A New Series of Pyridinone Derivatives as Potent Non-Nucleoside Human Immunodeficiency Virus Type 1 Specific Reverse Transcriptase Inhibitors

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4-(Arylthio)-pyridin-2(1*H*)-ones variously substituted in their 3-, 5-, and 6-positions have been synthesized as a new series of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT)–pyridinone hybrid molecules. Biological studies revealed that some of them show potent HIV-1 specific reverse transcriptase inhibitory properties. Compounds **16** and **7c**, the most active ones, inhibit the replication of HIV-1 at 3 and 6 nM, respectively.

Introduction

Among HIV-1 specific reverse transcriptase (RT) inhibitors, HEPT derivatives **1a,b** and pyridinones **2a,b** are interesting leads which emerged from various structure–activity relationship (SAR) studies in these series[1–8] (Figure 1).

Our continuing interest for the search of new anti-HIV drugs led us to design pyridinones **3** as potential specific human immunodeficiency virus type 1 (HIV-1) RT inhibitors. Indeed, the way considered toward these **1–2** hybrid molecules, which are new pyridinone derivatives, allowed us to conceive to obtain easily a large panel of 3–6-substituted compounds and therefore to study thoroughly the SAR in this series.

We report in this paper the synthesis and biological properties of this new series of **1–2b**-related compounds **3**.

Chemistry

Starting from 4-hydroxy-5-methyl-pyridin-2(1*H*)-one (**4a**),[9] the nitro derivative **5a** and the chloronitropyridinone **6a** were prepared as previously described.[10] Condensation with 3,5-dimethylthiophenol led to 4-[(3′,5′-dimethylphenyl)thio]-5-methyl-3-nitropyridin-2(1*H*)-one (**7a**) in 92% yield, and reduction with stannous chloride dihydrate in boiling ethyl acetate gave 87% of the amine **8a** (Scheme 1). In the same manner and in order to study the SAR in this new 5-methylpyridin-2(1*H*)-one series, variously 3- and 4-substituted derivatives have been synthetized. Compounds **7d,e** and **8e** were obtained by condensation of the chloronitropyridinone **6a** with thiophenol or *m*-thiocresol followed by the reduction of the nitro function (in the case of **8e**). According to Scheme 2, compounds **10a–h** were obtained from 2-mercapto derivatives of pyrimidines, benzimidazole, benzoxazole, benzothiazole, thiazoline, imidazole, and pyridine with a yield ranging from 10% to 98%. The 4-anilinopyridinone derivative **9** was also prepared in the standard method using 3,5-dimethylaniline instead of the mercapto reagent, and aminopyridinone **11** was obtained by reduction of **10d**, in the usual conditions.

Upon the model of the 3-amino-substituted pyridinone **2b**, modifications of the amino function of 3-amino-5-methyl-4-[(3′,5′-dimethylphenyl)thio]pyridin-2(1*H*)-one (**8a**) and 3-amino-5-ethyl-6-methyl-4-[(3′,5′-dimethylphenyl)thio]pyridin-2(1*H*)-one (**8c**) newly synthesized have been performed. Thus, in the usual amidification conditions, using ethyl formate, acetic anhydride, propionyl chloride, heptanoic anhydride, and phenylacetyl chloride, the amides **12a–e,g** were obtained in 50–90% yields (Scheme 3). The 3-(*N*-ethoxycarbamoyl) derivative **12f** was also synthesized in the same conditions with ethyl chloroformate in 26% yield.

It is worth mentioning that in various conditions no condensation occurred from this 3-aminopyridinone **8a** and 4,5-dimethyl-2-methoxybenzaldehyde, contrary to that described by Wai *et al.* in the case of the 3-amino-5-ethyl-6-methylpyridin-2(1*H*)-one.[6] This failure could be explained by the steric hindrance of the neighboring thiophenyl group and the basicity of this 3-amino group which seems to be weak since its methylation in the Eschweiler–Clark reaction[11,12] using formic acid and formaldehyde in various conditions was unsuccessfull.

At this stage, all new 3- and 4-substituted 5-methylpyridin-2(1*H*)-one derivatives were submitted to biological evaluation. Results were interesting and revealed that the most active compounds fitted a nitro or amino function and a (3,5-dimethylphenyl)thio group at their 3- and 4-positions, respectively. These characteristic structural requirements were then extended to the 5-ethyl- and 5-ethyl-6-methylpyridinone analogues, starting either from the known 5-ethyl-4-hydroxypyridin-2(1*H*)-one (**4b**)[13] or from 5-ethyl-4-hydroxy-6-methylpyridin-2(1*H*)-one (**4c**) which was prepared in a two-step sequence from ethyl 2-ethylaminocrotonate **13** and diethyl malonate (Scheme 4). Then, nitration, monochlorination, substitution with 3,5-dimethylthiophenol, and reduction of the nitro function occurred smoothly (Scheme 1) leading to the 3-nitro- and 3-aminopyridinone derivatives **7b**, **8b** and **7c**, **8c**, respectively. The 5,6-benzopyridinone analogues, **7f** and **8f** were also obtained in the

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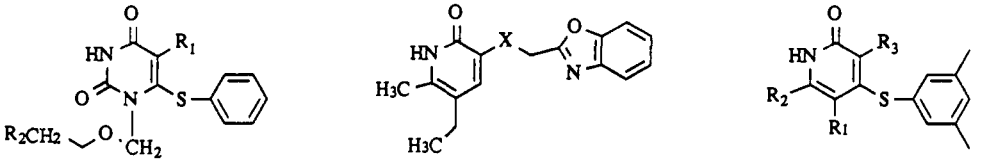


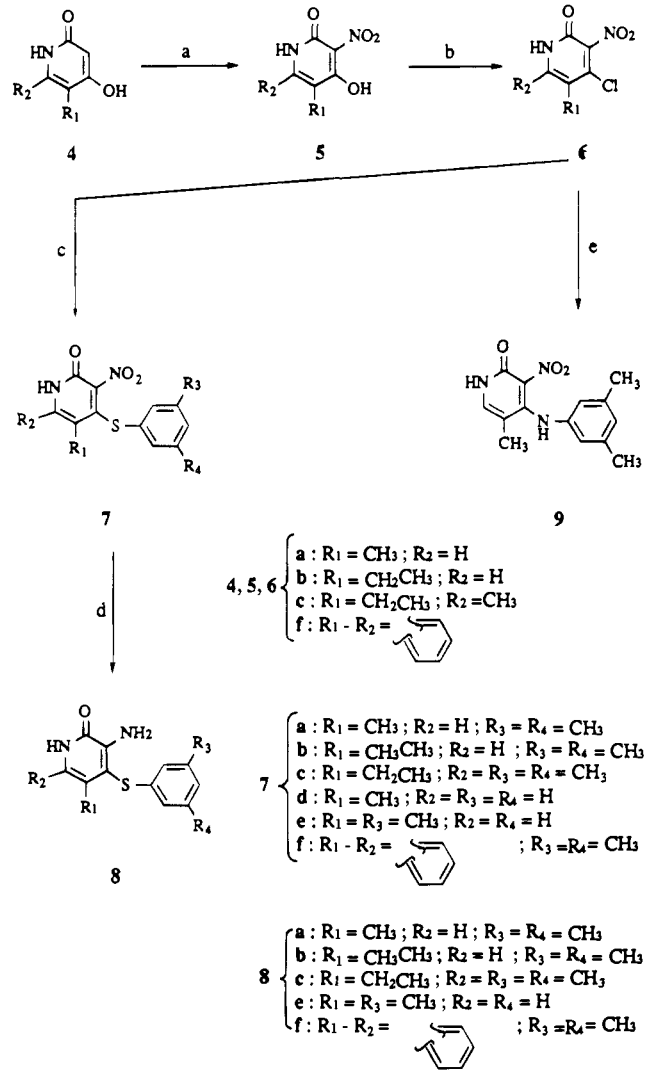
1a (HEPT) : $R_1 = CH_3$ and $R_2 = OH$
1b (E-EPU) : $R_1 = CH_2CH_3$ and $R_2 = H$

