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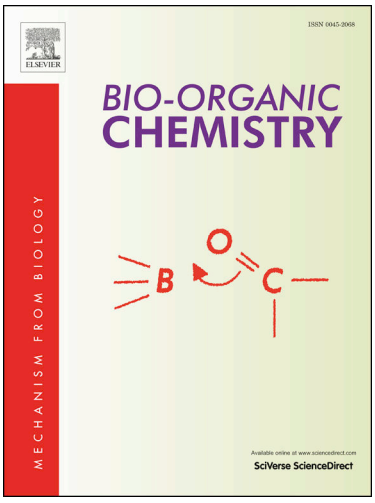

Structure-activity relationship of Human Carbonic Anhydrase-II inhibitors: Detailed Insight for future development as anti-glaucoma agents

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Structure-activity relationship of Human Carbonic Anhydrase-II inhibitors: Detailed Insight for future development as anti-glaucoma agents

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Abstract

Human carbonic anhydrase-II (hCA-II) is the most dominant physiologic isoform amongst the sixteen reported hCA isoforms. Because of its high availability in the different anatomical, and cellular sites of the eye like retina and lens, it plays a more prominent role in the regulation of intraocular pressure than the other twelve catalytically active hCA isoforms. This isoform is also located in the brain, kidney, gastric mucosa, osteoclasts, RBCs, skeletal muscle, testes, pancreas, lungs, etc. Earlier, hCA-II inhibitors were designed based on the sulfonamides e.g. acetazolamide, dichlorphenamide, methazolamide, ethoxzolamide, etc. and they were used systemically in antiglaucoma therapy. Many successful attempts have been made by the researchers in order to design more potent and effective inhibitors by incorporating various moieties in sulphonamides. Some novel scaffolds like chalcones, thiophenes, organotellurium compounds, dithiocarbamate, selenide, and 2-benzylpyrazine, etc. were also designed as hCA-II inhibitors and their inhibitory efficacy was proved in the nanomolar range. In order to obtain relevant information from the insights of their structure-activity relationship, the reported hCA-II inhibitors from the year 1989 to 2019 were critically analysed. It gave a complete insight into the relationship between their structure-activity and hCA-II inhibition. The broad spectrum of our investigation may help researchers to summarize all the crucial structural information required for the development of more potent hCA-II inhibitors for glaucoma.

Keywords: Human carbonic anhydrase, hCA-II inhibitors, structure-activity relationship (SAR), sulfonamides, dithiocarbamate, selenide, organotellurium, 2-benzylpyrazine.

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Introduction

Glaucoma is the second leading cause of blindness that affects nearly 67 million people worldwide. The development of highly potent antiglaucoma agents with less adverse effects is a great challenge in the area of ophthalmic drug discovery [1-3]. Often it is called the "silent thief of sight" as glaucoma gradually damages the eyes resulting in irretrievable impairment of vision and affecting the elderly in particular [4]. The retinal ganglion cells in the optic nerve gradually degenerate under increased pressure in the eye, and this leads to irreversible blindness [5-9]. In normal humans, the ciliary body of the eye secrets a transparent liquid called aqueous humor that is rich in bicarbonate and is drained through the trabecular meshwork (TM) pathway (~90%), and by the uveoscleral pathway (~10%) [10-13]. The intraocular pressure (IOP) is maintained by the tissues of trabecular meshwork located in between the cornea and iris which allow the aqueous humor of eye to flow through the pupil of the iris into the anterior chamber. In open or wide-angle glaucoma, these tissues lose their normal rhythm of activity resulting in an elevated, chronic, painless buildup of intraocular pressure in the eye [5-9]. The imbalance between the aqueous humor inlet and outlet causes increased IOP levels often associated with the excessive inflow or obstruction in drainage of aqueous humor through iridocorneal angle (juxtacanalicular region of Schlemm's canal) [14]. In the present scenario, IOP reduction therapy, surgical operation is performed for lowering ocular hypertension [5,8,9]. Topical prostaglandins, β-blockers, carbonic anhydrase inhibitors (CAIs) or combinations are prescribed as initial medical therapy for IOP management [15].

There is an established relationship between glaucoma, and human carbonic anhydrase enzyme (EC 4.2.1.1). The human carbonic anhydrases are the zinc-containing metalloenzymes known for their ability in catalyzing the hydration of Carbon dioxide (CO_2) to bicarbonate, and protons [16-23]. They play an important role in various physiological processes associated with respiration, and transport of CO_2/bicarbonate between metabolizing tissues, and the lungs, pH, and CO_2 homeostasis, electrolyte excretion in different tissues/organs, biosynthetic reactions, bone resorption, calcification, tumorigenicity, and several other physiologic or pathologic processes. Till date, carbonic anhydrases are broadly classified into six distinct genetic families (α, β, γ, δ, ξ, and η CAs) [24,25]. Among the sixteen hCA isoforms reported so far only twelve isoforms are catalytically active. Each isoform differs with respect to their location, kinetic properties, and inhibitor profiles. In the cell, the hCA isoforms, hCA-I, hCA-II, hCA-III, hCA-VII, and hCA-XIII are located in the cytosol. The hCA-VA and hCA-VB isoforms are predominant in the mitochondria and are involved mainly in ureagenesis,

gluconeogenesis, and lipogenesis. On the cell membrane, hCA-IV, hCA-IX, hCA-XII, and hCA-XIV, isoforms are present, and hCA-VI is secreted through saliva and milk [23, 26]. G. De Simone *et al.* investigated the structural differences among the hCA classes with the help of X-ray crystallography [27-36]. The enzyme catalytic core (Fig. 1A) of all the α-CA isoforms consists of a metal coordinated complex that interacts with the Glu106 carboxylate residue through the hydrogen bonding with the hydroxyl moiety of the Thr199 [17,27,44]. The coordinated metal ion complex plays an important role by generating nucleophilic hydroxide species(ii), that triggers the catalytic cycle of Human Carbonic Anhydrases (Fig. 1B) [37].

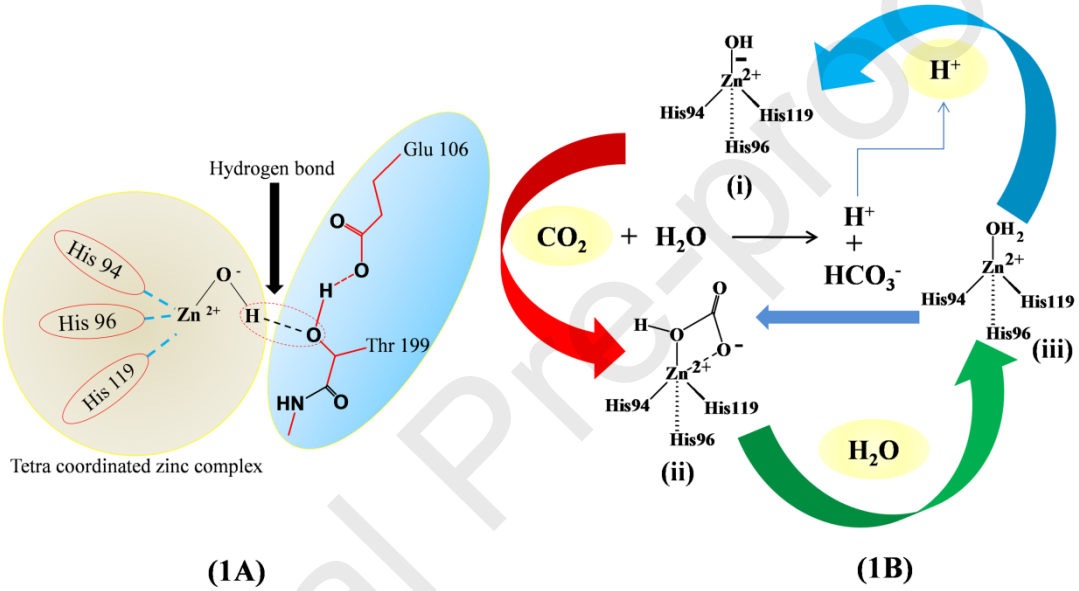


Fig. 1. Structure and mechanism of action of hCAs. **(A)**. Mechanism of the formation of the catalytic core of hCAs. **(B)** Catalytic cycle of hCAs

The most common known mechanism of action of anti-glaucoma agents is through the inhibition of hCA isozymes such as hCA-I, II, IV, and XII which decrease bicarbonate and subsequently, aqueous humor secretion and high Intraocular pressure [5, 23, 28]. Among all the known isozymes, hCA-II appears to play a prominent and efficient role in the regulation of intraocular pressure. Besides its high occurrence in the different anatomical, and cellular sites of the eye like retina, and the lens, it is also found in the kidney, brain, pancreas, gastric mucosa, RBCs, skeletal muscle, lungs, testes, osteoclasts [38-41]. It is known to regulate the acid-base homeostasis, and fluid movements by exchanging the protons, bicarbonates and other solutes across membranes [15]. The X-ray crystal structure of human hCA-II was first reported by Liljas and colleagues [42]. The active site of this isozyme contains a single zinc ion together

with catalytically active residues Thr 199, Glu 106, and His 64. His 94 acts like "proton shuttle" that transfers a proton from zinc-bound water to bulk solvent [43, 44]. For the formation of hydrogen bond between the water molecules of His 94 and Zn-OH_2, proton transfer takes place by Grotthuss diffusion [45, 46]. Two distinct His 94 conformations play an imperative role in the binding pattern with zinc. When the imidazole ring of His 94 directed towards the zinc, it adopts the "in" conformation or the "out" conformation takes place when its imidazole ring is directed away from zinc. The X-ray crystal structure of human hCA-II, at pH 8.5 shows that His 94 predominantly adopts the 'in' conformation [45] whereas the crystal structure at pH 5.7 (at which His 94 is protonated) shows that His 94 adopts the 'out' conformation [47].

The design of inhibitors targeting hCA-II is important in developing antiglaucoma agents. The backbone of all the CA inhibitors (hCA-II) is constituted by molecules containing the primary sulfonamide group (R-SO_2NH_2) [28]. Sulfonamide hCA-II inhibitors such as acetazolamide, dichlorphenamide, methazolamide, ethoxzolamide etc are used systemically in antiglaucoma therapy [3, 5, 16, 44]. They showed significant inhibition in typically low nanomolar concentrations against most hCA isoforms in humans [17]. At present, they become an alternative component of regimens for the treatment of refractory glaucoma, which does not respond to the topically acting adrenergic antagonists, or PG analogs [3, 5]. Moreover, Nitrous Oxide (NO) donating sulfonamide compounds demonstrate IOP lowering effects in rabbits and show improved antiglaucoma effect *in vivo* by hCA-II isoenzyme [48]. These NO derivatives may improve blood supply to optic nerve artery by regulating systolic and diastolic velocities [49]. Many undesired side effects such as numbness, and tingling of extremities; metallic taste, depression, fatigue, malaise, weight loss, decreased libido, gastrointestinal irritation, metabolic acidosis, renal calculi, and transient myopia are observed for the inhibition of CA [17].

However, the primary sulfonamide group does not contain any structural features that are responsible only for specific hCA-II enzyme inhibition. Therefore, researchers focused on some alternative molecules in order to explore the structural features of specific hCA-II inhibitors and they established ring and tail approaches for the specific inhibition of hCAs by incorporating different aromatic/heteroaromatic fragments into the active core of the zinc-binding groups (ZBGs) like sulfonamides, sulfamides, sulfamates, DTCs, Xanthates, etc (Fig. 2). The detailed description of different molecules along with its important structure-activity relationships is illustrated scaffold wise to offer an insight into the design of inhibitors. In this way, in the hydrophobic pocket (Val 121, Val 143, & Leu 198), the tetrahedral adduct of Zn

(II) ion associated with His 94, His 96 and His 119 can be selectively inhibited to develop specific hCA-II inhibitor [17].

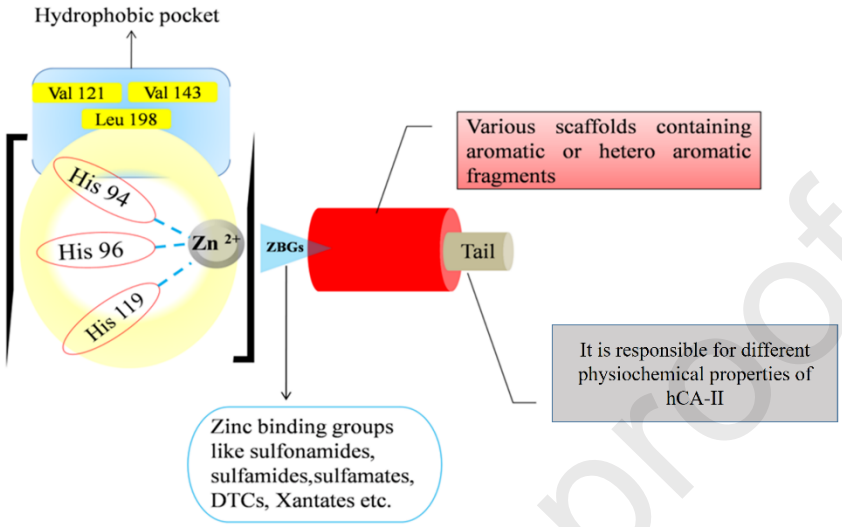


Fig. 2. Common structural features of hCA-II

A. *N*-benzenesulfonamide with cyclic imide scaffolds.

Abdel-Aziz *et al* reported [50] the inhibition profile of hCA-II inhibitors in the nanomolar range of 5 to 693 nM of K_i. Under this scaffold, sulfonamide incorporating tetrahydro-phthalimido ring (compound **A3)** showed the most prominent inhibition against hCA-II with a K_i value of 5 nM (Fig. 3). A similar effect was observed when the norbornene-imido moiety was introduced with an appropriate linker (compound **A13**; K_i value of 23.2 nM). Except compound **A3,** no significant changes were observed with regard to the replacement of monocyclic imido ring with a bicyclic ring. However, the presence of bicyclic imido scaffold such as pyrazine-2,3-dicarboxamide moiety (compound **A4**) resulted in a decreased inhibitory potency (K_i of 426 nM). Moreover, bulky groups such as *tertiary*-butyl in bicyclic imido ring (compound **A7**) slightly decreased the inhibition potency against this isoform. Another important aspect reported was that the substitution on the phthalimido scaffold by bromine (compound **A10**) lowered the inhibitory potency significantly being the least effective hCA-II inhibitor of all (K_i 693 nM). Furthermore, the inhibitory potency of compound **A12 (**K_i 140 nM) was found to be enhanced twice more than the compound **A11** (K_i 85.4 nM) due to the substitution position of the nitro group.

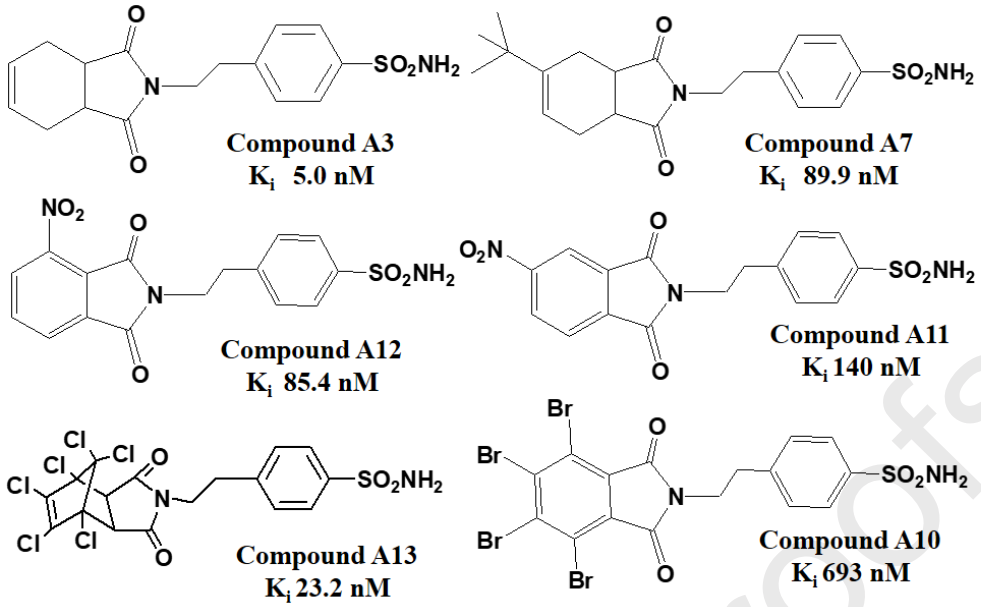


Fig. 3. Important SARs of *N*-benzenesulfonamide with cyclic imides

B. *N*-benzenesulfonamide with polycyclic imides

Under this scaffold, the presence of the nitro group in the 5th position of the isoquinoline moiety (compounds **B4-B6**) improved the inhibitory potency from moderate to high level of significant action. The introduction of linker significantly changed the potency of the scaffolds than those without linker, e.g, compound **B4**. (Fig. 4). However, the presence of one and two atoms of carbon in the methylene linker in the benzylisoquinoline (compounds **B2** and **B3** with K_i value of 32.7 nM) facilitated a slight increase in the inhibition potency over the ones without a linker (compound **B1**; K_i value of 63 nM). Moreover, the linker had a great impact on the inhibition when it was present in the bulky scaffolds like dibenzoazepine, dihydro-pyrroloisoindole and tetrahydrobenzo phenanthroline (compound **B7-B15**). Compound **B12** (K_i of 9188.4 nM) was found to be the least active compound that had ethylene group as linker. Thus, it can be inferred that the presence of an appropriate linker, as well as the nitro group at the 5th position of polycyclic imide scaffold, is necessary for the inhibition potency against this isoform. It is also noted that the bis-sulfonamides (compounds **B8-B15**) are generally poor hCA-II inhibitors compared to the mono-sulfonamides (compounds **B1-B7**) [51].

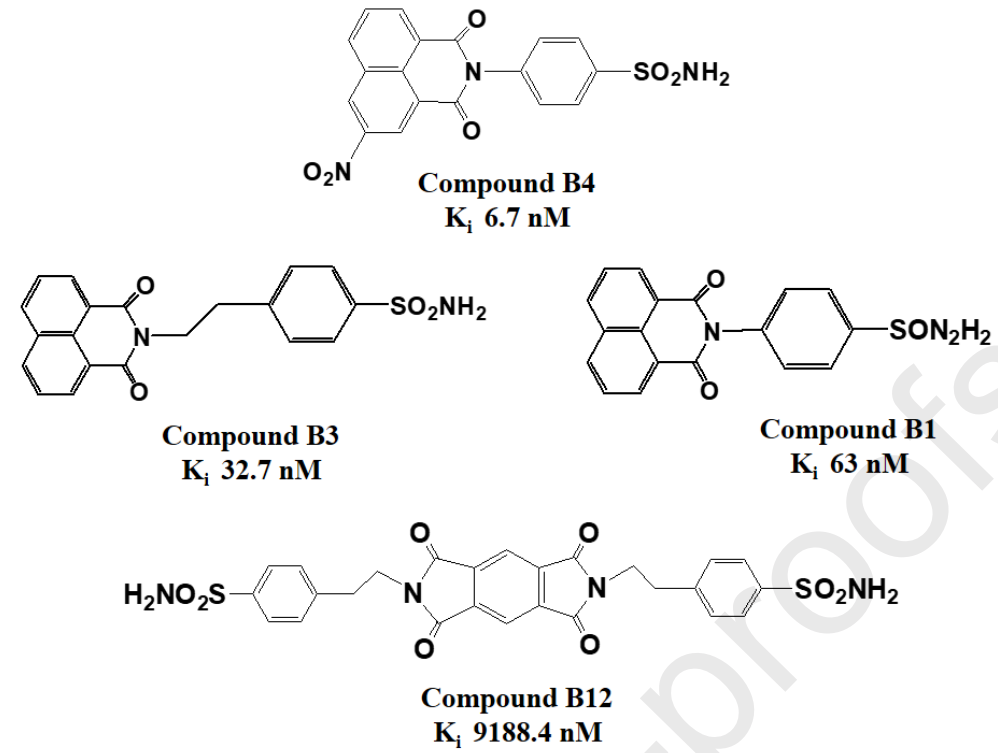


Fig. 4. SAR of *N*-benzenesulfonamide with polycyclic imides.

C. Sulfonamides and carboxylic acids with cyclic imides

Substitution in phthalimide moiety was not suitable for the inhibitory activity against hCA-II as it was confirmed from the compound **C7** (K_i 2.3 nM) and the best active compound **C8** (K_i 2.2 nM) (Fig. 5). Compound **C19** (K_i value of 27.7 nM) was found to be the best evidence of it. Except for compounds **C6** and **C9,** all the carboxylates (compounds **C3**, **C12**, **C15**, **C21**, **C24**, **C27**, and **C30**) do not much inhibit this hCA-II as compared to the sulfonamides. Moreover, sulfonamides containing moieties such as monocyclic succinimides, tetrahydro-phthalimide, phthalimide, substituted phthalimides (compounds **C10-C11**, **C13-C14**, **C16-C17**, **C19-C20**, **C22**) are shown to be a moderate hCA-II inhibitor except pyrazine-2,3-dicarboxamide (compound **C26**) or naphthalene-1,8-dicarboximide (compound **C28-C29**). However, the moderate inhibition of compound **C26** and compound **C23** might be due to the absence of linker in their structures. When 5-nitrophthalimide group is placed in the sulfonamide moiety with a linker, the resultant compound **C22** (K_i value of 238 nM) becomes the least active inhibitor of hCA-II. Therefore, it may be inferred that the presence of linker with a substitution in phthalimide ring is highly unfavorable for potently inhibiting hCA-II [52]**.**

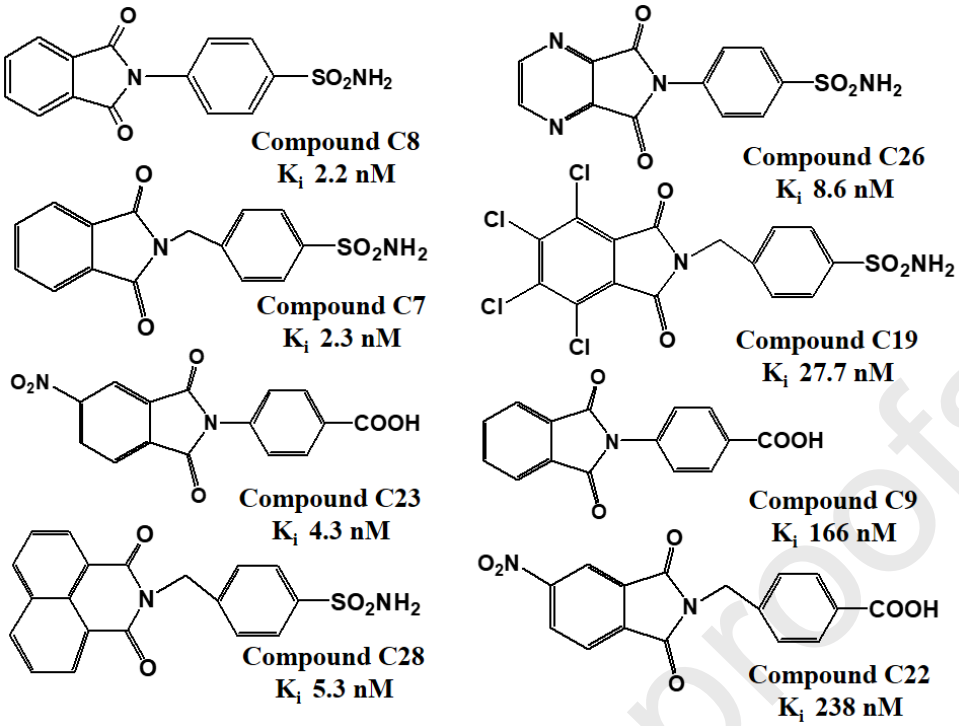


Fig. 5. SAR of *N*-benzenesulfonamide with polycyclic imides.

D. Nitrophthalimide

The position of the nitro group in the phthalimide ring played a very crucial role in the inhibitory profile for all the compounds under this scaffold. It was observed that the presence of nitro group at the 4th position of the phthalimide ring (compounds **D6-D8**) was highly favorable than the 5th position (compounds **D1-D5**) (Fig. 6). However, compound **D1**(K_i value of 4.3 nM) irrespective of the position of nitro group, showed a very promising inhibition probably because of the presence of sulfonamide group at the 4th position of the benzene ring in the absence of the linker. Moreover, the presence of sulfonamide at the 2nd position of benzene ring resulted in the best active compound **D8** with K_i value of 1.7 nM as compared to its presence at 3rd position as seen in the compound **D7** (K_i value of 3.9 nM). The increased carbon chain length between the phthalimide and benzenesulfonamide led to decreased activities of compounds **D1**>**D2**>**D3** with K_i of 4.3, 238 and 589 nM, respectively. The 4th position of nitro group and the 2nd position of sulfonamide group without a linker were found to be important for hCA-II inhibition activity [53].

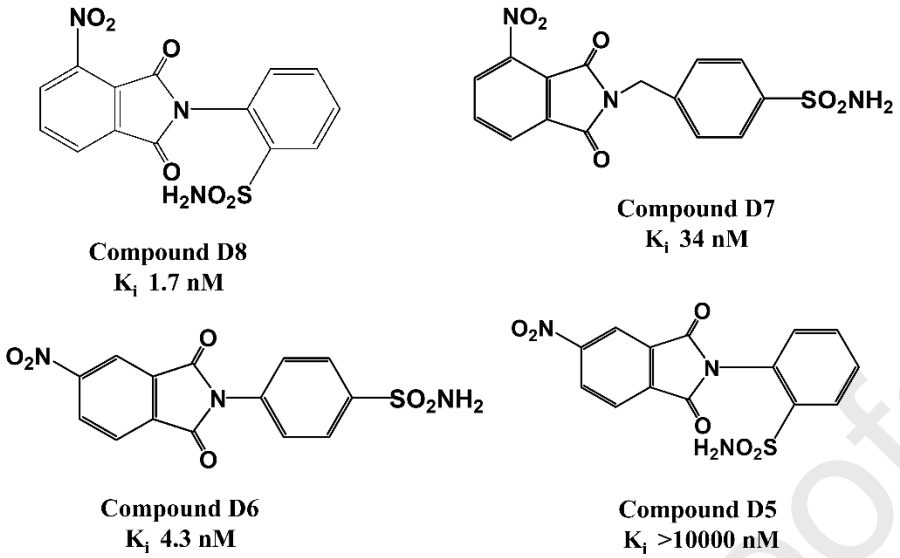


Fig. 6. SAR of Nitrophthalimide with sulfonamides

E. 4,5,6,7-tetrachloro-phthalimide moiety with sulfonamides

Sulfonamides with tetrachloro-phthalimide moiety exerted a moderate to high level of inhibition against hCA-II with K_i range of 2.4 - 4515 nM. Compound **E8** showed the highest inhibition potency with a K_i of 2.4 nM probably because of less steric hindrance effects in the 4th position of sulfonamide group. Not only the absence of linker was useful but also the presence of halogen in the 2nd position of benzene ring produced a profound inhibitory effect which was confirmed from the inhibitory concentration of compounds **E6** (K_i of 3.4 nM), **E7** (K_i of 4.9 nM) and **E8**. Irrespective of the above fact, compound **E2** having ethylene linker showed a prominent inhibition with a K_i of 2.9 nM. As seen in the compounds **E4** (K_i of 27.7 nM) and **E5** (K_i of 4515 nM) the position of sulfonamide at 2nd or 3rd position of the benzene ring resulted in the poor inhibitory activity (Fig. 7). Compounds **E1**, **E2** and **E3** exerted their action at moderate level in the presence of sulfonamide group at the 4th position with no halogen atom. Hence, the SAR above necessitates the presence of sulfonamide at the 4th position of benzene ring along with the influence of halogen-substituted benzene sulfonamides for better inhibition of hCA-II. [54].

