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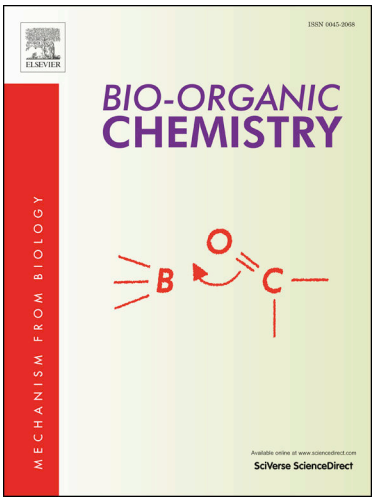

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Synthesis of some *N*-aroyl-2-oxindole benzenesulfonamide conjugates with carbonic anhydrase inhibitory activity

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Declarations of interest: none

Abstract

Implication of carbonic anhydrases (CAs) in many physiological functions made them attractive therapeutic targets. Herein, we report the synthesis of three series of benzenesulfonamide-based compounds (**5a-e**, **9a-e** and **10a-e**) as potential ligands to four of the human CA isoforms (hCA I, hCA II, hCA IX and hCA XII). All synthesized compounds were evaluated for their CA inhibitory activity. Most of the compounds preferentially inhibited the tumor-associated isoforms IX and XII. Series **9a-e** and **10a-e** showed the highest activity. Of particular interest was compound **10a** which demonstrated the highest activity among all compounds with K_i of 68.3 and 21.5 nM against hCA IX and hCA XII, respectively, in addition to its highest selectivity index. To get deep insight on the interaction of compound **10a** with CA, docking experiment was run to study the binding interaction with key amino acids and zinc ion in the catalytic site of the four isoforms studied.

Keywords: benzenesulfonamide; carbonic anhydrase inhibitors; synthesis; molecular docking.

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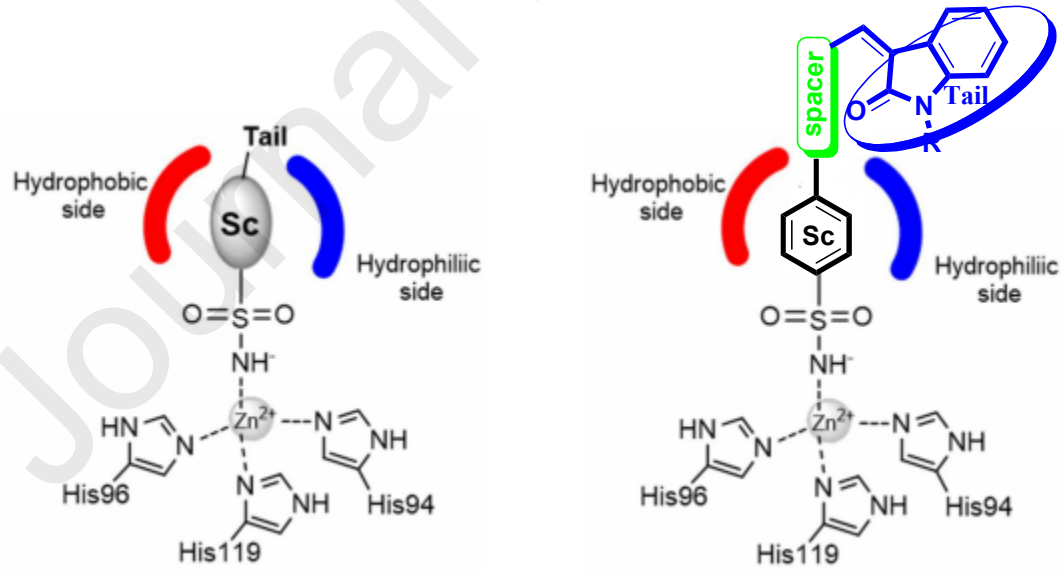
1. Introduction

Carbonic anhydrases (CAs) constitute an important family of metalloenzymes that catalyse the reversible CO_2 hydration to bicarbonate and protons which is a crucial reaction for pH regulatory mechanisms [1-3]. The CAs are widespread in many living organisms and accordingly they are classified into distinct genetic families, such as: α-CAs that are present in vertebrates, protozoa, algae, corals, bacteria and green plants, [1,4] the β-CAs have been identified in bacteria, fungi, Archaea, algae and chloroplasts of both mono- and dicotyledons, [4-6] the γ-CAs are encoded in Archaea, bacteria and plants, [7] the δ-CAs were found in marine phytoplankton, such as haptophytes, dinoflagellates, diatoms and chlorophyte prasinophytes [8], whereas ζ-CAs seem to be present only in marine diatoms [9]. A unique η-CA has been identified to date in the protozoa *Plasmodium falciparum* [10]. Recently, θ-CAs were detected in the marine diatom *Phaeodactylum* tricornutum [11] and the first iota-CA was identified in the marine diatom *Thalassiosira pseudonana* [12].

The human carbonic anhydrases (hCAs) belonging to the α-subfamily are subdivided into 16 diverse isoforms that have different kinetic properties, cellular and tissue distribution. According to their cellular distribution, they can be categorized as cytosolic (hCA I, hCA II, hCA III, hCA VII, and hCA XIII), membrane-bound (hCA IV, hCA IX, hCA XII, and hCA XIV), secreted (hCA VI), mitochondrial (hCA Va and hCA Vb), in addition to the acatalytic isoforms also known as the CA related-proteins (CARP), hCA VIII, hCA X and hCA XI which are located in the cytosol [1-3, 13, 14]. From a physiopathological aspect, CA isozymes are involved in many

medical conditions such as glaucoma (hCA II, hCA IV and hCA XII), edema (hCA II, hCA IV, hCA XIV), central nervous system pathologies (hCA VII and hCA XIV) and tumors (hCA IX and hCA XII), and therefore, they are considered as significant therapeutic targets for a wide range of disorders [15,16].

Primary sulfonamide group (RSO_2NH_2) is one of the most widely encountered functionality of carbonic anhydrase inhibitors (CAIs) as it is responsible for coordination to the enzyme's zinc ion [17]. The group carrying the sulfonamide function is therefore considered the scaffold of CAIs. Different approaches have been adopted to design sulfonamide-based CAIs; of which the "tail approach" being the most efficient and investigated one. It depends on attaching different molecular fragments (tails) to the aromatic/heterocyclic ring bearing the sulfonamide scaffold, in order to modulate the binding interaction with the active site cavity, which in turn affects potency and selectivity (Figure 1A) [18,19].



(A) (B)

Figure 1. (A) Schematization of the interaction between a generic sulfonamide and the Zn^{2+} in the CA active site. (Sc = scaffold). (B) Illustration of an isatin/ benzene sulfonamide hybrid incorporated in CA active site.

In this respect, benzene-sulfonamides are the most widely prominent scaffold of CAIs that can be diversely substituted to achieve isozyme specificity. Isatin represents a privileged molecular fragment in the design of biologically active agents. Recently, they have been utilized as the tail moiety in several CAIs and many isatin/benzene sulfonamide hybrid molecules have been reported (Figure 1B) [20]. The structure of these hybrids is discriminated based on the attachment between the benzene sulfonamide scaffold and the isatin tail, whether it is direct or through a spacer group. Also, these compounds vary in the substitution observed on the isatin ring. Compounds **I-IV** are selected examples illustrating the structure diversity of the isatin/benzenesulfonamide hybrids (Figure 2) [21-24].

