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Acetylcholinesterase Inhibitor Activity of Some 5-Nitrothiophene-Thiazole Derivatives

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ABSTRACT

The potential anticholinesterase characteristics of some thiazole derivatives (2a–2j), including the 5-nitrothiophene moiety, were examined in this work. 1H-NMR, 13C-NMR, and HRMS spectral data were used to determine the structure of the compounds. Using a modified Ellman's spectrophotometric approach, each compound was tested for its ability to inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) enzymes. It was determined that the compounds exhibited inhibition of between 33.66–47.96 % against AChE and 13.03–63.29 % against BuChE at 80 µg/mL concentration.

Keywords: Thiazole, Thiophene, Achetycolinesteras inhibition, Galantamine.

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Introduction

Alzheimer's Disease (AD), which belongs to the category of degenerative nervous system disorders, refers to the different difficulties that arise in the central nervous system, such as various behavioral abnormalities and cognitive regression problems [1]. Neurotransmitters are strongly linked to neurodegenerative illness, with cholinergic pathway dysfunction being one of the acknowledged causes of AD [2]. The number of people with Alzheimer's disease is expected to exceed 131 million by 2050 [3]. As a result, Alzheimer's disease has become one of the most pressing public health problems we face. Increasing cholinergic neurotransmission while decreasing ACh hydrolysis is the most effective therapy available right now for Alzheimer's disease [4]. Acetylcholinesterase (AChE) and butyryl cholinesterase (BuChE) are the cholinesterases that hydrolyze ACh, with AChE having a 10-fold higher hydrolytic activity than BuChE [5].

However, BuChE inhibitors may produce peripheral side effects since they are mostly found in peripheral systems such as plasma, liver, and muscle tissue [6]. Tacrine, for example, displayed significant hepatotoxicity and other peripheral adverse effects as a dual inhibitor of AChE and BuChE [7]. Accordingly, efficient and selective AChE inhibitors with low side effects are beneficial in the treatment of Alzheimer's disease and diminish peripheral cholinergic adverse effects [8]. Three medications have been licensed for usage in the United States (by the FDA): galantamine, donepezil, and rivastigmine, the mechanism of which is to raise the quantity of acetylcholine at synapses by blocking acetylcholinesterase while they promote neurotransmission. Despite the fact that AChE inhibitors only provide symptomatic relief, they are still the first-line treatment for cognitive impairment, Alzheimer's disease, and Parkinson's disease [9]. As a result, researchers are still looking for AChE inhibitors to use in drug development [7].

The unique structure of the thiazole derivatives makes these compounds with high potential for biological activities. Thiazole derivatives shows numerous biological activities such as MAO inhibition[10], anticancer [11, 12], anticonvulsant [13], antidiabetic [14], antimicrobial [15-17], anti-inflammatory [18], analgesic [19]. The thiazole ring also has been reported to have anticholinesterase activity in many studies [10, 20-23]. Acotiamide hydrochloride is a thiazole-primarily based totally selective AChEI for the remedy of functional dyspepsia that was recently discovered (as a prokinetic drug) and has been applied therapeutically in Japan. Moreover, previous research has identified thiazolylhydrazone derivatives as having potential for the therapy of neurodegenerative diseases. All of these factors suggest that the final compounds may have potential against AD. In fact, it was found that acetylcholinesterase (AChE) enzyme inhibition decreased cell growth in lung tumor cell lines in the literature [24-28]. Thus, it can be concluded that for antiproliferation activity, there is a link between mitochondrial potential loss and apoptosis.

According to the above information, we have designed and synthesized thiazole derivatives originated from 5-nitrothiophene and determined anticancer activity on lung cancer cell lines in our previous study [29]. This study is a continuation of our aforementioned study in which the anticholinesterase activities of the compounds were investigated.

Materials and Methods

Chemistry

Merck Chemicals (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich Chemicals provided all of the chemicals utilized in the syntheses (Sigma-Aldrich Corp., St. Louis, MO, USA). The reactions and thus the purity of the compounds was determined using thin-layer chromatography (TLC) on Merck's silica gel 60 F254 aluminum sheets (Darmstadt, Germany). The MP90 digital melting point equipment was used to get the uncorrected melting points of the produced compounds (Mettler Toledo, Ohio, USA). The ^{1}H-NMR and ^{13}C-NMR spectra in DMSO-*d6* were recorded using Bruker 300 MHz and 75 MHz digital FT-NMR spectrometers (Bruker Bioscience, Billerica, MA, USA). The following cleavage patterns were identified in the NMR spectra: s: singlet; d: doublet; t: triplet; m: multiplet. The coupling constants (*J*) are expressed in Hertz. An LC/MS-IT-TOF system was used to undertake high resolution mass spectrometric (HRMS) experiments (Shimadzu, Kyoto, Japan).

