Bioorganic Chemistry 83 (2019) 549–558

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

SLC-0111 enaminone analogs, 3/4-(3-aryl-3-oxopropenyl) aminobenzenesulfonamides, as novel selective subnanomolar inhibitors of the tumor-associated carbonic anhydrase isoform IX

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ARTICLE INFO

Keywords:
Benzenesulfonamide
Carbonic anhydrase
Enaminones
SLC-0111
Selective CA IX inhibitors

ABSTRACT

SLC-0111, an ureido substituted benzenesulfonamide, is a selective carbonic anhydrase (CA, EC 4.2.1.1) IX inhibitor that is currently in Phase I/II clinical trials for the treatment of advanced hypoxic tumors complicated with metastases. Herein we report the synthesis of two series of 3/4-(3-aryl-3-oxopropenyl) aminobenzenesulfonamides **5a–i** and **6a–j** as SLC-0111 enaminone congeners. The prepared enaminones were *in vitro* investigated as inhibitors of the metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1) isoforms hCA I, II, IV and IX, using a stopped-flow CO_2 hydrase assay. All these isoforms were inhibited by the enaminones reported here in variable degrees. The target tumor-associated isoform hCA IX was undeniably the most affected one (K_Is: 0.21–7.1 nM), with 6- to 21-fold enhanced activity than SLC-0111 (K_I = 45 nM). All the prepared enaminones displayed interesting selectivity towards hCA IX over hCA I (SI: 32 – > 35714), hCA II (SI: 2 – 1689) and hCA IV (SI: 11 – > 45454). Of particular interest, bioisosteric replacement of phenyl tail with the bulkier 2-naphthyl tail, sulfonamide **6h**, achieved the higher II/IX selectivity herein reported with SI of 1689.

1. Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) are prevalent metalloenzymes in all life kingdoms. In humans, the CA-catalyzed reaction encompasses three simple chemical entities, CO_2, HCO_3^-, and H^+ essential in several physiological and pathological processes, such as the pH and CO2 homeostasis, bone resorption, electrolyte secretion, respiration, biosynthetic reactions (like lipogenesis and gluconeogenesis) and tumorigenicity, to name few [1–3].

The human (h) CA isoforms displayed diverse tissue distribution and location patterns; thus cytosolic (I, II, III, VII, and XIII), membrane-bound (IV, IX, XII, and XIV), secreted (VI) and mitochondrial (VA and VB) forms have been described in mammals. These CA isozymes are important therapeutic targets for a wide range of disorders such as glaucoma (hCA II, IV and XII), edema (hCA II, IV, XIV), and tumors (hCA IX and hCA XII) [3–5]. In particular, hCA IX is an extracellular transmembrane tumor-related protein that overexpressed in a wide variety of human malignancies and appears to be tightly regulated by microenvironmental hypoxia. During the last years, this CA isozyme had been stood out as an innovative anticancer drug target and recognized as a marker of tumor hypoxia and prognostic factor for several cancer types [6–8]. It is noteworthy, that ubiquitous hCA I and CA II, which are involved in many physiological processes, are considered to be off-target in the cancer therapies [1], as they are responsible of most of the side effects of non-selective inhibitors. The “tail approach” represents the most efficient and exploited tactic to overcome the poor selectivity of sulfonamide-like CAIs towards the different human isozymes [9–11].

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https://doi.org/10.1016/j.bioorg.2018.11.014
Received 25 August 2018; Received in revised form 6 November 2018; Accepted 13 November 2018
Available online 14 November 2018

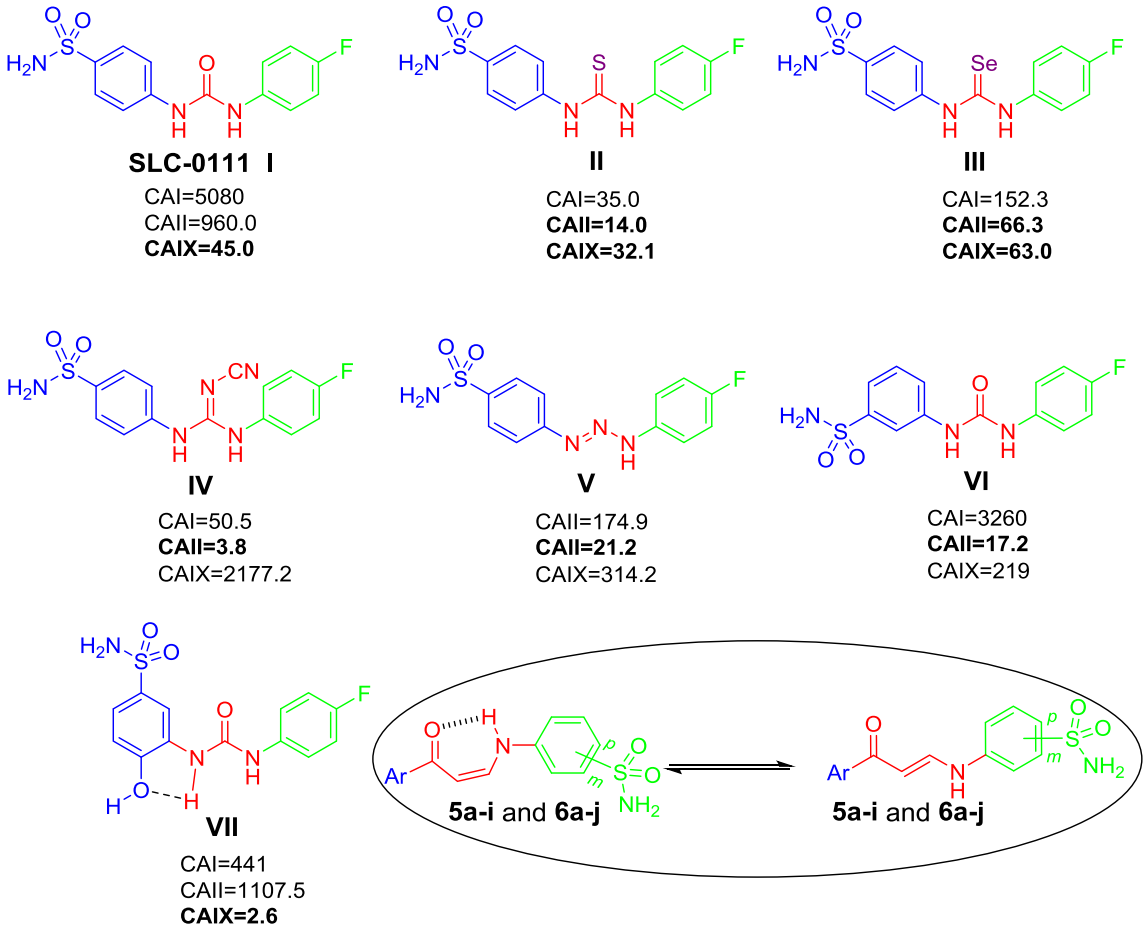


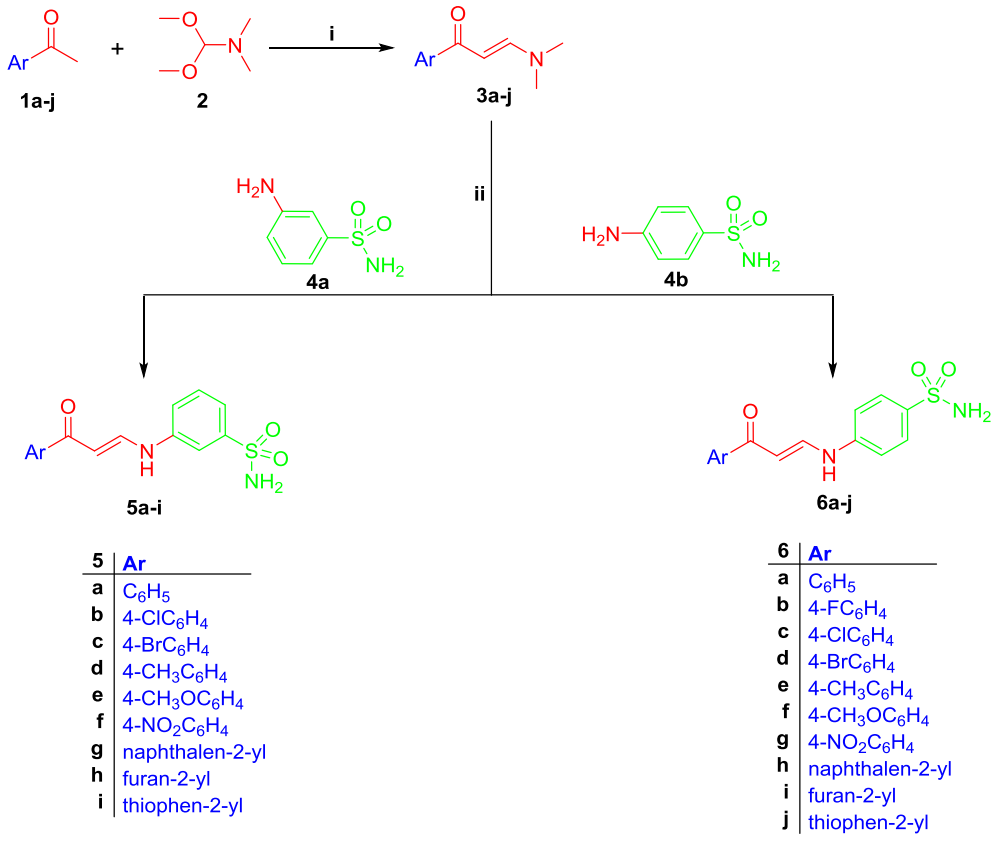
Fig. 1. Structures of some reported SLC-0111 analogs **I–VII** and the target enaminones **5a–i** and **6a–j**.

