



# Comparative analysis of stability of tricyclic analogues of acyclovir in an acidic environment

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## Abstract

In the presented studies, the effect of hydrogen ions on the stability of the tricyclic aciclovir derivative (i.e. 3,9-dihydro-3-[(2-hydroxyethoxy)methyl]-6-(4-methoxyphenyl)-9-oxo-5*H*-imidazo[1,2-*a*]purine; 6-(4-MeOPh)-TACV) and its esters (acetyl (Ac-), *iso*-butyryl (*i*But-), pivaloyl (Piv-), ethoxycarbonyl (Etc-) and nicotinoyl (Nic-)) has been assessed. The HPLC chromatographic method (Lichrospher RP-18 column, 5  $\mu$ m, 250 mm  $\times$  4 mm) with UV detection (262 nm) was used to observe changes in the concentration of the tested compounds. The mobile phase consisted of acetonitrile–phosphate buffer (pH 6; 20 mM; 17 mM  $\text{KH}_2\text{PO}_4$ , 3 mM  $\text{K}_2\text{HPO}_4$ ) (35:65, v/v). The studies were carried out in the pH range 0.42–1.38 in the water-organic environment at constant ionic strength (0.50 M). The observed rate constants of the degradation reactions of the tested compounds were determined, kinetic equations describing the dependence of  $k_{\text{pH}}$  as a function of pH for the proper acid catalysis were determined and the parameters of the equations describing the reaction of formation the observed product were determined. The dependence of durability on the chemical structure of the tested compounds was indicated and the mechanism of observed reactions was proposed.

**Keywords** HPLC · Prodrugs · Stability · Tricyclic analog of acyclovir · pH profiles

## Introduction

Acyclovir manifests antiviral activity mainly with regard to the viruses of labial herpes (HSV-1), genital herpes (HSV-2) as well as varicella zoster virus (VZV). The efficiency of the eradication of other viruses from the *Herpesviridae* family, such as

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Epstein-Barr virus and cytomegalovirus, is much lower [1]. The bioavailability of acyclovir after oral administration amounts to 15–30%. It is not a high value; however, the use of sufficiently high doses allows for obtaining therapeutic concentrations of the medication in the blood [2]. It is also characterized by a shorter half-life of 2–3 h. Such fast elimination from the body causes problems with the maintenance of a sufficient concentration of the medication in the body [2, 3]. Furthermore, ACV poorly exceeds the blood–brain barrier, which limits its application, e.g. in encephalitis caused by HSV [1, 4].

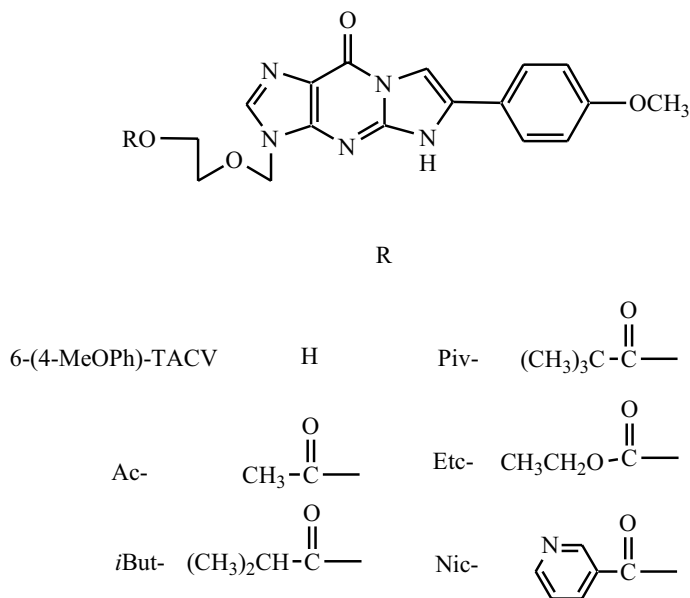
These inconveniences and limitations lead to continuous search for acyclovir analogues and prodrugs. Concerning the prodrugs, numerous esters have been synthesized: acyl derivatives [5], compounds modified with amino acids [6, 7] (including clinically used valacyclovir [6]), dipeptide [8, 9], fatty acids and biotin [10] and polyethylene glycol derivatives [11].

Linking the N<sup>2</sup> and N1 atoms *via* an etheno bridge resulted in the formation of tricyclic compound (TACV), a derivative of 3,9-dihydro-9-oxo-5*H*-imidazo[1,2-*a*]purine, which was devoid of antiviral activity [12]. Introduction of a methyl substituent in the 6 position of the tricyclic system of TACV enhances its activity: 6-Me-TACV exhibited only 10–100-fold lower activity than ACV (depending on the virus strain and the kind of cells used) [13]. Generally, as is reviewed [14], the substituents in the appended ring allow to modulate the biological and physical properties of the resulting compounds. The structure-activity studies on a series of 6- or/and 7-substituted TACV analogues led to finding the fluorescent 6-phenyl and 6-(4-methoxyphenyl) congeners displaying considerably enhanced activities [15, 16]. The latter one, 6-(4-MeOPh)-TACV, showed antiherpetic potency and selectivity comparable to those of parent acyclovir. Both compounds exhibit increased lipophilicity as compared with ACV which can contribute to more efficient crossing of blood–brain barriers [17, 18].

In the existing literature references, there is no information concerning the kinetics of reaction of decomposition of ACV tricyclic derivatives in an acidic environment. That is why, the purpose of the presented research was to assess the influence of such factors as hydrogen ions on the decomposition of the studied compounds: 3,9-dihydro-3-[(2-hydroxy-ethoxy)methyl]-6-(4-methoxyphenyl)-9-oxo-5*H*-imidazo[1,2-*a*]purine (6-(4-MeOPh)-TACV) and its esters. In the framework of the research, it was decided to describe the observed reactions with a kinetic equation and to use the HPLC method for assessment of changes in concentration of the studied compounds in time.

