Bioorganic Chemistry 104 (2020) 104272

Contents lists available at ScienceDirect

Bioorganic Chemistry

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

Synthesis and biological evaluation of some coumarin hybrids as selective carbonic anhydrase IX and XII inhibitors

Pavitra S. Thacker[a,1], Nerella Sridhar Goud[a,1], Omkar S. Argulwar[a], Jyothsna Soman[a], Andrea Angeli[c], Mallika Alvala[a], Mohammed Arifuddin[a,b,*], Claudiu T. Supuran[c,*]

[a] Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research (NIPER), Balanagar, Hyderabad 500037, India
[b] Department of Chemistry, Anwarul Uloom College, 11-3-918, New Malleypally, Hyderabad 500001, T. S., India
[c] Università degli Studi di Firenze, Neurofarba Dept., Sezione di Scienze Farmaceutiche e Nutraceutiche, Via Ugo Schiff 6, 50019 Sesto Fiorentino, Florence, Italy

ARTICLE INFO

Keywords:
Carbonic anhydrases
hCA IX & XII isoform
Coumarin
Pyrazole
Thiazolidinone
1, 2, 3-triazole

ABSTRACT

Two series, coumarin-linked to thiazolidinone via a pyrazole linker (**6a-m**, Series 1) and coumarin-linked 1,2,3-triazoles (**5a-j**, Series 2) were synthesized and the synthesized compounds were subjected for evaluation against the four physiologically and pharmacologically relevant hCA isoforms, hCA I, II, IX and XII. The results indicated selective inhibition of tumor-associated isoforms hCA IX and XII over the off-target isoforms, hCA I and II. The compounds of series 1 exhibited better hCA IX inhibition compared to hCA XII, with compounds **6i**, **6h**, **6a** and **6k**, exhibiting notable K_i values of less than 100 nM. Among all the compounds, compound **6i** showed the best inhibition with a K_i value of 61.5 nM. Among the compounds of series 2, compounds **5a**, **5b**, **5c**, **5d**, **5f** and **5j** exhibited notable hCA IX inhibition. Compound **5d** showed the best inhibition with a K_i value of 32.7 nM. In the case of hCA XII, compound **5i** showed the best inhibition with a K_i value of 84.2 nM. Hence, compound **6i** from Series 1 and **5d** from Series 2 could be taken as lead compounds for the further development of selective and potent hCA IX inhibitors, whereas the compound **5i** from Series 2 can be explored further for the design of selective and potent hCA XII inhibitors.

1. Introduction

The living organisms on earth are broadly classified into two major classes namely prokaryotes and eukaryotes. These two classes, in spite of being distinct in their own ways, are still related to each other by the presence of certain common molecules/ions like CO_2, bicarbonate and protons. Carbonic anhydrases (CAs, EC 4.2.1.1) are a superfamily of enzymes which utilize these simple molecules/ions as substrates and thereby aid in catalyzing the reversible conversion of CO_2 to bicarbonate and proton as depicted in the equation below:-

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

The expression of these enzymes is widespread across various tissues wherein they play a key role in many physiological processes such as acid base balance, transport of ions and gases, respiration, ureagenesis, gluconeogenesis and lipogenesis to name a few. These enzymes elicit their diverse activities with the help of various metals present within their active sites. The metal ions which are typically present are Zn(II), Fe(II), Co(II) and cadmium [1–4].

The carbonic anhydrases are divided into eight genetically distinct classes namely α-, β-, γ-, δ-, ζ-, η-, θ and ι. All animals, including humans, mainly contain the α-CAs (denoted as human carbonic anhydrases, hCAs, in humans). The α-CA class is in turn comprised of 16 isoforms. These isoforms possess distinct catalytic activities as well as physiological functions [5,6].

Amongst these 16 isoforms, hCA IX and hCA XII are transmembrane isoforms which have been characterized as biomarkers for several types of tumors. The hCA IX is expressed in healthy tissues. However, it is overexpressed in tumor microenvironment on account of concurrent conditions like hypoxia and acidity. This in turn promotes the survival and growth of tumor cells, cancer cell migration, invasion and also allows the cells to maintain stem-cell like properties. The hCA XII, originally identified from human renal clear cell carcinoma, also assists in maintenance of acid-base homoeostasis in normal as well as tumor cells. The overexpression of both these isoforms in cancer results in the

* Corresponding authors at: Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research (NIPER), Balanagar, Hyderabad 500037, India (M. Arifuddin).
E-mail addresses: arif@niperhyd.ac.in (M. Arifuddin), claudiu.supuran@unifi.it (C.T. Supuran).
[1] Equal contribution by the authors.

https://doi.org/10.1016/j.bioorg.2020.104272
Received 1 May 2020; Received in revised form 30 August 2020; Accepted 7 September 2020
Available online 08 September 2020

poor prognosis as well as chemoresistance to weakly basic anti-cancer drugs [7–9].

This warrants the need for discovering selective hCA IX and XII inhibitors. In recent years, several classes of compounds have been identified as showing selective and potent inhibition against both of the aforementioned isoforms. Amongst them, coumarins are a class of naturally occurring compounds which have been shown to inhibit hCA IX and XII via a non-classical mechanism, by occlusion of the active-site entrance [10,11]. Taking a cue from this, several research groups, including ours, have reported various derivatives of coumarin as selective CA IX and XII inhibitors [12–15].

Pyrazole is one of the key privileged scaffolds in medicinal chemistry which has been shown to possess diverse pharmacological profiles such as anticancer, anti-inflammatory, antipyretic, analgesic, antipsychotic, anti-anxiety and anti-obesity [16]. In addition to that, pyrazole has also been shown to possess potent carbonic anhydrase inhibition especially against tumor-associated isoforms hCA IX and XII [17,18]. On the other hand, the 4-thiazolidinone pharmacophore is a key motif possessing a wide array of pharmacological activities such as anticancer, anti-diabetic, antimicrobial, cardiovascular, anti-hyperlipidemic and anticonvulsant. It's effectiveness for CA inhibition has also been explored by various research groups [19,20]. Similarly, the 1,2,3-triazole scaffold represents another key scaffold which possesses a broad range of pharmacological activities such as anticancer, antimicrobial and anti-inflammatory. It also serves as an effective amide surrogate in bioactive compounds due to its strong dipole moments. Apart from that, it has been shown to elicit potent CA inhibitory activity particularly against hCA IX and XII [21,22].

Hence, taking into consideration the potency of coumarin, pyrazole, thiazolidinone and 1,2,3-triazole nuclei to inhibit the tumor-associated isoforms CA IX and XII, it was decided to synthesize two different series of compounds through amalgamation of the above said nuclei by applying molecular hybridization strategy. The first series encompassed the synthesis of coumarin linked to 4-thiazolidinone hybrids via a pyrazole linker (**6a-m**), whereas, the second series consisted of coumarin-linked 1,2,3-triazole hybrids (**5a-j**). The highly selective and potent hCA IX and hCA XII moiety coumarin, was kept common in both the series (Fig. 1).

2. Result and discussion

2.1. Chemistry

2.1.1. Synthesis of series 1 compounds

The synthesis of coumarin linked to 4-thiazolidinone via a pyrazole linker (**6a-m**) was performed as per the general synthetic scheme as illustrated in Scheme 1. Firstly, the intermediate **3** was synthesized by the reaction of salicylaldehyde with ethylacetoacetate in the presence of piperidine as a catalyst. Consequently, the intermediate **3** was further reacted with phenylhydrazine using glacial acetic acid as a solvent to afford intermediate **4** which upon subjection to Vilsmeier-Haack reaction afforded the intermediate **5** as per reported literature method [23]. Finally, the Intermediate **5** was reacted with various anilines and thioglycollic acid in the presence of silica gel (100–200 mesh) and DCM as a solvent to afford the final compounds (**6a-m)** by following the reported literature method [24].

2.1.2. Synthesis of series 1 compounds

The synthesis of coumarin-linked 1,2,3-riazoles (**5a-j**) was performed as per the general synthetic scheme as illustrated in Scheme 2. Initially, the intermediate **3** was synthesized by the reaction of resorcinol **(1)** with ethyl-4-chloroacetoacetate **(2)** using sulphuric acid as solvent under cold conditions through Pechmann condensation mechanism [25]. Thereafter, the intermediate **4** was synthesized by O-alkylation of intermediate **3** with propargyl bromide in the presence of potassium carbonate as a base and acetone as a solvent. Finally, the terminal alkyne of intermediate **4** was cyclized with various benzyl azides furnishing the target compounds **5a-j** through copper (I)-catalyzed 1,3-dipolar cycloaddition reaction in moderate to good yields.

