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Synthesis and exploration of 2-morpholino-4-phenylthiazol-5-yl acrylamide derivatives for their effects against carbonic anhydrase I, II, IX and XII isoforms as a non-sulfonamide class of inhibitors

Baijayantimala Swain[a], Chander Singh Digwal[a], Andrea Angeli[b], Mallika Alvala[a], Priti Singh[a], Claudiu T. Supuran[b,*], Mohammed Arifuddin[a,*]

[a] Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research (NIPER), Balanagar, Hyderabad 500037, India
[b] Università degli Studi di Firenze, Neurofarba Dept., Sezione di Scienze Farmaceutiche e Nutraceutiche, Via Ugo Schiff 6, 50019 Sesto Fiorentino, Florence, Italy

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ABSTRACT

Novel series of 2-morpholino-4-phenylthiazol-5-yl acrylamide derivatives (**8a–s**) have been synthesized and explored as a non-sulfonamide class of carbonic anhydrase (CA, EC 4.2.1.1) inhibitors. The newly synthesized molecules were evaluated for their CA inhibitory potency against four isoforms: the cytosolic isozyme hCA I, II as well as trans-membrane tumor associated isoform hCA IX and hCA XII taking acetazolamide (AAZ) as standard drug. The results revealed that most of the compounds showed good activity against hCA II, IX, and XII whereas none of them were active against hCA I (K_i > 100 μM). It is observed that the physiologically most important cytosolic isoform hCA II was inhibited by these molecules in the range of K_i 9.3–77.7 μM. It is also found the both the transmembrane isoforms hCA IX and XII were also inhibited with K_is ranging between 54.7–96.7 μM and 4.6–8.8 μM, respectively. The binding modes of the active compounds within the catalytic pockets of hCA II, IX and XII were evaluated by docking studies. This new non-sulfonamide class of selective inhibitors of hCA II, IX and XII over the hCA I isoform may be used for further understanding the physiological roles of some of these isoforms in various pathologies.

1. Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) are the essential enzymes that effectively catalyze the reversible hydration of CO_2 to bicarbonate and proton, an important reaction for many physiological processes.[1] This Zn(II) containing metalloenzyme (α-CA) has sixteen different isoforms (I–XV, VA and VB) in mammals (Fig. 1), which may promote pathological situations under their malfunctioning and/or altered expression.[2] Therefore, it is important to develop an effective approach that can selectively inhibit isoforms involved in different diseases. Although, there are several commercially available CAIs, such as acetazolamide, ethoxzolamide, dorzolamide, well known for their anticonvulsant, anti-glaucoma, and anti-infective activities,[3] none of them shows selectivity for a specific isoenzyme, therefore causing side effects. Nowadays, the major concern of the researchers working in this field is to develop new concept for designing new families of compounds that are capable to block specifically the process catalyzed by one or two isoforms of these enzymes.

In this perspective, privileged scaffolds such as thiazole, morpholine, phenyl acrylamide etc., are frequently used for the synthesis of various heterocyclic compounds with wide ranges of pharmacological activities. As an important class of heterocyclic compounds, the thiazoles are associated with almost all the biological and pharmacological activities such as antimicrobial (Sulfathiazole) (Fig. 2A), antiretroviral (Ritonavir) (Fig. 2B), antifungal (Abafungin) (Fig. 2C), and antineoplastic (Tiazofurin) (Fig. 2D) with recent applications for the treatment of allergies, hypertention, inflammation, schizophrenia, bacterial, HIV infections, hypnotics, and more recently for the treatment of pain, as fibrinogen receptor antagonists with antithrombotic activity and as new inhibitors of bacterial DNA gyrase B.[4]

The morpholine linked thiazole moiety was found to be relevant in the structure of inhibitors of the DNA binding domain of the androgen receptor (Fig. 2E),[5] PI3K inhibitors (Fig. 2F, G)[6] and as antifungal agents (Fig. 2H).[7] On the hand acrylamide derivatives were explored by various researchers for numerous biological activities such as anticancer (Fig. 2I),[8] antiparasite (Fig. 2J),[9] antioxidant (Fig. 2K),[10] and also as a lipid lowering agent (Fig. 2L).[11]

Coming to CA inhibition, sulfonamides were the most investigated

* Corresponding authors.
E-mail addresses: claudiu.supuran@unifi.it (C.T. Supuran), arif.niperhyd@gov.in (M. Arifuddin).

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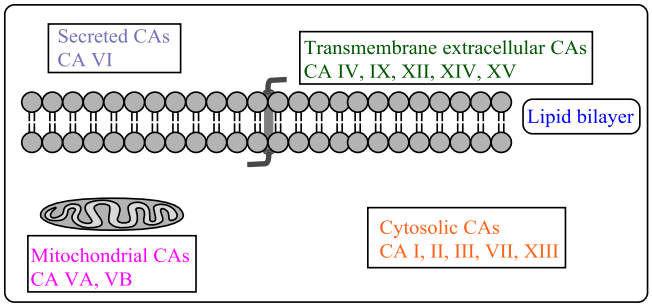


Fig. 1. The subcellular localization of catalytically active human CA isoenzymes.

class of CA inhibitors (CAIs) that bind to the metal ion from the enzyme active site by displacing the metal-bound hydroxide ion/water which is situated at the bottom of a (15 Å) deep active site pocket and coordinated by three amino acid residues, His94, His96 and His119 in a tetrahedral geometry (Fig. 3A).[12,13] On the other hand, the carboxylates were found to be the most complicated family of inhibitors, showing a variety of inhibition mechanism such as monodentate coordination of carboxylate to the metal ion, or anchoring to the zinc coordinated water, or binding at the entrance or even outside the active site cavity (Fig. 3).[14–17]

Recently, one of us presented[17] a comparison study of carbon versus sulfur based zinc binding groups where the carbon based compounds showed an interesting inhibition profile against all the mammalian isoforms of carbonic anhydrase CA I–XV, although with a lower efficacy compared to the sulfonamides (Fig. 4A). It has also been explained that the inhibition depends on: the pKa of the ZBG, its geometry (tetrahedral, i.e. sulfur-based, versus trigonal, i.e. carbon-based ZBGs), orientation of the organic scaffold induced by the nature of the ZBG where the carboxamide has shown very good inhibition potency against CA isoforms.[18] Di Fiore et al. showed that the hydroxamate have inhibited all 12 CA isoforms with inhibition constants in the range of 0.94–179 μM and are less effective with compared to the sulfonamide but exhibiting a comparable activity with reference to that of the *N*-substituted sulfonamides (Fig. 4B).[19] Also Carradori et al. has described the amide derivatives of Probenecid as selective inhibitors of carbonic anhydrase IX and XII (Fig. 4C).[20] Based on these literatures findings on carbonic anhydrase and the diverse activity associated with thiazole our endeavor to discover novel CA inhibitors and hence this particular scaffold non-sulfonamide based 2-morpholinothiazolophenylacrylamide has been synthesized and evaluated for CA inhibitions.

