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Enhancement of aqueous solubility of tricyclic acyclovir derivatives by their complexation with hydroxypropyl-β-cyclodextrin

Wojciech Zielenkiewicz · Małgorzata Koźbiał · Bożenna Golankiewicz · Jarosław Poznański

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Abstract Solubilities of tricyclic acyclovir derivatives in buffered aqueous solutions of hydroxypropyl-β-cyclodextrin (HP-β-CD) at pH 5.5 and 7.0 were determined at 25 and 37 °C. Complexation of these compounds with HP-β-CD resulted in a noticeable increase of their solubility; nevertheless it was limited to tricyclic derivatives of acyclovir carrying an aryl substituent. Combination of ^{1}H NMR and DSC techniques demonstrated the existence of inclusion complexes between acyclovir derivatives and HP-β-CD. The stability constants, estimated using the Higuchi–Connors method, were found in the range of 10–100 M^{-1}. Additionally, the pK_a values at 25 °C and molar extinction coefficients in aqueous buffered solutions were also determined for all studied compounds.

Keywords Acyclovir derivatives · Hydroxypropyl-β-cyclodextrin · Solubility enhancement

Introduction

Acyclovir, ACV, (9-[2-hydroxy)methyl] guanine) is well known as a selective antiherpetic drug [1]. Some substitutions at the TACV system have proven to result in desirable physicochemical properties, e.g., fluorescence with biological activity somewhat changed but still present [2–4].

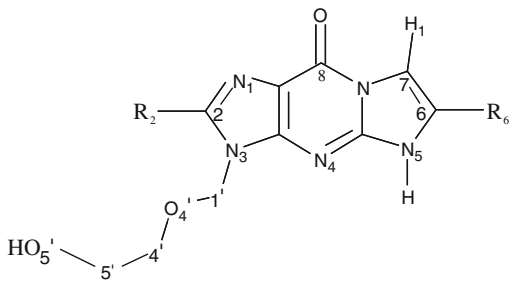
W. Zielenkiewicz · M. Koźbiał · J. Poznański (✉)
Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warszawa, Poland
e-mail: jarek@ichf.edu.pl

B. Golankiewicz
Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznan, Poland

The modification of its guanine moiety by linking an etheno bridge to nitrogen atoms was led to tricyclic analog **1**, TACV, (3,9-dihydro-3-[(2-hydroxyetoxy)methyl]-9-oxo5H-imidiazo]1,2a] purine). Some substitutions at the TACV system have proven to result in desirable physicochemical properties, e.g., fluorescence with biological activity somewhat changed but still present. The magnitude of the antiviral effect depended upon: the position and type of the substituent, the nature of the virus, and the type of the exocyclic moiety attached to the N_3 atom located in the heterocycle. Especially, the substitution in the 6th position by a phenyl or 4-biphenylyl group afforded the greatest increase in antiviral activity. In principle, numerous 6-phenyl congeners of ACV display decreased cytotoxicity and relatively high activity against HSV-1 and HSV-2 [5]. In our previous study, we have determined some thermodynamic properties of this group of compounds [6–8], including aqueous and octanol solubility, as well as octanol–water partition coefficient. These experiments have demonstrated extremely low aqueous solubility of a majority of these compounds (10^{-3} to 10^{-5} mol kg^{-1}). In the present study, we focused to find a method for enhancing the solubility of tricyclic analogs of acyclovir in aqueous solutions. We have carried out a number of experiments on complexation of the studied compounds by cyclodextrin. Complexation of drugs by cyclodextrins frequently results in aqueous solubility enhancement. There are few reports on the complexation of acyclovir and its derivatives by cyclodextrins [9–15], and the enhancements of solubility of the examined compounds were reported rather small. The complexation of drugs by cyclodextrin may also change their physicochemical and biochemical properties. The stability and bioactivity of a complexed drug may increase. It is very important for acyclovir and its derivatives, because their low solubility makes problems in

preparing required doses. The overall oral bioavailability of acyclovir derivatives is thereby strongly affected by their solubility, reaches only 15–30%. Luengo et al. [12] used β-cyclodextrin to enhance bioavailability by forming inclusion complexes with acyclovir. The results obtained in vivo on male rats indicated the higher bioavailability of complexed drug. Analogous results were obtained by Chavanpatil and Vavia [11] in the studies on influence of cyclodextrins on nasal absorption of acyclovir. Their results indicated that hydroxypropyl-β-cyclodextrin (HP-β-CD) was more effective for enhancing the nasal absorption of acyclovir than the other ones. The hydroxylpropyl derivatives of cyclodextrin are very often used in pharmacy due to their better complexation ability than that of natural cyclodextrin and superior behavior in human body. HP-β-CD was used by Tirucherai and Mitra [10] to enhance the aqueous solubility, stability, and in vitro corneal permeation of acyl ester prodrugs of ganciclovir. Considerable improvement in chemical and enzymatic of ganciclovir derivatives was observed in the presence of HP-β-CD.

