Chapter 13

Carbonic Anhydrase Inhibitors: Molecular Structures, Mechanistic Features and Therapeutic Applications

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Abstract

Carbonic anhydrases (CAs) are a large family of zinc-dependent metalloenzymes that catalyse the reversible hydration of carbon dioxide to bicarbonate and protons, a reaction fundamental to acid–base homeostasis, gas exchange, ion transport and metabolic regulation. Multiple CA isoforms exhibit distinct cellular localisations and tissue-specific expression patterns, reflecting their diverse physiological and pathological roles. Carbonic anhydrase inhibitors (CAIs) have long been used in the management of conditions such as glaucoma, altitude sickness and epilepsy; however, advances in structural biology, medicinal chemistry and molecular pharmacology have significantly expanded their therapeutic relevance. Detailed crystallographic and computational studies have revealed multiple inhibitor binding modes within the zinc-coordinated active site, enabling the rational design of isoform-selective compounds with improved efficacy and reduced systemic side effects. In particular, tumour-associated isoforms such as CA IX and CA XII have emerged as prominent targets due to their involvement in pH regulation, cellular adaptation to hypoxia and disease progression. Beyond oncology, CAIs are increasingly investigated for their potential roles in neurological, inflammatory, metabolic and infectious diseases. This chapter provides a comprehensive overview of carbonic anhydrase isoenzymes, their structural and biochemical characteristics, mechanisms of inhibition and

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the diversity of inhibitor classes. Emphasis is placed on structure–activity relationships and isoform selectivity as key determinants for the development of next-generation carbonic anhydrase inhibitors with broad therapeutic potential.

Introduction

Carbonic anhydrase inhibitors (CAIs) have attracted attention in recent years not only for their classical clinical indications, but also as alternative therapeutic approaches against global health threats such as increasing antibiotic resistance. Carbonic anhydrase (CA) enzymes play an important role in maintaining the balance of carbon dioxide and bicarbonate in microorganisms, making them important target molecules. In particular, classical CAIs such as acetazolamide have shown antibacterial activity against multidrug-resistant pathogens such as vancomycin-resistant Enterococcus spp. suggesting that CAIs may be effective beyond conventional antibiotics. Furthermore, the flexibility of active CA sites allows for multiple binding modes, enabling the development of isoform-selective inhibitors and creating potential for targeted therapies. This book chapter is organised to highlight the cellular distribution, biochemical functions, structure and diversity of inhibitors as well as the therapeutic potential of carbonic anhydrases.

Definition ofCarbonic Anhydrase

Carbonic anhydrase is a metalloenzyme that catalyses the reversible hydration of carbon dioxide to bicarbonate and protons and is of central importance for many basic physiological processes such as respiration, acid-base balance and electrolyte regulation. CA has a highly conserved protein structure that carries a zinc (Zn^{2+}) ion in its active centre and is subdivided into different isoenzyme classes such as α, β and γ. The Zn^{2+} ion normally coordinates with three histidine residues (His94, His96 and His119) to form the catalytic centre that enables hydration of the substrate (CO_2) (Figure 1). This family of enzymes, which has numerous isoforms in humans, contributes to the maintenance of systemic homeostasis by performing specific tasks in different tissues. Some CA isoforms show very efficient catalytic activity with kcat values of up to 10^6 s^{-1}. This high efficiency has led to potential use of the enzymes in biotechnological applications, such as carbon capture technologies. In addition, structural biology studies using advanced technologies such as native mass spectrometry have detailed the binding dynamics between human carbonic anhydrase I and its inhibitors, which has helped us to better understand the strategic value of CAs as drug targets (Supuran CT, 2016).