## Materials and methods

3,9-Dihydro-3-[(2-hydroxyethoxy)methyl]-6-(4-methoxyphenyl)-9-oxo-5*H*-imidazo[1,2-*a*]purine (6-(4-MeOPh)-TACV) and its esters: acetyl (Ac), *iso*-butyryl (*i*But), pivaloyl (Piv), ethoxycarbonyl (Etc) and nicotinoyl (Nic) (Fig. 1), were synthesized as previously reported in a laboratory scale [16, 19]. Acetonitrile for HPLC (isocratic basis) was from Avantor Performance Materials Poland S.A. (Gliwice, Poland). Ethyl 4-hydroxybenzoate (99%) and methyl 4-hydroxybenzoate (>99%)



**Fig. 1** The chemical structure of derivatives of 9-oxo-5*H*-imidazo[1,2-*a*]purine: 6-(4-MeOPh)-TACV, Ac-6-(4-MeOPh)-TACV, *i*But-6-(4-MeOPh)-TACV, Piv-6-(4-MeOPh)-TACV, Etc-6-(4-MeOPh)-TACV and Nic-6-(4-MeOPh)-TACV

were from Sigma-Aldrich (Steinheim, Germany). Deionized water was acquired from Millipore Simplicity UV water purification system (Waters Corporation, Milford, MA, USA). All other chemicals of the highest purity were commercially available.

Chromatographic separation and quantitative analysis were performed by using the high performance liquid chromatography method. The HPLC system consisted of a Rheodyne 7120, 20  $\mu\text{L}$  fixed-loop injector, an LC 3-UV detector (Pye Unicam, Cambridge, England), an L-6000A pump (Merck-Hitachi, Darmstadt, Germany), and an A/C transmitter with Chromed software (Medson, Poznan, Poland).

The pH values of the buffer solutions were measured on a HI 110 pH-Meter (HANNA Instruments, Cluj-Napoca, Romania).

### Chromatographic conditions (HPLC-UV)

As the stationary phase a Lichrospher RP-18 column, 5  $\mu\text{m}$  particle size, 250 mm  $\times$  4 mm, (Merck, Darmstadt, Germany) and isocratic elution were used. The mobile phase consisted of acetonitrile–phosphate buffer (pH 6; 20 mM; 17 mM  $\text{KH}_2\text{PO}_4$ , 3 mM  $\text{K}_2\text{HPO}_4$ ) (35:65, v/v). The flow rate of the mobile phases was 1.0 or 1.5 mL/min (Table 1) and the injection volume was 20  $\mu\text{L}$ . The UV detection was carried out at 262 nm [19].

**Table 1** The type and concentration of internal standards along with the retention times of test compounds and their potential degradation products observed on HPLC chromatograms

Flow rate (mL/min)	Internal standard (i.s.)			Compound	$t_R$ (min)
	4-Hydroxybenzoate	c ( $\mu\text{g/mL}$ )	$t_R$ (min)		
1.5	Ethyl	80	6.18	ACV	1.20
				Ac-	3.87
				<i>i</i> But-	8.12
1.0	Ethyl	80	9.42	Nicotinic acid	1.20
				6-(4-MeOPh)-TACV	3.27
				Nic-	6.20
				Piv-	17.68
1.0	Methyl	60	5.81	Etc-	8.93

The internal standard (i.s.) was an ethyl 4-hydroxybenzoate solution (0.08 mg/mL) or methyl 4-hydroxybenzoate solution (0.06 mg/mL) (Table 1) in a mobile phase.

## Kinetic procedure

### Influence of the dimethyl sulfoxide and propylene glycol on the acidic hydrolysis

An influence of the dimethyl sulfoxide on the degradation of compounds (ca. 120  $\mu\text{g/mL}$ ) was evaluated in the concentration range from 2.5 to 7.5 V%, at the temperature of 310 K, in hydrochloric acid environment (0.50 M) in the presence of a constant concentration of propylene glycol (45 V%). Aqueous-organic hydrochloric acid solutions were prepared by mixing 2.5 mL of hydrochloric acid solutions (2 M), 4.5 mL of propylene glycol and appropriate amounts of water. The solutions were equilibrated at the temperature of the study prior to initiation of the reaction. The reaction was initiated by adding 0.25–0.75 mL of the ester solution in the dimethyl sulfoxide.

An influence of the propylene glycol on the degradation of compounds (ca. 120  $\mu\text{g/mL}$ ) was evaluated in the concentration range from 35 to 55 V%, at the temperature of 310 K, in hydrochloric acid environment (0.50 M) in the presence of a constant concentration of dimethyl sulfoxide (5 V%). Aqueous-organic hydrochloric acid solutions were prepared by mixing 2.5 mL of hydrochloric acid solutions (2 M) and appropriate amounts of propylene glycol and water. The solutions were equilibrated at the temperature of 310 K prior to initiation of the reaction. The reaction was initiated by adding 0.5 mL of the ester solution in the dimethyl sulfoxide.

At specified time points, 0.2 mL of the reaction mixture was transferred into 5 mL tubes and neutralized by adding 0.2 mL of acetate buffer solution (pH 5.57; 0.5 M). Then, the mixture was cooled in ice water to stop the reaction. To each mixture 0.2 mL of the internal standard solution was added and the contents of the tested compounds were determined by HPLC method.

## Compounds degradation in an acidic environment

Aqueous-organic hydrochloric acid solutions (0.05–0.50 M) were prepared while maintaining a constant ionic strength ( $\mu = 0.50$  M) by mixing of the propylene glycol (4.5 mL), water (2.5 mL) and varying amounts of the hydrochloric acid (2 M) and sodium chloride solutions (2 M). The solutions (9.5 mL) were equilibrated at the temperature of the study (310 K) prior to initiation of the reaction. The reaction was initiated by adding 0.5 mL of the ester solution in the dimethyl sulfoxide (ca. 2.4 mg/mL for 6-(4-MeOPh)-TACV, Ac and Nic or 4.8 mg/mL for *i*But, Piv and Etc, respectively). Then, to analyze the loss of the test substances by HPLC, the samples were prepared according to the procedure described at the end of the chapter 2.4.1. The final concentration of tested substances in the mixture applied to the column, were ca. 40 or 80  $\mu\text{g/mL}$ , depending on the initial concentration.

