HYDROLYTIC STABILITY OF ADAMANTANE HYBRID MOLECULES

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ABSTRACT

Herein, the hydrolytic stability of new hybrid adamantane molecules modified with amino acid cysteine (Cys) at different pH is reported. Cys is a rare proteinogenic amino acid but it is a key structural unit in proteins. Cys is the only amino acid containing thiol group in the lateral chain which make it important source of sulphur for human organism. In addition, Cys has many biological functions such as antioxidant properties, immunomodulation activity by influence of the levels of the glutathione hormone, support liver function to eliminate toxins, help the breakdown of mucus in the lungs and improve breathing, etc. Adamantane derivatives are organic compounds largely used as antiviral therapeutics for treatment of influenza virus type A as well as neurodegenerative illnesses such as Parkinson's and Alzheimer's diseases. The adamantane motif assures high thermic stability and resistance. The modification of many adamantane derivatives such as amantadine, rimantadine and memantine with proteinogenic amino acid Cys could lead to increasing of activity and bioavailability of newly designed molecules. It is well known that hydrolytic stability is important feature for prodrug molecules related to the ability to penetrate cell membranes and to reach the specific receptors. A series of prodrugs based on adamantane motif including Cys-S-tert.-butylamantadine, Cys-S-tert.-butylrimantadine and Cys-S-tert.-butylmemantine was studied. The hydrolytic stability was determined at two different pH 1.0 and 7.4 at 37°C, similar to these in the human stomach and blood plasma. Kinetic of hydrolysis is monitored spectrophotometrically by specifically created UV-VIS method following the concentration of non-hydrolyzed part of the compounds. The most stable compound at pH 7.4 was Cys-S-tert.-butylamantadine with $t_{1/2}$ = 8.5 h. The compound Cys-S-tert.-butylmemantine also has good hydrolytic stability with $t_{1/2}$ = 6.7 h and Cys-S-tert.-butylrimantadine has $t_{1/2}$ = 6.2 h. Almost identical are $t_{1/2}$ values at acid pH 1.0: the most stable is Cys-S-tert.-butylamantadine with $t_{1/2}$ = 4.7 h, followed by Cys-S-tert.-butylrimantadine with $t_{1/2}$ = 3.9 h and Cys-S-tert.-butylmemantine with $t_{1/2}$ = 3.5 h. However, it was revealed that hydrolytic stability of tested compounds in the two model systems at acid pH is relatively lower than those in neutral conditions.

Keywords: cysteine, adamantane derivatives, hydrolytic stability, prodrug, physiological pH, non-linear calibration, first order kinetics.

INTRODUCTION

The remarkable structural and chemical properties of adamantane provide exceptional opportunities for the design of various adamantane derivatives for biomedical applications. A wide range of monofunctional adamantane derivatives have been studied, mostly as antiviral agents. The four bridgehead positions of adamantane provide many opportunities for the design of polyfunctional derivatives, and the recent concept of multi-substituted adamantane building blocks has shown promising applications in several fields [1].

The adamantane molecule, containing $C_{10}H_{16}$, is a rigid structure consisting of four cyclohexane rings fused in a "chair" conformation. The arrangement in space of the carbon atoms of this polycyclic cell is similar to that of the unit cell of the diamond crystal lattice with an almost identical carbon-carbon bond length of 1.54 Å [2, 3].

Adamantane could be isolated from different natural sources such as petroleum, natural gas and sediments. Firstly, adamantane has been identified from petroleum fraction obtained next to Hodonin in the South Moravian Region of the Czech Republic in 1933 by Landa and Macháček [4, 5]. Today, adamantane is synthesized in a large amount by thermal cracking process of high molecular weight fractions of crude petroleum. However, adamantane could be also synthesized in laboratory conditions. First artificial synthesis is done in 1941 by Prelog and Steinwerth and later by Stetter et al. [6 - 8]. Adamantane is highly reactive which allows the synthesis of many derivatives which are found different applications, especially in medicinal chemistry. The discovery of powerful inhibitory effect of 1-aminoadamantane, named amantadine, against many viruses including influenza A virus [9] and rubella [10] in the 60th years of the last century leads to creation of a new area in the study of adamantane. The well known 3D structure as well as hydrophobicity and lipophilicity of adamantane suppose good conditions for their transport trough biological membranes. Many studies for different activities of large scale of adamantane derivatives have been realized but the most interesting are their antiviral properties [11 - 16].

