Dihydro(alkylthio)(naphthylmethyl)oxopyrimidines: Novel Non-Nucleoside Reverse Transcriptase Inhibitors of the *S*-DABO Series

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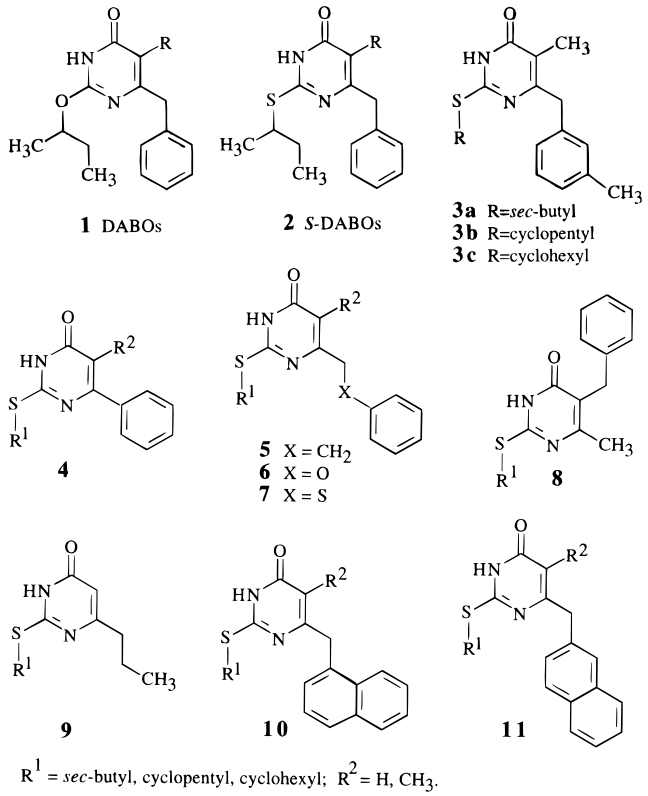
Novel compounds related to 2-(cyclohexylthio)-3,4-dihydro-5-methyl-6-(3-methylbenzyl)-4-oxopyrimidine (**3c**, MC 639) have been synthesized and tested as inhibitors of human immunodeficiency virus type-1 (HIV-1). Reaction of thiourea with ethyl arylmethylacetoacetates furnished 5-alkyl-6-(arylmethyl)-3,4-dihydro-2-mercapto-4-oxopyrimidines which were then alkylated at the sulfur atom to afford the required 2-alkylthio or 2-cycloalkylthio derivatives (*S*-DABOs). Chemical modifications at N-3, C-4, and C-6 of the pyrimidine ring were attempted with the aim of improving antiretroviral activity. In particular, replacement of the benzyl group with the 1-naphthylmethyl moiety enhanced the activity of *S*-DABOs, whereas N-3 alkylation and C═O transformation into C═S at position 4 of the pyrimidine ring led to compounds devoid of anti-HIV-1 activity. Lower activity was generally observed when 1-naphthylmethyl was replaced by the isomeric 2-naphthylmethyl moiety. The most active compounds showed activity in the low micromolar range with EC_{50} values comparable to that of nevirapine.

Dihydroalkoxybenzyloxopyrimidines **1** (DABOs) are a new class of specific inhibitors of human immunodeficiency virus type 1 (HIV-1) which possess a benzyl moiety and an alkyl (cycloalkyl) chain linked through an oxygen bridge to the uracil or thymine base.[1−3] Replacement of the side-chain oxygen with a sulfur atom furnished thio-DABOs **2** (*S*-DABOs), which showed increased anti-HIV-1 activity.[4] Structure−activity relationship (SAR) studies on the *S*-DABO series have led to the selection of structures of formula **3a**−**c** as lead compounds for further investigations.

With the aim to improve the anti-HIV-1 activity of *S*-DABOs, we planned various chemical modifications. These included the replacement of the benzyl group at position 6 of the pyrimidine ring with phenyl (**4**), phenylethyl (**5**), phenoxymethyl (**6**), or (phenylthio)-methyl (**7**) moieties; the shift of the benzyl group from the C-6 to the C-5 of the pyrimidine ring (reversed *S*-DABOs) (**8**); the replacement of benzyl with smaller (propyl) (**9**) or bulkier [1- (**10**) and 2- (**11**) naphthylmethyl] substituents; the introduction of a variety of substituents at the C-4 position of the pyrimidine ring. *sec*-Butylthio, cyclopentylthio, and cyclohexylthio chains were chosen as substituents at the C-2 of the pyrimidine ring because of the highest anti-HIV-1 activity shown by these pharmacophores in the *S*-DABO series. Introduction of a methyl group at C-5 was also planned for a comparison of structure−activity relationships between the uracil and thymine series.

Chart 1



Chemistry

The synthesis of the newly designed products is described in Scheme 1. Derivatives obtained by chemical modifications of **3c** at position 4 of the pyrimidine ring are reported in Scheme 2. Novel 4-oxo-1,2,3,4-tetrahydro-2-thioxopyrimidines **14c**−**f,h**−**l** and known pyrimidines **14a,b**,[5] **g,**[6] **m,**[7] which are key intermediates for the synthesis of new DABO derivatives, were prepared starting from ethyl benzoylacetate (**13a**), ethyl (2-benzylacetylacetate) (**13m**),[7] ethyl 2-acylacetates **13b**,[8] **c,d**,[9] **e,f,** and 2-acylpropionates **13g**,[10] **h**,[11] **i**−**l** (Scheme 1). *β*-Oxo ester derivatives **13b**−**l** were prepared by reaction between potassium ethyl malonate

