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A. D. Ocheretniuk, O. L. Kobzar, O. P. Kozachenko, V. S. Brovarets, A. I. Vovk

Institute of Bioorganic Chemistry and Petrochemistry of the National Academy of Sciences of Ukraine
1, Murmanska str., Kyiv, 02660, Ukraine. E-mail: oleksanrkobzar@gmail.com

Synthesis and the activity assessment of adamantyl-containing thiazolium inhibitors of butyrylcholinesterase

Cholinesterase inhibitors can be used for treatment of neuropsychiatric symptoms and functional impairments in neurodegenerative pathologies such as Alzheimer's and Parkinson's diseases.

Aim. To synthesize and assess the inhibitory activity of adamantyl-containing 5-substituted *N*-benzyl and *N*-phenacylthiazolium salts against butyrylcholinesterase and acetylcholinesterase.

Results and discussion. The synthesis of 3-aroylmethyl- and 3-arylmethyl-5-(2-acyloxyethyl)-4-methylthiazolium salts included preparation of 5-acyloxyethyl thiazole derivatives by the reaction of 5-(2-hydroxyethyl)-4-methyl-1,3-thiazole with the corresponding adamantoyl- or adamantylacetyl chlorides. The derivatives of 5-acyloxyethyl thiazole were quaternized in the reaction with benzyl or phenacyl halides. The studies *in vitro* have shown that the compounds synthesized inhibit butyrylcholinesterase with IC_{50} values in the micromolar range. Some of them exhibited selectivity over acetylcholinesterase. The molecular docking was performed for understanding the mechanisms of the enzyme-inhibitor complex formation.

Experimental part. The synthesis of the intermediate and target compounds was carried out by the classical methods. The structures of compounds were proven by NMR ^{1}H-spectroscopy and elemental analysis. The methods of enzymatic kinetics were used for determination of the inhibitory effects of the compounds synthesized. Calculations by molecular docking were carried out using Autodock 4.2 program.

Conclusions. 3-Aroylmethyl- and 3-arylmethyl-5-(2-acyloxyethyl)-4-methylthiazolium salts with adamantyl-containing substituents in position 5 can selectively inhibit butyrylcholinesterase compared to their effect on acetylcholinesterase.

Key words: butyrylcholinesterase; acetylcholinesterase; adamantan; inhibitor; thiazolium salt; molecular docking

А. Д. Очеретнюк, О. Л. Кобзар, О. П. Козаченко, В. С. Броварець, А. І. Вовк

Синтез та оцінка активності адамантиловмісних тіазолієвих інгібіторів бутирилхолінестерази

Відомо, що інгібітори холінестераз можуть використовуватись для лікування нейродегенеративних захворювань, таких як хвороба Альцгеймера і хвороба Паркінсона.

Мета роботи. Метою роботи був синтез та оцінка активності адамантиловмісних 5-заміщених *N*-бензильних та *N*-фенацильних солей тіазолію як інгібіторів бутирилхолінестерази і ацетилхолінестерази.

Результати та їх обговорення. Синтези 3-ароїлметил- і 3-арилметил-5-(2-ацилоксіетил)-4-метилтіазолієвих солей включали одержання 5-ацилоксіетильних похідних тіазолу при взаємодії 5-(2-гідроксіетил)-4-метил-1,3-тіазолу з відповідними адамантоїл- чи адамантилацетилхлоридами, які надалі кватернізували в реакції з бензил- або фенацилгалогенідами. Результати дослідження *in vitro* показали, що синтезовані сполуки інгібують бутирилхолінестеразу зі значеннями IC_{50} в мікромолярному діапазоні. Деякі з них демонстрували селективність дії у порівнянні з інгібуванням ацетилхолінестерази. Для з'ясування механізмів формування комплексів інгібіторів з бутирилхолінестеразою було проведено молекулярний докінг.

Експериментальна частина. Синтези проміжних і цільових сполук були виконані класичними способами. Структури сполук підтверджено методом ЯМР ^{1}H-спектроскопії та даними елементного аналізу. Для визначення інгібувального впливу синтезованих сполук на активність бутирилхолінестерази та ацетилхолінестерази були застосовані методи ферментативної кінетики. Для розрахунків методом молекулярного докінгу використано програму Autodock 4.2.

Висновки. 3-Ароїлметил- і 3-арилметил-5-(2-ацилоксіетил)-4-метилтіазолієві солі з адамантиловмісними замісниками в положенні 5 можуть селективно інгібувати бутирилхолінестеразу у порівнянні з їх впливом на ацетилхолінестеразу.

Ключові слова: бутирилхолінестераза; ацетилхолінестераза; інгібування; солі тіазолію; адамантан; молекулярний докінг

А. Д. Очеретнюк, А. Л. Кобзарь, А. П. Козаченко, В. С. Броварец, А. И. Вовк

Синтез и оценка активности адамантилсодержащих тиазолиевых ингибиторов бутирилхолинэстеразы

Известно, что ингибиторы холинэстераз могут использоваться для лечения нейродегенеративных заболеваний, таких как болезнь Альцгеймера и болезнь Паркинсона.

