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Research Article

Synthesis of novel thiazole derivatives against Alzheimer's disease and investigation of their cholinesterase inhibition and antioxidant properties

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ABSTRACT

In this study, 7 new thiazole derivatives were synthesized. Cholinesterase inhibition and antioxidant properties were examined to understand whether the synthesized compounds were anti-Alzheimer drug candidates. The antioxidant properties of these newly synthesized thiazole derivatives and their enzyme inhibition values for acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) were evaluated. According to the data, these substances inhibited the AChE and BuChE enzymes at deficient levels. Compound **2e** showed the highest AChE inhibition effect with a value of 20.32 ± 0.005% at 50 µM concentration. Although high activity against BuChE was not observed, compound **2d** was an exception with a value of 32.54 ± 0.021% at 50 µM concentration. Values that were comparable to the reference medication gallic acid were found when the antioxidant qualities were investigated using DPPH and ferric ion chelation studies. Ferrous ion-chelating and DPPH radical scavenging consistent with all of the previously reported information, the compounds' antioxidant properties were very high, despite their modest cholinesterase enzyme inhibitory capabilities. In terms of AChE inhibition and antioxidant activity, respectively, compounds **2e** and **2f** were shown to be promising prospective agents among these compounds'.

Keywords: AChE, BuChE, Antioxidant, Thiazole

1. INTRODUCTION

Alzheimer's Disease (AD) is one of the most common diseases among older people. It was first reported in 1906, and information about the progression of the disease was given [1]. AD is the leading cause of dementia worldwide. Characterized by neurodegenerative disorders of the brain, the disease has a prevalence of more than 10,000 per million people and continues to increase with the increasing elderly population. Globally, dementia is causing a major public health crisis. AD accounts for 70% of all dementia cases. It is a disease characterized by neuropathological events such as neuronal cell loss, amyloid β peptide (Aβ) accumulation in extracellular plaques, and intracellular tau (τ) protein accumulation [2]. The amyloid cascade theory of Aβ deposition is a widely recognised hypothesis that posits a causal relationship between the accumulation of Aβ peptides in the brain and the development of AD [3]. There is no problem with the production and

metabolism of Aβ in healthy individuals. However, in the case of AD, this process is disrupted and Aβ accumulates between neuronal cells. Consequently, due to this accumulation, disturbances in nerve conduction, decline in cognitive activities and memory loss occur [4]. The dementia formation pathway is shown in Figure 1.

The etiopathogenesis of the disease is not entirely explained by the Aβ hypothesis. In this case, neurodegeneration is caused by the τ protein, which manifests as a secondary pathogenic event. In neuronal cells, Aβ causes τ protein changes. This protein is primarily responsible for maintaining microtubule stability, undergoes hyperphosphorylation in the case of AD, and hyperphosphorylated protein structures accumulate in neuronal regions and lose their functions. As a result, disruptions in axon transmission are observed [5]. In addition to the pathophysiological events mentioned above, AD is also related to the dysregulation in the cholinergic system. The cholinergic hypothesis has been put forward to explain these dysregulations. This hypothesis is one of the most basic approaches accepted for the treatment of AD [6]. Acetylcholine (ACh) is found in many regions of the brain and plays a role in many events such as learning, memory, stress management, and regulation of cognitive functions [7]. ACh is found in important brain regions

