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Trends in drug discovery for carbonic anhydrases: FDA approvals, clinical trials, molecular functions, and therapeutic potential

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ABSTRACT

Human carbonic anhydrases (CAs) catalyse the rapid conversion of CO_2 and bicarbonate, supporting pH balance, ion transport and many metabolic processes. All 15 human CA family proteins share a conserved structure, but they differ in activity, localisation and tissue distribution. Several CA isozymes are directly linked to distinct clinical conditions. Although CA-inhibiting drugs have long been clinically used, research across individual isozymes, chemical compound classes, and therapeutic uses has progressed unevenly. This review brings together current structural, biochemical and pharmacological knowledge of human CAs. It summarises catalytic properties, expression patterns and disease associations across the isozyme family. Loss-of-function variants in CA II and CA VA cause well-defined inherited metabolic disorders. At the same time, the strong and selective overexpression of CA IX and CA XII in tumours provides clear targets for cancer therapy. We outline the development of CA-directed drugs, from early non-selective sulphonamides to modern treatments used in ophthalmology, neurology and metabolic medicine. We also review ongoing clinical trials of isozyme-selective small molecules, antibodies, and radiopharmaceuticals, with particular focus on CA IX-targeting agents. Classical sulphonamides inhibit CAs by binding the catalytic zinc ion and displacing the Zn-bound water. Newer inhibitors achieve selectivity by engaging isozyme-specific pockets and surrounding regions. Antibody- and small-molecule-based radiolabelled ligands targeting CA IX are now advancing as promising tools for precision oncology, with several candidates demonstrating excellent clinical trial results and entering late-stage clinical development.

1. Introduction

Carbon dioxide is a central molecule in biology, with multiple roles in different organisms. It serves as the primary substrate for photosynthesis in plants, acts as a metabolic by-product of cellular respiration in animals, contributes to pH regulation in tissues and body fluids, and participates in the mineralisation of calcium carbonate-based structures. To fulfil these diverse roles, organisms need mechanisms that allow rapid interconversion between carbon dioxide and bicarbonate. Although the spontaneous reaction is relatively fast ($0.0375 \pm 0.002\ s^{-1}$ for CO_2 hydration [1,2]), it is not quick enough for most biological processes. To overcome this kinetic limitation, nearly all organisms have at least one carbonic anhydrase (CA) enzyme. Carbonic anhydrase (EC 4.2.1.1) increases the rate of reversible hydration of carbon dioxide up to 10^6-10^7 times [3,4].

Carbonic anhydrases are an ancient and highly diverse enzyme family with a long evolutionary history. They evolved independently multiple times, resulting in at least eight structurally distinct enzyme classes (α, β, γ, δ, ζ, η, θ, and ι) across the domains of life [5]. These classes share no common ancestry but perform the same catalytic function, highlighting the importance of efficient CO_2/HCO_3^- interconversion. Most CAs are metalloenzymes that use Zn^{II} as a cofactor, but some utilise alternative metals such as Cd^{II}, Fe^{II}, Co^{II}, or Mn^{II}, depending on environmental availability. In rare cases, catalytic activity can occur in the absence of a metal cofactor [6]. Among these distinct classes, only the α-class CA family is found in mammals.

In mammals, α-CAs are present as multiple isozymes with distinct tissue distributions, subcellular localisations, and biological functions. In humans, these enzymes play a central role in CO_2/bicarbonate transport, regulate acid-base balance, ion transport, bone resorption,

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and the secretion of gastric, cerebrospinal, and other fluids. They also provide bicarbonate as a substrate for biosynthetic pathways, including gluconeogenesis, lipogenesis, and ureagenesis. Due to their essential physiological roles, several human CA isozymes have been validated as drug targets. Pharmacological inhibition of CA is already exploited in therapy for glaucoma, epilepsy, altitude sickness, and certain oedematous states, and is under investigation for oncology and other indications [7].

The rapidly evolving field of human CAs and their inhibitors receives constant attention; however, reviews covering the field are often fragmented by isozyme, compound class, or indication. Recent reviews have provided detailed analyses of CA-inhibitor interactions, structural details, and multiple binding modes of diverse inhibitor classes [8,9], as well as a wide range of chemical diversity and possible new applications of experimental CA inhibitors identified in recent years [10–12]. An excellent review by S. Zamanova et al. precisely summarised the use of CA isozymes as biomarkers for various diseases and syndromes [13], and S. Hallaj et al. reviewed the clinical use of systemic CA inhibitors [14].

This review focuses on human carbonic anhydrases and their relevance to medicinal chemistry, pharmacology and clinical development. Here, we present each human CA family member, discuss their physiological and pathological relevance, structural and isozyme-specific features, and therapeutic potential. We categorise diseases that are directly related to CA genetic changes or alterations in expression, and those that can be managed with CA inhibitors. Finally, we review FDA-approved carbonic anhydrase inhibitors and active clinical trials. Experimental and purely preclinical compounds are outside the scope of this article.

2. Approach

To compile a comprehensive picture of the human CA family, we retrieved protein sequence and annotation data for human CA proteins from UniProt [15] and protein expression data from the Human Protein Atlas [16] and the IUPHAR/BPS Guide to Pharmacology [17]. We obtained information on clinical development and regulatory status from ClinicalTrials.gov for registered interventional studies and from the FDA's Drugs@FDA resource for FDA-approved indications and labels, using search terms “carbonic anhydrase” and separate symbols for each isozyme. To describe the evolution of the CA field, we assembled a timeline covering almost a century of important pharmacologically relevant events in CA research (see Fig. 1), which will be discussed in further sections of this review.

CA research began in the 1930s with the isolation of CA from mammalian erythrocytes and the discovery of sulphanilamide as the first CA inhibitor [18–20]. The 1950s–1960s saw the development of classical systemic inhibitors such as acetazolamide and others, used for diuretic and antiglaucoma therapy, and antiepileptic sulthiame – most of these drugs are still used nowadays [21,22]. Structural elucidation [23] of CA and the discovery of 15 CA family proteins, beginning in the 1970s, revealed the complexity of the CA family and underscored the need for isozyme-selective pharmacology. However, despite these advances, truly isozyme-specific CA inhibitors are still not clinically available. From the 1990s onward, topical antiglaucoma agents dorzolamide and brinzolamide were introduced, representing significant progress in reducing systemic adverse effects associated with earlier inhibitors [24]. Further antiepileptic agents, topiramate and zonisamide, were introduced, but both were subsequently found to weakly inhibit multiple carbonic anhydrase isozymes, with topiramate in particular causing metabolic acidosis through renal CA inhibition [25]. The generation of the CA IX-specific antibody G250 and subsequent advancements in antibody engineering and radiochemistry enabled late-stage clinical evaluation of CA IX-directed diagnostic and therapeutic constructs [26]. SLC-0111 was the first orally active small-molecule therapeutic inhibitor developed to block CA IX enzymatic activity and alter tumour pH biology [27]. Most other small-molecule agents are directed towards the visualisation of CA IX-expressing tumours. Although no CA IX-specific theranostic agent has yet received regulatory approval, several candidates are in advanced clinical testing and approaching potential clinical translation. Thus, the last decade could be called a new era of isozyme-specific drug discovery with multiple antibody-based and small-molecule agents entering clinical trials.

