Taylor & Francis
Taylor & Francis Group

Journal of Biomolecular Structure and Dynamics

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tbsd20

The impact of some phenolic compounds on serum acetylcholinesterase: kinetic analysis of an enzyme/inhibitor interaction and molecular docking study

Mesut Işık & Şükrü Beydemir

To cite this article: Mesut Işık & Şükrü Beydemir (2021) The impact of some phenolic compounds on serum acetylcholinesterase: kinetic analysis of an enzyme/inhibitor interaction and molecular docking study, Journal of Biomolecular Structure and Dynamics, 39:17, 6515-6523, DOI: 10.1080/07391102.2020.1801509

To link to this article: https://doi.org/10.1080/07391102.2020.1801509

JOURNAL OF BIOMOLECULAR STRUCTURE AND DYNAMICS
2021, VOL. 39, NO. 17, 6515–6523
https://doi.org/10.1080/07391102.2020.1801509

Taylor & Francis
Taylor & Francis Group

The impact of some phenolic compounds on serum acetylcholinesterase: kinetic analysis of an enzyme/inhibitor interaction and molecular docking study

Mesut Işık[a] and Şükrü Beydemir[b,c]

[a]Department of Pharmacy Services, Vocational School of Health Services, Harran University, Şanlıurfa, Turkey; [b]Department of Biochemistry, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey; [c]The Rectorate of Bilecik Şeyh Edebali University, Bilecik, Turkey

Communicated by Ramaswamy H. Sarma

ABSTRACT

In the treatment of Alzheimer's disease (AD), it is important to develop alternative cholinesterase inhibitors with antioxidant properties that will reduce acetylcholine deficiency and free radical formation. The aim of this study was to investigate the effect of hydroquinone, 4-hydroxybenzoic acid, 3,5-dihydroxybenzoic acid, caffeic acid, vanillic acid and chlorogenic acid against acetylcholinesterase (AChE), partially purified from serum. Binding of compounds with effective inhibitory potential to the AChE active site as competitive was illuminated by molecular docking. Hydroquinone, chlorogenic acid and 4-hydroxybenzoic acid have been found to have higher inhibitory potential than others against the AChE. IC_{50} and K_I values of the phenolic compounds against AChE were found in the range of 0.26 ± 0.01–36.34 ± 2.72 mM and 0.72 ± 0.00–29.23 ± 2.62 mM, respectively. The effectiveness of the compounds has been associated with its structure. Consequently, the phenolic compounds, which have AChE inhibitory potential and antioxidant properties, can be considered as alternative drugs in the treatment of AD.

ARTICLE HISTORY
Received 14 May 2020
Accepted 21 July 2020

KEYWORDS
Alzheimer disease; phenolic compounds; molecular docking; acetylcholinesterase inhibitor

1. Introduction

Studies have reported that oxidative stress, defined as disruption of balance of the oxidant-antioxidant system due to oxidative metabolic reactions and their byproducts, has an important role in the formation of neurodegenerative diseases such as Alzheimer's disease (AD) (Işık & Beydemir, 2019; Praticò & Delanty, 2000). The nervous system and brain are considered to be particularly vulnerable to oxidative stress due to metabolic oxygen consumption of 20%, limited antioxidant capacity, and insufficient glutathione synthesis of neurons. According to the cholinergic hypothesis, AD is also associated with the exchange and degeneration of cholinergic markers such as acetylcholinesterase (AChE) and choline acetyltransferase, which are involved in cholinesterase metabolism (Işık, 2019; Oboh et al., 2013). The AChE (E.C. 3.1.1.7), the choline esters hydrolyzer, is located in the brain, lymphocytes, serum, erythrocytes and neuromuscular junction, and plays an active role in the regulation of physiological events involving the acetylcholine (ACh) cycle in cholinergic metabolism (Anglister et al., 2008; Anwar et al. 2012; Işık et al., 2017; Taslimi et al., 2020). This enzyme is one of the most important enzymes that rapidly hydrolyze neurotransmitter ACh in the neuromuscular junction and cholinergic synapses in the cycle (Anglister et al., 2008; Rao et al., 2007). Also, AChE has been reported to increase the excess aggregation of β-amyloid to growing fibrils. Many studies have stated that there is an important relationship between these B-amyloid fibrils and AD (Szwajgier, 2013). Therefore, it is important to use compounds with AChE inhibitory effects in the treatment of AD (Durgun et al., 2020; Işık, Akocak et al., 2020). It is known that most of the drugs used today have side effects. Therefore, it is demanded to develop and use AChE inhibitors that are new and known for their antioxidant properties (Scozzafava et al., 2015).

Phenolic compounds that form the most important groups of antioxidants contain hydroxyl groups bound to the benzene ring and its derivative (Kähkönen et al., 1999; Moure et al., 2001). These compounds containing aromatic rings prevent the oxidation of biomolecules such as protein, lipid, carbohydrate, nucleic acids by free radicals (Burda & Oleszek, 2001; Isik et al., 2015). These phenolic acids, which are abundant in plants, are also used as preservatives to extend the shelf life of foods (Robbins, 2003; Takım & Işık, 2020). This property of phenolic acids and their derivatives depends on the location and number of hydroxyl groups bound to the aromatic ring (Gülcin, 2012). In many studies, it has been stated that phenolic acids can be used in the treatment of diseases due to their important bioactivities such as anti-allergic, anti-inflammatory, anticancer, antimicrobial, antioxidant, antithrombotic and vasodilatory (Balasundram et al., 2006; Mattila & Kumpulainen, 2002). Moreover, phenolic acids also have anti-Alzheimer's properties (Cheng et al., 2008; Yan et al., 2001).

The study was investigated the inhibitory effect of caffeic acid, 3,5-dihydroxybenzoic acid, 4-hydroxybenzoic acid,

CONTACT Şükrü Beydemir sukrubeydemir@anadolu.edu.tr; beydemirs@gmail.com Department of Biochemistry, Faculty of Pharmacy, Anadolu University, Eskişehir 26470, Turkey.

vanillic acid, hydroquinone and chlorogenic acid on serum AChE, and then determined kinetics properties of its. The binding mechanism of compounds that are actively bound to the enzyme as competitive was explained by molecular docking. Moreover, relationship between the structure ve activity of the phenolic compounds was evaluated.

2. Materials and methods

2.1. Chemicals

All commercially available reagents required for the study were obtained from Merck and Sigma.

2.2. Partial purification of AChE from human serum

The precipitation was carried out by slowly adding $(NH_4)_2S0_4$ to human serum at 0–20%, 20–40%, 40–60%, 60–80% and 80–100% ranges, respectively. After each precipitation, it was centrifuged for 20 min at +4 °C, 10,000 rpm. The precipitates obtained were dissolved in 0.1 M sodium phosphate (pH 7.4) buffer, containing 0.5% Triton X-100 and 1 mM EDTA. The activity of precipitate and supernatant were determined at each interval and then the precipitation range was determined as 20–80% as in our previous assays (Işık, 2019).