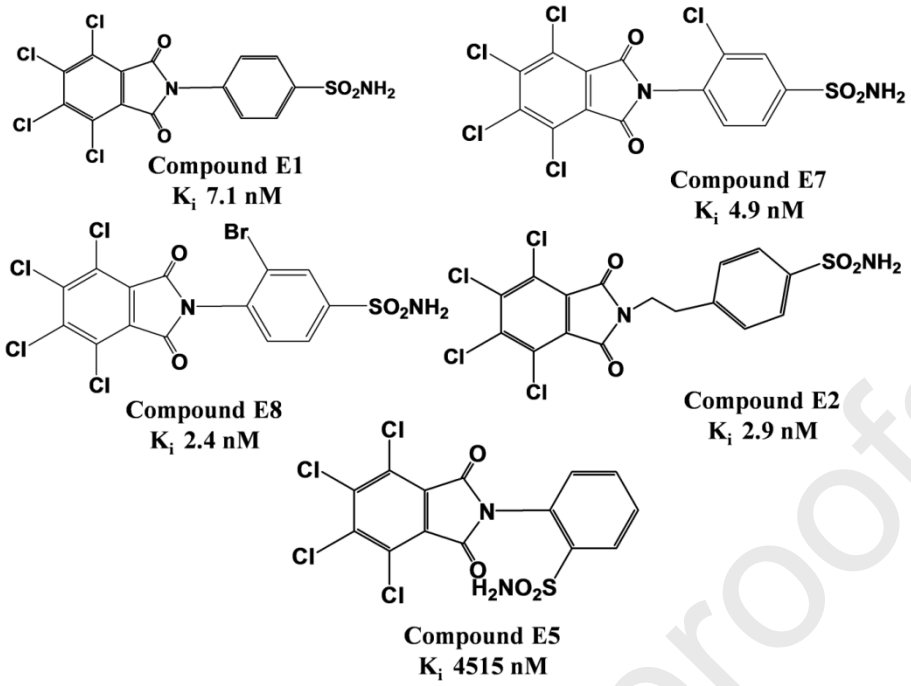


Fig. 7 SAR of 4,5,6,7 -tetrachloro-phthalimide moiety with sulfonamides

F. Sulfamides

The inhibitory activity (K_i range of 7-890 nM) of sulfamides relies on the type of substituent groups. Moderate inhibition was observed when the phenyl ring was attached with sulfamides (compound **F8**). On the other hand, the presence of some electronegative groups on phenyl ring (compounds **F10**-**F13**) exerted their inhibitory action at low nanomolar concentration (K_i range of 7-21 nM). The best inhibition was shown by the compound **F13** with K_i value of 7 nM. Although compound **F20** contained electronegative atom like fluorine, it showed less potency due to its unfavorable occupancy with the active binding site. Substitution in phenyl ring by other groups like -OMe (compound **F14**; K_i value of **11** nM), -OH (compound **F15**; K_i value of 12 nM), -NO_2 (compound **F16**; K_i value of 13 nM), -EtO_2C (compound **F17**; K_i value of 19 nM), -NC (compound **F18**; K_i value of 16 nM), -Me_2N (compound **F19**; K_i value of 21 nM), -benzoyl (compound **F21**; K_i value of 49 nM), led to a similar effect like that of halogen-substituted sulfamides (Fig. 8). Replacement of the phenyl ring by bulky groups like 2-adamantyl (compound **F3** is the least active hCA-II inhibitors under this scaffold), and long alkyl chain (compounds **F1**, **F6,** and **F7**) resulted in a very low inhibition due to their less accommodation with the active site of this enzyme. So, both the presence of substituted phenyl ring and the absence of bulky group favor the inhibition of these sulfamides [55].

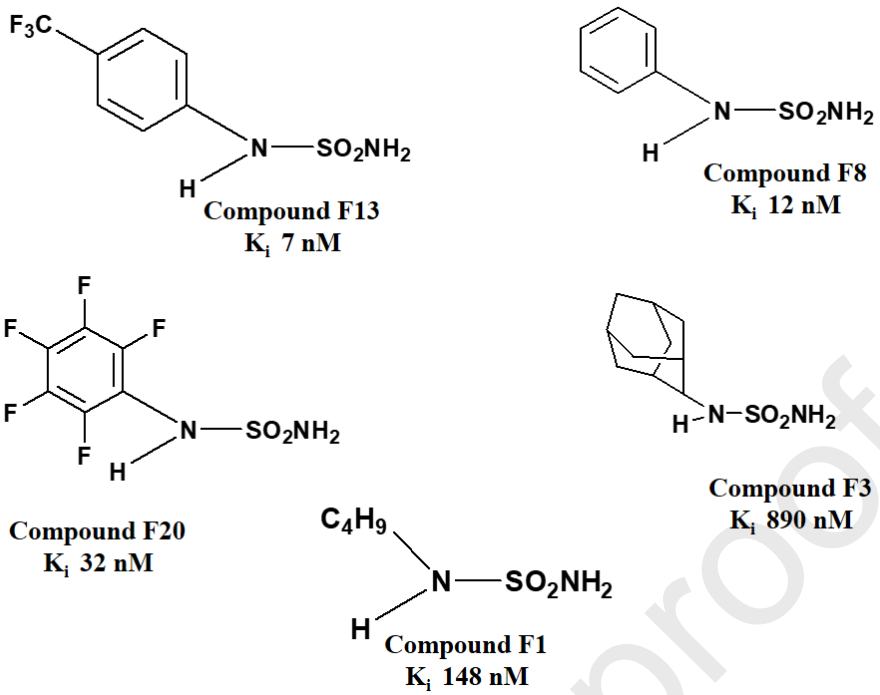


Fig. 8.SAR of Sulfamides

G. Thiadiazole, thienothiopyran, benzothiazole and substituted benzene with sulfonamides

An almost similar type of inhibition was observed for the sulfonamides - incorporated moieties like thiadiazole (compounds **G1-G18**), thienothiopyran (compounds **G19-G23**), benzothiazole (compounds **G24-G34**) and substituted benzene (compounds **G35-G73**). These compounds exerted a very effective inhibition with $K_i \leq 8.275$ nM. Their inhibitory action depends on the presence or absence of the electronegative atom together with its position of substitution in different heterocyclic moieties. For example, the presence of fluorine on benzene ring (compound **G41**; K_i value of 8.275 nM; the least active compound) has less inhibitory activity when compared to fluorine substituted thiadiazole (compounds **G3**, **G4**, **G13,** and **G14**) and fluorine substituted benzothiazole (compounds **G26**, **G27,** and **G31-G33**). Similar results were noticed when chlorine was attached with the thiadiazole moiety (compound **G8**; K_i value of 8.085 nM). Likewise, the presence of NH_2 (compounds **G10**; K_i value of 8.06 nM), and NO_2 (compounds **G11**; K_i value of 8 nM; the best active compound) (Fig. 9a) in this moiety significantly enhanced the inhibition potency when compared to others.

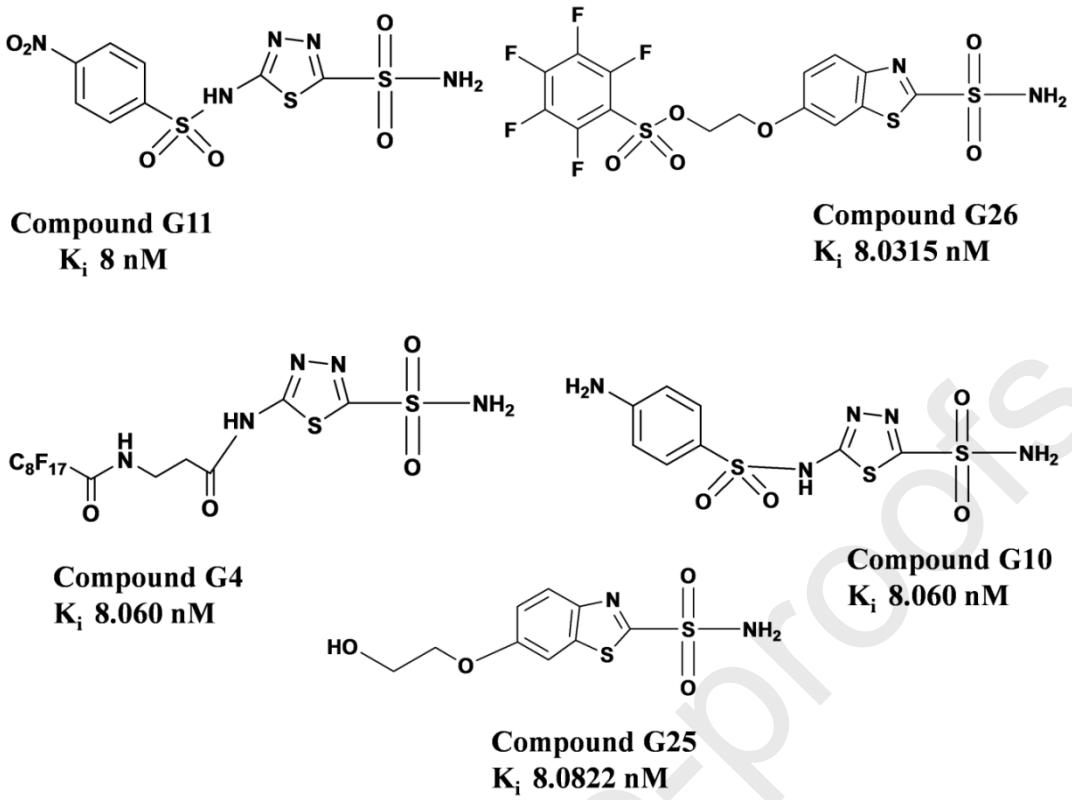


Fig. 9a. SAR of various heterocyclic moieties with sulfonamides.

The inhibitory activity is slightly decreased when amino group is present in a branched-chain of the benzenesulfonamides (compounds **G36-G38**). Hydroxyl group may bring an effective inhibition when it is present in the benzothiazole (compounds **G24** and **G25**). Likewise, carboxylic acid group might bring an effective inhibition when it is present in substituted benzene sulfonamides (compounds **G64-G68**) with the exception of compounds **G39** (K_i value of 8.157 nM) **G60-G62** (Fig. 9b). Therefore, it can be concluded that not only the electronegative atom substituted heterocyclic rings are important for the effective inhibition but also the presence of other essential groups like -NO_2,-NH_2 in the unbranched chain facilitates more potent inhibitory activity in case of sulfonamides incorporated with heterocyclics. Therefore, order of inhibition towards hCA-II can be summarized as benzothiazole > thiadiazole > thienothiopyranesulfonamides > benzenesulfonamides [56]. In another study [57], 4-functionalized 1,3-diarylpyrazoles having 6-aminosulfonyl benzothiazole were also shown to have potential inhibition profile (K_i) in the range of 2.3 nM to 4.8 µM, and pyrazolecarboxylic acids type compounds were found to be the most potent compounds in this class of inhibitors.

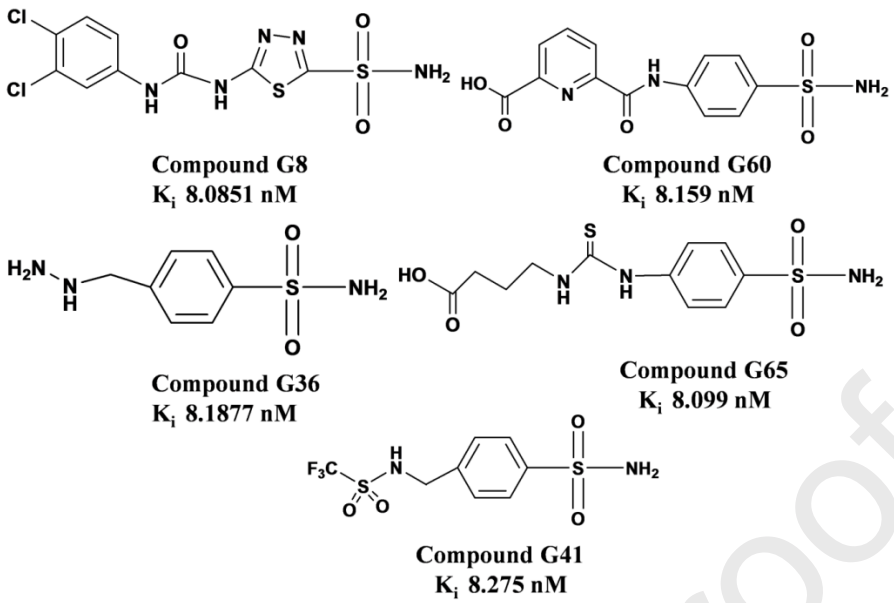


Fig. 9b. SAR of various moieties with sulfonamides

H. Scaffolds containing sulfonamides with hydroxamates and a hydroxyl group

Sulfonamides, when attached with the hydroxamates, brought about a similar pattern of inhibition with K_i range of 8.091–8.116 nM. The presence of fluorine either in simple alkyl chain (compound **H5**; K_i value 8.123 nM, compound **H6**; K_i value 8.106 nM, compound **H7**; K_i value 8.094 nM) or in the benzene ring (compound **H1**; K_i value 8.091 nM, the best active compound) (Fig. 10) favoured the inhibition against this isoform. The same K_i value was observed for compound **H10** where a deactivating group like -NO_2 is present. Unsubstituted benzene rings incorporated sulfonamides (compound **H11**; K_i value 8.119 nM) exerted a slightly less inhibition. The presence of chlorine (compound **H4**; K_i value of 8.166 nM, the least active compound) resulted in moderate inhibition against this enzyme with hydroxysulfonamides [56].

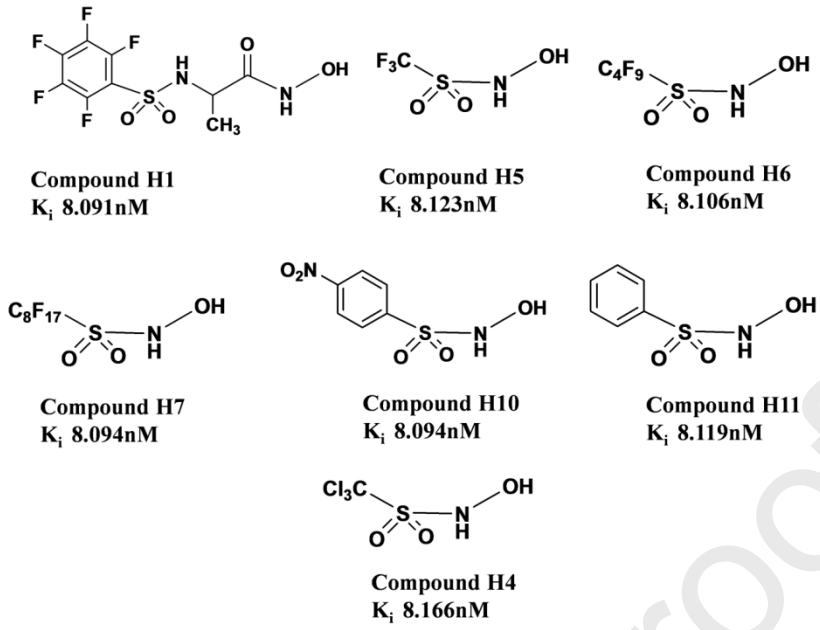


Fig. 10. SAR of hydroxamates and hydroxysulfonamides with sulfonamides

I. 8-Quinoline sulfonyl moiety with sulfonamides

For the 8-quinoline sulfonyl moiety incorporated sulfonamides, the presence of either non-fused or fused five-membered ring was found to be most crucial in the inhibition profile. Thiadiazole (compound **I13**; K_i value of 1.351 nM), and benzothiazole (compound **I17**; K_i value of 1.351 nM) moieties played the most prominent role in the absence, and presence of ether linker, respectively (Fig. 11). A moderate inhibition was noted due to the presence of only a six-membered ring, whereas halogen substitution enabled this scaffold to significantly inhibit this cytosolic dominant isoform (compound **I7**; K_i value of 4.504 nM). The order of inhibition for halogen-substituted benzenesulfonamide was as follows F >I >Cl >Br. A similar effect was observed for the alkylamine linked 8-QS moiety (compound **I6**; K_i value of 5.919 nM). Furthermore, the length of linker between 8-QS, and benzene ring contributed to the inhibition as in the case of the compounds **I19,** and **I20** with K_i value of 6.324 nM, and 6.168 nM, respectively. A significant decrease in the inhibitory potency was seen for the compound **I21** (K_i value of 20.11 nM, the least active compound) having bulky group in its interior. Finally, it can be inferred that the presence of heterocyclics along with halogen substitution, and an appropriate linker efficiently potentiates their inhibitory action [58].

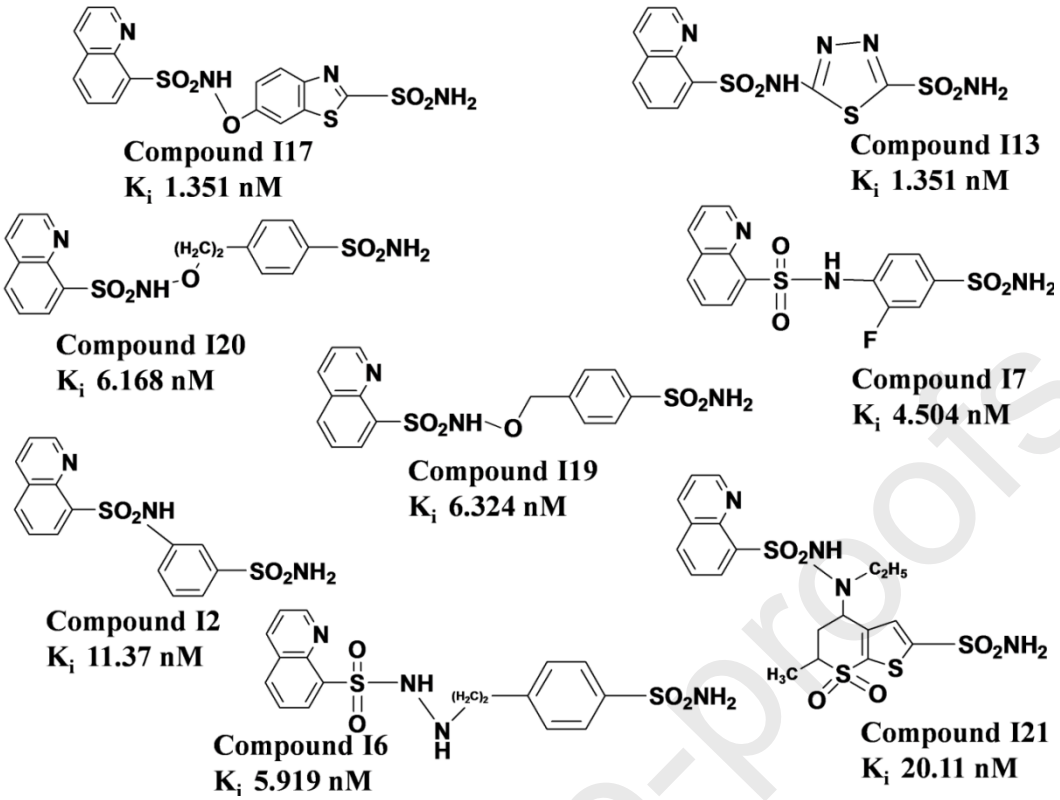


Fig. 11. SAR of 8-Quinoline sulfonyl moiety with sulfonamides

J. Phthalimide with sulfonamides.

Under this scaffold, the listed compounds showed moderate to high inhibition against hCA-II with K_i range of 2.1-17.4 nM. From the SAR, it was quite evident that the iodo-substituted derivatives influenced the inhibitory potency compared to the corresponding dimethoxy substituted, and the 5-methyl-substituted compounds. Compound **J2** was the most effective inhibitor amongst all with K_i value of 2.1 nM. Moreover, methyl substitution at 5th position in the benzoic acid attached with phthalimide incorporated sulfonamides (compound **J3**; K_i value of 9.1 nM, compound **J6**; K_i value of 8.0 nM, and compound **J9**; K_i value of 8.5 nM) resulted in a similar activity irrespective of the presence or absence of linker. However, absence of linker was found to be very crucial for enhanced activity of inhibition as in the case of iodo-substituted compounds (**J2**, **J5**; K_i value of 2.5 nM, and **J8**; K_i value of 8.4 nM) (Fig. 12) [59].

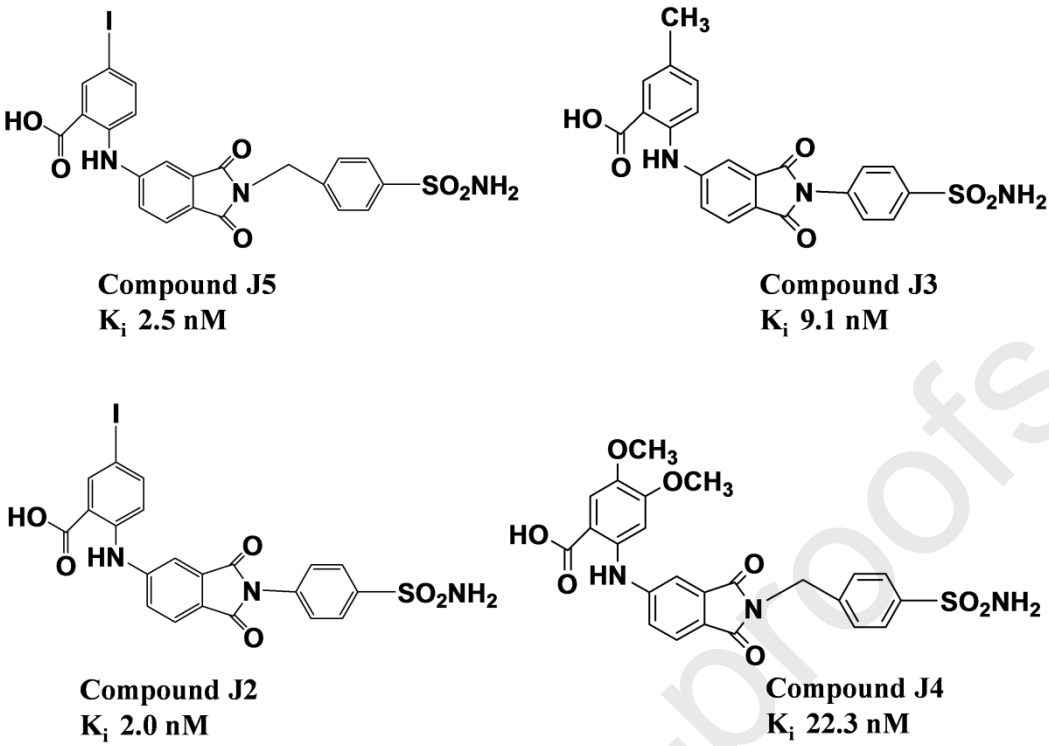


Fig. 12. SAR of Phthalimide moiety with sulfonamides

K. 1,4,5-trisubstituted -1,2,3-triazoles moiety with sulfonamides.

R. Kumar *et al*., 2017 [60] investigated the inhibition profile of some triazoles incorporated sulfonamides (compounds **K1**-**K20**). Out of all the functional groups attached in the 1,2,3-triazoles incorporated sulfonamides, the hydrazino-carbonyl functionality was found to play a very prominent role when it contained (compound **K16**; K_i value of 1.6 nM, the best active compound) 2-naphthyl group at the 4th position of 1,2,3-triazole. A similar activity was observed when methyl substitution was at the same position of 1,2,3-triazole moiety having carboxylic group (compound **K5**; K_i value of 1.6 nM, another the best active compound). This scenario can be different when these substituents are present with different functional groups. In case of 2-naphthyl substituent, the order of inhibition observed was -$CONHNH_2$ (compound **K16**; K_i value of 1.6 nM) > -COOEt (compound **K4**; K_i value of 5.8 nM) > -$CONH_2$ (compound **K12**; K_i value of 6 nM) > -COOH (compound **K8**; K_i value of 8.9 nM) whereas in case of -CH_3 substituent the order of inhibition observed was >-COOH (compound **K5**; K_i value of 1.6 nM) > -$CONH_2$ (compound **K9**; K_i value of 1.9 nM)> -COOEt (compound **K1**; K_i value of 3.2 nM)> -$CONHNH_2$ (compound **K13**; K_i value of 7.6 nM) > -COOH(compound **K6**; K_i value of 97.9 nM; the least active compound) (Fig. 13). Thus, not only the position of particular substitution but also the nature of functional group present in this moiety is important for

effective inhibition. All the other substituents groups like -C_6H_5, 4-C_6H_4-OCH_3 together with the presence of different functionalities exerted moderate to low inhibitory activity towards this isoform. Except three compounds **K6 (**K_i value of 97.9 nM**)**, **K14 (**K_i value of 38 nM**)**, and **K17**, all the synthesized compounds (**K1-K4**, **K5-K8**, **K9-K12**, **K13-K16**, and **K17-K20**) exerted better inhibitory potency with low K_i range of 1.6-9.4 nM.

Further, the work was extended by the joint efforts of L. Vats*et al*., 2018 [61], and they synthesized compounds **K21-K50** which showed a moderate to low inhibition with a K_i value in the range of 21.8 - 807.5 nM. With all the above functional groups remaining the same, they incorporated some new groups like -CH_3 C_6H_4, halogens (F, Cl, Br), and heterocyclics like 2-pyridyl, 2-thienyl, and observed their inhibition. Here, presence of -CH_2OH together with thienyl group at 2nd position (compound **K50**; K_i value of 21.8 nM), facilitated better inhibition than all the other compounds investigated here. Moreover, all the different substituents brought a comparable good inhibition when these were present with this functional group (compound **K45-K50**; K_i value in the range of 21.8 -84.9 nM).