Adopting "tail approach" has led to the development of SLC-0111 (**I**) (Fig. 1). SLC-0111, an ureido substituted benzenesulfonamide, is a selective carbonic anhydrase IX inhibitor that is currently in Phase I/II clinical trials for the treatment of advanced hypoxic tumors complicated with metastases [12–14]. Being the first-in-class CAI, SLC-0111 has been used as a lead molecule for designing other compounds with a selective CA IX inhibitory activity [15–24].

A. Angeli et al. [15] reported the synthesis of novel SLC-0111 congeners *via* adopting a divalent bioisosteric replacement approach of the ureido oxygen on SLC-0111 with a sulfur or selenium atom, compounds **II** and **III** (**Fig. I**). Such replacement determined dramatic enhancements of the inhibition potencies towards the hCA I and II isoforms (**Fig. I**). While, the thioureido derivative **II** resulted in only a slight improvement of the inhibition potency towards hCA IX (K_I = 45.0 and 32.1 nM for SLC-0111 and **II**, respectively), the introduction of the selenium, compound **III**, was found to be detrimental for the activity (K_I = 63.0 nM). Accordingly, the inhibition profiles of both thioureido **II** and selenoureido **III** congeners of SLC-0111 displayed loss of selectivity for the inhibition of the tumor-associated over the cytosolic CA isoforms. Akocak and co-workers reported two studies about the development of *N,N′*-diaryl cyanoguanidines and 1,3-diaryltriazenes as novel SLC-0111 analogues, through bioisosteric replacement of urea linker in SLC-0111 with cyanoguanidine or 1,3-triazene linker, compounds **IV** and **V** (**Fig. I**) [16,17]. These structural modifications switched the selectivity of cyanoguanidines and 1,3-diaryltriazenes counterparts to be highly selective for hCA II over the tumor-associated hCA IV and IX.

Last year, F. Carta et al. [18] explored the synthesis and biological evaluation of SLC-0111 regioisomers, compound **VI** (**Fig. I**). The study outcomes revealed that shifting primary sulfonamide ($-SO_2NH_2$) functionality from the *para*-position, SLC-0111, to *meta*-position, compound **VI**, resulted in a significant improvement of the inhibitory activity against hCA II concurrently with worsening of effectiveness towards hCA IX which massively spoiled the selectivity ratio hCA II/hCA IX for this regioisomer. Recently, M. Bozdag et al. [19] reported the design and synthesis of ureido containing compounds based on the 2-aminophenol-4-sulfonamide as new SLC-0111 congeners with controlled degrees of tail flexibility, compound **VII** (**Fig. I**). The phenolic OH was mainly intended to form a stable intramolecular five-membered ring with the ureido N*H* moiety at 2-position (**Fig. I**), which thought to induce C2-N′ rotational restriction, that may determine preferential rotational isomers to interact within the hCAs enzymatic sites thus resulting in improvement of CAs selectivity. Interestingly, the rotational restriction within compound **VII**, resulted in 20-fold enhanced selectivity ratio hCA II/hCA IX in comparison to SLC-0111 (**Fig. I**).

In view of the facts mentioned above and as part of our ongoing effort towards developing effective CAIs [25–29], herein we report two series of 3/4-(3-aryl-3-oxopropenyl) aminobenzenesulfonamides (**5a–i** and **6a–j**) as SLC-0111 enaminone analogs (Fig. 1), with the prime aim of developing potent and selective hCA IX inhibitors. Firstly, the bioisosteric replacement approach was utilized to replace the urea linker within SLC-0111 with an enaminone one, followed by introduction of two structural modifications. The first modification focuses on the replacement on 4-fluorophenyl tail of SLC-0111 with different phenyl, 4-substituted-phenyl, the bulky 2-naphthyl, and the small 5-membered heterocycles (2-furyl and 2-thienyl). The second modification includes the development of the regioisomers, through moving the sulfonamide ($-SO_2NH_2$) functionality from the *para*-position to the *meta*-position. All the newly synthesized enaminones were examined *in vitro* for their inhibitory activity towards a panel of hCA I, II, IV and IX isoforms, using stopped-flow CO_2 hydrase assay.



5	Ar
a	C_6H_5
b	4-ClC_6H_4
c	4-BrC_6H_4
d	4-$CH_3C_6H_4$
e	4-$CH_3OC_6H_4$
f	4-$NO_2C_6H_4$
g	naphthalen-2-yl
h	furan-2-yl
i	thiophen-2-yl

6	Ar
a	C_6H_5
b	4-FC_6H_4
c	4-ClC_6H_4
d	4-BrC_6H_4
e	4-$CH_3C_6H_4$
f	4-$CH_3OC_6H_4$
g	4-$NO_2C_6H_4$
h	naphthalen-2-yl
i	furan-2-yl
j	thiophen-2-yl

Scheme 1. Synthesis of target enaminones **5a–i** and **6a–j**; *Reagents and conditions*: (**i**) Dry xylene/reflux 8 h, (**ii**) Glacial acetic acid/reflux 4 h.

2. Results and discussion

2.1. Chemistry

The synthetic strategy for the development of the target sulfonamides **5a–i** and **6a–j** was depicted in Scheme 1. Different acetophenones **1a–j** were refluxed with DMF-DMA **2** in xylene to afford enaminones **3a–j** which reacted with *m*- and *p*-aminobenzenesulfonamides **4a,b** in refluxing glacial acetic acid to furnish the target sulfonamides **5a–i** and **6a–j**, respectively.

The structures of target sulfonamides **5a–i** and **6a–j** were confirmed under the basis of their spectral data and elemental analyses. Their IR spectra showed the absorption bands due to the (NH, NH_2) and (C═O) groups in the regions 3120–3400 cm^{-1} and 1720–1735 cm^{-1}, respectively, besides two bands of (SO_2) at 1150–1160 cm^{-1} and 1300–1320 cm^{-1}.

Furthermore, ^{1}H NMR spectra of sulfonamides **5a–i** and **6a–j** revealed presence of *E/Z* geometric conjugation forms for $H_aC = CH_b$. The existing of *E*-form confirmed by the coupling constant of the olefinic hydrogens H_a and H_b where $J_{Ha\text{-}Hb}$ = 12.0–12.8 Hz which calculated from the down-field doublet signal of *E*-H_a at δ 6.37–6.69 ppm (Fig. 2). Similarly, the existing of *Z*-form proved by the coupling constant of the olefinic hydrogens H_a and H_b (J = 7.2–8.4 Hz) which elucidated from the up-field doublets of *Z*-H_a at δ 6.00–6.40 ppm. Additionally, ^{1}H NMR spectra revealed two sets of doublet, integrated for a total one proton, each belonging to the NH group of the *E* and *Z* forms which observed at δ 10.24–10.59 and 11.72–12.18 ppm, respectively (Fig. 2). The downfield lines of −NH protons were assigned to *Z* form and up-field lines of the same group to *E* form. The coupling values of these doublets are in the range of 12.0–12.8 Hz which provides the existing of NH of sulfonamides **5a–i** and **6a–j** in *trans* direction for H_b around HN-CH_b bond in both forms *E* and *Z* (Fig. 3). The down-field appearance of *Z* NH attributed to its intra-molecular hydrogen bond with the oxygen of C═O group. Interestingly, x-ray single crystal analyses of **5a** showed the *Z* configuration around $H_aC = CH_b$ with *trans* HN-CH_b in solid state and it is also revealed the intra-molecular hydrogen bond between NH and C═O groups [30].

Moreover, their ^{1}H NMR spectra confirmed the presence of singlet signal of NH_2 of $-SO_2NH_2$ group around δ 7.20–7.37 ppm, also Compounds **5d** and **6e** confirmed with presence of single peak of $-CH_3$ at δ 2.38 ppm and compounds **5e** and **6f** revealed by presence of single peak of $-OCH_3$ at δ 3.81–3.84 ppm.

In addition, ^{13}C NMR spectra reveled the presence of *E* and *Z* forms as (−CO−CH═CH−) present in two chemical shift range 94.49–95.36 ppm and 98.77–100.00 ppm for *Z* and *E* forms, respectively, while C═O, present in range 177.10–188.08 ppm and 179.55–190.42 ppm. On the other hand, the chemical shift of OCH_3 group of compounds **5e**, **6f** and CH_3 group of compounds **5d**, **6e** appeared at 55.87, 55.90 ppm and 21.54, 21.55 ppm, respectively, for E/Z forms.

2.2. Single crystal X-ray crystallographic analysis

Single crystal of enaminone **5a** was obtained through the slow evaporation of its ethanol solution. A suitable crystal was selected and analyzed on a Bruker *APEX*-II CMOS diffractometer. The data was collected at 293(2) K.

