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New Compounds

Synthesis and antiviral properties of novel analogues of monophosphate and diphosphate bioactive forms of acyclovir

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Abstract

New analogues (compounds **6**, **7** and **9**) of the mono- (**8**) and diphosphate (**10**) bioactive forms of the antiherpes drug acyclovir are described. In compound **6**, the monophosphate moiety of **8** was replaced by an aminosulfonyloxy group, while in compounds **7** and **9**, a phosphonoacetamidoxy and an *O*-ethyl phosphonoacetamidoxy moiety are, respectively present instead of the diphosphate one of **10**. None of the compounds synthesized proved to possess an appreciable activity on herpes simplex virus (HSV) or human immunodeficiency virus (HIV).

Keywords: Antiviral; HSV-1; HIV-1; Acyclovir-monophosphate; Acyclovir-diphosphate

1. Introduction

The nucleoside analogues currently used in therapy against herpes simplex virus (HSV) or human immunodeficiency virus (HIV) infections, such as acyclovir (ACV) [1], ganciclovir (DHPG) [2] or Zidovudine (AZT) [3], Lamivudine (3TC) [4] and Stavudine (D4T) [5] are not active per se: their antiviral activity is due to their transformation to the corresponding triphosphate bioactive forms, which are formed through three distinct intracellular phosphorylation steps by the action of cellular kinases. The resulting triphosphate nucleotide analogues then block viral replication through the inhibition of enzymes such as DNA polymerases or reverse transcriptases [1,6].

The process of activation to triphosphates represents the limiting step for the efficacy of such drugs, especially against the resting viral cells which possess only one low level of kinase [7,8]; nucleotide mono- di- and triphosphate themselves would partially or totally bypass this requirement for intracellular activation, but they are poor drug candidates as they are metabolically unstable and become degraded rapidly to the parent nucleosides.

For this reason, over the last few years various chemically and metabolically stable analogues of nucleotide and acyclonucleotide mono-, di- and triphosphate have been studied as a good approach for antiviral therapy against HSV and HIV. Examples of this class include monophosphate acyclonucleotide analogues such as 9-[(3-hydroxy-2-(phosphonylmethoxy)propyl]-adenine (HPMPA) [9], and 9-[2-(phosphonylmethoxy)-ethyl]adenine (PMEA) [10], in which the monophosphate moiety of the metabolite of acyclonucleotides is replaced by a stable phosphonomethyloxy group. HPMPA and PMEA proved to possess a broad-spectrum anti-HSV activity and a potent anti-HIV activity, respectively (Fig. 1).

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In previous papers, we described the synthesis and the antiviral properties of compounds **1**, **2** and **4** [11,12], designed as stable analogues of the mono- (**3**) and diphosphate (**5**) metabolites of 5-iodo-2′-deoxyuridine (IDU), a drug actually used in therapy against HSV whose activity is due to its bioactive triphosphate form.

In particular, while compound **4**, in which a phosphonoacetamido moiety is present instead of the diphosphate one of **5**, was practically devoid of any appreciable antiviral activity, compounds **1** and **2**, in which the mono- and diphosphate moiety of **3** and **5** were replaced, respectively by an aminosulfonyloxy and aminosulfonylaminocarbonyloxy moiety, proved to possess an appreciable activity against herpes virus type 1 (HSV-1), even if slightly lower than that of IDU. The latter results indicated that the aminosulfonyloxy and aminosulfonylaminocarbonyloxy moieties of **1** and **2** might be considered, at least for HSV-1, as partial bioisosters, respectively of the mono- and diphosphate portions of the metabolite of IDU **3** and **5** (Fig. 2).

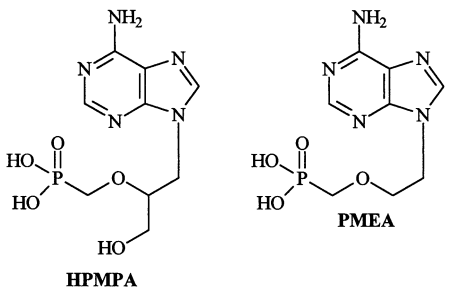


Fig. 1.