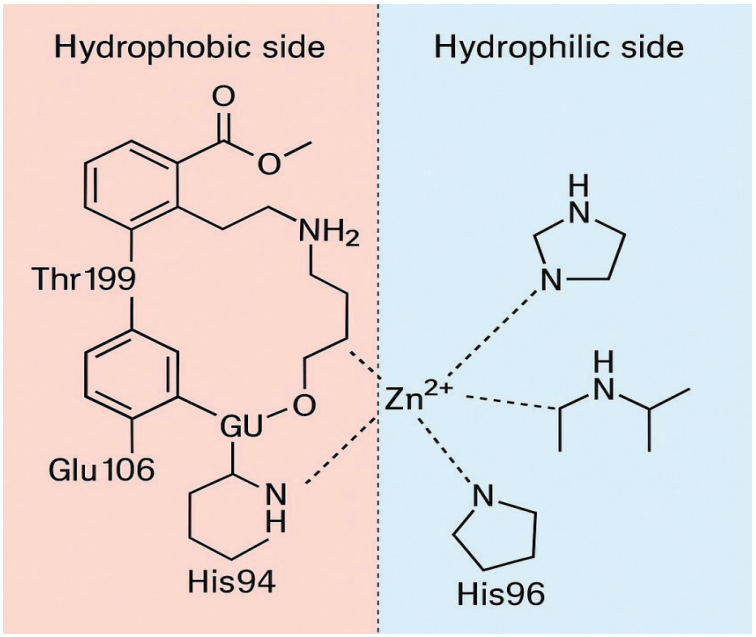

Figure 1. Schematic representation of a benzenesulfonamide inhibitor that binds to the active centre of human carbonic anhydrase II (hCA II). The active centre is divided into a hydrophobic (left, red) and a hydrophilic (right, blue) region. The central Zn^{2+} ion is coordinated by three histidine residues (His94, His96 and His119), while important hydrogen bonds and interactions with residues such as Thr199 and Glu106 occur on the hydrophobic side. This interaction is crucial for the inhibitory effect of sulphonamide-based drugs.

ImportanceofCarbonicAnhydraseinBiologicalSystems

Carbonicanhydrasesregulatevital biochemical functions not only in the respiratory and renal systems, but also in numerous organs and cell types. These enzymes are involved in systemic processes such as cell metabolism, gas exchange, fluid-electrolyte balance and ion transport through bicarbonate production and pH buffering (Table 1). Disruption of CA activity plays an important role in numerous pathological conditions, includingglaucoma, epilepsy, cancerandneurodegenerativediseases (Supuran CT, 2020). In recent years, CA isoforms have also been shown to be important in obstetric physiology. CAs have been reported to be involved in processes such as regulation of placental pH, uteroplacental blood flow and fetal gas exchange, and accordingly, enzyme imbalance may be associated with obstetric complicationssuch as pre-eclampsiaandintrauterinegrowthretard ation (Kumar M et al., 2023;Van Dyke JU et al., 2015). These findings

highlight the importance of understanding the physiological role of CA enzymes, not only in terms of basic research, but also for the development of clinical applications.

Table1. Physiological and Clinical Characteristics of Major CA Isoforms

CA Isoform	Tissue Distribution	Physiological Function	Clinical Role
CA I	Red blood cells, kidney	pH buffering; regulation of acid–base balance	Contribution to systemic acid–base homeostasis
CA II	Kidney, brain, bone	Rapid CO_2 hydration–dehydration cycle	Target in glaucoma therapy; involvement in renal tubular acidosis and neurological disorders
CA IX	Placenta; hypoxic tumours	Cellular adaptation to hypoxia; regulation of extracellular pH	Biomarker and therapeutic target in cancer
CA XII	Breast, ovary, various tumours	Regulation of acidic tumour microenvironment	Therapeutic target in oncology

Overview of Carbonic Anhydrase Inhibitors

Carbonic anhydrase inhibitors are pharmacological agents that are used to treat many clinical diseases, especially in glaucoma and hypertension. These compounds disrupt the acid-base balance by inhibiting the enzyme carbonic anhydrase, thereby lowering intraocular pressure and reducing aqueous humour production. These effects of CAIs have shown comparable efficacy to established therapies (e.g. beta-blockers), particularly in the treatment of glaucoma. Current pharmacotherapeutic studies are not limited to the traditional indications; the neuroprotective properties of CAIs, their potential for combination with Rho kinase inhibitors and their role in inflammatory processes are also being investigated (Futterknecht Set al.,2024).

On the other hand, new generation compounds, such as polmakoxib, inhibit the enzyme cyclooxygenase together with carbonic anhydrase and offer a therapeutic contribution in inflammatory diseases with a dual mechanism of action. Such pharmacological innovations strengthen the role of CAIs in modern medicine. The increasing knowledge of the tissue-specific distribution and physiological functions of CA isoforms offers a major advantage for the development of isomer-selective inhibitors. In particular, studies on the CA IX isoform have shown that CAIs can be promising agents for oncological treatments by targeting the adaptation process of tumour cells to acidic microsystems (McDonald et al., 2012).

