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Novel 3-substituted coumarins as selective human carbonic anhydrase IX and XII inhibitors: Synthesis, biological and molecular dynamics analysis

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ABSTRACT

In this study, diverse series of coumarin derivatives were developed as potential carbonic anhydrase inhibitors (CAIs). A "tail" approach was adopted by selecting the coumarin motif as a tail that is connected to the ZBG benzenesulfonamide moiety *via* a hydrazine (**4a**,**b**) or hydrazide (**5a**,**b**) linker. Thereafter, an aryl sulfone tail was incorporated to afford the dual tailed coumarin-sulfonamide arylsulfonehydrazones (**13a-d**) and hydrazides (**14a**,**b**). Then, the ZBG were removed from compounds **13** and **14** to furnish coumarin arylsulfonehydrazones (**11a-d**) and hydrazides (**12a**,**b**). Coumarin-sulfonamides **4** and **5** emerged as non-selective CAIs as they displayed good inhibitory activities toward all the examined CA isozymes (I, II, IX and XII) in the nanomolar ranges. Interestingly, the "dual-tail" approach (compounds **13** and **14**) succeeded in achieving a good activity and selectivity toward CA IX/XII over the physiologically dominant CA I/II. In particular, compounds **13d** and **14a** were the most selective coumarin-sulfonamide counterparts. Concerning non-sulfonamide coumarin derivatives, coumarins **8** exhibited excellent activity and selectivity profiles against the target *h*CA IX/XII, whereas, coumarins **11** and **12** reported excellent selectivity profile, but they barely inhibited *h*CA IX/XII with K_Is spanning in the micromolar ranges. Furthermore, molecular modelling studies were applied to get a deep focus about the feasible affinities and binding interactions for target coumarin-sulfonamides **4**, **5**, **13** and **14** with the active site for CA II, IX and XII isoforms.

1. Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) are metalloenzymes, carrying a zinc (II) ion which is essential for catalysis of hydration of CO_2 and dehydration of HCO_3^- [1–3]. Seven families have been discovered within carbonic anhydrases from which α-CAs exist in vertebrates, the cytoplasm of green plants, bacteria, and algae [4]. Regarding hCAs, fifteen different CA isozymes belonging to the α class have been identified, among them isozymes hCA IX and XII are important in the cancer cells pH regulatory machine, as their inhibition was considered to be an effective antitumor strategy [3]. The expression of hCA IX is strongly related to poor prognosis in cancers, whereas hCA XII isozyme is expressed in plenty of normal tissues and organs, like endometrium, eye kidney, and colon and

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overexpressed in a wide range of human cancers. Additionally, hCA I and II are ubiquitous cytosolic isozymes and their inhibition is to be avoided to overcome the upsetting side effects when treatment of cancer is the main target [5].

Classical CAIs are primarily sulfonamide-based compounds. The sulfonamide function is the most widely used zinc-binding group (ZBG) among the CAIs which include the long-lasting clinically used CAI- drugs as acetazolamide, furosemide and indisulam. Non-classical CAIs are on contrast not based on ZBG. Non-classical CAIs as polyamines, phenols, carboxylic acids and coumarins could inhibit CA catalytic activity by binding directly to the catalytic zinc moiety, through anchoring the zinc-bound water/hydroxide ion or binding to the amino acid residues outside the active site, occluding the entry of substrate and preventing catalysis [6].

In 2009, Supuran CT. et al., have reported coumarin derivatives **I** as a promising novel class of non-zinc mediated CAIs and suggested that the product of coumarin hydrolysis, *cis*-2-hydroxy-cinnamic acid, but not the coumarin moiety bound with the CA active site [7]. Interestingly, the high selective inhibitory action toward cancer-related isozymes (hCA IX and XII) exerted by coumarin-based CAIs is attributed to the binding of the coumarin hydrolysis product at the entrance for the active site cavity; the region which significantly differ amongst the diverse hCAs [8,9]. The discovery of this CAI class paved the way to develop a considerable number of structurally diverse coumarin-based CAIs with efficient and selective profiles toward the cancer-associated isozymes IX and XII over the ubiquitous CA I and II isozymes [10–12], such as umbelliferone **II** and compound **III** [13,14] (Fig. 1).

Recently our research group adopted the "tail" approach, a successful strategy to design isoform-selective CAIs, to develop several sulfonamide-based CAIs with good potency and selectivity profiles toward the cancer-related isozymes hCA IX and XII [15–18], such as compounds **IV** [19] and **V** [20,21] (Fig. 1).

In this research work, our aim is to explore the CA inhibitory activities for novel series of coumarin-based small molecules. Our design is based on bioisosteric replacement for the *para*-substituted phenyl and isatin tails, in CAIs **IV** and **V**, by a coumarin motif to furnish target coumarin-sulfonamide hydrazones (**4a**,**b**) and hydrazides (**5a**,**b**) (Fig. 1). Thereafter, a "dual-tail" approach was utilized through merging an aryl sulfone tail within compounds **4** and **5** to afford target coumarin-sulfonamide arylsulfonehydrazones (**13a-d**) and hydrazides (**14a**,**b**), respectively, which expected to modulate the interactions at both hydrophobic and hydrophilic regions of CA active site. Moreover, the ZBG were removed from compounds **13** and **14** affording target coumarin arylsulfonehydrazones (**11a-d**) and hydrazides (**12a**,**b**), respectively, in order to explore the CA inhibitory impact of the coumarin motif only. In addition, the coumarin moiety was decorated with 6-bromo substituent in order to furnish a lipophilic environment which should be suitable for the hydrophobic nature of the *h*CA IX active site and to explore a valuable SAR.

2. Result and discussion

2.1. Chemistry

The proposed synthetic approaches to develop the target coumarins in this manuscript have been shown in Schemes 1, **2** and **3**. First, synthesis of coumarin-sulfonamide hydrazones (**4a**,**b**) and hydrazides (**5a**,**b**) has been accomplished by condensation of 4-hydrazinobenzenesulfonamide **2** or 4-sulfamoylbenzoic acid hydrazide **3** with 3-acetylcoumarins **1a,b** in boiling glacial acetic acid (Scheme 1).

In Scheme 2, 3-acetylcoumarins **1a**,**b** were subjected to bromination through reaction with Br_2 in glacial acetic acid to yield 3-(bromoacetyl)coumarins **6a**,**b**, which subsequently treated with sodium benzenesulfinates **7a**,**b** in refluxing absolute ethanol to afford 3-(2-(phenylsulfonyl)acetyl)coumarin derivatives **8a-d**. Thereafter, reaction of compounds **8a-d** with phenylhydrazine **9** or benzoic acid hydrazide **10** in absolute ethyl alcohol with addition of catalytic amount of glacial acetic acid furnished coumarin arylsulfonehydrazones (**11a-d**) and hydrazides (**12a**,**b**), respectively, in

