Contents lists available at GrowingScience

Current Chemistry Letters

homepage: www.GrowingScience.com

O-Substituted *N*(3)-benzyl analogs of vitamin B_1 as inhibitors of acetylcholinesterase or butyrylcholinesterase

Oleksandr Kobzar[a], Alla Ocheretniuk[a], Vladyslav Buldenko[a], Lubov Babiy[a], Oleksandr Kozachenko[a], Volodymyr Brovarets[a] and Andriy Vovk[a*]

[a]*V. P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry of the National Academy of Sciences of Ukraine, Kyiv-94, 02094, Ukraine*

C H R O N I C L E

Article history:
Received June 20, 2019
Received in revised form
June 30, 2019
Accepted July 19, 2019
Available online
July 19, 2019

Keywords:
Thiazolium salts
Acetylcholinesterase
Butyrylcholinesterase
Enzyme inhibition
Molecular docking

A B S T R A C T

O-Acyl substituted derivatives of 3-benzyl-5-(2-hydroxyethyl)-4-methyl-1,3-thiazolium chloride which is a structural analog of vitamin B_1 were synthesized and evaluated towards acetylcholinesterase and butyrylcholinesterase *in vitro*. The inhibition properties of the *O*-substituted compounds depend on nature of substituents at position 3 and 5 of the thiazolium ring. Some of the thiazolium salts showed high potency in the inhibition of only one of the two enzymes. The selective effects of these inhibitors are governed by substituent at position 5. Kinetic studies and molecular docking simulation were performed for elucidating mechanisms of enzyme-inhibitor complex formation.

1. Introduction

Thiazolium ring is one of the two heterocyclic fragments of thiamine (vitamin B_1) which can exhibit coenzyme and non-coenzyme activity in living cells and is necessary for energy metabolism and nervous system functioning.[1-3] Severe deficiency of vitamin B_1 leads to beriberi, Wernicke–Korsakoff syndrome, and can be one of the reasons of Alzheimer's and Parkinson's diseases.[4,5] Thiamine, its derivatives and structural analogs may target specific and other proteins including mitochondrial enzymes which are involved in regulation of acetylcholine biosynthesis.[2,6] It was reported that thiamine and thiamine-like compounds exhibit inhibitory properties against carbonic anhydrase isozymes.[7] *N*-Phenacylthiazolium bromide and some other thiazolium compounds can cleave the crosslinks of AGEs (advanced glycation end products).[8] Mono- and bis-thiazolium salts were described as potential therapeutic agents for malaria.[9,10]

Acetylcholinesterase (AChE) catalyzes hydrolysis of acetylcholine playing a key role in termination of cholinergic neurotransmission.[11] Butyrylcholinesterase (BChE), which has 65% identity in amino acid sequence to AChE, is able to compensate the lack of AChE activity[12] and may be involved in formation of amyloid plaques.[13] Donepezil (Fig. 1), pyridostigmine, galantamine, and rivastigmine as AChE inhibitors can be used for treatment of Alzheimer's disease,[14] myasthenia gravis,[15] and

* Corresponding author.
E-mail address: vovk@bpci.kiev.ua (A. Vovk)

doi: 10.5267/j.ccl.2019.007.002

glaucoma.[16] They also showed beneficial effects in therapy of Lewy body dementias,[17] Parkinson's disease,[18] and schizophrenia.[19]

Different classes of AChE and BChE inhibitors have been designed previously including 2,4-substituted thiazoles,[20-21] thiazolotriazinones,[23] and benzothiazoles.[24] However, thiamine (compound **1**, Fig. 1) and some thiazolium salts were reported to have only weak inhibitory effects on AChE.[25] Our interest in searching for effective inhibitors of both AChE and BChE has been focused on *O*-substituted structural analogues of thiamine (compounds **3**, Fig. 1). *O*-Benzoylthiamine[26] **2** is known as a metabolite of benfotiamine,[27] the most widely used synthetic precursor of vitamin B_1. We supposed that *N*-benzylthiazolium moiety of the compounds **3** can mimic the binding of *N*-benzylpiperidine part of donepezil to the active site of cholinesterase, and different acyl groups linked to the thiazolium ion were expected to exert influence of the inhibitors on the enzyme.

Here, we designed a series of *O*-substituted derivatives of 3-benzyl-5-(2-hydroxyethyl)-4-methyl-1,3-thiazolium chloride, among which some compounds were identified as potential inhibitors with selectivity for either AChE or BChE.

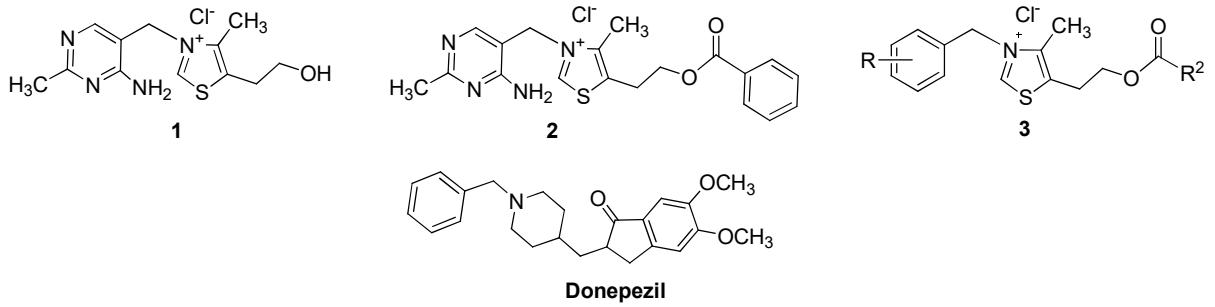
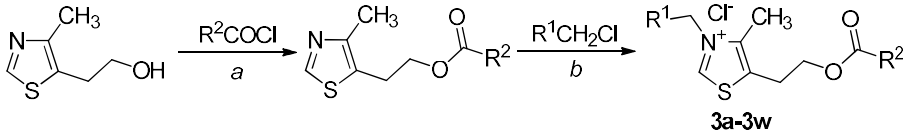


Fig. 1. Thiamine (**1**), *O*-benzoylthiamine (**2**), *O*-substituted derivatives of 3-benzyl-5-(2-hydroxyethyl)-4-methyl-1,3-thiazolium chloride (**3**), and donepezil

2. Results and Discussion

2.1. Synthesis

O-Acyl-substituted thiazolium salts were prepared using known synthetic methods. On the first stage, 5-(2-hydroxyethyl)-4-methyl-1,3-thiazole was converted to the *O*-substituted derivatives by reaction with appropriate benzoyl or other acyl chlorides[28] in dichloromethane in the presence of triethylamine. Then, the *O*-substituted thiazoles were quaternized with corresponding benzyl chlorides in anhydrous acetonitrile giving thiazolium salts **3a**-**w** (Scheme 1). All compounds were obtained in moderate to good yield. After crystallization of the crude products, the thiazolium salts were characterized by ^{1}H NMR, ^{13}C NMR and MS.



R^1 = C_6H_5 (**3a-3k, 3u, 3v**), 2-$CH_3C_6H_4$ (**3m**), 3-$CH_3C_6H_4$ (**3n**), 4-$CH_3C_6H_4$ (**3o**), 2-ClC_6H_4 (**3l**, **3p**, **3t**, **3w**), 4-ClC_6H_4 (**3q**), 2-FC_6H_4 (**3r**)

R^2 = C_6H_5 (**3a**), 2-ClC_6H_4 (**3b**), 4-ClC_6H_4 (**3c**), 4-$CH_3OC_6H_4$ (**3d**), 3-Cl-4-$CH_3OC_6H_3$ (**3f**), 3,5-Cl_2-4-$C_2H_5OC_6H_2$ (**3g**), 2-$NO_2C_6H_4$(**3h**), 3-$NO_2C_6H_4$ (**3i**), 4-$NO_2C_6H_4$ (**3j**, **3m-3r**), 2-Cl-5-$NO_2C_6H_3$ (**3k**, **3l**),4-$O(C_2H_4)_2$N-3-$NO_2C_6H_3$ (**3s**, **3t**), 4-$C_6H_5C_6H_4$ (**3u**), $(C_6H_5)_2CH$ (**3v**, **3w**)

Scheme 1. Synthesis of *O*-acyl substituted thiazolium salts **3a**-**3w**. Reagents and conditions: (*a*) dry dichloromethane, Et_3N, 0-5 °C; (*b*) CH_3CN, 82 °C

