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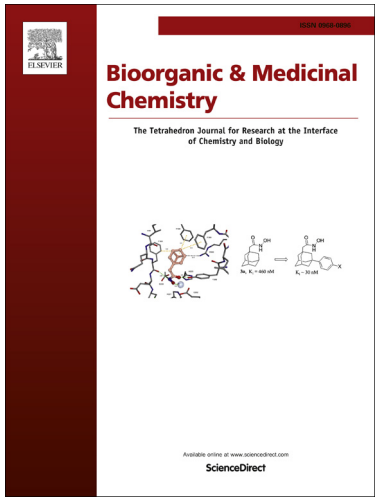

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Benzenesulfonamide bearing 1,2,4-triazole scaffolds as potent inhibitors of tumor associated carbonic anhydrase isoforms hCA IX and hCA XII

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Abstract:

Three series of novel heterocyclic compounds (**3a-3g**, **4a-4g** and **5a-5g**) containing benzenesulfonamide moieties and incorporating a 1,2,4-triazole ring, have been synthesized and investigated as inhibitors against four isomers of the α-class carbonic anhydrases (CAs, EC 4.2.1.1), comprising hCAs I and II(cytosolic, ubiquitous isozymes) and hCAs IX and XII (transmembrane, tumor associated isozymes). Against the human isozymes hCA I and II, compounds of two series (**3a-3g** and **4a-4g**) showed K_i values in the range of 84-868 nM and 5.6-390 nM respectively whereas compounds of series **5a-5g** were found to be poor inhibitors (K_i values exceeding 10000 nM in some cases). Against hCA IX and XII, all the tested compounds exhibited excellent to moderate inhibitory potential with K_i values in the range of 2.8-431 nM and 1.3-63 nM respectively. Compounds **3d**, **3f** and **4f** exhibited excellent inhibitory potential against all of the four isozymes hCA I, II, IX and XII, even better than the standard drug acetazolamide (AZA) whereas compound of the series **5a-5g** were comparatively less potent but more selective towards hCA IX and XII.

Keywords: triazoles, benzenesulfonamide, carbonic anhydrase isoforms 1, II, IX, XII, acetazolamide

1. Introduction:

Carbonic anhydrase is the omnipresent class of metalloenzymes which catalyzes the reversible hydration and dehydration of carbon dioxide.[1] This is a rather simple but essential reaction as the products of this reaction are involved in crucial physiological processes such as respiration and transport of CO_2/bicarbonate between metabolizing tissues and the lungs, pH and CO_2 homeostasis, electrolyte secretion, gluconeogenesis, lipogenesis and ureagenesis, bone resorption, calcification, tumorigenicity and many other physiologic or pathologic processes. [2-7]

There are five main classes of these enzymes viz. α, β, γ, δ and ζ-CAs. Out of the 16 different CA isoforms discovered so far in the α-class, human CA isoforms hCA I and II are cytosolic enzymes that are widespread throughout the human body and are drug targets for clinically used diuretics, antiglaucoma drugs and anticonvulsants.[8-10] Further, dimeric transmembrane glycoproteins hCA IX and XII are also human associated CA isoforms having extracellular active site and are marker for a broad spectrum of hypoxic tumor types.[11-13] The overexpression of these isoforms contributes to the increased acidification of extracellular hypoxic environment (pH = 6.8) in contrast to normal tissues (pH = 7.4) thus promoting tumor cell survival in an acidic condition by decreasing uptake of weakly basic anticancer drugs.[14] They also help tumors by providing bicarbonate to be used as substrate for cell growth as bicarbonate is required in the synthesis of pyrimidine nucleotides.[15] Thus specifically targeting the tumor associated isoforms hCA IX and XII over the main off target isoforms hCA I and II, which have a physiological relevance,[16-20]using specific inhibitors is considered to be a promising strategy in the cancer therapy.

Sulfonamides are the most widely investigated class of CA inhibitors possessing significant inhibitory power against many isoforms. Among the interesting class of such sulfonamide derivatives, acetazolamide (AZA), methazolamide (MZA), ethoxzolamide (EZA) etc. are widely used CAIs as clinical drugs (Figure 1). Recently it has also been reported that many benzenesulfonamide incorporating compounds possessing significant anti-tumor activity like indisulam (IND) (Figure 1) also exhibited excellent CA inhibitory activity against a number of isoforms.[21] Possessing a wide spectrum of biological activities, indeed the class of heterocyclic compounds containing 1,2,4-triazole scaffold has been attracting the attention of researchers for a long time. 1,2,4-Triazole moiety has been found to be present in the skeleton of various natural products[22] and a large number of compounds containing this

moiety exhibits antimicrobial, antitubercular, analgesic, anti-inflammatory, anti-convulsant, antiviral and antidepressant activities.[23,24] In addition to the wide range of bioactivities, compounds possessing such scaffold have also shown potent antitumor effects[25-27] and recently triazolothiadiazoles **1**[28] and triazolothiadiazines **2**[29] were reported to possess good antitumor activity (Figure 1).

Motivated by the above findings coupled with our ongoing interest in the field of exploring benzenesulfonamide containing heterocycles as potential biologically active agents,[30-33] we envisioned to synthesize three series of compounds containing benzenesulfonamide moiety bearing 1,2,4-triazole scaffold (**3a-3g**, **4a-4g** and **5a-5g**) for evaluating their CA activity profile (Scheme 1 and 2).

