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Mini-review

Tricyclic nucleoside analogues as antiherpes agents

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Dedicated to Prof. Erik De Clercq on the occasion of reaching the status of Emeritus-Professor at the Katholieke Universiteit Leuven in September 2006.

Abstract

Tricyclic (T) guanine analogues are a class of compounds in which the N1 and N^2 atoms of the guanine system are linked by etheno bridge to form the 3,9-dihydro-9-oxo-5H-imidazo[1,2-a]purine system. Almost 70 tricyclic derivatives of guanine-type potent antiherpetic agents acyclovir (ACV), ganciclovir (GCV) and 9-{[cis-1′,2′-bis(hydroxymethyl)cycloprop-1′-yl]methyl}guanine were synthesized and evaluated for activity against viruses of the herpes family. Here, we review the most successful compounds in terms of their antiviral activity and physico-chemical properties. These features are modulated by the kind and position of additional substituents present in the appended third ring of aglycone. The best antiherpetic activity–fluorescence combinations as well as activity of compounds in comparison to parent congeners are summarized. The data presented indicate that compounds of the 6-(4-RPh) family are of particular importance because of their advantageous antiviral potency, increased lipophilicity and good or moderate fluorescence properties.

Keywords: Herpes simplex viruses; Antivirals; Acyclovir; Ganciclovir; Tricyclic analogues; Fluorescence

The discovery of acyclovir, 9-[(2-hydroxyethoxy)methyl]-guanine (ACV, **1**), potent and selective antiherpetic drug together with disclosure of its mechanism of action (Elion et al., 1977; Schaeffer et al., 1978; Elion, 1989) have generated much interest in the synthesis of new congeners. Acyclovir owes its antiherpetic selectivity to preferential phosphorylation by the virus-encoded thymidine kinase (TK) which confines further action to the virus-infected cell (Fyfe et al., 1978; Keller et al., 1981). The resulting acyclovir monophosphate is further phosphorylated to triphosphate form by some cellular kinases (Miller and Miller, 1980, 1982). The latter nucleotide interacts competitively with respect to deoxyguanosine triphosphate with the herpesvirus DNA polymerase, resulting in inhibition of viral DNA synthesis (Reardon and Spector, 1989).

Modifications of the sugar portion of parental guanosine have given rise to several new acyclic/carbocyclic analogues with significant selective anti-herpesvirus activity (De Clercq et al., 2001). Some of them have already been marketed as antiherpetic drugs. Among these, 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine, ganciclovir (GCV, formerly DHPG, **2**) has been the one most extensively studied for its antiviral properties (Ashton et al., 1982; Field et al., 1983; Martin et al., 1983; Ogilvie et al., 1982; Smith et al., 1982).

On the contrary, modifications of the guanine moiety of acyclovir have received much less attention and yielded few compounds with appreciable antiviral activity. Only the 8-substituted derivatives, i.e., 8-amino, 8-bromo, 8-iodo, and 8-methylacyclovir have proven to be active antiherpetic agents in vitro (Robins et al., 1984).

The study from the laboratory of the discoverers of acyclovir (Beauchamp et al., 1985) has provided convincing data that the viral enzyme does not tolerate much variations in the guanine moiety. A group of analogues was prepared which possessed some of the features of guanine molecule. The modifications included monocyclic (isocytosine, thiazole, imidazole), bicyclic (8-azapurine, pyrrolo[2,3-d]pyrimidine, pyrazolo[3,4-d]pyrimidine and tricyclic (linear benzoguanine) congeners. When evaluated against HSV-1, none of these compounds exerted significant antiherpes action.

In search to delineate the structural features of the base moiety of acyclovir which are crucial for its antiviral activity it seemed of interest to assess the importance of the nitrogen centers. In