2.2. Structure and activity relationship

The compounds **3a-3w** were tested *in vitro* as inhibitors of AChE from *Electric eel* and BChE from equine serum, which share significant similarity with the human enzymes. The activities of the enzymes were determined by a modified Ellman's method.[29] According to the obtained results (Table 1), compounds **3a-3w** exhibited inhibitory effects with IC_{50} values in the micromolar or nanomolar range depending on substituents at positions 3 and 5 of the thiazolium ring. The inhibiting potency of the *O*-substituted derivatives of 3-benzyl-5-(2-hydroxyethyl)-4-methyl-1,3-thiazolium chloride was sufficiently increased as compared to corresponding *O*-substituted derivatives of thiamine. As an example, *O*-benzoyl-containing thiazolium salt **3a** exhibited micromolar inhibitory activity for AChE with moderate selectivity over BChE, while *O*-benzoylthiamine **2** did not inhibit AChE and BChE at the concentration of 10 μM. It should also be noted that *N*-benzylthiazolium-based compound **3a** is more active inhibitor of AChE than corresponding *N*-phenacylthiazolium derivative.[30] The presence of more bulky *O*-adamantoyl or *O*-adamantylacetyl fragments instead of benzoyl one of compound **3a** decreased the inhibition of AChE.[31]

Table 1. Inhibitory activities of thiazolium salts **3a-3w** against AChE and BChE*

Inhibitor	R^1	R^2	IC_{50}, μM (AChE)	IC_{50}, μM (BChE)
3a	phenyl	phenyl	0.88±0.15	3.8±0.9
3b	phenyl	2-chlorophenyl	0.31±0.05	0.83±0.15
3c	phenyl	4-chlorophenyl	0.79±0.16	1.8±0.4
3d	phenyl	4-metoxyphenyl	0.32±0.09	2.0±0.5
3f	phenyl	3-chloro-4-metoxyphenyl	0.11±0.03	1.9±0.1
3g	phenyl	3,5-dichloro-4-ethoxyphenyl	0.26±0.07	0.38±0.07
3h	phenyl	2-nitrophenyl	0.053±0.010	0.96±0.27
3i	phenyl	3-nitrophenyl	0.056±0.012	7.1±1.3
3j	phenyl	4-nitrophenyl	0.096±0.030	6.7±1.4
3k	phenyl	2-chloro-5-nitrophenyl	0.055±0.014	3.5±0.9
3l	2-chlorophenyl	2-chloro-5-nitrophenyl	0.028±0.007	1.0±0.3
3m	2-methylphenyl	4-nitrophenyl	0.52±0.11	3.7±0.8
3n	3-methylphenyl	4-nitrophenyl	1.0±0.3	5.5±0.4
3o	4-methylphenyl	4-nitrophenyl	2.1±0.5	14±3
3p	2-chlorophenyl	4-nitrophenyl	0.19±0.05	1.7±0.3
3q	4-chlorophenyl	4-nitrophenyl	3.5±0.8	15±4
3r	2-fluorophenyl	4-nitrophenyl	0.36±0.11	4.9±1.5
3s	phenyl	4-morpholino-3-nitrophenyl	0.032±0.010	3.0±0.9
3t	2-chlorophenyl	4-morpholino-3-nitrophenyl	0.020±0.005	0.90±0.13
3u	phenyl	biphenyl-4-yl	0.25±0.07	0.21±0.48
3v	phenyl	diphenylmethyl	61±10	0.059±0.011
3w	2-chlorophenyl	diphenylmethyl	18±2	0.016±0.003
Donepezil			0.013±0.003	2.3±0.6

*The IC_{50} values are the means of 2-3 assays ± standard deviations. Concentrations of substrate were 0.1 mM for AChE and 0.5 mM in case of BChE.

Compounds **3b-3d** and **3g** with 2-chloro, 4-chloro, 4-methoxy, 3,5-dichloro-4-ethoxy groups at the benzoyl moiety exhibited only slightly enhanced potency against AChE and BChE compared with unsubstituted *O*-benzoyl derivative **3a**. Modification of the benzoyl fragment by 3-chloro and 4-methoxy groups gave compound **3f** having IC_{50} value of 0.11 μM for AChE and displaying selectivity over BChE (IC_{50} = 1.9 μM). Introduction of 2-nitro, 3-nitro, 4-nitro, or 2-chloro-5-nitro groups in the benzoyl fragment (compounds **3h-3k**) resulted in increasing AChE inhibition and improved selectivity over BChE. The AChE inhibitors **3i** (IC_{50} = 0.056 μM) and **3j** (IC_{50} = 0.096 μM) with significantly lower potency against BChE were selected to further study the relationship between the structure and activity. Unfortunately, smaller inhibitory effects on AChE and BChE were observed in case of compounds **3m-3o** bearing 2-methylbenzyl, 3-methylbenzyl, or 4-methylbenzyl substituents in position 3 of the thiazolium ring. Comparison of compounds **3k** and **3l**, as well as **3j** and **3p** showed a slight increase in BChE inhibition by *N*-(2-chlorobenzyl)-substituted thiazolium salts. However, *N*-(4-chlorobenzyl) derivative **3q** displayed a decreased capacity to inhibit both AChE and BChE. These

results suggest that among the compounds synthesized in this study, *N*-benzyl-containing derivatives and their 2-chlorosubstituted analogs can provide the best inhibition of AChE. High inhibitory activities toward AChE were observed for 5-(2-chloro-5-nitrobenzoyloxyethyl) and 5-(4-morpholino-3-nitrobenzoyloxyethyl) substituted thiazolium salts **3l**, **3s**, and **3t**, displaying IC_{50} values of 28 nM, 32 nM and 20 nM with approximately 35-fold, 90-fold and 45-fold selectivity over BChE, respectively. Under the assay conditions, the effects of these inhibitors are comparable with activity of donepezil, which showed IC_{50} values of 13 nM for AChE and 2.3 μM in case of BChE.

Replacing the 4-morpholino-3-nitrophenyl fragment by biphenyl-4-yl group resulted in much weaker inhibition of AChE, but showed some increased inhibition of BChE. A significant increment of inhibitory activity towards BChE was observed for compounds **3v** and **3w** containing diphenylmethylcarbonyloxyethyl substituent in position 5 of the thiazolium ring. These thiazolium salts were potent inhibitors of BChE with IC_{50} values of 59 nM and 16 nM, respectively, demonstrating about three orders of magnitude selectivity for the BChE over AChE.

Dose-dependent curves for AChE and BChE inhibition by compounds **3s** and **3w** indicated no significant differences in the Hill slopes which were close to 1. The further kinetic experiments were carried out with different substrate concentrations. The double reciprocal Lineweaver-Burk plots (Fig. 2) showed that compounds **3s** and **3w** act as mixed-type inhibitors. For the inhibition of AChE by compound **3s**, the values of K_i and K_i' are 14 ± 1 nM and 53 ± 8 nM, respectively. Thus the inhibitor can compete with the substrate for binding to the enzyme active site. Besides binding to the free enzyme, a mixed type inhibitor of AChE is supposed to interact with acyl-enzyme intermediate, which results in blocking the deacetylation step of acetylcholine hydrolysis.[32] For compound **3w** which exhibits potent inhibition of BChE, the calculated values of K_i and K_i' are of 17 ± 2 nM and 31 ± 4 nM, respectively.

