N-Aminoimidazole Derivatives Inhibiting Retroviral Replication via a Yet Unidentified Mode of Action

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The synthesis of a series of *N*-aminoimidazoles (NAIMs) with an uncommon spectrum of antiretroviral activity is described. From a group of 60 closely related molecules, we were able to subdivide the molecules in different groups based on their anti-HIV and anti-SIV activity in vitro: (i) molecules acting on a new, immediate postintegration step, (ii) molecules acting on both postintegration and HIV-1 reverse transcriptase (RT) as NNRTI, and (iii) molecules that mainly act at the HIV-1 RT according to an NNRTI-type mode of action.

Introduction

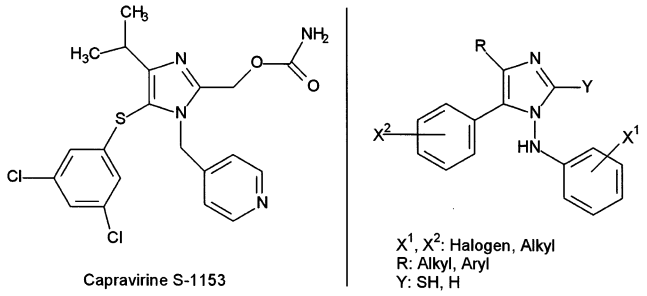
Replication of human immunodeficiency virus type 1 can be reduced in HIV-infected patients using a combination of antiviral drugs targeted at the reverse transcriptase (RT) and protease.[1] Unfortunately, because of the high mutation rate of HIV, treatment, even combination therapy, is required for drug-resistant variants.[2]

Shortly after the discovery of the nonnucleoside reverse transcriptase inhibitors (NNRTIs), it became clear that resistance to this class of compounds emerges rapidly.[3] Moreover, cross-resistance is very important. If a patient's virus becomes resistant to NNRTIs, no other member of the group can be used effectively. Capravirine (S-1153), a 1,2,4,5-substituted imidazole derivative, is an NNRTI[4] that is able to inhibit HIV-1 strains that are resistant to other NNRTIs. This ability to cope with typical NNRTI-induced mutations is based, at least in part, on an extensive network of hydrogen bonds involving the main chain of residues 101, 103, and 236 of the p66 RT subunit. Side chain mutations are not likely to disrupt these interactions.[5]

We synthesized a series of *N*-aminoimidazoles via a one-pot synthesis. The similarity of this series of compounds to S-1153 is depicted in Chart 1.

The in vitro antiviral evaluation of a series of 60 *N*-aminoimidazole and *N*-aminoimidazole thiones yielded unexpected antiviral activity findings. Out of a series of 60 compounds, 7 compounds were equipotent against the replication of HIV-1 (strains III_B and the molecular clone NL4.3), an NNRTI-resistant HIV-1 strain (with mutations K103N, Y181C in RT), HIV-2(ROD), and SIV(mac251). The most potent of these compounds were also active against MSV-induced transformation of C3H/3T3 embryo murine fibroblast in vitro.

Chart 1. Structure Analogy of S-1153 vs *N*-Aminoimidazole Derivatives **3** and **7**



This very unusual antiviral activity pattern suggests that the antiretroviral activity of these compounds is not based on an inhibition of the reverse transcriptase activity as might have been expected from the structural similarity with capravirine.

Other members of the series had an activity profile that coincided with that of the NNRTIs (no activity against HIV-2(ROD) and reduced activity against an NNRTI-resistant strain), but some of these compounds inhibited the replication of SIV(mac251) to the same extent as wild-type HIV-1 strains, unlike the classical NNRTIs nevirapine, delavirdine, and efavirenz.

These results prompted us to study the structure−activity relationship and potential mode of action of these *N*-aminoimidazoles (NAIMs) more thoroughly.

Chemistry

Starting from cheap, easily available compounds such as hydrazines **1**, α-bromoketones **2**, and potassium thiocyanate, N-substituted 1-amino-2,3-dihydro-1*H*-imidazole-2-thiones **3** were formed in good yield in a one-pot reaction (Scheme 1).[6] By use of strategies of parallel synthesis and combination of starting materials **1** and **2**, a broad range of differently substituted heterocycles **3** could be obtained.

This multistep reaction is considered to occur via the conversion of an α-thiocyanatoketone (formed by nucleophilic substitution reaction) to the thiocyanato-

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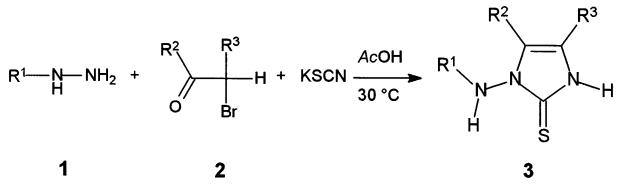
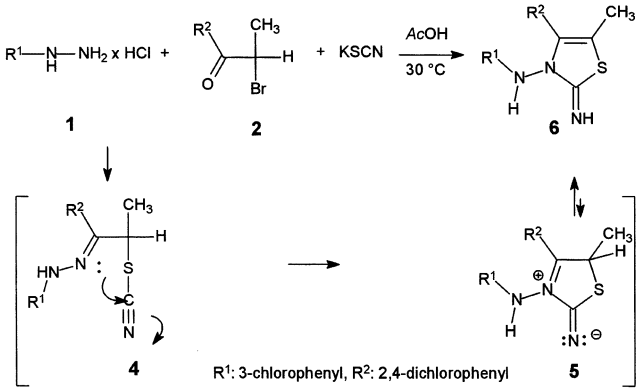
Scheme 1. Synthesis of *N*-Aminoimidazoline-2-thiones **3**



Scheme 2. Most Likely Pathway for the Formation of 2-Iminothiazoles **6**



hydrazone **4**. Subsequent 1,4-elimination led to the corresponding azoalkene and thiocyanic acid. The thus formed two intermediates can undergo a [3 + 2] cycloaddition reaction, and following hydrogen shift, the 1-amino-2,3-dihydro-1*H*-imidazole-2-thiones **3** were obtained.

In the case of ortho-substituted ketones, it was found that the reaction did not follow the pathway outlined in Scheme 1. Instead of the cycloaddition, a 5-exo-dig cyclization reaction (Scheme 2) took place. Likewise, in this pathway the α-thiocyanatohydrazone **4** is the key intermediate. In the case of substituents in the ortho position of the former ketone **2**, the 1,4-elimination giving rise to the formation of azoalkenes, which are planar, delocalized π-electron systems, is not favored. As shown in Scheme 2, a cyclization reaction yielding a 2-iminothiazole **6** took place instead. Attempts to convert the 2-iminothiazole **6** to the desired imidazoline-2-thione derivative **3** by way of a Dimroth rearrangement[7] failed.

The two isomers can be easily distinguished by NMR methods. The signal corresponding to the carbon 2 of the imidazoline-2-thione derivative **3** was found in the range δ 160−162 ppm, whereas the corresponding signal of the 2-iminothiazole **6** was found in the range δ 169−171 ppm.

In the case of isomer **3**, saturation of the 3-NH causes a 10% nuclear Overhauser enhancement (NOE) at the 4-substituent, whereas irradiation of the imino NH showed an NOE enhancement of 5% relative to the exocyclic NH. The results of structure elucidation are summarized in Chart 2.

