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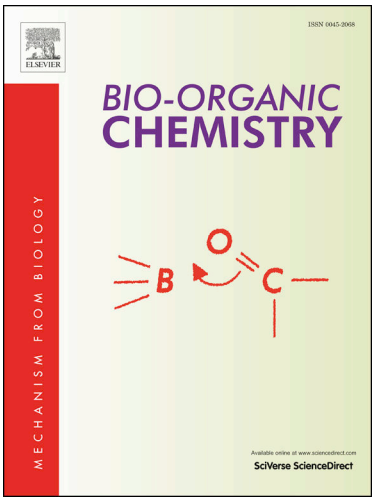

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Synthesis and carbonic anhydrase inhibition studies of sulfonamide based indole-1,2,3-triazole chalcone hybrids

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ABSTRACT. Sulfonamide is one of the most promising classes of classical carbonic anhydrase (CA, EC 4.2.1.1) inhibitors. A novel series of indolylchalcones incorporating benzenesulfonamide-1,2,3-triazole (**6a-q**) has been synthesized by click chemistry reaction and investigated for hCA inhibitory activity against a panel of human carbonic anhydrases (hCAs). Most of these newly synthesized compounds exhibited interesting inhibition constants, in the nanomolar range, with some derivatives being more potent than the standard drug acetazolamide (**AAZ**) on hCA I isoform. Among the tested compounds, the compounds **6d** (18.8 nM), **6q** (38.3 nM) and **6e** (50.4 nM) were 13, 6 and 5 times more potent than AAZ against hCA I isoform, respectively. Compounds **6o**, **6m** and **6f** efficiently inhibited isoform hCA XII, with K_Is in the range of 10 – 41.9 nM. Several compounds were also active against isoforms hCA II and hCA IX, with K_Is under 100 nM. These indolylchalcone-benzenesulfonamide-1,2,3-triazole hybrids may be considered as potential leads for hCA I-selective inhibitors.

Keywords: Carbonic anhydrase, Knoevenagel condensation, Acetazolamide, indolylchalcone-benzenesulfonamide-1,2,3-triazole hybrids, selective hCA I inhibition.

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1. Introduction

A massive research activity has been reported in the field of carbonic anhydrase inhibition. Carbonic anhydrases (CAs; also known as carbonate dehydratases EC 4.2.1.1), are metalloenzymes, as their active site contains a metal ion. The main function of this enzyme is to catalyze the reversible inter-conversion of carbon dioxide and water to bicarbonate and protons as shown by the following equation 1 [1].

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+ \qquad \text{(Eq. 1)}$$

Most of the CA isozymes are involved in important physiological functions such as respiration, pH regulation, ion transport, bone resorption and secretion of gastric juice and other fluids (e.g., aqueous humor in the eye, cerebrospinal fluid, etc.) [1]. Therefore, their inhibitors constitute an important class of therapeutic agents to treat a range of disorders including edema, glaucoma, obesity, cancer, epilepsy and osteoporosis [2].

There are several classes of CA inhibitors (CAIs): such as i) metal ion binders which include the sulfonamides and their isosteres sulfamates/sulfamides, dithiocarbamates, mercaptans and hydroxamates; ii) compounds anchoring to the zinc-coordinated water molecule/hydroxide ion like phenols, carboxylates, polyamines, esters and iii) coumarins and related compounds such as sulfocoumarins, which bind even further away from the metal ion [3].

The primary sulfonamide group plays an important role in CA inhibition mechanism, with more than 20 compounds in clinical use, with: “acetazolamide (AAZ)” **A**, methazolamide **B**, ethoxzolamide **C**, celecoxib **D** [4], dichlorophenamide **E**, dorzolamide **F**, brinzolamide (**Fig 1**) to cite a few. Furthermore, compounds such as sulpiride, zonisamide, topiramate etc., are in clinical use as anti-epileptics or antidepressant agents [1a]. They function by binding to the zinc metal ion, in their active deprotonated form, as sulfonamidate [5].

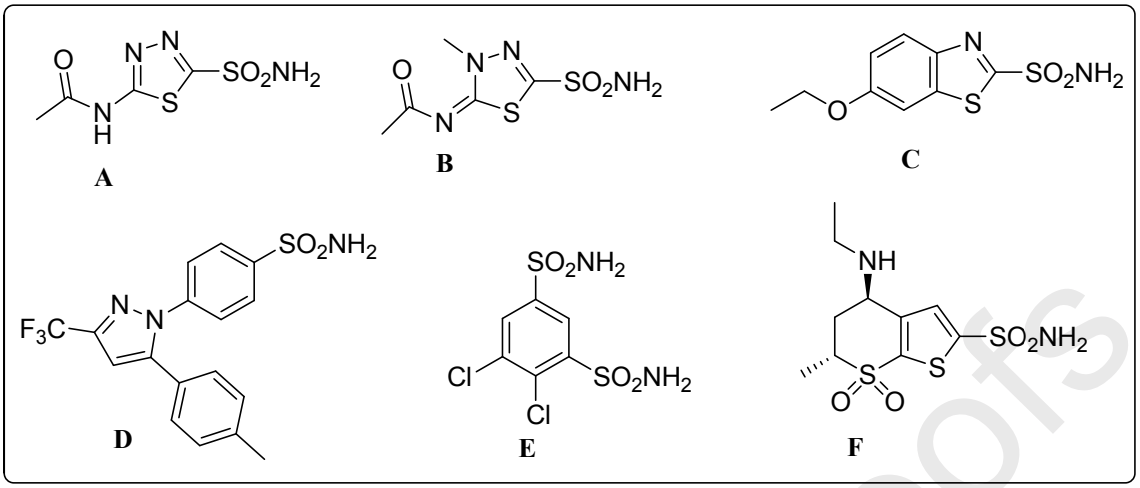


Fig-1: Structure of clinically used sulfonamide containing drugs

There are eight CA families identified up until now among various living species like bacteria, archaea and eukarya. The α-CAs are expressed primarily in vertebrates, plants, and algae; β in bacteria, algae, and fungi; γ in archaea and bacteria; two minor forms, δ and ζ, are expressed in marine diatoms; and η in protozoa and the θ- and ι-classes are found in marine diatoms such as *Phaeodactylum tricornutum*, and related species [6]. In humans, 15 CA isoforms are found which differ not only in tissue localization but also in enzymatic efficiency: CA I, CA II, CA III, CA VII, CA XIII are cytosolic; CA IV, CA IX, CA XII, CA XIV, membrane-bound; CA Va and CA Vb mitochondrial and CA VI secreted primarily in saliva.

