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Design, synthesis, and biological evaluation of 1,2,3-triazole-linked triazino[5,6-b]indole-benzene sulfonamide conjugates as potent carbonic anhydrase I, II, IX , and XIII inhibitors

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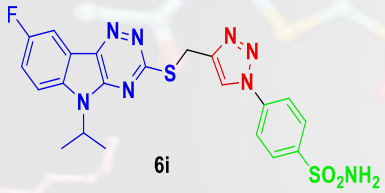
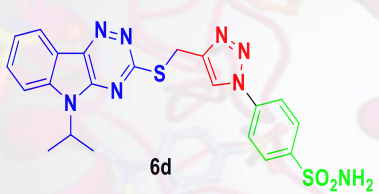


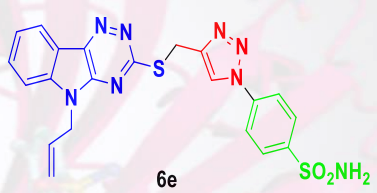
6i

hCAI K_i = 379.2nM
hCAII K_i = 7.7nM
hCAIX K_i = 34.9 nM
hCAXIII K_i = >500 nM



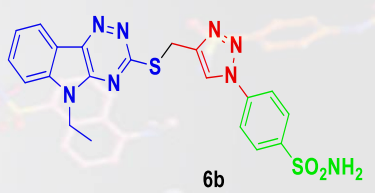
6d

hCAI K_i = 314.7nM
hCAII K_i = 20.9nM
hCAIX K_i = 37.8 nM
hCAXIII K_i = 65.8nM



6e

hCAI K_i = >500nM
hCAII K_i = 41.7nM
hCAIX K_i = 41.1nM
hCAXIII K_i = >500nM



6b

hCAI K_i = >500nM
hCAII K_i = 72.0nM
hCAIX K_i = 172.5nM
hCAXIII K_i = 69.8nM

Abstract:

A series of 1,2,3-triazole-linked triazino[5,6-b]indole-benzene sulfonamide hybrids (**6a–6o)** was synthesized and evaluated for carbonic anhydrase (CA, EC 4.2.1.1) inhibitory activity against the human (h) isoforms hCA I, II, XIII (cytosolic isoforms), and hCA IX (transmembrane tumor-associated isoform) The results revealed that the compounds **6a–6o exhibited Ki values in the low to medium** nanomolar range against hCA II and hCA IX (Kis ranging from 7.7nM to 41.3 nM) and higher Ki values against hCA I and hCA XIII. Compound **6i showed potent inhibition of hCA II (Ki = 7.7 nM),** being more effective compared to the standard inhibitor acetazolamide (AAZ) (Ki = 12.1 nM). Compounds, **6b and 6d showed moderate activity against hCA XIII (Ki = 69.8 and 65.8 nM). Hence, compound 6i** could be consider as potential lead candidate for the design of potent and selective hCA II inhibitors.

Keywords: 1,2,3-triazole; triazino[5,6-b]indole-benzene sulfonamide; carbonic anhydrase inhibitors

Introduction

- Respiration is one of the key physiological processes across all phyla, ranging from prokaryotes to eukaryotes.

- Several cellular metabolic reactions utilize carbon dioxide (CO_2) as their substrate and produce its hydrated form, bicarbonate (HCO_3^-), as the product.

- This bicarbonate plays a pivotal role physiologically by acting as a substrate for various carboxylating enzymes, which are involved in the biosynthesis of fatty acids, amino acids and nucleotides.

- The interconversion between CO_2 and HCO_3^- can be shown by the following two-step reaction:-

$$\text{E-M}^{2+}\text{-OH- + CO}_2 \rightleftharpoons \text{E-M}^{2+}\text{-HCO}_3^- \xrightleftharpoons{H_2O} \text{E-M}^{2+}\text{-H}_2\text{O + HCO}_3^- \quad (1)$$

$$\text{E-M}^{2+}\text{-H}_2\text{O + B} \rightleftharpoons \text{E-M}^{2+}\text{-OH- + BH}^+ \quad (2)$$

where M^{2+} = Zn(II), Cd(II), Fe(II) or Mn(II)

- The uncatalyzed reaction is slow at physiological pH.
- Hence in order to accelerate this reaction, a class of enzymes called carbonic anhydrases come into picture.
- Carbonic anhydrases (CAs, EC 4.2.1.1) are a superfamily of ubiquitous metalloenzymes, which facilitate the above said reaction across all phyla.
- Till date, seven genetically distinct families of CAs have evolved across all phyla, ranging from bacteria and archaea to eukarya.
- These seven families are the α-, β-, γ-, δ-, ζ-, η- and θ.
- These carbonic anhydrases cater a role in various biosynthetic processes and pH regulation.

α- CA	Vertebrates, Algae, Gram – ve bacteria
β- CA	Gram +ve bacteria, monocots & dicots
γ- CA	Archaea, Cyanobacteria
δ- CA	Marine diatoms
ζ- CA	Marine diatoms
η- CA	Protozoa
θ- CA	*Phaeodactylum tricornutum*

Humans and Carbonic anhydrases

- Humans primarily contain the α- class of carbonic anhydrases.

- Sixteen isozymes of this class have been identified in total and they differ in a variety of attributes like molecular features, oligomeric arrangement, cellular localization, distribution in organs and tissues, expression levels, kinetic properties and response to different inhibitor classes.

