Tricyclic Analogues of Acyclovir and Ganciclovir. Influence of Substituents in the Heterocyclic Moiety on the Antiviral Activity

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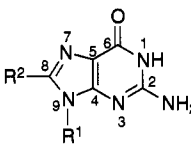
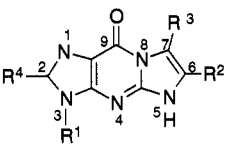
The effect of substitution in the tricyclic moiety of 3,9-dihydro-9-oxo-5*H*-imidazo[1,2-*a*]purine (1,*N*-2-ethenoguanine) analogues of acyclovir (**1**) and ganciclovir (**2**) on their physical properties and antiherpetic activity was investigated by synthesizing a series of compounds substituted in the 2, 6, or 7 position (**6**–**14**). Substitution in the 6-position with phenyl or 4-biphenylyl resulted in fluorescent compounds (**7**, **9**, **13**, **14**). In general, the substituent in the 6 position potentiated the antiviral activity. The fluorescent 6-phenyl derivatives: 3,9-dihydro-3-[(2-hydroxyethoxy)methyl]-9-oxo-6-phenyl–5*H*-imidazo[1,2-*a*]purine (**7**) and its 3-[(1,3-dihydroxy-2-propoxy)methyl] congener (**13**) were the most potent tricyclic analogues of **1** and **2**, respectively. Compound **7** was inhibitory to TK$^+$ HSV-1, TK$^+$ HSV-2, and TK$^+$ VZV within the concentration range of 0.2–2.0 μg/mL, well below the cytotoxicity threshold (50 to >100 μg/mL). Compound **13** was inhibitory to TK$^+$ HSV-1 and TK$^+$ HSV-2 within the concentration range of 0.005–0.3 μg/mL and to TK$^+$ and TK$^-$ VZV within the concentration range of 0.4–3 μg/mL (cytotoxicity threshold >200 μg/mL). Both **7** and **13** seem to be promising candidate compounds for the noninvasive diagnosis of herpesvirus infections.

We have previously found that when the 1 and N-2 positions of the guanine moiety in the two potent antivirals, acyclovir (**1**) and ganciclovir (**2**), are linked together with a prop-1-ene-1,2-diyl bridge, the resulting compounds **3** and **4** exhibit marked and selective antiherpetic activity.[1,2] In the thus formed 3,9-dihydro-9-oxo-5*H*-imidazo[1,2-*a*]purine system, the 6-methyl substituent is of importance: its absence results in a 6–100-fold decrease of antiviral activity.[2] We have now studied an expanded series of the tricyclic, 3,9-dihydro-9-oxo-5*H*-imidazo[1,2-*a*]purine analogues of **1** and **2**, bearing substituents in the 2, 6, or 7 positions (Chart 1).

Chart 1





	R^1	R^2
1:	a	H
2:	b	H
5:	a	Br

a = HO–⟨O⟩

b = HO–⟨O⟩–OH

	R^1	R^2	R^3	R^4
3:	a	CH_3	H	H
4:	b	CH_3	H	H
6:	a	$(CH_3)_3C$	H	H
7:	a	C_6H_5	H	H
8:	a	4-BrC_6H_4	H	H
9:	a	4-$C_6H_5C_6H_4$	H	H
10:	a	CH_3	H	Br
11:	a	H	CH_3	H
12:	b	$(CH_3)_3C$	H	H
13:	b	C_6H_5	H	H
14:	b	4-$C_6H_5C_6H_4$	H	H

Chemistry

The tricyclic analogues substituted at the 6 position were prepared by reacting the 1-sodium derivative of acyclovir, 8-bromoacyclovir (**5**), or ganciclovir in dimethylformamide with an appropriate bromo ketone according to a previously described method for an alkylation–condensation reaction using bromoacetone.[2]

The analogues of ganciclovir, 3-[(1,3-dihydroxy-2-propoxy)methyl] derivatives (**12**–**14**) showed strong tendency to form amorphous precipitates. They were finally obtained as crystalline hydrates from 2-propanol: neat or with admixture of water or ethanol. The crystalline form was indispensible for their analytical purity.

