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Graphical abstract

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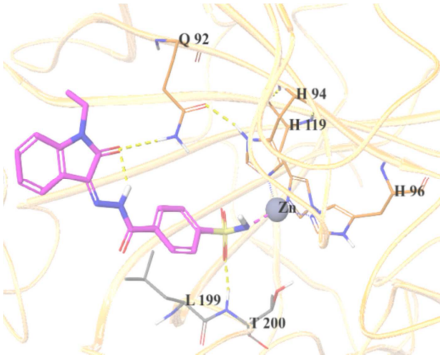

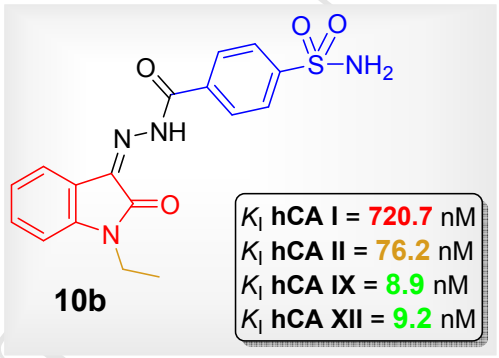

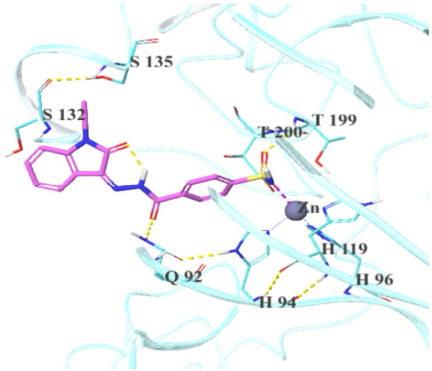

Novel hydrazido benzenesulfonamides-isatin conjugates: Synthesis, carbonic anhydrase inhibitory activity and molecular modeling studies

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ABSTRACT. As a part of our ongoing efforts towards developing novel carbonic anhydrase inhibitors based on the isatin moiety, herein we report the synthesis and biological evaluation of novel sulfonamides (**5a-h**, **10a-g** and **11a-c**) incorporating substituted 2-indolinone moiety (as tail) linked to benzenesulfonamide (as zinc anchoring moiety) through a hydrazide linker. 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, IX and XII. All these isoforms were inhibited by the sulfonamides reported here in variable degrees. hCA I was inhibited with K_Is in the range of 671.8 : 3549.5 nM, hCA II in the range of 36.8 : 892.4 nM; hCA IX in the range of 8.9 : 264.5 nM, whereas hCA XII in the range of 9.0 : 78.1 nM. In particular, compound **10b** emerged as a single-digit nanomolar hCA IX and XII inhibitor (8.9 and 9.2 nM, respectively). Molecular docking studies were carried out for compound **10b** within the hCA II, IX and XII active sites, allowed us to rationalize the obtained inhibition results.

Keywords: Benzenesulfonamides; Isatin; Carbonic anhydrase inhibitors; Synthesis; Molecular docking.

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1. Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) are ubiquitous metalloenzymes, present throughout most living organisms and encoded by seven evolutionarily unrelated gene families: the α-, β-, γ-, δ-, ζ-, η and θ-CAs. Conversion of CO_2 to the bicarbonate ion and protons is a plain physiological reaction that is effectively sped up by CAs [1-4]. This simple reaction is required in many physiological processes, including pH and CO_2 homeostasis, biosynthetic reactions, respiration and transport of CO_2 and bicarbonate between lungs and metabolizing tissues [1,2].

Many of the CA isozymes involved in these processes are significant therapeutic targets with the potential to be inhibited to treat a wide range of disorders. Indeed CA isoforms are involved in diverse physio/pathological conditions such as glaucoma (hCA II, IV and XII), edema (hCA II, IV, XIV), central nervous system (CNS)-related pathologies (hCA VII and XIV) and tumors (hCA IX and hCA XII) [1,2]. In detail, human CA isozymes IX and XII are two tumor-associated proteins, being overexpressed in many tumors and involved in serious processes related to cancer progression and response to therapy. These two CA isozymes had been the recent target for anticancer drug during last years [5-10].

Literature surveying revealed that isatin is a promising tail scaffold to design compounds with interesting carbonic anhydrase inhibitory activity profiles towards different CA isoforms. Several studies have developed diverse isatin-based derivatives as potent CA inhibitors, compounds **I-VI** (**Figure 1**) [11-18].

Based on the aforementioned findings and as a part of our ongoing effort to develop potent isatin-based CAIs [11-14], herein we report the synthesis and biological evaluation of three novel series of sulfonamides (**5a-h**, **10a-g** and **11a-c**, **Figure 1**) featuring benzenesulfonamide, a zinc anchoring moiety, conjugated with an isatin tail through hydrazide linker (-NNHC=O). Two strategies were adopted to design the target sulfonamides. The first one focused on grafting various substituents (compounds **5a-h**) at 5-position of the isatin tail to ensure different electronic and lipophilic environments which could manipulate the activity of the target sulfonamides, and to evaluate their

influence on activity. In the second strategy, different *N*-alkyl (compounds **10a-g**) and *N*-benzyl (compounds **11a-c**) substituents were incorporated into the isatin tail. The *N*-substitution pattern on the isatin moieties was varied in a systemic fashion to define the optimal length (methyl, ethyl, *n*-propyl, *n*-butyl), bulkiness (isopropyl) and unsaturation (allyl, benzyl) which confer the best CA inhibitory effect.

All the newly synthesized sulfonamides were *in vitro* evaluated for their inhibitory activity against a panel of hCA I, II, IX and XII isoforms, using stopped-flow CO2 hydrase assay. Molecular docking studies were carried out for compound **10b** to justify its activity through investigating its key interactions within the active site of hCA II (PDB 5LJT), hCA IX (PDB 5FL4) and hCA XII (PDB 1JD0) isoforms.