Цель работы. Целью работы был синтез и оценка активности адамантилсодержащих 5-замещенных *N*-бензильных и *N*-фенацильных солей тиазолия как ингибиторов бутирилхолинэстеразы и ацетилхолинэстеразы.

Результаты и их обсуждение. Синтезы 3-ароилметил- и 3-арилметил-5-(2-ацилоксиэтил)-4-метилтиазолиевых солей включали получение 5-ацилоксиэтильных производных тиазола при взаимодействии 5-(2-гидроксиэтил)-4-метил-1,3-тиазола с соответствующими адамантоил- или адамантилацетилхлоридами, которые в дальнейшем были кватернизированы в реакции с бензил- или фенацилгалогенидами. Результаты исследования *in vitro* показали, что синтезированные соединения ингибируют бутирилхолинэстеразу со значениями IC_{50} в микромолярном диапазоне. Некоторые из них демонстрировали селективность действия по сравнению с ингибированием ацетилхолинэстеразы. Для выяснения механизмов формирования комплексов ингибиторов с бутирилхолинэстеразой был применен молекулярный докинг.

Экспериментальная часть. Синтез промежуточных и целевых соединений был выполнен традиционными методами. Структуры соединений доказаны методом ЯМР ^{1}H-спектроскопии и данными элементного анализа. Для определения ингибирующего влияния синтезированных соединений на активность бутирилхолинэстеразы и ацетилхолинэстеразы использовали методы ферментативной кинетики. Для расчетов методом молекулярного докинга использовали програму Autodock 4.2.

Выводы. 3-Ароилметил- и 3-арилметил-5-(2-ацилоксиэтил)-4-метилтиазолиевые соли с адамантилсодержащими заместителями в положении 5 могут селективно ингибировать бутирилхолинэстеразу по сравнению с их влиянием на ацетилхолинэстеразу.

Ключевые слова: бутирилхолинестераза; ацетилхолинэстераза; ингибирование; соли тиазолия; адамантан; молекулярный докинг

Alzheimer's disease (AD), the most common cause of dementia in the elderly, is associated with β-amyloid aggregation, neuron dysfunction and decrease of the acetylcholine level in cholinergic synapses of the brain [1, 2]. This complex neurodegenerative disorder is characterized with progressive cognitive impairment, including the memory loss [3].

Cholinergic hypothesis is the most popular explanation of mechanisms of neurodegenerative disorders. According to it acetylcholinesterase (AChE; EC 3.1.1.7) as an enzyme, which is involved in the breakdown of acetylcholine in cholinergic synapses, can be a target for therapeutic intervention [2]. It should be also noted that AChE predominates in the healthy brain, while butyrylcholinesterase (BChE; EC 3.1.1.8) plays a minor role in regulation of the acetylcholine level [4]. However, the BChE activity in the brain of AD patients is increased, while the AChE activity remains constant or decreases. In case of the AChE knockout mice, BChE is able to hydrolyze acetylcholine and compensates the lack of the AChE activity [5]. The BChE exhibiting peptidase activity may be involved into AD progression due to production of β-amyloid plaques [6]. All these facts suggest that BChE can also be a therapeutic target in the treatment of neurodegenerative disorders. In addition, the inhibitors of BChE were found to increase the acetylcholine level in the cortex of the rat brain tissues, improve their learning [5], and they did not show significant adverse side effects.

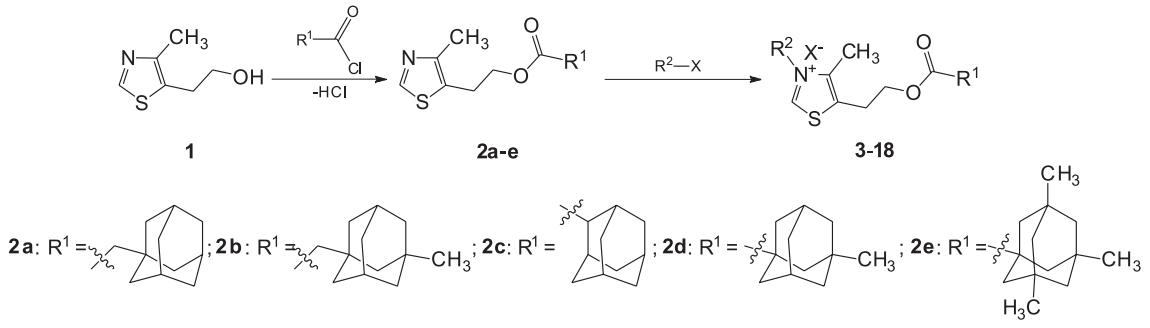
A broad number of compounds were described as selective inhibitors of AChE. Some of them are used for the treatment of Alzheimer's disease [7] and show the activity against Parkinson disease, Lewy Body Dementia [8], and schizophrenia [9]. However, only a few compounds were described as selective inhibitors of BChE, for example, cymserine and its analogs [10], carbamate derivatives of isosorbide [11], and antiparkinson drug profenamine [12]. Rivastigmine, which is used in clinical practice, is a non-selective inhibitor of AChE and BChE [7]. Tacrine, which shows the side effect of hepatotoxicity, is a selective inhibitor of BChE [13].

