Chemical Papers
https://doi.org/10.1007/s11696-019-00988-3

ORIGINAL PAPER

Molecular docking and investigation of 4-(benzylideneamino)- and 4-(benzylamino)-benzenesulfonamide derivatives as potent AChE inhibitors

Mesut Işık[1] · Yeliz Demir[2] · Mustafa Durgun[3] · Cüneyt Türkeş[4] · Adem Necip[1] · Şükrü Beydemir[5]

Received: 29 June 2019 / Accepted: 4 November 2019

Abstract

The discovery of acetylcholinesterase inhibitors is important for the treatment of Alzheimer's disease (AD), known as the most common type of dementia. Due to the side effects of commonly used acetylcholinesterase inhibitors, studies for the detection of new inhibitors are increasing day by day. In this study, we investigated the effects of some sulfonamide derivatives (S1–S4 and S1i–S4i) on AChE enzymes. The best pose of the active compounds to understand the mechanism of possible inhibition in interaction of enzyme-sulfonamide derivative were performed docking studies after in vitro experimental results. ADME-related physicochemical and pharmacokinetic properties of the synthesized 4-aminobenzenesulfonamide derivatives were the compatibility with Lipinski's rule of five. We found that the synthesized derivatives of sulfonamides show potential inhibitor properties for AChE with K_i constants in the range of 2.54 ± 0.22–299.60 ± 8.73 µM. The derivatives of sulfonamides exhibited different inhibition type. We determined that the derivatives (S1, S1i, S3, and S3i) showed a competitive inhibition effect, whereas others (S2, S2i, S4, and S4i) showed mixed-type inhibition. As a result, the sulfonamide derivatives can be used as an alternative acetylcholinesterase inhibitor due to this effect. Inhibitors with fewer side effects, are thought to be important in the treatment of AD.

Keywords Sulfonamide derivatives · Alzheimer · AChE inhibitor · Molecular docking · Pharmacokinetic properties

Introduction

Alzheimer's disease (AD) which is more common with the increase of the aging population is a neurodegenerative disorder that occurs in the central nervous system (Bag et al. 2015). The neurodegenerative disorder is thought to be associated with degeneration of cholinergic neurons that occur in subcortical structures and cerebral cortex (Davis

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11696-019-00988-3) contains supplementary material, which is available to authorized users.

✉ Mesut Işık
misik@harran.edu.tr

Yeliz Demir
yelizdemir2116@gmail.com

Mustafa Durgun
mustafadurgun@harran.edu.tr

Cüneyt Türkeş
cuneyt.turkes@erzincan.edu.tr

Adem Necip
ademnecip@harran.edu.tr

Şükrü Beydemir
sukrubeydemir@anadolu.edu.tr

[1] Department of Pharmacy Services, Vocational School of Health Services, Harran University, 63300 Şanlıurfa, Turkey

[2] Department of Pharmacy Services, Nihat Delibalta Göle Vocational High School, Ardahan University, 75700 Ardahan, Turkey

[3] Department of Chemistry, Faculty of Arts and Sciences, Harran University, 63290 Şanlıurfa, Turkey

[4] Department of Biochemistry, Faculty of Pharmacy, Erzincan Binali Yıldırım University, 24100 Erzincan, Turkey

[5] Department of Biochemistry, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey

Published online: 11 November 2019

1976). Plasma and serum biochemical markers known for Alzheimer's disease have appeared as a result of pathophysiological processes such as oxidative stress, lipid metabolism, amyloid plaque formation and vascular diseases (Işık et al. 2017). For example, many neurotransmitters such as cholinergic, noncholinergic, serotonergic, dopaminergic, amino acidergic, and Neuropeptidergic have been shown to change with this disease. Two basic hypotheses, amyloid cascade and cholinergic hypothesis, are accepted in the explanation of the molecular mechanism of AD (Parihar and Hemnani 2004). The AD is both associated with the change of cholinergic markers, such as acetylcholinesterase (AChE) and choline acetyltransferase (ChAT), according to the cholinergic hypothesis, is also related to the formation of β-amyloid peptide (Aβ) self-assemblies (oligomers and fibrils) and the development of neurotoxic effects, according to amyloid cascade hypothesis (Bag et al. 2015).

Many studies have been carried out to determine the new inhibitors (Aβ self-assembly and AChE inhibitors) to reduce the strong effect of multiple Aβ neurotoxic products on disease development and the treatment of AD, which may occur with the formation of low-level neurotransmitters, such as acetylcholine (ACh) (Göçer et al. 2015). The first generation of drugs used to treat AD patients is acetylcholinesterase (AChE) inhibitors because these inhibitors which are used to provide symptomatic treatment reduce the excessive hydrolysis of the neurotransmitter ACh (Bag et al. 2015). Development of cognitive impairment in transgenic mice is reported to occur with changed expression of acetylcholine. Conversely, the performance of cognitive tasks both in man and animals can enhance with cholinergic stimulation (Liston et al. 2004; Rusted and Warburton 1992). The critical role in the cognitive function of ACh is consistent with the studies conducted and it is possible to treat cognitive and memory loss associated with AD with cholinomimetic agents. Cholinesterase inhibitors, one of the cholinomimetic agents in the treatment of functional and cognitive symptoms of AD have played an important role (Liston et al. 2004; Weinstock 1999). The AChE, which is one of the cholinesterase group, is a membrane-dependent enzyme consisting of multiple subunits. The enzyme, which is found in muscles, cholinergic neurons and brain have terminated nerve conduction by hydrolyzing acetylcholine (ACh) in cholinergic synapses of the central nervous system and somatic system (Köksal et al. 2019; Yiğit et al. 2018).

The AChE inhibitors such as galantamine, rivastigmine, tacrine and donepezil, which are widely used in AD treatment have many side effects. Therefore, the identification of new AChE inhibitors (novel drugs) is important in terms of contributing to the treatment of AD. Recent research has expressed that some molecules as newly synthesized sulfonamide derivative exhibit very low concentrations of AChE inhibition (Köksal et al. 2019; Turkan et al. 2018).

The sulfonamides are important bioactive compounds that exhibit many biological effects such as diuretic, antitumor, antithyroid, antibacterial, anti-carbon anhydrase, antidiabetic, hypoglycemic, protease inhibitor activity and analgesic among others and are well tolerated in biomedical applications (Bag et al. 2015; Genç et al. 2008; Santos et al. 2006; Scozzafava et al. 2002). It has been reported that sulfonamides, which are systematically used for the prevention and treatment of various diseases, are among the precursors of chemotherapeutic agents, as well as that the compounds may be used for symptomatic treatment of AD (Kołaczek et al. 2014). Moreover, the formation of amyloid-β which occurred by a β-amyloid precursor protein (APP) is inhibited by sulfonamides. These molecules with low cost and low toxicity are important for applications in the synthesis of new derivatives (Bag et al. 2015; de Oliveira et al. 2016). In a previous study, we have demonstrated the design and synthesis of sulphonamide compounds (S1–S4 and S1i–S4i) and investigated inhibitory activities against carbonic anhydrase isoforms I, II, IX, and XII (Durgun et al. 2016). In the context of this information, we investigated the effects of synthesized sulfonamide derivatives (S1–S4 and S1i–S4i) on AChE enzymes. The possible mechanism for the interaction of the enzyme-sulfonamide derivative was performed with docking studies after in vitro experimental results.

