2	62% @ 5 μM	3	0.12
1v	3-Cl, 5-CH_3	1.76	16	1.7
1w	3-OCH_3, 5-CH_3	2	10	0.14
1x	3-OCH_3, 5-CF_3	5.1	9	13
18		93.5	>200	>20

[a] The protocols for obtaining the IC_{50} values are described in the Experimental Section. [b] Denotes no significant activity at concentrations up to 200 μM. [c] Not tested.

Table 10. Activity against HIV-1 and RT of 2-Amino-6-arylsulfinylbenzonitriles (**2a**−**q**)[a]

compd	R	anti-HIV-1 IC_{50} (μM)	$CCIC_{50}$ (μM)	HIV-1 RT IC_{50} (μM)
2a	2-OCH_3	4.8	>200	12
2b	3-OCH_3	16	>80	19
2c	4-OCH_3	>200	>200	>20
2d	2-CH_3	93	>200	>21
2e	3-CH_3	29.23	>200	10
2f	4-CH_3	49	>200	>21
2g	2-Br	39.2	168	>19
2h	3-Br	0.08	*b*	4.8
2i	4-Br	20.21	>200	>21
2j	2-CN	3.9	>200	9.9
2k	3-CN	14.2	>200	>15
2l	4-CN	>200	>200	>23
2m	3-CF_3	40	125	>22
2n	3,5-$(CH_3)_2$	0.34	200	0.5
2o	2,5-Cl_2	9.85	>200	6.2
2p	3-Cl, 5-CH_3	0.32	>200	0.52
2q	3-OCH_3, 5-CF_3	2.07	108	0.90
19		*c*	*b*	*b*

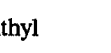
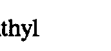
[a] The protocols for obtaining the IC_{50} values are described in the Experimental Section. [b] Not tested. [c] No significant activity at concentrations up to 200 μM.

flexibility of the methoxy groups, allowing for both analogues to occupy the same space in the pocket of the enzyme. The same level of antiviral activity shared by the 2-fluoro and 3-fluoro analogues, **3n** and **3o**, respectively, may be due to the small-sized fluoro group not appreciably affecting the hydrophobic region of the enzyme. This was further evidenced by the fact that both **3n** and **3o** and the unsubstituted **3a** showed equipotency against HIV-1. Differences in the electronic properties of the substituents did not seem to have a significant impact in the resultant antiviral activity. Thus, the 3-methoxy analogue **3c** had antiviral activity similar to that of the electron-withdrawing 3-chloro analogue **3i** and the 3-bromo analogue **3l**. However, strong electron-withdrawing groups such as the cyano group in **3q** and the trifluoromethyl group in **3s** led to reduced antiviral activities.

As was the case with the sulfide series, C-4 substituted analogues were also generally the least active in the sulfone series (**3d**, **3g**, **3j**, **3m**, and **3r**). Similar to the sulfide and sulfoxide series, the additional meta methyl substituent enhanced antiviral activity in the sulfone series. Compounds **3v** (739W94) and **3w**−**y** were among the most potent in the current series, and they were at least 20-fold more potent than that of the corresponding monosubstituted **3c**, **3f**, **3i**, and **3l** against HIV-1. Although not as pronounced as the addition of a second meta methyl group, the addition of another meta chloro group to a monosubstituted analogue **3i** also resulted in a more potent analogue **3u**. The additive effect of a C-3 substituent was also seen when a C-3 chloro group was added to the 2-chloro analogue **3h**, resulting in a compound (**3t**) that was approximately 7-fold more active against HIV-1 than **3h**.

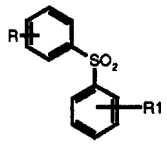
Demethylation of **3y** resulted in a compound **3aa** that, although still active, was 9-fold less active than **3y**, again suggesting that the environment within the

Table 11. Activity against HIV-1 of 2-Amino-6-arylsulfonylbenzonitriles (**3a**−**ff**)[a]

compd	R	anti-HIV-1 IC_{50} (μM)	CC IC_{50} (μM)	HIV-1 RT IC_{50} (μM)
3a	H	2	>5	6.9
3b	2-OCH_3	0.6	146	1.4
3c	3-OCH_3	0.9	>200	0.6
3d	4-OCH_3	25	>200	13
3e	2-CH_3	2.3	>200	4.5
3f	3-CH_3	0.4	179	0.2
3g	4-CH_3	9.5	>200	7.3
3h	2-Cl	4.1	>200	5.9
3i	3-Cl	0.59	>200	0.4
3j	4-Cl	3	>5	*b*
3k	2-Br	5.0	40	12
3l	3-Br	0.54	>200	0.2
3m	4-Br	20	>200	>14
3n	2-F	3.0	57	5.0
3o	3-F	3.0	>200	*b*
3p	2-CN	5.4	>200	6.0
3q	3-CN	2.4	>200	1.8
3r	4-CN	80.0	>200	>18
3s	3-CF_3	3.5	197	5.3
3t	2,5-Cl_2	0.3	>200	0.3
3u	3,5-Cl_2	0.07	>200	0.03
3v	3,5-$(CH_3)_2$	0.01	>200	0.007
3w	3-Br, 5-CH_3	0.02	133	0.003
3x	3-Cl, 5-CH_3	0.03	>200	0.005
3y	3-OCH_3, 5-CH_3	0.05	94	0.01
3z	3-OCH_3, 5-CF_3	0.09	164	0.04
3aa	3-OH, 5-CH_3	0.43	167	*b*
3bb	3-OCH_2CH_3, 5-CH_3	0.06	9	*b*
3cc	3-$O(CH_2)_2CH_3$, 5-CH_3	0.06	11	*b*
3dd	3-$O(CH_2)_3CH_3$, 5-CH_3	0.6	>2	0.4
3ee	 =1-Naphthyl	3.32	>200	1.0
3ff	 =2-Naphthyl	0.05	>200	0.03
4a		8.6	*b*	16
4b		2.2	*b*	14

[a] The protocols for obtaining the IC_{50} values are described in the Experimental Section. [b] Not tested.