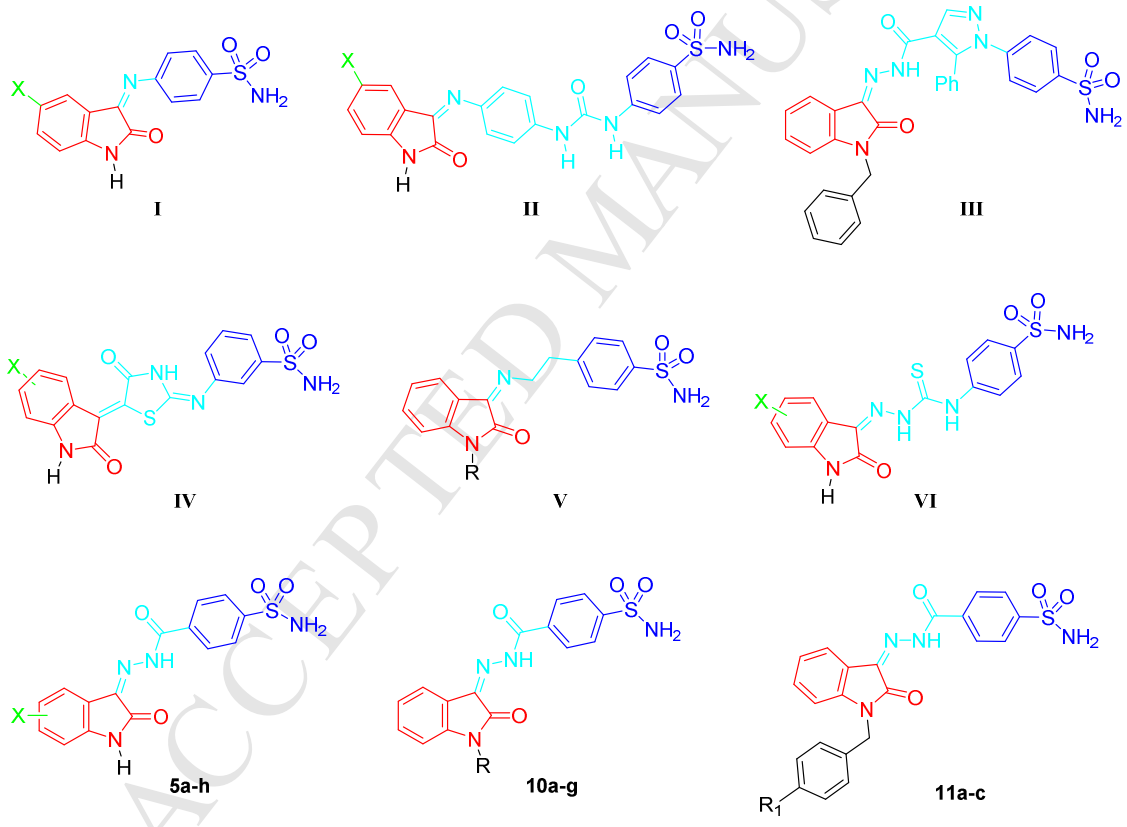
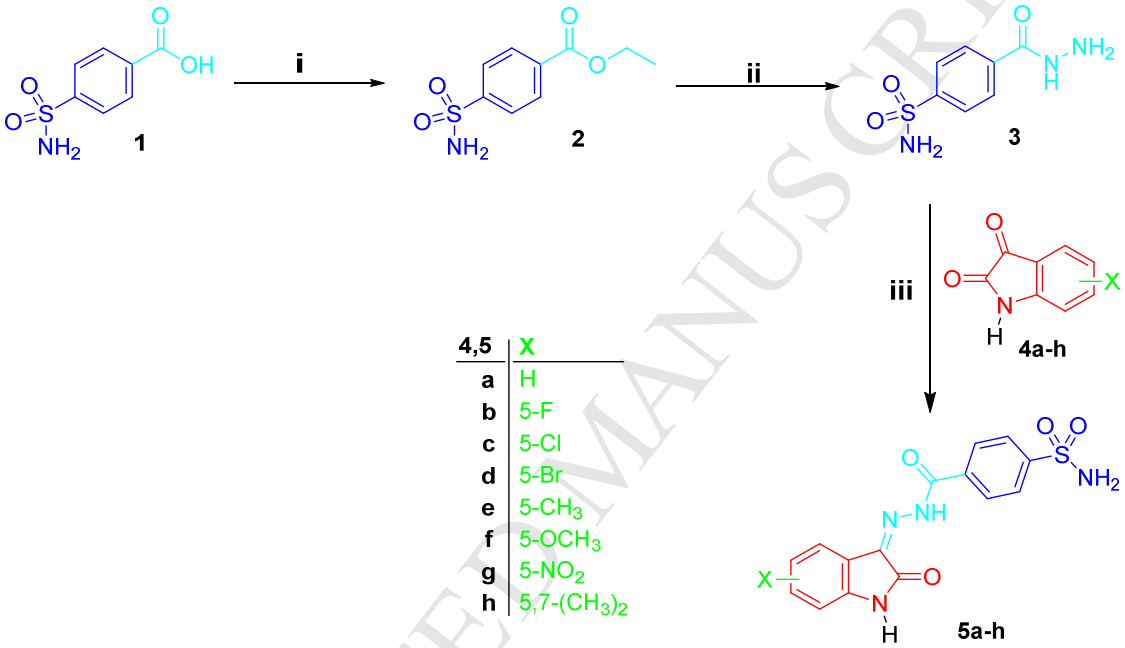


Figure 1. Structures of some reported isatin-based carbonic anhydrase inhibitors **I-VI** and the target sulfonamides **5a-h**, **10a-g** and **11a-c**.

2. Results and Discussion

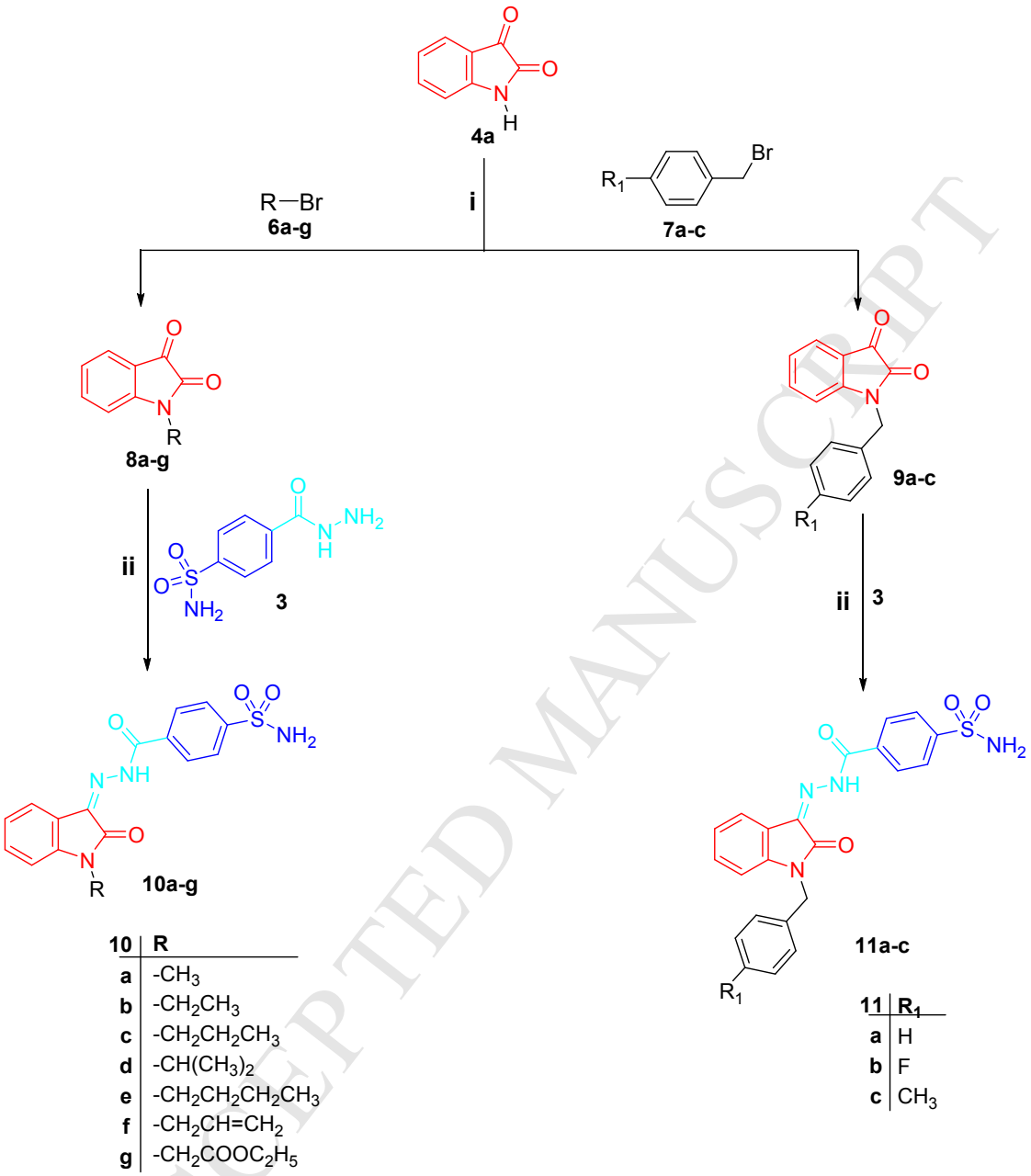
2.1. Chemistry

The synthesis of the new sulfonamides in this study is outlined in **Schemes 1** and **2**. In **Scheme 1**, esterification of 4-sulfamoylbenzoic acid using methanol with catalytic amount of H_2SO_4 under reflux afforded methyl 4-sulfamoylbenzoate **2** which converted to 4-(hydrazinecarbonyl)benzenesulfonamide **3** by refluxing with hydrazine hydrate in methanol. Hydrazide **3** condensed with different isatin derivatives **4a-h** to afford the target compounds **5a-h**.



Scheme 1. Synthesis of target sulfonamides **5a-h**; *Reagents and conditions*: (**i**) Methanol / H_2SO_4 (Cat.) / reflux 8 h, (**ii**) Hydrazine hydrate / methanol / reflux 4 h, (**iii**) Glacial acetic acid / reflux 6 h.

In **Scheme 2**, *N*-substituted isatins **8a-g** and **9a-c** were furnished through NH alkylation of isatin **4a** with different alkyl bromides **6a-g** or benzyl bromides **7a-c** in DMF in the presence of potassium carbonate, which subsequently reacted with the key intermediate 4-(hydrazinecarbonyl)benzene-sulfonamide **3** to give the target compounds **10a-g** and **11a-c** respectively.



