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Synthesis, biological evaluation and *in silico* studies of novel N-substituted phthalazine sulfonamide compounds as potent carbonic anhydrase and acetylcholinesterase inhibitors

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ABSTRACT

The synthesis, characterization and biological evaluation of a series of novel N-substituted phthalazine sulfonamide (**5a-l**) are disclosed. Phthalazines which are nitrogen-containing heterocyclic compounds are biologically preferential scaffolds, endowed with versatile pharmacological activity, such as anti-inflammatory, cardiotonic vasorelaxant, anticonvulsant, antihypertensive, antibacterial, anti-cancer action. The compounds were investigated for the inhibition against the cytosolic hCA I, II and AChE. Most screened sulfonamides showed high potency in inhibiting hCA II, widely involved in glaucoma, epilepsy, edema, and other pathologies (K_i*s* in the ranging from 6.32 ± 0.06 to 128.93 ± 23.11 nM). hCA I was inhibited with K_i*s* in the range of 6.80 ± 0.10–85.91 ± 7.57 nM, whereas AChE in the range of 60.79 ± 3.51–249.55 ± 7.89 nM. ADME prediction study of the designed N-substituted phthalazine sulfonamides showed that they are not only with carbonic anhydrase and acetylcholinesterase inhibitory activities but also with appropriate pharmacokinetic, physicochemical parameters and drug-likeness properties. Also, *in silico* docking studies were investigated the binding modes of selected compounds, to hCA I, II, and AChE.

1. Introduction

Nitrogen-containing heterocyclic compounds have been significant interest because of their common applicability in the past few decades. These compounds have an essential role in biological systems as therapeutic agents and found in many of the compounds in medicinal chemistry [1]. The compounds containing heterocyclic groups such as pyridines, piperazines, indoles, pyrazoles, thiazolidine-4-ones have received great importance because of their biological and pharmacological activities [2]. They are widely found in nature, and their utilization is increasing more and more with each passing day as functional, pharmaceutical, and agrochemicals materials [3].

Phthalazine derivatives are very important nitrogen-containing heterocyclic compounds that have variety of pharmacological activity. Heterocycles containing phthalazine moiety have been reported to possess different pharmacological properties, and they have been commonly employed as therapeutic agents owing to their anti-inflammatory, cardiotonic vasorelaxant, anticonvulsant [4], antihypertensive [5], antibacterial [6], anti-cancer features [7], and carbonic anhydrase enzyme activity [8]. Several commercially available phthalazine derivative drugs, for instance, hydralazine, azelastine, budralazine, zaleplon, luminal, zopolrestat, and MY5445 are used in the treatment of antihypertensive agent for pulmonary hypertension, allergic rhinitis, vasorelaxation, antihistaminic, insomnia and which are shown in Scheme 1 [9,10].

Sulfonamides are an essential compound for the synthesis of bacteriostatic antibiotics. They are known as sulfa drugs and were used against bacterial infections before discovering penicillin [11]. The compounds have a wide range of biological activities; for example, furosemide used as a diuretic, amprenavir used in HIV therapy, sulfathiazole as an antibacterial agent, and acetazolamide as a carbonic anhydrase inhibitor [12]. However, sulfonamides are also as effective lactoperoxidase inhibitors [13].

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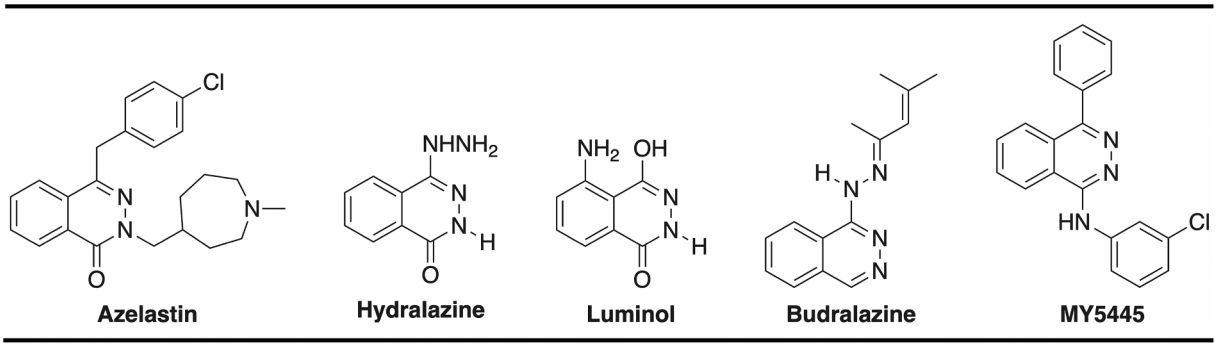


Scheme 1. Structure of some commercially available phthalazine derivative drugs.

Sulfonamide compounds inhibit carbonic anyhdrase (CA) enzymes in a large scale [14–16], and they have been employed for more than 50 years as agents to reduce blood pressure, in the treatment of diseases such as now potentially cancer, glaucoma, heart failure, and epilepsy. Some clinically used sulfonamides such as ethoxzolamide, acetazolamide, dichlorphenamide, and methazolamide have been employed as systemic CA inhibitors since the 1950s. [17].

Carbonic anhydrase isoenzymes (CAs) catalyze dehydration and hydration reactions of carbonic acid (H_2CO_3), and carbon dioxide (CO_2); in the dehydration reaction H_2CO_3 is converted to CO_2, although the hydration reaction produces hydrogen (H^+) and bicarbonate (HCO_3^-) ions from CO_2. [18–22]. This reaction has a crucial role in some vital physiologic functions linked to metabolic pathways involved in CO_2 [23]. Because of that, their modulation is employed for a long time in the treatment of several diseases such as epilepsy [24], edema [25], glaucoma [26]. Recently, inhibitors of CAs have been employed as a novel approach for the treatment of the cerebral ischemia [27] different forms of the tumor [28] and neuropathic pain [29].

Alzheimer's disease (AD) is a significant problem for old people worldwide. This disease can affect many aspects of a person's life [30–32]. Also, there is no cure for AD disease, but several drugs are employed for the cure [33]. Acetylcholinesterase inhibitors (AChEI) have been developed as an impact of the cholinergic assumption of cognitive decline [34]. Also, the effectiveness of these treatments has been investigated in a large number of randomized controlled tests between cognitive, global, neuropsychiatric domains, and functional [35]. AChEI compounds are employed for the treatment of mild-to-moderate AD. These compounds inhibit AChE, which is responsible for the separation of acetylcholine (ACh), a neurotransmitter molecule related to memory function [36].

In this study, a dozen novel N-substituted phthalazine sulfonamide compounds were synthesized, and their inhibitory effects on the activity of human carbonic anhydrase (hCA) I, II and acetylcholinesterase (AChE) were evaluated.

2. Results and discussion

2.1. Chemistry

4-Sulfonylamide ester was prepared from 4-Sulfamoylbenzoic acid in ethanol with a catalytic amount of sulfuric acid by refluxing for 24 hr. The ester group was converted to the hydrazide with hydrazine hydrate in ethanol at 70 °C for 24 hr. A mixture of 4-(hydrazinecarbonyl)benzen sulfonamide, phthalicanhydride derivatives in acetic acid was heated for 8 h at 100 °C. The prepared compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR, and elemental analysis. The proposed compounds (**5a-l**) were shown in Scheme 2.

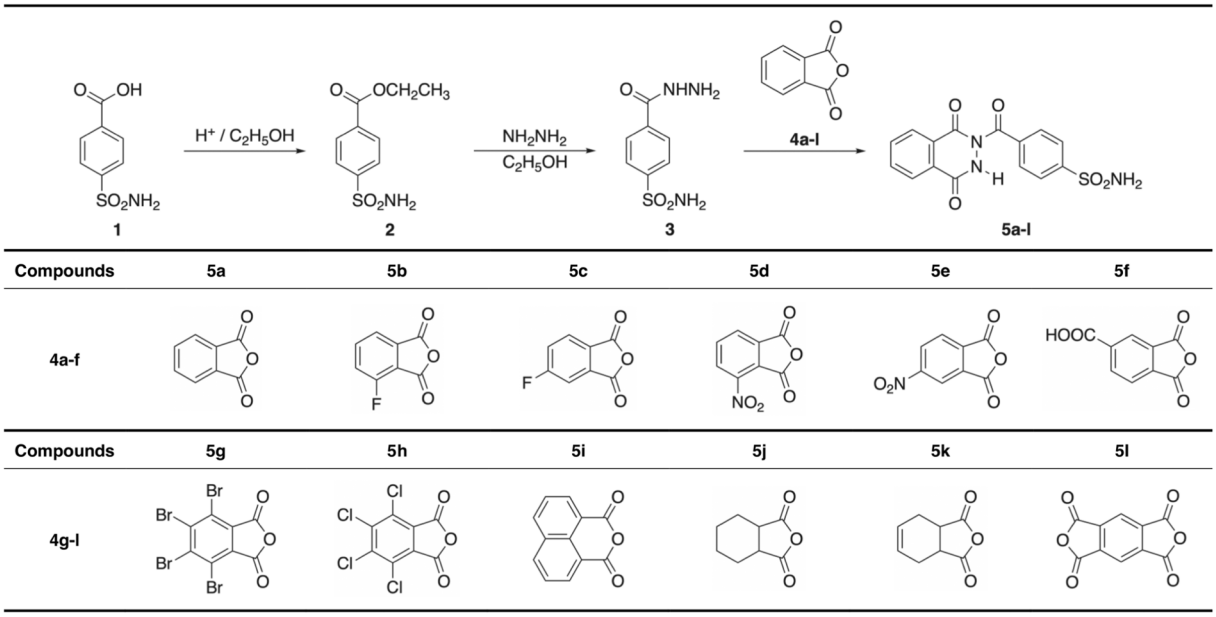
From the ^{1}H NMR spectra, sulfanilamide NH_2 and the =CH proton peaks on aromatic ring resonances at around 7.60 ppm and between 7.00 and 8.00 ppm respectively. From the ^{13}C NMR spectra, the signals of amide and ester carbonyl group are seen at around 165 ppm. In the infrared spectra of compounds **5a-l**, it was possible to observe the absorptions around 3300 cm^{-1} relating to NH_2 stretchings and absorptions around 1700 cm^{-1} from carbonyl moiety stretching. As can be seen in the literature [37], there are two peaks assigned to S=O as symmetric and asymmetric stretching. The peaks of asymmetric and symmetric stretch are appeared around 1300 and 1100 cm^{-1}, respectively.