Table 12. Anti-HIV-1 Activity of 2-Arylthio-6-fluoro and 6-Alkylaminobenzonitriles (**10U**−**Y**, **14b**−**d**)[a]



compd	R	R1	Anti-HIV-1 IC_{50} (μM)	CCIC_{50} (μM)
12u	3,5-Cl_2	2-CN, 3-F	42	188
12v	3,5-$(CH_3)_2$	2-CN, 3-F	1.5	>200
12y	3-OCH_3, 5-CH_3	2-CN, 3-F	51	8
20a	3,5-$(CH_3)_2$	2-CN, 3-NH-$(CH_2)_3CH_3$	>12	>200
20b	3,5-$(CH_3)_2$	2-CN, 3-cyclohexylamino	>200	>200
21	H	3-NH_2	>50	>50

[a] The protocol for obtaining the IC_{50} values is described in the Experimental Section.

enzyme that these meta substituents bind to was hydrophobic. Increasing the lipophilicity by extending the methylene group from one (**3y**) to two (**3bb**) and three (**3cc**) did not appear to increase antiviral activity. A four-methylene spacer unit (**3dd**) caused a decrease in antiviral activity. Introducing the bulky 1-naphthyl group gave a compound **3ee** that was still potent. However, a 2-naphthyl group (**3ff**) was detrimental to antiviral activity.

Similar to the sulfoxide series, the sulfone analogues generally showed high IC_{50} values in the cell cytotoxic assay, resulting in acceptable therapeutic indices. Antiviral potency in the sulfone series was well reflected in the potent activity of the compounds against HIV-1 RT. Compounds **3u**−**3z** with submicromolar IC_{50} values against the virus were also the most potent against HIV-1 RT.

Finally, an array of the compounds in Table 11 was more potent against HIV-1 than NPPS (**4a**) with 739W94 being >200-fold more potent than **4a** against HIV-1.

Our next SAR study focused on the aminocyanophenyl ring system, and the antiviral results are summarized in Table 12. Compounds **12u,v** and **12y** are the chemical precursors to the sulfones **3u,v** and **3y**. The requirement of the 3-amino group for antiviral activity was well demonstrated by compounds **12u,v** and **12y**. The only analogue with an acceptable level of antiviral activity, the 3,5-dimethyl-substituted **12v**, showed antiviral potency approximately 150-fold less than **3v**. The need for a 3-amino group was further amplified by the lack of activity against HIV-1 of compounds **20a,b**. The *n*-butylamino analogue **20a** and the cyclohexylamino analogue **20b** were designed to probe spatial requirements, and as can be seen from Table 12, both compounds showed a lack of antiviral activity. The need for a 2-cyano group was demonstrated by compound **21**, which showed a lack of antiviral activity at concentrations up to 50 μM. Thus, it was the cumulative effect of the 2-cyano and 3-amino groups that imparted antiviral activity.

Although there is a high degree of cross-resistance among NNRTIs, the emergence of drug resistance is generally treatment-specific. For example, in monotherapy with nevirapine, a rapid emergence of the Y181C mutant was observed that rendered the drug clinically ineffective. However, a therapy involving the simultaneous use of nevirapine and AZT resulted in the emergence of K103N, which was then followed by Y188C or G190A.[30] Likewise, the clinical failure of a therapy involving efavirenz was attributed to the appearance of the K103N.[31] Therefore, although combination therapy has been proven to be effective in prolonging the emergence of NNRTI-resistant viruses, selection ultimately occurs that leads to loss of clinical efficacy of the NNRTI being used. It is well accepted that a clinically useful next generation NNRTI should possess some levels of potency against a group of mutant strains that shows reduced susceptibility to currently approved NNRTIs. Some of the key amino acid substitutions around the NNRTI binding site that are selected in the clinic are the K103N, Y181C, Y188C, V106A, V108I, E138K, G190A, and P236L. In vitro and in vivo, a large number of the currently available NNRTIs are ineffective against these mutants.[30,32]

On the basis of the procedure of Kellam and Larder,[34] we have created a panel of recombinant, isogenic viruses containing the mutations mentioned above either singly or in combination within the HIV reverse transcriptase coding region. The genotypes corresponding to the substituted amino acid residues for each of these mutants are listed in Table 13, along with the antiviral

Table 13. Antiviral Activity of Representative 2-Amino-6-arylsulfonylbenzonitriles and Marketed NNRTIs against HIV-1 Wild-Type and Mutant Viruses in the HeLa MAGI Assay[a]

	IC_{50} values (μM) vs wild-type and mutant viruses								
virus/compds	**3u**	**3v**	**3w**	**3x**	**3y**	**3z**	**3ee**	EFV	NEV
wild-type	0.037	0.011	0.009	0.015	0.014	0.063	0.014	0.0009	0.089
K103N	8.3	1.1	0.97	3.6	1.8	10.5	2.3	0.024	7.1
V106A	0.0062	0.0024	0.0015	0.0028	0.0029	0.013	0.0054	0.0015	~10
Y181C	50	14	10	14	13	36	29	0.0018	>10
V106A/Y181C	1.8	0.49	0.077	0.21	0.42	2.1	0.23	0.0033	>10
E138K	0.085	0.017	0.015	0.015	0.029	0.08	0.068	0.0008	0.072
G190A	0.084	0.030	0.031	0.040	0.087	0.66	0.20	0.0064	>10
V108I/Y181C	58	13	18	13	14	36	66	0.0033	>10
V108I	0.047	0.017	0.011	0.017	0.029	0.12	0.03	0.0014	0.34
Y188C	0.14	0.21	1.7	0.4	0.3	0.4	0.18	0.0012	2.2
K103N/L100I	5.5	3	16	20	19	5	1.7	1.6	10
P236L	0.0025	0.005	0.011	0.01	0.008	0.007	0.002	0.0005	0.056
K103N/Y181C	>50	>50	>50	>50	>50	>50	>50	0.042	>50
K103N/V108I	4	4.8	15	33	16	10	3.5	0.074	21
K103N/P225H	3.8	3.3	13	19	7.8	5.5	2.8	0.13	10

[a] The protocol for obtaining the IC_{50} values is described in the Experimental Section.

data of a select group of the most potent disubstituted sulfones (**3u**−**z**, **3ee**). Included in the table are marketed drugs: the first generation nevirapine and efavirenz, which is a recent addition to the armamentarium of drugs against HIV.