3. Roles of human carbonic anhydrases in health and disease

In humans, carbonic anhydrase is a family of fifteen proteins, with their genes located on eight different chromosomes (Table 1). The human CAs are designated by Roman numerals from CA I to CA XIV, except for CA V, which has two isozymes, CA VA and CA VB. The fifteen proteins show high structural similarity but differ in catalytic activity, cellular localisation, oligomeric state (monomers or dimers), and expression patterns in healthy tissues. Some CA isozymes have been directly related to specific diseases (Table 1). In defining CA-disease associations, we restrict to cases in which a direct molecular link between an isozyme and a disease mechanism has been demonstrated. This includes genetic disorders caused by CA mutations, autoimmune

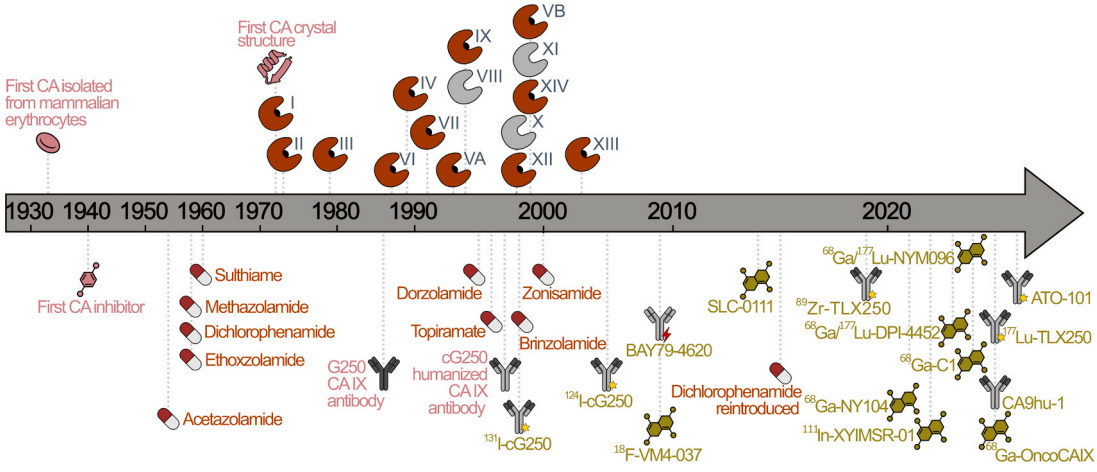

Fig. 1. Timeline of pharmacologically relevant events in CA research. This timeline integrates major developments in the CA field, including foundational biochemical discoveries, regulatory approvals of clinically used CA-inhibiting drugs, the chronological identification of human CA proteins based on the dates their amino acid sequences were first determined, and the clinical advancement of key next-generation CA-targeting molecules, plotted according to the study start dates reported on ClinicalTrials.gov.

Table 1
Members of human carbonic anhydrase: genes and their location in chromosomes, proteins and protein expression in healthy tissues, and key relation to specific diseases. Main protein expression sites in normal human tissues and cell types are summarised from the Human Protein Atlas [16].

Gene	Gene location	Protein	Expression in normal tissues/cells	Association with diseases
CA1	8q21.2	CA I	Erythrocytes, leukocytes, bone marrow, spleen, large intestine, and choroid plexus	Autoantibodies are reported in acute myeloid leukaemia and some autoimmune conditions [28,29]
CA2	8q21.2	CA II	Erythrocytes, gastrointestinal tract, renal tubules, gallbladder, glial cells, and choroid plexus	CA II Deficiency Syndrome (OMIM 259730), characterised by osteopetrosis, renal tubular acidosis, and cerebral calcification; autoantibodies are reported in many autoimmune conditions [28,30]
CA3	8q21.2	CA III	Skeletal muscle fibres, adipocytes	Autoantibodies are reported in rheumatoid arthritis, systemic lupus erythematosus, type 1 diabetes, and other diseases; a decrease in expression is linked to muscular diseases, such as *myasthenia gravis* and Duchenne muscular dystrophy [31]
CA4	17q23.1	CA IV	Gastrointestinal glandular epithelium; renal tubules; endothelial cells (several tissues)	Autoantibodies are reported in some autoimmune diseases; mutations are reported in association with *retinitis pigmentosa* [31,32]
CA5A	16q24.2	CA VA	Liver	CA VA deficiency causes neonatal/infant hyperammonemic metabolic crises (OMIM 615751) [33]
CA5B	Xp22.1	CA VB	Most tissues	No established direct disease association
CA6	1p36.23	CA VI	Salivary and mammary gland	Autoantibodies linked to Sjögren's syndrome; genetic polymorphisms associated with dental caries susceptibility [34,35]
CA7	16q22.1	CA VII	CNS	No established direct disease association
CA8	8q12.1	CARP VIII	Purkinje cells	CARP VIII loss-of-function variants cause cerebellar ataxia, mental retardation, and dysequilibrium syndrome 3 (CAMRQ-3, OMIM 613227) [36]
CA9	9p13.3	CA IX	Gastrointestinal mucosa and gall bladder	Overexpressed in many tumours. This overexpression, often triggered by hypoxic conditions in the tumour microenvironment, promotes tumour growth by regulating the pH [37]
CA10	17q21.33-q22	CARP X	Neuronal cells. CNS	No established direct disease association
CA11	19q13.33	CARP XI	CNS	No established direct disease association
CA12	15q22.2	CA XII	Several cell types; most abundant in the intestinal tract and renal tubules, kidney, intestine, reproductive epithelia, eye, tumours, and CNS	Biallelic *CA12* gene mutations cause isolated hyperchlorhidrosis/ salt-losing sweat disorder (HYCHL; OMIM 143860); CA XII protein upregulation is reported in some tumours; an increase in expression is reported in glaucoma [38]
CA13	8q21.2	CA XIII	Most abundant in the intestinal tract, also in the kidney, brain, lung, gut, and reproductive tract	No established direct disease association
CA14	1q21.2	CA XIV	A group of tissues, including skeletal muscle and CNS, also the gastrointestinal tract, kidney, brain, liver, and eye	No established direct disease association

diseases in which CA autoantibodies are part of the disease process, and diseases characterised by significant protein upregulation. In contrast, cases where CA inhibitors are used therapeutically but the enzyme is not directly implicated in pathogenesis were omitted and will be discussed later.

Carbonic anhydrase I is a key cytoplasmic isozyme primarily involved in respiration and acid/base balance, with high expression in human red blood cells. It is also present in the intestine, but at lower levels than CA II. CA I has been linked to various health conditions, including bipolar disorder, glaucoma, and retinal/cerebral oedema [39,40]. Later studies show that CA I promotes biocalcification, particularly in breast carcinoma, by enhancing calcium carbonate deposition [41,42].

Carbonic anhydrase II exhibits high catalytic activity and is found throughout the body. It plays several critical roles, including regulating bicarbonate levels in the aqueous humour of the eye, forming cerebrospinal fluid, and facilitating biochemical processes such as gluconeogenesis and ureagenesis [43]. CA II also contributes to urine formation and bicarbonate reabsorption in renal tubules [44] and is involved in processes related to bone resorption and calcification [45].

CA II cooperates with various membrane transporters, enhancing their functioning. For example, it enhances the sodium/bicarbonate cotransporter's capacity, with inhibitors restoring this transport activity to normal levels [46]. CA II also interacts with chloride/bicarbonate exchangers to aid CO_2 removal and with sodium/hydrogen exchangers to enhance transport efficiency [47,48].

Carbonic anhydrase III is a protein highly expressed in skeletal muscle, particularly in red fibres [49], and is also present in adipose tissue and the liver together with other isozymes like CA I and CA II [50,51]. Unlike other α-class CAs, CA III exhibits low catalytic activity and is resistant to sulphonamide inhibition due to steric restrictions imposed by bulky amino acids in its active site, as well as the absence of His64, which is replaced by lysine, reducing proton transfer efficiency [52].

The exact role of CA III remains uncertain. It is associated with *myasthenia gravis*, where decreased CA III expression is linked to the disease. It has antioxidant properties, potentially protecting cells from oxidative damage through its two sulfhydryl groups, which can reversibly bind to glutathione [53]. Autoantibodies against CA III and CA IV have been detected in patients with rheumatoid arthritis, systemic lupus erythematosus, diabetes, hypertensive renal disease, and heart failure [31].

Carbonic anhydrase IV is a glycoprotein anchored to the plasma membrane via a GPI anchor [54]. CA IV is widely distributed in the

body, found in the endothelial cells of the eye [55], kidney, and lung. In the kidney's proximal tubule, CA IV facilitates bicarbonate reabsorption [56]. In the lungs, it facilitates CO_2 exchange between blood and alveoli by catalysing the dehydration of bicarbonate to CO_2 [57]. CA IV helps regulate pH in the extracellular space of the hippocampus [58]. Additionally, it plays a role in ion and fluid transport in the intestine, mainly in the colon [57]. CA IV is responsible for the perception of the taste of carbonation [59]. CA IV interacts with transporters like the sodium/-bicarbonate cotransporter and the anion exchanger, collaborating with CA II to facilitate Cl^-/HCO_3^- transport [60].

CA IV's diverse functions are linked to various diseases. A missense mutation in the CA IV gene is associated with *retinitis pigmentosa*, a vision loss disorder [32]. CA IV also promotes keratinocyte migration during the early stages of wound healing by creating an acidic environment and accelerating re-epithelialization [61]. Increased CA IV and CA II expression correlates with cardiac hypertrophy progression by supporting Cl^-/HCO_3^- and Na^+/H^+ transporter activity [62]. CA IV acts as a tumour suppressor in colon cancer by inhibiting *Wnt* signalling; its gene silencing is observed in cancer patients [63].

