Bioorg. Med. Chem. Lett. 37 (2021) 127856

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

3-Functionalised benzenesulphonamide based 1,3,4-oxadiazoles as selective carbonic anhydrase XIII inhibitors: Design, synthesis and biological evaluation

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ARTICLE INFO

Keywords:
1,3,4-Oxadiazole
Carbonic anhydrase
hCA XIII isoform
Isoform selective inhibitors
Fluorescence

ABSTRACT

A new series of benzenesulphonamide linked-1,3,4-oxadiazole hybrids (**6a**–**s**) has been synthesized and tested for their carbonic anhydrase inhibition against human (h) carbonic anhydrase (CA) isoforms hCA I, II, IX, and XIII. Fluorescence properties of some of the synthesized molecules were studied. Most of the molecules exhibited significant inhibitory power, comparable or better than the standard drug acetazolamide (AAZ) on hCA XIII. Out of 19 tested molecules, compound **6e** (75.8 nM) was 3 times more potent than AAZ (250.0 nM) against hCA I, whereas compound **6e** (15.4 nM), **6g** (16.2 nM), **6h** (16.4 nM) and **6i** (17.0 nM) were found to be more potent than AAZ (17.0 nM) against isoform hCA XIII. It is anticipated that these compounds could be taken as the potential leads for the development of selective hCA XIII isoform inhibitors with improved potency.

Carbonic anhydrases (CAs, EC 4.2.1.1) are the superfamily of metalloenzymes with eight distinct families α, β, γ, δ, ζ, η, θ and ι, which are found in all life kingdoms from eukaryotes to prokaryotes.[1,2] The α-CAs are expressed in humans and are subdivided into 16 different isoforms that vary by localization and catalytic activity: CA I, CA II, CA III, CA VII, CA XIII are cytosolic; CA IV, CA IX, CA XII, CA XIV, CA XV membrane-bound; CA VA and CA VB mitochondrial; and CA VI secreted in saliva and colostrum. On the other hand, CA VIII, CA X, and CA XI are three catalytically inactive forms referred to as CA-related proteins (CARPs).[3,4] These enzymes catalyze the reversible inter-conversion of CO_2 and H_2O to bicarbonate and proton, for which, comparatively high amounts of CAs are present in different tissues of most studied organisms. They are associated with vital processes like respiration and transport of CO_2/bicarbonate between metabolizing tissues and the lungs, pH and CO_2 homeostasis, electrolyte secretion in a variety of tissues/organs, various biosynthetic reactions such as the gluconeogenesis, lipogenesis, and ureagenesis, bone resorption, calcification, tumorigenicity and many other physiologic or pathologic processes.[5,6]

The catalytically active species in all classes of CA, is a metal hydroxide derivative (L_3-M^{2+}-OH^-) of the enzyme, that act as a strong nucleophile (at neutral pH) on the CO_2 molecule bound in a hydrophobic pocket. The water coordinated to the metal ion, which is found at the bottom of the active site cavity generated this metal hydroxide species.[7] In addition, CAs also catalyze several other reactions, such as the aldehyde hydration to *gem*-diols, the hydration of cyanate to carbamic acid, or cyanamide to urea and the hydrolysis of carboxylic, sulphonic or phosphoric acids esters.[8] Basically there are two main classes of compounds that inhibit CAs: firstly, the metal complexing inorganic anions (e.g., cyanide, cyanate, thiocyanate, azide, hydrogensulphide etc.), which were important for understanding the catalytic and inhibitory mechanisms and secondly, the unsubstituted sulphonamides with the general formula RSO_2NH_2 (R = aryl; hetaryl; perhaloalkyl) that led to the development of several classes of pharmacological agents.[9] Out of the 16 isoforms of α CA, the cytosolic CA XIII was reported in 2000s and shows similar catalytic activity to that of mitochondrial isoenzyme CA V and cytosolic isoenzyme CA I, II, III. This enzyme is expressed both in mouse (mCA XIII) and several human (hCA XIII) tissues including salivary glands, kidney, small intestine,

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https://doi.org/10.1016/j.bmcl.2021.127856
Received 2 December 2020; Received in revised form 27 January 2021; Accepted 5 February 2021
Available online 18 February 2021

