



Research paper

Isatin-pyrazole benzenesulfonamide hybrids potently inhibit tumor-associated carbonic anhydrase isoforms IX and XII



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ABSTRACT

New series of benzenesulfonamide derivatives incorporating pyrazole and isatin moieties were prepared using celecoxib as lead molecule. Biological evaluation of the target compounds was performed against the metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1) and more precisely against the human isoforms hCA I, II (cytosolic), IX and XII (transmembrane, tumor-associated enzymes). Most of the tested compounds efficiently inhibited hCA I, II and IX, with K_{i} s of 2.5–102 nM, being more effective than the reference drug acetazolamide. Compounds **11e**, **11f**, **16e** and **16f** were found to inhibit hCA XII with K_{i} of 3.7, 6.5, 5.4 and 7.2 nM, respectively. Compounds **11e** and **16e**, with 5-NO₂ substitution on the isatin ring, were found to be selective inhibitors of hCA IX and hCA XII. Docking studies revealed that the NO₂ group of both compounds participate in interactions with Asp132 within the hCA IX active site, and with residues Lys67 and Asp130 in hCA XII, respectively.

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1. Introduction

The sulfonamides and their isosters such as the sulfamates and sulfamides, are well known carbonic anhydrase (CA, EC 4.2.1.1) inhibitors (CAIs) and are in clinical use for almost 70 years for the treatment of glaucoma, obesity, epilepsy and as diuretics [1]. The large use of CAIs for pharmaceutical applications relies on the wide distribution of the 15 human (h) CA isoforms within different tissues as well as on their involvement in many physiological/pathological conditions. Antiglaucoma CAI-drugs mainly target CA II, IV and XII; the diuretics CA II, IV, XII and XIV; the antiepileptics CA VII and XIV [2–5]. The selective inhibition of CA IX and XII produces significant antitumor and

antimetastatic effects. However the main drawback associated to the use of sulfonamide CAIs is represented by the lack of selectivity in inhibiting the various isoforms, thus leading to a plethora of side effects [6,7]. In this contest many efforts have been made for the development of isoform-selective CAIs, and some remarkable results have been achieved in the last 15 years since the introduction of the tail approach [6–8]. Currently a sulfonamide CA IX inhibitor (SLC-0111) entered in Phase I clinical studies for the treatment of hypoxic, advanced stage solid tumors [8–10]. Furthermore, novel CAI classes such as the polyamines, [11] phenols, [12] dithiocarbamates, [13] xanthates [14] coumarins, thiocoumarins, 2-thioxo-coumarins and coumarinoximes [1,4,15–17] were discovered and the inhibition mechanisms of these compounds were explained by using kinetic, spectroscopic and X-ray crystallographic techniques [2,6,12,13].

Recently, benzyl aniline sulfonamides such as **I** were reported as hCA IX inhibitors (K_{i} = 1.8–27 nM) [18]. The same group developed similar compounds such as the cyclic form **II**, which selectively inhibited hCA IX (with K_{i} of 13–27 nM) versus hCA I/II [19]. The lead molecule of these derivatives was celecoxib **III** which was

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demonstrated to be a strong hCA IX ($K_i = 16$ nM) and hCA II ($K_i = 21$ nM) inhibitor by one of our groups [20]. In addition, other studies were reported on five membered heterocyclic *N*-benzene sulfonamide **IV** possessing an amino group instead of the aryl one found in compounds **II** and **III**. Compound **IV** showed excellent inhibitory action against hCA IX ($K_i = 6.3$ nM) and hCA XII ($K_i = 0.74$ nM) [21] (Fig. 1).

A series of 4-(4,5-dihydro-5-thioxo-1,3,4-thiadiazol-2-yl)-1-(5-substituted-oxoindolin-3-ylidene)semicarbazides **V** was recently evaluated as carbonic anhydrase inhibitors and displayed interesting activity against hCA I and IX ($X = \text{NO}_2$, $K_i = 5.95$ and 1.25 μM , respectively) [22]. The Schiff base **VI** showed a potent inhibitory activity against hCA IX ($K_i = 1.1$ nM) and had a high selectivity for isoform hCA IX compared to the cytosolic isozymes hCA I and hCA II [23] (Fig. 1).

Based on these literature data, we report here the synthesis of three novel series of isatin-pyrazole-benzenesulfonamide hybrids **5a–d**, **11a–f** and **16a–f** as CAIs. The design of the new hybrids relies on grafting the oxindole hydrazine carbonyl moiety from the isatin semicarbazide derivatives **V** to the 4-(5-phenyl-1*H*-pyrazol-1-yl) benzenesulfonamide scaffold found compounds **II** and **III**, at either position 3 (**5a–d**) or 4 (**11a–f**) of the pyrazole ring. The additive effect of combining these pharmacophore moieties might produce compounds with high CA inhibitory activity. Furthermore, the structure modification in the third series **16a–f** involved replacement of the 5-phenyl ring in **11a–f** by an amino group in analogy to the potent CAI **IV** mentioned above (Fig. 1).

2. Results and discussion

2.1. Chemistry

The synthesis of the first series of sulfonamides, **5a–d**, is presented in Scheme 1. The classical Claisen condensation of acetophenone (**1**) with diethyl oxalate in the presence of sodium ethoxide gave ethyl 2,4-dioxo-4-phenylbutanoate (**2**). The regioselective cyclization of butanoate **2** with 4-aminosulfonylphenylhydrazine [24] was achieved in acetic acid, yielding ethyl 5-phenyl-1-(4-sulfamoylphenyl)-1*H*-pyrazole-3-carboxylate (**3**) which was then reacted with hydrazine hydrate to afford hydrazide **4**. Hydrazones **5a–d** were obtained by refluxing hydrazide **4** with the appropriate isatin derivative in ethanol, in the presence of catalytic amounts of acetic acid.

The IR spectrum of the unreported *hitherto* 4-(3-(hydrazine-carbonyl)-5-phenyl-1*H*-pyrazol-1-yl)benzenesulfonamide (**4**) showed absorption bands due to NH_2 and NH groups at 3334 – 3196 cm^{-1} , beside the absorption peak of $\text{C}=\text{O}$ group at 1674 cm^{-1} and two absorption bands of the SO_2 group at 1319 and 1153 cm^{-1} . Its $^1\text{H-NMR}$ spectrum revealed three D_2O exchangeable singlet signals corresponding to SO_2NH_2 , NH-NH_2 and NH-NH_2 at δ 7.51, 9.69 and 4.61 ppm, respectively. The characteristic singlet signal of H-4 of pyrazole appeared at δ 7.06 ppm.

The structure hydrazones **5a–d** was confirmed by their $^1\text{H-NMR}$ spectra which revealed the disappearance of the hydrazide NH_2 signal in compound **4** in addition to the appearance of the signal of

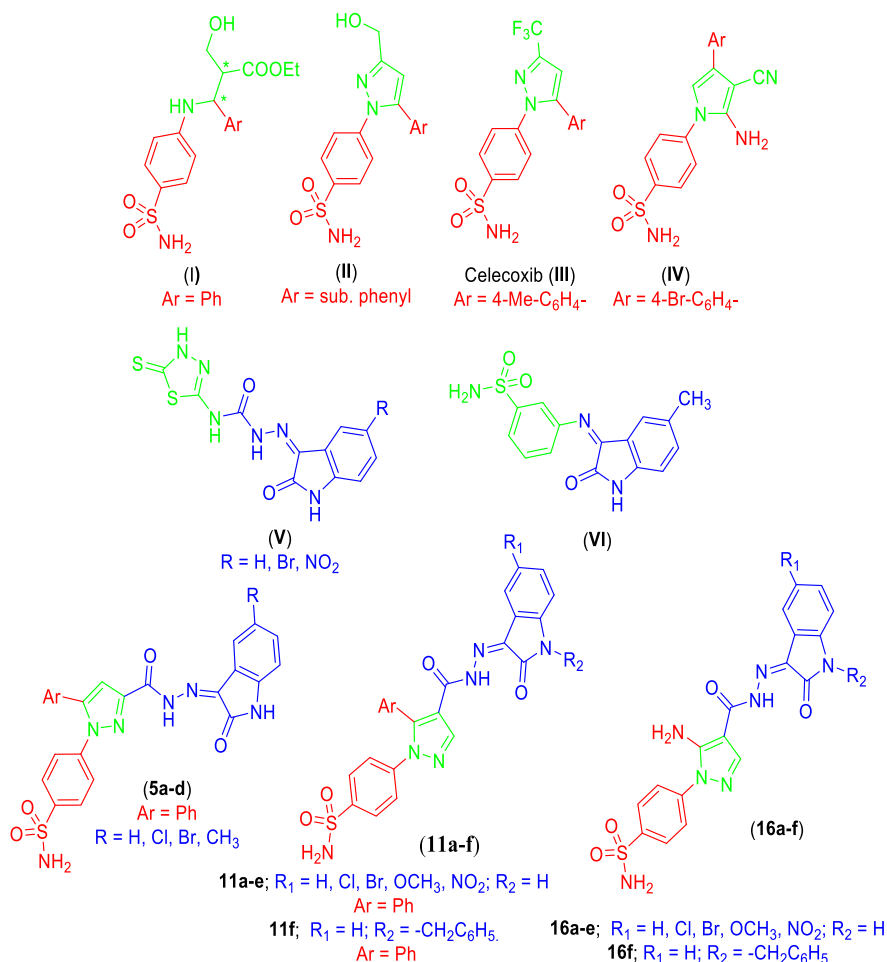
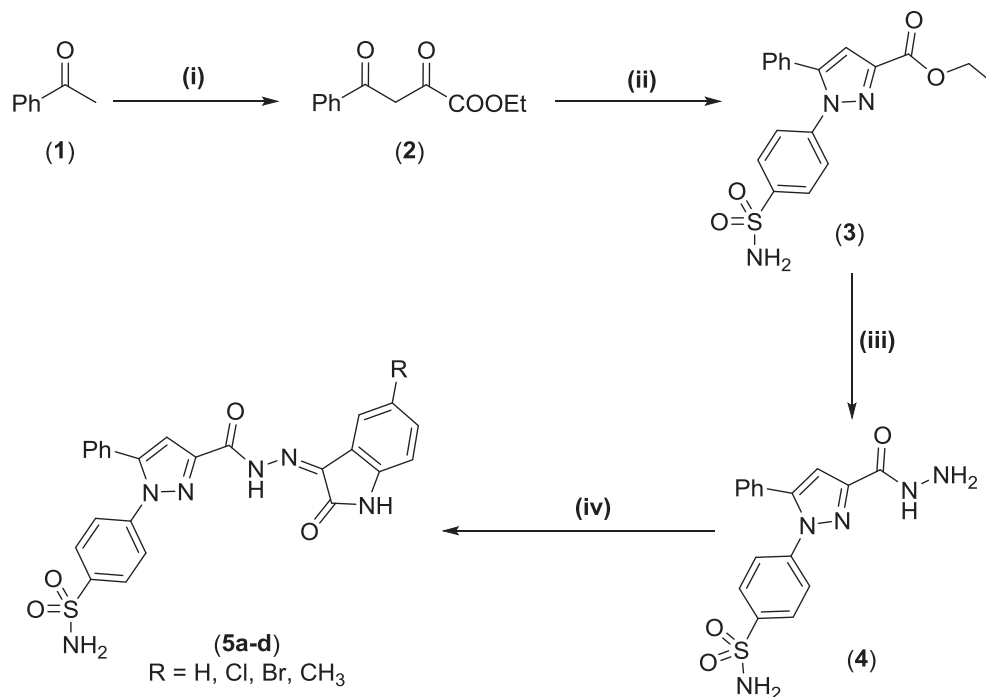