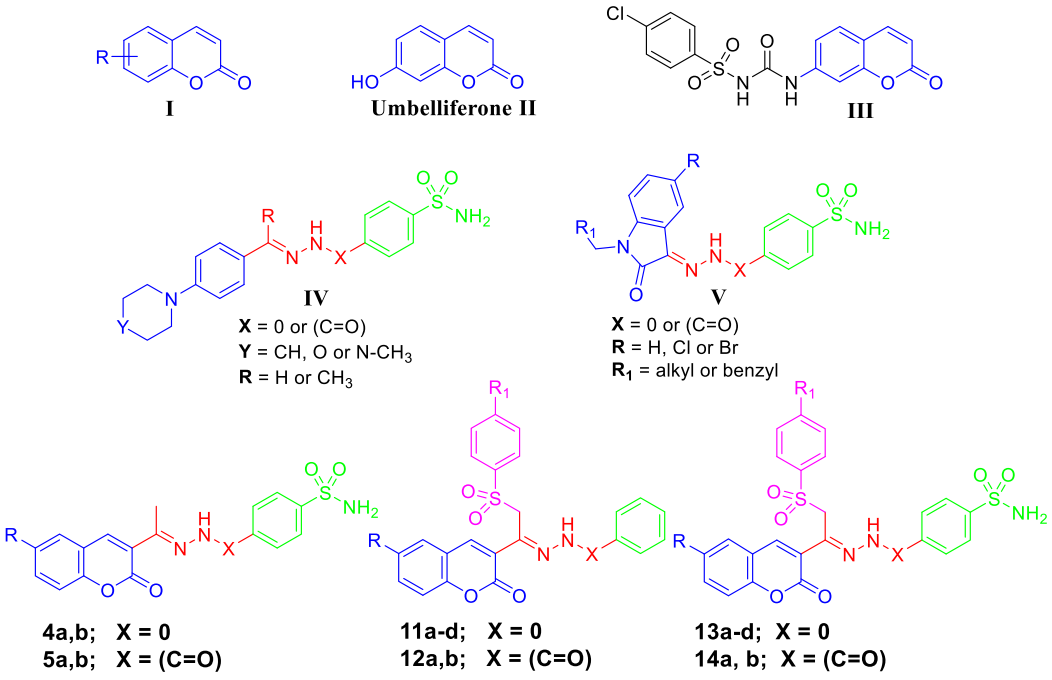
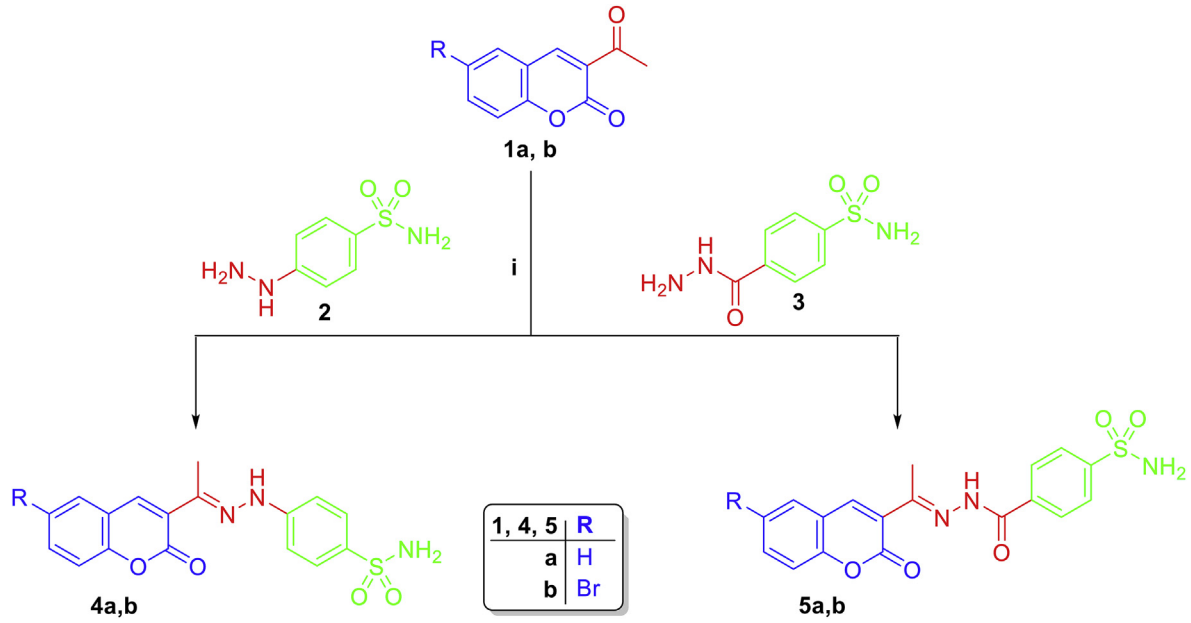
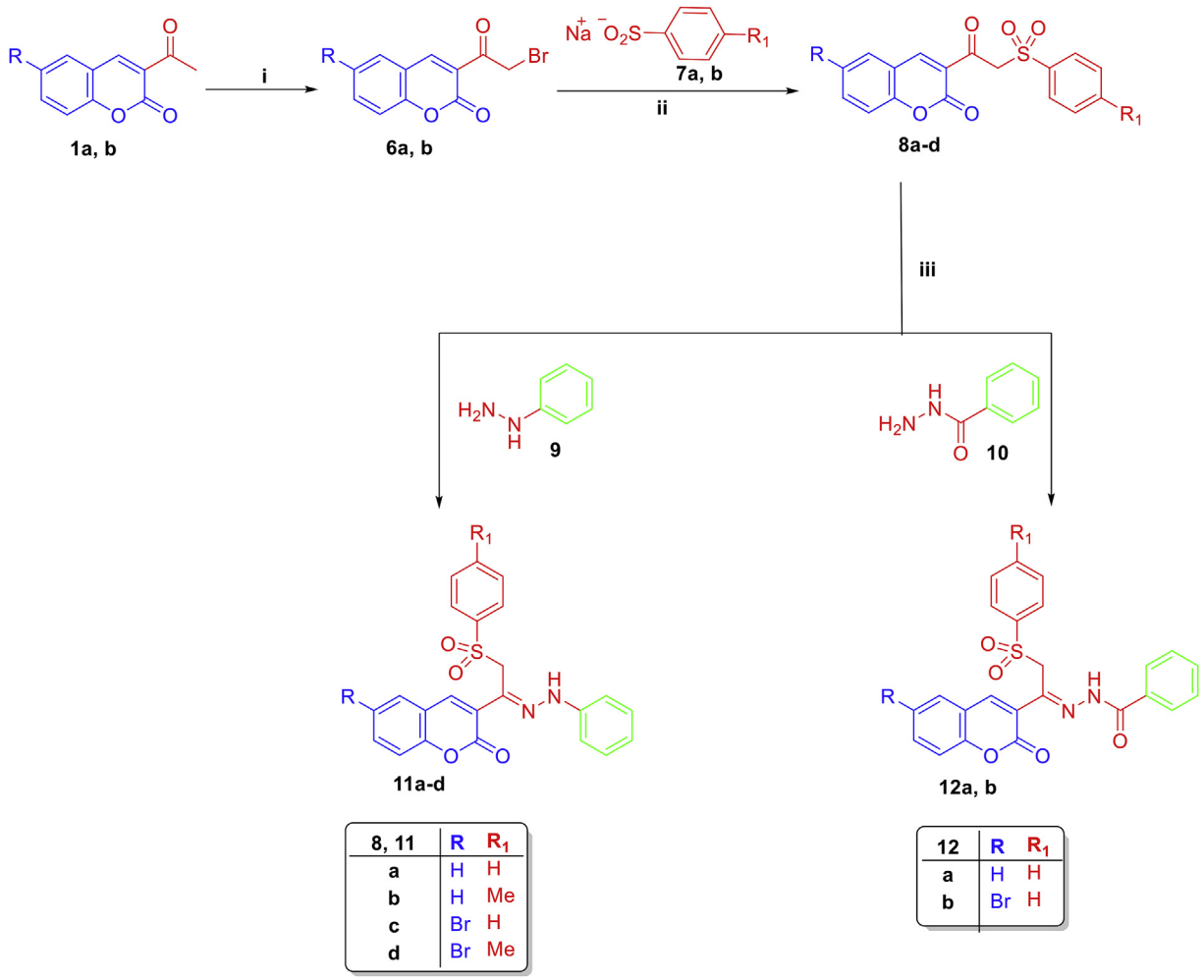


Fig. 1. Chemical structures for some coumarin-based (**I-III**) and sulphonamide-based (**IV** and **V**) CAIs, in addition to the structures of the target coumarin derivatives **4**, **5**, **11**, **12**, **13** and **14**.



Scheme 1. *Reagents and condition*: **(i)** Glacial acetic acid/reflux 2 h.



Scheme 2. *Reagents and condition*: **(i)** Br_2/Glacial acetic acid/Stirring r.t 5 h; **(ii)** Absolute ethyl alcohol/reflux 3 h; **(iii)** Ethyl alcohol/Acetic acid/reflux 5 h.

74–82% yield.

Finally, condensation of 3-(2-(phenylsulfonyl)acetyl)coumarins **8a-d** with 4-hydrazinobenzenesulfonamide **2** and 4-sulfamoylbenzoic acid hydrazide **3** produced target coumarin-sulfonamide arylsulfonehydrazones (**13a-d**) and hydrazides (**14a**,**b**), respectively, in 74–78% yield (Scheme 3).

Postulated structures for the target coumarins were in agreement with their various spectral and analytical data.

2.2. Biological evaluation

2.2.1. Carbonic anhydrase inhibition

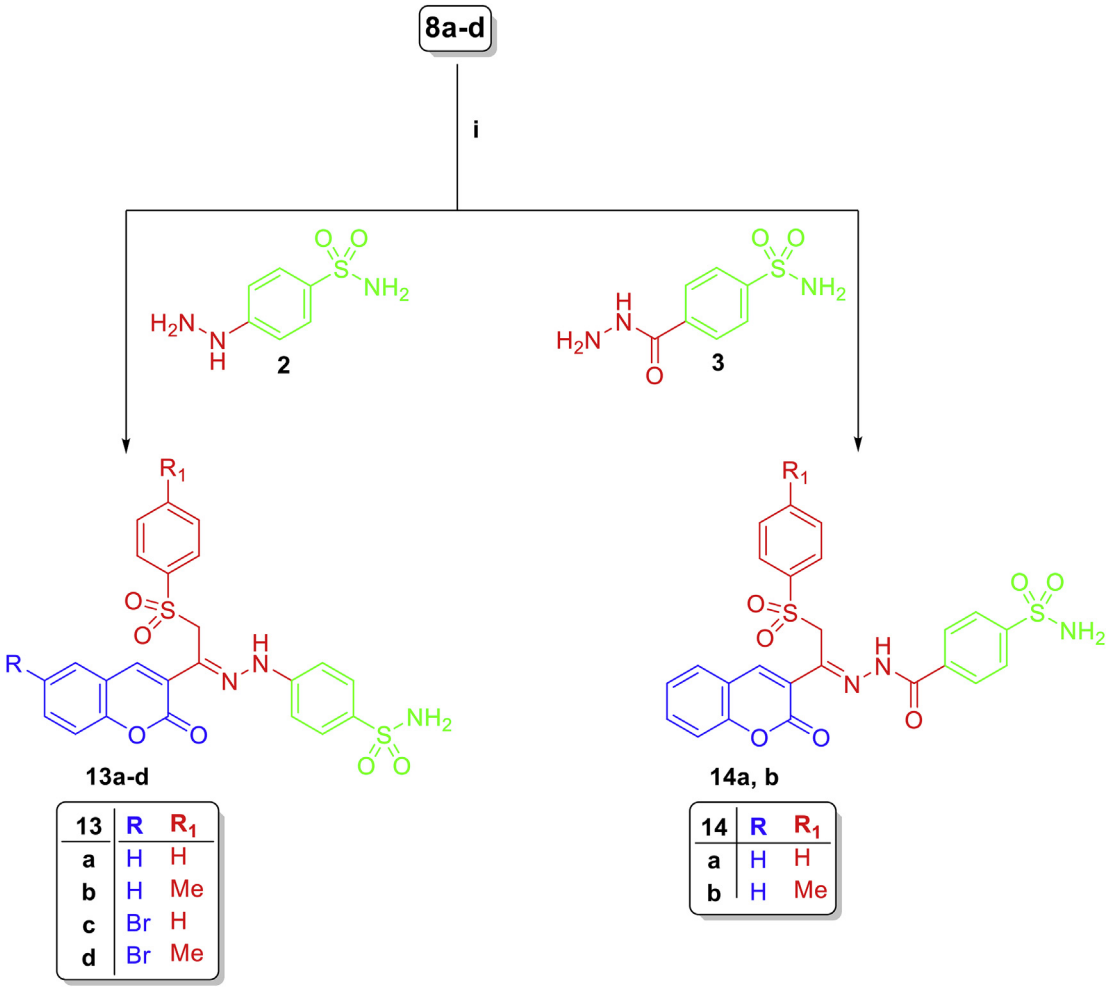
Target coumarins **4, 5, 8, 11, 12, 13** and **14** were assessed for their CA inhibitory actions toward the ubiquitous CA I and II (cytosolic) and CA IX and XII (tumor-related) isozymes by the use of a stopped flow CO_2 hydrase assay, utilizing acetazolamide (**AAZ**) as reference CAI. Several structure activity relationships (SARs) were highlighted from the inhibition data displayed in Table 1.

