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Research Article

Synthesis of new 1,3,4-thiadiazole derivatives, investigation of their AChE effects

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ABSTRACT

Alzheimer's disease (AD) is the most common cause of dementia and one of the most prevalent neurodegenerative diseases. It begins with mild cognitive impairment and progressively affects all aspects of the patient's life functions. Alzheimer's disease is more commonly seen in the elderly and has a progressive incidence. With the global increase in the elderly population, Alzheimer's disease poses a significant threat. Additionally, current medications do not prevent AD, highlighting the need for new drug molecules to be used in AD treatment. Although 1,3,4-thiadiazoles have many biological activities such as anticancer and antiviral, their activities on acetylcholinesterase (AChE) are also being investigated. For this purpose, three new 1,3,4-thiadiazole compounds were synthesized in this study. The structure determinations of these compounds were carried out using ^{1}H-NMR and HRMS spectrophotometric methods. Activity studies were conducted *in vitro* using the modified Ellman method. As a result of the activity tests, compound **3b** showed the closest effect to donepezil with an IC_{50}= 0.096±0.004 µM.

Keywords: Acetylcholinesterase, Alzheimer's Disease, Molecular docking, 1,3,4-Thiadiazole

1. INTRODUCTION

Alzheimer's disease (AD) is an irreversible neurodegenerative disease that starts with memory loss and affects cognitive skills [1-2]. It is the most common cause of dementia and is an age-related disease [3-4]. Given the aging of the world's population, AD has an increasing prevalence [5].

Among the pathologic causes of AD is the cholinergic hypothesis [6-7]. Behavioral and cognitive impairment in AD is due to low acetylcholine levels in different regions of the Central Nervous System (CNS) [8]. Acetylcholinesterase (AChE) inhibitors are targeted to increase cholinergic levels in the brain by inhibiting the biological activity of AChE [9]. AChE inhibitors have therefore been one of the key strategies in developing anti-AD drugs [3-10].

Thiadiazoles are five-membered heterocyclic rings containing hydrogen, sulfur, carbon and nitrogen. It has an important position in heterocyclic chemistry because it contains both electron-withdrawing (S) and electron-donating (-C=N) groups [11]. The presence of a sulfur atom in the thiadiazole increases the compound's liposolubility and, consequently,

its pharmacokinetics [12]. This ring system exists in nature in four isomeric forms: 1,2,5-thiadiazole, 1,3,4-thiadiazole, 1,2,3-thiadiazole, 1,2,4-thiadiazole [13]. 1,3,4-Thiadiazoles show many biological activities such as antiviral, antibacterial, anticonvulsant, anti-inflammatory, and antioxidant [14-16]. They also exhibit antitumor activity [12]. In addition to showing many biological activities, they also have distinctive physicochemical properties, which is the reason why they are preferred in new drug production studies [17].

In this study, 3 compounds containing 1,3,4-thiadiazole core were synthesized. The structures of these compounds were determined and their AChE inhibitory effects were investigated.

2. MATERIALS AND METHODS

2.1. Chemistry

In the synthesis studies described, the control of the reactions was carried out with TLC applications. Samples were taken from the test flasks at certain time intervals. Then, the ethanol solutions of the starting materials used in the syntheses were applied to the silica gel F_{60}-coated aluminum plates previously saturated with appropriate solvent mixtures. Entrainment in the mobile phases was ensured and ultraviolet light (254 nm and 366 nm) was used to detect the stains. Petroleum ether: Ethyl acetate (4 : 1) was used as the appropriate mobile phase for the control of these syntheses. The melting points (M.p) of the synthesized compounds were determined by filling ½ cm of the powdered substance into capillary tubes with one end open. Electrothermal melting point determination device was used. The values found were recorded and not corrected. Spectroscopic methods were used for structure determination of the synthesized compounds. ^{1}H-NMR was performed using Bruker DPX 300 FT-NMR spectrometer. LCMS-IT-TOF (Shimadzu, Kyoto, Japan) was used for High Resolution Mass Spectra (HRMS).