The main subject of our study was the determination of the solubility of tricyclic acyclovir analogs presented in Fig. 1 in different concentrations of HP-β-CD solutions at two physiological pH equal 5.5 and 7.0. The study of complexations was carried out using the Higuchi–Connors method [16] at 25 and 37 °C. The obtained results were analyzed in relation to the size of molecule and its dissociation pK_a values estimated using UV–VIS spectrophotometric titrations. The host–guest complexes were also analyzed by the combination of NMR and thermal analysis, which we found efficient in the study of the systems of similar size [17].



		R_2	R_6
1	TACV	H	H
2	2-Br-6-Me-TACV	Br	CH_3
3	6-t-But -TACV	H	$C(CH_3)_3$
4	6-Ph-TACV	H	C_6H_5
5	6-(2-Napht)-TACV	H	2-Naphtyl

Fig. 1 Structures of the studied tricyclic derivatives of acyclovir

Experimental

Materials

The subject of our studies was the following compounds presented in Fig. 1: 3,9-dihydro-3-[(2-hydroxyetoxy)methyl]-9-oxo-5H-imidazol[1,2-a]purine (TACV) **1** and its substituted derivatives: 2-bromo-6-methyl (2-Br-6-Me-TACV), **2**; 6-tert-butyl (6-t-Bu-TACV), **3**; 6-phenyl (6-Ph-TACV), **4**; 6-(2-nphtyl) ([6-(2-napht)-TACV]), **5**; synthesized by Golankiewicz et al. [2–4]. The physicochemical properties have been determined during our earlier study [5–8]. The HP-β-CD was purchased from Janssen Drug Delivery System (USA) R81216 no 30 221 54 and used without additional purification. KH_2PO_4 and Na_2HPO_4 salts used for preparation of buffer solutions were of analytical grade (POCh, Gliwice, Poland). Distilled and deionised water from a Milli-Q system (Millipore, USA) was used for solution preparation.

Solubility measurement

Solubility studies were carried out according to excessive method of Higuchi and Connors [16]. An excess amount of compounds **1–5** (2–3 mg) oversaturated solutions was added to 1.5 mL of buffered water solutions, pH = 5.5 or 7.0 containing various concentration of HP-β-CD. The cyclodextrin concentration varied in the range of 10^{-3} to 2×10^{-2} mol kg^{-1}. The suspensions were shaken in screw-capped vials in a water bath thermostat with the volume of 60 dm^3 (home made apparatus by IChF PAN) at 25 and 37 °C. After 7 days, the equilibrium was reached, and then the suspensions were centrifuged and the absorbance of the studied compounds was measured by UV–VIS spectrophotometry using Shimadzu UV 2401 PC Spectrometer equipped with a thermostatically controlled cell compartment. During the measurement of absorbance, in the reference cuvette there was always a buffered-solution of HP-β-CD of the same concentration as in the studied solution. The absorbance data used for calculation of the concentrations of the particular acyclovir derivatives were the average of at least three independent determinations. The molar extinction coefficients at the maximum of absorbance for all compounds studied in buffered aqueous solutions at pH = 5.5 and 7.0 were determined and used for the calculation of compounds **1–5** concentrations in cyclodextrin solutions. The pK_a was calculated using the absorbance measurement during the titration of acyclovir derivatives **1–5** aqueous solutions by 0.01 mol dm^{-3} HCl or NaOH.

Thermogravimetry

The determination of water content in HP-β-CD was performed using DuPont TGA 951 thermogravimetric

analyzer in the range of 25–200 °C or 25–300 °C, at a rate 10 °C min^{-1}. The samples of about 10 mg were dehydrated by heating two times up to 200 °C [18], and finally up to 300 °C.

