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Research Article

Synthesis of a new thiadiazole-benzodioxole derivative, investigation of acetylcholinesterase inhibition with *in vitro* and *in silico* studies

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ABSTRACT

Alzheimer's disease is a progressive and degenerative brain disease that negatively affects people's lives and reduces cognitive and sensory human functions. Today, there are active ingredients that work on Alzheimer's disease, containing benzodioxole and thiadiazole rings. Acetylcholinesterase terminates neurotransmission in the nervous system and leads to the accumulation of acetylcholine, overstimulation of various receptors and consequent impairment of neurotransmission. Thiadiazole and benzodioxole rings are compounds that exhibit a wide range of biological activities, especially known to be effective on acetylcholinesterase. A new compound containing benzodioxole and thiadiazole rings was designed, synthesized and its chemical structure was revealed using spectroscopic methods such as HRMS, ^{13}C-NMR and ^{1}H-NMR. Acetylcholinesterase inhibition activities were investigated using *in vitro* methods. To elucidate the acetylcholinesterase inhibition of compound **4a**, it was subjected to *in silico* insertion procedure with 4EY7. Compound **4a** exhibited 0.114±0.005 µM against AChE. The above data is compared with data for donepezil (0.0201±0.0014 µM), the reference compound in our study.

Keywords: AChE, ADME, Benzodioxole, Molecular Docking, Thiadiazole

1. INTRODUCTION

Acetylcholinesterase is a cholinesterase group enzyme that can catalyze the decomposition reaction of acetylcholine, a neurotransmitter [1]. Cholinesterases, which cleave choline esters with varying efficiency, are a ubiquitous group of serine hydrolases. When vertebrates are analyzed, we come across forms encoded by two different genes, acetylcholinesterase and butyrylcholinesterase. While the biological role of butyrylcholinesterase is not fully defined, acetylcholinesterase hydrolyzes acetylcholine at cholinergic synapses [2]. Acetylcholinesterase is found in muscle, lungs, spleen, neurons, brain gray matter, bone marrow and placenta [3]. This enzyme is an elliptical α/β protein polymer with a 12-stranded central mixed β-sheet surrounded by 14 α-helices. When the catalytic domains of other serine proteases are analyzed, there

are similarities and the catalytic domain has a serine-histidine-glutamate triplet structure [4].

When we look at its role in the brain, acetylcholinesterase is the enzyme that hydrolyzes acetylcholine, which is responsible for the passage of the stimulus between neurons and prevents the post-synapse passage of the stimulus [5].

Looking at this role of acetylcholinesterase, it is an important enzyme despite its secondary position after and before the synapse [6,7]. This enzyme terminates neurotransmission in the nervous system and leads to the accumulation of acetylcholine, overstimulation of various receptors and consequent impairment of neurotransmission. Synthesized acetylcholinesterase inhibitor drugs have attempted to reverse these conditions by inhibiting this enzyme. These inhibitory drugs are either reversible or irreversible. Reversible inhibitors are generally used in Alzheimer's disease, which we call neurodegenerative disorders. In today's pharmaceutical market, the approved active ingredients donepezil, rivastigmine and galantamine stand out [8].

Alzheimer's disease is the most common form of dementia. It is a progressive neurodegenerative disease that occurs at the onset of dementia. In this case, there is an initial cognitive decline, followed by speech, visual changes, and motor systems are affected. It is an extremely bad condition [9]. This disease is associated with aging and causes severe deficiencies in choline acetyltransferase activity in the cerebral cortex. These patients have reduced cholinergic activity, so acetylcholinesterase inhibitors are used to increase cholinergic activity and improve cognitive function. The most promising treatment for this disease is to try to increase the acetylcholine neurotransmitter in the brain. But of course, side effects such as hepatotoxicity develop with the drugs [10].

Thiadiazole and benzodioxole rings are compounds that exhibit a wide range of biological activities, especially known to be effective on acetylcholinesterase. It is found in the structure of compounds can be used in the treatment of Alzheimer disease [11-15]. When previous studies were examined, acetylcholinesterase inhibition activity was observed in many compounds containing thiadiazole and benzodioxole rings [16, 17].

In this study, a new compound containing thiadiazole and benzodioxole rings was synthesized, its structure was determined, molecular docking studies and biological evaluation of their acetylcholinesterase inhibition were carried out.

2. MATERIALS AND METHODS

2.1. Chemistry

All reagents are purchased from chemical suppliers (Sigma-Aldrich Corp., USA or Merck KGaA, Germany). NMR spectroscopy was recorded ^{1}H-NMR 300 MHz FT-NMR spectrometer; ^{13}C-NMR, 75 MHz spectrometer (Bruker Bioscience, Billerica, MA, USA) in DMSO-d_6. In the NMR spectra splitting patterns were represented as follows: singlet (s); doublet (d); triplet (t). *J* values were expressed as Hertz. Mass spectra were recorded on a LCMS-IT-TOF (Shimadzu, Kyoto, Japan) using ESI. Melting degree determination was determined with the Mettler Toledo-MP90 (Greifensee, Switzerland).

2.1.1. Synthesis of N-(benzo[d][1,3]dioxol-5-ylmethyl)hydrazinecarbothioamide (1)

5-(Isothiocyanatomethyl)benzo[*d*][1,3]dioxole (0.012 mol, 2.32 g) and hydrazine hydrate (0.015 mol) were dissolved in separate beakers by adding ethanol. The hydrazine hydrated mixture was added dropwise to the other mixture in an ice bath environment. At the end of the reaction, the precipitated product was filtered, washed with ethanol and dried.

