N-Phenacylthiazolium Salts as Inhibitors of Cholinesterases

Alla D. Ocheretniuk, Oleksandr L. Kobzar, Iryna M. Mischenko, Andriy I. Vovk*

Institute of Bioorganic Chemistry and Petrochemistry of the National Academy of Sciences of Ukraine, Murmanska Street, 1, Kyiv-94 02094, Ukraine

vovk@bpci.kiev.ua

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Inhibition of acetylcholinesterase is considered as a promising approach for treatment of neurodegenerative disorders including Alzheimer's disease. In this study, we demonstrated that 5-substituted *N*-phenacylthiazolium derivatives are capable of inhibiting acetylcholinesterase and butyrylcholinesterase activities with IC_{50} values in the micromolar range. Some of the new thiazolium-based inhibitiors showed more than 10-fold selectivity for butyrylcholinesterase. Kinetic experiments and molecular docking were performed for understanding the inhibition mechanisms.

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1. Introduction

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) belong to a class of serine hydrolases and have 65% identity in amino acid sequences [1, 2]. The function of AChE is termination of nerve impulses at the cholinergic synapses through acetylcholine hydrolysis. Reduced amount of the acetylcholine, because of abnormal activity of choline acetyltransferase [3, 4], along with extracellular deposits of β-amyloid in senile plaques is associated with cognitive function impairment in patients with Alzheimer's disease [5, 6]. According to the cholinergic hypothesis [7], consequences of the neurotransmitter deficit can be reduced by inhibition of AChE [5, 8]. Such approach is used in treatment of Alzheimer's disease and may be effective in Parkinson's disease and dementia with Lewy bodies [9-11]. The functions of BChE are still not fully understood. This enzyme is able to participate in fat metabolism [12], protect the AChE from inactivation by the anticholinergic agents [13], and compensate the lack of AChE activity [14, 15]. In addition, BChE is involved in the formation of amyloid plaques [16], and increased activity of the enzyme is observed in Alzheimer's disease [1, 17, 18].

2,4-Substituted thiazoles [19-21] including thiazole drug acotiamide [22] as well as derivatives of benzothiazole, [23, 24] thiazolopyrimidine, [25] and thiazolotriazinone [26, 27] can exhibit inhibitory properties against AChE and BChE. It should be noted that some of thiazolium salts may be considered as neuromuscular blocking agents [28, 29].

Thiamine pyrophosphate and thiazolium salts were shown to break protein crosslinks (AGEs) [30-32]. Vitamin B_1 (thiamine) and its derivatives are important regulators of metabolic processes in the central nervous system [33, 34] possessing a weak inhibitory action on cholinesterases [35].

We have suggested that the thiazolium scaffold may be used to design compounds with enhanced inhibitory potential against cholinesterases. In this paper, a series of *N*-phenacyl-4-methyl-5-(2-substituted) thiazolium salts has been synthesized and tested for their ability of inhibiting acetylcholinesterase and butyrylcholinesterase *in vitro*.