The structure of enaminone **5a** (Fig. 4) was unambiguously determined by X-ray crystallographic analysis. The molecular structure of **5a** ($C_{15}H_{14}N_2O_3S$) was crystallized in the monoclinic, space group $P2_1$, a = 5.2360 (5) Å, b = 9.4977 (8) Å, c = 14.1960 (14) Å, β = 98.628 (5) °, V = 697.98 (11) $Å^3$, Z = 2. The crystallographic data and refinement information are summarized in Table 1. The dihedral angle between the mean planes of the two phenyl rings is 4.78, which makes the whole structure nearly flat. In the crystal packing structure, molecules are linked *via* three intermolecular hydrogen bonds along the b

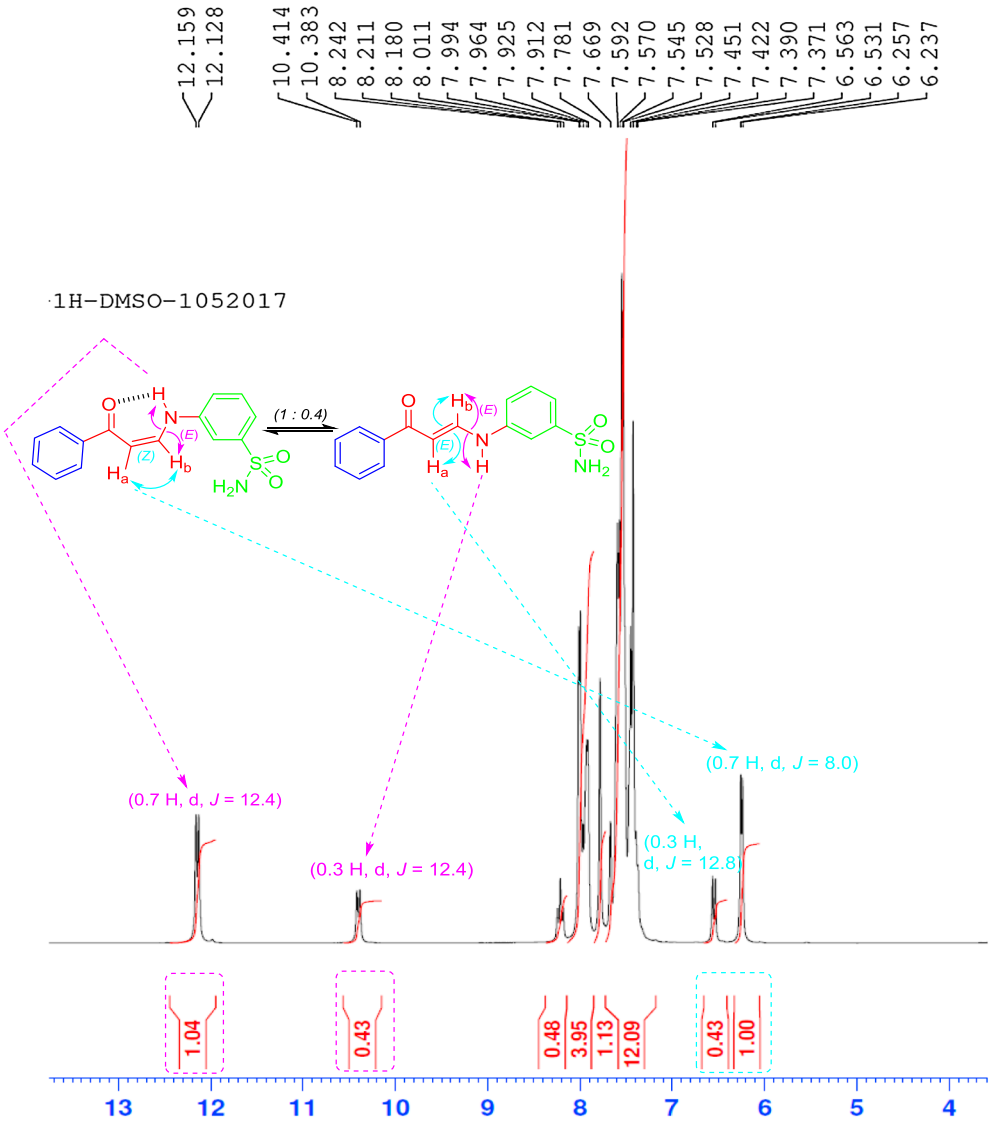


Fig. 2. ^{1}H NMR of compound **5a** which showed the existing of *E/Z* configurations around $H_aC = CH_b$ with *trans* HN-CH_b in DMSO.

axis between N2—H1N2···O3^{i}, N2—H1N···O2^{ii} and C5—H5A···O1^{iii}. Symmetry codes: (i) x + 1, y, z; (ii) −x + 1, y − 1/2, −z + 2; (iii) −x + 2, y + 1/2, −z + 1.

2.3. Biological evaluation

2.3.1. Carbonic anhydrase inhibition

All synthesized enaminones **5a–i** and **6a–j** were tested *in vitro* for their inhibitory properties against physiologically relevant hCA isoforms, hCA I, II (cytosolic) and hCA IV (transmembrane) as well as the tumor-associated hCA IX (transmembrane), by means of a stopped-flow carbon dioxide hydration assay [31]. Their inhibitory activities were compared to SLC-0111 and the clinically used standard carbonic anhydrase inhibitor acetazolamide (AAZ). The following structure–activity relationship (SAR) could be deduced from the inhibition data displayed in Table 2:

(i) The ubiquitous cytosolic isoform hCA I was inhibited by most of the prepared enaminones reported here with inhibition constants (K_Is) ranging in the low nanomolar - high micromolar range, in detail, between 8.5 nM and 9.09 μM, except compounds **6g** and **6h** which did not inhibit hCA I up to 10 μM. It is noteworthy that shifting the sulphonamide group from *para*-position (as compound **6a**; K_I = 68.8 nM) to the *meta*-position (as compound **5a**; K_Is = 626.7 nM) resulted in a sharp decrease in the inhibitory activity against hCA I, except enaminones **5d**, **5g** and **5h**. Whereas, bioisosteric replacement of the phenyl ring (compound **6a**; K_I = 68.8 nM) with 2-furyl or 2-thienyl moieties led to the most potent hCA I inhibitors in this study with remarkable increase in activity (compounds **6i** and **6j**; K_Is = 8.5 and 9.3 nM, respectively).

(ii) The physiologically dominant isoform hCA II was efficiently inhibited by most sulfonamides prepared in this study (K_Is in the range of 0.48–472.8 nM) (Table 2). It is worth highlighting that the *para*-substituted enaminones **6a–f, 6i** and **6j** showed a generally improved inhibitory profile against hCA II in comparison to their *meta*-substituted analogues **5a–e, 5h** and **5i**. In particular *para* sulphonamides **6b**, **6e**, **6i** and **6j** stood out as the best hCA II inhibitors with subnanomolar potencies (K_Is = 0.48, 0.53, 0.84 and 0.89 nM, respectively).

Noteworthy, the SAR outcomes highlighted that substitution of the pendant phenyl tail with larger lipophilic groups (Cl, Br, or NO_2) or its replacement with the bulkier 2-naphthyl tail elicited a worsening of effectiveness against hCA II both for the *meta*-substituted enaminones **5b**, **c**, **e–g** (K_I: 29.2–344.4 nM) and the *para*-substituted enaminones **6c**, **d**, **f–h** (K_Is: 6.3–472.8 nM) in comparison to unsubstituted counterparts **5a** and **6a** (K_Is = 21.7 and 5.9 nM, respectively). In addition, bioisosteric replacement of the phenyl tail for the *meta*-substituted sulphonamide **5a** (K_Is = 21.7 nM) with 2-furyl or 2-thienyl tail moieties led to about two- and four-fold diminished hCA II inhibition efficacy

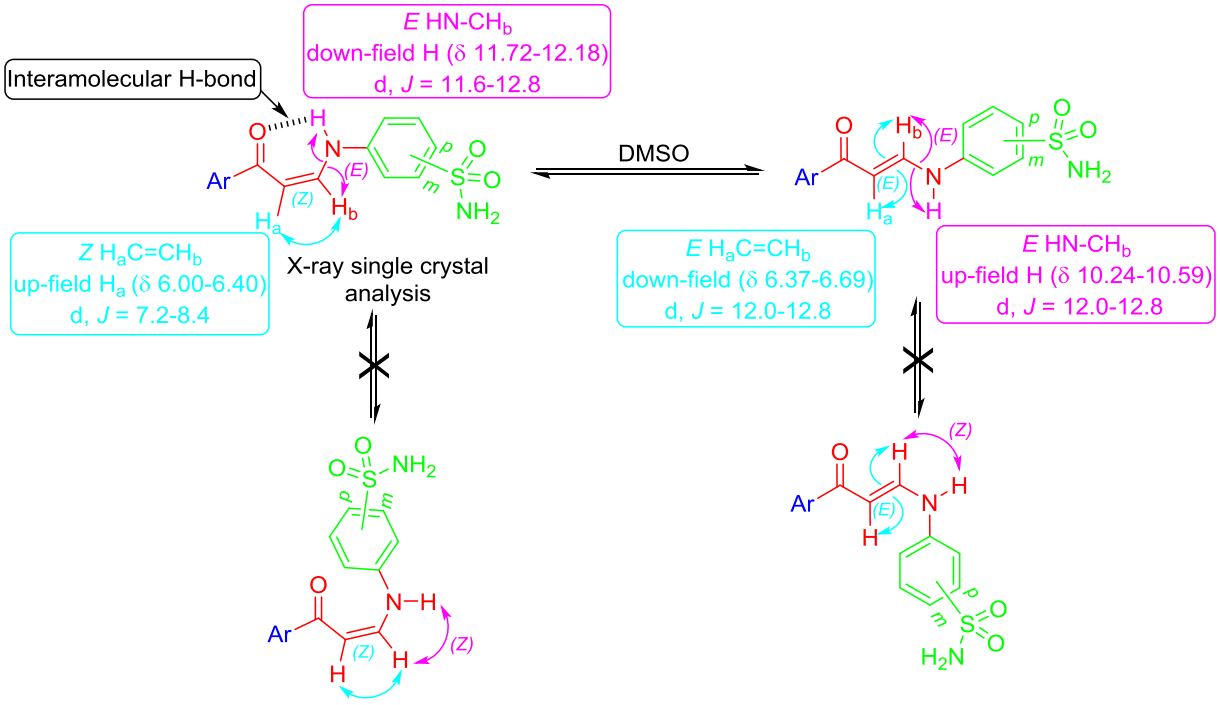


Fig. 3. The existing of compounds **5a** as *Z* configuration around $H_aC = CH$ with *E* HN-CH in solid state (X-ray single crystal analyses) and as *Z*/*E* $H_aC = CH_b$, *E* HN-CH_b configurations in DMSO (^{1}H NMR).