There are many medical drugs used currently in a medicinal practice that contain adamantane structure. Amantadine, rimantadine and tromantadine are aminocontaining derivatives of adamantane with antiviral activity. Some adamantane derivatives such as vildagliptin and saxagliptin have antidiabetic activity and they are currently used for treatment of the type 2 diabetes (T2DM). The adapalene is known as anti-inflammatory compound used for acne treatment [17 - 19]. Due to increasing of bacterial resistance against existing on the medicinal market antimicrobial substances in recent years, scientists have also paid particular attention to the antimicrobial properties of adamantane derivatives [17, 20, 21]. Thus, Al-Wahaibi et al. reported for the synthesis of thioisourea derivatives containing adamantane motif, which have high antibacterial activity against both Gram (+) and Gram (-) bacteria [22]. Moreover, the synthesized compounds significantly decrease glucose's levels in blood serum in comparison with gliclazide [22, 23].

In any cases when chemical modifications do not assure enough bioavailability, period of activity, membrane penetration or metabolic stability, a prodrug could be designed, an unactive or weakly active precursor which further will be transformed in a human body in an active form. After the process of sorption, the substance is transported to the liver where under the action of different enzymes it is transformed to water soluble molecule for easer excretion. The quantity of the substance which stays available after the first pass through the liver is named the bioavailable fraction [24].

In a search for new substances for the fight against COVID-19 virus after the world pandemia our attention was drawn to compounds known for their biological activity and especially those with antiviral effect. The idea was to modify the adamantane derivatives amantadine, rimantadine and memantine with proteinogenic amino acid Cys, which is a part from many natural molecules, and in addition it has several biological functions and activities as it was mentioned in the introduction section. Our hypothesis was that these two structural motifs could act with a synergic effect forming a kind of prodrug. Herein, based on a data from literature and previous our investigations, hybrid molecules between amantadine motif and Cys were created in order to study their hydrolytic activity. The obtained results will be further used for a decision to be made for study of some additional effects of targeted molecules.

The synthesized analogs are tested at different conditions corresponding to pH in the stomach (pH = 1.0) and blood plasma (pH = 7.4) at 37°C.

EXPERIMENTAL

Reagents and methods

The buffer components HCl, NaCl, Na_2HPO_4, KH_2PO_4 and H_3PO_4 are purchased from Sigma Aldrich. Kinetic study is done using Agilent 8453 UV/Vis spectrophotometer Agilent Technologies with UV-Visible ChemStation software at λ range 205 - 210 nm.

Buffer solutions used for hydrolytic stability determination are prepared according to European pharmacopoeia 11th edition and are described in our

previous [25]. Briefly:

(i) buffer with pH 1.0 - 8.33 mL of 0.1 M HCl is dissolved in 900 mL distilled H_2O (dH_2O). The obtained solution is diluted to 1000.0 mL with distilled H_2O (dH_2O);

(ii) buffer with pH 7.4 - 2.38 g Na_2HPO_4, 0.19 g KH_2PO_4 and 8.0 g NaCl are dissolved in dH_2O. The obtained solution is diluted to 1000.0 mL with distilled H_2O (dH_2O);

(iii) standard solutions for UV-VIS-NIR spectrophotometry.

For maximum precision, stock solutions of targeted compounds were prepared immediately prior to hydrolytic stability studies.

