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Indolylarylsulfones Bearing Natural and Unnatural Amino Acids. Discovery of Potent Inhibitors of HIV-1 Non-Nucleoside Wild Type and Resistant Mutant Strains Reverse Transcriptase and Coxsackie B4 Virus

Francesco Piscitelli,[†] Antonio Coluccia,[||] Andrea Brancale,[||] Giuseppe La Regina,[†] Anna Sansone,[†] Cesare Giordano,[⊥] Jan Balzarini,[∞] Giovanni Maga,[‡] Samantha Zanoli,[‡] Alberta Samuele,[‡] Roberto Cirilli,[#] Francesco La Torre,[#] Antonio Lavecchia,[§] Ettore Novellino,[§] and Romano Silvestri*[,†]

Istituto Pasteur-Fondazione Cenci Bolognetti, Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma, Piazzale Aldo Moro 5, I-00185 Roma, Italy, Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff, CF10 3NB, U.K., Istituto di Chimica Biomolecolare del CNR, Sapienza Università di Roma, Piazzale Aldo Moro 5, I-00185 Roma, Italy, Dipartimento di Chimica Farmaceutica e Tossicologica, Università di Napoli Federico II, Via Domenico Montesano 49, I-80131, Napoli, Italy, Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium, Istituto di Genetica Molecolare-Consiglio Nazionale delle Ricerche, Via Abbiategrasso 207, I-27100 Pavia, Italy, and Dipartimento del Farmaco, Istituto Superiore di Sanità, Viale Regina Elena 299, I-00161 Roma, Italy

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New potent indolylarylsulfone (IAS) HIV-1 NNRTIs were obtained by coupling natural and unnatural amino acids to the 2-carboxamide and introducing different electron-withdrawing substituents at position 4 and 5 of the indole nucleus. The new IASs inhibited the HIV-1 replication in human T-lymphocyte (CEM) cells at low/subnanomolar concentration and were weakly cytostatic. Against the mutant L100I, K103N, and Y181C RT HIV-1 strains in CEM cells, sulfones **3**, **4**, **19**, **27**, and **31** were comparable to EFV. The new IASs were inhibitors to Coxsackie B4 virus at low micromolar (2−9 μM) concentrations. Superimposition of PLANTS docked conformations of IASs **19** and **9** revealed different hydrophobic interactions of the 3,5-dimethylphenyl group, for which a staking interaction with Tyr181 aromatic side chain was observed. The binding mode of **19** was not affected by the L100I mutation and was consistent with the interactions reported for the WT strain.

Introduction

Acquired immunodeficiency syndrome (AIDS[a]) causes over 5700 deaths every year, and at the same time over 6800 people become infected with human immunodeficiency virus (HIV).[1] Both prevention and treatment of HIV are still inadequate. The challenge toward new anti-HIV/AIDS drugs is the discovery of (i) an effective vaccine, (ii) better tolerated drugs, and (iii) agents endowed with improved properties against both drug resistance and cross-resistance.[2]

Drugs currently approved for the treatment of HIV infection fall into six target classes. In addition to (i) nucleoside (NRTIs) and nucleotide (NtRTIs) reverse transcriptase inhibitors, (ii) non-nucleoside reverse transcriptase inhibitors (NNRTIs), (iii) protease inhibitors (PIs), and (iv) fusion inhibitors (FIs),[3] two new drugs, (v) the entry inhibitor CCR5 co-receptor antagonist maraviroc and (vi) the integrase inhibitor raltegravir, became available in 2007.[4] These drugs slow the viral infection and multiplication, affecting the progression of the disease. By combination of three (recommended) or four antiretroviral drugs, highly active antiretroviral therapy (HAART) produces an effective and prolonged reduction of morbidity and mortality in AIDS patients.[5] However, HAART does not completely eradicate the viral infection; thus, the required long-term drug administrations provoke drug resistance and unwanted side effects.[6]

NNRTIs showed low toxicity and favorable pharmacokinetic properties. The major problem of earlier inhibitors, namely, the rapid emergence of drug resistance, was overcome by new generation agents that succeeded in inhibiting both HIV-1 wild type (WT) and viral strains carrying NNRTI resistance mutations.[7] Etravirine was approved in 2008 by the U.S. FDA for the drug combination treatment of HIV-1 infected people who experienced drug resistance to other drugs of this class.[8]

The development of indolylarylsulfones (IASs) NNRTIs was based on the Merck derivative L-737,126 (**1**)[9] as reference compound. The potent activity of IAS **2** against the NNRTI-resistant mutants was correlated to the presence of a 3-(3,5-dimethylphenyl)sulfonyl moiety.[10] Potent IAS derivatives were obtained by adding to the 2-carboxamide chain of **2** simple amino acids such as glycine and D,L-alanine to give derivatives (for example, **3** and **4**) endowed with activities against HIV-1 WT and NNRTI-resistant mutants superior to that of the parent indole **1**.[11]

On the other hand, the activity against the K103N RT mutant virus exhibited by imidazole **5**[12] prompted the synthesis of derivatives bearing either the *N*-(2-hydroxyethyl)carboxamide functionality (**6**)[13] or substituted *N*′-carboxyhydrazide derivatives (**7**)[14] at position 2 of the indole. DuPont Merck synthesized analogues of efavirenz (**8**) containing two halogen atoms at positions 5 and 6 of the quinazolinone ring which showed

* To whom correspondence should be addressed. Phone: +39 06 4991 3800. Fax: +39 06 491 491. E-mail: romano.silvestri@uniroma1.it.
[†] Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma.
[||] Cardiff University.
[⊥] Consiglio Nazionale delle Ricerche, Roma.
[∞] Katholieke Universiteit Leuven.
[‡] Consiglio Nazionale delle Ricerche, Pavia.
[#] Istituto Superiore di Sanità, Roma.
[§] Università di Napoli Federico II.

[a] Abbreviations, IAS, indolylarylsulfone; HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome; RT, reverse transcriptase; NRTI, nucleoside/nucleotide reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; FI, fusion inhibitor; HAART, highly active antiretroviral therapy; WT, wild type; NVP, nevirapine; EFV, efavirenz.

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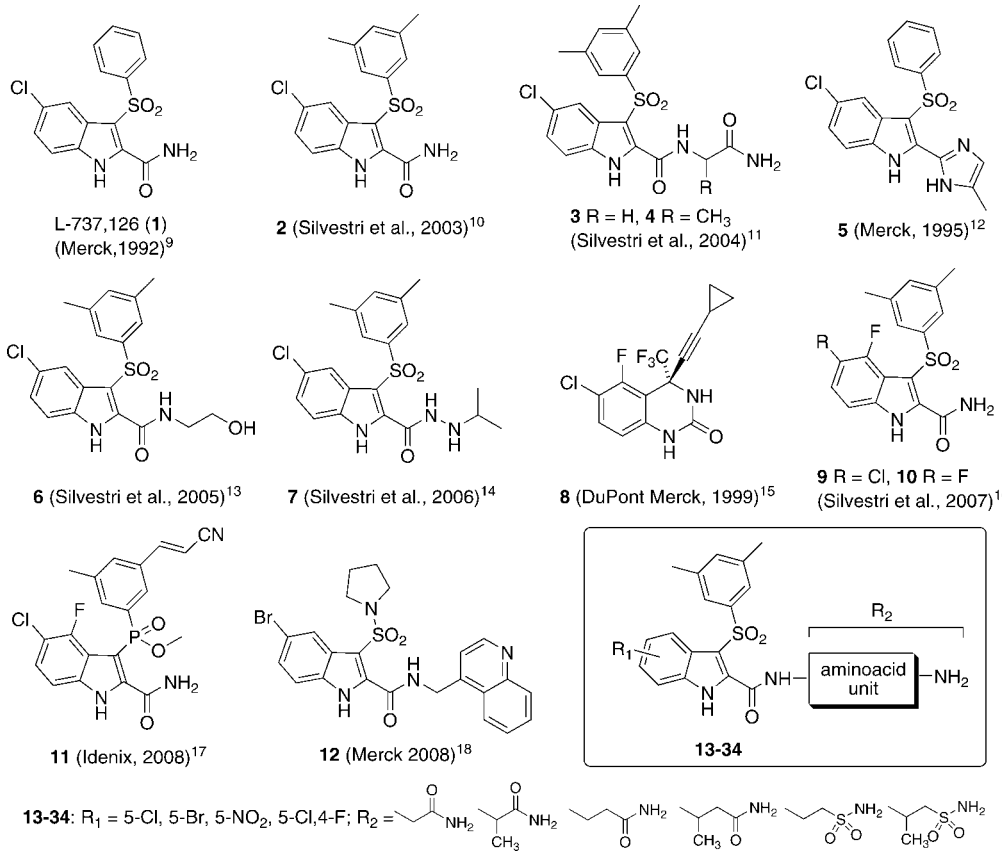
Chart 1. Structure of Reference Compounds **1**−**12** and New IASs **13**−**34**



broader spectrum than the monosubstituted counterparts.[15] Recently we synthesized IASs derivatives bearing two halogen atoms at the indole ring. The 5-chloro-4-fluoro- (**9**) and 4,5-difluoro- (**10**) IASs turned out to be potent inhibitors of HIV-1 WT and the NNRTI-resistant Y181C RT and K103N-Y181C RT HIV-1 strains. In particular, compound **10** was exceptionally potent against RT WT and RTs carrying the K103N, Y181I, L100I mutations.[16] In 2008, Idenix Pharmaceuticals and Merck & Co. showed a renewed interest in IASs and replaced the 3-arylsulfonyl group by either an arylphosphonyl[17] (**11**) or a sulfonamide[18] (**12**) group. Continuing our research project, we planned the synthesis of new IASs **13**−**34** characterized by (a) natural and unnatural amino acids at the 2-carboxamide and (b) different electron-withdrawing substituents at positions 4 and 5 of the indole (Chart 1).

Chemistry

The synthesis of new IASs is depicted in Scheme 1. Reaction of ethyl 5-chloro- (**35**), 5-bromo- (**36**), 5-nitro- (**37**), or 5-chloro-4-fluoroindole-2-carboxylate[19] (**38**) with *N*-(3,5-dimethylphenyl)succinimide in the presence of boron trifluoride diethyl etherate at room temperature in dichloromethane afforded the corresponding 3-(3,5-dimethylphenyl)indole-2-carboxylates **39**−**42**. The sulfides **39**−**42** were oxidized to sulfones **43**−**46** with 3-chloroperoxybenzoic acid in chloroform at 0 °C. Subsequent lithium hydroxyde hydrolysis of esters **43**−**46** at room temperature afforded the corresponding acids **47**−**50**. Coupling reaction of **47**−**50** with an appropriate amino acid in the presence of BOP reagent and triethylamine in DMF overnight provided IAS derivatives bearing an amino acid unit (**51**−**66**). Treatment of **51**−**66** with 28% ammonium hydroxyde in ethanol at 60 °C or microwave irradiation at 150 W and 130 °C for 10 min provided **3**, **4**, and **13**−**36**. Sulfonamides **27**−**34** were obtained by reaction of **47**−**50** with 2-aminoethanesulfonamide hydrochloride[20] or 2-aminopropanesulfonamide hydrochloride in the presence of BOP reagent and triethylamine (Scheme 1).

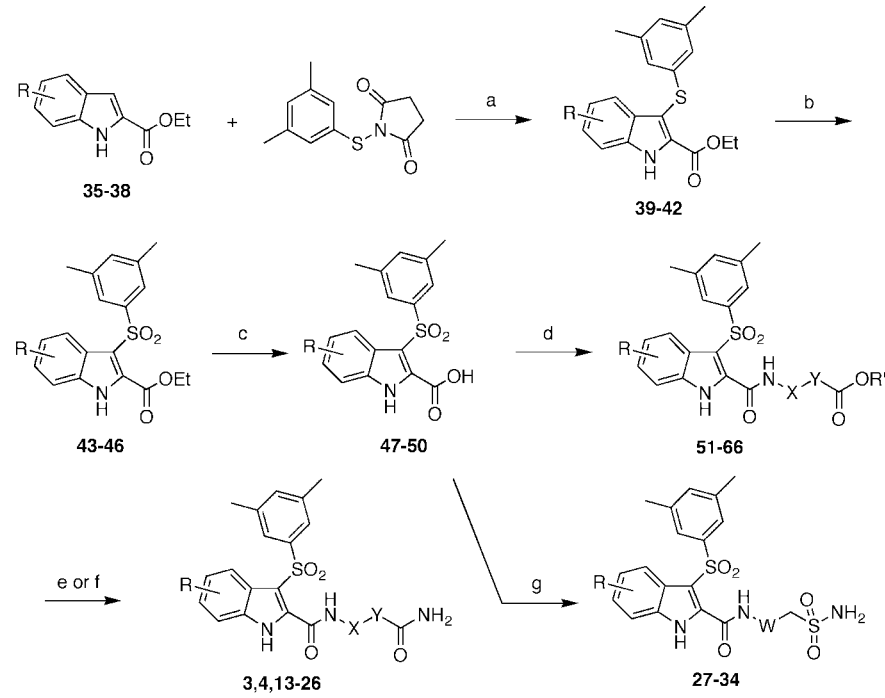
2-Aminopropanesulfonamide hydrochloride (**67**) was synthesized by reaction of mesylate **68**[21] with potassium thioacetate in DMF to afford **69**, which was oxidized with hydrogen peroxide to give the sulfonic acid **70**. Treatment of **70** with phosgene gave the corresponding sulfonyl chloride **71**, which was transformed into sulfonamide **72** with gaseous ammonia and then deprotected with hydrogen over carbon to provide **67** (Scheme 2).

