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Short communication

Indoles and pyridazino[4,5-*b*]indoles as nonnucleoside analog inhibitors of HIV-1 reverse transcriptase

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Summary — The synthesis and the study of the activity of new indol-2-carboxamides and pyridazino[4,5-*b*]indoles as inhibitors of HIV-1 reverse transcriptase (RT) are presented. The activity of the compounds synthesized as inhibitors of different types of HIV-1 RT (wild type enzyme and mutant forms P236L, Y181C and P236L/Y181C) was evaluated. The activity of the most active compounds was investigated in the syncytia reduction *in vitro* assay, in HIV-1_{IIIB}-infected HT4lacZ-1 cells. Their potential cytotoxicity was determined in parallel. Two lead compounds, *N*-[1-[2-(3-isopropylamino)pyridyl]piperazin]-5,6-methylenedioxy indol-2-carboxamide **7q** and *N*-[1-[2-(3-ethylamino)pyridyl]piperazin]-5,6-methylenedioxyindol-2-carboxamide **7s** have been identified.

indole / nonnucleoside RT inhibitor / syncytia assay/ HIV-1_{IIIB} HT4lacZ-1 cells

Introduction

Within the wide range of therapeutic targets involved in the understanding of the vital cycle of the HIV-1 virus, the design of new nonnucleoside reverse transcriptase enzyme inhibitors continues to be an objective of great interest, especially if the problems of toxicity and resistance to the utilization of the antiretrovirals are taken into account [1–3].

In an attempt to obtain new compounds that act as inhibitors of the HIV-1 reverse transcriptase (HIV-1 RT), compounds with the general structure indol-2-carboxamide **I** and pyridazino[4,5-*b*]indole **II** (fig 1) have been synthesized. Compounds of general structure **I** are structural analogs of bis(heteroaryl) piperazine (BHAP) reverse transcriptase inhibitors [4], a new series of compounds that were discovered by directed broad screening of the Upjohn Company chemical library and subsequent structure–activity relationship (SAR) investigation for RT inhibitors [5–7], and also of L-737126 developed by Merck (fig 2) [8]. Compounds of general structure **II** are analogs which rigidify the model and no similar described structures have been found to possess this activity.

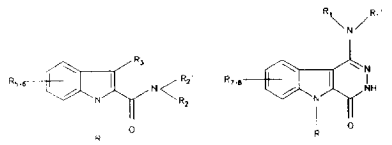

Fig 1. Structures **I** and **II**.

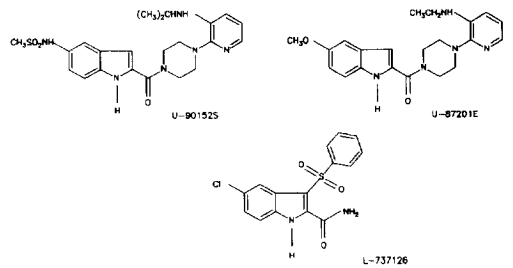

Fig 2. Compounds related to general structure **I**.

The activity of derivatives **I** and **II** as inhibitors of wild type HIV-1 RT was determined. Active compounds identified *via* this screen were further examined to evaluate their inhibition of HIV-1 repli-

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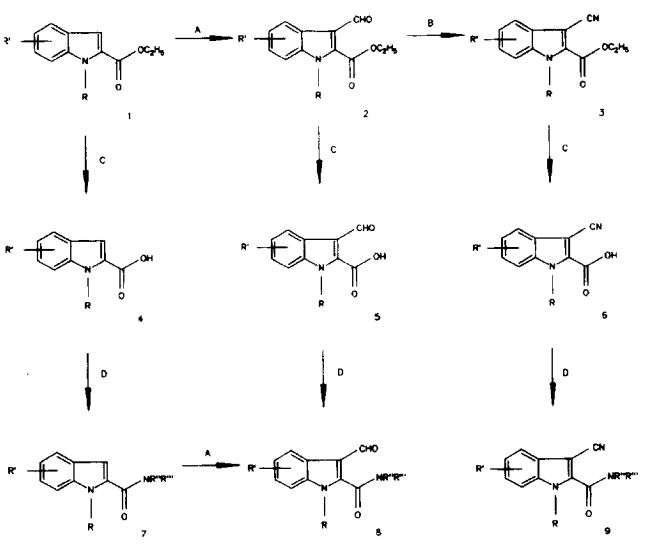
cation in infected HIV-1_{IIIB} HT4lacZ-1 cells. Parallelly, their potential cytotoxicity was determined *in vitro*, using the same cellular type, but uninfected. Their activity as inhibitors of mutated HIV-1 RT enzymes (P236L, Y181C and P236L/Y181C) was determined for the compounds that stood out in the above assays.

These types of mutants were selected bearing in mind the structural analogy of the new series proposed with the BHAPs. The mutation P236L is specifically linked to the treatment with the BHAPs, while the mutation Y181C appears after treatment with different antiretrovirals such as TIBO, nevirapine, pyridinones or BHPAs, thereby confirming the role that the amino acids at the positions 181 and 236, among others, play in the appearance of resistance [9, 10].

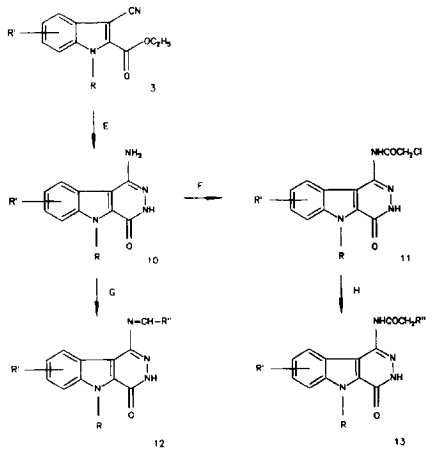
Chemistry

The synthetic routes followed for obtaining the compounds of this SAR investigation are summarized in schemes 1 and 2.

Preparation of ethyl 5,6-methylenedioxyindole-2-carboxylate **1c** started from the diazotation of 3,4-methylenedioxaniline. Condensation of the resulting diazonium salt with ethyl 2-methylacetylacetate, according to the Japp–Klingemann procedure, and subsequent cyclization in ethanol saturated with HCl, according to the Fischer method, produced **1c**. Ethyl *N*-methylindole-2-carboxylate **1b** was obtained by



Scheme 1. Reagents: **A** *N,N*-dimethylformamide, $POCl_3$; **B** $CH_3CH_2NO_2$, acetic acid, sodium acetate; **C** 1) EtOH, KOH; 2) HCl (d); **D** 1) $SOCl_2$; 2) amines. R = H; CH_3. R' = H; -O-CH_2-O-.