2.1.2. Synthesis of 5-((benzo[d][1,3]dioxol-5-ylmethyl)amino)-1,3,4-thiadiazole-2-thiol (2)

N-(Benzo[*d*][1,3]dioxol-5-ylmethyl) hydrazinecarbotioamide (**1**) (0.009 mol, 2.025 g) was dissolved in ethanol. Sodium hydroxide (0.012 mol) and carbon disulfide (0.012 mol) were added and this mixture was refluxed for 12h. Afterwards, 20% HCl was added dropwise in an ice bath and the pH was adjusted to 4. At the end of the reaction,

the precipitated product was filtered, washed with ethanol and dried.

2.1.3. Synthesis of 2-chloro-N-(4-trifluoromethylphenyl)acetamide (3)

Chloroacetyl chloride (0.009 mol) was added dropwise to a mixture of 4-(trifluoromethyl) aniline (0.009 mol) and triethanolamine (10 mL) in tetrahydrofuran (10 mL) in an ice bath. After the reaction occurred, the resulting substance was purified and washed with water to remove the salt.

2.1.4. Synthesis of the target compound (4a)

5-((Benzo[*d*][1,3]dioxol-5-ylmethyl)amino)-1,3,4-thiadiazole-2-thiol (**2**) (0.007 mol, 1.87 g) and 2-chloro-*N*-(4-trifluoromethylphenyl)acetamide (**3**) (0.007 mol) in acetone were dissolved. K_2CO_3 was added to the mixture and was refluxed for 12 h. At the end of the reaction, the precipitated product was filtered, washed with ethanol and dried.

*2-((5-((Benzo[d][1,3]dioxol-5-ylmethyl)amino)-1,3,4-thiadiazol-2-yl)thio)-N-(4-(trifluoromethyl) phenyl)acetamide (**4a**)*

Yield: 83%, M.p: 120-123°C ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 4.04 (2H, s, -CH_2), 4.34 (2H, s, -CH_2), 5.92 (1H, s, -NH), 5.98 (2H, s, -CH_2, 1,3-dioxole), 6.77 (1H, d, *J*= 4.6 Hz, benzodioxole), 6.81 (1H, s, benzodioxole), 6.85 (1H, d, *J*= 8.0 Hz, benzodioxole), 6.89 (1H, s, -CONH), 7.68 (2H, d, *J*= 8.7 Hz, trifluoromethylphenyl), 7.78 (2H, d, *J*= 8.5 Hz, trifluoromethylphenyl). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 37.28, 48.09, 48.92, 99.02, 101.35, 103.67, 107.42, 109.61, 118.40, 118.47, 120.30, 120.67, 122.42, 125.54, 127.68, 142.98, 146.90, 166.99, 170.07. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{15}F_3N_4O_3S_2$: 469.0622; found 469.0610.

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

Compound	AChE IC_{50} (μM)	BChE IC_{50} (μM)
4a	0.114±0.005	>100
Donepezil	0.0201±0.0014	-
Tacrine	-	0.0064±0.0002

2.2. Acetylcholinesterase Inhibition Assay

The inhibitory activities of compound **4a** against AChE and BChE were determined by modified Ellman method using 96-well plate. The reference drugs in the method were Donepezil and Tacrine. Pipetting in the method was performed by Biotek Precision XS robotic system (USA). Percent inhibition values were measured at 412 nm by BioTek-Synergy H1 microplate reader (USA) [18]. First, compound **4a** was prepared at two different concentrations (10^{-3} and 10^{-4} M) using 2% DMSO and the inhibition potentials were measured. Then the compound was tested at higher concentrations (10^{-5}-10^{-9} M). Inhibition potencies of synthesized compound and IC_{50} of selected derivatives were calculated as reported previously. The results obtained are shown as mean ± standard deviation (SD) [19] (Table 1).

2.3. Prediction of ADME Parameters

SwissADME (online) were used for the prediction of ADME parameters of our synthesized compound [20] (Table 2).

Table 2. Predicted ADME parameters of compound **4a**

Comp	Physicochemical Properties							Lipo.	Druglikeness					Water Solubility		Pharmacokinetics	
	MW	Fsp3	RB	HBA	HBD	MR	TPSA	cLogP	Lipinski	Ghose	Veber	Egan	Muegge	LogS	Class	GI abs.	F
4a	468.47	0.21	9	8	2	110.32	138.91	4.13	+	+	+	-	+	-5.47	Moderately	Low	0.55

Comp: Compounds, MW: Molecular weight, Fsp3: Fraction Fsp3, RB: Number of rotatable bonds, HBA: Number of hydrogen bond acceptors, HBD: Number of hydrogen bond donors, MR: Molar refractivity, TPSA: Total polar surface area, Lipo: Lipophilicity, GI abs: Gastrointestinal absorption, F: Bioavailability score.