2.2. Biological evaluation

The novel N-substituted phthalazine sulfonamides obtained here were tested as inhibitors of pharmacologically and physiologically related the cytosolic hCA I, and II by esterase assay. Also, it was investigated the inhibition effects on AChE enzyme activity of these compounds. Their activities were compared to the acetazolamide (AAZ) and tacrine (TAC) which is standard reference inhibitors for hCA and AChE.

(i) hCA I is expressed in normal colorectal mucosa and found at the highest level in erythrocytes [38]. As for hCA I, IC_{50} values for novel synthesized N-substituted phthalazine sulfonamide derivatives showed in the ranging from 12.42 ± 0.23 to 32.80 ± 0.49 nM and K_i constants in the range of 6.80 ± 0.10–85.91 ± 7.57 nM. It exhibited that K_i constants order of synthesized sulfonamides inhibitory strength was **5b** (K_i: 6.80 ± 0.10 nM) > **5 h** (K_i: 9.26 ± 0.16 nM) > **5 k** (K_i: 9.84 ± 0.13 nM) > **5e** (K_i: 10.35 ± 0.10 nM) > **5c** (K_i: 10.59 ± 0.14 nM) > **5i** (K_i: 15.10 ± 0.16 nM) > **5 g** (K_i: 16.85 ± 0.20 nM) > **5j** (K_i: 20.01 ± 0.31 nM) > **5d** (K_i: 20.69 ± 0.35 nM) > **5a** (K_i: 49.31 ± 0.25 nM) > **5l** (K_i: 54.23 ± 4.62 nM) > **5f** (K_i: 85.91 ± 7.57 nM). It is well known that incorporation of electron-donating groups resulted in a lessen hCA isoenzymes activity. Unlike this impact, addition of electron-withdrawing groups in the inhibitor improved the inhibition impacts. The best inhibitor was *2-(4-sulfamoylbenzoyl)-8-flouro-2,3-dihydrophthalazine-1,4-dione* (**5b**) (Figs. 1 and 2) when compared to AAZ (K_i: 434.55 ± 11.91 nM), which is used for positive control. Also, the least active was *2-(4-sulfamoylbenzoyl)-6-carboxy-2,3-dihydrophthalazine-1,4-dione* (**5f**) for hCA I. Compound **5b** decreased the enzyme activity ~ 13 fold compared to **5f** (Tables 1 and 2).

hCA II is a cytosolic enzyme and highly active [39]. As shown in Table 1, the inhibition profile of the novel synthesized sulfonamides was very active compared to the hCA I. As for hCA II, the isolated compounds showed IC_{50} and K_i values in the low nanomolar range which gave IC_{50} values in the range of 10.14 ± 0.13–28.57 ± 0.50 nM and K_i constants in the ranging between 6.32 ± 0.06 and 128.93 ± 23.11 nM. The results exhibited that hCA II was effectively inhibited by novel sulfonamide derivatives compared to hCA I. All studied sulfonamide derivatives exhibited potent inhibition profile against hCA II. The best inhibitor was *2,8-di(4-sulfamoylbenzoyl)-1,2,3,4,6,7,8,9-octahydropyridazino[4,5-g] phthalazine-1,4,6,9-tetraone* (**5l**) (Figs. 1 and 2), whereas the least effective one *2-(4-sulfamoylbenzoyl)-6-nitro-2,3-dihydrophthalazine-1,4-dione* (**5e**) for hCA II. On the other hand, AAZ, which is used a clinical



Scheme 2. Synthesis of phthalazine substituted sulfonamide derivatives. Reagents and yields: 4-sulfonylamidebenzohydrazide, phthalic anhydride, CH_3COOH, reflux, 100 °C 16 h.

medication had a K_i constant of 47.60 ± 0.56 nM against hCA II. Compound **5l** which is a more potent inhibitor in comparison with AAZ reduced the hCA II activity approx.7 fold (Tables 1 and 2).

In literature, it was observed that the sulfonamides inhibit hCA isozymes and the concentration of these compounds was found in the micromolar to the nanomolar range [40–42]. The novel ureido benzenesulfonamides incorporating 1,3,5-triazine moieties were investigated as inhibitors of hCA I, II, IX, and XII. All compounds exhibited strong inhibition effect for hCA isoenzymes [43]. Another study, novel benzenesulfonamide bearing 1,2,3-triazole linked hydroxy-trifluoromethylpyrazolines were synthesized and tested inhibitory effects towards hCA I, II, IX, and XII isoforms [44]. 3-aminobenzenesulfonamides incorporating acylthiourea moieties showed potent inhibitory effect against hCA I, II, IV, and IX [45].

The results showed that novel synthesized N-substituted phthalazine sulfonamide derivatives were an excellent inhibitor for hCA isoenzymes. hCA inhibitors have been employed for the treatment of a great deal of disorder containing epilepsy, glaucoma, cancer, obesity, and high-altitude sickness [46].

(ii) AChEIs or anti-cholinesterases inhibit cholinesterase, enhancing the level and length of ACh action. The variety of use of AChEI compounds is popular in medicine and agriculture [47]. These compounds traditionally employed for medical aims include organophosphates and carbamates. In myasthenia gravis and AD diseases in which cholinergic function is impaired, carbamates/organophosphates offer therapeutic choices because of high effect as AChEI compounds. The inhibitory effects of the synthesized novel N-substituted phthalazine sulfonamide compounds had IC_{50} values in the range of 48.69 ± 0.57–221.10 ± 7.14 nM for AChE. These compounds had K_i constants in ranging between 60.79 ± 3.51 and 249.55 ± 7.89 nM. *2-(4-sulfamoylbenzoyl)-8-flouro-2,3-dihydrophthalazine-1,4-dione* (**5b**) (Figs. 1 and 2) which is one of the best potent inhibitors in this study had more effective inhibition profile than that of tacrine (K_i: 107.96 ± 2.38 nM) as standard AChE inhibitor. Additionally, *2-(4-sulfamoylbenzoyl)-7-flouro-2,3-dihydrophthalazine-1,4-dione* (**5c**) was one of the least effective inhibitors for AChE (Tables 1 and 2).

There are many studies about the inhibition of AChE by sulfonamides. For instance, 4-(3-Substitutedphenyl-5-polymethoxyphenyl-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamides were synthesized and studied the inhibition effect on AChE enzyme activity. It was found that K_i constants in ranging from 37.7 ± 14.4 to 89.2 ± 30.2 nM [48]. Another study, novel b-benzylphenethylamines and their sulfamide derivatives were synthesized and K_i constants were found in the range of 0.127–2.452 nM [49].

Novel sulfonamide derivatives displayed effective inhibition profile towards AChE. These compounds might be used as precursor compounds for AD. Because of critical adverse impacts of the present AChE inhibitors, there is the try to find safer and more effective AChE inhibitors for the treatment of neurodegenerative disease. Bioassay-guided isolation and ethnopharmacological approaches have determined as potential in identifying effective AChE inhibitors.