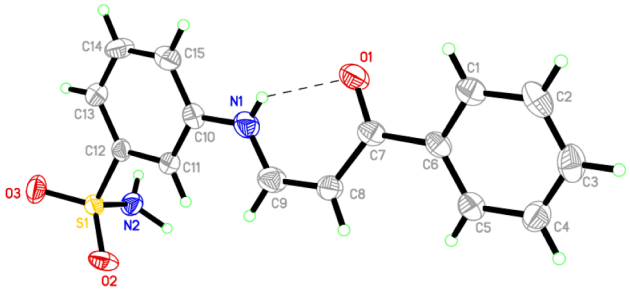


Fig. 4. An ORTEP diagram of final X-ray structure of enaminone **5a**. Displacement ellipsoids are plotted at the 40% probability level for non-H atoms.

(enaminones **5h** and **5i**; K_Is = 34.6 and 76.6 nM, respectively). Conversely, adopting this replacement for the *para*-substituted derivative **5a** (K_Is = 5.9 nM) resulted in a seven-fold efficacy enhancement (enaminones **6i** and **6j**; K_Is = 0.84 and 0.89 nM, respectively).

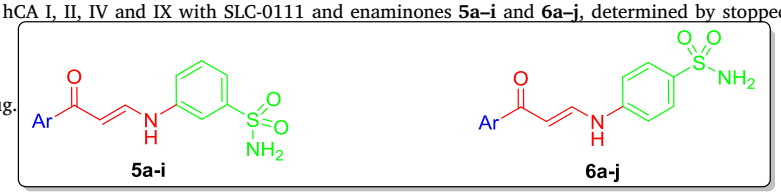
(**iii**) The *in vitro* kinetic data reported in Table 2 showed that inhibition of *trans*-membrane isoform hCA IV by the prepared enaminones was ranged from weak to potent inhibition, with K_I values ranging from 6.5 to 6140.8 nM, except compound **6a** (K_I > 10000 nM). Interestingly, enaminones **5h**, **5i**, **6i** and **6j**, possessing 2-furyl/thienyl tail, displayed potent inhibitory activity against hCA IV (K_Is = 64.5, 8.7, 22.40 and 6.5 nM, respectively), hinting that replacement of the phenyl tail with the 2-furyl or 2-thienyl tails is advantageous for inhibitory activity toward hCA IV. Moreover, sulphonamides **5f** and **6g**, possessing 4-nitrophenyl tail, beside **6h** effectively inhibited hCA IV with inhibition constants equals 80.70, 56.30 and 64.2 nM, respectively.

(**iv**) The tumor-associated isoform hCA IX was potently inhibited by all enaminones reported here. The hCA IX inhibition profile was found to be rather flat, since the measured K_Is ranged between 0.21 and 1.5 nM, aside from enaminones **5b** and **5c** whose efficacy raised at slightly higher concentrations (K_Is = 7.1 and 7.0 nM, respectively). Superiorly, all the investigated enaminones emerged as subnanomolar hCA IX inhibitors (K_Is: 0.21–0.31 nM), except sulfonamides **5b**, **5c** and **5g** displayed single-digit nanomolar inhibitory activity towards hCA IX (K_Is: 1.5–7.1 nM). All the examined enaminones **5a–i** and **6a–j** were 6–21 times more potent than SLC-0111 (K_I = 45 nM). It is worth noting that a decrease of potency (2.7–33 folds) was obtained upon shifting the sulphonamide group from *para*-position to the *meta*-position.

Table 1
Crystallographic data and refinements for enaminone **5a**.

Crystal data	**5a**
Chemical formula	$C_{15}H_{14}N_2O_3S$
Mr	302.34
Crystal system, space group	Monoclinic, $P2_1$
Temperature (K)	293
a, *b*, *c* (Å)	5.2360 (5), 9.4977 (8), 14.1960 (14)
β (°)	98.628 (5)
V (Å3)	697.98 (11)
Z	2
Radiation type	Mo *Kα*
μ (mm^{-1})	0.24
Crystal size (mm)	0.21 × 0.12 × 0.05
Data collection	
Diffractometer	Bruker APEX-II D8 venture diffractometer
Absorption correction	Multi-scan SADABS Bruker 2014
Tmin, Tmax	0.951, 0.987
No. of measured, independent and observed [I > 2σ(I)] reflections	27,807, 5834, 2695
R_{int}	0.145
Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.076, 0.189, 0.98
No. of reflections	5834
No. of parameters	202
No. of restraints	1
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e $Å^{-3}$)	0.56, −0.34
Flack parameter	0.14 (11)

Table 2
Inhibition data of human CA isoforms hCA I, II, IV and IX with SLC-0111 and enaminones **5a–i** and **6a–j**, determined by stopped-flow CO_2 hydrase assay, using acetazolamide (AAZ) as a standard drug.



Comp.	Ar	K_I (nM)*			
		hCA I	hCA II	hCA IV	hCA IX
5a	C_6H_5	626.7	21.70	1355.80	0.90
5b	4-ClC_6H_4	2985	29.20	4391.50	7.10
5c	4-BrC_6H_4	3803	183.20	6140.80	7.00
5d	4-$CH_3C_6H_4$	693	6.70	2616.80	0.82
5e	4-$CH_3OC_6H_4$	916	265.50	5266.70	0.96
5f	4-$NO_2C_6H_4$	9095	190.40	80.70	0.90
5g	naphthalen-2-yl	8143	344.40	793.0	1.50
5h	furan-2-yl	155.6	34.60	64.5	0.73
5i	thiophen-2-yl	566.5	76.60	8.7	0.77
6a	C_6H_5	68.80	5.90	< 10,000	0.22
6b	4-FC_6H_4	72.40	0.48	772.40	0.27
6c	4-ClC_6H_4	323.10	8.70	874.10	0.22
6d	4-BrC_6H_4	7888	88.90	893.40	0.21
6e	4-$CH_3C_6H_4$	30.80	0.53	798.60	0.21
6f	4-$CH_3OC_6H_4$	70.90	6.30	589.0	0.23
6g	4-$NO_2C_6H_4$	< 10,000	464.40	56.30	0.31
6h	naphthalen-2-yl	< 10,000	472.80	64.20	0.28
6i	furan-2-yl	8.50	0.84	22.40	0.27
6j	thiophen-2-yl	9.30	0.89	6.50	0.22
SLC-0111	–	5080	960.0	286.0	45.0
AAZ	–	250	12.10	74.0	25.80

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

(**v**) As a result of the inhibitory trends extrapolated from the data in Table 2, interesting selectivity index (SI) arose for all enaminones herein reported (Table 3). Interestingly, all the examined sulfonamides **5a–i** and **6a–j** displayed interesting selectivity towards hCA IX over hCA I (SI: 32 – > 35714), hCA II (SI: 2 – 1689) and hCA IV (SI: 11 – > 45454). Of particular interest, bioisosteric replacement of phenyl tail with the the bulkier 2-naphthyl tail, sulfonamide **6h**, achieved the higher II/IX selectivity herein reported with SI of 1689 (Table 3).

As discussed above, the *Z* configuration around $H_aC = CH_b$ resulted in an intra-molecular hydrogen bond between NH and C=O groups, affording stable intra-molecular five-membered ring. It's suggested that this intra-molecular ring furnished a rotational restriction with a controlled degree for flexibility of the tail, which in turn led to a preferential interaction within the hCAs active sites resulting in an improvement for CAs selectivity.

Table 3
Selectivity ratios for the inhibition of hCA IX over hCA I, II and hCA IV for enaminones **5a–i** and **6a–j** reported in the paper, SLC-0111 and acetazolamide.

