Eur J Med Chem (1996) 31, 111–122

Novel C-organosilicon derivatives as leads for reverse-transcriptase-mediated anti-HIV-1 activity

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(Received 24 July 1995; accepted 23 October 1995)

Summary — In order to prepare novel antiretroviral lead compounds active against human immunodeficiency virus type 1 reverse transcriptase (HIV-1 RT), a family of C-silylalkylated heterocyclic derivatives were synthesized. One of these derivatives **3** inhibited HIV-1 replication in cell culture (IC_{50} = 12 ± 4 μM) without significant cytotoxic effects. This compound did not exhibit antiviral activity against herpes simplex virus type 1 and poliovirus type 1. Biochemical studies showed that **3** inhibited the DNA polymerase activity of a recombinant HIV-1 RT. This derivative was shown to be a non-competitive inhibitor with respect to dNTP substrate incorporation. Different levels of inhibition were obtained depending on the template-primer used. Compound **3** did not inhibit either the RNase H activity of HIV-1 RT, or the RNA-directed DNA polymerase activity of HIV-2 RT.

human immunodeficiency virus / reverse transcriptase inhibitor / C-silylalkylated nucleobase / C-silylalkylated heterocycle

Introduction

Reverse transcriptase (RT) is essential for retroviral replication. Thus, there is interest in human immunodeficiency virus (HIV) reverse transcriptase as a target for AIDS chemotherapy [1–3]. The nucleoside analogs 3'-azido-2',3'-dideoxythymidine (AZT), 2',3'-dideoxyinosine (ddI) and 2',3'-dideoxycytidine (ddC) are at present the only drugs approved for the clinical treatment of AIDS, and all three act through this target. However, prolonged use of these molecules results in severe problems of cellular toxicity, including bone marrow suppression (AZT), peripheral neuropathy (ddC) or pancreatitis (ddI) [4, 5]. These compounds are also terminators of DNA chain elongation during the HIV reverse transcription process.

A second class of inhibitors, the non-nucleoside RT inhibitors, comprises compounds with very different structures. Key lead compounds are tetrahydroimidazobenzodiazepinone (TIBO) [6], 1-[(2-hydroxyethoxy)-methyl]-6-(phenylthio)thymine (HEPT) [7, 8], dipyridodiazepinone [9], pyridinone [10], bis(heteroaryl)-piperazines (BHAP) [11], *tert*-butyldimethylsilyl spiroamino oxathioledioxide nucleoside analogs (TSAO) [12] and α-anilinophenylacetamide (α-APA) [13].

Under the pressure of both classes of inhibitors rapid mutations occur in the viral genome leading to the emergence of drug-resistant strains. This problem necessitated the search for new compounds that are more efficient in their anti-HIV activity.

One strategy that could provide inhibitors with large selectivity indexes and for which mutant strains do not evolve, is to address biochemical mechanistic considerations in order to target molecules towards the active site of the enzyme.

Indeed, the transition state of the reaction catalyzed by RT proceeds through a nucleophilic attack of the 3'-terminal hydroxyl group of DNA, on the α-phosphorus of the newly added nucleoside triphosphate (scheme 1). In this pattern, the phosphorus atom undergoes a valence extension from the tetravalent to the pentavalent state, before the expulsion of the diphosphate moiety [14]. Two nucleic bases are present in the catalytic center, throughout this pathway.

In an attempt to find new lead anti-HIV agents we describe here the synthesis and evaluation of the biochemical and biological properties of C-silylated organosilicon derivatives, which were designed to

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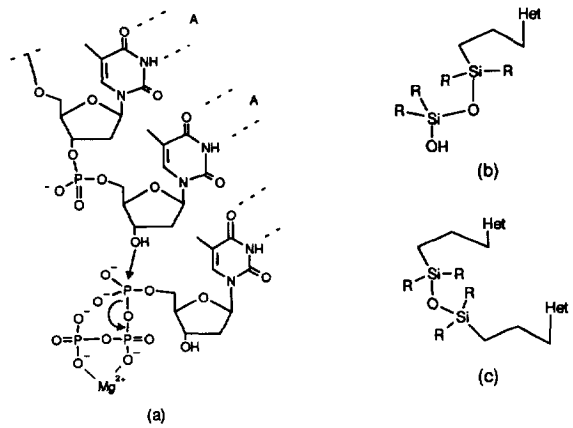


Scheme 1. (a) Incorporation of a nucleotide in the growing DNA strand catalyzed by the retroviral DNA polymerase (reverse transcriptase); A = adenine. (b, c) General structure of the organosilicon compounds synthesized (R = Me, Phe; Het = heterocycle).

interact with the catalytic site of the RT. Two series of compounds were studied in which the silicon atom could afford advantages due to the low energy state of its 3*d* orbitals and its similarity with phosphorus in terms of bond lengths and angles. First, transition state analogs [15] (scheme 1b) require a heterocycle able to generate hydrogen bonds with the complementary RNA strand, and an atom which readily reacts with a nucleophile (as does phosphorus) while remaining relatively stable when pentavalent. The second series comprises bisubstrate analogs [16] (scheme 1c). Silicon could also allow a greater synthetic flexibility than pure carbon compounds and easier modulation of the physico-chemical properties (eg, lipophilicity or charge) of the synthesized molecules.

Chemistry

We focused our attention on the preparation of C-silylated nucleobases and their heterocyclic analogs. Thus a series of silanols, siloxanes or silanol-siloxanes bearing one or two heterocycles with a variable length of hydrocarbon chain between the heterocycle and the silicon atom was synthesized.

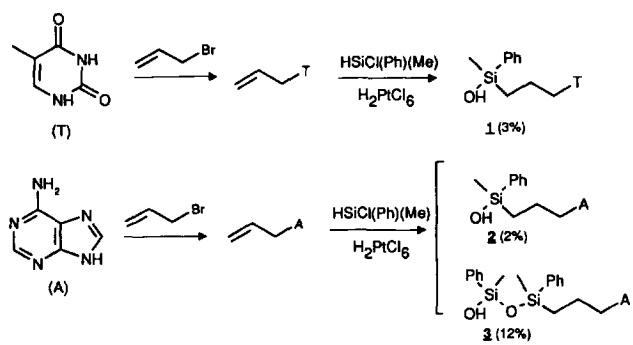
Molecules bearing one thymine or adenine nucleobase were obtained by a hydrosilylation reaction. Condensation of allylbromide on thymine and adenine sodium salts [17] yielded *N*-1-allylthymine and *N*-7-allyladenine, which upon hydrosilylation with phenylmethylchlorosilane in the presence of Speier's catalyst [18] gave the silylated compounds **1–3** (scheme 2). The low yields observed may result from the poor solubility of the nucleobases in the non-polar solvents required to dissolve organochlorosilanes. Furthermore, we could not prevent the reduction of *N*-allyladenine and *N*-allylthymine to *N*-propyladenine and *N*-propylthymine during the reaction.

Molecules with two heterocycles were obtained by condensation of various heterocyclic sodium salts with either 3-(chloropropyl)dimethylchlorosilane or 1,3-bis(3-chloropropyl)-1,1,3,3-tetramethyldisiloxane as illustrated in scheme 3. The results obtained for these transformations are described in table I.

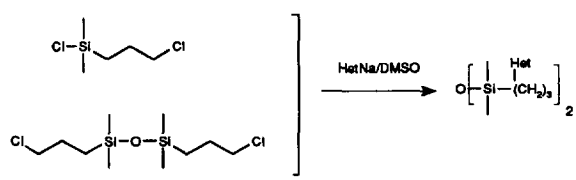
For thymine, competition between N-1 and N-3 alkylation was observed, as previously mentioned in the literature [19]. Thus even though the crude yield was excellent (100% with an equimolar ratio), a dramatic decrease of the yield (18%) was observed after purifying the compound.

The corresponding reaction of adenine yielded both N-7 and N-9 regioisomers. As previously described [20], the N-9/N-7 ratio for alkylation is close to 9:1. Other heterocycles like imidazole, 2-mercaptopyridine, 5-chloro-2-mercaptobenzothiazole or 1*H*-indole were similarly used and led to single compounds.

Finally, 1,3-bis(3-aminopropyl)-1,1,3,3-tetramethyldisiloxane was directly reacted with various isatoic anhydrides [21] yielding *N*-silylalkyl orthoamino benzamides (scheme 4).