Carbonic anhydrase V comprises two mitochondrial isozymes: **CA VA** and **CA VB**. The first mitochondrial isozyme, collectively termed CA V, was isolated from guinea pig liver [64]. Later studies identified CA V in other species, leading to the recognition of CA VB as distinct from CA VA due to its broader tissue distribution [65]. Human CA VA and CA VB exhibit significant sequence differences [66]. CA VA is primarily found in liver hepatocytes, while CA VB is expressed in various tissues, including heart, skeletal muscle, pancreas, salivary glands, and spinal cord [65].

Mitochondrial CA isozymes are essential to various metabolic pathways, notably gluconeogenesis, lipogenesis, ureagenesis, and the Krebs cycle, by providing mitochondrial bicarbonate (HCO_3^-) as a substrate [67]. The absence of CA VA significantly impairs ureagenesis, as low liver expression of CA VB cannot compensate for the required substrate for carbamoyl phosphate synthase, leading to hyperammonaemia [68]. Mitochondrial CA isozymes are essential for lipogenesis; inhibiting them reduces lipid synthesis [69]. The carbonic anhydrase inhibitors topiramate and zonisamide have been shown to induce weight loss, suggesting their potential as anti-obesity agents [70,71].

CA VA may also be a target for preventing diabetic cerebrovascular pathology by modulating ROS production and pericyte apoptosis. Overexpression of CA VA increased ROS and apoptosis [72]. CA VA knockdown reduced glucose-induced ROS and apoptosis in pericytes, while CA VB did not affect these parameters [73]. More recently, CA VB has been implicated in amyloid beta-induced cerebrovascular pathology, as silencing CA VB prevented endothelial apoptosis without affecting CA VA or CA II. Notably, carbonic anhydrase inhibitors also reduced amyloid beta accumulation in cerebral vascular and glial tissues [72].

Carbonic anhydrase VI is the only secreted isozyme of catalytically active mammalian carbonic anhydrases. Purified from human saliva in 1987, it was named CA VI [74]. CA VI is also known as gustin, as it was initially believed to be a distinct enzyme [75]. CA VI is secreted by major salivary glands and has been found in human milk, where it acts as a growth factor for the developing digestive tract of infants [76,77].

One of the physiological functions of CA VI in organisms is to act as an anti-caries protein in saliva, contributing to the neutralisation of plaque acid [78]. Lower concentrations of CA VI have been linked to an increased risk of dental caries [79]. Additionally, CA VI is involved in taste perception; reduced levels and the use of inhibitors, such as topiramate, can lead to taste loss, potentially explaining the weight loss associated with these medications [80].

Carbonic anhydrase VII, a cytosolic carbonic anhydrase, was first identified through the human genome [81]. It is predominantly expressed in mammalian brain tissue, though traces can also be found in the stomach, duodenum, colon, liver, and skeletal muscle. CA VII plays a role in maintaining the bicarbonate gradient established by GABA receptors. As one of two isozymes found in the somata and dendrites of the CA1 pyramidal neuron, it contributes to integrative functions, long-term plasticity, and susceptibility to epileptogenesis [82,83]. Similar to CA III, CA VII helps protect proteins under oxidative stress, with its expression increasing in response to oxidative stress, making cells expressing the isozyme less susceptible to apoptosis [53]. CA VII's most promising application lies in the treatment of epilepsy, given its expression in the brain and its role in bicarbonate regulation. Yet, no selective CA VII-targeting drugs have been developed so far.

Carbonic anhydrase IX is a hypoxia-inducible protein whose expression has been extensively investigated in cancer. It is strongly upregulated in many aggressive tumours. In contrast, its presence in normal tissues is very limited in normal adult tissues, primarily within the gastrointestinal tract – specifically in the stomach, epithelial tissues of the intestine, the colon, and the base of intestinal glands, also detected in the outer sheath of hair follicles, and during skin wound healing [61, 84]. CA IX is important for tumour cell growth and survival under both normoxic and hypoxic conditions [37]. Inhibition of CA IX expression or activity has been shown to reduce tumour growth and increase sensitivity to anticancer therapies. Moreover, CA IX expression correlates with chemotherapy resistance, as extracellular acidosis can hinder drug uptake and radiation efficacy [85]. More recently, CA IX has been shown to protect tumour cells from ferroptosis by regulating iron metabolism, redox balance, and glutathione-dependent antioxidant pathways, thereby further contributing to therapy resistance [86].

Carbonic anhydrase XII. Unlike CA IX, CA XII is widely expressed in many normal tissues, notably the pancreas, colon, rectum, kidney, prostate, and intestine, with traces found in the oesophagus, oral mucosa, urinary bladder, breast, cervix, endometrium, and skin [87]. CA XII is predominantly expressed in the principal cells of the kidney collecting duct, where it plays a crucial role in sodium and water absorption [88]. It also regulates electrolyte homeostasis by activating the ductal Cl^-/HCO_3^- exchanger AE2 and associating with the Na^+/HCO_3^- cotransporter kNBC1 [89,90]. In the intestine, CA XII facilitates transcellular water transport by converting extracellular water and carbon dioxide into bicarbonate and protons, thereby acidifying extracellular fluid [91]. Additionally, it plays a key role in chloride and sodium resorption in sweat ducts; loss-of-function mutations can lead to an autosomal recessive disorder [92]. Furthermore, CA XII influences pH-dependent spermatozoa activities in the endometrial epithelium, contributing to morphological changes throughout the menstrual cycle [93].

CA XII is overexpressed in glaucoma patients compared to normal levels, while CA IX does not show such notable increases in glaucoma [38]. Both CA XII and CA II are overexpressed in advanced atherosclerosis, although their specific roles in this process remain unclear [94].

Carbonic anhydrase XIII is the most recently discovered isozyme of cytosolic carbonic anhydrases. This isozyme exhibits high conservation across species [95]. CA XIII is expressed in numerous tissues and organs, including the kidney, brain, lung, heart, gastrointestinal tract, skin, adipose tissue, soft tissue, and reproductive organs, with particularly high expression levels observed in the gastrointestinal tract. In the submandibular gland, CA XIII is present in both serous acinar cells and ductal epithelial cells [95].

The physiological role of CA XIII remains partially unclear. It is hypothesised to contribute to pH regulation during reproductive processes, including the maintenance of sperm motility and normal fertilisation. However, direct evidence supporting this hypothesis is still lacking [96]. There have been reported instances of CA XIII downregulation in colorectal cancer, with some minimal signals detected in carcinoma samples, but these observations have not demonstrated significant clinical relevance [97].

Carbonic anhydrase XIV is localised in specific neurons and axons in both mouse and human brains, where it contributes to pH regulation in the hippocampus, alongside CA IV [58,98]. In the kidneys, its expression is linked to bicarbonate reabsorption [99]. Furthermore, CA

XIV is found in the retina, specifically in Müller cells and the retinal pigmented epithelium, where it regulates pH, facilitates CO_2 removal, and modulates photoreceptor function [100,101]. In the heart, it works with other isozymes to rapidly buffer H^+ ions released during Ca^{2+} uptake and interacts with the Cl^-/HCO_3^- exchanger to form a complex [102]. It is also present in skeletal muscle, accounting for about 50% of the total carbonic anhydrase activity [103].

A deficiency of CA XIV in mouse models can impair retinal light response, as evidenced in knockout mouse studies showing significant deficits, particularly in double CA IV/CA XIV knockout mutants [104]. Additionally, CA XIV knockout leads to more severe liver dysfunction, hepatic fibrosis, and altered bile acid levels, indicating its protective role against bile acid toxicity. However, no pathogenic *CA14* has been described and linked to human diseases.

Carbonic anhydrase-related proteins (CARP) cannot catalyse the CO_2 hydration reaction because Zn^{2+}-coordinating histidine residues are replaced by other amino acids in their active centre. [105]. Although lacking catalytic CO_2-hydration activity, these proteins are conserved across species and are predominantly expressed in the brain. CARP VIII also exhibits relatively high expression levels in the liver and lung [106]. CARP proteins interact with other proteins, such as the inositol 1,4,5-trisphosphate receptor type 1. This interaction, observed in Purkinje cells where CARP protein expression is significant, reduces the receptor's affinity for its ligand IP3, thereby affecting cellular signalling [107]. Coexpression of one of the CARP proteins with the monocarboxylate transporter (MCT1) has also been found to enhance transport activity [108].