Hydrolytic stability determination

The kinetic study of hydrolysis is done by measurement of the concentration of adamantane derivatives and their products from the hydrolysis in a range of 30 min during 5 h period in the described in the methods subsection conditions. UV-VIS spectrophotometry is used for quantitative determination of analytes at λ range 205 - 210 nm. During experiment adamantane derivatives containing Cys were incubated at pH 1.0 and pH 7.4 at 37ºC. Aliquots of 9.8 mL of the corresponding buffer were placed in a screw-capped vial and incubated at 37°C. The stock solution of the test compound (0.2 mL) was further added to the buffer. The vial was placed on a magnetic stirrer in a thermostat at 37ºC and stirred at 60 rpm for 300 min. Each sample was further directly analyzed by UV-Vis-NIR spectrometry.

RESULTS AND DISCUSSION

The hydrolytic stability of all tested adamantane derivatives is realized in experimental conditions with biological importance, i.e. pH 1.0 and pH 7.4, at 37°C. All targeted compounds are synthesized according by Knorr [26], (Fig. 1) using 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU) as a condensation agent.

In order to calibrate the used UV-Vis spectrophotometer six standard solutions of all tested compounds were prepared covering the working range. Mathematical model is based on polynomial of second order and they are presented in Table 1. All experiments were performed under repeatability conditions, immediately after the corresponding calibration, to avoid the effect of any potential inter-day variability.

The corresponding second-order polynomial calibration plots for the same examples are presented in Fig. 2.

The main problem for the reliability of the planned kinetic studies was the best choice of model for calibration and evaluation of concentration of the studied compound. The definition is that when the lowest and highest points on the calibration curve are on the same side of the most likely straight line, while the middle part is on the other side, this is a clear indication that the real function is nonlinear.

The corresponding linear calibration curve for the same examples are presented in Fig. 3.

In all three cases for calibration function seams to

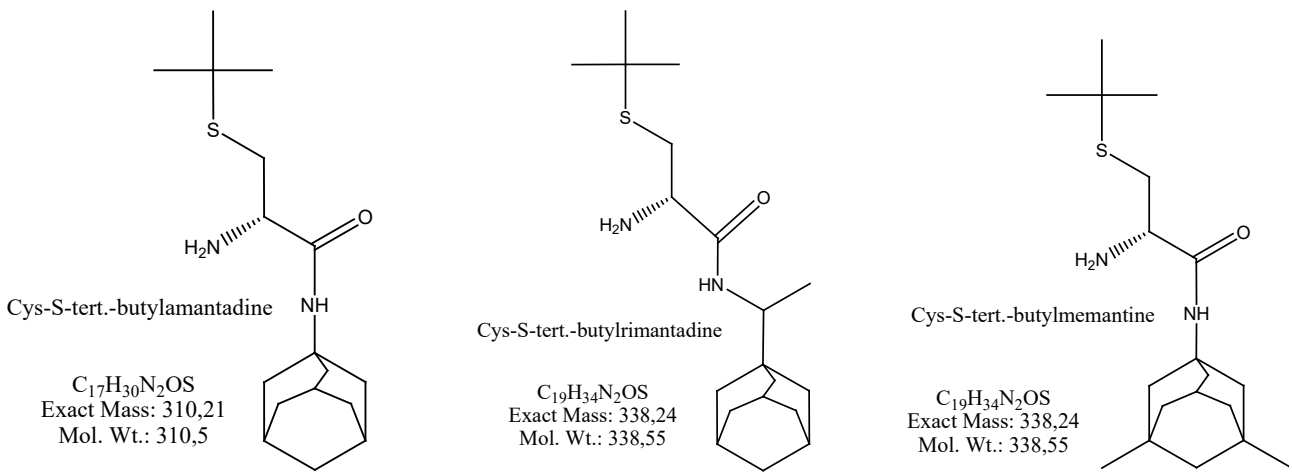
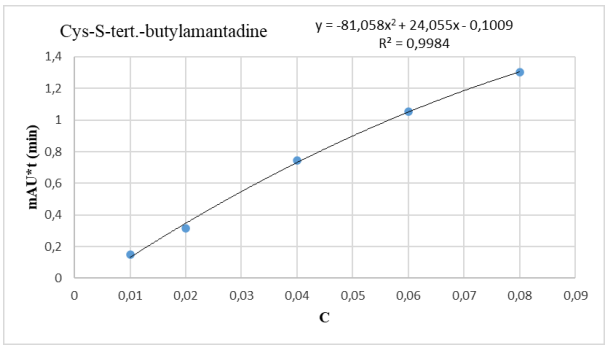
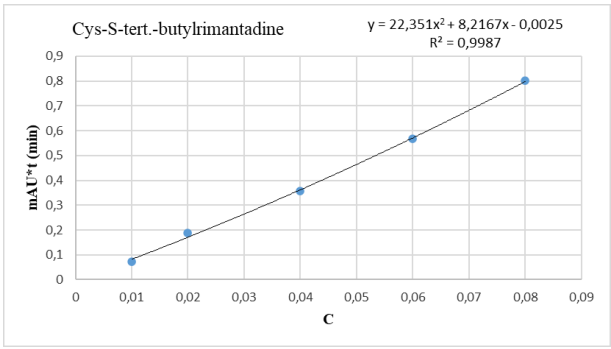