2.3. In silico studies

2.3.1. ADMET prediction and drug likeness study

ADME (absorption, distribution, metabolism, and excretion)-related pharmacokinetic and physicochemical parameters of the synthesized N-substituted phthalazine sulfonamides derivatives were computed. As could be seen from Table 3, the suitability of the novel compounds was evaluated based on human oral absorption parameters and Lipinski's rule of five [50], which is an indication of the drug-likeness of a molecule. According to these results, all compounds (**5a-l**) complied with the Lipinski's rule of five (recommend value is max. 4). Among the properties and descriptors, molecular weight (MW), dipole moment (Dipole), total solvent-accessible volume, van der Waals surface area of polar nitrogen and oxygen atoms (except for **5l**) (PSA), number of property or descriptor values (except for **5l**) (#Stars), number of likely metabolic reactions (#Metab), number of non-trivial, non-hindered rotatable bonds (#Rotor), octanol/water partition coefficient (QPlogPo/w), aqueous solubility (QPlogS) values were calculated within the ranges for drug-like chemical space. On the other hand, the predicted Caco-2 cell (QPPCaco), and MDCK cell (QPPMDCK) permeability were below 25.

2.3.2. Molecular docking study

We performed *in silico* studies to understand the molecular mechanism underlying inhibitory activities of N-substituted phthalazine sulfonamides (**5a-l**). Therefore, compounds **5a-l** were subjected to the docking analysis against hCA I, II, and AChE receptor proteins. As an initial level of *in silico* molecular docking computations, the reliability

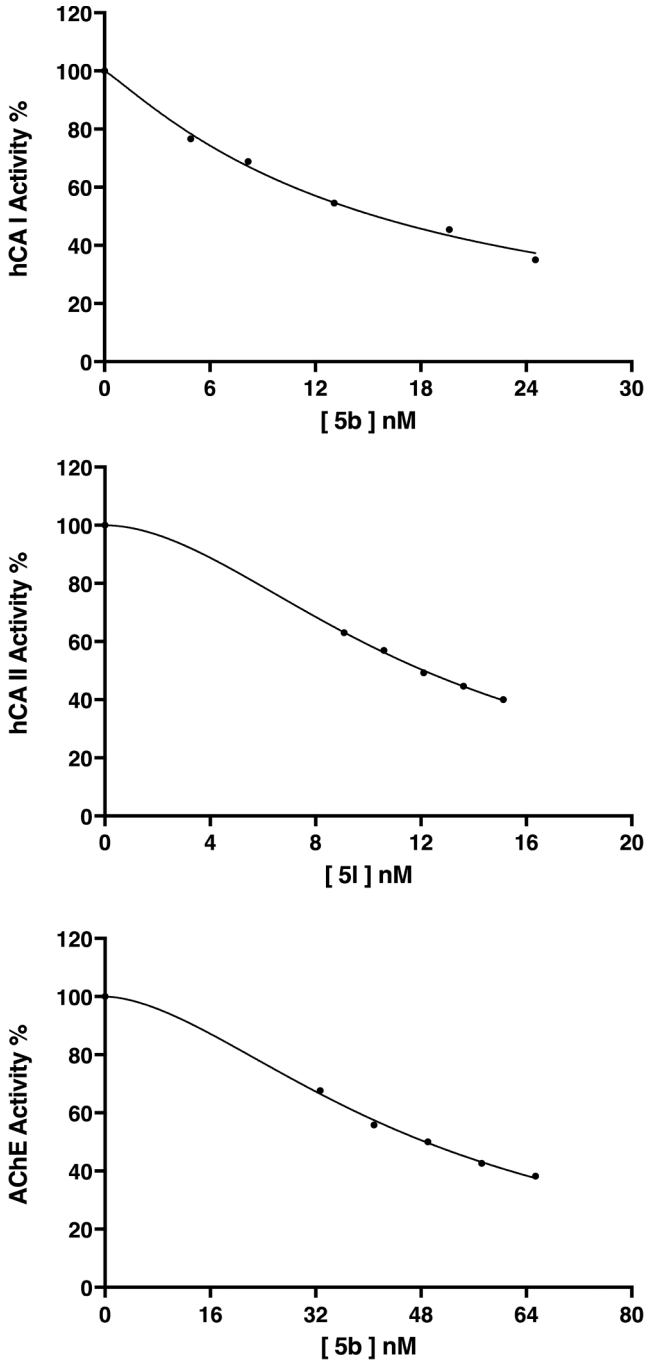


Fig. 1. *In vitro* effects of compounds **5b** (for hCA I and AChE) and **5l** (for hCA II) on enzyme activities at five different concentration. Percent activity versus inhibitor concentrations graphs.

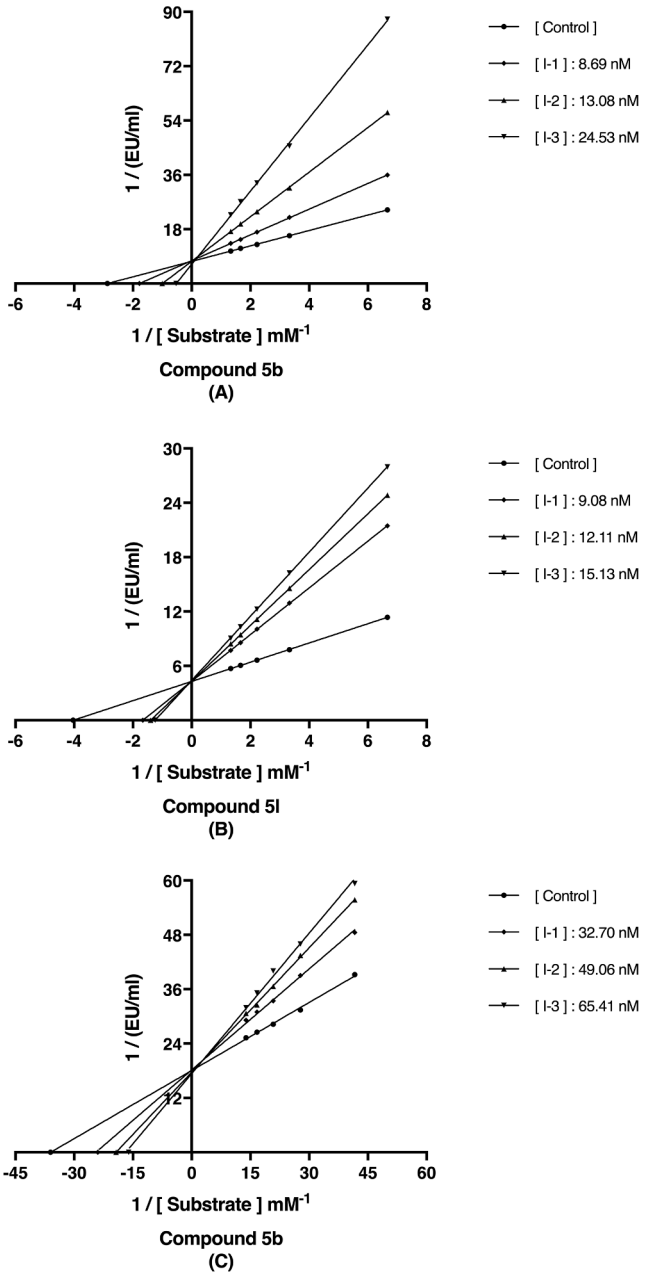


Fig. 2. K_i constants and inhibition types determination were performed by using Lineweaver-Burk plots for compounds **5b** and **5l** was numbered as A (**5b** against hCA I), B (**5l** against hCA II), and C (**5b** against AChE).

for the Glide SP docking was estimated by docking the native ligands; 2,3,5,6-tetrafluoro-4-piperidin-1-ylbenzenesulfonamide (3UG) into the binding site of hCA I, N-[(2Z)-1,3-oxazolidin-2-ylidene]sulfuric diamide (OVX) into the binding site of hCA II, and donepezil (E20) into the binding site of AChE. The binding structures of native ligands with receptors were successfully reproduced yielding the RMSD between the predicted binding pose and the experimental data pose as 0.295 Å, 0.259 Å, and 0.115 Å for 3UG, OVX, and E20, respectively.

The hydrogen bonding interactions are between 3UG and OVX and polar regions of the active sites of hCA I, and II, respectively. The major amino acid residues included in these regions of the hydrogen bonds are Thr199, and Thr200. Moreover, the critical hydrogen bonding interaction exists between E20 and hydrophobic part of the binding site of AChE. Herein, the amino acid residue which is hydrogen bonding interaction is Phe295 (Fig. 3).

In the next step, N-substituted phthalazine sulfonamides (**5a-l**) and reference inhibitors (AAZ and TAC) were docked into the binding pocket. Molecular docking study successfully generated biologically active conformations of the derivatives (Tables 4–6). Nevertheless, *in silico* molecular docking results should not be expected to have similar correlations with the experimental activity, e.g., IC_{50} values and K_i constants [51]. Compounds **5b** and **5l** were selected for *in silico* molecular docking simulations to justify its activity by investigating its key interactions within the binding sites of hCA I (PDB ID: 4WUQ), hCA II (PDB ID: 4FU5), and AChE (PDB ID: 4EY7) enzymes. The top poses for **5b** and **5l** docked within the active site of enzymes were illustrated in Fig. 4.

