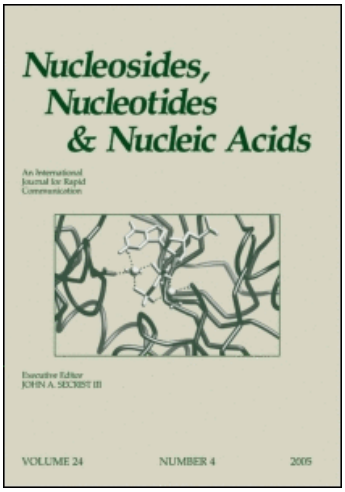

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Synthesis and Anti-HIV Evaluation of 7-Deaza Analogues of Carbovir

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SYNTHESIS AND ANTI-HIV EVALUATION OF 7-DEAZA ANALOGUES OF CARBOVIR

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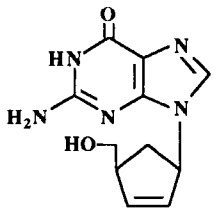
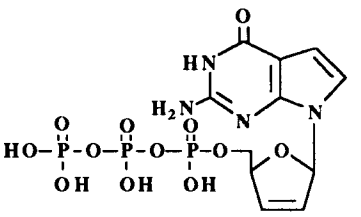
Abstract: Three analogues of Carbovir **1** have been synthesized and evaluated for antiviral activity *in vitro*. Anti-HIV-1 and anti-HIV-2 activities have been observed with 7-deaza analogues **3** and **5** of **1**. Compound **5** was about ten times more potent than **3** against HIV-1 and HIV-2 on different cell lines.

Introduction

Carbovir (**1**) has emerged as the first carbocyclic nucleoside analogue with potential as a therapeutic agent for the treatment of AIDS[1]. Furthermore some 2,3-dideoxy-2,3-didehydro-pyrrolo[2,3-d] pyrimidine nucleosides in their triphosphate form such as **2**[2] have been shown to be potent and specific inhibitors of the reverse transcriptase (RT) of HIV-1.



CARBOVIR (1)



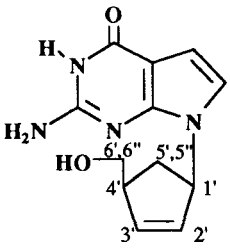
(**2**)

IC_{50}(RT, HIV-1)=0.09 μ M
IC_{50}(ADN Pol. α)=680 μ M
IC_{50}(ADN Pol. γ) =1216 μ M

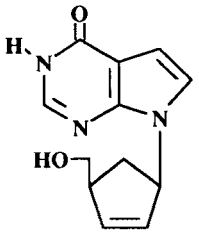
Therefore, in an attempt to increase selectivity and potency against HIV RT, we have been interested to combine their structural features in the same molecule .

Chemistry

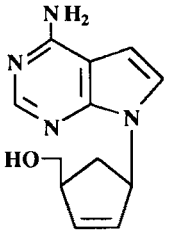
We have synthesized three new analogues of Carbovir as racemic mixtures, in which the 7-nitrogen atom of the purine ring has been deleted, providing 7-deaza guanine (**3**), 7-deazahypoxanthine (**4**) and 7-deazaadenine (**5**) analogues of Carbovir (**1**).



(3)