Differential scanning calorimetry (DSC)

The thermal behavior of all studied compounds, their physical mixtures with cyclodextrin, and the corresponding complexes were studied by DSC measurements using Du Pont Instruments model 9900A Thermal Analyzer equipped with a model 910 DSC cell. Rate of heating was 10 °C min^{-1}. The complexes in the solid state were prepared by evaporating of aqueous mixtures containing HP-β-CD and acyclovir derivative in molar ratio 10:1. Samples of pure compounds under study, cyclodextrin complexes in solid state, cyclodextrin and physical mixtures (5–10 mg) were placed in Al pans. Measurements were made using flow of argon. The DSC device was calibrated using indium samples.

NMR spectroscopy

^{1}H NMR spectra were recorded on 500 MHz spectrometer (Unity Plus Varian) at 25 °C in D_2O. The tricyclic acyclovir **1** and its derivatives, **3**, **4**, were dissolved at concentration of 5×10^{-5} mol kg^{-1} either in D_2O or HP-β-CD solution (5×10^{-5} mol kg^{-1}). All spectra were processed with the aid of MestRe-C 2.3a [19]. Zero-filling up to 32 k data points and Lorenzian filter resulting in 1 Hz line broadening were applied prior to Fourier transformation.

Results and discussion

The absorbance spectra of the studied compounds, **1–5**, changed with the pH of solution due to protonation/deprotonation of nitrogen atoms in the drug skeleton. The pK_a values at 25 °C and the molar extinction coefficient, ε, at λ_{max} of all compounds were determined at pH = 5.5 and 7.0 and are summarized in Table 1. The absorption spectra of compounds **1–5** depend on pH of solution. With increase of pH, the bands shift to red, and a new band appears. As expected, upon titrations the spectra showed isosbestic points. The nonsubstituted tricyclic analog of acyclovir showed the same character of absorbance changes (two isosbestic points) as alkyl-substituted compounds. The compounds containing the aromatic substituents showed more complicated changes (four isosbestic points). These results are similar to those obtained earlier for other tricyclic analogs of acyclovir with alkyl or aromatic substituents [20]. The pK_a values for the compound with an aromatic group are close to 2.0 and 8.0, and with an alkyl group 2.0 and 9.0, respectively. The differences are connected with the deprotonation of the nitrogen atom, which is located at 5-position, near the substituents at 6-position. The variation of UV–VIS spectra of unsubstituted compound, TACV, and its phenylated congener, 6-Ph-TACV, undergoing upon pH changes are presented in Fig. 2.

The aqueous solubility of the studied compounds was found to increase almost linearly with the HP-β-CD concentration. The one exception is compound **3** possessing a *tert*-butyl constituent, for which the relation was found nonlinear. The solubility of compounds **1–5** in pure aqueous solution, the stability constants estimated for their complexes with HP-β-CD, and the enrichment of solubility estimated for 20 mM HP-β-CD aqueous solution are presented in Table 2. As demonstrated in Fig. 3, the aqueous solubility of the studied compounds depends both on pH and the temperature of the solutions. The solubility data collected in Table 2 indicate that the largest enhancement of the solubility and highest stability constants values were obtained for compounds **4–5** carrying an aromatic

Table 1 Molar extinction coefficients, ε, at wavelength maxima, λ_{max}, determined for aqueous solutions of compounds **1–5** at pH = 5.5 and 7.0, and protonation constants, pK_a, of these compounds estimated at 25 °C

Compound	λ_{max}	$\varepsilon/10^4$ M^{-1} cm^{-1}			pK_a
		Water	pH = 5.5	pH = 7.0	
1. TACV	226	3.21	3.29	3.12	2.27(±0.20); 9.07(±0.04)
	284	1.10	1.28	1.19	
2. 2-Br-6-Me-TACV	235	3.27	3.32	3.30	–; 9.14(±0.06)
	284	1.34	1.36	1.35	
3. 6-t-But-TACV	230	3.62	3.63	3.72	1.85(±0.17); 9.60(±0.06)
	284	1.17	1.22	1.26	
4. 6-Ph-TACV	250	1.79	2.20	2.38	1.71(±0.25); 7.97(±0.07)
	306	0.55	0.78	0.76	8.0 [12]
5. 6-(2-Napht)-TACV	254	3.13	3.44	3.42	1.87(±0.13); 8.10(±0.10)
	315	0.76	0.94	0.94	