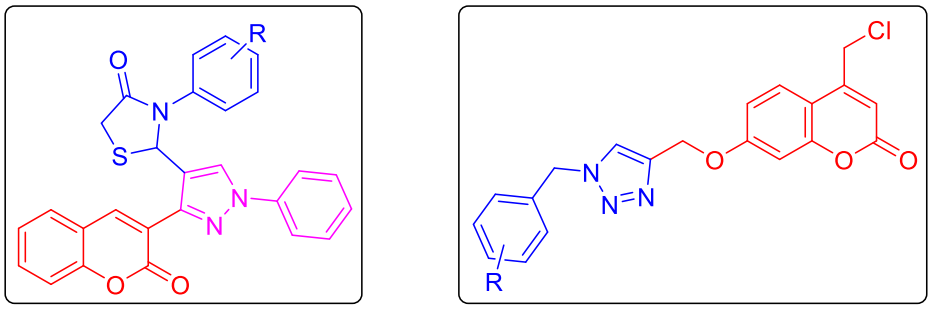
2.2. Carbonic anhydrase inhibition

The compounds synthesized in series 1 (**6a-m**) and series 2 (**5a-j**) were evaluated for their activity against the four physiologically and pharmacologically relevant isoforms, namely, the cytosolic isoforms, hCA I and hCA II and the trans-membrane tumor associated isoforms, hCA IX and hCA XII, by a stopped flow CO_2 hydrase assay method. Acetazolamide (AAZ) was taken as the standard drug. The following inferences regarding the structure–activity relationship of the synthesized compounds can be drawn for both the series:-

2.2.1. Series 1

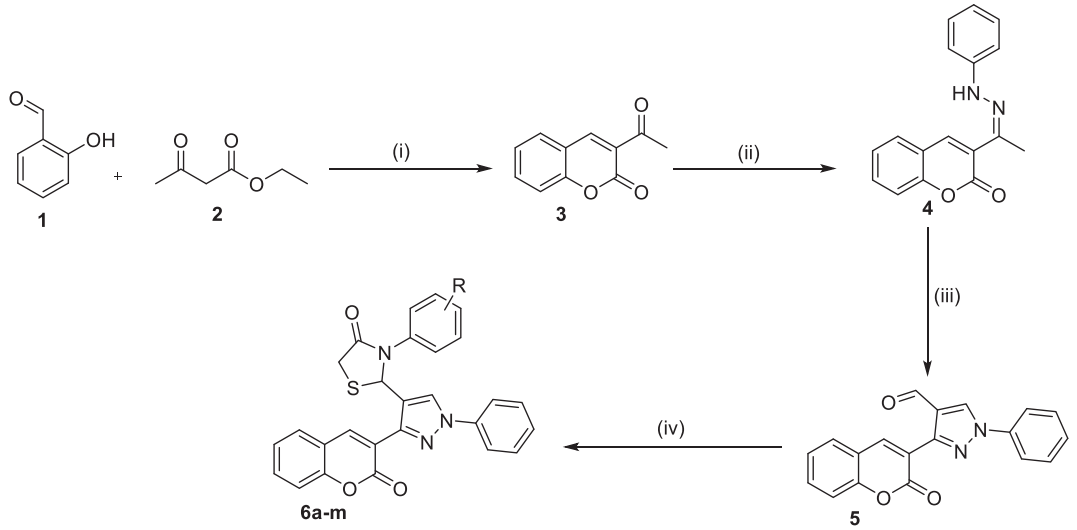
The inferences derived for the compounds synthesized in series 1 (**6a-m**) (Table 1) are summarized below:-

i. The two cytosolic isoforms, hCA I and hCA II, were not at all inhibited by the synthesized compounds **6a-m** (K_i > 10000 nM).
ii. The tumor-associated isoform hCA IX, was inhibited by the compounds, **6a-m,** in a low to high nanomolar range, with the K_i values ranging from 61.5 to 788.4 nM. The best inhibition constant i.e. 61.5 nM was shown by compound **6i**, possessing a methyl group at the meta position of the phenyl ring located at the 3rdposition of 4-thiazolidinone ring. Apart from this, the other compounds found to exhibit a low inhibition on this isoform were compounds **6 h** (K_i = 71.1 nM),**6a** (K_i = 72.1 nM), **6c** (K_i = 72.6 nM), and **6 k** (K_i = 72.7 nM). Barring compounds **6b**, **6e** and **6j**, all the remaining compounds showed K_i values below 100 nM.
iii. The compounds, **6a-m** exhibited a comparatively weaker inhibitory profile for the tumor-associated isoform hCA XII with K_i values ranging between 389.2 and 945.4 nM. The best inhibition constant i.e. 389.2 nM was shown by compound **6 l**, possessing a chloro group at the meta position of the phenyl ring located at the 3rd

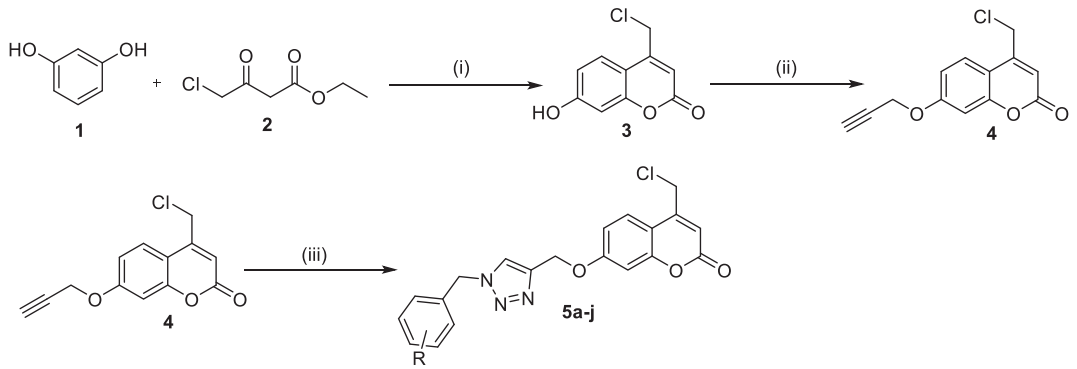


Series 1 Series 2

Fig. 1. General structure of the designed compounds.



Scheme 1. Synthesis of coumarin linked 4-thiazolidinones via pyrazole linker (**6a-m**)-Reagents and conditions: (i) Catalytic piperidine, neat, 90% (ii) Phenylhydrazine, Glacial acetic acid, rt, 30 min, 95% (iii) DMF/$POCl_3$, 0 °C-rt, 24 h, 73% (iv) Substituted anilines, thioglycolic acid, silica gel (100–200 mesh), DCM, rt, 3–12 h, 42–64%.



Scheme 2. Synthesis of coumarin-linked triazoles (**5a-j**)-Reagents and conditions: (i) H_2SO_4, ice bath, 15 min, 95% (ii) Propargyl bromide, K_2CO_3, acetone, rt, 3–4 h 90% (iii) Substituted benzyl azides, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, EtOH:H_2O::1:1, rt, overnight, 72–90%.

position of 4-thiazolidinone ring.

2.2.2. Series 2

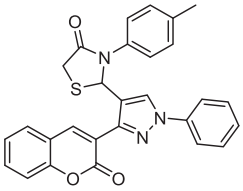
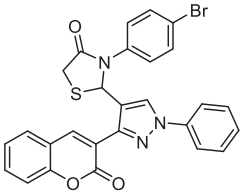
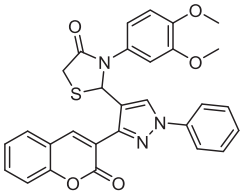
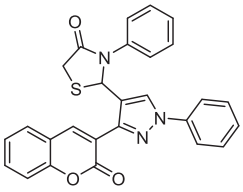
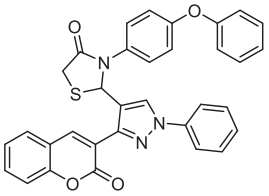
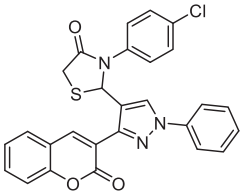
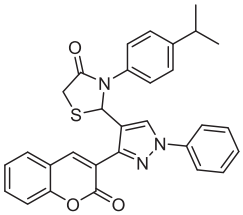
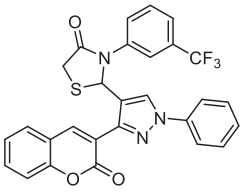
The inferences derived for the compounds of series 2 (**5a-j**) are summarized below (Table 2):-

i. The two cytosolic isoforms, hCA I and hCA II were not at all inhibited by the synthesized compounds **5a-j** (K_i > 10000 nM).
ii. The tumor-associated isoform hCA IX was inhibited by the compounds **5a-j** in a low to high micromolar range with the K_i values ranging from 32.7 to 2152 nM. The best inhibition constant i.e. 32.7 nM was shown by compound **5d** possessing a cyano group at the para position of the benzyl ring located at the 1stposition (N-1) of 1,2,3-triazole ring. Apart from this, the other compounds found to exhibit a low micromolar inhibition on this isoform were compounds **5c** (K_i = 54.2 nM), **5j** (K_i = 60.7 nM), **5f** (K_i = 62.6 nM), **5a** (K_i = 75.3 nM) and **5b** (K_i = 92.6 nM).
iii. The compounds **5a-j** exhibited a comparatively weaker inhibitory profile for the tumor-associated isoform hCA XII with K_i values ranging from 84.2 to 717.1 nM. The best inhibition constant i.e. 84.2 nM was shown by compound **5i** possessing a methoxy group at the para position of the benzyl ring located at the 1st position (N-1) of 1,2,3-triazole ring.