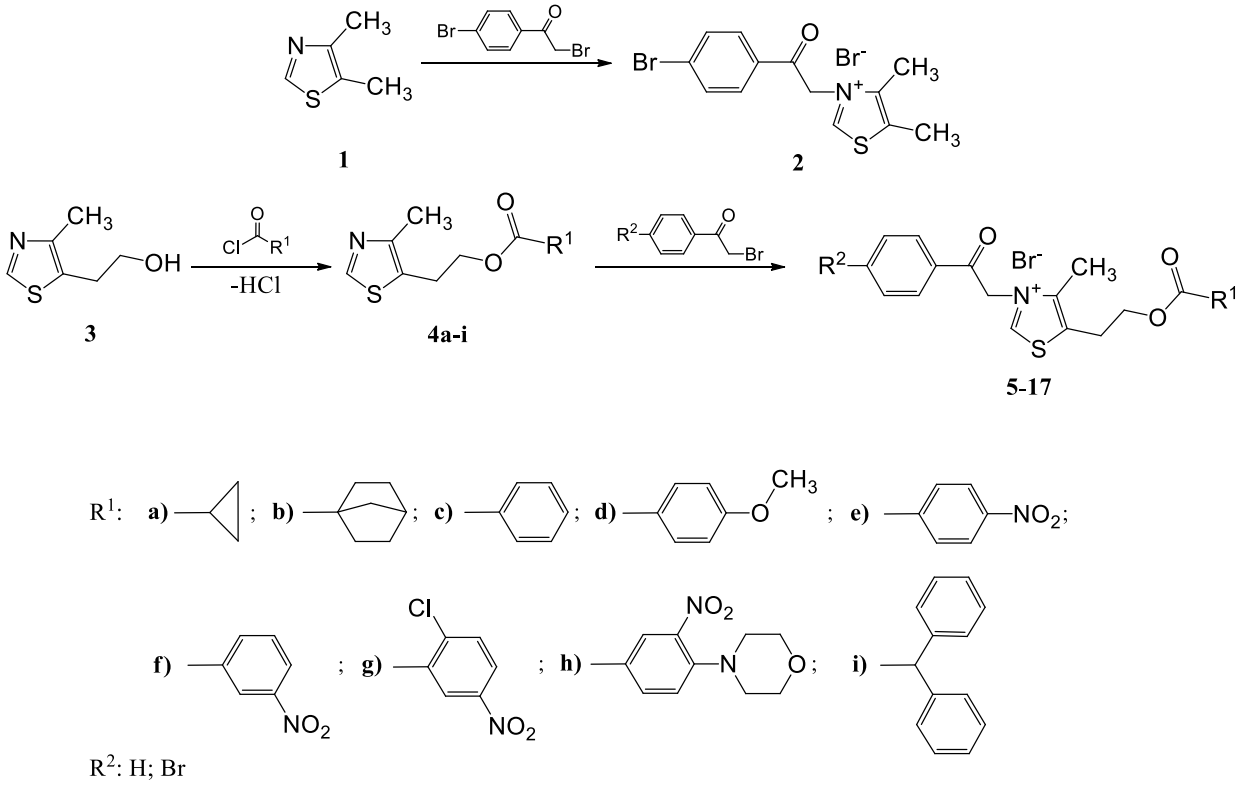
2. Results and discussion

Chemistry

Target *N*-phenacylthiazolium salts **5-17** were obtained in two steps (Scheme 1). The synthetic procedure involves the preparation of compounds **4a-i** by reaction of 5-(2-hydroxyethyl)-4-methyl-1,3-thiazole (**3**) with the appropriate acyl chlorides [36-38]. Then, the *O*-acylated compounds were reacted with corresponding phenacyl bromides, giving thiazolium salts **5-17**. Compound **2** was synthesized by quaternization of 4,5-dimethyl-1,3-thiazole (**1**) [32].

Inhibitory activity

The activities of the thiazolium salts were evaluated *in vitro* using AChE from *E. electricus* and BChE from equine serum. The



Scheme 1. Synthesis of *N*-phenacylthiazolium salts **2**, **5-17**.

results in Table 1 show that the nature of R^1 substituent modulates inhibitory activity of the compounds tested. Thiazolium salts **2** and **5-10** exhibited weak inhibitory effects on activity of AChE, while BChE was more inhibited, especially by compounds **6-9** bearing norbornyl, phenyl or 4-methoxyphenyl groups. Among them, the highest inhibitory activity toward BChE was observed for 5-(4-methoxybenzoyloxyethyl) substituted thiazolium salt **9** (IC_{50} = 0.94 μM) with more than 30-fold selectivity over AChE. 3-Nitrophenyl derivatives **12-14** were found to be more potent inhibitors of both AChE and BChE as compared to their 4-nitrophenyl analogs **10-11.** Compound **14** exhibits IC_{50} of 0.85 μM for AChE and IC_{50} of 1.4 μM in case of BChE. Further modification of substituent R^1 with morpholino groups did not improve significantly the inhibitory properties of compounds **15** and **16.** Introduction of bromine atom as R^2 resulted in decreasing the inhibitory effects of compounds **10** and **15** on AChE whereas the inhibitory ability of compounds **7**

Table 1. Compounds **2**, **5-17** as inhibitors of cholinesterases[a,b,c]

№	R^2	R^1	IC_{50}, μM	
			AChE	BChE
2	-	-	52.0±12.8	59.7±9.9
5	Br	cyclopropanyl	59.2±14.9	66.7±14.1
6	H	bicyclo[2.2.1]heptane-1-yl	51.3±15.4	4.2±0.5
7	Br	phenyl	57.8±13.4	6.7±1.2
8	H	phenyl	44.2±6.3	4.5±0.9
9	H	4-metoxyphenyl	33.1±7.9	0.94±0.28
10	Br	4-nitrophenyl	22.0±4.9	15.7±4.6
11	H	4-nitrophenyl	8.8±1.0	16.3±4.7
12	Br	3-nitrophenyl	2.8±0.5	2.8±0.4
13	H	3-nitrophenyl	2.7±0.6	2.4±0.5
14	H	2-chlor-5-nitrophenyl	0.85±0.23	1.9±0.55
15	Br	4-morpholino-3-nitrophenyl	22.9±3.7	1.0±0.18
16	H	4-morpholino-3-nitrophenyl	7.7±1.2	2.1±0.44
17	H	2,2-diphenylmethyl	10.4±0.99	0.14±0.03

[a] IC_{50} values are the means of 2-3 assays ± standard deviations; [b] under assay conditions the substrates concentration were 0.1 mM and 0.5 mM for AChE and BChE, respectively; [c] The determined K_m values were 0.31 mM (AChE) and 0.26 mM (BChE).

and **12** was unchanged. Among the inhibitors tested, compound **17** demonstrated the most potent BChE inhibition (IC_{50} = 0.14 μM) with more than 50-fold selectivity over AChE (IC_{50} = 10.4 μM).