In the present paper, we synthesized a series of 3-aroylmethyl- and 3-arylmethyl-5-(2-acyloxyethyl)-4-methylthiazolium salts with adamantoyloxyethyl or adamantylacetoxyethyl substituents in position 5 and assessed their inhibitory activity towards AChE and BChE. These compounds may be considered as structural analogs of the thiazolium part of vitamin B_1 which exhibits only a weak activity towards AChE [14] and BChE. The change of the substituent in position 3 of thiamine and introduction of a bulky adamantyl fragment in position 5 of the thiazolium ring was expected to improve the inhibitory properties of the compounds synthesized. Different adamantane derivatives are known to demonstrate the antiviral, antibacterial and anticancer activities [15-17]. Among them, amantadine, rimantadine and memantine show the anti-Parkinson's and anti-Alzheimer's effects [18-20], but their activities are not associated with inhibition of cholinesterases. At the same time, 6-*O*-demethyl-6-*O*-[(adamantan-1-yl)carbonyl]galanthamine and adamantine-substituted guanylhydrazones are described as inhibitors of AChE and BChE, respectively [21, 22].

Results and discussion

1. Chemistry

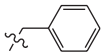
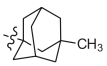
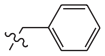
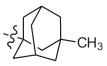
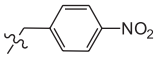
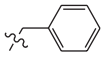
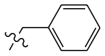
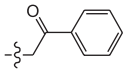
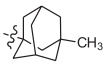
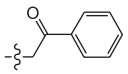
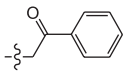
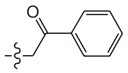
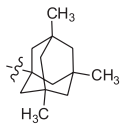
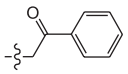
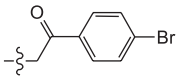
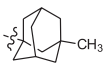
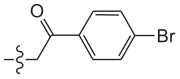
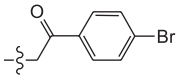
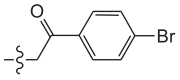
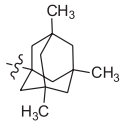
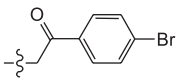
Adamantyl-containing *N*-benzyl- and *N*-phenacylthiazolium salts **3**-**18** were synthesized according to the procedure described in Scheme. The trivial synthetic route [23-25] involves preparation of interme-



Scheme. The synthesis of adamantyl-containing N-benzyl- and N-phenacylthiazolium salts **3-18**

Table

Compounds **3**-**18** as inhibitors of acetylcholinesterase and butyrylcholinesterase*/**

Compound	R^1	R^2	IC_{50}, μM	
			AChE	BChE
3		H	n. a.***	n. a.
4			4.9 ± 1.2	1.6 ± 0.5
5			6.6 ± 1.8	2.0 ± 0.33
6			6.0 ± 1.1	6.9 ± 0.5
7			2.4 ± 0.7	1.4 ± 0.4
8			2.8 ± 0.5	0.7 ± 0.1
9			15 ± 3	1.6 ± 0.5
10			16 ± 4	2.8 ± 0.7
11			23 ± 6	2.5 ± 0.7
12			19 ± 5	0.9 ± 0.2
13			19 ± 4	0.42 ± 0.07
14			29 ± 8	2.7 ± 0.6
15			24 ± 6	9.9 ± 2.8
16			57 ± 9	6.4 ± 1.1
17			> 60	4.3 ± 1.1
18			> 60	2.7 ± 0.7

Notes: * – IC_{50} values are the means of 2-3 assays ± standard deviations; ** – The substrate concentrations were of 0.1 mM for AChE and 0.5 mM for BChE; *** – no activity under the assay conditions

diate compounds **2a**-**e** by the reaction of 5-(2-hydroxyethyl)-4-methyl-1,3-thiazole (**1**) with the appropriate adamantyl-containing acyl chlorides. At the second stage, thiazole compounds **2a**-**e** were quaternized with anhydrous hydrogen chloride, or benzyl chlorides, or phenacyl halogenides, giving salts **3**-**18**.

