Brief Articles

Simple, Short Peptide Derivatives of a Sulfonylindolecarboxamide (L-737,126) Active in Vitro against HIV-1 Wild Type and Variants Carrying Non-Nucleoside Reverse Transcriptase Inhibitor Resistance Mutations

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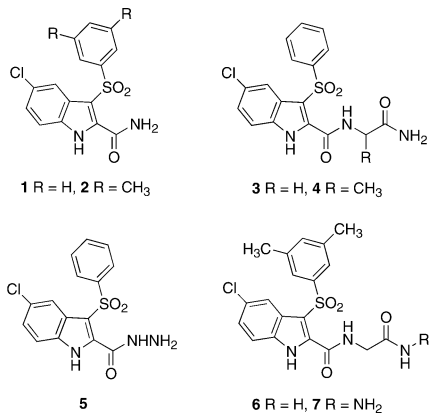
Non-nucleoside reverse transcriptase inhibitors (NNRTIs) active against NNRTI-resistant mutants were obtained by introducing two methyl groups at positions 3 and 5 of the benzenesulfonyl moiety of L-737,126 (**1**) and coupling one to three glycinamide/alaninamide units to its carboxyamide function. In cell-based assays, the new derivatives showed activities against HIV-1 wild type and NNRTI-resistant mutants [Y181C, K103N−Y181C, and triple mutant (K103R, V179D, P225H) highly resistant to efavirenz] superior to that of the parent indole derivative **1**.

Introduction

The emergence of drug-resistant variants is a dramatic problem in the management of the HIV infection AIDS. Because of the fact that monotherapy with any of the currently approved antiretroviral drugs leads to rapid selection of drug-resistant mutants, combination therapy protocols have been devised. The most commonly used combinations include two nucleoside reverse transcriptase inhibitors (NRTI) and one protease inhibitor (PRI). More recently, protocols including two NRTIs and one non-nucleoside reverse transcriptase inhibitor (NNRTI) have been increasingly recommended as first-line therapy.[1−6] Among the latter are nevirapine (NVP, Viramune) and delavirdine (DLV, Rescriptor), two first-generation NNRTIs, and efavirenz (EFV, Sustiva), a potent second-generation NNRTI that, retaining potent activity against the mutants selected by nevirapine and delavirdine, has been successfully employed as a key drug in combination therapies.[2] However, even with triple drug combination therapy, the high error rate of HIV replication rapidly leads to the selection of resistant variants. Therefore, new NNRTIs with improved properties in terms of spectrum of activity, tolerability, and pharmacokinetics parameters are needed, and studies in this direction are being pursued.[7,8]

In 1993 colleagues from Merck Laboratories reported on the potent activity of L-737,126 (**1**), a sulfonylindolecarboxyamide displaying anti-HIV-1 activity in the low nanomolar range[9,10] and cytoxicity (CC_{50}) for MT-4 cells at 45 μM.[10] Unfortunately, L-737,126 was poorly active against HIV-1 strains carrying NNRTI mutations (Chart 1). Recent structure−activity relationship (SAR) studies[10] on the indolylarylsulfone (IAS) family strictly related to previously described pyrrolylarylsulfones (PASs)[11] led us to introduce methyl groups at positions 3 and 5 of the benzene ring of **1**. The best derivative of the IAS series (**2**) showed the following interesting characteristics: (i) retained the same potency of **1** and efavirenz against both the wild type (WT) and the Y181C resistant strain; (ii) proved to be 10-fold more potent than **1** (and only 4-fold less potent than efavirenz) against the double mutant K103N−Y181C; (iii) showed a 10- and 20-fold better activity than **1** and efavirenz, respectively, against the K103R−V179D−P225H triple mutant, a strain highly resistant to efavirenz.[10]

Chart 1



During previous studies on IAS derivatives we prepared **3** and **4** having glycinamide or alanylamide moieties as chain elongators of the 2-carboxyamide function. When tested in cell-based assays, these compounds showed significant anti-HIV-1 activity.[12] This finding urged us to synthesize derivatives **6**, **8**, **10**, **12**, **13**, and **15**, which differed from **3** and **4** in having methyl groups at positions 3 and 5 of the benzenesulfo-