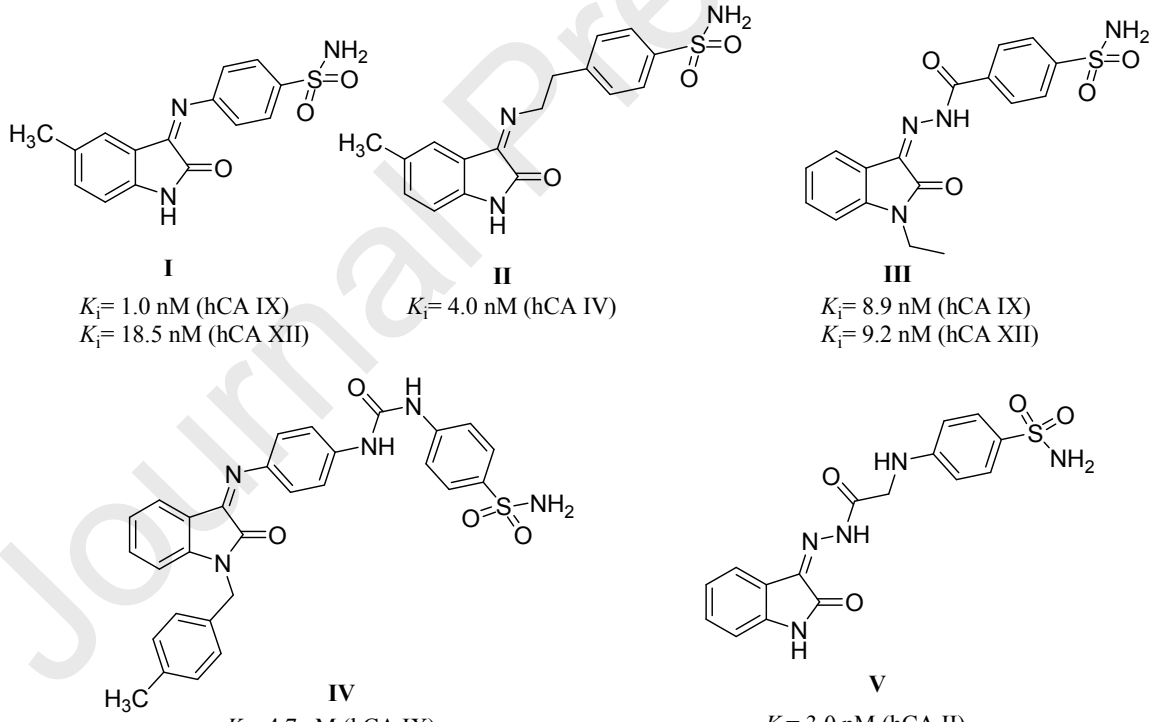
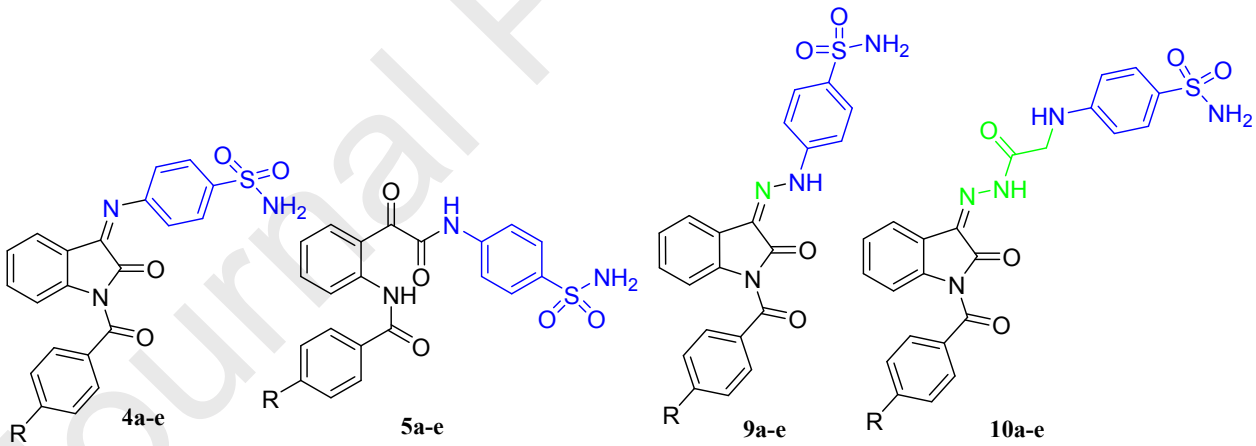


Figure2. Selected examples of isatin/benzene sulfonamide hybrids as CAIs.

Considering the aforementioned facts, and in continuation of our project to develop potent and selective CAIs as compounds **V** [25], it was thought worthwhile to design new isatin-based benzene sulfonamide hybrids and explore their CA inhibitory activity. The design of the new target compounds relies on two structural modifications. Firstly, *N*-substitution of the isatin moiety with a benzoyl ring grafting groups of diverse size, lipophilicity and electronic property (viz., H, F, Cl, Br, OCH_3) was adopted to modulate the interaction of the tail fragment with the CA active site. Secondly, the isatin ring was merged with the benzene sulfonamide scaffold either through direct attachment (compounds **4a-e**) or via a hydrazinyl (compounds **9a-e**) or hydrazinyl-2-oxoethylamino spacers (compounds **10a-e**) aiming to investigate the effect of this structure elongation on the coordination of the tail fragment with the CA active site. Unexpectedly, compounds **4a-e** were synthetically inaccessible, and the sulfonylbenzamides **5a-e** were rather obtained. Interestingly, these compounds can be regarded as the open isosteres of compounds **4a-e** which makes them significantly exceptional due to their high conformational flexibility compared to the other target compounds, a feature which may allow more efficient orientation of the tail moiety into the active site (Figure 3).



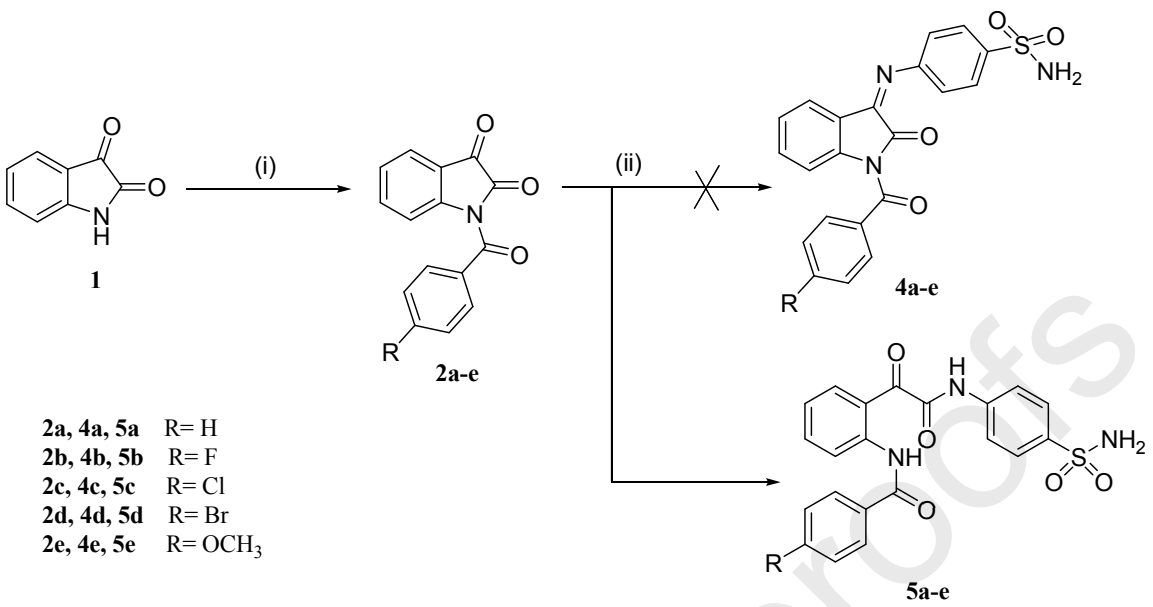
a R=H, **b** R=F, **c** R=Cl, **d** R=Br, **e** R=OCH_3

Figure 3. General structures of the designed isatin/benzenesulfonamides **4a-e**, **5a-e**, **9a-e** and **10a-e**.

2. Results and discussion

2.1. Chemistry

The synthetic pathways adopted for the preparation of the target compounds were depicted in Schemes 1 and 2. Benzoylation of isatin (**1**) was carried out as reported using different benzoyl chlorides in dry tetrahydrofuran (THF) in the presence of triethylamine (TEA) affording *N*-aroyl-isatin derivatives **2a-e** [26]. Synthesis of Schiff's bases **4a-e** using the usual condensation reaction between **2a-e** and sulfanilamide **(3)** in refluxing glacial acetic acid failed to afford the expected products **4a-e** and produced instead the ring opened *N*-(2-(2-Oxo-2-((4-sulfamoylphenyl)amino)acetyl)phenyl)benzamides (**5a-e)**. Several trials were performed to obtain **4a-e** through reacting **2a-e** with sulfanilamide **(3)** in glacial acetic acid, absolute ethanol or *N*,*N*-dimethylformamide at room temperature or heating the reactants in absolute ethanol or methanol. However, all these trials failed to produce the corresponding Schiff bases **4a-e**, and the unreacted **2a-e** were separated. Opening of the pyrrole ring and formation of **5a-e** could be explained on the basis of increased basicity of the aromatic amino group of the sulfanilamide (**3**) in the presence of glacial acetic acid which in turn attacked the carbonyl group at position 2 of the isatin ring being the most electron deficient centre. This resulted in nucleophilic addition followed by ring opening rather than the regular condensation with carbonyl at position 3 (Figure 4). The structure of **5a-e** was confirmed by ^{1}H NMR spectra that revealed three singlet exchangeable signals at 7.30-7.31, 11.09-11.11 and 11.21-11.28 ppm assigned to NH_2 and 2 NH protons along with increased number of aromatic protons due to the inserted benzenesulfonamide moiety. Moreover, ^{13}C NMR of **5a-e** showed signals at 162.4-162.8, 165.2-166.2 and 190.2-190.8 ppm corresponding to the three carbonyl groups (Scheme 1).



Reagents and reaction conditions: (i) appropriate benzoyl chloride, TEA, dry THF, RT, 6h; (ii) sulfanilamide (**3)**, glacial acetic acid, 80°C, 3h.

Scheme 1. Synthetic pathway for compounds **5a-e**.

