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Enzymes inhibition profiles and antibacterial activities of benzylidenemalononitrile derivatives

Pınar Güller[a,*], Ziya Dağalan[a], Uğur Güller[b], Ulaş Çalışır[c,1], Bilal Nişancı[a]

[a] Department of Chemistry, Faculty of Science, Atatürk University, Erzurum, Turkey
[b] Department of Food Engineering, Faculty of Engineering, Iğdır University, Iğdır, Turkey
[c] Vocational School of Health Services, Iğdır University, Iğdır, Turkey

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ABSTRACT

The activities of enzymes can be targeted in the treatment of some diseases. Recently, this strategy has been used frequently in the development of new drugs. Carbonic anhydrase isozymes (CA-I and CA-II) and acetylcholine esterase (AChE) are some of these enzymes. So, in the present study, we aimed to investigate primer effects of presynthesized benzylidenemalononitrile derivatives (**1-11**) on the enzymes via *in vitro* inhibition study, to clarify inhibition profiles of derivatives through molecular docking, and to examine their effects against some bacterial strains.

For this purpose, firstly the benzylidenemalononitrile derivatives were synthesized starting from readily available aldehydes and malononitrile with catalyst free conditions in aqueous medium just in 15 minutes (11 examples). Then, biochemical and molecular docking studies were performed.

CA-I, CA-II and AChE was isolated from the human erythrocyte and the inhibition effects of synthesized derivatives were examined through both *in vitro* and *in silico* approaches. While compound **5** was found as the most effective inhibitor on hCA-I and hCA-II with K_i constant of 7.51±2.25 and 11.92±2.22 μM respectively, it was determined that compound **3** showed the highest inhibition effect on hAChE with K_i of 0.058±0.014 μM. From the antibacterial studies, it was found that while molecule **5** is the most effective compound against *K. pneumonia* molecule **7** is the most effective compound against *S. aureus.*

Introduction

Malononitriles are highly reactive compounds. The reactive methylene group is actively used in condensation reactions as a nucleophile in the preparation of carbo- and heterocyclic products. This property makes them an important chemical in medical, industrial and agricultural applications [1,2]. Malononitrile has been reported to have antithyroid effect after *in vivo* experiments on rats and humans. Besides, its biological activities such as causing an increase of nucleoproteins in cells, regulating RNA synthesis in neurons and nerve tissues, promoting nerve tissue regeneration, neurotransmitter acetylcholine (ACh) biosynthesis and learning and memory processes, and reducing amnesia after electric shock were described [3–5].

ACh, synthesized from cholinergic neurons of the brain, is a significant neurotransmitter involved in signal transmission and delivery of messages. Some literature has been shown that it degrades in a short time in diseases related to memory loss such as Alzheimer's disease (AD) [6–9]. Acetylcholine esterase (AChE, E.C. 3.1.17) catalyses the hydrolysis of ACh to form choline and acetate ions and it performs hydrolysis of approximately 80% of ACh in a healthy brain [8,10]. It was declared that anticholinesterase drugs can significantly reduce patients' behavioural disorders by inhibiting the AChE enzyme. Therefore, one of the potential therapeutic strategy is cholinergic hypothesis via inhibiting the activity of acetylcholinesterase (AChE) [11].

Carbonic anhydrase inhibitors (CAIs) are a class of pharmaceuticals and inhibit the catalytic activity of carbonic anhydrases (CAs). CAs (EC 4.2.1.1) containing Zn^{2+} in the active site, catalyse the reversible conversion of carbon dioxide to bicarbonate and proton [12,13]. hCA-I and hCA-II are two isoforms (among sixteen CA isozymes in human) known to be abundantly expressed in erythrocytes and they are mainly associated with important physiological processes such as the regulation of respiratory and acid/base

* Corresponding author at: Atatürk University, Faculty of Science, Department of Chemistry, 25240, Erzurum, Turkey
E-mail address: ptaser@atauni.edu.tr (P. Güller).
[1] He passed away at the date of December 31, 2019.

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homeostasis, and have been the target of retinal pathologies, brain oedema, glaucoma and epilepsy [14]. CAIs are known to be used in the clinic as diuretics, antiglaucoma agents and antiepileptic [15–18]. Besides, they are used in the treatment of high altitude disease, idiopathic intracranial hypertension, neurological disorders, osteoporosis, gastric and duodenal ulcers, etc. [13, 19–21].

Following the strategy that AChEIs and CAIs are effective in the treatment of some diseases, it was aimed to clarify the inhibition profiles of benzylidenemalononitrile derivatives on AChE and CA isozymes through both *in vitro* and *in silico approaches* and investigate the antibacterial properties of these derivatives.

2. Experimental

2.1. Chemicals

DE-52 anion exchange gel, dialysis bag, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide, Sepharose-4B, p-nitrophenyl acetate (PNF), L-tyrosine and all other chemicals were procured from Sigma Chem. Co. and E. Merk AG. The chemicals used for the synthesis of benzylidenemalononitrile derivatives including organic solvents were purchased from Sigma-Aldrichs and used without any purification. All test microorganisms (*Staphylococcus aureus, Escherichia coli and Klebsiella pneumonia*) used in the determination of antibacterial activity were isolated from clinical samples from Iğdır State Hospital microbiology laboratory. The expired waste human blood was obtained from the Erzurum Turkish Red Crescent, Turkey.

2.2. General procedure for the synthesis of benzylidenemalononitrile derivatives

Benzylidenemalononitrile derivatives were synthesized in previous study [22]. Benzaldehyde (0.25 mmol) and malononitrile (0.25 mmol) were added in a round bottom flask with 3 mL MeOH / H_2O (2: 1) and the mixture was magnetically stirred for 15 minutes at room temperature. After the completion of the reaction, the solvent removed under reduced pressure and the product was purified by recrystallization from dichloromethane/hexanes (3:1) at room temperature. Finally, the samples were prepared for ^{1}H NMR analysis. NMR spectra were recorded using a 400 MHz Bruker NMR instrument (^{1}H NMR at 400 MHz) in deuterated chloroform unless stated otherwise and the Topspin (Topspin 2.1) software was used in NMR.

2.3. Biochemical studies

2.3.2. Enzyme assays

Esterase activity of CA was measured according to the method described by Verpoorte, Mehta [23]. The carbonic anhydrases catalyse the hydrolysis of p-nitrophenylacetate to p-nitrophenol or p-nitrophenylate ion which gives maximum absorption at 348 nm.

The AChE activity was assayed at 436 nm with a spectrophotometer, according to Worek, Mast [24] method, a modified method of the Ellman's procedure.

2.2.3. Isolation of enzymes from human erythrocytes

To prepare hemolysate, erythrocyte pellets were haemolysis by stirring with 5 volume of ice water and cell membrane debris was removed by centrifugation at 10 000 rpm for 15 min.

For isolation of CA isozymes, the pH of hemolysate (25 ml) was adjusted to 8.7 with solid Tris and applied to the Sepharose 4B-L-tyrosine-sulfanylamide affinity column as previously described by Arslan, Nalbantoğlu [25]. CA-I and CA-II isozymes were eluted sequentially from the column with different elution buffer. All experiments were carried out at 4°C. The active eluates were combined and dialyzed against the 0.05 M Tris-SO_4 buffer at pH:7.4, then they were stored at -20 °C up to use in inhibition studies.

For isolation of AChE enzyme, the pH of hemolysate (25 ml) was adjusted to 7.8 with 0.1 M K_2HPO_4 solution and applied to DE-52 anion exchange chromatography column as previously described by Güller et al., 2020 [26]. Active eluates were combined and dialyzed against the 20 mM KH_2PO_4 (pH 7.5) buffer. All these process were carried out at about + 4°C by using ice blocks.

In order to calculate specific activity quantitative protein assay was carried out according to the method of Bradford 1976 at 595 nm [27].

2.3.3. Inhibition assays

In order to investigate the inhibition effects of benzylidenemalononitrile derivatives (Fig. 1), firstly, enzyme activities were assayed at the various concentrations of compounds. Control enzyme activity (in the absence of the compound) was taken as 100%. For each derivative, an activity%- [compound] graphs were drawn using conventional polynominal regression software (Microsoft Office 2010, Excel). Benzylidenemalononitrile derivatives concentrations that produced 50% inhibition (IC_{50}) were calculated from the equation of these graphs. Secondly, for molecules, that showed inhibition effects, inhibition types and constants of the breakdown of enzyme-inhibitor complexes (K_i) were determined by drawing Lineweaver Burk plots. For this purpose, at three different inhibitor concentrations, enzyme activities were measured with five various substrate concentrations.

2.2.5. In silico *studies*

Possible docking modes between molecules and isozymes were studied using the AutoDock4 [28]. The crystal structures of hCA-I (PDB code: 3LXE) [29] hCA-II (PDB code: 5AML) [30], and hAChE (PDB code: 4EY7) [31] were used in docking calculations and their pdb file format were downloaded from protein data bank (http://www.rcsb.org/pdb). Three dimensional structures of the derivatives were obtained by using ChemDraw software as the sdf file and turned into pdb file by using Avogadro software.

