Brief Articles

Fluorescent Tricyclic Analogues of Acyclovir and Ganciclovir. A Structure−Antiviral Activity Study

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Of a series of new guanine base modified tricyclic analogues of acyclovir (ACV, **1**) and ganciclovir (GCV, **2**), derivatives of the 3,9-dihydro-9-oxo-5*H*-imidazo[1,2-*a*]purine system, evaluated for activity against herpes simplex virus type 1 and 2, several fluorescent analogues, 6-(4-MeOPh)-TACV (**8**), 7-Me-6-Ph-TACV (**17**), 6-(4-MeOPh)-TGCV (**27**), and 7-Me-6-Ph-TGCV (**28**), were obtained that showed similar potency and selectivity as the parent compounds. The activity was found to be strongly dependent on the nature and steric demands of the substituents in the 6 and/or 7 position.

Introduction

We have previously reported that linking the 2-NH_2 and N-1 positions of guanine moiety of acyclovir (ACV, **1**) and ganciclovir (GCV, **2**) with an etheno bridge to form derivatives of the tricyclic 3,9-dihydro-9-oxo-5*H*-imidazo[1,2-*a*]purine system, TACV (**3**) and TGCV (**4**) [For the systematic names 3,9-dihydro-3-[(2-hydroxyethoxy)methyl]-9-oxo-5*H*-imidazo[1,2-*a*]purine and 3,9-dihydro-3-[(1,3-dihydroxy-2-propoxy]-9-oxo-5H-imidazo-[1,2-*a*]purine the abbreviations TACV and TGCV, respectively, are used throughout this paper.], allows for the modulation of physical and biological properties of the parent antivirals.[1−3] The appended ring by itself diminishes the antiherpetic activity, depending on the type and strain of the virus, from 100- to more than 2000-fold. Further substitutions in the 6 or 7 position of this ring potentiate the activity and make the compounds more selective toward particular herpes viruses. The magnitude of the antiviral activity has been found to depend on the position and type of the substituent, the virus type, and the nature of the acyclic moiety in the 3 position of the heterocycle. Appropriate substituents in the appended ring may simultaneously endow **3** and **4** with advantageous physical properties: e.g. a 6-methyl function improves solubility[4] and 6-aryl groups may confer fluorescence. Of a series of several tricyclic analogues evaluated so far,[1−3] the fluorescent 6-phenyl-TACV (**5**) and 6-phenyl-TGCV (**6**) have appeared as the most promising. In particular, the activity of compound **6** against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), thymidine kinase deficient (TK^-) HSV-1, and varicella-zoster virus (VZV) is very similar to that of parent ganciclovir; only its activity against cytomegalovirus (CMV) is lower by 1 order of magnitude. Compound **5** is on the average approximately 15-fold less active than parent acyclovir.[3]

The fact that some tricyclic analogues show selective activity against herpes virus infections suggests that they are preferentially metabolized by the virus-infected cells and/or show a preferential affinity for virus-specific enzymes. This assumption has recently been substantiated by an NMR experiment in solution which demonstrated the formation of 6-ethyl-TACV−HSV-1TK complex.[5] Fluorescent, antivirally active tricyclic analogues related to TACV and TGCV may prove useful in the noninvasive diagnosis of herpes virus infections. These compounds and their metabolites could be monitored as "tags" for the virus-infected cells and/or virus-specified enzymes.

The fact that aryl substitution may confer on the tricyclic analogues simultaneously fluorescence and antiherpetic activity together with the continuous need for the development of new antiviral drugs resulting from the emergence of drug−resistant herpesviruses[6] encouraged us to study a further series of 22 derivatives of 3,9-dihydro-9-oxo-5*H*-imidazo[1,2-*a*]purine.