Besides the restriction of having a substituent in the ortho position of ketone **2**, the method of generating *N*-aminoimidazoline-2-thiones **3** in a one-pot-reaction starting from hydrazines **1**, ketones **2**, and potassium thiocyanate is suitable for all substituents, R^1, R^2, and R^3 being either aryl or alkyl. The method is also suitable

Chart 2. Comparison between the NMR Data of 2-Iminothiazoles **6** and *N*-Aminoimidazoline-2-thiones **3**

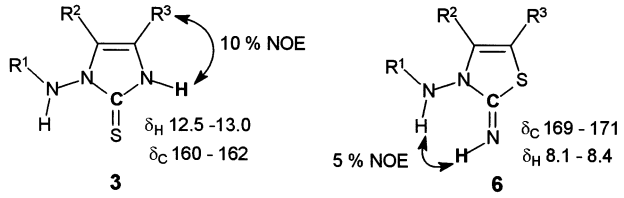
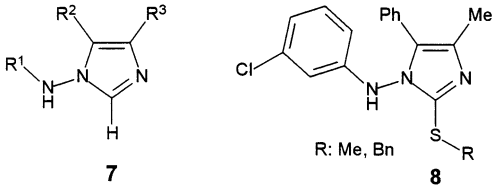


Chart 3. Structures of *N*-Aminoimidazoles **7** and *S*-Alkylaminoimidazoles **8**



for the conversion of N,N-disubstituted hydrazines **1**, obtaining the corresponding imidazoline thione **3** with an N,N-disubstituted side chain. Further derivatization of heterocycle **3** could be achieved by desulfurization with hydrogen peroxide in acetic acid,[8] yielding the corresponding *N*-aminoimidazole derivatives **7**.

Alkylation of the imidazoline-2-thiones **3** quantitatively formed the *S*-alkyl product **8** (Chart 3), even under Mitsunobu[9] conditions. Substitution patterns, yields, melting points, R_f values and HRMS of the imidazole derivatives **3** and **7** are shown in Table 1.

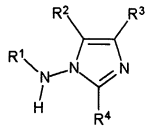
Biological Results and Discussion

Inhibition of HIV and SIV Replication in Cell Culture by *N*-Aminoimidazoles 3 and 7. We tested a series of *N*-arylaminoimidazoline-2-thione derivatives **3** and *N*-arylaminoimidazoles **7** for their potential to inhibit the replication of HIV and SIV in a cell culture model for acute infection. The cytotoxicity of the compounds was determined in parallel. The antiviral activity and cytotoxicity data are presented in Table 2. From a series of 60 compounds, 26 compounds were active against the replication of at least one of the included virus strains. Seven compounds (**3.01**, **3.05**, **3.07**, **3.39**, **7.01**, **7.02**, and **7.03**) were equally potent inhibitors of the HIV-1, HIV-2, and SIV replication in MT-4 cells. Similar results were obtained in peripheral blood mononuclear cells (PBMCs) (data not shown).

Some molecules showed reduced activity against a clinically relevant NNRTI-resistant strain (mutations in RT K103N and Y181C), but unlike established NNRTIs as nevirapine, delavirdine, and efavirenz, they remained potent inhibitors of SIV(mac251) replication in vitro. Moreover, the most potent broad-spectrum *N*-aminoimidazole derivatives **3** and **7** inhibited MSV-induced transformation of C3H/3TC embryo murine fibroblasts in vitro (Table 3).

Structure−Activity Relationship (SAR) for *N*-Aminoimidazole Derivatives 3 and 7. All the antiretroviral-active *N*-aminoimidazoles **3** and **7** have in common the basic molecule depicted in Chart 4. No marked difference in antiviral activity and cytotoxicity was observed between the imidazoles **7** and their 2-thiono homologue **3** series. However, alkylation (methylation or benzylation) of the sulfur led to complete

Table 1. Analytical Data of *N*-Aminoimidazole-2-thiones **3** (Written as Thiole Tautomer with R^4 = SH) and *N*-Aminoimidazoles **7** (R^4 = H)[a]