10	R
a	-CH_3
b	-CH_2CH_3
c	-$CH_2CH_2CH_3$
d	-$CH(CH_3)_2$
e	-$CH_2CH_2CH_2CH_3$
f	-$CH_2CH{=}CH_2$
g	-$CH_2COOC_2H_5$

11	R_1
a	H
b	F
c	CH_3

Scheme 2. Synthesis of target sulfonamides **10a-g** and **11a-c**; *Reagents and conditions*: (**i**) DMF / K_2CO_3 / KI (Cat.) / reflux 4 h, (**ii**) Glacial acetic acid / reflux 6 h.

The elemental and spectral data supported the structures of the target compounds **5a-h**, **10a-g** and **11a-c**. IR spectra of **5a-h**, **10a-g** and **11a-c** revealed the presence of bands of (NH_2, NH) at 3405-3017 cm^{-1}, also two bands of (C=O) around 1695-1650 cm^{-1}, in addition to two bands of (SO_2) at 1023-1050 and 1308-1390 cm^{-1}. Furthermore, the ^1H

NMR spectra of compounds **5a-h**, **10a-g** and **11a-c** displayed singlet signal of D_2O exchangeable NH_2 of sulfamoyl group and D_2O exchangeable NH of hydrazide at δ 7.60-7.62 *ppm* and δ 13.67-14.02 *ppm* respectively. While, compounds **5a-h** were confirmed with the presence of an additional signal of D_2O exchangeable NH of isatin at δ 11.23-12.01 *ppm*. The protons of aliphatic substituents in compounds **10a-g** were detected at expected chemical shift and integration, meanwhile compounds **11a-c** were confirmed with appearance of benzylic protons at δ 5.02-5.13 *ppm.* On the other hand, ^{13}C NMR spectra of compounds **5a-h**, **10a-g** and **11a-c** revealed the presence of two signals of (C=O) of hydrazid and isatin between δ 160-.07-162.67 and 163.10-163.95 *ppm* respectively. While, compounds **11a-c** were confirmed with appearance of benzylic carbon at δ 42.34-43.20 *ppm.*

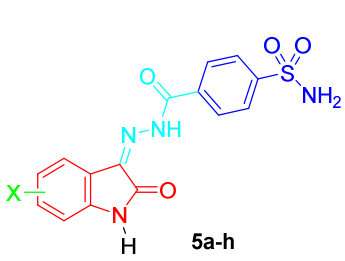
2.2. Carbonic anhydrase inhibition

The newly synthesized sulfonamides **5a-h, 10a-g** and **11a-c** were evaluated for their ability to inhibit the physiologically relevant hCA isoforms, hCA I, II (cytosolic) as well as hCA IX and XII (trans membrane, tumor associated isoforms) using acetazolamide (AAZ) as standard inhibitor by a stopped flow CO_2 hydras assay [19]. The following structure–activity relationship (SAR) can be compiled from the inhibition data reported in **Table 1**:

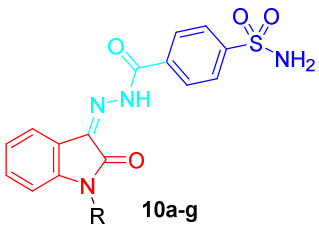
(**i**) The off-target isoform hCA I was weakly inhibited by sulfonamides **5a-h**, **10a-g** and **11a-c** reported here, with inhibition constants (K_Is) ranging from high nanomolar to low micromolar concentration, between 671.8 and 3.549 μM. Indeed, compounds **5a**, **5b**, **5e**, 5**h**, **10a**, **10e**, **10f** and **11a** arose as the least hCA I inhibitors in this study with K_Is in the range (1.485-3.549 μM).

(**ii**) Inhibition of isoform hCA II was ranged from weak to potent inhibitory activity, with K_I values ranging between 36.8 and 892.4 nM. The 5-NO_2 oxoindole derivative **5g** was the most active compound, followed by *N*-ethyl acetate derivative **10g** with K_I values of 36.8 and 38.1 nM, respectively. On the other hand, the *N*-benzyl derivatives **11b** and **11c** displayed the modest activity with K_I values of 784.1 and 892.4 nM, respectively.

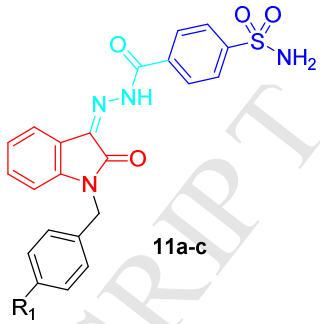
Table 1: Inhibition data of human CA isoforms hCA I, hCA II, hCA IX and hCA XII with sulfonamides **5a-h**, **10a-g** and **11a-c** determined by stopped-flow CO_2 hydrase assay, using acetazolamide (AAZ) as a standard drug.



5a-h



10a-g



11a-c

Comp.	X	R/R_1	K_I (nM)			
			hCA I	hCA II	hCA IX	hCA XII
5a	H	-	2587.3	741.7	173.6	43.9
5b	5-F	-	3289.5	235.1	32.3	9.1
5c	5-Cl	-	843.6	465.0	31.5	15.3
5d	5-Br	-	872.1	488.1	101.2	40.2
5e	5-CH_3	-	1864.2	530.3	196.9	48.7
5f	5-OCH_3	-	899.7	95.1	264.5	24.7
5g	5-NO_2	-	896.3	36.8	23.8	9.0
5h	5,7-$(CH_3)_2$	-	3549.5	598.6	715.6	53.9
10a	-	CH_3	1832.6	160.9	30.6	65.6
10b	-	CH_2CH_3	720.7	76.2	8.9	9.2
10c	-	$CH_2CH_2CH_3$	900.9	244.7	30.1	24.7
10d	-	$CH(CH_3)_2$	991.9	347.9	177.2	16.7
10e	-	$CH_2CH_2CH_2CH_3$	1485.6	565.1	27.4	44.1
10f	-	$CH_2CH{=}CH_2$	1816.0	94.0	77.9	43.3
10g	-	$CH_2COOC_2H_5$	979.3	38.1	17.7	9.6
11a	-	H	2335.2	595.9	25.2	70.4
11b	-	F	671.8	784.1	148.3	53.1
11c	-	CH_3	822.8	892.4	81.2	78.1
AAZ	-	-	250	12	25	5.7

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

(**iii**) All the investigated sulfonamides acted as good inhibitors for the tumor-associated isoform hCA IX (K_I values ranging between 8.9 and 264.5 nM). Superiorly, conjugate **10b** emerged as a single-digit nanomolar hCA IX inhibitor with K_I value of 8.9 nM, which is 3-fold more potent than the standard drug AAZ (K_I = 25 nM against hCA IX). Also, compound **10g** (K_Is = 17.7 nM) was 1.5 times more active than AAZ. In addition, compounds **5b**, **5c**, **5g**, **10a**, **10c**, **10e** and **11a** were equipotent to AAZ with K_Is in the range 23.8-32.3 nM.

It is worth stressing that the inhibitory activity was affected the type of substituent at position 5 of the isatin moiety. Grafting of electron-withdrawing groups enhanced the activity against hCA IX (compounds **5b-d** and **5g**; K_Is range 23.8-101.2 nM) compared to unsubstituted analogue **5a** (K_Is =173.6 nM). Whereas, incorporation of electron-donating groups resulted in a decreased activity (**5e** and **5f**; K_Is = 196.9 and 264.5 nM, respectively) compared to the unsubstituted **5a**. Thus, the order of activities of the 5-substituted isatins **5b-g**, was decreased in the order of NO_2 > F ≈ Cl (small halogens) > Br > CH_3 > OCH_3. In the same context, 5,7-disubstitution with methyl group, compound **5h**, led to the weakest hCA IX inhibitor in this study with remarkable decrease in activity (K_Is =715.6 nM).