As a result, the sulfonamide group played the usual role as a zinc-binding moiety for compound **5b** with the hCA I by accepting a

Table 1
Enzyme inhibition data (IC_{50} values, K_i constants, and inhibition types) of the compounds (**5a-5l**).

Compound	hCA I				hCA II				AChE				
	IC_{50} (nM)	R^2	K_i (nM)	R^2	Inhibition type	IC_{50} (nM)	R^2	K_i (nM)	R^2	Inhibition type	IC_{50} (nM)	R^2	K_i (nM)	R^2	Inhibition type
5a	18.17 ± 0.22	0.9988	49.31 ± 0.25	0.9999	Noncompetitive	28.57 ± 0.50	0.9976	76.14 ± 0.38	0.9999	Noncompetitive	174.10 ± 4.41	0.9957	94.87 ± 3.65	0.9978	Competitive
5b	15.47 ± 0.56	0.9943	6.80 ± 0.10	0.9997	Competitive	13.78 ± 0.10	0.9996	34.39 ± 0.21	0.9999	Noncompetitive	48.69 ± 0.57	0.9982	60.79 ± 3.51	0.9938	Competitive
5c	16.05 ± 0.09	0.9998	10.59 ± 0.14	0.9997	Competitive	21.26 ± 0.26	0.9985	34.89 ± 1.01	0.9999	Mixed	221.10 ± 7.14	0.9910	249.55 ± 7.89	0.9981	Competitive
5d	31.59 ± 0.66	0.9955	20.69 ± 0.35	0.9996	Competitive	17.84 ± 0.10	0.9996	24.66 ± 0.79	0.9998	Mixed	57.96 ± 0.56	0.9992	75.00 ± 2.87	0.9972	Competitive
5e	15.07 ± 0.37	0.9972	10.35 ± 0.10	0.9999	Competitive	10.14 ± 0.13	0.9989	128.93 ± 23.11	0.9998	Mixed	147.70 ± 1.17	0.9992	88.75 ± 2.64	0.9986	Competitive
5f	27.20 ± 0.47	0.9976	85.91 ± 7.57	0.9995	Mixed	25.69 ± 0.27	0.9987	25.07 ± 1.00	0.9999	Mixed	193.60 ± 5.95	0.9934	248.69 ± 8.92	0.9975	Competitive
5g	28.22 ± 0.33	0.9990	16.85 ± 0.20	0.9998	Competitive	16.67 ± 0.16	0.9989	30.55 ± 1.53	0.9996	Mixed	200.20 ± 3.41	0.9970	142.04 ± 4.34	0.9984	Competitive
5h	15.91 ± 0.35	0.9950	9.26 ± 0.16	0.9996	Competitive	35.36 ± 0.58	0.9967	43.89 ± 1.39	0.9998	Mixed	142.40 ± 4.25	0.9940	134.10 ± 3.90	0.9984	Competitive
5i	32.80 ± 0.49	0.9983	15.10 ± 0.16	0.9999	Competitive	23.41 ± 0.52	0.9950	12.07 ± 0.14	0.9998	Competitive	162.60 ± 0.82	0.9998	184.34 ± 7.85	0.9966	Competitive
5j	16.51 ± 0.50	0.9945	20.01 ± 0.31	0.9997	Competitive	17.10 ± 0.35	0.9974	15.61 ± 0.16	0.9999	Competitive	108.00 ± 1.62	0.9986	88.59 ± 2.89	0.9982	Competitive
5k	17.24 ± 0.32	0.9981	9.84 ± 0.13	0.9998	Competitive	13.39 ± 0.26	0.9973	7.15 ± 0.08	0.9998	Competitive	133.90 ± 3.30	0.9955	249.35 ± 7.08	0.9985	Competitive
5l	12.42 ± 0.23	0.9974	54.23 ± 4.62	0.9997	Mixed	12.12 ± 0.06	0.9995	6.32 ± 0.06	0.9999	Competitive	75.67 ± 0.50	0.9997	83.22 ± 2.73	0.9980	Competitive
AAZ[a]	220.10 ± 2.42	0.9996	434.55 ± 11.91	0.9980		94.46 ± 0.98	0.9996	47.60 ± 0.56	0.9999		–	–	–	–	–
TAC[b]	–	–	–	–	–	–	–	–	–	–	425.60 ± 1.70	0.9997	107.96 ± 2.38	0.9999	

[a] Acetazolamide.
[b] Tacrine.

Table 2
Selectivity index values of the compounds (**5a-5l**).

Compound	K_i (hCA II)/K_i (hCA I)	K_i (AAZ)/K_i (hCA I)	K_i (AAZ)/K_i (hCA II)	K_i (TAC)/K_i (AChE)
5a	1.54	8.81	0.63	1.14
5b	5.06	63.89	1.38	1.78
5c	3.29	41.02	1.36	0.43
5d	1.19	21.00	1.93	1.44
5e	12.46	41.99	0.37	1.22
5f	0.29	5.06	1.90	0.43
5g	1.81	25.80	1.56	0.76
5h	4.74	46.92	1.08	0.81
5i	0.80	28.79	3.94	0.59
5j	0.78	21.72	3.05	1.22
5k	0.73	44.17	6.66	0.43
5l	0.12	8.01	7.54	1.30

coordinate bond with Zn metal and hydrogen bond from Thr199. Carbonyl group was involved in the interaction within the active site of hCA I by forming a hydrogen bond with Gln92. Also, the benzene ring was found to have pi-pi stacking with His200. The subnanomolar inhibitor **5l** coordinates the prosthetic Zn ion of the enzyme through the sulfonamide group of phthalazine, also forms two H-bonds with Gln92 and Thr199 in the binding site of hCA II. The carbonyl moiety of compound **5b** establishes an H-bond with Phe295 in the active site of AChE, the phthalazine ring is involved in pi-pi stacking and hydrophobic interactions with Trp286. Second pi-pi stacking was composed between the benzene ring of the sulfonamide moiety and Phe338. Above mentioned contacts explained the inhibitory activities for compounds **5b** (for the hCA I, and AChE), and **5l** (for the hCA II).

3. Conclusion

In conclusion, we reported a novel N-substituted phthalazine sulfonamide derivatives. The novel sulfonamides were studied as inhibitors of two pharmacologically and physiologically relevant isoforms, hCA I, and II, as well as AChE related to AD. All studied compounds exhibited potent inhibition against these enzymes. K_i constants were found in the range of 6.32 ± 0.06–128.93 ± 23.11 nM for hCA isoenzymes and 60.79 ± 3.51–249.55 ± 7.89 nM for AChE. N-substituted phthalazine sulfonamide derivatives might be of interest for further pharmacologic and medicinal research. Finally, the inhibitory potency of the synthesized N-substituted phthalazine sulfonamide derivatives was also researched carrying out *in silico* molecular docking for compounds **5b** and **5l** to rationalize the found biological study results. These derivatives might be of interest for further pharmacologic, and medicinal research.

4. Experimental

4.1. Chemistry

4.1.1. General

Melting points were determined by Yanagimoto micro-melting point apparatus and uncorrected. IR spectra were measured on a SHIMADZU Prestige-21 (200 VCE) spectrometer. ^{1}H, and ^{13}C NMR spectra were obtained using VARIAN Infinity Plus in 300 and 75 Hz, respectively. ^{1}H and ^{13}C chemical shifts were referenced to the internal deuterated solvent. The elemental analysis was carried out with a Leco CHNS-932 instrument. All chemicals were purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany).

4.1.2. General procedure for the preparation of 4-sulfonylamide ester (1)

4-Sulfamoylbenzoic acid (10 mmol) was refluxed for 24 hr in 50 mL of ethanol and 1.0 mL of sulfuric acid. The solvent was evaporated, and the obtained product was washed with cold water and dried.

Table 3
ADME-related pharmacokinetic and physicochemical parameters of compounds (**5a-l**).