Literature data report only 18% yield of 3,9-dihydro-7-methyl-9-oxo-3-(β-D-ribofuranosyl)-5*H*-imidazo[1,2-*a*]-purine when guanosine is subjected to reaction with α-bromopropionaldehyde.[3] The reason for such low yield is mainly the instability of α-bromopropionaldehyde. In our hands, α-bromopropionaldehyde obtained by bromination of propionaldehyde with bromine–dioxane complex according to ref 4 gave much better yield (53%) of 3,9-dihydro-3-[(2-hydroxyethoxy)methyl]-7-methyl-9-oxo-5*H*-imidazo[1,2-*a*]purine (**11**) than, as used in ref 3, α-bromopropionaldehyde prepared from silyl enol ether of propionaldehyde and bromine.[5]

The conditions used to synthesize the novel tricyclic analogues (**6**–**14**) as well as their physical properties and elemental analyses are presented in Table 1. The structures of these compounds were also characterized and confirmed by TLC chromatographic mobility, proton magnetic resonance, and ultraviolet and fluorescence spectra (Table 2). The presence of the appended ring together with a phenyl or 4-biphenylyl group in the 6 position endowed acyclovir and ganciclovir with relatively strong fluorescence, a property known to be advantageous for various analytical goals.

The regiochemistry of the reaction of bromoacetone with substituted guanines was established some time

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Table 1. Reaction Conditions, Physical Properties, and Analytical Data of Compounds **6**–**14**

compd	substrate	condensation–cyclization reagent	yield,[a] %	recryst solvent	mp, °C	formula	anal.[b]
6	1	$(CH_3)_3CCOCH_2Br$	66	EtOAc–MeOH (5:1)	207–209	$C_{14}H_{19}N_5O_3$	C, H, N
7	1	$C_6H_5COCH_2Br$	56	MeOH	242–244 dec	$C_{16}H_{15}N_5O_3$	C, H, N
8	1	$4\text{-}BrC_6H_4COCH_2Br$	52	MeOH	244–245 dec	$C_{16}H_{14}N_5O_3Br$	C[c], H, N
9	1	$4\text{-}C_6H_5C_6H_4COCH_2Br$	53	$CHCl_3$–MeOH (9:1)	252 dec	$C_{22}H_{19}N_5O_3{\cdot}0.5H_2O$	C, H, N[d]
10	5	CH_3COCH_2Br	58	$CHCl_3$–MeOH (7:1)	244 dec	$C_{11}H_{12}N_5O_3Br$	C, H, N
11	1	$CH_3CHBrCHO$	53	MeOH	252–254 dec	$C_{11}H_{13}N_5O_3$	C, H, N
12	2	$(CH_3)_3CCOCH_2Br$	73	2-PrOH	232 dec	$C_{15}H_{21}N_5O_4{\cdot}H_2O$	C, H, N
13	2	$C_6H_5COCH_2Br$	75	2-PrOH–H_2O (1:1)	>300 dec	$C_{17}H_{17}N_5O_4{\cdot}0.5H_2O$	C, H, N[e]
14	2	$4\text{-}C_6H_5C_6H_4COCH_2Br$	72	2-PrOH–EtOH (2:1)	>300 dec	$C_{23}H_{21}N_5O_4{\cdot}0.25H_2O$	C, H, N

[a] After chromatography. [b] Elemental compositions (%) were found to be within ±0.4% of the theoretical values for C, H, and N unless stated otherwise. [c] C: calcd, 47.54; found, 47.00. [d] N: calcd, 17.06; found, 17.52. [e] N: calcd, 19.22; found, 18.62.

