Cumhuriyet Sci. J., 45(3) (2024) 503-509
DOI: https://doi.org/10.17776/csj.1449622

Cumhuriyet Science Journal

| csj.cumhuriyet.edu.tr | Founded: 2002 ISSN: 2587-2680 e-ISSN: 2587-246X Publisher: Sivas Cumhuriyet University

Design, Synthesis and Cholinesterase Inhibitory Activity of Novel 1,3,4-Thiadiazole Derivatives

Betül Kaya[1,a,*], Ulviye Acar Çevik[2,b], Bilge Çiftçi[3,c], Mesut Işık[4,d], Zafer Asım Kaplancikli[2,5,e], Namık Kılınç[6,f], Şükrü Beydemir[7,g]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Zonguldak Bulent Ecevit University, 67600 Zonguldak, Türkiye*
[2]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Türkiye*
[3]*Vocational School of Health Services, Bilecik Şeyh Edebali University, 11230, Bilecik, Türkiye*
[4]*Department of Bioengineering, Faculty of Engineering, Bilecik Şeyh Edebali University, 11230, Bilecik, Türkiye*
[5]*The Rectorate of Bilecik Şeyh Edebali University, 11230, Bilecik, Türkiye*
[6]*Department of Medical Services and Techniques, Vocational School of Health Services, Igdir University, Igdir, Türkiye*
[7]*Department of Biochemistry, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Türkiye*
**Corresponding author*

Research Article

History
Received: 11/03/2024
Accepted: 22/07/2024

ABSTRACT

Inhibition of the cholinesterases (AChE and BChE) plays a pivotal role in the symptomatic treatment of Alzheimer's disease. The present study reports the synthesis and anticholinesterase activity of five novel thiadiazole analogs in search of anti-Alzheimer agents. The structures of the newly synthesized compounds were characterized using ^{1}H NMR, ^{13}C NMR and HRMS. Tested compounds inhibited acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes with IC_{50} values in the range of 8.250-20.382 µM and 14.143-0.986 µM, respectively. *N*-(4-Chlorophenyl)-2-[(5-(allylamino)-1,3,4-thiadiazol-2-yl)thio]-*N*-(3-nitrobenzyl)acetamide (6e) was determined as the most potent inhibitor against both tested enzymes when compared with standard drug tacrine. Molecular docking study was carried out to reveal the binding interactions between compound 6e and the active site of AChE.

Keywords: Acetylcholinesterase, Butyrylcholinesterase, Cholinesterase inhibitors, 1,3,4-thiadiazole, Alzheimer's disease.

[a] *betul.kaya@beun.edu.tr* *https://orcid.org/0000-0002-1713-9485*
[b] *uacar@anadolu.edu.tr* *https://orcid.org/0000-0003-1879-1034*
[c] *bilge.ciftci@bilecik.edu.tr* *https://orcid.org/0000-0002-4153-1209*
[d] *mesut.isik@bilecik.edu.tr* *https://orcid.org/0000-0002-4677-8104*
[e] *zakaplan@anadolu.edu.tr* *https://orcid.org/0000-0003-2252-0923*
[f] *namik.kilinc@igdir.edu.tr* *https://orcid.org/0000-0002-9102-1370*
[g] *sukrubeydemir@anadolu.edu.tr* *https://orcid.org/0000-0003-3667-6902*

Introduction

Alzheimer's disease (AD) is a common type of neurodegenerative brain disease that affects over 55 million people globally and people with AD is estimated to increase to 78 million by 2030 [1]. Acetylcholine (ACh) is critical for cognitive processes and, in the brain of people suffering from AD, ACh is decreased, cholinergic pathway is noticed to be irreversibly impaired, resulting in cognitive dysfunction. The therapeutic strategies in the treatment of AD are mainly focused on reduced cholinergic neurotransmission and β-amyloid protein aggregation that leads to progressive loss of structure and/or function of neurons [2,3]. Cholinesterase inhibitors exert their activity by inhibiting acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) which are both types of cholinesterase enzymes, eventually increase the concentration of the ACh in the brain of AD patients. Currently licensed drugs used for AD are tacrine, donepezil, rivastigmine and galantamine. However, their effectiveness is often limited by the appearance of central and peripheral side effects. For instance, tacrine has been withdrawn from the market due to hepatotoxicity [4,5].

Thiadiazole nucleus is a well-known five-membered heterocyclic scaffold in medicinal chemistry. The thiadiazole moiety functions as a "two-electron donor system" and "hydrogen-binding domain, which provide the thiadiazole ring its biological activity [6]. The sulfur atom also contributes lipo-solubility, resulting in more lipophilic analogues with enhanced blood–brain barrier (BBB) permeability. Thiadiazole ring has four isomers, 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole, and 1,3,4-thiadiazole, depending on the positions of heteroatoms. 1,3,4-Thiadiazole is the main isomer that has a wide range of pharmacological effects [7]. There are currently numerous marketed pharmaceutical products containing thiadiazole scaffold including acetazolamide, methazolamide (carbonic anhydrase inhibitors), megazole (antitrypanosomal), sulfamethizole (anti-microbial), cefazolin, cefazedone (antibiotics) and timolol (antihypertensive) [8,9].