Cpd.	Selectivity ratio						
	I/IX	II/IX	IV/IX	Cpd.	I/IX	II/IX	IV/IX
5a	696	24.1	1506	**6a**	313	27	< 45,454
				6b	268	2	2861
5b	420	4	619	**6c**	1469	40	3973
5c	543	26	877	**6d**	37,561	**423**	4254
5d	846	8	3191	**6e**	147	3	3803
5e	959	**277**	5486	**6f**	308	27	2561
5f	10,106	**212**	90	**6g**	< 32,258	**1498**	182
5g	5429	**230**	529	**6h**	< 35,714	**1689**	229
5h	213	47	88	**6i**	32	3	83
5i	736	100	11	**6j**	42	4	30
AAZ	9.7	0.5	2.9	**SLC-0111**	112.9	21.3	6.4

3. Conclusion

In summary, this study reports the design and synthesis of two series of 3/4-(3-aryl-3-oxopropenyl) aminobenzenesulfonamides **5a–i** and **6a–j** as SLC-0111 enaminone congeners. The synthesized derivatives incorporated an enaminone moiety, instead of the ureido moiety in SLC-0111, as a linker between the sulfonamide hCA inhibitor (CAI) warhead and the molecular tail. Furthermore two structural modifications were introduced. The first modification focuses on replacement on 4-fluorophenyl tail of SLC-0111 with different phenyl, 4-substituted-phenyl, the bulky 2-naphthyl, and the small 5-membered heterocycles (2-furyl and 2-thienyl). The second modification includes development of the regioisomers, through moving the sulfonamide (-SO_2NH_2) functionality from the *para*-position to the *meta*-position. The prepared enaminones were *in vitro* investigated as inhibitors of the metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1) isoforms hCA I, II, IV and IX, using a stopped-flow CO_2 hydrase assay. All the examined isoforms were inhibited by the sulfonamides reported here in variable degrees; hCA I was inhibited with K_Is in the range of 8.5–9095 nM, hCA II in the range of 0.48–472.8 nM; hCA IV in the range of 6.5–6140.8 nM, whereas hCA IX in the range of 0.21–7.1 nM. Superiorly, all the investigated enaminones emerged as subnanomolar hCA IX inhibitors (K_Is: 0.21–0.31 nM), except sulfonamides **5b**, **5c** and **5g** displayed single-digit nanomolar inhibitory activity towards hCA IX (K_Is: 1.5–7.1 nM). All sulfonamides **5a–i** and **6a–j** displayed interesting selectivity towards hCA IX over hCA I (SI: 32 – > 35714), hCA II (SI: 2 – 1689) and hCA IV (SI: 11 – > 45454). Of particular interest, bioisosteric replacement of phenyl tail with the the bulkier 2-naphthyl tail, sulfonamide **6h**, achieved the highest II/IX selectivity herein reported with SI of 1689.

4. Experimental

4.1. Chemistry

4.1.1. General

Melting points were measured with a Stuart melting point apparatus and were uncorrected. Infrared spectra were recorded on Schimadzu FT-IR 8400S spectrophotometer. The NMR spectra were recorded by Bruker spectrophotometer at 400 MHz. ^{13}C NMR spectra were run at 100 MHz in deuterated dimethylsulfoxide (DMSO-d_6). Chemical shifts (δ_H) are reported relative to TMS as internal standard. All coupling constant (J) values are given in hertz. Chemical shifts (δ_C) are reported relative to DMSO-d_6 as internal standards. High-resolution mass spectra were recorded using a Bruker MicroTOF spectrometer. Elemental analyses were carried out at the Regional Center for Microbiology and Biotechnology, Al-Azhar University, Cairo, Egypt. Unless otherwise noted, all solvents and reagents were commercially available and used without further purification.

4.1.2. General procedure for preparation of enaminone intermediates **3a–j**

Compounds **3a–j** were prepared as reported [32,33].

4.1.3. General procedure for preparation of target sulfonamides **5a–i** and **6a–j**

The appropriate enaminone intermediate **3a–j** (1 mmol) was added to a hot stirred solution of *p*- or *m*-aminobenzenesulfonamide **4a, b** (0.17 gm, 1 mmol) in glacial acetic acid (15 mL). The reaction mixture was heated under reflux for 4 h. The formed solid upon cooling was filtered off, washed with cold methanol, dried and recrystallized from dioxane to afford the target enaminones **5a–s**.

*4.1.3.1. 3-((3-Oxo-3-phenylprop-1-en-1-yl)amino)benzenesulfonamide (**5a**).* Yellow crystals (yield 65%), m.p. 175–177 °C; IR (KBr, ν cm^{-1}): 3122–3395 broad band (NH, NH_2), 1728 (C=O), 1300, 1155 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 6.23 (d, 2/3H, Ar-H, J = 8 Hz), 6.53 (d, 1/3H, Ar-H, J = 12.8 Hz), 7.37–7.66 (m, 8H, 6H of Ar-H and 2H of NH_2, D_2O exchangeable), 7.78 (s, 1H, Ar-H), 7.91–8.01 (m, 2.73H, Ar-H), 8.12 (t, 1/3H, Ar-H, J = 12.4 Hz), 10.38 (d, ½ H, NH exchanged with D_2O, J = 12.4 Hz), 12.12 (d, ½ H, NH exchanged with D_2O, J = 12.4 Hz); ^{13}C NMR (DMSO-d_6,100 MHz)δ *ppm*: 94.78, 99.21 (−CO−CH=CH−, cis and trans conformers), 112.58, 113.68, 119.24, 119.42, 119.83, 120.45, 127.73 (2C), 127.77 (2C), 129.04 (2C), 129.11 (2C), 130.85 (2C), 132.22, 132.46, 138.90, 139.66, 141.13, 142.09, 144.09, 145.70, 145.97, 146.05, 188.08, 190.42 (C=O, cis and trans conformers); MS *m/z* [%]: 302 [M$^+$, 13.99], 301 [100]; Anal. Calcd. for $C_{15}H_{14}N_2O_3S$ (302.35): C, 59.59; H, 4.67; N, 9.27; found C, 59.72; H, 4.70; N, 9.40.

*4.1.3.2. 3-((3-(4-Chlorophenyl)-3-oxoprop-1-en-1-yl)amino) benzenesulfonamide (**5b**).* Yellow crystals (yield 74%), m.p. 156–157 °C; IR (KBr, ν cm^{-1}): 3122–3392 broad band (NH, NH_2), 1724 (C=O), 1300, 1160 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 6.23 (d, 1/3H, Ar-H, J = 8 Hz), 6.53 (d, 2/3H, Ar-H, J = 12 Hz), 7.37–7.66 (m, 7H, 5H of Ar-H and 2H of NH_2, D_2O exchangeable), 7.76 (s, 1H, Ar-H), 7.90–8.02 (m, 2.73H, Ar-H), 8.12 (t, 1/3H, Ar-H, J = 12.4 Hz), 10.44 (d, ½ H, NH exchanged with D_2O, J = 12 Hz), 12.09 (d, ½ H, NH exchanged with D_2O, J = 12 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz)δ *ppm*: 94.49, 98.77 (−CO−CH=CH−, cis and trans conformers), 112.66, 113.77, 119.32, 119.56, 119.97, 120.59, 129.13, 129.20, 129.64, 129.67, 130.87, 136.99, 137.31, 137.54, 138.29, 140.99, 141.92, 144.58, 145.93, 146.01, 146.23 (Aromatic carbons), 186.74, 188.91 (C=O, cis and trans conformers); HRMS (ESI) for $C_{15}H_{14}ClN_2O_3S$, calcd 337.04082, found 337.04088 [M+H]$^+$; Anal. Calcd. for $C_{15}H_{13}ClN_2O_3S$ (336.79): C, 53.49; H, 3.89; N, 8.32; found C, 53.65; H, 3.79; N, 8.50.

*4.1.3.3. 3-((3-(4-Bromophenyl)-3-oxoprop-1-en-1-yl)amino) benzenesulfonamide (**5c**).* Beige crystals (yield 73%), m.p. 211–213 °C; IR (KBr, ν cm^{-1}): 3125–3390 broad band (NH, NH_2), 1720 (C=O), 1307, 1154 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 6.20 (d, ½ H, Ar-H, J = 7.6 Hz), 6.52 (d, ½ H, Ar-H, J = 12.4 Hz), 7.04 (d, 2H, Ar-H, J = 7.2 Hz), 7.36–7.56 (m, 5H, 3H of Ar-H and 2H of NH_2, D_2O exchangeable), 7.64 (s, ½ H, Ar-H), 7.73 (s, ½ H, Ar-H), 7.84–7.99 (m, 2.5H, Ar-H), 8.16 (br s, ½ H, Ar-H), 10.27 (d, ½ H, NH exchanged with D_2O, J = 10 Hz), 12.09 (d, ½ H, NH exchanged with D_2O, J = 11.6 Hz); MS *m/z* [%]: 383 [M$^+$+2, 50.95], 381 [M$^+$, 56.26], 332 [100]; Anal. Calcd. for $C_{15}H_{13}BrN_2O_3S$ (381.24): C, 47.26; H, 3.44; N, 7.35; found C, 47.32; H, 3.61; N, 7.49.

*4.1.3.4. 3-((3-Oxo-3-(p-tolyl)prop-1-en-1-yl)amino)benzenesulfonamide (**5d**).* Yellow crystals (yield 76%), m.p. 270–271 °C; IR (KBr, ν cm^{-1}): 3127–3396 broad band (NH, NH_2), 1725 (C=O), 1302, 1151 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.38 (s, 3H, CH_3), 6.21 (d, 1/3H, Ar-H, J = 8 Hz), 6.50 (d, 2/3H, Ar-H, J = 12 Hz), 7.31–7.63 (m, 7.5H, 5.5H of Ar-H and 2H of NH_2, D_2O exchangeable), 7.74 (s, ½ H, Ar-H), 7.80 (d, 1H, Ar-H, J = 8 Hz), 7.88–7.93 (m, 1.5H, Ar-H), 8.13 (t, ½ H, Ar-H, J = 12 Hz), 10.33 (d, ½ H, NH exchanged with D_2O, J = 12 Hz), 12.09 (d, ½ H, NH exchanged with D_2O, J = 12 Hz); ^{13}C NMR (DMSO-d_6,100 MHz)δ *ppm*: 2 (21.55) (CH_3, cis and trans conformers), 94.71, 99.15 (−CO−CH=CH−, cis and trans conformers), 112.39, 113.49, 119.14, 119.26, 119.73, 120.28, 127.84, 127.88, 129.60, 129.69, 130.85, 136.27, 136.99, 141.17, 142.14, 142.34, 142.66, 143.70, 145.34, 145.93, 146.01 (Aromatic carbons), 187.64, 190.17 (C=O, cis and trans conformers); HRMS (ESI) for $C_{16}H_{17}N_2O_3S$, calcd 317.09544, found 317.09525 [M+H]$^+$; Anal. Calcd. for $C_{16}H_{16}N_2O_3S$ (316.37): C, 60.74; H, 5.10; N, 8.85; found C, 61.01; H, 5.03; N, 8.97.