compd	R^1	R^2	R^3	yield (%)	R_f [b]	mp (MeOH, °C)	exact mass
3.01[1]	3-ClC_6H_4	C_6H_5	CH_3	82	0.55	215−217	Calcd: 316.0675 $[M + H]^+$. Found: 316.0660
3.02[11]	2-ClC_6H_4	C_6H_5	CH_3	86	0.54	220−221	Calcd: 316.0675 $[M + H]^+$. Found: 316.0703
3.03	4-FC_6H_4	C_6H_5	CH_3	68	0.53	228−231	Calcd: 300.0971 $[M + H]^+$. Found: 300.0996
3.04[6]	4-ClC_6H_4	C_6H_5	CH_3	85	0.54	212−214	Calcd: 316.0675 $[M + H]^+$. Found: 316.0662
3.05	3-ClC_6H_4	3-BrC_6H_4	CH_3	86	0.52	196−200	Calcd: 393.9790 $[M + H]^+$. Found: 393.9839
3.06	3-ClC_6H_4	4-BrC_6H_4	CH_3	84	0.52	220−222	Calcd: 393.9790 $[M + H]^+$. Found: 393.9779
3.07	3-ClC_6H_4	3-ClC_6H_4	CH_3	85	0.52	204−207	Calcd: 350.0855 $[M + H]^+$. Found: 350.0271
3.08	3-ClC_6H_4	4-ClC_6H_4	CH_3	82	0.52	225−226	Calcd: 350.0855 $[M + H]^+$. Found: 350.0356
3.09	3-ClC_6H_4	4-$CH_3OC_6H_4$	CH_3	88	0.53	226−229	Calcd: 346.0781 $[M + H]^+$. Found: 350.0831
3.10	3-ClC_6H_4	CH_3	C_6H_5	83	0.53	238−240	Calcd: 316.0675 $[M + H]^+$. Found: 316.0684
3.11	3-ClC_6H_4	$-(CH_2)_4-$		79	0.52	148−151	Calcd: 280.0675 $[M + H]^+$. Found: 280.0733
3.12[6]	C_6H_5	C_6H_5	CH_3	92	0.49	224−226	Calcd: 282.1065 $[M + H]^+$. Found: 282.1037
3.13	3-CH_3−4-CH_3−C_6H_3	C_6H_5	CH_3	62	0.53	228−230	Calcd: 310.1378 $[M + H]^+$. Found: 310.1373
3.14	3-BrC_6H_4	C_6H_5	CH_3	52	0.52	190−192	Calcd: 360.0170 $[M + H]^+$. Found: 360.0168
3.15	3-Cl-4-$CH_3C_6H_3$	C_6H_5	CH_3	32	0.52	190−193	Calcd: 330.0832 $[M + H]^+$. Found: 330.0834
3.16	2-Cl−5-ClC_6H_3	C_6H_5	CH_3	60	0.54	266−270	Calcd: 350.0285 $[M + H]^+$. Found: 350.0248
3.17	3-$NO_2C_6H_4$	C_6H_5	CH_3	48	0.49	214−116	Calcd: 327.0916 $[M + H]^+$. Found: 327.0928
3.18	3-FC_6H_4	C_6H_5	CH_3	36	0.53	226−229	Calcd: 300.0971 $[M + H]^+$. Found: 300.0933
3.19	3-$CH_3C_6H_4$	C_6H_5	CH_3	58	0.53	206−207	Calcd: 296.1221 $[M + H]^+$. Found: 296.1208
3.20	3-ClC_6H_4	CH_3	CH_3	87	0.54	234−236	Calcd: 254.0519 $[M + H]^+$. Found: 254.0521
3.21	C_6H_5	CH_3	CH_3	78	0.53	217−219	Calcd: 220.0908 $[M + H]^+$. Found: 220.0844
3.22	3-$CH_3C_6H_4$	CH_3	CH_3	85	0.54	230−232	Calcd: 234.1065 $[M + H]^+$. Found: 234.1078
3.23	3-$CH_3C_6H_4$	C_6H_5	$(CH_3)_2CH$	46	0.50	220−222	Calcd: 324.1534 $[M + H]^+$. Found: 324.1524
3.24	3-ClC_6H_4	C_6H_5	CH_3CH_2	68	0.52	116−118	Calcd: 330.0831 $[M + H]^+$. Found: 330.0833
3.25	3-$CH_3C_6H_4$	C_6H_5	CH_3CH_2	72	0.53	202−204	Calcd: 310.1377 $[M + H]^+$. Found: 310.1375
3.26	3-ClC_6H_4	C_6H_5	C_6H_5	56	0.57	178−180	Calcd: 378.0832 $[M + H]^+$. Found: 378.0863
3.27	3-ClC_6H_4	CH_3OCO	CH_3	68	0.49	194−196	Calcd: 298.0417 $[M + H]^+$. Found: 298.0421
3.28	3-ClC_6H_4	COOH	CH_3	52	0.30	166−168	Calcd: 284.0261 $[M + H]^+$. Found: 284.0261
3.29	3-ClC_6H_4	CH_3	CH_3OCO	75	0.45	160−162	Calcd: 298.0417 $[M + H]^+$. Found: 298.0410
3.30	3-CH_3−5-$CH_3C_6H_3$	C_6H_5	CH_3	82	0.53	240−242	Calcd: 310.1378 $[M + H]^+$. Found: 310.1394
3.31	3-$CH_3OC_6H_4$	C_6H_5	CH_3	80	0.53	184−186	Calcd: 312.1171 $[M + H]^+$. Found: 312.1188
3.32	3-ClC_6H_4	3-CNC_6H_4	CH_3	84	0.53	168−170	Calcd: 341.0628 $[M + H]^+$. Found: 341.0675
3.33	3-MeC_6H_4	3-CNC_6H_4	CH_3	88	0.53	160−162	Calcd: 321.1174 $[M + H]^+$. Found: 321.1152
3.34	3-ClC_6H_4	3-$MeOCOC_6H_4$	CH_3	90	0.54	170−172	Calcd: 374.0730 $[M + H]^+$. Found: 374.0757
3.35	3-$CH_3C_6H_4$	3-$MeOCOC_6H_4$	CH_3	80	0.52	164−166	Calcd: 354.1276 $[M + H]^+$. Found: 354.1305
3.36	3-ClC_6H_4	3-$HOCOC_6H_4$	CH_3	70	0.34	170−172	Calcd: 360.0573 $[M + H]^+$. Found: 360.0608
3.37	3-$CH_3C_6H_4$	3-$HOCOC_6H_4$	CH_3	61	0.33	166−168	Calcd: 340.1119 $[M + H]^+$. Found: 340.1147
3.38	3-ClC_6H_4	3-$NH_2COC_6H_4$	CH_3	73	0.35	164−166	Calcd: 359.0733 $[M + H]^+$. Found: 359.0702
3.39	α-naphthyl	C_6H_5	CH_3	47	0.56	194−196	Calcd: 332.1143 $[M + H]^+$. Found: 332.1224
3.40	4-$CH_3CH_2C_6H_4$	C_6H_5	CH_3	58	0.50	180−182	Calcd: 310.1378 $[M + H]^+$. Found: 310.1361
3.41	4-$CH_3SC_6H_4$	C_6H_5	CH_3	59	0.48	215−217	Calcd: 328.0942 $[M + H]^+$. Found: 328.0928
3.42[8]	C_6H_5, CH_3	C_6H_5	CH_3	56	0.62	223−225	Calcd: 296.1221 $[M + H]^+$. Found: 296.1205
7.01	3-ClC_6H_4	C_6H_5	CH_3	88	0.82	216−218	Calcd: 284.0945 $[M + H]^+$. Found: 284.0955
7.02	3-ClC_6H_4	3-BrC_6H_4	CH_3	52	0.85	164−166	Calcd: 362.0059 $[M + H]^+$. Found: 362.0062
7.03	3-ClC_6H_4	3-ClC_6H_4	CH_3	66	0.83	168−170	Calcd: 318.0565 $[M + H]^+$. Found: 318.0616
7.04	3-ClC_6H_4	$-(CH_2)_4-$		76	0.76	179−180	Calcd: 248.0954 $[M + H]^+$. Found: 248.0953
7.05	C_6H_5	C_6H_5	CH_3	86	0.84	165−167	Calcd: 250.1344 $[M + H]^+$. Found: 250.1322
7.06	C_6H_5	CH_3	CH_3	67	0.76	176−178	Calcd: 188.1188 $[M + H]^+$. Found: 188.1153
7.07	3-ClC_6H_4	CH_3	CH_3	82	0.77	146−148	Calcd: 222.0798 $[M + H]^+$. Found: 222.0793.
7.08	3-$CH_3C_6H_4$	CH_3	CH_3	86	0.78	144−146	Calcd: 202.1344 $[M + H]^+$. Found: 202.1356.
7.09	3-$CH_3C_6H_4$	C_6H_5	CH_3	75	0.84	159−160	Calcd: 264.1500 $[M + H]^+$. Found: 264.1536.
7.10	4-FC_6H_4	C_6H_5	CH_3	79	0.76	153−155	Calcd: 268.1250 $[M + H]^+$. Found: 268.1237
7.11	3-$CH_3C_6H_4$	C_6H_5	CH_3CH_2	81	0.85	158−160	Calcd: 278.1657 $[M + H]^+$. Found: 278.1641
7.12	3-ClC_6H_4	CH_3OCO	CH_3	81	0.85	138−140	Calcd: 278.1657 $[M + H]^+$. Found: 278.1641
7.13	3-CH_3−5-$CH_3C_6H_3$	C_6H_5	CH_3	66	0.86	154−156	Calcd: 278.1657 $[M + H]^+$. Found: 278.1636.
7.14	3-$CH_3OC_6H_4$	C_6H_5	CH_3	68	0.84	156−158	Calcd: 280.1450 $[M + H]^+$. Found: 280.1449.
7.15	3-ClC_6H_4	3-CNC_6H_4	CH_3	75	0.84	140−142	Calcd: 309.0907 $[M + H]^+$. Found: 309.0911
7.16	3-MeC_6H_4	3-CNC_6H_4	CH_3	70	0.84	138−140	Calcd: 289.1453 $[M + H]^+$. Found: 289.1456
7.17	3-ClC_6H_4	3-$NH_2COC_6H_4$	CH_3	84	0.67	228−230	Calcd: 327.1013 $[M + H]^+$. Found: 327.1017
7.18	3-MeC_6H_4	3-$NH_2COC_6H_4$	CH_3	70	0.66	220−222	Calcd: 307.1556 $[M + H]^+$. Found: 307.1544
7.19	3-ClC_6H_4	3-$MeOCOC_6H_4$	CH_3	65	0.80	172−174	Calcd: 342.1009 $[M + H]^+$. Found: 342.1015
7.20	3-ClC_6H_4	3-$HOCOC_6H_4$	CH_3	77	0.63	176−178	Calcd: 328.0853 $[M + H]^+$. Found: 328.0854
7.21[8]	C_6H_5, CH_3	C_6H_5	CH_3	54	0.82	110−112	Calcd: 264.1501 $[M + H]^+$. Found: 264.1517

[a] The results of elemental analyses (C, H, N) of all active compounds were within ±0.4% of the theoretical values. [b] Solvent system for *N*-aminoimidazoline-2-thiones **3**: CH_2Cl_2/MeOH, 99:1. Solvent system for *N*-aminoimidazoles **7**: CH_2Cl_2/MeOH, 9:1.