Synthesis of 2-[(5-nitrothiophen-2-yl)methylene]hydrazinecarbothioamide (1)

At room temperature, a solution of 4-nitrothiophene-2-carbaldehyde (4.17 g, 0.03 mol) in ethanol (50 mL) was treated with thiosemicarbazide (2.52 g, 0.03 mol). The reaction mixture was swirled for 2 hours at 80 °C. TLC was used to monitor the reaction. The precipitate was filtered out and rinsed with cold ethanol once the reaction was done.

m. p. 255–258 °C [30], yield 75%.

General synthesis of 2-thiazole derivatives (2a-2j)

In a solution of 2-[(5-nitrothiophen-2-yl)methylene]hydrazinecarbothioamide (**1**) (0.3 g, 1.30 mmol), 2-Bromo-1-phenylethanone derivatives (1.30 mmol) were added in ethanol (30 mL). At 80 °C, the mixture was stirred. TLC was used to monitor the reaction. The product was filtered when the reaction was finished. The final compounds were obtained by recrystallizing ethanol.

2-{2-[(5-Nitrothiophen-2-yl)methylene]hydrazinyl}-4-(p-tolyl)thiazole (2a)

m. p. 221–222 °C, yield 79 %, ^{1}HNMR (300 MHz, DMSO-d_6, ppm) δ = 3.42 (s, 3H, -CH_3), 7.22 (d, *J*= 8.08 Hz, 1H, Ar-H), 7.40 (m, H, Ar-H), 7.54 (d, *J*= 4.41 Hz, H, Ar-H), 7.74 (d, *J*= 8.12 Hz, 1H, Ar-H), 8.06–8.10 (m, 2H, thiophene-H), 8.19 (s, 1H, thiazole-H), 8.47 (s, 1H, H-C=N), and 11.83 (brs, 1H, N-N-H). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 21.27 (CH_3), 125.93, 128.19, 129.70, 130.92, 131.40, 135.71, 137.52, 147.23, 151.20, and 178.59 (S-C=N). HRMS (m/z): $[M+H]^+$ calculated for $C_{15}H_{12}N_4O_2S_2$: 345.0474; found 345.0460.

4-(4-Nitrophenyl)-2-{2-[(5-nitrothiophen-2-yl) methylene]hydrazinyl}thiazole (2b)

m. p. 252–254 °C, yield 82%, ^{1}HNMR (300 MHz, DMSO-d_6, ppm) δ = 7.42 (d, *J*= 4.34 Hz, 1H, Ar-H), 7.78 (s, 1H, thiazole-H), 8.05–8.09 (m, 3H, Ar-H), 8.18 (s, 1H, H-C=N), 8.25 (d, *J*= 8.83 Hz, 2H, thiophene-H), and 12.82 (brs, 1H, N-N-H). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 110.22, 124.62, 126.83 (-CH=N), 128.49, 131.32, 135.24, 140.81, 146.76, 147.46, 149.21, 150.23, and 168.05 (S-C=N). HRMS (m/z): $[M+H]^+$ calculated for $C_{14}H_9N_5O_4S_2$: 376.0169; found 376.0172.

2-{2-[(5-Nitrothiophen-2-yl)methylene]hydrazinyl}-4-phenylthiazole (2c)

m. p. 209–210 °C, yield 75%, ^{1}HNMR (300 MHz, DMSO-d_6, ppm) δ = 7.31 (d, *J*= 7.19 Hz, 1H, Ar-H), 7.37–7.40 (m, 3H, Ar-H), 7.42 (s, 1H, thiazole-H) 7.84 (d, *J*= 8.17Hz, 2H, thiophene-H), 8.03 (dd, J_1= 1.44Hz, J_2= 4.32 Hz, 1H, Ar-H), 8.16 (s, 1H, H-C=N), and 12.75 (brs, 1H, N-N-H). ^{13}C-NMR (75 MHz, DMSO-d6, ppm) δ 105.29, 125.97 (-CH=N), 128.05, 128.15, 129.09, 131.23, 134.68, 147.80, 150.02, 151.01, and 167.69 (S-C=N). HRMS (m/z): $[M+1]^+$ calculated for $C_{14}H_{10}N_4O_2S_2$: 331.0318; found 331.0310.

