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Research Article

Synthesis of thiazole derivatives as cholinesterase inhibitors with antioxidant activity

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ABSTRACT

In the present research, we synthesized two unique series of thiazole compounds having 5-bromothiophene and 3-methylthiophene (**2a-2f**) in their structure. After that, spectroscopic methods were used to analyze the chemical compositions of the newly synthesized molecules. Then *in vitro* evaluation was done to determine acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activity of the synthesized compounds using galantamine as reference standard. The compounds' antioxidant properties were assessed using DPPH radical scavenging and ferrous ion-chelating techniques. The results of the study showed weak anticholinesterase activity against AChE and BuChE enzymes for all the final compounds. The synthesized analogs also showed significant DPPH radical scavenging activities with IC_{50} values in the range of 29.16 ± 0.009 to 33.09 ± 0.004 µM (for DDPH) incomparison to standard gallic acid with IC_{50} = 31.13 ± 0.008 µM (for DDPH). Especially, compound **2c** showed the best antioxidant activity with IC_{50} value of 29.16 ± 0.009 µM.

Keywords: AChE, Antioxidant, BuChE, Thiazole

1. INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disease at the moment, which causes loss of memory, behavioral problems, and a reduction in cognitive function eventually leading to death [1,2]. The disease is most commonly caused by cholinergic hypothesis, Amyloid- β (Ab) plague formation, N-methyl-D-aspartate (NMDA) receptor (NMDAR) antagonism hypothesis, The accumulation of thin protein after their hyperphosphorylation, biometal dyshomeostasis, and oxidative stress [3-5].

Currently, the traditional "cholinergic hypothesis" is mostly agreed upon by academics [6]. According to the cholinergic hypothesis, the main biochemical features of AD are reported to be loss and dysfunctions of cholinergic transmission and reductions in acetylcholine neurotransmitters [7]. Cholinesterase enzymes encompass two distinct isozymes, namely AChE (E.C.3.1.1.7) and BuChE (E.C.3.1.1.8), which hydrolyze acetylcholine (ACh) and influence cholinergic neuron activity [8,9]. AChE hydrolyzes the neurotransmitter acetylcholine, which is present at cholinergic synapses, whereas BuChE co-regulates AChE's activity. Cholinesterase inhibitors increase the quantity of acetylcholine required for the neurotransmission process by counteracting the effects of these enzymes [10]. Four cholinesterase (ChE) inhibitors have so far received FDA approval for use in the treatment of AD (**Figure 1**): donepezil, tacrine, galantamine, and rivastigmine [11-12].

By scavenging and stabilizing free radicals, antioxidants are chemicals that lessen the oxidative damage caused by free radicals. Additionally, antioxidants have a protective effect on macromolecules such as proteins, nucleic

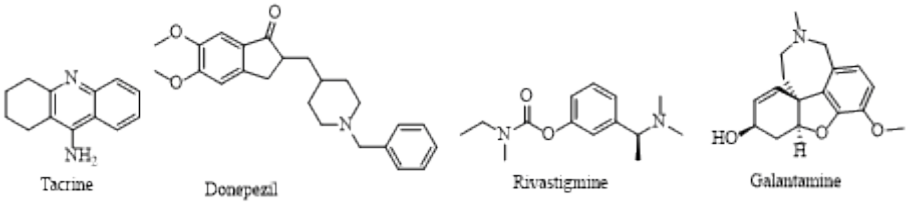


Figure 1. The structures of some commercially available AChE inhibitors.

acids and lipids. As a result, molecules with both cholinergic inhibitor and antioxidant properties provide advantages in the treatment of AD for potential therapeutic purposes [8].

In order to develop effective innovative drugs for the central nervous system, thiazole has been found as a potential scaffold. A number of thiazole derivatives are currently being investigated in clinical studies, and thiazole-based CNS medications are now used as therapeutic agents for a variety of CNS disorders [13-14].

In this study, thiazole derivatives were synthesized and their structure characterized using ^{1}H-NMR and ^{13}C-NMR, and HRMS. The compounds' antioxidant properties were assessed using Ferrous ion-chelating and DPPH Radical Scavenging methods. Furthermore, this study investigated these derivatives for *in vitro* inhibition on AChE and BuChE.

2. MATERIALS AND METHODS

2.1. Chemistry

Synthesis of 2-((3-Methyl/5-bromothiophene-2-yl)methylene)hydrazine-1-carbothioamide (1): In ethanol, 3-methylthiophene-2-carbaldehyde or 5-bromothiophene-2-carbaldehyde and thiosemicarbazide were dissolved. Following that, the mixture was refluxed for three hours. After the completion of reaction, the mixture was placed in an ice bath to chill down. The resultant precipitate was then removed by filtering.