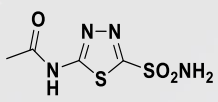
- Among all isozymes, CA I, CA II, CA III, CA VII, CA VIII, CA X, CA XI and CA XIII are cytosolic, CA IV, CA IX, CA XII and CA XIV are membrane-bound, CA VA and VB are mitochondrial and CA VI is secreted in saliva and mammary glands.

- CAs VIII, X and XI are devoid of any enzymatic activity and are designated as CA-related proteins (CARPs).

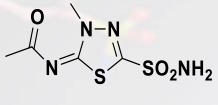
Enzyme	Organ/tissue distribution	Disease in which enzyme is involved
CA I	Erythrocytes, gastrointestinal tract, eye	Retinal/cerebral edema
CA II	Erythrocytes, eye, gastrointestinal tract, bone osteoclasts, kidney, lung, testis, brain	Glaucoma, edema, epilepsy, altitude sickness
CA III	Skeletal muscle, adipocytes Skeletal muscle, adipocytes	Oxidative stress
CA IV	Kidney, lung, pancreas, brain capillaries, colon, heart muscle, eye	Glaucoma, retinitis pigmentosa, stroke
CA VA	Liver	Obesity
CA VB	Heart and skeletal muscle, pancreas, kidney, spinal cord, gastrointestinal Tract	Obesity
CA VI	Salivary and mammary glands	Cariogenesis
CA VII	Central nervous system	Epilepsy Oxidative stress
CA IX	Tumors, gastrointestinal mucosa	Cancer
CA XII	Renal, intestinal, reproductive epithelia, eye, tumors	Cancer Glaucoma
CA XIII	Kidney, brain, lung, gut, reproductive tract	Sterility
CA XIV	Brain, liver, eye, skeletal muscle	Epilepsy, retinopathies

Classical carbonic anhydrase inhibitors

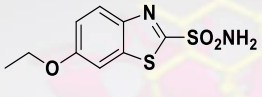
- **Benzene sulphonamide** based compounds are most potent and most utilized among carbonic anhydrase inhibitor classes



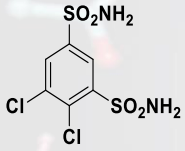
Acetazolamide



Methazolamide

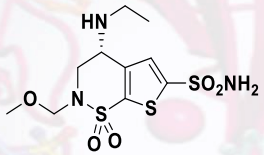


Ethoxzolamide

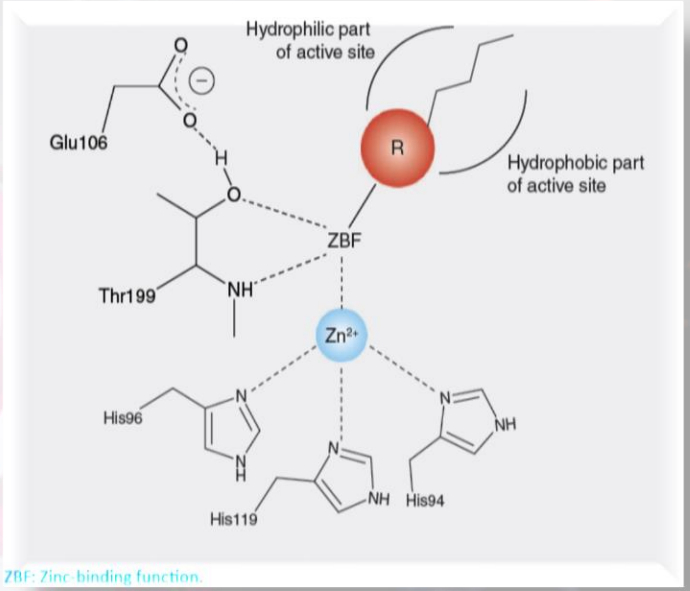


Dichlorophenamide

Dorzolamide



Brinzolamide



ZBF: Zinc-binding function.

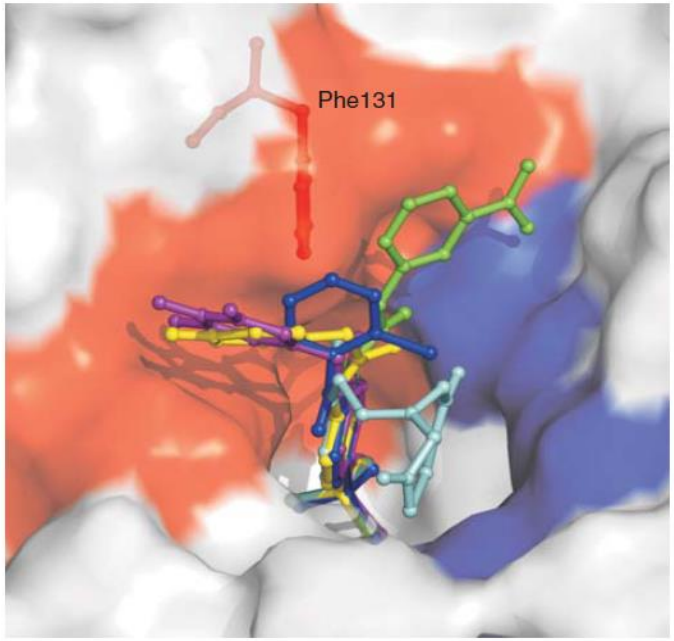
- These compounds bind to zinc ion via sulphonamide as Zinc binding group (ZBG) in deprotonated form displacing zinc bound water/hydroxide molecule.