Among above-mentioned thiadiazoles, 2-amino-1,3,4-thiadiazole derivatives were studied for their antibacterial, antifungal, antitubercular, antiparasitic activities, antidepressant, anticancer and cholinesterase inhibitory activities [10-15]. The activity of 1,3,4-thiadiazoles is probably due to the presence of =N–C–S moiety [16]. In view of these facts, in this paper, we synthesized new series of 2-subsitutedamino-1,3,4-

thiadiazole derivatives and evaluated their *in vitro* cholinesterase inhibitory activity.

Materials and Methods

Chemistry

All Chemicals used in the synthesis pathway of the compounds were purchased from Sigma-Aldrich (Sigma-Aldrich Corp., St. Louis, MO, USA) or Merck (Merck KGaA, Darmstadt, Germany) chemical companies and were used without further chemical purifications. Melting points (MP) were determined by an automatic melting point apparatus (MP90, Mettler-Toledo, OH, USA). The NMR spectra (^{1}H and ^{13}C) were recorded in DMSO-d_6 by a Bruker digital FT-NMR spectrometer (Bruker Bioscience, MA, USA) at 300 MHz and 75 MHz, respectively. HRMS studies were performed on an LCMS-IT-TOF system (Shimadzu, Tokyo, Japan). Chemical purities of the compounds were checked by classical TLC applications performed on silica gel 60 F254 (Merck KGaA, Darmstadt, Germany).

Synthesis of 4-chloro-*N*-(3-nitrobenzylidene)aniline (1) [17]

Yield 69%. 4-Chloroaniline (33 mmol, 4.22 g), 3-nitrobenzaldehyde (33 mmol, 5 g) and catalytic amount of glacial acetic acid (0.5 ml) were dissolved in ethanol (100 ml) and refluxed for 9 h. After TLC screening, the mixture was cooled, the product was filtered and the crude product was recrystallized from ethanol.

Synthesis of 4-chloro-*N*-(3-nitrobenzyl)aniline (2) [17]

Yield 70%. Compound 1 (27 mmol, 7.1 g) was dissolved in methanol (150 mL) and sodium borohydride was added in four portions (4×0.5 g) 15 min intervals. After addition, reaction mixture was allowed to stir for 1 h at room temperature. The solvent was evaporated and the crude product was washed with water, dried and recrystallized from ethanol.

Synthesis of 2-chloro-*N*-(4-chlorophenyl)-N-(3-nitrobenzyl)acetamide (3) [17]

Yield 67%. To a mixture of compound 2 (24 mmol, 6.2 g) and triethylamine (29 mmol, 4.04 mL) in tetrahydrofuran (100 mL), chloroacetyl chloride (29 mmol, 2.31 mL) was added dropwise with stirring at 0–5 °C. The reaction was mixed for one hour at room temperature, after dripping of $ClCH_2COCl$. To gain product, tetrahydrofuran was evaporated. The product was recrystallized from ethanol, after washing with water.

Synthesis of 4-substituted thiosemicarbazides (4a-4e) [18]

Yield 72%. Various substituted isothiocyanates (20 mmol) and hydrazine hydrate (40 mmol) were dissolved in ethanol (50 mL) and stirred at room temperature for 4 h. The precipitated compound filtered and crystallized from ethanol.

Synthesis of 5-substituted amino-1,3,4-thiadiazole-2(3*H*)-thiones (5a-5e) [18]

Yield 65%. To a solution of 4-substitutedthiosemicarbazide derivative (4a-4e) (23 mmol) in ethanol (80 mL), carbon disulfide (27 mmol, 1.6 mL) and potassium hydroxide (27 mmol) were added and the mixture was refluxed for 12 h. The solution was cooled and acidified to pH 4–5 with a hydrochloric acid solution and crystallized from ethanol.

Synthesis of *N*-(4-chlorophenyl)-2-[(5-substitutedamino-1,3,4-thiadiazol-2-yl)thio]-*N*-(3-nitrobenzyl)acetamide derivatives (6a-6e) [18]

Yield 77%. A mixture of appropriate 5-substitutedamino-1,3,4-thiadiazole-2(3*H*)-thione derivative (5a-5e) (4 mmol), compound 3 (4 mmol) and potassium carbonate (5 mmol, 0.66 g) were stirred was stirred at room temperature for 9 h in acetone (40 mL). After that, acetone was evaporated, the residue was washed and crystallized from ethanol.

