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Novel benzenesulfonamides aryl and arylsulfone conjugates adopting tail/dual tail approaches: Synthesis, carbonic anhydrase inhibitory activity and molecular modeling studies

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ABSTRACT

New series of benzenesulfonamide and benzoic acid derivatives were designed and synthesized using tail/dual tail approach to improve potency and selectivity as carbonic anhydrase inhibitors. The synthesized compounds evaluated as CAIs against isoforms hCA I, II, IV and IX with acetazolamide (**AAZ**) as standard inhibitor. The benzenesulfonamide derivatives **7a-d**, **8a-h**, **12a-c**, **13a** and **15a-c** showed moderate to potent inhibitory activity with selectivity toward isoform hCA II, especially, compound **13a** with ($K_i = 7.6$ nM), while the benzoic acid analogues **12d-f**, **13b** and **15d-f** didn't show any activity except compounds **12d,f** and **15**e that showed weak activity. Additionally, molecular docking was performed for compounds **7a**, **8a**, **8e**, **12a**, **13a** and **15a** on isoform hCA I, II to illustrate the possible interaction with the active site to justify the inhibitory activity.

1. Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) are pervasive metalloenzymes present in prokaryotes and eukaryotes [1]. 16 α-CA isozymes were identified in mammals with various catalytic activity and subcellular localization [2]. Up till now, 15 different isoenzymes of hCAs have been detected. Among these, 12 isoenzymes are catalytically active with different cellular localizations (I-III,VII, and XIII are cytosolic; IV, IX, XII, and XIV are membrane-bounded; VA and VB are mitochondrial; and VI is secreted in milk and saliva), while the CARPs VIII, X, and XI are catalytically inactive [3,4]. The Zn^{2+} active site is essential for acid-base homeostasis^{-} by enzymatically catalyzing the conversion of carbon dioxide to carbonic acid [5,6]. Human CAs are involved in a vast range of physiopathological processes, including electrolytes secretion, pH and CO_2 homeostasis, biosynthetic reactions, bone resorption and oncogenesis, as a result, carbonic anhydrase suppressors are used for management of glaucoma, edema, epilepsy, obesity and tumors [7–14].

Sulphanilamide is one of the most classical classes acting as CAIs [15,16]. During last few years, many approaches was adopted to synthetize potent and selective carbonic anhydrase inhibitors as tail/dual tail approaches[16–18]. In tail approach, sulphanilamide was conjugated with different moieties to increase the interaction with hydrophobic or hydrophilic parts of the active site[19,20]. Compounds **I**–**V** (Fig. 1) were designed as CAIs depending on the last approach[21–24]. While dual tail approach depended on

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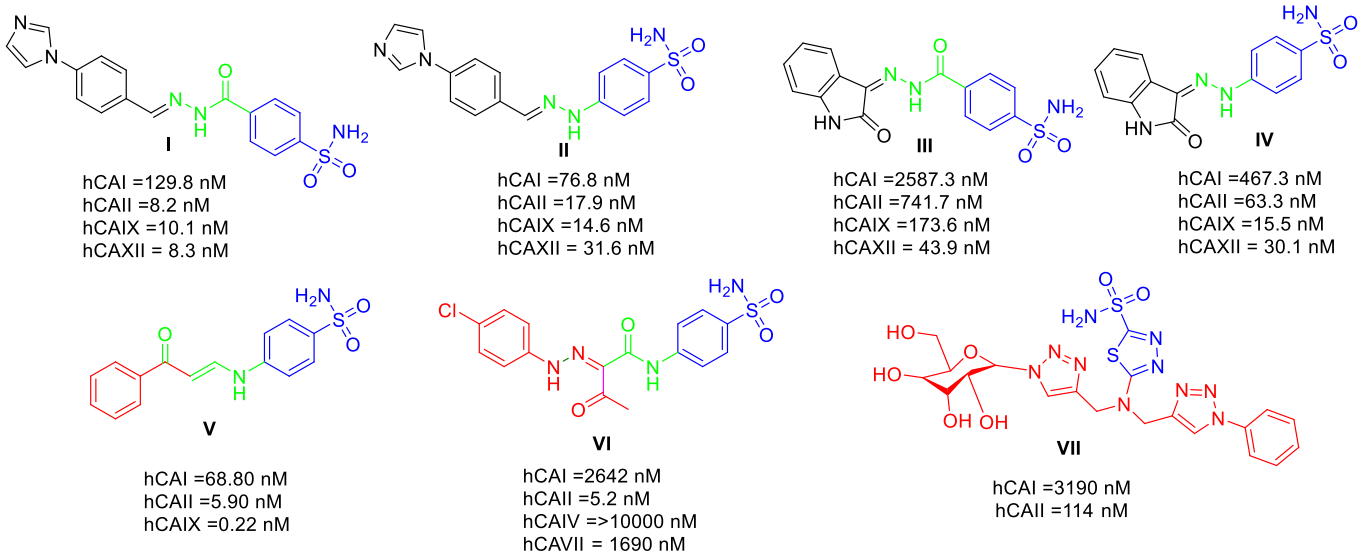


Fig. 1. Structures of some benzenesulfonamides as potent and selective CAIs.

interaction of the ligand with both the hydrophilic and hydrophobic parts of the active site seeking for selectivity and potency as compounds **VI-VII** (Fig. 1)[17,18].

Based on the previous finding, this study presents some sulphanilamide derivatives conjugated with aryl and arylsulfone moieties to synthesize novel potent and selective CAIs.

Firstly, the 4-(hydrazinecarbonyl)benzenesulfonamide **6** was conjugated with different with aryl and arylsulfone moieties to verify their effect on selectivity, then, the phenyl group was grafted with (F, Cl and Br) seeking enhancement the hydrophobic interaction (**7a-d** and **8a-h**). Moreover, SO_2NH_2 group was replaced with COOH moiety as a biological isostere because of its different binding manner to act as CAI to assess its effect (**12d-**f, **13b** and **15d-f**). Additionally, the hydrazino linker replaced the hydrazido to investigate its effect on the inhibitory activity as seen in compounds **I-IV** replacement of hydrazino linker with hydrazido had a great effect on the activity (**12a-f**). Furthermore, a positional replacement carried out for phenyl hydrazine derivatives from the C═O moiety to the active methylene to explore the ability of carbonyl group to bind with the active site (**13a,b**). Finally, the reported enaminone sulphanilamide conjugates **V** showed potent activity with moderate selectivity on isoform IX, so, benzenesulfonyl group were added as a hybrid to increase it (**15a-f**). (Fig. 2).

2. Results and discussion

2.1. Chemistry

The synthetic approaches implemented for design the last targeted products (**7a-d**, **8a-h**, **12a-f**, **13a,b** and **15a-f**) were elucidated in Schemes 1–3. In Scheme 1, various acetophenone derivatives **1a-d** were brominated in AcOH using Br_2 to produce the phenylethyl bromide analogues **2a-d**, where the bromide moiety replaced with sodium benzenesulfinate **3** *via* refluxing in absolute EtOH to furnish the key intermediates 1-phenyl-2-(phenylsulfonyl)ethan-1-one derivatives **4a-d**. The carbonyl moiety of the key intermediates **4a-d** was condensed with 4-(hydrazinecarbonyl)benzenesulfonamide **6** by refluxing in AcOH to produce the final novel compounds **7a-d**. On the other hand, the appropriate analogue of acetophenone or aldehydes **1a-h** was heated under reflux with 4-(hydrazinecarbonyl) benzenesulfonamide **6** in AcOH to furnish the final compounds **8a-h**.

In Scheme 2, sulfanilamide **9a** or PABA **9b** were dissolved in HCl and diatzotized with $NaNO_2$ to get compounds **10a,b** which were reduced by $SnCl_2$ in HCl to furnish 4-hydrazinylbenzenesulfonamide **11a** and 4-hydrazinylbenzoic acid **11b**, respectively. The key intermediates **4a-c** were refluxed with the hydrazinyl compounds **11a,b** in AcOH to produce the targeted compounds **12a-f**.

In Scheme 3, 1-phenyl-2-(phenylsulfonyl)ethan-1-one **4a** was reacted with diazonium salt of sulfanilamide **9a** or PABA **9b** in pyridine at 0–5 °C to furnish the final compounds **13a,b**. on the other hand, the key intermediates **4a-c** were refluxed with DMFDMA in dry xylene to get 3-(dimethylamino)-1-phenyl-2-(phenylsulfonyl)prop-2-en-1-one derivatives **14a-c** where, the dimethylamino group was replaced with sulfanilamide **9a** and PABA **9b** *via* heating in AcOH to furnish the final compounds **15a-f**.

The proposed structure for compounds **7a-d, 8a-h, 12a-f, 13a,b** and **15a-f** were entirely consistent with the data from their spectral and elemental analyses. The ^{1}H NMR spectra of compounds **7a-d**, **8a-h**, **12a-c**, **13a** and **15a-c** were confirmed with appearance of D_2O exchangeable peak of SO_2NH_2 at δ 7.3–7.5 *ppm*, while compounds **7a-d**, **8a-h**, **12a-f**, **13a,b** and **15a-f** revealed with presence of extra D_2O exchangeable peak for NH at δ 10–12 *ppm*. Further, compounds **12d-f**, **13b** and **15d-f** were approved with presence peak of OH acidic at δ 12.4–12.9 *ppm*. Also, compounds **7a-d** and **12a-f** were confirmed with presence of active methylene peak at δ 5.2–5.5 *ppm*, which its absence also, confirm compounds **13a,b** and **15a-f**, while compounds **8a-d** showed aliphatic peak for CH_3 at δ 2.38–2.40 *ppm* that confirm its proposed structure. It is noteworthy that the spectra of enaminones **15a-f** revealed the presence of *E/Z* geometric conjugation forms as previously reported[24].

On the other hand, ^{13}C NMR spectra of compounds **8a-d** confirmed with presence of aliphatic peaks of –CH_3 at δ 14.99–15.22 *ppm*. While compounds **7a-d** and **12a-f** reveled with appearance of presence of active methylene peak at δ 52.17–53.19 *ppm*. Further, compounds **7a-d** and **8a-h** were approved with presence C═O peak of hydrazide linker at δ 162.75-163.87 *ppm*, also, compounds **13a,b** and **15a-f** showed C═O of 1-phenyl-2-(phenylsulfonyl)ethan-1-one derivatives at δ 186.84–191.35 *ppm*, in addition, compounds **12d-f, 13b** and **15d-f** approved with presence of C═O of COOH at δ 167.11–167.80 *ppm*.