- For all catalytic isoforms, three histidine residues coordinating the zinc**, Thr199 and Glu106** are conserved. Both T199 and E106 play a crucial role in catalysis

- Small molecular weight carbonic anhydrase inhibitors utilize ZBG tend to bind deep inside the active site cavity **make extensive interactions with amino acid residues** thus contributing **indiscriminate inhibition profiles.** Therefore alternative approaches have been developed for better isoform specific carbonic anhydrase inhibitors

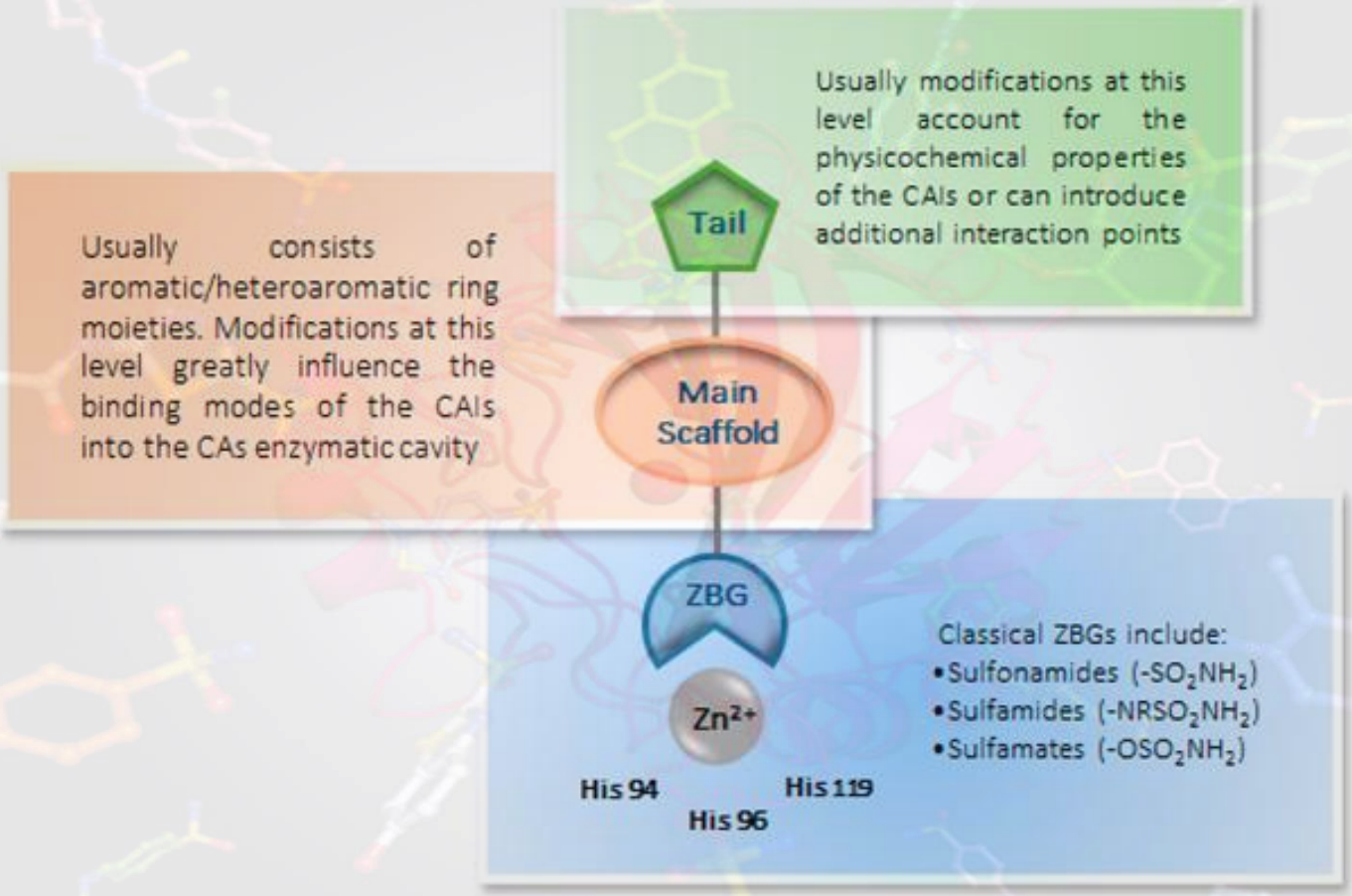
"Tail approach" in designing isoform specific carbonic anhydrase inhibitors

- It is one of the most successful approach in designing isoform specific CAIs. In this approach a chemical moiety known as tail appended on to a organic scaffold (usually aromatic/Heterocyclic) of a ZBG
- This tail elongates the inhibitor allowing it to make extensive interactions with amino acids towards outside of the active site, mainly at hydrophobic and hydrophilic halves.



Structural superposition of Tails of different compounds towards hydrophobic(red) and hydrophilic(blue) halves.

