European Journal of Medicinal Chemistry 44 (2009) 3313–3317

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Synthesis and anti-VZV activity of 6-heteroaryl derivatives of tricyclic acyclovir and 9-{[*cis*-1′,2′-bis(hydroxymethyl)cycloprop-1′-yl]methyl}guanine analogues

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ARTICLE INFO

Article history:
Received 3 November 2008
Received in revised form
4 March 2009
Accepted 6 March 2009
Available online 21 March 2009

Keywords:
Acyclovir
A-5021
6-Heteroaryl tricyclic analogues
Synthesis
Anti-VZV activity

ABSTRACT

A series of tricyclic analogues of acyclovir and 9-{[*cis*-1′,2′-bis(hydroxymethyl)cycloprop-1′-yl]methyl}guanine substituted in the 6 position with thien-2-yl, 5-bromothien-2-yl or furan-2-yl group were synthesized. The new compounds **5a–f** were evaluated for their activity in vitro against varicella-zoster virus (VZV) and cytomegalovirus (CMV). The marked anti-VZV activities of **5a–f** remained comparable to those of their previously described 6-phenyl-substituted counterparts.

1. Introduction

Purine and pyrimidine derivatives including nucleoside analogues, bearing thienyl or furanyl substituents, have been reported to exhibit interesting biological properties. 5-(Thien-2-yl)- and 5-(furan-2-yl)-2′-deoxyuridine and 5-(thien-2-yl)-1-(β-D-arabinofuranosyl)uracil have been found to exhibit marked activity against herpes simplex virus type 1 (HSV-1). Further substitution of the heterocyclic ring of 5-(thien-2-yl)-2′-deoxyuridine has resulted in 5-halogenated analogues, equipotent to (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU, brivudin) against HSV-1 and significantly active against varicella-zoster virus (VZV) [1,2]. 8-(Thien-2-yl)adenosine displayed at least a 100-fold improvement, as compared with the lead compound adenosine, in inhibition of glycosomal glyceraldehyde 3-phosphate dehydrogenase of *Trypanosoma brucei* and *Leishmania mexicana* [3]. 2-(Furan-2-yl)- and 2-(thien-2-yl)-5,6-dihydroxy-4-carboxypyrimidine, and especially the latter one, additionally modified by substituted urea present in the 3 position of thiophene ring, have been described as potent inhibitors of the hepatitis C virus (HCV) NS5B RNA-dependent RNA polymerase [4]. 6-(Furan-2-yl)- and 6-(thien-2-yl)-9-(β-D-ribofuranosyl)purine have shown anti-HCV activity exceeding that of 2′-C-methyladenosine [5]. 6-(Furan-2-yl)- and 6-(thien-2-yl)-9-(4-methoxybenzyl)purines, carrying small hydrophobic substituents in the 2 position, have been identified as potent inhibitors of *Mycobacterium tuberculosis*, comparable in terms of activity to rifampin [6,7]. In all the above-mentioned cases the activities of heteroaryl compounds proved superior to those of their phenyl counterparts.

We have previously modified the guanine part of antiviral drugs acyclovir (ACV, **1a**) and ganciclovir (GCV, **1b**) by introduction of a 1,N^2-(ethene-1,2-diyl) bridge to form the tricyclic analogues (TACV, **2a** and TGCV, **2b**) as derivatives of 3,9-dihydro-9-oxo-5*H*-imidazo[1,2-*a*]purine (Fig. 1). Generally, as is reviewed [8], the substituents in the appended ring of TACV and TGCV enabled us to modulate the biological and physical properties of the compounds. Out of an early series of tricyclic analogues, 6-phenyl-TACV (**2c**) and its TGCV counterpart **2d** exhibited activities similar to those of parent **1a** and **1b**, as well as relatively strong fluorescence properties [9]. Therefore they have been selected as the lead compounds for the synthesis of further analogues, modified in different positions of the phenyl ring and/or 6,7-disubstituted [10–12]. We have subsequently extended our studies to tricyclic analogues of 9-{[*cis*-1′,2′-bis(hydroxymethyl)cycloprop-1′-yl]methyl}guanine (**3a**). The 1′*S*,2′*R* enantiomer of **3a** (A-5021, **3b**) has been described as more active than ACV against HSV-1, HSV-2 and VZV [13,14], more cytostatic and selective than GCV against HSV-1 thymidine kinase (TK) gene-transfected human osteosarcoma cells