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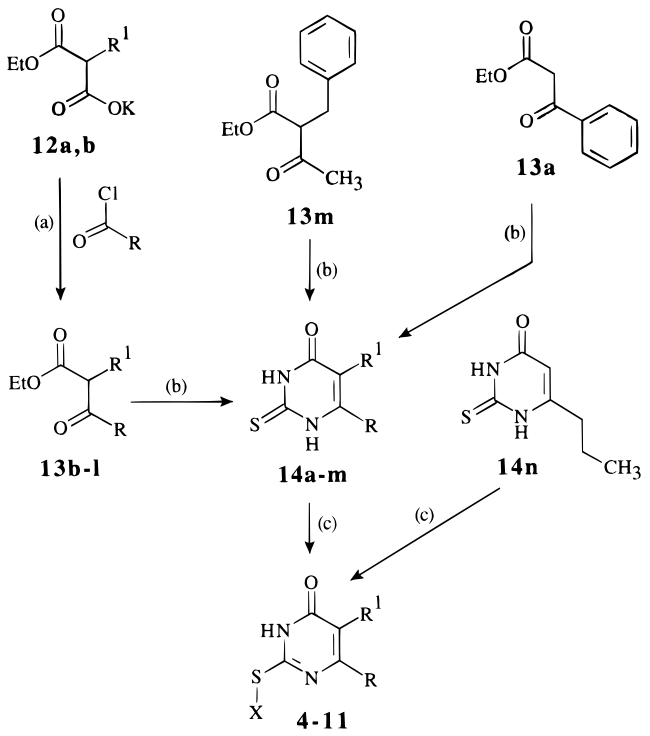
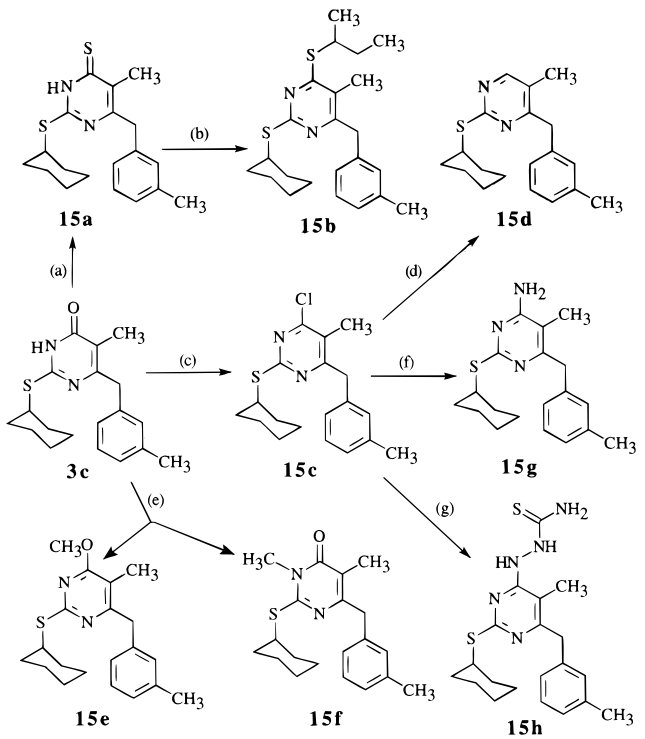
Scheme 1[a]



[a] (a) $MgCl_2$, Et_3N, MeCN; (b) $CS(NH_2)_2$, EtONa, EtOH; (c) X-Hal, K_2CO_3, DMF.

Scheme 2[a]



[a] Lawesson's reagent; (b) 2-iodobutane, K_2CO_3; (c) DMF, $POCl_3$; (d) Zn, NH_4OH; (e) CH_3I, K_2CO_3; (f) NH_4OH; (g) thiosemicarbazide.

12a[12] or potassium ethyl 2-methylmalonate **12b** and the corresponding acyl chloride, in acetonitrile as solvent, in the presence of magnesium chloride−triethylamine system according to the Clay procedure.[13] This method was simpler and more reddititious than previously reported procedures[7−11,14] [compare yields of **13b** (90%), **13c** (90%), **13d** (89%), **13g** (92%), and **13h** (87%) with those of literature (64%, 79%, 75%, 83%, and 73%, respectively)]. Condensation of ethyl 2-acylacetates **13a**−**f**, 2-acylpropionates **13g**−**l**, or (2-benzyl)acetylacetate (**13m**) with thiourea in the presence of sodium ethoxide in boiling ethanol gave 4-oxo-1,2,3,4-tetrahydro-2-thioxopyrimidines **14a**−**f**, their 5-methyl analogues **14g**−**l**, and the 5-benzylpyrimidyl derivative **14m**, respectively. Selective *S*-alkylation of derivatives **14a**−**m** and 4-oxo-6-propyl-1,2,3,4-tetrahydro-2-thioxopyrimidine (**14n**) in anhydrous DMF with the proper alkyl or cycloalkyl halide in the presence of potassium carbonate afforded the required DABO derivatives **4**−**11** (Scheme 1).

Treatment of **3c** with Lawesson's reagent afforded **15a**, which was alkylated with 2-iodobutane to yield **15b**. Preparation of 4-chloro derivative **15c** was achieved by reaction of **3c** with phosphorus oxychloride−DMF complex. Dehalogenation of **15c** to obtain **15d** was performed with zinc dust in ammonium hydroxide. Alkylation of **3c** with methyl iodide in the presence of potassium carbonate gave a mixture of 4-*O*-methyl (**15e**) and 3-*N*-methyl (**15f**) derivatives. Displacement of chlorine from **15c** with ammonium hydroxide and thiosemicarbazide afforded **15g**,**h**, respectively.

Results and Discussion

The cytotoxicity and capability of derivatives **4**−**11**, **14**, and **15** to inhibit the HIV-1-induced cytopathogenicity was tested in MT-4 cells (Table 3). In these *in vitro* assays selected dihydro(alkylthio)benzyloxopyrimidines **3a**−**c**, AZT, and nevirapine were used as reference drugs, and all were confirmed to be both potent and selective HIV-1 inhibitors. The majority of compounds were noncytotoxic for MT-4 cells at doses as high as 300 μM, and only a few showed CC_{50} values at concentrations around 50 μM or lower (**6d**, **11a**, **15f**,**h**).

Maximum activity was obtained with compounds of series **10** and related derivatives **11**, among which **10a**,**b**,**j** were endowed with the highest potency (EC_{50} = 1.0, 0.3, and 1.0, respectively) and selectivity (SI = >300, >900, and >300, respectively). Compounds belonging to the other series were considerably less potent and selective (**5**−**7**) or totally inactive (**4**, **8**, **9**, **14**, and **15**).

SAR studies allow to identify several structural features of the title compounds which are essential for anti-HIV activity. The first one relates to the substituent at position 6 of the pyrimidine ring. Replacement of the benzyl group with phenyl (**4a**−**f**) or alkyl (**9a**,**b**) moieties results in compounds totally devoid of anti-HIV-1 activity. The introduction of phenylethyl (**5a**−**f**), phenoxymethyl (**6a**−**f**), and (phenylthio)methyl (**7a**-**f**) groups leads to compounds with modest antiviral activity, whereas the introduction of 1-naphthylmethyl (**10a**−**l**) and 2-naphthylmethyl (**11a**−**f**) substituents led to numerous compounds endowed with potencies in the low-micromolar range. It is noteworthy that, when the substituents at positions 5 and 6 of the pyrimidine ring (see reference compounds **3a**−**c**[4]) are interchanged (**8a**−**c**), loss of activity ensues.