The main aim of this book chapter is to provide a comprehensive review of the therapeutic roles, biochemical mechanisms and current research directions of CAIs in different diseases. In this direction, especially glaucoma, cancer, inflammatory diseases and clinical pictures related to the reproductive system are among the primary focal points. In addition, this study analyses the structure-activity relationships of CAIs and details compounds such as thiourea and sulfonamide derivatives, which have low toxicity but show strong inhibitory properties (Eldehna et al., 2017).

Recently developed glycosyl- and carbohydrate-based CAIs have great potential in terms of selectivity and bioavailability in drug design, which supports the inclusion of CAIs among the next generation of pharmacotherapy agents. Our goal in this chapter is to provide readers with a scientific basis for CAI research by presenting the molecular functioning of CAIs, their pharmacological diversity and their potential for use in modern clinical medicine from both classical and innovative perspectives.

Mechanismof Action

The mechanism of action of CAI has significant effects on basic physiological processes, particularly in organs such as the kidneys, lungs and placenta. By inhibiting the enzymes ofCA, these agents prevent the conversion of carbon dioxide into bicarbonate, resulting in a disturbance of the acid-base balance, reduced reabsorption of bicarbonate in the kidneys and consequently increased bicarbonate excretion in the urine. This mechanism plays an active role in the treatment of diseases such as glaucoma and metabolic alkalosis, but also influences systemic functions such as electrolyte balance and the regulation of fluid volume.However, given the role of CA isoforms in pregnancy-specific processes such as uteroplacental circulation, placental pH regulation, fetal oxygenation and nutrient diffusion, the use of CAIs in pregnancy should be carefully evaluated for fetal safety. Recent studies show that CAIs also have potential in infectious diseases. It has been reported that inhibitors targeting the carbonic anhydrase of Mycobacterium tuberculosis could offer an alternative route in the anti-tuberculosis treatment strategy. In addition, the development of penicillin-based CAI hybrid compounds represents an innovative approach in the fight against antibiotic resistance and demonstrates the potential of these inhibitors to interact with multiple bacterial targets (SupuranCT, 2020).

The Role of Carbonic Anhydrase in Physiological Processes

CAs are metalloenzymes that catalyse a reversible reaction in which carbon dioxide and water are converted into bicarbonate and protons. This

reaction is crucial for many vital processes such as pH balance, respiration, renal acid-base regulation, neuronal signalling and intracellular homeostasis. Since pH changes directly affect neurotransmission and neuronal activity, especially in brain tissue, CAs also play a key role in neurological functions. Recent studies show that CAs are involved in memory, learning and the pathogenesis of neurodegenerative diseases. CAIs have been shown to attenuate neurotoxicity in Alzheimer's disease by reducing amyloid-beta deposition. In addition, isoforms such as CA IX and CA XII promote the adaptation of cancer cells to the acidic tumour microenvironment. Therefore, the development of selective inhibitors against these isoforms is of great importance, especially for oncological therapies.There is also clear evidence that overexpression of CA IX in placental tissues under hypoxic stress can be used as a biomarker for the diagnosis of obstetric complications such as intrauterine growth retardation and pre-eclampsia. (Monti SM et al., 2012) .This suggests that carbonic anhydrases may be therapeutic targets not only in systemic homeostasis but also in pregnancy physiology.

Inhibitor-Enzyme Interaction

CAIs modulate enzyme activity by binding to active sites or allosteric sites of the enzyme and thus influence catalytic efficiency. These binding patterns are key factors that determine the selectivity and therapeutic efficacy of inhibitors. In particular, the isoform-selective binding capabilities of some inhibitors are of great importance for clinical targeting. For example, 2-(benzylsulfinyl)benzoic acid derivatives can inhibit specific isoforms such as human carbonic anhydrase IX (hCA IX) with high affinity, suggesting that structure-activity relationships (SAR) play a central role in drug design (Buemi MR et al., 2015)