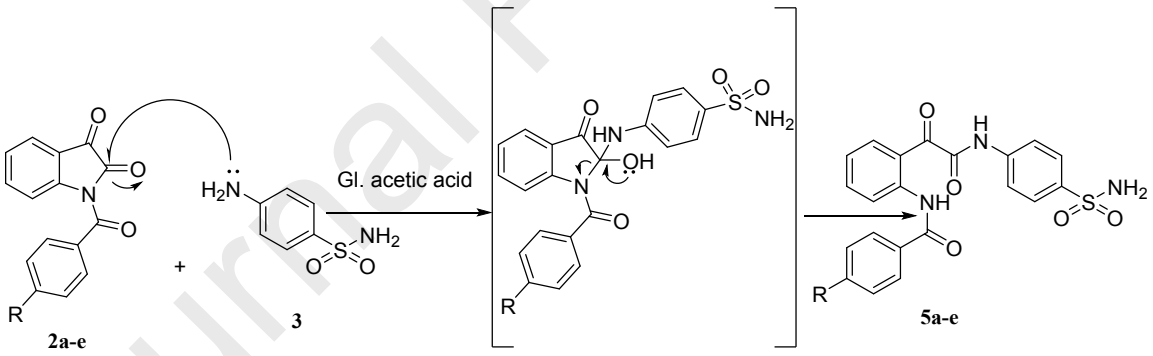
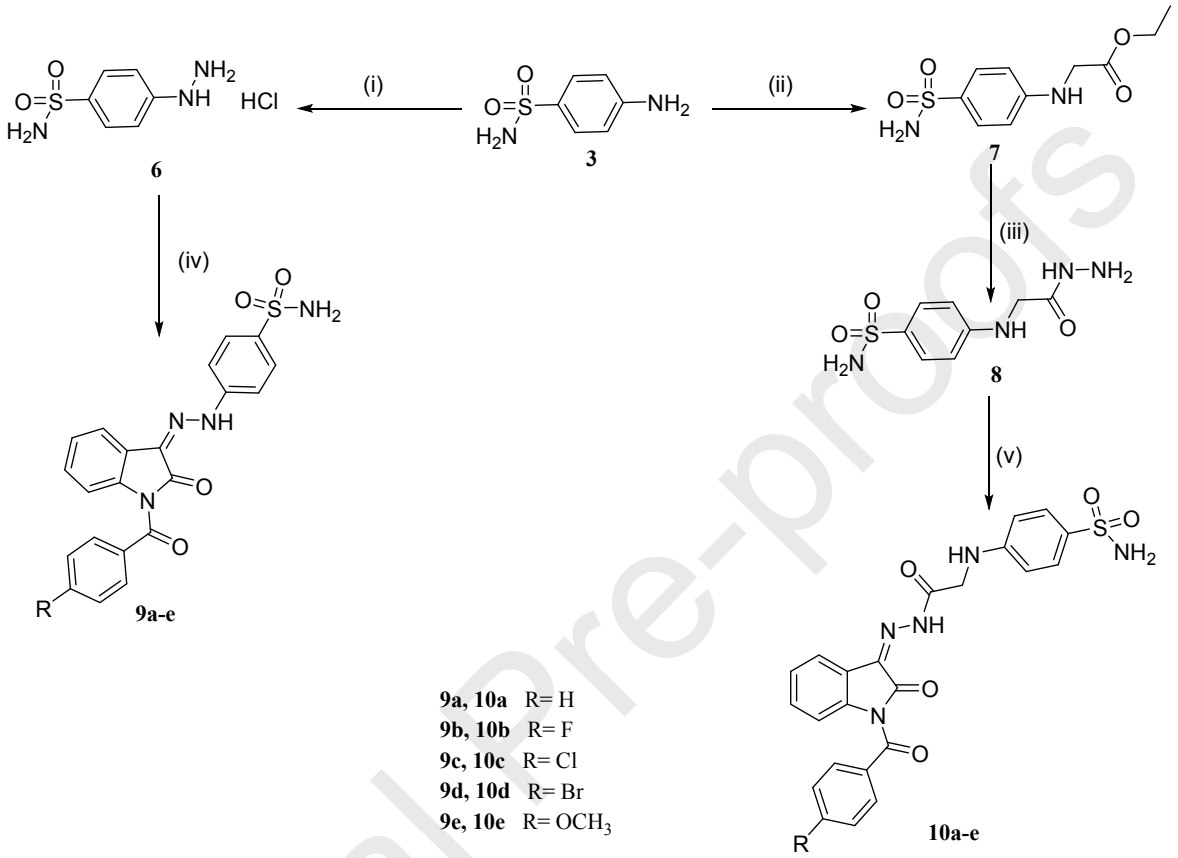


Figure 4. Proposed mechanism for formation of compounds **5a-e.**

On the other hand, Schiff's bases **9a-e** and **10a-e** were obtained via condensation of *N*-aroyl-isatins **2a-e** with hydrazinylbenzenesulfonamide hydrochloride (**6**) and 4-((2-hydrazinyl-2-oxoethyl)amino)benzenesulfonamide (**8**), respectively. Reactants **6** and **8** were prepared according to the reported methods [27,28]. The reaction for compounds **9a-e** proceeded in methanol at room temperature, the acidity of the reactant **6** was sufficient to

catalyze the reaction. While for compounds **10a-e**, two drops of HCl was required to render the medium acidic to activate the hydrazinyl moiety (Scheme 2).



Reagents and reaction conditions: (i) $NaNO_2$, dil. HCl, $SnCl_2$; (ii) ethyl bromoacetate, $NaHCO_3$, aqueous ethanol, RT, overnight; (iii) $NH_2NH_2.H_2O$, absolute ethanol, reflux, 3h; (iv) **2a-e**, methanol, RT, 3h; (v) **2a-e**, methanol, 2drops HCl, RT, 3h.

Scheme 2. Synthetic pathways of compounds **9a-e** and **10a-e**.

The structure of **9a-e** was confirmed by ^{1}H NMR which revealed two exchangeable singlet signals at 7.28-7.29 and 12.39-12.46 ppm attributed to NH_2 and NH protons, respectively, in addition to increase in the integration of aromatic protons due to the incorporated benzenesulfonamide fragment. Moreover, ^{13}C NMR spectra of **9a-e** displayed signals for two carbonyl groups at 160.3-160.4 and 167.7-168.9 ppm. Regarding compounds **10a-e**, ^{1}H NMR spectra showed a singlet at 4.36-4.37 ppm corresponding to CH_2 protons along with three exchangeable singlet signals at 6.96-6.98, 8.32 and 11.57-11.61 ppm due to NH_2

and 2 NH protons, respectively. Furthermore, their ^{13}C NMR spectra exhibited a signal at 44.8-45.0 ppm due to the methylene carbon in addition to three signals at the range of 160.4- 169.0 ppm corresponding to three C=O carbons. It was noteworthy that the ^{1}H and ^{13}C NMR spectra of the flouro-containing compounds **5b**, **9b** and **10b** showed multiplet signals as expected due to ^{1}H-^{19}F and ^{13}C-^{19}F coupling, ^{19}F being the main isotope, therefore, the splitting are clearly visible in both spectra.

2.2.Carbonic anhydrase inhibition.

The CA inhibitory activity of all the newly synthesized compounds **5a-e**, **9a-e** and **10a-e** was evaluated against hCA I, hCA II (cytosolic) as well as hCA IX and hCA XII (transmembrane, tumor-associated isoforms) using the stopped-flow CO_2 hydrase assay [29-33]. The inhibitory activities were compared to acetazolamide (AAZ) (Table 1). The following SAR was deduced:

(i) Activity against hCA I is the least among all the other isoforms, lying in the micromolar range (*Ki* =1555.8-8429.2 nM). The benzamide derivatives **5a-e**, with relatively flexible geometry, were more potent than the other isatin-based compounds. Moreover, the isatin hybrids with shorter hydrazine spacer **9a-e** were more potent than compounds **10a-e** which have the longer hydrazinyl-2-oxoethylamino spacer. Concerning the effect of the substitution on the benzoyl group on activity, it was observed that electron withdrawing substitution of suitable size, -Cl, (**5c**, **9c**), an exception was observed in the fluorine derivative **10b**, was associated with the highest activity followed by the unsubstituted derivatives (**5a**, **9a** and **10a**). The compounds with the large -Br group, or electron donating substitution, $-OCH_3$, were the least in potency.

(ii) Regarding the activity against hCA II, the tested compounds demonstrated a slightly better overall potency; K_i values ranged from 89.2 to 634.1 nM. Based on average potencies, the benzamide series, **5a-e**, showed the highest inhibitory activity followed by compounds **10a-e** then compounds **9a-e**. Interestingly, it was noticed that hybrids with the benzoyl moiety substituted with the electron donating $–OCH_3$ group (**5e**, **9e** and **10e**) revealed the highest potency in all series. Derivatives with the unsubstituted benzoyl or chloro/fluorobenzoyl group

came next in activity, while the bromobenzoyl compounds were the least in activity.

(iii) As per the activity of the compounds against hCA IX, a gradual shift in selectivity started to be noticed where the activity ranged from 68.3-421.3 nM. Unlike their activity towards hCA I and hCA II, derivatives **5a-e** revealed the least potency against hCA IX. The hybrids with the hydrazinyl-2-oxoethylamino spacer **10a-e** were the most potent followed by the hybrids with the shorter hydrazino spacer **9a-e**. Among the latter series **9a-e** and **10a-e**, the unsubstituted benzoyl derivatives **9a** and **10a** were the most potent followed by the chlorobenzoyl compounds **9c** and **10c**. The fluorobenzoyl and methoxybenzoyl hybrids were of moderate potency while the bromobenzoylisatins **9d** and **10d** were the least potent. The compound possessing the highest inhibitory activity against hCA IX was the unsubstituted benzoyl isatin derivative **10a**.