After pdb files were obtained, ligand and protein were prepared by using Autodock tool. To prepare the protein, water molecules and other unnecessary atoms were deleted, polar H atoms were added, missing atoms were checked, and Kollman charge was added. The grid box dimension (60 × 60 × 60 Å) and grid spacing (0.375 Å) were adjusted manually to keep the ligand flexible around the protein active site. Grid boxes were centred as x= -5.425, y= 30.918, z= 50.152 for hCA-I, x= -0.183, y= -3.555, z= 7.192 for hCA-II. In docking procedure of AChE, grid boxes were centred as x= 1.165, y= -63.640, z= -24.967 for active site and x= -2.892, y= -40.113, z= 14.866 for the allosteric site. The Lamarckian genetic algorithm was used to determine the appropriate binding positions, orientations, and conformations of ligands. To prepare ligand, rotatable torsions in the ligands were generated with the Autodock tool. Acetazolamide (AZA) was used as standard inhibitor for hCA isozymes and tacrine was used as standard inhibitor for hAChE. The results files were analysed using Discovery Studio Visualizer.

Before molecular docking studies, the validity of the protocol was analysed through re-docking of co-crystallized ligand into the receptors [32]. To test the scoring power, a dataset of known inhibitors and non-inhibitors of enzymes was constructed, and Receiver operating characteristic (ROC) curves were drawn. Active ligands and decoys were generated using the DUD-E web service for hCA isozymes and hAChE [33–35].

2.3.1. Antibacterial assay

All benzylidenemalononitrile derivatives were dissolved in dimethyl sulfoxide (DMSO), reported as not being antibacterial

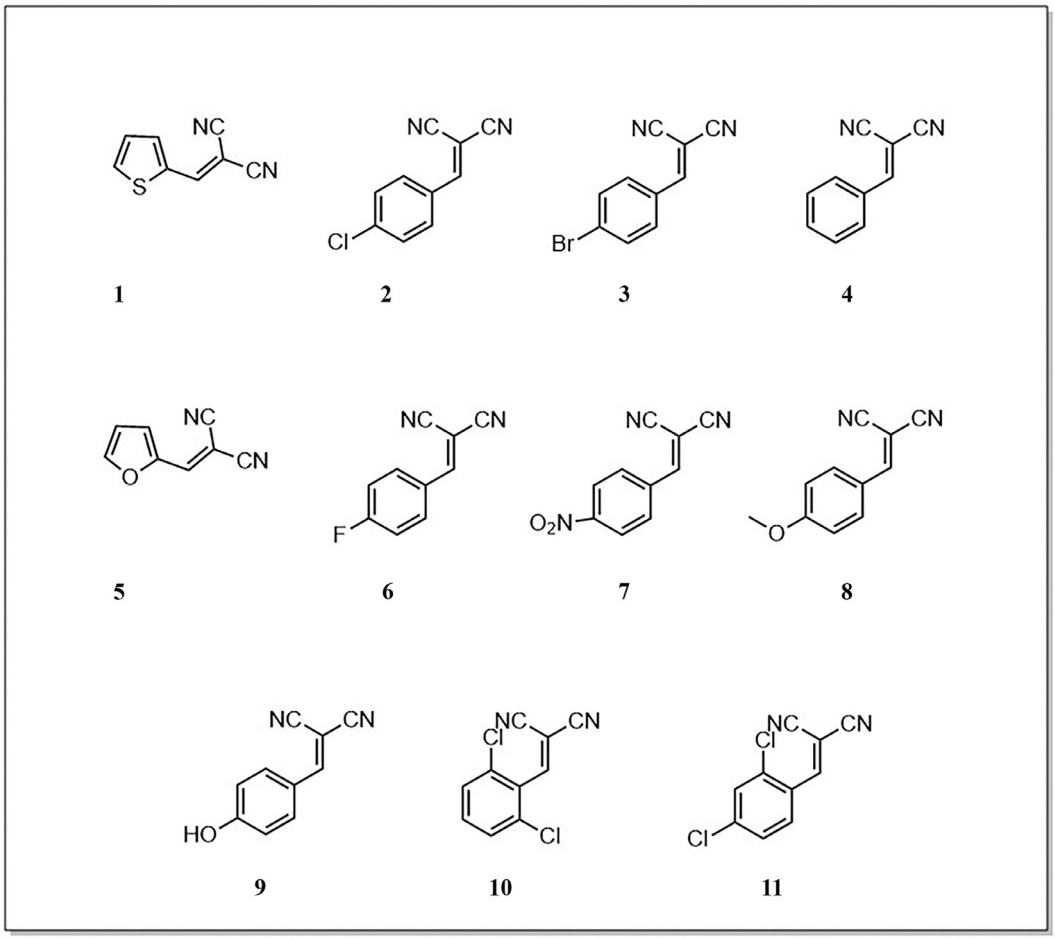

Fig. 1. Structure of benzylidenemalononitrile derivatives.

[36–38]. For the antibacterial analysis, agar diffusion method was carried out [39].

All test microorganisms (*Staphylococcus aureus, Escherichia coli and Klebsiella pneumonia*) were identified using VITEK® 2 identification system.

The bacteria to be tested were inoculated in Mueller Hinton broth media and grown at 37°C overnight and also revived. The microorganism suspensions at 10^6 cfu/ml (colony forming unit/ml) concentrations were inoculated to the Mueller Hinton agar media and spread consistently on the plates. 5 mm diameter wells at 2 cm intervals were made in the plate with the help of sterile glass tube. 50 µL (containing 25, 50, 75, 100, 125, and 150 μg of substance) of chemical solutions was placed into the wells. The plates were incubated at 37 °C for 24 hours and after incubation clear zones of inhibition surrounded the well/disc, mm in diameter, were measured by using a Vernier calliper [40,41]. Commercial discs were used for standard antibacterial [42]. For Gr (-) bacteria Penicillin G (10 U), Clindamycin (2 µg), Erythromycin (15 µg), Chloramphenicol (30 µg), Cephalexin (30 µg), for Gr (+) bacteria Gentamicin (10), Tetracycline (30 µg), Vancomycin (30 µg), Methicillin (5 µg), Bacitracin (0.04 U) disk were used as standard control drug.

3. Results

3.1. Synthesis of benzylidenemalononitrile derivates

The benzylidenemalononitrile analogues were synthesized efficiently at room temperature by known method in the literature [22]. The spectral data were given at supplementary file 1.

3.2. Biochemical studies

3.2.1. In vitro inhibition studies

After isolation of human erythrocyte CA-I, CA-II and AChE, *in vitro* inhibitory effects of benzylidenemalononitrile derivatives on enzymes were measured spectroscopically. And inhibition types and K_i constants of derivatives were determined by means of Lineweaver-Burk graphs.

As indicated in Table 1, it was found out that all molecules inhibited hCA-I and hCA-II noncompetitively. Most effective inhibitor was found as compound **5** for both isozymes with K_i constants of 7.51±2.25 and 11.92±2.22 µM respectively. For the most effective compounds on hCA-I and hCA-II, Lineweaver-Burk graphs were given in Fig. 2a and b.

As for results regarding hAChE, it was seen that molecules **2, 3, 6, 7, 8**, and **9** inhibited hAChE and compound **3** showed the highest inhibition potency with the K_i value of 0.058±0.014 µM (see Fig. 2c). IC_{50} values, K_i constants and inhibition types of inhibitor molecules were summarized in Table 2.

3.2.2. Molecular docking studies

Molecular docking studies were performed by using AutoDock4.2 to elucidate the interactions between the compounds and enzymes. Firstly, the validity of the protocol was analysed through re-docking of co-crystallized ligands into the receptors. Topiramate for hCA-I, 2-(But-2-yn-1-ylsulfamoyl)-4-sulfamoylbenzoic acid for hCA-II and Donepezil for hAChE were used. Docking analysis of hCA isozymes showed that derivative **7** had the smallest binding energy. Molelecule **3** showed the lowest

Table 1
IC_{50} values, K_i constants, and inhibition types of benzylidenemalononitrile derivatives on hCA-I and hCA-II.