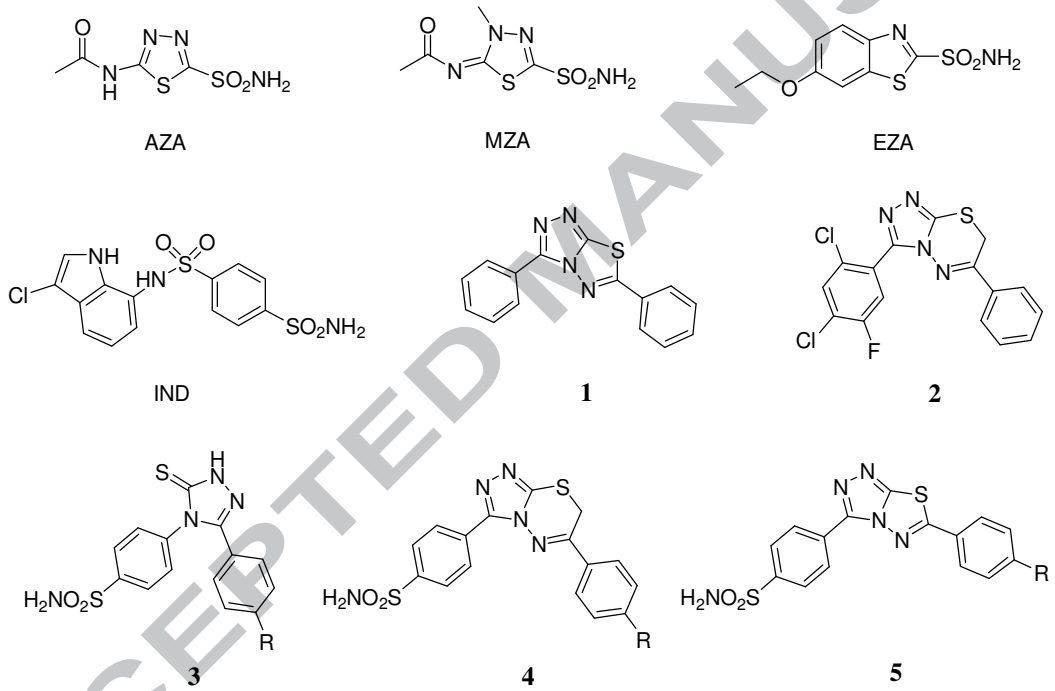


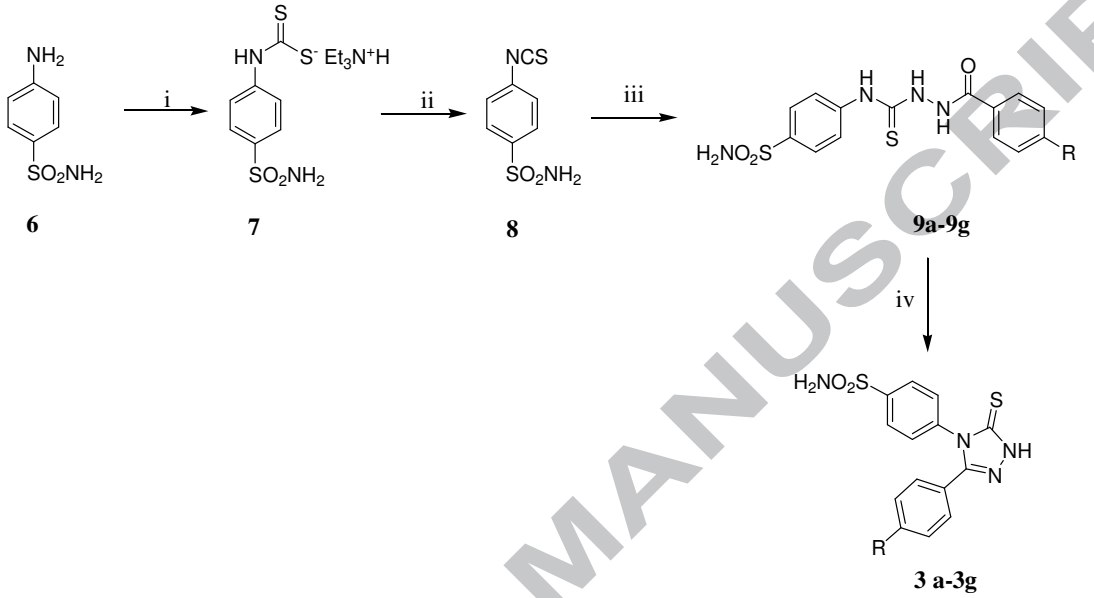
Figure 1

2. Results and discussion

2.1. Chemistry:

The synthetic route for preparing all the target compounds is outlined in Scheme 1 and 2. The structures of newly synthesized target compounds were elucidated on the basis of their spectral (MS, IR and NMR) data. 4-Aminosulfonylphenyl isothiocyanate **8** was the key precursor for realizing the synthesis of target compounds **3**. The only literature procedure reported till date for the synthesis of **8** requires thiophosgene[34] which is a well-known highly

toxic chemical. It is pertinent to mention here that we could successfully obtain the target compound **8** by adapting a recently reported green methodology.[35] Refluxing **8** with variously substituted aromatic hydrazides in anhydrous ethanol resulted in the formation of thiosemicarbazides (**9a-9g**) which on further refluxing in aqueous NaOH transformed into the target compounds (**3a-3g**)[36] (Scheme 1).



6 **7** **8** **9a-9g** **3 a-3g**

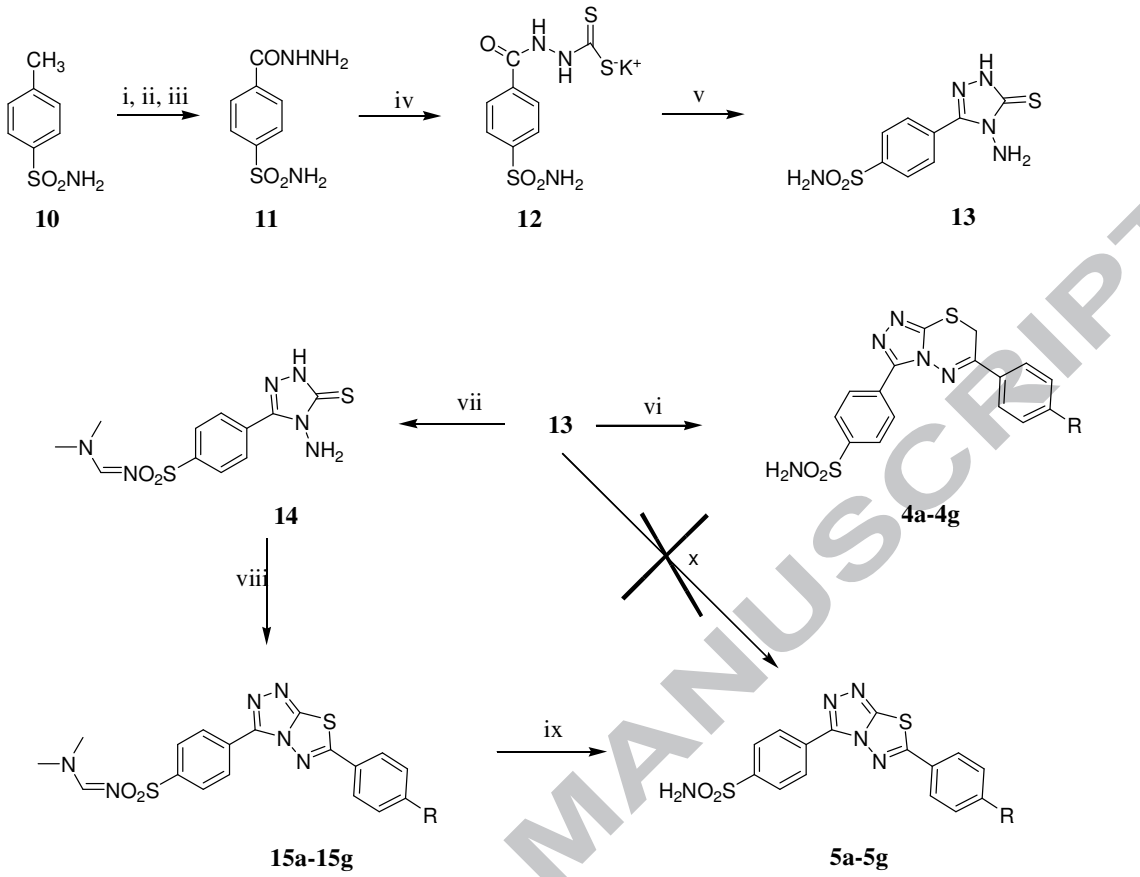
Compounds	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**
R	H	CH_3	OCH_3	F	Cl	Br	SO_2NH_2

Scheme 1. Reagents and conditions: (i) a.CS_2, TEA, acetone, (ii) I_2, $NaHCO_3$, ethyl acetate:H_2O (iii) 4-R-$C_6H_4CONHNH_2$, dry ethanol, reflux (iv) Reflux in aq. NaOH solution.

The key intermediate in the preparation of triazolothiadiazines (**4a-4g**) and triazolothiadiazoles (**5a-5g**) was aminotriazole **13**, which in turn was prepared from 4-aminosulfonyl benzoic acid hydrazide**11**. The later was prepared by refluxing an aqueous suspension of tolylsulfonamide **10** with $KMnO_4$ to generate the corresponding carboxylic acid followed by refluxing its methanolic solution firstly in acidic condition and then in excess of hydrazine hydrate (Scheme 2). Then the required dithiocarbazinate **12** was prepared by reacting hydrazide **11** with carbondisulfide and potassium hydroxide in anhydrous ethanol. The salt **12** underwent ring closure on refluxing with an excess of 99% hydrazine hydrate to give aminotriazole **13**. While 4-amino-3-mercapto-1,2,4-triazoles may exist in thione–thioltautomeric forms, in our study, the proton NMR data showed it to be only thione without any trace of thiol. The compound **13** thus obtained was treated with variously substituted

phenacyl bromides in ethanol under reflux conditions to obtain the final compounds triazolothiadiazines **4a-4g** (Scheme 2). The ^{1}H NMR data of these compounds showed a characteristic singlet at around δ 4.5 for ring SCH_2 protons and SO_2NH_2 protons appeared at δ 7.52 as an exchangeable singlet.

We attempted to prepare fused triazolothiadiazoles (**5a-5g**) from **13** by refluxing it with variously substituted aromatic carboxylic acids in $POCl_3$, but could not isolate the desired products. Instead, a mixture of products was obtained and proton NMR did not show any peak corresponding to free SO_2NH_2 group probably due to the reaction of this NH_2 group with carboxylic acid in the presence of $POCl_3$. Therefore, we first protected the sulfonamide group as its formamidine following a green methodology developed recently by our research group[37] which involved stirring of **13** in neat DMFDMA at room temperature followed by a simple work up in water resulting into the formation of **14** in excellent yield (Scheme 2). A characteristic singlet of =CH proton of protected sulfonamide group was obtained at around δ 8.28 in proton NMR. Two singlets at δ 2.95 and δ 3.18 for $-N(CH_3)_2$ also evidenced the protection of sulfonamide group. Then the compound **14** was reacted with aromatic acids under the conditions of reflux in $POCl_3$ which resulted into successful conversion to **15a-15g**. The ^{1}H-NMR data of the compounds **15a-15g** showed complete disappearance of singlets for NH_2 as well as SH protons which confirmed the cyclization. The final compounds **5a-5g** were obtained by deprotection of sulphonamide group in **15a-15g** under acidic conditions (Scheme 2). The deprotection was further confirmed by proton NMR which showed a singlet for SO_2NH_2 group at δ 7.51.



