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Graphical abstract

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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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.
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Synthesis, in vitro biological evaluation against carbonic anhydrases 
isoforms I, II, IV and VII and molecular docking studies

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\end{itemize}

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.
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 CO₂ 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 (1H-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-3H-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](image)

**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, K₂CO₃, 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 NH₄SCN 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 / CICOCH₂Cl / r.t. 3 h, (ii) NH₄SCN / 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 (NH₂), (C=O) and (SO₂) groups in the ranges of 3367-3159, 1739-1693 and 1346-1153 cm⁻¹, respectively. Their ¹H NMR spectra revealed the appearance of two triplicate signals due to the aliphatic protons of -CH₂-CH₂- around δ 3.10 and 4.20 ppm, also, their ¹H NMR spectra revealed the presence of D₂O exchangeable protons (NH₂) of sulfonamide group around δ 7.30 ppm. Additionally, ¹H NMR spectra of compounds 3a-f and 9a-f showed a singlet D₂O-exchangeable signal attributable to NH protons of the indolin-2-one moiety at a δ range 10.65-11.21 ppm. Also, the ¹H NMR spectra of 6a-c showed the signals of the aliphatic (N-CH₂) protons around δ 3.15 ppm, while the benzylic (CH₂) protons in 6d-f appeared around δ 4.90 ppm.

On the other hand, ¹³C NMR spectra of the newly prepared sulfonamides 3a-f, 6a-f and 9a-f showed two characteristic signals due to the carbons of the -CH₂-CH₂- between δ 36.88-37.26 and 52.69-55.10 ppm. Moreover, the ¹³C NMR spectra for such derivatives showed a signal resonating in the range δ 162.39-164.59 ppm attributable for the carbon of carbonyl group.
(C=O), while the carbons of (N-CH$_3$) and benzylic (CH$_2$) 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 $\mu$M) (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 ($K_{IS} = 875.4$, 4450.2 and 3603.7 nM, respectively) than their corresponding analogues 3a-c ($K_{IS} = 92.5$, 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 $K_{IS}$ 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 ($K_{IS} = 17.9$ nM) with 2.3-folds increased activity than the unsubstituted counterpart 3a ($K_{IS} = 41.4$ nM). Furthermore, incorporation of the more lipophilic 5-Br substituent, compound 3c, significantly enhanced the activity against hCA II ($K_{IS} = 9.4$ nM). Otherwise, grafting methyl or methoxy groups at 5-position maintained the activity ($K_{IS} = 33.8$ 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 ($K_{IS} = 6.8$, 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 $K_{IS}$ 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 CO₂ hydrase assay, using acetazolamide (AAZ) as a standard drug.