As seen in Table 13, sulfones **3u**−**z** and **3ee** showed single-digit nanomolar activity against the V106A and P236L strains and submicromolar to low nanomolar activity against strains E138K, V108I, and Y188C. Additionally, **3u**−**y** manifested low nanomolar activity against the G190A. These sulfones in general were more potent than nevirapine against this panel of mutant viruses. However, they were less potent than efavirenz in this panel. Unfortunately, they showed a lack of activity against two key resistant strains, the K103N and Y181C, and double mutants based on those two strains. As described below, we were able to use the X-ray crystal structure of **3v** (739W94) in complex with HIV-1 RT to rationalize the activity (or lack of) of these sulfones against the mutants described above.

The HIV-1 RT/739W94 complex has been refined to an overall *R* factor of 0.211 for all data from 30 to 3.0 Å resolution with the retention of excellent stereochemistry (Table 14). The binding mode of 739W94 to HIV-1 RT is revealed unambiguously from the omit map shown in Figure 1a. Comparisons of the bound conformation of 739W94 and the structurally related compound GCA-186, an emivirine analogue,[35] and the first generation NNRTI, nevirapine,[29] are shown in parts b and c of Figure 1. The 3,5-dimethylphenyl of 739W94 overlapped well with one of the pyridine rings of nevirapine at the "top" of the NNRTI pocket but only very marginally with the equivalent 3,5-dimethylphenyl ring of GCA-186. There was also a greater degree of overlap of the 2-aminobenzonitrile ring of 739W94 and the second pyridine ring of nevirapine than with the pyrimidine ring of GCA-186. Thus, the 739W94 inhibitor was significantly displaced from the binding position of GCA-186 and showed a greater overlap with the more structurally dissimilar nevirapine molecule. As a result of this displacement, one of the sulfone oxygens of 739W94 was in a position to maintain the Tyr181 side chain in the "up" position, a structural feature necessary for tight binding for compounds of the HEPT/GCA-186 series.[35,36] For this latter class of compounds Tyr181 is positioned by an entirely different moiety, the 5-isopropyl substituent of the pyrimidine ring. 739W94 formed a hydrogen bond to the main-chain oxygen of Lys101 via its amino group and in this respect was more similar to GCA-186 than nevirapine, which has no main-chain hydrogen bonding. The contribution of this hydrogen bond to the binding energy of the compound was indicated by compound **12v**, which has the amino group replaced by fluorine, abolishing hydrogen bond and reducing activity 150-fold. Despite the relative displacement of 739W94 and GCA-186, the angle between the two rings of each compound was similar, a feature noted for many NNRTI compound series.[29]

The RT/739W94 model can be used to interpret some of the structure−activity and drug-resistance data for 739W94 presented here (Tables 9−13) in terms of the stereochemistry of inhibitor−protein interactions (Figure 1a). The weak binding of the sulfide (**1**) and sulfoxide (**2**) analogues in contrast to the sulfone-

Table 14. Statistics for Crystallographic Structure Determinations

Data Collection Details	
data collection site	KEK BL-6A2
wavelength (Å)	1.000
unit cell (*a*,*b*,*c* in Å)	141.1, 110.9, 73.1
resolution range (Å)	30.0−2.98
observations	74 167
unique reflections	23 124
completeness (%)	96.1
average $I/\sigma(I)$	7.49
r_{merge} (%)[a]	14.6
outer resolution shell	
resolution range (Å)	3.09−2.98
unique reflections	2219
completeness (%)	93.7
average $I/\sigma(I)$	0.86
Refinement Statistics	
resolution range (Å)	30.0−3.0
unique reflections (working/test)	22606 (21508/1098)
R [b] ($R_{working}/R_{free}$)	0.211 (0.221/0.265)
protein atoms	7706
inhibitor atoms	20
rms bond length deviation (Å)	0.0096
rms bond angle deviation (°)	1.61
mean *B* factor[c] (Å^2)	87/91/57

[a] $R_{merge} = \Sigma|I - \langle I\rangle|/\Sigma\langle I\rangle$. [b] $R = \Sigma|F_o - F_c|/\Sigma F_o$. [c] Mean *B* factor for main-chain, side chain, and inhibitor atoms, respectively.