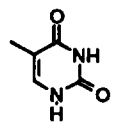
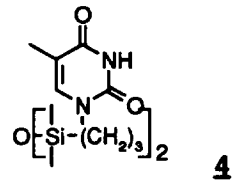
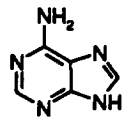
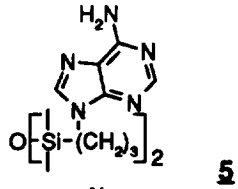
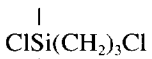
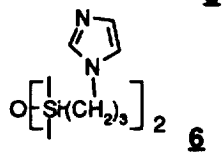
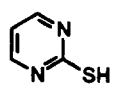
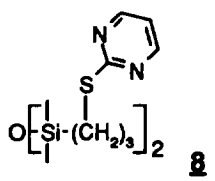
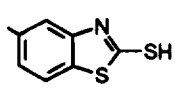
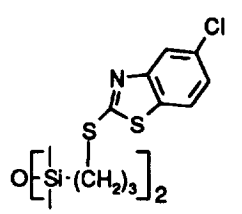
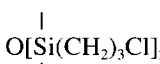
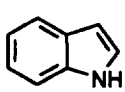
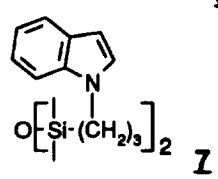


Scheme 2.



Scheme 3.

Table I. Products and yields for the reactions in scheme 3.

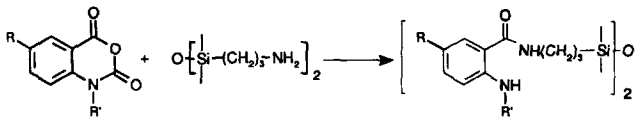
Organosilicon derivative	*Heterocycle*	*Products*	*Yield*
		 **4**	18%
		 **5**	40%
		 **6**	59%
		 **8**	47%
		 **9**	21%
		 **7**	27%

Results and discussion

Enzyme inhibitory activities

In order to perform a preliminary screening of the drugs as anti-viral agents, we tested their effect on recombinant HIV-1 RT activity. The majority of the anti-retroviral agents that inhibit RT also inhibit cellular DNA polymerases. Thus, it seemed important to determine the effect of the drugs on animal DNA polymerase α. This enzyme was moderately sensitive to inhibition by compounds **3** (IC_{50} = 500 μM) and **11** (IC_{50} = 125 μM). The IC_{50} values were at least sixfold higher than those needed for HIV-1 RT inhibition (table II).

The concentration inhibiting 50% of the HIV-1 RT activity (IC_{50}) for each compound is shown in table II. Only compounds **3** and **11** behaved as RT inhibitors,



10 (R= R'= H) yield: 96%
11 (R= Cl, R'= H) yield: 74%
12 (R= H, R'= Me) yield: 64%

Scheme 4.

Table II. HIV-1 RT inhibitory activity (HIV-1 RT and DNA polymerase α were tested as described in the *Experimental protocols*.

Compound	*HIV-1 RT* IC_{50} *(μM)*	*DNA polymerase-α* IC_{50} *(μM)*
1	> 2000	> 2000
2	2000	> 2000
3	50	500
4	2000	> 2000
5	> 2000	> 2000
6	> 2000	500
7	> 2000	> 2000
8	> 2000	> 2000
9	1000	1000
10	125	400
11	20	125
12	> 2000	> 2000

with IC_{50} values of 50 and 20 μM respectively, which can be compared with the TIBO activity (20 μM in the same system). For the other compounds, higher concentrations were necessary to obtain 50% inhibition.

The effect of these two compounds on the activities of RT, DNA polymerase and RNase H was examined. Dose–response curves are presented in figure 1. Both compounds **3** and **11** were found to strongly inhibit the DNA polymerase activity of recombinant RT. In the same experimental conditions, the RNase H activity from RT was less affected. Only 20% inhibition of RNase H activity was obtained with concentrations at least ten times higher than those inhibiting the RT-associated DNA polymerase activity.

Although compound **11** was a good inhibitor of recombinant RT, it was cytotoxic in MT-2 cells (see fig 6 below). Thus, we focused our studies on compound **3**.

It has been reported previously that non-nucleoside RT inhibitors show different levels of inhibition depending on the template-primer used to measure the RT activity [22]. This is possibly due to a major conformational effect on the binding site of the inhibitor.

We determined the inhibition produced by compound **3** on RT using two template-primers, poly A-oligo dT and poly C-oligo dG. Better inhibition was obtained in the presence of poly C-oligo dG with an IC_{50} that is 30-fold lower than the one obtained with poly A-oligo dT (fig 2). Similar IC_{50} values were obtained with HEPT [7, 8] tested in the same conditions and used as a reference of non-nucleoside inhibitors.

All non-nucleoside inhibitors thus far identified are specific for HIV-1 and do not inhibit HIV-2 RT. Compound **3** was tested with both RTs. As shown in figure 3, at concentrations where HIV-1 RT was well inhibited, HIV-2 RT was not. This discriminatory behaviour of compound **3** towards HIV-1 vs HIV-2 RT makes this compound share another common property of non-nucleoside inhibitors.

This series of organosilicon compounds was synthesized in order to obtain inhibitors targeted towards the active site of the enzyme. It was therefore important to perform studies allowing an insight into the nature of the inhibition. The results show that the inhibition of HIV-1 RT by compound **3** is non-competitive with respect to the substrate dGTP in the presence of the template-primer poly C-oligo dG (fig 4). The same type of inhibition (ie, non-competitive) was observed for dTTP using poly A-oligo dT as primer-template (fig 5).

Antiviral activities

Antiviral activity and cytotoxicity in MT-2 cells

The organosilicon derivatives were tested for their anti-HIV-1 activity by determining their ability to inhibit the cytophatic effect in MT-2 cells infected with HIV-1/HTLV-III-B strain, the production of reverse transcriptase and the p24 antigen expression. As shown in figure 6, compound **3** could inhibit the RT activity in the culture supernatant with an IC_{50} of 12 ± 4 μM without a significant cytotoxic effect (CC_{50} > 100 μM). Among the other compounds, some were toxic (**2**, **4**, **6**, **7**, **11** and **12**) or had low solubility in culture medium (**2**, **5**, **7**, **9**, **10** and **12**).

Compound **3** added to MT-2 cells infected with HIV-1 could inhibit the HIV-1 induced cytopathic effect (fig 7). The intracellular expression of p24 antigen was also inhibited in the presence of compound **3** (data not shown).

Antiviral activity of compound 3 added at different times after infection of MT-2 cells

Experiments were performed at different times to determine which stage in the HIV-1 life cycle could be

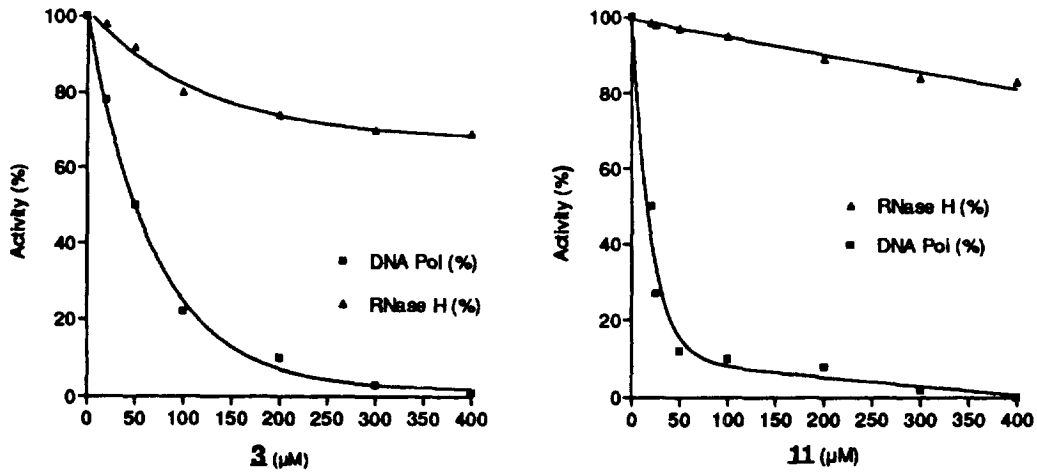


Fig 1. Effect of compounds **3** and **11** on recombinant HIV-1 RT. RT (50 ng) was added to increasing amounts of **3** or **11** dissolved in DMSO. DNA polymerase (measured in the presence of poly A-oligo dT) and RNase H activities were then tested for 10 min at 37 °C. Controls, corresponding to 100% activity, were performed in the presence of DMSO.

inhibited by **3**. Thus, MT-2 cells were: i) pretreated for 24 h with different concentrations of the drug prior to washing, and then infected with HIV-1 and maintained 4 days without the drug; ii) treated with different concentrations of the drug during the viral adsorption period (2 h) prior to washing, and then maintained without the drug; or iii) treated after the viral adsorption and maintained with different concentrations of the drug over 4 days.

