Synthesis and Anti-HIV-1 Activity of Thio Analogues of Dihydroalkoxybenzyloxopyrimidines

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Received February 28, 1995[⊗]

Various thio analogues of dihydroalkoxybenzyloxopyrimidines (DABOs), a new class of non-nucleoside reverse transcriptase inhibitors, were found to selectively inhibit the HIV-1 multiplication *in vitro*. Among the C-5 H-substituted 6-benzyl-3,4-dihydro-4-oxopyrimidines, the introduction of alkylthio or cycloalkylthio substituents at C-2 of the pyrimidine ring led to derivatives (S-DABOs) which were up to 10-fold more potent than the alkyloxy or cycloalkyloxy counterparts. The further introduction of a methyl group at the 3′-position of the benzyl portion of 2-(alkylthio)-6-benzyluracils reduced the cytotoxicity leading to more selective compounds. Among C-5 methyl-substituted S-DABOs, numerous derivatives showed EC_{50} values as low as 0.6 μM and lacked cytotoxicity at doses as high as 300 μM. In the C-5 double methyl-substituted series, a more pronounced cytotoxicity was observed and the further introduction of a methyl at the 3′-position in the benzylidene group resulted in total loss of antiviral activity. S-DABOs, namely 2-(alkylthio)-6-benzyl-3,4-dihydro-4-oxopyrimidines, were synthesized by reacting proper methyl (phenylacetyl)acetates or their 2-methyl compounds with thiourea to afford 6-benzyl-4-oxo-1,2,3,4-tetrahydro-2-thiaoxopyrimidines or the related 5-methyl derivatives. Treatment of the latter derivatives with alkyl or cycloalkyl halides in alkaline medium gave the required title compounds.

Several steps in the HIV growth cycle have been identified as potential targets for drug design.[1-4] Among them, retrotranscription of single-stranded genomic RNA into double-stranded DNA by the reverse transcriptase (RT) has been one of the most widely studied. Essentially, two types of RT inhibitors have been described so far: nucleoside analogues[5-10] and non-nucleoside compounds (NNRTIs).[11-17] Unfortunately, the former exhibit different degrees of toxic side effects, and viral mutant strains that are drug resistant emerge following prolonged therapy.[18] On the other hand, NNRTIs select mutant resistant viruses very rapidly both *in vitro* and *in vivo*,[19] and thus are not effective as single therapeutic agents.

Recent reports have suggested the possibility of using NNRTIs alternating or in combination with nucleoside analogues or with other NNRTIs, in order to reduce toxicities and delay or prevent selection of drug-resistant viruses.[20-24]

Moreover, the fact that NNRTIs, alone or in combination with nucleoside analogues, may be able to suppress the HIV replication[25-28] *in vitro* gives more clues to the search for this type of compounds.

Recently, we have reported on the synthesis of a new class of NNRTIs, *i.e.*, 3,4-dihydro-2-alkoxy-6-benzyl-4-oxopyrimidines (DABOs).[29,30] In the attempt to obtain more potent and selective compounds, a new series of derivatives was synthesized and assayed for anti-HIV activity. The new derivatives, which differ from previous DABOs in the nature of the side-chain linked at the C-2 position that presents a sulfur atom instead of an oxygen, will be referred to as S-DABOs.

Uracils (**7a–o**), thymines (**8a–l**), and the related 5,5-dimethyl analogues (**9a–l**) having alkylthio or cycloalkylthio moieties at C-2 and benzyl or benzylidene groups at C-6 of the pyrimidine ring were synthesized and tested as potential anti-HIV agents. Some S-unsubstituted derivatives (**4b, 5a,b,** and **6a,b**) were also synthesized for a better understanding of structure–activity relationships.

Chemistry

The synthesis of 6-(arylmethyl)-2-thiouracils **4a,b**,[31-33] 6-(arylmethyl)-2-thiothymines **5a,b**, and the related hexahydropyrimidines **6a,b**, which are key intermediates for the preparation of the new DABO derivatives, was achieved starting from methyl (phenylacetyl)-acetates **1a,b** (Scheme 1). The latter compounds were prepared by acylation of 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) with the proper phenylacetyl chloride in the presence of anhydrous pyridine and subsequent methanolysis of the resulting intermediates.

Reaction of **1a,b** with 1 and 2 mol of methyl iodide in the presence of sodium methoxide furnished the corresponding 2-methyl (**2a,b**)[29,30] and 2,2-dimethyl (**3a,b**) β-keto esters, respectively.