Synthesis of Target Compounds (2a-2f): Ethanol was used to dissolve compound 1 and derivative of 2-bromoacetophenone. After that, the mixture was refluxed for four hours. After the reaction is complete, the mixture is placed in an ice bath to chill down. The resultant precipitate is removed by filtering. After chilling, the drying and crystallization of precipitates is done using ethanol.

4-(4-Cyanophenyl)-2-(2-((3-methylthiophenyl-2-yl)methylene)hydrazineyl)thiazole (2a): Yield: 75 %, M.P.= 248.6 °C. ^{1}H-NMR (300 Mega Hz, Dimethylsulfoxide-d_6): δ: 2.18 (3H, s, CH_3), 7.75-7.76 (1H, m, Aromatic CH), 7.81-7.82 (2H, m, Aromatic CH), 7.90 (1H, s, Aromatic CH), 8.11 (1H, s, CH=N), 8.31 (3H, d, *J*=8.60 Hz, Aromatic CH), 11.33 (1H, s, NH). ^{13}C-NMR (75 Mega Hz, Dimethylsulfoxide-d_6): δ= 15.59 (CH_3), 109.40, 120.43, 123.37, 125.18, 127.94, 128.79, 131.55, 132.31, 133.83, 136.39, 139.25, 152.08 (thiazole C), 170.14 (thiazole C). Calculated HRMS (m/z): [M+H] for $C_{16}H_{12}N_4S_2$: 325.0576; found: 325.0586.

4-(3,4-Dichlorophenyl)-2-(2-((3-methylthiophenyl-2-yl)methylene)hydrazineyl) thiazole (2b): Yield: 78 %, M.P.= 240.5 °C. ^{1}H-NMR (300 Mega Hz, Dimethylsulfoxide-d_6): δ: 2.27 (3H, s, CH_3), 7.01 (1H, s, Aromatic CH), 7.37 (1H, s, Aromatic CH), 7.72-7.80 (4H, m, Aromatic CH), 8.08 (1H, s, CH=N), 11.17 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ= 17.01 (CH_3), 102.65, 113.58, 119.19, 123.37, 124.70, 128.03, 129.84, 130.03, 132.31, 133.26, 136.58, 138.77, 151.03 (thiazole C), 170.42 (thiazole C). Calculated HRMS (m/z): [M+H] for $C_{15}H_{11}N_3O_2S_2Cl_2$: 367.9844; found: 367.9856.

4-(2,4-Difluorophenyl)-2-(2-((3-methylthiophenyl-2-yl)methylene)hydrazineyl) thiazole (2c): Yield: 76 %, M.P.= 200.2 °C. ^{1}H-NMR (300 Mega Hz, Dimethylsulfoxide-d_6): δ: 2.19 (3H, s, CH_3), 7.74 (1H, s, Aromatic CH), 7.88-7.90 (2H,

m, Aromatic CH), 8.03-8.05 (3H, m, Aromatic CH), 8.14 (1H, s, CH=N), 11.34 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ= 17.58 (CH_3), 108.45, 110.54, 112.54, 122.14, 124.04, 127.74, 128.80, 130.99, 132.40, 134.30, 135.54, 138.58, 150.18 (thiazole C), 170.23 (thiazole C). Calculated HRMS (m/z): [M+H] for $C_{15}H_{11}N_3F_2S_2$: 336.0435; found: 336.0446.

4-(4-Cyanophenyl)-2-(2-((5-bromothiophenyl-2-yl)methylene)hydrazineyl)thiazole (2d): Yield: 80 %, M.P.= 207.5 °C. ^{1}H-NMR (300 Mega Hz, Dimethylsulfoxide-d_6): δ: 6.89-7.05 (2H, m, Aromatic CH), 7.37 (1H, s, Aromatic CH), 7.82-7.92 (4H, m, Aromatic CH), 8.18 (1H, s, CH=N), 11.38 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ= 109.63, 120.14, 122.34, 125.08, 126.93, 127.54, 131.35, 132.20, 132.83, 135.29, 138.15, 152.11 (thiazole C), 170.18 (thiazole C). Calculated HRMS (m/z): [M+H] for $C_{15}H_9N_4S_2Br$: 388.9525; found: 388.9536.