As with previous DABOs, the absence of bulky substituents at the C-2 position of uracil and thymine rings correlates with the absence of antiviral activity;[1,3,4] in fact, derivatives **14c**−**m**, lacking substituents at the thio group, are devoid of anti-HIV-1 activity.

Table 1. Physical and Chemical Data for Compounds **4**−**11**

compd	R	R_1	X	mp (°C)	cryst[a] solvent	reagents[b]	yield (%)
4a	phenyl	H	*sec*-butyl	197−198 dec	A	c	42
4b	phenyl	H	cyclopentyl	229−230	A	c	35
4c	phenyl	H	cyclohexyl	217−220	B	d	39
4d	phenyl	Me	*sec*-butyl	152−155	C	c	49
4e	phenyl	Me	cyclopentyl	187−189	B	c	41
4f	phenyl	Me	cyclohexyl	190−192	B	d	30
5a	phenylethyl	H	*sec*-butyl	95−96	C	c	58
5b	phenylethyl	H	cyclopentyl	115−116	C	c	59
5c	phenylethyl	H	cyclohexyl	125−127	C	d	47
5d	phenylethyl	Me	*sec*-butyl	90−93	C	c	53
5e	phenylethyl	Me	cyclopentyl	153−155	D	c	49
5f	phenylethyl	Me	cyclohexyl	177−180	D	d	60
6a	phenoxymethyl	H	*sec*-butyl	115−117	A	c	65
6b	phenoxymethyl	H	cyclopentyl	183−185	A	c	69
6c	phenoxymethyl	H	cyclohexyl	189−190	A	c	59
6d	phenoxymethyl	Me	*sec*-butyl	135−137	B	c	64
6e	phenoxymethyl	Me	cyclopentyl	168−170	A	c	57
6f	phenoxymethyl	Me	cyclohexyl	166−168	B	d	42
7a	(phenylthio)methyl	H	*sec*-butyl	85−87	D	c	59
7b	(phenylthio)methyl	H	cyclopentyl	106−109	D	c	64
7c	(phenylthio)methyl	H	cyclohexyl	130−132	B	d	61
7d	(phenylthio)methyl	Me	*sec*-butyl	105−106	B	c	71
7e	(phenylthio)methyl	Me	cyclopentyl	140−142	B	c	63
7f	(phenylthio)methyl	Me	cyclohexyl	160−162	B	d	54
8a	Me	benzyl	*sec*-butyl	139−142	B	c	58
8b	Me	benzyl	cyclopentyl	157−159	B	c	49
8c	Me	benzyl	cyclohexyl	174−176	B	d	45
9a	*n*-propyl	H	*sec*-butyl	148−151	B	c	70
9b	*n*-propyl	H	cyclohexyl	122−123	C	d	43
10a	1-naphthylmethyl	H	isopropyl	187−188	G	c	58
10b	1-naphthylmethyl	H	*sec*-butyl	161−162	A	c	54
10c	1-naphthylmethyl	H	2-pentyl	111−112	B	c	64
10d	1-naphthylmethyl	H	3-pentyl	136−137	D	c	40
10e	1-naphthylmethyl	H	cyclopentyl	172−173	F	c	72
10f	1-naphthylmethyl	H	cyclohexyl	175−176	F	d	43
10g	1-naphthylmethyl	H	cycloheptyl	166−167	F	d	77
10h	1-naphthylmethyl	H	benzyl	179−180	G	c	62
10i	1-naphthylmethyl	H	*n*-undecyl	94−95	C	c	60
10j	1-naphthylmethyl	Me	*sec*-butyl	176−177	B	c	52
10k	1-naphthylmethyl	Me	cyclopentyl	187−188	G	c	54
10l	1-naphthylmethyl	Me	cyclohexyl	212−213	F	d	48
11a	2-naphthylmethyl	H	*sec*-butyl	133−134	B	c	62
11b	2-naphthylmethyl	H	cyclopentyl	182−184	G	c	60
11c	2-naphthylmethyl	H	cyclohexyl	175−176	F	d	52
11d	2-naphthylmethyl	Me	*sec*-butyl	161−162	D	c	40
11e	2-naphthylmethyl	Me	cyclopentyl	218−219	G	c	51
11f	2-naphthylmethyl	Me	cyclohexyl	213−214	F	d	47

[a] A = benzene; B = cyclohexane; C = *n*-hexane; D = *n*-hexane/cyclohexane; E = ethanol; F = ethyl acetate; G = benzene/cyclohexane; H = diethyl ether/petroleum ether. [b] See Scheme 1.

Interestingly, when compared to uracil counterparts, thymine derivatives bearing the *sec*-butyl substituent are slightly more cytotoxic; the sole exceptions to this rule are the derivatives bearing 1-naphthylmethyl and 2-naphthylmethyl substituents. As far as the anti-HIV-1 activity is concerned, the introduction of a methyl group at the C-5 position of the pyrimidine ring leads to an increase in potency in the case of phenylethyl and phenoxyethyl derivatives, which thus behaved like the benzyl counterparts.[4] In this respect, (phenylthio)-methyl, 1-naphthylmethyl, and 2-naphthylmethyl derivatives have an opposite behavior, showing a significant decrease in potency. Finally, the variety of modifications at the C-4 position of the pyrimidine ring, including N-3 alkylation, C═O transformation into C═S, etc., leads to compounds devoid of anti-HIV-1 activity.

In order to directly prove that, like the parent benzyl DABOs, they were targeted at the HIV-1 reverse transcriptase (RT), title compounds were tested against a homodimeric HIV-1 recombinant enzyme using poly(rC)-oligo$(dG)_{12-18}$ as template primer (Table 4). While **3a**−**c** and nevirapine remained active at concentrations comparable to those active in cell culture-based assays, compounds **6**, **10**, and **11** were inhibitory to the enzyme, although at concentrations 10−30-fold higher.

Active compounds representative of the various types of chemical modifications were tested for their capability to inhibit the HIV-2 multiplication in acutely infected C8166 cells and the infectious HIV-1 yield in chronically infected $H9/III_B$ cells, but none was found effective (Table 4).

