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PII: S0223-5234(17)30025-9

DOI: 10.1016/j.ejmech.2017.01.017

Reference: EJMECH 9168

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 28 December 2016

Revised Date: 8 January 2017

Accepted Date: 10 January 2017

Please cite this article as: W.M. Eldehna, G.H. Al-Ansary, S. Bua, A. Nocentini, P. Gratteri, A. Altoukhy, H. Ghabbour, H.Y. Ahmed, C.T. Supuran, Novel indolin-2-one-based sulfonamides as carbonic anhydrase inhibitors: Synthesis, *in vitro* biological evaluation against carbonic anhydrases isoforms I, II, IV and VII and molecular docking studies, *European Journal of Medicinal Chemistry* (2017), doi: 10.1016/j.ejmech.2017.01.017.

# Graphical abstract

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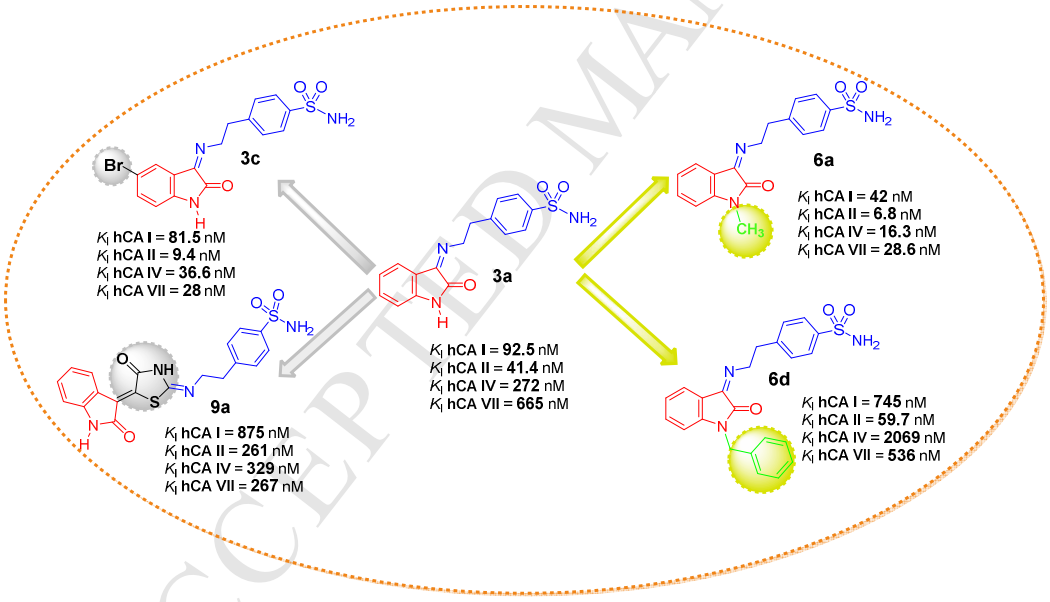
Three different series of novel sulfonamides incorporating substituted indolin-2-one moieties linked to benzenesulfonamide through aminoethyl or (4-oxothiazolidin-2-ylidene)aminoethyl linkers, were synthesized and evaluated for their inhibitory activity against a panel of carbonic anhydrase isoforms, hCA I, II, IV and VII.



# Novel indolin-2-one-based sulfonamides as carbonic anhydrase inhibitors: Synthesis, *in vitro* biological evaluation against carbonic anhydrases isoforms I, II, IV and VII and molecular docking studies

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**Abstract**

Herein we present the design, synthesis, and biological evaluation of three different series of novel sulfonamides (**3a-f**, **6a-f** and **9a-f**) incorporating substituted indolin-2-one moieties (as tails) linked to benzenesulfonamide (as zinc anchoring moieties) through aminoethyl or (4-oxothiazolidin-2-ylidene)aminoethyl linkers. The synthesized sulfonamides were evaluated *in vitro* for their inhibitory activity against the following human (h) carbonic anhydrase (hCA, EC 4.2.1.1) isoforms, hCA I, II, IV and VII. All these isoforms were inhibited by the sulfonamides reported here in variable degrees. hCA I was inhibited with $K_I$s in the range of 42–8550.9 nM, hCA II in the range of 5.9–761 nM; hCA IV in the range of 4.0–2069.5 nM, whereas hCA VII in the range of 13.2–694 nM. Molecular docking studies were carried out for some of the tested compounds within the hCA II active site, allowed us to rationalize the obtained inhibition results.

*Keywords: Synthesis; Carbonic anhydrase; 4-(2-Aminoethyl)benzenesulfonamide; Isatin.*

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## 1-Introduction

The carbonic anhydrases (CAs, EC 4.2.1.1) represent a superfamily of metalloenzymes, with seven different genetic families known to date, the α-, β-, γ-, δ-, ζ-, η- and θ-CAs, all of the which efficiently catalyze the reaction between $CO_2$ and water, with formation of bicarbonate and protons [1-5]. The inhibition and activation of CAs are well-understood processes: most types of classical inhibitors bind to the metal center within the enzyme active site, but recently a multitude of alternative inhibition mechanisms have been reported, with many diverse classes of inhibitors binding in different parts of the active site, without directly interacting with the catalytic metal ion. However, primary sulfonamides and their isosteres remain the main class of CA inhibitors (CAIs), with many such derivatives in clinical use for decades as diuretics or for the treatment of glaucoma, epilepsy, obesity and more recently cancer [1-5].

Recently, isatin (1*H*-indole-2,3-dione) has been emerged as a promising and attractive nucleus that possesses diverse interesting activity profiles, to name just a few, anticancer [6-10] and carbonic anhydrase inhibition activities [11-14]. In 2015, a novel series of 2/3/4-[(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)amino]benzenesulfonamides was developed as potential carbonic anhydrase inhibitors, compound **I** (Figure 1) displayed excellent inhibitory activity against the tumor associated CA isoforms IX and XII [11]. One year later, a molecular hybridization approach was adopted to design and synthesize two novel series of amido/ureidosubstituted benzenesulfonamides incorporating substituted-isatin moieties [12]. The most promising activity was observed against the tumor associated CA isoforms IX and XII. It is noteworthy that sulfonamide **II** (Figure 1) exhibited outstanding selectivity profile for the tumor associated isoforms CAs IX and XII. Also, Ibrahim *et al*. [13] explored the inhibitory activity of new series of isatinpyrazole-benzenesulfonamide hybrids against several CA isoforms. Compound **III** (Figure 1), with *N*-substituted isatin moiety, elicited better activity than its unsubstituted counterpart.

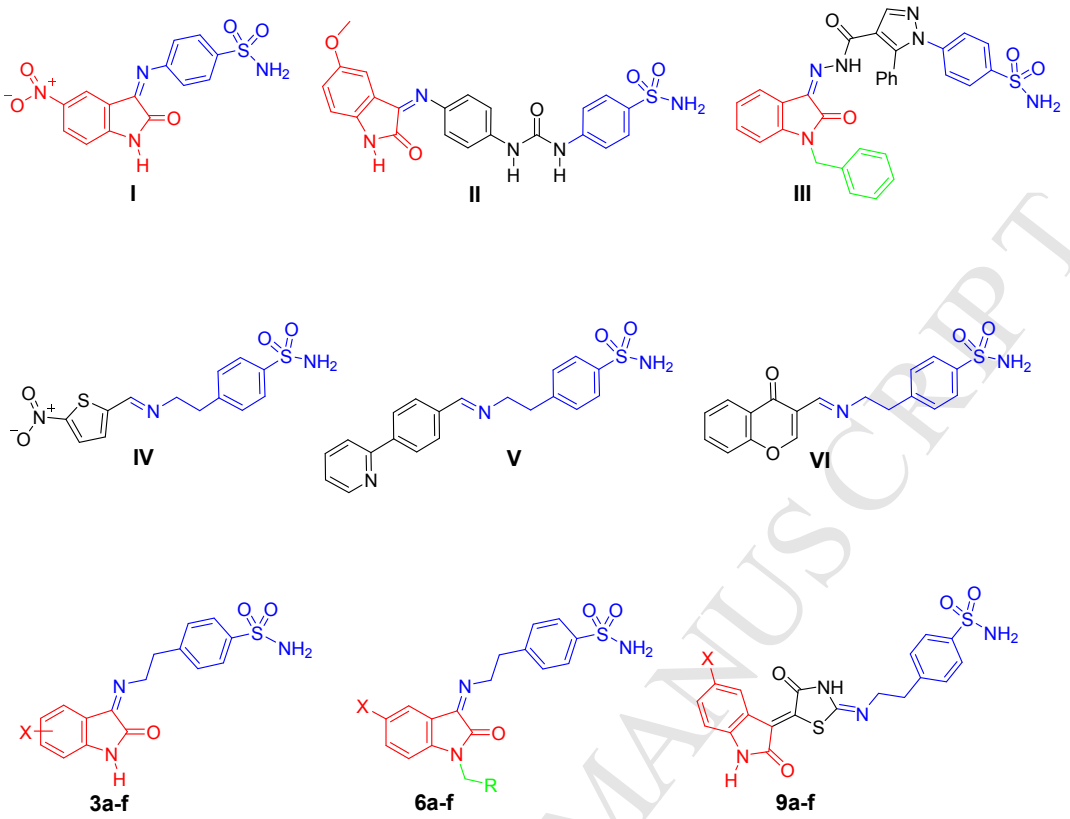



**Figure 1**. Structures of the reported carbonic anhydrase inhibitors **I-VI** and the target sulfonamides **3a-f**, **6a-f** and **9a-f**.