4-{2-{2-[(5-Nitrothiophen-2-yl)methylene]hydrazinyl} thiazol-4-yl} benzonitrile (2d)

m. p. 249–251 °C, yield 81%, ^{1}HNMR (300 MHz, DMSO-d_6, ppm) δ = 7.47 (d, *J*= 4.43 Hz,1H, Ar-H), 7.76 (s, 1H, thiazole-H), 7.88 (d, *J*= 8.54 Hz, 2H, Ar-H), 8.03 (d, *J*= 8.50Hz, 2H, thiophene-H), 8.10 (d, *J*= 4.37 Hz, 1H, Ar-H), 8.22 (s, 1H, H-C=N), and 12.83 (brs, 1H, N-N-H). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 109.29, 110.24, 119.42 (-CN), 126.60 (-CH=N), 128.49, 131.36, 133.24, 135.19, 138.94, 147.49, and 167.95 (S-C=N). HRMS (m/z): $[M+1]^+$ calculated for $C_{15}H_9N_5O_2S_2$: 356.0270; found 356.0272.

4-(4-Fluorophenyl)-2-{2-[(5-nitrothiophen-2-yl)methylene]hydrazinyl}thiazole (2e)

m. p. 218–221 °C, yield 76 %, ^{1}HNMR (300 MHz, DMSO-d_6, ppm) δ = 7.25 (t, *J*= 8.91 Hz, 2H, Ar-H), 7.42–7.46 (m, 2H, Ar-H), 7.87–7.91 (m, 2H, Ar-H), 8.10 (d, *J*= 4.86 Hz, 1H, Ar-H), 8.20 (s, 1H, H-C=N), and 12.77 (brs, 1H, N-N-H). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) 105.21, 115.85, 116.14, 127.94 (-CH=N), 128.05, 128.27, 131.37, 134.84, 147.70, 150.08, 160.53, and 163.77 (S-C=N). HRMS (m/z): $[M+1]^+$ calculated for $C_{14}H_9FN_4O_2S_2$: 349.0224; found 349.0229.

2-{2-[(5-Nitrothiophen-2-yl)methylene]hydrazinyl}-4-(pyridin-4-yl)thiazole (2f)

m. p. 278–279 °C, yield 84 %, ^{1}HNMR (300 MHz, DMSO-d_6, ppm) δ = 7.50 (d, *J*= 4.44 Hz, 1H, Pyridine-H), 8.11 (d, *J*= 4.35 Hz, 1H, pyridine-H), 8.27 (s, 1H, thiazole-H), 8.34 (d, *J*= 6.76 Hz, 2H, thiophene-H), 8.37 (s, 1H, H-C=N), 8.90 (d, *J*= 6.80 Hz, 2H, pyridine-H), and 12.99 (brs, 1H, N-N-H). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 117.12, 122.45, 128.93 (-CH=N), 131.34, 136.09, 143.32, 146.64, 147.10, 148.74, 150.46, and 168.67 (S-C=N). HRMS (m/z): $[M+1]^+$ calculated for $C_{13}H_9N_5O_2S_2$: 332.0270; found 332.0272.

4-(Naphthalen-2-yl)-2-{2-[(5-nitrothiophen-2-yl) methylene]hydrazinyl}thiazole (2g)

m. p. 216–217 °C, yield 80%, ^{1}HNMR (300 MHz, DMSO-d_6, ppm) δ = 7.43–7.56 (m, 4H, Ar-H), 7.92–8.06 (m, 6H, Ar-H), 8.21 (s, 1H, H-C=N), and 8.37 (brs, 1H, N-N-H). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 106.01, 124.29, 124.62, 126.49 (-CH=N), 126.88, 128, 128.59, 131.09, 132.12, 132.91, 133.54, 134.72, 147.75, 150.01, 150.79, and 167.74 (S-C=N). HRMS (m/z): $[M+1]^+$ calculated for $C_{18}H_{12}N_4O_2S_2$: 381.0474; found 381.0471.