Compounds	**4a/5a**	**4b/5b**	**4c/5c**	**4d/5d**	**4e/5e**	**4f/5f**	**4g/5g**
R	H	CH_3	OCH_3	F	Cl	Br	NO_2

Scheme 2. Reagents and conditions: (i) a. $KMnO_4$, H_2O, reflux (ii)MeOH, H^+, reflux (iii) $NH_2NH_2.H_2O$, MeOH, reflux (iv) CS_2, KOH (v) $H_2NH_2.H_2O$, reflux (vi) $ArCOCH_2Br$, EtOH, reflux (vii) DMFDMA, stirring, r.t. (viii) ArCOOH, $POCl_3$, reflux (ix) Conc. HCl, reflux, (x) ArCOOH, $POCl_3$, reflux.

2.2. CA inhibition:

The CA inhibitory potential of all the 21 compounds (**3a-3g**, **4a-4g**, **5a-5g**) against cytosolic isoforms hCA I and II as well as the membrane associated isoforms hCA IX and XII was assayed by using stopped flow assay method and the results are presented in Table 1. Clinically used acetazolamide (AZA) was the reference drug chosen for the assay. Inhibition data of the compounds let the following insights regarding CAs inhibitory properties.

(i) The cytosolic isoform hCA I was moderately inhibited by compounds **3a-3g** and **4a-4g**, with the inhibition constants in the range of 84-868 nM and mainly the compounds **3b**, **3d**, **3e**, **3f**, **3g**, **4c**, and **4f** were shown to be more efficient inhibitors than AZA. The compounds of series **3a-3g** exhibited almost similar activities and a remarkable difference was found only in the case of chloro substituted compound **3e** which showed almost three times greater potential than the reference acetazolamide (AZA) with inhibition constant, K_I = 86 nM. Other two halogensubstituents (F and Br) also show an in increase in potential as compared to unsubstituted derivative **3a**.In case of series of **4a-4g**, *p*-methoxy and *p*-bromo substituted compounds (**4c** and **4f**) showed maximum range of activity i.e. 84 and 89 nM respectively while all other compounds were weakly efficient against this enzyme. Compounds **5a-5g** proved to be much weaker inhibitors as most of the compounds crossed 10 μM range and none of them was even near to the AZA range. Thus, it is of great interest to relate this behaviour of these compounds toward hCA I and it can be concluded that both nuclear triazoles as well as triazole-fused with six membered thiadiazines are moderate inhibitors of hCA I while fusing triazoles with five membered thiadiazole ring leads to a steep decrease in the inhibitory potency of these compounds against hCA I.

(ii) The tested compounds in general showed better activity profile against hCA II than hCA I and compounds **3d**, **3f** and **4f** were better inhibitors of hCA II as compared to AZA. However being a ubiquitous, housekeeping isoform, this may not be a valuable property in another context if compounds targeting other isoforms (hCA IX and hCA XII) should also possess activity against hCA II. Thus it is interesting to note that some of the novel compounds showed poor inhibition against hCA II while retaining potent inhibition against hCA IX and hCA XII. Compounds **5a-5g** in which the triazole ring is fused with another 5-memebered thiadiazole ring showed a highly reduced potential for hCA II.This again leads to the conclusion that reducing the size of heteroyclic ring fused to the triazole ring leads to important differences in hCA II inhibitiory potency. The relative effect of substitution on activity profile was almost similar to that against hCA I.

(iii) The tumor associated target isoform hCA IX was highly inhibited by all the compounds of series **3a-3g** and **4a-4g** except compounds **4e** and **4g** which were

less efficient than AZA. The compound **3d** having fluoro substitution showed around 10 fold activity as compared to AZA. The activity pattern of compounds **3a-3g** shows that both electron donating as well as electron withdrawing groups enhance the inhibitory potential. The sulfonamide substituted compound **3g** was rather a less efficient inhibitor than halogen substituted compounds, probably due to large bulky nature of the group. The compounds of **4a-4g** series also showed similar pattern to that of **3a-3g** and again compound possessing sulfonamide group was weaker inhibitor. Compounds **5a-5g**were less effective as hCA IX inhibitors, with K_Is in the range of 41-431 nM.

(iv) The second tumor-associated isoform, hCA XII, was the most inhibited isoform by all the compounds investigated here, with many of them being low nanomolar (**3c-3g**, **4b**, **4c** and **4f**) CAIs. The least effective hCA XII inhibitors were **4g**, **5e** and **5f** (K_Is of 43-63 nM). Eight compounds **3c**, **3d**, **3e**, **3f**,**3g**, **4b**, **4c**, and **4f** were more potent inhibitors than the standard AZA. The best results were shown by compounds **3f** with a bromo substitution which was found to be four-fold efficient than AZA with inhibition constant 1.3 nM.

(v) Selectivity for inhibiting the tumor-associated isoforms (hCA IX and XII) over the widespread cytosolic forms (hCA I and II) is a key issue when designing CAIs. As seen from data in Table 2, where these ratios are provided, none of the compounds reported here showed uniformity in their inhibitory properties against all four CAs investigated (hCA I, II, IX and XII, Tables 1 and 2). It is satisfying to note that all the compounds showed better activity profile against hCA IX and XII over I and II which is highly desirable when only the tumor-associated isoforms would be targeted. It was observed that the compounds which showed excellent inhibition of isoform IX and XII were also shown to be highly selective. Compound **5g** showed maximum selectivity for IX and XII over II with the selectivity ratio of 164.63 and 758.43 respectively and have potential almost half to that of AZ**A**. All other compounds of this series i.e. **5a-5f** were also shown to be excellently selective having selectivity ratio close to 10 or more. These compounds **5a-5g** also showed excellent selectivity profile for both tumor associated isoforms over hCA I. All the compounds of series **3a-3g** and **4a-4g** were also more selective in their activity profile as compared with the chosen standard. Compound **3d** being the most efficient inhibitor of hCA IX was about five-fold more selective over hCAII than AZA. Similarly compound **3f** being the

most efficient inhibitor of hCA XII, was about four fold more selective over hCAII. Thus, the new class of sulfonamides reported here may indeed lead to isoform-selective CAIs targeting the tumor-associated CAs.

Table 1. Inhibitory potency data for compounds **3a-3g**, **4a-4g**, and **5a-5g** against isozymes hCAI, hCAII, hCAIX, and hCAXII

Inhibitors	K_i* **(nM)**			
	hCA I	**hCA II**	**hCA IX**	**hCA XII**
3a	288	31	22	10.4
3b	166	20	16	9.5
3c	271	27	8.1	4.9
3d	170	6.6	2.8	4.9
3e	86	24	5.7	3.6
3f	132	6.7	7.6	1.3
3g	220	37	9.0	4.5
4a	711	158	21	12.7
4b	292	73	13	4.9
4c	84	44	6.5	3.8
4d	507	93	25	18
4e	868	223	76	45
4f	89	5.6	3.7	3.0
4g	463	390	115	63
5a	4480	831	114	18
5b	>10000	3780	356	24
5c	>10000	6585	431	20
5d	>10000	8760	64	21
5e	6630	885	89	43
5f	8450	3615	104	56
5g	>10000	6750	41	8.9
AZA	250	12.1	25	5.7

AZA = acetazolamide, reference compound, a standard sulfonamide CAI, is also provided for comparison.
* Mean from 3 diferent assays, errors were in the range of ± 5-10 % of the reported value.

Table 2.Selectivity ratios for the inhibition of the tumor-associated isozymes hCA IX and hCAXII over the cytosolic isozymes hCA I and hCA II for compounds **3a-3g**, **4a-4g**, and **5a-5g**

Inhibitors	Selectivity ratio*			
	hCA I/hCA IX	hCA II/hCA IX	hCA I/XII	hCA II/hCAXII
3a	13.0	1.4	27.6	2.9
3b	10.3	1.2	17.4	2.1
3c	33.4	3.3	55.3	5.5
3d	60.7	2.3	34.6	1.3
3e	15.0	4.2	23.8	6.6
3f	17.3	0.8	101.5	5.1
3g	24.4	4.1	48.8	8.2
4a	33.8	7.5	55.9	12.4
4b	22.4	5.6	59.5	14.9
4c	12.9	6.7	22.1	11.5
4d	20.2	3.7	28.1	5.1
4e	11.4	3.0	19.2	4.9
4f	24.0	1.5	29.6	1.8
4g	4.0	3.3	7.3	6.1
5a	74.3	7.2	471.1	46.1
5b	>28.0	10.6	>416.0	157.5
5c	>23.0	15.2	>500.0	329.2
5d	>156.0	136.8	>476.0	417.1
5e	74.4	9.9	154.1	20.5
5f	81.2	34.7	150.8	64.5
5g	>243.0	164.6	>1123.0	758.4
AZA	10.0	0.4	43.8	2.1

AZA = acetazolamide, reference compound, a standard sulfonamide CAI, is also provided for comparison.
* The K_i ratios are indicative of isozyme selectivity: a weak selective inhibitor is characterized by a low value ratio..