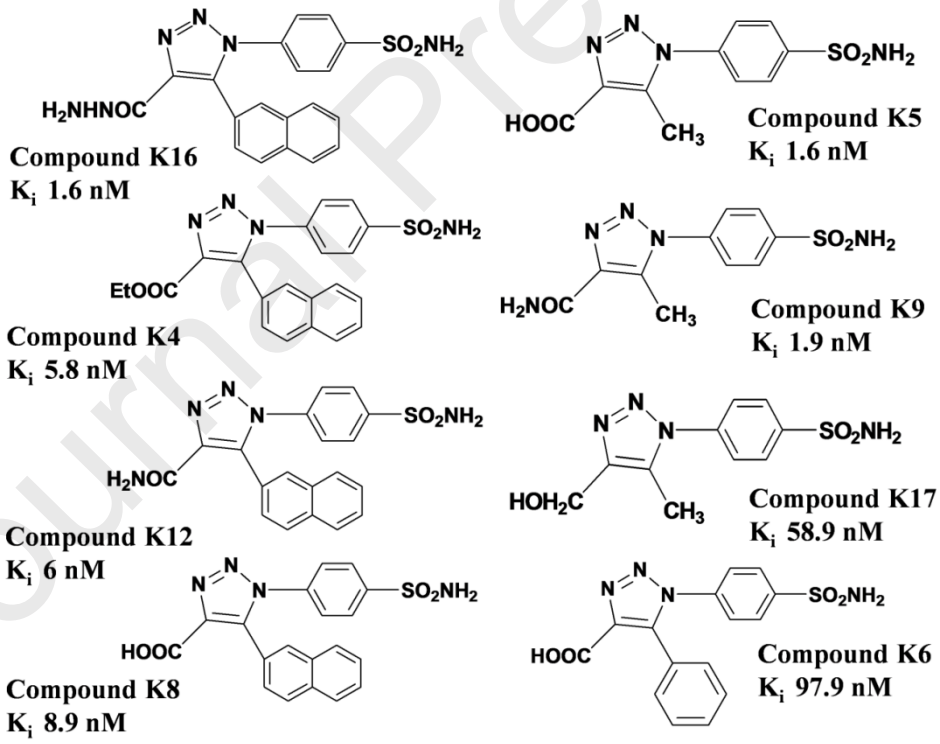


Fig. 13. SAR of 1,4,5-trisubstituted -1,2,3-triazoles moiety with sulfonamides

R. Kumar et al., 2018, [62] continued their previous study on 1,2,3-triazoles by placing some aromatic segments at the 4th position and examined the inhibitory profile for the compounds **K51-K70** by varying the 5th position of the triazole moiety either by placing only hydrogen or

by trifluoromethyl group (**Fig. 14**). For the presence of halogen-substituted aromatic fragments together with hydrogen at the 5th position of this moiety, the order of inhibition was followed as Cl (compound **K55**; K_i value of 40.4 nM)> F(compound **K54**; K_i value of 48.8 nM)>Br(compound **K56**; K_i value of 80 nM) whereas trifluoromethyl substituted triazoles produced more promising inhibition in comparison to the unsubstituted triazoles, and the order of inhibition was as follows, F (compound **K64**; K_i value of 23 nM)> Cl(compound **K65**; K_i value of 31.1 nM)> Br(compound **K66**; K_i value of 48.9 nM)**.** Similarly, a greater inhibitory activity was observed by these trifluoromethyl substituted triazoles when they contained different heterocyclics like 2-naphthyl (compound **K67**; K_i value of 55.3 nM), 2-Picolyl (compound **K68**; K_i value of 67.3 nM), 2-thienyl (compound **K69**; K_i value of 59 nM), and 2-furyl(compound **K70**; K_i value of 35.8 nM) at the 5th position. Very recently twenty two new benzenesulfonamides having triazole and dual triazole moieties were synthesized and evaluated against the hCA II enzyme. They showed interesting inhibition (K_i) profile in the range of 8 nM – 0.903 µM [63]. 3-nitrophenacyl group appended dual triazole containing benezenesulfonamide was the most potent compound (K_i value of 8 nM) in the series.

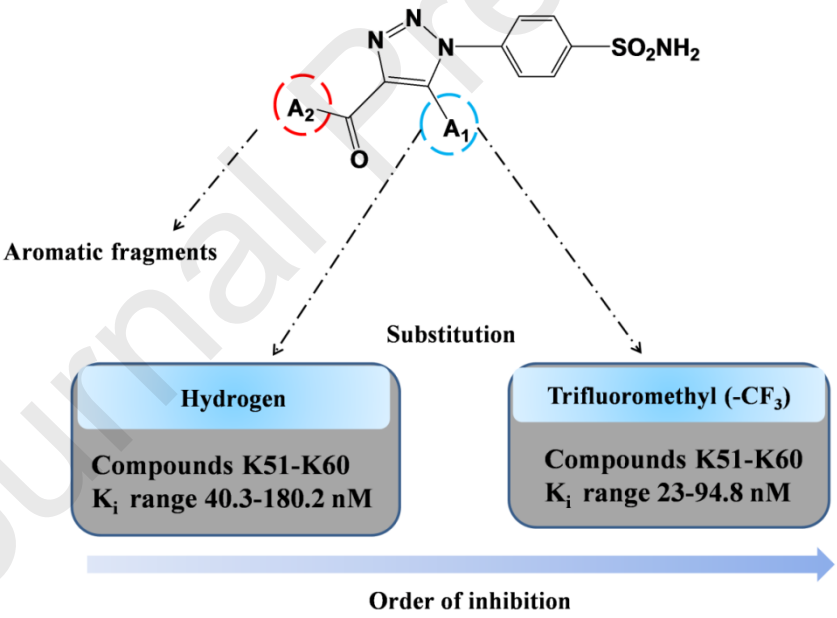


Fig. 14. Effect of substituents on the order of inhibition in the presence of different aromatic fragments for the sulfonamides with polycyclic imides

L. 1,4-disubstituted-1,2,3-triazoles moiety with sulfonamides.

Under this scaffold, the influence of different types of linkers such as oxymethylene linker, sulfur methylene linker, aminoethyl linker incorporated in the 1,2,3-triazole was observed.

From the SAR, it was evident that the presence of a hydrophilic amino methylene linker (compound **L7**; K_i value of 0.83 nM) in the first series of derivatives was the most effective inhibitor. The order of inhibition varied for the presence of different linkers: amino methylene linker >oxymethylene linker (compound **L1**; K_i value of 1.0 nM, compound **L5**; K_i value of 1.4 nM) >sulfermethylene linker (compound **L6**; K_i value of 1.4 nM) (Fig. 15). In case of oxymethylene linker, the nature of substituent, and its position also influenced their activity as noticed that the compound **L3** (K_i value of 1.5 nM) produced greater inhibition than the compound **L4** (K_i value of 4.3 nM). A similar effect was observed when the triazole moiety was attached to the benzene sulfonamides by means of a suitable oxymethylene linker. However, their inhibitory action can be significantly varied for the substitution in the benzene ring incorporated in triazole moiety. Unsubstituted benzene ring (compound **L8**; K_i value of 1.2 nM) produced more prominent inhibition than the substituted benzene ring, except compound **L12** (K_i value of 1.0 nM) that had hydroxy substitution. Incorporation of methoxy or trifluoromethyl group in meta and para positions of the attached benzene moiety resulted in a 10-fold decrease in the inhibitory concentration in compound **L9** (K_i value of 12.4 nM), and compound **L11** (K_i value of 15.7 nM; the least active compound), respectively [64].

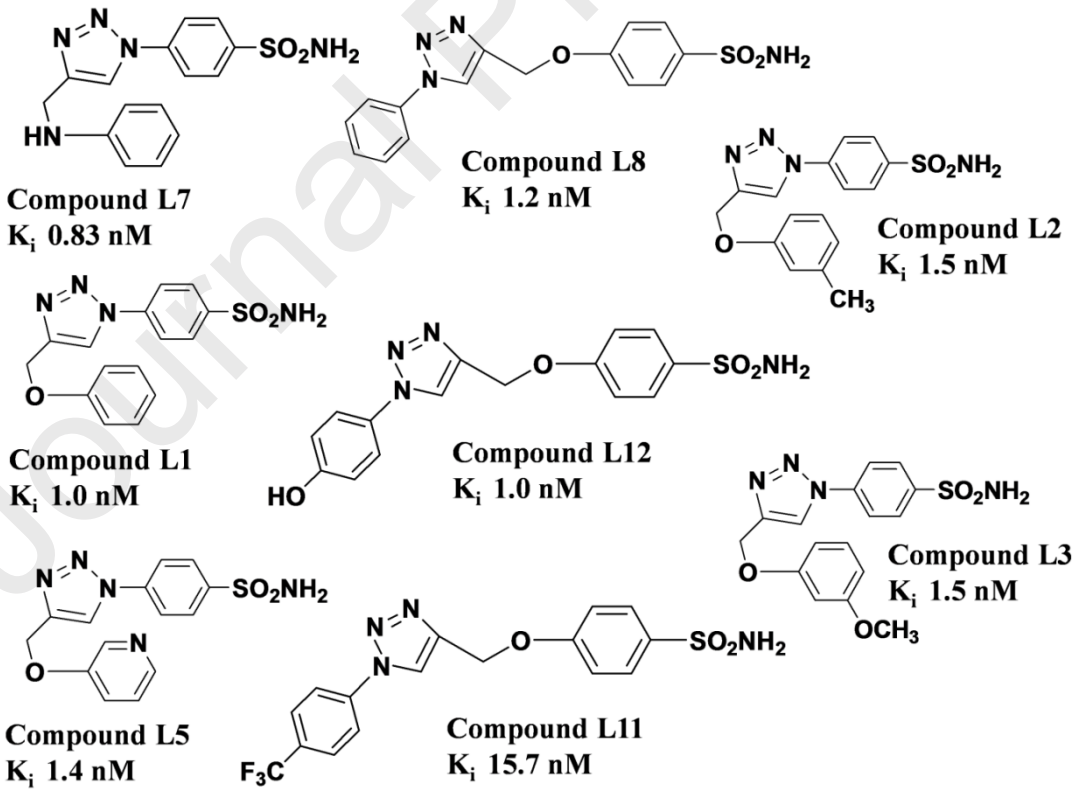


Fig. 15. SAR of 1,4-disubstituted-1,2,3-triazoles moiety with sulfonamides

M. *N*-protected amino acids with sulfonamides.

Sulfonamides with N-protected amino acids were found to exert inhibitory activity in K_i range of 8.6-694 nM. The presence of a suitable linker like - $(CH_2)_4$-O- between the benzenesulfonamide moiety, and the primary amine group facilitated more potent inhibition. In this series, the more effective compounds were **M17**-**M19** with K_i range of 8.6-11.9 nM (Fig. 16). The protecting groups may contribute significantly to the presence of appropriate linker. It was observed that compound **M11** (K_i value of 35.9 nM) was less potent than the compound **M18** (K_i value of 18 nM) due to the presence of unsuitable linker like - $(CH_2)_2$. In the absence of linker, the inhibitory concentration of these compounds decreased (compound **M3**; K_i value of 18 nM). However, compound **M20** (K_i value of 694 nM) became the least active compound due to the presence of a hydrophobic moiety like -CH_2Ph. Therefore, it may be concluded that the N-protected amino acids adjoined with sulfonamides by an appropriate linker favor the inhibitory activity of these compounds. In addition, the substitution in **A2** position is not favorable for inhibition of hCA-II [65].

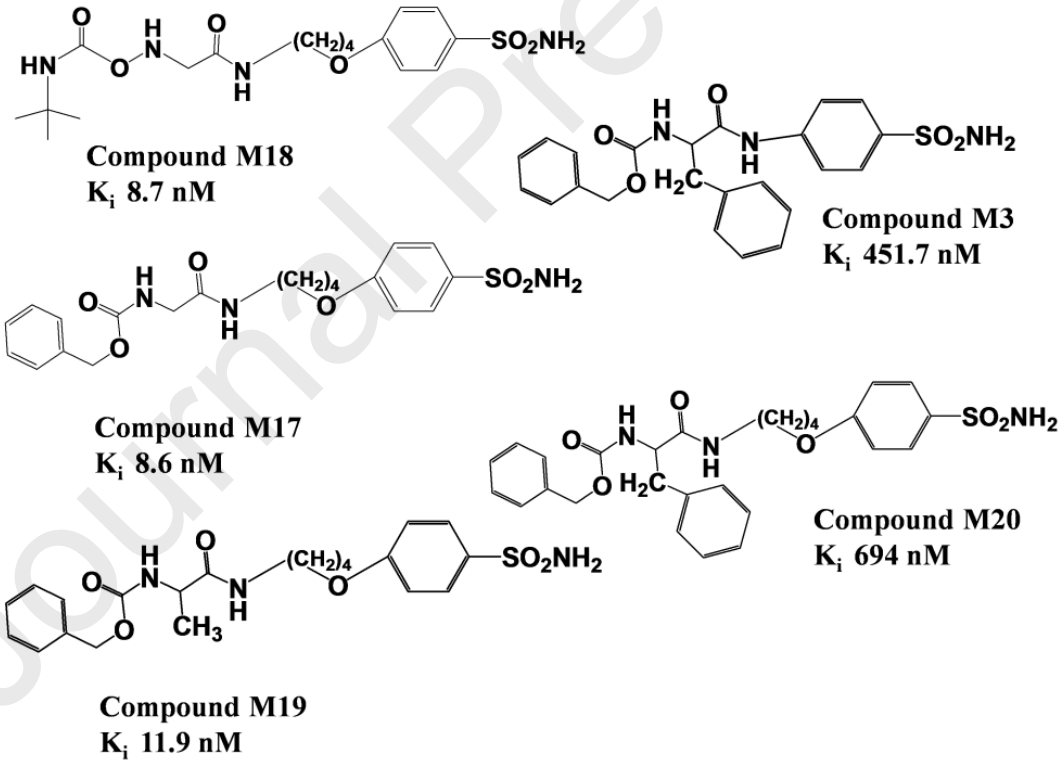


Fig. 16. SAR of *N*-protected amino acids with sulfonamides

N. 1,3-dioxo-2-substituted phenyl isoindoline-5-carboxamide moiety with sulfonamides

All the compounds under this scaffold brought very poor inhibition against this isoform except compounds **N1**-**N3** which showed a reasonable inhibition with K_i range of 8.9-13.8 nM (Fig. 17). The substitution at the 2nd position of the 1,3-dioxo-phenyl isoindole-5-carboxamide moiety by benzenesulfonamide (compounds **N1**-**N3**) provided more enhanced activity than the benzoic acid moiety (compounds **N7**-**N8**). The absence of benzenesulfonamide made these compounds (compounds **N4**-**N8**) totally ineffective. From the SAR of three best active compounds (**N1**-**N3**), it was clear that greater the length of the linker, lesser was the inhibition. So, it can be inferred that the absence of linker and the presence of benzenesulfonamide core favored their inhibition action [66].

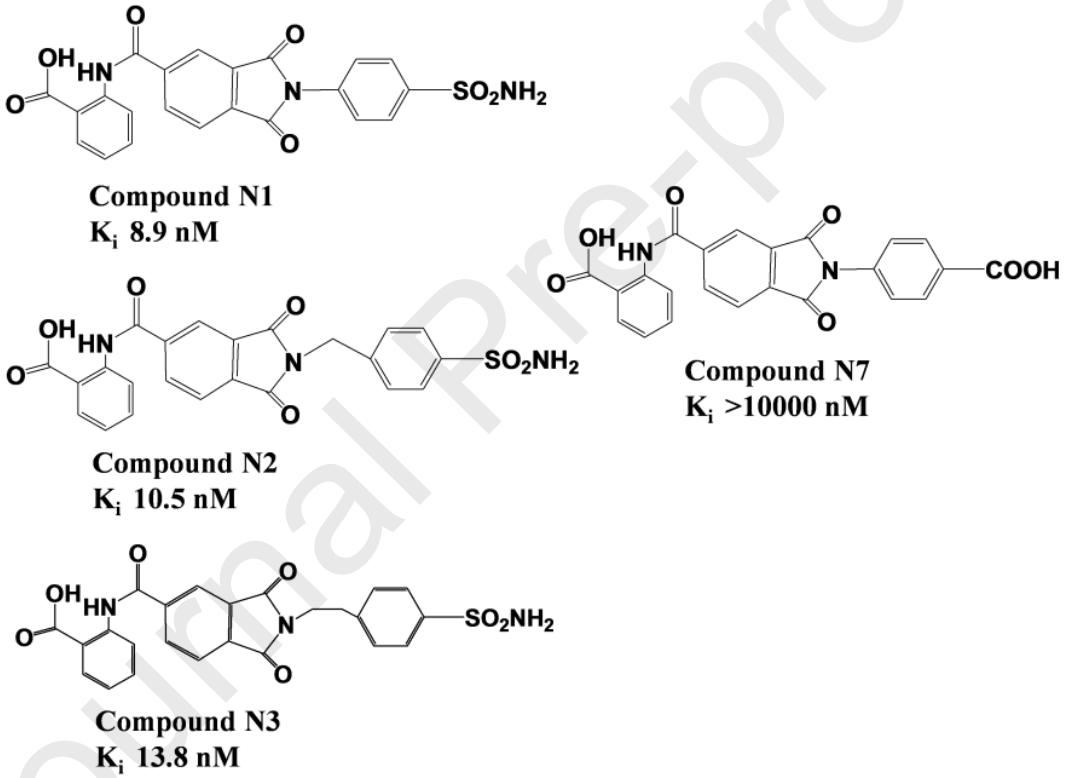
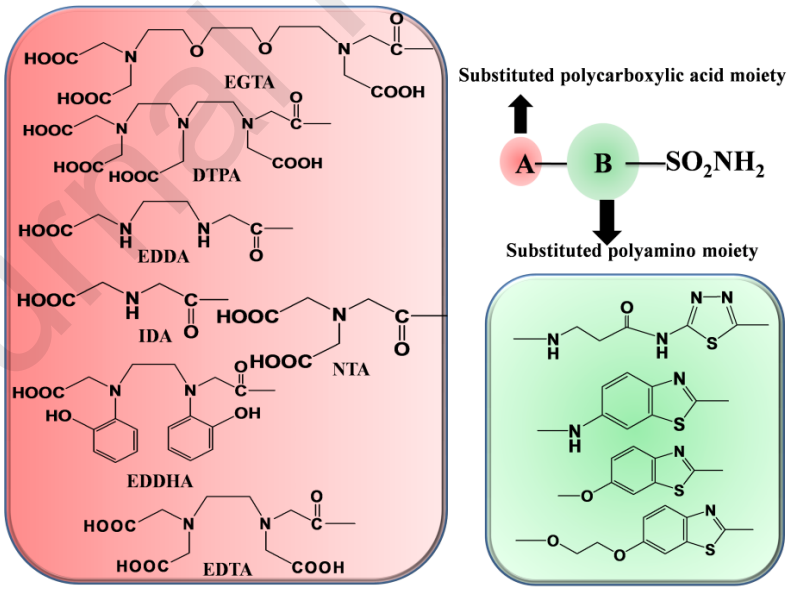


Fig. 17. SAR of 1,3-dioxo-2-substituted phenyl isoindoline-5-carboxamide moiety with sulfonamides

O. Polyamino-polycarboxylate moiety with sulfonamides.

All the bis-sulfonamides (compounds **O29-O40**) showed profound inhibition as compared to the monoamides (compounds **O1-O28**). These polyamine polycarboxylic group incorporated sulfonamides exerted a reasonable inhibitory concentration with a K_i range of 0.5-7 nM. The presence of this moiety enhanced their water solubility significantly contributing to the increase

in the duration of IOP lowering action. The inhibitory concentration of these compounds slightly varied irrespective of the presence of different heterocyclic attached amino acids. However, the opposite effect was observed for the introduction of different carboxylic acid chains in the mono-sulfonamides. EGTA (compounds **O21-O24**; K_i range of 0.8-1.5 nM) and DTPA (compounds **O17-O20**; K_i range of 0.6-1 nM) containing mono-sulfonamides were more potent than the other mono-sulfonamides (compounds **O1-O16**, **O25-O28**; K_i range of 1.5-7 nM). Similar inhibition was observed for the bis-sulfonamides (compounds **O33-O40**; K_i value is ≤ 0.6 nM) although their core contains variable polycarboxylic acid functionality. When mono-sulfonamides were acylated, the order of their inhibition was followed as IDA derivatives (compounds **O1-O4**) < NTA derivatives (compounds **O5-O8**) < EDDA derivatives (compounds **O9-O12**) < EDDHA derivatives (compounds **O25-O28**) < EDTA derivatives (compounds **O13-O16**) < EGTA derivatives (compounds **O21-O24**) = DTPA derivatives (compounds **O17-O20**). The most effective inhibition was reflected in the introduction of 2-(benzo[d]thiazol-6-yloxy) group with an ethoxy linker present in the most effective derivatives like DTPA derivative (compound **O36**), and EGTA derivative (compound **O40**). Substitution in the place of ethoxy linker by the amino linker made NTA derivatives (compound **O6**; K_i value of 7 nM) and EDDA derivatives (compound **O10**; K_i value of 7 nM) the least active in this series (Fig. 18) [67].



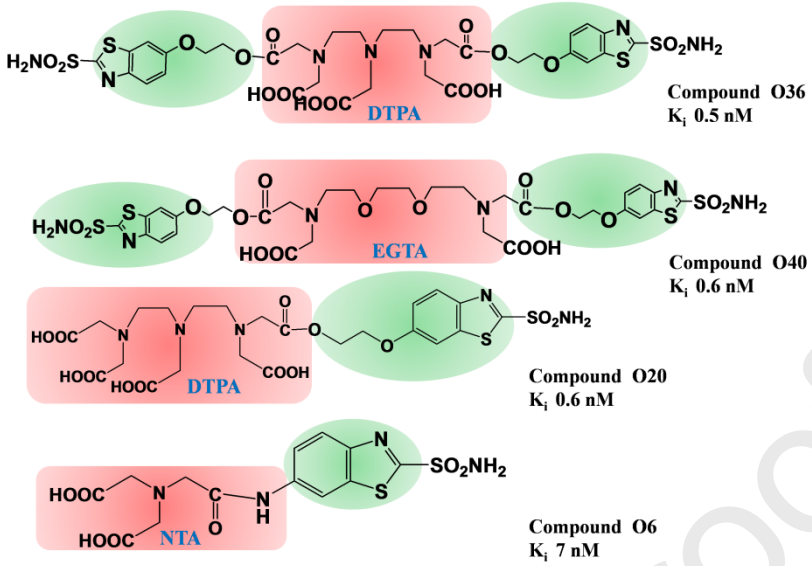


Fig. 18. SAR of Polyamino-polycarboxylate moiety with sulfonamides

P. 4-sulfamoyl phenylmethyl thiourea moiety with sulfonamides.

The most ineffective inhibition was approached by the heterocyclic and aromatic amines incorporated sulfonamides (compounds **P1-P4**; K_i range of 30-43 nM). A moderate inhibition was observed for the substituted benzoic acids (compounds **P5-P7**; K_i range of 9-11 nM). But the whole context of these compounds could be turned into prominent inhibition (K_i range of 1-10 nM) when amino acid moiety was directly attached to the sulfamoyl phenylmethyl thiourea. Incorporation of serine (compound **P13**; K_i value of 2 nM), threonine (compound **P15**; K_i value of 2 nM) and cysteine (compound **P16**; K_i value of 3 nM), methionine (compound **P17**; K_i value of 3 nM), valine (compound **P18**; K_i value of 4 nM), leucine (compound **P19**; K_i value of 3 nM), isoleucine (compound **P20**; K_i value of 4 nM), histidine (compound **P26**; K_i value of 6 nM), phenylalanine (compound **P27**; K_i value of 5 nM), tyrosine (compound **P28**; K_i value of 3 nM), dopamine (compound **P29**; K_i value of 6 nM) and aspartic acid (compound **P21**; K_i value of 7 nM), aspartame (compound **P22**; K_i value of 5 nM) showed a very good inhibition (Fig. 19). For the incorporation of aminobenzoic acids (**P5-P7**; K_i range of 9-11 nM), glycine (compound **P8**; K_i value of 17 nM), alanine **(**compound **P9**; K_i value of 10 nM**)**, beta-Alanine (compound **P10**; K_i value of 8 nM) and GABA (compound **P11**; K_i value of 10 nM), as well as those tryptophan (compound **P30**; K_i value of 9 nM), lysine (compound **P31**; K_i value of 10 nM), arginine (compound **P32**; K_i value of 18 nM), and glutamine (compound **P23**; K_i value of 10 nM) resulted in a slightly less inhibition with K_i range of 10–18 nM against hCA-II. Bulky amino acids like proline (compound **P25**; K_i value of 21 nM)

produced comparatively less inhibition than the other amino acids. The incorporation of another amino acid in the peptide chain significantly enhanced the inhibitory activity when compared to the single amino acids. Except compound **P33** (Gly-Gly derivatives) and compound **P39** (Asp-Asp derivatives), all the other oligopeptide derivatives (compounds **P33-P41)** exhibited a significant increase in inhibition (K_i range of 1-4 nM). The most potent inhibitory action was caused by the compound **P36** (K_i value of 1 nM) whereas least inhibition was seen for the compound **P1** (K_i value of 43 nM) [68].

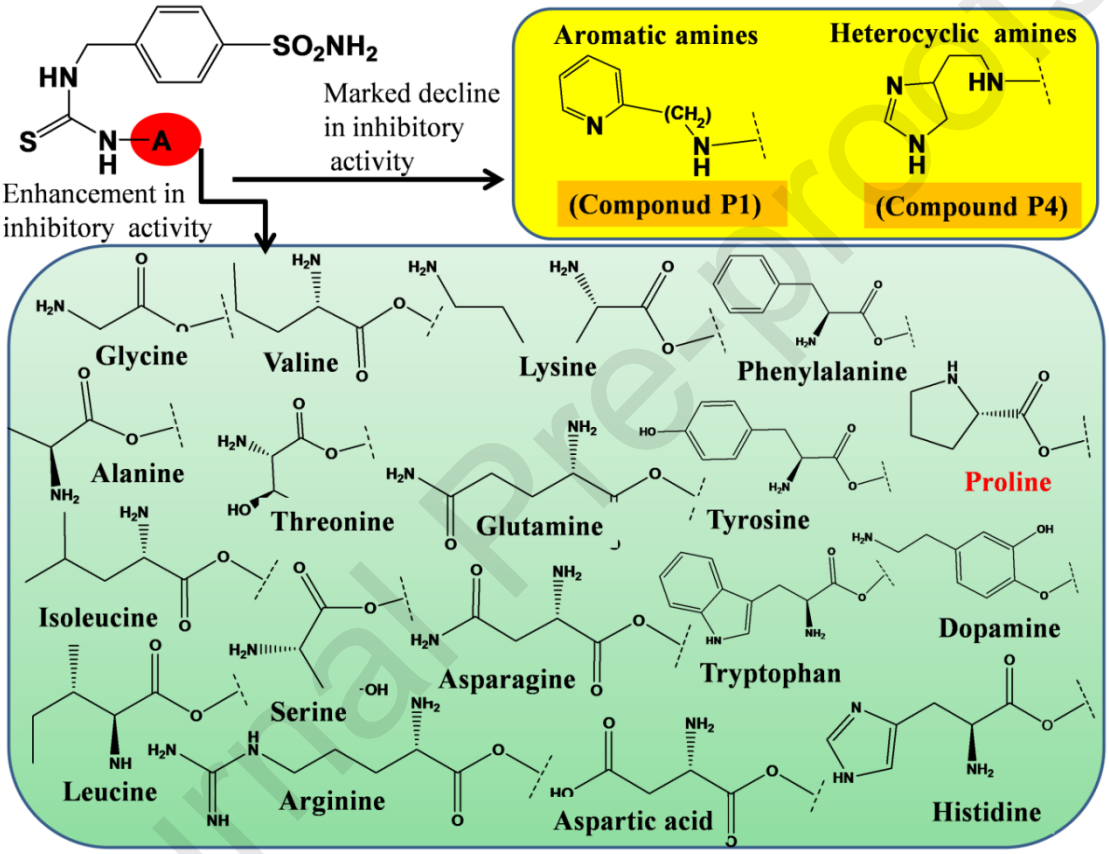


Fig. 19. SAR of 4-sulfamoyl phenylmethyl thiourea moiety with sulfonamides

Q. 7-chloro-4-ethyl-2H-chromen-2-one substituted secondary amines with sulfonamides

Under this series, a profound inhibitory activity was possessed by the heterocyclic moiety containing sulfonamides (compounds **Q17-Q23**; with K_i range of 0.5-8 nM). The exception was observed for the compound **Q24 (**K_i value of 8 nM**)** due to the presence of its bulky group. An ineffective inhibition was exerted by the mono-substituted aromatic sulfonamides (compounds **Q1-Q8**; with K_i range of 120-270 nM) whereas a moderate inhibition was reflected from the di-and tri-substituted aromatic sulfonamides (compounds **Q1-Q8**; K_i range of 12-45 nM). The order of inhibition for these aromatic compounds was tri-substituted

sulfonamides>di-substituted sulfonamides>mono-substituted sulfonamides. In here, halogen substitution did not play any significant role in the inhibition like the number of substitutions did. Due to the preferential accommodation in the binding site of hCA-II, compound **Q21 (**K_i value of 0.5 nM**)** became the most effective inhibitor. It was reported that the presence of both hydrophilic as well as hydrophobic structural elements in 7-chloro-4-ethyl-2H-chromen-2-one (Ccm moiety) provided significantly high water solubility and increased liposolubility for these compounds. Therefore, it can be inferred that existence of heterocyclic moiety in the 7-chloro-4-ethyl-2H-chromen-2-one (Ccm) incorporated sulfonamides is necessary for better inhibition of this cytosolic dominant isoform (Fig. 20) [69].