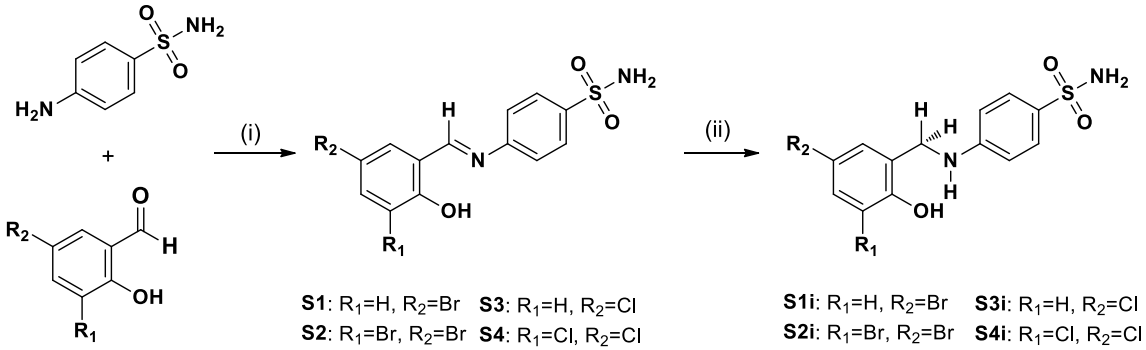
Experimental

Chemicals

All commercially available reagents required for synthesis and enzyme inhibition were obtained from Merck and Sigma.

Synthesis of sulphonamide derivatives (S1–S4 and S1i–S4i)

The synthesis of sulphonamide derivatives, S1–S4 and S1i–S4i, was outlined in Scheme 1. First, for the synthesis of sulphonamides containing imine group (S1–S4), sulphanilamide, and 4-aminobenzenesulfonamide, was reacted with substituted aromatic aldehyde (5-bromo-2-hydroxybenzaldehyde, 3,5-dibromo-2-hydroxybenzaldehyde, 5-chloro-2-hydroxybenzaldehyde and 3,5-dichloro-2-hydroxybenzaldehyde) in methanol with catalytic amounts of formic acid, respectively. In the second step, the obtained sulphonamide compounds were reacted with $NaBH_4$ to give the final corresponding secondary amine derivative (S1i–S4i) in methanol. These products are air stable and slightly soluble in most polar organic solvents (e.g., tetrahydrofuran, methanol, acetone, and acetonitrile). Experimental details, data, and spectral analysis of synthesized sulphonamide derivatives



Scheme 1 Synthesis of the sulphonamide derivatives (S1–S4 and S1i–S4i); reagents and conditions: (i) CH_3OH, reflux, 3–4 h., (ii) CH_3OH, $NaBH_4$, H_2O, 0–25 °C, 24 h

have been presented in our previous studies and in supplementary materials (Durgun et al. 2016).

In general, the FT-IR spectrum of imine compounds (S1–S4) were characterized by the presence of a strong band at about 1620 cm^{-1} due to the stretching of the C=N bond, while the FT-IR spectrum of amine compounds (S1i–S4i) was not observed in this strong band (C=N). Besides, in the ^{1}H NMR spectra of S1–S4, a singlet peak at about 8.50 ppm was observed that attributed to azomethine (CH=N) chemical shift, while in the ^{1}H NMR spectrum of S1i–S4i, this peak (CH=N) was not observed. The derivatives S1i–S4i showed a peak at about 4.25 ppm due to the presence of $–CH_2–$ group. In the ^{13}C-NMR spectrum, the signals at about 163 ppm were assigned to azomethine group (CH=N) for S1–S4. These signals were not observed for S1i–S4i (see supplementary materials for details).

AChE activity determination

The inhibitory effects of some sulfonamides compounds (S1–S4 and S1i–S4i) on AChE enzymes were tested by Ellman's spectrophotometric method (Ellman et al. 1961) as described in previous studies (Caglayan et al. 2019; Türkan et al. 2019). Briefly, 50 µl of 5,5′-dithio-bis(2-nitro-benzoic) acid compound (DTNB) and 100 µl of Tris–HCl solution (1 M, pH 8.0), and 50 µl AChE (5.32×10^{-3} U) solution were incubated and mixed for 15 min at 30 °C. Finally, the reaction was started by adding 50 µl of acetylthiocholine iodide (AChI), which was used as substrates conforming to our previous studies (Çağlayan et al. 2019; Erdemir et al. 2019). The enzymatic hydrolysis of the substrate was recorded spectrophotometrically at a wavelength of 412 nm (Kucukoglu et al. 2019).

In vitro inhibition studies

The study investigated the inhibitory effect of some synthesized sulfonamide derivatives (S1–S4 and S1i–S4i) on the acetylcholinesterase (AChE) enzyme at various concentrations. The percent inhibition values for each compound, the inhibition types (Türkeş et al. 2014, 2015, 2016), and the K_i values with Lineweaver–Burk curves (Lineweaver and Burk 1934) were determined as described in previous studies (Demir et al. 2017; Türkeş et al. 2019c; Yamali et al. 2018).

ADMET analysis

The physicochemical and pharmacokinetic properties of these 4-aminobenzenesulfonamide derivatives were predicted by the QikProp and ADME, and Molecular Properties Panel of Schrödinger Suite as in previous assay (Türkeş et al. 2019a). These properties involved frontier molecular orbital energies (HOMO and LUMO), dipole moment (Dipole), octanol/water partition coefficient (QPlogPo/w), aqueous solubility (QPlogS), IC_{50} value for blockage of HERG K^+ channels (QPlogHERG), Caco-2 cell permeability (QPPCaco), brain/blood partition coefficient (QPlogBB), MDCK cell permeability (QPPMDCK), number of likely metabolic reactions (#metab), binding to human serum albumin (QPlogKhsa), percent human oral absorption, and Lipinski's rule of five (Lipinski et al. 1997).

Molecular docking analysis

The docking studies were performed using the Protein Preparation Wizard (Sastry et al. 2013), LigPrep (Türkeş 2019b), Receptor Grid Generation (Halgren et al. 2004), Ligand Docking (Friesner et al. 2004), and panels implemented in Schrodinger Suite (Schrödinger Release 2019-1: Glide, Schrödinger, LLC, New York, NY, 2019). The crystal structure of acetylcholinesterase complexed with the co-crystallized dihydrotanshinone-I (PDB ID: 4M0E) with the resolution of 2 Å obtained from the protein data bank (https://www.rcsb.org/pdb) as in our previous assays (Türkeş 2019a; Türkeş and Beydemir 2019). The ligands were prepared using LigPrep at pH 7.0. The energy minimization was

done using OPLS3e force field (Harder et al. 2015; Türkeş 2019c). The Glide standard precision mode (Glide SP) was used for ligand docking according to our previous studies (Beydemir et al. 2019; Türkeş et al. 2019b). Based on the calculated Glide Gscore, the best pose was rank based.