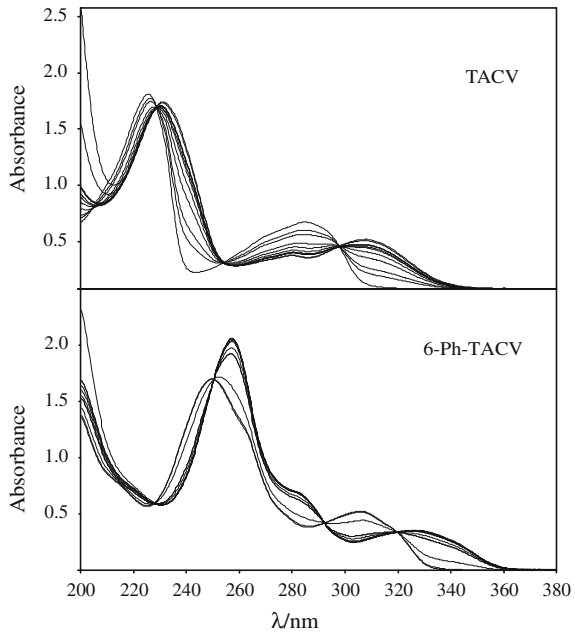


Fig. 2 The absorption spectra recorded upon titration of 5×10^{-5} mol kg^{-1} aqueous solutions of TACV and 6-Ph-TACV

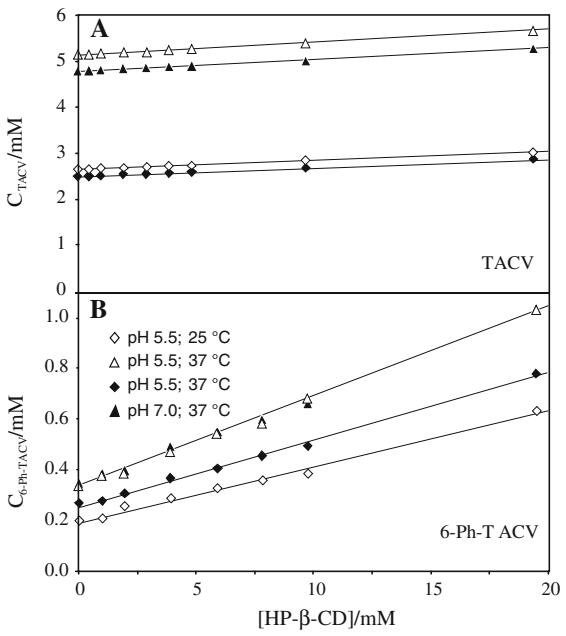


Fig. 3 The dependence of solubility of of TACV (**a**) and 6-Ph-TACV (**b**) as a function of HP-β-CD concentration. The data were recorded at 25 and 37 °C; for aqueous solution of pH = 5.5 and 7.0

substituent. The observed increase in solubility should be attributed to the formation of inclusion complexes between acyclovir derivatives and HP-β-CD, probably by protecting the aryl substituents from water accessibility by their inclusion inside the cyclodextrin hydrophobic cavity. The enhancements of aqueous solubility of these species are in the range from 3.0 to 8.1, depending on the temperature and solution pH. It should be pointed that the increase of aqueous solubility of compounds without an aromatic group is significantly lower both at 25 and 37 °C. The process of complexation of acyclovir derivatives by HP-β-CD was connected with the protonation of guest molecules and association between guests or cyclodextrins molecules in solutions, so for all the acyclovir derivatives under study the stability of complexes with HP-β-CD was higher at pH 5.5. The architecture of tricyclic derivatives of acyclovir–HP-β-CD complexes was studied by using DSC and ^{1}H NMR measurements.

Table 2 DSC-derived melting temperature, T_m, and the associated heat, H_m of acyclovir derivatives, their solubility in aqueous solution, c_{aq}, the stability constants of their complexes with HP-β-CD, K_s, and the relative enhancement of their solubility in the presence of 20 mM cyclodextrin aqueous solution, c/c_{aq}

