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Review Article

A review on Carbonic Anhydrase IX and XII Inhibitors

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Abstract

Carbonic Anhydrases are metalloenzymes that catalyse the carbon di-oxide hydration/ dehydration reaction. Crucial biological processes in most organisms are related to such a reversible reaction viz. respiration, photosynthesis, pH regulation, CO2 and HCO3 transportation etc. Excess production of CA IX and CA XII are fraught with production of malignant tumours and, therefore, the two varieties need selective inhibition. Indiscriminate inhibition leads to suppression of those varieties also that are required for metabolic activities of organisms. Isoform specific CA inhibition is a priority of research in this direction.

Key words: carbonic anhydrases; cytosolic proteins; hypoxic tumours; off-target inhibition

Introduction

Carbonic Anhydrases [CAs,EC 4.2.1.1] are a super family of metalloenzymes that catalyse the Carbon di oxide hydration / dehydration reaction which is of utmost importance amongst living beings. The hydration / dehydration reaction goes on in absence of the metalloenzyme catalyst also but the rate of reaction is not enough so as to support the physiological requirements of organisms. Carbonic Anhydrases possess Zn (II) ion which is coordinated by three histidine residues (His_{94}, His_{96} and His_{119}) and a water molecule at their active sites, which acts as the nucleophile required for the catalytic activity. [1-6]. These enzymes play important role in many physiological andpatological processes like transport of carbon dioxide/ bicarbonate ion pH homeostatis, respiration, electrolytic secretion in various tissues, gluconeogenesis, lipogenesis, ureagenesis and development of tumours [6-9]. CAs I and II play roles in many normal and routine physiological activities of organisms such as maintaining equilibrium between acid and base. Development of many drugs with different pharmacological properties like diuretic [10], antiglaucoma [11], antiepileptic [12] and antiobesity [13] are the result of inhibition of these two isoforms. Expression of CAIX and CAXII is restricted in normal tissues as these two isoforms are mainly detected in tumour cells and induced by tumour hypoxia. This is why these membranes bound isozymes have become targets for antitumour activity. [14-18]. Carbonic Anhydrases are classified into seven genetically different families namely, α, β, γ, δ,ζ, η and θ [19-21]. All human (h) Carbonic Anhydrases are α class enzymes [21]. Fifteen different isoforms of human Carbonic Anhydrases have been identified and characterized, so far. Of these, twelve are catalytically active viz. hCAs I- IV, VA, VB, VI, VII, IX, XII-XIV. Human Carbonic Anhydrases can be further divided into four subsets depending upon their location in the cell organnels. Among these, identified as hCA I, II, III, VII, VIII, X, XI, XIII are cytosolic proteins, hCA VA and VB are present in the mitochondria, hCA VI is a secreted enzyme, hCA IV is a glycosylphosphatidylinositol (GPI) – anchored protein and hCA IX, XII and XIV are trans -membrane protein isoforms [19,21]. These enzymes are present in different organs and tissues and are responsible for very important physiological functions. Dysregulated expression or abnormal activity of hCAs can transform into pathological conditions [19, 22-25]. The hCA IX and hCA XII, two trans-membrane isoforms, are found overexpressed in tumour cells. In turn, it aids to cancer progression, metastatis and also impairs certain therapeutic responses. Both these isoforms have been used as makers of the disease progression in many hypoxic tumours and their inhibition is related to the reduction of growth of both the primary tumours and metastases. The validation of hCAIX has been done as an imaging agent for hypoxic as well as metastatic hypoxic tumours. hCA XII has also been found predominantly expressed in a variety of cancers. It has been reported that hCAXII expression can be correlated to the type of cancer.

Inhibition of Carbonic Anhydrases has clinical importance for treatment of a variety of diseases like glaucoma, epilepsy, obesity and gastric ulcers etc. Studies on the subject have shown that CA inhibition has an important role in carcinogenesis and metastatis. The selectivity of clinically used inhibitors designed for various isoforms is important for targeting a certain disease without undesired effects. During past few years, search for isoform specific CA inhibitors has gained momentum as off-target inhibition has been found associated with undesired effects. The off-target

inhibition is brought about by the *classical* inhibitors like sulphonamides.The sulphonamide group interacts with the zinc ion in the active site of the CA enzyme and is the main pharmacophoric group for studies related to CA inhibition. No doubt, sulphonamide derivatives with aromatic or hetero- aromatic rings are strong inhibitors. Non-selective primary sulphonamides like acetazolamide (AAZ) have been in use for treatment of glaucoma on regular basis for more than sixty years. Second generation antiglaucoma drugs viz. dorzolamide and brinzolamide are highly selective CAII inhibitors. They are used for topical application with side effects reduced substantially.