We first attempted to develop fluorescent tricyclic analogues of acyclovir, with antiviral activity similar to that of the parent compound, and then to synthesize the ganciclovir counterparts of the most active ones. The antivirally active TACV and TGCV derivatives described so far[3] consist mostly of 6-substituted compounds with 7-Me-TACV as the only one example of different substitution site. The present series (Chart 1) included 6-substituted (**7**−**16**, **25**−**27**), 6,7-disubstituted (**17**−**22**, **28**), and 7-substituted (**23**, **24**) derivatives. 6-Et-TACV (**25**) was included in our studies as an active reference compound; it corresponds to the only HSV-1 TK substrate for which complex formation with the enzyme was shown in solution.[5]

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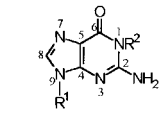
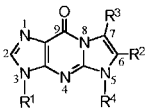
Chart 1

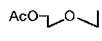


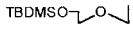


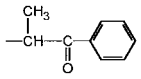
	R^1	R^2
1	a	H
2	b	H
17'	a	e
28'	b	e

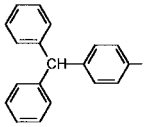
a 

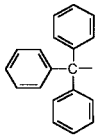
b 

c 

d 

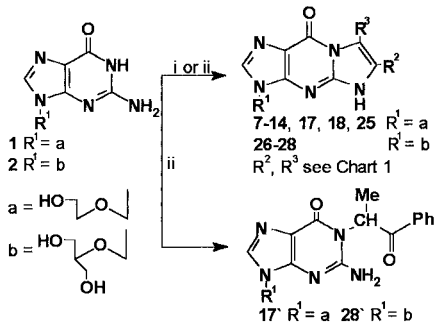
e 

BzhPh 

Tr 

	R^1	R^2	R^3	R^4
3	a	H	H	H
4	b	H	H	H
5	a	Ph	H	H
6	b	Ph	H	H
7	a	4-MePh	H	H
8	a	4-MeOPh	H	H
9	a	2-MeOPh	H	H
10	a	3-MeOPh	H	H
11	a	2,4-diMeOPh	H	H
12	a	2,5-diMeOPh	H	H
13	a	4-NO_2Ph	H	H
14	a	2-napht	H	H
15	c	2-napht	H	H
16	a	Ph	H	CH_2Ph
17	a	Ph	Me	H
18	a	Ph	Ph	H
19	a	Ph	4-BzhPh	H
20	a	4-MeOPh	4-BzhPh	H
21	a	Me	Tr	H
22	d	Me	Tr	H
23	a	H	CH_2OH	H
24	a	H	Tr	H
25	a	Et	H	H
26	b	4-MePh	H	H
27	b	4-MeOPh	H	H
28	b	Ph	Me	H

Scheme 1[a]



[a] Reagents: (i) 1. NaH, DMF, R^2 $COCH_2Br$ or PhCOCH(Br)Ph; 2. NH_4OH. (ii) 1. NaH, DMF, $PhCOCH(Br)CH_3$; 2. NH_4OH.

Chemistry

The tricyclic analogues **7**−**14**, **17**, **18**, and **25**−**28** were prepared by reacting the 1-sodium derivative of acyclovir or ganciclovir in dimethylformamide with an appropriate bromo ketone (Scheme 1) according to a previously described method for an alkylation-condensation reaction using other bromo ketones.[2,3] In the case of the reaction of **1** and **2** with 2-bromopropiophenone, we isolated, in addition to the expected 6-Ph-7-Me-TACV **17** and 6-Ph-7-Me-TGCV **28**, the intermediate N-1 alkylation products: 1-(1-benzoylethyl)-ACV **17′** and 1-(1-benzoylethyl)-GCV **28′**. The structures of **17′** and **28′** were identified on the basis of their HR MS and ^1H and ^{13}C NMR spectra. The mechanistic sequence of the reaction of bromoacetone[7] and other α-bromoketones[3] with substituted guanines involving first alkylation at N-1 of the guanine moiety followed by ring closure and elimination has been established on the basis of the structures of the tricyclic products. However, the initial intermediate had never been isolated. Compound **15** with the blocked OH function in the acyclic moiety was obtained by routine acetylation of **14** with acetic anhydride in pyridine. Compound **16** has been described previously.[8] The 7-hydroxymethyl derivative **23** was synthesized from acyclovir and glycidaldehyde following the procedure described for guanosine.[9] As in the case of guanosine, compound **23** was accompanied by the 2-NH_2, N-1-etheno derivative of the parent compound.

