Erzincan Üniversitesi
Fen Bilimleri Enstitüsü Dergisi
2024,17(1), 174-195
ISSN: 1307-9085, e-ISSN: 2149-4584
Araştırma Makalesi

Erzincan University
Journal of Science and Technology
2024,17(1), 174-195
DOI: 10.18185/erzifbed.1377310
Research Article

Exploring Novel Schiff Base Compounds Derived from Benzothiophene-3-carboxaldehyde Hydrazones: In vitro and In silico Evaluation as Potential Inhibitors of Cholinesterases and Carbonic Anhydrases I-II

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Received: 17/10/2023, **Revised:** 07/03/2024, **Accepted:** 07/03/2024, **Published:** 28/03/2024

Abstract

In this study, we evaluated the inhibition of several cytosolic enzymes, aiming to shed light on and potentially treat various associated diseases such as Alzheimer's, Parkinson's, and Glaucoma. Our goal is to minimize drug side effects by combining multiple effects in a single molecule. To achieve this, we investigated the *in vitro* effects of two new benzothiophene Schiff bases on cholinesterases (AChE and BuChE) as well as human carbonic anhydrase isoforms (CAI and CAII). Molecular modeling studies were also conducted to elucidate the inhibition mechanism of these two compounds on these enzymes. Subsequently, both compounds (1a and 1b) were tested *in vitro* on the aforementioned enzymes. Furthermore, the *in vitro* results were supported by data obtained from in silico studies. Our findings indicate that benzothiophene derivatives significantly inhibited these enzymes. Compound 1b exhibited a stronger inhibitory effect against CAI and CAII compared to the AZA control compound. Additionally, both compounds demonstrated more potent inhibitory effects on cholinesterases (AChE and BuChE) compared to the control compound Tacrine.

Keywords: Benzothiophene, Schiff bases, cholinesterases, carbonic anhydrase, inhibition.

Schiff Baz Bileşikleri olarak yeni benzotiyofen-3-karboksaldehid hidrazon türevleri: Potansiyel kolinesteraz (AChE-BuChE) ve karbonik anhidraz (CAI-CAII) inhibitörleri olarak *in vitro* ve *in silico* değerlendirmesi

Öz

Bu çalışmada, birçok sitozolik enzimin inhibisyonunu değerlendirdi ve Alzheimer, Parkinson ve Glokom gibi çeşitli ilişkili hastalıkları aydınlatılması ve potansiyel olarak tedavi edilmesi amaçlanmıştır. Amacımız, tek bir molekülde birden fazla etkiyi birleştirerek ilaç yan etkilerini en aza indirmektir. Bunun için, iki yeni benzotiyofen Schiff bazının kolinesterazlar (AChE ve BuChE) ile insan karbonik anhidraz izoformları (CAI ve CAII) üzerindeki *in vitro* etkileri araştırılmıştır. Bu iki bileşiğin enzimler üzerindeki inhibisyon mekanizmasını aydınlatmak için moleküler modelleme çalışmaları da yürütülmüştür. Daha sonra, her iki bileşiği (1a ve 1b) söz konusu enzimler üzerinde *in vitro* olarak test edildi. Ayrıca, *in vitro* sonuçlarımızı in silico çalışmalardan elde edilen verilerle desteklendi. Bulgular, benzotiyofen türevlerinin bu enzimleri önemli ölçüde inhibe ettiğini göstermektedir. Bileşik 1b, AZA kontrol bileşiğine kıyasla CAI ve CAII üzerinde daha güçlü bir inhibisyon etkisi sergiledi. Ayrıca, her iki bileşiğin de kolinesterazlar (AChE ve BuChE) üzerinde kontrol bileşiği takrin'e kıyasla daha güçlü inhibisyon etkileri gösterdiği belirlenmiştir.

Anahtar Kelimeler: Benzotiyofen, Schiff Bazı, kolinesteraz, karbonik anhidraz, inhibisyon.

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1. Introductıon

Different derivatives of benzothiophenes has been reported as compounds which demonastrate various biolgical and pharmacological activities like anti-inflammatory (1), antibacterial (2-4), antiviral (5) and anticancer agents (6, 7). Also, Schiff bases are an important class of organic compounds that have antifungal and antimalarial activity, among others, and are widely used and researched for their numerous applications in many fields including analytical, biological and inorganic chemistry (8-16). The synthesis of benzothiophene-derived Schiff bases has been made by some researchers and antimicrobial, antioxidant, antileishmanial activities of them have been examined. (17-20). Activity studies of such compounds, especially against cholinesterase and carbonic anhydrase enzymes, have not been found more in the literature. Therefore, molecular docking studies of new synthesized compounds and enzymes were carried out to gain an idea about the possible interaction between enzymes and synthesized compounds. Data obtained as a result of molecular docking motivated us to make this study about the preparation and characterization of Schiff base with benzothiophene derivatives and also their anti-cholinesterase and anti-carbonicanhydrase activities.

Enzymes act as catalysts in many biochemical reactions and are important in regulating, controlling and accelerating these reactions. In the drug development process, the inhibition of enzymes plays an important role in the development of drugs used for the treatment of many diseases. Acetylcholine esterase (AChE) and butyrylcholinesterase (BuChE), which are enzymes that terminate the effects of molecules known as neurotransmitters on the nervous system, are used as targets in the treatment of nervous system disorders such as Alzheimer's disease. In addition, this enzyme, which has effects on its functions in the respiratory muscles, has an important place in the treatment of diseases such as myasthenia gravis (21). Another group of enzymes associated with the respiratory system are the carbonicanhydrases (CAs). CAs are also responsible for respiration and CO_2/(HCO_3^-) transport, pH and CO_2 homeostasis, electrolyte release in various organs and tissues, biosynthesis reactions (such as gluconeogenesis, lipogenesis, and ureagenesis), bone resorption, calcification and tumorigenesis. Additionally they also play an important role in many physiological and pathological processes in different organisms(22). Therefore, enzyme inhibition is an important strategy to be selected as a target in the drug development process.