3. Conclusion:

Three novel series of compounds **3a-3g**, **4a-4g** and **5a-5g** containing 1,2,4-triazole scaffold have been synthesized and investigated as inhibitors against four of the isomers of α class of carbonic anhydrases comprising cytosolic, ubiquitous isozymes hCA I and II as well as the transmembrane, tumor-associated isoforms hCA IX and XII which are validated

antitumor targets. In this study, compounds **3c-3g**, **4b**, **4c** and**4f** showed excellent CAs inhibitory profile. Compounds **3d** and **3f** being the most potent inhibitors of hCA IX and XII were associated with manyfold selectivity over hCA I and II as compared to the standard drug AZA. The activity profile also revealed that both nuclear triazole scaffold as well as triazole fused with six membered thiadiazines are moderate inhibitors of hCA I andII and substitution of thiadiazine ring by a 5-membered thiadiazole leads to a steep decrease in the inhibitory property of these compounds.Similarly, **5g** was the most selective inhibitor of tumor associated hCA XII over hCA II of all the investigated compound with nearly same range of activity comparable to the reference compound. As it has been evidenced that isoforms IX and XII are responsible for tumours and are also potential targets for diagnosis and treatment of cancers, discovery of potent selective IX and XII inhibitors will be a promising step in the strategy for an effective cancer therapy.

4. Experimental protocols:

4.1. General

Melting points were determined in open glass capillaries in an electrical melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on Shimadzu-21 FT-IR or Perkine Elmer IR Spectrophotometer using the KBr pellet technique. ^{1}H NMR and ^{13}C NMR spectra were recorded either in pure DMSO-d_6 or in $CDCl_3$/DMSO-d_6 mixture on Bruker NMR spectrometers at 300/400 MHz and 75.5/100 MHz respectively using tetramethylsilane (TMS) as internal standard. Chemical shifts are expressed in δ ppm. Mass spectra (DART-MS) were recorded on a JEOL-AccuTOFJMS-T100LC Mass spectrometer having a DART (Direct Analysis in Real Time) source in ESþ mode. The purity of the compounds was checked by ^{1}H NMR and thin layer chromatography (TLC) on silica gel plates using a mixture of petroleum ether and ethyl acetate as eluent. Iodine or UV lamp was used as a visualizing agent. Abbreviations‘s’ for singlet, ‘m’ for multiplet, ‘ex’ for exchangeable proton are used for NMR assignments; ‘s’ for strong, ‘m’ for medium for IR and ‘br’ for broad in NMR as well as IR assignments. ‘d’ stands for decomposition in melting point data.

4.2. Synthesis of 4-isothiocyanatobenzenesulfonamide (8)

To a well cooled mixture of methanol and triethylamine to 0 $^{\circ}$C was added carbon disulphide dropwise and stirred for 10 minutes. Then the amine was added in small lots with constant stirring and allowed the reaction mixture to stirr for further 15-16 hours when

triethyl ammonium dithiocarbamate salt precipitated completely. The solid thus precipitated was filtered, washed with diethyl ether and air dried. Then to stirred and ice-cooled biphasic solvent system, water/ethylacetate (1:1, 10 ml) was added the dithiocarbamate salt **7** (1 mmol) followed by sodium bicarbonate (2 mmol). To this stirring solution was then added iodine (2 mmol) pinch wise over a period of 15-20 minutes. After the completion of reaction, aqueous solution of sodium thiosulfate was added to neutralize the excess of iodine and then the ethylacetate layer was separated, filtered to remove precipitated sulphur, washed with water and dried over anhydrous sodium Sulphate. The ethylacetate was evapourated under reduced pressure yielding pure. Yield 79%, Lit. mp: 212-214 ^{o}C, Obs. mp: 209-211^{o}C, ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 7.87 (d, *J* = 7.5 Hz, 2H, Ar), 7.58 (d, *J* = 7.5 Hz, 2H, Ar), 7.49 (s, 2H, SO_2NH_2); ^{13}C NMR (DMSO-d_6, 75.5 Hz) δ (ppm): 143.38 (NCS), 133.63, 127.85, 126.91, 122.78.

4.3. Synthesis of hydrazino carbothiamide compounds (9a-9g):

General procedure

A mixture of **8** (1 mmol) and aromatic acid hydrazide was refluxed in absolute ethanol for 3-4 hours. After the completion of the reaction the solid precipitated was filtered off, washed with cold ethanol, dried and recrystalized from ethanol which afforded the pure products **9a-9g**.

4.3.1. *N*-[4-(aminosulfonyl)phenyl]-2-benzoyl-1-hydrazinecarbothioamide (9a)

Yield 84%, mp: 198-200 ^{o}C, IR(KBr) (υ, cm^{-1}), 3333, 3240, 3132 and 3101 (NH), 1643 (CO), 1335, 1157 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 10.38, 9.92, 9.68 (3s, 3H, 3NH, exch. D_2O), 7.96 (d, *J* = 6.9 Hz, 2H, Ar), 7.76 (d, *J* = 8.4 Hz, 2H, Ar), 7.70 (s, 1H, Ar), 7.58 (d, *J* = 6.9 Hz, 2H, Ar), 7.52 (d, *J* = 7.5 Hz, 2H, Ar), 7.31 (s, 2H, SO_2NH_2); ^{13}C NMR (75.5 Hz, DMSO-d_6) δ (ppm): 166.46, 142.85, 132.87, 132.42, 128.77, 128.32, 126.15.

4.3.2. *N*-[4-(aminosulfonyl)phenyl]-2-(4-methylbenzoyl)-1-hydrazinecarbothioamide (9b)

Yield 87%, mp: 202-204 ^{o}C, IR(KBr) (υ, cm^{-1}), 3333, 3202 and 3109 (NH), 1659 (CO), 1335, 1157 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 10.42, 9.92, 9.84 (3s, 3H, 3NH, exch. D_2O), 7.88 (d, *J* = 8.7 Hz, 2H, Ar), 7.47 (m, 4H, Ar), 7.31 (s, 2H, SO_2NH_2), 7.22 (d, *J* = 8.7 Hz, 2H, Ar), 2.34 (s, 3H, CH_3); ^{13}C NMR (75.5 Hz, DMSO-d_6) δ (ppm): 166.44, 142.89, 142.48, 130.10, 129.30, 128.35, 126.16, 21.51.

4.3.3. *N*-[4-(aminosulfonyl)phenyl]-2-(4-methoxybenzoyl)-1-hydrazinecarbothioamide (9c)

Yield 92%, mp: 200-202 ^{o}C, IR(KBr) (υ, cm^{-1}), 3340, 3202 and 3194 (NH), 1659 (CO), 1335, 1157 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 10.44, 9.96, 9.87 (3s, 3H, 3NH, exch. D_2O), 7.94 (d, *J* = 8.7 Hz, 2H, Ar), 7.50 (m, 4H, Ar), 7.31 (s, 2H, SO_2NH_2), 7.04 (d, *J* = 8.7 Hz, 2H, Ar), 3.82 (s, 3H, OCH_3); ^{13}C NMR (75.5 Hz, DMSO-d_6) δ (ppm): 166.07, 162.65, 142.86, 130.28, 126.11, 125.06, 114.02, 55.91.

4.3.4. *N*-[4-(aminosulfonyl)phenyl]-2-(4-fluorobenzoyl)-1-hydrazinecarbothioamide (9d)

Yield 78%, mp: 172-174 ^{o}C, IR(KBr) (υ, cm^{-1}), 3325, 3202 (NH), 1666 (CO), 1335, 1157 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 10.62 (1s, 1H, NH, exch. D_2O), 9.95 (2s, 2H, 2NH, exch. D_2O), 8.02 (m, 2H, Ar), 7.78 (m, 4H, Ar), 7.70 (m, 2H, Ar), 7.30 (s, 2H, SO_2NH_2); ^{13}C NMR (75.5 Hz, DMSO-d_6) δ (ppm): 166.41, 142.83, 130.97, 126.89, 126.08, 122.19, 115.90, 115.62.

4.3.5. *N*-[4-(aminosulfonyl)phenyl]-2-(4-chlorobenzoyl)-1-hydrazinecarbothioamide (9e)

Yield 85%, mp: 194-196 ^{o}C, IR(KBr) (υ, cm^{-1}), 3310, 3202 (NH), 1666 (CO), 1335, 1157 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 10.67 (1s, 1H, NH, exch. D_2O), 9.94 (2s, 2H, 2NH, exch. D_2O), 7.96 (d, *J* = 8.4 Hz, 2H, Ar), 7.76 (d, *J* = 8.4 Hz, 2H, Ar), 7.69 (m, 2H, Ar), 7.60 (d, *J* = 8.4 Hz, 2H, Ar), 7.31 (s, 2H, SO_2NH_2); ^{13}C NMR (75.5 Hz, DMSO-d_6) δ (ppm): 165.58, 142.78, 137.29, 131.70, 130.25, 128.89, 126.20.