2a : X = CH_2 (L-696,229)
2b : X = NH

3

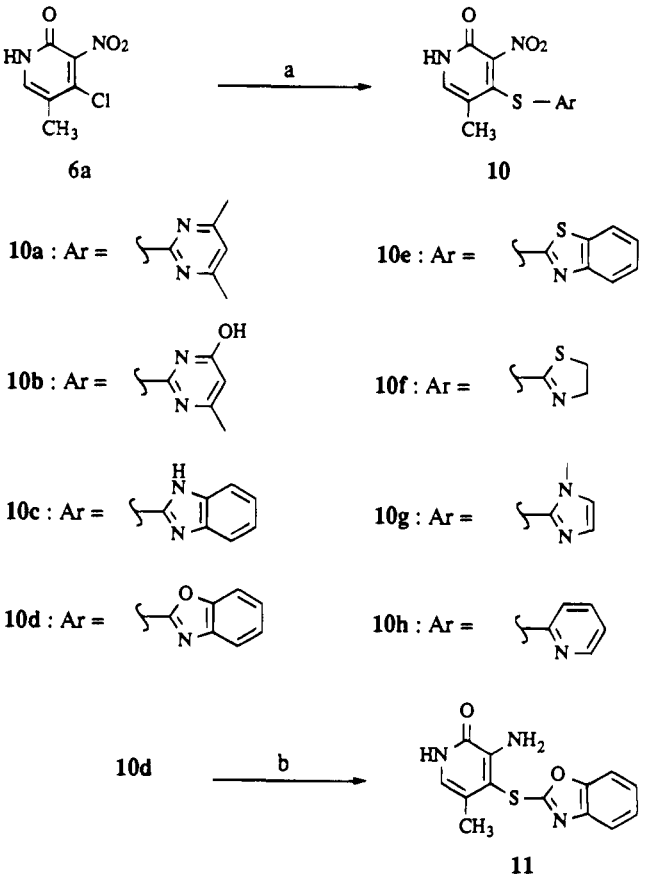
Figure 1.

Scheme 1[a]



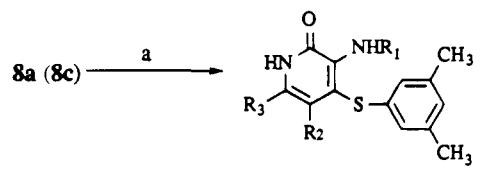
[a] Reagents: (a) HNO_3/Δ; (b) $POCl_3$/benzyltriethylammonium chloride/CH_3CN/Δ_{reflux}; (c) thiophenol/Et_3N/EtOH/room temperature; (d) $SnCl_2 \cdot 2H_2O$/EtOAc/Δ_{reflux}; (e) 3,5-dimethylaniline/Et_3N/EtOH/Δ_{reflux}.

Scheme 2[a]



[a] Reagents: (a) mercaptoaromatic/Et_3N/EtOH/Δ; (b) $SnCl_2 \cdot 2H_2O$/EtOAc/Δ_{reflux}.

Scheme 3[a]



[a] Reagents: (a) **12a**, R_1 = CHO, $R_2 = CH_3$, R_3 = H, ethyl formate/formic acid/Δ_{reflux}/12 h; **12b**, $R_1 = COCH_3$, $R_2 = CH_3$, R_3 = H, acetic anhydride/acetic acid/Δ_{reflux}/1.5 h; **12c**, $R_1 = COCH_2CH_3$, $R_2 = CH_3$, R_3 = H, propionyl chloride/NEt_3/CH_2Cl_2/room temperature/5 h; **12d**, $R_1 = CO(CH_2)_5CH_3$, $R_2 = CH_3$, R_3 = H, heptanoic anhydride/toluene/100 °C/1.5 h; **12e**, $R_1 = COCH_2C_6H_5$, $R_2 = CH_3$, R_3 = H, phenylacetyl chloride/NEt_3/CH_2Cl_2/room temperature/2 h; **12f**, $R_1 = COOCH_2CH_3$, $R_2 = CH_3$, R_3 = H, ethyl chloroformate/NEt_3/EtOH/room temperature/48 h; **12g**, $R_1 = COCH_3$, $R_2 = CH_2CH_3$, $R_3 = CH_3$, acetic anhydride/acetic acid/Δ_{reflux}/5.5 h.

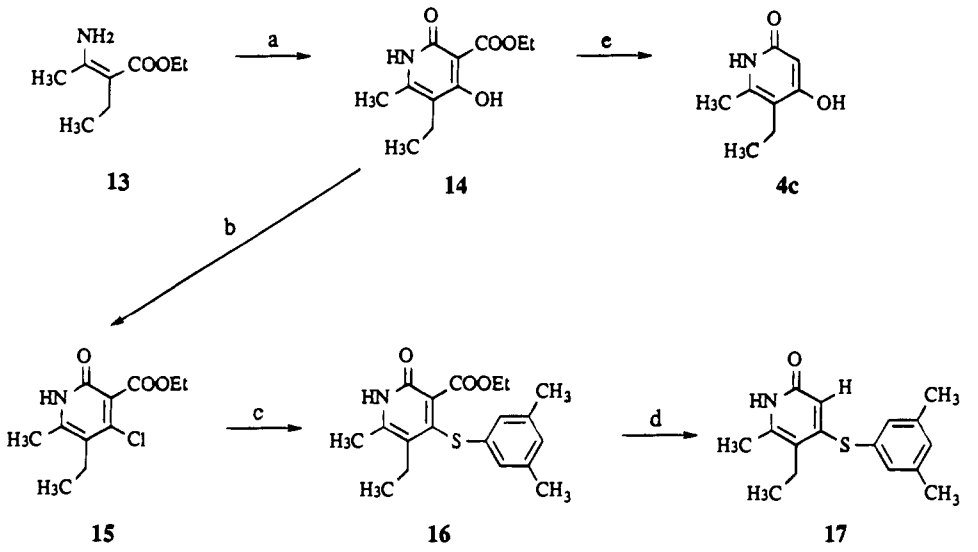
same way using the commercially available quinoline-2,4-diol (**4f**) as starting material.