2.1.1. General synthesis of N-substituted-hydrazinecarbothioamides (1a-c)

The hydrazine hydrate (0.04 mol) was reacted with an isothiocyanate derivative (0.02 mol) in ethanol at 80°C for 4 hours under reflux. After the reaction, the precipitated product was filtered and washed with ethanol.

2.1.2. General synthesis of 5-(substituted-amino)-1,3,4-thiadiazole-2-thiols (2a-c)

Compound 1a-c was reacted in ethanol with carbon disulfide (0.019 mol) and sodium hydroxide (0.019 mol) under reflux for 8 hours. After the reaction was completed, the solution was cooled and acidified to pH 4-5 with hydrochloric acid, then crystallized from ethanol.

2.1.3. Synthesis of target compounds (3a-c)

Compounds **2**(**a-c**) and 2-bromo-4′-(trifluoromethyl) acetophenone (0.0007 mol) were reacted in acetone with potassium carbonate (0.0007 mol). After the reaction, which occurred at room temperature, the mixture was filtered and washed with ethanol.

*2-((5-(Phenylamino)-1,3,4-thiadiazol-2-yl)thio)-1-(4-trifluoromethyl)phenylethan-1-one (**3a**)*

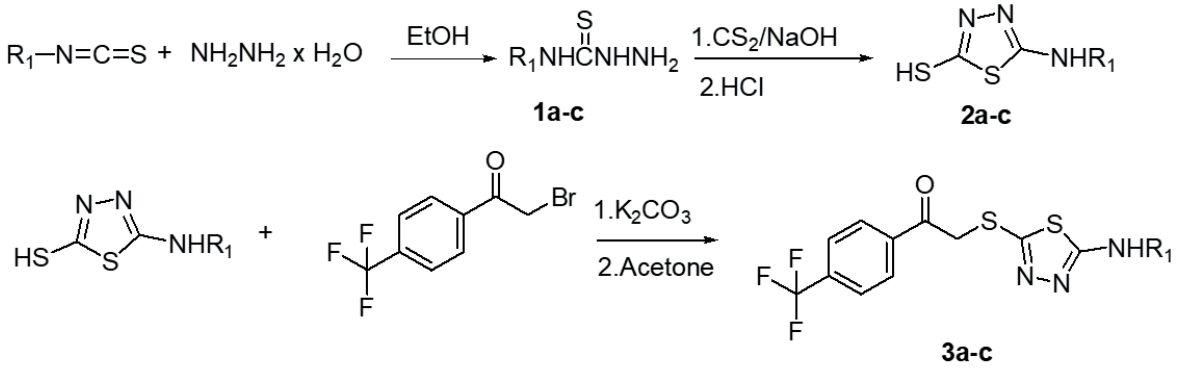
Yield: 80%, M.p: 171.2°C-172.8°C, ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 4.99 (2H, s, -$COCH_2$), 6.96-7.01 (1H, m), 7.28-7.32 (2H, m), 7.52-7.55 (2H, m), 7.93-7.96 (2H, m), 8.21-8.25 (2H, m), 10.38 (1H, brs). HRMS (*m/z*): $[M+H]^+$ calcd for $C_{17}H_{12}OF_3N_3S_2$: 396.0447; found 396.0465.

*2-((5-(Benzylamino)-1,3,4-thiadiazol-2-yl)thio)-1-(4-trifluoromethyl)phenylethan-1-one (**3b**)*

Yield: 77%, M.p: 162.7°C-165.1°C, ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 4.42-4.44 (2H, m, -$NHCH_2$), 4.86 (2H, s, -$COCH_2$), 7.32-7.34 (5H, m), 7.90-7.94 (2H, m), 8.17-8.20 (2H, m), 8.34 (1H, brs). HRMS (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{14}OF_3N_3S_2$: 410.0603; found 410.0598.

