REVIEW

Therapeutic potential of carbonic anhydrase inhibitors

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Abstract: Enzymes are biological catalyst involve in different biochemical reactions. But over activation of these biomolecules can cause disease thus different inhibitors and knockout therapies are use in current clinical practice. Carbonic anhydrases (CAs), a group of ubiquitously expressed metalloenzymes, are involved in numerous physiological and pathological processes, including gluconeogenesis, lipogenesis, ureagenesis, tumorigenicity and the growth and virulence of various pathogens. In addition to the established role of CA inhibitors (CAIs) as diuretics and antiglaucoma drugs, it has recently emerged that CAIs could have potential as novel anti-obesity, anticancer and anti-infective drugs. Furthermore, recent studies suggest that CA activation may provide a novel therapy for Alzheimer's disease. This article discusses the biological rationale for the novel uses of inhibitors or activators of CA activity in multiple diseases, and highlights progress in the development of specific modulators of the relevant CA isoforms, some of which are now being evaluated in clinical trials.

Keywords: Carbonic anhydrase, inhibitors, potential targets, therapeutic uses.

INTRODUCTION

Carbonic anhydrase (EC 4.2.1.1) are metalloenzymes ubiquitously present in eukaryotes as well as prokaryotes. Thus, classified in 4 families α-carbonic anhydrases, β-carbonic anhydrases, γ-carbonic anhydrases and δ-carbonic anhydrases. The α-CA are present in algae, bacteria, vertebrates, and green plants, β-CA are present in chloroplast of dicotyledons and monocotyledons, γ-CA are in archaea, while as δ-CA are found to be in marine (Scozzafava *et al*., 2006; Supuran *et al*., 2003). In mammals 16 isoforms of alpha carbonic anhydrase are performing its function in the catalytic activity of tissue distribution and subcellular localization (Nishimori, 2007a; Alterio, 2006). Five isoforms (CA-II, III, VII, XIII) are present in the cytosol, two (CA-VA, CA-VB) are in mitochondria, five (CA-IV, IX, XII, XIV, XV) are membrane bounded and CA-VI is secretory isoform of carbonic anhydrase (Kohler, 2007; Saczewski, 2006).

Carbonic anhydrases are responsible for the conversion of CO_2 to proton and bicarbonate. As for metalloenzyme Zn is present in the active site of carbonic anhydrase enzyme. This catalysis of CO_2 is involved in many physiological and pathological reaction I-e transformation of CO_2 and transformation of bicarbonate between lungs and metabolizing tissues, CO_2 hemostasis, pH and respiration, electrolytes balance in various organs and tissues, bone resorption tumorigenicity, anabolic reactions like lipogenesis, glycogenesis and ureagenesis and calcification (Abbate, 2004b; Nishimori, 2004).

Almost all the carbonic anhydrase isozymes are potential therapeutic targets for the treatment of glaucoma, cancer, osteoporosis, edema, obesity and epilepsy. For the inhibition activity of carbonic anhydrase two classes are mainly involve are sulfonamides and metal complexes. These compounds bind to the Zn^{2+} ion in the active site of enzyme and form a trigonal bipyramidal geometry with zinc. Almost 25 carbonic anhydrase inhibitors are use in current clinical practice most of them are of sulfonamide family (Innocenti, 2004; Saczewski, 2006; Scozzafava *et al*., 2013 ; Pastorekova, 2004).

Moreover, carbonic anhydrase inhibitors are also having potential activity to treat bacterial, protozoal and fungal infections such as helicobacter pylori, candida albicans, plasmodium falciparum, mycobacterium tuberculosis and

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Cryptococcus neoformans (Krungkrai, 2005; Nishimori, 2006; Nishimori, 2007c; Klengel, 2005; Bahn, 2005). 26-34 in addition to that carbonic anhydrase inhibitors may give pharmacological effect for the treatment of memory disorders and Alzheimer's disease (Sun and Alkon, 2002).