4-(3,4-Dichlorophenyl)-2-(2-((5-bromothiophenyl-2-yl)methylene)hydrazineyl) thiazole (2e): Yield: 79 %, M.P.= 220.5 °C. ^{1}H-NMR (300 Mega Hz, Dimethylsulfoxide-d_6): δ: 6.94-7.08 (2H, m, Aromatic CH), 7.39 (1H, s, Aromatic CH), 7.89-7.94 (3H, m, Aromatik CH), 8.20 (1H, s, CH=N), 11.40 (1H, s, NH). ^{13}C-NMR (75 Mega Hz, Dimethylsulfoxide-d_6): δ= 109.18, 112.45, 118.57, 123.62, 125.78, 126.99, 129.80, 131.28, 133.44, 124.32, 136.58, 138.42, 150.28 (thiazole C), 170.12 (thiazole C).

4-(2,4-Difluorophenyl)-2-(2-((5-bromothiophenyl-2-yl)methylene)hydrazineyl) thiazole (2f): Yield: 74 %, M.P.= 197.2 °C. ^{1}H-NMR (300 Mega Hz, Dimethylsulfoxide-d_6): δ: 6.98-7.10 (2H, m, Aromatic CH), 7.42 (1H, s, Aromatic CH), 7.87-7.91 (3H, m, Aromatik CH), 8.18 (1H, s, CH=N), 11.42 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ= 110.42, 111.48, 114.63, 121.14, 123.44, 128.70, 128.84, 131.19, 132.44, 135.30, 136.62, 139.20, 150.24 (thiazole C), 170.22 (thiazole C). Calculated HRMS (m/z): [M+H] for $C_{14}H_8N_3F_2S_2Br$: 399.9384; found: 399.9394.

2.2. Assay for inhibition of cholinesterase enzyme

The ability of the synthesized compounds to inhibit the BuChE and AChE enzyme was examined. Ellman's modified spectrophotometric technique [15] was used to measure the inhibition potential of synthesized compounds against AChE and BuChE. Cholinesterase activity experiments were conducted using "equine serum BuchE" (EC 3.1.1.8, Sigma) and electric eel AChE (Type-VI-S, EC 3.1.1.7, Sigma) enzymes. The reaction's substrates were butyrylthiocholine chloride and acetylthiocholine iodide obtained from Sigma Aldrich at Saint Louis, USA. To test the cholinesterase activity, 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB, Sigma Aldrich at Saint Louis, United States America) was utilized. In a 96-well microplate, additional reagents were added in the following order: 50 mM Tris HCl buffer (pH 8.0), 6.8 mM DTNB, 10 µl of BuChE/AChE solution, and 2 µl of sample solutions and multichannel automated pipette obtained from Thermo Fisher Scientific, USA). Next, butyrylthiocholine chloride/acetylthiocholine iodide was added in 10 µl amount to start the reaction. The formation of the yellow 5-thio-2-nitrobenzoate anion, which results from the reaction of DTNB with thiocholines, was employed to track the acetylthiocholine iodide/butyrylthiocholine chloride hydrolysis. Using a 96-well plate, the following reaction was catalyzed by enzymes at a wavelength of 412 nm. The plate was obtained from Varioskan Flash, Thermo Scientific, USA). The incubation of microplate was done for 15 minutes at 27°C. Periodic test lasting 75 seconds was obtained. The Varioskan Flash software's SkanIt Software 2.4.5 RE was used to assess the measurements and computations. By comparing the sample reaction rates to those of the blank sample (DMSO and methanol) and applying the formula (E-S)/E x 100, the percentage of AChE and BChE inhibition was calculated. Three replicates of each experiment were conducted. Galantamine hydrochloride obtained from the Sigma-Al, USA has been utilized as a reference material.

In the formula;

E: the activity of the enzyme without the test sample.

S: the activity of the enzyme with the test sample.

2.3. Antioxidant Activity

2.3.1. Ferrous ion-chelating effect

Using Chua et al.'s (2008) approach, the ferrous ion-chelating impact of the reference compound and all the extracts was evaluated. In summary, 200 µL of a 2 mM $FeCl_2$ solution was used to incubate different dilutions of ethanol dissolved extracts (80%). Then, we added 5 mM ferrozine concentration of 5 mM ferrozine in 800 µL amount to the mixture to start the reaction, which was then allowed to stand for 10 minutes at room temperature. Using a spectrophotometer, the reaction mixture's absorbance was determined at 562 nm (Varioskan Flash, Thermo Scientific, USA) against ethanol (80%) as blank. The following formula was used to determine the ratio of inhibition of ferrozine-Fe^{2+} complex formation:

$$I\% = \left[\frac{A_{blank} - A_{sample}}{A_{blank}}\right] \times 100$$

where A_{sample} is the absorbance of the extracts/reference, and A_{blank} is the absorbance of the control reaction (which only contained $FeCl_2$ and ferrozine). In this test, butylated hydroxytoluene (BHT) and rutin served as the reference. Both were purchased from Sigma Aldrich in the USA. Three duplicate analyses were performed, and the average values with S.E.M. were reported as the results [16,17].