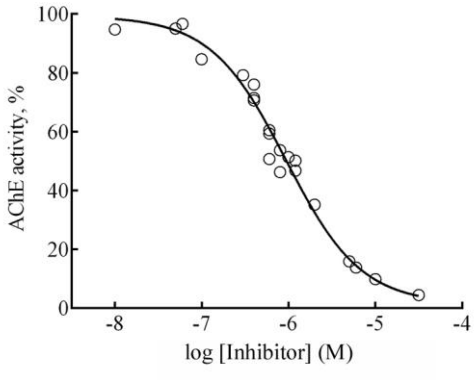


Figure 1. Dose-dependent curve of AChE inhibition by compound **14**.

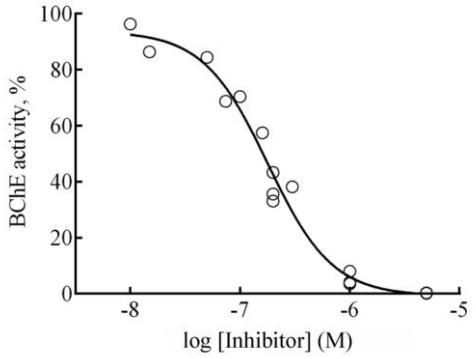


Figure 2. Dose-dependent curve of BChE inhibition by compound **17**.

The dose-dependent curves of AChE and BChE inhibition by compounds **14** and **17** showed in Figures 1 and 2, respectively. The Hill coefficient for the inhibition of AChE by compound **14** is 1.01 ± 0.13. This suggests that only one binding site on the enzyme molecule surface may be involved in the inhibition mechanism. In case of BChE the Hill coefficient was 1.48 ± 0.29 indicating possibility of more than one binding site for compound **17**.

A Lineweaver–Burk plot for inhibition of AChE by compound **14** (Figure 3) showed a mixed type mechanism. The calculated values of K_i and K_i' are 0.78 ± 0.09 μM and 2.11 ± 0.22 μM, respectively. According to this, the compound binds to the free enzyme and the enzyme-substrate complex. The mixed type inhibition of AChE (Scheme 2) can include interaction of the inhibitor with covalent acyl-enzyme intermediate, which results in block of the deacetylation step [39-41].

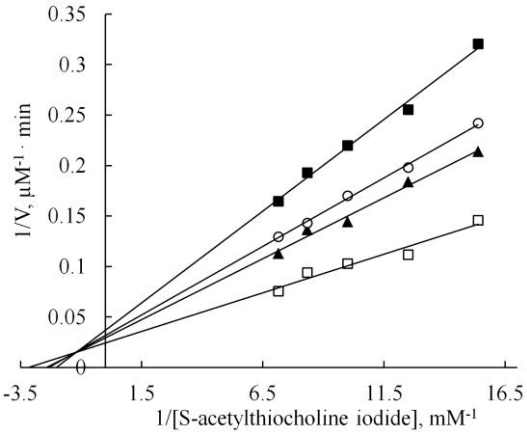
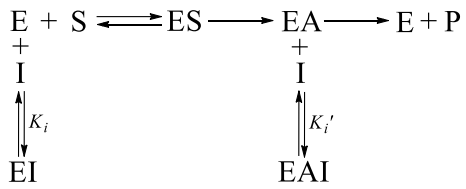


Figure 3. Lineweaver-Burk plots for inhibition of AChE by compound **14**. Concentrations of the inhibitor were: □ - 0, ▲ – 0.4 μM, ○ – 0.6 μM and ■ – 1.2 μM.



Scheme 2. Probable mechanism of AChE inhibition by compound **14**.

Regarding BChE, Lineweaver-Burk reciprocal plot (Figure 4) showed that compound **17** is competitive inhibitor of the enzyme with K_i value of 0.098 ± 0.057 μM. This suggests that the inhibitor is positioned in the active site, preventing the substrate binding (Scheme 3).

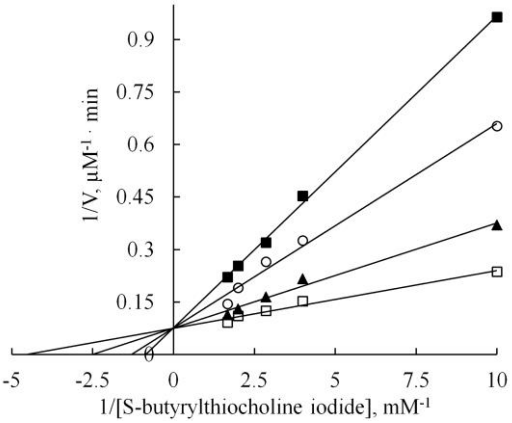
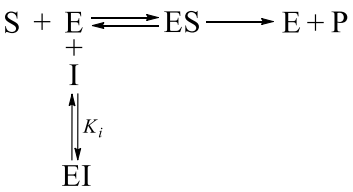


Figure 4. Lineweaver-Burk plots for inhibition of BChE by compound **17**. Concentration of the inhibitor were: □ - 0, ▲ – 0.01 μM, ○ – 0.016 μM and ■ – 0.02 μM.