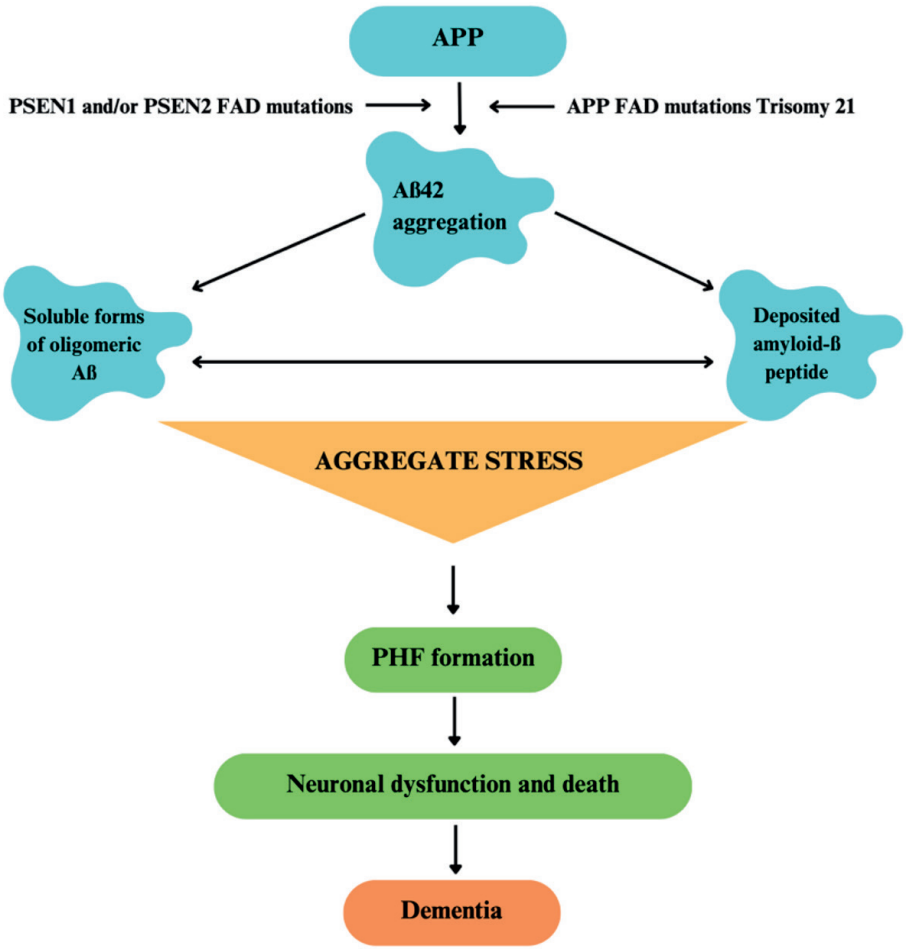


Figure 1. Mutations in presenilin 1 (PSEN1), presenilin-2 and amyloid precursor protein (APP) genes are the main genetic causes of AD. The concept of Aβ derived soluble ligands or soluble toxic oligomers has been proposed to explain the neurotoxicity of Aβ peptide. We would prefer to refer to this as "aggregate stress" in order to highlight potential mechanisms that may result in the formation of paired helical filaments (PHFs) of τ protein aggregates, Aβ aggregation, and ultimately neuronal loss because the mechanisms of action of these species are not fully understood [3].

such as the basal forebrain, cerebral neocortex and hippocampus. It is degraded by the enzyme acetylcholinesterase (AChE) to choline and acetate [8]. In order to normalize the decreasing ACh levels in the later stages of the disease, the basic approach followed in the treatment is to suppress the enzymes AChE, which breaks down the neurotransmitter, and butyrylcholinesterase (BuChE), which is activated when AChE cannot fulfill its function [9].

In this study, thiazole derivatives were synthesized and their structure was clarified using HRMS, ^{1}H-NMR, and ^{13}C-NMR. Ferrous ion-chelating and DPPH radical scavenging methods were used to evaluate the compounds' antioxidant qualities. Additionally, this study aimed at these derivatives' ability to inhibit AChE and BuChE *in vitro*.

2. MATERIALS AND METHODS

2.1. Chemistry

Synthesis of (*E*)-2-((1-methyl-1*H*-pyrrol-2-yl) methylene)hydrazine-1-carbothioamide (1): Ethanol solvent was employed to dissolve 1-methyl-1*H*-pyrrole-2-carbaldehyde and thiosemicarbazide. Three hours were spent refluxing the resultant mixture. After the reaction was finished, the resultant solution was placed in a bath of ice to chill. Filtration was then used to isolate the resultant precipitate.

Synthesis of Target Compounds (2a-2g): Ethanol was utilized to dissolve Compound 1 and a derivative of 2-bromoacetophenone. Four hours were spent refluxing the resultant mixture. Following the completion of the reaction, the mixture was transferred to an ice bath to cool. The precipitate that is produced was isolated by filtration. Subsequently, the precipitate was dried and crystallized by ethanol.