The pH values of the hydrochloric acid at the temperature of the study were calculated from the activity coefficient data [20].

## Calculation

Statistical analyses of results were performed using a spreadsheet of MS Excel program. The errors of the values were estimated and presented as a confidence interval for a level of significance  $p < 0.05$ .

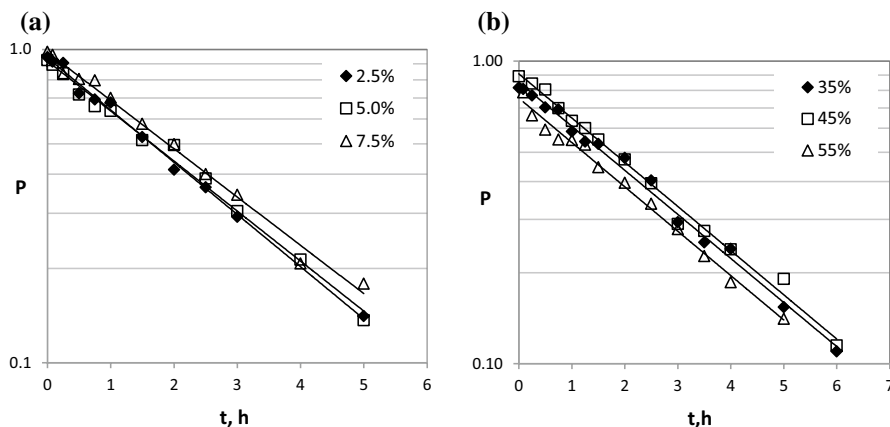
Changes in the concentration of individual compounds over time have been described by the kinetic equation of the pseudo-first order reaction:

$$\ln P_t = \ln P_0 - k_{obs.} \cdot t, \quad (1)$$

where  $P_t$  and  $P_0$  are the ratio of the areas of the peak of the test substance and the peak of the internal standard, respectively determined at time  $t$  and  $t_0$ ;  $k_{obs.}$  is the observed rate constant of the decomposition of the test compound and  $t$  is the time of the reaction.

## Results and discussion

The kinetic study was carried out in a hydrochloric acid environment (0.05–0.50 M) with pH 0.42–1.38, at a temperature of 37 °C considered as suitable for the study due to the value that is similar to the temperature of the human body. All solutions were led to a constant value of ionic strength ( $\mu$ ) equal to 0.50 M through the addition of a proper volume of sodium carbonate solution (2.0 M). In this way, the potential salt effect that may have an influence on the observed hydrolysis reaction rate was eliminated.



**Fig. 2** Semi-logarithmic plots of the  $P = f(t)$  for the Ac-6-(4-MeOPh)-TACV hydrolysis reaction in hydrochloric acid at a concentration of 0.50 M ( $\mu = 0.50$  M, 37 °C) for various concentrations of DMSO (a) and propylene glycol (b)

**Table 2** The statistical analysis of the effect of DMSO and propylene glycol concentration on the Ac-6-(4-MeOPh)-TACV ester degradation rate

DMSO			Propylene glycol		
c, V%	$k_{\text{obs.}} \pm \Delta k_{\text{obs.}}, \text{ s}^{-1}$	Regression parameters	c, V%	$k_{\text{obs.}} \pm \Delta k_{\text{obs.}}, \text{ s}^{-1}$	Regression parameters
2.50	$(1.07 \pm 0.05) \times 10^{-4}$	$a = (1.68 \pm 2.35) \times 10^{-6}$	35	$(9.31 \pm 0.47) \times 10^{-5}$	$a = (3.50 \pm 3.67) \times 10^{-8}$
5.00	$(1.02 \pm 0.06) \times 10^{-4}$	$b = (1.11 \pm 0.13) \times 10^{-4}$ $t_a = -9.093$	45	$(9.34 \pm 0.52) \times 10^{-5}$	$b = (9.19 \pm 0.17) \times 10^{-5}$
7.50	$(9.86 \pm 0.68) \times 10^{-5}$	$t_{0.05}(1) = 12.706$ $-r = 0.9940$	55	$(9.38 \pm 0.57) \times 10^{-5}$	$t_a = 12.124$ $t_{0.05}(1) = 12.706$ $-r = 0.9966$

### Effect of the DMSO and propylene glycol at the esters hydrolysis

Due to the limited solubility of 6-(4-MeOPh)-TACV and its esters in hydrochloric acid, the study of their stability was planned in an aqueous-organic environment. The organic solvents used in the study were aimed to dissolve the test compounds (DMSO, 5 V%) and to protect them against their precipitation (propylene glycol, 45 V%) in an acidic environment. On the example of Ac- ester, we studied the influence of changes in the concentration of both DMSO and propylene glycol on the acid hydrolysis of this ester within the scope  $5 \pm 2.5\%$  of the DMSO and  $45 \pm 5\%$  of the propylene glycol.

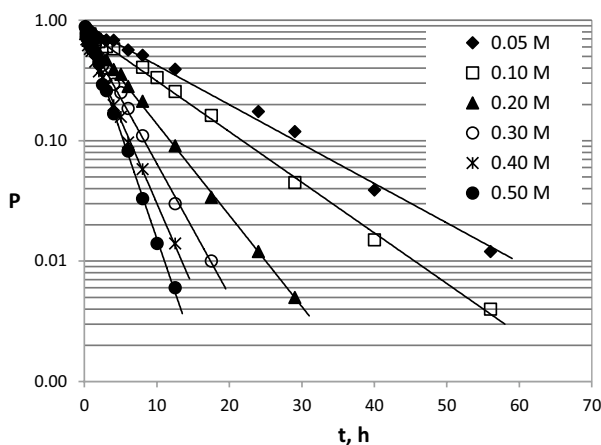
From Eq. 1, the  $k_{\text{obs.}}$  values of the decomposition of Ac- at the temperature of 37°C in the hydrochloric acid environment with a concentration of 0.50 M within the scope of concentrations from 2.5 to 7.5% of the DMSO at the presence of propylene glycol with concentration of 45% (Fig. 2a) were determined, and the parameters of linear regression of the  $k_{\text{obs.}}$  correlation as a function of the DMSO concentration (Table 2) was calculated. Similarly, the observed decomposition

rate constants of the compound within a scope of concentrations 35–55% of the propylene glycol at the presence of DMSO with a concentration of 5.0% were determined (Fig. 2b) and parameters of the observed regression of linear  $k_{\text{obs}}$  correlation as a function of propylene glycol concentration (Table 2) were calculated.