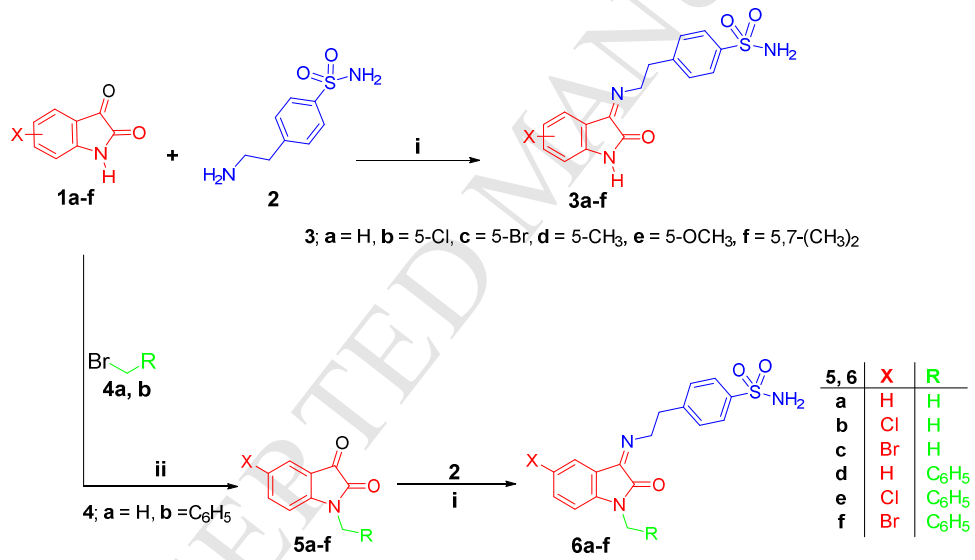
On the other hand, numerous studies pointed out the importance of incorporating the 4-(2-aminoethyl)benzenesulfonamide moiety in the design of several derivatives with potent CA inhibitory activity [15-20]. Among such derivatives, compound **IV** (Figure 1) [21], compound **V** (Figure 1) [22] and compound **VI** (Figure 1) [23] displayed good activities against different CA isoforms.

In view of the literature data mentioned above and as part of our ongoing effort to develop potent CAIs [24-26], herein we present the design, synthesis, and biological evaluation of different three series of novel sulfonamides incorporating un/substituted isatino moieties (as tails) linked to benzenesulfonamide (as zinc anchoring moieties) through aminoethyl or (4-oxothiazolidin-2-ylidene)aminoethyl linker. The synthesized sulfonamides will be *in vitro* evaluated for their inhibitory activity against a panel of hCA I, II, IV and VII isoforms, using stopped-flow CO2 hydrase assay.

## 2. Results and Discussion

### *2.1. Chemistry*

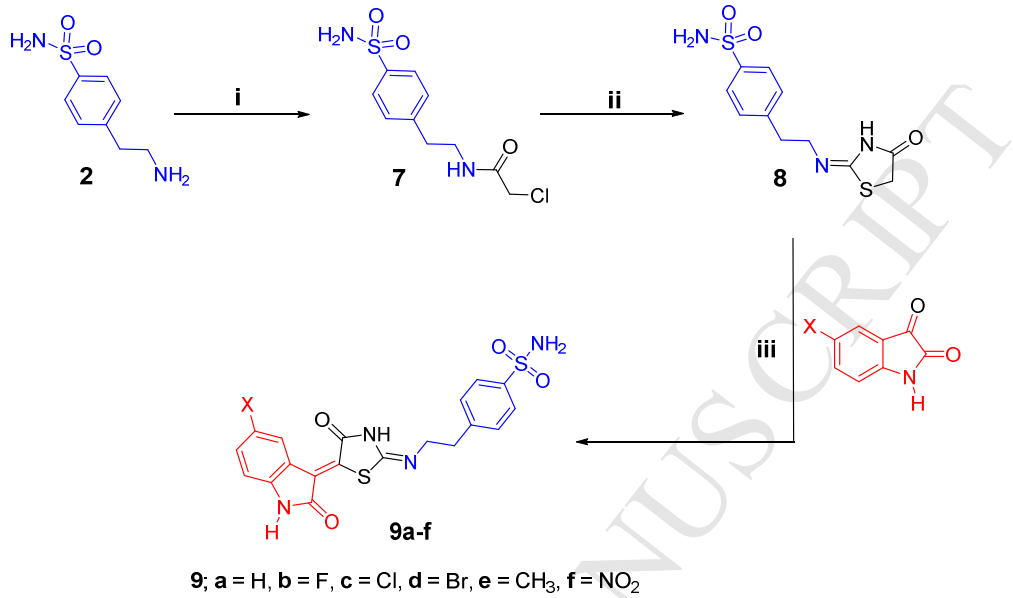
The synthetic strategies for the preparation of the target sulfonamides **3a-f**, **6a-f** and **9a-f** were depicted in Schemes **1** and **2**. The reaction of isatin derivatives **1a-f** with 4-(2-aminoethyl)benzenesulfonamide **2** in ethanol in the presence of a catalytic amount of glacial acetic acid furnished the target sulfonamides **3a-f**. While, the reaction of isatins **1a-c** with methyl bromide **4a** and benzyl bromide **4b** yielded N-substituted isatins **5a-f**, which was then reacted with 4-(2-aminoethyl)benzenesulfonamide **2** to afford the target sulfonamides **6a-f** (Scheme **1**).




**Scheme 1.** Synthesis of target sulfonamides **3a-f** and **6a-f**; Reagents and conditions: (**i**) Absolute ethyl alcohol / catalytic amount of glacial acetic acid / reflux 2 h, (**ii**) DMF, $K_2CO_3$, reflux 3 h.

In scheme 2, the amino function of 4-(2-aminoethyl)benzenesulfonamide **2** was acylated with 2-chloroacetyl chloride to give compound **3**, which underwent heterocyclization with $NH_4SCN$ in ethanol to furnish the key intermediate 4-(2-((4-oxothiazolidin-2-ylidene)amino)ethyl)benzenesulfonamide **8**. The later was reacted with different isatins in

refluxing glacial acetic acid in the presence of sodium acetate to afford the target sulfonamides **9a-f** (Scheme **2**).




**Scheme 2.** Synthesis of target sulfonamides **9a-f**; Reagents and conditions: (**i**) Dioxane / $ClCOCH_2Cl$ / r.t. 3 h, (**ii**) $NH_4SCN$ / Ethanol / reflux 3 h, (**iii**) Glacial acetic acid / Sodium acetate / reflux 5 h.

Postulated structures of the newly prepared sulfonamides were in full agreement with their spectral and elemental analyses data. IR spectra of the prepared sulfonamides **3a-f**, **6a-f** and **9a-f** showed the absorption bands of ($NH_2$), (C=O) and ($SO_2$) groups in the ranges of 3367-3159, 1739-1693 and 1346-1153 $cm^{-1}$, respectively. Their $^1$H NMR spectra revealed the appearance of two triplicate signals due to the aliphatic protons of the -$CH_2$-$CH_2$- around $\delta$ 3.10 and 4.20 *ppm*, also, their $^1$H NMR spectra revealed the presence of $D_2O$ exchangable protons ($NH_2$) of sulfonamide group around $\delta$ 7.30 *ppm*. Additionally, $^1$H NMR spectra of compounds **3a-f** and **9a-f** showed a singlet $D_2O$-exchangeable signal attributable to NH protons of the indolin-2-one moiety at a $\delta$ range 10.65-11.21 *ppm*. Also, the $^1$H NMR spectra of **6a-c** showed the signals of the aliphatic (N-C$\underline{H_3}$) protons around $\delta$ 3.15 *ppm*, while the benzylic (C$\underline{H_2}$) protons in **6d-f** appeared around $\delta$ 4.90 *ppm*.

On the other hand, $^{13}$C NMR spectra of the newly prepared sulfonamides **3a-f**, **6a-f** and **9a-f** showed two characteristic signals due to the carbons of the -$CH_2$-$CH_2$- between $\delta$ 36.88-37.26 and 52.69-55.10 *ppm*. Moreover, the $^{13}$C NMR spectra for such derivatives showed a signal resonating in the range $\delta$ 162.39-164.59 *ppm* attributable for the carbon of carbonyl group

(C=O), while the carbons of (N-C<u>H</u>$_3$) and benzylic (C<u>H</u>$_2$) groups of compounds **6a-c** and **6d-f** appeared around $\delta$ 26.08 and 42.95 *ppm*, respectively.