4-(4-Cyanophenyl)-2-(2-((1-methylpyrrol-2-yl) methylene)hydrazineyl)thiazole (2a): Yield: 78 %, M.P.= 195.0 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ: 3.86 (3H, s, CH_3), 6.08 (1H, s, Aromatic CH), 6.42 (1H, s, Aromatic CH), 6.95 (1H, s, Aromatic CH), 7.59 (1H, s, Aromatic CH), 7.86 (2H, d, *J*=7.84 Hz, 1,4-disubstituted benzene), 7.99 (1H, s, CH=N), 8.02 (2H, d, *J*=7.40 Hz, 1,4-disubstituted benzene), 11.91 (1H, s, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ= 36.92 (CH_3), 107.40, 108.52, 109.97, 114.71, 119.47, 126.56, 127.39, 128.31, 133.13, 136.26, 139.28, 149.22, 169.20. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{16}H_{13}N_5S$: 308.0964; found: 308.0966.

4-([1,1'-biphenyl]-4-yl)-2-(2-((1-methylpyrrol-2-yl)methylene)hydrazineyl)thiazole (2b): Yield: 79 %, M.P.= 220.7 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ: 3.88 (3H, s, CH_3), 6.09 (1H, s, Aromatic CH), 6.42 (1H, s, Aromatic CH), 6.95 (1H, s, Aromatic CH), 7.34 (1H, s, Aromatic CH), 7.38 (1H, d, *J*=6.92 Hz, Aromatic CH), 7.48 (2H, t, *J*=6.72 Hz, Aromatic CH), 7.72 (4H, d, *J*=7.48 Hz, Aromatic CH), 7.94 (2H, d, *J*=7.64 Hz, Aromatic CH), 7.99 (1H, s, CH=N), 11.84 (1H, s, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ= 36.93 (CH_3), 103.07, 108.48, 114.49, 119.28, 120.70, 126.53, 126.93, 127.28, 127.52, 127.92, 128.17, 129.43, 135.92, 139.44, 140.14, 168.95. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{21}H_{18}N_4S$: 359.1325; found: 359.1336.

4-(3,4-Dichlorophenyl)-2-(2-((1-methylpyrrol-2-yl)methylene)hydrazineyl)thiazole (2c): Yield: 69 %, M.P.= 198.5 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ: 3.86 (3H, s, CH_3), 6.09 (1H, s, Aromatic CH), 6.42 (1H, s, Aromatic CH), 6.95 (1H, s, Aromatic CH), 7.50 (1H, s, Aromatic CH), 7.66 (1H, d, *J*=8.32 Hz, Aromatic CH), 7.83 (1H, d, *J*=8.32 Hz, Aromatic CH), 7.97 (1H, s, CH=N), 8.07 (1H, s, Aromatic CH), 11.86 (1H, s, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ= 36.94 (CH_3), 107.55, 108.15, 114.20, 116.12, 119.89, 126.06, 127.58, 128.28, 129.25, 131.31, 132.08, 137.30, 142.72, 169.33. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{15}H_{12}N_4SCl_2$: 351.0232; found: 351.0236.

4-(2,4-Difluorophenyl)-2-(2-((1-methylpyrrol-2-yl)methylene)hydrazineyl)thiazole (2d): Yield: 76 %, M.P.= 162.4 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ: 3.86 (3H, s, CH_3), 6.08 (1H, s, Aromatic CH), 6.42 (1H, s, Aromatic CH), 6.95 (1H, s, Aromatic CH), 7.16-7.20 (2H, m, Aromatic CH), 7.32-7.37 (1H, m, Aromatic CH), 8.01-8.05 (2H, m, Aromatic CH, CH=N). ^{13}C-NMR (100 MHz, DMSO-d_6): δ= 36.92 (CH_3), 104.99, 105.33, 107.66, 108.51, 112.20, 112.36, 114.67, 122.36, 127.41, 128.29, 130.84, 136.25, 144.66, 168.33. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{15}H_{12}N_4F_2S$: 319.0824; found: 319.0825.