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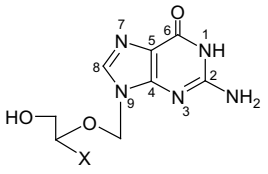
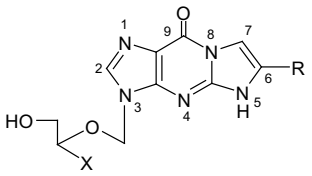
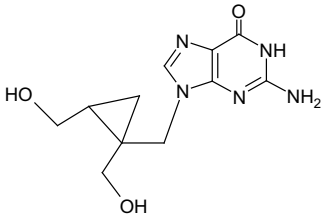
doi:10.1016/j.ejmech.2009.03.005



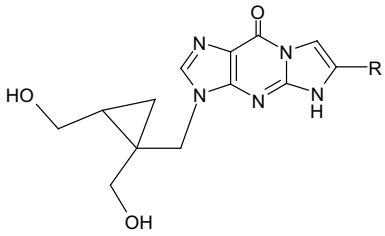
1a ACV X = H
1b GCV X = CH_2OH



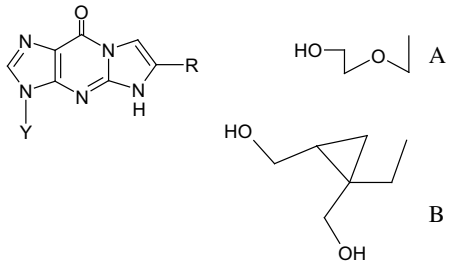
2a X = H, R = H
2b X = CH_2OH, R = H
2c X = H, R = Ph
2d X = CH_2OH, R = Ph



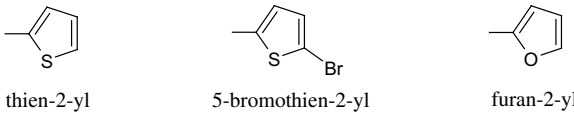
3a[a] RA-5021
3b[b] A-5021



4a[a] R = Ph
4b[a] R = 4-MeOPh



5a Y = A, R = thien-2-yl
5b Y = B[a], R = thien-2-yl
5c Y = A, R = 5-bromothien-2-yl
5d Y = B[a], R = 5-bromothien-2-yl
5e Y = A, R = furan-2-yl
5f Y = B[a], R = furan-2-yl

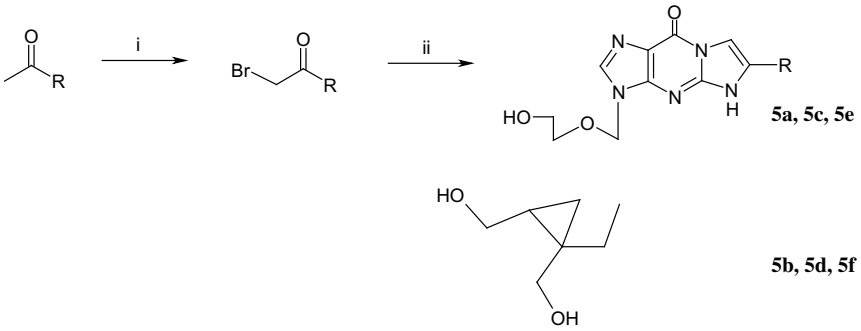


thien-2-yl 5-bromothien-2-yl furan-2-yl

Fig. 1. Structures of compounds discussed. [a] The mixture of 1′*S*,2′*R* and 1′*R*,2′*S* forms. [b] 1′*S*,2′*R* form.

[15], and less toxic to bone marrow cells as compared with GCV [16]. The racemic form (RA-5021) exhibited only slightly lower antiviral potency than A-5021 [13], whereas the synthesis of RA-5021 is much less expensive. Among tricyclic analogues of RA-5021 tested, we found that the antiviral activities of the fluorescent 6-phenyl-T(RA-5021) (**4a**) and 6-(4-methoxyphenyl)-T(RA-5021) (**4b**) were from 2- to 8-fold higher than those of ACV [17]. Compounds **4a** and **4b** virtually retained the potent activity of the parent **3a** against VZV.