The ubiquitous cytosolic *h*CA I isozyme was affected by the synthesized coumarin derivatives in a variable degree. While coumarin-sulfonamide hydrazones (**4a**,**b**) and hydrazides (**5a**,**b**) showed potent *h*CA I inhibitory action with inhibition constant (K_I) values in the nanomolar range (K_Is = 98.8, 159.7, 77.6 and 92.5 nM, respectively), coumarin-arylsulfone sulfonamide hydrazones (**13a-d**) and sulfonamide hydrazides (**14a**,**b**) weakly inhibited *h*CA I isoform with K_I values in the low micromolar range, as mentioned, between 1.35 and 3.96 μM, except **13a** which displayed sub-micromolar potency (K_I = 0.82 μM). On the contrary, coumarin-arylsulfones **8a-d**, **11a-d** and **12a,b** failed to inhibit CA I isoform up to 100 μM (Table 1).

The *in vitro* kinetic data presented in Table 1 suggested that inhibition profile for the physiologically dominant CA II isozyme is analogous to the inhibition profile for CA I. Coumarin-sulfonamide hydrazones (**4a**,**b**) and hydrazides (**5a**,**b**) potently inhibited CA II with K_I values equal 29.1, 40.0, 26.3 and 12.1 nM, respectively, whereas coumarin-arylsulfone-sulfonamide hydrazones (**13a-d**) and hydrazides (**14a**,**b**) displayed moderate *h*CA II inhibitory action with K_I values in the high nanomolar range (224.8–559.7 nM). On the other hand, coumarin-arylsulfones **8a-d**, **11a-d** and **12a**,**b** didn't inhibit *h*CA II isoform up to 100 μM.

It is worthy to mention that, presence of sulfonamide functionality is essential for inhibitory activity toward both *h*CA I and II isoforms. Moreover, replacement of the hydrazine linker in coumarin-sulfonamide **4a**,**b** with the hydrazide one furnished coumarin-sulfonamide **5a**,**b** with slightly enhanced *h*CA I and *h*CA II inhibitory actions, whereas, incorporation of the arylsulfone moiety illustrated a lowering of effectiveness toward *h*CA I and *h*CA II isoforms both for the hydrazones **13** and the hydrazides **14** in comparison to their counterparts **4** and **5** (Table 1).

The investigated coumarin-sulfonamide hydrazones/hydrazides (**4a**,**b** and **5a**,**b**), coumarin-arylsulfones (**8a-d**) and coumarin-arylsulfone-sulfonamide hydrazones/hydrazides (**13a-d** and **14a**,

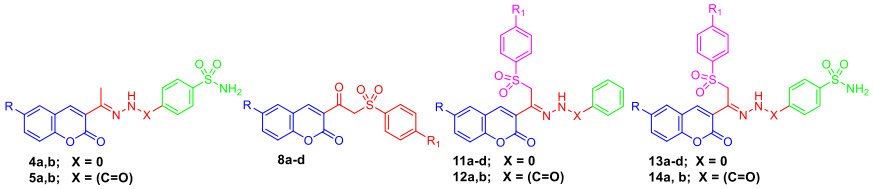


13	R	R_1
a	H	H
b	H	Me
c	Br	H
d	Br	Me

14	R	R_1
a	H	H
b	H	Me

Scheme 3. *Reagent and conditions*: **(i)** Ethanol/Acetic acid/reflux 5 h.

Table 1
Inhibition data for *h*CA isozymes I, II, IX and XII for target coumarins **4, 5, 8, 11, 12, 13, 14** using **AAZ** as a standard CAI.



Cmp	R	R_1	X	K_I (nM)[a]			
				CA I	CA II	CA IX	CA XII
4a	H	–	–	98.8	29.1	43.8	10.1
4b	Br	–	–	159.7	40.0	24.2	55.8
5a	H	–	C=O	77.6	26.3	31.6	19.7
5b	Br	–	C=O	92.5	12.1	19.8	34.6
8a[b]	H	H	–	>100 μM	>100 μM	41.4	60.1
8b[b]	H	CH_3	–	>100 μM	>100 μM	66.3	51.5
8c[b]	Br	H	–	>100 μM	>100 μM	76.6	45.7
8d[b]	Br	CH_3	–	>100 μM	>100 μM	87.2	83.7
11a[b]	H	H	–	>100 μM	>100 μM	1.50 μM	1.81 μM
11b[b]	H	CH_3	–	>100 μM	>100 μM	3.94 μM	1.05 μM
11c[b]	Br	H	–	>100 μM	>100 μM	4.64 μM	7.21 μM
11d[b]	Br	CH_3	–	>100 μM	>100 μM	8.11 μM	4.77 μM
12a[b]	H	H	C=O	>100 μM	>100 μM	16.0 μM	6.59 μM
12b[b]	Br	H	C=O	>100 μM	>100 μM	8.86 μM	9.34 μM
13a	H	H	–	824.2	442.4	89.1	74.4
13b	H	CH_3	–	2596	559.7	76.0	91.1
13c	Br	H	–	3455	276.0	63.6	36.2
13d	Br	CH_3	–	3962	538.3	29.2	12.0
14a	H	H	C=O	1537	224.8	18.6	32.3
14b	H	CH_3	C=O	1352	365.2	42.1	76.8
AAZ	–	–	–	250.0	12.0	25.0	5.7

[a] Mean from three different assays (errors were calculated in the range of ± 5–10% of the aforementioned values).
[b] Incubation time of 6 h (for coumarin derivatives only).

b) stood out as efficient inhibitors for the cancer-related *h*CA IX isozyme (K_I values: 18.6–89.1 nM), whereas coumarin arylsulfone-hydrazones/hydrazides (**11a-d** and **12a**,**b**) exerted weak *h*CA IX inhibitory action with K_I values in the micromolar range (1.5–16.0 μM). In particular, hydrazides **5b** and **14a** stood out as the best CA IX inhibitors in the current study with low nanomolar potencies (K_Is = 19.8 and 18.6 nM, respectively).

Remarkably, the deduced SAR pointed out that utilizing the hydrazide linker (compounds **5a**, **5b** and **14a**, **14b**; K_Is = 31.6, 19.8, 18.6 and 42.1 nM, respectively) is more profitable for inhibitory action toward *h*CA IX than the hydrazine linker (compounds **4a**, **4b** and **13a**, **13b**; K_Is = 43.8, 24.2, 89.1 and 76.0 nM, respectively), Table 1. Notably, the increased activities of 6-Br substituted coumarins **4b**, **5b**, **13c** and **13d** (K_Is = 24.2, 19.8, 63.6 and 29.2 nM, respectively) than their corresponding unsubstituted derivativess **4a**, **5a**, **13a** and **13b** (K_Is = 43.8, 31.6, 89.1 and 76.0 nM, respectively), highlighted that grafting of 6-Br group within coumarin moiety is advantageous for *h*CA IX inhibitory activity. The obtained SAR toward *h*CA II and IX isoforms was illustrated in Fig. 2.

The second cancer-associated isozyme *h*CA XII was potently inhibited by all coumarin derivatives prepared here with K_Is spanned in the range 10.1–91.1 nM, except coumarins **11** and **12** which barely inhibited *h*CA XII (K_Is: 1.05–9.34 μM). Superiorly, compound **4a** exerted the best *h*CA XII inhibition here reported with K_I = 10.1 nM.

On the contrary to the *h*CA IX inhibition profile, 6-Br substitution of the coumarin scaffold resulted in a worsening of effectiveness toward *h*CA XII within coumarin-sulfonamide hydrazones/hydrazides **4** and **5**, whereas such substitution within coumarin-arylsulfone- sulfonamide hydrazones **13** led to an improvement of the activity with about 2- and 7.5-fold potency enhancement likened to the unsubstituted derivatives.

Regarding target/off-target *h*CAs selectivity ratio of action for herein reported coumarin derivatives, all the examined coumarins, except **4** and **5**, displayed significant I/IX and I/XII inhibitory selectivity, with calculated SI spanning the ranges 9.3 – >2415.5 and 11.1 – >2188.2, respectively, Table 2. Moreover, all compounds, except **4a**, **b** and **5a**, **b**, exhibited a preferred inhibition action for target CA IX and XII isozymes over the main off-target CA II isoform with SIs spanning between 4.3 and >2415.5 and 4.8 – >2188.2, respectively, Table 2.