Fig. 1. Chemical structure of compounds I–VI and the targeted hybrids **5a–d**, **11a–f** and **16a–f**.



Scheme 1. Synthesis of compounds **5a–d**. Reagents and conditions: (i) $(\text{COOEt})_2/\text{NaOC}_2\text{H}_5/\text{EtOH}/0^\circ\text{C}$; (ii) $p\text{-SO}_2\text{NH}_2\text{C}_6\text{H}_4\text{NHNH}_2/\text{AcOH}/\text{reflux } 1\text{ h}$; (iii) $\text{N}_2\text{H}_4\cdot\text{H}_2\text{O}/\text{reflux } 3\text{ h}$; (iv) 5-(Un)substituted isatin/ $\text{EtOH}/\text{AcOH}/\text{reflux } 1\text{ h}$.

NH group of isatin moiety in the range δ 10.74–11.33. The $^1\text{H-NMR}$ spectra of **5a–d** showed downfield shifts of the hydrazide NH signal, which appeared in the range δ 11.59–14.16 ppm. Moreover, the $^1\text{H-NMR}$ spectrum of **5d** showed a signal of aliphatic protons (CH_3 group) at δ 2.30 ppm. The $^{13}\text{C-NMR}$ spectrum of **5d** showed the signals of $\text{C}=\text{O}$ groups at δ 157.50 and 162.61 ppm, in addition to the signal of the aliphatic carbon (CH_3 group) at δ 20.48 ppm.

Preparation of the second series of sulfonamides (**11a–f**) was achieved as illustrated in Scheme 2. Ethyl benzoyl acetate (**7**) was synthesized by condensation of ethyl acetoacetate (**6**) with benzoyl chloride in sodium ethoxide followed by hydrolysis in the presence of aqueous NH_3 and NH_4Cl . Refluxing **7** with DMF-DMA afforded ethyl 2-benzoyl-3-(dimethylamino)acrylate (**8**) which was employed in the next step without further purification. The reaction of the latter enaminone with 4-aminosulfonylphenylhydrazine hydrochloride in refluxing ethanol produced the pyrazole ester **9**. The ester **9** was subjected to hydrazinolysis by fusion with hydrazine hydrate to give the corresponding hydrazide **10**. Hydrazones **11a–f** were synthesized by the reaction of hydrazide **10** with the appropriate isatin derivative in ethanol and in the presence of catalytic amount of acetic acid.

The $^1\text{H NMR}$ spectra of ester **9**, hydrazide **10** and hydrazones **11a–f** showed the characteristic signal of H-3 of the pyrazole ring in the region δ = 8.19–8.83 ppm. However, single crystal X-ray analysis of hydrazide **10** gave an absolute confirmation for the structure of the latter compound and excluded the other possible positional isomer (Fig. 2) and (see also Supplementary Figure 1).

The $^1\text{H-NMR}$ spectrum of **11d** revealed the appearance of a signal due to the aliphatic proton of the OCH_3 group at δ 3.77 ppm, whereas the carbon of the same group appeared at δ 55.58 ppm in the $^{13}\text{C-NMR}$ spectrum. The $^1\text{H-NMR}$ spectrum of **11f** presented one singlet signal characteristic of benzylic protons (at δ 4.93 ppm), whilst the carbon of the same group appeared at δ 42.96 ppm in the $^{13}\text{C NMR}$ spectrum.

The synthetic pathway of the third series of sulfonamides,

16a–f, is depicted in Scheme 3. The reaction of ethyl cyanoacetate (**12**) with triethyl orthoformate in the presence of acetic anhydride generated ethyl (ethoxyethylene)cyanoacetate (**13**) which was then converted to pyrazole **14** by treatment with 4-aminosulfonylphenylhydrazine in a mixture of acetic acid and water (5:1). The structure of compound **14** was confirmed by X-ray crystallography (Fig. 3) and (Supplementary Figure 2). Consequently, hydrazinolysis of the latter ester led to the formation of hydrazide **15** which reacted with different isatins in refluxing ethanol to yield hydrazones **16a–f**.

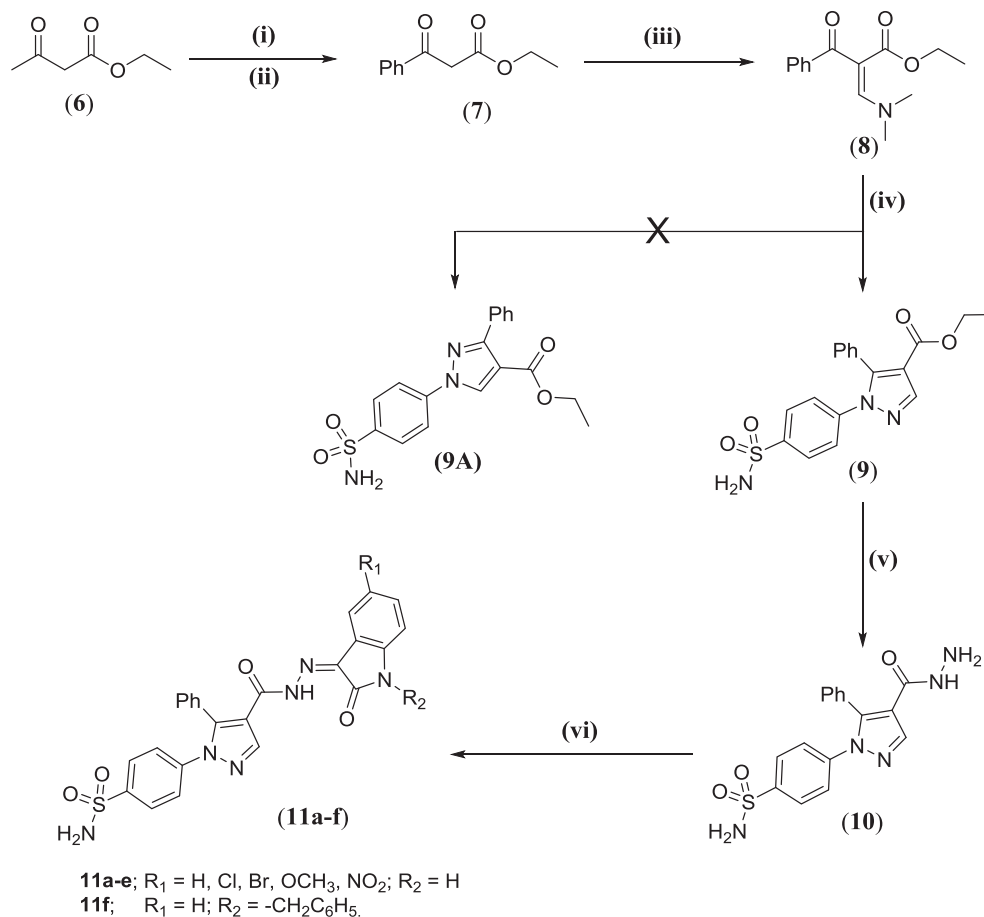
The IR spectra of compounds **16a–f** contained bands of the NH_2 and NH groups in the range of $3120\text{--}3421\text{ cm}^{-1}$. Their $^1\text{H-NMR}$ spectra had D_2O exchangeable signals related to 5-amino protons at δ 6.52–7.07 ppm, in addition to D_2O exchangeable singlet signals attributed to protons of the sulfonamide group at around δ 7.49 ppm, isatin NH proton (in **16a–e**) in the range δ 10.55–11.50 ppm and the hydrazide NH proton in the range δ 11.24–13.10 ppm. In this series, $^1\text{H-NMR}$ spectroscopy revealed the characteristic signal of H-3 of the pyrazole in the range δ 8.20–9.18 ppm, while the $^{13}\text{C-NMR}$ spectra showed a characteristic signal due to the C-5 of pyrazole ring between δ 94.24–95.72 ppm.