Compound (T_m; H_m)	pH	c_{aq}/mM		K_s/M^{-1}		c/c_{aq}	
		25 °C	37 °C	25 °C	37 °C	25 °C	37 °C
TACV	5.5	2.64	5.16	8(±1)	6(±1)	1.1	1.1
(255.6 °C; 121.8 J/g)	7.0	2.48	4.79	8(±1)	6(±1)	1.1	1.1
2-Br-6-Me-TACV	5.5	1.18	2.36	27(±2)	26(±2)	1.4	1.5
	7.0	1.03	2.02	26(±2)	17(±1)	1.5	1.3
(207.9 °C; 137.8 J/g)							
6-t-But-TACV	5.5	3.90	7.02	–	–	1.5	1.6
	7.0	4.26	7.75	–	–	1.2	1.3
(208.2 °C; 85.8 J/g)							
6-Ph-TACV	5.5	0.19	0.34	122(±2)	113(±10)	3.3	3.0
(234.5 °C; 122.9 J/g)	7.0	0.26	0.34	107(±8)	105(±6)	3.1	3.0
6-(2-Napht)-TACV	5.5	0.026	0.035	297(±7)	279(±9)	7.7	6.4
	7.0	0.026	0.042	311(±9)	305(±6)	8.1	6.9
(235.0 °C; 41.8 J/g)							

The data were determined at 25 °C and 37 °C for neutral (pH 7) and acidic (pH 5.5) solutions

DSC measurements demonstrated that heat flow observed for the physical mixtures of compounds **1**–**5** and HP-β-CD represent the superposition of the experiments recorded separately for TACV congeners and HP-β-CD (data not shown), while no endothermic peaks were observed for the putative complexes (c.f Fig. 4 for the DSC thermograms recorded for TACV and 6-Ph-TACV). This indicates the absence of microcrystals of TACV analogs in the sample.

^{1}H NMR data were recorded for TACV (**1**) and its derivatives, **3**, **4**, in aqueous solution, and in the presence of HP-β-CD in a 1:1 and 1:10 (TACV:HP-β-CD) concentration ratio. The analysis of the ^{1}H NMR spectrum of HP-β-CD (c.f. Fig. 5, bottom trace) demonstrated the divergence in hydrodroxypropyl substitution pattern, what results in the

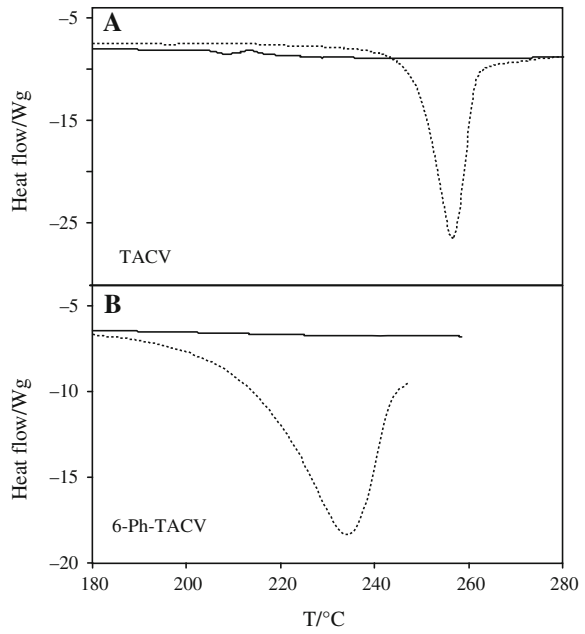


Fig. 4 DSC thermograms obtained for TACV (**a**) and 6-Ph-TACV(**b**) for the pure solid states (*dotted lines*) and their complexes with HP-β-CD (*solid lines*). The absence of endothermic peaks characteristic for the pure compounds indicate the interactions between the compounds and the HP-β-CD

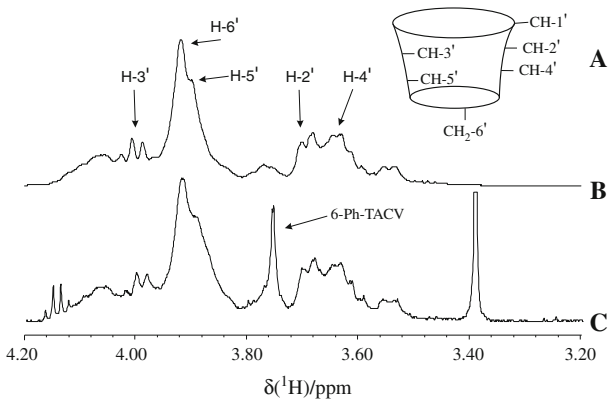


Fig. 5 Schematic location of HP-β-CD aliphatic protons on the macrocyclic ring (**a**) and ^{1}H NMR spectra of HP-β-CD (5×10^{-5} mol kg^{-1}) measured in pure D_2O at 25 °C (**b**), and for its 1:1 mixture with 6-Ph-TACV (**c**)