The CA II is an important target in the treatment of glaucoma. CA inhibitors with high selectivity of CAII and having strong inhibition potency are focus of interest in the development of new antiglaucoma medicines. 4-sulfamoylphenylthiourea derivatives carrying amines, amino acids and oligopeptides have been reported to have hCA I and IV isoenzymes inhibitory properties. The water-soluble derivatives incorporating hydroxyl and mercapto amino acids displayed, in particular, selective inhibition in the low nanomolar range. The compounds with balanced hydrophilic and lipophilic properties also showed in vivo interesting pharmacological properties. These finding lead to the clue that the 4-sulfamoylphenyl derivative selective hCA II inhibitors may be good candidates for developing new antiglaucoma medicines with the least or no ocular side effects. It is reported that cyanoguanidine moiety has been used as a bioisosteric group to reduce side effects and improve pharmacological and pharmacokinetic properties. The cynoguanine derivatives containing a 4-sulfamoylphenyl moiety showed potent inhibition of hCA II isoenzyme in low nanomolar range as well as high selectivity for hCA II isozyme over hCA I, IV, and IX isoenzymes.

Many classes of *non-classical* inhibitors have been developed and studied during past few years and Coumarins are one such important class [26]. Coumarins are a type of heterocyclic compounds and occupy place of a privileged scaffold in medicinal chemistry. These are widely distributed in nature and possess a variety of pharmacological activities like antibacterial, anticonvulsant, antifungal, antihyperglycemic, anticancer, inhibitors of enzymes. In addition, Coumarins have been found to be potent and highly selective CA IX and CA XII inhibitors. These have been found to inhibit CAs not by binding to the zinc metal in the active site cavity but by occlusion of the active site entrance. Thus, the binding mechanism being different, Coumarins are categorised as *non-classical* CA inhibitors. Coumarin linked 1,2,4-oxadiazoles are still more selective inhibitors of CA IX and CA XII. The five membered heterocycle, 1,2,4-oxadiazole is an important scaffold in medicinal chemistry and acts as bio isostere for amides and carboxylic acids. It is reported to have diverse activities like anthelmintic, antileishmanial, antitrypanosomial, antispasmodic, anti-tussive, analgesic and anti-inflammatory. In the light of selectivity of coumarins for hCAIX and XII and to explore 1,2,4-oxadiazoles for CA inhibition, it was decided to club both the heterocycles and synthesize coumarin-1,2,4-oxadiazole hybrid. The synthesized compounds, having various substituents at the phenyl ring, were evaluated against four physiologically and pharmacologically relevant isoforms, hCAI, hCAII, hCAIX and hCAXII with Acetazolamide (AAZ) taken as the standard. The results were as under with regard to the structure-activity relationship of synthesized compounds.

1.The two cytosolic isoforms, CAI and CAII were not at all inhibited by the synthesized compounds (Ki > 10000 nM)

2. The tumour associated isoform CAIX was inhibited in a low to moderate nano molar range with the Ki values in the range of 23.6 to 315.6 nM. The best inhibition was shown by the compound having a methoxy group in para position of the phenyl ring located at the 5th position of 1,2,4-oxadiazole ring. (Ki 23.6 nM).

3. The other tumour related isoform, hCAXII was inhibited by synthesized compounds in a low to moderate nano molar range. The best inhibition was achieved with the compound having a tertiary butyl group in the para position of the phenyl ring located at the 5th position of the 1,2,4-oxadiazole ring. It showed a Ki value of 1.00 nM which is 5.7 times more than that of the standard compound, AAZ.

There are many more which fall under this category viz. Sulfocoumarins (1,2-Bezoxathiine-2,2-dioxides), coumarin inspired sulphonamide derivatives, N- substituted saccharin derivatives and the latest one being the 1H-indole-2,3-dione 3-thiosemicarbazones with 3-sulfamoylphenyl moiety as reported by C.T.Supuran and co- workers vide a paper accepted for publication by the journal, Arch Pharma DPhG on April 1, 2022.