The sample obtained as a result of precipitation was centrifuged at 10,000 rpm about 20 min. The pellet obtained from here was dissolved in sodium phosphate buffer (pH 7.4; 0.1 M), containing 0.5% Triton X-100 and 1 mM EDTA (Işık, 2019; Wright & Plummer, 1973). The obtained sample was first placed in the dialysis bag and then dialyzed for 1 h in dialysis buffer (50 mM Na-phosphate pH: 7.4) at +4 °C for three hours.

2.3. Measurement of AChE activity

The inhibitory effects of caffeic acid, 3,5-dihydroxybenzoic acid, 4-hydroxybenzoic acid, vanillic acid, hydroquinone and chlorogenic acid on AChE enzymes as *in vitro* were tested by Ellman's spectrophotometric method (Ellman et al., 1961). First, 100 µl of Tris–HCl solution (1 M, pH 8.0), 50 µl 5,5′-dithio-bis(2-nitro-benzoic)acid compound (DTNB) and 50 µl of partially purified AChE solution were incubated and mixed for 15 min at 30 °C. Next, the reaction was started by adding 50 µl of substrates acetylthiocholine iodide (AChI). The enzymatic hydrolysis of the substrate was determined as spectrophotometrically at 412 nm (Işık, Demir et al., 2020; Taslimi et al., 2019).

2.4. Kinetic study

Inhibitory effects of the phenolic compounds on AChE partially purified from serum were screened. The kinetic studies were determined by using five concentrations of AChI and three concentrations of the phenolic compounds. The inhibition types for each compound, the IC_{50} values (Akbaba et al., 2013; Demir, 2019; Türkeş et al., 2013) and the K_I values with Lineweaver-Burk curves (Demir, 2020; Demir et al., 2017; Türkeş et al., 2015; Yamali et al., 2018) were determined as in the previous studies of our group (Işık, Beydemir et al., 2020; Türkeş, Demir et al., 2019; Türkeş et al., 2014; Türkeş et al., 2016).

Table 1. Kinetic values and types of inhibition of some phenolic compounds on AChE.

Inhibitor	IC_{50} (mM)	R^2	K_I (mM)	R^2
Hydroquinone	0.26 ± 0.01	0.978	0.72 ± 0.01	0.971
Chlorogenic acid	0.41 ± 0.01	0.964	0.90 ± 0.01	0.968
4-Hydroxybenzoic acid	11.04 ± 1.53	0.962	1.45 ± 0.10	0.954
Vanillic acid	9.23 ± 1.37	0.951	9.94 ± 0.09	0.967
Caffeic acid	16.80 ± 1.43	0.933	12.42 ± 1.04	0.945
3,5-Dihydroxybenzoic acid	36.34 ± 2.72	0.942	29.23 ± 2.62	0.968

2.5. Molecular docking study

3D structure of AChE (PDB ID: 4EY5; Resolution: 2.30 Å, R-Value Free: 0.21, R-Value Work: 0.18, R-Value Observed: 0.18) (Cheung et al., 2012) was retrieved from Protein Data Bank (Türkeş, Arslan et al., 2019) (www.rcsb.org) as a docking protein. The X-ray crystal structure of 4EY5 was present in the form of a homodimer chain; hence, its chain A was chosen for *in silico* studies. Molecular docking studies were performed on Schrödinger Suite Release 2019-4 for Mac (Schrödinger, LLC, New York, NY, 2019). Firstly, the structure of the ligands was sketched in the ChemDraw software (Lolak et al., 2020) version 19.0 for Mac (PerkinElmer, Inc., Waltham, MA, USA). Then, Protein Preparation Wizard (Beydemir et al., 2019) and LigPrep (Türkeş, 2019a) were utilized to prepare enzymes and ligands, respectively. Protein-energy was minimized with the Root Mean Square Deviation (RMSD) value of 0.3 Å using the OPLS3e force field (Türkeş et al., 2020). The protonation states of ligands were generated utilizing Epik (Demir et al., 2020) at pH value 7.4 ± 0.5 (Türkeş, 2019b; Türkeş & Beydemir, 2020). The grid box for 4EY5 was set to using the Receptor Grid Generation module (Türkeş, 2019c) to cover all residues locating within 10 Å in the centroid of native ligand (HUP: Huperzine A, $C_{15}H_{18}N_2O$). Finally, the Glide standard precision (SP) mode (Gündoğdu et al., 2019; Türkeş, Beydemir et al., 2019) was employed for the investigation of potential bioactive mechanisms of these inhibitors. Also, the Prime MM-GBSA module (Istrefi et al., 2020) was utilized to calculate binding affinities between the inhibitors' superior conformations and protein.

2.6. Statistical study

Analysis of the data were realized using GraphPad Prism version 6, GraphPad Software, La Jolla, CA, USA. The results were exhibited as mean ± standard deviation (95% confidence intervals).

3. Results and discussion

Alzheimer's disease (AD), a neurodegenerative disease, disrupts the balance in the cholinergic system, leading to lower levels of acetylcholine (ACh) release. One of the methods to be used to increase ACh levels is the suppression of the AChE enzyme that

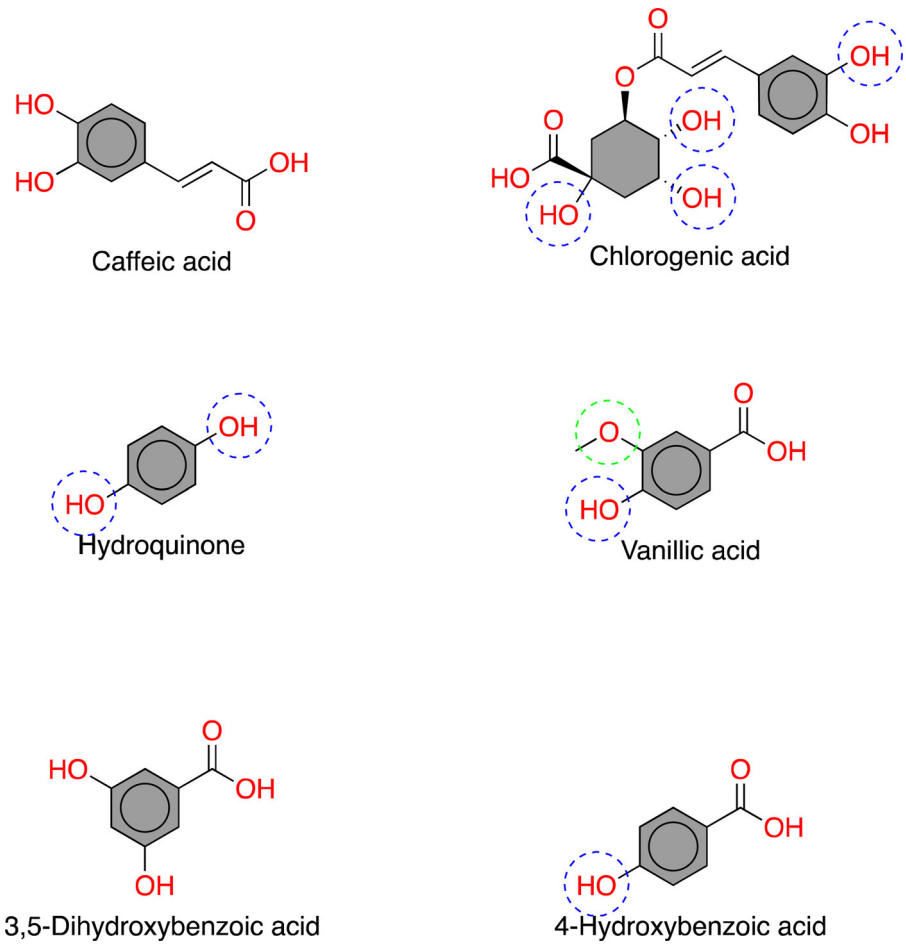

Figure 1. Chemicals structures of phenolic compounds.