Table 3. Molecular docking scores, interaction types and estimated inhibition constants of synthesized compound and AChE (PDB ID: 4EY7)

Comp.	Autodock Results				Vina Results
	Interacting Residues	Interaction Types	Estimated Inhibition Constant, K_i	Best Docking Score	Best Docking Score
4a	Trp86	Pi-Pi Stacking	358.32 nM	-8.79	-10.6
	Tyr337	Pi-Pi Stacking			

nM: nanomolar, Docking Score: Estimated Free Energy of Binding (kcal/mol)

2.4. Molecular Docking Study

Molecular docking studies were performed using *in silico* procedure to define the binding modes of compound **4a** in the active regions of enzyme X-ray crystal structures of acetylcholinesterase (PDB ID: 4EY7) [21] were retrieved from Protein Data Bank server (https://www.rcsb.org/structure/4ey7, accessed 20.05.2024). Molecular docking studies were performed as previously reported [22-24]. For docking validation, co-crystallized ligand were re-docked onto target site of 4EY7 and RMSD value have been determined to be less than 0.3 for existing ligand E20 in macromolecule. In the receptor, waters around the previously identified active site E20 (8Å) were left and all other water molecules were removed. Preprocessing and H-Bond optimization for the macromolecule was performed using the Maestro program. Then, the resulting pdb formatted macromolecule was edited with the AutoDock program and saved in pdbqt format. The regular spacing of the grid boxes was determined to be 0.375 Å centered on E20 (40*40*40 Å3). Lamarckian Genetic Algorithm was preferred in the study, detailed results such as docking scores were obtained using both AutoDock 4.2 and AutoDock Vina software [25, 26] and are presented in Table 3 and Figure 1, 2.

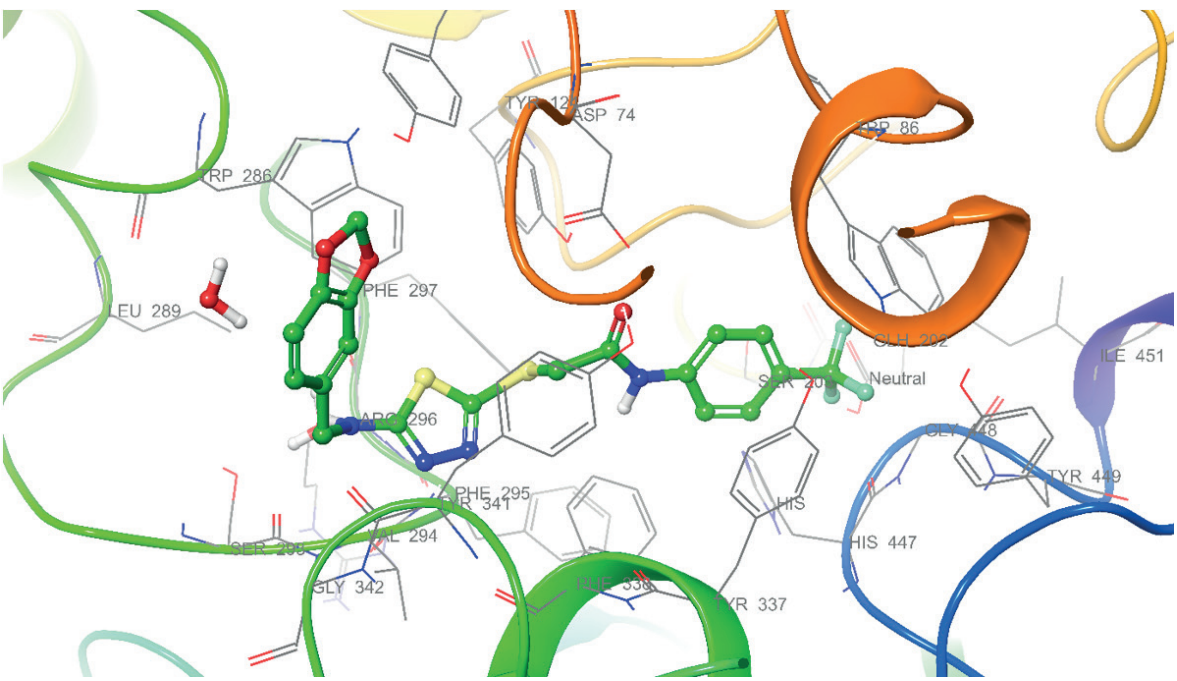


Figure 1. Localization of compound **4a** in the enzyme active site (PDB ID: 4EY7)

