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A Review on Toxicity Prediction of Carbonic Anhydrase Inhibitors

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Abstract: Carbonic anhydrases (CAs, EC 4.2.1.1) are the metalloenzymes that are widespread in living organisms. Several CA isoforms found in humans, bovine, murine are competent therapeutic targets to regress various diseases like cancer, edema, glaucoma, epilepsy, obesity, stroke, sterility, & neural disorders. Similarly, in photosynthetic organisms CAs maintains carbon concentration around RuBisCo & regulates photosynthetic pathways which results growth & development. The abundant growth of harmful strains of algae & cyanobacteria form blooms that are associated with the release of fatal toxins. Along with that the biofilm-producing cyanobacteria colonize various historical monuments, caves, & pre-historic sites. They stain the surfaces by secreting biogenic pigments significantly reducing their aesthetic value.In this review we discuss several synthetic and natural CA inhibitors, their role,mechanism of action, structural arrangement,side effects& potency in the form of IC_{50} against wide classes of CA isozymes. Preliminary ADMET investigations of synthetic molecules as well as phytochemicals were performed to identify the most effective & non-toxic molecules. Following synthetic molecules (acetazolamide, dichlorophenamide, methazolamide, ethoxzolamide)&phytochemicals such as phenols (p-Hydroxybenzoic acid, p-Coumaric acid), polyphenols (curcumin, catechin, rosmarinic acid), flavonoids (luteolin, fisetin, rhamnetin)& coumarin are potent non-toxic molecules& may be competent for the treatment of a variety of human disorders as well as helpful to inhibit the toxic cyanobacterial strains.

Keywords: Carbonic anhydrase; CA, s Isoforms; CA Inhibitors; Phytochemicals & Synthetic; ADMET

1. Introduction

Carbonic anhydrase (CA) is a metalloenzyme that is ubiquitous &catalysesthe interconversion of carbon dioxide & bicarbonate (1).They are globally distributed among Archaea, prokaryotes,& eukaryotes (2) and encoded by three evolutionarily unrelated gene families: α, β, & γ. αCAs are mainly present in vertebrates, plants, algae, & eubacteria; β CAs in bacteria, algae, & fungi; & γ CAs in archaea & bacteria. In addition, δ & ζ CAs have been reported in marine diatoms, & η CAs in protozoa(3,4). CAs serve the vital role in all physiological, bio-synthetic & pathological processes carried out in vertebrates (5,6). In algae, plants, & certain bacteria, CAs utilize CO_2/HCO_3^- as substrates to carry out

photosynthesis & other biosynthetic processes (7,8), whereas in marine diatoms, δ, ζ CAs facilitate CO_2 fixation (Xu et al., 2008)(Figure 2).

Zn (II) metal ions are present in all classes of CAs (α, β, γ, δ, & ζ) but γ CAs are bound to Fe (II) or Co (II) & ζ CAs to Cd (II) in their active sites (9,10) in some cases (Figure 3). Sixteen isoforms of α-CA have been reported in mammals (CA I – CA XVI) & fifteen isoforms in primates (lack CA XV)(9,11). In many tissues or organs, the isoforms of CAs serve their functions collectively, such as CAs IX & XII in hypoxic tumor cells (5) & CAs VA & XIV in liver cells (9). Of the CAs isoforms, five are cytosolic (I, II, III, VII, XIII), five are membrane-bound (IV, IX, XII, XIV, XV), two are mitochondrial (VA, VB), one secretory (VI) & three cytosolic acatalytic carbonic anhydrase-related proteins (CARP VIII, CARP X, CARP XI) (9)(Figure 1).

