

## **Multi- and poly-pharmacology of carbonic anhydrase inhibitors**

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## Running Title Page

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d) Non standard abbreviations

ACC – acetyl-coenzyme A carboxylase

AD – Alzheimer's disease

A $\beta$  – amyloid  $\beta$

CA – carbonic anhydrase

CAA – CA activator

CAI – CA inhibitor

DNL – *de novo* lipogenesis

GIT – gastrointestinal tract

hCA – human CA

IOP – intraocular pressure

PD – Parkinson’s disease

pMCAO – permanent middle cerebral artery occlusion

PC – pyruvate carboxylase

VRE – vancomycin-resistant enterococci

ZBG – zinc-binding group

**Abstract.** Eight genetically distinct families of the enzyme carbonic anhydrase (CA, EC 4.2.1.1) were described in organisms all over the phylogenetic tree. They catalyze the hydration of CO<sub>2</sub> to bicarbonate and protons, and are involved in pH regulation, chemosensing and metabolism. The 15  $\alpha$ -CA isoforms present in humans are pharmacological drug targets known for decades, their inhibitors being used as diuretics, antiglaucoma, antiepileptic or antiobesity drugs, as well as for the management of acute mountain sickness, idiopathic intracranial hypertension and recently, as antitumor theragnostic agents. Other potential applications include the use of CA inhibitors (CAIs) in inflammatory conditions, cerebral ischemia, neuropathic pain, or for Alzheimer's/Parkinson's disease management. CAs from pathogenic bacteria, fungi, protozoans and nematodes started to be considered as drug targets in recent years, with notable advances registered ultimately. CAIs have a complex multipharmacology probably unique to this enzyme, which has been exploited intensely but may lead to other relevant applications in the future, due to the emergence of drug design approaches which afforded highly isoform-selective compounds for most  $\alpha$ -CAs known to date. They belong to a multitude of chemical classes (sulfonamides and isosteres, (iso)coumarins and related compounds, mono- and dithiocarbamates, selenols, ninhydrines, boronic acids, benzoxaboroles, etc). The polypharmacology of CAIs will also be discussed since drugs originally discovered for the treatment of non-CA related conditions (topiramate, zonisamide, celecoxib, pazopanib, thiazide and high-ceiling diuretics) show effective inhibition against many CAs, which led to their repurposing for diverse pharmacological applications.

## **Significance Statement**

Carbonic anhydrase inhibitors have multiple pharmacologic applications as diuretics, antiglaucoma, antiepileptic, antiobesity, anti-acute mountain sickness, anti-idiopathic intracranial hypertension and as antitumor drugs. Their use in inflammatory conditions, cerebral ischemia, neuropathic pain, or neurodegenerations started to be investigated recently. Parasite carbonic anhydrases are also drug targets for antiinfectives with novel mechanisms of action which can bypass drug resistance to commonly used such agents. Drugs discovered for the management of other conditions that effectively inhibit these enzymes exert interesting polypharmacologic effects.

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## 1. Introduction

CO<sub>2</sub> is one of the simplest molecules involved in crucial physiological processes and also a very stable and abundant form of carbon, the central element connected with life processes on earth. This gas reacts slowly with water, generating carbonic acid, which is unstable and spontaneously dissociates to bicarbonate and a proton (equation 1 in Fig. 1A) (Maren, 1967; Supuran, 2008; Supuran, 2023a,b). In this way, from two neutral molecules, CO<sub>2</sub> and water, a weak base (bicarbonate) and a strong acid (H<sup>+</sup> ions) are generated. In order to use this system as a biological buffer, the reaction must occur much more efficiently than the uncatalyzed one mentioned above, which has a  $k_{\text{cat}}$  of 0.15 s<sup>-1</sup> at pH 7.4 (Supuran, 2023a), and for this reason, catalysts acting on CO<sub>2</sub> hydration evolved, which are enzymes called carbonic anhydrases (CAs, EC 4.2.1.1), discovered already in 1933 (Meldrum and Roughton, 1933). This process, the interconversion between CO<sub>2</sub> and bicarbonate catalyzed by CA, which may occur with huge  $k_{\text{cat}}$  values (in the range of 10<sup>4</sup>-10<sup>6</sup> s<sup>-1</sup> for different such enzymes) (Lindskog, 1997; Mishra et al., 2020; Supuran, 2016a), was thereafter shown to be physiologically relevant for many systems in organisms throughout the phylogenetic tree, being connected not only with pH regulation (in most cells, tissues and organs), but also with CO<sub>2</sub>/bicarbonate sensing as well as several metabolic pathways (Arechederra et al., 2013; Capasso and Supuran, 2015; Maren, 1967; Neri and Supuran, 2011; Santi et al., 2013; 2021; 2022; 2023c,d). The extremely high catalytic efficiency of the CAs (an increase of the conversion rate of CO<sub>2</sub> to bicarbonate of 10<sup>5</sup> – 10<sup>7</sup> times over the non-catalytic process) probably explains why these enzymes are present in most living organisms, with the exception of few bacteria and one archeon, which do not express them (Smith et al., 1999; Ueda et al., 2012). Presently, eight genetically different CA families are known, which represent an interesting and unique case of convergent evolution at the molecular level (Aspatwar et al., 2022b; Supuran, 2023a,b). Indeed, the CA enzyme families described so far are the  $\alpha$ -,  $\beta$ -,  $\gamma$ -,  $\delta$ -,  $\zeta$ -,  $\eta$ -,  $\theta$ - and  $\iota$ -CAs (Alber and Ferry, 1994; Alterio et al., 2021; Cox et al., 2000; Del Prete et al., 2014; Di Fiore et al., 2022; Hirakawa et al., 2021; Jin et al.,



2020; Kikutani et al., 2016; Nocentini et al., 2021b; Xu et al., 2008), with a new class being discovered each 2-3 years over the past decade (Supuran, 2023a).

Fig. 1 here

The first seven CA families are metalloenzymes and they use a metal hydroxide mechanism to achieve the very efficient catalytic process of CO<sub>2</sub> hydration to bicarbonate (and obviously also the reverse reaction), as shown schematically in equations 2 and 3 in Fig. 1A. The metal hydroxide species of the enzyme is situated at a bottom of a rather spacious active site, as illustrated for the human isoform hCA II in Fig. 2A. This metal hydroxide species has a strong nucleophilic character and acts on the CO<sub>2</sub> bound in a hydrophobic pocket nearby (Fig. 2B), as demonstrated by X-ray crystallography (Domsic et al., 2008; Sjöblom et al., 2009), leading to the formation of bicarbonate bound to the metal ion (Fig. 2B), which is thereafter replaced by an incoming water molecule, leading to the formation of the “acidic” species of the enzyme, EM<sup>2+</sup>—OH<sub>2</sub> (Lindskog and Coleman, 1973; Lindskog and Malmstrom, 1962). This is a catalytically ineffective species, and in order to form the nucleophilic species, the acidic form EM<sup>2+</sup>—OH<sub>2</sub> transfers one proton from the metal coordinated water molecule to the environment (eq. 3 in Fig. 1A), usually with the assistance of active site residues (His in α-CAs, and presumably a Glu or Asp in β- and γ-CAs) (Steiner et al., 1975; Tu et al., 1989; Smith et al., 2002; Tripp and Ferry, 2000), regenerating thus the catalytically effective metal hydroxide species. In most CAs this is also the rate-determining step of the entire catalytic cycle (Steiner et al., 1975; Lindskog, 1997). This structural feature is shown in Fig. 2A for one of the most studied such enzyme, the human (h) isoform hCA II, by evidencing residue His64, the proton shuttle in this and many other hCAs, and the His cluster (comprising residues His4, 3, 10, 15 and 17) which all participate to proton transfer processes for generating the nucleophilic, zinc hydroxide species of the enzyme (Briganti et al., 1997).

Fig. 2 here

In the seven CA families which use metal ions in their active site, the nature of the cation is variable (Fig 1A), but most CAs are active with Zn(II) bound at the active site (Lindskog and Malmstrom, 1962; Supuran, 2023a). The  $\gamma$ -CAs might contain Fe(II) in their natural (reducing) environment (Macauley et al, 2009) although they are active also with Zn(II) or Co(II) bound (Ferry, 2010). The  $\zeta$ -CAs contain Cd(II) which may be substituted with Zn(II), both enzymes showing similar activity (Alterio et al., 2012; Xu et al., 2008). The other classes ( $\delta$ -,  $\eta$ - and  $\theta$ -) seem to be zinc enzymes (Del Prete et al., 2014; Kikutani et al., 2016) but no detailed studies regarding their metal ion preferences are available so far. The metal ion ligands are three His residues in  $\alpha$ -,  $\gamma$ - and  $\delta$ -CAs, two Cys and one His residue in  $\beta$ -,  $\zeta$ - and  $\theta$ -CAs, and two His and a Gln in  $\eta$ -CAs, whereas the geometry of the cation is usually tetrahedral, distorted tetrahedral and rarely trigonal bipyramidal (Supuran, 2016a,b).

The most recently described class, the  $\iota$ -CA was investigated in two different organisms, the cyanobacterium *Anabaena* sp. PCC7120, and the chlorarachniophyte alga *Bigeloviella natans* (Hirakawa et al. 2021). Surprisingly, it has been demonstrated by using X-ray crystallography that no metal ions are present in these enzymes. However, similar to the remaining seven CA genetic families discussed above, the CO<sub>2</sub> hydration catalyzed by  $\iota$ -CAs is achieved by an active site activated water molecule (Fig. 1B). But for this class, the water activation for the nucleophilic attack is performed by three amino acid residues conserved in all  $\iota$ -CAs investigated so far: Thr106, Ser199 and Tyr124 without the need of metal ions (Nocentini et al., 2021b), although, initially these enzymes were reported to contain Mn(II) at their active site (Jensen et al., 2019).

Thus, the ubiquity of CO<sub>2</sub> which is generated in most metabolic processes in all types of organisms, its gaseous nature and the need to be transformed in water soluble products, reaction which in turn is connected with pH changes, led to the emergence of CAs as widespread, versatile and highly effective catalysts (second only to superoxide dismutase as efficiency, when considering  $k_{cat}/K_M$  values of all enzymes) (Supuran, 2008; Hekmat et al., 2024).

The presence of CAs in vertebrates, humans included, is exploited pharmacologically for almost seven decades nowadays (Maren, 1967; Supuran 2008,2021). However, one of the most intriguing aspects related to CAs is the fact that a large number of isoforms is present in humans (15), with a varied localization and functions in many cells, tissues and organs, which leads to a multiplicity of pharmacological and clinical applications of their inhibitors, and to a lower extent also of their activators (Supuran, 2018a). Thus, the multi-pharmacology of CA inhibitors (CAIs) will be discussed in this review in the context of the recent developments in the field of both classical applications of these drugs (diuretics, antiglaucoma, antiepileptic, antiobesity, anti-acute mountain sickness and antitumor agents) but also newer applications, till recently not closely associated with these enzymes (such as the management of neuropathic pain, cerebral ischemia and inflammation, among others) will be dealt with (Supuran, 2021b). Furthermore, the presence of CAs in many pathogenic agents (bacteria, fungi, protozoans, nematodes, etc.) opened new possibilities for the management of drug resistant such infections and the design of anti-infectives with a new mechanism of action (Capasso and Supuran, 2015; Supuran and Capasso, 2021; Flaherty et al., 2021; Supuran, 2023e). On the other hand, several clinically used agents, discovered for other pathologies/drug targets than CAs, were shown to possess relevant CA inhibitory effects and a rather interesting polypharmacology due to their interaction with various CAs, leading in several cases to drug repurposing (Supuran, 2024a). These agents (topiramate, zonisamide, sulpiride, veralipride, celecoxib, polmacoxib, pazopanib and famotidine) will be also discussed in the review article.

## **2. CAs as drug targets: overview of mammalian, eukaryotic and prokaryotic enzymes**

$\alpha$ -CAs are present in many prokaryotic and eukaryotic organisms, among which vertebrates, protozoans, some fungi, algae, cytoplasm of green plants and in many bacteria (Aspatwar et al., 2022b; Capasso and Supuran, 2015; Supuran, 2016a), whereas the  $\beta$ -CAs are found in algae,

chloroplasts of mono- and dicotyledon plants, in many fungi, *Archaea* and most Gram negative and positive bacteria (Capasso and Supuran, 2015; DiMario et al., 2018; Schlicker et al., 2009; Supuran, 2016a). The  $\gamma$ -CAs are present in cyanobacteria, bacteria and *Archaea* (Alber and Ferry, 1994; Capasso and Supuran, 2015; De Luca et al., 2016), the  $\delta$ -,  $\zeta$ -, and  $\theta$ -CAs were described at the moment only in marine diatoms (Cox et al., 2000; Kikutani et al., 2016; Xu et al., 2008), whereas the  $\eta$ -CAs were found to date only in some protozoan species (Del Prete et al., 2014). The  $\iota$ -CAs are present in bacteria (Nocentini et al., 2021b), diatoms (Jensen et al., 2018), cyanobacteria and algae (Hirakawa et al. 2021).

Fig. 3 here

Fig. 3 shows an example of a phylogenetic tree for a restricted number of CAs belonging to the mainly investigated genetic families, the  $\alpha$ -,  $\beta$ - and  $\gamma$ -CAs from several bacteria, algae, plants, fungi and humans. As mentioned earlier, the different CA classes evolved separately through convergent evolution and are not much related with each other. On the other hand, in each specific genetic family, the enzymes are rather conserved and even between highly distant organisms, such as *Homo sapiens* and bacteria encoding  $\alpha$ -CAs, there is a high level of conserved amino acid residues, and tight structural similarities (Aspatwar et al., 2022b). The same may be said for the  $\beta$ -CAs from various organisms, although the plant, bacterial and fungal enzymes cluster on different branches of the tree (Fig. 3). The  $\alpha$ - and  $\gamma$ -CAs seem to have a more recent common ancestor compared to the  $\beta$ -CAs (Capasso and Supuran, 2015), as obvious from data of Fig. 3 (Supuran, 2023a).

Vertebrates encode only for  $\alpha$ -CAs (Aspatwar et al., 2022b), and for a long period only such enzymes were considered as drug targets (Supuran, 2008). This is of course still a reality, but over the last two decades the CAs present in other organisms, among which pathogenic bacteria, fungi and protozoans, many of which belonging to the  $\beta$ -,  $\gamma$ - and  $\eta$ -classes, started to be considered and in some cases validated as drug targets (Capasso and Supuran, 2015; Flaherty et al., 2021; Supuran,

2023e; Supuran and Capasso, 2021). Thus, the family of possible CA drug targets is greatly enlarged compared to the first years of drug design research for obtaining CAIs (Maren, 1967). This offered interesting opportunities, but also many challenges, since as mentioned above, bacterial, fungal or protozoan  $\alpha$ -CAs show a great homology and similar active site architecture to human enzymes (Supuran, 2016a), of which, as already mentioned, 15 different isoforms are known, 12 possessing catalytic activity for CO<sub>2</sub> hydration (Aspatwar et al., 2022b; Supuran, 2008) - Table 1.

Table 1 here

Table 1 shows the 12 catalytically active hCA isoforms, hCA I – XIV. It should be mentioned that isoforms VIII, X and XI (also called CA-related proteins, or CARPs) are devoid of catalytic activity since one (or two) of their zinc-binding His ligands are absent and as thus, they do not bind Zn(II) and are catalytically inactive for the CO<sub>2</sub> hydration reaction (Aspatwar et al., 2022b). Although they seem to be involved in some pathologies (Aspatwar et al., 2013; Karjalainen et al., 2018) they will be not discussed here.

As seen from Table 1, some isoforms are widespread in many tissues, such as for example hCA I and II, whereas others have a more restricted distribution in few organs and tissues (e.g., hCA III in skeletal muscle and adipocytes; hCA VII in the CNS, hCA VA in the liver and brain). Furthermore, their subcellular localization is also variable, with cytosolic, mitochondrial, membrane-bound, transmembrane and secreted isoforms (Table 1) (Nocentini et al., 2021a; Supuran, 2021b). Their catalytic activity (for the CO<sub>2</sub> hydration or bicarbonate dehydration) is also variable, with hCA III being around 1% as active as a catalyst for CO<sub>2</sub> hydration compared to hCA II and IX, which are the catalytically most effective hCAs, with the other isoforms having an activity between these two extremes (Nocentini et al., 2021a; Supuran, 2008; 2016a,b). The role of these isoforms in the cells, tissues and organs where they are present is multiple, and range from pH regulation, electrolyte secretion, sensing and transport of CO<sub>2</sub>/bicarbonate, to biosynthetic reactions (lipogenesis, gluconeogenesis, urea synthesis) and internal/external pH homeostatic control (Theparambil et al.,

2024). CAs are thus involved in fundamental physiologic processes, among which respiration, excretion of cations and anions, pH homeostasis, electrolytes secretion, bone resorption, calcification, etc. (Aspatwar et al., 2022b; Maren, 1967; Supuran, 2021b;2022; 2023,a,b). Dysregulation of such physiological activities due to a too high (or too low) activity of these enzymes may lead to a range of disorders, which are shown in Table 1. They include edema, glaucoma, epilepsy, altitude sickness, retinis pigmentosa, obesity, cerebrovascular disease, oxidative stress, cariogenesis, sterility, but also inflammation, retinopathies, Alzheimer's disease and tumors (Aspatwar et al., 2022b; Maren, 1967; Provensi et al., 2019; Supuran, 2021b,2022, 2023,a,b). In most cases the precise isoform(s) involved in each disorder is clear and the targeting well understood. For example, the renal isoforms hCA I, II and IV are involved in the diuretic effects of the sulfonamide CAIs (Maren, 1967; Supuran 2016c; 2021), whereas targeting the overexpressed hCA IX/XII in hypoxic tumors (McDonald et al., 2022) leads to anticancer effects of these pharmacological agents. However, in other cases the involvement of more than one isoform seems to be associated with a disease, e.g., hCA I, II, IV and XIV in some retinopathies; hCA II and VII in neuropathic pain; hCA VII and XIV in epilepsy; hCA IV, IX and XII in some inflammatory pathologies (Bua et al., 2020; Margheri et al., 2016; Mishra et al., 2021; Supuran, 2016c). In few cases, such as idiopathic intracranial hypertension (IIH) (Supuran, 2015) or Alzheimer's disease (Provensi et al., 2019) for which increasing evidences demonstrate the efficacy of CAIs in their management, the involved isoform(s) and the precise mechanism of action of their inhibition are still largely unknown and/or debated (Supuran, 2021b).

A crucial question thus raises: why are there 12 catalytically active CAs, with a rather superimposable distribution (in many tissues/organs) and even similar catalytic activity, when most other pharmacologically relevant enzymes have usually only 2 or 3 different isoforms? The monoamine oxidase, MAO, has just two human isoforms MAO-A and MAO-B which metabolize the three major monoamine neurotransmitters (Giovannuzi et al., 2024); the cyclooxygenase (COX)

three isoforms (Romanelli, 2024), but only COX-1 and COX-2 are involved in pharmacological effects of the NSAIDs; the angiotensin-converting enzyme ACE has again only two isoforms, ACE-1 and ACE-2 (Arrighi et al., 2024) and the list may continue with many other such examples. These enzymes (MAO, COX or ACE) play highly relevant physiological functions in many cells, tissues and organs, which are carried out by a limited number of 2-3 isoforms. Why is the situation for the CAs so different? For the moment no clear-cut reply to this question emerged, and this may represent a serious complication from the pharmacological viewpoint, due to the fact that isoform-selective inhibitors for 12 similar enzymes are needed (Alterio et al., 2012) in order to have useful pharmacological agents, which is not a facile task for medicinal chemists and pharmacologists. On the other hand, the large number of isoforms and their slightly different properties, offer the opportunity for many different pharmacological and clinical applications for inhibitors of these enzymes, in diseases not at all related to each other, as shown in this discussion and illustrated in Table 1. This is probably a unique case in which the same drug target, an enzyme possessing 12 slightly different isoforms, leads to so many pharmacological applications in very diverse fields, ranging from acid base balance and metabolism, to epilepsy, obesity, cancer, inflammatory conditions, neurodegeneration and all the other pathologies mentioned above.

When considering CAs from pathogenic organisms, the targeting is apparently easier, but also in these cases many complications may emerge, which are outlined below: (i) pathogenic organisms may encode for CAs belonging to more than one class and frequently possess different isoforms (but usually not as many as the vertebrates ones mentioned above for hCAs) with different inhibition mechanisms or sensitivity to inhibitors (Capasso and Supuran CT, 2015); (ii) in case of organisms possessing  $\alpha$ -CAs, the homology with some of the human enzymes is quite high, which may lead to inhibitors targeting both the host and the parasite enzyme, and as thus, to side effects of the potential drugs; (iii) most CAIs are highly polar molecules which do not penetrate membranes easily, and thus do not arrive in high enough concentrations at the parasite enzyme, for exerting

their antiinfective action; (iv) many fungal or protozoan parasites have different developmental stages which are not particularly easy to target by using CAIs since the expression of these enzymes in all such stages is not known or highly variable (Capasso and Supuran, 2023; Fisher et al., 2017; Vermelho et al., 2020); (v) few animal models for studying antiinfective CAIs are available to date, although relevant progress has been registered ultimately for some bacterial infections (Abutaleb et al., 2022a,b).

Thus, apart the 12 hCAs, considered as the classical drug targets among these enzymes (and which continue to be highly investigated to date), the last decade saw the emergence of many CAs from other organisms, belonging to the  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\eta$ -families as potential antiinfective targets. Many of these drug targets and the pharmacological agents modulating them will be discussed in this review.

### 3. CA inhibitor (CAI) classes

CAs are inhibited by a large number of different chemotypes, shown in Table 2 (Angeli et al., 2022a; Schulze Wischeler et al., 2010; Supuran, 2016a,b; Mueller et al., 2021; Chrysanthopoulos et al., 2017; Pustenko et al., 2020;2023; Grandane et al., 2020; Winum and Supuran, 2015; Winum et al., 2009).

Table 2 here

There are in fact four CA inhibition mechanisms (for the metallo-CAs, i.e.,  $\alpha$ - $\theta$ -class enzymes), which have been rationalized by considering the interaction (or the lack of interaction) between the inhibitor and the metal ion from the active site of the enzyme (Supuran, 2016b). They are:

(i) binding to the active site metal ion, these inhibitors being termed metal ion binders, as they incorporate a metal-binding group (usually a zinc-binding group, ZBG, as most CAs investigated in detail for pharmacological applications contain this cation). The coordination is usually achieved as



anions (for example the primary sulfonamides bind as sulfonamidates,  $\text{RSO}_2\text{NH}^-$ ), monodentately (the metal ion being in tetrahedral geometry) or more rarely, bidentately (the active site cation is in trigonal bipyramidal geometry) (Alterio et al., 2012). At least 25 different chemotypes bind CAs in this way (Table 2) and for most of them there are X-ray crystal structures of complexes of the inhibitors with many human or non-human enzymes belonging to the  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\zeta$ -CA classes (around 1000 different crystallographic structures were deposited in the Protein Data Base) (Alterio et al., 2012; Angeli et al., 2019; Costa et al., 2016; De Simone and Supuran, 2012; De Simone et al., 2013; Tanini et al., 2020). The primary sulfonamides and their isosteres (sulfamates and sulfamides) are by far the most investigated such CAIs (Supuran, 2021b), also due to their pharmacological activity and clinical use for decades, see later in the text;

(ii) inhibitors that anchor to the metal ion coordinated water molecule/hydroxide ion. The phenol was the first inhibitor for which this mechanism has been reported for hCA II (Simonsson et al., 1982; Nair et al., 1994), followed thereafter by many other chemotypes, such as polyamines (Carta et al., 2010), sulfocoumarins (Tars et al., 2013) and all other derivatives mentioned in Table 2, second column, for which X-ray crystal structures are available mainly in adduct with hCAs (Nair et al., 1994; Carta et al., 2013; Supuran, 2016b). These 7 chemotypes possess an anchoring moiety (e.g., phenol/alcohol OH, primary amine, sulfonate, etc.) which hydrogen bonds to the solvent molecule coordinated to the metal cation and participates in other favorable interactions with residues from the enzyme active site (Supuran, 2016b; D'Ambrosio et al., 2020);

(iii) the third CA inhibition mechanism, the occlusion of the active site entrance, has been reported in 2009 (Maresca et al., 2009) for coumarins, which have been shown to act as “prodrug inhibitors” of hCAs, undergoing an active site mediated hydrolysis of the lactone ring, followed by the binding of the obtained 2-hydroxy-cinnamic acids at the entrance of the active site (crystal structures of two different coumarins bound to hCA II were reported so far) (Maresca et al., 2009; 2010). Other CAIs of this type were thereafter reported (see the third column of Table 2), most of which are coumarin

derivatives, but also monocyclic (thio)lactones (Carta et al., 2012); isocoumarins (Onyilmaz et al., 2022; 2024); homocoumarins (Grandane et al., 2020), phosphocoumarins (Pustenko et al., 2023) and also the antiepileptic drug lacosamide (Temperini et al., 2010), which although not structurally related to the coumarins, binds in the same active site region as the hydrolyzed coumarins, as demonstrated by kinetic and X-ray crystallographic studies (Temperini et al., 2010);

(iv) the fourth inhibition mechanism was observed only for one compound, and it was named “out of the active site binding” (D’Ambrosio et al., 2015). Indeed, 2-benzylsulfinylbenzoic acid was observed bound out of the active site of hCA II, in an adjacent hydrophobic pocket near the entrance to the active site, position in which allows the inhibitor to participate to hydrogen bonds, through a bridging water molecule, with His64, the proton shuttling residue of this enzyme, thus leading to the collapse of the catalytic cycle, as demonstrated again by kinetic and crystallographic studies (D’Ambrosio et al., 2015).

Thus, more than 40 different chemotypes (Table 2) show significant CA inhibitory properties against enzymes belonging to all 8 genetic families, since a large number of representative enzymes belonging to various classes (including the  $\iota$ -CAs) were investigated in detail for their inhibition with different such classes of inhibitors (Supuran 2008; 2016a,b; 2023a). It is interesting to note that for the  $\iota$ -CAs, Hirakawa et al (2021) observed by X-ray crystallography bicarbonate and iodide (anion inhibitors of metallo-CAs) bound in the enzyme active site, although no metal ion is present in this class of enzymes, as mentioned above. Furthermore, other studies demonstrated that both inorganic/organic anions and sulfonamides, do inhibit bacterial  $\iota$ -CAs, although the inhibition mechanism is not well understood at the moment (De Luca et al., 2021; Petreni et al., 2021).

It should be also mentioned that there are inhibitors for which the CA inhibition mechanism is unknown (Supuran, 2016b). They include some tertiary sulfonamides (Le Darz et al., 2015), probenecid amide derivatives (Carradori et al., 2015) and kinase inhibitors such as imatinib or nilotinib (Parkkila et al., 2009), for which solution (kinetic) studies revealed relevant inhibitory

activity (against hCAs of physiological relevance) but no X-ray crystal structures of such inhibitors bound to any CAs are available to date.

Not all CA inhibitory chemotypes of Table 2 were investigated for their detailed pharmacological activity in cell or animal models so far, but some of them, among which the primary sulfonamides, sulfamates and sulfamides, several dithiocarbamates and benzoxaboroles, phenols/catechols and coumarins are present in clinically used drugs interacting with these enzymes and they will be discussed in detail in the next sections.

#### **4. The tail approach for obtaining isoforms-selective CAIs**

Sulfanilamide (4-amino-benzenesulfonamide) was the first organic CAI ever discovered (Mann and Keilin, 1940). Other aromatic and heterocyclic (primary) sulfonamides were soon thereafter investigated, being noted that heterocyclic derivatives were more effective CAIs compared to aromatic compounds (Krebs, 1948). The first drug design studies in the field were shortly thereafter reported (Miller et al., 1950) which led to the discovery and launch of the first clinically used CAI diuretic, acetazolamide in 1954, and in the next years of the other first generation sulfonamides (Maren, 1967) that will be discussed in the Diuretics/Antiglaucoma sections shortly. However, all drugs of the first (and actually also second) generation of CAIs were aromatic/heterocyclic sulfonamides incorporating simple and compact scaffolds that assured a potent binding to all known CA isoforms, but as thus, they were pan-inhibitors, presenting a large number of side effects when used systemically (Maren, 1967; Supuran, 2008). This considerably limited the use of the entire class of such pharmacological agents which were considered problematic drugs, with a too wide range of undesired side effects, due to the inhibition of CAs in many organs, other than the target one (Maren, 1967; Wistrand, 1984). Thus, the need to design isoform-selective CAIs was stringent in the late '90s, when an increasing number of human isoforms was constantly being discovered and

the available CAIs at that time were inhibiting indiscriminately most of them (Lindskog, 1997; Supuran and Scozzafava, 2000).

Fig. 4. here

The tail approach thus emerged as a solution for resolving the problem of obtaining isoform-selective CAIs for all human isoforms (Scozzafava et al., 1999a,b). The original idea was to obtain elongated molecules (actually primary sulfonamides) which could interact not only with the bottom and middle parts of the CA active site (similar to the clinically used agents, e.g., acetazolamide, methazolamide), but also with the entrance in the cleft (Fig. 4A). The attachment of moieties named “tails” on the scaffold of simple aromatic/heterocyclic sulfonamides (easily derivatizable at their amino or hydroxyl moieties) may thus induce not only the desired physico-chemical properties (e.g., enhanced hydro- or liposolubility to the CAI) but also the possibility that the tail interacts with the middle and outer rim (the entrance) of the active site cavity (Scozzafava et al., 1999b). It has been known for a long time that the bottom and partially also the middle parts of the hCA active sites are rather conserved among the isoforms, with many identical amino acid residues being involved in the catalytic and inhibition mechanisms (Lindskog, 1997). However, the rim part of the active site is more variable among the different hCAs, differentiating them in their interaction with modulators of activity, as shown by a large number of solution and crystallographic studies (Scozzafava et al, 1999a,b; Menchise et al., 2005; Alfterio et al., 2006; Di Fiore et al., 2007; Winum et al., 2006). Indeed, the rather high variability of many amino acid residues in those parts of the active site make possible favorable or clash interactions between the inhibitor and the enzyme, potentially leading to isoform-selective inhibitors, as again demonstrated in a multitude of kinetic and crystallographic studies on various human isoforms (Alterio et al., 2012; Pacchiano et al., 2010; 2011; Winum et al., 2006). Detailed kinetic (and in some cases X-ray crystallographic) studies were thereafter performed on over 10,000 different sulfonamide derivatives obtained according to the tail approach in our and many other laboratories worldwide, which demonstrated

not only that the initial hypothesis was correct but also that it is a facile and versatile modality to generate a large number of CAIs by tailoring the dimensions (length, bulkiness, etc.) as well as the chemical nature of the various tails, leading to compounds with a good selectivity for all human isoforms of interest (reviewed in Supuran, 2023a and Kumar et al., 2022). Furthermore, the tail approach was shown to be extendable to many other chemotypes apart the sulfonamides acting as CAIs (Table 2), both belonging to the metal ion binders or to the inhibitors which anchor to the metal ion coordinated water molecule (Supuran, 2023a). As the active sites of many CA isoforms are rather spacious, the idea to introduce more than one tail, of different natures (e.g., one hydrophobic and one hydrophilic; two hydrophobic, two hydrophilic, more than two, etc.) appeared of interest, and resulted in highly effective and isoform-selective CAIs, by the so-called “two tails” (Tanpure et al., 2015; Fares et al., 2020) – see Fig. 4B and “three tails approaches (Bonardi et al., 2020;2022) – Fig. 4C. After its discovery in 1999 (Scozzafava et al., 1999b), the tail approach and its spinoffs (two- and three tails) was virtually the only drug design strategy used to obtain CAIs, being adopted by research groups all over the world (Supuran, 2023a; Kumar et al., 2022). For space reasons the many hundreds of such drug design strategies and the huge number of effective CAIs thus obtained, cannot be mentioned here.

## **5. Classical pharmacological applications of the CAIs used clinically**

In this section I will review the classical pharmacological applications and the available drugs with CA inhibitory action, acting on different isoforms and possessing thus various actions, as diuretics, antiglaucoma/ocular drugs, antiepileptics, antiobesity agents, drugs for the management of acute mountain sickness, or idiopathic intracranial hypertension agents. I will thereafter deal in the next section with the latest developments in the field, i.e., the developments of CAIs acting as antitumor agents targeting hypoxic tumors as well as “less classical” applications of these pharmacological agent, in the management of neuropathic pain, cerebral ischemia and some forms of inflammation.

## 5.1. Diuretics

Acetazolamide (compound **1**, Fig. 5) was the first modern diuretic ever, being launched for clinical use in 1954, replacing the rather toxic mercurials employed till then (Maren, 1967). It is still a clinically used compound, as it will be mentioned in the review, albeit more rarely as a diuretic, nowadays. However, it was the lead molecule which led to the discovery of many widely used diuretics, such as the thiazides **2**, the thiazide-like drugs **3-6** as well as the high-ceiling diuretics furosemide **7**, azosemide **8** and bumethanide **9** (Fig. 5), all of which incorporate the primary sulfonamide moiety, responsible for CA inhibition (Supuran, 2021b; Ferraroni et al., 2022b; Temperini et al, 2008;2009; Carta and Supuran, 2013).