The interactions of inhibitors with enzymes are not limited to the binding sites. The conformational dynamics of enzyme-inhibitor complexes can change depending on the binding mode of the inhibitor. In this context, advanced structural analysis methods such as circular dichroism spectroscopy (CD) show how the binding of an inhibitor leads to changes in the enzyme structure (Supuran CT, 2008). Such data contribute to the development of more targeted and effective therapeutic strategies, particularly in CA-related diseases such as cancer, neurodegeneration and acid-base disorders. Understanding the interaction between enzymes and inhibitors is essential for the development of next-generation selective CA inhibitors. Figure 2 shows the cellular localisation of CA isoforms and their importance for therapeutic targeting.

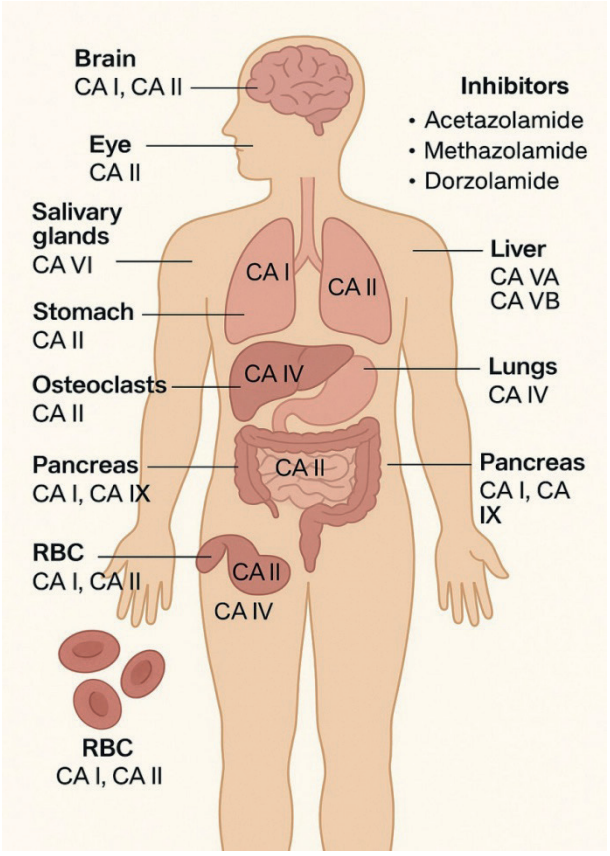

Figure 2. Anatomical distribution of CA isoforms and their primary therapeutic inhibitor targets.

This schematic diagram shows the distribution of the isoforms of carbonic anhydrase (CA I, II, IV, IX, XII) in the most important organs and the CAIs that are active against these isoforms. The isoforms CA II and IV are found in the kidney, the eye and the central nervous system, while the isoforms CA IX and XII are mainly found in hypoxic tumour tissue and in the placenta. Specific CAIs (e.g. acetazolamide, dorzolamide, SLC-0111) inhibit the target enzymes in the relevant tissues and influence physiological and pathological processes such as pH regulation, fluid secretion and modulation of the tumour microenvironment. This mapping illustrates the therapeutic vulnerability of CA isoforms and the importance of tissue selectivity for clinical pharmacology.

Mechanisms of Carbonic Anhydrase Inhibition and Isoform Selectivity

Understanding the mechanisms by which carbonic anhydrase inhibitors interact with their target enzymes is essential for the rational design of selective therapeutic agents. Beyond classical kinetic classifications, structural features and binding conformations play a critical role in determining inhibitor selectivity among carbonic anhydrase isoforms. Recent structural and computational studies on benzoxaborole derivatives have demonstrated that conformational flexibility can be exploited to enhance isoform selectivity by modulating enzyme–inhibitor interactions (Langella et al., 2019). Such insights highlight the importance of integrating structural biology with inhibition mechanism analysis to optimise pharmacological profiles and support the development of targeted carbonic anhydrase inhibitors with reduced off-target effects.