4.3.6. *N*-[4-(aminosulfonyl)phenyl]-2-(4-bromobenzoyl)-1-hydrazinecarbothioamide (9f)

Yield 74%, mp: 188-190 ^{o}C, IR(KBr) (υ, cm^{-1}), 3310, 3202 (NH), 1659 (CO), 1335, 1157 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 10.67 (1s, 1H, NH, exch. D_2O), 9.96 (2s, 2H, 2NH, exch. D_2O), 7.89 (d, *J* = 7.8 Hz, 2H, Ar), 7.75 (m, 6H, Ar), 7.30 (s, 2H, SO_2NH_2); ^{13}C NMR (75.5 Hz, DMSO-d_6) δ (ppm): 165.54, 142.98, 137.47, 131.79, 131.36, 128.46, 126.18.

4.3.7 2-[4-(aminosulfonyl)benzoyl]-*N*-[4-(aminosulfonyl)phenyl]-1-hydrazinecarbothioamide (9g)

Yield 78%, mp: 194-196 ^{o}C, IR(KBr) (υ, cm^{-1}), 3325, 3256 and 3202 (NH), 1666 (CO), 1335, 1157 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 10.78 (1s, 1H, NH, exch. D_2O), 9.98 (2s, 2H, 2NH, exch. D_2O), 7.87 (d, *J* = 8.1 Hz, 2H, Ar), 7.64 (d, *J* = 8.4 Hz, 2H, Ar), 7.46 (m, 2H, Ar), 7.35 (d, *J* = 8.4 Hz, 2H, Ar), 7.31 (s, 2H, SO_2NH_2), 7.29 (s, 2H, SO_2NH_2);

^{13}C NMR (75.5 Hz, DMSO-d_6,) δ (ppm): 165.58, 142.78, 137.29, 131.70, 130.25, 128.89, 126.20.

4.4. Synthesis of thioxotriazolo benzensulfonamide compounds (3a-3g)

General procedure: Compound **9** was dissolved in 2% aq. NaOH solution and refluxed for 4 hrs. Then cooled the solution to room temperature, added ice to it and neutralized with conc. HCl which resulted into the precipitation of a white solid. The solid was filtered off, washed with water, dried and recrystallized from appropriate solvent which afforded the pure products **3a-3g.**

4.4.1. 4-(3-phenyl-5-thioxo-1,5-dihydro-4*H*-1,2,4-triazol-4-yl)benzenesulfonamide (3a)

Yield 78%, mp: 294 ^{o}C, IR(KBr) (υ, cm^{-1}), 3381, 3278, 3094, 1557, 1512, 1335, 1165 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 14.2 (s, 1H, ring NH, exch. D_2O), 7.91 (d, *J* = 7.8 Hz, 2H, Ar), 7.58 (d, *J* = 8.1 Hz, 2H, Ar), 7.53 (s, 2H, SO_2NH_2), 7.43 (m, 2H, Ar), 7.36 (m, 3H, Ar); ^{13}C NMR (75.5 Hz, DMSO-d_6) δ (ppm): 169.02 (C=S), 150.92, 145.15, 137.65, 130.99, 130.00, 129.13, 128.91, 127.13, 126.04; DART MS m/z 333.05 $(M+H)^+$, $C_{14}H_{12}N_4O_2S_2H^+$, calcd. 332.04.

4.4.2. 4-[3-(4-methylphenyl)-5-thioxo-1,5-dihydro-4*H*-1,2,4-triazol-4-yl]benzenesulfonamide (3b)

Yield 79%, mp: 276 ^{o}C, IR(KBr) (υ, cm^{-1}), 3379, 3279, 3094, 1551, 1512, 1335, 1165 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 14.2 (s, 1H, ring NH), 7.91 (d, *J* = 8.1 Hz, 2H, Ar), 7.56 (d, *J* = 8.1 Hz, 2H, Ar), 7.53 (s, 2H, SO_2NH_2), 7.19 (m, 4H, Ar), 2.27 (s, 3H, CH_3); ^{13}C NMR (75.5 Hz, DMSO-d_6) δ (ppm): 168.92 (C=S), 151.00, 145.11, 140.92, 137.73, 130.01, 129.69, 128.80, 127.13, 123.18, 21.31 (CH_3); DART MS m/z 347.19 $(M+H)^+$, $C_{15}H_{14}N_4O_2S_2H^+$, calcd. 346.43.

4.4.3. 4-[3-(4-methoxyphenyl)-5-thioxo-1,5-dihydro-4*H*-1,2,4-triazol-4-yl]benzenesulfonamide (3c)

Yield 82%, mp: 274 ^{o}C, IR(KBr) (υ, cm^{-1}), 3360, 3287, 3078, 1551, 1512, 1335, 1165 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 7.91 (d, *J* = 6.9 Hz, 2H, Ar), 7.57 (d, *J* = 7.5 Hz, 2H, Ar), 7.53 (s, 2H, SO_2NH_2), 7.25 (d, *J* = 7.2 Hz, 2H, Ar), 6.92 (d, *J* = 7.5 Hz, 2H, Ar), 3.74 (s, 3H, OCH_3); ^{13}C NMR (75.5 Hz, DMSO-d_6) δ (ppm): 168.77 (C=S), 161.21, 150.84, 145.06, 137.82, 130.44, 130.03, 127.15, 118.16, 114.62, 55.78 (OCH_3); DART MS m/z 362.87 $(M+H)^+$, $C_{15}H_{14}N_4O_3S_2H^+$, calcd. 362.43.

4.4.4. 4-[3-(4-fluorophenyl)-5-thioxo-1,5-dihydro-4*H*-1,2,4-triazol-4-yl]benzenesulfonamide (3d)

Yield 77%, mp: 282 ^{o}C, IR(KBr) (υ, cm^{-1}), 3371, 3286, 3094, 1512, 1335, 1165 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 14.2 (s, 1H, ring NH), 7.91 (d, *J* = 7.8 Hz, 2H, Ar), 7.58 (d, *J* = 8.1 Hz, 2H, Ar), 7.52 (s, 2H, SO_2NH_2), 7.40 (m, 2H, Ar), 7.25 (m, 2H, Ar); ^{13}C NMR (75.5 Hz, DMSO-d_6) δ (ppm): 168.96 (C=S), 150.20, 145.15, 137.48, 131.63, 130.00, 127.16, 122.62, 116.49, 116.19; DART MS m/z 348.86 $(M+H)^+$, $C_{14}H_{11}FN_4O_2S_2H^+$, calcd. 350.39.

4.4.5. 4-[3-(4-chlorophenyl)-5-thioxo-1,5-dihydro-4*H*-1,2,4-triazol-4-yl]benzenesulfonamide (3e)

Yield 76%, mp: 288 ^{o}C, IR(KBr) (υ, cm^{-1}), 3354, 3264, 3094, 1512, 1335, 1165 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 14.3 (s, 1H, ring NH), 7.90 (d, *J* = 6.3 Hz, 2H, Ar), 7.58 (d, *J* = 5.8 Hz, 2H, Ar), 7.52 (s, 2H, SO_2NH_2), 7.46 (d, 2H, Ar), 7.33 (d, 2H, Ar); ^{13}C NMR (75.5 Hz, DMSO-d_6) δ (ppm): 169.02 (C=S), 150.03, 145.17, 137.40, 135.88, 130.78, 129.97, 129.30, 127.17, 124.94; DART MS m/z 367.13/369.13 $(M+H)^+/(M+H+2)^+$, $C_{14}H_{11}ClN_4O_2S_2H^+$, calcd. 366.85/368.85.

4.4.6. 4-[3-(4-bromophenyl)-5-thioxo-1,5-dihydro-4*H*-1,2,4-triazol-4-yl]benzenesulfonamide (3f)

Yield 71%, mp: 268 ^{o}C, IR(KBr) (υ, cm^{-1}), 3348, 3225, 1497, 1339, 1149 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 14.2 (s, 1H, ring NH), 7.91 (d, *J* = 7.8 Hz, 2H, Ar), 7.59 (m, 4H, Ar), 7.52 (s, 2H, SO_2NH_2), 7.26 (d, *J* = 8.1 Hz, 2H, Ar); ^{13}C NMR (75.5 Hz, DMSO-d_6) δ (ppm): 169.08 (C=S), 150.14, 145.19, 137.40, 132.22, 130.93, 129.96, 127.19, 125.27, 124.72; DART MS m/z 411.88/413.88 $(M+H)^+/(M+H+2)^+$, $C_{14}H_{11}BrN_4O_2S_2H^+$, calcd. 410.55/412.55.

