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Design, Synthesis and Evaluation of Pyrrol-thiazole Derivatives as AChE and BuChE Inhibitory and Antioxidant Activities

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ABSTRACT

Thiazole rings are one of the most frequently used heterocyclic moieties and are found in a wide variety of biologically active chemicals. In this research project, we report the synthesis and biological activities of some new thiazole derivatives (2a-2c) as potent anti-Alzheimer's agents. These final compounds' structures were characterized by spectral (^{1}H NMR, ^{13}C NMR, and MS spectra) analyses. The highest inhibitory activity against AChE was demonstrated by compound 2c (23.73 ± 0.018 %) with chloro substitution at the *meta* and *para* positions of the phenyl ring, while the highest inhibitory activity against BuChE was produced by compound 2a (28.87± 0.003 %) with cyano substitution at the *ƒ* position of the phenyl ring. Ferrous ion-chelating and DPPH techniques were also used to assess the compounds' antioxidant properties. Compound 2a showed antioxidant effect according to the DPPH method with an IC_{50} value of 27.18 ± 0.009 µM.

Keywords: Thiazole, Imidazole, AChE, BuChE, Antioxidant.

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Introduction

Alzheimer's disease (AD) is a neurological condition that progresses and is characterized by a permanent loss of memory. Although the etiology of the disease is not fully known, many factors seem to play a role. These; decline in acetylcholine (ACh) levels (cholinergic theory), the β-amyloid peptide (Aβ) deposition, hyperphosphorylated tau-protein deposition and the increase in oxidative stress [1]. According to the cholinergic theory, the death of cholinergic neurons in AD causes a deficiency of acetylcholine (ACh) in particular brain regions, which causes severe memory impairments and irreversible cognitive function impairment [2, 3]. The two main cholinesterase isoenzymes that break down ACh and stop its activities are acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). AChE hydrolyzes the bulk of ACh in the brain under physiologically normal conditions. As a result, the most popular methods for treating AD have focused on raising acetylcholine levels by decreasing the activity of the AChE enzyme [4-7]. AChE's sister enzyme, BChE, has recently drawn more attention due to a potential connection to Alzheimer's disease [8]. Inhibiting BuChE is a promising method for treating advanced AD, according to numerous research [9].

The European and American regulatory authorities have now approved tacrine, memantine, galantamine, and donepezil as four commonly used medications for the treatment of Alzheimer's disease (Figure 1) [10].

Thiazole nuclei are one of the most frequently used heterocyclic moieties and are found in a wide variety of biologically active chemicals [11, 12]. Thiazole is an essential structural component of many commercially marketed medications, including as bacitracin, penciling, and ritonavir [8].

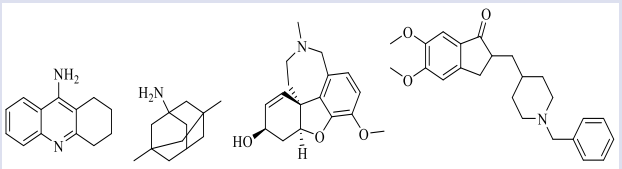


Figure 1. Chemical structure of drugs used for Alzheimer's disease

One of the first and most important pathophysiologies of AD is oxidative stress, according to several researchers. Oxidative stress also plays a significant part in the development of AD. By the deterioration of biological molecules like DNA, proteins, and lipids, it contributes to neurodegeneration. Antioxidant-active substances therefore appear to be helpful for the treatment of AD [13].

In this study, we synthesized new pyrrol-thiazole derivatives and their structures were elucidated by ^{1}H-NMR, ^{13}C-NMR and HRMS. With the purpose of treating Alzheimer's disease, the AChE and BuChE inhibitory activities of these recently synthesized compounds was examined. In addition, the antioxidant effects of the compounds were evaluated by Ferrous ion-chelating and DPPH methods.

Materials and Methods

Chemistry

Synthesis of 2-((1H-pyrrol-2-yl)methylene)hydrazine-1-carbothioamide (1): 1H-pyrrol-2-carbaldehyde and thiosemicarbazide are dissolved in ethanol. After that, it undergoes 3 hours of reflux stirring. The mixture is chilled in an ice bath after the conclusion of the reaction. It is filtered to remove the precipitated product [18].

