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Discovery of a novel series of indolylchalcone-benzenesulfonamide hybrids acting as selective carbonic anhydrase II inhibitors

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ABSTRACT

The primary sulfonamide group is one of the most efficient zinc binding group (ZBG) for designing carbonic anhydrase (CA, EC 4.2.1.1) inhibitors. In the present study primary sulfonamide linked with indolylchalcone were designed. The newly synthesized molecules (**5a-r**) were examined against four human (h) CA isoforms (hCA I, hCA II, hCA IX and hCA XIII). These sulfonamides showed good inhibition activity against isoforms hCA I, hCA II and hCA XIII. Compound **5i** (2.3 nM), **5m** (2.4 nM), **5o** (3.6 nM) and **5q** (7.0 nM) were more potent than standard drug AAZ (12.1 nM) against isoform hCA II, respectively. Most of the other compounds in the present series inhibited hCA XIII and hCA IX in the range of 50 nM – 100 nM.

1. Introduction

Carbonic anhydrase (CA, EC 4.2.1.1) is a well-explored enzyme in the pharmacological research field. Inhibition of the different isoforms of carbonic anhydrase (CA) has been used in the treatment of a variety of disorders such as edema, osteoporosis, glaucoma, epilepsy, and obesity for decades, whereas applications in the field of cancer started to be considered in the last 15 years. These is due to the fact that CAs are involved in various important physiological functions such as metabolism, respiration, pH regulation, ion transport, bone resorption and secretion of gastric juice and other fluids (e.g., aqueous humor in the eye, cerebrospinal fluid, etc.) [1–3]. A total of 16 isozymes have been previously reported as members of the mammalian α-CA family. CAs I, II, III, VII, and XIII are cytosolic isoenzymes, CAs VA and VB are localized in the mitochondria, CA VI is a unique secreted isozyme, CAs IX, XII, and XIV are transmembrane proteins, and CAs IV and XV are GPI-anchored to the cell membrane [4]. Exploration of CA inhibitors has been done by many researchers but still many aspects have remained to be investigated.

At least 25 clinically used drugs have been reported to possess significant CA inhibitory properties [5]. Indisulam is an indole-containing anti-cancer drug in clinical trials which strongly inhibits CA, having strong affinities for isoforms II, VA, VB, VI, VII, IX, XII, XIII and XIV [6–7]. Various other reports also claim that indole containing moieties have significant inhibitory properties against CA (Fig. 1) [8–10]. Similarly, the chalcone is a well-known moiety which posses various biological properties. [11]

Sulfonamides represent a significant class of biologically active compounds and most of the human carbonic anhydrase isoforms (hCA) are inhibited by aromatic sulfonamides [12]. Aromatic sulfonamides are known as specific and strong inhibitors of CA isoforms [13–14] and considered as prototype drugs for additional modification and development of new and more potent inhibitors (Fig. 1) [14] But lack of selectivity in inhibiting various isoforms is the major drawback with the use of CAIs, that results from structural similarity and identical subcellular localization of these isoforms producing undesirable side effects [15,16].

In the last two decades, CAIs research has been focused on designing isoform-selective sulfonamide inhibitors by employing two principal methods: the ring and the tail approaches. The first resides in modulating the ring (chiefly its chemical nature) directly connected to the sulfonamide ZBG, whereas the other consists of attaching different tails to the aromatic/heterocyclic ring carrying various ZBGs like sulfonamide, sulfamide, sulfamate, carboxylate, hydroxamate, or

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dithiocarbamate. This enabled modulation of the interactions that the ligand establishes with the middle and outer parts of the active site cavity, which are the most variable regions among the 16 hCA isoforms mentioned above, and led to a variety of isoform-selective CAIs.

In this article we explore indolylchalcone moiety using tail approach as CA inhibitors. In our previous research work [17], we have explored indolylchalcone linked to benzsulfonamide moiety through a triazole linker and they were extremely good inhibitor of selectively hCA I in subnanomolar range. In addition, various reports were found which stated that presence of an amide bond showed potent inhibition of CA isoforms (Fig. 1). In the present study the target molecules were designed by replacing the triazole linker with an amide linker in such a way that it contains benzsulfonamide as head, indolylcalcone as tail and an amide bond as linker and to test these synthesized compounds against CA isoforms, hCAs I, II, IX and XIII for their inhibitory activity (Fig. 2).

2. Results and discussion

2.1. Chemistry

The synthetic strategy employed for the synthesis of the target compounds is depicted in Scheme 1. The chalcones (**2a-r**) were synthesized by Knoevenagel condensation by reacting indole-3-carboxaldehyde with different acetophenones using piperidine as the base and ethanol as solvent [18]. On the other hand the sulfanilamide (**3**) was *N*-acetylated with chloroacetyl chloride to get (**4**) [19] which was subsequently subjected to nucleophilic substitution with indolylchalcones (**2a-r**) in the presence of K_2CO_3 in DMF as solvent to afford the final target compounds (**5a-r**) by using reported literature method [20]. The structures of all the compounds (**5a-r**) were elucidated on the basis of ^{1}H NMR, ^{13}C NMR and HRMS.