Finally, the intermediate 3-carbethoxy-4-hydroxypyridinone **14**, obtained as an intermediate during the preparation of the 5-ethyl-6-methylpyridinone **4c**, was successively transformed into 4-chloropyridinone **15** and 4-(phenylthio)pyridinone **16** in the standard conditions. Attempts to perform hydrolysis of the 3-carbethoxypyridinone **16** were however followed by the unavoidable decarboxylation, leading to the 3-unsubstituted pyridinone **17** in acidic conditions or to the partial degradation of the starting material in ethanol with 5% sodium hydroxide.

Biological Results

Inhibition of HIV-1 Multiplication. Thirty newly synthesized pyridinones were studied for their anti-HIV-1 biological activity. Several molecules showed significant antiviral properties. For example, the most

Scheme 4[a]



[a] Reagents: (a) (1) diethyl malonate/EtONa/EtOH/Δ_{reflux}, (2) **13**/Δ_{reflux}; (b) $POCl_3$/benzyltriethylammonium chloride/CH_3CN/Δ_{reflux}; (c) 3,5-dimethylthiophenol/Et_3N/EtOH/Δ_{reflux}; (d) THF/H_2O/HCl (37%) (18:3:4)/75 °C; (e) HCl(1 N)/Δ_{reflux}.

Table 1. Anti-HIV-1 Biological Activity of the Pyridinones on HIV-1_{IIIB} Wild Type

compd	IC_{50} (nM)	CC_{50} (nM)	SI
AZT	3	>100 000	>33333
7a	120	>10 000	>83
7b	≈90	>10 000	>111
7c	≈6	>10 000	>1666
7d	>10 000	>10 000	
7e	2800	>10 000	>3
7f	3800	>100 000	>26
8a	10	>10 000	>1000
8b	10	>10 000	>1000
8c	14	>10 000	>714
8e	1400	78 000	>55
8f	250	>9000	>36
9	45 000	>100 000	>2
10a	17 500	19 500	>1
10b	>100 000	>100 000	
10c	43 500	54 500	>1
10d	5500	16 000	>2
10e	14 500	13 500	
10f	17 000	21 500	>1
10g	56 000	68 500	>1
10h	18 000	28 500	>1
11	8000	7500	
12a	6600	>100 000	>15
12b	67	>100 000	>1492
12c	41 400	>100 000	>2
12d	>10 000	>10 000	
12e	>10 000	>10 000	
12f	7500	>100 000	>13
12g	50	80 000	1600
16	3	>10 000	>3333
17	500	>10 000	>20

actives ones are the compounds **16** and **7c** with IC_{50}s of 3 and 6 nM, respectively (see Table 1). The best inhibitors, namely, those which display a selective index (SI) superior to 20, were tested on a Nevirapine resistant strain (see Table 2). It was found that compound **7c** has good anti-HIV-1 activity with an IC_{50} of 260 nM on this resistant strain.

Inhibition of RT. Compounds found active against HIV-1 in cell culture were tested on recombinant HIV-1 RT. The concentration inhibiting 50% of the RT activity (IC_{50}) for each compound is given in Table 3. Compounds **7c** and **8c** were the best inhibitors with IC_{50} values of 30 and 15 nM, respectively. A HEPT derivative, 1-[(benzyloxy)methyl]-6-(phenylthio)thymine or

Table 2. Anti-HIV-1 Activity of the Pyridinones on HIV-1 Nevirapine Resistant Strain

compd	IC_{50} (nM)	CC_{50} (nM)	SI
TIBO R82913	>10 000	>10 000	
7a	>10 000	>10 000	
7b	6700	>10 000	>1
7c	260	>10 000	>38
7f	>10 000	>10 000	
8a	>10 000	>10 000	
8b	6700	>10 000	>1
8c	2100	>10 000	>4
8e	41 500	78 000	>1
8f	6600	8200	>1.5
12b	>100 000	>100 000	
12g	4300	>100 000	>23
16	2200	>10 000	>4.5

Table 3. Inhibition of HIV-1 Reverse Transcriptase (RT)[a]

compd	IC_{50} (nM)	compd	IC_{50} (nM)
HEPT	>6000	**8c**	15
BPT	400	**8e**	>4000
7a	>10 000	**12b**	>4000
7c	30	**12c**	>4000
7e	>10 000	**16**	600
8a	100	**17**	400

[a] RT activity was measured in the presence of poly(C)-oligo(dG), as described in the Experimental Section.

BPT,[14] tested in the same conditions and used as a reference of non-nucleoside inhibitor gave an IC_{50} value of 400 nM.

As all the derivatives were synthesized to obtain inhibitors targeted toward RT, it was important to perform studies allowing an insight on the nature of the inhibition. These studies were done mainly with **7c** and **8c**.

The inhibition of RT was determined using different template-primers. It has been previously described that non-nucleoside RT inhibitors show different levels on inhibition depending on the template-primer used to measure the RT activity.[15] Dose–response curves for **7c** in the presence of two different template-primers are shown in Figure 2. Better inhibition was obtained in the presence of poly(C)-oligo(dG) as compared to that obtained with poly(A)-oligo(dT). Compound **8c** gave the same result (not shown). With HIV-1 RNA used as natural template, a strong inhibition was also obtained

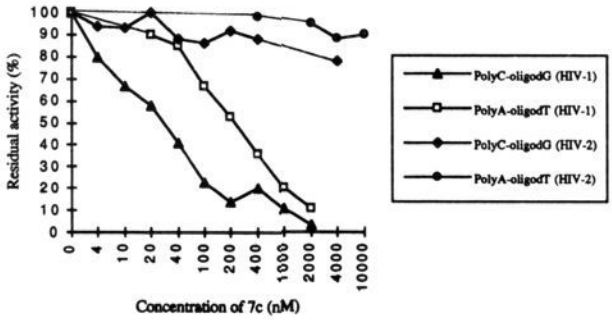


Figure 2. Effect of compound **7c** on HIV-1 and HIV-2 RT.

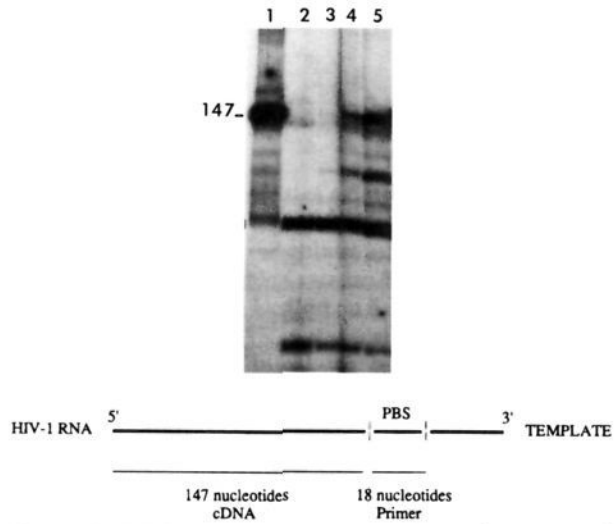


Figure 3. Inhibition of reverse transcription by compound **7c**. Reverse transcription was performed as described in the Experimental Section. The expected cDNA product (147 nucleotides long) is indicated in the left of the panel. Lane 1: control in the presence of the whole system. Lanes 2–5: same as lane 1 but in the presence of different concentrations of compound **7c**. Lane 2: 400 nM **7c**. Lane 3: 300 nM **7c**. Lane 4: 100 nM **7c**. Lane 5: 40 nM **7c**.