Sulfocoumarins i.e., 1,2-bezoxathiine 2,2-dioxides have been investigated for their inhibitory activity against for human (h) CA isoforms viz. the cytosolic and wide spread hCAI and II (off-target isoforms) as well as the transmembrane and tumour associated hCAIX and XII (anti-cancer drug targets). It has been found that they have a similar mechanism of CA inhibition as the Coumarins and are effective inhibitors of this enzyme. The sulfocoumarins were hydrolysed by the esterase CA ativity to 2-hydroxyphenyl vinyl sulfonic acids which later bound within the enzyme active site in a region rarely occupied by other classes of inhibitors such as sulphonamides or dithiocarbamates which are Zn (II) ion binders. The X-ray crystal structure of one of the investigated 6-hydroxy-1,2-benzoxathiine 2,2-dioxides in the adduct with a modified CAII enzyme, having two amino acid residues from the CAIX active site, helped in working out the mechanism of inhibition. The working group observed that vinylsulfonic acid formed by the hydrolysis of the sulfocoumarin was anchored to the zinc –coordinated water molecule making favourable interactions with Thr200 and Pro201. Simple coumarins were not found effective against hCAI and hCAII, these being micromolar inhibitors of the same. Compounds with 1,2,3-triazole moieties showed nanomolar inhibitory action against tumour associated hCAIX and hCAXII and proved less effective against cytosolic isoforms, hCAI and II. Thus, sulfocoumarins like coumarins are effective and isoform selective class of inhibitors targeting tumour associated isoforms, CAIX and CAXII.

The most common approach to design small molecules targeting Carbonic Anhydrases is to insert zinc binding moiety into the structure of the inhibitor. The sulphonamide group is one of the most important and widely used such moiety.[27]. It was earlier reported through crystallographic and molecular modelling studies that the primary sulphonamide derivatives bind to the catalytic zinc ion in the active site of the Carbonic Anhydrase in their deprotonated form. Later, it was also demonstrated that secondary and even tertiary sulphonamides can selectively inhibit the cancer related CAIX and XII isoforms. The group of scientists, Melissa D'Ascenzio, Simone Carradori, Celeste De Monte, Daniela Secci, Mariangela Ceruco and Claudiu T. Supuran chose N-substituted derivatives of saccharin to obtain tertiary sulphonamide derivatives and investigated them for their ability to inhibit four human CA isoforms viz. the two cancer related isoforms, CAIX and XII and the most common off- targets of antitumoral CAIs, CA I and II. All investigated compounds showed no affinity for CAI while all of them inhibited CAXII in low micromolar/ nanomolar range. Some of the new compounds synthesized were not active as CAI and II inhibitors, showing effective inhibition of CAXII. The results obtained with these compounds have put up a promising start for the development of new selective inhibitors of this less investigated isoform of CA as novel anticancer agents based on toxicologically safe scaffold of saccharin.

Curcumin inspired sulphonamide derivatives have been investigated and found to act as CA inhibitors of isoforms, I, II, IX, XII. The investigating group comprised scientists from NIPER, Hyderabad, India and Claudiu T.Supuran of Italy. In view of lack of selectivity and side effects with existing drugs, exhaustive search for superior CAIs is an ongoing process. The focus is on synthesis of new derivatives of existing drugs or finding new molecular bases including natural products. Natural products such as resveratrol, catechin, silymarin, dobutamin and curcumin are found to possess CA inhibitory activity. Curcumin is the main ingredient of the

popular Indian spice turmeric (*Curcuma longa*) and reported to have neuroprotective properties, which may be effective in treatment of glaucoma. Curcumin and its analogues and 4'-(phenylurenyl) chalcones have been found to be Carbonic Anhydrase inhibitors [28-29]. The group prepared various derivatives of curcumin containing sulphonamides checked them for their purity and tested *in vitro* for their activity against physiologically relevant hCA isoforms, I, II, IX and XII by means of stopped flow carbon di-oxide hydration assay and their activities were compared to the standard CA inhibitor, acetazolamide (AAZ). Interesting inhibitory activities were found against all these isoforms. These synthetic compounds inhibited hCAI (found responsible for some eye diseases) moderately with Kis in the range of 191.8 to 904.2 nM, hCAII (an antiglaucoma drug target) very potently with Kis in the range of 0.75 to 8.8 nM, hCAIX (an isoform related to cancer) significantly with Kis in the range of 2.3 to 87.3 nM and hCAXII (antiglaucoma and anticancer drug target) with Kis in the range of 6.1 to 71.8 nM.