*2.2. Carbonic anhydrase inhibition*

The newly prepared sulfonamides **3a-f, 6a-f and 9a-f** were evaluated for their ability to inhibit four physiologically relevant hCA isoforms, hCA I, II, IV and VII by a stopped-flow CO2 hydrase assays [27]. The inhibition data of the prepared compounds and the sulfonamide acetazolamide **AAZ** (as a standard inhibitor) against the four isoforms are summarized in Table 1. The following structure-activity relationship (SAR) should be noted regarding the inhibition data of Table 1:

(i) The cytosolic isoform hCA I was efficiently inhibited by most sulfonamides prepared in this study ($K_I$s in the range of 42–899.4 nM), except compounds **6e**, **6f**, **9c** and **9d** ($K_I$ > 1 µM) (Table 1). Compounds **8**, **3a-c, 6a** and **6c** showed better activity ($K_I$s = 93.9, 92.5, 64.1, 81.5, 42.0 and 64.9 nM, respectively) than that of the standard drug **AAZ** ($K_I$ = 250 nM against hCA I). Concerning the activity of the first series (**3a-f**) with aminoethyl linker, it was found that substitution of the 5-position of the indolin-2-one moiety has an impact on their activity against hCA I. Incorporation of unsubstituted indolin-2-one moiety led to compound **3a** with good activity against hCA I ($K_I$ = 92.5 nM). While, introduction of chlorine or bromine atoms at the 5-position, compounds **3b** and **3c**, increased the activity ($K_I$s = 64.1 and 81.5 nM, respectively), suggesting that substitution with halogens as 5-Cl or 5-Br is advantageous. Otherwise, grafting methyl or methoxy substituents resulted in a slight decrease in the activity, hinting that grafting lipophilic electron withdrawing substituents, like halogens, is more beneficial for the activity than the lipophilic electron donating ones, like methyl or methoxy.

We then investigate the effect of *N*-alkylation of the indolin-2-one moiety in the second series **6a-f**. Notably, *N*-methylated derivatives **6a** and **6c** displayed better activity ($K_I$s = 42 and 64.9 nM, respectively) than their corresponding counterparts **3a** and **3c** ($K_I$s = 92.5 and 81.5 nM, respectively), suggesting that *N*-methylation may be useful for activity against hCA I. Whereas, *N*-benzylation led to compounds **6d-f** with much decreased activity ($K_I$s = 745.6, 8550.9 and 6149.3 nM, respectively), than their corresponding members **3a-c**.

Comparing the activity of compounds **9** and **3** revealed that, the (4-oxothiazolidin-2-ylidene)aminoethyl linker dramatically decreased the activity. Compounds **9a,c,d** elicited

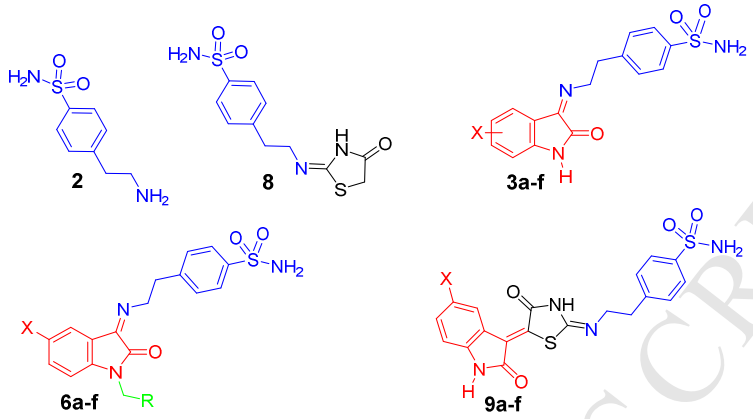
elevated $K_I$ values ($K_I$s = 875.4, 4450.2 and 3603.7 nM, respectively) than their corresponding analogues **3a-c** ($K_I$s = 92.5, 64.1 and 81.5 nM, respectively).

(ii) All the investigated sulfonamides acted as good inhibitors for the physiologically dominant isoform hCA II ($K_I$ values ranging between 5.9 and 761.9 nM). Superiorly, sulfonamides **8**, **3c** and **6a-c** emerged as single-digit nanomolar hCA II inhibitors with $K_I$s values of 6.6, 9.4, 6.8, 7.7 and 5.9 nM, respectively, which are more potent than the standard drug **AAZ** ($K_I$ = 12 nM against hCA II). In the same fashion, the SAR observed for hCA II inhibition is similar to that of hCA I. Generally substitution of the 5-position of the indolin-2-one moiety is beneficial for activity. Introduction of 5-Cl substituent led to compound **3b** ($K_I$s = 17.9 nM) with 2.3-folds increased activity than the unsubstituted counterpart **3a** ($K_I$s = 41.4 nM). Furthermore, incorporation of the more lipophilic 5-Br substituent, compound **3c**, significantly enhanced the activity against hCA II ($K_I$s = 9.4 nM). Otherwise, grafting methyl or methoxy groups at 5-position maintained the activity ($K_I$s = 33.8 and 34.4 nM, respectively), suggesting that substitution with halogens as 5-Cl or 5-Br is more advantageous than methyl or methoxy substituents. The order of activities of the substituted indolin-2-one derivatives in this series was decreased in the order of 5-Br > 5-Cl > 5-$CH_3$ > 5-O $CH_3$ > 5,7-$(CH_3)_2$.

On the other hand, *N*-methylation of first series **3**, resulted in considerable improvement of the activity and led to the strongest inhibitors reported here **6a-c** with their single-digit nanomolar activity for this isoform ($K_I$s = 6.8, 7.7 and 5.9 nM, respectively). Remarkably, the brominated derivative **6c**, displayed the better inhibitory activity in this series also. Finally, *N*-benzylation and usage of (4-oxothiazolidin-2-ylidene)aminoethyl linker had negative effect on inhibition of hCA II.

(iii) The membrane-bound isoform hCA IV was potently inhibited by all substituted sulfonamides of the first series **3b-f** with $K_I$s ranging between 4.0 and 46.1 nM. Contrariwise, the unsubstituted analogue **3a** displayed moderate inhibitory activity towards hCA IV ($K_I$ = 272.3 nM), suggesting that substitution of the indolin-2-one moiety is indispensable for activity of this series against hCA IV. The order of activities of the substituted indolin-2-one members in such series was decreased in the order of 5-$CH_3$ > 5,7-$(CH_3)_2$ > 5-Br > 5-O $CH_3$ > 5-Cl.

**Table 1**: Inhibition data of human CA isoforms hCA I, II, IV and VII with sulfonamides **2**, **8**, **3a-f**, **6a-f** and **9a-f** determined by stopped-flow $CO_2$ hydrase assay, using acetazolamide (AAZ) as a standard drug.




| Comp. | X | R | $K_I$ (nM) | | | |
|---|---|---|---|---|---|---|
| | | | hCA I | hCA II | hCA IV | hCA VII |
| **2** | - | - | 94.7 | 36.8 | 348.8 | 432.2 |
| **8** | - | - | 93.9 | 6.6 | 79.4 | 13.2 |
| **3a** | H | - | 92.5 | 41.4 | 272.3 | 665.3 |
| **3b** | Cl | - | 64.1 | 17.9 | 46.1 | 43.6 |
| **3c** | Br | - | 81.5 | 9.4 | 36.6 | 28.0 |
| **3d** | $CH_3$ | - | 316.0 | 33.8 | 4.0 | 121.4 |
| **3e** | $OCH_3$ | - | 206.2 | 34.4 | 42.8 | 88.6 |
| **3f** | 5,7-$(CH_3)_2$ | - | 255.9 | 50.5 | 26.6 | 90.9 |
| **6a** | H | H | 42.0 | 6.8 | 16.3 | 28.6 |
| **6b** | Cl | H | 667.9 | 7.7 | 31.2 | 91.0 |
| **6c** | Br | H | 64.9 | 5.9 | 33.1 | 177.6 |
| **6d** | H | $C_6H_5$ | 745.6 | 59.7 | 2069.5 | 536.5 |
| **6e** | Cl | $C_6H_5$ | 8550.9 | 713.9 | 1799.5 | >10000 |
| **6f** | Br | $C_6H_5$ | 6149.3 | 761.9 | 1433.5 | 694.3 |
| **9a** | H | - | 875.4 | 261.6 | 329.3 | 267.6 |
| **9b** | F | - | 899.4 | 249.3 | 270.2 | 347.5 |
| **9c** | Cl | - | 4450.2 | 302.1 | 44.7 | 605.0 |
| **9d** | Br | - | 3603.7 | 157.4 | 40.7 | 79.3 |
| **9e** | $CH_3$ | - | 845.2 | 63.9 | 36.3 | 67.2 |
| **9f** | $NO_2$ | - | 264.9 | 28.6 | 36.7 | 16.7 |
| **AAZ** | - | - | 250 | 12 | 74 | 2.5 |

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

On the other hand, the *N*-methylated sulfonamides **6a-c** belonging to the second series showed improved hCA IV inhibitory action ($K_I$s = 16.3, 31.2 and 33.13 nM, respectively), with respect to their analogues in the first series **3a-c** ($K_I$s = 272.3, 46.1 and 36.6 nM, respectively). However, the *N*-benzylated congeners **6d-f**, belonging to the same series, showed diminished inhibitory activity against hCA IV. Regarding the activity of the third series **9**, incorporation of unsubstituted indolin-2-one moiety resulted in sulfonamide **9a** with moderate activity against hCA IV ($K_I$ = 329.3 nM). While appending a small fluorine substituent at 5 position as in compound **9b** slightly improved the activity ($K_I$ = 270.2 nM), grafting of larger chloro, bromo, methyl, nitro or methoxy groups at the 5-position of indolin-2-one moiety as in compounds **9c-f**, greatly enhanced the activity against hCA IV ($K_I$s = 44.7, 40.7, 36.3 and 36.7 nM, respectively). This suggests that substitution at 5-positions with large lipophilic group is more favorable to the inhibitory activity towards hCA IV.