	Compounds	Compound No	IC_{50} (μM)	K_i (μM)	Inhibition Type
CA-I	2-(thiophen-2-ylmethylene) malononitrile	1	22.26	17.24±2.81	Noncompetitive
	2-(4-chlorobenzylidene) malononitrile	2	26.54	14.51±7.47	Noncompetitive
	2-(4-bromobenzylidene) malononitrile	3	16.04	11.33±2.79	Noncompetitive
	2-benzylidene malononitrile	4	230	142.33±58.25	Noncompetitive
	2-(furan-2-ylmethylene) malononitrile	5	7.11	7.51±2.25	Noncompetitive
	2-(4-fluorobenzylidene) malononitrile	6	115	104.29±35.62	Noncompetitive
	2-(4-nitrobenzylidene) malononitrile	7	24.64	20.37±2.75	Noncompetitive
	2-(4-methoxybenzylidene) malononitrile	8	40.59	41.42±12.99	Noncompetitive
	2-(4-hydroxybenzylidene) malononitrile	9	138	370.66±41.14	Noncompetitive
	2-(2,6-dichlorobenzylidene) malononitrile	10	345	397.84±70.01	Noncompetitive
	2-(2,4-dichlorobenzylidene) malononitrile	11	14.37	8.30±0.70	Noncompetitive
	Acetazolamide*	AZA*	0.76	0.74±0.094	Noncompetitive
CA-II	2-(thiophen-2-ylmethylene) malononitrile	1	16.43	23.69±3.95	Noncompetitive
	2-(4-chlorobenzylidene) malononitrile	2	27.6	85.65±22.27	Noncompetitive
	2-(4-bromobenzylidene) malononitrile	3	26.54	91.42±14.59	Noncompetitive
	2-benzylidene malononitrile	4	172.5	169.92±68.03	Noncompetitive
	2-(furan-2-ylmethylene) malononitrile	5	10.95	11.92±2.22	Noncompetitive
	2-(4-fluorobenzylidene) malononitrile	6	230	202.06±35.10	Noncompetitive
	2-(4-nitrobenzylidene) malononitrile	7	17.25	12.1±3.4	Noncompetitive
	2-(4-methoxybenzylidene) malononitrile	8	23.79	36.75±2.77	Noncompetitive
	2-(4-hydroxybenzylidene) malononitrile	9	76.67	146.05±15.50	Noncompetitive
	2-(2,6-dichlorobenzylidene) malononitrile	10	690	430.82±98.88	Noncompetitive
	2-(2,4-dichlorobenzylidene) malononitrile	11	14.68	13.27±3.43	Noncompetitive
	Acetazolamide*	AZA*	0.28	0.252 ± 0.007	Noncompetitive

* Acetazolamide (AZA) was used as standard inhibitor for both isozymes.

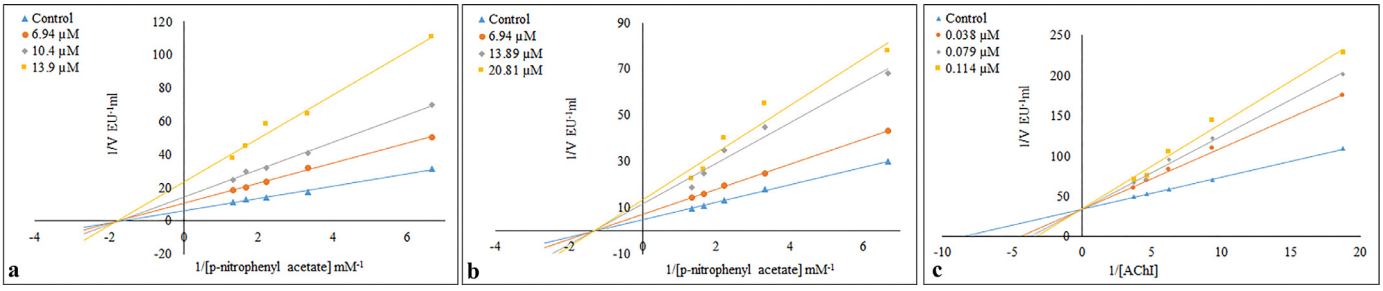

Fig. 2. Lineweaver-Burk plots showing the highest inhibition effects on hCA-I (a), on hCA-II (b), and on hAChE (c).

Table 2
IC_{50} values, K_i constants, and inhibition types of malanontrille derivatives on hAChE.

Compounds	IC_{50} (μM)	K_i (μM)	Inhibition Type
1	-	-	NI
2	76.67	11.91 ± 1.27	Competitive
3	0.134	0.058 ± 0.014	Competitive
4	-	-	NI
5	-	-	NI
6	172.5	34.88 ± 2.68	Competitive
7	69	50.05 ± 13.05	Noncompetitive
8	3.27	5.66 ± 1.04	Noncompetitive
9	23.79	37.18 ± 18.96	Noncompetitive
10	-	-	NI
11	-	-	NI
Tacrine	0.015	0.011 ± 0.001	Noncompetitive

estimated binding energy against hAChE receptor. Estimated Free Energies of Binding were summarized in Table 3.

3.2.3. Antibacterial studies

The benzylidenemalononitrile derivatives were tested against two Gram-negative (*Escherichia coli* and *Klebsiella pneumonia)* and a Gram-positive (*Staphylococcus aureus)* microorganisms. Fig. 3 presents the inhibition zone diameters in mm formed by the compounds against the microbial strains. It was determined that while molecule **5** is the most effective compound against *K. pneumonia* and molecule **7** is the most effective compound against *S. aureus*, derivatives at issue did not affect *E. coli*.

4. Discussion

4.1. Inhibition studies

This paper focused on the *in vitro* inhibition of hCA-I, hCA-II, and AChE activity with pre-synthesized benzylidenemalononitrile derivatives. Furthermore, inhibition profiles were clarified by molecular docking analysis. Because these enzymes act in the regulation of many different biological functions, they have become the therapeutic target for drugs used in the treatment of many diseases. Enzyme inhibition is frequently used for drug design in medicine, so it is important to determine the type of inhibition correctly [43,44]. For example, irreversible inhibitors limit competition with high concentrations of endogenous ligands and achieve desired pharmacological effects even at lower drug concentrations/doses [44].

For the first step of inhibition studies, we partially isolated enzymes from human erythrocytes. *In vitro* inhibition studies of CA isozymes were performed based on the esterase activity considering the nature of inhibition with respect to PNF. The most effective inhibitor for both hCA-I and hCA-II is furan analogue **5** with the IC_{50} constant of 7.11 and 10.95 μM respectively. According to our results about the type of inhibition, it can be predicted that the inhibitors that we studied inhibit the enzymes by binding to a site

Table 3
Results of binding energies, and ligand interaction types of the studied benzylidenemalononitriles with hCA-I, hCA-II, and hAChE.

Compounds	hCA-I Estimated Free Energy of Binding (kcal/mol)	hCA-II Estimated Free Energy of Binding (kcal/mol)	hAChE Estimated Free Energy of Binding (kcal/mol)
1	-5.64	-5.51	NI[a]
2	-5.99	-6.14	-7.02
3	-6.21	-6.04	-7.39
4	-5.72	-5.69	NI
5	-5.69	-5.51	NI
6	-5.63	-5.68	-5.57
7	-7.85	-7.05	-5.69
8	-5.64	-5.51	-5.74
9	-6.49	-6.08	-6.30
10	-6.44	-6.31	NI
11	-6.79	-6.67	NI
AZA[b]	-7.16	-6.19	-
Tacrine[c]	-	-	-6.72

[a] NI: Not inhibitor according to the experimental study.
[b] Acetazolamide (AZA) was used as standard inhibitor for both hCA-I and hCA-II.
[c] Tacrine was used as standard inhibitor for hAChE.

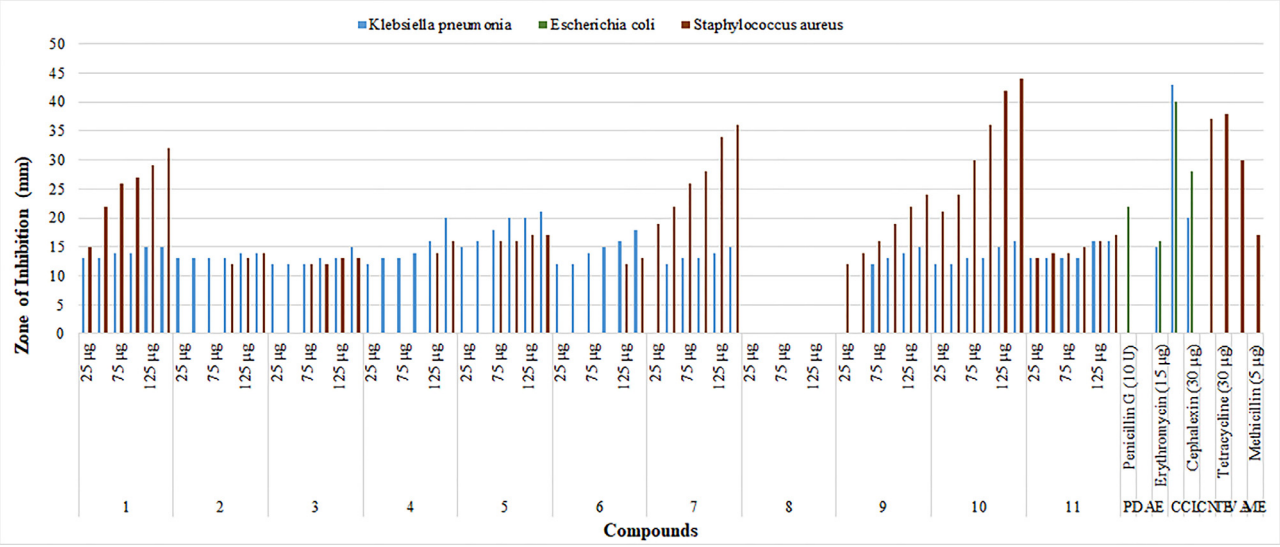

Fig. 3. The inhibition zone diameters formed by the compounds against the microbial strains.

that is remote from the active site, and caused a conformational change within the enzyme. So this conformational change blocked the binding of substrate. Because inhibitor and substrate do not compete the degree of inhibition depends solely on the concentration of the inhibitor.