The remaining 6,7-disubstituted (**19**−**22**) and 7-substituted (**24**) TACV derivatives were obtained *via* unusual tritylation reactions, as previously described.[10,11]

Structure−Activity Relationship

The substituent introduced in the 6-phenyl group of **5** had a pronounced influence on its antiviral activity and cytotoxicity (Table 1). The effect depended upon the nature of the substituent and the site of its attachment. Electron donating groups [methyl (compound **7**); methoxy (compound **8**)] in the 4 position of 6-phenyl-TACV increased its potency to that of acyclovir. Compound **9** with a 6-(2-methoxyphenyl) substituent and compound **10** with a 6-(3-methoxyphenyl) substituent had some antiviral activity but 10-fold weaker than for the unsubstituted 6-phenyl-TACV. Introduction of an additional ortho-methoxy group in the 6-phenyl substituent of compounds **8** and **10**, to form compounds **11** and **12**, respectively, decreased antiherpetic activity and increased cytotoxicity. The effect was especially pronounced for the highly effective **8**, for which the selectivity indices against HSV-1 and HSV-2 decreased from 1500 to 40 and from 1000 to 8, respectively. Introduction of an electron withdrawing nitro group in the 4 position of the 6-phenyl moiety was unfavorable and resulted in the weakly active compound **13**. This may not, necessarily, be attributed to the electron withdrawing character of the substituent but rather to steric reasons, since the previously examined 6-(4-bromophenyl)-TACV showed antiviral activity pattern very similar to that of unsubstituted 6-Ph-TACV.[3] In contrast to the replacement of the phenyl group in the 6 position with the linear 4-biphenylyl substituent, which on average resulted only in about 5-fold decrease of activity against HSV-1 and HSV-2,[3] introduction of angular, condensed 2-naphthyl group (compd **14**) resulted in approximately 15-fold decrease of activity.

The complete loss of selectivity of compound **14** after blocking its side-chain hydroxy group (compound **15**) suggests that, as in acyclovir, antiviral activity requires phosphorylation of the acyclic moiety. As observed for 6-Me-TACV,[1] an unsubstituted N-5 position was crucial for the activity of 6-aryl derivatives. 5-Benzyl-6-phenyl-TACV (**16**) was totally inactive.

Table 1. Activity Against Human Herpes Simplex Virus Type 1 and 2 and Cytotoxicity of Novel Tricyclic Analogues of Acyclovir and Ganciclovir