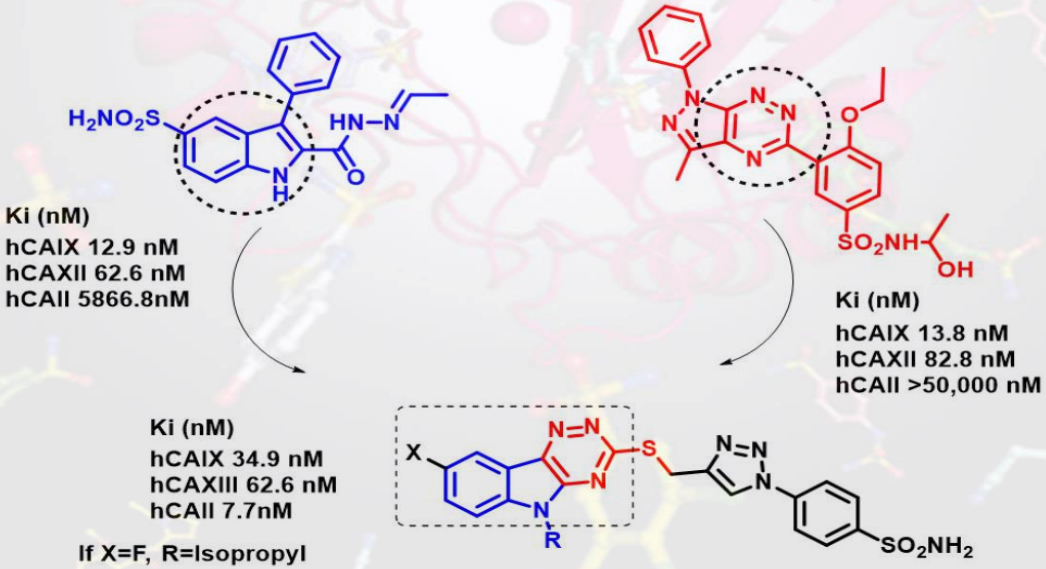
Expert Opin. Drug Discov. (2013) 8(7)



Bioorganic & Medicinal Chemistry 22 (2014) 334-340.

In the present work, new series of compounds were designed based on Tail approach via the fusion of **indole scaffold** with **1,2,4-triazine** which were reported as potent scaffolds for carbonic anhydrase activity.

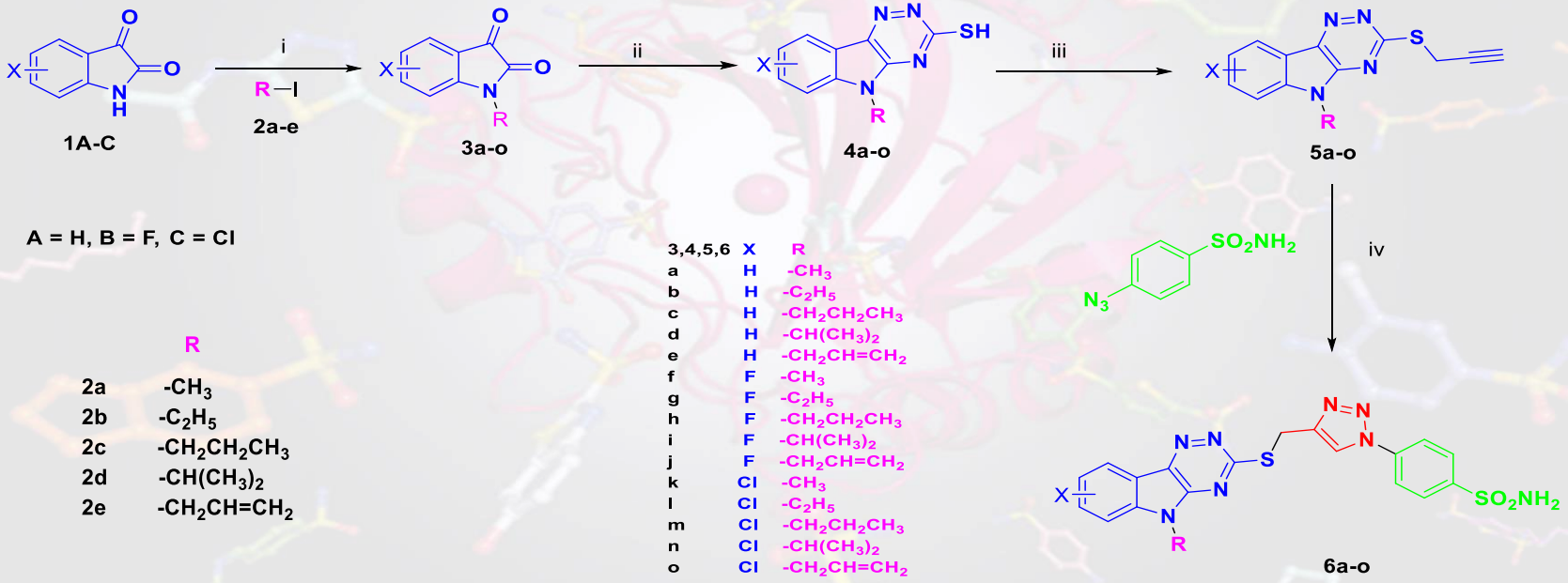
The present design of compounds based on two strategies, The first one was fusion of two potent Scaffolds i.e. Indole & 1,2,4-triazine in order to develop a flexible tail with better interactions in the Enzymatic active site and the second one was to incorporate different N-alkyl substituents in the Indole tail in systematic fashion to enhance hydrophobic interactions.



Results and Discussion

The current Design of experiment (DOE) based on molecular hybridization approach

We synthesized molecular hybrids of bulky triazno[5,6-b] indole, used as a tail, conjugated to Benzene sulfonamide through a flexible 1,2,3-triazole as al inker.



Scheme:1 Synthesis of target sulfonamides 6a-o; Reagents and conditions: (i) K_2CO_3, KI(0.05 mole%), DMF, reflux, 4-6h, Yield: 72-75%, (ii) Thiosemicarbazide, Cs_2CO_3, 1,4-dioxane, reflux, Overnight, Yield: 68-70% (iii) Propargylbromide, K_2CO_3, DMF, rt, Overnight, Yield 86-90% (iv) $CuSO_4.5H_2O$, Sodiumascorbate, tBuOH:H_2O(1:1), 60°C,Overnight, Yield: 65-70%.