Alzheimer's disease (AD) is a progressive, irreversible, incurable, neurodegenerative disease and is the most common form of dementia. In addition, AD is a problematic and expensive disease for humanity, and it is also known as a 'silent threat' (23,24). Acetylcholine esterase inhibitors are drugs used to increase acetylcholine (ACh) levels in the brain. Alzheimer's disease causes a decrease in the level of acetylcholine in the brain, and as a result, neural transmission processes are disrupted (25). Acetylcholine esterase inhibitors can help relieve the symptoms of this disease by increasing the levels of acetylcholine in the brain.

On the other hand, carbonic anhydrase (CA) plays a crucial role in signal processing, long-term synaptic conversion, and the careful overhaul of memory storage. Abnormalities in carbonic anhydrase enzyme activity impair cognition and are associated with mental retardation,

Alzheimer's disease, and aging (26). CA function has been associated with AD pathology as well as stroke. CAs are important potential mediators and inhibition of CA is essential in AD. The aim of several recent studies is to further elucidate the association of CAs with both AD and stroke (27).

In this study, the in vitro activity of synthesized new benzothiophene rings against AChE, BuChE, hCAI and hCAII enzymes was evaluated and molecular docking studies were carried out.

2. Material and Methods

2.1 Chemicals

The Stuart melting point SMP30 apparatus was employed for determining uncorrected melting points, while ^{1}H and ^{13}C NMR spectra were recorded using a Varian 400 MHz spectrometer located in Palo Alto, CA. TMS served as the internal standard, and DMSO-d6 was utilized as the solvent during this process. ESI mass spectra were generated using a Waters Micromass ZQ device, and elemental analyses were conducted using the CHNS-932 instrument from Leco Corporation in St. Joseph, MI. Merck silica gel 60 (230–400 mesh ASTM) was employed for chromatography procedures. All spectral analyses were carried out at the Central Laboratory of the Faculty of Pharmacy at Ankara University. With the exception of L-tyrosine (E.Merck), Sepharose-4B, indazole molecules, and chemicals for electrophoresis, all other chemical reagents used in synthesis and other procedures were obtained from Sigma (Germany) and Aldrich (USA). Furthermore, AChE (CAS no. 9000-81-1) and BuChE (CAS no. 9001-08-5) were procured from Sigma-Aldrich.

2.2 Synthesis of compounds 1a and 1b

The general procedure for synthesizing compounds 1a and 1b involved the condensation of bromo-substituted phenylhydrazine hydrochloride with benzothiaphene-3-carboxaldehyde (Fig. 1). The new imines were obtained using a methodology adapted from Kidwai (28).

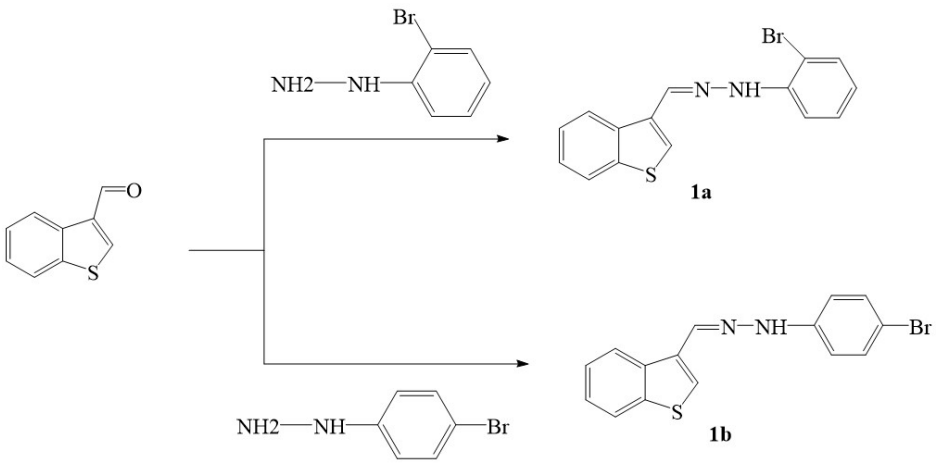

Figure 1. Synthesis of benzothiophene-3-carboxaldehyde hydrazones

2.3 Synthesis of 1-(benzo[b]thiophen-3-ylmethylene)-2-(2-bromophenyl)hydrazine (1a)

Benzothiophene-3-carboxaldehyde (1 mmol) was reacted with 2-bromophenyl hydrazine hydrochloride (1.2 mmol) in 20 ml of absolute ethanol in the presence of 0.4 g CH_3COONa for 2 hours on a hot water bath. Upon completion of the reaction, the mixture was cooled to room temperature. The resulting precipitate was collected, washed with cold water, and recrystallized from EtOH to obtain compound 1a. Yield 70%; m.p. 129–131°C; ^{1}H-NMR: 6.76(td, 1H, J=8 & 1.6 Hz), 7.37(td, 1H, J=8 & 1.6 Hz), 8.01(s, 1H), 7.44-8.72(m, 6H, Ar-H) 8.66(s, 1H, azomethine-CH), 9.61(s, 1H, hydrazon-NH); ^{13}C-NMR: 106.13; 114.10; 120.23; 123.05; 124.46; 125.12; 125.16; 128.79; 129.24; 131.66; 132.68; 135.67; 137.89; 140.18; 142.44 (azomethine C); ESI MS m/z 331(M+,%100),333(M+2, %90).

2.4 Synthesis of 1-(benzo[b]thiophen-3-ylmethylene)-2-(4-bromophenyl)hydrazine (1b)

Benzothiophene-3-carboxaldehyde (1 mmol) was reacted with 4-bromophenyl hydrazine hydrochloride (1.2 mmol) in 20 ml of absolute ethanol in the presence of 0.4 g CH_3COONa for 2 hours on a hot water bath. Upon completion of the reaction, the mixture was cooled to room temperature. The resulting precipitate was collected, washed with cold water, and recrystallized from EtOH to obtain compound 1b. Yield 84%; m.p. 133–135°C; ^{1}H-NMR: 7.05(d, 2H, J=11.8 Hz), 7.41(d, 2H, J=8.8 Hz), 7.43-8.73-8.7(m, 4H), 7.99(s, 1H) 8.22(s, 1H, azomethine-CH), 10.44(s, 1H, hydrazon-NH); ^{13}C-NMR: 109.38; 113.78; 123.00; 124.61; 125.05; 125.12; 128.53; 131.79; 131.87; 134.90; 135.62; 140.17; 144.63 (azomethine C); ESI MS m/z 331(M+,%100),333(M+2, %90).