4.4.7. 4-{3-[4-(aminosulfonyl)phenyl]-5-thioxo-1,5-dihydro-4*H*-1,2,4-triazol-4-yl}benzenesulfonamide (3g)

Yield 74%, mp: 294 ^{o}C IR(KBr) (υ, cm^{-1}), 3225, 3063, 1535, 1342, 1165 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 14.45 (s, 1H, ring NH), 7.92 (d, *J* = 8.4 Hz, 2H, Ar), 7.18 (d, *J* = 8.4 Hz, 2H, Ar), 7.62 (d, *J* = 8.4 Hz, 2H, Ar), 7.53 (s, 2H, SO_2NH_2), 7.51 (d, *J* = 8.7Hz, 2H, Ar), 7.45 (s, 2H, SO_2NH_2); ^{13}C NMR (75.5 Hz, DMSO-d_6) δ (ppm): 169.56 (C=S), 149.88, 146.02, 145.30, 137.37, 130.00, 129.61, 129.06, 127.23, 126.33; DART MS m/z 411.99 $(M+H)^+$, $C_{14}H_{13}N_5O_4S_3H^+$, calcd. 411.48.

4.5 Synthesis of 4-(hydrazinocarbonyl)benzenesulfonamide (11):

Tolylsulfonamide **10** (1.00 mM) was first converted to 4-aminosulfonyl benzoic acid by refluxing its aqueous suspension with $KMnO_4$ (3.00 mM) to generate the corresponding carboxylic acid followed by refluxing its methanolic solution firstly in acidic condition and then in excess of hydrazine hydrate.[38] Yield 74%, Lit. mp: 230-232 ^oC, Obs. mp: 228-230 ^oC.

4.6 Synthesis of 4-(4-amino-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)benzenesulfonamide (13):

To a well cooled and stirring ethanolic solution of KOH (1.00 mM) was added **11** (1.00 mM) followed by dropwise addition of carbondisulfide (1.20 mM). The reaction mixture was stirred for overnight and the dithiocarbamate salt precipitated as yellow solid was filtered, washed with diethyl ether and dried. Then to the aqueous suspension of dithiocarbamate salt was added excess of hydrazine hydrate and was refluxed for 20 h. To the cooled reaction mixture was added glacial acetic acid just to neutralize it when aminotriazole **13** was precipitated as white solid which was filtered, washed with water, dried and recystallized from alcohol.[39] Yield 72%, mp: 238-240 ^oC, IR(KBr) (υ, cm^{-1}), 1565, 1342, 1165 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 14.12 (s, 1H, ring NH), 8.22 (d, *J* = 6.9 Hz, 2H, Ar), 7.95 (d, *J* = 6.9 Hz, 2H, Ar), 7.51 (s, 2H, SO_2NH_2), 5.82 (s, 1H, NH_2); ^{13}C NMR (75.5 Hz, DMSO-d_6) δ (ppm): 167.78 (C=S), 148.85, 145.89,128.97, 127.13, 126.25; DART MS m/z 272 $(M+H)^+$, $C_8H_9N_5O_2S_2H^+$, calcd. 271.32.

4.7 Synthesis of 4-(4-amino-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)-*N*-[(*Z*)-(dimethylamino)methylidene]benzenesulfonamide (14):

A suspension of **13** (1.00 mM) in DMFDMA (1.20 mM) was stirred at 40 ^oC for 7-8 hours and then reaction mixture was poured in cold water. The solid separated was filtered, washed with water, dried and recrystallized from alcohol. Yield 83%, mp: 220-222 ^oC IR(KBr) (υ, cm^{-1}), 1342, 1165 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 14.12 (s, 1H, ring NH), 8.24 (s, 1H, =CH), 8.19 (d, *J* = 8.7 Hz, 2H, Ar), 7.91 (d, *J* = 8.7 Hz, 2H, Ar), 5.80 (s, 1H, NH_2), 3.16 (s, 3H, N-CH_3), 2.92 (s, 3H, N-CH_3); ^{13}C NMR (75.5 Hz, DMSO-d_6) δ (ppm): 167.78 (C=S), 160.43, 151.38, 150.74, 130.45, 128.82, 127.80, 126.76, 41.50, 35.18.

4.8. Synthesis of triazolothiadiazine compounds (4a-4g)

General procedure: A mixture of **9** (1.00 mM) and variously substituted phenacyl bromides (1.00 mM) was refluxed in absolute ethanol on water bath for 9-11 hrs and a solid

precipitated out while reflux. The reaction mixture was allowed to cool to room temp. and the solid was filtered off, dried and recrystallized from suitable solvent which afforded the pure title compounds.

4.8.1. 4-(6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)benzenesulfonamide (4a)

Yield 74%, mp: 264-266 ^{o}C, IR(KBr) (υ, cm^{-1}), 3294, 2970 and 2839 (CH_2), 1697, 1651, 1558, 1319, 1157 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.20 (d, *J* = 8.1 Hz, 2H, Ar), 8.03 (m, 3H), 7.62 (m, 4H, Ar), 7.49 (s, 2H, SO_2NH_2), 4.44 (s, 2H, CH_2); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ (ppm): 156.77, 151.21, 145.69, 143.76, 133.78, 132.54, 129.62, 129.34, 128.86, 128.13, 127.51, 127.16, 126.60, 23.31; DART MS m/z 372 $(M+H)^+$, $C_{16}H_{13}N_5O_2S_2H^+$, calcd. 371.44.

4.8.2. 4-[6-(4-methylphenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl]benzenesulfonamide (4b)

Yield 77%, mp: 264-266 ^{o}C, IR(KBr) (υ, cm^{-1}), 3317, 3040, 1597, 1528, 1335, 1157 (SO_2). ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.20 (d, *J* = 8.7 Hz, 2H, Ar), 8.02 (d, *J* = 8.7 Hz, 2H, Ar), 7.93 (d, *J* = 8.1 Hz, 2H, Ar), 7.50 (s, 2H, SO_2NH_2), 7.40 (d, *J* = 8.1 Hz, 2H, Ar), 4.44 (s, 2H, CH_2), 2.40 (s, 3H, CH_3); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ (ppm): 156.69, 151.09, 145.64, 143.80, 142.88, 130.93, 130.21, 129.37, 128.80, 128.10, 126.58, 23.16, 21.53; DART MS m/z 386 $(M+H)^+$, $C_{17}H_{15}N_5O_2S_2H^+$, calcd. 385.46.

4.8.3. 4-[6-(4-methoxyphenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl]benzenesulfonamide (4c)

Yield 71%, mp: 264-266^{o} C, IR(KBr) (υ, cm^{-1}), 3225, 3032, 1705, 1597, 1551, 1327, 1157 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.20 (d, *J* = 8.4 Hz, 2H, Ar), 8.00 (m, 4H, Ar), 7.52 (s, 2H, SO_2NH_2), 7.12 (d, *J* = 9.0 Hz, 2H, Ar), 4.40 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ (ppm): 156.73, 151.09, 145.99, 143.86, 142.91, 130.88, 130.22, 129.32, 128.82, 128.10, 126.57, 23.10, 55.67; DART MS m/z 402 $(M+H)^+$, $C_{17}H_{15}N_5O_3S_2H^+$, calcd. 401.46.

4.8.4. 4-[6-(4-fluorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl]benzenesulfonamide (4d)

Yield 73%, mp: 274-276 ^{o}C, IR(KBr) (υ, cm^{-1}), 3217, 3047, 1690, 1597, 1512, 1335, 1157 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.20 (d, *J* = 8.4 Hz, 2H, Ar), 8.11 (m, 2H, Ar), 8.02 (d, *J* = 8.4 Hz, 2H, Ar), 7.51 (s, 2H, SO_2NH_2), 7.42 (m, 2H, Ar), 4.47 (s, 2H, CH_2); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ (ppm): 164.82 (d, $^{1}J_{CF}$ = 251.41 Hz), 155.85, 151.19,

145.69, 143.68, 130.83 (d, $^{3}J_{CF}$ = 9.06 Hz), 130.29, 129.29, 128.86, 126.59, 116.74 (d, $^{2}J_{CF}$ = 22.65 Hz), 23.28; DART MS m/z 390 $(M+H)^+$, $C_{16}H_{12}FN_5O_2S_2H^+$, calcd. 389.43.

4.8.5. 4-[6-(4-chlorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl]benzenesulfonamide (4e)

Yield 78%, mp: 276-278 ^{o}C, IR(KBr) (υ, cm^{-1}), 3209, 3047, 1697, 1589, 1335, 1157 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.19 (d, *J* = 8.1 Hz, 2H, Ar), 8.04 (m, 4H, Ar), 7.67 (d, *J* = 8.4 Hz, 2H, Ar), 7.52 (s, 2H, SO_2NH_2), 4.46 (s, 2H, CH_2); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ (ppm): 155.79, 151.21, 145.71, 143.69, 137.45, 132.62, 129.94, 129.71, 129.24, 128.86, 126.60, 23.19; DART MS m/z 406/408 $(M+H)^+/(M+H+2)^+$, $C_{16}H_{12}ClN_5O_2S_2H^+$, calcd. 405.88/407.88.