hydrolyzes the ACh. Studies have shown that reducing neurotransmitter ACh hydrolysis can improve cognitive impairment in the early stages of AD. For this purpose, cholinesterase enzyme inhibitors are mostly used to balance the amount of ACh (Ha, 2002; Işık, 2020; Thacker, 2003). The AChE has an effect on coronary artery disease as it hydrolyzes ACh, which provides flexibility within blood vessels (Collins et al., 1995). According to some studies, cholinergic systems have been reported to be related to the regulation of angiogenesis. It has been stated that some AChE inhibitors that have a regulatory effect in the cholinergic system used for therapeutic purposes in AD also play a role in the regulation of angiogenesis in cardiovascular patients (Kakinuma et al., 2010). Since Alzheimer's and coronary artery diseases are associated with ACh deficiency, some drugs that inhibit AChE enzyme are used in the treatment of the diseases. Drugs that inhibit the AChE enzyme are called cholinesterase inhibitors or anticholinesterases. Cholinesterase inhibitors regulate angiogenesis and peripheral cholinergic function by inhibiting AChE activity. The inhibitors inhibit the enzyme reversibly or irreversibly and prevent ACh hydrolysis.

Oxidative stress is known to be an important neurodegenerative factor that increases with age and leads to neuronal loss (Butterfield & Lauderback, 2002). According to the amyloid cascade hypothesis, it is known that there is a relationship between increased oxidative stress and amyloid β (Aβ) formation in AD patients. This Aβ interacts with vascular endothelial cells, producing superoxide radicals and oxidizing agents that cause lipid peroxidation. This indicates that Aβ contributes to free radical production and can cause neurodegenerative diseases (Christen, 2000). Therefore, recently, studies on the development of inhibitors that both reduce Aβ fibril formation and regulate the cholinergic system are important.

Phenolic acids may appear to be weak inhibitors, compared to compounds used for therapeutic purposes in AD (Giacobini, 2004; Sabbagh et al., 2006). Eserine, the known AChE inhibitor acts at nM level, while phenolic acids are effective at mM level. Although their effectiveness is low, they have some advantages over well-known inhibitors used in current therapy: these compounds with antioxidant properties are thought to play an important role in reducing the formation of Aβ caused by oxidative stress. Moreover, they are abundant in foods (Szwajgier, 2013). Therefore, it is important to know the effects of the compounds found in foods in the medical treatment of AD.

In this study, the inhibition effects of vanillic acid, 3,5-dihydroxybenzoic acid, caffeic acid, 4-hydroxybenzoic acid, hydroquinone and chlorogenic acid on AChE were scanned. IC_{50} and K_I values of the compounds are given in Table 1. All of the compounds showed an inhibition effect against the enzyme. IC_{50} values of some some phenolic compounds against the AChE; the following order: hydroquinone (0.26 ± 0.01 mM, R^2: 0.978) > chlorogenic acid (0.41 ± 0.00 mM, R^2: 0.964) > vanillic acid (9.23 ± 1.37 mM, R^2: 0.951) > 4-hydroxybenzoic acid (11.04 ± 1.53 mM, R^2: 0.962) >

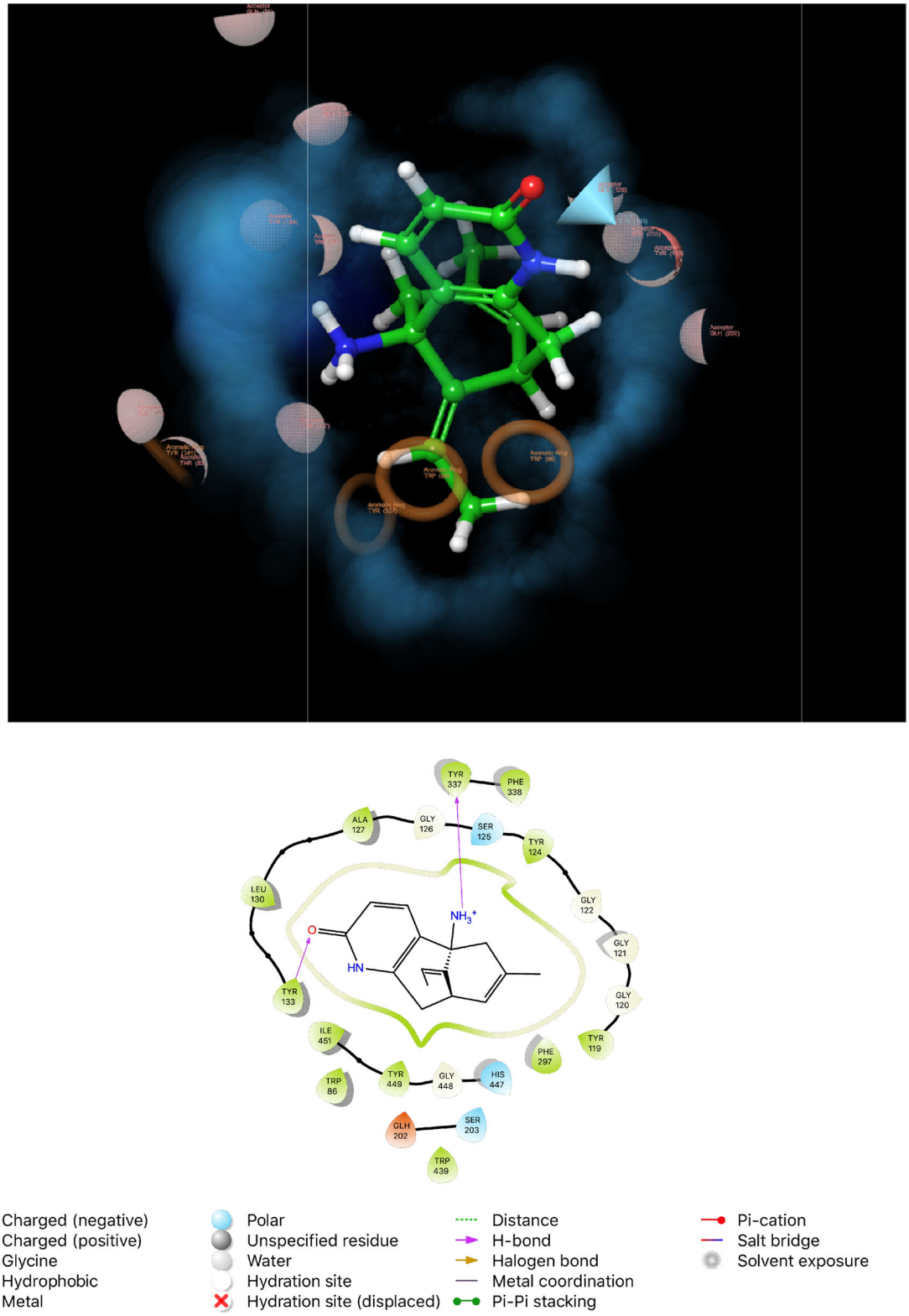

Figure 2. 3D and 2D schematic views of huperzine A in the binding site with AChE (PDB ID: 4EY5).