Investigation of the calculated SIs for coumarin-sulfonamides **4**, **5**, **13** and **14** (Table 2) revealed that the dual tailed compounds **13d** and **14a** were the most selective counterparts among these series toward *h*CA IX/XII isoforms. Concerning non-sulfonamide coumarin derivatives, coumarins **8a-d** stood out as the most efficient tumor-associated isoforms *h*CA IX/XII inhibitors reported here in terms of both activity and selectivity. Coumarins **8a-d** could not inhibit *h*CA I and II lower than 100 μM, whereas, *h*CA IX and XII were inhibited in low nanomolar ranges: 41.4–87.2 and 45.7–83.7 nM, respectively and possessed excellent selectivity profile (SI = >1146.8 – >2415.5). In contrast, coumarin arylsulfone-hydrazones/hydrazides **11a-d** and **12a**,**b** reported good selectivity profile (SI = >6.3 – >95.2), but they barely inhibited *h*CA IX/XII with K_I values in the micromolar ranges: 1.5–16.0 and 1.05–9.34 μM, respectively.

3. *In silico* analysis

Investigation on the binding mode of coumarin-sulfonamides **4a,b**, **5a,b**, **13a-d** and **14a,b** within the active sites for *h*CA II (pdb code 5LJT), *h*CA IX (pdb code 5FL4) and *h*CA XII (pdb code 1JD0) was

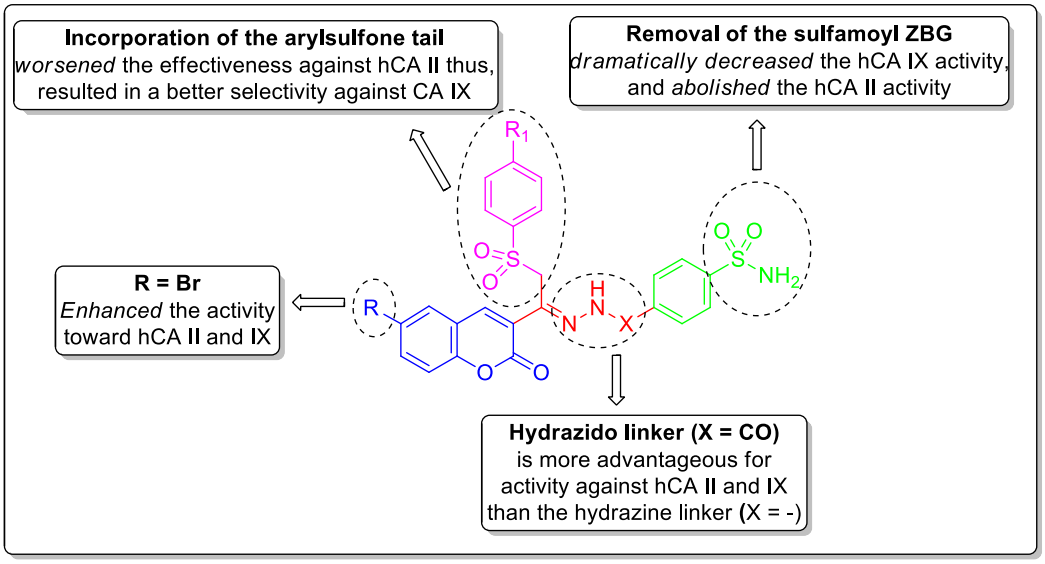


Fig. 2. SAR for the target CAIs toward *h*CA II and IX isoforms.

Table 2
Selectivity index (SI) calculated for hCA (IX and XII) over off-targets hCA isozymes (I and II) as ratio between K_Is.

Cmpd	I/IX	I/XII	II/IX	II/XII
4a	2.3	9.8	0.7	2.9
4b	6.6	2.9	1.7	0.7
5a	2.5	3.9	0.8	1.3
5b	4.7	2.7	0.6	0.3
8a	>2415.5	>1663.9	>2415.5	>1663.9
8b	>1508.3	>1941.7	>1508.3	>1941.7
8c	>1305.5	>2188.2	>1305.5	>2188.2
8d	>1146.8	>1194.7	>1146.8	>1194.7
11a	>66.7	>55.2	>66.7	>55.2
11b	>25.4	>95.2	>25.4	>95.2
11c	>21.6	>13.9	>21.6	>13.9
11d	>12.3	>21.0	>12.3	>21.0
12a	>6.3	>15.2	>6.3	>15.2
12b	>11.3	>10.7	>11.3	>10.7
13a	9.3	11.1	5.0	5.9
13b	34.2	28.5	7.4	6.1
13c	54.3	95.4	4.3	7.6
13d	135.7	330.2	18.4	44.9
14a	82.6	47.6	12.1	7.0
14b	32.1	17.6	8.7	4.8
AAZ	10.0	43.9	0.5	2.2

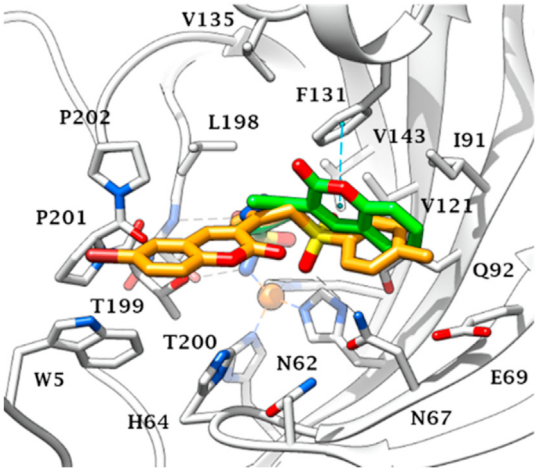


Fig. 3. Predicted binding orientation of **4a** (green) and **13d** (orange) within CA II active site. H-bonds and π-π interactions are represented as black and blue dashed lines. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

carried out by docking simulations. In general, all docking solutions revealed that benzenesulfonamide moiety was located in-depth into the CA active sites, where the sulfamoyl group was coordinated to the zinc ion trough the deprotonated nitrogen atom and it engaged two hydrogen bonds with the OG1 and backbone NH of T199. Moreover, the phenyl moiety located in an area defined by V121, V143, L198 residues where it was stabilized by the formation of hydrophobic contacts.

The reduced size of the tail of derivatives **4a,b** and **5a,b** enables a better accommodation of the ligand in the narrow binding site of CA II, when compared to the bulkier dual tailed **13a-d** and **14a,b**, inducing a greater CA II inhibition (Fig. 3).

In CA IX and XII, the coumarin moiety is placed in a cleft lined by the amino acids V131, L91, Q92, Q71 and A131, T91, Q92 and K67, respectively, while the arylsulfonyl moiety is oriented toward the hydrophilic residues Q92, Q71, N67, H64, W5 (CA IX) and Q92, K67, N62, H64, W5 (CA XII) engaging H-bonds with the $-SO_2-$ group.

To study the solvation effect and conformational behavior over the time, for the most selective coumarin-sulfonamides, against the tumor-associated isoforms (**13d** and **14a**), the docking was performed with a 100 ns long molecular dynamic (MD) simulations performed on the predicted binding poses. While the proteins are even subjected to small conformational diversions, the plots in Fig. 4 (red line) and Fig. 6 (violet) report variable dynamic evolution of the docked ligands complexed with CA IX and XII during the 100 ns.

In particular, **13d** reached a stable conformation (pose 5, Figs. 4A and 5A) within CA IX active site after 50.0 ns with RMSD fluctuating around 4.5 Å (Fig. 4A). As observed in Fig. S1A (Supporting Information), the two tails of the ligand undergo small fluctuations induced by the conformational evolution of the active site: from 50 ns onwards, the coumarin framework stably accommodates in the hydrophobic area of the cavity, whereas the sulfonyltolyl portion fits into the area between the metal-coordination pattern and residues Q92, Q71, N67, H64, W5.

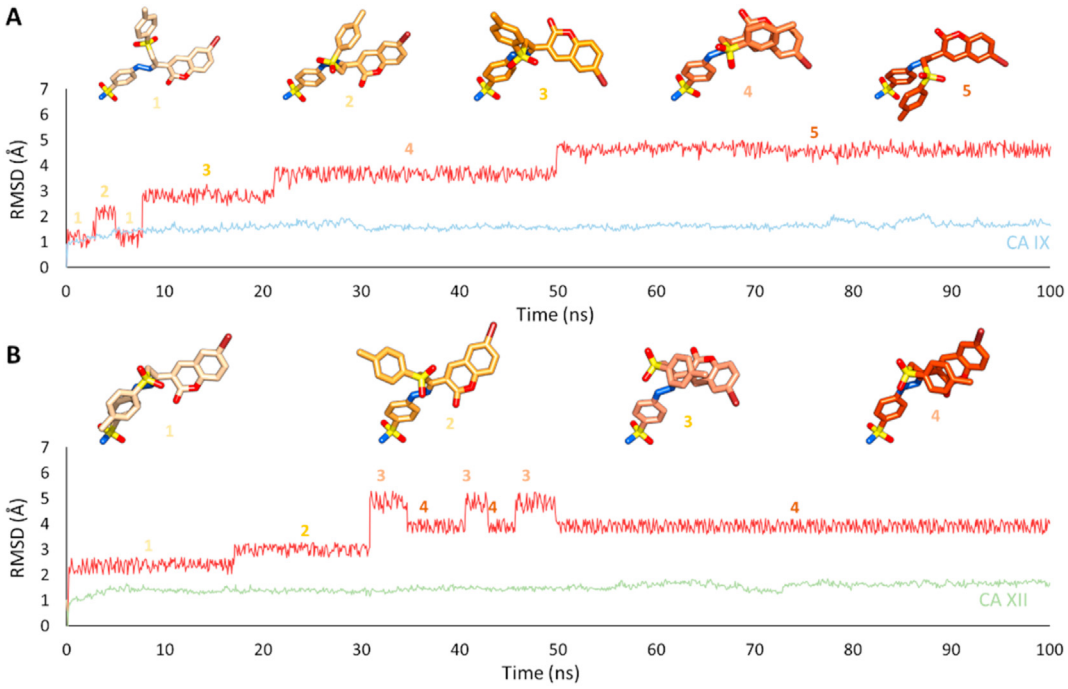


Fig. 4. RMSD analysis of ligand **13d** heavy atoms and A) CA IX and B) CA XII backbone over the 100 ns MD simulation. The ligand color darkens over the dynamic simulation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