Bearing in mind the antiviral activity of compounds **2c**, **2d**, **4a** and **4b**, we examined the effects of heteroaryl substituents attached to the 6 position of the tricycle. Herein we present the synthesis and the results of anti-VZV evaluations of 6-(thien-2-yl), 6-(5-bromothien-2-yl) and 6-(furan-2-yl) derivatives of TACV and T(RA-5021).



5a, 5c, 5e

5b, 5d, 5f

Scheme 1. Schematic synthesis of compounds **5a**–**f**. Reagents and conditions: (i) Br_2, $AlCl_3$, THF, 0 °C; (ii) **1a** or **3a**, NaH, DMF, r.t., then NH_4OH, r.t.

2. Chemistry

All 6-substituted tricyclic analogues were synthesized by an alkylation–condensation reaction of ACV or RA-5021 with the appropriate bromoketones, according to a previously described method for other tricyclic derivatives [9–12,17] (Scheme 1). Compounds **5a**–**f** were characterized by ^{1}H and ^{13}C NMR, and by elemental analysis. The bromination of ketones was carried out using bromine in tetrahydrofuran solution, in the presence of aluminium chloride [18]. The ^{1}H NMR data of the resulting 2-(2-bromoacetyl)thiophene, 2-(2-bromoacetyl)-5-bromothiophene and 2-(2-bromoacetyl)furan were in good agreement with data reported earlier for these bromoketones prepared by diverse methods [19–21].

3. Antiviral evaluations

The newly synthesized compounds **5a**–**f** as well as compound **3a** and other pertinent standards were screened for their inhibitory effect on the replication of VZV (strains YS and OKA), VZV TK^- (TK-deficient VZV, strain 07/1) and cytomegalovirus (CMV, strains AD-169 and Davis) in human embryonic lung (HEL) cell cultures.

4. Results and discussion

All tested tricyclic analogues of ACV and RA-5021 exhibited slightly decreased anti-VZV activity as compared with the respective parent bicyclic compounds **1a** and **3a** (Table 1). We have previously described the reduction of activity for 6-phenyl-TACV (**2c**) in comparison with ACV to be 2.5-fold against YS strain and 5-fold against OKA strain [9]. We found now a similar i.e. 3- to 9-fold reduction for its 6-heteroaryl congeners **5a**, **5c** and **5e** in comparison with ACV against the same strains. 6-Phenyl-T(RA-5021) (**4a**) was found to be only 2- and 3-fold less active than RA-5021 against the YS and OKA strains, respectively [17]. The activities of its 6-heteroaryl analogues **5b**, **5d** and **5f** likewise showed 1.3- to 3-fold lower activity than the parent **3a**. Only the activity of **5d** against OKA strain was a little more reduced (7-fold). In summary, it may be concluded that the replacement of a 6-phenyl substituent by 6-heteroaryl ones in the tricyclic (T) derivatives of ACV and RA-5021 did not bring about a significant change in anti-VZV activity.

Compound **3a** was shown to be 7- and 14-fold more active than ACV against VZV YS and OKA strains, respectively. The 1′*S*,2′*R* enantiomer of **3a** (A-5021, **3b**) has been reported previously to exceed ACV 20- to 30-fold in activity against both VZV strains in HEL cells [15]. From these data the strong similarity between **3a** and **3b** in inhibiting VZV replication may be indirectly postulated. The new tricyclic analogues derived from **3a** (i.e. **5b**, **5d** and **5f**) exhibited 2- to 6-fold more potent anti-VZV activity than ACV. The activities of all compounds against the TK^- deficient 07/1 VZV strain were, as expected, much lower than those against the TK-positive strains. Compounds **3a** and **5a**–**f** were also inactive against CMV (AD-169 and Davis strains).

The 6-(thien-2-yl) and 6-(furan-2-yl) compounds **5a**, **5b**, **5e** and **5f** retained the fluorescence properties previously observed for the 6-phenyl derivatives **2c**, **2d** and **4a**, although the intensities were generally somewhat reduced. Only 6-(5-bromothien-2-yl) compounds **5c** and **5d** were virtually not fluorescent.