Carbonic anhydrase inhibitors

Some classical carbonic anhydrase are given below as compound 1 (Acetazolamide), compound 2 (Methazolamide), compound 3 (Ethoxzolamide), compound 4 (Sulthiame) and compound 5 (Dichlorophenamide). Some of the recently investigated drugs are compound 6 (Dorzolamide), compound 7 (Brinzolamide), compound 8 (Indisulam), compound 9 (Topiramate), compound 10 (Zonisamide), compound 11 (phentermine) compound 12 (Sulpiride). Compound 13 and 14 are involve in CAIX inhibition for the treatment of tumor. Several of these composites were developed initially for the exploration of diuretics, amongst those thiazides derivatives, compound 15, compound 16, compound 17, compound 18 and compound 19 are of the same nucleus, are restudied after the exploration of new isozymes, and compound 20-25 are currently of extensive use clinically (Supuran, 2004; Supuran *et al*.; 2003; Supuran, 2008). As particular isozyme is involved in a particular pathology thus an extended pharmacological role of therapeutic agent is explainable for each isozyme inhibition, that's why carbonic anhydrase inhibitors are involved in a wide range of therapeutic application starting from anti-glaucomic agent to anti-obesity, and anticancer to anti-epileptic agents. Fig. 1 represents the general structure of CA, however, selectivity and localization is still an issue for carbonic anhydrase inhibitors. Likewise, sulfonamide derivatives showed potential inhibition to carbonic anhydrase XIII, XII, IX, VII, VI and II in lower Nano-molar concentration, the remaining isozymes like XIV, VA, VB, IV, and CAI did not show potential inhibition against these drugs (Pastorekova, 2004; Nishimori, 2007a; Nishimori, 2005b; Nishimori, 2007b; Nishimori, 2006; Vullo, 2003; Vullo, 2005; Lehtonen, 2004; Nishimori, 2005a).

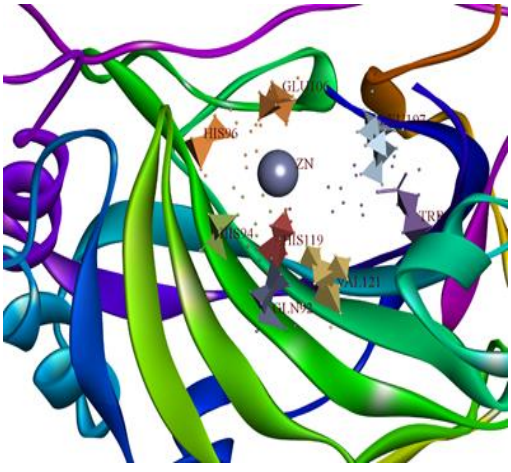


Fig. 1: General structure of carbonic anhydrase

MATERIALS AND METHODS

For the review of literature regarding this study, different key words are used I-e "carbonic anhydrase inhibitors, carbonic anhydrase isoforms in different diseases, clinically available carbonic anhydrase inhibitors". Data was collected from science direct, PubMed and google scholar using chemical and biological abstracts. Compounds were selected on the bases of their clinical importance and application as a potential inhibitor of different isozymes and its therapeutic involvements in specific diseases. Different mechanisms and pathophysiology of diseases were also searched out with respect to specific isozyme of carbonic anhydrase. The resulted data was then rechecked and comparisons were drawn to its literature and binding poses were predicted using AutoDock.

Molecular docking protocol

For all the potent clinically used carbonic anhydrase inhibitors, molecular docking was carried out using MGL tools V1.5.6 and AutoDock v4.2. All the structures were drawn using ChemDraw ultra 12.0 and converted to 3D using chem-3D pro 12.0 using MM2 the energies of the ligand structures were minimized. The carbonic anhydrase II (PDB ID: 5je7), IX (PDB ID: 5sz6), IV (PDB ID: 5jn8), and XII (PDB ID: 4ww8) were downloaded from RCSB protein data bank. All the protein structures were having less than 2.0 Å resolution. The protein structures were prepared for docking by adding water molecules, by removing co-crystallized ligand and adding charges and hydrogens to the enzyme. Prior to the deletion of ligands, the active site was defined by selecting the grids around the co-crystallized ligands for carrying out molecular docking. Using Lamarkian Genetic Algorithm, 100 poses were generated for docking the compound. The possible docked poses and binding sites were selected by visualizing carefully and from the calculated binding free energies. Visualizer discovery studio was used to generate the possible binding pose figs. (Santos-Martins *et al*., 2014).

Mechanism of carbonic anhydrase inhibition

For the activity of carbonic anhydrase His3, His4, His 10, His15, His 17, His 64, Glu 106, Thr 199, Val 121, Val 143, Leu 198 are mainly involve in the reaction. As carbonic anhydrase is a metallic enzyme and Zinc Is present as a metal, which is responsible for the boning to the carboxylate moiety of Glu 106 which intern results a nucleophilic attack to the CO_2 as a substrate, the hydroxyl part of water bound to zinc and activate the enzyme and thus create the nucleophilic attack on the CO_2 thus resulting in the bicarbonates that's why the sulfonamides moiety show potential binding because of the Sulfoxide hydrophobicity and the amine part alkylation (Pastorekova, 2004).