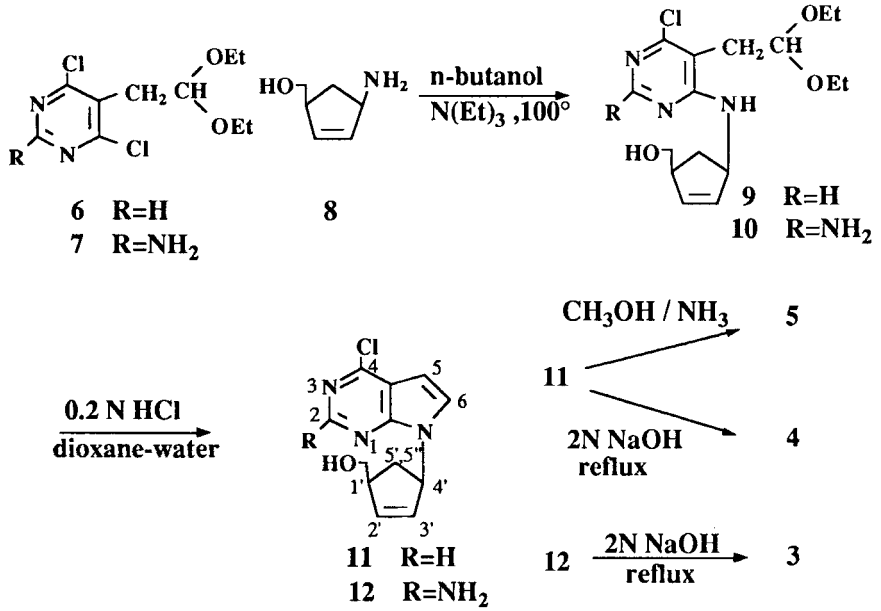


(4)



(5)

Pyrimidines **6**[3] and **7**[4] were reacted with cyclopentenylamine **8**[5] in butanol to provide the carbocyclic pyrimidines **9** and **10** respectively (Scheme 1). Cyclisation was then performed in dilute hydrochloric acid at room temperature to give **11** and **12**. Nucleophilic displacement of the chlorine atom in **11** and **12** was then carried out with sodium hydroxide to give **4** and **3**, whereas treatment of **11** with liquid ammonia gave **5**.



SCHEME 1

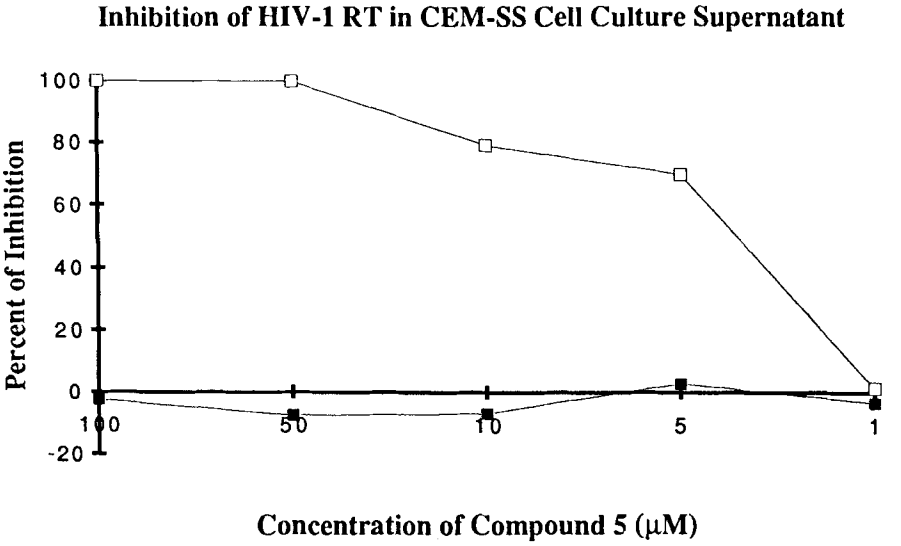


FIG.1.The percentage of RT inhibition is expressed in comparison with untreated cells which were incubated in the presence the solvent (DMSO); -□-: RT inhibition; -■-:cytoxicity.

Biology

Compounds **3**, **4**, **5**, **11** and **12** were tested for anti-HIV-1 activity on CEM-SS cells. Compound **5** exhibited a significant inhibitory property and no toxicity as shown on Fig. 1 while compound **3** also inhibited HIV replication but was less active.

These two compounds were further analyzed for their activity on different isolates of HIV-1 and HIV-2 and on different cells including primary cultures of PBMC. Non. of these molecules were toxic on these cells at the highest concentration used (10^{-4} M). Table 1 indicates the IC_{50} measured for HIV-1 and HIV-2. Compounds **5** and **3** were 10 times more active on PBMC than on cell lines and they inhibited HIV-1 and HIV-2 with the same efficiency. Compound **5** with an IC_{50} of 3 10^{-6}M on CEM-SS cells is slightly less potent than Carbovir **1** (IC_{50} of 6 10^{-7}M)[1].

Compounds **3**, **4**, **5**, **11** and **12** were found inactive against HSV-1 HSV-2, HCMV, Vaccinia virus, VSV, and Sindbis virus. Compound **3** has been reported recently by W. Schneller et al[6] and was also found inactive against HSV-1, HSV-2, HCMV, and HIV-1 in CEM cells, different from the CEM-SS used in this work.