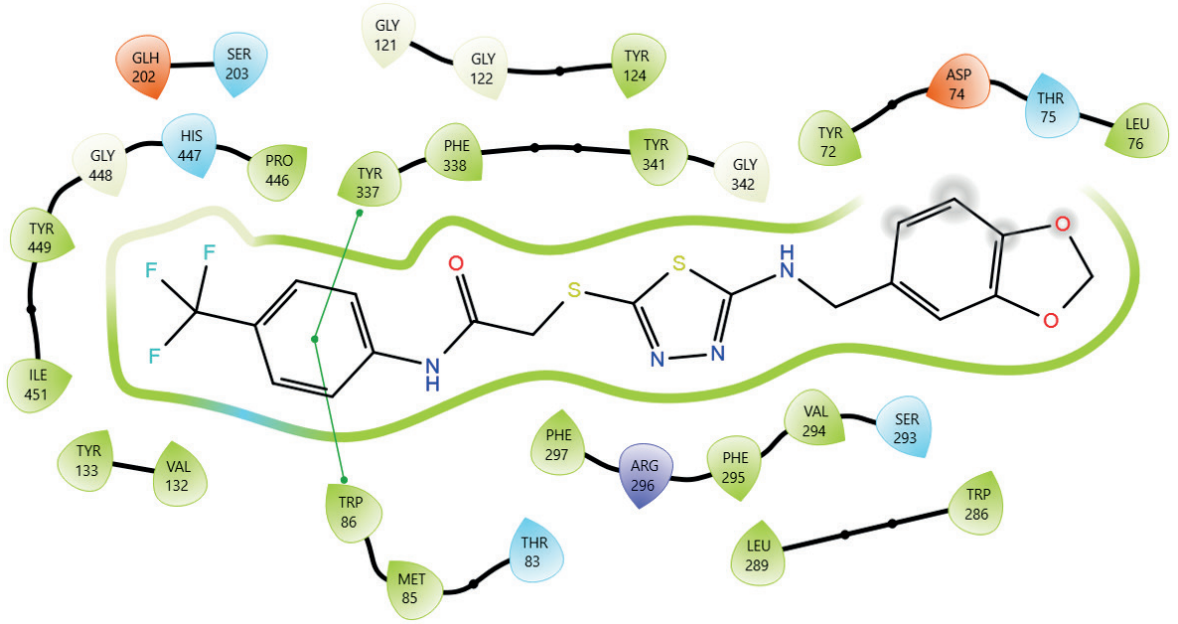
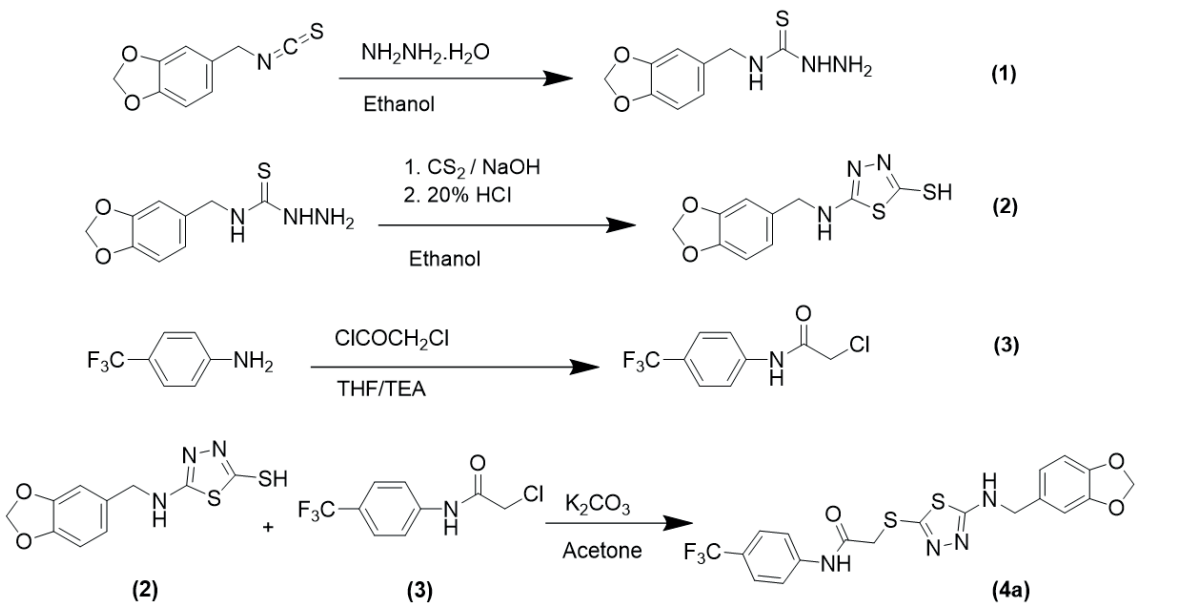


Figure 2. The three-dimensional interacting mode of compound **4a** in the active region of acetylcholinesterase enzyme (PDB ID: 4EY7)



Scheme 1. Synthesis pathway for compound **4a**

3. RESULTS AND DISCUSSION

3.1. Chemistry

Compound **4a** was obtained as presented in Scheme 1. Initially, hydrazinecarbothioamide derivative **(1)** was obtained as a result of the reaction of 5-(isothiocyanato-methyl)benzo[*d*][1,3]dioxol with hydrazine hydrate. Secondly, *N*-(Benzo[*d*][1,3]dioxol-5-ylmethyl)hydrazinecarbothioamide underwent ring closure reaction to obtain thiadiazole derivative (**2**). As the third, 4-trifluoromethylphenyl was reacted with chloroacetylchloride to obtain

2-Chloro-*N*-(4-trifluoromethyl phenyl)acetamide (**3**). Target compound **4a**, 5-((Benzo[*d*][1,3]dioxol-5-ylmethyl)amino)-1,3,4-thiadiazole-2-thiol (**2**) and 2-Chloro-*N*-(4-trifluoromethylphenyl)acetamide (**3**) was obtained by reaction. The structure of compound **4a** was evaluated using spectroscopic methods (HRMS, ^{1}H-NMR and ^{13}C-NMR) (Figures 3-5).

When the NMR data of the compound are examined, it is seen that the protons of methylene groups observed between 4.04 ppm and 4.34 ppm with 2H and 2H. Proton of the amine group were recorded singlet 5.92 ppm. Methyl group of inside benzodioxole come 5.98 ppm and 2H singlet. When benzodioxole ring was examined 3H protons were observed between 6.77 and 6.85 ppm. Trifluoromethylphenyl ring protons were detected as 2H-2H between 7.68-7.68 ppm. When carbon NMR peaks are examined its seen that all carbon peaks are detected as we expect. Aromatic carbon peaks were observed between 110-170 ppm. Moreover, the carbonyl peak we expected around 165 ppm was observed at 166.99 ppm. Most importantly our compound was examined with mass spectra, which is equipped with high resolution liquid chromatography system, and its HRMS data was found to be compatible with its molecular weight.

3.2. Acetylcholinesterase Inhibition Assay

Synthesized compounds were tested at 10^{-3} M and 10^{-4} M concentrations. The inhibition of acetylcholinesterase and butyrylcholinesterase at the initial concentrations of the resulting compound **4a**, donepezil and tacrine is shown in Table 1. According to the activity results, the obtained compound showed higher inhibition activity on AChE than BChE. The IC_{50} value was calculated as 0.0201±0.0014 µM for donepezil. The synthesized compound **4a** gave an IC_{50} value of 0.114±0.005 µM. The 4-substituted phenyl group activity also played an important role.