Carbonic anhydrase as diuretics

Physiology and pathophysiology

Carbonic anhydrases are immensely abundant in kidney, three main physiological functions are because of carbonic anhydrase different isoforms in the kidney: that are; the homeostasis equilibrium of acid–base balance (by concealing and expelling protons, because of the hydration of CO_2 response, catalyzed by these isoforms); the reabsorption of bicarbonate progression; and the $NH4^+$ yield (Splendiani and Condo, 2006; Kyllonen, 2003). The excretion of bicarbonate increases in the urine, with K^+ and Na^+ as complementary cations, although the quantity of chloride defecation is reduced. This categorization of on the base of the inhibition of carbonic anhydrase in the proximal tubule, thus a prime reduction in the H^+ excretion occurs by the upper loop of nephron. Subsequent to the introduction of a carbonic anhydrase inhibitor, I-e acetazolamide, the urine volume increases and turn into alkaline (Kyllonen, 2003; Supuran, 2004). Fig. 2 shows all compounds involved in active site of CAII, while Fig. 3 shows possible poses of all compounds.

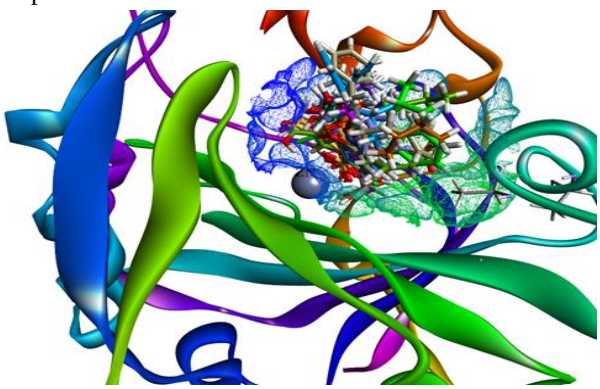


Fig. 2: Figure shows all the compounds involved in the diuresis, in carbonic anhydrase II (Pdb. id: 5je7) active site.

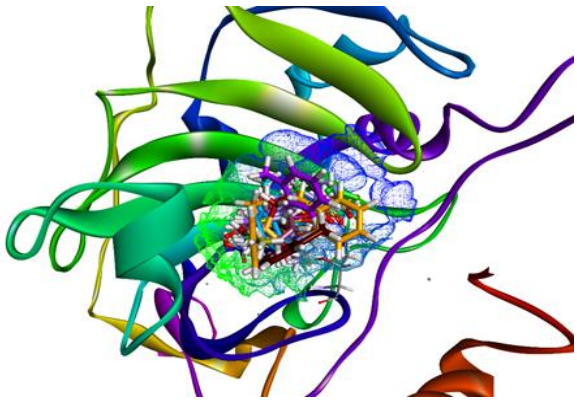


Fig. 3: Figure shows docked poses of all the compounds involved in the diuresis in CAXII PDB ID: 4ww8

Carbonic anhydrase in glaucoma

Physiology and pathophysiology

CAIs as therapeutic for ocular ailments mainly treat Glaucoma which is a chronic, degenerative ophthalmic disorder, characterized by increased intraocular pressure (IOP) that reasons irretrievable damage to the head of optic nerve, subsequent in the progressive damage of pictorial role and finally impaired vision (Mincione, 2007; Sugrue, 2000; Scozzafava, 1999a). Previously reported suggested that this is because of the sodium bicarbonate secretion (Splendiani and Condo, 2006; Kyllonen, 2003). Carbonic anhydrase were found to be responsible for this release of bicarbonates. Thus, CAIs signifies the treatment of glaucoma, as via preventing the ciliary-course enzyme the sulfonamide vulnerable isozyme CA II decrease the degree of bicarbonate and aqueous humor excretion, ensuing in a 25-30% reduction in IOP (Scozzafava, 1999b; Ilies, 2000; Scozzafava, 2000; Scozzafava, 2002; Winum, 2004).

Carbonic anhydrase inhibitors for obesity

Physiology and pathology

Carbonic anhydrases are potentially use for obesity, two isozymes (VA and VB) of carbonic anhydrase are actively present in the mitochondria and are responsible for biochemical processes like gluconeogenesis, lipogenesis and ureagenesis. The substrate for the production of bicarbonates are provided by carbamoyl phosphate synthases I and II, acetyl-CoA carboxylase (ACC), and pyruvate carboxylase (PC) are mainly assured by the metabolic reaction of mitochondria which involve the mitochondrial isozymes (VA and VB) and probably assisted by CAII which is a cytosolic isozyme but highly active (Supuran, 2003).

The actual role of CA-VA and CA-VB (mitochondrial CAs) is to pursue the pyruvate carboxylase enzyme to offer enough carbon moieties in the form of bicarbonates in mitochondria. Which are required by the pyruvate conversion to oxaloacetate and then reacting with acetyl coenzyme-A to form citrate. And then with the help of tricarboxylate transporter, citrate move to cytosol and then converted back to oxaloacetate and coenzyme-A with the catalytic activity of ATP citrate-lisase. Here the oxaloacetate decarboxylate the to aid the transfer of pyruvate to mitochondria with the help of pyruvate decarboxylase, and the coenzyme-A in the cytosol convert in to malonyl coenzyme-A with cytosolic acetyl coenzyme-A carboxylase which is a utilizer of bicarbonates whom are produced by carbonic anhydrase II (cytosolic CA), the malonyl-CoA is an initiator of fatty acids (Supuran, 2003; Supuran, 2008). Thus, this conversion of bicarbonates from CAII are observed to be inhibited by sulfamate/sulfonamide *in vitro* as well as *in-vivo* (Supuran, 2003).