2.2. Carbonic anhydrase inhibition

The newly synthesized compounds (**5a-r**) have been assayed for their inhibition against the four isoforms, the cytosolic isoforms, hCA I, hCA II and hCA XIII as well as the *trans*-membrane tumor associated isoform, hCA IX by means of stopped flow carbon dioxide assay [21]. Here acetazolamide (AAZ) was taken as the standard drug. The result of the synthesized compounds against different hCA isoforms are summarized in Table 1:-

From the data given in the Table 1 the following inferences were made:-

1) The ubiquitous cytosolic isoform hCA I was weakly inhibited by all the synthesized compounds with K_i values ranging from 166.9 nM – 6.6 μM. The 2-bromo substituted derivative **(5 m)** and 3,4,5-trimethoxy substituted derivative **(5o)** exhibited the best inhibition amongst all the compounds with K_i values of 166.9 nM and 673 nM respectively.
2) All the synthesized compounds were strongly inhibited the physiologically dominant isoform hCA II with K_i values ranging between 2.3 nM and 136.8 nM. Among all the synthesized compounds, the compounds **5i** (2.3 nM), **5 m** (2.4 nM), **5o** (3.6 nM) and **5q** (7.0 nM) were found to be more potent hCA II inhibitors compared to the standard AAZ. The compound **5i** (K_i = 2.3 nM) and **5 m** (2.4 nM)

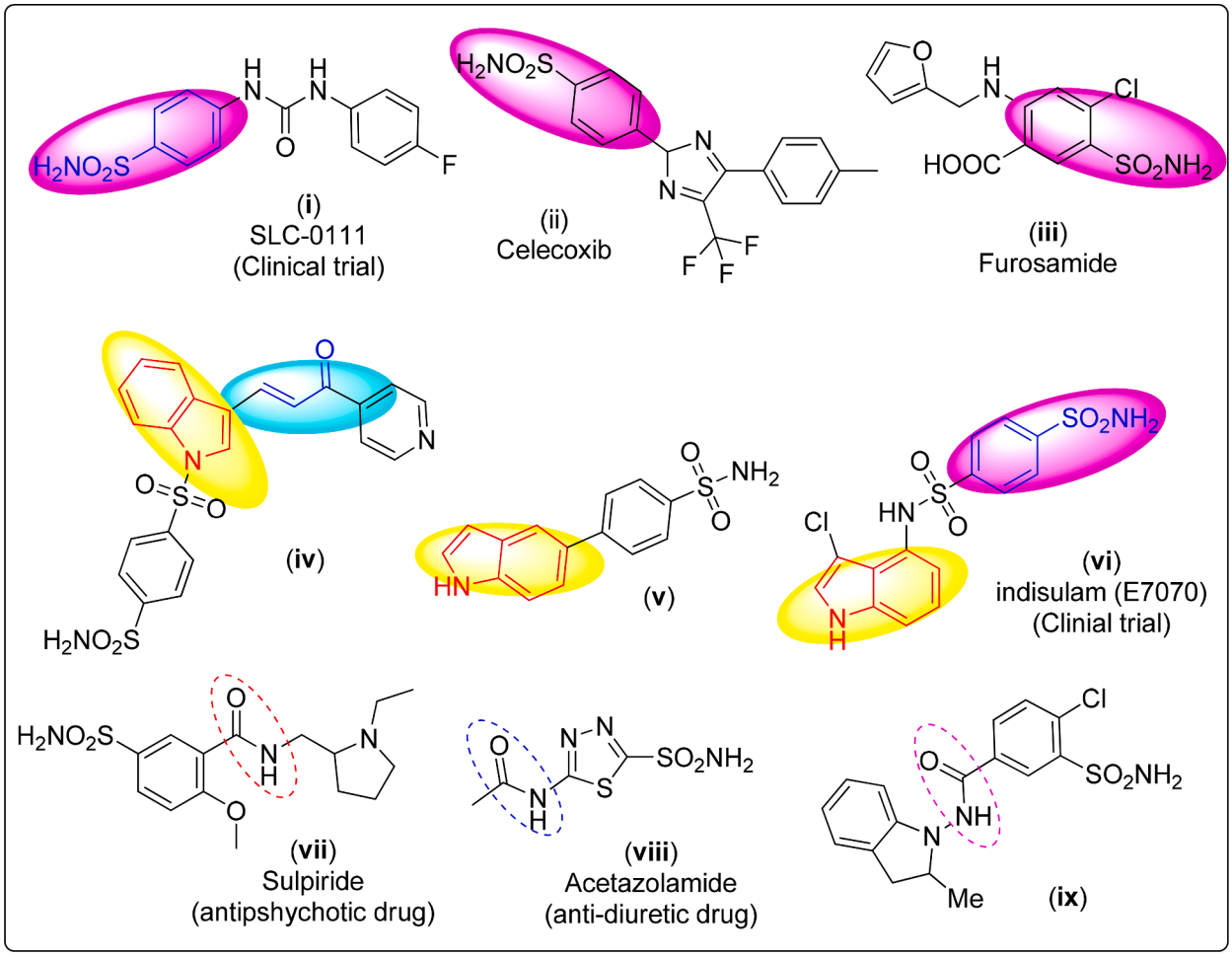


Fig. 1. Structure of clinically used drugs containing amide bond.