Compound **16d** had a signal of aliphatic protons (OCH_3 group) at δ 3.79 ppm in its $^1\text{H-NMR}$ spectrum and at δ 56.09 ppm in its $^{13}\text{C-NMR}$ spectrum. The benzylic protons of **16f** appeared at δ 5.02 ppm in $^1\text{H NMR}$ and the benzylic carbon was detected at δ 43.00 ppm in the $^{13}\text{C-NMR}$ spectrum.

2.2. Carbonic anhydrase inhibition

Inhibition data against four physiologically relevant hCA isoforms, hCA I, II (cytosolic) as well as hCA IX and XII (transmembrane, tumor-associated isoforms), are shown in Table 1 and were determined by a stopped-flow CO_2 hydrase assays. [25].

The following SAR is evident from the data of Table 1:



Scheme 2. Synthetic pathway of compounds **11a–f**. Reagents and conditions: (i) PhCOCl , NaOC_2H_5 ; (ii) NH_3 , NH_4Cl , H_2O ; (iii) DMF-DMA / Reflux / 2h; (iv) $p\text{-SO}_2\text{NH}_2\text{C}_6\text{H}_4\text{NHNH}_2\text{HCl}$ / EtOH / Reflux 4 h; (v) $\text{N}_2\text{H}_4\cdot\text{H}_2\text{O}$ /reflux 3 h; (vi) 5-(Un)substituted isatin or *N*-benzyl isatin / EtOH / AcOH /reflux 1 h.

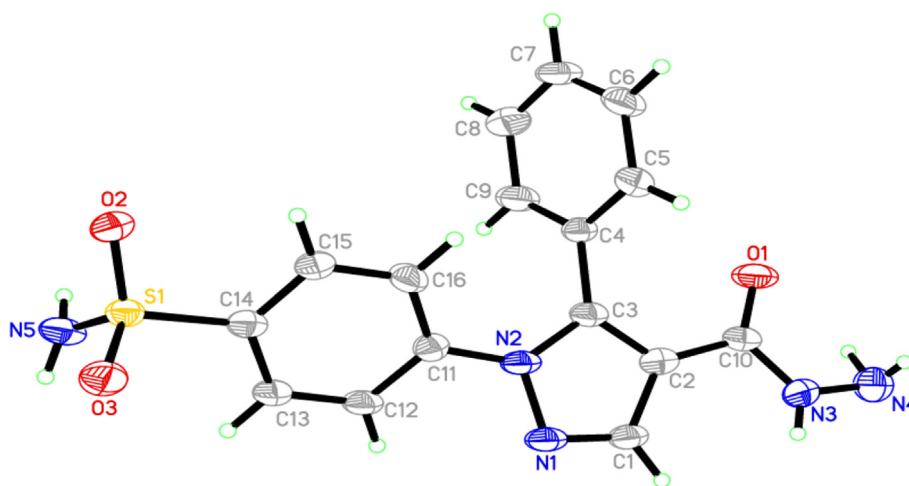
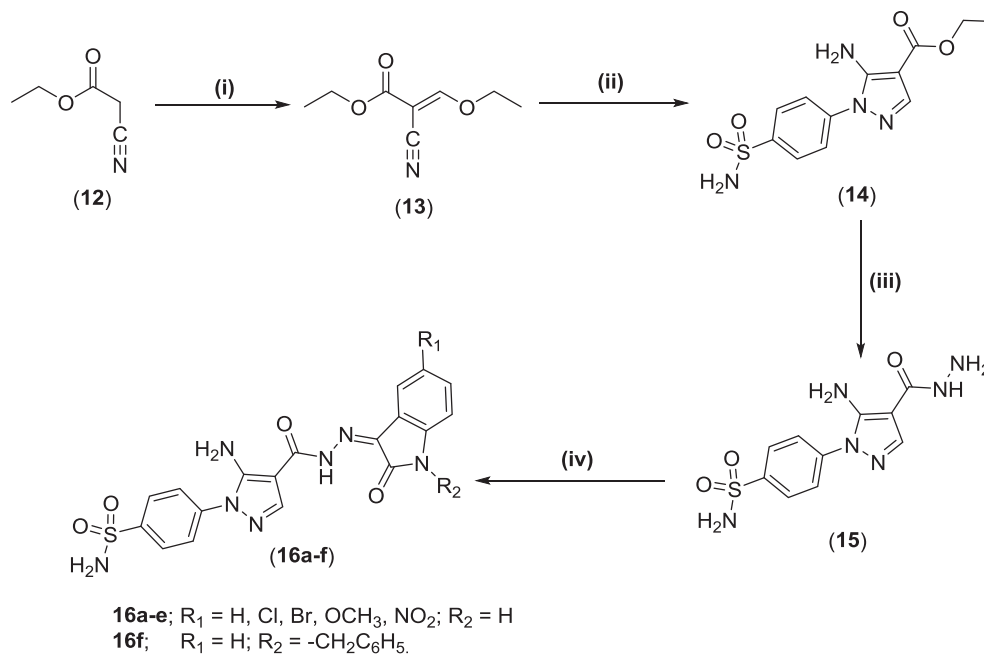


Fig. 2. ORTEP diagram of compound **10**.

(i) The slow cytosolic isoform hCA I was effectively inhibited by sulfonamides **5**, **11** and **16** reported here, with K_i s ranging between 5.2 and 102 nM. Otherwise, **5b** which was slightly less effective (K_i of 102 nM) all the other compounds were low nanomolar inhibitors of this isoform whose physiologic function is still not well understood. Acetazolamide, a

clinically used sulfonamide, was a much weaker CAI compared to the new compounds reported here (K_i of 250 nM).

(ii) hCA II, the physiologically dominant isoform was highly inhibited by all the compounds reported here, with low nanomolar efficacy (K_i s ranging between 2.9 and 31.3 nM),



Scheme 3. Synthetic pathway of compounds **16a–f**. Reagents and conditions: (i) Triethylorthoformate/acetic anhydride/reflux 10 h; (ii) *p*-SO₂NH₂C₆H₄NHNH₂.HCl/Acetic acid/H₂O/Reflux 4 h; (iii) N₂H₄.H₂O/reflux 4 h; (iii) 5-(Un)substituted isatin or *N*-benzyl isatin/EtOH/AcOH/reflux 1 h.

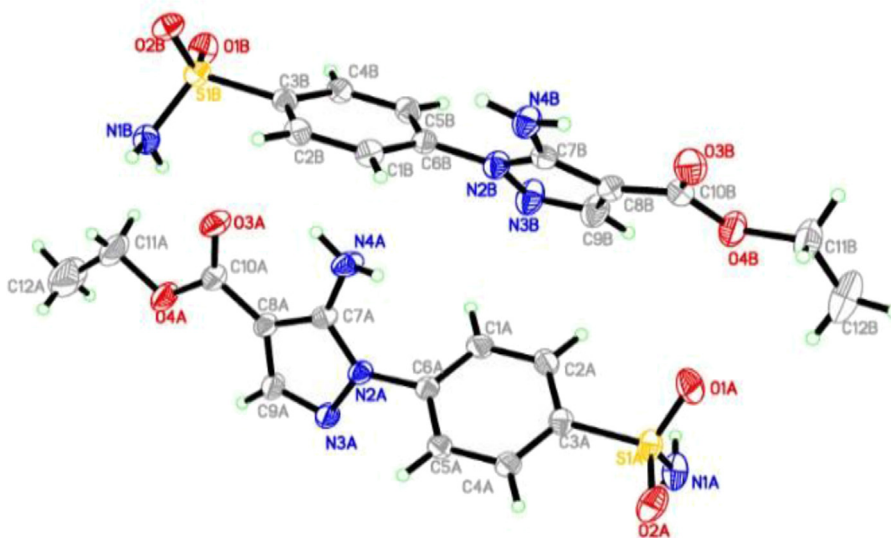


Fig. 3. ORTEP diagram of compound **14**.

making the SAR discussion almost impossible since all scaffolds led to extremely effective hCA II inhibitors (Except for compounds **11e**, **11f**, **16e** and **16f** which were fairly less active than **AAZ** and **5a** that had the same efficacy as **AAZ**, the other compounds were much better hCA II inhibitors compared to the standard drug, **Table 1**).

(iii) The tumor-associated hCA IX was also a highly inhibited by sulfonamides reported here, with K_i s ranging between 2.5 and 52.9 nM. A part for **11a** which was slightly less effective as hCA IX inhibitors, all other synthesized derivatives showed inhibition constants ≤ 20 nM, being thus highly effective for inhibiting this tumor-associated enzyme, a validated antitumor target.

(iv) hCA XII was also inhibited by sulfonamides reported here with K_i s ranging between 3.7 and 244 nM (**Table 1**). Except for compounds **11e**, **11f**, **16e** and **16f**, hCA XII was less efficiently inhibited by the new derivatives than the other three isoforms. In fact compound **11e** showed higher activity than **AAZ** ($K_i = 3.7$ and 5.7 nM, respectively), whereas **11f**, **16e** and **16f** had comparable potency to the reference drug ($K_i = 6.5$, 5.4 and 7.2 nM, respectively).

It can be observed that moving the oxindole hydrazine carbonyl moiety on the pyrazole ring from position 3 in **5a–d** to position 4 in **11a–d** and **16a–d** enhanced the inhibitory activity against hCA I, II and XII isoforms. On the other hand, no clear relationship was

Table 1
Inhibition data of human carbonic anhydrase isoforms hCA I, II, IX and XII with the sulfonamide derivatives **5**, **11** and **16** determined by stopped-flow CO₂ hydrase assay [33], using acetazolamide (AAZ) as standard drug.