(Figure 3). The pyridinone derivatives show marked template-primer preferences, poly(C)-oligo(dG) being the most efficient. With poly(C)-oligo(dG), the IC_{50}s of **7c** and **8c** were respectively 7- and 13-fold lower than with poly(A)-oligo(dT).

All non-nucleoside inhibitors so far identified are specific for HIV-1 and do not inhibit RT from HIV-2. Compounds **7c** and **8c** were tested with both RTs. As shown in Figure 2, at concentrations where HIV-1 RT was well inhibited, the activity of HIV-2 RT was not affected. This discriminatory behavior toward HIV-1 vs HIV-2 RT makes these compounds share another common property of non-nucleoside inhibitors.

Enzyme kinetic analyses show that the inhibition of HIV-1 RT by compound **7c** (or **8c**) was noncompetitive with respect to the substrate dGTP in the presence of the template-primer poly(C)-oligo(dG) (Figure 4). When poly(A)-oligo(dT) was used as template-primer, a competitive type of inhibition was obtained (Figure 5). The competitive type of inhibition has also been shown for other non-nucleoside inhibitors. It is the case, for example, of a HEPT derivative (E-EPU) that competitively inhibits the HIV-1 RT reaction (with respect to dTTP) if the reaction is directed by poly(A)-oligo(dT).[14] These data point to the possibility of the existence of two functionally (and possibly also spatially) related RT-binding sites differing in their affinity to the derivatives: a first binding site, different from the substrate-binding site, that leads to a noncompetitive type of inhibition and a second binding site that causes a competitive type of inhibition.[14]

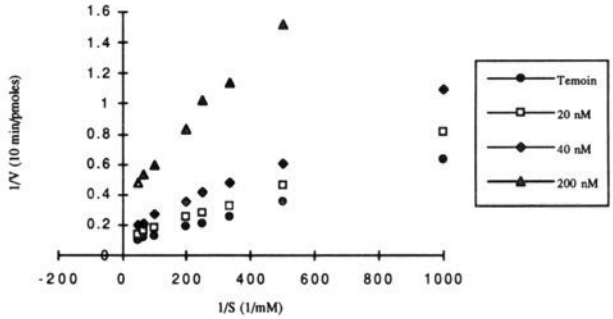


Figure 4. Double-reciprocal (Lineweaver–Burk) plot for inhibition of HIV-1 RT by compound **7c** with poly(C)-oligo(dG) as the template-primer and dGTP as substrate.

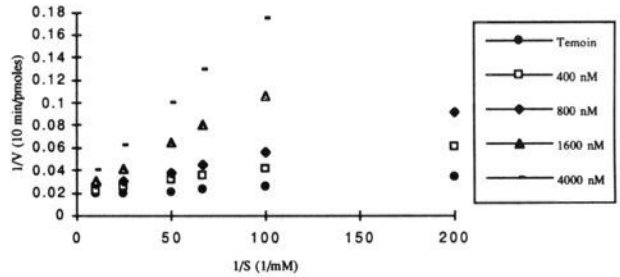


Figure 5. Double-reciprocal (Lineweaver–Burk) plot for inhibition of HIV-1 RT by compound **7c** with poly(A)-oligo(dT) as the template-primer and dTTP as substrate.

Discussion

In this work, studies were first realized on a variety of 3- and 4-substituted 5-methylpyridin-2(1*H*)-one derivatives in order to define the characteristic structural requirements for biological activities. The results arising from these studies were then extented to the 5-ethyl- and 5-ethyl-6-methylpyridinones, and to the quinolin-2(1*H*)-one analogues as well.

Thus, from these initial SAR studies of 20 new derivatives belonging to the 5-methylpyridin-2(1*H*)-one series, some general comments can be drawn (see Table 1). (a) Like in the HEPT series,[16,17] the two methyl groups at the 3- and 5-positions of the thiophenyl substituent play an important role for biological activity. Thus, in our tests, 4-(thiophenyl)pyridinone **7d** was inactive, the 3-methylphenyl analogue **7e** displayed moderate activity (IC_{50} = 2800 nM) similar to that of the HEPT derivative **1a** (IC_{50} = 6000 nM), but the 3,5-dimethylphenyl derivative **7a** exhibited a more pronounced inhibitory effect (IC_{50} = 120 nM). (b) When the sulfur atom in the thioether function was replaced by an NH group as in the case of the compound **9**, with respect to its analogue **7a**, RT inhibitory properties were totally abolished. (c) If various heterocycles took place at the 4-position of the 5-methylpyridinones, the same results were observed (compounds **10a–h** and **11**) (Scheme 2). Contrary to results described by Pan *et al.*[18] for 2-pyridylthio HEPT derivatives, this group is inadequate for our pyridinone analogues (compare **10h** vs **7a**). (d) A striking decrease or an abolishment of biological activity was also observed for amides and carbamates derived from amino derivative **8a**, with IC_{50}s which reach values 1000-fold higher than that of their amino counterparts (compare **8a** vs **12a,c–f**).

3-Acetamidopyridinone derivative **12b** was yet a particular exception to these findings. Indeed, this compound displayed an IC_{50} value equal to 67 nM, which is only 6-fold higher than that of amine **8a** (10 nM). Since the cytotoxicity of **12b** was significantly lower (*ca.* 10-fold), its selectivity index was better.

For 5-methylpyridinone derivatives, it was obvious that the characteristic structural requirements to display significant biological activity are the presence of a (3,5-dimethylphenyl)thio group at their 4-position together with a 3-nitro or, at the best, a 3-amino function (compare **7a** vs **8a**, **7b** vs **8b**, **7e** vs **8e**, and **7f** vs **8f**).

The substituents present at the 5- and 6-positions also play a very important role in this series. Thus, quinolone derivatives corresponding to compounds with a fuzed benzo ring at these positions resulted in the weak inhibitors **7f** and **8f**. On the contrary, a 5-ethyl group (**7b** and **8b**) and 5-ethyl plus 6-methyl substituents (**7c** and **8c**), led to highly active compounds, with IC_{50} values ranging from 90 (**7b**), 14 (**8c**), 10 (**8b**), and 6 (**7c**) nM.

Finally, a complementary result which clearly showed the importance of the 3-substituent group in this series was given by the comparison of biological activities of 5-ethyl-6-methyl-4-[(3′,5′-dimethylphenyl)thio]pyridin-2(1*H*)-one (**17**) (IC_{50} = 500 nM) to those of its 3-carbethoxy analogue **16** (IC_{50} = 3.1 nM) which is the most efficient compound in this series, at least up to date, but less active on RT. To explain this difference, one may hypothesize that compound **16** may act as a prodrug which can be activated in the culture medium or the cell, activation which would not occur during the RT assay (see Table 3). Another possibility is that the molecule **16** may have, in addition to RT, other viral targets during the replication cycle, and the effects could be additive or synergistic.