The most common approach to design small molecules for targeting this family of metalloenzymes is to insert zinc binding moieties into the structure of the inhibitor. The sulphonamide group is one such important and widely used moiety. Studies show that the primary sulphonamide derivatives bind to the catalytic zinc ion in the active site of Carbonic Anhydrase in its deprotonated form. However, it has been demonstrated that even secondary and tertiary sulphonamides also inhibit selectively the cancer related isoforms, CA IX and CA XII. The group consisting of Melissa D'Ascenzio, Simone Carradori, C.T.Supran and others have worked on this project and reported their findings in the journal, Bioorganic & Medicinal Chemistry {22 (2014) 1821-1831 }.

The group prepared a series of tertiary sulphonamides by functionalizing nitrogen atom of saccharin, a well-known sweetener, characterized by a safe toxicological profile and a peculiar ability of inhibiting Carbonic Anhydrse isoforms in high nanomolar/low micromolar range. The group synthesized a number of compounds in which the nitrogen atom saccharin was converted to tertiary N atom substituted by a variety of groups/ moieties. The compounds synthesized were tested for identification and purity. Then, these were evaluated for their biological activity against tumour related CA IX and CA XII and corresponding off-target analogues, CA I and CA II. For this purpose, a photophysics stopped – flow instrument was pressed in service for assaying Carbonic Anhydrase catalyzed CO_2 hydration reaction. Phenol red in 0.2mM concentration was used as indicator, working at absorbance maxima 557 nm, with20 nM Hepes (pH 7.5, for α-CAs) as buffer and 20 mM Sodium Chlorate for maintaining constant ionic strength. The reaction time was chosen from 10 to 100 seconds. For determining kinetic parameters and inhibition constants, the CO_2 concentration was kept 1.7 mM to 17 mM. The rates of uncatalyzed reactions were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor of 1μM concentration were prepared in distilled and deionised water and diluted to 0.1nM concentration with the assay buffer. Both inhibitor and enzyme solutions were preincubated together at room temperature for 15 minutes prior to assay to allow formation of the E1 complex or for the active site mediated hydrolysis of the inhibitor. The inhibition constants were worked out by **non-linear least squares method using PRISM 3 and Cheng-Prusoff equation.The group's findings were as follows.**

1. All of the compounds tested were found inactive (K_i >50 μM) against hCA I, the common off-target isoform of anticancer CAIs. The hCA I is wide spread in the body and is found in high concentration in blood and the gastro-intestinal tract. The hCA II isoform was also poorly inhibited (K_i > 50μM) by most of the compounds tried in the experiment.
2. All the compounds investigated showed inhibition of hCA XII in low micromolar/ nanomalar range with the exception of three compounds designated as 11a,PH010918 and S763217 which were not active at concentrations higher than 50 μM. Introduction of small unsaturated carbon chains on the sulphonamide nitrogen of saccharin showed an increase of activity of the obtained compounds against CA XII though no selectivity was obtained against the off-target isoform CA II. The compound with substituent R of four carbons with double bond between second and third Carbon atoms was found to be more active against CA II (K_i = 36.7 nM) than against the cancer related isoforms CA IX and XII (K_i =97 nM and 0.21 μM, respectively).When the unsaturated substituent was replaced by a cyano group, the derivative produced showed a slight loss of activity against CA IX and XII (K_i =0.29 μM and 0.71 μM, respectively) with a gain of selectivity (K_i > 50 μM towards CA I and II). Introduction of a three-methylene long spacer between the sulfonamidic nucleus of saccharin and the cyano group, however, caused loss of activity against CA XII (Ki > 50 μM) and selectivity against CA II (Ki =1.26 μM). The group of workers also prepared saccharin derivatives having electron poor benzyl groups at the sulfonamidic nitrogen. Of these compounds, those in which the aromatic ring was substituted with an ortho/ meta nitro group or bromine atom showed the highest activity against CA XII (21.1 nM < Ki <28.1 nM) in spite of their low selectivity (39.1 nM < Ki < 86.8 nM towards CA II). Moreover, compounds containing $–NO_2$ in ortho or para position of the benzyl ring were found to be good inhibitors of the other Cancer related isoform, CA IX (Ki = 91 nM and 11 nM, respectively). However, the exchange of bromine with fluorine or chlorine on the benzyl ring showed a significant loss of the obtained compounds against both CA IX and XII. The substitution by bulky groups like naphthyl or phthalyl groups had a similar effect, though these compounds showed a promising selectivity against CA XII with reference to all of the other examined isoforms: CA I, II, and IX (Ki >50 μM), while compound with benzyl ring having Cl atoms at 3,4 positions inhibited both CA IX and XII. A similar effect was noticed on introducing an extra carbonyl group between the methylene and the aromatic ring of the benzyl group. Benzoyl derivatives were found to inhibit CA XII only in low micro molecular range (Ki =1.78 μM, 0.97 μM and 2.01 μM,for the three derivatives prepared,respectively) and were inactive towards CA I, II and IX at concentrations higher than 50 μM.