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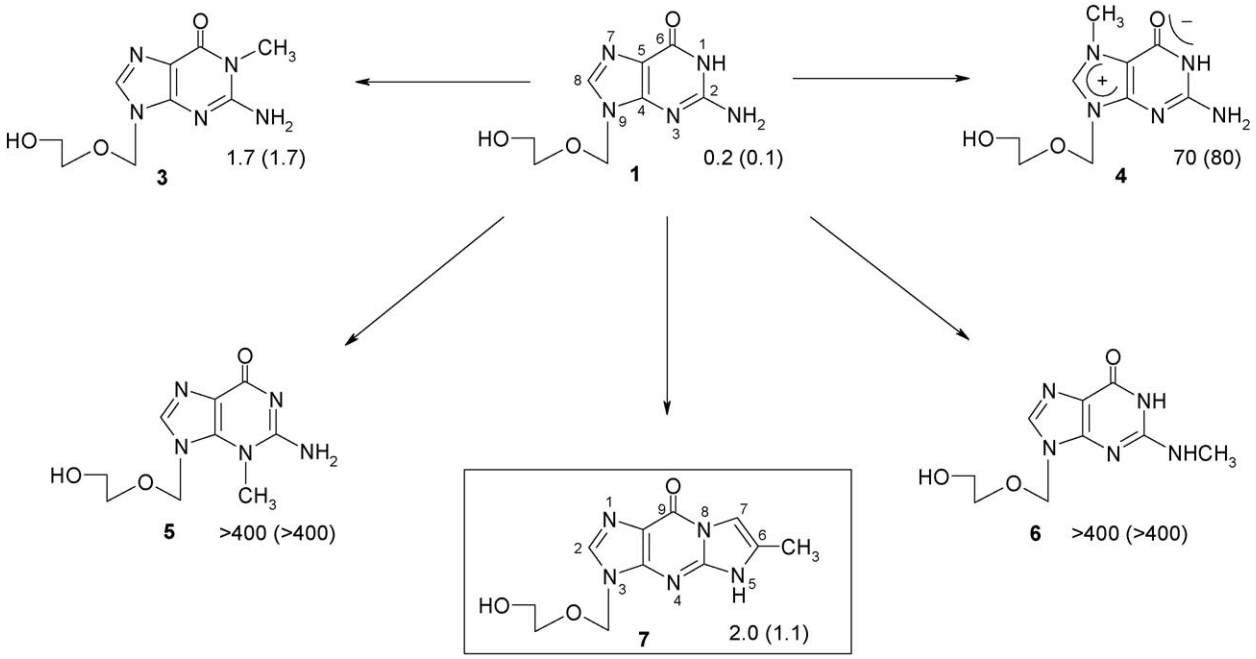
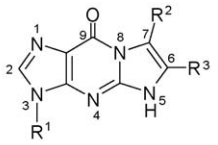
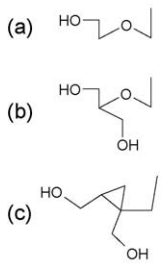


Fig. 1. N-substituted derivatives of acyclovir. Antiviral activity against HSV-1 (HSV-2), minimal inhibitory concentration (μg/mL), mean values for 3 strains.

this perspective, we prepared several new acyclovir derivatives in which one or more nitrogen centers were blocked by methylation or incorporation into an additional ring (**3-7**, Fig. 1). They were examined for their inhibitory effects on the replication of a wide variety of DNA viruses, including herpes simplex virus type 1 (HSV-1) (strains KOS, F, McIntyre), herpes simplex virus type 2 (HSV-2) (strains G, 196, Lyons), thymidine kinase deficient (TK^-) HSV-1 mutants (B2006, VMW 1837), varicella-zoster virus (VZV) (strains YS, OKA), TK^- VZV mutants (YSR, 07-01) and cytomegalovirus (CMV) (strains Davis, AD-169) (Boryski et al., 1988; Golankiewicz et al., 1991). The antiviral data of compounds **3–6** (Fig. 1) indicated the following order of decreasing importance of nitrogen centers in the antiviral activity of acyclovir: N3 $\geq N^2 >$ N7 > N1. As in the tricyclic 1,N^2-(prop-1-ene-1,2-diyl)acyclovir {according to the IUPAC systematic nomenclature 3,9-dihydro-3-[(2-hydroxyethoxy)methyl]-6-methyl-9-oxo-5H-imidazo-[1,2-a]purine} (7) the exocyclic NH_2 group at C-2 is blocked, the compound could have been expected to be inactive. However, that was not the case, the tricyclic analogue 7 exhibited a potent and selective antiherpetic activity. The spectrum of its activity was narrower than that of acyclovir limited to HSV-1 and HSV-2; lower cytotoxicity resulted in a higher selectivity index than that of acyclovir itself (Boryski et al., 1988). The studies were therefore extended to additional tricyclic analogues bearing either a 3-[(1,3-dihydroxy-2-propoxy)methyl] side chain – that are ganciclovir analogues, or 6-unsubstituted appended imidazole ring or both (Boryski et al., 1991). We found that the enhancement of activity with introduction of (1,3-dihydroxy-2-propoxy)methyl residue was higher than that for the pair acyclovir – ganciclovir. Besides, the 6-methyl substituent appeared to be of importance: its absence resulted in 6–100-fold decrease of antiviral activity which implied that substitution might become a way to shape the physical and biological properties of the tricyclic analogues of acyclovir and ganciclovir (Fig. 2).