Regarding the impact of *N*-alkylation of isatin moiety in compounds **10**; the obtained results highlighted that incorporating different alkyl groups, except **10d**, enhanced the activity (K_Is range 8.9-77.9 nM), in comparison to unsubstituted derivative **5a** (K_Is =173.6 nM). It is noteworthy that ethyl group represented the optimal length which confer the best hCA IX inhibitory effect in this study. Otherwise, both branching (as compound **10d**; K_Is =177.2 nM) and unsaturation (as compound **10f**; K_Is =77.9 nM) decreased activity, compared to compound **10c** (K_Is =30.1 nM). Finally, grafting *N*-benzyl group, compound **11a**, was well-tolerated (K_Is =25.2 nM). Whereas, *p*-substitution on the benzyl group with electron-withdrawing or electron-donating group, decreased the inhibitory activity against hCA IX (Compounds **11b** and **11c**; K_Is = 148.3 and 81.2 nM, respectively).

(**iv**) The tumor-associated isoform hCA XII was undeniably the highly affected isoform amongst the hCA isoforms considered here, with K_Is in the range of 9.0-78.1 nM. The most effective compounds were **5b, 5c, 5f, 5e** and **10b, 10c, 10d** and **10g** with K_Is in

the range of 9.0-24.7 nM. Similar to the SAR for hCA IX inhibition; 5-substitution with electron-withdrawing NO_2 group and small halogens (as F and Cl) displayed an improved activity (compounds **5b**, **5c** and **5g**; K_Is range 9.0-15.3 nM). In addition, 5-substitution with electron-donating methyl group displayed a slight decreased activity (compound **5e**; K_Is =48.7 nM) compare to unsubstituted analogue **5a** (K_Is =43.9 nM). In contrary, grafting electron-donating methoxy group (compound **5f**; K_Is =24.7 nM) enhanced the inhibitory activity towards hCA XII.

Interestingly, *N*-ethyl isatin counterpart **10b** emerged as the most potent hCA XII inhibitor (K_Is =9.2 nM) among the *N*-substituted isatin series **10**, as highlighted for hCA IX inhibition. Moreover, unsaturated derivative **10f** possessed lower inhibitory activity (K_Is =43.3 nM) than its saturated analogue **10c** (K_Is =24.7 nM). Unlike hCA IX, the utilization of branched alkyl group enhanced the activity (K_Is =16.7 nM) against hCA XII. Finally, the *N*-benzyl derivatives **11a-c** failed to improve the inhibitory activity against hCA XII.

3. Molecular docking studies

Compound **10b** was selected for molecular docking simulations to justify its activity by investigating its key interactions within the active site of hCA II (PDB 5LJT [20]), hCA IX (PDB 5FL4 [21]) and hCA XII (PDB 1JD0 [22]) isoforms. The top poses for **10b** docked within the active site of hCA II, hCA IX and hCAXII were illustrated in **Figures 2**, **3** and **4,** respectively**.** As a result, the sulfonamide group played the usual role as zinc-binding group (ZBG) for compound **10b** with the three different hCA isoforms by forming a metallic bond with Zn metal and hydrogen bond with Thr200. Carbonyl group of oxindole moiety was involved in the interaction within the active site of hCA II and IX isoforms by accepting a hydrogen bond from Gln92. The same interaction took place in hCA XII active site by carbonyl group of hydrazide moiety. All of the aforementioned contacts explained the inhibitory activity for compound **10b** for the three different isoforms.

Regarding the low inhibitory activity of compound **10b** towards hCAII isoform, this could be explained through the behavior of *N*-ethyl moiety of oxindole ring inside the active site of the three isoforms. Within hCA II active site, the *N*-ethyl moiety was

engaged to unfavorable interactions with Ile91 and Gln 92. Free rotation of this moiety would not compensate this bad interaction, as it would result in another unfavorable interaction with Asn67 (**Figure 2**). In contrast, *N*-ethyl moiety was not surrounded by any clashes with amino acid in the active site of hCA IX as Leu91 was 4.06 Å away from *N*-ethyl moiety (**Figure 3**). Moreover, within the active site of hCA XII, polar Ser132 and Ser135 were facing *N*-ethyl moiety from one side, but free rotation of this group would assure the absence of any unfavorable interactions with these amino acids **(Figure 4**).

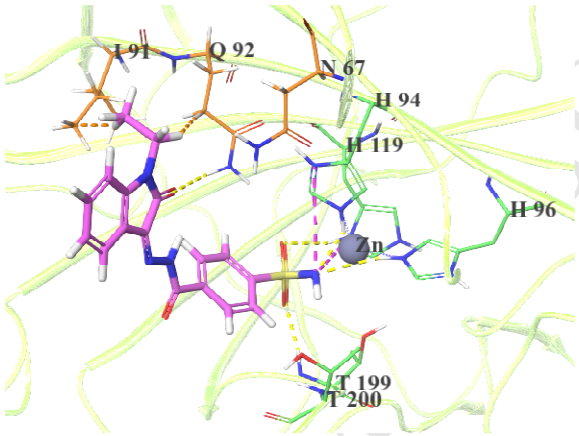


Figure 2. Docking pose of compound **10b** with the active site of hCA II (PDB 5LJT) showing the unfavorable interactions of *N*-ethyl moiety of oxindole group with amino acids Ile91 and Gln92 which is assigned by orange dashed lines.

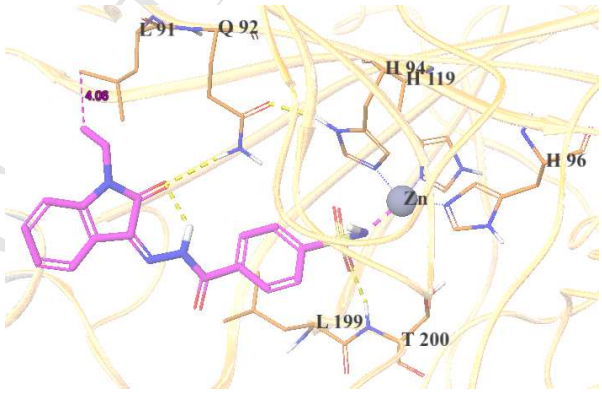


Figure 3. Simulated binding pose of compound **10b** with the active site of hCA IX (PDB 5FL4) illustrating the common features for activity with no clashes around *N*-ethyl moiety of oxindole group.

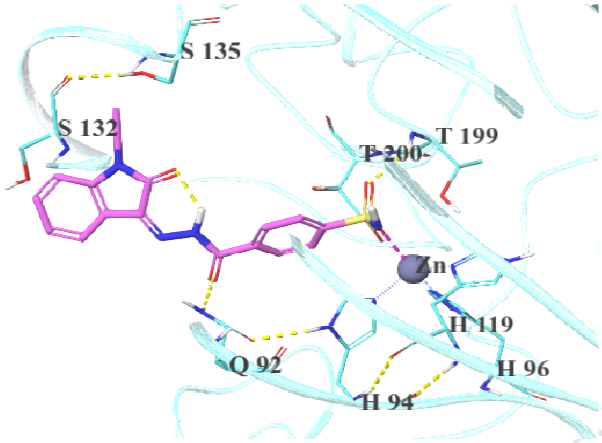


Figure 4. Interaction of compound **10b** with the key amino acids and Zn metal within the active site of hCA XII (PDB 1JD0) showing that *N*-ethyl moiety of oxindole group is hindered from one side only. Hydrogen bonds were represented as yellow dashed lines.

4. Conclusion

In summary, we report here the synthesis of three novel series of sulfonamides (**5a-h**, **10a-g** and **11a-c**), incorporating different isatin moieties linked to benzenesulfonamide (as zinc anchoring moieties) through hydrazide linker. The structure of the novel sulfonamides was confirmed by the different spectral and elemental analyses methods. Biological evaluation of the newly prepared sulfonamides was performed against hCA I, II, IX and XII. All the tested isoforms were inhibited by the synthesized sulfonamides **5a-h**, **10a-g** and **11a-c**, in variable degrees. Best activity was observed against both isoforms hCA IX and XII. Sulfonamide **10b** emerged as single-digit nanomolar hCA IX and XII inhibitor (K_Is = 8.9 and 9.2 nM, respectively). Finally, the inhibitory potency of the synthesized sulfonamides was further investigated carrying out molecular docking for sulfonamide **10b** to rationalize the obtained inhibition results.