Scheme 3. Mechanism of competitive inhibition of BChE by compound **17**.

It should be noted that rivastigmine, galantamine and donepezil are widely used today in symptomatic treatment of neurodegenerative diseases [7]. Rivastigmine exhibits a dual action with inhibiting both of the cholinesterases. Donepezil is more effective inhibitor of AChE, with lower activity against BChE [1]. In our experiments, IC_{50} values of donepezil were of 0.013 μM and 2.29 μM for AChE and BChE, respectively.

Molecular docking

Molecular docking calculations were carried out by using Autodock 4.2 in order to elucidate possible binding modes of the thiazolium derivatives with AChE and BChE. In case of compound **14**, the inhibitor occupies the gorge of active site of AChE (Figure 5) with the estimated binding energy of -9.13 kcal/mol. The inhibitor is involved in aromatic-aromatic interactions with Trp86 in the anionic subsite as well as Trp286 in peripheral anionic site, and Phe338 at acyl binding pocket, which is consistent with the known mechanisms of AChE inhibition [42]. The oxygen atom of carbonyl group participates in hydrogen bonds with NH-groups of backbone chains of Phe295 and Arg296. The NO_2 group of the inhibitor forms H-bond with Tyr72 and Tyr124 whereas the chlorine atom has weak interaction with Ser293.

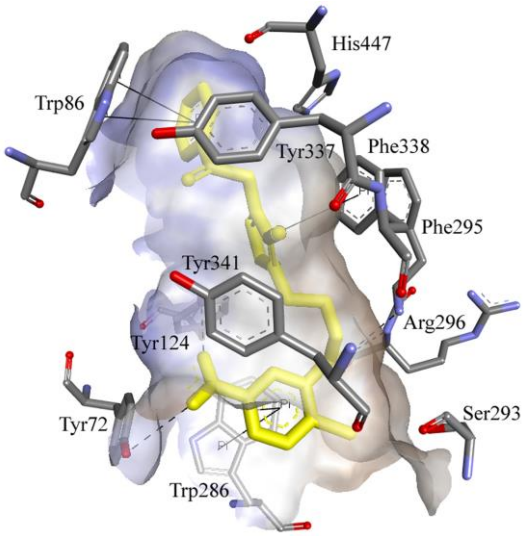


Figure 5. Possible binding mode of compound **14** at the active site of human AChE.

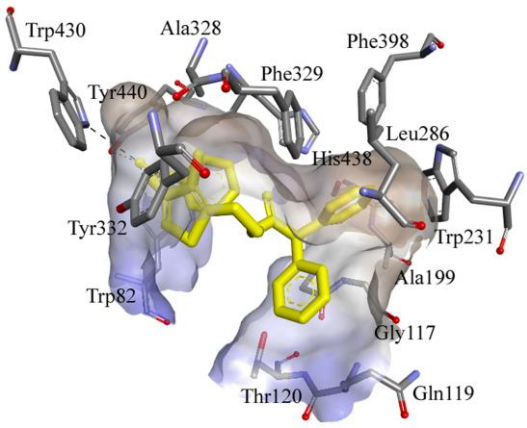


Figure 6. Possible binding mode of compound **17** at the active site of human BChE.

In case of BChE, compound **20** occupies the anionic and esterase subsites (Figure 6) that is consistent with the mechanism of competitive inhibition. The binding energy was calculated to be -8.25 kcal/mol. *N*-Phenacyl fragment is located between Trp82 and His438, while carbonyl atom of oxygen forms hydrogen bonds with Trp82, Trp430, and Tyr440. The thiazolium ring of the inhibitor is close to Tyr332. One phenyl ring of the bulky diphenylmethyl fragment is located in esterase subsite and surrounded by Trp231, Leu286, Phe329, Phe398, and His438. Another phenyl ring is located close to Gly116 and Gly117 of the oxyanion hole. The hydrophobic, van der Waals, and electrostatic interactions can be important for stabilization of this enzyme-inhibitor complex.

This study showed that 5-substituted *N*-phenacylthiazolium derivatives **6-9**, **15**, and **17** can selectively inhibit BChE as compared with AChE. The differences between the inhibition profiles can be interpreted on a structural basis. The catalytic sites of both enzymes are located at the bottom of the gorge on the depth of about 20 Å and are surrounded at the exit by amino acid residues of peripheral anionic sites [43, 44]. Some of aromatic amino acid residues at the active site gorge of AChE are replaced by aliphatic ones in BChE [45]. Therefore, the acyl pocket of BChE is wider which enables to bind of bulky ligands [46] whereas AChE is structurally adapted to acetylcholine. According to this, acyl binding pocket of BChE accommodates phenyl ring of diphenylmethyl fragment of inhibitor **17** (Figure 6). The aromatic amino acid residues of Tyr72, Tyr124 and Trp286 at the peripheral anionic site of AChE are represented by Asn68, Gln119 and

Ala277 in case of BChE, which also promotes the binding of bulky molecules.