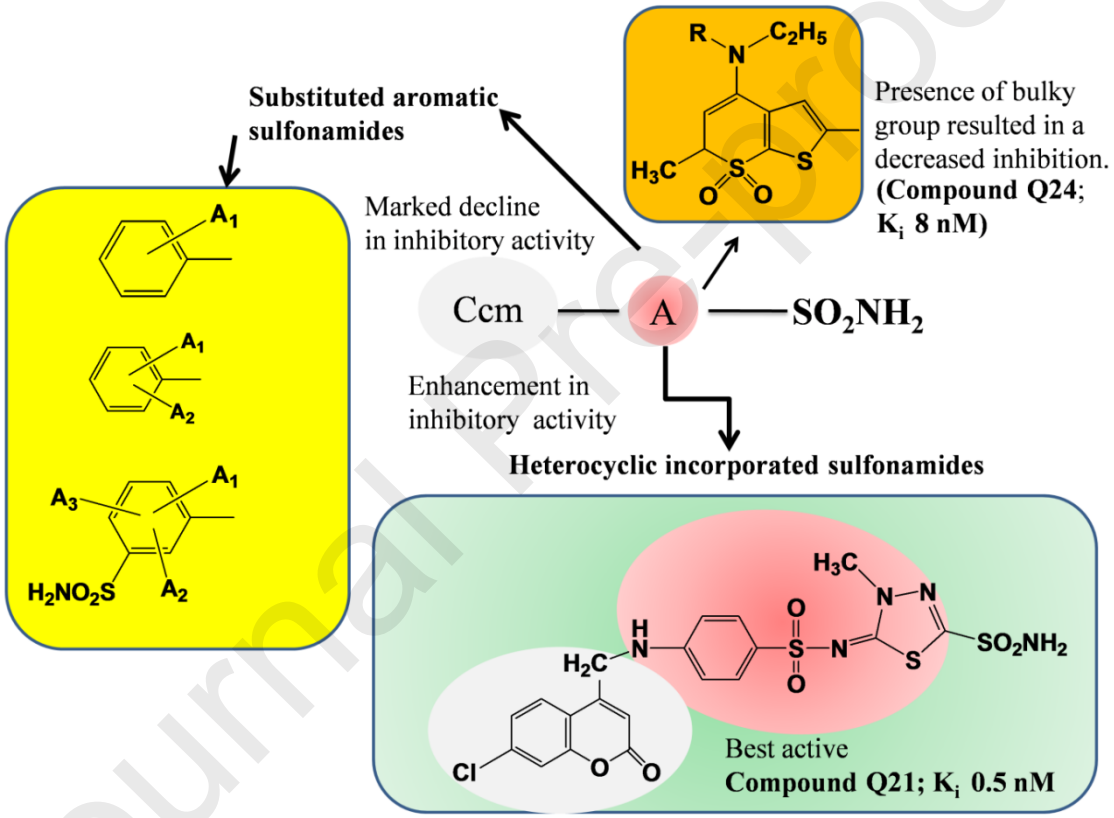


Fig. 20. SAR of 7-chloro-4-ethyl-2H-chromen-2-one substituted secondary amines with sulfonamides.

R. Thienothiopyran-2-sulfonamides

The 5,6-dihydro-4H-thieno [2,3-*b*] thiopyran-7,7-dioxide introduced sulfonamides showed a reasonable good inhibitory activity with a K_i range of 0.61 nM - 71 nM. Substituents such as unbranched or branched alkyl amine significantly enhanced the inhibitory profile of these compounds (compounds **R4-R14**) when compared to other substituents (compounds **R1-R3**). An important aspect noted here was that the presence of secondary amino groups (compounds

R5-R9, **R10-R14)** favored the inhibition more than the primary amines (compound **R5**; K_i value of 3.7 nM) and tertiary amines (compound **R9**; K_i value of 9.3 nM). The way of inhibition depended on the stereochemical configuration. The meso-configuration (compound **R6**; K_i value of 0.69 nM, the most potent compound) and S absolute stereochemical configuration (compound **R7;** K_i value of 0.82 nM) facilitate better inhibition of hCA-II than the R absolute stereochemical configuration (compound **R8**; K_i value of 16.0 nM). Furthermore, the extent of inhibition was significantly improved for the more bulkiness in secondary amino group (compound **R10**; K_i value of 1.1nM, compound **R11**; K_i value of 1.8 nM compound **R12**; K_i value of 0.7 nM**,** compound **R13**; K_i value of 0.61 nM**)** (Fig. 21). Compound **R14** was the least active compound with a K_i value of 71 nM [70].

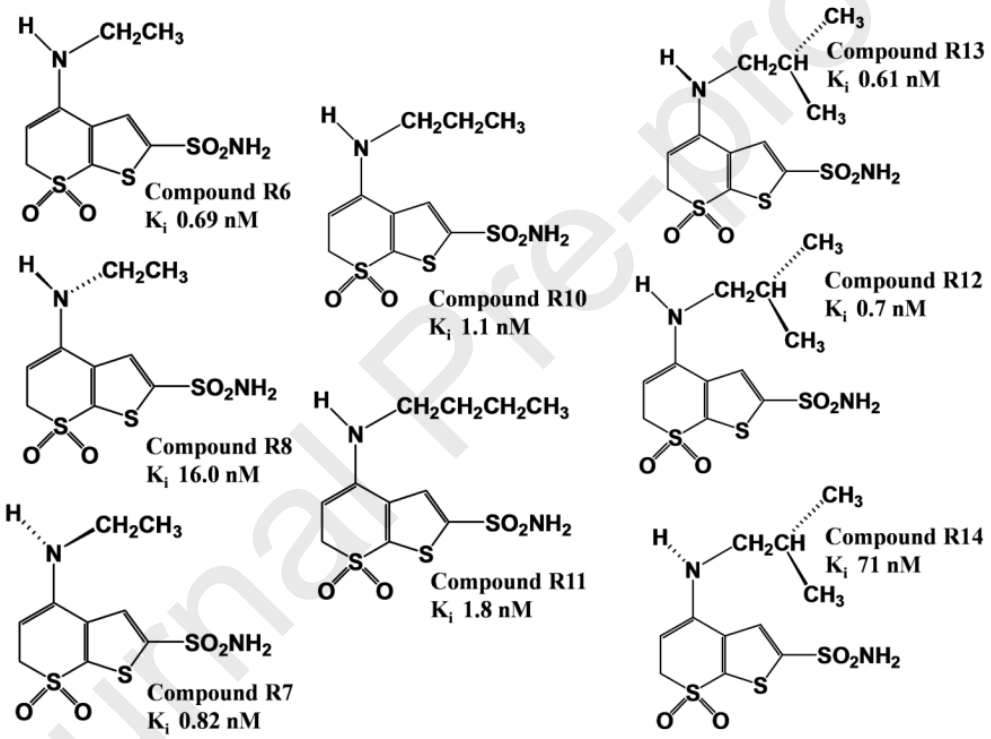


Fig. 21. SAR of Thienothiopyran-2-sulfonamides

S. 5,6-dihydro-4H-thieno [2,3-*b*] thiopyran-7,7-dioxide with sulfonamides

The most prominent inhibition was found due to the presence of hydrazine functionality in the 4th position of the thiophene ring (compound **S5**; K_i value of 2 nM). From their SAR depicted in Fig. 22, it was observed that replacement of this functional group by the other groups like ketone (compound **S2**; K_i value of 5 nM, compound **S7**; K_i value of 6.3 nM, compound **S10**; K_i value of 16 nM and compound **S12**; K_i value of 6.8 nM), hydroxide (compound **S3**; K_i value

of 5.8 nM), hydroxyl (compound **S1**; K_i value of 13 nM), secondary amino group (compound **S11**; K_i value of 590 nM), resulted in a significant decrease in their inhibitory action. Moreover, the presence of bulkier group enhanced their action as it was confirmed form the compound **S3** (K_i value of 5.8 nM) and compound **S9** (K_i value of 15 nM). Due to the absence of necessary functional group and less bulkiness, compound **S9** became the least active compound [71]. Therefore, we may take the hydrazine group can be taken as the beneficiary structural facts while incorporating the 5,6-dihydro-4H-thieno [2,3-*b*] thiopyran-7,7-dioxide moiety into the sulfonamides with an appropriate unbranched alkyl chain.

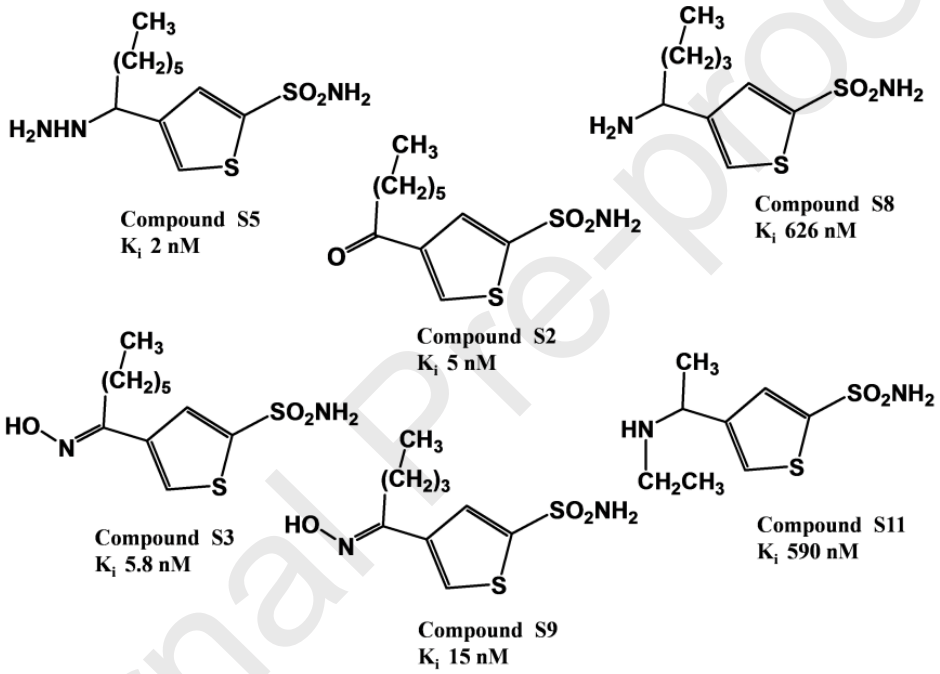


Fig. 22. SAR of 5,6-dihydro-4H-thieno [2,3-*b*] thiopyran-7,7-dioxide with sulfonamides

T. Sulfonamides with heterocyclic moieties like mercaptans, sulfenamides, and metal complexes

The inhibitory activity of the reported compounds [72] was greatly influenced by the nature of incorporating groups. A significant variance in their inhibitory concentration was observed for the substitution pattern of the 1,2,4-triazole moiety. The order of inhibition followed as sulfonamides (compounds **T10-T13**; K_i range of 4 ± 0.3 nM – 27 ± 3 nM) >sulfonamides (compounds **T6-T9**; K_i range of 10 ± 1 nM – 49 ± 3 nM) > mercaptans (compounds **T6-T9**; K_i range of 96 ± 8 nM – 210 ± 8 nM). The presence of long alkyl chain produced detrimental effect for these compounds (compound **T4**; K_i value of 210 ± 8 nM**,** compound **T9**; K_i value of

49±3 nM - compound **T13**; K_i value of 27 ± 3 nM). However, presence of halogen with an ethyl substitution at the 1st position of this moiety facilitated a more potent inhibition for the compounds. It was observed that bromine as a halogen substitution in this moiety (compound **T3**; K_i value of 162 ± 4 nM**,** compound **T8**; K_i value of 10 ± 1 nM compound **T12**; K_i value of 4 ± 0.3 nM), enhances inhibitory activity significantly as compared to the other halogens. Moreover, a different scenario took place when these compounds contained metals like Zn, Cu in their interior (Fig. 23). Compounds **T14-T21** showed a more effective inhibitory profile (K_i range of 0.2±0.8 nM - 10±0.07 nM) against this isoform. As an example, compound **T12** exerts most profound inhibition when it gets complexed either Zn (compound **T16**; K_i value of 0.5±1 nM) or with Cu (compound **T20**; K_i value of 0.2±0.8 nM, the best active compound). Therefore, it can be concluded that both the presence of metals and the presence of bromine with an ethyl substitution at 1st position of this moiety may produce favourable outcomes for their inhibitory action.

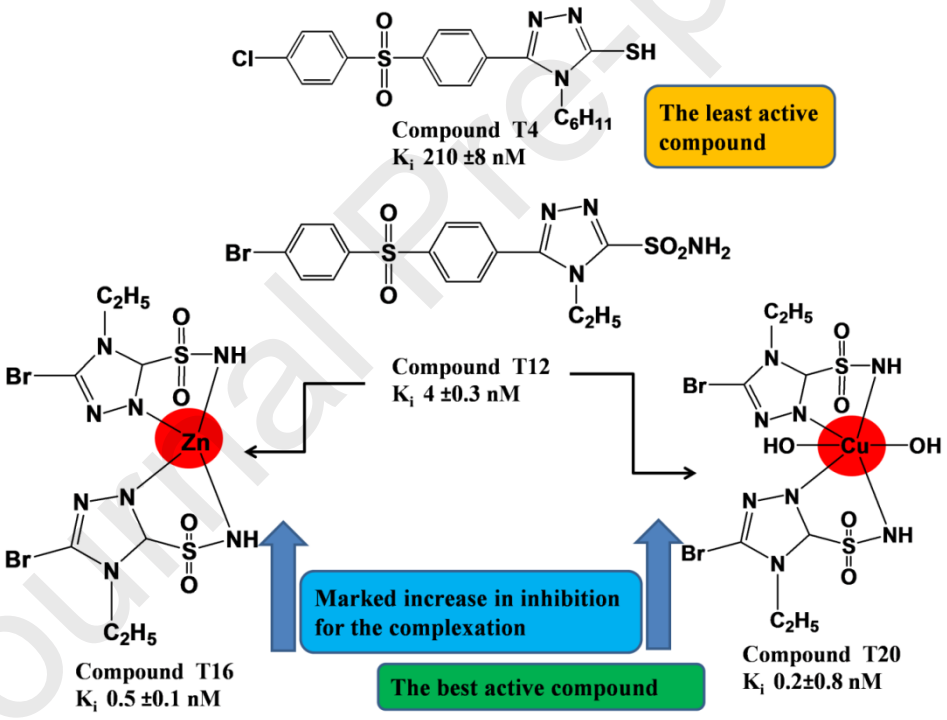


Fig. 23. SAR of various heterocyclic moieties with sulfonamides

U. N-hydroxy sulfonamides

A moderate to high inhibitory profile was addressed by this class of sulfonamides. Compounds **U1-U20** produced a more prominent effect with a K_i range of 0.8±0.1 - 190 ± 4 nM. Depending on the substitution in the benzene ring attached to *N*-hydroxy sulfonamides, their action became significantly varied. Under this scaffold, compound **U16** (K_i value of 0.8±0.1 nM) becomes the

most potent inhibitor for the presence of unsubstituted benzene ring. Substituted benzene decreased their potency as observed in the least active compound **U2** (K_i value of 190±4 nM). Halogen substitution in benzene ring significantly improved their inhibitory action. The order of inhibition observed for the presence of different halogens was: -I (compound **U7**; K_i value of 13±2 nM) > -Br (compound **U6**; K_i value of 18±2 nM) > -F (compound **U4**; K_i value of 19±1 nM) > -Cl (compound **U5**; K_i value of 21±0.8 nM). Deactivating group (-O_2N) substituted benzene helped these compounds (compound **U9**-**U12**; K_i range of 4±0.3 - 9 ±0.3 nM) for exerting more enhanced inhibition than the activating groups (-CH_3 -, -H_2N) substituted benzene (compound **U8**; K_i value of 70±4 nM, compound **U14**; K_i value of 37±1 nM, and compound **U15**; K_i value of 45±3 nM). Their inhibitory concentration slightly varied due to the presence of deactivating group in different positions of the benzene ring. Normally ortho-substituted (compound **U11**; K_i value of 5 ± 0.4 nM), and meta- substituted *N*-hydroxy sulfonamides (compound **U10**; K_i value of 5 ± 0.4 nM) were more potent than the para-substituted ones (compound **U9**; K_i value of 9 ± 0.3) (Fig. 24). Moreover, their action slightly increased in the presence of halogen along with the deactivating group substituted benzene ring (compound **U12**; K_i value of 4 ± 0.3 nM). A similar effect was seen for the presence of the trifluoromethyl group in the place of benzene ring (compound **U3**; K_i value of 3 ± 0.3 nM). Compounds **U17** (K_i value of 9 ± 0.1 nM) and **U18 (**K_i value of 8 ± 0.1 nM**)** produced moderate inhibition for the presence of carboxyl group in ortho position. Besides these twenty compounds, some other types of compounds (compounds **U21**-**U25**) also showed a better inhibition with a low K_i range of 1 ± 0.1 nM – 18 ± 2 nM [73].

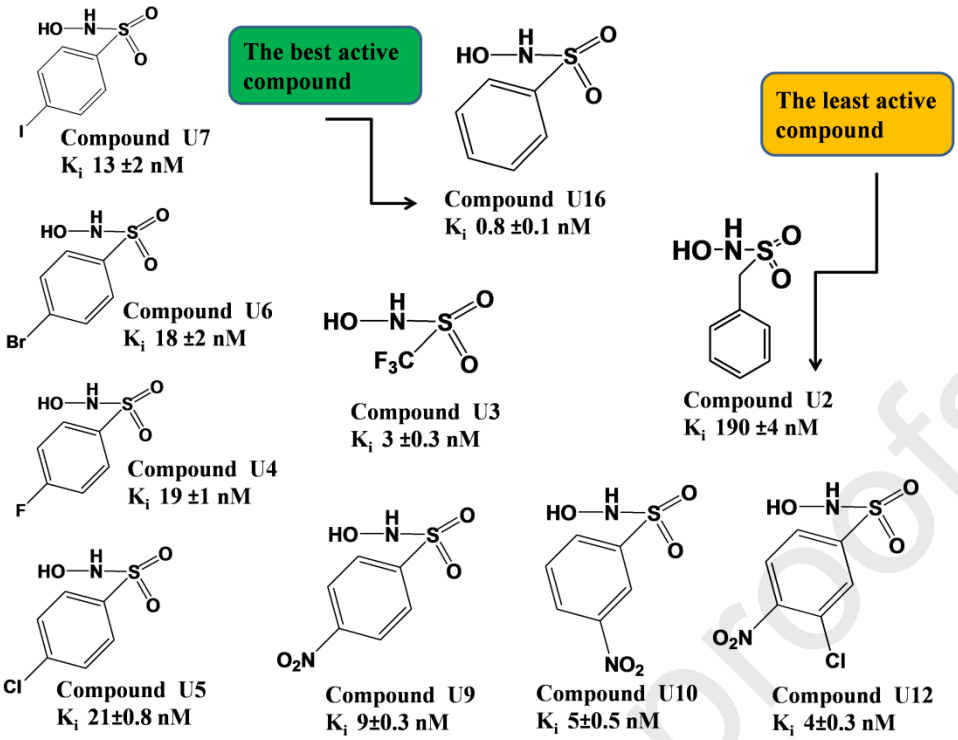


Fig. 24. SAR of *N*-hydroxy sulfonamides

V. Sulfenamido with sulfonamides

Under this scaffold, the effect of attachment of various groups like 2-nitrophenyl sulfane, 4-nitrophenyl sulfane, 1-sulfonyl-2-nitrobenzene, and 1-sulfonyl-4-nitrobenzene with the sulfenamido-sulfonamides (compounds **V1-V40**; K_i value in the range of 10-320 nM), the bis-sulfonamides (compounds **V41-V50**; K_i value in the range of 9-93 nM) and1,3,4-thiadiazole-sulfonamides (compounds **V51-V60**; K_i value in the range of 0.1-19 nM) on the inhibitory potency, was studied (Fig. 26). Their order of inhibition was followed as mentioned in Fig. 25. Monohalogen substituted sulfenyl amido-mono sulfonamides (compounds **V11-V25**; K_i value in the range of 10-110 nM) were slightly more effective than the di-halogen substituted sulfenamido-monosulfonamides (compounds **V26-V40**; K_i value in the range of 15-220 nM). Moreover, the inhibitory effect of fluorine was observed to be greater as compared to chlorine and bromine for nitrophenyl sulfane derivatives (compounds **V14-V19**), and 1-sulfonyl nitrobenzene derivatives (compounds **V20-V25**). However, diiodo-substituted sulfenamido monosulfonamides **V28**, **V31**, **V34,** and **V37** exhibited more promising inhibition against this isoform than the other di-halogen substituted sulfonamides under the same class.

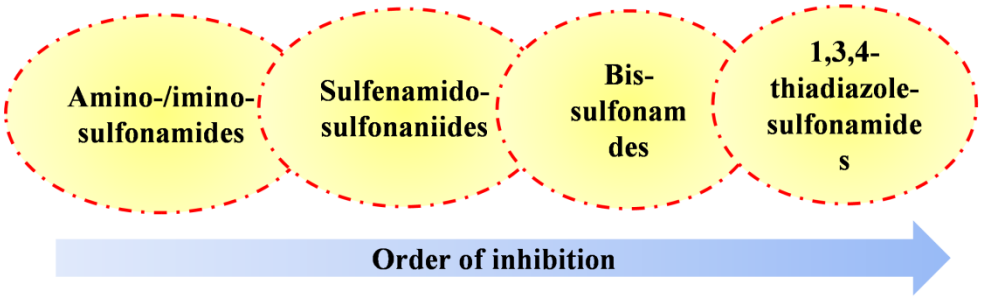


Fig. 25. The relation between the order of inhibition of hCA-II and the different with heterocyclic moieties

4-nitrophenyl-derivatives produced a significant potent inhibition as compared to the corresponding 2-nitrophenyl derivatives. The orthanilamide (compounds **V1-V5**), as well as 4-hydrazino-benzenesulfonamide (compounds V6-V10) derivatives, were generally the least active CA inhibitors as compared to the 1,3,4-thiadiazole-sulfonamides (compounds **V51-V60**; K_i range of 0.1-19 nM). A prominent nanomolar inhibition (K_i value in the range of 0.1-0.2 nM) was reflected from the compounds **V54**, **V55**, **V59,** and **V60** due to the presence of both 1-sulfonyl-2-nitrobenzene, and 1-sulfonyl-4-nitrobenzene in their preferable substitution positions [74].

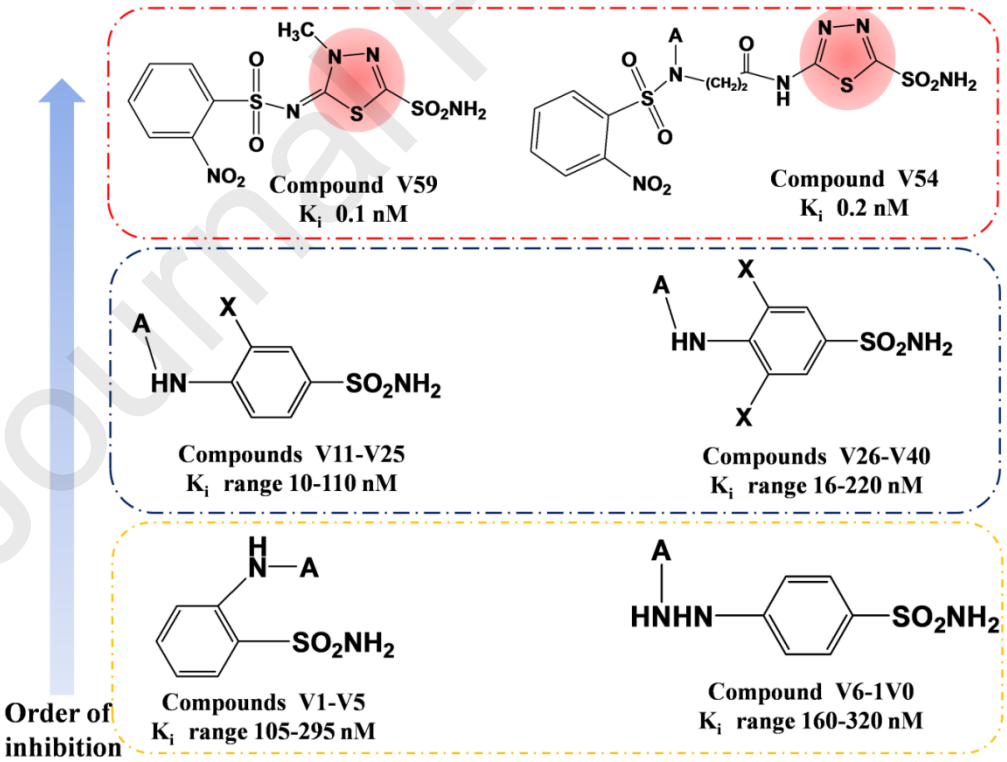


Fig. 26. SAR of sulfenamido-sulfonamides

W. Sulfonamides with furan, thiophene, and pyrrole carboxamide groups

With a K_i range of 3-105 nM, heterocyclic substituted sulfonamides proved to be one of the effective inhibitors of hCA-II. Depending on the nature of heterocyclic moiety, the inhibitory action of these compounds significantly varied. The presence of fluorine in the benzene ring directly linked with the pyrrole carboxamide group (compound **W2**; K_i value of 10 nM) enhanced the extent of inhibition 10 times than the unsubstituted benzene (compound **W1**; K_i value of 105 nM, the least active compound) (Fig. 27). However, in the absence of substitution in benzene ring, the linker played a vital role in inhibition. Compound **W6** (K_i value of 8 nM) and compound **W7** (K_i value of 7 nM) showed more prominent effect than the compound **W1** due to the presence of methylene and ethylene linker, respectively. It was also noted that fluorine substituted benzene showed similar effect in furan (compound **W9**; K_i value of 10 nM), pyrrole (compound **W2**), and thiophene (compound **W16**; K_i value of 8 nM) carboxamide group incorporated sulfonamides. Moreover, the order of inhibitory action for thiophene attached sulfonamides having different halogen substitution was: Cl (compound **W17**; K_i value of 7 nM) > F (compound **W16**; K_i value of 8 nM) > I (compound **W19**; K_i value of 9 nM) > Br (compound **W18**; K_i value of 12 nM). The inhibition can be dramatically changed if 1,3,4-thiadiazoline-2-sulfonamide moiety is introduced in the core of heterocyclics (compounds **W22-W25**). Methyl substitution at 3rd position (compound **W24**; K_i value of 3 nM, compound **W25**; K_i value of 4 nM) of this moiety brought more favorable outcomes than the unsubstituted moiety (compound **W22**; K_i value of 6 nM, compound **W23**; K_i value of 6 nM) [75].