Along this line we investigated the effect of substitution in the imidazopurine moiety of T analogues on their antiherpetic activity and physical characteristics by synthesizing a series of 9 ACV and GCV congeners substituted in the 2, 6 or 7 position. We found that generally substitution in the appended ring enhanced the antiviral activity. The magnitude of the antiviral effect depended upon: (i) the position and type of the substituent, (ii) the nature of the virus, and (iii) the kind of the acyclic moiety in the 3 position of the heterocycle. 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 6-Ph-TACV **(8)** or 6-PhPh-TACV **(9)** to 3-[(1,3-dihydroxy-2-propoxy)methyl] as in 6-Ph-TGCV **(10)** or 6-PhPh-TGCV **(11)**, gave a 100-fold enhancement of activity, whereas for the conversion of ACV to GCV this increase was approximately 10-fold. Compound **10** was also distinguished by its anti-VZV and anti-CMV activity comparable (within the same order of magnitude) to that of parental GCV. In addition, substitution in the 6 position with phenyl or 4-biphenylyl gave rise to relatively strong fluorescence (Golankiewicz et al., 1994). Fluorescent, antivirally active tricyclic analogues related to ACV and GCV 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. Intrinsically strong fluorescence properties may also allow simple and sensitive monitoring of drug concentrations in biological fluids and tissues. The advantageous fluorescence properties for the above biological applications include emission in visible region, little overlap with absorption and strong to moderate intensity. The





	R^1	R^2	R^3
TACV	a	H	H
TGCV	b	H	H
T(RA-5021)	c	H	H
7	a	H	Me
8	a	H	Ph
9	a	H	4-PhPh
10	b	H	Ph
11	b	H	4-PhPh
12	a	H	4-MeOPh
13	a	Me	Ph
14	b	H	4-MeOPh
15	b	Me	Ph
16	a	H	2-MeOPh
17	a	H	2-napht
18	a	H	4-HOPh
19	a	H	4-H_2NPh
20	a	H	4-(PhOCOO)Ph
21	b	H	4-(PhOCOO)Ph
22	a	H	4-AcOPh
23	a	H	4-IbuOPh
24	b	H	4-HOPh
25	b	H	4-HOCH$_2$PhPh
28	c	H	Ph
29	c	H	4-MeOPh
30	a	Me	4-FPh

Fig. 2. Structures of compounds discussed and abbreviations used throughout the article. TACV, tricyclic acyclovir, 3,9-dihydro-3-[(2-hydroxyethoxy)-methyl]-9-oxo-5H-imidazo[1,2-a]purine; TGCV, tricyclic ganciclovir, 3,9-dihydro-3-[(1,3-dihydroxy-2-propoxy)methyl]-9-oxo-5H-imidazo[1,2-a]purine; A-5021, (1′S,2′R)-9-{[cis-1′,2′-bis(hydroxymethyl)cycloprop-1′-yl]methyl}guanine; RA-5021, racemic form of A-5021; T(RA-5021), tricyclic RA-5021, 3-{[cis-1′,2′-bis(hydroxymethyl)cycloprop-1′-yl]methyl}-3,9-dihydro-9-oxo-5H-imidazo[1,2-a]purine.

above perspective together with continuous need for the development of new antiviral drugs resulting from the emergence of drug-resistant herpesviruses (Field and Biron, 1994) encouraged us to study further series of 6-aryl-substituted TACV and TGCV analogues to find the most advantageous combination of antiherpes activity and fluorescence properties. The consecutive steps towards that goal are shown in Table 1.