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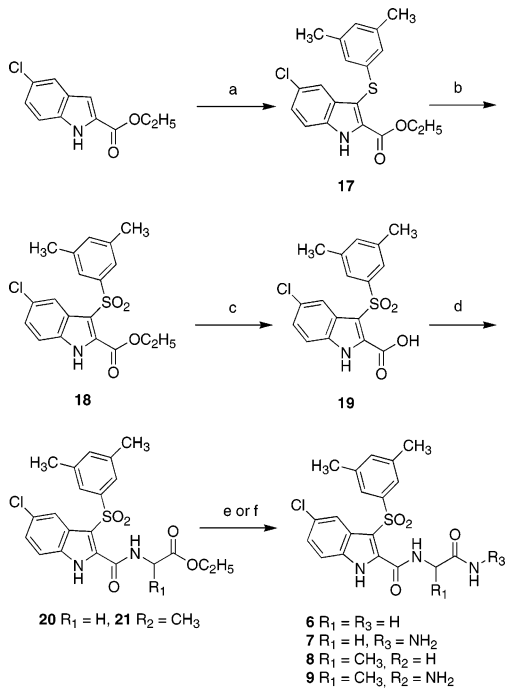
Scheme 1[a]



[a] Reagents: (a) DMPTS, BF_3·Et_2O, CH_2Cl_2, room temp, 2 h, then 45 °C, 2 h, Ar stream; (b) MCPBA, $CHCl_3$, room temp, 1 h; (c) LiOH, THF·H_2O, room temp, 24 h; (d) $H_2NCH(R_1)COOEt$, BOP, NEt_3, DMF, room temp, 72 h; (e) NH_4OH, EtOH, 60 °C, 3 h; (f) NH_2NH_2, EtOH, room temp, 3 h.

nyl moiety. Counterparts of the above compounds characterized by a hydrazide function as a terminus (**7**, **9**, **11**, **14**, and **16**) were also synthesized on the basis of the valuable antiretroviral activity and low cytotoxicity found with **5**.[10]

Chemistry

Compounds **6** and **7** were prepared by reacting the corresponding ethyl ester **20** with ammonium hydroxide or hydrazine hydrate, respectively. The starting ester was obtained by condensation of ethyl 5-chloro-1*H*-indole-2-carboxylate with *N*-(3,5-dimethylphenylthio)-succinimide (DMPTS) using boron trifluoride diethyl etherate as a catalyst, followed by oxidation of the 3,5-dimethylphenylthiosulfide **17** to sulfone **18** with MCPBA.[10] Subsequent hydrolysis of the ester **18** to acid **19** with lithium hydroxide and condensation with glycine ethyl ester in the presence of BOP and triethylamine afforded the required ethyl ester of *N*-{3-[(3,5-dimethylphenyl)sulfonyl]-5-chloro-1*H*-indole-2-carbonyl}-glycine (**20**) (Scheme 1). A similar reaction with D,L-alanine ethyl ester gave the *N*-(indole-2-carbonyl)-D,L-alanine ethyl ester **21**, which was reacted with ammonium hydroxide or with hydrazine to afford amide **8** and hydrazide **9**, respectively. Further elongation of the 2-indole chain with identical reagents and under the same reaction conditions afforded compounds with side chains containing dipeptide or tripeptide moieties as exemplified by derivatives **10**−**16**. (Scheme 2).