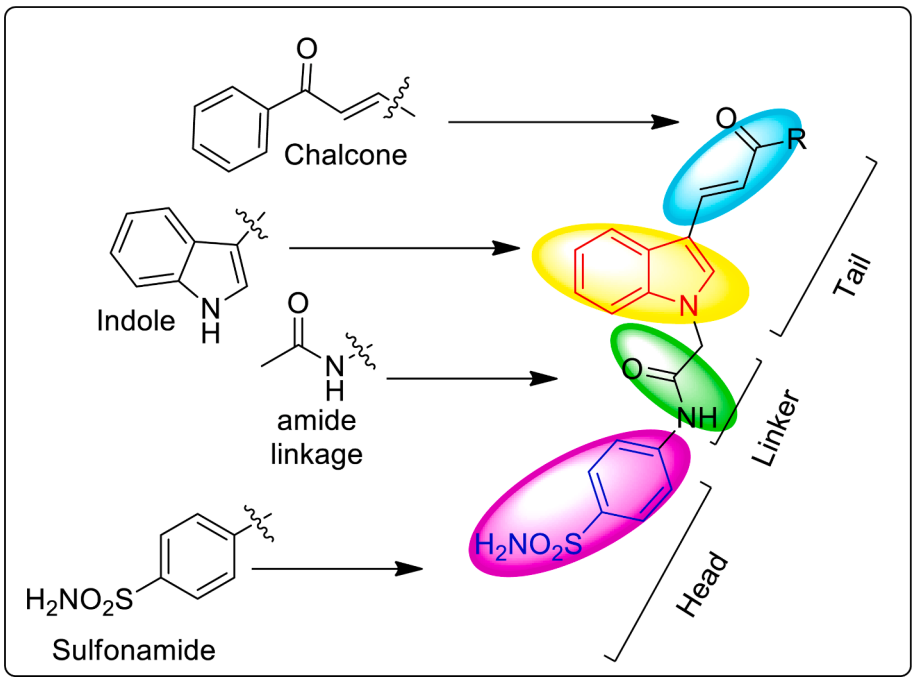
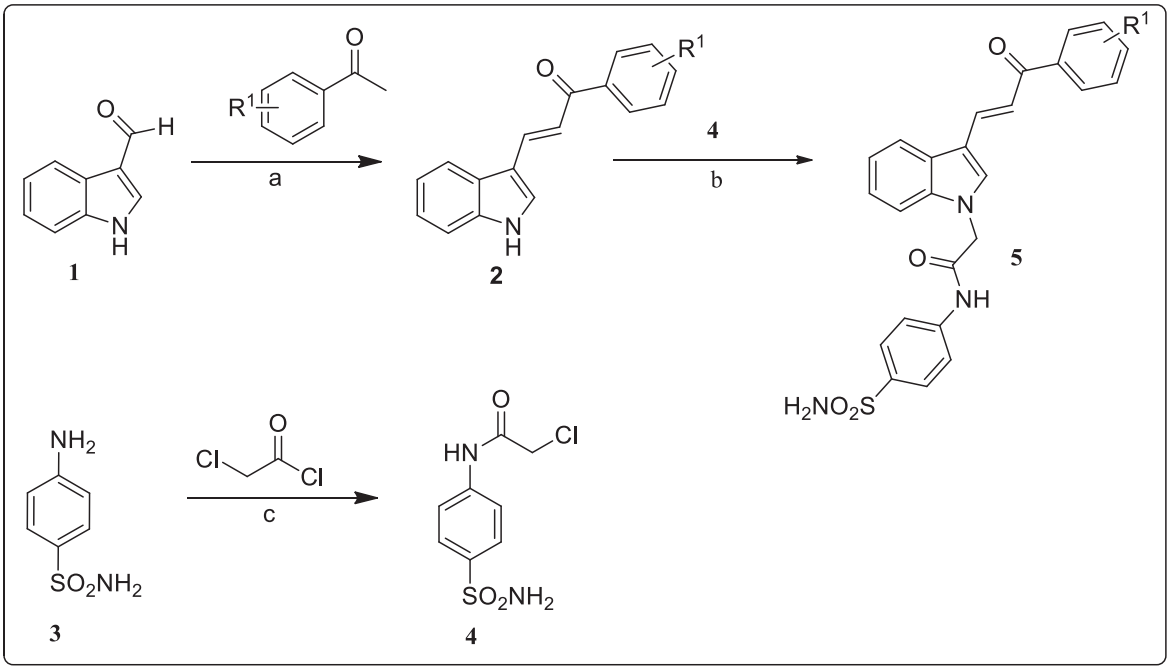


Fig. 2. Rational to design target molecule.



Scheme 1. Synthetic Route for (E)-2-(3-(3-oxo-3-phenylprop-1-en-1-yl)-1*H*-indol-1-yl)-*N*-(4-sulfamoylphenyl)acetamides**.** Reagents and conditions: a) Piperidine (0.5 equiv), EtOH, reflux, 24 h, b) 2-chloro-*N*-(4-sulfamoylphenyl)acetamide **4** (1 equiv), K_2CO_3 (3 equiv), DMF, 60 °C, C) K_2CO_3, acetone, 0 °C 1 h.

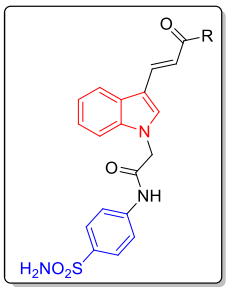
were 5 times more potent than AAZ (K_i = 12.1 nM) against hCA II. Similarly, the compound **5q** (7.0 nM) was 2 times more active than AAZ. Apart from them other compounds also inhibited hCA II with K_i values < 100 nM (except **5f** {136.6 nM}).

3) The tumor-associated isoform hCA IX was also inhibited by all the synthesized compounds **(5a-r)** in the 29 nM to 160 nM range. Among all the compounds, the 9 compounds i.e., **5a** (32.4 nM), **5e** (30.3 nM), **5f** (32.1 nM), **5 g** (29.9 nM), **5i** (31.0 nM), **5 m** (32.4 nM), **5p** (31.5 nM), **5q** (29.8 nM) and **5r** (31.0 nM) inhibition constant values with compare to standard AAZ (25.8 nM).
4) The cytosolic isoform hCA XIII was also inhibited by the compounds **5o, 5p** and **5r** in a low to moderate nanomolar range with Ki values of 18.4 nM, 18.5 nM and 19.4 nM respectively compared to standard AAZ (17 nM). The most potent inhibitor against this isoform was found to be the compound **5o**, **5p** and **5r** with 3,4,5-trimethoxy, 4-trifluoromethyl and 3-chloro substitution respectively.