Scheme 2. Reagents: **E** NH_2NH_2·H_2O; **F** $ClCH_2COCl$; **G** aldehydes; **H** amines; R = H; CH_3. R' = -O-CH_2-O-.

methylation of the commercially available ethyl indole-2-carboxylate **1a** with dimethylsulfate.

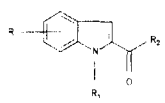
Treatment of **1** with KOH in EtOH, subsequent acidification and reaction with thionyl chloride yielded the corresponding acid chlorides, which were not isolated but were used directly in the subsequent reactions with different amines to afford indole-2-carboxamides **7** (table I). 3-Formyl indole-2-carboxylates **2** were obtained from the corresponding indoles **1** by Vilsmeier–Hack formylation with $POCl_3$ and *N,N*-dimethylformamide. The hydrolysis of ester and conversion of the acid to the acid chloride followed by reaction with the appropriate amine led to compounds **8** (table II). Nitrile derivatives **3** were synthesized from compounds **2** by reaction with nitroethane in an acetic acid/sodium acetate medium [11]. As in the aforementioned series, the acid chloride was obtained and the reaction of these compounds with selected amines provided compounds **9** (scheme 1; table II).

The pyridazino[4,5-*b*]indoles were synthesized by reaction of **3** (scheme 2) with hydrazine hydrate. In this way, the intermediate 1-amino-3,4-dihydropyridazino[4,5-*b*]indole-4-ones **10** were reacted with aldehydes to afford the imino derivatives **12**. From **10** and by reaction with chloroacetyl chloride, the 2-chloramide analogs **11** were obtained. Treatment of **11** with amines produced **13**. Physical data for compounds **10–13** are reported in table III.

Biology

Initially, the enzyme inhibitory activity of each compound was evaluated in an *in vitro* recombinant HIV-1 RT wild type screening assay [12–14] at a

Table I. Physical properties and HIV-1 RT inhibitory activity of indole analogs.



No	R_1	R_2	*R*	*Yield*[a] *(%)*	*Mp (°C) (recrystallization solvent)*[b]	*Inhibition of HIV-1RT* % *Inhibition*[c]	IC_{50} *(μM)*[d]	*Formula*[e]
7a	H	-N[CH(CH$_3$)$_2$]$_2$	H	21	168–173 (A)	I[f]		$C_{15}H_{20}N_2O$
7b	H	-N(CH$_2$CH$_3$)$_2$	-OCH$_2$O-	11	190–191 (B)	30.8 ± 8.1		$C_{14}H_{16}N_2O_3$
7c	H	Adamantanamin-1-yl	H	4	221–223 (B)	I		$C_{19}H_{22}N_2O$
7d	CH$_3$	Adamantanamin-1-yl	H	10	174–176 (B)	I		$C_{20}H_{24}N_2O$
7e	H	Morpholin-1-yl	-OCH$_2$O-	15	189–191 (B)	I		$C_{14}H_{14}N_2O_4$
7f	H	*N*-Furoylpiperazin-1-yl	H	9	166–167 (A)	39.4 ± 6.86		$C_{18}H_{17}N_3O_3$
7g	CH$_3$	*N*-Furoylpiperazin-1-yl	H	11	102–105 (A)	I		$C_{19}H_{19}N_3O_3$
7h	H	*N*-Furoylpiperazin-1-yl	-OCH$_2$O-	37	230–231 (C)	27.7 ± 10.3		$C_{19}H_{17}N_3O_5$
7i	H	*N*-(4-Cl)-Phenylpiperazin-1-yl	H	15	226–228 (A)	I		$C_{19}H_{18}N_3OCl$
7j	CH$_3$	*N*-(4-Cl)-Phenylpiperazin-1-yl	H	22	211–212 (A)	I		$C_{20}H_{20}N_3OCl$
7k	H	*N*-(2-Ethoxy)phenylpiperazin-1-yl	H	30	138–141 (D)	89.6 ± 3.62	2.0	$C_{21}H_{23}N_3O_2$
7l	CH$_3$	*N*-(2-Ethoxy)phenylpiperazin-1-yl	H	21	109–111 (B)	I		$C_{22}H_{25}N_3O_2$
7m	H	*N*-(2-Ethoxy)phenylpiperazin-1-yl	-OCH$_2$O-	42	126–130 (C)	98.5 ± 0.07	1.4	$C_{22}H_{23}N_3O_4$
7n	H	*N*-(2-Methoxy)phenylpiperazin-1-yl	H	25	193–194 (A)	54.7 ± 5.80	65.0	$C_{20}H_{21}N_3O_2$
7o	H	*N*-(2-Methoxy)phenylpiperazin-1-yl	-OCH$_2$O-	8	187–188 (E)	96.9 ± 0.90	2.5	$C_{21}H_{21}N_3O_4$
7p	H	*N*-(4-Nitro)phenylpiperazin-1-yl	-OCH$_2$O-	42	>300 (F)	I		$C_{20}H_{18}N_4O_5$
7q	H	*N*-[(3-Isopropylamin)pyrid-2-yl]piperazin-1-yl	-OCH$_2$O-	28	214–215 (C)	99.9 ± 0.01	0.23	$C_{22}H_{25}N_5O_3$·HCl
7s	H	*N*-[(2-Ethylamin)pyrid-2-yl]piperazin-1-yl	-OCH$_2$O-	34	212–213 (C)	99.1 ± 2.0	0.44	$C_{21}H_{23}N_5O_3$
U-90152S						99.4 ± 0.5	0.40	

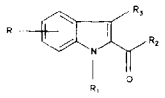
[a]Value of the final transformation is expressed. [b]Recrystallization solvent: A, MeOH; B, MeOH/H_2O; C, EtOH; D, isolated by column chromatography, packed with silica gel and $CHCl_3$/MeOH (99:1) as mobile phase; E, EtOH/H_2O; F, acetone. [c]% inhibition at 50 μM, wild type (n = 3–5). [d]Concentration–activity curves are carried out with four or more concentrations of test compounds; IC_{50} values are calculated from log curve. [e]All compounds were analyzed for C,H,N and results agree to ±0.4% of theoretical values. [f]I = inactive, % Inhibition ≤ 20 %.

concentration of 50–100 μM. The most active compounds were subsequently assayed as inhibitors of the replication of the HIV-1 virus in HLT4lacZ-1 cells, and their potential toxicity was determined simultaneously [15, 16]. Finally, these selected compounds were assayed as inhibitors of the mutant enzymes P236L, Y181C and P236L/Y181C, applying the same screening system that was used for the wild type enzyme.