4.8.6. 4-[6-(4-bromophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl]benzenesulfonamide (4f)

Yield 74%, mp: 280-282 ^{o}C, IR(KBr) (υ, cm^{-1}), 3225, 3032, 1705, 1597, 1551, 1327, 1157 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.19 (d, *J* = 8.1 Hz, 2H, Ar), 8.02 (d, *J* = 8.4 Hz, 2H, Ar), 7.97 (d, *J* = 8.7 Hz, 2H, Ar), 7.80 (d, *J* = 8.4 Hz, 2H, Ar), 7.52 (s, 2H, SO_2NH_2), 4.46 (s, 2H, CH_2); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ (ppm): 155.96, 151.20, 145.65, 143.75, 132.94, 132.66, 130.08, 129.18, 128.88, 126.60, 126.42, 23.09; DART MS m/z 451/453 $(M+H)^+/(M+H+2)^+$, $C_{16}H_{12}BrN_5O_2S_2H^+$, calcd. 405.88/407.88.

4.8.7. 4-[6-(4-nitrophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl]benzenesulfonamide (4g)

Yield 64%, mp: 286-288 ^{o}C, IR(KBr) (υ, cm^{-1}), 3232, 3063, 1643, 1574, 1512, 1342, 1157 (SO_2); .^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.40 (d, *J* = 8.7 Hz, 2H, Ar), 8.27 (d, *J* = 8.7 Hz, 2H, Ar), 8.19 (d, *J* = 8.1 Hz, 2H, Ar), 8.02 (d, *J* = 8.1 Hz, 2H, Ar), 7.51 (s, 2H, SO_2NH_2), 4.53 (s, 2H, CH_2); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ (ppm): 155.14, 151.40, 149.72, 145.81, 143.61, 139.71, 129.56, 129.08, 128.94, 126.64, 124.58, 23.42; DART MS m/z 417 $(M+H)^+$, $C_{16}H_{12}N_6O_4S_2H^+$, calcd. 416.43.

4.9. Synthesis of *N*-protected triazolothiadiazole compounds (15a-15g)

General procedure: A mixture of **10** (10 mmol) and aromatic acids (10 mmol) in the presence of $POCl_3$ (15mL) was refluxed for 4–6 h. After the completion of the reaction the reaction mixture was cooled to room temperature and then slowly added to crushed ice and neutralized with aq. NaOH solution. A solid was precipitated out which was filtered off, washed with excess of water, dried and recrystalized from appropriate solvent to afford the title compounds.

4.9.1. *N*-[(*Z*)-(dimethylamino)methylidene]-4-(6-phenyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)benzenesulfonamide (15a)

Yield 88%, mp: 262 ^{o}C, IR(KBr) (υ, cm^{-1}), 2978, 1705, 1620 (C=N), 1342, 1149 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.47 (d, *J* = 8.1 Hz, 2H, Ar), 8.25 (s, 1H, CH=N), 8.01-8.10 (m, 4H, Ar), 7.68 (m, 3H, Ar), 3.18 (s, 3H, N-CH_3), 2.95 (s, 3H, N-CH_3); ^{13}C NMR (75.5 Hz, DMSO-d_6) δ (ppm): 167.78, 160.43, 144.73, 133.57, 130.17, 129.35, 128.69, 127.83, 127.28, 126.87, 41.48, 35.64.

4.9.2. *N*-[(*Z*)-(dimethylamino)methylidene]-4-[6-(4-methylphenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]benzenesulfonamide (15b)

Yield 84%, mp: 266 ^{o}C , IR(KBr) (υ, cm^{-1}), 2978, 1705, 1620 (C=N), 1342, 1149 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.51 (d, *J* = 8.4 Hz, 2H, Ar), 8.25 (s, 1H, CH=N), 8.24 (d, *J* = 8.4 Hz, 2H, Ar), 7.77 (d, *J* = 8.1 Hz, 2H, Ar), 7.43 (d, *J* = 8.1 Hz, 2H, Ar), 3.17 (s, 3H, N-CH_3), 2.95 (s, 3H, N-CH_3), 2.40 (s, 3H, CH_3); ^{13}C NMR (75.5 Hz, DMSO-d_6) δ (ppm): 160.27, 144.45, 143.9, 130.43, 128.68, 127.48, 127.09, 126.48, 41.52, 35.28, 21.71.

4.9.3. *N*-[(*Z*)-(dimethylamino)methylidene]-4-[6-(4-methoxyphenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]benzenesulfonamide (15c)

Yield 89%, mp: 274 ^{o}C, IR(KBr) (υ, cm^{-1}), 2978, 1705, 1620 (C=N), 1342, 1149 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.46 (d, *J* = 8.1 Hz, 2H, Ar), 8.27 (s, 1H, CH=N), 8.00-8.04 (m, 4H, Ar), 7.19 (d, *J* = 8.7 Hz, 2H, Ar), 3.89 (s, 3H, OCH_3), 3.17 (s, 3H, N-CH_3), 2.95 (s, 3H, N-CH_3); ^{13}C NMR (75.5 Hz, DMSO-d_6) δ (ppm): 161.13, 144.47, 142.7, 129.57, 129.18, 127.78, 127.29, 126.32, 55.63, 41.52, 35.28.

4.9.4. *N*-[(*Z*)-(dimethylamino)methylidene]-4-[6-(4-fluorophenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]benzenesulfonamide (15d)

Yield 83%, mp: 278 ^{o}C, IR(KBr) (υ, cm^{-1}), 2978, 1705, 1620 (C=N), 1342, 1149 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.46 (d, *J* = 8.4 Hz, 2H, Ar), 8.28 (s, 1H, CH=N), 8.17 (m, 2H, Ar), 8.01 (d, *J* = 8.4 Hz, 2H, Ar), 7.51 (m, 2H, Ar), 3.18 (s, 3H, N-CH_3), 2.95 (s, 3H, N-CH_3); ^{13}C NMR (75.5 Hz, DMSO-d_6) δ (ppm): 160.44, 148.62, 127.24, 126.73, 41.49, 35.26.

4.9.5. 4-[6-(4-chlorophenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]-*N*-[(*Z*)-(dimethylamino)methylidene]benzenesulfonamide (15e)

Yield 81%, mp: 282 °C, IR(KBr) (υ, cm^{-1}), 2978, 1705, 1628 (C=N), 1342, 1149 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.45 (d, *J* = 8.1 Hz, 2H, Ar), 8.28 (s, 1H, CH=N), 8.11 (d, *J* = 8.1 Hz, 2H, Ar), 8.01 (d, *J* = 8.1 Hz, 2H, Ar), 7.73 (d, *J* = 8.1 Hz, 2H, Ar), 3.18 (s, 3H, N-CH_3), 2.95 (s, 3H, N-CH_3); ^{13}C NMR (75.5 Hz, DMSO-d_6) δ (ppm): 159.89, 129.94, 128.89, 127.01, 126.50, 41.54, 35.54.

4.9.6. 4-[6-(4-bromophenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]-*N*-[(*Z*)-(dimethylamino)methylidene]benzenesulfonamide (15f)

Yield 79%, mp: 286 °C, IR(KBr) (υ, cm^{-1}), 2978, 1705, 1628 (C=N), 1342, 1149 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.44 (d, *J* = 8.4 Hz, 2H, Ar), 8.28 (s, 1H, CH=N), 8.01 (m, 4H, Ar), 7.85 (d, *J* = 8.4 Hz, 2H, Ar), 3.18 (s, 3H, N-CH_3), 2.95 (s, 3H, N-CH_3); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ (ppm): 166.76, 160.46, 145.16, 144.78, 133.18, 129.66, 128.64, 128.59, 127.28, 127.19, 126.86, 41.48, 35.64.

4.9.7. *N*-[(*Z*)-(dimethylamino)methylidene]-4-[6-(4-nitrophenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]benzenesulfonamide (15g)

Yield 87%, mp: 294 °C, IR(KBr) (υ, cm^{-1}), 3078, 1705, 1636 (C=N), 1342, 1142 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.46 (m, 4H, Ar), 8.36 (d, *J* = 9 Hz, 2H, Ar), 8.30 (s, 1H, CH=N), 8.02 (d, *J* = 8.4 Hz, 2H, Ar), 3.18 (s, 3H, N-CH_3), 2.95 (s, 3H, N-CH_3); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ (ppm): 166.23, 160.49, 145.06, 143.58, 133.18, 128.96, 128.43, 128.47, 127.29, 128.17, 127.76, 41.44, 35.62.