*2-((5-((1,3-Benzodioxol-5-yl)methylamino)-1,3,4-thiadiazol-2-yl)thio)-1-(4-trifluoromethyl) phenylethan-1-one (**3c**)*

Yield: 79%, M.p: 182.6°C-183.9°C, ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 4.31-4.33 (2H, s, -$NHCH_2$), 4.86 (2H, s, -$COCH_3$), 5.98 (2H, s), 6.78-6.89 (3H, m), 7.91-7.94 (2H, m), 8.18-8.21 (2H, m). HRMS (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{14}O_3F_3N_3S_2$: 454.0401; found 454.0503.



Compounds	R_1
3a	-phenyl
3b	-benzyl
3c	-1,3-benzodioxole

Scheme 1. Synthesis pathway for obtained compounds **(3a-3c)**

2.2.Cholinesterase Enzymes Inhibition Assay

All synthesized compounds **(3a-c)** were subjected to the modified Ellman's method, previously prepared by our team, to evaluate their potency in inhibiting ChE enzymes [18].

2.3. Molecular Docking Study

Molecular docking studies of the synthesized compounds were performed using AChE (PDB: 4EY7 [19]) crystals. Docking studies were performed using standard procedures with the Schrödinger Suite 2020 Update 2 program [20]. The docking process was carried out with single precision (SP) using the LigPrep 3.8 [21] and Glide 7.1 [22] interfaces.

Table 1. The IC_{50} (µM) values of the obtained compounds against AChE and BChE enzymes

Compound	AChE IC_{50} (µM)	BChE IC_{50} (µM)
3a	>100	>1000
3b	0.096±0.004	>1000
3c	0.302±0.014	>1000
Donepezil	0.0201±0.0014	-
Tacrine	-	0.0064±0.0002

3. RESULTS AND DISCUSSION

3.1. Chemistry

The preparation of compounds **3a-3c** is as shown in Scheme 1. 1,3,4-Thiadiazole derivatives were obtained and their interactions with AChE were investigated by docking studies. The structure-determination of the obtained compounds was elucidated using spectroscopic methods. When the ^{1}H-NMR results were examined, it was seen that the protons of the aromatic rings were H, 2H, 3H between 6.80 ppm-7.94 ppm. The protons of methyl attached to the carbonyl group were 3.33 ppm-3.83 ppm 2H. The protons of methyl attached to the amino group in compound **3b** were shown to be 4.44 ppm 2H and 4.27 ppm 2H in compound **3c**. Mass spectra were performed using high resolution liquid chromatography and all compounds were recorded in excess of their molecular weights in the mass spectra obtained using electron sputtering method.