Conclusion

This work led us to obtain a new series of potent non-nucleoside HIV-1 RT inhibitors. They are 4-(arylthio)-3,5,6-trisubstitutedpyridin-2(1*H*)-one derivatives related to both HEPT and the Merck pyridinone series. Though totally novel, they can be considered as a hybrid of these two lead models. The worked out pathway to these molecules will probably allow us to obtain a large panel of analogues and related compounds which could be useful for a more elaborate SAR study.

From the biological point of view, biochemical studies showed that compounds **7c** and **8c** strongly inhibited the activity of a recombinant HIV-1 RT. The derivatives showed different levels of inhibition depending on the template-primer used. Better levels of inhibition were obtained with a template-primer of poly(C)-oligo(dG), as compared to poly(A)-oligo(dT). Enzyme kinetic analysis of RT inhibition by these compounds indicated that they were noncompetitive with respect to the substrate dGTP. Compounds **7c** and **8c** did not inhibited HIV-2 RT. All these properties enable us to classify compounds **7c** and **8c** as HIV-1 specific non-nucleoside RT inhibitors.

Besides trying to use our findings to develop new and more efficient HIV-RT inhibitors, our results could also serve for a better understanding of the main parameters involved in the interactions of these compounds with the "allosteric" site of RT.

Table 4. 4-Hydroxy-3-nitropyridin-2(1*H*)-ones **5b,c** and 4-Hydroxy-3-nitroquinolin-2(1*H*)-one (**5f**): Physical Data

no.	yield (%)	mp (°C)	formula	anal.
5b	82	213–214 (water)[a]	$C_7H_8N_2O_4 \cdot 0.25H_2O$	C, H, N
5c	85	255–256 (water)[a]	$C_8H_{10}N_2O_4$	C, H, N
5f	98	250 (none)[a]	$C_9H_6N_2O_4$	C, H, N

[a] Recrystallization solvent.

Table 5. 4-Chloro-3-nitropyridin-2(1*H*)-ones **6b,c**, 4-Chloro-3-nitroquinolin-2(1*H*)-one (**6f**), and 4-Chloro-5-ethyl-6-methyl-3-carbethoxypyridin-2(1*H*)-one (**15**): Experimental Conditions and Physical Data

no.	$POCl_3$[a] (mol equiv)	rfx[b] (h)	yield (%)	mp (°C)	formula	anal.
6b	4.4	1	74	*c*	*c*	*c*
6c	4.4	1	34	*c*	*c*	*c*
6f	2.4	0.3	67	*c*	*c*	*c*
15	4.4	6	71	167[d]	$C_{11}H_{14}NO_3Cl$	C, H, N, Cl

[a] Optimized proportion. [b] Rfx = heating (reflux) times in hours (h). [c] See caution in the Experimental Section. [d] The crude product was crystallized in ethyl acetate to give white crystals.

Experimental Section

Chemistry. TLC was carried out on precoated plates of silica gel 60F254 (Merck). In order to reveal the compounds, TLC plates were exposed to UV light. Purifications were performed on silica gel (40–60 μm, SDS) columns by medium pressure chromatography. All melting points were measured on an Electrothermal 9200 apparatus and are uncorrected. ^1H NMR spectra were recorded in the given solvents with a Bruker AC 200 apparatus with $CHCl_3$ (δ = 7.25 ppm) or DMSO (δ = 2.54 ppm) as internal standard (* and # = interchangeable assignments). Elemental analyses, performed by the Service Central de Microanalyses du CNRS, 91190 Gif-sur-Yvette, France, were within 0.3% of the theoretical values calculated for C, H, N, S, and Cl.

CAUTION: Because of the strong allergic effects on the skin of the chloronitropyridinone derivatives **6b,c,f**, their manipulation should be carried out in a ventilated hood and the use of gloves is recommended. This is the reason why they were used without further purification and the elemental analyses and the meltings points were not performed for these compounds.

5-Ethyl-4-hydroxy-6-methylpyridin-2(1*H*)-one (4c). The ester **14** prepared as described below (17.2 g, 76.4 mmol) was dissolved in 1.2 L of an aqueous solution of HCl (1 N), and the mixture was heated under reflux for 36 h. After evaporation of the solvent, 100 mL of water was added, and the mixture was neutralized with aqueous ammonia. The precipitate was filtered off and washed with water. The product **4c** was obtained (11.2 g, 96%) as a white solid: mp 360 °C; ^1H NMR (DMSO-d_6) δ 10.75 (1H, s, NH-1), 5.46 (1H, s, H-3), 2.32 (2H, q, J = 7 Hz, CH_2CH_3), 2.13 (3H, s, CH_3-6), 0.99 (3H, t, J = 7 Hz, CH_2CH_3). Anal. ($C_8H_{11}NO_2$) C, H, N.

4-Hydroxy-3-nitropyridin-2(1*H*)-ones 5b,c and 4-Hydroxy-3-nitroquinolin-2(1*H*)-one (5f) (Table 4). Preparation of 5-Ethyl-4-hydroxy-3-nitropyridin-2(1*H*)-one (5b): Example of the General Method. A suspension of **4b**[13] (5.00 g, 36.0 mmol) in 40 mL of nitric acid (d = 1.33) was stirred for 10 min at room temperature and at 75 °C during 12 min; 150 mL of ice water was added immediately, and the yellow precipitate was filtered off and recrystallized from water giving the nitropyridinone **5b** (5.4 g, 82%) as yellow crystals: mp 213–214 °C; ^1H NMR (DMSO-d_6) δ 11.85 (1H, s, NH-1), 7.39 (1H, s, H-6), 2.42 (2H, q, J = 7 Hz, CH_2), 1.10 (3H, t, J = 7 Hz, CH_3). Anal. ($C_7H_8N_2O_4 \cdot 0.25H_2O$) C, H, N.

Chlorination Reaction for the Production of the 4-Chloro-3-nitropyridin-2(1*H*)-ones 6b,c, 4-Chloro-3-nitroquinolin-2(1*H*)-one (6f), and 4-Chloro-5-ethyl-6-methyl-3-carbethoxypyridin-2(1*H*)-one (15) (Table 5). Preparation of 4-Chloro-5-ethyl-3-nitropyridin-2(1*H*)-one (6b): Example of the General Method. To a solution of **5b** (3.00 g, 16.3 mmol) and benzyltriethylammonium chloride (14.90 g, 65.2 mmol) in acetonitrile (60 mL) was added phosphorus oxychloride (6.7 mL, 71.9 mmol). The obtained mixture was