3. Conclusion

Novel indolychalcone-benzsulfonamide hybrids linked with an

Table 1
Inhibition of hCA isoforms I, II, IX and XIII with compounds **5a-r** and acetazolamide (AAZ) as standard inhibitor



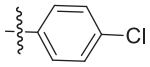
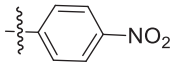
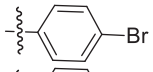
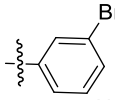
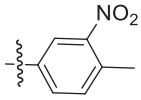
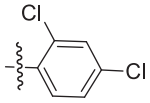
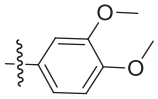
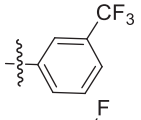
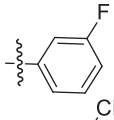
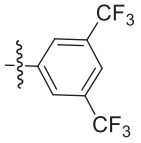
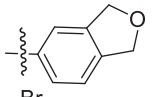
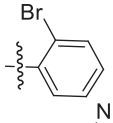
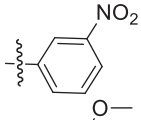
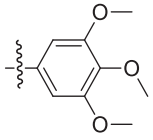
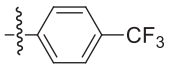
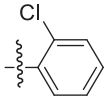
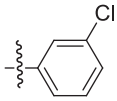
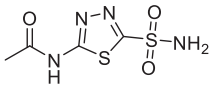
		K_I (nM)*			
Cmp	R	hCA I	hCAII	hCA IX	hCA XIII
5a		5702	97.6	32.4	50.0
5b		2498	27.5	72.1	114.3
5c		1520	26.6	61.2	47.0
5d		3505	30.7	159.9	146.6
5e		3765	53.5	30.3	117.6
5f		5764	136.8	32.1	110.8
5 g		964.1	37.5	29.9	127.9
5 h		3463	21.4	139.5	51.4
5i		3744	2.3	31.0	120.2
5j		2019	30.7	106.1	163.8
5 k		4086	62.1	88.3	63.8
5 l		840.0	18.9	106.8	74.3
5 m		166.9	2.4	32.4	184.4
5n		4640	94.4	90.0	40.4
5o		672.3	3.6	107.8	18.5

Table 1 (*continued*)

		K_I (nM)*			
Cmp	R	hCA I	hCAII	hCA IX	hCA XIII
5p		3625	60.2	31.5	18.4
5q		775.3	7.0	29.8	42.9
5r		6630	66.1	31.0	19.4
AAZ		250.0	12.1	25.8	17.0

* Mean from 3 different assays, by a stopped flow technique (errors were i31.0n the range of ± 5–10% of the reported values)

amide bond (**5a-r**) were synthesized. All the newly synthesized compounds were evaluated for their CA inhibitory profile against cytosolic isoforms (hCA I, II and XIII) and membrane-bound isoform (hCA IX). Most of them were found to be more selective inhibitors of hCA II with compare to other hCA isoforms hCA I, hCA IX and hCA XIII. The results displayed 4 compounds **5i** (2.3 nM), **5 m** (2.4 nM), **5o** (3.6 nM) and **5q** (7.0 nM)) displayed more potencies than AAZ against hCA II and remaining were active under 100 nM range except **5f** (K_i 136.8 nM). In the case of hCA XIII, the compounds **5o**, **5p** and **5r** showed the best inhibition with K_i values of 18.4 nM, 18.5 nM and 19.4 nM respectively. In the case of hCA IX, nine compounds exhibited the best inhibition with K_i values $<$ 50 nM. Whereas, all these compounds showed weak potencies against hCA I with submicromolar range. Thus, **5i** (2.3 nM), **5 m** (2.4 nM), **5o** (3.6 nM) and **5q** (7.0 nM)) will be taken as lead for further development of potent hCA II inhibitors that target ocular disorders and cancer *via* their structural modifications.

4. Experimental section

4.1. General

All solvents were purified and dried using standard methods prior to use. Commercially available reagents were used without further purification. All reactions involving air- or moisture sensitive compounds were performed under a nitrogen atmosphere using dried glassware and syringe techniques to transfer solutions. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel 60F-254 aluminium plates. Melting points were determined on Stuart digital melting-point apparatus/SMP 30 in open capillary tubes and uncorrected. Nuclear magnetic resonance (1H NMR, 13C NMR) spectra were recorded using an Avance Brucker 500 MHz, 125 MHz spectrometer in DMSO-d_6. Chemical shifts reported in parts per million (ppm) with TMS as an internal reference, and the coupling constants (J) expressed in hertz (Hz). Splitting patterns are denoted as follows: s, singlet; d, doublet; t, triplet; m, multiplet; dd, doublet of doublet. HRMS were determined with Agilent QTOF mass spectrometer 6540 series instrument and were performed in the ESI techniques at 70 eV.

4.2. General procedures for the synthesis of compounds

4.2.1. General procedure for synthesis of indolylchalcone (2a-r)

A mixture of indole-3-carboxaldehyde **1** (1 equiv) was treated with substituted acetophenones (1 equiv), piperidine (0.5 equiv) in ethanol and refluxed for 24 h at 60 °C. The progress of the reaction was monitored by TLC. After the completion of the reaction the crushed-ice was added into the reaction mixture and neutralized with glacial acetic acid

to obtian solid product **2.** The solid formed was filtered under high vacuum and washed with hexane and dried over to get the product in 80–90% yield**.**

4.2.2. General procedure for synthesis of 2-chloro-(4-sulfamoylphenyl) acetamide (4)

To a solution of Sulfanilamide **3** (1 equiv) and K_2CO_3 (2 equiv) in 20 ml acetone, 2-chloroacetylchloride (2 equiv) was added drop-wise. The reaction mixture was stirred at 0 ^{0}C for 1 h and then left to warm at room temperature. After completion of reaction filter and dry it washed with ethanol yields 88%.

*4.2.3. General procedure for synthesis of (E)-2-(3-oxo-phenylprop-1-en-yl)-1H-indolyl)-N-(4-sulfamoylphenyl)acetamide **(5a-r)***

To a mixture of indolylchalcone (**2a-r**) in DMF solvent add K_2CO_3 (3 equiv) and heated for 30 min at 50 °C. After dissolving add 2-chloro-(4-sulfamoylphenyl)acetamide **4** (1 equiv). Then stir it at rt for 6 h, after the completion of the reaction the crushed-ice was added into the reaction mixture. The solid formed was filter out and dry it to get desired product in 70–86% yields.