The previous research also reported that so many synthesized compounds such as tetra-pyridine-triazole-substituted phthalocyanines, hydroxy and phenolic compounds, acridine bis-sulfonamides, 1,3-bis-chalcone derivatives, pyrazole derivatives, some uracil derivatives, 5-methyl-2,4-dihydro-3H-1,2,4-triazole-3-one's aryl schiff base derivatives, ureido benzenesulfonamides, chalcone substituted benzenesulfonamides, chalcones derivatives bearing morpholine moiety, Schiff bases of Sulfa drugs, and alicilaldehyde-N-methylp-toluenesulfonylhydrazone were inhibited hCAs [45–56]. It was concluded that the inhibition potencies of benzylidenemalononitrile derivatives was higher than some benzothiazole derivatives and 7-amino-3,4-dihydroquinolin-2(1H)-one derivatives [57,58]. Acetazolamide (AAZ), methazolamide (MZA), ethoxzolamide (EZA), celecoxib (CLX) drugs, common CAIs, have been used for many years in the treatment of many diseases such as glaucoma, edema, osteoporosis, idiopathic intracranial hypertension [59].

In recent years, the development of new AChE inhibitors has become popular among the organic chemists and pharmacologists. The chromone-2-carboxamido-alkylamines, carbamates, new 4-arylthiazole-2-amine derivatives, thiazole-piperazines and xanthones showed inhibitory effect on electric eel AChE at micromolar range [60–64]. The previous research also reported inhibition of mammals AChE, coumarinyl thiazoles and coumarinyl oxadiazoles showed inhibitory effects on horse serum AChE with IC_{50} ranging 0.87 ± 0.09- 36.34 ± 0.18 µM [65] and indanone derivatives inhibited mouse brain AChE (IC_{50} values as, 12.01-79.71 µM). Novel chromone-2-carboxamido-alkylamines designed and synthesized for cholinesterase inhibitory activity. The compounds exhibited inhibitory activities on human erythrocytes AChE at micromolar range (IC_{50} values of 0.09 ± 0.02- 6.38 ± 0.67) [61]. As summarized in Table 2, in the current study, the IC_{50} values of the chemicals that exhibited an inhibition effect ranged from 0.134 to 172.5 µM. Compounds **2,3** and **6** (monohalogenated malononitriles) inhibited hAChE competitively with the K_i values of 11.91 ± 1.27, 0.058 ± 0.014, and 34.88 ± 2.68, respectively and it was observed that the derivative **3** showed the highest inhibition effect on the hAChE. H Ferreira-Vieira, M Guimaraes [8] reported that mostly competitive inhibitors of AChE are used in AD treatment. When we look at the structure of these three competitive inhibitor, it is seen that all three include mono-halogen such as Cl, Br, or F respectively in the para-position.

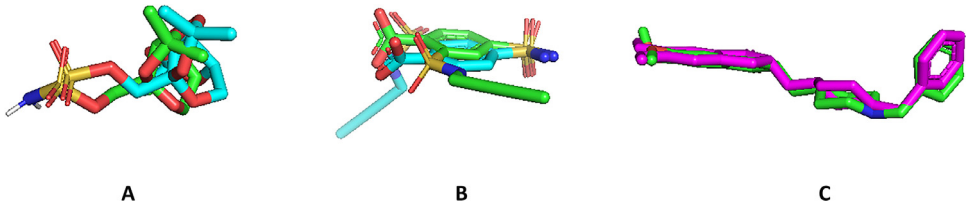

Fig. 4. Docking validation.
*The poses of co-crystallized ligands, (A) Topiramate for hCA-I, (B) 2-(But-2-yn-1-ylsulfamoyl)-4-sulfamoylbenzoic acid for hCA-II, and (C) Donepezil for hAChE.

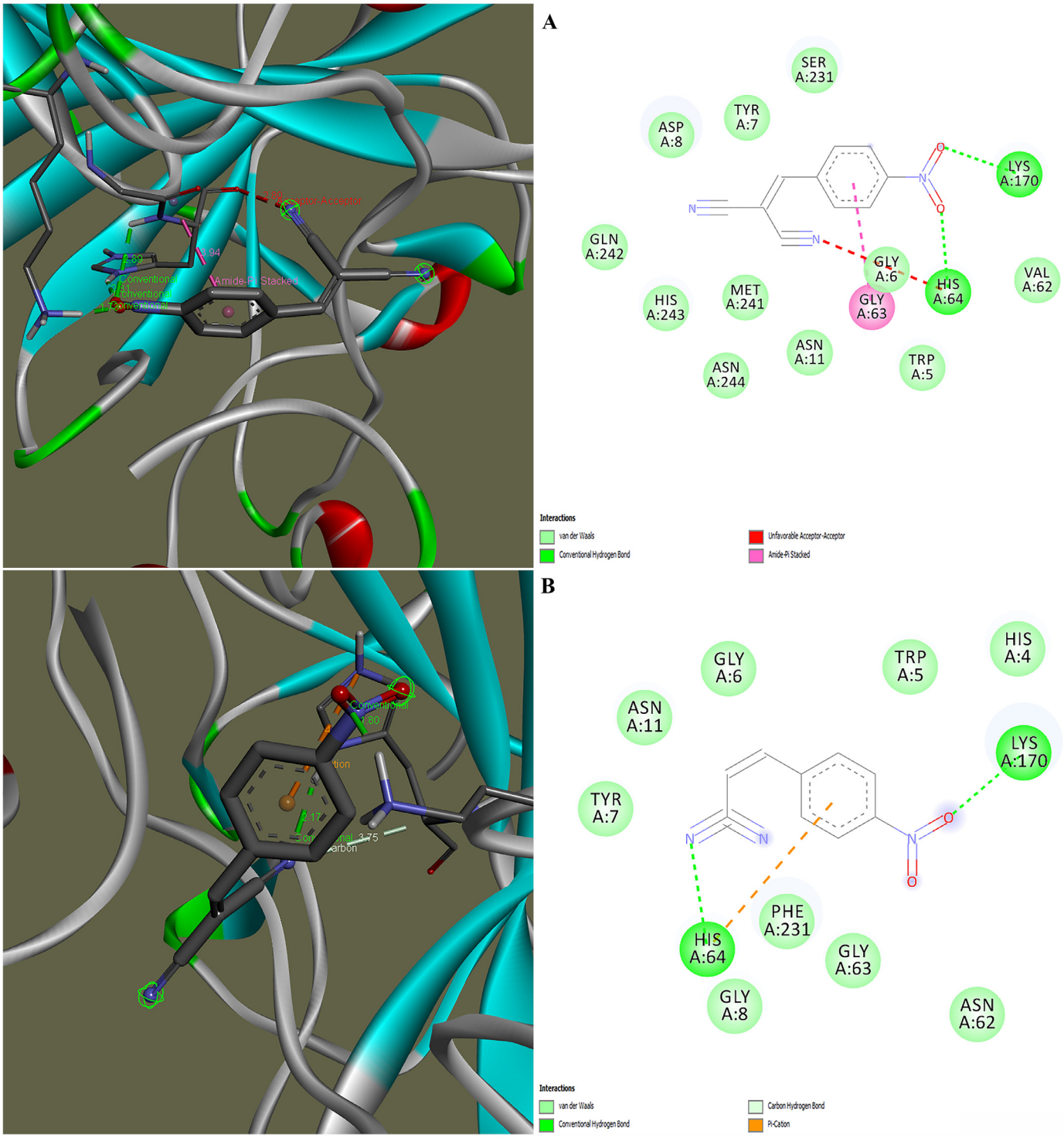

Fig. 5. Potential binding modes and 2D ligand-receptor interaction diagrams of compound 7 with hCA-I (A) and compound 7 with hCA-II (B).