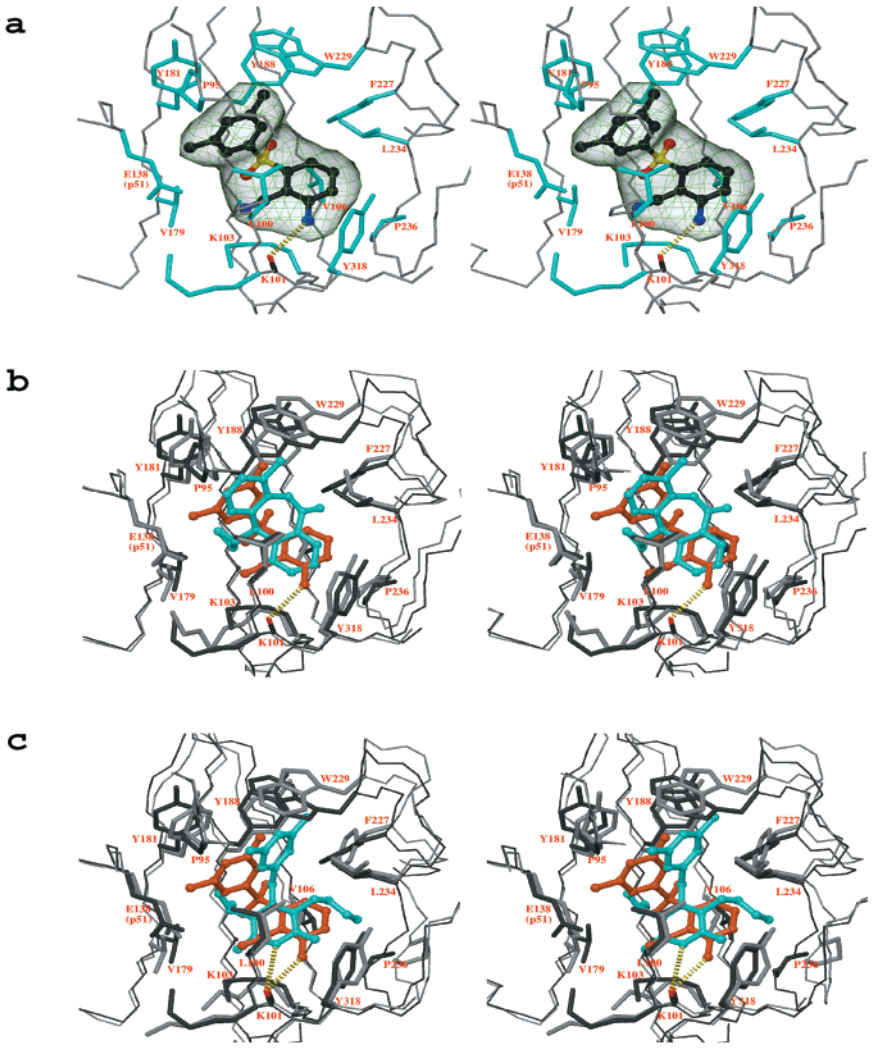


Figure 1. Position and orientation of 739W94 in the NNRTI pocket and comparison with nevirapine and GCA-186. (a) Stereoview of simulated annealing that omits $|F_o| - |F_c|$ maps contoured at 3σ, showing the electron density for 739W94 bound to HIV-1 reverse transcriptase. The 739W94 is shown with conventional atom colors, protein side chains as cyan ball-and-stick representation and protein backbone as thin gray sticks. The H-bond is shown as a yellow dashed line. (b) Stereodiagram showing the relative positions and orientations of RT-bound nevirapine and 739W94. The superimposition was carried out using the Cα atoms of amino acid residues around the NNRTI pocket to compensate for different crystal forms and domain rearrangements. Residues used included 94−118, 156−215, and 225−243 from the palm domain, 317−319 from the connection domain of p66, and 137−139 from the fingers domain of p51. (c) Stereodiagram showing the relative positions and orientations of RT-bound GCA-186 and 739W94. The inhibitor and protein are shown in brown and dark-gray, respectively, for the RT/739W94 complex, cyan and light-gray for RT/nevirapine in (b) and RT/GCA-186 in (c).

containing 739W94 can be explained by the absence of a group to correctly position the side chain of Tyr181 in the "up" position as discussed above. The detrimental effect of most 4-position substituents on the phenyl ring was due to a steric clash with adjacent residues, particularly Pro95. Such a clash could lead to a displacement of the inhibitor away from Tyr181 and thus to weakened interactions that appeared to be a significant contribution to the binding energy for this series. It can be seen from Figure 1c that the positioning of the phenyl ring of 739W94 meant that the 2-naphthyl-containing compound (**3ff**) could be accommodated within the site with only slight loss of potency because the second ring would be positioned approximately coincident with that of the GCA-186 phenyl ring.

The drug-resistant mutations that showed the greatest effects on 739W94 binding were Y181C and K103N (Table 13). Examination of the model of 739W94/RT helped to explain these effects. 739W94 showed many close contacts with Tyr181, and mutation to the smaller nonaromatic cysteine will abolish most of these interactions. We have previously reported that the emivirine analogue GCA-186, which has 3,5-dimethyl substituents on its phenyl ring, showed greater resilience to the presence of the Y181C mutation than did emivirine itself. Such substituents allowed GCA-186 to be in contact with the highly conserved Trp229 at the top of the NNRTI pocket.[35] In the case of 739W94 its phenyl ring was positioned lower in the pocket, and hence, addition of 3,5-dimethyl groups to the ring did not give rise to the same contacts with Trp229.

In contrast to its close contacts with Tyr181, 739W94 interacted with Tyr188 only via the β carbon; thus, mutation at this position showed only a 10-fold weaker binding compared to ~100-fold reduction in binding for the Tyr181 mutant. In the case of the K103N mutation,

we observed van der Waals contacts between the nitrile group of 739W94 and the aliphatic portion of the side chain of Lys103. On mutation to the shorter asparagine side chain, it seemed plausible that this favorable interaction would be disrupted. An additional effect of the K103N mutation might be derived from the potential of the asparagine side chain to hydrogen-bond to the hydroxyl of Tyr188, leading to the stabilization of unliganded reverse transcriptase.[3] For two of the mutant RTs studied (V106A and P236L) there was evidence for hypersensitivity to 739W94 and analogues (Table 13). For the wild-type RT the side chain of Val106 had a close contact between the 5′ position of the 2-aminobenzonitrile ring and one sulfone oxygen (2.9 Å). The reduction in bulk on mutation of V106A would relieve this close contact and hence lead to tighter binding for this mutant form. Pro236 had no van der Waals contact with 739W94, while mutation of P236L would give a longer and more flexible side chain that appeared to point away from the inhibitor. It is possible that the polypeptide chain might rearrange on mutation to leucine, allowing some main-chain contacts to be made with the inhibitor (this loop region has previously been shown to be highly flexible).[36,37]

The pharmacokinetic profiles of sulfones **3u**−**y** were next evaluated primarily to explore their potentials as drug candidates. In a single 2.5 mg/kg dose by intravenous injection to male Wistar rats, analogue **3u** had the most acceptable pharmacokinetic profile (with a mean half-life of 5.2 h and a mean clearance of 18 mL min^{-1} kg^{-1}). The other analogues, **3v**−**y** were rapidly cleared from the systemic circulation (mean clearance values ranged from 40 to 70 mL min^{-1} kg^{-1}; mean half-life values ranged from 0.8 to 1.9 h). In a single 7.5 mg/kg dose by oral administration to rats, analogues **3u**−**y** gave bioavailability values in the range 4−18% with **3v** having the lowest bioavailability and **3w** and **3v** the highest bioavailability.