Results and Discussion

The antiretroviral activity (EC_{50} values) of IAS derivatives **3**, **4**, and **13**−**34** was evaluated against the HIV-1 WT in human T-lymphocyte (CEM) cells, using IASs **2**, **7**, and **9** as reference compounds. Compounds **3**, **4**, and **13**−**34** proved to be highly potent against HIV-1 in human T-lymphocyte (CEM) cells and showed inhibitory potencies in low/subnanomolar range of concentrations which were comparable with the previously reported lead compounds **2**,[10] **7**,[14] and **9**.[16] Against HIV-1 WT, the inhibitory activity was only marginally affected by the substituent introduced on the indole nucleus, and with only one exception (**23**), 5-chloro-IASs were the most potent inhibitors (Table 1).

The derivatives were also evaluated for their antiproliferative activities against murine leukemia cells (L1210) and human T-lymphocyte cells (Molt4/C8, CEM). Generally, the IASs inhibited the cell proliferation in the micromolar range, that is, at compound concentrations that are usually 3−4 orders of magnitude higher than their antivirally active concentrations. 5-Nitro-IASs bearing an unbranched amino acid unit were often devoid of any

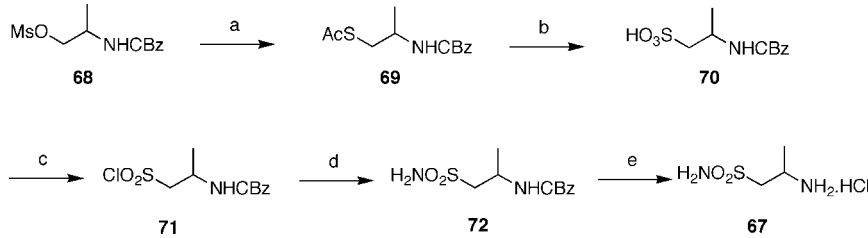
Scheme 1. Synthesis of Compounds **3**, **4**, and **13**−**34**[a]



3,[11] R = 5-Cl, X = 0, Y = CH_2; **13**, R = 5-Br, X = 0, Y = CH_2; **14**, R = 5-NO_2, X = 0, Y = CH_2; **15**, R = 5-Cl,4-F, X = 0, Y = CH_2; **4**,[11] R = 5-Cl, X = 0, Y = CHMe; **16**, R = 5-Br, X = 0, Y = CHMe; **17**, R = 5-NO_2, X = 0, Y = CHMe; **18**, R = 5-Cl,4-F, X = 0, Y = CHMe; **19**, R = 5-Cl, X = Y = CH_2; **20**, R = 5-Br, X = Y = CH_2; **21**, R = 5-NO_2, X = Y = CH_2 **22**, R = 5-Cl,4-F, X = Y = CH_2; **23**, R = 5-Cl, X = CHMe, Y = CH_2; **24**, R = 5-Br, X = CHMe, Y = CH_2; **25**, R = 5-NO_2, X = CHMe, Y = CH_2; **26**, R = 5-Cl,4-F, X = CHMe, Y = CH_2; **27**, R = 5-Cl, W = CH_2; **28**, R = 5-Br, W = CH_2; **29**, R = 5-NO_2, W = CH_2; **30**, R = 5-Cl,4-F, W = CH_2; **31**, R = 5-Cl, W = CHMe; **32**, R = 5-Br, W = CHMe; **33**, R = 5-NO_2, W = CHMe; **34**, R = 5-Cl,4-F, W = CHMe; **35**, **39**,[10] **43**,[10] **47**,[11] R = 5-Cl; **36**, **40**[10], **44**,[10] **48**, R = 5-Br; **37**, **41**, **45**, **49**, R = 5-NO_2; **38**,[19] **42**,[16] **46**,[16] **50**, R = 5-Cl,4-F; **51**,[11] R = 5-Cl, X = 0, Y = CH_2, R' = Et; **52**, R = 5-Br, X = 0, Y = CH_2, R' = Et; **53**, R = 5-NO_2, X = 0, Y = CH_2, R' = Et; **54**, R = 5-Cl,4-F, X = 0, Y = CH_2, R' = Et; **55**,[11] R = 5-Cl, X = 0, Y = CHMe, R' = Et; **56**, R = 5-Br, X = 0, Y = CHMe, R' = Et; **57**, R = 5-NO_2, X = 0, Y = CHMe, R' = Et; **58**, R = 5-Cl,4-F, X = 0, Y = CHMe, R' = Et; **59**, R = 5-Cl, X = Y = CH_2, R' = Me; **60**, R = 5-Br, X = Y = CH_2, R' = Me; **61**, R = 5-NO_2, X = Y = CH_2, R' = Me; **62**, R = 5-Cl,4-F, X = Y = CH_2, R' = Me; **63**, R = 5-Cl, X = CHMe, Y = CH_2, R' = Et; **64**, R = 5-Br, X = CHMe, Y = CH_2, R' = Et; **65**, R = 5-NO_2, X = CHMe, Y = CH_2, R' = Et; **66**, R = 5-Cl,4-F, X = CHMe, Y = CH_2, R' = Et.

[a] Reagents and reaction conditions: (a) $BF_3 \cdot Et_2O$, dichloromethane, room temp to 45 °C, 4 h; (b) 3-chloroperoxybenzoic acid, chloroform, 0 °C, 2 h; (c) LiOH, aqueous tetrahydrofuran, room temp, 5 h; (d) amino acid methyl or ethyl ester hydrochloride, BOP reagent, triethylamine, dimethylformamide, room temp, overnight; (e) 28% NH_4OH, ethanol, 60 °C, 3 h; (f) 28% NH_4OH, closed vessel, 130 °C, 150 W, 10 min; (g) 2-aminoethanesulfonamide hydrochloride or 2-aminopropane sulfonamide hydrochloride, BOP reagent, triethylamine, dimethylformamide, room temp, overnight.

Scheme 2. Synthesis of Compound **67**[a]



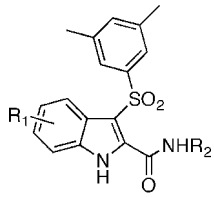
[a] Reagents and reaction conditions: (a) potassium thioacetate, DMF, room temp, overnight; (b) hydrogen peroxide, acetic acid, room temp, overnight; (c) phosgene, DMF, dichloromethane, 2 h, overnight, nitrogen atmosphere; (d) gaseous NH_3, benzene, 60 °C, 2 h; (e) hydrogen, palladium over carbon, aqueous methanol, 37% HCl, room temp, 3 h.

significant cytostatic effect at 500 μM (i.e., **14**, **21**, and **29**). On the contrary, the bromine atom at position 5 of the indole had the tendency to strengthen the cytotoxicity (Table 2).

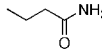
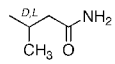
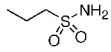
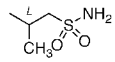
The most active compounds **3**, **4**, **19**, **27**, and **31** have been evaluated against mutant HIV-1 strains that harbor the L100I, K103N, Y181C, Y181I, and Y188L mutations in their RT (Table 3). The compounds showed high antiviral potency against the mutant L100I and K103N RT HIV-1 strains. Against these mutant strains, the compounds were always superior to EFV (Table 3, footnote b) and were comparable with data reported for MK-4965[22] and TMC-120;[23] however, rilpivirine (TMC-278) was overall 10-fold more potent.[23]

The compounds lost antiviral potency against the mutant Y181C RT HIV-1 strain. In this respect, EFV was comparable

Table 1. Structure, and Anti-HIV Activitiy of IAS Derivatives **3**, **4**, and **13**−**34** and Reference Compounds **2**, **7**, and **9** in Human T-Lymphocyte (CEM) Cells[a]



3, **4**, **13-36**

Compd	R_1	R_2	HIV-1 (III_B) EC_{50}[b] (nM)	HIV-2 (ROD) EC_{50}[b] (nM)
3	5-Cl		1.9 ± 1.3	>50000
13	5-Br		2.6 ± 2.0	43000 ± 9900
14	5-NO_2		2.4 ± 1.8	>50000[c]
15	5-Cl,4-F		3.1 ± 1.9	>10000
4	5-Cl		1.6 ± 1.3	>10000[c]
16	5-Br		1.9 ± 1.1	>10000[c]
17	5-NO_2		3.8 ± 0.98	>10000[c]
18	5-Cl,4-F		3.7 ± 1.7	>10000
19	5-Cl		1.0 ± 0.6	>10000[c]
20	5-Br		1.1 ± 0.21	23000 ± 8500
21	5-NO_2		1.6 ± 1.4	>10000[c]
22	5-Cl,4-F		2.0 ± 1.0	>10000
23	5-Cl		26 ± 8.7	30000 ± 3500
24	5-Br		1.5 ± 0.57	>10000[c]
25	5-NO_2		5.1 ± 0.81	>10000[c]
26	5-Cl,4-F		4.6 ± 2.2	>10000[c]
27	5-Cl		1.4 ± 1.5	>10000[c]
28	5-Br		1.0 ± 0.14	>10000[c]
29	5-NO_2		2.7 ± 1.3	>10000[c]
30	5-Cl,4-F		2.3 ± 2.3	>10000[c]
31	5-Cl		2.3 ± 1.1	>10000[c]
32	5-Br		2.3 ± 5.9	>10000[c]
33	5-NO_2		4.2 ± 0.89	≥10000
34	5-Cl,4-F		3.9 ± 2.4	>10000[c]
2	5-Cl	H	1.1 ± 0.0	>2000[c]
7	5-Cl	$NHCH(CH_3)_2$	6.4 ± 0.8	>50000
9	5-Cl,4-F	H	1.0 ± 0.0	>10000

[a] Data are mean values of two experiments performed in triplicate. [b] EC_{50}: effective concentration (nM) or concentration required to protect CEM cells against the cythopathicity of HIV by 50%, as monitored by giant cell formation. [c] Compound precipitation was detected at higher compound concentrations.

Table 2. Inhibitory Effects of Compounds **3**, **4**, and **13**−**34** on the Proliferation of Murine Leukemia Cells (L1210) and Human T-Lymphocyte Cells (Molt4/C8, CEM)[a]

	IC_{50} (μM)		
compd	L1210	Molt4/C8	CEM
3	220 ± 2	15 ± 7	92 ± 13
13	40 ± 5	17 ± 0	31 ± 15
14	>500	>500	≥500
15	53 ± 3	34 ± 1	31 ± 19
4	12 ± 1	3.8 ± 1.3	11 ± 1
16	10 ± 0	8.7 ± 1.4	10 ± 0
17	96 ± 4	44 ± 13	66 ± 17
18	30 ± 10	22 ± 11	21 ± 3
19	26 ± 2	9 ± 1	16 ± 2
20	46 ± 4	25 ± 12	36 ± 1
21	>500	>500	≥500
22	42 ± 3	33 ± 0	30 ± 2
23	48 ± 1	36 ± 1	32 ± 1
24	16 ± 4	16 ± 11	18 ± 9
25	37 ± 13	41 ± 1	36 ± 11
26	42 ± 3	25 ± 0	27 ± 4
27	20 ± 7	12 ± 2	14 ± 5
28	17 ± 2	11 ± 0	14 ± 1
29	408 ± 131	244 ± 1	200 ± 73
30	42 ± 3	36 ± 3	31 ± 0
31	15 ± 0	8.8 ± 0.7	13 ± 3
32	11 ± 1	9.3 ± 0.5	12 ± 1
33	20 ± 3	11 ± 0	15 ± 1
34	18 ± 6	12 ± 1	16 ± 1
2	≥500	20 ± 2	301 ± 211
7	>500	432 ± 96	≥500
9	46 ± 27	14 ± 6	>28 ± 18

[a] Data are mean values of at least two independent experiments.