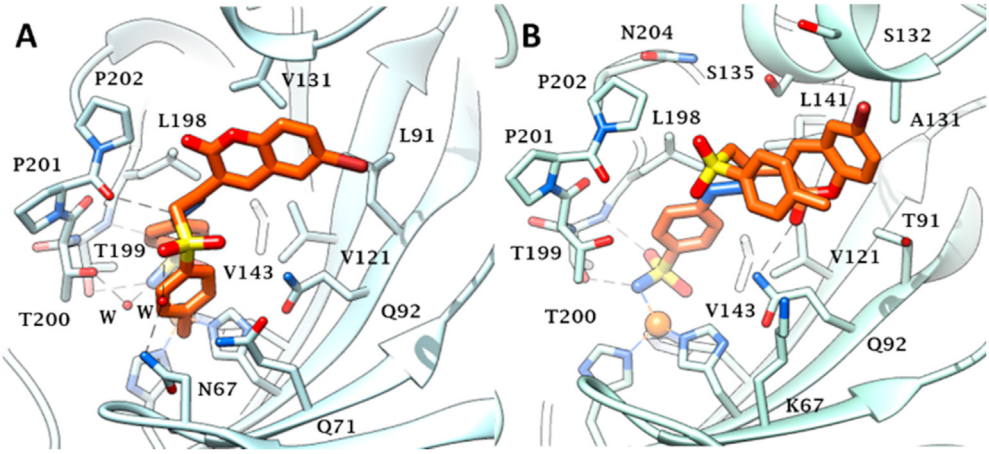


Fig. 5. Final stable binding mode of ligand **13d** into A) CA IX and B) CA XII active site after dynamic simulations of 100 ns. Water molecules are represented as red spheres. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Likewise, from about 50 ns onwards the binding conformation of **13d** stably settles within CA XII active site (Figs. 4B–5B, RMSD: 3.8 Å). In detail, the coumarin and tolyl rings move to stack one onto the other additionally forming VdW interactions with the CA XII area lined by S135, A131 and T91 and a H-bond with Q92 (Fig. S1B).

Two main binding conformations, which quickly interchange one into the other for the entire dynamic simulation, are found for **14a** within CA IX active site (Fig. 6A, lower RMSD: 2.7 Å). In all poses the hydrazide NH engages a water-bridged H-bond with the side chain C=O of Q92, while the NH_2 donates a H-bond to the exocyclic coumarin C=O (Fig. S2A). It is worth noting that moving from the hydrazone (**13d**) to hydrazide (**14a**) there is an inversion in the positioning of the two ligand tails within CA IX active site (Figs. 5A and 7A), as the coumarin framework of **14a** accommodates into the hydrophilic area previously occupied by **13d**.

As well, in CA XII, the conformation of derivative **14a** alternates between pose 1 and 2 and then stabilizes definitively in pose 3 (Figs. 6B and 7B, RMSD: 2.9 Å). In this latter pose the coumarin scaffold restores a docking-like conformation engaging a H-bond with the OH of S135 side chain whereas the methylenesulfonylphenyl pendant folds accommodating in the hydrophilic pocket nearby H64. Moreover, a water-bridged H-bond occurs between the S=O group and N204 side chain NH_2 (Fig. 7B).

4. Conclusion

In summary, the current research work described the design and synthesis of novel coumarin-sulfonamides (**4a**,**b**, **5a**,**b**, **13a-d** and **14a**,**b**) and coumarins (**8a-d**, **11a-d**, and **12a**,**b**) as efficient *h*CAIs. All the tested CA isoforms were affected by the synthesized coumarin

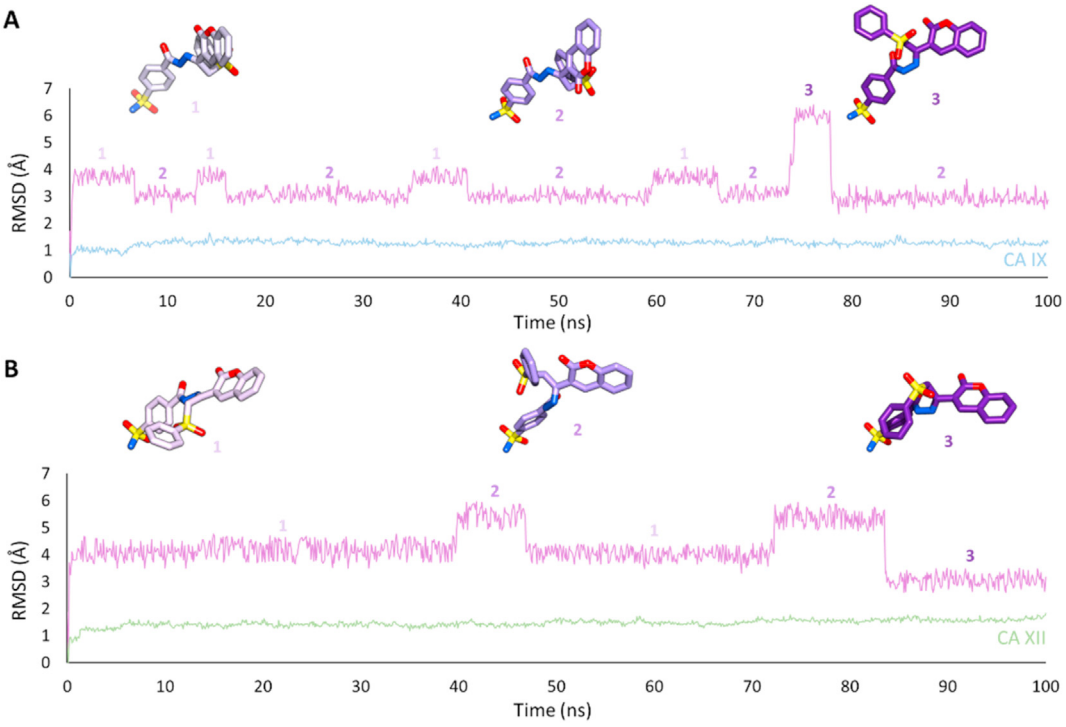


Fig. 6. RMSD analysis of ligand **14a** heavy atoms and A) CA IX and B) CA XII backbone over the 100 ns MD simulation. The ligand color darkens over the dynamic simulation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

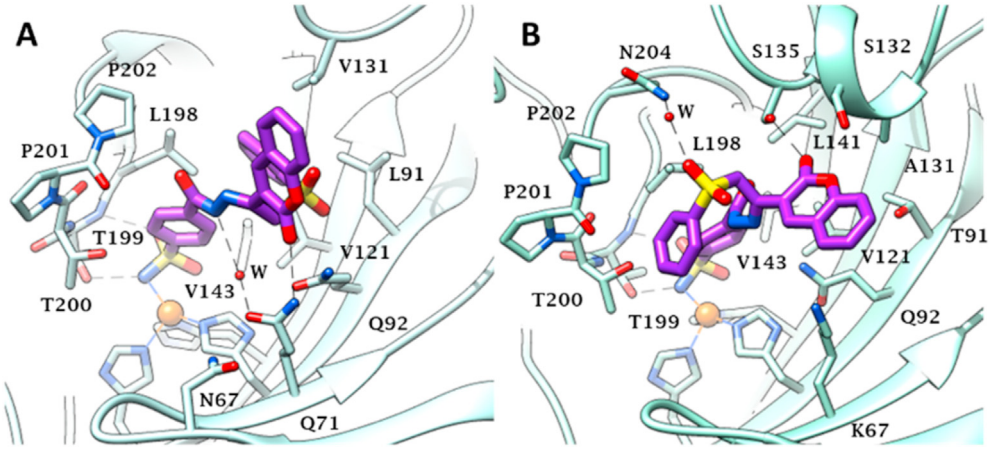


Fig. 7. Final stable binding mode of ligand **14a** into A) CA IX and B) CA XII active site after dynamic simulations of 100 ns. Water molecules are represented as red spheres. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