2.5 Molecular docking studies

In the molecular docking study, hCA I (PDB: 2FOY) (29) and hCA II (PDB: 1IF7) (29), as well as AChE and BChE (30), were retrieved from the Protein Data Bank in pdb format. Ions, water molecules, and ligands were removed from the downloaded proteins, and polar hydrogens and Gasteiger charges were added to the proteins. The proteins were saved in pdbqt format using AutoDockTools 1.5.6 (31). Ligands were drawn in ChemDraw3D 19.0, minimized, and saved in pdb format. After conversion to pdbqt format using AutoDockTools 1.5.6, molecular docking was carried out using the latest AutoDock Vina program (32). The results were visualized in 2D and 3D using PyMOL (33) and the Discovery Studio Visualizer (34).

2.6 Biological activity studies

Inhibition Studies of Cholinesterase Enzymes

AChE (CAS no. 9000-81-1) and BuChE (CAS no. 9001-08-5) used in the study were purchased from Sigma-Aldrich. The inhibitory effects of, two benzothiophene Schiff bases (1a and 1b compounds) on AChE and BuChE activities were determined by IC_{50} and Ki values under *in vitro* conditions, following the methodology outlined by Ellman et al. (35). IC_{50} means the

inhibitor concentration that reduces the enzyme activity by half, and a low IC_{50} value indicates high inhibition power. To determine the IC_{50} values of (**1a and 1b**) compounds, AChE and BChE activities were measured at least five different concentrations of each molecule and %Activities were calculated. The activity of the enzymes without inhibitor was accepted as 100%. %Activity versus inhibitor concentrations were then plotted for each molecule. From these graphs, the IC_{50} values of each molecule for AChE and BChE were determined. Lineweaver–Burk graphs were drawn using three different concentrations of 1a and 1b compounds and six different concentrations of substrate (Acetylthiocholine iodide: ACh and Butyrylthiocholine iodide: BuCh) for the calculation of K_i values. The same procedures were done for tacrine, the standard inhibitor of AChE and BChE enzymes, and both IC_{50} and K_i values were calculated. The enzyme unit was calculated using the molar absorption coefficient (13.600 $M^{-1}.cm^{-1}$) of 5-thio-2-nitrobenzoic acid (DTNB) at 412 nm (36).

Carbonic Anhydrase Isoenzymes Inhibition Studies

All purification procedures were conducted following protocols outlined in our previous studies (37-40). Protein quantification at each purification step was performed spectrophotometrically at 595 nm using the Bradford method (41). Carbonic anhydrase (CA) isoenzyme activities were determined according to the method described by Verpoorte et al. (42). Enzyme activities were assessed by measuring the increase in absorbance spectrophotometrically at 348 nm. A concentration-response curve of inhibitor concentration versus percentage activity was generated to assess the inhibitory potency of each benzothiophene-derived Schiff base compound (1a-1b) against both hCA isoenzymes. IC50 values were derived from these curves. Ki values were calculated using three different concentrations of the tested compounds and five different concentrations of substrate. Additionally, inhibition curves of the most potent compounds were plotted and presented. Both IC50 and Ki values were determined using the same procedures applied to AZA, which served as the standard inhibitor of CA isoenzymes.

3. Results and Discussion

In this study, first, in silico studies of novel benzothiophene derived Schiff bases compounds and then in vitro effects were studied on hCAI, hCAII isoenzymes, and cholinesterases (AChE and BuChE) activities.

After CAI and CAII isoenzymes were purified by CNBr-activated Sepharose-4B-L-tyrosine sulfanilamide affinity chromatography method, the effects of novel benzothiophene derived Schiff bases compounds on these isoenzymes were examined. 1a and 1b showed strong inhibitiory effects at very low concentrations on hCAI and hCAII isoenzymes. Inhibitor concentration versus %Activity for 1a and 1b compounds showing inhibition effect on hCAI and hCAII isoenzymes were graphed (Fig.2). Ki values were determined by three different concentrations of each compound. The compounds were found to showed inhibitory effects at all three different concentrations. Lineweaver-Burk curves was drawn for identifying of Ki values and inhibition type. For hCAI and hCAII, the obtained IC_{50} and Ki values were given in Table 1. Ki values were determined to be in the range of 58.82 ± 7.96-126.28 ± 26.22 nM for

hCAI and 27.86 ± 3.76-74.30 ± 7.89 nM for hCAII. Compared to the control compound AZA, both 1a and 1b represent potent inhibitory effect on carbonicanhydrase isoenzymes (Fig 1.) while 1b demonstrated more strong inhibition effect on these isoenzymes (Fig 2.) (Table 1).