Table 2. Inhibition of Viral Replication in MT-4 Cells by *N*-Aminoimidazole Derivatives **3** and **7**

	IC_{50} [a] (μM)					
	HIV-1					
compd	III_B	NL4.3	NNRTI(res)[b]	HIV-2 ROD	SIV mac251	CC_{50} (μM)[c]
3.01	6.39 ± 0.94	8.99 ± 6.14	9.72 ± 4.99	8.11 ± 0.38	8.20 ± 4.62	66.65 ± 19.00
3.05	8.54 ± 1.06	5.60 ± 4.81	3.00 ± 1.20	10.46 ± 6.71	1.75 ± 0.20	43.98 ± 20.60
3.07	5.00 ± 1.17	2.48 ± 0.91	1.86 ± 1.20	3.91 ± 0.65	1.22 ± 0.77	30.95 ± 11.93
3.39	5.55 ± 2.14	6.58 ± 4.80	11.89 ± 5.31	5.10 ± 3.77	4.68 ± 0.30	32.05 ± 9.17
7.01	1.59 ± 0.32	1.90 ± 0.60	1.73 ± 0.74	1.65 ± 1.55	2.15 ± 0.42	17.48 ± 7.58
7.02	0.58 ± 0.08	1.30 ± 0.77	0.97 ± 0.14	0.91 ± 0.19	0.66 ± 0.25	16.41 ± 7.94
7.03	0.72 ± 0.59	0.63 ± 0.03	0.38 ± 0.28	0.72 ± 0.03	0.31 ± 0.13	11.94 ± 9.93
3.13	9.30 ± 0.55	3.54 ± 0.81	>55.58	>55.58	10.31 ± 3.59	55.58 ± 18.68
3.14	9.96 ± 1.61	4.52 ± 3.80	5.77 ± 0.28	>44.24	2.72 ± 0.17	44.24 ± 14.15
3.17	7.29 ± 1.53	11.25 ± 4.57	42.18 ± 2.39	>48.26	7.54 ± 1.59	48.26 ± 22.49
3.19	1.56 ± 0.61	2.17 ± 0.88	26.10 ± 3.96	>35.71	6.50 ± 4.60	88.25 ± 35.71
3.25	17.64 ± 13.02	12.80 ± 3.39	≥53.55	>53.55	>53.55	53.55 ± 28.73
3.30	1.03 ± 0.58	0.87 ± 0.52	>194.21	>194.21	>194.21	194.21 ± 132.79[d]
3.31	4.78 ± 1.38	9.79 ± 0.22	>38.02	>38.02	>38.02	38.02 ± 5.34
7.05	≥11.67	20.98 ± 2.64	>52.18	>52.18	>52.18	52.18 ± 11.52
7.09	3.26 ± 0.91	5.20 ± 0.04	3.99 ± 0.04	>27.04	3.08 ± 0.41	27.04 ± 11.81
7.15	6.96 ± 2.00	8.91 ± 3.01	22.57 ± 5.05	>36.79	7.22 ± 0.027	36.79 ± 5.15
3.02	>161.81	23.27 ± 8.49	>161.81	>161.81	>161.81	161.81 ± 52.84
3.08	>40.80	>40.80	>40.80	>40.80	7.71 ± 3.88	40.80 ± 13.90
3.09	>38.69	>38.69	>38.69	>38.69	10.12 ± 11.65	38.69 ± 13.01
3.13	>44.29	10.85 ± 0.82	>44.29	>44.29	10.88 ± 8.45	44.29 ± 14.34
3.18	>108.23	18.44 ± 9.39	≥43.42	>108.23	33.80 ± 20.36	108.23 ± 71.38
3.23	>42.63	≥42.63	>42.63	>42.63	>42.63	42.63 ± 16.79
3.24	9.06 ± 1.55	>38.96	>38.96	>38.96	3.88 ± 3.27	38.96 ± 15.86
3.32	≥99.40	41.96 ± 2.08	>99.40	>99.40	>99.40	99.40 ± 42.13
7.13	>20.44	10.99 ± 2.85	>20.44	>20.44	>20.44	20.44 ± 9.55
3.03	>220.13	>220.13	ND	>220.13	>220.13	220.13 ± 60.29
3.04	>134.41	>134.41	ND	>134.41	>134.41	134.41 ± 33.82
3.06	>33.24	>33.24	ND	>33.24	>33.24	33.24 ± 14.31
3.10	>49.96	>49.96	ND	>49.96	>49.96	49.96 ± 27.07
3.11	>126.42	>126.42	ND	>126.42	>126.42	126.42 ± 40.14
3.12	>191.27	>191.27	ND	>191.27	>191.27	191.27 ± 72.25
3.16	>147.12	>147.12	ND	>147.12	>147.12	147.12 ± 78.80
3.20	>259.42	>259.42	ND	>259.42	>259.42	259.42 ± 25.69
3.21	>180.79	>180.79	ND	>180.79	>180.79	180.79 ± 29.78
3.22	>359.86	>359.86	ND	>359.86	>359.86	359.86 ± 77.44
3.26	>36.31	>36.31	>36.31	>36.31	>36.31	36.31 ± 14.87
3.27	>190.31	>190.31	ND	>190.31	>190.31	190.31 ± 41.98
3.28	>265.56	>265.56	ND	>265.56	>265.56	265.56 ± 41.94
3.29	>48.80	>48.80	ND	>48.80	>48.80	48.80 ± 28.14
3.33	>45.63	>45.63	ND	>45.63	>45.63	45.63 ± 36.45
3.34	>67.36	>67.36	ND	>67.36	>67.36	67.36 ± 59.70
3.36	>120.03	>120.03	ND	>120.03	>120.03	120.03 ± 40.10
3.37	>187.13	>187.13	ND	>187.13	>187.13	187.13 ± 45.74
3.38	>42.16	>42.16	ND	>42.16	>42.16	42.16 ± 12.49
3.40	>34.45	>34.45	ND	>34.45	>34.45	34.45 ± 2.37
3.41	>53.04	>53.04	ND	>53.04	>53.04	53.04 ± 14.87
7.04	>67.29	>67.29	>67.29	>67.29	>67.29	67.29 ± 16.95
7.06	>351.42	>351.42	ND	>351.42	>351.42	351.42 ± 53.09
7.07	>213.68	>213.68	ND	>213.68	>213.68	213.68 ± 55.17
7.08	>312.12	>312.12	ND	>312.12	>312.12	312.12 ± 29.36
7.10	>30.82	>30.82	ND	>30.82	>30.82	30.82 ± 9.39
7.11	>27.87	>27.87	ND	>27.87	>27.87	27.87 ± 13.45
7.12	>106.70	>106.70	ND	>106.70	>106.70	106.70 ± 70.83
7.14	>34.44	>34.44	ND	>34.44	>34.44	34.44 ± 10.81
7.16	>44.70	>44.70	ND	>44.70	>44.70	44.70 ± 9.68
7.17	>260.50	>260.50	ND	>260.50	>260.50	260.50 ± 62.76
7.18	>162.31	>162.31	ND	>162.31	>162.31	162.31 ± 56.76
7.19	>33.15	>33.15	ND	>33.15	>33.15	33.15 ± 3.83
7.20	>160.57	>160.57	ND	>160.57	>160.57	160.57 ± 21.14
8.01	>42.11	>42.11	ND	>42.11	>42.11	42.11 ± 17.19
8.02	>39.66	>39.66	ND	>39.66	>39.66	39.66 ± 17.29
R89439 (α-APA)	0.014	0.006	>10	>10	>10	>10

[a] 50% inhibitory concentration, or concentration required to inhibit the viral cytopathic effect by 50% in MT-4 cells. Data represent average values ± SD for at least two independent experiments. [b] S0561945. [c] 50% cytotoxic concentration, or concentration that reduced the MT-4 cell viability by 50%. [d] Crystals observed at 16.15 μM.