Antiviral therapy with non-nucleoside RT inhibitors is often compromised by the appearance of drug-resistant strains of HIV-1. Improved activity against mutant enzymes may be beneficial in terms of suppressing the emergence of drug-resistant strains of virus. Therefore, we evaluated the inhibiting activity of derivatives **6d**, **10b**,**e**,**j**,**k,** and **11a**,**c**,**d** against two resistant HIV-1 variants generated by serial passage in the presence of either nevirapine or **3c**. The first mutant virus (Nev^R) is known to carry, in the *pol* gene, a tyrosine to cysteine substitution at amino acid 181 (Y181C) that is known to confer broad cross-resistance

Table 2. Physical and Chemical Data for Compounds **12**−**15**

compd	R	R_1	mp (°C)	cryst[a] system	reagents[b]	yield (%)
12b			134−137 dec			80
13e	1-naphthylmethyl	H	oil		a	100
13f	2-naphthylmethyl	H	43−44	C	a	90
13i	phenoxymethyl	Me	oil		a	88
13j	(phenylthio)methyl	Me	oil		a	85
13k	1-naphthylmethyl	Me	oil		a	100
13l	2-naphthylmethyl	Me	98−100	H	a	88
14c	phenoxymethyl	H	252−253	E	b	62
14d	(phenylthio)methyl	H	203−204	E	b	67
14e	1-naphthylmethyl	H	>280	E	b	78
14f	2-naphthylmethyl	H	264−266 dec	E	b	74
14h	phenylethyl	Me	262−265	E	b	58
14i	phenoxymethyl	Me	247−250	E	b	65
14j	(phenylthio)methyl	Me	163−166	A	b	69
14k	1-naphthylmethyl	Me	220−221	E	b	52
14l	2-naphthylmethyl	Me	230−231	E	b	65
15a			187−188	B	a	80
15b			oil		b	91
15c			58−59	E	c	100
15d			oil		d	57
15e			oil		e	48
15f			oil		e	40
15g			117.5−118.5	D	f	94
15h			164−165	I	g	52

[a] A = benzene; B = cyclohexane; C = *n*-hexane; D = *n*-hexane/cyclohexane; E = ethanol; F = ethyl acetate; G = benzene/cyclohexane; H = diethyl ether/petroleum ether; I = diethyl ether. [b] See Scheme 1 or 2.

to most NNRTIs.[15] The second one (MC639^R) carries a valine to aspartic acid substitution at amino acid 179 (V179D) of the *pol* gene (unpublished results). Like nevirapine, none of the *S*-DABO derivatives was inhibitory to the nevirapine-resistant virus (Table 4). However, the new compounds were inhibitory to the **3c**-resistant virus (MC639^R), although at doses 7−11 times higher than the respective EC_{50}s against the wt strain. The sole 2-naphthylmethyl derivative **11a** was equally effective against both the wt and the MC639^R strain.

Conclusion

DABOs have emerged as a new class of non-nucleoside reverse transcriptase inhibitors.[1−3] Initially, their anti-HIV-1 activity has been significantly increased by substituting the 2-alkoxy chain with a corresponding 2-alkylthio chain (*S*-DABOs).[4] Now we report that further improvement in potency can be obtained through the substitution of the benzyl moiety with a 1-naphthylmethyl group (DATNOs).

Interestingly, following studies on the crystal structure of the anti-HIV-1 RT complexed with MKC-442 and TNK-651, Hopkins *et al.*[16] have recently concluded that their compounds interact with the enzyme in such a way that it could be possible to accommodate a 6-naphthylmethyl substituent as a replacement of the 6-benzyl group. Whether this substitution effectively leads to active HEPT derivatives is not known at present; however, if this will occur, further evidence will be gained that HEPT and DABO derivatives share similar chemical requirements for anti-HIV activity.

When the EC_{50} values obtained in viral spread assays are compared to the IC_{50} values obtained in enzyme assays, DATNOs behave unlike all the other DABO derivatives. Their EC_{50}s are, in fact, 10−30 times lower than IC_{50}s, whereas under the same experimental conditions, **3c** and nevirapine show very similar values. Although differences between EC_{50} and IC_{50} values of up to 3 orders of magnitude have been obtained with NNRTIs,[17−19] the possibility cannot be excluded that DATNOs target an additional step of the HIV-1 multiplication cycle. However, besides being specific inhibitors of the HIV-1 acute infection, DATNOs do not inhibit the multiplication of HIV-2 or a nevirapine-resistant mutant, suggesting that the main target of DATNOs is the HIV-1 reverse transcriptase.

It is finally worth noting that DATNOs, although inactive against nevirapine-resistant mutants, retain substantial activity against an *S*-DABO-resistant variant which carries a valine to aspartic acid substitution at amino acid 179 of RT (V179D) and is cross-resistant to all previous DABO derivatives (unpublished results). Such a phenomenon, already described for new derivatives of delarvidine and atervidine,[20] reinforces the concept that it is possible to develop derivatives retaining activity against variants resistant to close congeners.

Experimental Section

Melting points were determined on a Büchi 530 melting point apparatus and are uncorrected. Infrared (IR) spectra (Nujol mulls) were recorded on a Perkin-Elmer 297 instrument. ^{1}H-NMR spectra were recorded at 90 MHz on a Varian EM-390 spectrometer. Chemical shifts are reported in δ (ppm) units relative to the internal reference tetramethylsilane (TMS). All compounds were routinely checked by TLC and ^{1}H-NMR. NMR spectral data were consistent with the indicated structures. TLC was performed by using aluminum-backed silica gel plates (Merck DC-Alufolien Kieselgel 60 F_{254}). Developed plates were visualized by UV light. Chromatographic purifications were performed with Merck silica gel 60. Solvents were reagent grade and, when necessary, were purified and dried by standard methods. Concentration of solutions after reactions and extractions involved the use of a rotary evaporator operating at a reduced pressure of approximately 20 Torr. Organic solutions were dried over anhydrous sodium sulfate. Analytical results agreed to within ±0.40% of the theoretical values. 2-Iodopropane, 2-iodobutane, 2-bromopentane, 3-bromopentane, cyclopentyl bromide, cyclohexyl bromide, cycloheptyl bromide, benzyl bromide, and 1-bromoundecane were purchased from Aldrich Chimica, Milan, Italy. Specific examples presented below illustrate general synthetic procedures. Samples prepared for physical data (Tables 1 and 2) and biological studies (Tables 3 and 4) were