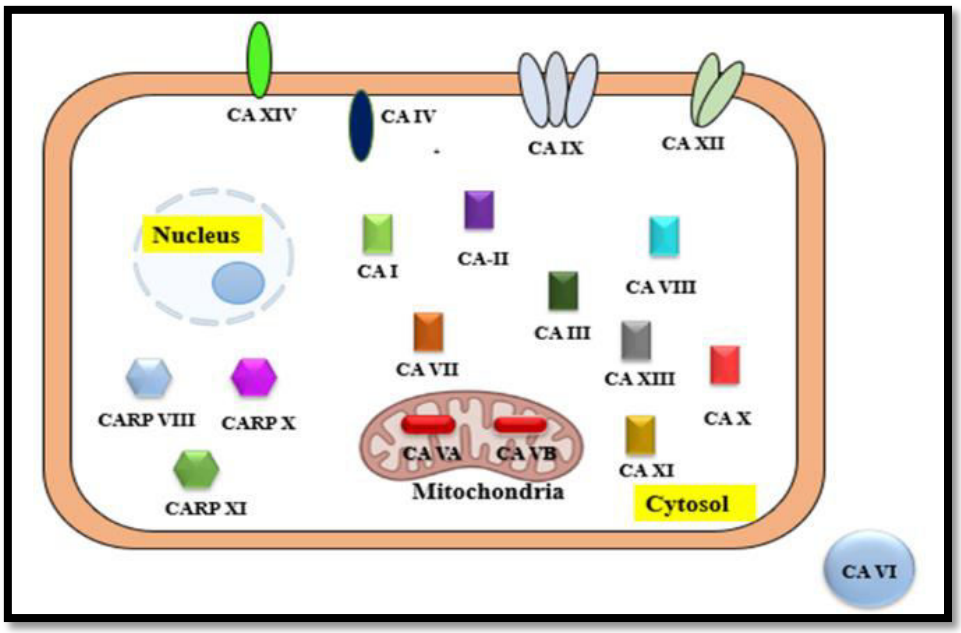


Figure 1: Isoforms of CAs

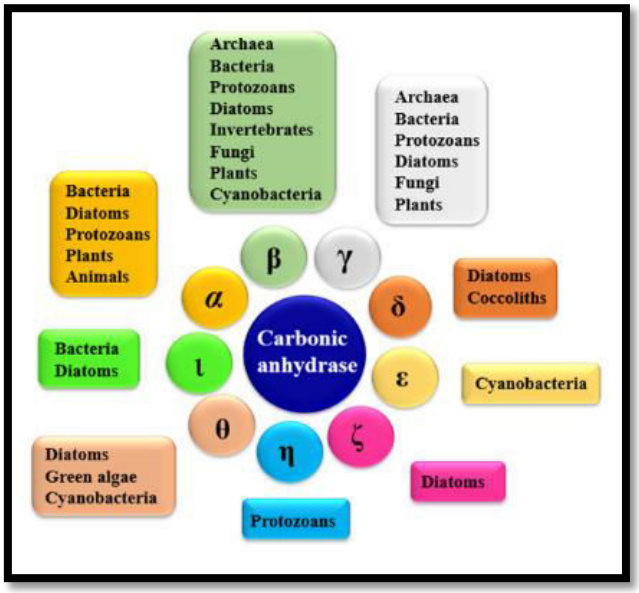


Figure 2:The distribution of carbonic anhydrase classes in various organisms

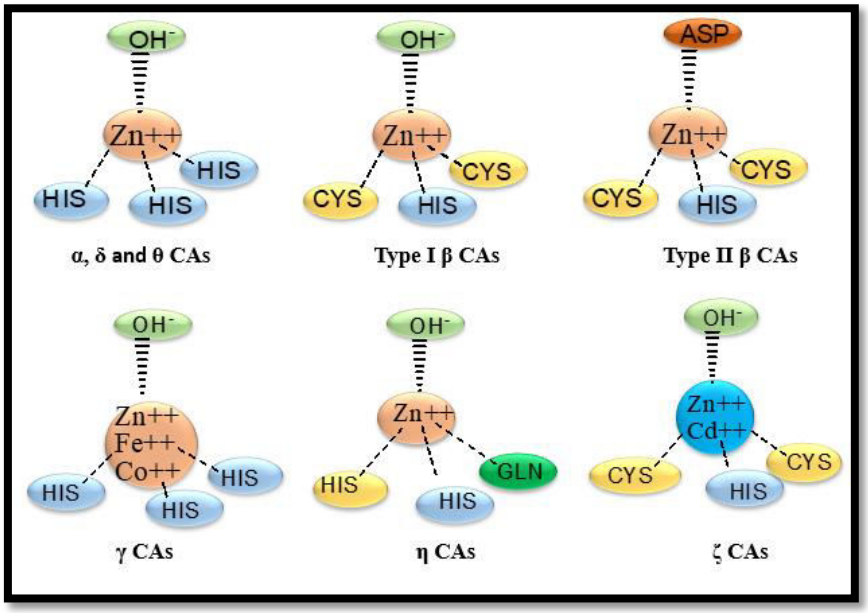
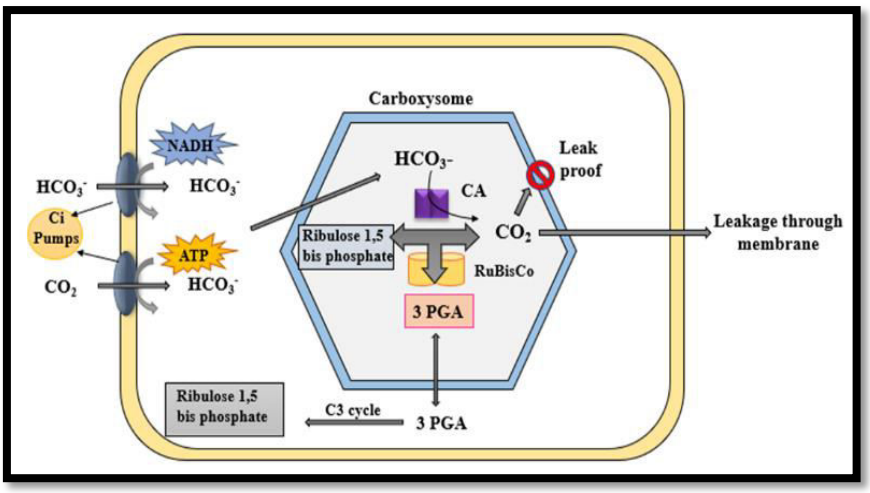


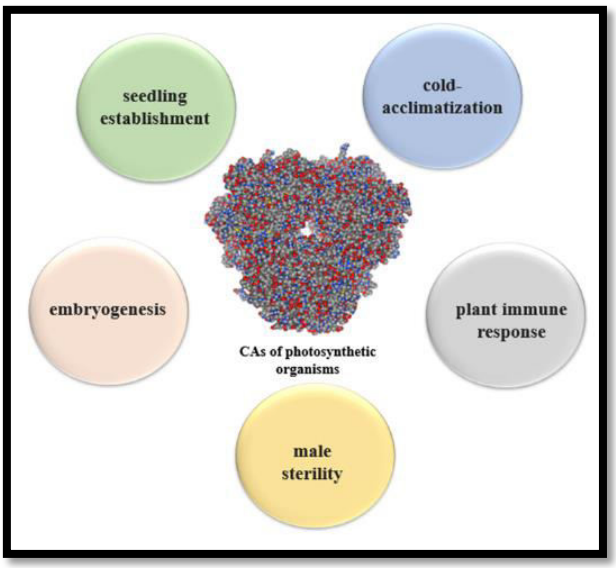
Figure 3:Representation ofmetal co-factors &differential adjacent amino acids in catalytically sites of various CA classes

2.Role of carbonic anhydrase

The inter-conversion of CO_2 to HCO_3^- is catalysed by CAs, which is closely related to RuBisCo (12).Thus, it increases the CO_2 availability at Rubisco's active site, which is the main enzyme responsible for controlling the main photosynthetic pathways (C3/C4/CAM)(12). CAs consistently supplies HCO_3^- to the phosphoenolpyruvate carboxylase (PEPC) carboxylation site in C4 & CAM plants (13). In addition to their involvement in photosynthetic regulation, CAs also perform respiration, fatty acid biosynthesis, & pH regulation (14,15). Other investigations have suggested that CAs can assist in theprocess of seedling establishment, embryogenesis, male sterility, cold-acclimatization & plant immune response (16)&γ-CAs helps in maintaining the physiology of Complex I of mitochondrial electron transport chain (17). Animal CAs perform a variety of tasks including cell development, brain metabolism, ureagenesis, gluconeogenesis, lipogenesis, & carboxylation processes (18) (Figure 4 a & b).Thus, CAs isoforms are used as therapeutic targets (20)Figure 5.



(a)



(b)

Figure 4: (a) Mechanism of action of carbonic anhydrase & (b) role in photosynthetic organisms

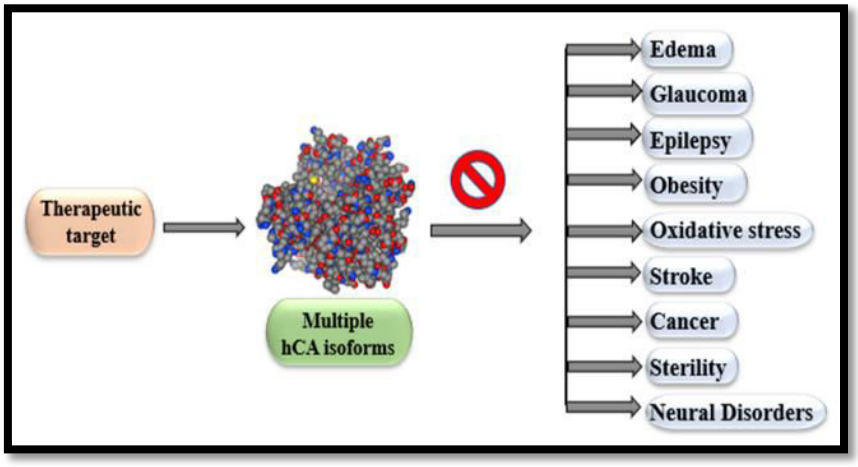


Figure 5: Multiple human (h) CA isoforms are therapeutic targets for a variety of disease ailments

The mitochondrial CA isoforms VA & VB are involved in metabolic processes like ureagenesis, gluconeogenesis & lipogenesis (18). Several studies have shown that CAs IX & XII are the initiators of tumor, metastasis, & cancer (21). CA IX is limited to hypoxic tumors, whereas CA XII is expressed in both tumor cells & normal tissues (22).Comparison of the expression of CA IX & CA XII in tumors indicated that CA IX was more lethal than CA XII in patients (22). Predominantly, the transmembrane bound CA-IX & CA-XII maintain the acid-base balance within the tumor microenvironment by the reversible hydration of carbon dioxide to carbonic acid, which results in hypoxia (23).Under hypoxic conditions, the master regulator hypoxia-inducible transcription factor (HIF-1) induces the expression of target genes associated with angiogenesis, growth factors, glycolytic enzyme metabolism, & glycolysis (24)Figure 6.

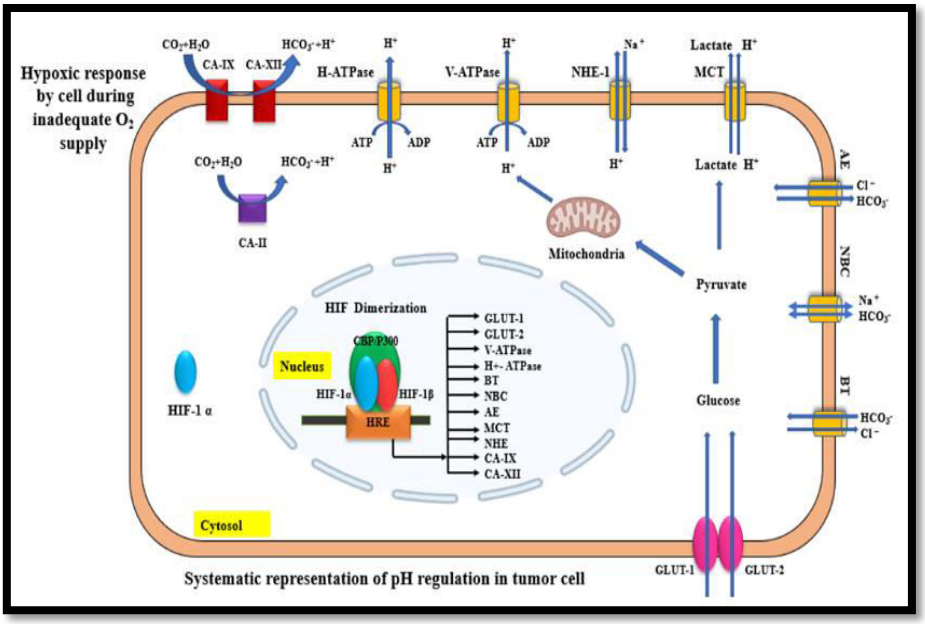


Figure 6: Representation of hypoxia-inducible genes.