Compound	<i>K_i</i> (nM) ^a			
	hCA I	hCA II	hCA IX	hCA XII
5a	53.7	11.8	8.8	91.5
5b	102	9.9	7.4	65.9
5c	9.0	6.4	20.0	83.6
5d	52.4	5.9	4.7	244
11a	9.7	4.3	52.9	73.5
11b	38.3	4.6	9.7	44.5
11c	6.7	5.5	7.8	91.4
11d	7.6	3.5	3.3	74.6
11e	49.5	31.3	15.7	3.7
11f	61.9	17.9	13.6	6.5
16a	5.7	2.9	2.8	37.7
16b	7.1	3.8	2.5	22.8
16c	5.2	3.2	9.4	56.8
16d	7.1	4.5	3.5	82.8
16e	70.4	23.1	7.4	5.4
16f	14.9	19.5	20.0	7.2
AAZ^b	250	12	25	5.7

^a *K_i* presented is the mean from 3 different assays; errors are in the range of ± 5–10% of the reported values (data not shown).

^b Acetazolamide (AAZ) was used as a standard inhibitor for all CAs investigated here.

observed between the enzyme inhibitory activity of different isoforms and replacement of the phenyl ring at position 5 of the pyrazole moiety in **11a–f** with an amino group in **16a–f**.

Regarding the effect of the substitution pattern on the isatin moiety, it was observed that the introduction of a NO₂ group to position 5 of the isatin led to compounds which preferentially inhibited hCA IX and hCA XII over hCA I and hCA II, as evident for derivatives **11e** and **16e**. Meanwhile, *N*-benzyl substitution on the isatin moiety, as in **11f** and **16f**, led to an increased affinity of these derivatives for hCA XII.

2.3. Molecular docking studies

Docking studies were employed to analyze the binding pattern of compounds **11e** and **16e** to the tumor associated hCA IX and hCA

XII isoforms. These studies revealed significant information about the binding mode of these compounds, and showed the crucial role of the sulfonamide as a zinc binding group [19]. Docking of compound **11e** within the active site of hCA IX revealed the same important role of the deprotonated sulfonamide moiety, which interacts with zinc ion and the neighbor residues Leu198 and Thr199 (Fig. 4A), whereas for **16e**, the sulfonamide group forms H-bond with Thr199 beside the coordination bond to the Zn(II) ion (Fig. 4B). Moreover, the 5-NO₂ substituent of isatin moiety presented an important role in the interaction of both compounds with hCA IX by forming an electrostatic bond with Asp132 (Fig. 4A and B).

As for hCA IX; the binding of **11e** and **16e** to hCA XII is mainly influenced by the deprotonated sulfonamide group acting as zinc binding moiety and H-bonds with the conserved residue in all α -CAs, Thr199. The NO₂ group showed its significant role by participating in electrostatic interactions with Lys67 (compound **11e**) or Asp130 (compound **16e**) (Fig. 5A and B). In contrast to the binding to hCA IX, the isatin moiety of these compounds displayed extra H-bond interactions with the hCA XII active site. For example, the isatin moiety in **11e** was able to H-bond with Ser132 (Fig. 5A). In compound **16e**, the isatin moiety formed two hydrogen bonds with Asn62 and Gln92 (Fig. 5B). The additional interaction of the isatin moiety with hCA XII active site may explain the higher inhibition of these compounds against hCA XII compared to hCA XI.

Both compounds displayed CDOCKER interaction energy (**11e**; –50.23 Kcal/mol and –44.67 Kcal/mol) (**16e**; –49.78 Kcal/mol and –45.64 Kcal/mol) higher than **AAZ** (–24.63 Kcal/mol and –24.67 Kcal/mol) upon interaction with hCA IX and hCA XII, respectively.

3. Conclusion

Stimulated by the reported activity of several *N*-benzene sulfonamide pyrazoles and isatins as CAIs, new series of isatin-pyrazole-benzenesulfonamide hybrids **5a–d**, **11a–f** and **16a–f** were designed and synthesized as inhibitors of several CA isoforms. The structure of the new compounds was confirmed by spectral methods and intermediates **10** and **14** were confirmed by using X-ray crystallography. Biological evaluation of the new compounds was performed against hCA I, II, IX and XII. Most of the tested

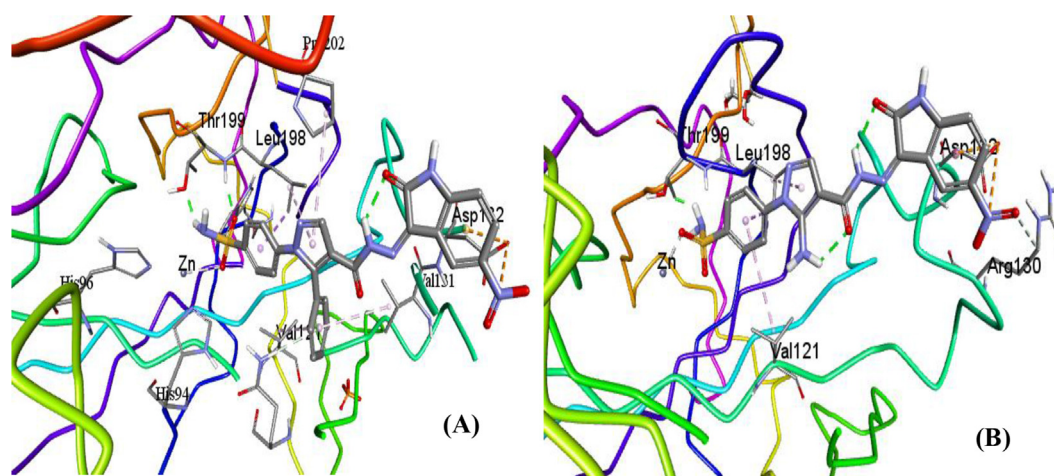


Fig. 4. (A) 3D diagram for interaction of compound **11e** with hCA IX (PDB id: 3IAI) showing sulfonamide as zinc binding group and nitro group interacting with Asp132 with electrostatic bond. (B) 3D diagram for interaction of compound **16e** with hCA IX (PDB id: 3IAI) showing sulfonamide as zinc binding group and nitro group interacting with Asp132 with electrostatic bond. In these diagrams, the whole protein was displayed as a tube except the interacting amino acids were displayed as stick. Hydrogen bond was represented by green dots, electrostatic bond was represented by orange dots, Pi-hydrophobic interaction was represented by pink dots and metallic bond was represented by gray dots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

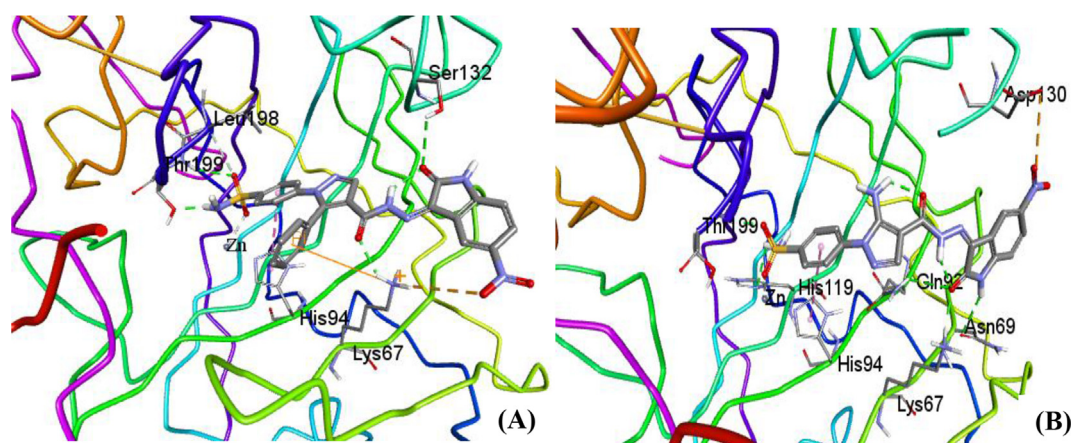


Fig. 5. (A) 3D diagram for interaction of compound **11e** with hCA XII (PDB id: 1JD0) showing sulfonamide as zinc binding group and nitro group interacting with Lys67 with electrostatic bond. (B) 3D diagram for interaction of compound **16e** with hCA XII (PDB id: 1JD0) showing sulfonamide as zinc binding group and nitro group interacting with Asp130 with electrostatic bond. In these diagrams, the whole protein was displayed as a tube except the interacting amino acids were displayed as stick. Hydrogen bond was represented by green dots, electrostatic bond was represented by orange dots, Pi-hydrophobic interaction was represented by pink dots and metallic bond was represented by gray dots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

compounds efficiently inhibited hCA I, II and IX ($K_i = 2.5\text{--}102$ nM) being more effective than the reference drug acetazolamide (AAZ). On the other hand, they inhibited hCA XII to a lesser extent except for compounds **11e**, **11f**, **16e** and **16f** ($K_i = 3.7, 6.5, 5.4$ and 7.2 nM, respectively). Sulfonamides **11e** and **16e** with a 5- NO_2 substituent on the isatin ring were found to preferentially inhibit hCA IX and hCA XII. Docking studies were performed to investigate the role of the NO_2 group and revealed that it can form electrostatic interaction with Asp132 in hCA IX, and with Lys67/Asp130 during inhibition of hCA XII. These results indicate that, the new hybrids provide an efficient pharmacophore to design CAIs, yet further investigations are required to improve their selectivity toward the tumor-associated isoforms hCA IX and XII.