4.2.3.1. (E)-2-(3-(3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)-N-(4-sulfamoylphenyl). acetamide **(5a):** Yellow solid (yield 82%); m. p: 289–290 °C; FT-IR (ν cm^{-1}): 3267, 1681, 1651, 1588, 1564, 1524, 1400, 1205, 1150; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm): 10.84–10.77 (m, 1H), 8.20–8.03 (m, 5H), 7.82–7.73 (m, 4H), 7.70–7.61 (m, 3H), 7.60–7.54 (m, 1H), 7.35–7.23 (m, 4H), 5.28–5.18 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 188.1, 166.6, 141.9, 139.2, 138.6, 137.8, 137.5, 130.5, 129.2, 127.3, 126.0, 123.5, 122.0, 121.0, 119.3, 116.1, 112.9, 111.3, 49.8; HRMS (ESI) *m/z* calculated for $C_{25}H_{20}ClN_3O_4S$ 494.0941 [M + H$^+$]; found 494.0955.

4.2.3.2. (E)-2-(3-(3-(4-nitrophenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)-N-(4-sulfamoylphenyl). acetamide **(5b):** Orange solid (yield 84%); m.p: 260–261 °C; FT-IR (ν cm^{-1}): 3272, 1681, 1651, 1583, 1520, 1343,1155; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm): 10.86 (s, 1H), 8.37 (q, J = 8.8 Hz, 4H), 8.22 (s, 1H), 8.19–8.15 (m, 1H), 8.11 (d, J = 15.4 Hz, 1H), 7.78 (q, J = 9.0 Hz, 4H), 7.68 (d, J = 15.4 Hz, 1H), 7.60–7.57 (m, 1H), 7.35–7.30 (m, 2H), 7.28 (d, J = 11.7 Hz, 2H), 5.24 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ (ppm): 188.2, 166.6, 149.9, 143.9, 141.9, 140.4, 139.3, 138.6, 138.3, 129.9, 127.2, 126.0, 124.2, 123.6, 122.23, 121.1, 119.3, 116.1, 112.9, 49.9; HRMS (ESI) *m/z* calcd for $C_{25}H_{20}N_4O_6S$ [M + H$^+$] 505.1182; found 505.1177.

4.2.3.3. (E)-2-(3-(3-(4-bromophenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)-N-(4-sulfamoylphenyl). acetamide **(5c):** Yellow solid (yield 82%); m. p: 270–272 °C; FT-IR (ν cm^{-1}): 3266, 1681, 1650, 1586, 1525, 1397, 1290, 1156; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm): 10.81 (s, 1H), 8.18 (s, 1H), 8.15 (d, J = 6.9 Hz, 1H), 8.07 (t, J = 11.7 Hz, 3H), 7.78 (q, J = 8.9 Hz, 6H), 7.66 (d, J = 15.4 Hz, 1H), 7.57 (d, J = 7.3 Hz, 1H), 7.30 (dd, J = 15.5, 9.9 Hz, 4H), 5.23 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ (ppm): 188.3, 166.6, 141.9, 139.3, 138.6, 137.9, 137.6, 132.2, 130.7, 127.3, 126.9, 126.1, 123.5, 122.0, 121.0, 119.3, 116.1, 112.9, 111.3, 49.9; HRMS (ESI) *m/z* calcd for $C_{25}H_{20}BrN_3O_4S$ [M + H$^+$] 540.0416; found 540.0446.

*4.2.3.4. (E)-2-(3-(3-oxo-3-(p-tolyl)prop-1-en-1-yl)-1H-indol-1-yl)-N-(4-sulfamoylphenyl)acetamide **(5d)***. Yellow solid (yield 82%); m.p: 268–270 °C; FT-IR (ν cm^{-1}): 3272, 1657, 1651, 1591, 1524, 1393, 1290, 1156; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm): 11.00–10.71 (m, 1H), 8.19–8.11 (m, 2H), 8.10–8.00 (m, 3H), 7.85–7.75 (m, 4H), 7.72–7.65 (m, 1H), 7.61–7.55 (m, 1H), 7.43–7.25 (m, 6H), 5.32–5.16 (m, 2H), 2.45–2.37 (m, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ (ppm): 188.8, 166.7, 143.2, 141.9, 139.2, 138.5, 138.2, 137.1, 136.3, 129.7, 128.7, 127.3, 126.1, 123.4, 121.9, 120.9, 119.3, 116.5, 112.8, 111.3, 49.8, 21.6; HRMS (ESI) *m/z* calcd for $C_{26}H_{23}N_3O_4SNa$ [M + Na$^+$] 496.1320, found 496.1307.

4.2.3.5. (E)-2-(3-(3-(3-bromophenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)-N-(4-sulfamoylphenyl). acetamide **(5e):** Yellow solid, (yield 82%); m. p 270–272 °C; FT-IR (ν cm^{-1}): 3269, 1679, 1650, 1591, 1526, 1394, 1289, 1156; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm): 10.80 (s, 1H), 8.22 (d, J = 11.3 Hz, 2H), 8.15 (dd, J = 8.8, 4.9 Hz, 2H), 8.08 (d, J = 15.4 Hz, 1H), 7.85 (d, J = 8.4 Hz, 1H), 7.80–7.75 (m, 4H), 7.66 (d, J = 15.4 Hz, 1H), 7.59–7.53 (m, 2H), 7.33–7.29 (m, 2H), 7.26 (s, 2H), 5.23 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ (ppm): 188.26, 166.61, 149.94, 143.99, 141.93, 140.40, 139.30, 138.68, 138.36, 129.98, 127.29, 126.04, 124.26, 23.65, 122.23, 121.15, 122.10, 119.36, 116.18, 112.92, 112.32, 49.9; HRMS (ESI) *m/z* calcd for $C_{25}H_{20}BrN_3O_4S$ [M + H$^+$]; 538.0436, found 538.0432.