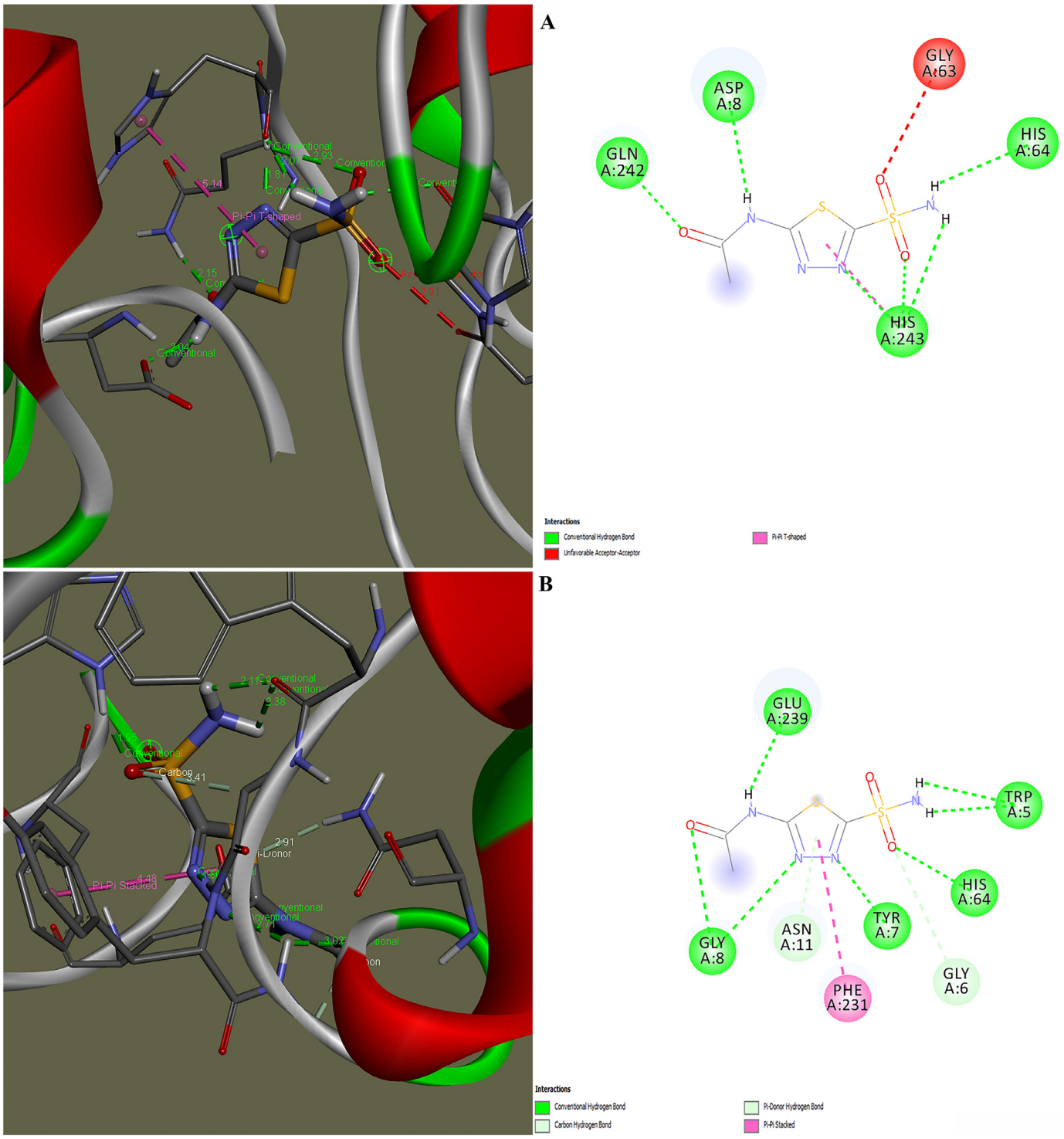

Fig. 6. Potential binding modes and 2D ligand-receptor interaction diagrams of AZA with hCA-I (A) and hCA-II (B).

4.2. In silico *studies*

Molecular docking studies were performed to get insight the interactions between derivatives and enzymes (hCA-I, hCA-II, and hAChE). Firstly docking validations were performed and it was seen that co-crystallized and re-docked ligands were located in a very closet region of the receptor (see Fig. 4). As the result of our experimental studies, we found that derivatives and standard inhibitor (AZA) inhibited hCA-I and II isoenzymes non-competitively. Therefore, we searched different pockets other than the active site where inhibitors can bind to enzymes in the literature and in the CASTp web server. We docked the molecules inside the assigned pockets and chose the best binding sites according to the lowest binding energies [66,67].

The results given in Table 3 indicated that **7** had the lowest binding energy for hCA-I receptor. Each nitro moiety of the molecule was found to form a hydrogen bond with His64 and Lys170 residues. Benzene ring of **7** had the hydrophobic interac-

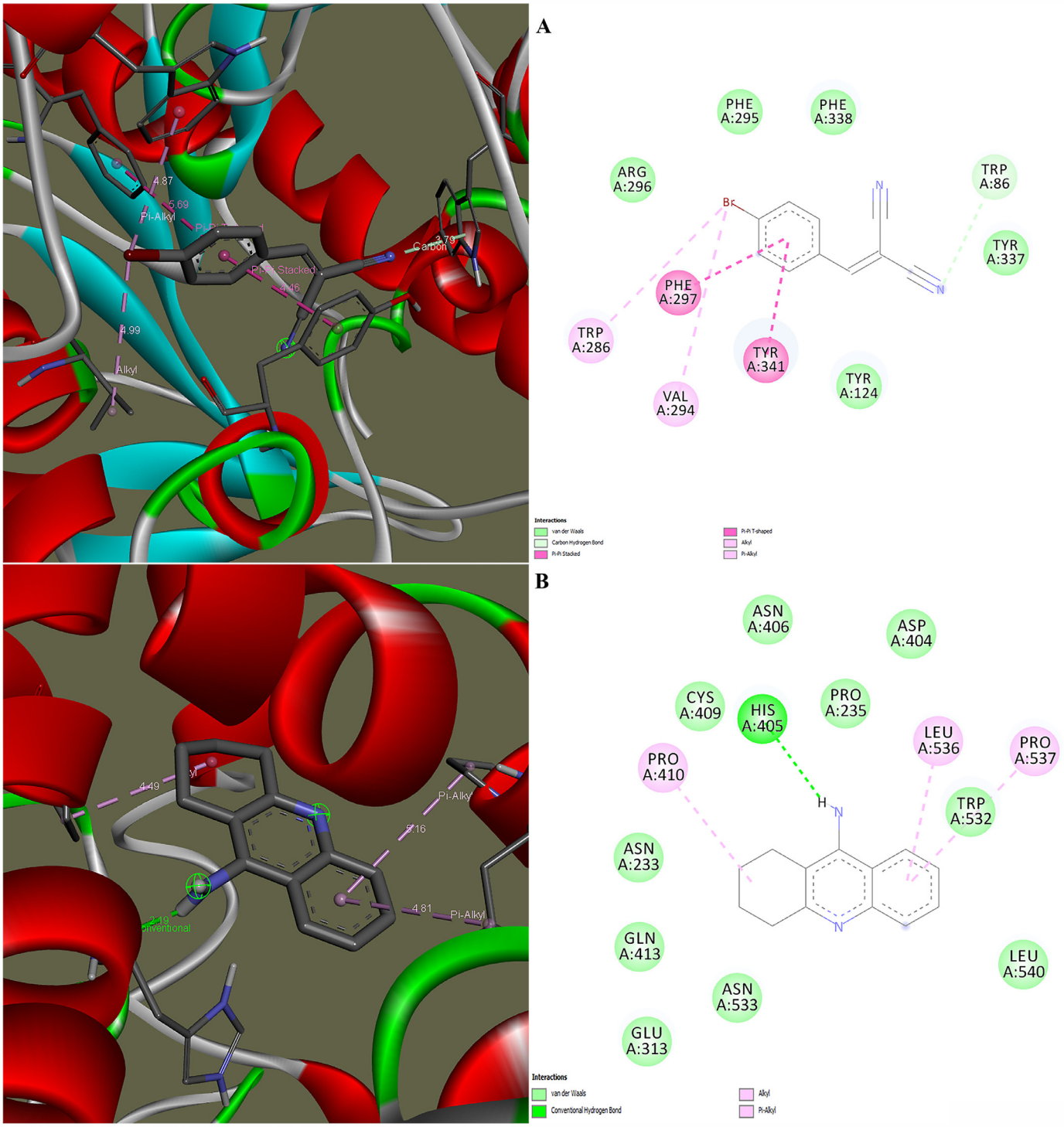

Fig. 7. Potential binding modes and 2D ligand-receptor interaction diagrams of compound 3 (A) and tacrine (B) with hAChE.

tions of the amide-pi stacked with Gly6. Nitrile group showed unfavorable acceptor-acceptor interactions with His64. Besides, several Van der Waals interactions in the deep binding pocket contributed inhibitory effects of molecule (Fig. 5A). When considering the binding energies of molecules, it was figured out that the inhibitory effect of **7** is higher than the standard inhibitor, AZA. In Fig. 6A, it was seen that the inhibition effect of AZA was mainly due to hydrogen bonds formed with Asp8, His64, Gln242, His243 residues. The interactions of molecule **7** with hCA-I were found to be similar to those for thiophene–based sulfonamides and 9-Butyl-N-(2,3,4-trimethoxybenzylidene)-9H-carbazole-3-amine [66,68].