4. Experimental

4.1. Chemistry

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Melting points were determined on Stuart SMP3 version 5 digital melting point apparatus and were uncorrected. Elemental microanalyses were performed at the Regional Center for Mycology and Biotechnology, Al-Azhar University. The NMR spectra were recorded for some compounds on a Varian Mercury VX-300 NMR spectrometer. ^1H spectra were run at 300 MHz and ^{13}C spectra were run at 75 MHz. For other compounds, the NMR spectra were recorded on Bruker Avance III 400 MHz high performance digital FT-NMR spectrophotometer (^1H : 400, ^{13}C : 100 MHz). Chemical shifts are quoted in δ and were related to that of the solvents. Mass spectra were recorded using Hewlett Packard Varian (Varian, Palo, USA), Shimadzu Gas Chromatograph Mass spectrometer-QP 1000 EX (Shimadzu, Kyoto, Japan) and Finnegan MAT, SSQ 7000 mass spectrophotometer at 70 eV. IR spectra were recorded on Bruker FT-IR spectrophotometer as potassium bromide discs. Compounds **2** [26], **7** [27], **8** [28], **9** [19], **13** [29], **15** [30] were prepared and confirmed as reported.

4.1.1. Synthesis of ethyl 5-phenyl-1-(4-sulfamoylphenyl)-1H-pyrazole-3-carboxylate (**3**)

The diketoester, ethyl 2,4-dioxo-4-phenylbutanoate (**2**) (4 mmol, 0.88 g) was dissolved in acetic acid (15 mL) and then a

solution of 4-aminosulfonylphenylhydrazine (4 mmol, 0.75 g) in ethanol (20 mL) was added. The reaction mixture was refluxed for 1 h. The formed precipitate was filtered, dried and recrystallized from ethanol to yield compound **3**. All spectral data coincide with those reported [19].

4.1.2. Synthesis of 4-(3-(hydrazinecarbonyl)-5-phenyl-1H-pyrazol-1-yl)benzenesulfonamide (**4**)

The ester **3** (10 mmol, 3.71 g) was refluxed in hydrazine hydrate (10 mL) and the reaction was followed by TLC. After complete reaction (3 h), the mixture was poured onto ice and stirred for 1 h with addition of few drops of acetic acid. The formed precipitate was filtered off, washed with diethyl ether, dried and recrystallized from ethanol. Beige crystals, 61% yield; mp 265 °C. IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$ 3334–3196 (NH_2 , NH), 1674 ($\text{C}=\text{O}$), 1593 ($\text{C}=\text{N}$), 1319, 1153 (SO_2). ^1H NMR (DMSO- d_6 , 400 MHz) δ 4.61 (s, 2H, NH_2 hydrazide, D_2O exchangeable), 7.06 (s, 1H, H-4 of pyrazole), 7.29–7.31 (m, 2H, Ar-H), 7.38–7.46 (m, 3H, Ar-H), 7.51 (s, 2H, SO_2NH_2 , D_2O exchangeable), 7.53 (d, 2H, $J = 8.7$ Hz, Ar-H), 7.88 (d, 2H, $J = 8.7$ Hz, Ar-H), 9.69 (s, 1H, NH hydrazide, D_2O exchangeable). ^{13}C NMR (DMSO- d_6 , 100 MHz) δ 108.69, 125.99, 127.16, 129.18, 129.32, 129.49, 129.56, 142.07, 143.81, 144.64, 147.32, 161.07. MS m/z [%] 357 [M^+ , 9.84], 326 [100]. Anal. Calcd for $\text{C}_{16}\text{H}_{15}\text{N}_5\text{O}_3\text{S}$ (357.39): C, 53.77; H, 4.23; N, 19.60; S, 8.97. Found: C, 53.93; H, 4.29; N, 19.78; S, 9.04.

4.1.3. General procedure for synthesis of compounds (**5a–d**)

To a solution of hydrazide **4** (10 mmol, 0.36 g) in ethanol (20 mL), the appropriate isatin (10 mmol) was added followed by a catalytic amount of acetic acid (0.5 mL) then the mixture was refluxed for 1 h. The formed precipitate was filtered off, washed with hot ethanol and recrystallized from DMF/ EtOH to give the targeted compounds **5a–d**.

4.1.3.1. 4-(3-(2-(2-Oxoindolin-3-ylidene)hydrazine-1-carbonyl)-5-phenyl-1H-pyrazol-1-yl)benzenesulfonamide (**5a**). Yellow powder, 89% yield; mp > 300 °C. IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$ 3361–3184 (NH_2 , NH), 1732, 1701 ($\text{C}=\text{O}$), 1516 ($\text{C}=\text{N}$), 1325, 1155 (SO_2). ^1H NMR (DMSO- d_6 , 400 MHz) δ 6.95 (d, 1H, $J = 7.8$ Hz, H-7 isatin), 7.10 (t, 1H, $J = 7.8$ Hz, H-5 isatin), 7.28 (s, 1H, H-4 of pyrazole), 7.34–7.38 (m, 2H, Ar-H), 7.40–7.45 (m, 4H, Ar-H), 7.53 (s, 2H, SO_2NH_2 , D_2O exchangeable), 7.57 (d, 2H, $J = 8.7$ Hz, Ar-H), 7.65 (d, 1H, $J = 7.8$ Hz, H-4 isatin), 7.93 (d, 2H, $J = 8.7$ Hz, Ar-H), 10.88, 11.22 (2s, 1H, NH

isatin, D₂O exchangeable), 11.50, 14.16 (2s, 1H, NH hydrazone, D₂O exchangeable). ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 109.61, 111.54, 116.10, 120.37, 121.44, 122.52, 123.10, 126.09, 126.44, 127.35, 129.03, 129.32, 129.72, 132.22, 133.42, 138.45, 141.84, 143.05, 144.40, 145.95, 146.09, 158.02, 163.05. MS *m/z* [%] 486 [M⁺, 8.62], 326 [100]. Anal. Calcd for C₂₄H₁₈N₆O₄S (486.51): C, 59.25; H, 3.73; N, 17.27; S, 6.59. Found: C, 59.37; H, 3.76; N, 17.41; S, 6.67.

4.1.3.2. 4-(3-(2-(5-Chloro-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl)-5-phenyl-1H-pyrazol-1-yl)benzenesulfonamide (**5b**)

Yellow powder, 56% yield; mp > 300 °C. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3396–3221 (NH₂, NH), 1720, 1697 (C=O), 1517 (C=N), 1340, 1157 (SO₂). ¹H NMR (DMSO-*d*₆, 400 MHz) δ 6.97 (dd, 1H, *J* = 8.3, 3.6 Hz, H-7 of isatin), 7.31 (s, 1H, H-4 of pyrazole), 7.34–7.49 (m, 6H, Ar–H), 7.54 (s, 2H, SO₂NH₂, D₂O exchangeable), 7.58 (d, 1H, *J* = 8.9 Hz, H-4 isatin), 7.64 (d, 1H, *J* = 8.6 Hz, Ar–H), 7.90 (d, 2H, *J* = 8.6 Hz, Ar–H), 11.00, 11.34 (2s, 1H, NH isatin, D₂O exchangeable), 11.69, 14.13 (2s, 1H, NH hydrazone, D₂O exchangeable). ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 109.98, 112.84, 117.26, 120.97, 122.12, 125.82, 126.16, 126.31, 126.46, 127.20, 127.35, 129.14, 129.34, 129.42, 129.48, 131.26, 132.72, 137.61, 141.72, 143.12, 144.25, 145.63, 162.89, 164.77. MS *m/z* [%] 522 [M⁺+2, 6.34], 520 [M⁺, 18.60], 222 [100]. Anal. Calcd for C₂₄H₁₇ClN₆O₄S (520.95): C, 55.33; H, 3.29; N, 16.13; S, 6.15. Found: C, 55.51; H, 3.27; N, 16.29; S, 6.22.

4.1.3.3. 4-(3-(2-(5-Bromo-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl)-5-phenyl-1H-pyrazol-1-yl)benzenesulfonamide (**5c**)

Yellow powder, 87% yield; mp > 300 °C. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3367–3219 (NH₂, NH), 1725, 1699 (C=O), 1516 (C=N), 1325, 1157 (SO₂). ¹H NMR (DMSO-*d*₆, 300 MHz) δ 6.94 (dd, 1H, *J* = 8.4, 4.4 Hz, H-7 of isatin), 7.29 (s, 1H, H-4 of pyrazole), 7.35–7.49 (m, 6H, Ar–H), 7.48 (s, 2H, SO₂NH₂, D₂O exchangeable), 7.54–7.89 (m, 5H, Ar–H), 10.94, 11.30 (2s, 1H, NH isatin, D₂O exchangeable), 11.61, 14.08 (2s, 1H, NH hydrazone, D₂O exchangeable). MS *m/z* [%] 566 [M⁺+2, 5.41], 564 [M⁺, 6.16], 326 [100]. Anal. Calcd for C₂₄H₁₇BrN₆O₄S (565.40): C, 50.98; H, 3.03; N, 14.86; S, 5.67. Found: C, 51.16; H, 3.04; N, 14.95; S, 5.72.