3. Conclusion

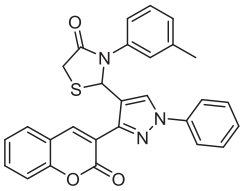
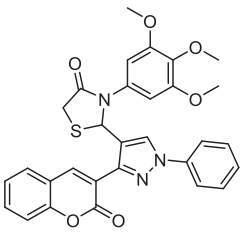
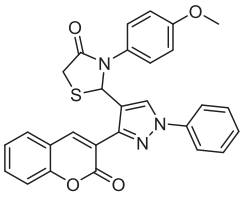
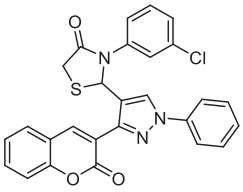
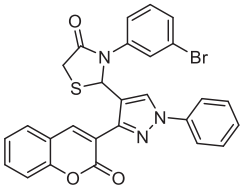
In conclusion, two series, coumarin linked 4-thiazolidinone via a pyrazole linker **(6a-m**, **Series 1)** and coumarin-linked 1,2,3-triazoles (**5a-j**, **Series 2**) were synthesized. The synthesized molecules were screened against the four physiologically and pharmacologically relevant isoforms namely the cytosolic isoforms hCA I and II as well as the transmembrane tumor-associated isoforms, hCA IX and XII. All the compounds showed selective inhibition for hCA IX and XII over hCA I and II. In the case of **series 1**, for hCA IX, the values of the inhibition constants (K_is) ranged from 61.5 to 788.4 nM with the best inhibition constant i.e. 61.5 nM shown by compound **6i**. The other compounds of this series showing notable hCA IX inhibition were compounds **6 h** (K_i = 71.1 nM), **6a** (K_i = 72.1 nM), **6c** (K_i = 72.6 nM), and **6 k** (K_i = 72.7 nM). None of the compounds of this series were able to elicit note worthy hCA XII inhibition. In the case of **series 2** the inhibtion data for hCA IX, the values of the inhibition constants (K_is) ranged from 32.7 to 2152 nM, with the best inhibition constant i.e. 32.7 μM shown by compound **5d**. The other compounds of this series showing notable hCA IX inhibition were compounds **5c** (K_i = 54.2 nM),**5j** (K_i = 60.7 nM), **5f** (K_i = 62.6 nM),**5a**(K_i = 75.3 nM) and **5b** (K_i = 92.6 nM). For hCA XII, the values of the inhibition constants (K_is) ranged between 84.2 and 717.1 nM with the best inhibition constant i.e. 84.2 nM was shown by compound **5i**. Hence, compounds **6i** from **Series 1** and **5d** from **Series 2** could be taken as lead compounds for the further development of selective and potent hCA IX inhibitors, whereas

Table 1
Inhibition data of compounds (**6a-m**) for hCA I, II, IX and XII isoforms and acetazolamide (**AAZ**) as a standard drug.

		K_I (nM)* (IC_{50})			
Compound	**Structure**	**hCA I**	**hCAII**	**hCA IX**	**hCA XII**
6a		> 10000	> 10000	72.1 ± 2.1 (84.6)	945.4 ± 23.4 (599.7)
6b		> 10000	> 10000	788.4 ± 24.5 (915.0)	863.1 ± 25.8 (547.6)
6c		> 10000	> 10000	72.6 ± 3.7 (85.2)	891.0 ± 16.9 (565.3)
6d		> 10000	> 10000	84.8 ± 2.7 (99.3)	906.3 ± 22.3 (575.0)
6e		> 10000	> 10000	672.7 ± 25.8 (780.9)	728.0 ± 27.3 (462.0)
6f		> 10000	> 10000	90.6 ± 3.1 (106.0)	850.6 ± 13.5 (539.7)
6g		> 10000	> 10000	94.3 ± 1.5 (110.3)	674.8 ± 18.3 (428.4)
6h		> 10000	> 10000	71.1 ± 1.3 (83.4)	722.3 ± 15.3 (458.4)

(*continued on next page*)

Table 1 (*continued*)

Compound	Structure	K_I (nM)* (IC_{50})			
		hCA I	hCAII	hCA IX	hCA XII
6i		> 10000	> 10000	61.5 ± 2.0 (72.3)	586.8 ± 17.8
6j		> 10000	> 10000	404.0 ± 9.5 (469.4)	502.7 ± 7.2 (372.6)
6k		> 10000	> 10000	72.7 ± 1.1 (85.3)	618.8 ± 11.8 (392.9)
6l		> 10000	> 10000	91.1 ± 3.0 (106.6)	389.2 ± 8.4
6m		> 10000	> 10000	90.9 ± 2.7 (106.4)	623.4 ± 8.8 (247.5)
AAZ		250	12.1	25.8	5.7

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

compound **5i** from **Series 2** can be explored further for the design of selective and potent hCA XII inhibitors.

4. Experimental section

4.1. General

All the chemicals and solvents were procured and utilized as such from the suppliers. Wherever necessary, anhydrous solvents were used. Thin Layer Chromatography (TLC) analysis was done by utilizing Merck silica gel 60 F_{254} aluminum plates. Stuart Digital Melting Point Apparatus (SMP 30) was used in determining the melting points of the compounds, which are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded using Bruker Avance 500 MHz and 125 MHz respectively using DMSO-d_6 as the solvent. Chemical Shift values are recorded in ppm using TMS as the internal standard. HRMS were determined by Agilent QTOF mass spectrometer 6540 series instrument and were performed using ESI techniques at 70 eV.

4.2. General procedures for the synthesis of series 1 compounds

4.2.1. Synthesis of 3-acetylcoumarin **(3)**

To an equimolar mixture of salicylaldehyde (1equiv.) and ethylacetoacetate (1equiv.) were added few (5–6) drops of piperidine. The mixture was stirred for 30 min at rt. resulting in the formation of a yellowish solid. On completion of the reaction as indicated by the disappearance of staring materials on TLC, crushed ice was added and the solid was filtered off. The crude product was purified via column chromatograpy using EtOAc:Hexane (1:5) as eluent to afford 3-acetylcoumarin **(3)** as a yellow solid in 90% yield.

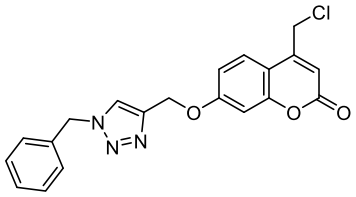
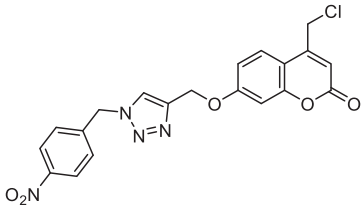
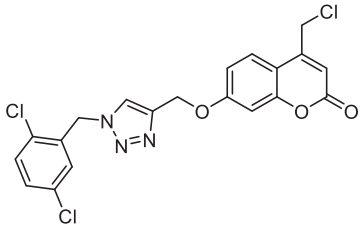
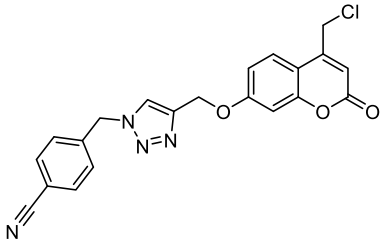
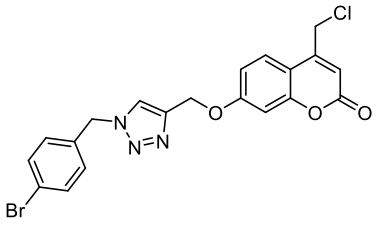
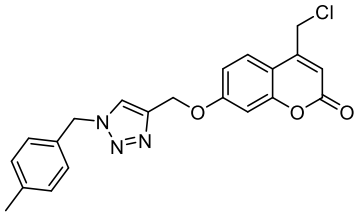
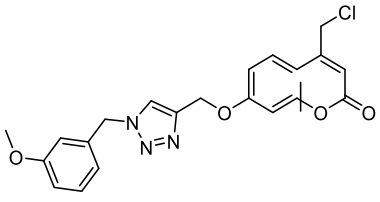
4.2.2. Synthesis of 3-(1-(2-phenylhydrazono)ethyl)-2H-chromen-2-one **(4)**

To a stirred solution of 3-acetylcoumarin **3** (1 equiv.) in acetic acid was added phenylhydrazine (1 equiv.). The solution was allowed to stir at rt. for half an hour. Thereafter, the resultant suspension was added in ice-water and filtered off to afford the crude product **4** in 95% yield.

4.2.3. Synthesis of 1,3-disubstituted-4-formylpyrazole **(5)**

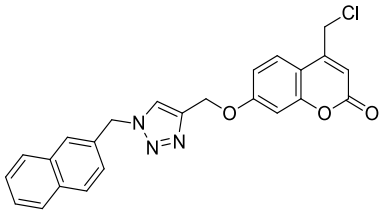
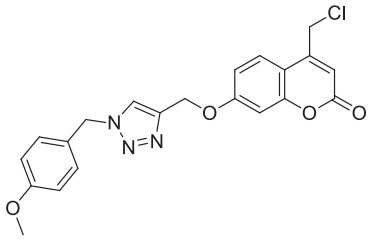
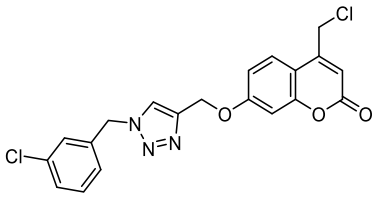
To a stirred solution of DMF (25 mL) and $POCl_3$ (5 mL) cooled to

Table 2
Inhibition data of compounds **5a-j** against hCA isoforms I, II, IX and XII and acetazolamide (**AAZ**) as a standard drug.