Synthesis of Target Compounds (2a-c): In ethanol, 2-((1H-pyrrol-2-yl)methylene)hydrazine-1-carbothioamide (1) and its derivative, 2-bromoacetophenone, are dissolved. After that, it is stirred for 4 hours of reflux. The mixture is chilled in an ice bath after the conclusion of the reaction. It is filtered to remove the precipitated product. From ethanol, it is crystallized and dried [18].

4-(4-Cyanophenyl)-2-(2-((1H-pyrrol-2-yl)methylene) hydrazineyl)thiazole (2a):

Yield: 75 %, M.P.= 194.7 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ: 7.74-7.75 (1H, m, Aromatic CH), 7.81-7.82 (2H, m, Aromatik CH), 7.91 (1H, s, Aromatic CH), 8.11-8.15 (5H, m, Aromatic CH, CH=N), 13.22 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ= 115.40, 116.78 (CN), 123.65, 125.65, 126.40, 127.28, 127.90, 128.78, 131.05, 133.28, 138.78, 139.41 (CH=N). HRMS (m/z): $[M+H]^+$ calcd for $C_{15}H_{11}N_5S$: 294.0808; found: 294.0814.

4-([1,1'-biphenyl]-4-yl)-2-(2-((1*H*-pyrrol-2-yl)methylene) hydrazineyl)thiazole (2b):

Yield: 78 %, M.P.= 179.2 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ: 7.38-7.41 (1H, m, Aromatic CH), 7.46-7.51 (3H, m, Aromatic CH), 7.59 (1H, s, Aromatic CH), 7.72-7.77 (5H, m, Aromatic CH), 7.81-7.82 (1H, m, Aromatic CH), 7.96-7.98 (2H, m, Aromatic CH), 8.14 (1H, s, CH=N), 13.17 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ= 123.56, 123.76, 125.69, 126.09, 126.56, 127.21, 127.37, 127.73, 128.04, 128.89, 130.25, 131.09, 132.06, 135.33, 141.62 (CH=N), 148.20. HRMS (m/z): $[M+H]^+$ calcd for $C_{20}H_{16}N_4S$: 345.1168; found: 345.1181.

4-(3,4-Dichlorophenyl)-2-(2-((1*H*-pyrrol-2-yl)methylene) hydrazineyl)thiazole (2c):

Yield: 74 %, M.P.= >350 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ: 7.57-7.62 (3H, m, Aromatic CH), 7.74 (1H, s, Aromatic CH), 7.80-7.84 (3H, m, Aromatic CH), 8.14 (1H, s, CH=N), 13.11 (1H, s, NH).^{13}C-NMR (75 MHz, DMSO-d_6): δ= 123.06, 127.14, 127.43, 127.59, 128.93, 130.38, 130.81, 131.96, 133.49, 135.07, 138.10, 139.84 (CH=N), 144.32, 147.24. HRMS (m/z): $[M+H]^+$ calcd for $C_{14}H_{10}N_4SCl_2$: 337.0076; found: 337.0091.

Cholinesterase Enzymes Inhibition Assay

The ability of each of the initially produced substances to obstruct AChE and BChE enzymes was evaluated. Using a modified version of Ellman's spectrophotometric technique, the compounds' AChE and BChE inhibitory activities were assessed [14].

Antioxidant Activity

Ferrous ion-chelating effect

By using the Chua et al. approach, the ferrous ion-chelating effect of each extract and the reference was calculated (2008). In a nutshell, different concentrations of the ethanol (80%)-dissolved extracts were incubated with a 200 L solution of 2 mM $FeCl_2$. 800 L of 5 mM ferrozine (Sigma, St. Louis, MO, USA) was added to the mixture to start the reaction, which was then given 10 minutes to stand at room temperature. With ethanol (80%) serving as a blank, the reaction mixture's absorbance was measured at 562 nm using a spectrophotometer (Varioskan Fast, Thermo Scientific, USA). The following formula was used to determine the ratio of inhibition of ferrozine-Fe^{2+} complex formation:

$I\% = [(A_{blank}-A_{sample})/A_{blank}]x100$

where Asample is the absorbance of the extracts/reference, and Ablank is the absorbance of the control reaction (consisting solely of ferrozine and $FeCl_2$). In this test, rutin served as the reference, while butylated hydroxytoluene (BHT) was purchased from Sigma Aldrich (USA). The findings of the analyses were presented as average values with standard error of the mean (S.E.M.) [15, 16].

