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Synthesis and antiviral properties of 9-[(2-methyleneaminoxyethoxy)methyl]guanine derivatives as novel Acyclovir analogues

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Abstract

This paper reports the synthesis and the antiviral properties of a series of 9-[(2-methyleneaminoxyethoxy)methyl]guanine derivatives, which can be viewed as analogues of the antiherpes drug Acyclovir (ACV) from which they differ in the replacement of its hydroxy group with variously substituted methyleneaminoxy moieties. Some of the newly synthesized compounds proved to possess a certain activity against HSV-1, albeit lower than that of ACV. © 2000 Elsevier Science S.A. All rights reserved.

Keywords: Antiviral; HSV-1; HIV-1; Acyclovir; 9-[(2-Methyleneaminoxyethoxy)methyl]guanine derivatives

1. Introduction

Modified nucleosides still represent one of the main approaches in antiviral therapy. Over the last few years, a variety of strategies have been devised to design nucleoside analogues capable of interfering with viral replication, without affecting cellular processing. These strategies include several formal modifications of the naturally occurring nucleosides, such as alterations to the heterocyclic base, or replacement of the carbohydrate moiety with acyclic, carbocyclic or more or less complex heterocyclic moieties [1].

In the majority of cases, nucleoside analogues are not active per se, but they undergo intracellular activation by phosphorylation to the bioactive triphosphate form, which then blocks viral replication through the inhibition of enzymes such as DNA polymerases or reverse transcriptases [2,3]. This particular mechanism of action is due to the presence of a hydroxy group on their sugar mimics, which simulates the 5′-hydroxy group of natural nucleosides.

However, it has also been demonstrated that some nucleoside analogues do not require this kind of biotransformation in order to exert their antiviral effect. For example, 9-(2-aminoethoxy)methylguanine (**1**), which differs from the antiherpes drug Acyclovir (ACV) in the replacement of its hydroxy group with an amino moiety, proved not to be phosphorylated to the bioactive triphosphate form, but demonstrated an appreciable antiherpes activity, even if lower than that of ACV [4]. Furthermore, the anti-HIV-1 agent HEPT [5] proved to be totally inactive against reverse transcriptases in its triphosphate form, thus suggesting that it does not need to be phosphorylated intracellularly in order to exert its inhibitory properties [6]. This result was further confirmed by a series of HEPT analogues, such as MKC-442, lacking the hydroxy group but still possessing good anti-HIV-1 activity [7,8].

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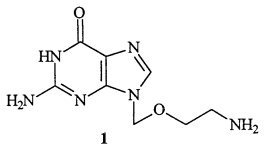

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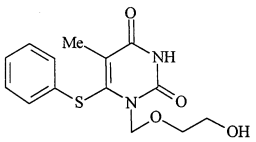
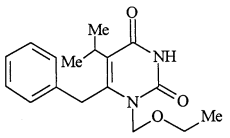
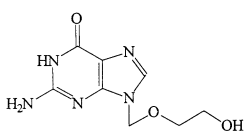


HEPT

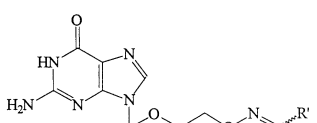


MKC-442

As a part of our search for new antiviral drugs, we report here the synthesis and the antiviral properties of a series of 9-[(2-methyleneaminoxyethoxy)methyl]-guanine derivatives (**2a**–**f**); these can be viewed as analogues of ACV, from which they differ in the replacement of its hydroxy group with variously substituted methyleneaminoxy moieties, with the result that they cannot be phosphorylated to the bioactive triphosphate form. This oxime functionality was chosen in view of its versatility in making it possible to modulate the steric and lipophilic characteristics of the compounds through the insertion of appropriate substituents on the oximic carbon.