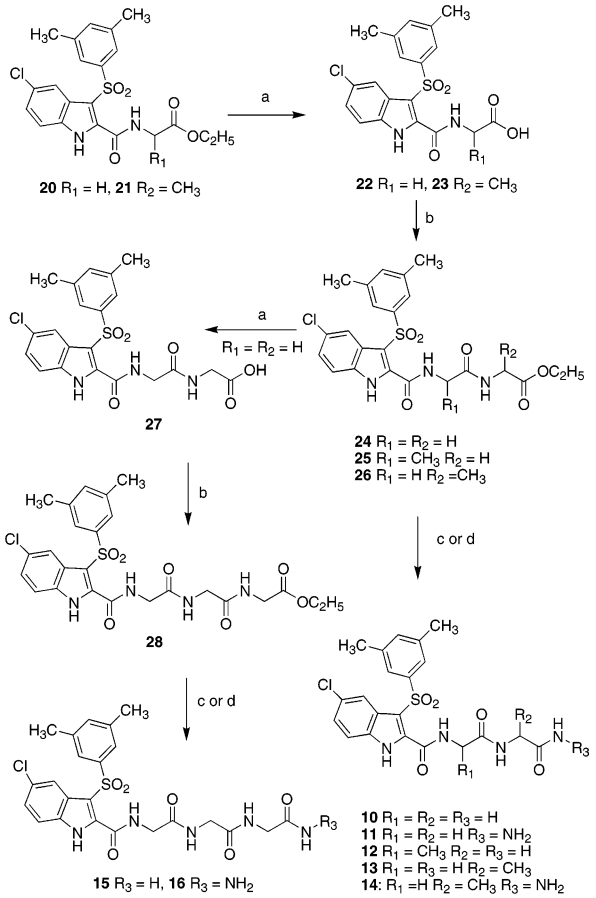
Results and Discussion

Test derivatives were evaluated for cytotoxicity and antiretroviral activity against HIV-1 WT and NNRTI resistant strains. L-737,126 (**1**), derivatives **2**−**5**, nevirapine, and efavirenz were used as reference compounds. The efficacy of test compounds against HIV-1 WT, Y181C, and the double mutant K103N−Y181C was evaluated in MT-4 cells by means of an MTT assay (EC_{50} values). On the other hand, the efficacy against the triple mutant K103R−V179D−P225H (highly resistant to EFV in vitro) was carried out in C8166 cells by means of a p24 assay, in parallel with the WT strain (EC_{90} values) (Table 1).

In general, with only one exception (**15**), derivatives bearing an amide terminus were more cytotoxic than hydrazide counterparts (compare **6** with **7**, **8** with **9**, **10** with **11**, and **13** with **14**). On the other hand, amides were more potent than hydrazides against the HIV-1 WT and the NNRTI resistant strains. Compounds **6**−**16** were more potent and/or selective than nevirapine against the WT strain and, most important, far more potent against all mutant strains. Interestingly, many of the new derivatives showed a potency comparable to that of efavirenz against the WT, the Y181C, and the triple mutant, being 4- to 10-fold less potent against the double mutant.

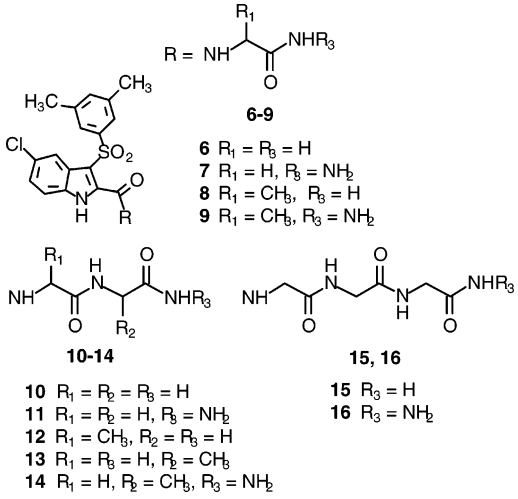
Among the new amides, the glycinamide **6**, the alanylamide **8**, and the diglycinamide **10** emerged as the most potent against all test viruses, favorably compared with **2**. When compared to efavirenz, **10** was 5-fold more potent against HIV-1 WT and the Y181C mutant, 18-fold more potent against the triple mutant, but 8-fold less potent against the double mutant. On

Scheme 2[a]



[a] Reagents: (a) LiOH, THF·H_2O, room temp, 24 h; (b) $H_2NCH(R_1, R_2)COOEt$, BOP, NEt_3, DMF, room temp, 72 h; (c) NH_4OH, EtOH, 60 °C, 3 h; (d) NH_2NH_2, EtOH, room temp, 3 h.

Table 1. Cytotoxicity and Antiviral Activities of Derivatives **1**−**16**[a]



compd	CC_{50} [b]	WT_{IIIB} EC_{50} [c]	WT_{IIIB} EC_{90} [d]	SI[e]	Y181C[f] EC_{50}	K103N−Y181C[g] EC_{50}	EFV^R,[h] EC_{90}
6	30	0.006	0.01	5000	0.03	0.8	0.1
7	>100	0.01	0.03	>10000	0.05	2.0	1.0
8	4	0.003	0.004	1333	0.01	0.7	0.04
9	20	0.03	0.07	666	0.14	4.7	0.7
10	7	0.0007	0.001	10000	0.005	1.2	0.1
11	52	0.06	0.1	875	0.08	2.8	0.15
12	17	0.013		1307	0.2	5.3	1.0
13	13	0.014	0.01	929	0.03	2.2	0.4
14	100	0.08	0.12	1250	0.16	3.5	1.0
15	>100	0.12	0.1	>833	0.2	6.0	1.9
16	>100	0.18	0.2	>556	0.3	>11	2.3
1[i]	45	0.001	0.007	45000	0.02	8.0	0.9
2[i]	15	0.004	0.006	3750	0.03	0.6	0.08
3	65	0.013		5000	0.18	>20	2.7
4	64	0.01		6400	0.36	16	3.0
5[i]	>100	0.01	0.02	>10000	0.4	≥100	3.0
NVP	>100	0.4	0.12	>270	>30	>30	>30
EFV	35	0.004	0.008	8750	0.025	0.15	1.8