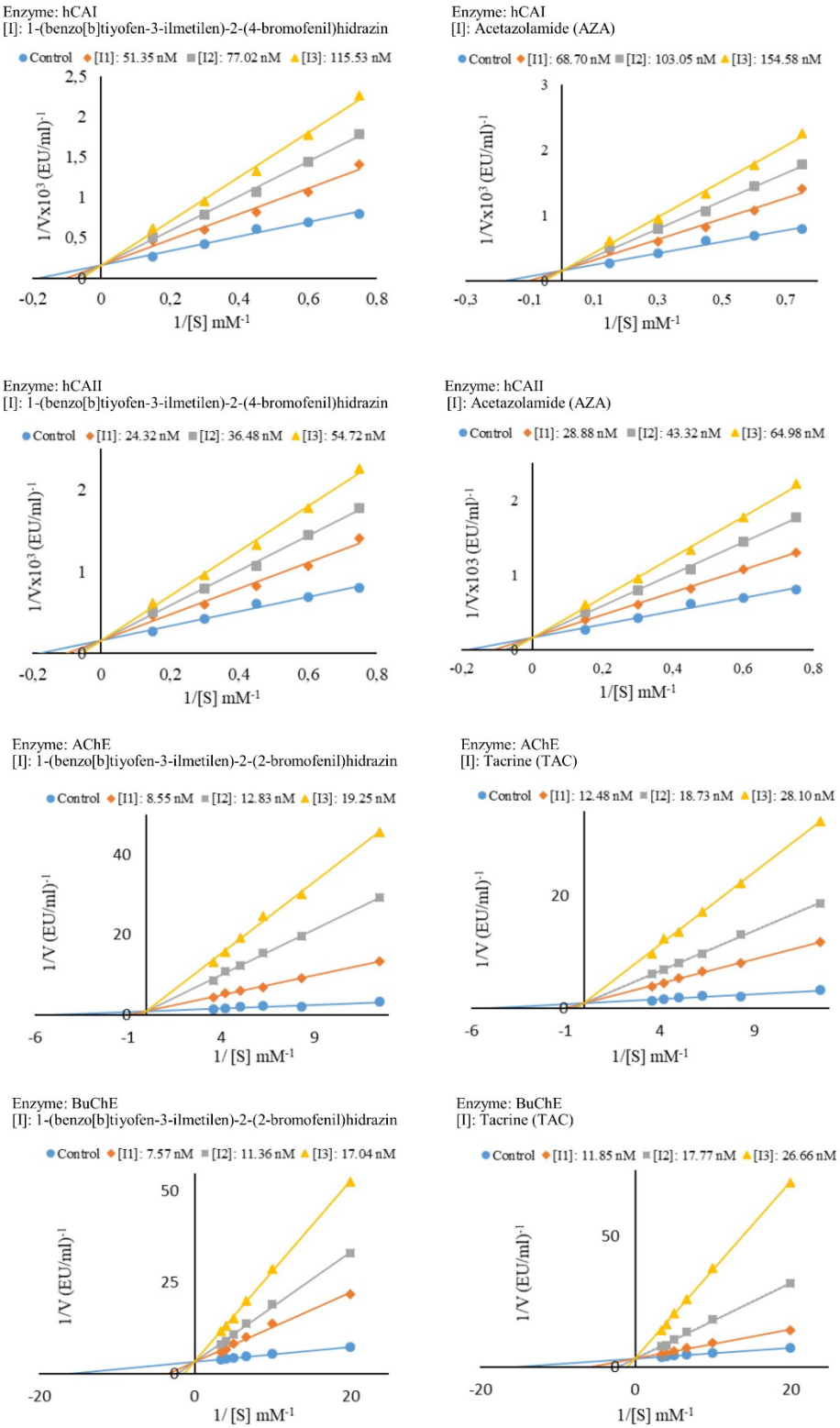

Figure 2. Ki constants and inhibition types were determined by using Lineweaver-Burk graphs for

excellent inhibitors of hCAI, hCAII, AChE and BuChE enzymes. (Each enzyme was given in comparison with its specific inhibitor.)

Table 1. Experimental IC_{50} and Ki values of hCAI and hCAII for thiophene derivative schiff bases (1a-1b) and AZA as standard inhibitors.

	IC_{50} (nM)				K_i (nM)	
Compounds	**hCA I**	**R^2**	**hCA II**	**R^2**	**hCA I**	**hCA II**
1a	86.64	0.9970	38.51	0.9960	126.28 ± 26.22	74.30 ± 7.89
1b	77.02	0.9968	36.48	0.9959	58.82 ± 7.96	27.86 ± 3.76
Acetazolamide (AZA)	103.05	0.9979	43.32	0.9937	78.70 ± 10.64	33.63 ± 5.41

On the other hand, the in vitro effects of 1a and 1b compounds on AChE and BuChE activity were investigated. Compared with the control compound Tacrine, both compounds showed the best inhibitory effect for cholinesterases (AChE and BuChE). Ki values were determined to be in the range of 1.31 ± 0.39-2.16 ± 1.01 nM for acetylcholinesterase; in the range of 1.80 ± 0.27-2.01 ± 1.67 nM for butyrylcholinesterase. In addition, to explain the inhibition mechanism Ki values and inhibition types were determined for 1a and 1b compounds on whole enzymes. Except that 1a non-competitively inhibited CAI, other enzymes were competitively inhibited by the all compounds. (Table 2, Fig.1).

Table 2. Experimental IC_{50} and Ki values of AChE and BuChE for thiophene derivative schiff bases (1a-1b) and Tacrine as standard inhibitors.

	IC_{50} (nM)				K_i (nM)	
Compounds	**AChE**	**R^2**	**BuChE**	**R^2**	**AChE**	**BuChE**
1a	12.83	0.9977	11.36	0.9951	1.31 ± 0.39	1.80 ± 0.27
1b	13.86	0.9909	13.08	0.9916	2.16 ± 1.01	2.01 ± 1.67
Tacrine (TAC)	18.73	0.9974	17.77	0.9908	2.57 ± 0.39	3.92 ± 2.76

Selectivity index values of the compounds were calculated to assess their preference for targeting specific enzymes. According to these values, compound 1b, which exhibited higher potency compared to AAZ, reduced hCAI activity approximately 1.34-fold and hCAII activity approximately 1.21-fold, with a higher affinity towards hCAII (Ki (hCAI)/ Ki (hCAII): 2.11). Conversely, compound 1a, with greater potency compared to TAC, decreased AChE activity approximately 2.17-fold. Compound 1b, surpassing TAC in potency, reduced BuChE activity approximately 1.07-fold. Moreover, compound 1a, which outperformed BuChE, reduced AChE activity approximately 1.96-fold (Table 3).

Table 3. Selectivity index values of the compounds (1a-1b).

Compounds	Ki (hCAI)/ Ki (hCAII)	Ki(AAZ)/ Ki (hCAI)	Ki (AAZ)/ Ki(hCAII)	Ki(AChE)/ Ki (BuChE)	Ki(TAC)/ Ki (AChE)	Ki(TAC)/ Ki (BuChE)
1a	1.68	0.62	0.45	1.96	2.17	0.73
1b	2.11	1.34	1.21	1.19	1.95	1.07

In molecular docking studies, it was observed that the binding energies of 1a and 1b to hCA-I and hCA-II enzymes were lower than the reference drug acetazolmide. Likewise, compounds 1a and 1b were found to bind to AChE and BChE enzymes with lower binding energies than the reference drug tacrine (Table 4).