2. Assessment of the anticholinesterase activity

Compounds **3**-**19** were tested as inhibitors of AChE and BChE using the Ellman's method [26]. The absence of the inhibitory activity of compound **3** suggests the importance of a benzyl or phenacyl substituent in position 3. The data represented in Table

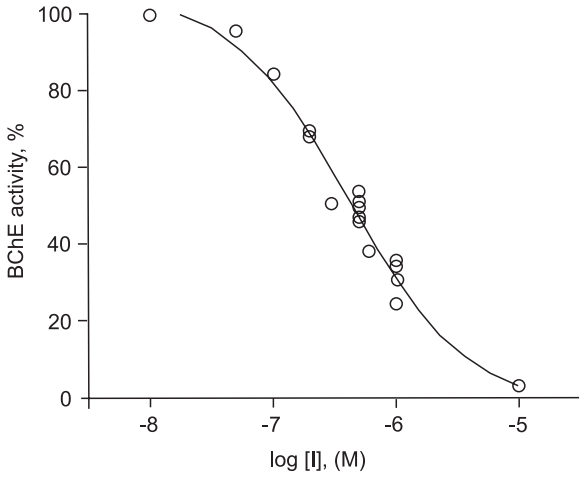


Fig. 1. The dose-dependent curve of BChE inhibition by compound **13**

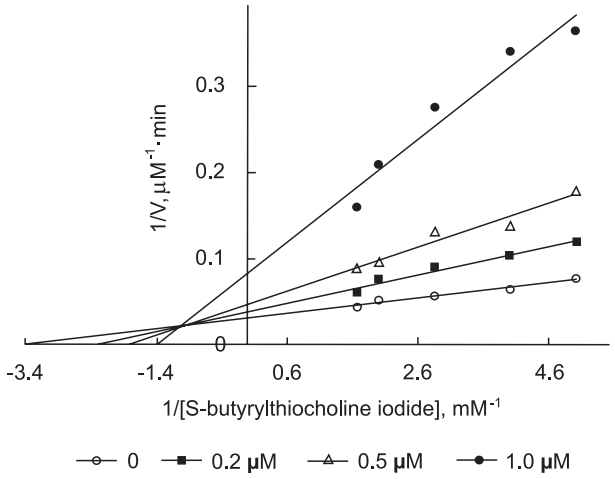


Fig. 2. The Lineweaver-Burk plots for inhibition of BChE by compound **13**

show that compounds **4**-**18** exhibit inhibition of BChE with IC_{50} values in the range from 0.42 to 10 μM. In case of AChE, this inhibition was less effective with IC_{50} values higher than 2 μM. *N*-Benzylthiazolium derivatives **4**-**8**, including compound **6** with the *p*-nitrobenzyl group, showed the micromolar inhibitory activity towards BChE and a modest selectivity over AChE. *N*-Phenacylthiazolium salts **9**-**13** were more effective inhibitors of BChE with greater selectivity over AChE. Among them, compounds **12** and **13** bearing the 3-methyl-1-adamantyl and 3,5,7-trimethyl-1-adamantyl group had IC_{50} values of 0.9 μM and 0.42 μM, respectively, and showed more than the 10-fold selectivity over AChE. *p*-Bromophenacyl derivatives **14**-**18** did not show better inhibitory effects and selectivity.

The dose-dependent curve of BChE inhibition by compound **13** is shown in Fig. 1. The calculated Hill slope is 0.90 ± 0.14. According to the Lineweaver-Burk plot (Fig. 2) compound **13** is a mixed inhibitor of BChE. This suggests that the inhibitor interacts with free enzyme and the enzyme-substrate complex. The apparent K_i and K_i' values are 0.23 ± 0.05 μM and 0.76 ± 0.16 μM, respectively.

3. Molecular docking

The molecular docking was performed for understanding the mechanisms of the enzyme-inhibitor complex formation. Compound **13** was docked into the active site of human BChE (PDB code 4BDS [27]). The result obtained showed that compound **13** could be positioned at the active site of BChE (Fig. 3) with the estimated binding energy of -9.60 kcal/mol. According to the possible binding mode the 3,5,7-trimethyl-1-adamantyl fragment was located in the anionic binding site and surrounded by amino acid residues of Asp70, Gly78, Ser79, Trp82, Tyr332, Trp430, Met437, and Tyr440. The phenacyl fragment of the inhibitor occupied the ester binding site and provided electrostatic and van der Waals interactions with amino acid residues of Gly117, Trp231, Leu286, Val288, Phe329, Phe398, His438. The oxygen atom of the phenacyl substituent could form the hydrogen bond with Ser198 belonged to the catalytic triad (Ser198, His438 and Glu325). The binding mode suggests that hydrophobic, van der Waals and electrostatic interactions are responsible for stabilization of the enzyme-inhibitor complex.