2. Results and discussion

2.1. Chemistry

The current work was designed to selectively inhibit hCA I, II, IX, XII isoforms, with derivatives which do not incorporate a sulfonamide moiety. The designed molecule 2-morpholino-4-phenylthiazol-5-yl cinnamamide (**8a–s**) was synthesized according to the general synthetic plan depicted in Scheme 1. Acyl chloride (**1a–d**) was refluxed with KSCN in dry CH_3CN, cooled to room temperature and followed by addition of a solution of morpholine to give *N*-(morpholine-4-carbonothioyl)benzamide (**2a–d**). Compound **2a–d** was treated with TEA followed by ethyl bromoacetate at room temperature in ethanol to afford the thiazole ester derivative (**3a–d**). The compound **3a–d** on reduction with lithium aluminum hydride produces the corresponding thiazole alcohol (**4a–d**) which in turn was subjected to Swern oxidation to provide compound **5a–d**. Furthermore, compound **5a–d** was subjected to Wittig reaction to give the corresponding acryl ester **6a–d**. In the next step the compound **6** was saponified to give the corresponding acrylic acid (**7a–d**) derivatives which were finally subjected to acid-amine coupling in the presence of EDCI-HOBt as coupling reagent to afford the target compound (**8a–s**) in good yield. It selectively yields E-isomer and were found to be geometrically pure with *trans* configuration (J = 13–16 Hz) from ^{1}H NMR (i.e. in compound **8a** the unsaturated proton at 6.20 showing a doublet with J = 14.9 Hz). The newly synthesized compounds (**8a–s**) are shown in the Table 1.

2.2. Carbonic anhydrase inhibition

The inhibition data against four CA isoenzymes, hCA I, II (cytosolic isoforms involved in glaucoma and other pathogenic conditions) and the transmembrane tumor-associated hCA IX, and XII (anticancer drug target) with the newly synthesized molecules **8a–s** along with the standard drug AAZ, are shown in Table 2. The screening was done by a stopped flow CO_2 assay.[20]

The following structure activity relationship can be figured out from the inhibition data of Table 2:

- All the synthesized compounds (**8a–s**) were very weak or ineffective inhibitors for isoform hCA I, which is a slow cytosolic and off-target isozyme for anti-epileptics.
- The anti-glaucoma drug target hCA II was inhibited by five compounds (**8k**, **8m**, **8n**, **8o and 8r**) in moderate to low micro molar range (K_i = 9.3–77.7 μM) whereas other compounds were ineffective. It is assumed that these compounds i.e. **8k** (K_i = 39.2 μM), **8m** (K_i = 9.3 μM), **8n** (K_i = 77.7 μM), **8o** (K_i = 50.4 μM), **8r** (K_i = 67.8 μM) were showing inhibition might be due to the presence of 4-NO_2, 4-Cl as R_1 group and 4-OMe, 4-Br and 2-NH_2 as R_2 group.
- hCA IX, the tumor associated transmembrane isoform was inhibited in low micro molar range by some of the synthesized molecules and the rest of the molecules were ineffective. The molecules (**8m**, **8n**, **8o** and **8r**) which contain -NO_2 and -Cl substitution at R_1 position were showing inhibition with K_i ranging 54.7–96.7 μM.
- In the case of tumor associated isoform hCA XII the compounds **8k**, **8m** and **8o** exhibited moderate to weak inhibition properties with K_i in the range 4.6–8.8 μM.
- Thus in brief the results summarised from the screening data against the four isoforms: The molecules have showed good inhibition when R_1 substituted with -NO_2 and -Cl as compared to 4-OMe and -H. Hence the electron withdrawing groups were facilitating the good inhibition. It was observed that the compounds with the substitution on R_2 as 4-OMe, 4-Br and 2-NH_2 exhibiting better inhibitory potency when compared to the di- and tri -OMe derivatives. It was also noticed that the compounds have shown inhibitory potency selectivity towards hCA II, IX, XII over hCA I.

2.3. Docking studies

Molecular docking calculations were performed to understand the interaction of synthesized analogues at active site pocket of CA isoforms. The docking scores of **8a–s** against various isoforms of CA were shown in Table 3. All the synthesized compounds shown lower docking scores in comparison to standard co-crystals of hCA II, IX, XII, but some of them were showing interactions with amino acid (AA) residues within the binding site. All the docking scores and interacting AA are displayed in Table 4.

With respect to SAR, it has been observed that the compounds with electron donating groups are less interactive than electron withdrawing groups. The compounds **8k**, **8m**, **8n**, **8o**, **8r** which are showing good inhibition against several hCA isoforms demonstrated strong interaction. These results are supporting the in-vitro enzymatic assay results. Furthermore, these new class of compounds also not belongs to any non-classical CA inhibitors. It is anticipated that these new class of

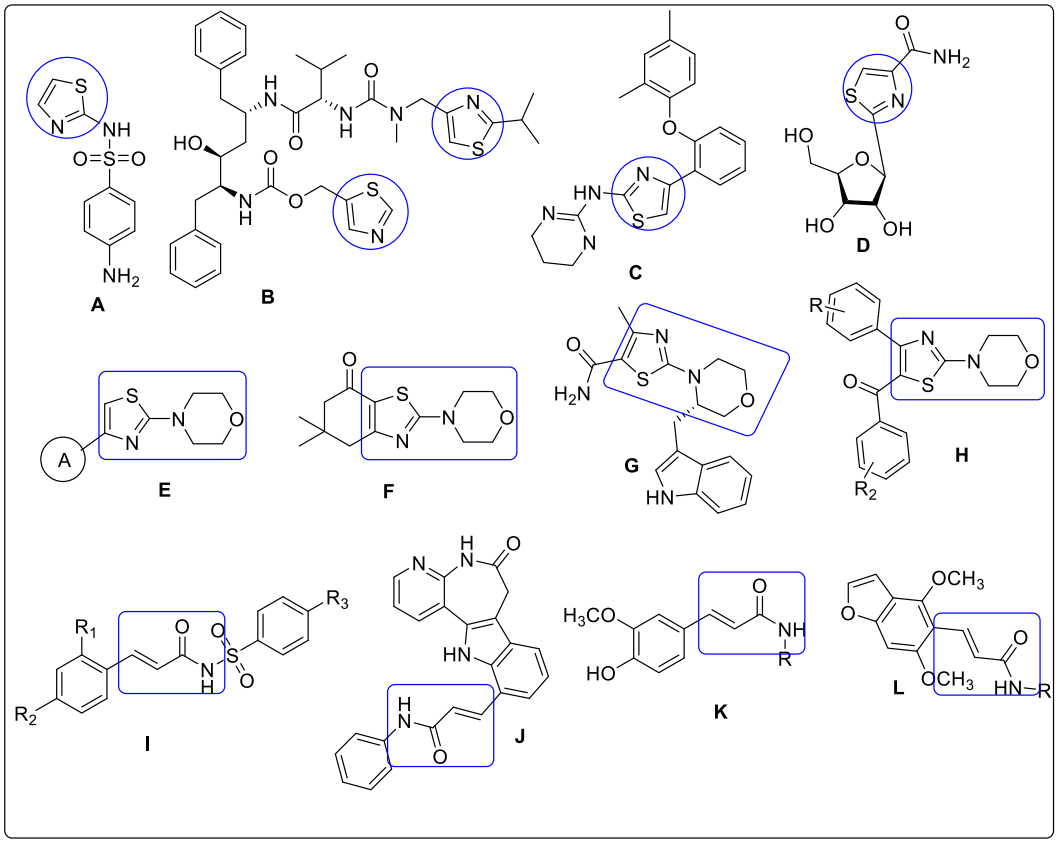


Fig. 2. Thiazoles, 2-morpholinothiazole and acrylamides derivatives used for the treatment of various diseases.