4-(2,4-Dimethoxyphenyl)-2-(2-((1-methylpyrrol-2-yl)methylene)hydrazineyl)thiazole (2e): Yield: 69 %, M.P.= 219.5 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ: 3.81 (3H, s, CH_3), 3.87 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 6.10 (1H, s, Aromatic CH), 6.46 (1H, s, Aromatic CH), 6.62 (1H, d, *J*=8.68 Hz, Aromatic CH), 6.67 (1H, s, Aromatic CH), 6.98 (1H, s, Aromatic CH), 7.16 (1H, s, Aromatic CH), 7.84 (1H, d, *J*=8.56 Hz, Aromatic CH), 8.05 (1H, s, CH=N). ^{13}C-NMR (100 MHz, DMSO-d_6): δ= 36.89 (CH_3), 55.77, 56.03, 99.13, 100.39, 105.17, 105.53, 106.90, 108.60, 114.89, 118.83, 121.72, 127.33, 128.45, 130.33, 158.20, 160.57. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{17}H_{18}N_4O_2S$: 343.1223; found: 343.1228.

4-(2,4-Dichlorophenyl)-2-(2-((1-methylpyrrol-2-yl)methylene)hydrazineyl)thiazole (2f): Yield: 70 %, M.P.= 158.9 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ: 3.86 (3H, s, CH_3), 6.09 (1H, s, Aromatic CH), 6.46 (1H, s, Aromatic CH), 6.97 (1H, s, Aromatic CH), 7.32 (1H, s, Aromatic CH), 7.51 (1H, d, *J*=8.28 Hz, Aromatic CH), 7.70 (1H, s, Aromatic CH), 7.84-7.86 (1H, m, CH=N), 8.09-8.13 (1H, m, Aromatic CH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ= 36.94 (CH_3), 108.65, 109.10, 114.98, 115.17, 127.22, 127.96, 128.63, 130.14, 132.38, 132.88, 133.36, 137.15, 137.54, 168.01. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{15}H_{12}N_4SCl_2$: 351.0232; found: 351.0238.

4-(3-Nitrophenyl)-2-(2-((1-methylpyrrol-2-yl) methylene)hydrazineyl)thiazole (2g): Yield: 71 %, M.P.= 200.6 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ: 3.87 (3H, s, CH_3), 6.08 (1H, s, Aromatic CH), 6.43 (1H, s, Aromatic CH), 6.95 (1H, s, Aromatic CH), 7.60 (1H, s, Aromatic CH), 7.70 (1H, t, *J*=7.84 Hz, Aromatic CH), 7.98 (1H, s, CH=N), 8.12 (1H, d, *J*=8.00 Hz, Aromatic CH), 8.29 (1H, d, *J*=7.44 Hz, Aromatic CH), 8.67 (1H, s, Aromatic CH), 11.95 (1H, s, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ= 36.94 (CH_3), 106.22, 108.51, 114.75, 120.36, 122.41, 127.39, 128.32, 130.66, 132.01, 136.20, 136.72, 148.56, 148.74, 169.24. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{15}H_{13}N_5O_2S$: 328.0863; found: 328.0867.

2.2. Assay for inhibition of cholinesterase enzyme

Inhibition of cholinesterase enzyme assay was performed as mentioned in previous studies [10,11].

2.3. Antioxidant Activity

2.3.1. Ferrous ion-chelating effect

Ferrous ion-chelating effect activity was performed as mentioned in previous studies [10,11].

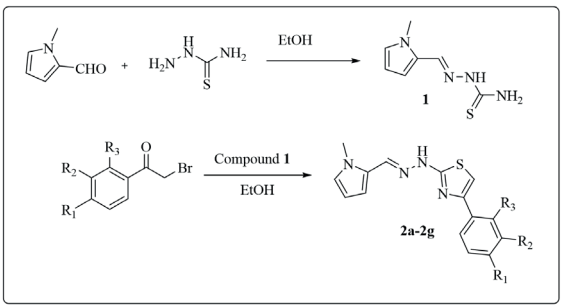
2.3.2. DPPH radical scavenging activity

DPPH radical scavenging activity was performed as mentioned in previous studies [10,11].