5. Experimental

5.1. Chemistry

5.1.1. General

Melting points were measured with a Stuart melting point apparatus and were uncorrected. Infrared (IR) Spectra were recorded as KBr disks using Schimadzu FT-IR

8400S spectrophotometer. Mass spectral data are given by GCMS-QP1000 EX spectrometer at 70 e.V. NMR Spectra were recorded on Bruker AVF-400 spectrometer. ^{1}H spectrum was run at 400 MHz and ^{13}C spectrum was run at 100 MHz in deuterated dimethylsulfoxide (DMSO-d_6). Chemical shifts are expressed in values (*ppm*) using the solvent peak as internal standard. All coupling constant (*J*) values are given in hertz. The abbreviations used are as follows: s, singlet; d, doublet; m, multiplet. Elemental analyses were carried out at the Regional Center for Microbiology and Biotechnology, Al-Azhar University, Cairo, Egypt. High-resolution mass spectra (EI and ESI) were recorded using a Bruker MicroTOF spectrometer. Analytical thin layer chromatography (TLC) on silica gel plates containing UV indicator was employed routinely to follow the course of reactions and to check the purity of products.

5.1.2. Synthesis of compounds 2 and 3

Compounds **2** and **3** were prepared according to the literature procedures [23].

5.1.3. Synthesis of N-substituted isatin derivatives 8a-g and 9a-c.

Isatin **4a** (0.5 gm, 3.4 mmol) was stirred in DMF (15 mL) with (3.7 mmol) the appropriate alkyl halide **6a-g** or benzyl bromide derivative **9a-c** in the presence of potassium carbonate (7.4 mmol) and catalytic amount of potassium iodide at reflux temperature for 4 hrs. After the reaction is completed, the mixture was poured into ice water, the formed solid was collected, washed with water and recrystallized from ethanol to furnish compounds **8a-g** or **9a-c**, respectively [24-27].

5.1.4. Synthesis of 4-[2-(2-oxoindolin-3-ylidene)hydrazine-1-carbonyl]benzenesulfonamide derivatives 5a-h, 10a-g and 11a-c

To a hot solution of 4-(hydrazinecarbonyl)benzenesulfonamide **3** (0.25g, 1.16 mmol) in glacial acetic acid (15 mL), the appropriate isatin derivative **4a-h, 8a-g** or **9a-c** (1.2 mmol) was added. This mixture was heated under reflux for 6 hrs. The formed precipitate was filtered off while hot and washed with ethanol and petroleum ether then recrystallized from DMF/ethanol to obtain the target compounds **5a-h**, **10a-g** and **11a-c**.

*5.1.4.1. 4-(2-(2-Oxoindolin-3-ylidene)hydrazine-1-carbonyl)benzenesulfonamide **5a.***

Yellow powder (yield 75%), m.p. Over 300 °C; IR(KBr, ν cm^{-1}): 3210, 3150, 3018 (NH_2, 2NH), 1690, 1652 (2C=O) and 1337, 1023 (SO_2); ^{1}H NMR (DMSO-d6, 400 MHz) *δ ppm*: 6.97 (d, 1H, H-7 of isatin, *J* = 8 Hz), 7.11 (t, 1H, H-5 of isatin, *J* = 7.6 Hz), 7.40 (t, 1H, H-5 of isatin, *J* = 7.6 Hz), 7.61 (br s, 3H, H-4 of isatin, 2H, NH_2, D_2O exchangeable), 8.02-8.09 (m, 4H, H-2,3,5 and 6 of phenyl ring of benzenesulfonamide), 11.33 (s, 1H, isatin NH, D_2O exchangeable), 13.97 (s, 1H, hydrazide NH, D_2O exchangeable); ^{13}C NMR (DMSO-d6,100 MHz) *δ ppm*: 111.79, 116.22, 120.16, 121.60, 123.32, 126.84, 128.79, 132.55, 134.35, 135.47, 139.28, 143.10, 147.96 (Aromatic carbons), 162.67 (hydrazide C=O), 163.47 (isatin C=O); HRMS (ESI) for $C_{15}H_{11}O_4N_4S$, calcd 343.05065, found 343.05029 $[M+H]^+$.

5.1.4.2. 4-[2-(5-Fluoro-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl]benzenesulfonamide ***5b****.*

Yellow powder (yield 65%), m.p. Over 300 °C; IR (KBr, ν cm^{-1}): 3395, 3210, 3018 (NH_2, 2NH), 1695, 1667 (2 C=O) and 1331, 1023 (SO_2); ^{1}H NMR (DMSO-d6, 400 MHz) *δ ppm*: 6.95 (dd, 1H, H-6 of isatin, *J* = 8.4 Hz), 7.23 (dt, 1H, H-7 of isatin, *J* = 8.8), 7.43 (d, 1H, H-4 of isatin, *J* = 6.8 Hz), 7.61 (s, 2H, NH_2, D_2O exchangeable), 8.03-8.09 (m, 4H, H-2,3,5 and 6 of phenyl ring of benzenesulfonamide), 11.43 (s, 1H, isatin NH, D_2O exchangeable), 13.94 (s, 1H, hydrazide NH, D_2O exchangeable); ^{13}C NMR (DMSO-d6, 100 MHz) *δ ppm*: 108.57, 108.83, 112.87, 112.95, 118.76, 119.00, 121.35, 126.86, 128.92, 135.26, 139.35, 148.03, 157.70 (aromatic carbons), 160.07 (hydrazide C=O), 163.58 (isatin C=O); HRMS (ESI) for $C_{15}H_{10}O_4N_4FS$, calcd 361.04123, found 361.04106 $[M+H]^+$.

5.1.4.3. 4-(2-(5-Chloro-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl)benzenesulfonamide ***5c.***

Red powder (yield 60%), m.p. Over 300 °C; IR (KBr, ν cm^{-1}): 3395, 3232, 3018 (NH_2,2NH), 1690, 1652 (2 C=O) and 1337, 1023 (SO_2); ^{1}H NMR (DMSO-d6, 400 MHz) *δ ppm*: 6.97 (d, 1H, H-6 of isatin, *J* = 8.4 Hz), 7.44 (d, 1H, H-7 of isatin, *J* = 8.8 Hz), 7.59-7.61 (m, 3H, 1H of H-4 of isatin and 2H, NH_2, D_2O exchangeable), 8.03-8.09 (m, 4H, H-2,3,5 and 6 of phenyl ring of benzenesulfonamide), 11.52 (s, 1H, isatin NH, D_2O

exchangeable), 13.88 (s, 1H, hydrazide NH, D_2O exchangeable); ^{13}C NMR (DMSO-d6,100 MHz) *δ ppm*: 111.39, 113.32, 117.98, 121.08, 121.87, 126.85, 127.42, 128.86, 129.02, 131.90, 135.22, 141.76, 148.03 (aromatic carbons), 162.07 (hydrazide C=O), 163.26 (isatin C=O), HRMS (ESI) for $C_{15}H_{10}O_4N_4ClS$, calcd 377.01168, found 377.01167 $[M+H]^+$.

5.1.4.4. 4-[2-(5-Bromo-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl]benzenesulfonamide ***5d****.*

Yellow powder (yield 70%), m.p. Over 300 °C; IR (KBr, ν cm^{-1}): 3408, 3210, 3017 (NH_2,2NH), 1690, 1671 (2C=O) and 1337, 1023 (SO_2); ^1H NMR (DMSO-d6, 400 MHz) *δ ppm*: 6.92 (d, 1H, H-6 of isatin, *J* = 8.4 Hz), 7.56-7.61 (m, 3H, 1H of H-7 of isatin and 2H, NH_2, D_2O exchangeable), 7.69 (s, 1H, H-4 of isatin, Ar-H), 8.03-8.09 (m, 4H, H-2,3,5 and 6 of phenyl ring of benzenesulfonamide), 11.50 (s, 1H, isatin NH, D_2O exchangeable), 13.88 (s, 1H, hydrazide NH, D_2O exchangeable); ^{13}C NMR (DMSO-d6, 100 MHz) *δ ppm*: 111.76, 113.76, 114.99, 118.76, 122.26, 123.79, 126.85, 128.77, 134.69, 135.21, 138.16, 142.13, 148.02 (aromatic carbons), 162.07 (hydrazide C=O), 163.10 (isatin C=O); HRMS (ESI) for $C_{15}H_{10}O_4N_4BrS$, calcd 420.96116, found 420.96126 $[M+H]^+$.