Table 2. Spectral and Thin-Layer Chromatography Data of Compounds **3**, **4**, and **6**–**14**

	^1H NMR (DMSO-d_6)[a]								UV (H_2O) λ^{max}, m ($\epsilon \times 10^{-3}$ dm^{-3} mol^{-1} cm^{-1})	fluorescence (H_2O) emission λ^{max}, nm (excitation at 305 nm) (φ, %)	R_f values × 100 in system[c]	
compd	N-5-H	H-2	7-R^3	NCH_2O	OH	$CH(CH_2)_2$	CH_2CH_2	6-R^2			A	B
3	12.42 (brs, 1)	8.03 (s, 1)	7.36 (d, 1)	5.49 (s, 2)	4.69 (br, 1)	–	3.50 (brs, 4)	2.27 (d, 3)	231 (27.3), 285 (10.1)	378 (0.23)	45	17
4	12.38 (brs, 1)	8.00 (s, 1)	7.35 (d, 1)	5.58 (s, 2)	4.58 (t, 2)	3.62 (p, 1), 3.45 (m, 4)	–	2.26 (d, 3)	231 (28.5), 284 (10.7)	*d*	25	09
6	12.54 (brs, 1)	8.02 (s, 1)	7.26 (s, 1)	5.50 (s, 2)	4.64 (t, 1)	–	3.52 (brs, 4)	1.32 (s, 9)	231 (29.6), 285 (9.0)	*d*	57	29
7	13.09 (brs, 1)	8.06 (s, 1)	8.21 (s, 1)	5.52 (s, 2)	4.65 (t, 1)	–	3.53 (brs, 4)	7.42, 7.88 (m, 3, m, 2)	251 (39.3), 306 (12.2)	392 (7.95)	57	29
8	13.44 (br, 1)	8.05 (s, 1)	8.26 (s, 1)	5.52 (s, 2)	4.66 (br, 1)	–	3.53 (brs, 4)	7.66, 7.86 (2 × d, 4)	255 (32.3), 310 (11.2)	*d*	55	27
9	13.12 (s, 1)	8.06 (s, 1)	8.26 (s, 1)	5.53 (s, 2)	4.63 (t, 1)	–	3.55 (brs, 4)	7.42–7.97 (m, 9)	271 (45.7), 314 (28.1)	424 (7.91)	57	30
10	12.60 (brs, 1)	–	7.39 (d, 1)	5.45 (s, 2)	4.70 (br, 1)	–	3.48–3.57 (m, 4)	2.27 (d, 3)	236 (21.7), 286 (9.5)	*d*	67	40
11	12.11 (brs, 1)	7.96 (s, 1)	2.63 (d, 3)	5.44 (s, 2)	4.67 (t, 1)	–	3.42–3.52 (m, 4)	7.03 (d, 1)	228 (20.4), 280 (7.2)	*d*	44	17
12	12.53 (brs, 1)	8.01 (s, 1)	7.27 (s, 1)	5.58 (s, 2)	4.61 (t, 2)	3.60 (p, 1), 3.20–3.50 (m, 4)	–	1.32 (s, 9)	231 (29.1), 285 (9.0)	*d*	35	13
13	nd[e]	8.00 (s, 1)	8.12 (s, 1)	5.60 (s, 2)	4.62 (t, 2)	2.80–3.80 (m, 5)	–	7.38 (m, 3), 7.87 (m, 2)	251 (38.9), 305 (12.1)	396 (8.37)	35	13
14	13.18 (brs, 1)	8.06 (s, 1)	8.31 (s, 1)	5.62 (s, 2)	4.63 (t, 2)	3.64 (p, 1), 3.26–3.50 (m, 4)	–	7.36–7.54 (m, 3), 7.74–7.82 (m, 4), 8.00–8.05 (m, 2)	271 (45.9), 314 (28.4)	428 (12.81)	36	14

[a] Parts per million downfield from TMS. [b] Fluorescence intensity of 2-aminopurine taken as 100% standard. [c] A, $CHCl_3$–MeOH (4:1); B, $CHCl_3$–MeOH (9:1). [d] Very weak fluorescence comparable to that observed for **3**. [e] nd, not detected.