The diuretic effects of acetazolamide are due to the inhibition of renal CA isoforms, since both cytosolic (CA I, II) as well as membrane-bound (CA IV and XII) such enzymes are abundant in all segments of the nephron, being involved in urine acidification (Maren, 1967; Wistrand, 1980). When the renal enzyme is inhibited, an increased amount of bicarbonate is excreted, which leads to urine alkalinization, together with an increased loss of  $\text{Na}^+$ ,  $\text{K}^+$  and osmotically obligated water (Garvey and Maude, 1981) with the concomitant instauration of a mild metabolic acidosis. This phenomenon is due to the fact that bicarbonate eliminated in the urine, is formed from  $\text{CO}_2$  in equivalent amounts with  $\text{H}^+$  ions in the CA catalyzed process, and the acid is taken by the blood, leading thus to acidosis (Maren, 1967; Tsikas, 2024). This is also the reason why the diuretic effect of acetazolamide alone is low and tolerance develops in few weeks, although the precise mechanisms by which this occurs is still debated (Maren, 1967; Tsikas, 2024). However, very recently, this diuretic has been “resurrected”, since it has been observed that the combination of acetazolamide and high ceiling diuretics of types **7-9** was synergistic and efficient in the management of patients with acute decompensated heart failure (Mullens et al., 2022). This randomized, multicenter, double-blind, placebo-controlled trial, demonstrated that in patients

suffering of acute decompensated heart failure, a condition with few therapeutic options, the i.v. administration of acetazolamide plus the loop diuretics mentioned above, was more efficient in decongestion of the patients, compared to each drug alone, leading also to a higher cumulative natriuresis and urine output (Mullens et al., 2022). These findings were confirmed in other studies (Lim, 2022; Cuthbert and Cleland, 2023; Meekers et al., 2023) demonstrating thus the clinical utility and “renaissance” of this old diuretic. This discovery is not only clinically relevant, but also counter-intuitive, since it has been considered for a long period that the combination of acetazolamide with other sulfonamide diuretics (thiazides, loop diuretics, etc.) may lead to serious drug-drug interaction manifested by electrolyte abnormalities and enhanced metabolic acidosis (Supuran, 2020a; 2024).

Fig. 5 here

It is well-known that the sulfonamide drugs **2-9** (Fig. 5) exert their diuretic action by targeting other proteins than the CAs (e.g.,  $\text{Na}^+/\text{Cl}^-$  transporters,  $\text{Na}^+/\text{K}^+/\text{Cl}^-$  cotransporters, such as NKCC1, etc.) (Supuran, 2018b,c), but they also possess significant inhibitory action against many CA isoforms (Temperini et al., 2008,2009), in some cases in the low nanomolar range (see Supuran, 2008, for the precise  $K_I$  values of all drugs discussed here against all human isoforms) which might be useful for their repurposing for other therapeutic applications, such as antitumor agents, as already proposed for some of them, e.g., benzthiazide (Lee et al., 2012).

It has recently been demonstrated that acute ingestion of acetazolamide **1** (250 mg) temporarily increased also the excretion of two amino acids (Leu and Ile), not only of bicarbonate and cations, as well as that of nitrite and nitrate, with a concomitant decreased excretion of other amino acids (Tsikas, 2024). In the same study, acetazolamide was shown to decrease the urinary excretion of malondialdehyde, a biomarker of oxidative stress (Tsikas, 2024). The urine alkalization induced by acetazolamide is on the other hand useful also for the management of kidney injury in patients treated with methotrexate (Reed et al., 2019; Truong et al., 2024), for the reduction of lithium-

induced nephrogenic diabetes insipidus (de Groot et al., 2016), for the management of cystinuria (Tiselius, 2010), or in patients with uric acid/cystine kidney stones (Sterrett et al., 2008).

## 5.2. Antiglaucoma agents and other applications of CAIs in ocular diseases

Glaucoma, an eye condition characterized by elevated intraocular pressure (IOP) is a neuropathy leading to blindness if untreated (Mincione et al., 2021). CAIs constitute a cornerstone in its treatment, starting with the '50s, when it has been discovered that aqueous humor in the anterior chamber of the eye is rich in bicarbonate, which in turn is generated through the activity of ocular CAs (isoforms I, II, IV, XII), highly abundant in the ciliary processes (Wistrand, 1951; Maren, 1967).

Fig. 6 here

Inhibition of CA isoforms within the ciliary processes of the eye (with acetazolamide **1**) has been shown to decreased bicarbonate formation in the anterior chamber (Becker, 1955) which was responsible of the pharmacologic effect of decreased IOP, with **1** and several other CAIs approved for clinical use in the first decades of CA research, e.g., methazolamide **10**, ethoxzolamide **11** and dichlorophenamide **12** – Fig. 6 (Kinsey and Reddy, 1955; Maren, 1967; Carta et al., 2012; Masini et al., 2013; Bua and Supuran, 2019). The lowered IOP is beneficial for the patients, as the damage of the optic nerve is directly proportional to the high pressure in the eye (Mincione et al., 2021). Drugs **1** and **10-12** are effective only by systemic administration as antiglaucoma agents, due to their improper physico-chemical properties, among which a low water solubility, too low lipophilicity, and the impossibility to be administered as eye drops (Maren 1967, 1997; Supuran, 2008). Methazolamide **10**, ethoxzolamide **11** and dichlorophenamide **12** are highly effective *in vitro* CAIs (similar to acetazolamide **1**) but are more lipophilic compared to acetazolamide and also show more CNS side effects compared to acetazolamide, due to their easier penetration through membranes



(Maren, 1967). However, like **1**, they are also administered only systemically in order to exert their ocular pharmacological activity. This administration modality led to serious side effects due to the inhibition of CAs in other organs than the eye, such as the gastro-intestinal tract, kidneys, lungs, blood, etc. (Maren 1967, 1997; Supuran, 2008) limiting thus their usefulness. In the '90s, the second generation antiglaucoma agents dorzolamide **13** and brinzolamide **14** (Fig. 6) have been developed, which are water-soluble, can be administered as eye drops, and are efficient IOP lowering agents (Ponticello et al., 1998; Silver, 1998). Both drugs are used alone or more frequently in combination with other antiglaucoma agents, such as  $\beta$ -blockers,  $\alpha$ -adrenergic agonists, prostaglandin receptor antagonists, rho-kinase inhibitors or nitric oxide donors (Angeli and Supuran, 2019; Berrino and Supuran, 2019; Nocentini and Supuran, 2019; Mincione et al., 2021; Supuran et al., 2019). Thus, acetazolamide **1** and the remaining first generation CAIs **10-12** are no longer used as systemic drugs for the treatment of glaucoma, being replaced by the topically-acting agents dorzolamide **13** and brinzolamide **14**, but there are many recent reports highlighting their usefulness (alone, or more frequently in combination with other drugs) for the management of other ocular diseases, among which X-linked retinoschisis (Wey et al., 2023); macular edema secondary to retinal vein occlusion, in combination with the VEGF drug bevacizumab (Karimi et al., 2023); macular edema, in combination with topical NSAIDs (Aljundi et al., 2022) as well as age-related macular degeneration (Supuran, 2019), cases in which antagonistic interactions between these CAIs and NSAIDs did not manifest (Supuran, 2020a;2024).

### 5.3. Antiepileptics

Acetazolamide **1**, sulthiame **15**, topiramate **16** and zonisamide **17** (Fig. 7) are clinically used agents for the management of several types of epilepsy (Supuran, 2021b).

Fig. 7 here

Acetazolamide **1** has a long story in the treatment of epilepsy, dating back to the '50s (Merlis, 1954) although its mechanism of action and its efficacy in various epilepsy forms were and are rather controversial (Ansel and Clarke, 1956; Oles et al., 1989; Reiss and Oles, 1996). pH regulation in the brain is potently correlated with the neuronal function, and since 11 CA isoforms are present in the CNS (Table 1), their inhibition/activation as well as the activity of other voltage- and ligand-gated ion channels/gap junctions, may synergistically lead to pH changes which affect neuron excitability (Ruusuvuori and Kaila K, 2014). Indeed, alkaline shifts were shown to induce an increase in excitability which may trigger epileptiform activity, whereas acidosis had the opposite effect (Ruusuvuori and Kaila K, 2014). The highly CNS-abundant cytosolic isoforms hCA II and VII, as well as the transmembrane hCA XIV (Table 1) are considered the most probable targets on which CAIs act exerting an antiepileptic activity (Thiry et al., 2008; Aggarwal et al., 2013; De Simone et al., 2009). Indeed, it is ascertained that acetazolamide is effective in patients with refractory epilepsy to other medical treatments (Oles et al., 1989; Reiss and Oles, 1996; Aribi and Stringer, 2002). Sulthiame **15** is a highly potent CAI for all brain CA isoforms (Leniger et al., 2002; Temperini et al., 2007) and was shown to interfere with the pH regulation in CNS inducing a modest but pharmacologically significant intracellular acidosis of central neurons (Leniger et al., 2002) together with CO<sub>2</sub> retention, which probably also interferes with pH regulation in this organ, and explains its antiepileptic effect (Shi et al., 2017). Topiramate **16**, a sulfamate and zonisamide **17**, an aliphatic sulfonamide (Fig. 7), are two newer, widely used antiepileptics which show significant CA inhibitory effects (Leniger et al., 2004; Leppik, 2004; Thiry et al., 2008; Aggarwal et al., 2013; De Simone et al., 2009). However, both of them have a rather complex pharmacology (similar to many other antiepileptics), since their anti-seizure effects are due to interaction with other targets apart the brain CAs (Leniger et al., 2004; Leppik, 2004; Thiry et al., 2008), among which voltage-gated sodium channels, high-voltage-activated calcium channels, GABA<sub>A</sub> receptors, AMPA/kainate receptors, for topiramate (Leniger et al., 2004) whereas for zonisamide sodium and T-type calcium channels (Leppik, 2004). The diuretic bumethanide **9** (Fig. 5) was also shown

recently to possess antiepileptic action in neonatal seizure (Kaila and Löscher, 2022) although, as for the previously discussed agents, it targets not only brain CAs but also the Na-K-2Cl cotransporter, NKCC1. These drugs and other antiseizure CAIs, primarily sulfonamides and carbamates acting by inhibiting the CNS isoforms CA II, VII and XIV (Bibi et al., 2019; Odi et al., 2021; Mishra et al., 2021) were investigated experimentally in various seizure models, leading to effective anticonvulsant effects. However, similar to many other antiepileptics, CAIs **1, 9, 15-17** may show a considerable number of drug-drug interactions with other antiepileptics and different other pharmacological agents (Arman and Haghshenas, 2022; Ji et al., 2022; Pan et al., 2021; Sarayani et al., 2023; Willems et al., 2023).

#### **5.4. Antiobesity agents**

Obesity is considered nowadays as a chronic, degenerative disease, characterized by excessive fat accumulation, and constitutes a challenging medical problem worldwide, with a large number of affected people and few available efficient drugs, which in addition possess a large number of side effects (Müller et al., 2022; Supuran, 2022). CAs were demonstrated to possess a relevant role in several metabolic processes in various cells, including fatty acid biosynthesis and *de novo* lipogenesis (DNL) (Supuran, 2022). Both fatty acid biosynthesis and DNL involve mitochondrial and cytosolic steps, in which participate several enzymes implicated in the Krebs cycle as well as DNL, such as pyruvate carboxylase (PC) and acetyl-coenzyme A carboxylase (ACC), which both employ bicarbonate and not CO<sub>2</sub> as substrate (De Simone et al., 2008; Supuran, et al., 2008; Supuran, 2012; Scozzafava et al., 2013). In order to achieve the rapid interconversion between these two species (CO<sub>2</sub> and bicarbonate), highly catalytically active CA isoforms (among which hCA II in the cytosol and hCA VA/VB in the mitochondria) are needed (Aldred and Reilly, 1997; Atwood, 1995). It has been demonstrated already in the 90s that these CAs participate in biosynthetic pathways, also being observed that inhibition of mitochondrial/cytosolic CAs interferes with both

fatty acid biosynthesis and DNL in various cells, tissues and animal models (Lynch et al., 1995; Chegwiddden and Spencer, 1996; Hazen et al., 1996). The metabolism of Krebs cycle intermediates, such as pyruvate, acetate, and succinate were examined thereafter in the presence of specific sulfonamide hCA VA/B – selective inhibitors using electrode wired mitochondria, and measuring metabolic energy conversion (Arechederra et al., 2013). Several potent, nanomolar hCA VA/B inhibitors showed a broad spectrum inhibition of metabolism, whereas others only had significant effects on pyruvate and fatty acid metabolism, but such data conclusively demonstrated the crucial role of mitochondrial CAs in the biosynthesis of fatty acid, and as thus in lipogenesis. Evenso, the idea to use CAIs for obtaining antiobesity drugs started to be considered only in 2000s (Antel and Hebebrand, 2012) by examining weight loss side effects of some antiepileptics, such as topiramate and zonisamide (compounds **16** and **17**, Fig. 7). The use of **16** and **17** (and to a lower extent also of acetazolamide **1**) as anti-obesity drugs may be thus considered as one of the first example of drug repurposing among clinically used CAIs (Supuran, 2022). The use of such CAIs alone or in combination with other agents (phentermine + topiramate, bupropion + zonisamide, metformin in combination with both CAIs, etc.) was demonstrated to induce weight loss in many patients, also improving their blood glucose levels (Aronne et al., 2013; Gadde et al., 2003; 2011; Garvey et al., 2012; Lévy et al., 2007; Mahmood et al., 2013; Schneiderhan and Marvin, 2007; Muñoz et al., 2018; Sari et al., 2021).

How do these agents exert their antiobesity effects? Although the pharmacology of **16** and **17** is rather complex (see discussion in the previous section), both of them and also acetazolamide **1**, are effective CAIs against hCA isoforms involved in fatty acid biosynthesis/DNL, exerting probabaly in this way their antiobesity action (Supuran, 2022). The increased use of topiramate (and zonisamide) as antiobesity agents in the last 5 years also led to the observation of side effects typical of systemic CAIs, such as abnormalities in the serum bicarbonate concentration (Naps et al., 2023); dysgeusia with carbonated drinks, presumably due to salivary CA VI inhibition

(Charbonneau et al., 2020), as well as type II renal tubular acidosis (Chahin et al., 2020). However, several different studies demonstrated that the elevated glucose-induced mitochondrial respiration followed by formation of reactive oxygen species (ROS), typical of type II diabetes, may be reversed by pharmacological inhibition of mitochondrial CAs with topiramate **16**, in a mouse cerebral pericytes model of the disease, allowing thus for potential therapeutic applications of the CAIs in other metabolic disorders, e.g., diabetes (Gamal et al., 2024; Shah et al., 2013; Salameh et al., 2019).

### **5.5. Acute mountain sickness drugs**

Acute mountain sickness commonly affects high altitudes travelers, with debilitating symptoms such as dizziness, headache, insomnia, nausea, anorexia, dyspnea, vomiting, peripheral edema, and retinal hemorrhage (Bradwell et al., 1992; Porcelli and Gugelchuk, 1995). Pulmonary and/or cerebral edema may also be rather common in severe cases. The reduced oxygen supply at high altitudes seems to be the triggering event of this condition, which induces hypoxemia and hypoxia in many tissues and organs. CA inhibition has been reported several decades ago to represent one of the few useful therapeutic approaches for the management of acute mountain sickness (Bradwell et al., 1992). The usefulness of CA inhibition in the treatment of this condition is not fully understood, but the diuresis and bicarbonate excretion induced by CAIs, such as acetazolamide **1** or methazolamide **10**, leading also to a mild metabolic acidosis, might explain the phenomenon (West, 2004). In addition, CA inhibition in peripheral chemoreceptors mediates ventilatory optimization by receding the hypoxic and hypercapnic sensitivity (Ainslie et al., 2013). This also leads to an alteration in cerebral blood flow, that in turn is responsible for the control of cerebral oxygenation, leading to the relieve of many symptoms associated with this condition, which may become life-threatening if not treated (Teppema et al., 2007). It has been also demonstrated that cerebral intraventricular administration of acetazolamide **1** induced an enhancement of cerebrospinal fluid

(CSF) bicarbonate concentrations, with consequences in the modulating of CSF pH values during respiratory acidosis in experimental animals (Kazemi and Choma, 1977). Acetazolamide **1** was among the most popular and well-studied drug for the management of this condition (Carlsten et al., 2004), but methazolamide **10** seems to be even more effective due to its increased lipophilicity (Doherty et al., 2023). At 250 mg/day acetazolamide **1** significantly reduced symptoms of high altitude sickness (Carlsten et al., 2004; Burtscher et al., 2016) whereas methazolamide may be used at a lower dosage of 100 mg/day (Doherty et al., 2023; Swenson, 2022).

### **5.6. Idiopathic intracranial hypertension agents**

Idiopathic intracranial hypertension (IIH) is characterized by an increased intracranial pressure in the absence of brain tumors, condition that occurs through a poorly understood mechanism, but presumably involves cerebrospinal fluid (CSF) secretion, process in which several brain CA isoforms are involved (Maren, 1967; Supuran, 2015; Uldall et al., 2017). Acetazolamide **1** is known for decades to constitute one of the few effective therapies for its treatment (Maren, 1967). Acetazolamide, a low nanomolar inhibitor of all CA isoforms involved in CSF formation, e.g., hCA II, IV, VA and XII (Supuran, 2008) leads to a decreased CSF secretion and an efficient control of the intracranial pressure in many of the affected patients after systemic administration (Maren, 1967; Supuran, 2015; Uldall et al., 2017). Apart **1**, currently used for the treatment of IIH (although it has many side effects, as mentioned above), the more lipophilic CAIs methazolamide **10** (Nia et al., 2022) and topiramate **16** (Goyal and Zarroli, 2023) were recently shown to lead to an even more effective control of the increased intracranial pressure compared to **1**, presumably due to their enhanced lipophilicity compared to acetazolamide, and easier access to the brain enzymes. The combination of acetazolamide **1** with glucagon-like peptide-1 (GLP-1) receptor agonists, such as semaglutide, tirzepatide, and other similar drugs, was on the other hand very recently shown to be synergistic [118] and to be more effective for the management of IIH than **1** alone, also leading to a

significant weight loss, which is a beneficial “side effect” for patients suffering of this disease, who frequently are obese (Krajnc et al., 2023). Thus, there seem to be highly synergistic interactions between CAIs and GLP-1 mimic drugs, which need to be studied in more detail, considering that these are relatively new drugs, highly effective for the management of diabetes and obesity, but their safety profile and especially drug-drug interactions are poorly investigated at the moment (Supuran, 2024a).

## **6. Pharmacological applications of CAIs in clinical trials/preclinical development**

### **6.1. Antitumor agents targeting hypoxic tumors**

Connection between CA inhibition and cancer started to emerge in the ‘90s, when Teicher et al (1993) showed that acetazolamide **1** may sensitize cancer cells to other chemotherapeutic agents, whereas other researchers observed that this sulfonamide inhibits the growth of gastric cancers in patients treated with high doses of the drug for the management of ulcers (Puscas et al., 1994). Such reports were considered anecdotal until 1994 when a cancer-associated CA isoforms, CA IX has been discovered (Pastorek et al., 1994), followed by the report of a second such enzyme, CA XII, in 1998 (Tureci et al., 1998). Both of them are multi-domain transmembrane isoforms with the CA domain situated outside the cell, are present in many tumors and possess a rather limited distribution in normal tissues (Kivelä et al., 2000; Leppilampi et al., 2003; McDonald et al., 2020, 2022; Angeli et al., 2020; Supuran, 2020b). Both proteins are under the control of a transcription factor, hypoxia inducible factor (isoforms HIF-1 or HIF-2), which, by acting as O<sub>2</sub> sensors, induce CA IX/XII overexpression in hypoxic tumors as a consequence of an activation cascade which upregulates several proteins involved in pH regulation, metabolism, angiogenesis, ferroptosis and other physiologic/pathologic processes (Semenza, 2019; Neri and Supuran, 2011; Pugh and Ratcliffe, 2017; Pettersen et al., 2015; Chafe et al., 2021). As shown schematically in Fig. 8, CA IX (and also CA XII, which is however less widespread in tumors compared to isoform IX) is involved

not only in intra-/extracellular pH regulation and metabolism, but also in survival, proliferation, and migration of the cancer cells, as well as in angiogenesis and ferroptosis, phenomena which cancer cells exploit in order to overcome the Darwinian competition with their normal counterparts and thus, thrive (Semenza, 2019; Neri and Supuran, 2011; Pugh and Ratcliffe, 2017; Pettersen et al., 2015; Chafe et al., 2021).

Work from several laboratories demonstrated that inhibition of CA IX, CA XII or both enzymes, with compounds acting as potent (usually low nanomolar inhibitors of both enzymes), such as derivatives **18-26** shown in Fig. 9, leads to a potent inhibition of growth of primary tumors and metastases, in several cell cultures/animal models of cancer (Svastová et al., 2004; Ahlskog et al., 2009; Pacchiano et al., 2011; Lou et al., 2011; Lounnas et al., 2013; Gieling et al., 2012). As seen from Fig. 9, all types of CAIs (sulfonamides, such as **18, 19, 25** and **26**; sulfamates, such as **22-24**, and coumarins, such as **20** and **21**) possess significant antitumor activity (reviewed in Supuran, 2020b, 2021, 2023d).

Figs. 8 and 9 here

CA IX/XII inhibition with such compounds (and also with monoclonal antibodies, mAbs, which will be not discussed here, as they were reviewed recently, see Supuran, 2020b) leads to interference with pH regulation (Neri and Supuran, 2011; Svastova et al., 2004), affects the metabolism of the cancer cells, as less bicarbonate is available for biosynthetic reactions (Santi et al., 2013), enhances ferroptosis (Chafe et al., 2021) and also diminishes the number of cancer stem cells (Lock et al., 2013), all antitumor/antimetastatic mechanisms which explain the significant biological effects observed with them. One of the main hurdles in validating CAIs as antitumor/antimetastatic agents was the lack of CA IX/XII-selective inhibitors till recent dates (Kumar et al., 2022; Supuran, 2023a; Angeli and Supuran, 2023; Bendi et al., 2024). However, by using the tail approach (discussed above) and the many new chemotypes with CA inhibitory action discovered (e.g., coumarins, benzoxaboroles, etc.), many highly isoform-selective such compounds



were obtained (Supuran, et al., 2018), among which the sulfonamide **19** (SLC-0111), which is one such interesting example, acting as a low nanomolar hCA IX/XII inhibitor, without potently inhibiting the remaining hCAs (Pacchiano et al., 2010,2011). SLC-0111 is the first new generation CAI to progress to clinical trials for the management of hypoxic metastatic tumors (Table 3) (McDonald et al., 2020).

Table 3 here

In fact apart SLC-0111, the pan-inhibitors acetazolamide **1**, and benzolamide (in combination with various other anticancer drugs, see Table 3) are nowadays investigated in several clinical trials, but as they are strong inhibitors of all human CAs, most of the preclinical and clinical work has been performed with compound **19** (Andreucci et al., 2023; Boyd et al., 2017; Eloranta et al., 2023; Grajek and Poleszczuk, 2023; McDonald et al., 2019; 2020; 2022; McDonald and Dedhar, 2023; Mussi et al., 2022; Peppicelli et al., 2020; Sarnella et al., 2022). SLC-0111 is nowadays in Phase Ib/II clinical trials for pancreatic cancer in combination with gemcitabine (NCT03450018) but several other Phase II clinical trials are scheduled for the near future. The potential of CAIs for the imaging of hypoxic tumors is also relevant, and the field has been reviewed recently and will be not discussed in detail here (see Burianova et al., 2021; Nerella et al., 2023).

It should be also mentioned that a large number of CA IX/XII inhibitors were reported by several groups worldwide in the last decade, which used SLC-0111 as a lead molecule, but they are not discussed here as they were reviewed recently (Kumar et al., 2022; Bendi et al., 2024; Elsawi et al., 2023). Nowadays there is a large number of CAIs (mainly sulfonamides, coumarins and sulfocoumarins) for which significant antitumor effects were proved in many cell/animal models, and presumably some of them will enter in clinical trials soon.

It should be mentioned that several groups reported aplastic anemia-like syndromes in patients with various malignancies (Lakota et al., 2012; Jankovicova, 2013; Menteşe et al., 2015).

In all cases it has been discovered that an immune response against red blood cell isoforms hCA I

and II developed, leading to auto-antibodies against these isoforms which interfere with their physiological function, whereas isoforms CA IX and CA XII seem to be not implicated in these forms of anemia (Lakota et al., 2012; Jankovicova, 2013; Menteş et al., 2015).

## 6.2. New pharmacological applications of CAIs

In the last decade, several proof-of-concept studies have emerged on the applications of CAIs in conditions usually not associated with CA modulation, such as neuropathic pain (Supuran, 2016c), cerebral ischemia (Bulli et al., 2021), rheumatoid arthritis (Bua et al., 2017), neurodegenerative conditions, such as Alzheimer's (Provensi et al., 2019) and Parkinson's diseases (Murata et al., 2020) and more generally, oxidative stress (Del Giudice, 2013).

### 6.2.1. Neuropathic pain

Advances in understanding pathological mechanisms related to neuropathic pain, for which few therapies are available (Supuran, 2016c) and the biochemical/pharmacological characterization of novel drug targets, provided evidence that CA inhibition might be a novel approach for obtaining agents useful for the management of this condition (Assiedu et al., 2010; Carta et al. 2015). The rationale for using CAIs for the management of neuropathic pain is based on the fact that peripheral nerve injury negatively influences spinal GABA-ergic networks via a reduction in the neuron-specific potassium-chloride ( $K^+$ -Cl<sup>-</sup>) cotransporter (KCC2) which induces neuropathic allodynia (Assiedu et al., 2010). CAIs inhibit enzymes present in these tissues and reduce the bicarbonate-dependent depolarization of GABA<sub>A</sub> receptors by diminishing the production of bicarbonate in SNC and peripheral nervous system, thus leading to an analgesic effect (Assiedu et al., 2010). hCA II and VII are most probably the enzymes involved in these phenomena, both at central and peripheral levels (Supuran, 2016c; Carta et al., 2015). Sulfonamide hCA II/VII inhibitors such as

derivative **27** (Fig. 10) showed efficacy in animal models of neuropathic pain (Carta et al., 2015), better than acetazolamide **1** (Assiedu et al., 2010), putting the basis for the development of a specific therapy based on selective hCA II/VII inhibition. Many other CAIs apart **27** were investigated to date for their efficacy in the management of neuropathic pain, with promising and long lasting effects being noticed (Angeli et al., 2023), but no derivative reached clinical trials at the moment.

### 6.2.2. Cerebral ischemia

The current pharmacological options for cerebral ischemia are few and have a limited impact on ischemic damage. Hypoxia, which is a consequence of the ischemic event, triggers overexpression of isoforms hCA IX/XII (as discussed above), and their inhibition was recently demonstrated to represent a new possibility for the management of this condition (Di Cesare Manelli et al., 2016; Bulli et al., 2021). The pharmacological evaluation of several sulfonamide and coumarin CAIs (such as compounds **28** and **29**, Fig. 10) in an animal model of cerebral ischemia, i.e., rats with a permanent middle cerebral artery occlusion (pMCAO), afforded the proof-of-concept study that CA IX/XII inhibition is useful to treat ischemia, since the neurological score of pMCAO rats was dramatically reduced 24 h after occlusion, and repeated subcutaneous injections of CAIs **28** or **29** (at 1 mg/kg) increased the neurological score by 40% (Di Cesare Manelli et al., 2016). Coumarin **28**, acting as a low nanomolar hCA IX/XII-selective inhibitor also reduced the volume of hemisphere infarction, whereas acetazolamide **1** used as standard drug was completely ineffective. The ability of novel CAIs to improve neurological functionalities after cerebral ischemic events may thus open the path to a novel pharmacologic treatment of the condition (Dettori et al., 2021).

Fig. 10 here

### 6.2.3. Rheumatoid arthritis

Margheri et al (2016) reported that isoforms hCA IX and XII are also overexpressed in some forms of arthritis. Thus, the rationale was that inhibition of these enzymes with CAIX/XII-selective inhibitors might provide the the proof-of-concept that these isoforms may be new drug targets for the management of arthritis (Margheri et al., 2016). The drug design involved the synthesis of hybrid compounds incorporating non-steroidal anti-inflammatory drugs (NSAIDs) belonging to the carboxylic acid derivatives (Fig. 10) and sulfonamide or coumarins CAIs (Akgul et al., 2018; Bua et al., 2017) leading to compounds of types **30** and **31**. These hybrids showed effective inhibitory profiles against the target isoforms hCA IX and XII, and had a potent and long-lasting antihyperalgesic effects in a rat model of arthritis and may thus have a future for the management of this disease (Akgul et al., 2018; Bua et al., 2017).

### 6.2.4. Neurodegenerative diseases and oxidative stress

Fossati's group demonstrated that classical CAIs such as acetazolamide **1** and methazolamide **10** show effectiveness in the prevention of mitochondrial dysfunction, caspase activation and cell death associated with amyloid  $\beta$  ( $A\beta$ ) formation in animal models of Alzheimer's disease (AD) (Fossati et al., 2016; Solesio et al., 2018). Acetazolamide was 10 times more effective than methazolamide in inhibiting such toxic mitochondrial mechanisms induced by  $A\beta$  in neuronal and vascular cells in an *in vitro* model of the disease showing also neuroprotective activity. The two compounds reduced memory impairment and  $A\beta$  pathology in a transgenic mouse model of amyloidosis, also ameliorating cerebrovascular health and glial fitness (Canepa et al., 2023; Fossati et al., 2016; Solesio et al., 2018), which led to the proposal of clinical trials to be scheduled for their evaluation in patients with AD (Canepa et al., 2023). Another approach recently proposed consisted in the conjugation of reversible MAO-B inhibitors of the coumarin/chromone types with sulfonamide CAIs, leading to hybrids which showed efficacy in preventing  $A\beta$ -related neurotoxicity, reverting

ROS formation, and restoring mitochondrial functionality in an SH-SY5Y cell model of AD (Giovannuzzi et al., 2024).

Recently, Murata et al. (2020) reported that zonisamide **17** is useful in the management of Parkinson's disease (PD), in combination with levodopa or other PD drugs (Murata et al., 2020; Tsuboi et al., 2021). Zonisamide significantly improved wearing off without exacerbating dyskinesia in patients with PD, presumably due to its inhibitory effects on MAO-B and brain CA isoforms, and several clinical trials nowadays investigate in detail these drug regimens for a neurodegeneration for which few pharmacological options are available in late stages of the disease (Tohge et al., 2021; Odawara et al., 2022).

More generally speaking, some CA isoforms among which hCA III and VII, were shown to be involved in oxidative stress, being associated with inflammatory diseases such as systemic lupus erythematosus, diabetes, hypertensive renal disease, myasthenia gravis, rheumatoid arthritis, all conditions in which the redox state of the cell is perturbed (Del Giudice, 2013). hCA III, although exhibiting a very low CO<sub>2</sub> hydratase activity, possesses a relevant phosphatase activity, is abundant in skeletal muscles, where free radical production is increased during physical exercise, and probably plays a role in shielding cells against oxidative damage and free radicals formation through reaction of an exposed cysteine residues (Cys186) with glutathione (Cabiscol and Levine, 1996). A similar role has been demonstrated for hCA VII, which undergoes S-glutathionylation at two residues, Cys183 and Cys217, without any loss of its high catalytic activity for CO<sub>2</sub> hydration to bicarbonate, being also proposed that the enzyme may function as an oxygen radical scavenger for protecting cells from oxidative stress (Monti et al., 2017; Truppo et al., 2012).

## **7. Antiinfective CAIs**

As discussed in the Introduction (see Fig. 3 and Table 4), CAs belonging to diverse genetic families are widespread in many organisms, including pathogenic bacteria, fungi, protozoans and invertebrates (nematodes, for example) (Capasso and Supuran, 2015; 2023; Supuran, 2021b). In the last decade, several proof-of-concept studies demonstrated the possibility to consider CAs present in such organisms as antiinfective drug targets, with relevant results being obtained mainly for antibacterials and antifungals, with some interesting data being also obtained on antiprotozoan effects of some CAIs.

### 7.1. Antibacterials

Bacteria encode for four CA classes, the  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\iota$ -CAs (Capasso and Supuran, 2015; Nocentini et al., 2021b), but presently only enzymes belonging to the first three classes have been investigated in detail and validated as antiinfective drug targets (Capasso and Supuran, 2024; Supuran, 2021b; 2024b). Table 4 shows some bacterial pathogenic species which produce a range of diseases in humans, some of which mild or relatively easy to be treated, whereas others (among which gonorrhoea, tuberculosis, but also the infections due to *Helicobacter pylori*, *Enterococcus* spp., *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*) characterized in the last decades by the emergence of drug resistant strains to most classes of available antibiotics. This leads to significant difficulties in treating the infected patients and a worldwide medical emergency with unpredictable consequences, considering that the phenomenon is in expansion (Salam et al., 2023). In species shown in Table 4 (as well as in many others which will be not discussed here) the various CAs have been biochemically characterized in detail in the last decade, and for some of them effective and selective inhibitors were detected. In few cases, the *in vitro* and *in vivo* validation that bacterial CAs may be alternative antiinfective targets to classical antibiotics, have also been reported (Abutaleb et al., 2022b; Flaherty et al., 2021).

Table 4 here

*N. gonorrhoeae* encodes for two CAs, an  $\alpha$ - and a  $\beta$ -CA, of which the first one (NgCA $\alpha$ ) was the most investigated enzyme. Acetazolamide (**1**), the clinically used sulfonamide CAI discussed extensively in the article was used as starting point in the design of compounds (sulfonamides **32**) targeting this bacterial enzyme– Table 5 (Hewitt et al., 2021; Marapaka et al., 2022).

Table 5 here

Some of the sulfonamides **32** showed low nanomolar activity against NgCA $\alpha$ , and MIC values for the inhibition of the bacterial growth of 0.25 – 4  $\mu\text{g/mL}$  (for the CDC 181 strain of *N. gonorrhoeae*, see Table 5, and for some drug resistant strains, data not shown but available in Hewitt et al., 2021). Furthermore, FDA-approved sulfonamides, such as **1** but also ethoxzolamide **11** (Fig. 6) showed effective *in vitro* inhibition of NgCA $\alpha$  and MIC values in the range of 0.015 – 4  $\mu\text{g/mL}$  against a range of various drug-resistant strains of *N. gonorrhoeae* (Abutaleb et al., 2022a) leading to the proposal of the repurposing of these sulfonamides as antibacterials for the management of this infection. Seleem's group thereafter also demonstrated the *in vivo* efficacy of **1**, using a mouse model of *N. gonorrhoeae* infection (Abutaleb et al., 2022b). Administration of **1** for three days reduced by 90% the gonococcal burden in vagina of infected mice when 50 mg/kg drug was used, and as **1** is a safe, inexpensive and well-known sulfonamide CAI drug, these data represent a strong basis for using such agents in the management of drug-resistant gonorrhea (Abutaleb et al., 2022b). It should be mentioned that the  $\beta$ -CA from this pathogen was less investigated for the moment (as a drug target), but it seems to be involved in the drug resistance of the pathogen to many classes of antibiotics (Rubin et al., 2023) and presumably detailed investigation will be performed soon on its inhibition.