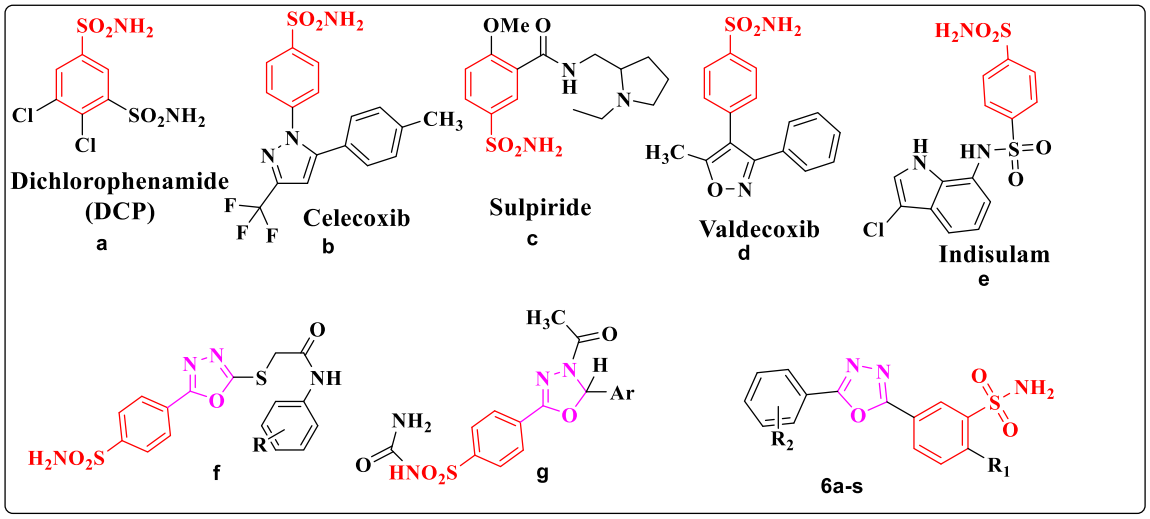
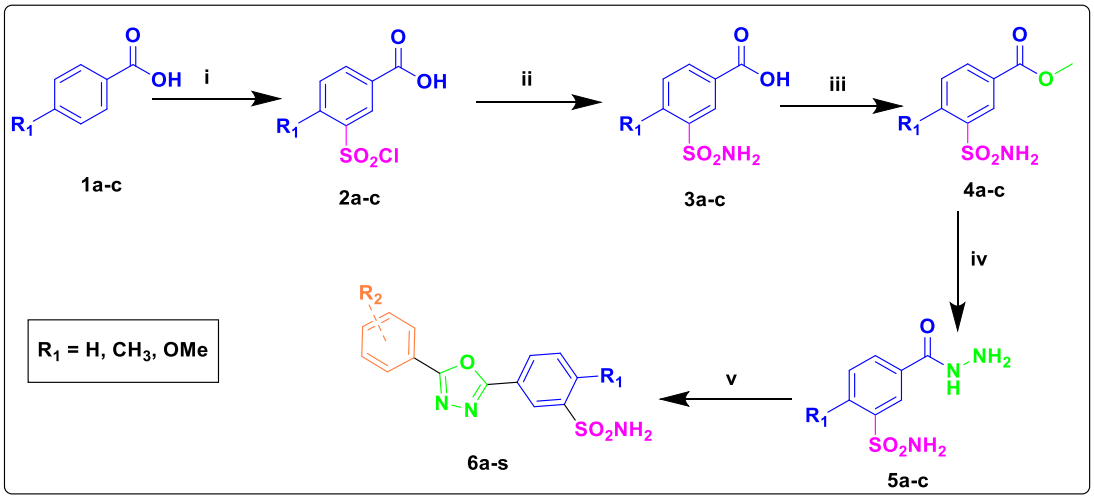


Fig. 1. Representative examples of benzenesulphonamide and 1,3,4-oxadiazole as carbonic anhydrase inhibitors and rationale for designed molecules (**6a**–**s**).



colon, uterus and testis. These findings suggest that the widespread distribution of hCA XIII leads to the development of inhibitors for treatment and prevention of various disorders.[10–14]