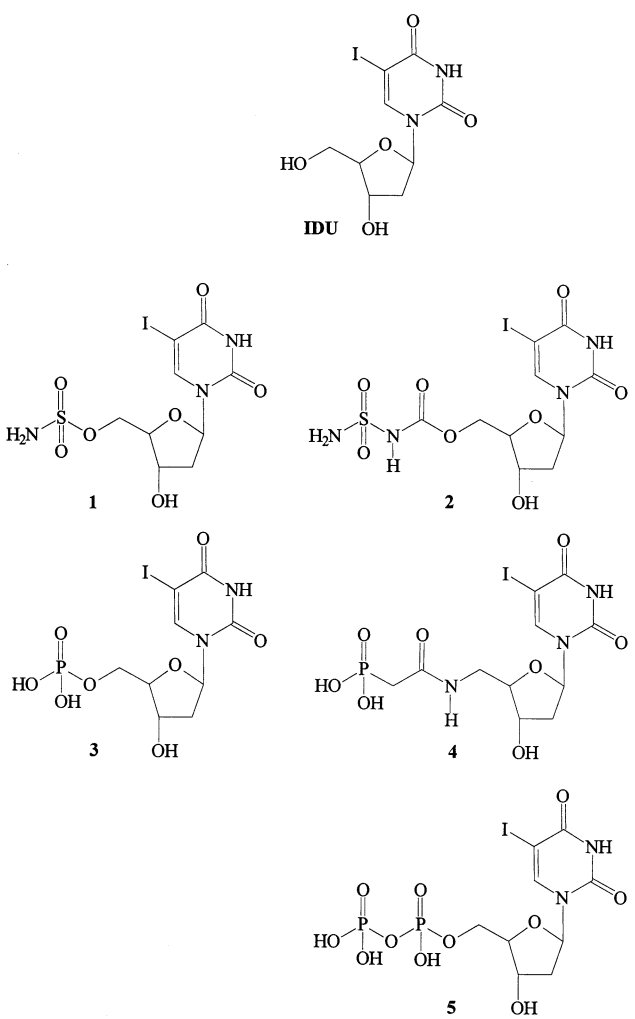


Fig. 2.

As a part of our search for new chemically and metabolically stable analogues of nucleotide and acyclonucleotide mono-, di- and triphosphate, we here report the synthesis and the antiviral properties of new analogues (compounds **6**, **7** and **9**) of the mono- (**8**) and diphosphate (**10**) bioactive forms of the antiherpes drug acyclovir. In particular, compound **6**, the monophosphate moiety of **8** was replaced by an aminosulfonyloxy group, as in the case of the previously reported stable analogue **1** of IDU-monophosphate **3**, while compounds **7** and **9** contain, respectively a phosphonoacetamidoxy and an *O*-ethyl phosphonoacetamidoxy moiety instead of the diphosphate one of **10**. The choice to use a phosphonoacetamidoxy and an *O*-ethyl phosphonoacetamidoxy portion as potential bioisosters of the diphosphate moiety of **10**, was based on our previous studies in the field of antitumour agents [13–15] in which we found that, in the case of a series of analogues of geranylgeranyldiphosphate, an intermediate of the cholesterol biosynthetic pathway involved in the isoprenylation of RAS superfamily GTP-binding proteins such as Rho, Rac and CdC42, these moieties were able to act as stable isosters of the diphosphate group (Fig. 3).

2. Chemistry

The aminosulfonyl derivative **6** was synthesized as reported in Scheme 1. Reaction of 2-benzoylamino-9-(2-hydroxyethoxymethyl)-1,9-dihydropurin-6-one (**11**) [16] with sodium hydride and aminosulfonyl chloride in anhydrous DMF afforded the intermediate **12**, which was then treated with a saturated methanolic solution of ammonia, to obtain the *N*-debenzoylated final product **6**.

Phosphonic derivatives **7** and **9** were prepared using the synthetic procedure shown in Scheme 2. 2-Benzoylamino-9-[2-(phthalimidoxy)ethoxymethyl]-1,9-dihydropurin-6-one (**13**) [16] was subjected to hydrazinolysis to give the aminoxy derivative **14**, which was then treated with diethylphosphonoacetic acid in the pres-ence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBt), affording the phosphonated intermediate **15**.