Research indicates that CARP proteins are involved in the transmission of neuro-signals and may also play a role in tumour formation, especially in gliomas [109]. For example, CARP VIII has been associated with neurodegeneration and various pathological conditions in both humans and mice [110,111]. Furthermore, CARP VIII is highly expressed in gastrointestinal stromal tumours, and overexpression of CARP XI in cancer cells has been correlated with increased cell proliferation [112].

4. Structural features of human carbonic anhydrases

The fifteen human CAs are globular proteins that share a conserved antiparallel beta-sheet fold, which forms the core of the catalytic domain (Fig. 2). They exhibit a high degree of sequence homology, with pairwise sequence identities reaching up to 60% [113]. Despite their high structural similarity, human CA family proteins exhibit distinct properties. The most catalytically active CA isozymes (CA II, CA IX, CA VII) reach the limit of substrate diffusion, while other isozymes have lower enzymatic activity, and CA III is the least active one [114]. Proteins VIII, X, and XI lack CA activity due to mutations in the His residues that bind Zn^{II} in their active sites. These proteins are referred to as carbonic anhydrase-related proteins (CARP VIII, CARP X, and CARP XI) [106]. Three isozymes (CA VI, CA IX, and CA XII) exist as dimers, while others are monomeric.

Some isozymes consist primarily of the catalytic domain. However, others include additional sequence elements, namely signal or transit peptide sequences that are cleaved during maturation, or structural features retained in the mature form (Fig. 2). The cytosolic isozymes CA I, CA II, CA III, CA VII, and CA XIII are composed solely of the catalytic domain, CA IV is anchored to the outer cell membrane via a glycosylphosphatidylinositol (GPI) anchor, and its C-terminal propeptide is removed during serine lipidation. Three isozymes (CA IX, CA XII, and CA XIV) are type I membrane proteins with an extracellular catalytic domain, a single transmembrane helix, and a short intracellular tail containing potential phosphorylation sites. In CA IX, these sites regulate interaction with the bicarbonate transporter NBC1, whereas in CA XII and CA XIV, their functional roles remain less well-defined [116–118]. The CA IX isozyme contains a unique N-terminal proteoglycan-like domain [114]. The CARP VIII has an N-terminal, while CARP XI has a

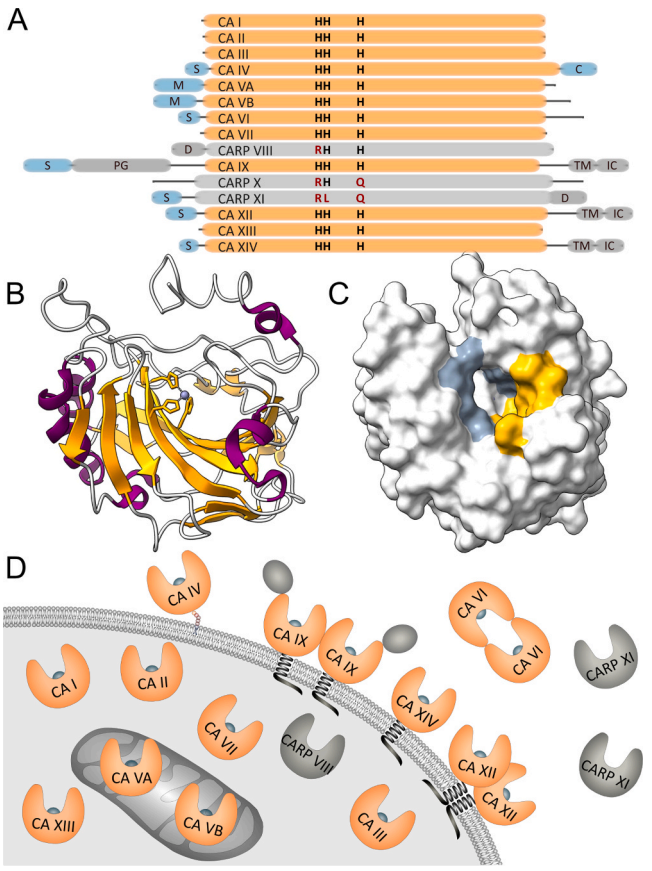

Fig. 2. Structure and cellular localisation of human CA proteins. **A**. Comparison of human CAs' primary structure. All proteins contain a catalytic domain (CA or CARP) with histidines that coordinate the Zn^{2+} ion (or alternative residues in CARPs). Signal sequences (S) direct proteins to the extracellular space, while transit peptides (M) target them to mitochondria. The C-terminal propeptide of CA IV (C) is cleaved during GPI anchoring. CA IX, CA XII, and CA IV have transmembrane helices (TM) and short intracellular domains (IC), while CA IX uniquely possesses an N-terminal proteoglycan-like domain (PG). Some CARPs have disordered regions (D). **B**. Typical CA catalytic domain structure – here in CA II, the secondary structure elements and Zn^{II} coordinating histidines are highlighted. **C**. The surface of CA II, hydrophobic (gold) and hydrophilic (grey) active site residues highlighted. ChimeraX 1.10.1 software [115] and PDB ID 4PYY were used for visualisation. **D**. Cellular localisation: proteins are shown as horseshoe-shaped figures, with the grey sphere marking the Zn^{II} coordinated in the active site. Catalytically active isozymes are indicated in orange, while catalytically inactive CA-related proteins (CARPs), which lack Zn^{II} coordination, are shown in grey. CA I, CA II, CA III, CA VII, CA XIII, and CARP VIII are localised in the cytosol; CA VA and CA VB are mitochondrial; CA IX, CA XII, and CA XIV contain a transmembrane domain with the catalytic site facing the extracellular space. CA IV is attached to the outer cell membrane via a glycosylphosphatidylinositol (GPI) anchor. CA VI, CARP X, and CARP XI are secreted.

C-terminal disordered sequence fragment.

All catalytically active human CA isozymes possess a cone-shaped active site cleft approximately 15 Å deep. At the bottom of this cleft, a catalytic zinc ion is coordinated by three histidine residues (His94, His96, and His119, following the standard amino acid numbering of CA II). Carbonic anhydrase-related proteins (CARPs) lack one or more of these active-site histidines, resulting in their inability to coordinate a metal ion. Specifically, in CARP VIII, His94 is replaced by arginine; in CARP X, His94 is replaced by arginine and His119 by glutamine; and in CARP XI, all three histidines are replaced by other amino acids: His94 by arginine, His96 by leucine, and His119 by glutamine [106].

The active site of human CAs has a distinctive feature: it consists of two halves, one of which is dominated by hydrophobic residues, while

the other half consists of hydrophilic amino acid residues (Fig. 2). This unique "bipolar" architecture of the active site was first observed in the crystal structure of the human isozyme CA II [23]. This active site design serves an important function: the hydrophobic side is thought to trap carbon dioxide, a relatively hydrophobic, poorly water-soluble gas. Conversely, the hydrophilic half of the active site likely facilitates the release of the polar products generated during CO_2 hydration (namely, bicarbonate and protons) from the enzyme active site cavity into the surrounding environment. In particular, it has been well demonstrated that the release of protons from CO_2 hydration involves several water molecules and polar histidine residues, thereby significantly enhancing proton release. Additionally, the active site contains a proton shuttle system, typically involving a histidine residue (His64 in CA II), which facilitates rapid proton transfer during the catalytic cycle.

5. Catalytic cycle and inhibition of enzymatic activity

The catalytic cycle of human CAs follows a two-step ping-pong mechanism (Fig. 3) [119]. The process begins with the entry of the substrate, carbon dioxide, into the enzyme's active site. Here, the catalytically active species, a Zn^{II} hydroxide complex, acts as a potent nucleophile, targeting the carbon dioxide molecule to form the bicarbonate ion. The bicarbonate ion is then released into the surrounding medium and replaced by a water molecule from the solvent. To regenerate the active form of the enzyme, the Zn^{II}-bound water molecule must be deprotonated to form the Zn^{II} hydroxide complex. This proton release is the rate-limiting step in the overall catalytic process. Catalytically active human CA isozymes (except CA III, CA VA, and CA VB) contain a so-called proton shuttle residue, His64, which assists in proton transfer by changing its conformation. Unlike most CAs that use His64 as a proton shuttle, CA VA and CA VB have Tyr64 in this position and employ Tyr100, Lys91, and Tyr131 for proton transfer [120].