Carbonic anhydrase Inhibition

The newly synthesized 1,2,3-triazole linked triazino[5,6-b]indole-benzene sulfonamide hybrids (**6a–6o**) were evaluated for their carbonic anhydrase inhibitory activity against a panel of carbonic anhydrases, i.e., hCA I, hCA II, hCA IX, and hCA XIII, by the stopped-flow CO_2 hydrase assay method.

Highly purified CA isoforms were employed, for which the kinetic parameters for the physiologic reaction (CO_2 hydration) were measured , monitoring the color change produced by the formation of H^+ ions (and bicarbonate).

For all the pure enzymes, the kinetic parameters (k_{cat} and k_{cat}/K_M) are measured and these values are given in the below table. These activities were highly inhibited by the clinically used sulfonamide inhibitor acetazolamide (AAZ), as shown in table below. It was observe that all these enzymes are highly efficient catalysts with $k_{cat}/K_M > 10^7\ M^{-1}x\ s^{-1}$.

Organisms	CA Class	Acronym	K_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1} \times s^{-1}$)	K_I (Acetazolamide) (nM)
Homo sapiens	α	hCA I	2.0×10^5	5.0×10^7	250
	α	hCA II	1.4×10^6	1.5×10^8	12.1
	α	hCA IX [a]	3.8×10^5	5.5×10^7	25.8
	α	hCA_XIII	1.5×10^5	1.1×10^7	17.0

[a] Catalytic domain.

Inhibition of hCA isoforms I, II, IX & XIII by the compounds 6a to 6o

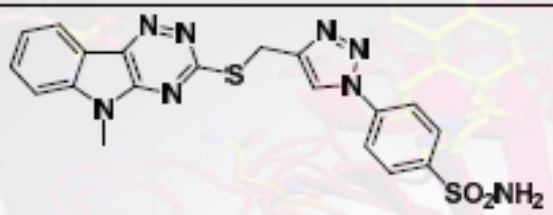
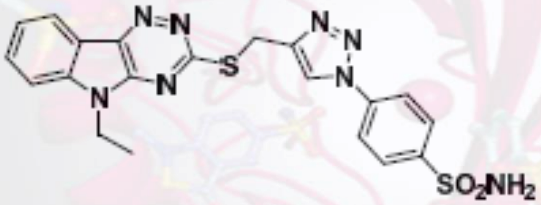
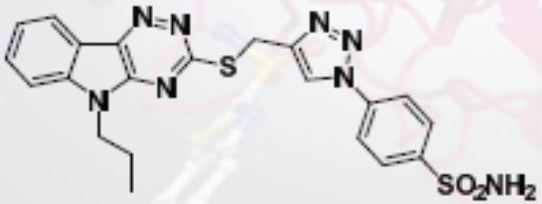
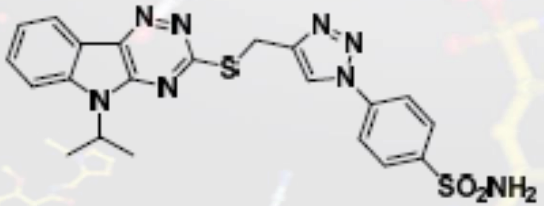
Compound	Structure	K_I (nM) hCA I	hCA II	hCA IX	hCA XIII
6a		910.1	65.5	285.6	77.8
6b		642.2	72.0	172.5	69.8
6c		3960	88.7	219.4	364.8
6d		314.7	20.9	37.8	65.8

Table 3. *Cont.*

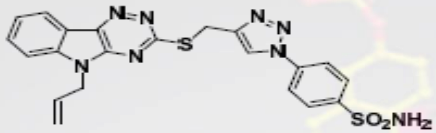
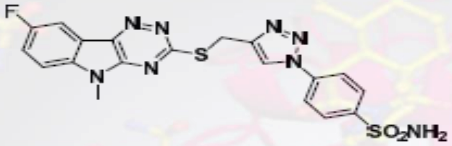
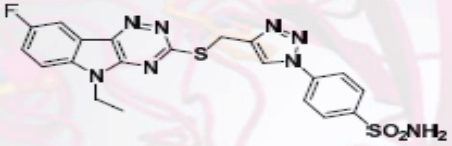
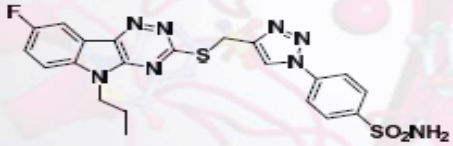
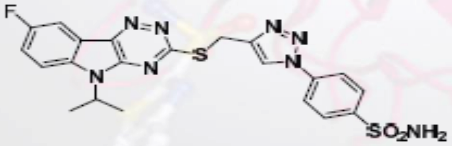
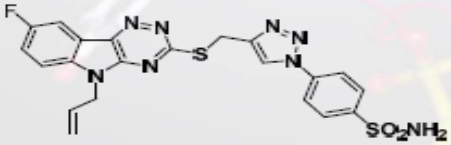
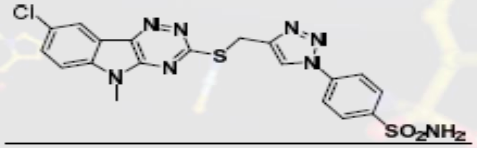
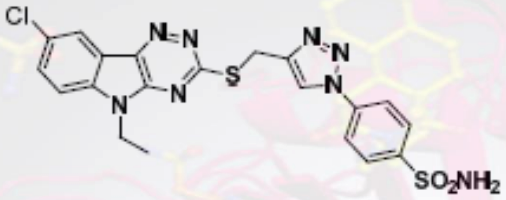
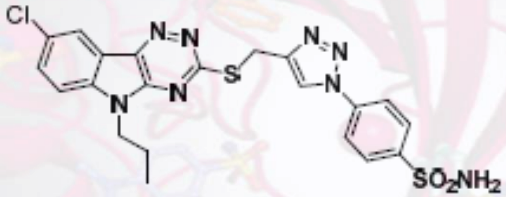
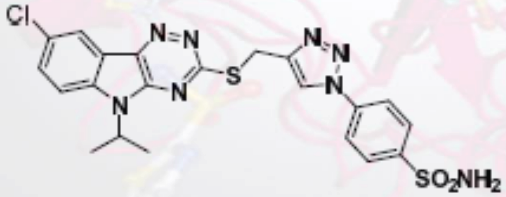
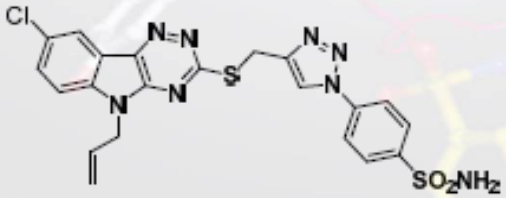
Compound	Structure	K_I (nM) hCA I	hCA II	hCA IX	hCA XIII
6e		535.8	41.7	41.1	626.7
6f		766.2	59.6	41.3	834.8
6g		698.5	63.9	193.1	675.0
6h		764.2	682.7	118.6	1815
6i		379.2	7.7	34.9	736.2
6j		4592	73.7	401.7	793.6
6k		5140	252.7	330.7	867.0