Experimental Protocols

1. Chemistry

The ^{1}H NMR-spectra were recorded on a Varian M400 (400 MHz/100 MHz) spectrometer in DMSO-d_6. 4-Methyl-5-(2-hydroxyethyl)thiazole (**1**), benzyl chloride, phenacyl bromide, and *p*-bromophenacyl bromide were commercially available. Compounds **2a**-**e** were prepared by the reaction of 5-(2-hydroxyethyl)-4-methyl-1,3-thiazole (**1**) with the appropriate acyl chlorides in benzene in the presence of triethylamine (Scheme). Adamantyl-containing acyl chlorides were obtained from the corresponding carboxylic acids.

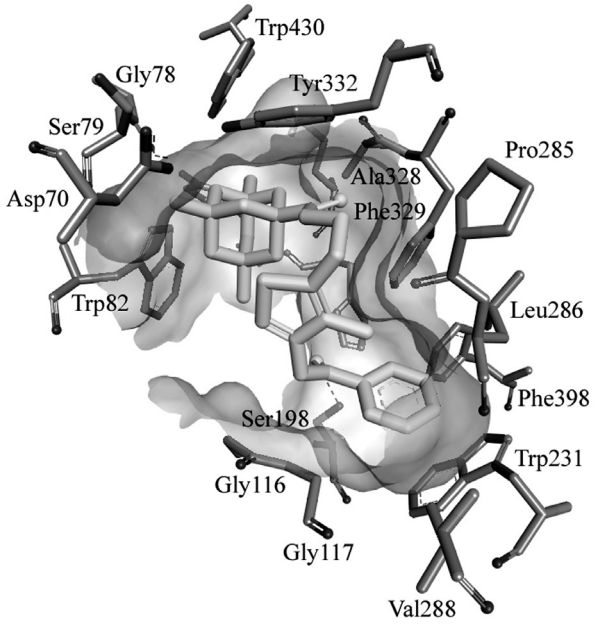


Fig. 3. A possible binding mode of compound **13** at the active site of human BChE

The synthesis of compounds 3, 9-18. Add benzyl chloride, or phenacyl halogenide, or *p*-bromophenacyl bromide (5 mmol) to the solution of 4.5 mmol of 4-methyl-5-substituted thiazole **2a-e** in acetone. Reflux the reaction mixture for 3-5 h and allow it to stand at the room temperature. Treat the product with acetone and diethyl ether, filter and recrystallize from the methanol-acetone-diethyl ether mixture to give compounds **9-18**. Compound **3** was synthesized by the interaction of **2a** with anhydrous hydrogen chloride in benzene at the room temperature.

The synthesis of compounds 4-8. Heat the mixture of benzyl chloride or *p*-nitrobenzyl chloride (5 mmol) and 4.5 mmol of 4-methyl-5-substituted thiazole (compounds **2a-d**) at 100-115 °C for 1.5-4.5 h. After that add acetone and diethyl ether to wash the solidified product, and allow the mixture to stand at the room temperature. Recrystallize the product from the mixture of methanol, acetone, and diethyl ether to give compounds **4-8**.

2-(4-Methyl-1,3-thiazol-5-yl)ethyl 1-adamantylacetate hydrochloride (**3**). Yield – 46 %, a white solid. M. p. – 135-137 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 1.40-1.65 (m, 12H), 1.82 (s, 3H), 2.00 (s, 2H), 2.37 (s, 3H), 3.13 (t, 2H, *J* = 5.60 Hz), 4.17 (t, 2H, *J* = 5.60 Hz), 6.72 (s, 1H), 9.34 (s, 1H). Anal. calcd. for $C_{18}H_{26}ClNO_2S$: C, 59.72; H, 7.08; N, 4.10. Found: C, 60.07; H, 6.94; N, 3.91.

5-{2-[(2-Adamantyl)carbonyloxy]ethyl}-3-benzyl-4-methyl-1,3-thiazolium chloride (**4**). Yield – 50 %, a white solid. M. p. – 192-194 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 1.45 (d, 2H, *J* = 12.55 Hz), 1.81-1.55 (m, 11H), 2.12 (s, 2H), 2.35 (s, 3H), 3.28 (t, 2H, *J* = 6.02 Hz), 4.24 (t, 2H, *J* = 6.02 Hz), 5.89 (s, 2H), 7.33 (d, 2H, *J* = 8.03 Hz), 7.45-7.39 (m, 3H), 10.53 (s, 1H). Anal. calcd. for $C_{24}H_{30}ClNO_2S$: C, 66.72; H, 7.00; N, 3.24. Found: C, 67.42; H, 6.53; N, 2.94.

3-Benzyl-4-methyl-5-{2-[(3-methyl-1-adamantyl)carbonyloxy]ethyl}-1,3-thiazolium chloride (**5**). Yield – 31 %, a white solid. M. p. – 173-175 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 0.77 (s, 3H), 1.30-1.70 (m, 12H), 1.97 (s, 2H), 2.38 (s, 3H), 3.27 (s, 2H), 4.22 (t, 2H, *J* = 5.03 Hz), 5.88 (s, 2H), 7.33 (t, 2H, *J* = 8.76 Hz), 7.43 (d, 3H, *J* = 7.46 Hz), 10.50 (s, 1H). Anal. calcd. for $C_{25}H_{32}ClNO_2S$: C, 67.32; H, 7.23; N, 3.14. Found: C, 67.88; H, 6.89; N, 2.86.