Table 4. Enzyme binding energies of compounds and reference drugs

Bileşik Adı	Binding energy for hCA-I (kcal/mol)	Binding energy for hCA-II (kcal/mol)	Binding energy for AChE (kcal/mol)	Binding energy for BChE (kcal/mol)
1a	-6.5	-7.1	-9.7	-8.6
1b	-6.9	-7.1	-9.6	-8.1
Asetazolamide	-5.7	-6.1	-	-
Tacrine	-	-	-8.5	-8.0

When the 2D images are examined, it is seen that the benzothiophene rings of the compounds make a pi-sulfur bond with the asparagine amino acid, The nitrogen atom in the schiff base is connected to the serine amino acid in compound 1a, while it is connected to the lysine amino acid in compound 1b. The reference drug, acetazolamide, binds with threonine, histamine and glycine amino acids (Fig 3.).

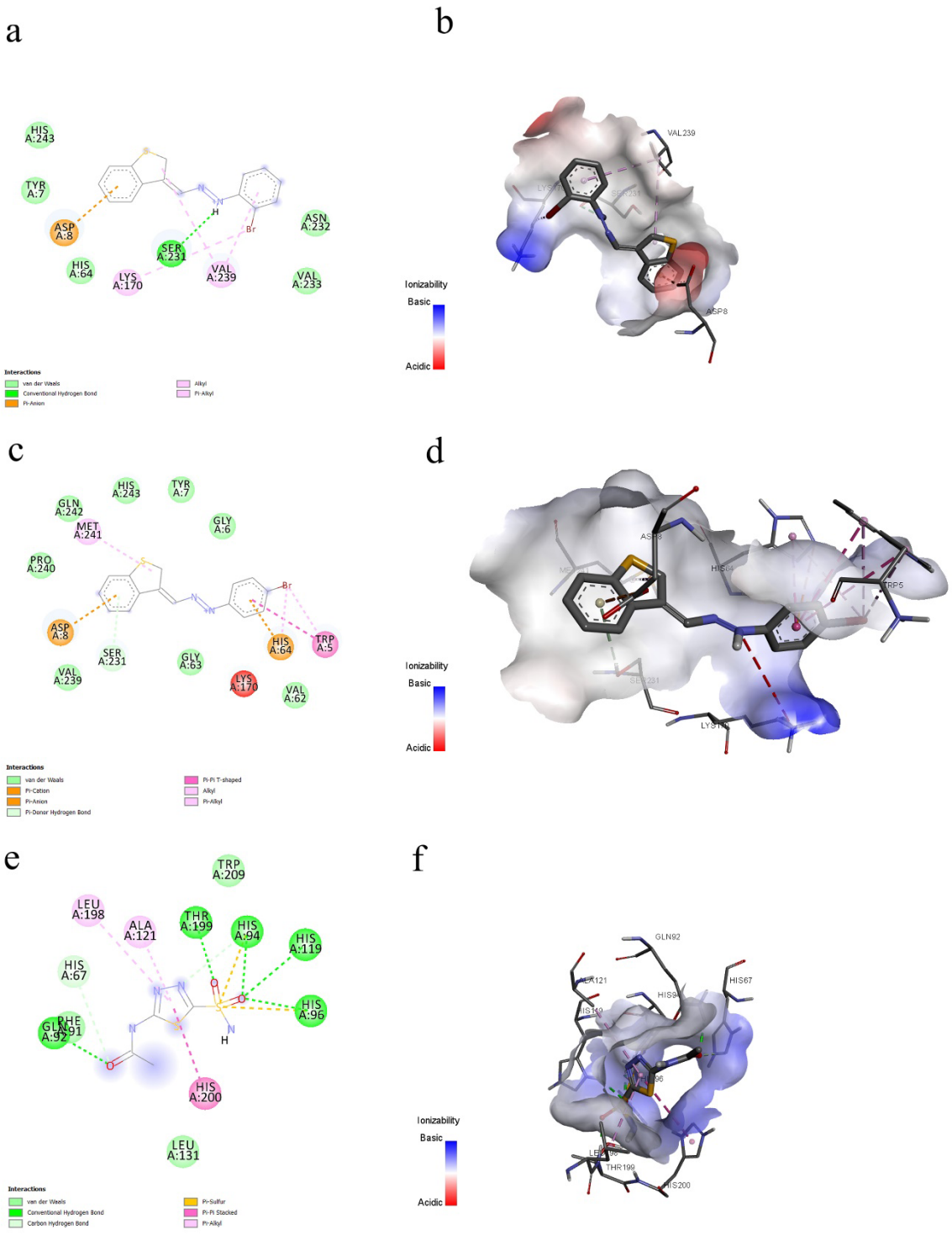

Figure 3. 2D molecular docking images of 1a (a), compound 1b (c) and reference drug acetazolamide (e) located in the active site of the hCA I enzyme and 3D molecular docking images of 1a (b), compound 1b (d) and reference drug acetazolamide (f) located in the active site of the hCA I enzyme

In compounds 1a and 1b the benzothiophene ring, makes pi-pi bonds with tryptophan and phenylalanine amino acids. In addition, the N atom in the Schiff base is also bonded with the amino acids glutamine and phenylalanine. Moreover in both compounds, it is seen that the phenyl group attached to the Schiff base is bonded with glutamine in compound 1a and tyrosine in compound 1b. The reference drug acetazolamide binds with tyrosine, histamine, gultamine and glycine amino acids (Fig 4.).