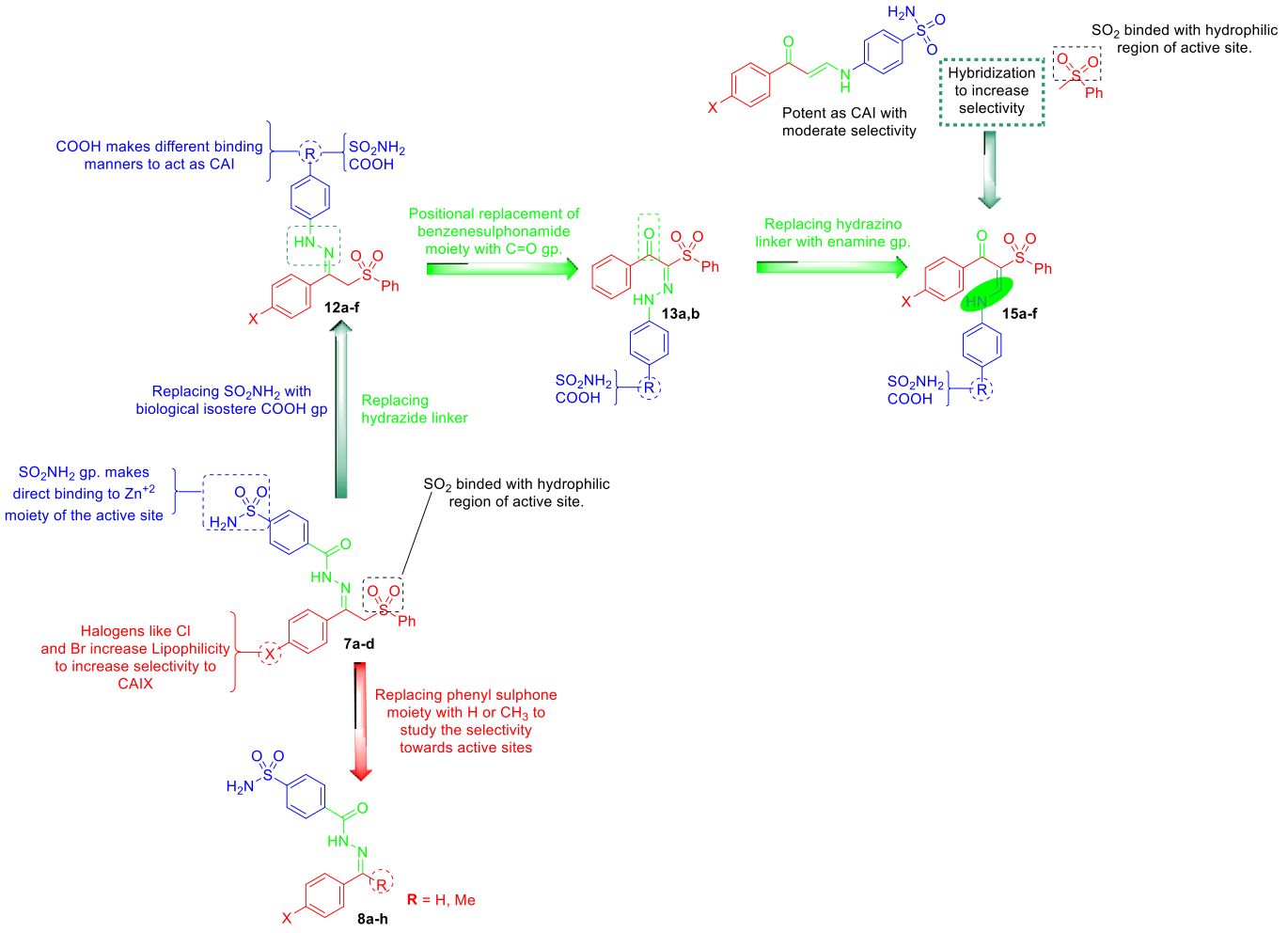


Fig. 2. Design and structures of the targeted compounds **7a-d, 8a-h, 12a-f, 13a,b** and **15a-f**.

2.2. Biological evaluation

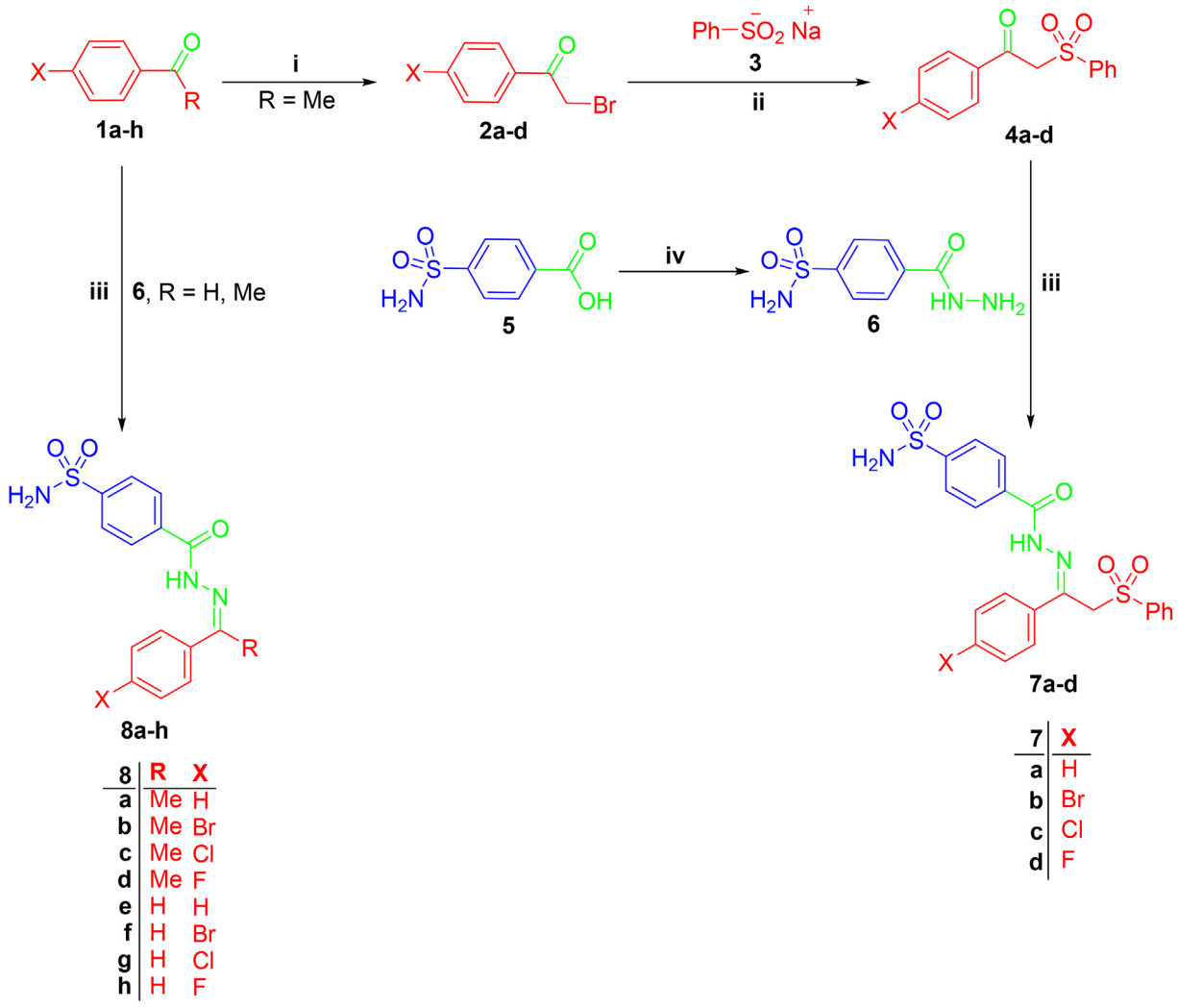
2.2.1. Carbonic anhydrase inhibition

All synthesized compounds **7a-d, 8a-h, 12a-f, 13a,b** and **15a-f** were screened for their ability to suppress the physiologically relevant hCA isoforms, hCA I, II, IV and IX were measured using the standard inhibitor acetazolamide (**AAZ**) *via* a stopped flow CO_2 hydras assay[25]. The following structure activity relationship (SAR) can be assembled from the inhibition data mentioned in Table 1:

(i) The pervasive isoform CA I was heartily inhibited by the synthetized compounds **7a-d, 8a-h, 12a-c, 13a** and **15a-c** with inhibition constants ranging between 26.9 nM and 7.4 μM, especially, compounds **8a,d,e** (K_i = 26.9–65.2 nM) showed potent activity ranging from (4–9) times more active than **AAZ** (K_i = 250 nM), also, compounds **7a** and **8c,h** were equipotent to **AAZ.** On the other hand, replacement of ($-SO_2NH_2$) moiety with its isostere (COOH) abolished the activity as seen in compounds **12e,f**, **13b** and **15d-f**, while, compound **12d** showed weak activity with K_i = 93.21 μM. Noteworthy, there are many factors that had great impact on the activity:

- Grafting with diverse moieties at *p*-position for the phenyl ring had awesome effect on the inhibitory activity with the following order F>H>Cl>Br for compounds **8a-d**, H>F>Br>Cl for compounds **8e-h**, H>F>(Cl═Br) for **7a-d** and H>Cl>Br for **12a-c** and **15a-c**
- Replacement of the olefinic proton with ($-CH_3$) or ($-SO_2C_6H_5$) moieties increased the activity for Br and Cl derivatives, while, decreased the activity for unsubstituted derivative.
- Change the hydrazido linker with hydrazino diminished the inhibitory effect [compounds **7a-d** (K_i = 283.5–882.8 nM) and **12a-c** (K_i = 951.2–7383 nM)].
- Positional replacement of benzensulfonamide from the carbonyl moiety (compounds **12a** K_i = 951.2 nM) to the active methylene enhanced the inhibitory activity (compound **13a** K_i = 425.6 nM).
- While substitution of (-NHN =) linker with (-NHCH =) improved the activity (compounds **13a** K_i = 425.6 nM and **15a** K_i = 186.7 nM).