2.3.2. DPPH Radical Scavenging Activity

Radical-scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was screened using Blois's UV technique. Using this procedure, 20 µL of methanol was mixed with the compounds having 40 micro molar and 100 micro molar concentrations, as well as gallic acid. Then, in each solution180 µL of a 0.15 mM DPPH solution dissolved in methanol was added. Then incubation was done at room temperature for 20 minutes and amount of DPPH was measured at 520 nm (Varioskan Flash, Thermo Scientific, USA). The following formula was employed to determine radical scavenging capacity of DPPH.

$I\% = [(A_{control} - A_{sample})/A_{control}] \times 100$, where

$A_{control}$ = Absorbance of the control reaction

A_{sample} = Absorbance of the extracts/reference.

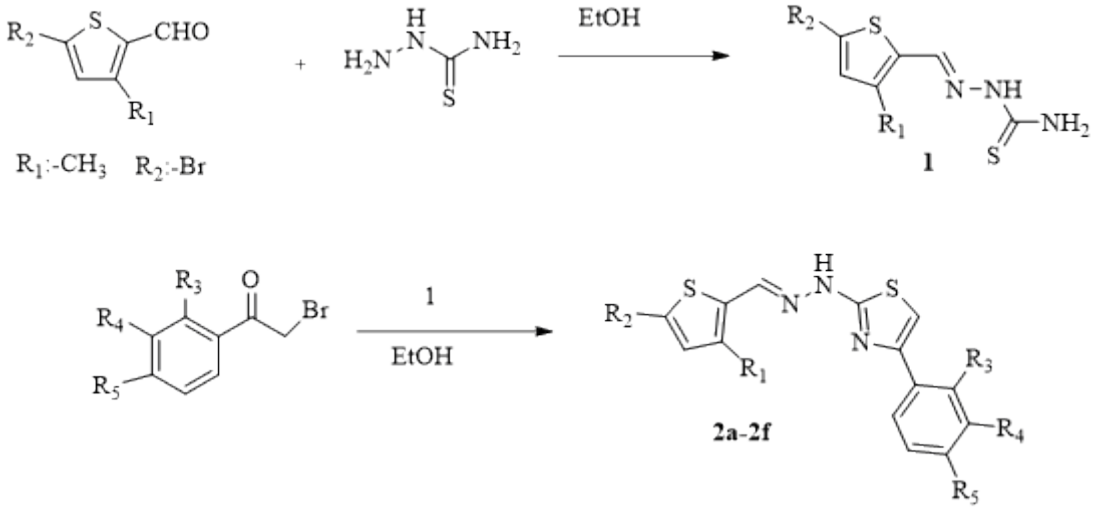
The experiments were performed as replicates of three, and the average was taken with standard error mean [18].

3. RESULTS AND DISCUSSION

3.1. Chemistry

In this study, three new thiazole derivatives were synthesized, as shown in **Scheme 1.** The synthesis of the compounds was carried out in two stages. At the first step, 3-methylthiophene-2-carbaldehyde or 5-bromothiophene-2-carbaldehyde compound was reacted with thiosemicarbazide and thiosemicarbazone compound was obtained. In the second step, the thiosemicarbazone compound obtained in the first step was reacted with 2-bromoacetophenone derivative compounds and thiazole derivative compounds were obtained. Thiazole compounds are made by the reaction between α-halo-ketones and thioamides, a procedure known as Hantzsch thiazole synthesis. The reaction is driven by the intense nucleophilic nature of the S in thioamides, which is enhanced by electron resonance from the amide group. Because halogen is a good leaving group, sulfur attacks the α-carbon of α-halo-ketones as a nucleophile instead of the nearby carbonyl group. This encourages the thiazole ring to form and cyclize.