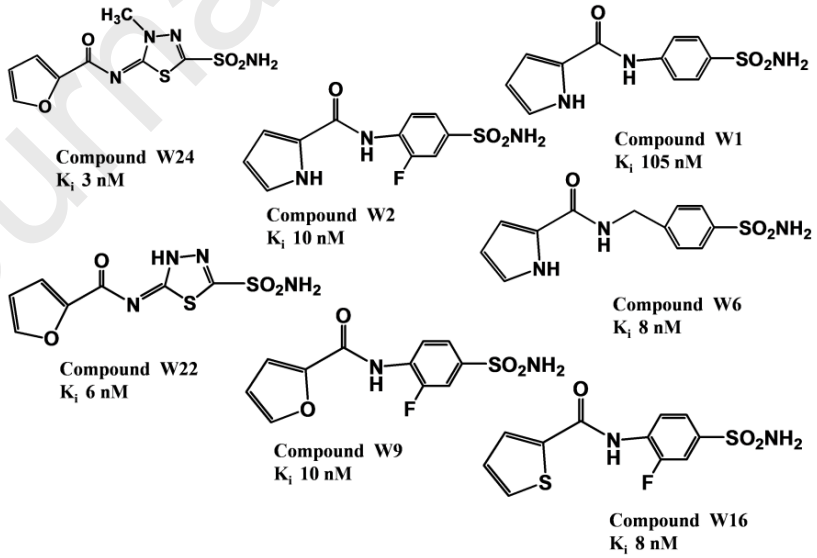


Fig. 27. SAR of sulfonamides with furan, thiophene, and pyrrole carboxamide groups

X. Isonicotinoyl moiety with sulfonamides

Inclusion of iso-nicotinoyl group into the active core of the sulfonamides brought a low to high nanomolar range of inhibition with a K_i value in the range of 1-320 nM. Their inhibitory activity greatly varied with the presence of amino group, hydrazine group, aminoethyl group, alkoxy group, halogens along their different substitution position of benzenesulfonamide. In the case of anilamide derivatives, *para*-substitution (compound **X4**; K_i value of 130 nM) was more preferable than the *ortho*-substitution (compound **X1**; K_i value of 290 nM) and *meta*-substitution (compound **X3**; K_i value of 280 nM). For sulfanilamides, presence of halogens like F, Cl, Br, and I made these derivatives (compounds **X9-X12**; K_i value in the range of 31-42 nM) more effective towards inhibition of this isoform. Another substitution brought out by the second halogen resulted in a slight increase in the inhibitory action of these 1,3-benzene disulfonamides (compounds **X13-X14**; K_i value in the range of 30-37 nM). Moreover, another important aspect observed, like in the case of other scaffolds (Scaffolds O, Q, V, W, Y, Z, AA, & AB) was that the incorporated heterocyclics have influenced the inhibitory profile of these derivatives (compounds **X15-X21**; K_i value in the range of 1-5 nM). In comparison to the thiadiazoline containing sulfonamides (compounds **X15-X17**; K_i value in the range of 2-4 nM), benzothiazole moiety (compounds **X18-X20**; K_i value in the range of 1-5 nM) exerted more prominent inhibition (Fig. 28). Under this series, compound **X20** was the most effective inhibitor whereas compound **X4** was seen to be the least active compound [76].

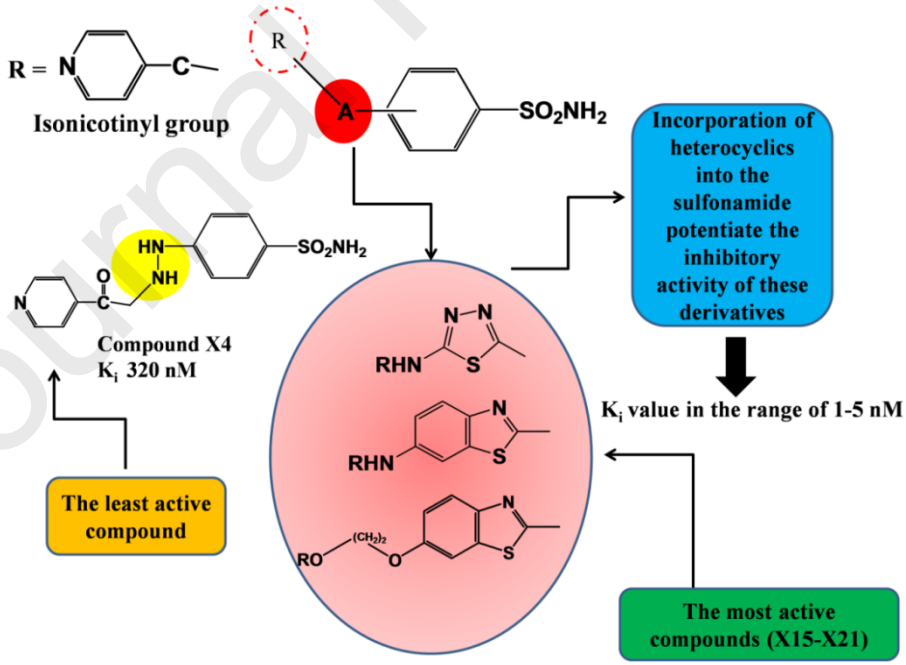


Fig. 28. SAR of iso-nicotinoyl moiety with sulfonamides

Y. Aminoacyl/dipeptidyl moiety with sulfonamides

The inhibition profile of these aminoacyl or dipeptidyl moiety incorporated sulfonamides were turned to be more effective towards this physiologic dominant isoform of CA than those of simple aromatic sulfonamides (compounds **Y1**, **Y6**, **Y11**, **Y16**, **Y21**, **Y26**, **Y31**, **Y36**, **Y41**, **Y46**, **Y51**, **Y56**, **Y61,** and **Y66**; K_i value in the range of 40-320 nM). Depending on the nature of amino acids from which the aminoacyl or dipeptidyl moiety were obtained, the order of inhibition was followed as Gly derivatives (**R_1** type) <GlyGly derivatives (**R_4** type) < Ser derivatives (**R_2** type) < Creatine derivatives (**R_3** type). The presence of different substituents like amino group, hydrazine group, aminoethyl group, alkoxy group as well as the presence of halogens in their definite substitution position of these sulfonamides, significantly led in a variable inhibition. The influence of fluorine in the inhibition was comparatively higher than that of the other halogens. Except compound **Y91** (K_i value 0.6 nM, the most effective inhibitor),as previously mentioned, here also the simple aromatic sulfonamide derivatives (compounds **Y71**, **Y76**, **Y81**, **Y86**, **Y91**, **Y96**, **Y101**, **Y106**, **Y111**, **Y116**, **Y121,** and **Y126**; K_i value in the range of 2-60 nM) showed slightly less potency than the heterocyclic sulfonamides.

With the presence of the heterocyclic fragments like 1,3,4-thiadiazoles, 1,3,4-thiadiazolines, benzothiazoles, and thieno-thiopyransulfonamides, these aminoacyl/ dipeptidyl incorporated sulfonamides exert their promising potent inhibitory action. Slightly less inhibition was observed for the 1,3-benzenedisulfonamide and 3-fluorosulfanilamide derivatives, together with the pyrimidine substituted sulfanilamides (compounds **Y106-110**; K_i value in the range of 12-23 nM), and the sulfanilyl sulfanilamides (compounds **Y96-100**; K_i value in the range of 6-12 nM), and sulfanilyl metanilamides (compounds **Y101-105**; K_i value in the range of 8-13 nM). The order of inhibition was followed as the 1,3,4- thiadiazole-2-sulfonamides (compounds **Y71**, **Y81**, and **Y86**) = the 4-methyl- *ä*2-1,3,4-thiadiazoline-2-sulfonamides (compounds **Y76** and **Y91**) = the benzothiazole-2-sulfonamides (compounds **Y111**, **Y116,** and **Y121**) = the dorzolamide derivatives (compound **Y126**) (Fig. 29) [77].

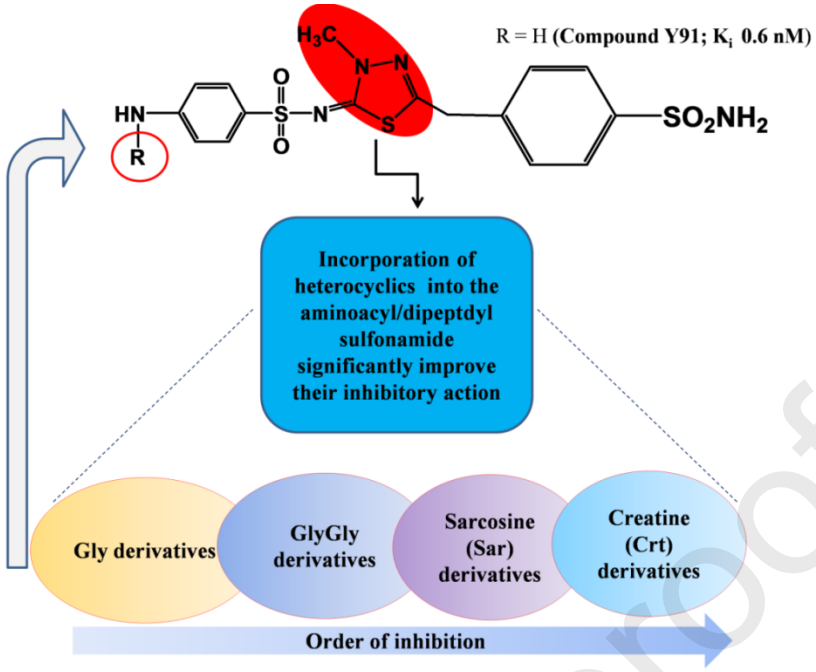


Fig. 29. SAR of aminoacyl/dipeptidyl moiety with sulfonamides having 1,3,4-thiadiazole heterocyclic

Z. Perfluoroalkyl/aryl-substituted derivatives of sulfonamides

The biological activity of perfluoroalkyl-aryl-substituted derivatives was greatly influenced by the nature of the acylating/sulfonylating moiety having perfluoroalkyl/aryl groups. For the attachment of these groups, the order of inhibition was followed as $C_6F_5SO_2Cl$ (**R_3**)> $C_4F_9SO_2Cl$ (**R_2**) = C_6F_5COCl (**R_6**)> $C_8F_{17}SO_2Cl$ (**R_4**) = $C_8F_{17}COCl$ (**R_5**)> CF_3SO_2Cl (**R_1**) (Fig. 30).

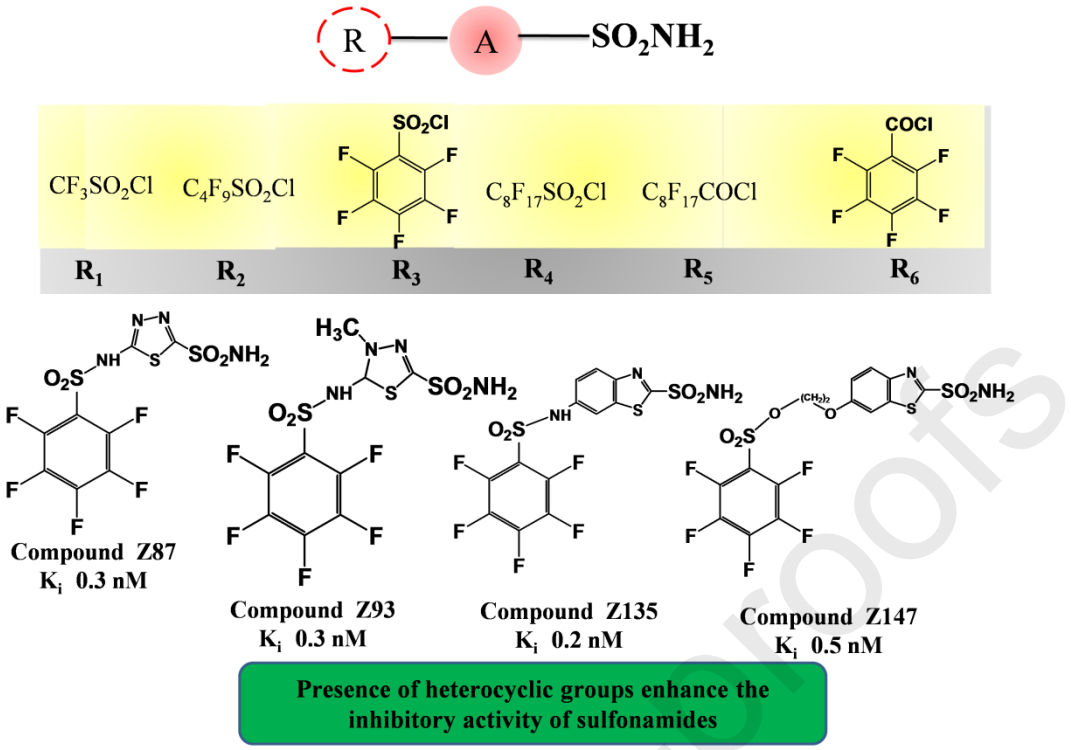


Fig. 30. Inhibitory activity profile of different heterocyclic moiety with perfluoroalkyl/aryl-substituted derivatives of sulfonamides

As like the previously discussed scaffolds (Scaffolds **O**, Q, **V**, **W**, **Y**), inhibitory activity shown by these derivatives varied with the presence of the similar substituents. The most efficient inhibitors included the heterocyclic sulfonamide derivatives, such as the 1,3,4-thiadiazole-2-sulfonamides (compound **Z87**; K_i value of 0.3 nM, compound **Z99**; K_i value of 0.4 nM) and the corresponding thiadiazolines (compound **Z93**; K_i value of 0.3 nM), as well as the benzothiazole-2-sulfonamide derivatives (compounds **Z133-Z150**; K_i value in the range of 0.2 - 3 nM). In the aromatic sulfonamide series, a moderately active inhibition was shown by the derivatives of orthanilamide (compounds **Z1-Z6**; K_i value in the range of 24 - 20450 nM), metanilamide (compounds **Z7-Z12**; K_i value in the range of 10-18700 nM) and sulfanilamide (compounds **Z13-Z18**; K_i value in the range of 10 - 10900 nM), whereas an effective inhibition was shown by the derivatives of halogen sulfanilamides (compounds **Z49-Z72**; K_i value in the range of 9 - 1300 nM), and by the benzene-1,3- disulfonamides (compounds **Z73-Z84**; K_i value in the range of 5 - 430 nM). The most prominent inhibitory action emerged from the derivatives containing benzothiazole functionality in its active core [78].

AA. Sulfonamides with diethylenetriaminepenta acetic acid (dtpa) tails and of their zinc complexes

Incorporation of diethylenetriaminepenta acetic acid (dtpa) moiety into the core of sulfonamides led to a significant enhancement in the inhibitory activity of these synthesized compounds. Among all the derivatives investigated here, heterocyclic sulfonamides were found to be the most effective inhibitors. The variance in the inhibition was usually observed due to the attachment of different groups and it is followed as *p*-hydrazino-benzenesulfonamide (compound **AA4**, K_i value of 250 nM) < the orthanilamides (compound **AA1**, K_i value of 210 nM) < the metanilamides (compound **AA2**, K_i value of 180 nM)< the sulfanilamides (compound **AA3**, K_i value of 75 nM) < the *p*-aminomethylbenzenesulfonamides (compound **AA5**, K_i value of 23 nM) < the *p*-aminoethyl-benzenesulfonamide (compound **AA6**, K_i value of 15 nM) <the halogen-substituted sulfonamides (compounds **AA9-AA12**, K_i value in the range of 12-20 nM) <the 1,3-benzene-disulfonamides (compounds **AA13-AA14**, K_i value in the range of 10-12 nM) < the 1,3,4-thiadiazole-2-sulfonamides (compounds **AA15** and **AA17**, K_i value of 0.9 nM and 1 nM, respectively), 4-methyl-1,3,4-thiadiazoline-2-sulfonamide (compounds **AA16**, K_i value of 2 nM), the benzothiazole-2-sulfonamides (compounds **AA18-AA20**, K_i value in the range of 0.6-0.8 nM).

Moreover, the scenario became more favourable towards more effective inhibition due to the attachment of another substituted benzenesulfonamides with the active core. As a result, heterocyclic containing bis-sulfonamides (**AA35-AA40**) showed more prominent action in the nanomolar level (K_i value in the range of 0.5-1 nM) as compared to the heterocyclic substituted mono-sulfonamides (compounds **AA15-AA20**; K_i value in the range of 0.6-2 nM).

The presence of metal complex like zinc in the center of the bis sulfonamides (compounds **AA41-AA45**; K_i value in the range of 0.4-16 nM), produced the most efficient inhibition as compared to normal bis sulfonamides. Therefore, it may be concluded that metal complexes incorporated in the bis sulfonamides together with the presence of appropriate heterocyclic functionality can exhibit more prominent inhibitory activity than the reported scaffolds [79].

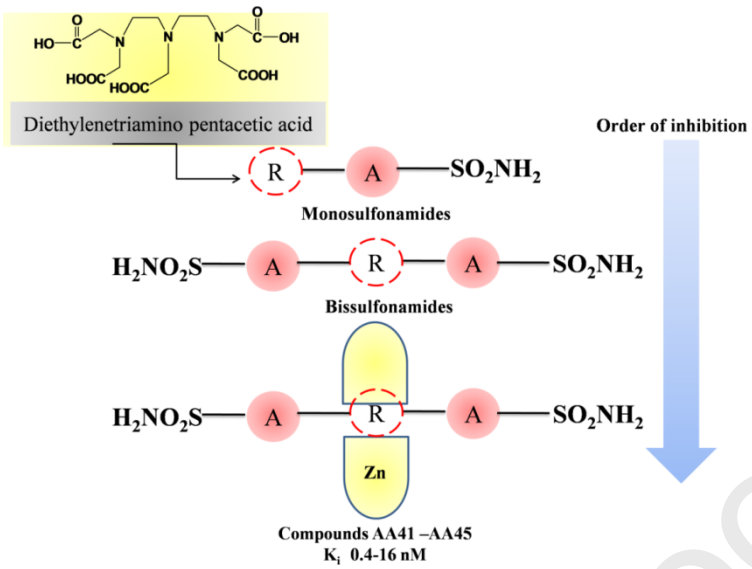


Fig. 31. SAR of sulfonamides with diethylenetriaminepenta acetic acid (dtpa) tails and of their zinc complexes

AB Dithiocarbamate moiety

The scientific reports from S. Avram*et al*., 2013, revealed that dithiocarbamates can effectively induce the potential inhibition of hCA-II in low micromolar or submicromolar range besides its prominent inhibitory effect on other hCA isoforms (hCA-I, IX, and XII). Except for compound **AB12** (K_i value of 6910 nM) and **AB13** (K_i value of 3100 nM), all other investigated compounds from **AB1**-**AB25** had shown an effective inhibition with a K_i value in the range of 0.70 -325nM. From their SAR, it was observed that benzyl group (compound **AB6**; K_i value of 0.70 nM), iso-butyl group (compound **AB15**; K_i value of 0.95 nM), di-ethyl ether group (compound **AB23**; K_i value of 0.95 nM) mostly favoured their inhibitory action. Phenyl substituted dithiocarbamate (compound **AB1**; K_i value of 4.5 nM) showed a slight decrease in inhibition as compared to the benzyl substituted one (compound **AB6**). Similar effect was seen from heterocyclic moiety like 2-thiazolyl containing compound (compound **AB9**; K_i value of 4.6 nM). Presence of branched alkyl chain in these derivatives (compound **AB15**) resulted in a more favorable outcome as compared to the unbranched alkyl chain (compound **AB16**; K_i value of 55.5 nM, compound **AB17**; K_i value of 50.9 nM, compound **AB18**; K_i value of 51.3 nM) (Fig. 32). Therefore, it may be concluded that the presence of hydrophobic moiety like branched alkyl chain or simply the benzyl substitution in the core of dithiocarbamates is beneficial for the enhancement of their inhibitory action against this isoform [80].

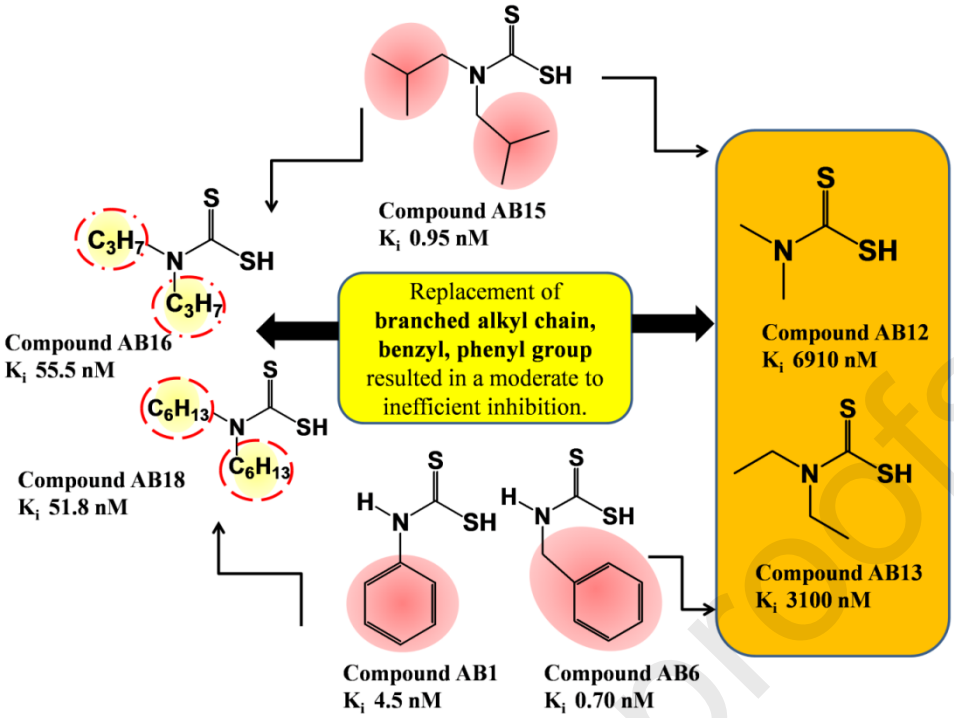


Fig. 32. SAR of dithiocarbamate moiety

AC. Selenide moiety

Selenides incorporated sulfonamides showed a reasonably good inhibition in the low nanomolar range of K_i except compound **AC5** which exerted its inhibitory activity at 920.8 nM. Incorporation of another selenium in the place of cyanate (compound **AC2**; K_i value of 7.9 nM) contributed six times increase in the inhibitory activity as compared to the selenocyanate derivative (compound **AC1**; K_i value of 53.1 nM). Inclusion of β-hydroxyl group in the selenides (compound **AC3-AC9**; K_i range of 0.18- 8.8 nM) except compound **AC5** produced a profound inhibitory potency for these derivatives as it is described in their SAR (Fig. 33). Attachment of various groups like benzyl (compound **AC3**; K_i value of 1.4 nM), propyl (compound **AC4**; K_i value of 4.4 nM) etc by means of ether linkage, produced slight increase in the inhibition than the direct attachment of butyl (compound **AC3**; K_i value of 8.8 nM), bromine (compound **AC3**; K_i value of 4.9 nM). Introduction of the alkyl-substituted cyclohexyl group together with the presence of hydroxyl group at the 2nd position makes compound **AC9** (K_i value of 0.18 nM), the most potent inhibitor of hCA-II in this series. When the N terminal of these compounds (compound **AC10-AC12**; K_i range of 14.0- 90.2 nM) are protected by Tosyl or Boc group, they produced a marked decrease in their inhibition potency as compared to the unprotected β-amino selenides (compound **AC8**; K_i value of 0.51 nM) [81]. Therefore, it may be assumed that for selenides incorporated sulfonamides, an unprotected amino group having branched alkyl chain or a cyclohexyl group having hydroxyl functionality can be taken

into consideration while designing these type of effective and more potent hCA-II inhibitors in near future.

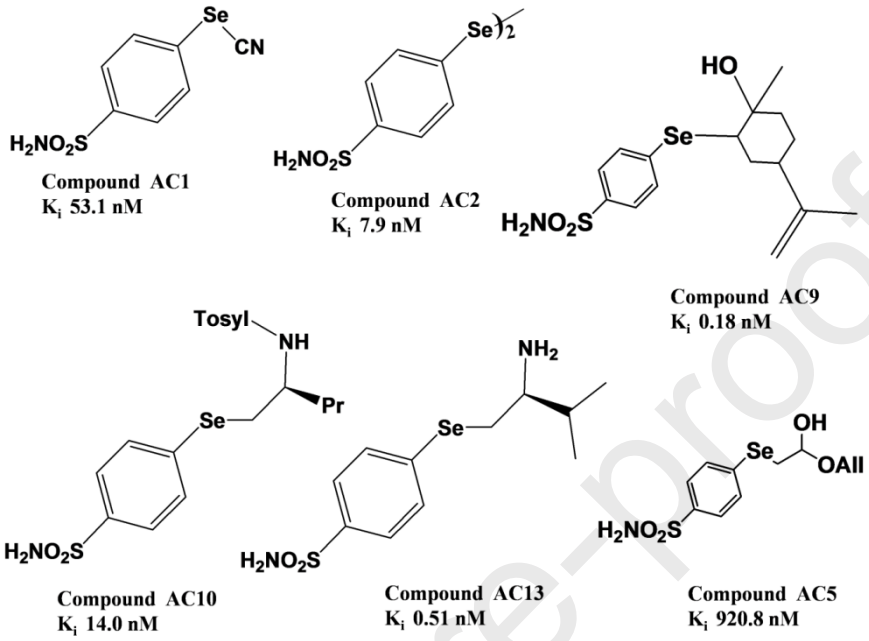


Fig. 33. SAR of selenide moiety

AD. 1,3-diaryltriazene moiety with sulfonamides

An outstanding inhibitory potency was reflected from all the investigated compounds except compound **AD1 (**K_i value of 21.5 nM). Their proficiency of inhibition depended on the nature of substituents and their substitution position. Incorporation of the activating groups into the 1,3-diaryltriazenemoiety, like-OMe (compounds **AD5** and **AD10**; K_i value of 0.4 nM, and 0.2 nM, respectively), -OBu (compound **AD4**; K_i value of 1.7 nM), -$COCH_3$ (compound **AD6**; K_i value of 0.3 nM) etc improved the inhibition significantly than the deactivating groups like -NO_2 **(**compound **AD7;** K_i value of 3.2 nM), -COOH (compound **AD2**; K_i value of 7.4 nM), halogen (compound **AD11**, **AD12,** and **AD1**; K_i value of 7.2 nM, 2.1 nM, and 21.5 nM, respectively), -CN. However, irrespective of the presence of -CN group, compound **AD13** exerted the most potent inhibitory action on K_i value of 0.2 nM (Fig. 34). This might have been due to the presence of this group at 2nd position of benzene ring which facilitated favorable ligand-binding interactions as compared to its 4th position of substitution in benzene ring (compound **AD3**; K_i value of 3.7 nM). Dramatic enhancement in the inhibitory concentration was observed for the presence of four fluorine atoms in benzene ring (compound **AD12**; K_i

value of 2.1 nM) in place of single fluorine (compound **AD1**; K_i value of 21.5 nM). A similar effect was noticed when there is a 3,5-diMe substituted benzene ring present in this moiety (compound **AD9**; K_i value of 0.7 nM) instead of 4-Me substituted benzene (compound **AD5**; K_i value of 0.4 nM). The presence of hydrophobic alkyl chain such as isopropyl group (compound **AD8**; K_i value of 3.1 nM) showed a moderate action [82].