(iv) The third cytosolic isoform examined here was hCA VII. It was obvious from the obtained results, in Table 1, that most the synthesized sulfonamides displayed excellent to modest inhibitory activity against hCA VII. In particular, compounds **8** and **9f** emerged as the most active sulfonamides against hCA VII with $K_I$ values of 13.2 and 16.7 nM, respectively. Indeed only compound **6e** could not inhibit hCA VII up to 10000 nM.

Likewise the other cystolic hCA I and hCA II isoforms, substitution with halogens at 5-position (**3b** and **3c**) has positive effect for the activity towards hCA VII in the first series. Thence, the order of activity of the first series derivatives was decreased in the order of 5-Br > 5-Cl > 5-$OCH_3$ > 5,7-$(CH_3)_2$ > 5-$CH_3$ >>>> unsubstituted. Moreover, *N*-methylation (**6a-c**) maintained the activity ($K_I$s in the range of 28.6–177.6 nM) but *N*-benzylation (**6d-f**) negatively affected the activity against hCA VII ($K_I$s in the range of 536.5– >10000 nM). Comparing the activity of compounds **3** and **9** revealed that, the (4-oxothiazolidin-2-ylidene)aminoethyl linker seemed to show better activity than aminoethyl linker for unsubstituted (**9a**) and 5-$CH_3$ substituted (**9e**) sulfonamides. On the other hand, the (4-oxothiazolidin-2-ylidene)aminoethyl linker showed decreased activity in case of 5-Cl and 5-Br derivatives (**9c** and **9d**).

*2.3. Molecular modeling studies*

In order to rationalize the tendency of the inhibitory activities with the substitution patterns of the synthesized benzenesulfonamides, compound **8** and the 5-bromo-2-oxoindolin **3c**, **6c**, **6f** and **9d** were submitted to modelling investigations within hCAII binding cavity (PDBID: 5JLT). The compounds were shown to orient the aromatic sulfonamide moiety deeply into the active site region establishing two hydrogen bonds with the T199 residue, the nitrogen atom of the $NH^-$ coordinates the zinc ion and the phenyl ring is involved in several hydrophobic contacts (V121, H94 and L198).

The 4-oxothiazolidin-2-ylidene tail of the nanomolar inhibitor **8** establishes a H-bond with the side chain group of Q92 and a set of hydrophobic interactions with F131 and I91 (Fig. 2a).
On the contrary, two favorable, iso-energetic, poses were predicted for compounds **3c**, **6c**, **6f**, which differ for the substituent on the N atom of the 5-bromo-2-oxoindolin moiety**.**

In one of these orientations (Fig. 2a) the isatin moiety lies in the same region described for the derivative **8**: the aromatic core is at π- π stacking distance with the F131 side chain and forms, in addition, hydrophobic interactions with I91, whereas the bromine atom is accommodated in a small lipophilic pocket formed by P202, L204 and V135.
In the second orientation (Fig. 2b) the carbonyl group of the oxoindolin moiety establishes a H-bond with N67 side chain, the aromatic moiety is involved in π- π stacking and hydrophobic interactions with H64 and the bromine atom establishes a halogen bond with the W5 NH side chain and lipophilic contacts with P201 and P202.

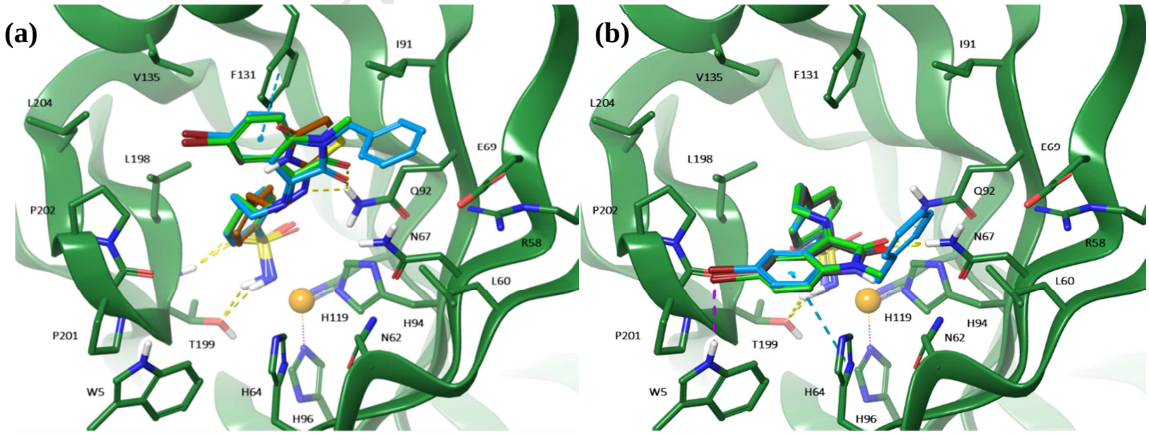



**Figure 2.** Simulated binding modes of compound **8** (panel (a), brown), **3c** (dark grey), **6c** (green) and **6f** (light blue) within hCA II active site (PDB ID 5JLT). The two isoenergetic orientations of the 5-bromo-2-oxoindolin derivatives are shown in panel (a) and (b). The hydrogen bonds are shown as yellow dashed lines.

A quite satisfying degree of agreement is found between the simulated binding modes and the hCAII inhibitory trend of derivatives **3c**, **6c**, **6f** (Table 1). The intense network of interactions described above reflects the nanomolar inhibition data found for compounds **3c** and **6c**. On the contrary, the lipophilic benzyl group present on the N atom of the isatin scaffold (**6f**) is forced to position towards the unfavourable hydrophilic region of the active site, thus explaining the lower inhibitory potency found for the compound.

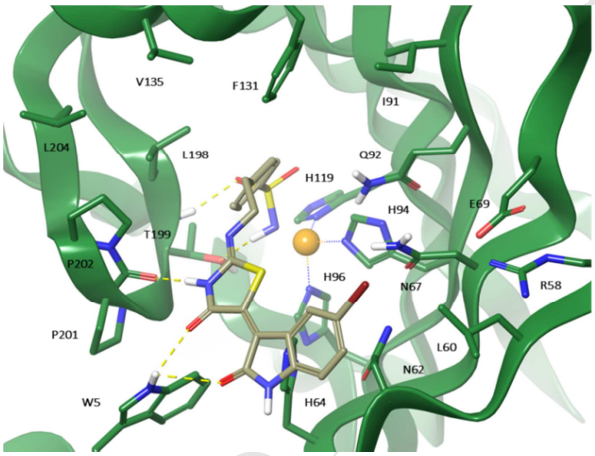



**Figure 3.** Simulated binding mode of compound **9d** (light grey) within hCA II active site. The hydrogen bonds are shown as yellow dashed lines.

Docking findings for derivative **9d** (Fig. 3) highlight that the NH and carbonyl moiety of the 4-oxothiazolidin-2-ylidene are in H-bond distance respectively with the P201backbone carbonyl group and the W5 NH side chain. In addition, the isatin core forms a H-bond with the same Trp5 side chain. Further lipophilic interactions with P201, W5, H64, N62 and L60 stabilize the pose. The effectiveness of these interactions is expressed most clearly (Table 1) when the bromine at the 5 position is replaced by more hydrophobic substituent such as methyl (**9e**) and nitro (**9f**) group.

## 3. Conclusion

In conclusion, we report here the design and synthesis of three novel series of sulfonamides **3a-f, 6a-f and 9a-f**, incorporating un/substituted indolin-2-one moieties linked

to benzenesulfonamide (as zinc anchoring moieties) through aminoethyl or (4-oxothiazolidin-2-ylidene)aminoethyl linker. The structure of the novel derivatives was confirmed by the different spectral and elemental analyses methods. Biological evaluation of the newly prepared sulfonamides was performed against hCA I, II, IV and VII. All the tested isoforms were inhibited by the synthesized sulfonamides **3a-f, 6a-f and 9a-f**, in variable degrees. Best activity was observed against both isoforms hCA II and IV. Sulfonamides **8**, **3c** and **6a-c** emerged as single-digit nanomolar hCA II inhibitors ($K_I$s in the range of 5.9–9.4 nM). While, compounds **3b-f**, **6a-c** and **9c-f** displayed potent potency with $K_I$s in a range of 16.3–46.1 nM.

## 4. Experimental

### *4.1. Chemistry*

#### *4.1.1. General*

Melting points were measured with a Stuart melting point apparatus and were uncorrected. The NMR spectra were recorded by Varian Mercury at 400 MHz or Bruker spectrophotometer at 400 MHz. $^{13}$C NMR spectra were run at 100 MHz in deuterated dimethylsulfoxide (DMSO-$d_6$). Chemical shifts ($\delta_H$) are reported relative to TMS as internal standard. All coupling constant ($J$) values are given in hertz. Chemical shifts ($\delta_C$) are reported relative to DMSO-$d_6$ as internal standards. Infrared spectra were recorded on Schimadzu FT-IR 8400S spectrophotometer. Elemental analyses were carried out in the Regional Center for Microbiology and Biotechnology, Al-Azhar University, Cairo, Egypt. Reaction courses and product mixtures were routinely monitored by thin layer chromatography (TLC) on silica gel precoated $F_{254}$ Merck plates.