DPPH radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was screened using Blois' UV technique. The reference molecule (gallic acid) and the compound concentrations of 40 M and 100 M were created using this approach in 20 L of methanol. Each solution received 180 L of a 0.15 mM DPPH solution in methanol. The amount of residual DPPH was measured at 520 nm following a 20-minute incubation at room temperature (Varioskan Flash, Thermo Scientific, USA). Using the following formula, the % DPPH radical scavenging activity was determined;

$I\% = [(Acontrol - Asample)/Acontrol] \times 100$,

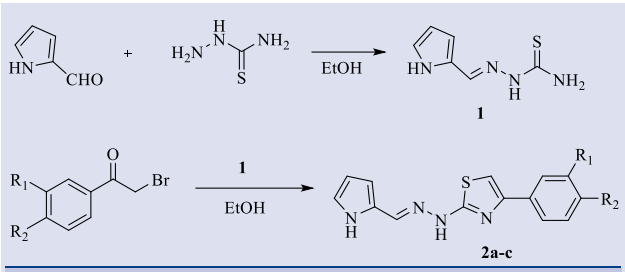
Acontrol: absorbance of the control reaction

Asample: absorbance of the extracts/reference

The findings of the experiments were reported as average values with S.E.M. (standard error mean) [17]. The experiments were carried out in triplicate.

Results and Discussion

In this study, three new compounds with pyrrol-thiazole structure were synthesized. The synthetic strategy for target compounds 2a-c was depicted in Figure 2. The synthesis of the planned compounds was carried out in two steps. First of all, in the first step, 1H-pyrrol-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.



Comp.	R1	R2
2a	-H	-CN
2b	-H	-Phenyl
2c	-Cl	-Cl

Figure 2. Synthesis pathway of compounds 2a-c

Using ^{1}H-NMR, ^{13}C-NMR, and HRMS, the structures of all compounds were clarified. The ^{1}H-NMR spectrum of final compounds show singlet in the range of 13.11-13.22 ppm, which was attributed to the NH group. Aromatic protons appeared as multiplets in the range of 7.57-8.15 ppm. The predicted chemical changes were followed by the appearance of the ^{13}C-NMR signals. The bulk of the produced compounds included M+1 peaks.