(ii) The CA II was affected efficiently by the synthetized compounds, as compounds **7a-d**, **8a-h**, **12a**, **13a** and **15a** showed potent activity inhibition constants ranging between 7.6 nM to 89.2 nM, also, compounds **12b-d,f** and **15b,c,e** showed moderate to weak activity (K_i = 0.42-65.44 μM), while compounds **12e**, **13b** and **15d,f** didn't show any inhibitory activity up to 100 μM. Regarding to SAR:-



8	R	X
a	Me	H
b	Me	Br
c	Me	Cl
d	Me	F
e	H	H
f	H	Br
g	H	Cl
h	H	F

7	X
a	H
b	Br
c	Cl
d	F

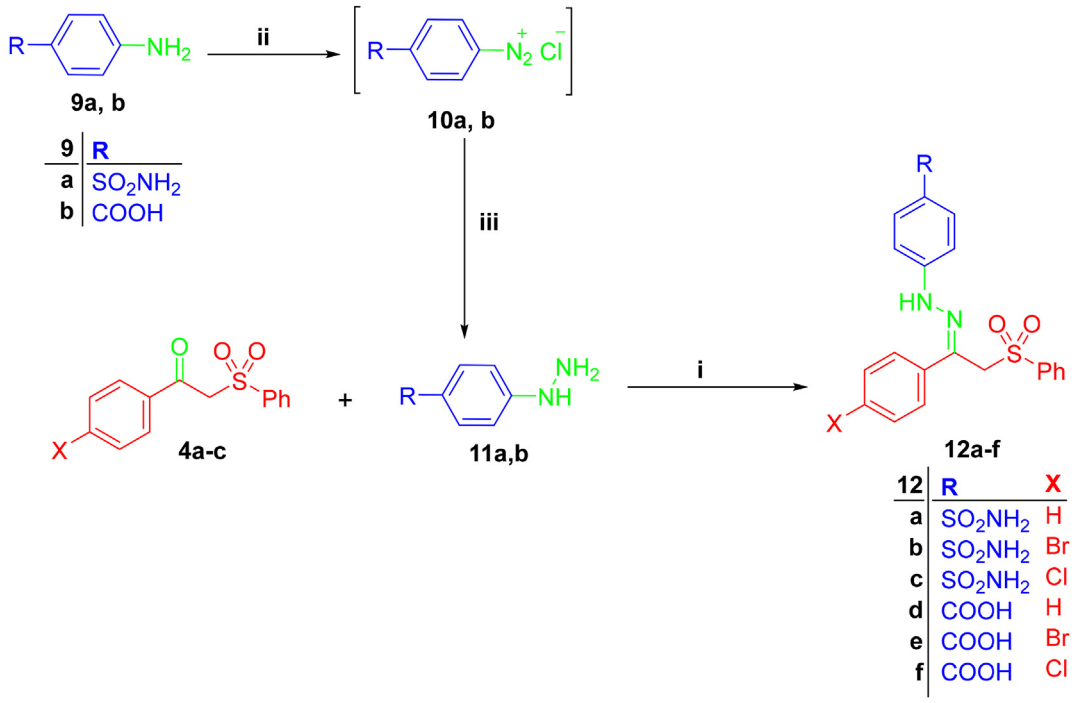
Scheme 1. Reagents and conditions: **i**) Br_2/AcOH/stirring 2 h; **ii**) EtOH/reflux 2 h; **iii**) AcOH/reflux 4 h; **iv**) a- MeOH/H_2SO_4/reflux 10 h, b- $NH_2NH_2 \cdot H_2O$ (99%, 3 equ.)/reflux 4 h.

- Similar to CAI, grafting with diversified moieties at *p*-position for the phenyl ring had great effect on the inhibitory activity with the following order H>F>Cl>Br for **7a-d.** While, for compounds **8a-d** Br moiety showed better activity than Cl and F better than H with the following order F>H>Br>Cl. whilst, compounds **8e-h** showed different pattern as *p*-Cl derivative was the most active one in this series ($K_i = 21.4$ nM) and the unsubstituted analogue showed the least inhibitory activity and the order was Cl>Br>F>H. on the other hand, the order for compounds **12a-c** and **15a-c** was identical to CAI as following H>Cl>Br.
- On the contrary to CAI, replacement of the olefinic proton with (-CH_3) or (-$SO_2C_6H_5$) moieties decreases the inhibitory activity for Br and Cl derivatives, while, increases activity for unsubstituted derivative.
- Similar to CAI, change the hydrazido linker with hydrazino decreased the activity (compounds **7a-d** $K_i = 26.9–89.2$ nM and **12a-c** $K_i = 80.5–794.2$ nM).
- Positional replacement of benzensulfonamide from the carbonyl moiety (**12a** $K_i = 80.5$ nM) to the active methylene (**13a** $K_i = 7.6$ nM) enhanced the inhibitory activity.
- Also, replacement of (-NHN =) linker with (-NHCH =) decreased the inhibitory activity (compounds **13a** $K_i = 7.6$ nM and **15a** $K_i = 40.1$ nM).

(iii) The membrane bound isoform CA IV showed moderate inhibitory activity for compounds **7a-d, 8a-h, 12a-c, 13a** and **15a-c** ($K_i = 0.09–8.33$ μM). While, replacement of (-SO_2NH_2) moiety with its isostere (COOH) diminished the activity from weak (compound **12f** with $K_i = 18.07$ μM) to inactive up to 100 μM (compounds **12d,e**, **13b** and **15d-f**).

According to SAR:-

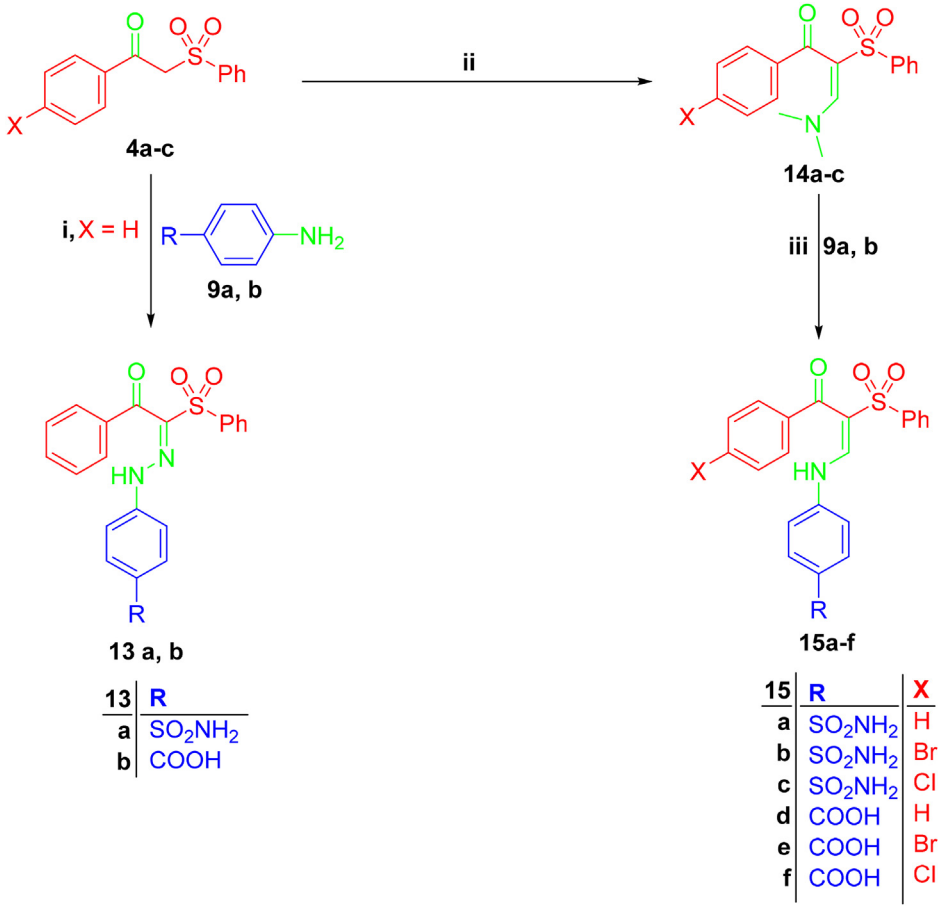
- Compounds **7a-d** showed the same order of activity as CAII while, for compounds **12a-c** Br moiety showed better activity than Cl with the following order H>Br>Cl. whilst, compounds **15a-c** showed different pattern as *p*-Br derivative was the most active one in this series ($K_i = 6.7$ μM) and the un-substituted analogue showed the least inhibitory activity and the order was Br>Cl>H.
- Interestingly, changing the olefinic proton showed different pattern as replace the previous proton with (-CH_3) decreased the inhibitory activity. While, its replacement with (-$SO_2C_6H_5$) group improved the activity for unsubstituted and *p*-Br substituted derivatives, whilst, it decreased the inhibitory activity for *p*-F and *p*-Cl substituted analogues.
- On the contrary to CAI and CAII, positional replacement of benzensulfonamide from the carbonyl moiety to the active



9	R
a	SO_2NH_2
b	COOH

12	R	X
a	SO_2NH_2	H
b	SO_2NH_2	Br
c	SO_2NH_2	Cl
d	COOH	H
e	COOH	Br
f	COOH	Cl

Scheme 2. Reagents and conditions: **i**) AcOH/reflux 4 h; **ii**) $NaNO_2$/HCl/stirring 0.5 h/0–5 °C; **iii**) $SnCl_2$/HCl/stirring 6 h/0–5 °C.

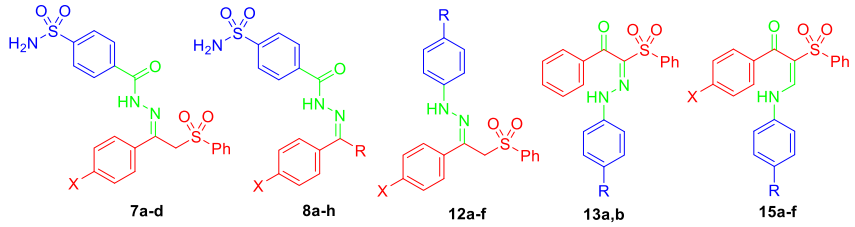


13	R
a	SO_2NH_2
b	COOH

15	R	X
a	SO_2NH_2	H
b	SO_2NH_2	Br
c	SO_2NH_2	Cl
d	COOH	H
e	COOH	Br
f	COOH	Cl

Scheme 3. Reagents and conditions: **i**) a- $NaNO_2$/HCl/stirring 0.5 h/0–5 °C, b-pyridine/stirring 12 h/0–5 °C; **ii**) DMFDMA/Xylene/reflux 15 h; **iii**) AcOH/reflux 4 h.