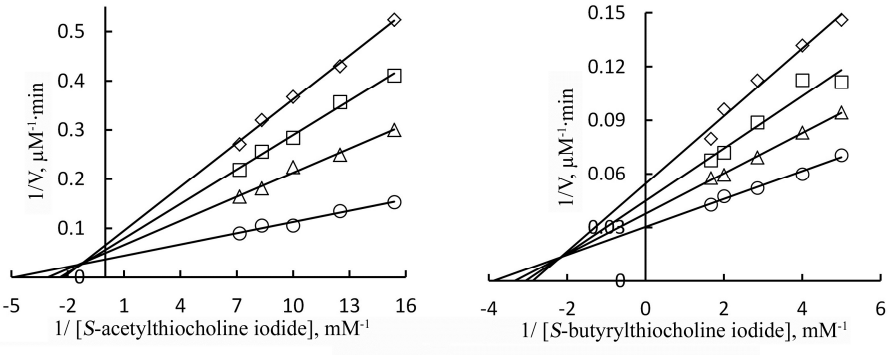


Fig. 2. Lineweaver-Burk plots for inhibition of AChE by compound **3s** (left) and inhibition of BChE by compound **3w** (right). The concentrations of inhibitor **3s** were 15 nM, 30 nM, and 45 nM. The concentrations of inhibitor **3w** were 7 nM, 14 nM, and 28 nM

Molecular docking modeling was performed to understand the possible binding modes of compounds **3s** and **3w** in complexes with AChE and BChE. Compound **3s** was docked into the active site region of human AChE (PDB code 4EY7[33]). Before the simulation, molecules of water, ligands and the B chain were removed from the enzyme crystal structure. According to obtained model (Fig. 3), benzyl fragment of the inhibitor **3s** is involved in aromatic-aromatic interaction with Trp86 at the anionic subsite of catalytic site, and thiazolium ring shows such interaction with phenyl ring of Tyr341. 4-Morpholino-3-nitrobenzoyloxyethyl fragment is oriented towards peripheral anionic site, and its carbonyl oxygen participates in hydrogen bond formation with NH-group of backbone chain of Phe295, whereas phenyl ring of this substituent is involved in interaction with Val294. Electrostatic and van der

Waals contacts are observed between morpholine part of compound **3s** and the surrounding amino acid residues of Trp286, Leu289, and Ser293.

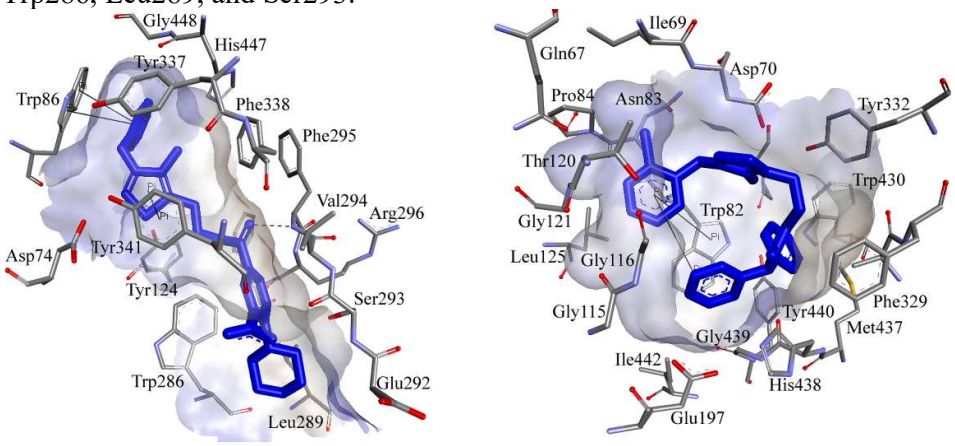


Fig. 3. Possible binding modes of compound **3s** in the active site of human AChE (left) and compound **3w** in the active site of human BChE (right)

The differences between the inhibition profiles of AChE and BChE can be explained by structural features of these enzymes. The catalytic sites of both AChE and BChE are located at the bottom of the gorge on the depth of about 20 Å.[34] Several aromatic amino acid residues of AChE active site are represented by aliphatic ones in the structure of BChE. In addition, Tyr72, Tyr124, and Trp286 of the AChE peripheral anionic site are represented by Asn68, Gln119 and Ala277 in case of BChE. This provides BChE with a larger active site and free access for more bulky inhibitors.[35]

Compound **3w** was docked to the active site of human BChE (PDB code 4BDS[36]). The inhibitor is located at the bottom of the gorge occupying the catalytic anionic site (Fig. 3). 2-Chlorophenyl fragment of substituent in position 3 showed weak aromatic-aromatic interactions with the indole part of Trp82. The aromatic rings of bulky substituent in position 5 of the thiazolium ion are located near the Trp82 residue. The diphenylmethyl fragment participates in hydrophobic, van der Waals, and electrostatic interactions with Gly78, Glu197, Ala328, Trp430, Met437, His438, Gly439, and Tyr440, which contributes to stabilization of the enzyme-inhibitor complex.

3. Conclusions

This study demonstrated potential of *O*-substituted derivatives of 3-benzyl-5-(2-hydroxyethyl)-4-methyl-1,3-thiazolium chloride as potent and selective inhibitors of AChE and BChE. Inhibitory effects of the thiazolium-based compounds on the cholinesterases were found to depend on substituents at positions 3 and 5 of the thiazolium ring. Among the tested compounds, *N*-benzylthiazolium salt **3s** bearing 4-morpholino-3-nitrobenzoyl substituent at position 5 showed IC_{50} value for inhibition of AChE in nanomolar range with approximately two orders of magnitude selectivity over BChE. At the same time, compound **3w** containing 2,2-diphenylacetyl fragment demonstrated strong inhibitory effect on BChE and three orders of magnitude selectivity over AChE. The mixed type of inhibition suggests that the inhibitors may compete with the substrate for binding to the enzyme active site. Results of molecular docking indicate that the efficient coordination of thiazolium ring and substituents at position 3 and 5 can be responsible for activity and high selectivity of the inhibitors. The obtained data create a background for further designing cholinesterase inhibitors based on structural analogs of vitamin B_1.

Acknowledgements

This research was supported by the National Academy of Sciences of Ukraine (project 0117 U000096) and President's of Ukraine grant for competitive projects Ф75/126-2018 of the State Fund for Fundamental Research.

4. Experimental

4.1. Instruments and Reagents

^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on Bruker Avance DRX 500 spectrometer with TMS as an internal standard. The IR spectra were measured with a Vertex 70 spectrometer from KBr pellets. Melting points were measured with a Buchi melting point apparatus and are uncorrected. LC-MS spectra were obtained using an HPLC apparatus, Agilent 1100 Series, equipped with the diode-matrix and mass-selective detector Agilent LC/MSD SL. Spectrophotometric measurements were carried out with a Specord 210 Plus spectrophotometer.

Acetylcholinesterase from *Electric eel* (Type V-S, lyophilized powder, 200 units/mg protein) and butyrylcholinesterase from equine serum (lyophilized powder, ≥10 units/mg protein) were purchased from Sigma-Aldrich.

*4.2. Synthetic procedure for compounds **3a-3w***

To a solution of 1.44 g (10 mmol) 2-(4-methyl-1,3-thiazol-5-yl)ethanol and 1.7 ml (12 mmol) of triethylamine in 20 ml of dry dichloromethane was added dropwise a solution of 11 mmol corresponding aroyl or other acyl chloride in 10 ml of dichloromethane at 0-5 °C. The mixture was mixed overnight, then 50 ml of water was added, organic layer was separated, dried with anhydrous Na_2SO_4, and solvent evaporated under vacuum. The obtained esters were used without purification for further synthesis. To a solution of 10 mmol of ester in anhydrous acetonitrile was added 15 mmol of benzyl chloride or its derivatives, and the reaction mixture was refluxed for 20 h. Then the solvent was evaporated under vacuum, the residue was treated with hot acetone, and precipitate was filtered and recrystallized from methanol-acetone-diethyl ether mixture to give corresponding thiazolium salts. 5-(Chloromethyl)-2-methylpyrimidin-4-amine hydrochloride was used for quaternization of the *O*-benzoyl derivative of 2-(4-methyl-1,3-thiazol-5-yl)ethanol to obtain compound **2**.

*5-[2-(Benzoyloxy)ethyl]-3-benzyl-4-methyl-1,3-thiazolium chloride (**3a**)*
Yield 56%, a light gray solid. Mp = 156-158 °C. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 2.38 (s, 3H), 3.40 (br s, 2H+H_2O), 4.50 (br s, 2H), 5.88 (br s, 2H), 7.30 (br s, 2H), 7.40 (br s, 3H), 7.52-7.53 (m, 2H), 7.67 (m, 1H), 7.90-7.91 (m, 2H), 10.47 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 12.0, 26.2, 56.3, 64.3, 128.3, 129.3, 129.3, 129.6, 133.5, 134.0, 135.4, 143.3, 158.5, 165.9. IR (KBr), ν, cm^{-1}: 699, 716, 1023, 1071, 1091, 1119, 1252, 1278, 1450, 1598, 1705, 3029. LC-MS, *m/z* (%): 338.0 (100) [M-CI]$^{+}$. Anal. calcd. for $C_{20}H_{20}ClNO_2S$: C, 64.25; H, 5.39; N, 3.75. Found: C, 64.92; H, 5.61; N, 3.79.

