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Design, synthesis, and molecular docking studies of benzimidazole-1,3,4-triazole hybrids as carbonic anhydrase I and II inhibitors

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Abstract

In this study, with an aim to develop novel heterocyclic hybrids as potent enzyme inhibitors, we synthesized a series of 10 novel 2-(4-(4-ethyl-5-(2-(substitutedphenyl)-2-oxo-ethylthio)-4*H*-1,2,4-triazol-3-yl)-phenyl)-5,6-dimethyl-1*H*-benzimidazole (**5a–5j**) derivatives and characterized by ^{1}H-NMR, ^{13}C-NMR, and HRMS. These compounds were evaluated for their inhibitory activity against hCA I and hCA II. All the compounds exhibited good hCA I and hCA II inhibitory activities with IC_{50} values in range of 1.288 μM–3.122 μM. Among all these compounds, compound **5e,** with an IC_{50} value of 1.288 μM is the most active against carbonic hCA I. Compound **5h** with an IC_{50} value of 1.532 μM is the most active against carbonic hCA-II. Compounds **5a–5j** were also evaluated for their cytotoxic effects on the L929 mouse fibroblast (normal) cell line. The compounds were also analyzed for their antioxidant capacity by TAS, FRAP, and DPPH activity. Enzyme inhibition kinetics showed all compounds **5a–5j** to inhibit the enzyme by non-competitive. The most active compound **5e** for hCA I and compound **5h** for hCA-II were subjected to molecular docking, which revealed their binding interactions with the enzyme's active site, confirming the experimental findings.

KEYWORDS

1,3,4-triazole, antioxidant, benzimidazole, carbonic anhydrase, molecular docking

1 | INTRODUCTION

The researchers have been focusing on the biological functions of carbonic anhydrase enzymes and their pharmaceutical uses in the pharmaceutical industry since 1933 (Stadie & O'Brien, 1933). Carbonic anhydrases (CA; EC. 4.2.1.1) that are involved in the metalloenzyme family catalyze the reversible hydration of CO_2 to a bicarbonate anion (HCO_3^-) and a proton (H^+) (Supuran, 2013). They are also available in mammals, other animals, and plants. Hitherto, eight CA classes are identified: α-, β-, γ-, δ-, ζ-, η-, θ-, and ι-CAs (Scozzafava et al., 2015). The class found mainly in mammals is the α-CAs and 16 isoforms having different catalytic activity, subcellular localization, and tissue distribution have been discovered so far. Of these, CA I, CA II, CA III, CA VII, and CA XIII are cytosolic forms, CA IV, CA IX, CA XII, CA XIV, and CA XV are membrane bound, CA VA and CA VB are mitochondrial, and CA VI is secretory isoforms (Alterio et al., 2012; Supuran, 2008, 2016, 2018). pH and bicarbonate homeostasis, respiration, lipogenesis, signal transduction, bone metabolism, gluconeogenesis, calcification, sodium ion retention, ureagenesis, tumorigenesis are biochemical processes involving CA

isozymes (Supuran & Capasso, 2018; Winum et al., 2008; Thiry et al., 2007). Acetazolamide, ethoxzolamide, dorzolamide, and methazolamide inhibiting CA isozymes have been used in clinics for many years (Supuran & Scozzafava, 2000). It was thought that the development of new agents with the ability to inhibit CA isoforms will be beneficial in the treating some diseases, such as glaucoma, ulcers, osteoporosis, obesity, and cancer (Supuran, 2008; Supuran & Scozzafava, 2007).

Reactive oxygen species (ROS) and free radicals that are produced in excess during living metabolism can harm various cellular macromolecules, particularly nucleic acids, membrane lipids, and proteins, ultimately leading to cell death. Antioxidants reduce the production of free radicals, shielding living things from the harmful effects of ROS. Additionally, it has been suggested that ROS and free radicals have a role in a number of serious illnesses that affect people all over the world, including cancer, atherosclerosis, rheumatoid arthritis, neurodegenerative, autoimmune, cardiovascular, and age-related diseases (Aytac et al., 2023). It is thought that the synthesized compounds showing both carbonic anhydrase and antioxidant properties will provide an advantage in the treatment of diseases for which ROS, free radicals, and carbonic anhydrases are responsible.

Benzimidazole scaffold formed by the fusion of imidazole and benzene rings has been widely studied in medicinal chemistry. This ring shows an amphoteric character because it has acidic and basic NH groups. Furthermore, it has the ability to create salts rapidly (Kamanna, 2019). Benzimidazole derivatives have been shown to have many biological activities such as antimicrobial (Özkay et al., 2011), antidiabetic (Bansal & Silakari, 2012), anticancer (Vemana et al., 2019), analgesic (Gaba et al., 2014), antihistaminic (Wang et al., 2012), anti-HIV (Pan et al., 2015), and anthelmintic (Sethi et al., 2018) activities.

Triazole is a five-member heterocyclic ring with two carbon and three nitrogen atoms (Potts, 1961). Triazole is of great importance due to its synthetic utility and different pharmacological activities in the last few decades (Mansoory & Rajput, 2015). The antifungal fluconazole, antitumoral letrozole, antiviral ribavirin, anti-migraine rizatriptan, and anxiolytic estazolam are commercially available drugs with triazole skeleton (Dixit et al., 2021).