*4.2.3.6. (E)-2-(3-(3-(4-methyl-2-nitrophenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)-N-(4-sulfamoylphenyl)acetamide **(5f)***. Red solid (yield 82%); m.p: 275–277 °C; FT-IR (ν cm^{-1}): 3266, 1685, 1596, 1526, 1524, 1396, 1334, 1159; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm): 10.82 (s, 1H), 8.62 (d, J = 1.5 Hz, 1H), 8.40 (dd, J = 8.0, 1.5 Hz, 1H), 8.23 (s, 1H), 8.18 (dd, J = 6.1, 2.5 Hz, 1H), 8.13 (d, J = 15.4 Hz, 1H), 7.78 (q, J = 9.0 Hz, 4H), 7.72 (s, 1H), 7.70 (d, J = 5.6 Hz, 1H), 7.58 (dd, J = 6.4, 2.3 Hz, 1H), 7.35 – 7.29 (m, 2H), 7.28 (s, 2H), 5.24 (s, 2H), 2.62 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ (ppm): 188.3, 166.6, 141.9, 139.3, 138.6, 137.9, 137.6, 132.2, 130.7, 127.3, 126.9, 126.1, 123.5, 122.0, 121.0, 119.3, 116.1, 112.9, 111.37, 49.9; HRMS (ESI) *m/z* calcd for $C_{26}H_{22}N_4O_6S$ [M + H$^+$]; 519.1338, found 519.1347.

4.2.3.7. (E)-2-(3-(3-(2,4-dichlorophenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)-N-(4-sulfamoylphenyl). acetamide **(5 g):** Yellow solid (yield 82%); m.p: 269–271 °C; FT-IR (ν cm^{-1}):3268, 1683, 1591, 1522, 1395, 1254, 1156; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm): 10.80 (s, 1H), 8.10 (s, 1H), 7.98 (d, J = 7.6 Hz, 1H), 7.77 (dd, J = 20.0, 8.1 Hz, 5H), 7.66 (d, J = 15.9 Hz, 1H), 7.58 (dd, J = 12.6, 6.2 Hz, 3H), 7.33–7.25 (m, 4H), 7.06 (d, J = 16.0 Hz, 1H), 5.20 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ (ppm): 188.1, 166.6, 141.9, 139.2, 138.6, 137.8, 137.5, 130.5, 129.2, 127.3, 126.0, 123.5, 122.0, 121.0, 119.3, 116.1, 112.9, 111.3, 49.8; HRMS (ESI) *m/z*: calcd for $C_{25}H_{19}Cl_2N_3O_4S$ [M + H$^+$] 528.0552, found 528.0549.

4.2.3.8. (E)-2-(3-(3-(3,4-dimethoxyphenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)-N-(4-sulfamoylphenyl). acetamide **(5 h):** Yellow solid (yield 82%); m.p: 268–270 °C; FT-IR (ν cm^{-1}): 3321, 1689, 1646, 1596, 1529, 1388, 1273, 1126; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm): 10.82 (s, 1H), 8.15 (s, 1H), 8.11 (dd, J = 6.2, 2.3 Hz, 1H), 8.01 (d, J = 15.4 Hz, 1H), 7.88 (dd, J = 8.4, 1.8 Hz, 1H), 7.78 (q, J = 9.1 Hz, 4H), 7.71 (d, J = 15.5 Hz, 1H), 7.61 (d, J = 1.7 Hz, 1H), 7.58 – 7.55 (m, 1H), 7.31 – 7.26 (m, 4H), 7.12 (d, J = 8.5 Hz, 1H), 5.22 (s, 2H), 3.88 (d, J = 3.2 Hz, 6H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ (ppm): 188.2, 166.6, 149.9, 143.9, 141.9, 140.4, 139.3, 138.6, 138.3, 129.9, 127.2, 126.0, 124.2, 123.6, 122.2, 121.1, 119.3, 116.1, 112.9, 49.9; HRMS (ESI) *m/z* calcd for $C_{27}H_{25}N_3O_6S$ [M + H$^+$] 520.1542; found 520.1557.

*4.2.3.9. (E)-2-(3-(3-oxo-3-(3-(trifluoromethyl)phenyl)prop-1-en-1-yl)-1H-indol-1-yl)-N-(4-sulfamoyl-phenyl)acetamide **(5i)***. Yellow solid (yield 82%); m.p: 284–286 °C; FT-IR (ν cm^{-1}): 3268, 1680, 1650, 1525, 1390, 1156; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm): 10.83 (s, 1H), 8.49 (d, J = 7.6 Hz, 1H), 8.35 (s, 1H), 8.24 (s, 1H), 8.14 (dd, J = 23.0, 11.0 Hz, 2H), 8.02 (d, J = 7.6 Hz, 1H), 7.86–7.71 (m, 6H), 7.58 (d, J = 8.1 Hz, 1H), 7.34–7.26 (m, 4H), 5.24 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ (ppm): 188.3, 166.6, 141.9, 139.3, 138.6, 137.9, 137.6, 132.2, 130.7, 127.3, 126.9, 126.1, 123.5, 122.0, 121.0, 119.3, 116.1, 112.9, 111.3, 49.9; HRMS (ESI) *m/z* calcd for $C_{26}H_{20}F_3N_3O_4S$ [M + H$^+$] 528.1205; found 528.1215.