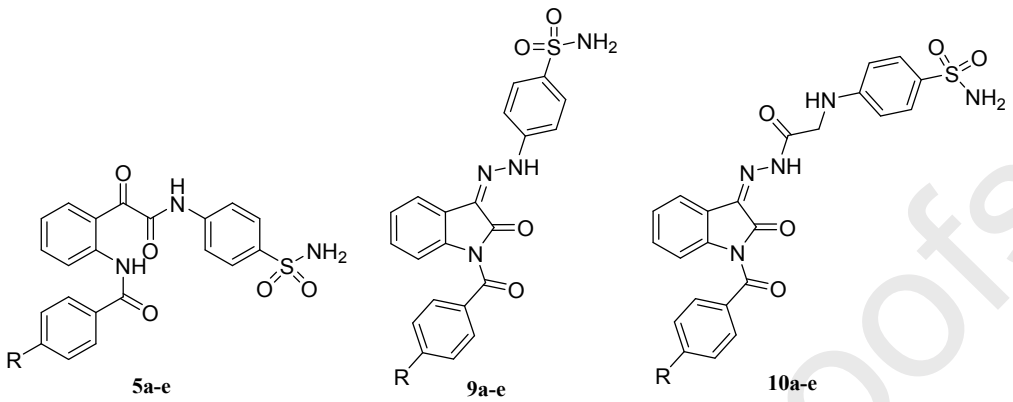
(iv) Finally, a significant shift in selectivity toward the inhibition of hCA XII was observed by all the tested compounds. K_i values spanned in the range of 21.5-175.1 nM. The hydrazinoisatin derivatives **9a-e** and the hydrazinyl-2-oxoethylamino derivatives **10a-e** displayed the highest potency compared to the benzamide derivatives **5a-e**. Regarding the influence of the substitution on the benzoyl moiety on activity, the order of activity was -OCH_3 >H >Cl> F > Br in series **5a-e** and **9a-e**, while in series **10a-e**, OCH_3 and unsubstituted benzoyl isatins **10e** and **10a**, respectively, were almost equipotent and preceded the halogenated benzoyl derivatives. The most active compounds against hCA XII belonged to the hydrazinyl-2-oxoethylamino isatins bearing the unsubstitued benzoyl and methoxybenzoyl groups, **10a** and **10e**, possessing K_i values of 21.5 and 22.3 nM, respectively.

(v) In summary, the tested compounds showed moderate selectivity to the human isoform IX but high selectivity toward the isoform XII with significantly low potency against hCA I. This selectivity profile suggested that the designed compounds might be potentially useful in cancer treatment. Focusing on the activity on hCA XII, being the highest inhibited isoform, the isatin derivatives, **9a-e** and **10a-e** were more efficient in the enzyme inhibition than their non-cyclic

benzamide bioisosteres **5a-e**. Also, the derivatives with the longer hydrazinyl-2-oxoethylamino spacer **10a-e** were more potent than their shorter hydrazine congeners **9a-e**. Furthermore, the unsubstituted benzoyl and methoxybenzoyl hybrids **10a** and **10e**, respectively, were almost equipotent and the most potent of all tested compounds.

(vi) Since this investigation is a continuation of the previous work on developing isatin-based carbonic anhydrase derivatives (compounds **V**, Figure 1) [25], it seems essentially useful to outline the progress of our findings. In the previous study, where the compounds possessed *N*-unsubstituted isatins or *N*-substituted derivatives bearing small alkyl or benzyl groups, there was no consistent relation between the activity against a specific isoform and the size of *N*-substitution. Concerning the effect of the spacer group on activity, the longer hydrazinyl-2-oxoethylamino linker shifted selectivity towards the tumor associated isoform hCA IX but not hCA XII. In the current investigation, a more informative and consistent SAR was developed in terms of the effect of the benzoyl substitution on potency which can be attributed to the fact that the carbonyl group of the benzoyl moiety might be involved in steric hindrance with the carbonyl group at position 2 of the isatin ring leading to a restricted rotation of the benzoyl group and thus locking the bioactive conformation of the molecule. On the other hand, and in accordance with the previous study, the increase in the length of the spacer group shifted selectivity towards the tumor-associated isoform hCA IX and additionally to hCA XII.

Table 1. Inhibition data of human CA isoforms hCA I, II, IX and XII with compounds **5a-e**, **9a-e**, **10a-e** and the standard inhibitor acetazolamide (AAZ) by a stopped flow CO_2 hydrase assay [29].



Compd. ID	R	K_i* (nM)				Selectivity ratio			
		hCAI	hCAII	hCAIX	hCAXII	I/IX	II/IX	I/XII	II/XII
5a	H	1891.8	150.6	362.9	88.6	5.21	0.41	21.35	1.70
5b	F	1962.5	163.1	421.3	164.4	4.66	0.39	11.94	0.99
5c	Cl	1555.8	192.4	274.3	140.9	5.67	0.70	11.04	1.36
5d	Br	2024.6	332.0	417.2	175.1	4.85	0.79	11.56	1.90
5e	CH_3	2456.7	89.2	221.8	60.4	11.08	0.40	40.67	1.48
9a	H	3447.1	418.4	84.9	32.9	40.60	4.93	104.77	12.72
9b	F	3604.3	458.2	172.4	48.2	20.91	2.66	74.78	9.51
9c	Cl	3417.7	390.2	145.7	42.4	23.46	2.68	80.61	9.20
9d	Br	5926.1	634.1	265.1	88.9	22.35	2.39	66.66	7.13
9e	OCH_3	5277.3	185.6	192.2	31.4	27.46	0.96	168.07	5.91
10a	H	6413.1	254.3	68.3	21.5	93.90	3.72	298.28	11.83
10b	F	6173.9	340.9	142.9	79.1	43.20	2.38	78.05	4.31
10c	Cl	6409.4	309.6	93.2	86.2	68.77	3.32	74.35	3.59
10d	Br	8429.2	549.7	197.1	130.2	42.77	2.79	64.74	4.22
10e	OCH_3	7898.6	171.5	164.8	22.3	47.93	1.04	354.20	7.69
AAZ	------	250	12	25	5.7	10	0.48	43.86	2.10

* Mean from 3 different assays, by a stopped flow technique (errors were in the range of ± 5-10 % of the reported values).

2.3. Molecular docking study

In order to rationalize the obtained CA inhibition results and the relative selectivity towards CA IX and CA XII isoforms, compound **10a**, which revealed the highest activity and selectivity for these isoforms, was selected for docking into the active pocket of CA I (PDBID: 3W6H), CA II (PDBID: 3HS4), CA IX (PDB ID: 3IAI) and CA XII (PDB ID: 1JD0) co-crystallized with acetazolamide (AAZ) [34]. The molecular docking was

performed using Molecular Operating Environment (MOE, 2015.10) and the results were presented in Figure 5 and Figures 1-6 in the supplementary materials.

(i) It was observed that the high activity and selectivity of compound **10a** to CA IX compared to the other isoforms resulted from its binding to the active site of the crystal structure of the enzyme through most of the fundamental binding interactions of the co-crystallized ligand, AAZ. Thus, it interacted through Zn^{2+} metal coordination with sulfonamide anion ($S\text{-}O^{-}$, S=NH), hydrogen bonding with Thr199 (similar to AAZ) and additionally to His94 through arene-arene interaction. (Figure 5C, Figures 1C, 4 and 6C in supplementary materials).

(ii) Regarding its interaction with CA XII, compound **10a** revealed binding mode through the same hot spots of AAZ, i.e. Zn^{2+}, Thr199, Thr200 and Leu198 via metal coordination and hydrogen bondings, respectively (Figure 5D, Figure 1D, 5 and 6D in supplementary materials). In addition to these fundamental interactions, three additional hydrogen bonds were observed between C=O of isatin core, C=O of benzoyl group and NH=N with Lys67, Asn69 and Gln92, respectively.

(iii) As per the interaction with the crystal structure of CA I, compound **10a** showed only Zn^{2+}coordination and hydrogen bonding with his200 explaining its mild inhibitory activity in the enzyme assay (Figure 5A, Figures 1A, 2 and 6A in supplementary materials).

(iv) Similarly, compound **10a** interacted with the binding site of CA II only via Zn^{2+}coordinate bond and hydrogen bond with leu198 (Figure 5B, Figures 1B, 3 and 6B in supplementary materials).

(v) Accordingly, the observed binding mode of compound **10a** to the crystal structures of CA I, CA II, CA IX and CA XII, were consistent and explicit to its activity and selectivity profile.