N-(4-Chlororophenyl)-2-[(5-(methylamino)-1,3,4-thiadiazol-2-yl)thio]-N-(3-nitrobenzyl) acetamide (6a): Yield 77%. M.P.= 145.9 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ 2.83 (3H, s, CH_3), 3.91 (2H, s, CO-CH_2), 5.01 (2H, s, N-CH_2), 7.33 (2H, d, *J*= 8.6 Hz, Ar-H), 7.46 (2H, d, *J*= 8.6 Hz, Ar-H), 7.46 (1H, t, *J*= 7.8 Hz, Ar-H), 7.67-7.74 (2H, m, Ar-H), 8.08-8.13 (2H, m, Ar-H and NH). ^{13}C NMR (75 MHz, DMSO-d_6, ppm) δ 31.53, 38.33, 52.17, 122.81, 123.22, 130.19, 130.38, 130.53, 133.36, 135.13, 139.68, 140.31, 148.24, 149.27, 167.47, 170.77. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{16}ClN_5O_3S_2$: 450.0456; found 450.0434.

N-(4-Chlororophenyl)-2-[(5-(ethylamino)-1,3,4-thiadiazol-2-yl)thio]-N-(3-nitrobenzyl) acetamide (6b): Yield 67%. M.P.= 161.5 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ 1.14 (3H, t, *J*= 7.2 Hz, CH_2-C$\underline{H}_3$), 3.19-3.28 (2H, m, C$\underline{H}_2$-CH_3), 3.91 (2H, s, CO-CH_2), 5.01 (2H, s, N-CH_2), 7.33 (2H, d, *J*= 8.6 Hz, Ar-H), 7.47 (2H, d, *J*= 8.6 Hz, Ar-H), 7.59 (1H, t, *J*= 7.8 Hz, Ar-H), 7.69 (1H, d, *J*= 7.6 Hz, Ar-H), 7.76 (1H, t, *J*= 5.2 Hz, Ar-H), 8.08-8.13 (2H, m, Ar-H and NH). ^{13}C NMR (75 MHz, DMSO-d_6, ppm) δ 14.66, 38.32, 39.86, 52.17, 122.81, 123.21, 130.19 (2C), 130.38, 130.53 (2C), 133.36, 135.13, 139.68, 140.31, 148.24, 149.12, 167.48, 169.83. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{18}ClN_5O_3S_2$: 464.0612; found 464.0606.

N-(4-Chlorophenyl)-2-[(5-(propylamino)-1,3,4-thiadiazol-2-yl)thio]-N-(3-nitrobenzyl) acetamide (6c): Yield 72%. M.P.= 144.0 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ 0.88 (3H, t, *J*= 7.4 Hz, CH_3), 1.48-1.60 (2H, m, CH_3-C$\underline{H}_2$-CH_2-), 3.14-3.21 (2H, m, CH_3-CH_2-C$\underline{H}_2$-), 3.91 (2H, s, CO-CH_2), 5.01 (2H, s, N-CH_2), 7.33 (2H, d, *J*= 8.6 Hz, Ar-H), 7.46 (2H, d, *J*= 8.6 Hz, Ar-H), 7.59 (1H, t, *J*= 7.8 Hz, Ar-H), 7.69 (1H, d, *J*= 7.7 Hz, Ar-H), 7.79 (1H, t, *J*= 5.5 Hz, Ar-H), 8.08-8.13 (2H, m, Ar-H and NH). ^{13}C NMR (75 MHz, DMSO-d_6, ppm) δ 11.82, 22.22, 38.32, 46.84, 52.18, 122.80, 123.21, 130.18 (2C), 130.37, 130.52 (2C), 133.36, 135.13,

139.69, 140.31, 148.24, 148.99, 167.49, 170.06. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{20}ClN_5O_3S_2$: 478.0769; found 478.0749.

N-(4-Chlorophenyl)-2-[(5-(isopropylamino)-1,3,4-thiadiazol-2-yl)thio]-N-(3-nitro benzyl)acetamide (6d): Yield 69%. M.P.= 118.9 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ 1.15 (6H, d, *J*= 6.5 Hz, $2CH_3$), 3.68-3.79 (1H, m, CH), 3.91 (2H, s, CO-CH_2), 5.01 (2H, s, N-CH_2), 7.33 (2H, d, *J*= 8.6 Hz, Ar-H), 7.46 (2H, d, *J*= 8.6 Hz, Ar-H), 7.59 (1H, t, *J*= 7.9 Hz, Ar-H), 7.70 (2H, t, *J*= 5.0 Hz, Ar-H), 8.08-8.13 (2H, m, Ar-H and NH). ^{13}C NMR (75 MHz, DMSO-d_6, ppm) δ 22.57 (2C), 38.32, 46.98, 52.17, 122.81, 123.21, 130.18 (2C), 130.38, 130.53 (2C), 133.36, 135.13, 139.68, 140.30, 148.24, 148.97, 167.49, 169.01. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{20}ClN_5O_3S_2$: 478.0769; found 478.0749.