In conclusion, this series of compounds is non-cytotoxic and two of them, the 7-deaza adenine (**5**) and the 7-deaza guanine (**3**) analogues were found active against HIV-1 and HIV-2 *in vitro*, although slightly less active than Carbovir.

TABLE 1

ANTI-HIV ACTIVITY
INHIBITORY CONCENTRATION 50 (IC_{50}) OF ACTIVE MOLECULES

VIRUS/CELLS	3	5
HIV-1 LAI/CEM-SS	1.5 10^{-5} M	3 10^{-6}M
HIV-1 LAI/CEMX174	2.5 10^{-5} M	2.5 10^{-6}M
HIV-1 IIIB/PBMC	7 10^{-6} M	4 10^{-7} M
HIV-2 D194/PBMC	6 10^{-6} M	3.5 10^{-7}M
HIV-2 D205/PBMC	9 10^{-6} M	3.5 10^{-7} M

Experimental section

Chemistry

The melting points were taken on a Kofler hot stage apparatus and were uncorrected. Elemental analyses were performed by the "Service de Microanalyse", CNRS, ICSN, 91198 Gif-sur-Yvette, France. Fast atom bombardment (FAB) mass spectra (MS) were obtained from the "Laboratoire de Spectrometrie de Masse", CNRS, ICSN, 91198 Gif-sur-Yvette, France. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded at 200 MHz on a Bruker AC 200 spectrometer. Numbering of cyclopentene methanol in compounds **5**, **9**, **10**, **11**, and **12** and of the pyrrolo[2,3-d]pyrimidine ring is indicated for **11** and **12** on scheme 1 as well as the numbering of cyclopentenyl derivatives of pyrrolo[2,3-d]pyrimidinones **3** and **4** which is mentioned for **3**.

(±)-[*cis*-4 -(4-Chloro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-cyclopent-2 -enyl]-methanol (11).

A solution of **6** (9.7 g, 36.6 mmol) in n-butanol (50 ml) was treated with **8**[5] (3.2 g, 28.31 mmol) in the presence of an excess of triethylamine (4 ml) at 100°C for 3 days under nitrogen. The mixture was then cooled, evaporated to dryness and coevaporated several times in the presence of n-heptane. The residue was partitioned between chloroform and water. The organic layer was washed twice with water, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude product (11.34 g) was purified by column chromatography (silica gel, CH_2Cl_2-EtOH 19 : 1) to give 5.4 g of

unreacted pyrimidine **6** and 5.5 g of **9** as an oil which was contaminated with about 10 % of **11** according to its ^1H NMR spectrum in $CDCl_3$. **9** : ^1H NMR ($CDCl_3$) δ 8.27 (s, 1H, H-2) ; 6.41 (d, 1H, NH, $J_{NH-4'}$ = 7.4 Hz) ; 5.87 (m, 2H, CH = CH) ; 5.19 (m, 1H, H-4') ; 4.59 (t, 1H, CH $(OEt)_2$, $J_{CH-CH_2\text{-pyrimidine}}$ = 5.4 Hz) ; 3.57 (m, 6H, 2 x OCH_2CH_3, CH_2OH) ; 2.91 (m, 3H, CH_2-pyrimidine, H-1') ; 2.65 (m, 1H, H5') ; 1.46 (m, 1H, H5") ; 1.21 (m, 6H, 2 x OCH_2CH_3). FAB-MS m/e = 342