*4.1.3.5. 3-((3-(4-Methoxyphenyl)-3-oxoprop-1-en-1-yl)amino) benzenesulfonamide (**5e**).* Yellow crystals (yield 63%), m.p. 200–201 °C; IR (KBr, ν cm^{-1}): 3121–3392 broad band (NH, NH_2), 1723 (C=O), 1304, 1155 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 3.84 (s, 3H, OCH_3), 6.20 (d, ½ H, Ar-H, J = 7.2 Hz), 6.52 (d, ½ H, Ar-H, J = 12.4 Hz), 7.04 (d, 2H, Ar-H, J = 7.6 Hz), 7.36–7.56 (m, 5H, 3H of Ar-H and 2H of NH_2, D_2O exchangeable), 7.64 (s, ½ H, Ar-H), 7.73 (s, ½ H, Ar-H), 7.84–7.99 (m, 2.5H, Ar-H), 8.12 (t, ½ H, Ar-H, J = 12 Hz), 10.27 (d, ½ H, NH exchanged with D_2O, J = 12 Hz), 12.08 (d, ½ H, NH exchanged with D_2O, J = 11.6 Hz); ^{13}C NMR (DMSO-d_6,100 MHz)δ *ppm*: 55.87, 55.90 (OCH_3), 94.65, 99.06 (−CO−CH=CH−, cis and trans conformers), 112.35, 113.40, 114.25 (2C), 114.34 (2C), 119.07, 119.15, 119.57, 120.14, 129.90 (2C), 130.81 (2C), 130.84 (2C), 131.58, 132.24, 141.27, 142.26, 143.28, 144.84, 145.96, 146.04, 162.65, 162.85, 186.72, 189.49 (C=O, cis and trans conformers); MS *m/z* [%]: 331 [M$^+$, 61.03], 332 [100]; Anal. Calcd. for $C_{16}H_{16}N_2O_4S$ (332.37): C, 57.82; H, 4.85; N, 8.43; found C, 57.82; H, 4.79; N, 8.50.

*4.1.3.6. 3-((3-(4-Nitrophenyl)-3-oxoprop-1-en-1-yl)amino) benzenesulfonamide (**5f**).* Yellow crystals (yield 68%), m.p. 195–197 °C; IR (KBr, ν cm^{-1}): 3126–3397 broad band (NH, NH_2), 1728 (C=O), 1309, 1150 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 6.26 (d, 1/3H, Ar-H, J = 7.6 Hz), 6.48 (d, 2/3H, Ar-H, J = 12.4 Hz), 7.37–7.68 (m, 5.33H, 3.33H of Ar-H and 2H of NH_2, D_2O exchangeable), 7.81 (s, 2/3H, Ar-H), 8.02–8.34 (m, 5H, Ar-H), 10.59 (d, ½ H, NH exchanged with D_2O, J = 12.4 Hz), 12.16 (d, ½ H, NH exchanged with D_2O, J = 12.4 Hz); ^{13}C NMR (DMSO-d_{6100} MHz)δ *ppm*: 94.87, 99.07 (−CO−CH=CH−, cis and trans conformers), 113.07, 114.19, 119.53, 119.97, 120.28, 121.05, 123.83, 124.25 (2C), 129.02 (2C), 129.06 (2C), 130.88 (2C), 140.77, 141.68, 144.00, 144.94, 145.60, 145.97, 146.05, 147.29, 149.50, 149.65, 186.45, 188.02 (C=O, cis and trans conformers); MS *m/z* [%]: 347 [M$^+$, 96.23], 346 [100]; Anal. Calcd. for $C_{15}H_{13}N_3O_5S$ (347.35): C, 51.87; H, 3.77; N, 12.10; found C, 52.03; H, 3.78; N, 12.17.

*4.1.3.7. 3-((3-(Naphthalen-2-yl)-3-oxoprop-1-en-1-yl)amino) benzenesulfonamide (**5g**).* Yellow crystals (yield 64%), m.p. 183–185 °C; IR (KBr, ν cm^{-1}): 3120–3391 broad band (NH, NH_2), 1722 (C═O), 1303, 1154 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 6.24 (d, ½ H, Ar-H, J = 8 Hz), 6.69 (d, ½ H, Ar-H, J = 12 Hz), 7.37–7.45 (m, 3H, 1H of Ar-H and 2H of NH_2, D_2O exchangeable), 7.51–7.67 (m, 4.5H, Ar-H), 7.78 (s, ½ H, Ar-H), 7.79–8.15 (m, 4.5H, Ar-H), 8.22 (t, ½ H, Ar-H, J = 12 Hz), 8.52 (s, ½ H, Ar-H), 8.66 (s, ½ H, Ar-H), 10.44 (d, ½ H, NH exchanged with D_2O, J = 12 Hz), 12.18 (d, ½ H, NH exchanged with D_2O, J = 12 Hz); ^{13}C NMR (DMSO-d_6 100 MHz)δ *ppm*: 95.00, 99.41 (−CO−CH═CH−, cis and trans conformers), 112.54, 113.62, 119.25, 119.40, 119.88, 120.44, 124.18, 124.52, 127.18, 127.21, 128.07, 128.39, 128.47, 128.54, 128.63, 128.69, 128.72, 129.82, 129.87, 130.88, 132.81, 132.86, 134.96, 135.03, 136.18, 136.98, 141.13, 142.09, 144.05, 145.67, 145.95, 146.03 (Aromatic carbons) 187.91, 190.24 (C═O, cis and trans conformers); HRMS (ESI) for $C_{19}H_{17}N_2O_3S$, calcd 353.09544, found 353.09537 $[M+H]^+$; Anal. Calcd. for $C_{19}H_{16}N_2O_3S$ (352.40): C, 64.76; H, 4.58; N, 7.95; found C, 64.54; H, 4.67; N, 8.10.

*4.1.3.8. 3-((3-(Furan-2-yl)-3-oxoprop-1-en-1-yl)amino) benzenesulfonamide (**5h**).* Yellow crystals (yield 70%), m.p. 195–197 °C; IR (KBr, ν cm^{-1}): 3125–3396 broad band (NH, NH_2), 1727 (C═O), 1308, 1159 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 6.00 (d, 1/3H, Ar-H, J = 7.6 Hz), 6.37 (d, 2/3H, Ar-H, J = 12.8 Hz), 6.69 (s, 1H, Ar-H), 7.22 (s, 1H, Ar-H), 7.33–7.56 (m, 5H, 3H of Ar-H and 2H of NH_2, D_2O exchangeable), 7.64 (s, 2/3H, Ar-H), 7.72 (s, 1/3H, Ar-H), 7.86–7.94 (m, 1.33H, Ar-H), 8.12 (t, 2/3H, Ar-H, J = 12.8 Hz), 10.35 (d, ½ H, NH exchanged with D_2O, J = 12.8 Hz), 11.77 (d, ½ H, NH exchanged with D_2O, J = 12.4 Hz); ^{13}C NMR (DMSO-d_6 100 MHz) δ *ppm*: 94.81, 99.00 (−CO−CH═CH−, cis and trans conformers), 112.53, 112.82, 112.96, 113.53, 115.29, 115.81, 119.24, 119.42, 119.67, 120.38, 130.83 (2C), 141.08, 142.00, 143.15, 145.34, 145.95, 146.02, 146.55, 147.04, 153.41, 154.25, 177.11, 179.55 (C═O, cis and trans conformers); MS *m/z* [%]: 292 [M^+, 41.49], 184.49 [100]; Anal. Calcd. for $C_{13}H_{12}N_2O_4S$ (292.31): C, 53.42; H, 4.14; N, 9.58; found C, 53.08; H, 4.22; N, 9.65.

*4.1.3.9. 3-((3-Oxo-3-(thiophen-2-yl)prop-1-en-1-yl)amino) benzenesulfonamide (**5i**).* Yellow crystals (yield 65%), m.p. 208–210 °C; IR (KBr, ν cm^{-1}): 3120–3391 broad band (NH, NH_2), 1722 (C═O), 1303, 1154 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 6.12 (d, 1/3H, Ar-H, J = 8 Hz), 6.41 (d, 2/3H, Ar-H, J = 12.0 Hz), 7.21 (m, 1H, Ar-H), 7.35–7.43 (m, 4H, 2H of Ar-H and 2H of NH_2, D_2O exchangeable), 7.49–7.56 (m, 1.5H, Ar-H), 7.63 (s, ½ H, Ar-H), 7.73 (s, 1/3H, Ar-H), 7.77 (br s, 2/3H, Ar-H), 7.85–7.92 (m, 1.33H, Ar-H), 8.11 (t, 2/3H, Ar-H, J = 12 Hz), 10.38 (d, ½ H, NH exchanged with D_2O, J = 12 Hz), 11.77 (d, ½ H, NH exchanged with D_2O, J = 12 Hz); ^{13}C NMR (DMSO-d_6 100 MHz) δ *ppm*: 94.88, 99.01 (−CO−CH═CH−, cis and trans conformers), 112.47, 113.47, 119.25, 119.39, 119.72, 120.33, 128.98, 129.14, 130.30, 130.78, 130.85, 133.07, 133.68, 141.09, 142.00, 143.34, 145.13, 145.92, 145.99, 146.27, 147.00, 180.80, 183.66 (C═O, cis and trans conformers); HRMS (ESI) for $C_{13}H_{13}N_2O_3S_2$, calcd 309.03621, found 309.03589 $[M+H]^+$; Anal. Calcd. for $C_{13}H_{12}N_2O_3S_2$ (308.37): C, 50.63; H, 3.92; N, 9.08; found C, 50.89; H, 3.98; N, 9.13.