derivatives in variable degrees with K_I ranges: 77.6 nM−100 μM for CA I, 12.1 nM−100 μM for CA II, 19.8 nM−8.86 μM for CA XI, and 10.1 nM−9.34 μM for XII. Concerning selectivity of our target coumarins, all compounds except **4a**,**b** and **5a**,**b** showed good selectivity toward CA IX and XII over CA I (SI ranges: 6.3−2415.5 and 10.7−2188.2, respectively, respectively) and over CA II (SI ranges: 4.3−2415.5 and 4.8−2188.2, respectively). The SAR outcomes highlighted that incorporation of the arylsulfone moiety led to a worsening of effectiveness against CA I and II isoforms for the dual tailed hydrazones **13** and hydrazides **14** in comparison to their counterparts **4** and **5**, which resulted in a better selectivity profile for series **13** and **14**. This worsening of effectiveness could be attributed to the worse accommodation of the bulkier dual tailed **13** and **14** than the reduced size coumarin-tailed derivatives **4** and **5** in the narrow binding site of *h*CA I and II. Furthermore, removal of the ZBG from series **13** and **14** resulted in coumarins **11** and **12** that barely inhibited *h*CA IX/XII with K_I values in the micromolar ranges. In addition, utilizing the hydrazide linker (compounds **5a**, **5b** and **14a**, **14b**; K_Is = 31.6, 19.8, 18.6 and 42.1 nM, respectively) was found to be more profitable for inhibitory action against *h*CA IX than the hydrazine linker (compounds **4a**, **4b** and **13a**, **13b**; K_Is = 43.8, 24.2, 89.1 and 76.0 nM, respectively). The molecular modeling for **13d** revealed that the coumarin tail stably accommodated in the hydrophobic area of the cavity, whereas, the aryl sulfone tail oriented toward the hydrophilic pocket. Replacement of hydrazino linker (**13**) with the hydrazide one (**14**) resulted in an inversion in the positioning of the ligand's two tails within CA IX active site where the coumarin framework accommodated into the hydrophilic area.

5. Experimental

5.1. Chemistry

5.1.1. General

NMR spectra have been recorded by Varian Gemini-400BB 400 MHz spectrometer. ^{1}H and ^{13}C spectra were carried out at 400 and 100 MHz, respectively, 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. IR spectra have been recorded with a Bruker FT-IR spectrophotometer as KBr disks and expressed in wave number (cm^{-1}). Unless otherwise noted, all solvents and reagents were commercially available and used without further purification.

5.1.2. General procedures for synthesis of coumarin-sulfonamide hydrazones/hydrazides **4a,b** and **5a,b**

To a solution of 3-acetylcoumarin derivatives **1a,b** (1 mmol) in glacial acetic acid (5 mL), 4-hydrazinobenzenesulfonamide **2** (0.187 g, 1 mmol) or 4-sulfamoylbenzoic acid hydrazide **3** (0.215 g, 1 mmol) was added. The resulting reaction mixture was allowed to stir under reflux for 2 h. The precipitated solid was collected by filtration while hot, washed with cold ethanol, dried and recrystallized from dioxan to produce the titled coumarin-based sulfonamides **4a,b** and **5a,b**.

*5.1.2.1. 4-(2-(1-(2-Oxo-2H-chromen-3-yl)ethylidene)hydrazineyl) benzenesulfonamide (**4a**).* Yellow crystals (yield 88%), m.p. 223–225 °C; IR: 3437, 3324, 3242 (NH, NH_2), 1725 (C=O) and 1334, 1168 (SO_2); ^{1}H NMR δ *ppm*: 2.28, (s, 3H, CH_3), 7.12 (s, 1H, Ar–H), 7.36–7.45 (m, 5H, Ar–H and NH_2 D_2O exchangeable), 7.61–7.70 (m, 3H, Ar–H), 7.86 (d, 1H, J = 7.8 Hz, Ar–H), 8.26 (s, 1H, H-4 coumarin), 9.99 (s, 1H, NH D_2O exchangeable); Anal. calcd. for $C_{17}H_{15}N_3O_4S$ (357.38): C, 57.13; H, 4.23; N,11.76. Found C, 57.23; H, 4.19; N, 11.67.

*5.1.2.2. 4-(2-(1-(6-Bromo-2-oxo-2H-chromen-3-yl)ethylidene) hydrazineyl)benzenesulfonamide (**4b**).* Yellow crystals (yield 77%), m.p. 232–234 °C; IR: 3421, 3329, 3243 (NH, NH_2), 1720 (C=O) and, 1341, 1163 (SO_2); ^{1}H NMR δ *ppm*: 2.27, (s, 3H, CH_3), 7.12 (s, 2H, Ar–H), 7.37–7.42 (m, 3H, Ar–H and NH_2 D_2O exchangeable), 7.68 (d, 2H, J = 8.6 Hz, Ar–H), 7.75 (d, 1H, J = 8.8 Hz, Ar–H), 8.13 (s, 1H, Ar–H), 8.25 (s, 1H, H-4 coumarin), 10.04 (s, 1H, NH D_2O exchangeable); Anal. calcd. for $C_{17}H_{14}BrN_3O_4S$ (336.28): C, 46.80; H, 3.23; N, 9.63. Found C, 46.73; H, 3.26; N, 9.71.

*5.1.2.3. 4-(2-(1-(2-Oxo-2H-chromen-3-yl)ethylidene)hydrazine-1-carbonyl)benzenesulfonamide (**5a**).* Yellow crystals (yield 78%), m.p. 220–222 °C; IR: 3427, 3321, 3246 (NH, NH_2), 1724, 1655 (2C=O) and 1344, 1166 (SO_2); ^{1}H NMR δ *ppm*: 2.26, 2.33 (2s, 3H, CH_3), 7.38–7.52 (m, 5H, Ar–H and NH_2 D_2O exchangeable), 7.64–7.73 (m, 1H, Ar–H), 7.78–7.96 (m, 2H, Ar–H), 8.01–8.07 (m, 2H, Ar–H), 8.17, 8.25 (2s, 1H, H-4 coumarin), 10.91, 10.98 (2s, 1H, NH D_2O exchangeable); Anal. calcd. for $C_{18}H_{15}N_3O_5S$ (385.38): C, 56.10; H, 3.92; N, 10.90. Found C, 56.23; H, 3.89; N, 10.97.

*5.1.2.4. 4-(2-(1-(6-Bromo-2-oxo-2H-chromen-3-yl)ethylidene)hydrazine-1-carbonyl)benzenesulfonamide (**5b**).* Yellow crystals (yield 76%), m.p. 230–232 °C; IR: 3430, 3353, 3249 (NH, NH_2), 1722, 1639 (2C=O) and 1320, 1193 (SO_2); ^{1}H NMR δ *ppm*: 2.25, 2.32 (2s, 3H, CH_3), 7.40–7.52 (m, 4H, Ar–H and NH_2 D_2O exchangeable), 7.77 (m, 1H, Ar–H), 7.89–7.93 (m, 2H, Ar–H), 7.98–8.03 (m, 2H, Ar–H), 8.14, 8.20 (2s, 1H, H-4 coumarin), 10.91, 10.98 (2s, 1H, NH D_2O exchangeable); Anal. calcd. for $C_{18}H_{14}BrN_3O_5S$ (464.29): C, 46.57; H, 3.04; N, 9.05. Found C, 46.73; H, 3.00; N, 9.10.

5.1.3. Synthesis of 3-(bromoacetyl)coumarins **6a, b**

3-Acetylcoumarins **1a**, **b** were brominated according to the reported procedures [22,23].

5.1.4. General procedures for preparation of key intermediate of **8a-d**

The appropriate sodium benzenesulfinate derivative **7a**,**b** (15 mmol) was added to a hot stirred solution of 3-(bromoacetyl) coumarin derivatives **6a,b** (15 mmol) in absolute ethanol (15 mL), then the resulting mixture was stirred under reflux for 3 h. The formed precipitate was collected by filtration while hot, washed with methyl alcohol, dried and recrystallized dioxan/ethanol to yield 3-(2-(phenylsulfonyl)acetyl)coumarins **8a-d**.

*5.1.4.1. 3-(2-(Phenylsulfonyl)acetyl)-2H-chromen-2-one (**8a**).* Gray crystals (yield 86%), m.p. 138–140 °C; IR: 1727, 1689 (2C=O) and 1318, 1144 (SO_2); ^{1}H NMR δ *ppm*: 5.42 (s, 2H, CH_2), 7.43 (d, 1H, J = 7.9 Hz, Ar–H), 7.55–7.58 (m, 3H, Ar–H), 7.68 (t, 1H, J = 7.9 Hz, Ar–H), 7.89–7.99 (m, 4H, Ar–H), 8.15 (s, 1H, H-4 coumarin); Anal. calcd. for $C_{17}H_{12}O_5S$ (328.34): C, 62.19; H, 3.68. Found C, 62.04; H, 3.71.

*5.1.4.2. 3-(2-Tosylacetyl)-2H-chromen-2-one (**8b**).* Gray crystals (yield 73%), m.p. 152–154 °C; IR: 1733, 1684 (2C=O) and 1320, 1146 (SO_2); ^{1}H NMR δ *ppm*: 2.39 (s, 3H, CH_3), 5.41 (s, 2H, CH_2), 7.30–7.37 (m, 4H, Ar–H), 7.65–7.69 (m, 2H, Ar–H), 7.79–7.81 (m, 2H, Ar–H), 8.17 (s, 1H, H-4 coumarin); Anal. calcd. for $C_{18}H_{14}O_5S$ (342.37): C, 63.15; H, 4.12. Found C, 63.29; H, 4.10.