However, the best results were obtained with regard to activity and selectivity with the ethyl carboxylic ester as R in the saccharin molecule. Its hydrolysed product, the acid, inhibited CA XII isoform in nanomolar concentrations (Ki =76.5 nM and 41.9 nM respectively) with the free carboxylic group containing derivative being the most selective compound in the whole series. It is for the well-known hydrolytic activity of some CA isoenzymes that the ethyl carboxylic ester derivative could be considered a prodrug. However, conversion of the free acid to the N-methylamide did not cause any loss of activity against CA XII (Ki =28.4 nM) though it significant loss of selectivity towards CA II. As against this, the change of the carboxylic group to carbonyl or an oxime caused a switch of selectivity towards CA II.

3. The tumour related isoform hCA IX was inhibited by some of the saccharin derivatives prepared by the study group and some of these were also found to be inactive. These were compounds with R as benzene ring, and having some substituents on it (Ki =50 μM). The derivative 9, having R with NO_2 group attached in meta position, was found to be effective hCA IX inhibitor: inhibition constant ,11 nM (better than acetazolamide). Other derivatives were found to be inhibitors of medium potency, with inhibition constants between 91 and 400 nM. Modifications in the structures of these inhibitors brought about drastic changes in their hCA IX inhibition power viz. regioisomers having $NO_{2\ group}$ in ortho and meta positions of benzene ring of R, the substituent on the saccharin molecule,

differed in their affinity for hCA IX by a factor of 8.3. The same kind of behaviour was observed for regioisomers with bromine atom as substituent on the benzene ring in place of NO_2.

4. N-substitution vs. O-substitution of saccharin: Alkylation of saccharin under alkaline conditions is known to take place at both the nitrogen and oxygen atoms of the lactam ring, giving a mixture product [30]. However, derivatization products generally reported for saccharin are all N-alkylated products, which are believed too more stable thermodynamically. It was observed by this study group that only N-alkylated products are able to inhibit the four isoforms of CAs under consideration. (The Ki values for O-alkylated products in case of all four isoforms viz. hCA I, II, IX, XII were above 50000 nM.)

Based on the above observations, it can be concluded that none of the compounds under investigation showed affinity for CA I while they inhibited CA XII in low micromolar/ nanomolar quantities. Some of the new compounds were not active as CA I and II inhibitors but showing effective inhibition of CA XII. This group of compounds is a promising lot for developing new and selective inhibitors of CAs as novel anticancer agents based on toxicologically safe frame of saccharin molecule. The latter was discovered in 1879 by Constantin Fahlberg in the laboratory of Ira Remsen at Johns Hopkins university, USA. It is about 600 times as sweet as sucrose. It has no food value and is used as sweetening agent by diabetic patients.

Cabonic Anhydrase Inhibitors (CAIs) can be classified into different groups based on their binding mode to the enzyme active site and zinc binders are most effective and also most investigated for drug design purpose. Within this sub-class, sulphonamides are the ideal zinc binding group due to a peculiar combination of interactions this moiety can establish with the zinc ion and the residues nearby. Amongst others, pyrazoline sulphonamides have also been reported as CA inhibitors as well as inhibitors of AchE. Replacement of sulphonamide by sulfamate group, a cogener of sulphonamide, presented interesting examples of selective inhibitors of CAs. These considerations led the group comprising Davide Moi, Alessio Nocentini, Alessandro Deplano, Gianfranco Balboni, Claudiu T. Supuran and Valentina Onnis to work on the prospective CA inhibitors of this class of compounds. The group also carried out full Structure Activity relationship of these new-found CAIs.