[a] Data represent mean values for three separate experiments. Variation among triplicate samples was less than 15%. [b] Cytotoxicity: compound concentration (μM) required to reduce the viability of mock-infected cells by 50% (MTT method). [c] Compound concentration (μM) required to achieve 50% protection of infected MT-4 cells from WT_{IIIB}-HIV-1-induced cytopathicity (MTT method). [d] Compound concentration (μM) required to reduce the amount of p24 by 90% in WT_{IIIB} infected C8166 cells. [e] Selectivity index: CC_{50}/EC_{50} ratio. [f] Compound concentration (μM) required to achieve 50% protection of infected MT-4 cells from Y181C-HIV-1-induced cytopathicity (MTT method). [g] Compound concentration (μM) required to achieve 50% protection of infected MT-4 cells from K103N−Y181C−HIV-1-induced cytopathicity (MTT method). [h] Compound concentration (μM) required to reduce the amount of p24 by 90% in EFV^R (K103R, V179D, P225H) infected C8166 cells. [i] Reference 10.

the other hand, whereas the glycine alanylamide **13** retained fairly good potency only against the Y181C mutant, the alanylglycinamide **12** and the triglycinamide **15** showed a net decrease of activity against all test viruses.

As far as hydrazides are concerned, although the addition of methyl groups at positions 3 and 5 of the phenyl ring considerably improved their activity against mutants with respect to the previously described counterparts, they turned out to be less potent than the corresponding amides (compare **7**, **9**, **11**, **14**, and **16** with **6**, **8**, **10**, **13**, and **15**) against all test viruses. In this series, the glycine hydrazide **7** showed the best selectivity index (>10000) because of its low cytotoxicity (CC_{50} ≥ 100 μM) and the most potent activity against the WT, the Y181C, and the double mutant K103N−Y181C, whereas the diglycine hydrazide **11** was the most potent against the triple mutant K103R−V179D−P225H.

In conclusion we have developed a series of analogues of L-737,126 (**1**), an indolylarylsulfone derivative endowed with high selectivity and potent activity against HIV-1 WT and Y181C mutant, although poorly active against the double mutant K103N−Y181C and the highly EFV-resistant triple mutant K103R−V179D−P225H. From this point of view, title derivatives can be considered a potent second generation of compounds related to the previously described indolecarboxyamide L-737,126 (**1**).[9]

Generally, improvement of activity against resistant mutants was obtained not only by introducing two methyl groups at positions 3 and 5 of the benzenesulfonyl moiety of **1**[10] but also by adding to the 2-carboxyamide chain of **2** simple amino acids such as glycine and D,L-alanine to give mono-, di-, and tripeptide derivatives. Subsequent modification of the elongated chain terminus into amide or hydrazide produced new derivatives characterized by an interesting chemical biodiversity (**6**−**16**) other than potent and selective activity against HIV-1 WT and NNRTI resistant mutants. Interestingly, the title compounds compare favorably not only with their congener **2** but also with efavirenz, especially as far as the activity against the highly EFV-resistant triple mutant is concerned.

Derivative **8**, bearing a D,L-alanine unit, was the most active compound against the double and triple mutants. This result could warrant the chiral resolution of the racemate aimed at evaluating the cytotoxicity and the antiretroviral activity of both enantiomers (+)-**8** and (−)-**8**. As a matter of course, the interesting biological profiles shown by the title compounds are a stimulus for further studies on this novel series of IAS NNRTIs.