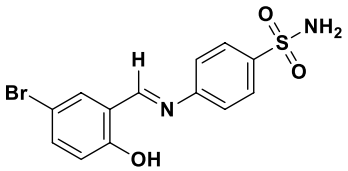
Statistical analysis

The determination of K_i constants was conducted using Systat SigmaPlot12 software. The results were presented as mean ± standard deviation (95% confidence intervals). Differences between datasets were considered statistically significant when the p value was less than 0.05.

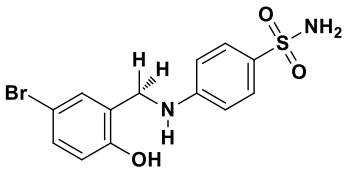
Results

These compounds were re-prepared according to the general procedure described in our previous study. Sulphonamides containing imine group, S1–S4, were obtained by condensation of the sulphanilamide (4-aminobenzenesulfonamide) with the corresponding aromatic aldehydes in methanol, with catalytic amounts of formic acid (Fig. 1). The secondary amine sulphonamides, S1i–S4i, were subsequently prepared by reduction of the imine derivatives S1–S4 with $NaBH_4$ in methanol (Durgun et al. 2016). Some data of these compounds are summarized below.

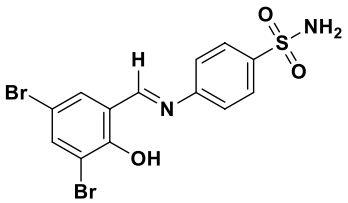
Fig. 1 The molecular structure of sulfonamide compounds (S1–S4 and S1i–S4i) with inhibitory effect on AChE



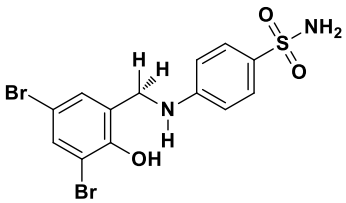
S1: 4-((5-bromo-2-hydroxybenzylidene)amino)benzenesulfonamide



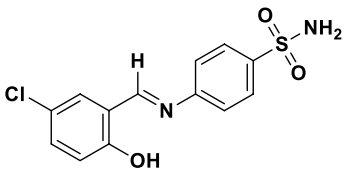
S1i: 4-((5-bromo-2-hydroxybenzyl)amino)benzenesulfonamide



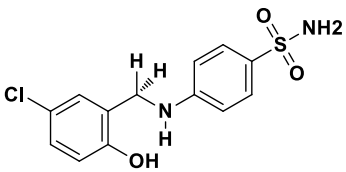
S2: 4-((3,5-dibromo-2-hydroxybenzylidene)amino)benzenesulfonamide



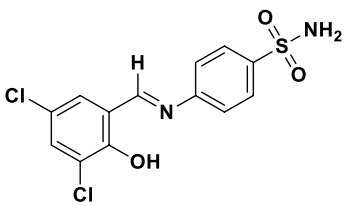
S2i: 4-((3,5-dibromo-2-hydroxybenzyl)amino)benzenesulfonamide



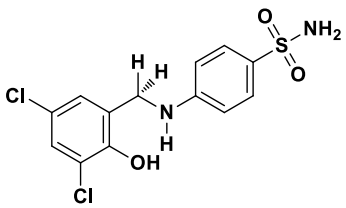
S3: 4-((5-chloro-2-hydroxybenzylidene)amino)benzenesulfonamide



S3i: 4-((5-chloro-2-hydroxybenzyl)amino)benzenesulfonamide



S4: 4-((3,5-dichloro-2-hydroxybenzylidene)amino)benzenesulfonamide



S4i: 4-((3,5-dichloro-2-hydroxybenzyl)amino)benzenesulfonamide

S1: yield: 85%; color: orange; mp: 217–219 °C; $C_{13}H_{11}BrN_2O_3S$ (355.21 g/mol). S2: yield: 85%; color: bright red; mp: 246–248 °C; $C_{13}H_{10}Br_2N_2O_3S$ (434.10 g/mol). S3: yield: 85%; color: bright orange; mp: 199–201 °C; $C_{13}H_{11}ClN_2O_3S$ (310.76 g/mol). S4: yield: 85%; color: bright red; mp: 242–244 °C; $C_{13}H_{10}Cl_2N_2O_3S$ (345.20 g/mol). S1i: yield: 60%; color: white; mp: 213–215 °C; $C_{13}H_{13}BrN_2O_3S$ (357.22 g/mol). S2i: yield: 55%; color: white; mp: 163 °C; $C_{13}H_{12}Br_2N_2O_3S$ (436.12 g/mol). S3i: yield: 60%; color: white; mp: 195–197 °C; $C_{13}H_{13}ClN_2O_3S$ (312.77 g/mol). S4i: yield: 85%; color: white; mp: 174–176 °C; $C_{13}H_{12}Cl_2N_2O_3S$ (347.22 g/mol).

The effect of the sulfonamide derivatives (S1–S4 and S1i–S4i) on AChE was determined by K_i values. The compounds showed competitive inhibition type, and K_i constants were calculated as 299.60 ± 8.04, 116.25 ± 2.12, 203.76 ± 4.97, and 298.94 ± 7.49 μM, respectively. On the other hand, S2, S2i, S4, and S4i have shown a mixed-type inhibition and K_i values were found to be 2.54 ± 0.22, 5.12 ± 0.10, 5.19 ± 0.44, and 4.50 ± 0.31 μM, respectively (Table 1).

Table 1 K_i constants of compounds and inhibition types for AChE

Inhibitor	K_i (μM)	R^2	Inhibition type
S1	299.60 ± 8.04	0.9988	Competitive
S1i	116.25 ± 2.12	0.9994	Competitive
S2	2.54 ± 0.22	0.9988	Mixed
S2i	5.12 ± 0.10	0.9994	Mixed
S3	203.76 ± 4.97	0.9989	Competitive
S3i	298.94 ± 7.49	0.9989	Competitive
S4	5.19 ± 0.44	0.9988	Mixed
S4i	4.50 ± 0.31	0.9990	Mixed

The analysis results were exhibited as mean ± standard deviation

In silico prediction of the physicochemical and pharmacokinetic properties of each compound is an essential step in drug development. ADME properties of the synthesized 4-aminobenzenesulfonamide derivatives were calculated by the help of QikProp. In addition, the suitability of the compounds was evaluated based on Lipinski's rule of five. The results are presented in Table 2. The brain/blood partition coefficients (QPlogBB) of all the synthesized derivates were found less than − 1.28. The percent human oral absorption of compounds were determined in the range of 72.76–78.83%. The values of a binding to human serum albumin (QPlogKhsa) of drug molecules were observed as less than 1.5 (i.e., in the range from − 0.40 to − 0.25).