Anticancer effect of Carbonic anhydrase

Pathophysiology of c Carbonic anhydrase

Carbonic anhydrase IX is an isoform of human alpha carbonic anhydrases (αCA), which is important for the CO_2 hydrolyses reaction. In tumors, the over expression of

carbonic anhydrase occurs because of the hypoxic condition through HIF cascade. Which thus cause pH imbalance and in most of the tumors the pH become acidic (pH 6) with respect to the pH of normal tissues (pH 7.5), this reduction in pH is supposed to be caused by the production of HCO_3^+ and pyrimidine nucleotides by CA IX over expression (Thiry, 2006; Pastorekova and Pastorek, 2004; Dubois, 2007) which is thus needed to be reduced to normalize the pH of tumor. The pH of tumor is also decreased by the production of lactic acid (produced by the glycolysis) and the hydration of CO_2 associated by CA IX. Acidic pH can cause chromosomal rearrangement, migration and invasion, tumorigenic transformation, breakdown of extracellular matrix, protease inactivation and cell growth factors expression.
Fig. 4 depicts drugs responsible for diuresis in active site of CA XIV (pdb. ID: 5jn8).

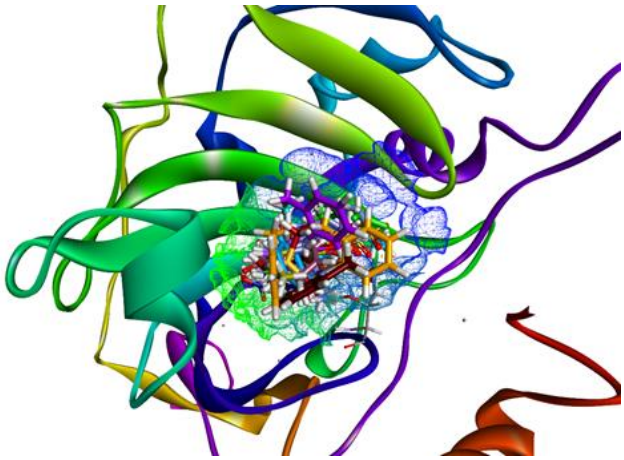


Fig. 4: Figure shows the drugs responsible for diuresis in active site of carbonic anhydrase XIV (pdb. ID: 5jn8)

Carbonic anhydrase inhibitors in Anti- osteoporosis
Physiology and Pathophysiology
The carbonic anhydrase II (CA II) is ubiquitous in almost every tissue. It is also abundant in bones, especially in osteoclasts and its concentration is almost similar to that of kidney concentration (Riihonen, 2007). For the mobilization of calcium in bones; an ATPase proton pump is involved and for this pump the hydrogen is produced by the hydration of CO_2, as the hydrolysis of carbon dioxide is done by carbonic anhydrase. For the organic matrix of bone removal with the aid of enzyme the same ATPase pump is used in which the membrane bound isozymes (CA IV and XIV) expression is observed in osteoclasts *in vitro* as well as *in vivo* (Scozzafava *et al*., 2000). Fig. 5 depicts CA II inhibitors in the active site of pdb ID: 5je7.

It has been observed that release of H^+ cause a decrease in the pH, from which a hypothesis is drawn that osteoclasts are responsible for the metabolons transport, and carbonic anhydrase in low concentration are observed to increase the osteoclasts and increase the bone resorption activity in rats, but a high concentration of these inhibitors affect the survival of the cells (Riihonen, 2007). Expression of Carbonic anhydrase IX is strongly augmented in numerus forms of tumors. I-e papillary or follicular carcinomas, ependymomas or gliomas , uterine cervix, (Swietach, 2007; Hutchison, 2004) head and neck(Koukourakis, 2004) , breast, (Pouyssegur, 2006; Potter and Harris, 2003; Hussain, 2007) nasopharyngeal carcinoma,(Sung, 2007) oesophagus, mesotheliomas, (Pastorekova and Pastorek, 2004) brain, kidney, (Dorai, 2006) bladder carcinomas,(Trastour, 2007) lungs,(Hussain, 2007) squamous/basal cell carcinomas, vulva, and other tumors. Up to 150-fold increase of CA IX expression is proposed to be because of VHL gene mutation and upregulation because of HIF activation (Thiry, 2006; Potter and Harris, 2003). Fig. 6 represents the potential docked pose of compound 17 in CAIX active domain.