5.1.4.5. 4-[2-(5-Methyl-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl]benzenesulfonamide ***5e****.*

Yellow powder (yield 66%), m.p. Over 300°C; IR (KBr, ν cm^{-1}): 3386, 3210, 3018 (NH_2, 2NH), 1695, 1652 (2C=O) and 1337, 1023 (SO_2); ^1H NMR (DMSO-d6, 400 MHz) *δ ppm*: 2.31 (s, 3H, CH_3), 6.84 (d, 1H, H-6 of isatin, *J* = 8 Hz), 7.19 (d, 1H, H-7 of isatin, *J* = 7.6 Hz), 7.43 (s, 1H, H-4 of isatin), 7.60 (s, 2H, NH_2, D_2O exchangeable), 8.02-8.08 (m, 4H, H-2,3,5 and 6 of phenyl ring of benzenesulfonamide), 11.29 (s, 1H, isatin NH, D_2O exchangeable), 13.97 (s, 1H, hydrazide NH, D_2O exchangeable); ^{13}C NMR (DMSO-d6, 100 MHz) *δ ppm*: 20.98 (CH_3), 111.54, 115.07, 120.13, 121.91, 126.83, 128.70, 132.41, 132.95, 135.46, 139.44, 140.82, 147.91, 153.50 (aromatic carbons), 162.48 (hydrazide C=O), 163.53 (isatin C=O); HRMS (ESI) for $C_{16}H_{13}O_4N_4S$, calcd 357.06630, found 357.06635 $[M+H]^+$.

5.1.4.6. 4-[2-(5-Methoxy-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl]benzenesulfonamide ***5f.***

Red powder (yield 60%), m.p. Over 300 °C; IR (KBr, *ν* cm^{-1}): 3393, 3210, 3018 (NH_2, 2NH), 1692, 1654 (2C=O) and 1308, 1023 (SO_2); ^{1}H NMR (DMSO-d6, 400 MHz) *δ ppm*: 3.79 (s, 3H, OCH_3), 6.88 (d, 1H, H-6 of isatin, *J* = 8.4 Hz), 6.99 (d, 1H, H-7 of isatin, *J* = 8.4 Hz), 7.15 (s, 1H, H-4 of isatin), 7.60 (s, 2H, NH_2, D_2O exchangeable), 8.02-8.08 (m, 4H, H-2,3,5 and 6 of phenyl ring of benzenesulfonamide), 11.23 (s, 1H, isatin NH, D_2O exchangeable), 14.02 (s, 1H, hydrazide NH, D_2O exchangeable); ^{13}C NMR (DMSO-d6,100 MHz) *δ ppm*: 56.13 (O-CH_3), 106.38, 111.85, 112.67, 118.84, 120.86, 126.87, 128.73, 135.40, 136.75, 140.61, 145.51, 148.01, 155.96 (aromatic carbons), 161.58 (hydrazide C=O), 163.59 (isatin C=O); HRMS (ESI) for $C_{16}H_{13}O_5N_4S$, calcd 373.06121, found 373.06106 $[M+H]^+$.

5.1.4.7. 4-[2-(5-Nitro-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl]benzenesulfonamide ***5g***.

Yellow powder (yield 70%), m.p. Over 300 °C; IR (KBr, ν cm^{-1}): 3393, 3210, 3018 (NH_2, 2NH), 1694, 1655 (2C=O) and 1382, 1048 (SO_2); ^{1}H NMR (DMSO-d6, 400 MHz) *δ ppm*: 7.13 (d, 1H, H-7 of isatin, *J* = 8.4 Hz), 7.62 (s, 2H, NH_2, D_2O exchangeable), 8.04-8.09 (m, 4H, H-2,3,5 and 6 of phenyl ring of benzenesulfonamide), 8.24 (s, 1H, H-4 of isatin), 8.28 (d, 1H, H-6 of isatin, *J* = 8.8 Hz), 12.01 (s, 1H, isatin NH, D_2O exchangeable), 13.67 (s, 1H, hydrazide NH, D_2O exchangeable); ^{13}C NMR (DMSO-d6,100 MHz) *δ ppm*: 110.29, 112.06, 116.35, 120.83, 126.87, 128.23, 128.99, 134.99, 137.48, 140.52, 143.35, 148.10, 148.15 (aromatic carbons), 160.34 (hydrazide C=O), 163.71 (isatin C=O); HRMS (ESI) for $C_{15}H_{10}O_6N_5S$, calcd 388.03573, found 388.03564 $[M+H]^+$.

5.1.4.8. 4-[2-(5,7-Dimethyl-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl]benzenesulfonamide ***5h***.

Yellow powder (yield 85%), m.p. Over 300 °C; IR (KBr, ν cm^{-1}): 3387, 3210, 3018 (NH_2, 2NH), 1692, 1653 (2C=O) and 1361, 1023 (SO_2); ^{1}H NMR (DMSO-d6, 400 MHz) *δ ppm*: 2.20 (s, 3H, CH_3 of position 7 of isatin), 2.27 (s, 3H, CH_3 of position 5 of isatin),

7.04 (s, 1H, H-6 of isatin), 7.26 (s, 1H, H-4 of isatin), 7.60 (s, 2H, NH_2, D_2O exchangeable), 8.02-8.08 (m, 4H, H-2,3,5 and 6 of phenyl ring of benzenesulfonamide), 11.31 (s, 1H, isatin NH, D_2O exchangeable), 13.97 (s, 1H, hydrazide NH, D_2O exchangeable); ^{13}C NMR (DMSO-d6, 100 MHz) *δ ppm*: 16.31 (CH_3 of position 7 of isatin), 20.89 (CH_3 of position 5 of isatin), 116.33, 119.31, 119.88, 120.94, 126.84, 128.71, 131.47, 132.32, 134.38, 135.51, 139.47, 146.22, 147.96 (aromatic carbons), 162.67 (hydrazide C=O), 163.95 (isatin C=O); HRMS (ESI) for $C_{17}H_{17}O_4N_4S$, calcd 373.09650, found 373.09634 $[M+H]^+$.

5.1.4.9. 4-[2-(1-Methyl-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl]benzenesulfonamide ***10a****.*

Orange powder (yield 71%), m.p. 300 °C; IR (KBr, ν cm^{-1}): 3400, 3019 (NH, NH2), 16935, 1652 (C=O) and 1380, 1043 (SO_2); ^1H NMR (DMSO-d6, 400 MHz) *δ ppm*: 3.35 (s, 3H, *N*-CH_3), 7.16-7.20 (m, 2H, H-5,7 of isatin), 7.46 (t, 1H, H-6 of isatin, *J* = 7.6 Hz), 7.61 (br s, 3H,1H of H-4 of isatin, 2H of NH_2, D_2O exchangeable), 8.04-8.9 (m, 4H, H-2,3,5 and 6 of phenyl ring of benzenesulfonamide), 13.88 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d6,100 MHz) *δ ppm:* 26.21 (CH_3), 110.55, 116.60, 119.41, 121.20, 123.82, 126.85, 128.77, 132.41, 135.31, 138.42, 144.27, 149.02, 154.22 (aromatic carbons), 161.59 (hydrazide C=O), 162.49 (isatin C=O); Anal. Calcd. for $C_{16}H_{14}N_4O_4S$ (358.37): C, 53.62; H, 3.94; N, 15.63; found C, 53.80; H, 3.90; N, 15.57.

5.1.4.10. 4-(2-(1-Ethyl-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl)benzenesulfonamide ***10b****.*

Yellow powder (yield 70%), m.p. 270-273 °C; IR (KBr, ν cm^{-1}): 3395, 3017 (NH, NH2), 1693, 1652 (C=O) and 1345, 1043 (SO_2); ^1H NMR (DMSO-d6, 400 MHz) *δ ppm*: 1.20 (t, 2H, *N*-CH_2, *J* = 7.2 Hz), 3.77 (q, 3H, CH_2-$C\underline{H}_3$, *J* = 7.2), 7.14 (t, 1H, H-5 of isatin, *J* = 7.6 Hz), 7.20 (d, 1H, H-7 of isatin, *J* = 8 Hz), 7.44 (t, 1H, H-6 of isatin, *J* = 7.6 Hz), 7.62 (br s, 3H,1H of H-4 of isatin and 2H of NH_2, D_2O exchangeable), 8.04-8.09 (m, 4H, H-2,3,5 and 6 of phenyl ring of benzenesulfonamide), 13.88 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d6,100 MHz) *δ ppm*: 13.01 ($\underline{C}H_3$-CH_2), 34.62 (*N*-$\underline{C}H_2$), 110.56, 116.60, 119.56, 121.40, 123.71, 126.84, 128.78 132.42, 135.30, 138.42, 143.19, 148.02, 154.22 (aromatic carbons), 161.21 (hydrazide C=O), 162.49 (isatin

C=O); Anal. Calcd. for $C_{17}H_{16}N_4O_4S$ (372.40): C, 54.83; H, 4.33; N, 15.05; found C, 54.72; H, 4.29; N, 15.11.