3. Conclusion

We found that the derivatives 3-phenacyl-4-methyl-5-substituted thiazolium salts can inhibit activity of BChE and AChE exhibiting micromolar IC_{50} values. The nature of substituent in position 5 of the thiazolium salts may influence sufficiently their inhibitory potency. Among the compounds tested, the best inhibitor of BChE with more then 50-fold selectivity over AChE was compound **17** bearing bulky diphenylmethyl fragment (IC_{50} = 0.14 μM). Compound **14** with 2-chlor-5-nitrophenyl moiety was found to be dual AChE and BChE inhibitors (IC_{50} of 0.85 μM and 1.4 μM, respectively). Molecular docking results indicate that inhibitor **14** in the active site of AChE and compound **17** in the active site of BChE are stabilized by hydrogen bonds and hydrophobic interactions. The data obtained suggest that *N*-phenacylthiazolium salt can be promising scaffold for designing inhibitors of the cholinesterases.

4. Materials and methods

4.1. Chemistry

^{1}H NMR spectra were recorded on a Varian M400 (400 MHz/100 MHz) spectrometer in DMSO-d_6. 4,5-Dimethylthiazole (**1)** and 4-methyl-5-(2-hydroxyethyl)thiazole (**3**) were commercially available and used after distillation. Compounds **4a**-**i** were prepared by reaction of 5-(2-hydroxyethyl)-4-methyl-1,3-thiazole (**3**) with the appropriate acyl chlorides [36-38] in benzene in the presence of triethylamine. Acyl chlorides were produced by standard methods from corresponding carboxylic acids. Phenacyl bromide and *p*-bromophenacyl bromide were obtained from Sigma-Aldrich. 3-[2-(4-Bromophenyl)-2-oxoethyl]-4,5-dimethyl-1,3-thiazolium bromide **2** was synthesized as described previously [32].

4.1.1. General procedure for the synthesis of N-phenacylthiazolium salts 5-17

*Synthesis of compounds **5-13**, **17***. Phenacyl bromide or *p*-bromophenacyl bromide (5 mmol) was added to a solution of 5 mmol of 4-methyl-5-substituted thiazole (compounds **4a-f,i**) in acetone. The reaction mixture was heated under reflux for 3-5 h. After that, the reaction was left standing at room temperature. The product was treated with acetone and diethyl ether, filtered and recrystallized from methanol-acetone-diethyl ether mixture to give compounds **5-13**, **17**.

*Synthesis of compounds **14-16***. A mixture of phenacyl bromide or *p*-bromophenacyl bromide (5 mmol) and 5 mmol of 4-methyl-5-substituted thiazole (compounds **4g,h**) was heated at 100-110 ^{o}C for 1.5-3 h. After that, acetone and diethyl ether were added to wash the solidified product and the mixture left standing at room

temperature. The product was recrystallized from methanol-acetone or *i*-propanol to give compounds **14**-**16**.

3-[2-(4-bromophenyl)-2-oxoethyl]-4,5-dimethyl-1,3-thiazolium bromide (**2**). Yield of 65 %, a white solid, m.p. 189–191 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 9.98 (s, 1H), 7.99 (d, 2H, *J* = 8.4 Hz), 7.88 (d, 2H, *J* = 8.4 Hz), 6.41 (s, 2H), 2.53 (s, 3H), 2.31 (s, 3H). Anal. calcd. for $C_{13}H_{13}Br_2NOS$: C, 39.92; H, 3.35; N, 3.58; S, 8.20. Found: C, 39.99; H, 3.44; N, 3.59; S, 8.35.

3[2-(4-bromophenyl)-2-oxoethyl]-5-{2-[(cyclopropylcarbonyl)oxy]ethyl}-4-methyl-1,3-thiazolium bromide (**5**). Yield of 52 %, a white solid, m.p. 176–178 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 10.03 (s, 1H), 7.99 (d, 2H, *J* = 8.7 Hz), 7.89 (d, 2H, *J* = 8.7 Hz), 6.40 (s, 2H), 4.25 (t, 2H, *J* = 5.6 Hz), 3.33 (t, 2H, *J* = 5.6 Hz), 2.36 (s, 3H), 1.65-1.59 (m, 1H), 0.92-0.82 (m, 4H). Anal. calcd. for $C_{18}H_{19}Br_2NO_3S$: C, 44.19; H, 3.91; N, 2.86; S, 6.55. Found: C, 44.43; H, 3.93; N, 2.81; S, 6.43.