ACV



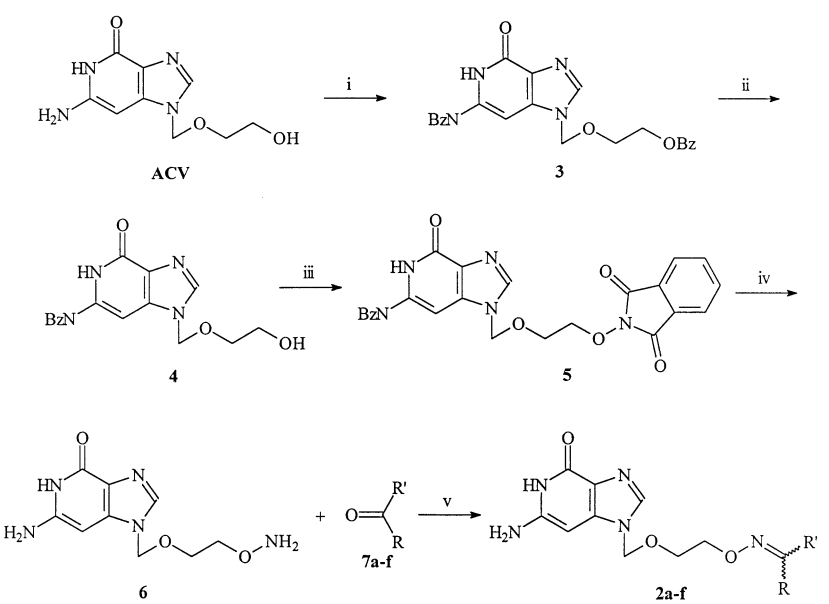
2a-f

a, R = H, R' = Et; **b**, R = R' = Me; **c**, R = Me, R' = Et; **d**, R, R' = *c*-C_4H_8; **e**, R = H, R' = Ph; **f**, R = Me, R' = Ph

2. Chemistry

The 9-[(2-methyleneaminoxyethoxy)methyl]guanine derivatives **2a**–**f** were prepared using the synthetic procedure shown in Scheme 1. Reaction of ACV with benzoyl chloride in anhydrous pyridine afforded the dibenzoyl derivative **3**. Subsequent partial hydrolysis of the most sensitive benzoic ester group in **3** with MeONa–MeOH produced the alcohol **4**, which was transformed into the *N*-alkoxy-phthalimide (**5**) upon reaction with *N*-hydroxyphthalimide in the presence of triphenylphosphine and diethylazodicarboxylate (DEAD). Hydrazinolysis of **5** with hydrazine in refluxing methanol caused the removal of both the phthalimido and the *N*-benzoyl groups, affording the completely deprotected intermediate **6**. Compound **6** was then reacted with carbonyl compounds **7a**–**f** in methanol, producing the target molecules **2a**–**f** in moderate-to-good yields. In the case of compounds **2a**,**c**,**e**,**f**, in which the possibility of the *E*/*Z* isomerism exists, compounds **2c** and **2f** were obtained as *E*/*Z* diastereomeric mixtures in a ratio of 73:27 and 86:14, respectively, while compounds **2a** and **2e** consisted only of the diastereomer possessing the *E* configuration.

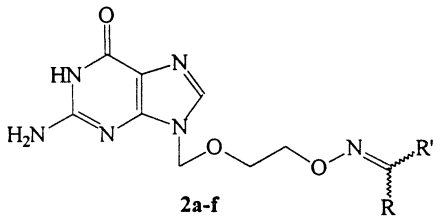
The configuration around the N=C double bond of **2a**,**c**,**e**,**f** was assigned by ^{1}H NMR spectrometry [9]. For both compounds **2c** and **2f**, in the diastereomer with the *E* configuration, the methyl group linked to the carbon of the oximethereal portion resonates at lower fields with respect to the corresponding methyl group of the diastereomer with the *Z* configuration, due to the para-



a, R = H, R' = Et; **b**, R = R' = Me; **c**, R = Me, R' = Et; **d**, R, R' = *c*-C_4H_8; **e**, R = H, R' = Ph; **f**, R = Me, R' = Ph

Scheme 1. Reagents and conditions. (i) Benzoyl chloride, Py, 40°C, 4 h. (ii) MeONa, MeOH, r.t., 2 h. (iii) *N*-Hydroxyphthalimide, triphenylphosphine, diethylazodicarboxylate, Py, r.t., 48 h. (iv) Hydrazine monohydrate, MeOH, reflux, 24 h. (v) MeOH, r.t., 24 h.