Discussion

The preliminary results obtained for compounds **7** are summarized in table I. It is noteworthy that the inhibitory activity is favored by the progressive increase of the size of the amine substituents introduced at position 2. Compounds containing the heterocyclic-substituted piperazine moiety at position 2 of the indole nucleus are more active than those substituted by

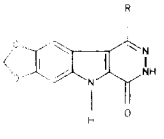
Table II. Physical properties of indole analogs **8** and **9**.



No	R_1	R_2	R_3	*R*	*Yield*[a] *(%)*	*Mp (°C) (recrystallization solvent)*[b]	*Formula*[c]
8c	H	Adamantanamin-1-yl	CHO	H	22	251–252 (A)	$C_{20}H_{22}N_2O_2$
8d	CH_3	Adamantanamin-1-yl	CHO	H	21	264–265 (B)	$C_{21}H_{24}N_2O_2 \cdot H_2O$
8h	H	*N*-Furoylpiperazin-1-yl	CHO	$-OCH_2O-$	6	130–131 (C)	$C_{20}H_{17}N_3O_6$
8i	H	*N*-(4-Cl)-Phenylpiperazin-1-yl	CHO	H	23	232–233 (B)	CHNO
8m	H	*N*-(2-Ethoxy)phenylpiperazin-1-yl	CHO	$-OCH_2O-$	22	151–152 (D)	$C_{23}H_{23}N_3O_5 \cdot 1/2H_2O$
8o	H	*N*-(2-Methoxy)phenylpiperazin-1-yl	CHO	$-OCH_2O-$	9	148–149 (E)	$C_{22}H_{21}N_3O_5$
8p	H	*N*-(4-Nitro)phenylpiperazin-1-yl	CHO	$-OCH_2O-$	20	> 300 (F)	$C_{21}H_{18}N_4O_6$
9c	H	Adamantanamin-1-yl	CN	$-OCH_2O-$	36	> 300 (H)	$C_{21}H_{21}N_3O_3$
9f	H	*N*-Furoylpiperazin-1-yl	CN	H	23	169–172 (G)	$C_{19}H_{16}N_4O_3 \cdot H_2O$
9h	H	*N*-Furoylpiperazin-1-yl	CN	$-OCH_2O-$	54	210–215 (E)	$C_{20}H_{16}N_4O_5$
9i	H	*N*-(4-Cl)-Phenylpiperazin-1-yl	CN	H	36	245–246 (B)	$C_{20}H_{17}ClN_4O$
9j	CH_3	*N*-(4-Cl)-Phenylpiperazin-1-yl	CN	H	10	226–227 (B)	$C_{21}H_{19}ClN_4O$
9k	H	*N*-(2-Ethoxy)phenylpiperazin-1-yl	CN	H	27	148–149 (G)	$C_{22}H_{22}N_4O_2 \cdot HCl$
9m	H	*N*-(2-Ethoxy)phenylpiperazin-1-yl	CN	$-OCH_2O-$	26	228–229 (E)	$C_{23}H_{22}N_4O_4$
9o	H	*N*-(2-Methoxy)phenylpiperazin-1-yl	CN	$-OCH_2O-$	37	224–225 (E)	$C_{22}H_{20}N_4O_4$
9p	H	*N*-(4-Nitro)phenylpiperazin-1-yl	CN	$-OCH_2O-$	5	> 300 (G)	$C_{21}H_{17}N_5O_5$

[a]Value of the final transformation is expressed. [b]Recrystallization solvent: A, dioxane/MeOH; B, MeOH; C, EtOH/H_2O; D, MeOH/H_2O; E, EtOH; F, *N*,*N*-dimethylformamide/H_2O; G, toluene/MeOH; H, dioxane. [c]All compounds were analyzed for C, H, N and results agree to ±0.4% of theoretical values.

Table III. Physical properties of pyridazino[4,5-*b*]indole analogs.



No	*R*	*Method*	*Yield*[a] *(%)*	*Mp (°C) (recrystallization solvent)*[b]	*Formula*[c]
10	NH_2	–	72	> 300 (A)	$C_{11}H_8N_4O_3$
11	$NHCOCH_2Cl$	–	54	> 300 (B)	$C_{13}H_9ClN_4O_2$
12a	$N=CHN(CH_3)_2$	–	33	> 300 (C)	$C_{14}H_{13}N_5O_3$
12b	$N=CH(4\text{-}OH)C_6H_4$	–	10	> 300 (B)	$C_{18}H_{12}N_4O_4$
12c	$N=CH(4\text{-}COOH)C_6H_4$	–	25	> 300 (C)	$C_{19}H_{12}N_4O_5$
12d	$N=CH(4\text{-}NO_2)C_6H_4$	–	16	> 300 (D)	$C_{18}H_{11}N_5O_5$
12e	$N=CH(4\text{-}COOCH_3)C_6H_4$	–	43	> 300 (B)	$C_{20}H_{14}N_4O_5 \cdot 1/2H_2O$
13a	Acetamidoimidazol-1-yl	A	23	> 300 (C)	$C_{16}H_{12}N_6O_4 \cdot 1/2H_2O$
13b	Acetamidopiperidin-1-yl	B	20	> 300 (C)	$C_{18}H_{19}N_5O_4$
13c	Acetamidomorpholin-1-yl	B	27	> 300 (C)	$C_{17}H_{17}N_5O_5$
13d	Acetamido-*N*-(methyl)piperazin-1-yl	B	19	> 300 (C)	$C_{18}H_{20}N_6O_4 \cdot 1/2H_2O$
13e	Acetamido-*N*-(2-methoxy)phenylpiperazin-1-yl	C	9	> 300 (C)	$C_{24}H_{24}N_6O_5$
13f	Acetamido-*N*-(2-ethoxy)phenylpiperazin-1-yl	C	9	> 300 (B)	$C_{25}H_{26}N_6O_5$
13g	Acetamido-*N*-(4-nitro)phenylpiperazin-1-yl	C	20	> 300 (B)	$C_{23}H_{21}N_7O_6$

[a]Value of the final transformation. [b]Recrystallization solvent: A, dimethylsulfoxide (DMSO); B, DMSO/EtOH; C, dimethylformamide (DMF); D, DMF/dioxane. [c]All compounds were analyzed for C, H, N and results agree to ±0.4% of theoretical values.

aliphatic amines. Of the piperazine-containing derivatives, those with phenyl substituents (**7k**, **7m**, **7n** and **7o**) and especially compounds with pyridylpiperazine substituents (**7q** and **7s**) stand out. In general, the most active compounds are those that contain electron-donating substituents in the aromatic ring, particularly the ethoxy-substituted phenylpiperazine analogs (**7k** and **7m**) and the isopropylamine-substituted pyridylpiperazine analogs (**7q**). These SAR observations are comparable to those originally developed by scientists at the Upjohn Company in their BHAP series of analogs (*eg*, U-90152S) [5–7].