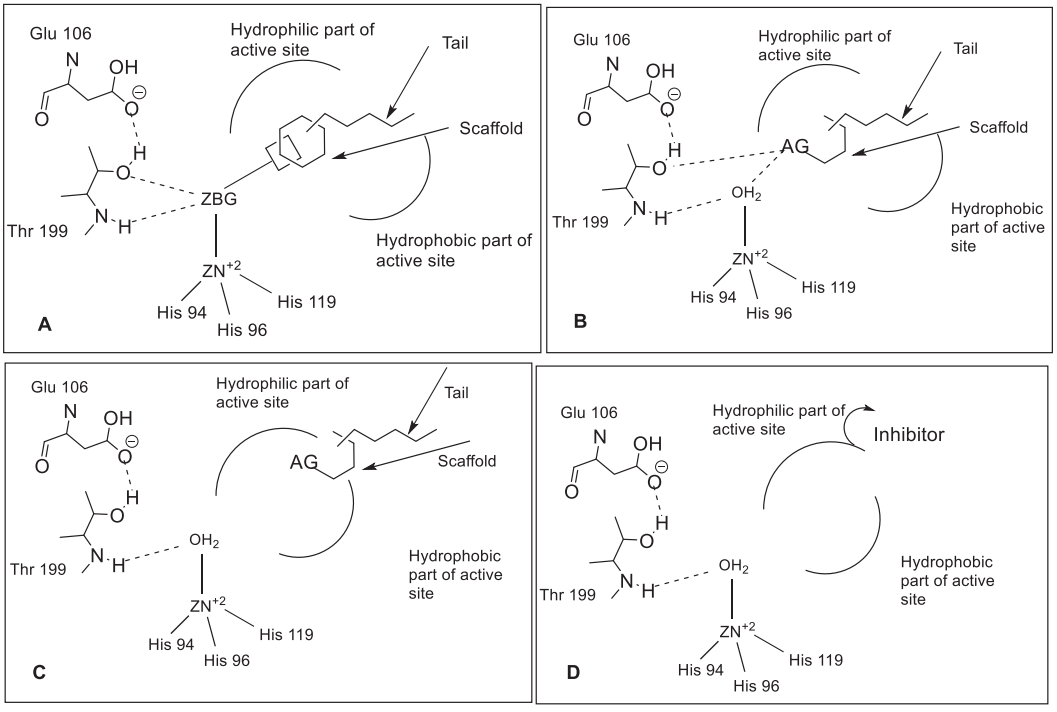


Fig. 3. CA inhibition mechanisms: A. Zinc-binders; B. Compounds which anchor to the metal ion coordinated water/hydroxide ion; C. Occlusion of the active site entrance. D. Out of the active site binding. Adapted from Ref. 3b.

compounds might be interacting with a CA via different mechanism which is beyond the present scope of work. Some docking poses of compounds synthesized in this work, when bound to the active site of isoforms hCA II, IX and XII are shown in Fig. 5. It should be mentioned that the suggested binding modes of Fig. 5 were not yet validated by means of crystallographic studies.

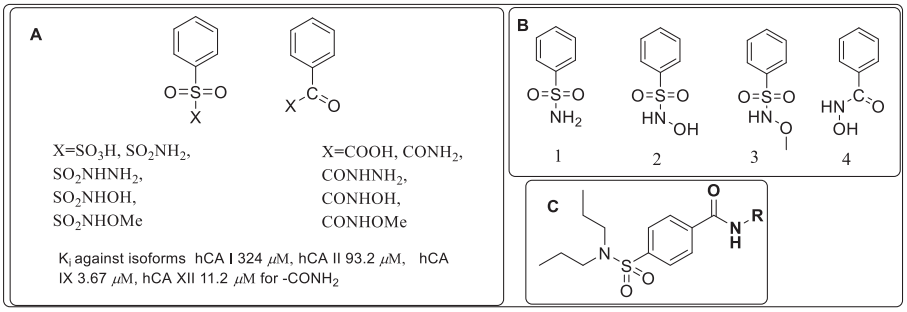


Fig. 4. Carbon and sulfur based CAIs investigated in Ref. 17.

3. Conclusion

In conclusion, we have synthesized 2-morpholinothiazole phenylacrylamide derivatives (**8a–s**) and screened against the various CA isoforms. Among them compound **8k**, **8m**, **8n**, **8o** and **8r** are shown notable inhibitory activity against three isoforms hCA II, IX and XII with K_i in the range of 4.6–96.7 μM. Since these compounds are not belonging to neither classical nor non-classical CAIs functionality, thus it is anticipated that further optimization and exploration of such kind of novel scaffolds will lead to structurally diverse ZBGs and play important role in the design and development of new isoform selective CAIs with better inhibition potency and less side effects. Hence it may be concluded that the further modification of this newly designed 2-morpholinothiazole phenylacrylamide derivatives (**8a–s**) will emerges as isoform selective CAIs.

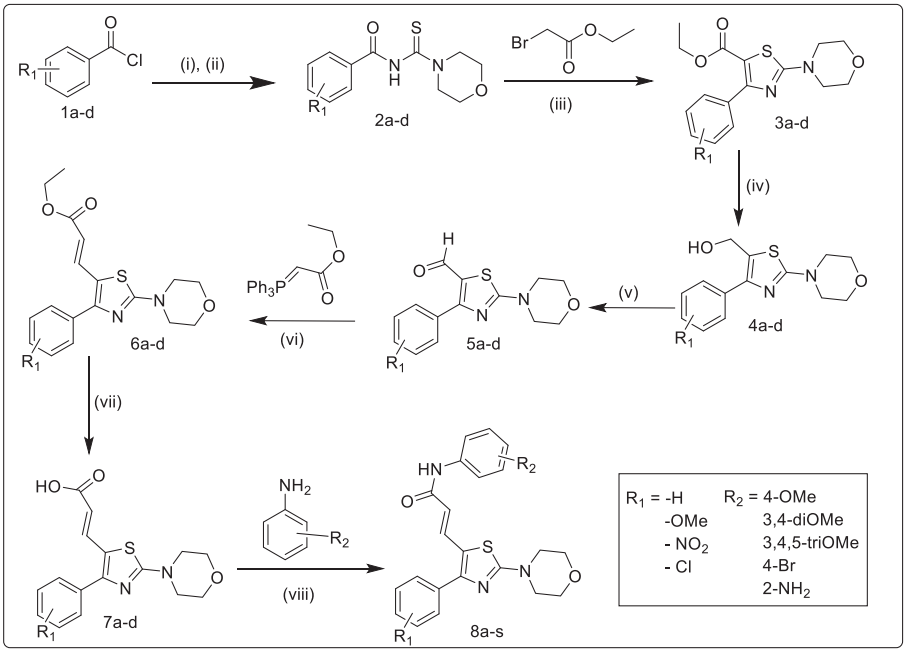
4. Experimental

4.1. General

All the reagents implemented here in the present work were purchased from commercial suppliers and used without further purification. All the solvents were purified and dried using standard methods prior to use. All reactions were performed under atmospheric pressure and reactions involving air- or moisture-sensitive compounds were performed under a nitrogen atmosphere using dried glassware and syringe techniques to transfer solutions. Melting points (mp) were measured on Stuart digital melting-point apparatus/SMP-30 in open capillary tubes and uncorrected. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel 60 F-254 aluminum plates. Column chromatography purifications were performed on silica gel (60–120 mesh) as the stationary phase and ethyl acetate /*n*-hexane (3:7) were used as eluents. Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were recorded using an Advanced Brucker NMR spectrometer at 500 MHz, 125 MHz in DMSO-d_6. Chemical shifts (δ) values reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal reference, and the coupling constants (J) expressed in hertz (Hz). Splitting patterns are symbolized as follows: s, singlet; d, doublet; t, triplet; m, multiplet; dd, doublet of doublet; q, quartet. HRMS were conformed with Agilent QTOF mass spectrometer 6540 series instrument and were carried out in the ESI techniques at 70 eV.