Compound **7** showed the highest inhibition potential against hCA-II such as hCA-I. The benzene ring of the molecule showed a pi-cation interaction between and the imidazole ring of the amino acid His64 (Fig. 5B). While nitro moiety made hydrogen bond with Lys170, nitrile group of compound **7** made hydrogen bond with His64. In addition, molecule exhibited several Van der Waals interactions. Molecule **7** showed higher inhibition potency than AZA with estimated binding energy of -7.05 kcal/mol. AZA showed sev-

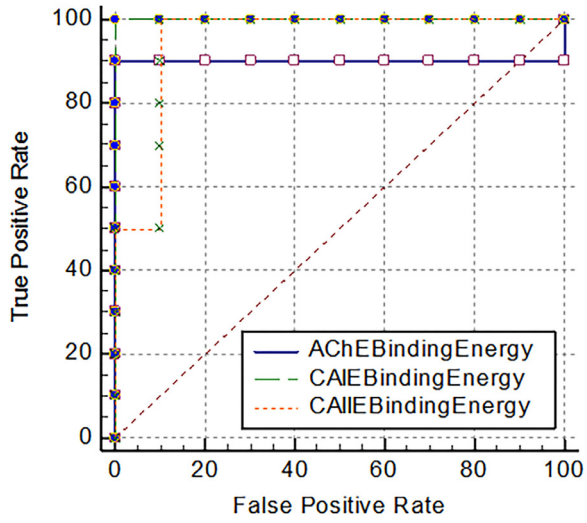

Fig. 8. ROC curves for validation of docking method.

eral hydrogen bonds with Trp5, Tyr7, Gly8, His64, and Glu239 residues of hCA-II. It formed pi-pi stacked interaction between the benzene ring of the molecule and the benzyl ring of the amino acid Phe231. The interaction was displayed in Fig. 6B. It was seen that molecule **7** was interacted with same amino acid residues with hCA-II inhibitors specified in the study performed by Camadan et al. (2020) [68].

As for hAChE, the lowest free binding energy was estimated for derivative **3** as -7.39 kcal/mol. Molecule **3** was found to be the most effective inhibitor for the hAChE enzyme in both *in vitro* and *in silico* studies. It was predicted that the interactions at the benzene ring of it were largely responsible for the effects of derivative **3** on AChE. Notably, it was involved in forming a pi-pi T-shaped and pi-pi stacked hydrophobic interaction with Phe297 and Tyr341. It had pi-alkyl hydrophobic interaction with Trp286 and alkyl interaction with Val294 at Br atom. Molecule **3** formed a carbon hydrogen bond with Trp86 residue. And it showed several Van der Waals interactions too (see Fig. 7A). It was seen from the Fig. 7B that derivative **3** interacted with the enzyme in a different manner than standard inhibitor, tacrine. Free binding energy of tacrine was estimated as -6.72 kcal/mol. The inhibitory potency of **3** was found higher than tacrine. Tacrine interacted with the amino acid residue Leu536 and Pro537 of hAChE by forming pi-alkyl interaction. Besides, it formed several Van der Waals interactions with hAChE receptor.

Furthermore, the accuracy and predictability of the generated models were checked by Receiver operating characteristic (ROC) curves for each three enzymes (Fig. 8). The identity of the compounds of the test set were given in the Supporting Information. The Area Under The Curve (AUC) values of hCA-I, hCA-II, and hAChE were determined as 1.00, 0.90, and 0.95 respectively which is very close to the optimal value.

4.3. Antibacterial studies

In addition to *in vitro* inhibition studies and *in silico* screening of inhibition mechanism, the research team try to explore the antibacterial properties of benzylidenemalononitriles.

The effects of benzylidenemalononitrile derivatives on bacteria were analysis on the basis of well-diffusion method and zone of inhibitions is also shown in Fig. 3 as diameter in millimetre. The structure and activity relationships (SAR) of the compounds were evaluated. It was seen that none of the malononitrile derivatives was effective against *E. coli* strain, a gram-negative bacterium, and molecule **8** was effective against neither *K. pneumonia* nor *S. aureus* strains as well. An inhibition zone diameter ≥10mm indicated that the tested compound is active against the indicator cultures [69]. Most of the compounds showed a zone diameter greater than 10 mm at a lower concentration (25 µg). Despite their structural similarities, against the *K. pneumonia* strain, compound **7** showed a zone diameter greater than 10 mm at 50 µg, **9** showed a zone diameter greater than 10 mm at 75 µg. This is thought to be caused by nitro and hydroxy group at the para position in the structure of compound **7** and **9**. Molecule **5** showed a zone diameter 15 mm at even 25 µg since it contains the furane heterocyclic ring. Effectiveness of compound **5** at 25 µg was found almost as efficient as erythromycin and cephalexin. Against the strain of *Staphylococcus aureus*, a gram-positive bacterium, molecule **1, 7**, and **10** caused a zone diameter of high than 15 mm at the lowest concentration (25 µg). Most effective compound against this strain was found as molecule **10** containing dichloro as a functional group. At minimum concertation (25 µg), it showed a 21 mm zone diameter. At high concentration, these derivatives were almost as effective as standards. Benzylidenemalononitrile derivatives that examined in this study were found more sovereign against *K. pneumonia* and *S. aureus* than those reported by Beyzaei, Deljoo [70]. Besides, other authors manifested that some malononitrile derivatives did not affected *B. cereus* and *S. typhimurium* strains [71].

5. Conclusion

Consequently, this study contains *in vitro* and *in silico* inhibition profiles of the benzylidenemalononitrile derivatives on human erythrocyte CA-I, CA-II, and AChE. Besides antibacterial effects of them were also investigated. All malononitrile derivative was found to be of potential CA inhibitor. The structures of these compounds can be useful to give an idea in the design of drugs for diseases in which CA is targeted in the treatment. In the current study, derivatives with mono-halogen such as Cl, Br, or F at the para-position were found to inhibit AChE competitively. It was reported that competitive AChE inhibitors were preferred in the treatment of neurodegenerative diseases such as AD. On this basis, we think that the derivatives **2,3** and **6** can be a guide in drug development. Besides, the primer effects of compounds were enlightened and in order to identify the effects of metabolites of these compounds and evaluate safety and bioavailability, further *in vivo* experiments are also required.

Credit author statement

Investigation: P. Güller, U. Güller, and B. Nişancı. Methodology: Z. Dağalan, U. Çalışır, U. Güller, and P. Güller. Formal analysis: P. Güller and U. Güller. Writing - original draft: P. Güller. Writing - review & editing: U. Güller, and B. Nişancı. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Pınar Güller: Investigation, Methodology, Formal analysis, Writing - original draft. **Ziya Dağalan:** Methodology. **Uğur Güller:** Investigation, Methodology, Formal analysis, Writing - review & editing. **Ulaş Çalışır:** Methodology. **Bilal Nişancı:** Investigation, Writing - review & editing.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2021.130498.