Likewise, and in support of earlier unpublished observations by Upjohn scientists, methylation of the indole nitrogen leads to a total loss of inhibitory activity. Whereas **7k** has an IC_{50} of 2.0 μM, its methylated analog **7l** is inactive at the highest dose tested.

The introduction of double substitution on the benzene ring of the indole nucleus also turns out to be advantageous, as can be observed upon comparing the inhibitory activities of **7k** (IC_{50} = 2.0 μM) or **7n** (IC_{50} = 65.0 μM), with the activities of their methylenedioxy analogs **7m** (IC_{50} = 1.4 μM) or **7o** (IC_{50} = 2.5 μM), respectively.

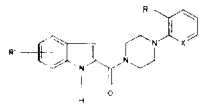
Introduction of formyl or cyano substituents leads to a total loss of inhibitory activity. The increase in the rigidity and size of the molecule has a negative effect upon the inhibitory activity, especially for compounds **13**, with acetamide substituents at position 1 of the ring system. Compounds **12** with imine substituents at position 1, are also inactive. Only modest inhibitory activity is found for **12a** (approximately 53%), in which the amine substituent is the smallest of the series, at the highest dose tested. The elevated insolubility of these compounds hindered the attempts to carry out an in-depth study of their biological activities and the formation of salt derivatives was impeded by the instability of the compounds, due to the presence of the imine bond.

In view of these results, and in agreement with earlier SAR investigations carried out by the Upjohn scientists [5–7], a greater inhibitory activity is observed for indoles with a methylenedioxy substituent, or those without substituents in positions 1 and 3 but with a carbonylpiperazinpyridine substituent at position 2. Analogs of this type are as active as U-90152S in this assay.

Compounds **7k**, **7m**, **7o**, **7q** and **7s** were selected for subsequent assays. The assays for determining the capacity to inhibit the replication of the $HIV\text{-}1_{IIIB}$ virus have been carried out *in vitro* with HLT4lacZ-1 cells. The results obtained are summarized in table IV. The activity shown by the derivative **7s** (IC_{50} = 0.0098 μM) is comparable to that found in our assays for U-90152S. This compound and the other more active compounds have low cytotoxicity.

In tables IV and V, a difference is observed in the activity of the selected compounds. The activity is dependent on the concentration of the substrate used (deoxythymidine triphosphate, dTTP, 0.25 or 10 μM). Better correlation is found between data concerning the inhibition of the enzymes and the results concerning whole cells when a low concentration of substrate (dTTP, 0.25 μM) is used.

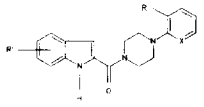
Table IV. Biological activities on HIV-1 RT types of selected compounds.



No	*X*	*R*	*R'*	*Inhibition of HIV-1 RT*[a]		
				Wild type IC_{50} *(μM)*[b]	*P236L* IC_{50} *(μM)*[d]	*HT4lacZ-1/IIIb* ED_{50} *(μM)*[c]
7k	C	OC_2H_5	H	2.0 ± 0.5	> 50	4.85
7m	C	OC_2H_5	$-OCH_2O-$	1.5 ± 0.1	31.3 ± 7.5	1.62
7o	C	OCH_3	$-OCH_2O-$	2.8 ± 0.5	>> 50	11.76
7q	N	$NHCH(CH_3)_2$	$-OCH_2O-$	0.44 ± 0.07	3.3 ± 0.9	0.072
7s	N	NHC_2H_5	$-OCH_2O-$	0.23 ± 0.02	4.7 ± 0.47	0.0098
U-90152S				0.35 ± 0.1	3.91 ± 1.1	0.014

[a]Inhibition of Poly(rA):(dT_{10}) directed poly(dT) synthesis with 0.25 μM dTTP as substrate. [b]Concentration–activity curves are carried out with four or more concentrations of tested compounds. IC_{50} values are calculated from log curve. [c]ED_{50} = 50% effective antiviral dose, syncytia assay. CC_{50} values for all compounds are > 15 μM. [d]A greater than (>) symbol indicates that the IC_{50} is not reached at the highest concentration tested (50 μM).

Table V. Inhibition on wild and mutant HIV-1 RT types of selected compounds.



No	*X*	*R*	*R'*	*Inhibition of HIV-1 RT*[a]			
				Wild type IC_{50} *(μM)*[b]	*P236L* IC_{50} *(μM)*[c]	*Y181C* IC_{50} *(μM)*	*Y181C/P236L* IC_{50} *(μM)*
7k	C	OC_2H_5	H	8.2 ± 0.4	> 50	> 50	> 50
7m	C	OC_2H_5	$-OCH_2O-$	5.6 ± 0.4	> 50	> 50	> 50
7o	C	OCH_3	$-OCH_2O-$	15.9 ± 0.8	> 50	> 50	> 50
7q	N	$NHCH(CH_3)_2$	$-OCH_2O-$	0.56 ± 0.04	11.1 ± 0.22	10.9 ± 2.1	> 50
7s	N	NHC_2H_5	$-OCH_2O-$	1.1 ± 0.13	55.2 ± 3.0	> 50	> 50
U-90152S				0.21 ± 0.01	8.13 ± 0.3	4.5 ± 0.17	> 50

[a]Inhibition of Poly(rA):(dT_{10}) directed poly(dT) synthesis with 10 μM dTTP as substrate. [b]Concentration–activity curves are carried out with four or more concentrations of tested compounds. IC_{50} values are calculated from log curve. [c]A greater than (>) symbol indicates that the IC_{50} is not reached at the highest concentration tested (50 μM).

As regards the results corresponding to the inhibition of the mutant forms of HIV RT (table V), it can be said that a notable loss of activity is generally observed at the highest substrate concentration. This is especially true for the double mutant Y181C/P236L. Compound **7q** maintains significant activity for the P236L mutant as well as for Y181C. Therefore, subsequent structural modifications will be carried out on this compound, selected as the base molecule, so as to optimize the activity found.