3. Harmful aspects of cyanobacterial growth

CAs sustain carbon concentration around RuBisCo, which regulates various pathways in photosynthetic organisms, leading to their growth (13,12). The abundant growth of algae & cyanobacteria in aquatic bodies causes cyanobacterial blooms. Some strains in the bloom contain genes associated with the production of harmful toxins such as hepatotoxins, neurotoxins, &dermatotoxins(25,26).Hepatotoxins (microcystin, nodularin, & cylindrospermopsin) inhibit protein phosphatases which can result in accumulation of proteins in the liver, cell necrosis, hemorrhage, & death; neurotoxins (anatoxin-a, anatoxin-a (s), homoanatoxin-a, saxitoxin, & β-methylamino-L-alanine) inhibit acetylecholinesterase that may lead to

intraneuronal protein misfolding, numbness, muscular paralysis, respiratory arrest, & death. Dermatotoxins (aplysiatoxins&lynbyatoxins) cause dermatitis & gastrointestinal inflammation (26). Hepatotoxins &dermatotoxins are also associated with tumor initiation & progression (27).Biofilm-producing cyanobacteria colonize various historical monuments, caves, & pre-historic sites via adhesion mechanisms,and their acidolytic&oxido-reductive potential cause disfigure ofmonuments (28,29). These biofilms are stress tolerators & can adapt to high temperatures, desiccation, humidity, & secrete biogenic pigments, resulting in the staining & deterioration of stone surfaces, which significantly reduces the aesthetic value of prehistoric site & monuments (29).

Thus, to eradicate the harmful cyanobacterial strains, we have reviewed natural CA inhibitors such as polyphenolic compounds (furano-flavonoid, furano-flavonoid, coumarins, thio-coumarin, coumaronochromone, ellagitannins), polyamines (spermine, spermidine), & coumarins since most of these substances are non-toxic & do not negatively impact the environment. Derived CA inhibitors from sulphonamide, sulphamates, & isothiocyanates have also been investigated to determine the potential dangers of these artificial substances. Further discussion has been held regarding their chemical structures, half-maximal inhibitory concentration (IC_{50}) against various CA isozyme classes, & their precise mode of action.

4. Derived carbonic anhydrase inhibitors

The most prominent class of CA inhibitors includes aromatic sulfonamides, which have been reported to possess therapeutic ability against tumors, glaucoma, obesity, epilepsy, & diuretics (9).

4.1. Sulphonamide compounds

Sulphonamides (R-NH-SO_2NH_2)/sulphamate (R-O-SO_2NH_2) drugs are potent CA inhibitors(11, 9). Their mechanism involves the formation of a tetrahedral adduct with zinc ions by substituting the non-protein zinc ligand in sulfonamides, whereas trigonal-bipyramidal adducts are formed in thiocyanates (Figure 7 a, b & 8). In higher vertebrates, the affinity of sulfonamides for CAII & CAVII is very high(30)Table 1.

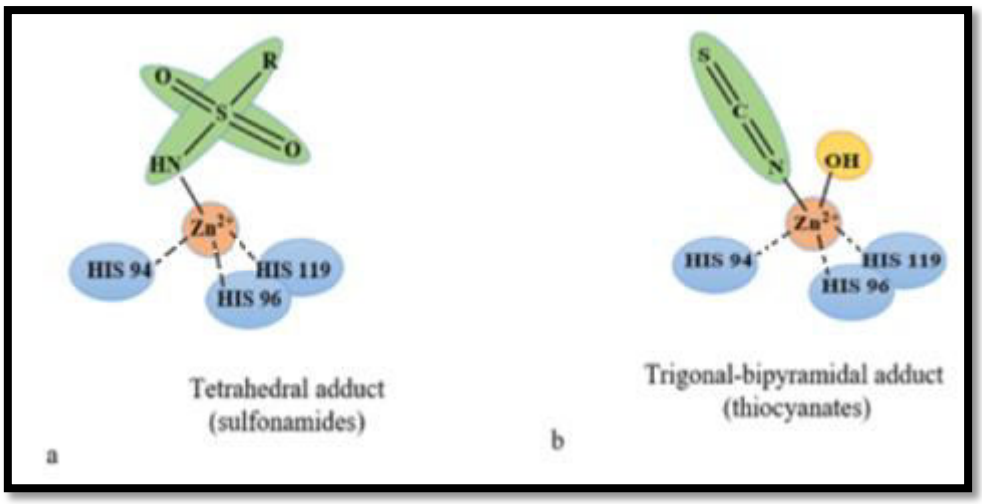


Figure 7: Interactions by (a) sulphonamides & (b) thiocyanates with Zn co-factor of CA following tetrahedral adduct & trigonal-bipyramidal adduct.

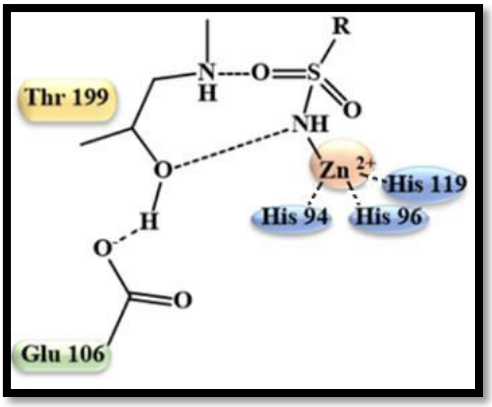


Figure 8: Schematic representation of binding of sulphonamide with cofactor of carbonic anhydrase occupying in the binding site.

Table 1: Affinity of sulphonamide inhibitors with CA isozymes (Supuran, 2008)

	CA isozymes							
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**IX**
Sulphonamide inhibitors	***	*****	*	****	****	**	*****	****
* Very low **Medium low ***Medium ****High *****Very high								

Innocenti et al., 2004 (31) used sulphonamides to inhibit α-class hCA II, γ-class Zn-Cam & Co-Cam from M. thermophila&β-class Cab-type CA from M. thermoautotrophicum. The most effective CA inhibitors were ethoxzolamide, acetaozolamide, topiramate, compound 19 (Ki <= 23.5 μM) as well as simple

substituted sulphonamide derivatives compound 1-7. Sulphonamides effectively inhibited the **α** class, **β**-cab class, &**β**-type plant CA in a similar manner. It was also concluded that sulphonamides are much efficient in inhibiting γ class Cam type CA as compared to α class or β class CA (Table 2).

Table 2: Inhibition data of commercially available sulphonamides with α type (hCA II), β -type (Cab-CA) & γ type (Cam-CA) (Innocenti et al., 2004)

Sulphonamides	**Ki (μM)**				
	α type	**β type**	**γ type**		
	hCA II	Cab	Co-Cam	Zn-Cam	
Acetazolamide	12	12.1	1.43	0.063	
Methazolamide	14	32.1	0.17	0.14	
Ethoxzolamide	8	5.35	0.74	0.20	
Topiramate	5	23.5	0.12	1.02	
Valdecoxib	43	61	0.24	0.13	
Celecoxib	21	38.5	1.01	0.14	
Dorzolamide	9	30.7	1.71	0.41	
Sulphanilamide	300	57.8	3.93	0.25	

Sulfonamides have greater inhibitory efficiency towards plant beta-CA than microbial beta-CA (32).Sulphonamide drugs have been clinically approved for the treatment of various diseases (9). Acetazolamide is a first-generation sulfonamide-based diuretic that has been clinically adopted since 1956, followed by other first-generation diuretics, such as methazolamide, ethoxzolamide, & dichlorphenamide, which have proven effective against human& murine CA (33, 34).These reported diuretic molecules were also effective against glaucoma. The first molecules used for glaucoma were acetazolamide &dichlorophenamide, which were later discontinued due to their emerging side effects & were replaced by dorzolamide & brinzolamide (9,35). Later, these molecules also showed side effects that led to the development of other derived sulfonamide drugs as CA inhibitors against glaucoma (35)

Table 3: Inhibition data of certain classical sulphonamides against human (h) & murine (m) CA isozymes (I-XV) (Carta& Supuran, 2013; Masini et al., 2013).