4-Methyl-5-{2-[(3-methyl-1-adamantyl)carbonyloxy]ethyl}-3-(4-nitrobenzyl)-1,3-thiazolium bromide (**6**). Yield – 41 %, a white solid. M. p. – 197-199 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 0.73 (s, 3H), 1.27-1.40 (m, 6H), 1.49-1.63 (m, 6H), 1.92 (s, 2H), 2.31 (s, 3H), 3.27 (t, 2H, *J* = 6.02 Hz), 4.19 (t, 2H, *J* = 5.52 Hz), 6.02 (s, 2H), 7.59 (d, 2H, *J* = 8.53 Hz), 8.27 (s, 1H), 8.29 (s, 1H), 10.38 (s, 1H). Anal. calcd. for $C_{25}H_{31}BrN_2O_4S$: C, 56.07; H, 5.84; N, 5.23. Found: C, 56.63; H, 5.03; N, 4.98.

5-{2-[(1-Adamantyl)acetyloxy]ethyl}-3-benzyl-4-methyl-1,3-thiazolium chloride (**7**). Yield – 29 %, a white solid. M. p. – 170-172 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 1.47 (s, 6H), 1.55 (d, 3H, *J* = 11.54 Hz), 1.62 (d, 3H, *J* = 11.05 Hz), 1.87 (s, 3H), 1.96 (s, 2H), 2.35 (s, 3H), 3.25 (t, 2H, *J* = 5.02 Hz), 4.19 (t, 2H, *J* = 5.02 Hz), 5.81 (s, 2H), 7.30 (d, 2H, *J* = 6.02 Hz), 7.42 (d, 3H, *J* = 7.03 Hz), 10.28 (s, 1H). Anal. calcd. for $C_{25}H_{32}ClNO_2S$: C, 67.32; H, 7.23; N, 3.14. Found: C, 67.71; H, 6.58; N, 2.74.

3-Benzyl-4-methyl-5-{2-[(3-methyl-1-adamantyl)acetyloxy]ethyl}-1,3-thiazolium chloride (**8**). Yield – 42 %, a white solid. M. p. – 147-149 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 0.74 (s, 3H), 1.18-1.52 (m, 12H), 1.93 (s, 2H), 1.99 (s, 2H), 2.36 (s, 3H), 3.26 (s, 2H), 4.21 (t, 2H, *J* = 5.60 Hz), 5.84 (s, 2H), 7.32 (d, 2H, *J* = 7.46 Hz), 7.19-7.38 (m, 3H), 10.35 (s, 1H). Anal. calcd. for $C_{26}H_{34}ClNO_2S$: C, 67.88; H, 7.45; N, 3.04. Found: C, 68.34; H, 7.13; N, 2.86.

5-{2-[(2-Adamantyl)carbonyloxy]ethyl}-4-methyl-3-(2-oxo-2-phenylethyl)-1,3-thiazolium bromide (**9**). Yield – 30 %, a white solid. M. p. – 187-189 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 1.58 (d, 2H, *J* = 12.55 Hz), 1.63-1.88 (m, 10H), 2.24 (s, 2H), 2.35 (s, 3H), 2.66 (s, 1H), 3.33 (s, 2H), 4.30 (t, 2H, *J* = 6.03 Hz), 6.42 (s, 2H), 7.65 (t, 2H, *J* = 7.53 Hz), 7.79 (t, 1H, *J* = 7.03 Hz), 8.07 (d, 2H, *J* = 7.52 Hz), 10.03 (s, 1H). Anal. calcd. for $C_{25}H_{30}BrNO_3S$: C, 59.52; H, 5.99; N, 2.78. Found: C, 60.46; H, 5.38; N, 2.54.

4-Methyl-5-{2-[(3-methyl-1-adamantyl)carbonyloxy]ethyl}-3-(2-oxo-2-phenylethyl)-1,3-thiazolium bromide (**10**). Yield – 71 %, a white solid. M. p. – 142-144 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 0.79 (s, 3H), 1.36-1.76 (m, 12H), 2.01 (s, 2H), 2.36 (s, 3H), 3.32 (s, 2H), 4.25 (s, 2H), 6.42 (s, 2H), 7.65 (t, 2H, *J* = 7.53), 7.78 (t, 1H, *J* = 7.53 Hz), 8.06 (d, 2H, *J* = 7.53 Hz), 10.03 (s, 1H). Anal. calcd. for $C_{26}H_{32}BrNO_3S$: C, 60.23; H, 6.22; N, 2.70. Found: C, 60.91; H, 6.04; N, 2.34.