Experimental protocols

Chemistry

General laboratory chemicals were purchased from Merck, Sigma, Janssen, and Scharlau. All the new compounds were characterized by elemental analysis, IR and ^{1}H-NMR. The IR spectra were recorded on a Perkin Elmer FT 681 using KBr pellets. The ^{1}H-NMR spectra were obtained on a Bruker AC-200E (200 MHz) instrument with Me_4Si as the internal standard and at a concentration of about 0.1 g/ml. The mass spectra were obtained on a Hewlett-Packard HP-5890 (GC/HPLC/DIP) instrument. All spectra were consistent with assigned structures. Melting points were determined on a Mettler FP82 hot stage apparatus equipped with an FP800/FP80 processor and an Olympus 8091 microscope provided with a video system, and were uncorrected. Elemental analyses of vacuum-dried samples (over P_2O_5 at 1–2 mmHg, 24 h at 60–80°C) were obtained on a Carlo Erba Elemental Analyzer. Results were within 0.4% of theoretical values.

Ethyl 1-methylindol-2-carboxylate **1b** and ethyl 5,6-methylenedioxyindol-2-carboxylate **1c** were obtained according to previously reported methods [17, 18].

*Ethyl 3-formylindol-2-carboxylates **2**. General method*

$POCl_3$ (12 ml, 0.16 mol) was added dropwise to DMF (45 ml, 0.56 mol) in an ice bath, with stirring. A solution of **1** in DMF (mimimum volume) was added. The mixture was stirred at room temperature for 30 min and then heated at 40–60°C for 4 h. The mixture was poured onto ice-water (100 g), and neutralized with aqueous NaOH. The solid obtained was isolated, washed with water (3 x 25 ml), dried and recrystallized from the appropriate solvent. **2a**: yield 85%, mp 189–190°C (EtOH/dioxane). **2b**: yield 85%, mp 110°C (EtOH). **2c**: yield 98%, mp 280°C (dioxane/DMF).

*Ethyl 3-cyanoindol-2-carboxylates **3**. General method*

A mixture of the appropriate **2** (9.2 mmol), nitroethane (3.4 ml, 48 mmol), anhydrous sodium acetate (2.96 g, 36 mmol) and acetic acid (8 ml) was refluxed for 9–10 h. The mixture was treated with water (25 ml) and the solid obtained was filtered, washed with water (3 x 5 ml) and recrystallized from the appropriate solvent. **3a**: yield 80%, mp 167°C (EtOH/H_2O); Anal $C_{12}H_{10}N_2O_2$ (C, H, N). **3b**: yield 64%, mp 239–240°C (EtOH/H_2O); Anal $C_{13}H_{12}N_2O_2$ (C, H, N). **3c**: yield 63%, mp >300°C (2-propanol); Anal $C_{13}H_{10}N_2O_4$ (C, H, N).

*Indol-2-carboxylic acid **4**. General method*

The appropriate **1** (4.29 mmol) was suspended in EtOH (15 ml), and refluxed for 10 min. A solution of KOH (8.5 mmol) in water (6 ml) was added dropwise and the mixture was refluxed for 30 min. The solvent was eliminated, the solid obtained was dissolved in water (40 ml), and the solution was acidified with HCl. The solid that precipitated was recrystallized from the appropriate solvent. **4b**: yield 86%, mp 196–197°C (dioxane); Anal $C_{10}H_9NO_2$ (C, H, N). **4c**: yield 80%, mp 241°C (EtOH/H_2O); Anal $C_{10}H_7NO_4$ (C, H, N).

*3-Formylindol-2-carboxylic acid **5**. General method*

The appropriate **2** (1.19 mmol) was suspended in EtOH (5 ml) and the mixture was refluxed for 10 min. A solution of KOH

(1.3 mmol) in water (2 ml) was added dropwise. The mixture was allowed to cool and the solvent was eliminated by rotary evaporation. The solid residue was dissolved in water (20 ml) and acidified with HCl (conc). The solid obtained was filtered, dried, and recrystallized from the appropriate solvent. **5a**: yield 82%, mp 235–238°C (dioxane); Anal $C_{10}H_7NO_3$ (C, H, N). **5b**: yield 90%, mp 109–111°C (EtOH/dioxane); Anal $C_{11}H_9NO_3$ (C, H, N). **5c**: yield 89%, mp 140°C (EtOH); Anal $C_{11}H_7NO_5$ (C, H, N).

*3-Cyanoindol-2-carboxylic acid **6**. General method*

A mixture of the appropriate **3** (1.72 mmol), H_2O (2 ml), EtOH (5 ml) and KOH (3.56 mmol) was heated at 60°C for 1 h with magnetic stirring. The solvents were eliminated and the residue was dissolved in water (60 ml) and filtered. HCl (conc) (3 ml) was added to the filtered solution and the solid obtained was collected and recrystallized from the appropriate solvent. **6a**: yield 85%, mp 231°C (toluene/MeOH, 9:1); Anal $C_{10}H_6N_2O_2$ (C, H, N). **6b**: crude yield 85% (this compound was used as the starting product without further purification). **6c**: yield 85%; mp 231°C (toluene/MeOH, 9:1); Anal $C_{10}H_6N_2O_2$ (C, H, N).