#### *4.1.2. General procedure for preparation of the target 4-(2-((2-oxoindolin-3-ylidene)amino) ethyl)benzenesulfonamides* **3a-f**.

To a stirred solution of the appropriate indolin-2,3-dione derivative **1a-f** (1 mmol) in absolute ethyl alcohol (10 mL), 4-(2-aminoethyl)benzenesulfonamide **2** (0.2 gm, 1 mmol) and catalytic amount of glacial acetic acid were added. After refluxing for 2 h, the formed

precipitate was collected by filtration while hot, washed with methanol, dried and crystallized from ethanol to furnish compounds **3a-f** with 62-75% yield.

*4.1.2.1. 4-(2-((2-Oxoindolin-3-ylidene)amino)ethyl)benzenesulfonamide* (**3a**). Yellow powder (yield 65%); m.p. > 280 °C; IR (KBr, $\nu$ $cm^{-1}$): 3259, 3209 (NH, $NH_2$), 1724 (C=O), 1330, 1153 ($SO_2$); $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ *ppm*: 3.08, 3.21 (2t, 2H, =N-$CH_2$-C$\underline{H_2}$-, $J$ = 6.84 Hz), 4.22, 4.52 (2t, 2H, =N-C$\underline{H_2}$-$CH_2$-, $J$ = 6.48 Hz), 6.83, 6.89 (2d, 1H, H-7 isatin, $J$ = 7.64 Hz), 7.00 (t, 1H, H-5 isatin, $J$ = 7.40 Hz), 7.28 (s, 2H, $SO_2NH_2$, $D_2O$ exchangeable), 7.36-7.76 (m, 6H, Ar-H), 10.80 and 10.89 (2s, 1H, $D_2O$ exchangeable, NH isatin); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) $\delta$: 36.90, 37.19, 52.77, 54.91, 111.04, 111.52, 117.13, 121.92, 122.07, 122.65, 122.69, 126.10, 127.76, 129.72, 129.81, 133.49, 133.98, 142.41, 142.45, 144.83, 144.95, 146.34, 154.44, 155.37, 160.04, 164.02; MS, *m/z* [%]: 329 [$M^+$, 9.45]; Anal. Calcd. for $C_{16}H_{15}N_3O_3S$: C, 58.35; H, 4.59; N, 12.76; Found C, 58.49; H, 4.67; N, 13.02.

*4.1.2.2. 4-(2-((5-Chloro-2-oxoindolin-3-ylidene)amino)ethyl)benzenesulfonamide* (**3b**). Yellow powder (yield 68%); m.p. > 280 °C; IR (KBr, $\nu$ $cm^{-1}$): 3309, 3290 (NH, $NH_2$), 1739 (C=O), 1340, 1157 ($SO_2$); $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ *ppm*: 3.07, 3.19 (2t, 2H, =N-$CH_2$-C$\underline{H_2}$-, $J$ = 7.20 Hz), 4.23, 4.49 (2t, 2H, =N-C$\underline{H_2}$-$CH_2$-, $J$ = 7.20 Hz), 6.84, 6.88 (2d, 1H, H-7 isatin, $J$ = 8.40 Hz), 7.24 (s, 1H, H-4 isatin), 7.41 (s, 2H, $SO_2NH_2$, $D_2O$ exchangeable), 7.42-7.56 (m, 3H, Ar-H), 7.72 (d, 2H, H-2 and H-6 of -$C_6H_4$-$SO_2NH_2$, $J$ = 8.40 Hz), 10.90, 11.00 (2s, 1H, $D_2O$ exchangeable, NH isatin); Anal. Calcd. for $C_{16}H_{14}ClN_3O_3S$: C, 52.82; H, 3.88; N, 11.55; Found C, 53.01; H, 3.92; N, 11.78.

*4.1.2.3. 4-(2-((5-Bromo-2-oxoindolin-3-ylidene)amino)ethyl)benzenesulfonamide* (**3c**). Yellow powder (yield 72%); m.p. > 280 °C; IR (KBr, $\nu$ $cm^{-1}$): 3290, 3170 (NH, $NH_2$), 1739 (C=O), 1327, 1157 ($SO_2$); $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ *ppm*: 3.05, 3.18 (2t, 2H, =N-$CH_2$-C$\underline{H_2}$-, $J$ = 7.20 Hz), 4.22, 4.49 (2t, 2H, =N-C$\underline{H_2}$-$CH_2$-, $J$ = 7.20 Hz), 6.79, 6.83 (2d, 1H, H-7 isatin, $J$ = 8.00 Hz), 7.24 (s, 2H, $SO_2NH_2$, $D_2O$ exchangeable), 7.45-7.58 (m, 3H, Ar-H), 7.84 (s, 1H, H-4 isatin), 7.72 (d, 2H, H-2 and H-6 of -$C_6H_4$-$SO_2NH_2$, $J$ = 8.00 Hz), 10.91, 11.00 (2s, 1H, $D_2O$ exchangeable, NH isatin); Anal. Calcd. for $C_{16}H_{14}BrN_3O_3S$: C, 47.07; H, 3.46; N, 10.29; Found C, 47.31; H, 3.41; N, 10.52.

*4.1.2.4. 4-(2-((5-Methyl-2-oxoindolin-3-ylidene)amino)ethyl)benzenesulfonamide* (**3d**). Yellow powder (yield 70%); m.p. > 280 °C; IR (KBr, $\nu$ $cm^{-1}$): 3305, 3159 (NH, $NH_2$), 1732 (C=O), 1327, 1157 ($SO_2$); $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ *ppm*: 2.24 (s, 3H, $CH_3$), 3.05, 3.18 (2t, 2H, =N-$CH_2$-C$\underline{H_2}$-, $J$ = 7.20 Hz), 4.23, 4.49 (2t, 2H, =N-C$\underline{H_2}$-$CH_2$-, $J$ = 7.20 Hz), 6.70, 6.76 (2d, 1H, H-7 isatin, $J$ = 8.00 Hz), 7.16-7.25 (m, 4H, Ar-H), 7.45 (d, 1H, Ar-H, $J$ = 8.40 Hz), 7.50 (d, 1H, Ar-H, $J$ = 8.40 Hz), 7.71-7.74 (m, 2H, Ar-H), 10.65, 10.75 (2s, 1H, $D_2O$ exchangeable, NH isatin); Anal. Calcd. for $C_{17}H_{17}N_3O_3S$: C, 59.46; H, 4.99; N, 12.24; Found C, 59.62; H, 5.06; N, 12.37.

*4.1.2.5. 4-(2-((5-Methoxy-2-oxoindolin-3-ylidene)amino)ethyl)benzenesulfonamide* (**3e**). Orange powder (yield 62%); m.p. > 280 °C; IR (KBr, $\nu$ $cm^{-1}$): 3284, 3180 (NH, $NH_2$), 1729 (C=O), 1332, 1157 ($SO_2$); $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ *ppm*: 3.20 (t, 2H, =N-$CH_2$-C$\underline{H_2}$-, $J$ = 6.80 Hz), 3.74 (s, 3H, $OCH_3$), 4.24 (t, 2H, =N-C$\underline{H_2}$-$CH_2$-, $J$ = 6.80 Hz), 6.81 (d, 1H, H-7 isatin, $J$ = 8.40 Hz), 7.02 (dd, 1H, H-6 isatin, $J$ = 7.40, 2.40 Hz), 7.27 (s, 1H, H-4 isatin), 7.28 (s, 2H, $SO_2NH_2$, $D_2O$ exchangeable), 7.53 (d, 2H, H-3 and H-5 of -$C_6H_4$-$SO_2NH_2$, $J$ = 8.40 Hz), 7.74 (d, 2H, H-2 and H-6 of -$C_6H_4$-$SO_2NH_2$, $J$ = 8.40 Hz), 10.78 (s, 1H, $D_2O$ exchangeable, NH isatin); MS, *m/z* [%]: 359 [$M^+$, 1.12]; Anal. Calcd. for $C_{17}H_{17}N_3O_4S$: C, 56.81; H, 4.77; N, 11.69; Found C, 56.97; H, 4.73; N, 11.94.