Table 3. Anti-HIV-1 Activity of Compounds **3**, **4**, **19**, **27**, and **31** against Mutant HIV-1 Strains in CEM Cell Cultures[a]

	IC_{50} (nM)				
compd	L100I	K103N	Y181C	Y181I	Y188L
3	8 ± 7	29 ± 18	74 ± 18	>1000	690 ± 440
4	9 ± 7	14 ± 6	96 ± 34	>1000	660 ± 480
19	7 ± 2	19 ± 9	78 ± 23	>1000	≥1000
27	1.2 ± 1	40 ± 32	230 ± 190	>1000	>1000
31	2.0 ± 8	36 ± 32	390 ± 120	>1000	>1000
NVP	60 ± 4	2900 ± 1320	11000 ± 4100	>10000	>10000
EFV[b]	22 ± 14	130 ± 180	160 ± 180	12 ± 14	760 ± 630

[a] Data are mean values of two to three independent experiments. [b] The determinations of EFV showed a high degree of variability.

to the tested compounds against the mutant Y181C RT HIV-1 strain and showed a better inhibition profile against the Y181I strain. Against the mutant Y188L RT HIV-1 strain, compounds **3** and **4** were equipotent to EFV. These results were in agreement with the inhibitory effect of the compounds noted for the corresponding mutant RT enzymes (Table 4).

The compounds were potent inhibitors of the RT of HIV-1 WT, and they were always superior to nevirapine (NVP) and often comparable to efavirenz (EFV) (Table 4). Against the RT WT, the sulfonamides **27** and **31** showed inhibitory concentrations comparable to the reference compounds **2**[10] and **9**[16] and 1000 and 200 times superior to NVP and EFV, respectively. Fifteen compounds inhibited in the submicromolar range the mutant K103N RT HIV-1, which is the major mutation emerging in patients treated with EFV, whose viral loads rebound after an initial response to the drug.[7a] Against the K103N RT HIV-1 strain, the most active compounds **3**, **4**, **19**, **23**, **24**, and **31** were notably more active than NVP and EFV, albeit less active than the references **2** and **9**. Both 5-chloro and 5-bromo-IASs were potent inhibitors of the L100I RT mutant, and compound **31** was exceptionally effective against this mutant. We identified compound **31** containing the 2-aminopropane-1-sulfonamide motif as a potent inhibitor of WT and L100I RTs.

To evaluate the influence of the chiral center at the amino acid unit, the racemates **4**, **16**, and **23** were separated by chiral HPLC. Against HIV-1 WT RT, the enantiomers **4a** and **4b**, **16a** and **16b**, and **23a** and **23b** obtained from the corresponding racemic mixtures **4**, **16**, and **23**, respectively, showed negligible differences of activity (Table 5). Against the K103N mutation, the D-enantiomers **4b** and **16b** were about 5 times more potent than the corresponding L-enantiomers **4a** and **16a**, and the enantiomers **23a** and **23b** did not show significant difference of activity.

Compounds **3**, **4**, **13**−**34** were evaluated for their antiviral activity against vesicular stomatitis virus, Coxsackie B4 virus, and respiratory syncytial virus in HeLa cell cultures (Table 6). The IASs showed no activity against vesicular stomatitis virus in HeLa cells. Several compounds (**3**, **4**, **13**, **15**, **16**, **22**, **23**, **26, 28**, **29**, and **30**−**32**) were inhibitors to Coxsackie B4 virus at low micromolar (2−9 μM) concentrations. Seven compounds (**16**, **23**, **24**, **28**, and **31**−**33**) inhibited this viral strain at EC_{50} = 2−5 μM, a concentration that is 25 times lower than required

Table 4. HIV-1 RT Inhibitory Activity of Compounds **3**, **4**, and **13**−**34** against the WT and Mutant Enzymes Carrying Single Amino Acid Substitutions[a]

	IC_{50}[b] (nM)			
compd	WT	L100I	K103N	Y181I
3	26	161	280	1645
13	26	199	782	2211
14	34	206	417	2909
15	25	319	1042	2459
4	23	112	190	2493
16	28	41	509	1810
17	22	209	362	4211
18	62	270	1522	6277
19	27	143	351	5055
20	86	169	420	8566
21	27	204	474	5534
22	34	130	783	7165
23	120	252	288	4137
24	81	296	321	15830
25	33	191	1088	11920
26	359	390	3387	>40000
27	0.4	25	584	>40000
28	2	13	529	>40000
29	61	388	787	>40000
30[c]	nd[d]	nd[d]	nd[d]	nd[d]
31	0.4	4	233	1944
32	3	125	1328	8550
33	28	364	3277	26220
34	36	267	3	>40000
2	0.3	60	3	140
7	10	nd[d]	800	nd[d]
9	3	2	6	29
NVP[e]	400	9000	7000	>20000
EFV[e]	80	nd[d]	>20000	400

[a] Data represent mean values of at least three separate experiments. [b] Compound concentration (IC_{50}, nM) required to inhibit by 50% the RT activity of the indicated strain. [c] Insoluble in the aqueous medium during the experiment. [d] nd: no data. [e] Data from literature.[16]

Table 5. Inhibitory Activity of Racemates **4**, **16**, and **23** and Enantiomers **4a**, **4b**, **16a**, **16b**, **23a**, and **23b** against HIV-1 RT WT and RTs Carrying Single Amino Acid Substitutions[a]

		IC_{50}[b](nM)	
compd	chirality	WT	K103N
4	D, L	20	477
4a	L	26	1005
4b	D	17	253
16	D, L	16	555
16a	L	13	1504
16b	D	16	324
23	D, L	490	1690
23a	L	315	1340
23b	D	147	1417

[a] Data represent mean values of at least three separate experiments. [b] Compound dose (IC_{50}, nM) required to inhibit by 50% the RT activity of the indicated strain.

for ribavirin. The compounds were also evaluated against Coxsackie B4 virus in Vero cell cultures. The antiviral potency was in general less pronounced in Vero than in HeLa cell cultures. Compounds **20**, **23**, **28**, **31**, and **32** showed anti-Coxsackie B4 virus activity in both HeLa and Vero cell cultures. The inhibitory concentrations of compounds **16** and **32** against respiratory syncytial virus were ∼4 μM. The activity against Coxsackie B4 virus seemed to be positively affected by the presence of the bromine atom at position 5 of the indole and an amino acid unit bearing a α- or β-methyl group. It is noted that the antivirally active compounds have a limited selectivity index (ratio MIC/EC_{50}) that is several orders of magnitude lower than the selectivity index for HIV, and it can be questioned whether the observed antiviral activity is due to a direct anti-Coxsackie virus effect or caused by the cytotoxic activity of

Table 6. Cytotoxicity and Antiviral Activity of Compounds **3**, **4**, **13**, **15**−**17**, **20**, **22**−**26**, and **28**−**33** against Vesicular Stomatitis Virus, Coxsackie B4 Virus, and Respiratory Syncytial Virus in HeLa Cell Cultures[a]

	MCC[b] (μM)		EC_{50}[c] (μM), virus			
					Coxsackie B4	
compd	HeLa	Vero	vesicular stomatitis	respiratory syncytial	HeLa	Vero
3	100	>100	>100	>100	≥20	>100
13	100	100	>20	20	4	>20
14	>100	>100	>100	>100	>100	>100
15	100	>100	>20	16	9	>20
4	100	100	>100	>100	6	>20
16	20	20	>4	4	2	>4
17	>100	>100	>100	>100	≥20	>100
18	20	100	>4	>4	>4	20
19	20	100	>4	>4	>4	12
20	≥20	100	>20	≥20	11	16
21	>100	>100	>100	>100	>100	>100
22	100	100	>20	16	9	>20
23	≥20	100	>20	>20	4.5	16
24	≥20	≥20	>20	>20	≥20	>20
25	100	100	>20	>20	≥20	>20
26	100	100	>20	20	9	>20
27	20	100	>4	>4	>4	9
28	20	≥20	>4	>4	≥4	12
29	≥100	100	≥100	20	≥20	>20
30	100	100	>20	20	7	>20
31	100	≥20	>20	>20	3	12
32	20	≥20	>4	≥4	3	16
33	≥20	≥20	≥20	>20	≥20	≥20
34	20	100	>4	>4	>4	12
2	>100	>100	>100	>100	>100	>100
7	>100	>100	>100	>100	>100	>100
9	>100	>100	>100	>100	27	100
(*S*)-DHPA[d]	>250	>250	>250	>250	>250	>250
ribavirin	>250	>250	22	10	50	≥250

[a] Data are mean values of two independent experiments performed in triplicate. [b] MCC: minimum cytotoxic concentration (μM) required to cause microscopically detectable alteration of normal cell morphology. [c] EC_{50}: effective dose (μM) required to reduce virus-induced cytopathogenicity by 50%. [d] Acyclonucleoside (*S*)-9-(2,3-dihydroxypropyl)adenine.

the compounds. Several substituted benzimidazole-based structures have been identified in the past to be endowed with anti-Coxsackie virus activity in cell culture (i.e., MRL-1237, TBZE-029, enviradene, and enviroxime) (for an overview see ref 24). One of these compounds (e.g., TBZE-029) was, like the present structures, initially discovered as an NNRTI active against HIV-1. TBZE-029 is active at an EC_{50} value of 1.2 μg/mL, a concentration close to the EC_{50} of the most inhibitory indole derivatives (i.e., **13**, **16**, **31**, **32**). The TBZE-029 compound affects viral RNA synthesis by targeting the nonstructural protein 2C. Therefore, it would be most interesting to investigate the most active indole derivatives against this virus-specific protein to reveal a potential viral target for this novel class of compounds. Therefore, we believe that this study allowed disclosure of a new lead class of Coxsackie B4 virus and respiratory syncytial virus inhibitors. Coxsackie B virus may be the causative agent of viral polymyositis and rhabdomyolysis, a pathological condition that may be associated with immunodepressed conditions.[25] Such agents may provide a starting point for the development of new effective inhibitors of both HIV-1 RT and Coxsackie B4 virus.

The new IASs were also evaluated against a wide panel of other viral strains including parainfluenza virus 3, reovirus 1, sindbis virus, and Punta Toro virus in Vero cell cultures, herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), vaccinia virus, herpes simplex virus-1 TK^- (KOS ACV) in HEL cell cultures, influenza A virus (H1N1 and H3N2 subtypes) and

Table 7. The rmsd Values for Co-Crystallized Inhibitors by PLANTS, FlexX, and MOE

	rmsd		
PDB code	PLANTS 1.0	FlexX 3.0	MOE 2007/09
1vrt	0.23	1.18	1.53
2rf2	0.80	1.59	1.20
2zd1	0.40	2.01	5
1fk0	0.85	1.28	1.47
1s1t	0.26	1.04	4.60
2opq	0.48	0.90	2.10

influenza B virus in MDCK cell cultures, and feline corona virus (FIPV) and feline herpes virus in CRFK cell cultures. No marked inhibitory activity was detected (Tables 1S−4S, Supporting Information).

Molecular Modeling Studies

The binding mode of the IASs was extensively studied by means of docking experiments into the non-nucleoside binding site (NNBS) of the RT. The latest available **12a**/RT cocrystal structure (PDB code 2rf2) solved by Merck[18] was used as a new starting point for docking studies because of the high structural correlation between the cocrystallized inhibitor **12a** and IASs. In our previous studies,[13a,14] we used Autodock 3.0[26] software to generate IASs docking poses. However, Autodock 3.0 software consistently yielded two different clusters of poses, and the crystallographic conformation was often the lower scoring pose. To obtain more accurate docking results and also to validate the generated poses with crystallographic information, we decided to test the docking softwares PLANTS,[27] FlexX,[28] and MOE[29] docking algorithm.

We selected seven PDB structures of inhibitors cocrystallized with either HIV-1 WT RT or mutated RTs (2rf2,[18] 1vrt,[30] 2zd1,[31] 1fk0,[32] 1s1t,[33] 2opq,[34] and 1jkh[35]). The best scoring poses predicted by the different algorithms were compared with the crystallographic conformations obtained from each complex in terms of the rmsd. Our experiments showed that only PLANTS was able to correctly dock each molecule with a rmsd of <1 (Table 7). The other docking software always achieve bigger rmsd values than PLANTS on the whole test set. Therefore, we decided to use PLANTS to dock the new IAS series. Interestingly, the best scoring conformation obtained by PLANTS clustering algorithm was always superimposed to Merck's crystal (Figure 1S, Supporting Information).