4-(Naphthalen-1-yl)-2-{2-[(5-nitrothiophen-2-yl) methylene] hydrazinyl} thiazole (2h)

m. p. 226–227 °C, yield 77 %, ^{1}HNMR (300 MHz, DMSO-d_6, ppm) δ = 7.20 (s, 1H, thiazole-H), 7.45 (d, *J*= 4.42 Hz, 1H, thiophene-H), 7.52–7.57 (m, 3H, naphthalene-H), 7.68–7.71 (m, 1H, naphthalene-H), 7.94–7.99 (m, 2H, naphthalene-H), 8.09 (d, *J*= 4.36 Hz, 1H, thiophene-H), 8.22 (s, 1H, H-C=N), 8.39–8.42 (m, 1H, naphthalene-H), and 12.81 (brs, 1H, N-N-H). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 108.95, 125.94 (-CH=N), 126.43, 126.66, 127.43, 128.15, 128.73, 128.92, 131.02, 131.36, 133.09, 133.95, 134.83, 147.87, 150.03, and 167.51 (S-C=N). HRMS (m/z): $[M+1]^+$ calculated for $C_{18}H_{12}N_4O_2S_2$: 381.0474; found 381.0485.

4-(2,5-Dimethoxyphenyl)-2-{2-[(5-nitrothiophen-2-yl) methylene]hydrazinyl}thiazole (2i)

m. p. 217–218 °C, yield 71 %, ^{1}HNMR (300 MHz, DMSO-d_6, ppm) δ = 3.73 (s, 3H, -OCH_3), 3.85 (s, 3H, -OCH_3), 6.84 (d, *J*= 3.21 Hz, 1H, Ar-H), 6.87 (d, *J*= 3.21 Hz, 1H, Ar-H), 7.02–7.05 (m, 1H, Ar-H), 7.42–7.45 (m, 1H, N-N-H), 7.50 (s, 1H, thiazole-H), 7.58 (d, *J*= 3.19 Hz, 1H, thiophene-H), 8.07–8.09 (m, 1H, thiophene-H), and 8.19 (s, 1H, H-C=N). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 55.79 (CH3), 56.28 (CH3), 109.74, 113.20, 114.25, 116.69, 123.40, 128.13 (-CH=N), 131.35, 134.69, 147.84, 151.35, 153.37, and 166.09 (S-C=N). HRMS (m/z): $[M+1]^+$ calculated for $C_{16}H_{14}N_4O_4S_2$: 391.0529; found 391.0532.

4-(4-Methoxyphenyl)-2-{2-[(5-nitrothiophen-2-yl) methylene]hydrazinyl}thiazole (2j)

m. p. 214–215 °C, yield 89 %, ^{1}HNMR (300 MHz, DMSO-d_6, ppm) δ = 3.78 (s, 3H, -OCH_3), 6.95–6.98 (m, 2H, Ar-H), 7.24 (s, 1H, thiazole-H), 7.42 (s, 1H, H-C=N), 7.77 (d, *J*= 3.88 Hz, 2H, Ar-H), 8.07 (d, *J*= 4.25 Hz, 1H, thiophene-H), 8.17 (d, *J*= 1.82 Hz, 1H, thiophene-H), and 12.72 (brs, 1H, N-N-H). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 55.58 (CH_3), 103.18, 114.46, 127.32 (-CH=N), 127.64, 128.10, 131.35, 134.62, 147.85, 149.98, 159.36, and 167.56 (S-C=N). HRMS (m/z): $[M+1]^+$ calculated for $C_{15}H_{12}N_4O_3S_2$: 361.0424; found 361.0428.