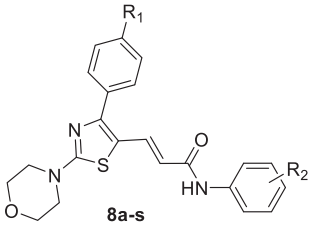
4.1.1. General procedure for the synthesis of N-(morpholine-4-carbonothioyl)benzamide derivatives (2a–d)

A stirred solution of acyl chloride **1** (10 mmol) and KSCN (12 mol) in dry CH_3CN (150 mL) was refluxed for 3 h. After the mixture was cooled to room temperature, a solution of the morpholine (12 mol) in CH_3CN (40 mL) was added drop wise (temperature maintained below



Scheme 1. Reagents and conditions: (i) KSCN, CH_3CN, reflux, 3 h; (ii) Morpholine, CH_3CN, rt, 1–3 h; (iii) Ethyl bromoacetate, TEA, EtOH, rt (iv) $LiAlH_4$, dry THF, 0 °C (v) MnO_2, DCM, 0 °C (vi) $Ph_3PCHCOOEt$, benzene, reflux (vii) EtOH: 10% NaOH (1:1), 50 °C (viii) EDCI, HOBt, TEA, DMF, rt.

Table 1
Structure of compounds **8a–s**.



8a-s

Compound	R_1	R_2	Isolated yield
8a	-H	-4-OMe	80%
8b	-H	-3,4-OMe	82%
8c	-H	-3,4,5-OMe	86%
8d	-H	-4-Br	42%
8e	-H	-2-NH_2	66%
8f	-OMe	-4-OMe	83%
8g	-OMe	-3,4-OMe	83%
8h	-OMe	-3,4,5-OMe	88%
8i	-OMe	-4-Br	44%
8j	-OMe	-2-NH_2	69%
8k	-NO_2	-4-OMe	78%
8l	-NO_2	-3,4-OMe	80%
8m	-NO_2	-4-Br	36%
8n	-NO_2	-2-NH_2	56%
8o	-Cl	-4-OMe	80%
8p	-Cl	-3,4-OMe	82%
8q	-Cl	-3,4,5-OMe	85%
8r	-Cl	-4-Br	38%
8s	-Cl	-2-NH_2	64%

Table 2
Inhibitory potency K_I (μM) data for compounds **8a–s** against hCA I, II, IX, XII.

Compounds	hCA I	hCAII	hCA IX	hCAXII
8a	> 100	> 100	> 100	> 100
8b	> 100	> 100	> 100	> 100
8c	> 100	> 100	> 100	> 100
8d	> 100	> 100	> 100	> 100
8e	> 100	> 100	> 100	> 100
8f	> 100	> 100	> 100	> 100
8g	> 100	> 100	> 100	> 100
8h	> 100	> 100	> 100	> 100
8i	> 100	> 100	> 100	> 100
8j	> 100	> 100	> 100	> 100
8k	> 100	39.2	> 100	4.6
8l	> 100	> 100	> 100	> 100
8m	> 100	9.3	54.7	8.8
8n	> 100	77.7	96.7	> 100
8o	> 100	50.4	94.9	7.5
8p	> 100	> 100	> 100	> 100
8q	> 100	> 100	> 100	> 100
8r	> 100	67.8	96.5	> 100
8s	> 100	> 100	> 100	> 100
AAZ	0.250	0.012	0.026	0.006

*Mean from 3 different assays, by a stopped flow technique (errors were in the range of ± 5–10% of the reported values), K_I = inhibitor constant in μM.

50 °C). The mixture was stirred for 30 min and poured into an ice/water mixture (500 mL) with stirring. The precipitate was collected and recrystalized from EtOH/H_2O to provide white solid product **2**.

*4.1.2. General procedure for the synthesis of thiazole esters (**3a–d**)*

To a stirred solution of compound **2** (10 mmol, 1 equiv.) in ethanol (100 mL), was added TEA (12 mmol, 1.2 equiv.) followed by addition of ethyl bromoacetate (12 mmol, 1.2 equiv.) at room temperature. The reaction mixture was stirred overnight and then poured into an ice/water mixture (500 mL) with stirring. The precipitate was collected and recrystallized from EtOH/H_2O to provide white solid of thiazole ester derivatives **3a–d**.

Table 3
Docking scores for the compounds **8a–s** against hCA I, II, IX, XII.

S. No	Compound	hCA II	hCA IX	hCA XII
1	**8a**	−4.322	−4.865	−4.394
2	**8b**	−3.794	−4.906	−4.86
2	**8c**	−3.762	−1.605	−4.219
4	**8d**	−3.217	−1.032	−2.549
5	**8e**	−4.492	−4.408	−4.43
6	**8f**	−4.028	−1.728	−3.384
7	**8g**	−4.384	−1.104	−3.832
8	**8h**	−3.277	−1.544	−3.76
9	**8i**	−3.82	−0.975	−2.916
10	**8j**	−4.251	−4.431	−4.515
11	**8k**	−4.045	−4.911	−3.762
12	**8l**	−3.591	−4.765	−3.233
13	**8m**	−3.945	−5.075	−4.513
14	**8n**	−5.359	−4.802	−4.013
15	**8o**	−4.187	−3.405	−3.403
16	**8p**	−4.709	−3.935	−3.733
17	**8q**	−2.859	−3.418	−3.658
18	**8r**	−2.062	−1.838	−3.502
19	**8s**	−5.174	−4.79	−4.482

*4.1.3. Synthesis of (2-morpholino-4-phenylthiazol-5-yl)methanol (**4a–d**)*

To a suspension of Lithium aluminum hydride (2 equiv.) in THF, a solution of compound **3a–d** (1 equiv.) in THF (13 mL) was added dropwise at 0 °C. The reaction mixture was slowly warmed up to rt and was stirred for 30 min. It was then cooled to 0 °C. Water, aq. NaOH (1 N), and water were added successively. The mixture was stirred at rt for 15 min, then filtered through Celite. The filtrate was extracted with DCM and the combined DCM layer was concentrated to provide crude compound (**4a–d**) which was used without further purification.

*4.1.4. Synthesis of 2-morpholino-4-phenylthiazole-5-carbaldehyde (**5a–d**)*

To a suspension of MnO_2 (30 equiv) in DCM maintained at 0 °C, a solution of compound **4a–d** in DCM (13 mL) was added dropwise through dropping funnel over period of 1 h. After completion of the reaction, the reaction mixture was filtered through Celite and celite. The filtrate was concentrated to provide crude compounds **5a–d** which were purified through column chromatography (30% EA:Hexane).