The structure–activity studies on a series of 20 diverse 6-aryl-substituted TACV and TGCV analogues (Golankiewicz et al., 2001) led to the finding of 6-(4-MeOPh)-TACV **(12)**, 7-Me-6-Ph-TACV **(13)**, 6-(4-MeOPh)-TGCV **(14)** and 7-Me-6-Ph-TGCV **(15)** that showed similar potency and selectivity as the parent ACV and GCV. The activity was found to be strongly dependent on the structure and steric demands of the substituents in the 6 and 7 positions. The tolerance of the 6-aryl substituent to modifications was quite low. Only variations in the 4 (para) position of the phenyl ring seemed to be permitted, e.g., 6-(2-MeOPh)-TACV **(16)** was at least over 20-fold less active than its 4-MeOPh isomer. The observed high antiviral potency of 6-(4-MeOPh) and 7-Me-6-Ph substituted analogues was not accompanied by an increase in fluorescence intensity. On the other hand, 6-(2-napht)-TACV **(17)** with desired fluorescence characteristics practically lost antiviral activity.

We envisioned that the introduction of an ester or amide group in the 4 position of phenyl substituent may enhance the intensity of fluorescence. A series of 12 strongly fluorescent tricyclic analogues derived from 6-(4-HOPh)-TACV **(18)** and 6-(4-H_2NPh)-TACV **(19)** followed by the TGCV counterparts of the former was synthesized and evaluated for the antiherpetic activity (Goslinski et al., 2002). The amides were only weakly active. The 6-[4-(phenoxycarbonyloxy)phenyl] substituted compounds 6-[4-(PhOCOO)Ph]-TACV and -TGCV (compounds **20** and **21**) displayed the best combination of the fluorescence and antiviral potency.

We anticipated that the ester derivatives **20–23** may be split by esterases into the parental substituted TACV or TGCV analogues. While in this case their fluorescence may be reduced to the level of the weakly fluorescent 6-(4-HOPh) substituted compounds **18** and **24**, they could still be of interest because they might serve as prodrugs of parent compounds. They would then constitute a new type of prodrug that, in contrast to the majority of the prodrugs of acyclovir and ganciclovir (Gao and Mitra, 2000) would not be blocked at the side chain hydroxyl group involved in phosphorylation and would be crucial for converting this class of compounds to their antivirally active metabolites.

In order to further improve the fluorescence characteristics of the tricyclic analogues in terms of emission extended into visible region, a series of 6 6-[(4′-R)-4-biphenylyl]-TACV and –TGCV analogues was synthesized and evaluated (Goslinski et al., 2003). Replacement of phenyl with biphenylyl substituent resulted in ∼10 nm shift of the absorption maximum to the longer wavelength region and significant (∼2×) increase of absorption intensity. The similar changes of λ^{F}_{max} were observed in fluorescence spectra. Among the 6-bifenylyl derivatives, the 6-[(4′-hydroxymethyl)-4-biphenylyl]-TGCV **(25)** showed a high (∼1000) selectivity index against HSV-1 together with advantageous fluorescence in aqueous media, with moderate yield ($\phi^F = 0.15$), emission relatively long lived ($\tau^F = 3.9$ ns) and significant intensity in visible spectral region.

The 1′S,2′R enantiomer of 9-{[cis-1′,2′-bis(hydroxymethyl)cycloprop-1′-yl]methyl}guanine (A-5021, **26**) is a guanosine analogue which exhibits the activities against HSV-1, HSV-2 and VZV exceeding those of acyclovir (Sekiyama et al., 1998; Iwayama et al., 1998). Its racemic form, RA-5021 (**27**), is only 2-fold less potent than A-5021, whereas the synthesis of **27** is much less expensive. A special feature of two fluorescent tricyclic analogues of **27**, 6-Ph- and 6-(4-MeOPh)-T(RA-5021) (compounds **28** and **29**) is their specific activity against VZV, comparable with that of parent compound (Ostrowski et al., 2006).

In connection with perspective applications of fluorescent analogues spectral properties of representative lead compound 6-Ph-TACV **(8)** were studied in more detail (Wenska et al., 2004).