5-{2-[(1-Adamantyl)acetyloxy]ethyl}-4-methyl-3-(2-oxo-2-phenylethyl)-1,3-thiazolium bromide (**11**). Yield – 40 %, a white solid. M. p. – 169-171 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 1.50-1.68 (m, 12H), 1.91 (s, 3H), 2.05 (s, 2H), 2.37 (s, 3H), 3.32 (t, 2H, *J* = 6.52 Hz), 4.25 (t, 2H, *J* = 5.60 Hz), 6.45 (s, 2H), 7.65 (t, 2H, *J* = 8.39 Hz), 7.79 (t, 1H, *J* = 7.46 Hz), 8,05 (d, 2H, *J* = 7.46 Hz), 10.08 (s, 1H). Anal. calcd. for $C_{26}H_{32}BrNO_3S$: C, 60.23; H, 6.22; N, 2.70. Found: C, 61.10; H, 5.87; N, 2.23.

4-Methyl-5-{2-[(3-methyl-1-adamantyl)acetyloxy]ethyl}-3-(2-oxo-2-phenylethyl)-1,3-thiazolium bromide (**12**). Yield – 42 %, a white solid. M. p. – 170-172 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 0.75 (s, 3H), 1.23-1.53 (m, 12H), 1.95 (s, 2H), 2.07 (s, 2H), 2.36 (s,3H), 3.30 (s, 2H), 4.24 (s, 2H), 6.48 (s, 2H), 7.65 (t, 2H, 7.53 Hz), 7.79 (t, 1H, *J* = 6.52),

8.05 (d, 2H, *J* = 6.02 Hz), 10.13 (s, 1H). Anal. calcd. for $C_{27}H_{34}ClNO_3S$: C, 66.44; H, 7.02; N, 2.87. Found: C, 66.76; H, 6.67; N, 2.56.

4-Methyl-3-(2-oxo-2-phenylethyl)-5-{2-[(3,5,7-trimethyl-1-adamantyl)carbonyloxy]ethyl}-1,3-thiazolium bromide (**13**). Yield – 49 %, a white solid. M. p. – 195-197 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 0.81 (s, 9H), 1.01-1.09 (m, 6H), 1.35 (s, 6H), 2.36 (s, 3H), 3.33 (s, 2H), 4.25 (s, 2H), 6.42 (s, 2H), 7.66 (t, 2H, *J* = 7.53 Hz), 7.79 (t, 1H, *J* = 7.03 Hz), 8.04 (d, 2H, *J* = 7.03 Hz), 10.03 (s, 1H). Anal. calcd. for $C_{28}H_{36}BrNO_3S$: C, 61.53; H, 6.64; N, 2.56. Found: C, 61.95; H, 6.07; N, 2.18.

5-{2-[(2-Adamantyl)carbonyloxy]ethyl}-3-[2-(4-bromophenyl)-2-oxoethyl]-4-methyl-1,3-thiazolium bromide (**14**). Yield – 33 %, a white solid. M. p. – 247-249 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 1.58 (d, 2H, *J* = 12.55 Hz), 1.65-1.86 (m, 10H), 2.24 (s, 2H), 2.35 (s, 3H), 2.65 (s, 1H), 3.33 (t, 2H, *J* = 6.03 Hz), 4.30 (t, 2H, *J* = 6.03 Hz), 6.37 (s, 2H), 7.90 (d, 2H, *J* = 7.53 Hz), 7.97 (d, 2H, *J* = 7.03 Hz), 9.99 (s, 1H). Anal. calcd. for $C_{25}H_{29}Br_2NO_3S$: C, 51.47; H, 5.01; N, 2.40. Found: C, 52.06; H, 4.87; N, 2.03.

3-[2-(4-Bromophenyl)-2-oxoethyl]-4-methyl-5-{2-[(3-methyl-1-adamantyl)carbonyloxy]ethyl}-1,3-thiazolium bromide (**15**). Yield – 55 %, a white solid. M. p. – 223-225 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 0.79 (s, 3H), 1.35-1.75 (m, 12H), 2.01 (s, 2H), 2.36 (s, 3H), 3.31 (s, 2H), 4.24 (s, 2H), 6.41 (s, 2H), 7.90 (d, 2H, *J* = 7.53 Hz), 7,96 (d, 2H, *J* = 7.53 Hz), 10.02 (s, 1H). Anal. calcd. for $C_{26}H_{31}Br_2NO_3S$: C, 52.27; H, 5.23; N, 2.34. Found: C, 52.85; H, 4.91; N, 1.98.