For both analyzed  $k_{\text{obs}}$  correlations =  $f(c)$ , the relevance of  $a$  regression coefficient was assessed on the basis of the Student  $t$  test ( $t_a < t_{0.05}(1)$ ) (Table 2). On this basis, it was determined that the DMSO and propylene glycol concentrations have no influence on the compound decomposition rate within the adopted scope. Therefore, further studies on the stability of the tricyclic ACV analogue and its esters was carried out in an aqueous-organic environment containing 5% of the DMSO and 45% of the propylene glycol, which ensured its solubility. The applied solvents do not participate in the hydrolysis reaction of the studied compounds.

### Stability of 6-(4-MeOPh)-TACV and its esters in the hydrochloric acid ( $\log k_{\text{pH}} - \text{pH}$ profile)

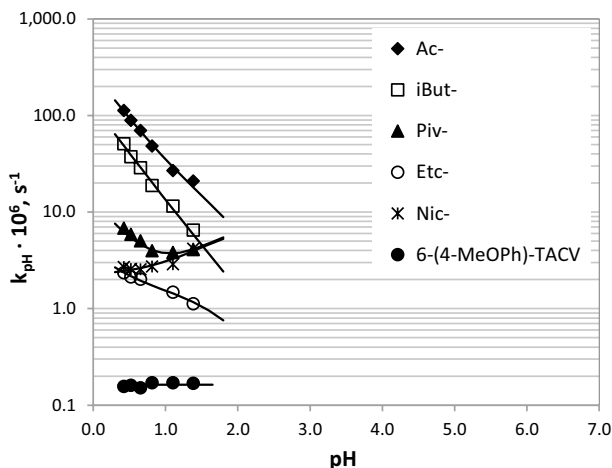
Knowing the values of  $\text{pK}_{\text{a}}$  of the parent compound (6-(4-MeOPh)-TACV;  $\text{pK}_{\text{a}1} = 1.75$ ;  $\text{pK}_{\text{a}2} = 8.20$ ; [21, 22]), it was stated that within the studied scope of the pH, all the studied compounds occur in monoprotonated ( $\text{BH}^+$ ) and neutral (B) forms. In the studied conditions, the neutral form constitutes from 0.000002% (pH 0.42) to 29.9% (pH 1.38). The  $\text{BH}^+$  form of tricyclic compounds is formed as a result of protonation of the nitrogen atom in the N1 position (Fig. 1) [23]. The values of  $k_{\text{obs}}$  of the reactions of hydrolysis of the studied esters in solutions with appropriate pH values were determined from a linear correlation described by Eq. 1 proving that the observed reaction is of the pseudo-first-order-reaction nature in terms of the substrate concentration (Fig. 3, Table 3).



**Fig. 3** Semi-logarithmic plots of the  $P = f(t)$  for the hydrolysis of Ac-6-(4-MeOPh)-TACV in hydrochloric acid at a concentration of 0.05–0.50 M ( $\mu = 0.50$  M, 37 °C), in aqueous-organic solutions

**Table 3** Observed rate constants of the pseudo-first order of the hydrolysis reaction of 6-(4-MeOPh)-TACV and its esters in hydrochloric acid ( $\mu = 0.50$  M, 37 °C)

$c_{\text{HCl}}$ [M]	pH	$k_{\text{obs.}} \pm \Delta k_{\text{obs.}}, \text{s}^{-1}$		
		Ac-	iBut-	Piv-
0.05	1.38	$(2.09 \pm 0.09) \times 10^{-5}$	$(6.50 \pm 0.46) \times 10^{-6}$	$(4.08 \pm 0.14) \times 10^{-6}$
0.10	1.10	$(2.69 \pm 0.07) \times 10^{-5}$	$(1.15 \pm 0.03) \times 10^{-5}$	$(3.83 \pm 0.22) \times 10^{-6}$
0.20	0.81	$(4.85 \pm 0.09) \times 10^{-5}$	$(1.88 \pm 0.04) \times 10^{-5}$	$(3.99 \pm 0.13) \times 10^{-6}$
0.30	0.65	$(6.99 \pm 0.21) \times 10^{-5}$	$(2.88 \pm 0.06) \times 10^{-5}$	$(5.05 \pm 0.16) \times 10^{-6}$
0.40	0.52	$(8.90 \pm 0.22) \times 10^{-5}$	$(3.73 \pm 0.12) \times 10^{-5}$	$(5.87 \pm 0.20) \times 10^{-6}$
0.50	0.42	$(1.13 \pm 0.03) \times 10^{-4}$	$(5.09 \pm 0.15) \times 10^{-5}$	$(6.81 \pm 0.23) \times 10^{-6}$
$c_{\text{HCl}}$ [M]	pH	$k_{\text{obs.}} \pm \Delta k_{\text{obs.}}, \text{s}^{-1}$		
		Etc-	Nic-	6-(4-MeOPh)-TACV
0.05	1.38	$(1.12 \pm 0.11) \times 10^{-6}$	$(4.17 \pm 0.42) \times 10^{-6}$	$(1.69 \pm 0.09) \times 10^{-7}$
0.10	1.10	$(1.48 \pm 0.11) \times 10^{-6}$	$(2.89 \pm 0.11) \times 10^{-6}$	$(1.71 \pm 0.11) \times 10^{-7}$
0.20	0.81	$(2.07 \pm 0.05) \times 10^{-6}$	$(2.74 \pm 0.12) \times 10^{-6}$	$(1.71 \pm 0.08) \times 10^{-7}$
0.30	0.65	$(2.02 \pm 0.08) \times 10^{-6}$	$(2.55 \pm 0.08) \times 10^{-6}$	$(1.51 \pm 0.09) \times 10^{-7}$
0.40	0.52	$(2.13 \pm 0.10) \times 10^{-6}$	$(2.57 \pm 0.09) \times 10^{-6}$	$(1.61 \pm 0.11) \times 10^{-7}$
0.50	0.42	$(2.36 \pm 0.09) \times 10^{-6}$	$(2.69 \pm 0.12) \times 10^{-6}$	$(1.57 \pm 0.13) \times 10^{-7}$