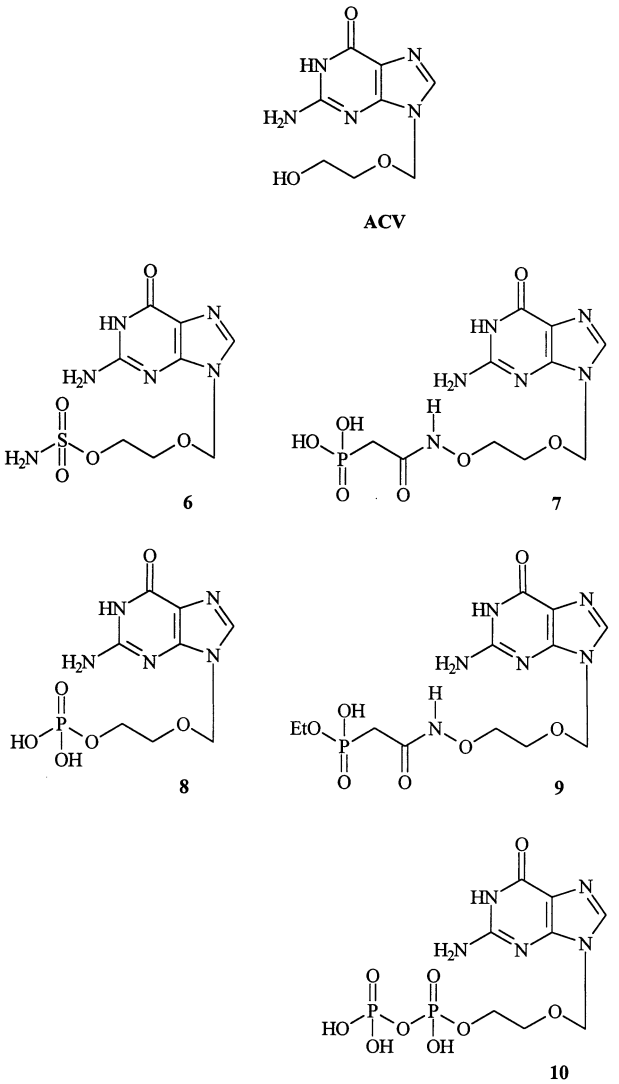


Fig. 3.

Reaction of **15** with trimethylsilyl bromide (TMSBr) and collidine followed by treatment with aqueous sodium hydroxide, led to the removal of the ethyl groups from the phosphonate moiety of **15** to yield compound **16**. The *N*-benzoyl group in **16** was removed by alkaline hydrolysis with sodium hydroxide in dioxane, to give the final phosphonoacetamidoxy-substituted compound **7**.

The *O*-ethylphosphonoacetamidoxy derivative **9** was directly obtained from intermediate **15** by alkaline hydrolysis with NaOH in dioxane.

3. Biological results and conclusions

Compounds **6**, **7** and **9** were tested for their antiviral activity against herpes virus type 1 (HSV-1), using VERO cells infected with the HSV-1 strain HF and for their HIV-1 inhibitory activity, using lymphoblastoid CD4+ cells (C8166), infected with the HIV strain HTLV-IIIB derived from chronically infected H9 cells.

At a concentration of 50 μM all the compounds were found to be practically devoid of any appreciable antiviral activity on both HSV-1 and HIV-1.

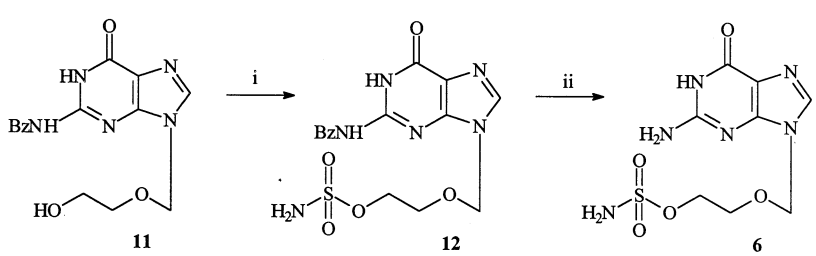
These data indicate that the aminosulfonyloxy moiety of compound **6**, which was used as a potential bioisoster of the monophosphate portion of the bioactive form of ACV **8**, is not able to confer any antiviral activity to ACV, contrary to previous findings in the case of IDU-monophosphate analogue **1**, for which such type of modification led to an appreciable activity against herpes virus type 1 (HSV-1), even if slightly lower than that of IDU.

Furthermore, also the phosphonoacetamidoxy and the *O*-ethyl phosphonoacetamidoxy moieties of compounds **7** and **9** were not able to act as bioisosters of the diphosphate portion of the bioactive form of ACV **10**; however the possibility cannot be excluded that the inactivity found for compounds **7** and **9** might be ascribed to distribution problems.