Ki (nM) **CA isozymes**													
Compounds	**I**	**II**	**III**	**IV**	**VA**	**VB**	**VI**	**VII**	**IX**	**XII**	**XIV**	**mCA XIII**	**mCA XV**
Acetazolamide	250	12	2.10^5	74	63	54	11	2.5	25	5.7	41	17	72
Methazolamide	50	14	7.10	6200	65	62	10	2.1	27	3.4	43	19	65
Ethoxzolamide	25	8	1.10^6	93	25	19	43	0.8	34	22	25	50	58
Dichlorphenamide	1200	38	$6.8.10^5$	15000	630	21	79	26	50	50	345	23	95
Benzolamide	15	9	$1.4.10^5$	*	37	34	93	0.45	49	3.5	33	*	70
Hydrochlorothiazide	328	290	$7.9.10^5$	427	4225	603	3655	5010	367	355	4105	3885	135
Hydroflumethiazide	2840	435	$8.7.10^5$	4780	10200	429	8250	433	412	305	360	15400	141
Quinethazone	35000	1260	*	*	*	*	*	*	*	*	*	*	*
Metholazone	54000	2000	$6.1.10^5$	216	750	312	1714	2.1	320	5.4	5432	15	79
Chlorthalidone	348	138	$1.1.10^4$	196	917	9	1347	2.8	23	4.5	4130	15	143
Indapamide	51900	2520	$2.3.10^5$	213	890	274	1606	0.23	36	10	4950	13	234
Furosemide	62	65	$3.2.10^6$	564	499	322	245	513	420	261	52	550	176
Bumetanide	4930	6980	$3.4.10^6$	303	700	*	*	*	25.8	21.1	250	2570	431

*** Data not available**

In comparison to the classical CAIs (generally low nanomolar CA II inhibitors), only furosemide proved to be an effective CA II inhibitor (Ki 65 nM) as

compared to the remaining sulphonamide inhibitors. Many nano-molar or sub-nanomolar CA inhibitors have been identified among these diuretics, including metholazone against CA VII, XII, & XIII; chlorthalidone against CA VB, VII, IX, XII, & XIII; indapamide against CA VII, IX, XII, & XIII; & furosemide against CA I, II, & XIV. Bumetanide is a tumor-specific CAI that has the same potency as acetazolamide but lacks the promiscuity of acetazolamide, which is a powerful CAI against the majority of mammalian isozymes. Bumetanide has a mild inhibitory effect on all other isoforms except CA IX & XII, which are overexpressed in tumours(33,36)(Table 3).

Observing the roles of CA inhibitors as diuretics & anti-glaucoma agents it was further used against obesity (37). The sulfamate derivative, topiramate (TPM), leads to weight loss in patients undergoing anticonvulsant treatment, experimentally proven in murine models (9). TPM is an effective CA inhibitor as the crystallographic structure of TPM-CA II complex reveals the formation of tetrahedral structure with the Zn ion within the enzymes active site (38) The mechanism behind the weight loss could be the action of TPM against mitochondrial CAs that are involved in the lipogenesis process (37). In addition to its role against obesity, TPM also aids in the treatment of partial & generalized epilepsy (39).Inspite of the use of TPM for the treatment of epilepsy it is also associated with side effects like neural in-coordination (40). The other sulphonamide/sulphamate derivatives used for the treatment of epilepsy are sulthiame& zonisamide respectively (39).ZNS is a more potent hCA VA inhibitor than hCA II whereas TPM is a stronger hCA II inhibitor than hCA VA (38)(Table 4).

Table 4: Inhibition data of Topiramate (TPM) & Zonisamide (ZNS) against human (h), rat (r) murine (m) CA isozymes

	Ki (μM)												
	hCA isozymes						**rCA isozymes**					**mCA isozymes**	
	I	**II**	**IV**	**VA**	**VB**	**VI**	**I**	**II**	**III**	**IV**	**V**	**II**	**IV**
TPM	90	0.01	6	0.063	0.03	>100	180	0.1	>100	0.2	18	1.0	20
ZNS	*	0.0138	*	0.0254	*	*	*	*	*	*	*	*	*

***Data not available**

Apart from all these therapeutic roles, sulpha drugs have been widely studied as anti-cancer agent(21).

Among the commercially available sulpha drugs, the systemic CA inhibitors include Acetazolamide, Dichlorophenamide, Methazolamide

&Ethoxzolamide/ Ethoxyzolamide& the topical CA inhibitors includeDorzolamide&Brinzolamide.

5. Natural inhibitors

The naturally available compounds that have inhibitory potential are polyphenols, phenolic acids, coumarins, ellagitannins & polyamines

5.1 Phenolic compounds

Phenolic compounds are secondary metabolites found in plants of the Pteridophyta& Spermatophyta families. These include simple phenols & polymers derived from these groups (41,42) These naturally obtained simple & polyphenolic acids have shown interactions & inhibitory potency with 13 catalytically active mammalian CA isozymes (41,42). Phenolic compounds bind to CA in a non-classical manner, where the OH moiety of phenol anchors to the fourth zinc ligand of CA via hydrogen bonding (9) (Figure 9a). This mechanism is different from the binding mechanisms of sulfonamide inhibitors. Simple phenolic molecules& polyphenolic moleculespossess strong inhibitory potency against CA I-XV at low micromolar concentrations (41-43) (Table 5).

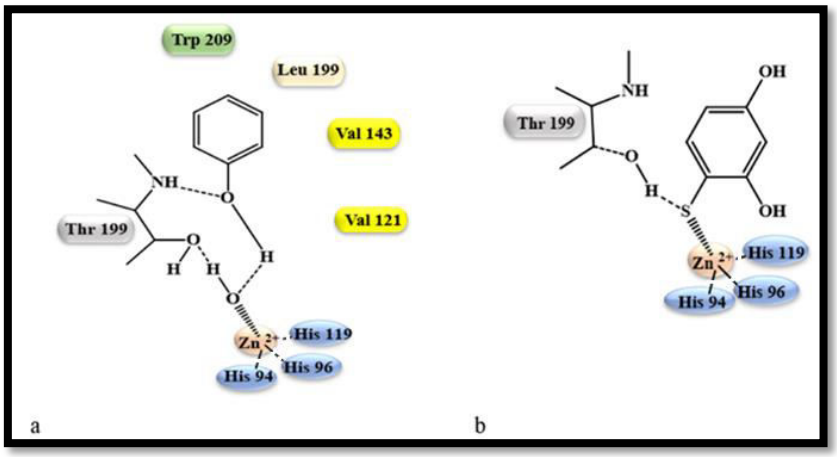


Figure 9: Schematic representation of binding of (a) phenols & (b) coumarins to the zinc cofactor of CA

Table 5: Inhibition data of simple & polyphenolic compounds against human (h) &murine (m) CA isozymes (Sentürk et al., 2009; Innocenti et al., 2010; Bayram et al., 2008)