Classical sulphonamide inhibitors interrupt the CA catalytic cycle by displacing the Zn^{II}-bound water molecule (Fig. 3**I**) [121]. The binding only occurs when the sulphonamide is in its deprotonated, negatively charged form [122,123]. However, since the pK_a of typical sulphonamides is in the basic region (pH 8–11), at neutral pH they exist as neutral protonated substances. To bind, they first have to deprotonate, which requires energy and thus diminishes their affinity. To evaluate the contribution of the binding-linked deprotonation of the inhibitor, the term *intrinsic* binding affinity is introduced. This should be distinguished from the binding affinity observed by any experimental technique [124,125].

A major target-specific constraint in developing isozyme-selective CA inhibitors is the high structural similarity among isozymes. Nevertheless, substantial progress has been achieved at the experimental and preclinical levels [9], and several CA IX-selective compounds have advanced to clinical trials (discussed in subsequent chapters). More broadly, drug development remains challenged by the limited translation of *in vitro* potency into *in vivo* efficacy, reflecting the impact of pharmacokinetics, tissue distribution, and target accessibility [126].

6. Carbonic anhydrase inhibitors in current clinical practice

While altered carbonic anhydrase expression or activity is rarely the primary cause of disease, with exceptions in previously described CA deficiencies (Table 1), these enzymes are useful tools for controlling diseases and disorders linked to fluid balance, pH regulation, and ion homeostasis. Over time, the use of CA inhibitors has evolved: early applications focused on their diuretic properties, while current use is more specialised to treat conditions in the eye, kidney, and central nervous system. These drugs, which are generally broad-spectrum inhibitors of CA isozymes, produce therapeutic effects by interfering with proton transfer, fluid balance, and electrochemical gradients across tissues and organ systems. Although they are not first-line agents in most conditions, they remain crucial in specific clinical situations where their unique pharmacological action is necessary [14,127].

There are currently many types of CA inhibitors and proposed inhibition mechanisms described in the literature [128]; however, all clinically approved CA inhibitors share either a sulphonamide (-SO_2NH_2) or a sulphamate (-NH-SO_2NH_2) functional group (Table 2). These groups bind directly to the zinc ion in the enzyme's active site, displacing the hydroxide ion, which normally participates in the enzyme's catalytic activity (Fig. 2).

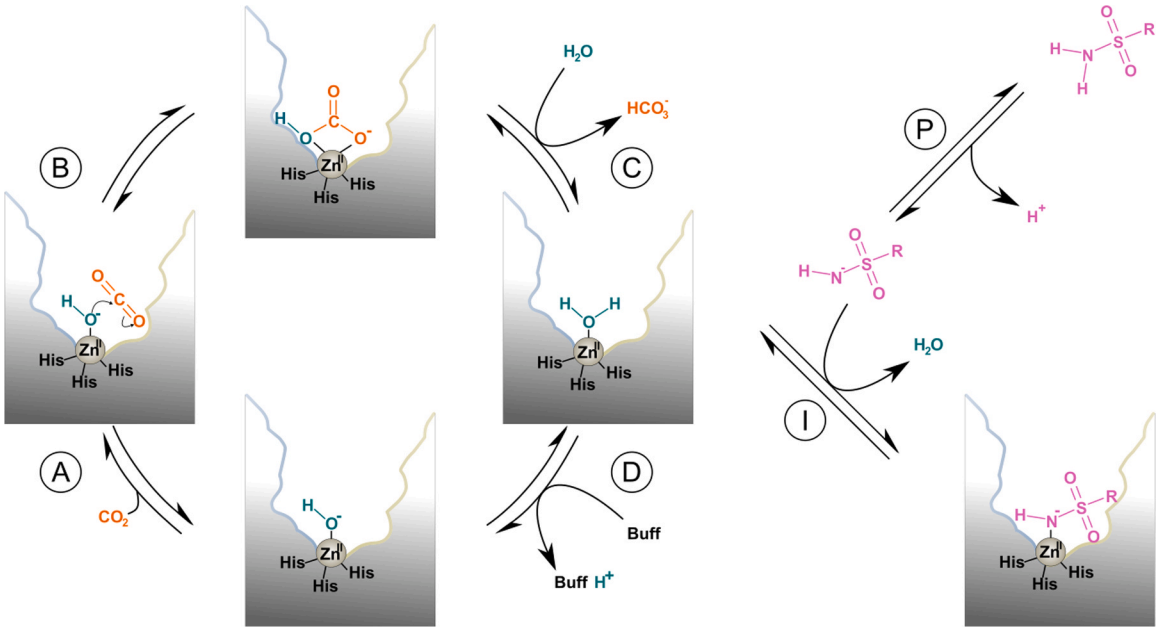

Fig. 3. Catalytic cycle and inhibition of CA enzymes by sulphonamide inhibitors. In the catalytic cycle, the CO_2 molecule binds to the hydroxide form of CA (**A**), forming a bicarbonate anion (**B**), which is then displaced by a water molecule (**C**) that needs to be deprotonated to reform an active enzyme molecule (**D**). Sulphonamide inhibitors bind carbonic anhydrase by replacing the water molecule (not the hydroxide anion, as does CO_2) (**I**). To bind the CA, the sulphonamide inhibitor has to undergo deprotonation **(P)**.

Table 2
Clinically used small-molecule CA inhibitors (A) and key CA-targeting agents used in recent clinical trials (B).

A. CA inhibitors in clinical practice

Generic name	Brand name(s) examples	Type	Year of approval	Primary indication	Often used in combination with
Acetazolamide	Diamox	Small molecule	1953 (FDA)	Glaucoma, altitude sickness, epilepsy, IIH, and diuretic	Carbamazepine, Furosemide
Ethoxzolamide	Ethamide, Cardrase	Small molecule	1950s (FDA)	Glaucoma, diuretic (historical use)	
Dichlorphenamide	Keveyis, Ormalvi	Small molecule	1958 (FDA) and 2015 (FDA)	Periodic paralysis	
Methazolamide	Neptazane	Small molecule	1959 (FDA)	Glaucoma	
Sulthiame	Ospolot	Small molecule	1950s	Epilepsy	
Dorzolamide	Trusopt	Small molecule	1995 (FDA)	Glaucoma	Timolol, Latanoprost
Topiramate	Topamax, Trokend XR,	Small molecule	1996 (FDA), 1997 (EMA)	Epilepsy, migraine prevention	Valproate, Lamotrigine
Brinzolamide	Azopt	Small molecule	1998 (FDA)	Glaucoma	Timolol, Brimonidine
Zonisamide	Zonegran	Small molecule	2000 (FDA), 2005 (EMA)	Epilepsy	

B. CA-targeting agents in recent clinical trials

Name	Clinical trial No	Type	Study start	Primary indication	Combination with
SLC-0111	NCT02215850	Small molecule	2014 Ph1	Advanced solid tumours that over-express CAIX	
SLC-0111	NCT03450018 (terminated)	Small molecule	2019 Ph1/Ph2	Cancer	Gemcitabine
^{89}Zr-TLX250	NCT03849118, NCT06750419	Antibody conjugate	2019 Ph3, 2024 Ph3 in progress	Non-invasive detection of clear cell renal cell cancer	
^{68}Ga-NY104	NCT05728515	Small-molecule	2021 Early Ph1	ccRCC imaging	
^{111}In-XYIMSR-01	NCT07062549	Small-molecule	2022 Ph1	Metastatic ccRCC	
^{177}Lu-TLX250	NCT05868174	Antibody conjugate	2023 Ph1 in progress	CA IX-expressing solid tumours	Peposertib
^{177}Lu-girentuximab	NCT05663710	Antibody conjugate	2023 Ph1b/2 in progress	Advanced ccRCC	Cabozantinib, Nivolumab
^{68}Ga-DPI-4452 ^{177}Lu-DPI-4452	NCT05706129	CAIX-targeting cyclic peptide with a DOTA cage	2023 Ph1/2 in progress	Unresectable locally advanced or metastatic solid tumours	
^{68}Ga-NY104	NCT06613269	Small-molecule	2024 Ph2 in progress	ccRCC guided radiation therapy	18F-FDG PET/CT
^{68}Ga-NYM096/ ^{177}Lu-NYM096	NCT06649682 (suspended)	Small molecule	2024 Ph1	Metastatic ccRCC	
^{68}Ga-C1 PET/CT	NCT06519760	Small molecule	2024 in progress Ph N.A.	CA IX-specific PET imaging in ccRCC patients	
CA9-hu1	NCT05698238	Antibody	2024 Ph1 in progress	Advanced solid tumours	
^{68}Ga-NYM096	NCT07144748	Small molecule	2025 in progress Ph N.A.	Various kinds of tumours	
^{177}Lu-TLX250	NCT07197580	Antibody conjugate	2025 Ph3 in progress	CAIX expressing advanced relapsed or recurrent ccRCC	
^{68}Ga-OncoCAIX	NCT06840548	Small molecule	2025 Ph1	ccRCC patients	
^{211}At-girentuximab	NCT07260162	Antibody conjugate	2026 Ph1 in progress	Bladder cancer	