Based on the aforementioned rationale, we designed and synthesized a novel series of benzimidazole-triazole hybrid compounds and evaluated their inhibitory effects on hCA I and hCA II isoforms. Compounds **5e** and **5h** were screened to determine their binding potential at target protein PDB ID:3W6H (hCA I) and PDB ID:4G0C (hCA II). Cytotoxicity of the synthesized compounds **5a–5j** was determined using a healthy mouse fibroblast cell line (L929). Furthermore, these compounds were also analyzed for their antioxidant capacity by TAS, FRAP, and DPPH activity.

1.1 | Experimental study

Synthetic procedures and characterization of compounds are included in Data S1.

1.2 | hCA inhibition assay

The in vitro hCA inhibition assay was performed as mentioned in previous studies (Küçükoğlu et al., 2022).

1.3 | Molecular docking

All phases of molecular docking studies were carried out using Schrödinger software Maestro version 12.8. Three-dimensional structures of target proteins hCA I (PDB ID: 3W6H, Resolution: 2.96 Å) and hCA II (PDB ID: 4G0C, Resolution: 2.00 Å) were obtained from the protein data bank (PDB) https://www.rcsb.org/. Water and other heteroatoms other than Zn^{2+} were removed and target proteins were prepared with the 'Protein Preparation Wizard' default settings. The 3D minimizing structures of the compounds were prepared with the 'LigPrep' module at pH:7 ± 2. The active site coordination file for both target proteins hCA I (x: 33.6, y: −1.33, z: 9.01) and hCA II (x: −4.98, y: 3.81, z: 14.7) were created as 20*20*20 Å3 with the 'Receptor Grid Generation' module based on the cocrystal ligand acetohexamide. To validate the molecular docking work, re-docking was performed with Glide SP and the cocrystal ligand acetohexamide. Then, molecular docking of all compounds with Glide SP ligand was performed.

1.4 | Antioxidant activity

Many methods have been developed to determine antioxidant capacity. These methods differ according to the radicals or the target molecule. No single method of determining antioxidant capacity can fully assess antioxidant capacity. Therefore, in future studies, it tried to determine the antioxidant capacity by using several different methods. Among the antioxidant capacity determination methods, DPPH, ABTS, CUPRAC, FRAP, FCR can be given as examples (Pellegrini et al., 2003).

1.4.1 | TAS activity

The total antioxidant status (TAS) is determined by a commercial kit which is manufactured by Rel Assay Diagnostics. According to this method, the sample's potential antioxidant structures are reduced the dark blue-green

ABTS radical form to the colorless reduced ABTS form. The alteration of absorbance at 660 nm is related to the total antioxidant capacity of the sample. The assay was calibrated with the reference substance used as the stable standard antioxidant solution, which is the vitamin E analog called the Trolox equivalent. TAS measurement was performed according to the kit procedure. After calculating the difference between absorbance values, the equation given below is calculated according to Equation 1 (Erel, 2004).

$$A2 - A1 = \Delta\text{Abs of standard or sample or } H_20$$
$$\text{Results} = \frac{\left[\Delta\text{Abs } H_2O - \Delta\text{Abs Sample}\right]}{\left[\Delta\text{Abs } H_2O - \Delta\text{Abs Standard}\right]}. \quad (1)$$

1.4.2 | DPPH

2,2-Diphenyl-1-picrylhydrazil (DPPH) is a stable organic radical and is commercially available (Huang et al., 2005). DPPH radical scavenging capacity analysis, which is frequently used to measure the antioxidant capacity of natural extracts, is also used to measure the antioxidant capacity of the synthesized structures (Moţ et al., 2011). This method can be defined by the change of absorbance at 517 nm with proton transfer to the DPPH free radical by the potential antioxidant. The methanol solution of the DPPH radical at 517 nm shows maximum absorbance. Since it is a simple and fast method, it is used in many studies. However, its sensitivity to oxygen, light, and pollution are the disadvantages of the method.

1.4.3 | FRAP

This method, in which antioxidants determine the reducing capacity of iron (III), was developed by Benzei and Strain. Fe(III)-TPTZ complex is formed as a result of the reaction of Fe(III) with tripyridyltriazine (TPTZ), and this complex is reduced to Fe(II)-TPTZ complex with the antioxidant in the environment. The color of this complex is dark blue and gives maximum absorbance at 593 nm (Pellegrini et al., 2003). Incubation is performed for up to 30 min to complete the reaction and read the correct absorbance values. The results obtained can be given as Trolox equivalent or as IC_{50} values.

1.5 | Cytotoxicity assay

The effect of the compounds **5a–5j** on the viability of L929 cell line was analyzed by MTT assay. The MTT method was performed as previously described (Ayşen et al., 2022).