*Helicobacter pylori* is a Gram-negative neutralophilic bacterium, considered to be the principal cause of gastric ulcer, gastritis and gastric cancer (Buzas and Birinyi, 2023). It is adapted to live in the highly acidic environment of the stomach, and it was observed already more than two decades ago to encode for three CAs belonging to the  $\alpha$ -,  $\beta$ - and  $\gamma$ -classes, but so far, only HpCA $\alpha$

and HpCA $\beta$  were cloned and investigated in detail (Campestre et al., 2021; Nishimori et al., 2006, 2007). Acetazolamide **1** has been used for at least two decades as an antiulcer drug by Puscas and his group starting with the '70s, with the rationale that inhibition of gastric mucosa CAs induces a reduction of gastric acid secretion (Puscas, 1984; Buzas and Supuran, 2016). However, decades later, it has been demonstrated that the antiulcer effects are due to the inhibition of the bacterial enzymes HpCA $\alpha/\beta$  (not of the human ones present in the gastric mucosa), which impairs the ability of the bacterium to survive in the acidic environment within the stomach (Marcus et al., 2005; Buzas and Supuran, 2016). HpCAs are involved together with the urease in the acclimation of *H. pylori* in the acidic environment in which it lives, whereas the inhibition of these enzymes disturbs the process, leading to the death and decolonization of the stomach from the pathogen (Marcus et al., 2005; Buzas and Supuran, 2016). Roujeinikova's group evaluated ethoxzolamide **11** on various *H. pylori* strains *ex vivo*, in cell cultures, in order to understand whether drug resistance to this sulfonamide may emerge after repeated passages (Modak et al., 2019; Rahman et al., 2020). For strains SS1 and 26695, it has been observed that resistance did not develop easily, and a quite low rate of spontaneous resistance acquisition of  $< 10^{-8}$  has been reported (Modak et al., 2019). Acquisition of resistance was associated with mutations in three genes in strain SS1, and six different genes in strain 26695, but the bacterial CA genes were not among them. In fact, the resistant isolates had mutations in genes involved in bacterial cell wall synthesis, gene expression control but remained susceptible to inhibition by ethoxzolamide (Modak et al., 2019; Rahman et al., 2020).

Acetazolamide **1** was again used as lead molecule for drug design studies of HpCA $\alpha/\beta$  inhibitors, also considering the fact that the first enzyme has been crystallized, alone or in complex with several inhibitors, including acetazolamide (Modak et al., 2015,2016) – Table 6. Compound **35** (and acetazolamide **1**) were the most effective inhibitors of both bacterial enzymes, ( $K_{iS} < 50$  nM) but it has been observed that only **35** was a more effective HpCA $\alpha$  than hCA II inhibitor (Modak et al.,



2016). The compounds were medium-high nanomolar bacterial CA inhibitors, apart deacetylated acetazolamide (compound **33**) which was the most ineffective inhibitor (micromolar inhibitor for the bacterial  $\alpha$ - and  $\beta$ -CAs). These differences in inhibition between these sulfonamides were rationalized by the report of their X-ray crystal structures in adduct with the bacterial (and human) enzymes (Modak et al., 2015,2016; Supuran, 2024b). The importance of these studies was that for the first time it has been demonstrated that it is possible to design bacterial CA-selective inhibitors (over the human CAs) and that some of these compounds do have anti-*H. pylori* effects, without leading easily to the development of drug resistance in the bacterial CA genes (Supuran, 2024b).

Table 6 here

Different species belonging to the genus *Enterococcus*, among which *E. faecium* and *E. faecalis*, gram-positive bacteria commonly found in the gastrointestinal tract (GIT) microbioma, cause disease in hospitalized/immunosuppressed patients, ranging from mild to serious, with few therapeutic options for their treatment, as the pathogens became highly multi-drug resistant to most antibiotics used clinically, including vancomycin (Geraldès et al., 2022; O'Toole et al., 2023). These bacteria are commonly referred to as vancomycin-resistant enterococci (VRE) and only two therapies for their treatment, i.e., linezolid, and a combination of quinupristin and dalfopristin (which leads to serious toxicity problems) are available to date (Geraldès et al., 2022; O'Toole et al., 2023).

CAs present in these bacteria ( $\alpha$ - and a  $\gamma$ -class enzymes) were recently proposed as alternative antimicrobial drug targets, with some remarkable results being reported by inhibiting them with sulfonamide CAIs (Kaur et al., 2020; An et al., 2022). *E. faecium* CAs (EfCA $\alpha$  and EfCA $\gamma$ ) have been used as model enzymes, being cloned, purified and characterized biochemically and for their inhibition profiles with sulfonamides and other CAIs (An et al., 2022). Acetazolamide **1** has been used again as investigational inhibitor, being observed that it possesses a rather effective MIC against *E. faecium* strain HM-965 (of 2  $\mu\text{g/mL}$ ) as well as against several multidrug-resistant strains

of the bacterium (Kaur et al., 2020; An et al., 2022) Analogs of **1** in which the acetamide moiety of the lead molecule has been modified to a variety of other amides (compounds **36a-h**), led to CAIs which showed both effective MIC values for the inhibition of *E. faecium* HM-965 strain, and also inhibited *in vitro* the two CAs from the pathogen (Table 7).

Table 7 here

The lowest MIC values against *E. faecium* strain HM-965 were observed for the *tert*-butylacetamido (**36g**), cyclohexylcarboxamido (**36a**), cyclohexylmethylcarboxamido (**36c**), cyclohexylethylcarboxamido (**36e**), and phenethylcarboxamido (**36d**) derivatives, for which MIC-s in the range of 0.007 – 0.25 µg/mL were determined. Acetazolamide **1**, the benzoyl derivative **36b** or the morpholinyl-methyl- derivative **36h** were also active, with MIC-s in the range of 1-2 µg/mL (Kaur et al., 2020; An et al., 2022). The possibility of using such bacterial CAIs for the gut decolonization of VRE in an animal model of the disease has recently been reported (Abutaleb et al., 2023). Acetazolamide **1** and three of its derivatives (**36e**, **36g** and **36h**) were used in such experiments, all of which had MIC-s in the range of 0.007 -0.060 µg/mL and were also effective inhibitors of the two CAs present in *E. faecium*. Mice infected with *E. faecium* strain HM-952 have been used in the experiments and their GIT bioburden was quantified in the fecal pellets after treatment for 8 days with 20 mg/kg CAIs. The treated animals showed significantly reduced *E. faecium* fecal bioburden compared to those treated with the vehicle or linezolid as control drug. After three days of treatment with CAIs, the *E. faecium* fecal bioburden was already reduced by 90 %, whereas the highest effect was observed after 5 days of treatment, with the bioburden being reduced by 92.6–99.3%, with **1** which was the most effective inhibitor among the four investigated compounds (Abutaleb et al., 2023). Of note, the effect of linezolid was of only 86.5 % reduction of the fecal bioburden (after 8 days of treatment), demonstrating thus that sulfonamide CAIs were by far more effective as decolonization agents against VRE as compared to the standard, clinically used drug (Abutaleb et al., 2023).

*Vibrio cholerae* is a Gram-negative bacillus possessing several serogroups, of which two, the O1 and O139, cause cholera. The disease is spread in poor countries, being characterized by a massive loss of water and electrolytes, which leads to death, if untreated. *V. cholerae* encodes for three CAs, belonging to the  $\alpha$ -,  $\beta$ - and  $\gamma$ -classes, denominated VchCA $\alpha$ , VchCA $\beta$  and VchCA $\gamma$ , which were cloned and characterized biochemically over the last decade, with many effective *in vitro* inhibitors being discovered (Del Prete et al., 2012; 2016a,b; Fantacuzzi et al., 2023). Sulfonamide CAIs structurally related to SLC-0111 (compound **19**, Fig. 9), which efficiently inhibited the three *V. cholerae* CAs *in vitro*, were recently investigated *in vivo* for their antibacterial effects on different clinical isolates of the bacterium, i.e., SI-Vc22, SI-Vc71, and SI-Vc912, but their MICs were > 64  $\mu\text{g/mL}$  (although inhibition of growth of the bacterium has been observed, without the possibility to determine the precise MIC values) (Fantacuzzi et al., 2023). Thus, more detailed studies of the three CAs present in this pathogen and better inhibitors are needed in order to validate these enzymes as antibacterial drug targets.

*M. tuberculosis*, the pathogen provoking tuberculosis, encodes for three  $\beta$ -CAs which have been well characterized [99-103], and also a  $\gamma$ -class enzyme, which has not yet been cloned and characterized (Minakuchi et al., 2009; Nishimori et al., 2009; Carta et al., 2009). The three  $\beta$ -CAs, sometimes called with names derived from the genes encoding them, as Rv1284, Rv3588c and Rv3273, but also as mtCA1, mtCA2 and mtCA3, have been investigated in detail for their catalytic activity and inhibition with sulfonamides and other classes of compounds, such as phenols, carboxylic acids, natural products, dithiocarbamates, with many effective, low nanomolar *in vitro* inhibitors detected (Aspatwar et al., 2019). However, many such compounds were ineffective *in vivo/ex vivo*, especially the sulfonamides, presumably due to their low penetrability through the complex membranes of the bacterium, whereas the dithiocarbamates, such as derivatives **37** and **38** (Fig. 11) showed significant anti-mycobacterial activity *in vivo*, in zebra fish larvae infected with *M. marinum* (Aspatwar et al., 2017, 2018).

Fig. 11 here

Apart the enzymes discussed above, many other CAs have been described in other pathogenic bacterial species, such as *Brucella suis*, *Salmonella enterica* serovar *Typhimurium*, *Legionella pneumophila*, *Porphyromonas gingivalis*, *Clostridium perfringens*, *Streptococcus mutans*, *Burkholderia pseudomallei*, *Francisella tularensis*, *Escherichia coli*, *Mammaliicoccus (Staphylococcus) sciuri*, *Acinetobacter baumannii*, etc. (Capasso and Supuran, 2015, 2024; De Luca et al., 2024b). For many of them, detailed catalytic activity and inhibition data with various classes of compounds are available, but *in vivo/ex vivo* validation studies are missing. Future studies and the growing problem of resistance to clinically used drugs might contribute to considering some of them as potential antiinfective drug targets.

## 7.2. Antifungals

A number of pathogenic fungi/yeasts, such as *Cryptococcus neoformans*, *Candida albicans*, *C. glabrata*, *Malassezia globosa*, *M. restricta*, *M. pachydermatis*, *M. furfur*, *Saccharomyces cerevisiae*, etc., encode for  $\beta$ -CAs (one of the most relevant such gene being denominated Nce103, Aguilera et al., 2005) which are involved in CO<sub>2</sub>-sensing mechanisms together with an adenylate cyclase enzyme (Klengel et al., 2005; Martin et al., 2017; Mogensen et al., 2006; Supuran and Capasso, 2021). This bi-component enzyme system is crucial for fungal growth, differentiation, but also determines the expression of phenotypic features essential for virulence of these pathogens (Klengel et al., 2005; Innocenti et al., 2009; Isik et al., 2010; Martin et al., 2017; Supuran and Capasso, 2021; Schlicker et al., 2009). Some non-pathogenic fungi, such as *Sordaria macrospora* also encode for  $\beta$ -CAs which have also been investigated (Lehneck et al., 2014; Vullo et al., 2020). In fact, the genomes of basidiomycetous and hemiascomycetous yeasts encode only for one  $\beta$ -CA, as mentioned above, whereas filamentous ascomycetes contain multiple  $\beta$ -CA genes (*S.*

*macrospora* has three such enzymes, Vullo et al., 2020; Lehneck et al., 2014). Furthermore, in some ascomycetes, genes encoding for  $\alpha$ -CAs were also reported and such enzymes characterized (Cuesta-Seijo et al., 2011). However, at the moment only fungal  $\beta$ -CAs have been investigated in detail as potential antifungal drug targets (Supuran and Capasso, 2021; Angeli et al., 2022c). These enzymes have been investigated for their inhibition with many classes of CAIs, such as sulfonamides, dithiocarbamates, carboxylates, boronic acids, etc., and many low nanomolar *in vitro* inhibitors were thus detected for many enzymes from various pathogens (*C. albicans*, *C. glabrata*, *Malassezia* spp., etc.) (Supuran and Capasso, 2021; Angeli et al., 2022c). The most investigated fungal CAs were however those from *Malassezia* spp., as potential anti-dandruff agents (Hewitson et al., 2012; Supuran and Capasso, 2021; Angeli et al., 2022b). Simple aromatic sulfonamides such as **39** and **40** (Fig. 12) acted as low nanomolar *in vitro* inhibitor for the CA from *M. globosa* (MgCA) and also showed MIC values in the medium potency range, of 10 – 120  $\mu\text{g/mL}$  for inhibiting the growth of various *Malassezia* spp. (Hewitson et al., 2012). In a mouse model of *M. pachydermatis* skin infection, compound **40** showed an efficacy similar to that of the azole antifungal drug ketoconazole, leading to hypha fragmentation, indicative of an effect impairing the fungal growth of the CAI (Hewitson et al., 2012).

Fig. 12 here

More recently, a large series of selenoureas of which **41a,b** (Fig. 12) are two representatives, showed much better *in vitro* fungal CA inhibition and MIC values (in the range of 0.25 – 32  $\mu\text{g/mL}$ ) against various strains of *M. pachydermatis*, *C. albicans* and *C. glabrata* (Angeli et al., 2022c). These seleno-containing sulfonamides showed appreciable antifungal activity, comparable to the standard-of-care antifungal drugs for which drug resistance emerged, which was suppressed when the selenium was replaced with its cognate isosteric elements sulfur or oxygen. Furthermore, some of these compounds showed excellent selectivity against *M. pachydermatis* over its related genera

*M. furfur* and *M. globosa* (Angeli et al., 2022c), making them of interest for the development of novel antifungals.

### 7.3. Antiprotozoal agents

Protozoans encode for CAs belonging to several genetic families, with  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\eta$ - classes enzymes described so far in several pathogenic species (Supuran 2023e; Capasso and Supuran, 2023; Caroli et al., 2023; Mansoldo et al., 2020; Vermelho et al., 2020; D'Ambrosio et al., 2018; De Simone et al., 2015; Vermelho et al., 2017). CAs were characterized in the following protozoans, producing diseases with various degrees of danger, from mild to very serious: *Trichomonas vaginalis* (Urbański et al., 2020,2021,2022); *Plasmodium falciparum*, the most difficult to treat malaria-provoking protozoan (Krungkrai et al., 2005; De Simone et al., 2015; Fisher et al., 2017; D'Agostino et al., 2023); *Leishmania donovani* the parasite provoking visceral leishmaniasis (Syrjänen et al., 2013; da Silva Cardoso et al., 2017), *Trypanosoma cruzi*, the etiological agents of Chagas disease, for which few therapeutic options are available (Pan et al., 2013; Vermelho et al., 2018; 2020; Mansoldo et al., 2020) and *Entamoeba histolytica* (Bua et al., 2018; Haapanen et al., 2018). As for bacterial and fungal CAs, also in the case of the protozoan enzymes mentioned above, a large number of effective *in vitro* inhibitors were detected, but it has been more challenging to evidence antiprotozoal action *in vivo* (Supuran, 2023e). The reasons are multiple, and are mainly due to the fact that most protozoans have complicated life cycles, with many different stages and also more than one host, with the human one being generally just one of the links in their cycle. For example *Plasmodia* have at least 6 different stages/phases during their life cycle, with the various forms of the pathogen present in different organs and tissues, but also with many different genes which are expressed/silenced in the different phases, and thus, with a substantial capability to evade the host immune defences (Supuran, 2023e). However, some successful studies have recently emerged for protozoan CAs as potential anti-infective drug targets. Sulfonamide CAI

nanoformulations for the management of *Leishmania* and *T. cruzi* infections have been obtained in clove (*Eugenia caryophyllus*) oil, which showed low micromolar efficacy in inhibiting the growth of *Leishmania infantum* MHOM/BR/1974/PP75 and *Leishmania amazonensis* IFLA/BR/1967/PH8 strains (da Silva Cardoso et al., 2017) and *T. cruzi* strains Y and DM28c (Vermelho et al., 2018). Both benzenesulfonamide and 1,3,4-thiadiazole-2-sulfonamides obtained by the tail approach showed such promising activity (da Silva Cardoso et al., 2017; Vermelho et al., 2018), although they were ineffective in normal formulation not envisaging the use of nanoemulsions (Vermelho et al., 2018). Many other pathogenic protozoans, among which *Cryptosporidium* spp., *Giardia lamblia*, *Entamoeba* spp. or *Acanthamoeba castellanii* encode for CAs (Supuran, 2023e) and they started to be investigated very recently, with some promising results being reported for example for the ocular infections caused by the last pathogen, *A. castellanii* (Haapanen et al., 2024). Although this amoeba encodes for at least eight CAs belonging to the  $\alpha$ -,  $\beta$ - and  $\gamma$ -classes, all attempts to clone them were not successful for the moment (Haapanen et al., 2024). However, a drug screening assay recently developed, evidenced that clinically used CAIs such as acetazolamide **1**, ethoxzolamide **11**, and dorzolamide **13** showed promising antiamoebic effects *in vivo* (Haapanen et al., 2024). *Toxoplasma gondii* also encodes for an  $\alpha$ -CA which was recently cloned and characterized, being observed that the enzyme was effectively inhibited by acetazolamide, sulfamide and sulfamic acid (De Luca et al., 2024a). Thus, the more detailed investigation of protozoan CAs may lead to innovative approaches for the management of these difficult to treat diseases.

#### 7.4. Antinematode agents

CAs are probably present in all nematodes, but they have been investigated in some detail only in few species, among which the model organism *Caenorhabditis elegans*, which encodes both for  $\alpha$ - (Hall et al., 2008) and  $\beta$ -CAs (Fasseas et al., 2010) and in several parasitic organisms, among which

*Ascaris lumbricoides* (Zolfaghari Emameh et al., 2015,2016), *Schistosoma mansoni* (Da'dara et al., 2019; Haapanen et al., 2023) and *Gyrodactylus salaris* (Aspatwar et al., 2022a, 2023). The role of these enzymes in worms are poorly understood, but in *C. elegans* it has been demonstrated that they are involved in pH regulation and adaptation to the environment/niches in which these organisms live. Indeed, unlike many other organisms, this nematode is able to maintain > 90% survival in pH conditions ranging from 3 to 10 (Hall et al., 2008). A transcriptional analysis identified genes differentially regulated by pH, and among them a gene encoding for two (splicing variants)  $\alpha$ -CAs (*cah-4*), was observed to be five-fold upregulated in alkaline environment. CAH-4b was also shown to be a highly active enzyme for the CO<sub>2</sub> hydration reaction, being effectively inhibited by sulfonamide CAIs (Hall et al., 2008; Crocetti et al., 2009; Güzel et al., 2009). Such data constituted the proof-of-concept that nematode CAs might be considered as drug targets. Indeed, in the intravascular parasitic worm *Schistosoma mansoni* which causes schistosomiasis, a disease of great global public health significance, two CAs were identified, belonging to the  $\alpha$ - and  $\beta$ -classes (Da'dara et al., 2019; Haapanen et al., 2023). The first enzyme has been investigated in detail, with several effective *in vitro* inhibitors discovered (sulfonamides, boronic and arsonic acids) which showed a modest but significant inhibition of growth of the pathogen (Angeli et al., 2021,2022b; Ferraroni et al., 2022a). The salmon plathelminth parasite *Gyrodactylus salaris* encodes for a  $\beta$ -CA which has been cloned and characterized in detail recently (Aspatwar et al., 2022a, 2023). Several effective *in vitro* inhibitors of this enzyme were also detected, belonging to the sulfonamide class, but no *in vivo* studies were performed to date for validating this protein as a potential antiinfective drug target (Aspatwar et al., 2022a, 2023). The challenges to obtain a potent antiinfective effect with such CAIs is mainly due to the highly polar nature of these molecules and their difficulty to cross the rather thick tegument (and the membranes it contains) of nematodes.

## 8. Polypharmacology of CAIs



Some drugs which have been discovered for treating conditions normally not associated with CA inhibition, were subsequently shown to possess potent inhibitory action against some isoforms of this enzyme, and are thus considered to possess a polypharmacology in which CA may play a relevant role which explains their activity and in some cases, also their side effects. The examples which will be discussed here are the antiepileptics topiramate **16** and zonisamide **17** (Fig. 7), the antipsychotic acting as dopamine D<sub>2</sub> receptors antagonists sulpiride **42** and verapride **43**, the NSAIDs acting as COX-2 selective inhibitors celecoxib **44** and polmacoxib **45**, the pan-kinase inhibitor pazopanib **46** and the histamine H<sub>2</sub> receptor antagonist famotidine **47** (Fig. 13). As it will be discussed shortly, all these clinically used drugs were shown to possess relevant CA inhibitory activity against most human CA isoforms, and in all cases except one (pazopanib), X-ray crystal structures of their adducts with some hCAs were also reported (see discussion in the next paragraphs).

Fig. 13 here

### 8.1. Topiramate and zonisamide

Topiramate **16**, an antiepileptic drug belonging to the sugar sulfamates class was discovered by Maryanoff's group in the 80s by using the maximal electroshock seizure (MES) test, an empirical approach widely employed for testing anticonvulsants (Maryanoff et al., 1987), Topiramate is an orally active agent, useful for the management of various forms of epilepsy, refractory to other medication, has a high bioavailability, rapid absorption and long duration of action (Rosenfeld, 1997). The drug is not structurally related to any other antiepileptic used clinically, such as the phenytoins, carbamazepines, barbiturates, benzodiazepines or GABA-scaffold derived antiepileptics. It has some similarity to zonisamide **17** (Fig. 7), as both drugs contain the sulfamate/sulfonamide ZBG known to coordinate to the Zn(II) ion of CAs (Alterio et al., 2012). The mechanism of action of this drug is not fully understood yet (Supuran, 2008; De

Simone et al., 2009) and seems to be rather complex, including apart CA inhibition, enhancement of GABA-ergic transmission (Rosenfeld, 1997; Herrero et al., 2002), antagonism of kainate/AMPA receptors (Gibbs et al., 2000; Zullino et al., 2003), inhibition of action potentials creation in neurons via antagonizing the activation of Na<sup>+</sup> channels (Perucca, 1997; Taverna et al., 1999). Initially topiramate was reported to be a very weak (millimolar) CAI (Maryanoff et al., 1987) but later it has been ascertained by several techniques that topiramate is a potent CAI against most CA isoforms present in the SNC (Casini et al., 2003; Supuran, 2008), and its crystal structure in complex with several isoforms has also been reported (Casini et al., 2003; Alterio et al., 2010). These findings match with many clinically observed side effects of topiramate, which are in agreement with the typical pharmacological profiles of strong sulfonamide CAIs used as systemic antiglaucoma agents/diuretics and include paresthesias, nephrolithiasis, metabolic acidosis, weight loss, carbonation dysgeusia (bitter taste perception of carbonated drinks, due to salivary CA VI inhibition), etc. (Charbonneau et al., 2020; Chahin et al., 2020; Naps et al., 2023). Topiramate inhibits eight CA isoforms with inhibition constants < 65 nM, it inhibits hCA I with a K<sub>I</sub> of 250 nM whereas only three isozymes are less inhibited (hCA III, IV and XIV, with K<sub>I</sub>s in the range of 1460 nM – 0.78 mM) (Supuran, 2008) - see Table 8. Many CA isozymes involved in crucial physiologic functions, such as hCA II, hCA VA, hCA VB, hCA VI, hCA VII, hCA IX, hCA XII and hCA XIII are highly inhibited by this drug, sometimes with K<sub>I</sub>s in the subnanomolar range (e.g., hCA VII, K<sub>I</sub> of 0.9 nM, Supuran, 2008). Apart its use as an antiepileptic, topiramate has been repurposed as an antioesity agent (Supuran, 2022) as discussed earlier in this review, but also as an anti-migraine drug (Pearl et al., 2023).

Zonisamide **17** (Fig. 7), is another widely used antiepileptic drug (Leppik, 2004). Zonisamide, as discussed above, is an efficient sulfonamide CAI, but also blocks repetitive firing of voltage-sensitive sodium channels and reduces voltage-sensitive T-type calcium currents without affecting L-type calcium currents (Perucca, 1997; Leppik, 2004). This rather complex mechanism of action probably explains its efficacy in patients resistant to other antiepileptic drugs, and its

pharmacokinetic profile is also favorable, since the drug is rapidly and completely absorbed, has a long half-life (63-69h), which allows twice- or once-daily dosing (Perucca, 1997; Leppik, 2004). Zonisamide has been investigated for the inhibition of CA already by its discoverers (Masuda and Karasawa, 1993), being concluded that although it binds significantly to erythrocytes (where two CA isozymes, hCA I and II are highly abundant – Maren, 1967) its CA inhibitory properties are rather weak, and thus, this phenomenon does not play any role in the anticonvulsant activity of the drug (Masuda et al., 1994). However subsequent studies showed zonisamide to be a potent CAI for many CNS-present CA isoforms such as hCA I, II, VA, VI, VII and IX (which are inhibited with  $K_{iS} < 120$  nM) (De Simone et al., 2005; Supuran, 2008). The X-ray crystal structure of zonisamide bound to hCA II has also been reported, which explained its potent inhibition of this and other CA isoforms (De Simone et al., 2005). Apart its use as an antiepileptic, as for topiramate, it has been proposed a repurposing of zonisamide as an antiobesity agent (Gadde et al., 2003; Supuran, 2022) and for the management of Parkinson's disease (Murata et al., 2020), a conditions in which CAs seem definitely to be involved (Supuran, 2008; 2023a).

## 8.2. Sulpiride and veralipride

Sulpiride **42** and veralipride **43** (Fig. 13) are atypical antipsychotics acting as dopamine  $D_2$  receptors antagonist and belong to the benzamide class of such drugs (Furtado and Srihari, 2008; Masdrakis and Baldwin, 2021). They are widely employed for the management of schizophrenia, depression but also as anticonvulsants (Furtado and Srihari, 2008; Masdrakis and Baldwin, 2021). Both compounds possess a primary sulfonamide moiety, which is known to be involved in CA inhibition as one of the most effective ZBGs described to date (Alterio et al., 2012; Supuran, 2008). Thus, both drugs have been tested as potential inhibitors of all hCAs and their X-ray crystal structures in adducts with the major isoforms, hCA I and II, were also reported (Abbate et al., 2004; Angeli et al., 2024). Sulpiride **42** is a highly effective inhibitor of hCA II, VB, VI, IX, XII

and XIV ( $K_{IS}$  of 0.8 – 110 nM), being less potent against hCA I, III, IV and VII, with  $K_{IS}$  in the micromolar range (Abbate et al., 2004; Supuran, 2008). Veralipride **43** also showed effective inhibitory activity against hCA VB, VI, IX, XII and XIII ( $K_{IS}$  ranging between 24 – 92 nM) being less effective against the remaining isoforms ( $K_{IS}$  of 0.15 – 2.7  $\mu$ M against hCA I, II, IV, VA, VII and XIV, and > 10  $\mu$ M against hCA III, Angeli et al., 2024). It is not known at the moment whether the CA inhibitory effects of these drugs may have a relevance for their clinical efficacy in schizophrenia. In fact, older studies showed acetazolamide to possess some effectiveness for the management of this diseases (Inoue et al., 1984) whereas a more recent study proposed the repurposing of CAIs, including **1** (as well as topiramate), for the management of schizophrenia based on interactome analysis (Karunakaran et al., 2019). Thus, we hypothesize that CAIs may have a future for the management of this condition too.

### 8.3. Celecoxib and polmacoxib

COXs catalyze a committed step in the conversion of arachidonic acid to prostaglandins (PGs) and thromboxane, with at least three distinct isozymes (COX-1 - COX-3) known to date (Romanelli et al., 2024). Isoforms COX-1 and COX-2 have been considered to be responsible for different effects, beneficial or not, of PGs in various tissues (Fitzgerald, 2003; Romanelli et al., 2024). The development of COX-2 specific inhibitors, collectively called coxibs in the late '90s, was initially considered as a breakthrough in anti-inflammatory therapy due to the fact that this inducible isoform was considered to play a major role in inflammation (Fitzgerald, 2003), but the withdrawal of some of these drugs in 2004/2005 due to severe adverse side effects completely redimensioned the field (Dognè et al., 2005). In fact, the development of the coxibs, was based on the erroneous hypothesis that only COX-2 mediates inflammation in several organs via the biosynthesis of prostaglandins  $E_2$  and  $I_2$  and that COX-1 was the source of prostaglandins in the gastric epithelium, acting as cytoprotective mediators (Dognè et al., 2005). The severe cardiac side

effects of some of the coxibs (such as rofecoxib) demonstrated that this was not the real situation (Epstein, 2001), as both COX-1 and COX-2 participates to the inflammatory PG biosynthesis, and thus, the only two drugs of the class still in clinical use are those which were less COX-2 selective, i.e., celecoxib **44** and polmacoxib **45** (Dogné et al., 2008; Kim et al., 2016; Supuran, 2020a). However, celecoxib **44** and polmacoxib **45** contain a primary sulfamoyl moiety which is known to interact with the zinc ion from CAs (Alterio et al., 2012). Celecoxib has been investigated as CAI against all human enzymes in 2004, and its X-ray crystal structure in adduct with hCA II was also reported (Weber et al., 2004). Isoforms hCA II, VB, IX and XII were inhibited with  $K_{Is} < 100$  nM by celecoxib, with CA IX and XII showing the most effective inhibition, in the low nanomolar range ( $K_{Is} < 40$  nM) (Weber et al., 2004). Based on the potent inhibitory activity of celecoxib **44** on the tumor-associated isoforms hCA IX/XII, there are various studies suggesting its repurposing as an antitumor agent (Supuran et al., 2004; Alian et al., 2024; Berckmans et al., 2023; Xu et al., 2023). Polmacoxib (also known as GCG100649) was reported to show high affinity for hCA I and II (Hirankarn et al., 2013). The drug, considered a dual inhibitor of COX-2 and hCA I/II, was approved in 2022 for the treatment of osteoarthritis in South Korea (Cho et al., 2022).

#### 8.4. Pazopanib

Pazopanib **46** (Fig. 13) is an antitumor drug belonging to the class of tyrosine kinase inhibitors, approved for clinical use for the treatment of solid tumors in 2010 (Schutz et al., 2011; Pick and Nystrom, 2012). It is a pan-kinase inhibitor, binding to several different proteins involved in angiogenesis, tumor growth and cell survival, such as the vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), c-Kit, and it is mainly used for the management of renal cell cancer, breast cancer, soft tissue sarcoma, thyroid cancer, hepatocellular cancer and cervical cancer (Jones et al., 2022; Schutz et al., 2011; Peerzada et al., 2021; Pick and Nystrom, 2012, Wang et al., 2024). As many drugs discussed in this section, also

pazopanib incorporates the primary sulfonamide moiety associated with effective CA inhibitory action. Thus, the drug was investigated for its interaction with all catalytically active hCAs (Winum et al., 2012). Pazopanib was observed to be a highly effective CAI of all hCAs except for hCA III, which is anyhow inhibited in the low micromolar range ( $K_I$  of 4.5  $\mu\text{M}$ ). The remaining isoforms were inhibited with  $K_{IS}$  ranging between 0.88 nM to 78 nM, and the most pazopanib-avid isoforms were hCA VA, VB, IX and XII ( $K_{IS}$  of 0.88-9.1 nM) (Winum et al., 2012). Thus, it has been hypothesized that the effective antitumor effects of the compounds are due to both its kinase and CAs inhibitory activities (Winum et al., 2012; Supuran, 2021a)

### 8.5. Famotidine

Famotidine **47** (Fig. 13) is a  $H_2$  receptor antagonists of third generation, used for the treatment of gastric and duodenal ulcers, gastroesophageal reflux, pathological acid hypersecretory conditions (e.g., Zollinger-Ellison syndrome) and NSAIDs-induced gastric injury (Campoli-Richards et al., 1986; Sakurai et al., 2012; Zhang et al., 2021). Like other drugs discussed in this section, famotidine incorporates a putative ZBG present in CAIs, not of the sulfonamide but of the sulfamide type. Thus, the drug has been assayed for the inhibition of human and bacterial CAs, including those found in the gastric pathogen *H. pylori* (Angeli et al., 2018). Famotidine was observed to be an effective inhibitor of hCA II, VI, VII, IX, XII and XIII, with  $K_{IS}$  in the range of 3.0 – 171 nM, whereas it was a submicromolar or micromolar inhibitor of hCA I, IV, VA, VB and XIV ( $K_{IS}$  of 0.67 – 5.3  $\mu\text{M}$ ), with only hCA III being poorly inhibited ( $K_I > 10 \mu\text{M}$ ) (Angeli et al., 2018). The drug was highly effective as an inhibitor of the two CAs from *H. pylori* with  $K_{IS}$  of 21-50 nM (Angeli et al., 2018). The X-ray crystal structures of famotidine bound to hCA I and II were also reported, explaining at molecular level the binding efficacy of the drug to these targets (Angeli et al., 2018). As discussed in this article, bacterial CA inhibition may constitute a novel antibacterial mechanism, and considering that other CAIs were demonstrated to inhibit the growth of *H. pylori*

(Modak et al., 2019) it has been proposed a polypyarmacological effect also for explaining the action of this drug, which probably exerts its antiulcer action both by inhibiting hydrochloric acid secretion through the H<sub>2</sub> receptors blockade, as well as the growth of *H. pylori* by interfering with mechanisms of acid acclimation of the bacterium within the stomach (Angeli et al., 2018).