5-{2-[(1-Adamantyl)acetyloxy]ethyl}-3-[2-(4-bromophenyl)-2-oxoethyl]-4-methyl-1,3-thiazolium bromide (**16**). Yield – 32 %, a white solid. M. p. – 202-204 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 1.46-1.66 (m, 12H**),** 1.91 (s, 3H), 2.05 (s, 2H), 2.36 (s, 3H), 3.31 (t, 2H, *J* = 6.02 Hz), 4.25 (t, 2H, *J* = 5.02 Hz), 6.38 (s, 2H), 7.90 (d, 2H, *J* = 7.53 Hz), 7.97 (d, 2H, *J* = 8.03 Hz), 10.01 (s, 1H). Anal. calcd. for $C_{26}H_{31}Br_2NO_3S$: C, 52.27; H, 5.23; N, 2.34. Found: C, 53.03; H, 4.86; N, 2.02.

3-[2-(4-Bromophenyl)-2-oxoethyl]-4-methyl-5-{2-[(3-methyl-1-adamantyl)acetyloxy]ethyl}-1,3-thiazolium bromide (**17**). Yield – 69 %, a white solid. M. p. – 196-198 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 0.75 (s, 3H), 1.20-1.58 (m, 12H), 1.95 (s, 2H), 2.07 (s, 2H), 2.37 (s, 3H), 3.36 (s, 2H), 4.25 (s, 2H), 6.45 (s, 2H), 7.89 (t, 2H, *J* = 8.40 Hz), 8,01 (d, 2H, *J* = 6.02 Hz), 10.09 (s, 1H). Anal. calcd. for $C_{27}H_{33}Br_2NO_3S$: C, 53.04; H, 5.44; N, 2.29. Found: C, 53.55; H, 5.02; N, 2.12.

3-[2-(4-Bromophenyl)-2-oxoethyl]-4-methyl-5-{2-[(3,5,7-trimethyl-1-adamantyl)carbonyloxy] ethyl}-1,3-thiazolium bromide (**18**). Yield – 41 %, a white solid. M. p. – 246-248 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 0.81 (s, 9H), 1.01-1.09 (m, 6H), 1.35 (s, 6H), 2.35 (s, 3H), 3.32 (s, 2H), 4.25 (s, 2H), 6.40 (s, 2H), 7.90 (d, 2H, *J* = 7.02 Hz), 7.96 (d, 2H, *J* = 8.54 Hz), 10.02 (s, 1H). Anal. calcd. for $C_{287}H_{35}Br_2NO_3S$: C, 53.77; H, 5.64; N, 2.24. Found: C, 54.23; H, 5.17; N, 2.06.

2. *In vitro* study of AChE and BChE inhibition

Acetylcholinesterase from electric eel, butyrylcholinesterase from the equine serum, and Ellman's reagent (DTNB) were purchased from Sigma-Aldrich. *S*-Acetylthiocholine iodide (Sigma-Aldrich) and *S*-butyrylthiocholine iodide (Fluka) were used as substrates for AChE and BChE, respectively.

Spectrophotometric detection of 5-thio-2-nitrobenzoate (TNB^{2-}) was performed at the wavelength of 412 nm using the molar extinction coefficient of TNB^{2-} of 14150 M^{-1} cm^{-1} [28]. Before the *in vitro* study the inhibitor was dissolved in dimethyl sulfoxide. The assay solution (0.5 mL) contained of 25 mM phosphate buffer (pH 7.48), 0.1 mM *S*-acetylthiocholine iodide, 1 % DMSO, 1 mM DTNB, inhibitor, and water. AChE was added after incubation of the reaction mixture at 25 °C for 5 min. The inhibition of BChE was studied in the similar conditions with 0.5 mM *S*-butyrylthiocholine iodide.

The Hill coefficient for compound **13** was calculated from the dose-dependent inhibition curve (Fig. 1) using the equation with four parameters. The values of IC_{50} presented in Table are concentrations of inhibitors, which reduce the enzyme activity by 50 %. The kinetic data of the enzymatic transformation of *S*-butyrylthiocholine iodide in the absence and the presence of compound **13** were calculated from Lineweaver-Burk plots (Fig. 2).

3. Molecular docking

The molecular docking calculations were performed by Autodock 4.2 using the Lamarckian Genetic Algorithm. Compound **13** was docked into the active site of human BChE (PDB code 4BDS [27]). Before the docking experiment the ligand and water molecules were removed from the protein structure. The three-dimensional structure of compound **13** was optimized by the AM1 semi-empirical quantum mechanical method in MOPAC program. MGLTools was used to prepare the docking files.

Conclusions

1. Adamantyl-containing *N*-benzyl- and *N*-phenacylthiazolium salts can exhibit the inhibitory properties against BChE with IC_{50} values in the micromolar range and selectivity over AChE.

2. The most effective compound **13** is a mixed inhibitor of BChE.

3. The molecular docking calculations indicate that the compound occupies the anionic and esterase binding sites of the enzyme providing hydrophobic, van der Waals and electrostatic interactions.

Conflict of Interests: authors have no conflict of interests to declare.

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