*4.1.5. Synthesis of ethyl (E)-3-(2-morpholino-4-phenylthiazol-5-yl)acrylate (**6a–d**)*

To a solution of compound **5a–d** (1 equiv.) in benzene (30 mL) was added ethyl (triphenylphosphoranylidene)acetate (2 equiv.) at room temperature. The reaction mixture was then refluxed overnight and then concentrated. The residue was purified by column chromatography to give compounds **6a–d**.

*4.1.6. Synthesis of (E)-3-(2-morpholino-4-phenylthiazol-5-yl)acrylic acid (**7a–d**)*

A solution of compound **6a–d** (1 equiv.) in 10% aq. NaOH:EtOH (5 mL) was heated at 50 °C until the complete consumption of starting material. Water (20 mL) was added and the reaction mixture was acidified using 1 N HCl to provide compound **7a–d** as solid. The solid was collected after filtration, air dried and used without further purification.

*4.1.7. Synthesis of (E)-3-(2-morpholino-4-phenylthiazol-5-yl)-N-phenylacrylamide (**8a–s**)*

To a solution of **7a–d** (1 equiv.) and aniline (1.2 equiv.) in DMF (5 mL) was added EDCI (1.2 equiv.) and HOBt (0.1 equiv.). The mixture was stirred at room temperature until complete consumption of starting materials. The reaction mixture was then added to H_2O and extracted

Table 4
Docking interactions with important amino acids for the compounds **8a–s** against hCA I, II, IX, XII.

Compound	Substitution		H bond interaction		
	R1	R2	hCA II	hCA IX	hCA XII
8a	H	OMe	TRP 5, π-π HIS94	GLN 92	π-π HIE 66, TRP 4
8b	H	Di-OMe	ASN 62	x	π-π HIE 66, TRP 4
8c	H	Tri-OMe	ASN 62	x	ASN 64
8d	H	Br	π-π TRP 5	HIE 64, GLN 92, π-π HIS94	π-π LYS 69
8e	H	NH_2	THR 199, THR 200, π-π HIS94	VAL 19	ASN 71, LYS 69, π-cat LYS 69
8f	OMe	OMe	GLN 94, π-π HIS94	x	x
8g	OMe	Di-OMe	GLN 94, π-π HIS94	GLN 94, π-π HIS94	LYS 69
8h	OMe	Tri-OMe	x	x	LYS 69
8i	OMe	Br	x	x	ASN 64, π-π HIS91
8j	OMe	NH_2	GLU 69, π-π HIS94	THR 199	ASN 64, HIE 66, TRP 4, π-π HIE 66
8k	NO_2	OMe	π-π HIS64, HIS 94	THR 199, GLN 92, π-π HIS64, HIS 94, Salt bridge Zn	TRP 4
8l	NO_2	Di-OMe	TRP 5	x	x
8m	NO_2	Br	TRP 5	GLN 92, THR 199, TRP 5, Salt brdg Zn	LYS 69, TRP 4
8n	NO_2	NH_2	ASN 67, GLN 92, π-π HIS94, Salt brdg Zn	GLN 92, TRP 5	TRP 4, π-cat LYS 69
8o	Cl	OMe	GLN 92, π-π HIS94	x	ASN 64
8p	Cl	Di-OMe	TRP 5, π-π HIS94, PHE 131	GLN 92, π-π HIS94	x
8q	Cl	Tri-OMe	x	π-π TRP 5	LYS 69, π-cat LYS 69
8r	Cl	Br	x	HIE 64, GLN 92, π-π HIS64, HIS94, Salt brdg. Zn	x
8s	Cl	NH_2	HIS 64, π-π HIS94	THR 199, THR 200, π-π HIE 64, TRP 5	THR 88, π-cat LYS 69

X = no interaction.

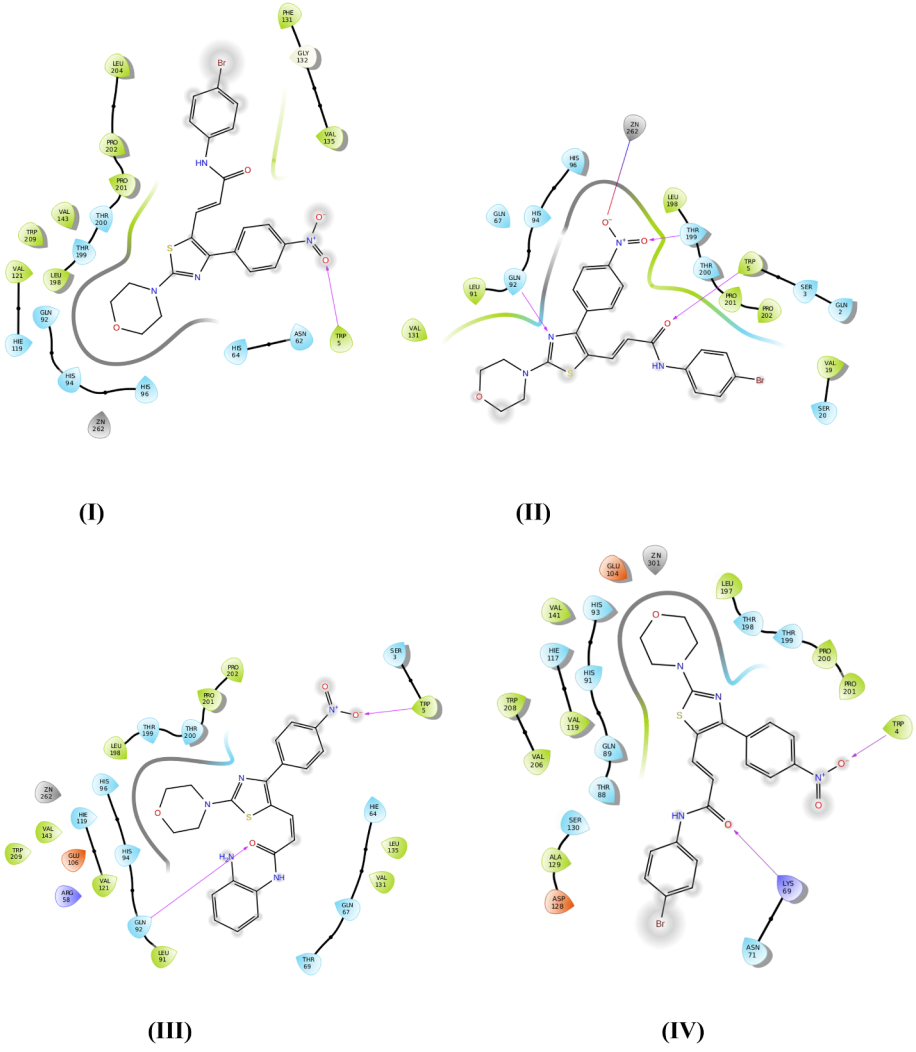


Fig. 5. (I) Docking pose of compound **8m** with hCA II (II) docking pose of compound **8m** with hCA IX (III) docking pose of compound **8n** with hCA IX (IV) docking pose of compound **8m** with hCA XII. The pink arrows in the figure indicate hydrogen bonding interactions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with CH_2Cl_2 (3×). The combined organic phases were washed with H_2O, dried over $NaSO_4$, and concentrated in vacuo. The crude products were purified using silica gel chromatography in EA:Hexane gradients.