		K_I (nM)*			
Compound	**Structure**	**hCAI**	**hCAII**	**hCA IX**	**hCA XII**
5a		> 10000	> 10000	75.3 ± 1.6 (88.3)	466.6 ± 10.3 (296.7)
5b		> 10000	> 10000	92.6 ± 2.2 (108.4)	597.8 ± 9.3 (379.6)
5c		> 10000	> 10000	54.2 ± 1.9 (63.8)	717.1 ± 14.8 (455.1)
5d		> 10000	> 10000	32.7 ± 2.4 (38.9)	623.5 ± 16.3 (395.9)
5e		> 10000	> 10000	787.7 ± 19.6 (914.2)	632.5 ± 8.4 (401.6)
5f		> 10000	> 10000	62.6 ± 1.9 (73.6)	590.9 ± 6.9 (375.2)
5g		> 10000	> 10000	2152 ± 20.0	571.7 ± 15.3 (363.0)

(*continued on next page*)

Table 2 (*continued*)

Compound	Structure	K_I (nM)*			
		hCAI	hCAII	hCA IX	hCA XII
5h		> 10000	> 10000	558.7 ± 13.5 (648.7)	333.0 ± 7.7 (211.9)
5i		> 10000	> 10000	268.5 ± 1.5 (312.3)	84.2 ± 2.0 (54.3)
5j		> 10000	> 10000	60.7 ± 2.0 (71.4)	437.0 ± 6.0 (277.8)
AAZ		250.0	12.1	25.8	5.7

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

0–5 °C was added compound **4** (5 mmol) portion wise. The resulting solution was allowed to stir at rt. for 24 h and the progress of the reaction was monitored by TLC. On the completion of the reaction as indicated by TLC using 100% chloroform as mobile phase, the reaction mixture was poured onto crushed ice and neutralized with conc. NaOH to pH 7. The resulting solid was filtered off and dried to afford a crude yellowish solid which was subjected to column chromatography using silica gel 60–120 mesh as the stationary phase and 100% chloroform as the mobile phase to afford a crystalline whitish solid **5** in 73% yield.

*4.2.4. Synthesis of 1,3-disubstituted-4-thiazolidinonepyrazole **(6)***

To a stirred solution of aldehyde **5** (1equiv.) in DCM at rt was added aniline (1 equiv.) and the mixture was stirred for 5 min. Thioglycolic acid (2 equiv.) was then added to the above reaction mixture and stirring was continued for another 5 min followed by addition of silica gel 100–200 mesh (0.5 g for 1 mmol aldehyde). On completion of the reaction as indicated by TLC the solvent was removed under reduced pressure. The obtained crude product were subjected to column chromatography using silica gel 60–120 mesh as the stationary phase and Hexane: EtOAc : 5:1 as mobile phase to afford the final compound **5** as a white solid.

2-(3-(2-Oxo-2H-chromen-3-yl)-1-phenyl-1H-pyrazol-4-yl)-3-(p-tolyl) thiazolidin-4-one **(6a)**: Yield: 64%; Color: Pale brown solid; mp: 190–192 °C ; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.87 (s, 1H), 7.95 (d, J = 11.6 Hz, 1H), 7.91 (d, J = 8.2 Hz, 2H), 7.80 (d, J = 7.5 Hz, 1H), 7.69 (t, J = 7.4 Hz, 1H), 7.56 – 7.48 (m, 3H), 7.42 (t, J = 7.5 Hz, 1H), 7.36 (t, J = 7.3 Hz, 1H), 7.12 (d, J = 8.2 Hz, 2H), 6.95 (d, J = 8.2 Hz, 2H), 6.45 (s, 1H), 4.09 (d, J = 12.1 Hz, 1H), 3.80 (d, J = 15.6 Hz, 1H), 2.01 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.3, 160.0, 153.7, 145.8, 143.1, 139.4, 136.8, 135.3, 132.8, 130.0, 129.6, 129.3, 129.0, 127.3, 126.8, 125.2, 124.1, 120.2, 119.4, 118.8, 116.5, 56.8, 32.7, 20.8; FT-IR (cm^{-1}): 3098, 3048, 1715, 1668, 1289, 1212, 760; HR-MS (ESI-QTOF): m/z calculated for $C_{28}H_{21}N_3O_3S$; 480.1382; found 480.1392 $[M + H]^+$.

3-(4-Bromophenyl)-2-(3-(2-oxo-2H-chromen-3-yl)-1-phenyl-1H-pyrazol-4-yl)thiazolidin-4-one **(6b)**: Yield: 51%; Color: Off white solid; mp: 188–190 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.12 (s, 1H), 7.89 (d, J = 7.9 Hz, 2H), 7.83 (d, J = 7.6 Hz, 1H), 7.69 (dd, J = 11.4, 4.2 Hz, 1H), 7.54 – 7.49 (m, 3H), 7.44 – 7.40 (m, 3H), 7.35 (dd, J = 15.7, 8.1 Hz, 3H), 6.50 (s, 1H), 4.08 (d, J = 15.6 Hz, 1H), 3.81 (d, J = 15.7 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.5, 160.0, 153.8, 145.6, 143.4, 139.4, 137.4, 132.9, 132.0, 130.0, 129.5, 128.8, 128.4, 127.3, 125.3, 123.9, 120.3, 119.8, 119.4, 118.9, 116.5, 56.6, 32.6; FT-IR (cm^{-1}): 2924, 1709, 1670, 1342, 1213, 758; HR-MS (ESI-QTOF): m/z calculated for $C_{27}H_{18}BrN_3O_3S$; 546.0310, found 546.0309 $[M + 2H]^+$.

3-(3,4-Dimethoxyphenyl)-2-(3-(2-oxo-2H-chromen-3-yl)-1-phenyl-1H-pyrazol-4-yl)thiazolidin-4-one **(6c)**: Yield: 64%; Color: Off white solid, mp: 232–234 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.91 (s, 1H), 7.92 (d, J = 5.4 Hz, 3H), 7.75 (d, J = 7.4 Hz, 1H), 7.68 (t, J = 7.5 Hz, 1H), 7.53 (t, J = 7.8 Hz, 2H), 7.47 (d, J = 8.2 Hz, 1H), 7.40 (t, J = 7.5 Hz, 1H), 7.36 (t, J = 7.3 Hz, 1H), 6.87 (s, 1H), 6.70 (q, J = 8.6 Hz, 2H), 6.42 (s, 1H), 4.05 (d, J = 15.5 Hz, 1H), 3.81 (d, J = 15.5 Hz, 1H), 3.54 (s, 3H), 3.52 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.2, 160.0, 153.8, 149.0, 148.0, 146.1, 143.1, 139.5, 132.7, 130.5, 130.0, 129.2, 129.1, 127.3, 125.2, 124.0, 120.1, 119.8, 119.2, 118.9, 116.4, 111.5, 111.3, 56.9, 55.8, 55.6, 32.7; FT-IR (cm^{-1}): 2971, 2935, 1686, 1600, 1506, 1238, 765; HR-MS (ESI-QTOF): m/z calculated for $C_{29}H_{23}N_3O_5S$; 526.1437; found 526.1436 $[M + H]^+$.

2-(3-(2-Oxo-2H-chromen-3-yl)-1-phenyl-1H-pyrazol-4-yl)-3-phenylthiazolidin-4-one **(6d)**: Yield: 50%; Color: Off white solid; mp:209–211 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.05 (s, 1H), 7.89 (d, J = 8.6, 1.0 Hz, 2H), 7.80 (d, J = 7.7, 1.4 Hz, 1H), 7.69 (t, J = 8.6, 7.4, 1.6 Hz, 1H), 7.54 – 7.49 (m, 3H), 7.42 (t, J = 7.6, 1.0 Hz, 1H), 7.37 – 7.33 (m, J = 11.1, 3.9 Hz, 3H), 7.22 (t, J = 10.8, 5.1 Hz, 2H), 7.06 (t, 1H), 6.50 (s, 1H), 4.08 (d, 1H), 3.80 (d,

J = 15.6 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.4, 160.0, 153.9, 145.6, 143.4, 139.4, 138.1, 132.8, 130.0, 129.4, 129.1, 128.8, 127.3, 127.0, 126.5, 125.2, 124.2, 120.4, 119.4, 118.9, 116.5, 56.8, 32.7; FT-IR (cm^{-1}): 3132, 3062, 1714, 1675, 1389, 1225, 749, 686; HR-MS (ESI-QTOF): *m/z* calculated for $C_{27}H_{19}N_3O_3S$; 466.1225; found 466.1223 [M + H]$^+$.

2-(3-(2-Oxo-2H-chromen-3-yl)-1-phenyl-1H-pyrazol-4-yl)-3-(4-phenoxyphenyl)thiazolidin-4-one **(6e)**: Yield: 47%; Color: Off white solid; mp: 168–170 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.88 (s, 1H), 8.08 (s, 1H), 7.91 (d, J = 7.9 Hz, 2H), 7.82 (d, J = 7.3 Hz, 1H), 7.69 (t, J = 7.3 Hz, 1H), 7.55 – 7.48 (m, 3H), 7.43 (t, J = 7.4 Hz, 1H), 7.36 (dd, J = 9.4, 6.2 Hz, 3H), 7.28 (d, J = 8.8 Hz, 2H), 7.14 (t, J = 7.4 Hz, 1H), 6.90 (d, J = 7.9 Hz, 2H), 6.79 (d, J = 8.8 Hz, 2H), 6.44 (s, 1H), 4.08 (d, J = 15.6 Hz, 1H), 3.80 (d, J = 15.6 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.4, 160.0, 156.3, 155.8, 153.8, 145.7, 143.4, 139.4, 133.0, 132.8, 130.5, 130.0, 129.4, 128.9, 128.5, 127.3, 125.3, 124.3, 124.1, 120.3, 119.6, 119.3, 118.9, 118.4, 116.5, 56.8, 32.6; FT-IR (cm^{-1}): 2970, 1720, 1680, 1229, 754; HR-MS (ESI-QTOF): *m/z* calculated for $C_{33}H_{23}N_3O_4S$ 558.1488; found 558.1488 [M + H]$^+$.