4.1.3.4. 4-(3-(2-(5-Methyl-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl)-5-phenyl-1H-pyrazol-1-yl)benzenesulfonamide (**5d**)

Yellow powder, 77% yield; mp > 300 °C. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3344–3215 (NH₂, NH), 1714, 1685 (C=O), 1517 (C=N), 1340, 1166 (SO₂). ¹H NMR (DMSO-*d*₆, 300 MHz) δ 2.30 (s, 3H, CH₃), 6.83 (d, 1H, *J* = 7.9 Hz, H-7 of isatin), 7.17 (d, 1H, *J* = 7.9 Hz, H-6 of isatin), 7.26 (s, 1H, H-4 of pyrazole), 7.27–7.35 (m, 5H, Ar–H), 7.49 (s, 2H, SO₂NH₂, D₂O exchangeable), 7.57 (d, 2H, *J* = 8.1 Hz, Ar–H), 7.65 (d, 1H, *J* = 7.8 Hz, H-4 isatin), 7.91 (d, 2H, *J* = 8.7 Hz, Ar–H), 10.81, 11.22 (2s, 1H, NH isatin, D₂O exchangeable), 11.54, 14.13 (2s, 1H, NH hydrazone, D₂O exchangeable). ¹³C NMR (DMSO-*d*₆, 75 MHz) δ 20.48 (CH₃), 109.07, 110.87, 119.88, 121.29, 125.37, 125.90, 126.69, 128.54, 129.21, 131.68, 132.13, 138.08, 140.28, 141.34, 143.91, 145.43, 157.50, 162.61. MS *m/z* [%] 500 [M⁺, 5.60], 222 [100]. Anal. Calcd for C₂₅H₂₀N₆O₄S (500.53): C, 59.99; H, 4.03; N, 16.79; S, 6.41. Found: C, 60.23; H, 4.11; N, 16.93; S, 6.46.

4.1.4. Synthesis of 4-(4-(hydrazinecarbonyl)-5-phenyl-1H-pyrazol-1-yl)benzenesulfonamide (**10**)

Ethyl 5-phenyl-1-(4-sulfamoylphenyl)-1H-pyrazole-4-carboxylate (**9**) (10 mmol, 3.71 g) was refluxed with 15 mL of hydrazine hydrate for 3 h. After checking the end of the reaction using TLC, the mixture was poured on ice, stirred for 1 h with addition of few drops of acetic acid. The formed precipitate was filtered off, washed with diethyl ether, dried and recrystallized from ethanol. Violet crystals, 77% yield; mp 263–265 °C. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3398–3219 (2NH₂, NH), 1647 (C=O), 1560 (C=N), 1328, 1157 (SO₂). ¹H NMR

(DMSO-*d*₆, 400 MHz) δ 4.42 (s, 2H, NH₂ hydrazone, D₂O exchangeable), 7.31–7.33 (m, 2H, Ar–H), 7.35–7.40 (m, 5H, Ar–H), 7.46 (s, 2H, SO₂NH₂, D₂O exchangeable), 7.79 (d, 2H, *J* = 8.7 Hz, Ar–H), 8.21 (s, 1H, H-3 of pyrazole), 9.40 (s, 1H, NH hydrazone, D₂O exchangeable). ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 117.13, 125.82, 126.59, 128.54, 129.23, 129.45, 130.98, 140.35, 141.96, 143.49, 143.70, 162.23. MS *m/z* [%] 357 [M⁺, 10.98], 326 [100]. Anal. Calcd for C₁₆H₁₅N₅O₃S (357.39): C, 53.77; H, 4.23; N, 19.60; S, 8.97. Found: C, 53.91; H, 4.30; N, 19.76; S, 9.06.

4.1.5. General procedure for synthesis of compounds (**11a–f**)

To a solution of 4-(4-(hydrazinecarbonyl)-5-phenyl-1H-pyrazol-1-yl)benzenesulfonamide (**10**) (10 mmol, 0.36 g) in 20 mL ethanol, 10 mmol of 5-(un)substituted isatin or *N*-benzyl isatin was added followed by catalytic amount of acetic acid (0.5 mL). The reaction mixture was refluxed for 1 h. The formed precipitate, in case of **11a–e**, was filtered, washed with hot ethanol and recrystallized from DMF/ EtOH to give the targeted compounds **11a–e**. Concerning compound **11f**, the precipitate formed after cooling was filtered and recrystallized from DMF/ EtOH.

4.1.5.1. 4-(4-(2-(2-Oxoindolin-3-ylidene)hydrazine-1-carbonyl)-5-phenyl-1H-pyrazol-1-yl)benzenesulfonamide (**11a**)

Yellow powder, 83% yield; mp > 300 °C. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3278–3136 (NH₂, 2NH), 1710–1681 (C=O), 1552 (C=N), 1320, 1153 (SO₂). ¹H NMR (DMSO-*d*₆, 300 MHz) δ 6.85 (d, 1H, *J* = 7.8 Hz, H-7 isatin), 7.05 (t, 1H, *J* = 7.8, H-5 isatin), 7.30–7.53 (m, 9H, Ar–H), 7.50 (s, 2H, SO₂NH₂, D₂O exchangeable), 7.81 (d, 2H, *J* = 8.4 Hz, Ar–H), 8.34, 8.47 (2s, 1H, H-3 of pyrazole), 10.74, 11.20 (2s, 1H, NH isatin, D₂O exchangeable), 10.95, 13.10 (2s, 1H, NH hydrazone, D₂O exchangeable). ¹³C NMR (DMSO-*d*₆, 75 MHz) δ 110.43, 111.06, 115.21, 119.71, 120.77, 121.58, 122.58, 125.77, 125.88, 126.47, 128.01, 129.54, 130.34, 131.52, 132.41, 141.13, 142.11, 143.37, 162.46, 164.60. MS *m/z* [%] 486 [M⁺, 7.98], 222 [100]. Anal. Calcd for C₂₄H₁₈N₆O₄S (486.51): C, 59.25; H, 3.73; N, 17.27; S, 6.59. Found: C, 59.37; H, 3.76; N, 17.39; S, 6.64.

4.1.5.2. 4-(4-(2-(5-Chloro-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl)-5-phenyl-1H-pyrazol-1-yl)benzenesulfonamide (**11b**)

Yellow powder, 61% yield; mp > 300 °C. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3410–3186 (NH₂, NH), 1712–1698 (C=O), 1506 (C=N), 1350, 1165 (SO₂). ¹H NMR (DMSO-*d*₆, 400 MHz) δ 6.87 (d, 1H, *J* = 8.4 Hz, H-7 of isatin), 6.93 (d, 1H, *J* = 8.4 Hz, H-6 of isatin), 7.33–7.42 (m, 8H, Ar–H), 7.47 (s, 2H, SO₂NH₂, D₂O exchangeable), 7.77 (d, 2H, *J* = 8.7 Hz, Ar–H), 8.38, 8.48 (2s, 1H, H-3 of pyrazole), 10.88, 11.33 (2s, 1H, NH isatin, D₂O exchangeable), 11.35, 13.02 (2s, 1H, NH hydrazone, D₂O exchangeable). ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 112.26, 113.09, 116.73, 120.81, 121.92, 126.08, 126.28, 126.41, 127.04, 127.22, 128.80, 128.96, 129.79, 130.71, 130.84, 131.40, 132.26, 141.38, 141.71, 142.81, 143.84, 143.96, 162.76, 164.92. MS *m/z* [%] 522 [M⁺+2, 3.22], 520 [M⁺, 9.01], 326 [100]. Anal. Calcd for C₂₄H₁₇ClN₆O₄S (520.95): C, 55.33; H, 3.29; N, 16.13; S, 6.15. Found: C, 55.71; H, 3.34; N, 16.29; S, 6.21.

4.1.5.3. 4-(4-(2-(5-Bromo-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl)-5-phenyl-1H-pyrazol-1-yl)benzenesulfonamide (**11c**)

Yellow powder, 87% yield; mp > 300 °C. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3412–3178 (NH₂, NH), 1712–1683 (C=O), 1506 (C=N), 1350, 1166 (SO₂). ¹H NMR (DMSO-*d*₆, 400 MHz) δ 6.82 (d, 1H, *J* = 8.4 Hz, H-7 of isatin), 6.88 (d, 1H, *J* = 8.4 Hz, H-6 of isatin), 7.38–7.45 (m, 8H, Ar–H), 7.51 (s, 2H, SO₂NH₂, D₂O exchangeable), 7.82 (d, 2H, *J* = 8.7 Hz, Ar–H), 8.38, 8.47 (2s, 1H, H-3 of pyrazole), 10.89, 11.23 (2s, 1H, NH isatin, D₂O exchangeable), 11.35, 13.01 (2s, 1H, NH hydrazone, D₂O exchangeable). ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 112.74, 113.53, 113.74, 114.79, 117.20, 122.33, 123.55, 126.28, 126.41, 127.04, 128.87, 128.96, 129.76, 130.69, 130.83, 134.18, 135.06, 141.60, 141.75, 142.74, 143.84, 143.95,

162.61, 164.79. MS *m/z* [%] 566 [$M^+ + 2$, 3.32], 564 [M^+ , 3.56], 326 [100]. Anal. Calcd for $C_{24}H_{17}BrN_6O_4S$ (565.40): C, 50.98; H, 3.03; N, 14.86; S, 5.67. Found: C, 51.08; H, 3.09; N, 14.97; S, 5.73.

4.1.5.4. 4-(4-(2-(5-Methoxy-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl)-5-phenyl-1H-pyrazol-1-yl)benzenesulfonamide (**11d**). Orange powder, 81% yield; mp > 300 °C. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3414–3190 (NH₂, NH), 1722–1690 (C=O), 1512 (C=N), 1322, 1156 (SO₂). ¹H NMR (DMSO-*d*₆, 300 MHz) δ 3.77 (s, 3H, OCH₃), 6.81 (dd, 1H, *J* = 11.4, 8.6 Hz, H-7 of isatin), 6.91–7.08 (m, 1H, Ar–H), 7.30–7.43 (m, 6H, Ar–H), 7.45 (s, 2H, SO₂NH₂, D₂O exchangeable), 7.80 (d, 2H, *J* = 8.7 Hz, Ar–H), 8.34, 8.49 (2s, 1H, H-3 of pyrazole), 10.55, 11.02 (2s, 1H, NH isatin, D₂O exchangeable), 11.31, 13.12 (2s, 1H, NH hydrazone, D₂O exchangeable). ¹³C NMR (DMSO-*d*₆, 75 MHz) δ 55.58, 105.73, 110.91, 111.91, 112.39, 115.65, 118.23, 120.46, 125.74, 126.50, 128.29, 129.23, 130.23, 135.90, 137.35, 141.14, 142.19, 143.31, 154.45, 155.32, 162.60, 164.74. MS *m/z* [%] 516 [M^+ , 7.62], 326 [100]. Anal. Calcd for $C_{25}H_{20}N_6O_5S$ (516.53): C, 58.13; H, 3.90; N, 16.27; S, 6.21. Found: C, 58.30; H, 3.96; N, 16.38; S, 6.32.