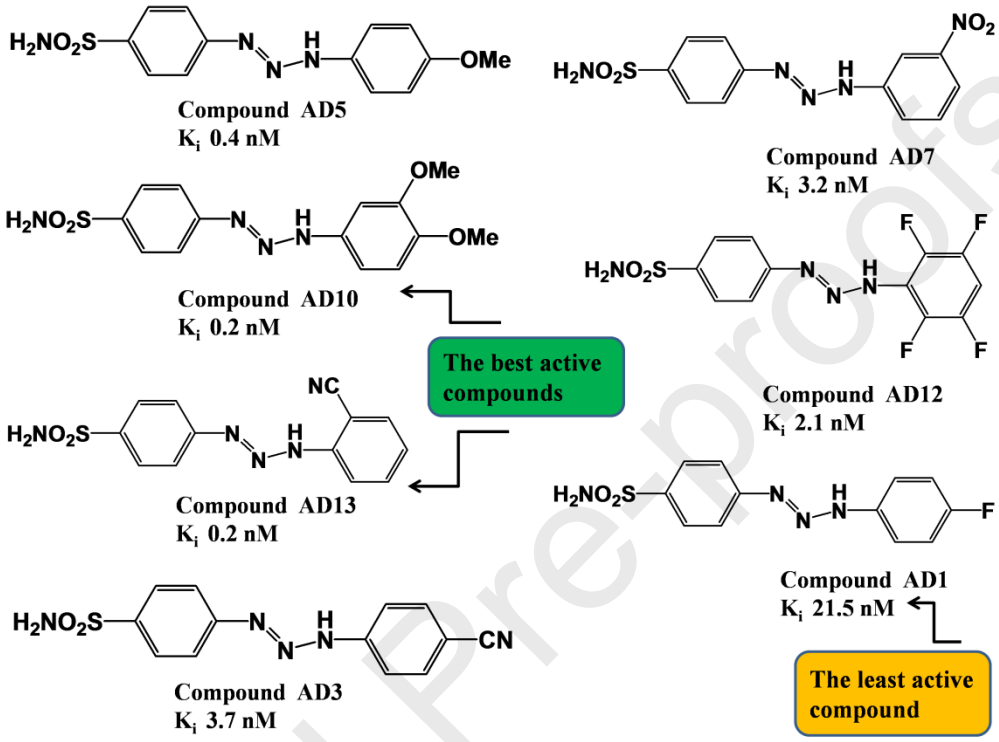


Fig. 34. SAR of 1,3-diaryltriazene moiety with sulfonamides

AE. Iminothiazolidinone moiety with sulfonamides

A very potent inhibition (K_i values in the range of 0.41-37.8 nM) was reflected from the inhibitory profile of the iminothiazolidinone incorporated sulfonamides. Substitution at various positions of benzenesulfonamide brought significant changes in the inhibitory concentration. For the presence of deactivating groups like -NO_2, the order of inhibition was: *ortho* (compound **AE5**; K_i value of 0.53 nM) >*para* (compound **AE7**; K_i value of 4.6 nM) > *meta* (compound **AE6**; K_i value of 7.23 nM). However, meta substituted benzenesulfonamide (compound **AE3**; K_i value of 4.3 nM) containing activating group was comparatively more effective inhibitor than the *para* (compound **AE4**; K_i value of 7.6 nM) and *ortho*-substituted (compound **AE2**; K_i value of 37.8 nM; the least active compound) ones. Substitution by halogens (compound **AE9**; K_i value of 0.46 nM) made an effective contribution in the inhibition. This effect can be significantly enhanced for the presence of both **-NO_2** and halogen

like chlorine in these compounds, leading to the most prominent inhibitor of hCA-II (compound **AE10**; K_i value of 0.41 nM) under this scaffold (Fig. 35) [83].

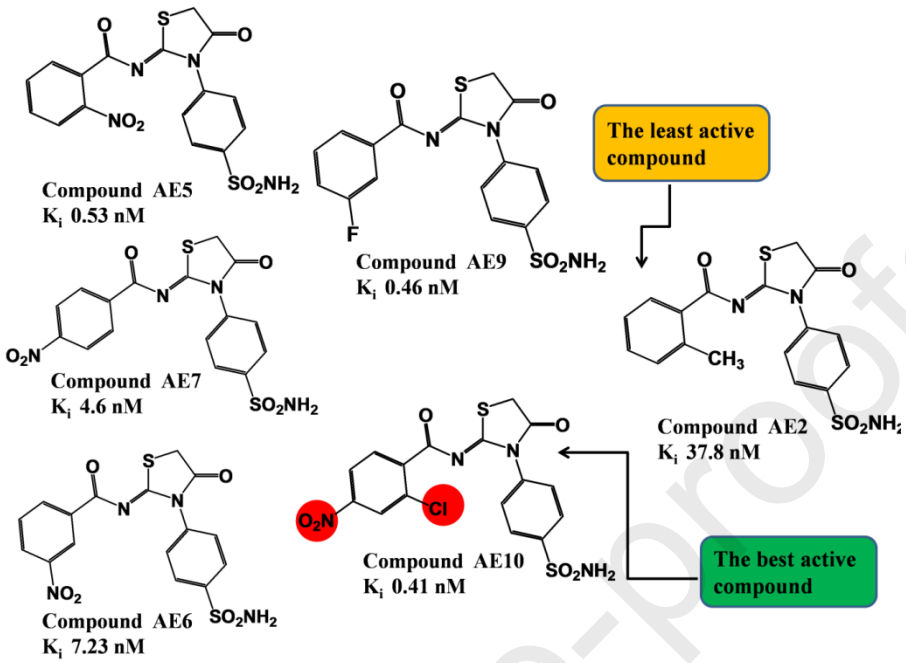


Fig. 35. SAR of iminothiazolidinone moiety with sulfonamides

AF. Organotellurium moiety

Except for compounds **AF4** (K_i value of 30 nM) and **AF5** (K_i value of 90 nM), an ineffective inhibition was addressed from all the other compounds. From their SAR, it was quite obvious that the incorporation of dithiatellurepane (compound **AF6**; K_i value of >100000 nM), β -Phenyltelluro thiol (compound **AF7**; K_i value of >100000 nM), disulfide of β -Phenyltelluro thiol (compound **AF8**; K_i value of >100000 nM), epoxides (compound **AF9**, and **AF10**; same K_i value of >100000 nM) and amines (compound **AF11, AF12,** and **AF13**; K_i value of 35800 nM, 56800 nM, 61600 nM) resulted in such an inhibition that cannot be considered as an effective inhibition of this dominant physiologic isoform (K_i range of 31400 - 56300 nM) containing a methyl-substituted benzene or methoxy substituted benzene. In case of tellurides, replacement of β-hydroxyl group (compound **AF1** and **AF2**) by the β-amino group (compound **AF4** and **AF5**) significantly improved their inhibitory activity almost greater than 100000 times as compared to others. The presence of isopropyl group further improved the inhibitory activity of β-amino-tellurides in place of methyl substitution of it (Fig. 36) [84].

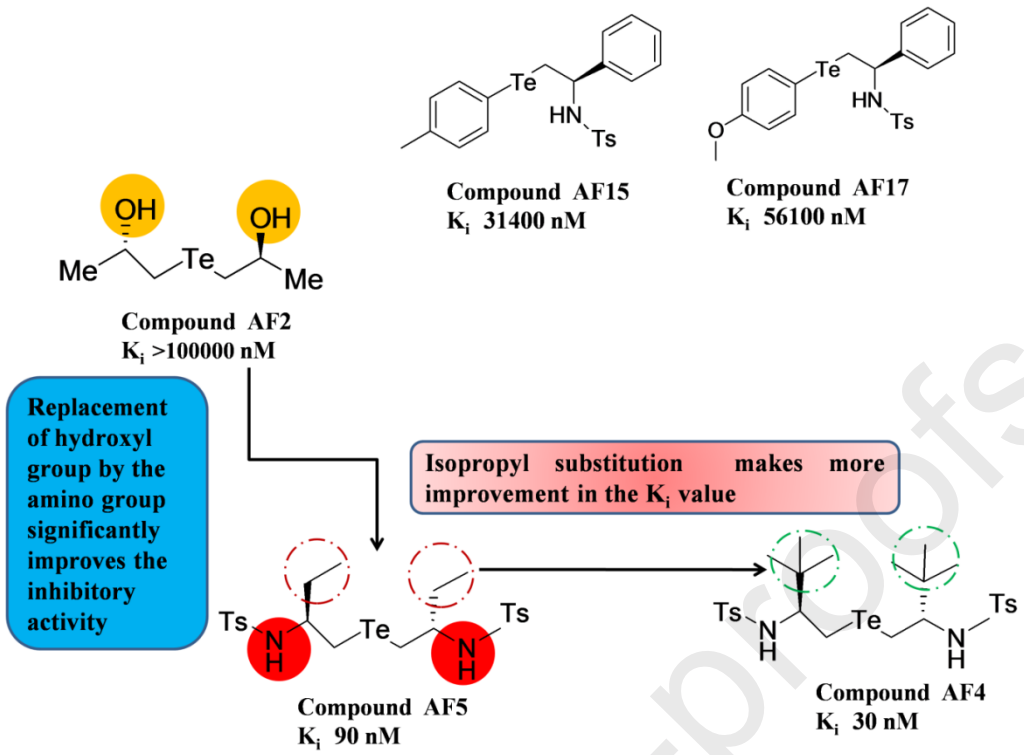


Fig. 36. SAR of an organotellurium moiety

AG. Sulfamoylphenyl and sulfocoumarin carboxamide derivatives

Sulfamoylphenyl carboxamide derivatives (compounds **AG1-AG19**) showed a prominent inhibition with K_i value in the range of 5.2-417 nM, which was far better than the inhibitory profile of ineffective sulfocoumarin derivatives (compounds **AG20-AG36**; K_i value >10000 nM) (Fig. 37). Extent of inhibition may vary from one compound to another compound due to the presence of different heterocyclics together with different groups like H, OCH_3, CH_3, Cl, and F. In the presence of these heterocyclics the order of inhibition is followed as pyrrolidine (compound **AG4**; K_i value of 5.3 nM) > piperidine (compound **AG3**; K_i value of 8.6 nM) >morpholine (compound **AG1**; K_i value of 17.1 nM) >*cis*-2,6-dimethylmorpholine (compound **AG2**; K_i value of 37.5 nM) for sulfamoyl phenyl carboxamide derivatives having hydrogen as its substituent (Fig. 38). When these derivatives contain a methoxy substituent, inverse order of inhibition followed by pyrrolidine (compound **AG8**; K_i value of 92.1 nM) < piperidine (compound **AG7**; K_i value of 49.3 nM) <morpholine (compound **AG5**; K_i value of 8.3 nM) <*cis*-2,6-dimethylmorpholine (compound **AG6**; K_i value of 6.3 nM) was observed. Thiomorpholine exhibited a moderate inhibition (K_i value of13.5 nM) for the compound **AG9**. Further, their inhibitory activity can be significantly improved in the presence of halogen like

fluorine (compound **AG14**; K_i value of 5.2 nM; the best active compound) together with ***Cis***-2,6-dimethylmorpholine moiety.

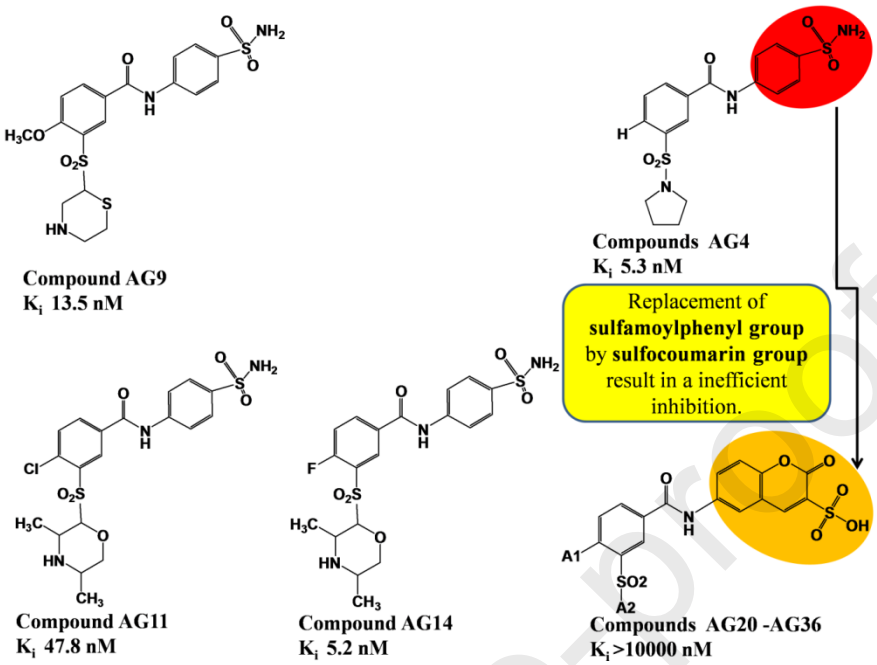


Fig. 37. SAR of sulfamoylphenyl and sulfocoumarin carboxamides derivatives.

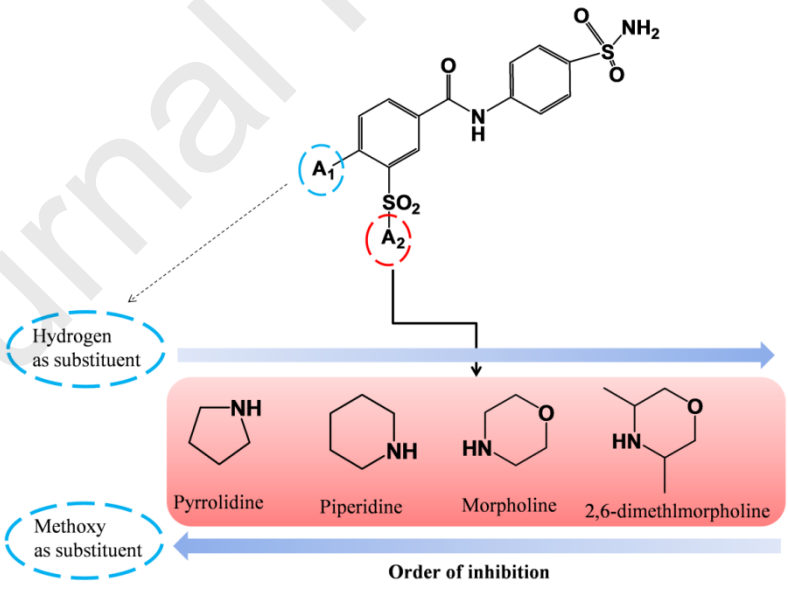


Fig. 38. Comparison of K_i values with heterocyclic rings, the two different substituents in case of sulfamoylphenyl, and sulfocoumarin carboxamides derivatives

Although compound **AG11** contains this moiety, still it showed a moderate inhibition **(**K_i value of 47.8 nM**)** due to the presence of chlorine as its substituent. Another fact observed was, pyrrolidine incorporated sulfamoylphenyl carboxamide derivative having hydrogen as its substituent, (compound **AG4**) exerted a very promising inhibition with a K_i value of 5.3 nM [85].

AH. 2-Benzylpiperazine derivatives

Incorporation of zinc-binding group (ZBG) into the 2-benzylpiperazine moiety helped these derivatives (compounds **AH1**-**AH34)** show a promising inhibition with a K_i value in the range of 2.0 -358.6 nM (Fig. 39). In the presence of ZBG, not only the nature of different substituents influenced their inhibitory action but also their substitution pattern at different positions made important contributions towards the effective inhibition of this isoform of CA. They have also studied the impact of stereoisomers on the inhibitory profile of these compounds. It was observed that except some derivatives (compound **AH7**; K_i value of 7.7 nM, compound **AH21**; K_i value of 8.3 nM, compound **AH25**; K_i value of 15.0 nM, compound **AH29**; K_i value of 6.0 nM and compound **AH33**; K_i value of 2.0 nM) the R-configuration of these inhibitors more prominently inhibit this isoform as compared to the S-configuration. Out of all the substituents present at 4th position of this moiety, -$PhCH_2CO$ group significantly enhanced their activity as observed in both stereo configuration (compound **AH11**; K_i value of 6.8 nM for S-configuration, compound **AH12**; K_i value of 4.8 nM for R-configuration). However, a different scenario was observed when the methyl group occupied in the 4th position of this moiety (compound **AH20**) and it resulted in the most ineffective inhibition with a K_i value of 358.6 nM for S-configuration only. Whereas for R-configuration, methyl substitution at N-1 of the scaffold brings a more favourable outcome as it was seen with compound **AH20** (K_i value of 5.4 nM). The most potent inhibition was seen in the compound **AH33 (**K_i value of 2.0 nM, S-configuration) having two ZBG in its core, which can correlate their effective interactions with the zinc metal [86, 87].

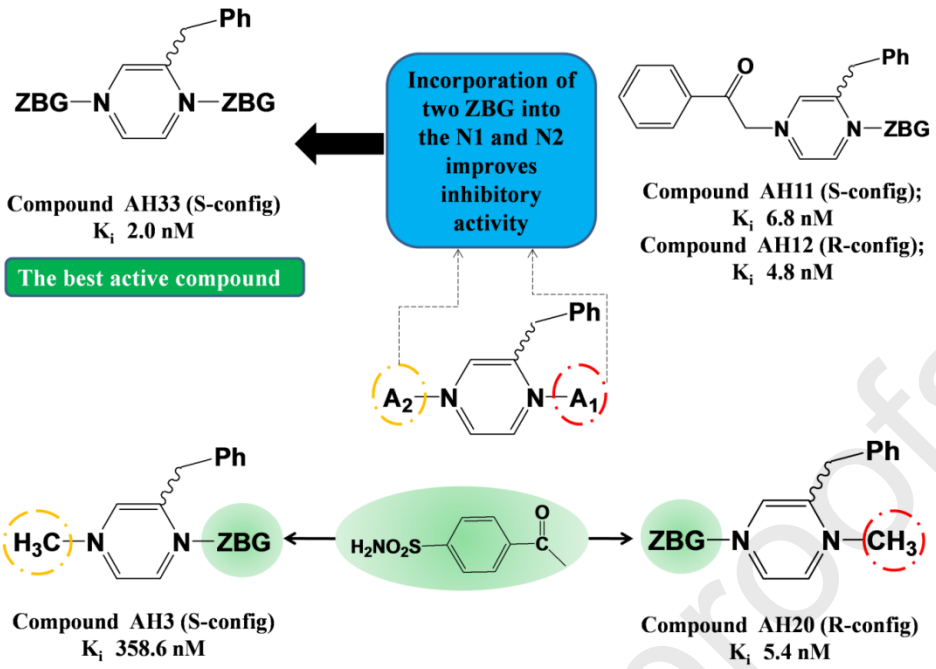


Fig. 39. SAR of 2-benzylpiperazine derivatives

AI. 4-sulfamoyl benzamide with cyclic imide derivatives

From the outcomings of the inhibitory result for the Scaffold No. A, A.M. Alaa Abdel-Aziza *et al.* 2018 [88] further investigated their inhibitory profile by incorporating 4-sulfamoyl benzamide into cyclic imides and they designed a new series of 14 compounds that showed more effective inhibition (K_i in the range of 0.7-128.1 nM) against HCA-II as compared to their past results. The best inhibition was reflected from tertiary-butyl substituted compound **AI4** (K_i 0.7 nM) whereas the methyl-substituted compound **AI3** (K_i 9.1 nM) and unsubstituted cyclic imide incorporated sulfonyl benzamide scaffold (compound **AI2;** K_i 7.7 nM) were found to be moderate inhibitors. Substitution by chlorine atoms (compound **AI9**) into cyclic imide moiety facilitates also similar inhibition (K_i 5 nM). But this scenario becomes totally changed when bromine atoms were added in place of chlorine atoms resulting in an ineffective inhibition (compound **AI10** K_i 62.3 nM). The presence of bulky groups like polychlorinated norborneneimido moiety decreased their effectiveness mostly (compound **AI14** K_i 128.1 nM). Isoquinoline moiety incorporated sulfonyl benzamide compounds also show the potent inhibitory action (compound **AI12** K_i 7.7 nM). But, their inhibitory potency may be markedly decreased when nitro group was present (compound **AI13** K_i 20.6 nM). Therefore, it may be concluded that presence of branched alkyl chain facilitates the required hydrophobicity in the

ring structure for appropriate binding with the active site of receptor, and it is important in the 5th position of phthalimide group incorporated 4- sulfonyl benzamide derivatives.

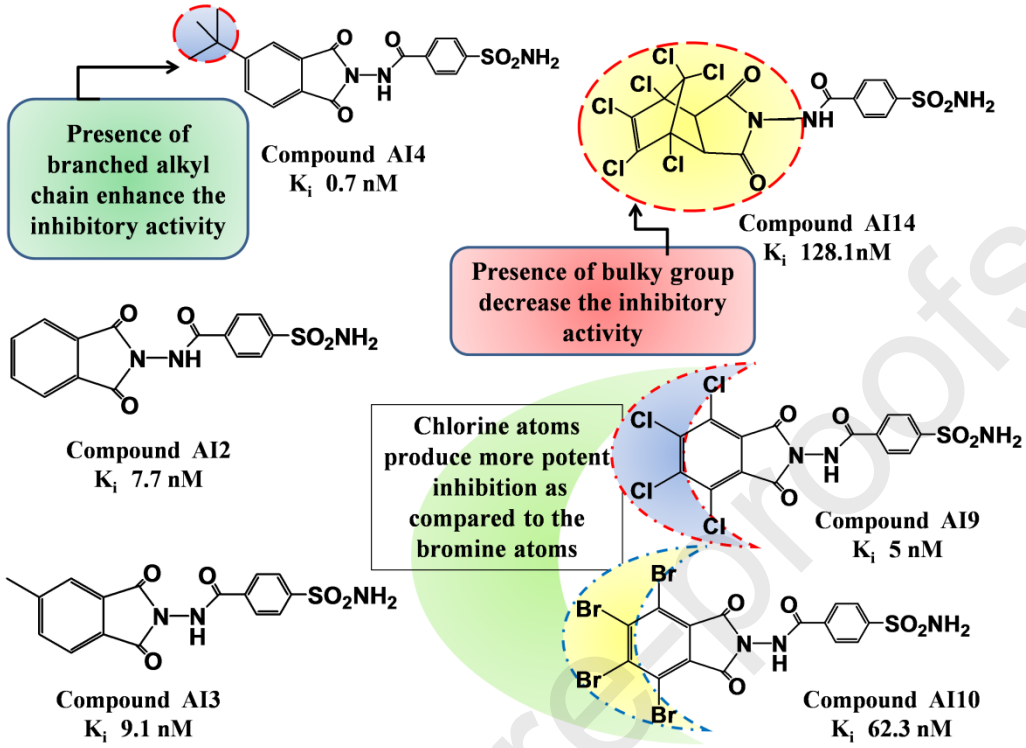


Fig. 40. SAR of 4-sulfamoyl benzamide with cyclic imide derivatives

AJ. Unsubstituted Methanoisoindole-1,3(2H)-dione with chalcone derivatives

Unsubstituted methano isoindole-1,3(2H)-dione moiety showed a profound inhibition against HCA-II (K_i range of 0.245±0.09- 0.489±0.09 nM) when it is incorporated in the chalcone scaffold. Based on the substituting groups in the chalcone derivatives, their inhibitory action is slightly varied. The presence of halogen in chalcones (compounds **AJ6-AJ11)** produces remarkably more potent effect than the methoxy substituted chalcones (compounds **AJ1-AJ2**) or methyl-substituted chalcones (compounds **AJ3-AJ5**). The best active compound is **AJ9** with K_i value of 0.245 ± 0.09 nM in where bromine is present at 4th position of benzene ring. Due to the presence of heterocyclic groups in this scaffold (compounds **AJ12-AJ14**), the potency of these compounds is moderately decreased. The least activity was reflected from the compound **AJ14** with K_i value of 0.489 ± 0.09 nM [89].

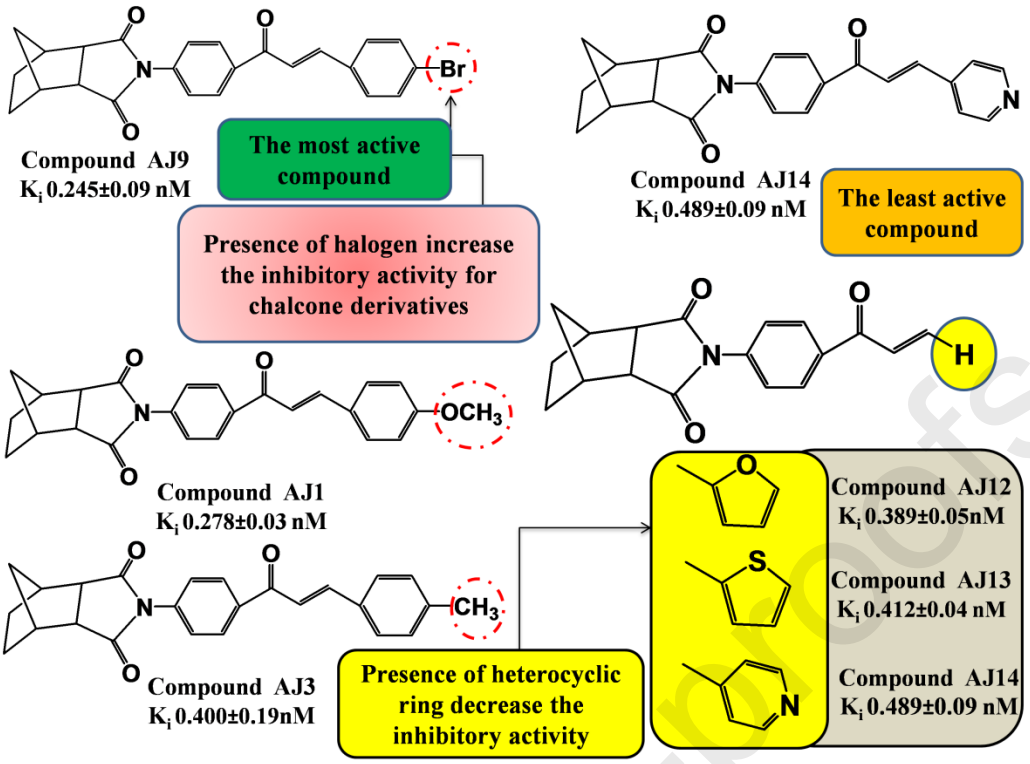


Fig. 41. SAR of unsubstituted Methanoisoindole-1,3(2H)-dione with chalcone derivatives

AK. Substituted Methanoisoindole-1,3(2H)-dione with tetrabromo chalcone derivatives

Compared to the previous scaffold, when dibromo substituted methanoisoindole-1,3(2H)-dione moiety was incorporated into the dibromo substituted chalcone derivatives, moderate inhibition (K_i range of 8.20 ± 1.62-12.86 ± 1.98 nM) was observed against this isoform. U.M. Kocyigit *et al.* 2017 reported that the nature of halogen substitutions and its presence in the benzene ring were very crucial for these tetrabromo Chalcone derivatives. The best inhibition was shown by compound **AK3 (**K_i value of 8.20 ± 1.62 nM) due to the presence of Cl atom at 4th position of benzene ring. But, this scenario became completely different when Br atom was present in the 4th position of benzene ring, resulting in the least active compound **AK4 (**K_i value of 12.86 ± 1.98 nM) as well as for compound **AK7 (**K_i value of 12.45 ± 3.18 nM) in where Cl atom is present in 3rd position of benzene ring. Moreover, the presence of heterocyclic groups like furan and thiophene (compound **AK8;** K_i value of 10.02 ± 1.90 nM and compound **AK9;** K_i value of 9.21 ± 2.22 nM) had influenced the inhibitory potency. Therefore, it may be concluded that presence of chlorine at 4th position of benzene ring will produce more favourable outcome in the inhibitory profile of these derivatives [90].