Table 1
Antiherpes activity of compounds **2a**–**f** against HSV-1



2a-f

Comp.	R	R′	% Inhibition (10 μM)	IC_{50} (μM)
2a	H	Et	50	10
2b	Me	Me	0	
2c	Me	Et	55	7.3
2d		*c*-C_4H_8	60	5.1
2e	H	Ph	0	
2f	Me	Ph	40	
Acyclovir				0.05

magnetic effect of the spatially proximal oximethereal oxygen. In the case of **2a** and **2e** the *E* configuration was assigned on the basis of the low-field resonance of the proton linked to the oximic carbon, with chemical shift values in agreement with those reported in literature for oximethereal compounds with an analogous configuration [9].

3. Biological results

Compounds **2a**–**f** were tested for their antiviral activity against herpes virus type 1 (HSV-1), using VERO cells infected with the HSV-1 strain HF. The results obtained are shown in Table 1, together with the result for ACV, taken as the reference drug. The antiherpes activity was expressed as the percentage inhibition of viral replication at a concentration of 10 μM and, for compounds possessing an inhibition percentage $\geq 50\%$, also as IC_{50}.

Compounds **2a**,**c**,**d**,**f** showed a certain antiherpes activity, albeit two orders of magnitude lower than ACV, with a percentage inhibition of viral replication varying from 40 up to 60%; in particular compound **2d** proved to be the most active of the series with an IC_{50} value of 5.1 μM. Compounds **2b**,**e**, in which the oximic carbon is either symmetrically (**2b**) or asymmetrically substituted (**2e**), at the concentration used, proved to be inactive.

Furthermore, compounds **2a**–**f** were also tested 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 inhibitory properties.

4. Conclusions

The results obtained on HSV-1 strains indicate that, at least for this type of virus, the replacement of the hydroxy group of ACV with methyleneaminoxy moieties leads to compounds, like **2a**,**c**,**d**,**f**, still possessing a certain antiherpes activity, albeit lower than that of ACV.

The fact that these compounds show a certain activity, even if they lack the hydroxy group responsible for the bioactivation of ACV to its triphosphate form, might lead us to hypothesize that the guanine derivatives of type **2** might exert their antiherpes activity thanks to a mode of action different from that of ACV. However, this hypothesis needs to be confirmed by specific studies, in order to clarify the mechanism of action of these new guanine derivatives.

A comparison between the structure and the activity of compounds **2a**–**f** does not allow us to make any hypothesis about the influence of the substituents linked to the methyleneaminoxy moiety on the antiherpes activity.

5. Experimental

5.1. 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 in DMSO-d_6 solution. The proton magnetic resonance assignments were established on the basis of the expected chemical shifts and the multiplicity of the signals. For ^{1}H NMR spectra, only the most significant details are reported. Mass spectra were recorded on an HP-5988 A spectrometer. Analytical TLC was carried out on 0.25-mm layer silica gel plates containing a fluorescent indicator; spots were detected under UV light (254 nm). Column chromatography was performed using 230–400 mesh silica gel (Macherey–Nagel silica gel 60 Art. Nr. 815381). Sodium sulfate was used as the drying agent in all cases. Evaporations were performed in vacuo (rotating evaporator).