*4.1.2.6. 4-(2-((5,7-Dimethyl-2-oxoindolin-3-ylidene)amino)ethyl)benzenesulfonamide* (**3f**). Orange powder (yield 75%); m.p. > 280 °C; IR (KBr, $\nu$ $cm^{-1}$): 3275, 3174 (NH, $NH_2$), 1724 (C=O), 1330, 1157 ($SO_2$); $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ *ppm*: 2.13 (s, 3H, $CH_3$), 2.21 (s, 3H, $CH_3$), 3.07, 3.20 (2t, 2H, =N-$CH_2$-C$\underline{H_2}$-, $J$ = 6.60 Hz), 4.18, 4.51 (2t, 2H, =N-C$\underline{H_2}$-$CH_2$-, $J$ = 6.64 Hz), 6.99, 7.02 (2s, 1H, H-6 isatin), 7.09, 7.34 (2s, 1H, H-4 isatin), 7.29 (s, 2H, $SO_2NH_2$, $D_2O$ exchangeable), 7.47, 7.53 (2d, 2H, H-3 and H-5 of -$C_6H_4$-$SO_2NH_2$, $J$ = 7.96 Hz), 7.75-7.78 (m, 2H, H-2 and H-6 of -$C_6H_4$-$SO_2NH_2$), 10.69 and 10.81 (2s, 1H, $D_2O$ exchangeable, NH isatin); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) $\delta$: 16.07, 16.35, 19.01, 20.91, 37.01, 37.26, 52.69, 54.69, 116.95, 119.82, 120.06, 120.43, 121.65, 125.44, 126.10,129.31, 129.68, 129.84, 130.29, 131.33, 131.55, 135.09, 135.54,140.49, 141.21, 142.39, 142.43, 142.51, 144.88, 144.93, 154.93, 155.87, 160.67, 164.59; MS, *m/z* [%]: 357 [$M^+$, 7.38]; Anal. Calcd. for $C_{18}H_{19}N_3O_3S$: C, 60.49; H, 5.36; N, 11.76; Found 60.72; H, 5.41; N, 11.98.

*4.1.3. N-Substitutedindoline-2,3-diones* **5a-f**.

Compounds **5a-f** were prepared according to the literature procedures [6].

*4.1.4. General procedure for preparation of the target compounds* **6a-f**.

Following the same procedures described for preparation of compounds **3a-f**, using N-Substitutedindoline-2,3-diones **5a-f** instead of indoline-2,3-dione **1a-f** (60-75% yield).

*4.1.4.1. 4-(2-((1-Methyl-2-oxoindolin-3-ylidene)amino)ethyl)benzenesulfonamide* (**6a**). Yellow powder (yield 73%); m.p. > 280 °C; IR (KBr, $\nu$ $cm^{-1}$): 3213 ($NH_2$), 1724 (C=O), 1338, 1157 ($SO_2$); $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ *ppm*: 3.08, 3.22 (2t, 2H, =N-$CH_2$-C$\underline{H_2}$-, $J$ = 7.28 Hz), 3.13, 3.15 (2s, 3H, -N-$CH_3$), 4.25, 4.54 (2t, 2H, =N-C$\underline{H_2}$-$CH_2$-, $J$ = 7.32 Hz), 7.04-7.12 (m, 2H, H-5 and H-7 isatin), 7.28 (s, 2H, $SO_2NH_2$, $D_2O$ exchangeable), 7.46-7.55 (m, 4H, H-4 & H-6 isatin and H-3 & H-5 of -$C_6H_4$-$SO_2NH_2$), 7.74 (d, 2H, H-2 and H-6 of -$C_6H_4$-$SO_2NH_2$, $J$ = 8.20 Hz); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) $\delta$: 26.08, 26.44, 36.88, 37.12, 52.99, 54.95, 109.79, 110.10, 116.47, 121.11, 121.65, 123.15, 123.25, 125.79, 125.94, 126.10, 127.43, 129.49, 129.72, 129.80, 130.24, 133.44, 133.91, 142.44, 142.46, 144.77, 146.10, 147.37, 153.60, 154.63, 158.36, 162.71; MS, *m/z* [%]: 343 [$M^+$, 1.22]; Anal. Calcd. for $C_{17}H_{17}N_3O_3S$: C, 59.46; H, 4.99; N, 12.24; Found C, 59.71; H, 5.02; N, 12.41.

*4.1.4.2. 4-(2-((5-Chloro-1-methyl-2-oxoindolin-3-ylidene)amino)ethyl)benzenesulfonamide* (**6b**). Yellow powder (yield 70%); m.p. > 280 °C; IR (KBr, $\nu$ $cm^{-1}$): 3259 ($NH_2$), 1701 (C=O), 1346, 1165 ($SO_2$); $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ *ppm*: 3.27 (t, 2H, =N-$CH_2$-C$\underline{H_2}$-, $J$ = 7.32 Hz), 3.15 (s, 3H, -N-$CH_3$), 4.28 (t, 2H, =N-C$\underline{H_2}$-$CH_2$-, $J$ = 7.20 Hz), 7.11 (d, 1H, H-7 isatin, $J$ = 8.00 Hz), 7.30 (s, 2H, $SO_2NH_2$, $D_2O$ exchangeable), 7.53 (d, 2H, H-3 and H-5 of -$C_6H_4$-$SO_2NH_2$, $J$ = 6.80 Hz), 7.72-7.77 (m, 3H, H-6 isatin and H-2 & H-6 of -$C_6H_4$-$SO_2NH_2$), 7.97 (s, 1H, H-4 isatin); Anal. Calcd. for $C_{17}H_{16}ClN_3O_3S$: C, 54.04; H, 4.27; N, 11.12; Found C, 54.18; H, 4.33; N, 11.37.

*4.1.4.3. 4-(2-((5-Bromo-1-methyl-2-oxoindolin-3-ylidene)amino)ethyl)benzenesulfonamide* (**6c**). Yellow powder (yield 60%); m.p. > 280 °C; IR (KBr, $\nu$ $cm^{-1}$): 3264 ($NH_2$), 1705 (C=O), 1341, 1160 ($SO_2$); $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ *ppm*: 3.24 (t, 2H, =N-$CH_2$-C$\underline{H_2}$-, $J$ = 7.20 Hz), 3.14 (s, 3H, -N-$CH_3$), 4.30 (t, 2H, =N-C$\underline{H_2}$-$CH_2$-, $J$ = 7.20 Hz), 7.08 (d, 1H, H-7

isatin, *J* = 8.00 Hz), 7.28 (s, 2H, $SO_2NH_2$, $D_2O$ exchangeable), 7.53 (d, 2H, H-3 and H-5 of -$C_6H_4$-$SO_2NH_2$, *J* = 6.80 Hz), 7.70-7.76 (m, 3H, H-6 isatin and H-2 & H-6 of -$C_6H_4$-$SO_2NH_2$), 7.90 (s, 1H, H-4 isatin); MS, *m/z* [%]: 420 [$M^+$, 11.29]; 422 [$M^+$+2, 11.37]; Anal. Calcd. for $C_{17}H_{16}BrN_3O_3S$: C, 48.35; H, 3.82; N, 9.95; Found C, 48.51; H, 3.89; N, 10.08.

*4.1.4.4. 4-(2-((1-Benzyl-2-oxoindolin-3-ylidene)amino)ethyl)benzenesulfonamide* (**6d**). Yellow powder (yield 63%); m.p. > 280 °C; IR (KBr, *v* $cm^{-1}$): 3363 ($NH_2$), 1693 (C=O), 1338, 1161 ($SO_2$); $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ *ppm*: 3.13, 3.25 (2t, 2H, =N-$CH_2$-C$\underline{H_2}$-, *J* = 6.84 Hz), 4.28, 4.61 (2t, 2H, =N-C$\underline{H_2}$-$CH_2$-, *J* = 6.92 Hz), 4.91, 4.94 (2s, 2H, benzylic $CH_2$), 6.94 (d, 1H, H-7 isatin, *J* = 7.72 Hz), 7.06 (t, 1H, H-5 isatin, *J* = 7.36 Hz), 7.30-7.44 (m, 8H; 5H of $C_6H_5$ and H-6 isatin and 2H of $SO_2NH_2$), 7.51-7.57 (m, 3H, H-4 isatin and H-3 & H-5 of -$C_6H_4$-$SO_2NH_2$), 7.76 (d, 2H, H-2 and H-6 of -$C_6H_4$-$SO_2NH_2$, *J* = 7.36 Hz); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) $\delta$: 36.91, 37.12, 42.95, 43.17, 53.13, 55.10, 110.36, 110.68, 116.72, 121.29, 121.91, 123.35, 123.43, 126.12, 127.69, 127.76, 127.99,129.18, 129.75, 129.84, 133.37,133.82, 136.40, 136.50, 142.47, 142.50, 144.76, 145.12, 146.30, 153.36, 154.42, 158.31, 162.85; Anal. Calcd. for $C_{23}H_{21}N_3O_3S$: C, 65.85; H, 5.05; N, 10.02; Found C, 66.02; H, 5.12; N, 10.23.

*4.1.4.5. 4-(2-((1-Benzyl-5-chloro-2-oxoindolin-3-ylidene)amino)ethyl)benzenesulfonamide* (**6e**). Yellow powder (yield 75%); m.p. > 280 °C; IR (KBr, *v* $cm^{-1}$): 3281 ($NH_2$), 1711 (C=O), 1339, 1161 ($SO_2$); $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ *ppm*: 3.12 (t, 2H, =N-$CH_2$-C$\underline{H_2}$-, *J* = 7.20 Hz), 4.60 (t, 2H, =N-C$\underline{H_2}$-$CH_2$-, *J* = 7.20 Hz), 4.94 (s, 2H, benzylic $CH_2$), 6.96 (d, 1H, H-7 isatin, *J* = 8.00 Hz), 7.28 (s, 2H, $SO_2NH_2$, $D_2O$ exchangeable), 7.31-7.36 (m, 5H, 5H of $C_6H_5$), 7.45 (dd, 1H, H-6 isatin, *J* = 8.40, 2.40 Hz), 7.50 (s, 1H, H-4 isatin), 7.51 (d, 2H, H-3 and H-5 of -$C_6H_4$-$SO_2NH_2$, *J* = 8.40 Hz), 7.75 (d, 2H, H-2 and H-6 of -$C_6H_4$-$SO_2NH_2$, *J* = 8.40 Hz); Anal. Calcd. for $C_{23}H_{20}ClN_3O_3S$: C, 60.86; H, 4.44; N, 9.26; Found C, 61.09; H, 4.51; N, 9.44.