5.1.4.11. 4-[2-(2-Oxo-1-propylindolin-3-ylidene)hydrazine-1-carbonyl]benzenesulfonamide ***10c****.*

Yellow powder (yield 80%), m.p. 285-286 °C; IR (KBr, ν cm^{-1}): 3405, 3017 (NH, NH2), 1693, 1654 (C=O) and 1382, 1047 (SO_2); ^{1}H NMR (DMSO-d6, 400 MHz) *δ ppm*: 0.90 (t, 3H, C<u>H</u>$_3$-CH$_2$, *J* = 7.6 Hz), 1.63-1.73 (m, 2H, CH$_3$-C<u>H</u>$_2$), 3.73 (t, 2H, *N*-C<u>H</u>$_2$, *J* = 6.8 Hz), 7.17 (t, 1H, H-5 of isatin, *J* = 7.6 Hz), 7.25 (d, 1H, H-7 of isatin, *J* = 8 Hz), 7.47 (t, 1H, H-6 of isatin, *J* = 8 Hz), 7.61 (s, 2H, NH_2, D_2O exchangeable), 7.65 (d, 1H, H-4 of isatin, *J* = 6.8 Hz), 8.03-8.10 (m, 4H, H-2,3,5 and 6 of phenyl ring of benzenesulfonamide), 13.91 (s, 1H, hydrazide NH, D_2O exchangeable); ^{13}C NMR (DMSO-d6,100 MHz) *δ ppm:* 11.66 (CH$_2$-<u>C</u>H$_3$), 20.88 (<u>C</u>H$_2$-CH$_3$), 41.32 (*N*-<u>C</u>H$_2$), 110.81, 116.60, 119.51, 121.41, 123.76, 126.86, 128.81, 132.47, 135.31, 140.38, 143.69, 148.03, 154.22 (aromatic carbons), 161.62 (hydrazide C=O), 162.57 (isatin C=O); HRMS (ESI) for $C_{18}H_{19}O_4N_4S$, calcd 387.11215, found 387.11266 $[M+H]^+$.

5.1.4.12. 4-[2-(1-Isopropyl-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl]benzenesulfonamide ***10d****.*

Yellow powder (yield 77%), m.p. 295-296 °C; IR (KBr, ν cm^{-1}): 3400, 3017 (NH, NH2), 1690, 1650 (C=O) and 1380, 1040 (SO_2); ^{1}H NMR (DMSO-d6, 400 MHz) *δ ppm*: 1.47 (d, 6H, C<u>H</u>$_3$-CH-C<u>H</u>$_3$, *J* = 7.2 Hz), 4.55-4.63 (m, 1H, *N*-CH), 7.15 (t, 1H, H-5 of isatin, *J* = 7.6 Hz), 7.34 (d, 1H, H-7 of isatin, *J* = 8 Hz), 7.45 (t, 1H, H-6 of isatin, *J* = 7.6 Hz), 7.61 (s, 2H, NH_2, D_2O exchangeable), 7.64 (d, 1H, H-4 of isatin, *J* = 7.2 Hz), 8.04-8.10 (m, 4H, H-2,3,5 and 6 of phenyl ring of benzenesulfonamide), 13.95 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d6,100 MHz) *δ ppm:* 19.56 (<u>C</u>H$_3$-CH-<u>C</u>H$_3$), 44.41 (*N*-<u>C</u>H), 111.69, 119.86, 116.60, 121.45, 123.48, 126.86, 128.80, 132.34, 135.38, 138.36, 142.88, 148.01, 154.22 (aromatic carbons), 161.40 (hydrazide C=O), 162.40 (isatin C=O); Anal. Calcd. for $C_{18}H_{18}N_4O_4S$ (386.43): C, 55.95; H, 4.70; N, 14.50; found C, 55.73; H, 4.66; N, 14.57.

*5.1.4.13. 4-[2-(1-Butyl-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl]benzenesulfonamide **10e**.*

Yellow powder (yield 70%), m.p. 272 °C; IR (KBr, ν cm^{-1}): 3989, 3017 (NH, NH2), 1693, 1655 (C=O) and 1378, 1042 (SO_2); ^{1}H NMR (DMSO-d6, 400 MHz) *δ ppm*: 0.89 (t, 3H, CH_2-C<u>H</u>$_3$, *J* = 7.6 Hz), 1.32-1.40 (m, 2H, C<u>H</u>$_2$-CH_3), 1.60-1.67 (m, 2H, *N*-C<u>H</u>$_2$-CH_2), 3.75 (t, 2H, *N*-CH_2, *J* = 7.2 Hz), 7.16 (t, 1H, H-5 of isatin, *J* = 7.6 Hz), 7.22 (d, 1H, H-7 of isatin, *J* = 8 Hz), 7.46 (t, 1H, H-6 of isatin, *J* = 8 Hz), 7.61 (s, 2H, NH_2, D_2O exchangeable), 7.64 (d, 1H, H-4 of isatin, *J* = 7.2 Hz), 8.04-8.10 (m, 4H, H-2,3,5 and 6 of phenyl ring of benzenesulfonamide), 13.89 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d6,100 MHz) *δ ppm*: 14.05 (<u>C</u>H$_3$-CH_2), 20.03 (CH_3-<u>C</u>H$_2$), 29.55 (*N*-<u>C</u>H$_2$-CH_2), 41.18 (*N*-<u>C</u>H$_2$-), 110.73, 116.60, 119.54, 121.38, 123.73, 126.85, 128.78, 138.36, 132.44, 135.32, 143.60, 148.03, 154.22 (aromatic carbons), 161.52 (hydrazide C=O), 162.40 (isatin C=O); Anal. Calcd. for $C_{19}H_{20}N_4O_4S$ (400.45): C, 56.99; H, 5.03; N, 13.99; found C, 56.85; H, 4.99; N, 13.93.

*5.1.4.14. 4-[2-(1-Allyl-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl]benzenesulfonamide **10f**.*

Yellow powder (yield 67%), m.p. 253-255 °C; IR (KBr, ν cm^{-1}): 3405, 3017 (NH, NH2), 1693, 1654 (C=O) and 1390, 1050 (SO_2); ^{1}H NMR (DMSO-d6,400MHz) *δ ppm*: 4.42 (br s, 2H, *N*-CH), 5.21-5.30 (m, 2H, C<u>H</u>$_2$=CH), 5.86-5.96 (m, 1H, *N*-CH-C<u>H</u>), 7.11 (d, 1H, H-7 of isatin, *J* = 8 Hz), 7.17 (t, 1H, H-5 of isatin, *J* = 7.6 Hz), 7.45 (t, 1H, H-6 of isatin, *J* = 7.6 Hz), 7.62 (s, 2H, NH_2, D_2O exchangeable), 7.64 (d, 1H, H-4 of isatin, *J* = 6.4 Hz), 8.04-8.10 (m, 4H, H-2,3,5 and 6 of phenyl ring of benzenesulfonamide), 13.85 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d6,100 MHz) *δ ppm*: 41.91 (*N*-<u>C</u>H), 111.05, 116.60, 118.09, 119.56, 121.37, 123.86, 126.86, 128.81, 131.72, 132.34, 135.29, 138.18, 143.38, 148.04, 154.15 (aromatic carbons), 161.32 (hydrazide C=O), 162.40 (isatin C=O); Anal. Calcd. for $C_{18}H_{16}N_4O_4S$ (384.41): C, 56.24; H, 4.20; N, 14.58; found C, 56.45; H, 4.17; N, 14.52.