## 9. Conclusions and future prospects

CAIs are pharmacological agents with a multitude of clinical applications, some of them known for decades (as diuretics, antiglaucoma, anti-acute mountain sickness or antiepileptic drugs – Maren, 1967), others, more recent, such as for the management of obesity or hypoxic tumors (Supuran, 2021b;2022). Even newer pharmacological applications have recently become feasible, such as for example in the management of neuropathic pain, cerebral ischemia, inflammation, and neurodegenerative conditions, e.g., Alzheimer's and Parkinson's disease (Supuran, 2021b). The presence and detailed biochemical/pharmacological investigations of CAs in pathogens (bacteria, fungi, protozoans, nematodes) opened the possibility to design antiinfectives with a new mechanism of action, considering the crucial roles these enzyme play in such organisms (Supuran, 2023a,b,c). Relevant progress has been reached indeed in the last few years in the validation of some of these enzymes as antibacterials, mainly for drug resistant pathogens such as *Neisseria gonorrhoea* or vancomycin resistant enterococci (Flaherty et al., 2021; Abutaleb et al., 2022a,b). However, only  $\alpha$ - and few  $\beta$ -/ $\gamma$ - bacterial CAs have been investigated in detail for obtaining antibacterials, whereas the number of pathogenic bacteria encoding such enzymes is high, and presumably the future will see the emergence of effective inhibitors for some of them. The  $\iota$ -CAs are also present in many pathogenic bacteria and were scarcely investigated for the moment (Nocentini et al., 2021b). Furthermore, the bacterial CAs present in microbiota organisms, as well as the differences between healthy/diseased microbiotas (and their enzymes), started to be investigated only recently (Amedei et al., 2021), but the field may lead to relevant discoveries for the pharmacology of inhibitors of

these enzymes and more generally for the management of antibiotics, which may damage the “good” bacteria from the microbiota, with deleterious effects for the patient (Amedei et al., 2021).

Although viruses do not encode for CAs, there may be interesting connections between some of them, such as for example SARS CoV-2, the causative agent of COVID-19, and CAs. Indeed, it has been reported already in 2021 that blood CA activity was 2-3 times higher than normal in patients with acute COVID-19 as well as in the first weeks after infection, leading to the proposal that CAIs such as acetazolamide **1** may be useful in the management of these patients (Deniz et al., 2021). These results were validated in another study, which reported CA I as a candidate biomarker in platelets of COVID-19 patients, with a significant increase of the level of this isoform in such patients (Wolny et al., 2023) or in those who were dead of COVID-19 infection (Eltobgy et al., 2024), although the mechanisms responsible for this increased CA I expression are not known at the moment. Kugler et al (2024) reported on the other hand that CA XIV levels were decreased in patients with worsening SARS CoV-2 infection compared to those whose infection improved or compared to non-infected subjects. Muñoz-Prieto et al. (2022) observed downregulation of CA VI in saliva of patients with COVID-19, which may explain the abnormalities in taste perception observed for some infected subjects with SARS CoV-2.

Many advances in the CAI field were possible because highly isoform-selective inhibitors started to be available in the last two decades, most of which emerged after the tail-approach has been discovered (Scozzafava et al., 1999b) for generating large libraries of CAIs but also through the discovery of various new chemotypes possessing efficient inhibitory action against these enzymes (Supuran, 2016b). In fact, apart sulfonamides and their isosteres, known since the ‘40s and dominating the field till the 2000s, which are still pharmacologically relevant and clinically used CAIs, nowadays more than 40 different chemotypes with effective CA inhibitory patterns were disclosed, many of which amenable to drug development programs.

Table 8 here



Table 8 shows the CA inhibition data of 9 clinically used drugs and one compound in clinical trials against the twelve catalytically active human isoforms, hCA I – XIV (Table 8) (Supuran, 2008). It may be observed that the first and second generation drugs (from acetazolamide **1** to zonisamide **17**) potently inhibit almost all isoforms, except hCA III, which is less prone to inhibition by sulfonamides/sulfamates. For example acetazolamide **1** has  $K_{iS}$  ranging from 2.5 to 74 nM for 10 of the 12 considered isoforms, which explain the many side effects of the drug, that have been discussed here. The other drugs presented in the table show more or less the same range of promiscuous inhibition as acetazolamide (Table 8). On the other hand, the third generation compound **19** (SLC-0111) which has been designed as a selective agent for targeting CA IX and XII by the tail approach, is a potent inhibitor of these two isoforms ( $K_{iS}$  of 4.5 – 45 nM) whereas it acts as a poor inhibitor of the remaining 10 CAs. Most probably, the future drugs belonging to this class of pharmacological agents will be designed by using this type of strategy, for potently inhibiting only the isoforms of interest and not the offtarget ones, e.g., CA I and II, which are abundant in many organs and their inhibition is responsible for many of the side effects of the first and second generation such drugs (Supuran, 2021a,b).

Another much investigated drug design strategy in the last decade was that of obtaining hybrid drugs incorporating CAI and other pharmacological agents, by the so-called multitargeting approach (Supuran, 2021c). Indeed, sulfonamides, coumarins or sulfocoumarins were hybridized with NO donors, CO donors, prostaglandin analogs,  $\beta$ -adrenergic blockers, non-steroidal anti-inflammatory drugs, and a variety of anticancer agents (cytotoxic drugs, kinase/telomerase inhibitors, P-gp and thioredoxin inhibitors, etc.) leading to compounds with an interesting pharmacology and biological activity, most of the times better than that of the parent drugs or their combinations. These derivatives were not dealt with in this review, as the field is very ample and was reviewed recently (Supuran, 2021c), but this strategy may lead to relevant progress, mainly for

developing more effective anticancer drugs and antiinfectives (De Simone and Supuran, 2024; Capasso and Supuran, 2024).

Considering the many topics treated here, a hyperkink to CAs in the the International Union of Basic and Clinical Pharmacology (IUPHAR) /British Pharmacological Society (BPS) database is provided here <https://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=842>, which may be helpful for those interested to read more on this argument.

CAs have as substrate CO<sub>2</sub>, a hot house gas which by accumulating in the atmosphere may induce irreversible climate change in a planet of > 8 billion inhabitants, and there are recent attempts for using these enzymes for carbon capture (Migliardini et al., 2014). Plant/algal CAs are involved in photosynthesis and there are attempts of crop engineering consisting in inserting CA genes in cultivars such as rice, corn and other cereals, for boosting agricultural yields (Findinier and Grossman, 2023; Supuran, 2023b). Although these aspects are not directly connected with the CA pharmacological applications discussed in this review, the detailed knowledge acquired by studying these enzymes as drug targets and the design of their inhibitors with clinical applications led to relevant discoveries that may relieve (or resolve, if one is optimistic) some of the huge problems which humankind faces in the 21 century: climate change, insufficient resources, pandemics. Whether this will be feasible depends not only of our scientific achievements for understanding in more details these enzymes and their inhibitors, but also of the wisdom of our globalized society, which hopefully will be good enough for the purpose.

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## References

Abbate F, Coetzee A, Casini A, Ciattini S, Scozzafava A and Supuran CT (2004) Carbonic anhydrase inhibitors: X-ray crystallographic structure of the adduct of human isozyme II with the antipsychotic drug sulphiride. *Bioorg Med Chem Lett* 14:337-41.

Abutaleb NS, Elhassanny AEM, Nocentini A, Hewitt CS, Elkashif A, Cooper BR, Supuran CT, Seleem MN, and Flaherty DP (2022a) Repurposing FDA-approved sulphonamide carbonic anhydrase inhibitors for treatment of *Neisseria gonorrhoeae*. *J Enzyme Inhib Med Chem* 37:51-61.

Abutaleb NS, Elhassanny AEM, Seleem MN (2022b) In vivo efficacy of acetazolamide in a mouse model of *Neisseria gonorrhoeae* infection. *Microb Pathog* 164:105454.

Abutaleb NS, Shrinidhi A, Bandara AB, Seleem MN, and Flaherty DP (2023) Evaluation of 1,3,4-Thiadiazole Carbonic Anhydrase Inhibitors for Gut Decolonization of Vancomycin-Resistant Enterococci. *ACS Med Chem Lett* 14:487-492.

Aggarwal M, Kondeti B, and McKenna R (2013) Anticonvulsant/antiepileptic carbonic anhydrase inhibitors: a patent review. *Expert Opin Ther Pat* 23:717-24.

Aguilera J, Van Dijken JP, De Winde JH, and Pronk JT (2005) Carbonic anhydrase (Nce103p): an essential biosynthetic enzyme for growth of *Saccharomyces cerevisiae* at atmospheric carbon dioxide pressure. *Biochem J* 391:311-6.

Ahlskog JK, Dumelin CE, Trüssel S, Mårlind J, and Neri D (2009) In vivo targeting of tumor-associated carbonic anhydrases using acetazolamide derivatives. *Bioorg Med Chem Lett* 19:4851-6.

Ainslie PN, Lucas SJ, and Burgess KR (2013) Breathing and sleep at high altitude. *Respir Physiol Neurobiol* 188: 233-256.

Akgul O, Di Cesare Mannelli L, Vullo D, Angeli A, Ghelardini C, Bartolucci G, Alfawaz Altamimi AS, Scozzafava A, Supuran CT, and Carta F. (2018) Discovery of novel nonsteroidal anti-inflammatory drugs and carbonic anhydrase inhibitors hybrids (NSAIDs-CAIs) for the management of rheumatoid arthritis. *J Med Chem* 61: 4961-4977.

Alber BE and Ferry JG (1994) A carbonic anhydrase from the archaeon *Methanosarcina thermophila*. *Proc Natl Acad Sci U S A* 91:6909-13.

Allred JB and Reilly KE (1997) Short-term regulation of acetyl CoA carboxylase in tissues of higher animals. *Prog Lipid Res* 35: 371-85.

Alian DME, Helmy MW, Haroun M, and Moussa N (2024) Modulation of autophagy and apoptosis can contribute to the anticancer effect of Abemaciclib/Celecoxib combination in colon cancer cells. *Med Oncol* 41:43.

Aljundi W, Daas L, Abu Dail Y, Käsmann-Kellner B, Seitz B, and Abdin AD (2022) Topical NSAIDs and Oral Acetazolamide for Macular Edema after Uncomplicated Phacoemulsification: Outcome and Predictors of Non-Response. *J Clin Med* 11:5537.

Alterio V, Di Fiore A, D'Ambrosio K, Supuran CT, and De Simone G (2012) Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms? *Chem Rev* 112:4421-68.

Alterio V, Langella E, Buonanno M, Esposito D, Nocentini A, Berrino E, Bua S, Polentarutti M, Supuran CT, Monti SM, and De Simone G (2021) Zeta-carbonic anhydrases show CS<sub>2</sub> hydrolase activity: A new metabolic carbon acquisition pathway in diatoms? *Comput Struct Biotechnol J* 19:3427-3436.

Alterio V, Langella E, Viparelli F, Vullo D, Ascione G, Dathan NA, Morel FM, Supuran CT, De Simone G, and Monti SM (2012) Structural and inhibition insights into carbonic anhydrase CDCA1 from the marine diatom *Thalassiosira weissflogii*. *Biochimie* 94: 1232-1241.

Alterio V, Monti SM, Truppo E, Pedone C, Supuran CT, and De Simone G (2010) The first example of a significant active site conformational rearrangement in a carbonic anhydrase-inhibitor adduct: the carbonic anhydrase I-topiramate complex. *Org Biomol Chem* 8:3528-33.

Alterio V, Vitale RM, Monti SM, Pedone C, Scozzafava A, Cecchi A, De Simone G, Supuran CT (2006) Carbonic anhydrase inhibitors: X-ray and molecular modeling study for the interaction of a fluorescent antitumor sulfonamide with isozyme II and IX. *J Am Chem Soc* 128:8329-35.

Amedei A, Capasso C, Nannini G, and Supuran CT (2021) Microbiota, Bacterial Carbonic Anhydrases, and Modulators of Their Activity: Links to Human Diseases? *Mediators Inflamm* 2021:6926082.

An W, Holly KJ, Nocentini A, Imhoff RD, Hewitt CS, Abutaleb NS, Cao X, Seleem MN, Supuran CT, Flaherty DP (2022) Structure-activity relationship studies for inhibitors for vancomycin-resistant *Enterococcus* and human carbonic anhydrases. *J Enzyme Inhib Med Chem* 37:1838-1844.

Andreucci E, Biagioni A, Peri S, Versienti G, Cianchi F, Staderini F, Antonuzzo L, Supuran CT, Olivo E, Pasqualini E, Messerini L, Massi D, Lulli M, Ruzzolini J, Peppicelli S, Bianchini F, 61

Schiavone N, Calorini L, Magnelli L, and Papucci L (2023) The CAIX inhibitor SLC-0111 exerts anti-cancer activity on gastric cancer cell lines and resensitizes resistant cells to 5-Fluorouracil, taxane-derived, and platinum-based drugs. *Cancer Lett* 571:216338.

Angeli A, Carta F, Nocentini A, Winum JY, Zalubovskis R, Akdemir A, Onnis V, Eldehna WM, Capasso C, Simone G, Monti SM, Carradori S, Donald WA, Dedhar S, Supuran CT (2020) Carbonic Anhydrase Inhibitors Targeting Metabolism and Tumor Microenvironment. *Metabolites* 10:412.

Angeli A, Ferraroni M, Capasso C, and Supuran CT (2024) The dopamine D2 receptors antagonist Veralipride inhibits carbonic anhydrases: solution and crystallographic insights on human isoforms. *Chem Asian J* 19:e202400067.

Angeli A, Ferraroni M, Capperucci A, Tanini D, Costantino G, Supuran CT (2022a) Selenocarbamates As a Prodrug-Based Approach to Carbonic Anhydrase Inhibition. *ChemMedChem* 17:e202200085.

Angeli A, Ferraroni M, Carta F, Häberli C, Keiser J, Costantino G, and Supuran CT (2022b) Development of Praziquantel sulphonamide derivatives as antischistosomal drugs. *J Enzyme Inhib Med Chem* 37:1479-1494.

Angeli A, Ferraroni M, Da'dara AA, Selleri S, Pinteala M, Carta F, Skelly PJ, and Supuran CT (2021) Structural Insights into *Schistosoma mansoni* Carbonic Anhydrase (SmCA) Inhibition by Selenoureido-Substituted Benzenesulfonamides. *J Med Chem* 64:10418-10428.

Angeli A, Ferraroni M, and Supuran CT (2018) Famotidine, an Antiulcer Agent, Strongly Inhibits *Helicobacter pylori* and Human Carbonic Anhydrases. *ACS Med Chem Lett* 9:1035-1038.

Angeli A, Velluzzi A, Selleri S, Capasso C, Spadini C, Iannarelli M, Cabassi CS, Carta F, and Supuran CT (2022c) Seleno Containing Compounds as Potent and Selective Antifungal Agents. *ACS Infect Dis* 8:1905-1919.

Angeli A, Micheli L, Turnaturi R, Pasquinucci L, Parenti C, Alterio V, Di Fiore A, De Simone G, Monti SM, Carta F, Di Cesare Mannelli L, Ghelardini C, and Supuran CT (2023) Discovery of a novel series of potent carbonic anhydrase inhibitors with selective affinity for  $\mu$  Opioid receptor for Safer and long-lasting analgesia. *Eur J Med Chem* 260:115783.

Angeli A and Supuran CT (2019) Prostaglandin receptor agonists as antiglaucoma agents (a patent review 2013 - 2018). *Expert Opin Ther Pat* 29:793-803.

Angeli A and Supuran CT (2023) Click chemistry approaches for developing carbonic anhydrase inhibitors and their applications. *J Enzyme Inhib Med Chem* 38:2166503.

Angeli A, Tanini D, Nocentini A, Capperucci A, Ferraroni M, Gratteri P, and Supuran CT (2019) Selenols: a new class of carbonic anhydrase inhibitors. *Chem Commun (Camb)* 55(5):648-651.

Ansell B, and Clarke E (1956) Acetazolamide in Treatment of Epilepsy. *Br Med J* 1:650-4.

Antel J and Hebebrand J (2012) Weight-reducing side effects of the antiepileptic agents topiramate and zonisamide. *Handb Exp Pharmacol* 209:433-66.

Arechederra RL, Waheed A, Sly WS, Supuran CT, and Minter SD (2013) Effect of sulfonamides as carbonic anhydrase VA and VB inhibitors on mitochondrial metabolic energy conversion. *Bioorg Med Chem* 21:1544-8.

Aribi AM and Stringer JL (2002) Effects of antiepileptic drugs on extracellular pH regulation in the hippocampal CA1 region in vivo. *Epilepsy Res* 49:143-51.

Arman S and Haghshenas M (2022) Metabolic effects of adding Topiramate on Aripiprazole in bipolar patients aged between 6-18 years, a randomized, double-blind, placebo-controlled trial. *J Res Med Sci* 27:23.

Aronne LJ, Wadden TA, Peterson C, Winslow D, Odeh S, Gadde KM (2013) Evaluation of phentermine and topiramate versus phentermine/topiramate extended-release in obese adults. *Obesity* 21:2163–71.

Arrighi F, Berrino E, Secci D (2024) Angiotensin-converting enzyme. In *Metalloenzymes from Bench to Bedside* (Supuran CT, Donald WA Eds.), pp. 239-253, Elsevier/Academic Press, London.

Aspatwar A, Barker H, Aisala H, Zueva K, Kuuslahti M, Tolvanen M, Primmer CR, Lumme J, Bonardi A, Tripathi A, Parkkila S, and Supuran CT (2022a) Cloning, purification, kinetic and anion inhibition studies of a recombinant  $\beta$ -carbonic anhydrase from the Atlantic salmon parasite platyhelminth *Gyrodactylus salaris*. *J Enzyme Inhib Med Chem* 37:1577-1586.

Aspatwar A, Becker HM, Parvathaneni NK, Hammaren M, Svorjova A, Barker H, Supuran CT, Dubois L, Lambin P, Parikka M, Parkkila S, and Winum JY (2018) Nitroimidazole-based inhibitors DTP338 and DTP348 are safe for zebrafish embryos and efficiently inhibit the activity of human CA IX in *Xenopus* oocytes. *J Enzyme Inhib Med Chem* 33:1064-1073.

Aspatwar A, Bonardi A, Aisala H, Zueva K, Primmer CR, Lumme J, Parkkila S, and Supuran CT (2023) Sulphonamide inhibition studies of the  $\beta$ -carbonic anhydrase GsaCA $\beta$  present in the salmon platyhelminth parasite *Gyrodactylus salaris*. *J Enzyme Inhib Med Chem* 38:2167988.

Aspatwar A, Hammarén M, Koskinen S, Luukinen B, Barker H, Carta F, Supuran CT, Parikka M, and Parkkila S (2017)  $\beta$ -CA-specific inhibitor dithiocarbamate Fc14-584B: a novel antimycobacterial agent with potential to treat drug-resistant tuberculosis. *J Enzyme Inhib Med Chem* 32:832-840.

Aspatwar A, Kairys V, Rala S, Parikka M, Bozdag M, Carta F, Supuran CT, and Parkkila S (2019) *Mycobacterium tuberculosis*  $\beta$ -Carbonic Anhydrases: Novel Targets for Developing Antituberculosis Drugs. *Int J Mol Sci* 20:5153.



Aspatwar A, Tolvanen MEE, Barker H, Syrjänen L, Valanne S, Purmonen S, Waheed A, Sly WS, and Parkkila S (2022b) Carbonic anhydrases in metazoan model organisms: molecules, mechanisms, and physiology. *Physiol Rev* 102:1327-1383.

Aspatwar A, Tolvanen ME, and Parkkila S (2013) An update on carbonic anhydrase-related proteins VIII, X and XI. *J Enzyme Inhib Med Chem* 28:1129-42.

Asiedu M, Ossipov MH, Kaila K, Price TJ (2010) Acetazolamide and midazolam act synergistically to inhibit neuropathic pain. *Pain* 148: 302-308

Atwood PV (1995) The structure and mechanism of action of pyruvate carboxylase. *Int J Biochem Cell Biol* 27: 231-49.

Becker B (1955) The mechanism of the fall in intraocular pressure by the carbonic anhydrase inhibitor Diamox. *Am J Ophthalmol* 39:177-83.

Bendi A, Taruna, Rajni, Kataria S, Singh L, Kennedy JF, Supuran CT, and Raghav N (2024) Chemistry of heterocycles as carbonic anhydrase inhibitors: A pathway to novel research in medicinal chemistry review. *Arch Pharm (Weinheim)*. (in press) Apr 29:e2400073 (doi: 10.1002/ardp.202400073).

Berckmans Y, Hoffert Y, Vankerckhoven A, Dreesen E, and Coosemans A (2023) Drug Repurposing for Targeting Myeloid-Derived Suppressor-Cell-Generated Immunosuppression in Ovarian Cancer: A Literature Review of Potential Candidates. *Pharmaceutics* 15:1792.

Berrino E and Supuran CT (2019) Rho-kinase inhibitors in the management of glaucoma. *Expert Opin Ther Pat* 29:817-827.

Bibi D, Shusterman B, Nocentini A, Supuran CT, and Bialer M (2019) Stereoselective pharmacokinetic and pharmacodynamic analysis of a CNS-active sulphamoylphenyl carbamate derivative. *J Enzyme Inhib Med Chem* 34:1078-1082.

Bonardi A, Nocentini A, Bua S, Combs J, Lomelino C, Andring J, Lucarini L, Sgambellone S, Masini E, McKenna R, Gratteri P, and Supuran CT (2020) Sulfonamide Inhibitors of Human

Carbonic Anhydrases Designed through a Three-Tails Approach: Improving Ligand/Isoform Matching and Selectivity of Action. *J Med Chem* 63:7422-7444

Bonardi A, Bua S, Combs J, Lomelino C, Andring J, Osman SM, Toti A, Di Cesare Mannelli L, Gratteri P, Ghelardini C, McKenna R, Nocentini A, and Supuran CT (2022) The three-tails approach as a new strategy to improve selectivity of action of sulphonamide inhibitors against tumour-associated carbonic anhydrase IX and XII. *J Enzyme Inhib Med Chem* 37:930-939.

Boyd NH, Walker K, Fried J, Hackney JR, McDonald PC, Benavides GA, Spina R, Audia A, Scott SE, Landis CJ, Tran AN, Bevensee MO, Griguer C, Nozell S, Gillespie GY, Nabors B, Bhat KP, Bar EE, Darley-USmar V, Xu B, Gordon E, Cooper SJ, Dedhar S, and Hjelmeland AB (2017) Addition of carbonic anhydrase 9 inhibitor SLC-0111 to temozolomide treatment delays glioblastoma growth in vivo. *JCI Insight* 2:e92928.

Bradwell AR, Wright AD, Winterborn M, and Imray C (1992) Acetazolamide and high altitude disease. *Int J Sports Med* 13, 63–64.

Briganti F, Mangani S, Orioli P, Scozzafava A, Vernaglione G, and Supuran CT (1997) Carbonic anhydrase activators: X-ray crystallographic and spectroscopic investigations for the interaction of isozymes I and II with histamine. *Biochemistry* 36:10384-92.

Bua S, Di Cesare Mannelli L, Vullo D, Ghelardini C, Bartolucci G, Scozzafava A, Supuran CT, Carta F (2017) Design and synthesis of novel nonsteroidal anti-inflammatory drugs and carbonic anhydrase inhibitors hybrids (NSAIDs-CAIs) for the treatment of rheumatoid arthritis. *J Med Chem* 60: 1159-1170.

Bua S, Haapanen S, Kuuslahti M, Parkkila S, and Supuran CT (2018) Sulfonamide Inhibition Studies of a New  $\beta$ -Carbonic Anhydrase from the Pathogenic Protozoan *Entamoeba histolytica*. *Int J Mol Sci* 19:3946.

Bua S, Lucarini L, Micheli L, Menicatti M, Bartolucci G, Selleri S, Di Cesare Mannelli L, Ghelardini C, Masini E, Carta F, Gratteri P, Nocentini A, and Supuran CT (2020) Bioisosteric Development of Multitarget Nonsteroidal Anti-Inflammatory Drug-Carbonic Anhydrases Inhibitor Hybrids for the Management of Rheumatoid Arthritis. *J Med Chem* 63:2325-2342.

Bua S and Supuran CT (2019) Diagnostic markers for glaucoma: a patent and literature review (2013-2019). *Expert Opin Ther Pat* 29:829-839.

Bulli I, Dettori I, Coppi E, Cherchi F, Venturini M, Di Cesare Mannelli L, Ghelardini C, Nocentini A, Supuran CT, Pugliese AM, and Pedata F (2021) Role of Carbonic Anhydrase in Cerebral Ischemia and Carbonic Anhydrase Inhibitors as Putative Protective Agents. *Int J Mol Sci* 22:5029.

Burianova V, Kalinin S, Supuran CT, and Krasavin M (2021) Radiotracers for positron emission tomography (PET) targeting tumour-associated carbonic anhydrase isoforms. *Eur J Med Chem* 213:113046.

Burtscher J, Gatterer H, Faulhaber Martin, and Burtscher M (2016) Acetazolamide pre-treatment before ascending to high altitudes: sustained effect on systemic blood pressure during submaximal exercise. *Int J Clin Exp Med* 9 :6656-6662.

Buzas GM and Birinyi P (2023) Newer, older, and alternative agents for the eradication of *Helicobacter pylori* infection: A narrative review. *Antibiotics* 12: 946.

Buzás GM and Supuran CT (2016) The history and rationale of using carbonic anhydrase inhibitors in the treatment of peptic ulcers. In memoriam Ioan Pușcaș (1932-2015). *J Enzyme Inhib Med Chem* 31:527-33.

Cabiscol E and Levine RL (1996) The phosphatase activity of carbonic anhydrase III is reversibly regulated by glutathiolation. *Proc Natl Acad Sci U S A* 93:4170-4.

Campestre C, De Luca V, Carradori S, Grande R, Carginale V, Scaloni A, Supuran CT, and Capasso C (2021) Carbonic Anhydrases: New Perspectives on Protein Functional Role and Inhibition in *Helicobacter pylori*. *Front Microbiol* 12:629163.

Campoli-Richards DM, Clissold SP. Famotidine (1986) Pharmacodynamic and pharmacokinetic properties and a preliminary review of its therapeutic use in peptic ulcer disease and Zollinger-Ellison syndrome. *Drugs* 32:197-221.

Canepa E, Parodi-Rullan R, Vazquez-Torres R, Gamallo-Lana B, Guzman-Hernandez R, Lemon NL, Angiulli F, Debure L, Ilies MA, Østergaard L, Wisniewski T, Gutiérrez-Jiménez E, Mar AC, and Fossati S (2023) FDA-approved carbonic anhydrase inhibitors reduce amyloid  $\beta$  pathology and improve cognition, by ameliorating cerebrovascular health and glial fitness. *Alzheimers Dement* 19:5048-5073.

Capasso C and Supuran CT (2015) An overview of the alpha-, beta- and gamma-carbonic anhydrases from Bacteria: can bacterial carbonic anhydrases shed new light on evolution of bacteria? *J Enzyme Inhib Med Chem* 30:325-332.

Capasso C and Supuran CT (2023) The management of Babesia, amoeba and other zoonotic diseases provoked by protozoa. *Expert Opin Ther Pat* 33:179-192.

Capasso C and Supuran CT (2024) Carbonic anhydrase and bacterial metabolism: a chance for antibacterial drug discovery. *Expert Opin Ther Pat* 34:465-474.

Carlsten C, Swenson ER, Ruoss S (2004) A dose-response study of acetazolamide for acute mountain sickness prophylaxis in vacationing tourists at 12,000 feet (3630 m). *High Alt Med Biol* 5:33-9.

Caroli AP, Mansoldo FRP, Cardoso VS, Lage CLS, Carmo FL, Supuran CT, Beatriz Vermelho A (2023) Are patents important indicators of innovation for Chagas disease treatment? *Expert Opin Ther Pat* 33:193-209.

Carradori S, Mollica A, Ceruso M, D'Ascenzio M, De Monte C, Chimenti P, Sabia R, Akdemir A, Supuran CT (2015) New amide derivatives of Probenecid as selective inhibitors of carbonic

anhydrase IX and XII: biological evaluation and molecular modelling studies. *Bioorg Med Chem*. 23:2975-81.

Carta F, Di Cesare Mannelli L, Pinard M, Ghelardini C, Scozzafava A, McKenna R, and Supuran CT. (2015) A class of sulfonamide carbonic anhydrase inhibitors with neuropathic pain modulating effects. *Bioorg Med Chem* 23: 1828-1840.

Carta F, Maresca A, Covarrubias AS, Mowbray SL, Jones TA, and Supuran CT (2009) Carbonic anhydrase inhibitors. Characterization and inhibition studies of the most active beta-carbonic anhydrase from *Mycobacterium tuberculosis*, Rv3588c. *Bioorg Med Chem Lett* 19:6649-54.

Carta F, Maresca A, Scozzafava A, Supuran CT (2012) 5- and 6-membered (thio)lactones are prodrug type carbonic anhydrase inhibitors. *Bioorg Med Chem Lett* 22:267-70.

Carta F and Supuran CT (2013) Diuretics with carbonic anhydrase inhibitory action: a patent and literature review (2005 - 2013). *Expert Opin Ther Pat* 23:681-91.

Carta F, Supuran CT, and Scozzafava A (2012) Novel therapies for glaucoma: a patent review 2007-2011. *Expert Opin Ther Pat* 22: 79-88.

Carta F, Temperini C, Innocenti A, Scozzafava A, Kaila K, Supuran CT (2010) Polyamines inhibit carbonic anhydrases by anchoring to the zinc-coordinated water molecule. *J Med Chem* 53: 5511-22.

Casini A, Antel J, Abbate F, Scozzafava A, David S, Waldeck H, Schäfer S, and Supuran CT (2003) Carbonic anhydrase inhibitors: SAR and X-ray crystallographic study for the interaction of sugar sulfamates/sulfamides with isozymes I, II and IV. *Bioorg Med Chem Lett* 13:841-5.

Chafe SC, Vizeacoumar FS, Venkateswaran G, Nemirovsky O, Awrey S, Brown WS, McDonald PC, Carta F, Metcalfe A, Karasinska JM, Huang L, Muthuswamy SK, Schaeffer DF, Renouf DJ, Supuran CT, Vizeacoumar FJ, and Dedhar S (2021) Genome-wide synthetic lethal screen unveils

novel CAIX-NFS1/xCT axis as a targetable vulnerability in hypoxic solid tumors. *Sci Adv* 7:eabj0364.

Chahin M, Oye M, Jadeja S, Sanders K, and Reddy P (2020) Topiramate causing type II renal tubular acidosis: A case and review of the mechanism. *Clin Case Rep* 8:731-733.

Charbonneau M, Doyle-Campbell C, Laskey C, and Capoccia K (2020) Carbonation dysgeusia associated with topiramate. *Am J Health Syst Pharm* 77(14):1113-1116.

Chegwidden WR and Spencer IM (1996) Carbonic anhydrase provides bicarbonate for de novo lipogenesis in the locust. *Comp Biochem Physiol* 115B: 247-54.

Cho YS, Bae KS, Choi SC, Cho JM, and Lim HS (2022) Population Pharmacokinetic and Pharmacodynamic Analysis of Polmacoxib in Healthy Volunteers and Patients With Osteoarthritis. *Clin Ther* 44:67-80.

Chrysanthopoulos PK, Mujumdar P, Woods LA, Dolezal O, Ren B, Peat TS, and Poulsen S (2017) Identification of a New Zinc Binding Chemotype by Fragment Screening. *J Med Chem* 60:7333-7349.

Costa G, Gidaro MC, Vullo D, Supuran CT, Alcaro S (2016) Active Components of Essential Oils as Anti-Obesity Potential Drugs Investigated by in Silico Techniques. *J Agric Food Chem* 64:5295-300.

Cox EH, McLendon GL, Morel FM, Lane TW, Prince RC, Pickering IJ, and George GN (2000) The active site structure of *Thalassiosira weissflogii* carbonic anhydrase 1. *Biochemistry* 39: 12128-12130.

Crocetti L, Maresca A, Temperini C, Hall RA, Scozzafava A, Mühlischlegel FA, and Supuran CT (2009) A thiabendazole sulfonamide shows potent inhibitory activity against mammalian and nematode alpha-carbonic anhydrases. *Bioorg Med Chem Lett* 19:1371-5.

Cuesta-Seijo JA, Borchert MS, Navarro-Poulsen JC, Schnorr KM, Mortensen SB, Lo Leggio L (2011) Structure of a dimeric fungal  $\alpha$ -type carbonic anhydrase. *FEBS Lett* 585:1042-8.

Cuthbert JJ, Cleland JGF (2023) Should we resurrect acetazolamide as a diuretic for congestion due to heart failure? *Cardiovasc Res* 119:e149-e151.

D'Agostino I, Zara S, Carradori S, De Luca V, Capasso C, Kocken CHM, Zeeman AM, Angeli A, Carta F, and Supuran CT (2023) Antimalarial Agents Targeting Plasmodium falciparum Carbonic Anhydrase: Towards Artesunate Hybrid Compounds with Dual Mechanism of Action. *ChemMedChem* 18:e202300267.

D'Ambrosio K, Carradori S, Cesa S, Angeli A, Monti SM, Supuran CT, De Simone G (2020) Catechols: a new class of carbonic anhydrase inhibitors. *Chem Commun (Camb)* 56:13033-13036.

D'Ambrosio K, Carradori S, Monti SM, Buonanno M, Secci D, Vullo D, Supuran CT, De Simone G (2015) Out of the active site binding pocket for carbonic anhydrase inhibitors. *Chem Commun* 51: 302-305.

D'Ambrosio K, Supuran CT, and De Simone G (2018) Are Carbonic Anhydrases Suitable Targets to Fight Protozoan Parasitic Diseases? *Curr Med Chem* 25:5266-5278.