Indisulam (E7070), an anticancer agent incorporating a chloro-indolyl sulfonamide (**Fig 2**) was in phase II clinical studies till recently, and it inhibits several CA isoform, including the tumor-associated one, CA IX (CAIX) [7,8]. The ureido benzenesulfonamide compound SLC-0111 (**Fig 2**), is currently in Phase I/II clinical trials for the treatment of advanced metastatic solid tumors [9,10]. The 1,2,3-triazole scaffold has received considerable attention due to its diverse biological activities such as antimicrobial, anti-allergic, analgesic, anti-HIV, anti-inflammatory, anticancer, anti-malarial and anti-tuberculosis [11]. In addition, many research groups have also explored carbonic anhydrase inhibitory activity of 1,2,3-triazole [12,13,14]. Chalcone has also found to be active against hCAs [15,16]. Hence, in the present study we decided to incorporate these 4 scaffolds i.e., indole, chalcone, triazole and sulfonamide, to synthesize our target molecules.

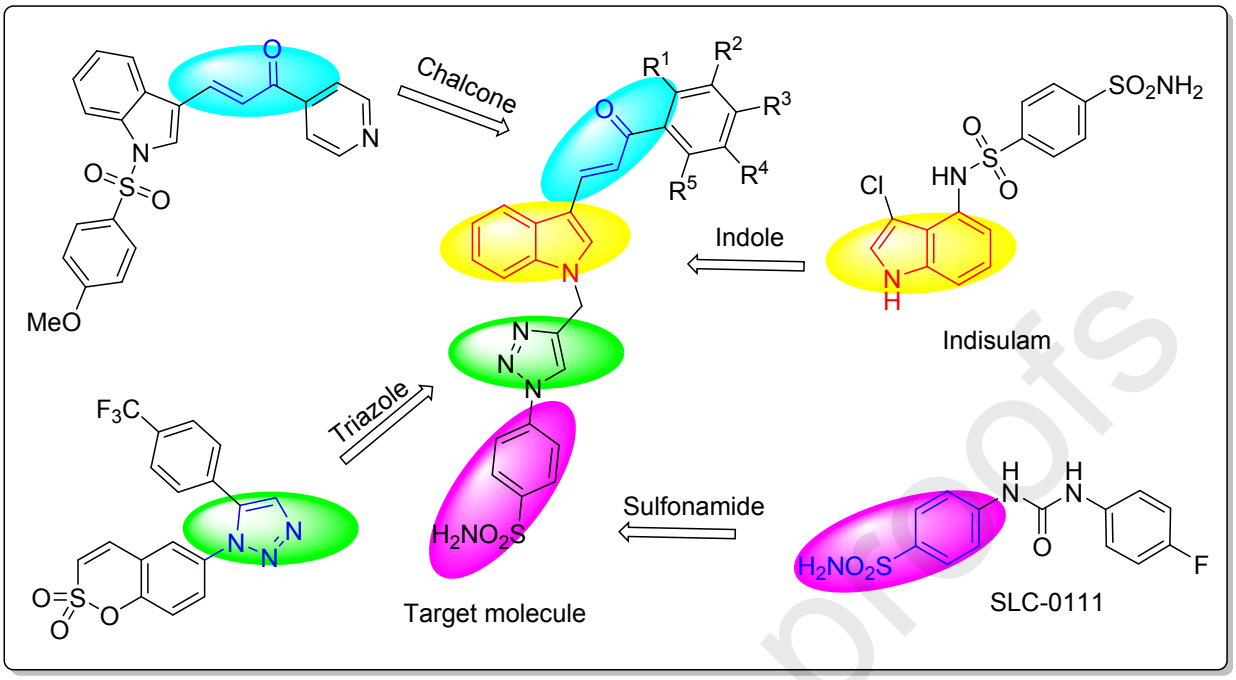
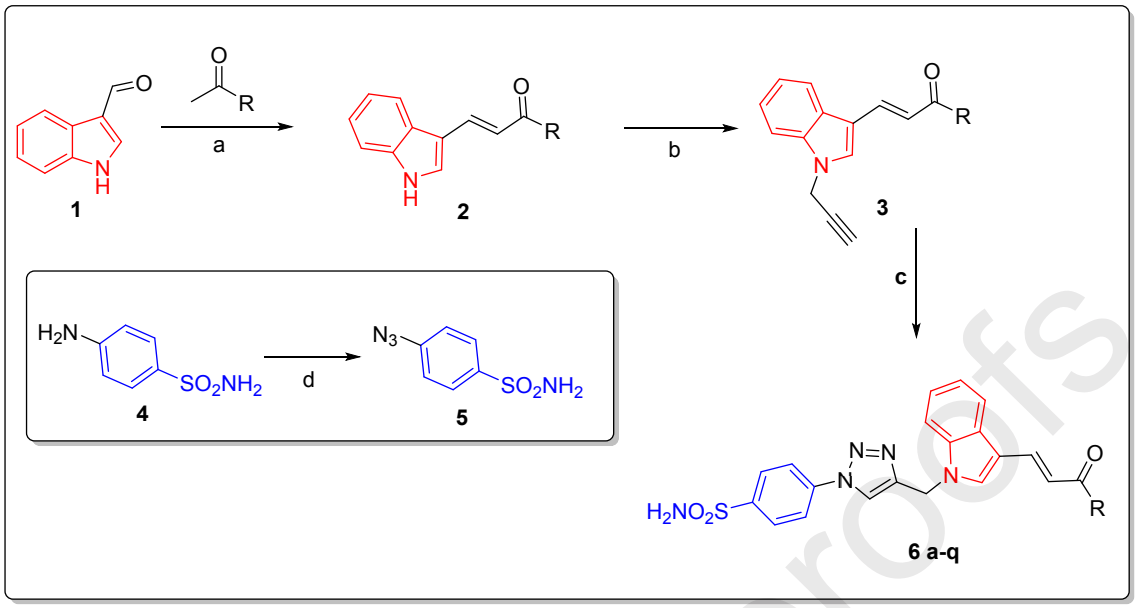


Fig-2: Rationale for designing the target molecules

2. Result and discussion

2.1. Chemistry

The synthetic strategy employed for the synthesis of the target compounds is depicted in Scheme **1**. The chalcones (**2a-q**) were synthesized by Knoevenagel condensation by reacting indole-3-carboxaldehyde with different acetophenones using piperidine as the base and ethanol as solvent [17]. In the next step the obtained chalcones (**2a-q**) were propargylated using propargyl bromide and anhydrous K_2CO_3 in dry DMF to get intermediates (**3a-q**) [18]. On the other side the benzenesulfonamide azide (**5**) was prepared from sulfanilamide through diazotization reaction using conc. HCl, $NaNO_2$ and NaN_3, which was finally subjected to click reaction with substituted intermediates (**3a-q**) in the presence of $CuSO_4$ and sodium ascorbate in ^{t}BuOH and H_2O (1:1) as solvent to afford the final target products (**6a-q**) by using reported literature method [19]. The structures of all the compounds (**6a-q**) were elucidated on the basis of ^{1}H NMR, ^{13}C NMR, and HRMS.