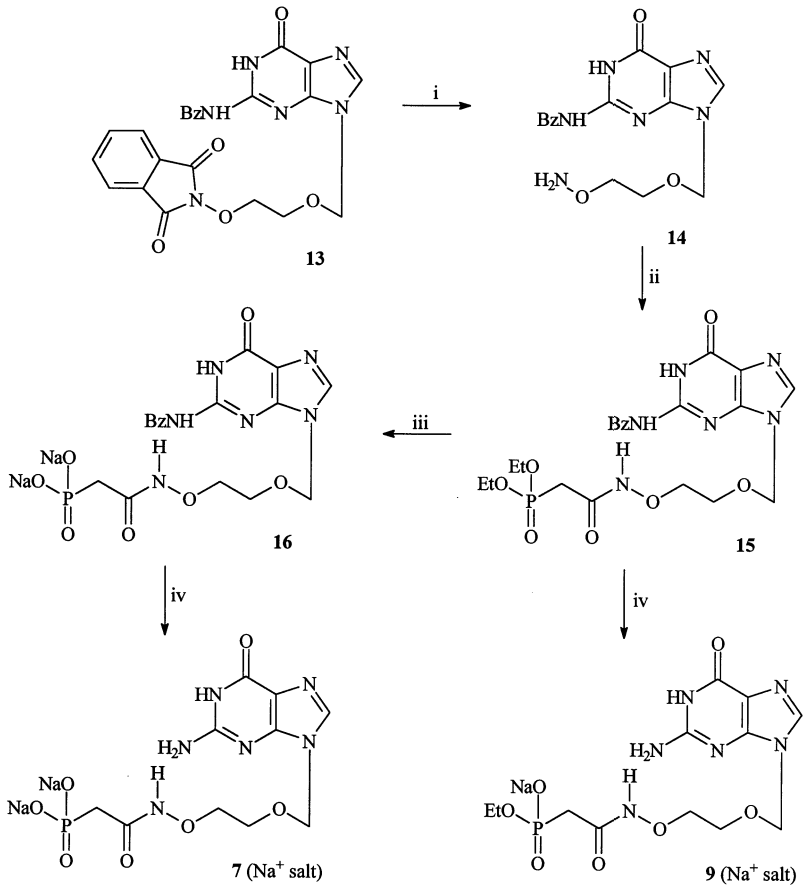
4. Experimental

4.1. Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra for comparison of compounds were taken as paraffin oil mulls or as liquid films on a Mattson 1000 FTIR spectrometer. ^{1}H NMR spectra of all compounds were obtained with a Varian Gemini-200 instrument operating at 200 MHz. For ^{1}H NMR spectra, only the most



Scheme 1. Reagents and conditions. (i) NH_2SO_2Cl, NaH, DMF, r.t., 48 h; (ii) sat. NH_3/MeOH, 0°C, 72 h.



Scheme 2. Reagents and conditions. (i) NH_2NH_2, MeOH, r.t., 1 h; (ii) diethylphosphonoacetic acid, EDC, HOBt, DMF, r.t., 24 h; (iii) (a) TMSBr, collidine, CH_2Cl_2, r.t., 12 h; (b) aqueous NaOH; (iv) NaOH, dioxane, 30°C, 24 h.

significant details are reported. Mass spectra were recorded on a VG 70-250S mass spectrometer. Analytical TLCs were carried out on 0.25-mm layer silica gel plates containing a fluorescent indicator; spots were detected under UV light (254 nm). Column chromatographies were performed using 230–400 mesh silica gel (Macherey-Nagel Silica Gel 60 Art. no. 815381). Sodium sulfate was always used as the drying agent. Evaporations were performed in vacuo (rotating evaporator).

4.1.1. Synthesis of 2-benzoylamino-9-(2-aminosulfonyloxyethoxymethyl)-1,9-dihydropurin-6-one ***(12)***

A solution of compound **11** [16] (1.40 g, 4.25 mmol) in anhydrous DMF (85 ml) was treated with 0.51 g (12.8 mmol) of NaH under nitrogen and stirred at 0°C for 15 min. Then, a solution of aminosulfonyl chloride (1.22 g, 10.6 mmol) in anhydrous THF (15 ml) was added and stirring was continued for 48 h at room temperature. The excess of NaH was quenched with EtOH (2 ml) and the resulting suspension was concentrated to give a solid which was recrystallized from MeOH, yielding 1.18 g (2.89 mmol, 68% yield) of **12** as a white solid. ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.35 (s, 1H), 11.96 (s, 1H), 8.21 (s, 1H), 8.14–7.50 (m, 7H), 5.52 (s, 2H), 4.55–4.38 (m, 2H), 3.85–3.70 (m, 2H). MS (FAB): m/z 409 $[M+H]^+$.