Compound	MW[a]	HOMO Energy[b]	LUMO Energy[c]	Dipole [d]	Volume[e]	PSA[f]	#Stars [g]	Human Oral Absorption[h]						Rule of Five [o]
								#Metab [i]	#Rotor [j]	QPlogPo/w [k]	QPlogS [l]	QPPCaco [m]	QPPMDCK [n]	
5a	345.33	−2.12	4.51	2.67	968.13	159.11	2	0	3	−0.92	−3.11	22.27	8.32	0
5b	363.32	−2.07	4.45	4.73	983.48	158.15	2	0	3	0.23	−3.44	19.46	11.22	0
5c	363.32	−2.22	4.28	5.44	982.75	159.06	2	0	3	0.12	−3.46	22.31	15.00	0
5d	390.33	−2.49	3.76	9.04	1047.67	201.39	4	1	4	−0.65	−3.25	4.15	1.35	1
5e	390.33	−2.55	2.97	9.33	1040.39	204.03	4	1	4	−0.81	−3.23	2.72	0.86	1
5f	389.34	0.16	7.05	5.23	1047.77	208.48	5	0	4	−0.79	−3.27	0.48	0.17	0
5g	660.91	−2.47	3.38	2.96	1158.26	154.62	3	0	3	1.80	−5.40	35.20	256.93	1
5h	483.11	−2.38	3.58	3.94	1127.93	154.95	3	0	3	1.54	−5.09	33.49	196.60	0
5i	367.38	−6.01	0.59	8.41	1036.82	132.06	1	0	2	0.54	−3.72	63.27	25.55	0
5j	351.38	−2.00	5.58	6.70	991.87	156.32	0	2	3	−0.30	−3.16	27.73	10.49	0
5k	349.36	−1.99	5.41	5.99	1001.93	157.00	0	4	3	−0.75	−3.23	19.55	7.20	0
5l	611.56	0.00	4.04	7.16	1559.92	308.68	6	2	8	−1.78	−4.80	0.44	0.10	3

[a] Molecular weight of the molecule (recommended value: 130–725).
[b] Highest occupied molecular orbital (eV).
[c] Lowest unoccupied molecular orbital (eV).
[d] Computed dipole moment of the molecule (recommended value: 1.0–12.5).
[e] Total solvent-accessible volume in cubic angstroms using a probe with a 1.4 Å Radius (recommended value: 500–2000).
[f] Van der Waals surface area of polar nitrogen and oxygen atoms (recommended value: 7–200).
[g] Number of property or descriptor values that fall outside the 95% range of similar values for known drugs (recommended value: 0–5).
[h] Predicted qualitative human oral absorption (recommended value: 1, 2, or 3 for low, medium, or high).
[i] Number of likely metabolic reactions (recommended value: 1–8).
[j] Number of non-trivial (not CX3), non-hindered (not alkene, amide, small ring) rotatable bonds (recommended value: 0–15).
[k] Predicted octanol/water partition coefficient (recommended value: −2.0 to 6.5).
[l] Predicted aqueous solubility, log S. S in mol dm^{-3} is the concentration of the solute in a saturated solution that is in equilibrium with the crystalline solid (recommended value: −6.5 to 1.5).
[m] Predicted apparent Caco-2 cell permeability in nm/s. Caco-2 cells are a model for the gut-blood barrier (< 25 is poor and > 500 is great).
[n] Predicted apparent MDCK cell permeability in nm/s (< 25 is poor and > 500 is great).
[o] Number of violations of Lipinski's rule of five (max. is 4).

4.1.3. General procedure for the preparation of 4-sulfonylamidebenzohydrazide (2)

4-sulfonylamide ester (10 mmol) and hydrazine hydrate (25 mmol) in ethanol were refluxed for 24 hr at 70 °C. The reaction mixture was cooled to room temperature, and the solid was filtered then washed with water and dried.

4.1.4. General procedure for the synthesis of 2-(4-sulfamoylbenzoyl)-2,3-dihydrophthalazine 1,4-dione derivatives (3a-l)

A mixture of 4-(hydrazinecarbonyl)benzen sulfonamide (1 mmol), Phthalic anhydride derivatives (1 mmol) in acetic acid (10 ml) was heated for 8 h at 100 °C. After cooling to room temperature, the product was poured into ice-cold water. Then, filtered and dried. The prepared compounds shown in Scheme 2 were characterized by ^{1}H NMR, ^{13}C NMR, IR, and elemental analysis.

4.1.5. 2-(4-sulfamoylbenzoyl)-2,3-dihydrophthalazine-1,4-dione (5a)

Yield 81%, recrystallized from ethanol to give as white powder, m.p. 280 °C; IR (KBr, υ, cm^{-1}): 3350 (N−H), 3220 (NH_2), 1698 (C═), 1640 (C═C, aromatic), 1355 and 1170 (SO_2); ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 11.60(1H, s, NH), 8.10 (2H, d, Ar−H). 8.04 (2H, d, Ar−H), 7.90–7.80 (4H, m, Ar−H), 7.60 (2H, s, $-NH_2$). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 165.9, 165.3, 165.2, 148.3, 136.2, 134.2, 133.8, 130.1, 129.6, 129.3, 126.8, 125.5, 124.7. Anal. Calcd. for $C_{15}H_{11}N_3O_5S$ (%): C, 52.17; H, 3.21; N, 12.17; O, 23.17; S, 9.29. Found: C, 51.38; H, 3.33; N, 12.33; O, 23.92; S, 9.42.

4.1.6. 2-(4-sulfamoylbenzoyl)-8-flouro-2,3-dihydrophthalazine-1,4-dione (5b)

Yield 75%, recrystallized from ethanol to give as white crystals, m.p. 288 °C; IR (KBr, υ, cm^{-1}): 3350 (N−H), 3250 (NH_2), 1700 (C═O), 1640 (C═C), 1145 (C−F), 1355 and 1160 (SO_2); ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 11.80 (1H,s, NH), 8.12 (2H, d, Ar−H). 8.0 (2H, d, Ar−H), 7.8–8.0 (3H, m, Ar−H), 7.60 (2H, s, $-NH_2$). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 165.3, 163.9, 161.3, 148.4, 145.2, 137.9, 134.0, 131.8, 130.2, 129.4, 128.6, 126.8, 121.9. Anal. Calcd. for $C_{15}H_{10}FN_3O_5S$ (%): C, 49.59; H, 2.77; N, 11.57; F, 5.23; O, 22.02; S, 8.83. Found: C, 49.63; H, 2.94; N, 11.72; F, 5.30; O, 22.19; S, 8.90.

4.1.7. 2-(4-sulfamoylbenzoyl)-7-flouro-2,3-dihydrophthalazine-1,4-dione (5c)

Yield 78%, recrystallized from ethanol to give as white powder, m.p. 284 °C; IR (KBr, υ, cm^{-1}): 3320 (N−H), 3220 (NH_2), 1680 (C═O), 1640 (C═C), 1310 and 1165 (SO_2); ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 11.76 (1H,s, NH), 8.10 (2H, d, Ar−H), 7.90 (2H, d, Ar−H), 7.8–7.5 (3H, m, Ar−H), 7.33 (2H, s, $-NH_2$). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 165.3, 164.9, 164.8, 148.3, 134.1, 133.1, 133.0, 129.3, 127.8, 127.6, 126.8, 126.4, 123.2. Anal. Calcd. for $C_{15}H_{10}FN_3O_5S$ (%): C, 49.59; H, 2.77; N, 11.57; F, 5.23; O, 22.02; S, 8.83. Found: C, 49.63; H, 2.85; N, 11.68; F, 5.28; O, 22.19; S, 8.92.

4.1.8. 2-(4-sulfamoylbenzoyl)-8-nitro-2,3-dihydrophthalazine-1,4-dione (5d)

Yield 72%, recrystallized from ethanol to give as white powder, m.p. 270 °C; IR (KBr, υ, cm^{-1}): 3325 (N−H, stretch), 3230 (NH_2), 1698 (C═O), 1650 (C═C), 1540 (NO_2), 1315 and 1158 (SO_2); ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 11.80 (1H, s, NH), 8.42 (1H, d, Ar−H), 8.40 (1H, d, Ar−H), 8.10 (1H, t, Ar−H), 8.08 (2H, d, Ar−H), 8.0 (2H, d, Ar−H), 7.61 (2H, s, $-NH_2$). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 165.3, 164.9, 164.8, 155.9, 148.3, 139.4, 139.3, 134.1, 132.0, 129.4, 126.8, 124.4, 124.2. Anal. Calcd. for $C_{15}H_{10}N_4O_7S$ (%): C, 46.16; H, 2.58; N, 14.35; O, 28.69; S, 8.21. Found: C, 46.31; H, 2.69; N, 14.49; O, 28.71; S, 8.30.