In order to investigate the possible binding mode of the new IASs, we selected the highly potent compound **19** (IC_{50} = 26 nM; EC_{50} = 0.70 nM) for docking studies into the NNBS of the HIV-1 WT RT. From docking, feature interactions of **19** into the NNBS were (i) the H-bond between the indole NH and the carbonyl oxygen of Lys101 (in stick in the Figure 1), (ii) the interactions of the *N*-(3-amino-3-oxopropyl)carboxamide moiety with the residues Lys101 and Glu138B at the bottom of the binding pocket (they seemed to be particularly important for the anti-HIV activity), (iii) the sulfone group allowing **19** to assume a butterfly-like conformation that is common to many other NNRTIs, (iv) the chlorine atom at position 5 of the indole occupying a hydrophobic pocket formed by Val106 and Leu234 and forming steric rather than electrostatic interaction, and (v) the 3,5-dimethylphenyl moiety forming hydrophobic interactions with an aromatic cleft formed by the side chains of Tyr181, Tyr188, Trp229, and Pro95 residues (Figure 1).

Worthy of note, a superimposition of PLANTS docked conformations of **19** and the highly active derivative **9**[16] (IC_{50} = 3 nM; EC_{50} = 1.0 nM) showed different hydrophobic interactions of the 3,5-dimethylphenyl group, for which a

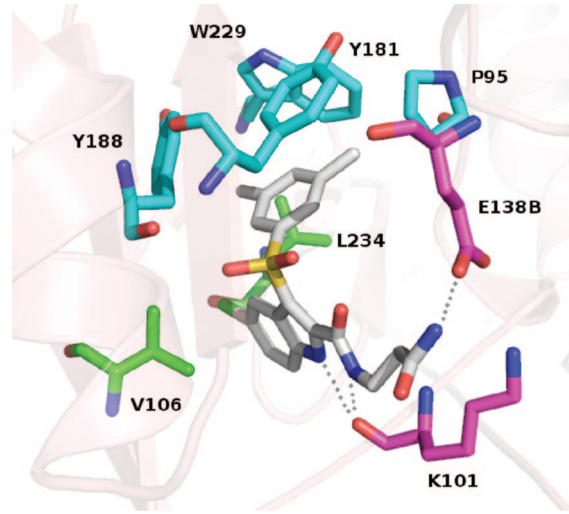


Figure 1. Binding conformation of **19** in the NNBS of HIV-1 WT RT.

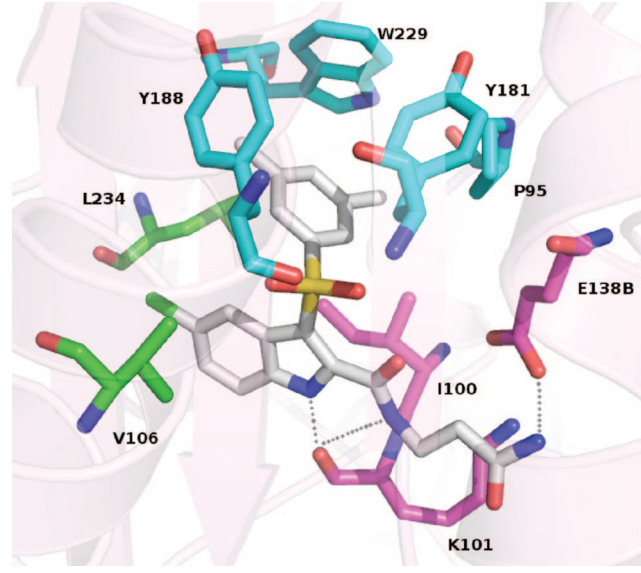


Figure 2. Binding conformation of **19** in the NNBS of HIV-1 L100I RT.

stacking interaction with the Tyr181 aromatic side chain was observed (Figure 2S, Supporting Information).

The IASs binding mode was also extensively studied in mutated RTs. The results obtained by docking the IASs analogues with PLANTS in L100I mutated RT (PDB codes 1s1t, 2opq) showed that the mutation of leucine to isoleucine does not affect the binding mode and were consistent with the interactions reported for the WT strain. Furthermore, all compounds show a similar binding mode, consistent with the low EC_{50} values (Figure 2).

We have also repeated the docking simulations on the K103N and Y181I mutated RTs, and the results obtained did not show significant differences with the IASs binding mode observed with the wild type. However, the lower antiviral activity of the IASs against these mutants could be the consequence of the reduced interaction between **19** and the mutated residues. In particular, for the Y181I RT HIV-1 strain, the limited anti-HIV efficiency could be caused by the loss of the favorable protein−ligand $\pi-\pi$ interactions, while in the mutant K103N RT, the loss of the favorable hydrophobic interaction between the ligand and the side chain of the lysine 103 might be

responsible for the reduced activity. In both cases the geometry of the H-bonds between the carbonyl oxygen of the E138 with unsubstituted amidic nitrogen of **19** is worst than that in the wild type (Figure 3S, Supporting Information). Molecular modeling was also unable to explain the differences of activity of the enantiomers against the K103N mutant.

Conclusions

Compounds **3**, **4**, and **13**−**34** proved to be highly potent against HIV-1 replication in human T-lymphocyte (CEM) cells and showed inhibitory potencies in low/subnanomolar range that were comparable with those of the previously reported lead compounds **2**,[10] **7**,[14] and **9**.[16] Against HIV-1 WT, the inhibitory activity seemed only marginally affected by the substituent introduced on the indole nucleus. In general, none of the IASs proved to be markedly cytostatic. Against the L100I and K103N RT HIV-1 strains, **3**, **4**, **19**, **27**, and **31** were always superior to EFV and had similar activity to that reported for MK-4965[22] and TMC-120.[23] These IASs were also equipotent to EFV against the mutant Y181C RT HIV-1 strain. The D-enantiomers **4b** and **16b** were about 5 times more potent than the corresponding L-enantiomers **4a** and **16a** against RT. Several compounds were inhibitory to Coxsackie B4 virus at EC_{50} = 3−5 μM but were much less selective in their anti-Coxsackie virus activity than observed for HIV. In view that for picornaviruses, no single antiviral drug has ever been approved,[36] these agents may serve as basis for the development of drugs endowed with both anti-HIV-1 RT and Coxsackie B4 virus inhibitory activities.

In order to investigate the possible binding mode of the new IASs, we selected the highly potent compound **19**. Superimposition of PLANTS docked conformations of **19** and the highly active derivative **9** revealed different hydrophobic interactions of the 3,5-dimethylphenyl group, for which a stacking interaction with Tyr181 aromatic side chain was observed. The IAS binding mode was also extensively studied in mutated RTs. In L100I mutated RT, the mutation of leucine to isoleucine does not affect the binding mode, and it was consistent with the interactions reported for the WT strain. The lower antiviral activity against the mutant K103N and Y181I RT HIV-1 strains could be the consequence of the reduced interaction between **19** and the mutated residues. For the Y181I RT HIV-1 strain, the limited anti-HIV efficiency could also be caused by the loss of the favorable protein−ligand $\pi-\pi$ interactions, while in the K103N mutant, the loss of the hydrophobic interaction between the ligand and the side chain of the lysine 103 might be responsible for the reduced activity.

Experimental Section

Chemistry. Microwave (MW)-assisted reactions were performed on Discover S-Class (CEM), setting temperature, irradiation power, maximum pressure (Pmax), PowerMAX (*in situ* cooling during the MW irradiation), ramp and hold times, and open and closed vessel modes as indicated. Melting points (mp) were determined on a Büchi 510 apparatus and are uncorrected. Infrared spectra (IR) were obtained using Perkin-Elmer 1310 and SpectrumOne spectrophotometers. Band position and absorption ranges are given in cm^{-1}. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Bruker AM-200 (200 MHz) and Bruker Avance 400 MHz FT spectrometers in the indicated solvent. Chemical shifts are expressed in δ units (ppm) from tetramethylsilane. Column chromatographies were packed with alumina (Merck, 70−230 mesh) and silica gel (Merck, 70−230 mesh). Aluminum oxide TLC cards (Fluka, aluminum oxide precoated aluminum cards with fluorescent indicator at 254 nm) and silica gel TLC cards (Fluka, silica gel precoated aluminum cards with fluorescent indicator at 254 nm) were used for thin layer chromatography (TLC). Developed plates were visualized with a Spectroline ENF 260C/F UV apparatus. Organic solutions were dried over anhydrous sodium sulfate. Evaporation of the solvents was carried out on a Büchi rotavapor R-210 equipped with a Büchi V-850 vacuum controller and Büchi V-700 (∼5 mbar) and V-710 vacuum (∼2 mbar) pumps. Elemental analyses were found to be within ±0.4% of the theoretical values. Purity of tested compounds was >95%. Ethyl 5-chloroindole-2-carboxylate (**35**) was purchased from Sigma-Aldrich. Ethyl 5-nitroindole-2-carboxylate (**37**) was purchased from Acros Organics. Ethyl 5-bromoindole-2-carboxylate (**36**)[10] and ethyl 5-chloro-4-fluoroindole-2-carboxylate (**38**)[19] were prepared as previously reported.

***N*-(2-Amino-2-oxoethyl)-5-chloro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxamide (3). 3** was prepared as previously reported.[11]

***N*-(1-Amino-1-oxopropan-2-yl)-5-chloro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxamide (4). 4** was prepared as previously reported.[11]

General Procedure for the Synthesis of Derivatives 13−26. Example. *N*-(3-Amino-3-oxopropyl)-5-chloro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxamide (19). A sample of 28% ammonium hydroxide (28 mL) was added to a mixture of **59** (0.48 g, 0.001 mol) in ethanol (48 mL). Reaction mixture was stirred at 60 °C for 3 h. After the mixture was cooled, water was added and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried, and evaporated to furnish a residue which was purified by silica gel column chromatography (ethyl acetate as eluent) to give **19**. Yield 40%, mp >290 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 2.30 (s, 6H), 2.42 (t, J = 7.1 Hz, 2H), 3.52 (t, J = 6.4 Hz, 2H), 6.90 (broad s, 1H, disappeared on treatment with D_2O), 7.24 (s, 1H), 7.32 (dd, J = 8.7 and 1.9 Hz, 1H), 7.41 (broad s, 1H, disappeared on treatment with D_2O), 7.51 (d, J = 8.7 Hz, 1H), 7.62 (s, 2H), 7.93 (d, J = 1.7 Hz, 1H), 9.03 (broad s, 1H, disappeared with treatment with D_2O), 12.92 ppm (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1637, 3222 cm^{-1}. Anal. ($C_{20}H_{20}ClN_3O_4S$ (433.91)) C, H, N, Cl, S.

***N*-(2-Amino-2-oxoethyl)-5-bromo-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxamide (13). 13** was synthesized as for **19** but using **52**. Yield 57%, mp 274−276 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 2.32 (s, 6H), 4.01 (s, 2H), 7.27 (s, 1H), 7.33 (broad s, 1H, disappeared on treatment with D_2O), 7.47−7.49 (m, 2H), 7.52 (d, J = 8.2 Hz, 1H), 7.73 (s, 2H), 8.17 (s, 1H), 9.39 (broad s, 1H, disappeared on treatment with D_2O), 13.08 ppm (s, 1H, disappeared on treatment with D_2O). IR: ν 1639, 1693, 3243, 3445 cm^{-1}. Anal. ($C_{19}H_{18}BrN_3O_4S$ (464.33)) C, H, Br, N, S.

***N*-(2-Amino-2-oxoethyl)-3-[(3,5-dimethylphenyl)sulfonyl]-5-nitro-1*H*-indole-2-carboxamide (14). 14** was synthesized as for **19** but using **53**. Yield 42%, mp 259−261 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 2.29 (s, 6H), 4.00 (s, 2H), 7.26 (s, 1H), 7.31 (s, 1H, disappeared on treatment with D_2O), 7.46 (s, 1H, disappeared on treatment with D_2O), 7.70−7.71 (m, 3H), 8.16 (d, J = 8,3 Hz, 1H), 8.89 (s, 1H), 9.33 (s, 1H, disappeared on treatment with D_2O), 13.42 ppm (s, 1H, disappeared on treatment with D_2O). IR: ν 1681, 3291 cm^{-1}. Anal. ($C_{19}H_{18}N_4O_6S$ (430.43)) C, H, N, S.