*4.1.3.10. 4-((3-Oxo-3-phenylprop-1-en-1-yl)amino)benzenesulfonamide (**6a**).* Yellow crystals (yield 70%), m.p. 223–225 °C (reported: 220–222 °C [34]); IR (KBr, ν cm^{-1}): 3122–3395 broad band (NH, NH_2), 1724 (C═O), 1300, 1150 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm:* 6.24 (d, ½ H, Ar-H, J = 8 Hz), 6.54 (d, ½ H, Ar-H, J = 16 Hz), 7.26 (s, 2H, NH_2, D_2O exchangeable), 7.31 (d, 1H, Ar-H, J = 8.8 Hz), 7.51–7.59 (m, 4H, Ar-H), 7.77 (t, ½ H, Ar-H, J = 8.8 Hz), 7.89 (d, 2H, Ar-H, J = 7.6 Hz), 7.99 (d, 2H, Ar-H, J = 7.6 Hz), 8.17 (t, ½ H, Ar-H, J = 12.4 Hz), 10.38 (d, ½ H, NH exchanged with D_2O, J = 12 Hz), 12.05 (d, ½ H, NH exchanged with D_2O, J = 12 Hz); ^{13}C NMR (DMSO-d_6,100 MHz) δ *ppm*: 94.96, 99.58 (−CO−CH═CH−, cis and trans conformers), 115.58, 115.87, 115.97, 116.09, 116.19, 116.53, 127.91, 128.10, 130.48, 130.53, 130.57, 130.62, 135.42, 136.14, 137.64, 138.76, 143.34,143.99, 144.29, 145.59, 163.46, 163.67, 165.94 (Aromatic carbons), 186.67,189.04 (C═O, cis and trans conformers); MS *m/z* [%]: 302 [M^+, 31.74], 301 [100]; Anal. Calcd. for $C_{15}H_{14}N_2O_3S$ (302.35): C, 59.59; H, 4.67; N, 9.27; found C, 59.81; H, 4.73; N, 9.44.

*4.1.3.11. 4-((3-(4-Fluorophenyl)-3-oxoprop-1-en-1-yl)amino) benzenesulfonamide (**6b**).* Pale yellow crystals (yield 65%), m.p. 225–227 °C; IR (KBr, ν cm^{-1}): 3120–3390 broad band (NH, NH_2), 1720, (C═O), 1310, 1155 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm:* 6.23 (d, ½ H, Ar-H, J = 8 Hz), 6.50 (d, ½ H, Ar-H, J = 12 Hz), 7.27–7.36 (m, 5H, 3H of Ar-H and 2H of NH_2, D_2O exchangeable), 7.51 (d, 1H, Ar-H, J = 8 Hz), 7.75 (t, 2H, Ar-H, J = 8 Hz), 7.95–7.99 (m, 1.5H, Ar-H), 8.06–8.10 (m, 1H, Ar-H), 8.17 (d, ½ H, Ar-H, J = 12 Hz), 12.0 (d, 1H, NH exchanged with D_2O, J = 12 Hz); HRMS (ESI) for $C_{15}H_{14}FN_2O_3S$, calcd 321.07037, found 321.07037 $[M+H]^+$; Anal. Calcd. for $C_{15}H_{13}FN_2O_3S$ (320.34): C, 56.24; H, 4.09; N, 8.75; found C, 56.41; H, 4.18; N, 8.97.

*4.1.3.12. 4-((3-(4-Chlorophenyl)-3-oxoprop-1-en-1-yl)amino) benzenesulfonamide (**6c**).* Reddish yellow crystals (yield 80%), m.p. 257–259 °C; IR (KBr, ν cm^{-1}): 3122–3395 broad band (NH, NH_2), 1735 (C═O), 1320, 1160 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 6.21 (d, ½ H, Ar-H, J = 8 Hz), 6.49 (d, ½ H, Ar-H, J = 12.8 Hz), 7.27 (s, 2H, NH_2, D_2O exchangeable), 7.31 (d, 1H, Ar-H, J = 10.8 Hz), 7.51 (d, 1H, Ar-H, J = 8.4 Hz), 7.56 (d, 2H, Ar-H, J = 8 Hz), 7.77 (t, ½ H, Ar-H, J = 8.8 Hz), 7.89 (d, 2H, Ar-H, J = 8 Hz), 7.99 (d, 2H, Ar-H, J = 8 Hz), 8.18 (t, ½ H, Ar-H, J = 12.8 Hz), 10.43 (d, ½ H, NH exchanged with D_2O, J = 12.8 Hz), 12.02 (d, ½ H, NH exchanged with D_2O, J = 12.4 Hz); MS *m/z* [%]: 338 [M^+ + 2, 24.15], 336 [M^+, 58.57], 335 [100]; Anal. Calcd. for $C_{15}H_{13}ClN_2O_3S$ (336.79): C, 53.49; H, 3.89; N, 8.32; found C, 53.72; H, 3.95; N, 8.45.

*4.1.3.13. 4-((3-(4-Bromophenyl)-3-oxoprop-1-en-1-yl)amino) benzenesulfonamide (**6d**).* White crystals (yield 74%), m.p. 268–270 °C; IR (KBr, ν cm^{-1}): 3130–3400 broad band (NH, NH_2), 1730 (C═O), 1300, 1160 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 6.22 (d, ½ H, Ar-H, J = 8 Hz), 6.48 (d, ½ H, Ar-H, J = 12 Hz), 7.62–7.33 (m, 3H, 1H of Ar-H and 2H of NH_2, D_2O exchangeable), 7.52 (d, 1H, Ar-H, J = 8 Hz), 7.72–7.84 (m, 5H, Ar-H), 7.93 (d, 1H, Ar-H, J = 8 Hz), 7.98–8.03 (m, ½ H, Ar-H), 8.19 (d, ½ H, Ar-H, J = 12 Hz), 10.47 (s, ½ H, NH exchanged with D_2O), 12.02 (d, ½ H, NH exchanged with D_2O, J = 12 Hz); ^{13}C NMR (DMSO-d_6,100 MHz)δ *ppm*: 94.90, 99.52, 115.68, 116.64, 126.08, 126.49, 127.90, 128.09, 129.84, 129.87, 132.10, 132.17, 137.75, 137.83, 138.61, 138.89, 143.26, 144.20, 144.33, 145.94, 187.01 (Aromatic carbons), 189.19 (C═O); HRMS (ESI) for $C_{15}H_{14}BrN_2O_3S$, calcd 380.99030, found 380.99020 $[M+H]^+$; Anal. Calcd. for $C_{15}H_{13}BrN_2O_3S$ (381.24): C, 47.26; H, 3.44; N, 7.35; found C, 47.08; H, 3.41; N, 7.45.

*4.1.3.14. 4-((3-Oxo-3-(p-tolyl)prop-1-en-1-yl)amino)benzenesulfonamide (**6e**).* Buff crystals (yield 65%), m.p. 240–242 °C; IR (KBr, ν cm^{-1}); 3125–3395 broad band (NH, NH_2), 1728 (C═O), 1315, 1150 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 2.38 (s, 3H, CH_3), 6.21 (d, ½ H, Ar-H, J = 8.4 Hz), 6.53 (d, ½ H, Ar-H, J = 12.4 Hz), 7.25 (s, 2H, NH_2, D_2O exchangeable), 7.29–7.33 (m, 3H, Ar-H), 7.49 (d, 1H, Ar-H, J = 8.8 Hz), 7.76–7.82 (m, 2.5H, Ar-H), 7.89–7.96 (m, 2H, Ar-H), 8.14 (t, ½ H, Ar-H, J = 12.8 Hz), 10.32 (d, ½ H, NH exchanged with D_2O, J = 12.8 Hz), 12.03 (d, ½ H, NH exchanged with D_2O, J = 12 Hz); ^{13}C NMR (DMSO-d_6,100 MHz)δ *ppm*: 21.54, 21.55 (CH_3), 95.22, 100.00 (−CO−CH═CH−, cis and trans conformers), 115.44 (2C), 116.35 (2C), 127.87 (2C), 127.91 (2C), 127.95 (2C), 128.13 (2C), 129.61 (2C),

129.70 (2C), 136.26, 137.00, 137.48, 138.60, 142.38, 142.74, 143.39, 143.47, 144.46, 144.98, 187.76, 190.32 (C=O, cis and trans conformers); MS *m/z* [%]: 316 [M^+, 28.81], 301 [100]; Anal. Calcd. for $C_{16}H_{16}N_2O_3S$ (316.38): C, 60.74; H, 5.10; N, 8.85; found C, 60.98; H, 5.13; N, 8.99.