Table 1
Inhibitory data of human CA isoforms hCA I, hCA II, hCA IV, hCA IX with the synthesized compounds **7a-d, 8a-h, 12a-f, 13a,b** and **15a-f** determined by stopped-flow CO_2 hydrase assay, using the standard inhibitor acetazolamide (**AAZ**).



Comp.	R	X	K_I (nM) hCA I	hCA II	hCA IV	hCA IX
7a	–	**H**	283.5	26.9	574.5	768.8
7b	–	**Br**	874.0	89.2	2289	289.2
7c	–	**Cl**	882.8	71.2	1830	818.0
7d	–	**F**	677.6	53.9	765.5	1124
8a	**CH_3**	**H**	65.2	23.1	2936	321.7
8b	**CH_3**	**Br**	629.7	42.3	7991	961.9
8c	**CH_3**	**Cl**	291.0	45.8	8335	318.9
8d	**CH_3**	**F**	48.9	21.2	699.3	1447
8e	**H**	**H**	26.9	30.4	1974	869.0
8f	**H**	**Br**	4722	23.0	7184	2205
8g	**H**	**Cl**	6162	21.4	660.7	2092
8h	**H**	**F**	260.9	24.4	158.1	2369
12a	**SO_2NH_2**	**H**	951.2	80.5	97.0	2487
12b	**SO_2NH_2**	**Br**	7383	794.2	8167	908.7
12c	**SO_2NH_2**	**Cl**	7373	421.5	8302	2910
12d	**COOH**	**H**	93211	32633	>100000	>100000
12e	**COOH**	**Br**	>100000	>100000	>100000	>100000
12f	**COOH**	**Cl**	>100000	9190	18070	>100000
13a	**SO_2NH_2**	–	425.6	7.6	2206	1605
13b	**COOH**	–	>100000	>100000	>100000	>100000
15a	**SO_2NH_2**	**H**	186.7	40.1	8381	2756
15b	**SO_2NH_2**	**Br**	7447	9672	6730	3035
15c	**SO_2NH_2**	**Cl**	7443	3016	6950	3012
15d	**COOH**	**H**	>100000	>100000	>100000	>100000
15e	**COOH**	**Br**	>100000	65439	>100000	>100000
15f	**COOH**	**Cl**	>100000	>100000	>100000	>100000
AAZ	–	–	250.0	12.1	74.0	25.8

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

methylene decreased the inhibitory activity as shown in compounds **12a** (K_i = 97.0 nM) and **13a** (K_i = 2.206 μM).

- While, replacement of (-NHN =) linker with (-NHCH =) let to decrease of the inhibitory activity as compounds **13a** (K_i = 2.206 μM) and **15a** (K_i = 8.381 μM).

(iv) The isoform CAIX was also, moderately inhibited with the synthetized compounds **7a-d, 8a-h, 12a-c, 13a** and **15a-c** (K_i = 0.28–3.03 μM). In addition, replacement of (-SO_2NH_2) moiety with its isostere (COOH) rescinded the inhibitory activity up to 100 μM (compounds **12d-f**, **13b** and **15d-f**).

Concerning to SAR:-

- Replacement of the olefinic proton with (-CH_3) or (-$SO_2C_6H_5$) moieties improved the activity for all derivatives.
- Also, similar to CAI and CAII, change the hydrazido linker with hydrazino decreased the activity (**7a-d** with K_i = 289.2–1124 nM and **12a-c** with K_i = 908.7–2910 nM).
- In addition, positional replacement of benzensulfonamide from the carbonyl moiety to the active methylene led to increase the

Table 2
Selectivity ratios for the inhibition of hCA II over hCA I, IV and hCA IX for the synthetized benzensulfonamide compounds **7a-d, 8a-h, 12a-c, 13a**, **15a-c** and **AAZ**.

Comp.	I/II	IV/II	IX/II
7a	10.54	21.36	28.58
7b	**9.80**	**25.66**	3.24
7c	**12.40**	**25.70**	**11.49**
7d	**12.57**	14.20	**20.85**
8a	2.82	**127.10**	13.93
8b	**14.89**	**188.91**	**22.74**
8c	6.35	**181.99**	6.96
8d	2.31	**32.99**	**68.25**
8e	0.88	**64.93**	**28.59**
8f	**205.30**	**312.35**	**95.87**
8g	**287.94**	**30.87**	**97.76**
8h	**10.69**	6.48	**97.09**
12a	**11.82**	1.20	**30.89**
12b	**9.30**	**10.28**	1.14
12c	**17.49**	**19.70**	6.90
13a	**56.00**	**290.26**	**211.18**
15a	4.66	**209.00**	**68.73**
15b	0.77	0.70	0.31
15c	2.47	2.30	1.00
AAZ	20.66	6.12	2.13

Table 3
Anti-proliferative impact of sulfonamides **7a-d, 8a-h, 12a-c, 13a**, **15a-c** against breast cancer (**MCF-7**) cell line, and human normal breast epithelia (**MCF-10A**) cell line.

Comp.	IC_{50} (μM)[a]		Selectivity Index
	MCF-7	MCF-10A	
7a	10.73 ± 0.6	84.76 ± 4.3	7.90
7b	19.1 ± 1.0	34.08 ± 1.7	1.78
7c	57.0 ± 3.1	25.57 ± 1.3	0.45
7d	**0.62 ± 0.02**	**63.7 ± 3.2**	**102.74**
8a	20.1 ± 1.1	31.05 ± 1.6	1.54
8b	**2.29 ± 0.1**	**37.64 ± 1.9**	**16.44**
8c	**3.75 ± 0.2**	**62.74 ± 3.2**	**16.73**
8d	31.86 ± 1.7	21.99 ± 1.1	0.69
8e	14.87 ± 0.8	39.42 ± 2.2	2.65
8f	**1.37 ± 0.1**	**27.73 ± 1.4**	**20.24**
8g	36.71 ± 2.1	81.97 ± 4.2	2.23
8h	9.17 ± 0.5	35.89 ± 1.8	3.91
12a	**3.97 ± 0.2**	**46.08 ± 2.3**	**11.61**
12b	17.33 ± 0.9	12.87 ± 0.7	0.74
12c	8.01 ± 0.4	28.63 ± 1.5	3.57
13a	**1.74 ± 0.1**	**14.05 ± 0.7**	**8.07**
15a	3.44 ± 0.2	17.93 ± 0.9	5.21
15b	62.71 ± 3.4	20.36 ± 1.6	0.32
15c	15.11 ± 0.8	27.96 ± 1.4	1.85
5-FU	12.5 ± 0.7	17.2 ± 0.9	1.38

[a] IC_{50} values are the mean ± S.D. of three experiments.

activity like CAI and CAII (compounds **12a** $K_i = 2.487$ μM and **13a** $K_i = 1.605$ μM).

- While replacement of (-NHN =) linker with (-NHCH =) decreased of the inhibitory activity (**13a** $K_i = 1.605$ μM and **15a** $K_i = 2.756$ μM).

Interestingly, all the synthetized benzensulfonamide compounds **7a-d, 8a-h, 12a-c, 13a** and **15a-c** showed good to highly selectivity to isoform hCA II over hCA I, IV and IX (Table 2). Especially, compound **13a** which considered as the most potent derivative against hCA II with $K_i = 7.6$ nM, also, it showed highly selectivity ratio = 56.0 over hCAI, 290.26 over hCA IV and 211.18 over hCA IX.

2.2.2. Anti-proliferative activity

All herein synthetized sulfonamide derivatives **7a-d, 8a-h, 12a-c, 13a** and **15a-c** were evaluated for their ability to induce cytotoxic effect against breast cancer (**MCF-7**) cell line, under hypoxic condition, adopting the colorimetric MTT assay (Table 3).

Regarding **MCF-7** breast cancer cells, the tested sulfonamide compounds **7a-d, 8a-h, 12a-c, 13a**, **15a-c** showed potent to weak activity compared with **5-FU** ($IC_{50} = 12.5$ μM). Compounds **7a**,**d**, **8b**,**c**,**f**,**h**, **12a**,**c**, **13a** and **15a** were the most active derivatives with IC_{50}= (0.62−10.73) μM. In particular, sulfonamide **7d** was the most active one with $IC_{50} = 0.62$ μM that is approximately 20-fold more than **5-FU**, followed with compound **8f** with $IC_{50} = 1.37$ μM that is approximately 9-fold more than **5-FU**. Moreover, compounds **7b**, **8a**,**e**,**d**,**g**, **12b** and **15c** displayed moderate activity with IC_{50} range: 14.87−36.71 μM. While, compounds **7c** and **15b** had weak activity with $IC_{50} = 57$ and 62.71 μM, respectively.

On the other hand, all the synthetized sulfonamide compounds **7a-d, 8a-h, 12a-c, 13a**, **15a-c** were evaluated for their ability to induce cytotoxic effect towards human normal breast epithelia (**MCF-10A**) cell line under normal condition, to investigate their safety. Thereafter, the selectivity index for each compound was calculated (Table 3). The tested compounds showed non-significant cytotoxic action with IC_{50} range (12.87−84.76 μM). Moreover, compounds **7a**,**d**, **8b**,**c**,**f**,**h**, **12a**,**c**, **13a** and **15a** had good selectivity index range (3.57−102.74), especially compound **7d** that was the most active and safe compounds (selectivity index = 102.74), thereby providing a high safety profile as anticancer agents.

3. Molecular modeling studies

To explore in depth the binding mode of the synthesized compounds within the hCA I and II active sites, docking studies were performed on derivatives **7a**, **8a**, **8e**, **12a**, **13a** and **15a**.

Outcomes from docking showed that all compounds coordinated the zinc ion with the deprotonated nitrogen atom of the sulfonamide moiety. Moreover, the binding was strengthened by two H-bonds, engaged by the sulfonamide group with the backbone NH and the side chain OH of T199, and further supported by hydrophobic contacts between the phenyl ring of benzenesulfonamide and the lipophilic residues L198, A121 and F91.

Both the presence of two bulky tails in derivative **7a** and the small dimension of the hCA I active site forced the ligand towards the lipophilic half of the binding pocket (Fig. 3A), leading the S═O group of the sulfonic bridge in H-bond distance with the side chain OH of Y204, a peculiar residue of this isoform. Moreover, the phenyl rings is well stabilized by the lipophilic residues A135, A132 and L131, and W5.