References

[1] A.W. Erian, The chemistry of. beta.-enaminonitriles as versatile reagents in heterocyclic synthesis, Chem. Rev. 93 (6) (1993) 1991–2005.
[2] F. Freeman, Chemistry of malononitrile, Chem. Rev. 69 (5) (1969) 591–624.
[3] K.S. Dhindsa, Histological changes in the thyroid gland of the mouse following treatment with 1, 1, 3-tricyano-2-amino-1-propene, Cells Tissues Organs 106 (4) (1980) 468–472.
[4] S.H. Ingbar, The action of 1, 1, 3-tricyano-2-amino-1-propene (U-9189) on the thyroid gland of the rat and its effects in human thyrotoxicosis, J. Clin. Endocrinol. Metab. 21 (2) (1961) 128–139.
[5] J.W. Paul, J.P. DaVanzo, 1, 1, 3 Tricyano-2-amino-1-propene (Triap) stimulates choline acetyltransferase activity in vitro and in vivo, Dev. Brain Res. 67 (2) (1992) 113–120.
[6] A. Blokland, Acetylcholine: a neurotransmitter for learning and memory? Brain Res. Rev. 21 (3) (1995) 285–300.
[7] P.T. Francis, A.M. Palmer, M. Snape, G.K. Wilcock, The cholinergic hypothesis of Alzheimer's disease: a review of progress, J. Neurol. Neurosurg. Psychiatry 66 (2) (1999) 137–147.
[8] T. H Ferreira-Vieira, I. M Guimaraes, F. R Silva, F. M Ribeiro, Alzheimer's disease: targeting the cholinergic system, Curr. Neuropharmacol. 14 (1) (2016) 101–115.
[9] C.N. Pope, S. Brimijoin, Cholinesterases and the fine line between poison and remedy, Biochem. Pharmacol. 153 (2018) 205–216.
[10] N.H. Greig, T. Utsuki, Q.-s. Yu, X. Zhu, H.W. Holloway, T. Perry, B. Lee, D.K. Ingram, D.K. Lahiri, A new therapeutic target in Alzheimer's disease treatment: attention to butyrylcholinesterase, Curr. Med. Res. Opin. 17 (3) (2001) 159–165.
[11] Y. Sun, M.S. Lai, C.J. Lu, R.C. Chen, How long can patients with mild or moderate Alzheimer's dementia maintain both the cognition and the therapy of cholinesterase inhibitors: a national population-based study, Eur. J. Neurol. 15 (3) (2008) 278–283.
[12] M. Aggarwal, R. McKenna, Update on carbonic anhydrase inhibitors: a patent review (2008-2011), Expert Opin. Ther. Pat. 22 (8) (2012) 903–915.
[13] V. Alterio, A. Di Fiore, K. D'Ambrosio, C.T. Supuran, G. De Simone, Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms? Chem. Rev. 112 (8) (2012) 4421–4468.
[14] C.T. Supuran, Carbonic Anhydrases as drug targets - an overview, Curr. Top. Med. Chem. 7 (9) (2007) 825–833.
[15] A. Nocentini, C.T. Supuran, Carbonic anhydrase inhibitors as antitumor/antimetastatic agents: a patent review (2008-2018), Expert Opin. Ther. Pat. 28 (10) (2018) 729–740.
[16] S. Pastorekova, A. Casini, A. Scozzafava, D. Vullo, J. Pastorek, C.T. Supuran, Carbonic anhydrase inhibitors: the first selective, membrane-impermeant inhibitors targeting the tumor-associated isozyme IX, Bioorg. Med. Chem. Lett. 14 (4) (2004) 869–873.
[17] C.T. Supuran, Carbonic anhydrase inhibitors, Bioorg. Med. Chem. Lett. 20 (12) (2010) 3467–3474.
[18] C.T. Supuran, A. Scozzafava, Carbonic anhydrase inhibitors and their therapeutic potential, Expert Opin. Ther. Pat. 10 (5) (2000) 575–600.
[19] D. Neri, C.T. Supuran, Interfering with pH regulation in tumours as a therapeutic strategy, Nat. Rev. Drug Discovery 10 (10) (2011) 767–777.
[20] A.M. Shabana, U.K. Mondal, M.R. Alam, T. Spoon, C.A. Ross, M. Madesh, C.T. Supuran, M.A. Ilies, pH-sensitive multiligand gold nanoplatform targeting carbonic anhydrase IX enhances the delivery of doxorubicin to hypoxic tumor spheroids and overcomes the hypoxia-induced chemoresistance, ACS Appl. Mater. Interfaces 10 (21) (2018) 17792–17808.
[21] C.T. Supuran, Carbonic anhydrases: novel therapeutic applications for inhibitors and activators, Nat. Rev. Drug Discovery 7 (2) (2008) 168–181.
[22] B. Nişanci, Katalizörsüz Ortamda Benzaldehit Türevlerinin Malononitril Eşliğinde Knoevenagel Kondenzasyonu: Disiyano Bileşiklerinin Sentezi İçin Yeşil Kimya Yasalarına Uygun Etkin Bir Yöntem, Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi 9 (1) (2019) 500–511.
[23] J.A. Verpoorte, S. Mehta, J.T. Edsall, Esterase activities of human carbonic anhydrases B and C, J. Biol. Chem. 242 (1967) 4221–4229.
[24] F. Worek, U. Mast, D. Kiderlen, C. Diepold, P. Eyer, Improved determination of acetylcholinesterase activity in human whole blood, Clin. Chim. Acta 288 (1-2) (1999) 73–90.
[25] O. Arslan, B. Nalbantoğlu, N. Demir, H. Özdemir, Ö.İ. Küfrevioğlu, A new method for the purification of carbonic anhydrase isozymes by affinity chromatography, Turkish J.Med. Sci. 21 (1996) 159–162.
[26] U. Güller, P. Güller, M. Çiftci, Radical Scavenging and Antiacetylcholinesterase Activities of Ethanolic Extracts of Carob, Clove, and Linden, Alternative Therapies in Health and Medicine, 2020.
[27] M.M. Bradford, Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein-dye binding, Anal. Biochem. 72 (1-2) (1976) 248–254.
[28] G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A.J. Olson, AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility, J. Comput. Chem. 30 (16) (2009) 2785–2791.
[29] V. Alterio, S.M. Monti, E. Truppo, C. Pedone, C.T. Supuran, G. De Simone, The first example of a significant active site conformational rearrangement in a carbonic anhydrase-inhibitor adduct: the carbonic anhydrase I–topiramate complex, Org. Biomol. Chem. 8 (15) (2010) 3528–3533.
[30] J. Ivanova, J. Leitans, M. Tanc, A. Kazaks, R. Zalubovskis, C.T. Supuran, K. Tars, X-ray crystallography-promoted drug design of carbonic anhydrase inhibitors, Chem. Commun. 51 (33) (2015) 7108–7111.
[31] J. Cheung, M.J. Rudolph, F. Burshteyn, M.S. Cassidy, E.N. Gary, J. Love, M.C. Franklin, J.J. Height, Structures of human acetylcholinesterase in complex with pharmacologically important ligands, J. Med. Chem. 55 (22) (2012) 10282–10286.
[32] Shivanika, C., Kumar, D., Ragunathan, V., Tiwari, P., Sumitha, A., 2020. Molecular docking, validation, dynamics simulations, and pharmacokinetic prediction of natural compounds against the SARS-CoV-2 main-protease. Journal of biomolecular structure & dynamics: p. 1.
[33] M.M. Mysinger, M. Carchia, J.J. Irwin, B.K. Shoichet, Directory of useful decoys, enhanced (DUD-E): better ligands and decoys for better benchmarking, J. Med. Chem. 55 (14) (2012) 6582–6594.
[34] N. Triballeau, F. Acher, I. Brabet, J.-P. Pin, H.-O. Bertrand, Virtual screening workflow development guided by the "receiver operating characteristic" curve approach. Application to high-throughput docking on metabotropic glutamate receptor subtype 4, J. Med. Chem. 48 (7) (2005) 2534–2547.
[35] M.A. Llanos, M.L. Sbaraglini, M.L. Villalba, M.D. Ruiz, C. Carrillo, C. Alba Soto, A. Talevi, A. Angeli, S. Parkkila, C.T. Supuran, A structure-based approach towards the identification of novel antichagasic compounds: Trypanosoma cruzi carbonic anhydrase inhibitors, J. Enzyme Inhib. Med. Chem. 35 (1) (2020) 21–30.
[36] B. Gleeson, J. Claffey, D. Ertler, M. Hogan, H. Mueller-Bunz, F. Paradisi, D. Wallis, M. Tacke, Novel organotin antibacterial and anticancer drugs, Polyhedron 27 (18) (2008) 3619–3624.
[37] S. Patil, J. Claffey, A. Deally, M. Hogan, B. Gleeson, L.M.M. Mendez, H. Muller-Bunz, F. Paradisi, M. Tacke, Synthesis, cytotoxicity and antibacterial studies of p-methoxybenzyl-substituted and benzyl-substituted n-heterocyclic carbene-silver complexes, Eur. J. Inorg. Chem. (7) (2010) 1020–1031.
[38] C.R. Shahini, G. Achar, S. Budagumpi, M. Tacke, S.A. Patil, Non-symmetrically p-nitrobenzyl-substituted N-heterocyclic carbene-silver(I) complexes as metallopharmaceutical agents, Appl. Organomet. Chem. 31 (12) (2017).
[39] G. Kaur, M. Kaur, L. Sharad, M. Bansal, Theoretical molecular predictions and antimicrobial activities of newly synthesized molecular hybrids of norfloxacin and ciprofloxacin, J. Heterocycl. Chem. 57 (1) (2020) 225–237.
[40] E.J. Threlfall, I.S.T. Fisher, L.R. Ward, H. Tschape, P. Gerner-Smidt, Harmonization of antibiotic susceptibility testing for Salmonella: Results of a study by 18 national reference laboratories within the European Union-funded enter-net group, Microb. Drug Resis.-Mech. Epidemiol. Dis. 5 (3) (1999) 195–200.
[41] R.D. Walker, J.F. Prescott, J.D. Baggot, Antimicrobial therapy in veterinary medicine, Iowa State 12 (2000) University Press.
[42] D. Staneva, E. Vasileva-Tonkova, R. Kukeva, R. Stoyanova, I. Grabchev, Synthesis, spectral characteristics and microbiological activity of benzanthrone derivatives and their Cu(II) complexes, J. Mol. Struct. 1197 (2019) 576–582.
[43] J.M. Berg, J.L. Tymoczko, L. Stryer, Biochemistry, W H Freeman, New York, 2000 5th edition.
[44] D.S. Johnson, E. Weerapana, B.F. Cravatt, Strategies for discovering and derisking covalent, irreversible enzyme inhibitors, Future Med. Chem. 2 (6) (2010) 949–964.
[45] S. Alyar, S. Adem, Synthesis, characterization, antimicrobial activity and carbonic anhydrase enzyme inhibitor effects of salicilaldehyde-N-methyl p-toluenesulfonylhydrazone and its Palladium(II), Cobalt(II) complexes, Spectrochim. Acta Part a-Mol. Biomol. Spectrosc. 131 (2014) 294–302.
[46] S. Alyar, C.H. Sen, H. Alyar, S. Adem, A. Kalkanci, U.O. Ozdemir, Synthesis, characterization, antimicrobial activity, carbonic anhydrase enzyme inhibitor effects, and computational studies on new Schiff bases of Sulfa drugs and their Pd(II), Cu(II) complexes, J. Mol. Struct. 1171 (2018) 214–222.
[47] T. Arslan, M.B. Ceylan, H. Bas, Z. Biyiklioglu, M. Senturk, Design, synthesis, characterization of peripherally tetra-pyridine-triazole-substituted phthalocyanines and their inhibitory effects on cholinesterases (AChE/BChE) and carbonic anhydrases (hCA I, II and IX), Dalton Trans. 49 (1) (2020) 203–209.
[48] T. Arslan, E.A. Turkoglu, M. Senturk, C.T. Supuran, Synthesis and carbonic anhydrase inhibitory properties of novel chalcone substituted benzenesulfonamides, Bioorg. Med. Chem. Lett. 26 (24) (2016) 5867–5870.
[49] I. Esirden, R. Ulus, B. Aday, M. Tanc, C.T. Supuran, M. Kaya, Synthesis of novel acridine bis-sulfonamides with effective inhibitory activity against the car-