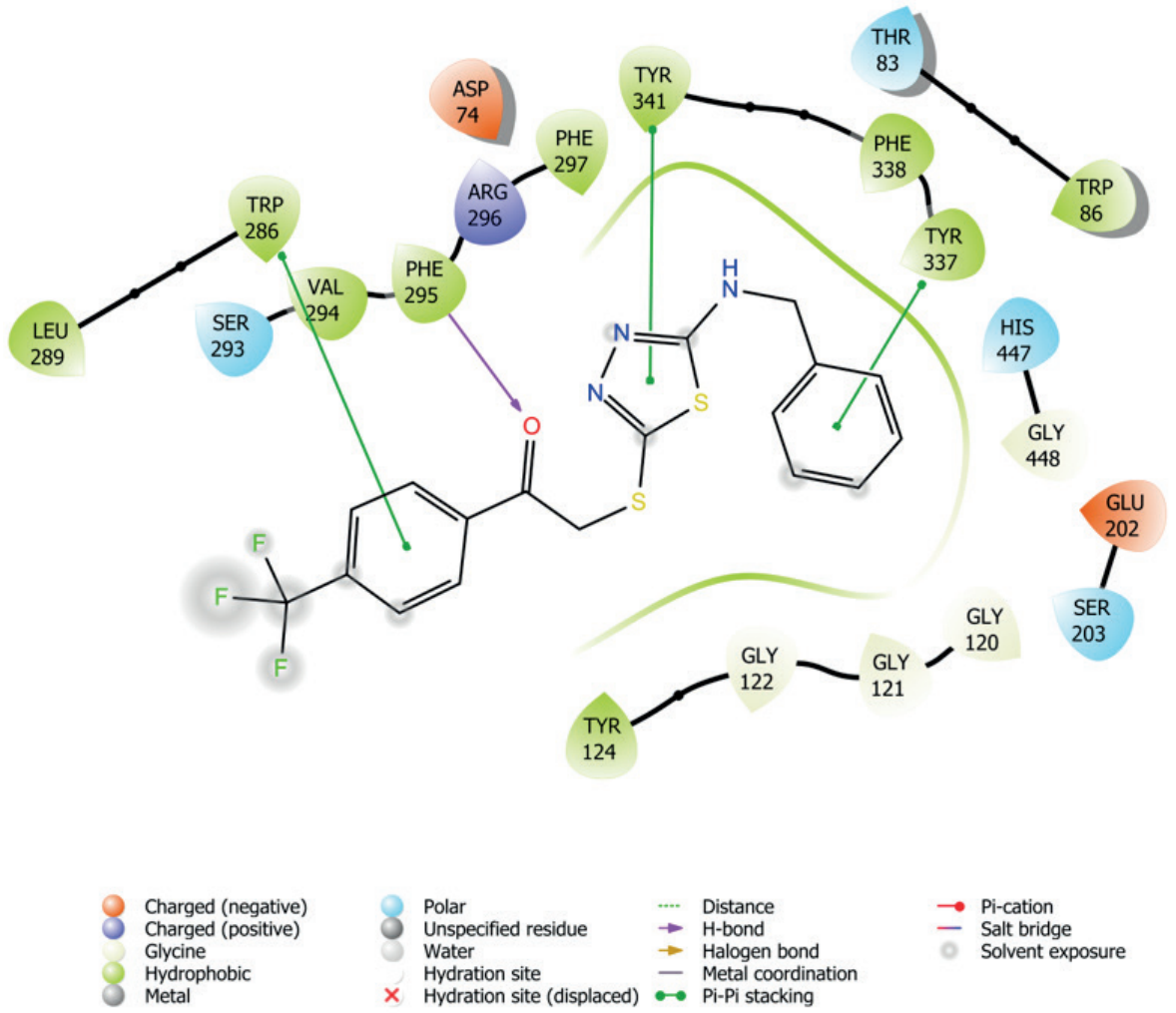


Figure 1. Two-dimensional interaction mode of compound **3b** in the active site of acetylcholinesterase enzyme (PDB: 4EY7)

3.2. Cholinesterase Enzymes Inhibition Assay

The synthesized compounds **(3a-c)** were assessed for their *in vitro* AChE and BChE inhibitory potencies using the modified Ellman's spectrophotometric technique. The results are shown in Table 1. The compound that showed the closest activity to donepezil was **3b**, with an IC_{50} value of 0.096±0.004 µM.

3.3. Molecular Docking Study

Molecular docking studies of the synthesized compounds were carried out using AChE (PDB: 4EY7 [13]) crystals and the interaction of compound **3b** with the active site of the AChE enzyme is best observed. Figure 1 shows the 2D localization of 3b in enzyme active site and Figure 2 shows the 3D localization of **3b** in the enzyme active site. The bonds formed by compound **3b** at the enzyme active site have been studied and can be summarized as follows. It forms a π-π interaction between the 1,3,4-thiadizole ring and the phenyl ring of Tyr341. The benzyl ring attached to the amino group formed a π-π interaction with the phenyl ring of Tyr337. The other phenyl ring in the structure formed a π-π interaction with the indole ring of Trp286. The hydroxyl group in the structure formed an H-bond with the amino acid Phe295.