Experimental Section

Chemistry. Melting points were determined on a Büchi 510 apparatus and are uncorrected. Infrared spectra were run on Perkin-Elmer 1310 and SpectrumOne spectrophotometers. Band position and absorption ranges are given in cm^{-1}. Proton nuclear magnetic resonance spectra were recorded on Bruker A300 (200 MHz) Fourier transform spectrometer in the indicated solvent. Chemical shifts are expressed in δ units (ppm) from tetramethylsilane. Chromatographic columns were packed with alumina (Merck, 70−230 mesh) and silica gel (Merck, 70−230 mesh). Aluminum oxide TLC cards from Fluka (aluminum oxide precoated aluminum cards with fluorescent indicator at 254 nm) and silica gel TLC cards from Fluka (silica gel precoated aluminum cards with fluorescent indicator at 254 nm) were used for thin-layer chromatography (TLC). Developed plates were visualized by a Spectroline ENF 260C/F UV apparatus. Organic solutions were dried over anhydrous sodium sulfate. Concentration and evaporation of the solvent after reaction or extraction were carried out on a rotary evaporator (Büchi Rotavapor) operating at reduced pressure. Elemental analyses were found to be within ±0.4% of the theoretical values.

General Procedure for the Preparation of Amides 6, 8, 10, 12, 13, and 15. Example. *N*-{3-[(3,5-Dimethylphenyl)sulfonyl]-5-chloro-1*H*-indole-2-carbonyl}glycinamide (6). A mixture of **20** (0.80 g, 0.0018 mol), ethanol (80 mL), and 32% NH_4OH (80 mL) was heated at 60 °C for 3 h. After dilution with water, the amide was extracted with ethyl acetate. The organic solution was washed with brine and dried. Removal of the solvent gave the crude product, which was purified by column chromatography (ethyl acetate as eluent) to afford 0.36 g (48%) of **6**, mp 265−267 °C (from ethanol). ^1H

NMR (DMSO-d_6): δ 2.31 (s, 6H), 4.01 (m, 2H), 7.27 (s, 1H), 7.30−7.41 (m, 2H, on treatment with D_2O showed dd, J = 1.8 and 8.8 Hz, 1H), 7.47 (br s, 1H, disappeared on treatment with D_2O), 7.57 (d, J = 8.8 Hz, 1H), 7.74 (s, 2H), 8.00 (d, J = 1.8 Hz, 1H), 9.39 (br t, J = 5.5 Hz, 1H, disappeared on treatment with D_2O), 13.06 ppm (br s, 1H, disappeared on treatment with D_2O). IR (Nujol): ν 1640, 1680, 3280, 3300, 3400 cm^{-1}. Anal. ($C_{19}H_{18}ClN_3O_4S$ (419.88)), C, H, N, Cl, S.

General Procedure for the Preparation of Hydrazides 7, 9, 11, 14, and 16. Example. *N*-{3-[(3,5-Dimethylphenyl)-sulfonyl]-5-chloro-1*H*-indole-2-carbonyl}glycine hydrazide (7). A mixture of **20** (0.50 g, 0.0011 mol), ethanol (5 mL), and hydrazine hydrate (2.5 mL) was stirred at room temperature for 3 h. After dilution with water, the crude product was filtered and dried to afford 0.36 (75%) of pure **7**, mp 224−227 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 2.33 (s, 6H), 4.03 (m, 2H), 4.38 (br s, 2H, disappeared on treatment with D_2O), 7.28 (s, 1H), 7.37 (dd, J = 1.8 and 8.7 Hz, 1H), 7.58 (d, J = 8.7 Hz, 1H), 7.75 (s, 2H), 8.00 (d, J = 1.8 Hz, 1H), 9.16 (br s, 1H, disappeared on treatment with D_2O), 9.39 (br m, 1H, disappeared on treatment with D_2O), 13.00 ppm (very br, 1H, disappeared on treatment with D_2O). IR (Nujol): ν 1640, 3300 cm^{-1}. Anal. ($C_{19}H_{19}ClN_4O_4S$ (434.89)), C, H, N, Cl, S.

General Procedure for the Preparation of Acids 19, 22, 23, and 27. Example. 3-[(3,5-Dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxylic acid (19). Lithium hydroxide monohydrate (0.24 g, 0.0057 mol) was added to a solution of **18** (0.70 g, 0.0019 mol) in THF (20 mL) and water (20 mL). Then the reaction mixture was stirred at room temperature for 24 h. After dilution with water, the mixture was acidified with 1 N HCl until pH 2 was reached. The acid was extracted with ethyl acetate, washed with brine, and dried. Removal of the solvent furnished 0.63 g (94%) of satisfactorily pure **19**, mp 277−278 °C (from ethanol). Anal. ($C_{17}H_{14}ClNO_4S$ (363.81)), C, H, N, Cl, S.