2 | RESULTS AND DISCUSSION

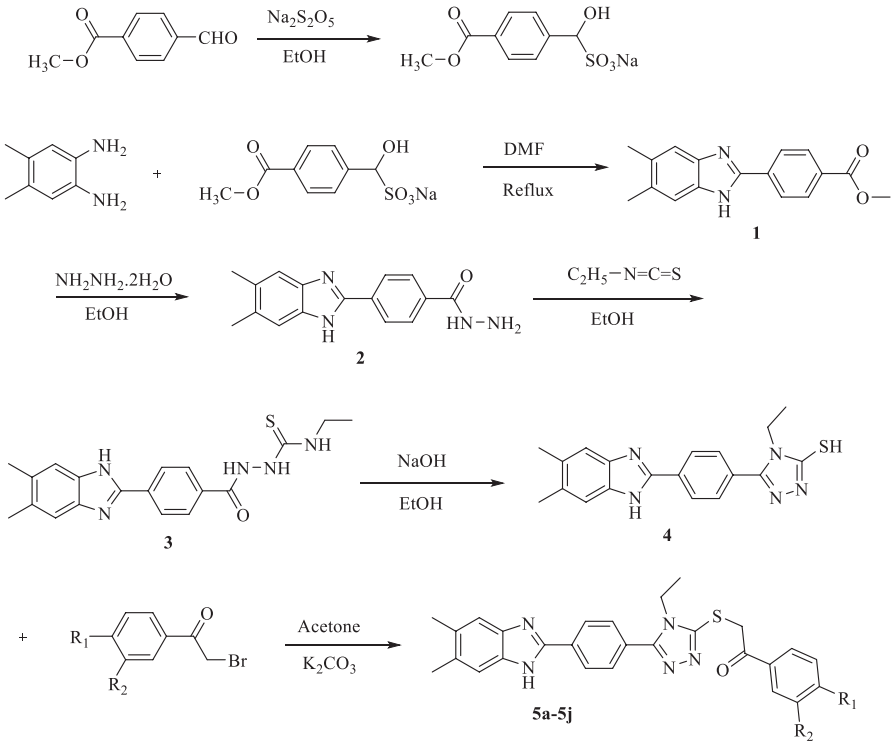
2.1 | Chemistry

The synthesis schema of 2-(4-(4-ethyl-5-(2-(substitute dphenyl)-2-oxo-ethylthio)-4*H*-1,2,4-triazol-3yl)-pheny l)-5,6-dimethyl-1*H*-benzimidazole (**5a–5j**) derivatives is summarized in Schema 1. The synthesis of the compounds was carried out in five steps. In the first step, the salt of methyl 4-formyl benzoate compound with sodium meta-bisulfite was synthesized. In the second step, the benzimidazole ring was synthesized by reacting 4,5-dimethylbenzene 1,2-diamine compound with benzaldehyde salt in DMF. In the third step, a hydrazide compound was obtained by reacting the ester structure of 4-(5,6-dimethyl-1*H*-benzimidazol-2-yl)benzoic acid methyl ester (**1**) with hydrazine hydrate. In order to synthesize the triazole ring from the hydrazide compound, first, the hydrazide compound (**2**) was reacted with ethyl isocyanate, the obtained compound was reflux in ethanol with NaOH solution and the product was precipitated with HCl at the end of the reaction. In the last step, the final compounds were obtained by reacting the thiol group in the second position of the triazole ring with various phenacyl bromide derivatives. Structures of synthesized compounds were proved by ^{1}H-NMR, ^{13}C-NMR, and HRMS spectroscopic analysis methods.

2.2 | In vitro hCA activity

Benzimidazole-1,3,4-triazole hybrid molecules, **5a–5j,** were tested for their inhibition properties against the two physiologically relevant CA isoforms (hCA I and hCA II). The inhibition effects of compounds **5a–5j** are presented in Table 1. Acetazolamide (AAZ) was used as a standard inhibitor for hCA I and hCA II in this work. Inhibition effects of compounds **5a–5j** were between 1.288 and 2.622 μM against hCA I isoenzyme and most of the compounds had higher inhibitor activity than that of AAZ (IC_{50} = 2.26 μM), except compounds **5d** and **5i.** The activity results obtained from this study clearly pointed out that the most potent compound was **5e**, which is a 4-chloro derivative, with an IC_{50} value of 1.288 μM against hCA I. The other derivatives showed notable inhibitory effects on hCA I isoform were **5a, 5c, 5f–5h,** and **5j** with IC_{50} values ranging between 1.591 and 1.816 μM.

It was found that inhibition properties of compounds **5a–5j** were between 1.532 and 3.122 μM on hCA II enzyme and they had no more activity than that of AAZ (IC_{50} = 1.17 μM).



Comp.	5a	5b	5c	5d	5e	5f	5g	5h	5i	5j
R_1	-Br	$-CH_3$	-CN	-Cl	-Cl	-H	-F	$-OCH_3$	$-NO_2$	-Phenyl
R_2	-H	-H	-H	-Cl	-H	-H	-H	-H	-H	-H

SCHEMA 1 General procedure for synthesis of the final compounds **5a–5j**.

TABLE 1 IC_{50} and K_i values of the compounds **5a–5j** toward hCA I and hCA II isoforms.

	hCA I inhibition			hCA II inhibition		
Compounds	**IC_{50} (μM)**	**K_i (μM)**	**Type of inhibition**	**IC_{50} (μM)**	**K_i (μM)**	**Type of inhibition**
5a	1.6003	1.599 ± 0.14	Noncompetitive	2.248	2.1692 ± 0.036	Noncompetitive
5b	2.113	1.706 ± 0.09	Noncompetitive	3.064	2.308 ± 0.078	Noncompetitive
5c	1.721	1.381 ± 0.011	Noncompetitive	2.065	1.838 ± 0.014	Noncompetitive
5d	2.622	2.1125 ± 0.063	Noncompetitive	3.122	2.385 ± 0.11	Noncompetitive
5e	**1.288**	1.225 ± 0.02	Noncompetitive	1.6195	1.369 ± 0.0	Noncompetitive
5f	1.591	1.219 ± 0.022	Noncompetitive	2.563	1.923 ± 0.032	Noncompetitive
5g	1.654	1.599 ± 0.012	Noncompetitive	2.051	1.538 ± 0.020	Noncompetitive
5h	1.801	1.463 ± 0.011	Noncompetitive	**1.532**	1.154 ± 0.06	Noncompetitive
5i	2.477	1.95 ± 0.013	Noncompetitive	2.13996	1.654 ± 0.01	Noncompetitive
5j	1.816	1.463 ± 0.08	Noncompetitive	1.765	1.308 ± 0.014	Noncompetitive
Acetazolamide	2.26	1.63 ± 0.011	Noncompetitive	1.17	0.812 ± 0.01	Noncompetitive

The values of the most active compounds are emphasized in bold.