*5.1.4.3. 6-Bromo-3-(2-(phenylsulfonyl)acetyl)-2H-chromen-2-one (**8c**).* Gray crystals (yield 82%), m.p. 188–190 °C; IR: 1734, 1683 (2C=O) and 1349, 1156 (SO_2); ^{1}H NMR δ *ppm*: 5.29 (s, 2H, CH_2), 7.46 (d, 1H, J = 8.9 Hz, Ar–H), 7.63–7.68 (m, 2H, Ar–H), 7.73–7.76 (m, 1H, Ar–H), 7.91–7.96 (m, 3H, Ar–H), 8.22 (d, 1H, J = 2.4 Hz, Ar–H), 8.68 (s, 1H, H-4 coumarin); ^{13}C NMR δ *ppm*: 64.95 (CH_2), 117.13, 119.04, 120.25, 124.60, 128.53, 129.79, 133.35, 134.62, 137.89, 139.87, 147.76, 154.19, 158.26, 186.27; Anal. calcd. for $C_{17}H_{11}BrO_5S$ (407.23): C, 50.14; H, 2.72. Found C, 50.31; H, 2.69.

*5.1.4.4. 6-Bromo-3-(2-tosylacetyl)-2H-chromen-2-one (**8d**).* Gray crystals (yield 75%), m.p. 168–170 °C; IR: 1740, 1686 (2C=O) and 1350, 1158 (SO_2); ^{1}H NMR δ *ppm*: 2.36 (s, 3H, CH_3), 5.24 (s, 2H, CH_2), 7.41 (d, 2H, J = 8.0 Hz, Ar–H), 7.45 (d, 1H, J = 8.9 Hz, Ar–H), 7.76 (d, 2H, J = 8.4 Hz, Ar–H), 7.91 (dd, 1H, J = 2.5 Hz, J = 8.8 Hz, Ar–H), 8.19 (d, 1H, J = 2.4 Hz, Ar–H), 8.63 (s, 1H, H-4 coumarin); Anal. calcd. for $C_{18}H_{13}BrO_5S$ (421.26): C, 51.32; H, 3.11. Found C, 51.23; H, 3.13.

5.1.5. General procedures for preparation of target coumarins **11a-d, 12a, b, 13a-d** and **14a, b**

To a hot solution of the appropriate key intermediate 3-(2-(phenylsulfonyl)acetyl)coumarins **8a-d** (4 mmol) in absolute ethanol (10 mL) containing few drops of glacial acetic acid, phenylhydrazine **9** (0.43 g, 4 mmol) or benzoic acid hydrazide **10** (0.54 g, 4 mmol) or 4-hydrazinobenzenesulfonamide **2** (0.74 g, 4 mmol) or 4-sulfamoylbenzoic acid hydrazide **3** (0.86 g, 4 mmol) was added. The resulting reaction mixture was heated under reflux for 5 h with stirring. Thereafter, the reaction mixture was filtered while hot, and the collected precipitate was washed with ethyl alcohol and recrystallized from ethanol/DMF mixture (5:1) to afford the target coumarins **11a-d, 12a,b, 13a-d, and 14a,b**, respectively.

*5.1.5.1. 3-(1-(2-Phenylhydrazono)-2-(phenylsulfonyl)ethyl)-2H-chromen-2-one (**11a**).* Orange crystals (yield 81%), m.p. 210–212 °C; IR:

3452 (NH), 1712 (C=O) and 1349, 1149 (SO_2); ^{1}H NMR δ *ppm*: 5.29 (s, 2H, CH_2), 6.85 (t, 1H, J = 7.2 Hz, Ar−H), 7.15 (d, 2H, J = 8.0 Hz, Ar−H), 7.24−7.28 (m, 2H, Ar−H), 7.37−7.41 (m, 2H, Ar−H), 7.46−7.49 (m, 3H, Ar−H), 7.61−7.64 (m, 1H, Ar−H), 7.83−7.86 (m, 3H, Ar−H), 8.11 (s, 1H, H-4 coumarin), 10.17 (s, 1H, NH D_2O exchangeable); ^{13}C NMR δ *ppm*: 52.51, 113.79, 116.18, 119.58, 121.10, 125.26, 125.29, 126.24, 128.54, 129.38, 129.50, 132.37, 134.45, 139.36, 140.80, 144.55, 153.16, 160.07; Anal. calcd. for $C_{23}H_{18}N_2O_4S$ (418.47): C, 66.02; H, 4.34; N, 6.69. Found C, 66.10; H, 4.39; N, 6.61.

*5.1.5.2. 3-(1-(2-Phenylhydrazono)-2-tosylethyl)-2H-chromen-2-one (**11b**).* Orange crystals (yield 79%), m.p. 215−217 °C; IR: 3423 (NH), 1725 (C=O) and 1347, 1150 (SO_2); ^{1}H NMR δ *ppm*: 1.99 (s, 3H, CH_3), 5.23 (s, 2H, CH_2), 6.85 (t, 1H, J = 7.3 Hz, Ar−H), 7.16 (d, 2H, J = 8.0 Hz, Ar−H), 7.20 (d, 2H, J = 7.9 Hz, Ar−H), 7.25 (t, 2H, J = 7.7 Hz, Ar−H), 7.37 (t, 2H, J = 7.8 Hz, Ar−H), 7.59 (t, 1H, J = 7.8 Hz, Ar−H), 7.66 (d, 2H, J = 7.9 Hz, Ar−H), 7.81 (d, 1H, J = 7.6 Hz, Ar−H), 8.00 (s, 1H, H-4 coumarin), 10.17 (s, 1H, NH D_2O exchangeable); ^{13}C NMR δ *ppm*: 21.11 (CH_3), 52.63 (CH_2), 113.77, 116.14, 119.57, 119.98, 120.33, 121.02, 125.06, 125.24, 126.52, 128.67, 129.31, 129.48, 129.69, 129.83, 130.04, 132.26, 136.28, 140.89, 144.58, 145.38, 153.11, 160.00; Anal. calcd. for $C_{24}H_{20}N_2O_4S$ (432.49): C, 66.65; H, 4.66; N, 6.48. Found C, 66.58; H, 4.69; N, 6.55.

*5.1.5.3. 6-Bromo-3-(1-(2-phenylhydrazono)-2-(phenylsulfonyl) ethyl)-2H-chromen-2-one (**11c**).* Orange crystals (yield 77%), m.p. 230−232 °C; IR: 3428 (NH), 1705 (C=O) and 1350, 1141 (SO_2); ^{1}H NMR δ *ppm*: 5.27 (s, 2H, CH_2), 6.86 (t, 1H, J = 7.2 Hz, Ar−H), 7.16 (d, 2H, J = 8.0 Hz, Ar−H), 7.24 (t, 2H, J = 7.7 Hz, Ar−H), 7.35 (d, 1H, J = 8.8 Hz, Ar−H), 7.49−7.51 (m, 3H, Ar−H), 7.74 (dd, 1H, J = 2.4 Hz, J = 8.8 Hz, Ar−H), 7.81−7.84 (m, 2H, Ar−H), 8.12−8.13 (m, 2H, H-4 coumarin and Ar−H), 10.23 (s, 1H, NH D_2O exchangeable); Anal. calcd. for $C_{23}H_{17}BrN_2O_4S$ (497.36): C, 55.54; H, 3.45; N, 5.63. Found C, 55.57; H, 3.49; N, 5.71.

*5.1.5.4. 6-Bromo-3-(1-(2-phenylhydrazono)-2-tosylethyl)-2H-chromen-2-one (**11d**).* Orange crystals (yield 74%), m.p. 233−235 °C; IR: 3437 (NH), 1722 (C=O) and 1348, 1143 (SO_2); ^{1}H NMR δ *ppm*: 2.04 (s, 3H, CH_3), 5.21 (s, 2H, CH_2), 6.86 (t, 1H, J = 7.2 Hz, Ar−H), 7.16 (d, 2H, J = 8.0 Hz, Ar−H), 7.21 (d, 2H, J = 8.0 Hz, Ar−H), 7.25 (t, 2H, J = 7.7 Hz, Ar−H), 7.35 (d, 1H, J = 8.8 Hz, Ar−H), 7.65 (d, 2H, J = 8.0 Hz, Ar−H), 7.73 (dd, 1H, J = 2.4 Hz, J = 8.8 Hz, Ar−H), 8.00 (s, 1H, H-4 coumarin), 8.09 (d, 1H, J = 2.4 Hz, Ar−H), 10.22 (s, 1H, NH D_2O exchangeable); Anal. calcd. for $C_{24}H_{19}BrN_2O_4S$ (511.39): C, 56.37; H, 3.75; N, 5.48. Found C, 56.43; H, 3.70; N, 5.54.

*5.1.5.5. N'-(1-(2-Oxo-2H-chromen-3-yl)-2-(phenylsulfonyl)ethylidene)benzohydrazide (**12a**).* Yellow crystals (yield 81%), m.p. 270−272 °C; IR: 3428 (NH), 1641, 1630 (2C=O) and, 1337, 1160 (SO_2); ^{1}H NMR δ *ppm*: 5.43 (s, 2H, CH_2), 7.42 (d, 2H, J = 7.7 Hz, Ar−H), 7.54−7.56 (m, 4H, Ar−H), 7.64−7.71 (m, 3H, Ar−H), 7.78−7.80 (m, 2H, Ar−H), 7.84−7.91 (m, 3H, Ar−H), 8.12 (s, 1H, H-4 coumarin), 11.31 (s, 1H, NH D_2O exchangeable); Anal. calcd. for $C_{23}H_{18}N_2O_4S$ (446.48): C, 64.56; H, 4.06; N, 6.27. Found C, 64.49; H, 4.10; N, 6.33.