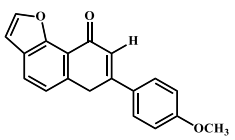
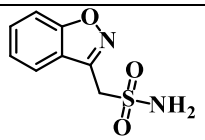
	Ki (μM) **hCA isozymes**												
	I	**II**	**III**	**IV**	**VA**	**VB**	**VI**	**VII**	**IX**	**XII**	**XIII**	**XIV**	**mCA XV**
	10.2	5.5	2.7	9.5	218	543	208	710	8.8	9.2	*	11.5	10.5

techol	4003	9.9	13.0	10.9	55.1	4.2	606	714	115	8.9	*	48.9	*
xybenzoic	0.92	0.87	6.61	7.78	3.67	7.13	5.70	4.08	3.73	6.27	*	6.16	*
naric	1.07	0.98	7.57	9.60	5.96	7.76	6.72	5.23	5.33	8.01	*	6.68	*
acid	2.38	1.61	10.0	10.1	6.49	9.08	7.33	6.42	7.87	9.06	*	8.71	*
acid	2.89	2.40	11.1	10.8	7.04	10.5	8.45	7.41	9.87	9.78	*	9.43	*
acid	3.20	2.25	7.49	9.80	4.08	9.97	6.13	6.07	6.99	7.78	*	7.03	*
c acid	4.15	3.19	8.58	10.6	6.34	35.4	7.55	7.81	8.20	9.01	*	9.41	*
etin	2.68	2.54	8.10	7.89	6.81	11.9	6.17	4.84	7.00	9.39	*	5.41	*
acid	2.32	2.18	10.5	9.08	7.59	12.7	7.06	6.32	9.37	10.1	*	8.91	*
atrol	2.21	2.77	9.09	4.47	4.75	4.64	8.07	4.35	0.81	0.95	4.09	0.83	9.36
mine	1.92	0.48	7.40	8.98	0.73	0.89	9.47	4.30	9.82	4.35	9.53	12.02	0.39
nin	2.41	0.38	11.30	4.97	10.25	9.46	9.94	9.30	4.05	3.48	6.85	11.73	5.09
in	2.42	1.84	3.58	4.90	4.21	4.02	4.91	0.45	5.03	4.72	10.51	11.55	7.68
rin	1.49	2.51	6.43	8.96	4.08	4.56	9.70	4.71	10.15	9.05	4.82	11.64	0.65
oxy ic acid	3.1	9.2	>1000	62.3	>1000	578	>1000	>1000	>1000	>1000	>1000	>1000	>1000

*** Data not available**

Another furanoflavonoid compound isolated from the bark of Millettiaovalifolia showed inhibition of cytosolic bovine CA II having IC_{50} value 17.86 ± 0.09 μM against zonisamide (IC_{50} value 1.86 ± 0.03 μM) (44) (Table 6).

Table 6: Inhibition data of furanoflavonoid compound isolated from Millettiaovalifolia& standard inhibitor zonisamide against bCA II

Compound	**Plant source**	**IC_{50} (μM ±SEM) bCA II**	**Structure**
Furano flavonoid	Millettiaovalifolia	17.86±0.09	
Zonisamide (st&ard)		1.86±0.03	

A series of substituted flavonoids (C4 carbonyl group absent) showed effective but diverse inhibitory potencies against human (h) & bovine (b) alpha-CAs, (Table 7). This suggests that the substitution of groups also plays an

important role in inhibition efficacy(45). As suggested, fisetin & rhamnetin showed better inhibitory potency due to the structural changes in ring A; however, rutin was more active than the aglycone quercetin & rhamnetin because of its sugar moiety (45) (Table 8).

Table 7: Inhibition of human & bovine CAs by flavonoids (Karioti et al., 2016)

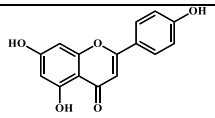
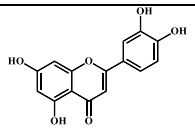
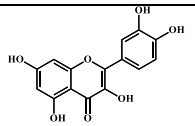
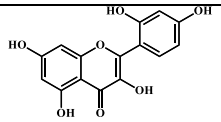
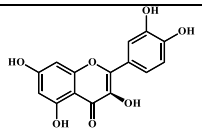
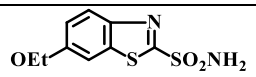
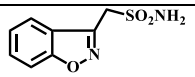
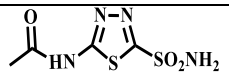
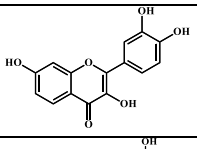
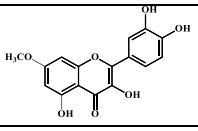
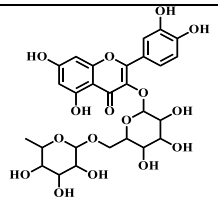
Flavonoids	**IC_{50} (μM)** **hCA I** **hCA II** **hCA IV** **bCA II**				**Structure**
Apigenin	4.1	2.7	9.1	11.6	
Luteolin	2.2	0.74	4.4	5.4	
Quercetin	3.6	2.4	13.8	9.1	
Morin	12.8	4.4	15.7	21.3	
Catechin	6.8	6.2	5.6	2.2	
EZA	3.7	0.32	0.84	9.4	
ZNA	14.8	1.1	38.4	8.5	
AZA	36.2	0.37	0.58	263	

Table 8: Inhibition of hCA I & II by flavonoids via CO_2 hydration assay (Karioti et al., 2016)

Flavonoids	**IC_{50} (μg/ml)** **hCA I** **hCA II**		**Structure**
Fisetin	0.72	0.55	
Rhamnetin	0.95	0.86	

Rutin	1.44	0.52	

Recent studies have also demonstrated the action of coumarins as CA inhibitors. The first naturally obtained compound 6-(1S-hydroxy-3-methylbutyl)-7-methoxy-2H-chromen-2-one (compound 1)isolated from Leionemaellipticum (Table 9) showed inhibitory activity against all CA investigated by Maresca et al. (2009)(46), except CA III. The best inhibitory efficacy reported by compound I was against cytosolic hCA I & II(46)(Table 10).

Similarly, thiocoumarin has been shown to be an efficient CA inhibitor (46). The hydrolysed product of compound 1 (cis-2-hydroxy-cinnamic derivative 4Z) effectively blocked the entrance of the enzyme active site, showing no interaction with the catalytically active zinc cofactor (46) (Figure 9b). A series of monohydroxy, chloro, & chloromethyl derivatives attached to the coumarin ring were investigated as CA inhibitors, & they showed that these moieties are better sub-micromolar inhibitors of transmembrane tumor-associated CA IX & XII (47).

Table 9: The first naturally obtained compound1 isolated from **Leionemaellipticum**

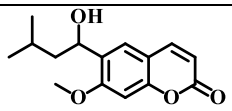
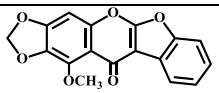
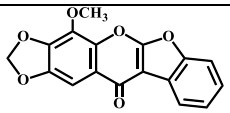
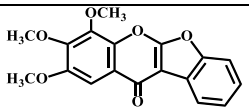
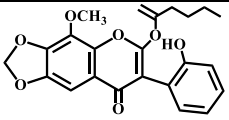
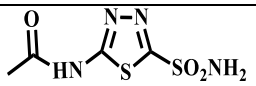
	Compound	**Source**	**Structure**
1	6-(1S-hydroxy-3-methylbutyl)-7-methoxy-2H-chromen-2-one	Leionemaellipticum	

Table 10: Inhibition data of human (h) & murine (m) CA isozymes with compound 1 isolated from Leionemaellipticum& coumarin (2) (Maresca et al., 2009)

	Ki (μM)												
	hCA isozymes											**mCA isozymes**	
	I	**II**	**III**	**IV**	**VA**	**VB**	**VI**	**VII**	**IX**	**XII**	**XIV**	**XIII**	**XV**
1	0.08	0.06	>1000	3.8	96.0	17.7	35.7	27.9	54.5	48.6	7.8	7.9	93.1
2	3.1	9.2	>1000	62.3	>1000	578	>1000	>1000	>1000	>1000	>1000	*	*

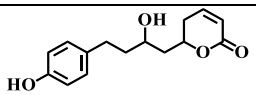
Coumaronochromone derivatives, aervin 1-4 derived from Aerva persica, likewise inhibited CA with IC_{50} values of 19.01, 18.24, 18.65, & 12.92 M, respectively, against the conventional inhibitor acetazolamide. where compound 4 showed better catalytic activity (48) (Table 11).

Table11: Inhibition data of coumaronochromone (aervin 1-5) compound &acetozolamide against b CAII **(**Imran et al., 2021)

Plant Source	**Compounds**	**IC_{50} ±SEM (μM) bCA II**	**Structures**
Aerva persica	Aervin 1	19.01±0.33	
	Aervin 2	18.24±0.23	
	Aervin 3	18.65±0.21	
	Aervin 4	12.92±0.79	
	AZA (standard)	1.12±0.02	

Another active molecule dodoneine isolated from Agelanthusdodoneifolius showed inhibitory effects against several hCA isoforms with Ki values 5.5-10.4 μM, (Table 12) further in vivo & in vitro studies of dodoneine in rat vascular smooth muscles & aorta, induced vasorelaxation by blocking L-type calcium channels, inhibiting the expression of hCA isoforms (49).