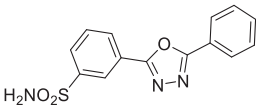
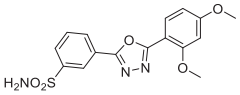
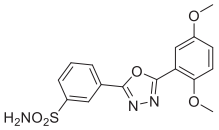
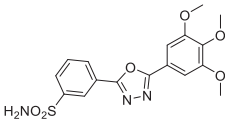
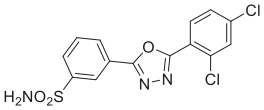
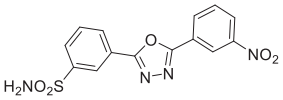
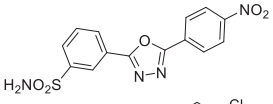
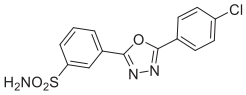
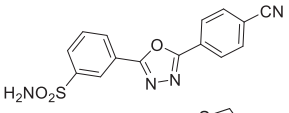
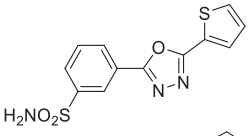
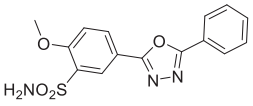
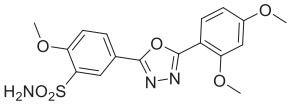
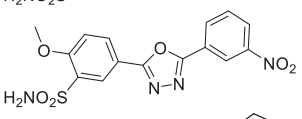
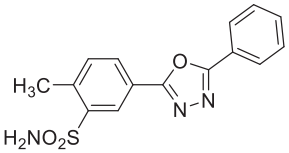
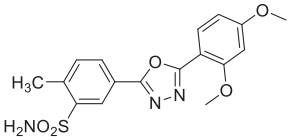
However, substitution on the tail part of benzenesulphonamide with various heterocyclic or aromatic rings enhance the inhibitory activity by modifying absorption and gastro-intestinal tolerance. Dichlorphenamide, celecoxib, sulpiride, valdecoxib, indisulam (Fig. 1a–e) are some of the most popular marketed drug which contains the benzenesulphonamide moiety.[15] However, the heterocycles with 1,3,4-oxadiazole moieties are also exhibiting wide range of biological activities that included anticancer, antibacterial, antifungal, analgesic, anti-inflammatory, anticonvulsant, antihypertensive, antiviral, anti HIV, and antidiabetic properties.[16] Sharma et al. have reported that 1,3,4-oxadiazole benzenesulphonamide hybrids showed potential inhibition against hCA I, II, IX (Fig. 1f).[17] Bianco et al. have reported selectivity of *N*-acylbenzenesulphonamide dihydro-1,3,4-oxadiazole hybrids against hCA IX and XII (Fig. 1g).[18] In view of the importance of these scaffold in the present work, we designed and synthesized a series of new molecules with 3-functionalised benzenesulphonamide incorporated 1,3,4-oxadiazoles which could inhibit different CAs isoforms with improved selectivity.

Considering the inhibitory potency of the 3-functionalised benzene sulphonamide from our previous paper, here we extend our investigations in the design of novel CA inhibitors containing oxadiazole moieties linked to 3-functionalised benzene sulphonamide. The synthesis of the target compounds start with reaction of commercially available benzoic acids (**1a**–**c**) and chlorosulphonic acid were reacted at 110 °C to afford the 3-(chlorosulphonyl)benzoic acids (**2a**–**d**) which were in the second step treated with ammonium hydroxide solution at 0 °C to give the corresponding 3-sulphamoylbenzoic acids (**3a**–**c**). Compounds **3a**–**c** were subjected to esterification with $SOCl_2$ and MeOH forming **4a**–**c**, followed by hydrazide formation **5a**–**c** using $NH_2NH_2{\cdot}H_2O$ under reflux. Finally, the hydrazides underwent transition-metal-free oxidative cyclization forming 1,3,4-oxadiazoles **6a**–**s** in the presence of potassium carbonate, molecular I_2 with different aromatic aldehydes.

Reagent and reaction conditions: i) HSO_3Cl (5 equiv.), 110 °C, 6–8 h, 70–80%; ii) NH_4OH sol., 0 °C, 2 h; iii) MeOH, $SOCl_2$, reflux, overnight, 90–98%; iv) $NH_2NH_2{\cdot}H_2O$ (3 equiv.), EtOH, reflux, 4 h, 75–80%; v) Substituted benzaldehyde (1 equiv.), I_2 (1.2 equiv.), K_2CO_3 (3 equiv.), DMSO, 100 °C, N_2, reflux, 74–80%.