The inhibitory effect of compound **3** was evaluated using different criteria such as protection of virus induced cytopathicity, RT activity released in the supernatant, and p24 Ag levels in the supernatant. Results indicated that the inhibitory effect of compound **3** was detected only after viral infection and in a dose-dependent manner (with IC_{50} = 10–16 μM irrespective of the test used). No effect of the solvent (DMSO conc < 2%) on HIV-1 replication in infected cells or on growth of the mock-infected cells was observed.

Antiviral activity of compound 3 in human PBMCs

Besides being able to inhibit HIV-1 replication in MT-2 cells, compound **3** was also a good inhibitor of viral

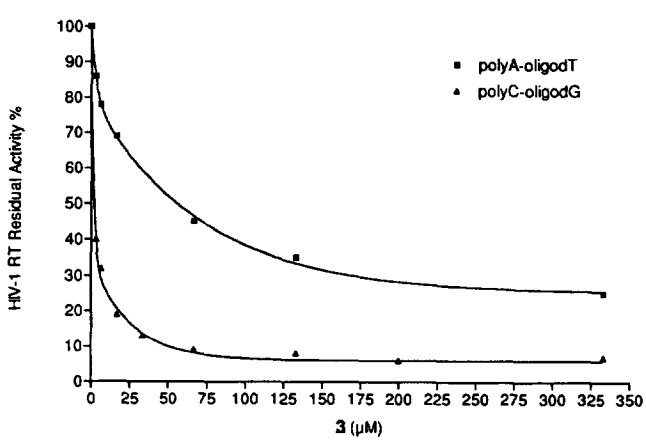


Fig 2. Template-primer influence on the level of inhibition of HIV-1 RT.

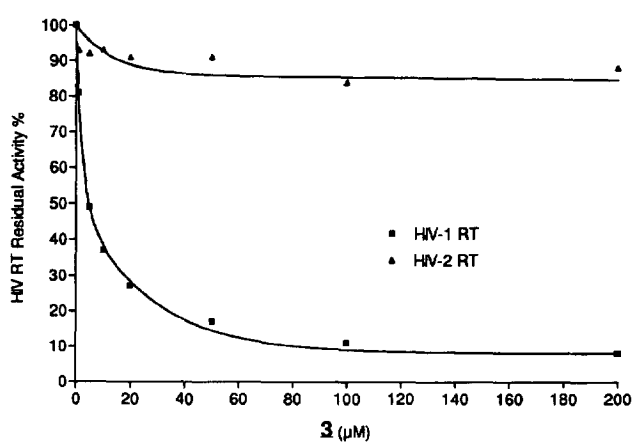


Fig 3. Effect of **3** on HIV-1 and HIV-2 RT.

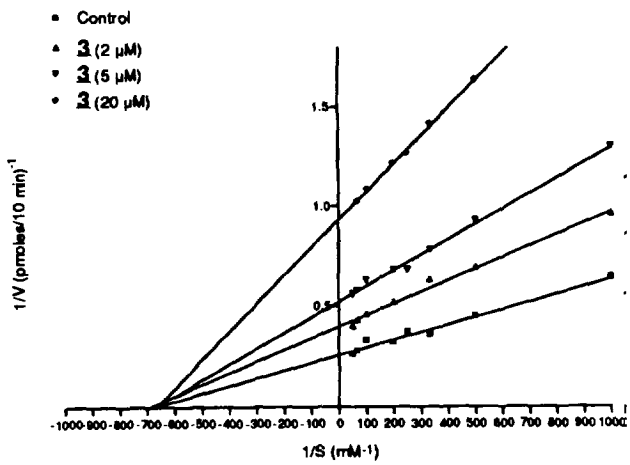


Fig 4. Double-reciprocal plot (Lineweaver–Burk) for inhibition by 3. The activity was measured with poly C-oligo dG as template-primer and dGTP as substrate.

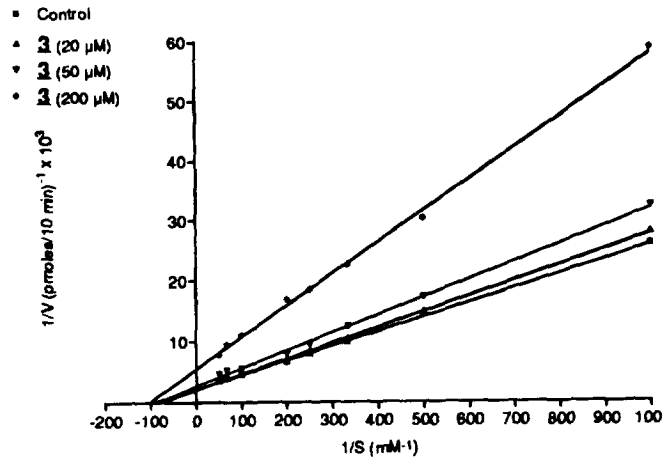


Fig 5. Double reciprocal plot (Lineweaver–Burk) for inhibition by 3. The activity was measured with poly A-oligo dT as template-primer and dTTP as substrate.

replication in human peripheral blood mononuclear cells (PBMCs). Normally these are one of the target cells of HIV-1 in human organism. When PBMCs were treated over 5 days after viral infection with HIV-1/HTLV-III-B strain, an inhibition of RT activity was observed in the supernatant (IC_{50} = 6 µM) without affecting cell growth (CC_{50} > 100 µM) (table III).

To screen for cross-reactivity, PBMCs were infected by different doses of BGL (AZT-susceptible HIV-1) and BRC (AZT-resistant HIV-1) viral strains and were treated by increasing concentrations of compound 3. Compound 3 exhibited an antiviral effect on both AZT-resistant and AZT-sensitive HIV-1 strains. AZT was used as a reference compound in parallel experiments.

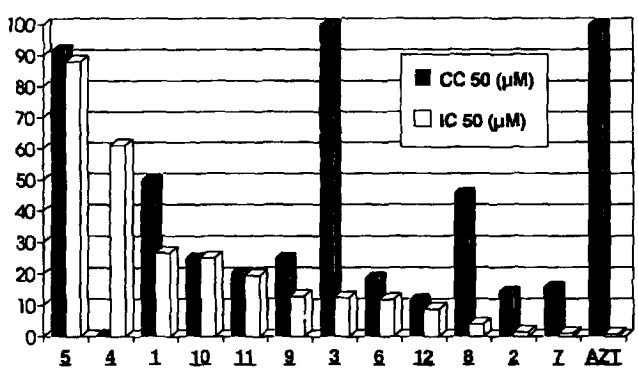


Fig 6. Antiviral activities and cytotoxicities of the synthesized compounds. Virus production was determined by measuring the endogeneous RT, and the number of viable cells was evaluated by MTT assay. CC_{50} is defined as the compound concentration required to decrease cell viability by 50%. IC_{50} is the concentration of compound that inhibits RT activity in supernatant culture by 50%.

Spectrum of activity of compound 3

The antiviral activity of compound 3 against DNA and RNA viruses (other than retroviruses) was examined in Vero cells infected at 10 and 100 $TCID_{50}$ (see *Experimental protocols).* Compound 3 did not show significant antiviral activity (IC_{50} > 100 µM) against herpes simplex virus type 1 and poliovirus type 1 as determined by inhibition of cytopathic effect and protection of cells. Furthermore, compound 3 did not show any cytotoxicity at the highest concentrations used in the assays.

Conclusion

A series of new lead compounds, the C-silylalkylated nucleobases (or heterocyclic analogs) were synthesized for interaction with the catalytic site of HIV-1 RT. Among these derivatives, compound 3 was a good inhibitor both of HIV-1 replication and of recombinant HIV-1 RT activity. Retroviral replication was inhibited by compound 3, as evidenced by the ability to inhibit the cytopathic effect of HIV-1 in MT-2 cells, the decrease in p24 antigen and virion-associated RT activity. At concentrations inhibiting viral replication, compound 3 did not produce significant cytotoxic effects.