<table>
<thead>
<tr>
<th>Comp.</th>
<th>X</th>
<th>R</th>
<th>(K_i) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>hCA I</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>94.7</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>93.9</td>
</tr>
<tr>
<td>3a</td>
<td>H</td>
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<td>64.1</td>
</tr>
<tr>
<td>3c</td>
<td>Br</td>
<td>-</td>
<td>81.5</td>
</tr>
<tr>
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<td>-</td>
<td>316.0</td>
</tr>
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<td>3e</td>
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</tr>
<tr>
<td>3f</td>
<td>5,7-(CH₃)₂</td>
<td>-</td>
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</tr>
<tr>
<td>6a</td>
<td>H</td>
<td>H</td>
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<tr>
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<td>H</td>
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<tr>
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<tr>
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<tr>
<td>9a</td>
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</tr>
<tr>
<td>9b</td>
<td>F</td>
<td>-</td>
<td>899.4</td>
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<tr>
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<td>Cl</td>
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<td>Br</td>
<td>-</td>
<td>3603.7</td>
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<tr>
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<td>-</td>
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<tr>
<td>9f</td>
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<td>264.9</td>
</tr>
<tr>
<td>AAZ</td>
<td>-</td>
<td>-</td>
<td>250</td>
</tr>
</tbody>
</table>

* 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 modeling 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 P201 backbone 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 ($K_{\text{I}s}$ in the range of 5.9–9.4 nM). While, compounds 3b-f, 6a-c and 9c-f displayed potent potency with $K_{\text{I}s}$ 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, ν cm⁻¹): 3259, 3209 (NH, NH₂), 1724 (C=O), 1330, 1153 (SO₂); ¹H NMR (DMSO-d₆, 400 MHz) δ ppm: 3.08, 3.21 (2t, 2H, =N-CH₂-CH₂-, J = 6.84 Hz), 4.22, 4.52 (2t, 2H, =N-CH₂-CH₂-, 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, SO₂NH₂, D₂O exchangeable), 7.36-7.76 (m, 6H, Ar-H), 10.80 and 10.89 (2s, 1H, D₂O exchangeable, NH isatin); ¹³C NMR (DMSO-d₆, 100 MHz) δ: 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 C₁₆H₁₅N₃O₃S: 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, ν cm⁻¹): 3309, 3290 (NH, NH₂), 1739 (C=O), 1340, 1157 (SO₂); ¹H NMR (DMSO-d₆, 400 MHz) δ ppm: 3.07, 3.19 (2t, 2H, =N-CH₂-CH₂-, J = 7.20 Hz), 4.23, 4.49 (2t, 2H, =N-CH₂-CH₂-, 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, SO₂NH₂, D₂O exchangeable), 7.42-7.56 (m, 