AChE/BuChE Inhibition Assay

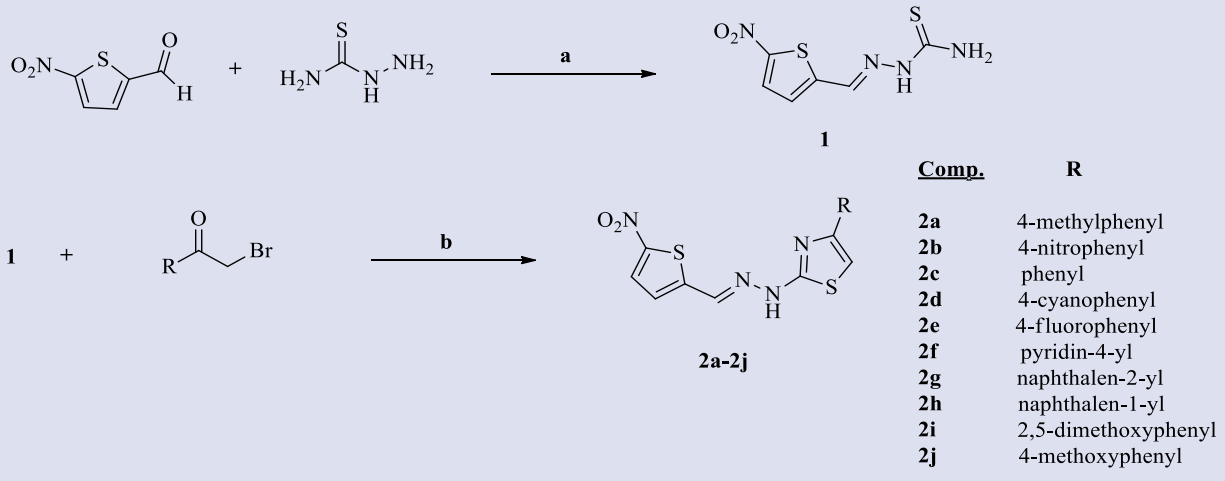
The inhibitory effects of title compounds on AChE/BuChE were studied using Ellman's approach[31], which was slightly modified. The reagents and materials used in the enzyme inhibition assay were supplied commercially by Sigma-Aldrich (St. Louis, MO, USA) and Fluka (Steinheim, Germany). A 10 L solution of studied compounds dissolved in 2 % dimethyl sulfoxide (DMSO, dilute with water) at final concentrations of 5, 10, 20, 40, and 80 g/mL, as well as a 20 L (1 unit/mL) solution of AChE, received a 2.4 mL 0.1 M phosphate buffer (from Electrophorus electricus, electric eel). After 15 minutes, the mixture was added to with 50 L of 0.01 M 5,5-dithio-bis(2-nitrobenzoic) acid (DTNB) and 20 L of 75 mM acetylthiocholine iodide (ATCI) or 25 mM butrylthiocholine iodide (BTCI). Using polystyrol cuvettes and a spectrophotometer, the absorbance at 412 nm and 37 °C was measured after 30 minutes at room temperature (Shimadzu, UV-1700).

$$I\ (\%) = 100\text{-}(OD_{sample}/OD_{control}) \times 100$$

Results and Discussion

Chemistry

The synthetic protocol of compounds 2a–2j was represented in Scheme 1 [29].



Scheme 1. The synthesis of the compounds 2a–2j. Reagents and conditions: (a) EtOH, 80 °C, 2 h; (b) EtOH, 80 °C.

The core structures of ten compounds with the 2-2-[(5-nitrothiophen-2-yl)methylene]hydrazinylthiazole nucleus were synthesized in this work. To make 2-[(5-nitrothiophen-2-yl)methylene]hydrazinecarbothio-amide (1), the first stage involved reacting 5-nitrothiophene-2-carbaldehyde and thiosemicarbazide for 2 hours at 80 oC. Compound 1 and 2-bromo-1-phenylethanone derivatives were then reacted in ethanol to yield 2-2-[(5-nitrothiophen-2-yl)methylene]hydrazinylthiazole derivatives (2a–2j), as shown in Scheme 1 [29]. The structures of produced compounds (2a–2j) were verified using 1H-NMR, 13C-NMR, and high-resolution mass spectroscopy (HRMS). Peaks were seen in the aromatic and aliphatic regions of the 1H-NMR spectra. The peaks in the 13C-NMR spectra were evident in the expected aromatic and aliphatic regions. The peaks in the mass spectra (ESI-MS) of the compounds [M+1] corresponded to their predicted chemical formula (2a–2j).