Table 3. Selected ^{13}C NMR Data[a] of Tricyclic Analogues of Acyclovir **3**, **6**, **7**, **10**, and **11**

compd	chemical shifts (DMSO-d_6)[b] C-6	C-7
3	126.01	103.25
	Sm[c]	Dq
6	139.44	100.36
	Sm	Ds
7	129.03	103.22
	Sm	Ds
10	126.20	103.59
	Sm	Dq
11	112.97	119.99
	Dq	Sm
15[8]	116.49	106.90

[a] Complete ^{13}C NMR data are available as supplementary material. [b] Parts per million downfield from TMS. [c] Multiplicity in the coupled ^{13}C NMR spectra.

ago.[6,7] The assumption that the same regiochemistry would apply in the case of various additional α-bromo ketones used in the present work was confirmed by C-6 and C-7 signals in fully coupled ^{13}C NMR spectra of selected acyclovir derivatives (Table 3). The ^{13}C resonances of the appended ring of the newly synthesized compounds, exemplified by **6**, **7**, and **11**, closely resembled those of the model 6-substituted compound **3** but not its 7-substituted isomer **8**. Some variations in chemical shifts and multiplicities were in accord with different structures of particular substituents. The ^{13}C chemical shift values when compared to those reported in the literature for 6,7-unsubstituted 1,*N*-2-ethenoguanosine **15**[8] obeyed general rules of the chemical shift changes upon substitution at the double bond.

Biological Activity

The compounds **3**, **4**, and **6**–**14** were evaluated against a broad range of viruses, including herpes simplex virus type 1 (HSV-1) and 2 (HSV-2), varicella-zoster virus (VZV), cytomegalovirus (CMV), vesicular stomatitis virus (VSV), vaccinia virus (VV), and thymidine kinase-deficient (TK^-) strains of HSV and VZV. The effects of the compounds on cell morphology and cell growth were tested in parallel with their antiviral activity.

Compounds **4** and **13** emerged as the most potent inhibitors of HSV-1 and HSV-2. Their potency was comparable to that of GCV (ganciclovir). Compound **14** had an anti-HSV activity comparable to that of ACV (acyclovir). Substantial antiviral activity was also noted for compounds **3**, **5**, **7**, **8**, **11**, and **12** (Table 4). As a rule, the compounds were inactive against TK^- herpesviruses (HSV, VZV), CMV, VSV, and VV, except for compound **13**, which showed marked activity against the TK^- VZV strains. In terms of toxicity, none of the compounds significantly altered cell morphology or impaired cell growth.

Structure–Activity Relationship

From a previous work,[2] it is known that linking the 1 and N-2 positions of guanine moiety of acyclovir and ganciclovir with a 1,*N*-2-etheno bridge lowers the activity against HSV-1, HSV-2, VZV, and CMV by a factor of 10^2 or more. We show here that further substitutions in the resulting ring enhance the antiviral activity. The magnitude of the antiviral effect depended upon (i) the position and type of the substituent, (ii) the nature of

Table 4. Activity against Human Herpesviruses and Cytotoxicity of Compounds **3**, **4**, and **6**–**14**