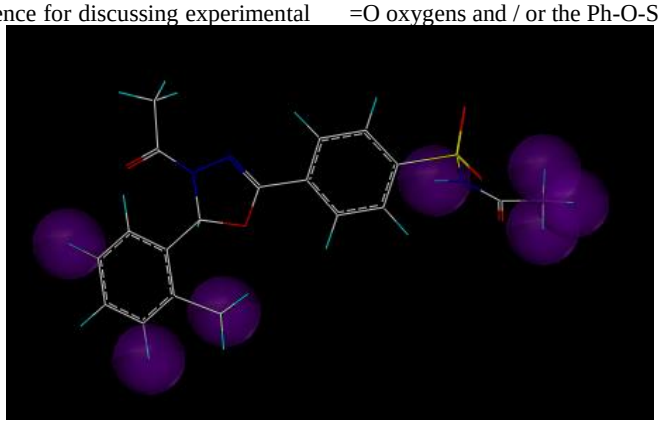
The inhibitory strength of sulfamate derivatives was tested by the above group who first synthesized sulfamates and established their identity and structure.For elucidation of structures of various sulfamates , they recorded NMR spectra on an Inova 500 spectrometer (Varian, Palo Alto, CA, USA).The chemical shifts (δ) were recrded on parts per million downfield from tetramethylsilane (TMS)used as internal standard. The spectra were recorded in hexadeuteriodimethylsulphoxide (DMSO-d_6). IR spectra were recorded on a Vector 22 spectrometer (Bruker, Bremen, Germany) in Nujol mulls. IR bands were recorded in cm^{-1}. Positive- ion electrospray ionization (ESI) mass spectra were recorded on double – focusing MAT 95 instrument with BE geometry. Melting Points were determined on SMP 1 Melting Point apparatus (Stuart Scientific, Stone, UK) and reported as such. All products analysed showed ^1H NMR spectra in agreement with the assigned structures. The purity of the tested compounds was determined by combustion elemental analysis carried out at the Microanalytical Laboratory of the Chemistry Department of the University of Ferrara with a MT-5 CHN recoder elemental analyser (Yanagimoto, Kyoto, Japan) and the values obtained were witin 0.4% of theoretical values. Then, they used the stopped flow instrument with CO_2 hydrase assay in the presence of acetazolamide as a standard inhibitor.

Their findings have been as under.