caffeic acid (16.80 ± 1.43 mM, R^2: 0.933) > 3,5-dihydroxybenzoic acid (36.34 ± 2.72 mM, R^2: 0.942). K_I values; the following order: hydroquinone (0.72 ± 0.00 mM, R^2: 0.971) > chlorogenic acid (0.9 ± 0.01 mM, R^2: 0.968) > 4-hydroxybenzoic acid (1.45 ± 0.1 mM, R^2: 0.954) > vanillic acid (9.94 ± 0.09 mM, R^2: 0.967) > caffeic acid (12.42 ± 1.04 mM, R^2: 0.945) > 3,5-dihydroxybenzoic acid (29.23 ± 2.62 mM, R^2: 0.968). Hydroquinone, chlorogenic acid and 4-hydroxybenzoic acid have been found to have higher inhibitory potential than others against the AChE. Hydroquinone, chlorogenic acid, vanillic acid and 3,5-dihydroxybenzoic acid showed an inhibition effect on AChE as noncompetitive, while caffeic acid and 4-hydroxybenzoic acid showed competitive inhibition. In noncompetitive inhibition, the compounds reduce the catalytic activity of the enzyme by binding to

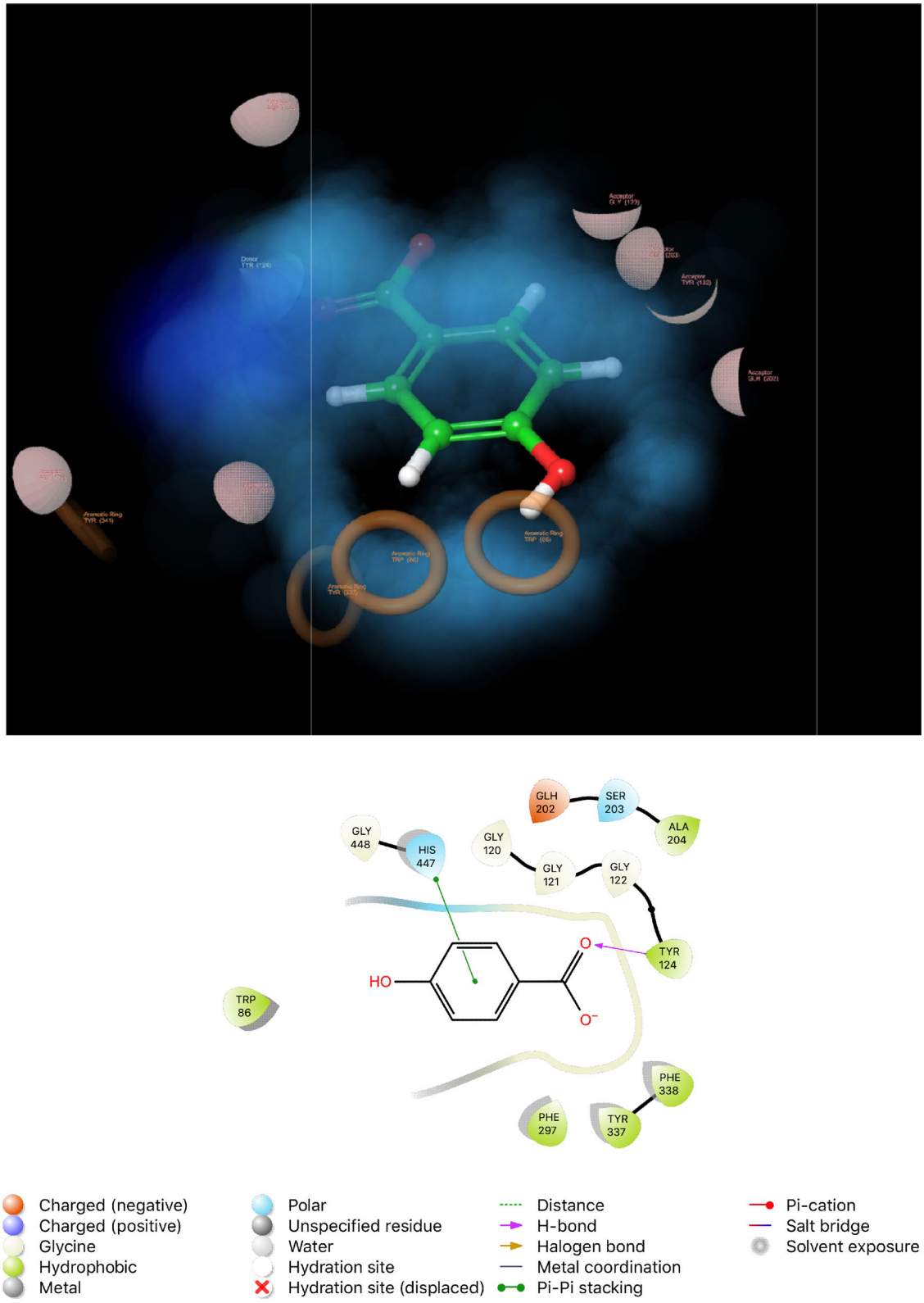

Figure 3. 3D and 2D schematic views of 4-hydroxybenzoic acid in the binding site with AChE (PDB ID: 4EY5).

an area outside the active region of the enzyme, while in competition, it causes the reduction of the catalytic activity by binding to the active region of the enzyme.

Studies have reported that the electron density in the ortho or para-position substitution –OH group and the bond energy between oxygen and hydrogen contribute significantly to the bioactivity of phenolic compounds. It is known that the functional group in the structure of benzoic acid and its derivatives has an impact on the effectiveness of the compound. The bioactivity of the compounds has been