Table 6. 3-Nitro-4-(phenylthio)pyridin-2(1*H*)-ones **7a–f**, 3-Nitro-5-methyl-4-[(3′,5′-dimethylphenyl)amino]pyridin-2(1*H*)-one (**9**), 3-Nitro-4-(arylthio)-pyridin-2(1*H*)-ones **10a–h**, and 5-Ethyl-6-methyl-3-carbethoxy-4-[(3′,5′-dimethylphenyl)thio]pyridin-2(1*H*)-one (**16**): Experimental Conditions and Physical Data

no.	*T* (°C) (time, h)	yield (%)	purification technique[a]	mp (°C)	formula	anal.
7a	20 (1)	92	cyclohexane*	235	$C_{14}H_{14}N_2O_3S \cdot 0.25H_2O$	C, H, N, S
7b	20 (4)	53	ethanol#	197	$C_{15}H_{16}N_2O_3S$	C, H, N, S
7c	20 (3)	81	ethanol#	236–237	$C_{16}H_{18}N_2O_3S$	C, H, N, S
7d	20 (15)	47	ethanol#	214–216	$C_{12}H_{10}N_2O_3S \cdot 0.10H_2O$	C, H, N, S
7e	20 (15)[b]	71	water*, ethanol#	222–223	$C_{13}H_{12}N_2O_3S \cdot 0.25H_2O$	C, H, N, S
7f	50 (2.5)[b]	59	water/hexane* ethanol#	294	$C_{17}H_{14}N_2O_3S$	C, H, N, S
9	reflux (1)	79	ethanol#	273–275	$C_{14}H_{15}N_3O_3$	C, H, N
10a	reflux (3)[b]	58	water*	238–239	$C_{12}H_{12}N_4O_3S \cdot 0.125H_2O$	C, H, N, S
10b	reflux (4.5)	30	water* chromatography[c]	>350	$C_{11}H_{10}N_4O_4S$	C, H, N, S
10c	reflux (6.5)[b]	66	water*	272–275	$C_{13}H_{10}N_4O_3S \cdot 0.25H_2O \cdot 0.25C_2H_5OH$	C, H, N
10d	reflux (3)	27	ethanol#	180–181	$C_{13}H_9N_3O_4S \cdot 0.5C_2H_5OH$	C, H, N, S
10e	reflux (4)	60	water*	240	$C_{13}H_9N_3O_3S_2$	C, H, N
10f	reflux (8)[b]	7	water* chromatography[d]	177	$C_9H_9N_3O_3S_2 \cdot 0.25C_2H_5OH$	C, H, N
10g	reflux (6)[b]	85	water*	260–265	$C_{10}H_{10}N_4O_3S$	C, H, N, S
10h	20 (48)	66	water*	195–196	$C_{11}H_9N_3O_3S$	C, H, N, S
16	reflux (12)	82	ethyl acetate#	202	$C_{19}H_{23}NO_3S$	C, H, N, S

[a] Asterisk = washing and pound sign = recrystallization. [b] The mixture was evaporated to dryness before the treatment. [c] After extraction with ethyl acetate, the crude mixture was chromatographed on a silica gel column using hexane–ethyl acetate (1:1–0:1) and ethyl acetate–ethanol (95:5–9:1) as eluants. [d] Flash chromatography on a silica gel column using dichloromethane–ethanol (1:0–9:1) as eluant.

Table 7. 3-Amino-4-(phenylthio)pyridin-2(1*H*)-ones **8a–c,e,f** and 3-Amino-5-methyl-4-(benzoxazol-2-ylthio)pyridin-2(1*H*)-one (**11**): Experimental Conditions and Physical Data

no.	*T* (°C) (time, h)	yield (%)	purification technique	mp (°C)	formula	anal.
8a	reflux (1)	87	chromatography[a]	208	$C_{14}H_{16}N_2OS$	C, H, N, S
8b	70 (1)	64	chromatography[a]	208–209	$C_{15}H_{18}N_2OS \cdot 0.25H_2O$	C, H, N, S
8c	70 (1)	88	chromatography[b]	188–189	$C_{16}H_{20}N_2OS$	C, H, N, S
8e	80 (20)	56	chromatography[c]	170–171	$C_{13}H_{14}N_2O_3S$	C, H, N, S
8f	70 (1)	52	recrystallization in ethyl acetate	235–236	$C_{17}H_{16}N_2OS \cdot 0.25H_2O$	C, H, N, S
11	reflux (6)	10	dichloromethane washings	296–297	$C_{13}H_{11}N_3O_2S \cdot H_2O$	C, H, N

[a] Flash chromatography on a silica gel column using dichloromethane–ethanol (9:1) as eluant. [b] Flash chromatography on a silica gel column using dichloromethane–ethanol (95:5–9:1) and ethyl acetate as eluants. [c] Flash chromatography on a silica gel column using ethyl acetate–heptane (2:1–4:1) as eluant.

stirred at 40 °C for 30 min and heated under reflux for 1 h. After evaporation of the solvent, 60 mL of water was added, and the mixture was stirred at room temperature for 3 h. The yellow precipitate was collected, washed with cyclohexane (3 × 6 mL), and dried to give **6b** (2.4 g, 74%) as pale yellow crystals: ^{1}H NMR (DMSO-d_6) δ 13.19 (1H, s, NH-1), 7.73 (1H, s, H-6), 2.56 (2H, q, J = 7.5 Hz, CH_2CH_3), 1.16 (3H, t, J = 7.5 Hz, CH_2CH_3).

3-Nitro-4-(phenylthio)pyridin-2(1*H*)-ones 7a–f, 3-Nitro-5-methyl-4-[(3′,5′-dimethylphenyl)amino]pyridin-2(1*H*)-one (9), 3-Nitro-4-(arylthio)pyridin-2(1*H*)-ones 10a–h, and 5-Ethyl-6-methyl-3-carbethoxy-4-[(3′,5′-dimethylphenyl)thio]pyridin-2(1*H*)-one (16) (Table 6). Preparation of 5-Methyl-3-nitro-4-[(3′,5′-dimethylphenyl)thio]pyridin-2(1*H*)-one (7a): Example of the General Method. A mixture of the compound **6a**[10] (1.88 g, 10.0 mmol) in 20 mL of ethanol and 2 mL of triethylamine was stirred until homogeneity. 3,5-Dimethylthiophenol (1.39 g, 10.1 mmol) was added dropwise. After 1 h under stirring at room temperature, the precipitate was filtered off and washed with cyclohexane (20 mL). The product **7a** was obtained (2.66 g, 92%) as a yellow solid: mp 235 °C; ^{1}H NMR (DMSO-d_6) δ 12.27 (1H, s, NH-1), 7.63 (1H, s, H-6), 7.01 (1H, s, H-4′), 6.96 (2H, s, H-2′,6′), 2.27 (6H, s, CH_3-3′,5′), 1.89 (3H, s, CH_3-5). Anal. ($C_{14}H_{14}N_2O_3S \cdot 0.25H_2O$) C, H, N, S.

3-Amino-4-(phenylthio)pyridin-2(1*H*)-ones 8a–c,e,f and 3-Amino-5-methyl-4-(benzoxazol-2-ylthio)pyridin-2(1*H*)-one (11) (Table 7). Preparation of 3-Amino-5-methyl-4-[(3′,5′-dimethylphenyl)thio]pyridin-2(1*H*)-one (8a): Example of the General Method. To a suspension of **7a** (2.50 g, 8.6 mmol) in ethyl acetate (150 mL) was added tin(II) chloride dihydrate (9.75 g, 43.0 mmol). The mixture was heated under reflux under argon for 1 h. After cooling at 0 °C, adding ice water (110 mL), and basifing with a saturated solution of sodium carbonate, the precipitate was eliminated by filtration and washed with water. The filtrate was separated and extracted with ethyl acetate. The combined organic layers were washed with brine (3 × 120 mL), dried over magnesium sulfate, and evaporated. The residue was purified by column chromatography using dichloromethane–ethanol (9:1) as eluant giving the product **8a** (2.02 g, 87%) as a pale yellow solid: mp 208 °C; ^{1}H NMR (DMSO-d_6) δ 11.46 (1H, s, NH-1), 6.82 (1H, s, H-4′), 6.72 (2H, s, H-2′,6′), 6.62 (1H, s, H-6), 5.46 (2H, s, NH_2-3), 2.22 (6H, s, CH_3-3′,5′), 1.97 (3H, s, CH_3-5). Anal. ($C_{14}H_{16}N_2OS$) C, H, N, S.