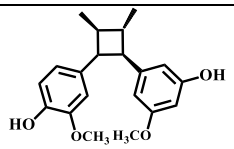
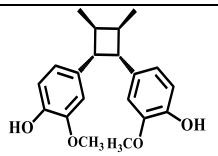
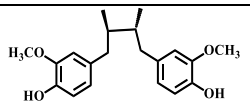
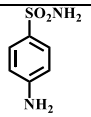
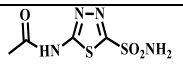
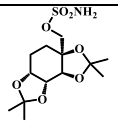
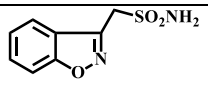
Table 12: Inhibition data of human (h) & murine (m) CA isozymes with dodoneine

Compound	**Plant Source**	**Ki (μM)**					
		hCA Structure I	**hCA III**	**hCA IV**	**hCA XIII**	**mCA XIV**	**hCA**
Dodoneine	Agelanthus dodoneifolius	5.48	10.35	9.61	9.27	9.34	

Davis et al., (2011)(50) used a series of naturally obtained phenolic compounds to inhibit a panel of CAs including beta-CA of M. tuberculosis

(Rv3273, Rv1284), beta-CA of C. albicans (Nce103) &C. neoformans (Can2) & human CA I & II. Plant based phenolic compounds endiandrin A & endiandrin B were 10-fold weaker Rv3273 inhibitor than (-)-dihydroguaiaretic. All of the compounds inhibited CA Rv1284, Nce103 & Can2 more efficiently. These compounds on the other hand were weak hCA I & hCA II inhibitors (Table 13).

Table 13: Inhibition of CA Rv3273, Rv1284, Nce103, Can2 & hCA I & II with endiandrin A, endiandrin B & (-)-dihydroguaiaretic acid (Davis et al., 2011)

Compounds	**Ki (μM)**						
	Rv3273	**Rv1284**	**Nce103**	**Can2**	**hCA I**	**hCA II**	**Structure**
Endiandrin A	8.92	0.82	0.73	0.77	368	11.7	
Endiandrin B	0.89	0.80	0.70	0.95	354	12.1	
(-)-dihydroguaiaretic acid	9.10	0.85	0.62	0.81	307	230	
Phenol	79.0	64.0	17.3	25.9	10.1	5.5	
SA	7.11	9.84	7.63	0.77	25.0	0.24	
AZA	0.10	0.48	0.13	0.01	0.25	0.012	
TPM	3.02	0.61	1.11	0.37	0.25	0.010	
ZNS	0.21	286.8	0.94	0.97	0.056	0.035	

The polyphenolic compound Rosmarinic acid, found in the Lamiaceace family belonging to class depsides, was reported to be an inhibitor of hCA I & II(51)(Table 14). On the other hand, salvianolic acid (depsides 43-45) isolated from plant Salvia miltiorrhiza were weak inhibitors of hCA I & II but proved effective against hCA IV (45). Lithospermic acid proved to be a potent inhibitor of hCA XIII as compared to salvianolic acids A & B. As salvianolic acid A & B contain catechol moieties, they are more effective inhibitors than lithospermic acid (45)(Table 15).

Table 14: Inhibition of hCA I & II with Rosmarinic acid (Topal& GÜLÇİN,2014).

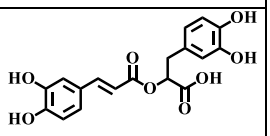
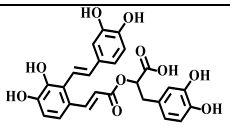
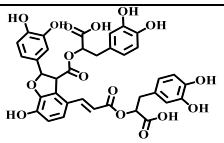
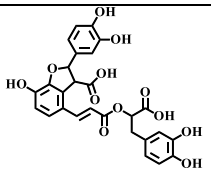
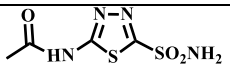
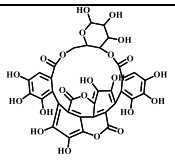
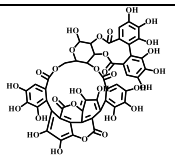
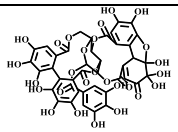
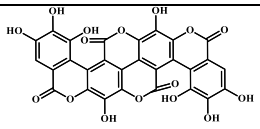
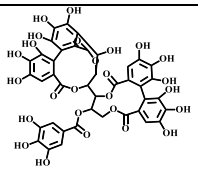
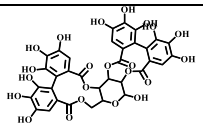
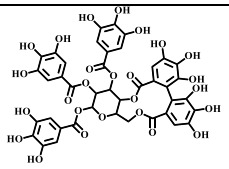
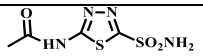
Compound	**Plant Source**	**IC_{50}**		**Ki**		**Structure**
		hCA I	**hCA II**	**hCA I**	**hCA II**	
Rosmarinic acid	Lamiaceae	8.0x10^{-5} M	47.0x10^{-5} M	86.0x10^{-5} M	57.0x10^{-5} M	

Table 15: Inhibition of hCA isozymes with salvianolic (A, B) & lithospermic acid (Karioti, et al., 2016)

Compound	**Plant Source**	**Ki (nM) hCA I**	**hCA II**	**hCA IV**	**hCA VII**	**hCA XII**	**Structure**
Salvianolic acid A	Salvia miltiorrhiza	>10,000	9594.4	66.6	71.4	39.8	
Salvianolic acid B		>10,000	>10,000	65.6	35.5	453.6	
Lithospermic acid		>10,000	>10,000	101.2	268.3	4.8	
AZA		250	12	74	2.5	5.7	

The ellagitannins isolated from the pericarp of Punica granatum L. inhibited carbonic anhydrase well out of which gallagyldilactone was the most active (52)(Table 16).

Table 16: Inhibition of carbonic anhydrase with ellagitannins isolated from pericarp of Punica granatum L. (Satomi et al., 1993)

Source	**Ellagitannins**	**IC_{50} (M)**	**Structures**
Punica granatum L.	Punicalin	1.0×10^{-6}	
	Punicalagin	2.3×10^{-7}	
	Granatin B	3.7×10^{-7}	
	Gallagyldilactone	2.2×10^{-7}	
	Casuarinin	2.7×10^{-7}	
	Pedunculagin	5.5×10^{-7}	
	Tellimagr&in	3.2×10^{-7}	
	AZA	2.0×10^{-7}	

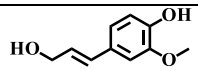
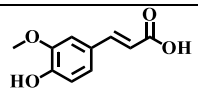
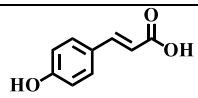
Similarly, phenolic compounds isolated from the ethyl acetate fractioninLuffa acutangula (L.), inhibited bCA II with an IC_{50} value of 286.0 ± 2.41 μg/mL, which was statistically significant when compared to the reference positive control acetazolamide (53)(Table 17).

Table 17: Inhibition of bCA II with phenolic compounds isolated from ethyl acetate fraction (Chanda et al., 2019)

	Compounds	**IC_{50} (μg/mL) bCA II**
Ethyl acetate fractions	Phenolic compounds	286.0 ± 2.41
AZA		203.6 ± 2.08

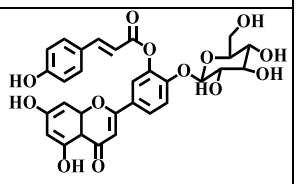
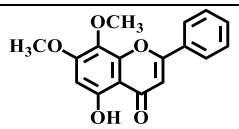
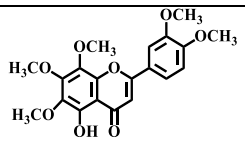
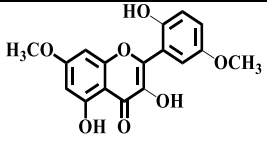
The phenolic compounds isolated from Lagenaria siceraria using LC-QTOF-MS/MS were found to inhibit bCA II in a dose dependent manner. Coniferyl alcohol had the highest inhibition potency when compared to ferulic acid & p-coumaric acid (54)(Table 18).