***N*-(2-Amino-2-oxoethyl)-5-chloro-3-[(3,5-dimethylphenyl)sulfonyl]-4-fluoro-1*H*-indole-2-carboxamide (15). 15** was synthesized as for **19** but using **54**. Yield 95%, mp 255−257 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 2.32 (m, 6H), 3.92 (s, 2H), 7.26 (s, 1H), 7.28−7.42 (m, 4H), 7.66 (s, 2H), 9.47 (broad s, 1H, disappeared on treatment with D_2O), 13.27 (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1662, 1703, 3280 cm^{-1}. Anal. ($C_{19}H_{17}ClFN_3O_4S$ (437.87)) C, H, Cl, F, N, S.

***N*-(1-Amino-1-oxopropan-2-yl)-5-bromo-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxamide (16). 16** was synthesized as for **19** but using **56**. Yield 43%, mp 248−251 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.37 (d, J = 7.1 Hz, 3H), 2.29 (s, 6H), 4.46−4.54 (qn, J = 7.1 Hz, 1H), 7.26 (s, 2H), 7.46 (dd, J = 8.7 and 1.7 Hz, 1H), 7.49−7.52 (m, 2H), 7.68 (s, 2H), 8.15 (d, J = 1.7 Hz, 1H), 9.32 (s, 1H, disappeared on treatment with D_2O), 13.05 ppm (s, 1H, disappeared on treatment with D_2O). IR: ν 1662, 1672, 3254, 3465 cm^{-1}. Anal. ($C_{20}H_{20}BrN_3O_4S$ (478.36)) C, H, Br, N, S.

***N*-(1-Amino-1-oxopropan-2-yl)-3-[(3,5-dimethylphenyl)sulfonyl]-5-nitro-1*H*-indole-2-carboxamide (17). 17** was synthesized as for **19** but using **57**. Yield 99%, mp 245−250 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.37 (d, J = 7.0 Hz, 3H), 2.29 (s, 6H), 4.46−4.54 (qn, J = 7.1 Hz, 1H), 7.27 (s, 1H), 7.50 (s, 1H, disappeared on treatment with D_2O), 7.66 (s, 1H, disappeared on treatment with D_2O), 7.68 (s, 2H), 7.71 (d, J = 9.1 Hz, 1H), 8.17 (dd, J = 9.0 and 2.0 Hz, 1H), 8.90 (d, J = 1.9 Hz, 1H), 9.29 (s, 1H, disappeared on treatment with D_2O), 12.95 ppm (s, 1H, disappeared on treatment with D_2O). IR: ν 1673, 3275, 3409 cm^{-1}. Anal. ($C_{20}H_{20}N_4O_6S$ (444.46)) C, H, N, S.

***N*-(1-Amino-1-oxopropan-2-yl)-5-chloro-3-[(3,5-dimethylphenyl)sulfonyl]-4-fluoro-1*H*-indole-2-carboxamide (18). 18** was synthesized as for **19** but using **58**. Yield 79%, mp 249−250 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.39 (d, J = 7.1 Hz, 3H), 2.32 (m, 6H), 4.44 (q, J = 7.2 Hz, 1H), 7.26 (s, 1H), 7.29 (broad s, 1H, disappeared on treatment with D_2O), 7.35 (broad s, 1H, disappeared on treatment with D_2O), 7.36−7.40 (m, 2H), 7.68 (s, 2H), 9.37 (broad s, 1H, disappeared on treatment with D_2O), 13.26 (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1655, 1698, 3245 cm^{-1}. Anal. ($C_{20}H_{19}ClFN_3O_4S$ (451.90)) C, H, Cl, F, N, S.

***N*-(3-Amino-3-oxopropyl)-5-bromo-3-[(3,5-dimethylphenyl)-sulfonyl]-1*H*-indole-2-carboxamide (20). 20** was synthesized as for **19** but using **60**. Yield 99%, mp 258−260 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 2.32 (s, 6H), 2.47 (t, J = 7.1 Hz, 2H), 3.58 (t, J = 7.1 Hz, 2H), 6.98 (broad s, 1H, disappeared on treatment with D_2O), 7.26 (s, 1H), 7.44−7.49 (m, 3H), 7.64 (s, 2H), 8.10 (s, 1H), 9.03 (s, 1H, disappeared on treatment with D_2O), 13.07 ppm (s, 1H, disappeared on treatment with D_2O). IR: ν 1635, 1665, 3212, 3273, 3473 cm^{-1}. Anal. ($C_{20}H_{20}BrN_3O_4S$ (478.36)) C, H, Br, N, S.

***N*-(3-Amino-3-oxopropyl)-3-[(3,5-dimethylphenyl)sulfonyl]-5-nitro-1*H*-indole-2-carboxamide (21). 21** was synthesized as for **19** but using **61**. Yield 99%, mp 260 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 2.29 (s, 6H), 2.44 (t, J = 7.1 Hz, 2H), 3.54 (q, J = 6.6 Hz, 2H), 6.69 (broad s, 1H, disappeared on treatment with D_2O), 7.25 (s, 1H), 7.45 (broad s, 1H, disappeared on treatment with D_2O), 7.64 (s, 2H), 7.68 (d, J = 9.1 Hz, 1H), 8.16 (dd, J = 9.1 and 2.3 Hz, 1H), 8.84 (d, J = 2.2 Hz, 1H), 9.06 (broad s, 1H, disappeared on treatment with D_2O), 13.44 ppm (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1639, 11656, 3190, 3288, 3436 cm^{-1}. Anal. ($C_{20}H_{20}N_4O_6S$ (444.46)) C, H, N, S.

***N*-(3-Amino-3-oxopropyl)-5-chloro-3-[(3,5-dimethylphenyl)-sulfonyl]-4-fluoro-1*H*-indole-2-carboxamide (22). 22** was synthesized as for **19** but using **62**. Yield 94%, mp 240−242 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 2.32 (m, 6H), 2.43 (t, J = 7.3 Hz, 2H), 3.49 (t, J = 7.1 Hz, 2H), 6.88 (broad s, 1H, disappeared on treatment with D_2O), 7.25 (s, 1H), 7.31−7.39 (m, 3H), 7.65 (s, 2H), 9.05 (broad s, 1H, disappeared on treatment with D_2O), 13.13 (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1649, 1704, 3267, 3496 cm^{-1}. Anal. ($C_{20}H_{19}ClFN_3O_4S$ (451.90)) C, H, Cl, F, N, S.

***N*-(4-Amino-4-oxobutan-3-yl)-5-chloro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxamide (23). 23** was synthesized as for **19** but starting from **63**. Yield 43%, mp 237−240 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.26 (d, J = 6.6 Hz, 3H), 2.32 (s, 6H), 2.44 (dd, J = 15.7 and 7.9 Hz, 1H), 2.67 (dd, J = 15.8 and 5.0 Hz, 1H), 4.33−4.40 (m, 1H), 7.26 (s, 1H), 7.31 (broad s, 1H disappeared on treatment with D_2O), 7.34 (d, J = 8.7 Hz, 1H), 7.51 (broad s, 1H, disappeared on treatment with D_2O), 7.54 (d, J = 8.6 Hz, 1H), 7.64 (s, 2H), 7.95 (s, 1H), 9.02 (broad s, 1H, disappeared with treatment with D_2O), 12.72 ppm (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1647, 1711, 3204, cm^{-1}. Anal. ($C_{21}H_{22}ClN_3O_4S$ (447.94)) C, H, N, Cl, S.

***N*-(4-Amino-4-oxobutan-3-yl)-5-bromo-3-(3,5-dimethylphenylsulfonyl)-1*H*-indole-2-carboxamide (24). 24** was synthesized as for **19** but using **64**. Yield 8%, mp 225−228 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.22 (d, J = 6.6 Hz, 3H), 2.29−2.32 (m, 7H), 2.45−2.49 (m, 1H), 4.27−4.32 (m, 1H), 6.90 (broad s, 1H, disappeared on treatment with D_2O), 7.26 (s, 1H), 7.41−7.46 (m, 2H), 7.48 (d, J = 8.7 Hz, 1H), 7.62 (s, 2H), 8.08 (d, J = 1.5 Hz, 1H), 8.9 (broad s, 1H, disappeared on treatment with D_2O), 13.00 ppm (s, 1H, disappeared on treatment with D_2O). IR: ν 1610, 1644, 3299, 3425 cm^{-1}. Anal. ($C_{21}H_{22}BrN_3O_4S$ (492.39)) C, H, Br, N, S.

***N*-(4-Amino-4-oxobutan-3-yl)-3-[(3,5-dimethylphenyl)sulfonyl]-5-nitro-1*H*-indole-2-carboxamide (25).** A mixture of **65** (0.30 g, 0.0006 mol) and 28% ammonium hydroxide (2 mL) was placed into the MW cavity (closed vessel, Pmax = 250 PSI). MW irradiation of 150 W was used, the temperature being ramped from 25 °C to 130 °C, while stirring. Once 130 °C was reached, taking about 3 min, the reaction mixture was held at this temperature for 10 min. The reaction mixture was neutralized with 1 N HCl and extracted with ethyl acetate. The organic layer was washed with brine, dried, and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate−ethanol 9:1 as eluent) to give **25**. Yield 65%, mp 264−268 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.25 (d, J = 6.6 Hz, 3H), 2.29−234 (m, 8H), 4.32−4.44 (m, 1H), 6.94 (broad s, 1H, disappeared on treatment with D_2O), 7.29 (s, 1H), 7.47 (broad s, 1H, disappeared on treatment with D_2O), 7.66 (s, 2H), 7.71 (d, J = 9.1 Hz, 1H), 8.19 (dd, J = 9.0 and 2.0 Hz, 1H), 8.85 (d, J = 1.4 Hz, 1H), 9.00 (broad s, 1H, disappeared with treatment with D_2O), 13.46 ppm (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1639, 1657, 3232, 3437 cm^{-1}. Anal. ($C_{21}H_{22}N_4O_6S$ (458.49)) C, H, N, S.

***N*-(4-Amino-4-oxobutan-2-yl)-5-chloro-3-[(3,5-dimethylphenyl)sulfonyl]-4-fluoro-1*H*-indole-2-carboxamide (26). 26** was synthesized as for **19** but from **66**. Yield 22%, mp 217−219 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.19 (d, J = 6.7 Hz, 3H), 2.17−2.20 (m, 1H), 2.31 (s, 6H), 2.44−2.49 (m, 1H), 4.29−4.37 (m, 1H), 6.89 (broad s, 1H, disappeared on treatment with D_2O), 7.24 (s, 1H), 7.31−7.38 (m, 2H), 7.43 (broad s, 1H, disappeared on treatment with D_2O), 7.66 (s, 2H), 8.96 (broad s, 1H, disappeared on treatment with D_2O), 13.22 (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1625, 1691, 3194 cm^{-1}. Anal. ($C_{21}H_{21}ClFN_3O_4S$ (465.93)) C, H, Cl, F, N, S.

General Procedure for the Synthesis of Derivatives 26−34, 52−54, and 56−66. Example. Methyl 3-[*N*-[5-chloro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxamido]]propanoate (59). A mixture of **47**[11] (0.70 g, 0.0019 mol), methyl β-alanine hydrochloride (0.52 g, 0.0037 mol), triethylamine (0.57 g, 0.79 mL, 0.0056 mol), and BOP (0.80 g, 0.0019 mol) in DMF (10 mL) was stirred at room temperature overnight. Water was added, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried, and evaporated to dryness. The residue was purified by silica gel column chromatography (ethyl acetate as eluent) to give **59**. Yield 81%, mp 197−200 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 2.29 (s, 6H), 2.65 (t, J = 6.7 Hz, 2H), 3.58 (t, J = 6.3 Hz, 2H), 3.61 (s, 3H), 7.24 (s, 1H), 7.31 (dd, J = 8.8 and 1.9 Hz, 1H), 7.51 (d, J = 8.8 Hz, 1H), 7.60 (s, 2H), 7.90 (d, J = 2.0 Hz, 1H), 9.08 (broad s, 1H, disappeared with treatment with D_2O), 13.00 ppm (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1738, 3204 cm^{-1}. Anal. ($C_{21}H_{21}ClN_2O_5S$ (448.92)) C, H, N, Cl, S.

***N*-(4-Amino-4-oxobutan-2-yl)-5-chloro-3-[(3,5-dimethylphenyl)sulfonyl]-4-fluoro-1*H*-indole-2-carboxamide (26). 26** was synthesized as for **19** but from **66**. Yield 22%, mp 217−219 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.19 (d, J = 6.7 Hz, 3H), 2.17−2.20 (m, 1H), 2.31 (s, 6H), 2.44−2.49 (m, 1H), 4.29−4.37 (m, 1H), 6.89 (broad s, 1H, disappeared on treatment with D_2O), 7.24 (s, 1H), 7.31−7.38 (m, 2H), 7.43 (broad s, 1H, disappeared on treatment with D_2O), 7.66 (s, 2H), 8.96 (broad s, 1H, disappeared on treatment with D_2O), 13.22 (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1625, 1691, 3194 cm^{-1}. Anal. ($C_{21}H_{21}ClFN_3O_4S$ (465.93)) C, H, Cl, F, N, S.