	minimal inhibitory concn (μg/mL)[a]										
	HSV-1			HSV-2					HSV-1 TK$^-$	HSV-1 TK$^-$/TK$^+$	minimal[b] cytotoxic
compd	KOS	F	McIntyre	G	196	Lyons	VV	VSV	ACV^r	VMW 1837	concn (μg/mL)
ACV (**1**)	0.13	0.08	0.13	0.19	0.07	0.28	>80	>80	19	7.1	≥400
GCV (**2**)	0.006	0.004	0.006	0.01	0.02	0.04	>100	>100	0.48	0.07	>100
BVDU	0.02	0.02	0.01	>100	>100	>100	9.2	>200	90	>100	≥400
3	20	4	20	20	70	10	400	ND[c]	400	ND	400
4	0.7	0.7	0.7	2	2	0.7	400	ND	400	ND	400
5	0.4	0.7	0.2	1.3	0.2	0.7	>100	>100	ND	>70	>100
6	0.02	0.005	0.005	0.3	0.02	0.005	>90	>175	ND	0.5	>175
7	0.43	0.07	0.07	0.93	0.2	0.4	>40	>40	9.2	82	250
8	0.1	0.07	0.03	0.15	0.2	0.2	>40	>40	5.9	21	150
9	1.92	1.92	1.92	1.92	9.6	9.6	>80	>80	>80	9.6	400
10	1.92	1.92	0.64	1.92	3.2	3.2	>400	240	>80	9.6	>400
11	1.92	1.92	1.92	3.2	1.92	1.92	16	16	>16	1.92	80
12	9.6	16	16	9.6	>16	>16	>16	>16	>16	>16	80
13	9.6	16	16	9.6	>16	>16	>16	>16	>16	>16	80
14	5.8	9.6	9.6	6.4	16	9.6	>16	>16	16	12.8	≥80
15	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	≥80
16	>100	ND	ND	>100	ND	ND	>100	>100	>100	>100	≥100
17	0.11	0.04	0.06	0.16	0.1	0.07	>200	>200	3.2	3.2	300
18	1.92	9.6	9.6	1.92	9.6	16	>16	>16	>16	>16	≥16
19−**21**, **24**	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	80
22	>10	ND	ND	>10	ND	ND	2	>10	>10	10	40
23	>3.2	>3.2	>3.2	>3.2	>3.2	>3.2	>3.2	>3.2	>3.2	>3.2	≥16
25	1.92	9.6	9.6	9.6	9.6	9.6	240	>400	240	48	>400
26	0.02	0.01	0.08	0.13	0.13	0.08	>16	>16	>16	0.1	80
27	0.005	0.005	0.005	0.03	0.08	0.02	>80	>80	>16	0.04	≥80
28	0.02	0.005	0.005	0.03	0.005	0.02	>400	>240	3.2	0.02	>400

[a] Concentration required to reduce virus-induced cytopathicity by 50%. [b] Concentration required to cause a microscopically detectable change in normal cell morphology. The results listed are mean values of two or more independent determinations. [c] Not determined.

Introduction of a methyl group in the 7 position of the 6-phenyl derivative gave rise to compound **17**, the most potent fluorescent tricyclic analogue of acyclovir found so far. It turned out to be even slightly more active against various strains of TK$^+$ HSV-1 and HSV-2 and distinctly more active against TK$^-$ HSV-1 than acyclovir. Unfortunately none of the other 6-aryl-7-substituted TACV derivatives (**18**−**20**) showed any selective antiviral activity. For compounds **19** and **20**, bearing bulky benzhydrylphenyl group in the 7-position this may be due to steric reasons, which, as discussed above, proved important for mono 6-substituted TACV derivatives. Another bulky group, trityl, in the 7 position of compound **21** similarly inactivated 6-methyl-TACV. When the OH group of the acyclic moiety of **21** was blocked with TBDMS group, compound **22** thus formed became quite toxic but displayed anti-VV activity (SI 20). The substituent in the 7 position may have a significant role in the biological activity of TACV. The activity is very sensitive to structural changes of substituents in this position. Replacement of 7-methyl of **17** with 7-phenyl group resulted in compound **18**, which was nonselective and highly cytotoxic. The effect was not a steric one because replacement of the 7-methyl group of 7-Me-TACV (moderately active against HSV-1 and HSV-2, on average ∼100 fold less than ACV)[3] by hydroxymethyl resulted in **23**, which was totally nonselective and toxic. 6-Et TACV[5] turned out to be about 10−100-fold less active than ACV and nontoxic. The tricyclic ganciclovir congeners **27** and **28**, corresponding to **8** and **17,** were active against TK$^+$HSV-1 and TK$^+$HSV-2 similarly to parent GCV.