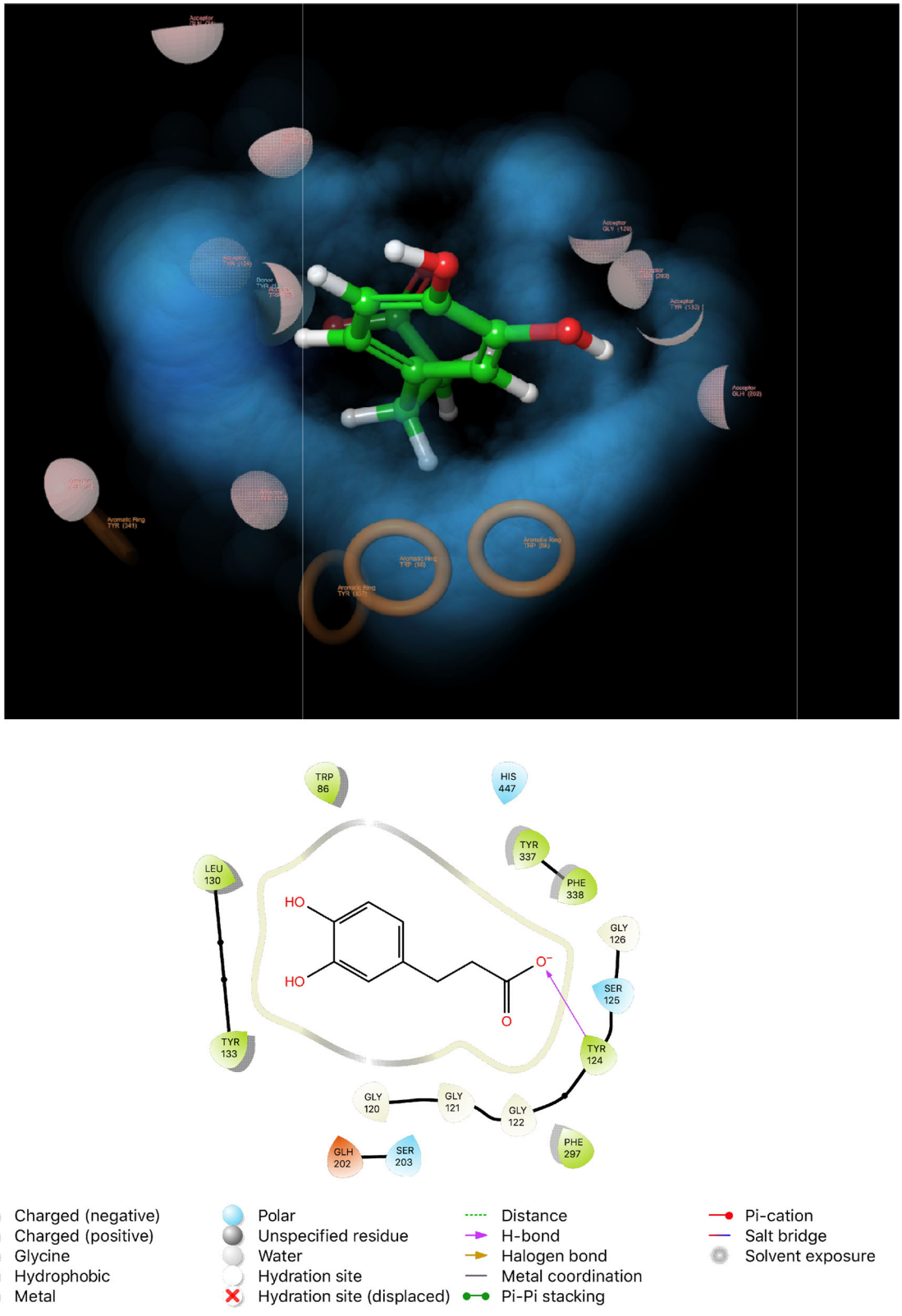

Figure 4. 3D and 2D schematic views of caffeic acid in the binding site with AChE (PDB ID: 4EY5).

reported to be related to the existing functional groups and the positions of the substituents on the aromatic ring. Hydroquinone, which contains two -OH groups, has high bioactivity. Molecules including 4-hydroxy-3-methoxyl or ortho-dihydroxyl groups have significantly higher activity than nonfunctional ones (Barclay et al., 1993; Cheng et al., 2007; Legrand et al., 2004). In our study, the reason why hydroquinone and 4 hydroxybenzoic acid are active AChE inhibitors may be due to OH groups in the ortho or para-position, as stated in the literature. The 4-hydroxybenzoic acids are thought to have a lower inhibition effect than hydroquinone due to activity-reducing properties of the

compound-bound carboxyl group. In addition, the methoxy group in vanillic acid structure has a partially activity-reducing effect, so this compound may have lower bioactivity than hydroquinone. Chlorogenic acid can have high inhibition activity because it contains a large number of functional groups to be attached to functional groups on the enzyme (Figure 1).

To detail the possible binding modes between phenolic compounds exhibiting competitive inhibition with AChE, the molecular docking study was performed using the Schrödinger Suite. Firstly, the native ligand HUP ($C_{15}H_{18}N_2O$, Huperzine A) was docked into the 4EY5, to verify whether the docking protocol was suitable for ligands docking. The RMSD value of re-docked co-ligand was calculated as 0.05 Å. According to the literature, the HUP displays two strong interactions, H-bond interaction with Tyr133 (1.87 Å) and Tyr337 (1.88 Å), and the docking score was −10.34 kcal/mol in the catalytic domain of 4EY5 (Figure 2). Then, the 4-hydroxybenzoic acid and caffeic acid determined as competitive inhibitors were docked into the active site of the enzyme AChE, to investigate the binding interactions of the compounds with the enzyme.

4-hydroxybenzoic acid displayed the best docking score result (−5.98 kcal/mol) compared to other compounds. Such lower values demonstrate good fitness of inhibitor or activator in the binding site of the target enzyme and formation of a stable ligand-receptor complex. 4-hydroxybenzoic acid interacted with Tyr124 (1.82 Å) through a strong H-binding and His447 through pi-pi stacking interaction at the 4EY5. Also, it composed hydrophobic contact with the amino acid residues Trp86, Ala204, Phe297, Tyr337 and Phe338 in the active site of AChE (Figure 3). Caffeic acid (docking score: −5.90 kcal/mol) showed only a strongly H-binding formation at the 4EY5 with Tyr124 (1.97 Å). Additionally, it showed hydrophobic interactions with Tyr119, Leu130, Tyr133, Phe297, Tyr337 and Phe338 (Figure 4).

Many studies have reported that phenolic compounds, reducing oxidative stress due to antioxidant properties have inhibitory effects on AChE. The inhibitory effect on the AChE of phenolic compounds strongly depends on the structure of a particular compound, especially the position and/or number of the C = O and OH groups (Szwajgier, 2013). Kwon et al reported that chlorogenic acid has an inhibition effect on AChE in the hippocampus and frontal cortex (IC_{50}: 98.17 μg/ml). It is also stated that chlorogenic acid plays a role in preventing lipid peroxidation in the hippocampus and frontal cortex (Kwon et al., 2010). *In vitro*, caffeic acid has an activation effect on AChE in the cerebral cortex, cerebellum, hypothalamus, whole blood and lymphocytes, while it has an inhibition effect at the concentrations of 0.5, 1.0, 1.5 and 2 mM in the muscles. *In vivo*, 50 and 100 mg/kg caffeic acid decreased AChE activity in the striatum and cerebral cortex, while increasing its activity in the lymphocytes, hypothalamus, hippocampus, cerebellum and muscles compared to the control group (Anwar et al. 2012). As stated in the literature, it is understood that the compounds have different effects on the AChE enzyme found in different tissues. In other words, while the compound causes enzyme inhibition in one tissue, it can activate the same enzyme in another tissue. In our study, the phenolic compounds showed an inhibition effect on AChE partially purified from serum.