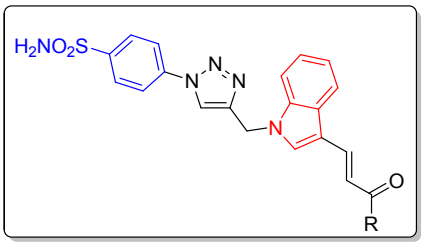


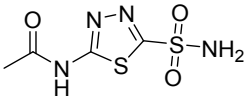
Scheme 1: General synthetic scheme for the synthesis of benzenesulfonamide triazole linked to indolylchalcone. Reagents and conditions: a) Piperidine (0.5 equiv), EtOH, reflux, 24 h; b) (i) K_2CO_3 (3 equiv), DMF, 70^0C, 3-4 h; (ii) Propargyl bromide (1 mmol); (c) **5**, $CuSO_4$ (5 mol%), Sodium ascorbate (10 mol%), ^{t}BuOH:H_2O (1:1); d) (i) HCl, $NaNO_2$ (1 equiv), 0^0C, 30 min; (ii) Sodium azide (1.6 equiv), 0^0C, 4 h.

2.2. Carbonic anhydrase inhibition

The 17 newly synthesized compounds (**6a-q**) have been tested for the inhibition against the four physiologically and pharmacologically significant isoforms, the cytosolic isoforms, hCA I and hCA II and the trans-membrane tumor associated isoforms, hCA IX and hCA XII, by means of stopped flow carbon dioxide assay [20]. Here acetazolamide (AAZ) was taken as the standard drug. The screening result of the synthesized compounds against different hCA isoforms can be drawn, from the results shown in **Table 1**:-

Table 1. K_i value of compounds (**6a- q)** obtained from stopped flow carbon dioxide assay against hCA isoforms I, II, IX and XII.



		K_I (nM)*			
Compound	**R**	**hCA I**	**hCA II**	**hCAIX**	**hCAXII**
6a	Phenyl	**89.0±7**	468.4±24	69.3±4	522.4±27
6b	4-Chlorophenyl	596.3±32	861.2±49	73.3±5	554.0±38
6c	4-Fluorophenyl	501.5±24	341.2±17	94.5±3	73.8±4
6d	4-Bromophenyl	**18.8**±1.1	1721±124	375.1±21	283.9±21
6e	4-Methylphenyl	**50.4**±4	36.2±3	615.1±34	54.5±3
6f	3-Bromophenyl	5500±214	88.1±7	240.9±16	41.9±1
6g	4-Methoxyphenyl	771.7±25	416.5±22	1397±121	81.1±4
6h	4-Methyl-3-nitrophenyl	548.5±30	88.5±6	187.0±9	450.7±21
6i	2,4-Dichlorophenyl	241.4±15	881.9±62	763.6±31	257.8±17
6j	3,4-Dimethoxyphenyl	**54.7**±4	84.7±5	490.3±41	50.3±3
6k	3-(Trifluoromethyl)phenyl	2800±126	7738±340	1439±78	82.0±4
6i	3-Fluorophenyl	**60.9**±4	192.3±15	888.0±54	70.6±2
6m	3,5-Bis(trifluoromethyl)phenyl	2247±157	7589±420	500.7±29	36.9±1.5
6n	3,4-(Methylenedioxy)phenyl	**78.7**±3.6	722.5±31	85.0±5	92.4±5
6o	2-Bromophenyl	829.8±45	9688±310	757.8±20	10.0±0.4
6p	3,4,5-Trimethoxyphenyl	405.2±21	943.0±30	767.5±41	1534±120
6q	3-Methoxyphenyl	**38.3**±2	716.4±27	940.1±40	192.8±9.5
AAZ		250±11	12.1±0.5	25.8±1.1	5.7±0.3

* Mean ± standard error (from 3 different assays, by a stopped flow technique).

From the data given in the Table 1 the following inferences were made:-

1) The cytosolic isoform hCA I was strongly inhibited by all the synthesized compounds with K_is ranging between 18.8 nM to 5.5 μM. Among all the synthesized compounds, **6d, 6q, 6e** and **6j** were found to be more potent hCA I inhibitors compared to the standard AAZ. The compound **6d** (K_i = 18.8 nM) was 13 times more active than AAZ (K_i = 250 nM) whereas compound **6q** (K_i = 38.3 nM) was found to be 6 times more active than AAZ against hCA I. Similarly, the compound **6e** (K_i= 50.4 nM) was 5 times more active than AAZ against hCA I isoforms. It was also noticed that the 4-bromo (**6d**), 4-methyl (**6e**), and 3-methoxy (**6q**) substituted derivatives were showing better inhibition as compared to electron withdrawing derivatives (**Table-1**).
2) The other cytosolic isoform hCA II was also inhibited by few compounds in moderate nanomolar range with K_i values ranging between 36.2-88 nM. Compound **6e** having a p-methyl substitution was found to be more potent amongst all with K_i= 36.2 nM. Three other compounds i.e., **6j**, **6f** and **6h** were also inhibited hCA II isoform with K_i values of 84.7 nM, 88.1 nM and 88.5 nM respectively.
3) The tumor associated isoform hCA IX was weakly inhibited by all the synthesized compounds with K_i values ranging from 69.3 nM-1.4 μM. The 4-chloro substituted derivative **(6b)** and 3,4-(Methylenedioxy) substituted derivative **(6n)** exhibited the best inhibition amongst all the compounds with K_i values of 73.3 and 85 nM respectively (**Table-1**).
4) The other tumor-associated isoform hCA XII, was also inhibited by the compounds **6o**, **6m** and **6f** in a low to moderate nanomolar range with K_i values of 10nM, 36.9 nM and 41.9 nM respectively. The most potent inhibitor against this isoform was found to be the compound **6o** with 2-bromo substitution.