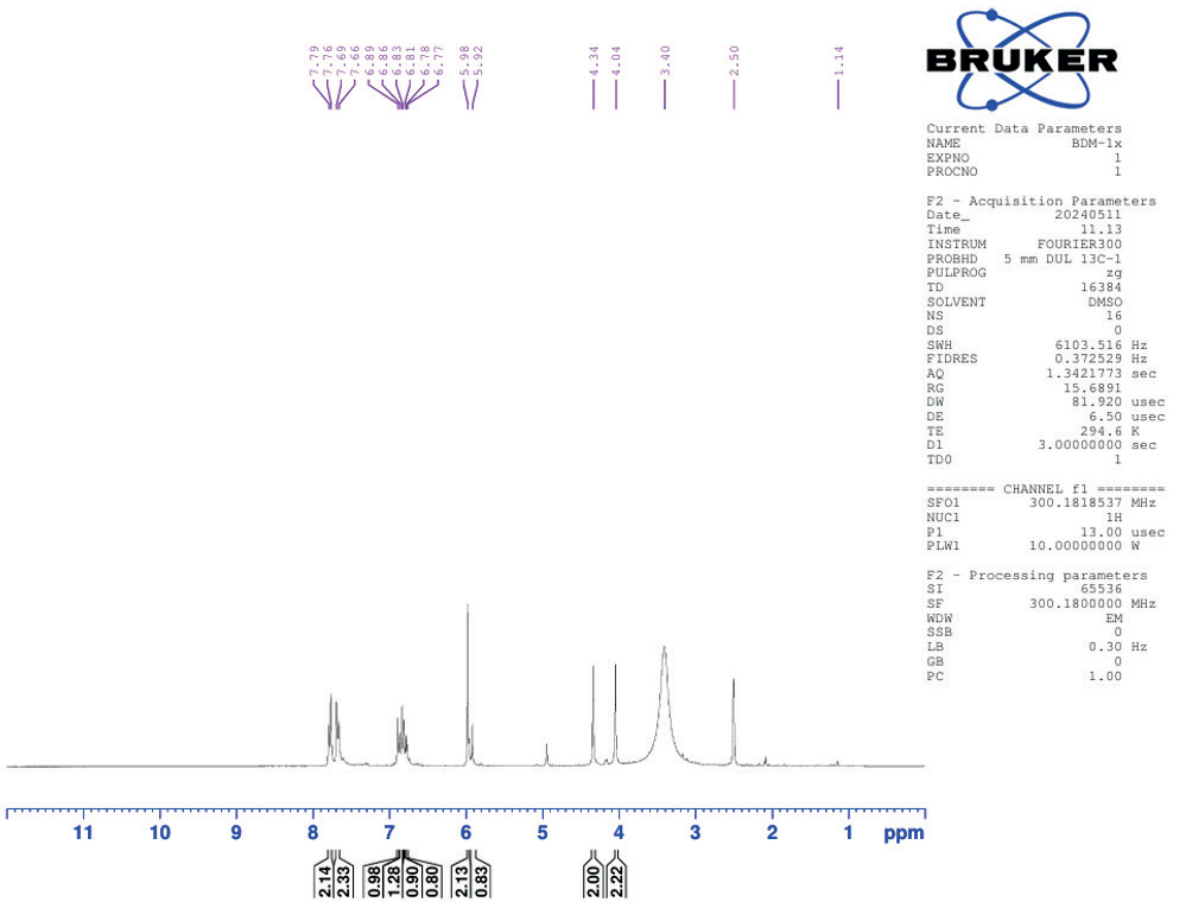


Figure 3. ^{1}H-NMR spectrum of compound **4a**

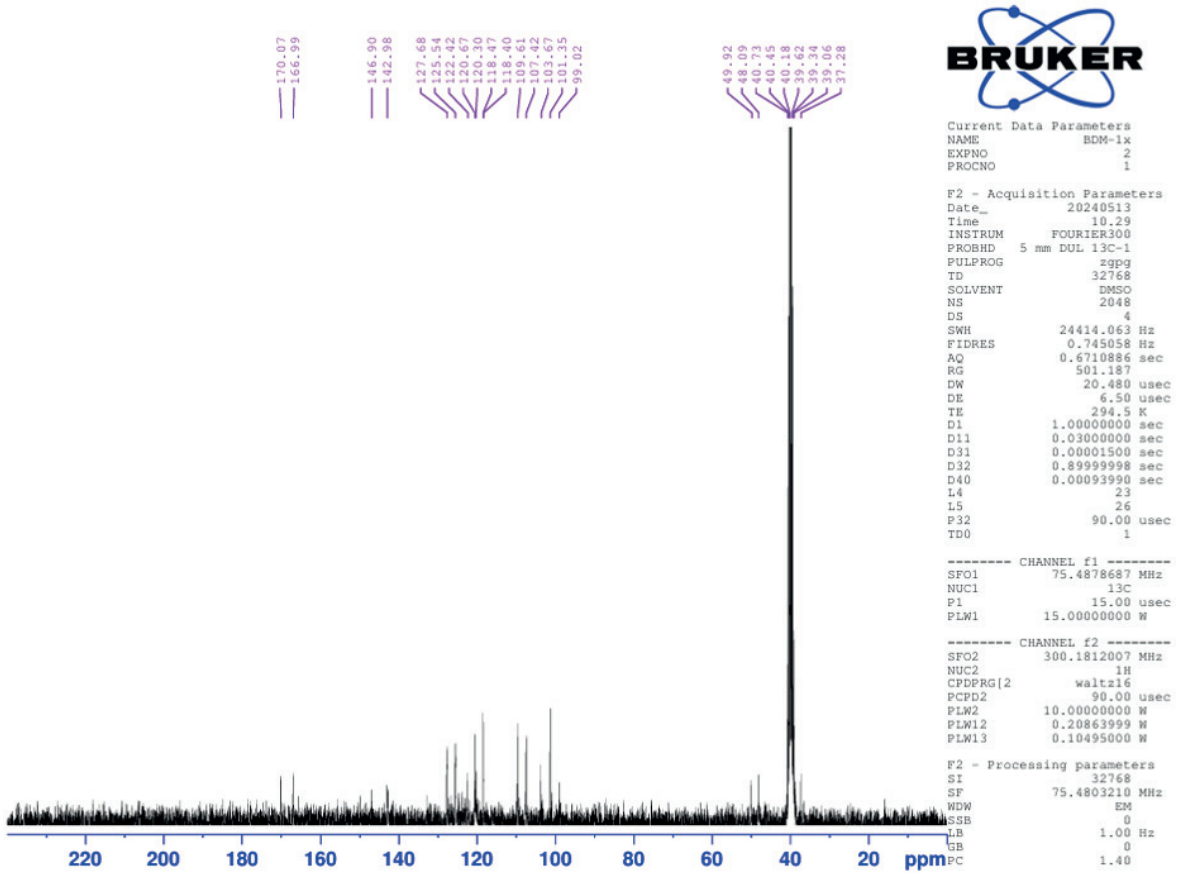


Figure 4. ^{13}C-NMR spectrum of compound **4a**

The results demonstrated that our compound IC_{50} values similar activities compared to the reference drug against AChE.

3.3. Prediction of ADME Parameters

The online SwissADME was used and the estimated ADME parameters of the obtained compound were calculated [20]. Looking at Table 2 showing the results, It was observed that none of the synthesized compounds violated any other drug rules except Egan's rule [27]. Absorption from the gastrointestinal tract provides a preliminary idea about the oral use of the synthesized compound. When the table was examined, it was seen that the absorption of the compound was low. Log S values of the compound is -5.47, and their solubility is estimated to be moderate. The F value, which shows the oral bioavailability of the compound, is 0.55, which is the ideal value [28].

3.4. Molecular Docking Studies

To elucidate the acetylcholinesterase inhibition of compound **4a**, it was subjected to *in silico* insertion procedure with 4EY7 [21]. Active site interactions of the synthesized compound are shown Figure 1-2.

The interaction domain of acetylcholinesterase and its cocrystal ligand Donepezil (PDB ID: E20) has been previously revealed, Tyr72, Trp86, Tyr124, Glu202, Trp286, Ser293, Phe295, Phe297, Tyr337, Phe338, Tyr341, His447 were emphasis to be important for the interaction (https://www.ebi.ac.uk/pdbe/entry/pdb/4ey7/bound/E20#604A).

When the docking poses of compound **4a** were examined, it was clearly seen that it had many interactions such as H-bond and pi-pi stacking with macromolecule. The pi-pi stacking was detected in the 4-trifluoromethylphenyl (Trp86 and Tyr337)