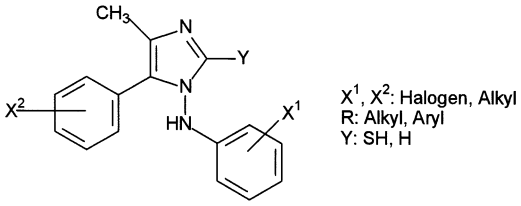
abolishment of the antiviral activity (**8.01**, **8.02** vs **3.01**, **3.19**). Comparison of the antiviral activity of a series of compounds bearing methyl, ethyl, propyl, or phenyl at position 4 of the imidazole moiety revealed that the smaller the substituent, the more pronounced was the antiviral activity. The nature of the different substituents at position 5 of the imidazole ring suggested the need of an aromatic group with certain limitations to

Table 3. Inhibitory effects of *N*-Aminoimidazoles **3** and **7** on MSV-Induced Transformation of C3H/3T3 Embryo Murine Fibroblast in Vitro

compd	IC_{50} [a] (μM)	MIC [b] (μM)
3.01	38.0 ± 14.2	>63.3 (316.6)
3.02	>12.7	≥12.7
3.05	>10.1	>10.1 (50.7)
3.07	>11.4	>11.4 (57.1)
3.13	>64.6	>64.6 (323.2)
3.19	>67.7	>67.7 (338.5)
3.24	>12.13	>12.13 (60.63)
3.39	>12.07	>12.07 (60.35)
7.01	6.9 ± 3.8	>14.1 (70.5)
7.02	6.89 ± 1.10	>11.03 (55.15)
7.03	5.37 ± 1.04	≥12.6
7.09	13.37 ± 2.05	>15.2 (75.95)

[a] 50% effective dose. [b] Minimal inhibitory concentration.

Chart 4. General Structural Requirements of the *N*-Aminoimidazole Derivatives **3** and **7**



its substitution pattern. Chloro or bromo being well accepted in the meta position, the same halogens introduced at the para position reduced the antiviral properties of the molecules.

The nature of the substitutions on the anilino substitutent at position 1 of the imidazole has serious repercussions on the antiviral activity of the *N*-aminoimidazoles and 2-thiones (NAIMs) **3** and **7**. Not only do these substitutions influence the potency, more importantly they do influence the activity spectrum of the molecules. The most active molecules were found among meta-substituted anilino derivatives. The active molecules bearing a chlorine at position 3 of the anilino phenyl ring had a broader activity spectrum [HIV-1 (strains III_B and NL4.3), HIV-1 (NNRTI-resistant strain with mutations K103N, Y181C in the RT), HIV-2(ROD), SIV(mac251), and MSV(Moloney)]. The activity of these molecules is mainly due to a yet unidentified mode of action taking place immediately after the integration is finished. Replacement of the *m*-chloro on the aniline moiety by a methyl increases the NNRTI action of the molecules (see **3.01** vs **3.19**).

Time of Intervention. A time of addition (TOA) experiment was carried out to investigate which step of the replicative cycle was inhibited by different *N*-aminoimidazoles **3** and **7**. Briefly, this experiment determines how long the addition of an anti-HIV compound can be postponed before losing its antiviral activity in the viral replication cycle. Virus was added at a high multiplicity of infection (moi = 0.5) to synchronize all steps of the viral replication. Reference compounds with a known mode of action were included. Dextran sulfate, a polyanion, is known to interfere with the binding of the virus to the cell. The nucleoside analogue AZT inhibits the reverse transcription process. L-708,906 is an established inhibitor of the strand transfer step of the integration process.[10] Ritonavir is an inhibitor of the proteolytic cleavage.

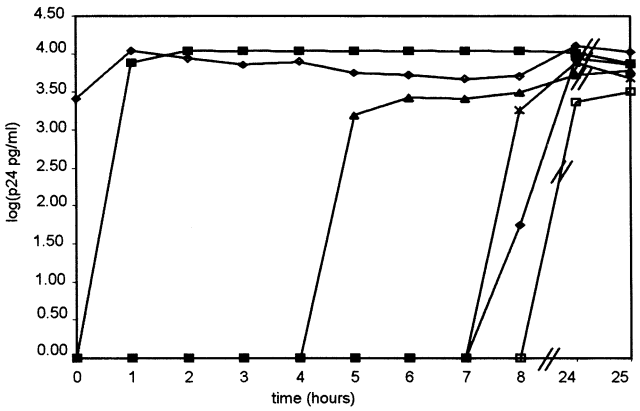


Figure 1. Time of addition experiment. MT-4 cells were infected with HIV-1(IIIB) at a multiplicity of infection of 0.5, and the test compounds were added at different times postinfection. Viral p24 Ag production was determined at 31 h postinfection and is expressed as the log 10 of the p24 Ag content in pg/mL. Symbols: (◇) control; (■) dextran sulfate; (▲) AZT; (◆) L-708,906; (∗), 7.03; (□) ritonavir.

Addition of these compounds can be delayed for 0, 4, 7, and 18−19 h (Figure 1). Addition of **3.19** or **7.03** can be postponed for up to 7 h postinfection, suggesting a possible interaction at the moment of integration. These results should be interpreted with care, not excluding the possibility of an interaction with earlier processes in the HIV replication cycle because TOA experiments only reveal the last step targeted by the inhibitor.

Reverse Transcriptase. Several compounds from the **3** and **7** series have been evaluated against recombinant HIV-1 reverse transcriptase). **3.19** was inhibiting at 125 μM using polyrA.oligodT as the template primer and at 105 μM when using polyrC.oligodG. This compound was, however, inactive against Maloney murine leukaemia virus RT using endogenous RNA as the template at 1000 μM.

Because the in vitro inhibition of the HIV-1 RT activity was observed for **3.19** and typical NNRTI-resistance mutations were observed when genotypically analyzing the HIV-1(III_B) strain selected in the presence of increasing concentrations of **3.19**, it is clear that **3.19** acts as an NNRTI in addition to its action at a later process in the viral replication cycle.

Inhibition of HIV-1 Production from Chronically Infected Cells. When **7.03** was evaluated for its influence on the production of virus from chronically HIV-1(NL4.3)-infected HUT-78 cells, we noticed an inhibitory effect. From TOA experiments, in vitro RT assays suggested the possibility of an action at the reverse transcription, integration, or later step in the replicative cycle of HIV. AZT, ritonavir, and L-708,906 were also included in the experiment. For molecules acting before the termination of the integration process, AZT and L-708,906, no inhibitory effect on release of virus could be observed (Figure 2).

As expected, for ritonavir an inhibition of the virus production was observed. **7.03** inhibited virus production up to the level of ritonavir. This fact reveals that although the unknown target of molecule **7.03** is situated around the moment of integration, it must coincide with a process taking place immediately after integration.