3-Formamido-5-methyl-4-[(3′,5′-dimethylphenyl)thio]pyridin-2(1*H*)-one (12a). To a solution of the amine **8a** (0.10 g, 0.4 mmol) in ethyl formate (previously distilled on calcium hydride) (8 mL) was added formic acid (2 mL). The mixture was heated under reflux for 12 h. After evaporation of the volatile materials, the residue was washed twice with ethanol and once with ethyl acetate. It was purified by column chromatography using dichloromethane–ethanol (95:5) and pure ethyl acetate as eluants giving **12a** (0.06 g, 58%) as a white powder: mp 221–222 °C; ^{1}H NMR (DMSO-d_6) δ 11.96 (1H, s, NH-1), 8.28 (1H, s, C*H*O), 7.23 (1H, s, H-6), 6.88 (1H, s, H-4′), 6.78 (2H, s, H-2′,6′), 2.23 (6H, s, CH_3-3′,5′), 1.85 (3H, s, CH_3-5). Anal. ($C_{15}H_{16}N_2O_2S$) C, H, N, S.

3-Amido-4-[(3′,5′-dimethylphenyl)thio]pyridin-2(1*H*)-ones 12b–e,g (Table 8). Method A: 3-Acetamido-5-methyl-4-[(3′,5′-dimethylphenyl)thio]pyridin-2(1*H*)-one (12b). A solution of the amine **8a** (0.10 g, 0.4 mmol) and acetic anhydride (0.05 mL, 0.39 mmol) in acetic acid (20 mL) was heated to reflux for 1.5 h. After evaporation of the solvents, 10 mL of water was added, and the mixture was neutralized at 0 °C with an aqueous diluted solution of ammonia. After filtration, the residue was washed with cyclohexane (2 × 5 mL). The product **12b** was obtained (0.10 g, 86%) as a beige solid: mp 138 °C; ^{1}H NMR (DMSO-d_6) δ 11.84 (1H, s, NH-1), 9.41 (1H, s, NH-3), 7.20 (1H, s, H-6), 6.87 (1H, s, H-4′), 6.81

Table 8. 3-Amido-4-[(3′,5′-dimethylphenyl)thio]pyridin-2(1*H*)-ones **12b–e,g**: Experimental Conditions and Physical Data

no.	reagents and solvents	T (°C) (time, h)	yield (%)	purification technique	mp (°C)	formula	anal.
12b	acetic anhydride, acetic acid (method A)	reflux (1.5)	86	cyclohexane washings	138	$C_{16}H_{18}N_2O_2S·H_2O$	C, H, N, S
12c	propionyl chloride, NEt_3, CH_2Cl_2 (method B)	20 (5)	90	chromatography[a]	186–188	$C_{17}H_{20}N_2O_2S·1.25H_2O$	C, H, N, S
12d	heptanoic anhydride, toluene (method A)	100 (1.5)	50	diethyl ether washings, recrystallization in ethyl acetate	212–213	$C_{21}H_{28}N_2O_2S$	C, H, N, S
12e	phenylacetyl chloride, NEt_3, CH_2Cl_2 (method B)	20 (2)	56	chromatography[b]	200–202	$C_{22}H_{22}N_2O_2S$	C, H, N
12g	acetic anhydride, acetic acid (method A)	reflux (5.5)	74	cyclohexane washings	238–239	$C_{18}H_{22}N_2O_2S·0.45H_2O$	C, H, N, S

[a] Flash chromatography on a silica gel column using dichloromethane–ethanol (94:6) and ethyl acetate as eluants. [b] Flash chromatography on a silica gel column using dichloromethane–ethanol (95:5) as eluant.

(2H, s, H-2′,6′), 2.22 (6H, s, CH_3-3′,5′), 1.99 (3H, s, CH_3-5), 1.81 (3H, s, CH_3CO). Anal. ($C_{16}H_{18}N_2O_2S·H_2O$) C, H, N, S.

Method B: 5-Methyl-3-propionamido-4-[(3′,5′-dimethylphenyl)thio]pyridin-2(1*H*)-one (12c). To a solution of the amine **8a** (0.10 g, 0.40 mmol) and triethylamine (0.04 mL, 0.38 mmol) in dichloromethane (5 mL) was added freshly distilled propionyl chloride (0.04 mL, 0.41 mmol) at 0 °C (flask was fitted with a $CaCl_2$ drying tube). The mixture was stirred at room temperature during 5 h. The solvent was evaporated, water was added, and the solid was filtered off. The residue was purified by column chromatography using dichloromethane–ethanol (94:6) and then pure ethyl acetate as eluants giving **12c** (0.10 g, 90%) as a white powder: mp 186–188 °C; ^{1}H NMR (DMSO-d_6) δ 11.86 (1H, s, NH-1), 8.60 (1H, s, NH-3), 7.21 (1H, s, H-6), 6.88 (1H, s, H-4′), 6.80 (2H, s, H-2′,6′), 4.04 (2H, q, J = 7 Hz, $COCH_2CH_3$), 2.22 (6H, s, CH_3-3′,5′), 1.82 (3H, s, CH_3-5), 1.20 (3H, t, J = 7 Hz, $COCH_2CH_3$). Anal. ($C_{17}H_{20}N_2O_2S·1.25H_2O$) C, H, N, S.

5-Methyl-3-[*N*-(ethoxycarbonyl)amino]-4-[(3′,5′-dimethylphenyl)thio]pyridin-2(1*H*)-one (12f). Triethylamine (0.10 g, 0.50 mmol) was added to a solution of the amine **8a** (0.05 g, 0.20 mmol) in ethanol (2 mL). To this mixture, cooled in ice water, was added dropwise freshly distilled ethyl chloroformate (0.65 g, 6.00 mmol), and the mixture was stirred at room temperature for 48 h. After evaporation of the solvent, 5 mL of water was added. After filtration, the red solid was purified by column chromatography using dichloromethane–ethanol (98:2) as eluant to give the recovered amine **8a** (0.005 g, 10%) and the compound **12f** (0.01 g, 26%) as a white solid: mp 208–210 °C; ^{1}H NMR (DMSO-d_6) δ 11.83 (1H, s, NH-1), 8.60 (1H, s, NH-3), 7.21 (1H, s, H-6), 6.88 (1H, s, H-4′), 6.80 (2H, s, H-2′,6′), 4.04 (2H, q, J = 7 Hz, OCH_2CH_3), 2.22 (6H, s, CH_3-3′,5′), 1.82 (3H, s, CH_3-5), 1.19 (3H, t, J = 7 Hz, OCH_2CH_3). Anal. $C_{17}H_{20}N_2O_3S$ (C, H, N, S).