Drugs that inhibit CA activity can be classified into two main categories: 1) compounds designed to inhibit CAs and used as CA inhibitors, and 2) compounds that can inhibit CAs according to *in vitro* data, but have other molecular targets and CA inhibition is not their primary function. In the latter category are drugs such as $Na^+/K^+/2Cl^-$ cotransporter inhibitors (NKCC inhibitors, also known as loop diuretics, including furosemide) and Na^+/Cl^- cotransporter inhibitors (NCC inhibitors, also referred to as thiazide/thiazide-like diuretics, including hydrochlorothiazide, indapamide, and chlorthalidone). These drugs demonstrate affinities for multiple CA isozymes *in vitro*, typically in the low micromolar to submicromolar range [25,129]. Similarly, certain COX-2-selective inhibitors, such as celecoxib and valdecoxib, inhibit several CA isozymes with nanomolar potency *in vitro*; however, their therapeutic effects primarily result from their action on cyclooxygenase rather than from CA inhibition [130,131]. Overall, while many clinically used sulphonamide-containing drugs can bind to CA isozymes under experimental conditions, CA inhibition is not their primary pharmacological mechanism at therapeutic doses [25].

The primary clinical applications of carbonic anhydrase inhibitors include:

1) Ophthalmology (glaucoma and ocular hypertension). The CA inhibitors lower intraocular pressure by slowing aqueous humour production. Systemic drugs (acetazolamide, methazolamide) were among the first effective treatments, but their frequent side effects (e.g., polyuria, paraesthesia, and metabolic acidosis) led to the development of topical versions. Topical CA inhibitors (dorzolamide and brinzolamide) are often used in combination with other medications, such as beta-blockers and alpha-agonists, and are generally second- or third-line agents and can reduce intraocular pressure by 15–20% with fewer systemic side effects [14,24].

2) In renal disorders (oedema) and metabolic disorders (alkalosis, refractory cases), CA inhibitors (acetazolamide, methazolamide) help correct acid-base imbalances by inducing metabolic acidosis and leading to mild diuresis due to inhibition of bicarbonate reabsorption in the kidney proximal tubule. Inhibition of CA reduces bicarbonate formation and reabsorption, leading to increased urinary excretion of bicarbonate, sodium, and water [132,133]. This results in systemic acidification and a transient natriuretic effect.
3) Respiratory and altitude medicine (acute mountain sickness, high-altitude pulmonary oedema prevention, and central sleep apnoea). Acetazolamide adjusts acid-base balance by inducing mild metabolic acidosis, thereby promoting bicarbonate excretion by the kidneys. This triggers a compensatory increase in ventilation, elevating blood oxygen levels and reducing the risk of sleep- or altitude-related complications. Inhibition of CA reduces sodium and water reabsorption in the kidneys, thereby helping prevent fluid buildup [134,135].
4) In neurological disorders (idiopathic intracranial hypertension (IIH) and epilepsy), CA inhibitors are used to reduce cerebrospinal fluid (CSF) production in IIH; they also can alter ion balance, thus stabilising neuronal activity. In IIH, acetazolamide is the most commonly used agent. It reduces CSF production by inhibiting CA in the choroid plexus, lowering intracranial pressure [136]. Sulthiame, which is specifically developed for epilepsy, is used in Europe, particularly for paediatric forms of the disease [137]. Other antiepileptic drugs with a broad mechanism of action that also exhibit inhibitory activity against CA are zonisamide and topiramate. Zonisamide, approved for partial seizures, is a sulphonamide anticonvulsant with weak CA inhibitory activity and also affects sodium and calcium channels. Topiramate, a broad-spectrum antiepileptic, modulates GABA and glutamate receptors and also inhibits CA. Topiramate is effective in preventing migraines, as it can reduce both the frequency and intensity of migraine attacks by nearly 50% [138].
5) Periodic paralysis (both hypokalaemic and hyperkalaemic forms) is managed with CA inhibitors, which help regulate potassium balance and prevent episodes of muscle weakness. Acetazolamide has been used for this indication since 1968 [139]. Initially approved in 1958, Dichlorphenamide was discontinued in 2002 and reintroduced in 2015 for primary periodic paralysis following new clinical evidence of its efficacy [140]. It is a potent inhibitor of CA II and is currently marketed in the U.S. under orphan drug status for this rare neurological condition.

Systemic CA inhibitors, administered orally or by injection, are used to control renal, neurological, and metabolic disorders; however, they frequently cause adverse effects because most clinically used agents simultaneously inhibit multiple CA isozymes [141]. In some cases, broad inhibition can be therapeutically beneficial (e.g., acetazolamide inhibiting CA II, CA IV, and CA XII to lower intraocular pressure in glaucoma); however, inhibition of off-target CA isozymes can also cause dose-limiting side effects. For instance, inhibition of CA II and CA IV in the kidney by systemic sulphonamides causes metabolic acidosis, paraesthesia, and electrolyte disturbances. A partial solution to this challenge is the use of topical inhibitors in ophthalmology, where administration as eye drops allows local action in the ciliary body to reduce intraocular pressure while minimising systemic absorption and off-target effects. Nevertheless, current research efforts are shifting toward the design of more selective CA inhibitors that target specific isozymes to enhance therapeutic efficacy while limiting adverse effects [142].

Although non-selective systemic inhibition of CA isozymes can cause characteristic adverse effects, CA inhibitors are generally considered safe and well-tolerated [141]. This favourable profile reflects the fact that clinically used doses never achieve full inhibition of all CA activity and that humans express 12 catalytically active CA isozymes capable of partial functional compensation. The most common systemic side effects, such as fatigue, nausea, abdominal discomfort, diarrhoea, paraesthesia, and taste alteration, are typically dose-dependent and reversible. In contrast, metabolic acidosis, hyponatraemia, or hypokalaemia occur less frequently [25]. Although rare immune-mediated reactions such as Stevens-Johnson syndrome or toxic epidermal necrolysis have been reported even with topical sulphonamide-based therapies, their incidence is exceedingly low [143]. Moreover, true cross-reactivity with sulphonamide allergies is rare because CA inhibitors lack the aniline structure responsible for classic sulphonamide hypersensitivity. However, they should still be avoided in individuals with a history of severe reactions [144,145]. Importantly, systemic CA inhibitors are contraindicated in patients with liver cirrhosis, significant renal impairment, or advanced pulmonary disease [25]. The recommended maximal daily doses (e.g., 1000 mg acetazolamide, 300 mg methazolamide, 200 mg dichlorphenamide) should be followed to minimise risk [14]. In contrast, topical ocular CA inhibitors deliver very low systemic exposure and therefore have a substantially lower adverse-event burden. Overall, while systemic agents require individualised risk-benefit assessment, especially during long-term use, the safety and tolerability of CA inhibitors remain well established across clinical applications.

7. Emerging indications and novel applications of carbonic anhydrase inhibitors

Although CA inhibitors have established therapeutic roles in clinical practice, recent research is exploring their potential in new disease areas, particularly in oncology. There is a growing interest in targeting the hypoxia-associated isozymes CA IX and CA XII, which play significant roles in tumour growth, invasion, and metastatic progression [146]. In preclinical studies, inhibition of CA IX and CA XII has been shown to normalise extracellular pH levels, enhance the effectiveness of chemotherapy, radiotherapy, and immunotherapy, and decrease invasion by disrupting the interaction with monocarboxylate transporters in hypoxic environments [147]. Preclinical studies consistently report that blocking CA IX and CA XII reduces tumour proliferation, migration, and growth in xenograft models. However, achieving complete and sustained tumour regression solely through CA IX inhibition remains a challenge [148,149]. This has led to consensus that targeting CA IX and CA XII is most effective when used in combination with other therapies rather than as a standalone treatment, and in clinical trials, CA inhibitors (e.g., SLC-0111, acetazolamide) are being pursued in combination regimens [27,150].