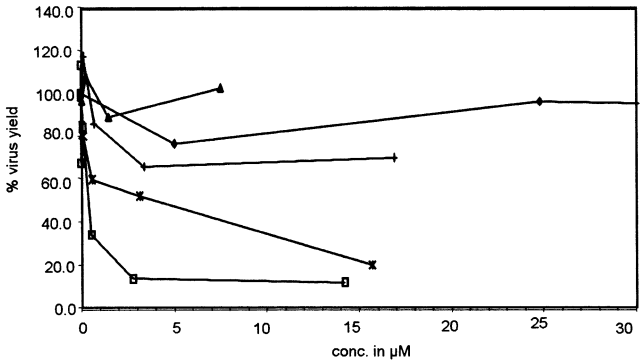


Figure 2. Percentage of virus (HIV-1 NL4.3) produced from chronically infected HUT78 cells in the presence of varying concentrations of compounds. After incubation for 44 h, virus p24 Ag production was determined. Symbols: (▲) AZT; (◆) L-708,906; (+) **3.19**; (∗) **7.03**; (□) ritonavir.

Conclusion

From a study to discover new NNRTIs with anti-HIV activity against clinically relevant mutants, a group of molecules with a new antiretroviral mode of action was identified. Although the exact molecular target remains to be resolved, the experiments clearly show that some of the *N*-aminoimidazole derivatives interfere with an (immediate) postintegrational event occurring after integration of the viral DNA into the host cell genome.

On the basis of a limited structure−activity relationship, it was possible to identify some structural requirements that confer antiretroviral activity via a new mode of action to these molecules.

Experimental Section

Materials and General Chemical Methods. NMR spectra were recorded on a Varian, Gemini 200 spectrometer (for ^{1}H (200 MHz) and ^{13}C). ^{13}C and ^{1}H are referenced to TMS. All NH and OH protons were assigned by exchange with D_2O. Exact mass measurements were performed on a quadrupole time-of-flight mass spectrometer (Q-Tof-2, Micromass, Manchester, U.K.) equipped with a standard electrospray ionization (ESI) interface. Samples were infused in a 2-propanol/water (1:1) mixture at 3 μL/min. TLC was performed with TLC aluminum sheets (Merck, silica gel 60 F254), and silica (200−425 mesh) was used for column chromatography. Melting points (mp [°C]) were determined with a Büchi-SMP-20 capillary melting apparatus. For all reactions, analytical grade solvents were used.

Syntheses of 1-Amino-2,3-dihydro-1*H*-imidazole-2-thiones 3. General Procedure. To a stirred solution of the α-haloketone **2** (2.5 mmol) in acetic acid (10 mL), potassium thiocyanate (0.37 g, 3.8 mmol) was added at ambient temperature. After 30 min the hydrazine **1** (2.5 mmol) or hydrazine hydrochloride was added. Following stirring for 4 h at 30 °C, the reaction mixture was evaluated by TLC (CH_2Cl_2/MeOH = 99 :1). In case no product formation could be observed, the mixture was warmed to 80 °C for 2 h. After addition of water (30 mL), the precipitate was filtered off and washed with water. In general, recrystallization from methanol afforded pure products. Only in some cases, further purification by column chromatography (silica, CH_2Cl_2/MeOH = 99 :1) was necessary. When *N*,*N*-methylphenylhydrazine was used as a starting material, 2,3-dihydro-4-methyl-1-(*N*-methyl-*N*-phenylamino)-5-phenyl-1*H*-imidazole-2-thione **3.42** could be obtained in an analogous way.

Synthesis of 1-(3-Chlorophenylamino)-2,3-dihydro-4,5-diphenyl-1*H*-imidazole-2-thione (3.26). A mixture of benzoine (1.0 g, 7.1 mmol), KSCN (0.68 g, 7.1 mmol), and 3-chlorophenylhydrazine·HCl (1.26 g, 7.1 mmol) was stirred in acetic acid (10 mL) for 2 days. The white precipitate formed was filtered off (0.4 g). Water (50 mL) was added, and the additional precipitate formed was likewise filtered off. Recrystallization from MeOH/water (1:1) yielded 1.5 g (56%) of **3.26**.

Conversion of Methoxycarbonyl Groups into the Free Acids. General Procedure. The corresponding methoxycarbonyl-substituted imidazoline-2-thione derivative (1.33 mmol) (**3.27**, **3.34**, **3.35**) was stirred in a 10% solution of NaOH (25 mL) for 6 h. After washing with CH_2Cl_2, the aqueous layer was acidified. The precipitate formed was filtered off and dried.

Synthesis of 5-(3-Carboxamidophenyl)-1-(3-chlorophenylamino)-2,3-dihydro-4-methyl-1*H*-imidazole-2-thione (3.38). A mixture of the corresponding methoxycarbonyl-substituted imidazoline-2-thione **3.34** (1.33 mmol) was stirred in NH_3/MeOH fore 2 days. After removal of the solvent, the residue was recrystallized from MeOH/water (1:3) to yield **3.38** in 73% yield.

Preparation of 1-(3-Chlorophenylamino)-4-methyl-2-methylsulfanyl-5-phenyl-1*H*-imidazole (8.01). A mixture of 1-(3-chlorophenylamino)-2,3-dihydro-4-methyl-5-phenyl-1*H*-imidazoline-2-thione (**3.01**) (0.1 g, 0.37 mmol) and methyl iodide (1.19 g, 8.40 mmol) in dichloromethane (5 mL) was heated under reflux for 1 h. Excess of methyl iodide and solvent were removed in vacuo. Further purification was achieved by recrystallization from CH_2Cl_2/*n*-hexane, 1:1.

Preparation of 2-Benzylsulfanyl-1-(3-chlorophenylamino)-4-methyl-5-phenyl-1*H*-imidazole (8.02). A mixture of 1-(3-chlorophenylamino)-2,3-dihydro-4-methyl-5-phenyl-1*H*-imidazoline-2-thione (**3.01**) (0.20 g, 0.62 mmol) and benzyl chloride (0.08 g, 0.62 mmol) in pyridine (5 mL) was heated under reflux for 3 h. After removal of the solvent, the crude product was purified by column chromatography (silica gel, ethyl acetate/hexane = 2:1.

Synthesis of 1-Arylamino-1*H*-imidazoles 7. General Procedure. A suspension of the respective 1-amino-2,3-dihydro-1*H*-imidazole-2-thione (**3**) (2 mmol) in glacial acetic acid (10 mL) was stirred in an ice bath. Upon dropwise addition of 30% hydrogen peroxide (1 mL, 9.76 mmol), the reaction mixture cleared up, resulting in a clear-brown solution. After stirring for another 15 min, the reaction mixture was made alkaline (pH 8−9) with 10% NaOH. The resulting precipitate was filtered off, washed with cold water, and recrystallized from water.

Experimental data concerning the syntheses of the starting hydrazines **1** and α-bromo compounds **2**, the NMR data of all NAIMs (*N*-aminoimidazole-2-thiones **3**, *N*-aminoimidazoles **7**, and imidazole derivatives **8**−**10**), and elemental analyses are available as Supporting Information.

Reference Compounds. Dextran sulfate with an average molecular weight of 5000 was purchased from Sigma. AZT was synthesized as previously described.[11] Loviride (α-APA, R89439) was obtained from Janssen Research Foundation (Beerse, Belgium). The diketo acid L-708,906[13a] was kindly provided by Drs. N. Neamati and T. R. Burke (NIH, Bethesda, MD). Ritonavir (ABT538) was kindly provided by J. M. Leonard, Abbott Laboratories (Abbott Park, IL).