Condensation between thiourea and the above β-keto esters **1a,b**, **2a,b**, and **3a,b** in the presence of sodium methoxide gave the related pyrimidines **4a,b**, **5a,b**, and **6a,b**, respectively.

Reaction of the latter compounds with the proper alkyl or cycloalkyl halide in alkaline medium afforded the required 2-(alkylthio)-6-benzyl-3,4-dihydro-4-oxopyrimidines **7a–o** and the corresponding 5-methyl analogues **8a–l**. Similar reaction starting from **6a,b** led to the benzylidene pyrimidines **9a–l** (Scheme 1).

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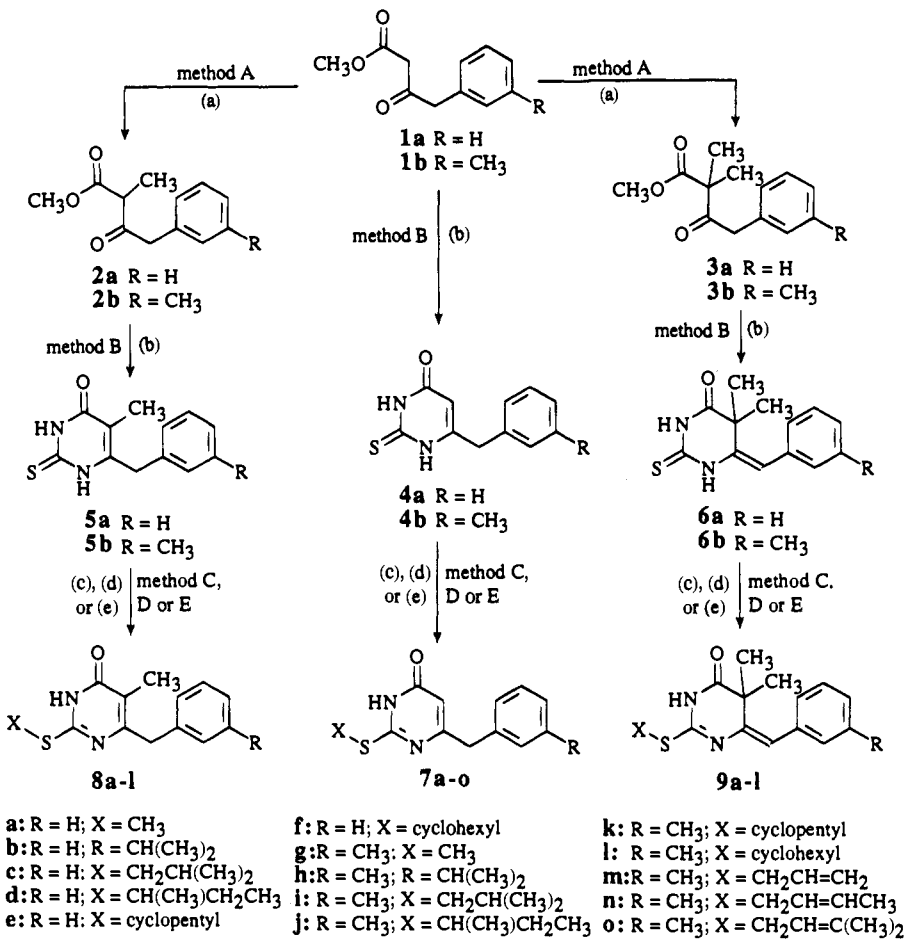
[⊗] Abstract published in *Advance ACS Abstracts*, July 1, 1995.

0022-2623/95/1838-3258$09.00/0

Scheme 1[a]



[a] (a) CH_3ONa, CH_3I, CH_3OH, reflux; (b) CH_3ONa, NH_2CSNH_2, CH_3OH, reflux; (c) CH_3I, DMF, room temperature; (d) X-halide, K_2CO_3, DMF, room temperature; (e) $C_6H_{11}I$, K_2CO_3, DMF, 80 °C.

Antiviral Activity

The ability of derivatives **4**–**9** to inhibit the HIV-1-induced cytopathogenicity was assayed in MT-4 cells together with their cytotoxicity (Table 2). In these *in vitro* assays, AZT and nevirapine were used as reference compounds.