Fig. 1. Structure of tested adamantane analogs.

Table 1. Squared regression coefficients, correlation coefficients and concentration ranges for calibration functions $y = a_2x^2 + a_1x = a_0$.

№	Compound	Calibration equations			R^2	Concentration Range, g L^{-1}
		a^2	a^1	a^0		
1.	Cys-S-tert.-butylamantadine	-81.058	24.055	0.1009	0.9984	0.01 - 0.10
2.	Cys-S-tert.-butylrimantadine	22.351	8.2167	0.0025	0.9987	0.01 - 0.10
3.	Cys-S-tert.-butylmemantine	125.42	11.085	0.0655	0.9987	0.01 - 0.10





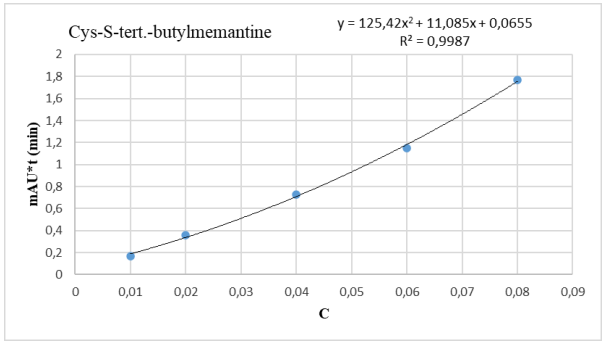
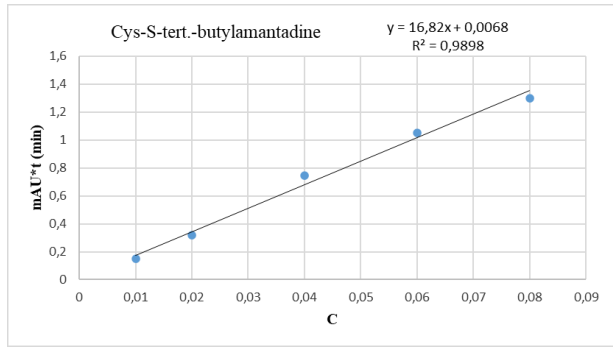
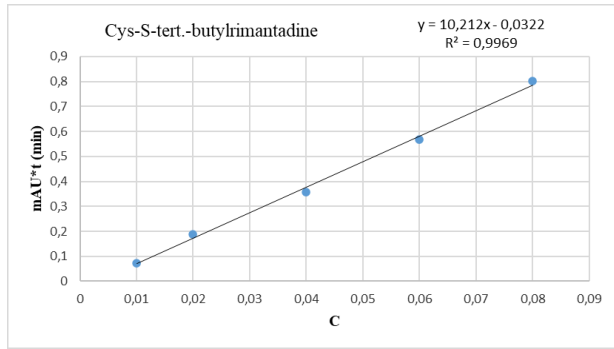


Fig. 2. Calibration plots of tested compounds.