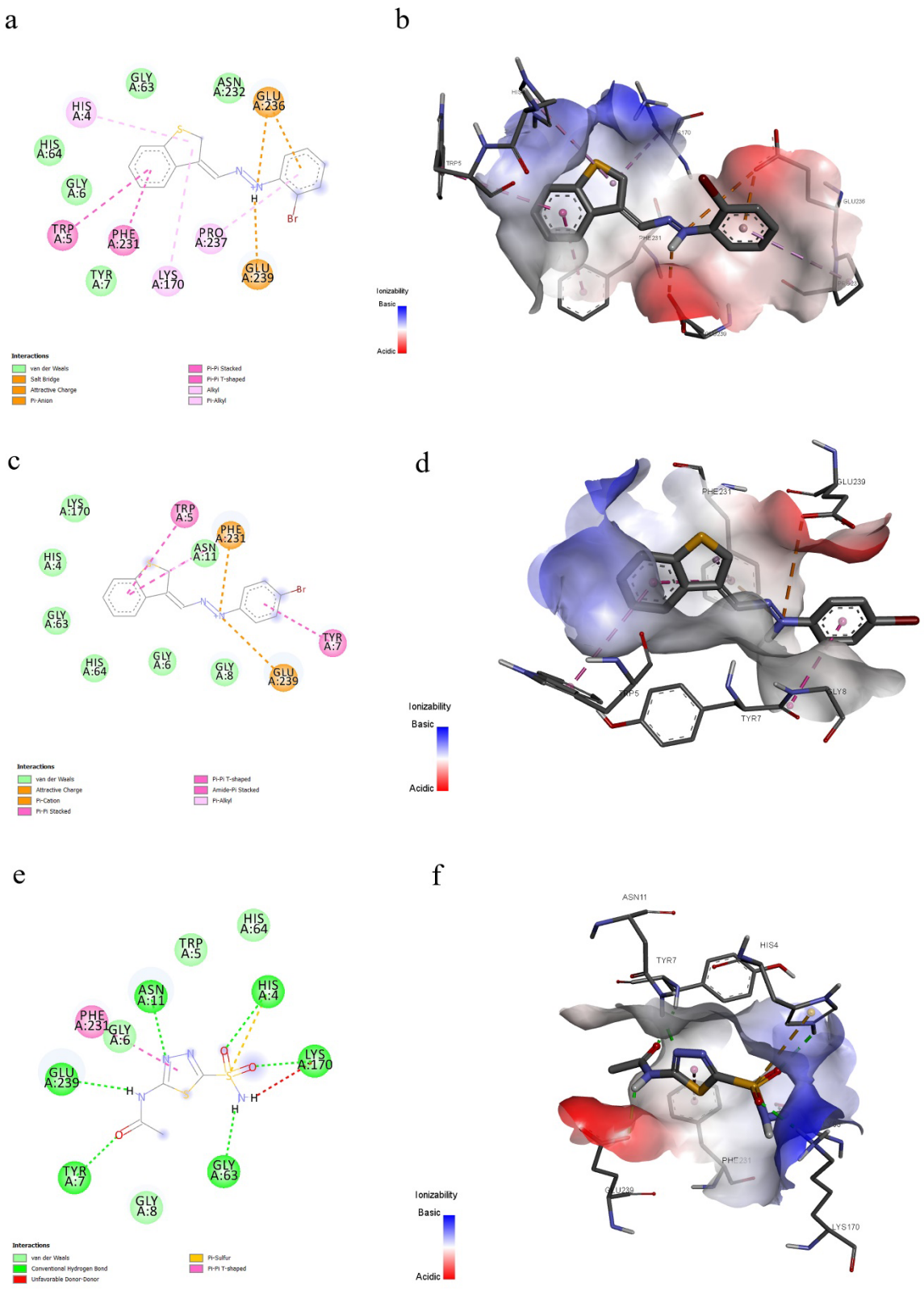

Figure 4. 2D molecular docking images of 1a (a), compound 1b (c) and reference drug acetazolamide (e) located in the active site of the hCA II enzyme and 3D molecular docking images of 1a (b), compound 1b (d) and reference drug acetazolamide (f) located in the active site of the hCA II enzyme

When the 2D molecular docking images of the 1a compound bound to the AChE enzyme are examined, it is observed that the benzothiophene ring in the compound makes a pi-pi bond with the tryptophan, phenylalanine and tyrosine amino acids in the enzyme.

Also, it has been revealed that the bromine atom in the 2nd position and the phenyl ring attached to the schiff base form pi-sigma, pi-pi bonds with the tyrosine amino acid. The reference drug, tacrine, was observed to bind only to tryptophan and tyrosine amino acids via pi-pi bonds (Fig 5.).