Based on the results obtained from in vitro enzymatic assays, molecular modeling studies were performed to understand the binding mode of all the synthesized derivatives into the active site of the 4M0E receptor (Fig. 2). The docked molecules were ranked according to the Glide Gscore (Table 3). S2 is the most active compound in the synthesized library. S2 formed four hydrogen bonds with His287, Leu289, Ser293, and Phe295. The amino group of the benzenesulfonamide formed two hydrogen bonds with His287 and Leu289. Third hydrogen bond was composed between the hydroxyl group of the phenol and Ser293, and fourth hydrogen bond was observed between the bromine

Table 2 Analysis of physicochemical and pharmacokinetic properties of 4-aminobenzenesulfonamide derivatives

Compound	HOMO energy[a] (eV)	LUMO energy[b] (eV)	Dipole[c]	QPlogPo/w[d]	QPlogS[e]	QPlogHERG[f]	QPPCaco[g]	QPPMDCK[h]	#Metab[i]
S1	− 9.32	− 1.32	8.96	1.48	− 3.54	− 5.65	141.43	161.67	1
S1i	− 9.24	− 0.55	11.66	1.33	− 3.49	− 5.56	144.93	166.13	4
S2	− 9.60	− 1.39	5.48	2.04	− 4.25	− 5.60	170.09	493.59	1
S2i	− 9.28	− 0.67	10.26	1.92	− 4.20	− 5.51	171.05	498.72	4
S3	− 4.27	1.91	6.08	1.41	− 3.43	− 5.62	146.90	156.73	1
S3i	− 9.25	− 0.53	11.70	1.22	− 3.31	− 5.53	144.91	154.49	4
S4	− 9.49	− 1.28	7.53	1.90	− 4.08	− 5.55	163.29	409.17	1
S4i	− 9.15	− 0.66	9.80	1.63	− 4.12	− 5.46	167.41	422.13	4

[a]Highest occupied molecular orbital

[b]Lowest unoccupied molecular orbital

[c]Computed dipole moment of the molecule (recommended value: 1.0–12.5)

[d]Predicted octanol/water partition coefficient (recommended value: − 2.0 to 6.5)

[e]Predicted aqueous solubility (recommended value: − 6.5 to 1.5)

[f]Predicted IC_{50} value for blockage of HERG K^+ channels (concern below − 5)

[g]Predicted apparent Caco-2 cell permeability in nm/s (< 25 is poor and > 500 is great)

[h]Predicted apparent MDCK cell permeability in nm/s (< 25 is poor and > 500 is great)

[i]Number of likely metabolic reactions (recommended value: 1–8)

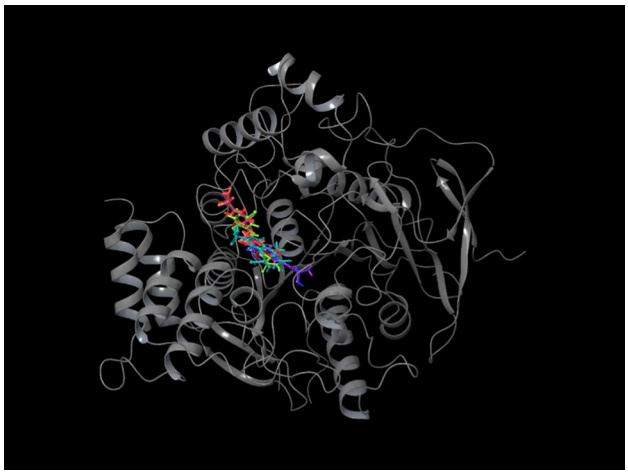


Fig. 2 Alignment of the binding conformations of 4-aminobenzenesulfonamide derivatives

of the benzene and Phe295. In addition, the S2 composed a hydrophobic cloud with Tyr72, Trp286, Val288, Leu289, Val294, Phe295, Phe297, Phe338, and Tyr341. Moreover, the phenol ring was found to have pi–pi stacking with Trp286 (Fig. 3a). In compound S4i (second most potent derivative), functional groups (–OH and Cl) of the phenol ring showed four hydrogen bonds formation with amino acids Ser293, Phe295, and Arg296. Moreover, two hydrogen bonds were observed between the amino group of the benzenesulfonamide and His287 and Leu289 residues. Additionally, it showed hydrophobic interactions with Tyr72, Tyr124, Trp286, Val288, Leu289, Val294, Phe295, Phe297, Phe338, and Tyr341. pi–pi interaction appeared between the phenol ring and Trp286 residue (Fig. 3b). Compound S4 (third most potent derivative) showed five hydrogen bond interactions. Three hydrogen bonds were formed between the phenol ring and amino acids Asp74, Ser293, and Phe295. Two hydrogen bonds were observed between the amino groups of the benzenesulfonamide and amino acids His287 and Leu289 and pi–pi stacking were composed between the phenol ring and Trp286 amino acid. Furthermore, S4 formed hydrophobic contact with Tyr72, Tyr124, Trp286, Val288, Leu289, Val294, Phe295, Phe297, Phe338 and Tyr341 (Fig. 3c).

Discussion

Many researches showed that some neurotransmitter systems in the brain of Alzheimer's patients (AD) are selectively modified. The most striking abnormalities resulting from the change are the cholinergic system, which is the basis for the cholinergic hypothesis of AD. The etiology and exact pathogenesis of AD is still unknown. The cholinergic hypothesis constituted the rationale for a symptomatic treatment aimed at strengthening the central cholinergic function of AD, hoping to improve cognitive function (Benzi and Moretti 1998). The AD which is related to the cholinergic hypothesis is associated with the change of cholinesterases such as acetylcholinesterase (AChE) and choline acetyltransferase (ChAT). In the brain of AD patients, acetylcholinesterase and butyrylcholinesterase have been reported to be in around tangles and plaques. Biochemical properties of cholinesterase in AD brain are sensitive to inhibitors, the optimum pH has changed, and appears to be different from those in the normal brain (Geula and Mesulam 1989; Schätz et al. 1990; Weinstock 1999). The findings suggest that agents which inhibit cholinesterase may have advantages over selective AChE inhibitors (Weinstock 1999). The agents which have the common mechanism of AChE inhibition shows a

Table 3 Binding free energy calculation results for 4-aminobenzenesulfonamide derivatives bound with 4M0E

Inhibitor	Glide Gscore[a]	ΔG vdW (kcal/mol)	ΔG Coulomb (kcal/mol)	Glide energy (kcal/mol)	Glide emodel (kcal/mol)	H-bonds	pi–pi stacking
S2	−8.24	−34.50	−11.451	−46.02	−62.38	His287, Leu289, Ser293, Phe295	Trp286
S4i	−7.84	−34.38	−10.96	−45.34	−63.06	His287, Leu289, Ser293, Phe295, Arg296	Trp286
S4	−7.83	−35.51	−9.92	−45.43	−59.07	Asp74, His287, Leu289, Ser293, Phe295	Trp286
S2i	−7.25	−36.94	−10.15	−47.09	−63.36	Asp74, His287, Leu289, Ser293, Phe295	Trp286
S3	−7.17	−37.18	−3.20	−40.38	−56.05	Phe295	Tyr337
S1	−6.57	−36.21	−4.94	−41.15	−55.51	Tyr124, Ser293	
S3i	−6.54	−37.88	−3.66	−41.54	−53.32	Phe295	Tyr337
S1i	−5.78	−30.26	−8.62	−38.88	−50.92	Asp74, Ser293, Phe295	Trp286, Tyr341