In compounds **5a–5j**, **5a–5g** exhibited more inhibitor activity against hCA I than hCA II, whereas the inhibitory effects of three derivatives (**5h, 5i, 5j**) were more significant on hCA II than on hCA I. Compound **5j** showed slightly more inhibitory activity on hCA II than on hCA I with IC_{50} values of 1.765 μM and 1.816 μM, respectively.

K_i constants of benzimidazole-1,3,4-triazole hybrids derivatives **5a–5j** were calculated between 1.219 and 2.1125 μM for hCA I and are presented in Table 1. All of

the compounds showed noncompetitive inhibition and most of them were found to be greater inhibition effects than that of AAZ ($K_i = 1.63\,\mu M$), except compounds **5b, 5d,** and **5i.** Among all the compounds, **5e** and **5f** were determined as the most active derivatives against hCA I enzyme with K_i values of 1.225 and 1.219 μM, respectively. Other compounds with same/fairly close K_i values against hCA I are: **5a** and **5g** ($K_i = 1.599\,\mu M$), **5h**, and **5j** ($K_i = 1.463\,\mu M$).

All of the compounds showed noncompetitive inhibitory activity exhibited higher K_i value of AAZ ($K_i = 1.17\,\mu M$) against hCA II, the physiologically dominant isoform (Table 1), and their K_i values were found between 1.154 and 2.385 μM.

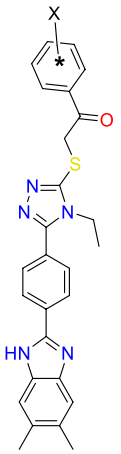
The structure–activity relationship studies revealed that the presence of chloro group at *para* position of phenyl ring (**5e**) is beneficial for hCA I activity. However, the presence of the chloro substituent (**5d**) in both the third and fourth positions decreased activity. While the nonsubstituted (**5f**) phenyl ring was effective in increasing the hCA I activity, this activity increased with the chloro substituent in the *para* position. However, the presence of substituents such as bromo, cyano, fluoro in the *para* position decreases the activity compared to the previous compound (**5f**). The structure–activity relationship studies revealed that the presence of methoxy (**5h**), chloro (**5e**), and phenyl (**5j**) group at *para* position of phenyl ring is beneficial for hCA II activity.

2.3 | Molecular docking

Molecular docking studies are used to predict how designed or synthesized compounds interact with the target protein/enzyme. In this study, in silico molecular docking studies of compounds whose in vitro hCA I and hCA II inhibitory activities were evaluated were performed. PDB ID:3W6H for hCA I and PDB ID:4G0C for hCA II were preferred because they contain AAZ, which is used as a reference compound in in vitro studies, as a cocrystal ligand. To validate the molecular docking, cocrystal ligand AAZ was self-docking. The RMSD value of AAZ contained in PDB ID:3W6H for hCA I was measured as 1.357 Å, and for hCA II, the RMSD value of AAZ of PDB ID:4G0C was measured as 0.167 Å. As given in Table 2, the compounds produced Glide emodel: −48.973 to −69.676 kcal/mol binding energies against hCA I between Glide score: −4.700 and −5.934 kcal/mol. However, the compounds produced binding energies for hCA II between Glide score: −3.785 and −4.667 kcal/mol. AAZ, on the other hand, gave Glide binding energies of −7.893 and −7.097 kcal/mol against hCA I and hCA II, respectively. The glide gscores of the compounds are lower compared to AAZ, but the glide emodel interaction energies are closer together. The glide gscores of the compounds are generally better in hCA I than in hCA II.

The binding poses and protein–ligand interactions of compounds **5e** and **5h**, which showed the highest activity against hCA I and hCA II, were analyzed. The compounds did not interact directly with Zn^{2+} as in acetolazamide. As shown in Figure 1a, compound **5e** is located right next to Zn^{2+} in the active site of hCA I. Compound **5e** showed Pi–Pi stacking with His67 (4.84 Å), Pi-cation with His94 (5.26 Å), hydrophobic interactions with Trp5, Val62, Ile60, Phe91, Ala121, Leu141, Val143, Val207 and Trp209, Hie119, Thr199. It gave polar interactions with His200, Gln92, His94, and His67 and positively charged interactions with Lys170 and Arg173. Compound **5h** gave

TABLE 2 Molecular docking protein-ligand Glide score and Glide emodel interaction energies of hCA I and hCA II and compounds.