3-(4-Chlorophenyl)-2-(3-(2-oxo-2H-chromen-3-yl)-1-phenyl-1H-pyrazol-4-yl)thiazolidin-4-one **(6f)**: Yield: 58%; Color: Off white solid; mp: 180–182 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.09 (d, J = 19.4 Hz, 1H), 7.89 (d, J = 7.8 Hz, 2H), 7.82 (d, J = 7.3 Hz, 1H), 7.69 (t, J = 7.5 Hz, 1H), 7.51 (dd, J = 13.7, 7.8 Hz, 3H), 7.42 (t, J = 7.5 Hz, 1H), 7.38 (d, J = 8.6 Hz, 2H), 7.34 (d, J = 7.2 Hz, 1H), 7.27 (d, J = 8.6 Hz, 2H), 6.50 (s, 1H), 4.08 (d, J = 15.7 Hz, 1H), 3.81 (d, J = 15.7 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.6, 160.1, 153.8, 145.7, 143.4, 139.4, 137.0, 132.9, 131.4, 130.0, 129.4, 129.1, 128.8, 128.2, 127.3, 125.3, 123.9, 120.3, 119.4, 118.9, 116.5, 56.6, 32.6; FT-IR (cm^{-1}): 3098, 3055, 1710, 1672, 1213, 759, 698; HR-MS (ESI-QTOF): *m/z* calculated for $C_{27}H_{18}ClN_3O_3S$ 500.0836; found 500.0836 [M + H]$^+$.

3-(4-Isopropylphenyl)-2-(3-(2-oxo-2H-chromen-3-yl)-1-phenyl-1H-pyrazol-4-yl) thiazolidin-4-one **(6 g)**: Yield: 53%; Color: Off white solid; mp: 207–209 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.88 (s, 1H), 8.04 (s, 1H), 7.92 (d, J = 7.8 Hz, 2H), 7.79 (d, J = 7.7, 1.2 Hz, 1H), 7.69 (t, 1H), 7.55 – 7.48 (m, 3H), 7.41 (t, J = 7.5 Hz, 1H), 7.36 (t, J = 7.4 Hz, 1H), 7.21 (d, J = 8.4 Hz, 2H), 7.07 (d, J = 8.5 Hz, 2H), 6.47 (s, 1H), 4.09 (d, J = 13.4 Hz, 1H), 3.80 (d, J = 15.6 Hz, 1H), 2.71 – 2.61 (m, 1H), 1.07 (d, J = 6.9 Hz, 3H), 1.02 (d, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.4, 160.0, 153.8, 147.2, 145.5, 143.3, 139.4, 135.6, 132.8, 130.0, 129.3, 128.8, 127.3, 127.0, 126.5, 125.2, 124.4, 120.4, 119.3, 118.9, 116.5, 56.8, 33.1, 32.6, 23.9, 23.9.; FT-IR (cm^{-1}): 2957, 1688, 1606, 1326, 756; HR-MS (ESI-QTOF): *m/z* calculated for $C_{30}H_{25}N_3O_3S$ 508.1695; found 508.1692 [M + H]$^+$.

2-(3-(2-Oxo-2H-chromen-3-yl)-1-phenyl-1H-pyrazol-4-yl)-3-(3-(trifluoromethyl)phenyl) thiazolidin-4-one **(6 h)**: Yield: 51%; Color: Off white solid; mp: 142–144 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.92 (s, 1H), 8.09 (s, 1H), 7.86 (d, J = 7.4 Hz, 3H), 7.80 (d, J = 7.5 Hz, 1H), 7.73 – 7.64 (m, 2H), 7.54 – 7.47 (m, 4H), 7.43 (dd, J = 14.2, 7.1 Hz, 2H), 7.35 (t, J = 7.3 Hz, 1H), 6.61 (s, 1H), 4.08 (d, J = 15.7 Hz, 1H), 3.85 (d, J = 15.7 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.8, 160.1, 153.9, 145.7, 143.4, 139.3, 138.9, 132.9, 130.2, 130.1, 130.0, 129.7, 129.4, 129.0, 127.3, 125.3, 123.7, 123.5, 123.0, 123.0, 120.3, 119.3, 118.8, 116.6, 56.5, 32.7; FT-IR (cm^{-1}): 2924, 1709, 1690, 1326, 1116, 750; HR-MS (ESI-QTOF): *m/z* calculated for $C_{28}H_{18}F_3N_3O_3S$ 534.1099; found 534.1110 [M + H]$^+$.

2-(3-(2-Oxo-2H-chromen-3-yl)-1-phenyl-1H-pyrazol-4-yl)-3-(m-tolyl) thiazolidin-4-one **(6i)**: Yield: 43%; Color: Off white solid; mp: 165–167 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.86 (s, 1H), 7.91 (d, 2H), 7.80 (d, 1H), 7.70 (t, 1H), 7.52 (dd, J = 6.8 Hz, 3H), 7.40 (dt, J = 33.3 Hz, 3H), 7.15 – 7.01 (m, 3H), 6.82 (d, 1H), 6.46 (s, 1H), 4.09 (d, J = 14.8 Hz, 1H), 3.81 (d, J = 14.6 Hz, 1H), 2.06 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.3, 160.0, 153.8, 145.8, 143.3, 139.4, 138.5, 137.9, 132.8, 130.0, 129.3, 128.9, 128.9, 127.9, 127.3, 127.0, 125.3, 124.1, 123.8, 120.3, 119.4, 118.9, 116.5, 56.7, 32.8, 21.1; FT-IR (cm^{-1}): 1717, 1677, 1225, 752; HR-MS (ESI-QTOF): *m/z* calculated for $C_{28}H_{21}N_3O_3S$ 480.1382; found 480.1385 [M + H]$^+$.

2-(3-(2-Oxo-2H-chromen-3-yl)-1-phenyl-1H-pyrazol-4-yl)-3-(3,4,5-trimethoxyphenyl) thiazolidin-4-one **(6j)**: Yield: 56%; Color: Off white solid; mp: 228–230 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.31 (s, 1H), 8.28 (s, 1H), 7.96 (s, 1H), 7.82 (d, J = 7.8 Hz, 2H), 7.67 – 7.59 (m, 2H), 7.53 (t, J = 7.9 Hz, 2H), 7.42 – 7.30 (m, J = 18.3, 9.1, 5.7 Hz, 3H), 6.21 (s, 1H), 5.72 (s, 1H), 3.82 (s, 3H), 3.47 (d, J = 1.6 Hz, 6H), 3.23 (d, J = 12.3 Hz, 1H), 2.74 (d, J = 12.1 Hz, 1H).^{13}C NMR (125 MHz, DMSO-d_6) δ 169.6, 159.4, 153.6, 153.1, 150.8, 145.6, 142.4, 139.8, 139.5, 134.4, 132.4, 130.0, 129.0, 128.7, 126.8, 125.7, 125.1, 121.5, 119.5, 119.1, 118.8, 116.5, 104.6, 79.6, 61.7, 60.4, 55.8, 32.4; FT-IR (cm^{-1}): 2926, 1728, 1667, 1101, 749; HR-MS (ESI-QTOF): *m/z* calculated for $C_{30}H_{25}N_3O_6S$ 556.1542; found 556.1539 [M + H]$^+$.

3-(4-Methoxyphenyl)-2-(3-(2-oxo-2H-chromen-3-yl)-1-phenyl-1H-pyrazol-4-yl) thiazolidin-4-one **(6 k)**: Yield: 52%; Color: Off white solid; mp: 184–186 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.89 (s, 1H), 7.95 – 7.90 (m, 3H), 7.77 (d, J = 7.3 Hz, 1H), 7.69 (t, J = 7.5 Hz, 1H), 7.53 (t, J = 7.5 Hz, 2H), 7.48 (d, J = 8.2 Hz, 1H), 7.41 (t, J = 7.3 Hz, 1H), 7.37 (t, J = 7.2 Hz, 1H), 7.11 (d, J = 8.4 Hz, 2H), 6.68 (d, J = 8.4 Hz, 2H), 6.38 (s, 1H), 4.07 (d, J = 15.5 Hz, 2H), 3.80 (d, J = 15.5 Hz, 1H), 3.49 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.3, 160.0, 158.3, 153.7, 145.9, 143.1, 139.4, 132.7, 130.4, 130.0, 129.3, 129.1, 128.6, 127.3, 125.2, 124.0, 120.1, 119.3, 118.9, 116.4, 114.3, 56.9, 55.3, 32.6; FT-IR (cm^{-1}): 2923, 1719, 1683, 1224, 756; HR-MS (ESI-QTOF): *m/z* calculated for $C_{28}H_{21}N_3O_4S$ 496.1331; found 496.1327 [M + H]$^+$.