Effects of Inhibitionon Carbon Dioxide and Bicarbonate Levels

Inhibition of CA can lead to significant changes in the acid-base balance, as it has a direct effect on the carbon dioxide and bicarbonate levels in the body. This enzyme plays a critical role in maintaining physiological pH balance by catalysing the conversion between CO_2 and HCO_3- .In case of inhibition, bicarbonate production decreases and pH regulation, especially at the level of the renal tubules, may be impaired. This can limit the body's ability to buffer excess acids, which can lead to metabolic acidosis. In addition, some studies suggest that blocking carbonic anhydrase with acetazolamide may not only reduce bicarbonate levels, but may also affect polyp development and tumourprogression by modulating cytokine profiles (Occhipinti R et al., 2019).

Effectsof Altered Enzyme Activity

Carbonic anhydrase inhibitors exert effects not only at the biochemical level but also on systemic physiological processes. By suppressing the activity of carbonic anhydrase enzymes involved in acid–base balance and fluid and electrolyte regulation, these agents can alter key homeostatic mechanisms. In particular, impaired bicarbonate and sodium reabsorption in the renal tubules may predispose to electrolyte imbalance and acid–base disturbances. The tissue-specific distribution of carbonic anhydrase isoforms further influences the therapeutic efficacy and side-effect profile of these drugs, which is especially relevant in clinical settings such as glaucoma and oedema management (Occhipinti R et al., 2019).

Types of Carbonic Anhydrase Inhibitors

A. SulfonamidesasCarbonicAnhydraseInhibitors

Sulfonamides, synthetic antimicrobial agents, have an important place in medicinal chemistry thanks to their ability to inhibit carbonic anhydrase. These compounds, which usually have an A-SO_2NHR structure, play an important role in maintaining acid-base balance and supporting cellular respiration (Supuran CT, 2017). For example, some sulfonamide derivatives such as SLC-0111 have anti-cancer potential by inhibiting CA isoenzymes that are overexpressed in the hypoxic tumour microenvironment (Zhang et al., 2021). These inhibitors can suppress tumour growth, particularly by targeting CA IX. Therefore, sulfonamides are thought to have therapeutic value not only in infections but also in oncological processes. A better understanding of the physiological functions of CA activity at the level of the nephron emphasises the importance of these compounds for renal therapy.

B. Non-SulfonamideInhibitors

The development of non-sulfonamide inhibitors has gained great importance, especially in the search for selectivity against cancer-related isoforms such as CA IX and CA XII. These compounds can disrupt metabolic adaptation and suppress tumour growth by interfering with hypoxia-induced pH regulation in the tumour microenvironment (Zhang et al., 2021). Although structures such as ureido-substituted sulphamates are not yet fully used in the clinic, preliminary studies show that these agents are promising in aggressive tumour types such as triple-negative breast cancer (Williams KJ et al., 2019). In contrast to classic sulphonamides, these molecules can specifically suppress the activity of the enzyme by targeting allosteric or alternative binding sites. Clarification of the structure-activity relationships further increases the potential of this class.

C. Selective Inhibitors Targeting Specific Isoforms

The development of highly selective inhibitors against tumour-associated isoenzymes such as CA IX and CA XII has become particularly important in pharmacological research in the field of oncology. Ureido-substituted benzenesulfonamides, which are currently undergoing clinical trials, are valuable in this context, and molecules such as SLC-0111 (U-104) show promising results for targeted cancer therapies (Williams KJ et al., 2019). In addition, derivatives based on the 2-(benzylsulfinyl)benzoic acid structure have enabled the development of selective hCA-IX inhibitors with high affinity (Zhang et al., 2021). These developments underpin the strategic role of isoform-specific inhibitors in cancer treatment. Understanding the

extensive functions of carbonic anhydrase in cell physiology provides a scientific basis for the effective use of these drugs.

D. Natural Inhibitors from Plants

Plant-derived natural products are a potential source for drug discovery as they offer a rich and diverse range of phytochemicals with inhibitory activity on carbonic anhydrase. The inhibition profiles of these natural compounds have been investigated for use in diseases such as glaucoma and epilepsy. For example, essential oils from Mentha longifolia (wild mint) were found to have significant effects on the inhibition of CA (Karaçelik AA et al., 2022).

In addition, selenoester derivatives of natural origin have been found to show strong inhibitory effects, especially on the CA-IX isoform, and the potential use of these agents in oncological treatments has attracted attention (Astrain-Redin N et al., 2023). The structural diversity of phytochemicals contributes to the development of new pharmacophores by determining their interactions with CA enzymes. The role of carbonic anhydrase in the cellular environment allows us to better understand the effects of these natural inhibitors in a physiological context.