*5.1.5.6. N'-(1-(6-bromo-2-oxo-2H-chromen-3-yl)-2-(phenylsulfonyl) ethylidene)benzohydrazide (**12b**).* Yellow crystals (yield 82%), m.p. above 300 °C; IR: 3431 (NH), 1649, 1628 (2C=O) and 1334, 1154 (SO_2); ^{1}H NMR δ *ppm*: 5.41 (s, 2H, CH_2), 7.50 (d, 2H, J = 8.0 Hz, Ar−H), 7.54−7.66 (m, 4H, Ar−H), 7.74−7.77 (m, 2H, Ar−H), 7.79−7.90 (m, 2H, Ar−H), 7.98−8.01 (m, 3H, Ar−H), 8.13 (s, 1H, H-4 coumarin), 11.39 (s, 1H, NH D_2O exchangeable); Anal. calcd. for $C_{24}H_{17}BrN_2O_5S$ (525.37): C, 54.87; H, 3.26; N, 5.33. Found C, 54.77; H, 3.31; N, 5.41.

*5.1.5.7. 4-(2-(1-(2-Oxo-2H-chromen-3-yl)-2-(phenylsulfonyl)ethylidene)hydrazinyl)benzenesulfonamide (**13a**).* Yellow crystals (yield 77%), m.p. 255−257 °C; IR: 3421, 3327, 3257 (NH, NH_2), 1747 (C=O), 1310 and 1156 (SO_2); ^{1}H NMR δ *ppm*: 5.31 (s, 2H, CH_2), 7.15 (s, 2H, NH_2 D_2O exchangeable), 7.25 (d, 2H, J = 8.9 Hz, Ar−H), 7.39−7.43 (m, 2H, Ar−H), 7.45−7.51 (m, 3H, Ar−H), 7.63 (t, 1H, J = 8.0 Hz, Ar−H), 7.69−7.72 (m, 2H, Ar−H), 7.83−7.89 (m, 3H, Ar−H), 8.18 (s, 1H, H-4 coumarin), 10.48 (s, 1H, NH D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 52.73 (CH_2), 113.17, 116.27, 119.49, 124.93, 125.29, 127.71, 128.60, 129.13, 129.57, 129.61, 132.67, 134.53, 135.95, 139.27, 141.67, 147.20, 153.33, 159.89; Anal. calcd. for $C_{23}H_{19}N_3O_6S_2$ (497.54): C, 55.52; H, 3.85; N, 8.45. Found C, 55.43; H, 3.90; N, 8.51.

*5.1.5.8. 4-(2-(1-(2-Oxo-2H-chromen-3-yl)-2-tosylethylidene)hydrazinyl)benzenesulfonamide (**13b**).* Yellow crystals (yield 74%), m.p. 240−242 °C; IR: 3430, 3340, 3259 (NH, NH_2), 1731 (C=O), 1320 and 1148 (SO_2); ^{1}H NMR δ *ppm*: 2.00 (s, 3H, CH_3), 5.25 (s, 2H, CH_2), 7.19 (d, 4H, J = 7.6 Hz, Ar−H and NH_2 D_2O exchangeable), 7.25 (d, 2H, J = 8.8 Hz, Ar−H), 7.39 (t, 2H, J = 8.0 Hz, Ar−H), 7.61 (dd, 1H, J = 1.7 Hz, J = 8.0 Hz, Ar−H), 7.66 (d, 2H, J = 8.2 Hz, Ar−H), 7.71 (d, 2H, J = 8.8 Hz, Ar−H), 7.83 (d, 1H, J = 9.4 Hz, Ar−H), 8.08 (s, 1H, H-4 coumarin), 10.51 (s, 1H, NH D_2O exchangeable); ^{13}C NMR δ *ppm*: 21.15 (CH_3), 52.78 (CH_2), 113.14, 11,621, 119.46, 124.66, 125.29, 127.69, 128.72, 129.35, 130.07, 132.57, 135.84, 136.14, 141.77, 145.46, 147.21, 153.25, 159.84; Anal. calcd. for $C_{24}H_{21}N_3O_6S_2$ (511.57): C, 56.35; H, 4.14; N, 8.21. Found C, 56.43; H, 4.19; N, 8.16.

*5.1.5.9. 4-(2-(1-(6-Bromo-2-oxo-2H-chromen-3-yl)-2-(phenylsulfonyl)ethylidene)hydrazineyl)benzenesulfonamide (**13c**).* Yellow crystals (yield 78%), m.p. 215−217 °C; IR: 3420, 3330, 3249 (NH, NH_2), 1721 (C=O), 1332 and 1146 (SO_2); ^{1}H NMR δ *ppm*: 5.30−5.36 (m, 2H, CH_2), 7.04 (d, 2H, J = 8.5 Hz, NH_2 D_2O exchangeable), 7.21 (s, 2H, Ar−H), 7.46−7.53 (m, 2H, Ar−H), 7.63 (t, 2H, J = 7.7 Hz, Ar−H), 7.71−7.75 (m, 3H, Ar−H), 7.91−7.96 (m, 2H, Ar−H), 8.20−8.22 (m, 1H, Ar−H), 8.69 (s, 1H, H-4 Coumarin), 10.48 (s, 1H, NH D_2O exchangeable); Anal. calcd. for $C_{23}H_{18}BrN_3O_6S_2$ (574.44): C, 47.92; H, 3.15; N, 7.29. Found C, 47.87; H, 3.19; N, 7.37.

*5.1.5.10. 4-(2-(1-(6-Bromo-2-oxo-2H-chromen-3-yl)-2-tosylethylidene)hydrazineyl)benzenesulfonamide (**13d**).* Yellow crystals (yield 75%), m.p. 250−252 °C; IR: 3378, 3321, 3272 (NH, NH_2), 1721 (C=O), 1330 and 1149 (SO_2); ^{1}H NMR δ *ppm*: 1.99, 2.04 (2s, 3H, CH_3), 5.24 (s, 2H, CH_2), 7.21−7.28 (m, 5H, Ar−H and NH_2 D_2O exchangeable), 7.36−7.48 (m, 2H, Ar−H), 7.65−7.84 (m, 5H, Ar−H), 8.08−8.18 (m, 2H, H-4 coumarin and Ar−H), 10.56 (s, 1H, NH D_2O exchangeable); Anal. calcd. for $C_{24}H_{20}BrN_3O_6S_2$ (590.46): C, 48.82; H, 3.41; N, 7.12. Found C, 48.92; H, 3.36; N, 7.24.

*5.1.5.11. 4-(2-(1-(2-oxo-2H-chromen-3-yl)-2-(phenylsulfonyl)ethylidene)hydrazine-1-carbonyl)benzenesulfonamide (**14a**).* Yellow crystals (yield 78%), m.p. 225−227 °C; IR: 3422, 3287, 3206 (NH, NH_2), 1718, 1691 (2C=O), 1318 and 1156 (SO_2); ^{1}H NMR δ *ppm*: 5.43 (s, 2H, CH_2), 7.40−7.44 (m, 2H, Ar−H), 7.54−7.60 (m, 5H, Ar−H and NH_2 D_2O exchangeable), 7.67−7.71 (m, 1H, Ar−H), 7.90−8.01 (m, 7H, Ar−H), 8.15 (s, 1H, H-4 coumarin), 11.42 (s, 1H, NH D_2O exchangeable); ^{13}C NMR δ *ppm*: 53.72 (CH_2), 116.46, 119, 124.52, 125.51, 126.21, 128.66, 129.32, 129.68, 129.96, 131.66, 133.50, 134.41, 134.87, 136.43, 136.90, 138.96, 143.92, 143.96, 153.71, 159.70, 163.87; Anal. calcd. for $C_{24}H_{19}N_3O_7S_2$ (525.55): C, 54.85; H, 3.64; N, 8.00. Found C, 54.72; H, 3.59; N, 8.11.

*5.1.5.12. 4-(2-(1-(2-Oxo-2H-chromen-3-yl)-2-tosylethylidene)hydrazine-1-carbonyl)benzenesulfonamide (**14b**).* Yellow crystals (yield 75%), m.p. 240−242 °C; IR: 3421, 3298, 3220 (NH, NH_2), 1696, 1663 (2C=O), 1319 and 1159 ($2SO_2$); ^{1}H NMR δ *ppm*: 2.03 (s, 3H, CH_3),

5.39 (s, 2H, CH_2), 7.26 (d, 2H, J = 7.9 Hz, Ar−H), 7.41−7.44 (m, 2H, NH_2 D_2O exchangeable), 7.61 (s, 2H, Ar−H), 7.66 (t, 1H, J = 7.2 Hz, Ar−H), 7.73 (d, 2H, J = 7.9 Hz, Ar−H), 7.84−7.85 (m, 1H, Ar−H), 7.94−8.03 (m, 5H, Ar−H and H-4 coumarin), 11.41 (s, 1H, NH D_2O exchangeable); ^{13}C NMR δ *ppm*: 21.20 (CH_3), 53.73 (CH_2), 116.42, 116.57, 118.98, 124.14, 125.49, 126.18, 128.72, 129.28, 129.86, 130.16, 130.40, 133.40, 135.91, 136.48, 136.91, 140.27, 144.00, 145.09 145.18, 145.81, 147.55, 153.64, 159.64, 163.80; Anal. calcd. for $C_{25}H_{21}BrN_3O_7S_2$ (439.58): C, 55.65; H, 3.92; N, 7.79. Found C, 55.53; H, 3.89; N, 7.87.

5.2. Biological evaluation

The experimental procedures adopted for CA inhibitory assay for the target compounds were performed as descriped eariler [24,25], and presented in the Supplementary Materials.

5.3. In silico study

The procedures adopted for the *in silico* were presented in the Supplementary Materials.

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.

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Appendix A. Supplementary data

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