Compounds		X	hCA I		hCA II	
			Glide gscore	Glide emodel	Glide gscore	Glide emodel
	**5a**	4-Br	−4.809	−64.119	−4.243	−50.881
	5b	4-CH_3	−5.310	−60.774	−3.835	−65.784
	5c	4-CN	−4.792	−60.920	−4.091	−62.424
	5d	3,4-diCl	−5.601	−57.051	−4.043	−60.076
	5e	4-Cl	−4.641	−48.973	−4.054	−57.441
	5f	4-H	−4.700	−59.114	−3.612	−61.828
	5g	4-F	−5.314	−53.756	−3.785	−55.951
	5h	4-OCH_3	−4.904	−58.092	−3.967	−55.515
	5i	4-NO_2	−4.874	−61.534	−4.606	−67.527
	5j	4-Ph	−4.832	−69.676	−4.224	−68.052
	Acetazolamide		−7.893	−70.685	−7.097	−67.269

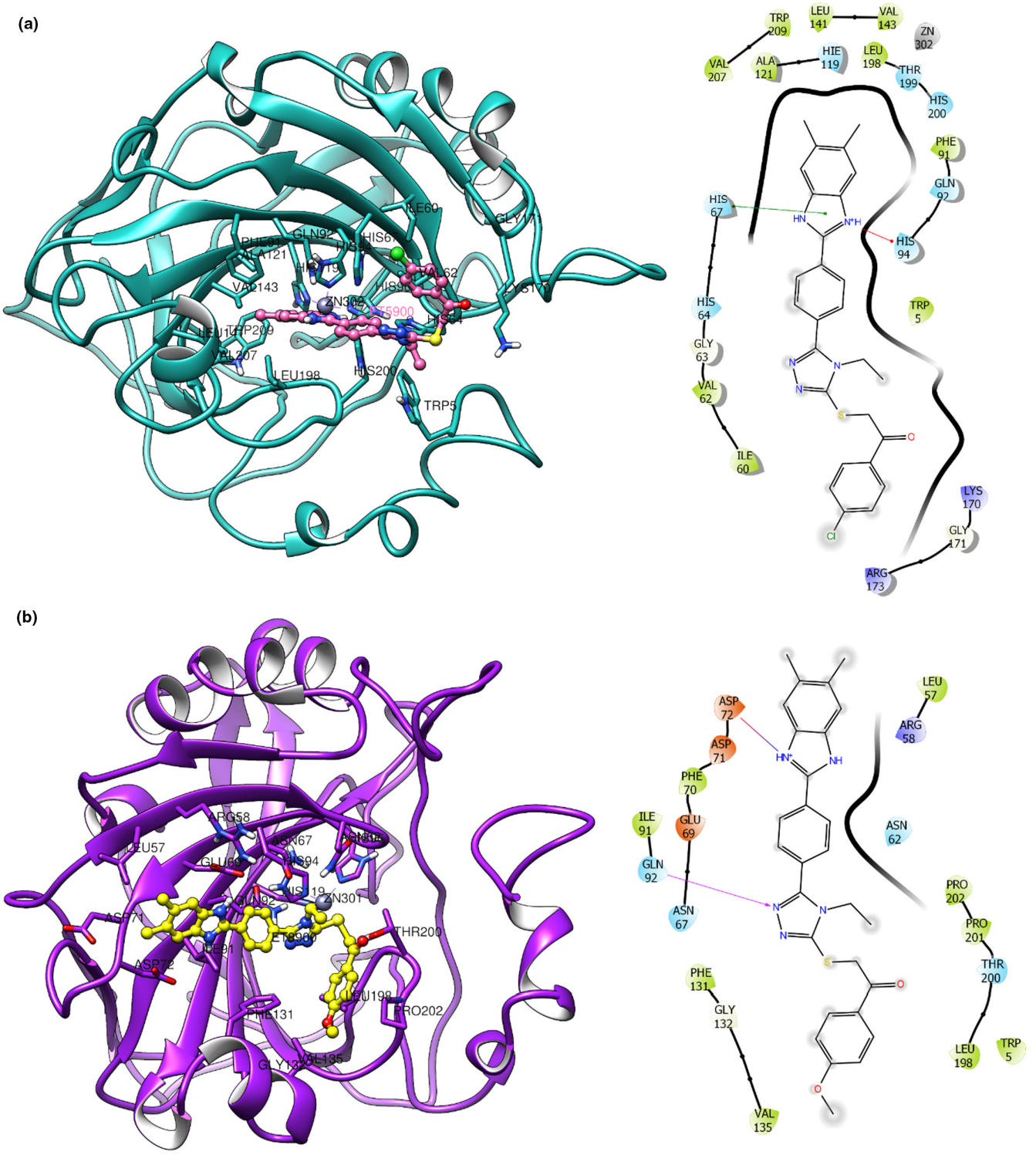


FIGURE 1 Molecular docking results of hCA I & **5e** and hCA II & **5h** enzyme ligand complexes obtained from Glide SP ligand docking. (a) Binding poses and protein–ligand interaction diagrams of hCA I & **5e** and (b) hCA II & **5h** complex.

binding mode and interactions at the active site of hCA II as shown in Figure 1b. Compound **5h** showed that H bond with Gln92 (2.29 Å), salt bridge with Asp72 (4.64 Å), positive charge with Arg58, polar interactions with Asn62, Asn67, and Thr200, hydrophobic interactions with Trp5, Phe131, Leu57, Val135, Leu198, Pro201, and Pro202. Red-docking poses of AAZ with CA I (PDB ID: 3W6H) and CA II (PDB ID: 4G0C) are given in Figure 2.

2.4 | Antioxidant activity

2.4.1 | TAS

The total antioxidant capacity values greater than or equal to 1.0 mmol Trolox equivalent/L are considered as high and desired levels. Total antioxidant capacity values of the compounds between **5a-5j** were found low which is shown in Table 3. Although compounds **5i** and **5d** show partially high antioxidant capacity values, this amount is not at the desired level.