[a]Glide Gscore: standard precision Glide

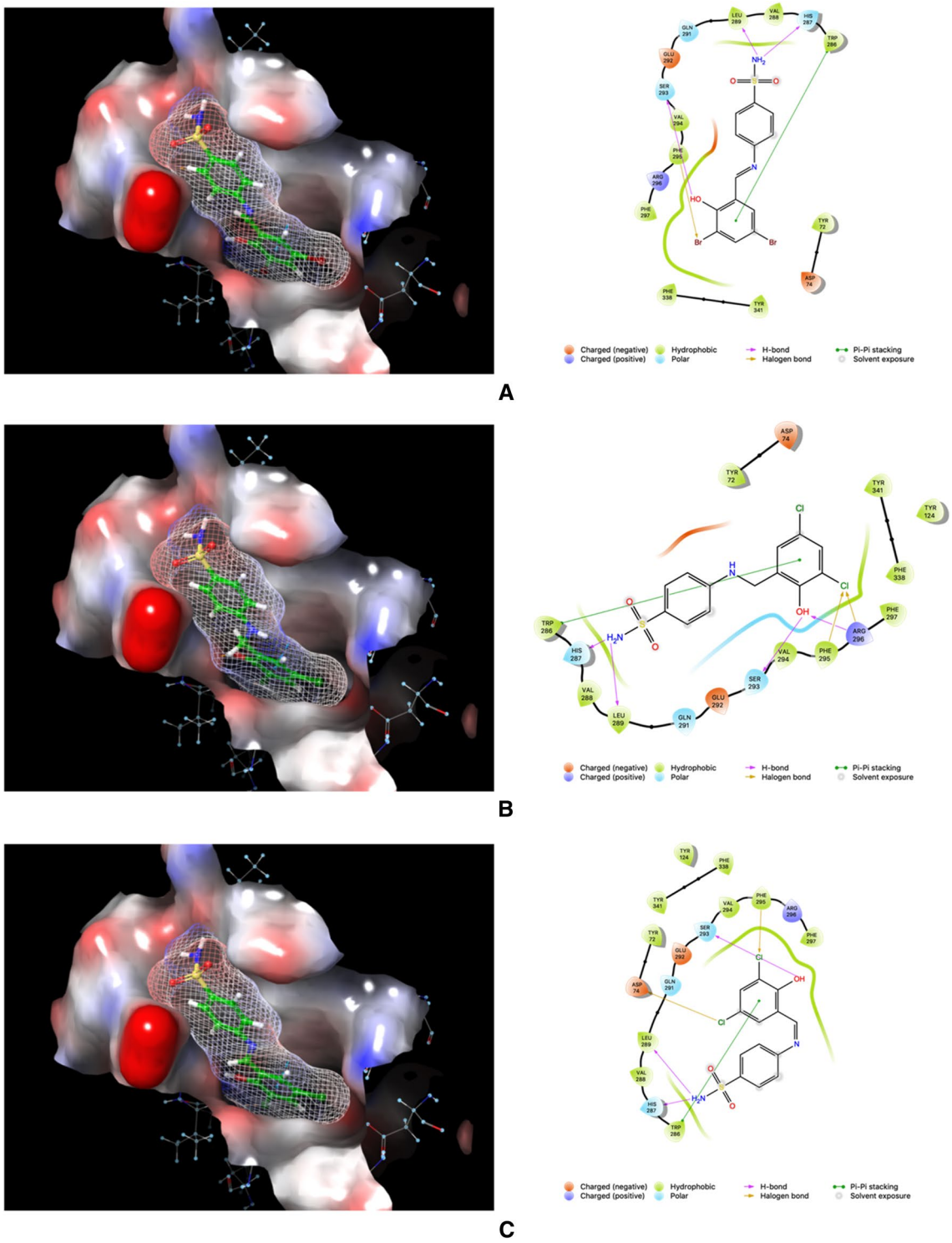


Fig. 3 3D and 2D ligand interaction diagrams of docking pose of S2 (**a**), S4i (**b**), and S4 (**c**) at the binding pocket of AChE (PDB: 4M0E)

positive correlation with cognitive changes in both patients with AD and laboratory animals (Imbimbo 2001; Liston et al. 2004).

The main role of AChE is to end nerve impulse conduction in cholinergic synapses by reducing ACh concentration in presynaptic region with rapid hydrolysis of acetylcholine (ACh). Therefore, AChE inhibitors that inhibit or slow down the hydrolysis of ACh are great importance for the treatment of many diseases such as AD, myasthenia gravis, Parkinson, senile dementia and ataxia (Choudhary 2001; Mukherjee

et al. 2007). For the treatment of cognitive dysfunction and AD-associated memory loss, there are a number of synthetic drugs currently available, which are the AChE inhibitors such as rivastigmine, tacrine, and donepezil (Mukherjee et al. 2007; Oh et al. 2004). According to the literature results, the commonly used the AChE synthetic inhibitors have been shown to have side effects such as gastrointestinal disturbance and hepatotoxicity, while the sulfonamides used have no side effects (Köksal et al. 2019). Sulfonamides, which are important bioactive compounds with many biological effects such as diuretic, antitumor, antithyroid, antibacterial, anti-carbon anhydrase, antidiabetic, hypoglycemic, and protease inhibitor activity, have been found to be beneficial in AD. The sulfonamide derivatives used for symptomatic treatment of AD has inhibited the formation of amyloid-β which occurred by a β-amyloid precursor protein (APP) (Bag et al. 2015).

In the context of this information, we investigated the effects of synthesized sulfonamide derivatives (S1–S4 and S1i–S4i) on AChE enzymes. The molecular docking studies were performed to determine the probable binding mechanism. In this study, the K_i parameters of the sulfonamide derivatives (S1–S4 and S1i–S4i) were determined with Lineweaver–Burk graphs ($1/V - 1/[S]$).

In our results, 4-((3,5-dibromo-2-hydroxybenzylidene) amino)benzenesulfonamide (S2) has a high inhibitory effect than other derivatives. Inhibition type was found for sulfonamides derivatives (S1–S4 and S1i–S4i). The S1, S1i, S3, and S3i derivatives have shown competitive inhibition type, and K_i values were found to be 299.60 ± 8.04, 116.25 ± 2.12, 203.76 ± 4.97, and 298.94 ± 7.49 µM, respectively. According to the results, the sulfonamide derivatives (S1, S1i, S3, and S3i) may be attached to the functional ends of the amino acids in the active site of the AChE enzyme. The S2, S2i, S4, and S4i have shown a mixed-type inhibition and K_i values were found to be 2.54 ± 0.22, 5.12 ± 0.10, 5.19 ± 0.44, and 4.50 ± 0.31 µM, respectively (Table 1). According to this result, the derivatives (S2, S2i, S4, and S4i) can bind to the enzyme substrate complex or free enzyme and lead to inhibition. In this study, the molecular docking studies were performed to determine the probable binding mechanism of S1–S4 and S1i–S4i derivatives into the active site of the AChE.