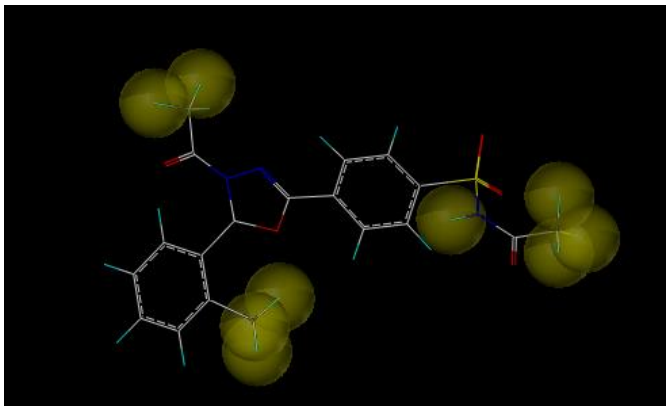
1. All pyrazoline sulfamates showed low inhibitory activity in general towards CA I except a few analogues having H, OCH_3, and F in para position of phenyl ring as substituent R of the main compound. These analogues rather, showed better inhibitory values as compared to the reference compound, acetazolamide.
2. As regards CA II isozyme, the 5- Aryl substituted analogue (8a-1) showed high activity (Ki in the 0.8-13.2 range). The compound 8e, bearing a 4-trifluoromethylphenyl ring showed best results with Ki value of 0.8 nM. Substitution of the 4-trifluoromethyl with a chlorine atom viz. compound 8i reduced the activity while displacement of the chlorine atom from position-4 to position-3 (compound 8h) restored the activity (Ki =0.87 nM). Displacement of the chlorine atom to 2-position (compound 8g) as also the introduction of two chlorine atoms (compounds 8j, 8l) led to a reduction in activity as compared to the analogue 8h. Comparison of sulfamates 8 and 9 and their respective activities showed that the displacement of the 4-sulfamate group to 3-position resulted in reduction of activity. However, analogues 9e and 9i bearing 4-trifluromethyl and 4-chlorine moieties maintained high inhibitory activity. In the 17th series of the of pyrazoline sulfamates, the activity was found related to the presence of substituents on the 3-aryl ring. The compound 17a with H atom as substituent R showed the lowest activity in the series. The best analogue was 17c having a 4-methoxyphenyl group attached to the main pyrazoline molecule. The presence of 4-methyl, 4-nitro and 2,4-dichloro substituents was favourable to the activity in the pyrazoline 17 series whereas the presence of the same groups 5-aryl of pyrazoline 8 did not increase the activity. Pyrazoline sulfamates 18diplayed high activity as compared to their analogues 9 except for 18f, where the substituent R contains one Cl atom in 4 positions. The general observation was that displacement of the 4-sulfamate group of pyrazolines 17 into3-position to give pyrazolines 18 resulted in slight reduction in activity when substituents are present on the 5-aryl ring.
3. With regard to inhibition of CA IX, the 3- chlorine derivative (8h) of the pyrazoline sulfamates put up the best show (Ki =0.72 nM) The displacement of the chlorine atom to 4-position (8i) led to a ten- fold reduction in activity. The replacement of the 4-chlorine of compound 8i with a methyl group (compound 8b) maintained the same activity while the replacement with a fluorine atom, sulfamate 8f, enhanced the activity. As against this, the presence of two chlorine atoms (8j and 8l) reduced the activity as compared to both the 8h and 8i analogues. The displacement of the 4-sulfamate group to 3-position to give the isomeric pyrazolines 9 induced similar or better activity in comparison to the corresponding analogues 8 except for three compounds,9g,9k and 9l. In the pyrazoline sulfamate 17 series the best activity was shown by the chlorine substituted compound, 17g whose Ki value was very similar to the analogue 8i as well as for the compounds 17a and 17c as compared to their analogues 8a and 8c.
4. CA XII: The pyrazoline sulfamates, 8f,8h,9c,9f and 9i confirmed their good inhibition potency against this CA isoform. Thus, the presence of a halogen atom or a trifluoromethyl group in 4-position increased the activity in the series 9 while in the series 8 the best activity was correlated to the presence of 4-fluorine or 3-chlorine atom on the 5-aryl ring. The pyrazoline 17d showed the best activity of all four series (Ki =0.88 nM). The removal of the 4-nitro group of the sulfamate, 17d or its replacement with a methoxy group led to a ten-fold reduction in activity. Pyrazoline sulfamates of the series 18 showed reduced activity as compared to both pyrazolines, 17 and 9.

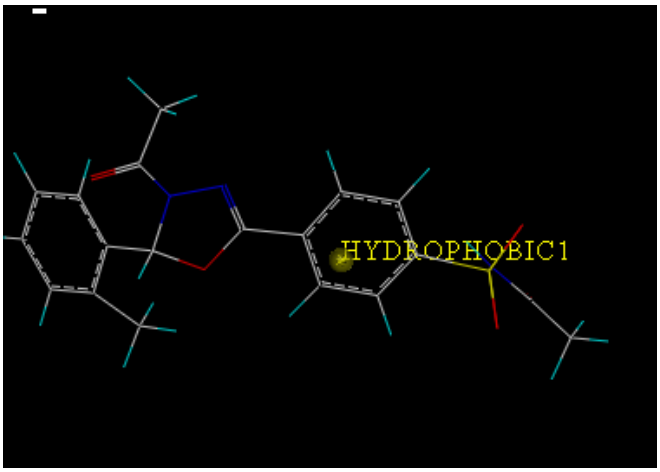
The investigating group of scientists also carried out molecular docking experiment with all the four CA isoforms to understand the binding patterns. Docking is a kind of bioinformatic modelling which involves the interaction of two or more molecules to give a stable adduct. Depending

upon binding properties of ligand and the target, it predicts the three-dimensional structure of any complex. Molecular docking generates different possible adduct structures that are ranked and grouped together using scoring function in the software. Docking simulations predict optimized docked conformer based upon total energy of the system. In the present case, the crystal structures were selected for the presence of AAZ as co-crystallized ligand used as a reference for discussing experimental activities. It was found that the sulphate moiety of all compounds reproduced almost the same interactions with the sulphonamide group of AAZ. In detail the sulfamate group fit deeply into the active site with the negatively charged nitrogen coordinating with the zinc. Apart from this, the hydrogen of the sulfamate establish H-bond with T 199 oxygen of the hydroxyl group and the amidic hydrogen of the same residue binds the S =O oxygens and / or the Ph-O-S oxygen.