2.4.2 | DPPH

In order to determine the total free radical scavenging capacity of each sample, solutions of different concentrations (50–1000 μM) were prepared and compared with vitamin C using stable DPPH according to the procedure previously used by Yu et al (Küçükoğlu et al., 2022). A freshly prepared 0.1 mM DPPH solution with ethanol was added at a ratio of 1/1 to all the different concentrations of samples prepared and to the different concentrations of vitamin C solutions used as standard. As a negative control, DPPH solution was added to the ethanol solution and the measurement was made in this way. After all the solutions were added to the microplates, they were incubated for 60 min in a dark environment. Then, UV absorbance values were measured at 517 nm in a spectrophotometer (BMG LABTECH SPECTROstar Nano) at room temperature. Results are expressed as mean ± standard deviation ($n = 3$) and compared with ascorbic acid used as a natural antioxidant. IC_{50} values were calculated by determining the radical scavenging activity of the samples and standards compared to the negative control (Table 3). As a result of the study, it was determined that compounds **5f**, **5g**, **5h**, **5i**, and **5j** showed less scavenging properties for DPPH than ascorbic acid. However, it was determined that compounds **5a**, **5b**, **5c**, **5d**, **5e,** and **5f** had more scavenging properties than ascorbic acid.

2.4.3 | FRAP

Like some reducing agents, antioxidants also cause the Fe^{3+} ferricyanide complex to be reduced to Fe^{2+}. In this method, the color of the test solution changes from yellow to green, depending on the reducing power of the sample tested. This green color gives maximum absorbance at 700 nm and increasing absorbance indicates increasing reduction strength. According to this method, trolox was used as the standard antioxidant compound, and measurements were made in accordance with the procedure determined by Benzie and Strain (Pellegrini et al., 2003). With the IC_{50} (Table 3) results obtained as a result of the study, it was determined that the compounds **5a**, **5b**, **5c**, **5d,** and **5g** showed more antioxidant properties than vitamin E for iron reduction.

2.5 | Cytotoxicity assay

Cytotoxicity of compounds **5a–5j** was evaluated against L929 cell line. For preliminary screening, the cytotoxic bioactivity of synthesized compounds was evaluated in vitro against L929 cell line with the MTT assay. Cell viability percentages were calculated after the treatment of cells for 48 h. The preliminary cytotoxic effect results of compounds **5a–5j** against L929 fibroblast are presented in Table 4. The data obtained at the end of the study showed that almost all compounds **5a–5j** showed low cytotoxic effects. As a result of the maximum dose applied, all compounds except compounds **5a** and **5b** showed 80% and more viability. However, compound **5a** showed an IC_{50} value above 100 μM, but cell viability decreased to 61% at the maximum dose. As a result of the calculations, the

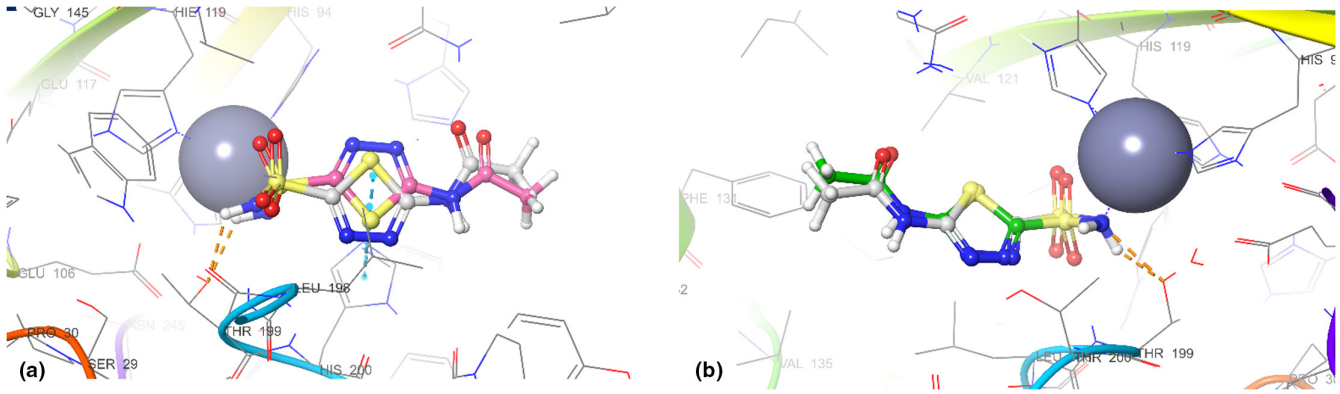


FIGURE 2 (a) Redocking poses of acetazolamide with CA I (PDB ID: 3W6H) and (**b**) CA II (PDB ID: 4G0C).

Compounds	TAS mmol Trolox equivalent/L	Reducing for Fe^{+3} IC_{50} (μM)	DPPH IC_{50} (μM)
5a	0.148 ± 0.009	0.481 ± 0.013	113.71 ± 0.019
5b	0.059 ± 0.003	0.567 ± 0.021	116.96 ± 0.021
5c	0.058 ± 0.001	0.513 ± 0.039	106.4 ± 0.005
5d	0.282 ± 0.026	0.525 ± 0.038	73.10 ± 0.002
5e	0.256 ± 0.016	0.955 ± 0.001	74.32 ± 0.001
5f	0.049 ± 0.010	1.227 ± 0.065	190.07 ± 0.003
5g	0.138 ± 0.010	0.455 ± 0.041	263.58 ± 0.010
5h	0.172 ± 0.018	1.529 ± 0.032	482.48 ± 0.019
5i	0.343 ± 0.018	3.653 ± 0.012	974.29 ± 0.002
5j	0.258 ± 0.011	2.061 ± 0.008	663.2 ± 0.012
Vitamin E	1.000 ± 0.078	0.629 ± 0.017	-
Ascorbic acid	-	-	227.08 ± 0.186

TABLE 3 Total antioxidant status (TAS) values of vitamin E and synthesized compounds **5a**–5**j**, vitamin E and compounds **5a**–5**j** against reducing for Fe^{3+}, and ascorbic acid and compounds **5a**–5**j** against DPPH.