Ethyl 2-Ethyl-3-aminocrotonate (13). Ethyl 2-ethylacetoacetate (150 g, 0.95 mol) and ammonium nitrate (84 g, 1.04 mol) were dissolved in dry tetrahydrofuran (1.1 L). The mixture was stirred for 5 days with a blow of ammonia bubbles. The solvent was evaporated at room temperature under reduced pressure, 1 L of water was added, and the mixture was stirred for 30 min further. The colorless residue was filtered off and crystallized from hexane to give the product **13** (107 g, 72%) as colorless crystals: mp 61 °C; ^{1}H NMR ($CDCl_3$) δ 4.11 (2H, q, J = 7 Hz, OCH_2CH_3), 2.17 (2H, q, J = 7 Hz, CH_2CH_3), 1.93 (3H, s, CH_3), 1.24 (3H, t, J = 7 Hz, OCH_2CH_3), 0.94 (3H, t, J = 7 Hz, CH_2CH_3). Anal. ($C_8H_{15}NO_2$) C, H, N.

4-Hydroxy-5-ethyl-6-methyl-3-carbethoxypyridin-2(1*H*)-one (14). Sodium (48.34 g, 2.10 mol, lump in kerosene) was dissolved slowly in 530 mL of ethanol under nitrogen. The mixture was heated under reflux, and freshly distilled diethyl malonate (335 mL, 2.20 mol) was added dropwise for 30 min. Still under reflux, the aminocrotonate **13** (150 g, 0.96 mol) in 200 mL of ethanol was added dropwise. The mixture was stirred under reflux for 72 h to give a pale yellow suspension which was cooled at room temperature, and the precipitate was filtered off. The solid was dissolved in water, cooled at 0 °C, and acidified to pH 1 with an aqueous solution of hydrochloric acid. The precipitate was filtered off, washed with water, and recrystallized from toluene to give the product **14** (109.6 g, 51%) as white crystals: mp 196–197 °C; ^{1}H NMR (DMSO-d_6) δ 11.52 (1H, s, NH*-1), 11.32 (1H, s, OH*), 4.34 (2H, q, J = 7 Hz, $COOCH_2CH_3$), 2.39 (2H, q, J = 7.5 Hz, CH_2CH_3), 2.23 (3H, s, CH_3-6), 1.31 (3H, t, J = 7 Hz, $COOCH_2CH_3$), 1.02 (3H, t, J = 7 Hz, CH_2CH_3). Anal. ($C_{11}H_{15}NO_4$) C, H, N.

5-Ethyl-6-methyl-4-[(3′,5′-dimethylphenyl)thio]pyridin-2(1*H*)-one (17). The compound **16** (120 mg, 0.34 mmol) was dissolved in 6 mL of tetrahydrofuran–H_2O–37% HCl (18:3:4). The mixture was stirred at 75 °C for 10 days. The tetrahydrofuran was evaporated, and 5 mL of water was added. The mixture was stirred, and the water was eliminated. The residue was crystallized in ethanol (25 mL) giving the product **17** (50 mg, 59%) as colorless paillettes: mp 277–278 °C; ^{1}H NMR (DMSO-d_6) δ 11.26 (1H, s, NH-1), 7.22 (3H, s, H-2′,4′,6′), 5.25 (1H, s, H-3), 2.36 (6H, s, CH_3-3′,5′), 2.21 (3H, s, CH_3-6), 1.13 (3H, t, J = 7.5 Hz, CH_2CH_3), CH_2CH_3 signal was overlapped by DMSO signal. Anal. ($C_{16}H_{19}NOS·0.25H_2O$) C, H, N, S. (In ethanol and 5% sodium hydroxide, the starting material was recovered in majority. Traces of compound were isolated and showed by ^{1}H NMR the loss of the arylthio part.)

Biology. Evaluation of Antiviral Activity of the Compounds. The effects of the compounds on the replication of HIV-1 were evaluated (see Table 1), as previously described, in CEM-SS cells (a cell line of the lymphocytic lineage) acutely infected with HIV-1 LAI.[19] CEM-SS cells were obtained from Peter Nara and Nevirapine resistant HIV-1 (N119) cells bearing a point mutation at RT codon 181 from D. Richman through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH.

The production of virus was measured by quantification of the RT activity associated with the virus particles released in the culture supernatant. Briefly, cells were infected with 100 $TCID_{50}$ for 30 min; after virus adsorption, unbound particles were eliminated by two washes, and cells were cultured in the presence of different concentrations of test compounds for 5 days before virus production determination. The 50% inhibitory concentration of virus multiplication (IC_{50}) was derived from the computer-generated median effect plot of the dose–effect data.[20] In parallel experiments, cytotoxicity of the molecules for uninfected cells was measured after an incubation of 5 days in their presence using a colorimetric assay (MTT test) based on the capacity of mitochondrial dehydrogenases of living cells to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide into formazan.[21] The 50% cytotoxic concentration (CC_{50}) is the concentration at which OD_{540} was reduced by one-half and was calculated using the program mentioned above.

Expression and Purification of the Recombinant HIV-1 RT Enzyme. Yeast cells, transformed with the vector pAB24/RT-4 were used to purify the recombinant HIV-1 RT enzyme as described previously.[22] The system for the expression of recombinant HIV-2 RT in *Escherichia coli*[23] was a kind gift of Dr. R. Goody. HIV-2 RT was purified as HIV-1 RT.

RT Assays. Incubation was carried out at 37 °C for 10 min in the presence of different template-primers. (a) Poly(C)-oligo(dG): the reaction mixture contained, in a final volume

of 0.05 mL, 50 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, 4 mM dithiothreitol, 0.48 A_{260}/mL poly(C)-oligo(dG) (5:1), 1.0 μCi [^{3}H]-dGTP (28 Ci/mmol), 2 μM dGTP, 80 mM KCl, 1 μg of bovine serum albumin, and 20–50 nM RT. (b) Poly(A)-oligo(dT): same conditions as in a, except that 0.48 A_{260}/mL poly(A)-oligo-(dT) (5:1), 0.5 μCi [^{3}H]dTTP (46 Ci/mmol), and 20 μM dTTP were used.

Reactions were stopped by the addition of 1 mL of cold 10% trichloroacetic acid plus 0.1 M sodium pyrophosphate. The precipitates were filtered through nitrocellulose membranes, washed with 2% trichloroacetic acid, dried, and counted in a PPO/POPOP/toluene scintillation mixture.

Reverse Transcription. The plasmid pmCG6 containing the nucleotide fragment 1-4005 of HIV-1 (pmal) in psP64, under the control of the bacteriophage T7 promoter was a kind gift from Dr. J. L. Darlix. *E. coli* HB101(1035)recA$^-$ was used for plasmid amplification. After digestion of this clone with *Hin*cII and *in vitro* transcription using T7 RNA polymerase, RNAs were obtained starting at position +50 of the pmal sequence. *In vitro* transcription and reverse transcription were performed as described in ref 24.

Inhibition Experiments. All compounds were dissolved in dimethyl sulfoxide (DMSO). Controls were made in the presence of the same final concentration of DMSO. IC_{50} is the concentration required to inhibit recombinant HIV-1 RT activity by 50%.

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