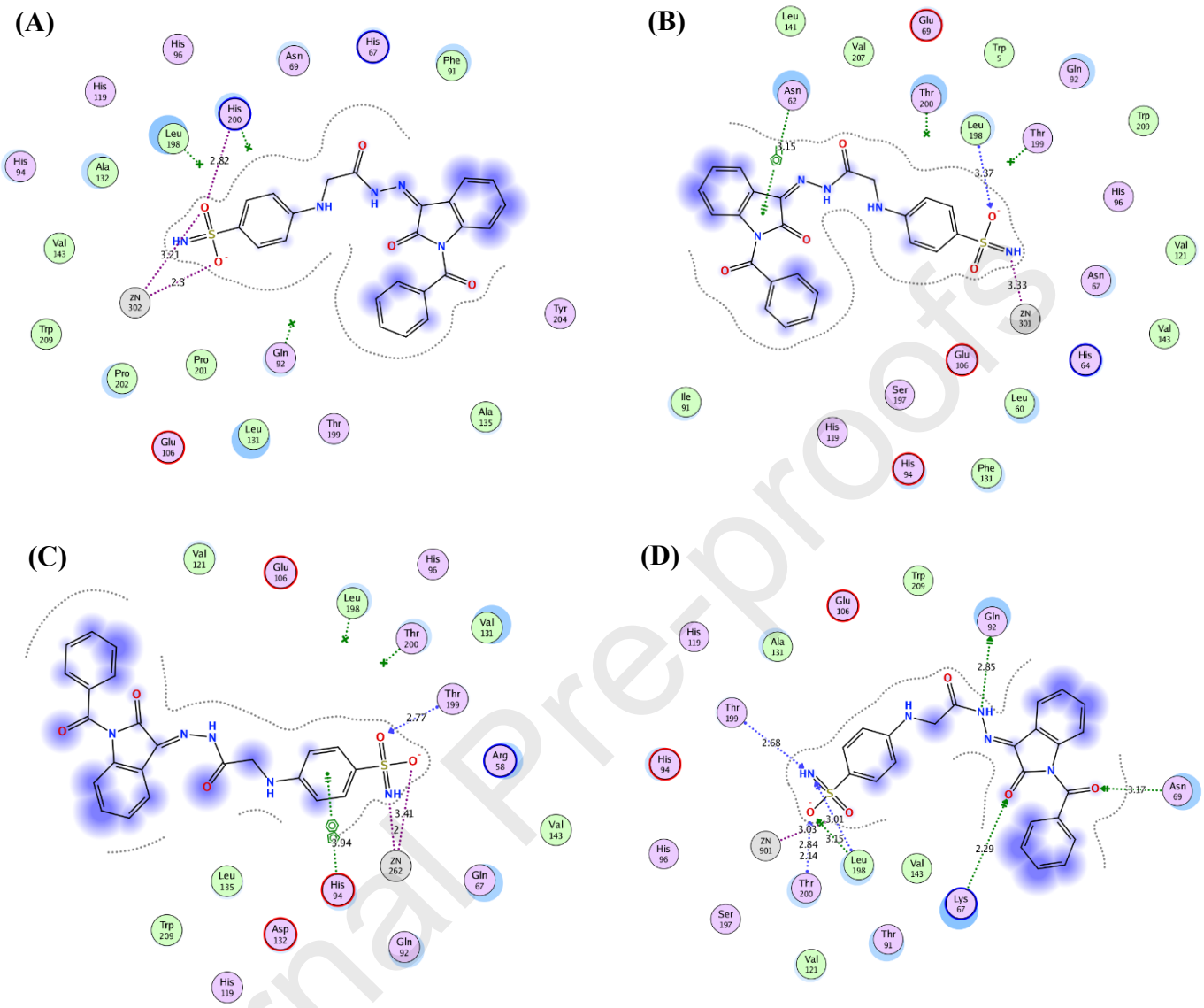


Figure 5. 2D diagram of compound **10a** showing its interaction with the CA I (A), CA II (B), CA IX (C) & CA XII (D) binding site.

3. Conclusion

Three series of benzenesulfonamide-based carbonic anhydrase inhibitors were synthesized. The design of the new compounds involved a hybrid approach combining the benzenesulfonamide zinc binding fragment to the privileged isatin fragment as the tail portion. Linking groups were either the shorter hydrazinyl linker (**9a-e**) or the longer hydrazinyl-2-oxoethylamino linker (**10a-e**). These compounds were synthesized by

condensation of the *N*-benzoylisatins **2a-e** with either hydrazinylbenzenesulfonamide **6**, or 4-((2-hydrazinyl-2-oxoethyl)amino)benzenesulfonamide **8**. Direct attachment of the pharmacophoric fragments via reaction of the *N*-benzoylisatins and benzenesulfonamide was synthetically inaccessible and instead open chain benzenesulfonamide isosteres (**5a-e**) were obtained. All compounds were subjected to enzyme inhibitory assay against four human CA isoforms (I, II, IX and XII). The most susceptible isoforms were IX and XII. Series **9a-e** and **10a-e** showed potent inhibitory activity preferentially against hCA IX, and selectively against hCA XII. Compounds **10a-e** were generally more potent than **9a-e** against these isoforms. Compounds **10a** and **10e** were the highest in potency against hCA XII (K_i = 21.5 and 22.3 nM, respectively). Interestingly, compound **10a** combined the highest activity against both tumor-associated isoforms IX and XII, together with the highest selectivity index. Concerning the substitution on the *N*-benzoyl moiety, it was observed that highest activity was associated with the absence of any substitution or with the presence of Cl and OCH_3 groups. To have a deep insight on the interaction with the four tested CA isoforms, compound **10a** was selected to perform docking experiments with the crystal structures of the four isoforms. A consistent correlation was observed between potency of the compound in the enzyme assay and its binding modes. Interactions with isoform XII, to which **10a** is highly potent, included the zinc ion, Thr199, Thr200, Leu198, Lys67, Asn69 and Gln92. Fewer interactions were observed with isoform IX, including the zinc ion, Thr199 and His94. The poor activity of **10a** against isoforms I and II was consistent with poor interactions where **10a** only interacted with zinc ion and His200 in isoform I, and in isoform II with zinc ion and Leu198.

4. Experimental

4.1. Chemistry

Melting Points are uncorrected and were carried out by open capillary tube method using Stuart SMP3 Melting Point apparatus. Infrared spectra were recorded on Shimadzu Infrared spectrometer IR Affinity-1 (FTIR- 8400S-Kyoto-Japan), and expressed in wave number (cm^{-1}). ^1H NMR and ^{13}C NMR Spectra were recorded on Bruker High Performance Digital FT-NMR Spectrometer Avance III 400 MHz, ^{13}C, 100 MHz NMR spectrometer. Chemical shifts were expressed in δ units and were related to that of the solvents. Mass

Spectra were recorded using ISQLT Thermo Scientific Mass spectrometer at The Regional Center for Mycology and Biotechnology, Al-Azhar University. Elemental Microanalyses were carried out at the Regional Center for Mycology and Biotechnology, Al-Azhar University. All the reactions were monitored by TLC using silica gel F254 plates (Sigma-Aldrich), using chloroform: methanol 9.5:0.5 as eluting system and were visualized by UV-lamp. Compounds **2a-e** [26], **6** [27], **7** and **8** [28] were prepared according to their reported methods.

*4.1.1. General procedure for preparation of compounds **5a-e***

A mixture of equimolar amounts of the appropriate 1-benzoylisatin derivative **(2a-e)** and sulfanilamide **(3)** (10 mmol) in glacial acetic acid (5 mL) was heated at 80°C for 3h. The obtained solid was filtered while hot, washed with ethanol and dried to obtain **5a-e** in a pure form.

4.1.1.1. *N-(2-(2-Oxo-2-((4-sulfamoylphenyl)amino)acetyl)phenyl)benzamide* **(5a)**

Yellow crystals, yield 89%, m.p. 250-252°C (decomposition); IR (KBr, υ cm^{-1}): 3389, 3314, 3277, 3111 (2NH and NH_2), 3069-3032 (CH aromatic), 1697, 1663 (3C=O), 1628 (bending NH), 1599-1522 (C=C), 1346, 1163 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.31 (s, 2H, NH_2, D_2O exchangeable), 7.37 (t, J = 7.60 Hz, 1H, aromatic H), 7.53 (t, J = 7.64 Hz, 2H, aromatic H), 7.61 (t, J = 7.12 Hz, 1H, aromatic H), 7.74-7.79 (m, 3H, aromatic H), 7.85-7.88 (m, 3H, aromatic H), 7.93 (d, J = 7.56 Hz, 2H, aromatic H), 8.09 (d, J = 8.24 Hz, 1H, aromatic H), 11.11 (s, 1H, NH, D_2O exchangeable), 11.28 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 120.3, 122.8, 124.6, 124.7, 127.1, 127.9, 129.2, 132.4, 132.7, 134.2, 135.0, 139.2, 139.9, 141.2 (aromatic carbons), 162.6 (C=O), 166.2 (C=O), 190.6 (C=O); MS, m/z [%]: 423 [M^+, 22], 77 [100]; Anal. Calcd. for $C_{21}H_{17}N_3O_5S$ (423.44): C, 59.57; H, 4.05; N, 9.92. Found: C, 59.31; H, 4.28; N, 10.14.

4.1.1.2. *4-Fluoro-N-(2-(2-oxo-2-((4-sulfamoylphenyl)amino)acetyl)phenyl) benzamide* **(5b)**

Yellow crystals, yield 68%, m.p. 246-248°C; IR (KBr, υ cm^{-1}): 3319, 3252, 3238, 3119 (2NH and NH_2), 3060 (CH aromatic), 1693, 1672 (3C=O), 1624 (bending NH), 1599-1526 (C=C), 1340, 1157 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.31 (s, 2H, NH_2, D_2O exchangeable), 7.34-7.39 (m, 3H, aromatic H), 7.75-7.79 (m, 3H, aromatic H), 7.84-7.87 (m, 3H, aromatic H), 7.99-8.02 (m, 3H, aromatic H), 11.09 (s, 1H, NH, D_2O exchangeable), 11.21 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 116.0, 116.3, 120.3, 123.1, 124.9, 125.3, 127.1, 130.7, 130.8, 132.2, 134.8, 138.9, 139.9, 141.2, 163.6, 166.1 (aromatic carbons), 162.5 (C=O), 165.2 (C=O), 190.3 (C=O); MS, m/z [%]: 441 [M^+, 5], 442 [(M^+ + 1), 28], 443 [(M^++ 2), 12], 123 [100]; Anal. Calcd. for $C_{21}H_{16}FN_3O_5S$ (441.43): C, 57.14; H, 3.65; N, 9.52. Found: C, 57.35; H, 3.83; N, 9.78.