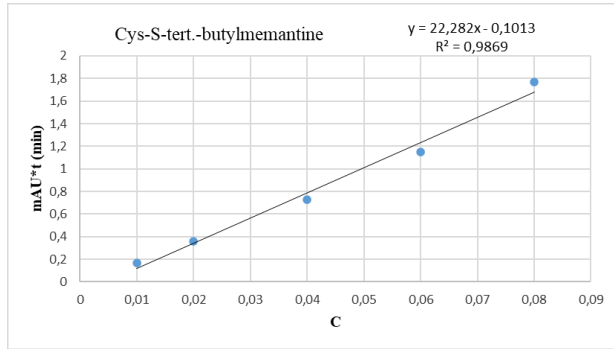


Fig. 3. Graphics for linear calibration.

be nonlinear. Thus, finally, according to the F-test of Fisher a nonlinear function for calibration was used for calibration and working procedure [27]. According to literature data as well as our previous studies in most cases hydrolysis of medical drugs follows kinetic of zero- or first-order [25]. Herein, as a kinetic of first-order

the rate of the studied reactions will be a function of the concertation of one of the reagents and the linearity could be achieved in the coordinates $\ln(C/C_0)$ against t. The linearity was evaluate using Pearson and t-test of Student coefficients. Practically, always when $R^2 \geq 0.99$ the application of all other test for linearity will also give positive results. The values of rate constants were obtained as slope of the linear regression line in coordinates $\ln(C/C_0)$ against *t*. Standard deviation (SD) of the slope (Sa_1) was calculated according to the Eq. (1) [28]:

$$S_{a_1} = S_R \sqrt{\frac{1}{\sum_{i=1}^{n} x_i^2 - \frac{1}{n}(\sum_{i=1}^{n} x_i)^2}}, \quad (1)$$

where: x_i is a time value for the point i of the data of any kinetic, and S_R is a regressive SD calculated according to the Eq. (2):

$$S_R = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n-c}}, \quad (2)$$

where: y_i and $\hat{y}_i$ are measured and estimated according to the regression equation values for absorption in the point **i** of data of any of kinetic curves, n is the number of points and **c** is number of coefficients in the regression equation.

Since the measured value is the slope of the linear regression curve, the SD could be accepted to be the combined standard uncertainty of the rate constant measurement ($u(k_r)$) [29]. Thus, the reaction half-time ($t_{1/2}$) can be calculated according to the Eq. (3):

$$t_{1/2} = ln\frac{2}{k_r} \quad (3)$$

where k_r is a rate constant of the reaction. Taking into account the low for spread of uncertainty to the Eq. (3) the following dependence presented in the Eq. (4) could be derived to estimate the combined standard uncertainty of the measurement of the reaction half-life [29].

$$u_c(t_{1/2}) = ln\frac{k_r}{2}.\left(\frac{u(k_r)}{k_r}\right) \quad (4)$$

The expanded uncertainty for the rate constant (kr) and the half-life of the reaction ($t_{1/2}$) can be calculated according to the presented Eq. (5):

$$U = k.u_c \quad (5)$$

where k is the coverage factor and и u_c is the corresponding combined standard uncertainty of the measurement. All results for the kinetic determination are summarized in Table 2.

It was revealed that in the described reaction conditions all adamantane derivatives are hydrolyzed. The hydrolytic reaction followed a first-order kinetic and the rate constants (k) were obtained as slopes from semi-logarithmic plots of unchanged concentration vs the time. Chemical stability was assessed using the decomposition half-life of the compounds expressed by Eq. (3) (Table 2).