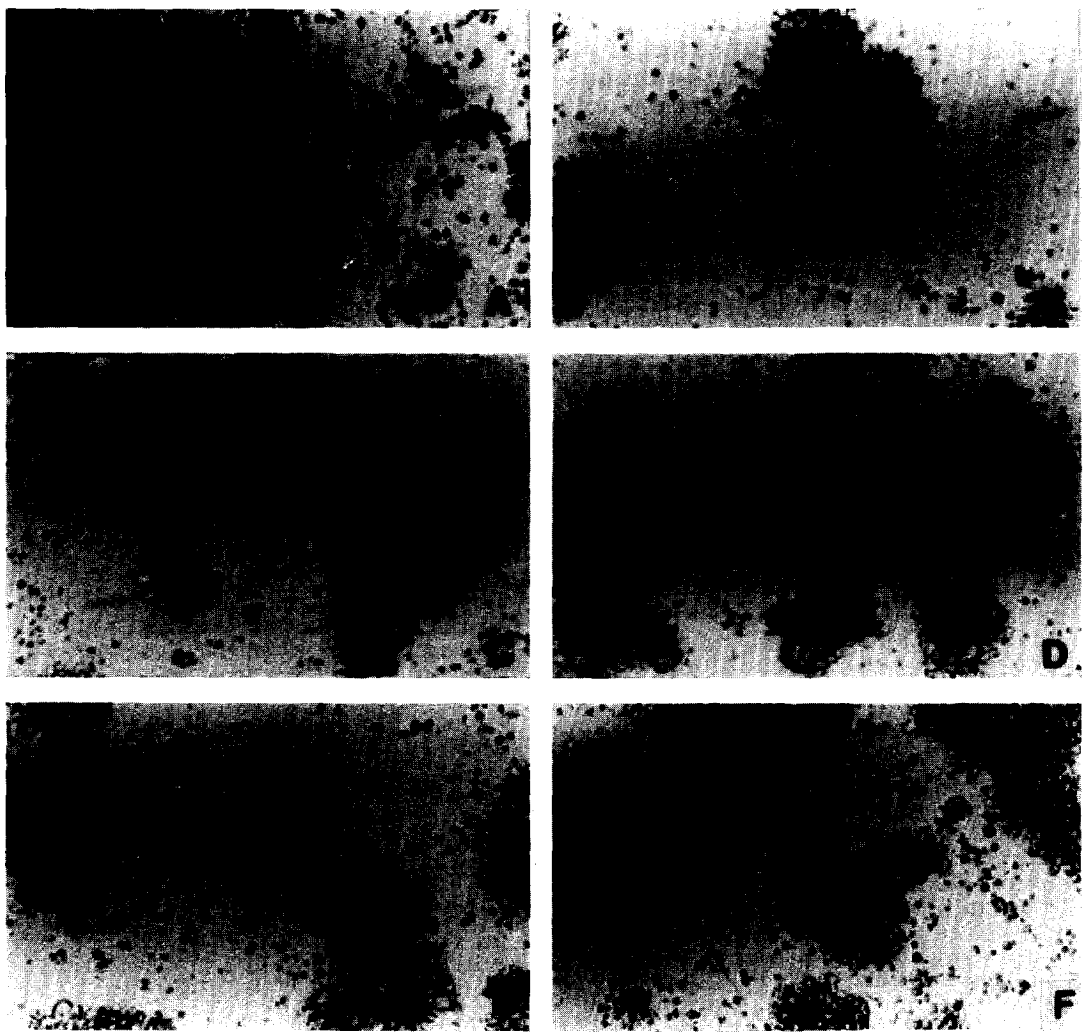


Fig 7. Inhibition of HIV-1 infection in cultured MT-2 cells. **A** to **D**: virus-infected cells treated with: 100, 20, 4 and 0.8 μM of compound **3**. **E**: untreated HIV-1 infected cells (positive control). **F**: uninfected cells (negative control). Incubation time: 4 days.

Table III. Antiviral activity of **3** in different cell systems.

Virus and strain	*Cells*	*Assay*	*Day of analysis*	***3***		*AZT*	
				CC_{50} (μM)	IC_{50} (μM)	CC_{50} (μM)	IC_{50} (μM)
HTLV III-B	MT-2	CPE/MTT	3/4	> 100	10	> 100	< 0.8
		RT Activity	3		14		< 0.8
		p24 antigen	3		16		< 0.8
HTLV III-B	PBMCs	MTT	5	> 100		> 100	
		RT Activity	5		6	ND	< 0.8
BGL	PBMCs	MTT	7	80		> 100	
		RT Activity	7		14		0.03
		p24 antigen	7		12		ND
BRC	PBMCs	MTT	7	80		> 100	
		RT Activity	7		14		3.4
		p24 antigen	7		15		ND

Compound **3** was targeted at the RT as demonstrated by experiments designed to identified the drug-sensitive phase of the replicative cycle. Our results indicated that there was inhibition only after viral infection and in a dose-dependent manner. While compound **3** was active against HIV-1 in acutely infected cells after adsorption, it did not interfere either with the binding of the virus to cells, or in preventing the reactivation of HIV-1 in MT-2 cells, when tested before or during infection. This organosilicon derivative did not show activity in chronically infected H9 cells at concentrations up to 50 μM. Moreover, no antiviral activity of compound **3** was observed on DNA and RNA viruses other than HIV-1.

The main characteristics of compound **3** as an inhibitor of HIV-1 RT are: a) it is a non-competitive inhibitor; b) the degree of inhibition depended on the template-primer used (better inhibition levels were obtained with a poly C-oligo dG than with a poly A-oligo dT); and c) the specific inhibition of HIV-1 RT compared to HIV-2 RT. All these properties allow us to classify compound **3** into the class of HIV-1 specific non-nucleoside RT inhibitors.

Experimental protocols

Chemistry

Melting points were taken on a Kofler hot stage apparatus and are uncorrected. ^{1}H, ^{13}C and ^{29}Si NMR spectra were recorded on Bruker AC 200 (^{1}H: 200.13 MHz, ^{13}C: 50.32 MHz) or Bruker AMX 500 (^{1}H: 500.13 MHz, ^{13}C: 125.76 MHz, ^{29}Si: 99.36 MHz) instruments. Chemical shifts are given in ppm from tetramethylsilane as an internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, mutiplet. Multiplicities in ^{13}C NMR spectra were derived from DEPT or JMOD experiments. When necessary, COSY, HMQC and HMBC experiments were used. The chain between silicon atom and heteroatom (N or S) of the symmetrical compounds was numbered as follows, N(S)-1'-2'-3'-Si, while current nomenclature was used for heterocycles. Infrared spectra were recorded on a Bruker IFS-25 FTIR instrument as KBr pellets or as solutions in the cited solvent. Patterns are designed as follows: br b, broad band; w b, weak band; sh, shoulder. Mass spectra were recorded on a Finnigan Mat TSQ 70 triple quadrupole mass spectrometer in electron impact ionization mode (70 eV). Thin-layer chromatography was performed on Merck silica gel 60-F-254 (0.25 mm thickness) plates. Column chromatography was performed on silica gel 60 (230–400 mesh). All elemental analyses were performed by the Laboratoires du CNRS, BP 22, 69390 Vernaison, France.

[3-(N-1-Thyminyl)propyl]methylphenylsilanol ***1***

A solution of N-1 allylthymine (2 g, 12 mmol) in anhydrous THF (10 mL) was added dropwise to a few drops of a 0.1 N chloroplatinic acid solution in isopropyl alcohol heated to 50 °C, followed by addition of phenylmethylchlorosilane (1.88 g, 12 mmol). The mixture was stirred at 50 °C for 48 h under inert atmosphere, allowed to cool to room temperature, and then poured into a mixture of $NaHCO_3$ (1.0 g, 12 mmol) and NH_4Cl (2.0 g, 37 mmol) and ice water (50 mL). The product was extracted with $CHCl_3$. The combined extracts dried over $MgSO_4$, filtered and concentrated under reduced pressure yielded an oil chromatographed on a silica-gel column (CH_2Cl_2/MeOH 95:5) to afford **8** as an oil (83 mg, 3%) R_f = 0.40, dichloromethane/ methanol 90:10.