The antiherpetic activity of nucleoside analogues is principally dependent on their affinities for the phosphorylating enzymes, the intracellular stability of triphosphates formed and the inhibitory effects of the latter on the viral DNA polymerases. Virus-specific thymidine kinases are key enzymes in the metabolic activation of these agents in the virus-infected cells. The diverse affinities of 5-substituted 2′-deoxyuridine derivatives for HSV-1 TK have been demonstrated to result from geometric and electronic factors that influence the energies of interaction with the enzyme and from the hydrophobicity of the substituents [22]. The flexibility of the compounds which facilitates binding within the active site also is of considerable importance [23]. The same combination of factors most likely contributes to the affinity for herpesviral TKs of the 6-substituted and 6,7-disubstituted tricyclic analogues of ACV, GCV and RA-5021 described so far. Prediction of their affinity for TKs, or other metabolic enzymes, and for the target viral DNA polymerase, seems to be difficult. At this stage, the best way to assess the antiviral activity of new tricyclic derivatives of either ACV, GCV or RA-5021, is to evaluate them empirically.

5. Experimental protocols

5.1. Chemistry

Melting points were determined on MEL-TEMP II capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Microanalytical Laboratories of the A. Mickiewicz University, Poznan. Fluorescence spectra were measured on a Perkin Elmer MPF-3 fluorescence spectrophotometer (excitation at 305 nm); quantum yields were calculated relative to quinine sulphate in 0.1 N H_2SO_4 as a standard ($\phi_F = 0.52$). ^{1}H and ^{13}C NMR spectra were recorded on a Unity 300 Varian spectrometer operating at 299.95 MHz; tetramethylsilane was used as

Table 1
Antiviral activity and cytotoxicity of compounds **3a** and **5a**–**f** and of reference drugs.

Compound	EC_{50}[a] (μM)			EC_{50}[b] (μM)		MCC (μM)[c]	CC_{50}[d] (μM)
	VZV (YS)	VZV (OKA)	TK^- VZV (07/1)	CMV (AD-169)	CMV (Davis)		
3a	0.3	0.13	25.8	100	44.7	>100	>100
5a	7.9	5.8	>100	>100	100	>100	>100
5b	0.38	0.32	71.5	>100	50.2	>100	>100
5c	10	8.7	>100	>100	100	>100	>100
5d	0.87	0.93	>100	>100	76.5	>100	>100
5e	–	16.4	>100	>100	>100	>100	>100
5f	0.64	0.42	>100	>100	>100	>100	>100
1a (ACV)	2.0	1.8	62.2	178	45	>1778	782
1b (GCV)	1.1	0.59	2.5	12.6	2.5	>1575	221
Brivudin	0.026	0.015	157	–	–	>1201	432
Cidofovir	–	–	–	1.6	0.41	≥254	54

[a] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 20 plaque forming units (PFUs).
[b] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFUs).
[c] Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.
[d] Cytotoxic concentration required to reduce cell growth by 50%.

the internal standard; the chemical shifts are reported in ppm (δ scale). Thin-layer chromatography (TLC) was performed on Merck precoated 60 F_{254} gel plates. Column chromatography was carried out on Merck silica gel 60H (40–63 μm).

5.1.1. 2-(2-Bromoacetyl)thiophene

Purified by silica gel column chromatography using toluene for elution, followed by rechromatography using hexane–EtOAc 9:1 to give colorless oil in 73% yield. ^{1}H NMR ($CDCl_3$) δ 7.84 (dd, 1H, 3′-*H*, $J = 4.0$, 1.2 Hz), 7.75 (dd, 1H, 5′-*H*, $J = 5.2$, 1.2 Hz), 7.20 (dd, 1H, 4′-*H*, $J = 5.2$, 4.0 Hz), 4.39 (s, 2H, CH_2Br).

5.1.2. 2-(2-Bromoacetyl)-5-bromothiophene

Chromatographed on silica gel column using toluene to obtain white foam in 79% yield. ^{1}H NMR ($CDCl_3$) δ 7.55 (d, 1H, 3′-*H*, $J = 3.9$ Hz), 7.14 (d, 1H, 4′-*H*, $J = 3.9$ Hz), 4.28 (s, 2H, CH_2Br).