*5.1.4.15. Ethyl-2-[2-oxo-3-(2-(4-sulfamoylbenzoyl)hydrazono)indolin-1-yl]acetate **10g**.*

Yellow powder (yield 72%), m.p. 253-255 °C; IR (KBr, ν cm^{-1}): 3405, 3018 (NH, NH2), 1706, 1693, 1654 (C=O) and 1381, 1023 (SO_2); ^{1}H NMR (DMSO-d6, 400 MHz) *δ ppm*: 1.22 (t, 3H, CH_2-C<u>H</u>$_3$, *J* = 7.2 Hz), 4.16 (q, 2H, C<u>H</u>$_2$-CH_3, *J* = 7.6 Hz), 4.74 (s, 2H, *N*-CH_3), 7.20-7.24 (m, 2H, H-5,7 of isatin), 7.47 (t, 1H, H-6 of isatin, *J* = 7.6 Hz), 7.61 (s, 2H, NH_2, D_2O exchangeable), 7.64 (d, 1H, H-4 of isatin, *J* = 7.2 Hz), 8.04-8.11 (m, 4H, H-2,3,5 and 6 of phenyl ring of benzenesulfonamide), 13.85 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d6,100 MHz) *δ ppm*: 14.48 (CH_3), 56.51 (*N*-CH_2), 61.94 (<u>C</u>H_2-CH_3), 110.91, 116.60, 119.34, 121.42, 124.17, 126.82, 128.90, 132.46, 135.24, 137.49, 143.31, 148.06, 154.22 (aromatic carbons), 161.40 (hydrazide C=O), 162.40 (isatin C=O), 167.85 (ester C=O); Anal. Calcd. for $C_{19}H_{18}N_4O_6S$ (430.44): C, 53.02; H, 4.22; N, 13.02; found C, 52.83; H, 4.24; N, 12.95.

5.1.4.16. 4-[2-(1-Benzyl-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl]benzenesulfonamide ***11a****.*

Yellow powder (yield 56%), m.p. 243-245 °C; IR (KBr, ν cm^{-1}): 3955, 3017 (NH, NH2), 1701, 1654 (C=O) and 1385, 1023 (SO_2); ^{1}H NMR (DMSO-d6,400MHz) *δ ppm*: 5.03 (s, 2H, C<u>H</u>$_2$-Ar), 7.07 (d, 1H, Ar-H, *J* = 7.6 Hz), 7.15 (t, 1H, Ar-H, *J* = 7.6 Hz), 7.29 (d, 1H, Ar-H, *J* = 7.2 Hz), 7.34-7.43 (m, 5H, Ar-H), 7.62 (s, 2H, NH_2, D_2O exchangeable), 7.66 (d, 1H, H Ar-H, *J* = 6.4 Hz), 8.05-8.13 (m, 4H, H-2,3,5 and 6 of phenyl ring of benzenesulfonamide), 13.85 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d6,100 MHz) *δ ppm*: 43.2 (CH_2), 111.07, 116.60, 119.68, 121.44, 123.97, 126.87, 127.63, 127.87 (2C), 128.15, 128.84, 129.21(2C), 132.33, 135.32, 136.01, 138.18, 143.32, 148.06 (aromatic carbons), 161.40 (hydrazide C=O), 162.70 (isatin C=O); Anal. Calcd. for $C_{22}H_{18}N_4O_4S$ (434.47): C, 60.82; H, 4.18; N, 12.90; found C, 60.61; H, 4.13; N, 12.96.

5.1.4.17. 4-[2-(1-(4-Fluorobenzyl)-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl]benzenesulfonamide ***11b****.*

Yellow powder (yield 75%), m.p. 242-243 °C; IR (KBr, ν cm^{-1}): 3398, 3017 (NH, NH2), 1690, 1654 (C=O) and 1314, 1023 (SO_2); ^{1}H NMR (DMSO-d6, 400 MHz) *δ ppm*: 5.02 (s, 2H, C<u>H</u>$_2$-Ar), 7.10 (d, 1H, Ar-H, *J* = 7.6 Hz), 7.16-7.21 (m, 3H, Ar-H), 7.41-7.49 (m, 3H, Ar-H), 7.61-7.69 (m, 3H, 1H of Ar-H and 2H, NH_2, D_2O exchangeable), 8.04-8.12

(m, 4H, H-2,3,5 and 6 of phenyl ring of benzenesulfonamide), 13.81 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d6,100 MHz) δ *ppm*: 42.34 (CH_2), 110.88, 111.05, 115.89, 116.10, 119.73, 121.48, 124.01, 126.88, 130.04 (2C), 130.12, 132.28, 133.11, 135.32 (2C),141.78, 143.21, 148.02, 155.49 (aromatic carbons), 161.63 (hydrazide C=O), 162.81 (isatin C=O); HRMS (ESI) for $C_{22}H_{18}O_4N_4FS$, calcd 453.10273, found 453.10302 $[M+H]^+$.

5.1.4.18. 4-[2-(1-(4-Methylbenzyl)-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl]benzenesulfonamide ***11c****.*

Yellow powder (yield 70%), m.p. 260-261 °C; IR (KBr, ν cm^{-1}): 3405, 3017 (NH, NH2), 1693, 1654 (C=O) and 1382, 1047 (SO_2); ^1H NMR (DMSO-d6, 400 MHz) δ *ppm*: 2.51 (s, 3H, Ar-C$\underline{H_3}$), 5.13 (s, 2H, C$\underline{H_2}$-Ar), 7.04 (d, 1H, Ar-H, *J* = 8 Hz), 7.15 (t, 1H, Ar-H, *J* = 7.2 Hz), 7.39 (t, 1H, Ar-H, *J* = 7.6 Hz), 7.60-7.63 (m, 4H, 2H of Ar-H and 2H, NH_2, D_2O exchangeable), 7.66 (d, 1H, H-4 of isatin, *J* = 7.2 Hz), 7.82 (d, 2H, Ar-H, *J* = 8 Hz), 8.05-8.11 (m, 4H, H-2,3,5 and 6 of phenyl ring of benzenesulfonamide), 13.81 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d6,100 MHz) δ *ppm*: 21.30 (CH_3), 42.82 (CH_2), 110.88, 110.99, 119.09, 119.79, 121.51, 124.10, 126.88, 128.71 (2C), 130.12, 132.36, 133.11 (2C), 135.28, 138.14, 141.78, 143.08, 148.06, 155.49 (aromatic carbons), 161.40 (hydrazide C=O), 162.81 (isatin C=O); Anal. Calcd. for $C_{23}H_{20}N_4O_4S$ (448.50): C, 61.60; H, 4.50; N, 12.49; found C, 61.44; H, 4.53; N, 12.56.

4.2. Biological Evaluation

4.2.1. CA inhibitory assay

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalysed CO_2 hydration activity, as reported earlier [19]. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3 and the Cheng-Prusoff equation as reported earlier [28], and represent the mean from at least three different determinations. The four tested CA isofoms were recombinant ones obtained in-house as reported earlier [29].

4.3. Molecular docking simulation

The crystal structure of hCA II (PDB 5LJT), hCA IX (PDB 5FL4) and hCA XII (PDB 1JD0) was downloaded from protein databank and were prepared according to the Protein Preparation wizard in Maestro - Schrödinger suite 2017-1 [30], using the default values in the preprocess. The missing atoms appeared after this process and were completed using Prime. Consequently, optimization of the hydrogen bonds was done followed by refinement process by running a restrained minimization (OPLS3 force field) which is controlled by cutoff RMSD value of heavy atoms reached 0.30 Å. 3D ligand structures were drawn and prepared minimized using Maestro [31] (Schrödinger suite). LigPrep [32] was used to predict ionization states for the ligands using a pH of 7.0 ± 0.5. In docking simulations using Glide, the protein was treated as rigid, compounds were flexibly docked, and scoring was assigned according to the standard precision (SP) mode. Before docking process, the grid box was prepared according to the default size. The constrained were assigned using the metallic bond for Zn metal.

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Highlights

- Three novel series of isatin-based sulfonamides (**5a-h**, **10a-g** and **11a-c**) were synthesized.

- Inhibitory activity of these sulfonamides was evaluated toward hCA I, II, IX and XII isoforms.

- **10b** emerged as a single-digit nanomolar hCA IX and XII inhibitor (8.9 and 9.2 nM).

- Molecular docking studies were carried out for **10b** within the hCA II, IX and XII active sites.