*4.1.3.15. 4-((3-(4-Methoxyphenyl)-3-oxoprop-1-en-1-yl)amino) benzenesulfonamide (**6f**).* Yellow crystals (yield 68%), m.p. 250–252 °C; IR (KBr, ν cm^{-1}): 3127–3395 broad band (NH, NH_2), 1724 (C=O), 1310, 1155 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 3.81 (s, 3H, OCH_3), 6.18 (d, ½ H, Ar-H, J = 8 Hz), 6.50 (d, ½ H, Ar-H, J = 16 Hz), 7.01 (d, 2H, Ar-H, J = 8 Hz), 7.20 (s, 2H, NH_2, D_2O exchangeable), 7.24 (d, 2H, Ar-H, J = 12 Hz), 7.44 (d, 1H, Ar-H, J = 8 Hz), 7.71 (t, 1.5H, Ar-H, J = 8 Hz), 7.85–7.90 (m, 1H, Ar-H), 7.95 (d, 1H, Ar-H, J = 8 Hz), 8.08 (t, ½ H, Ar-H, J = 12 Hz), 10.24 (d, ½ H, NH exchanged with D_2O, J = 12 Hz), 11.98 (d, ½ H, NH exchanged with D_2O, J = 12 Hz); MS *m/z* [%]: 332 [M^+, 29.04], 331 [100]; Anal. Calcd. for $C_{16}H_{16}N_2O_4S$ (332.37): C, 57.82; H, 4.85; N, 8.43; found C, 57.60; H, 4.97; N, 8.50.

*4.1.3.16. 4-((3-(4-Nitrophenyl)-3-oxoprop-1-en-1-yl)amino) benzenesulfonamide (**6g**).* Yellow crystals (yield 70%), m.p. 235–237 °C; IR (KBr, ν cm^{-1}): 3122–3380 broad band (NH, NH_2), 1720 (C=O), 1300, 1150 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 6.27 (d, ½ H, Ar-H, J = 8 Hz), 6.49 (d, ½ H, Ar-H, J = 12.8 Hz), 7.27–7.37 (m, 3H, 1H of Ar-H and 2H of NH_2, D_2O exchangeable), 7.56 (d, 1H, Ar-H, J = 8.4 Hz), 7.77–7.82 (m, 2H, Ar-H), 8.06–8.10 (m, 1.5H, Ar-H), 8.19–8.35 (m, 3.5H, Ar-H), 10.59 (d, ½ H, NH exchanged with D_2O, J = 12.8 Hz), 12.08 (d, ½ H, NH exchanged with D_2O, J = 12.8 Hz); ^{13}C NMR (DMSO-d_6,100 MHz)δ *ppm*: 95.29, 99.88, (−CO−CH=CH−, cis and trans conformers), 115.99 (2C), 117.01 (2C), 123.84 (2C), 124.27 (2C), 127.91 (2C), 128.09 (2C), 129.07 (2C), 129.09 (2C), 138.05, 139.42, 143.03, 143.94, 143.95, 144.91, 145.36, 146.97, 149.52, 149.71, 186.66, 188.20 (C=O, cis and trans conformers); MS *m/z* [%]: 347 [M^+, 33.42], 346 [100]; Anal. Calcd. for $C_{15}H_{13}N_3O_5S$ (347.35): C, 51.87; H, 3.77; N, 12.10; found C, 52.04; H, 3.80; N, 12.15.

*4.1.3.17. 4-((3-(Naphthalen-2-yl)-3-oxoprop-1-en-1-yl)amino) benzenesulfonamide (**6h**).* White crystals (yield 73%), m.p. 271–272 °C; IR (KBr, ν cm^{-1}): 3120–3380 broad band (NH, NH_2), 1721 (C=O), 1300, 1150 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 6.40 (d, ½ H, Ar-H, J = 8 Hz), 6.68 (d, ½ H, Ar-H, J = 12.8 Hz), 7.22 (s, 2H, NH_2, D_2O exchangeable), 7.27 (s, 1H, Ar-H), 7.30 (d, 1H, Ar-H, J = 8 Hz), 7.51 (d, 1H, Ar-H, J = 8 Hz), 7.57–7.64 (m, 2H, Ar-H), 7.73–7.79 (m, 2H, Ar-H), 7.95–8.07 (m, 2.5H, Ar-H), 8.10 (d, 1H, Ar-H, J = 8 Hz), 8.18 (t, ½ H, Ar-H, J = 12 Hz), 8.48 (s, ½ H, Ar-H), 8.64 (s, ½ H, Ar-H), 10.40 (d, ½ H, NH exchanged with D_2O, J = 12 Hz), 12.08 (d, ½ H, NH exchanged with D_2O, J = 12 Hz); MS *m/z* [%]: 352 [M^+, 8.22], 303 [100]; Anal. Calcd. for $C_{19}H_{16}N_2O_3S$ (352.41): C, 64.76; H, 4.58; N, 7.95; found C, 64.90; H, 4.57; N, 8.01.

*4.1.3.18. 4-((3-(Furan-2-yl)-3-oxoprop-1-en-1-yl)amino) benzenesulfonamide (**6i**).* Yellow crystals (yield 81%), m.p. 263–265 °C; IR (KBr, ν cm^{-1}): 3125–3395 broad band (NH, NH_2), 1724 (C=O), 1318, 1150 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 6.01 (d, ½ H, Ar-H, J = 8 Hz), 6.39 (d, ½ H, Ar-H, J = 12.8 Hz), 6.69 (s, 1H, Ar-H), 7.22–7.36 (m, 4H, 2H of Ar-H and 2H of NH_2, D_2O exchangeable), 7.46 (d, 1H, Ar-H, J = 8.4 Hz), 7.75–7.79 (m, 2H, Ar-H), 7.89–7.95 (m, 1.5H, Ar-H), 8.13 (t, ½ H, Ar-H, J = 12.8 Hz), 10.36 (d, ½ H, NH exchanged with D_2O, J = 12.8 Hz), 11.72 (d, ½ H, NH exchanged with D_2O, J = 12 Hz); ^{13}C NMR (DMSO-d_6,100 MHz)δ *ppm*: 95.29, 99.74 (−CO−CH=CH−, cis and trans conformers), 112.84, 113.00, 115.42, 115.53 (2C), 116.02, 116.32 (2C), 127.93 (2C), 128.10 (2C), 137.61, 138.64, 142.86, 143.36, 144.29, 144.99, 146.63, 147.18, 153.36, 154.21, 177.10, 179.58 (C=O, cis and trans conformers); MS *m/z* [%]: 292.31 [M^+, 39.27], 42 [100]; Anal. Calcd. for $C_{13}H_{12}N_2O_4S$ (292.31): C, 53.42; H, 4.14; N, 9.58; found C, 53.19; H, 4.11; N, 9.45.

*4.1.3.19. 4-((3-Oxo-3-(thiophen-2-yl)prop-1-en-1-yl)amino) benzenesulfonamide (**6j**).* Yellow crystals (yield 80%), m.p. 255–257 °C (reported: 254.6 °C [35]); IR (KBr, ν cm^{-1}): 3125–3400 broad band (NH, NH_2), 1720 (C=O), 1300, 1150 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 6.14 (d, ½ H, Ar-H, J = 8 Hz), 6.44 (d, ½ H, Ar-H, J = 12 Hz), 7.22–7.32 (m, 4H, 2H of Ar-H and 2H of NH_2, D_2O exchangeable), 7.48 (d, 1H, Ar-H, J = 8 Hz), 7.75–7.78 (m, 3H, Ar-H), 7.88–7.94 (m, 1.5H, Ar-H), 8.12 (t, ½ H, Ar-H, J = 12 Hz), 10.39 (d, ½ H, NH exchanged with D_2O, J = 12 Hz), 11.72 (d, ½ H, NH exchanged with D_2O, J = 12 Hz); ^{13}C NMR (DMSO-d_6,100 MHz)δ *ppm*: 95.36, 99.76 (−CO−CH=CH−, cis and trans conformers), 115.52, 116.32, 127.90, 128.09, 129.01, 129.17, 130.39, 130.96, 133.18, 133.86, 137.57, 138.58, 143.04, 143.38, 144.29, 144.79, 146.24, 146.94 (Aromatic carbons), 180.81, 183.76 (C=O, cis and trans conformers); HRMS (ESI) for $C_{13}H_{13}N_2O_3S_2$, calcd 309.03621, found 309.03607 $[M+H]^+$; Anal. Calcd. for $C_{13}H_{12}N_2O_3S_2$ (308.37): C, 50.63; H, 3.92; N, 9.08; found C, 50.88; H, 4.01; N, 9.14.

4.2. Single crystal X-ray crystallographic analysis

Enaminone **5a** was obtained as single crystals by slow evaporation from ethanol solution of the pure compound at room temperature. Data were collected on a Bruker APEX-II D8 Venture area diffractometer, equipped with graphite monochromatic Mo Kα radiation, λ = 0.71073 Å at 293 (2) K. Cell refinement and data reduction were carried out by Bruker SAINT. SHELXT [36–38] was used to solve structure. The final refinement was carried out by full-matrix least-squares techniques with anisotropic thermal data for nonhydrogen atoms on F. CCDC 1856635 contains the supplementary crystallographic data for this compound can be obtained free of charge from the Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

4.3. Biological evaluation

4.3.1. CA inhibitory assay

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalysed CO_2 hydration activity, as reported earlier [31]. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3 and the Cheng-Prusoff equation as reported earlier [39], and represent the mean from at least three different determinations. The four tested CA isofoms were recombinant ones obtained in-house as reported earlier [40].

Acknowledgements

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through the Research Group Project no. RG-1439-65.

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