5.1.3. 2-(2-Bromoacetyl)furan

Purified by silica gel column chromatography using toluene–MeOH 99:1 → 98:2 for elution to obtain colorless oil in 75% yield. ^{1}H NMR ($CDCl_3$) δ 7.64 (dd, 1H, 5′-*H*, $J = 1.8$, 0.6 Hz), 7.34 (dd, 1H, 3′-*H*, $J = 3.6$, 0.6 Hz), 6.60 (dd, 1H, 4′-*H*, $J = 3.6$, 1.8 Hz), 4.32 (s, 2H, CH_2Br).

5.1.4. General procedure for the alkylation–condensation reactions

To a suspension of **1a** or **3a** (0.5 mmol) in anhydrous DMF (10 ml) was added sodium hydride as 60% suspension in oil (1.5 eq). After being stirred with exclusion of moisture for 1–2 h at room temperature, the resulting solution was treated with bromoketone (1.2 eq). The reaction mixture was stirred at room temperature for the next 2 h, made alkaline by addition of 25% aqueous ammonia (10 ml) and left overnight. The volatiles were evaporated and the oily residue was chromatographed on silica gel column using CH_2Cl_2–MeOH (8:1 → 7:1 or 8:1 → 6:1). Fractions containing the main product were evaporated, whereas the residual solid was subjected to further work-up.

*5.1.5. 3,9-Dihydro-3-[(2-hydroxyethoxy)methyl]-9-oxo-6-(thien-2-yl)-5*H*-imidazo[1,2-*a*]purine (**5a**)*

Crude product after chromatography (94 mg, 57% yield) was recrystallized from MeOH at +5 °C to afford colorless crystalline material: mp 240–242 °C (dec). Fluorescence emission (H_2O): λ_{max} 393 nm; $\phi_F = 0.075$. ^{1}H NMR (DMSO-d_6) δ 13.21 (br s, 1H, N*H*), 8.06 (s, 1H, C2–*H*), 7.94 (s, 1H, C7–*H*), 7.67 (dd, 1H, 5′-*H*, $J = 5.1$, 1.2 Hz), 7.62 (dd, 1H, 3′-*H*, $J = 3.6$, 1.2 Hz), 7.18 (dd, 1H, 4′-*H*, $J = 5.1$, 3.6 Hz), 5.51 (s, 2H, N–CH_2–O), 4.69 (t, 1H, O*H*), 3.45–3.56 (m, 4H, CH_2–CH_2). ^{13}C NMR (DMSO-d_6) δ 151.12 (*C*-9), 150.34 (*C*-3a), 146.14 (*C*-4a), 139.36 (*C*-2), 129.87, 124.32 (*C*-6, *C*-2′), 128.09 (*C*-4′), 127.08, 126.02 (*C*-3′, *C*-5′), 115.47 (*C*-9a), 102.38 (*C*-7), 72.36 (N–CH_2–O), 70.59 (HO–CH_2–CH_2), 59.90 (HO–CH_2). Anal. Calcd for $C_{14}H_{13}N_5O_3S$: C 50.75, H 3.95, N 21.14, S 9.68; found: C 50.52, H 4.15, N 20.97, S 9.35.

*5.1.6. 3-{[*cis*-1′,2′-Bis(hydroxymethyl)cycloprop-1′-yl]methyl}-3,9-dihydro-9-oxo-6-(thien-2-yl)-5*H*-imidazo[1,2-*a*]purine (**5b**)*