*4.1.4.6. 4-(2-((1-Benzyl-5-bromo-2-oxoindolin-3-ylidene)amino)ethyl)benzenesulfonamide* (**6f**). Yellow powder (yield 70%); m.p. > 280 °C; IR (KBr, *v* $cm^{-1}$): 3321 ($NH_2$), 1722 (C=O), 1330, 1157 ($SO_2$); $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ *ppm*: 3.14, 3.26 (2t, 2H, =N-$CH_2$-C$\underline{H_2}$-, *J* = 7.28 Hz), 4.33, 4.62 (2t, 2H, =N-C$\underline{H_2}$-$CH_2$-, *J* = 7.32 Hz), 4.91, 4.93 (2s, 2H, benzylic

$CH_2$), 6.91 (d, 1H, H-7 isatin, *J* = 7.72 Hz), 7.30-7.44 (m, 7H; 5H of $C_6H_5$ and 2H of $SO_2NH_2$), 7.51-7.63 (m, 3H, H-6 isatin and H-3 & H-5 of -$C_6H_4$-$SO_2NH_2$), 7.76 (d, 2H, H-2 and H-6 of -$C_6H_4$-$SO_2NH_2$, *J* = 6.96 Hz), 7.93 (s, 1H, H-4 isatin); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) *δ*: 36.85, 37.00, 43.02, 43.22, 53.38, 55.12, 112.54, 112.63,115.00, 115.32, 118.23, 123.23, 124.25, 126.09, 126.12, 127.64, 127.72, 128.04, 129.19, 129.79, 129.91, 135.58, 136.04, 136.15, 142.46, 142.49, 144.26, 144.65, 144.82, 145.43, 152.47, 153.33, 157.87, 162.39; Anal. Calcd. for $C_{23}H_{20}BrN_3O_3S$: C, 55.43; H, 4.04; N, 8.43; Found C, 55.59; H, 4.08; N, 8.49.

*4.1.5. 2-Chloro-N-(4-sulfamoylphenethyl)acetamide* **7**.

To a stirred solution of 4-(2-aminoethyl)benzenesulfonamide **2** (2 gm, 10 mmol) in dioxan (15 mL), 2-chloroacetyl chloride (1.13 g, 10 mmol) was added drop-wise at °C during 40 min. The reaction mixture was further stirred at room temperature (at 25 °C) for 3 h. The obtained precipitate was filtered off, washed with water (10 ml), dried and recrystallized from methanol to afford compound **7**. White crystals (yield 80%); m.p. 145-147 °C; IR (KBr, *v* cm$^{-1}$): 3315, 3282 (NH, $NH_2$), 1662 (C=O), 1334, 1157 ($SO_2$); $^1$H NMR (DMSO-$d_6$, 400 MHz) *δ ppm*: 2.77 (t, 2H, =N-$CH_2$-C$\underline{H_2}$-, *J* = 7.20 Hz), 3.32 (t, 2H, =N-C$\underline{H_2}$-$CH_2$-, *J* = 7.2 Hz), 4.00 (s, 2H, C$\underline{H_2}$-C=o), 7.27 (s, 2H, $SO_2NH_2$, $D_2O$ exchangeable), 7.36 (d, 2H, H-3 and H-5 of -$C_6H_4$-$SO_2NH_2$, *J* = 8.40 Hz), 7.71 (d, 2H, H-2 and H-6 of -$C_6H_4$-$SO_2NH_2$, *J* = 8.40 Hz), 8.27 (t, 1H, NH, $D_2O$ exchangeable, *J* = 5.20 Hz).

*4.1.6. 4-(2-((4-Oxothiazolidin-2-ylidene)amino)ethyl)benzenesulfonamide* **8**.

Ammonium thiocyanate (1.15 gm, 15 mmol) was added to a hot solution of 2-chloro-*N*-(4-sulfamoylphenethyl)acetamide **7** (2.76 gm, 10 mmol) in ethanol (20 mL). The reaction mixture was heated under reflux for 3 h then allowed to cool to the room temperature. The obtained precipitate was filtered off, washed several times with water and recrystallized from methanol to give the intermediate **8**. Beige crystals (yield 55%); m.p. > 280 °C; IR (KBr, *v* cm$^{-1}$): 3329, 3217 (NH, $NH_2$), 1647 (C=O), 1327, 1149 ($SO_2$); $^1$H NMR (DMSO-$d_6$, 400 MHz) *δ ppm*: 2.80 (t, 2H, =N-$CH_2$-C$\underline{H_2}$-, *J* = 7.00 Hz), 3.35 (q, 2H, =N-C$\underline{H_2}$-$CH_2$-, *J* = 6.86 Hz), 3.87 (s, 2H, C$\underline{H_2}$-C=o), 7.30 (s, 2H, $SO_2NH_2$, $D_2O$ exchangeable), 7.41 (d, 2H, H-3 and H-5 of -$C_6H_4$-$SO_2NH_2$, *J* = 8.12 Hz), 7.75 (d, 2H, H-2 and H-6 of -$C_6H_4$-$SO_2NH_2$, *J* = 8.12

Hz), 8.42 (t, 1H, NH, *J* = 5.20 Hz); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) $\delta$: 35.03, 36.66, 49.09, 113.37, 126.20, 129.64, 142.60, 143.82, 165.94; Anal. Calcd. for $C_{11}H_{13}N_3O_3S_2$: C, 44.13; H, 4.38; N, 14.04; Found C, 44.38; H, 4.45; N, 14.23.

*4.1.7. General procedure for preparation of the target compounds* **9a-f**.

To a hot stirred solution of the intermediate **8** (0.3 gm, 1 mmol) in glacial acetic acid (10 mL), the appropriate indoline-2,3-dione derivative and sodium acetate (0.16 gm, 2 mmol) were added. The reaction mixture was refluxed for 5 h. The formed solid was filtered off while hot, washed, dried and crystallized from DMF/methanol to furnish the target sulfonamides **4a-m**, in 60-72% yield.

*4.1.7.1. 4-(2-((4-Oxo-5-(2-oxoindolin-3-ylidene)thiazolidin-2-ylidene)amino)ethyl) benzenesulfonamide* (**9a**). Red powder (yield 60%); m.p. > 280 °C; IR (KBr, $\nu$ cm$^{-1}$): 3313, 3275 (NH, $NH_2$), 1695 (C=O), 1352, 1156 ($SO_2$); $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ *ppm*: 3.05 (t, 2H, =N-$CH_2$-C$\underline{H_2}$-, *J* = 7.24 Hz), 4.11 (t, 2H, =N-C$\underline{H_2}$-$CH_2$-, *J* = 7.16 Hz), 6.93 (d, 1H, H-7 isatin, *J* = 7.76 Hz), 7.06 (t, 1H, H-5 isatin, *J* = 7.64 Hz), 7.31 (s, 2H, $SO_2NH_2$, $D_2O$ exchangeable), 7.39 (t, 1H, H-6 isatin, *J* = 7.56 Hz), 7.46 (d, 2H, H-3 and H-5 of -$C_6H_4$-$SO_2NH_2$, *J* = 8.24 Hz), 7.75 (d, 2H, H-2 and H-6 of -$C_6H_4$-$SO_2NH_2$, *J* = 8.24 Hz), 8.82 (d, 1H, H-4 isatin, *J* = 7.76 Hz), 11.21 (s, 1H, NH, $D_2O$ exchangeable), 11.23 (s, 1H, NH, $D_2O$ exchangeable); MS, *m/z* [%]: 428 [M$^+$, 5.97]; Anal. Calcd. for $C_{19}H_{16}N_4O_4S_2$: C, 53.26; H, 3.76; N, 13.08; Found C, 53.48; H, 3.80; N, 13.21.

*4.1.7.2. 4-(2-((5-(5-Fluoro-2-oxoindolin-3-ylidene)-4-oxothiazolidin-2-ylidene)amino)ethyl) benzenesulfonamide* (**9b**). Red powder (yield 68%); m.p. > 280 °C; IR (KBr, $\nu$ cm$^{-1}$): 3305, 3271 (NH, NH2), 1697 (C=O), 1357, 1157 ($SO_2$); $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ *ppm*: 3.04 (t, 2H, =N-$CH_2$-C$\underline{H_2}$-, *J* = 7.08 Hz), 4.06 (t, 2H, =N-C$\underline{H_2}$-$CH_2$-, *J* = 7.16 Hz), 6.80-6.83 (m, 1H, H-4 isatin), 7.10 (t, 1H, H-6 isatin, *J* = 8.60 Hz), 7.31 (s, 2H, $SO_2NH_2$, $D_2O$ exchangeable), 7.39 (d, 2H, H-3 and H-5 of -$C_6H_4$-$SO_2NH_2$, *J* = 8.24 Hz), 7.71 (d, 2H, H-2 and H-6 of -$C_6H_4$-$SO_2NH_2$, *J* = 8.24 Hz), 8.33 (d, 1H, H-7 isatin, *J* = 8.12 Hz), 11.14 (s, 1H, NH, $D_2O$ exchangeable), 11.22 (s, 1H, NH, $D_2O$ exchangeable); Anal. Calcd. for $C_{19}H_{15}FN_4O_4S_2$: C, 51.11; H, 3.39; N, 12.55; Found C, 51.29; H, 3.44; N, 12.79.