**Fig. 4** Semi-logarithmic relationships  $k_{\text{pH}} = f(\text{pH})$  of the hydrolysis reaction of 6-(4-MeOPh)-TACV and its esters in a hydrochloric acid medium ( $\mu = 0.50$  M) at 37 °C

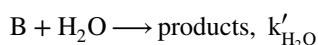
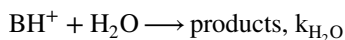
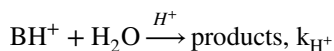
Semi-logarithmic  $k_{\text{pH}} = f(\text{pH})$  correlations (Fig. 4) allow to state that the hydrolysis of the studied compounds at the temperature of 37 °C in the hydrochloric acid environment with a pH of 0.42–1.38 is composed of:

- a hydrolysis of protonated forms under the influence of hydrogen ions;



- a spontaneous hydrolysis of protonated forms under the influence of water and/or;
- a spontaneous hydrolysis of neutral forms under the influence of water.

The general scheme of the observed reactions can be represented as follows:



For individual compounds, the following equations describing the dependence of  $k_{\text{pH}} = f(\text{pH})$  in the tested pH range were proposed:

$$\text{Ac-}, i\text{But-}, \text{Etc-}: k_{\text{pH}} = k_{\text{H}^+} \cdot a_{\text{H}^+} \cdot f_1 + k_{\text{H}_2\text{O}} \cdot f_1 \quad (2)$$

$$\text{Piv-}: k_{\text{pH}} = k_{\text{H}^+} \cdot a_{\text{H}^+} \cdot f_1 + k_{\text{H}_2\text{O}} \cdot f_1 + k'_{\text{H}_2\text{O}} \cdot f_2 \quad (3)$$

$$\text{Nic-}: k_{\text{pH}} = k_{\text{H}_2\text{O}} \cdot f_1 + k'_{\text{H}_2\text{O}} \cdot f_2 \quad (4)$$

$$6-(4\text{-MeOPh})\text{-TACV}: k_{\text{pH}} = k_{\text{H}_2\text{O}}, \quad (5)$$

where  $k_{\text{H}^+}$  is the catalytic rate constant describing the catalytic effect of hydrogen ions,  $a_{\text{H}^+}$  is the hydrogen ion activity,  $f_1$  is the fraction of the ionic form  $\text{BH}^+$  and  $f_2$ —fraction of the ionic form B,  $k_{\text{H}_2\text{O}}$  is the catalytic rate constant describing spontaneous hydrolysis under the influence of water in a monoprotated form  $\text{BH}^+$ ,  $k'_{\text{H}_2\text{O}}$  is the catalytic rate constant describing spontaneous hydrolysis under the influence of water in a neutral form B.

Fractions of individual forms ( $\text{BH}^+$ , B) were calculated using the following equations:

$$f_1 = \frac{(a_{\text{H}^+})^2}{(a_{\text{H}^+})^2 + K_{a1} \cdot a_{\text{H}^+} + K_{a1} \cdot K_{a2}} \quad (6)$$

$$f_2 = \frac{K_{a1} \cdot a_{\text{H}^+}}{(a_{\text{H}^+})^2 + K_{a1} \cdot a_{\text{H}^+} + K_{a1} \cdot K_{a2}}, \quad (7)$$

where  $K_{a1}$  and  $K_{a2}$  are dissociation constants.

The determined values of the observed  $k_{\text{obs}}$  rate constants (Table 3) corresponding to the  $k_{\text{pH}}$  values (specific acid-base catalysis) will serve to determine the catalytic rate constants describing the catalytic effect of hydrogen ions ( $k_{\text{H}^+}$ ) and spontaneous hydrolysis under the influence of water ( $k_{\text{H}_2\text{O}}$  and  $k'_{\text{H}_2\text{O}}$ ) on the hydrolysis of the studied esters (the  $\text{BH}^+$  and B forms) (Table 4).

**Table 4** Catalytic rate constants for the acid hydrolysis reaction of 6-(4-MeOPh)-TACV and its esters in hydrochloric acid medium ( $\mu = 0.50$  M,  $37^\circ\text{C}$ )

Compound	$k_{\text{H}^+}$ , $\text{mol}^{-1} \text{L s}^{-1}$	$k_{\text{H}_2\text{O}}$ , $\text{s}^{-1}$	$k'_{\text{H}_2\text{O}}$ , $\text{s}^{-1}$
Ac-	$(2.68 \pm 0.28) \times 10^{-4}$	$(1.46 \pm 0.65) \times 10^{-5}$	
<i>i</i> But-	$(1.26 \pm 0.17) \times 10^{-4}$	$(3.14 \pm 3.81) \times 10^{-6}$	
Piv-	$(1.16 \pm 0.27) \times 10^{-5}$	$1.72 \times 10^{-6}$	$8.60 \times 10^{-6}$
Etc-	$(2.41 \pm 0.76) \times 10^{-6}$	$(1.57 \pm 0.18) \times 10^{-6}$	
Nic-		$8.06 \times 10^{-6}$	$2.19 \times 10^{-6}$
6-(4-MeOPh)-TACV		$(1.63 \pm 0.08) \times 10^{-7}$	

The individual catalytic rate constants  $k_{\text{H}^+}$  and  $k_{\text{H}_2\text{O}}$  were determined using the following linear equation:

$$\frac{k_{\text{pH}}}{f_1} = k_{\text{H}^+} \cdot a_{\text{H}^+} + k_{\text{H}_2\text{O}} \quad (8)$$