5-{2-[(bicyclo[2.2.1]hept-1-ylcarbonyl)oxy]ethyl}-4-methyl-3-(2-oxo-2-phenylethyl)-1,3-thiazolium bromide (**6**). Yield of 56 %, a white solid, m.p. 180–182 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 10.05 (s, 1H), 8.06 (d, 2H, *J* = 7.5 Hz), 7.79 (t, 1H, *J* = 7.5 Hz), 7.65 (t, 2H, *J* = 7.5 Hz), 6.44 (s, 2H), 4.28 (t, 2H, *J* = 5.6 Hz), 3.33 (t, 2H, *J* = 5.6 Hz), 2.36 (s, 3H), 2.27 (s, 1H), 1.82-1.75 (m, 2H), 1.67-1.62 (m, 2H), 1.50-1.45 (m, 4H), 1.35-1.26 (m, 2H). Anal. calcd. for $C_{22}H_{26}BrNO_3S$: C, 56.90; H, 5.64; N, 3.02; S, 6.89. Found: C, 56.94; H, 5.75; N, 3.11; S, 6.95.

5-[2-(benzoyloxy)ethyl]-3-[2-(4-bromophenyl)-2-oxoethyl]-4-methyl-1,3-thiazolium bromide (**7**). Yield of 60 %, a white solid, m.p. 190–192 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 10.01 (s, 1H), 7.99-7.96 (m, 4H), 7.88 (d, 2H, *J* = 8.4 Hz), 7.69 (t, 1H, *J* = 7.5 Hz), 7.55 (t, 2H, *J* = 7.5 Hz), 6.38 (s, 2H), 4.52 (t, 2H, *J* = 5.6 Hz), 3.48 (t, 2H, *J* = 5.6 Hz), 2.38 (s, 3H). Anal. calcd. for $C_{21}H_{19}Br_2NO_3S$: C, 48.02; H, 3.65; N, 2.67; S, 6.10. Found: C, 48.37; H, 3.74; N, 2.75; S, 6.52.

5-[2-(benzoyloxy)ethyl]-4-methyl-3-(2-oxo-2-phenylethyl)-1,3-thiazolium bromide (**8**). Yield of 58 %, a white solid, m.p. 180–182 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 10.06 (s, 1H), 8.06 (d, 2H, *J* = 7.5 Hz), 7.99 (d, 2H, *J* = 7.5 Hz), 7.78 (t, 1H, *J* = 7.5 Hz), 7.70-7.63 (m, 3H), 7.55 (t, 2H, *J* = 7.5 Hz), 6.44 (s, 2H), 4.53 (t, 2H, *J* = 5.6 Hz), 3.49 (t, 2H, *J* = 5.6 Hz), 2.39 (s, 3H). Anal. calcd. for $C_{21}H_{20}BrNO_3S$: C, 56.51; H, 4.52; N, 3.14; S, 7.18. Found: C, 56.67; H, 4.43; N, 3.24; S, 7.19.

5-{2-[(4-methoxybenzoyl)oxy]ethyl}-4-methyl-3-(2-oxo-2-phenylethyl)-1,3-thiazolium bromide

(**9**). Yield of 70 %, a white solid, m.p. 199–201 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 10.05 (s, 1H), 8.06 (d, 2H, *J* = 7.5 Hz), 7.94 (d, 2H, *J* = 9.3 Hz), 7.79 (t, 1H, *J* = 7.5 Hz), 7.65 (t, 2H, *J* = 7.5 Hz), 7.06 (d, 2H, *J* = 8.4 Hz), 6.43 (s, 2H), 4.49 (t, 2H, *J* = 5.6 Hz), 3.83 (s, 3H), 3.46 (t, 2H, *J* = 5.6 Hz), 2.38 (s, 3H). Anal. calcd. for $C_{22}H_{22}BrNO_4S$: C, 55.47; H, 4.65; N, 2.94; S, 6.73. Found: C, 55.69; H, 4.73; N, 2.89; S, 7.07.

3-[2-(4-bromophenyl)-2-oxoethyl]-4-methyl-5-{2-[(4-nitrobenzoyl)oxy]ethyl}-1,3-thiazolium bromide (**10**). Yield of 55 %, a white solid, m.p. 228–230 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 10.01 (s, 1H), 8.36 (d, 2H, *J* = 8.4 Hz), 8.20 (d, 2H, *J* = 8.4 Hz), 7.97 (d, 2H, *J* = 8.4 Hz), 7.88 (d, 2H, *J* = 8.4 Hz), 6.37 (s, 2H), 4.58 (t, 2H, *J* = 5.6 Hz), 3.50 (t, 2H, *J* = 5.6 Hz), 2.38 (s, 3H). Anal. calcd. for $C_{21}H_{18}Br_2N_2O_5S$: C, 44.23; H, 3.18; N, 4.91; S, 5.63. Found: C, 44.46; H, 3.23; N, 5.04; S, 5.74.