Da'dara AA, Angeli A, Ferraroni M, Supuran CT, and Skelly PJ (2019) Crystal structure and chemical inhibition of essential schistosome host-interactive virulence factor carbonic anhydrase SmCA. *Commun Biol* 2:333.

da Silva Cardoso V, Vermelho AB, Ricci Junior E, Almeida Rodrigues I, Mazotto AM, and Supuran CT (2018) Antileishmanial activity of sulphonamide nanoemulsions targeting the  $\beta$ -carbonic anhydrase from Leishmania species. *J Enzyme Inhib Med Chem* 33:850-857.

de Groot T, Sinke AP, Kortenoeven ML, Alsady M, Baumgarten R, Devuyst O, Loffing J, Wetzels JF, and Deen PM (2016) Acetazolamide Attenuates Lithium-Induced Nephrogenic Diabetes Insipidus. *J Am Soc Nephrol* 27:2082-91.

De Luca V, Giovannuzzi S, Capasso C, and Supuran CT (2024a) Cloning, expression, and purification of an  $\alpha$ -carbonic anhydrase from *Toxoplasma gondii* to unveil its kinetic parameters and anion inhibition profile. *J Enzyme Inhib Med Chem* 39:2346523.

De Luca V, Giovannuzzi S, Supuran CT, Capasso C (2024b) A comprehensive investigation of the anion inhibition profile of a  $\beta$ -carbonic anhydrase from *Acinetobacter baumannii* for crafting innovative antimicrobial treatments. *J Enzyme Inhib Med Chem* 39:2372731.

De Luca V, Del Prete S, Vullo D, Carginale V, Di Fonzo P, Osman SM, AlOthman Z, Supuran CT, and Capasso C (2016) Expression and characterization of a recombinant psychrophilic  $\gamma$ -carbonic anhydrase (NcoCA) identified in the genome of the Antarctic cyanobacteria belonging to the genus *Nostoc*. *J Enzyme Inhib Med Chem* 31:810-7.

De Luca V, Petreni A, Nocentini A, Scaloni A, Supuran CT, Capasso C (2021) Effect of Sulfonamides and Their Structurally Related Derivatives on the Activity of  $\iota$ -Carbonic Anhydrase from *Burkholderia territorii*. *Int J Mol Sci* 22:571.

De Simone G, Alterio V, and Supuran CT (2013) Exploiting the hydrophobic and hydrophilic binding sites for designing carbonic anhydrase inhibitors. *Expert Opin Drug Discov* 8:793-810.

De Simone G, Di Fiore A, Supuran CT (2008) Are carbonic anhydrase inhibitors suitable for obtaining antiobesity drugs? *Curr Pharm Des* 14:655-60.

De Simone G, Di Fiore A, Capasso C, Supuran CT (2015) The zinc coordination pattern in the  $\eta$ -carbonic anhydrase from *Plasmodium falciparum* is different from all other carbonic anhydrase genetic families. *Bioorg Med Chem Lett* 25:1385-9.



De Simone G, Di Fiore A, Menchise V, Pedone C, Antel J, Casini A, Scozzafava A, Wurl M, and Supuran CT (2005) Carbonic anhydrase inhibitors. Zonisamide is an effective inhibitor of the cytosolic isozyme II and mitochondrial isozyme V: solution and X-ray crystallographic studies. *Bioorg Med Chem Lett* 15:2315-20.

De Simone G, Scozzafava A, Supuran CT (2009) Which carbonic anhydrases are targeted by the antiepileptic sulfonamides and sulfamates? *Chem Biol Drug Des* 74:317-21.

De Simone G and Supuran CT (2012) (In)organic anions as carbonic anhydrase inhibitors. *J Inorg Biochem* 111:117-29.

De Simone G and Supuran CT (2024). Anticancer drugs: where are we now? *Expert Opin Ther Pat* (in press) doi:10.1080/13543776.2024.2353625.

Del Giudice R, Monti DM, Truppo E, Arciello A, Supuran CT, De Simone G, and Monti SM (2013) Human carbonic anhydrase VII protects cells from oxidative damage. *Biol Chem* 394:1343-8.

Del Prete S, Isik S, Vullo D, De Luca V, Carginale V, Scozzafava A, Supuran CT, and Capasso C (2012) DNA cloning, characterization, and inhibition studies of an  $\alpha$ -carbonic anhydrase from the pathogenic bacterium *Vibrio cholerae*. *J Med Chem* 55:10742-8.

Del Prete S, Vullo D, De Luca V, Carginale V, Ferraroni M, Osman SM, AlOthman Z, Supuran CT, Capasso C (2016a) Sulfonamide inhibition studies of the  $\beta$ -carbonic anhydrase from the pathogenic bacterium *Vibrio cholerae*. *Bioorg Med Chem* 24:1115-20.

Del Prete S, Vullo D, De Luca V, Carginale V, Osman SM, AlOthman Z, Supuran CT, Capasso C (2016b) Comparison of the sulfonamide inhibition profiles of the  $\alpha$ -,  $\beta$ - and  $\gamma$ -carbonic anhydrases from the pathogenic bacterium *Vibrio cholerae*. *Bioorg Med Chem Lett* 26:1941-6.

Del Prete S, Vullo D, Fisher GM, Andrews KT, Poulsen SA, Capasso C, and Supuran CT (2014) Discovery of a new family of carbonic anhydrases in the malaria pathogen *Plasmodium falciparum*-the  $\eta$ -carbonic anhydrases. *Bioorg Med Chem Lett* 24:4389-4396.

Deniz S, Uysal TK, Capasso C, Supuran CT, and Ozensoy Guler O (2021) Is carbonic anhydrase inhibition useful as a complementary therapy of Covid-19 infection? *J Enzyme Inhib Med Chem* 36:1230-1235.

Dettori I, Fusco I, Bulli I, Gaviano L, Coppi E, Cherchi F, Venturini M, Di Cesare Mannelli L, Ghelardini C, Nocentini A, Supuran CT, Pugliese AM, and Pedata F (2021) Protective effects of carbonic anhydrase inhibition in brain ischaemia in vitro and in vivo models. *J Enzyme Inhib Med Chem* 36:964-976.

Di Cesare Mannelli L, Micheli L, Carta F, Cozzi A, Ghelardini C, and Supuran CT. (2016) Carbonic anhydrase inhibition for the management of cerebral ischemia: in vivo evaluation of sulfonamide and coumarin inhibitors. *J Enzyme Inhib Med Chem* 31: 894-899.

Di Fiore A, De Luca V, Langella E, Nocentini A, Buonanno M, Monti SM, Supuran CT, Capasso C, De Simone G (2022) Biochemical, structural, and computational studies of a  $\gamma$ -carbonic anhydrase from the pathogenic bacterium *Burkholderia pseudomallei*. *Comput Struct Biotechnol J* 20:4185-4194.

Di Fiore A, Scozzafava A, Winum JY, Montero JL, Pedone C, Supuran CT, and De Simone G (2007) Carbonic anhydrase inhibitors: binding of an antiglaucoma glycosyl-sulfanilamide derivative to human isoform II and its consequences for the drug design of enzyme inhibitors incorporating sugar moieties. *Bioorg Med Chem Lett* 17:1726-31.

DiMario RJ, Machingura MC, Waldrop GL, and Moroney JV (2018) The many types of carbonic anhydrases in photosynthetic organisms. *Plant Sci* 268:11-17.

Dogné JM, Supuran CT, and Pratico D (2005) Adverse cardiovascular effects of the coxibs. *J Med Chem* 48:2251-7.

Dogné JM, Thiry A, and Supuran CT (2008) Carbonic anhydrase inhibition: insight into non-COX-2 pharmacological effect of some coxibs. *Curr Pharm Des* 14:679-84.

Doherty CJ, Chang JC, Thompson BP, Swenson ER, Foster GE, and Dominelli PB (2023) The Impact of Acetazolamide and Methazolamide on Exercise Performance in Normoxia and Hypoxia. *High Alt Med Biol* 24:7-18.

Domsic JF, Avvaru BS, Kim CU, Gruner SM, Agbandje-McKenna M, Silverman DN, McKenna R (2008) Entrapment of carbon dioxide in the active site of carbonic anhydrase II. *J Biol Chem* 283:30766-71.

Eloranta K, Pihlajoki M, Liljeström E, Nousiainen R, Soini T, Lohi J, Cairo S, Wilson DB, Parkkila S, and Heikinheimo M (2023) SLC-0111, an inhibitor of carbonic anhydrase IX, attenuates hepatoblastoma cell viability and migration. *Front Oncol* 13:1118268.

Elsawi AE, Elbadawi MM, Nocentini A, Almahli H, Giovannuzzi S, Shaldam M, Salem R, Ibrahim TM, Abdel-Aziz HA, Supuran CT, and Eldehna WM (2023) 1,5-Diaryl-1,2,4-triazole Ureas as New SLC-0111 Analogues Endowed with Dual Carbonic Anhydrase and VEGFR-2 Inhibitory Activities. *J Med Chem* 66:10558-10578.

Eltobgy M, Johns F, Farkas D, Leuenberger L, Cohen SP, Ho K, Karow S, Swoope G, Pannu S, Horowitz JC, Mallampalli RK, Englert JA, and Bednash JS (2024) Longitudinal transcriptomic analysis reveals persistent enrichment of iron homeostasis and erythrocyte function pathways in severe COVID-19 ARDS. *Front Immunol* 15:1397629.

Epstein M (2001) Cardiovascular and renal effects of COX-2-specific inhibitors: recent insights and evolving clinical implications. *Am J Ther* 8:81-3.

Fantacuzzi M, D'Agostino I, Carradori S, Liguori F, Carta F, Agamennone M, Angeli A, Sannio F, Docquier JD, Capasso C, and Supuran CT (2023) Benzenesulfonamide derivatives as *Vibrio cholerae* carbonic anhydrases inhibitors: a computational-aided insight in the structural rigidity-activity relationships. *J Enzyme Inhib Med Chem* 38:2201402.

Fares M, Eldehna WM, Bua S, Lanzi C, Lucarini L, Masini E, Peat TS, Abdel-Aziz HA, Nocentini A, Keller PA, and Supuran CT (2020) Discovery of Potent Dual-Tailed Benzenesulfonamide Inhibitors of Human Carbonic Anhydrases Implicated in Glaucoma and in Vivo Profiling of Their Intraocular Pressure-Lowering Action. *J Med Chem* 63:3317-3326.

Fasseas MK, Tsikou D, Flemetakis E, and Katinakis P (2010) Molecular and biochemical analysis of the beta class carbonic anhydrases in *Caenorhabditis elegans*. *Mol Biol Rep* 37:2941-50.

Ferraroni M, Angeli A, Carradori S, and Supuran CT (2022a) Inhibition of *Schistosoma mansoni* carbonic anhydrase by the antiparasitic drug clorsulon: X-ray crystallographic and in vitro studies. *Acta Crystallogr D Struct Biol* 78:321-327.

Ferraroni M, Angeli A, Pinteala M, and Supuran CT (2022b) Sulfonamide diuretic azosemide as an efficient carbonic anhydrase inhibitor. *J Mol Struct* 1268: 133672.

Ferry JG (2010) The gamma class of carbonic anhydrases. *Biochim Biophys Acta* 1804:374-81.

Findinier J and Grossman AR. One step further toward a crop CO<sub>2</sub>-concentrating mechanism. *J Exp Bot* 74:3402-3405.

Fisher GM, Bua S, Del Prete S, Arnold MS, Capasso C, Supuran CT, Andrews KT, and Poulsen SA (2017) Investigating the antiplasmodial activity of primary sulfonamide compounds identified in open source malaria data. *Int J Parasitol Drugs Drug Resist* 7:61-70.

Fitzgerald GA (2003) COX-2 and beyond: Approaches to prostaglandin inhibition in human disease. *Nat Rev Drug Discov* 2: 879-90.

Flaherty DP, Seleem MN, and Supuran CT (2021) Bacterial carbonic anhydrases: underexploited antibacterial therapeutic targets. *Future Med Chem* 13:1619-1622.

Fossati S, Giannoni P, Solesio ME, Cocklin SL, Cabrera E, Ghiso J, and Rostagno A (2016) The carbonic anhydrase inhibitor methazolamide prevents amyloid beta-induced mitochondrial dysfunction and caspase activation protecting neuronal and glial cells in vitro and in the mouse brain. *Neurobiol Dis* 86: 29-40.

Furtado VA and Srihari V (2008) Atypical antipsychotics for people with both schizophrenia and depression. *Cochrane Database Syst Rev* 1:CD005377.

Gadde KM, Allison DB, Ryan DH, Peterson CA, Troupin B, Schwiers ML, and Day WW (2011) Effects of low-dose, controlled-release, phentermine plus topiramate combination on weight and associated comorbidities in overweight and obese adults (CONQUER): a randomised, placebo-controlled, phase 3 trial. *Lancet* 377:1341-52.

Gadde KM, Franciscy DM, Wagner HR 2nd, and Krishnan KR (2003) Zonisamide for weight loss in obese adults: a randomized controlled trial. *JAMA* 289:1820-5.

Gamal MA, Fahim SH, Giovannuzzi S, Fouad MA, Bonardi A, Gratteri P, Supuran CT, and Hassan GS (2024) Probing benzenesulfonamide-thiazolidinone hybrids as multitarget directed ligands for efficient control of type 2 diabetes mellitus through targeting the enzymes:  $\alpha$ -glucosidase and carbonic anhydrase II. *Eur J Med Chem* 271:116434.

Garvey MH and Maude DL (1981) Acid excretion by bicarbonate-free perfused rat kidney. *Am J Physiol.* 240:F306-10.

Garvey WT, Ryan DH, Look M, Gadde KM, Allison DB, Peterson CA, Schwiers M, Day WW, Bowden CH (2012) Two-year sustained weight loss and metabolic benefits with controlled-release phentermine/topiramate in obese and overweight adults (SEQUEL): a randomized, placebo-controlled, phase 3 extension study. *Am J Clin Nutr* 95:297-308.

Geraldes C, Tavares L, Gil S, and Oliveira M (2022) Enterococcus Virulence and Resistant Traits Associated with Its Permanence in the Hospital Environment. *Antibiotics (Basel)* 11(7):857.

Gibbs JW 3rd, Sombati S, DeLorenzo RJ, and Coulter DA (2000) Cellular actions of topiramate: blockade of kainate-evoked inward currents in cultured hippocampal neurons. *Epilepsia* 41:10-6.

Gielsing RG, Babur M, Mamnani L, Burrows N, Telfer BA, Carta F, Winum JY, Scozzafava A, Supuran CT, and Williams KJ (2012) Antimetastatic effect of sulfamate carbonic anhydrase IX inhibitors in breast carcinoma xenografts. *J Med Chem* 55:5591-600

Giovannuzzi S, Chavarria D, Provensi G, Leri M, Bucciantini M, Carradori S, Bonardi A, Gratteri P, Borges F, Nocentini A, Supuran CT (2024) Dual Inhibitors of Brain Carbonic Anhydrases and Monoamine Oxidase-B Efficiently Protect against Amyloid- $\beta$ -Induced Neuronal Toxicity, Oxidative Stress, and Mitochondrial Dysfunction. *J Med Chem* 67(5):4170-4193.

Goyal A and Zarroli K (2023) Should topiramate be initial therapy in the management of idiopathic intracranial hypertension?: A literature review. *Medicine (Baltimore)* 102:e35545.

Grajek J and Poleszczuk J (2023) Carbonic Anhydrase IX Suppression Shifts Partial Response to Checkpoint Inhibitors into Complete Tumor Eradication: Model-Based Investigation. *Int J Mol Sci* 24:10068.

Grandane A, Nocentini A, Werner T, Zalubovskis R, Supuran CT (2020) Benzoxepinones: A new isoform-selective class of tumor associated carbonic anhydrase inhibitors. *Bioorg Med Chem.* 2020 28:115496.

Güzel O, Innocenti A, Hall RA, Scozzafava A, Mühlischlegel FA, and Supuran CT (2009) Carbonic anhydrase inhibitors. The nematode alpha-carbonic anhydrase of *Caenorhabditis elegans* CAH-4b is highly inhibited by 2-(hydrazinocarbonyl)-3-substituted-phenyl-1H-indole-5-sulfonamides. *Bioorg Med Chem* 17:3212-5.

Haapanen S, Angeli A, Tolvanen M, Emameh RZ, Supuran CT, and Parkkila S (2023) Cloning, characterization, and inhibition of the novel  $\beta$ -carbonic anhydrase from parasitic blood fluke, *Schistosoma mansoni*. *J Enzyme Inhib Med Chem* 38:2184299.

Haapanen S, Barker H, Carta F, Supuran CT, and Parkkila S (2024) Novel Drug Screening Assay for *Acanthamoeba castellanii* and the Anti-Amoebic Effect of Carbonic Anhydrase Inhibitors. *J Med Chem* 67:152-164.

Haapanen S, Bua S, Kuuslahti M, Parkkila S, and Supuran CT (2018) Cloning, Characterization and Anion Inhibition Studies of a  $\beta$ -Carbonic Anhydrase from the Pathogenic Protozoan *Entamoeba histolytica*. *Molecules* 23:3112.

Hall RA, Vullo D, Innocenti A, Scozzafava A, Supuran CT, Klappa P, Mühlischlegel FA (2008) External pH influences the transcriptional profile of the carbonic anhydrase, CAH-4b in *Caenorhabditis elegans*. *Mol Biochem Parasitol* 161:140-9.

Hazen SA, Waheed A, Sly WS, LaNoue KF, and Lynch CJ (1996) Differentiation-dependent expression of CA V and the role of carbonic anhydrase isozymes in pyruvate carboxylation in adipocytes. *FASEB J* 10:481-90.

Hekmat A, Saboury AA, and Saso L (2024) Superoxide dismutase inhibitors. In *Metalloenzymes from Bench to Bedside* (Supuran CT, Donald WA Eds.), pp. 523-531, Elsevier/Academic Press, London.

Herrero AI, Del Olmo N, González-Escalada JR, Solís JM (2002) Two new actions of topiramate: inhibition of depolarizing GABA(A)-mediated responses and activation of a potassium conductance. *Neuropharmacology* 42:210-20.

Hewitson KS, Vullo D, Scozzafava A, Mastrolorenzo A, and Supuran CT (2012) Molecular cloning, characterization, and inhibition studies of a  $\beta$ -carbonic anhydrase from *Malassezia globosa*, a potential antidandruff target. *J Med Chem* 55:3513-20.

Hewitt CS, Abutaleb NS, Elhassanny AEM, Nocentini A, Cao X, Amos DP, Youse MS, Holly KJ, Marapaka AK, An W, Kaur J, Krabill AD, Elkashif A, Elgammal Y, Graboski AL, Supuran CT, Seleem MN, and Flaherty DP (2021) Structure-Activity Relationship Studies of Acetazolamide-Based Carbonic Anhydrase Inhibitors with Activity against *Neisseria gonorrhoeae*. *ACS Infect Dis*. 7:1969-1984.

Hirakawa Y, Senda M, Fukuda K, Yu HY, Ishida M, Taira M, Kinbara K, and Senda T (2021) Characterization of a novel type of carbonic anhydrase that acts without metal cofactors. *BMC Biol*. 19:105.

Hirankarn S, Barrett JS, Alamuddin N, FitzGerald GA, and Skarke C (2013) GCG100649, A Novel Cyclooxygenase-2 Inhibitor, Exhibits a Drug Disposition Profile in Healthy Volunteers Compatible With High Affinity to Carbonic Anhydrase-I/II: Preliminary Dose-Exposure Relationships to Define Clinical Development Strategies. *Clin Pharmacol Drug Dev* 2:379-86.

Innocenti A, Hall RA, Schlicker C, Scozzafava A, Steegborn C, Mühlischlegel FA, and Supuran CT (2009) Carbonic anhydrase inhibitors. Inhibition and homology modeling studies of the fungal beta-carbonic anhydrase from *Candida albicans* with sulfonamides. *Bioorg Med Chem* 17:4503-9.

Inoue H, Hazama H, Hamazoe K, Ichikawa M, Omura F, Fukuma E, Inoue K, and Umezawa Y (1984) Antipsychotic and prophylactic effects of acetazolamide (Diamox) on atypical psychosis. *Folia Psychiatr Neurol Jpn* 38:425-36.

Isik S, Guler OO, Kockar F, Aydin M, Arslan O, and Supuran CT (2010) *Saccharomyces cerevisiae*  $\beta$ -carbonic anhydrase: inhibition and activation studies. *Curr Pharm Des* 16: 3327-36.

Jankovicova B, Skultety L, Dubrovackova M, Stern M, Bilkova Z, and Lakota J (2013) Overlap of epitopes recognized by anti-carbonic anhydrase I IgG in patients with malignancy-related aplastic anemia-like syndrome and in patients with aplastic anemia. *Immunol Lett* 153:47-9.



Jensen EL, Clement R, Kosta A, Maberly SC, and Gontero B (2019) A new widespread subclass of carbonic anhydrase in marine phytoplankton. *ISME J* 13:2094-2106.

Ji ZY, Huang YQ, and He WZ (2022) Sodium Valproate Combined With Topiramate vs. Sodium Valproate Alone for Refractory Epilepsy: A Systematic Review and Meta-Analysis. *Front Neurol* 12:794856.

Jin S, Vullo D, Bua S, Nocentini A, Supuran CT, and Gao YG (2020) Structural and biochemical characterization of novel carbonic anhydrases from *Phaeodactylum tricornutum*. *Acta Crystallogr D Struct Biol* 76:676-686.

Jones RL, Ravi V, Brohl AS, Chawla S, Ganjoo KN, Italiano A, Attia S, Burgess MA, Thornton K, Cranmer LD, Cheang MCU, Liu L, Robertson L, Adams B, Theuer C, and Maki RG (2022) Efficacy and Safety of TRC105 Plus Pazopanib vs Pazopanib Alone for Treatment of Patients With Advanced Angiosarcoma: A Randomized Clinical Trial. *JAMA Oncol* 8:740-747.

Kaila K and Löscher W (2022) Bumetanide for neonatal seizures: No light in the pharmacokinetic/dynamic tunnel. *Epilepsia* 63:1868-1873.

Karimi S, Nikkhah H, Nafisi H, Nouri H, Ansari I, Barkhordari S, Samnejad S, and Abtahi SH (2023) Acetazolamide and bevacizumab combination therapy versus bevacizumab monotherapy in macular edema secondary to retinal vein occlusion. *J Fr Ophtalmol* 46:322-326.

Karjalainen SL, Haapasalo HK, Aspatwar A, Barker H, Parkkila S, and Haapasalo JA (2018) Carbonic anhydrase related protein expression in astrocytomas and oligodendroglial tumors. *BMC Cancer* 18:584.

Karunakaran KB, Chaparala S, and Ganapathiraju MK (2019) Potentially repurposable drugs for schizophrenia identified from its interactome. *Sci Rep* 9:12682.

Kaur J, Cao X, Abutaleb NS, Elkashif A, Graboski AL, Krabill AD, AbdelKhalek AH, An W, Bhardwaj A, Seleem MN, and Flaherty DP (2020) Optimization of Acetazolamide-Based Scaffold as Potent Inhibitors of Vancomycin-Resistant Enterococcus. *J Med Chem* 63:9540-9562.

Kazemi H and Choma LH (1977) transport from CNS in hypercapnia and regulation of CSF  $[\text{HCO}_3^-]$ . *J Appl Physiol* 42: 667–672.

Kikutani S, Nakajima K, Nagasato C, Tsuji Y, Miyatake A, and Matsuda Y. (2016) Thylakoid luminal theta-carbonic anhydrase critical for growth and photosynthesis in the marine diatom *Phaeodactylum tricornutum*. *Proc Natl Acad Sci U S A* 113:9828-9833.

Kim HT, Cha H, and Hwang KY (2016) Structural insight into the inhibition of carbonic anhydrase by the COX-2-selective inhibitor polmacoxib (CG100649). *Biochem Biophys Res Commun* 478:1-6.

Kinsey VE and Reddy DVN (1959) Turnover of total carbon dioxide in aqueous humors and the effect thereon of acetazolamide. *Arch Ophthalmol* 62:78-83.

Kivelä AJ, Parkkila S, Saarnio J, Karttunen TJ, Kivelä J, Parkkila AK, Pastoreková S, Pastorek J, Waheed A, Sly WS, Rajaniemi H (2000) Expression of transmembrane carbonic anhydrase isoenzymes IX and XII in normal human pancreas and pancreatic tumours. *Histochem Cell Biol* 114: 197-204.

Klengel T, Liang WJ, Chaloupka J, Ruoff C, Schröppel K, Naglik JR, Eckert SE, Mogensen EG, Haynes K, Tuite MF, Levin LR, Buck J, Mühlshlegel FA (2005) Fungal adenylyl cyclase integrates CO<sub>2</sub> sensing with cAMP signaling and virulence. *Curr Biol* 15:2021-6.

Krajnc N, Itariu B, Macher S, Marik W, Harreiter J, Michl M, Novak K, Wöber C, Pemp B, and Bsteh G (2023) Treatment with GLP-1 receptor agonists is associated with significant weight loss and favorable headache outcomes in idiopathic intracranial hypertension. *J Headache Pain* 24:89.

Krebs HA (1948) Inhibition of carbonic anhydrase by sulphonamides. *Biochem J.* 43:525-528.

Krungkrai J, Scozzafava A, Reungprapavut S, Krungkrai SR, Rattanajak R, Kamchonwongpaisan S, and Supuran CT (2005) Carbonic anhydrase inhibitors. Inhibition of *Plasmodium falciparum* carbonic anhydrase with aromatic sulfonamides: towards antimalarials with a novel mechanism of action? *Bioorg Med Chem* 13:483-9.

Kugler S, Hahnefeld L, Kloka JA, Ginzl S, Nürnberg-Goloub E, Zinn S, Vehreschild MJ, Zacharowski K, Lindau S, Ullrich E, Burmeister J, Kohlhammer J, Schwäble J, Gurke R, Dorochow E, Bennett A, Dauth S, Campe J, Knappe T, Laux V, Kannt A, Köhm M, Geisslinger G, Resch E, and Behrens F (2024) Short-term predictor for COVID-19 severity from a longitudinal multi-omics study for practical application in intensive care units. *Talanta* 268:125295.

Kumar A, Siwach K, Supuran CT, Sharma PK (2022) A decade of tail-approach based design of selective as well as potent tumor associated carbonic anhydrase inhibitors. *Bioorg Chem* 126:105920.

Lakota J, Lanz A, Dubrovackova M, Jankovicova B, Gonzalez A, and Stern M (2012) Antibodies against carbonic anhydrase in patients with aplastic anemia. *Acta Haematol* 128:190-4.

Le Darz A, Mingot A, Bouazza F, Castelli U, Karam O, Tanc M, Supuran CT, Thibaudeau S (2015) Fluorinated pyrrolidines and piperidines incorporating tertiary benzenesulfonamide moieties are selective carbonic anhydrase II inhibitors. *J Enzyme Inhib Med Chem* 30:737-45.

Lee HS, Bae T, Lee JH, Kim DG, Oh YS, Jang Y, Kim JT, Lee JJ, Innocenti A, Supuran CT, Chen L, Rho K, and Kim S (2012) Rational drug repositioning guided by an integrated pharmacological network of protein, disease and drug. *BMC Syst Biol* 6:80.

Lehneck R, Neumann P, Vullo D, Elleuche S, Supuran CT, Ficner R, and Pöggeler S (2014) Crystal structures of two tetrameric  $\beta$ -carbonic anhydases from the filamentous ascomycete *Sordaria macrospora*. *FEBS J* 281:1759-72.

Leniger T, Thöne J, and Wiemann M (2004) Topiramate modulates pH of hippocampal CA3 neurons by combined effects on carbonic anhydrase and Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchange. *Br J Pharmacol* 142:831-42.

Leniger T, Wiemann M, Bingmann D, Widman G, Hufnagel A, and Bonnet U (2002) Carbonic anhydrase inhibitor sulthiame reduces intracellular pH and epileptiform activity of hippocampal CA3 neurons. *Epilepsia*. 43:469-74.

Leppilampi M, Saarnio J, Karttunen TJ, Kivelä J, Pastoreková S, Pastorek J, Waheed A, Sly WS, Parkkila S (2003) Carbonic anhydrase isozymes IX and XII in gastric tumors. *World J Gastroenterol* 9:1398-403

Leppik IE (2004) Zonisamide: chemistry, mechanism of action, and pharmacokinetics. *Seizure* 13 Suppl 1:S5-9.

Lévy E, Agbokou C, Ferreri F, Chouinard G, and Margolese HC (2007) Topiramate-induced weight loss in schizophrenia: a retrospective case series study. *Can J Clin Pharmacol* 14:e234-9.

Lim GB (2022) Acetazolamide improves decongestion in acute heart failure. *Nat Rev Cardiol*19:720.

Lindskog S (1997) Structure and mechanism of carbonic anhydrase. *Pharmacol Ther* 74:1-20.

Lindskog S and Coleman JE (1973) The catalytic mechanism of carbonic anhydrase. *Proc Natl Acad Sci U S A* 70: 2505-2508.

Lindskog S and Malmstrom BG (1962) Metal binding and catalytic activity in bovine carbonic anhydrase. *J Biol Chem* 237:1129-1137.

Lock FE, McDonald PC, Lou Y, Serrano I, Chafe SC, Ostlund C, Aparicio S, Winum JY, Supuran CT, and Dedhar S (2013) Targeting carbonic anhydrase IX depletes breast cancer stem cells within the hypoxic niche. *Oncogene* 32:5210-9.

Lou Y, McDonald PC, Oloumi A, Chia S, Ostlund C, Ahmadi A, Kyle A, Auf dem Keller U, Leung S, Huntsman D, Clarke B, Sutherland BW, Waterhouse D, Bally M, Roskelley C, Overall CM, Minchinton A, Pacchiano F, Carta F, Scozzafava A, Touisni N, Winum JY, Supuran CT, and Dedhar S (2011) Targeting tumor hypoxia: suppression of breast tumor growth and metastasis by novel carbonic anhydrase IX inhibitors. *Cancer Res* 71:3364-76.

Lounnas N, Rosilio C, Nebout M, Mary D, Griessinger E, Neffati Z, Chiche J, Spits H, Hagenbeek TJ, Asnafi V, Poulsen SA, Supuran CT, Peyron JF, and Imbert V (2013) Pharmacological inhibition of carbonic anhydrase XII interferes with cell proliferation and induces cell apoptosis in T-cell lymphomas. *Cancer Lett* 333:76-88.

Lynch CJ, Fox H, Hazen SA, Stanley BA, Dodgson S, and Lanoue KF (1995) Role of hepatic carbonic anhydrase in de novo lipogenesis. *Biochem J* 310:197-202.

Macauley SR, Zimmerman SA, Apolinario EE, Evilia C, Hou YM, Ferry JG, and Sowers KR (2009) The archetype gamma-class carbonic anhydrase (Cam) contains iron when synthesized in vivo. *Biochemistry* 48:817-9.

Mahmood S, Booker I, Huang J, and Coleman CI (2013) Effect of topiramate on weight gain in patients receiving atypical antipsychotic agents. *J Clin Psychopharmacol* 33:90-4.

Mann T, and Keilin D (1940) Sulphanilamide as a specific carbonic anhydrase inhibitor Carbonic anhydrase. Purification and nature of the enzyme. *Nature* 146: 164–165.

Mansoldo FRP, Carta F, Angeli A, Cardoso VDS, Supuran CT, and Vermelho AB (2020) Chagas Disease: Perspectives on the Past and Present and Challenges in Drug Discovery. *Molecules* 25:5483.

Marapaka AK, Nocentini A, Youse MS, An W, Holly KJ, Das C, Yadav R, Seleem MN, Supuran CT, and Flaherty DP (2022) Structural Characterization of Thiadiazolesulfonamide Inhibitors Bound to *Neisseria gonorrhoeae*  $\alpha$ -Carbonic Anhydrase. *ACS Med Chem Lett* 14:103-109.

Marcus EA, Moshfegh AP, Sachs G, and Scott DR (2005) The periplasmic alpha-carbonic anhydrase activity of *Helicobacter pylori* is essential for acid acclimation. *J Bacteriol* 187:729-38

Maren TH (1967) Carbonic anhydrase: chemistry, physiology, and inhibition. *Physiol Rev* 47: 595-781.

Maren TH (1997) Sulfonamides and secretion of aqueous humor. *J Exp Zool* 279:490-7.

Maresca A, Temperini C, Vu H, Pham NB, Poulsen SA, Scozzafava A, Quinn RJ, Supuran CT (2009) Non-zinc mediated inhibition of carbonic anhydrases: coumarins are a new class of suicide inhibitors. *J Am Chem Soc* 131: 3057-62.

Maresca A, Temperini C, Pochet L, Masereel B, Scozzafava A, Supuran CT (2010) Deciphering the mechanism of carbonic anhydrase inhibition with coumarins and thiocoumarins. *J Med Chem* 53: 335-44

Margheri F, Ceruso M, Carta F, Laurenzana A, Maggi L, Lazzeri S, Simonini G, Annunziato F, Del Rosso M, Supuran CT, and Cimaz R (2016) Overexpression of the transmembrane carbonic anhydrase isoforms IX and XII in the inflamed synovium. *J Enzyme Inhib Med Chem*. 31(sup4):60-63.

Martin R, Pohlers S, Mühlischlegel FA, and Kurzai O (2017) CO<sub>2</sub> sensing in fungi: at the heart of metabolic signaling. *Curr Genet* 63:965-972.

Maryanoff BE, Nortey SO, Gardocki JF, Shank RP, and Dodgson SP (1987) Anticonvulsant O-alkyl sulfamates. 2,3:4,5-Bis-O-(1-methylethylidene)-beta-D-fructopyranose sulfamate and related compounds. *J Med Chem* 30:880-7.

Masdrakis VG and Baldwin DS (2021) Anticonvulsant and antipsychotic medications in the pharmacotherapy of panic disorder: a structured review. *Ther Adv Psychopharmacol* 11:20451253211002320.