Numerous S-DABOs were noncytotoxic in MT-4 cells at doses higher than 200 μM, and most of them resulted selective inhibitors of the HIV-1 multiplication. Maximum potency and selectivity was obtained with derivatives belonging to the uracil (**7f,j**) and thymine (**8a,e,f,k,l**) series, whereas the 5,5-dimethyl derivatives and compounds with S not substituted were considerably less potent and selective (**9a**–**f**) or totally inactive (**9g**–**l**, **4b**, **5a,b**, and **6a,b**).

Like what has been found with previous DABOs,[29,30] also in the new series the antiviral activity correlated with the presence of bulky substituents at the C-2 position of uracil or thymine rings. Therefore, *sec*-butyl, cyclopentyl, and cyclohexyl derivatives were among the most potent and selective S-DABOs. The sole exception was compound **8a**, which was very potent against HIV-1 although bearing a methyl substituent.

Among uracil derivatives **7a**–**o**, the introduction of a methyl group at position 3′ of the benzyl portion significantly increased the potency and/or reduced the cytotoxicity of the isopropyl (compare **7b** and **7h**), *sec*-butyl (compare **7d** and **7j**), and cyclopentyl (compare **7e** and **7k**) derivatives, while reducing the potency of the cyclohexyl derivative (compare **7f** and **7l**). Allyl-substituted compounds **7m**–**o** were moderately active.

When compared with the uracil counterparts, thymine derivatives bearing isopropyl (**8b**), isobutyl (**8c**), and *sec*-butyl (**8d**) substituents were slightly more cytotoxic, whereas the cyclopentyl derivative **8e** showed a lower cytotoxicity; the methyl (**8a**) and cyclohexyl (**8f**) derivatives remained non-cytotoxic. As far as the anti-HIV-1 activity was concerned, the introduction of a methyl at the C-5 of the pyrimidine ring led to a significant increase in potency, independently of the alkylthio substituent. The further introduction of a methyl group at position 3′of the benzyl moiety of compounds **8a**–**f** significantly modified neither potencies nor selectivities (compare **8a**–**f** with **8g**–**l**).

Derivatives **9a**–**f**, with no substituents at the benzylidene portion, showed some inhibitory activity but lacked selectivity, being cytotoxic at doses slightly higher than those effective. The introduction of a methyl group at the 3′ position of the benzylidene ring (compounds **9g**–**l**) completely abolished the antiviral activity.

Since previous DABO derivatives were found to target the HIV-1 RT, the title compounds were also tested in enzyme assays against highly purified recombinant HIV-1 RT using homopolymeric template primers. All the compounds which were found active in cell culture were also found active in enzyme assays (Table 2), although the IC_{50} values obtained on the recombinant