Crude solid (104 mg, 56% yield) was recrystallized from MeOH at room temperature to obtain colorless spangles: mp 295–298 °C (dec). Fluorescence emission (H_2O): λ_{max} 410 nm; $\phi_F = 0.030$. ^{1}H NMR (DMSO-d_6) δ 13.12 (br s, 1H, N*H*), 7.98 (s, 1H, C2–*H*), 7.91 (s, 1H, C7–*H*), 7.66 (dd, 1H, 5′-*H*, $J = 5.1$, 1.2 Hz), 7.62 (dd, 1H, 3′-*H*, $J = 3.6$, 1.2 Hz), 7.17 (dd, 1H, 4′-*H*, $J = 5.1$, 3.6 Hz), 4.69 (t, 1H, O*H*), 4.59 (t, 1H, O*H*), 4.15 (d, 1H, N–C*H*H–C1″, $J = 14.1$ Hz), 4.01 (d, 1H, N–CH*H*–C1″, $J = 14.4$ Hz), 3.61 (m, 1H, C2″–C*H*H–OH), 3.47 (dd, 1H, C2″–CH*H*–OH, $J = 12.0$, 6.0 Hz), 3.28–3.42 (m, 2H, C1″–C*H*H–OH), 1.32 (m, 1H, C2″–*H*), 0.93 (dd, 1H, C3″–*H*H, $J = 8.7$, 5.1 Hz), 0.44 (t, 1H, C3″–H*H*, $J = 5.1$ Hz). ^{13}C NMR (DMSO-d_6) δ 151.14 (*C*-9), 150.50 (*C*-3a), 145.95 (*C*-4a), 139.27 (*C*-2), 129.96, 124.19 (*C*-6, *C*-2′), 128.10 (*C*-4′), 126.99, 125.96 (*C*-3′, *C*-5′), 115.29 (*C*-9a), 102.24 (*C*-7), 60.84, 60.65 (C1″–*C*H_2–OH, C2″–*C*H_2–OH), 47.74 (C1″–*C*H_2–N), 26.82 (*C*-1″), 24.44 (*C*-2″), 13.94 (*C*-3″). Anal. Calcd for $C_{17}H_{17}N_5O_3S \cdot 0.25\ H_2O$: C 54.32, H 4.69, N 18.63, S 8.53; found: C 54.50, H 4.69, N 18.56, S 8.45.

*5.1.7. 6-(5-Bromothien-2-yl)-3,9-dihydro-3-[(2-hydroxyethoxy)methyl]-9-oxo-5*H*-imidazo[1,2-*a*]purine (**5c**)*

Product isolated by chromatography (138 mg, 67% yield) was subjected to crystallization from MeOH–EtOAc 4:1 to afford yellowish material. It was next recrystallized from MeOH at room temperature resulting in white crystals: mp 226–227 °C (dec). Fluorescence emission (H_2O): λ_{max} 414 nm; $\phi_F = 0.007$. ^{1}H NMR (DMSO-d_6) δ 13.24 (br s, 1H, N*H*), 8.06 (s, 1H, C2–*H*), 8.04 (s, 1H, C7–*H*), 7.44, 7.32 (2×d, 2H, 3′-*H*, 4′-*H*, $J = 3.9$, 3.9 Hz), 5.50 (s, 2H, N–CH_2–O), 4.68 (t, 1H, O*H*), 3.44–3.56 (m, 4H, CH_2–CH_2). ^{13}C NMR (DMSO-d_6) δ 151.11 (*C*-9), 150.38 (*C*-3a), 146.15 (*C*-4a), 139.43 (*C*-2), 131.72, 123.22 (*C*-6, *C*-2′), 131.38 (*C*-4′), 126.54 (*C*-3′), 115.48 (*C*-9a), 112.19 (*C*-5′), 103.26 (*C*-7), 72.37 (N–*C*H_2–O), 70.61 (HO–CH_2–*C*H_2), 59.91 (HO–*C*H_2). Anal. Calcd for $C_{14}H_{12}N_5O_3SBr \cdot 0.25\ H_2O$: C 40.54, H 3.04, N 16.89, S 7.73; found: C 40.68, H 3.20, N 16.58, S 7.78.

*5.1.8. 3-{[*cis*-1′,2′-Bis(hydroxymethyl)cycloprop-1′-yl]methyl}-6-(5-bromothien-2-yl)-3,9-dihydro-9-oxo-5*H*-imidazo[1,2-*a*]purine (**5d**)*