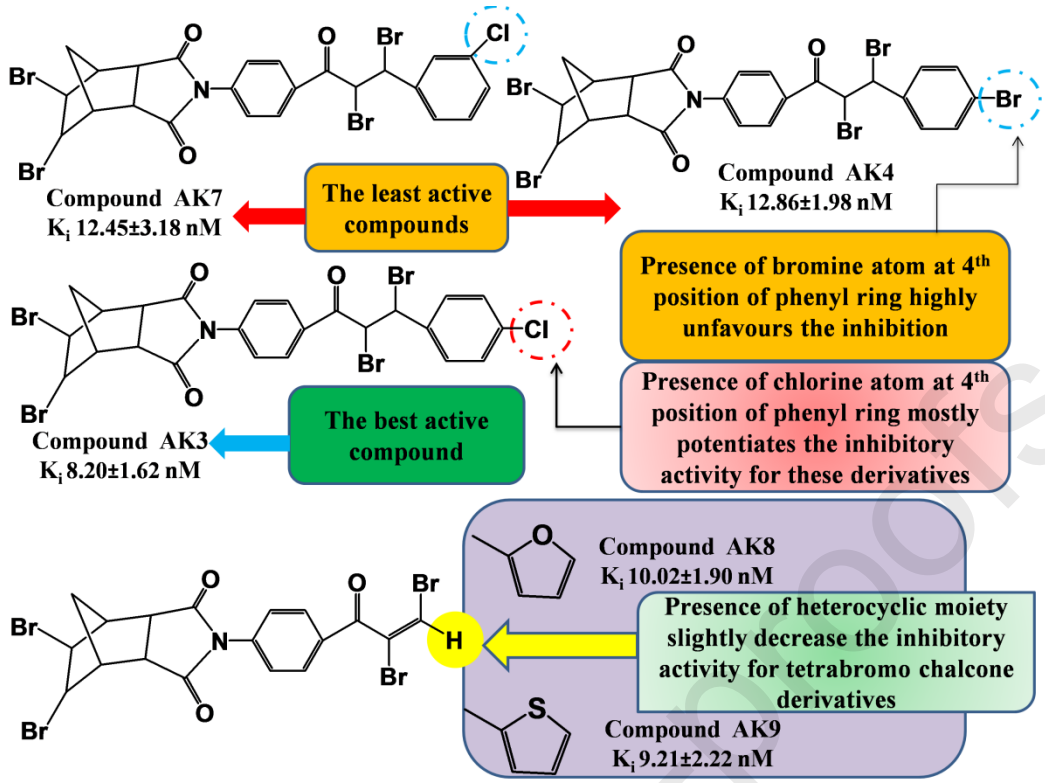
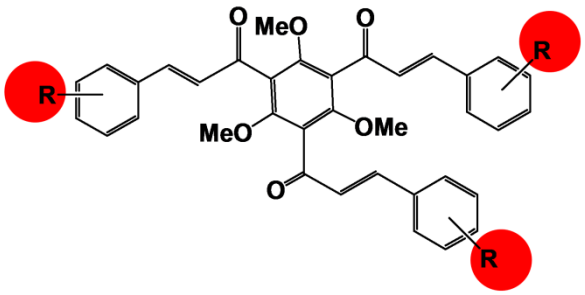


Fig. 42. SAR of Substituted Methanoisoindole-1,3(2H)-dione with tetrabromo chalcone derivatives

AL. Tris-Chalcone derivatives

Observing the inhibitory profile of chalcone containing scaffolds (Scaffold **AJ** & Scaffold **AK)**, S. Burmaoglu *et al.* 2018 [91] further pioneered their research for new molecules based on the chalcones and synthesized nine compounds which exerted their inhibitory activity against this isoform in the K_i range of 12.23 ± 2.43 nM - 41.70 ± 9.10 nM. The previous results showed an effective inhibition when halogen atom is used as a substituting group in the benzene group of chalcone moiety, so that they only focused on the effects of halogen (fluorine atom only) substitution in different position. Except compound **AL3 (**K_i value of 12.23 ± 2.43 nM), all the mono-fluoro substituted tris chalcones (compound **AL1** & **AL2;** K_i range of 37.35 ± 4.31 nM - 41.70 ± 9.10 nM) are less effective inhibitors than the difluoro substituted tris chalcones (compound **AL4-AL8;** K_i range of 17.86 ± 6.30 nM - 25.94 ± 7.41 nM) and trifluoro substituted tris chalcone (compound **AL9;** K_i value of 17.72 ± 2.28 nM). The presence of fluorine atom at the 4th position of benzene ring of chalcone in the most potent compound AL3 can be well correlated to the structure of previous scaffold AK (compound AK3). Therefore, we may conclude that the presence of halogens like chlorine or fluorine at 4th position enhanced

more favourable outcomes for the inhibition of this isoform in case of chalcone incorporated scaffolds.



Presence of halogen like **chlorine or fluorine** atom at **4th position** enhances more potent inhibition for chalcone derivatives

R –(F)	Compound	Position	Ki (nM)	Inhibitory action
Monofluoro	**AL1**	2	**41.70±9.10**	Least
	AL2	3	37.35±4.31	Moderate
	AL3	4	**12.23±2.43**	Most potent
Difluoro	**AL8**	3,5	17.86±6.30	Good
	AL5	2,3	19.18±3.45	Good
	AL4	2,4	20.02±4.17	Fair
	AL7	3,4	25.56±5.74	Fair
	AL6	2,5	25.94±7.41	Fair
Trifluoro	**AL9**	2,4,5	17.72±2.28	Good

Fig. 43. SAR of Substituted Tris-Chalcone derivatives

AM. Substituted Thiophene derivatives

Adnan Cetin *et al.,* 2018 [92] investigated the effectiveness of their designed compounds where they had incorporated a novel thiophene moiety as the main scaffold. All the molecules exerted good inhibition of this isoform hCA-II with K_i value in the range of 309.44 ± 97.04 to 935.93 ± 167.04 nM. When substituted pyrazole is introduced in the active moiety of thiophene, their inhibitory profile became more potent as compared to the non-pyrazole attached thiophene derivative (Compound **AM6**; K_i value of 904.37 ± 264.34 nM). Moreover, the substitution in pyrazole ring also influenced the inhibition. The order of inhibition was: thiamide (Compound **AM7**; K_i value of 309.44 ± 97.04 nM)> 4-bromophenyl (Compound **AM4**; K_i value of 423.84 ± 50.06 nM) >thioxazole (Compound **AM8**; K_i value of 465.28 ± 100.04 nM) >2,5-dimethyl phenyl (Compound **AM2**; K_i value of 506.37 ± 91.00 nM) >2-nitrophenyl (Compound **AM5**; K_i value of 768.34 ± 203.85 nM). Under this scaffold, the least active inhibitor was compound **AM3** having K_i value of 935.93 ± 167.04 nM while the presence of thiamide group in the thiophene moiety made the compound **AM7** best active. Therefore, it may be concluded that

the presence of thiamide group or presence of halogen simply in the substituted pyrazole**,** made these thiophene derivatives more potent against this isoform.

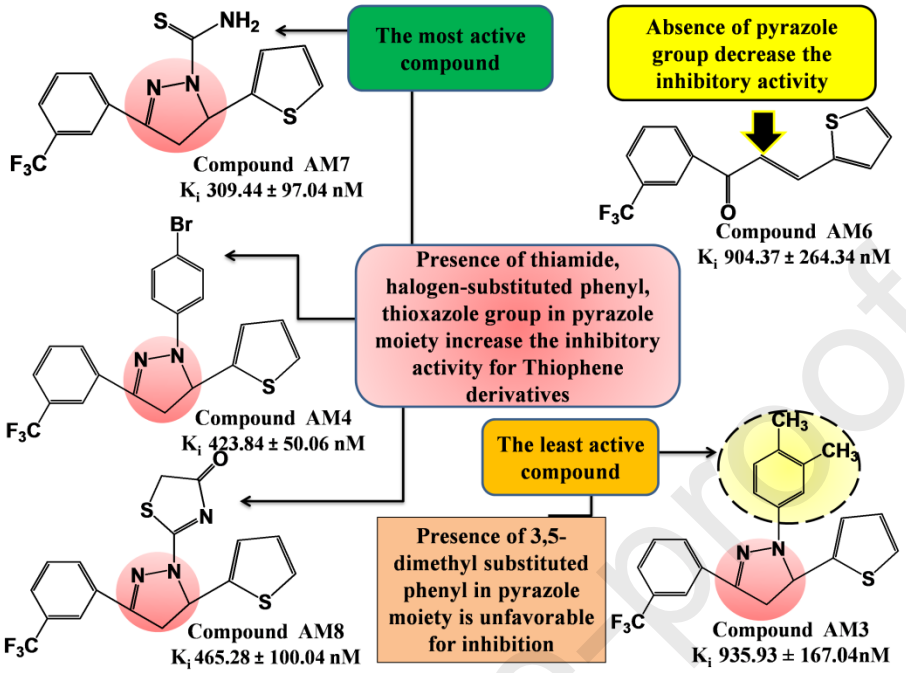


Fig. 44. SAR of Substituted Thiophene derivatives

AN. Ureido benzenesulfonamides with 1,3,5-triazine moieties

N. Lolak *et al.,* 2018 [93] investigated the efficacy of their designed ureido benzenesulfonamides by incorporating 1,3,5-triazine moieties against this physiologic dominant isoform of CA and their inhibitory activity was reported with K_i value in the range of 0.69 nM - 420.9 nM (S.T.No. **AN**). In the structure of their novel molecules, the presence of primary, secondary, tertiary amines together with chlorine or another heterocyclic ring-like morpholine or piperidine were correlated with their inhibitory activities. From the inhibitory profile of compound **AN2 (**K_i value of 178.6 nM**)**, compound **AN7 (**K_i value of 420.9 nM, the least active compound), compound **AN15 (**K_i value of 299.4 nM**)**, it was observed that the primary amino group strongly unfavourable for the inhibition, whereas, the presence of tertiary group along with chlorine atom **(**compound **AN4**; K_i value of 12.4 nM**)** or morpholine group **(**compound **AN9;** K_i value of **3.9** nM**)** or piperidine group **(**compound **AN13;** K_i value of 8.5 nM) favoured more potent inhibition. When this tertiary amine group was present with secondary amine, more profound inhibition was observed (compound **AN16**; K_i value of 3.1 nM**).** The potentiating power of heterocyclic groups like morpholine and piperidine was found to be more crucial when they were present with chlorine atom as another substituent of incorporated 1,3,5-triazine moieties, and they produced best active compounds like compound

AN5; K_i value of 1.5 nM and compound **AN6;** K_i value of 0.69 nM, respectively. It was also clear that dimorpholine substitution (compound **AN11**; K_i value of 6.9 nM**)** was more preferable than the dipiperidine substitution (compound **AN14**; K_i value of 78.9 nM**)** in the 1,3,5-triazines incorporated ureido benzenesulfonamides. Therefore, we may suggest that presence of tertiary amino group together with chlorine atom or morpholine or piperidine group may enhance the inhibitory potency of these ureido benzenesulfonamides.

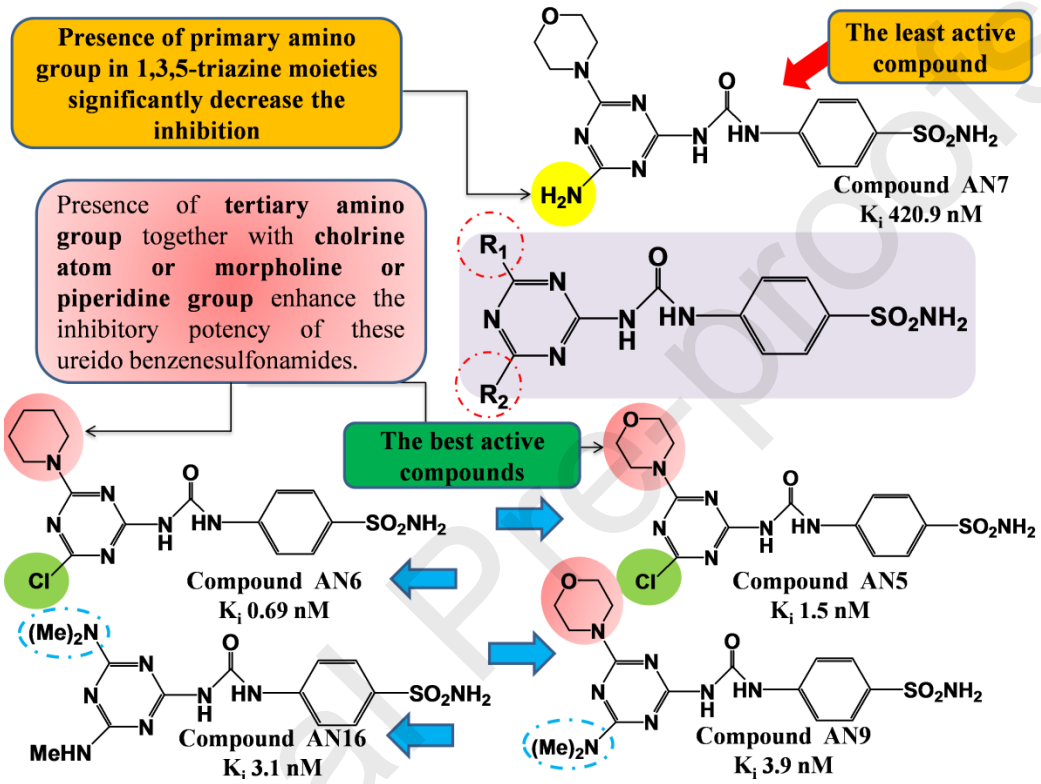


Fig. 45. SAR of Ureido benzenesulfonamides with 1,3,5-triazine moieties

Conclusion

In the current context, we have reviewed all the hCA-II inhibitors which were synthesized and reported till now and the brief outline of their structural activity relationship. Based on the nature of their interiors for all these scientifically reported physiologic dominant CA inhibitors, novel scaffolds like sulfonamides, dithiocarbamate, selenide, organotellurium, 2-benzylpyrazine, thiophene and chalcones, etc. were extracted and the information can be used further for the search of more potent hCA-II inhibitors.

Among all the antiglaucoma agents, sulfonamides are the most common scaffold where different types of moieties are attached in their active core. In the molecular structures,

substituents and their substitution positions have contributed a variable effects of inhibition of this dominant physiologic isoform of hCA-II. The effect for the presence of linker as well as the presence of heterocyclics along with the different functionalities was correlated with their observed SAR. Moreover, the role of halogens present in the different substitution positions of this scaffold is thoroughly discussed in the prominent inhibition of hCA-II. These important facts may be considered for the design and development of newer more potential antiglaucoma agents in future.

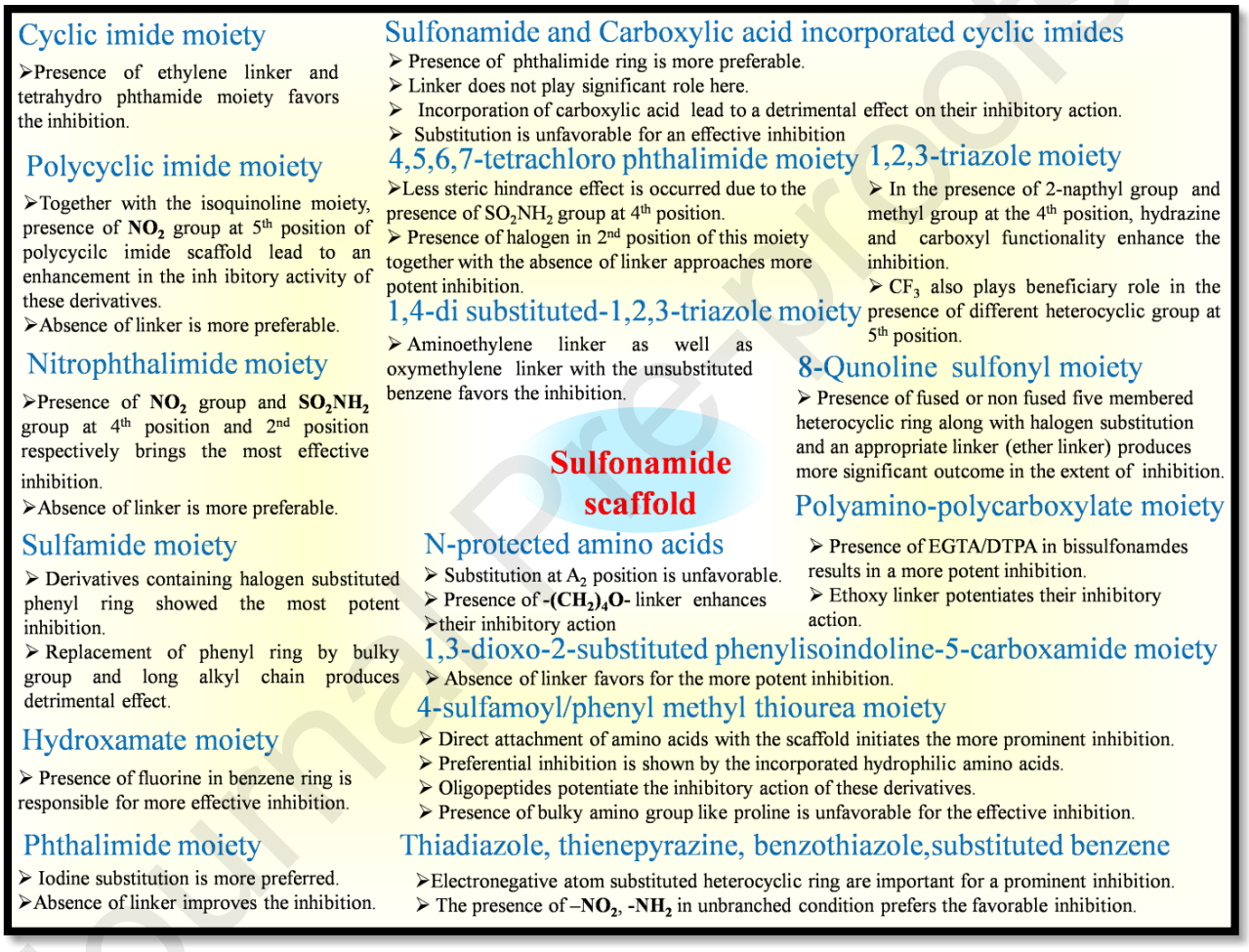


Fig. 46A. A brief outline for SAR of sulfonamide derivatives

We have also investigated the impact of the individual role of specific stereoisomers in the effective inhibition of hCA-II. Heterocyclic groups like furan, thiophene, thieno pyrazine, pyrrole, benzothiazole, thiadiazoline, pyrrolidine, pyridine, etc. showed a more promising outcome as compared to the other incorporated groups.

From the structural insights of various best active compounds under different scaffolds, all the beneficial and detrimental structural attributes are depicted in Fig. 46A, Fig. 46B and Fig. 47. This may help us to focus on synthesizing more effective inhibitors of this hCA in future.

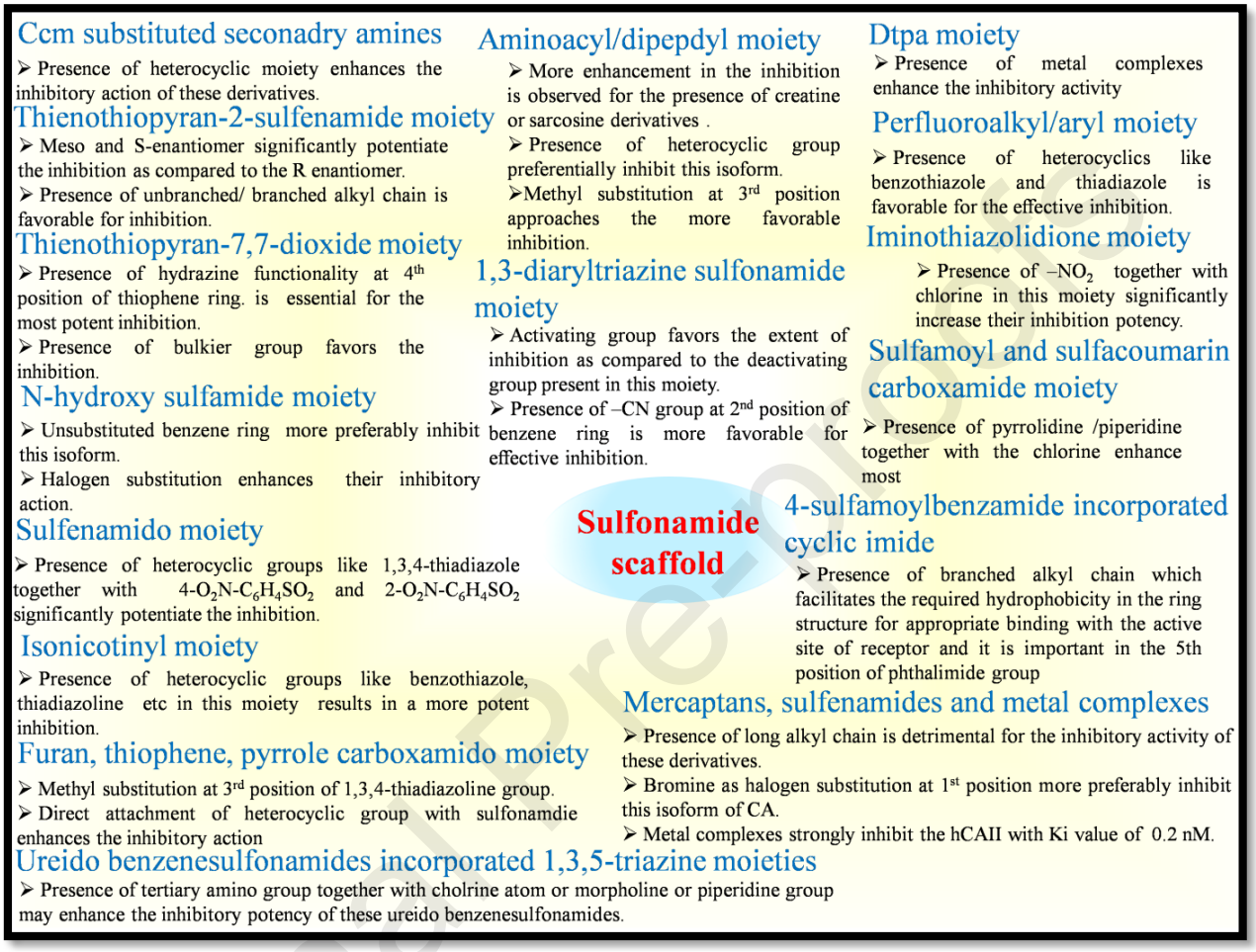


Fig. 46B. A brief outline for SAR of sulfonamide derivatives

Much recently, researchers have put their focus on other novel scaffolds like dithiocarbamate, selenides, benzylpyrazine, organotellurium, thiophene, chalcones (Fig. 47), and also in view of some common structural features like hydrophobicity, the presence of halogen or heterocyclic groups in their active core has been established.

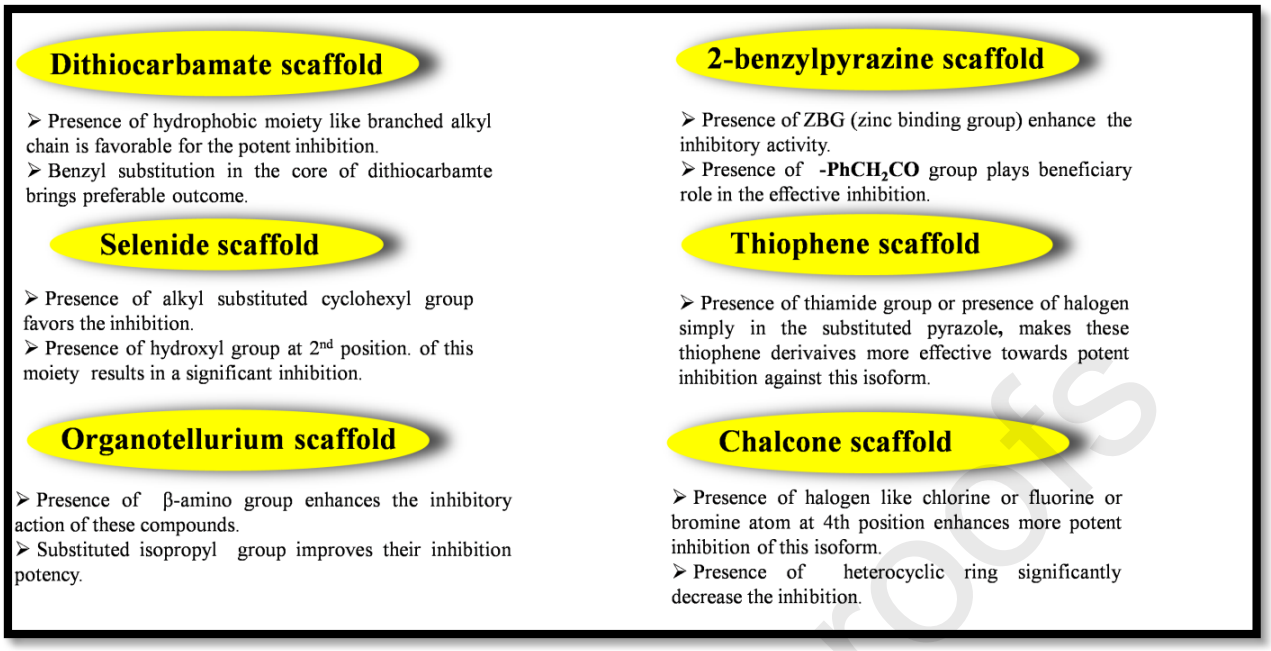


Fig. 47. A brief outline for SAR of other reported novel scaffolds

Furthermore, this comprehensive review reveals the importance of the presence of metal complexes and zinc-binding group ($-COC_6H_4SO_2NH_2$) incorporated in most of the investigated scaffolds, and this study may become a useful tool to all the researchers working on the hCA-II inhibitors preferably for anti-glaucoma therapy in upcoming future. Here, we have attempted for a complete summary of detailed features towards a prominent inhibition which may be achieved if all these above-mentioned facts are considered before designing the novel potent scaffolds.

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References

[1] S. Kingman, Glaucoma is second leading cause of blindness globally, Bulletin of the World Health Org. 82 (2004) 887-888.

[2] A.V. Mantravadi, N. Vadhar, Glaucoma. Primary Care: Clinics in Office Practice. 42 (2015) 437-449.

[3] K. Zhang, L. Zhang, R.N. Weinreb, Ophthalmic drug discovery: novel targets and mechanisms for retinal disease and glaucoma, Nat. Rev. Drug Discov. 11 (2012) 541-559.

[4] https://nei.nih.gov/news/pressreleases/012114 (acess on 26/12/2018).

[5] F. Carta, C.T. Supuran, A. Scozzafava, Novel therapies for glaucoma: a patent review 2007-2011, Expert Opin. Ther. Pat. 22 (2012) 79-88.

[6] F. Mincione, A. Scozzafava, C.T. Supuran, Antiglaucoma Carbonic Anhydrase Inhibitors as Ophthalomologic Drugs. In *Drug Design of Zinc-Enzyme Inhibitors*, Supuran, C. T.; Winum, J.-Y., Eds. John Wiley & Sons, Inc.: 2009. 139-154.