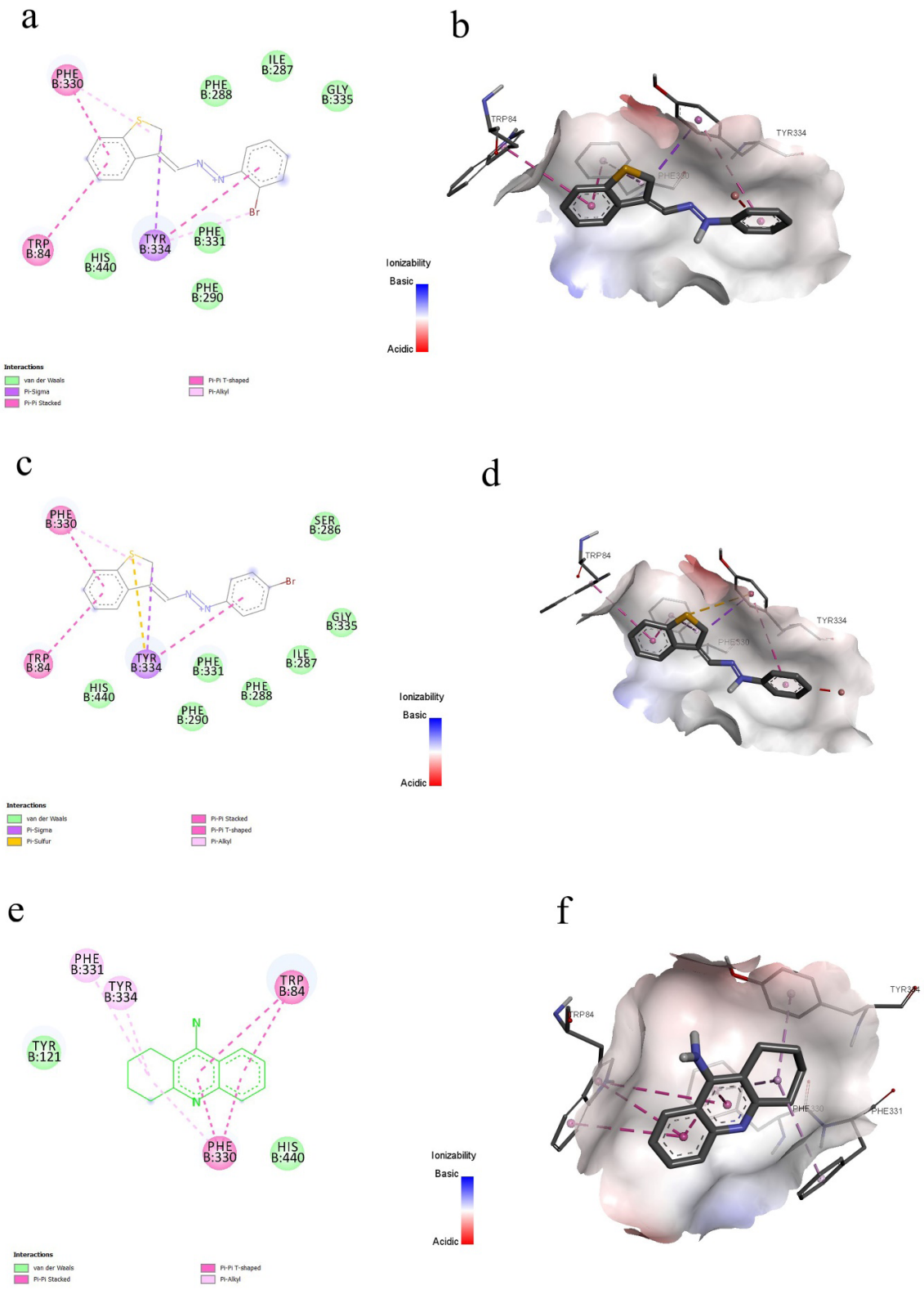

Figure 5. 2D molecular docking images of 1a (a), compound 1b (c) and reference drug tacrine (e) located in the active site of the AChE enzyme and 3D molecular docking images of 1a (b), compound 1b (d) and reference drug tacrine (f) located in the active site of the AChE enzyme

Considering the interactions present in the regions where the compounds bind to the BChE enzyme; It was determined that the benzothiophene ring in compound 1a makes a pi-pi bond with the tryptophan amino acid and the S atom makes a pi-sulfur bond with the phenylalanine amino acid. However, the same benzothiophene ring in compound 1b was found to form a pi-sulfur bond with phenylalanine and a pi-pi bond with tryptophan.

Nitrogen atoms contained in the schiff base in both compounds were observed to interact with histamine in compound 1a, while it was observed to interact with serine in compound 1b. It is detected that the reference drug tacrine molecule interacts with tryptophan and histamine amino acids (Fig.6.)