E. Synthetic Compounds and Their Development

The development of more effective and selective versions of carbonic anhydrase inhibitors is currently an important area of research, particularly in the treatment of cancer. The new generation of synthetic inhibitors aims to increase anti-tumour activity by targeting specific CA isoforms (in particular hCA IX and hCA XII). The development of inhibitors that bind with high affinity to the active sites of the enzyme through the use of small molecule ligands has the potential to influence the adaptive physiology of the tumour cell (Bonardi A, et al., 2018). In addition, it is possible to obtain potent and isoform-selective compounds by chemically modifying existing molecular scaffolds. This approach clearly shows that molecular design is directly linked to pharmacological action. The three-dimensional structural features of CA enzymes highlight how synthetic chemistry can be a strategic tool for targeted therapies.

Side Effects and Limitations

While CA inhibitors provide substantial therapeutic benefits in conditions such as glaucoma and macular oedema, their clinical use is limited by a range of adverse effects and pharmacological constraints. Common side effects include metabolic acidosis, electrolyte disturbances and gastrointestinal complaints, largely resulting from impaired renal bicarbonate reabsorption

following enzyme inhibition. These effects may be particularly pronounced in patients with compromised renal function and can negatively affect treatment adherence. In addition, interindividual variability in pharmacokinetics and pharmacodynamics may influence therapeutic efficacy, underscoring the need for careful patient-specific treatment selection and monitoring (Supuran CT, 2017).

Common Side Effects and Limitations

CA inhibitors are associated with a range of adverse effects that may limit their clinical utility. Metabolic acidosis, electrolyte disturbances such as hypokalaemia, and gastrointestinal or hypersensitivity reactions are among the most frequently reported side effects, particularly during long-term treatment. These effects primarily arise from disruption of acid–base homeostasis following inhibition of carbonic anhydrase activity. In addition, interindividual variability in pharmacokinetics and pharmacodynamics, as well as differences in comorbid conditions, can influence therapeutic efficacy and tolerability. Collectively, these limitations highlight the need for careful patient selection, dose optimisation and the development of improved formulations to enhance the safety and effectiveness of CA inhibitors (Supuran CT, 2017).

PotentialforDrug Interactions

CAIs should be carefully monitored for drug interactions due to their effects on acid-base balance and electrolyte homeostasis. Especially in patients treated with multiple drugs, the pharmacological effect of CAIs in combination with other agents may cause unexpected toxicities. For example, agents targeting the protozoan enzyme TgCA have been shown to be cross-active on human CA I and CA II isoforms (Giovannuzzi S et al, 2024). In such situations, dose adjustments and pharmacovigilance measures are required to minimise off-target effects. In addition, the role of renal clearance mechanisms in these interactions requires careful evaluation of drug elimination pathways.

MechanismsofResistanceinTarget Cells

In cancer treatment in particular, resistance mechanisms that develop in the target cells are an important factor limiting the efficacy of CAIs. Isoforms such as CA IX are known to facilitate cell survival by creating an acidic environment in the tumour microenvironment, and adaptation to this environment leads to pharmacological resistance over time. Recent studies show that increased CA IX expression is associated with stem cell-

like properties and plays a role in the development of therapy resistance (Mc Donald et al., 2012). In this context, it is of great importance to understand the resistance mechanisms at the molecular level and to develop new strategies targeting these mechanisms.

Challenges in Drug Formulation and Distribution

The pharmaceutical formulation of carbonic anhydrase inhibitors presents several challenges related to bioavailability and tissue distribution. Many compounds exhibit limited lipid solubility and rapid metabolism, which may restrict efficient access to target tissues. These pharmacokinetic constraints highlight the need for improved formulation strategies to enhance drug stability, optimise tissue penetration and achieve controlled therapeutic effects. Addressing such challenges remains an important aspect of ongoing research aimed at improving the clinical performance of carbonic anhydrase inhibitors (Supuran CT, 2017).