*3-Benzyl-5-{2-[(2-chlorobenzoyl)oxy]ethyl}-4-methyl-1,3-thiazolium chloride (**3b**)*
Yield 61%, a light brown solid. Mp = 127-129°C. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 2.36 (s, 3H), 3.43 (t, J_{HH}= 5.65 Hz, 2H), 4.50 (t, J_{HH} =5.65 Hz, 2H), 5.87 (s, 2H), 7.29-7.30 (m, 2H), 7.37-7.45 (m, 4H), 7.56-7.60 (m, 2H) 7.74-7.75 (m, 1H), 10.44 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 12.2, 26.2, 56.4, 65.0, 128.0, 128.4, 129.4, 129.7, 130.1, 131.5, 131.7, 132.5, 133.6, 134.1, 135.4, 143.5, 158.6, 165.2. IR (KBr), ν, cm^{-1}: 702, 752, 803, 1014, 1030, 1050, 1075, 1099, 1139, 1257, 1302, 1438, 1587, 1705, 3405, 3478. LC-MS, *m/z* (%): 372.0 (100) [M-CI]$^{+}$. Anal. calcd. for $C_{20}H_{19}Cl_2NO_2S$: C, 58.83; H, 4.69; N, 3.43. Found: C, 58.66; H, 4.78; N, 3.34.

*3-Benzyl-5-{2-[(4-chlorobenzoyl)oxy]ethyl}-4-methyl-1,3-thiazolium chloride (**3c**)*
Yield 60%, a light brown solid. Mp = 165-167 °C. 1H NMR (500 MHz, DMSO-d6), δ: 2.38 (s, 3H), 3.43 (t, J_{HH} = 5.35 Hz, 2H), 4.51 (t, J_{HH} = 5.35 Hz, 2H), 5.86 (s, 2H), 7.28-7.30 (m, 2H), 7.40 (m, 3H), 7.57 (d, J_{HH} = 8.0 Hz, 2H), 7.89 (d, J_{HH} = 8.0 Hz, 2H), 10.45 (s, 1H). 13C NMR (125 MHz, DMSO-d6), δ: 12.1, 26.3, 56.4, 64.6, 128.4, 129.4, 129.6, 129.7, 131.6, 133.6, 135.4, 139.1, 143.5, 158.6,

165.2. IR (KBr), ν, cm-1: 756, 1111, 1270, 1722. LC-MS, *m/z* (%): 372.0 (100) $[M\text{-}CI]^+$. Anal. calcd. for $C_{20}H_{19}Cl_2NO_2S$: C, 58.83; H, 4.69; N, 3.43. Found: C, 58.75; H, 4.83; N, 3.32.

*3-Benzyl-5-{2-[(4-methoxybenzoyl)oxy]ethyl}-4-methyl-1,3-thiazolium chloride (***3d***)*
Yield 63%, a light brown solid. Mp = 169-171 °C. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 2.36 (s, 3H), 3.40 (t, J_{HH} = 5.65 Hz, 2H), 3.84 (s, 3H), 4.46 (t, J_{HH} = 5.65 Hz, 2H), 5.82 (s, 2H), 7.02 (d, J_{HH} = 8.5 Hz, 2H), 7.27-7.28 (m, 2H), 7.40-7.42 (m, 3H), 7.85 (d, J_{HH} = 8.5 Hz, 2H), 10.31 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 12.1, 26.4, 56.2, 56.5, 64.0, 114.7, 122.0, 128.4, 129.4, 129.8, 131.9, 133.6, 135.6, 143.4, 158.4, 163.9, 165.7. IR (KBr), ν, cm^{-1}: 705, 1112, 1156, 1252, 1273, 1599, 1707. LC-MS, *m/z* (%): 368.0 (100) $[M\text{-}CI]^+$. Anal. calc. for $C_{21}H_{22}ClNO_3S$: C, 62.44%; H, 5.49%; N, 3.47%. Found: C, 62.58%; H, 5.62%; N, 3.55%.

*3-Benzyl-5-{2-[(3-chloro-4-methoxybenzoyl)oxy]ethyl}-4-methyl-1,3-thiazolium chloride (***3f***)*
Yield 76%, a white solid. Mp = 184-185 °C. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 2.36 (s, 3H), 3.43 (t, J_{HH} = 5.65 Hz, 2H), 3.95 (s, 3H), 4.47 (t, J_{HH} = 5.65 Hz, 2H), 5.82 (s, 2H), 7.24-7.27 (m, 3H), 7.37-7.40 (m, 3H), 7.84-7.86 (m, 1H), 7.89 (m, 1H), 10.31 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 12.1, 26.3, 56.4, 57.3, 64.4, 113.4, 121.9, 122.8, 128.4, 129.4, 129.8, 130.7, 131.2, 133.6, 135.5, 143.4, 158.5, 159.1, 164.7. IR (KBr), ν, cm^{-1}: 702, 1015, 1057, 1110, 1224, 1264, 1496, 1594, 1719, 2936. LC-MS, *m/z* (%): 402.0 (100) $[M\text{-}CI]^+$. Anal. calc. for $C_{21}H_{21}Cl_2NO_3S$: C, 57.54%; H, 4.83%; N, 3.20%. Found: C, 57.39%; H, 4.75%; N, 3.31%.

*3-Benzyl-5-{2-[(3,5-dichloro-4-ethoxybenzoyl)oxy]ethyl}-4-methyl-1,3-thiazolium chloride (***3g***)*
Yield 74%, a white solid. Mp = 157-159 °C. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 1.39 (t, J_{HH}= 5.5 Hz, 3H), 2.36 (s, 3H), 3.43 (t, J_{HH} = 5.6 Hz, 2H), 4.14 (q, J_{HH} = 5.5 Hz, 2H), 4.49 (t, J_{HH} = 5.6 Hz, 2H), 5.83 (s, 2H), 7.25-7.26 (m, 2H), 7.35-7.38 (m, 3H), 7.91 (s, 2H), 10.37 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 12.0, 15.9, 26.1, 56.3, 65.0, 70.6, 127.1, 128.2, 129.2, 129.6, 129.6, 130.3, 133.5, 135.2, 143.3, 155.4, 158.5, 163.5. IR (KBr), ν, cm^{-1}: 703, 798, 1018, 1134, 1238, 1278, 1385, 1453, 1719, 3370. LC-MS, *m/z* (%): 450.0 (100) $[M\text{-}CI]^+$. Anal. calc. for $C_{22}H_{22}Cl_3NO_3S$: C, 54.28%; H, 4.55%; N, 2.88%. Found: C, 54.12%; H, 4.58%; N, 2.74%.

*3-Benzyl-4-methyl-5-{2-[(2-nitrobenzoyl)oxy]ethyl}-1,3-thiazolium chloride (***3h***)*
Yield 58%, a brown solid. Mp = 100-102°C. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 2.33 (s, 3H), 3.38 (br s, 2H+H_2O), 4.50 (t, J_{HH} = 5.65 Hz, 2H), 5.87 (s, 2H), 7.29-7.34 (m, 3H), 7.36-7.44 (m, 3H), 7.79-7.85 (m, 3H), 8.05-8.07 (m, 1H), 10.45 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 12.0, 25.9, 56.3, 65.6, 124.7, 126.2, 128.4, 128.5, 129.3, 129.6, 129.7, 130.4, 133.5, 133.7, 134.3, 135.0, 143.5, 148.3, 158.5, 164.8. IR (KBr), ν, cm^{-1}: 702, 741, 1014, 1073, 1133, 1258, 1301, 1357, 1445, 1530, 1583, 1722, 3078, 3405, 3481. LC-MS, *m/z* (%): 383.0 (100) $[M\text{-}CI]^+$. Anal. calc. for $C_{20}H_{19}ClN_2O_4S$: C, 57.35%; H, 4.57%,; N, 6.69%. Found: C, 57.43%; H, 4.51%; N, 6.83%.