5-Chloro-3-[(3,5-dimethylphenyl)sulfonyl]-*N*-(2-sulfamoylethyl)-1*H*-indole-2-carboxamide (27). 27 was synthesized as **59** using 2-aminoethanesulfonamide hydrochloride.[20] Yield 21%, mp 245−247 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 2.32 (s, 6H), 3.27−3.35 (m, 2H), 3.72−3.77 (m, 2H), 7.05 (broad s, 2H, disappeared on treatment with D_2O), 7.26 (s, 1H), 7.35 (dd, J = 8.8 and 2.1 Hz, 1H), 7.55 (d, J = 8.8 Hz, 1H), 7.67 (s, 2H), 7.92 (d, J = 2.1 Hz, 1H), 9.19 (broad s, 1H, disappeared with treatment with D_2O), 13.05

ppm (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1641, 3228 cm^{-1}. Anal. ($C_{19}H_{20}ClN_3O_5S_2$ (469.96)) C, H, N, Cl, S.

5-Bromo-3-[(3,5-dimethylphenyl)sulfonyl]-*N*-(2-sulfamoylethyl)-1*H*-indole-2-carboxamide (28). 28 was synthesized as for **59** but using **48** and 2-aminoethanesulfonamide hydrochloride.[20] Yield 32%, mp 260 °C (from ethanol). ^1H NMR (DMSO-d_6): δ 2.32 (s, 6H), 3.31 (t, J = 7.5 Hz, 2H), 3.73 (t, J = 6.8 Hz, 2H), 7.06 (broad s, 2H, disappeared on treatment with D_2O), 7.26 (s, 1H), 7.45 (dd, J = 8.7 and 1.7 Hz, 1H), 7.50 (d, J = 8.7 Hz, 1H), 7.67 (s, 2H), 8.06 (d, J = 1.7 Hz, 1H), 9.19 (broad s, 1H, disappeared with treatment with D_2O), 13.08 ppm (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1646, 3226 cm^{-1}. Anal. ($C_{19}H_{20}BrN_3O_5S_2$ (514.41)) C, H, N, Br, S.

3-[(3,5-Dimethylphenyl)sulfonyl]-5-nitro-*N*-(2-sulfamoylethyl)-1*H*-indole-2-carboxamide (29). 29 was synthesized as for **59** but using **49** and 2-aminoethanesulfonamide hydrochloride.[20] Yield 14%, mp 265−268 °C (from ethanol). ^1H NMR (DMSO-d_6): δ 1.06 (t, J = 7.0 Hz, 2H), 2.32 (s, 6H), 3.72−3.76 (m, 2H), 7.06 (broad s, 2H, disappeared on treatment with D_2O), 7.28 (s, 1H), 7.68 (s, 2H), 7.73 (d, J = 9.1 Hz, 1H), 8.19 (dd, J = 9.1 and 2.2 Hz, 1H), 8.82 (d, J = 1.8 Hz, 1H), 9.24 (broad s, 1H, disappeared on treatment with D_2O), 13.48 ppm (broad s, 1H, disappeared on treatment with D_2O). IR: ν 3205, 1637 cm^{-1}. Anal. ($C_{19}H_{20}N_3O_5S_2$ (514.41)) C, H, N, S.

5-Chloro-3-[(3,5-dimethylphenyl)sulfonyl]-4-fluoro-*N*-(2-sulfamoylethyl)-1*H*-indole-2-carboxamide (30). 30 was synthesized as for **59** but using **50** and 2-aminoethanesulfonamide hydrochloride.[20] Yield 28%, mp 238−240 °C (from ethanol). ^1H NMR (DMSO-d_6): δ 2.32 (s, 6H), 3.30 (t, J = 7.8 Hz, 2H), 3.69 (t, J = 6.8 Hz, 2H), 7.03 (broad s, 2H, disappeared on treatment with D_2O), 7.26 (s, 1H), 7.33−7.41 (m, 2H), 7.65 (s, 2H), 9.25 (broad s, 1H, disappeared with treatment with D_2O), 13.22 ppm (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1646, 3242 cm^{-1}. Anal. ($C_{19}H_{19}ClFN_3O_5S_2$ (487.95)) C, H, N, Cl, F, S.

5-Chloro-3-[(3,5-dimethylphenyl)sulfonyl]-*N*-(1-sulfamoylpropan-2-yl)-1*H*-indole-2-carboxamide (31). 31 was synthesized as for **59** but using **67**. Yield 12%, mp 212−214 °C (from ethanol). ^1H NMR (DMSO-d_6): δ 1.42 (d, J = 6.6 Hz, 3H), 2.32 (s, 6H), 3.19 (dd, J = 13.9 and 8.3 Hz, 1H), 3.42 (dd, J = 13.8 and 4.4 Hz, 1H), 4.48−4.51 (m, 1H), 7.02 (broad s, 2H, disappeared on treatment with D_2O), 7.26 (s, 1H), 7.34 (dd, J = 8.6 and 1.8 Hz, 1H), 7.54 (d, J = 8.8 Hz, 1H), 7.66 (s, 2H), 7.91 (d, J = 1.8 Hz, 1H), 9.09 (broad s, 1H, disappeared with treatment with D_2O), 12.98 ppm (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1643, 3255 cm^{-1}. Anal. ($C_{20}H_{22}ClN_3O_5S_2$ (483.99)) C, H, N, Cl, S.

5-Bromo-3-[(3,5-dimethylphenyl)sulfonyl]-*N*-(1-sulfamoylpropan-3-yl)-1*H*-indole-2-carboxamide (32). 32 was synthesized as for **59** but using **48** and **67**. Yield 8%, mp 155−159 °C (from ethanol). ^1H NMR (DMSO-d_6): δ 1.41 (d, J = 6.7 Hz, 3H), 2.32 (s, 6H), 3.17−3.23 (m, 1H), 3.38−3.43 (m, 1H), 4.46−4.53 (m, 1H), 7.03 (broad s, 2H, disappeared on treatment with D_2O), 7.27 (s, 1H), 7.46 (dd, J = 8.7 and 1.8 Hz, 1H), 7.50 (d, J = 8.7 Hz, 1H), 7.66 (s, 2H), 8.06 (d, J = 1.8 Hz, 1H), 9.11 (broad s, 1H, disappeared with treatment with D_2O), 13.00 ppm (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1637, 3257 cm^{-1}. Anal. ($C_{20}H_{22}BrN_3O_5S_2$ (528.44)) C, H, N, Br, S.

3-[(3,5-Dimethylphenyl)sulfonyl]-5-nitro-*N*-(1-sulfamoylpropan-2-yl)-1*H*-indole-2-carboxamide (33). 33 was synthesized as for **59** but using **49** and **67**. Yield 21%, mp 226−229 °C (from ethanol). ^1H NMR (DMSO-d_6): δ 1.42 (d, J = 6.7 Hz, 3H), 2.32 (s, 6H), 3.16−3.22 (m, 1H), 3.37−3.42 (m, 1H), 4.47−4.52 (m, 1H), 7.05 (broad s, 2H, disappeared on treatment with D_2O), 7.29 (s, 1H), 7.67 (s, 2H), 7.73 (d, J = 9.0 Hz, 1H), 8.19 (dd, J = 9.0 and 2.2 Hz, 1H), 8.81 (d, J = 1.9 Hz, 1H), 9.15 (broad s, 1H, disappeared with treatment with D_2O), 13.44 ppm (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1655, 3239 cm^{-1}. Anal. ($C_{20}H_{22}N_4O_7S_2$ (494.54) C, H, N, S.

5-Chloro-3-[(3,5-dimethylphenyl)sulfonyl]-4-fluoro-*N*-(1-sulfamoylpropan-2-yl)-1*H*-indole-2-carboxamide (34). 34 was synthesized as for **59** but using **50** and **67**. Yield 20%, mp 193−197 °C (from ethanol). ^1H NMR (DMSO-d_6): δ 1.40 (d, J = 6.5 Hz, 3H), 2.31 (s, 6H), 3.13 (dd, J = 13.5 and 9.0 Hz, 1H), 3.45 (dd, J = 13.5 and 3.6 Hz, 1H), 4.43−4.50 (m, 1H), 7.01 (broad s, 2H, disappeared on treatment with D_2O), 7.25 (s, 1H), 7.31−7.41 (m, 2H), 7.65 (s, 2H), 9.16 (broad s, 1H, disappeared with treatment with D_2O), 13.19 ppm (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1629, 3293 cm^{-1}. Anal. ($C_{20}H_{21}ClFN_3O_5S_2$ (501.98)) C, H, N, Cl, F, S.

Ethyl 2-[*N*-[5-Chloro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxamido]]acetate (51). 51 was prepared as previously reported.[11]

Ethyl 2-[*N*-[5-Bromo-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxamido]]acetate (52). 52 was synthesized as for **59** but using **48** and ethyl glycine hydrochloride. Yield 48%, mp 199−201 °C (from ethanol). ^1H NMR (DMSO-d_6): δ 1.25 (t, J = 7.1 Hz, 3H), 2.31 (s, 6H), 4.17−4.26 (m, 4H), 7.27 (s, 1H), 7.47−7.53 (m, 2H), 7.70 (s, 2H), 8.18 (d, J = 1.7 Hz, 1H), 9.48 (broad s, 1H, disappeared on treatment with D_2O), 13.13 (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1639, 1732, 3213 cm^{-1}. Anal. ($C_{21}H_{21}BrN_2O_5S$ (493.37)) C, H, Br, N, S.

Ethyl 2-[3-[(3,5-Dimethylphenyl)sulfonyl]-5-nitro-1*H*-indole-2-carboxamido]acetate (53). 53 was synthesized as for **59** but using **49** and ethyl glycine hydrochloride. Yield 48%, mp 224−226 °C (from ethanol). ^1H NMR (DMSO-d_6): δ 1.24 (t, J = 7.1 Hz, 3H), 2.29 (s, 6H), 4.21−4.23 (m, 4H), 7.27 (s, 1H), 7.69−7.70 (m, 3H), 8.18 (dd, J = 9.1 and 2.3 Hz, 1H), 8.91 (d, J = 2.2 Hz, 1H), 9.43 (broad s, 1H, disappeared on treatment with D_2O), 13.88 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (Nujol): ν 1647, 1749, 3215 cm^{-1}. Anal. ($C_{21}H_{21}N_3O_7S$ (459.47)) C, H, N, S.

Ethyl 2-[5-Chloro-3-[(3,5-dimethylphenyl)sulfonyl]-4-fluoro-1*H*-indole-2-carboxamido)acetate (54). 54 was synthesized as for **59** but using **50** and ethyl glycine hydrochloride. Yield 86%, mp 221−223 °C (from ethanol). ^1H NMR (DMSO-d_6): δ 1.23 (t, J = 7.1 Hz, 3H), 2.30 (m, 6H), 4.12−4.20 (m, 4H), 7.24 (s, 1H), 7.33−7.40 (m, 2H), 7.62 (s, 2H), 9.51 (broad s, 1H, disappeared on treatment with D_2O), 13.24 (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1638, 1745, 3212 cm^{-1}. Anal. ($C_{21}H_{20}ClFN_2O_5S$ (466.91)) C, H, Cl, F, N, S.

Ethyl 2-[*N*-[5-Chloro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxamido]]propanoate (55). 55 was prepared as previously reported.[11]

Ethyl 2-[*N*-[5-Bromo-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxamido]]propanoate (56). 56 was synthesized as for **59** but using **48** and ethyl alanine hydrochloride. Yield 85%, mp 211−214 °C (from ethanol).^1H NMR (DMSO-d_6): δ 1.47 (d, J = 7.2 Hz, 3H), 2.32 (s, 6H), 3.73 (s, 3H), 4.63 (q, J = 7.1 Hz, 1H), 7.27 (s, 1H), 7.48 (dd, J = 8.8 and 1.8 Hz, 1H), 7.52 (d, J = 8.7 Hz, 1H), 7.67 (s, 2H), 8.16 (d, J = 2.9 Hz, 1H), 9.49 (broad s, 1H, disappeared on treatment with D_2O), 13.11 (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1644, 1752, 3216 cm^{-1}. Anal. ($C_{21}H_{21}BrN_2O_5S$ (493.37)) C, H, Br, N, S.