IR (KBr), ν_{max} cm^{-1}: 3360 (br b, Si-O-H) and 3170 (br b, N-H), 3040 (C-H olefinic), 2950–2900 (C-H aliphatic), 1670 (br b, C=O and C=C), 1465, 1350; 1250, 850 and 760 (Me-Si); 1420 and 1110 (Ph-Si). ^{1}H NMR ($CDCl_3$): 0.40 (s, 3H, Me-Si), 0.82 (m, 2H, H3'), 1.77 (m, 2H, H2'), 1.89 (s, 3H, Me-thy), 2.73 (br s, 1H, Si-OH), 3.68 (t, 2H, J = 7.3 Hz, H1'), 6.90 (s, 1H, H6), 7.47 (m, 3H ar), 7.55 (d, 2H ar), 8.45 (br s, 1H, H-N). ^{13}C NMR ($CDCl_3$): –1.60 (Me-Si), 12.21 (Me-thy), 13.14 (C3'), 22.90 (C2'), 50.80 (C1'), 110.69 (C5), 127.86, 129.60, 133.17 (C-H ar), 137.87 (Si-C quat ar), 140.50 (C6), 151.25 (C2), 164.40 (C4). MS, *m/z* (%): 590 (M^+, 2). The spectrum reveals only siloxane resulting from the silanol self condensation in the spectrometer source.

[3-(N-9-Adenyl)propyl]methylphenylsilanol ***2*** *and 1-[3-(N-9-adenyl)propyl]methylphenyl-3-(methylphenyl)disiloxan-3-ol* ***3***

To a solution of N-9 allyladenine (1.93 g, 11 mmol) in dry 1,2-dichloroethane was added under inert atmosphere phenylmethylchlorosilane (5.65 g, 36 mmol) at 40 °C followed, after 10 min, by a few drops of chloroplatinic acid (0.10 N in isopropyl alcohol). The reaction was heated at 70–80 °C for 7 days. After evaporation of the solvent, the remaining solution was washed with hot hexanes to remove excess chlorosilane, and then poured into a cold solution of saturated ammonium chloride (50 mL). The product was extracted with $CHCl_3$, dried over $MgSO_4$ and filtered. The filtrate was evaporated and applied to a silica-gel column (CH_2Cl_2/MeOH 90:10) to yield a white powder (**2**, 70 mg, 2%) and an oil (**3**, 590 mg, 12%).

2: mp: 84 °C; R_f 0.20, dichloromethane/methanol 90:10. IR (KBr), ν_{max} cm^{-1}, 3540 (sh Si-O-H), 3325 and 3175 (br b N-H), 2920 (C-H aliphatic), 1650, 1590, 1480, 1250, 860 and 736 (Me-Si); 1415 and 1110 (Ph-Si). ^{1}H NMR ($CDCl_3$): 0.30 (s, 3H, Me-Si), 0.73 (m, 2H, H3'), 1.84 (m, 2H, H2'), 4.07 (m, 2H, H1'), 5.57 (br s, 2H, NH_2), 7.35 (m, 5H Ar), 7.64 (d, H, H2 Ad), 8.33 (s, H, H8 Ad). ^{13}C NMR ($CDCl_3$): –1.32 (Me-Si), 14.18 (C3'), 23.97 (C2'), 46.45 (C1'), 119.50 (C5), 127.87, 129.71, 133 (C-H ar), 137.41 (Si-C ar), 140.25(C8), 150 (C4), 152.71 (C2), 155.63 (C6). ^{29}Si NMR ($CDCl_3$): –1.13. MS, *m/z* (%): 609 (M^+, 7), 474 (5), 432 (100), 382 (15), 313 (2), 256 (3), 149 (17), 134 (7); this spectrum corresponds to the siloxane resulting from the silanol self condensation in the spectrometer source.

3: two diastereoisomers R_f 0.43, dichloromethane/methanol 90:10. IR (KBr), ν_{max} cm^{-1}: 3540 (sh Si-O-H), 3325 and 3175 (br b N-H), 2920 (C-H aliphatic), 1650, 1590, 1480, 1250, 860 and 736 (Me-Si); 1428 and 1110 (Ph-Si), 1100–1000 (br b, Si-O-Si), 900 (Si-O-Si-OH). ^{1}H NMR ($CDCl_3$): 0.33, 0.37, 0.40 and 0.42 (4s, 6H, CH_3-Si), 0.81 (m, 2H, H3'), 1.93 (m, 2H, H2'), 4.06 (m, 2H, H1'), 6.30 (s, 2H, NH_2), 7.32 (m, 6H, H ar), 7.60 (m, 4H ar, H8), 8.22 (2s, 1H, H2). ^{13}C NMR ($CDCl_3$): –0.94/–0.81 and –0.77/–0.74 (2 × 2 CH_3-Si), 13.89 (C3'), 24.07 (C2'), 46.37/46.39 (2 C1'), 119.00 (C5), 127.69, 127.71, 127.77, 127.80, 129.50, 129.52, 129.70, 133.05, 133.26, 133.30 (C-H ar), 137.52, 137.63, 137.75, 137.84 (Si-C ar quat), 140.10 (C8), 149.00 (C4), 152.58 (C2), 155.60 (C6). ^{29}Si NMR ($CDCl_3$): –1.66 and –1.73 (Si-C3'), –24.57 and –24.72 (Si-OH). MS, *m/z* (%): 449 (M^+, 42), 434 (15), 194 (77), 149 (100), 136 (21).

1,3-Bis-3-(N-1 thyminyl)propyl-1,1,3,3-tetramethyl disiloxane ***4***

NaH (1.59 g, 97% in oil, 64.25 mmol) was slowly added to a solution of thymine (8.47 g, 67.15 mmol) in dry DMSO (80 mL)

and the mixture was stirred for 2 h at 50 °C. 3-(Chloropropyl)dimethylchlorosilane (4.8 mL, 29.21 mmol) was then added, and the mixture heated for 3 days at 100 °C. The resulting solution was hydrolyzed with water (300 mL) and extracted with CH_2Cl_2. The combined organic layers were washed with water and dried over $MgSO_4$. Evaporation gave a white powder washed with ether and recrystallized in MeOH to yield a mixture in a 80:20 ratio of regioisomers (1.20 g, yield 18%).

R_f 0.55, dichloromethane/methanol 90:10. IR (KBr), ν_{max} cm^{-1}: 3160 (N-H amide), 3050 (C-H olefinic), 2950–2800 (C-H aliphatic), 1680 (br b, C=O), 1350; 1250, 846 and 760 (CH_3-Si); 1100-1000 (br b, Si-O-Si). ^1H NMR ($CDCl_3$): 0.03 (s, 6H, Me-Si), 0.46 (m, 2H, H3'), 1.65 (m, 2H, H2'), 1.90 (s, 3H, Me-thy), 3.65 (t, J = 7 Hz, 2H, H1'), 6.95 (s, 1H, H6). ^{13}C NMR ($CDCl_3$): 0.16 (Me-Si), 12.19 (Me-thy), 14.89 (C3'), 22.97 (C2'), 51.20 (C1'), 110.28 (C5), 140.59 (C6), 150.96(C2), 164.59 (C4). MS, *m/z* (%): 466 (M^+, 2), 451 (4), 299 (87), 225 (99). Anal calc for $C_{20}H_{34}N_4O_5Si_2$: C, 51.47; H, 7.29; N, 12.00. Found: C, 51.16; H, 7.33; N, 11.94.

1,3-Bis-3-(N-9-adenyl) propyl-1,1,3,3-tetramethyl disiloxane ***5***
NaH (0.75 g, 97% in oil, 26.4 mmol) was slowly added to a solution of adenine (3.72 g, 27.6 mmol) in dry DMSO (15 mL) and the mixture was stirred for 2 h at 50 °C. (3-Chloropropyl)dimethylchlorosilane (2 mL, 12 mmol) was then added, and the mixture was heated for 3 days at 50 °C. The resulting medium was hydrolyzed with water (150 mL), and extracted with $CHCl_3$, the combined organic layers washed with water and dried over $MgSO_4$. Evaporation gave a white powder corresponding to the N-alkylation products (1.16 g, yield 40%). Several attempts at crystallization did not increase the 9:1 ratio.