Conclusions

From the 22 compounds synthesized, the fluorescent tricyclic analogues of acyclovir 6-(4-MeOPh)-TACV **8** and 7-Me-6-Ph-TACV **17** as well as of ganciclovir 6-(4-MeOPh)-TGCV **27** and 7-Me-6-Ph-TGCV **28** showed similar antiherpetic potency as the parent compounds, acyclovir and ganciclovir. The antiherpetic activity was found to be strongly dependent on the nature and steric demands of the substituents in the 6 and/or 7 position. To obtain 6-Ar-TACV and 6-Ar-TGCV with improved properties (stronger fluorescence, better solubility, better transport), variations in the 4 position of phenyl ring should be investigated. Present data and further studies on the modulation of biological activity of TACV system with nonsteric demanding substituents in the 7 position may contribute to a better understanding of the structure−activity relationships of the tricyclic analogues of ACV and GCV.

Experimental Section

General Methods. The methods are as described previously.[11]

Synthetic Procedures. General procedure for the preparation of 3,9-dihydro-3-[(2-hydroxyethoxy)methyl]-9-oxo-5*H*-imidazo[1,2-*a*]purines **7**−**14**, **17**, **18**, and 3,9-dihydro-3-[(1,3-dihydroxy-2-propoxy)methyl]-9-oxo-5*H*-imidazo[1,2-*a*]-purines **25**−**28** substituted in the 6 or 6,7 positions was according to ref 3. 3,9-Dihydro-3-[(2-hydroxyethoxy)methyl]-7-hydroxymethyl-9-oxo-5*H*-imidazo[1,2-*a*]purine **23** was prepared from **1** following the procedure described for guanosine.[9]

In the case of the reaction of **1** and **2** with 2-bromopropiophenone additional reaction products (**17′** and **28′**) were isolated chromatographically as fractions moving faster than the main product. 1-(1-Benzoylethyl)-9-[(2-hydroxyethoxy)-

methyl]guanine (**17′**): yield 16%; mp 73−75 °C (propanol-2); UV (MeOH) λ_{max} 245 nm (20.9), 283 nm (10.6); ^{1}H NMR (DMSO-d_6) δ 1.57 (d, 3H, CH_3), 3.43−3.50 (m, 4H, CH_2CH_2), 4.67 (t, 1H, OH), 5.42 (s, 2H, NCH_2O), 6.25 (brs, 2H, NH_2), 6.47 (q, 1H, CH), 7.54−7.71, 8.02−8.05 (2 × m, 5H, Ph), 8.03 (s, 1H, 8-H); ^{13}C NMR (DMSO-d_6) δ 17.41 (CH_3), 59.71 (CH_2-OH), 70.34 (CH_2CH_2OH), 72.01 (NCH_2O), 72.73 ($CH-CH_3$), 113.29 (*C*-5), 128.26, 128.90, 133.52, 134.33 (Ph), 140.37 (*C*-8), 154.55 (*C*-4), 159.02, 159.32 (*C*-2, *C*-6), 197.67 (*C*=O); MS (nba): calcd for $(M + 1)^+$ 358.15151, found 358.15152. 1-(1-Benzoylethyl)-9-[(1,3-dihydroxy-2-propoxy)methyl]guanine **(28′)**: yield 19%; mp 142−145 °C (propanol-2); UV (MeOH) λ_{max} 245 nm (15.2), 283 nm (7.7); ^{1}H NMR (DMSO-d_6) δ 1.57 (d, 3H, CH_3), 3.27−3.47 (m, 4H, 2 × CH_2), 3.52−3.58 (m, 1H, CH), 4.64 (t, 2H, 2 × OH), 5.52 (s, 2H, NCH_2O), 6.23 (brs, 2H, NH_2), 6.48 (q, 1H, CH), 7.54−7.71, 8.03−8.06 (2 × m, 5H, Ph), 8.03 (s, 1H, 8-H); ^{13}C NMR (DMSO-d_6) δ 17.48 (CH_3), 60.86 (CH_2CHCH_2), 71.52 (NCH_2O), 72.59 ($CH-CH_3$), 80.16 (CH_2CH-CH_2), 113.38 (*C*-5), 128.38, 128.94, 133.48, 134.56 (Ph), 140.32 (*C*-8), 154.68 (*C*-4), 159.03, 159.50 (*C*-2, *C*-6), 197.60 (*C*=O); MS (nba): calcd for $(M + 1)^+$ 388.15950, found 388.16208.