4.1.5.5. 4-(4-(2-(5-Nitro-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl)-5-phenyl-1H-pyrazol-1-yl)benzenesulfonamide (**11e**). Yellow powder, 92% yield; mp > 300 °C. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3367–3105 (NH₂, NH), 1716–1685 (C=O), 1523 (C=N), 1338, 1165 (SO₂). ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.08 (dd, 1H, *J* = 19.2, 8.7 Hz, H-7 of isatin), 7.33–7.46 (m, 5H, Ar–H), 7.47 (s, 2H, SO₂NH₂, D₂O exchangeable), 7.82 (d, 2H, *J* = 8.7 Hz, Ar–H), 8.16–8.42 (m, 4H, Ar–H), 8.49, 8.83 (2s, 1H, H-3 of pyrazole), 10.47, 11.86 (2s, 1H, NH isatin, D₂O exchangeable), 11.90, 12.86 (2s, 1H, NH hydrazone, D₂O exchangeable). ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 111.02, 111.84, 115.44, 116.15, 120.94, 122.07, 126.30, 126.43, 127.03, 128.74, 129.02, 129.65, 130.70, 130.82, 141.57, 141.68, 142.35, 143.22, 143.86, 144.00, 147.78, 149.60, 163.20, 165.40. MS *m/z* [%] 531 [M^+ , 9.67], 324 [30], 125 [100]. Anal. Calcd for $C_{24}H_{17}N_7O_6S$ (531.50): C, 54.24; H, 3.22; N, 18.45; S, 6.03. Found: C, 54.51; H, 3.28; N, 18.63; S, 6.11.

4.1.5.6. 4-(4-(2-(1-benzyl-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl)-5-phenyl-1H-pyrazol-1-yl)benzenesulfonamide (**11f**). Orange powder, 89% yield; mp = 210–212 °C. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3313–3194 (NH₂, NH), 1674–1612 (C=O), 1554 (C=N), 1342, 1168 (SO₂). ¹H NMR (DMSO-*d*₆, 400 MHz) δ 4.93 (s, 2H, benzylic CH₂), 7.04 (d, 1H, *J* = 8.7 Hz, H-7 of isatin), 7.11 (t, 1H, *J* = 7.5 Hz, H-5 of isatin), 7.25–7.40 (m, 11H, Ar–H), 7.47 (d, 2H, *J* = 8.4 Hz, Ar–H), 7.52 (s, 2H, SO₂NH₂, D₂O exchangeable), 7.56 (d, 1H, *J* = 7.4 Hz, Ar–H), 7.85 (d, 2H, *J* = 8.4 Hz, Ar–H), 8.39, 8.57 (2s, 1H, H-3 of pyrazole), 12.00, 13.02 (2s, 1H, NH hydrazone, D₂O exchangeable). ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 42.96, 110.82, 115.85, 119.69, 121.13, 123.74, 126.37, 127.02, 127.96, 128.15, 128.30, 128.99, 129.18, 130.05, 130.89, 131.84, 136.10, 141.62, 142.89, 143.97, 145.40, 161.08, 172.52. MS *m/z* [%] 576 [M^+ , 2.03], 144 [100]. Anal. Calcd for $C_{31}H_{24}N_6O_4S$ (576.63): C, 64.57; H, 4.20; N, 14.57; S, 5.56. Found: C, 64.74; H, 4.27; N, 14.74; S, 5.61.

4.1.6. Synthesis of ethyl 5-amino-1-(4-sulfamoylphenyl)-1H-pyrazole-4-carboxylate (**14**)

Ethyl 2-cyano-3-ethoxyacrylate (**13**) (10 mmol, 1.69 g) and 4-aminobenzenesulfonamide hydrochloride (10 mmol, 2.23 g) were refluxed in a mixture of acetic acid and water (5:1) for 4 h. The reaction mixture was poured on ice and stirred for 1 h. The given precipitate was filtered, washed with water, dried and recrystallized from ethanol. The experimental data were given as reported [31].

4.1.7. General procedure for synthesis of compounds (**16a–f**)

In 50 mL round flask, 4-(4-(hydrazinecarbonyl)-5-amino-1H-

pyrazol-1-yl)benzenesulfonamide **15** (10 mmol, 0.3 g) was dissolved in ethanol (20 mL) followed by the addition of the appropriate isatin derivative (10 mmol). Reflux was performed after the addition of a catalytic amount of acetic acid (0.5 mL) for 1 h. The formed precipitate, in case of **16a–e**, was filtered washed with hot ethanol and recrystallized from DMF / EtOH to give the targeted compounds **16a–e**. Concerning compound **16f**, the precipitate formed after cooling was filtered and recrystallized from DMF / EtOH.

4.1.7.1. 4-(5-Amino-4-(2-(2-oxoindolin-3-ylidene)hydrazine-1-carbonyl)-1H-pyrazol-1-yl)benzenesulfonamide (**16a**). Yellow powder, 76% yield; mp > 300 °C. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3385–3182 (NH₂, NH), 1718–1690 (C=O), 1531 (C=N), 1321, 1153 (SO₂). ¹H NMR (DMSO-*d*₆, 300 MHz) δ 6.52 (s, 2H, NH₂, D₂O exchangeable), 6.87–7.17 (m, 3H, Ar–H), 7.37 (t, *J* = 7.8 Hz, 1H, Ar–H), 7.45, 7.50 (2s, 2H, SO₂NH₂, D₂O exchangeable), 7.57 (d, 2H, *J* = 7.5 Hz, Ar–H), 7.76–8.12 (m, 2H, Ar–H), 8.53, 9.18 (2s, 1H, H-3 of pyrazole), 10.79, 11.14 (2s, 1H, NH isatin, D₂O exchangeable), 11.24, 12.96 (2s, 1H, NH hydrazone, D₂O exchangeable). ¹³C NMR (DMSO-*d*₆, 75 MHz) δ 95.48, 110.51, 111.04, 115.43, 120.01, 120.55, 121.71, 122.55, 123.52, 125.97, 126.93, 127.03, 131.12, 132.11, 138.77, 140.20, 142.02, 143.57, 151.60, 162.79, 165.02. MS *m/z* [%] 425 [M^+ , 30.01], 265 [100]. Anal. Calcd for $C_{18}H_{15}N_7O_4S$ (425.42): C, 50.82; H, 3.55; N, 23.05; S, 7.54. Found: C, 51.04; H, 3.53; N, 23.28; S, 7.63.

4.1.7.2. 4-(5-Amino-4-(2-(5-chloro-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl)-1H-pyrazol-1-yl)benzenesulfonamide (**16b**). Yellow powder, 70% yield; mp > 300 °C. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3390–3182 (NH₂, NH), 1725–1664 (C=O), 1521 (C=N), 1305, 1165 (SO₂). ¹H NMR (DMSO-*d*₆, 300 MHz) δ 6.52 (s, 2H, NH₂, D₂O exchangeable), 6.83–7.05 (m, 2H, Ar–H), 7.34 (t, *J* = 7.8 Hz, 1H, Ar–H), 7.50, 7.63 (2s, 2H, SO₂NH₂, D₂O exchangeable), 7.82 (t, *J* = 6.6 Hz, 2H, Ar–H), 7.99 (d, *J* = 8.4 Hz, 2H, Ar–H), 8.20, 8.46 (2s, 1H, H-3 of pyrazole), 10.80, 11.34 (2s, 1H, NH isatin, D₂O exchangeable), 12.85 (s, 1H, NH hydrazone, D₂O exchangeable). ¹³C NMR (DMSO-*d*₆, 75 MHz) δ 94.86, 112.43, 120.12, 121.66, 122.64, 123.25, 126.74, 127.01, 130.39, 133.30, 140.18, 140.58, 142.60, 151.68, 162.53. MS *m/z* [%] 461 [$M^+ + 2$, 3.22], 459 [M^+ , 9.10], 265 [100]. Anal. Calcd for $C_{18}H_{14}ClN_7O_4S$ (459.87): C, 47.01; H, 3.07; N, 21.32; S, 6.97. Found: C, 47.14; H, 3.09; N, 21.57; S, 7.04.

4.1.7.3. 4-(5-Amino-4-(2-(5-Bromo-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl)-1H-pyrazol-1-yl)benzenesulfonamide (**16c**). Yellow powder, 79% yield; mp > 300 °C. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3412–3178 (NH₂, NH), 1712–1683 (C=O), 1506 (C=N), 1350, 1166 (SO₂). ¹H NMR (DMSO-*d*₆, 300 MHz) δ 6.52 (s, 2H, NH₂, D₂O exchangeable), 6.86–7.02 (m, 3H, Ar–H), 7.49 (s, 2H, SO₂NH₂, D₂O exchangeable), 7.70–7.85 (m, 2H, Ar–H), 7.99 (d, 2H, *J* = 8.4 Hz, Ar–H), 8.20, 8.57 (2s, 1H, H-3 of pyrazole), 10.89, 11.33 (2s, 1H, NH isatin, D₂O exchangeable), 12.84 (s, 1H, NH hydrazone, D₂O exchangeable). ¹³C NMR (DMSO-*d*₆, 75 MHz) δ 94.24, 112.87, 114.35, 122.05, 122.84, 123.45, 127.01, 133.17, 140.18, 140.95, 142.58, 151.68, 162.38. MS *m/z* [%] 505 [$M^+ + 2$, 3.67], 503 [M^+ , 3.69], 222 [100]. Anal. Calcd for $C_{18}H_{14}BrN_7O_4S$ (504.32): C, 42.87; H, 2.80; N, 19.44; S, 6.36. Found: C, 42.99; H, 2.79; N, 19.62; S, 6.39.