Table 3. Cytotoxicity and Anti-HIV Activity of Derivatives **4**−**11**, **14**, and **15**[a]

compd	R	R_1	X	CC_{50}[b] (μM)	EC_{50}[c] (μM)	SI[d]
4a	phenyl	H	*sec*-butyl	>300	>300	
4b	phenyl	H	cyclopentyl	>300	>300	
4c	phenyl	H	cyclohexyl	>300	>300	
4d	phenyl	Me	*sec*-butyl	126	>126	
4e	phenyl	Me	cyclopentyl	175	>175	
4f	phenyl	Me	cyclohexyl	>300	>300	
5a	phenylethyl	H	*sec*-butyl	180	125	1.4
5b	phenylethyl	H	cyclopentyl	112	81.5	1.4
5c	phenylethyl	H	cyclohexyl	133	68	1.9
5d	phenylethyl	Me	*sec*-butyl	105	18	6
5e	phenylethyl	Me	cyclopentyl	>300	19	>16
5f	phenylethyl	Me	cyclohexyl	>300	27	>11
6a	phenoxymethyl	H	*sec*-butyl	>300	52	>6
6b	phenoxymethyl	H	cyclopentyl	>300	148	>2
6c	phenoxymethyl	H	cyclohexyl	>300	>300	
6d	phenoxymethyl	Me	*sec*-butyl	60	8.9	7
6e	phenoxymethyl	Me	cyclopentyl	>300	24	>13
6f	phenoxymethyl	Me	cyclohexyl	>300	22	>14
7a	(phenylthio)methyl	H	*sec*-butyl	288	53	5.4
7b	(phenylthio)methyl	H	cyclopentyl	300	66	4.5
7c	(phenylthio)methyl	H	cyclohexyl	>300	32	>9.4
7d	(phenylthio)methyl	Me	*sec*-butyl	75	23	3.3
7e	(phenylthio)methyl	Me	cyclopentyl	>300	150	>2
7f	(phenylthio)methyl	Me	cyclohexyl	>300	150	>2
8a	Me	benzyl	*sec*-butyl	>300	>300	
8b	Me	benzyl	cyclopentyl	>300	>300	
8c	Me	benzyl	cyclohexyl	>300	>300	
9a	*n*-propyl	H	*sec*-butyl	246	>246	
9b	*n*-propyl	H	cyclohexyl	210	>210	
10a	1-naphthylmethyl	H	isopropyl	>300	1.0	>300
10b	1-naphthylmethyl	H	*sec*-butyl	>300	0.33	>909
10c	1-naphthylmethyl	H	2-pentyl	158	4.6	34
10d	1-naphthylmethyl	H	3-pentyl	200	4.0	50
10e	1-naphthylmethyl	H	cyclopentyl	>300	2.0	>150
10f	1-naphthylmethyl	H	cyclohexyl	>300	3.1	>96
10g	1-naphthylmethyl	H	cycloheptyl	>300	3.0	>100
10h	1-naphthylmethyl	H	benzyl	>300	12.6	>24
10i	1-naphthylmethyl	H	*n*-undecyl	>300	>300	
10j	1-naphthylmethyl	Me	*sec*-butyl	>300	1.0	>300
10k	1-naphthylmethyl	Me	cyclopentyl	>300	6.0	>50
10l	1-naphthylmethyl	Me	cyclohexyl	>300	63	>4.7
11a	2-naphthylmethyl	H	*sec*-butyl	40	2.8	14
11b	2-naphthylmethyl	H	cyclopentyl	>300	3.7	>8.1
11c	2-naphthylmethyl	H	cyclohexyl	>300	1.7	>128
11d	2-naphthylmethyl	Me	*sec*-butyl	80	1.7	47
11e	2-naphthylmethyl	Me	cyclopentyl	>300	15	>20
11f	2-naphthylmethyl	Me	cyclohexyl	>300	125	>2.4
14c	phenoxymethyl	H		>300	>300	
14d	(phenylthio)methyl	H		>300	>300	
14e	1-naphthylmethyl	H		>300	>300	
14f	2-naphthylmethyl	H		>300	>300	
14i	phenylethyl	Me		>300	>300	
14j	phenoxymethyl	Me		>300	>300	
14k	(phenylthio)methyl	Me		>300	>300	
14l	1-naphthylmethyl	Me		145	>145	
14m	2-naphthylmethyl	Me		91	>91	
15a				176	14.6	12
15b				>300	>300	
15c				133	>133	
15d				128	>128	
15e				>300	>300	
15f				44	>44	
15g				>200	>200	
15h				24	>24	
3a				100	1	100
3b				>300	0.6	>500
3c				>300	0.6	>500
nevirapine				>300	0.25	>1200
AZT				>80	0.01	>2000

[a] Data represent mean values for three separate experiments. Standard errors average ≤10% of the respective means. [b] Compound dose required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method. [c] Compound dose required to achieve 50% protection of MT-4 cells from the HIV-1-induced cytopathogenicity, as determined by the MTT method. [d] Selectivity index, CC_{50}/EC_{50}.

dried in high vacuum over P_2O_5 for 20 h at temperatures ranging from 25 to 90 °C, depending on the melting point of the sample considered.

2-(Cyclohexylthio)-3,4-dihydro-5-methyl-6-(3′-methylbenzyl)-4-thioxopyrimidine (15a). A mixture of **3c** (0.20 g, 0.61 mmol) and 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-

Table 4. Activity of Derivatives **6**, **10**, and **11** against wt and Drug-Resistant HIV and HIV-1 rRT[a]

	IC_{50}[b]	EC_{50}[c]			
compd	HIV-1 rRT	HIV-2	HIV-1/ $H9/III_B$	Nev^R	$MC639^R$
6d	>100	>100	>100	>100	>100
10b	8.1	>100	>100	>100	2.1
10e	48.7	>100	>100	>100	15.3
10j	22.5	>100	>100	>100	6.0
10k	45.0	>100	>100	>100	16.2
11a	7.4	>100	>100	>100	4.1
11c	33.1	>100	>100	>100	20.0
11d	29.7	>100	>100	>100	17.8
3c	0.9	>100	>100	>100	>100
nevirapine	0.27	>100	>100	>100	0.31
AZT		0.015	>20	0.013	0.003

[a] Data represent mean values for three separate experiments. Standard errors average ≤10% of the respective means. [b] Compound concentration (μM) required to inhibit the HIV-1 rRT activity by 50%. [c] Compound dose (μM) required to achieve 50% protection of C8166 (HIV-2) or MT-4 (Nev^R, $MC639^R$) cells from the HIV-induced cytopathogenicity or to decrease the virus titer by 1 log (HIV-1 in chronically infected $H9/III_B$ cells).