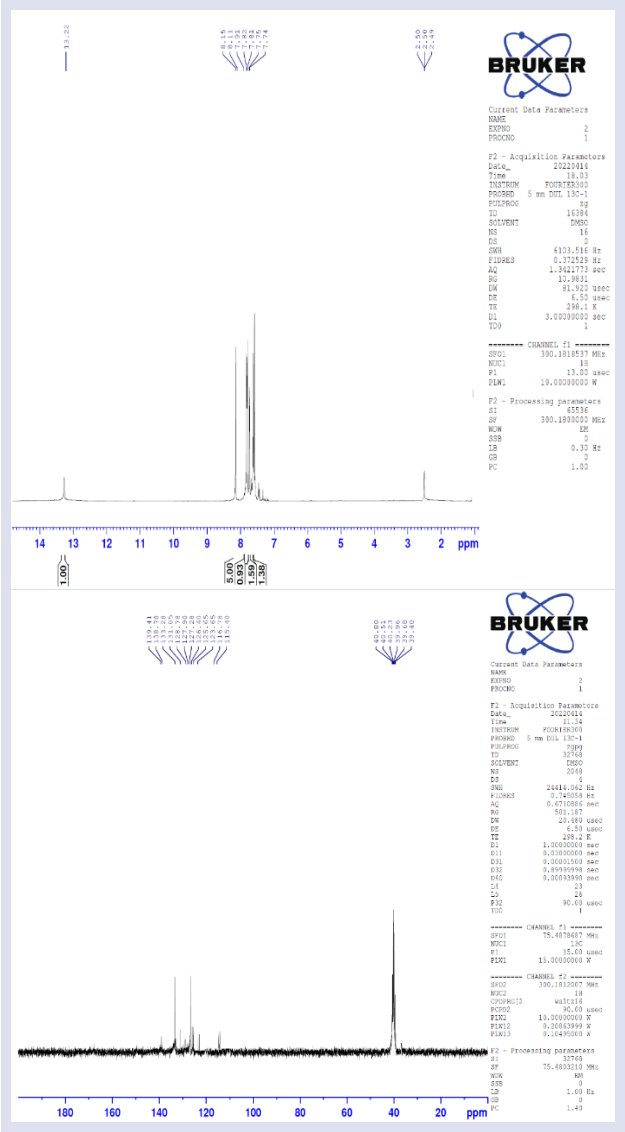


Figure 3. ^{1}H-NMR and ^{13}C-NMR spectrum of compound 2a

The Ellman technique was used to assess the inhibitory effects of compounds 2a–c against AChE and BuChE *in vitro*. Galantamine was used as a reference drug. The % inhibition values of compounds and reference drug at 50 µm concentration are shown in Table 1. The results showed that all substances had an inhibitory effect on the AChE enzyme, with a range of 7.22-23.73 %. According to the results, the inhibitory effect of all compounds against the BuChE enzyme ranged between 0.25-28.87 %. Compound 2a having cyano group at *para* position on phenyl ring were found to be the most active compound for BuChE among the series. Compound 2c having dichloro group at *para* and *meta* position on phenyl ring were found to be the most active for AChE among the series. Increased inhibition of compound 2c may be caused by the aryl ring's electron-withdrawing -Cl moiety.

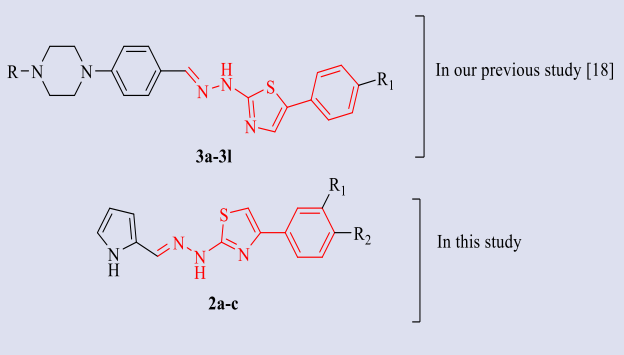


Figure 4. Design of synthesized compounds containing thiazole structure

In our previous study, compounds containing thiazole rings were synthesized and their effects against AChE and BuChE enzymes were examined [18]. As a result of the study, promising values were obtained. In this study, a smaller pyrrole ring was used instead of the piperazine-phenyl ring. However, as a result of the study, it was determined that the activity decreased with the introduction of the pyrrole ring.

Table 1. Inhibitory activity results of synthesized derivatives (2a-c) against AChE and BuChE enzymes

Comp.	% Inhibition (AChE) 50 µM	% Inhibition (BuChE) 50 µM
2a	7.22 ± 0.006	28.87 ± 0.003
2b	17.01 ± 0.014	0.25 ± 0.001
2c	23.73 ± 0.018	6.26 ± 0.008
Gal HBr	97.89 ± 0.01	62.48 ± 0.01

Table 2 shows the antioxidative activity results for produced compounds from Ion Chelating and DPPH methods. Among the compounds, compound 2a was found to be more effective than the reference drug gallic acid, with a value of 71.90 ± 0.004 µM. Compound 2b, on the other hand, was found to have an antioxidant effect close to the reference drug.

Table 2. DPPH and Ion Chelating results of the synthesized compounds

Comp.	IC_{50} µM (DPPP)	DPPH	ION CHELATING
2a	27.18 ± 0.009	71.90 ± 0.004	4.26 ± 0.001
2b	31.91 ± 0.011	64.69 ± 0.011	10.20 ± 0.018
2c	> 60 µM	NA	NA
RUTIN 50 µM	-	-	13.21 ± 0.007
RUTIN 100 µM	-	-	28.14± 0.011
BHT 50 µM	-	-	2.57 ± 0.004
BHT 50 µM	-	-	7.06 ± 0.009
GALLIC ACID	-	70.29 ± 0.005	-

Conclusion

In this study, three new compounds with pyrrole-thiazole structure were synthesized and their structures were elucidated by spectroscopic methods. The activities of the compounds against AchE and BuChE enzymes were evaluated. In addition, since oxidative stress is known to be effective in Alzheimer's disease, the antioxidant effects of the compounds were evaluated with two different methods. When the activity results were evaluated, it was found that compound 2a was the most effective compound against both BuChE enzyme in the series. In addition, it is seen that this compound shows more effective antioxidant activity than the reference drug according to the DPPH method.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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