4.1.9. 2-(4-sulfamoylbenzoyl)-6-nitro-2,3-dihydrophthalazine-1,4-dione (5e)

Yield 75%, recrystallized from ethanol to give as white powder, m.p. 252 °C; IR (KBr, υ, cm^{-1}): 3540 (N−H), 3370 (NH_2), 1720 (C═O),

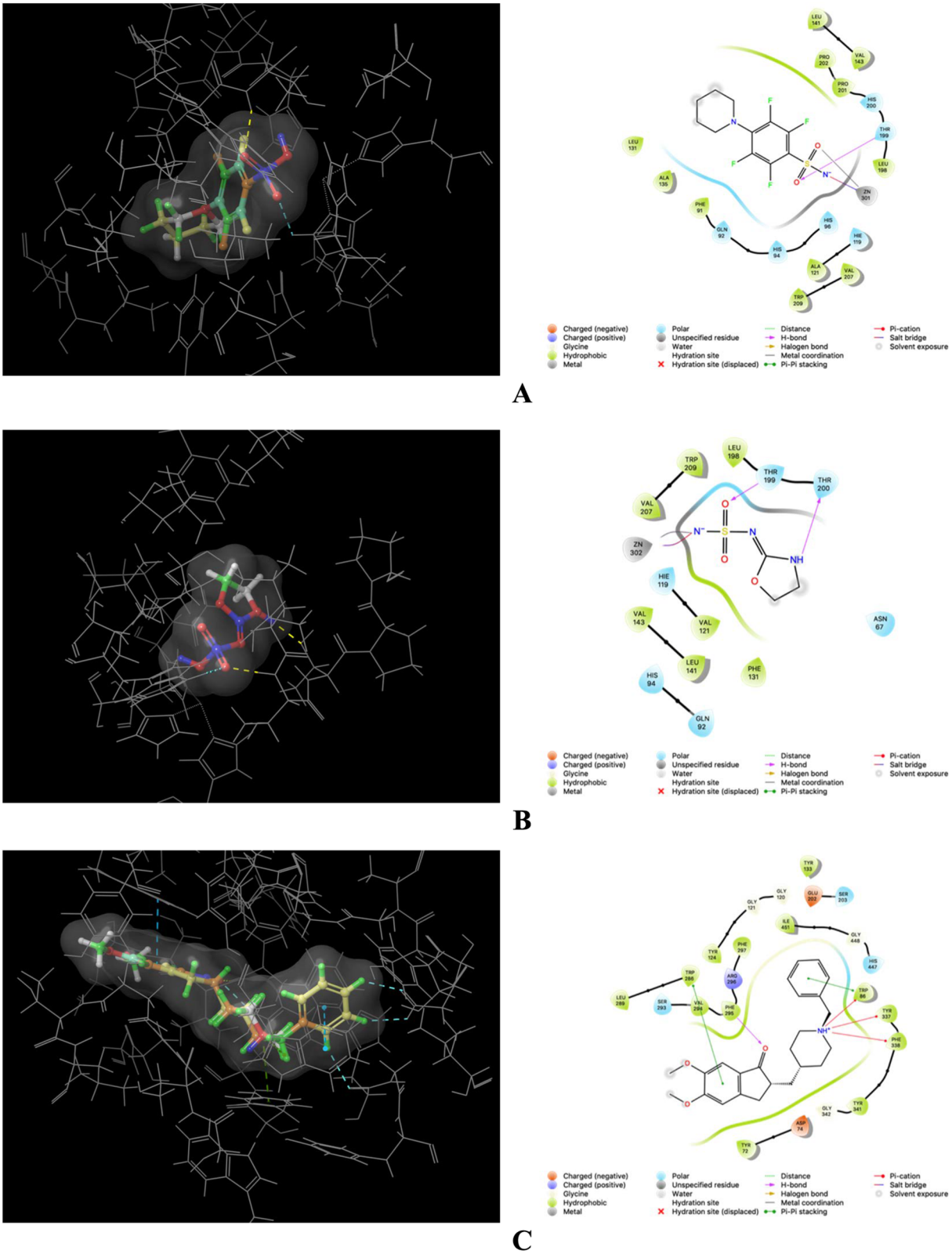


Fig. 3. Interaction of native ligands with the key amino acids within the binding sites of hCA I, hCA II, and AChE. (A) Docking pose of 2,3,5,6-tetrafluoro-4-piperidin-1-ylbenzenesulfonamide (3UG) with the active site of hCA I (PDB ID: 4WUQ). (B) Docking pose of N-[(2Z)-1,3-oxazolidin-2-ylidene]sulfuric diamide (OVX) with the active site of hCA II (PDB ID: 4FU5). (C) Docking pose of donepezil (E20) with the active site of AChE (PDB ID: 4EY7).

1640 (C═C), 1550 (NO_2), 1355 and 1170 (SO_2—NH_2); ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 11.82 (1H,s, NH), 8.78 (1H, s, Ar—H), 8.60 (1H, s, Ar—H), 7.69 (1H, s, Ar—H), 8.10 (2H, d, Ar—H), 7.90 (2H, d, Ar—H), 7.58 (2H, s, —NH_2). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 165.3, 164.3, 164.1, 152.7, 148.4, 134.6, 134.0, 131.4, 131.2, 129.4, 126.8, 126.3, 119.7. Anal. Calcd. for $C_{15}H_{10}N_4O_7S$ (%): C, 46.16; H, 2.58; N, 14.35; O, 28.69; S, 8.21. Found: C, 46.33; H, 2.65; N, 14.47; O, 28.82; S, 8.32.

Table 4
Molecular docking scores and binding modes of compounds (**5a-l**) and acetazolamide (AAZ) with hCA I (PDB ID: 4WUQ, 1.75 Å).

Compound	Glide GScore [a]	ΔG vdW[b]	ΔG Coulomb [b]	Glide Energy [b]	Glide emodel[b]	H-bonds	Pi-pi stacking
5a	−8.44	−30.79	−12.09	−42.88	−81.98	–	His64
5b	−7.82	−29.83	−15.44	−45.27	−78.00	Gln92, Thr199	His200
5c	−8.51	−31.00	−13.32	−44.32	−83.07	Gln92, Thr199	His200
5d	−8.33	−32.23	−13.83	−46.06	−84.51	Gln92, Thr199	His200
5e	−8.52	−33.13	−15.35	−48.48	−89.68	Gln92, Thr199	His67, His200
5f	−8.53	−25.44	−19.38	−44.83	−85.59	Trp5, Thr199	–
5g	−8.03	−35.18	−12.98	−48.16	−86.69	Gln92, Thr199	His200
5h	−8.10	−34.04	−13.28	−47.32	−86.07	Gln92, Thr199	His200
5i	−8.62	−35.91	−11.40	−47.31	−83.65	Gln92, Thr199	–
5j	−8.20	−29.05	−11.53	−40.58	−84.08	Gln92, Thr199	–
5k	−9.10	−30.06	−16.64	−46.70	−97.17	Gln92, Thr199	His200
5l	−10.55	−47.56	−23.07	−70.63	−137.84	Lys57, Leu131, Ala132, Thr199	His67, His200
AAZ[c]	−7.24	−24.89	−8.71	−33.60	−62.83	His200	His94

[a] Glide standard precision mode.
[b] kcal/mol.
[c] Acetazolamide.

*4.1.10. 2-(4-sulfamoylbenzoyl)-6-carboxy-2,3-dihydrophthalazine-1,4-dione (**5f**)*

Yield 82%, recrystallized from ethanol to give as white crystals, m.p. 268 °C; IR (KBr, υ, cm^{-1}): 3100 (carboxylic acid O—H), 3350 (N—H), 3210 (NH_2), 1700 (C═O), 1650 (C═C), 1352 and 1155 (SO_2); ^{1}H NMR (300 MHz, $CDCl_3$, *δ*, ppm): 11.90 (1H,s, NH), 8.20 (2H, d, Ar—H), 8.0 (2H, d, Ar—H), 7.81 (1H, s, Ar—H), 7.61 (2H, s, —NH_2), 7.71–7.58 (2H, dd, Ar—H). ^{13}C NMR (75 MHz, $CDCl_3$, *δ*, ppm): 166.3, 165.3, 165.2, 165.1, 148.3, 137.8, 136.9, 134.2, 133.3, 130.6, 129.3, 126.8, 125.2, 124.6. Anal. Calcd. for $C_{16}H_{11}N_3O_7S$ (%): C, 49.36; H, 2.85; N, 10.79; O, 28.77; S, 8.24. Found: C, 49.45; H, 2.97; N, 10.83; O, 28.94; S, 8.32.

*4.1.11. 2-(4-sulfamoylbenzoyl)-5,6,7,8-tetrabromo-2,3-dihydrophthalazine-1,4-dione (**5g**)*

Yield 82%, recrystallized from ethanol to give as white powder, m.p. 292 °C; IR (KBr, υ, cm^{-1}): 3352 (N—H), 3240 (NH_2), 1700 (C═O), 1665 (C═C), 1355 and 1175 (SO_2), 600 (C—Br); ^{1}H NMR (300 MHz, $CDCl_3$, *δ*, ppm): 11.70 (1H,s, NH), 8.18 (2H, d, Ar—H). 8.08 (2H, d, Ar—H), 7.62 (2H, s, —NH_2), ^{13}C NMR (75 Hz, $CDCl_3$, *δ*, ppm): 165.3, 162.0, 161.9, 148.4, 138.4, 134.0, 130.0, 129.7, 129.5, 129.4, 128.8, 126.8, 25.2. Anal. Calcd. for $C_{15}H_7Br_4N_3O_5S$ (%): C, 27.26; H, 1.07; Br, 48.36; N, 6.36; O, 12.10; S, 4.85. Found: C, 27.43; H, 1.15; Br, 48.49; N, 6.50; O, 12.25; S, 4.96.