N-(4-Chlorophenyl)-2-[(5-(allylamino)-1,3,4-thiadiazol-2-yl)thio]-N-(3-nitrobenzyl) acetamide (6e): Yield 75%. M.P.= 130.2 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ 3.86-3.92 (4H, m, CH_2=CH-C$\underline{H}_2$ and CO-CH_2), 5.01 (2H, s, N-CH_2), 5.10-5.25 (2H, m, C$\underline{H}_2$=CH-CH_2), 5.82-5.95 (1H, m, CH_2=C$\underline{H}$-CH_2), 7.33 (2H, d, *J*= 8.7 Hz, Ar-H), 7.46 (2H, d, *J*= 8.6 Hz, Ar-H), 7.59 (1H, t, *J*= 7.7 Hz, Ar-H), 7.69 (1H, d, *J*= 7.5 Hz, Ar-H), 7.95 (1H, t, *J*= 5.6 Hz, Ar-H), 8.08-8.12 (2H, m, Ar-H and NH). ^{13}C NMR (75 MHz, DMSO-d_6, ppm) δ 38.28, 47.12, 52.19, 116.65, 122.80, 123.21, 130.19 (2C), 130.38, 130.52 (2C), 133.37, 134.75, 135.13, 139.67, 140.31, 148.24, 149.71, 167.46, 169.82. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{18}ClN_5O_3S_2$: 476.0612; found 476.0604.

Cholinesterase Inhibitory Activity

The anticholinesterase (AChE and BChE) activities were assayed by a modified version of the Ellman method.The inhibitory effect of novel 2-subsitutedamino-1,3,4-thiadiazole derivatives (6a-6e) on AChE (from electric eel; Sigma) and BChE (from equine serum) was investigated using acetylthiocholine iodide (ATChI) and butrylcholine iodide (BChI) as substrates at 37°C. Stock solutions of the compounds synthesized as inhibitors were prepared in 20 % DMSO. The inhibition effects of novel 2-subsitutedamino-1,3,4-thiadiazole derivatives (6a-6e) was determined in the presence of at least five different inhibitor concentrations, usually in the 10^{-2}-10^2 µM range, to obtain between 0% and 100% inhibition of cholinesterase activity. Enzyme solution (5.32×10^{-3} U, 50 µL) and inhibitor solution (20 µL) were added to a cuvette containing Tris–HCl (100 µL, 1 M; pH= 8.0). After 5 min incubation, DTNB solution (0.01 M, 100 µL) and ATChI/BChI (0.050 M, 50 µL) were added. Immediately after brief stirring, 3 replicate measurements were monitored spectrophotometrically at 412 nm [19-22]. The IC_{50} values of the derivatives were calculated from Activity (%)-[Ligand] graphs for derivatives according to our previous studies [23,24].

Molecular Docking

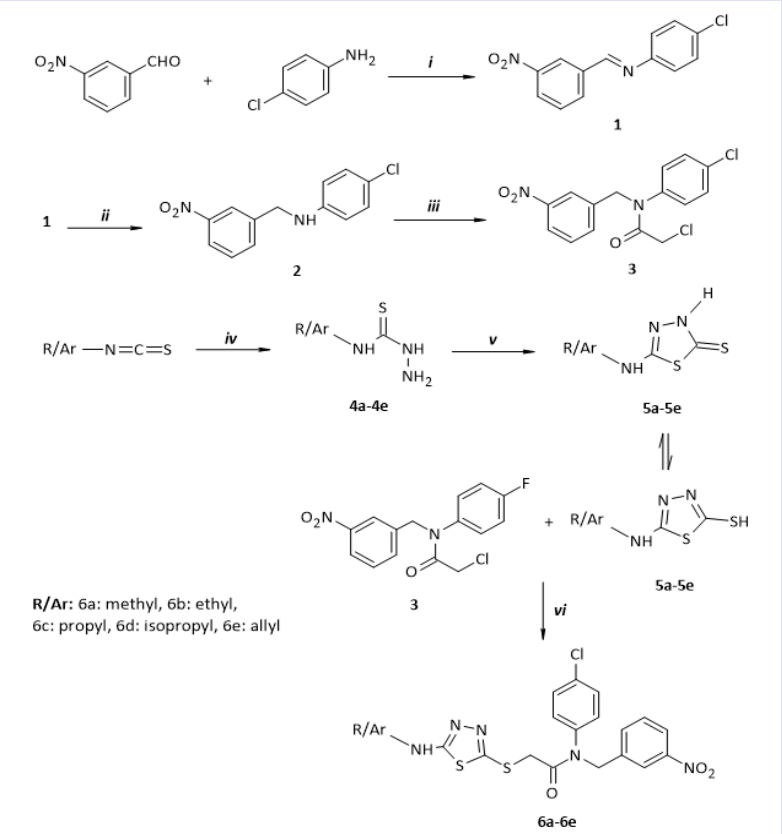
In silico investigations were conducted to study the interactions between the compound 6e and the AChE and BChE enzymes using molecular docking simulations, as described in our earlier research [25-27]. These simulations utilized Maestro 13.9 from the Schrödinger Molecular Modeling Suite [28]. The crystal structures of AChE and BChE, with PDB IDs 4TVK and 5NN0 respectively, were sourced from the RCSB Protein Data Bank. The receptors were prepared at physiological pH using the Protein Preparation Wizard [29], followed by optimization and minimization with the OPLS4 force field. A receptor grid was established around the natural ligands within the protein structures. Ligands were prepared and protonated at pH 7.0 ± 2.0 using the LigPrep module.