The aromatic tail of the most flexible ligands **8a** and **8e** accomodates in the pocket lined by hydrophobic residues A135, V207 and L131. However, the strain determined by the presence in **8a** of the methyl group pointing towards L198, is the probable cause of **8a** worse activity than **8e.**

Instead, the hCA I/hCA II residue mutations make the hCA II active site larger (F91/I91 and H67/N67) and more hydrophilic (V62/N62) compred to hCA I, allowing the derivative **7a** to orient towards the hydrophilic half of the binding pocket. Here the compounds engaged an H-bond between the sulfonic bridge S═O and the side chain NH_2 of N62 and vdW interactions between the aromatic rings and the side chains of I91 and W5 (Fig. 3B). The phenyl ring of derivatives **8a** and **8e** established π-π staking interactions with the phenyl ring of F131 side chain, hydrophobic contacts with the side chain of I91, F131 and Q92, whereas the methyl portion of **8a** is inevitably pushed towards V121 (Fig. 3B).

The shortening of the linker from hydrazido (**7a**, **8a**, **8e**) to hydrazino (**12a**, **13a**) and methylamino (**15a**) led the sulfonic bridge S═O in H-bond contact with the inner side chain NH_2 of Q92 or N67 (Fig. 3C), instead of N62 (**7a**, Fig. 3B) and derivative **12a** to position the aromatic ring in a cleft lined by an ensemble of hydrophobic residues (I91, V121, F131, Q92 and P202). The elongation from phenyl (**12a**) to benzoyl pendant, allow the compounds **13a** and **15a** to reach the area of W5, H64 and F131 where the two aromatic rings form π-π staking and VdW interactions (Fig. 3C) that overall stabilize the compounds.

4. Conclusion

This study reports the design and synthesis of five series of benzenesulfonamide derivatives **7a-d**, **8a-h**, **12a-c**, **13a** and **15a-c** and its isostere benzoic acid analogues **12d-f**, **13b** and **15d-f** adopting dual tail approach seeking for increasing the potency and selectivity. All synthetized compounds were evaluated as CAIs against hCA I, II, IV and IX with **AAZ** as stander inhibitor. The benzenesulfonamide derivatives showed moderate to potent inhibitory activity with selectivity toward isoform hCA II, especially, compound **13a** with $K_i = 7.6$ nM and selectivity index = 56.0 over hCAI, 290.26 over hCA IV and 212.18 over hCA IX. On the other hand, it's found that replacement of SO_2NH_2 moiety with its isostere COOH abolished the inhibitory activity except compounds **12d,e** and **15f** that showed weak activity. In addition, the anti-

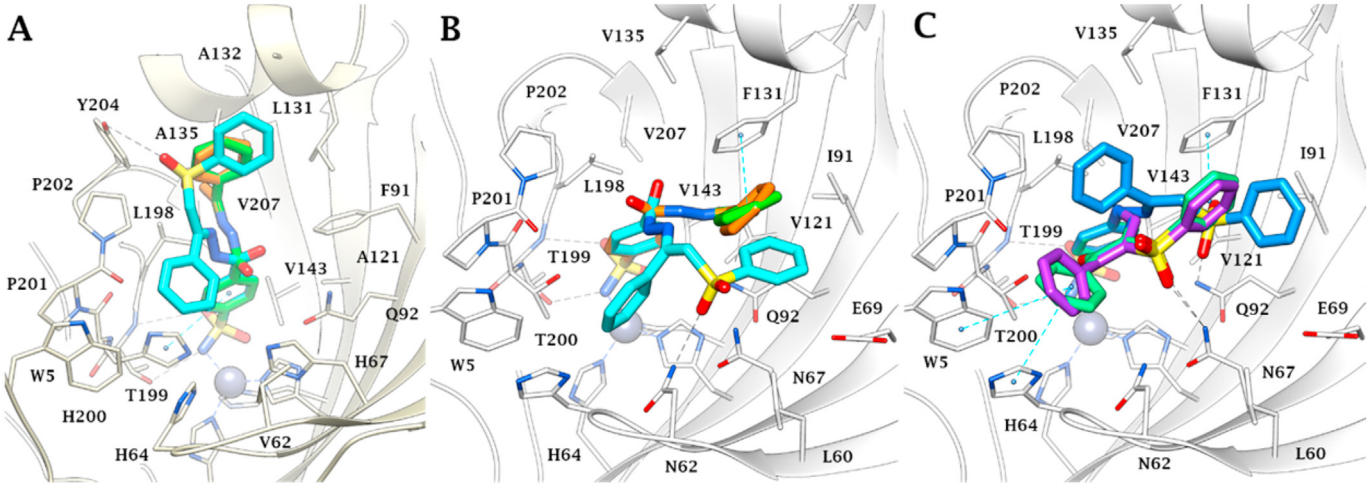


Fig. 3. Predicted binding mode of derivatives **7a** (cyan), **8a** (orange), **8e** (green) within A) hCA I and B) hCA II active sites, and of C) **12a** (dodger blue), **13a** (spring green), **15a** (purple) within hCA II active site.

proliferative study revealed that compound **7d** was the most active and safe compounds, thereby providing a high safety profile as anticancer agents. Finally, molecular docking was performed for compounds **7a**, **8a**, **8e**, **12a**, **13a** and **15a** to illustrate the different binding pattern within the active site of hCA I and II.

5. Experimental

5.1. Chemistry

5.1.1. General

A Stuart digital apparatus was used for determining melting points. Shimadzu FT-IR 8400S infrared spectrophotometer was used for recording infrared spectra. Both ^{1}H NMR and ^{13}C NMR were measured using deuterated dimethylsulfoxide (DMSO-d_6) as solvent but with variable frequency (100MHZ for ^{13}C NMR, 400MHZ for ^{1}H NMR) *via* Bruker spectrophotometer, at the Faculty of Pharmacy, Ain-shams and Cairo Universities. Mass spectra were recorded by TLC-MS Advion compact mass spectrometer (CMS). Elemental analyses were implemented at the Regional Center for Microbiology and Biotechnology, Al-Azhar University, Cairo, Egypt. Compounds **4a-d**, **6**, **11a,b**, **12b,c** and **14a-c** were prepared depending on their reported procedure[23,26–29].

5.1.2. Synthesis of targeted compounds 7a-d

In round conical flask, different phenyl sulfone derivative **4a-d** (1.21 mmol) was dissolved in 17 mL of acetic acid, to the previous solution, equivalent amount of 4-(hydrazinecarbonyl)benzenesulfonamide **6** (0.27g, 1.23 mmol) was added. The reaction mixture was heated under reflux for 4h, the formed precipitate was filtrated while hot, washed with ethoxyethan and recrystallized from DMF/MeOH mixture to furnish the targeted final compounds **7a-d**.

*5.1.2.1. 4-(2-(1-phenyl)-2-(phenylsulfonyl)ethylidene)hydrazine-1-carbonyl)benzenesulfonamide **7a**.* White crystals, (yield 84%), m.p. 284–285 °C; ^{1}H NMR (400 MHz) δ *ppm*: 5.43 (2H, s, CH_2), 7.34–7.36 (2H, d, J = 8.0 Hz, ArH), 7.57–7.60 (4H, m, 2H, SO_2NH_2, 2H, ArH), 7.67–7.70 (2H, m, ArH), 7.78 (2H, d, J = 8.0 Hz, ArH), 7.91 (3H, d, J = 8.0 Hz, ArH), 8.0 (3H, brs, ArH), 11.18 (1H, s, NH); ^{13}C NMR (100 MHz) δ *ppm*: 53.19 (CH_2), 126.22, 127.54, 128.31, 128.62, 128.64, 129.13, 129.77, 130.09, 131.54, 134.82, 136.81, 137.51, 139.28, 143.14, 147.32, 150.22, 155.10, 158.22, 163.42 (C=O); IR (KBr, ν cm^{-1}): 3317, 3237 (NH_2, NH), 1677 (C=O) and 1344, 1299, 1149, 1079 ($2SO_2$); EA Calcd.: C, 55.13; H, 4.19; N, 9.18; found C, 55.56; H, 4.28; N, 8.97.

*5.1.2.2. 4-(2-(1-(4-bromophenyl)-2-(phenylsulfonyl)ethylidene)hydrazine-1-carbonyl)benzenesulfonamide **7b**.* White crystals, (yield 87%), m.p. 277–278 °C; ^{1}H NMR (400 MHz) δ *ppm*: 4.60, 5.48 (2H, 2s, CH_2), 7.36 (1.7H, d, J = 8.0 Hz, ArH), 7.48 (2H, s, SO_2NH_2), 7.58 (1.3H, s, ArH), 7.76 (2H, d, J = 8.0 Hz, ArH), 7.88 (2H, d, J = 8.0 Hz, ArH), 7.96–8.01 (5H, m, ArH), 8.18 (1H, brs, ArH), 11.32 (1H, s, NH); IR (KBr, ν cm^{-1}): 3321, 3249 (NH_2, NH), 1679 (C=O) and 1337, 1304, 1158, 1081 ($2SO_2$); (ESI) m/z 534 $[M-H]^+$; EA Calcd.: C, 47.02; H, 3.38; N, 7.83; found C, 47.34; H, 3.58; N, 7.56.

*5.1.2.3. 4-(2-(1-(4-chlorophenyl)-2-(phenylsulfonyl)ethylidene)hydrazine-1-carbonyl)benzenesulfonamide **7c**.* White crystals, (yield 87%), m.p. 290–292 °C; ^{1}H NMR (400 MHz) δ *ppm*: 5.44 (2H, s, CH_2), 7.40 (2H, d, J = 8.0 Hz, ArH), 7.56–7.61 (4H, m, 2H, SO_2NH_2, 2H, ArH), 7.68 (2H, t, J = 8.0 Hz, ArH), 7.81 (1H, brs, ArH), 7.90 (3H, d, J = 8.0 Hz, ArH), 7.99 (3H, brs, ArH), 11.20 (1H, s, NH); ^{13}C NMR (100 MHz) δ *ppm*: 52.93 (CH_2), 126.17, 127.45, 128.65, 128.68, 129.05, 129.29, 129.79, 130.93, 132.04, 134.83, 135.79, 139.27, 141.75, 142.34, 145.67, 148.96, 151.19, 154.37, 163.42 (C=O); IR (KBr, ν cm^{-1}): 3327, 3239 (NH_2, NH), 1674 (C=O) and 1341, 1303, 1158, 1091 ($2SO_2$); (ESI) m/z 490 $[M-H]^+$; EA Calcd.: C, 51.27; H, 3.69; N, 7.21; found C, 51.33; H, 3.53; N, 9.07.