Table 3. *Cont.*

Compound	Structure	K_I (nM) hCA I	hCA II	hCA IX	hCA XIII
6l		2837	184.6	150.4	3980
6m		6513	61.7	204.5	823.8
6n		571.9	179.2	320.8	91.3
6o		694.4	97.1	324.6	2300
AAZ		250.0	12.1	25.8	17.0

Structural activity relationships

- The cytosolic hCA II isoform was strongly inhibited by all the synthesized compounds **6a–o**, in a low to medium nanomolar range (K_is = 7.7 nM to 0.2527 µM).

- The best activity against hCA II was shown by compound **6i** (K_i = 7.7 nM), possessing a fluoro group attached at the 5th position of the indole ring and an isopropyl group anchored to the nitrogen of indole. It was almost twofold more active than the standard AAZ (K_i = 12.1 nM).

- Compounds **6d–6g**, were found to have potent activity at the nanomolar concentration against hCA II, with K_i ranging from 20.9 to 63.9 nM.

- Compounds **6k–6o**, containing a chloro group at the 5th position of indole, showed lower activity in the range of 61.7 to 252.7 nM, compared to compounds containing a fluoro group and unsubstituted indole.

Continue….

- The transmembrane hCA IX isoform, which is expressed exclusively in tumors, was also strongly inhibited by the synthesized compounds in the medium nanomolar range (K_is = 34.9 nM to 0.3246 µM).

- Compounds **6d**, **6e**, **6f**, and **6i** showed equipotent nanomolar activity with AAZ, with K_is ranging from 34.9 nM to 41.3 nM. Among these compounds, **6i** showed the best activity (K_i = 34.9 nM) against hCA IX isoform.

- The cytosolic hCA I and hCA XIII isoforms were inhibited by all synthesized compounds in the high nanomolar range (K_is > 500 nM). However, compounds **6b** and **6d** showed moderate activity with K_is of 69.8 nM and 65.8 nM respectively against hCA XIII isoform.

From the above structure–activity relationship, it was found that compound 6i was the most potent compound with a K_i values of 7.7 nM against hCAII and 34.9 nM against hCA IX.

Conclusions

- In conclusion, we report here the synthesis of a series of 1,2,3-triazole-linked triazino[5,6-b]indole-benzene sulfonamide hybrids (**6a–6o**).