Viruses and Cells. The origin of HIV-1(III_B) virus stock has been described.[12] The HIV-1(NL4.3) strain[13] is a molecular clone obtained from the National Institutes of Health (Bethesda, MD). HIV-1 and HIV-2(ROD)[14] stocks were obtained from the culture supernatant of HIV-1- or HIV-2-infected MT-4 cells, respectively. Simian immunodeficiency virus, SIV(mac251), was originally isolated by Daniel et al.[15] and was obtained from C. Bruck (Smith Kline-RIT, Rixensart, Belgium). SIV(mac251) stocks were prepared from the supernatant of SIV-infected MT-4 cells.[16] S0561945 is an HIV-1(III_B) strain possessing the K103N and Y181C mutations in its reverse transcriptase gene, resulting in resistance toward nonnucleoside reverse transcriptase inhibitors. MSV was prepared from tumors induced following intramuscular inoculation of 3-day-old NMRI mice with MSV, as described previously.[17]

Antiviral Activity and Cytotoxicity Assays. The inhibitory effects of the *N*-aminoimidazole derivatives on HIV-1,

HIV-2, and SIV replication were monitored by measuring the viability of MT-4 cells 5 days after infection.[18] Cytotoxicity of the compounds was determined in parallel by measuring the viability of mock-infected cells on day 5. The number of viable cells was quantified semiautomatically by a tetrazolium-based colorimetric method using 3-(4,5-dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium (MTT), as described by Pauwels et al.[20]

Peripheral blood mononuclear cells (PBMCs) from healthy donors were isolated by density centrifugation (Lymphoprep, Nycomed Pharma AS, Diagnostics, Oslo, Norway) and stimulated with phytohemagglutinin (PHA, 2 μg/mL final concentration) (Sigma Chemical Co., Bornem, Belgium) for 3 days. The activated cells (PHA-stimulated blasts) were washed three times with PBS, and viral infections were done as described by the ACTG protocols.[19] HIV-infected or mock-infected PHA-stimulated blasts were cultured in the presence of 25 U/mL of IL-2 and varying concentrations of drugs. The supernatant was collected at day 7, and HIV-1 core antigen (p24 Ag) in the supernatant was analyzed by enzyme linked immunosorbent assay (ELISA) (NEN, Brussels, Belgium).

C3H/3T3 cells were seeded at 20 000 cells per milliliter into wells of tissue culture cluster plates (48 wells per plate). Following a 24 h incubation period, cell cultures were infected with 80 focus-forming units of MSV for 120 min, whereafter the culture medium was replaced by 1 mL of fresh medium containing appropriate concentrations of the test compound. After 6 days, transformation of the cells was examined microscopically.[20]

Time-of-Addition Experiment. MT-4 cells were infected with HIV-1(III_B) at a multiplicity of infection (moi) of 0.5. The test compounds were added at different times after infection.[21] Viral p24 Ag production was determined at 31 h postinfection by ELISA (NEN, Brussels, Belgium). The reference compounds were added at 100 times their 50% inhibitory concentration (IC_{50}) obtained in the MT-4/MTT assay.

Inhibition of HIV-1 Production from Chronically Infected Cells. Persistently infected (NL4.3) HUT-78 cells were washed three times in order to remove cell-free virus. The test compounds at the required concentrations were transferred into the cups of a 48-well plate. To each cup 200 000 cells were added (final volume of 1 mL). The cultures were allowed to grow for 44 h. The supernatant was collected, and viral p24 Ag production was determined by ELISA (NEN, Brussels, Belgium).

HIV-1 RT Assay. Poly(rC).oligo(dG) and [^{3}H]dGTP and poly(rA).oligo(dT) and [^{3}H]dTTP were used as a template primer and radiolabeled substrate, respectively. The final [^{3}H]dGTP and [^{3}H]dTTP concentrations in the reaction mixture were both 2.5 μM.

Selection of 3.19- and 7.03-Resistant Virus Strains. HIV-1(III_B) was subjected to passages in MT-4 cells (4×10^5 cells/2 mL) in the presence of increasing concentrations of the compounds. Passages were performed every 3−4 days.

Determination of the Amino Acid Sequence of the 3.19- and 7.03-Resistant HIV-1 Strains RT. The procedures for the MT-4 cells infection with resistant HIV-1, preparation of the samples for the PCR assays, amplification of the proviral DNA, and sequencing of the 727-bp fragment covering amino acid residues 50−270 have been reported elsewhere.[22]

HIV Integrase Assays. Wild type His-tagged recombinant HIV-1 integrase and the substrate and target DNA were as previously described.[23] The following high-performance-liquid-chromatography-purified deoxynucleotides were purchased from Amersham-Pharmacia Biotech: INT1, 5′-TGTGGAAAATCTCTAGCAGT; INT2, 5′-ACTGCTAGAGATTTTCCACA; T35, 5′-ACTATACCAGACAATAATTGTCTGGCCTGTACCGT; SK70, 5′-ACGGTACAGGCCAGACAATTATTGTCTGGTATAGT. The oligodeoxynucleotides INT1 and INT2 correspond to the U5 end of the HIV-1 LTR. The 3′-processing, overall integration, and strand-transfer assay were slightly modified from published procedures. The final reaction mixture of the 3′-processing assays contained 20 mM HEPES, pH 7.5, 5 mM dithiothreitol, 10 mM $MgCl_2$, 75 mM NaCl, 5% (v/v) poly(ethylene glycol) 8000, 15% dimethyl sulfoxide, 30 nM oligodeoxynucleotide substrate (INT1/INT2), and 230 nM His-tag IN in a volume of 10 μL. Inhibitors were incubated briefly with the reaction components before the addition of IN. Reactions were started by addition of the enzyme. All the reactions were stopped by addition of formamide loading dye, and the products were separated in a 15% denaturating polyacrylamide/urea gel. Autoradiography was performed by exposing the wet gel to X-ray film (CURIX RP1, Agfa). Quantification of the results was performed using the Cyclone (Canberra Packard, Zellik, Belgium).

To evaluate inhibition of 3′-processing, the reaction was allowed to proceed at 37 °C for 7 min. Inhibition of formation of the −2 band was determined. In the overall integration assay, the reaction was allowed to proceed for 60 min. Both inhibition of formation of the −2 band and DNA strand transfer products were scored. The DNA strand transfer was assayed in the following way. DNA substrate (INT1/INT2, 30 nM) was preincubated with 230 nM IN at 37 °C for 5 min to allow the cleavage reaction to occur. The composition of the reaction mixture was identical to that in the processing assay. After 5 min, 1 μL of excess target DNA (SK70/T35, at a final concentration of 250 nM) was added with or without inhibitor and the samples were incubated at 37 °C for 1 h. The excess of unspecific target DNA competitively blocked further binding of IN to the viral substrate so that inhibition of DNA strand transfer in the preformed complexes can be monitored independently of DNA binding and 3′-processing.

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Supporting Information Available: Syntheses and NMR data of NAIM derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) De Clercq, E. Toward improved anti-HIV chemotherapy: Therapeutic strategies for intervention with HIV infections. *J. Med. Chem.* **1995**, *38*, 2491−2517.

(2) (a) Coffin, J. M. HIV population dynamics in vivo: implications for genetic variation, pathogenesis, and therapy. *Science* **1995**, *267*, 483−489. (b) Vandamme, A.-M.; Van Vaerenbergh, K.; De Clercq, E. Anti-human immunodeficiency virus drug combination strategies. *Antiviral Chem. Chemother.* **1998**, *9*, 187−203.

(3) Richman, D. D.; Havlir, D.; Corbeil, J.; Looney, D.; Ignacio, C.; Spector, S. A.; Sullivan, J.; Cheeseman, S.; Barringer, K.; Pauletti, D.; Shih, C.-K.; Myers, M.; Griffin, J. Nevirapine resistance mutations of human immunodeficiency virus type 1 selected during therapy. *J. Virol.* **1994**, *68*, 1660−1666.