In summary, a series of 6-arylthio-2-aminobenzonitriles **1** and 6-arylsulfinyl-2-aminobenzonitriles **2** were identified as having antiviral activity in a cell-based assay against HIV-1. Modifications of these first groups of compounds led to 6-arylsulfonyl-2-aminobenzonitriles **3**, a series of compounds that showed potent antiviral activity against HIV-1, some with IC_{50} values in the nanomolar range. SAR studies on the arylsulfonyl ring system of **3** suggested that the placement of substituent for optimal antiviral activity was in the C-3 position. Another hydrophobic meta substituent, particularly a meta methyl group, added to an existing C-3 substituted analogue invariably resulted in an enhancement of antiviral activity. A good example of this was compound **3v** (739W94), which was approximately 40-fold more potent against HIV-1 than its monosubstituted analogue **3f** was. The requirement for a C-3 amino group in the other ring system of **3** was established by the lack of antiviral activity of compounds **12u**−**y** and **20a,b**. Likewise, the need for a C-2 nitrile group for antiviral activity was demonstrated by analogue **21**, which showed a lack of activity against HIV-1. A number of the analogues within the 6-arylsulfonyl-2-aminobenzonitriles **3** were more potent than NPPS (**4a**) and **4b**, two compounds in the bisarylsulfone series that received extensive evaluation as agents against HIV-1. In an assay to gauge the antiviral activity of the more potent sulfones in a panel of mutant viruses, **3u**−**z** and **3ee** were generally active against the panel of mutants listed in Table 13. However, these sulfones were inactive against two key mutants, the K103N and Y181C, and double mutants containing these strains. Their potency against the panel of mutants in Table 13 was in general better than that of the first-generation nevirapine but was less than that of efavirenz. X-ray crystallographic elucidation of the complex of **3v** (739W94) in HIV-1 RT suggested that the 6-arylsulfonyl-2-aminobenzonitriles **3** shared the same space in the RT binding pocket as nevirapine. The lack of activity against the K103N and Y181C and the activity of V106A and P236L mutants to 739W94 could also be rationalized on the basis of the crystal structure. Finally, bioavailability in rats ranged from 4 to 18% for compounds **3u**−**y**. Compound **3u** had the most acceptable pharmacokinetic profile with a mean half-life of 5.2 h and a mean clearance of 18 mL min^{-1} kg^{-1}.

Experimental Section

Melting points were taken on a Thomas-Hoover or a Mel-Temp apparatus and are uncorrected. ^1H NMR spectra were recorded on a Varian XL200 and Varian Unity Plus 400 or 200 MHz spectrometers. Elemental analyses were carried out by Atlantic Microlabs, Inc., Atlanta, GA. 2,6-Dinitrobenzonitrile and 2,6-difluorobenzonitrile were purchased from Lancaster Synthesis, Inc., Windham, NH, and Aldrich, respectively. All purchased starting materials were used without further purifications. All solvents were reagent grades. Dimethylformamide (DMF) and dimethylsulfoxide (DMSO) were dried over 4 Å molecular sieves. Flash column chromatography was performed according to ref 38. 3-Methoxy-5-methylthiophenol for the synthesis of **10y** was prepared according to a procedure described in ref 39.

Method A: 2-Arylthio-6-nitrobenzonitrile (8). This procedure has been reported in ref 23. Briefly, molar equivalents of 2,6-dinitrobenzonitrile (**6**), arylthiol (**7**), and anhydrous K_2CO_3 in DMF was cooled to 0 °C and stirred for 0.5 h. The reaction mixture was made basic by the addition of pyridine or 1 N NaOH, and this was followed by dilution with a copious amount of water. The precipitate formed was collected by filtration, washed with 1 N NaOH and water, and dried. **8** was obtained either analytically pure or after purification by flash column chromatography on silica gel using the solvent system noted in Table 1.

Method B: 2-Amino-6-arylthiobenzonitrile (1). This procedure followed that reported in ref 23. Briefly, intermediate **8** in diglyme was stirred with 3 molar equiv of $SnCl_2 \cdot 2H_2O$ in concentrated HCl (∼2 mL per mmol of **8**) cooled in a water bath. After being stirred for 0.5 h, the reaction mixture was poured into a vigorously stirring mixture of 50% NaOH and crushed ice (1:3 v/v, ∼5 mL per mmol of **8**). Precipitate was collected by filtration, washed with 1 N NaOH and water, and dried. **1** was obtained after purification by methods noted in Table 2.

Method C: 2-Arylthio-6-fluorothiobenzonitrile (10). The procedure is similar to that reported in ref 23. Thus, to an ice-bath-cooled suspension of potassium *t*-butoxide or potassium carbonate in dry DMSO or DMF was added 1−1.1 molar equiv of arylthiol **7**, followed by a molar equivalent of 2,6-difluorobenzonitrile (**9**). The resultant mixture was stirred for 1 h and was then poured into H_2O. This was followed by stirring for about 30 min. Intermediate **10** was collected by filtration and dried. This intermediate was either used without purification or subjected to flash column chromatography on silica gel using solvents summarized in Table 3.

Method D: 2-(2-Cyanophenylthio)-6-fluorobenzonitrile (10p). A mixture of **10k** (0.2 g, 0.6 mmol) and copper cyanide (0.07 g, 0.8 mmol) was refluxed in DMF for 3 h. The

reaction mixture was concentrated under high vacuum and subjected to flash column chromatography on silica gel with 30% hexane in CH_2Cl_2 as the solvents. This gave 0.13 g (85%) of **10p** as a white solid, mp 118−120 °C. ^{1}H NMR (Me_2SO-d_6): δ 7.1 (d, 1H, aromatic), 7.5 (t, 1H, aromatic), 7.5−7.7 (m, 2H, aromatic), 7.7−8 (m, 2H, aromatic), 8.0 (d, 1H, aromatic). Anal. ($C_{14}H_7N_2FS$) C, H, N, S.

Method E: 2-Arylsulfinyl-6-fluorobenzonitrile (11). To a solution of **10** in CH_2Cl_2 was added 1.2 equiv of *m*-chloroperbenzoic acid or OXONE.[27] The resultant mixture was stirred for 24 h, after which the mixture was diluted with additional CH_2Cl_2 or EtOAc. This mixture was then washed with concentrated sodium bisulfite, 1 N NaOH, water, and brine. After drying over $MgSO_4$ and solvent removal, the resultant crude product **11** was purified by flash column chromatography on silica using solvents summarized in Table 4.