*4.1.8. Characterization of compounds **8a–s***

*4.1.8.1. (E)-N-(4-Methoxyphenyl)-3-(2-morpholino-4-phenylthiazol-5-yl) acrylamide (**8a**)*. Light yellow solid, yield: 80%, mp: 302–304 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.95 (s, 1H), 7.65–7.44 (m, 8H), 6.90 (t, J = 14.5 Hz, 2H), 6.20 (d, J = 14.9 Hz, 1H), 3.78–3.73 (m, 4H), 3.72 (s, 3H), 3.58–3.51 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.86, 163.53, 155.66, 155.26, 134.80, 133.07, 131.15, 129.45, 129.21, 129.02, 120.92, 119.86, 119.06, 114.41, 65.83, 55.64, 48.39. HRMS (ESI) *m/z*: $[M+H]^+$ calculated for $C_{23}H_{24}N_3O_3S^+$ 422.1533, found 422.1534.

*4.1.8.2. (E)-N-(3,4-Dimethoxyphenyl)-3-(2-morpholino-4-phenylthiazol-5-yl)acrylamide (**8b**)*. Light yellow solid, yield: 82%, mp: 285–287 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.96 (s, 1H), 7.64–7.45 (m, 7H), 7.43 (d, J = 2.2 Hz, 1H), 7.10 (dd, J = 8.7, 2.3 Hz, 1H), 6.89 (d, J = 8.8 Hz, 1H), 6.20 (d, J = 14.9 Hz, 1H), 3.77–3.74 (m, 4H), 3.72 (d, J = 4.0 Hz, 6H), 3.57–3.53 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.89, 163.58, 155.34, 149.03, 145.26, 134.75, 133.55, 131.21, 129.44, 129.23, 129.04, 119.75, 119.01, 112.66, 111.30, 104.58, 65.83, 56.21, 55.78, 48.38. HRMS (ESI) *m/z*: $[M+Na]^+$ calculated for $C_{24}H_{25}N_3NaO_4S$ 474.1664, found 474.1669.

*4.1.8.3. (E)-3-(2-Morpholino-4-phenylthiazol-5-yl)-N-(3,4,5-trimethoxyphenyl)acrylamide (**8c**)*. Light yellow solid, yield: 86%, mp: 297–299 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.03 (s, 1H), 7.64 (d, J = 14.9 Hz, 1H), 7.59 (d, J = 1.5 Hz, 1H), 7.57 (s, 1H), 7.55–7.45 (m, 3H), 7.04 (s, 2H), 6.19 (d, J = 14.9 Hz, 1H), 3.78–3.72 (m, 10*H*), 3.62 (s, 3H), 3.58–3.53 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.95, 163.87, 155.58, 153.22, 135.99, 134.74, 133.94, 131.59, 129.44, 129.24, 129.03, 119.49, 118.95, 97.29, 65.83, 60.58, 56.17, 48.41. HRMS (ESI) *m/z*: $[M+H]^+$ calculated for $C_{25}H_{28}N_3O_5S^+$ 482.1744, found 482.1758.

*4.1.8.4. (E)-N-(4-Bromophenyl)-3-(2-morpholino-4-phenylthiazol-5-yl) acrylamide (**8d**)*. Light Yellow solid, yield 42%, mp: 299–301 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.20 (s, 1H), 7.65–7.61 (m, 3H), 7.59–7.56 (m, 2H), 7.54–7.47 (m, 5H), 6.20 (d, J = 14.9 Hz, 1H), 3.77–3.73 (m, 4H), 3.58–3.54 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.04, 164.12, 155.91, 139.22, 134.69, 132.05, 130.26, 129.48, 129.34, 129.06, 128.81, 121.36, 119.06, 118.84, 115.13, 65.82, 48.38. HRMS (ESI) *m/z*: $[M+H]^+$ calculated for $C_{22}H_{21}BrN_3O_2S^+$ 470.0532, found 472.0512.

*4.1.8.5. (E)-N-(2-Aminophenyl)-3-(2-morpholino-4-phenylthiazol-5-yl) acrylamide (**8e**)*. Light yellow solid, yield: 66%, mp: 280–282 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.26 (s, 1H), 7.59–7.56 (m, 3H), 7.52 (dd, J = 10.0, 4.7 Hz, 2H), 7.47 (dt, J = 5.2, 2.0 Hz, 1H), 7.32 (d, J = 7.7 Hz, 1H), 6.89 (t, J = 7.4 Hz, 1H), 6.73 (dd, J = 7.9, 1.1 Hz, 1H), 6.57–6.53 (m, 1H), 6.31 (d, J = 15.0 Hz, 1H), 4.91 (s, 2H), 3.77–3.74 (m, 4H), 3.57–3.53 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.87, 163.96, 155.21, 141.87, 134.82, 131.12, 129.44, 129.19, 129.02, 126.03, 124.89, 124.12, 119.84, 119.08, 116.71, 116.43, 65.83, 48.39. HRMS (ESI) *m/z*: $[M+Na]^+$ calculated for $C_{22}H_{22}N_4NaO_2S$ 429.1361, found 429.1361.

*4.1.8.6. (E)-N-(4-Methoxyphenyl)-3-(4-(4-methoxyphenyl)-2-morpholinothiazol-5-yl) acrylamide (**8f**)*. Light yellow solid, yield: 83%, mp: 295–297 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.92 (s, 1H), 7.66–7.46 (m, 5H), 7.10–7.05 (m, 2H), 6.92–6.85 (m, 2H), 6.17 (d, J = 14.9 Hz, 1H), 3.83 (d, J = 5.3 Hz, 3H), 3.77–3.73 (m, 4H), 3.72 (s, 3H), 3.55 (dd, J = 14.6, 9.6 Hz, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.72 (s), 163.64 (s), 160.18 (s), 155.62 (s), 155.20 (s), 131.45 (s), 130.81 (s), 120.89 (s), 119.30 (s), 118.00 (s), 114.45 (d, J = 10.0 Hz), 65.84 (s), 55.71 (d, J = 14.7 Hz), 48.38 (s). HRMS (ESI) *m/z*: $[M+H]^+$ calculated for $C_{24}H_{26}N_3O_4S^+$ 452.1639, found 452.1652.

*4.1.8.7. (E)-N-(3,4-Dimethoxyphenyl)-3-(4-(4-methoxyphenyl)-2-morpholinothiazol-5-yl) acrylamide (**8g**)*. Light yellow solid, yield: 83%, mp: 302–303 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.94 (s, 1H), 7.62 (d, J = 14.9 Hz, 1H), 7.52 (d, J = 8.6 Hz, 2H), 7.44 (s, 1H), 7.09 (dd, J = 12.9, 9.0 Hz, 3H), 6.89 (d, J = 8.7 Hz, 1H), 6.16 (d, J = 14.9 Hz, 1H), 3.83 (s, 3H), 3.77–3.71 (m, 10*H*), 3.58–3.51 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.73, 163.72, 160.18, 155.26, 149.11, 133.66, 131.53, 130.80, 127.26, 119.23, 117.99, 114.49, 112.81, 65.84, 56.29, 55.84, 55.76, 48.39. HRMS (ESI) *m/z*: $[M+H]^+$ calculated for $C_{25}H_{28}N_3O_5S^+$ 482.1744, found 482.1753.