(4) Fujiwara, T.; Sato, A.; El-Farrash, M.; Miki, S.; Abe, K.; Isaka, Y.; Kodama, M.; Wu, Y.; Chen, L. B.; Harada, H.; Sugimoto, H.; Hatanaka, M.; Hinuma, Y. S-1153 inhibits replication of known drug-resistant strains of human immunodeficiency virus type 1. *Antimicrob. Agents Chemother.* **1998**, *42*, 1340−1345.

(5) Ren, J.; Nichols, C.; Bird, L. E.; Fujiwara, T.; Sugimoto, H.; Stuart, D. I.; Stammers, D. K. Binding of the second generation non-nucleoside inhibitor S-1153 to HIV-1 reverse transcriptase involves extensive main chain hydrogen bonding. *J. Biol. Chem.* **2000**, *275*, 14316−14320.

(6) Schantl, J. G.; Lagoja, I. Direct synthetic approach to *N*-substituted 1-amino-2,3-dihydro-1*H*-imidazole-2-thiones. *Heterocycles* **1997**, *45*, 691−700.

(7) Sitte, A.; Wessel, R.; Paul, H. Umwandlung von 1,3,4-Thiadiazolinen in 1,2,4-Triazole durch Dimroth-Umlagerung. *Monatsh. Chem*. **1975**, *106*, 1291−1296.
(8) Schantl, J. G.; Lagoja, I. M. Oxidative Reduction of Cyclic Thiosemicarbazides: Conversion of 1-Arylamino-2,3-dihydro-1*H*-imidazole-2-thiones into 1-Arylamino-1*H*-imidazoles. *Heterocycles* **1998**, *48* (5), 929−938.
(9) Dodd, D. S.; Kozikowski, Conversion of Alcohols to Protected Guanidines Using the Mitsunobu Protocol. *Tetrahedron Lett*. **1994**, *35* (7), 977−980.
(10) (a) Hazuda, D. J.; Felock, P.; Witmer, M.; Wolfe, A.; Stillmock, K.; Grobler, J. A.; Espeseth, A.; Gabryelski, L.; Schleif, W.; Blau, C.; Miller, M. D. Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. *Science* **2000**, *287*, 646−650. (b) Pluymers, W.; Pais, G.; Van Maele, B.; Pannecouque, C.; Fikkert, V.; Burke, T.; De Clercq, E.; Witvrouw, M.; Neamati, N.; Debyser, Z. Inhibition of HIV-1 integrase activity and replication by a series of diketo derivatives. *Antimicrob. Agents Chemother*., submitted.
(11) Horwitz, J. P.; Chua, J.; Noel, M. Nucleoside V, the monomesylates of 1-(2′-deoxy-β-D-lyxyfuranosyl)thymine. *J. Org. Chem*. **1964**, *29*, 2076.
(12) Popovic, M.; Sarngadharan, M. G.; Read, E.; Gallo, R. C. Detection, Isolation and Continuous Production of Cytopathic Retroviruses (HTLV-III) from patients with AIDS or pre-AIDS. *Science* **1984**, *224*, 497−500.
(13) Adachi, A.; Gendelman, H.; Koenig, S.; Folks, T.; Willey, R.; Rabson, A.; Martin, M. Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. *J. Virol*. **1986**, *59*, 284−291.
(14) Barré-Sinoussi, F.; Chermann, J. C.; Nugeyre, M. T.; Chamaret, S.; Gruest, J.; Dauguet, C.; Axler-Blin, C.; Vézinet-Brun, F.; Rouzioux, C.; Rozenbaum, W.; Montagnier, L. Isolation of a T-Lymphotropic Retrovirus from a Patient at Risk for AIDS. *Science* **1983**, *220*, 868−871.
(15) Daniel, M. D.; Letvin, N. L.; Sehgal, P. K.; Hunsmann, G.; Schmidt, D. K.; King, N. W.; Desrosiers, R. C. Long-term persistent infection of macaque monkeys with the simian immunodeficiency virus. *J. Gen. Virol*. **1987**, *68*, 3183−3189.
(16) Harada, S.; Koyanagi, Y.; Yamamoto, N. Infection of HTLV-III/LAV in HTLV-I carrying cells MT-2 and MT-4: an application in a plaque assay. *Science* **1985**, *229*, 563−566.
(17) De Clercq, E.; Merigan, T. C. Moloney sarcoma virus-induced tumors in mice: inhibition or stimulation by (polyrI):poly(rC). *Proc. Soc. Exp. Biol. Med*. **1971**, *137*, 590−594.
(18) Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. Rapid and Automated Tetrazolium-Based Colorimetric Assay for Detection of anti-HIV Compounds. *J. Virol. Methods* **1988**, *20*, 309−321.
(19) Japour, A. J.; Mayers, D. L.; Johnson, V. A.; Kuritzkes, D. R.; Beckett, L. A.; Arduino, J. M.; Lane, J.; Black, R. J.; Reichelderfer, P. S.; D'Aquila, R. T.; Crumpacker, C. S. Standardized peripheral blood mononuclear cell culture assay for determination of drug susceptibilities of clinical human immunodeficiency virus type 1 isolates. The RV-43 Study Group, the AIDS Clinical Trials Group Virology Committee Resistance Working Group. *Antimicrob. Agents Chemother.* **1993**, *37*, 1095−1101.
(20) Balzarini, J.; Holý, A.; Jindrich, J.; Naesens, L.; Snoeck, R.; Schols, D.; De Clercq, E. Differential antiherpesvrius and antiretrovirus effects of the (*S*) and (*R*) enantiomers of acyclic nucleoside phosphonates: potent and selective in vitro and in vivo antiretrovirus activities of (*R*)-9-52-phosphonomethoxypropyl)-2,6-diaminopurine. *Antimicrob. Agents Chemother*. **1993**, *37*, 332−338.
(21) Pauwels, R.; Andries, K.; Desmyter, J.; Schols, D.; Kukla, M. J.; Breslin, H. J.; Raeymaeckers, A.; Van Gelder, J.; Woestenborghs, R.; Heykants, J. Potent and selective inhibition of HIV-1 replication by a novel series of TIBO derivatives. *Nature* **1990**, *343*, 470−474.
(22) (a) Balzarini, J.; Karlsson, A.; Pérez-Pérez, M.-J.; Vrang, L.; Walbers, J.; Zhang, H.; Öberg, B.; Vandamme, A.-M.; Camarasa, M.-J.; De Clercq, E. HIV-1-specific reverse transcriptase inhibitors show differential activity against HIV-1 mutant strains containing different amino acids substitutions in the reverse transcriptase. *Virology* **1993**, *192*, 246−253. (b) Balzarini, J.; Karlsson, A.; De Clercq, E. Human immunodeficiency virus type 1 drug resistance patterns with different 1-(2-hydroxyethoxy)-methyl-6-(phenylthio)thymine derivatives. *Mol. Pharmacol*. **1993**, *44*, 694−701.
(23) (a) Cherepanov, P.; Esté, J. A.; Rando, R. F.; Ojwang, J. O.; Reekmans, G.; Steinfeld, R.; David, G.; De Clercq, E.; Debyser, Z. Mode of action of G-quartets with the integrase of human immunodeficiency virus type 1. *Mol. Pharmacol*. **1997**, *52*, 771−780. (b) Debyser, Z.; Cherepanov, P.; Pluymers, W.; De Clercq, E. Assays for the evaluation of HIV-1 integrase inhibitors. In *Methods in Molecular Biology. Nucleases*. Humana Press: Clifton, NJ, 2001.

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