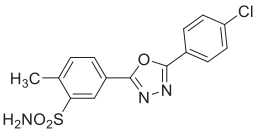
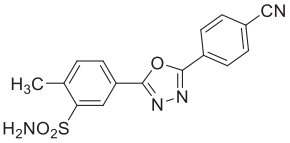
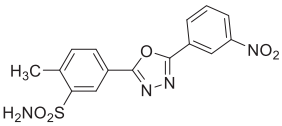
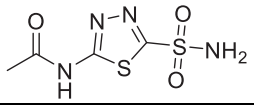
The target benzenesulphonamide linked-1,3,4-oxadiazole hybrids (**6a**–**s**) were screened for their potentials against four different hCA isoforms, namely, hCA I, II, XIII (the cytosolic) and hCA IX, (the tumor-associated) by a stopped-flow CO_2 hydrase assay, taking acetazolamide

Table 1
Inhibition of hCA isoforms I, II, IX and XIII with target compounds (**6a**–**s**) and acetazolamide (AAZ) as a standard drug (Ki = nM).*

Compound	Structure	hCA I	hCAII	hCA IX	hCA XIII
6a		629.3 ± 51.1	288.6 ± 16.8	108.2 ± 4.5	30.9 ± 1.7
6b		754.7 ± 46.5	1550 ± 153	82.2 ± 5.9	36.7 ± 2.4
6c		2568 ± 138	833.5 ± 61.7	76.8 ± 7.2	31.4 ± 1.5
6d		1392 ± 131	904.9 ± 72.8	68.3 ± 5.6	38.5 ± 3.2
6e		75.8 ± 5.6	89.8 ± 6.1	76.3 ± 4.2	15.4 ± 0.7
6f		861.6 ± 64.4	52.4 ± 2.2	44.0 ± 2.9	19.1 ± 1.6
6g		5023 ± 482	285.9 ± 26.6	57.1 ± 3.3	16.2 ± 1.1
6h		915.9 ± 78.9	608.0 ± 36.2	54.5 ± 4.5	16.4 ± 0.9
6i		887.9 ± 77.2	407.5 ± 32.1	76.3 ± 4.8	17.0 ± 1.1
6j		268.3 ± 18.5	217.3 ± 13.7	85.3 ± 4.0	18.4 ± 0.9
6k		>10,000	>10,000	>10,000	>10,000
6l		>10,000	>10,000	>10,000	>10,000
6m		>10,000	>10,000	>10,000	>10,000
6n		>10,000	>10,000	>10,000	>10,000
6o		>10,000	>10,000	>10,000	>10,000
6p		>10,000	>10,000	>10,000	>10,000

(*continued on next page*)

Table 1 (*continued*)

Compound	Structure	hCA I	hCAII	hCA IX	hCA XIII
6q		>10,000	>10,000	>10,000	>10,000
6r		>10,000	>10,000	>10,000	>10,000
6s		>10,000	>10,000	>10,000	>10,000
AAZ		250.0	12.1	25.8	17.0

* Mean from 3 different assays, by a stopped flow technique (from 3 different determinations).

(AAZ), as a standard drug. The following Structure-Activity Relationship (SAR) can be inferred from the inhibition data of benzenesulphonamide linked −1,3,4-oxadiazole hybrids (**6a**–**s**) as shown in Table 1:

- The target compounds **6a**–**s** had shown weak inhibition towards the cytosolic isoform hCA I except one molecule having an excellent inhibition potency that was **6e** with Ki = 75.8 nM as compared to AAZ (Ki = 250 nM). However, the compounds without substitution on the benzenesulphonamide ring (**6a**–**j**) have shown moderate inhibition with Ki ranging 0.268 μM–5.0 μM, whereas the compounds with substitution on the benzenesulphonamide ring (**6k**–**s**) were ineffective towards the isoform (Ki > 10000 nM).
- All the synthesized compounds (**6a**–**s**) were less potent than the standard drug AAZ for both cytosolic isoform hCA II and the transmembrane tumor-associated isoform hCA IX. Here also the compounds without substitution on benzenesulphonamide ring (**6a**–**j**) were moderate to weak inhibitors of hCA II with Ki ranging 52.4 nM-1.5 μM and hCA IX with Ki ranging 44.0 nM- 0.10 μM while compounds with substitution ($\mathbf{R_1}$) on benzenesulphonamide ring (**6k**–**s**) were ineffective and exhibited inhibitory potencies with Ki > 10 μM as compared to standard AAZ for both hCA II (Ki = 12.1 nM) and IX (Ki = 25.8 nM).
- The molecules investigated for the inhibition of the new isoenzyme CA XIII were quite excellent. Out of total nineteen molecules ten (**6a**–**j**) were showing very good inhibitory potencies with Ki ranging 15.4–38.5 nM as compared to AAZ (Ki = 17.0 nM). Compounds **6e**–**j** where $\mathbf{R_2}$ was substituted with electron withdrawing group (–**NO_2**, –**Cl**, –**CN**) and heteroaromatic ring (–thiophene) have shown better activity than that of the molecules (**6a**–**d**) with $\mathbf{R_2}$ containing electron donating group (–**H**, –**OMe**) and in all these $\mathbf{R_1}$ was without any substitution. Similarly, the molecules (**6k**–**s**) have not shown any inhibition because of the substitution on benzenesulphonamide ring.

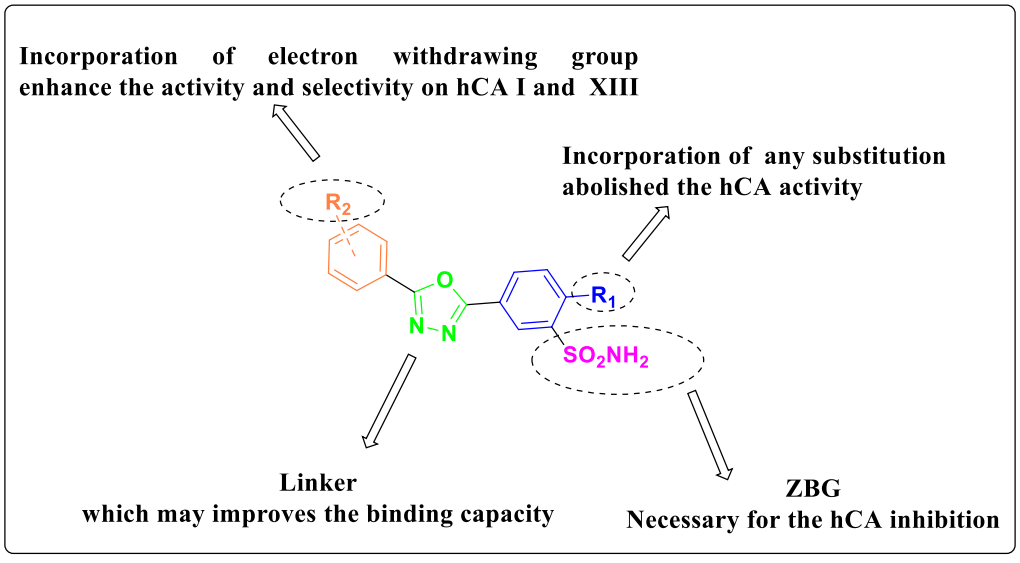


Fig. 2. SAR for the target CAIs toward *h*CA I and XIII isoforms.

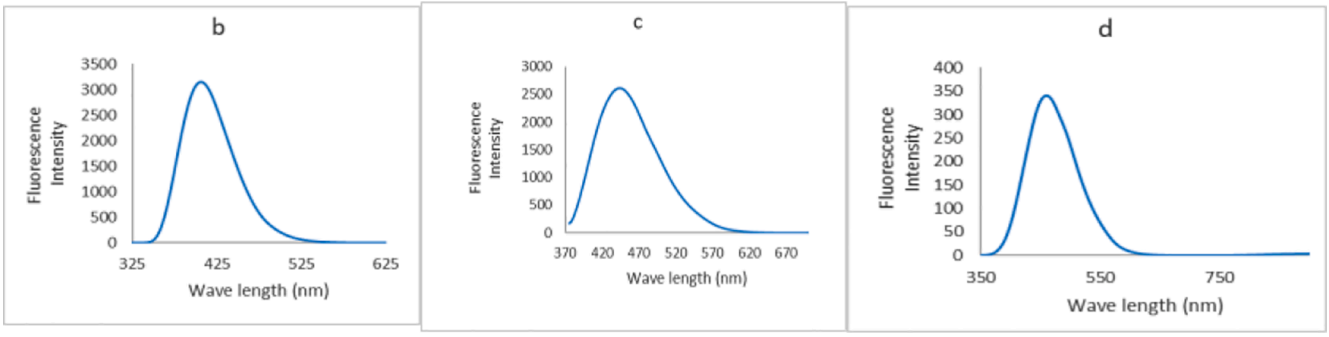


Fig. 3. Fluorescence emission spectra of compounds **6b**, **6c**, and **6d** in DMSO.