Masini E, Carta F, Scozzafava A, and Supuran CT (2013) Antiglaucoma carbonic anhydrase inhibitors: a patent review *Expert Opin Ther Pat* 23: 705-16.

Masuda Y, Noguchi H, and Karasawa T (1994) Evidence against a significant implication of carbonic anhydrase inhibitory activity of zonisamide in its anticonvulsive effects. *Arzneimittelforschung* 44: 267-269.

Masuda Y and Karasawa T (1993) Inhibitory effect of zonisamide on human carbonic anhydrase in vitro. *Arzneimittelforschung* 43: 416-418.

McDonald PC, Chafe SC, Brown WS, Saberi S, Swayampakula M, Venkateswaran G, Nemirovsky O, Gillespie JA, Karasinska JM, Kalloger SE, Supuran CT, Schaeffer DF, Bashashati A, Shah SP, Topham JT, Yapp DT, Li J, Renouf DJ, Stanger BZ, and Dedhar S (2019) Regulation of pH by Carbonic Anhydrase 9 Mediates Survival of Pancreatic Cancer Cells With Activated KRAS in Response to Hypoxia. *Gastroenterology* 157:823-837.

McDonald PC, Chafe SC, Supuran CT, and Dedhar S (2022) Cancer Therapeutic Targeting of Hypoxia Induced Carbonic Anhydrase IX: From Bench to Bedside. *Cancers (Basel)* 14(14):3297.

McDonald PC, Chia S, Bedard PL, Chu Q, Lyle M, Tang L, Singh M, Zhang Z, Supuran CT, Renouf DJ, Dedhar S (2020) A Phase 1 Study of SLC-0111, a Novel Inhibitor of Carbonic Anhydrase IX, in Patients With Advanced Solid Tumors. *Am J Clin Oncol* 43:484-490.

McDonald PC and Dedhar S (2023) Co-vulnerabilities of inhibiting carbonic anhydrase IX in ferroptosis-mediated tumor cell death. *Front Mol Biosci* 10:1327310.

Meekers E, Dauw J, Martens P, Dhont S, Verbrugge FH, Nijst P, Ter Maaten JM, Damman K, Mebazaa A, Filippatos G, Ruschitzka F, Tang WHW, Dupont M, and Mullens W (2023) Renal function and decongestion with acetazolamide in acute decompensated heart failure: the ADVOR trial. *Eur Heart J* 44:3672-3682.

Meldrum NU, Roughton FJ (1933) Carbonic anhydrase. Its preparation and properties. *J Physiol* 80: 113-142.

Menchise V, De Simone G, Alterio V, Di Fiore A, Pedone C, Scozzafava A, and Supuran CT (2005) Carbonic anhydrase inhibitors: stacking with Phe131 determines active site binding region of inhibitors as exemplified by the X-ray crystal structure of a membrane-impermeant antitumor sulfonamide complexed with isozyme II. *J Med Chem* 48:5721-7.

Menteşe A, Erkut N, Sümer A, Us Altay D, Alver A, and Sönmez M (2015) Anti-carbonic anhydrase antibodies in iron deficiency anemia. *Hematology* 20:363-7.

Merlis S (1954) Diamox; a carbonic anhydrase inhibitor; its use in epilepsy. *Neurology* 4:863-8.

Migliardini F, De Luca V, Carginale V, Rossi M, Corbo P, Supuran CT, and Capasso C (2014) Biomimetic CO<sub>2</sub> capture using a highly thermostable bacterial  $\alpha$ -carbonic anhydrase immobilized on a polyurethane foam. *J Enzyme Inhib Med Chem* 29:146-50.

Miller WH, Dessert AM, and Roblin RO Jr. (1950) Heterocyclic sulfonamides as carbonic anhydrase inhibitors. *J Am Chem Soc* 72: 4893–4896.

Minakuchi T, Nishimori I, Vullo D, Scozzafava A, and Supuran CT (2009) Molecular cloning, characterization, and inhibition studies of the Rv1284 beta-carbonic anhydrase from *Mycobacterium tuberculosis* with sulfonamides and a sulfamate. *J Med Chem* 52:2226-32.

Mincione F, Nocentini A, and Supuran CT (2021) Advances in the discovery of novel agents for the treatment of glaucoma. *Expert Opin Drug Discov* 16:1209-1225.

Mishra CB, Kumari S, Angeli A, Bua S, Mongre RK, Tiwari M, and Supuran CT (2021) Discovery of Potent Carbonic Anhydrase Inhibitors as Effective Anticonvulsant Agents: Drug Design, Synthesis, and In Vitro and In Vivo Investigations. *J Med Chem* 64:3100-3114.



Mishra CB, Tiwari M, and Supuran CT (2020) Progress in the development of human carbonic anhydrase inhibitors and their pharmacological applications: Where are we today? *Med Res Rev* 40: 2485-2565.

Modak JK, Liu YC, Machuca MA, Supuran CT, and Roujeinikova A (2015) Structural Basis for the Inhibition of *Helicobacter pylori*  $\alpha$ -Carbonic Anhydrase by Sulfonamides. *PLoS One* 10:e0127149.

Modak JK, Liu YC, Supuran CT, and Roujeinikova A (2016) Structure-Activity Relationship for Sulfonamide Inhibition of *Helicobacter pylori*  $\alpha$ -Carbonic Anhydrase. *J Med Chem* 59:11098-11109.

Modak JK, Tikhomirova A, Gorrell RJ, Rahman MM, Kotsanas D, Korman TM, Garcia-Bustos J, Kwok T, Ferrero RL, Supuran CT, and Roujeinikova A (2019) Anti-*Helicobacter pylori* activity of ethoxzolamide. *J Enzyme Inhib Med Chem* 34: 1660-1667.

Mogensen EG, Janbon G, Chaloupka J, Steegborn C, Fu MS, Moyrand F, Klengel T, Pearson DS, Geeves MA, Buck J, Levin LR, and Mühlshlegel FA (2006) *Cryptococcus neoformans* senses CO<sub>2</sub> through the carbonic anhydrase Can2 and the adenylyl cyclase Cac1. *Eukaryot Cell* 5:103-11.

Monti DM, De Simone G, Langella E, Supuran CT, Di Fiore A, and Monti SM (2017) Insights into the role of reactive sulfhydryl groups of Carbonic Anhydrase III and VII during oxidative damage. *J Enzyme Inhib Med Chem*. 32:5-12.

Mueller SL, Chrysanthopoulos PK, Halili MA, Hepburn C, Nebl T, Supuran CT, Nocentini A, Peat TS, and Poulsen SA (2021) The Glitazone Class of Drugs as Carbonic Anhydrase Inhibitors-A Spin-Off Discovery from Fragment Screening. *Molecules* 26:3010.

Mullens W, Dauw J, Martens P, Verbrugge FH, Nijst P, Meekers E, Tartaglia K, Chenot F, Moubayed S, Dierckx R, Blouard P, Troisfontaines P, Derthoo D, Smolders W, Bruckers L, Droogne W, Ter Maaten JM, Damman K, Lassus J, Mebazaa A, Filippatos G, Ruschitzka F,

Dupont M; and ADVOR Study Group (2022) Acetazolamide in Acute Decompensated Heart Failure with Volume Overload. *N Engl J Med* 387:1185-1195.

Muñoz W, Lamm A, Poppers D, and Lamm S (2018) Acetazolamide promotes decreased consumption of carbonated drinks and weight loss. *Oxf Med Case Reports* 2018:omy081.

Muñoz-Prieto A, Rubić I, Gonzalez-Sanchez JC, Kuleš J, Martínez-Subiela S, Cerón JJ, Bernal E, Torres-Cantero A, Vicente-Romero MR, Mrljak V, and Tvarijonaviciute A (2022) Saliva changes in composition associated to COVID-19: a preliminary study. *Sci Rep* 12:10879.

Murata M, Odawara T, Hasegawa K, Kajiwara R, Takeuchi H, Tagawa M, and Kosaka K (2020) Effect of zonisamide on parkinsonism in patients with dementia with Lewy bodies: A phase 3 randomized clinical trial. *Parkinsonism Relat Disord* 76:91-97.

Mussi S, Rezzola S, Chiodelli P, Nocentini A, Supuran CT, and Ronca R (2022) Antiproliferative effects of sulphonamide carbonic anhydrase inhibitors C18, SLC-0111 and acetazolamide on bladder, glioblastoma and pancreatic cancer cell lines. *J Enzyme Inhib Med Chem* 37:280-286.

Müller TD, Blüher M, Tschöp MH, and DiMarchi RD (2022) Anti-obesity drug discovery: advances and challenges. *Nat Rev Drug Discov* 21:201-223.

Nair SK, Ludwig PA, and Christianson DW (1994) Two-site binding of phenol in the active site of human carbonic anhydrase II: structural implications for substrate association. *J Am Chem Soc* 116: 3659-3660.

Naps MS, Leong SH, Hartwell EE, Rentsch CT, and Kranzler HR (2023) Effects of topiramate therapy on serum bicarbonate concentration in a sample of 10,279 veterans. *Alcohol Clin Exp Res* (Hoboken) 47:438-447.

Nerella SG, Singh P, Thacker PS, Arifuddin M, and Supuran CT (2023) PET radiotracers and fluorescent probes for imaging human carbonic anhydrase IX and XII in hypoxic tumors. *Bioorg Chem* 133:106399.

Neri D and Supuran CT (2011) Interfering with pH regulation in tumours as a therapeutic strategy. *Nat Rev Drug Discov* 10:767-77.

Nia AM, Srinivasan VM, Lall R, and Kan P (2022) Dural Venous Sinus Stenting in Idiopathic Intracranial Hypertension: A National Database Study of 541 Patients. *World Neurosurg* 167:e451-e455.

Nishimori I, Minakuchi T, Kohsaki T, Onishi S, Takeuchi H, Vullo D, Scozzafava A, and Supuran CT (2007) Carbonic anhydrase inhibitors: the beta-carbonic anhydrase from *Helicobacter pylori* is a new target for sulfonamide and sulfamate inhibitors. *Bioorg Med Chem Lett* 17:3585-94.

Nishimori I, Minakuchi T, Morimoto K, Sano S, Onishi S, Takeuchi H, Vullo D, Scozzafava A, Supuran CT (2006) Carbonic anhydrase inhibitors: DNA cloning and inhibition studies of the alpha-carbonic anhydrase from *Helicobacter pylori*, a new target for developing sulfonamide and sulfamate gastric drugs. *J Med Chem* 49:2117-26.

Nishimori I, Minakuchi T, Vullo D, Scozzafava A, Innocenti A, and Supuran CT (2009) Carbonic anhydrase inhibitors. Cloning, characterization, and inhibition studies of a new beta-carbonic anhydrase from *Mycobacterium tuberculosis*. *J Med Chem* 52:3116-20

Nocentini A, Angeli A, Carta F, Winum JY, Zalubovskis R, Carradori S, Capasso C, Donald WA, Supuran CT (2021a) Reconsidering anion inhibitors in the general context of drug design studies of modulators of activity of the classical enzyme carbonic anhydrase. *J Enzyme Inhib Med Chem*. 2021;36(1):561-580

Nocentini A and Supuran CT (2019) Adrenergic agonists and antagonists as antiglaucoma agents: a literature and patent review (2013-2019). *Expert Opin Ther Pat* 29:805-815.

Nocentini A, Supuran CT, and Capasso C (2021b) An overview on the recently discovered iotacarboxylic anhydroses. *J Enzyme Inhib Med Chem* 36:1988-1995.

O'Toole RF, Leong KWC, Cumming V, and Van Hal SJ (2023) Vancomycin-resistant *Enterococcus faecium* and the emergence of new sequence types associated with hospital infection. *Res Microbiol.* 174:104046.

Odawara T, Hasegawa K, Kajiwara R, Takeuchi H, Tagawa M, Kosaka K, and Murata M (2022) Long-Term Efficacy and Safety of Zonisamide for Treatment of Parkinsonism in Patients With Dementia With Lewy Bodies: An Open-Label Extension of a Phase three Randomized Controlled Trial. *Am J Geriatr Psychiatry* 30:314-328.

Odi R, Bibi D, Shusterman B, Erenburg N, Shaul C, Supuran CT, Nocentini A, and Bialer M (2021) Synthesis and Enantioselective Pharmacokinetic/Pharmacodynamic Analysis of New CNS-Active Sulfamoylphenyl Carbamate Derivatives. *Int J Mol Sci* 22:3361.

Oles KS, Penry JK, Cole DL, and Howard G (1989) Use of acetazolamide as an adjunct to carbamazepine in refractory partial seizures. *Epilepsia* 30:74-8.

Onyilmaz M, Koca M, Ammara A, Degirmenci M, Supuran CT (2024) Isocoumarins incorporating chalcone moieties act as isoform selective tumor-associated carbonic anhydrase inhibitors. *Future Med Chem* 16:1347-1355.

Onyilmaz M, Koca M, Bonardi A, Degirmenci M, Supuran CT (2022) Isocoumarins: a new class of selective carbonic anhydrase IX and XII inhibitors. *J Enzyme Inhib Med Chem* 37:743-748.

Pacchiano F, Aggarwal M, Avvaru BS, Robbins AH, Scozzafava A, McKenna R, and Supuran CT (2010) Selective hydrophobic pocket binding observed within the carbonic anhydrase II active site accommodate different 4-substituted-ureido-benzenesulfonamides and correlate to inhibitor potency. *Chem Commun (Camb)* 46:8371-3.

Pacchiano F, Carta F, McDonald PC, Lou Y, Vullo D, Scozzafava A, Dedhar S, and Supuran CT (2011) Ureido-substituted benzenesulfonamides potently inhibit carbonic anhydrase IX and show antimetastatic activity in a model of breast cancer metastasis. *J Med Chem* 54:1896-902.

Pan D, Li X, Jiang J, and Luo L (2021) Effect of levetiracetam in combination with topiramate on immune function, cognitive function, and neuronal nutritional status of children with intractable epilepsy. *Am J Transl Res* 13:10459-10468.

Pan P, Vermelho AB, Capaci Rodrigues G, Scozzafava A, Tolvanen ME, Parkkila S, Capasso C, and Supuran CT (2013) Cloning, characterization, and sulfonamide and thiol inhibition studies of an  $\alpha$ -carbonic anhydrase from *Trypanosoma cruzi*, the causative agent of Chagas disease. *J Med Chem* 56:1761-71.

Parkkila S, Innocenti A, Kallio H, Hilvo M, Scozzafava A, Supuran CT (2009) The protein tyrosine kinase inhibitors imatinib and nilotinib strongly inhibit several mammalian alpha-carbonic anhydrase isoforms. *Bioorg Med Chem Lett* 19:4102-6.

Pastorek J, Pastoreková S, Callebaut I, Mornon JP, Zelník V, Opavský R, Zat'ovicová M, Liao S, Portetelle D, Stanbridge EJ (1994) Cloning and characterization of MN, a human tumor-associated protein with a domain homologous to carbonic anhydrase and a putative helix-loop-helix DNA binding segment. *Oncogene* 9: 2877-88.

Pearl NZ, Babin CP, Catalano NT, Blake JC, Ahmadzadeh S, Shekoohi S, and Kaye AD (2023) Narrative Review of Topiramate: Clinical Uses and Pharmacological Considerations. *Adv Ther* 40:3626-3638.

Peerzada MN, Hamel E, Bai R, Supuran CT, and Azam A (2021) Deciphering the key heterocyclic scaffolds in targeting microtubules, kinases and carbonic anhydrases for cancer drug development. *Pharmacol Ther* 225:107860.

Peppicelli S, Andreucci E, Ruzzolini J, Bianchini F, Nediani C, Supuran CT, and Calorini L (2020) The Carbonic Anhydrase IX inhibitor SLC-0111 as emerging agent against the mesenchymal stem cell-derived pro-survival effects on melanoma cells. *J Enzyme Inhib Med Chem* 35:1185-1193.

Perucca E (1997) A pharmacological and clinical review on topiramate, a new antiepileptic drug. *Pharmacol Res* 35: 241-256.

Petreni A, De Luca V, Scaloni A, Nocentini A, Capasso C, Supuran CT (2021) Anion inhibition studies of the Zn(II)-bound  $\alpha$ -carbonic anhydrase from the Gram-negative bacterium *Burkholderia territorii*. *J Enzyme Inhib Med Chem* 36:372-376.

Pettersen EO, Ebbesen P, Gieling RG, Williams KJ, Dubois L, Lambin P, Ward C, Meehan J, Kunkler IH, Langdon SP, Ree AH, Flatmark K, Lyng H, Calzada MJ, Peso LD, Landazuri MO, Görlach A, Flamm H, Kieninger J, Urban G, Weltin A, Singleton DC, Haider S, Buffa FM, Harris AL, Scozzafava A, Supuran CT, Moser I, Jobst G, Busk M, Toustrup K, Overgaard J, Alsner J, Pouyssegur J, Chiche J, Mazure N, Marchiq I, Parks S, Ahmed A, Ashcroft M, Pastorekova S, Cao Y, Rouschop KM, Wouters BG, Koritzinsky M, Mujcic H, Cojocari D (2015) Targeting tumour hypoxia to prevent cancer metastasis. From biology, biosensing and technology to drug development: the METOXIA consortium. *J Enzyme Inhib Med Chem* 30:689-721.

Pick AM and Nystrom KK (2012) Pazopanib for the treatment of metastatic renal cell carcinoma. *Clin Ther* 34:511-20.

Ponticello GS, Sugrue MF, Plazonnet B, and Durand-Cavagna G (1998) Dorzolamide, a 40-year wait. From an oral to a topical carbonic anhydrase inhibitor for the treatment of glaucoma. *Pharm Biotechnol* 11:555-74.

Porcelli MJ and Gugelchuk GM (1995) A trek to the top: a review of acute mountain sickness. *J Am Osteopath Assoc* 95, 718–720.

Provensi G, Carta F, Nocentini A, Supuran CT, Casamenti F, Passani MB, and Fossati S (2019) A New Kid on the Block? Carbonic Anhydrases as Possible New Targets in Alzheimer's Disease. *Int J Mol Sci* 20:4724.

Pugh CW and Ratcliffe PJ (2017) New horizons in hypoxia signaling pathways. *Exp Cell Res* 356: 116-121.

Puşçaş I (1984) Treatment of gastroduodenal ulcers with carbonic anhydrase inhibitors. *Ann N Y Acad Sci* 429:587-91.

Puscas I, Coltau M, Farcau D, Puscas C, Supuran CT (1994) Carbonic anhydrase and cancer. In *"Carbonic anhydrase and modulation of physiologic and pathologic processes in the organism"*, Puscas I, Ed., Helicon Press, Timisoara (Romania), 1994, pp. 551-558.

Pustenko A, Balašova A, Nocentini A, Supuran CT, Žalubovskis R (2023) 3H-1,2-Benzoxaphosphepine 2-oxides as selective inhibitors of carbonic anhydrase IX and XII. *J Enzyme Inhib Med Chem* 38:216-224.

Pustenko A, Nocentini A, Gratteri P, Bonardi A, Vozny I, Žalubovskis R, and Supuran CT (2020) The antibiotic furagin and its derivatives are isoform-selective human carbonic anhydrase inhibitors. *J Enzyme Inhib Med Chem* 35:1011-1020.

Rahman MM, Tikhomirova A, Modak JK, Hutton ML, Supuran CT, and Roujeinikova A (2020) Antibacterial activity of ethoxzolamide against *Helicobacter pylori* strains SS1 and 26695. *Gut Pathog* 12:20.

Reed DR, Pierce EJ, Sen JM, Keng MK (2019) A prospective study on urine alkalization with an oral regimen consisting of sodium bicarbonate and acetazolamide in patients receiving high-dose methotrexate. *Cancer Manag Res* 11:8065-8072.

Reiss WG and Oles KS (1996) Acetazolamide in the treatment of seizures. *Ann Pharmacother* 30:514-9.

Romanelli MN (2024) Cyclooxygenase. In *Metalloenzymes from Bench to Bedside* (Supuran CT, Donald WA Eds.), pp. 431-447, Elsevier/Academic Press, London.

Rosenfeld WE (1997) Topiramate: a review of preclinical, pharmacokinetic, and clinical data. *Clin Ther* 19: 1294-1308.

Rubin DHF, Ma KC, Westervelt KA, Hullahalli K, Waldor MK, and Grad YH. CanB is a metabolic mediator of antibiotic resistance in *Neisseria gonorrhoeae*. *Nat Microbiol.* 8:28-39.

Ruusuvuori E and Kaila K (2014) Carbonic anhydrases and brain pH in the control of neuronal excitability. *Subcell Biochem* 75:271-90.

Sakurai K, Nagahara A, Inoue K, Akiyama J, Mabe K, Suzuki J, Habu Y, Araki A, Suzuki T, Satoh K, Nagami H, Harada R, Tano N, Kusaka M, Fujioka Y, Fujimura T, Shigeto N, Oumi T, Miwa J, Miwa H, Fujimoto K, Kinoshita Y, and Haruma K (2012) Efficacy of omeprazole, famotidine, mosapride and teprenone in patients with upper gastrointestinal symptoms: an omeprazole-controlled randomized study (J-FOCUS). *BMC Gastroenterol* 12:42

Salam MA, Al-Amin MY, Salam MT, Pawar JS, Akhter N, Rabaan AA, and Alqumber MAA (2023) Antimicrobial Resistance: A Growing Serious Threat for Global Public Health. *Healthcare (Basel).* 11:1946.

Salameh TS, Mortell WG, Logsdon AF, Butterfield DA, Banks WA (2019) Disruption of the hippocampal and hypothalamic blood-brain barrier in a diet-induced obese model of type II diabetes: prevention and treatment by the mitochondrial carbonic anhydrase inhibitor, topiramate. *Fluids Barriers CNS* 16:1.



Santi A, Caselli A, Paoli P, Corti D, Camici G, Pieraccini G, Taddei ML, Serni S, Chiarugi P, and Cirri P (2013) The effects of CA IX catalysis products within tumor microenvironment. *Cell Commun Signal* 11:81.

Sarayani A, Winterstein A, Cristofolletti R, Vozmediano V, Schmidt S, and Brown J (2023) Real-world effect of a potential drug-drug interaction between topiramate and oral contraceptives on unintended pregnancy outcomes. *Contraception* 120:109953.

Sari C, Seip RL, and Umashanker D (2021) Case Report: Off Label Utilization of Topiramate and Metformin in Patients With BMI  $\geq 50$  kg/m<sup>2</sup> Prior to Bariatric Surgery. *Front Endocrinol (Lausanne)* 12:588016.

Sarnella A, Ferrara Y, Auletta L, Albanese S, Cerchia L, Alterio V, De Simone G, Supuran CT, and Zannetti A (2022) Inhibition of carbonic anhydrases IX/XII by SLC-0111 boosts cisplatin effects in hampering head and neck squamous carcinoma cell growth and invasion. *J Exp Clin Cancer Res* 41:122.

Schlicker C, Hall RA, Vullo D, Middelhaufe S, Gertz M, Supuran CT, Mühlischlegel FA, and Steegborn C (2009) Structure and inhibition of the CO<sub>2</sub>-sensing carbonic anhydrase Can2 from the pathogenic fungus *Cryptococcus neoformans*. *J Mol Biol* 385:1207-20.

Schneiderhan ME and Marvin R (2007) Is acetazolamide similar to topiramate for reversal of antipsychotic-induced weight gain? *Am J Ther* 14:581-4.

Schulze Wischeler J, Innocenti A, Vullo D, Agrawal A, Cohen SM, Heine A, Supuran CT, Klebe G (2010) Bidentate Zinc chelators for alpha-carbonic anhydrases that produce a trigonal bipyramidal coordination geometry. *ChemMedChem* 5:1609-15.

Schutz FA, Choueiri TK, and Sternberg CN (2011) Pazopanib: Clinical development of a potent anti-angiogenic drug. *Crit Rev Oncol Hematol* 77:163-71.

Scozzafava A, Briganti F, Mincione G, Menabuoni L, Mincione F, Supuran CT (1999a) Carbonic anhydrase inhibitors: synthesis of water-soluble, aminoacyl/dipeptidyl sulfonamides possessing long-lasting intraocular pressure-lowering properties via the topical route. *J Med Chem* 42:3690-700

Scozzafava A, Menabuoni L, Mincione F, Briganti F, Mincione G, and Supuran CT (1999b) Carbonic anhydrase inhibitors. Synthesis of water-soluble, topically effective, intraocular pressure-lowering aromatic/heterocyclic sulfonamides containing cationic or anionic moieties: is the tail more important than the ring? *J Med Chem*. 1999; 42: 2641-2650.

Scozzafava A, Supuran CT, Carta F (2013) Antiobesity carbonic anhydrase inhibitors: a literature and patent review. *Expert Opin Ther Pat* 23:725-35.

Semenza GL (2019) Pharmacologic Targeting of Hypoxia-Inducible Factors. *Annu Rev Pharmacol Toxicol* 59:379-403.

Shah GN, Morofuji Y, Banks WA, and Price TO (2013) High glucose-induced mitochondrial respiration and reactive oxygen species in mouse cerebral pericytes is reversed by pharmacological inhibition of mitochondrial carbonic anhydrases: Implications for cerebral microvascular disease in diabetes. *Biochem Biophys Res Commun* 440:354-8.

Shi XY, Hu LY, Liu MJ, and Zou LP (2017) Hypercapnia-induced brain acidosis: Effects and putative mechanisms on acute kainate induced seizures. *Life Sci* 176:82-87.

Silver LH (1998) Clinical efficacy and safety of brinzolamide (Azopt), a new topical carbonic anhydrase inhibitor for primary open-angle glaucoma and ocular hypertension. Brinzolamide Primary Therapy Study Group. *Am J Ophthalmol* 126:400-8.

Simonsson I, Jonsson BH, and Lindskog S (1982) Phenol, a competitive inhibitor of CO<sub>2</sub> hydration catalyzed by carbonic anhydrase. *Biochem Biophys Res Commun* 108:1406-12.

Sjöblom B, Polentarutti M, Djinovic-Carugo K (2009) Structural study of X-ray induced activation of carbonic anhydrase. *Proc Natl Acad Sci U S A* 106:10609-10613

Smith KS, Ingram-Smith C, and Ferry JG (2002) Roles of the conserved aspartate and arginine in the catalytic mechanism of an archaeal beta-class carbonic anhydrase. *J Bacteriol* 184:4240-5.

Smith KS, Jakubzick C, Whittam TS, and Ferry JG (1999) Carbonic anhydrase is an ancient enzyme widespread in prokaryotes. *Proc Natl Acad Sci U S A* 96:15184-9.

Solesio ME, Peixoto PM, Debure L, Madamba SM, de Leon MJ, Wisniewski T, Pavlov EV, and Fossati S (2018) Carbonic anhydrase inhibition selectively prevents amyloid  $\beta$  neurovascular mitochondrial toxicity. *Aging Cell* 17: e12787

Steiner H, Jonsson BH, and Lindskog S (1975) The catalytic mechanism of carbonic anhydrase. *Eur J Biochem* 59: 253-259.

Sterrett SP, Penniston KL, Wolf JS Jr, and Nakada SY (2008) Acetazolamide is an effective adjunct for urinary alkalization in patients with uric acid and cystine stone formation recalcitrant to potassium citrate. *Urology* 72: 278-81.

Supuran CT (2008) Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. *Nat Rev Drug Discov* 7:168-81.

Supuran CT (2012) Carbonic anhydrase inhibitors as emerging drugs for the treatment of obesity. *Expert Opin Emerg Drugs* 17:11-5.

Supuran CT (2015) Acetazolamide for the treatment of idiopathic intracranial hypertension. *Expert Rev Neurother* 15:851-6.

Supuran CT (2016a) Structure and function of carbonic anhydrases. *Biochem J* 473: 2023-2032.

Supuran CT (2016b) How many carbonic anhydrase inhibition mechanisms exist? *J Enzyme Inhib Med Chem* 31:345-60.

Supuran CT (2016c) Carbonic anhydrase inhibition and the management of neuropathic pain. *Expert Rev Neurother* 16:961-8.

Supuran CT (2018a) Carbonic anhydrase activators. *Future Med Chem* 10:561-573.

Supuran CT (2018b) Carbonic anhydrase inhibitors and their potential in a range of therapeutic areas. *Expert Opin Ther Pat* 28:709-712.

Supuran CT (2018c) Applications of carbonic anhydrases inhibitors in renal and central nervous system diseases. *Expert Opin Ther Pat* 28:713-721.

Supuran CT (2019) Agents for the prevention and treatment of age-related macular degeneration and macular edema: a literature and patent review. *Expert Opin Ther Pat*. 29:761-767.

Supuran CT (2020a) An update on drug interaction considerations in the therapeutic use of carbonic anhydrase inhibitors. *Expert Opin Drug Metab Toxicol* 16:297-307.

Supuran CT (2020b) Experimental Carbonic Anhydrase Inhibitors for the Treatment of Hypoxic Tumors. *J Exp Pharmacol* 12:603-617.

Supuran CT (2021a) Carbonic anhydrase inhibitors: an update on experimental agents for the treatment and imaging of hypoxic tumors. *Expert Opin Investig Drugs* 30:1197-1208.

Supuran CT (2021b) Emerging role of carbonic anhydrase inhibitors. *Clin Sci (Lond)*. 135:1233-1249.

Supuran CT (2021c) Multitargeting approaches involving carbonic anhydrase inhibitors: hybrid drugs against a variety of disorders. *J Enzyme Inhib Med Chem* 36:1702-1714.

Supuran CT (2022) Anti-obesity carbonic anhydrase inhibitors: challenges and opportunities. *J Enzyme Inhib Med Chem* 37:2478-2488.

Supuran CT (2023a) A simple yet multifaceted 90 years old, evergreen enzyme: Carbonic anhydrase, its inhibition and activation. *Bioorg Med Chem Lett* 93:129411.

Supuran CT (2023b) Carbonic anhydrase versatility: from pH regulation to CO<sub>2</sub> sensing and metabolism. *Front Mol Biosci* 10:1326633.

Supuran CT (2023c) An overview of novel antimicrobial carbonic anhydrase inhibitors. *Expert Opin Ther Targets*. 27:897-910.

Supuran CT (2023d) Targeting carbonic anhydrases for the management of hypoxic metastatic tumors. *Expert Opin Ther Pat* 33:701-720.

Supuran CT (2023e) Antiprotozoal drugs: challenges and opportunities. *Expert Opin Ther Pat*. 33: 133-136.

Supuran CT (2024a) Drug interactions of carbonic anhydrase inhibitors and activators. *Expert Opin Drug Metab Toxicol* 20:143-155.

Supuran CT (2024b) Novel carbonic anhydrase inhibitors for the treatment of *Helicobacter pylori* infection. *Expert Opin Investig Drugs* (in press) Mar 27:1-10. doi: 10.1080/13543784.2024.2334714.

Supuran CT, Altamimi ASA, and Carta F (2019) Carbonic anhydrase inhibition and the management of glaucoma: a literature and patent review 2013-2019. *Expert Opin Ther Pat* 29:781-792.

Supuran CT, Alterio V, Di Fiore A, D' Ambrosio K, Carta F, Monti SM, and De Simone G. (2018) Inhibition of carbonic anhydrase IX targets primary tumors, metastases, and cancer stem cells: Three for the price of one. *Med Res Rev* 38:1799-1836.

Supuran CT and Capasso C (2021) A Highlight on the Inhibition of Fungal Carbonic Anhydrases as Drug Targets for the Antifungal Armamentarium. *Int J Mol Sci*. 2021 22:4324.

Supuran CT, Casini A, Mastrolorenzo A, and Scozzafava A (2004) COX-2 selective inhibitors, carbonic anhydrase inhibition and anticancer properties of sulfonamides belonging to this class of pharmacological agents. *Mini Rev Med Chem* 4:625-32.

Supuran CT, Di Fiore A, and De Simone G (2008) Carbonic anhydrase inhibitors as emerging drugs for the treatment of obesity. *Expert Opin Emerg Drugs* 13:383-92.

Supuran CT, Scozzafava A (2000) Carbonic anhydrase inhibitors and their therapeutic potential. *Expert Opin Ther Pat* 10: 575-600.

Svastová E, Hulíková A, Rafajová M, Zat'ovicová M, Gibadulinová A, Casini A, Cecchi A, Scozzafava A, Supuran CT, Pastorek J, and Pastoreková S (2004) Hypoxia activates the capacity of tumor-associated carbonic anhydrase IX to acidify extracellular pH. *FEBS Lett* 577: 439-45

Swenson ER (2022) Chronic Mountain Sickness Evolving Over Time: New Data From on High. *Chest* 161:1136-1137.

Syrjänen L, Vermelho AB, Rodrigues Ide A, Corte-Real S, Salonen T, Pan P, Vullo D, Parkkila S, Capasso C, and Supuran CT (2013) Cloning, characterization, and inhibition studies of a  $\beta$ -carbonic anhydrase from *Leishmania donovani chagasi*, the protozoan parasite responsible for leishmaniasis. *J Med Chem.* 56:7372-81.

Tanini D, Capperucci A, Ferraroni M, Carta F, Angeli A, Supuran CT (2020) Direct and straightforward access to substituted alkyl selenols as novel carbonic anhydrase inhibitors. *Eur J Med Chem* 185:111811.

Tanpure RP, Ren B, Peat TS, Bornaghi LF, Vullo D, Supuran CT, and Poulsen SA (2015) Carbonic anhydrase inhibitors with dual-tail moieties to match the hydrophobic and hydrophilic halves of the carbonic anhydrase active site. *J Med Chem* 58:1494-501

Tars K, Vullo D, Kazaks A, Leitans J, Lends A, Grandane A, Zalubovskis R, Scozzafava A, Supuran CT (2013) Sulfocoumarins (1,2-benzoxathiine-2,2-dioxides): a class of potent and isoform-selective inhibitors of tumor-associated carbonic anhydrases. *J Med Chem* 56: 293-300.

Taverna S, Sancini G, Mantegazza M, Franceschetti S, Avanzini G (1999) Inhibition of transient and persistent Na<sup>+</sup> current fractions by the new anticonvulsant topiramate. *J Pharmacol Exp Ther* 288:960-8.