4.1.1.3. 4-Chloro-N-(2-(2-oxo-2-((4-sulfamoylphenyl)amino)acetyl)phenyl)benzamide **(5c)**

Off white crystals, yield 77%, m.p. 264-266°C (decomposition); IR (KBr, υ cm^{-1}): 3337, 3294, 3252, 3190 (2NH and NH_2), 3081 (CH aromatic), 1678 (3C=O), 1636 (bending NH), 1593-1531 (C=C), 1335, 1161 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.30 (s, 2H, NH_2, D_2O exchangeable), 7.38 (t, J = 7.60 Hz, 1H, aromatic H), 7.61 (d, J = 8.44 Hz, 2H, aromatic H), 7.73 (d, J = 8.12 Hz, 1H, aromatic H), 7.78 (d, J = 8.88 Hz, 2H, aromatic H), 7.85 (t, J = 8.82 Hz, 3H, aromatic H), 7.94 (d, J = 8.48 Hz, 2H, aromatic H), 7.99 (d, J = 8.20 Hz, 1H, aromatic H), 11.09 (s, 1H, NH, D_2O exchangeable), 11.24 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 120.3, 123.1, 124.9, 125.3, 127.1, 129.2, 129.8, 132.2, 133.0, 134.8, 137.6, 138.7, 140.0, 141.2 (aromatic carbons), 162.4 (C=O), 165.2 (C=O), 190.3 (C=O); MS, m/z [%]: 457 [M^+, 2], 458 [(M^++ 1), 7], 459 [(M^+ + 2), 2], 139 [100]; Anal. Calcd. for $C_{21}H_{16}ClN_3O_5S$ (457.89): C, 55.09; H, 3.52; N, 9.18. Found: C, 55.32; H, 3.66; N, 9.40.

4.1.1.4. *4-Bromo-N-(2-(2-oxo-2-((4-sulfamoylphenyl)amino)acetyl)phenyl)benzamide* **(5d)**

Off white crystals, yield 46%, m.p. 256-258°C (decomposition); IR (KBr, υ cm^{-1}): 3300, 3294, 3260, 3190 (2NH and NH_2), 3078 (CH aromatic), 1678 (3C=O), 1636 (bending NH), 1589, 1531 (C=C), 1331, 1161 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.30 (s, 2H, NH_2, D_2O exchangeable), 7.38 (t, J = 7.56 Hz, 1H, aromatic H), 7.73-7.80 (m, 5H, aromatic H), 7.83-7.88 (m, 5H, aromatic H), 8.00 (d, J = 8.20 Hz, 1H, aromatic H), 11.09 (s, 1H, NH, D_2O exchangeable), 11.24 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 120.3, 123.0, 124.9, 125.3, 126.5, 127.1, 130.0, 132.2, 133.4, 134.8, 138.7, 140.0, 141.2 (aromatic carbons), 162.4 (C=O), 165.3 (C=O), 190.2 (C=O); MS, m/z [%]: 502 [M^+, 8], 381 [100]; Anal. Calcd. for $C_{21}H_{16}BrN_3O_5S$ (502.34): C, 50.21; H, 3.21; N, 8.37. Found: C, 50.49; H, 3.48; N, 8.61.

4.1.1.5. *4-Methoxy-N-(2-(2-oxo-2-((4-sulfamoylphenyl)amino)acetyl)phenyl)benzamide* **(5e)**

Yellow crystals, yield 47%, m.p. 221-223°C; IR (KBr, υ cm^{-1}): 3321, 3252 (2NH and NH_2), 3098-3009 (CH aromatic), 2963-2916 (CH aliphatic), 1682 (3C=O), 1628 (bending NH), 1585, 1531 (C=C), 1327, 1157 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.84 (s, 3H, OCH_3), 7.07 (d, J = 8.76 Hz, 2H, aromatic H), 7.30 (s, 2H, NH_2, D_2O exchangeable), 7.33 (t, J = 7.64 Hz, 1H, aromatic H), 7.73-7.79 (m, 3H, aromatic H), 7.84-7.87 (m, 3H, aromatic H), 7.93 (d, J = 8.76 Hz, 2H, aromatic H), 8.14 (d, J = 8.28 Hz, 1H, aromatic H), 11.11 (s, 1H, NH, D_2O exchangeable), 11.23 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 55.9 (OCH_3), 114.4, 120.3, 122.5, 124.0, 124.3, 126.3, 127.1, 129.9, 132.5, 135.2, 139.7, 139.9, 141.2, 162.9 (aromatic carbons), 162.8 (C=O), 165.5 (C=O), 190.8 (C=O); MS, m/z [%]: 453 [M^+, 23], 119 [100]; Anal. Calcd. for $C_{22}H_{19}N_3O_6S$ (453.47): C, 58.27; H, 4.22; N, 9.27. Found: C, 58.50; H, 4.45; N, 9.13.

4.1.2. General procedure for preparation of ***9a-e***

A mixture of the appropriate 1-benzoylisatin derivative **(2a-e)** (10 mmol) and 4-hydrazinylbenzenesulfonamide hydrochloride **(6)** (0.22 g, 11 mmol) in methanol (5 mL)

was stirred at room temperature for 3h. The obtained precipitate was filtered, washed with ethanol, dried and recrystallized from ethanol.

4.1.2.1. *4-(2-(1-Benzoyl-2-oxoindolin-3-ylidene)hydrazinyl)benzenesulfonamide* **(9a)**

Yellow crystals, yield 50%, m.p. 254-256°C (decomposition); IR (KBr, υ cm^{-1}): 3418, 3372, 3306 (NH and NH_2), 3086-3059 (CH aromatic), 1747, 1690 (2C=O), 1597 (bending NH), 1570, 1508 (C=C), 1331, 1150 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.28 (s, 2H, NH_2, D_2O exchangeable), 7.35 (t, J = 7.52 Hz, 1H, aromatic H), 7.46 (t, J = 7.78 Hz, 1H, aromatic H), 7.54 (t, J = 7.60 Hz, 2H, aromatic H), 7.66 (d, J = 7.40 Hz, 1H, aromatic H), 7.70 (d, J = 8.84 Hz, 2H, aromatic H), 7.78-7.82 (m, 3H, aromatic H), 7.86-7.89 (m, 3H, aromatic H), 12.41 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 115.0, 115.5, 119.4, 122.8, 125.5, 127.7, 128.6, 129.5, 129.9, 133.1, 134.8, 138.7, 138.8, 145.6, (aromatic carbons), 160.3 (C=O), 168.9 (C=O); MS, m/z [%]: 420 [M^+, 100], 421[(M^++ 1), 16], 422 [(M^++ 2), 2]; Anal. Calcd. for $C_{21}H_{16}N_4O_4S$ (420.44): C, 59.99; H, 3.84; N, 13.33. Found: C, 60.13; H, 3.96; N, 13.59.

4.1.2.2. *4-(2-(1-(4-Fluorobenzoyl)-2-oxoindolin-3-ylidene)hydrazinyl)benzene-sulfonamide* **(9b)**

Yellow crystals, yield 45%, m.p. 284-286°C (decomposition); IR (KBr, υ cm^{-1}): 3414, 3298, 3252 (NH and NH_2), 3074 (CH aromatic), 1709, 1686 (2C=O), 1601 (bending NH), 1570, 1504 (C=C), 1331, 1150 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.29 (s, 2H, NH_2, D_2O exchangeable), 7.33-7.40 (m, 3H, aromatic H), 7.46 (t, J = 7.74Hz, 1H, aromatic H), 7.71 (d, J = 8.72 Hz, 2H, aromatic H), 7.80 (d, J = 8.40 Hz, 3H, aromatic H), 7.86 (d, J = 8.08 Hz, 1H, aromatic H), 7.97-8.00 (m, 2H, aromatic H), 12.40 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 115.0, 115.5, 115.6, 115.8, 119.4, 122.8, 125.5, 127.6, 127.8, 129.5, 131.2, 131.3, 132.9, 133.0, 138.7, 138.8, 145.6, 163.9, 166.4 (aromatic carbons), 160.3 (C=O), 167.7 (C=O); MS, m/z [%]: 438 [M^+, 28], 440 [(M^++ 2), 21], 167 [100]; Anal. Calcd. for $C_{21}H_{15}FN_4O_4S$ (438.43): C, 57.53; H, 3.45; N, 12.78. Found: C, 57.80; H, 3.67; N, 12.62.