Crude isolated product (99 mg, 44% yield) was dissolved in EtOAc–MeOH 3:1, concentrated and kept at +5 °C to give a yellowish crystalline precipitate: mp 261–263 °C (dec). Fluorescence emission (H_2O): λ_{max} 412 nm; $\phi_F = 0.002$. ^{1}H NMR (DMSO-d_6) δ 13.15 (br s, 1H, N*H*), 8.01 (s, 1H, C7–*H*), 7.98 (s, 1H, C2–*H*), 7.44, 7.31 (2×d, 3′-*H*, 4′-*H*, $J = 3.9$, 3.9 Hz), 4.69 (t, 1H, O*H*), 4.59 (t, 1H, O*H*), 4.14 (d, 1H, N–C*H*H–C1″, $J = 13.8$ Hz), 4.00 (d, 1H, N–CH*H*–C1″, $J = 14.1$ Hz), 3.61 (m, 1H, C2″–C*H*H–OH), 3.46 (dd, 1H, C2″–CH*H*–OH, $J = 11.7$, 5.1 Hz), 3.29–3.42 (m, 2H, C1″–C*H*H–OH), 1.31 (m, 1H, C2″–*H*), 0.92 (dd, 1H, C3″–*H*H, $J = 8.7$, 4.8 Hz), 0.43 (t, 1H, C3″–H*H*, $J = 5.4$ Hz). ^{13}C NMR (DMSO-d_6) δ 151.13 (*C*-9), 150.52 (*C*-3a), 145.99 (*C*-4a), 139.32 (*C*-2), 131.89, 123.17 (*C*-6, *C*-2′), 131.38 (*C*-4′), 126.44 (*C*-3′), 115.28 (*C*-9a), 112.06 (*C*-5′), 103.10 (*C*-7), 60.85, 60.65 (C1″–*C*H_2–OH, C2″–*C*H_2–OH), 47.76 (C1″–*C*H_2–N), 26.84 (*C*-1″), 24.45 (*C*-2″), 13.94 (*C*-3″). Anal. Calcd for $C_{17}H_{16}N_5O_3SBr \cdot 1.25\ H_2O$: C 43.18, H 3.94, N 14.81, S 6.78; found: C 43.42, H 4.05, N 14.40, S 6.96.

*5.1.9. 3,9-Dihydro-3-[(2-hydroxyethoxy)methyl]-6-(furan-2-yl)-9-oxo-5*H*-imidazo[1,2-*a*]purine (**5e**)*

Chromatographically purified product (117 mg, 74% yield) was recrystallized from MeOH at +5 °C to afford colorless crystals: mp 219–221 °C (dec). Fluorescence emission (H_2O): λ_{max} 388 nm; $\phi_F = 0.026$. ^{1}H NMR (DMSO-d_6) δ 13.22 (br s, 1H, N*H*), 8.07 (s, 1H, C2–*H*), 7.86 (d, 1H, 5′-*H*, $J = 1.5$ Hz), 7.83 (s, 1H, C7–*H*), 7.00 (d, 1H, 3′-*H*, $J = 3.3$ Hz), 6.68 (dd, 1H, 4′-*H*, $J = 3.6$, 1.8 Hz), 5.51 (s, 2H, N–CH_2–O), 4.69 (t, 1H, O*H*), 3.44–3.56 (m, 4H, CH_2–CH_2). ^{13}C NMR (DMSO-d_6) δ 151.17 (*C*-9), 150.39 (*C*-3a), 146.08 (*C*-4a), 144.04 (*C*-5′), 143.04 (*C*-2′), 139.42 (*C*-2), 121.24 (*C*-6), 115.48 (*C*-9a), 112.02 (*C*-4′), 108.60 (*C*-3′), 101.73 (*C*-7), 72.35 (N–*C*H_2–O), 70.61 (HO–CH_2–*C*H_2), 59.90 (HO–*C*H_2). Anal. Calcd for $C_{14}H_{13}N_5O_4 \cdot 1.25\ H_2O$: C 49.78, H 4.63, N 20.73; found: C 50.16, H 4.84, N 20.31.