TherapeuticApplications

CAIs (Table 2) are among the most promising therapeutic agents, particularly in the field of oncology. Some tumour cells are known to overexpress isoenzymes such as CA IX and CA XII, making them ideal targets for CAIs. The detailed understanding of the molecular structures of these isoenzymes has made an important contribution to the development of selective inhibitors. Selective CA IX inhibitors such as SLC-0111 (U-104) have shown positive results in resistant tumour models such as triple negative breast cancer and small cell lung cancer (Williams et al., 2019). Similarly, research on hCA IX inhibitors has expanded our understanding of structure–activity relationships and is paving the way for the development of novel targeted therapeutic strategies (Zhang et al., 2021).

Table2.Selected Carbonic Anhydrase Inhibitors: Clinical Applications and Mechanisms

Inhibitor Name	Clinical Applications	Mechanism of Action
Acetazolamide	Glaucoma; acute-mountainsickness; idiopathicintracranial-hypertension	Systemicinhibition of carbonicanhydrase (mainly CA II and CA IV), resulting in decreasedaqueoushumourproductionandincreased renal bicarbonateexcretion
Methazolamide	Glaucoma	Systemiccarbonicanhydraseinhibitionwithsimilarefficacytoacetazolamide, but with a longerhalf-life andfewer renal sideeffects
Dorzolamide	Open-angleglaucoma; ocularhypertension	Topicalinhibition of carbonicanhydrase II in theciliaryprocesses, leadingtoreducedaqueoushumoursecretion

A. UseinGlaucoma Treatment

The treatment of glaucoma is one of the areas where CAIs are most clinically effective. These drugs reduce elevated intraocular pressure (IOP) and help prevent disease progression and associated vision loss. Topically applied agents such as dorzolamide and brinzolamide have been shown to be effective in lowering IOP and are commonly used in the treatment of open-angle glaucoma. Systemic formulations, such as acetazolamide, have a stronger effect on IOP but carry a higher risk of systemic side effects. Therefore, topical CAIs are often preferred at the beginning of treatment (Kurysheva N, 2020). In clinical practice, CAIs are often used in combination with other ophthalmic medications in a "mix-and-match" strategy that provides a holistic approach to the patient's overall eye health. In addition, the potential antioxidant effects of these agents have been investigated in recent years and combination drugs are being developed to treat not only the pressure but also the neurodegenerative aspects of glaucoma. In summary, CAIs continue to play an important role in the treatment of glaucoma and are considered one of the most important pharmacological tools to slow disease progression and improve quality of life in most clinical scenarios(Kurysheva N, 2020).

B. Roleinthemanagementofaltitude sickness

Altitude sickness (acute mountain sickness) is a physiological stress response to hypobaric hypoxia and can lead to severe symptoms, especially with rapid changes in altitude. CAIs, particularly acetazolamide, play an important therapeutic role in the prevention and treatment of this condition.

Acetazolamide increases renal excretion of bicarbonate by inhibiting the CA enzyme. This leads to a mild metabolic acidosis, stimulates the respiratory centre and increases oxygen uptake through hyperventilation. In cases of hypoxaemia at high altitude, taking acetazolamide allows the body to adapt more quickly, reducing the severity of symptoms such as headaches, dizziness and fatigue. This pharmacological intervention is often referred to as prophylaxis, especially for people who are mountaineering, practising high altitude sports or working at high altitudes. It has also been reported that CA inhibitors can help prevent the development of more serious altitude-related complications, such as pulmonary and cerebral oedema. In conclusion, carbonic anhydrase inhibitors are clinically valuable agents for the understanding and treatment of high altitude physiology, and acetazolamide is one of the most preferred drugs in this context (Swenson ER,2014).

C. ApplicationinCancer Treatment

CAIs have an important potential in cancer therapy as they contribute to the regulation of the intratumoral pH balance, especially in tumours with a hypoxic microenvironment. CA IX and CA XII isoforms are overexpressed in many solid tumours and are associated with poor prognosis and treatment resistance. These properties make them attractive targets for pharmacological intervention (Xiao-Qun Z et al., 2021). In ongoing studies, U-104 (SLC-0111), one of the ureido-substituted benzenesulfonamide derivatives, has shown therapeutic efficacy, particularly in triple-negative breast cancer models. In addition, the potential for synergistic effects has been reported when used in combination with chemotherapeutic agents such as cisplatin (Xiao-Qun Z et al., 2021). In addition, research on 2-(benzylsulfinyl) benzoic acid derivatives has contributed to the development of selective hCA IX inhibitors, deepening the understanding of structure-activity relationships. These developments emphasise the increasing importance of CAIs in targeted cancer therapies.