Over several decades, much effort has been dedicated to the discovery of new isozyme-specific CA inhibitors, especially targeting tumour-associated isozymes CA IX and CA XII. The first-in-class small-molecule compound, SLC-0111 (**Table 2**), has advanced to clinical trials. In a first-in-human Phase 1 trial (NCT02215850) in previously treated advanced solid tumours, SLC-0111 showed an acceptable safety profile and pharmacokinetics supporting 1000 mg once daily as the recommended Phase 2 dose [27]. A Phase 1b/2 study combining SLC-0111 with gemcitabine in CA IX-positive metastatic pancreatic ductal adenocarcinoma (NCT03450018) is currently listed as Terminated on ClinicalTrials.gov.

Limited CA IX expression in normal tissues and high upregulation in hypoxic tumours have led to the development of antibody-based CA IX-targeting agents, both for therapeutic and cancer-imaging applications. The foundation for this was laid in 1986, when an antibody G250 was generated by immunising mice with ccRCC (clear cell renal cell carcinoma) cells [26]. Later, its molecular target was identified: the catalytic domain of the cancer-associated human CA IX. To reduce immunogenicity, a chimeric version of cG250 was designed. cG250, also known as girentuximab (Rencarex), entered Phase III clinical trials (NCT00087022) and was well tolerated and safe; however, it did not improve disease-free or overall survival compared with placebo.

Clinical development of CA IX antibodies subsequently focused on

radiolabelled variants for therapy and imaging [151]. ^{131}I-girentuximab was the first attempt at CA IX-targeted radioimmunotherapy – the antibody delivers a therapeutic beta-particle from ^{131}I to the tumour. Trial NCT00003102 (phase 1/2) demonstrated the concept and stabilised disease in some patients, but had limited efficacy and required patient isolation. The ^{124}I-girentuximab was tested as a diagnostic PET reagent in a phase 3 trial (NCT00606632). The results showed high sensitivity and specificity for the identification of ccRCC. However, later ^{124}I-girentuximab was replaced by ^{89}Zr-based conjugate. TLX250-CDx (Zircaix®, ^{89}Zr-DFO-girentuximab) is the current most advanced antibody-based investigational kidney cancer PET imaging agent, a ccRCC (Phase 3 trial NCT03849118) and mTNBC Metastatic Triple Negative Breast Cancer (phase 2 trial NCT04758780) diagnostic tool, and TLX250 (^{177}Lu-girentuximab, NCT07197580) is being developed as a therapeutic agent targeting CA-IX expressing cancers [152]. Recently, ^{211}At-girentuximab (ATO-101™) entered clinical trials (NCT07260162) for patients with bladder cancer [153].

BAY 79-4620 and CA9hu-1 represent alternative CA IX-targeting biologics that have been or are being explored clinically. BAY 79-4620 is an antibody–drug conjugate built on the fully human monoclonal antibody 3ee9 and linked to monomethyl auristatin E through a cleavable valine–citrulline linker; although it produced strong antitumour activity in CA IX-positive xenograft models, its development did not progress beyond Phase 1 studies (NCT01028755, NCT01065623) [154]. CA9hu-1, in contrast, is a humanised monoclonal antibody directed against a catalytic-domain epitope of CA IX. It demonstrated inhibitory activity across several hypoxic solid tumour models and is currently under evaluation in a Phase 1 trial for advanced solid tumours (NCT05698238) [155].

The slow pharmacokinetics of antibody derivatives can be overcome by smaller molecules currently in clinical trials, such as the CAIX-targeting cyclic peptidomimetic DPI-4452, which carries a DOTA cage (NCT05706129) [156], and the small-molecule-based NY104 (NCT06613269) [157], NYM096 (NCT07144748) [158], XYIMSR-01 (NCT07062549) [159], and OncoCAIX (NCT06840548) [160], some of which have undisclosed, proprietary structures.

Another emerging application in the CA inhibitor field is its potential use in managing obesity and metabolic disorders. Clinical observations of weight loss in patients treated with the anticonvulsants topiramate and zonisamide sparked interest in their possible role in obesity therapy [70]. CA inhibitors may reduce *de novo* lipogenesis by inhibiting mitochondrial CA isozymes; however, CA inhibition is most likely only one component of a broader mechanism that includes appetite suppression and neurological effects. These findings ultimately led to the development of the phentermine/topiramate extended-release combination (Qsymia), the first FDA-approved obesity pharmacotherapy containing a CA inhibitor in 2012.

The use of CA inhibitors is widening in the area of neurological diseases; researchers are exploring possibilities for treating Alzheimer's disease, sleep apnoea, migraines, and other neurological conditions. Several Phase II-IV clinical trials evaluate the use of acetazolamide as a potential treatment for obstructive sleep apnoea, either as a single agent or in combination [161,162].

The role of carbonic anhydrases in pain modulation is a promising yet clinically unrealised area. Mechanistically, CA inhibition in the spinal cord reduces abnormal neuronal excitability that arises following peripheral nerve injury, restoring normal inhibitory signalling and reducing pain hypersensitivity in preclinical models [163,164]. This effect is isozyme-dependent: selective inhibitors targeting CA II/VII, mitochondrial CA VA/VB, or CA IV have each shown analgesic effectiveness in different pain models: neuropathic, chemotherapy-induced, and visceral inflammatory pain, respectively, generally outperforming the non-selective CA inhibitor acetazolamide [165–167]. Recently, hybrid compounds combining CA inhibition with opioid receptor activity have shown sustained analgesia with reduced tolerance in animal models, suggesting a potential strategy to address a major limitation of current opioid therapy [168]. However, clinically used anticonvulsants such as topiramate and zonisamide, although weak CA inhibitors, appear to exert their analgesic effects mainly through other mechanisms, suggesting the CA-pain axis remains compelling but unvalidated in humans.

8. Future directions for CA-targeting

Carbonic anhydrases are essential drug targets due to their central roles in bicarbonate metabolism, ion transport, and pH regulation. CA inhibitors have therefore become essential tools in ophthalmology, nephrology, neurology, emergency medicine, and high-altitude physiology. Despite the large number of human CA isozymes, only a limited number of CA-inhibiting drugs are in clinical use. As illustrated in the timeline in Fig. 1, many of the currently used drugs were approved before all human CA isozymes were discovered. Among systemic inhibitors, acetazolamide is the best-established agent, employed for conditions such as idiopathic intracranial hypertension, altitude sickness, certain epilepsies, and oedema [133]. Methazolamide, a more lipophilic analogue with improved tolerability, is used less frequently but can be preferable in some cases, particularly for ocular and neurological indications [14]. In glaucoma therapy, systemic CA inhibitors have largely been replaced by topical drugs. Dorzolamide and brinzolamide are now widely used to lower intraocular pressure, offering comparable efficacy with markedly fewer systemic side effects [24]. This therapeutic advance contributed to the discontinuation of older systemic agents such as ethoxzolamide.

Other CA inhibitors serve niche indications. Dichlorphenamide was reintroduced and subsequently approved by the FDA for the treatment of primary periodic paralyses [140]. Sulthiame remains in clinical use for paediatric epilepsy in parts of Europe, Japan, and Australia [137]. Two antiepileptic agents – topiramate and zonisamide – were initially developed without CA inhibition in mind, but later shown to inhibit several CA isozymes. This off-target effect is now recognised as a contributor to both therapeutic actions (such as weight reduction) and adverse effects, including paraesthesias and metabolic acidosis. The weight-loss effect is thought to involve inhibition of mitochondrial CA isozymes (CA VA and VB), which regulate fatty acid synthesis and energy metabolism [169].

The use of CA inhibitors in disorders such as glaucoma, epilepsy, or altitude sickness illustrates a broader pharmacological principle: drugs are used to modulate physiological processes rather than to correct a primary defect in the target enzyme **(**Table 3**)**. This concept is not unique to CAs. For instance, diuretics that inhibit the $Na^+/K^+/2Cl^-$ cotransporter or the Na^+/Cl^- symporter are used in hypertension and oedema, conditions not caused by transporter dysfunction but where altering renal ion transport is therapeutically beneficial [170]. Similarly, statins inhibit HMG-CoA reductase not because of pathological enzyme overactivity, but because reducing cholesterol biosynthesis lowers cardiovascular risk [171]. These examples highlight how well-characterised molecular targets can be exploited in clinical contexts beyond their direct causal role.