virus (strain)	cell	minimal inhibitory concentration (μg/mL) **3**	**4**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	ACV (1)	GCV (2)	IDU	BVDU	ribavirin
HSV-1(KOS)	E6SM	0.8	0.015	4.5	0.4	0.6	1.3	95	2.1	0.035	0.02	0.02	0.02	0.003	20	0.003	46
HSV-1(F)	E6SM	0.58	0.005	4	0.7	2	2	150	0.1	0.1	0.005	0.01	0.004	0.004	7	0.005	23
HSV-1(McIntyre)	E6SM	1	0.02	2	0.2	0.2	0.7	150	0.5	0.1	0.005	0.001	0.07	0.004	4	0.006	16
HSV-2(G)	E6SM	3	0.1	13	1.3	2	7	350	4.5	2.2	0.3	0.2	0.09	0.02	20	220	250
HSV-2(196)	E6SM	1.5	0.2	7	0.2	0.4	2	70	2	0.6	0.02	0.35	0.01	0.04	7	170	120
HSV-2(Lyons)	E6SM	1	0.05	20	0.7	0.7	2	150	1	0.1	0.005	0.04	0.009	0.004	20	90	103
TK+/TK-HSV-1-(VMW1837)	E6SM	40	1.6	>125	>70	>70	>55	>125	13	0.7	0.5	2.7	5	0.08	20	52	115
TK-HSV-1(B2006)	E6SM	127	31	ND[b]	ND	ND	ND	ND	>120	>70	>30	>60	70	4.5	ND	15	53
VZV(YS)	HEL	9.4	12	2.2	1.4	4.0	40	220	>50	>20	3	>50	0.38	1.4	ND	0.003	ND
VZV(OKA)	HEL	4.6	1	1	1.3	1.0	65	215	>50	5	0.4	10	0.18	0.5	ND	0.005	ND
TK-VZV(YS-R)	HEL	70	15	127	67	230	<400>100	185	>50	50	1	20	4.7	1.4	ND	20	ND
TK-VZV(07/1)	HEL	>50	15	40	67	125	100	180	>50	50	3	25	13.7	1.5	ND	17.5	ND
CMV(Davis)	HEL	>50	>50	<400>100	166	<100>40	<400>100	<400>100	>50	>50	7	>50	ND	0.9	ND	>20	ND
CMV(AD-169)	HEL	>50	>50	<400>100	<400>100	<100>40	<400>100	<400>100	>50	>50	20	>50	13.2	1.5	ND	>20	ND
VSV	E6SM	190	>175	>125	>100	>70	>70	>250	>175	>160	>175	>70	>220	>120	>200	>180	43
VV	E6SM	>190	>175	>250	>100	>70	>70	>250	>150	>135	>90	>70	>180	>80	20	0.22	28
morphological alteration	E6SM	>190	>175	>250	>100	≥70	≥70	>250	>100	>175	>175	>85	350	>270	≥400	>350	400
cell growth	HEL	>200	>200	167	45	>200	>200	>200	>200	>200	>200	>200	>200	>200	ND	60	ND

[a] Concentration required to reduce virus-induced cytopathicity (HSV, VSV, VV), plaque formation (VZV, CMV), or cell growth by 50%; for morphologic alteration, it corresponds to the minimal concentration required to cause a microscopically detectable change in normal cell morphology. The results listed are the mean values of two to six independent determinations. [b] Not determined.

the virus, and (iii) the kind of the acyclic moiety in the 3 position of the heterocycle.

The increase in activity following introduction of a methyl group in either the 6 or 7 position of the acyclovir congeners varied depending on the HSV-1 or HSV-2 strain used, e.g., **3** being 3 times more potent than **11** against HSV-1 (KOS), but **11** being 5 times more potent than **3** against HSV-1 (F). Against the VZV strains tested, **3** was at least 6 times more potent than **11**.

Introduction of a bromine atom in the C-2 position of the 6-methyl derivative **3** (to form compound **10**) resulted in a significant decrease of activity. Against HSV-1 and HSV-2 the activity decreased from 35 to 350 times, depending upon the virus strain; for VZV it decreased by 35-fold. For acyclovir itself only a 10-fold decrease in activity was noted.[9]

An alkyl substituent in the 6 position confined the activity of the tricyclic acyclovir derivatives toward particular viruses: the 6-methyl derivative **3** was 3–30 times more potent against HSV-1 and HSV-2 than the 6-*tert*-butyl derivative **6**, whereas the reverse (3–6 times) was true for VZV. In the case of the ganciclovir analogues, the 6-methyl derivative **4** was more potent than the 6-*tert*-butyl **12** against HSV-1, HSV-2 and VZV.