The development of alternative cholinesterase inhibitors, which also have antioxidant properties, can eliminate ACh deficiency and free radical formation in AD. Therefore, it is thought that knowing the inhibition effect and binding mechanism of phenolic compounds on this enzyme may be clinically important in the treatment of neurodegenerative diseases.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Research Fund of Atatürk University (grant number 2014/182) and the Research Fund of Anadolu University (grant number 1610S681).

References

Akbaba, Y., Türkeş, C., Polat, L., Söyüt, H., Sahin, E., Menzek, A., Göksu, S., & Beydemir, S. (2013). Synthesis and paroxonase activities of novel bromophenols. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *28*(5), 1073–1079. https://doi.org/10.3109/14756366.2012.715287

Anglister, L., Etlin, A., Finkel, E., Durrant, A. R., & Lev-Tov, A. (2008). Cholinesterases in development and disease. *Chemico-Biological Interactions*, *175*(1-3), 92–100. https://doi.org/10.1016/j.cbi.2008.04.046

Anwar, J., Spanevello, R. M., Thomé, G., Stefanello, N., Schmatz, R., Gutierres, J., Vieira, J., Baldissarelli, J., Carvalho, F. B., da Rosa, M. M., Rubin, M. A., Fiorenza, A., Morsch, V. M., & Schetinger, M. R. C. (2012). Effects of caffeic acid on behavioral parameters and on the activity of acetylcholinesterase in different tissues from adult rats. *Pharmacology Biochemistry and Behavior*, *103*(2), 386–394. https://doi.org/10.1016/j.pbb.2012.09.006

Balasundram, N., Sundram, K., & Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry*, *99*(1), 191–203. https://doi.org/10.1016/j.foodchem.2005.07.042

Barclay, L. R. C., Vinqvist, M. R., Mukai, K., Itoh, S., & Morimoto, H. (1993). Chain-breaking phenolic antioxidants: Steric and electronic effects in polyalkylchromanols, tocopherol analogs, hydroquinones, and superior antioxidants of the polyalkylbenzochromanol and naphthofuran class. *The Journal of Organic Chemistry*, *58*(26), 7416–7420. https://doi.org/10.1021/jo00078a020

Beydemir, Ş., Türkeş, C., & Yalçın, A. (2019). Gadolinium-based contrast agents: In vitro paraoxonase 1 inhibition, in silico studies. *Drug and Chemical Toxicology*, 1–10. https://doi.org/10.1080/01480545.2019.1620266

Burda, S., & Oleszek, W. (2001). Antioxidant and antiradical activities of flavonoids. *Journal of Agricultural and Food Chemistry*, *49*(6), 2774–2779. https://doi.org/10.1021/jf001413m

Butterfield, D. A., & Lauderback, C. M. (2002). Lipid peroxidation and protein oxidation in Alzheimer's disease brain: Potential causes and consequences involving amyloid beta-peptide-associated free radical oxidative stress. *Free Radical Biology & Medicine*, *32*(11), 1050–1060. https://doi.org/10.1016/s0891-5849(02)00794-3

Cheng, C.-Y., Su, S.-Y., Tang, N.-Y., Ho, T.-Y., Chiang, S.-Y., & Hsieh, C.-L. (2008). Ferulic acid provides neuroprotection against oxidative stress-related apoptosis after cerebral ischemia/reperfusion injury by inhibiting ICAM-1 mRNA expression in rats. *Brain Research*, *1209*, 136–150. https://doi.org/10.1016/j.brainres.2008.02.090

Cheng, J.-C., Dai, F., Zhou, B., Yang, L., & Liu, Z.-L. (2007). Antioxidant activity of hydroxycinnamic acid derivatives in human low density lipoprotein: Mechanism and structure–activity relationship. *Food Chemistry*, *104*(1), 132–139. https://doi.org/10.1016/j.foodchem.2006.11.012

Cheung, J., Rudolph, M. J., Burshteyn, F., Cassidy, M. S., Gary, E. N., Love, J., Franklin, M. C., & Height, J. J. (2012). Structures of human acetylcholinesterase in complex with pharmacologically important ligands. *Journal of Medicinal Chemistry*, *55*(22), 10282–10286. https://doi.org/10.1021/jm300871x

Christen, Y. (2000). Oxidative stress and Alzheimer disease. *The American Journal of Clinical Nutrition*, *71*(2), 621S–629S. https://doi.org/10.1093/ajcn/71.2.621s

Collins, P., Rosano, G. M. C., Sarrel, P. M., Ulrich, L., Adamopoulos, S., Beale, C. M., McNeill, J. G., & Poole-Wilson, P. A. (1995). 17β-estradiol attenuates acetylcholine-induced coronary arterial constriction in women but not men with coronary heart disease. *Circulation*, *92*(1), 24–30. https://doi.org/10.1161/01.CIR.92.1.24

Demir, Y. (2019). The behaviour of some antihypertension drugs on human serum paraoxonase-1: An important protector enzyme against atherosclerosis. *The Journal of Pharmacy and Pharmacology*, *71*(10), 1576–1583. https://doi.org/10.1111/jphp.13144

Demir, Y. (2020). Naphthoquinones, benzoquinones, and anthraquinones: Molecular docking, ADME and inhibition studies on human serum paraoxonase-1 associated with cardiovascular diseases. *Drug Development Research*. https://doi.org/10.1002/ddr.21667

Demir, Y., Işık, M., Gülçin, İ., & Beydemir, Ş. (2017). Phenolic compounds inhibit the aldose reductase enzyme from the sheep kidney. *Journal of Biochemical and Molecular Toxicology*, *31*(9), e21936. https://doi.org/10.1002/jbt.21935

Demir, Y., Türkeş, C., & Beydemir, Ş. (2020). Molecular docking studies and inhibition properties of some antineoplastic agents against paraoxonase-I. *Anti-Cancer Agents in Medicinal Chemistry*, *20*(7), 887–896. https://doi.org/10.2174/1871520620666200218110645

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. (2020). Synthesis, characterization, biological evaluation and in silico studies of sulfonamide Schiff bases. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *35*(1), 950–962. https://doi.org/10.1080/14756366.2020.1746784

Ellman, G. L., Courtney, K. D., Andres, V., & Feather-Stone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, *7*(2), 88–95. https://doi.org/10.1016/0006-2952(61)90145-9

Giacobini, E. (2004). Cholinesterase inhibitors: New roles and therapeutic alternatives. *Pharmacological Research*, *50*(4), 433–440. https://doi.org/10.1016/j.phrs.2003.11.017

Gülcin, I. (2012). Antioxidant activity of food constituents: An overview. *Archives of Toxicology*, *86*(3), 345–391. https://doi.org/10.1007/s00204-011-0774-2

Gündoğdu, S., Türkeş, C., Arslan, M., Demir, Y., & Beydemir, Ş. (2019). New isoindole-1, 3-dione substituted sulfonamides as potent inhibitors of carbonic anhydrase and acetylcholinesterase: Design, synthesis, and biological evaluation. *ChemistrySelect*, *4*(45), 13347–13355. https://doi.org/10.1002/slct.201903458

Ha, S. (2002). Asetilkolin, kolinesterazlar ve Alzheimer hastalığı. *Demans Dergisi*, *2*, 69–73.