Table 1. Physical and Chemical Data of Compounds **4–9**

compd	R	X	mp, °C	recryst solvent	method of synthesis	% yield	formula[a]
4b	Me		238–239[b]	ethanol	B	52	$C_{12}H_{12}N_2OS$
5a	H		229–230	ethanol	B	48	$C_{12}H_{12}N_2OS$
5b	Me		213–214	ethyl acetate	B	49	$C_{13}H_{14}N_2OS$
6a	H		116–120	benz/cyclohex	B	49	$C_{13}H_{14}N_2OS$
6b	Me		144–145	benz/cyclohex	B	44	$C_{14}H_{16}N_2OS$
7a	H	methyl	183–184[c]	benzene	C	98	$C_{12}H_{12}N_2OS$
7b	H	isopropyl	123.5–124.5	cyclohexane	D	97	$C_{14}H_{16}N_2OS$
7c	H	isobutyl	131.5–132.5	cyclohexane	D	90	$C_{15}H_{18}N_2OS$
7d	H	*sec*-butyl	100–102	cyclohexane	D	82	$C_{15}H_{18}N_2OS$
7e	H	cyclopentyl	147–148	cyclohexane	D	84	$C_{16}H_{18}N_2OS$
7f	H	cyclohexyl	172–173	benz/cyclohex	E	72	$C_{17}H_{20}N_2OS$
7g	Me	methyl	159.5–160[d]	benzene	C	94	$C_{13}H_{14}N_2OS$
7h	Me	isopropyl	122–123	cyclohexane	D	86	$C_{15}H_{18}N_2OS$
7i	Me	isobutyl	111–112	*n*-hexane	D	78	$C_{16}H_{20}N_2OS$
7j	Me	*sec*-butyl	76–78	*n*-hexane	D	69	$C_{16}H_{20}N_2OS$
7k	Me	cyclopentyl	157–158	benz/cyclohex	D	76	$C_{17}H_{20}N_2OS$
7l	Me	cyclohexyl	177–178	benz/cyclohex	E	68	$C_{18}H_{22}N_2OS$
7m	Me	allyl	118–119	cyclohexane	D	82	$C_{14}H_{16}N_2OS$
7n	Me	3-methylallyl	131–133	cyclohexane	D	74	$C_{15}H_{18}N_2OS$
7o	Me	3,3-dimethylallyl	87–88	*n*-hexane	D	78	$C_{16}H_{20}N_2OS$
8a	H	methyl	199–200	benzene	C	98	$C_{13}H_{14}N_2OS$
8b	H	isopropyl	150–151	cyclohexane	D	95	$C_{15}H_{18}N_2OS$
8c	H	isobutyl	114.5–115	*n*-hexane	D	92	$C_{16}H_{20}N_2OS$
8d	H	*sec*-butyl	127.5–128	*n*-hexane	D	90	$C_{16}H_{20}N_2OS$
8e	H	cyclopentyl	166–167	cyclohexane	D	85	$C_{17}H_{20}N_2OS$
8f	H	cyclohexyl	180–182	benz/cyclohex	E	70	$C_{18}H_{22}N_2OS$
8g	Me	methyl	195–196	benz/cyclohex	C	97	$C_{14}H_{16}N_2OS$
8h	Me	isopropyl	135–136	cyclohexane	D	94	$C_{16}H_{20}N_2OS$
8i	Me	isobutyl	110.5–111	*n*-hexane	D	90	$C_{17}H_{22}N_2OS$
8j	Me	*sec*-butyl	121–122	*n*-hexane/cyclohex	D	82	$C_{17}H_{22}N_2OS$
8k	Me	cyclopentyl	169–170	cyclohexane	D	89	$C_{18}H_{22}N_2OS$
8l	Me	cyclohexyl	179–180	benz/cyclohex	E	62	$C_{19}H_{24}N_2OS$
9a	H	methyl	171–172	benz/cyclohex	C	98	$C_{14}H_{16}N_2OS$
9b	H	isopropyl	130–131	*n*-hexane	D	86	$C_{16}H_{20}N_2OS$
9c	H	isobutyl	125–126	*n*-hexane	D	88	$C_{17}H_{22}N_2OS$
9d	H	*sec*-butyl	115–117	petroleum ether	D	76	$C_{17}H_{22}N_2OS$
9e	H	cyclopentyl	140–141	*n*-hexane	D	75	$C_{18}H_{24}N_2OS$
9f	H	cyclohexyl	154–155	*n*-hexane	E	69	$C_{19}H_{26}N_2OS$
9g	Me	methyl	142–143	cyclohexane	C	98	$C_{15}H_{18}N_2OS$
9h	Me	isopropyl	134–135	*n*-hexane	D	88	$C_{17}H_{22}N_2OS$
9i	Me	isobutyl	142–143	*n*-hexane	D	84	$C_{18}H_{24}N_2OS$
9j	Me	*sec*-butyl	85–87	*n*-hexane	D	79	$C_{18}H_{24}N_2OS$
9k	Me	cyclopentyl	133–134	cyclohexane	D	80	$C_{19}H_{26}N_2OS$
9l	Me	cyclohexyl	152–155	cyclohexane	E	64	$C_{20}H_{28}N_2OS$

[a] All compounds were analyzed for C, H, N, and S; analytical results were within ±0.4% of the theoretical values. [b] Literature[32,33] value 231 °C. [c] Literature[36] value 180 °C (EtOH). [d] Literature[33] value 167 °C.

homodimeric enzyme were 2–6-fold higher than the EC_{50} values obtained in cell culture. The reasons for this difference remain to be established, but the above results were not surprising since a 3–10-fold difference between IC_{50} and EC_{50} values has also been found[17] with TIBO R82150 and nevirapine.

Several of the S-DABO derivatives which were active against HIV-1, namely compounds **7f,j–l** and **8a,e,f,k,l**, were also tested for their capability to prevent the HIV-2-induced cytopathogenicity in MT-4 cells. None of them was found active at doses as high as 300 μM (data not shown), indicating a specific inhibition toward the HIV-1 multiplication. Furthermore, none of the above compounds was effective in reducing the infectious HIV-1 yield in chronically infected H9/IIIb cells.