4.10. Synthesis of triazolothiadiazole benzenesulfonamide compounds (5a-5g):

General procedure: The compounds **5a-5g** were synthesized by refluxing a mixture of **11a-11g** in conc. hydrochloric acid for 3 hrs. This leads to the deprotection of sulfonamide under acidic condition. After the completion of reaction the reaction mixture was cooled to room temperature and a solid was precipitated out which was filtered off, washed with excess of water, dried and recrystalized from suitable solvent to afford the title compounds.

4.10.1 4-(6-phenyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)benzenesulfonamide(5a)

Yield 81%, mp: 306 °C, IR(KBr) (υ, cm^{-1}), 3217, 3063, 1520, 1342, 1165 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.51 (d, *J* = 8.1 Hz, 2H, Ar), 8.08 (m, 4H, Ar), 7.69 (m, 3H, Ar), 7.54 (s, 2H, SO_2NH_2); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ (ppm): 167.79, 155.26,

145.67, 145.09, 133.58, 130.18, 129.33, 128.76, 127.86, 127.02, 126.84; DART MS m/z 358 $(M+H)^+$, $C_{15}H_{11}N_5O_2S_2H^+$, calcd. 357.41.

4.10.2. 4-[6-(4-methylphenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]benzenesulfonamide (5b)

Yield 79%, mp: 312 °C, IR(KBr) (υ, cm^{-1}), 3217, 3063, 1520, 1342, 1165 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.48 (d, *J* = 8.4 Hz, 2H, Ar), 8.06 (d, *J* = 8.4 Hz, 2H, Ar), 7.95 (d, *J* = 8.1 Hz, 2H, Ar), 7.56 (s, 2H, SO_2NH_2), 7.43 (d, *J* = 8.1 Hz, 2H, Ar), 2.40 (s, 3H, CH_3); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ (ppm): 167.62, 155.39, 145.56, 145.01, 133.32, 130.45, 129.54, 128.76, 127.86, 127.02, 123.64, 21.48; DART MS m/z 372 $(M+H)^+$, $C_{16}H_{13}N_5O_2S_2H^+$, calcd. 371.44.

4.10.3. 4-[6-(4-methoxyphenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]benzenesulfonamide (5c)

Yield 83%, mp: 314 °C, IR(KBr) (υ, cm^{-1}), 3217, 3063, 1520, 1342, 1165 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.51 (d, *J* = 8.4 Hz, 2H, Ar), 8.06 (d, *J* = 8.4 Hz, 2H, Ar), 8.05 (d, *J* = 9.0 Hz, 2H, Ar), 7.53 (s, 2H, SO_2NH_2), 7.19 (d, *J* = 9.0 Hz, 2H, Ar), 3.89 (s, 3H, OCH_3); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ (ppm): 167.04, 156.34, 145.18, 144.79, 133.32, 130.45, 129.54, 128.76, 127.86, 127.02, 123.74, 21.67; DART MS m/z 388 $(M+H)^+$, $C_{16}H_{13}N_5O_3S_2H^+$, calcd. 387.44.

4.10.4. 4-[6-(4-fluorophenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]benzenesulfonamide (5d)

Yield 77%, mp: 310 °C, IR(KBr) (υ, cm^{-1}), 3225, 3047, 1605, 1558, 1335, 1165 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.49 (d, *J* = 8.1 Hz, 2H, Ar), 8.17 (m, 2H, Ar), 8.06 (d, *J* = 8.1 Hz, 2H, Ar), 7.55 (s, 2H, SO_2NH_2), 7.49 (m, 2H, Ar); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ (ppm): 166.67, 145.73, 130.65, 130.53, 128.74, 127.01, 126.84, 125.95, 117.53, 117.23; DART MS m/z 376 $(M+H)^+$, $C_{15}H_{10}FN_5O_2S_2H^+$, calcd. 375.40.

4.10.5. 4-[6-(4-chlorophenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]benzenesulfonamide(5e)

Yield 78%, mp: 318 °C, IR(KBr) (υ, cm^{-1}), 3240, 3078, 1597, 1342, 1165 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.50 (d, *J* = 8.1 Hz, 2H, Ar), 8.13 (d, *J* = 8.4 Hz, 2H, Ar), 8.06 (d, *J* = 8.1 Hz, 2H, Ar), 7.73 (d, *J* = 8.4 Hz, 2H, Ar), 7.54 (s, 2H, SO_2NH_2); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ (ppm): 166.66, 145.77, 138.25, 130.53, 129.63, 128.72, 128.24, 127.02, 126.87; DART MS m/z 392/394 $(M+H)^+/(M+H+2)^+$, $C_{15}H_{10}ClN_5O_2S_2H^+$, calcd. 391.86/393.86.

4.10.6. 4-[6-(4-bromophenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]benzenesulfonamide(5f)

Yield 73%, mp: 304 ^{o}C, IR(KBr) (υ, cm^{-1}), 3286, 3063, 1589, 1342, 1165 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.48 (d, *J* = 8.1 Hz, 2H, Ar), 7.99 (m, 4H, Ar), 7.89 (d, *J* = 8.1 Hz, 2H, Ar), 7.55 (s, 2H, SO_2NH_2); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ (ppm): 166.80, 145.75, 145.14, 138.25, 129.63, 128.72, 128.24, 127.02, 126.87; DART MS m/z 438/440 $(M+H)^+/(M+H+2)^+$, $C_{15}H_{10}BrN_5O_2S_2H^+$, calcd. 436.31/438.31.

4.10.7. 4-[6-(4-nitrophenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]benzenesulfonamide(5g)

Yield 69%, mp: 302 ^{o}C, IR(KBr) (υ, cm^{-1}), 3217, 3063, 1520, 1342, 1165 (SO_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.50 (d, *J* = 8.1 Hz, 2H, Ar), 8.44 (d, *J* = 8.7 Hz, 2H, Ar), 8.37 (d, *J* = 8.7 Hz, 2H, Ar), 8.07 (d, *J* = 8.4 Hz, 2H, Ar), 7.58 (s, 2H, SO_2NH_2); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ (ppm): 166.21, 146.24, 145.36, 133.29, 129.09, 128.63, 128.29, 126.90, 126.59, 124.91; DART MS m/z 403 $(M+H)^+$, $C_{15}H_{10}N_6O_4S_2H^+$, calcd. 402.41.

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Benzenesulfonamide bearing 1,2,4-triazole scaffolds as potent inhibitors of tumor associated carbonic anhydrase isoforms hCA IX and hCA XII

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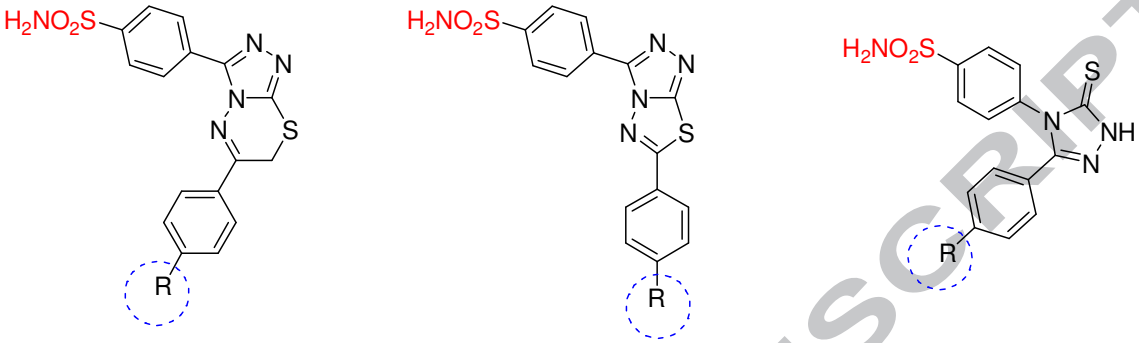

3c, 3d, 3e, 3f, 3g, 4c, 4f K_I(nM) < 10 against hCA IX

3c, 3d, 3e, 3f, 3g, 4b, 4c, 4f 4f K_I(nM) < 5 against hCA XII

Novel heterocyclic compounds containing benzenesulfonamide moiety bearing 1,2,4-triazole scaffold showed excellent carbonic anhydrase hCA IX and hCA XII inhibitory efficiency and also promising selectivity over hCA I and hCA II.

Research Highlights

- Library of novel hCA I, II, IX and XII inhibitors was synthesized.
- Benzene sulphonamide incorporated to 1,2,4-triazole scaffold.
- Compounds were screened against hCA isoforms I, II, IX and XII.
- Tested compounds showed excellent, low nanomolar affinity for CA isozymes.
- Some of the tested compounds showed high degree of selectivity for hCA IX and XII.