Substitution in the 6 position of a phenyl or 4-biphenylyl group afforded the greatest increase in antiviral activity. Especially the ganciclovir derivatives gained marked activity. Converting the 3-[(2-hydroxyethoxy)methyl] side chain of **7** and **9** to 3-[(1,3-dihydroxy-2-propoxy)methyl], as in **13** and **14**, gave a 100-fold enhancement of activity, whereas for the conversion of acyclovir to ganciclovir this increase was approximately 10-fold. Unlike the 6-phenyl derivatives, which were active against HSV-1, HSV-2, and VZV, the 6-(4-biphenylyl) derivatives were active only against HSV-1 and HSV-2.

Substitution in the 6 position with a phenyl or 4-biphenylyl group gave rise to relatively strong fluorescence. The intensity of fluorescence was somewhat higher for the 6-(4-biphenylyl) derivatives than for the 6-phenyl derivatives. Of the different molecules presented here, the fluorescent 3,9-dihydro-3-[(1,3-dihydroxy-2-propoxy)methyl]-9-oxo-6-phenyl-5*H*-imidazo-[1,2-*a*]purine **13** is the most promising. Its activity against HSV-1, HSV-2, TK^- HSV-1, TK^+ VZV, and TK^- VZV is very similar to that of the parent ganciclovir; only its activity against CMV is 1 order of magnitude lower.

Conclusion

The fluorescent compounds **7** and **13** may prove useful in the noninvasive diagnosis of herpesvirus infections. The fact that they show selective activity against herpesvirus infections suggests that they are preferentially metabolized by the virus-infected cells and/or show a particular affinity for virus-specific enzymes. Because of their fluorescence, compounds **7** and **13** and their metabolites could be monitored as "tags" for the virus-infected cells and/or virus-specified enzymes.

Experimental Section

General Methods. Melting points were determined on a Laboratory Devices Mel-Temp II micromelting point apparatus in open capillaries and are uncorrected. Elemental analyses were perforemd on a Perkin-Elmer 240 elemental analyzer, and the results are within 0.4% of the theoretical values unless states otherwise. The ultraviolet spectra were measured on a Beckman DU-65 spectrophotometer. The ^1H NMR spectra were recorded on a Varian Unity 300 FT NMR spectrometer in DMSO-d_6 at 299.949 MHz. Chemical shifts are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. Fluorescence spectra were measured on a Perkin-Elmer MPF-3 fluorescence spectrophotometer. Thin-layer chromatography (TLC) was conducted on Merck precoated silica gel F_{254} Type 60 plates in the following solvent systems (measured by volume): A, chloroform–methanol (4:1); B, chloroform–methanol (9:1). For a preparative short-column chromatography, Merck TLC gel HF_{254} Type 60 was used.

General Procedure for the Preparation of 3,9-Dihydro-3-[(2-hydroxyethoxy)methyl]-9-oxo-5*H*-imidazo-[1,2-*a*]purines and 3,9-Dihydro-3-[(1,3-dihydroxy-2-propoxy)methyl]-9-oxo-5*H*-imidazo[1,2-*a*]purines Substituted in the 2, 6, or 7 Position. To an anhydrous suspension of 1 mmol of acyclovir (in the case of **6–9** and 11), 8-bromoacyclovir (in the case of **10**), or ganciclovir (in the case of **12–14**) in dimethylformamide was added sodium hydride in 60% suspension in oil (1.3 mmol). After stirring with exclusion of moisture for 1–2 h at room temperature, the resulting solution was treated with bromo ketone (1.1 mmol) or bromo aldehyde (1.4 mmol), respectively (Table 1). The reaction mixture was stirred for the next 2–3 h, made alkaline by addition of concentrated aqueous ammonia, and left overnight at room temperature. Volatile materials were evaporated, the residual oil was dissolved in chloroform–methanol, 9:1, applied onto a silica gel short column, and chromatographed in $CHCl_3$–MeOH gradient 9:1 → 6:1. Fractions containing the main product were evaporated to dryness and recrystallized.

Details on the reaction conditions and physical properties of the compounds **6–14** are given in Table 1; their spectral and TLC chromatographic characteristics are presented in Table 2.

Antiviral Activity Assays. Viruses, cells, antiviral assay methods, and abbreviations are as described previously.[10]

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