4.1.7.4. 4-(5-Amino-4-(2-(5-methoxy-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl)-1H-pyrazol-1-yl)benzenesulfonamide (**16d**). Orange powder, 85% yield; mp > 300 °C. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3414–3190 (NH₂, NH), 1722–1690 (C=O), 1512 (C=N), 1322, 1156 (SO₂). ¹H NMR (DMSO-*d*₆, 400 MHz) δ 3.80 (s, 3H, OCH₃), 6.82 (d, 1H, *J* = 8.4 Hz, H-7 isatin), 6.96 (d, 2H, *J* = 10.2 Hz, Ar–H), 7.07 (s, 2H, NH₂, D₂O exchangeable), 7.49 (s, 2H, SO₂NH₂, D₂O exchangeable), 7.83 (d, 2H, *J* = 8.4 Hz, Ar–H), 8.00 (d, 2H, *J* = 8.4 Hz, Ar–H), 8.61 (s,

1H, H-3 of pyrazole), 10.61, 11.07 (s, 1H, NH isatin, D₂O exchangeable), 11.38, 13.02 (2s, 1H, NH hydrazone, D₂O exchangeable). ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 56.41, 95.92, 111.50, 112.13, 116.20, 118.52, 121.22, 123.18, 123.97, 127.51, 137.77, 140.83, 142.96, 143.31, 152.46, 155.11, 155.83, 165.69, 166.47. MS *m/z* [%] 455 [M⁺, 9.06], 223 [100]. Anal. Calcd for C₁₉H₁₇N₇O₅S (455.45): C, 50.11; H, 3.76; N, 21.53; S, 7.04. Found: C, 50.27; H, 3.83; N, 21.75; S, 7.13.

4.1.7.5. 4-(5-Amino-4-(2-(5-Nitro-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl)-1H-pyrazol-1-yl)benzenesulfonamide (**16e**). Yellow powder, 92% yield; mp > 300 °C. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3421–3120 (NH₂, NH), 1732–1666 (C=O), 1523 (C=N), 1327, 1165 (SO₂). ¹H NMR (DMSO-*d*₆, 400 MHz) δ 6.79–7.14 (m, 2H, Ar–H), 7.07 (s, 2H, NH₂, D₂O exchangeable), 7.51 (s, 2H, SO₂NH₂, D₂O exchangeable), 7.82 (d, 2H, *J* = 8.0 Hz, Ar–H), 7.99 (d, 2H, *J* = 8.0 Hz, Ar–H), 8.10–8.39 (m, 1H, Ar–H), 8.57, 9.16 (2s, 1H, H-3 of pyrazole), 10.98, 11.50 (2s, 1H, NH isatin, D₂O exchangeable), 11.95, 12.78 (2s, 1H, NH hydrazone, D₂O exchangeable). ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 95.72, 110.97, 115.64, 121.89, 123.44, 124.02, 124.07, 127.51, 128.48, 133.07, 140.75, 142.52, 143.03, 143.19, 149.49, 152.61, 165.94. MS *m/z* [%] 470 [M⁺, 8.97], 222 [100]. Anal. Calcd for C₁₈H₁₄N₈O₆S (470.42): C, 45.96; H, 3.00; N, 23.82; S, 6.82. Found: C, 46.31; H, 2.98; N, 23.94; S, 6.93.

4.1.7.6. 4-(5-Amino-4-(2-(1-benzyl-2-oxoindolin-3-ylidene)hydrazine-1-carbonyl)-1H-pyrazol-1-yl)benzenesulfonamide (**16f**). Yellow powder, 88% yield; mp = 270–272 °C. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3313–3194 (NH₂, NH), 1732–1674 (C=O), 1554 (C=N), 1342, 1168 (SO₂). ¹H NMR (DMSO-*d*₆, 400 MHz) δ 5.02 (s, 2H, benzylic CH₂), 7.03 (s, 2H, NH₂, D₂O exchangeable), 7.04 (d, 1H, *J* = 8.0 Hz, H-7 of isatin), 7.14 (t, 1H, *J* = 7.6 Hz, H-5 of isatin), 7.27–7.42 (m, 6H, Ar–H), 7.53 (s, 2H, SO₂NH₂, D₂O exchangeable), 7.70 (d, 1H, *J* = 7.4 Hz, Ar–H), 7.83 (d, 2H, *J* = 8.7 Hz, Ar–H), 8.00 (d, 2H, *J* = 8.7 Hz, Ar–H), 8.21 (s, 1H, H-3 of pyrazole), 12.88 (s, 1H, NH hydrazone, D₂O exchangeable). ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 43.00, 95.51, 110.81, 119.98, 120.90, 123.70, 124.01, 127.52, 127.86, 128.09, 129.19, 131.42, 133.89, 136.18, 140.66, 142.72, 143.13, 152.13, 161.41. MS *m/z* [%] 515 [M⁺, 10.12], 326 [100]. Anal. Calcd for C₂₅H₂₁N₇O₄S (515.55): C, 58.24; H, 4.11; N, 19.02; S, 6.22. Found: C, 58.43; H, 4.16; N, 19.26; S, 6.25.

4.2. Carbonic anhydrase inhibition

4.2.1. CA inhibitory assay

An SX.18 MV-R Applied Photophysics stopped-flow instrument was used for assaying the CA-catalyzed CO₂ hydration activity by using the method of Khalifah [33]. Inhibitor and enzyme were preincubated for 6 h. IC₅₀ values were obtained from dose response curves working at seven different concentrations of test compound (from 0.1 nM to 50 μ M), by fitting the curves using PRISM (www.graphpad.com) and non-linear least squares methods, values representing the mean of at least three different determinations, as described earlier by us. [32,33] The inhibition constants (*K*_i) were then derived by using the Cheng-Prusoff equation, as follows: $K_i = IC_{50}/(1 + [S]/K_m)$ where [S] represents the CO₂ concentration at which the measurement was carried out, and *K*_m the concentration of substrate at which the enzyme activity is at half maximal. All enzymes used were recombinant, produced in *E. coli* as reported earlier. [34,35] The concentrations of enzymes used in the assay were: hCA I, 1031 nM; hCA II, 8.4 nM; hCA IX, 7.8 nM and hCA XII, 10.4 nM.

4.3. X-ray crystallography

4.3.1. General data for compound **10**

Single crystals for compounds **10** were obtained by slow

evaporation from ethanol. A good crystal with a suitable size was selected for analysis. Crystallographic data for the structure **10** has been deposited with the Cambridge Crystallographic Data Center (CCDC) under the numbers CCDC 1053077. Data were collected on a Bruker APEX-II CCD diffractometer equipped with graphite monochromatic Cu *K* α radiation ($\lambda = 1.54178$ Å) at 296 (2) K. Cell refinement and data reduction were done by Bruker SAINT; program used to solve structure and refine structure is SHELXS-97 [36]. The final refinement was performed by full-matrix least-squares techniques with anisotropic thermal data for non-hydrogen atoms on F2. All the hydrogen atoms were placed in calculated positions and constrained to ride on their parent atoms. Multiscan absorption correction was applied by the use of SADABS software.

4.3.2. General data for compound **14**

Single crystals for compound **14** were obtained by slow evaporation from ethanol. A good crystal with a suitable size was selected for analysis. Crystallographic data for the structure **14** has been deposited with the Cambridge Crystallographic Data Center (CCDC) under the numbers CCDC 1063099. All diagrams and calculations were performed using maXus [37]. Data were collected on a KappaCCD diffractometer equipped with graphite monochromatic Mo *K* α radiation, $\lambda = 0.71073$ Å at 298 (2) K. Cell refinement and data reduction were done by HKL SCALEPACK (Otwinowski & Minor, 1997); program used to solve structure and refine structure is SHELXS-97 [36]. The final refinement was performed by full-matrix least-squares techniques with anisotropic thermal data for non-hydrogen atoms on F2. All the hydrogen atoms were placed in calculated positions and constrained to ride on their parent atoms. Multiscan absorption correction was applied by the use of SADABS software.

4.4. Molecular docking studies

The molecular docking of the tested compounds was performed using Discovery Studio 4 /CDOCKER protocol (Accelrys Software Inc.). The protein crystallographic structure, hCA IX (PDB id: 3IAI) and hCA XII (PDB id: 1JD0) was downloaded from the Protein Data Bank (PDB). The protein was prepared for docking process according to the standard protein preparation procedure integrated in Accelrys's discovery studio 4 and prepared by prepare protein protocol. Docked compounds were drawn and prepared by prepare ligand protocol to generate 3D structure and refined using CHARMM force field with full potential. Docking simulations were run using CDOCKER protocol where maximum bad orientations was 800 and orientation vdW energy threshold was 300. Simulated annealing simulation would be then carried out consisting of a heating phase 700 K with 2000 steps and a cooling phase back to 5000 steps. The binding energy was calculated as a score to rank the docking poses. The top 10 docking poses would be finally saved. Docking poses were ranked according to their –CDOCKER interaction energy, and the top pose was chosen for analysis of interactions for each compound.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejmech.2015.09.021>.

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