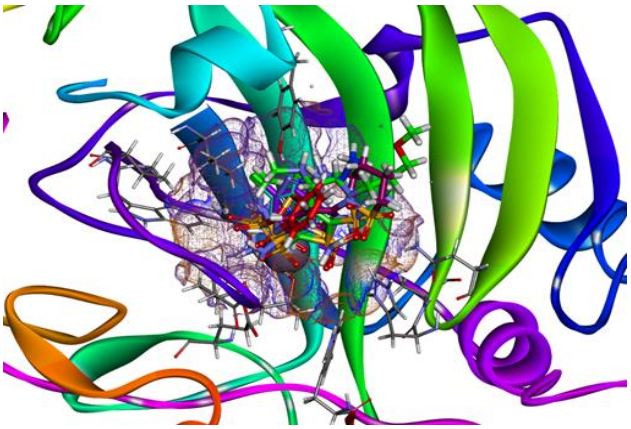


Fig. 5: Figure showed carbonic anhydrase II inhibitors in the active site of pdb ID: 5je7

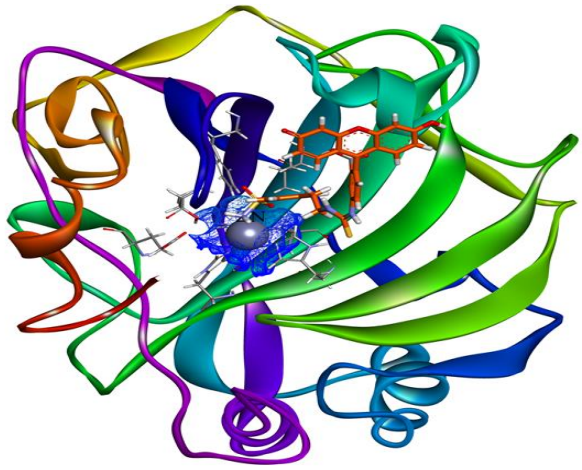


Fig. 6: Potential docked pose of compound 17 in CAIX active site.

RESULTS

Inhibitors and its mechanism of action as diuretics
Inhibition of cytosolic CA (CAII) and membrane bound CA (CA IV, XII and CA XIV) enzymes appeared to be

tangle in the diuretic possessions of the sulfonamides (Kyllonen, 2003; Supuran, 2004). The net conclusion of these progressions is the passage of HCO^{3+} from the tubular lumen to the interstitial fluid, afterward the transfer of the excess water isotonically and thus increase diuresis. Acetazolamide, dichlorophenamide (compound 4), ethoxzolamide (compound 3), and methazolamide (compound 2) are used to treat edema due to for drug-induced and congestive heart failure edema. Numerous other diuretics, for example, quinethazone (compound 20); metolazone (compound 21); bumetanide (compound 25); for instance the benzothiadiazines (compounds 15 to 19; chlorthalidone (compound 22); chlorothiazide and hydrochlorothiazide);indapamide (compound 23); and furosemide (compound 24); act as CA inhibitors with erratic effectiveness (Splendiani and Condo, 2006; Supuran, 2004; Supuran *et al*., 2003). This is to be anticipated, as they entirely have primary sulphonamide unsubstituted moieties which shows effective binding to zinc binding site. All detail is shown in figs. 2 and 3.

Inhibitors and its mechanism of action in glaucoma

Indeed, systemic dichlorphenamide (compound 5), ethoxzolamide (compound 3) methazolamide (compound 2), or acetazolamide (compound 1) are widely used to indulge glaucoma (Scozzafava, 2000). Whilst in all the above drugs, acetazolamide is used extensively for long period in the treatment of glaucoma because of it less toxicity and modeled pharmacokinetics.

Inhibitors and its mechanism of action in obesity

Topiramate (compound 9) is a potential anticonvulsant drug by different proposed mechanism like antagonistic effect of the sodium dependent voltage gated channels, and voltage gated calcium channels, increasing transmission of GABAergic transmission, and having negative effect on the isoxazolepropionic acid receptors (Shah *et al*., 2000; Gordon and Price, 1999; Picard, 2000) and Topiramate was also effective against several Cas amongst the mitochondrial CAs (VA and VB) are most prominently inhibited (Supuran, 2003).