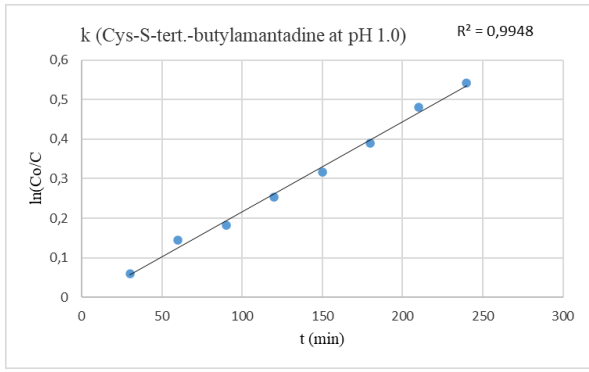
Study of the hydrolytic stability of the targeted compounds revealed that all compounds are relatively stable with $t_{1/2}$ in the range 3.5 to 4.7 h at pH 1.0 (Fig. 4).

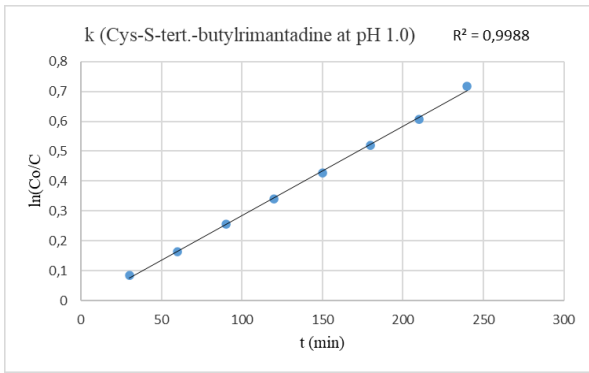
All tested compounds show better stability at pH 7.4 with half-time in the range 6.2 to 8.5 h (Fig. 5).

Table 2. Rate constants for the processes of decomposition of adamantane analogs, reactions' half-times and correlation coefficients of regression curves in coordinates $\ln(C/C_0)$ against *t*.

No	Compound	pH	Rate constant k_r	Reaction Half-time $t_{1/2}$, h	Correlation coefficient, R^2
1.	Cys-S-tert.-butylamantadine	1.0	- 0.00010 ± 0.00002	4.7 ± 0.1	0.9975
2.	Cys-S-tert.-butylrimantadine	1.0	- 0.00016 ± 0.00002	3.9 ± 0.1	0.9996
3.	Cys-S-tert.-butylmemantine	1.0	- 0.00008 ± 0.00001	3.5 ± 0.1	0.9915
4.	Cys-S-tert.-butylamantadine	7.4	- 0.00005 ± 0.00001	8.5 ± 0.2	0.9979
5.	Cys-S-tert.-butylrimantadine	7.4	- 0.00006 ± 0.00001	6.2 ± 0.2	0.9939
6.	Cys-S-tert.-butylmemantine	7.4	- 0.00006 ± 0.00001	6.7 ± 0.2	0.9921