*4.1.12. 2-(4-sulfamoylbenzoyl)-5,6,7,8-tetrachloro-2,3-dihydrophthalazine-1,4-dione (**5h**)*

Yield 76%, recrystallized from ethanol to give as white powder, m.p. 286 °C; IR (KBr, υ, cm^{-1}): 3350 (N—H), 3250 (NH_2), 1700 (C═O), 1655 (C═C), 1348 and 1155 (SO_2), 720 (C—Cl); ^{1}H NMR (300 MHz, $CDCl_3$, *δ*, ppm): 11.80 (1H, s, NH), 8.1 (2H, d, Ar—H), 8.01 (2H, d, Ar—H), 7.63 (2H, s, —NH_2), ^{13}C NMR (75 MHz, $CDCl_3$, *δ*, ppm): 165.3, 161.6, 161.4, 148.4, 140.1 133.9, 132.9, 131.3, 130.1, 129.7, 129.4, 126.8 124.1. Anal. Calcd. for $C_{15}H_7Cl_4N_3O_5S$ (%): C, 37.29; H, 1.46; Cl, 29.35; N, 8.70; O, 16.56; S, 6.64. Found: C, 37.38; H, 1.59; Cl, 29.83; N, 8.92; O, 16.58; S, 6.75.

*4.1.13. 2,3-dihydro-2-(4-sulfamoylbenzoyl)naphtha(1,8-de)(1,2)diazepine-1,4-dione (**5i**)*

Yield 76%, recrystallized from ethanol to give as white powder, m.p. 254 °C; IR (KBr, υ, cm^{-1}): 3320 (N—H), 3230 (NH_2), 1698 (C═O), 1655 (C═C), 1357 and 1175 (SO_2); ^{1}H NMR (300 MHz, $CDCl_3$, *δ*, ppm): 11.74 (1H,s, NH), 8.50 (4H, dd, Ar—H), 8.20 (2H, d, Ar—H), 8.02 (2H, d, Ar—H), 7.82 (2H, t, Ar—H), 7.62 (2H, s, —NH_2), ^{13}C NMR (75 MHz, $CDCl_3$, *δ*, ppm): 165.9, 165.3, 164.5, 148.3, 137.6, 136.2, 134.2, 130.5, 130.1, 129.6, 129.3, 128.9, 128.4, 127.5, 126.8, 124.7, 123.3. Anal. Calcd. for $C_{19}H_{15}N_3O_4S$ (%): C, 59.83; H, 3.96; N, 11.02; O, 16.78; S, 8.41. Found: C, 59.97; H, 4.11; N, 11.19; O, 16.90; S, 8.59.

Table 5
Molecular docking scores and binding modes of compounds (**5a-l**) and acetazolamide (AAZ) with hCA II (PDB ID: 4FU5, 0.98 Å).

Compound	Glide GScore [a]	ΔG vdW[b]	ΔG Coulomb [b]	Glide Energy [b]	Glide emodel [b]	H-bonds	Pi-pi stacking
5a	−5.84	−27.11	−42.57	−15.46	−60.68	Trp5, Asn62	Trp5
5b	−6.02	−26.19	−47.75	−21.56	−70.01	Trp5, Asn62	Trp5
5c	−4.57	−29.88	−40.28	−10.40	−54.32	Trp5, Gln92	–
5d	−6.31	−29.64	−50.25	−20.61	−71.81	Trp5, Asn62, Thr199	Trp5
5e	−5.75	−30.45	−46.51	−16.06	−65.37	Trp5, Asn62, Asn67	His64
5f	−8.51	−29.14	−48.92	−19.78	−91.48	Trp5, Asn62, Asn67, Thr199	–
5g	−4.34	−36.66	−44.86	−8.20	−54.34	Trp5, Asn62, Gln92, Thr199	Trp5
5h	−5.33	−34.65	−49.79	−15.14	−64.62	Trp5, Asn67, Thr199	–
5i	−5.36	−27.32	−43.99	−16.67	−65.18	Gln92, Thr199, Thr200	–
5j	−8.93	−29.38	−46.52	−17.14	−98.65	Asn67, Gln92, Thr199, Thr200	–
5k	−7.10	−29.90	−40.54	−10.64	−65.52	Thr199	–
5l	−4.11	−37.84	−48.00	−10.16	−64.32	Gln92, Thr199	–
AAZ[c]	−6.92	−20.74	−31.27	−10.52	−56.56	Asn67, Thr199, Thr200	–

[a] Glide standard precision mode.
[b] kcal/mol.
[c] Acetazolamide.

Table 6
Molecular docking scores and binding modes of compounds (**5a-l**) and tacrine (TAC) with AChE (PDB ID: 4EY7, 2.35 Å).

Compound	Glide GScore [a]	ΔG vdW[b]	ΔG Coulomb [b]	Glide Energy [b]	Glide emodel [b]	H-bonds	Pi-pi stacking	Pi-cation
5a	−7.71	−43.60	−1.64	−45.23	−61.86	Phe295	Trp286	–
5b	−7.75	−43.06	−1.25	−44.31	−63.66	Phe295	Trp286, Phe338	–
5c	−7.86	−43.44	−1.23	−44.67	−61.82	Phe295	Trp286, Phe338	–
5d	−6.95	−44.12	−4.65	−48.77	−66.84	Phe295	Trp286, Phe338, Tyr341	Trp286
5e	−8.00	−46.27	−1.53	−47.80	−67.05	Phe295	Trp286, Phe338	Trp286
5f	−7.35	−45.07	1.22	−43.85	−57.42	Phe295	Trp286, Phe338	Trp286
5g	−7.38	−50.36	−4.82	−55.18	−73.10	Ser293, Phe295, Arg296	Trp286, Tyr341	–
5h	−7.56	−48.35	−4.57	−52.92	−70.44	Trp286, Ser293, Phe295, Arg296	Tyr341	–
5i	−8.35	−40.02	−6.00	−46.01	−65.89	Trp286, Ser293, Phe295	Tyr124	–
5j	−9.40	−45.78	−6.76	−52.54	−74.67	Tyr124, Ser293, Tyr341	–	–
5k	−9.06	−48.18	0.48	−48.13	−68.78	Phe295	His447	–
5l	−8.75	−61.85	−4.00	−65.85	−90.03	Glu292, Phe295	Trp286, Tyr337, Tyr341	–
TAC[c]	−9.48	−29.00	−6.43	−35.43	−63.72	Ser125	Trp86	Trp86

[a] Glide standard precision mode.
[b] kcal/mol.
[c] Tacrine.

4.1.14. Octahydro-2-(4-sulfamoylbenzoyl)phthalazine-1,4-dione (5j)

Yield 70%, recrystallized from ethanol to give as white powder, m.p. 213 °C; IR (KBr, υ, cm^{-1}): 3320 (N—H), 3210 (NH_2), 1698 (C═O), 1610 (C═C, aromatic), 1351 and 1155 (SO_2); ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 11.40 (1H, s, NH), 8.05 (2H, d, Ar—H), 7.90 (2H, d, Ar—H), 7.60 (2H, s, $-NH_2$), 1.72–1.84 (2H, t), 1.30–1.49 (8H, m, $-CH_2$). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 177.3, 177.1, 164.8, 148.1, 134.5, 126.7, 129.2, 40.9, 38.4, 24.1 (2C), 23.9 (2C), Anal. Calcd. for $C_{15}H_{17}N_3O_5S$: C, 51.27; H, 4.88; N, 11.96; O, 22.77; S, 9.13. Found: C, 51.38; H, 5.11; N, 12.11; O, 25.75; S, 9.22.

4.1.15. 2,3,4a,5,8,8a-hexahydro-2-(4-sulfamoylbenzoyl)phthalazine-1,4-dione (5k)

Yield 66%, recrystallized from ethanol to give as white powder, m.p. 235 °C; IR (KBr, υ, cm^{-1}): 3350 (N—H), 3240 (NH_2), 1698 (C═O), 1610 (C═C), 1355 and 1160 (SO_2); ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 11.74 (1H,s, NH), 8.02 (2H, d, Ar—H), 7.80 (2H, d, Ar—H), 7.59 (2H, s, $-NH_2$), 5.90–5.99 (2H, q, ═CH, cyclohexenyl), 2.08–2.12 (2H, t, −CH, cyclohexenyl), 1.86–1.92 (4H, t, $-CH_2$). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 177.3, 177.0, 165.3, 148.3, 136.7, 131.7, 129.4, 127.6, 125.6, 41.4, 38.7, 27.2, 27.0. Anal. Calcd. for $C_{15}H_{15}N_3O_5S$: C, 51.57; H, 4.33; N, 12.03; O, 22.90; S, 9.18. Found: C, 51.62; H, 4.41; N, 12.15; O, 23.05; S, 9.28.