*3-Benzyl-4-methyl-5-{2-[(3-nitrobenzoyl)oxy]ethyl}-1,3-thiazolium chloride (***3i***)*
Yield 64%, a white solid. Mp = 167-169°C. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 2.39 (s, 3H), 3.48 (t, J_{HH} = 5.65 Hz, 2H), 4.55 (t, J_{HH} = 5.65 Hz, 2H), 5.89 (s, 2H), 7.26-7.28 (m, 2H), 7.32-7.36 (m, 3H), 7.84 (t, J_{HH} = 7.8 Hz, 1H), 8.29 (d, J_{HH} = 7.8 Hz, 1H), 8.50 (dd, J_{HH}= 8.2 Hz, 1H), 8.54 (s, 1H), 10.54 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 12.2, 26.2, 56.4, 65.3, 124.2, 128.4, 128.6, 129.3, 129.7, 131.4, 131.5, 133.6, 135.3, 135.8, 143.6, 148.5, 158.8, 164.3. IR (KBr), ν, cm^{-1}: 710, 1141, 1263, 1289, 1351, 1532, 1722. LC-MS, *m/z* (%): 383.0 (100) $[M\text{-}CI]^+$. Anal. calc. for $C_{20}H_{19}ClN_2O_4S$: C, 57.35%; H, 4.57%; N, 6.69%. Found: C, 57.28%; H, 4.29%; N, 6.81%.

*3-Benzyl-4-methyl-5-{2-[(4-nitrobenzoyl)oxy]ethyl}-1,3-thiazolium chloride (***3j***)*
Yield 63%, a light brown solid. Mp = 197-199°C. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 2.38 (s, 3H), 3.46 (t, J_{HH} = 5.35 Hz, 2H), 4.54 (t, J_{HH} = 5.35 Hz, 2H), 5.89 (s, 2H), 7.28-7.30 (m, 2H), 7.34-7.40 (m, 3H), 8.12 (d, J_{HH} = 8.8 Hz, 2H), 8.32 (d, J_{HH} = 8.8 Hz, 2H), 10.53 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6),

δ: 12.2, 26.3, 56.4, 65.2, 124.5, 128.4, 129.3, 129.7, 131.3, 133.7, 135.3, 143.5, 150.9, 158.7, 164.6. IR (KBr), ν, cm^{-1}: 703, 1091, 1124, 1256, 1277, 1347, 1455, 1518, 1720. LC-MS, *m/z* (%): 383.0 (100) $[M\text{-}CI]^+$. Anal. calc. for $C_{20}H_{19}ClN_2O_4S$: C, 57.35%; H, 4.57%; N, 6.69%. Found: C, 57.25%; H, 4.73%; N, 6.83%.

*3-Benzyl-5-{2-[(2-chloro-5-nitrobenzoyl)oxy]ethyl}-4-methyl-1,3-thiazolium chloride (***3k***)*
Yield 63%, a white solid. Mp = 185-187°C. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 2.38 (s, 3H), 3.46 (t, J_{HH} = 5.65 Hz, 2H), 4.54 (t, J_{HH} = 5.65 Hz, 2H), 5.85 (s, 2H), 7.26-7.28 (m, 2H), 7.35-7.36 (m, 3H), 7.89 (d, J_{HH} = 9.0 Hz, 1H), 8.40 (dd, J_{HH} = 9.0 Hz, 1H), 8.54 (d, J_{HH} = 9.0 Hz, 1H) , 10.42 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 12.0, 26.0, 56.3, 65.6, 126.5, 128.3, 128.3, 129.3, 129.6, 130.9, 133.2, 133.5, 135.1, 139.3, 143.6, 146.6, 158.5, 163.3. IR (KBr), ν, cm^{-1}: 704, 742, 920, 1044, 1137, 1248, 1307, 1348, 1451, 1463, 1534, 1613, 1731, 2997. LC-MS, *m/z* (%): 417.0 (100) $[M\text{-}CI]^+$. Anal. calc. for $C_{20}H_{18}Cl_2N_2O_4S$: C, 52.99%; H, 4.00%; N, 6.18%. Found: C, 53.07%; H, 4.12%; N, 6.04%.

*3-(2-Chlorobenzyl)-5-{2-[(2-chloro-5-nitrobenzoyl)oxy]ethyl}-4-methyl-1,3-thiazolium chloride (***3l***)*
Yield 64%, yellowish solid. Mp = 182-184°C. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 2.40 (s, 3H), 3.50 (t, J_{HH} = 5.5 Hz, 2H), 4.57 (t, J_{HH} = 5.5 Hz, 2H), 5.91 (s, 2H), 7.05 (d, J_{HH} = 7.8 Hz, 1H), 7.30 (t, J_{HH} = 7.6 Hz, 1H), 7.43 (t, J_{HH} = 7.6 Hz, 1H), 7.56 (d, J_{HH}= 7.8 Hz, 1H), 7.90 (d, J_{HH} = 9.0 Hz, 1H), 8.40 (dd, J_{HH} = 9.0 Hz, 1H), 8.56 (d, J_{HH} = 2.8 Hz, 1H), 10.23 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 11.9, 26.0, 54.4, 65.6, 126.5, 128.3, 128.5, 130.1, 130.5, 130.8, 131.0, 131.3, 132.9, 133.2, 135.1, 139.3, 143.7, 146.6, 159.2, 163.3. IR (KBr), ν, cm^{-1}: 759, 1144, 1274, 1347, 1526, 1722, 2955. LC-MS, *m/z* (%): 451.0 (100) $[M\text{-}CI]^+$. Anal. calc. for $C_{20}H_{17}Cl_3N_2O_4S$: C, 49.25%; H, 3.51%; N, 5.74%. Found: C, 49.03%; H, 3.63%; N, 5.57%.

*4-Methyl-3-(2-methylbenzyl)-5-{2-[(4-nitrobenzoyl)oxy]ethyl}-1,3-thiazolium chloride (***3m***)*
Yield 57%, a brown solid. Mp = 173-175°C. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 2.28 (s, 3H), 2.31 (s, 3H), 3.50 (t, J_{HH} = 5.35 Hz, 2H), 4.58 (t, J_{HH} = 5.35 Hz, 2H), 5.85 (s, 2H), 6.70 (d, J_{HH} = 7.5 Hz, 1H), 7.13 (t, J_{HH} = 7.5 Hz, 1H), 7.25-7.32 (m, 2H), 8.16 (d, J_{HH} = 8.5 Hz, 2H), 8.33 (d, J_{HH} = 8.5 Hz, 2H), 10.17 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 11.9, 19.2, 26.2, 54.8, 65.2, 124.5, 127.1, 127.3, 129.2, 131.2, 131.3, 131.9, 135.2, 135.2, 136.6, 143.7, 150.8, 158.6, 164.5. IR (KBr), ν, cm^{-1}: 715, 748, 1092, 1124, 1258, 1277, 1347, 1436, 1458, 1518, 1599, 1718. LC-MS, *m/z* (%): 397.0 (100) $[M\text{-}CI]^+$. Anal. calc. for $C_{21}H_{21}ClN_2O_4S$: C, 58.26%; H, 4.89%; N, 6.47%. Found: C, 58.33%; H, 4.76%; N, 6.59%.

*4-Methyl-3-(3-methylbenzyl)-5-{2-[(4-nitrobenzoyl)oxy]ethyl}-1,3-thiazolium chloride (***3n***)*
Yield 60%, a white solid. Mp = 189-191°C. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 2.25 (s, 3H), 2.38 (s, 3H), 3.46 (t, J_{HH} = 5.35 Hz, 2H), 4.54 (t, J_{HH} = 5.35 Hz, 2H), 5.82 (s, 2H), 7.05 (d, J_{HH} = 7.5 Hz, 1H), 7.12 (s, 1H), 7.16 (d, J_{HH} = 7.5 Hz, 1H), 7.27 (t, J_{HH} = 7.5 Hz, 1H), 8.10 (d, J_{HH} = 8.5 Hz, 2H), 8.31 (d, J_{HH} = 8.5 Hz, 2H), 10.46 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 12.1, 21.4, 26.1, 56.3, 65.1, 124.4, 125.4, 128.8, 129.5, 129.9, 131.1, 133.4, 135.1, 135.2, 139.0, 143.4, 150.8, 158.5, 164.5. IR (KBr), ν, cm^{-1}: 717, 1101, 1274, 1350, 1525, 1728, 2970. LC-MS, *m/z* (%): 397.0 (100) $[M\text{-}CI]^+$. Anal. calc. for $C_{21}H_{21}ClN_2O_4S$: C, 58.26%; H, 4.89%; N, 6.47%. Found: C, 58.09%; H, 4.53%; N, 6.35%.