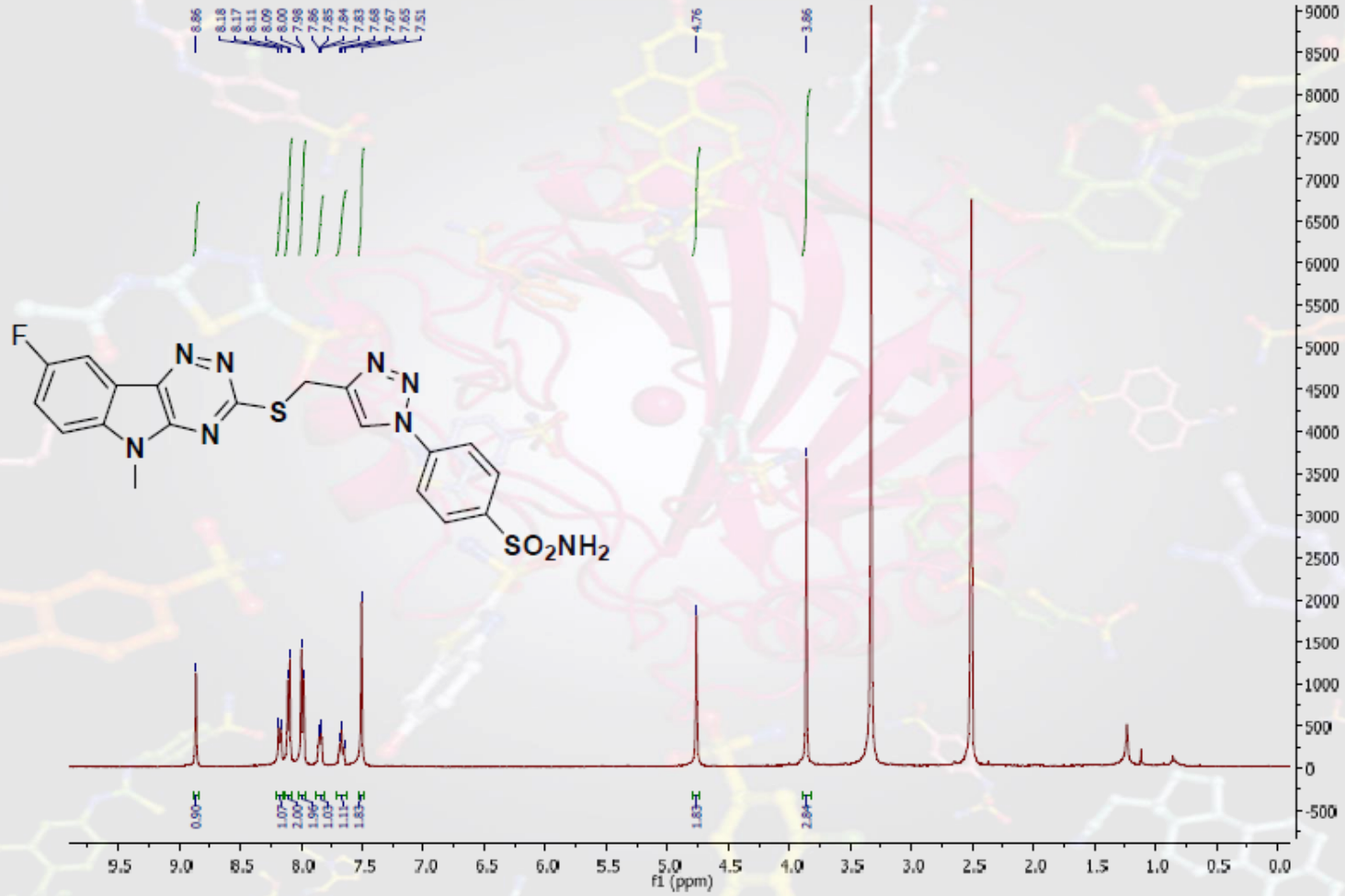
- The structures of these compounds where confirmed by different spectral and elemental analyses methods .

- The Biological evaluation of sulfonamides was performed against hCA I, hCA II, hCA IX, and hCA XIII. All compounds showed low to moderate inhibitory activity against hCA II and hCA IX isoforms, at concentrations in the range between 7.7 nM and 0.3246 µM.

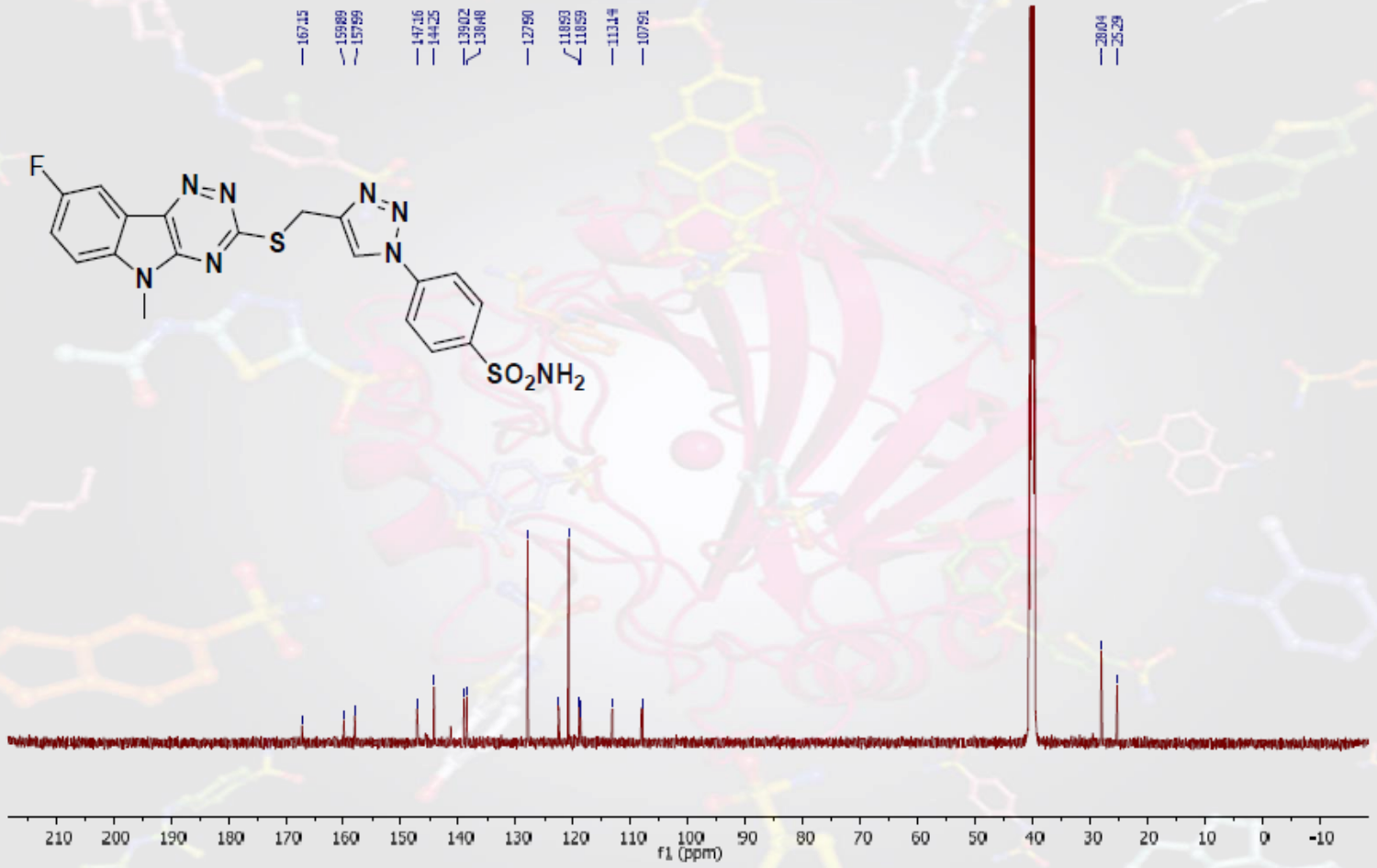
- Compound **6i** emerged as a potent hCA II and hCA IX inhibitor (K_i = 7.7 nM against hCA II and 34.9 nM against hCA IX).