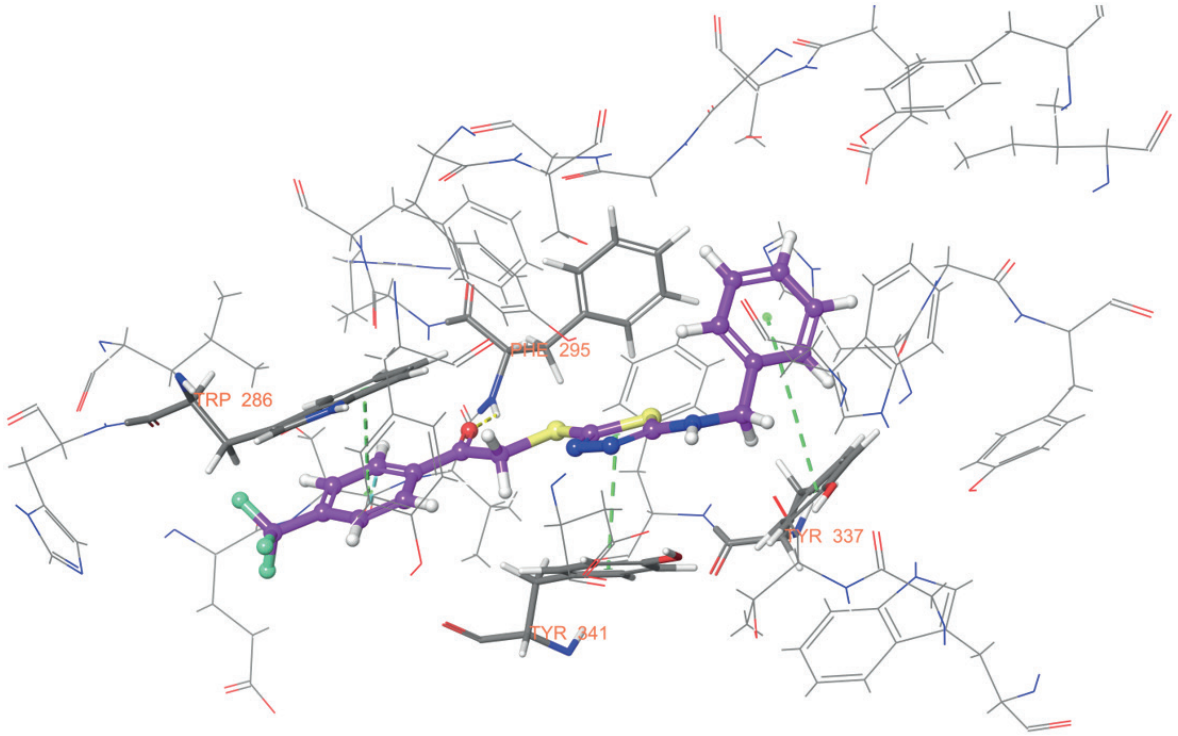


Figure 2. Three-dimensional interaction mode of compound **3b** in the active site of acetylcholinesterase enzyme (PDB: 4EY7)

4. CONCLUSION

When the studies carried out so far are examined, it has been revealed that 1,3,4-thiadiazole ring has many activities. Heterocyclic rings with electron acceptor and donor groups are used in many drug synthesis studies. 1,3,4-Thiadiazole ring is known to be used in many drug development studies. Therefore, in this study, 3 compounds bearing 1,3,4-thiadiazole ring were synthesized. ^{1}H-NMR and HRMS studies were carried out for structure determination of these synthesized compounds. 1,3,4-thiadiazole containing compounds have been shown to act as AChE inhibitors in previous studies. The molecular docking results with the AChE enzyme were then examined for each of the synthesized compounds. As a result of the docking study with AChE, it was observed that **3b** gave the best results. As a result of the activity study with the AChE enzyme, compound **3b** was found to have an IC_{50} value of 0.096±0.004 µM.

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Ethical approval

Not applicable, as this article does not involve any studies with human or animal subjects.

Author contribution

Conceptualization, D.O. and A.N.C.; Methodology, D.O.; Software, D.O.; Formal analysis, B.N.S.Ö.; Resources, A.N.C.; Data curation, A.N.C., D.O.; Writing—original draft preparation, A.N.C.; Writing—review and editing, A.N.C.; Supervision, Z.A.K. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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