*4.1.7.3. 4-(2-((5-(5-Chloro-2-oxoindolin-3-ylidene)-4-oxothiazolidin-2-ylidene)amino)ethyl) benzenesulfonamide* (**9c**). Red powder (yield 70%); m.p. > 280 °C; IR (KBr, $\nu$ $cm^{-1}$): 3319, 3237 (NH, NH2), 1696 (C=O), 1350, 1160 ($SO_2$); $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ *ppm*: 3.04 (t, 2H, =N-$CH_2$-C$\underline{H_2}$-, $J$ = 7.40 Hz), 4.10 (t, 2H, =N-C$\underline{H_2}$-$CH_2$-, $J$ = 7.16 Hz), 6.91-6.97 (m, 1H, H-7 isatin), 7.31 (s, 2H, $SO_2NH_2$, $D_2O$ exchangeable), 7.47 (d, 2H, H-3 and H-5 of -$C_6H_4$-$SO_2NH_2$, $J$ = 8.44 Hz), 7.60 (dd, 1H, H-6 isatin, $J$ = 2.24, 8.36 Hz), 7.75 (d, 2H, H-2 and H-6 of -$C_6H_4$-$SO_2NH_2$, $J$ = 8.20 Hz), 8.87 (s, 1H, H-4 isatin), 11.12 (s, 1H, NH, $D_2O$ exchangeable), 11.34 (s, 1H, NH, $D_2O$ exchangeable); Anal. Calcd. for $C_{19}H_{15}ClN_4O_4S_2$: C, 49.30; H, 3.27; N, 12.10; Found C, 49.53; H, 3.24; N, 12.37.

*4.1.7.4. 4-(2-((5-(5-Bromo-2-oxoindolin-3-ylidene)-4-oxothiazolidin-2-ylidene)amino)ethyl) benzenesulfonamide* (**9d**). Red powder (yield 65%); m.p. > 280 °C; IR (KBr, $\nu$ $cm^{-1}$): 3313, 3240 (NH, NH2), 1693 (C=O), 1354, 1165 ($SO_2$); $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ *ppm*: 3.04 (t, 2H, =N-$CH_2$-C$\underline{H_2}$-, $J$ = 7.36 Hz), 4.10 (t, 2H, =N-C$\underline{H_2}$-$CH_2$-, $J$ = 7.08 Hz), 6.86 (d, 1H, H-7 isatin, $J$ = 8.32 Hz), 7.32 (s, 2H, $SO_2NH_2$, $D_2O$ exchangeable), 7.50 (d, 2H, H-3 and H-5 of -$C_6H_4$-$SO_2NH_2$, $J$ = 8.08 Hz), 7.64 (s, 1H, H-4 isatin), 7.71 (dd, 1H, H-6 isatin, $J$ = 1.80, 8.32 Hz), 7.78 (d, 2H, H-2 and H-6 of -$C_6H_4$-$SO_2NH_2$, $J$ = 8.08 Hz), 11.24 (s br., 2H, NH, $D_2O$ exchangeable); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) $\delta$: 32.85, 44.21, 114.73, 120.02, 125.89, 126.28, 127.34, 129.77, 140.48, 142.69, 150.03, 159.03, 183.65; Anal. Calcd. for $C_{19}H_{15}BrN_4O_4S_2$: C, 44.98; H, 2.98; N, 11.04; Found C, 45.12; H, 3.01; N, 11.21.

*4.1.7.5. 4-(2-((5-(5-Methyl-2-oxoindolin-3-ylidene)-4-oxothiazolidin-2-ylidene)amino)ethyl) benzenesulfonamide* (**9e**). Red powder (yield 70%); m.p. > 280 °C; IR (KBr, $\nu$ $cm^{-1}$): 3394, 3224 (NH, NH2), 1693 (C=O), 1357, 1161 ($SO_2$); $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ *ppm*: 2.24 (s, 3H, $CH_3$), 3.03 (t, 2H, =N-$CH_2$-C$\underline{H_2}$-, $J$ = 7.44 Hz), 4.07 (t, 2H, =N-C$\underline{H_2}$-$CH_2$-, $J$ = 7.44 Hz), 6.69 (d, 1H, H-7 isatin, $J$ = 7.88 Hz), 7.05 (d, 1H, H-6 isatin, $J$ = 8.00 Hz), 7.31 (s, 2H, $SO_2NH_2$, $D_2O$ exchangeable), 7.39 (d, 2H, H-3 and H-5 of -$C_6H_4$-$SO_2NH_2$, $J$ = 8.08 Hz), 7.70 (d, 2H, H-2 and H-6 of -$C_6H_4$-$SO_2NH_2$, $J$ = 7.96 Hz), 8.37 (s, 1H, H-4 isatin), 11.10 (s, 1H, NH, $D_2O$ exchangeable), 11.63 (s, 1H, NH, $D_2O$ exchangeable); Anal. Calcd. for $C_{20}H_{18}N_4O_4S_2$: C, 54.29; H, 4.10; N, 12.66; Found C, 54.53; H, 4.16; N, 12.90.

*4.1.7.6. 4-(2-((5-(5-Nitro-2-oxoindolin-3-ylidene)-4-oxothiazolidin-2-ylidene)amino)ethyl) benzenesulfonamide* (**9f**). Red powder (yield 72%); m.p. > 280 °C; IR (KBr, $\nu$ $cm^{-1}$): 3367, 3271 (NH, NH2), 1705 (C=O), 1338, 1157 ($SO_2$); $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ *ppm*: 3.05 (t, 2H, =N-$CH_2$-C$\underline{H_2}$-, $J$ = 7.20 Hz), 4.07 (t, 2H, =N-C$\underline{H_2}$-$CH_2$-, $J$ = 7.16 Hz), 6.83 (d, 1H, H-7 isatin, $J$ = 7.96 Hz), 7.23 (d, 1H, H-6 isatin, $J$ = 8.00 Hz), 7.30 (s, 2H, $SO_2NH_2$, $D_2O$ exchangeable), 7.42 (d, 2H, H-3 and H-5 of -$C_6H_4$-$SO_2NH_2$, $J$ = 8.16 Hz), 7.72 (d, 2H, H-2 and H-6 of -$C_6H_4$-$SO_2NH_2$, $J$ = 8.12 Hz), 8.50 (s, 1H, H-4 isatin), 11.17 (s, 1H, NH, $D_2O$ exchangeable), 11.59 (s, 1H, NH, $D_2O$ exchangeable); Anal. Calcd. for $C_{19}H_{15}N_5O_6S_2$: C, 48.20; H, 3.19; N, 14.79; Found C, 48.37; H, 3.17; N, 15.02.

### 4.2. CA inhibitory assay

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalysed $CO_2$ hydration activity, as reported earlier [28]. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3 and the Cheng-Prusoff equation as reported earier [29], and represent the mean from at least three different determinations. All CA isofoms were recombinant ones obtained in-house as reported earlier [30].

### 4.3. Molecular modeling experimental

Crystal structures of hCAII (5JLT) was used in docking computation. Input 3D ligand structures were prepared by LigPrep (LigPrep, version 3.3, Schrödinger, LLC, New York, NY, 2015) and Epik (Epik, version 3.1, Schrödinger, LLC, New York, NY, 2015) for the evaluation of their ionization states. The target structures were prepared according to the recommended Protein Preparation module in Maestro - Schrödinger suite [Maestro, version 10.1, Schrödinger, LLC, New York, NY, 2015], assigning bond orders, adding hydrogens, deleting water molecules, and optimizing H-bonding networks Finally, energy minimization with a root mean square deviation (RMSD) value of 0.30 was applied using an Optimized Potentials for Liquid Simulation (OPLS_2005, Schrödinger, New York, NY, USA) force field. Docking experiments were carried out with Glide standard precision (SP) (Glide, version 6.6, Schrödinger, LLC, New York, NY, 2015) using grids prepared with default settings and centred in the centroid of the complexed

ligand. All computations were performed on an Intel® 2xCPU Xeon® 6-core E5-2620 v2 @ 2.10GHz (1200 MHz) 15MB processor running Linux.

**Acknowledgements**

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through the Research Group Project no. RG-1436-038.

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## Highlights

- Three different series of novel indolin-2-one-based sulfonamides were synthesized.

- Inhibitory activity of such sulfonamides was evaluated toward hCA I, II, IV and VII.

- **8**, **3c** and **6a-c** emerged as single-digit nanomolar CA II inhibitors (5.9–9.4 nM).

- Molecular docking studies were carried out for some compounds within the hCA II active site.