diphosphetane 2,4-disulfide (Lawesson's Reagent) (0.37 g, 0.92 mmol) in anhydrous benzene (50 mL) was refluxed for 1 h. After cooling, the solvent was removed to afford **15a**, which was purified by passing through a silica gel column eluting with chloroform: IR ν 3100, 1600 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.27−1.48 (m, 6H, C_3,C_4,C_5 cyclohexane-H), 1.72−1.76 (m, 2H, C_{2eq},C_{6eq} cyclohexane-H), 1.96−2.01 (m, 2H, C_{2ax},C_{6ax} cyclohexane-H), 2.32 (s, 3H, 3′-Me), 2.39 (s, 3H, C_5-Me), 3.75−3.80 (m, 1H, SCH), 3.93 (s, 2H, CH_2Ar), 7.03−7.04 (m, 3H, 2′,4′,6′-H Ar), 7.15−7.20 (m, 1H, 5′-H Ar). Anal. ($C_{19}H_{24}N_2S_2$, 344.53) C, H, S.

2-(Cyclohexylthio)-5-methyl-6-(3′-methylbenzyl)-4-[(2-methylpropyl)thio]pyrimidine (15b). A mixture of **15a** (40 mg, 0.120 mmol), 2-iodobutane (24.3 mg, 0.015 mL, 0.132 mmol) and potassium carbonate (18.2 mg, 0.132 mmol) in anhydrous *N*,*N*-dimethylformamide (1 mL) was stirred at room temperature for 8 h. The reaction content was poured on cold water (100 mL), and extracted with ethyl acetate (3 × 50 mL). The organic layers were collected, washed with brine (3 × 50 mL), dried, and evaporated to furnish crude **15b** which was then purified by column chromatography on silica gel (eluent: *n*-hexane/ethyl acetate, 10/1): ^{1}H-NMR ($CDCl_3$) δ 1.02−1.07 (t, 3H, CH_2CH_3), 1.28−1.55 (m, 6H, C_3,C_4,C_5 cyclohexane-H), 1.40−1.42 (d, 3H, $CHCH_3$, overlapped signal), 1.70−1.81 (m, 4H, C_{2eq},C_{6eq} cyclohexane-H, CH_2CH_3), 2.09−2.19 (m, 2H, C_{2ax},C_{6ax} cyclohexane-H), 2.09 (s, 3H, C_5-Me overlapped signal), 2.32 (s, 3H, 3′-Me), 3.74−3.80 (m, 1H, SCH cyclohexane), 3.96−4.02 (m, 1H, $CHCH_3$), 3.98 (s, 2H, CH_2Ar overlapped signal), 7.00−7.06 (m, 3H, 2′,4′,6′-H Ar), 7.16−7.19 (m, 1H, 5′-H Ar). Anal. ($C_{23}H_{32}N_2S_2$, 400.64) C, H, S.

4-Chloro-2-(cyclohexylthio)-5-methyl-6-(3′-methylbenzyl)pyrimidine (15c). A mixture of anhydrous *N*,*N*-dimethylformamide (0.20 g, 0.21 mL, 2.74 mmol) and phosphorus oxychloride (0.42 g, 0.25 mL, 2.74 mmol) was stirred at room temperature for 1 h; then a solution of **3c** (0.60 g, 1.83 mmol) in anhydrous chloroform (10 mL) was added. The resulting mixture was stirred at room temperature for a further 5 h; then the reaction was quenched with aqueous sodium hydrogen carbonate (50 mL), and the phases were separated. The aqueous layer was extracted twice with fresh chloroform (20 mL), and the organic extracts were collected, washed with brine (2 × 50 mL), dried, and evaporated to give pure **15c**: ^{1}H-NMR ($CDCl_3$) δ 1.27−1.52 (m, 6H, C_3,C_4,C_5 cyclohexane-H), 1.74−1.78 (m, 2H, C_{2eq},C_{6eq} cyclohexane-H), 2.06−2.10 (m, 2H, C_{2ax},C_{6ax} cyclohexane-H), 2.27 (s, 3H, C_5-Me), 2.32 (s, 3H, 3′-Me), 3.72−3.78 (m, 1H, SCH), 4.05 (s, 2H, CH_2Ar), 7.00−7.06 (m, 3H, 2′,4′,6′-H Ar), 7.16−7.20 (m, 1H, 5′-H Ar). Anal. ($C_{19}H_{23}ClN_2S$, 346.92) C,H,Cl,S.

2-(Cyclohexylthio)-5-methyl-6-(3′-methylbenzyl)-pyrimidine (15d). Zinc dust (1.0 g, 15.3 mmol) and 28% ammonium hydroxide (20 mL) were added to a solution of **15c** (0.20 g, 0.58 mmol) in tetrahydrofuran (5 mL), and the mixture was refluxed for 5 h. After cooling, the resulting mixture was filtered, diluted with water (100 mL), and extracted with ethyl acetate (3 × 50 mL). The organic layers were collected, washed with brine (3 × 50 mL), dried, and evaporated to give a residue, which was purified by column chromatography on silica gel (eluent: chloroform): ^{1}H-NMR ($CDCl_3$) δ 1.26−1.48 (m, 6H, C_3,C_4,C_5 cyclohexane-H), 1.74−1.78 (m, 2H, C_{2eq},C_{6eq} cyclohexane-H), 2.07−2.09 (m, 2H, C_{2ax},C_{6ax} cyclohexane-H), 2.16 (s, 3H, C_5-Me), 2.31 (s, 3H, 3′-Me), 3.72−3.78 (m, 1H, SCH), 4.00 (s, 2H, CH_2Ar), 7.02−7.04 (m, 3H, 2′,4′,6′-H Ar), 7.15−7.18 (m, 1H, 5′-H Ar), 8.20 (s, 1H, C_4-H). Anal. ($C_{19}H_{24}N_2S$, 312.47) C, H, S.

2-(Cyclohexylthio)-4-methoxy-5-methyl-6-(3′-methylbenzyl)pyrimidine (15e) and 2-(Cyclohexylthio)-3,4-dihydro-3,5-dimethyl-6-(3′-methylbenzyl)-4-oxopyrimidine (15f). A mixture of **3c** (0.20 g, 0.61 mmol), methyl iodide (0.13 g, 0.057 mL, 0.91 mmol), and potassium carbonate (0.13 g, 0.91 mmol) in anhydrous *N*,*N*-dimethylformamide (1 mL) was stirred at room temperature for 2 h and then poured on cold water (100 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layers were collected, washed with brine (3 × 50 mL), dried, and evaporated to afford a mixture of **15e,f**, which were separated by column chromatography on silica gel eluting with chloroform.