A solution of **9** obtained as described above (2.74 g, 10.33 mmol) in dioxane-1N aqueous hydrochloric acid (4 : 1) was stirred at room temperature for 2 days. An excess of concentrated ammonium hydroxide was then added and the mixture was evaporated under reduced pressure. The residue was partitioned between dichloromethane and water. The organic layer was washed twice with water, dried ($MgSO_4$), filtered and evaporated to dryness. The oily residue crystallized and was recrystallized from a mixture of cyclohexane-ethyl acetate (4 : 1) to give a 87 % yield of **11**; mp 95-96°C ; ^1H NMR (DMSO-d_6) δ 8.66 (s, 1H, H2) ; 7.69 (d, 1H, H6, J_{6-5}= 3.65 Hz) ; 6.69 (d, 1H, H5, J_{5-6} = 3.65 Hz) ; 6.19 (m, 1H, H2', $J_{3'-2'}$= 5.5 Hz, $J_{2'-4'}$= 2 Hz, $J_{2'-1'}$= 2 Hz) ; 5.95 (m, 1H, H4', $J_{4'-3'}$= 2.1 Hz, $J_{4'-5'}$= 5.85 Hz, $J_{4'-5"}$= 8.7Hz) ; 5.87 (m, 1H, H3', $J_{3'-1'}$= 2.1 Hz) ; 4.87 (t, 1H, OH, J_{OH-CH_2}= 5.5 Hz) ; 3.5 (m, 2H, CH_2OH) ; 2.94 (m, 1H, H1', $J_{1'-5'}$= 8.7 Hz, $J_{1'-5"}$= 5.85 Hz) ; 2.71 (m, 1H, H5', $J_{5'-5"}$=13.55 Hz) ; 1.60 (m, 1H, H5"). Anal. Calcd for $C_{12}H_{12}N_3O$ Cl : C, 57.72 ; H, 4.84 ; N, 16.83 ; Cl, 14.20. Found : C, 57.69 ; H, 4.66 ; N, 16.74 ; Cl, 14.43.

(±)-*cis*-7-[4 -(Hydroxymethyl)-cyclopent-2 -enyl]-*3H,7H*-pyrrolo[2,3-d] pyrimidin-4-one (4).

A solution of **11** (327 mg, 1.3 mmol) in 2N NaOH (20 ml) was refluxed for 5 hours. The mixture was neutralized with acetic acid, evaporated to dryness and coevaporated several times with toluene. The residue was adsorbed onto silica gel and purified by column chromatography eluting with dichloromethane-ethanol (19 : 1). Yield 220 mg (73 %) ; an analytical sample was obtained by crystallization from water. mp. 86-87°C; ^1H NMR (DMSO-d_6) δ 11.90 (s, 1H, NH); 7.93 (s, 1H, H2) ; 7.06 (d, 1H, H6, J_{6-5} = 3.5 Hz) ; 6.52 (d, 1H, H5, J_{5-6} = 3.5 Hz) ; 6.14 (m, 1H, H3', $J_{2'-3'}$ = 5.5 Hz, $J_{3'-1'}$ = 2 Hz, $J_{3'-4'}$ = 2Hz) ; 5.84 (m, 1H, H2', $J_{2'-1'}$ = 2.1 Hz ; $J_{2'-4'}$ = 2.1 Hz) ; 5.77 (m, 1H, H1', $J_{1'-5'}$= 8.6Hz, $J_{1'-5"}$= 6 Hz) ; 4.75 (t, 1H, OH, J = 5 Hz) ; 3.48 (t, 2H, CH_2OH, J_{CH_2-OH}= 5 Hz, $J_{CH_2-4'}$= 6 Hz) ; 2.90 (m, 1H, H4', $J_{4'-5'}$ = 8.6 Hz, $J_{4'-5"}$= 6 Hz) ; 2.65 (m, 1H, H5', $J_{5'-5"}$= 13.6 Hz) ; 1.54 (m, 1H, H5"). Anal. Calcd for $C_{12}H_{13}N_3O_2$: C, 62.33 ; H, 5.67 ; N, 18.17. Found : C, 62.01 ; H, 5.52 ; N, 18.01.

(±)-[*cis*-4 -(4-Amino-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-cyclopent-2 -enyl]-methanol (5).