3H, Ar-H), 7.72 (d, 2H, H-2 and H-6 of -C₆H₄-SO₂NH₂, J = 8.40 Hz), 10.90, 11.00 (2s, 1H, D₂O exchangeable, NH isatin); Anal. Calcd. for C₁₆H₁₄ClN₃O₃S: 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, ν cm⁻¹): 3290, 3170 (NH, NH₂), 1739 (C=O), 1327, 1157 (SO₂); ¹H NMR (DMSO-d₆, 400 MHz) δ ppm: 3.05, 3.18 (2t, 2H, =N-CH₂-CH₂-, J = 7.20 Hz), 4.22, 4.49 (2t, 2H, =N-CH₂-CH₂-, J = 7.20 Hz), 6.79, 6.83 (2d, 1H, H-7 isatin, J = 8.00 Hz), 7.24 (s, 2H, SO₂NH₂, D₂O 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 -C₆H₄-SO₂NH₂, J = 8.00 Hz), 10.91, 11.00 (2s, 1H, D₂O exchangeable, NH isatin); Anal. Calcd. for C₁₆H₁₄BrN₃O₃S: 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, 2.49 (s, 3H, CH\(_3\)), 3.05, 3.18 (2t, 2H, =N-CH\(_2\)-CH\(_2\)-, \(J = 7.20\) Hz), 4.23, 4.49 (2t, 2H, =N-CH\(_2\)-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\(_2\)O exchangeable, NH isatin); Anal. Calcd. for C\(_{17}\)H\(_{17}\)N\(_3\)O\(_3\)S: 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\(_2\)-CH\(_2\)-, \(J = 6.80\) Hz), 3.74 (s, 3H, OCH\(_3\)), 4.24 (t, 2H, =N-CH\(_2\)-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\(_2\)NH\(_2\), D\(_2\)O exchangeable), 7.53 (d, 2H, H-3 and H-5 of -C\(_6\)H\(_4\)-SO\(_2\)NH\(_2\), \(J = 8.40\) Hz), 7.74 (d, 2H, H-2 and H-6 of -C\(_6\)H\(_4\)-SO\(_2\)NH\(_2\), \(J = 8.40\) Hz), 10.78 (s, 1H, D\(_2\)O exchangeable, NH isatin); MS, \(m/z\) [%]: 359 [M\(^+\), 1.12]; Anal. Calcd. for C\(_{17}\)H\(_{17}\)N\(_3\)O\(_4\)S: 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, 2.21 (s, 3H, CH\(_3\)), 3.07, 3.20 (2t, 2H, =N-CH\(_2\)-CH\(_2\)-, \(J = 6.60\) Hz), 4.18, 4.51 (2t, 2H, =N-CH\(_2\)-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\(_2\)NH\(_2\), D\(_2\)O exchangeable), 7.47, 7.53 (2d, 2H, H-3 and H-5 of -C\(_6\)H\(_4\)-SO\(_2\)NH\(_2\), \(J = 8.40\) Hz), 7.75-7.78 (m, 2H, H-2 and H-6 of -C\(_6\)H\(_4\)-SO\(_2\)NH\(_2\), \(J = 7.96\) Hz), 10.69 and 10.81 (2s, 1H, D\(_2\)O 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\(_3\)O\(_3\)S: 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, ν cm⁻¹): 3213 (NH₂), 1724 (C=O), 1338, 1157 (SO₂); ¹H NMR (DMSO-d₆, 400 MHz) δ ppm: 3.08, 3.22 (2t, 2H, =N-CH₂-CH₂-, J = 7.28 Hz), 3.13, 3.15 (2s, 3H, -N-CH₃), 4.25, 4.54 (2t, 2H, =N-CH₂-CH₂-, J = 7.32 Hz), 7.04-7.12 (m, 2H, H-5 and H-7 isatin), 7.28 (s, 2H, SO₂NH₂, D₂O exchangeable), 7.46-7.55 (m, 4H, H-4 & H-6 isatin and H-3 & H-5 of -C₆H₄-SO₂NH₂), 7.74 (d, 2H, H-2 and H-6 of -C₆H₄-SO₂NH₂, J = 8.20 Hz); ¹³C NMR (DMSO-d₆, 100 MHz) δ 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 C₁₇H₁₇N₃O₃S: 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, ν cm⁻¹): 3259 (NH₂), 1701 (C=O), 1346, 1165 (SO₂); ¹H NMR (DMSO-d₆, 400 MHz) δ ppm: 3.27 (t, 2H, =N-CH₂-CH₂-, J = 7.32 Hz), 3.15 (s, 3H, -N-CH₃), 4.28 (t, 2H, =N-CH₂-CH₂-, J = 7.20 Hz), 7.11 (d, 1H, H-7 isatin, J = 8.00 Hz), 7.30 (s, 2H, SO₂NH₂, D₂O exchangeable), 7.53 (d, 2H, H-3 and H-5 of -C₆H₄-SO₂NH₂, J = 6.80 Hz), 7.72-7.77 (m, 3H, H-6 isatin and H-2 & H-6 of -C₆H₄-SO₂NH₂), 7.97 (s, 1H, H-4 isatin); Anal. Calcd. for C₁₇H₁₆ClN₃O₃S: 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, ν cm⁻¹): 3264 (NH₂), 1705 (C=O), 1341, 1160 (SO₂); ¹H NMR (DMSO-d₆, 400 MHz) δ ppm: 3.27 (t, 2H, =N-CH₂-CH₂-, J = 7.32 Hz), 3.14 (s, 3H, -N-CH₃), 4.30 (t, 2H, =N-CH₂-CH₂-, J = 7.20 Hz), 7.08 (d, 1H, H-7