4.1.2. Synthesis of 2-amino-9-(2-aminosulfonyloxyethoxymethyl)-1,9-dihydropurin-6-one ***(6)***

A suspension of compound **12** (0.30 g, 0.73 mmol) in a saturated solution of ammonia in methanol (6 ml) was stirred at 0°C for 72 h. The solvent was then removed under vacuum and the residue was recrystallized from water to give compound **6** as a white solid (0.085 g, 0.28 mmol, 38% yield). ^{1}H NMR (200 MHz, Py-d_5): δ 9.58 (s, 2H), 8.16 (s, 1H), 7.98-7.89 (br, 2H), 5.67 (s, 2H), 4.74–4.68 (m, 2H), 4.10–4.05 (m, 2H). MS (FAB): m/z 305 $[M+H]^+$.

4.1.3. Synthesis of 2-benzoylamino-9-(2-aminoxyethoxymethyl)-1,9-dihydropurin-6-one ***(14)***

A solution of compound **13** [16] (5.19 g, 10.9 mmol) in methanol (500 ml) was treated with hydrazine monohydrate (0.53 ml, 11 mmol), and the resulting mixture was stirred for 1 h at room temperature. The solvent was removed under vacuum and the residue was

purified by column chromatography on silica gel, using a 30:10:1 mixture of $CHCl_3$/MeOH/NH_3 as the eluant, yielding **14** as a white solid (1.69 g, 4.91 mmol, 45% yield). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.32 (s, 1H), 11.92 (s, 1H), 8.20–7.51 (m, 6H), 5.62 (s, 2H), 4.03 (br, 2H), 3.57 (s, 4H). MS (FAB): m/z 345 $[M+H]^+$.

*4.1.4. Synthesis of 2-benzoylamino-9-[2-(O,O′-diethylphosphonoacetamidoxy)ethoxymethyl]-1,9-dihydropurin-6-one (**15**)*

A solution of diethylphosphonoacetic acid (0.063 g, 0.33 mmol) and HOBt (0.043 g, 0.32 mmol) in anhydrous DMF (13 ml) was stirred at 0°C for 30 min. Compound **14** (0.10 g, 0.29 mmol) and EDC hydrochloride (0.061 g, 0.32 mmol) were then added to this solution. The reaction mixture was then stirred at room temperature for 24 h. The solvent was removed under vacuum and the oily residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH 9:1), yielding product **15** as a white solid (0.14 g, 0.26 mmol, 90% yield). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.36 (s, 1H), 11.91 (s, 1H), 8.21–7.48 (m, 7H), 5.54 (s, 2H), 4.27–3.87 (m, 4H), 3.58–3.43 (m, 4H), 2.90 (d, $J_{HP}=22$ Hz, 2H), 1.24 (t, $J=6.8$ Hz, 6H). MS (FAB): m/z 523 $[M+H]^+$.

*4.1.5. Synthesis of the disodium salt of 2-benzoylamino-9-[2-(phosphonoacetamidoxy)-ethoxymethyl]-1,9-dihydropurin-6-one (**16**)*

A solution of **15** (0.090 g, 0.17 mmol) in anhydrous dichloromethane (1 ml) was treated under nitrogen with trimethylsilyl bromide (0.14 ml, 1.0 mmol) and 2,4,6-collidine (0.05 ml, 0.3 mmol). The resulting solution was stirred for 12 h at room temperature. The solvent was removed under vacuum and the residue was treated with a solution of NaOH (0.052 g, 1.3 mmol) in water (3 ml) for 30 min under stirring. Final removal of water under vacuum afforded a crude product which was purified by reverse-phase column chromatography on Rp18-coated silica gel, using H_2O/MeOH 95:5 as the eluant, to give **16** as the disodic salt (0.077 g, 0.15 mmol, 91% yield). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.38 (s, 1H), 11.95 (s, 1H), 8.24–7.50 (m, 7H), 5.57 (s, 2H), 3.61–3.45 (m, 4H), 2.48 (d, $J_{HP}=20$ Hz, 2H). MS (FAB, anionic): m/z 487 $[M-Na]^-$, 232 $[M-2Na]^{2-}$.