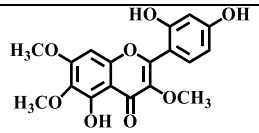
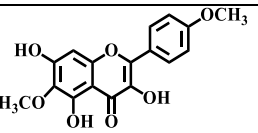
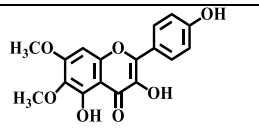
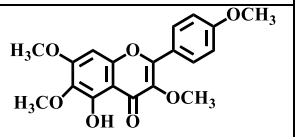
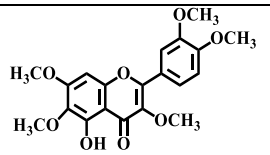
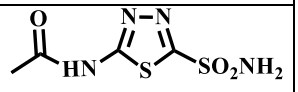
Table 18: Inhibition data of phenolic compounds isolated from Lagenaria siceraria against bCA II (Chanda et al., 2021)

Plant Source	**Compounds**	**IC_{50} (μM)**	**Structure**
Lagenaria siceraria	**Coniferyl alcohol**	80.38 ± 3.54	
	Ferulic acid	130.15 ± 5.38	
	p-coumaric acid	256.52 ± 7.16	

Both the ethyl acetate fraction & the chloroform extract of Oxalis corniculata L. contained flavonoid compounds (1-9) (55). The chloroform fraction had the highest CA II inhibition efficiency, with an IC_{50} value range of 17.11±25.18 & an inhibition efficiency of 65.21 ± 90.82% when compared to the reference (Table 19)(56).

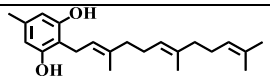
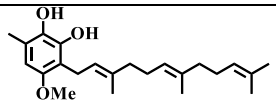
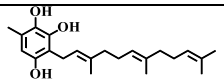
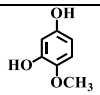
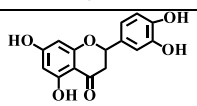
Table 19: Inhibition data of flavonoid compounds & acetazolamide against bCA II (Imran et al., 2020)

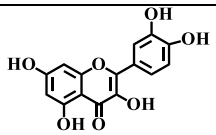
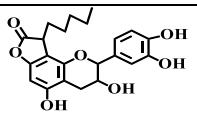
Plant Source	**Compound**	**IC_{50} (μM) b CAII**	**Inhibition %**	**Structure**
	1	17.11 ± 0.8	90.82 ± 1.1	
	2	25.18 ± 0.7	81.13 ±0.5	
	3	21.32 ± 0.5	78.72 ± 0.9	
	4	18.08 ± 0.4	65.21 ± 0.7	

Oxalis corniculata L.	**5**	17.12 ± 0.5	73.34 ± 0.9	
	6	18.27 ± 0.5	84.13 ± 0.6	
	7	19.85 ± 0.2	77.32 ± 1.0	
	8	24.71 ± 0.9	72.03 ± 0.5	
	9	27.01 ± 0.6	60.42 ± 0.8	
	AZA	1.57 ± 0.7	94.24 ± 0.5	

Natural phenolic compounds that were isolated from different plant sources demonstrated the ability to suppress CA IIhaving IC_{50} value ranging between 60.37-71.73 μmol/L. The active inhibitor was grifolic, with an IC_{50} value of 6.37 μmol/L (57)(Table 20).

Table 20: Plant sources & inhibitory efficacy of naturally obtained phenolic & flavone compounds (Huang et al., 2009).

	Compounds	**Plant Source**	**IC_{50} (μmol/L)**	**Structures**
Phenolic compounds	Grifolin	Albatrellus confluens	33.51± 2.94	
	4-O-methyl-grifolic acid	Polyporus dispansus	9.79± 0.21	
	Grifolic acid	Polyporus dispansus	6.37± 0.12	
	Isovanilic acid	Neonauclea sessilifolia	71.73± 1.51	
	Eriodictyol	Impatiens chungtienensis	39.10± 2.91	

Flavones	Quercitin	Cupressus duclouxiana	40.97± 0.61	
	Puerin A	Camelia sinensis var. assamica	28.51± 0.83	

5.2Polyamines

Polyamines (PA) are organic compounds that contain more than one amino group. Biogenic PA are linear & low-molecular-weight structures distributed throughout all life forms, including putrescine, spermidine (N-C3-N-C4-N-C3-N), & spermine (N-C3-N-C4-N) in mammals, & thermo-spermine in plants (58). Putrescine, spermidine, & spermine are obtained by the decarboxylation of ornithine or S-adenosyl amino acids catalyzed by the enzyme ornithine decarboxylase, whereas thermos-spermine is converted from spermidine & is an isomer of spermine (59).Generally, small molecules of polyamines act as carbonic anhydrase activators (CAAs) (60) but Carta et al., (2010) (61)discovered that biogenic polyamines spermine & spermidine were CA inhibitors rather than activators (Figure 11). Spermine showed better CA inhibitory potency for membrane bound hCA IV, mitochondrial hCA V(A,B) secretory isoenzyme hCA VI, CNS dominant isoforms hCA VII, XIV whereas spermidine showed better inhibition for all the hCAs taken in the study most preferably for hCAIV except tumor associated hCA XII (61) (Table 21). Due to the structural similarity of the active sites, both spermine & spermidine inhibited different CA isoenzymes with high efficiency (62). These findings were compared to those of the reference compounds acetazolamide & phenol.

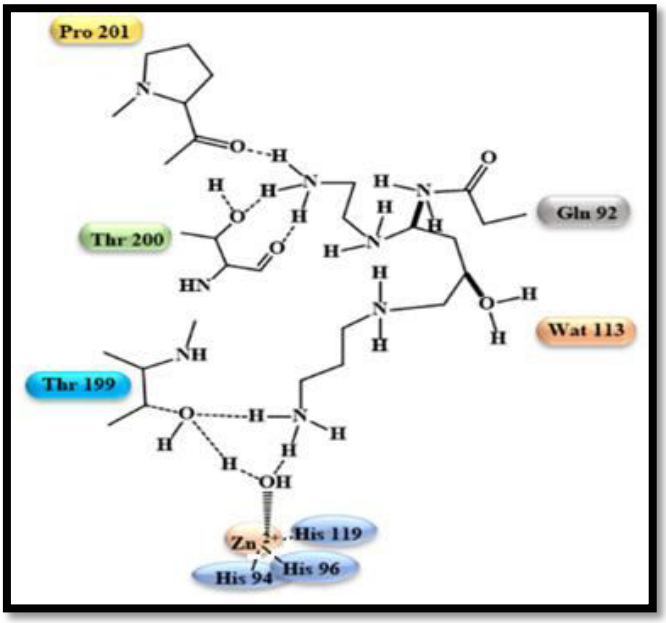


Figure 10: Schematic representation of binding of polyamines (spermine) to the zinc cofactor of carbonic anhydrase

Figure 11: Structure of natural polyamines spermidine & spermine

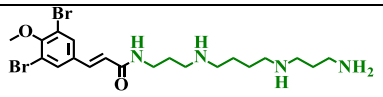
Table 21: Human (h) CA I-XII, XIV & murine (m) CA XIII & XV inhibition data with spermidine & spermine (Carta et al., 2010; Davis et al., 2014)

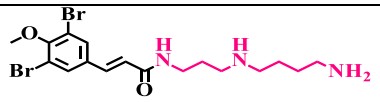
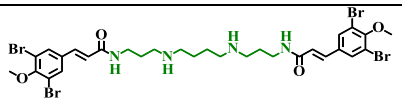
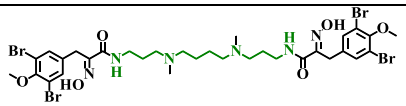
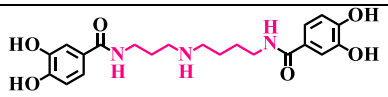
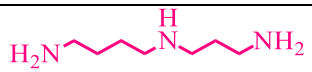
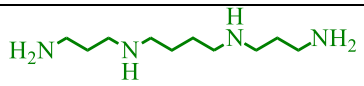
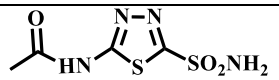
K_I (μM)

	hCA I	**II**	**III**	**IV**	**VA**	**VB**	**VI**	**VII**	**IX**	**XII**	**XIV**	**mCA XIII**	**mCA XV**
Spermidine	1.40	1.11	11.5	0.11	1.22	1.44	1.41	1.23	1.37	44.1	1.0	11.6	10.0
Spermine	231	84	167	0.01	0.84	0.83	0.99	0.71	13.37	27.6	0.86	22.6	74
AZA	0.25	0.012	200	0.074	0.063	0.054	0.011	0.0025	0.025	0.0057	0.041	0.017	0.072
Phenol	10.2	5.5	2.7	9.5	218	>500	208	>500	8.8	9.2	11.5	>500	10.5