*Indol-2-carboxamides **7**. General method*

A mixture of the appropriate **4** (4.35 mmol), thionyl chloride (20 mmol) and dioxane (20 ml) was stirred at 25–30°C for 3–8 h. The solvent and the excess reagent was eliminated under reduced pressure. A solution of the appropriate amine (2–10 mmol) in dioxane (10–20 ml) was added and the mixture was stirred for 2–6 h. Once this time had elapsed, the solvent was eliminated and the solid obtained was collected and recrystallized from the appropriate solvent. Yield: 10–40% (table I). ^{1}H-NMR: **7b** (DMSO-d_6): δ 1.17 (t, 6H, CH_3; J = 6.8–6.6 Hz); 3.54 (s, 4H, CH_2; J = 6.5 Hz); 5.96 (s, 2H, CH_2); 6.67 (s, 1H, H_3); 6.87 (s, 1H, H_4); 7.04 (s, 1H, H_7). **7e** (DMSO-d_6): δ 3.65 (d, 4H, CH_2; J = 3.6 Hz); 3.73 (d, 4H, CH_2; J = 4.0 Hz); 5.97 (s, 2H, CH_2); 6.71 (s, 1H, H_3); 6.88 (s, 1H, H_4); 7.03 (s, 1H, H_7); 11.41 (s, 1H, NH). **7h**: (DMSO-d_6) δ: 3.84 (s, 8H, CH_2); 6.66 (s, 1H, $H_{1'}$); 6.77 (s, 1H, $H_{4'}$); 6.90 (s, 1H, H_3); 7.04 (s, 1H, H_4); 7.07 (s, 1H, H_7); 7.87 (s, 1H, $H_{5'}$) 11.44 (s, 1H, NH). **7m** (DMSO-d_6) δ: 1.33 (t, 3H, CH_3; J = 6.9–7.0 Hz); 3.03 (s, 4H, CH_2); 3.88 (s, 4H, CH_2); 4.00 (q, 2H, CH_2; J = 7.0–7.1 Hz); 5.96 (s, 2H, CH_2); 6.73 (s, 1H, H_3); 6.88 (m, 5H, H_4, $H_{3'}$, $H_{4'}$, $H_{5'}$, $H_{6'}$); 7.02 (s, 1H, H_7); 11.39 (s, 1H, NH). **7o** (DMSO-d_6) δ: 3.00 (s, 4H, CH_2); 3.79 (s, 3H, CH_3); 3.88 (s, 4H, CH_2); 5.96 (s, 2H, CH_2-O); 6.73 (s, 1H, H_3); 6.89 (m, 5H, H_4, $H_{3'}$, $H_{4'}$, $H_{5'}$, $H_{6'}$); 7.02 (s, 1H, H_7); 11.42 (s, 1H, NH). **7p** (DMSO-d_6) δ: 3.64 (s, 4H, CH_2); 3.94 (s, 4H, CH_2); 5.98 (s, 2H, CH_2); 6.78 (s, 1H, H_3); 6.89 (s, 1H, H_4); 6.98 (s, 1H, H_7); 7.02 (d, 2H, $H_{3'}$, $H_{5'}$; J = 5.7 Hz); 8.08 (d, 2H, H_4); 6.98 (s, 1H, H_7); 7.02 (d, 2H, $H_{3'}$, $H_{5'}$; J = 5.7 Hz); 8.08 (d, 2H, $H_{2'}$, $H_{6'}$; J = 9.0 Hz); 11.44 (s, 1H, NH). **7q** (DMSO-d_6) δ: 1.06 (d, 6H, CH_3; J = 5.6 Hz); 3.08 (s, 4H, CH_2); 3.46 (m, 1H, CH); 3.96 (s, 4H, CH_2); 4.68 (s, 1H, NH); 5.97 (s, 2H, CH_2); 6.75 (s, 1H, H_3); 6.89 (s, 1H, H_4); 7.02 (m, 3H, H_7, $H_{3'}$, $H_{4'}$); 7.59 (s, 1H, $H_{5'}$); 11.43 (s, 1H, NH). **7s** (DMSO-d_6) δ: 1.15 (t, •H, CH_3; J = 7.0 Hz); 3.02 (s, 4H, CH_2); 3.10 (q, 2H, CH_2; J = 6.6 Hz); 3.94 (s, 4H, CH_2); 4.90 (m, 1H, NH); 5.96 (s, 2H, CH_2); 6.74 (s, 1H, H_3); 6.88 (m, 4H, H_4, H_7, $H_{3'}$, $H_{4'}$); 7.56 (s, 1H, $H_{5'}$); 11.40 (s, 1H, NH).

*3-Formylindol-2-carboxamides **8**. General method*

A mixture of **5** (2.1 mmol) with thionyl chloride (3 ml) was stirred for 3–5 h. The excess reagent was eliminated under reduced pressure. A mixture of the appropriate amine (4.2 mmol), dioxane (10 ml) and sodium carbonate (4.2 mmol) was added and the reaction mixture was stirred for 5 h at 30–40°C. When this time had elapsed, the mixture was allowed to cool and water (10 ml) was added. The oil obtained was removed with ethyl acetate (20 ml). The solvent was eliminated and the solid obtained was collected and recrystallized from the appropriate solvent. Yield: 10–30% (table III). ^{1}H-NMR: **8h** (DMSO-d_6) δ: 3.68 (br s, 8H, CH_2); 6.07 (s, 2H, CH_2); 6.65 (s, 1H, $H_{3'}$); 7.05 (s, 2H, $H_{4'}$, H_4); 7.55 (s, 1H, H_7); 7.86 (s, 1H, $H_{5'}$); 9.97 (s, 1H, CHO); 12.51 (s, 1H, NH). **8m** (DMSO-d_6) δ: 1.31 (t, 3H, CH_3; J = 6.9–6.7 Hz); 3.66 (s, 4H, CH_2); 3.86 (s, 4H, CH_2); 3.96 (q, 2H, CH_2; J = 6.8–6.9–6.8 Hz); 5.99 (s, 2H, CH_2); 6.90 (m, 5H, $H_{3'}$, $H_{4'}$, $H_{5'}$, $H_{6'}$, H_4); 7.54 (s, 1H, H_7); 10.79 (s, 1H, CHO); 11.7 (s, 1H, NH). **8o** (DMSO-d_6) δ: 2.96 (s, 3H, CH_3); 3.67 (s, 4H, CH_2); 3.97 (s, 4H, CH_2); 5.98 (s, 2H, CH_2-O); 6.88 (m, 4H, $H_{3'}$, $H_{4'}$, $H_{5'}$, $H_{6'}$); 7.03 (s, 1H, H_4); 7.48 (s, 1H, H_7); 9.87 (s, 1H, CHO); 12.50 (s, 1H, NH). **8p** (DMSO-d_6) δ: 3.59 (br s, 8H, CH_2); 6.05 (s, 2H, CH_2); 7.04 (m, 3H, $H_{3'}$, $H_{5'}$, H_4); 7.53 (s, 1H, H_7); 8.07 (d, 2H, $H_{2'}$, $H_{6'}$); 9.96 (s, 1H, CHO); 12.4 (s, 1H, NH).