In this study, we synthesized ten compounds with the 2-{2-[(5-nitrothiophen-2-yl)methylene]hydrazinyl} thiazole nucleus in their core structures. The first stage included reacting 5-nitrothiophene-2-carbaldehyde and thiosemicarbazide for 2 hours at 80 °C to produce 2-[(5-nitrothiophen-2-yl)methylene]hydrazinecarbothio-amide (1) [32]. Then, as illustrated in Scheme 1, compound 1 and 2-bromo-1-phenylethanone derivatives were reacted in ethanol to provide the end products: 2-{2-[(5-nitrothiophen-2-yl)methylene]hydrazinyl}thiazole derivatives (2a–2j). All of the generated substances were characterized using analytical and spectroscopic data. Compound ^{1}H-NMR spectra revealed doublets at δ 7.45–8.34 ppm for thiophene protons. The thiazole proton was detected as a singlet peak at δ 7.20–8.27 ppm. Azomethine proton (H-C=N) was detected as a singlet peak at δ 7.42–8.47 ppm. A broad singlet signal at δ 11.83–12.99 ppm identified the acetamide N–H proton. The aromatic protons of the aromatic rings caused the appearance of a pair of singlets, doublets, triplets, and/or multiplets at δ 6.84–8.10 ppm. Compound ^{13}C-NMR spectra revealed signals at δ 125.94–128.93 ppm for methylene proton (H-C=N) carbon and δ 103.18–178.59 ppm for aromatic. The calculated molecular weights of the target compounds (2a–2j) matched the M±1 peaks in the LC-MS/MS spectra.

Anticholinesterase Activity

To assess anticholinesterase activity, all ten compounds (2a-2j) were tested on the enzymes AChE and BuChE. In a previous study, blocking acetylcholinesterase (AChE) reduced cell proliferation in lung carcinoma cell lines [24-28]. It has also been observed that antiproliferation is associated with mitochondrial potential loss and apoptosis. Table 4 shows the inhibition percentages for the substances investigated at 80 µg/mL concentration. At the studied concentrations, chemicals 2e, 2f, 2g, 2h, and 2j did not inhibit AChE. The inhibition percentages for AChE for compounds 2a, 2b, 2c, 2d, and 2i ranged from 33.66 % to 47.96 %, with compounds 2b and 2d inhibiting the enzyme the most. On the BuChE enzyme, the inhibition percentages ranged from 13.03 % to 63.29 %. Compounds 2i and 2h inhibited BuChE the most, with 63.29 and 53.73 %, respectively, whereas compounds 2b and 2e inhibited it by more than 40 %.

Table 4. % AChE and BuChE enzyme inhibition percentages

Compounds	AChE%	BuChE%
2a	33.76±1.3	33.64±1.1
2b	45.71±1.5	41.08±1.5
2c	41.70±1.4	23.46±1.5
2d	47.96±1.0	23.09±1.2
2e	---	44.40±1.7
2f	---	13.96±1.6
2g	---	13.03±0.3
2h	---	53.73±1.3
2i	33.66±1.3	63.29±1.0
2j	---	---
Galantamin (IC_{50})	0.44±0.06	6.92±1.84

SAR for AChE and BChE inhibition

At the case of AChE inhibition, di-substitution of the phenyl ring with methoxy groups in the *ortho*-positions resulted in a significant drop-in inhibitory activity, as shown in compound 2i. Mono-substitution with a methyl group at the *para*-position of the phenyl ring reduced activity as well (compound 2a). The inhibitory action was boosted by adding to the phenyl ring highly electronegative species such as nitro (compound 2b) or cyano (compound 2d) group at the *para*-position.

In the case of BChE inhibition, naphthalene or pyridine resulted in a significant reduction in inhibitory activity, as shown in 2g and 2f, respectively. Mono-substitution with a methyl or cyano group at the *para*-position of the phenyl ring reduced activity as well (compound 2a and 2d, respectively). The highest inhibitory action was obtained by adding highly electronegative species on the phenyl ring, such as nitro group (compound 2b), fluoro group (compound 2e), and dimethoxy at *orto* positions (compound 2i).

Conclusions

2-{2-[(5-nitrothiophen-2-yl)methylene]hydrazinyl} thiazole derivatives (2a-2j) were synthesized and tested in vitro for their inhibitory effects on AChE and BuChE. The structures of the novel compounds were determined using spectroscopic methods such as ^{1}H-NMR, ^{13}C-NMR, and highresolution mass spectroscopy (HRMS). On AChE, the inhibition percentages ranged between 33.66%–47.96%, from where 4-cyanophenyl-substituted compound 2d was the most efficient AChE inhibitor (47.96 ±1.07%). On BuChE, the inhibition percentages ranged between 13.03%–63.29%, from where 2,5-dimethoxyphenyl-substituted compound 2i was the most effective BuChE inhibitor (63.29 ± 1.01%).

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Conflicts of Interest

The authors declare no conflict of interest.

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