The X-ray crystallographic structure of the spermine-CA II complex revealed that spermine was indirectly anchored to the zinc ion via the Zn-water coordinate system involving amino acid Thr 199 (61,62)). Thr 200 & Pro 201 are the amino acid residues involved in the interaction of the amine moiety of spermine (61) (Figure 10). Considering spermine & spermidine, Davis et al. (2014)(62) introduced five complex natural polyamines, ianthelliformisamine A (1), B (2), & C (3) isolated from a marine sponge, spermatinamine (4) isolated from the Pseudoceratinasp., &pistillarin (5) isolated from a variety of fungal species. Structurally 1,3,4 comprised polyamine fragment of spermine whereas 2 & 5 comprised the shorter polyamine chain of spermidine.These complex natural polyamines (1-5) showed greater & similar inhibition with CA I & II (Table 22). The length of polyamine chains as well as the substituted moieties & patterns, have an effect on carbonic anhydrase inhibition efficacy (61).

Table 22: Inhibition of hCA isoenzymes by naturally occurring polyamines, spermidine (magenta)& spermine (green) (Carta et al., 2010).

	Ki (μM)						
Natural polyamines	**CA I**	**CA II**	**CA IV**	**CA IX**	**CA XII**	**CA XIV**	**Structure**
Ianthelliformisam A	1.76	0.41	6.72	0.20	2.81	2.12	

Ianthelliformisami B	0.77	0.37	9.10	0.35	3.48	2.28	
Ianthelliformisami C	0.86	0.35	9.08	0.27	3.50	6.96	
Spermatinamine	0.85	0.48	>20	0.34	>20	2.72	
Pistillarin	0.79	0.34	7.03	0.36	4.21	1.52	
Spermidine	1.40	1.11	0.112	1.37	44.1	1.00	
Spermine	231	84	0.010	13.3	27.6	0.86	
AZA	0.25	0.012	0.074	0.025	0.006	0.041	

6. ADMET profiling of potent molecules (synthetic & phytochemicals)

The synthetic as well as phytochemicals were employed for ADMET analysis by using SwissADME (http://www.swissadme.ch/) &pkCSM (http://biosig.unimelb.edu.au/pkcsm/)webservers. Among the reviewed molecular scaffolds, thephytochemicalsbelongingto simple phenols (p-Hydroxybenzoic acid, p-Coumaric acid), polyphenols (curcumin, catechin, Rosmarinic acid), flavonoids (Luteolin, Fisetin, Rhamnetin) & coumarin (6-(1S-hydroxy-3-methylbutyl)-7-methoxy-2H-chromen-2-one) were found to be the non-toxic potent CAs inhibitors.These molecules followed Lipinski rule of five (RO5), which is commonly used to predict drug-likeness & oral suitability for human consumption (64). Furthermore, they showed no AMES toxicity, hepatotoxicity, herG I/II, & skin sensitization, indicating that these phytochemicals can be effective in the treatment of a variety of human disorders(Table 23). On the other hand, ADMET profiling demonstrated that synthetic molecules such as dorzolamide, brinzolamide, topiramate & Zonisamide are hepatotoxic compounds that can lead to acute &chronic liver diseases (Table 24).

Table 23: ADMET profiling of non-toxic phytochemicals obtained from SwissADME&pkCSM

Molecule	MW	iLOGP	H Donor	H	GI	BBB	CNS Permeat	Lipinski	Ghose	Veber	Egan	Muegge	AMES Toxicity	Hepatot	HerG I/II	Skin
p-Hydroxybenzoic acid	138.122	0.85	2	3	High	Yes	3.21	0	3	0	0	1	No	No	No	No
p-Coumaric acid	164.16	0.95	2	3	High	Yes	-2.418	0	0	0	0	1	No	No	No	No
Curcumin	368.385	3.27	2	6	High	No	-2.99	0	0	0	0	0	No	No	No	No
Catechin	290.271	1.47	5	6	High	No	-3.298	0	0	0	0	0	No	No	No	No
Luteolin	286.239	1.86	4	6	High	No	-2.251	0	0	0	0	0	No	No	No	No
Fisetin	286.239	1.5	4	6	High	No	-2.282	0	0	0	0	0	No	No	No	No
Rhamnetin	316.265	2.23	4	7	High	No	-3.235	0	0	0	0	0	No	No	No	No

6-(1S-hydroxy-3-methylbutyl)-7-methoxy-2H-chromen-2-one	262.305	2.91	1	4	High	Yes	-2.199	0	0	0	0	0	No	No	No	No
Rosmarinic acid	360.318	1.17	5	8	Low	No	-3.347	0	0	1	1	0	No	No	No	No

Table 24: ADMET profiling of synthetic CA inhibitors obtained from SwissADME&pkCSM

Molecule	MW	iLOGP	H Donor	H acceptor	GI absorption	BBB	CNS Permeation	Lipinski	Ghose	Veber	Egan	Muegge violations	AMES	Hepatotoxi	HerG I/II	Skin sensitizatio
Acetazolamide	222.251	-0.8561	2	6	Low	No	-3.249	0	1	1	1	2	No	No	No	No
Dichlorophenamide	305.164	0.2882	2	4	High	No	-3.119	0	0	0	1	0	No	No	No	No
Methazolamide	236.278	-1.4238	1	6	Low	No	-3.188	0	0	1	1	0	No	No	No	No
Ethoxzolamide	258.324	1.3424	1	5	High	No	-2.7	0	0	0	0	0	No	No	No	No
Dorzolamide	324.449	0.612	2	6	Low	No	-3.134	0	0	1	1	1	No	**Yes**	No	No

Brinzolamide	383.517	0.0869	2	7	Low	No	-3.248	0	0	1	1	1	No	**Yes**	No	No
Topiramate	339.366	-0.3954	1	8	High	No	-3.194	0	0	0	0	0	No	**Yes**	No	No
Zonisamide	212.23	0.6163	1	4	High	No	-2.59	0	0	0	0	0	No	**Yes**	No	No

7. Conclusions

The classical inhibitors (sulphonamides, sulfamates, thiocyanates) &phytochemicals (polyphenolic compounds & polyamines)against CAs were extensively reviewed. The majority of them are non-toxic & naturally isolated from living sources& therefore are competent to inhibit CAs. Sulphonamides & thiocyanates drugs have been broadly studied for their ability to inhibit various CA isozymes. They are clinically approved & commercially available for the treatment of a number of CA-targeted illnesses. Nonetheless, they are connected with a plethora of negative effects which limits its applicability in therapeutics. Besides synthetic CA inhibitors, phytochemicals such as polyphenolic compounds (furano-flavonoid, flavonoid, coumarin, thio-coumarin, coumaronochromone, & ellagitannins) & polyamines (spermine & spermidine) have been studied for their inhibitory efficacy against CAs of human, bovine, & murine isozymes. Out of which simple phenols (p-Hydroxybenzoic acid, p-Coumaric acid), polyphenols (curcumin, catechin, Rosmarinic acid), flavonoids (Luteolin, Fisetin, Rhamnetin) & coumarin (6-(1S-hydroxy-3-methylbutyl)-7-methoxy-2H-chromen-2-one)proved most potent & can be exploited medically with low toxic risks. They can also be employed as algaecides to treat toxin-producing algal or cyanobacterial strains because they have no negative side effects on non-targeted species. Furthermore, organically derived inhibitors do not exploit the stability of the environment, but rather positively influence the health of the environment.

8. Contributions

Archana Padhiary contributes to conceptualization, data curation, formal analysis, manuscript writing& structure preparation. Aiswarya Pati& S.S. Tete contributes to data curation & formal analysis. SAM &BN contribute to formal analysis, review editing manuscript, preparation & supervision. All authors read & accept the final version of manuscript for publication.

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