Results and Discussions

Chemistry

The target compounds were prepared as illustrated in Scheme 1. Firstly, 3-nitrobenzaldehyde and 4-chloroaniline was reacted in the presence of glacial acetic acid as a catalyst to afford compound 1, which further reacted with sodium borohydride for the reduction of imine bond to synthesize compound 2. Subsequently, compound 3 was obtained via the reaction between compound 2 and chloroacetyl chloride in the presence of triethylamine. Various substituted isothiocyanates were reacted with hydrazine hydrate to prepare compounds 4a-4e. In the next step, intermediate compounds 4a-4e were subjected to the ring closure reaction with carbon disulfide to give 5-substitutedamino-1,3,4-thiadiazole-2(3*H*)-thione derivatives (5a-5e). In the final step, compounds 5a-5e were treated with compound 3 in acetone in the presence of potassium carbonate to yield target compounds 6a-6e.

The structures of the synthesized analogues were confirmed using NMR and MS spectral analysis. In the ^{1}H NMR spectra, CH_2 protons bound to nitrogen and carbonyl group were observed at 5.01 ppm and at between 3.91-3.92 ppm, respectively. The rest aliphatic protons and all the aromatic protons were assigned at their expected area. In the ^{13}C NMR spectra, a peak at between 38.28-38.33 ppm was detected due to the signal of CH_2 carbon bound to carbonyl group. The signal belonging to the N-CH_2 carbon was determined at between 52.17-52.19 ppm. The rest aliphatic and aromatic carbons were assigned at their expected regions. In the HRMS spectra of final compounds (6a-6e), the M+1 peaks were observed in accordance with their molecular formula.



Scheme 1. The synthetic pathway of the compounds (6a-6e). Reagents and conditions; i: acetic acid, ethanol, reflux, 9 h; ii: sodium borohydride, methanol, r.t, 11 h; iii: Chloroacetyl chloride, triethylamine, tetrahydrofuran, ice-bath, 5 h; iv: hydrazine hydrate, ethanol, r.t., 4 h; v: (1) carbon disulfide/potassium hydroxide, ethanol, reflux, 12 h; (2) hydrochloric acid, pH 4–5; vi: potassium carbonate, acetone, r.t., 9 h

Biological Activity

The aim of this study was to investigate the *in vitro* effects of newly synthesized derivatives (referred to as 6a-6e) on cholinesterase enzymes. The inhibitory activities of the derivatives against key metabolic enzymes such as AChE and BChE were determined and analyzed in comparison with the known inhibitory effect of tacrine. The IC_{50} values of the derivatives were determined from Activity (%)-[Ligand] graphs for derivatives (Figure S1 and Figure S2). The inhibition data of IC_{50} values found to evaluate the potential inhibitory effects of all series of the new derivatives are presented in Table 1. The synthesized derivatives exhibited inhibitor activity in micromolar levels against AChE and BChE with the IC_{50} values in the range of 8.250-20.382 µM and 14.143-0.986 µM, respectively. The AChE inhibitory activities for the novel compounds reduced in the order of 6e (allyl-substituted) > 6d (isopropyl-substituted) > 6c (propyl-substituted) > 6b (ethyl-substituted) > 6a (methyl-substituted), as well as for BChE; 6e (allyl-substituted) > 6c (propyl-substituted) > 6d (isopropyl-substituted) > 6a (methyl-substituted) > 6b (ethyl-substituted). The 6e derivative shows a more selective inhibition potential for both cholinesterase enzymes, while the effect of compounds 6a and 6b is lower than the others. The allyl-substituted derivative 6e (IC_{50} for AChE and BChE; 8.250 µM and 0.986 µM, respectively) was identified as the most potent cholinesterase inhibitor in the series (6a-6e).

Although the inhibitory potential of the series was lower than the inhibitory potential of the standard tacrine (IC50 for AChE and BChE; 0.145 µM and 0.208 nM), they showed a more effective inhibitory effect than many compounds with similar structures given in the literature [30]. In a study, indole-based thiadiazole derivatives (1-18) were synthesized and their inhibition effects against AChE and BChE were evaluated. The IC_{50} values of the synthesized analogues against these enzymes ranging between 0.17 ± 0.05 to 33.10 ± 0.6 µM against AChE and 0.30 ± 0.1 to 37.60 ± 0.6 µM against BChE [31]. In a separate investigation, a novel set of benzimidazole-based thiadiazole hybrid analogues was synthesized, demonstrating diverse inhibitory capabilities against targeted enzymes AChE and BChE. The IC_{50} values ranged from 1.32 ± 0.10 µM to 19.26 ± 0.60 µM for AChE and from 1.94 ± 0.10 µM to 21.33 ± 0.70 µM for BChE [32]. The allyl-substituted derivative 6e has much more effective inhibitory potential than many thiadiazoles synthesized in many series in the literature and shown to have inhibitory effect on cholinesterase.