4.2.3.10. (E)-2-(3-(3-(3-fluorophenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)-N-(4-sulfamoylphenyl). acetamide **(5j):** Yellow solid (yield 84%); m. p: 268–270 °C; FT-IR (ν cm^{-1}): 3317, 1693, 1524, 1313, 1288, 1152; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm): 10.82 (s, 1H), 8.20 (s, 1H), 8.19–8.16 (m, 1H), 8.06 (s, 1H), 8.03–8.00 (m, 1H), 7.93–7.90 (m, 1H), 7.78 (dd, J = 15.2, 9.0 Hz, 5H), 7.67 (d, J = 15.5 Hz, 1H), 7.60–7.55 (m, 1H), 7.54–7.48 (m, 1H), 7.31 (d, J = 5.4 Hz, 2H), 7.27 (s, 2H), 5.23 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ (ppm): 188.1, 166.6, 141.9, 139.2, 138.6, 137.8, 137.5, 130.5, 129.2, 127.3, 126.0, 123.5, 122.0, 121.0, 119.3, 116.1, 112.9, 111.3, 49.8; HRMS (ESI) *m/z* calcd for $C_{25}H_{20}FN_3O_4S$ [M + H$^+$] 478.1237, found 478.1246.

4.2.3.11. (E)-2-(3-(3-(3,5-bis(trifluoromethyl)phenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)-N-(4-sulfamoylphenyl)acetamide ***(5 k)***. Yellow solid (yield 87%); m.p 290–292 °C; FT-IR (ν cm^{-1}): 3264, 1676, 1568, 1523, 1279, 1156, 1125; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm): 10.83 (s, 1H), 8.69 (s, 2H), 8.40 (s, 1H), 8.29 (s, 1H), 8.18 (dd, J = 9.6, 5.5 Hz, 2H), 7.78 (dd, J = 15.1, 8.0 Hz, 5H), 7.61–7.57 (m, 1H), 7.34–7.30 (m, 2H), 7.27 (s, 2H), 5.25 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ (ppm): 188.1, 166.6, 141.9, 139.2, 138.6, 137.8, 137.5, 130.5, 129.2, 127.3, 126.0, 123.5, 122.0, 121.0, 119.3, 116.1, 112.9, 111.3, 49.8; HRMS (ESI) *m/z* calcd for $C_{27}H_{19}F_6N_3O_4S$ [M + Na$^+$] 618.0898; found 618.0923.

4.2.3.12. (E)-2-(3-(3-(benzo[d][1,3]dioxol-5-yl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)-N-(4-sulfamoyl phenyl)acetamide ***(5 l)***. Yellow solid (yield 82%); m.p: 250–252 °C; FT-IR (ν cm^{-1}): 3268, 1686, 1525, 1288, 1244, 1155; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm): 10.82 (s, 1H), 8.17–8.13 (m, 2H), 8.01 (d, J = 15.4 Hz, 1H), 7.84 (dd, J = 8.2, 1.5 Hz, 2H), 7.81–7.74 (m, 3H), 7.68 (s, 1H), 7.66–7.64 (m, 1H), 7.56 (d, J = 7.2 Hz, 1H), 7.32–7.27 (m, 4H), 7.09 (d, J = 8.1 Hz, 1H), 6.17 (s, 2H), 5.22 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ (ppm): 188.1, 166.6, 141.9, 139.2, 138.6, 137.8, 137.5, 130.5, 129.2, 127.3, 126.0, 123.5, 122.0, 121.0, 119.3, 116.1, 112.9, 111.3, 49.8; HRMS (ESI) *m/z* calcd for $C_{26}H_{21}N_3O_6S$ [M + H$^+$] 504.1229, found 504.1240.

4.2.3.13. (E)-2-(3-(3-(2-bromophenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)-N-(4-sulfamoyl phenyl)acetamide ***(5 m)***. Yellow solid (yield 82%); m. p 251–252 °C; FT-IR (ν cm^{-1}): 3282, 1682, 1593, 1521, 1315, 1156; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm): 10.79 (s, 1H), 8.09 (s, 1H), 7.96 (d, J = 7.5 Hz, 1H), 7.81–7.72 (m, 5H), 7.62–7.51 (m, 4H), 7.48–7.44 (m, 1H), 7.29 (dt, J = 19.5, 9.7 Hz, 4H), 7.04 (d, J = 16.0 Hz, 1H), 5.19 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ (ppm): 188.1, 166.6, 141.9, 139.2, 138.6, 137.8, 137.5, 130.5, 129.2, 127.3, 126.0, 123.5, 122.0, 121.0, 119.3, 116.1, 112.9, 111.3, 49.8; HRMS (ESI) *m/z* calcd for $C_{25}H_{20}BrN_3O_4S$ [M + Na$^+$] 562.0235; found 562.0265.

4.2.3.14. (E)-2-(3-(3-(3-nitrophenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)-N-(4-sulfamoylphenyl). acetamide **(5n):** Orange solid (yield 86%); m. p 260–261 °C; FT-IR (ν cm^{-1}): 3272, 1676, 1586, 1525, 1393, 1290, 1157; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm): 10.83 (s, 1H), 8.78 (s, 1H), 8.62 (d, J = 7.6 Hz, 1H), 8.48 (d, J = 7.2 Hz, 1H), 8.25 (s, 1H), 8.17 (dd, J = 15.9, 11.9 Hz, 2H), 7.89 (t, J = 7.9 Hz, 1H), 7.76 (dt, J = 18.9, 12.2 Hz, 5H), 7.59 (d, J = 6.9 Hz, 1H), 7.31 (dd, J = 14.3, 10.7 Hz, 4H), 5.25 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ (ppm): 188.2, 166.6, 149.9, 143.9, 141.9, 140.4 139.3, 138.6, 138.3, 129.9, 127.2, 126.0, 124.2, 123.6, 122.2, 121.1, 119.3, 116.1, 112.9, 49.9; HRMS (ESI) *m/z* calcd for $C_{25}H_{20}N_4O_6S$ [M + H$^+$] 505.1182; found 505.1190.