*4-Methyl-3-(4-methylbenzyl)-5-{2-[(4-nitrobenzoyl)oxy]ethyl}-1,3-thiazolium chloride (***3o***)*
Yield 68%, a white solid. Mp = 188-190°C. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 2.28 (s, 3H), 2.40 (s, 3H), 3.47 (t, J_{HH} = 5.35 Hz, 2H), 4.56 (t, J_{HH} = 5.35 Hz, 2H), 5.85 (s, 2H), 7.21 (s, 4H), 8.11 (d, J_{HH} = 8.0 Hz, 2H), 8.30 (d, J_{HH} = 8.0 Hz, 2H), 10.57 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 12.2, 21.3, 26.3, 56.3, 65.2, 124.5, 128.5, 130.2, 130.6, 131.3, 135.2, 138.8, 143.5, 150.9, 158.6, 164.6. IR (KBr), ν, cm^{-1}: 711, 1100, 1120, 1144, 1283, 1349, 1522, 1708. LC-MS, *m/z* (%): 397.0 (100) $[M\text{-}CI]^+$. Anal. calc. for $C_{21}H_{21}ClN_2O_4S$: C, 58.26%; H, 4.89%; N, 6.47%. Found: C, 57.98%; H, 4.71%; N, 6.34%.

*3-(2-Chlorobenzyl)-4-methyl-5-{2-[(4-nitrobenzoyl)oxy]ethyl}-1,3-thiazolium chloride (**3p**)*
Yield 63%, a brown solid. Mp = 182-184°C. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 2.40 (s, 3H), 3.51 (t, J_{HH} = 5.65 Hz, 2H), 4.58 (t, J_{HH} = 5.65 Hz, 2H), 5.94 (s, 2H), 7.09 (d, J_{HH} = 7.5 Hz, 1H), 7.34 (t, J_{HH} = 7.5 Hz, 1H), 7.44 (t, J_{HH} = 7.5 Hz, 1H), 7.57 (d, J_{HH}= 7.5 Hz, 1H), 8.15 (d, J_{HH} = 8.5 Hz, 2H), 8.33 (d, J_{HH} = 8.5 Hz, 2H), 10.30 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 11.9, 26.2, 54.4, 65.1, 124.2, 124.5, 128.6, 130.1, 130.6, 131.0, 131.2, 131.3, 132.9, 135.2, 143.6, 150.8, 159.3, 164.5. IR (KBr), ν, cm^{-1}: 715, 760, 869, 1097, 1125, 1278, 1347, 1434, 1461, 1518, 1598, 1720. LC-MS, *m/z* (%): 417.0 (100) [M-CI]$^+$. Anal. calc. for $C_{20}H_{18}Cl_2N_2O_4S$: C, 52.99%; H, 4.00%; N, 6.18%. Found: C, 52.41%,; H, 3.79%; N, 6.06%.

*3-(4-Chlorobenzyl)-4-methyl-5-{2-[(4-nitrobenzoyl)oxy]ethyl}-1,3-thiazolium chloride (**3q**)*
Yield 69%, a white solid. Mp = 187-189 °C. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 2.35 (s, 3H), 3.45 (t, J_{HH} = 5.65 Hz, 2H), 4.54 (t, J_{HH} = 5.65 Hz, 2H), 5.88 (s, 2H), 7.32 (d, J_{HH} = 8.3 Hz, 2H), 7.42 (d, J_{HH} = 8.3 Hz, 2H), 8.10 (d, J_{HH} = 8.5 Hz, 2H), 8.32 (d, J_{HH} = 8.5 Hz, 2H), 10.50 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 12.1, 26.1, 55.5, 65.1, 124.4, 129.5, 130.3, 131.2, 132.5, 134.0, 135.1, 135.2, 143.4, 150.8, 158.8, 164.5. IR (KBr), ν, cm^{-1}: 720, 797, 824, 870, 1013, 1091, 1122, 1257, 1277, 1349, 1456, 1493, 1520, 1597, 1724, 3383. LC-MS, *m/z* (%): 417.0 (100) [M-CI]$^+$. Anal. calc. for $C_{20}H_{18}Cl_2N_2O_4S$: C, 52.99%; H, 4.00%; N, 6.18%. Found: C, 53.07%; H, 4.13%; N, 6.02%.

*3-(2-Fluorobenzyl)-4-methyl-5-{2-[(4-nitrobenzoyl)oxy]ethyl}-1,3-thiazolium chloride (**3r**)*
Yield 65%, a white solid. Mp = 208-210 °C. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 2.42 (s, 3H), 3.47 (t, J_{HH} = 5.65 Hz, 2H), 4.56 (t, J_{HH} = 5.65 Hz, 2H), 5.95 (s, 2H), 7.21-7.37 (m, 3H), 7.43-7.47 (m, 1H), 8.13 (d, J_{HH} = 8.5 Hz, 2H), 8.32 (d, J_{HH} = 8.5 Hz, 2H), 10.43 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 11.9, 26.1, 51.2, 65.1, 116.4, 116.6, 120.5, 120.7, 124.5, 125.7, 130.9, 131.2, 131.9, 132.0, 135.0, 135.1, 143.5, 150.8, 159.2, 159.4, 161.9, 164.5. IR(KBr), ν, cm^{-1}: 715, 759, 837, 870, 1073, 1096, 1125, 1224, 1259, 1278, 1346, 1457, 1494, 1516, 1595, 1721, 2997. LC-MS, *m/z* (%): 401.0 (100) [M-CI]$^+$. Anal. calc. for $C_{20}H_{18}ClFN_2O_4S$: C, 54.98%; H, 4.15%; N, 6.41%. Found: C, 54.65%,; H, 4.23%,; N, 6.56%.

*3-Benzyl-4-methyl-5-{2-[(4-morpholin-4-yl-3-nitrobenzoyl)oxy]ethyl}-1,3-thiazolium chloride (**3s**)*
Yield 59%, an orange solid. Mp = 148-150 °C. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 2.37 (s, 3H), 3.16 (br s, 4H), 3.43 (t, J_{HH} = 5.65 Hz, 2H), 3.70 (br s, 4H), 4.47 (t, J_{HH} = 5.65 Hz, 2H), 5.87 (s, 2H), 7.25-7.27 (m, 2H), 7.32-7.38 (m, 4H), 7.94 (dd, J_{HH} = 8.8 Hz, 1H), 8.24 (s, 1H), 10.47 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 12.0, 26.2, 50.9, 56.3, 64.4, 66.2, 120.1, 120.8, 128.2, 128.3, 129.2, 129.6, 133.5, 134.5, 135.3, 139.5, 143.4, 148.6, 158.5, 164.3. IR (KBr), ν, cm^{-1}: 702, 1109, 1129, 1234, 1269, 1293, 1526, 1612, 1716. LC-MS, *m/z* (%): 468.0 (100) [M-CI]$^+$. Anal. calc. for $C_{24}H_{26}ClN_3O_5S$: C, 57.19%; H, 5.20%; N, 8.34%. Found: C, 57.01%; H, 5.13%; N, 8.55%.

*3-(2-Chlorobenzyl)-4-methyl-5-{2-[(4-morpholin-4-yl-3-nitrobenzoyl)oxy]ethyl}-1,3-thiazolium chloride (**3t**)*
Yield 59%, a yellow solid. Mp = 122-124 °C. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 2.38 (s, 3H), 3.16 (br s, 4H), 3.46 (t, J_{HH} = 5.65 Hz, 2H), 3.70 (br s, 4H), 4.50 (t, J_{HH} = 5.65 Hz, 2H), 5.85 (s, 2H), 7.00 (d, J_{HH} = 7.3 Hz, 1H), 7.32-7.35 (m, 2H), 7.44 (t, J_{HH} = 7.3 Hz, 1H), 7.58 (d, J_{HH} = 7.8 Hz, 1H), 7.98 (d, J_{HH} = 7.8 Hz, 1H), 8.27 (s, 1H), 10.08 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 11.8, 26.2, 50.9, 54.4, 64.4, 66.2, 120.1, 120.7, 128.3, 128.5, 129.9, 130.5, 131.0, 131.2, 132.8, 134.5, 135.3, 139.4, 143.5, 148.6, 159.1, 164.3. IR (KBr), ν, cm^{-1}: 762, 1118, 1230, 1270, 1294, 1445, 1535, 1611, 1704, 3366, 3410. LC-MS, *m/z* (%): 502.0 (100) [M-CI]$^+$. Anal. calc. for $C_{24}H_{25}Cl_2N_3O_5S$: C, 53.54%; H, 4.68%; N, 7.80%. Found: C, 53.29%; H, 4.43%; N, 7.92%.