15e: ^{1}H-NMR ($CDCl_3$) δ 1.26−1.53 (m, 6H, C_3,C_4,C_5 cyclohexane-H), 1.75−1.82 (m, 2H, C_{2eq},C_{6eq} cyclohexane-H), 2.05 (s, 3H, C_5-Me), 2.07−2.14 (m, 2H, C_{2ax},C_{6ax} cyclohexane-H), 2.30 (s, 3H, 3′-Me), 3.72−3.78 (m, 1H, SCH), 3.93 (s, 3H, OCH_3), 3.97 (s, 2H, CH_2Ar), 7.00−7.05 (m, 3H, 2′,4′,6′-H Ar), 7.12−7.18 (m, 1H, 5′-H Ar). Anal. ($C_{20}H_{26}N_2OS$, 342.50) C,H,N,S.

15f: IR ν 1640 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.26−1.46 (m, 6H, C_3,C_4,C_5 cyclohexane-H), 1.72−1.78 (m, 2H, C_{2eq},C_{6eq} cyclohexane-H), 1.98−2.04 (m, 2H, C_{2ax},C_{6ax} cyclohexane-H), 2.14 (s, 3H, C_5-Me), 2.32 (s, 3H, 3′-Me), 3.44 (s, 3H, NCH_3), 3.72−3.83 (m, 1H, SCH), 3.83 (s, 2H, CH_2Ar overlapped signal), 7.00−7.08 (m, 3H, 2′,4′,6′-H Ar), 7.15−7.18 (m, 1H, 5′-H Ar). Anal. ($C_{20}H_{26}N_2OS$, 342.50) C,H,N,S.

4-Amino-2-(cyclohexylthio)-5-methyl-6-(3′-methylbenzyl)pyrimidine (15g). A mixture of **15c** (0.40 g, 1.15 mmol) in ethanol (5 mL) and ammonium hydroxide (5.0 N, 60 mL) was heated with stirring in a sealed tube at 120 °C for 10 h. After cooling, the reaction mixture was diluted with water (300 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layers were collected, washed with brine (100 mL), dried, and evaporated to give **15g**, which was purified by recrystallization from diethyl ether: IR ν 3460, 3290 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.33−1.51 (m, 6H, C_3,C_4,C_5 cyclohexane-H), 1.73−1.77 (m, 2H, C_{2eq},C_{6eq} cyclohexane-H), 1.98 (s, 3H, C_5-Me), 2.05−2.10 (m, 2H, C_{2ax},C_{6ax} cyclohexane-H), 2.30 (s, 3H, 3′-Me), 3.71−3.81 (m, 1H, SCH), 3.94 (s, 2H, CH_2Ar), 4.76 (br s, 2H, NH_2), 6.98−7.05 (m, 3H, 2′,4′,6′-H Ar), 7.11−7.19 (m, 1H, 5′-H Ar). Anal. ($C_{19}H_{25}N_3S$, 327.49) C,H,N,S.

2-(Cyclohexylthio)-5-methyl-6-(3′-methylbenzyl)-4-thiosemicarbazidopyrimidine (15h). A mixture of **15c** (0.13 g, 0.36 mmol) and thiosemicarbazide (0.06 g, 0.72 mmol) in anhydrous ethanol (2 mL) was refluxed for 2 h. After cooling, the reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3 × 20 mL). The organic layers were collected, washed with brine (3 × 50 mL), dried, and evaporated to give a residue which was purified by column chromatography on silica gel (eluent: chloroform/ethyl acetate, 1/1): IR ν 3400−3200, 1600 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.26−1.53 (m, 6H, C_3,C_4,C_5 cyclohexane-H), 1.60−1.68 (m, 2H, C_{2eq},C_{6eq} cyclohexane-H), 1.87−2.01 (m, 2H, C_{2ax},C_{6ax} cyclohexane-H), 1.92 (s, 3H, C_5-Me overlapped signal), 2.23 (s, 3H, 3′-Me), 3.75−3.83 (m, 4H, SCH, $NHCSNH_2$), 3.97 (s, 2H, CH_2Ar), 4.65 (br s, 1H, PyNH), 6.91−7.06 (m, 3H, 2′,4′,6′-H Ar), 7.11−7.19 (m, 1H, 5′-H Ar). Anal. ($C_{20}H_{27}N_5S_2$, 401.59) C,H,S.

General Procedure for the Preparation of Derivatives 13b−l. Example: Ethyl 2-[(2-Phenylthio)acetyl]propionate (13j). Triethylamine (14.6 mL, 100.0 mmol) and then magnesium chloride (7.05 g, 74.0 mmol) were added with stirring to a cooled (10−15 °C) suspension of potassium ethyl 2-methylmalonate (**12b**) (11.7 g, 63.5 mmol) in dry acetonitrile

(100 mL). After the addition, the mixture was stirred at room temperature for 2 h and cooled to 0 °C, and 2-(phenylthio)-acetyl chloride (5.0 g, 27.0 mmol) followed by triethylamine (2.0 mL, 13.7 mmol) was added dropwise. The reaction mixture was stirred overnight at room temperature; then 13% HCl (100 mL) was cautiously added while keeping the temperature below 25 °C. After stirring for 15 min more, the organic layer was separated, washed with brine (3 × 50 mL), dried, and evaporated to yield **13j** (5.0 g). The aqueous layer was extracted with ethyl acetate (2 × 50 mL), washed with brine (100 mL), dried, and concentrated to give additional **13j** (1.0 g). The collected residues were chromatographed on a silica gel column (eluent: chloroform) to yield pure **13j** (5.9 g): IR ν 1700, 1720 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.17−1.29 (m, 6H, CH_2CH_3, $CHCH_3$), 3.83 (s, 2H, SCH_2), 3.83−3.94 (q, 1H, $CHCH_3$ overlapped signal), 4.07−4.18 (q, 2H, CH_2CH_3), 7.18−7.34 (m, 5H, SPh). Anal. ($C_{13}H_{16}O_3S$, 252.33) C, H, S.