A solution of **11** (700 mg, 2.8 mmol) in methanol (70ml) was treated with liquid ammonia (70 ml) at 100°C in a stainless sealed vessel for 48 hours. The mixture was evaporated to dryness, adsorbed onto silica gel, and purified by silica gel column chromatography using dichloromethane-ethanol (19 : 1) as eluent affording 640 mg of a colorless oil (yield, 99 %). Analytically pure material was obtained by crystallization from water. mp 170-172°C; ^{1}H NMR ($CDCl_3$) δ 8.28 (s, 1H, H2) ; 7.04 (d, 1H, H6, $J_{6\text{-}5}$ = 3.6 Hz) ; 6.31 (d, 1H, H5) ; 6.09 (m, 1H, H2', $J_{2'\text{-}3'}$= 5.5 Hz, $J_{2'\text{-}1'}$ = 2.1 Hz); 5.83 (m, 1H, H3', $J_{3'\text{-}1'}$ =2.18); 5.73 (m, 1H, H4', $J_{4'\text{-}3'}$= 2.2 Hz ; $J_{4'\text{-}5'}$= 9.1 Hz; $J_{4'\text{-}5''}$= 6.7 Hz ; $J_{4'\text{-}2'}$= 2.1 Hz) ; 5.08 (s, 2H, NH_2) ; 3.78 (m, 2H, $\underline{CH_2}OH$, $^2J_{6'\text{-}6''}$= 10.66 Hz, $J_{6'\text{-}1'}$ = 4.25 Hz) ; 3.07 (m, 1H, H1', $J_{1'\text{-}2'}$ = 2.1 Hz, $J_{1'\text{-}3'}$= 2.18 Hz, $J_{1'\text{-}5'}$ = 9.1 Hz, $J_{1'\text{-}5''}$= 6.7 Hz, $J_{1'\text{-}6'}$= $J_{1'\text{-}6''}$= 4.25 Hz) ; 2.79 (m, 1H, H5',$^2J_{5'\text{-}5''}$= 14 Hz) ; 1.95 (m, 1H, H5''). Anal. Calcd. for $C_{12}H_{14}N_4O$: C, 62.59 ; H, 6.13 ; N, 24.33. Found : C, 62.73 ; H, 5.81 ; N, 23.97.

(±)-[*cis*-4 -[[2-Amino-6-chloro-5-(2,2-diethoxy-ethyl)-pyrimidin-4-yl]-amino]cyclopent-2 -enyl]-methanol (10).

A solution of **7** (2.77 g, 9.89 mmol) in n-butanol (30 ml) was treated with **8** (1.7 g, 15 mmol) according to the procedure used in the preparation of compound **9**. After extraction, a solid residue was obtained and purified by column chromatography (silica gel) eluting with dichloromethane-ethanol (49 : 1). Crystallization from ethanol afforded 1.4 g (40 %) of **10**; mp 153-154°C ; ^{1}H NMR ($CDCl_3$) δ : 6.19 (d, 1H, NH, $J_{NH\text{-}4'}$ = 7.54 Hz) ; 5.84 (m, 2H, CH = CH) ; 5.08 (m, 1H, H4') ; 4.84 (s, 2H, NH_2) ; 4.48 (t, 1H, $CH_2\underline{CH}(OEt)_2$; 3.75-3.40 (m, 6H, 2x$O\underline{CH_2}CH_3$, $\underline{CH_2}OH$); 2.90 (m, 1H, H1') ; 2.7 (m, 2H, $\underline{C}H_2CH(OEt)_2$; 2.54 (m, 1H, H5') ; 1.42 (m, 1H, H5''). Anal. Calcd for $C_{16}H_{25}N_4O_3Cl$: C, 53.85 ; H, 7.06 ; N, 15.70. Found : C, 54.11 ; H, 6.94 ; N, 15.87. FAB-MS m/e = 357.

(±)-[*cis*-4 -[2-Amino-4-chloro-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl]-cyclopent-2 -enyl]-methanol (12).

Compound **10** was treated according to the procedure used in the preparation of compound **11** to give **12** with a yield of 76% ; mp ($CHCl_3$) 144-145°C; ^{1}H NMR (DMSO-d_6) δ : 7.10 (d, 1H, H6, $J_{6\text{-}5}$ = 3.75 Hz); 6.67 (s, 2H, NH_2) ; 6.35 (d, 1H, H5) ; 6.13 (m, 1H, H2', $J_{3'\text{-}2'}$= 5.5 Hz ; $J_{2'\text{-}1'}$= 2 Hz) ; 5.82 (m, 1H, H3', $J_{3'\text{-}4'}$= 2 Hz) ; 5.69 (m,

1H, H4', $J_{4'-3'}$= 2 Hz, $J_{4'-5'}$= 6Hz, $J_{4'-5''}$= 8.7 Hz) ; 4.76 (t, 1H, OH, J_{OH-CH_2}= 5.3 Hz) ; 3.49 (m, 2H, $\underline{CH_2}OH$, $J_{1'-6'}$=$J_{1'-6''}$=2 Hz) ; 2.90 (m, 1H, H1', $J_{1'-5'}$= 8.7 Hz) ; 2.60 (m, 1H, H5') ; 1.55 (m, 1H, H5", $J_{5'-5''}$= 13.6 Hz). Anal. Calcd. for $C_{12}H_{13}N_4OCl$. : C, 54.45 ; H, 4.95 ; N, 21.16. Found : C, 54.32 ; H, 4.95 ; N, 20.91.