*4.1.8.8. (E)-3-(4-(4-Methoxyphenyl)-2-morpholinothiazol-5-yl)-N-(3,4,5 trimethoxyphenyl) acrylamide (**8h**)*. Light yellow solid, yield: 88%, mp: 327–329 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.02 (s, 1H), 7.64 (d, J = 14.9 Hz, 1H), 7.51 (t, J = 12.3 Hz, 2H), 7.12–7.02 (m, 4H), 6.15 (d, J = 14.9 Hz, 1H), 3.83 (d, J = 5.6 Hz, 3H), 3.76 (d, J = 20.0 Hz, 10*H*), 3.63 (d, J = 7.2 Hz, 3H), 3.54 (dd, J = 12.2, 7.6 Hz, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.81, 163.99, 160.21, 155.53, 153.22, 136.05, 131.91, 130.81, 127.20, 118.92, 117.90, 114.50, 97.28, 65.84, 60.58, 56.17, 55.77, 48.39. HRMS (ESI) *m/z*: $[M+H]^+$ calculated for $C_{26}H_{30}N_3O_6S^+$ 512.1850, found 512.1896.

*4.1.8.9. (E)-N-(4-Bromophenyl)-3-(4-(4-methoxyphenyl)-2-morpholinothiazol-5-yl) acrylamide (**8i**)*. Light yellow solid, yield: 44%, mp: 341–343 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.18 (s, 1H), 7.63 (dd, J = 11.7, 10.3 Hz, 3H), 7.49 (dt, J = 27.1, 13.5 Hz, 4H), 7.06 (t, J = 11.9 Hz, 2H), 6.16 (d, J = 14.9 Hz, 1H), 3.83 (d, J = 5.3 Hz, 3H), 3.79–3.71 (m, 4H), 3.60–3.50 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.88, 164.23, 160.26, 155.84, 139.29, 132.40, 132.02, 130.85, 127.18, 121.37, 118.52, 117.83, 115.06, 114.51, 65.83, 55.77, 48.38. HRMS (ESI) *m/z*: $[M+H]^+$ calculated for $C_{23}H_{23}BrN_3O_3S^+$ 500.0638, found 500.0624.

*4.1.8.10. (E)-N-(2-Aminophenyl)-3-(4-(4-methoxyphenyl)-2-morpholinothiazol-5-yl) acrylamide (**8j**)*. Light yellow solid, yield: 69%, mp: 292–294 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.24 (s, 1H), 7.60 (d, J = 14.9 Hz, 1H), 7.50 (dd, J = 20.1, 7.7 Hz, 2H), 7.32 (d, J = 7.8 Hz, 1H), 7.07 (d, J = 8.8 Hz, 2H), 6.89 (t, J = 7.4 Hz, 1H), 6.73 (d, J = 7.9 Hz, 1H), 6.56 (t, J = 7.5 Hz, 1H), 6.27 (d, J = 15.1 Hz, 1H), 4.93 (s, 2H), 3.83 (s, 3H), 3.75 (s, 4H), 3.53 (t, J = 8.2 Hz, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.73, 160.15, 155.19, 130.80, 127.25, 124.86, 117.99, 116.50, 114.48, 65.83, 55.75, 48.34. HRMS (ESI) *m/z*: $[M+H]^+$ calculated for $C_{23}H_{25}N_4O_3S^+$ 437.1642, found 437.1662.

*4.1.8.11. (E)-N-(4-Methoxyphenyl)-3-(2-morpholino-4-(4-nitrophenyl) thiazol-5-yl) acrylamide (**8k**)*. Orange solid, yield: 78%, mp: 305–307 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.02 (s, 1H), 8.31 (dd, J = 61.1, 8.5 Hz, 2H), 7.86 (d, J = 8.6 Hz, 2H), 7.71–7.45 (m, 3H), 6.89 (d, J = 8.8 Hz, 2H), 6.30 (d, J = 14.9 Hz, 1H), 3.75 (d, J = 4.4 Hz, 4H), 3.71 (d, J = 19.7 Hz, 3H), 3.55 (t, J = 14.5 Hz, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.93, 163.21, 155.75, 152.24, 147.66, 140.90, 132.96, 130.52, 130.07, 124.32, 121.62, 121.39, 120.96, 114.45, 65.81, 55.66, 48.42. HRMS (ESI) *m/z*: $[M+H]^+$ calculated for $C_{23}H_{23}N_4O_5S^+$ 467.1384, found 467.1380.

*4.1.8.12. (E)-N-(3,4-Dimethoxyphenyl)-3-(2-morpholino-4-(4-nitrophenyl)thiazol-5-yl) acrylamide (**8l**)*. Yellow solid, yield: 80%, mp: 334–336 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.03 (s, 1H), 8.39 (t, J = 14.4 Hz, 2H), 7.86 (d, J = 8.8 Hz, 2H), 7.61 (d, J = 14.9 Hz, 1H), 7.43 (d, J = 2.2 Hz, 1H), 7.11 (dd, J = 8.7, 2.2 Hz, 1H), 6.90 (d, J = 8.8 Hz, 1H), 6.29 (d, J = 14.9 Hz, 1H), 3.78–3.74 (m, 4H), 3.73 (s, 3H), 3.72 (s, 3H), 3.61–3.54 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6)

δ 168.95, 163.27, 152.31, 149.10, 147.66, 145.42, 140.88, 133.46, 130.52, 130.15, 124.33, 121.53, 121.36, 112.77, 111.42, 104.71, 65.81, 56.27, 55.83, 48.43. HRMS (ESI) *m/z*: $[M+H]^+$ calculated for $C_{24}H_{25}N_4O_6S^+$ 497.1489, found 497.1480.

*4.1.8.13. (E)-N-(4-Bromophenyl)-3-(2-morpholino-4-(4-nitrophenyl) thiazol-5-yl) acrylamide (**8m**)*. Brick red solid, yield: 36%, mp: 355–357 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.27 (s, 1H), 8.39–8.34 (m, 2H), 7.88–7.84 (m, 2H), 7.65–7.60 (m, 3H), 7.51–7.48 (m, 2H), 6.29 (d, *J* = 14.9 Hz, 1H), 3.79–3.73 (m, 4H), 3.61–3.53 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.09, 163.82, 152.83, 147.74, 140.81, 139.11, 132.08, 130.99, 130.57, 124.34, 121.43, 121.14, 120.85, 115.30, 65.80, 48.44. HRMS (ESI) *m/z*: $[M+H]^+$ calculated for $C_{24}H_{25}N_4O_6S^+$ 497.1489, found 497.1480.