R_f 0.35, dichloromethane/methanol 90:10. IR (KBr), ν_{max} cm^{-1}: 3300 and 3170 (NH_2), 3110 (C-H Ar), 2910 (C-H aliphatic), 1680, 1600, 1580; 1250, 840 and 760 (Si-Me), 1100–1040 (br b, Si-O-Si). ^1H NMR (DMSO-d_6): –0.06 (s, 6H, Me-Si), 0.35 (m, 2H, H3'), 1.74 (m, 2H, H2'), 4.09 (t, J = 6.7 Hz, 2H, H1'), 8.18 (s, 1H, H8 Ad), 8.19 (s, 1H, H2 Ad). ^{13}C NMR (DMSO-d_6): 0.14 (Me-Si), 14.56 (C3'), 23.50 (C2'), 45.68 (C1'), 118.77 (C5), 140.91 (C8), 149.53 (C4), 152.34 (C2), 155.94 (C6). ^{29}Si NMR (DMSO-d_6): 12.96. MS, *m/z* (%): 484 (M^+, 10), 335 (30), 308 (100), 234 (18), 149 (37).

1,3-Bis[3-(N-imidazolyl)propyl]-1,1,3,3-tetramethyldisiloxane ***6***
To a solution of imidazole (2.04 g, 30 mmol) in dry THF (50 mL) was slowly added NaH (0.70 g, 97% in oil, 28.69 mmol) and the mixture stirred for 80 min at room temperature. 3-(Chloropropyl)dimethylchlorosilane (2.1 mL, 13 mmol) was then added, and the mixture refluxed for 4 days. The resulting medium was hydrolyzed with water and extracted with CH_2Cl_2, the combined organic layers washed with water and dried over $MgSO_4$. After concentration under reduced pressure, the oily residue was applied to a silica-gel column (CH_2Cl_2/MeOH 95:5) to yield a clear oil (**6**, 1.34 g, 59%).

R_f 0.53, dichloromethane/ methanol 90:10. IR (KBr), ν_{max} cm^{-1}: 3400 (br b, NH), 3100 (C-H olefinic), 2950–2900 (C-H aliphatic), 1650 (br b, C=N Imidazole), 1510 (C=C Ar); 1250, 860 and 760 (Si-Me); 1100–1000 (br b, Si-O-Si). ^1H NMR ($CDCl_3$): 0.00 (s, 6H, Me-Si), 0.43 (m, 2H, H3'), 1.73 (m, 2H, H2'), 3.90 (t, J = 7.0 Hz, 2H, H1'), 6.88 (s, 1H, H4 or H5), 7.03 (s, 1H, H4 or H5), 7.47 (s, 1H, H2). ^{13}C NMR ($CDCl_3$): –0.16 (Me-Si), 14.67 (C3'), 24.94 (C2'), 49.37 (C1'), 118.36 (C5), 128.94 (C4), 136.71 (C2). MS, *m/z* (%): 350 (M^+, 29), 269 (99), 241 (74), 216 (26), 183 (6)), 147 (18). Anal calc for $C_{16}H_{30}N_4OSi_2$: C, 54.81; H, 8.62; N, 15.98. Found: C, 55.27; H, 8.73; N, 16.07.

1,3-Bis[3-(N-indolyl)propyl]-1,1,3,3-tetramethyldisiloxane 7
To a solution of indole (1.22 g, 10.41 mmol) in dry DMSO (50 mL) was slowly added NaH (0.25 g, 97%) in oil, 9.94 mmol and the mixture stirred for 2 h at 50 °C. 1,3-Bis(3-chloropropyl)-1,1,3,3-tetramethyldisiloxane (1.36 g, 4.73 mmol) was then added, and the mixture was heated 4 days at 100 °C. The resulting medium was hydrolyzed with water (200 mL), extracted with ether, the combined organic layers washed with water and dried over $MgSO_4$. After distillation of the unreacted starting material, the remaining oil was chromatographed over a silica-gel column to yield **7** as an orange-colored oil (**7**, 0.57 g, 27%).

R_f 0.75, dichloromethane. IR (KBr), $\nu_{max}$$cm^{-1}$: 3060 (C-H olefinic), 2950–2850 (C-H aliphatic), 1640 (weak, C=N conjugated), 1510 and 1450 (C=C Ar); 1250, 840 and 760 (Si-Me); 1100–1000 (br b, Si-O-Si). ^1H NMR ($CDCl_3$): 0.00 (s, 6H, Me-Si), 0.46 (m, 2H, H3'), 1.80 (m, 2H, H2'), 4.05 (t, J = 7.0 Hz, 2H, H1'), 7.10–7.65 (m, 6H, H Ar). ^{13}C NMR ($CDCl_3$): 0.0 (Me-Si), 15.5 (C3'), 24.7 (C2'), 49.5 (C1'), 101.0 (C3), 109.5 (C7), 119,0 120.5, 121.0 (C4,C5,C6), 128.0 (C2), 128.5 (C3a), 136.0 (C7a). MS, *m/z* (%): 448 (M^+, 100), 433 (4), 290 (27), 247 (32.5), 231 (15), 129 (45). Anal calc for $C_{26}H_{36}N_2OSi_2$: C, 69.59; H, 8.08; N, 6.24. Found: C, 69.51; H, 8.23; N, 6.39.

1,3-Bis[3-(2-mercaptopyrimidinyl)propyl]-1,1,3,3-tetramethyldisiloxane ***8***
To a solution of 2-mercaptopyrimidine (2.24 g, 20 mmol) in dry DMSO (50 mL) was slowly added NaH (0.46 g, 97% in oil, 19.13 mmol) and the mixture was stirred 40 min at 50 °C. 3-(Chloropropyl)dimethylchlorosilane (1.43 mL, 8.69 mmol) was then added, and the mixture heated at 50 °C for 3 days. The resulting medium was hydrolyzed with water (200 mL) and extracted with CH_2Cl_2, the combined organic layers washed with water and dried over $MgSO_4$. The remaining oily residue was chromatographed over a silica-gel column (CH_2Cl_2) to yield **8** as a clear oil (0.89 g, 47%).

R_f 0.65, dichloromethane. IR (KBr), ν_{max} cm^{-1}: 3050 (C-H olefinic), 2950–2850 (C-H aliphatic), 1560 and 1540 (C=N, C=C ar), 1380; 1250, 840 and 760 (Si-Me), 1190 (C-S), 1100–1000 (br b, Si-O-Si). ^1H NMR ($CDCl_3$): 0.05 (s, 6H, Me-Si), 0.65 (m, 2H, H-C3'), 1.72 (m, 2H, H-C2'), 3.12 (t, J = 7.0 Hz, 2H, H-C1'), 6.94 (dd, J = 5.0 Hz, 1H, H-C3), 8.49 (d, J = 5.0 Hz, 2H, H-C2). ^{13}C NMR ($CDCl_3$): 0.05 (Me-Si), 17.68 (C3'), 23.11 (C2'), 34 (C1'), 116 (C3), 156.50 (C2), 172.10 (C1). MS, *m/z* (%): 439 (M^+, 10), 424 (9), 326 (70), 285 (100), 242 (78), 210 (46), 173 (7), 133 (53). Anal calc for $C_{18}H_{30}N_4OS_2Si_2$: C, 49.27; H, 6.89; N, 12.76. Found: C, 49.46; H, 7.17; N, 12.33.

1,3-Bis[3-(5-chloro-2-mercaptobenzothiazolyl)propyl]-1,1,3,3-tetramethyldisiloxane ***9***
To a solution of 5-chloromercaptobenzothiazole (1 g, 5 mmol) in dry benzene (50 mL) was slowly added NaH (0.12 g, 97% in oil, 4.78 mmol) and the mixture was stirred at 90 °C for 3 h. 3-(Chloropropyl)dimethylchlorosilane (0.36 mL, 2.17 mmol) was then added and the mixture heated for 4 days under reflux. Solvent was then removed under reduced pressure. The solid residue was hydrolyzed with water (100 mL), extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$, and evaporated to dryness. The resulting solid was applied to a silica-gel column (hexane/CH_2Cl_2 50:50) to yield **9** (0.14 g, 21%).