3. RESULTS AND DISCUSSION

3.1. Chemistry

New thiazole compounds **2a-g** were created for this investigation, as indicated by Scheme 1. It took two stages to synthesize the chemicals. The first step was a reaction between thiosemicarbazide and 1-methyl-1*H*-pyrrole-2-carbaldehyde to create the thiosemicarbazone chemical. In the second stage, chemicals derived from 2-bromoacetophenone interacted with the thiosemicarbazone product from the first step to produce thiazole derivatives.



Comp.	R_1	R_2	R_3
2a	-CN	-H	-H
2b	-C_6H_5	-H	-H
2c	-Cl	-Cl	-H
2d	-F	-H	-F
2e	-OCH_3	-H	-OCH_3
2f	-Cl	-H	-Cl
2g	-H	-NO_2	-H

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

3.2. Cholinesterase Enzymes Inhibition Assay

The results of the *in vitro* inhibition experiments of the obtained thiazole derivative compounds were examined for AChE and BuChE activity. The results obtained for galantamine as the reference drug and all obtained compounds **(2a-2g)** are shown in Table 1. When Table 1 was examined, it was observed that the compounds showed low activity. Among the compounds, the compound **(2e)** with a methoxy group in the *orto* and *para* positions of the phenyl ring showed maximum activity in opposition to AChE by inhibiting 20.32 ± 0.005% at 50 µM concentration. The compound **(2d)** with a fluorine group in the *orto* and *para* positions of the phenyl ring showed maximum level of activity directed toward BuChE by inhibiting 32.54 ± 0.021% at 50 µM concentration.

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

Comp.	AChE	BuChE
2a	NA	7.22 ± 0.019
2b	18.16 ± 0.009	NA*
2c	NA	NA*
2d	11.44 ± 0.007	32.54 ± 0.021
2e	20.32 ± 0.005	NA*
2f	19.06 ± 0.004	NA*
2g	10.74 ± 0.006	23.56 ± 0.016
Gal HBr	97.89 ± 0.01	62.48 ± 0.01

* NA= non-active

3.3. Antioxidant Activity

Antioxidant properties of target compounds were determined by ferric ion chelation and DPPH methods using gallic acid as standard. The obtained values are given in Table 2. When Table 2 is examined, it is seen that the activities of compounds **2e** and **2f** are comparable to the reference drug. Their IC_{50} values are 30.02 ± 0.003 µM and 32.09 ± 0.006 µM, respectively. It is seen that especially compound **2f** shows an activity close to the reference drug gallic acid.

4. CONCLUSION

Seven new thiazole derivatives were synthesized as potential Alzheimer's drugs and their AChE, BuChE inhibition degrees and antioxidant properties were investigated. It was observed that the synthesized compounds did not show sufficient inhibition against AChE and BuChE when compared with the reference drug. The compound with the highest activity against AChE was compound **2e** with a value of 20.32 ± 0.005% at 50 µM concentration. In terms of the antioxidant moiety, compound **2f** showed antioxidant activity similar to the reference drug with an IC_{50} value of 32.09 ± 0.006 µM. According to the obtained results, the molecular docking study was not performed due to the weak activity of the compounds. However, all synthesized compounds can be a reference for new thiazole derivatives to be synthesized for Alzheimer's treatment in the future.

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)
2a	NA	NA	> 60 µM
2b	NA	NA	> 60 µM
2c	NA	NA	> 60 µM
2d	11.16 ± 0.007	NA	> 60 µM
2e	68.74 ± 0.003	NA	30.02 ± 0.003
2f	63.18 ± 0.008	NA	**32.09 ± 0.006**
2g	75.64 ± 0.026	NA	24.76 ± 0.008
Gallic Acid	70.29 ± 0.005	-	31.13 ± 0.008
Rutin 50 µM	-	13.21 ± 0.007	-
BHT 50 µM	-	7.06 ± 0.009	-

Ethical approval

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

Author contribution

Conceptualization, Y.Ö.; Supervision, Y.Ö.; Methodology, A.K., T.E. and U.A.Ç.; Data Collection and/or Processing, A.K., T.E. and U.A.Ç.; Analysis and/or Interpretation, U.A.Ç.; Investigation, A.K.; Writing—original draft preparation, Y.Ö., A.K., T.E. and U.A.Ç.; Critical Reviews, Y.Ö. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declared that there is no conflict of interest.

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