The structure of the 4-(substitutedphenyl)-2-(2-((3-methyl/5-bromothiophenyl-2-yl)methylene) hydrazineyl)thiazole (**2a-2f**) derivatives were confirmed by using ^{1}H-NMR, ^{13}C-NMR, and HRMS. The main structure of the target compounds constitute 3-methylthiophene and thiazole rings. The proton of methyl group in 3-methylthiophene was detected at 2.18-2.27 ppm range as singlet. Hydrazine (CH=N) protons have been detected around 8 ppm. The signals belonging to aromatic protons were found at 7.01–8.31 parts per million.



Comp.	2a	2b	2c	2d	2e	2f
R_1	$-CH_3$	$-CH_3$	$-CH_3$	-H	-H	-H
R_2	-H	-H	-H	-Br	-Br	-Br
R_3	-H	-H	-F	-H	-H	-F
R_4	-H	-Cl	-H	-H	-Cl	-H
R_5	-CN	-Cl	-F	-CN	-Cl	-F

Scheme 1. Chemical structure and general procedure for the synthesis of the final compounds **2a-2f**.

The carbon of methyl in the 3-methylthiophene ring resonated at 15.59–17.58 parts per million when the compounds' ^{13}C-NMR spectra were analyzed. All of the masses matched the expected M+H values.

3.2. Cholinesterase Enzymes Inhibition Assay

Using galantamine as reference drug, the **2a-2f** were analysed for their inhibitory effect against AChE and BuChE were assessed by employing Ellman's technique. **Table 1** is a summary of the findings. To verify the outcomes, three separate tests were run in duplicate. The results of our study showed weak results against AChE and BuChE enzymes.

Table 1. % Cholinesterase inhibitory activities of the synthesized compounds **2a-2f** at 50 µM reaction concentrations

Comp.	AChE	BuChE
2a	15.59 ± 0.006	5.07 ± 0.004
2b	3.39 ± 0.001	8.72 ± 0.001
2c	11.16 ± 0.005	3.94 ± 0.005
2d	NA	7.71 ± 0.002
2e	NA	NA
2f	NA	11.70 ± 0.006
Gal HBr	97.89 ± 0.01	62.48 ± 0.01

Table 2. DPPH free radical-scavenging activity and ferric ion chelating effect (inhibition % ± S.E.M) of synthesized compounds at 50 µM and IC_{50} values (**µm**)

Comp.	DPPH	ION CHELATING	IC_{50} (DPPH) µm
2a	44.55 ± 0.002	NA	-
2b	29.66 ± 0.003	1.80 ± 0.007	-
2c	64.31 ± 0.005	6.41 ± 0.004	29.16 ± 0.009
2d	63.42 ± 0.004	2.11 ± 0.008	32.08 ± 0.007
2e	65.96 ± 0.021	3.91 ± 0.013	31.94 ± 0.011
2f	60.83 ± 0.003	3.11 ± 0.006	33.09 ± 0.004
Gallic Acid	70.29 ± 0.005	-	31.13 ± 0.008
RUTIN 50 µM	-	13.21 ± 0.007	-
BHT 50 µM	-	7.06 ± 0.009	-

3.3. Antioxidant Activity

Test compounds for DPPH free radical scavenging and Ferrous ion-chelating effect were set at the concentration of 50 µM. We used gallic acid for reference. Based on control activities, the percentage of all substances evaluated as antioxidants was estimated (**Table 2**). The results showed the antioxidant activity of 70.29±0.005 % for gallic acid, and of 64.31 ± 0.005 % for **2c** at the concentration of 50 µm. Therefore, the compound **2c** can behave as a potential antioxidant agent.

4. CONCLUSION

Three novel thiazole-based compounds were synthesized and their potential as antioxidant and AChE inhibitors therapy was assessed. The three compounds showed minimal activity against AChE enzyme while compound **2c** showed antioxidant activity comparable to the reference drug. Based on the results and non-significant activities of the synthesized compounds **2a-2f**, no further molecular docking and ADMET studies were performed for these compounds. However, in the future, this synthetic scheme can be employed to synthesize a new series of thiazole derivatives as a strong candidate for the symptomatic relief of Alzheimer's disease.

Ethical approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Author contribution

Concept: UAÇ; Design: UAÇ; Supervision: UAÇ; Materials: AK, ZM, TE; Data Collection and/or Processing: UAÇ, ZM, TE; Analysis and/or Interpretation: UAÇ; Literature Search: AK; Writing: AK, ZM, TE, UAÇ; Critical Reviews: UAÇ.

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Conflict of interest

The authors declared that there is no conflict of interest.

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