Experimental study of UV absorption spectra and theoretical calculations (TD-DFT) showed that the lowest energy singlet excited state (S_1) of compound **8** has π,π^* configuration. The absorption and emission spectral properties as well as fluorescence quantum yields (ϕ^F) and lifetimes (τ^F) of 6-Ph-TACV were determined in several organic solvents with different polar-

Table 1
Selected fluorescent tricyclic analogues of ACV, GCV and RA-5021. Best combinations antiherpetic activity–fluorescence

Compound	MIC[a] (μM)								EC_{50}[b] (μM)		Fluorescence	
	HSV-1 (KOS)	HSV-1 (F)	HSV-1 (McIntyre)	HSV-2 (G)	HSV-2 (196)	HSV-2 (Lyons)	CMV (Davis)	CMV (AD-169)	VZV (YS)	VZV (OKA)	φ^{Fc}	λ^{em}_{max} (nm)
1; ACV[d]	0.089	0.018	0.311	0.400	0.044	0.040	nd	58.61	1.69	0.800	nf	
8; 6-Ph-TACV[d]	1.23	2.15	0.615	4.00	0.615	2.15	510	>300	4.30	4.00	0.079	392[e]
1; ACV[f]	0.577	0.355	0.577	0.844	0.311	1.24	nd	nd	nd	nd	nf	
12; 6-(4-MeOPh)-TACV[f]	0.281	0.197	0.084	0.422	0.563	0.563	nd	nd	nd	nd	0.095	359[g]
13; 7-Me-6-Ph-TACV[f]	0.324	0.118	0.177	0.471	0.295	0.206	nd	nd	nd	nd	0.007	366[g]
16; 6-(2-MeOPh)-TACV[f]	5.40	5.40	5.40	5.40	27.02	27.02	nd	nd	nd	nd	0.222	369[g]
17; 6-(2-napht)-TACV[f]	15.45	25.57	25.57	17.05	42.62	25.57	nd	nd	nd	nd	0.536	404[g]
1; ACV[h]	0.341	0.341	0.341	0.242	1.00	1.00	nd	nd	nd	nd	nf	
22; 6-(4-AcOPh)-TACV[h]	0.601	0.200	0.200	0.601	0.200	0.200	nd	nd	nd	nd	0.434	384[g]
23; 6-(4-IbuOPh)-TACV[h]	0.560	0.311	0.187	0.622	0.933	0.933	nd	nd	nd	nd	0.400	383[g]
20; 6-[4-(PhOCOO)Ph]-TACV[h]	0.832	0.166	0.166	0.830	0.832	0.832	nd	nd	nd	nd	0.541	388[g]
18; 6-(4-HOPh)-TACV[h]	0.675	0.225	0.225	0.675	0.375	0.225	nd	nd	nd	nd	0.070	369[g]
1; ACV[i]	0.568	0.342	1.71	1.71	0.342	0.342	nd	nd	nd	nd	nf	
30; 6-(4-FPh)-7-Me-TACV[i]	1.08	1.08	1.08	5.37	5.37	5.37	nd	nd	nd	nd	nf	
2; GCV[d]	0.012	0.016	0.016	0.078	0.157	0.016	3.53	5.88	5.49	1.96	nf	
10; 6-Ph-TGCV[d]	0.056	0.014	0.014	0.844	0.056	0.014	19.70	56.28	8.44	1.13	0.084	396[e]
2; GCV[f]	0.024	0.016	0.024	0.039	0.078	0.157	nd	nd	nd	nd	nf	
14; 6-(4-MeOPh)-TGCV[f]	0.013	0.013	0.013	0.078	0.208	0.052	nd	nd	nd	nd	0.037	357[g]
15; 7-Me-6-Ph-TGCV[f]	0.054	0.014	0.014	0.081	0.014	0.054	nd	nd	nd	nd	0.021	371[g]
2; GCV[h]	0.030	0.045	0.040	0.060	0.100	0.045	nd	nd	nd	nd	nf	
21; 6-[4-(PhOCOO)Ph]-TGCV[h]	0.008	0.031	0.781	0.417	0.781	0.156	nd	nd	nd	nd	0.480	388[g]
24; 6-(4-HOPh)-TGCV[h]	0.041	0.041	0.207	0.960	0.207	0.207	nd	nd	nd	nd	0.081	371[g]
2; GCV[j]	0.005	nd	nd	0.015	nd	nd	nd	nd	nd	nd	nf	
25; 6-(4-$HOCH_2$PhPh)-TGCV[j]	0.166	nd	nd	0.832	nd	nd	nd	nd	nd	nd	0.150	428[k]
1; ACV[l]	2.40	nd	nd	16.00	nd	nd	nd	nd	3.20	1.40	nf	
2, GCV[l]	0.480	nd	nd	3.20	nd	nd	nd	nd	nd	1.20	nf	
27; RA-5021[l]	0.130	nd	nd	0.960	nd	nd	nd	nd	0.370	0.310	nf	
28; 6-Ph-T(RA-5021)[l]	0.960	nd	nd	6.40	nd	nd	nd	nd	0.710	1.03	0.092	411[e]
29; 6-(4-MeOPh)-T(RA-5021)[l]	1.28	nd	nd	6.40	nd	nd	nd	nd	0.400	0.950	0.045	386[e]

nd: not determined; nf: non-fluorescent.