Inhibitors and mechanism in therapy of cancer

Among all carbonic anhydrase, CA IX is more prone to inhibit by sulfamates and sulfonamides, (Thiry, 2006; Pastorekova and Pastorek, 2004; Dubois, 2007) because of its interaction with the Zn ion in carbonic anhydrase active site and its interactions towards the hydrophilic and hydrophobic amino-acids in the cavity of carbonic anhydrase. Indisulam (compound 8), is a sulfonamide moiety having potential anticancer potential, which was recently identified as potent CA IX inhibitor in nanomoles (Abbate, 2004a; Owa, 2002; Owa, 1999; Talbot, 2007). Though its detail mechanism is not clearly known but it is supposed to be involved in the perturbation of G1 or/and G2 phases of cell cycle, the cyclin dependent kinase 2 inhibitor (CDK2), the cyclins downregulation, the retinoblastoma protein inhibitor (prb) immune responses, differential expression and phosphorylation of cell adhesion and signaling, with CA IX inhibition. CA-IX selective inhibitors are (type 13 and 14) reduce the acidity of the medium. Compound 13 is fluorescent sulfonamide which binds to CA IX in-vivo in hypoxic condition (Dubois, 2007; Svastova, 2004; Cecchi, 2005). That why this compound is also used as a fluorescent probe in tumor imaging. Compound 14 is membrane impermeable (positively charged) compound. That's why these compounds cannot inhibit carbonic anhydrase intracellularly. Thus, inhibit all isoforms of carbonic anhydrase generally (Ilies, 2000; Scozzafava, 2000; Scozzafava, 2002) and thus have less side effects (I-e acetazolamide). It has been recently reported that the crystal structure of CAII and CAIX is much similar.(Pastorekova and Pastorek, 2004) Compound 14 (positively charged pyridinium derivative) bind with in the active site of carbonic anhydrase and deprotonate the catalytic Zn^{2+}. The trimethyl pyridinium ring also show binding potential to phe131 (important amino-acid in binding site of CA) (Menchise, 2005).

Inhibitors and mechanism of action in the treatment of osteoporosis

A novel inhibitor (compound 14) of carbonic anhydrase has been observed to have effect on the membrane impermeable carbonic anhydrases. Thus, will result in decrease inhibition of membrane bound carbonic anhydrase CAIV and XIV. And will only affect the CAII (Riihonen, 2007).

Antimicrobial activity of carbonic anhydrase

Carbonic anhydrase is involved and important for every living specie thus the inhibitors of carbonic anhydrase I and II are utilized against plasmodium falciparum, in which4-(3,4-Dichlorophenylureido-ethyl)-benzene-sulfon amide produce very significant results in-vitro as well as ex-vivo in Nano molar range (80 nM) and in micro molar concentration (20 µM) respectively. So it clearly indicate that sulfonamide derivatives can show potential inhibition and are new target for malarial parasites (Krungkrai, 2005).

DISCUSSION

The initial diuretic which was used clinically was Acetazolamide in 1956. It signifies the model of a class of therapeutic agents with comparatively limited pharmacological use, but it played a key role in the expansion of ultimate renal pharmacology and physiology, and in the projection of numerous of the recent extensively used diuretic mediators, for instance the high-ceiling (loop) and the thiazide diuretic. CAs are far and widely expressed in vertebrates, the administration of sulfonamides systemically led to undesired side effect because of its non-selective inhibition of all carbonic

anhydrase isoforms such as tingling and numbness of extremities; fatigue malaise; decreased libido; metabolic acidosis; metallic taste; gastrointestinal irritation; transient myo-pia renal calculi; weight loss; and depression. Thus, in 1990s the water-soluble sulfonamide as CAIs were launched by MERK with the trade name Trusopt (dorzolamide (compound 6)) in eye drops (Sugrue, 2000). A second drug like that was launch by ALCON under the trade name AZOPT (brinzolamide (compound 7)) and is used for the treatment of glaucoma topically (Sugrue, 2000). Brinzolamide and dorzolamide are potent hydrophilic CAIs that are necessarily lipophilic to penetrate the cornea, and can be topically administered as a free base or as the Hydrochloride salt (Sugrue, 2000). These two drugs are very effective with few side effects I-e, reddening of the eye or burning, pruritus, stinging, and blurred vision, which may be because of the acidic pH of the dorzolamide. Likewise, An unpleasant taste by both topical as well as systemic CAIs is experienced, which can be possibly because of drug burdened lachrymal fluid dumping into the oropharynx and thus inhibiting CAs isoforms extant in the taste buds (CA VI and CA II) and saliva (CA VI) with the subsequent accumulation of bicarbonates (Scozzafava, 1999a). Another new approach is used to attach hydrophilic moieties and other derivatives, to heterocyclic and aromatic sulfonamides, which yields two to three times more effective derivatives than dorzolamide to reduce IOP in rodents (Scozzafava, 1999a; Scozzafava, 1999b; Ilies, 2000; Scozzafava, 2000; Scozzafava, 2002; Winum, 2004). These derivatives are potent human CA II inhibitors, which penetrated in to the cornea and potentially reduce IOP in both glaucomatous and normotensive rabbits. Thus, more efforts are necessary to improved understanding to words the immersion of numerous CA isoforms in optic pathologies such as macular degeneration, retinopathy and glaucoma.
Topiramate is an effective carbonic anhydrase inhibitor with Ki 10nM against CAII, 63nM against CA-VA and 30nM against CA-VB (Nishimori, 2005b; Winum, 2006). Crystallographic studies of Topiramate reveals that there is a classical tetrahedral geometry between the zinc ion and the sulfamte moiety while as scaffold of this molecule is ensnared in the enzymatic cleft with the means of hydrogen bonding and different wonder Waals forces. Using the Topiramate with carbonic anhydrase II molecular dynamics studies showed a similar binding mode with CA-VA (Casini, 2003; Vitale *et al*., 2007). This whole mechanism strongly suggest that CAI are involve in the inhibition of de-novo lipogenesis and thus as anti-obesity drugs.