Drug-likeness is a shred of evidence which defines an integrated equilibrium between many molecular properties and structural features, such as Lipinski's rule of five, IC_{50} value for blockage of HERG K^+ channels, Caco-2 cell permeability, MDCK cell permeability, and number of likely metabolic reactions, which describe whether a specific agent is comparable to already recognized medicates. In addition, HOMO (electron donor) and LUMO (electron acceptor) orbitals are responsible for charge transfer during a chemical reaction (Eroglu and Türkmen 2007). The ligands with the highest HOMO energy have the probability to strongly inhibiting the receptor. In addition, the compounds with smaller HOMO–LUMO energy gaps tend to be more active (Sakkiah and Lee 2012). Based on their high HOMO values and small energy gaps, S2 and S4 (−8.21 eV for S2 and S4) were assumed to be of interest. The MW of all the 4-aminobenzenesulfonamide derivatives was determined to be less than 500 and hence predicting their easy absorption, distribution, metabolism, and excretion (recommended value: 130–725). All these physicochemical and pharmacokinetic properties, as displayed in Table 2, are the compatibility with Lipinski's rule of five and thus making all these compounds as good orally potent AChE inhibitors. In addition, computed toxicity analysis results of compounds indicated that derivatives were relatively safe and it was displayed that there was a similarity between these agents and some known central nervous system drugs, such as galantamine, rivastigmine, tacrine, and donepezil (Wager et al. 2010). The results suggested that the derivatives had no risk as a potential drug temporarily.

The analysis of interactions between all derivatives and the binding site of 4M0E is shown in Table 3. All the derivatives in the series were active. Three compounds S2, S4i, and S4 showed the highest Glide Gscores (−8.24, −7.84, and −7.83, respectively) among all the derivatives. Docking study of compounds S2, S4i, and S4 into the active site of target indicated that several molecular interactions were supposed to be accountable for the remarkable affinity of these compounds.

Recently, new inhibition studies from newly synthesized derivatives have been performed in large numbers. In a study on Benzoxazole, substitution derivatives worked on the replacement of the indanone ring of donepezil with a benzisoxazole ring system. Some compounds synthesized from N-benzylpiperidine benzisoxazole derivatives showed a high effect of AChE inhibitory with the IC_{50} value of 0.8–14 nM. Moreover, the obtained compound, 1,2,4-thiadiazolidinone-substituted derivatives showed more selectivity to the AChE (IC_{50}: 0.14 µM) when the donepezil indanone system is replaced with the 1,2,4-thiadiazolidone ring (Martinez et al. 2000). Andreani et al. has been shown that alternative benzylpiperidinone substitution derivatives have AChE inhibitory potential (IC_{50}: 8 µM) when the donepezil indanone system is replaced with substitution indole or pyrol ring (Andreani et al. 2001). Benzophenone derivatives which contain the *N*,*N*-benzyl methylamine as tertiary amino function and benzophenone nucleus as an aromatic function also have high AChE inhibitory potential (Belluti et al. 2009; Singh et al. 2013). In another study on the inhibitory effect of sulfonamide derivatives on AChE and butyrylcholinesterase (BuChE), a series of new biphenyl bis-sulfonamide derivatives showed in vitro inhibition effect. The IC_{50} values of new biphenyl bis-sulfonamide derivatives were

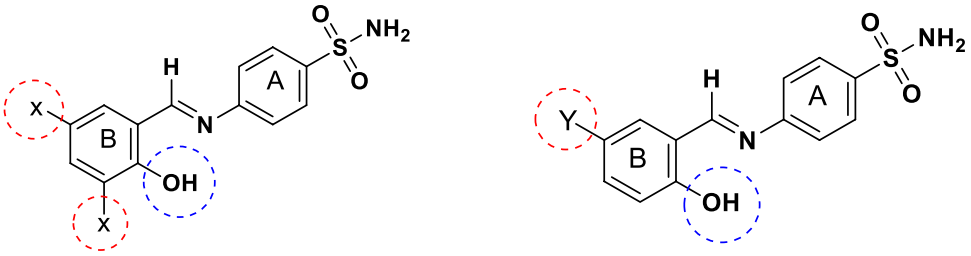


Fig. 4 Structure–activity relationship of sulfonamide-based agents

ranged from 2.27 ± 0.01 to 123.11 ± 0.04 µM for AChE and 7.74 ± 0.07 to < 400 µM for BuChE (Mutahir et al. 2016).

Furthermore, according to recent studies, it can be concluded that acetylcholinesterase inhibitors have a reducing effect on the formation of amyloid beta proteins and regulators cholinergic system. For example, a group of researchers showed that a new series of 2-(diethylaminoalkyl)-isoindoline-1,3-dione derivatives, which are designed using molecular modeling for the purpose of synthesizing cholinesterase inhibitors have AChE inhibitory activity with IC_{50} values ranging from 0.9 to 19.5 µM and weak Aβ anti-aggregation inhibitory activity (Ignasik et al. 2012).

Studies to identify novel and specific AChE inhibitors that both have a reducing effect on amyloid beta proteins and the formation of the regulatory cholinergic system are widely underway. We believe that this study may be important in determining the new inhibitory derivatives of sulfonamides and in using them as a new agent in the treatment of AD. When the results of the study are evaluated, in vitro effect studies have shown that bromine and chlorine derivatives inhibit the AChE activity. The locations where the different groups were bound to the chemical compound were important to understand the structure–activity relationship (SAR). When the structure of the compounds is examined, all compounds appear to contain the sulfonamide group in the para-position (Fig. 4). Thus, inhibition of AChE may be related to groups bound to the B ring. Nitrogen in the compounds is in imine form. Compounds S2 and S4 include imine groups and types of inhibition of non-competitive compounds. The amine group may have a inhibitory effect on AChE. The imine groups may have better binding interactions within the enzyme structure. The higher inhibitory effect of S2 and S4 than S1 and S3 may be due to the bonding of the bound electronegative atoms as mono and di. Two bromo groups in the para-ortho position of ring B facilitate the binding of the compounds to the active site of the enzyme.

Conclusions

The new derivatives of sulfonamides, which are systematically used for the prevention and treatment of various diseases, may be used for symptomatic treatment of AD as AChE inhibitor. The change in AChE activity plays an important role in determining the degree of neurotoxicity (Işık et al. 2015; von Bernhardi et al. 2005). Increased AChE activity is known to play a role in the formation of β-amyloid (Aβ) accumulated in extracellular toxic plaques in the brain of Alzheimer's patients. If this enzyme is inhibited, the formation of Aβ aggregation can be reduced (Bartolini et al. 2003; García-Ayllón et al. 2011; Işık 2019). It is thought that the newly synthesized derivatives of sulfonamides may inhibit the formation of amyloid-β which occurred by a β-amyloid precursor protein (APP). Moreover, the novel inhibiting compounds may have the potential to be an alternative drug in AD patients because it may have a cholinergic system regulatory effect.