General Procedure for the Preparation of Derivatives 14c−f,h−l. Example: 4-Oxo-6-(phenoxymethyl)-1,2,3,4-tetrahydro-2-thioxopyrimidine (14c). Thiourea (3.27 g, 43.0 mmol) and **13c** (6.9 g, 31.0 mmol) were added to a solution of sodium metal (1.43 g, 0.062 g-atom) in 30 mL of absolute EtOH, and the mixture was heated at reflux for 5 h. After evaporation at 40−50 °C the residue was dissolved in water (50 mL), neutralized with 0.5 N AcOH, and extracted with AcOEt (3 × 50 mL). The organic extracts were combined, washed with brine (100 mL), dried, and evaporated. The residue was recrystallized from ethanol to afford pure **14c**: IR ν 3000, 1660 cm^{-1}; ^{1}H-NMR (DMF-d_7) δ 4.94 (s, 2H, CH_2), 5.96 (s, 1H, C-5 H pyrimidine ring), 6.97−7.02 (m, 3H, C-2′,4′,6′ H phenyl ring), 7.29−7.35 (m, 2H, C-3′,5′ H phenyl ring), 12.48−12.51 (m, 2H, NH exchangeable with D_2O). Anal. ($C_{11}H_{10}N_2O_2S$, 234.27) C, H, N, S.

General Procedure for the Preparation of Derivatives 4a,b,d,e, 5a,b,d,e, 6a,b,d,e, 7a,b,d,e, 8a,b, 9a, 10a−e,g−k, and 11a,b,d,e. Example: 3,4-Dihydro-2-[(1-methylpropyl)thio]-4-oxo-6-(1-naphthylmethyl)pyrimidine (10b). A mixture of **14e** (0.54 g, 2.0 mmol), 2-iodobutane (0.40 g, 0.25 mL, 2.2 mmol) and potassium carbonate (0.29 g, 2.1 mmol), in anhydrous *N*,*N*-dimethylformamide (2 mL) was stirred at room temperature for 8 h. The mixture was poured on cold water (100 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layers were collected, washed with brine (3 × 50 mL), dried, and evaporated to give **10b**, which was chromatographed on a silica gel column (*n*-hexane/ethyl acetate/methanol, 12/3/1, as eluent) and then recrystallized from cyclohexane: IR ν 2900, 1640 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88−0.96 (t, 3H, CH_2CH_3), 1.25−1.29 (d, 3H, $CHCH_3$), 1.53−1.69 (m, 2H, CH_2CH_3), 3.78−3.88 (q, 1H, $CHCH_3$), 4.25 (s, 2H, CH_2-Naph), 7.37−7.95 (m, 7H, 1-Naph), 13.16 (s, 1H, NH exchangeable with D_2O). Anal. ($C_{19}H_{20}N_2OS$, 324.44) C, H, N, S.

General Procedure for the Preparation of Derivatives 4c,f, 5c,f, 6c,f, 7c,f, 8c, 9b, 10f,l, and 11c,f. Example: 2-(Cyclohexylthio)-3,4-dihydro-5-methyl-4-oxo-6-(phenoxymethyl)pyrimidine (6f). A mixture of **14i** (0.50 g, 2.0 mmol), cyclohexyl bromide (0.36 g, 0.27 mL, 2.2 mmol), and potassium carbonate (0.29 g, 2.1 mmol) in anhydrous *N*,*N*-dimethylformamide (2 mL) was heated at 80 °C for 15 h. After cooling, the mixture was poured on cold water (100 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layers were collected, washed with brine (3 × 50 mL), dried, and evaporated to furnish crude **6f** as a yellowish solid purified by column chromatography on silica gel (eluent: *n*-hexane/ethyl acetate/methanol 12/3/1) and recrystallization from cyclohexane: IR ν 2900, 1640 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.19−1.35 (m, 6H, C_3,C_4,C_5-cyclohexyl), 1.60−1.67 (m, 4H, C_2,C_6-cyclohexyl), 2.04 (s, 3H, CH_3), 3.89−3.91 (m, 1H, SCH), 4.91 (s, 2H, CH_2OPh), 6.86−6.95 (m, 3H, C-2′,4′,6′ H phenyl ring), 7.16−7.20 (m, 2H, C-3′,5′ H phenyl ring). Anal. ($C_{18}H_{22}N_2O_2S$, 330.44), C, H, N, S.

Antiviral Assay Procedures. 1. Compounds. Compounds were solubilized in DMSO at 200 μM and then diluted in culture medium.

2. Cells and Viruses. MT-4, C8166, H9/III_B, and CEM cells were grown at 37 °C in a 5% CO_2 atmosphere in RPMI 1640 medium, supplemented with 10% fetal calf serum (FCS), 100 IU/mL penicillin G, and 100 μg/mL streptomycin. Cell cultures were checked periodically for the absence of mycoplasma contamination with a MycoTect Kit (Gibco). Human immunodeficiency viruses type-1 (HIV-1 III_B strain) and type-2 (HIV-2 ROD strain; kindly provided by Dr. L. Montagnier, Paris) were obtained from supernatants of persistently infected H9/III_B and CEM cells, respectively. HIV-1 and HIV-2 stock solutions had titers of 4.5×10^6 and 1.4×10^5 50% cell culture infectious dose ($CCID_{50}$)/mL, respectively.

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

Anti-HIV Assays. Activity of the compounds against HIV-1 and HIV-2 multiplication in acutely infected cells was based on the inhibition of virus-induced cytopathicity in MT-4 and C8166 cells, respectively. Briefly, 50 μL of culture medium containing 1×10^4 cells was added to each well of flat-bottom microtiter trays containing 50 μL of culture medium with or without various concentrations of the test compounds. Then 20 μL of an HIV suspension containing 100 (HIV-1) or 1000 (HIV-2) $CCID_{50}$ (50% cell culture infective dose) was added. After a 4 day incubation (5 days for HIV-2) at 37 °C, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-1-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[21] Cytotoxicity of the compounds was evaluated in parallel with their antiviral activity. It was based on the viability of mock-infected cells, as monitored by the MTT method.

RT Assays. Assays were performed as previously described.[22] Briefly, purified rRT was assayed for its RNA-dependent polymerase-associated activity in a 50 μL volume containing 50 mM Tris-HCl (pH 7.8), 80 mM KCl, 6 mM $MgCl_2$, 1 mM DTT, 0.1 mg mL^{-1} BSA, 0.5 OD_{260} unit mL^{-1} template: primer [poly(rC)-oligo$(dG)_{12-18}$] and 10 μM [^{3}H]dGTP (1 Ci $mmol^{-1}$). After incubation for 30 min at 37 °C, the samples were spotted on glass fiber filters (Whatman GF/A), and the acid-insoluble radioactivity was determined.

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