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Chemical stability of thiazole analogues of rimantadine and amantadine

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At present, two classes antivirals of influenza virus are available: the neuraminidase inhibitors (oseltamivir, peramivir, zanamivir) and the M2 proton channel blockers (amantadine and rimantadine). Since vaccination and existing antiviral therapy and rapid emergence of M2 proton channel blockers resistance cannot guarantee protection against influenza, battling this virus remains important health care task that requires design and development of new drugs. In the search of new prodrugs effective against influenza virus were synthesized thiazole analogues with amantadine and rimantadine (RS) -1 - (1-adamantyl) ethanamine) and their antiviral activity was studied [1]. The chemical stability of them was studied at pH 1 and 7.4 temperature of 37°C. An HPLC method was developed for quantification of the unchanged ester concentration.

Keywords: adamantanes, thiazole, chemical stability

INTRODUCTION

Modification of antiviral agents by peptidomimetics, with chemical structures different from the natural peptides but maintaining the same ability to interact with specific receptors, is of great interest [2]. Based on the known structure/activity relationship we designed a new series of analogues of amantadine and rimantadine with peptidomimetics [3].

Novel rimantadine and amantadine analogues have been synthesized with amino acids containing thiazole and thiazole rings and their activity on the Influenza virus A/Hongkong/68 have been explored. The rimantadine analogues with thiazole ring showed moderate activity against influenza virus A/Hongkong. The remaining compounds were considerably less effective.

The object of this study was to assess the chemical stability of some of the synthesised adamantane esters with peptidomimetics at pH 1.0 and pH 7.4 at 37°C [4].

EXPERIMENTAL

General information

Chemicals

Acetonitrile for HPLC the buffer components HCI, $Na_2H_2PO_4$ of the purest grade, were purchased from Merck (Germany). The Grace Vidac chromatographic column was used (USA).

Chromatography

Chromatography was carried out isocratically, on a modular KNAUER HPLC system (Germany), consisting of a Smartline Pump 1000, a Smartline Manager 5000 solvent degasser, an injector with a 20 μl loop and a Smartline UV Detector 2600 diode array. The analyses were controlled and the data were acquired with EuroChrom software. The mobile phase consisted of acetonitrile/water in a ratio of 30:70 or 50:50 v/v depending on the polarity of the compound and a flow rate 1 ml/min was used. The detection was performed at relevant λmax for the respective compound (range 252–262 nm).

Kinetic study

A single chromatographic method was used to detect the studied adamantane esters with thiazole rings containing amino acid glycine in aqueous buffer solutions at pH 1.0 (0.1 M HCl) and pH 7.4 (phosphate buffer). Twenty microlitres of each sample were injected into a reverse phase HPLC C18 column. The mobile phase consisted of acetonitrile/water at a ratio of 30:70 or 50:50 v/v depending on the polarity of the compound. The analyses of the esters of amantadine and rimantadine with amino acid containing thiazole rings were validated. The specificity of the method was investigated by observing potential interference between the esters of amantadine and rimantadine and its parent drug. No interfering peaks were presented in the chromatograms. The linearity of the relationship between the peak area and concentration was determined by analysing six standard solutions in a concentration range of 0.1–1.0 mmol/l. For all analytes, the relationship

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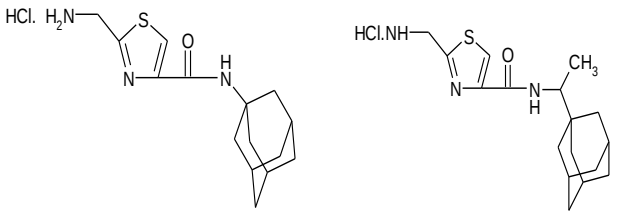
between the peak area ratio of the drug to the internal standard and concentration was linear over the entire examined concentration range. The correlation coefficients of the calibration curves were greater than 0.997. For all of the examined compounds the coefficient of variation calculated for the six analysed samples did not exceed 5%.

Hydrolysis of adamantane esters with thiazole rings containing amino acid Glycine was studied at pH 1.0 (HCl) and pH 7.4 (phosphate buffered saline). Stock solutions of the prodrugs were prepared and used immediately for stability studies. Aliquots (9.8 ml) of the buffer were placed in a screw-capped vial and allowed equilibrate at 37°C. A prodrug stock solution (0.2 ml) was added to the buffer. The vial was placed in a constant shaker bath set at 37°C and 60 rpm. Each sample was directly analysed by HPLC.

RESULTS AND DISCUSSION

The chemical stability of adamantane esters: HCI-2-aminomethyl-thiazole-amantadine (**1**), HCI-2-aminomethyl-thiazole-rimantadine (**2**) was studied under experimental conditions of biological relevance, i.e. at pH 1 and pH 7.4, at a temperature of 37°C. The compounds were synthesised as previously described. The structures of the compounds under investigation are presented on Fig. 1.