Acknowledgements The authors are grateful for the financial support provided by the Research Foundation of Harran University (Project no. 16180).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Andreani A et al (2001) Synthesis and screening for antiacetylcholinesterase activity of (1-benzyl-4-oxopiperidin-3-ylidene) methylindoles and-pyrroles related to donepezil. J Med Chem 44:4011–4014. https://doi.org/10.1021/jm0109356

Bag S et al (2015) Sulfonamides as multifunctional agents for Alzheimer's disease. Bioorg Med Chem Lett 25:626–630. https://doi.org/10.1016/j.bmcl.2014.12.006

Bartolini M, Bertucci C, Cavrini V, Andrisano V (2003) β-Amyloid aggregation induced by human acetylcholinesterase: inhibition studies. Biochem Pharmacol 65:407–416. https://doi.org/10.1016/S0006-2952(02)01514-9

Belluti F et al (2009) Design, synthesis, and evaluation of benzophenone derivatives as novel acetylcholinesterase inhibitors. Eur J Med Chem 44:1341–1348. https://doi.org/10.1016/j.ejmech.2008.02.035

Benzi G, Moretti A (1998) Is there a rationale for the use of acetylcholinesterase inhibitors in the therapy of Alzheimer's disease? Eur J Pharmacol 346:1–13. https://doi.org/10.1016/S0014-2999(98)00093-4

Beydemir Ş, Türkeş C, Yalçın A (2019) Gadolinium-based contrast agents: in vitro paraoxonase 1 inhibition, in silico studies. Drug Chem Toxicol. https://doi.org/10.1080/01480545.2019.1620266

Caglayan C, Demir Y, Kucukler S, Taslimi P, Kandemir FM, Gulçin İ (2019) The effects of hesperidin on sodium arsenite-induced different organ toxicity in rats on metabolic enzymes as antidiabetic and anticholinergics potentials: a biochemical approach. J Food Biochem 43:e12720. https://doi.org/10.1111/jfbc.12720

Çağlayan C, Taslimi P, Demir Y, Küçükler S, Kandemir FM, Gulçin İ (2019) The effects of zingerone against vancomycin-induced lung, liver, kidney and testis toxicity in rats: the behavior of some metabolic enzymes. J Biochem Mol Toxicol. https://doi.org/10.1002/jbt.22381

Choudhary MI (2001) Bioactive natural products as a potential source of new pharmacophores. A theory of memory. Pure Appl Chem 73:555–560. https://doi.org/10.1351/pac200173030555

Davis T (1976) Selective loss of central cholinergic neurons in Alzheimer's disease. Lancet 2:1403

de Oliveira AS et al (2016) New sulfonamides derived from carvacrol: compounds with high antibacterial activity against resistant staphylococcus aureus strains. J Biosci Med 4:105. https://doi.org/10.4236/jbm.2016.47011

Demir Y, Işık M, Gülçin İ, Beydemir Ş (2017) Phenolic compounds inhibit the aldose reductase enzyme from the sheep kidney. J Biochem Mol Toxicol 31:e21936. https://doi.org/10.1002/jbt.21935

Durgun M, Turkmen H, Ceruso M, Supuran CT (2016) Synthesis of 4-sulfamoylphenyl-benzylamine derivatives with inhibitory activity against human carbonic anhydrase isoforms I, II, IX and XII. Bioorg Med Chem 24:982–988. https://doi.org/10.1016/j.bmc.2016.01.020

Ellman GL, Courtney KD, Andres V Jr, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88–95. https://doi.org/10.1016/0006-2952(61)90145-9

Erdemir F et al (2019) Novel 2-aminopyridine liganded Pd (II) *N*-heterocyclic carbene complexes: synthesis, characterization, crystal structure and bioactivity properties. Bioorg Chem 91:103134. https://doi.org/10.1016/j.bioorg.2019.103134

Eroglu E, Türkmen H (2007) A DFT-based quantum theoretic QSAR study of aromatic and heterocyclic sulfonamides as carbonic anhydrase inhibitors against isozyme, CA-II. J Mol Graph Model 26:701–708. https://doi.org/10.1016/j.jmgm.2007.03.015

Friesner RA et al (2004) Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. J Med Chem 47:1739–1749. https://doi.org/10.1021/jm0306430

García-Ayllón M-S, Small DH, Avila J, Sáez-Valero J (2011) Revisiting the role of acetylcholinesterase in Alzheimer's disease: cross-talk with P-tau and β-amyloid. Front Mol Neurosci 4:22. https://doi.org/10.3389/fnmol.2011.00022

Genç Y, Özkanca R, Bekdemir Y (2008) Antimicrobial activity of some sulfonamide derivatives on clinical isolates of Staphylococus aureus. Ann Clin Microbiol Antimicrob 7:17. https://doi.org/10.1186/1476-0711-7-17

Geula C, Mesulam M (1989) Special properties of cholinesterases in the cerebral cortex of Alzheimer's disease. Brain Res 498:185–189. https://doi.org/10.1016/0006-8993(89)90419-8

Göçer H, Akincioğlu A, Göksu S, Gülçin İ, Supuran CT (2015) Carbonic anhydrase and acetylcholinesterase inhibitory effects of carbamates and sulfamoylcarbamates. J Enzyme Inhib Med Chem 30:316–320. https://doi.org/10.3109/14756366.2014.928704

Halgren TA, Murphy RB, Friesner RA, Beard HS, Frye LL, Pollard WT, Banks JL (2004) Glide: a new approach for rapid, accurate docking and scoring. 2. Enrichment factors in database screening. J Med Chem 47:1750–1759. https://doi.org/10.1021/jm030644s

Harder E et al (2015) OPLS3: a force field providing broad coverage of drug-like small molecules and proteins. J Chem Theory Comput 12:281–296. https://doi.org/10.1021/acs.jctc.5b00864

Ignasik M, Bajda M, Guzior N, Prinz M, Holzgrabe U, Malawska B (2012) Design, synthesis and evaluation of novel 2-(aminoalkyl)-isoindoline-1, 3-dione derivatives as dual-binding site acetylcholinesterase inhibitors. Arch Pharm 345:509–516. https://doi.org/10.1002/ardp.201100423

Imbimbo BP (2001) Pharmacodynamic-tolerability relationships of cholinesterase inhibitors for Alzheimer's disease. CNS Drugs 15:375–390. https://doi.org/10.2165/00023210-200115050-00004

Işık M (2019) The binding mechanisms and inhibitory effect of intravenous anesthetics on AChE in vitro and in vivo: kinetic analysis and molecular docking. Neurochem Res. https://doi.org/10.1007/s11064-019-02852-y

Işık M, Demir Y, Kırıcı M, Demir R, Şimşek F, Beydemir Ş (2015) Changes in the anti-oxidant system in adult epilepsy patients receiving anti-epileptic drugs. Arch Physiol Biochem 121:97–102. https://doi.org/10.3109/13813455.2015.1026912

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

Köksal Z, Alım Z, Bayrak S, Gülçin İ, Özdemir H (2019) Investigation of the effects of some sulfonamides on acetylcholinesterase and carbonic anhydrase enzymes. J Biochem Mol Toxicol 33:e22300. https://doi.org/10.1002/jbt.22300

Kołaczek A, Fusiarz I, Ławecka J, Branowska D (2014) Biological activity and synthesis of sulfonamide derivatives: a brief review. Chemik 68:620–628