*3-Benzyl-5-{2-[(biphenyl-4-ylcarbonyl)oxy]ethyl}-4-methyl-1,3-thiazolium chloride (**3u**)*
Yield 61%, a gray solid. Mp = 188-190 °C. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 2.39 (s, 3H), 3.44 (br m, 2H+H_2O), 4.52 (br m, 2H), 5.86 (s, 2H), 7.28-7.30 (m, 2H), 7.37-7.46 (m, 4H), 7.51-7.54 (m, 2H),

7.74-7.75 (m, 2H), 7.80-7.82 (m, 2H), 7.97-7.99 (m, 2H), 10.41 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 12.0, 26.3, 56.3, 64.3, 127.5, 127.5 128.3, 128.5, 129.0, 129.3, 129.7, 130.3, 133.5, 135.4, 139.2, 143.3, 145.4, 158.4, 165.7. IR (KBr), ν, cm^{-1}: 702, 748, 1118, 1281, 1714. LC-MS, *m/z* (%): 414.0 (100) [M-CI]$^+$. Anal. calc. for $C_{26}H_{24}ClNO_2S$: C, 69.40%; H, 5.38%; N, 3.11%. Found: C, 69.64%; H, 5.27%; N, 3.28%.

*3-Benzyl-5-{2-[(diphenylacetyl)oxy]ethyl}-4-methyl-1,3-thiazolium chloride (***3v***)*
Yield 65%, a light gray solid. Mp = 168-170 °C. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 2.22 (s, 3H), 3.25 (t, J_{HH} = 5.50 Hz, 2H), 4.33 (t, J_{HH} = 5.50 Hz, 2H), 5.14 (s, 1H), 5.76 (s, 2H), 7.24-7.31 (m, 12H), 7.43-7.45 (m, 3H), 10.29 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 11.8, 26.0, 56.3, 64.2, 127.7, 128.3, 128.9, 129.0, 129.4, 129.7, 133.4, 135.0, 139.1, 143.2, 158.0, 172.1. IR (KBr), ν, cm^{-1}: 613, 635, 699, 745, 982, 1025, 1076, 1149, 1153, 1190, 1245, 1251, 1309, 1450, 1729. LC-MS, *m/z* (%): 428.0 (100) [M-CI]$^+$. Anal. calc. for $C_{27}H_{26}ClNO_2S$: C, 69.89%; H, 5.65%; N, 3.02%. Found: C, 69.65%; H, 5.78%; N, 3.13%.

*3-(2-Chlorobenzyl)-5-{2-[(diphenylacetyl)oxy]ethyl}-4-methyl-1,3-thiazolium chloride (***3w***)*
Yield 63%, a light brown solid. Mp = 135-137 °C. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 2.21 (s, 3H), 3.29 (br m, 2H+H_2O), 4.33 (br m, 2H), 5.16 (s, 1H), 5.78 (s, 2H), 7.00-7.02 (m, 1H), 7.27-7.41 (m, 11H), 7.49 (m, 1H), 7.63 (m, 1H), 10.02 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 11.6, 26.1, 54.3, 56.3, 64.3, 127.7, 128.7, 128.9, 129.0, 130.1, 130.6, 130.9, 131.4, 132.9, 135.1, 139.2, 143.4, 158.8, 172.2. IR (KBr), ν, cm^{-1}: 695, 754, 1152, 1190, 1443, 1458, 1730. LC-MS, *m/z* (%): 462.0 (100) [M-CI]$^+$. Anal. calc. for $C_{27}H_{25}Cl_2NO_2S$: C, 65.06%; H, 5.06%; N, 2.81%. Found: C, 64.83%; H, 4.92%; N, 2.59%.

*3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-{2-[(benzoyl)oxy)ethyl]}-4-methyl-1,3-thiazolium chloride hydrochloride (**2**)*
Yield 50%, a white crystalls. Mp = 230 °C (dec.). ^{1}H NMR (500 MHz, DMSO-d_6), δ: 2.56 (s, 3H), 2.58 (s, 3H), 3.44 (s, 2H), 4.49 (s, 2H), 5.62 (s, 2H), 7.56 (br s, 2H), 7.69 (s, 1H), 7.98 (br s, 2H), 8.34 (s, 1H), 9.21 (br s, 2H), 10.03 (s, 1H) 15.05 (br s, 1H); LC-MS, *m/z* (%): 369.2 [M-H-2CI]$^+$. Anal. calc. for $C_{19}H_{22}N_4O_2SCl_2$: C, 51.70%; H, 5.02%; N, 12.69%. Found: C, 51.85%; H, 5.14%; N, 12.74%.

4.3. AChE and BChE inhibition assays

The tested compounds **3a-3w** were dissolved in DMSO and then were diluted with water to the required concentration. The solution, which consisted of 0.1 mM *S*-acetylthiocholine iodide, inhibitor, 25 mM phosphate buffer (pH 7.48), and 1% DMSO, was thermostated for 5 min at 25 °C. Then 10 μL of 50 mM ethanolic solution of DTNB was added for detection of AChE activity.[29] The reaction was started by adding the enzyme solution. The final assay volume was 0.5 mL. The activity of AChE was studied spectrophotometrically by measuring the absorbance of 5-thio-2-nitrobenzoate at 412 nm using molar extinction coefficient of 5-thio-2-nitrobenzoate of 14150 M^{-1}cm^{-1}.[37] The inhibition of BChE was studied under the same conditions with 0.5 mM *S*-butyrylthiocholine iodide as a substrate. The obtained K_m values were of 0.20 mM and 0.26 mM for AChE and BChE, respectively. The IC_{50} values represent the concentrations of compounds which reduces enzyme activity by 50 %.

4.4. Molecular docking

The PDB crystal files of human AChE (PDB code 4EY7[33]) and human BChE (PDB code 4BDS[36]) were obtained from RCSB PDB (www.rcsb.org).[38] Before the docking calculations, ligands and water molecules were removed from the crystal structures. Molecular structures of compounds **3s** and **3w** were drawn in MarvinSketch and optimized by AM1 semi-empirical quantum mechanical method in program MOPAC.[39] Docking files were prepared using the MGLTools 1.5.6. The Autodock 4.2 program was employed to perform the calculations with using the Lamarckian genetic algorithm (LGA)

method.[40] The program Discovery Studio 3.5 (Accelrys, San Diego, CA) was applied for analysis of inhibitor binding modes.