4-methyl-5-{2-[(4-nitrobenzoyl)oxy]ethyl}-3-(2-oxo-2-phenylethyl)-1,3-thiazolium bromide (**11**). Yield of 51 %, a white solid, m.p. 220–221 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 10.10 (s, 1H), 8.37 (d, 2H, *J* = 9.3 Hz), 8.20 (d, 2H, *J* = 8.4 Hz), 8.06 (d, 2H, *J* = 7.5 Hz), 7.78 (t, 1H, *J* = 7.5 Hz), 7.65 (t, 2H, *J* = 7.5 Hz), 6.47 (s, 2H), 4.59 (t, 2H, *J* = 5.6 Hz), 3.52 (t, 2H, *J* = 5.6 Hz), 2.39 (s, 3H). Anal. calcd. for $C_{21}H_{19}BrN_2O_5S$: C, 51.33; H, 3.90; N, 5.70; S, 6.53. Found: C, 51.56; H, 3.96; N, 5.71; S, 6.62.

3-[2-(4-bromophenyl)-2-oxoethyl]-4-methyl-5-{2-[(3-nitrobenzoyl)oxy]ethyl}-1,3-thiazolium bromide (**12**). Yield of 58 %, a white solid, m.p. 204–206 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 10.03 (s, 1H), 8.64 (s, 1H), 8.53 (d, 1H, *J* = 7.5 Hz), 8.38 (d, 1H, *J* = 7.5 Hz), 7.95 (d, 2H, *J* = 8.4 Hz), 7.87 (t, 3H, *J* = 7.5 Hz) 6.39 (s, 2H), 4.60 (t, 2H, *J* = 5.6 Hz), 3.52 (t, 2H, *J* = 5.6 Hz), 2.39 (s, 3H). Anal. calcd. for $C_{21}H_{18}Br_2N_2O_5S$: C, 44.23; H, 3.18; N, 4.91; S, 5.63. Found: C, 44.90; H, 3.12; N, 4.66; S, 5.81.

4-methyl-5-{2-[(3-nitrobenzoyl)oxy]ethyl}-3-(2-oxo-2-phenylethyl)-1,3-thiazolium bromide (**13**). Yield of 57 %, a white solid, m.p. 187–190 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 10.06 (s, 1H), 8.64 (s, 1H), 8.53 (d, 1H, *J* = 8.4 Hz), 8.38 (d, 1H, *J* = 7.5 Hz), 8.03 (d, 2H, *J* = 7.5 Hz), 7.87 (t, 1H, *J* = 7.5 Hz), 7.78 (t, 1H, *J* = 7.5 Hz), 7.64 (t, 2H, *J* = 7.5 Hz), 6.43 (s, 2H), 4.60 (t, 2H, *J* = 5.6 Hz), 3.53 (t, 2H, *J* = 5.6 Hz), 2.40 (s, 3H). Anal. calcd. for $C_{21}H_{19}BrN_2O_5S$: C, 51.33; H, 3.90; N, 5.70; S, 6.53. Found: C, 51.47; H, 3.98; N, 5.53; S, 6.60.

5-{2-[(2-chloro-5-nitrobenzoyl)oxy]ethyl}-4-methyl-3-(2-oxo-2-phenylethyl)-1,3-thiazolium bromide (**14**). Yield of 65 %, a light yellow solid, m.p. 177–178 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 10.16 (s, 1H), 8.61 (d, 1H, *J*

= 2.8 Hz), 8.41 (dd, 1H, *J* = 9.3, 2.8 Hz), 8.03 (d, 2H, *J* = 7.5 Hz), 7.92 (d, 1H, *J* = 8.4, Hz), 7.78 (t, 1H, *J* = 7.5 Hz), 7.64 (t, 2H, *J* = 7.5 Hz), 6.49 (s, 2H), 4.60 (t, 2H, *J* = 5.6 Hz), 3.52 (t, 2H, *J* = 5.6 Hz), 2.39 (s, 3H). Anal. calcd. for $C_{21}H_{18}BrClN_2O_5S$: C, 47.97; H, 3.45; N, 5.33; S, 6.10. Found: C, 48.24; H, 3.54; N, 5.45; S, 6.15.

3-[2-(4-bromophenyl)-2-oxoethyl]-4-methyl-5-{2-[(4-morpholin-4-yl-3-nitrobenzoyl)oxy]ethyl}-1,3-thiazolium bromide (**15**). Yield of 40 %, a yellow solid, m.p. 175–177 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 10.06 (s, 1H), 8.30 (s, 1H), 8.04 (d, 1H, *J* = 8.4), 7.97 (d, 2H, *J* = 7.5), 7.87 (d, 2H, *J* = 8.4 Hz), 7.37 (d, 1H, *J* = 8.4 Hz), 6.41 (s, 2H), 4.53 (t, 2H, *J* = 5.6 Hz), 3.70 (s, 4H), 3.48 (t, 2H, *J* = 5.6 Hz), 3.16 (s, 4H), 2.38 (s, 3H). Anal. calcd. for $C_{25}H_{25}Br_2N_3O_6S$: C, 45.82; H, 3.85; N, 6.41; S, 4.89. Found: C, 45.93; H, 3.89; N, 6.38; S, 4.78.