Table 1. IC_{50} values of novel 2-subsitutedamino-1,3,4-thiadiazole derivatives (6a-6e) against AChE and BChE enzymes.

Compound	AChE		BChE	
	IC_{50} (μM)	R^2	IC_{50} (μM)	R^2
6a	20.382	0.968	10.520	0.979
6b	17.769	0.974	14.143	0.987
6c	16.902	0.973	4.846	0.939
6d	13.075	0.963	5.331	0.983
6e	8.250	0.963	0.986	0.944
Tacrine	0.145	0.962	0.208	0.966

Molecular Docking

We employed the Extra Precision (XP) molecular docking method to perform detailed molecular docking studies with the 6e compound to understand the protein-ligand interactions. The 6e compound, recognized for its strong inhibitory effects on AChE and BChE enzymes, was analyzed using the XP docking protocol. Table 2 summarizes the docking scores of 6e compound against the AChE and BChE enzymes.

Table 2. The docking scores of 6e compound against the AChE and BChE enzymes.

Compound	Docking Score (Kcal/mol)	
	AChE	BChE
6e	-8.301	-5.008

Molecular docking studies of the 6e compound, which demonstrated significant inhibitory effects against AChE and BChE enzymes in *in vitro* assays, revealed high docking scores for both enzymes. Specifically, the docking simulations showed scores of -8.301 kcal/mol for AChE and -5.008 kcal/mol for BChE (Table 2), indicating a strong binding affinity between the 6e compound and the enzymes. Within the active site of the AChE enzyme, compound 6e established a hydrogen bond with TYR121. Additionally, it participated in pi-pi interactions with TRP279 and TRP84, key components of the enzyme's active site. In the BChE enzyme's active site, compound 6e formed hydrogen bonds with THR120 and SER287 residues and engaged in a halogen bond interaction with TRP82 (Figure 1).

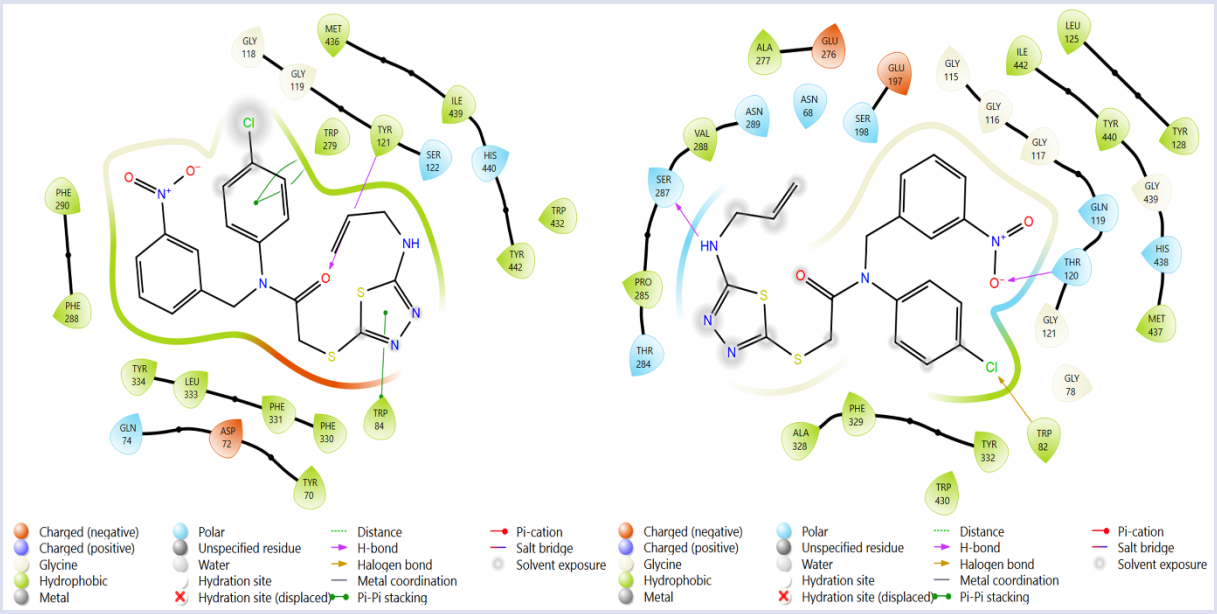


Figure 1. Ligand-enzyme interaction diagram of compound 6e with AChE (left) and BChE (right) enzymes.

Conclusions

In this study, a novel series of 2-subsitutedamino-1,3,4-thiadiazole derivatives (6a-6e) were synthesized as potential targets for treatment of AD. The structures were clarified by modern spectral techniques consisting of ^{1}H NMR, ^{13}CNMR and HRMS. Cholinesterase assay displayed that *N*-(4-chlorophenyl)-2-[(5-(allylamino)-1,3,4-thiadiazol-2-yl)thio]-*N*-(3-nitrobenzyl) acetamide (6e) was the most potent compounds against both AChE and BChE with the IC_{50} values of 8.250 μM and 0.986 μM, respectively. According to activity results, allyl group enhanced both acetylcholinesterase and butyrylcholinesterase inhibition. This might be due to the binding interactions between compound 6e and the active site of the cholinesterase enzymes.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgment

The authors thank the Anadolu University Faculty of Pharmacy Central Research Laboratory (MERLAB) for their support and contributions.