[a] Minimum inhibitory concentration or compound concentration required to reduce virus-induced cytopathogenicity by 50%.

[b] Effective concentration required to reduce virus plaque formation by 50%.

[c] Fluorescence quantum yield.

[d] From Golankiewicz et al. (1994).

[e] Spectrum measured in H_2O, excitation at 305 nm.

[f] From Golankiewicz et al. (2001).

[g] Spectrum measured in MeOH, excitation at 305 nm.

[h] From Goslinski et al. (2002).

[i] From Ostrowski et al. (2005).

[j] From Goslinski et al. (2003).

[k] Spectrum measured in H_2O-MeOH 10:1, excitation at 340 nm. nd: not determined. nf: non-fluorescent.

[l] From Ostrowski et al. (2006).

ity and in aqueous media. The absorption spectrum of **8** in all solvents studied consists of two broad band, of which the long wavelength band with maximum at $\lambda^{A}_{max} = 309$ nm (CH_3CN) is less intense ($\varepsilon_{max} \sim 10^4$ dm^3 mol^{-1} cm^{-1}). The position of the absorption bands maximum and the absorption intensity are not significantly dependent on the nature of the solvents. The room temperature emission spectra of 6-Ph-TACV in all solvents studied exhibit a single band, which are well separated from the absorption bands (Stokes shift ~5000–6000 cm^{-1}). On the other hand, the fluorescence quantum yield depends on polarity of the medium. In less polar solvent – dioxane, fluorescence is strong ($\phi^F = 0.25$). With increasing solvent polarity, in acetonitrile ($\phi^F = 0.17$) and in aqueous solution ($\phi^F = 0.053$), fluorescence becomes weaker. The decay of fluorescence intensity measured in organic solvents and in aqueous solution can be fitted with monoexponential function. The fluorescence lifetimes of 6-Ph-TACV determined from the time resolved measurements fall in nanosecond range ($\tau^F = 6.2$ ns in CH_3CN and 2.4 in H_2O pH 5.8).

The spectral properties and fluorescence quantum yield of 6-Ph-TACV are also dependent on pH of aqueous media. The pH dependence is a result of acid–base equilibria in the ground state. The values for neutral molecule **8**-anion and neutral molecule **8**-cation equilibrium constants determined from spectrophotometric titration amount to $pK_a = 8.0$ and 2.0, respectively. The study performed in aqueous buffers solution in the broad range (pH 0–12) revealed that in basic solution (pH 10) the maximum of the long wavelength absorption band is located at $\lambda_{max} = 327$ nm and is red shifted by 21 nm from the maximum in neutral solution. With increasing pH (pH > 5.8) the fluorescence intensity of 6-Ph-TACV decreases and emission maximum shifts towards longer wavelength. Weak ($\phi^F = 0.013$) fluorescence with maximum at $\lambda^{F}_{max} = 405$ nm observed at pH 10 was ascribed to the emission from an excited anionic species derived from 6-Ph-TACV. The cationic form of 6-Ph-TACV absorbs at longer wavelength ($\lambda_{max} = 315$ nm) than the neutral molecule but it is non-fluorescent. The above spectral study provided also important information about the stability of T analogues in aqueous buffers solution in the pH range 0–12.

Non-fluorescent 6-(4-FPh)-7-Me-TACV **(30)** included in Table 1 is the most promising out of 7 compound series of fluoro-substituted aryl derivatives of TACV and TGCV. It may be useful as a labeled substrate for ^{19}F NMR studies of the TK–ligand interaction and/or monitoring of its metabolites in cells expressing HSV TK (Ostrowski et al., 2005).

Tricyclic analogues, especially those bearing 6-aryl substituents, were found to be much more lipophilic than parent ACV and GCV. This was found by determination of their *n*-octanol/water partition coefficients (Zielenkiewicz et al., 1999) and *n*-octanol/phosphate buffered saline (PBS) pH 7.5 partition coefficients together with examining their retention times on a reverse phase HPLC column (Balzarini et al., 2002). This may allow better uptake of these derivatives from the blood into the central nervous system. The 6-methyl substitution in compound **7**, on the other hand, resulted in improved aqueous solubility in comparison with ACV (Zielenkiewicz et al., 1999).