*3-Cyanoindol-2-carboxamides **9**. General method*

A mixture of **6** (3.5 mmol), thionyl chloride (1.7 ml) and THF (15 ml) was stirred for 5–12 h. The excess reagent and the solvent were eliminated under reduced pressure. A solution of the appropriate amine (7 mmol) in THF (15 ml) was poured over the residue. The mixture was stirred at room temperature for 5 h and then filtered. EtOH was added over the filtered solution. The solid obtained was collected, dried and recrystallized from the appropriate solvent. Yield 15–30% (table II). ^{1}H-NMR: **9h** (DMSO-d_6) δ: 3.71 (s, 4H, CH_2); 3.76 (s, 4H, CH_2); 6.06 (s, 2H, CH_2); 6.63 (s, 1H, H_1); 7.03 (s, 2H, H_4, $H_{4'}$); 7.10 (s, 1H, H_7); 7.85 (s, 1H, $H_{5'}$); 12.3 (br s, 1H, NH). **9m** (DMSO-d_6) δ: 1.31 (t, 3H, CH_3; J = 6.8 Hz); 3.05 (s, 4H, CH_2); 3.74 (s, 4H, CH_2); 3.97 (q, 2H, CH_2; J = 6.8–7.6 Hz); 6.07 (s, 2H, CH_2); 6.88 (m, 4H, $H_{3'}$, $H_{4'}$, $H_{5'}$, $H_{6'}$); 7.03 (s, 1H, H_4); 7.12 (s, 1H, H_7); 12.5 (s, 1H, NH). **9o** (DMSO-d_6) δ: 3.03 (s, 4H, CH_2); 3.75 (s, 4H, CH_2); 3.79 (s, 3H, CH_3); 6.07 (s, 2H, CH_2); 6.90 (m, 4H, $H_{3'}$, $H_{4'}$, $H_{5'}$, $H_{6'}$); 7.02 (s, 1H, H_4); 7.11 (s, 1H, H_7); 12.5 (s, 1H, NH). **9p** (DMSO-d_6) δ: 3.62 (s, 4H, CH_2); 3.76 (s, 4H, CH_2); 6.07 (s, 2H, CH_2); 7.04 (m, 4H, $H_{2'}$, $H_{6'}$, H_4, H_7); 8.06 (d, 2H, $H_{3'}$, $H_{5'}$); 12.3 (br s, 1H, NH). **9q** (DMSO-d_6) δ: 1.69 (s, 6H, CH_2); 2.09 (s, 9H, CH_2, CH); 6.08 (s, 2H, CH_2); 7.07 (s, 2H, H_4, H_7); 7.59 (s, 1H, NH); 9.24 (s, 1H, NH).

*1-Amino-3,4-dihydro-7,8-methylenedioxypyridazino[4,5-b]-indol-4-one **10***

A mixture of **3c** (0.5 g; 1.9 mmol) with 90% hydrazine hydrate (15 ml) was refluxed for 9 h. The mixture was allowed to cool and the solid obtained was collected, washed with H_2O (5 x 10 ml), dried, and recrystallized. The product was obtained as white powder, with a yield of 72%, mp > 300°C (DMSO). ^{1}H-NMR (DMSO-d_6; 200 MHz) δ: 5.63 (s, 2H, NH_2); 6.08 (s, 2H, CH_2); 7.03 (s, 1H, H_6); 7.78 (s, 2H, H_9); 11.54 (s, 1H, NH); 12.42 (s, 1H, NH). IR 3465 (s, NH_2); 3176 (s, NH); 1653 (s, C=O); 1301 (s, C-O) cm^{-1}. Anal $C_{11}H_8N_4O_3$ (C, H, N).

*1-Chloroacetamido-3,4-dihydro-7,8-methylenedioxypyrid-azino[4,5-b]indol-4-one **11***

A mixture of **10** (0.5 g; 2.05 mmol) and chloroacetyl chloride (5 ml) was refluxed for 3 h. The mixture was allowed to cool and EtOH (5 ml) was added. The solid obtained was collected and washed with EtOH (5 x 5 ml), dried and recrystallized. The product was obtained as a dark-red solid, with a yield of 54%, mp > 300°C (DMF/EtOH). ^{1}H-NMR (DMSO-d_6; 200 MHz) δ: 4.47 (s, 2H, CH_2); 6.10 (s, 2H, CH_2); 7.05 (s, 1H, H_6); 7.19 (s, 2H, H_9); 10.72 (s, 1H, NH); 12.59 (s, 1H, NH); 12.77 (s, 1H, NH). IR 3248 (s, NH); 1675 (s, C=O); 1653 (s, C=O); 1290 (s, C-O); 1190–1051 (m, C-O) cm^{-1}. Anal $C_{13}H_9ClN_4O_4$ (C, H, N).

1-Imino-3,4-dihydro-7,8-methylenedioxypyridazino[4,5-b]-indol-4-one derivatives ***12****. General method*
A mixture of **10** (0.5 g; 2.04 mmol), the appropriate carbonyl derivative (10 mmol) and HCl (conc) (2 drops) was stirred for 10–15 min at 100–250°C. The mixture was allowed to cool and EtOH (10 ml) was added. The solid obtained was collected, dried and recrystallized from the appropriate solvent. Yield: 10–45% (table III).

1-Acetamido-3,4-dihydro-7,8-methylenedixopyridazino[4,5-b]-indol-4-one amino derivatives ***13***

Method A. A mixture of **11** (0.5 g; 1.56 mmol), the appropriate amine (4.7 mmol) and $NaHCO_3$ (4.7 mmol) was stirred at 100–120°C for 10–15 min. The mixture was allowed to cool and EtOH (10 ml) was added. The solid obtained was collected, dried and recrystallized from the appropriate solvent (table III).

Method B. A mixture of **11** (0.5 g; 1.56 mmol) and the appropriate amine (5 ml) was refluxed for 10–24 h. The excess amine was eliminated under reduced pressure. The solid obtained was collected, dried and recrystallized from the appropriate solvent. Yield: 10–25% (table III).

Method C. A mixture of **11** (0.5 g; 1.56 mmol), $NaHCO_3$ (4.7 mmol), the appropriate amine (4.7 mmol) and DMF (10 ml) was refluxed for 40 h. The mixture was allowed to cool and the solid obtained was collected, washed with EtOH (5 x 5 ml), dried and recrystallized from the appropriate solvent. Yield: 10–20% (table III).

Pharmacology

Poly-rA, oligo$(dT)_{10}$ and dTTP were obtained from Pharmacia LKB Biotechnology Inc. [^{35}S]-dTTPa was purchased from Amersham and DL-dithiothreitol was purchased from Sigma Chemical Co. Nonidet P-40 was obtained from Boehringer-Mannheim and the scintillation mixture used was Biogreen-11, from Scharlau. Glass microfiber filters (Filtermats) were purchased from Skatron Instruments.

U-90152S and recombinant HIV-1 reverse transcriptase (p66) (wild and mutant types), purified according to previously reported methods [14], were kindly provided by the Upjohn Company, Kalamazoo, USA.