*5.1.10. 3-{[*cis*-1′,2′-Bis(hydroxymethyl)cycloprop-1′-yl]methyl}-3,9-dihydro-6-(furan-2-yl)-9-oxo-5*H*-imidazo[1,2-*a*]purine (**5f**)*

Crude isolated product (91 mg, 51% yield) was subjected to crystallization from EtOAc–MeOH 3:1 at +5 °C to obtain colorless crystals: mp 260–261 °C (dec). Fluorescence emission (H_2O): λ_{max} 381 nm; $\phi_F = 0.026$. ^{1}H NMR (DMSO-d_6) δ 13.12 (br s, 1H, N*H*), 7.98 (s, 1H, C2–*H*), 7.85 (d, 1H, 5′-*H*, $J = 1.8$ Hz), 7.80 (s, 1H, C7–*H*), 7.00 (d, 1H, 3′-*H*, $J = 3.3$ Hz), 6.67 (dd, 1H, 4′-*H*, $J = 3.3$, 1.8 Hz), 4.69 (t, 1H,

O*H*), 4.59 (t, 1H, O*H*), 4.15 (d, 1H, N–C*H*H–C1″, $J = 14.1$ Hz), 4.00 (d, 1H, N–CH*H*–C1″, $J = 14.1$ Hz), 3.61 (m, 1H, C2″–C*H*H–OH), 3.46 (dd, 1H, C2″–CH*H*–OH, $J = 12.3$, 6.0 Hz), 3.28–3.41 (m, 2H, C1″–C*HH*–OH), 1.32 (m, 1H, C2″–*H*), 0.93 (dd, 1H, C3″–*H*H, $J = 8.7$, 4.8 Hz), 0.44 (t, 1H, C3″–H*H*, $J = 5.4$ Hz). ^{13}C NMR (DMSO-d_6) δ 151.19 (*C*-9), 150.54 (*C*-3a), 145.90 (*C*-4a), 143.98 (*C*-5′), 143.15 (*C*-2′), 139.30 (*C*-2), 121.16 (*C*-6), 115.28 (*C*-9a), 111.98 (*C*-4′), 108.49 (*C*-3′), 101.53 (*C*-7), 60.80, 60.64 (C1″–*C*H_2–OH, C2″–*C*H_2–OH), 47.76 (C1″–*C*H_2–N), 26.80 (*C*-1″), 24.45 (*C*-2″), 13.95 (*C*-3″). Anal. Calcd for $C_{17}H_{17}N_5O_4 \cdot 1.75\ H_2O$: C 52.78, H 5.34, N 18.10; found: C 53.11, H 5.09, N 17.73.

5.2. Antiviral assays

Human cytomegalovirus (CMV) AD-169 and Davis strains were exposed to human embryonic lung (HEL) cell cultures. Briefly, confluent cultures in microtiter plates were inoculated with 100 plaque forming units (PFU). After 2 h virus absorption, residual virus was removed and the cell cultures were incubated in the presence of varying concentrations of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures (usually at 7 days post-infection). Inhibition of CMV by the test compounds was compared with cidofovir, ganciclovir and acyclovir as the reference compounds. Varicella-zoster virus (VZV) Oka (TK+) and 07/1 (TK$^-$) strains were grown on HEL cells. For VZV, confluent cells were inoculated with 20 PFU/well and the different dilutions of the tested compounds were added as for CMV. After 5 days incubation at 37 °C in a 5% CO_2 atmosphere, the cells were fixed and stained. Viral plaque formation was recorded and compared to the untreated control. Acyclovir, ganciclovir and brivudin were used as reference drugs.

Antiviral activity is expressed as the concentration of the compound required to inhibit viral cytopathicity by 50% (EC_{50}).

5.3. Cytostatic activity assays

For the cytostatic assays 100-μL aliquots of HEL cell suspensions were added to the wells of a 96-well microtiter plate containing 100 μL of varying concentrations of the test compounds. After 3-day incubation period at 37 °C in a humidified CO_2-controlled incubator, the number of viable cells was determined using a Coulter Counter. Cytostatic activity is expressed as the compound concentration that reduced the number of viable cells by 50% (CC_{50}). The cytotoxicity measurement was based on microscopically visible morphological alterations of the HEL cell cultures: cytotoxicity was defined as the minimum cytotoxic concentration (MCC) required for causing a microscopically detectable alteration of cell morphology.

Acknowledgments

The authors thank Anita Camps, Lies Vandenheurck and Steven Carmans for excellent technical assistance. This work was supported by grants from the Belgian Fonds voor Wetenschappelijk Onderzoeck (FWO), Vlaanderen, and the Geconcerteerde Onderzoeksacties (GOA), Vlaamse Gemeenschap.

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