General Procedure for the Preparation of Ethyl Esters 20, 21, 24−26, and 28. Example. *N*-{3-[(3,5-Dimethylphenyl)sulfonyl]-5-chloro-1*H*-indole-2-carbonyl}-glycine Ethyl Ester (20). Benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) (0.61 g, 0.0014 mol) was added to a solution of **19** (0.50 g, 0.0014 mol), glycine ethyl ester hydrochloride (0.39 g, 0.0027 mol), and triethylamine (0.42 g, 0.0041 mol) in anhydrous DMF (25 mL). Then the reaction mixture was stirred at room temperature for 72 h. After dilution with water, the solid that formed was filtered, washed with water, and dried to afford 0.50 g (80%) of pure **20**, mp 209−211 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.25 (t, J = 7.1 Hz, 3H), 2.31 (s, 6H), 4.12−4.30 (m, 4H), 7.27 (s, 1H), 7.37 (dd, J = 1.7 and 8.8 Hz, 1H), 7.57 (d, J = 8.8 Hz, 1H), 7.72 (s, 2H), 8.03 (d, J = 1.7 Hz, 1H), 9.49 (t, J = 5.6 Hz, 1H, disappeared on treatment with D_2O), 13.13 ppm (br, 1H, disappeared on treatment with D_2O). IR (Nujol): ν 1635, 1740, 3200 cm^{-1}. Anal. ($C_{21}H_{21}ClN_2O_5S$ (448.92)), C, H, N, Cl, S.

Antiviral Assay Procedures. Compounds. Compounds were solubilized in DMSO at 200 mM and then diluted in culture medium.

Cells and Viruses. MT-4, C8166, and H9/IIIB cells were grown at 37 °C in a 5% CO_2 atmosphere in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 100 IU/mL penicillin G, and 100 μg/mL streptomycin. Cell cultures were checked periodically for the absence of mycoplasma contamination with a MycoTect Kit (Gibco). Human immunodeficiency viruses type 1 (HIV-1, IIIB strain) was obtained from supernatants of persistently infected H9/IIIB cells. The HIV-1 stock solutions had titers of 4.5 × 10^6 50% cell culture infectious dose ($CCID_{50}$)/mL. The K103R−V179D−P225H mutant was derived from an IIIB strain passaged in C8166 cells in the presence of efavirenz (up to 2 μM). The Y181C mutant (NIH N119) is derived from an AZT-sensitive clinical isolate passaged initially in CEM and then in MT-4 cells in the presence of nevirapine (10 μM). The K103N−Y181C (NIH A17) is derived from the IIIB strain passaged in H9 cells in the presence of BI-RG 587 (1 μM). K103R−V179D−P225H, Y181C, and K103N−Y181C stock solutions had titers of 3.0 × 10^5, 1.3 × 10^6, and 2.5 × 10^5 $CCID_{50}$/mL, respectively.

HIV Titration. Titration of HIV was performed in C8166 cells by the standard limiting dilution method (dilution 1:2, four replica wells per dilution) in 96-well plates. The infectious virus titer was determined by light microscope scoring of syncytia after 4 days of incubation. Virus titers were expressed as $CCID_{50}$/mL.

Anti-HIV Assays. The activity of test compounds against multiplication of WT HIV-1, Y181C, and K103N−Y181C in acutely infected cells was based on inhibition of virus-induced cytopathicity in MT-4 cells. The activity of the compounds against the K103R multiplication in acutely infected cells was based on inhibition of p24 antigen in C8166 cells. Briefly, an amount of 50 μL of culture medium containing 1 × 10^4 cells was added to each well of flat-bottom microtiter trays containing 50 μL of culture medium with or without various concentrations of test compounds. Then an amount of 20 μL of HIV suspensions (containing the appropriate amount of $CCID_{50}$ to cause complete cytopathicity at day 4) was added. After incubation at 37 °C, cell viability was determined by the 3-(4,5-dimethylthiazol-1-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[13] Alternatively, p24 levels were determined by an immunoenzymatic kit (Abbott). The cytotoxicity of test compounds was evaluated in parallel with their antiviral activity and was based on the viability of mock-infected cells, as monitored by the MTT method.

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Supporting Information Available: Chemical and physical data, elemental analysis results, and IR and ^{1}H NMR spectra for **8**−**16** and **21**−**28**. This material is available free of charge via the Internet at http://pubs.acs.org.

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