D. PotentialintheTreatmentofMetabolic Disorders

CA inhibitors are promising for the treatment of various metabolic disorders by specifically interfering with cellular metabolic processes. For example, inhibition of alpha-carbonic anhydrase, which Toxoplasma gondii requires for survival, shows that the parasite's energy metabolism can be disrupted without damaging the host cell (Mallia A, Broccaet al., 2025). Such selective targeting strategies suggest that CA inhibitors can both regulate metabolism at the cellular level and interfere with pathological processes. In addition, some preclinical studies comparing CA inhibition with corticosteroids have shown different efficacy profiles, particularly

in diseases such as macular oedema (L et al., 2024). These data open up potential opportunities for the use of CAIs in a wider range of metabolic diseases. Visual models showing the distribution of carbonic anhydrases at the cellular level contribute to our understanding of the importance of these enzymes in metabolic functions and the therapeutic efficacy of their inhibitors(Mallia A, Broccaet al., 2025).

E. UseinNeurologicalConditions

Carbonic anhydrase inhibitors are increasingly being explored as potential therapeutic agents in neurological conditions involving metabolic dysregulation and hypoxic injury, including ischaemic brain damage. The pathophysiology of such conditions is closely associated with hypoxia, pH imbalance and neuronal injury resulting from impaired cerebral perfusion. By inhibiting carbonic anhydrase activity, these agents can modulate intracellular and extracellular pH, thereby limiting acidosis-related neuronal damage. Integrative evidence derived from in vitro, in silico and in vivo studies suggests that carbonic anhydrase inhibition may exert neuroprotective effects and support functional recovery following neurological injury. Furthermore, mapping the distribution of carbonic anhydrase isoforms within the brain provides valuable insights into their role in neuronal metabolism and highlights the therapeutic relevance of carbonic anhydrase inhibitors in neurological disorders (Allam et al., 2025).

Conclusion and Future Directions

Due to their role in fundamental physiological processes and their versatile therapeutic potential, CAIs are being studied with increasing interest in modern medicine. The functional diversity of different isoforms such as CA III and CA VII under oxidative stress conditions emphasises their importance as drug targets, particularly in cancer, neurological disorders and renal diseases. However, assessments based only on in vitro enzyme activity may result in some effective compounds being missed; therefore, more comprehensive cellular and in vivo analyses are required. Research on CAIs provides important information for the optimisation of treatment strategies. For example, peptide-drug conjugates that simultaneously target EGFR and CA IX demonstrate the success of dual targeted strategies at the molecular level in cancer treatment. On the other hand, studies with agents such as flupirtine have shown that CAIs may be inadequate in complex conditions such as obstructive sleep apnoea. The use of CAIs during pregnancy is a specialised clinical area that should be handled with caution. The existing literature contains serious shortcomings regarding the safety of these

drugs in pregnant women. Therefore, more comprehensive clinical studies on maternal-fetal pharmacokinetics, placental transmission and effects on fetal development are required. In addition, follow-up studies of pregnant women using CAIs during lactation may provide important contributions to the literature. For the safe management of this process, multidisciplinary approaches should be developed that allow the integration of different health disciplines.

To summarise, ongoing research on CAIs is shedding light not only on their basic biochemical functioning, but also on their applicability and clinical impact in various disease groups. In addition to indications such as glaucoma, oedema and epilepsy, CAIs are playing an increasingly important role in a broad spectrum of diseases ranging from nephrology, oncology and neurology to perinatal medicine. It is an important pharmacological agent with the advantages of modulating apoptosis mechanisms, sensitive control of acid-base balance and isoform-based targeting. In the future, the importance of these molecules in the healthcare system will increase with the development of formulations that increase the efficacy of CAIs, the implementation of patient-specific, personalised treatment protocols and the updating of clinical guidelines. Further studies in the light of structural biology, pharmacodynamic interactions and clinical follow-up data could lead to CAIs becoming one of the most important therapeutic elements in modern medicine.

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