**All experiments are conducted at 37°C.*





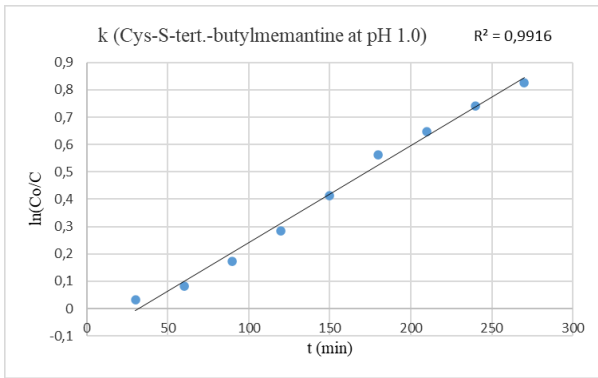
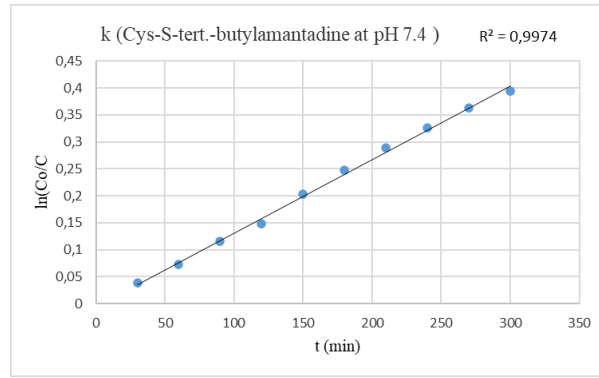
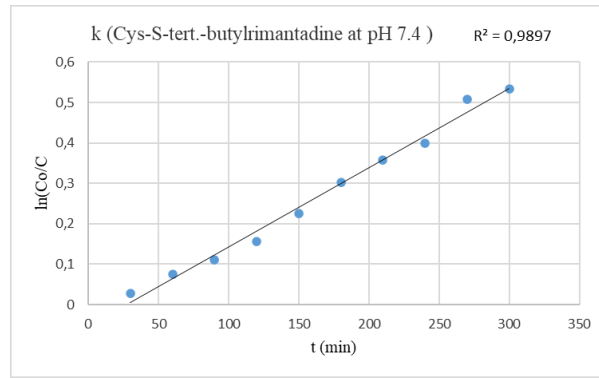


Fig. 4. Kinetic curves for the change in the concentration of tested adamantane derivatives with time at pH 1.0.





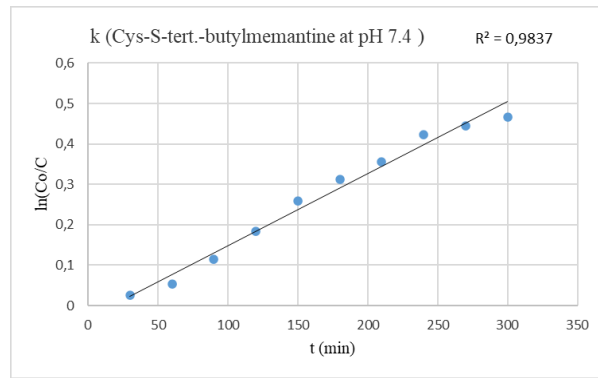


Fig. 5. Kinetic curves for the change in the concentration of tested adamantane derivatives with time at pH 7.4.

CONCLUSIONS

The hydrolytic stability of adamantane bioconjugates with Cys under experimental conditions simulating some suitable biological environments (pH 2.0 and pH 7.4 at 37°C) was studied using a specially developed UV-VIS spectrophotometric method. The obtained results revealed that Cys-S-tert.-butylamantadine is the most stable compound at neutral pH 7.4 with $t_{1/2}$ = 8.5 h. The analog Cys-S-tert.-butylmemantine has also good stability at the same conditions ($t_{1/2}$ = 6.7 h). The weaker stability but however close to this of Cys-S-tert.-butylmemantine has the third studied adamantane bioconjugate Cys-S-tert.-butylrimantadine. Thus, the stability at pH = 7.4 follows the order Cys-S-tert.-butylamantadine >> Cys-S-tert.-butylmemantine >> Cys-S-tert.-butylrimantadine.

Almost identical are the values at pH = 1.0. The order of stability is as follow: Cys-S-tert.-butylamantadine ($t_{1/2}$ = 4.7 h) >> Cys-S-tert. - butylrimantadine ($t_{1/2}$ =

3.9 h) >> Cys-S-tert.-butylmemantine ($t_{1/2}$ = 3.5 h).

The stability of all tested compounds in acid conditions is lower than this in the neutral ones. However, the stability at both tested conditions is good (more than 2 h), which make them suitable for application as prodrugs.

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