- The compounds **6b** and **6d** was showed activity at medium nanomolar concentrations, with K_i of 69.8 nM and 65.8 nM, respectively, against hCA XIII isoform. Thus, the compound **6i** can be emerged as a novel potential lead compound to develop selective carbonic anhydrase inhibitors against the hCA II isoform.

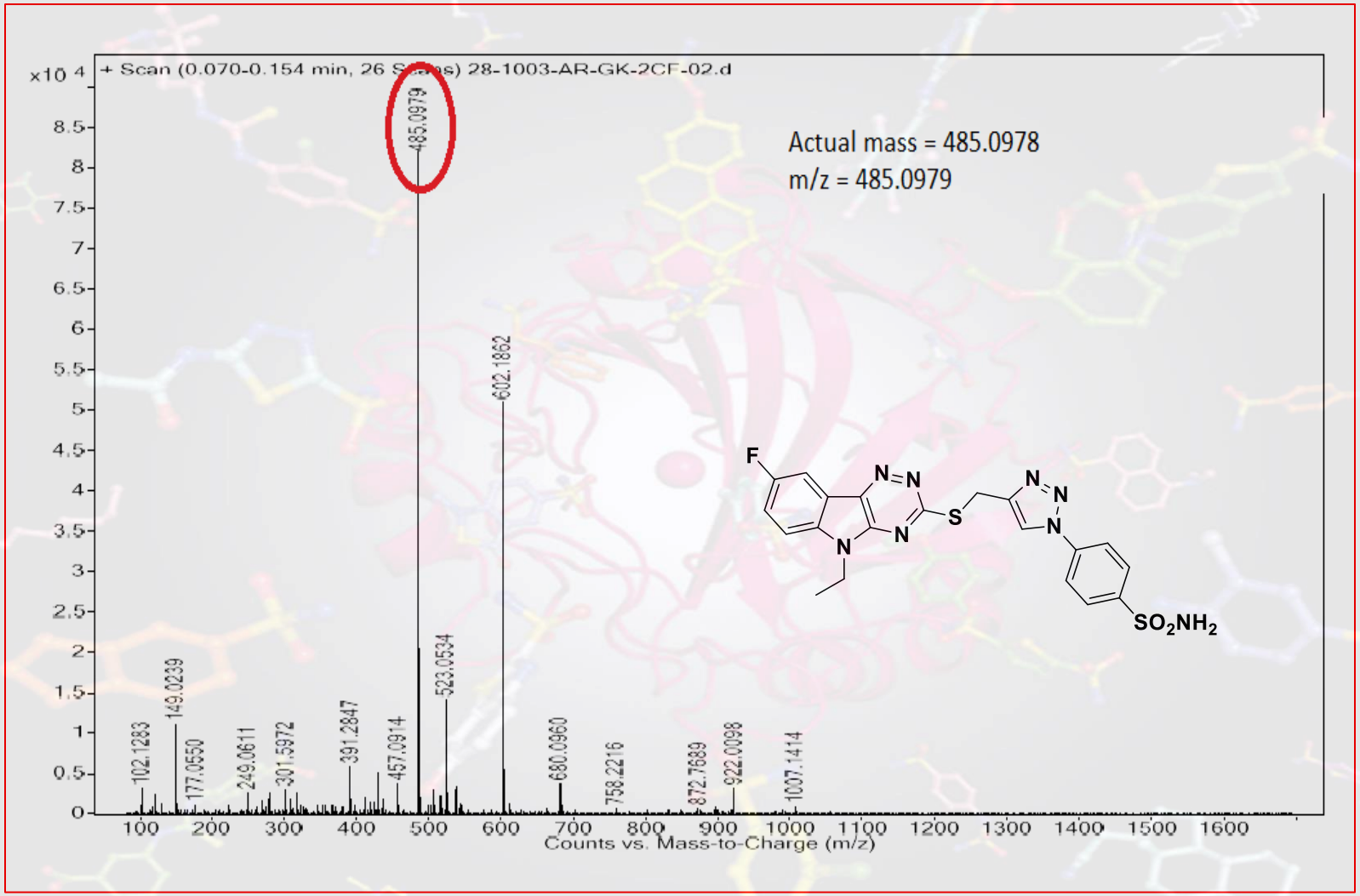
H1 NMR & C13 Spectra of some compounds



1H NMR spectra of 6f



13C NMR spectra of 6f



Mass spectra of compound 6g

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Thank You