References

[1] Gauthier S., José P.R.-N., Morais A., Webster C., World Alzheimer Report 2021: Journey Through the Diagnosis of Dementia, (2021).

[2] Giacobini E., Cholinesterases: New Roles in Brain Function and in Alzheimer's Disease, *Neurochem. Res.*, 28 (2003) 515–522.

[3] Bala M., Ankalgi, A., Piplani P., Design, Synthesis and Pharmacological Evaluation of Disubstituted 1, 3, 4 Thiadiazoles Derivatives for The Treatment of Cognitive Dysfunction, *J. Mol. Struct*., 1291 (2023) 135951.

[4] Sharma D., Bansal K.K., Sharma A., Pathak M., Sharma P.C., A Brief Literature and Review of Patents on Thiazole-Related Derivatives, *Curr. Bioact. Compd*., 15 (2019) 304–315.

[5] Skrzypek A., Matysiak J., Niewiadomy A., Bajda M., Szymański, P., Synthesis and Biological Evaluation of 1, 3, 4-Thiadiazole Analogues as Novel AChE and BChE Inhibitors, *Eur. J. Med. Chem*., 62 (2013) 311-319.

[6] Atmaram U.A., Roopan S.M., Biological Activity of Oxadiazole and Thiadiazole Derivatives, *Appl. Microbiol.*, 106 (2022) 3489-3505.

[7] Sharma B., Verma A., Prajapati S., Sharma U.K., Synthetic Methods, Chemistry, and the Anticonvulsant Activity of Thiadiazoles, *Int. J. Med. Chem*., 2013 (2013) 348948.

[8] Hatami M., Basri Z., Sakhvidi B.K., Mortazavi M., Thiadiazole–A Promising Structure in Design and Development of Anti-Alzheimer Agents, *Int. Immunopharmacol*., 118 (2023) 110027.

[9] Serban, G., Future Prospects in The Treatment of Parasitic Diseases: 2-Amino-1, 3, 4-Thiadiazoles in Leishmaniasis, *Molecules*, 24 (2019) 1557.

[10] Serban G., Stanasel O., Serban E., Bota S., 2-Amino-1, 3, 4-Thiadiazole as A Potential Scaffold for Promising Antimicrobial Agents, *Drug Des. Devel. Ther*., 12 (2018) 1545-1566.

[11] Talath S, Gadad AK., Synthesis, Antibacterial and Antitubercular Activities of Some 7-[4-(5-Amino-[1,3,4]thiadiazole-2-sulfonyl)-piperazin-1-yl]fluoroquinolonic Derivatives, *Eur. J. Med. Chem*., 41 (2006) 918–924.

[12] Serban G., 2-Amino-1,3,4-thiadiazoles as Prospective Agents in Trypanosomiasis and Other Parasitoses, *Acta Pharm*., 70 (2020) 259–290.

[13] Clerici F., Pocar D., Guido M., Loche A., Perlini V., Brufani M., Synthesis of 2-Amino-5-sulfanyl-1,3,4-thiadiazole Derivatives and Evaluation of Their Antidepressant and Anxiolytic Activity, *J. Med. Chem*., 44 (2001) 931–936.

[14] Çevik U.A., Osmaniye D., Levent S., Sağlik B. N., Çavuşoğlu B. K., Özkay Y., Kaplanciklı Z.A., Synthesis and Characterization of A New Series of Thiadiazole Derivatives as Potential Anticancer Agents, *Heterocycl. Commun*., 26 (2020) 6-13.

[15] Al-Sharabi A.A., Evren A.E., Sağlık B.N., Yurttaş L., Synthesis, Characterization, Molecular Docking and Molecular Dynamics Simulations of Novel 2, 5-Disubstituted-1, 3, 4-Thiadiazole Derivatives as Potential Cholinesterase/Monoamine Oxidase Dual Inhibitors for Alzheimer's Disease, *J. Biomol. Struct. Dyn*, (2023) 1-19.

[16] Obakachi V.A., Kushwaha B., Kushwaha N.D., Mokoena S., Ganai A.M., Pathan T.K., Karpoormath R., Synthetic and Anticancer Activity Aspects of 1, 3, 4-Thiadiazole Containing Bioactive Molecules: A Concise Review, *J. Sulphur Chem*., 42 (2021) 670-691.

[17] Kaya B., Sağlık B.N., Levent S., Özkay Y., Kaplancıklı, Z.A., Synthesis of Some Novel 2-Substituted Benzothiazole Derivatives Containing Benzylamine Moiety as Monoamine Oxidase Inhibitory Agents, *J. Enzyme Inhib. Med. Chem.*, 31 (2016), 1654-1661.