TABLE 4 Cell viability (%) of L929 fibroblast cell line against compounds for 48 h.

Compounds	48 h viability %
5a	61.09 ± 3.51
5b	41.3 ± 4.88
5c	81.21 ± 2.81
5d	86.94 ± 4.11
5e	81.62 ± 4.16
5f	82.13 ± 5.73
5g	84.22 ± 4.46
5h	97.51 ± 5.7
5i	91.25 ± 5.97
5j	96.76 ± 4.89
Control	100 ± 1.87

IC_{50} value of compound **5b** was found to be 85.18 ± 1.48 IC_{50} values of other compounds were not calculated because they were greater than 100 μM.

3 | CONCLUSION

In summary, a class of novel benzimidazole-1,3,4-triazole hybrids were designed, synthesized, and evaluated as carbonic anhydrase inhibitors. Although the synthesized compounds **5a–5j** do not carry a sulfonamide group, which is an important group in obtaining hCA inhibitory activity, most of them exhibited more significant inhibitory activity against hCA I isoform than AAZ. Except for compounds **5d** and **5i**, all other compounds showed better activity than AAZ against hCA I, especially compound **5e** showed the most activity against hCA I. For hCA II, compound **5h** showed a similar activity compared to the reference drug AAZ. It was determined that the presence of chlorine (electron withdrawing) substituent in the fourth position of the phenyl ring (**5e**) increased the activity against the hCA I enzyme, while the presence of the methoxy (electron donor) substituent in the fourth position (**5h**) increased the activity against the hCA II enzyme. Among these compounds, compounds **5e**, **5f** (for hCA I), and **5h** (for hCA II) may be developed in future studies. According to the antioxidant tests (TAS, DPPH, FRAP), it can be said that the compounds show good antioxidant properties in general. The effects of the compounds on the L929 cell line were investigated to determine their cytotoxicity. The data obtained at the end of the study showed that almost all compounds **5a–5j** showed low cytotoxic effects. Furthermore, molecular docking studies are used to predict how the designed or synthesized compounds interact with the target protein/enzyme.

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The data that supports the findings of this study are available in the supplementary material of this article

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REFERENCES

Alterio, V., Di Fiore, A., D'Ambrosio, K., Supuran, C. T., & De Simone, G. (2012). Multiple binding modes of inhibitors to carbonic anhydrases: How to design specific drugs targeting 15 different isoforms? *Chemical Reviews*, *112*(8), 4421–4468. https://doi.org/10.1021/cr200176r

Ayşen, I., Çevik, U. A., Çelik, I., Bostancı, H. E., Karayel, A., Gündoğdu, G., Ince, U., Koçak, A., Ozkay, Y., & Kaplancıklı, Z. A. (2022). Benzimidazole-hydrazone derivatives: Synthesis, in vitro anticancer, antimicrobial, antioxidant activities, in silico DFT and ADMET studies. *Journal of Molecular Structure*, *1270*, 133946. https://doi.org/10.1016/j.molstruc.2022.133946

Aytac, S., Gundogdu, O., Bingol, Z., & Gulcin, İ. (2023). Synthesis of Schiff bases containing phenol rings and investigation of their antioxidant capacity, anticholinesterase, butyrylcholinesterase, and carbonic anhydrase inhibition properties. *Pharmaceutics*, *15*(3), 779. https://doi.org/10.3390/pharmaceutics15030779

Bansal, Y., & Silakari, O. (2012). The therapeutic journey of benzimidazoles: A review. *Bioorganic & Medicinal Chemistry*, *21*, 6208–6236. https://doi.org/10.1016/j.bmc.2012.09.013.7

Dixit, D., Verma, P. K., & Marwaha, R. K. (2021). A review on 'triazoles': Their chemistry, synthesis and pharmacological potentials. *Journal of the Iranian Chemical Society*, *18*(10), 2535–2565.

Erel, O. (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clinical Biochemistry*, *37*(4), 277–285. https://doi.org/10.1016/j.clinbiochem.2003.11.015

Gaba, M., Singh, S., & Mohan, C. (2014). Benzimidazole: An emerging scaffold for analgesic and anti-inflammatory agents. *European Journal of Medicinal Chemistry*, *76*, 494–505. https://doi.org/10.1016/j.ejmech.2014.01.030

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, *53*(6), 1841–1856. https://doi.org/10.1021/jf030723c

Kamanna, K. (2019). Synthesis and pharmacological profile of benzimidazoles. In *Chemistry and applications of benzimidazole and its derivatives* (pp. 51–69). IntechOpen.

Küçükoğlu, K., Acar Çevik, U., Nadaroglu, H., Celik, I., Işık, A., Bostancı, H. E., … Kaplancıklı, Z. A. (2022). Design, synthesis and molecular docking studies of novel benzimidazole-1, 3, 4-oxadiazole hybrids for their carbonic anhydrase inhibitory and antioxidant effects. *Medicinal Chemistry Research*, *31*(10), 1771–1782. https://doi.org/10.1007/s00044-022-02943-6

Mansoory, J. H., & Rajput, S. S. (2015). Synthesis, reactivity and biological evaluation of triazole: Recent developments. *International Journal of Pharmaceutics*, *7*(5), 20–32.