Method F: 2-Arylsulfonyl-6-fluorobenzonitrile (12). To a solution of **10** in CH_2Cl_2 was added 2−3 equiv of *m*-chloroperbenzoic acid. The resultant mixture was stirred for 24 h, after which the mixture was diluted with additional CH_2Cl_2 or EtOAc. This mixture was then washed with concentrated sodium bisulfite, 1 N NaOH, water, and brine. After drying over $MgSO_4$ and solvent removal, the resultant crude product **12** was purified by flash column chromatography on silica using solvents summarized in Table 6.

Method G: 2-Amino-6-arylthiobenzonitrile (1), 2-Amino-6-arylsulfinylbenzonitrile (2), and 2-Amino-6-arylsulfonylbenzonitrile (3). Concentrated NH_4OH or gaseous ammonia was introduced into a solution of **10**, **11**, or **12** in EtOH, EtOH/THF, or MeOH/EtOH. The mixture was sealed in a glass-lined bomb and heated to 130−140 °C for 3−24 h. After solvent removal, the resultant crude product was subjected to flash column chromatography on silica as summarized in Tables 2, 5, and 7.

Method H: 2,6-Dibromo-4-methylaniline (14a). To an ice−water-cooled solution of 2-bromo-4-methylaniline (**13a**) (25 g, 134 mmol) in 60 mL of MeOH and 20 mL of AcOH was added dropwise bromine (6.9 mL, 134 mmol) in 60 mL of AcOH. The reaction mixture was allowed to stand for 2 h, after which the solvents were removed in vacuo. The resultant concentrate was suspended in 1 N NaOH (∼100 mL). This was extracted with EtOAc, dried over $MgSO_4$, and evaporated to give a reddish crude compound. Recrystallization from hexane gave 26 g (73%) of **14a** as an off-white solid, mp 71−73 °C. ^{1}H NMR (Me_2SO-d_6): δ 2.2 (s, 6H, CH_3), 5.1 (br s, 2H, NH_2), 7.3 (s, 2H, aromatic). Anal. ($C_7H_7NBr_2$) C, H, N, Br.

Method I: 3,5-Dibromotoluene (15a). To an ice−water-cooled and stirred solution of **14a** (10 g, 38 mmol) in a mixture of 70 mL of acetic acid, 30 mL of H_2O and 8 mL of concentrated HCl was added dropwise a solution of $NaNO_2$ (3.12 g, 45 mmol) in 10 mL of H_2O. The reaction mixture was stirred for 30 min and was then added to an 80 mL solution of 50% H_3PO_2 cooled at 0 °C. After being stirred for 8 h at 0 °C, the reaction mixture was allowed to stand at room temperature for 72 h. Following extraction with EtOAc (3 × 100 mL), drying ($MgSO_4$), and concentration of the solvent, 6.5 g (68%) of **15a** was obtained as off-white crystals, mp 37−40 °C. ^{1}H NMR (Me_2SO-d_6): δ 2.4 (s, 3H, CH_3), 7.5 (s, 2H, aromatic), 7.67 (s, 1H, aromatic). Anal. ($C_7H_6Br_2$) C, H, Br.

Method J: 2-(3-Bromo-5-methylphenylthio)-6-fluorobenzonitrile (10w). A stirred solution of **15a** (2.27 g, 9 mmol) in 20 mL of freshly distilled THF was cooled to −78 °C under an atmosphere of nitrogen. To this solution was added *sec*-BuLi (14 mL of a 1.3 M cyclohexane solution) via a syringe. The reaction mixture was stirred for 10 min, after which sulfur (0.32 g, 10 mmol) was added in one portion. The reaction mixture was gradually brought to room temperature, and stirring was continued for 12 h. The reaction mixture was cooled in an ice−water bath. To this was added 2,6-difluorobenzonitrile (**9**) (1.27 g, 9 mmol) in 10 mL of dry DMSO. After being stirred for 20 min, the mixture was poured into water (100 mL) and this aqueous solution was extracted with EtOAc (3 × 50 mL). The EtOAc extracts were combined and washed with 1 N NaOH and H_2O, dried ($MgSO_4$), and concentrated. Following flash column chromatography on silica with 10% EtOAc in hexane, 1.03 g (36%) of **10w** was obtained as a white solid, mp 87−90 °C. ^{1}H NMR (Me_2SO-d_6): δ 2.4 (s, 3H, CH_3), 7.1 (d, 1H, aromatic), 7.4 (s, 1H, aromatic), 7.4−7.6 (m, 3H, aromatic), 7.7 (dd, 1H, aromatic). Anal. ($C_{14}H_9NSBrF$) C, H, N, Br, S.

Method K: 2-Amino-6-(3-hydroxy-5-methylphenylsulfonyl)benzonitrile (3aa). A stirred solution of **3y** (0.1 g, 0.33 mmol) in 5 mL of CH_2Cl_2 was cooled to −78 °C under a nitrogen atmosphere. BBr_3 (0.27 g, 1.09 mmol) was added dropwise via a syringe. The reaction mixture was gradually brought to room temperature, and an additional 0.08 g of BBr_3 was added. Stirring was continued for 1 h. The reaction mixture was poured into ice−water, stirred for 10 minutes, saturated with NaCl, and extracted with CH_2Cl_2. After drying ($MgSO_4$) and solvent removal, the crude product was purified by flash column chromatography on silica gel with hexane/EtOAc (1:1) as the solvents. This gave 0.05 g (50%) of **3aa** as a white solid, mp 178−180 °C. ^{1}H NMR (Me_2SO-d_6): δ 2.3 (s, 3H, CH_3), 6.6 (br s, 2H, NH2), 6.9 (unresolved s, 1H, aromatic), 7−7.2 (m, 3H, aromatic), 7.3 (d, 1H, aromatic), 7.5 (t, 1H, aromatic), 10.4 (s, 1H, OH). Anal. ($C_{14}H_{12}N_2O_3S\cdot 0.2H_2O$) C, H, N, S.