- The inhibition order of the four most potent benzenesulphonamide linked-1,3,4-oxadiazole hybrids are as follows, hCA XIII inhibition: (**6e**) > (**6g**) > (**6h**) > (**6i**). Most active molecule **6e** has shown very good inhibition against both isoforms hCA I (75.8 nM) and hCA XIII (15.4 nM).
- Furthermore, it is interesting to note here that when the molecules contain any substitution ($\mathbf{R_1}$) on the benzenesulphonamide ring those molecules (**6k**–**s**) were devoid of any inhibitory activity against all the four isoforms with Ki > 10000 nM. Again the potent molecules were more selective towards hCA XIII over hCA I. The obtained SAR toward *h*CA I and XIII isoforms was illustrated in Fig. 2.

Fluorescence studies were performed for the synthesized molecules (**6a**–**s**) as 1,3,4-oxadiazoles are well known for exhibiting fluorescence phenopmena.[19] The molecules were diluted in DMSO and the excitation and emission spectra were investigated. Out of 19 molecules the excitation and emission spectra of three compounds **6b**, **6c** and **6d** showed prominent shifts to longer wavelengths, in contrast to the other cases in which the shifts were typically only marginal. Fig. 3. shows the fluorescence emission spectra of compounds **6b**, **6c** and **6d**. Fluorescence properties of these compounds suggest that they may hold a potential for applications as chemical probes.

In summary, the current research work explained the design and synthesis of new benzenesulphonamide linked-1,3,4-oxadiazole hybrids (**6a**–**s**) as significant human carbonic anhydrase inhibitors. The newly synthesized molecules in variable degrees of inhibition affected all the tested CA isoforms (hCA I, II, IX, XIII). Some of the synthesiz

ed molecules exhibited fluorescence properties, which specify their potential usefulness in the field of material chemistry. The present target sulfonamide molecules are selectivity as most of them showed good selectivity toward hCA XIII (Ki ranges 15.4–38.5 nM) over CA I (Ki ranges 75.8–5023 nM). SAR results revealed that incorporation of substitution ($\mathbf{R_1}$) on the benzenesulphonamide ring (**6k**–**s**) led to a lack of effectiveness against all the tested isoforms (Ki > 10,000 nM); whereas, molecules with substitution $\mathbf{R_2}$ on the tail part containing 1,3,4-oxadiazole and no substitution on head part ($\mathbf{R_1 = H}$) led to a better inhibition with Ki ranging: 268.3 nM–0.5 μM for hCA I, 52.4 nM–1.5 μM for hCA II, 44.0 nM–0.10 μM for hCA IX and 15.4 nM–38.5 nM hCA XIII. Furthermore, molecules with $\mathbf{R_2}$ containing electron withdrawing group (–**NO$_2$**, –**Cl**, –**CN**) and heteroaromatic ring (–furan) (**6e**–**j**) were more potent inhibitory action than those containing electron donating group (**6a**–**d**) against **hCA XIII**. In addition, molecule **6e** is the most potent one compared to the standard AAZ both in case of hCA I (Ki = 75.8 nM) and hCA XIII (Ki = 15.4 nM). Hence, it is anticipated that the molecules which are more potent could be taken as the lead for the design and development of selective hCA XIII inhibitor with better inhibition potency.

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.

Acknowledgements

The authors BS, A, PS and KA are thankful to Department of Pharmaceuticals, Ministry of Chemicals and Fertilizers, Govt. of India, New Delhi for the award of NIPER fellowship.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.127856.

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20 Khalifah RG. *J Biol Chem* **1971**, 246, 2561–2573. An SX.18MV-R applied photophysics (Oxford, UK) stopped-flow instrument has been used to assay the catalytic/inhibition of various CA isozymes.[20] 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 preincubated 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,[21] 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.[22,23] Their concentrations in the assay system were in the range of 4.5–12.3 nM

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