bonic anhydrase isoforms I, II, IX and XII, Bioorg. Med. Chem. 23 (20) (2015) 6573–6580.
[50] B.S. Kursun Aktar, E.E. Oruc-Emre, I. Demirtas, A. Sahin Yaglioglu, A. Karakucuk Iyidogan, C. Guler, S. Adem, Synthesis and biological evaluation of novel chalcones bearing morpholine moiety as antiproliferative agents, Turk. J. Chem. 42 (2) (2018) 482-+.
[51] N. Lolak, S. Akocak, S. Bua, R.K.K. Sanku, C.T. Supuran, Discovery of new ureido benzenesulfonamides incorporating 1,3,5-triazine moieties as carbonic anhydrase I, II, IX and XII inhibitors, Bioorg. Med. Chem. 27 (8) (2019) 1588–1594.
[52] M. Ozil, H.T. Balaydin, M. Senturk, Synthesis of 5-methyl-2,4-dihydro-3H-1,2,4-triazole-3-one's aryl Schiff base derivatives and investigation of carbonic anhydrase and cholinesterase (AChE, BuChE) inhibitory properties, Bioorg. Chem. 86 (2019) 705–713.
[53] R.E. Salmas, M. Mestanoglu, S. Durdagi, M. Senturk, A.A. Kaya, E.C. Kaya, Kinetic and in silico studies of hydroxy-based inhibitors of carbonic anhydrase isoforms I and II, J. Enzyme Inhib. Med. Chem. 31 (1) (2016) 31–37.
[54] F. Turkan, A. Cetin, P. Taslimi, M. Karaman, I. Gulcin, Synthesis, biological evaluation and molecular docking of novel pyrazole derivatives as potent carbonic anhydrase and acetylcholinesterase inhibitors, Bioorg. Chem. 86 (2019) 420–427.
[55] E.A. Turkoglu, M. Senturk, C.T. Supuran, D. Ekinci, Carbonic anhydrase inhibitory properties of some uracil derivatives, J. Enzyme Inhib. Med. Chem. 32 (1) (2017) 74–77.
[56] U. Tutar, U.M. Kocyigit, H. Gezegen, Evaluation of antimicrobial, antibiofilm and carbonic anhydrase inhibition profiles of 1,3-bis-chalcone derivatives, J. Biochem. Mol. Toxicol. 33 (4) (2019).
[57] D.Ü. Payaz, F.Z. Küçükbay, H. Küçükbay, A. Angeli, C.T. Supuran, Synthesis carbonic anhydrase enzyme inhibition and antioxidant activity of novel benzothiazole derivatives incorporating glycine, methionine, alanine, and phenylalanine moieties, J. Enzyme Inhib. Med. Chem. 34 (1) (2019) 343–349.
[58] H. Küçükbay, Z. Gönül, F.Z. Küçükbay, A. Angeli, G. Bartolucci, C.T. Supuran, Preparation, carbonic anhydrase enzyme inhibition and antioxidant activity of novel 7-amino-3, 4-dihydroquinolin-2 (1H)-one derivatives incorporating mono or dipeptide moiety, J. Enzyme Inhib. Med. Chem. 35 (1) (2020) 1021–1026.
[59] J.K.J. Ahlskog, C.E. Dumelin, S. Trussel, J. Marlind, D. Neri, In vivo targeting of tumor-associated carbonic anhydrases using acetazolamide derivatives, Bioorg. Med. Chem. Lett. 19 (16) (2009) 4851–4856.
[60] M.S. Alawi, T.A. Awad, M.A. Mohamed, A. Khalid, E.M. Ismail, F. Alfatih, S. Naz, Z. UL-Haq, Insights into the molecular basis of acetylcholinesterase inhibition by xanthones: an integrative in silico and in vitro approach, Mol. Simul. 46 (4) (2020) 253–261.
[61] P. Suwanhom, T. Nualnoi, P. Khongkow, V.S. Lee, L. Lomlim, Synthesis and evaluation of chromone-2-carboxamido-alkylamines as potent acetylcholinesterase inhibitors, Med. Chem. Res. 29 (3) (2020) 564–574.
[62] J. Wu, M. Pistolozzi, S. Liu, W. Tan, Design, synthesis and biological evaluation of novel carbamates as potential inhibitors of acetylcholinesterase and butyrylcholinesterase, Bioorg. Med. Chem. 28 (5) (2020) 115324.
[63] Y. Xu, M.-M. Jian, C. Han, K. Yang, L.-g. Bai, F. Cao, Z.-Y. Ma, Design, synthesis and evaluation of new 4-arylthiazole-2-amine derivatives as acetylcholinesterase inhibitors, Bioorg. Med. Chem. Lett. 30 (6) (2020) 126985.
[64] L. Yurttaş, Z.A. Kaplancıklı, Y. Özkay, Design, synthesis and evaluation of new thiazole-piperazines as acetylcholinesterase inhibitors, J. Enzyme Inhib. Med. Chem. 28 (5) (2013) 1040–1047.
[65] A. Ibrar, A. Khan, M. Ali, R. Sarwar, S. Mehsud, U. Farooq, S. Halimi, I. Khan, A. Al-Harrasi, Combined in vitro and in silico studies for the anticholinesterase activity and pharmacokinetics of coumarinyl thiazoles and oxadiazoles, Front. Chem. 6 (2018) 61.
[66] Z. Alım, Z. Köksal, M. Karaman, Evaluation of some thiophene-based sulfonamides as potent inhibitors of carbonic anhydrase I and II isoenzymes isolated from human erythrocytes by kinetic and molecular modelling studies, Pharmacol. Reports 72 (6) (2020) 1738–1748.
[67] W. Tian, C. Chen, X. Lei, J. Zhao, J. Liang, CASTp 3.0: computed atlas of surface topography of proteins, Nucleic Acids Res. 46 (W1) (2018) W363-W367.
[68] Y. Camadan, B. Çiçek, Ş. Adem, Ü. Çalişir, E. Akkemik, Investigation of in vitro and in silico effects of some novel carbazole Schiff bases on human carbonic anhydrase isoforms I and II, J. Biomol. Struct. Dyn. (2021) 1–10.
[69] S. Yordanova, E. Vasileva-Tonkova, D. Staneva, S. Stoyanov, I. Grabchev, Synthesis and characterization of new water soluble 9,10-anthraquinone and evaluation of its antimicrobial activity, J. Mol. Struct. 1168 (2018) 22–27.
[70] H. Beyzaei, M.K. Deljoo, R. Aryan, B. Ghasemi, M.M. Zahedi, M. Moghaddam–Manesh, Green multicomponent synthesis, antimicrobial and antioxidant evaluation of novel 5-amino-isoxazole-4-carbonitriles, Chem. Cent. J. 12 (1) (2018) 114.
[71] Behzad, G., Hamid, B., Mohsen, N., 2016. The antibacterial effects of the new derivatives of Thiazole, Imidazole and Tetrahydropyridine against Proteus vulgaris: An in vitro study.