Mp: 68 °C; R_f 0.82, dichloromethane/methanol 90:10. IR (KBr), ν_{max} cm^{-1}: 3050 (C-H olefinic), 2970–2800 (C-H aliphatic), 1420 (ar); 1250, 840 and 760 (Si-Me); 1100–1000 (br b, Si-O-Si). ^1H NMR ($CDCl_3$): 0.07 (s, 6H, Me-Si), 0.70 (m, 2H,

H3'), 1.82 (m, 2H, H2'), 3.30 (t, J = 7 Hz, 2H, H1'), 7.22 (m, 1H, H7, J_{ab} = 10.0), 7.62 (m, 1H, H6, J_{ab} = 10.0), 7.81 (s, 1H, H4). ^{13}C NMR ($CDCl_3$): 0.70 (Me-Si), 18 (C3'), 23.50 (C2'), 36.90 (C1'), 121.00, 121.50 and 124.50 (3 C-H ar), 131.85, 133.00, 154.00 (3 C quat Ar), 169.50 (C2). MS, *m/z* (%): 618 (M^+, 1), 375 (10), 373 (34), 331 (28), 301 (44), 300 (36), 298 (100). Anal calc for $C_{24}H_{30}Cl_2N_2OSSi_2$: C, 46.75; H, 4.91; N, 4.55. Found: C, 46.78; H, 5.05; N, 4.48.

General preparation of the 2-aminobenzamido compounds 1,3-bis[3-(2-aminobenzamido)propyl-1,1,3,3-tetramethyldisiloxane ***10*** *1,3-bis[3-(5-chloro-2-aminobenzamido)propyl]-1,1,3,3-tetramethyl-disiloxane* ***11*** *and 1,3-bis[3-(2-N-methylaminobenzamido)propyl]-1,1,3,3-tetramethyl-disiloxane* ***12***

To a stirred suspension of the isatoic anhydrides (80 mmol) in absolute ethanol (160 mL), was added 1,3-bis(3-aminopropyl)-1,1,3,3-tetramethyldisiloxane (9.90 g, 40 mmol). After stirring for 2 h at room temperature, the resulting mixture was poured into H_2O and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, evaporated and purified by chromatography over a silica-gel column (CH_2Cl_2/MeOH 90:10) to afford the desired compounds as white solids.

10: (18.75 g, 96%); mp: 100 °C; R_f 0.75, dichloromethane/methanol 90:10. IR (KBr), ν_{max} cm^{-1}: 3400 and 3300 (NH and NH_2), 3080 (C-H olefinic), 2980–2840 (C-H aliphatic), 1620 (C=O), 1590 and 1530 (C=C ar), 1250, 840 and 760 (Si-Me), 1100–1000 (br b, Si-O-Si). ^{1}H NMR ($CDCl_3$): 0.08 (s, 6H, Me-Si), 0.55 (m, 2H, H3'), 1.62 (m, 2H, H2'), 3.35 (q, J = 7.0 Hz, 2H, H1'), 6.35 (br, 1H, N-H), 6.60 (m, 2H, H4, H6)), 7.18–7.32 (m, 2H, H3, H5). ^{13}C NMR ($CDCl_3$): 0.16 (Me-Si), 15.36 (C3'), 23.44 (C2'), 42.34 (C1'), 116.49 (C3), 116.95 (C5), 127.12 (C6), 131.78 (C4), 148.30 (C2), 169.21 (C=O). MS, *m/z* (%): 481 (26, M^+), 308 (26), 235 (50), 499 (83), 120 (100), 114 (32), 92 (13). Anal calc for $C_{24}H_{38}N_4O_3Si_2$: C, 59.22; H, 7.87; N, 11.51. Found: C, 58.99; H, 7.54; N, 11.74.

11: (1.29 g, 74%); mp: 116 °C; R_f 0.80, dichloromethane/methanol 90:10. IR (KBr), ν_{max} cm^{-1}: 3420 and 3300 (NH sec amide and NH_2), 3080 (C-H ar), 2980–2840 (C-H aliphatic), 1620 (C=O), 1590 and 1540 (C=C ar), 1250, 840 and 760 (Si-Me), 1100–1000 (br b, Si-O-Si). ^{1}H NMR ($CDCl_3$): 0.06 (s, 6H, Me-Si), 0.55 (m, 2H, H3'), 1.61 (m, 2H, H2'), 3.34 (q, J = 7.0Hz, 2H, H1'), 6.37 (br, 1H, NH), 6.58 (d, J = 8.7 Hz, 1H, H3), 7.09 (dd, J = 8.7 and 2.3Hz, 1H, H4), 7.29 (d, J = 2.3 Hz, 1H, H6). ^{13}C NMR ($CDCl_3$): 0.31 (Me-Si), 15.54 (C3'), 23.58 (C2'), 42.68 (C1'), 117.54 (C1), 118.37 (C3), 120.84 (C2), 126.79 (C6), 131.80 (C4) 146.95 (C5), 168.21 (C=O). MS, *m/z* (%): 556 (M^+, 13), 555 (6), 554 (16), 342 (31), 269 (64), 148 (100). Anal calc for $C_{24}H_{36}Cl_2N_4O_3Si_2$: C, 51.88; H, 6.53; N, 10.08. Found: C, 51.90; H, 6.55; N, 10.02.

12: (8.11 g, 64%); mp: 78 °C; R_f 0.95, dichloromethane/methanol 90:10. IR (KBr), ν_{max} cm^{-1}: 3400–3200 (NH and NH_2), 3080 (C-H ar), 2980–2770 (C-H aliphatic), 1620 (C=O), 1570 and 1510 (C=C ar), 1250, 840 and 760 (Si-Me), 1100–1000 (br b, Si-O-Si). ^{1}H NMR ($CDCl_3$): 0.05 (s, 6H, Me-Si), 0.54 (m, 2H, H3'), 1.59 (m, 2H, H2'), 2.79 (s, 3H, N-Me), 3.34 (q, J = 7.0 Hz, 2H, H1'), 6.32 (br, 1H, N-H), 6.52 (td, J = 8.3 and < 1 Hz, 1H, H5), 6.62 (d, J = 8.0 Hz, 1H, H3), 7.33 (m, 2H, H4 H6). ^{13}C NMR ($CDCl_3$): 0.18 (Me-Si), 15.41 (C3'), 23.51 (C2'), 29.45 (N-Me), 42.39 (C1'), 110.78 (C3), 114.28 (C5), 115.34 (C1), 127.08 (C6), 132.40 (C4), 150.20 (C2), 169.72 (C=O). MS, *m/z* (%): 515 (M^+, 38), 514 (100), 499 (3), 298 (51), 148 (71), 133 (100). Anal calc for $C_{26}H_{42}N_4O_3Si_2$: C, 60.66; H, 8.22; N, 10.88. Found: C, 60.78; H, 8.08; N, 10.79.

Reagents

3'-Azido-2',3'-dideoxythymidine (AZT) was purchased from Sigma Chemical Company and was dissolved to a concentration of 50 mM in H_2O. All C-silylated organosilicon derivatives were first dissolved in DMSO to 10 mM concentration. Thereafter, stock solutions were serially diluted in RPMI 1640 culture medium. Unlabelled nucleotides, oligonucleotides or polynucleotides were obtained from Sigma or Pharmacia. [^{3}H]-dTTP was purchased from Commissariat à l'Energie Atomique-Saclay (CEA-France).

Enzyme assays

Recombinant HIV-1 RT, expressed in yeast, was obtained and purified as previously described [23]. The system for the expression of recombinant HIV-2 RT in *Escherichia coli* was a gift from R Goody [24]. HIV-2 RT was purified as HIV-1 RT. DNA polymerase-α from *Xenopus laevis* oocytes was isolated and purified as described previously [25].

Recombinant RT

DNA polymerase activity. In the presence of poly A-oligo dT, 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, 80 mM KCl, 0.48 A_{260} units/mL poly A-oligo dT (5:1), 0.5–1 μCi [^{3}H]dTTP (56 Ci/mmol), 20 μM dTTP and recombinant RT. In the presence of poly C-oligo dG, the reaction mixture (0.05 mL) contained 50 mM Tris-HCl pH 8.0, 5 mM $MgCl_2$, 4 mM dithiothreitol, 100 mM KCl, 0.48 A_{260} units/mL poly C-oligo dG (5:1), 0.5–1 μCi [^{3}H]dGTP (28 Ci/mmol), 2 μM dGTP and recombinant RT.