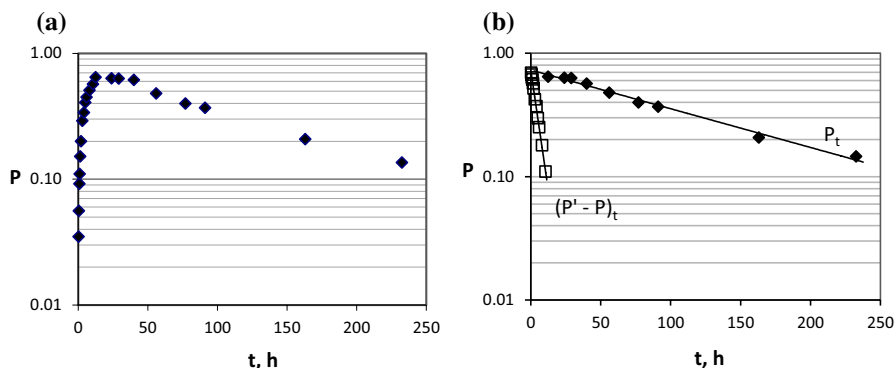
The catalytic  $k_{\text{H}^+}$  constant for pivaloyl ester (Piv-) was determined from Eq. 8. Next, the theoretical values of  $k'_{\text{pH}}$ , which were used to determine the values of  $k_{\text{H}_2\text{O}}$  and  $k'_{\text{H}_2\text{O}}$ , were calculated from the following transformation of Eq. 3:

$$k_{\text{pH}} - k'_{\text{pH}} = k_{\text{pH}} - (k_{\text{H}^+} \cdot a_{\text{H}^+} \cdot f_1) = k_{\text{H}_2\text{O}} \cdot f_1 + k'_{\text{H}_2\text{O}} \cdot f_2 \quad (9)$$

A graph of dependence  $k_{\text{pH}} - k'_{\text{pH}} = f(f_2)$  was drawn up, for which the ordinate value for  $f_2 = 0$  corresponds to the catalytic constant rate  $k_{\text{H}_2\text{O}}$ , and for  $f_2 = 1$  it corresponds to the catalytic constant rate  $k'_{\text{H}_2\text{O}}$  (Table 4).

Similarly, the  $k_{\text{H}_2\text{O}}$  and  $k'_{\text{H}_2\text{O}}$  catalytic rate constants describing a spontaneous hydrolysis of particular ionic forms of nicotinic ester (Nic-) under the influence of water were determined from the Eq. 4 on the basis of an analysis of the parameters of regression of the linear  $k_{\text{pH}} = f(f_2)$  correlation (Table 4). On the other hand, the  $k_{\text{H}_2\text{O}}$  catalytic constant describing a spontaneous hydrolysis under the influence of water of the  $\text{BH}^+$  form of the parent compound (6-(4-MeOPh)-TACV) was determined from the Eq. 5 as an average  $k_{\text{obs}}$  value (Table 4).

On the basis of Eqs. 2–5, the theoretical  $k_{\text{pH}}$  values were calculated and  $k_{\text{pH}} = f(\text{pH})$  correlation charts, on which the  $k_{\text{pH}} = k_{\text{obs}}$  values and a continuous line with  $k_{\text{pH}}$  theoretical values were marked, were drawn up (Fig. 4). The compliance of the experimentally determined  $k_{\text{pH}}$  values with the calculated values proves the correctness of the proposed mechanism of the hydrolysis reaction of particular esters in the studied conditions.



**Fig. 5** Semi-logarithmic plots of the  $P_t$  (a, b) and  $(P' - P_t)$  (b) relationship as a function of the reaction time for formation and decomposition of 6-(4-MeOph)-TACV from the Ac-ester in a hydrochloric acid medium (0.2 M,  $\mu = 0.50$  M, 37 °C)

**Table 5** Observed constants of reaction rates of formation ( $k_F$ ) and decomposition ( $k_D$ ) and catalytic constants of reaction rates of formation and degradation of 6-(4-MeOph)-TACV during hydrolysis of Ac-6-(4-MeOph)-TACV in a hydrochloric acid medium ( $\mu = 0.50$  M, 37 °C)

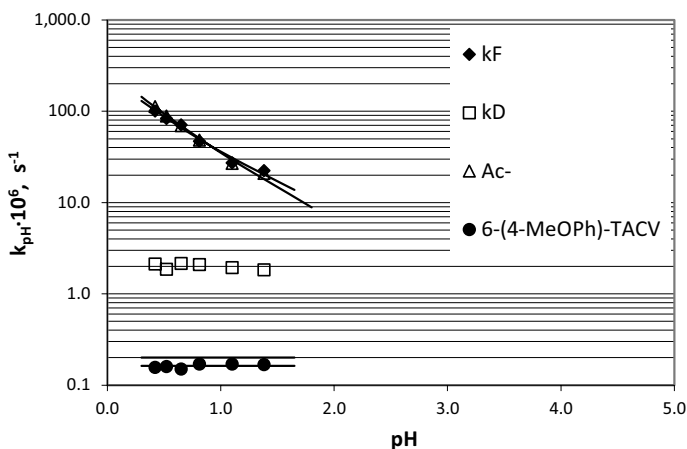
pH	Formation of 6-(4-MeOph)-TACV		Decomposition of 6-(4-MeOph)-TACV	
	$k_F$ , s <sup>-1</sup>	$k_{H^+}$ , mol <sup>-1</sup> L s <sup>-1</sup> $k_{H_2O}$ , s <sup>-1</sup>	$k_D$ , s <sup>-1</sup>	$k_{H_2O}$ , s <sup>-1</sup>
1.38	$2.25 \times 10^{-5}$	$k_{H^+} = (2.26 \pm 0.26) \times 10^{-4}$	$1.85 \times 10^{-6}$	$(1.93 \pm 0.13) \times 10^{-6}$
1.10	$2.74 \times 10^{-5}$		$1.95 \times 10^{-6}$	
0.81	$4.98 \times 10^{-5}$	$k_{H_2O} = (1.94 \pm 0.60) \times 10^{-5}$	$2.01 \times 10^{-6}$	$1.77 \times 10^{-6}$
0.65	$6.35 \times 10^{-5}$		$1.87 \times 10^{-6}$	
0.52	$8.33 \times 10^{-4}$		$2.13 \times 10^{-6}$	
0.42	$1.01 \times 10^{-4}$			

### Analysis of the formation and decomposition of 6-(4-MeOph)-TACV as a product of the degradation of tested esters

During the study of the hydrolysis of tricyclic esters, in the case of all compounds, the occurrence and changes in the size of the peak with the retention time compliant with the 6-(4-MeOph)-TACV retention time were observed. Since the separation of compounds was satisfactory, it was decided to conduct a quantitative analysis of the formation and decomposition of this compound. The studies were conducted for the Ac-ester in the aqueous-organic environment of the hydrochloric acid ( $\mu = 0.50$  M, 37 °C) (Fig. 5, Table 5).