4.1.2.3. *4-(2-(1-(4-Chlorobenzoyl)-2-oxoindolin-3-ylidene)hydrazinyl)benzene-sulfonamide* **(9c)**

Yellow crystals, yield 58%, m.p. 288-290°C (decomposition); IR (KBr, υ cm^{-1}): 3445 (NH and NH_2), 3050 (CH aromatic), 1732, 1697 (2C=O), 1639 (bending NH), 1597-1516 (C=C), 1350, 1153 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.28 (s, 2H, NH_2, D_2O exchangeable), 7.36 (t, J = 7.54 Hz, 1H, aromatic H), 7.47 (t, J = 7.82 Hz, 1H, aromatic H), 7.61 (d, J = 8.36 Hz, 2H, aromatic H), 7.71 (d, J = 8.72 Hz, 2H, aromatic H), 7.78-7.82 (m, 3H, aromatic H), 7.89-7.91 (m, 3H, aromatic H), 12.39 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 115.0, 115.6, 119.4, 122.8, 125.6, 127.6, 127.8, 128.7, 129.6, 131.7, 133.7, 137.8, 138.6, 138.7, 145.6 (aromatic carbons), 160.3 (C=O), 167.9 (C=O); MS, m/z [%]: 454 [M^+, 100], 455 [(M^++ 1), 32], 456 [(M^++ 2), 47]; Anal. Calcd. for $C_{21}H_{15}ClN_4O_4S$ (454.89): C, 55.45; H, 3.32; N, 12.32. Found: C, 55.62; H, 3.54; N, 12.60.

4.1.2.4. *4-(2-(1-(4-Bromobenzoyl)-2-oxoindolin-3-ylidene)hydrazinyl)benzene-sulfonamide* **(9d)**

Yellow crystals, yield 60%, m.p.> 300°C (decomposition); IR (KBr, υ cm^{-1}): 3375, 3263 (NH and NH_2), 3086 (CH aromatic), 1724, 1674 (2C=O), 1597 (bending NH), 1566-1520 (C=C), 1327, 1146 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.28 (s, 2H, NH_2, D_2O exchangeable), 7.36 (t, J = 7.54 Hz, 1H, aromatic H), 7.47 (t, J = 7.78 Hz, 1H, aromatic H), 7.71 (d, J = 8.76 Hz, 2H, aromatic H), 7.75 (d, J = 8.52 Hz, 2H, aromatic H), 7.79-7.83 (m, 4H, aromatic H), 7.85-7.88 (m, 1H, aromatic H), 7.91 (d, J = 8.12 Hz, 1H, aromatic H), 12.39 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 115.0, 115.4, 116.7, 119.4, 122.8, 125.6, 127.4, 127.7, 129.5, 131.6, 131.8, 133.5, 134.1, 138.6, 138.8, 145.6 (aromatic carbons), 160.3 (C=O), 168.0 (C=O); MS, m/z [%]: 499 [M^+, 27], 285 [100]; Anal. Calcd. for $C_{21}H_{15}BrN_4O_4S$ (499.34): C, 50.51; H, 3.03; N, 11.22. Found: C, 50.43; H, 3.19; N, 11.43.

4.1.2.5. *4-(2-(1-(4-Methoxybenzoyl)-2-oxoindolin-3-ylidene)hydrazinyl)benzene-sulfonamide* **(9e)**

Yellow crystals, yield 45%, m.p. 220-222°C; IR (KBr, υ cm^{-1}): 3406, 3317, 3240 (NH and NH_2), 3050 (CH aromatic), 2936 (CH aliphatic), 1674, 1655 (2C=O), 1605 (bending NH), 1560-1508 (C=C), 1327, 1149 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.88 (s, 3H, OCH_3), 7.07 (d, J = 8.64 Hz, 2H, aromatic H),7.29 (s, 2H, NH_2, D_2O exchangeable), 7.32 (t, J = 7.68 Hz, 1H, aromatic H), 7.44 (t, J = 7.74 Hz, 1H, aromatic H), 7.68-7.71 (m, 3H, aromatic H), 7.78-7.81 (m, 3H, aromatic H), 7.91 (d, J = 8.64 Hz, 2H, aromatic H), 12.46 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 56.1 (OCH_3), 114.1, 114.9, 115.1, 119.4, 122.5, 125.1, 126.3, 127.8, 127.9, 129.5, 132.9, 138.7, 139.1, 145.6, 163.8 (aromatic carbons), 160.4 (C=O), 167.9 (C=O); MS, m/z [%]: 450 [M^+, 9], 451 [(M^++1), 3], 77 [100]; Anal. Calcd. for $C_{22}H_{18}N_4O_5S$ (450.47): C, 58.66; H, 4.03; N, 12.44. Found: C, 58.74; H, 4.21; N, 12.69.

4.1.3. *General procedure for preparation of compounds* ***10a-e***

To a mixture of the appropriate 1-benzoylisatin derivative **(2a-e)** (10 mmol), 4-((2-hydrazinyl-2-oxoethyl)amino)benzenesulfonamide **(8)** (0.27 g, 11 mmol) in methanol (5mL) was added 2 drops of conc. hydrochloric acid. The reaction mixture was stirred at room temperature for 3h, the obtained precipitate was filtered, washed with ethanol, dried and recrystallized from ethanol to give **10a-e** in a pure form.

4.1.3.1. *4-((2-(2-(1-Benzoyl-2-oxoindolin-3-ylidene)hydrazinyl)-2-oxoethyl)amino) benzenesulfonamide* **(10a)**

Yellow crystals, yield 55%, m.p.244-246°C (decomposition); IR (KBr, υ cm^{-1}): 3383, 3240, 3105 (2NH and NH_2), 3067 (CH aromatic), 2916 (CH aliphatic), 1751, 1697

(3C=O), 1601 (bending NH), 1516 (C=C), 1346, 1150 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 4.36 (s, 2H, CH_2), 6.70 (d, J = 8.56 Hz, 2H, aromatic H), 6.96 (s, 2H, NH_2, D_2O exchangeable), 7.37 (t, J = 7.60 Hz, 1H, aromatic H), 7.51-7.56 (m, 5H, aromatic H), 7.61 (t, J = 7.84 Hz, 1H, aromatic H), 7.67 (t, J = 6.80 Hz, 1H, aromatic H), 7.84-7.91 (m, 3H, aromatic H), 8.32 (s, 1H, NH, D_2O exchangeable), 11.59 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 44.9 (CH_2), 112.0, 115.4, 115.7, 117.2, 121.1, 121.5, 125.1, 125.7, 127.8, 128.6, 128.7, 130.0, 131.5, 131.9, 132.8, 133.4, 134.6, 141.1, 151.4, 162.8 (aromatic carbons), 160.4 (C=O), 168.5 (C=O), 169.0 (C=O); MS, m/z [%]: 477 [M^+, 7], 478 [(M^++1), 2], 105 [100]; Anal. Calcd. for $C_{23}H_{19}N_5O_5S$ (477.50): C, 57.85; H, 4.01; N, 14.67. Found: C, 58.11; H, 4.27; N, 14.58.

4.1.3.2. *4-((2-(2-(1-(4-Fluorobenzoyl)-2-oxoindolin-3-ylidene)hydrazinyl)-2-oxoethyl) amino)benzenesulfonamide* **(10b)**

Yellow crystals, yield 45%, m.p. 259-261^{o}C (decomposition); IR (KBr, υ cm^{-1}): 3379, 3263, 3171, 3121 (2NH and NH_2), 3078, 3013 (CH aromatic), 2909 (CH aliphatic), 1744, 1693 (3C=O), 1601 (bending NH), 1508 (C=C), 1342, 1150 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 4.37 (s, 2H, CH_2), 6.70 (d, J = 8.44 Hz, 2H, aromatic H), 6.96 (s, 2H, NH_2, D_2O exchangeable), 7.34-7.39 (m, 3H, aromatic H), 7.53-7.56 (m, 3H, aromatic H), 7.61 (t, J = 7.86 Hz, 1H, aromatic H), 7.89 (d, J = 8.16 Hz, 1H, aromatic H), 7.94-7.98 (m, 2H, aromatic H), 8.32 (s, 1H, NH, D_2O exchangeable), 11.59 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 44.9 (CH_2), 112.0, 115.5, 115.6, 115.7, 115.8, 115.9, 117.3,121.0, 121.5, 125.1, 125.7, 126.2, 127.8, 130.7, 131.9, 133.0, 133.2, 133.3, 141.0, 141.5, 151.4, 164.0, 164.2, 166.6, 166.7 (aromatic carbons), 160.4 (C=O), 167.3 (C=O), 167.8 (C=O); MS, m/z [%]: 495 [M^+, 12], 497 [(M^++2), 2], 76 [100]; Anal. Calcd. for $C_{23}H_{18}FN_5O_5S$ (495.49): C, 55.75; H, 3.66; N, 14.13. Found: C, 55.97; H, 3.39; N, 14.29.