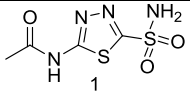
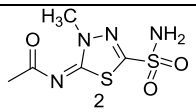
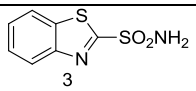
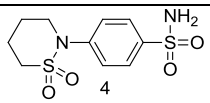
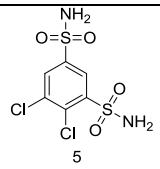
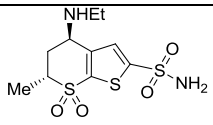
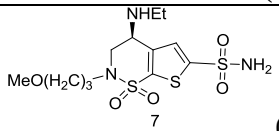
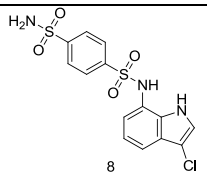
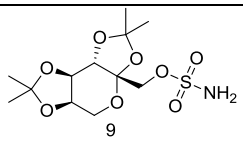
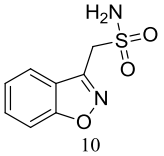
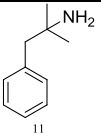
First patent related to CAI as anti-obesity was claimed in 2010, in which a sympathomimetic drug (phentermine) was reported for its anticonvulsant activity because of sulfamate moiety in the drug the patent incudes the treatment and prevention of obesity (Najarian, 2010) and the rationale of mitochondrial carbonic anhydrase (VA and VB) inhibition was also reported with the same pathway as discussed above (Supuran, 2003). In 2001 anticonvulsant agents like carbamazepine and valproic acid was also reported as prophylactic treatment for obesity.(Kozachuk, 2001) Novel sulfamides and sulfamates have also been reported by the same pharma company as carbonic anhydrase inhibitors for prophylactic treatment of obesity (Antel *et al*., 2007).

In 2015 Najarian *et al*. reported Topiramate with combination of sympathomimetic as anti-obesity agent (Najarian *et al*., 2015a; Najarian *et al*., 2015b). In 2012 phenteramine and Topiramate are approved by FDA as delayed release formulation with the market name Onexa (Heal *et al*., 2012) but marked reduction in weight loss is observed by Zonisamide (primary sylphonamide) (Zareba, 2005) the mechanism of action of this drug is also similar to Topiramate (mitochondrial CA-VA inhibition) and is studied via homology modeling kinetics and molecular dynamics (Vitale *et al*., 2007; Najarian, 2010). These patents of sulfonamides as anti-obesity drug are reported under US 2005/0026977, WO2008/153632, WO2009/017755, US2011/0098289 and US2008/ 0319036 (Najarian *et al*., 2008; Jennings, 2004; Murphy, 2011; Hauske, 2008). As for the docking studies, most of the researchers use CAII as a protein because CAIX crystal structure is not yet been identified and other used a modeled design of CAIX. Thus it has been notify that CAIX inhibitors are important for the hypoxic tumor management, because these kind of tumors do not respond to the classic radiotherapy or chemotherapy (Svastova, 2004; Thiry, 2006; Dubois, 2007).

Plasmodium falciparum is a malarial parasite, and is life threatening in human and currently the antimicrobial resistant is a global issue. H-pylori is a common microbe which reside in the acidic environment and carbonic anhydrase provide this acidity by hydrolysis of CO_2, so α and β class of carbonic anhydrase were isolated form different strains of H-pylori and different drug like acetazolamide, topiramate, ethoxzolamide and sulpiride was use for its inhibition potential against both α and β class of carbonic anhydrase and all the compounds shown significant activity against both the class of carbonic anhydrase thus it is also a new window for the treatment of gastric lesions of mucosa, gastric ulcer gastritis and gastric cancer (Nishimori, 2006; Nishimori, 2007c).

In the signaling pathway of fungus *Candida albicans* has been observed that the concentration of HCO_3^+ and CO_2 is important for the filamentation induced by adenylyl cyclase is maintained by the involvement of carbonic anhydrase. Because the balance between HCO_3^+ and CO_2 is due to carbonic anhydrase, and the cyclic AMP signaling is because of CO_2 and HCO_3^+ balance hence it is required by the fungal pathogenesis thus this can be control by carbonic anhydrase inhibitors and can be a new potential target for anti-fungal therapies (Klengel, 2005).