2.3. Molecular docking study

The molecular docking results along with the major interactions for **6d**, **6q** and co-crystallized ligand with human hCA I enzyme is depicted in **Table 2**. From the molecular docking studies, it was observed that **6d** and **6q** were well accommodated in the active pocket of hCA I. In case of the most active compound **6d** has shown two hydrogen bond interactions with the active residues

Thr199 and His200. **Fig 3** illustrates the predicted binding mode and the detailed protein-inhibitor interactions of compound **6d** within the active site of hCA I. One of the oxygen atom of sulfonamide moiety acts as hydrogen bond acceptor and formed two H-bond interactions with Thr199 and His200 with a distance of 1.93 and 3.98 Å, respectively. The other oxygen atom and nitrogen atom of sulfonamide moiety established a metal ion interaction with "Zn". The triazole core of **6d** has made one arene-arene (π-π) interaction with Phe91. Additionally, several hydrophobic interactions were observed between the compound **6d** and the active site residues, e.g., Ile60, Val62, Phe91, Ala121, Leu131, Ala132, Ala135, Leu141, Val143, Leu198, Pro202 and Trp209 that stabilized the binding of the compound **6d** in the active site of hCA I.

Similarly, Compound **6q** also showed good docking score and more protein-ligand interactions with the active site residues of hCA I. **Fig 4** demonstrates the possible binding mode and protein-ligand interactions of **6q** with the active site of hCA I. On the basis of our molecular docking study, it could be deduced that the active compounds elicited potency via occupying the active pocket of hCA I and making favourable interactions with its key residues.

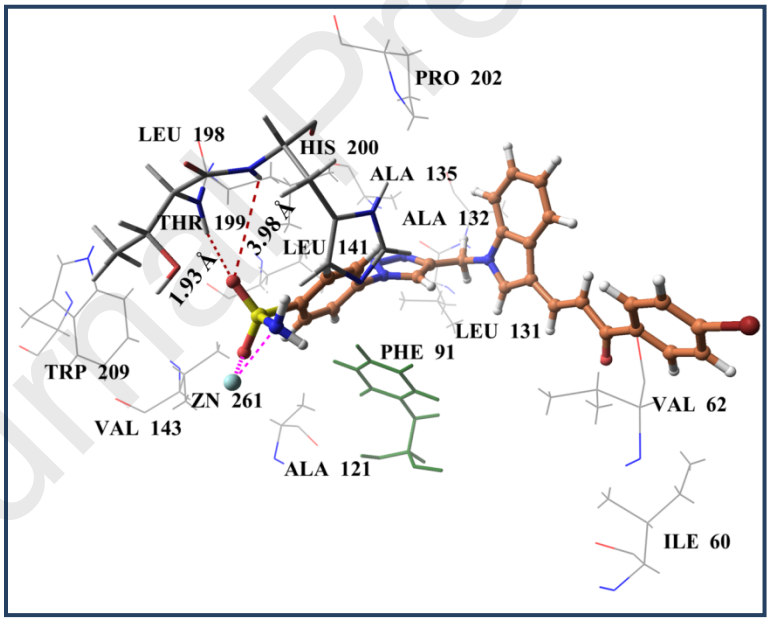


Fig 3. Binding pose of the most potent compound **6d** (orange colour stick) at the active site of hCA I (PDB ID: 1AZM). The red and purple dashed lines represent hydrogen bond and metal ion interactions, respectively.

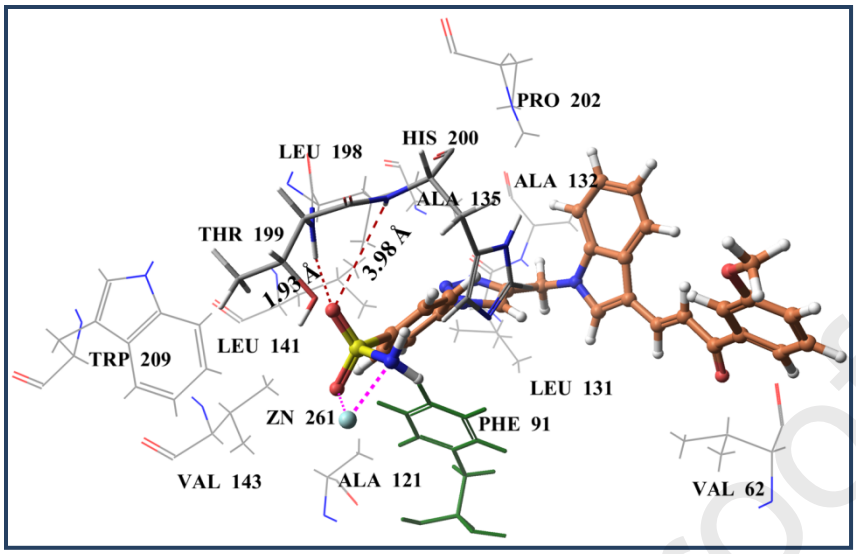


Fig 4. Binding pose of **6q** (orange colour stick) at the active site of hCA I (PDB ID: 1AZM). The red and purple dashed lines represent hydrogen bond and metal ion interactions, respectively.

Table 2. GLIDE docking results for compound **6d**, **6q** and co-crystallized ligand at the active site of human carbonic anhydrase I (PDB ID: 1AZM).

S. no	Ligand Name	Docking score	Interactions			
			H-bonds	π-π	Hydrophobic	Metal ion interaction with "Zn"
1	**6d**	-7.915	Thr199, His200	Phe91	Ile60, Val62, Phe91, Ala121, Leu131, Ala132, Ala135, Leu141, Val143, Leu198, Pro202, Trp209	Positive
2	**6q**	-7.269	Thr199, His200	Phe91	Val62, Phe91, Ala121, Leu131, Ala132, Ala135, Leu141, Val143, Leu198, Pro202, Trp209	Positive
3	**Co-crystal (acetazolamide)**	-8.520	Thr199, His200	His200	Phe91, Leu131, Ala 135, Leu141, Val143, Leu198, Pro202, Trp209	Positive

3. Conclusion

In the present work, novel indolylchalcone linked benzenesulfonamide-1,2,3-triazole hybrids **(6a-q)** were designed, synthesized and evaluated for their ability to inhibit pharmacologically significant human carbonic anhydrases hCA I, hCA II, hCA IX and hCAXII. Most of the synthesized compounds showed good inhibition profile against all the four isoforms, with K_I values less than 100 nM. Against hCA I the compounds **6d, 6e**, **6j** and **6q** were found to be more potent hCA I inhibitors compared to the standard AAZ with K_I values of 18.8 nM, 38.3 nM, 50.4 nM and 54.7 nM respectively. Against hCA II, compound **6e** showed the best inhibition with a K_I value of 36.3 nM. Against hCA IX the compounds **6b** and **6n** exhibited the best inhibition amongst all with K_i values of 73.3 and 85 nM respectively. Against hCA XII, compound **6o** showed the best K_i values of 10 nM. Hence based on the results, it can be concluded that the compounds containing electron donating groups (-CH_3 **(6d)** and -OCH_3 **(6e)**) as well as compounds containing bromo substitution on the phenyl ring **(6q)** of chalcone showed good inhibitory activity as compared to compounds containing electron withdrawing groups. Thus it is anticipated that the compounds **6d, 6q, 6e** and **6j** which are more potent than standard AAZ could be considered as potential lead molecules for the design and development of selective hCA I inhibitors.