Discussion

We have recently described DABOs[29,30] as a new class of non-nucleoside reverse transcriptase inhibitors. In an attempt to improve their potency, new alkylthio and cycloalkylthio derivatives (S-DABOs) were synthesized and their anti-HIV activity assessed.

S-DABOs were confirmed as specific inhibitors of HIV-1, targeted at the reverse transcriptase. In general, substitution of alkyloxy chain of DABOs with the corresponding alkylthio chain significantly increased the anti-HIV-1 activity. In fact, S-DABOs were more potent than the corresponding oxygen-substituted molecules,[29,30] and since their degree of cytotoxicity did not vary, they were also more selective. Structure–activity relationships suggest that, similarly to the previous DABO series, maximum potency correlates with the size of the alkyl chain rather than with the nature of the base (uracil or thymine). Again, the presence of a methyl group at the 3′-position in the benzyl moiety does not markedly influence the activity.

As with other NNRTIs, the main issue is to find compounds that can inhibit mutant HIV-1 strains resistant to other NNRTIs. The fact that we have identified S-DABOs derivatives with a favorable selectivity index and a potency comparable to that of nevirapine makes it now reasonable to further investigate this class of compounds in order to define (a) whether they inhibit HIV-1 strains resistant to other non-nucleoside and nucleoside reverse transcriptase inhibitors and (b) the rapidity of emergence of DABO-resistant mutants and which mutations appear in the RT gene.

Table 2. Cytotoxicity and anti-HIV-1 Activity of Compounds **4–9**[a]

compd	substituent R	substituent X	[μM] CC_{50}[b]	[μM] EC_{50}[c]	[μM] IC_{50}[d]	SI[e]
4b	Me		258	>258	>10	
5a	H		431	>108	>10	
5b	Me		284	>102	>10	
6a	H		>406	>406	ND[g]	
6b	Me		165	>165	ND	
7a	H	methyl	>431[f]	34.5	>10	>12
7b	H	isopropyl	332	7.7	>10	43
7c	H	isobutyl	186	5.1	9.4	36
7d	H	*sec*-butyl	150	1.2	2.7	125
7e	H	cyclopentyl	147	1.7	2.8	86
7f	H	cyclohexyl	>330	0.8	3	>412
7g	Me	methyl	317	17	>10	19
7h	Me	isopropyl	225	1.3	2.9	173
7i	Me	isobutyl	163	3.3	8.4	49
7j	Me	*sec*-butyl	>347	0.6	1.2	>578
7k	Me	cyclopentyl	>333	1.2	2.6	>278
7l	Me	cyclohexyl	>318	1.5	2.6	>212
7m	Me	allyl	188	3.6	ND	52
7n	Me	3-methylallyl	165	8	ND	21
7o	Me	3,3-dimethylallyl	193	13	ND	15
8a	H	methyl	>406	1.2	4.9	>338
8b	H	isopropyl	140	1.8	2.5	77
8c	H	isobutyl	130	0.8	2.2	162
8d	H	*sec*-butyl	86	0.6	2.4	140
8e	H	cyclopentyl	>333	0.6	3.4	>555
8f	H	cyclohexyl	>318	0.6	4.3	>530
8g	Me	methyl	385	3	2.5	128
8h	Me	isopropyl	100	1.3	2.5	77
8i	Me	isobutyl	330	1.6	4.6	206
8j	Me	*sec*-butyl	100	1	2.7	100
8k	Me	cyclopentyl	>318	0.6	3.4	>530
8l	Me	cyclohexyl	>304	0.6	1.3	>506
9a	H	methyl	160	3.1	6.1	53
9b	H	isopropyl	20	3.6	4.3	5.5
9c	H	isobutyl	20	3.6	4.6	5.5
9d	H	*sec*-butyl	16	3.3	4.3	4.8
9e	H	cyclopentyl	18	10	>10	1.8
9f	H	cyclohexyl	91	9.1	>10	10
9g	Me	methyl	5.2	>5.2	ND	
9h	Me	isopropyl	69	>69	ND	
9i	Me	isobutyl	>316	>316	ND	
9j	Me	*sec*-butyl	25	>25	ND	
9k	Me	cyclopentyl	>304	>304	ND	
9l	Me	cyclohexyl	>292	>292	ND	
Nevirapine			>376	0.3		>1250
AZT			>20	0.01		>2000

[a] Data represent mean values for three separate experiments. Variation among triplicate samples was less than 10%. [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 HIV-1-induced cytopathogenicity, as determined by the MTT method. [d] Compound dose required to inhibit the HIV-1 rRT activity by 50%. [e] Selectivity index, CC_{50}/EC_{50} ratio. [f] Higher concentrations could not be achieved because of crystallization of compounds in the culture medium. [g] Not determined.