Ethyl 2-[3-[(3,5-Dimethylphenyl)sulfonyl]-5-nitro-1*H*-indole-2-carboxamido)propanoate (57). 57 was synthesized as for **59** but using **49**and ethyl alanine hydrochloride. Yield 99%, mp 232−235 °C (from ethanol). ^1H NMR (DMSO-d_6): δ 1.23 (t, J = 7.1 Hz, 3H), 1.45 (d, J = 7.2 Hz, 3H), 2.29 (s, 6H), 4.18 (q, J = 7.1 Hz, 2H), 4.56−4.60 (m, 1H), 7.27 (s, 1H), 7.66 (s, 2H), 7.70 (d, J = 9.1 Hz, 1H), 8.18 (dd, J = 9.1 and 2.3 Hz, 1H), 8.88 (s, 1H), 9.45 (broad s, 1H, disappeared on treatment with D_2O), 13.82 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (Nujol): ν 1651, 1741, 3194 cm^{-1}. Anal. ($C_{22}H_{23}N_3O_7S$ (473.50)) C, H, N, S.

Ethyl 2-[5-Chloro-3-[(3,5-dimethylphenyl)sulfonyl]-4-fluoro-1*H*-indole-2-carboxamido)propanoate (58). 58 was synthesized as for **59** but using **50** and ethyl alanine hydrochloride. Yield 90%, mp 198−200 °C (from ethanol). ^1H NMR (DMSO-d_6): δ 1.25 (t, J = 7.1 Hz, 3H), 1.45 (d, J = 7.2 Hz, 3H), 2.33 (m, 6H), 4.18 (q, J = 7.0 Hz, 2H), 4.56 (q, J = 7.3 Hz, 1H), 7.26 (s, 1H), 7.35−7.43 (m, 2H), 7.68 (s, 2H), 9.51 (broad s, 1H, disappeared on treatment with D_2O), 13.31 (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1643, 1731, 3222 cm^{-1}. Anal. ($C_{22}H_{22}ClFN_2O_5S$ (480.94)) C, H, Cl, F, N, S.

Methyl 3-[*N*-[5-Bromo-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxamido]]propanoate (60). 60was synthesized as for **59** but using **48** and methyl β-alanine hydrochloride. Yield 95%, mp 208−211 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 2.31 (s, 6H), 2.68 (t, J = 6.7 Hz, 2H), 3.58−3.63 (m, 5H), 7.25 (s, 1H), 7.44−7.50 (m, 2H), 7.62 (s, 2H), 8.08 (s, 1H), 9.12 (broad s, 1H, disappeared on treatment with D_2O), 13.03 (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1644, 1736, 3291 cm^{-1}. Anal. ($C_{21}H_{21}BrN_2O_5S$ (493.37)) C, H, Br, N, S.

Methyl 3-[3-[(3,5-Dimethylphenyl)sulfonyl]-5-nitro-1*H*-indole-2-carboxamido)propanoate (61). 61 was synthesized as for **59** but using **49** methyl β-alanine hydrochloride. Yield 99%, mp 224−226 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 2.30 (s, 6H), 2.66 (t, J = 6.6 Hz, 2H), 3.56−4.62 (m, 5H), 7.26 (s, 1H), 7.62 (s, 2H), 7.68 (d, J = 9.0 Hz, 1H), 8.16 (dd, J = 9.0 and 2.12 Hz, 1H), 8.81 (d, J = 2.0 Hz, 1H), 9.11 (broad s, 1H, disappeared on treatment with D_2O), 14.57 ppm (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1656, 1732, 3223, 3322 cm^{-1}. Anal. (($C_{21}H_{21}N_3O_7S$ (459.47)) C, H, N, S.

Methyl 3-[5-Chloro-3-[(3,5-dimethylphenyl)sulfonyl]-4-fluoro-1*H*-indole-2-carboxamido)propanoate (62). 62 was synthesized as for **59** but using **50** and methyl β-alanine hydrochloride. Yield 73%, mp 205−268 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 2.34 (s, 6H), 2.68 (d, J = 6.6 Hz, 2H), 3.57−3.63 (m, 5H), 7.27 (s, 1H), 7.33−7.41 (m, 2H), 7.66 (s, 2H), 9.18 (broad s, 1H, disappeared on treatment with D_2O), 13.24 (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1639, 1734, 3221 cm^{-1}. Anal. ($C_{21}H_{20}ClFN_2O_5S$ (466.91)) C, H, Cl, F, N, S.

Ethyl 3-[*N*-[5-Chloro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2- carboxamido]butanoate (63). 63 was synthesized as for **59** but using ethyl 3-aminobutanoate. Yield 86%, mp 179−181 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.19 (t, J = 7.1 Hz, 3H), 1.28 (d, J = 6.7 Hz, 3H), 2.32 (s, 6H), 2.54 (dd, J = 15.7 and 7.4 Hz, 1H), 2.67 (dd, J = 15.6 and 6.0 Hz, 1H), 4.10 (q, J = 7.1 Hz, 2H), 4.36−4.42 (m, 1H), 7.27 (s, 1H), 7.35 (dd, J = 8.8 and 2.0 Hz, 1H), 7.54 (d, J = 8.7 Hz, 1H), 7.64 (s, 2H), 7.94 (d, J = 1.4 Hz, 1H), 9.03 (broad s, 1H, disappeared with treatment with D_2O), 13.00 ppm (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1643, 1732, 3212, 3285 cm^{-1}. Anal. ($C_{23}H_{25}ClN_2O_5S$ (476.97)) C, H, N, Cl, S.

Ethyl 3-[*N*-[5-Bromo-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxamido]]butanoate (64). 64 was synthesized as for **59** but using **48** and ethyl 3-aminobutanoate. Yield 99%, mp 181−185 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.19 (t, J = 7.1 Hz, 3H), 1.28 (d, J = 6.6 Hz, 3H), 2.30 (s, 6H), 2.49−2.53 (m, 1H), 2.69−2.74 (m, 1H), 4.01 (q, J = 7.1 Hz, 2H), 4.36−4.41 (m, 1H), 7.28 (s, 1H), 7.46 (dd, J = 8.8 and 1.8 Hz, 1H), 7.49 (d, J = 8.7 Hz, 1H), 7.64 (s, 2H), 8.09 (d, J = 1.7 Hz, 1H), 9.02 (broad s, 1H, disappeared on treatment with D_2O), 13.02 ppm (s, 1H, disappeared on treatment with D_2O). IR: ν 1643, 1730, 3193 cm^{-1}. Anal. ($C_{23}H_{25}BrN_2O_5S$ (521.42)) C, H, Br, N, S.

Ethyl 3-[3-[(3,5-Dimethylphenyl)sulfonyl]-5-nitro-1*H*-indole-2-carboxamido)butanoate (65). 65 was synthesized as for **59** but using **49** and ethyl 3-aminobutanoate. Yield 82%, mp 278−279 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.20 (t, J = 7.5 Hz, 3H), 1.29 (d, J = 6.6 Hz, 3H), 2.32 (s, 6H), 2.53−2.57 (m, 1H), 2.68−2.76 (m, 1H), 4.12 (q, J = 7.1 Hz, 2H), 4.36−4.41 (m, 1H), 7.29 (s, 1H), 7.65 (s, 2H), 7.72 (d, J = 9.1 Hz, 1H), 8.18 (dd, J = 8.3 and 2.3 Hz, 1H), 8.85 (d, J = 2.5 Hz, 1H), 9.05 (broad s, 1H, disappeared on treatment with D_2O), 13.46 ppm (s, 1H, disappeared on treatment with D_2O). IR: ν 1644, 1721, 3178 cm^{-1}. Anal. ($C_{23}H_{25}N_3O_7S$ (487.53)). C, H, N, S.

Ethyl 3-[5-Chloro-3-[(3,5-dimethylphenyl)sulfonyl]-4-fluoro-1*H*-indole-2-carboxamido)butanoate (66). 66 was synthesized as for **59** but using **50** and ethyl 3-aminobutanoate. Yield 88%, mp 172−174 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.19 (t, J = 7.1 Hz, 3H), 1.24 (d, J = 6.6 Hz, 3H), 2.31 (s, 6H), 2.41−2.47 (m, 1H), 2.71−2.77 (m, 1H), 4.09 (q, J = 7.1 Hz, 2H), 4.31−4.39 (m, 1H), 7.25 (s, 1H), 7.31−7.39 (m, 2H), 7.64 (s, 2H), 9.05 (broad s, 1H, disappeared on treatment with D_2O), 13.16 ppm (s, 1H, disappeared on treatment with D_2O). IR: ν 1643, 1731, 3218 cm^{-1}. Anal. ($C_{23}H_{24}ClFN_2O_5S$ (494.96)) C, H, Cl, F, N, S.

Ethyl 5-Chloro-3-[(3,5-dimethylphenyl)thio]-1*H*-indole-2-carboxylate (39). 39 was prepared as previously reported.[10]

Ethyl 5-Bromo-3-[(3,5-dimethylphenyl)thio]-1*H*-indole-2-carboxylate (40). 40 was prepared as previously reported.[10]

Ethyl 3-[(3,5-Dimethylphenyl)thio]-5-nitro-1*H*-indole-2-carboxylate (41). Boron trifluoride diethyletherate (0.14 g, 0.12 mL, 0.001 mol) was added to a mixture of ethyl 5-nitro-1*H*-indole-2-carboxylate (0.72 g, 0.0031 mol) and 1-(3,5-dimethylphenylthio)pyrrolidine-2,5-dione (0.77 g, 0.0033 mol) in anhydrous dichloromethane (20 mL) while cooling over an ice bath. After 2 h at room temperature, boron trifluoride diethyletherate (0.28 g, 0.24 mL, 0.002 mol) was added and the mixture was refluxed for 2 h. After the mixture was cooled, dichloromethane was added and the organic layer was washed with brine, dried, and evaporated. The residue was purified by silica gel column chromatography (dichloromethane−petroleum ether 1:1 as eluent) to give **41**. Yield 53%, mp 212−213 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.29 (t, J = 7.1 Hz, 3H), 2.16 (s, 6H), 4.36 (q, J = 7.1 Hz, 2H), 6.82 (s, 3H), 7.69 (d, J = 9.0 Hz, 1H), 8.17 (dd, J = 9.0 and 2.1 Hz, 1H), 8.25 (d, J = 2.1 Hz, 1H), 12.99 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (Nujol): ν 1660, 3270 cm^{-1}. Anal. ($C_{19}H_{18}N_2O_4S$ (370.42)) C, H, N, S.

Ethyl 5-Chloro-3-[(3,5-dimethylphenyl)thio]-4-fluoro-1*H*-indole-2-carboxylate (42). 42 was prepared as previously reported.[16]

Ethyl 5-Chloro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxylate (43). 43 was prepared as previously reported.[10]

Ethyl 5-Bromo-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxylate (44). 44 was prepared as previously reported.[10]

Ethyl 3-[(3,5-Dimethylphenyl)sulfonyl]-5-nitro-1*H*-indole-2-carboxylate (45). 3-Chloroperoxybenzoic acid (0.69 g, 0.004 mol) was added to an ice-cooled solution of **41** (0.50 g, 0.001 35 mol) in chloroform (22 mL). Reaction mixture was stirred at room temperature for 2 h. Water was added, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried, evaporated. The residue was purified by alumina chromatography (ethyl acetate as eluent) to give **45**. Yield 53%, mp 255−256 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.34 (t, J = 7.1 Hz, 3H), 2.37 (s, 6H), 4.43 (q, J = 7.1 Hz, 2H), 7.34 (s, 1H), 7.72 (s, 2H), 7.83 (d, J = 9.2 Hz, 1H), 8.29 (dd, J = 9.2 and 1.9 Hz, 1H), 9.21 (d, J = 1.9 Hz, 1H), 13.72 ppm (broad s, 1H, disappeared on treatment with D_2O, 1H). IR (Nujol): ν 1680, 3375 cm^{-1}. Anal. ($C_{19}H_{18}N_2O_6S$ (402.42)) C, H, N, S.