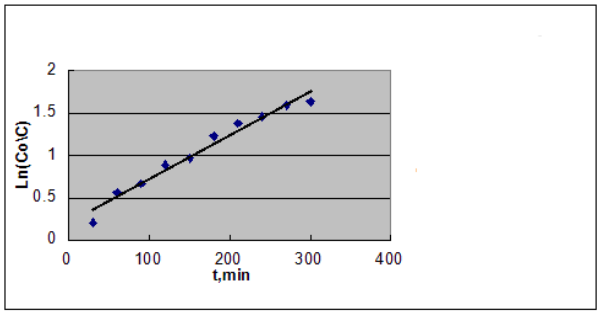
HCl.Gly-Thz-4-amantadine (**1**) HCl.Gly-Thz-4-rimantadine (**2**)

Fig.1.Adamantaneesters with peptidomimetics

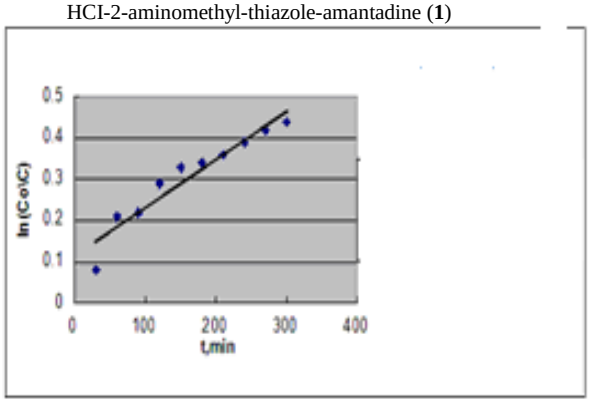
It was established that, under the described experimental conditions, some esters underwent decomposition by hydrolysis [5]. The hydrolysis followed apparent first order kinetics, and the rate constants (K) were obtained as slopes from the semi-logarithmic plots of the unchanged ester concentration versus time. The chemical stability was assessed by means of the decomposition half-lives:

$$t_{1/2} = \ln\frac{2}{K}$$

Chemical stability measurements revealed that the thiazolyl esters of amantadine and rimantadine were relatively unstable at acidic pH (Tabl.1., Fig. 2.).

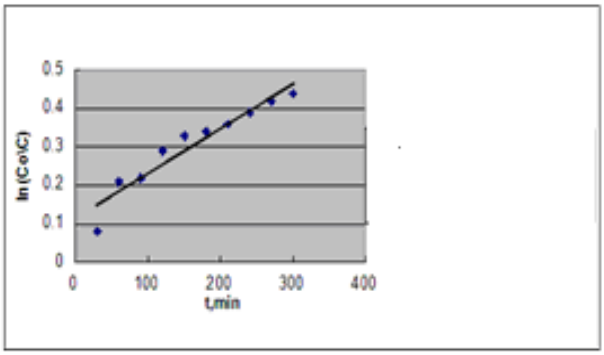


HCI-2-aminomethyl-thiazole-amantadine (**1**)

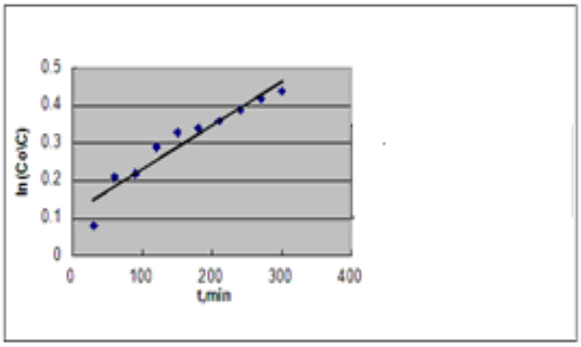


HCI-2-aminomethyl-thiazole-rimantadine (**2**)

Fig. 2. Decrease of the concentration of the esters at pH 1.0 (HCl)



HCI-2-aminomethyl-thiazole-amantadine (**1**)



HCI-2-aminomethyl-thiazole-rimantadine (**2**)

Fig. 3. Decrease of the concentration of the examined prodrugs in buffer solution at pH 7.4 (phosphate buffer)

Table 1. Half-lives (h) of thiazole analogues of amantadine and rimantadine at 37°C.

Compounds	pH=1	pH=7
1	0.53 h	1.03 h
2	1.48 h	6.05 h

The HCI-2-aminomethyl-thiazole-amantadine (1) and HCI-2-aminomethyl-thiazole-rimantadine (2) were less stable than the Boc-thiazole-OH at pH 1.0. Esters (1) and (2) manifest lower stability at pH 7.4. It was proved that the compound (2) is stable at the pH 7.4 (Fig. 3, Table 1.).

CONCLUSION

The chemical stability of thiazolyl esters of amantadine (1) and rimantadine (2) was studied in experimental conditions simulating some relevant biological medias (pH 1.0 and 7.4, 37°C). Test compounds are stable at pH 7.4 and 37°C where the highest stability manifests Boc-thiazole with rimantadine (t1/2=6.05 h) at pH 1.0 and 37°C the most stable is analogues of Boc-thiazole with rimantadine (t1/2=1.48 h).

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ХИМИЧНА СТАБИЛНОСТ НА ТИАЗОЛОВИ ПРОИЗВОДНИ НА РИМАНТАДИН И АМАНТАДИН

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(Резюме)

Нови аналози на амантадин и римантадин съдържащи тиазолов пръстен бяха синтезирани и бе изследвана противовирисната им активност спрямо грипен вирус [1].

Химичната стабилност на тиазоловите производни на амантадина и римантадина бе изследвана при pH=1, pH=7.4 и T=37°C с използване на високоефективна течна хроматография.