*4.1.8.14. (E)-N-(2-Aminophenyl)-3-(2-morpholino-4-(4-nitrophenyl) thiazol-5-yl)acrylamide (**8n**)*. Brick red solid, yield: 56%, mp: 302–304 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.34 (s, 1H), 8.42–8.33 (m, 2H), 7.86 (d, *J* = 8.8 Hz, 2H), 7.60 (d, *J* = 15.0 Hz, 1H), 7.33 (d, *J* = 7.8 Hz, 1H), 6.90 (t, *J* = 7.4 Hz, 1H), 6.74 (d, *J* = 7.0 Hz, 1H), 6.57 (t, *J* = 7.5 Hz, 1H), 6.42 (d, *J* = 14.9 Hz, 1H), 5.00 (s, 2H), 3.80–3.72 (m, 4H), 3.61–3.53 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.95, 163.63, 152.21, 147.67, 141.77, 140.93, 130.52, 130.03, 126.15, 124.92, 124.32, 121.61, 121.41, 116.83, 116.52, 65.81, 48.42. HRMS (ESI) *m/z*: $[M+H]^+$ calculated for $C_{22}H_{22}N_5O_4S^+$ 452.1387, found 452.1391.

*4.1.8.15. (E)-3-(4-(4-Chlorophenyl)-2-morpholinothiazol-5-yl)-N-(4-methoxyphenyl) acrylamide (**8o**)*. Light yellow solid, yield: 80%, mp: 255–257 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.96 (s, 1H), 7.62–7.54 (m, 7H), 6.89 (d, *J* = 9.0 Hz, 2H), 6.22 (d, *J* = 14.9 Hz, 1H), 3.77–3.71 (m, 7H), 3.58–3.52 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.62, 168.17, 160.44, 158.39, 138.69, 138.32, 137.78, 135.97, 135.78, 133.97, 133.79, 125.77, 125.60, 125.14, 124.21, 119.26, 119.11, 70.57, 60.40, 53.14. HRMS (ESI) *m/z*: $[M+H]^+$ calculated for $C_{23}H_{23}ClN_3O_3S^+$ 456.1143, found 456.1149.

*4.1.8.16. (E)-3-(4-(4-Chlorophenyl)-2-morpholinothiazol-5-yl)-N-(3,4-dimethoxyphenyl) acrylamide (**8p**)*. Light yellow solid, yield: 82%, mp: 279–281 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.98 (s, 1H), 7.63–7.55 (m, 5H), 7.43 (d, *J* = 2.2 Hz, 1H), 7.11 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.89 (d, *J* = 8.8 Hz, 1H), 6.22 (d, *J* = 14.9 Hz, 1H), 3.76–3.73 (m, 4H), 3.73 (d, *J* = 4.4 Hz, 6H), 3.57–3.52 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.89, 163.48, 153.71, 149.10, 145.35, 133.94, 133.55, 131.12, 130.74, 129.13, 120.31, 119.44, 112.79, 111.40, 104.73, 65.82, 56.28, 55.83, 48.40. HRMS (ESI) *m/z*: $[M+H]^+$ calculated for $C_{24}H_{25}ClN_3O_4S^+$ 486.1249, found 486.1251.

*4.1.8.17. (E)-3-(4-(4-Chlorophenyl)-2-morpholinothiazol-5-yl)-N-(3,4,5-trimethoxyphenyl) acrylamide (**8q**)*. Light yellow solid, yield: 85%, mp: 285–287 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.05 (s, 1H), 7.64–7.55 (m, 5H), 7.05 (s, 2H), 6.21 (d, *J* = 14.9 Hz, 1H), 3.77–3.71 (m, 10*H*), 3.63 (d, *J* = 6.9 Hz, 3H), 3.58–3.53 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.96, 163.76, 153.96, 153.23, 135.95, 133.98, 133.51, 131.12, 129.14, 120.01, 119.35, 97.33, 65.82, 60.58, 56.18, 48.42. HRMS (ESI) *m/z*: $[M+H]^+$ calculated for $C_{25}H_{27}ClN_3O_5S^+$ 516.1354, found 516.1342.

*4.1.8.18. (E)-N-(4-Bromophenyl)-3-(4-(4-chlorophenyl)-2-morpholinothiazol-5-yl) acrylamide (**8r**)*. Light yellow solid, yield: 38%, mp: 273–275 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.23 (s, 1H), 7.67–7.52 (m, 7H), 7.49 (d, *J* = 8.9 Hz, 2H), 6.22 (d, *J* = 14.9 Hz, 1H), 3.80–3.70 (m, 4H), 3.62–3.49 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.03, 164.02, 154.24, 139.20, 134.04, 133.49, 132.04, 131.59, 131.15, 129.15, 121.41, 119.64, 119.27, 115.18, 65.81, 48.41. HRMS (ESI) *m/z*: $[M+H]^+$ calculated for $C_{22}H_{20}BrClN_3O_2S^+$ 504.0143, found 506.0118.

*4.1.8.19. (E)-N-(2-Aminophenyl)-3-(4-(4-chlorophenyl)-2-morpholinothiazol-5-yl) acrylamide (**8s**)*. Light yellow solid, yield: 64%, mp: 291–293 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.28 (s, 1H), 7.57 (d, *J* = 18.3 Hz, 5H), 7.32 (d, *J* = 7.0 Hz, 1H), 6.90 (s, 1H), 6.73 (d, *J* = 7.6 Hz, 1H), 6.56 (t, *J* = 7.2 Hz, 1H), 6.33 (d, *J* = 14.8 Hz, 1H), 4.92 (s, 2H), 3.75 (s, 4H), 3.55 (s, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.88, 163.81, 153.59, 141.88, 133.92, 133.59, 131.12, 130.64, 129.13, 126.07, 124.90, 124.10, 120.36, 119.48, 116.73, 116.45, 65.82, 48.39. HRMS (ESI) *m/z*: $[M+H]^+$ calculated for $C_{22}H_{22}ClN_4O_2S^+$ 441.1147, found 441.1141.

4.2. Carbonic anhydrase inhibition assay

An SX.18MV-R Applied Photophysics (Oxford, UK) stopped-flow instrument has been used to assay the catalytic/inhibition of various CA isozymes.[21] Phenol Red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 10 mM Hepes (pH 7.4) as buffer, 0.1 M Na_2SO_4 or $NaClO_4$ (for maintaining constant the ionic strength; these anions are not inhibitory in the used concentration), following the CA-catalyzed CO_2 hydration reaction for a period of 5–10 s. Saturated CO_2 solutions in water at 25 °C were used as substrate. Stock solutions of inhibitors were prepared at a concentration of 10 μM (in DMSO-water 1:1, v/v) and dilutions up to 0.01 nM done with the assay buffer mentioned above. At least 7 different inhibitor concentrations have been used for measuring the inhibition constant. Inhibitor and enzyme solutions were preincubated together for 10 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. Triplicate experiments were done for each inhibitor concentration, and the values reported throughout the paper are the mean of such results. The inhibition constants were obtained by non-linear least-squares methods using the Cheng-Prusoff equation, as reported earlier,[22] and represent the mean from at least three different determinations. All CA isozymes used here were recombinant proteins obtained as reported earlier by our group.[23,24]

4.3. Molecular docking studies

The protein preparation wizard was used to prepare of hCA II, IX and XII downloaded from PDB (hCAII: 1BNM; hCA IX: 3IAI; hCA XII: 4Q0L),[25–27] ready for docking i.e. removing waters, adding missing side chains and energy minimization by OPLS-2005 force field.[28] The synthesized compound were sketched and converted to 3D using Ligprep.[29] The Glide XP docking algorithm was employed using a grid box volume of 10x 10x 10 Å at the centre of co-crystal as standard for all isoform docking.[30]

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmc.2019.115090.

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