On the semi-logarithmic  $P_t = f(t)$  correlation charts, simultaneously with a loss of the substrate, a growth (formation), and then, due to a follow-up reaction, a 6-(4-MeOph)-TACV decomposition was observed. Using the equations:

$$\ln P_t = \ln P_0 - k_D \cdot t (t > t_{max}) \quad (10)$$



**Fig. 6** Semi-logarithmic relationships  $k_{\text{pH}} = f(\text{pH})$  of the decomposition of the Ac-6-(4-MeOPh)-TACV (open triangle), formation (filled diamond) and degradation (open square) of 6-(4-MeOPh)-TACV during the hydrolysis of Ac-ester and during direct response degradation of 6-(4-MeOPh)-TACV (filled circle) in a hydrochloric acid solution (pH 0.42–1.38,  $\mu = 0.50 \text{ M}$ ) at  $37^\circ \text{C}$

$$\ln(P' - P)_t = \ln(P' - P)_0 - k_F \cdot t \quad (t < t_{\text{max}}), \quad (11)$$

where  $P_t$  and  $P_0$  are the values of the quotient  $P/P_{\text{i.s.}}$  accordingly in time  $t$  i  $t_0$ ,  $k_D$  is the observed rate constant of 6-(4-MeOPh)-TACV decomposition,  $k_F$  is the observed rate constant of 6-(4-MeOPh)-TACV formation and  $P'$  is the theoretical  $P$  value calculated from the Eq. (10), the observed constants of reaction rates of formation ( $k_F$ ) and decomposition ( $k_D$ ) of 6-(4-MeOPh)-TACV were calculated (Table 5). The  $k_{\text{pH}} = k_F = k_D = f(\text{pH})$  correlation profiles were subjected to an analysis (Fig. 6), and it was stated that within the examined pH scope, the 6-(4-MeOPh)-TACV formation rate during the hydrolysis of the Ac- ester is conditional on the catalytic influence of hydrogen ions and water. On the other hand, the hydrogen ions have no influence on the 6-(4-MeOPh)-TACV decomposition. Therefore, the equations describing the  $k_{\text{pH}} = f(\text{pH})$  correlation within the studied pH scope can be written as follows:

$$k_{\text{pH}} = k_F = k_{\text{H}^+} \cdot a_{\text{H}^+} \cdot f_1 + k_{\text{H}_2\text{O}} \cdot f_1 \quad (\text{formation of 6-(4-MeOPh)-TACV}) \quad (12)$$

$$k_{\text{pH}} = k_D = k_{\text{H}_2\text{O}} \quad (\text{decomposition of 6-(4-MeOPh)-TACV}) \quad (13)$$

The  $k_{\text{H}^+}$  and  $k_{\text{H}_2\text{O}}$  values were determined (Table 5), theoretical values  $k_{\text{pH}}$  were calculated and  $k_{\text{pH}} = f(\text{pH})$  correlation charts, on which we marked  $k_{\text{pH}}$  values and a continuous line with  $k_{\text{pH}}$  theoretical values, were drawn up on the basis of the above-described equations (Fig. 6). The compliance of the experimentally-determined  $k_{\text{pH}}$  values with the calculated values proves the correctness of the proposed mechanism of the formation and decomposition of 6-(4-MeOPh)-TACV during the Ac- ester in the studied conditions.

## Conclusions

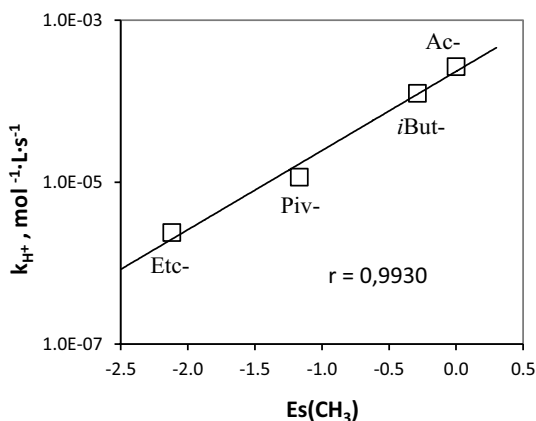
Studies on the stability of tricyclic derivatives were preceded by the determination of the influence of the DMSO and propylene glycol on the decomposition rate. It was stated that the concentration of the above-mentioned solvents within the analysed scope does not have an influence on the decomposition of the studied esters. The studies on decomposition of the analysed compounds in the acidic environment enabled to determine the kinetic parameters of the observed reactions. While comparing the results of the analysis of the ACV stability in analogous conditions [24], it was observed that the rate constant of analogous ACV and 6-(4-MeOPh)-TACV esters (except for nicotinic esters) are similar, and it was stated that the presence of an additional ring in the structure of tricyclic analogues does not have influence on the vulnerability of esters on the hydrolysis under the influence of hydrogen ions as catalytic factors. On the other hand, the spatial structure of substituents in the ester part has a significant influence on the rate of hydrolysis of the studied compounds in the acidic environment. A clear substituent steric effect described by Taft parameter ( $E_s$ ) is thus observed in the studied group of compounds [25]. According to the Taft analysis, only steric factors have an influence on the hydrolysis reaction rate in the acidic environment and the esterification reaction rate; however, in the case of basic hydrolysis, polar factors also have an influence [25]. In the analysed conditions, Taft equation can be written as follows:

$$\log \frac{k_s}{k_{CH_3}} = \delta E_s \quad (14)$$

Here  $k_s/k_{CH_3}$  is the quotient of the constant reaction rate of the compound with the analyzed substituent by the rate constant of the reference compound (with a methyl group as a substituent) and  $\delta$  is the factor of vulnerability of the reaction on steric effects (in the acid hydrolysis  $\delta = 1$ ) [26].