The crystal structure of 6-Me-TACV·$2H_2O$ was found to have three different conformations in the crystal, an indication of high conformation flexibility (Suwinska et al., 2001). Thermodynamic properties of aqueous solution of **7** were also studied. The determination of densities, the standard enthalpy of solution and the enthalpy of hydration of the antiviral agent can be useful in understanding of its interactions with enzymes (Zielenkiewicz et al., 1998).

It is still disputable whether the T analogues are intrinsically active or they are prodrugs which convert to ACV, GCV or RA-5021. The evidence supporting intrinsic activity of tricyclic analogues includes: (i) their stability at room temperature, pH various, (ii) NMR study using transferred NOE (nuclear Overhauser effect) of a tricyclic analogue of ACV bound to thymidine

Table 2
Selected fluorescent tricyclic analogues of ACV, GCV and RA-5021; antiherpetic activity as compared to parent congeners[a,b]

Compound	HSV-1 (KOS)	HSV-1 (F)	HSV-1 (McIntyre)	HSV-2 (G)	HSV-2 (196)	HSV-2 (Lyons)	VZV (YS)	VZV (OKA)
8; 6-Ph-TACV	14	120	2	10	14	54	2.5	5
12; 6-(4-MeOPh)-TACV	+2	+2	+7	+2	2	+2	nd	nd
13; 7-Me-6-Ph-TACV	+2	+3	+3	+2	1	+6	nd	nd
22; 6-(4-AcOPh)-TACV	2	+2	+2	2.5	+5	+5	nd	nd
23; 6-(4-IbuOPh)-TACV	2	1	+2	3	1	1	nd	nd
20; 6-[4-(PhOCOO)Ph]-TACV	2	+2	+2	3	1	1	nd	nd
18; 6-(4-HOPh)-TACV	2	+1.5	+1.5	3	+3	+4	nd	nd
30; 6-(4-FPh)-7-Me-TACV	2	3	+2	3	16	16	nd	nd
10; 6-Ph-TGCV	5	1	1	11	+3	1	1.5	+2
14; 6-(4-MeOPh)-TGCV	+2	1	+2	2	3	+3	nd	nd
15; 7-Me-6-Ph-TGCV	2	1	+2	2	+6	+3	nd	nd
21; 6-[4-(PhOCOO)Ph]-TGCV	+4	+1.5	20	7	8	3.5	nd	nd
25; 6-(4-HOCH$_2$PhPh)-TGCV	35	nd	nd	56	nd	nd	nd	nd
28; 6-Ph-T(RA-5021)	7.5	nd	nd	7	nd	nd	2	3
29; 6-(4-MeOPh)-T(RA-5021)	10	nd	nd	7	nd	nd	1	3

[a] The activities of the parent ACV **(1)**, GCV **(2)** and RA-5021**(27)** the same as in Table 1.

[b] Regular numbers are multiples showing the activity lower than that of parent compounds; higher activity is indicated by "+"; decimals are rounded up and down; e.g. HSV-1 (KOS) column: in comparison to **1**, **2**, **27** activity of **8**, **10**, **28** is respectively 14, 5, 7.5 times less, activity of **12**, **14** is respectively 2, 2 times more, activity of **29** is respectively 10 times less.

kinase (Czaplicki et al., 1996), (iii) an efficient conversion of 6-substituted and 6,7-disubstituted tricyclic derivatives of ACV and GCV to their triphosphates when exposed to purified HSV-1 TK, in a reaction mixture also containing purified GMP kinase and NDP kinase (Balzarini et al., 2002), (iv) the flexibility of the acyclic chains of acyclonucleosides and observed various modes of binding to thymidine kinase active site (Brown et al., 1995; Champness et al., 1998; Bennett et al., 1999) suggesting that the enzyme is able also to host the corresponding tricyclic analogues (v) the diverse magnitude of change of activity upon conversion of the bicyclic parent compounds **1**, **2** and **27** into their analogously 6-substituted tricyclic congeners (as shown in Table 2, e.g., for compounds **8**, **10** and **28**). It is a matter of discussion and further evaluation to explain the mechanism of action of tricyclic derivatives of ACV, GCV and RA-5021 in cells.

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