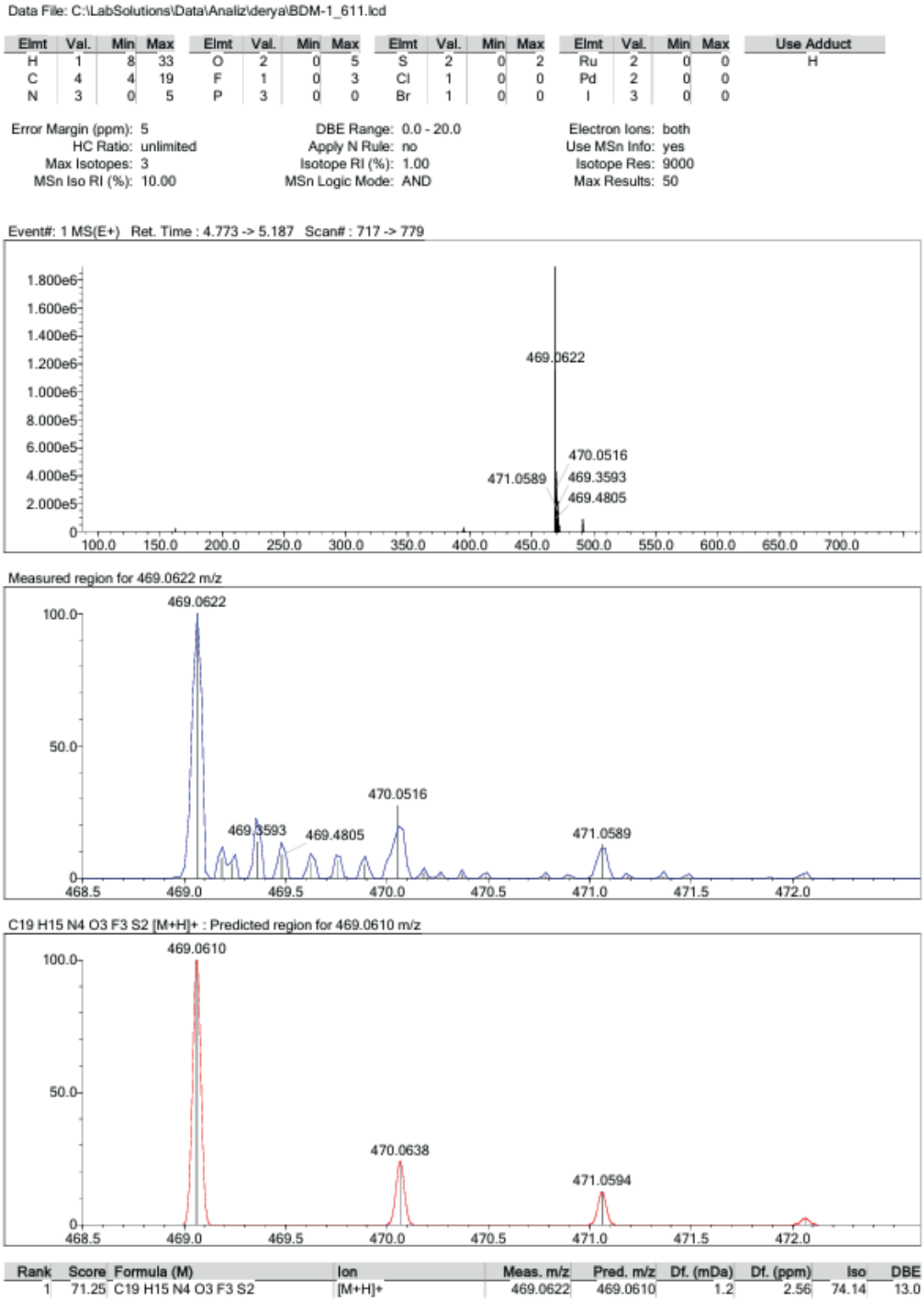


Figure 5. HRMS report of compound **4a**

ring of compound **4a**. Also, there was an H-bond interaction between the benzo[*d*][1,3]dioxol ring and HOH953 (Figures 1, 2). In this study, it was determined that compound **4a** interacted with these residues of acetylcholinesterase in a similar way.

4. CONCLUSION

A decrease in this neurotransmitter in the brain also causes Alzheimer's disease. Previous studies have shown that compounds containing thiadiazole rings have an acetylcholinesterase inhibition effect. In this study, a compound containing benzodioxole and thiadiazole rings (**4a**) was designed, and synthesized, and its acetylcholinesterase and butylcholinesterase inhibition activities were compared with the active ingredients donepezil and tacrine. Compound **4a** showed IC_{50} value of 0.114±0.005 µM against AChE (IC_{50} value of donepezil: 0.0201±0.0014 µM). No significant values were noted for compound **4a** against butyrylcholinesterase. *In silico* studies, the interactions of compound **4a** with the active site of acetylcholinesterease were examined and it was observed that it interacted with Trp86 and Tyr337 residues, which are known to be important in inhibition. In conclusion, this study provides a result that may be important for the development of new agents for neurodegenerative diseases such as Alzheimer's disease.

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Ethical approval

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

Author contribution

Conceptualization, D.O., H.U. and Y.Ö.; Methodology, S.P.G., S.L. and B.N.S.Ö.; Software, D.O., B.N.S.Ö. and H.U.; Formal analysis, S.P.G., D.O. and S.L.; Investigation, S.P.G.; Resources, B.G.; Writing—original draft preparation, S.P.G., D.O., B.G. and H.U.; Writing—review and editing, Y.Ö.; Supervision, Y.Ö. 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.

REFERENCES

1. Zou D, Liu R, Lv Y, Guo J, Zhang C, Xie Y. Latest advances in dual inhibitors of acetylcholinesterase and monoamine oxidase B against Alzheimer's disease. J Enzyme Inhib Med Chem. (2023); 38(1):2270781. https://doi.org/10.1080/14756366.2023.2270781
2. Talesa VN. Acetylcholinesterase in Alzheimer's disease. Mechanisms of ageing and development. (2001);122:1961-1969. https://doi.org/10.1016/S0047-6374(01)00309-8
3. Wilson IB, Nachmansohn D. Ion transport across membranes. Acedemic Pres Inc. New York, (1954); 35. https://doi.org/10.3390/molecules26237119
4. Osmaniye D, Kaplancıklı ZA. Synthesis and evaluation of biological activities of new piperazine derivatives against Alzheimer's Disease. J Health Sci. (2022);31:330-335. https://doi.org/10.34108/eujhs.979093
5. Demir Z, Türkan F. Association of acetylcholinesterase and butyrylcholinesterase enzymes with Alzheimer's disease. Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi. (2022); 12(4): 2386-2395. https://doi.org/10.21597/jist.1161271
6. Massoulié J, Sussman J, Bon S, Silman I. Structure and functions of acetylcholinesterase and butyrylcholinesterase. Prog Brain Res. 1993;98:139-146. https://doi.org/10.1016/S0079-6123(08)62391-2
7. Colović MB, Krstić DZ, Lazarević-Pašti TD, Bondžić AM, Vasić VM. Acetylcholinesterase Inhibitors: Pharmacology and toxicology. Curr Neuropharmacol. (2013);11:315-335. https://doi.org/10.2174/1570159X11311030006
8. Conger N, Osmaniye D, Sağlık BN, Levent S, Özkay Y, Kaplancıklı ZA. Design, synthesis, biological activities, and evaluation of molecular docking-dynamics studies of new thiosemicarbazones that may be effective against Alzheimer's disease. J Mol Recognit. (2023);36(12):e3059. https://doi.org/10.1002/jmr.3059

9. Kemal A, Şaik EB, Reddy GN, Kumar CG, Joseph J, Kumar GB, Purushotham U, Sastry GN. Synthesis, biological evaluation, and molecular modeling of (*E*)-2-aryl-5-styryl-1,3,4-oxadiazole derivatives as acetylcholine esterase inhibitors. Med Chem Res. (2014); 23:2080-2092. https://doi.org/10.1007/s00044-013-0786-y