4. Experimental:

4.1. General

All the commercially available reagents were used without further purification. Solvents were dried and distilled wherever necessary prior to use, using standard methods. All the air and moisture sensitive reactions were performed under inert conditions using clean and dried glassware and syringe technique to transfer solutions. Reactions were monitored by TLC using Merck silica gel 60F-254 plates. Melting points were acquired on Stuart digital melting point apparatus / SMP 30 in open capillary tubes and uncorrected. Nuclear Magnetic Resonance (^{1}H NMR and ^{13}C NMR) spectra were recorded by using an Avancebruker 500MHz and 125MHz spectrometer in DMSO-d_6 as solvent and trimethylsilane (TMS) as internal standard. Chemical shifts are reported as δ values in parts per million (ppm) and coupling constants (*J*) are expressed in Hz. Multiplicities are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublets). HRMS were determined with Agilent QTOF mass spectrometer 6540 series instrument and were performed using ESI techniques at 70 eV.

4.1.1 General procedure for synthesis of indolylchalcones **(2a-q):**

A mixture of indole-3-carboxaldehyde **1** (1 equiv) was refluxed with substituted acetophenones (1 equiv) in presence of piperidine (0.5 equiv) in ethanol for 24 h at 60^0C under stirring. The reaction was monitored by TLC and after completion of the reaction crushed-ice was added into the reaction mixture and neutralized with glacial acetic acid by monitoring with pH paper, which resulted in the formation of a solid product **2.** The solid formed was filtered using vacuum filtration and washed with hexane and dried to get the product in 80-90% yield**.**

4.1.2 General procedure for synthesis of (E)-3-(1-(prop-2-yn-1-yl)-1H-indol-3-ylchalcones **(3a-q):**

Anhydrous K_2CO_3 (3 equiv) was suspended in dry DMF (5 mL) followed by the addition of compound 2**a**-**q** (1 equiv). The resulting mixture was stirred for 1 h at 60^0C. Then propargyl bromide (1 equiv) was added drop wise. Reaction mixture was stirred for 2-4 h at rt. After the completion of the reaction as monitored by TLC, ice-cool water (10 mL) was added to the reaction mixture and the formed precipitate was filtered off to get the product in 85-90% yield.

4.1.3 General procedure for synthesis of **(6a- q):**

(E)-3-(1-(prop-2-yn-1-yl)-1*H*-indol-3-ylchalcones **3a-q** (1 equiv) and 4-azidobenzenesulfonamide **(5)** (1.08 equiv) were dissolved in ^{t}BuOH/H_2O (1:1, 5mL), followed by the addition of $CuSO_4.5H_2O$ (5 mol%) and sodium ascorbate (10 mol%). The resultant solution was stirred at 50^0C till completion of the reaction (TLC monitoring). Solvents were removed under vacuum and the residue was purified by column chromatography using silica gel (60-120 mesh) as the stationary phase and 1:3 Hex:Ethylacetate as mobile phase. Pure products (**6a-q)** were obtained as yellow solids with 63- 95% yield.

(E)-4-(4-((3-(3-oxo-3-phenylprop-1-en-1-yl)-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)benzenesulfonamide **(5a):** Yellow solid; yield 80%, m.p 225-227^0C; ^{1}H NMR (500 MHz, DMSO) δ 8.99 (s, 1H), 8.29 (s, 1H), 8.15 – 7.98 (m, 8H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.66 (dd, *J* = 20.3, 11.5 Hz, 2H), 7.57 (t, *J* = 7.5 Hz, 2H), 7.51 (s, 2H), 7.31 (m, 2H), 5.68 (s, 2H). ^{13}C NMR (125 MHz, DMSO) δ 189.34, 144.56, 144.44, 138.96, 138.89, 138.59, 137.65, 135.86, 132.91,

129.16, 128.61, 127.95, 126.39, 123.46, 122.76, 122.10, 121.06, 120.88, 116.65, 113.00, 111.68, 41.69. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{26}H_{21}N_5O_3S$ 484.1443, found 484.1441.

(E)-4-(4-((3-(3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)benzenesulfonamide **(5b):**Yellow solid; yield 95%, m.p 280-282^{0}C; ^{1}H NMR (500 MHz, DMSO) δ 9.00 (s, 1H), 8.31 (s, 1H), 8.21 – 7.96 (m, 8H), 7.77 (d, *J* = 7.8 Hz, 1H), 7.70 – 7.60 (m, 3H), 7.53 (s, 2H), 7.32 (dd, *J* = 14.1, 7.4 Hz, 2H), 5.68 (s, 2H). ^{13}C NMR (125 MHz, DMSO) δ 188.14, 144.51, 144.45, 139.12, 138.95, 137.81, 137.67, 137.53, 136.17, 130.56, 129.24, 127.95, 126.36, 123.52, 122.77, 122.15, 121.12, 120.88, 116.17, 113.01, 111.71, 41.70. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{26}H_{20}ClN_5O_3S$ 518.1054, found 518.1060.

(E)-4-(4-((3-(3-(4-fluorophenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)benzenesulfonamide **(5c):** Yellow solid; yield 89%, m.p 261-263^{0}C; ^{1}H NMR (500 MHz, DMSO) δ 9.00 (s, 1H), 8.30 (s, 1H), 8.26 – 8.20 (m, 2H), 8.13 (dd, *J* = 16.6, 8.2 Hz, 3H), 8.03 (dd, *J* = 20.7, 12.0 Hz, 3H), 7.77 (d, *J* = 8.1 Hz, 1H), 7.68 (d, *J* = 15.5 Hz, 1H), 7.53 (s, 2H), 7.42 – 7.27 (m, 4H), 5.68 (s, 2H). ^{13}C NMR (125 MHz, DMSO) δ 187.83, 166.15, 164.16, 144.54, 144.44, 138.95, 138.77, 137.65, 135.98, 135.47, 131.57, 131.49, 127.95, 126.36, 123.48, 122.76, 122.10, 121.12, 120.88, 116.27, 116.18, 116.00, 113.00, 111.67, 41.68. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{26}H_{20}FN_5O_3S$ 502.1349, found 502.1365.