Receptor site of CAIX and CA XII site





It can be inferred from the above that the pyrazoline sulfamates did not inhibit hCA I effectively whereas many low nanomolar inhibitors were found against hCA II (Kis in the range of 0.42 to 90.1 nM),hCA IX (Kis inthe range of 0.72 to 63.6 nM) and hCA XII (Kis in the range of 0.88 to 85.2 nM). The best substitution fragments at the pyrazoline ring included for CA II a 4-sufamic group on the 3-aryl and halogens on the 5-aryl or a metoxy group on the 3-aryl and a 4-sulfamic group on the 5-aryl. For CA IX and CA XII, the sulfamic group on the 3- or 4-position of the 5-aryl and an electron withdrawing group in the 4-position of the 3-aryl ring. The study group also took up the docking experiments which suggested that the selectivity and inhibition strength showed by some compounds may be related to the number of hydrogen bonds between the sulfamate compounds and the various CA isoforms.

Lately, a group of scientists namely, Nilgun Karali, Claudiu T.Supuran, Murat Bozdag, Emanuela Berrino, Atilla Akdemir and Pinar Eraslan-Elma have carried out investigation of 1H-indole-2,3-dione 3-thiosemicarbazones with 3-sulfamoylphenyl moiety as selective Carbonic Anhydrase inhibitors. A lot of work has been done in establishing role of Carbonic Anhydrases in development of cancer and finding

effectiveness of sulphonamide inhibitors towards mitigating the disease. Many Carbonic Anhydrase Inhibitors (CAIs) bearing benzene sulphonamide units have shown efficacy in inhibiting various tumour cell lines. These drugs are selective inhibitors of hCA IX and XII and it led to publication of several studies in which benzene sulphonamide derivatives have been synthesized and assayed as inhibitors of hCAIX and XII. [31-33].

1H-Indole-2,3-dione (Isatin) has been known as a versatile intermediate for designing compounds of pharmacological importance. Benzene sulphonamide derivatives bearing an isatin moiety revealed promising activity and different selectivity towards Carbonic Anhydrases. Many 3-imino/hydrazono-2-indolinone derivatives of benzene sulphonamides have shown different and highly selective inhibitory effects against CAs. 3-Imino-2Indolinone based benzene sulphonamides have shown selective inhibition of hCA IX (Ki 1.0-25.0 nM) and XII (Ki 2.8-53.8 nM) isoforms at low nanomolar levels.[34]. 3-Imino-2-Indolonone derivatives carrying amido/ureido- sustituted benzene sulphonamide groups have been found to inhibit hCA XII isoenzyme with Ki values in the range of 0.47-2.83 nM.[35]. The inhibitory effects of 1-alkyl/ benzyl-3-imino-2-indolinone derivatives incorporating an ureido-bezenesulfonamide moiety, which were intended for stronger hydrophobic interactions with the hCA IX active region were investigated against hCA IX. It was noticed that their inhibitory effects for hCA IX (Ki 4.7-86.1 nM) increased significantly as compared to N-substituted derivatives (Ki 192-239 nM). These derivatives were found to bind by van der Waals interactions inside a large hydrophobic pocket created by T73, P75, P76, L91, L123 and A128 in the hCA IX active region.

Based on this information the above working group synthesized 1H-indole-2,3-dione 3-thiosemicarbazone derivatives to investigate the contribution of the sulfamoyl residue at 3 positions of the phenyl ring to their hCA I, hCA II, hCA IX and hCA XII inhibitory activities and to establish structure –activity relationships involved. Findings of the group are summarised as under.

It has been observed that most of the compounds carrying a 3-sulfamoylphenyl moiety showed potent inhibitory activity against hCA II with high selectivity over other isoforms. It was also determined that 1-substituted compounds had higher inhibitory activity than those without substituents at position 1 against the hCA II isoform. Elongation of the alkyl chain and benzyl substitution increased the inhibitory activity. However, 1- benzyl substitution increased the activity against hCA IX and hCA XII isoforms while decreasing the selectivity for hCA II isoform as compared to 1-ethyl-sustituted compounds. The working group also established that both high inhibitory effect and selectivity for hCA II isoform were possible with chlorine atom at position 5 and the ethyl group at position 1 of the indole ring.

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