[7] H.A. Quigley, Glaucoma, Lancet. 377 (2011) 1367-1377.

[8] H.A. Quigley, A.T. Broman, The number of people with glaucoma worldwide in 2010 and 2020, Br. J. Ophthalmol. 90 (2006) 151-156.

[9] J. Pichake, P.S. Kharkar, M. Ceruso, C.T. Supuran, M.P. Toraskar, Carbonic Anhydrase Inhibitors: Design, Synthesis, and Biological Evaluation of Novel Sulfonyl Semicarbazide Derivatives, ACS Med. Chem. Lett. 5 (2014) 793-796.

[10] E. Masini, F. Carta, A. Scozzafava, C.T. Supuran, Antiglaucoma carbonic anhydrase inhibitors: a patent review, Expert Opin. Ther. Pat. 23 (2013) 705-716.

[11] J. Chen, S.A. Runyan, M.R. Robinson, Novel ocular antihypertensive compounds in clinical trials, Clin. Ophthalmol. *(Auckland, N.Z.)* 5 (2011) 667-677.

[12] M.P. Fautsch, D.H. Johnson, Aqueous humor outflow: what do we know? Where will it lead us? Invest Ophthalmol. & Visual Science. 47 (2006) 4181-4187.

[13] A. Alm, S.F. Nilsson, Uveoscleral outflow - a review, *Experimental Eye Research*. 88 (2009) 760-768.

[14] C. Kishore, M.T. Hoang, P. Dhananjay, K.M. Ashim, Discovery of novel inhibitors for the treatment of glaucoma, Expert Opin. Drug Discov. 10 (2015) 293-313.

[15] H. Bahrami, Causal inference in primary open angle glaucoma: specific discussion on intraocular pressure, Ophthalmic Epidemiol. 13 (2006) 283-289.

[16] C.T. Supuran, A. Scozzafava, Carbonic anhydrase inhibitors and their therapeutic potential, Expert Opin. Ther. Pat. 10 (2000) 575-600.

[17] C.T. Supuran, Carbonic anhydrases: novel therapeutic applications for inhibitors and activators, Nat. Rev. Drug Discov. 7 (2008) 168-181.

[18] C.T. Supuran, Carbonic anhydrase inhibitors, Bioorg. Med. Chem. Lett. 20 (2010) 3467-3474.

[19] C.T. Supuran, Carbonic anhydrase inhibition/activation: trip of a scientist around the world in the search of novel chemotypes and drug targets, Curr. Pharm. Des. 16 (2010) 3233-3245.

[20] C.T. Supuran, A. Scozzafava, A. Casini, Carbonic anhydrase inhibitors, Med. Res. Rev. 23 (2003) 146-189.

[21] M. Aggarwal, R. McKenna, Update on carbonic anhydrase inhibitors: a patent review (2008-2011), Expert Opin. Ther. Pat. 22 (2012) 903-915.

[22] A. Scozzafava, A. Mastrolorenzo, C.T. Supuran, Carbonic anhydrase inhibitors and activators and their use in therapy, Expert Opin. Ther. Pat. 16 (2006) 1627-1664.

[23] M. Aggarwal, C.D. Boone, B. Kondeti, Structural annotation of human carbonic anhydrases, J. Enz. Inhib. Med. Chem. 28 (2013) 267-277.

[24] E.R. Zolfaghari, H.R. Barker, L. Syrjänen, L. Urbański, C.T. Supuran, S. Parkkila, Identification and inhibition of carbonic anhydrases from nematodes, J. Enzyme. Inhib. Med. Chem. 31 (2016) 176-184.

[25] C. T. Supuran, how many carbonic anhydrase inhibition mechanisms exist? J. of Enz. Inhib. and Med. Chem. 31 (2016) 345-360.

[26] J.M. Purkerson, G.J. Schwartz, The role of carbonic anhydrases in renal physiology, Kidney Int. 71 (2007) 103-115.

[27] G. De Simone, V. Alterio, C.T. Supuran, Exploiting the hydrophobic and hydrophilic binding sites for designing carbonic anhydrase inhibitors, Expert Opin. Drug Discov. 8 (2013) 793-810.

[28] F. Carta, A. Scozzafava, C.T. Supuran, Sulfonamides: a patent review (2008-2012), Expert Opin. Ther. Pat. 22 (2012) 747-758.

[29] R. Liu, W. Bu, J. Xi, S.R. Mortazavi, J.C. Cheung-Lau, I.J. Dmochowski, P.J. Loll, Beyond the detergent effect: a binding site for sodium dodecyl sulfate (SDS) in mammalian apoferritin, Acta. Crystallogr. D. 68 (2012) 497-504.

[30] S. Breton, The cellular physiology of carbonic anhydrases, JOP. J Pancreas. 2 (2001) 159-164.

[31] A.J. Kivelä, J. Kivelä, J. Saarnio, S. Parkkila, Carbonic anhydrases in normal gastrointestinal tract and gastrointestinal tumours, World J. Gastroenterol. 11 (2005) 155-163.

[32] V.M. Krishnamurthy, G.K. Kaufman, A.R. Urbach, I. Gitlin, K.L. Gudiksen, D.B. Weibel, G.M. Whitesides, Carbonic anhydrase as a model for biophysical and physical-organic studies of proteins and protein-ligand binding, Chem Rev. 108 (2008) 946-1051.

[33] A.E. Eriksson, T.A. Jones, A. Liljas, Refined structure of human carbonic anhydrase II at 2.0 Å resolution, Proteins. 4 (1988) 274-282.

[34] C. Schlicker, R.A. Hall, D. Vullo, S. Middelhaufe, M. Gertz, C.T. Supuran, F.A. Mühlschlegel, C. Steegborn, Structure and inhibition of the CO_2-sensing carbonic anhydrase Can2 from the pathogenic fungus Cryptococcus neoformans, J. Mol. Biol. 385 (2009) 1207-1220.

[35] V. Alterio, E. Langella, F. Viparelli, D. Vullo, G. Ascione, N.A. Dathan, F.M. Morel, C.T. Supuran, G. De Simone, S.M. Monti, Structural and inhibition insights into carbonic anhydrase CDCA1 from the marine diatom Thalassiosira weissflogii, Biochimie. 94 (2012) 1232-1241.

[36] K.L. Peña, S.E. Castel, C. de Araujo, G.S. Espie, M.S. Kimber, Structural basis of the oxidative activation of the carboxysomal gamma-carbonic anhydrase, CcmM, Proc. Natl. Acad. Sci. USA. 107 (2010) 2455-2460.

[37] C.T. Supuran, Structure-based drug discovery of carbonic anhydrase inhibitors, J. Enzyme Inhib. Med. Chem. 27 (2012) 759-772.

[38] A. Liljas, K.K. Kannan, P.C. Bergsten, I. Waara, K. Fridborg, B. Strandberg, U. Carlbom, L. Jarup, S. Lovgren, M. Petef, Crystal structure of human carbonic anhydrase C, Nat. New Biol. 235 (1972) 131-137.

[39] D. Neri, C.T. Supuran, Interfering with pH regulation in tumors as a therapeutic strategy, Nat. Rev. Drug Discov. 10 (2011) 767–778.

[40] C. Tu, D.N. Silverman, C. Forsman, B.H. Jonsson, S. Lindskog, Role of histidine 64 in the catalytic mechanism of human carbonic anhydrase II studied with a site-specific mutant, Biochemistry. 28 (1989) 7913-7918.

[41] D.N. Silverman, S. Lindskog, The catalytic mechanism of carbonic anhydrase: implications of a rate-limiting protolysis of water, Acc. Chem. Res. 21 (1988) 30-36.

[42] K. Håkansson, M. Carlsson, L. A. Svensson, A. Liljas, Structure of native and apo carbonic anhydrase II and structure of some of its anion-ligand complexes, J. Mol. Biol. 227 (1992) 1192-1204.

[43] S.K. Nair, D.W. Christianson, Unexpected pH-dependent conformation of His-64, the proton shuttle of carbonic anhydrase II, J. Am. Chem. Soc. 113 (1991) 9455-9458.

[44] W.H. Miller, A.M. Dessert, R.O. Jr. Roblin, Heterocyclic sulfonamides as carbonic anhydrase inhibitors, J. Am. Chem. Soc. 72 (1950) 4893-4896.

[45] C.T. Supuran, C.W. Conroy, T.H. Maren. Is cyanate a carbonic anhydrase substrate?, Proteins: Structure, Function and Genetics. 27 (1997) 272-278.

[46] I. Bertini, C. Luchinat. A. Scozzafava, Carbonic anhydrase: An insight into the zinc binding site and into the active cavity through metal substitution, Structure and Bonding. 48 (1982) 45-92.

[47] S. Mangani, K. Hakansson, Crystallographic studies of the binding of protonated and unprotonated inhibitors to carbonic anhydrase using hydrogen sulphide and nitrate anions, Eur. J . Biochem. 210 (1992) 867-871.

[48] R.M. Steele, F. Benedini, S. Biondi, L. Carzaniga, F. Impagnatiello, D. Miglietta, W.K. Chong, R. Rajapakse, A. Cecchi, C. Temperini, C.T. Supuran, Nitric oxide-donating carbonic anhydrase inhibitors for the treatment of openangle glaucoma, Bioorg. Med. Chem. Lett. 19 (2009) 6565-6570.

[49] F. Fabrizi, F. Mincione, T. Somma, G. Scozzafava, F. Galassi, E. Masini, F. Impagnatiello, C.T. Supuran, A new approach to antiglaucoma drugs: carbonic anhydrase inhibitors with or without no donating moieties. Mechanism of action and preliminary pharmacology, J. Enzyme Inhib. Med. Chem. 27 (2012) 138-147.

[50] A.M. Alaa Abdel-Aziza, A. Angeli, A.S. El-Azaba, M.A. Abu El-Enin, C.T. Supuran, Synthesis and biological evaluation of cyclic imides incorporating benzenesulfonamide moieties as carbonic anhydrase I, II, IV and IX inhibitors, Bioorg. Med. Chem. 25 (2017) 1666-1671.

[51] A. Angeli, A.M. Alaa Abdel-Aziza, A. Nocentini, A.S. El-Azaba, P. Gratteri, C.T. Supuran, Synthesis and carbonic anhydrase inhibition of polycyclic imides incorporating N-benzenesulfonamide moieties, Bioorg. Med. Chem. 25 (2017) 5373-5379.

[52] A.M. Alaa Abdel-Aziza, A.S. El-Azaba, M. Ceruso, C.T. Supuran, Carbonic anhydrase inhibitory activity of sulfonamides and carboxylic acids incorporating cyclic imide scaffolds, Bioorg. Med. Chem. Lett. 24 (2014) 5185-5189.

[53] K.K. Sethi, S.M. Verma, M. Tanç, G. Purper, G. Calafato, F. Carta, C.T. Supuran, Carbonic anhydrase inhibitors: Synthesis and inhibition of the human carbonic anhydrase isoforms I, II, IX and XII with benzene sulfonamides incorporating 4- and 3-nitrophthalimide moieties, Bioorg. Med. Chem. 22 (2014) 1586-1595.

[54] K.K. Sethi, S.M. Verma, M. Tanç, F. Carta, C.T. Supuran, Carbonic anhydrase inhibitors: Synthesis and inhibition of the cytosolic mammalian carbonic anhydrase isoforms I, II and VII with benzene sulfonamides incorporating 4,5,6,7-tetrachlorophthalimide moiety, Bioorg. Med. Chem. 21 (2013) 5168-5174.

[55] S. Kumar, V. Singh, M. Tiwari, Quantitative structure activity relationship studies of sulfamide derivatives as carbonic anhydrase inhibitor: As Antiglaucoma Agents, Med. Chem. 3 (2017) 379-386.

[56] A. Weber, M. Bo¨hm, C.T. Supuran, A. Scozzafava, C.A. Sotriffer, G. Klebe, 3D QSAR Selectivity Analyses of Carbonic Anhydrase Inhibitors: Insights for the Design of Isozyme Selective Inhibitors, J. Chem. Inf. Model. 46 (2006) 2737-2760.

[57] M. Ceruso, P. Khloya, C.T. Supuran, P.K. Sharma, 4-Functionalized 1, 3-diarylpyrazoles bearing 6-aminosulfonylbenzothiazole moiety as potent inhibitors of carbonic anhydrase isoforms hCA I, II, IX and XII, Bioorgan. Med. Chem. 24 (2014) 6945-6952.

[58] V.K. Agrawal, S. Bano, C.T. Supuran, P.V. Khadikar, QSAR study on carbonic anhydrase inhibitors: aromatic/heterocyclic sulfonamides containing 8-quinoline-sulfonyl moieties, with topical activity as antiglaucoma agents, Euro. J. Med. Chem. 39 (2004) 593-600.

[59] M.A. Mohamed, A.M. Alaa Abdel-Aziza, H.M. Sakr, A.S. El-Azaba, S. Bua, C.T. Supuran, Synthesis and human/bacterial carbonic anhydrase inhibition with a series of sulfonamides incorporating phthalimido moieties, Bioorg. Med. Chem. 25 (2017) 2524-2529.

[60] R. Kumar, V. Sharma, S. Bua, C.T. Supuran, P.K. Sharma, Synthesis and biological evaluation of benzenesulphonamide-bearing 1,4,5- trisubstituted-1,2,3-triazoles possessing human carbonic anhydrase I, II, IV, and IX inhibitory activity, J. Enzyme Inhib. Med. Chem. 32 (2017) 1187-1194.

[61] L. Vats, V. Sharma, A. Angeli, R. Kumar, C.T. Supuran, P.K. Sharma, Synthesis of novel 4-functionalized 1,5-diaryl-1,2,3-triazoles containing benzenesulfonamide moiety as carbonic anhydrase I, II, IV and IX inhibitors, Eur J Med Chem. 150 (2018) 678-686.

[62] R. Kumar, L. Vats, S. Bua, C.T. Supuran, P.K. Sharma, Design and synthesis of novel benzenesulfonamide containing 1,2,3-triazoles as potent human carbonic anhydrase isoforms I, II, IV and IX inhibitors, Eur J Med Chem. 8 (2018) 545-551.

[63] L. Vats, R. Kumar, S. Bua, A. Nocentini, P. Gratteri, C.T. Supuran, P.K. Sharma, Continued exploration and tail approach synthesis of benzenesulfonamides containing triazole and dual triazole moieties as carbonic anhydrase I, II, IV and IX inhibitors, Eur. J. Med. Chem. 183 (2019) 111698.

[64] A. Nocentini, M. Ferraroni, F. Carta, M. Ceruso, P. Gratteri, C. Lanzi, E. Masini, C.T. Supuran, Benzenesulfonamides Incorporating Flexible Triazole Moieties Are Highly Effective Carbonic Anhydrase Inhibitors: Synthesis and Kinetic, Crystallographic, Computational, and Intraocular Pressure Lowering Investigations, J. Med. Chem. 59 (2016) 10692-10704.

[65] F.Z. Küçükbay, H. Küçükbay, M. Tanc, C.T. Supuran, Synthesis and carbonic anhydrase I, II., IV and XII inhibitory properties of N-protected amino acid sulfonamide conjugates, J. Enzyme Inhib. Med. Chem. 31 (2016) 1476-1483.

[66] A.S. El-Azaba, A.M. Alaa Abdel-Aziza, R.R. Ayyad, M. Ceruso, C.T. Supuran, Inhibition of carbonic anhydrase isoforms I, II, IV, VII and XII with carboxylates and sulfonamides incorporating phthalimide/phthalic anhydride scaffolds, Bioorg. Med. Chem. 24 (2016) 20-25.

[67] A. Scozzafava, L. Menabuoni, F. Mincione, C.T. Supuran, Carbonic Anhydrase Inhibitors. A General Approach for the Preparation of Water-Soluble Sulfonamides Incorporating Polyamino-Polycarboxylate Tails and of Their Metal Complexes Possessing Long-Lasting, Topical Intraocular Pressure-Lowering Properties, J. Med. Chem. 45 (2002) 1466-1476.

[68] A. Casini, A. Scozzafava, F. Mincione, L. Menabuoni, C.T. Supuran, Carbonic Anhydrase Inhibitors: Synthesis of Water Soluble Sulfonamides Incorporating a 4-sulfamoylphenylmethylthiourea Scaffold, with Potent Intraocular Pressure Lowering Properties, J. Enzyme Inhib. Med. Chem. 17 (2002) 333-343.

[69] G. Renzi, A. Scozzafava, C.T. Supuran, Carbonic Anhydrase Inhibitors: Topical Sulfonamide Antiglaucoma Agents Incorporating Secondary Amine Moieties, Bioorg. Med. Chem. Lett. 10 (2000) 673-676.

[70] J.J. Baldwin, G.S. Ponticellot, P.S. Anderson, M.E. Christy, M.A. Murck, W.C. Randall, H. Schwam, M.F. Sugrue, J.P. Springer, P. Gautheron, J. Grove, P. Mallorga, M.P. Viader, B.M. McKeever, M.A. Navia, Thienothiopyran-2-sulfonamides: Novel Topically

Active Carbonic Anhydrase Inhibitors for the Treatment of Glaucoma, J. Med. Chem. 32 (1989) 2510-2513.

[71] J.M. Holmes, G.C.M. Lee, M. Wijono, R. Weinkam, L.A. Wheeler, M.E. Garst, Synthesis and Carbonic Anhydrase Inhibitory Activity of 4-Substituted 2-Thiop henesulfonamfdes, J. Med. Chem. 37 (1994) 1646-1651.

[72] C.T. Supuran, A. Scozzafava, I. Saramet, M. Banciu, Carbonic anhydrase inhibitors: inhibition of isozymes I, II and IV with heterocyclic mercaptans, sulfenamides, sulfonamides and their metal complexes, J. Enzyme Inhib. 13 (1998) 177-194.

[73] F. Mincione, L. Menabuoni, F. Briganti, G. Mincione, A. Scozzafava, C.T. Supuran, Carbonic Anhydrase Inhibitors: Inhibition of Isozymes I, II and IV with N-Hydroxysulfonamides - A Novel Class of Intraocular Pressure Lowering Agents, J. Enzyme Inhib. 13(1998) 267-284.

[74] A. Scozzafava, C.T. Supuran, Carbonic anhydrase inhibitors: novel compounds containing s-nh moieties: sulfenamido-sulfonamides, sulfenimido-sulfonamides and their interaction with isozymes I, II and IV J. Enzyme Inhib. 13 (1998) 419-442.

[75] M. Ilies, C.T. Supuran, A. Scozzafava, A. Casini, F. Mincione, L. Menabuoni, M.T. Caproiu, M. Maganue, M.D. Banciu, Carbonic Anhydrase Inhibitors: Sulfonamides Incorporating Furan, Thiophene and Pyrrole-carboxamido Groups Possess Strong Topical Intraocular Pressure Lowering Properties as Aqueous Suspensions, Bioorg. Med. Chem. 8 (20000 2145-2155.

[76] L. Menabuoni, A. Scozzafava, F. Mincione, F. Briganti, G. Mincione, C.T. Supuran, Carbonic anhydrase inhibitors. water-soluble, topically effective intraocular pressure lowering agents derived from isonicotinic acid and aromatic/heterocyclic sulfonamides: is the tail more important than the ring? J. Enzyme Inhib. 14 (1999) 457-474.

[77] A. Scozzafava, F. Briganti, G. Mincione, L. Menabuoni, F. Mincione, C.T. Supuran, Carbonic Anhydrase Inhibitors: Synthesis of Water-Soluble, Aminoacyl/Dipeptidyl Sulfonamides Possessing Long-Lasting Intraocular Pressure-Lowering Properties via the Topical Route, J. Med. Chem. 42 (1999) 3690-3700.

[78] A. Scozzafava, L. Menabuoni, F. Mincione, F. Briganti, G. Mincione, C.T. Supuran, Carbonic Anhydrase Inhibitors: Perfluoroalkyl/Aryl-Substituted Derivatives of Aromatic/Heterocyclic Sulfonamides as Topical Intraocular Pressure-Lowering Agents with Prolonged Duration of Action, J. Med. Chem. 43 (2000) 4542-4551.

[79] A. Scozzafava, L. Menabuoni, F. Mincione, G. Mincione, C.T. Supuran, Carbonic Anhydrase Inhibitors: Synthesis of Sulfonamides Incorporating dtpa Tails of their Zinc

Complexes with Powerful Topical Antiglaucoma Properties, Bioorg. Med. Chem. Lett. 11 (2001) 575-582.

[80] S. Avram, A.L. Milac, F. Carta, C.T. Supuran, More effective dithiocarbamate derivatives inhibiting carbonic anhdrases, generated by QSAR and computational design, J. Enzyme Inhib. Med. Chem. 28 (2013) 350-359.

[81] A. Angeli, D. Tanini, A. Capperucci, C.T. Supuran, Synthesis of novel selenides bearing benzenesulfonamide moieties as carbonic anhydrase I, II, IV, VII and IX inhibitors, ACS Med. Chem. Lett. 8 (2017) 1213-1217.

[82] N. Lolak, S. Akocak, S. Bua, M. Koca, C.T. Supuran, Design and synthesis of novel 1,3-diaryltriazene-substituted sulfonamides as potent and selective carbonic anhydrase II inhibitors, Bioorg. Chem. 77 (2018) 542-547.

[83] S. Mahmood, A. Saeed, S. Bua, A. Nocentini, P. Gratteri, C.T. Supuran, Synthesis, biological evaluation and computational studies of novel iminothiazolidinone benzenesulfonamides as potent carbonic anhydrase II and IX inhibitors, Bioorg. Chem. 77 (2018) 381-386.

[84] A. Angeli, D. Tanini, A. Capperucci, C.T. Supuran, First evaluation of organotellurium derivatives as carbonic anhydrase I, II, IV, VII and IX inhibitors, Bioorg. Chem. 76 (2018) 268–272.

[85] S. Angapelly, A. Angeli, A.J. Khan, P.V. Sri Ramya, C.T. Supuran, M. Arifuddin, Synthesis and Biological Evaluation of 4-sulfamoylphenyl/sulfocoumarin carboxamides as selective inhibitors of carbonic anhydrase isoforms hCA-II, IX and XII, Chem. Med. Chem. 13 (2018) 1165-1171.

[86] N. Chiaramonte, S. Bua, M. Ferraroni, A. Nocentini, A. Bonardi, G. Bartolucci, M. Durante, L. Lucarini, D. Chiapponi, S. Dei, D. Manetti, E. Teodori, P. Gratteri, E. Masini, C.T. Supuran, M.N. Romanelli, 2-Benzylpiperazine: A new scaffold for potent human carbonic anhydrase inhibitors. Synthesis, enzyme inhibition, enantioselectivity, computational and crystallographic studies and in vivo activity for a new class of intraocular pressure lowering agents, Euro. J. Med. Chem. 151 (2018) 363-375.

[87] C.T. Supuran, J.Y. Winum, B. Wang, Carbonic anhydrases as drug targets: general presentation. In: Drug Design of Zinc-Enzyme Inhibitors: Functional, Structural, and Disease Applications, Wiley, NJ, USA, (2009) 473-486.

[88] A.M. Alaa Abdel-Aziza, A.S. El-Azab, A.H. Ghiaty, P. Gratteri, C.T. Supuran, A. Nocentini, 4-Substituted benzenesulfonamides featuring cyclic imides moieties exhibit

potent and isoform-selective carbonic anhydrase II/IX inhibition, Bioorg. Chem. 83 (2019) 198-204.

[89] U.M. Kocyigit, Y. Budak, M. Burcu Gürdere, S. Tekin, T. Kul Köprülü, F. Ertürk, K.Özcan, I. Gülçin, M. Ceylan, Synthesis, Characterization, Anticancer, Antimicrobial and Carbonic Anhydrase Inhibition Profiles of Novel (3aR,4S,7R,7aS)-2-(4-((E)-3-(3-aryl)acryloyl) phenyl)-3a,4,7,7a-tetrahydro-1H-4,7- methanoisoindole-1,3(2H)-dione Derivatives, Bioorg. Chem. 70 (2017), 118-125.

[90] U.M. Kocyigit, Y. Budak, F. Eliguzel, P. Taslimi, D. Kılıc, I. Gulcin, M. Ceylan, Synthesis and Carbonic Anhydrase Inhibition of Tetrabromo Chalcone Derivatives, Arch. Pharm. Chem. Life Sci, 12 (2017),350.

[91] S. Burmaoglu, A.O. Yilmaz, M. F. Polat, R. Kaya, I. Gulcin, O. Algul, Synthesis and Biological Evaluation of Novel Tris-Chalcones as Potent Carbonic Anhydrase, Acetylcholinesterase, Butyrylcholinesterase and α-Glycosidase Inhibitors, Bioorg. Chem. 85 (2019),191-197.

[92] A. Cetin, F. Türkan, P. Taslimi, İ. Gulçin, Synthesis and characterization of novel substituted thiophene derivatives and discovery of their carbonic anhydrase and acetylcholinesterase inhibition effects J Biochem. Mol. Toxicol. 3 (2018); 33, e22261.

[93] N. Lolak, S. Akocak, S. Bua, C.T. Supuran, Design, synthesis and biological evaluation of novel ureido benzenesulfonamides incorporating 1,3,5-triazine moieties as potent carbonic anhydrase IX inhibitors, Bioorg. Chem. 82 (2018), 117-122.

Structure-activity relationship of Human Carbonic Anhydrase-II inhibitors: Detailed Insight for future development as anti-glaucoma agents

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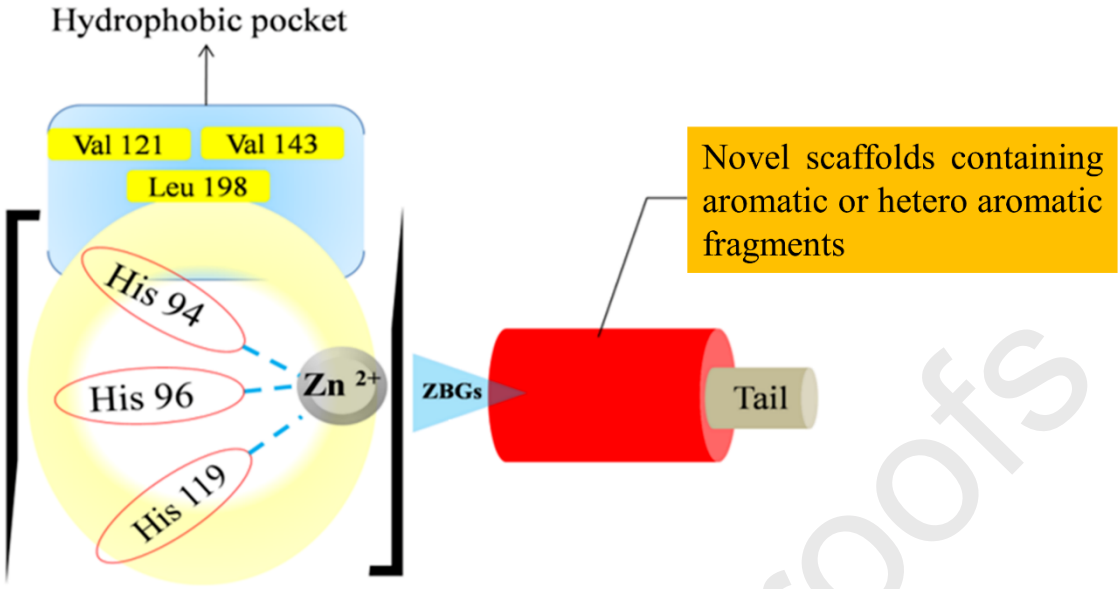

Development of novel hCA-II inhibitors as future anti-glaoma agents