4.1.16. 2,8-di(4-sulfamoylbenzoyl)-1,2,3,4,6,7,8,9-octahydropyridazino[4,5-g]phthalazine-1,4,6,9-tetraone (5l)

Yield 71%, recrystallized from ethanol to give as white powder, m.p. 298 °C; IR (KBr, υ, cm^{-1}): 3330 (N—H), 3230 (NH_2), 1700 (C═O), 1626 (C═C, aromatic), 1315 and 1165 (SO_2); ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 11.70 (1H,s, NH), 8.33 (2H, s, Ar—H), 8.20 (2H, d, Ar—H), 8.00 (2H, d, Ar—H), 7.58 (4H, s, $-NH_2$). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 165.1, 165.0, 162.4, 148.0, 136.1, 135.2, 132.4, 132.2, 129.2, 128.2, 127.9. Anal. Calcd. for $C_{24}H_{16}N_6O_{10}S_2$ (%): C, 47.06; H, 2.63; N, 13.72; O, 26.12; S, 10.47. Found: C, 47.32; H, 2.79; N, 13.95; O, 26.32; S, 10.52.

4.2. Biological studies

4.2.1. Purification of CA and activity assay

Carbonic anhydrase isoenzymes (I, and II) were purified from human erythrocytes in a shortly using Sepharose-4B-L-tyrosine-sulfanilamide affinity chromatography which is a rapid and straightforward chromatographic technique. The amount of the protein was determined according to the Bradford method which is a simple and quick analytical procedure at 595 nm wavelength, quantitatively as described previously [52,53]. Bovine serum albumin (BSA) was used as a standard protein for this method [54,55]. Enzyme purity and subunit composition were controlled with Laemmli's SDS-PAGE procedure (3–8%) [56,57]. PageRuler prestained protein ladder was used as the reference protein standard, a mixture of proteins ranging from 10 to 180 kDa [58,59] conforming to our previous studies [60,61]. The reaction mix (approx. 1 mL) consisted of p-nitrophenyl acetate (3 mM) as a substrate, p-acetoxynitrobenzene, purified enzyme, and Tris-sulfate buffer (50 mM, pH 7.4). The enzyme activity measurements were achieved with three times for each sample by spectrophotometer at 25 °C. Enzyme unit was calculated using the absorption coefficient ($\varepsilon = 5.4 \times 10^3\ M^{-1}\ cm^{-1}$) of p-nitrophenol at 348 nm [62] by using our earlier reported methods [63,64]. The inhibitory activities of the N-substituted phthalazine sulfonamide compounds (**5a-l**) were determined on the esterase activity of hCA I, and II isoenzymes. The activities were assayed in the presence of different inhibitor concentrations. The experiments were repeated in triplicate for each drug concentration used. IC_{50} values and K_i constants were computed as in our previous assays [65,66]. The inhibition type for each of the compounds was determined using Lineweaver-Burk curves [67,68].

4.2.2. AChE activity assay

Acetylcholinesterase activity measurement was carried out according to Ellman's method [69] by the 1.4 mL quartz cuvette at room temperature. The reaction mixture contained 10 mM of acetylthiocholine iodide (AChI), 2-(acetylthio)-N,N,N-trimethylethanaminium iodide, as substrate and 5 mM 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) in 1 mM Tris/HCl (pH 8.0) containing 5 mM EDTA as described previously [70]. AChE assay was depended on the performed of DTNB ($\varepsilon = 13{,}6\ M^{-1}\ cm^{-1}$) at 412 nm, spectrophotometrically according to our previous study [71]. The inhibition effects on AChE of these compounds (**5a-l**) were determined by Activity%-[Phthalazine] curves. The IC_{50} values were obtained from Activity% versus the inhibitors. Various concentrations of the phthalazines were utilized for the determination of K_i constants and inhibition types as described in a previous study [72].

4.3. In silico studies

4.3.1. ADMET study

The physicochemical and pharmacokinetic properties (i.e., ADMET prediction and drug-likeness) of the N-substituted phthalazine sulfonamide compounds (**5a-l**) were predicted using the QikProp [73,74] and Molecular Properties Panel of Maestro [75].

4.3.2. Molecular docking study

Protein Preparation Wizard [76] was used to pre-process the crystal structures of the hCA I, II, and AChE in complex with the ligands

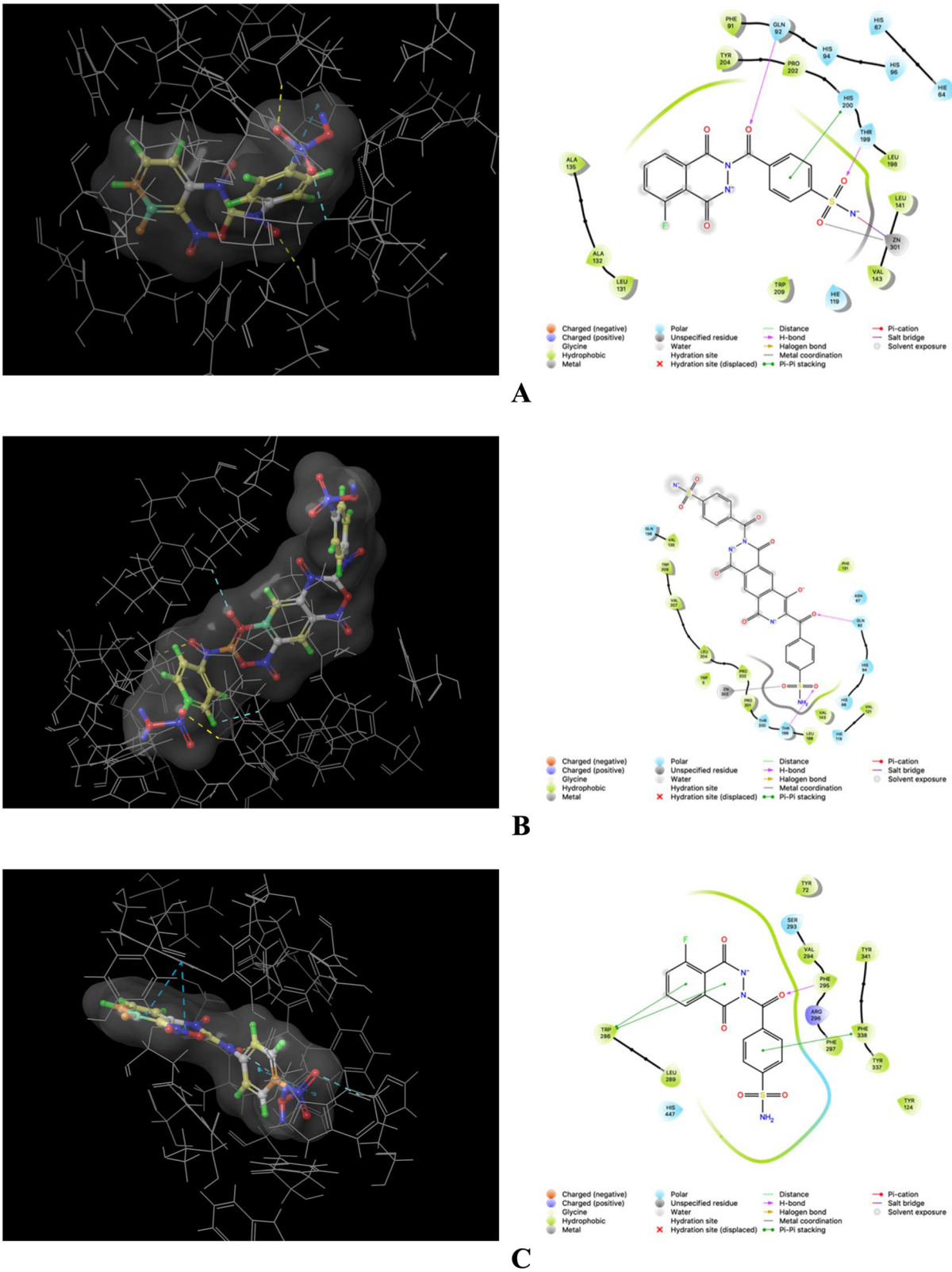


Fig. 4. 3D and 2D binding modes of compounds **5b** and **5l** in the active sites of hCA I (PDB ID: 4WUQ), hCA II (PDB ID: 4FU5), and AChE (PDB ID: 4EY7). (A) The binding mode of compound **5b** against hCA I. (B) The binding mode of compound **5l** against hCA II. (C) The binding mode of compound **5b** against AChE.

2,3,5,6-tetrafluoro-4-piperidin-1-ylbenzenesulfonamide (PDB ID: 4WUQ, 1.75 Å), N-[(2Z)-1,3-oxazolidin-2-ylidene]sulfuric diamide (PDB ID: 4FU5, 0.98 Å), and donepezil (PDB ID: 4EY7, 2.35 Å), respectively. The N-substituted phthalazine sulfonamide derivatives were prepared with the LigPrep module [77,78] as in our previous studies [79]. Protonation states at pH 7.0 ± 2.0 and tautomers were generated and minimizationed using OPLS3e force field [80,81]. The ligands were docked to the protein structure using the standard precision (SP) mode [82–84].

4.4. Statistical studies

Analysis of the data and drawing of graphs were implemented using GraphPad Prism6 and Systat SigmaPlot12 software. The results were shown as mean ± standard deviation (95% confidence intervals). Differences between data sets were considered statistically significant when the *p*-value was less than 0.05.

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