Experimental Section

Chemistry. 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 (Me_4Si). All compounds were routinely checked by TLC and ^{1}H NMR. NMR 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. Merck silica gel 60 was used for chromatographic purifications. Solvents were reagent grade and, when necessary, were purified and dried by standards 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. Iodomethane, 2-iodopropane, 2-iodo-2-methylpropane, 2-iodobutane, cyclopentyl bromide, and cyclohexyl iodide were purchased from Aldrich Chimica, Milan (Italy). Microanalyses (within ±0.4% of the theoretical values) were performed by the Microanalytical Laboratory of Prof. A. Pietrogrande, University of Padova, Italy.

Syntheses. Specific examples presented below illustrate general synthetic methods A–E. In general, samples prepared for physical (Table 1) and biological studies (Table 2) were dried in high vacuum over P_2O_5 for 20 h at temperatures ranging from 25 to 110 °C, depending on the sample melting point.

Method A Example. Methyl Dimethyl((3′-methylphenyl)acetyl)acetate (3b). Sodium metal (4.60 g, 0.20 mol) was dissolved in 250 mL of anhydrous methanol, and methyl ((3′-methylphenyl)acetyl)acetate (20.60 g, 0.10 mol) and methyl iodide (13.69 mL, 0.22 mol) were subsequently added. The resulting mixture was heated at reflux for 5 h. After cooling, the solvent was removed and the residue treated with water (200 mL) and extracted with chloroform (3 × 100 mL). The organic layer was washed with brine (2 × 100 mL), dried, and evaporated to give a residue which was purified by column chromatography (silica gel/chloroform). Pure **3b** was obtained as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.28 (s, 6H, 2,2-$(CH_3)_2$), 2.27 (s, 3H, 3′-CH_3), 3.58 (s, 5H, OCH_3 and CH_2), 6.92–7.12 (m, 4H, Ph); IR 1725, 1700 cm^{-1}.

Method B Example. 4-Hydroxy-2-mercapto-5-methyl-6-(3′-methylbenzyl)pyrimidine (5b). Sodium metal (2.30 g, 0.10 mol) was dissolved in 50 mL of anhydrous methanol, then thiourea (5.33 g, 0.07 mol) and methyl methyl((3′-methylphenyl)acetyl)acetate (11.01 g, 0.05 mol) were added to the clear solution, and the mixture was heated at reflux for 5 h. Removal of the solvent by distillation *in vacuo* at 40–50 °C gave a residue, which was dissolved in water (50 mL). The solution was neutralized with 0.5 N acetic acid and extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were washed with brine (100 mL), dried, and evaporated to dryness. The residue was purified by crystallization: ^{1}H NMR (DMF-d_7) δ 1.88 (s, 3H, 5-CH_3), 2.30 (s, 3H, 3′-CH_3), 3.97 (s, 2H, CH_2Ph), 7.10–7.30 (m, 4H, Ph); IR 3100, 1650, 1190 cm^{-1}.

Method C Example. 5,5-Dimethyl-2-(methylthio)-4-oxo-6-(phenylmethenyl)-3,4,5,6-tetrahydropyrimidine (9a). A mixture of 5,6-dihydro-5,5-dimethyl-4-hydroxy-2-mercapto-6-(phenylmethenyl)pyrimidine (**6a**) (0.30 g, 1.12 mmol) and methyl iodide (0.14 mL, 2.24 mmol) in 1 mL of anhydrous DMF was stirred at room temperature for 3 h. The solution was then diluted with 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 **9a**, which was purified by crystallization: ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 6H, 5,5-$(CH_3)_2$), 2.62 (s, 3H, SCH_3), 6.04 (s, 1H, C*H*Ph), 7.19–7.34 (m, 3H, 3′,4′,5′-Ph), 7.79–7.83 (m, 2H, 2′,6′-Ph), 8.53 (broad s, 1H, NH exchangeable with D_2O); IR 2950, 1675 cm^{-1}.