4.1.3.3. *4-((2-(2-(1-(4-Chlorobenzoyl)-2-oxoindolin-3-ylidene)hydrazinyl)-2-oxoethyl) amino)benzenesulfonamide* **(10c)**

Yellow crystals, yield 50%, m.p. 250-252°C (decomposition); IR (KBr, υ cm^{-1}): 3379, 3260, 3167, 3113 (2NH and NH_2), 3071, 3013 (CH aromatic), 2909 (CH aliphatic), 1747, 1693 (3C=O), 1597 (bending NH), 1520 (C=C), 1342, 1150 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 4.36 (s, 2H, CH_2), 6.70 (d, J = 8.36 Hz, 2H, aromatic H), 6.97 (s, 2H, NH_2, D_2O exchangeable), 7.37 (t, J = 7.58 Hz, 1H, aromatic H), 7.55 (d, J = 8.60 Hz, 2H, aromatic H), 7.59-7.61 (m, 3H, aromatic H), 7.83-7.89 (m, 3H, aromatic H), 7.94 (d, J = 8.16 Hz 1H, aromatic H), 8.32 (s, 1H, NH, D_2O exchangeable), 11.61 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 44.8 (CH_2), 112.0, 115.8, 115.9, 117.3,121.0, 121.5, 125.8, 127.8, 128.8, 129.2,131.6, 131.9, 133.1, 138.3, 140.9, 151.3, (aromatic carbons), 160.4 (C=O), 166.9 (C=O), 167.4 (C=O); MS, m/z [%]: 511 [M$^+$, 11], 438 [100]; Anal. Calcd. for $C_{23}H_{18}ClN_5O_5S$ (511.94): C, 53.96; H, 3.54; N, 13.68. Found: C, 54.12; H, 3.71; N, 13.87.

4.1.3.4. *4-((2-(2-(1-(4-Bromobenzoyl)-2-oxoindolin-3-ylidene)hydrazinyl)-2-oxoethyl) amino)benzenesulfonamide* **(10d)**

Yellow crystals, yield 50%, m.p. 245-247°C (decomposition); IR (KBr, υ cm^{-1}): 3379, 3352, 3256 (2NH and NH_2), 3094 (CH aromatic), 2905 (CH aliphatic), 1763, 1693 (3C=O), 1601 (bending NH), 1516 (C=C), 1346, 1150 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 4.37 (s, 2H, CH_2), 6.68-6.72 (m, 2H, aromatic H), 6.98 (s, 2H, NH_2, D_2O exchangeable), 7.37 (t, J = 7.54 Hz, 1H, aromatic H), 7.54-7.57 (m, 2H, aromatic H), 7.61 (t, J = 8.04 Hz, 1H, aromatic H), 7.70-7.83 (m, 5H, aromatic H), 7.94 (d, J = 8.12 Hz 1H, aromatic H), 8.32 (s, 1H, NH, D_2O exchangeable), 11.60 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 45.0 (CH_2), 112.0, 115.6, 115.8, 117.3, 121.0, 121.5, 125.9, 127.8, 129.8, 130.4, 131.5, 131.9, 132.8, 133.4, 133.9, 140.9, 141.3, 151.3 (aromatic carbons), 160.4 (C=O), 167.1 (C=O), 168.1 (C=O); MS, m/z [%]: 555 [M$^+$, 2], 50 [100]; Anal. Calcd. for $C_{23}H_{18}BrN_5O_5S$ (556.39): C, 49.56; H, 3.26; N, 12.59. Found: C, 49.81; H, 3.43; N, 12.62.

4.1.3.5. *4-((2-(2-(1-(4-Methoxybenzoyl)-2-oxoindolin-3-ylidene)hydrazinyl)-2-oxoethyl) amino)benzenesulfonamide* **(10e)**

Yellow crystals, yield 55%, m.p. 242-244ºC (decomposition); IR (KBr, υ cm^{-1}): 3387, 3352, 3233 (2NH and NH_2), 3097 (CH aromatic), 2970-2905 (CH aliphatic), 1743, 1693 (3C=O), 1601 (bending NH), 1516 (C=C), 1342, 1150 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.89 (s, 3H,OCH_3), 4.37 (s, 2H, CH_2), 6.71 (d, J = 8.36 Hz, 2H, aromatic H), 6.97 (s, 2H, NH_2, D_2O exchangeable), 7.06 (d, J = 8.68 Hz, 2H, aromatic H), 7.33 (t, J = 7.60 Hz, 1H, aromatic H), 7.54-7.60 (m, 4H, aromatic H), 7.72 (d, J = 8.12 Hz, 1H, aromatic H), 7.89 (d, J = 8.84 Hz, 2H, aromatic H), 8.32 (s, 1H, NH, D_2O exchangeable), 11.57 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 44.8 (CH_2), 56.1 (OCH_3), 111.8, 114.1, 115.1, 117.0, 121.1, 124.8, 125.4, 125.7, 126.1, 126.2, 127.8, 131.8, 133.1, 141.8, 151.4, 164.1 (aromatic carbons), 160.4 (C=O), 167.4 (C=O), 168.0 (C=O); MS, m/z [%]: 507 [M^+, 20], 64 [100]; Anal. Calcd. for $C_{24}H_{21}N_5O_6S$ (507.52): C, 56.80; H, 4.17; N, 13.80. Found: C, 56.63; H, 4.41; N, 13.57.

4.2. Carbonic anhydrase inhibition.

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalysed CO_2 hydration activity [29-33]. Phenol red (at a concentration of 0.2 mM) has been used as a pH indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as the buffer, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10-100 s at 25°C [29]. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor, at least six traces of the initial 5-10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled-deionized water and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3 and the

Cheng-Prusoff equation, as reported earlier [29-33], and represent the mean from at least three different determinations. All hCA isoforms were recombinant ones obtained in-house as reported earlier [1].

4.3. Molecular modeling.

All the molecular modeling studies were carried out using Molecular Operating Environment (MOE, 2015.10) software. All minimizations were performed with MOE until an RMSD gradient of 0.05 kcal·mol^{-1}Å^{-1} with MMFF94x force field and the partial charges were automatically calculated. The X-ray crystallographic structure of human carbonic anhydrase isoforms I, II, IX and XII co-crystalized with acetazolamide (PDB ID: 3W6H, 3HS4, 3IAI and 1JD0, respectively) were downloaded from the protein data bank [34]. Water molecules which are not involved in the binding were removed. The protein was prepared for the docking study using *Protonate 3D* protocol in MOE with default options. The 3D structures of the compounds were drawn by using the MOE of (Chemical Computing Group software, Canada) drawing tool bar. The Lowest energy conformer of the compounds (global-minima) was docked into the active site. All structures were drawn with deprotonation of their sulfonamide moiety and the negative charge was localized automatically by the program on S-O^{-}. The co-crystalized ligand (acetazolamide) was used to define the binding site for docking. Triangle Matcher placement method and London dG scoring function were used for docking. Docking setup was first validated by self-docking of the co-crystallized ligand (acetazolamide) in the vicinity of the binding site of the enzyme and by the ability of the docking pose to reproduce all the key interactions accomplished by the co-crystallized ligand with the hot spots in the active site. The validated setup was then used in predicting the ligand-receptor interactions at the binding site for the compounds of interest.

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Synthesis of some *N*-aroyl-2-oxindole benzenesulfonamide conjugates with carbonic anhydrase inhibitory activity

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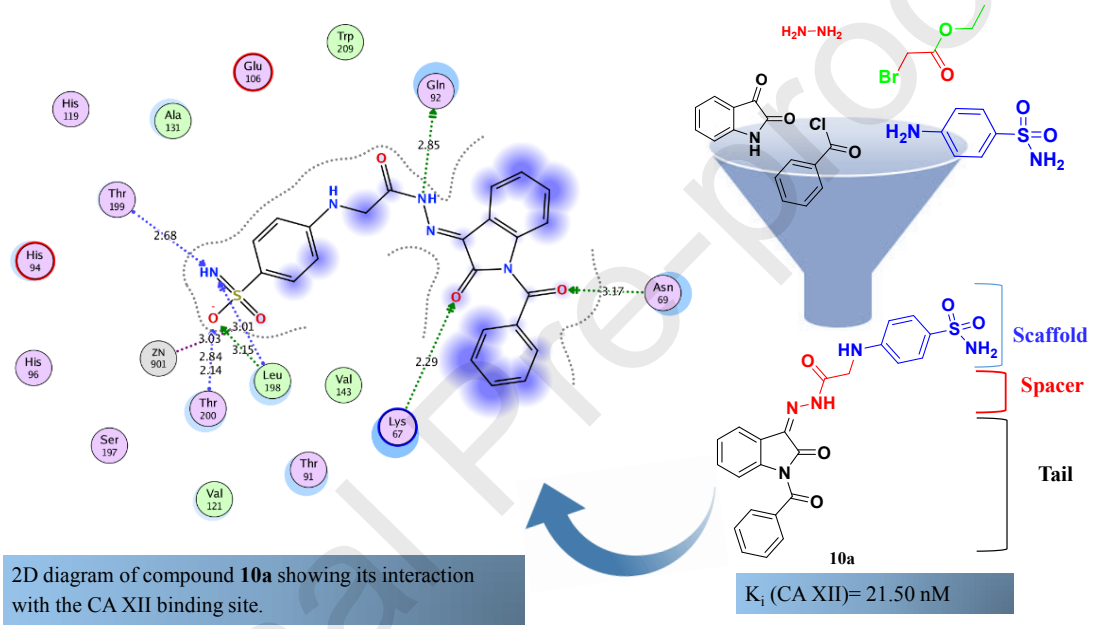
Graphical Abstract



2D diagram of compound **10a** showing its interaction with the CA XII binding site.

K_i (CA XII)= 21.50 nM

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Highlights

- Three series of benzenesulfonamide based compounds were prepared.

- All compounds were screened for CA inhibition against CA I, II, IX and XII isoforms.

- Compounds **9a-e** and **10a-e** preferentially inhibited the tumor-associated isoforms IX and XII.

- Compounds **10a** and **10e** were the highest in potency against hCA XII.

- Molecular docking of **10a** in the active sites of four CA isoforms confirmed its activity.