*5.1.1. Synthesis of 2-benzoylamino-9-[2-(benzoyloxy)-ethoxymethyl]-1,9-dihydro-purin-6-one (**3**)*

A suspension of Acyclovir (ACV) (10.0 g, 44.8 mmol) in anhydrous pyridine (250 ml) was treated with benzoyl chloride (26.0 ml, 224 mmol). The resulting mixture was stirred under argon at 40°C for 4 h. Pyridine was removed under vacuum, leaving a residue, which was diluted with $CHCl_3$ (500 ml). The organic phase was washed with a pre-cooled (0°C) 5% HCl solution (3×100 ml), saturated solution of $NaHCO_3$ (3×100 ml), and brine (3×100 ml); it was dried over Na_2SO_4 and concentrated under vacuum. The brownish solid residue was recrystallized from Et_2O, giving **3** (12.0 g, 27.8 mmol, 62% yield) as a white solid. M.p. 167°C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.29 (s, 1H), 11.96 (s, 1H), 8.20 (s, 1H), 8.01 (d, $J = 8.3$ Hz, 2H), 7.81 (d, $J = 8.3$ Hz, 2H), 7.68–7.45 (m, 6H), 5.56 (s, 2H), 4.36 (t, $J = 8.7$ Hz, 2H), 3.87 (t, $J = 8.8$ Hz, 2H). MS (EI, 70 eV): m/z 434 $[M + H]^+$.

*5.1.2. Synthesis of 2-benzoylamino-9-(2-hydroxyethoxy-methyl)-1,9-dihydro-purin-6-one (**4**)*

A suspension of compound **3** (15.8 g, 36.4 mmol) in anhydrous methanol (600 ml) was added to a solution of sodium methoxide (6.30 g, 116 mmol) in anhydrous methanol (300 ml). The resulting suspension was stirred at room temperature (r.t.) under argon for 2 h. The solvent was removed under vacuum and the residue was recrystallized from $CHCl_3$–MeOH, affording **4** (9.22 g, 28.0 mmol, 77% yield) as a white solid. M.p. 197–198°C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.39 (s, 1H), 11.98 (s, 1H), 8.15 (s, 1H), 8.05 (d, $J = 8.2$ Hz, 2H), 7.65–7.54 (m, 3H), 5.52 (s, 2H), 4.65 (br, 1H), 3.57-3.42 (m, 4H). MS (EI, 70 eV): m/z 330 $[M + H]^+$.

*5.1.3. Synthesis of 2-benzoylamino-9-[2(phthalimidoxy)-ethoxymethyl]-1,9-dihydro-purin-6-one (**5**)*

A suspension of compound **4** (0.432 g, 1.31 mmol) in anhydrous pyridine (20 ml) was treated with *N*-hydroxyphthalimide (0.215 g, 1.31 mmol), triphenylphosphine (0.690 g, 2.63 mmol) and diethylazodicarboxylate (0.503 g, 2.89 mmol). The resulting mixture was stirred at r.t. under argon for 48 h, until starting material **4** had completely reacted (determined by analytical TLC). Pyridine was removed under vacuum and the residue was purified by column chromatography (95:5 $CHCl_3$–MeOH), affording **5** (0.085 g, 0.28 mmol, 38% yield) as a white solid. M.p. 231°C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.35 (s, 1H), 11.97 (s, 1H), 8.15 (s, 1H), 8.04–7.99 (m, 2H), 7.79–7.63 (m, 5H), 7.54–7.46 (m, 2H), 5.52 (s, 2H), 4.28–4.26 (m, 2H), 3.85–3.84 (m, 2H). MS (EI, 70 eV): m/z 475 $[M + H]^+$.

*5.1.4. Synthesis of 2-amino-9-(2-aminoxyethoxy-methyl)-1,9-dihydro-purin-6-one (**6**)*

A suspension of compound **5** (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 refluxed for 24 h. The solvent was removed under vacuum and the residue was purified by column chromatography (6:1 $CHCl_3$–MeOH), affording **6** (1.07 g, 4.47 mmol, 41% yield) as a white solid. M.p. 250°C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.18 (s, 1H), 7.78 (s, 1H), 6.63 (br, 2H), 5.32 (s, 2H), 4.06 (br, 2H), 3.36 (s, 4H). MS (FAB): m/z 241 $[M + H]^+$.