4-methyl-5-{2-[(4-morpholin-4-yl-3-nitrobenzoyl)oxy]ethyl}-3-(2-oxo-2-phenylethyl)-1,3-thiazolium bromide (**16**). Yield of 43 %, a yellow solid, m.p. 178–180 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 10.06 (s, 1H), 8.31 (s, 1H), 8.04 (d, 3H, *J* = 7.5 Hz), 7.78 (t, 1H, *J*= 7.5 Hz), 7.65 (t, 2H, *J* = 7.5 Hz) 7.38 (d, 1H, *J* = 9.3 Hz) 6.42 (s, 2H), 4.53 (t, 2H, *J* = 5.6 Hz), 3.70 (t, 4H, *J* = 4.7 Hz), 3.49 (t, 2H, *J* = 5.6 Hz), 3.16 (t, 4H, *J* = 4.7 Hz), 2.38 (s, 3H). Anal. calcd. for $C_{25}H_{26}BrN_3O_6S$: C, 52.09; H, 4.55; N, 7.29; S, 5.56. Found: C, 51.98; H, 4.51; N, 7.21; S, 5.52.

5-{2-[(diphenylacetyl)oxy]ethyl}-4-methyl-3-(2-oxo-2-phenylethyl)-1,3-thiazolium bromide (**17**). Yield of 70 %, a white solid, m.p. 165–167 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 9.97 (s, 1H), 8.08 (d, 2H, *J* = 7.5 Hz), 7.80 (t, 1H, *J* = 7.5 Hz), 7.67 (t, 2H, *J* = 7.5 Hz), 7.35-7.24 (m, 10H), 6.35 (s, 2H), 5.19 (s, 1H), 4.36 (t, 2H, *J* = 6.0 Hz), 3.31 (t, 2H, *J* = 6.0 Hz), 2.23 (s, 3H). Anal. calcd. for $C_{28}H_{26}BrNO_3S$: C, 62.69; H, 4.88; N, 2.61; S, 5.98. Found: C, 62.83; H, 4.95; N, 2.70; S, 6.06.

4.2. In vitro study of acetylcholinesterase and butyrylcholinesterase inhibition

Acetylcholinesterase from *E. electricus*, butyrylcholinesterase from equine serum and Ellman's reagent (DTNB) were purchased from Sigma-Aldrich. S-Acetylthiocholine iodide and S-butyrylthiocholine iodide from Sigma-Aldrich and Fluka, respectively, were used as substrates. The inhibitory activities of compounds were determined by modified Ellman's method [47]. Spectrophotometric study was performed at a wavelength of 412 nm.

Reaction mixture was consisted of 25 mM phosphate buffer (pH 7.48), 0.1 mM S-acetylthiocholine iodide, 1 mM DTNB, 1 % dimethyl sulfoxide (DMSO), water, and inhibitor. Before used in the experiments, the

tested compounds **2** and **5**-**17** were dissolved in DMSO and then were diluted with water to the required concentration. The prepared mixture was incubated during 5 minutes at 25°C and substrate hydrolysis was started by adding the enzyme. The inhibitory activity of thiazolium salts toward BChE was study in similar assay conditions with the concentration of S-butyrylthiocholine iodide of 0.5 mM.

The Hill coefficients (values ± standard error) for compounds **14** and **17** were calculated from dose-dependent inhibition curves (Figures 1 and 2) with using of four parameters equation. The values of IC_{50} (Table 1) were the concentration of inhibitor which reduces enzyme activity by 50 %. The kinetic data of enzymatic transformation of S-acetylthiocholine iodide and S-butyrylthiocholine iodide in the absence and presence of compounds **16** and **20** were analyzed from Lineweaver-Burk plots (Figures 3 and 4). The molar extinction coefficient of 5-thio-2-nitrobenzoate (TNB^{2-}) of 14150 $M^{-1}cm^{-1}$ was used [48] for calculation.

Selectivity index (IC_{50} of AChE/IC_{50} of BChE) was estimated using the recalculated IC_{50} values [49] for BChE at 0.1 mM substrate concentration assuming competitive inhibition.

4.3. Molecular docking

Molecular docking was performed by using Autodock 4.2 program. The compound **14** was docked to the active site of A chain of human AChE (PDB code 4EY7 [50]). The compound **17** was docked to the active site gorge of human BChE (PDB code 4BDS [51]). Before starting the docking calculations, ligands and water molecules were removed from initial PDB crystals. The structures of inhibitors were optimized by AM1 semi-empirical quantum mechanical method in program MOPAC. MGLTools was used to prepare the docking files. The most probable binding modes were predicted by using the Lamarckian genetic algorithm (LGA) method.

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