The steric factors for the studied substituents were calculated, taking into consideration the  $k_{H^+}$  value determined for ACV esters. Their values were as follows:

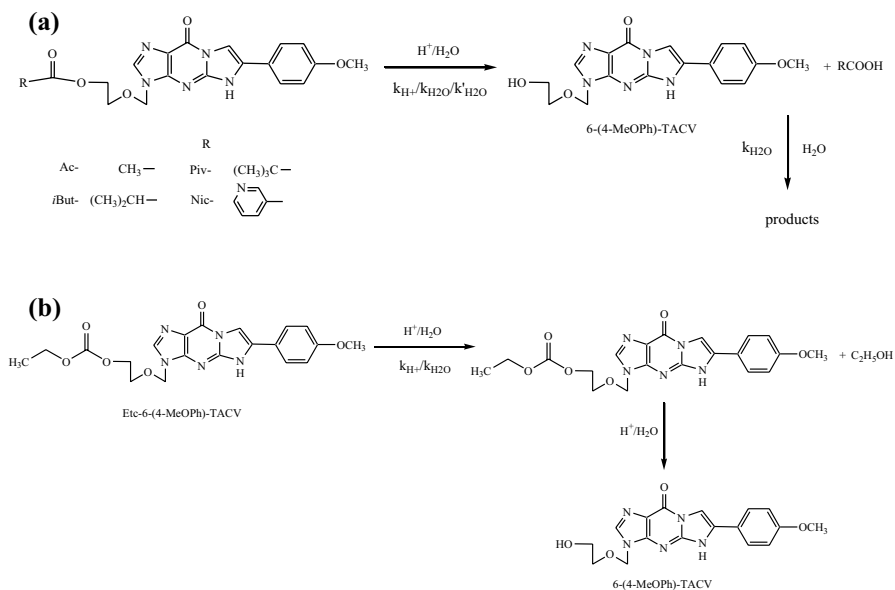
**Fig. 7** Semi-logarithmic relationship  $k_{H^+} = f(E_s(CH_3))$  of the studied compounds



–0.29 (isopropyl), –1.17 (*tert*-butyl), –2.12 (ethoxy), –2.09 (3-pyridine) [24]. Negative values demonstrate that the above-mentioned substituents decrease the esters hydrolysis rate. The analysis of steric parameters proved that the ethoxycarbonyl (Etc-) group stabilises the structure of the ACV tricyclic analogue ester [they have the lowest  $E_s(\text{CH}_3)$ ] value the most (Fig. 7). This conclusion was confirmed on  $\log k_{\text{pH}} = f(\text{pH})$  profiles of the hydrolysis reaction of the studied compounds (Fig. 4).

Furthermore, it was stated that the values of the catalytic rate constants of hydrolysis reaction of isobutyryl esters (*i*But-) are about 2-fold, of pivaloyl esters (Piv-) 10-fold and of ethoxycarbonyl (Etc-) and nicotinic (Nic-) esters 100-fold lower in comparison with acetyl esters (Ac-) (Table 3). This indicates differences in the energy of activation of the acid hydrolysis of the particular ester types. According to the data from the literature [27, 28], such differences in the stability of the studied compounds are a result of differences in the energy of activation of the observed reaction. This may constitute an additional explanation of a huge stability of ethoxycarbonyl and nicotinic esters in the analyzed conditions.

The hydrolysis of 6-(4-MeOPh)-TACV esters occurs in the acid environment under the influence of hydrogen ions and/or water within one stage according to a single scheme, and it leads to the formation of a parent compound and an appropriate acid (Scheme 1a). In the case of Etc- ester, a two-stage decomposition reaction with the formation of a non-durable intermediate product (Scheme 1b) has been proposed. The intermediate product was not observed on chromatograms during the analysis; therefore, the kinetic parameters of reaction of its formation and decomposition were not determined. The rate of the above-described reaction of the hydrolysis of Etc- ester is determined by the first stage that is slower than the second



**Scheme 1** Mechanism for the hydrolysis of esters of tricyclic ACV analogs in an acidic environment: **a** Ac-, *i*But-, Piv-, Nic-esters of 6-(4-MeOPh)-TACV; **b** Etc-ester of 6-(4-MeOPh)-TACV

one. A fast protonation occurs, then a slow, two-particle attack reaction of the water particle and fission of the C=O bond with ethanol release. At the second stage, a fast monoparticle reaction of the fission of the bond between the carbon and oxygen occurs. The result of such reaction is the formation of 6-(4-MeOPh)-TACV and carbonic acid. The above-mentioned reaction mechanism is compliant with the mechanism previously described for ACV esters [24].

The 6-(4-MeOPh)-TACV compound created as a product of the hydrolysis of tricyclic esters in the studied conditions is a subject to further decomposition in the follow-up reaction (Scheme 1a). The decomposition rate of 6-(4-MeOPh)-TACV, subjected to a hydrolysis in the acid hydrolysis, and of the 6-(4-MeOPh)-TACV, observed as a product of hydrolysis of Ac- ester, were compared. It was stated that the decomposition of 6-(4-MeOPh)-TACV, subjected to a hydrolysis, is about 10 times slower as compared to 6-(4-MeOPh)-TACV formed as a result of the decomposition of Ac- ester ( $k_{H_2O}$ , Tables 4, 5; Fig. 6). On this basis, it can be assumed that the acid formed during the hydrolysis of ester (e.g. acetic acid), although it has no influence on the change of pH of the solution, it catalyses the decomposition of 6-(4-MeOPh)-TACV (Scheme 1a).

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## Compliance with ethical standards

**Conflict of interest** The authors confirm that this article content has no conflict of interest.

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