Işık, M., Beydemir, Ş., Yılmaz, A., Naldan, M. E., Aslan, H. E., & Gülçin, İ. (2017). Oxidative stress and mRNA expression of acetylcholinesterase in the leukocytes of ischemic patients. *Biomedicine & Pharmacotherapy*, *87*, 561–567. https://doi.org/10.1016/j.biopha.2017.01.003

Işık, M. (2019). The binding mechanisms and inhibitory effect of intravenous anesthetics on AChE in vitro and in vivo: Kinetic analysis and molecular docking. *Neurochemical Research*, *44*(9), 2147–2155. https://doi.org/10.1007/s11064-019-02852-y

Işık, M. (2020). High stability of immobilized acetylcholinesterase on chitosan beads. *ChemistrySelect*, *5*(15), 4623–4627. https://doi.org/10.1002/slct.202000559

Işık, M., Akocak, S., Lolak, N., Taslimi, P., Türkeş, C., Gülçin, İ., Durgun, M., & Beydemir, Ş. (2020). Synthesis, characterization, biological evaluation, and in silico studies of novel 1,3-diaryltriazene-substituted sulfathiazole derivatives. *Archiv der Pharmazie*, *353*, e2000102. https://doi.org/10.1002/ardp.202000102

Işık, M., & Beydemir, Ş. (2019). AChE mRNA expression as a possible novel biomarker for the diagnosis of coronary artery disease and Alzheimer's disease, and its association with oxidative stress. *Archives of Physiology and Biochemistry*, 1–8. https://doi.org/10.1080/13813455.2019.1683584

Işık, M., Beydemir, Ş., Demir, Y., Durgun, M., Türkeş, C., Nasır, A., Necip, A., & Akkuş, M. (2020). Benzenesulfonamide derivatives containing imine and amine groups: Inhibition on human paraoxonase and molecular docking studies. *International Journal of Biological Macromolecules*, *146*, 1111–1123. https://doi.org/10.1016/j.ijbiomac.2019.09.237

Işık, M., Demir, Y., Durgun, M., Türkeş, C., Necip, A., & Beydemir, Ş. (2020). Molecular docking and investigation of 4-(benzylideneamino)-and 4-(benzylamino)-benzenesulfonamide derivatives as potent AChE inhibitors. *Chemical Papers*, *74*(5), 1395–1405. https://doi.org/10.1007/s11696-019-00988-3

Isik, M., Korkmaz, M., Bursal, E., Gulcin, I., Koksal, E., & Tohma, H. (2015). Determination of antioxidant properties of Gypsophila bitlisensis Bark. *International Journal of Pharmacology*, *11*(4), 366–337l. https://doi.org/10.3923/ijp.2015.366.371

Istrefi, Q., Türkeş, C., Arslan, M., Demir, Y., Nixha, A. R., Beydemir, Ş., & Küfrevioğlu, Ö. İ. (2020). Sulfonamides incorporating ketene N,S-acetal bioisosteres as potent carbonic anhydrase and acetylcholinesterase inhibitors. *Archiv der Pharmazie*, *353*(6), 1900383. https://doi.org/10.1002/ardp.201900383

Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J.-P., Pihlaja, K., Kujala, T. S., & Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry*, *47*(10), 3954–3962. https://doi.org/10.1021/jf990146l

Kakinuma, Y., Furihata, M., Akiyama, T., Arikawa, M., Handa, T., Katare, R. G., & Sato, T. (2010). Donepezil, an acetylcholinesterase inhibitor against Alzheimer's dementia, promotes angiogenesis in an ischemic hindlimb model. *Journal of Molecular and Cellular Cardiology*, *48*(4), 680–693. https://doi.org/10.1016/j.yjmcc.2009.11.010

Kwon, S.-H., Lee, H.-K., Kim, J.-A., Hong, S.-I., Kim, H.-C., Jo, T.-H., Park, Y.-I., Lee, C.-K., Kim, Y.-B., Lee, S.-Y., & Jang, C.-G. (2010). Neuroprotective effects of chlorogenic acid on scopolamine-induced amnesia via anti-acetylcholinesterase and anti-oxidative activities in mice. *European Journal of Pharmacology*, *649*(1-3), 210–217. https://doi.org/10.1016/j.ejphar.2010.09.001

Legrand, S., Nordlander, G., Nordenhem, H., Borg-Karlsonc, A.-K., & Uneliusa, C. R. (2004). Hydroxy-methoxybenzoic methyl esters: Synthesis and antifeedant activity on the pine weevil, Hylobius abietis. *Zeitschrift für Naturforschung B*, *59*(7), 829–835. https://doi.org/10.1515/znb-2004-0714

Lolak, N., Akocak, S., Türkeş, C., Taslimi, P., Işık, M., Beydemir, Ş., Gülçin, İ., & Durgun, M. (2020). 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. *Bioorganic Chemistry*, *100*, 103897. https://doi.org/10.1016/j.bioorg.2020.103897

Mattila, P., & Kumpulainen, J. (2002). Determination of free and total phenolic acids in plant-derived foods by HPLC with diode-array detection. *Journal of Agricultural and Food Chemistry*, *50*(13), 3660–3667. https://doi.org/10.1021/jf020028p

Moure, A., Cruz, J. M., Franco, D., Domínguez, J. M., Sineiro, J., Domínguez, H., José Núñez, M., & Parajó, J. C., (2001). Natural antioxidants from residual sources. *Food Chemistry*, *72*(2), 145–171. https://doi.org/10.1016/S0308-8146(00)00223-5

Oboh, G., Agunloye, O. M., Akinyemi, A. J., Ademiluyi, A. O., & Adefegha, S. A. (2013). Comparative study on the inhibitory effect of caffeic and chlorogenic acids on key enzymes linked to Alzheimer's disease and some pro-oxidant induced oxidative stress in rats' brain-in vitro. *Neurochemical Research*, *38*(2), 413–419. https://doi.org/10.1007/s11064-012-0935-6

Praticò, D., & Delanty, N. (2000). Oxidative injury in diseases of the central nervous system: Focus on Alzheimer's disease. *The American*

Journal of Medicine, *109*(7), 577–585. https://doi.org/10.1016/s0002-9343(00)00547-7

Rao, A. A., Sridhar, G. R., & Das, U. N. (2007). Elevated butyrylcholinesterase and acetylcholinesterase may predict the development of type 2 diabetes mellitus and Alzheimer's disease. *Medical Hypotheses*, *69*(6), 1272–1276. https://doi.org/10.1016/j.mehy.2007.03.032

Robbins, R. J. (2003). Phenolic acids in foods: An overview of analytical methodology. *Journal of Agricultural and Food Chemistry*, *51*(10), 2866–2887. https://doi.org/10.1021/jf026182t

Sabbagh, M. N., Farlow, M. R., Relkin, N., & Beach, T. G. (2006). Do cholinergic therapies have disease-modifying effects in Alzheimer's disease? *Alzheimer's & Dementia*, *2*(2), 118–125. https://doi.org/10.1016/j.jalz.2006.02.001