(E)-4-(4-((3-(3-(4-bromophenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)benzenesulfonamide **(5d):** Yellow solid; yield 70%, m.p 276-278^{0}C; ^{1}H NMR (500 MHz, DMSO) δ 9.00 (s, 1H), 8.31 (s, 1H), 8.16 – 7.99 (m, 8H), 7.78 (d, *J* = 8.3 Hz, 3H), 7.65 (d, *J* = 15.4 Hz, 1H), 7.52 (s, 2H), 7.32 (dd, *J* = 14.1, 7.7 Hz, 2H), 5.68 (s, 2H). ^{13}C NMR (125 MHz, DMSO) δ 188.32, 144.51, 144.44, 139.15, 138.94, 137.86, 137.67, 136.20, 132.19, 130.70, 127.95, 126.91, 126.35, 123.52, 122.77, 122.15, 121.12, 120.88, 116.13, 113.01, 111.71, 41.70. HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{26}H_{20}BrN_5NaO_3S$ 586.0347, found 586.0350

*(E)-4-(4-((3-(3-oxo-3-(p-tolyl)prop-1-en-1-yl)-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)benzenesulfonamide***(5e):** Yellow solid; yield 65%,m.p 265-267^{0}C; ^{1}H NMR (500 MHz, DMSO) δ 8.99 (s, 1H), 8.28 (s, 1H), 8.15 – 7.98 (m, 8H), 7.76 (d, *J* = 7.9 Hz, 1H), 7.68 (d, *J* = 15.5 Hz, 1H), 7.52 (s, 2H), 7.32 (ddd, *J* = 19.9, 17.5, 7.4 Hz, 4H), 5.67 (s, 2H), 2.42 (s, 3H). ^{13}C NMR (125 MHz, DMSO) δ 188.80, 144.59, 144.45, 143.19, 138.95, 138.13, 137.62, 136.31,

135.58, 129.72, 128.75, 127.94, 126.42, 123.41, 122.74, 122.04, 121.00, 120.88, 116.69, 112.99, 111.64, 41.68, 21.62. HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{27}H_{23}N_5NaO_3S$ 520.1419, found 520.1419.

(E)-4-(4-((3-(3-(3-bromophenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)benzenesulfonamide **(5f):** Yellow solid; yield 75%, m.p 222-224^{0}C; ^{1}H NMR (500 MHz, DMSO) δ 8.99 (s, 1H), 8.34 (s, 1H), 8.23 (s, 1H), 8.17 – 7.98 (m, 7H), 7.84 (d, *J* = 7.4 Hz, 1H), 7.77 (d, *J* = 7.8 Hz, 1H), 7.65 (d, *J* = 15.4 Hz, 1H), 7.57 – 7.48 (m, 3H), 7.31 (dd, *J* = 12.3, 7.8 Hz, 2H), 5.68 (s, 2H).^{13}C NMR (125 MHz, DMSO) δ 187.96, 144.45, 141.00, 139.38, 138.95, 137.64, 136.14, 135.51, 131.40, 131.03, 127.95, 127.70, 126.45, 123.53, 122.77, 122.69, 122.17, 121.08, 120.88, 116.11, 113.03, 111.70, 41.74. HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{26}H_{20}BrN_5O_3S$ 586.0347, found 586.0350

(E)-4-(4-((3-(3-(3-methoxyphenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)benzenesulfonamide **(5g):** Yellow solid; yield 70%, m.p 244-246^{0}C; ^{1}H NMR (500 MHz, DMSO) δ 8.98 (s, 10H), 8.26 (s, 10H), 8.12 (dd, *J* = 16.5, 8.7 Hz, 52H), 8.05 – 7.96 (m, 32H), 7.76 (d, *J* = 8.0 Hz, 11H), 7.69 (d, *J* = 15.5 Hz, 11H), 7.51 (s, 22H), 7.30 (dt, *J* = 14.6, 7.1 Hz, 22H), 7.09 (d, *J* = 8.6 Hz, 21H), 5.67 (s, 20H), 3.87 (s, 31H).^{13}C NMR (125 MHz, DMSO) δ 187.67, 163.23, 144.61, 144.45, 138.96, 137.60, 135.34, 131.65, 130.90, 127.95, 126.43, 123.37, 122.73, 121.97, 120.99, 120.88, 116.66, 114.40, 113.02, 111.61, 55.97, 41.66. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{27}H_{23}N_5O_3S$ 514.1549, found 514.1547.

(E)-4-(4-((3-(3-(4-chloro-3-nitrophenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)benzenesulfonamide **(5h):** Yellow solid; yield 76%, m.p 205-207^{0}C; ^{1}H NMR (500 MHz, DMSO) δ 9.01 (s, 1H), 8.61 (s, 1H), 8.38 (d, *J* = 14.0 Hz, 2H), 8.10 (t, *J* = 36.8 Hz, 6H), 7.75 (d, *J* = 27.2 Hz, 3H), 7.53 (s, 2H), 7.33 (s, 2H), 5.70 (s, 2H), 2.62 (s, 3H).^{13}C NMR (125 MHz, DMSO) δ 187.00, 149.81, 144.48, 144.41, 139.78, 138.93, 137.75, 137.65, 137.28, 136.52, 133.75, 132.81, 127.95, 126.38, 124.21, 123.59, 122.80, 122.23, 121.18, 120.88, 115.59, 113.04, 111.75, 41.73, 19.95. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{27}H_{23}N_6O_5S$ 543.1451, found 543.1446.

(E)-4-(4-((3-(3-(2,4-dichlorophenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)benzenesulfonamide **(5i):** Yellow solid; yield 83%, m.p. 236-238^{0}C; ^{1}H NMR (500

MHz, DMSO) δ 8.98 (s, 1H), 8.21 (s, 1H), 8.10 (d, *J* = 8.4 Hz, 2H), 7.99 (dd, *J* = 16.9, 8.0 Hz, 3H), 7.77 (d, *J* = 8.4 Hz, 2H), 7.59 (dd, *J* = 40.6, 20.6 Hz, 5H), 7.30 (dt, *J* = 31.4, 7.3 Hz, 2H), 7.04 (d, *J* = 16.0 Hz, 1H), 5.65 (s, 2H).^{13}C NMR (125 MHz, DMSO) δ 192.43, 144.44, 144.33, 141.85, 138.93, 138.79, 137.79, 136.86, 135.39, 131.55, 130.95, 129.97, 128.05, 127.95, 126.03, 123.66, 122.84, 122.41, 121.04, 120.88, 112.46, 111.84, 41.68. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{26}H_{20}Cl_2N_5O_3S$ 552.0664, found 552.0654.