Teicher BA, Liu SD, Liu JT, Holden SA, and Herman TS (1993) A carbonic anhydrase inhibitor as a potential modulator of cancer therapies. *Anticancer Res* 13: 1549-56.

Temperini C, Cecchi A, Scozzafava A, and Supuran CT (2008) Carbonic anhydrase inhibitors. Sulfonamide diuretics revisited--old leads for new applications? *Org Biomol Chem* 6:2499-506.

Temperini C, Cecchi A, Scozzafava A, and Supuran CT (2009) Carbonic anhydrase inhibitors. Comparison of chlorthalidone and indapamide X-ray crystal structures in adducts with isozyme II: when three water molecules and the keto-enol tautomerism make the difference. *J Med Chem* 52:322-8.

Temperini C, Innocenti A, Mastrolorenzo A, Scozzafava A, and Supuran CT (2017) Carbonic anhydrase inhibitors. Interaction of the antiepileptic drug sulthiame with twelve mammalian isoforms: kinetic and X-ray crystallographic studies. *Bioorg Med Chem Lett* 17:4866-72.

Temperini C, Innocenti A, Scozzafava A, Parkkila S, Supuran CT (2010) The coumarin-binding site in carbonic anhydrase accommodates structurally diverse inhibitors: the antiepileptic lacosamide as an example. *J Med Chem* 53: 850-4.

Teppema LJ, Balanos GM, Steinback CD, Brown AD, Foster GE, Duff HJ, Leigh R, and Poulin MJ (2007) Effects of acetazolamide on ventilatory, cerebrovascular, and pulmonary vascular responses to hypoxia. *Am J Respir Crit Care Med* 175: 277-281.

Theparambil SM, Begum G, and Rose CR (2024) pH regulating mechanisms of astrocytes: A critical component in physiology and disease of the brain. *Cell Calcium* 120:102882.

Thiry A, Dogné JM, Supuran CT, and Masereel B (2008) Anticonvulsant sulfonamides/sulfamates/sulfamides with carbonic anhydrase inhibitory activity: drug design and mechanism of action. *Curr Pharm Des.* 14:661-71.

Tiselius HG (2010) New horizons in the management of patients with cystinuria. *Curr Opin Urol* 20:169-73.

Tohge R, Kaneko S, Morise S, Oki M, Takenouchi N, Murakami A, Nakamura M, Kusaka H, and Yakushiji Y (2021) Zonisamide attenuates the severity of levodopa-induced dyskinesia via modulation of the striatal serotonergic system in a rat model of Parkinson's disease. *Neuropharmacology* 198:108771.

Tripp BC and Ferry JG (2000) A structure-function study of a proton transport pathway in the gamma-class carbonic anhydrase from *Methanosarcina thermophila*. *Biochemistry* 39:9232-40.

Truong HH, Reddy S, Charkviani M, Nikravangolsefid N, Ninan J, Hassett L, Kashani KB, Domecq JP (2024) Acetazolamide for acute kidney injury in patients undergoing high dose methotrexate therapy: a systematic review and meta-analysis. *J Nephrol* (in press) Jan 24. doi: 10.1007/s40620-023-01850-2.

Truppo E, Supuran CT, Sandomenico A, Vullo D, Innocenti A, Di Fiore A, Alterio V, De Simone G, and Monti SM (2012) Carbonic anhydrase VII is S-glutathionylated without loss of catalytic activity and affinity for sulfonamide inhibitors. *Bioorg Med Chem Lett* 22:1560-4.

Tsikas D (2024) Acetazolamide and human carbonic anhydrases: retrospect, review and discussion of an intimate relationship. *J Enzyme Inhib Med Chem.* 39:2291336.



Tsuboi Y, Nakamura M, Maruyama H, and Matsumoto Y (2021) Zonisamide improves wearing off in Parkinson's disease without exacerbating dyskinesia: Post hoc analysis of phase 2 and phase 3 clinical trials. *J Neurol Sci* 430:120026.

Tu C, Silverman DN, Forsman C, Jonsson BH, Lindskog S (1989) Role of histidine 64 in the catalytic mechanism of human carbonic anhydrase II studied with a site-specific mutant. *Biochemistry* 28: 7913-7918.

Türeci O, Sahin U, Vollmar E, Siemer S, Göttert E, Seitz G, Parkkila AK, Shah GN, Grubb JH, Pfreundschuh M, Sly WS (1998) Human carbonic anhydrase XII: cDNA cloning, expression, and chromosomal localization of a carbonic anhydrase gene that is overexpressed in some renal cell cancers. *Proc Natl Acad Sci U S A* 95: 7608-13.

Ueda K, Nishida H, and Beppu T (2012) Dispensabilities of carbonic anhydrase in proteobacteria. *Int J Evol Biol* 2012:324549.

Uldall M, Botfield H, Jansen-Olesen I, Sinclair A, and Jensen R (2017) Acetazolamide lowers intracranial pressure and modulates the cerebrospinal fluid secretion pathway in healthy rats. *Neurosci Lett* 645:33-39.

Urbański LJ, Angeli A, Hytönen VP, Di Fiore A, De Simone G, Parkkila S, and Supuran CT (2021) Inhibition of the  $\beta$ -carbonic anhydrase from the protozoan pathogen *Trichomonas vaginalis* with sulphonamides. *J Enzyme Inhib Med Chem* 36:329-334.

Urbański LJ, Angeli A, Mykuliak VV, Azizi L, Kuuslahti M, Hytönen VP, Supuran CT, and Parkkila S (2022) Biochemical and structural characterization of beta-carbonic anhydrase from the parasite *Trichomonas vaginalis*. *J Mol Med (Berl)* 100:115-124.

Urbański LJ, Di Fiore A, Azizi L, Hytönen VP, Kuuslahti M, Buonanno M, Monti SM, Angeli A, Zolfaghari Enameh R, Supuran CT, De Simone G, and Parkkila S (2020) Biochemical and

structural characterisation of a protozoan beta-carbonic anhydrase from *Trichomonas vaginalis*. J Enzyme Inhib Med Chem 35:1292-1299.

Vermelho AB, Capaci GR, Rodrigues IA, Cardoso VS, Mazotto AM, and Supuran CT (2017) Carbonic anhydrases from Trypanosoma and Leishmania as anti-protozoan drug targets. Bioorg Med Chem 25:1543-1555.

Vermelho AB, Capaci GR, Rodrigues IA, Cardoso VS, Mazotto AM, Supuran CT. Carbonic anhydrases from Trypanosoma and Leishmania as anti-protozoan drug targets. Bioorg Med Chem. 2017 Mar 1;25(5):1543-1555.

Vermelho AB, da Silva Cardoso V, Ricci Junior E, Dos Santos EP, and Supuran CT (2018) Nanoemulsions of sulfonamide carbonic anhydrase inhibitors strongly inhibit the growth of Trypanosoma cruzi. J Enzyme Inhib Med Chem 33:139-146.

Vermelho AB, Rodrigues GC, and Supuran CT (2020) Why hasn't there been more progress in new Chagas disease drug discovery? Expert Opin Drug Discov 15:145-158.

Vullo D, Lehneck R, Donald WA, Pöggeler S, and Supuran CT (2020) Sulfonamide Inhibition Studies of the  $\beta$ -Class Carbonic Anhydrase CAS3 from the Filamentous Ascomycete Sordaria macrospora. Molecules 25:1036.

Wang K, Liao PY, Chang WC, Yang CR, Su YT, Wu PC, Wu YC, Hung YC, Akhtar N, Lai HC, and Ma WL (2024) Linoleate-pazopanib conjugation as active pharmacological ingredient to abolish hepatocellular carcinoma growth. Front Pharmacol 14:1281067.

Weber A, Casini A, Heine A, Kuhn D, Supuran CT, Scozzafava A, and Klebe G (2004) Unexpected nanomolar inhibition of carbonic anhydrase by COX-2-selective celecoxib: new pharmacological opportunities due to related binding site recognition. J Med Chem 47:550-7.

Wey S, Brill DA, Miraldi Utz V, and Sisk RA (2023) Carbonic anhydrase inhibitors limit complications in X-linked retinoschisis. *Front Med (Lausanne)* 10:1281068.

West JB (2004) The physiologic basis of high-altitude diseases. *Ann Intern Med* 141: 789- 800.

Willems LM, van der Goten M, von Podewils F, Knake S, Kovac S, Zöllner JP, Rosenow F, and Strzelczyk A (2023) Adverse Event Profiles of Antiseizure Medications and the Impact of Coadministration on Drug Tolerability in Adults with Epilepsy. *CNS Drugs* 37:531-544.

Winum JY, Innocenti A, Scozzafava A, Montero JL, and Supuran CT (2009) Carbonic anhydrase inhibitors. Inhibition of the human cytosolic isoforms I and II and transmembrane, tumor-associated isoforms IX and XII with boronic acids. *Bioorg Med Chem* 17:3649-52.

Winum JY, Maresca A, Carta F, Scozzafava A, and Supuran CT (2012) Polypharmacology of sulfonamides: pazopanib, a multitargeted receptor tyrosine kinase inhibitor in clinical use, potently inhibits several mammalian carbonic anhydrases. *Chem Commun (Camb)* 48:8177-9.

Winum JY and Supuran CT (2015) Recent advances in the discovery of zinc-binding motifs for the development of carbonic anhydrase inhibitors. *J Enzyme Inhib Med Chem* 30:321-4.

Winum JY, Temperini C, El Cheikh K, Innocenti A, Vullo D, Ciattini S, Montero JL, Scozzafava A, and Supuran CT (2006) Carbonic anhydrase inhibitors: clash with Ala65 as a means for designing inhibitors with low affinity for the ubiquitous isozyme II, exemplified by the crystal structure of the topiramate sulfamide analogue. *J Med Chem* 49:7024-31.

Wistrand PJ (1951) Carbonic anhydrase in the anterior uvea of the rabbit. *Acta Physiol Scand* 24:144-8.

Wistrand PJ (1980) Human renal cytoplasmic carbonic anhydrase. Tissue levels and kinetic properties under near physiological conditions. *Acta Physiol Scand.* 109:239-48.

Wistrand PJ (1984) The use of carbonic anhydrase inhibitors in ophthalmology and clinical medicine. *Ann N Y Acad Sci* 429:609-19.

Wolny M, Rozanova S, Knabbe C, Pfeiffer K, Barkovits K, Marcus K, and Birschmann I (2023) Changes in the Proteome of Platelets from Patients with Critical Progression of COVID-19. *Cells*. 12:2191.

Xu Y, Feng L, Jeffrey PD, Shi Y, and Morel FM (2008) Structure and metal exchange in the cadmium carbonic anhydrase of marine diatoms. *Nature* 452: 56-61.

Xu H, Li P, Ma H, Tan Y, Wang X, Cai F, Xu J, Sun H, Zhuang H, and Hua ZC (2023) ADT-OH synergistically enhanced the antitumor activity of celecoxib in human colorectal cancer cells. *Cancer Med* 12:17193-17211.

Zhang WT, Wang MR, Hua GD, Li QY, Wang XJ, Lang R, Weng WL, Xue CM, and Zhu BC (2021) Inhibition of Aspirin-Induced Gastrointestinal Injury: Systematic Review and Network Meta-Analysis. *Front Pharmacol* 12:730681.

Zolfaghari Emameh R, Barker HR, Syrjänen L, Urbański L, Supuran CT, and Parkkila S (2016) Identification and inhibition of carbonic anhydrases from nematodes. *J Enzyme Inhib Med Chem* 31(sup4):176-184.

Zolfaghari Emameh R, Kuuslahti M, Vullo D, Barker HR, Supuran CT, Parkkila S. *Ascaris lumbricoides*  $\beta$  carbonic anhydrase: a potential target enzyme for treatment of ascariasis. *Parasit Vectors*. 2015 Sep 18;8:479.

Zullino DF, Krenz S, Besson J (2003) AMPA blockade may be the mechanism underlying the efficacy of topiramate in PTSD. *J Clin Psychiatry* 64:219-20.

Tables

Table 1. Human CA (hCA) isoforms, their distribution, localization/activity level, and involvement

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Isoform	Organ/tissue distribution	Subcellular localization/activity level	Diseases in which is involved
hCA I	Erythrocytes, gastrointestinal tract, eyes	Cytosolic/low	Retinal/cerebral edema
hCA II	Erythrocytes, eyes, gastrointestinal tract, bone osteoclasts, kidneys, lungs, testis, brain	Cytosolic/high	Glaucoma, edema, epilepsy, altitude sickness, gastric cancer
hCA III	Skeletal muscle, adipocytes	Cytosolic/very low	Oxidative stress, obesity
hCA IV	Kidneys, lungs, pancreas, brain capillaries, colon, heart muscle, eyes	Membrane-bound/high	Glaucoma, retinis pigmentosa
hCA VA	Liver, brain	Mitochondrial/moderate	Obesity, diabetic cerebrovascular disease; Alzheimer's disease ? Parkinson's diseases ?
hCA VB	Heart and skeletal muscle, pancreas, kidneys, spinal cord, gastrointestinal tract	Mitochondrial/high	Obesity Alzheimer's disease ?
hCA VI	Salivary and mammary glands	Secreted/moderate	Cariogenesis
hCA VII	Brain	Cytosolic/high	Epilepsy, neuropathic pain
hCA IX	Tumors, gastrointestinal mucosa	Transmembrane/high	Hypoxic tumors, inflammation
hCA XII	Kidneys, intestine, reproductive epithelia, eyes, tumors	Transmembrane/high	Cancer, glaucoma, inflammation
hCA XIII	Kidneys, brain, lung, gut, reproductive tract	Cytosolic/low	Sterility ?
hCA XIV	Kidneys, brain, liver, skeletal muscle	Transmembrane/medium	Epilepsy, rethinopathies

Table 2. CAI chemical classes belonging to three inhibition mechanisms: (i) metal binders, (ii) inhibitors which anchor to the metal coordinated water, and (iii) occluders of the active site entrance.

Metal ion binders	Anchoring to the metal coordinated water	Occlusion of the active site
Sulfonamides (primary) Sulfamates Sulfamides Thiols Boronic acids Dihiocarbamates Monothiocarbamates Xanthates Trithiocarbonates Carboxylates (Thio)hydroxamates Tropolones Selenols Ninhydrins Carbamates Secondary sulfonamides Phosphonamidates Benzoxaboroles Carbamimidothioates Hydantoins 2,4-Oxazolidinediones Thiazolidinediones Selenocarbamates Inorganic anions	Phenols Catechols Polyamines Sulfocoumarins 2-Thioxo-coumarins Alcohols Carboxylates	Coumarins Thiocoumarins Selenocoumarins Benzoxepinones Monocyclic lactones Monocyclic thiolactones Isocoumarins Phosphocoumarins 3 H-1,2-benzoxaphosphepine 2-oxides 1,2,3-benzoxathiazine 2,2-dioxides Lacosamide

Table 3. Clinical trials of anticancer/antimetastatic CAIs, alone or in combination with other agents.

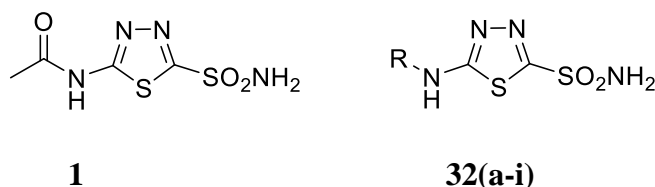
CAI	Combination drug	Phase	ClinicalTrials.gov Identifier
Acetazolamide	Temozolamide	I	NCT03011671 (malignant glioma)
Acetazolamide	Etoposide + Platinum derivatives	I	NCT03467360 (small cells lung cancer)
SLC-0111	Safety trial	I (completed)	NCT02215850 (advanced solid tumors)
SLC-0111	Gemcitabine	Ib/II	NCT03450018 (pancreas, metastatic)
Benzolamide	Temozolamide	II	NCT04209790 (glioblastoma)

Table 4. CAs present in several pathogenic bacterial species which have been investigated as antiinfective targets and the diseases/infections they produce (Capasso and Supuran, 2015; Supuran, 2023c).

<b>Bacterial pathogen</b>	<b>CA family</b>	<b>Disease</b>	<b>Infection(s)</b>
<i>Neisseria gonorrhoeae</i>	$\alpha, \beta$	gonorrhoea	sexually transmitted disease
<i>Helicobacter pylori</i>	$\alpha, \beta$ and $\gamma$	gastritis and gastric ulcers	inflammation of the stomach lining, may lead to gastric cancer
<i>Enterococcus</i> spp.	$\alpha$ and $\gamma$	no specific name	vancomycin resistant enterococci (VRE) involved in nosocomial infections
<i>Vibrio cholerae</i>	$\alpha, \beta$ and $\gamma$	cholera	severe diarrhea.
<i>Mycobacterium tuberculosis</i>	$\beta$ and $\gamma$	tuberculosis	infection attacking lungs or other organs.
<i>Francisella tularensis</i>	$\beta$ and $\gamma$	tularemia	debilitating febrile illness
<i>Clostridium perfringens</i>	$\beta$ and $\gamma$	food poisoning	death as a result of food poisoning.
<i>Streptococcus pneumoniae</i>	$\beta$ and $\gamma$	pneumonia	inflammatory condition of the lung
<i>Streptococcus mutans</i>	$\beta$ and $\gamma$	dental caries	infectious of the dental hard tissues
<i>Salmonella enterica</i>	$\beta$	salmonellosis	diarrhea, fever, vomiting, and abdominal cramps
<i>Haemophilus influenzae</i>	$\beta$	influenza	nausea, vomiting, gastroenteritis
<i>Porphyromonas gingivalis</i>	$\beta$ and $\gamma$	periodontitis, rheumatoid arthritis	inflammatory diseases affecting the tissues around teeth; rheumatoid arthritis
<i>Legionella pneumophila</i>	$\beta$	legionellosis	pneumonia
<i>Pseudomonas aeruginosa</i>	$\beta$ and $\gamma$	no specific name	infections in cystic fibrosis patients
<i>Escherichia coli</i>	$\beta$ and $\gamma$	no specific name	diarrhea (for some strains)
<i>Brucella suis</i>	$\beta$ and $\gamma$	brucellosis	contagious zoonosis (ingestion of unpasteurized milk or undercooked meat)

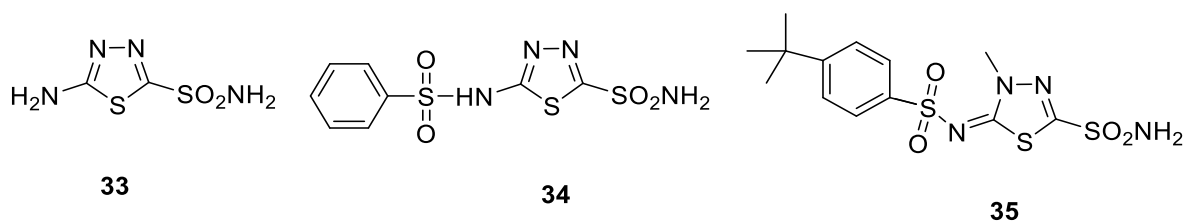


Table 5. Acetazolamide **1** and sulfonamides **32a-32i** effectively inhibit the bacterial enzyme NgCA $\alpha$  but also the human isoform hCA II. The minimum inhibitory concentration (MIC) against strain CDC 181 of *N. gonorrhoeae* are also shown (Abbutaleb et al., 2022a).



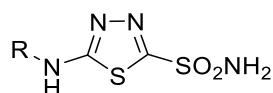
Compound	R	K <sub>i</sub> (nM)		MIC ( $\mu$ g/mL)
		hCA II	NgCA $\alpha$	
<b>1</b>	MeCO	12.5	74	4
<b>32a</b>	H	60	73	4
<b>32b</b>	Et	47	74	>64
<b>32c</b>	Cyclohexyl-CO	20	9.8	2
<b>32d</b>	Cyclohexyl-CH <sub>2</sub>	78	42	>64
<b>32e</b>	PhCO	29	79	4
<b>32f</b>	Cyclohexyl-CH <sub>2</sub> CO	24.5	37	0.5
<b>32g</b>	Cyclohexyl-CH <sub>2</sub> CH <sub>2</sub>	38	66	>64
<b>32h</b>	PhCH <sub>2</sub> CH <sub>2</sub> CO	8.1	8.3	0.25
<b>32i</b>	Cyclohexyl-CH <sub>2</sub> CH <sub>2</sub> CO	0.32	0.7	1

Table 6. HpCA $\alpha$ , HpCA $\beta$ , hCA I and hCA II inhibition data for acetazolamide **1**, methazolamide **10**, ethoxzolamide **11** and thiadiazole-sulfonamides **33-35** (Modak et al., 2016).



Compound	K <sub>i</sub> (nM)			
	HpCA $\alpha$	HpCA $\beta$	hCA I	hCA II
<b>1</b>	21	40	250	12
<b>10</b>	225	176	50	14
<b>11</b>	193	33	25	8
<b>33</b>	323	2590	8600	60
<b>34</b>	315	54	15	9
<b>35</b>	8	40	338	24

Table 7. Inhibitory effects of hCA II, EfCA $\alpha$  and EfCA $\gamma$  with sulfonamides **1**, **36a-h** incorporating the 1,3,4-thiadiazole-2-sulfonamide scaffold and their MIC values against *E. faecium* strain HM-965 is also shown (An et al., 2022).



**1, 36a-h**

Compound	R	K <sub>i</sub> (nM)			MIC ( $\mu$ g/mL)
		hCA II	EfCA $\alpha$	EfCA $\gamma$	
<b>1 (AAZ)</b>	MeCO	12.5	56.7	323	2
<b>36a</b>	Cyclohexyl-CO	20.2	49.3	131	0.25
<b>36b</b>	PhCO	29.0	11.7	310	2
<b>36c</b>	Cyclohexyl-CH <sub>2</sub> CO	24.5	14.5	305	0.06
<b>36d</b>	PhCH <sub>2</sub> CH <sub>2</sub> CO	8.1	6.4	148	0.06
<b>36e</b>	Cyclohexyl-CH <sub>2</sub> CH <sub>2</sub> CO	0.32	66.9	346	0.007
<b>36f</b>	EtCO	37.2	37.5	250	1
<b>36g</b>	<i>t</i> -Bu-CH <sub>2</sub> CO	7.3	69.6	390	0.015
<b>36h</b>	O[CH <sub>2</sub> CH <sub>2</sub> ] <sub>2</sub> NCH <sub>2</sub> CO	0.9	20.1	56.4	1

Table 8: Inhibition data with some of ten sulfonamides/sulfamates **1-19** in clinical use/clinical trials against hCA isozymes I – XIV (Supuran, 2008).

Isozyme	K <sub>i</sub> (nM)									
	<b>1</b> (acetazolamide)	<b>10</b> (metazolamide)	<b>11</b> (ethoxzolamide)	<b>12</b> (dichlorophenamide)	<b>13</b> (dorzolamide)	<b>14</b> (brinzolamide)	<b>15</b> (sulthiame)	<b>16</b> (topiramate)	<b>17</b> (zonisamide)	<b>19</b> (SLC-0111)
hCA I	250	50	25	1200	50000	45000	374	250	56	5080
hCA II	12	14	8	9	9	3	10	38	35	960
hCA III	2.10 <sup>5</sup>	7.10 <sup>5</sup>	1 10 <sup>6</sup>	6.8. 10 <sup>5</sup>	7.7.10 <sup>5</sup>	1.1.10 <sup>5</sup>	6.3.10 <sup>5</sup>	7.8. 10 <sup>5</sup>	2.2. 10 <sup>6</sup>	7920
hCA IV	74	6200	93	15000	8500	3950	95	4900	8590	286
hCA VA	63	65	25	630	42	50	81	63	20	2545
hCA VB	54	62	19	21	33	30	91	30	6033	910
hCA VI	11	10	43	79	10	0.9	134	45	89	6490
hCA VII	2.5	2.1	0.8	26	3.5	2.8	6	0.9	117	8550
hCA IX	25	27	34	50	52	37	43	58	5.1	45
hCA XII	5.7	3.4	22	50	3.5	3.0	56	3.8	11000	4.5
hCA XIII	17	19	50	23	18	10	1450	47	430	8755
hCA XIV	41	43	25	345	27	24	1540	1460	5250	257

## Figure legends

Fig. 1. A. Physiologic reaction catalyzed by the CAs (equation 1) and the catalytic mechanism of metallo-CAs, equations 2 and 3. B. Catalytic mechanism of  $\alpha$ -CAs: activation of the water molecule for the nucleophilic attack upon  $\text{CO}_2$  is achieved by hydrogen-bonds formation with a triad of hydrophilic amino acid residues, Thr106, Ser199 and Tyr124, whereas the proton transfer is achieved presumably by His197.

Fig. 2. A. Human (h) CA isoform II (hCA II) active site: the zinc ion (gold), its three His ligands (sky blue) and a coordinated water molecule (red) are shown, together with the proton shuttle residue His64 and the His cluster (in green) positioned at the exit of the active site cavity. The hydrophobic residues Phe131 and Gln92 involved in the binding of many inhibitor classes are also highlighted in green. B. hCA II with superimposed  $\text{CO}_2$  (in green) and bicarbonate (pink) bound within the active site, as determined by X-ray crystallography (pdb files 2VVA and 2VVB, respectively). The metal ion, which is zinc in  $\alpha$ -CAs is the gray sphere, and its three coordinated amino acid ligands (green), are His94, His96 and His119. Residues involved in the binding of the substrates/inhibitors (Glu106, Thr199, Leu198, Val121, Val143, Thr200 and Trp209) are also shown (hCA II numbering system).

Fig. 3. Phylogenetic tree of  $\alpha$ -,  $\beta$ - and  $\gamma$ -CAs from bacteria, algae, plants, fungi and vertebrates (left panel). The program PhyML 3.0 was used to build the tree and branch support values are reported at branch point as red figures. The enzyme family, organisms in which they are present and accession numbers of the genetic sequences are shown in the right panel.

Fig. 4. The tail approach (A), the two- (B) and three-tails (C) approaches exemplified for inhibitors binding to the zinc ion in the active site of a hCA isoform (hCA II numbering of amino acid residues); (ZBG = zinc-binding group).

Fig. 5. Chemical structures of sulfonamide CAIs used as diuretics: acetazolamide **1**, thiazides (**2a-e**), thiazide-like drugs (quinethazone **3**, metholazone **4**, chlorthalidone **5**, indapamide **6**) and the high-ceiling diuretics furosemide **7**, azosemide **8** and bumetanide **9**.

Fig. 6. Antiglaucoma CAIs: the first generation drugs include (apart acetazolamide **1**) methazolamide **10**, ethoxzolamide **11** and dichlorophenamide **12** whereas the second generation ones are dorzolamide **13** and brinzolamide **14**.

Fig. 7. Antiepileptics with CA inhibitory activity: sulthiame **15**, topiramate **16** and zonisamide **17**.

Fig. 8: Role of hypoxia-induced CA IX in pH regulation, ferroptosis, redox homeostasy, angiogenesis, survival, proliferation and migration of cancer cells.

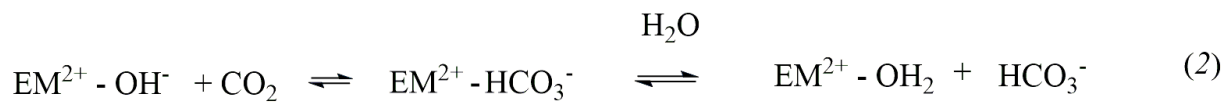
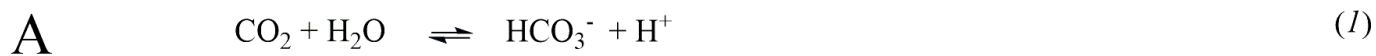
Fig. 9. Antitumor/antimetastatic CAIs **18-26**.

Fig. 10. CAIs used for the proof-of-concept studies of the involvement of these enzymes in neuropathic pain (**27**), cerebral ischemia (**28** and **29**) and rheumatoid arthritis (**30** and **31**).

Fig. 11. Dithiocarbamates **37** and **38** with antimycobacterial activity *in vivo*.

Fig. 12. Sulfonamides investigated as antifungals *in vitro/in vivo* (in **41a**, F in *para*; **41b**, F in *ortho*).

Fig. 13. Clinically used drugs **42-47** possessing polypharmacology connected to CA inhibition.



M may be Zn, Fe, Co, Cd

**B**

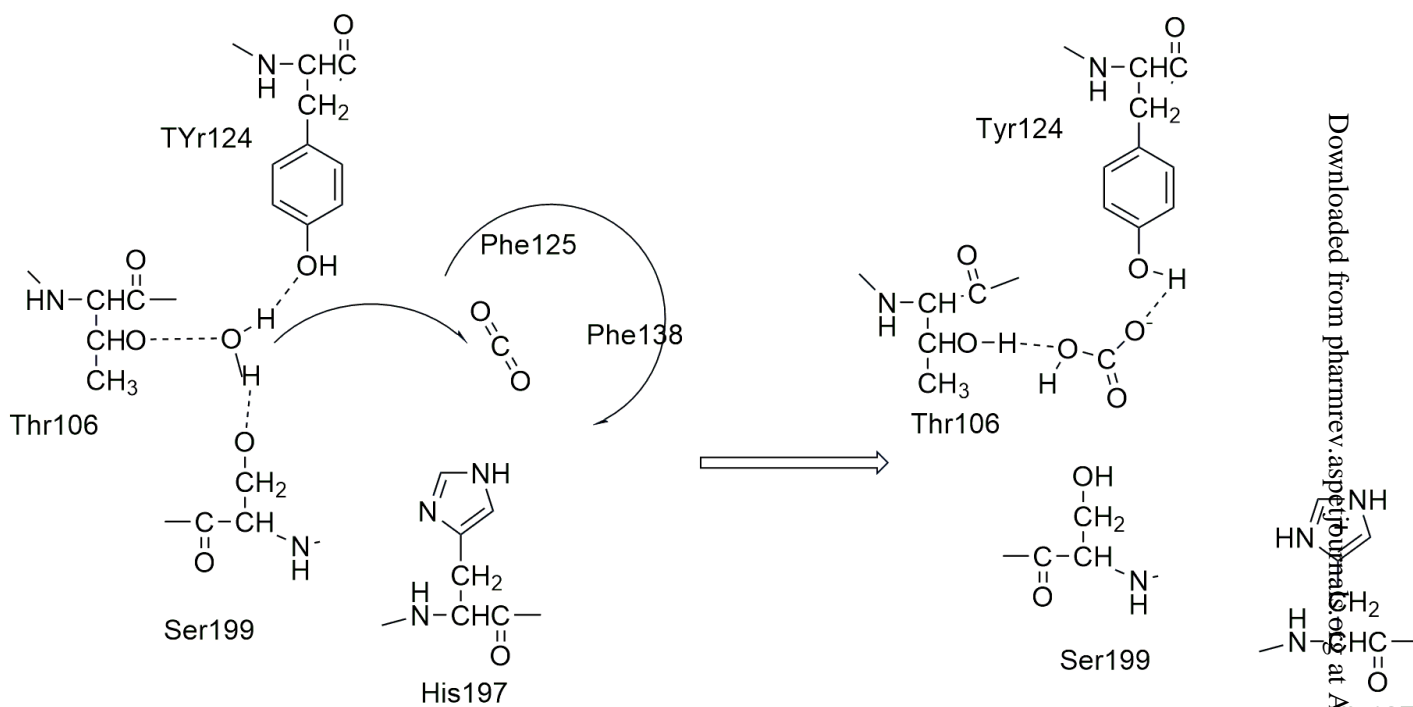


Fig. 1



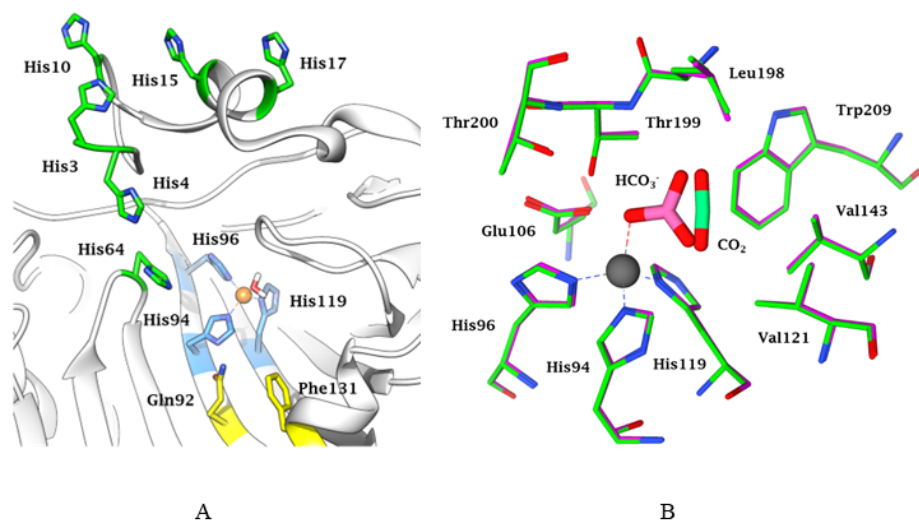
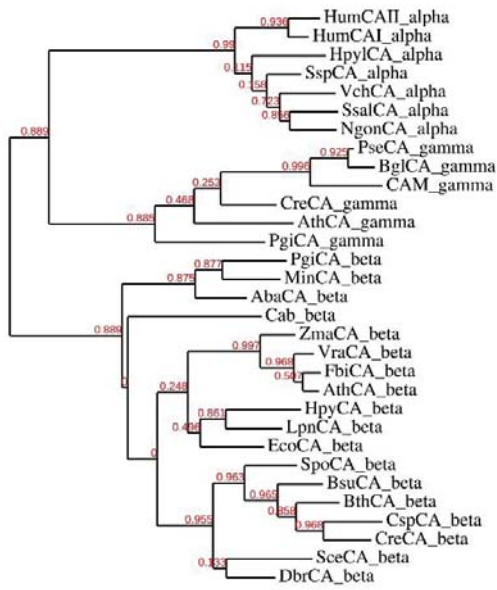