isatin, J = 8.00 Hz), 7.28 (s, 2H, SO\textsubscript{2}NH\textsubscript{2}, D\textsubscript{2}O exchangeable), 7.53 (d, 2H, H-3 and H-5 of -C\textsubscript{6}H\textsubscript{4}-SO\textsubscript{2}NH\textsubscript{2}, J = 6.80 Hz), 7.70-7.76 (m, 3H, H-6 isatin and H-2 & H-6 of -C\textsubscript{6}H\textsubscript{4}-SO\textsubscript{2}NH\textsubscript{2}), 7.90 (s, 1H, H-4 isatin); MS, m/z [%]: 420 [M\textsuperscript{+}], 11.29; 422 [M\textsuperscript{+}+2, 11.37]; Anal. Calcd. for C\textsubscript{17}H\textsubscript{16}BrN\textsubscript{3}O\textsubscript{3}S: 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, ν cm\textsuperscript{-1}): 3363 (NH\textsubscript{2}), 1693 (C=O), 1338, 1161 (SO\textsubscript{2}); \textsuperscript{1}H NMR (DMSO-\textsubscript{d}\textsubscript{6}, 400 MHz) δ ppm: 3.13, 3.25 (2t, 2H, =N-CH\textsubscript{2}-CH\textsubscript{2}-, J = 6.84 Hz), 4.28, 4.61 (2t, 2H, =N-CH\textsubscript{2}-CH\textsubscript{2}-, J = 6.92 Hz), 4.91, 4.94 (2s, 2H, benzylinc CH\textsubscript{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\textsubscript{6}H\textsubscript{5} and H-6 isatin and 2H of SO\textsubscript{2}NH\textsubscript{2}), 7.51-7.57 (m, 3H, H-4 isatin and H-3 & H-5 of -C\textsubscript{6}H\textsubscript{4}-SO\textsubscript{2}NH\textsubscript{2}), 7.76 (d, 2H, H-2 and H-6 of -C\textsubscript{6}H\textsubscript{4}-SO\textsubscript{2}NH\textsubscript{2}, J = 7.36 Hz); \textsuperscript{13}C NMR (DMSO-\textsubscript{d}\textsubscript{6}, 100 MHz) δ: 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\textsubscript{23}H\textsubscript{21}N\textsubscript{3}O\textsubscript{3}S: 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, ν cm\textsuperscript{-1}): 3281 (NH\textsubscript{2}), 1711 (C=O), 1339, 1161 (SO\textsubscript{2}); \textsuperscript{1}H NMR (DMSO-\textsubscript{d}\textsubscript{6}, 400 MHz) δ ppm: 3.12 (t, 2H, =N-CH\textsubscript{2}-CH\textsubscript{2}-, J = 7.20 Hz), 4.60 (t, 2H, =N-CH\textsubscript{2}-CH\textsubscript{2}-, J = 7.20 Hz), 4.94 (s, 2H, benzylinc CH\textsubscript{2}), 6.96 (d, 1H, H-7 isatin, J = 8.00 Hz), 7.28 (s, 2H, SO\textsubscript{2}NH\textsubscript{2}, D\textsubscript{2}O exchangeable), 7.31-7.36 (m, 5H, 5H of C\textsubscript{6}H\textsubscript{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\textsubscript{6}H\textsubscript{4}-SO\textsubscript{2}NH\textsubscript{2}, J = 8.40 Hz), 7.75 (d, 2H, H-2 and H-6 of -C\textsubscript{6}H\textsubscript{4}-SO\textsubscript{2}NH\textsubscript{2}, J = 8.40 Hz); Anal. Calcd. for C\textsubscript{23}H\textsubscript{20}ClN\textsubscript{3}O\textsubscript{3}S: C, 60.86; H, 5.05; N, 10.02; 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, ν cm\textsuperscript{-1}): 3321 (NH\textsubscript{2}), 1722 (C=O), 1330, 1157 (SO\textsubscript{2}); \textsuperscript{1}H NMR (DMSO-\textsubscript{d}\textsubscript{6}, 400 MHz) δ ppm: 3.14, 3.26 (2t, 2H, =N-CH\textsubscript{2}-CH\textsubscript{2}-, J = 7.28 Hz), 4.33, 4.62 (2t, 2H, =N-CH\textsubscript{2}-CH\textsubscript{2}-, J = 7.32 Hz), 4.91, 4.93 (2s, 2H, benzylinc