10. Yıldırım ER, Güzeldemirci NU. Recent advances of cholinesterase inhibitors playing a critical role in the treatment of Alzheimer's disease. Istanbul University Press. (2023);6(2):197-209. https://doi.org/10.26650/JARHS2023-1197992

11. Jeong GS, Kaipakasseri S, Lee SR, Marraiki N, Batiha GES, Dev S, Kim H. Selected 1,3-benzodioxine-containing chalcones as multipotent oxidase and acetylcholinesterase inhibitors. Chem Med Chem. (2020);15(23):2257-2263. https://doi.org/10.1002/cmdc.202000491

12. Fernandes TB, Cunha MR, Sakata RP, Candido TM, Baby AR, Tavares MT, Parise FR. Synthesis, molecular modeling, and evaluation of novel sulfonylhydrazones as acetylcholinesterase inhibitors for Alzheimer's disease. Archiv der Pharmazie. (2017);350(11):1700163. https://doi.org/10.1002/ardp.201700163

13. Lotfi S, Rahmani T, Hatami M, Pouramiri B, Kermani ET, Rezvannejad E, Zahedifar M. Design, synthesis and biological assessment of acridine derivatives containing 1,3,4-thiadiazole moiety as novel selective acetylcholinesterase inhibitors. Bioorg Chem. (2020);105:104457. https://doi.org/10.1016/j.bioorg.2020.104457

14. Altıntop MD, Temel HE, Sever B, Akalın Çiftçi G, Kaplancıklı ZA. Synthesis and evaluation of new benzodioxole-based thiosemicarbazone derivatives as potential antitumor agents. Molecules. (2016);21(11):1598. https://doi.org/10.3390/molecules21111598

15. Özdemir A, Sever B, Altıntop MD. New benzodioxole-based pyrazoline derivatives: Synthesis and anticandidal, *in silico* ADME, molecular docking studies. Lett Drug Des Discov. (2019);16(1):82-92. https://doi.org/10.2174/1570180815666180326152726

16. Koçak EA, Sağlık NB, Özkay Y, Palaska E. Synthesis and biological evaluation of benzoxazolone-thiosemicarbazide, 1,2,4-triazole, 1,3,4-thiadiazole derivatives as cholinesterase inhibitors. Chemistry Select. (2023); 8(35):e202302069. https://doi.org/10.1002/slct.202302069

17. Altıntop MD, Özdemir A, Zitouni GT, Ilgın S, Atlı Ö, Demirci F, Kaplancıklı ZA. Synthesis and *in vitro* evaluation of thiadiazole derivatives as AChE, BuChE and LOX inhibitors. Lett Drug Des Discov. (2014);11(9):1062-1069. https://doi.org/10.3390/moleküller190914809

18. Sağlık BN, Ilgın S, Özkay Y. Synthesis of new donepezil analogues and investigation of their effects on cholinesterase enzymes. Eur J Med Chem. (2016);124:1026-1040. https://doi.org/10.1016/j.ejmech.2016.10.042

19. Özkay ÜD, Can ÖD, Sağlık NP, Çevik UA, Levent S, Özkay Y. Design, synthesis, and AChE inhibitory activity of new benzothiazole–piperazines. Bioorg Med Chem Lett. (2016);26(22):5387-5394. https://doi.org/10.1016/j.bmcl.2016.10.041

20. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. (2017);7:42717. https://doi.org/10.1038/srep42717

21. Cheung J, Rudolph MJ, Burshteyn F, Cassidy MS, Gary EN, Love J, Height JJ. Structures of human acetylcholinesterase in complex with pharmacologically important ligands. J Med Chem. (2012); 55(22):10282-10286. https://doi.org/10.1021/jm300871x

22. Taslimi P, Işık M, Türkan F, Durgun M, Türkeş C, Gülçin İ, Beydemir Ş. Benzenesulfonamide derivatives as potent acetylcholinesterase, α-glycosidase, and glutathione S-transferase inhibitors: Biological evaluation and molecular docking studies. J Biomol Struct Dyn. (2021);39(15):5449-5460. https://doi.org/10.1080/07391102.2020.1790422

23. Lolak N, Akocak S, Türkeş C, Taslimi P, Işık M, Beydemir Ş, Durgun M. Synthesis, characterization, inhibition effects, and molecular docking studies as acetylcholinesterase, α-glycosidase, and carbonic anhydrase inhibitors of novel benzenesulfonamides incorporating 1,3,5-triazine structural motifs. Bioorg Chem. (2020);100:103897. https://doi.org/10.1016/j.bioorg.2020.103897

24. Yaşar Ü, Gönül İ, Türkeş C, Demir Y, Beydemir Ş. Transition-metal complexes of bidentate schiff-base ligands: *in vitro* and *in silico* evaluation as non-classical carbonic anhydrase and potential acetylcholinesterase inhibitors. ChemistrySelect. (2021);6(29):7278-7284. https://doi.org/10.1002/slct.202102082

25. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem. (2009);30(16):2785-2791. https://doi.org/10.1002/jcc.21256

26. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. (2010);31(2):455-461. https://doi.org/10.1002/jcc.21334

27. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. (2012);64:4-17. https://doi.org/10.1016/S0169-409X(00)00129-0

28. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. iLOGP: a simple, robust, and efficient description of n-octanol/water partition coefficient for drug design using the GB/SA approach. J Chem Inf Model. (2014);54(12):3284-3301. https://doi.org/10.1016/s0169-409x(00)00129-0