Ethyl 5-Chloro-3-[(3,5-dimethylphenyl)sulfonyl]-4-fluoro-1*H*-indole-2-carboxylate (46). 46 was prepared as previously reported.[16]

5-Chloro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxylic Acid (47). 47 was prepared as previously reported.[11]

5-Bromo-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxylic Acid (48). Lithium hydroxide monohydrate (1.79 g, 0.0428 mol) was added to a mixture of **44**[10] (6.07 g, 0.0139 mol) in tetrahydrofuran (16 mL) and water (16 mL). After the mixture was stirred at room temperature for 5 h, lithium hydroxide monohydrate (1.79 g, 0.0428 mol) was added and the mixture was maintained at room temperature for 3 days. Water and 1 N HCl were added (pH ∼ 2), and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried, filtered, and evaporated. The crude product was crystallized from ethanol to give **48**. Yield 96%, mp 280−284 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 2.30 (s, 6H), 7.21 (s, 1H), 7.46−7.53 (m, 2H), 7.70 (s, 2H), 8.37 (s, 1H), 13.06 (broad s, 1H, disappeared on treatment with D_2O), 14.02 (broad s, 1H, disappeared on treatment with D_2O). IR: ν 1708, 3169, 3386 cm^{-1}. Anal. ($C_{17}H_{14}BrNO_4S$ (408.27)) C, H, Br, N, S.

3-[(3,5-Dimethylphenyl)sulfonyl]-5-nitro-1*H*-indole-2-carboxylic Acid (49). 49 was synthesized as for **48** but using **45**. Yield 83%, mp 266−275 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 2.27 (s, 6H), 7.22 (s, 1H), 7.64 (s, 2H), 7.69 (d, J = 9.0 Hz, 1H), 8.18 (dd, J = 9.0 and 2.2 Hz, 1H), 9.11 (d, J = 2.17 Hz, 1H), 13.9 (broad s, 1H, disappeared on treatment with D_2O), 14.15 ppm (broad

s, 1H, disappeared on treatment with D_2O). IR (Nujol): ν 1608, 1730, 3191 cm^{-1}. Anal. ($C_{17}H_{14}N_2O_6S$ (402.43)) C, H, N, S.

5-Chloro-3-[(3,5-dimethylphenyl)sulfonyl]-4-fluoro-1*H*-indole-2-carboxylic Acid (50). 50 was synthesized as for **48** but starting from **46**.[16] Yield 99%, mp 255−257 °C (from ethanol). ^1H NMR (DMSO-d_6): δ 2.35 (s, 6H), 7.30 (s, 1H), 7.37−7.46 (m, 2H), 7.72 (s, 2H), 13.41 (broad s, 1H, disappeared on treatment with D_2O), 13.90 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (Nujol): ν 1668, 3236 cm^{-1}. Anal. ($C_{17}H_{13}ClFNO_4S$ (381.81)) C, H, N, S.

Cbz-Ala-ψ[CH_2SAc] (69). Potassium thioacetate (3.03 g, 26.5 mmol) was added in one portion to a solution of mesylate **68**[21] (1.51 g, 5.26 mmol) in DMF (26 mL), and stirring was maintained at room temperature overnight. The mixture was diluted with water (50 mL) and extracted with ethyl acetate. The pooled organic phases were washed with water, 5% sodium hydrogen sulfate, and brine. After removal of the solvent, the crude product was triturated with *n*-hexane to give **69** as a pale-yellow solid (yield 60%). ^1H NMR: δ 1.20 (d, $J = 6.6$ Hz, 3H), 2.40 (s, 3H), 3.02 (d, $J = 6.2$ Hz, 2H), 3.99−4.05 (m, 1H), 5.05 (s, 2H), 6.99 ppm (broad s, 1H, disappeared on treatment with D_2O), 7.39−7.41 (m, 5H). IR: ν 1715, 3435 cm^{-1}. Anal. ($C_{13}H_{17}NO_3S$ (267.34)) C, H, N, S.

Cbz-Ala-ψ[CH_2SO_2]-Cl (71). A solution of **69** (0.82 g, 3.06 mmol) in acetic acid (5 mL) was treated with a solution hydrogen peroxide [30% w/w in water (3 mL)] and acetic acid (7 mL). After the mixture was stirred overnight at room temperature, the excess peroxide was destroyed by addition of 10% Pd/C (0.050 g). The mixture was filtered on Celite, concentrated, and coevaporated with toluene. To the crude sulfonic acid, **70** was suspended in anhydrous dichloromethane (20 mL), and a solution of phosgene in toluene (20% m/m, 2.5 mL) and dry DMF (0.4 mL) was added under nitrogen atmosphere. After 1 h an additional 1 mL of phosgene solution was added and the mixture was stirred for 2 h under the same conditions. After removal of the solvent, the crude product was purified by silica gel flash chromatography (chloroform−methanol 97:3) to give **71** as a white solid (yield 70%). ^1H NMR: δ 1.50 (d, $J = 6.7$ Hz, 3H), 3.86−3.89 (m, 1H), 4.15−4.18 (m, 1H), 4.33−4.37 (m, 1H), 5.12 (s, 2H), 7.01 (broad s, 1H, disappeared on treatment with D_2O), 7.34−7.37 ppm (m, 5H). IR: ν 1719, 3439 cm^{-1}. Anal. ($C_{11}H_{14}ClNO_4S$ (291.75)) C, H, N, S.

Cbz-Ala-ψ[CH_2SO_2]-NH_2 (67). Gaseous ammonia was bubbled for 30 min through an ice-cooled solution of **71** (0.52 g, 1.8 mmol) in benzene (15 mL). The mixture was heated at 60 °C for 2 h. After the mixture was cooled, the precipitate was collected, washed with benzene, and dried to afford **72** as a white solid (yield 73%). ^1H NMR: δ 1.24 (d, $J = 6.6$ Hz, 3H), 3.13−3.18 (m, 2H), 4.04−4.09 (m, 1H), 5.08 (s, 2H), 6.95 (broad s, 2H, disappeared on treatment with D_2O), 7.34−7.39 ppm (m, 5H). IR: ν 1728, 3452 cm^{-1}. Anal. ($C_{11}H_{16}N_2O_4S$ (272.32)) C, H, N, S. The sulfonilamide hydrochloride **67** was obtained by treating **71** with hydrogen in methanol/water/37% HCl (10:10:1, 20 mL) in the presence of 10% Pd/C for 3 h at room temperature. The solution was filtered on Celite and the solvent removed under reduced pressure. The crude salt was triturated with diethyl ether and used without further purification in the next coupling step. Anal. ($C_3H_{11}ClN_2O_2S$ (174.65)) C, H, N, S.

Molecular Modeling. All molecular modeling studies were performed on a MacPro dual 2.66 GHz Xeon running Ubuntu 8. The RT structures were downloaded from the PDB Data Bank (http://www.rcsb.org/). Hydrogen atoms were added to the protein, using Molecular Operating Environment (MOE) 2007.09,[29] and minimized keeping all the heavy atoms fixed until a rmsd gradient of 0.05 kcal mol^{-1} $Å^{-1}$ was reached. Ligand structures were built with MOE and minimized using the MMFF94x force field until a rmsd gradient of 0.05 kcal mol^{-1} $Å^{-1}$ was reached. The docking simulations were performed using FlexX[28] with the MOE interface using default settings and getting 20 poses for compound with SurfleX[37] with the Sybyl8.0[38] interface, also in this case using default settings, and getting 10 poses for compound and PLANTS[27] with the Zodiac[39] interface. In the Zodiac GUI for PLANTS we set the binding lattice as a sphere of 12 Å binding site radius from the center of the 2zd1[31] cocrystallized inhibitor. Also in this case we used all default settings. The Y181I mutation was obtained by mutating the specific residue in the 1jkh[29] crystal using the rotamer explorer tool in MOE and using the lowest energy conformation obtained. The rmsd values between the cocrystallized and docked conformation were calculated using the MOE SVL script.[40] The images in the manuscript were created with Zodiac[39] and PyMOL.[41]

Antiviral Activity Assays. The compounds were evaluated against the following viruses: herpes simplex virus type 1 (HSV-1) strain KOS, thymidine kinase-deficient (TK^-) HSV-1 KOS strain resistant to ACV (ACVr), herpes simplex virus type 2 (HSV-2) strain, vaccinia virus Lederle strain, respiratory syncytial virus (RSV) strain Long, vesicular stomatitis virus (VSV), Coxsackie B4, parainfluenza 3 reovirus-1, sindbis, reovirus-1, Punta Toro, human immunodeficiency virus type 1 strain IIIB, and human immunodeficiency virus type 2 strain ROD. The antiviral, other than anti-HIV, assays were based on inhibition of virus-induced cytopathicity in human embryonic lung (HEL) fibroblasts, African green monkey kidney cells (Vero), and human cervix carcinoma cells (HeLa). Briefly, confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures). After a 1−2 h adsorption period, residual virus was removed and the cell cultures were incubated in the presence of varying concentrations of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. Antiviral activity was expressed as the EC_{50} or concentration required to reduce virus-induced cytopathogenicity by 50%.

Inhibition of HIV-Induced Cytopathicity in CEM Cells. The methodology for the anti-HIV assays had been described previously.[42] Briefly, human CEM cell cultures ($\sim 3 \times 10^5$ cells/mL) were infected with $\sim$100 $CCID_{50}$ HIV-1(IIIB) or HIV-2 (ROD) per milliliter and seeded in 200 μL well microtiter plates containing appropriate dilutions of the test compounds. After 4 days of incubation at 37 °C, syncytia cell formation was examined microscopically in the CEM cell cultures.

Cytostatic and Cytotoxicity Assays. The cytostatic activity of the test compounds was determined by seeding the murine leukemia L1210 or human lymphocyte Molt4/C8 or CEM cells at 5×10^4 to 7.5×10^4 cells/200 μL in 96-well microtiter plates in the absence or presence of serial dilutions of the test compounds. After 2 days (L1210) or 3 days (Molt4/C8 or CEM) of incubation at 37 °C, cell numbers were counted using a Coulter counter. The cytostatic concentration was calculated as the CC_{50}, or the compound concentration required to reduce cell proliferation by 50% relative to the number of cells in the untreated controls. CC_{50} values were estimated from graphic plots of the number of cells (percentage of control) as a function of the concentration of the test compounds. The cytotoxic activity of the test compounds was determined by administering serial dilutions of the test compounds on confluent monolayer cultures of human cervix carcinoma HeLa or monkey kidney Vero cells in 96-well microtiter plates. Cytotoxicity of the test compounds was expressed as the minimum cytotoxic concentration (MCC) or the compound concentration that caused a microscopically detectable alteration of cell morphology after 3 days of incubation.

Enzymatic Assay Procedures. Chemicals. [^3H]dTTP (40 Ci/mmol) was from Amersham and unlabeled dNTP from Boehringer. Whatman was the supplier of the GF/C filters. All other reagents were of analytical grade and purchased from Merck or Fluka.

Nucleic Acid Substrates. The homopolymer poly(rA) (Pharmacia) was mixed at weight ratios in nucleotides of 10:1 to the oligomer oligo$(dT)_{12-18}$ (Pharmacia) in 20 mM Tris-HCl (pH 8.0) containing 20 mM KCl and 1 mM EDTA, heated at 65 °C for 5 min, and then slowly cooled at room temperature.

Expression and Purification of Recombinant HIV-1 RT Forms. The coexpression vectors pUC12N/p66(His)/p51with the wild-type or the mutant forms of HIV-1 RT p66 were kindly provided by Dr. S. H. Hughes (NCI—Frederick Cancer Research

and Development Center). Proteins were expressed in *E. coli* and purified as described.[43]

HIV-1 RT RNA-Dependent DNA Polymerase Activity Assay. RNA-dependent DNA polymerase activity was assayed as follows: a final volume of 25 μL contained reaction buffer (50 mM Tris-HCl, pH 7.5, 1 mM DTT, 0.2 mg/mL BSA, 4% glycerol), 10 mM $MgCl_2$, 0.5 μg of poly(rA)/oligo(dT)$_{10:1}$ (0.3 μM 3′-OH ends), 10 μM [^{3}H]dTTP (1 Ci/mmol), and 2−4 nM RT. Mixtures were incubated at 37 °C for the indicated time. Then 20 μL aliquots were spotted on glass fiber filters GF/C which were immediately immersed in 5% ice-cold TCA. Filters were washed twice in 5% ice-cold TCA and once in ethanol for 5 min and dried, and acid-precipitable radioactivity was quantitated by scintillation counting.

Inhibition Assays. Reactions were performed under the conditions described for the HIV-1 RT RNA-dependent DNA polymerase activity assay. Incorporation of radioactive dTTP into poly(rA)/oligo(dT) at different substrate (nucleic acid or dTTP) concentrations was monitored in the presence of increasing fixed amounts of inhibitor. Data were then plotted according to Lineweaver−Burke and Dixon. For K_i determination, an interval of inhibitor concentrations between 0.2 K_i and 5 K_i was used.

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Supporting Information Available: Additional cytotoxicity, inhibitory, and antiviral data and elemental analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

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