Other 6,7-disubstituted (**19**−**22**) and 7-substituted (**24**) TACV derivatives were obtained as described previously.[10,11]

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Supporting Information Available: Preparation conditions, physical properties, spectral (^{1}H NMR, UV, fluorescence) characteristics and elemental analysis data of compounds **7**−**15**, **17**, **18**, **23**, **25**−**28**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Boryski, J.; Golankiewicz, B.; De Clercq, E. Synthesis and antiviral activity of novel N− substituted derivatives of acyclovir. *J. Med. Chem.* **1988**, *31*, 1351−1355.
(2) Boryski, J.; Golankiewicz, B.; De Clercq, E. Synthesis and antiviral activity of 3-substituted derivatives of 3,9-dihydro-9-oxo-5*H*-imidazo[1,2-a]purines, tricyclic analogues of acyclovir and ganciclovir. *J. Med. Chem.* **1991**, *34*, 2380−2383.
(3) Golankiewicz, B.; Ostrowski, T.; Andrei, G.; Snoeck, R.; De Clercq, E. Tricyclic analogues of acyclovir and ganciclovir. Influence of substituents in the heterocyclic moiety on the antiviral activity. *J. Med. Chem.* **1994**, *37*, 3187−3190, and references therein.
(4) Zielenkiewicz, W.; Golankiewicz, B.; Perlovich, G. L.; Koźbiał, M. Aqueous solubilities, infinite dilution activity coefficients and octanol−water partition coefficients of tricyclic analogues of acyclovir. *J. Solution Chem.* **1999**, *28*, 731−745.
(5) Czaplicki, J.; Bohner, T.; Habermann, A.-K.; Folkers, G.; Milon, A. A transferred NOE study of a tricyclic analogue of acyclovir bound to thymidine kinase. *J. Biomol. NMR*. **1996**, *8*, 261−272.
(6) Field, A. K.; Biron, K. K. 'The end of innocence' revisited: resistance of herpesviruses to antiviral drugs. *Clin. Microbiol. Rev.* **1994**, *7*, 1−13.
(7) Kasai, H.; Goto, M.; Ikeda, K.; Zama, M.; Mizuno, Y.; Takemura, S.; Matsuura, S.; Sugimoto, T.; Goto, T. Structure of wye (Yt base) and wyosine (Yt) from *Torulopsis utilis* phenyl transfer ribonucleic acid. *Biochemistry* **1976**, *15*, 898−904.
(8) Ostrowski, T.; Zeidler, J.; Goslinski, T.; Golankiewicz, B. Substituent-directed aralkylation and alkylation reactions of the tricyclic analogues of acyclovir and guanosine. *Nucleosides, Nucleotides Nucleic Acids* **2000**, *19*, 1911−1929.
(9) Nair, V.; Offerman, R. J. Ring-extended products from the reaction of epoxy carbonyl compounds and nucleic acid bases. *J. Org. Chem.* **1985**, *50*, 5627−5631.
(10) Zeidler, J.; Golankiewicz, B. A case of unusual sterically driven C-tritylation reaction of tricyclic analogues of acyclovir. *Tetrahedron* **1998**, *54*, 2941−2952.
(11) Goslinski, T.; Zeidler, J.; Golankiewicz, B. Unusual tritylation reactions of tricyclic analogues of acyclovir and an attempt to elucidate their mechanism. *Helv. Chim. Acta* **2000**, *83*, 373−379.

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