3-(3-Chlorophenyl)-2-(3-(2-oxo-2H-chromen-3-yl)-1-phenyl-1H-pyrazol-4-yl)thiazolidin-4-one **(6 l)**: Yield: 42%; Color: Off white solid; mp: 152–154 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.88 (s, 1H), 8.12 (s, 1H), 7.88 (d, J = 8.1 Hz, 2H), 7.83 (d, J = 7.7 Hz, 1H), 7.70 (t, 1H), 7.56 (t, 1H), 7.52 (t, J = 8.1 Hz, 3H), 7.43 (t, J = 7.5 Hz, 1H), 7.38 – 7.32 (m, J = 13.5, 7.2 Hz, 2H), 7.27 (t, J = 8.0 Hz, 1H), 7.14 (d, J = 7.2 Hz, 1H), 6.54 (s, 1H), 4.07 (d, J = 15.7 Hz, 1H), 3.82 (d, J = 15.7 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.6, 160.1, 153.9, 145.7, 143.5, 139.5, 139.4, 133.3, 132.9, 130.6, 130.0, 129.4, 128.8, 127.3, 126.8, 126.0, 125.3, 124.7, 123.8, 120.4, 119.4, 118.9, 116.6, 56.5, 32.7; FT-IR (cm^{-1}): 1717, 1682, 753, 683; HR-MS (ESI-QTOF): *m/z* calculated for $C_{27}H_{18}ClN_3O_3S$ 500.0836; found 500.0828 [M + H]$^+$.

3-(3-Bromophenyl)-2-(3-(2-oxo-2H-chromen-3-yl)-1-phenyl-1H-pyrazol-4-yl) thiazolidin-4-one **(6 m)**: Yield: 47%; Color: Off white solid; mp: 145–147 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.88 (s, 1H), 8.11 (s, 1H), 7.88 (d, J = 7.9 Hz, 2H), 7.83 (d, J = 7.4 Hz, 1H), 7.72 – 7.67 (m, 2H), 7.52 (t, J = 8.0 Hz, 3H), 7.43 (t, J = 7.5 Hz, 1H), 7.36 (d, J = 7.6 Hz, 2H), 7.27 (d, J = 8.0 Hz, 1H), 7.20 (t, J = 8.0 Hz, 1H), 6.53 (s, 1H), 4.06 (d, J = 15.7 Hz, 1H), 3.82 (d, J = 15.7 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.6, 160.1, 153.8, 145.9, 143.5, 139.6, 139.4, 132.9, 130.9, 130.0, 129.7, 129.4, 128.9, 127.3, 125.3, 125.1, 123.8, 121.6, 120.4, 119.4, 118.9, 116.6, 56.5, 32.7; FT-IR (cm^{-1}): 1717, 1683, 753, 893; HR-MS (ESI-QTOF): *m/z* calculated for $C_{27}H_{18}BrN_3O_3S$ 546.0310; found 546.0349 [M + 2H]$^+$.

4.3. General procedures for the synthesis of series 2 compounds

4.3.1. Synthesis of 4-(chloromethyl)-7-hydroxy-2H-chromen-2-one *(3)*

A solution of resorcinol (**1**) (9.08 mmol) in ethyl-4-chloroacetoacetate (**2**) (1.3 mL) was added drop wise to externally cooled conc. H_2SO_4 (10 mL) at 10 °C and the reaction mixture was then stirred at room temperature for 15 min. The mixture was poured into a beaker containing ice water. The precipitated compound was collected by suction filtration, washed with cold water and dried. It was recrystallized from ethanol. Yield 91%; Color: white solid; mp 186–188 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.66 (s, 1H), 7.68 (d, J = 7.8 Hz, 1H), 6.90 – 6.71 (m, 2H), 6.42 (s, 1H), 4.96 (s, 2H);^{13}C NMR (125 MHz, DMSO-d_6) δ 161.94, 160.61, 155.78, 151.45, 127.01, 113.55, 111.54, 109.83, 103.00, 41.83; HR-MS (ESI-QTOF): *m/z* calcd for [M + H] + $C_{10}H_7ClO_3$, 210.0084; found 211.0157.

*4.3.2. 4-(chloromethyl)-7-(prop-2-yn-1-yloxy)–2H-chromen-2-one **(4)***

To the solution of compound **3** (1 mmol) in freshly distilled acetone (10 mL), was added anhydrous K_2CO_3 (2.5 mmol) as a base and propargyl bromide (1 mmol) drop wise. The resulting reaction mixture was stirred under reflux for 2–3 hrs. On completion of the reaction as indicated by TLC, the solvent was evaporated completely and the obtained residue was extracted with ethyl acetate (3x20 mL) and dried over Na_2SO_4. The combined organic layer was concentrated in vacuo and the residue was purified by column chromatography on silica gel. Yield 85%; Color: Pale yellow solid; mp 123–125 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, J = 8.8 Hz, 1H), 6.99–6.87 (m, 2H), 6.40 (s, 1H), 4.77 (d, J = 2.3 Hz, 2H), 3.60 (s, 2H), 2.58 (t, J = 2.3 Hz, 1H), 2.55;^{13}C NMR (125 MHz, $CDCl_3$) δ 161.19, 160.34, 155.42, 151.53, 125.97, 113.05, 112.74, 112.47, 102.12, 78.42, 76.53, 59.51, 54.21; HR-MS (ESI-QTOF): *m/z* calcd for [M + H] + $C_{13}H_9ClO_3$; 248.0204, found 249.0324

4.3.3. General procedure for the preparation of the compounds (5a-j)

A 50 mL round bottom flask was charged with compound **4** (1 mmol), various benzyl azides (0.9 mmol), sodium ascorbate (0.3 mmol), 2 mL of ethanol and 2 mL of distilled water. Then, $CuSO_4{\cdot}5H_2O$ (0.15 mmol) was added. The reaction mixture was stirred at rt for overnight. After completion of the reaction by TLC analysis, the mixture was filtered and extracted with ethyl acetate (3x20 mL) and dried over Na_2SO_4. The combined organic layer was concentrated in vacuo and the residue was purified by column chromatography on silica gel to afford the final target compounds **5a-j** in moderate to good yields.

7-((1-Benzyl-1H-1,2,3-Triazol-4-yl)Methoxy)-4-(Chloromethyl)–2H-Chromen-2-one **(5a):** Yield: 66.2%; Color: Creamy white solid; mp: 96–124 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.35 (s, 1H), 7.79 (dd, J = 14.2, 8.9 Hz, 1H), 7.41 – 7.31 (m, 5H), 7.18 (d, J = 18.3 Hz, 1H), 7.09 (d, J = 11.6 Hz, 1H), 6.53 (d, J = 8.7 Hz, 1H), 5.63 (s, 2H), 5.29 (s, 2H), 5.00 (d, J = 8.6 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.6, 160.4, 155.5, 151.2, 133.8, 131.8, 129.6, 127.2, 126.8, 126.6, 125.7, 123.7, 113.3, 111.1, 102.4, 62.1, 51.3, 41.7; HR-MS (ESI-QTOF): *m/z* calc. for (M + H)$^+$ $C_{20}H_{16}ClN_3O_3$,382.0958; found 382.0969.

4-(Chloromethyl)-7-((1-(4-Nitrobenzyl)-1H-1,2,3-Triazol-4-yl) Methoxy)–2H-Chromen-2-one **(5b)**: 60.2%. Yield; Color: Greenish white solid; mp: 98–117 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.42 (s, 1H), 8.25 (d, J = 8.6 Hz, 2H), 7.78 (d, J = 8.9 Hz, 1H), 7.55 (d, J = 8.6 Hz, 2H), 7.21 (d, J = 2.3 Hz, 1H), 7.09 (dd, J = 8.9, 2.4 Hz, 1H), 6.53 (s, 1H), 5.82 (s, 2H), 5.32 (s, 2H), 5.01 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.7, 160.4, 155.5, 151.2, 138.0, 133.3, 129.7, 128.5, 126.8, 125.4, 113.3, 112.6, 111.1, 102.4, 102.1, 62.1, 53.1, 41.7; HR-MS (ESI-QTOF): *m/z* calc. for (M + H)$^+$ $C_{20}H_{15}ClN_4O_5$ 427.0809; found 427.0816.

4-(Chloromethyl)-7-((1-(2,5-Dichlorobenzyl)-1H-1,2,3-Triazol-4-yl) Methoxy)–2H-Chromen-2-one **(5c)**: Yield: 65.2%; Color: Pale yellow solid; mp:138–148 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.33 (s, 1H), 7.83 –7.78 (m, 1H), 7.71 (s, 1H), 7.49 (d, J = 8.1 Hz, 1H), 7.31 (d, J = 6.3 Hz, 1H), 7.20 (s, 1H), 7.10 – 7.07 (m, 1H), 6.53 (d, J = 7.1 Hz, 1H), 5.73 (s, 2H), 5.30 (s, 2H), 4.97 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.6, 160.4, 155.5, 151.2, 147.7, 143.7, 129.7, 129.5, 126.9, 125.9, 124.3, 124.2, 113.3, 112.6, 111.2, 102.4, 79.4, 62.1, 52.4, 41.7; HR-MS (ESI-QTOF): *m/z* calc. for(M + H)$^+$ $C_{20}H_{14}Cl_3N_3O_3$450.0179; found 451.0653.

4-((4-(((4-(Chloromethyl)-2-Oxo-2H-Chromen-7-yl) Oxy) Methyl)-1H-1,2,3-Triazol-1-yl) Methyl)Benzonitrile **(5d)**: Yield: 63.7%; Color: Pale yellow solid; mp: 113–140 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.41 (s, 1H), 7.87 (d, J = 7.2 Hz, 2H), 7.48 (d, J = 6.9 Hz, 2H), 7.22 – 7.07 (m, 3H), 6.53 (d, J = 5.8 Hz, 1H), 5.76 (s, 2H), 5.31 (s, 2H), 4.99 (d, J = 20.1 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.6, 160.4, 155.5, 151.2, 138.7, 133.7, 131.2, 128.6, 127.1, 125.7, 113.3, 111.1, 102.4, 62.1, 52.5, 41.8, 40.5, 40.3; HR-MS (ESI-QTOF): *m/z* calc. for (M + H)$^+$ $C_{21}H_{15}ClN_4O_3$407.0911; found 407.0923.