Method D Example. 3,4-Dihydro-6-(3′-methylbenzyl)-2-((1-methylpropyl)thio)-4-oxopyrimidine (7j). A mixture of 4-hydroxy-2-mercapto-6-(3′-methylbenzyl)pyrimidine (**4b**)[32] (0.40 g, 1.72 mmol), 1-methylpropyl iodide (0.35 g, 0.22 mL, 1.89 mmol), and potassium carbonate (0.25 g, 1.81 mmol) in 1 mL of anhydrous DMF was stirred at room temperature for 8 h. After treatment with cold water (100 mL), the solution was extracted with ethyl acetate (3 × 50 mL). The organic layers were collected, washed with brine (3 × 50 mL), dried, and evaporated to furnish crude **7j**, which was passed through a silica gel column (eluent: *n*-hexane/ethyl acetate/methanol, 12/3/1). The eluates were collected and evaporated to give a solid, which was recrystallized from suitable solvent: ^{1}H NMR ($CDCl_3$) δ 0.85–1.02 (t, 3H, CH_2CH_3), 1.26–1.33 (d, 3H, $CHCH_3$), 1.50–1.85 (m, CH_2CH_3), 2.25 (s, 3H, 3′-CH_3), 3.75 (s, 2H, CH_2Ph), 3.75–4.03 (s, 1H, SCH), 5.98 (s, 1H, 5-H), 7.00–7.27 (m, 4H, Ph): IR 2800, 1630 cm^{-1}.

Method E Example. 6-Benzyl-2-(cyclohexylthio)-3,4-dihydro-5-methyl-4-oxopyrimidine (8f). A mixture of 6-benzyl-4-hydroxy-2-mercapto-5-methylpyrimidine (**5a**) (0.80 g, 3.44 mmol), cyclohexyl iodide (0.83 g, 0.51 mL, 3.96 mmol),

and potassium carbonate (0.52 g, 3.78 mmol) in 2 mL of anhydrous DMF was heated at 80 °C for 15 h. After cooling, the reaction 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 a solid, which was purified by chromatography on silica gel column (eluent: *n*-hexane/ethyl acetate/methanol, 12/3/1). Recrystallization from suitable solvent afforded pure **8f**: ^{1}H NMR ($CDCl_3$) δ 1.22–1.73 (m, 6H, C_3, C_4, C_5-cyclohexyl), 1.85–2.02 (m, 4H, C_2, C_6-cyclohexyl), 2.10 (s, 3H, 5-CH_3), 3.67–3.95 (m, 1H, SCH), 3.88 (s, 2H, CH_2Ph overlapped signal), 7.27–7.37 (m, 5H, Ph); IR 2800, 1630 cm^{-1}.

Antiviral Assay Procedures. Activity of the compounds against HIV-1 (III_B strain) multiplication in acutely infected cells was based on the inhibition of virus-induced cytopathogenicity in MT-4 cells. Briefly, 50 μL of culture medium containing 1 × 10^4 cells were added to each well of flat-bottomed microtitre trays containing 50 μL of culture medium with or without various concentrations of the test compounds. Then, 20 μL of an HIV-1 suspension containing 100 $CCID_{50}$ (50% cell culture infective dose) were added. After a 4-day incubation at 37 °C, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-1-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[34] The 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 were performed as previously described.[35] Briefly, highly purified recombinant reverse transcriptase (rRT) was assayed for its RNA-dependent DNA polymerase associated activity in a 50 μL volume containing 50 mM Tris-HCl pH 7.8, 50 mM KCl, 6 mM $MgCl_2$, 1 mM DTT, 0.1 mg mL^{-1} BSA, 0.5 OD_{260} units mL^{-1} poly(rC)-oligo(dG)$_{12-18}$, 10 mM [^{3}H]dGTP (1 Ci mmol^{-1}). After incubation for 20 min, at 37 °C, the samples were spotted on glass fiber filters (Whatman GF/A), and the acid-insoluble radioactivity was determined.

Acknowledgment. This investigation was supported by Grants 9204-05 and 9204-68 from Ministero della Sanità, Istituto Superiore di Sanità, Progetto AIDS 1994, and by CNR grant "Progetto FATMA". One of us (S.M.) thanks Foundation "Istituto Pasteur-Fondazione Cenci-Bolognetti" for partial support.

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JM950147E