*5.1.2.4. 4-(2-(1-(4-flourophenyl)-2-(phenylsulfonyl)ethylidene)hydrazine-1-carbonyl)benzenesulfonamide **7d**.* White crystals, (yield 87%), m.p. 284–285 °C; ^{1}H NMR (400 MHz) δ *ppm*: 5.44 (2H, s, CH_2), 7.18 (2H, brs, ArH), 7.56–7.60 (4H, m, 2H, SO_2NH_2, 2H, ArH), 7.67 (2H, t, J = 8.0 Hz, ArH), 7.83 (1H, brs, ArH), 7.90 (3H, d, J = 8.0 Hz, ArH), 7.99 (3H, brs, ArH), 11.17 (1H, s, NH); ^{13}C NMR (100 MHz) δ *ppm*: 53.18 (CH_2), 115.45, 115.66, 126.20, 127.72, 128.65, 129.13, 129.78, 129.93, 133.35, 133.37, 134.83, 136.69, 138.07, 139.22, 142.20, 147.33, 152.00, 152.90, 163.42 (C=O); IR (KBr, ν cm^{-1}): 3317, 3220 (NH_2, NH), 1676 (C=O) and 1335, 1305, 1162, 1091 ($2SO_2$); EA Calcd.: C, 53.04; H, 3.82; N, 8.84; found C, 53.63; H, 3.89; N, 8.71.

5.1.3. Synthesis of targeted compounds 8a-h, 12a-f and 15a-f

As previously mentioned for compounds **7a-d**.

*5.1.3.1. 4-(2-(1-phenylethylidene)hydrazine-1-carbonyl)benzenesulfonamide **8a**.* White powder, (yield 89%), m.p. 272–273 °C; ^{1}H NMR (400 MHz) δ *ppm*: 2.40 (3H, s, CH_3), 7.45 (2H, brs, ArH), 7.55 (2H, s, SO_2NH_2), 7.89 (2H, brs, ArH), 7.96 (3H, d, J = 8.0Hz, ArH), 8.06 (2H, d, J = 8.0Hz, ArH), 10.96 (1H, s, NH); ^{13}C NMR (100 MHz) δ *ppm*: 15.22 (CH_3), 126.07, 126.98, 128.87, 129.08, 130.12, 132.25, 137.48,

138.43, 146.94, 149.67, 155.43, 156.76, 163.54 (C=O); IR (KBr, ν cm^{-1}): 3359, 3262 (NH_2, NH), 1634 (C=O) and 1330, 1158 (SO_2); EA Calcd.: C, 56.77; H, 4.76; N, 13.24; found C, 56.61; H, 4.85; N, 13.47.

5.1.3.2. 4-(2-(1-(4-bromophenyl)ethylidene)hydrazine-1-carbonyl) benzenesulfonamide ***8b***. White powder, (yield 89%), m.p. 295–296 °C; ^{1}H NMR (400 MHz) δ *ppm*: 2.38 (3H, s, CH_3), 7.53 (2H, s, SO_2NH_2), 7.64 (2H, d, J = 8.0 Hz, ArH), 7.82 (2H, d, J = 8.0 Hz, ArH), 7.94 (2H, d, J = 8.0 Hz, ArH), 8.04 (2H, d, J = 8.0 Hz, ArH), 10.97 (1H, s, NH); ^{13}C NMR (100 MHz) δ *ppm*: 14.99 (CH_3), 123.71, 126.10, 129.06, 131.83, 135.35, 137.50, 140.43, 145.10, 146.86, 151.18, 153.40, 155.78, 163.87 (C=O); IR(KBr, ν cm^{-1}): 3360, 3261 (NH_2, NH), 1644 (C=O) and 1310, 1158 (SO_2); (ESI) m/z 394 $[M - H]^+$; EA Calcd.: C, 45.47; H, 3.56; N, 10.60; found C, 45.27; H, 3.67; N, 10.84.

5.1.3.3. 4-(2-(1-(4-chlorophenyl)ethylidene)hydrazine-1-carbonyl) benzenesulfonamide ***8c***. White powder, (yield 89%), m.p. 275–277 °C; ^{1}H NMR (400 MHz) δ *ppm*: 2.38 (3H, s, CH_3), 7.51 (2H, brs, ArH), 7.53 (2H, s, SO_2NH_2), 7.88 (2H, d, J = 8.0 Hz, ArH), 7.94 (2H, d, J = 8.0 Hz, ArH), 8.04 (2H, d, J = 8.0 Hz, ArH), 10.98 (1H, s, NH); ^{13}C NMR (100 MHz) δ *ppm*: 15.04 (CH_3), 126.09, 128.77, 128.90, 129.10, 132.95, 134.87, 137.18, 137.30, 142.13, 146.91, 148.75, 152.58, 155.50, 163.77 (C=O); IR (KBr, ν cm^{-1}): 3358, 3261 (NH_2, NH), 1645 (C=O) and 1310, 1160 (SO_2); EA Calcd.: C, 51.21; H, 4.01; N, 11.94; found C, 50.98; H, 4.23; N, 11.81.

5.1.4. 4-(2-(1-(4-fluorophenyl)ethylidene)hydrazine-1-carbonyl) benzenesulfonamide ***8d***

White powder, (yield 89%), m.p. 280–282 °C; ^{1}H NMR (400 MHz) δ *ppm*: 2.39 (3H, s, CH_3), 7.26 (2H, t, J = 8.0 Hz, ArH), 7.54 (2H, s, SO_2NH_2), 7.91–7.97 (4H, m, ArH), 8.05 (2H, d, J = 8.0 Hz, ArH), 10.96 (1H, s, NH); ^{13}C NMR (100 MHz) δ *ppm*: 15.21 (CH_3), 115.64, 115.85, 126.08, 126.15, 128.11, 129.07, 131.66, 134.86, 134.89, 137.40, 146.93, 155.88, 157.41, 163.63 (C=O); IR (KBr, ν cm^{-1}): 3328, 3186 (NH_2, NH), 1659 (C=O) and 1335, 1160 (SO_2); (ESI) m/z 334 $[M - H]^+$; EA Calcd.: C, 53.72; H, 4.21; N, 12.53; found C, 53.94; H, 4.34; N, 12.49.

5.1.3.5. 4-(2-benzylidenehydrazine-1-carbonyl)benzenesulfonamide ***8e***. White powder, (yield 89%), m.p. over 300 °C; ^{1}H NMR (400 MHz) δ *ppm*: 7.47–7.50 (3H, m, ArH), 7.55 (2H, s, SO_2NH_2), 7.76 (2H, d, J = 8.0Hz, ArH), 7.99 (2H, d, J = 8.0 Hz, ArH), 8.1 (2H, d, J = 8.0 Hz, ArH), 8.5 (1H, s, =C–H), 12.03 (1H, s, NH); ^{13}C NMR (100 MHz) δ *ppm*: 126.28, 127.71, 128.84, 129.36, 130.81, 134.57, 136.77, 139.19, 147.14, 149.13, 153.90, 162.75 (C=O); IR (KBr, ν cm^{-1}): 3327, 3207 (NH_2, NH), 1630 (C=O) and 1336, 1156 (SO_2); (ESI) m/z 302 $[M - H]^+$; EA Calcd.: C, 55.43; H, 4.32; N, 13.85; found C, 53.64; H, 3.41; N, 4.41.

5.1.3.6. 4-(2-(4-bromobenzylidene)hydrazine-1-carbonyl)benzene-sulfonamide ***8f***. White powder, (yield 89%), m.p. over 300 °C; ^{1}H NMR (400 MHz) δ *ppm*: 7.55 (2H, s, SO_2NH_2), 7.66 (2H, d, J = 8.0 Hz, ArH), 7.70 (2H, d, J = 8.0 Hz, ArH), 7.98 (2H, d, J = 8.0 Hz, ArH), 8.09 (2H, d, J = 8.0 Hz, ArH), 8.45 (1H, s, =C–H), 12.08 (1H, s, NH); ^{13}C NMR (100 MHz) δ *ppm*: 124.06, 126.28, 128.86, 129.55, 130.64, 132.36, 133.86, 136.66, 147.19, 147.85, 150.15, 152.99, 162.78 (C=O); IR (KBr, ν cm^{-1}): 3313, 3209 (NH_2, NH), 1634 (C=O) and 1339, 1156 (SO_2); EA Calcd.: C, 43.99; H, 3.16; N, 10.99; found C, 44.16; H, 3.45; N, 10.74.

5.1.3.7. 4-(2-(4-chlorobenzylidene)hydrazine-1-carbonyl)benzene-sulfonamide ***8g***. White powder, (yield 89%), m.p. over 300 °C; ^{1}H NMR (400 MHz) δ *ppm*: 7.53 (2H, brs, ArH), 7.54 (2H, s, SO_2NH_2), 7.77 (2H, d, J = 8.0 Hz, ArH), 7.98 (2H, d, J = 8.0 Hz, ArH), 8.09 (2H, d, J = 8.0 Hz, ArH), 8.47 (1H, s, =C–H), 12.08 (1H, s, NH); ^{13}C NMR (100 MHz) δ *ppm*: 126.28, 128.85, 129.34, 129.44, 133.48, 135.28, 136.63, 138.45, 144.27, 147.15, 147.81, 150.77, 153.03, 162.84 (C=O); IR (KBr, ν cm^{-1}): 3311, 3211 (NH_2, NH), 1679 (C=O) and 1341, 1156 (SO_2); (ESI) m/z 336 $[M - H]^+$; EA Calcd.: C, 49.78; H, 3.58; N, 12.44; found C, 49.76; H, 3.79; N, 12.62.

5.1.3.8. 4-(2-(4-fluorobenzylidene)hydrazine-1-carbonyl)benzene-sulfonamide ***8h***. White powder, (yield 89%), m.p. over 300 °C; ^{1}H NMR (400 MHz) δ *ppm*: 7.29 (2H,t, J = 8.0 Hz, ArH), 7.54 (2H, s, SO_2NH_2), 7.8 (2H, t, J = 8.0 Hz, ArH), 7.97 (2H, d, J = 8.0 Hz, ArH), 8.08 (2H, d, J = 8.0 Hz, ArH), 8.48 (1H, s, =C–H), 12.03 (1H, s, NH); ^{13}C NMR (100 MHz) δ *ppm*: 116.30, 116.52, 126.29, 128.82, 129.93, 130.02, 131.07, 136.64, 144.65, 147.04, 148.16, 153.51, 155.07, 162.96 (C=O); IR (KBr, ν cm^{-1}): 3306, 3202 (NH_2, NH), 1636 (C=O) and 1345, 1159 (SO_2); EA Calcd.: C, 52.33; H, 3.76; N, 13.08; found C, 52.21; H, 3.56; N, 12.94.