7-((1-(4-Bromobenzyl)-1H-1,2,3-Triazol-4-yl)Methoxy)-4-(Chloromethyl)–2H-Chromen-2-one **(5e)**: Yield: 84.5%; Color: Creamy green solid; mp: 119–138 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.26 (s, 1H), 7.70 (t, J = 11.2 Hz, 1H), 7.50 (d, J = 8.2 Hz, 2H), 7.20 (d, J = 8.1 Hz, 2H), 7.12 (t, J = 10.2 Hz, 1H), 7.02 – 6.95 (m, 1H), 6.44 (d, J = 7.7 Hz, 1H), 5.53 (s, 2H), 5.20 (s, 2H), 4.96 – 4.87 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.9, 155.8, 151.5, 148.0, 144.0, 130.0, 129.8, 127.1, 126.2, 124.6, 124.5, 113.6, 112.9, 111.4, 102.7, 62.4, 52.7, 42.0; HR-MS (ESI-QTOF): *m/z* calc. for (M + 2)$^+$ $C_{20}H_{15}BrClN_3O$ 460.0064; found 462.0046.

4-(Chloromethyl)-7-((1-(4-Methylbenzyl)-1H-1,2,3-Triazol-4-yl) Methoxy)–2H-Chromen −2-one **(5f)**: Yield: 77.3%; Color: Creamy green solid; mp: 124–130 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.31 (d, J = 5.6 Hz, 1H), 7.78 (t, J = 7.9 Hz, 1H), 7.20 (dd, J = 13.2, 10.5 Hz, 5H), 7.11 – 7.05 (m, 1H), 6.53 (d, J = 9.1 Hz, 1H), 5.57 (s, 2H), 5.28 (s, 2H), 5.00 (s, 2H), 2.29 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.7, 160.4, 155.5, 151.2, 138.0, 133.4, 129.7, 128.5, 126.9, 125.4, 113.3, 112.6, 111.1, 102.4, 62.2, 53.1, 41.8, 21.1; HR-MS (ESI-QTOF): *m/z* calc. for (M + H)$^+$ $C_{21}H_{18}ClN_3O_3$ 396.1115;found 396.1124.

*4-(Chloromethyl)-7-((1-(3-Methoxybenzyl)-1H-1,2,3-Triazol-4-yl) Methoxy)–2H-Chromen-2-on***e (5 g)**: Yield: 74.8%; Color: Yellowish white solid; mp: 89–117 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.29 (s, 1H), 7.79 (dd, J = 16.2, 8.9 Hz, 1H), 7.31 (d, J = 8.6 Hz, 2H), 7.10 – 7.06 (m, 2H), 6.93 (d, J = 8.7 Hz, 2H), 6.53 (d, J = 9.6 Hz, 1H), 5.54 (s, 2H), 5.27 (s, 2H), 5.00 – 4.96 (s, 2H), 3.75 (d, J = 4.3 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.6, 160.8, 160.4, 155.5, 151.2, 141.8, 133.2, 129.2, 126.9, 125.9, 118.9, 113.3, 112.6, 111.4, 111.2, 102.6, 102.4, 79.4, 62.1, 52.7, 41.7; HR-MS (ESI-QTOF): *m/z* calc. for (M + H)$^+$ $C_{21}H_{18}ClN_3O_4$ 412.1064; found 412.1066.

4-(Chloromethyl)-7-((1-(Naphthalen-2-yl-Methyl)-1H-1,2,3-Triazol-4-yl)Methoxy)–2H-Chromen-2-one **(5 h)**: Yield: 86.8%; Color: Creamy pink solid; mp: 153–185 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.31 – 8.18 (m, 2H), 8.01 – 7.95 (m, 2H), 7.76 (d, J = 8.9 Hz, 1H), 7.62 – 7.53 (m, 3H), 7.46 (d, J = 6.8 Hz, 1H), 7.17 (d, J = 1.9 Hz, 1H), 7.06 (d, J = 8.8 Hz, 1H), 6.51 (s, 1H), 6.13 (s, 2H), 5.26 (s, 2H), 4.98 (d, J = 13.1 Hz, 2H);^{13}C NMR (125 MHz, DMSO-d_6) δ 160.1, 159.0, 154.0, 149.8, 132.3, 130.3, 128.1, 125.8, 125.3, 125.2, 124.2, 122.2, 111.8, 109.7, 100.9, 60.6, 49.8, 40.3; HR-MS (ESI-QTOF): *m/z* calc. for (M + H)$^+$ $C_{24}H_{18}ClN_3O_3$ 432.1115; found 432.1358.

4-(Chloromethyl)-7-((1-(4-Methoxybenzyl)-1H-1,2,3-Triazol-4-yl) Methoxy)–2H-Chromen-2-one **(5i)**: Yield: 79.9%; Color: Greenish white solid; mp: 151–157 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (s, 1H), 7.86 (d, J = 8.9 Hz, 1H), 7.43 – 7.37 (m, 2H), 7.23 – 7.13 (m, 3H), 7.01 (d, J = 8.8 Hz, 1H), 6.33 (s, 1H), 5.61 (s, 2H), 5.26 (s, 2H), 3.65 (s, 2H), 3.58 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.80, 159.93, 159.59, 154.71, 150.41, 140.97, 132.35, 128.34, 126.04, 125.07, 118.11, 112.48, 111.83, 110.59, 101.80, 78.56, 61.27, 51.86, 40.92; HR-MS (ESI-QTOF): *m/z* calc. for (M + 2)$^+$ $C_{21}H_{18}ClN_3O_4$ 412.1064; found 413.2665.

7-((1-(3-Chlorobenzyl)-1H-1,2,3-Triazol-4-yl)Methoxy)-4-(Chloromethyl) –2H-Chromen-2-one **(5j)**: Yield:84.4%; Color: Pale pink solid; mp: 97–142 °C;^{1}H NMR (500 MHz, DMSO-d_6) δ 8.32 (d, J = 8.7 Hz, 1H), 7.70 (d, J = 8.9 Hz, 1H), 7.34 (dd, J = 6.8, 3.4 Hz, 3H), 7.21 (t, J = 2.9 Hz, 1H), 7.12 (d, J = 2.4 Hz, 1H), 7.01 (dd, J = 8.9, 2.5 Hz, 1H), 6.44 (s, 1H), 5.58 (s, 2H), 5.22 (d, J = 6.6 Hz, 2H), 4.92 (s, 2H);^{13}C NMR (125 MHz, DMSO-d_6) δ 160.7, 159.5, 154.6, 150.3, 137.8, 132.8, 130.2, 127.7, 126.2, 126.1, 126.0, 125.9, 124.8, 112.4, 110.2, 101.4, 61.2, 51.6, 40.8; HR-MS (ESI-QTOF): *m/z* calc. for (M + H)$^+$ $C_{20}H_{15}Cl_2N_3O_3$ 416.0569; found 417.0564.

4.4. CA inhibition

An SX.18 V-R Applied Photophysics (Oxford, UK) stopped flow instrument has been used to assay the CA catalyzed CO_2 hydration activity. [26]. Phenol Red (at a concentration of 0.2 mM) has been used as an indicator, working at an absorbance maximum of 557 nm, with 10 mM Hepes (pH 7.4) as a 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 mM (in DMSO-water 1:1, v/v) and dilutions up to 0.01 nM done with the assay buffer mentioned above. The enzyme solution was prepared at a concentration of 0.1 μM. At least 7 different inhibitor concentrations have been used for measuring the inhibition constant. Inhibitor and enzyme solutions were pre-incubated together for 6 hr at 24 °C prior to assay, in order to allow for the formation of the E-I complex. In the case of coumarins the incubation is done for 6 h to allow the hydrolysis to occur. 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, 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 [27–30].

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

PST, NSG, OSA and JS would like to thank the Department of Pharmaceuticals, Ministry of Chemicals and Fertilizers, Govt. of India, New Delhi for awarding NIPER Fellowship.

Declaration of Competing Interest

The authors declare no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2020.104272.