[18] Sağlık B.N., Çavuşoğlu B.K., Çevik U.A., Osmaniye D., Levent S., Özkay Y., Kaplancıklı Z.A. Novel 1, 3, 4-thiadiazole compounds as potential MAO-A inhibitors–design, synthesis, biological evaluation and molecular modelling, *RSC Med. Chem*., 11 (2020) 1063-1074.

[19] Ellman G. L., Courtney K. D., Andres Jr V., Featherstone, R. M., A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity, *Biochem. Pharmacol*., 7(2) (1961) 88-95.

[20] Mughal E. U., Sadiq A., Murtaza S., Rafique H., Zafar M. N., Riaz T., Khan B.A., Hameed A., Khan K. M., Synthesis, Structure–Activity Relationship and Molecular Docking Of 3-Oxoaurones And 3-Thioaurones As Acetylcholinesterase And Butyrylcholinesterase Inhibitors, *Bioorg. Med. Chem*., 25(1) (2017) 100-106.

[21] Işık M., Akocak S., Lolak N., Taslimi P., Türkeş C., Gülçin İ., Durgun M., Beydemir Ş., Synthesis, Characterization, Biological Evaluation, And in Silico Studies of Novel 1, 3-Diaryltriazene-Substituted Sulfathiazole Derivatives, *Arch. Pharm*., 353 (9) (2020) 2000102.

[22] Tarikoğulları A., Çizmecioğlu M., Saylam M., Parlar S., Alptüzün V., Soyer Z., Synthesis and Cholinesterase Inhibitory Activity of Some Phenylacetamide Derivatives Bearing 1H-Pyrazole or 1H-1, 2, 4-Triazole, *Marmara Pharm. J*., 20 (1) (2016) 21-27.

[23] Akocak S., Taslimi P., Lolak N., Işık M., Durgun M., Budak Y., Türkeş C., Gülçin İ., Beydemir Ş., Synthesis, Characterization, And Inhibition Study of Novel Substituted Phenylureido Sulfaguanidine Derivatives As α-Glycosidase and Cholinesterase Inhibitors, *Chem. Biodivers*., 18 (4) (2021) e2000958.

[24] Durgun M., Türkeş C., Işık M., Demir Y., Saklı A., Kuru A., Güzel A., Beydemir Ş., Akocak S., Osman S.M., AlOthman Z., Supuran C. T., Synthesis, Characterisation, Biological Evaluation and In Silico Studies of Sulphonamide Schiff Bases, *J. Enzyme Inhib. Med. Chem*., 35 (1) (2020) 950-962.

[25] Akıncıoğlu A., Göksu S., Naderi A., Akıncıoğlu H., Kılınç N., Gülçin İ., Cholinesterases, Carbonic Anhydrase Inhibitory Properties and in Silico Studies of Novel Substituted Benzylamines Derived from Dihydrochalcones, *Comput. Biol. Chem*., 94 (2021) 107565.

[26] Alım Z., Şirinzade H., Kılınç N., Dilek E., Süzen S., Assessing Indole Derivative Molecules as Dual Acetylcholinesterase and Butyrylcholinesterase Inhibitors through in vitro Inhibition and Molecular Modelling Studies, *J. Mol. Struct*., 1311 (2024) 138276.

[27] Gök N., Akıncıoğlu A., Erümit Binici E., Akıncıoğlu H., Kılınç N., Göksu S. Synthesis of Novel Sulfonamides with Anti-Alzheimer and Antioxidant Capacities, *Arch. Pharm*., 354(7) (2021) 2000496.

[28] Schrödinger Release 2024-1: Maestro, Schrödinger, 2024 LLC, New York, NY.

[29] Sastry G.M., Adzhigirey M., Day T., Annabhimoju R., Sherman W., Protein and Ligand Preparation: Parameters, Protocols, and Influence on Virtual Screening Enrichments, *J. Comput. Aided Mol. Des*., 27 (3) (2013) 221-234.

[30] Shah S. A. A., Ashraf M., Rehman J., Saleem R. S. Z., Synthesis, in Vitro And in Silico Studies Of S-Alkylated 5-(4-

Methoxyphenyl)-4-Phenyl-4H-1, 2, 4-Triazole-3-Thiols as Cholinesterase Inhibitors, *Pak. J. Pharm. Sci.*, 31(6) (2018) 2697-2708.

[31] Taha M., Rahim F., Uddin N., Khan I. U., Iqbal N., Salahuddin M., Farooq R.K., Gollapalli M., Khan K.M., Zafar A., Exploring Indole-Based-Thiadiazole Derivatives as Potent Acetylcholinesterase and Butyrylcholinesterase Enzyme Inhibitors, *Int. J. Biol. Macromol*., 188 (2021) 1025-1036.

[32] Hussain R., Ashraf M., Khan S., Rahim F., Rehman W., Taha M., Sardar A., Khan Y., Khan I., Shah S. A.A., Molecular Modeling, Synthesis, and in vitro Acetylcholinesterase and Butyrylcholinesterase Inhibitory Activities of Novel Benzimidazole-Bearing Thiadiazole Derivatives, *J. Mol. Struct*., 1295 (2024) 136582.