5.1.3.9. 4-(2-(1-phenyl-2-(phenylsulfonyl)ethylidene)hydrazineyl) benzenesulfonamide ***12a***. White powder, (yield 79%), m.p. 250–252 °C; ^{1}H NMR (400 MHz) δ *ppm*: 5.29 (2H, s, CH_2), 7.15 (2H, s, SO_2NH_2), 7.23 (2H, d, J = 8.0 Hz, ArH), 7.27–7.33 (3H, m, ArH), 7.49 (2H, t, J = 8.0 Hz, ArH), 7.57 (1H, m, ArH), 7.67 (2H, d, J = 8.0 Hz, ArH), 7.74 (2H, d, J = 8.0 Hz, ArH), 7.89 (2H, d, J = 8.0 Hz, ArH), 10.30 (1H, s, NH); ^{13}C NMR (100 MHz) δ *ppm*: 52.41 (CH_2), 112.72, 126.55, 127.65, 128.40, 128.52, 128.68, 129.37, 131.67, 132.53, 134.44, 134.99, 137.69, 139.71, 143.10, 145.99, 147.81; IR (KBr, ν cm^{-1}): 3379, 3278 (NH_2, NH) and 1330, 1294, 1146, 1085 ($2SO_2$); EA Calcd.: C, 55.93; H, 4.46; N, 9.78; found C, 55.23; H, 4.69; N, 9.69.

5.1.3.10. 4-(2-(1-phenyl-2-(phenylsulfonyl)ethylidene)hydrazineyl) benzoic acid ***12d***. White powder, (yield 82%), m.p. 269–270 °C; ^{1}H NMR (400 MHz) δ *ppm*: 5.32 (2H, s, CH_2), 7.20 (2H, d, J = 8.8 Hz, ArH), 7.26–7.32 (3H, m, ArH), 7.47 (2H, t, J = 8.0 Hz, ArH), 7.54 (1H, t, J = 7.2 Hz, ArH), 7.74 (2H, d, J = 8.4 Hz, ArH), 7.81 (2H, d, J = 8.4 Hz, ArH), 7.89 (2H, d, J = 6.8 Hz, ArH), 10.39 (1H, s, NH), 12.41 (1H, s, OH); ^{13}C NMR (100 MHz) δ *ppm*: 52.42 (CH_2), 112.72, 121.82, 126.52, 128.36, 128.51, 128.70, 129.34, 131.36, 132.50, 134.39, 137.75, 139.75, 142.90, 146.24, 148.90, 151.67, 167.78 (C=O of COOH); IR (KBr, ν cm^{-1}): 3380-2410 (OH), 3337 (NH), 1679 (C=O) and 1290, 1154 (SO_2); (ESI) m/z 393 $[M - H]^+$; EA Calcd.: C, 63.95; H, 4.60; N, 7.10; found C, 63.73; H, 3.68; N, 4.68.

5.1.3.11. 4-(2-(1-(4-bromophenyl)-2-(phenylsulfonyl)ethylidene) hydrazineyl)benzoic acid ***12e***. White powder, (yield 82%), m.p. 273–274 °C; ^{1}H NMR (400 MHz) δ *ppm*: 5.27 (2H, s, CH_2), 7.18 (2H, d, J = 8.0 Hz, ArH), 7.48–7.54 (4H, m, ArH), 7.57 (1H, t, J = 8.0 Hz, ArH), 7.69 (2H, d, J = 8.0 Hz, ArH), 7.82 (2H, d, J = 8.0 Hz, ArH), 7.87 (2H, d, J = 8.0 Hz, ArH), 10.29 (1H, s, NH), 12.42 (1H, s, OH); ^{13}C NMR (100 MHz) δ *ppm*: 52.21 (CH_2), 112.80, 121.68, 122.14, 128.52, 128.73, 129.39, 131.36, 131.40, 134.39, 134.45, 137.06, 139.65, 148.64, 167.76 (C=O of COOH); IR (KBr, ν cm^{-1}): 3374-2354 (OH), 3336 (NH), 1674 (C=O) and 1288, 1151 (SO_2); EA Calcd.: C, 53.29; H, 3.62; N, 5.92; found C, 53.23; H, 3.80; N, 5.24.

5.1.3.12. 4-(2-(1-(4-chlorophenyl)-2-(phenylsulfonyl)ethylidene) hydrazineyl)benzoic acid ***12f***. White powder, (yield 79%), m.p. 275–276 °C; ^{1}H NMR (400 MHz) δ *ppm*: 5.27 (2H, s, CH_2), 7.18 (2H, d, J = 8.0 Hz, ArH), 7.34 (2H, d, J = 8.0 Hz, ArH), 7.49 (2H, t, J = 8.0 Hz, ArH), 7.57 (1H, t, J = 8.0 Hz, ArH), 7.75 (2H, d, J = 8.0 Hz, ArH), 7.83 (2H, d, J = 8.0 Hz, ArH), 7.87 (2H, d, J = 8.0 Hz, ArH), (1H, s, NH), 12.41 (1H, s, OH); ^{13}C NMR (100 MHz) δ *ppm*: 52.17 (CH_2), 112.81, 122.12, 123.03, 128.15, 128.49, 128.64, 129.46, 131.33, 131.44, 133.04, 134.51, 136.52, 139.48, 143.47, 148.59, 150.93, 167.80 (C=O of COOH); IR (KBr, ν cm^{-1}): 3387-2360 (OH), 3343 (NH), 1676 (C=O) and 1289, 1150 (SO_2); (ESI) m/z 427 $[M - H]^+$; EA Calcd.: C, 58.81; H, 4.00; N, 6.53; found C, 58.95; H, 3.86; N, 6.28.

*5.1.3.13. 4-((3-oxo-3-phenyl-2-(phenylsulfonyl)prop-1-en-1-yl) amino)benzenesulfonamide **15a***. White crystals, (yield 82%), m.p. 235–236 °C; ^{1}H NMR (400 MHz) *δ ppm*: 7.34–7.38 (3H, m, 2H, SO_2NH_2, 1H, ArH), 7.44–7.47 (2H, m, ArH), 7.49–7.52 (3H, m, ArH), 7.58–7.65 (3H, m, ArH), 7.68–7.75 (2H, m, ArH), 7.77–7.83 (2H, m, ArH), 8.05–8.13 (1.3H, m, ArH), 8.53 (0.7H, d, *J* = 13.2 Hz, ArH), 10.56 (0.3H, d, *J* = 14.0 Hz, NH), 10.74 (0.7H, d, *J* = 13.2 Hz, NH); ^{13}C NMR (100 MHz) *δ ppm*: 111.55, 113.72, 115.75, 118.21, 119.11, 127.61, 127.65, 127.78, 127.82, 128.53, 128.69, 128.94, 129.06, 129.33, 129.36, 129.39, 129.60, 131.60, 132.40, 132.48, 133.08, 133.44, 133.72, 134.73, 137.91, 138.62, 138.78, 140.21, 140.47, 140.76, 142.49, 142.87, 142.95, 143.17, 144.87, 146.59, 147.85, 150.99, 189.74, 191.32 (C═O); IR (KBr, *ν* cm^{-1}): 3318, 3229 (NH_2, NH), 1631 (C═O) and 1389, 1302, 1137, 1095 ($2SO_2$); (ESI) *m/z* 441 [M − H]$^+$; EA Calcd.: C, 57.00; H, 4.10; N, 6.33; found C, 58.19; H, 4.36; N, 6.31.

*5.1.3.14. 4-((3-(4-bromophenyl)-3-oxo-2-(phenylsulfonyl)prop-1-en-1-yl)amino)benzenesulfonamide **15b***. White powder, (yield 79%), m.p. 280–281 °C; ^{1}H NMR (400 MHz) *δ ppm*: 7.35–7.40 (3H, m, 2H, SO_2NH_2, 1H, ArH), 7.50–7.54 (3.3H, m, ArH), 7.56–7.58 (2H, m, ArH), 7.60–7.66 (1.7H, m, ArH), 7.67–7.71 (1H, m, ArH), 7.75–7.80 (2H, m, ArH), 7.82–7.84 (1.3H, m, ArH), 8.07 (1H, t, *J* = 7.6 Hz, ArH), 8.54 (0.7H, d, *J* = 13.2 Hz, ArH), 10.59 (0.3H, d, *J* = 14.0 Hz, NH), 10.74 (0.7H, d, *J* = 13.2 Hz, NH); ^{13}C NMR (100 MHz) *δ ppm*: 111.32, 113.03, 113.34, 118.38, 119.24, 126.21, 126.31, 127.31, 127.55, 127.65, 127.75, 127.82, 128.45, 129.40, 129.76, 130.70, 131.43, 131.46, 131.60, 132.08, 132.32, 132.61, 132.87, 133.22, 133.81, 134.61, 135.09, 135.68, 137.68, 137.84, 140.32, 140.78, 142.34, 142.75, 142.77, 143.01, 148.30, 151.14, 188.79, 190.26 (C═O); IR (KBr, *ν* cm^{-1}): 3327, 3235 (NH_2, NH), 1623 (C═O) and 1338, 1298, 1139, 1098 ($2SO_2$); EA Calcd.: C, 48.38; H, 3.29; N, 5.37; found C, 48.95; H, 3.41; N, 5.48.

*5.1.3.15. 4-((3-(4-chlorophenyl)-3-oxo-2-(phenylsulfonyl)prop-1-en-1-yl)amino)benzenesulfonamide **15c***. White powder, (yield 79%), m.p. 270–271 °C; ^{1}H NMR (400 MHz) *δ ppm*: 7.36 (2H, s, SO_2NH_2), 7.42–7.47 (2H, m, ArH), 7.48–7.51 (2H, m, ArH), 7.51–7.54 (2.3H, m, ArH), 7.61–7.65 (2H, m, ArH), 7.66–7.70 (0.7H, m, ArH), 7.76–7.79 (1.3H, m, ArH), 7.80–7.84 (2H, m, ArH), 8.07 (1H, t, *J* = 8.0 Hz, ArH), 8.53 (0.7H, d, *J* = 12.8 Hz, ArH), 10.59 (0.3H, d, *J* = 14.0 Hz, NH), 10.77 (0.7H, d, *J* = 13.2 Hz, NH); ^{13}C NMR (100 MHz) *δ ppm*: 111.34, 112.99, 113.36, 118.35, 119.21, 126.92, 127.55, 127.64, 127.75, 127.82, 128.45, 128.68, 129.15, 129.40, 129.76, 130.59, 131.28, 131.41, 133.22, 133.81, 137.20, 137.31, 137.48, 138.92, 140.30, 140.76, 141.38, 142.34, 142.76, 142.78, 143.02, 148.23, 148.92, 151.12, 188.65, 190.10 (C═O); IR (KBr, *ν* cm^{-1}): 3316, 3231 (NH_2, NH), 1624 (C═O) and 1336, 1295, 1144, 1090 ($2SO_2$); EA Calcd.: C, 52.88; H, 3.59; N, 5.87; found C, 53.13; H, 3.76; N, 5.98.