References

[1] C. Capasso, C.T. Supuran, An overview of the alpha-, beta- and gamma-carbonic anhydrases from Bacteria: can bacterial carbonic anhydrases shed new light on evolution of bacteria? J. Enzyme. Inhib. Med. Chem. 30 (2015) 325–332.
[2] D.M. Monti, G. De Simone, E. Langella, C.T. Supuran, A. Di Fiore, S.M. Monti, Insights into the role of reactive sulfhydryl groups of Carbonic Anhydrase III and VII during oxidative damage, J. Enzyme. Inhib. Med. Chem. 32 (2017) 5–12.
[3] C.T. Supuran, Carbon-versus sulphur-based zinc binding groups for carbonic anhydrase inhibitors? J. Enzyme. Inhib. Med. Chem. 33 (2018) 485–495.
[4] V. Alterio, A. Di Fiore, K. D'Ambrosio, C.T. Supuran, G. De Simone, Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms? Chem. Rev. 112 (2012) 4421–4468.
[5] C.T. Supuran, Exploring the multiple binding modes of inhibitors to carbonic anhydrases for novel drug discovery [published online ahead of print, 2020 Mar 25], Expert. Opin. Drug. Discov. (2020) 1–16.
[6] C.T. Supuran, An update on drug interaction considerations in the therapeutic use of carbonic anhydrase inhibitors, Expert. Opin. Drug. Metab. Toxicol. 16 (2020) 297–307.
[7] E. Andreucci, J. Ruzzolini, S. Peppicelli, F. Bianchini, A. Laurenzana, F. Carta, C.T. Supuran, L. Calorini, The carbonic anhydrase IX inhibitor SLC-0111 sensitises cancer cells to conventional chemotherapy, J. Enzyme. Inhib. Med. Chem. 34 (2019) 117–123.
[8] O. OzensoyGuler, C.T. Supuran, C. Capasso, Carbonic anhydrase IX as a novel candidate in liquid biopsy, J. Enzyme. Inhib. Med. Chem. 35 (2020) 255–260.
[9] A. Waheed, W.S. Sly, Carbonic anhydrase XII functions in health and disease, Gene 623 (2017) 33–40.
[10] C.L. Lomelino, C.T. Supuran, R. McKenna, Non-classical inhibition of carbonic anhydrase, Int. J. Mol. Sci. 17 (2016) 1150.
[11] C.T. Supuran, How many carbonic anhydrase inhibition mechanisms exist? J. Enzyme. Inhib. Med. Chem. 31 (2016) 345–360.
[12] P.S. Thacker, A. Angeli, O.S. Argulwar, P.L. Tiwari, M. Arifuddin, C.T. Supuran, Design, synthesis and biological evaluation of coumarin linked 1,2,4-oxadiazoles as selective carbonic anhydrase IX and XII inhibitors, Bioorg. Chem. 98 (2020) 103739.
[13] P.S. Thacker, M. Alvala, M. Arifuddin, A. Angeli, C.T. Supuran, Design, synthesis and biological evaluation of coumarin-3-carboxamides as selective carbonic anhydrase IX and XII inhibitors, Bioorg. Chem. 86 (2019) 386–392.
[14] S.G. Narella, M.G. Shaik, A. Mohammed, M. Alvala, A. Angeli, C.T. Supuran, Synthesis and biological evaluation of coumarin-1,3,4-oxadiazole hybrids as selective carbonic anhydrase IX and XII inhibitors, Bioorg. Chem. 87 (2019) 765–772.
[15] L. De Luca, F. Mancuso, S. Ferro, M.R. Buemi, A. Angeli, S. Del Prete, C. Capasso, C.T. Supuran, R. Gitto, Inhibitory effects and structural insights for a novel series of coumarin-based compounds that selectively target human CA IX and CA XII carbonic anhydrases, Eur. J. Med. Chem. 143 (2018) 276–282.
[16] M.F. Khan, M.M. Alam, G. Verma, W. Akhtar, M. Akhter, M. Shaquiq-uz-zaman, The therapeutic voyage of pyrazole and its analogs: a review, Eur. J. Med. Chem. 120 (2016) 170–201.
[17] (a) H.S. Ibrahim, S.M. Abou-Seri, M. Tanc, M.M. Elaasser, H.A. Abdel-Aziz, C.T. Supuran, Isatin-pyrazolebenzenesulfonamide hybrids potently inhibit tumor-associated carbonic anhydrase isoforms IX and XII, Eur. J. Med. Chem. 103 (2015) 583–593;
(b) I.N. Cvijetic, M. Tanç, I.O. Juranic, T.Z. Verbic, C.T. Supuran, B.J. Drakulic, 5-Aryl-1H-pyrazole-3-carboxylic acids as selective inhibitors of human carbonic anhydrases IX and XII, Bioorg. Med. Chem. 23 (2015) 4649–4659.
[18] (a) P. Khloya, G. Celik, SitaRam, D. Vullo, C.T. Supuran, P.K. Sharma, 4-Functionalized 1,3-diarylpyrazoles bearing benzenesulfonamide moiety as selective potent inhibitors of the tumor associated carbonic anhydrase isoforms IX and XII, Eur. J. Med. Chem. 76 (2014) 284–290;
(b) C. Yamali, H. I. Gul, A. Ece, S. Bua S, A. Angeli A, H. Sakagami H, E. Sahin, C. T. Supuran, Synthesis, biological evaluation and in silico modelling studies of 1,3,5-trisubstituted pyrazoles carrying benzenesulfonamide as potential anticancer agents and selective cancer-associated hCA IX isoenzyme inhibitors, Bioorg. Chem. 92 (2019) 103222.
[19] A. C. Tripathi, S. J. Gupta, G. N. Fatima P. K. Sonar, A. Verma, S. K. Saraf, 4-Thiazolidinones: the advances continue, Eur. J. Med. Chem. 72 (2014) 52–77.
[20] (a) S.U. Mahmood, A. Saeed, S. Bua, A. Nocentini, P. Gratteri, C.T. Supuran, Synthesis, biological evaluation and computational studies of novel iminothiazolidinonebenzenesulfonamides as potent carbonic anhydrase II and IX inhibitors, Bioorg. Chem. 77 (2018) 381–386;
(b) O. Guzel-Akdemir, A. Angeli, K. Demir, C.T. Supuran, A. Akdemir, Novel thiazolidinone-containing compounds, without the well-known sulphonamide zinc-binding group acting as human carbonic anhydrase IX inhibitors, J. Enzyme. Inhib. Med. Chem. 33 (2018) 1299–1308;
(c) Z. P. Zhang, Z. F. Yin, J. Y. Li, Z. P. Wang, Q. J. Wu, J. Wang, Y. Liu Y, M. S. Cheng, Synthesis, Molecular Docking Analysis, and Carbonic Anhydrase Inhibitory Evaluations of Benzenesulfonamide Derivatives Containing Thiazolidinone, Molecules.24 (2019) 2418.
[21] D. Dheer, V. Singh, R. Shankar, Medicinal attributes of 1,2,3-triazoles: Current developments, Bioorg. Chem. 71 (2017) 30–54.
[22] (a) P. Singh, B. Swain, P. S. Thacker, D. K. Sigalapalli, P. Y. Purnachander, A. Angeli A, C. T. Supuran, M. Arifuddin, Synthesis and carbonic anhydrase inhibition studies of sulfonamide based indole-1,2,3-triazole chalcone hybrids, Bioorg. Chem. 99 (2020)103839.
(b) A. Grandane, M. Tanc, R. Zalubovskis, C.T. Supuran, 6-Triazolyl-substituted sulfocoumarins are potent, selective inhibitors of the tumor-associated carbonic anhydrases IX and XII, Bioorg. Med. Chem. Lett. 24 (2014) 1256–1260;
(c) J. Leitans, A. Sprudza, M. Tanc, I. Vozny, R. Zalubovskis, K. Tars, C.T. Supuran, 5-Substituted-(1,2,3-triazol-4-yl)thiophene-2-sulfonamides strongly inhibit human carbonic anhydrases I, II, IX and XII: solution and X-ray crystallographic studies, Bioorg. Med. Chem. 21 (2013) 5130–5138
[23] D. Srikrishna P. K. Dubey, Synthesis of novel substituted 3-(4-((1H-benzo[d]imidazol-2-ylthio)methyl)-1-phenyl-1H-pyrazol-3-yl)-2H-chromen-2-ones: various approaches, Res. Chem. Intermed. 44 (2018) 4455–4468.
[24] M.P. Thakare, P. Kumar, N. Kumar, S.P. Pandey, Silica gel promoted environment friendly synthesis of 2,3-disubstituted 4-thiazolidinones, Tetrahydron. Lett. 55 (2014) 2463–2466.
[25] N.S. Goud, V.K. Kanth Makani, J. Pranay, R. Alvala, I.A. Qureshi, P. Kumar, R.D. Bharath, C. Nagaraj, S. Yerramsetty, M. Pal-Bhadra, M. Alvala, Synthesis, ^{18}F-radiolabeling and apoptosis inducing studies of novel 4, 7-disubstituted coumarins, Bioorg. Chem. 97 (2020) 103663.
[26] R. G. Khalifah, The carbon dioxide hydration activity of carbonic anhydrase. I. Stop-flow kinetic studies on the native human isoenzymes B and C, J. Biol. Chem. 246 (1971) 2561-2573.
[27] P.S. Thacker, P. Shaikh, A. Angeli, M. Arifuddin, C.T. Supuran, Synthesis and biological evaluation of novel 8-substituted quinoline-2-carboxamides as carbonic anhydrase inhibitors, J. Enzyme. Inhib. Med. Chem. 34 (2019) 1172–1177.
[28] W.M. Eldehna, G.H. Al-Ansary, S. Bua, A. Nocentini, P. Gratteri, A. Altoukhy, H. Ghabbour, H.Y. Ahmed, C.T. Supuran, Novel indolin-2-one-based sulfonamides as carbonic anhydrase inhibitors: Synthesis, in vitro biological evaluation against carbonic anhydrases isoforms I, II, IV and VII and molecular docking studies, Eur. J. Med. Chem. 127 (2017) 521–530.
[29] A. Nocentini, M. Ferraroni, F. Carta, M. Ceruso, P. Gratteri, C. Lanzi, E. Masini, C.T. Supuran, Benzenesulfonamides incorporating flexible triazole moieties are highly effective carbonic anhydrase inhibitors: synthesis and kinetic, crystallographic, computational, and intraocular pressure lowering investigations, J. Med. Chem. 59 (2016) 10692–10704.
[30] A.G. Uslu, T. GurMaz, A. Nocentini, E. Banoglu, C.T. Supuran, B. Calıskan, Benzimidazole derivatives as potent and isoform selective tumor-associated carbonic anhydrase IX/XII inhibitors, Bioorg. Chem. 95 (2020) 103544.