Fig. 2



CA class	Organism	Accession number	Cryptonym	
alpha	<i>Helicobacter pylori</i> J99	NP_223829.1	HpylCA_alpha	
	<i>Homo sapiens</i> , isoform II	AAH11949.1	HumCAI_alpha	
	<i>Homo sapiens</i> , isoform I	NP_001158302.1	humCAI_alpha	
	<i>Sulfolobus solfataricus</i> ATCC 35061	ACD66216.1	SspCA_alpha	
	<i>Streptococcus salivarius</i> P54	EIC81445.1	SsaICA_alpha	
	<i>Vibrio Cholerae</i>	AFC59768.1	VchCA_alpha	
	<i>Neisseria gonorrhoeae</i>	CAA72038.1	NgonCA_alpha	
	beta	<i>Schizosaccharomyces pombe</i>	CAA21790	SpoCA_beta
		<i>Brucella suis</i> 1330	NP_699962.1	BsuCA_beta
		<i>Burkholderia thailandensis</i> Bt4	ZP_02386321	BthCA_beta
<i>Coccomyxa</i> sp.		AAC33484.1	CspCA_beta	
<i>Chlamydomonas reinhardtii</i>		XP_001699151.1	CreCA_beta	
<i>Acinetobacter baumannii</i>		YP_002326524	AbaCA_beta	
<i>Porphyromonas gingivalis</i>		YP_001929649.1	PgiCA_beta	
<i>Myroides injenensis</i>		ZP_10784819	MinCA_beta	
<i>Zea mays</i>		NP_001147846.1	ZmaCA_beta	
<i>Vigna radiata</i>		AAD27876	VraCA_beta	
<i>Flaveria bidentis</i> , isoform I		AAA86939.2	FbiCA_beta	
<i>Arabidopsis thaliana</i>		AAA50156	AthCA_beta	
<i>Helicobacter pylori</i>		BAF34127.1	HpyCA_beta	
<i>Legionella pneumophila</i> 2300/99		YP_003619232	LpnCA_beta	
<i>Escherichia coli</i>		ACI70660	EcoCA_beta	
<i>Methanobacterium thermoautotrophicum</i>		GI:13786688	MtaCA_beta	
<i>Saccharomyces cerevisiae</i>		GAA26059	SeeCA_beta	
<i>Dekkera bruxellensis</i> AWRI1499		EIF49256	DbrCA_beta	
gamma		<i>Pseudomonas</i> sp. PAMC 25886	ZP_10427314.1	PseCA_gamma
		<i>Burkholderia gladioli</i> BSR3	YP_004359911.1	BglCA_gamma
	<i>Methanosarcina thermophila</i>	ACQ57353.1	CAM_gamma	
	<i>Chlamydomonas reinhardtii</i>	XP_001703237.1	CreCA_gamma	
	<i>Arabidopsis thaliana</i>	NP_564091.1	AthCA_gamma	
	<i>Porphyromonas gingivalis</i>	YP_001929649.1	PgiCA_gamma	

Fig. 3

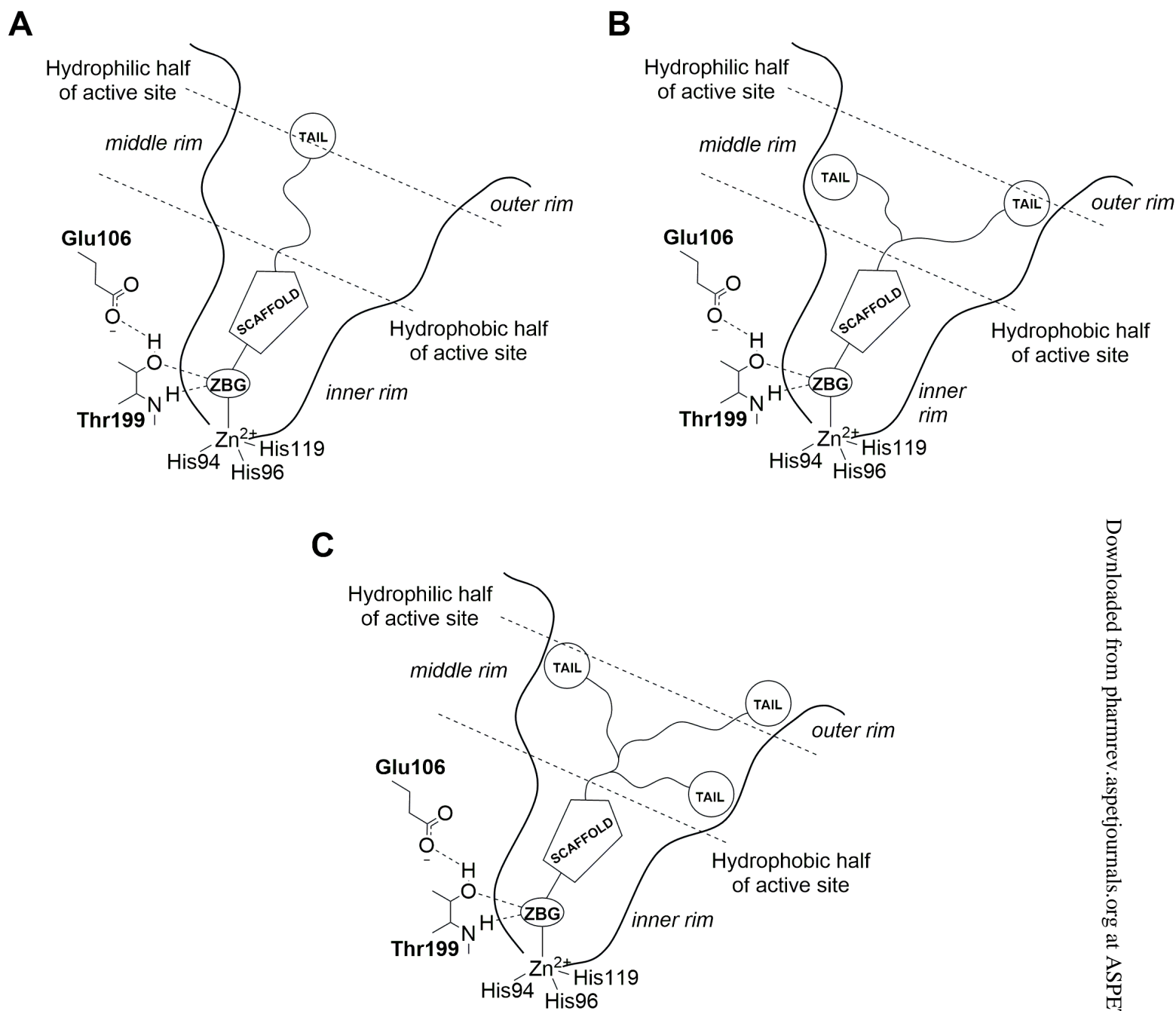
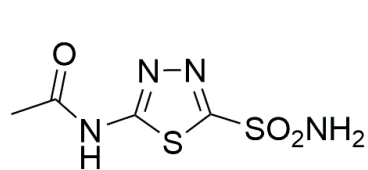
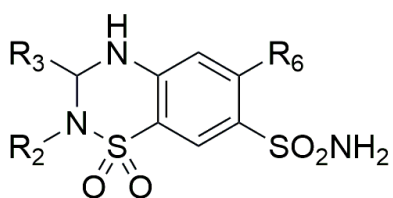


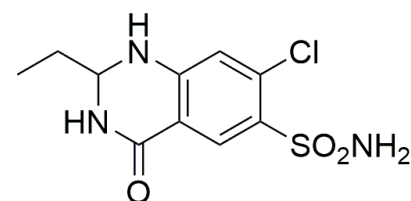
Fig. 4



1

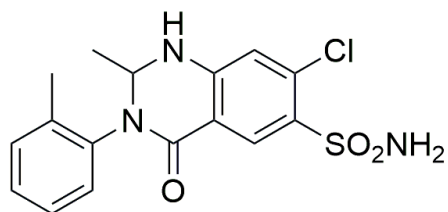


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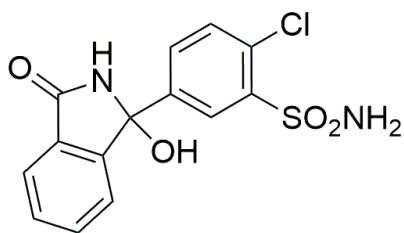


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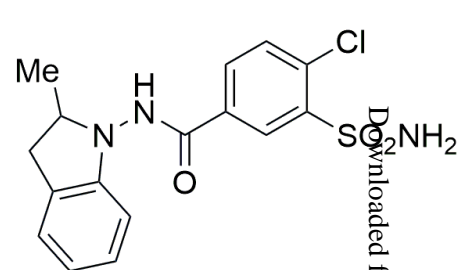
- a:  $R_2 = R_3 = H$ ,  $R_6 = Cl$ , Hydrochlorothiazide  
 b:  $R_2 = R_3 = H$ ,  $R_6 = CF_3$ , Hydroflumethiazide  
 c:  $R_2 = H$ ,  $R_3 = PhCH_2$ ,  $R_6 = CF_3$ , Bendroflumethiazide  
 d:  $R_2 = H$ ,  $R_3 = CHCl_2$ ,  $R_6 = Cl$ , Trichloromethiazide  
 e:  $R_2 = Me$ ,  $R_3 = CH_2SCH_2CF_3$ ,  $R_6 = Cl$ , Polythiazide



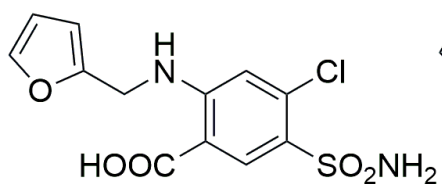
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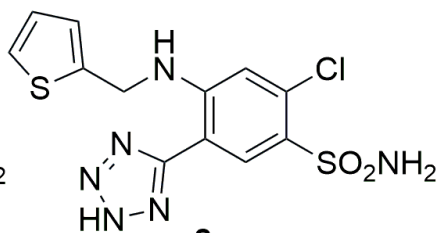
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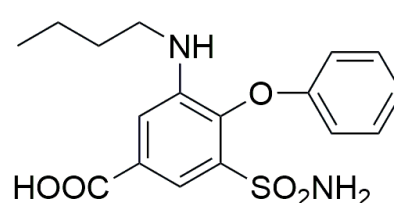
6



7



8



9

Fig. 5

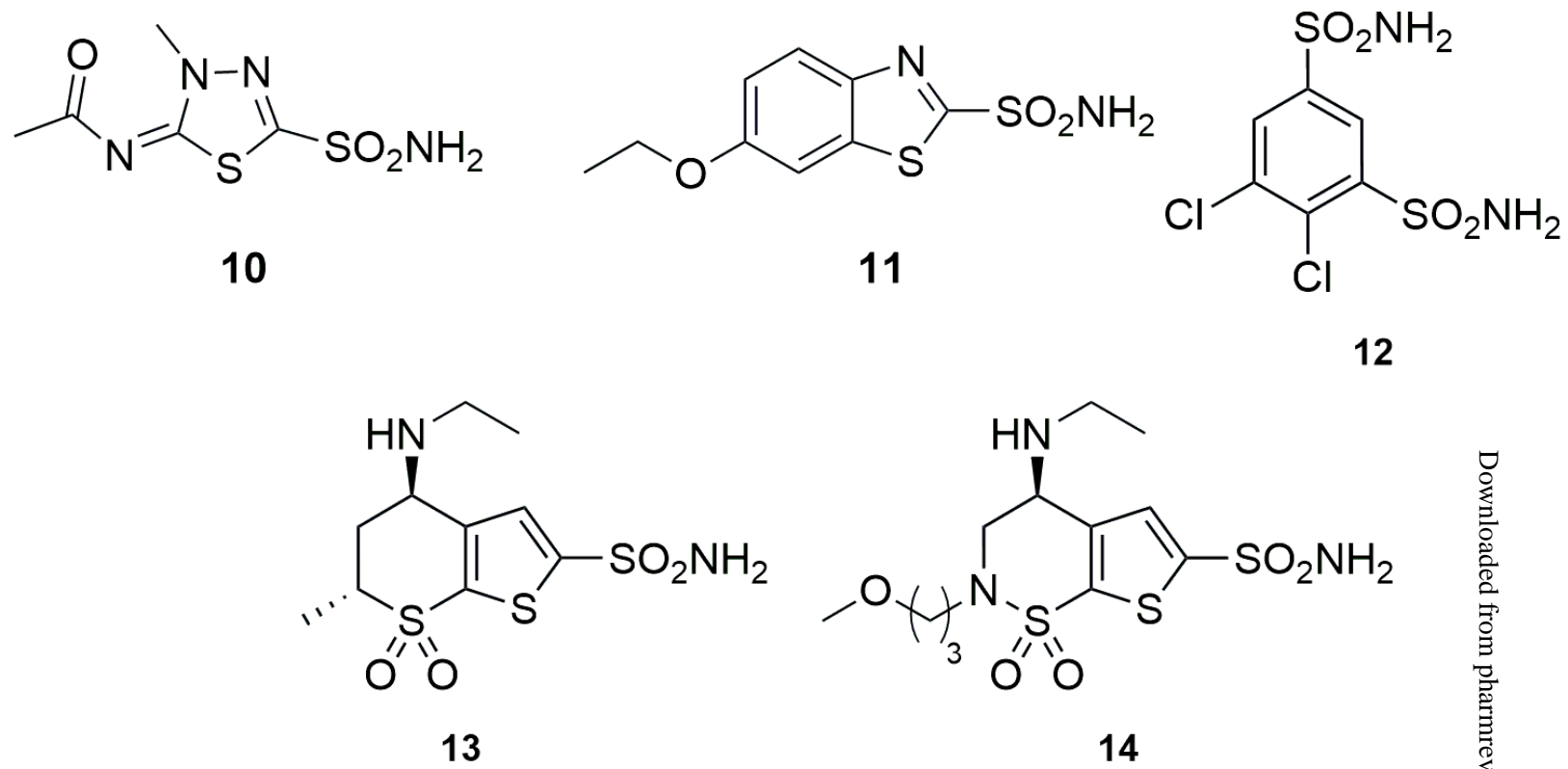


Fig. 6

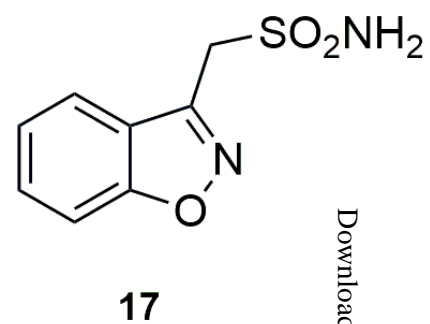
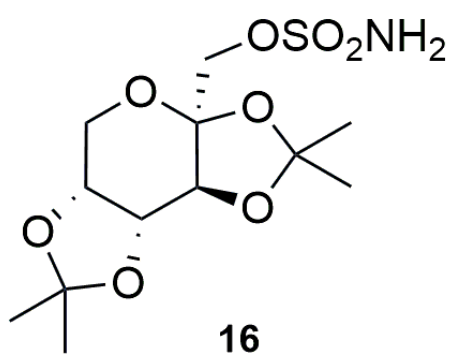
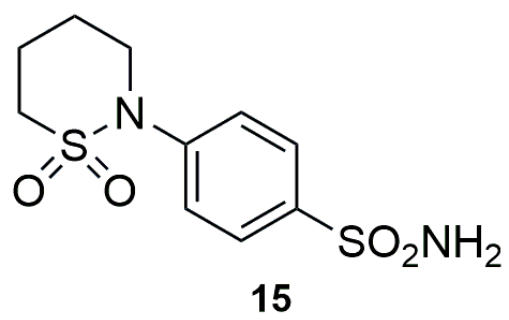


Fig. 7

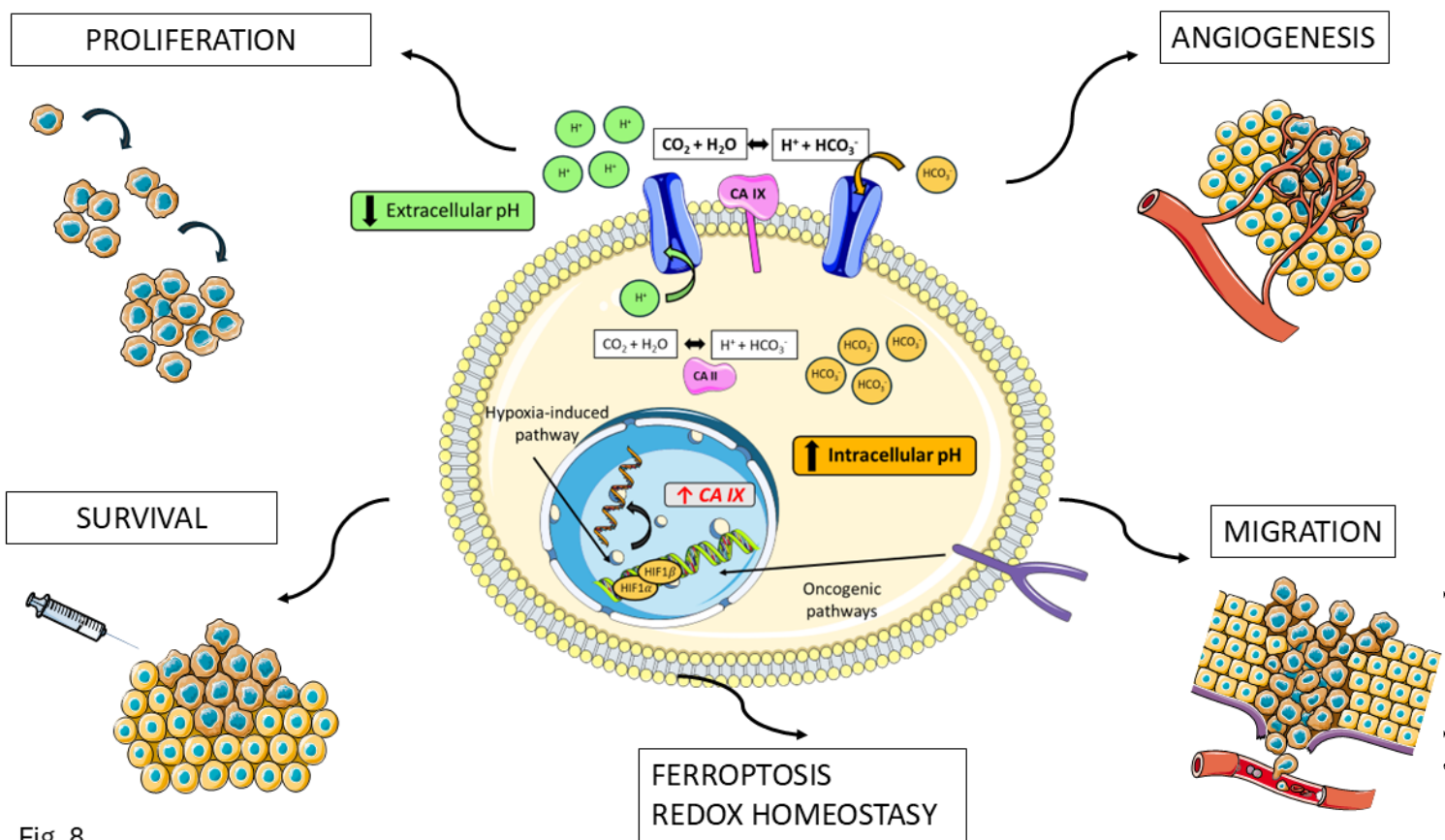
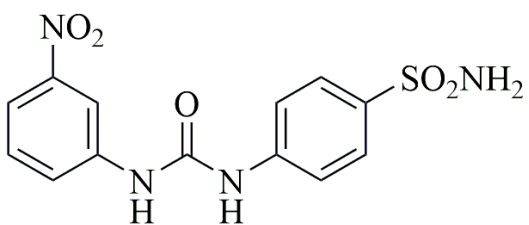
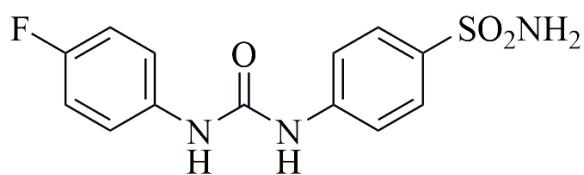


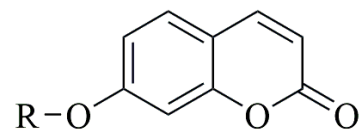
Fig. 8



**18**

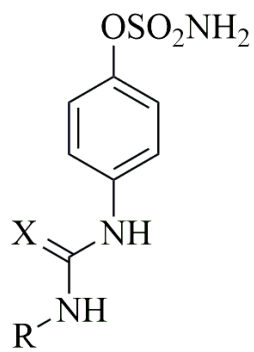


**19: SLC-0111**



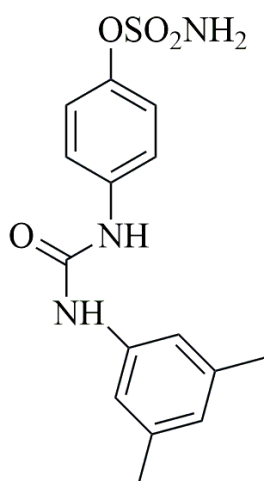
**20: R = glucosyl**

**21: R = galactosyl**

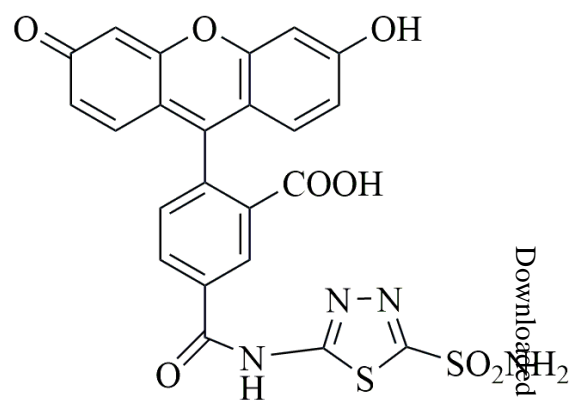


**22: X = O**

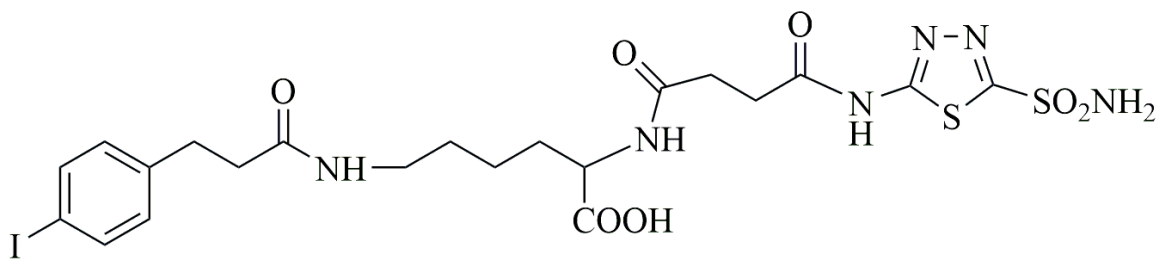
**23: X = S**



**24 (S4)**



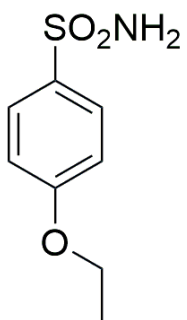
**25**



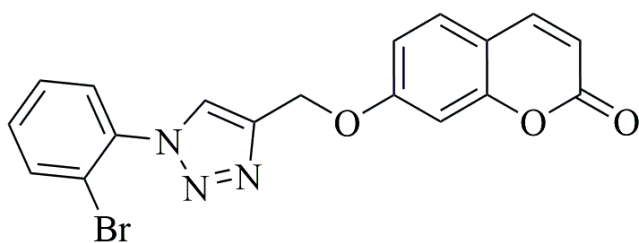
**26**

Fig. 9

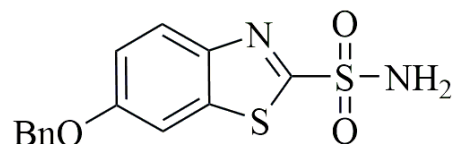




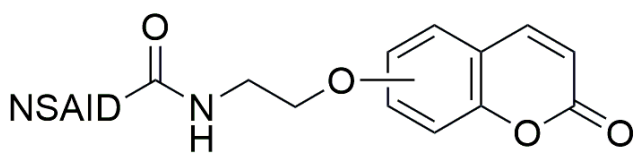
27



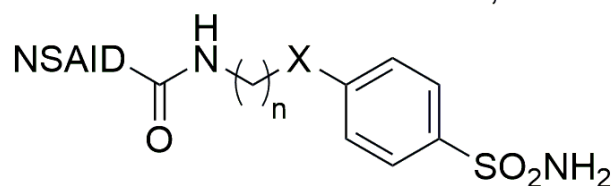
28



29



30

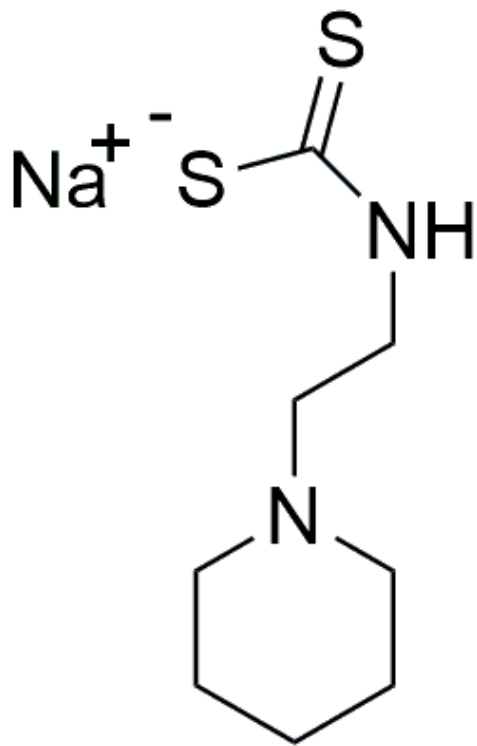


31

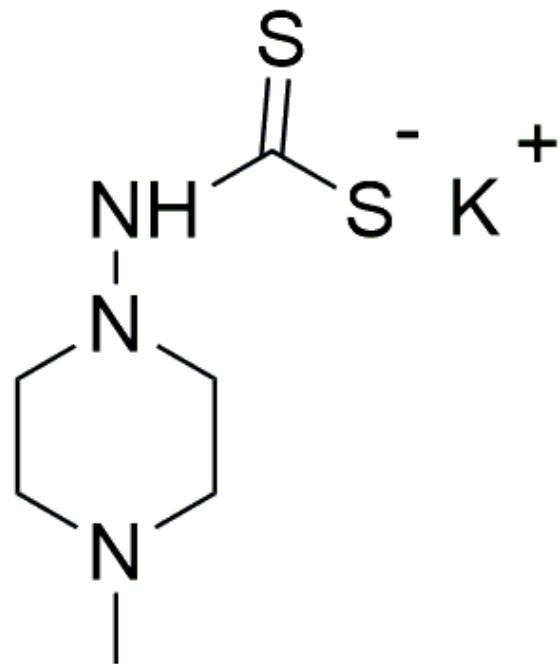
n = 0-4; X = none or C

**NSAID:** Indomethacin, Sulindac, Ketoprofen, Ibuprofen, Diclofenac, Flurbiprofen, Ketorolac, Naproxen

Fig. 10

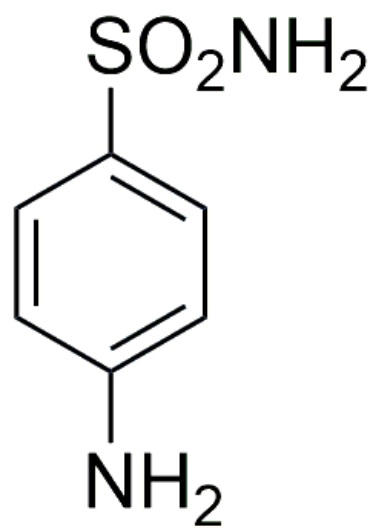


**37**

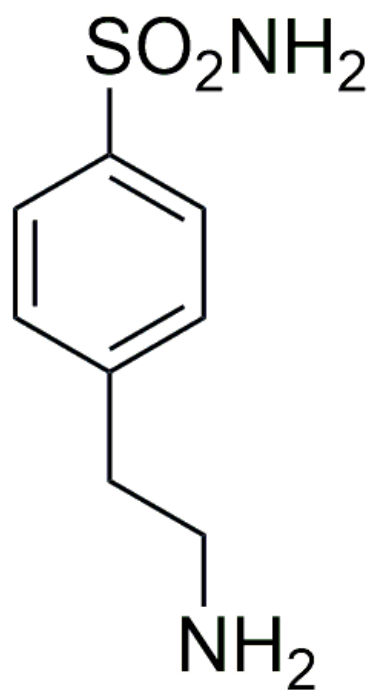


**38**

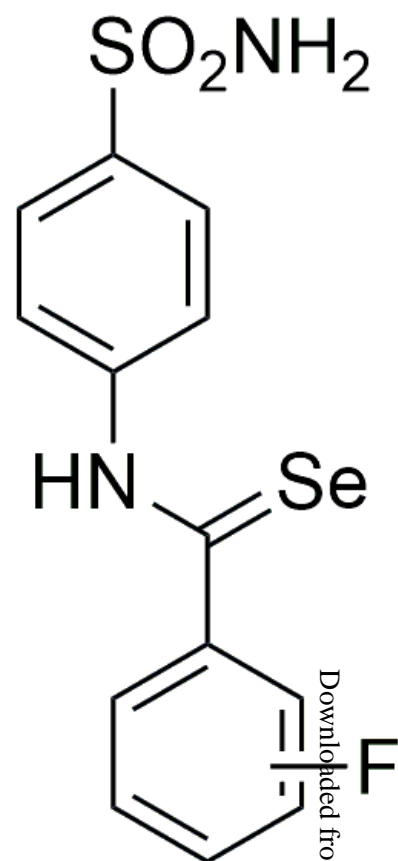
Fig. 11



**39**



**40**



**41a,b**

Fig. 12

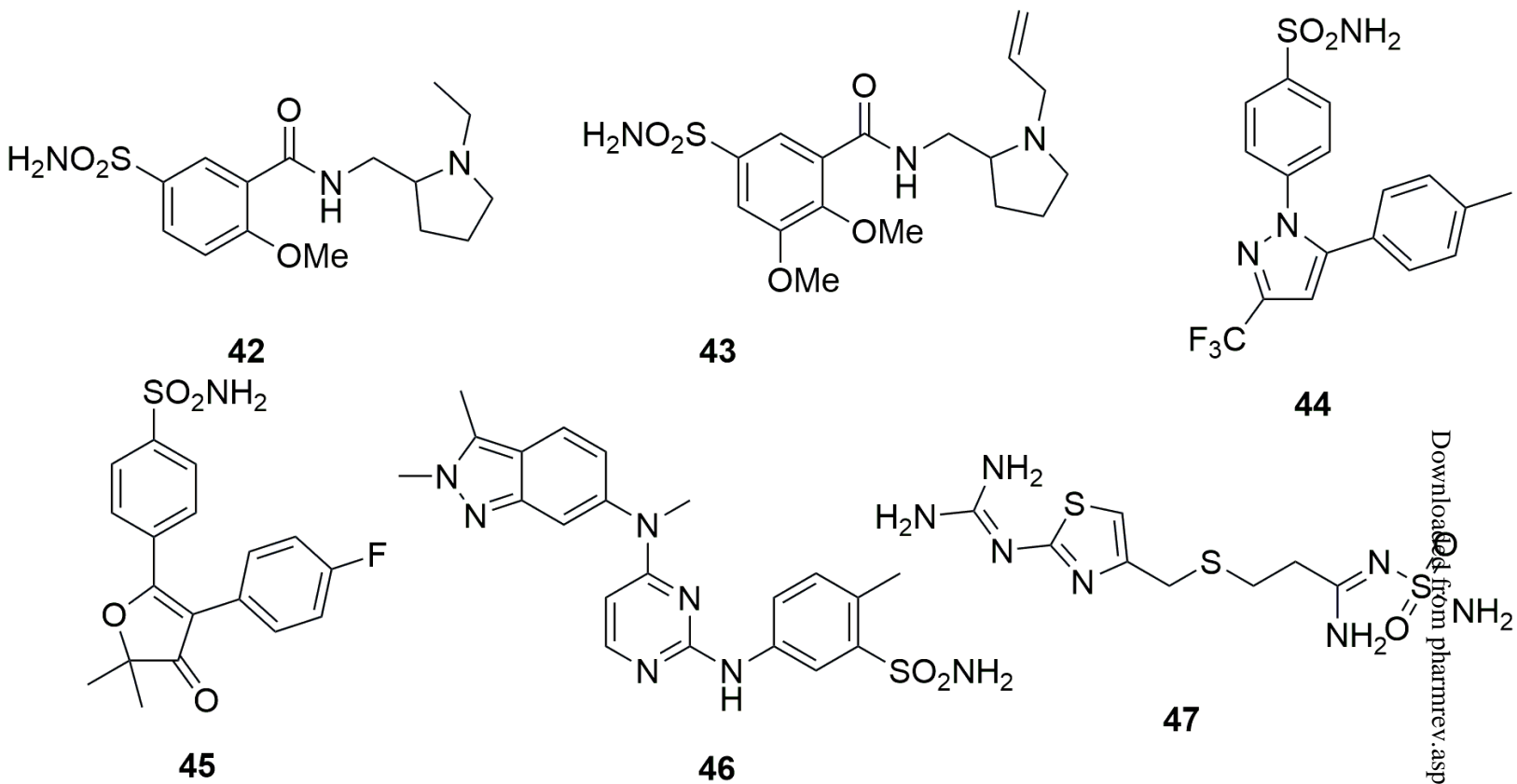


Fig. 13