Method L: 2-(3-Ethoxy-5-methylphenylsulfonyl)-2-fluorobenzonitrile (12bb). To a stirred suspension of sodium hydride (50% oil dispersed, 0.03 g, 0.82 mmol) in 5 mL of DMF was added **12aa** (0.2 g, 0.7 mmol). The mixture was stirred for 30 min, after which iodoethane (0.1 mL, 1.4 mmol) was added. After being stirred for 1 h, the reaction mixture was poured into water, which was in turn extracted with EtOAc. This EtOAc solution was washed with water, dried ($MgSO_4$), and concentrated. Purification using a silica gel preparative plate with CH_2Cl_2 gave 0.2 g (92%) of **12bb** as a white solid. ^{1}H NMR (Me_2SO-d_6): δ 1.4 (t, 3H, CH_3), 2.3 (s, 3H, CH_3), 4.0 (q, 2H, CH_2), 7.2 (s, 1H, aromatic), 7.3 (s, 1H, aromatic), 7.4 (s, 1H, aromatic), 7.9 (t, 1H, aromatic), 8−8.1 (m, 1H, aromatic), 8.2 (d, 1H, aromatic).

Method M: 2-Cyclohexylamino-6-(3, 5-dimethylphenylsulfonyl)benzonitrile (20b). A mixture of cyclohexylamine (0.3 g, 3 mmol), **12v** (0.5 g, 1.7 mmol), and K_2CO_3 (0.55 g, 4 mmol) in 10 mL of DMF was stirred at room temperature for 12 h. The reaction mixture was concentrated in vacuo, and the resultant concentrate was subjected to flash column chromatography on silica gel with hexane/EtOAc (1:1) as the eluent. A total of 0.45 g (71%) of **20b** was obtained as a white solid, mp 152−153 °C. ^{1}H NMR (Me_2SO-d_6): δ 1−2 (m, 10H, cyclohexyl), 2.3 (s, 6H, $2CH_3$), 3.3−3.5 (m, 1H, cyclohexyl), 5.8 (d, 1H, NH), 7.2 (d, 1H, aromatic), 7.3−7.4 (m, 2H, aromatic), 7.5−7.7 (m, 3H, aromatic). Anal. ($C_{21}H_{24}N_2SO_2$) C, H, N, S.

Method N: 2-(3, 5-Dimethylphenylsulfonyl)-6-butylaminobenzonitrile (20a). Following the procedure described for the synthesis of **20b** above using *n*-butylamine, a 78% yield of **20a** was obtained as a white solid after purification by flash column chromatography on silica gel with 40% EtOAc in hexane as the eluent, mp 117−119 °C. ^{1}H NMR (Me_2SO-d_6): δ 0.9 (t, 3H, CH_3), 1.1−1.4 (m, 2H, CH_2), 1.4−1.6 (m, 2H, CH_2), 3.2 (q, 2H, CH_2), 6.4 (t, 1H, NH), 7.1 (d, 1H, aromatic), 7.3−7.4 (m, 2H, aromatic), 7.5−7.7 (m, 3H, aromatic). Anal. ($C_{19}H_{22}N_2SO_2$) C, H, N, S.

Method O: Phenyl Nitrophenylsulfide (17). A mixture of 2-bromonitrobenzene (**16**) (4 g, 20 mmol), thiophenol (**7a**) (2.17 g, 20 mmol), and NaH (0.8 g, 20 mmol) in 20 mL of DMF was stirred for 12 h. The reaction mixture was concentrated and dry-packed in silica gel. Flash column chromatography on silica gel with 20% EtOAc in hexane gave 3.2 g (69%) of **17** as a yellow solid, mp 74−75 °C. ^{1}H NMR (Me_2SO-d_6): δ 6.9 (d, 1H, aromatic), 7.4 (t, 1H, aromatic), 7.5−7.7 (m, 6H, aromatic), 8.2 (d, 1H, aromatic). Anal. ($C_{12}H_9NO_2S$) C, H, N, S.

Method P: Phenylnitrophenylsulfone (4a, NPPS). A solution of $KMnO_4$ (1.89 g, 12 mmol) in 23 mL of water was added to a stirred solution of **17** in glacial AcOH. The reaction mixture was stirred for 0.5 h and was then filtered through

Celite with repeated washing with water. The filtrate was basified with concentrated NH_4OH. The precipitate formed was collected by filtration. **4a** was extracted from this precipitate with hot DMF. After treatment of the DMF solution with charcoal and filtration through Celite, the solution was concentrated to dryness. A total of 0.07 g (5%) of **4a** was obtained as a pale-yellow solid, mp 143−145 °C. ^{1}H NMR (Me_2SO-d_6): δ 7.6−7.9 (m, 2H, aromatic), 7.9−8.1 (m, 6H, aromatic), 8.3−8.5 (d, 1H, aromatic). Anal. ($C_{12}H_9NO_4S$) C, H, N, S.

Method Q: Bisnitrophenylsulfide 18. This procedure is similar to that described in method O using 2-nitrothiophenol. Following flash column chromatography on silica gel with 30% EtOAc in hexane as the eluent, a 60% yield of **18** was obtained as a yellow solid, mp 121−122 °C. ^{1}H NMR (Me_2SO-d_6): δ 7.4 (d, 2H, aromatic), 7.9−8.1 (m, 4H, aromatic), 8.32−8.3 (d, 2H, aromatic). Anal. ($C_{12}H_8N_2O_4S$) C, H, N, S.

Method R: Bisnitrophenylsulfoxide 19. To a solution of 18 (0.3 g, 1.1 mmol) in 50 mL of MeOH was added a solution of OXONE[27] (2.7 g, 4.3 mmol) in 50 mL of water. The resultant mixture was stirred for 12 h. The reaction mixture was filtered, and the precipitate was repeatedly washed with hot MeOH. The filtrate was concentrated and subjected to column chromatography on silica gel with 4% MeOH in CH_2Cl_2 as the eluent. This gave 0.2 g (77%) of **19** as a light-yellow solid, mp 185−187 °C. ^{1}H NMR (Me_2SO-d_6): δ 7.8−7.9 (m, 2H, aromatic), 7.9−8.1 (m, 4H, aromatic), 8.2−8.3 (m, 2H, aromatic). Anal. ($C_{12}H_8N_2O_5S$) C, H, N, S.