(E)-4-(4-((3-(3-(3,4-dimethoxyphenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)benzenesulfonamide **(5j):**Yellow solid; yield 71%,m.p. 194-196^{0}C; ^{1}H NMR (500 MHz, DMSO) δ 8.99 (s, 1H), 8.27 (s, 1H), 8.05 (d, *J* = 41.8 Hz, 6H), 7.86 (s, 1H), 7.78 – 7.66 (m, 2H), 7.59 (s, 1H), 7.51 (s, 2H), 7.30 (s, 2H), 7.11 (d, *J* = 7.1 Hz, 1H), 5.67 (s, 2H), 3.87 (s, 6H).^{13}C NMR (125 MHz, DMSO) δ 187.70, 153.23, 149.29, 144.45, 138.96, 137.58, 137.46, 135.20, 131.77, 127.94, 126.48, 123.35, 123.16, 122.73, 121.97, 120.88, 116.69, 113.01, 111.61, 111.44, 111.27, 56.24, 56.09, 41.67. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{28}H_{26}N_5O_5S$ 544.1655, found 544.1650.

(E)-4-(4-((3-(3-oxo-3-(3-(trifluoromethyl)phenyl)prop-1-en-1-yl)-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)benzenesulfonamide **(5k):** Yellow solid; yield 74%, m.p. 219-221^{0}C; ^{1}H NMR (500 MHz, DMSO) δ 8.99 (s, 1H), 8.47 (d, *J* = 7.0 Hz, 1H), 8.34 (d, *J* = 9.8 Hz, 2H), 8.12 (dd, *J* = 17.8, 9.9 Hz, 4H), 8.01 (s, 3H), 7.83 (d, *J* = 7.3 Hz, 1H), 7.74 (dd, *J* = 30.0, 11.4 Hz, 2H), 7.50 (s, 2H), 7.37 – 7.26 (m, 2H), 5.69 (s, 2H).^{13}C NMR (125 MHz, DMSO) δ 188.04, 144.49, 144.42, 139.71, 138.93, 137.64, 136.35, 132.71, 130.51, 129.25, 127.95, 126.43, 124.85, 123.56, 122.79, 122.22, 121.10, 120.88, 115.94, 113.01, 111.74, 41.74. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{27}H_{21}FN_5O_3S$ 552.1317, found 552.1315.

(E)-4-(4-((3-(3-(3-fluorophenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)benzenesulfonamide **(5l):** Yellow solid; yield 82%, m.p. 223-225^{0}C; ^{1}H NMR (500 MHz, DMSO) δ 8.99 (s, 1H), 8.32 (s, 1H), 8.15 (d, *J* = 7.7 Hz, 1H), 8.09 (dd, *J* = 15.1, 12.2 Hz, 3H), 8.04 – 7.96 (m, 3H), 7.90 (d, *J* = 9.6 Hz, 1H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.69 – 7.60 (m, 2H), 7.54 – 7.46 (m, 3H), 7.32 (dt, *J* = 21.1, 7.1 Hz, 2H), 5.68 (s, 2H). ^{13}C NMR (125 MHz, DMSO) δ 188.04, 163.81, 161.86, 144.51, 144.45, 141.26, 141.21, 139.32, 138.95, 137.67, 136.21, 131.34, 131.27, 127.95, 126.39, 124.81, 123.53, 122.77, 122.17, 121.17, 120.89, 119.85, 119.68, 116.17,

115.15, 114.98, 113.03, 111.68, 41.72. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{26}H_{21}FN_5O_3S$ 502.1349, found 502.1351.

(E)-4-(4-((3-(3-(3,5-bis(trifluoromethyl)phenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)benzenesulfonamide **(5m):** Yellow solid; yield 77%, m.p. 277-279^{0}C; ^{1}H NMR (500 MHz, DMSO) δ 9.00 (s, 1H), 8.68 (s, 2H), 8.40 (d, *J* = 11.5 Hz, 2H), 8.23 – 8.07 (m, 4H), 8.01 (d, *J* = 8.2 Hz, 2H), 7.85 – 7.72 (m, 2H), 7.50 (s, 2H), 7.41 – 7.28 (m, 2H), 5.70 (s, 2H). ^{13}C NMR (125 MHz, DMSO) δ 186.74, 144.42, 140.94, 140.55, 138.92, 137.59, 136.50, 131.62, 131.36, 131.09, 130.83, 129.10, 127.95, 127.55, 126.89, 126.56, 126.02, 124.72, 123.63, 122.81, 122.55, 122.27, 121.06, 120.38, 115.51, 113.09, 111.76, 41.79. HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{28}H_{19}F_6N_5O_3S$ 642.1001, found 642.1007

(E)-4-(4-((3-(3-(1,3-dihydroisobenzofuran-5-yl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)benzenesulfonamide **(5n):** Yellow solid; yield 80%, m.p. 241-243^{0}C; ^{1}H NMR (500 MHz, DMSO) δ 8.98 (s, 1H), 8.27 (s, 1H), 8.06 (d, *J* = 50.4 Hz, 6H), 7.68 (dd, *J* = 109.0, 45.9 Hz, 6H), 7.31 (s, 2H), 7.08 (s, 1H), 6.16 (s, 2H), 5.67 (s, 2H).^{13}C NMR (125 MHz, DMSO) δ 187.25, 151.47, 148.38, 144.44, 138.96, 137.88, 137.59, 135.42, 133.51, 127.94, 126.44, 124.80, 123.38, 122.73, 121.98, 121.05, 120.88, 116.49, 113.03, 111.59, 108.53, 108.22, 102.38, 41.67. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{27}H_{22}ClN_5O_5S$ 528.1342, found 528.1343.

(E)-4-(4-((3-(3-(2-bromophenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)benzenesulfonamide **(5o):** Yellow solid; yield 72%, m.p. 182-184^{0}C; ^{1}H NMR (500 MHz, DMSO) δ 8.99 (s, 1H), 8.34 (s, 1H), 8.23 (s, 1H), 8.17 – 7.98 (m, 7H), 7.84 (d, *J* = 7.4 Hz, 1H), 7.77 (d, *J* = 7.8 Hz, 1H), 7.65 (d, *J* = 15.4 Hz, 1H), 7.57 – 7.48 (m, 3H), 7.31 (dd, *J* = 12.3, 7.8 Hz, 2H), 5.68 (s, 2H).^{13}C NMR (125 MHz, DMSO) δ 187.96, 144.45, 141.00, 139.38, 138.95, 137.64, 136.14, 135.51, 131.40, 131.03, 127.95, 127.70, 126.45, 123.53, 122.77, 122.69, 122.17, 121.08, 120.88, 116.11, 113.03, 111.70, 41.74. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{26}H_{21}BrN_5O_3S$ 564.0528, found 564.0534.