Moţ, A. C., Silaghi-Dumitrescu, R., & Sârbu, C. (2011). Rapid and effective evaluation of the antioxidant capacity of propolis extracts using DPPH bleaching kinetic profiles, FT-IR and UV–vis spectroscopic data. *Journal of Food Composition and Analysis*, *24*(4–5), 516–522. https://doi.org/10.1016/j.jfca.2010.11.006

Özkay, Y., Tunalı, Y., Karaca, H., & Işıkdağ, İ. (2011). Antimicrobial activity of a new series of benzimidazole derivatives. *Archives of Pharmacal Research*, *34*, 1427–1435. https://doi.org/10.1007/s12272-011-0903-8

Pan, T., He, X., Chen, B., Chen, H., Geng, G., Luo, H., … Bai, C. (2015). Development of benzimidazole derivatives to inhibit HIV-1 replication through protecting APOBEC3G protein. *European Journal of Medicinal Chemistry*, *95*, 500–513. https://doi.org/10.1016/j.ejmech.2015.03.050

Pellegrini, N., Serafini, M., Colombi, B., Del Rio, D., Salvatore, S., Bianchi, M., & Brighenti, F. (2003). Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *The Journal of Nutrition*, *133*(9), 2812–2819. https://doi.org/10.1093/jn/133.9.2812

Potts, K. T. (1961). The chemistry of 1,2,4-triazoles. *Chemical Reviews*, *61*(2), 87–127.

Scozzafava, A., Passaponti, M., Supuran, C. T., & Gülçin, İ. (2015). Carbonic anhydrase inhibitors: Guaiacol and catechol derivatives effectively inhibit certain human carbonic anhydrase isoenzymes (hCA I, II, IX and XII). *Journal of Enzyme Inhibition and Medicinal Chemistry*, *30*(4), 586–591. https://doi.org/10.3109/14756366.2014.956310

Sethi, P., Bansal, Y., & Bansal, G. (2018). Synthesis and PASS-assisted evaluation of coumarin–benzimidazole derivatives as potential anti-inflammatory and anthelmintic agents. *Medicinal Chemistry Research*, *27*, 61–71. https://doi.org/10.1007/s00044-017-2036-1

Stadie, W. C., & O'Brien, H. (1933). The catalysis of the hydration of carbon dioxide and dehydration of carbonic acid by an enzyme isolated from red blood cells. *Journal of Biological Chemistry*, *103*(2), 521–529. https://doi.org/10.1016/S0021-9258(18)75831-6

Supuran, C. T. (2008). Carbonic anhydrases: Novel therapeutic applications for inhibitors and activators. *Nature Reviews Drug Discovery*, *7*, 168–181.

Supuran, C. T. (2013). Carbonic anhydrases: From biomedical applications of the inhibitors and activators to biotechnological use for CO_2 capture. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *28*(2), 229–230. https://doi.org/10.3109/14756366.2013.761876

Supuran, C. T. (2016). Structure and function of carbonic anhydrases. *Nature Reviews Drug Discovery*, *14*, 2023–2032. https://doi.org/10.1042/BCJ20160115

Supuran, C. T. (2018). Carbonic anhydrases and metabolism. *Metabolites*, *8*, 25. https://doi.org/10.3390/metabo8020025

Supuran, C. T., & Capasso, C. (2018). Biomedical applications of prokaryotic carbonic anhydrases. *Expert Opinion on Therapeutic Patents*, *28*(10), 745–754. https://doi.org/10.1080/13543776.2018.1497161

Supuran, C. T., & Scozzafava, A. (2000). Carbonic anhydrase inhibitors and their therapeutic potential. *Expert Opinion on Therapeutic Patents*, *5*, 575–600. https://doi.org/10.1517/13543776.10.5.575

Supuran, C. T., & Scozzafava, A. (2007). Carbonic anhydrases as targets for medicinal chemistry. *Bioorganic & Medicinal Chemistry*, *13*, 4336–4350. https://doi.org/10.1016/j.bmc.2007.04.020

Thiry, A., Dogne, J. M., Supuran, C. T., & Masereel, B. (2007). Carbonic anhydrase inhibitors as anticonvulsant agents. *Current Topics in Medicinal Chemistry*, *9*, 855–864. https://doi.org/10.2174/156802607780636726

Vemana, H. P., Barasa, L., Surubhotla, N., Kong, J., Ha, S. S., Palaguachi, C., … Dukhande, V. V. (2019). Benzimidazole scaffolds as potential anticancer agents: Synthesis and biological evaluation. *The FASEB Journal*, *33*, 646.18. https://doi.org/10.1096/fasebj.2019.33.1_supplement.646.18

Wang, X. J., Xi, M. Y., Fu, J. H., Zhang, F. R., Cheng, G. F., & You, Q. D. (2012). Synthesis, biological evaluation and SAR studies of benzimidazole derivatives as H1-antihistamine agents. *Chinese Chemical Letters*, *23*(6), 707–710. https://doi.org/10.1016/j.cclet.2012.04.020

Winum, J. Y., Rami, M., Scozzafava, A., Montero, J. L., & Supuran, C. (2008). Carbonic anhydrase IX: A new druggable target for the design of antitumor agents. *Medicinal Research Reviews*, *28*(3), 445–463. https://doi.org/10.1002/med.20112

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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