*5.1.3.16. 4-((3-oxo-3-phenyl-2-(phenylsulfonyl)prop-1-en-1-yl) amino)benzoic acid **15d***. White powder, (yield 79%), m.p. 287–288 °C; ^{1}H NMR (400 MHz) *δ ppm*: 7.34–7.42 (2.7H, m, ArH), 7.44–7.48 (2.3H, m, ArH), 7.50–7.54 (2H, m, ArH), 7.57–7.69 (3H, m, ArH), 7.73 (1.3H, d, *J* = 8.0 Hz, ArH), 7.91–7.97 (2H, m, ArH), 8.07–8.13 (1H, m, ArH), 8.53 (0.7H, d, *J* = 13.2 Hz, ArH), 10.55 (0.3H, d, *J* = 14.0 Hz, NH), 10.71 (0.7H, d, *J* = 13.2 Hz, NH), 12.90 (1H, s, OH); ^{13}C NMR (100 MHz) *δ ppm*: 111.50, 113.65, 117.82, 118.56, 127.00, 127.53, 127.59, 127.77, 128.43, 128.54, 128.68, 129.08, 129.23, 129.34, 129.36, 129.51, 129.71, 130.99, 131.38, 131.52, 132.40, 132.49, 133.08, 133.73, 138.60, 138.78, 142.89, 143.12, 143.30, 143.82, 147.67, 150.73, 167.13, 167.25 (C═O of COOH), 189.80, 191.35 (C═O); IR (KBr, *ν* cm^{-1}): 3363-2434 (OH), 3128 (NH), 1684, 1626 (2C = O) and 1357, 1174 (SO_2); EA Calcd.: C, 64.85; H, 4.21; N, 3.44; found C, 65.37; H, 4.37; N, 3.51.

*5.1.3.17. 4-((3-(4-bromophenyl)-3-oxo-2-(phenylsulfonyl)prop-1-en-1-yl)amino)benzoic acid **15e***. White powder, (yield 79%), m.p. over 300 °C; ^{1}H NMR (400 MHz) *δ ppm*: 7.39–7.47 (3.7H, m, ArH), 7.50–7.54 (1.3H, t, *J* = 7.6 Hz, ArH), 7.57 (2H, d, *J* = 8.8 Hz, ArH), 7.60 (1H, t, *J* = 8.0 Hz, ArH), 7.67 (1H, d, *J* = 8.0Hz, ArH), 7.76 (1.3H, d, *J* = 8.0 Hz, ArH), 7.93–7.98 (2H, m, ArH), 8.11 (1H, d, *J* = 8.0 Hz, ArH), 8.55 (0.7H, d, *J* = 13.2 Hz, ArH), 10.58 (0.3, d, *J* = 14.0 Hz, NH), 10.76 (0.7H, d, *J* = 13.2 Hz, NH), 12.9 (1H, s, OH); ^{13}C NMR (100 MHz) *δ ppm*: 111.29, 113.16, 113.31, 117.40, 117.98,118.70, 119.09, 126.19, 126.29, 127.17, 127.54, 127.61, 127.75,128.45, 129.08, 129.38,129.75, 130.70, 131.00, 131.36, 131.40, 131.46, 131.52, 131.59, 131.71, 132.07, 132.31, 133.19, 133.79, 134.59, 135.09, 137.70, 137.88, 139.63, 142.77, 143.02, 143.17, 143.70, 148.12, 150.85, 167.15, 167.24 (C═O of COOH), 188.82, 190.27 (C═O); IR (KBr, *ν* cm^{-1}): 3387-2360 (OH), 3119 (NH), 1685, 1630 (2C = O) and 1361, 1170 (SO_2); EA Calcd.: C, 54.33; H, 3.32; N, 2.88; found C, 54.21; H, 3.17; N, 2.97.

*5.1.3.18. 4-((3-(4-chlorophenyl)-3-oxo-2-(phenylsulfonyl)prop-1-en-1-yl)amino)benzoic acid **15f***. White powder, (yield 79%), m.p. over 300 °C; ^{1}H NMR (400 MHz) *δ ppm*: 7.42–7.44 (2H, m, ArH), 7.45–7.48 (2H, m, ArH), 7.49–7.54 (2.3H, m, ArH), 7.60–7.64 (1.7H, m, ArH), 7.64–7.71 (1H, m, ArH), 7.76 (1.3H, d, *J* = 8.0 Hz, ArH), 7.92–7.98 (2H, m, ArH), 8.11 (1H, d, *J* = 8.0 Hz, ArH), 8.54 (0.7H, d, *J* = 12.4 Hz, ArH), 10.58 (0.3H, d, *J* = 14.0 Hz, NH), 10.74 (0.7H, d, *J* = 13.2 Hz, NH), 12.90 (1H, s, OH); ^{13}C NMR (100 MHz) *δ ppm*: 111.34, 113.37, 117.94, 118.72, 127.12, 127.60, 127.62, 127.82, 128.35, 128.69, 129.13, 129.36, 130.22, 130.66, 131.12, 131.32, 131.48, 131.84, 132.25, 133.12, 133.72, 133.98, 134.50, 137.14, 137.31, 137.37, 137.62, 141.03, 142.09, 142.92, 143.18, 143.26, 143.79, 147.95, 150.81, 151.90, 167.11, 167.19 (C═O of COOH), 188.56, 190.01 (C═O); IR (KBr, *ν* cm^{-1}): 3313-2456 (OH), 3114 (NH), 1686, 1625 (2C = O) and 1359, 1171 (SO_2); (ESI) *m/z* 440 [M − H]$^+$; EA Calcd.: C, 59.80; H, 3.65; N, 3.17; found C, 59.96; H, 3.97; N, 3.38.

5.1.5. Synthesis of targeted compounds 13a,b

A solution of sulphanilamide **9a** or PAPA **9b** (5.5 mmol) in 15 mL of HCl was diazotized with equivalent amount of $NaNO_2$. The previous freshly prepared solution was added gradually to solution of 1-phenyl-2-(phenylsulfonyl)ethan-1-one **4a** (1.3g, 5.0 mmol) in 20 mL pyridine at (0–5) °C over 33 min. The reaction mixture was left for stirring for 12 h at 0 °C then poured onto ice water, the formed precipitate was collected and recrystallized form EtOH/dioxane mixture to furnish the targeted final compounds **13a,b**.

*5.1.4.1. 4-(2-(2-oxo-2-phenyl-1-(phenylsulfonyl)ethylidene)hydrazineyl)benzenesulfonamide **13a***. White powder, (yield 84%), m.p. 240–241 °C; ^{1}H NMR (400 MHz) *δ ppm*: 7.17 (2H, d, *J* = 8.0 Hz, ArH), 7.25 (2H, s, SO_2NH_2), 7.62 (2H, d, *J* = 8.0 Hz, ArH), 7.70–7.75 (3H, m, ArH), 7.79–7.82 (3H, m, ArH), 7.83 (2H, d, *J* = 8.0 Hz, ArH), 7.95 (2H, d, *J* = 8.0 Hz, ArH), 11.58 (1H, s, NH); ^{13}C NMR (100 MHz) *δ ppm*: 114.46, 116.72, 127.65, 127.82, 128.68, 129.80, 129.89, 130.11, 130.29, 134.73, 135.25, 135.88, 136.96, 138.43, 140.02, 140.28, 142.03, 145.79, 189.53 (C═O); IR (KBr, *ν* cm^{-1}): 3317, 3220 (NH_2, NH), 1642 (C═O) and 1332, 1301, 1155, 1138 ($2SO_2$); (ESI) *m/z* 442 [M − H]$^+$; EA Calcd.: C, 54.17; H, 3.86; N, 9.48; found C, 53.97; H, 3.59; N, 9.81.

*5.1.4.2. 4-(2-(2-oxo-2-phenyl-1-(phenylsulfonyl)ethylidene)hydrazineyl)benzoic acid **13b***. White powder, (yield 79%), m.p. 255–256 °C; ^{1}H NMR (400 MHz) *δ ppm*: 7.14 (2H, d, *J* = 8.0 Hz, ArH), 7.61 (2H, t, *J* = 8.0 Hz, ArH), 7.69 (2H, t, *J* = 8.0 Hz, ArH), 7.77–7.80 (2H, m, ArH), 7.83–7.88 (3H, m, ArH), 7.93–7.96 (3H, m, ArH), 11.75 (1H, s, NH), 12.67 (1H, s, OH); ^{13}C NMR (100 MHz) *δ ppm*: 114.29, 116.42, 117.84, 121.71, 125.28, 127.01, 127.28, 127.64, 128.64, 128.68, 128.71, 129.80, 129.88, 130.10, 130.29, 131.33, 131.47, 132.19, 133.30, 133.98, 134.70, 135.11, 135.30, 135.83, 136.97, 138.57, 140.09, 140.19,

140.38, 141.44, 142.85, 143.88, 144.51, 145.61, 146.80, 148.73, 167.19, 167.37 (C=O of COOH), 186.84, 189.59 (C=O); IR (KBr, ν cm^{-1}): 3324-2523 (OH), 3211 (NH), 1684, 1646 (2C = O) and 1312, 1166 (SO_2); (ESI) m/z 407 $[M - H]^+$; EA Calcd.: C, 61.76; H, 3.95; N, 6.86; found C, 61.68; H, 3.96; N, 6.95.

5.2. Biological evaluation

The used procedure performed as reported for carbonic anhydrase inhibitory activity [30–32] and anti-proliferative study [33–35] mentioned in the Supplementary Materials.

5.3. Molecular modelling

The procedures applied in the docking experiments for compounds **7a, 8a, 8e, 12a, 13a** and **15a** within CA I (PDB 3K34) and CA II (PDB 3K34) were provided in the supplementary materials [36–42].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2021.113486.

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