Molt 3/HIV-1_{IIIB} infected cells were gifts from F Barin and B Janvier from Université François-Rabelais, Tours, France; HLT4lacZ-1 cells were kindly provided by S Saragosti from Hôpital Cochin, Paris, France.

Reverse transcriptase assay
Enzyme activity was measured in a total volume of 50 µl using a standard reaction mixture containing 50 mM Tris/HCl (pH = 8.3), 20 mM DL-dithiothreitol, 60 mM NaCl, 0.05% Nonidet P-40, 10 mM $MgCl_2$, 10 µg/ml poly-rA, 5 µg/ml oligo $(dt)_{10}$ and 10 µM [^{35}S]-dTTPα (0.2 Ci/mmol). The mixture was preincubated at 37°C for 2 min and the reaction was started by adding the enzyme. The reaction was stopped after 10 min with 50 µl of ice-cold 10% trichloroacetic acid. The insoluble material was collected on glass microfiber filters with Skatron cell harvester and extensively washed with 5% trichloroacetic acid. Filters were dried in a microwave oven for 2 min, transferred to scintillation vials with 3 ml of cocktail and counted using a liquid scintillator counter. All the compounds tested were solubilized in pure DMSO, diluted in water and assayed, maintaining the final concentration of DMSO at 0.5% (v/v).

Cell cultures
HIV-1_{IIIB} chronically infected Molt-3 cells (Molt-3/HIV-1_{IIIB}) were cultured at 37°C and 5% CO_2 in RPMI 1640 (Gibco) supplemented with 20% FCS (fetal calf serum, Flow), 1% penicillin/streptomycin (Gibco), and 1% anti-PPLO (Gibco). HT4lacZ-1 cells were cultured in DMEM (Dulbecco's modification of Eagle medium, ICN Flow) supplemented with 2 mM L-glutamine (Gibco), 10% FCS (Flow) and 1% penicillin/streptomycin (Gibco).

Titration of the virus was carried out by means of a syncytia formation assay, co-cultivating serial dilutions from the viral stocks with uninfected HLT4lacZ-1. The concentration of the virus that caused 100–150 syncytia per well was used for the infection inhibition assays.

Syncytia formation assay
The characteristics and use of HLT4lacZ-1 cells have been described previously [15]. We adapted their use to perform a quantitative syncytia assay in 96-well plates, in order to quantify the inhibitory activity of the synthesized products.

Briefly, 10 000 cells (200 µl)/well were plated the day before the assay. On the following day the medium was removed and 100 µl of product solution and 100 µl of diluted virus were added. Controls with no product were also made. On the third day post-infection, the medium was removed and the cells were fixed for 5 min at room temperature with 200 µl of a PBS solution containing 1% formaldehyde (Merck) and 0.2% glutaraldehyde (Merck). After two washes with 0.9% NaCl (Merck), the cells were incubated for 1 h at 37°C with 200 µl of a reaction mixture containing X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, Boehringer-Mannheim) (400 µg/ml), 4 mM potassium ferrocyanide (Merck), and 2 mM $MgCl_2$ (Merck), in PBS. After two washes, 100 µl of 0.9% NaCl was added per well. The plates were examined under the microscope and only syncytia with three or more blue nuclei were counted in the entire well.

Cell toxicity
Viability of HLT4lacZ-1 cells in the presence of the synthesized products is evaluated using a modified cell lytic assay described previously [16]. It is performed parallelly with the syncytia formation assay.

Medium (200 µl) containing different concentrations of the products or medium (control wells) were added to 10 000 HLT4lacZ-1 cells plated the day before. On the third day, the wells were washed three times with PBS, and cell lysis was detected by staining the plate for 10 min at room temperature, with 20 µl/well of a methanol/water (1:4 v/v) solution containing 0.5% crystal violet (Merck). Controls of wells without cells were stained in order to provide the background (blank wells). Three washes were made by immersion of the plates in PBS, changing the PBS of the container each time. The plates were wiped and 100 µl/well of 0.1% SDS (sodium dodecyl sulfate, Sigma) were added. After complete disaggregation of the cell membranes, the OD at 540 nm was read using a Titertek Multiskan II autoreader (Flow) and the percentage of viability (%V) was calculated using the formula:

$$\%\ V = \frac{100 \times (\text{OD product wells} - \text{OD blank})}{(\text{OD control wells} - \text{OD blank})}$$

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References

1 Larder BA, Kemp SD (1989) *Science* 246, 1155–1158
2 Kellam P, Boucher CAB, Larder BA (1992) *Proc Natl Acad Sci USA* 89, 1934–1938
3 St Cair MH, Martin JL, Tudor-Williams G *et al* (1991) *Science* 253, 1557–1559
4 Romero DL, Busso M, Tan CK *et al* (1991) *Proc Natl Acad Sci USA* 88, 8806–8810
5 Dueweke TJ, Poppe SM, Romero DL *et al* (1993) *Antimicrob Agents Chemother* 37, 1127–1131
6 Romero DL, Morge RA, Genin MJ *et al* (1993) *J Med Chem* 36, 1505–1508
7 Romero DL, Morge RA, Biles C *et al* (1994) *J Med Chem* 37, 999–1014
8 Williams TM, Ciccarone TM, MacTough SC *et al* (1993) *J Med Chem* 36, 1291–1294
9 De Vreese K, Debyser Z, Vandamme AM *et al* (1992) *Virology* 188, 900–904
10 Sardana VV, Emini EA, Gotlib L *et al* (1992) *J Biol Chem* 267, 17526–17530
11 Blater HM, Lukaszewski H, Stevens G (1961) *J Am Chem Soc* 83, 2203–2205
12 Deibel MR, McQuade TJ, Brunner DP, Tarpley WG (1990) *AIDS Res Hum Retroviruses* 6, 329–340
13 Tan CK, Zhang J, Li ZY, Tarpley WG, Downey KM, So AG (1991) *Biochemistry* 30, 2651–2655
14 Sharma SK, Evans DB, Vosters AF, McQuade TJ, Tarpley WG (1991) *Biotechnol Appl Biochem* 14, 69–81
15 Rocancourt D, Bonnerot C, Jouin H, Emerman M, Nicolas JF (1990) *J Virol* 64, 2660–2668
16 Aggarwal BB, Kohr WJ, Hass PE *et al* (1985) *J Biol Chem* 260, 2345–2354
17 Adrisiano R, Vitale T (1957) *Gazz Chim Ital* 87, 949–954
18 Monge A, Aldana I, Alvarez T *et al* (1991) *J Med Chem* 34, 3023–3026