Scozzafava, A., Kalın, P., Supuran, C. T., Gülçin, İ., & Alwasel, S. H. (2015). The impact of hydroquinone on acetylcholine esterase and certain human carbonic anhydrase isoenzymes (hCA I, II, IX, and XII). *Journal of Enzyme Inhibition and Medicinal Chemistry*, *30*(6), 941–946. https://doi.org/10.3109/14756366.2014.999236

Szwajgier, D. (2013). Anticholinesterase activity of phenolic acids and their derivatives. *Zeitschrift für Naturforschung C*, *68*(3-4), 125–132. https://doi.org/10.1515/znc-2013-3-408

Takım, K., & Işık, M. (2020). Phytochemical analysis of Paliurus spina-christi fruit and its effects on oxidative stress and antioxidant enzymes in streptozotocin-induced diabetic rats. *Applied Biochemistry and Biotechnology*, 1–16. https://doi.org/10.1007/s12010-020-03287-w

Taslimi, P., Işık, M., Türkan, F., Durgun, M., Türkeş, C., Gülçin, İ., Beydemir, Ş. (2020). Benzenesulfonamide derivatives as potent acetylcholinesterase, α-glycosidase, and glutathione S-transferase inhibitors: Biological evaluation and molecular docking studies. *Journal of Biomolecular Structure and Dynamics*, 1–12. https://doi.org/10.1080/07391102.2020.1790422

Taslimi, P., Kandemir, F. M., Demir, Y., İleritürk, M., Temel, Y., Caglayan, C., & Gulçin, İ. (2019). The antidiabetic and anticholinergic effects of chrysin on cyclophosphamide-induced multiple organ toxicity in rats: Pharmacological evaluation of some metabolic enzyme activities . *Journal of Biochemical and Molecular Toxicology*, *33*(6), e22313. https://doi.org/1002/jbt.22313 https://doi.org/10.1002/jbt.22313

Thacker, P. D. (2003). Surprising discovery with Alzheimer's medication. *Drug Discovery Today*, *8*(9), 379–380. https://doi.org/10.1016/S1359-6446(03)02685-0

Türkeş, C. (2019a). Inhibition effects of phenolic compounds on human serum paraoxonase-1 enzyme. *Journal of the Institute of Science and Technology*, *9*(2), 1013–1022. https://doi.org/10.21597/jist.491054

Türkeş, C. (2019b). Investigation of potential paraoxonase-I inhibitors by kinetic and molecular docking studies: Chemotherapeutic drugs. *Protein & Peptide Letters*, *26*(6), 392–402. https://doi.org/10.2174/0929866526666190226162225

Türkeş, C. (2019c). A potential risk factor for paraoxonase 1: In silico and in-vitro analysis of the biological activity of proton-pump inhibitors . *The Journal of Pharmacy and Pharmacology*, *71*(10), 1553–1564. https://doi.org/10.1111/jphp.13141

Türkeş, C., Arslan, M., Demir, Y., Çoçaj, L., Rifati Nixha, A., & Beydemir, Ş. (2019). Synthesis, biological evaluation and in silico studies of novel N-substituted phthalazine sulfonamide compounds as potent carbonic anhydrase and acetylcholinesterase inhibitors. *Bioorganic Chemistry*, *89*, 103004. https://doi.org/10.1016/j.bioorg.2019.103004

Türkeş, C., & Beydemir, Ş. (2020). Inhibition of human serum paraoxonase-I with antimycotic drugs: In vitro and in silico Studies. *Biotechnology and Applied Biochemistry*, *190*(1), 252–269. https://doi.org/10.1007/s12010-019-03073-3

Türkeş, C., Beydemir, Ş., & Küfrevioğlu, Ö. İ. (2019). In vitro and in silico studies on the toxic effects of antibacterial drugs as human serum paraoxonase 1 inhibitor. *ChemistrySelect*, *4*(33), 9731–9736. https://doi.org/10.1002/slct.201902424

Türkeş, C., Demir, Y., & Beydemir, Ş. (2019). Anti-diabetic properties of calcium channel blockers: Inhibition effects on aldose reductase enzyme activity. *Applied Biochemistry and Biotechnology*, *189*(1), 318–329. https://doi.org/10.1007/s12010-019-03009-x

Türkeş, C., Demir, Y., & Beydemir, Ş. (2020). Calcium channel blockers: Molecular docking and inhibition studies on carbonic anhydrase I and II isoenzymes. *Journal of Biomolecular Structure and Dynamics*, 1–15. https://doi.org/10.1080/07391102.2020.1736631

Türkeş, C., Söyüt, H., & Beydemir, Ş. (2013). Inhibition effects of gemcitabine hydrochloride, acyclovir, and 5-fluorouracil on human serum paraoxonase-1 (hPON1): In vitro. *Open Journal of Biochemistry*, *1*, 10–15. https://doi.org/10.15764/BIOC.2014.01002

Türkeş, C., Söyüt, H., & Beydemir, Ş. (2014). Effect of calcium channel blockers on paraoxonase-1 (PON1) activity and oxidative stress. *Pharmacological Reports*, *66*(1), 74–80. https://doi.org/10.1016/j.pharep.2013.08.007

Türkeş, C., Söyüt, H., & Beydemir, Ş. (2015). Human serum paraoxonase-1 (hPON1): In vitro inhibition effects of moxifloxacin hydrochloride, levofloxacin hemihidrate, cefepime hydrochloride, cefotaxime sodium and ceftizoxime sodium. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *30*(4), 622–628. https://doi.org/10.3109/14756366.2014.959511

Türkeş, C., Söyüt, H., & Beydemir, Ş. (2016). In vitro inhibitory effects of palonosetron hydrochloride, bevacizumab and cyclophosphamide on purified paraoxonase-I (hPON1) from human serum. *Environmental Toxicology and Pharmacology*, *42*, 252–257. https://doi.org/10.1016/j.etap.2015.11.024

Wright, D. L., & Plummer, D. T. (1973). Multiple forms of acetylcholinesterase from human erythrocytes. *The Biochemical Journal*, *133*(3), 521–527. https://doi.org/10.1042/bj1330521

Yamali, C., Gul, H. I., Ece, A., Taslimi, P., & Gulcin, I. (2018). Synthesis, molecular modeling, and biological evaluation of 4-[5-aryl-3-(thiophen-2-yl)-4, 5-dihydro-1H-pyrazol-1-yl] benzenesulfonamides toward acetylcholinesterase, carbonic anhydrase I and II enzymes. *Chemical Biology & Drug Design*, *91*(4), 854–866. https://doi.org/10.1111/cbdd.13149

Yan, J. J., Cho, J. Y., Kim, H. S., Kim, K. L., Jung, J. S., Huh, S. O., Suh, H. W., Kim, Y. H., & Song, D. K. (2001). Protection against beta-amyloid peptide toxicity in vivo with long-term administration of ferulic acid. *British Journal of Pharmacology*, *133*(1), 89–96. https://doi.org/10.1038/sj.bjp.0704047