*5.1.5. General procedure for preparation of compounds **2a–f***

A suspension of amine **6** (0.134 g, 0.568 mmol) in methanol (10 ml) was treated with five equivalents of the appropriate carbonyl compound **7a–f**. The resulting mixture was stirred at r.t. for 24 h. The solvent was removed under vacuum and the crude product was purified by column chromatography ($CHCl_3$–MeOH) to yield compounds **2a–f**.

2a (73%): M.p. 223°C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 11.07 (s, 1H), 7.82 (s, 1H), 7.63 (s, 1H), 6.65 (br, 2H), 5.28 (s, 2H), 3.91 (t, $J = 5.1$ Hz, 2H), 3.66 (t, $J = 5.1$ Hz, 2H), 2.14 (q, $J = 7.3$ Hz, 2H), 0.91 (t, $J = 7.3$ Hz, 3H). MS (FAB): m/z 281 $[M + H]^+$.

2b (70%): M.p. 176-178°C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.00 (s, 1H), 7.77 (s, 1H), 6.63 (br, 2H), 5.32 (s, 2H), 3.94 (t, $J = 5.1$ Hz, 2H), 3.64 (t, $J = 5.1$ Hz, 2H), 1.72 (s, 3H), 1.68 (s, 3H). MS (FAB): m/z 281 $[M + H]^+$.

2c (*E*-isomer, 47%; *Z*-isomer, 17%): ^{1}H NMR (200 MHz, DMSO-d_6): δ 10.62 (s, 1H), 7.78 (s, 1H), 6.50 (br, 2H), 5.31 (s, 2H), 3.97 (t, $J = 5.0$ Hz, 2H), 3.63 (t, $J = 5.0$ Hz, 2H), 2.10 (q, $J = 7.4$ Hz, 2H, CH_3CH_2– *Z*-isomer), 2.07 (q, $J = 7.5$ Hz, 2H, CH_3CH_2- *E*-isomer), 1.71 (s, 3H, CH_3– *E*-isomer), 1.65 (s, 3H, CH_3– *Z*-isomer), 0.93 (t, $J = 7.4$ Hz, 3H, CH_3CH_2– *Z*-isomer), 0.89 (t, $J = 7.5$ Hz, 3H, CH_3CH_2– *E*-isomer). MS (FAB): m/z 295 $[M + H]^+$.

2d (58%): M.p. 235°C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 10.71 (s, 1H), 7.79 (s, 1H), 6.56 (br, 2H), 5.31 (s, 2H), 3.96 (t, $J = 4.9$ Hz, 2H), 3.63 (t, $J = 4.9$ Hz, 2H), 2.16 (m, 4H), 1.61 (m, 4H). MS (FAB): m/z 307 $[M + H]^+$.

2e (79%): M.p. 218°C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 10.62 (s, 1H), 8.18 (s, 1H), 7.82 (s, 1H), 7.56 (m, 2H), 7.40 (m, 3H), 6.50 (br, 2H), 5.36 (s, 2H), 4.18 (t, $J = 4.7$ Hz, 2H), 3.73 (t, $J = 4.7$ Hz, 2H). MS (FAB): m/z 329 $[M + H]^+$.

2f (*E*-isomer, 56%; *Z*-isomer, 9%): ^{1}H NMR (200 MHz, DMSO-d_6): δ 10.59 (s, 1H), 7.81 (s, 1H), 7.58 (m, 2H), 7.38 (m, 3H), 6.49 (br, 2H), 5.36 (s, 2H), 4.18 (t, $J = 4.6$ Hz, 2H), 3.75 (t, $J = 4.6$ Hz, 2H), 2.10 (s, 3H, CH_3– *E*-isomer), 2.07 (s, 3H, CH_3- *Z*-isomer). MS (FAB): m/z 343 $[M + H]^+$.

5.1.6. 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 back-titrate 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 the compounds was evaluated in terms of the 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 [10].

5.1.7. 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 [11]. These cells were maintained in RPMI supplemented with 10% fetal calf serum and gentamicin.

The antiviral activity of the substances was evaluated in terms of the 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. Later (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 previously described [12] 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 [10].

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