*4.1.6. Synthesis of the disodium salt of 2-amino-9-[2-(phosphonoacetamidoxy)ethoxymethyl]-1,9-dihydropurin-6-one (**7**)*

A solution of **16** (0.026 g, 0.048 mmol) in dioxane (1.3 ml) was treated with a 1 N aqueous solution of NaOH (0.3 ml, 0.3 mmol) and the resulting mixture was stirred at 30°C for 24 h. The solvent was removed under vacuum and the crude product was purified by reverse-phase column chromatography on Rp18-coated silica gel using water as the eluant, to give **7** as the disodic salt (0.015 g, 0.037 mmol, 77% yield). ^{1}H NMR (200 MHz, DMSO-d_6): δ 11.98 (s, 1H), 8.13–7.78 (m, 4H), 5.55 (s, 2H), 3.61–3.44 (m, 4H), 2.46 (d, $J_{HP}=20$ Hz, 2H). MS (FAB, anionic): m/z 383 $[M-Na]^-$, 180 $[M-2Na]^{2-}$.

*4.1.7. Synthesis of the monosodium salt of 2-amino-9-[2-(O-ethylphosphonoacetamidoxy)ethoxymethyl]-1,9-dihydropurin-6-one (**9**)*

A solution of **15** (0.200 g, 0.383 mmol) in dioxane (10 ml) was treated with a 1 N aqueous solution of NaOH (2.0 ml, 2.0 mmol) and the resulting reaction mixture was stirred at 30°C for 24 h. The solvent was then removed under vacuum and the crude product was purified by reverse-phase column chromatography on Rp18-coated silica gel, using water as the eluant, to obtain **9** as the monosodic salt (0.099 g, 0.24 mmol, 62% yield). ^{1}H NMR (200 MHz, DMSO-d_6): δ 11.91 (s, 1H), 8.15–7.81 (m, 4H), 5.54 (s, 2H), 3.93 (q, $J=7.2$ Hz, 2H), 3.60–3.45 (m, 4H), 2.19 (d, $J_{HP}=20$ Hz, 2H), 1.24 (t, $J=7.2$ Hz, 3H). MS (FAB, anionic): m/z 389 $[M-Na]^-$.

4.2. Assay of anti-HSV-1 activity

The HSV-1 strain HF, obtained from ATCC (Manassas VA), was used to infect VERO cells. These cells were maintained in MEM supplemented with 10% fetal calf serum, glutamine and gentamicin. RS cells, used to backtitrate the virus, were maintained in MEM supplemented with 10% fetal calf serum and sodium pyruvate. VERO and RS cells were originally purchased from Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia (Brescia, Italy).

The antiviral activity of compounds was evaluated in terms of inhibition of viral yield in the presence of the drugs. Briefly VERO cells were infected with HSV_{HF} at a multiplicity of infection (MOI) of 0.05, and treated with different dilutions of compounds. After 48 h, the virus released in the supernatant was harvested and then titrated on RS cells by the standard limiting dilution method (0.5 log ratio, three replicates per dilution) on 96-well microtitre plates. The infectious titre was calculated by the method of Reed and Muench [17].

4.3. Assay of anti-HIV-1 activity

The HTLV-IIIB strain of HIV-1 was derived from chronically infected H9 cells. Acute infection with HIV-1 was carried out in the CD4+ lymphoblastoid cell line C8166, containing the HTLV-I genome and expressing only the *tax* gene [18]. These cells were maintained in RPMI supplemented with 10% fetal calf serum and gentamicin.

The antiviral activity of substances was evaluated in terms of inhibition of virus yield in the presence of the drugs. Briefly C8166 cells were incubated at 0°C with HTLV-IIIB at a multiplicity of infection (MOI) of 0.001. After 72 h, the cells were subjected to three cycles of freeze–thawing; cells and cell debris were removed by low speed centrifugation and the supernatants were titrated as described previously [19] in C8166 cells by the standard limiting dilution method (0.5 log ratio, three replicates per dilution) on 96-well microtitre plates. The infectious titre, expressed as tissue culture infectious doses ($TCID_{50}$)/ml, was calculated by the method of Reed and Muench [17].

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