CH₂), 6.91 (d, 1H, H-7 isatin, \(J = 7.72\) Hz), 7.30-7.44 (m, 7H; 5H of C₆H₅ and 2H of SO₂NH₂), 7.51-7.63 (m, 3H, H-6 isatin and H-3 & H-5 of -C₆H₄-SO₂NH₂), 7.76 (d, 2H, H-2 and H-6 of -C₆H₄-SO₂NH₂, \(J = 6.96\) 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, 152.43, 152.47, 153.33, 157.87, 162.39; Anal. Calcd. for C₂₃H₂₀BrN₃O₃S: 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)benzenesulfonylamide 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, NH₂), 1662 (C=O), 1334, 1157 (SO₂); \(^1\)H NMR (DMSO-\(d_6\), 400 MHz) δ ppm: 2.77 (t, 2H, =N-CH₂-CH₂-, \(J = 7.20\) Hz), 3.32 (t, 2H, =N-CH₂-CH₂-, \(J = 7.2\) Hz), 4.00 (s, 2H, CH₂-C=O), 7.27 (s, 2H, SO₂NH₂, D₂O exchangeable), 7.36 (d, 2H, H-3 and H-5 of -C₆H₄-SO₂NH₂, \(J = 8.40\) Hz), 7.71 (d, 2H, H-2 and H-6 of -C₆H₄-SO₂NH₂, \(J = 8.40\) Hz), 8.27 (t, 1H, NH, D₂O 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, NH₂), 1647 (C=O), 1327, 1149 (SO₂); \(^1\)H NMR (DMSO-\(d_6\), 400 MHz) δ ppm: 2.80 (t, 2H, =N-CH₂-CH₂-, \(J = 7.00\) Hz), 3.35 (q, 2H, =N-CH₂-CH₂-, \(J = 6.86\) Hz), 3.87 (s, 2H, CH₂-C=O), 7.30 (s, 2H, SO₂NH₂, D₂O exchangeable), 7.41 (d, 2H, H-3 and H-5 of -C₆H₄-SO₂NH₂, \(J = 8.12\) Hz), 7.75 (d, 2H, H-2 and H-6 of -C₆H₄-SO₂NH₂, \(J = 8.12\) Hz).
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$_3$O$_3$S$_2$: 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$_2$-CH$_2$-, J = 7.24 Hz), 4.11 (t, 2H, =N-CH$_2$-CH$_2$-, J = 7.16 Hz), 6.93 (d, 1H, H-7 isatin, J = 7.67 Hz), 7.06 (t, 1H, H-5 isatin, J = 7.64 Hz), 7.31 (s, 2H, SO$_2$NH$_2$, D$_2$O exchangeable), 7.39 (t, 1H, H-6 isatin, J = 7.56 Hz), 7.46 (d, 2H, H-3 and H-5 of -C$_6$H$_4$SO$_2$NH$_2$, J = 8.24 Hz), 7.75 (d, 2H, H-2 and H-6 of -C$_6$H$_4$SO$_2$NH$_2$, J = 8.24 Hz), 8.82 (d, 1H, H-4 isatin, J = 7.76 Hz), 11.21 (s, 1H, NH, D$_2$O exchangeable), 11.23 (s, 1H, NH, D$_2$O exchangeable); MS, m/z [%]: 428 [M$^+$, 5.97]; Anal. Calcd. for C$_{19}$H$_{16}$N$_4$O$_4$S$_2$: 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, NH$_2$), 1697 (C=O), 1352, 1156 (SO$_2$); $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ ppm: 3.04 (t, 2H, =N-CH$_2$-CH$_2$-, J = 7.08 Hz), 4.06 (t, 2H, =N-CH$_2$-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$_2$NH$_2$, D$_2$O exchangeable), 7.39 (d, 2H, H-3 and H-5 of -C$_6$H$_4$SO$_2$NH$_2$, J = 8.24 Hz), 7.71 (d, 2H, H-2 and H-6 of -C$_6$H$_4$SO$_2$NH$_2$, J = 8.24 Hz), 8.33 (d, 1H, H-7 isatin, J = 8.12 Hz), 11.14 (s, 1H, NH, D$_2$O exchangeable), 11.22 (s, 1H, NH, D$_2$O exchangeable); Anal. Calcd. for C$_{19}$H$_{15}$FN$_4$O$_4$S$_2$: 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-Chloro-2-oxoindolin-3-ylidene)-4-oxothiazolidin-2-ylidene)amino)ethyl) benzenesulfonamide (9c). Red powder (yield 70%); m.p. > 280 °C; IR (KBr, ν cm⁻¹): 3319, 3237 (NH, NH2), 1696 (C=O), 1350, 1160 (SO₂); ¹H NMR (DMSO-d₆, 400 MHz) δ ppm: 3.04 (t, 2H, =N-CH₂-CH₂-, J = 7.40 Hz), 4.10 (t, 2H, =N-CH₂-CH₂-, J = 7.16 Hz), 6.91-6.97 (m, 1H, H-7 isatin), 7.31 (s, 2H, SO₂NH₂, D₂O exchangeable), 7.47 (d, 2H, H-3 and H-5 of -C₆H₄-SO₂NH₂, 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 -C₆H₄-SO₂NH₂, J = 8.20 Hz), 8.87 (s, 1H, H-4 isatin), 11.12 (s, 1H, NH, D₂O exchangeable), 11.34 (s, 1H, NH, D₂O exchangeable); Anal. Calcd. for C₁₉H₁₅ClN₄O₄S₂: 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-Bromo-2-oxoindolin-3-ylidene)-4-oxothiazolidin-2-ylidene)amino)ethyl) benzenesulfonamide (9d). Red powder (yield 65%); m.p. > 280 °C; IR (KBr, ν cm⁻¹): 3313, 3240 (NH, NH₂), 1693 (C=O), 1354, 1165 (SO₂); ¹H NMR (DMSO-d₆, 400 MHz) δ ppm: 3.04 (t, 2H, =N-CH₂-CH₂-, J = 7.36 Hz), 4.10 (t, 2H, =N-CH₂-CH₂-, J = 7.08 Hz), 6.86 (d, 1H, H-7 isatin, J = 8.32 Hz), 7.32 (s, 2H, SO₂NH₂, D₂O exchangeable), 7.50 (d, 2H, H-3 and H-5 of -C₆H₄-SO₂NH₂, 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 -C₆H₄-SO₂NH₂, J = 8.08 Hz), 11.24 (s br., 2H, NH, D₂O exchangeable); ¹³C NMR (DMSO-d₆, 100 MHz) δ 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 C₁₀H₁₅BrN₄O₄S₂: 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-Methyl-2-oxoindolin-3-ylidene)-4-oxothiazolidin-2-ylidene)amino)ethyl) benzenesulfonamide (9e). Red powder (yield 70%); m.p. > 280 °C; IR (KBr, ν cm⁻¹): 3394, 3224 (NH, NH₂), 1693 (C=O), 1357, 1165 (SO₂); ¹H NMR (DMSO-d₆, 400 MHz) δ ppm: 2.24 (s, 3H, CH₃), 3.03 (t, 2H, =N-CH₂-CH₂-, J = 7.44 Hz), 4.07 (t, 2H, =N-CH₂-CH₂-, 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, SO₂NH₂, D₂O exchangeable), 7.39 (d, 2H, H-3 and H-5 of -C₆H₄-SO₂NH₂, J = 8.08 Hz), 7.70 (d, 2H, H-2 and H-6 of -C₆H₄-SO₂NH₂, J = 7.96 Hz), 8.37 (s, 1H, H-4 isatin), 11.10 (s, 1H, NH, D₂O exchangeable), 11.63 (s, 1H, NH, D₂O exchangeable); Anal. Calcd. for C₂₀H₁₈N₄O₄S₂: 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, NH\(_2\)), 1705 (C=O), 1338, 1157 (SO\(_2\)); \(^1\)H NMR (DMSO-\(d_6\), 400 MHz) \( \delta \) ppm: 3.05 (t, 2H, =N-CH\(_2\)-CH\(_2\)-, \( J \) = 7.20 Hz), 4.07 (t, 2H, =N-CH\(_2\)-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\(_2\)NH\(_2\), D\(_2\)O exchangeable), 7.42 (d, 2H, H-3 and H-5 of -C\(_6\)H\(_4\)-SO\(_2\)NH\(_2\), \( J \) = 8.16 Hz), 7.72 (d, 2H, H-2 and H-6 of -C\(_6\)H\(_4\)-SO\(_2\)NH\(_2\), \( J \) = 8.12 Hz), 8.50 (s, 1H, H-4 isatin), 11.17 (s, 1H, NH, D\(_2\)O exchangeable), 11.59 (s, 1H, NH, D\(_2\)O exchangeable); Anal. Calcd. for C\(_{19}\)H\(_{15}\)N\(_5\)O\(_6\)S\(_2\): 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 earlier [29], and represent the mean from at least three different determinations. All CA isoforms 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.

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References:


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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.