(±)-2-Amino-7-[*cis*-4 -(hydroxymethyl)-cyclopent-2 -enyl]-*3H,7H*-pyrrolo[2,3-d]pyrimidin-4-one (3).

Compound **12** was treated according to the procedure used in the preparation of compound **4** to give **3** with a yield of 56%; The titled compound crystallized from water-ethanol (4 : 1) after column chromatography (silica gel), eluting with dichloromethane-ethanol (19 : 1). mp: decomposition started at 254-255°C and was complete at 270°C; ^1H NMR (DMSO-d_6) δ 10.30 (s, 1H, NH) ; 6.64 (d, 1H, H6, J_{6-5}= 3.6 Hz) ; 6.27 (d, 1H, H5) ; 6.22 (s, 2H, NH_2) ; 6.09 (m, 1H, H3', $J_{3'-2'}$= 5.6 Hz, $J_{3'-4'}$= 2.06 Hz, $J_{3'-1'}$ = 2.06 Hz) ; 5.77 (m, 1H, H2', $J_{2'-1'}$= 2.14 Hz, $J_{2'-4'}$= 2.14 Hz) ; 5.57 (m, 1H, H1', $J_{1'-5'}$= 8.7 Hz, $J_{1'-5''}$= 6.2 Hz) ; 4.74 (t, 1H, OH, J_{OH-CH_2}= 5 Hz) ; 3.46 (t, 2H, $\underline{CH_2}OH$, $J_{CH_2-H4'}$= 5.6 Hz) ; 2.86 (m, 1H, H4', $J_{4'-5'}$= 8.7 Hz, $J_{4'-5''}$= 6.2 Hz) ; 2.5 (m, 1H, H5') ; 1.48 (m, 1H, H5", $J_{5'-5''}$= 13.5 Hz). Anal. Calcd for $C_{12}H_{14}N_4O_2$: C, 58.53 ; H, 5.73 ; N, 22.75. Found : C, 58.64 ; H, 5.71 ; H, 22.44.

Biology

Cell cultures and viruses

The human T-cell lines CEM-SS[8] , CEM-X 174 fusion of a B-cell line (obtained through the AIDS Research and Reference Reagent Program-Division of AIDS, NIAID, NIH from Cresswell, P.) were maintained in RPMI 1640 medium with 2 mM glutamine and 10 % fœtal bovine serum (FBS, heated 30 min. at 56°C) in a CO_2 incubator at 37°C. Human peripheral blood mononuclear cells (PBMC) from HIV seronegative donors, separated on ficoll, were cultured in RPMI 1640 containing 10 % FBS in the presence of 4 µl/ml phytohaemagglutinin (PHA). After 3 days, PHA was removed, cells were washed with medium and reincubated, after infection, in medium containing interleukin-2 (20 U/ml). Several isolates of HIV were used ; HIV-1 LAI[9,10] , HIV-1 IIIB[11] , HIV-2 D194 and HIV-2 D205[12] .

Inhibition of virus production

Production of virus particles was evaluated by measurement of virion associated reverse transcriptase activity in the culture supernatants as described (Jonassen, T.O., Skatron Application Notes, April 1986).

Cells were infected with the HIV isolates (20 TC:D_{50}). After 0.5 h adsorption, the residual free virus was removed by centrifugation, the cultures were resuspended at the

concentration of 40 x 10^3 cells/ml for CEM-SS, 80 x 10^3 for CEM -X 174, 160 x 10^3 in the medium indicated above and distributed into microtitration plates (100 µl/well) containing 0.1 ml/well of different dilutions of the antiviral drugs. Reverse transcriptase activity was measured after 5 days for CEM-SS and CEM-X 174, or after 7 days for PBMC. In this later case, 100 µl of medium was removed at day 5 and replaced with fresh medium containing the same concentration of drug. Fifty per cent inhibitory concentration (IC_{50}) was defined as the concentration of drug that reduced the reverse transcriptase activity by 50 %. Cytotoxicity was determined by the MTT dye reduction assay[13].

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