In contrast to CA inhibitors, CA activators enhance the rate of proton transfer, the rate-limiting step in the catalytic cycle of carbonic anhydrase. Activators (typically amines, amino derivatives, or amino acids) bind near the entrance to the active site and act as proton shuttles, effectively accelerating turnover without altering substrate binding [172]. Preclinical findings show that activating human carbonic anhydrases improves cognitive function, glial health, cerebrovascular function, and pH balance in Alzheimer's disease experimental systems [173, 174]. Experiments in rodents showed that activating brain carbonic anhydrase enhances learning and memory in vivo [175]; however, no further clinical translation has yet been achieved.

On the other hand, while loss-of-function variants are currently known in CA II, CA VA, CA VIII and CA XII, none of these conditions can be corrected at the level of the defective isozyme **(**Table 3**)**. Treatment,

Table 3
Mechanistic functions of human carbonic anhydrases and their inhibitors across diverse disease contexts. The table distinguishes three categories of conditions: those in which CA does not contribute to disease progression and CA inhibitors are used symptomatically via modulation of ion and fluid physiology; those in which CA participates in disease processes and inhibition is therapeutically beneficial; and those in which CA dysfunction is the primary cause of pathology but CA inhibition is not a viable therapeutic approach.

Symptomatic inhibitor use: CA inhibition modulates ion/fluid physiology			
Disease/disorder	**Isozyme**	**CA function**	**Mechanism**
Glaucoma, ocular hypertension	CA II, CA IV, CA XII	CA in non-pigmented ciliary epithelium generate bicarbonate that drives aqueous humour secretion	Inhibition slows fluid production, lowering IOP
Oedema, metabolic alkalosis	CA II, CA IV	Renal CAs catalyse $NaHCO_3$ reabsorption in the proximal tubule	Inhibition of proximal tubule CA blocks $NaHCO_3$ reabsorption, resulting in bicarbonaturia, natriuresis, mild diuresis, and corrective metabolic acidosis
Altitude sickness	CA II, CA IV	CAs in the renal proximal tubule regulate bicarbonate reabsorption, maintaining systemic acid-base balance	CA inhibition induces mild metabolic acidosis via bicarbonaturia, stimulating compensatory hyperventilation and raising blood O_2
Intracranial hypertension	CA II, CA IV, CA XII	CAs in the choroid plexus support bicarbonate-dependent CSF secretion	CA inhibition in the choroid plexus reduces CSF secretion, lowering intracranial pressure
Epilepsy	CA II, CA VII, CA XIV	CAs modulate neuronal bicarbonate flux and pH-dependent excitability	CA inhibitors alter neuronal ion balance and stabilise membrane excitability as an adjunct mechanism
Periodic paralysis	CA II	CA participates in pH-dependent ion homeostasis	CA inhibition reduces attack frequency, likely via pH-mediated membrane stabilisation and K^+ handling
CA participates in disease processes: inhibition is beneficial			
Hypoxic solid tumours	CA IX, CA XII	HIF-1α transcriptionally induces CA IX in hypoxic tumour cells. CA IX catalyses the extracellular hydration of CO_2, directly acidifying the extracellular space. Extracellular acidosis drives invasion, suppresses anti-tumour immunity, and confers chemoresistance	Inhibition reduces extracellular acidification, decreases invasiveness, and improves response to therapy
Obesity, Metabolic disease	CA VA, CA VB	Mitochondrial CAs supply HCO_3^- to pyruvate carboxylase and CPS-I, rate-controlling enzymes in gluconeogenesis and the urea cycle	Inhibition reduces hepatic glucose output; the weight-loss effect of topiramate is partly attributed to CA V inhibition
Neuropathic/ inflammatory pain	CA VII	In spinal dorsal horn neurons, CA VII generates intracellular HCO_3^- that exits through GABA-A receptors during sustained nociceptive activation, converting inhibitory GABAergic signalling into excitatory depolarisation — a direct driver of central sensitisation	Inhibition restores inhibitory GABAergic drive by limiting bicarbonate accumulation
Osteoporosis, bone metastases	CA II	Osteoclasts use CA II to acidify the resorption lacuna, dissolving hydroxyapatite. CA II is functionally central to resorption	Inhibition reduces osteoclast activity and bone loss in preclinical models
CA participates in disease processes: CA inhibition is not beneficial			
CA II deficiency syndrome	CA II	Loss of CA II impairs renal acid handling and osteoclast function	Inhibitor application will provide no therapeutic benefit
CA IV mutation - retinitis pigmentosa type 17 (RP17)	CA IV	Missense mutations (R14W) cause CA IV misfolding in photoreceptors, resulting in ER stress via the unfolded protein response and apoptosis	
CA VA deficiency	CA VA	Loss of mitochondrial CA VA blocks HCO_3^- supply to carbamoyl phosphate synthetase I, resulting in hyperammonaemia and metabolic acidosis in infancy	
CA XII deficiency	CA XII	Loss of CA XII impairs Cl^-/HCO_3^- exchange, resulting in hyponatraemia and salt wasting	

therefore, focuses on managing downstream consequences, such as metabolic acidosis in CA II deficiency, hyperammonaemia in CA VA deficiency, neurological impairment in CARP VIII-related ataxia, or salt loss in CA XII deficiency, rather than restoring enzymatic activity [30,33,36,92].

Advances in the identification, structural characterisation, and functional understanding of the 12 catalytically active human carbonic anhydrase isozymes have enabled the development of isozyme-selective small-molecule inhibitors and antibody-based therapeutics. Recent structure-based analyses have further refined the understanding of the binding-site features that distinguish the cancer-associated CA IX and CA XII from the ubiquitous CA II, providing additional context for selective inhibitor design [176]. These efforts have been particularly impactful for CA IX, not only as a target for anticancer therapy but also as a focus for molecular imaging and theranostic applications. The sulphonamide SLC-0111 was the first isozyme-selective small molecule to complete a first-in-human Phase 1 study, demonstrating an acceptable safety and pharmacokinetic profile, and subsequently advanced into a Phase 1b/2 trial in combination with gemcitabine, which was later discontinued. In parallel, several low-molecular-weight CA IX–targeting ligands, including DPI-4452, NY104, NYM096, XYIMSR-01, and Onco-CAIX, have entered early clinical imaging studies, showing encouraging preliminary diagnostic performance while larger validation trials are ongoing. In addition, a Phase 3 trial of radiolabelled girentuximab (^{89}Zr-TLX250) demonstrated high sensitivity and specificity for clear cell renal cell carcinoma (ccRCC), supporting regulatory submissions. Nevertheless, further validation and regulatory documentation are required before these agents can be implemented in clinical practice.

Looking ahead, carbonic anhydrase drug discovery is expected to shift toward greater isozyme selectivity, precision targeting, the use of biologicals, and expanded therapeutic applications. Advances in structural biology, computational design, and chemical biology will facilitate the development of non-classical inhibitors, allosteric modulators, and tissue-selective delivery strategies that minimise systemic side effects. In oncology, CA IX- and XII-directed small molecules, antibodies, and radiopharmaceuticals are likely to mature from diagnostic tools into integrated theranostic platforms. Beyond cancer, emerging insights into mitochondrial and non-canonical CA functions may open new opportunities in metabolic, neurological, and inflammatory diseases, broadening the clinical relevance of this well-established enzyme family.

CRediT authorship contribution statement

Lina Baranauskiene: Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Helgi B. Schiöth:** Writing – review & editing, Writing – original draft, Validation, Formal analysis, Conceptualization. **Daumantas Matulis:** Writing – review & editing, Supervision, Resources, Formal analysis, Conceptualization. **Tautvydas Kojis:** Writing – original draft, Methodology, Investigation.

Declaration of Competing Interest

We, the authors of the manuscript “Trends in drug discovery for

carbonic anhydrases: FDA approvals, clinical trials, molecular functions, and therapeutic potential", declare that LB and DM own patents and have submitted patent applications on carbonic anhydrase inhibitors and their use for pharmaceutical purposes.

Data availability

No data was used for the research described in the article.

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