Incubation was carried out for 10 min at 37 °C. Reactions were stopped by the addition of 1 mL cold 10% trichloroacetic acid (TCA) in 0.1 M sodium pyrophosphate. The precipitates were filtered through nitrocellulose membranes, washed with 2% TCA, dried and counted in a PPO/POPOP/toluene scintillation mixture.

RNase H activity. The reaction mixture contained in a final volume of 0.05 mL, 50 mM Tris-HCl pH 8.0, 10 mM dithiothreitol, 5 mM $MgCl_2$, 60 mM KCl and [^{3}H]RNA-M13 DNA. The reaction mixture was incubated in the presence of RT for 10 min at 37 °C and stopped as described above. [^{3}H]RNA-M13 DNA was prepared as described by Andreola et al [26].

Animal DNA polymerase α

The reaction mixture contained in a final volume of 0.05 mL, 50 mM Tris-HCl pH 8.0, 10 mM dithiothreitol, 5 mM $MgCl_2$, 0.05 mM each dATP, dCTP, dGTP, 0.01 mM dTTP, 1 μCi [^{3}H]dTTP (56 Ci/mmol), and 20 μg/mL activated DNA. The incubation in the presence of DNA polymerase α was carried out at 37 °C and stopped as described above.

Cells, virus strains, and cell culture assays

T-cells lines were kindly provided by D Dormont (CEA, Fontenay-aux-Roses). MT-2 cells were chosen for their rapid susceptibility to most strains of HIV-1. The H9 cell line chronically infected with HIV-1/HTLV-III-B strain was used to produce HIV-1 working stocks. All cells were grown at 37 °C in RPMI 1640 medium (Gibco-BRL), supplemented with 10% heat-inactivated fetal calf serum (Boehringer), 2 mM L-glutamine, penicillin (100 IU/mL), streptomycin (0.1 ng/mL) and anti-interferon alpha (700 IU/mL) (Boehringer). Human peripheral blood mononuclear cells (PBMCs) were isolated by

Ficoll-Hypaque (Eurobio) gradient centrifugation of blood collected from HIV-1 seronegative donors. 48 h before infection, the PBMCs were stimulated with 0.2% phytohemagglutinin (Sigma) in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum and then maintained in the presence of natural interleukin-2 (20 IU/mL, Boehringer). Vero cells (a cell line derived from African green monkey kidney) were used for the spectrum activity assay against DNA and RNA viruses other than retroviruses.

HIV-1 stock (strain HTLV-III-B) was obtained from cell-free supernatants of HIV-1 infected H9 cells (H9/HTLV-III-B). The 50% cell culture infective dose ($CCID_{50}$) of cell-free virus stock were determined on day 4 by endpoint titration with MT-2 cells in 96-well microculture plates using the Reed and Muench method. The titer of virus stock was about 10^6 $TCID_{50}$/mL. In some experiments, virus were pelleted by ultracentrifugation from clarified culture supernatants of H9/HTLV-III-B cells and titer was calculated as 1.6×10^8 $TCID_{50}$/mL. AZT-susceptible (BGL) and AZT-resistant (BRC) clinical isolates of HIV-1 (IC_{50} = 0.029 and 3.37 μM, respectively, determined in PBMCs culture) were obtained from an AIDS patient prior to and following initiation of AZT therapy. BRC strain was considered as AZT-resistant by determination of the mutations at amino acid positions 41 and 215 of viral reverse transcriptase (gene RT) by sequencing after polymerase chain reaction [27]. Herpes simplex virus type 1 (HSV) was recovered from a patient with acute HSV infection. Poliovirus type 1 (vaccine strain SABIN, Diagnostic Pasteur) was amplified in Vero cells. Virus titers were respectively 10^8 and 10^6 $TCID_{50}$/mL. Virus stocks were stored at –80 °C.

All culture experiments were performed in duplicate to allow simultaneous evaluation of their effects on HIV- and mock-infected cells. Uninfected and untreated samples (negative control) and infected and untreated samples (positive control) were included in each experiment. AZT and DMSO were tested in parallel.

Infection of MT-2 cells

Pretreatment inhibitor experiments. A total of 6×10^4 MT-2 cells per well were pretreated with serial five-fold dilutions of compounds in triplicate of 96-well microtiter plates. After 24 h of incubation at 37 °C in a CO_2 incubator, cells were washed twice and exposed to HIV-1 for 2 h at 37 °C. Cells were infected with one $TCID_{50}$/cell. Infection at high virus multiplicity allowed the virus replicative steps to be better synchronized in the whole cell population. Cells were washed again and cultured in complete medium for 4 days.

Virus adsorption inhibitor experiments. Dilutions of compounds were added simultaneously to HIV-1 and cells, and then washed off after 2 h incubation at 37 °C. Thereafter, cultures were kept in drug-free medium for 4 days.

Post-treatment inhibitor experiments. Untreated MT-2 cells were infected as indicated above and incubated 2 h at 37 °C. After washing off unadsorbed virus, cells were resuspended in fresh medium and were mixed with different concentrations of compounds in 96-well plates. Cultures were incubated at 37 °C for 4 days. Experiments with various $TCID_{50}$ /cell (0.01 to 1) led to similar results.

Infection of human PBMCs

Phytohemagglutinin (PHA) stimulated human PBMCs (10^6 cells per mL) were exposed to 0.04 $TCID_{50}$/cell. After virus adsorption for 2 h, the cells were washed, suspended in RPMI medium, supplemented as described above, and then transfered to microtiter plates in the presence or absence of various concentrations of the compound tested. All dilutions of samples were tested in triplicate. Uninfected PBMCs, either treated or untreated with the drug, were grown in parallel. On day 5 of culture, supernatants and aliquots of cells were stored at –80 °C for subsequent analysis. Remaining cells were washed, and further cultured with the same concentrations of the compounds in fresh medium. On day 8, all cultures were stopped and subjected to different tests (RT assay in supernatant, p24 detection by ELISA).

Furthermore, PHA-stimulated donor PBMCs (10^6 cells) were infected with serial tenfold dilutions (0.01–100 $TCID_{50}$/well) of AZT-susceptible or AZT-resistant virus strains (BGL or BCR) and incubated for 2 h at 37 °C. After 2 washings, the cells were resuspended in RPMI medium and 125 μL of the infected cell suspensions were distributed in a 96-well plate containing 125 μL of various concentrations of the compound and of the solvent (DMSO, 0.032–4.0 μL/mL). Every dilution was tested in quadruplicate. Untreated, infected cells were included in each plate. Uninfected PHA stimulated PBMCs were grown in parallel to determine the cytotoxicity of the compound. On days 5 and 8, supernatants were assayed for RT activity and p24 antigen production.

Antiviral assays in cell systems

The activity of the organosilicon compounds against HIV-1/HTLV-III-B replication was determined following several criteria: appearance of cytopathic effect (CPE); protection of virus-induced cytopathicity in MT-2 cells; inhibition of reverse transcriptase activity; and specific antigen expression.

Protection of virus induced cytopathicity

Compounds were tested for their ability to inhibit the cytopathic effect induced by HIV-1 infection. Cells were examined microscopically for the development of CPE. Cytotoxicity and protective effect of compounds were tested as follow. The viability of uninfected cells and cells that had been infected with HIV-1, which had been exposed to various concentrations of the compounds was measured spectrophotometrically *via* the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) as described previously [28].

Virion RT activity assay

RT assay was performed as described previously [29]. Supernatant samples (50 μL) from each culture were incubated with 10 μL of virus-disrupting buffer (500 mM KCl; 50 mM dithiothreithol; 0.5% Triton X-100) for 15 min at 4 °C. Thereafter a solution (40 μL), containing 125 mM Tris-HCl pH 7.8, 1.25 mM EGTA, 12.5 mM $MgCl_2$, 0.5 A_{260} units/mL poly A-oligo dT, 3 μCi [^{3}H]dTTP at 30 Ci/mmol was added and incubated for 1 h at 37 °C. The reaction was stopped by addition of 20 μL of 60% TCA in 120 mM sodium pyrophosphate. Acid-insoluble nucleic acids were precipitated 15 min at 4 °C, and collected on glass fiber filters. The filters were washed with 5% TCA, dried, placed in scintillation vials containing 1 mL of scintillation fluid and the radioactivity counted.

Acknowledgments

This work was supported by the Conseil Régional d'Aquitaine (Bordeaux, France), the Association pour la Recherche sur le Cancer (ARC) and the Agence Nationale de Recherches sur le SIDA (ANRS).

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