References

1. Manzetti S., Zhang J., and van der Spoel D. (**2014**) Thiamin function, metabolism, uptake, and transport. *Biochemistry,* 53 (5) 821–835.
2. Bettendorff L. (**2013**) Thiamine, in: Zempleni J., Suttie J., Gregory J.F. 3rd, and Stover P. (Eds.) Handb. Vitam., fifth ed., CRC Press, Boca Raton, 268–323.
3. Bunik V. I. (**2014**) Benefits of thiamin (vitamin B_1) administration in neurodegenerative diseases may be due to both the coenzyme and non-coenzyme roles of thiamin. *J. Alzheimer's Dis. Parkinson*., 4 (6) 173–177.
4. Butterworth R. F. (**2003**) Thiamin deficiency and brain disorders. *Nut Res Rev*., 16, 277–284.
5. Liu D., Ke Z., and Luo J. (**2017**). Thiamine deficiency and neurodegeneration: the interplay among oxidative stress, endoplasmic reticulum stress, and autophagy. *Molecular neurobiology*, *54* (7) 5440-5448.
6. Mkrtchyan G., Aleshin V., Parkhomenko Y., Kaehne T., Di Salvo M. L., Parroni A., Contestabile R., Vovk A., Bettendorff L., and Bunik V. (**2015**) Molecular mechanisms of the non-coenzyme action of thiamin in brain: biochemical, structural and pathway analysis. *Sci Rep*., 5 12583.
7. Ozdemir Z. O., Senturk M., and Ekinci D. (**2013**) Inhibition of mammalian carbonic anhydrase isoforms I, II and VI with thiamine and thiamine-like molecules. *J. Enzyme Inhib. Med. Chem*., 28 (2) 316–319.
8. Vasan S., Zhang X., Zhang X., Kapurniotu A., Bernhagen J., Teichberg S., Basgen J., Wagle D., Shih D., Terlecky I., Bucala R., Cerami A., Egan J., and Urlich P. (**1996**) An agent cleaving glucose-derived protein crosslinks *in vitro* and *in vivo*. *Nature*, 382 (6588) 275–278.
9. Hamze A., Rubi E., Arnal P., Boisbrun M., Carcel C., Salom-Roig X., Maynadier M., Wein S., Vial H., and Calas M. (**2005**) Mono-and bis-thiazolium salts have potent antimalarial activity. *J. Med. Chem*., 48 (10) 3639–3643.
10. Caldarelli S. A., Fangour S. E., Wein S., van Ba C. T., Perigaud C., Pellet A., Vial H. J., and Peyrottes S. (**2013**) New bis-thiazolium analogues as potential antimalarial agents: design, synthesis, and biological evaluation. *J. Med. Chem*., 56 (2) 496–509.
11. Soreq H., Seidman S. (**2001**) Acetylcholinesterase – new roles for an old actor. *Nat Rev Neurosci*., 2 (4) 294–302.
12. Mesulam M.-M., Guillozet A., Shaw P., Levey A., Duysen E.G., and Lockridge O. (**2002**) Acetylcholinesterase knockouts establish central cholinergic pathways and can use butyrylcholinesterase to hydrolyze acetylcholine. *Neuroscience*, 110 (4) 627–639.
13. Darvesh S., Cash M., Reid G. A., Martin E., Mitnitski A., and Geula C. (**2012**) Butyrylcholinesterase is associated with β-amyloid plaques in the transgenic APPSWE/PSEN1dE9 mouse model of Alzheimer disease. *J. Neuropathol. Exp. Neurol*., 71 (1) 2–14.
14. Singh M., Kaur M., Kukreja H., Chugh R., Silakari O., and Singh D. (**2013**) Acetylcholinesterase inhibitors as Alzheimer therapy: from nerve toxins to neuroprotection. *Eur. J. Med. Chem*., 70 165–188.
15. Maggi L., Mantegazza R. (**2011**) Treatment of myasthenia gravis: focus on pyridostigmine. *Clin. Drug Invest*., 31 691–701.
16. Alward W.L. (**1998**) Medical management of glaucoma. *N. Engl. J. Med*. 339 (18) 1298–1307.
17. Simard M., van Reekum R. (**2004)** The acetylcholinesterase inhibitors for treatment of cognitive and behavioral symptoms in dementia with Lewy bodies. *J. Neuropsychiatry Clin. Neurosci*., 16 (4) 409–425.
18. Pagano G., Rengo G., Pasqualetti G., Femminella G. D., Monzani F., Ferrara N., and Tagliati M. (**2015**) Cholinesterase inhibitors for Parkinson's disease: a systematic review and meta-analysis. *J. Neurol. Neurosurg. Psychiatry,* 86, 767–773.
19. Ribeiz S. R., Bassitt D. P., Arrais J. A., Avila R., Steffens D. C., and Bottino C. M. (**2010**) Cholinesterase inhibitors as adjunctive therapy in patients with schizophrenia and schizoaffective disorder. *CNS Drugs,* 24 (4) 303–317.
20. Matsunaga Y., Tanaka T., Yoshinaga K., Ueki S., Hori Y., Eta R, Kawabata Y., Yoshii K., Yoshida K., Matsumura T., Furuta S., Takei M., Tack J., and Itoh Z. (**2011**) Acotiamide hydrochloride (Z-338), a new selective acetylcholinesterase inhibitor, enhances gastric motility without prolonging QT interval in dogs: comparison with cisapride, itopride, and mosapride. *J. Pharmacol. Exp. Ther*., 336 (3) 791–800.

21. Sun Z.-Q., Tu L.-X., Zhuo F.-J., and Liu S.-X. (**2016**) Design and discovery of novel thiazoleacetamide derivatives as anticholinesterase agent for possible role in the management of Alzheimer's. *Bioorg. Med. Chem. Lett*., 26 (3) 747–750.
22. Rahim F., Javed M. T., Ullah H., Wadood A., Taha M., Ashraf M., Qurat-ul-Ain, Khan M. A., Khan F., Mirza S., and Khan K. M. (**2015**) Synthesis, molecular docking, acetylcholinesterase and butyrylcholinesterase inhibitory potential of thiazole analogs as new inhibitors for Alzheimer disease. *Bioorg. Chem*., 62 106–116.
23. Liu S., Shang R., Shi L., Wan D. C. C., and Lin H. (**2014**) Synthesis and biological evaluation of 7*H*-thiazolo[3,2-b]-1,2,4-triazin-7-one derivatives as dual binding site acetylcholinesterase inhibitors. *Eur. J. Med. Chem*., 81 237–244.
24. Mohsen U. A., Kaplancikli Z. A., Ozkay Y., and Yurttaş L. (**2015**) Synthesis and evaluation of anti-acetylcholinesterase activity of some benzothiazole based new piperazine-dithiocarbamate derivatives. *Drug Res. (Stuttg)*, 65 (4) 176–183.
25. Alspach J. D., Ingraham L. L. (**1977**) Inhibition of acetylcholinesterase by thiamine. A structure-function study. *J. Med. Chem*. 20 (1) 161–164.
26. Inoue K., Itokawa Y. (**1973**). Metabolism of *O*-benzoylthiamine in animal body. *Biochem. Med.*, 8 (3) 450-463.
27. Hurt J. K., Coleman J. L., Fitzpatrick B. J., Taylor-Blake B., Bridges A. S., Vihko P., and Zylka, M. J. (**2012**). Prostatic acid phosphatase is required for the antinociceptive effects of thiamine and benfotiamine. *PloS one*, *7*(10), e48562.
28. Sano T. (**1944**) Vergleich der wirksamkeit der verschiedenen aneurinester von organischen sauren. *Bull. Chem. Soc. Jpn*., 19 (11)185–205.
29. Ellman G. L., Courtney K. D., Andres V. jr., and Featherstone R. M. (**1961**) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol*., 7 (2) 88–95.
30. Ocheretniuk A., Kobzar O., Mischenko I., and Vovk A. (**2017***) N*-Phenacylthiazolium salts as inhibitors of cholinesterases. *French-Ukrainian J. Chem*., 5 (2) 1–14.
31. Ocheretniuk A. D., Kobzar O. L., Kozachenko O. P., Brovarets V. S., and Vovk A. I. (**2017**) Synthesis and the activity assessment of adamantyl-containing thiazolium inhibitors of butyrylcholinesterase. *Ž org farm hìm*., 15 (4) 48–55.
32. Krupka R. M., Laidler K. J. (**1961**) Molecular mechanisms for hydrolytic enzyme action. I. Apparent non-competitive inhibition, with special reference to acetylcholinesterase. *J. Am. Chem. Soc*., 83 (6) 1445–1447.
33. Cheung J., Rudolph M. J., Burshteyn F., Cassidy M. S., Gary E. N., Love J., Franklin M. C., and Height J. J. (**2012**) Structures of human acetylcholinesterase in complex with pharmacologically important ligands. *J. Med. Chem*., 55 (22) 10282–10286.
34. Sussman J. L., Harel M., Frolow F., Oefner C., Goldman A., Toker L., and Silman I. (**1991**) Atomic structure of acetylcholinesterase from Torpedo californica: a prototypic acetylcholine-binding protein. *Science,* 253 (5022) 872–879.
35. Zhan C.-G., Zheng F., and Landry D. W. (**2003)** Fundamental reaction mechanism for cocaine hydrolysis in human butyrylcholinesterase. *J. Am. Chem. Soc*., 125 (9) 2462–2474.
36. Nachon F., Carletti E., Ronco C., Trovaslet M., Nicolet Y., Jean L., and Renard P.-Y. (**2013**) Crystal structures of human cholinesterases in complex with huprine W and tacrine: elements of specificity for anti-Alzheimer's drugs targeting acetyl-and butyryl-cholinesterase. *Biochem. J*., 453 (3) 393–399.
37. Eyer P., Worek F., Kiderlen D., Sinko G., Stuglin A., Simeon-Rudolf V., and Reiner E. (**2003**) Molar absorption coefficients for the reduced Ellman reagent: reassessment. *Anal. Biochem.,* 312 (2) 224-227.
38. Berman H. M., Westbrook J., Feng Z., Gilliland G., Bhat T. N., Weissig H., Shindyalov I. N., and Bourne P. E. (**2000**) The Protein Data Bank. *Nucleic Acids Res*., 28 (1) 235–242.
39. Stewart J. J. P. MOPAC2016. Stewart Computational Chemistry, Colorado Springs, CO, USA. http://OpenMOPAC.net.
40. Morris G. M., Goodsell D. S., Halliday R. S., Huey R., Hart W. E., Belew R. K., and Olson A. J. (**1998**) Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *J. Comput. Chem*., 19 (14) 1639-1662.