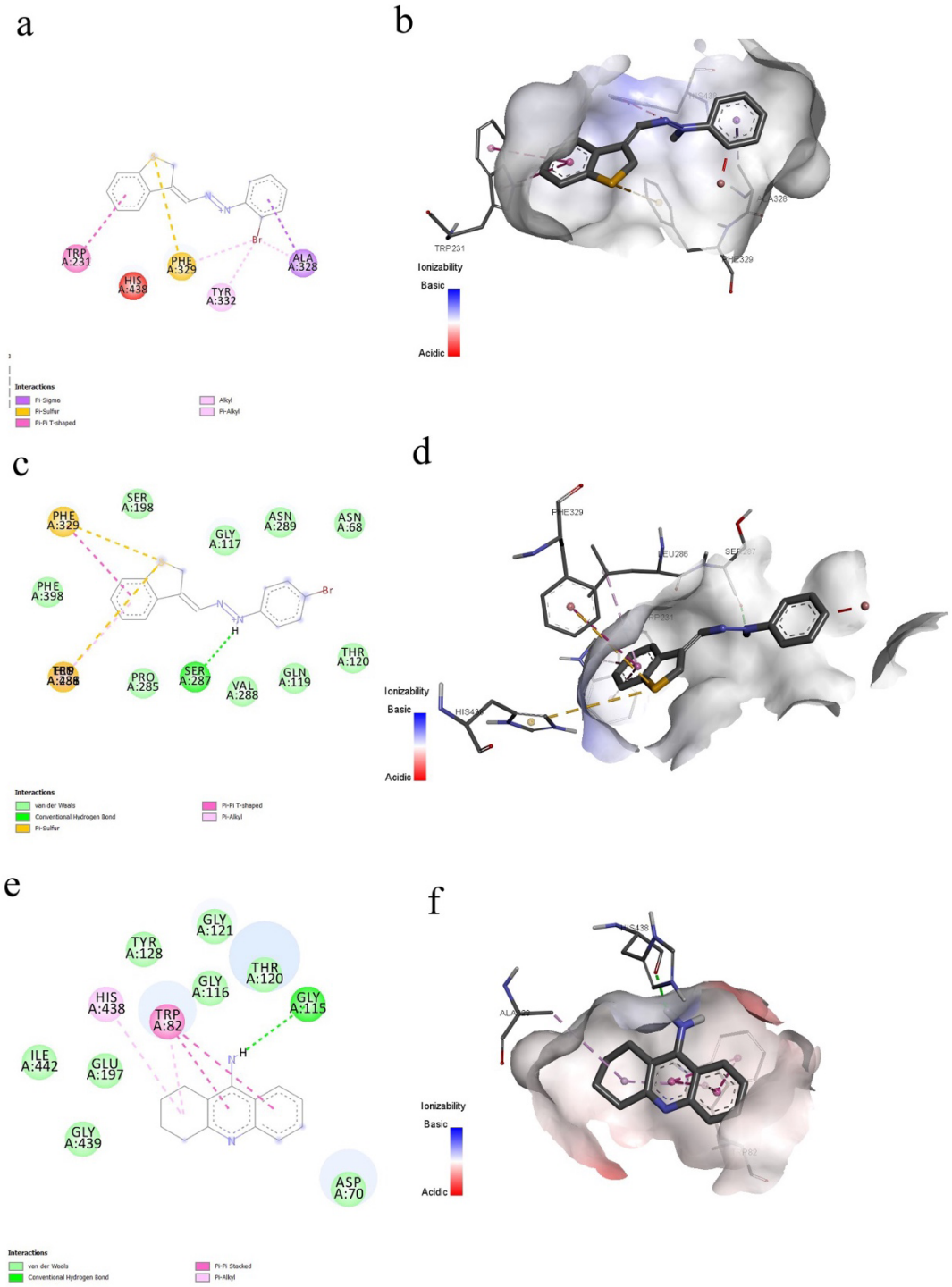

Figure 6. 2D molecular docking images of 1a (a), compound 1b (c) and reference drug tacrine (e) located in the active site of the BChE enzyme and 3D molecular docking images of 1a (b), compound 1b (d) and reference drug tacrine (f) located in the active site of the BChE enzyme

Schiff base compounds are both stable and easily synthesized compounds. Because of these features they have wide usage areas in many fields of chemistry (43), industry (44,45), medicine and pharmacy (46) has increased the interest in these compounds and made them widely used in different fields. In addition, some activities of schiff bases like anti-inflammatory,

antimicrobial, anticancer, antioxidant, antimalarial, antifungal, antiviral, analgesic, anticonvulsant, antituberculous, anthelmintic (17-20, 47,48) have been studied by many researchers. The fact that Schiff bases and derivatives can be used especially as drugs (49) has inspired many researches working in the field of health. It has been reported that different derivatives of benzothiophenes have biological and pharmacological activities, used as anti-inflammatory (1), antibacterial (2-4), antiviral (5) and anticancer agents (6, 7).

In the light of this information, benzothiophene derivative Schiff bases were designed, synthesized and characterized by spectroscopic methods such as ^{1}H-NMR, ^{13}C-NMR, LC-MS/MS. Molecular docking of the compounds into active site of AChE, BuChE, hCAI and hCAII was performed in order to understand ligand–protein interactions and calculate to bindng energy. Compounds 1a and 1b bind to the hCA I, hCAII, AChE, BuChE isoenzyms with a lower binding energy than the reference drug acetazolamide.

To understand ligand-protein interactions, molecular docking studies of compounds into the active site of AChE, BuChE, hCAI and hCAII was evaluated.

It has been observed in our data that the benzothiophene rings of the compounds interact with the Asparagine amino acid in the active site of the AchE enzyme and the tryptophan, phenylalanine and histidine amino acids in the BuChE enzyme.

Phenylalanine and tryptophan amino acids which have exist in the different active site of the hCA I and hCA II enzymes have been interacted with benzothiophene ring. Moreover, the bromine atom in the ortho position contributed to the interaction.

Inhibition of enzymatic activity, besides creating a control mechanism in biological systems, various drugs and toxic compounds exert their effects by enzyme inhibition. Many drugs that are widely used today also act as enzyme inhibitors. In order to determine how inhibitory substances interact with enzymes, IC_{50} values are determined first. Then Ki values are calculated by using the IC_{50} values. Inhibition types can also be determined from Lineweaver Burk graphs drawn for calculating K_i values. To determine the interaction of our synthesized benzothiophene derivative schiff bases with these enzymes, Lineweaver Burk graphs were drawn and K_i values were calculated. According to our results, compound 1b demonstrated excelent inhibition effect on hCAI and hCAII isoenzymes. Compound 1a inhibited both enzymes non-competitively, whereas compound 1b competitively inhibited both isoenzymes. Compared to the control compound AZA, the compound with small Ki value shows strong inhibitor feature (Table 1, Fig.2). On the other hand, compounds 1a and 1b demonstrated excelent inhibition effect on on AChE and BuChE activity. Compared with the control compound Tacrine, both compounds showed the best inhibitory effect for cholinesterases (AChE and BuChE). Both compounds competitively inhibited cholinesterase enzymes (Table 2., Fig.2). Competitive inhibition indicates that the inhibitor acts as a substrate and binds to the active site of the enzyme. It also means that the inhibition effect can be eliminated when the amount of substrate is increased.

4. Conclusion

In conclusion, inhibition of both carbonicanhydrase isoenzymes (hCAI and CAII) and cholinesterases (AChE and BuChE) is important in the treatment of Alzheimer's disease. This study aimed to design and synthesize benzothiophene derivative Schiff bases and test their inhibition activities against AChE and BuChE, hCAI and hCAII. Compounds 1a and 1b demonstrate perfect inhibition activities against to AChE, BChE, hCAI and hCAII. It has been seen at in silico studies that 1a and lb compounds can bind to the active site of the enzyme in a similar way and at appropriate positions, and these results are also supported by in vitro studies. The presence of the bromine atom in the 4th position in the 1b compound caused it to interact more with the active site of the hCAI enzyme. This situation is also confirmed by in-vitro activity results.The position of the bromine atom did not contribute to its interaction with the hCA II enzyme, however, it showed different interactions with the enzyme by affecting the conformations of the molecule.The bromine atom attached to the 2nd position in compound 1a interacted only with tyrosine in the AChE enzyme While it interacted with phenylalanine, tyrosine and alanine amino acides in the BuChE enzyme.In conclusion to evaluate all obtained data, has been considered that these compounds can be evaluated as lead compounds for the development of new drugs for neurodegenerative disorders and may be suitable for further studies.

Acknowledgments

This study received support from project number 1919B012109379 under the auspices of the 2209-A University Students Research Projects Support Program 2021, spanning 2 semesters, administered by the TÜBITAK Scientist Support Programs Presidency (BIDEB). We extend our gratitude to TUBITAK for their financial assistance.

Conflicts of Interest

The authors declare no conflict of interest.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

Author Contributions

Dilek E. written manuscript; Gürsoy Ş., Dilek E. and Şirinzade H. designed the study, prepared protocols, analyzed the data; Gürsoy Ş., Dilek E., Çaka Z., Faydalı N., and Şirinzade H.; performed the experiments and participated in discussions. All the authors were responsible for the data acquisition, review and editing of the paper.

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