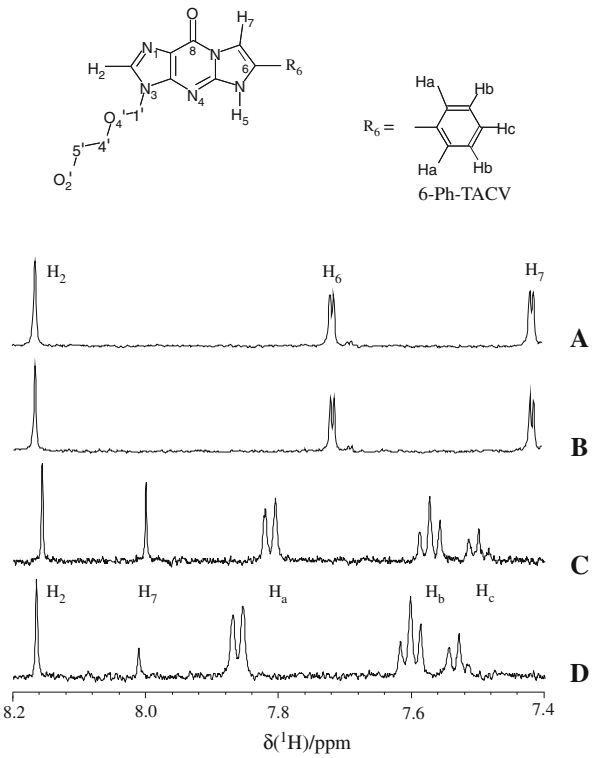


Fig. 6 ^{1}H NMR spectra of TACV and 6-Ph-TACV (both at 5×10^{-5} mol kg^{-1} concentration) recorded at 25 °C in D_2O for pure compounds (**a**, **c**), and their 1:1 mixtures with HP-β-CD (**b**, **d**, respectively)

significant broadening of the resonance lines, which practically disables the quantitative analysis of TACV complexation. A small variation in the shape of the spectrum was observed mainly for H3′ and H5′ resonances, whereas no significant changes were observed for H2′, H4′, and H6′ signals. That suggest that the inclusion undergoes from the H1′ side. This is mostly evident for the complex of HP-β-CD with **4**, while the complexation of the two others TACV derivatives (**1**, **3**) induces smaller changes in ^{1}H spectrum of cyclodextrin. Moreover, the spectra of HP-β-CD complexed with either **1** or **3** are almost identical, while that one of complex with **4** differs by the location of H3′, shifted upfield by $\sim$0.01 ppm. The complexation phenomenon was also monitored on the basis of the changes in TACV resonances. The most significant HP-β-CD-induced variations were observed for the resonances of benzene ring of **4** (Ha, Hb, Hc), as presented in Fig. 6. This clearly indicates that this fragment of TACV derivative is included inside the cyclodextrin cavity. The changes in the resonances of tricyclic ring were found at least 10 times smaller. Additionally, the splitting of H4′, H5′ resonances of methylene groups were observed for **4** as a result of interaction of the sugar-mimicking N_3 substituent with the rim of cyclodextrin macrocycle. The HP-β-CD-induced shifts observed for **1** and **3** were found negligible (c.f. Fig. 6).

Based on the NMR data we may propose a general model of the complex. Thus, the phenyl group of 6-Ph-TACV is placed deeply inside the CD cavity, while the analog of sugar exocyclic C5′ group is weakly interacting with the edge of cyclodextrin ring. The two other TACV (**1**, **3**), which do not carry a phenyl group, were found to interact with CD significantly weaker. The phenyl group attached to TACV ring seems to be crucial for the efficient complex formation. For the other compounds, the transfer of a relatively polar ring carrying dissociable proton H(N_5)

from aqueous solution towards the apolar cyclodextrin cavity is so unfavorable, that it almost disables complex formation.

The results of ^{1}H NMR measurement confirm that significant enhancement of aqueous solubility of tricyclic acyclovir derivatives was mainly caused by complexation of drug molecules by HP-β-CD. The aqueous solubility of TACV and its aryl congeners were found log-dependent on the molecular volume of the molecules (results not shown), what, according to the LCW theory [21], indicates the hydrophobic solvation as the factor limiting the dissolvation phenomena.

It must be stressed that the stability constants found for the aryl derivatives of TACV of the range $\sim 100\ M^{-1}$ are sufficient for the improvement the therapeutic properties of the carried drug—too high values may shift the dissociation equilibrium toward the bound state, decreasing the effective drug concentration in the bio-fluids.

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