Method S: Bisnitrophenylsulfone 4b. To a solution of **19** (0.17 g, 0.6 mmol) in 2 mL of trifluoroacetic (0.6 mL, 6 mmol) acid was added dropwise 30% H_2O_2 (0.19 g, 5.7 mmol). The resultant mixture was stirred for 12 h. Water was added to the reaction mixture, and the precipitate formed was collected by filtration. After the mixture was washed with a copious amount of water and oven-dried at 60 °C, 0.14 g (82%) of **4b** was obtained as a white solid, mp 180−182 °C. ^{1}H NMR (Me_2SO-d_6): δ 8−8.1 (m, 4H, aromatic), 8.1−8.3 (m, 4H, aromatic). Anal. ($C_{12}H_8N_2O_6S$) C, H, N, S.

HeLa MAGI Assay. The assay described in this report is a modified version of the HeLa MAGI system[40] in which HIV-1 infection is detected by the activation of an HIV-LTR driven β-galactosidase reporter in HeLa CD4-LTR-b-gal cells (obtained from Dr. Michael Emerman through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH). Quantitation of β-galactosidase is achieved by measuring the activation of a chemiluminescent substrate (Tropix) 3 days after infection. The concentration of each compound required to inhibit 50% (IC_{50}) of the HIV-1-induced β-galactosidase signal, relative to untreated controls, was determined for each isogenic recombinant virus.

Activity against HIV-1 in an Acute Infection Assay Using MT4 Cells. The human T-cell lymphotropic virus type 1 transformed cell line MT4 was infected with HIV-1 (strain 3B) at 100 times the amount necessary to cause a 50% reduction in cell growth. The infected cells were incubated for 5 days in the presence of various concentrations of each of the compounds to be tested. HIV-1-mediated cytopathic effect (CPE) is expressed as an inhibition of MT4 cell growth. A compound that expresses activity against HIV-1 reverses this CPE. The extent of the CPE reduction was monitored by a propidium iodide stain for DNA (1), allowing for the calculation of IC_{50} values (50% inhibitory concentration). The antiviral effect of a compound is reported as an IC_{50} value, which is the inhibitory concentration that would produce a 50% decrease in the HIV-induced CPE. This effect is measured by the amount of drug required to restore 50% of the cell growth of HIV-1-infected MT4 cells, compared to uninfected MT4 cell controls. Each value represents the mean ± standard error (SE) from a single experiment performed in triplicate.

HIV Reverse Transcriptase Assay. Assays were conducted in black round-bottomed 96-well plates. Biotinylated DNA primer and RNA template were custom-synthesized by Oligos Etc. (Wilsonville, OR). The primer template used in the reaction (sequence 1) was labeled with Eu−chelate (Wallac, CR28−100) via a streptavidin−biotin linkage at the 5′ end of the primer.

Sequence 1

DNA: 5′-**Eu**-SA-B-GTC ATA GCT GTT TCC TG-3′

RNA: 3′-CAG UAU CGA CAA AGG ACA CAC UUU A

Reactions (50 μL) contained 100 nM Cy5-dUTP (Amersham Life Science, PA55022), 40 nM Eu-labeled primer template, 1 nM recombinant HIV reverse transcriptase, 50 mM Tris-HCl, pH 8.0, 80 mM KCl, 10 mM $MgCl_2$, 0.0032% NP40, 10 mM DTT, test compound, and 2% DMSO. The reaction was initiated by the addition of reverse transcriptase to each well. Reaction progress was monitored by fluorescence energy transfer from the Eu−chelate to the Cy5-dUMP incorporated into the template primer. The Eu−chelate was excited at 340 nm, and time-resolved fluorescence emission of the incorporated Cy5-dUMP was monitored at 665 nm with a Victor[41] 1420 multilabel counter (Wallac) over 30 min.

Crystallization and X-ray Data Collection. Crystals of the complex of HIV-1 RT with compound 739W94 (**3v**) were grown at 4 °C from citrate/phosphate buffer, pH 5.0, with PEG3400 (6−10% w/v) as precipitant, using the protocols described previously.[42] Crystals were soaked in increasing concentrations of PEG 3400, with citrate/phosphate buffer to a maximum PEG 3400 concentration of 50% (w/v). This latter procedure increases the order of the crystals and results in a series of closely related cell forms.[42,43] Crystals were resoaked in 0.2 mM compound for 2 days prior to data collection. X-ray diffraction data were collected using a Weissenberg camera on beamline BL6-A2[44] at the Photon Factory, KEK, Japan, utilizing procedures described previously[29,45] Data were collected at 16 °C with a 0.1 mm collimator, using 3.5° frames with a coupling constant of 1.5°/mm on pairs of 200 mm × 400 mm Fuji Bas-IIIB imaging plates positioned 429.7 mm from the crystal. The exposure time was 112 s per frame. The collimator, crystal enclosure, and camera cassette were flushed with helium to reduce background scattering. Exposed image plates were scanned off-line using Fuji BA 100 IP scanners. A single large crystal of RT/739W94, exposed to X-rays at three different positions, was used to collect a data set to 2.98 Å resolution.

Data images were indexed and integrated using DENZO[46] and merged with SCALEPACK.[46] Statistics are given in Table 1.

Structure Solution and Refinement. The structure was solved by molecular replacement using the RT-1051U91 complex (refined at 2.2 Å resolution to an R factor of 0.214; PDB 1RTH[29]). The molecular orientation and position were optimized by rigid-body refinement (program CNS[47]). The models were initially treated as single rigid bodies, then as two subunits, and finally as separate rigid groups corresponding to the nine domains of the heterodimer. The data used were gradually extended from 12 to 6.0 to 3.0 Å. During the refinement, anisotropic B factor scaling, solvent correction, strong stereochemical restraints to all atoms, and positional restraints to those lying greater than 25 Å from the Cα atom of residue 188 (approximately defining the NNRTI binding pocket) were applied. Manual model rebuilding used the program "O"[48] on an SGI O2 workstation. Further rounds of restrained refinement and manual rebuilding resulted in the current models (Table 1). Illustrations were prepared using BOBSCRIPT[49] and RASTER3D.[50]

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