Kucukoglu K, Gul HI, Taslimi P, Gulcin I, Supuran CT (2019) Investigation of inhibitory properties of some hydrazone compounds on hCA I, hCA II and AChE enzymes. Bioorg Chem 86:316–321. https://doi.org/10.1016/j.bioorg.2019.02.008

Lineweaver H, Burk D (1934) The determination of enzyme dissociation constants. J Am Chem Soc 56:658–666. https://doi.org/10.1021/ja01318a036

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 23:3–25. https://doi.org/10.1016/S0169-409X(96)00423-1

Liston DR et al (2004) Pharmacology of selective acetylcholinesterase inhibitors: implications for use in Alzheimer's disease. Eur J Pharmacol 486:9–17. https://doi.org/10.1016/j.ejphar.2003.11.080

Martinez A, Fernandez E, Castro A, Conde S, Rodriguez-Franco I, Baños J-E, Badia A (2000) *N*-Benzylpiperidine derivatives of 1, 2, 4-thiadiazolidinone as new acetylcholinesterase inhibitors. Eur J Med Chem 35:913–922. https://doi.org/10.1016/S0223-5234(00)01166-1

Mukherjee PK, Kumar V, Mal M, Houghton PJ (2007) Acetylcholinesterase inhibitors from plants. Phytomedicine 14:289–300. https://doi.org/10.1016/j.phymed.2007.02.002

Mutahir S et al (2016) Novel biphenyl bis-sulfonamides as acetyl and butyrylcholinesterase inhibitors: synthesis, biological evaluation and molecular modeling studies. Bioorg Chem 64:13–20. https://doi.org/10.1016/j.bioorg.2015.11.002

Oh M, Houghton P, Whang W, Cho J (2004) Screening of Korean herbal medicines used to improve cognitive function for anticholinesterase activity. Phytomedicine 11:544–548. https://doi.org/10.1016/j.phymed.2004.03.001

Parihar MS, Hemnani T (2004) Alzheimer's disease pathogenesis and therapeutic interventions. J Clin Neurosci 11:456–467. https://doi.org/10.1016/j.jocn.2003.12.007

Rusted JM, Warburton DM (1992) Facilitation of memory by post-trial administration of nicotine: evidence for an attentional explanation. Psychopharmacology 108:452–455. https://doi.org/10.1007/BF02247420

Sakkiah S, Lee KW (2012) Pharmacophore-based virtual screening and density functional theory approach to identifying novel butyrylcholinesterase inhibitors. Acta Pharmacol Sin 33:964. https://doi.org/10.1038/aps.2012.21

Santos MA, Marques SM, Tuccinardi T, Carelli P, Panelli L, Rossello A (2006) Design, synthesis and molecular modeling study of iminodiacetyl monohydroxamic acid derivatives as MMP inhibitors. Bioorg Med Chem 14:7539–7550. https://doi.org/10.1016/j.bmc.2006.07.011

Sastry GM, Adzhigirey M, Day T, Annabhimoju R, Sherman W (2013) Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. J Comput Aided Mol Des 27:221–234. https://doi.org/10.1007/s10822-013-9644-8

Schätz CR, Geula C, Mesulam M (1990) Competitive substrate inhibition in the histochemistry of cholinesterase activity in Alzheimer's disease. Neurosci Lett 117:56–61. https://doi.org/10.1016/0304-3940(90)90119-T

Scozzafava A, Mastrolorenzo A, Supuran CT (2002) Sulfonamides and sulfonylated derivatives as anticancer agents. Curr Cancer Drug Targets 2:55–75. https://doi.org/10.2174/1568009023334060

Singh M, Kaur M, Kukreja H, Chugh R, Silakari O, Singh D (2013) Acetylcholinesterase inhibitors as Alzheimer therapy: from nerve toxins to neuroprotection. Eur J Med Chem 70:165–188. https://doi.org/10.1016/j.ejmech.2013.09.050

Turkan F, Cetin A, Taslimi P, Gulçin İ (2018) Some pyrazoles derivatives: potent carbonic anhydrase, α-glycosidase, and cholinesterase enzymes inhibitors. Arch Pharm 351:1800200. https://doi.org/10.1002/ardp.201800200

Türkan F, Huyut Z, Demir Y, Ertaş F, Beydemir Ş (2019) The effects of some cephalosporins on acetylcholinesterase and glutathione *S*-transferase: an in vivo and in vitro study. Arch Physiol Biochem 125:235–243. https://doi.org/10.1080/13813455.2018.1452037

Türkeş C (2019a) A potential risk factor for paraoxonase 1: in silico and in-vitro analysis of the biological activity of proton-pump inhibitors. J Pharm Pharmacol 71:1553–1564. https://doi.org/10.1111/jphp.13141

Türkeş C (2019b) Investigation of potential paraoxonase-I inhibitors by kinetic and molecular docking studies: chemotherapeutic drugs. Protein Pept Lett 26:392–402. https://doi.org/10.2174/0929866526266619022622 5

Türkeş C (2019c) Inhibition effects of phenolic compounds on human serum paraoxonase-1 enzyme. J Inst Sci Technol 9:1013–1022. https://doi.org/10.21597/jist.491054

Türkeş C, Beydemir Ş (2019) Inhibition of human serum paraoxonase-I with antimycotic drugs: in vitro and in silico studies. Appl Biochem Biotechnol. https://doi.org/10.1007/s12010-019-03073-3

Türkeş C, Söyüt H, Beydemir Ş (2014) Effect of calcium channel blockers on paraoxonase-1 (PON1) activity and oxidative stress. Pharmacol Rep 66:74–80. 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. J Enzyme Inhib Med Chem 30:622–628. 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. Environ Toxicol Pharmacol 42:252–257. https://doi.org/10.1016/j.etap.2015.11.024

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

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

Türkeş C, Demir Y, Beydemir Ş (2019c) Anti-diabetic properties of calcium channel blockers: inhibition effects on aldose reductase enzyme activity. Appl Biochem Biotechnol 189:318–329. https://doi.org/10.1007/s12010-019-03009-x

von Bernhardi R, Alarcón R, Mezzano D, Fuentes P, Inestrosa NC (2005) Blood cells cholinesterase activity in early stage Alzheimer's disease and vascular dementia. Dement Geriatr Cogn Disord 19:204–212. https://doi.org/10.1159/000083500

Wager TT, Chandrasekaran RY, Hou X, Troutman MD, Verhoest PR, Villalobos A, Will Y (2010) Defining desirable central nervous system drug space through the alignment of molecular properties, in vitro ADME, and safety attributes. ACS Chem Neurosci 1:420–434. https://doi.org/10.1021/cn100007x

Weinstock M (1999) Selectivity of cholinesterase inhibition. CNS Drugs 12:307–323. https://doi.org/10.2165/00023210-199912040-00005

Yamali C, Gul HI, 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. Chem Biol Drug Des 91:854–866. https://doi.org/10.1111/cbdd.13149

Yiğit B, Yiğit M, Taslimi P, Gök Y, Gülçin İ (2018) Schiff bases and their amines: synthesis and discovery of carbonic anhydrase and acetylcholinesterase enzymes inhibitors. Arch Pharm 351:1800146. https://doi.org/10.1002/ardp.201800146