(E)-4-(4-((3-(3-oxo-3-(3,4,5-trimethoxyphenyl)prop-1-en-1-yl)-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)benzenesulfonamide **(5p):** Yellow solid; yield 73%, m.p. 244-246^{0}C; ^{1}H NMR (500 MHz, DMSO) δ 9.00 (s, 1H), 8.32 (s, 1H), 8.04 (dd, *J* = 33.2, 15.7 Hz, 6H), 7.76 (d, *J* = 7.4 Hz, 1H), 7.68 (s, 1H), 7.52 (s, 2H), 7.42 – 7.24 (m, 4H), 5.68 (s, 2H), 3.91 (s, 6H), 3.77 (s, 3H).^{13}C

NMR (125 MHz, DMSO) δ 188.34, 153.34, 144.57, 144.45, 142.03, 138.95, 138.15, 137.55, 135.27, 134.39, 127.94, 126.58, 123.39, 122.74, 122.05, 120.88, 116.79, 112.98, 111.64, 106.40, 60.66, 56.69, 56.55, 41.71. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{29}H_{28}N_5O_6$ 574.1760, found 574.1762

(E)-4-(4-((3-(3-(3-methoxyphenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)benzenesulfonamide **(5q):** Yellow solid; yield 80%, m.p. 203-205^{0}C; ^{1}H NMR (500 MHz, DMSO) δ 8.98 (s, 1H), 8.26 (s, 1H), 8.12 (dd, *J* = 16.5, 8.7 Hz, 52H), 8.05 – 7.96 (m, 32H), 7.76 (d, *J* = 8.0 Hz, 11H), 7.69 (d, *J* = 15.5 Hz, 11H), 7.51 (s, 22H), 7.30 (dt, *J* = 14.6, 7.1 Hz, 22H), 7.09 (d, *J* = 8.6 Hz, 21H), 5.67 (s, 20H), 3.87 (s, 31H).^{13}C NMR (125 MHz, DMSO) δ 187.67, 163.23, 144.61, 144.45, 138.96, 137.60, 135.34, 131.65, 130.90, 127.95, 126.43, 123.37, 122.73, 121.97, 120.99, 120.88, 116.66, 114.40, 113.02, 111.61, 55.97, 41.66. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{27}H_{23}N_5O_3S$ 514.1549, found 514.1540.

4.1.4. 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^{0}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]

4.2 Computational studies

To rationalize the experimental results obtained, molecular docking studies were performed on representative compounds **6d**, **6q** and co-crystallized ligand (acetazolamide) on the active site of human carbonic anhydrase I (hCA I) enzyme by employing GLIDE docking module of Schrödinger suite 2019-1 [25]. The protein crystal structure of hCA I in complex with acetazolamide was retrieved from the RCSB Protein Data Bank (PDB code: 1AZM, resolution 2 Å) [26]. Next, protein was prepared by using Protein Preparation Wizard of Maeströ. Missing amino acid residues and side chains were added using Prime module of Maeströ. The bound co-crystallized ligand was used to define the active site in hCA I. All the ligand molecules were built and optimized using Maestro Molecule Builder and OPLS_2005 force field in LigPrep modules of Schrödinger software, respectively. Further, the prepared ligands were docked at the active site of hCA I.

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Conflict of Interest

The authors declare no conflict of interest.

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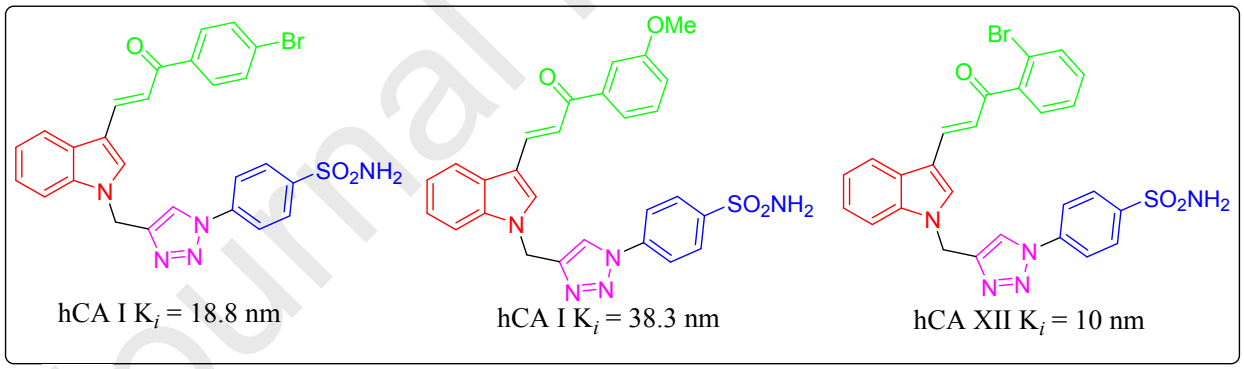
Highlights

- 17 novel compound of indole-1,2,3-triazole chalcones **(6a-q)** hybrid were synthesized
- The synthesized compounds were screened for their CA inhibition potency against four human CA isoforms hCA I, II, IX, XII
- Compound **6d** (18.8 nm)**, 6q** (38.3 nm) **and 6e** (50.4 nm) are 13, 6 and 5 times more potent than standard AAZ against hCA I.
- Compound **6o**, **6m** and **6f** also inhibit isoform hCA XII in the range of 10 nm, 36.9 nm and 41.9 nm respectively

A novel series of indolylchalcone linked with benzenesulfonamide-1,2,3-triazoles (**6a-q**) has been synthesized by click chemistry reaction and investigated for their hCA inhibitory activity against a panel of human carbonic anhydrases (CA; EC 4.2.1.1). The hCA inhibition assay results shows that these compounds were inhibiting all the four isoforms, hCA I, II, IX and XII. Among all the compounds tested the compound **6d** (18.8 nM), **6q** (38.3 nM) and **6e** (50.4 nM) are 13, 6 and 5 times more potent than standard AAZ respectively against hCA I isoform. The compound **6o**, **6m** and **6f** also inhibited isoform hCA XII in the range of 10 nM, 36.9 nM and 41.9 nM respectively. Few of the compounds are also active in against isoform hCA II and isoform hCA IX under 100 nM ranges.



All other are agreed for this work to be published and have no conflict of interest