Table 1: List of various carbonic anhydrase inhibitors against its various isoforms

Name	Structures (Compound number).	Activity against
Acetazolamide	 (1)	hCA II, hCA VI
Methazolamide	 (2)	hCAII, hCAVI, hCAVII, hCAXII
Ethoxzolamide	 (3)	hCAII
Sulthiame	 (4)	hCAII, hCAVII
Dichlorophenamide	 (5)	hCAVB
Dorzolamide	 (6)	hCAII, hCAVI, hCAXII
Brinzolamide	 (7)	hCAII, hCAVI, hCAVII, hCAXII
Indisulam	 (8)	hCAII, hCAXII
Topiramate	 (9)	hCAII, hCAVII, hCAXII, hCAVA, hCAVB
Zonisamide	 (10)	hCAVA, hCAIX
Phentermine	 (11)	hCAVA, hCAVB

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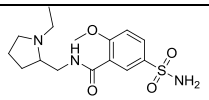
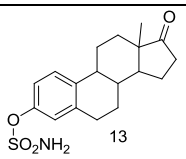
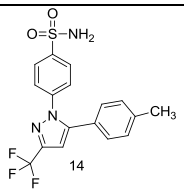
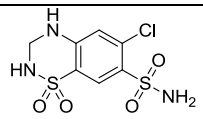
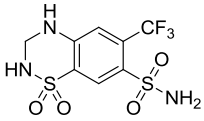
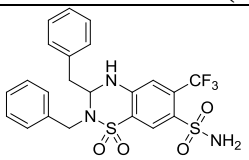
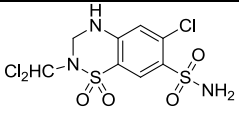
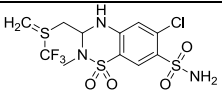
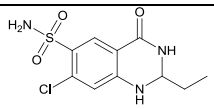
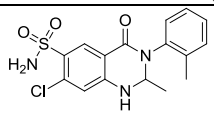
Name	Structures (Compound number).	Activity against
Sulpiride	 12 (12)	hCAVI, hCAXII
EMATE	 13 (13)	hCAII, hCAXII
Celecoxib	 14 (14)	hCAII, hCAIX, hCAXII
Chlorothiazide	 15 (15)	hCAII, hCAIX, hCAXII
Hydroflumethiazide	 16 (16)	hCAII, hCAIX, hCAXII
Bendroflumethiazide	 17 (17)	hCAII, hCAIX, hCAXII
Trichloromethiazide	 18 (18)	hCAII, hCAIX, hCAXII
Polythiazide	 19 (19)	hCAII, hCAIX, hCAXII
Quinethazone	 20 (20)	hCAII
Metolazone	 21 (21)	hCAVII, hCAXII

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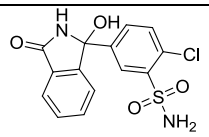
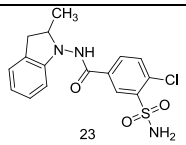
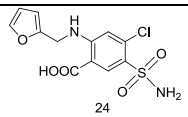
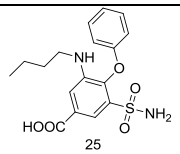
Name	Structures (Compound number).	Activity against
Chlorthalidone	 22 (22)	hCAVII, hCAXII
\Indapamide	 23 (23)	hCAVII, hCAXII
Furosemide	 24 (24)	hCAI, hCAII
Bumetanide	 25 (25)	hCAIX, hCAXII

CONCLUSIONS

The last decade has been very productive and dynamic for the research of carbonic anhydrase inhibitors. Certainly, infectious disease need anti-microbial agents and resistance the most common problem in today medical ailments caused by Mycobacterium tuberculosis, plasmodium falciparum and *H. pylori*, thus carbonic anhydrase inhibitors can overcome this problem. Different isozymes have been isolated characterized and reported, due to which the catalytic role of carbonic anhydrase is evolving day by day and importance for the inhibitors are increasing with it, and selective inhibitors are required for each isozyme to treat different disease irrespective of other complications and adverse effects. Nonselective inhibitors of carbonic anhydrase II inhibitors show selectivity for carbonic anhydrase XIII, IX, and VA that is due to the resemblance of structure of CAII to all the other isoforms.

The fluorescently activated carbonic anhydrase inhibitors show selectivity towards CAIX and thus show anti-tumor activity as these are membrane-impermeable compounds and active of membrane associated carbonic anhydrase (CAIX) due to this impermeability selective CAIX inhibitors have few to non-side effects.Numerous anti-obesity and ophthalmic applications of carbonic anhydrase inhibitors has been reported and these inhibitors are also having anti-convalescent application but their selectivity is still questioned thus they are prone to other adverse effect as well because of the non-selective inhibition.

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