4.2.3.15. (E)-2-(3-(3-oxo-3-(3,4,5-trimethoxyphenyl)prop-1-en-1-yl)-1H-indol-1-yl)-N-(4-sulfamoyl phenyl)acetamide ***(5o)***. Brown solid, yield 83%; m.p 262–264 °C; FT-IR (ν cm^{-1}):3272, 1657, 1651, 1591, 1524, 1393, 1290, 1156; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm): 10.91–10.77 (m, 1H), 8.20 (s, 1H), 8.06 (s, 2H), 7.78 (d, J = 5.6 Hz, 4H), 7.69 (s, 1H), 7.58 (d, J = 5.6 Hz, 1H), 7.40 (s, 2H), 7.29 (s, 4H), 5.24 (s/'', 2H), 3.93 (s, 6H), 3.78 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ (ppm): 188.2, 166.6, 149.9, 143.9, 141.9, 140.4, 139.3, 138.6, 138.3, 129.9, 127.2, 126.0, 124.2, 123.6, 122.2, 121.1, 119.3, 116.1, 112.9, 49.9; HRMS (ESI) *m/z* calcd for $C_{28}H_{27}N_3O_7S$ [M + H$^+$] 550.1648; found 550.1675.

4.2.3.16. (E)-2-(3-(3-oxo-3-(4-(trifluoromethyl)phenyl)prop-1-en-1-yl)-1H-indol-1-yl)-N-(4-sulfamoyl phenyl)acetamide ***(5p)***. Yellow solid, (yield 83%); m.p: 253–255 °C; FT-IR (ν cm^{-1}): 3264, 1681, 1590, 1527, 1321, 1158, 1123; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm): 10.90 (s, 1H), 8.32 (s, J = 8.0 Hz, 1H), 8.22 (s, 1H), 8.16 (s, J = 6.9 Hz, 1H), 8.10 (d, J = 15.5 Hz, 1H), 7.94 (d, J = 8.2 Hz, 2H), 7.79 (q, J = 9.2 Hz, 4H), 7.74 (s, 1H), 7.69 (d, J = 15.4 Hz, 2H), 7.59 (d, J = 7.1 Hz, 1H), 7.33–7.27 (t, 3H), 5.25 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ (ppm): 188.3, 166.6, 141.9, 139.3, 138.6, 137.9, 137.6, 132.2, 130.7, 127.3, 126.9, 126.1, 123.5, 122.0, 121.0, 119.3, 116.1, 112.9, 111.3, 49.9; HRMS (ESI) *m/z* calcd for $C_{26}H_{20}F_3N_3O_4S$ [M + H$^+$] 528.1205; found 528.1226.

4.2.3.17. (E)-2-(3-(3-(2-chlorophenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)-N-(4-sulfamoylphenyl). acetamide **(5q):** Yellow solid (yield 82%); m. p: 272–274 °C; FT-IR (ν cm^{-1}):3272, 1657, 1651, 1591, 1524, 1393, 1290, 1156; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm): 10.79 (s, 1H), 8.09 (s, 1H), 7.96 (d, J = 7.4 Hz, 1H), 7.77 (dd, J = 19.9, 8.1 Hz, 4H), 7.66–7.49 (m, 6H), 7.29 (d, J = 18.1 Hz, 4H), 7.07 (d, J = 16.0 Hz, 1H), 5.19 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ (ppm): 188.1, 166.6, 141.9, 139.2, 138.6, 137.8, 137.5, 130.5, 129.2, 127.3, 126.0, 123.5, 122.0, 121.0, 119.3, 116.1, 112.9, 111.3, 49.8; HRMS (ESI) *m/z* calcd for $C_{25}H_{20}ClN_3O_4S$ [M + H$^+$] 494.0941; found 494.0967.

4.2.3.18. (E)-2-(3-(3-(3-chlorophenyl)-3-oxoprop-1-en-1-yl)-1H-indol-1-yl)-N-(4-sulfamoylphenyl). acetamide **(5r):** Yellow solid (yield 81%); m. p: 264–266 °C; FT-IR (ν cm^{-1}): 3260, 1679, 1522, 1391, 1287, 1152; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm):10.87–10.78 (s, 1H), 8.24–8.20 (s, 1H), 8.19–8.15 (s, 1H), 8.15–8.09 (d, 2H), 8.09–8.05 (s, 1H), 7.82–7.75 (m, 4H), 7.74–7.71 (s, 1H), 7.70–7.65 (s, 1H), 7.64–7.60 (s, 1H), 7.60–7.56 (s, 1H), 7.35–7.27 (m, 4H), 5.27–5.21 (d, 2H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ (ppm): 187.9, 166.6, 141.9, 140.7, 139.5, 139.2, 138.5, 137.7, 128.1, 127.3, 126.1, 123.5, 122.1, 121.1, 119.3, 115.9, 112.9, 111.3, 49.8; HRMS (ESI) *m/z* calcd for $C_{25}H_{20}ClN_3O_4S$ [M + H$^+$] 494.0941, found 494.0952.

4.3. Carbonic anhydrase inhibition assay

An SX.18MV-R Applied Photophysics (Oxford, UK) stopped-flow instrument has been used to assay the catalytic/inhibition of various CA isozymes.[22] Phenol Red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 10 mM Hepes (pH 7.4) as buffer, 0.1 M Na_2SO_4 or $NaClO_4$ (for maintaining constant the ionic strength; these anions are not inhibitory in the used concentration), following the CA-catalyzed CO_2 hydration reaction for a period of 5–10 s. Saturated CO_2 solutions in water at 25 °C were used as substrate. Stock solutions of inhibitors were prepared at a concentration of 10 μM (in DMSO-water 1:1, v/v) and dilutions up to 0.01 nM done with the assay buffer mentioned above. At least 7 different inhibitor concentrations have been used for measuring the inhibition constant. Inhibitor and enzyme solutions were pre-incubated together for 10 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. Triplicate experiments were done for each inhibitor concentration, and the values reported throughout the paper are the mean of such results. The inhibition constants were obtained by non-linear least-squares methods using the *Cheng-Prusoff* equation, as reported earlier [23] and represent the mean from at least three different determinations. All CA isozymes used here were recombinant proteins obtained as reported earlier by our group [24–26].

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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