1 Obesity and Carbonic Anhydrase Inhibition: Potential Pathways and Therapeutic Challenges

Yogesh Matta[1*] & Neha Arora[2]

[1,2]Associate Professor, School of Pharmacy, Suresh Gyan Vihar University, Jaipur, Rajasthan, India.

*Corresponding Author: yogesh.matta@rediffmail.com

Abstract

Clinically defined as a pathological buildup of body fat with a body mass index (BMI) ≥ 30 kg/m2, obesity is a serious global health issue that is becoming more and more common in both developed and developing nations. According to epidemiological data, almost two-thirds of people in the United States alone suffer from excess body weight, with almost one in three adults and 20% of adolescents fitting the criteria for obesity. Because of its complicated origin and vast range of related comorbidities, obesity—which is currently recognized as a multifactorial and chronic degenerative disorder—presents a special therapeutic challenge.

Keywords: Obesity, Carbonic Anhydrase Inhibition, Therapeutic Challenges, Global Health Issue, Adolescents.

Introduction

Metabolic disorders (such as type 2 diabetes mellitus, non-alcoholic fatty liver syndrome, dyslipidemia, gallstone disease, and gout), cardiovascular diseases (such as hypertension, atherosclerosis, atrial fibrillation, and heart failure), and an increased risk of cancer (particularly colorectal, breast, and pancreatic cancers) are among these comorbidities that affect several organ systems. Furthermore, the burden of disease is further increased by respiratory issues like asthma and obstructive sleep apnea as well as neuropsychiatric symptoms like depression, anxiety disorders, cognitive impairment, and panic attacks. Although pharmacological therapy options are still limited, management techniques for obesity generally focus on lifestyle modification through hypocaloric dietary regimes and, in extreme situations, bariatric surgical operations. The therapeutic arsenal of anti-obesity medications is limited by significant safety issues in addition to their weak efficacy.

In fact, a number of medications that were once authorized for clinical use were pulled off the market soon after they were introduced because a sizable percentage of patients experienced severe side effects. These restrictions highlight how urgently safer and more potent pharmaceuticals must be developed in order to combat the world's obesity crisis.

Carbonic Anhydrases(S) Role in Obesity

A family of metalloenzymes known as carbonic anhydrases (CAs, EC 4.2.1.1) are essential to pH regulation because they catalyze the reversible hydration of carbon dioxide to bicarbonate and a proton. Currently categorized into at least eight different genetic groups, these enzymes are found throughout the evolutionary range, spanning from organisms such as bacteria and to Eukaryotes. Only the α-class CAs are expressed in humans and other animals. Fifteen humans isoforms (hCA I–XIV, including the two mitochondrial variants VA and VB) have been found and well characterized thus far; several of these have been confirmed therapeutic targets in the creation of anticancer, antiglaucoma, antiepileptic, and diuretic medications.

Traditionally, CAs have been studied primarily for their role in pH buffering and regulation; however, more recent evidence highlights their importance as enzymes that regulate metabolism In particular, they have been shown to contribute to diverse metabolic processes in both healthy and tumor cells, including fatty acid biosynthesis and newly generated lipogenesis (DNL). These pathways, which involve both the mitochondrial and cytosolic reactions, rely on enzymes such as pyruvate carboxylase (PC) and acetyl-CoA carboxylase (ACC) that utilize bicarbonate, rather than CO_2, as a substrate.

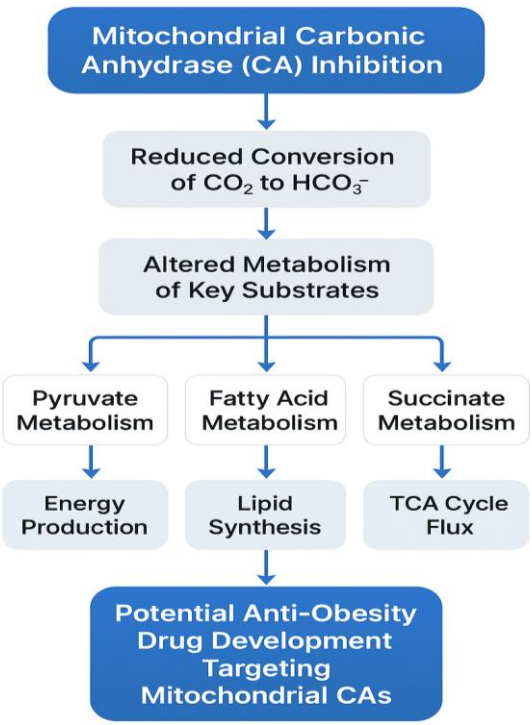
Highly active CA isoforms, particularly CA II in the cytosol and CA VA/VB in the mitochondria, are essential for maintaining the quick interconversion of CO_2 and bicarbonate needed in these activities. Their functional importance in intermediary metabolism was highlighted by research conducted as early as the 1990s, which showed that suppression of the mitochondrial and cytosolic CA isoforms impairs fatty acid biosynthesis and DNL in a variety of cells, organs, and experimental animal models.

Using selective respiratory CA inhibitors (CAIs) and mitochondria linked to electrodes, Minteer and colleagues (2013) carried out one of the most thorough studies on the metabolic function of mitochondrial carbonic hydrate anhydrases (CAs). Through the monitoring of metabolic energy conversion and the comparison of results across structurally distinct but potent primary sulfonamide-type CAIs, the metabolism of pyruvate, sodium acetate, which and succinate was examined in the presence of particular CA VA/VB inhibitors. The findings showed that the effects of inhibiting mitochondrial CAs varied according to the metabolic substrate: the metabolism of pyruvate was most severely hampered, followed by that of fatty acids, while the metabolism of succinate was least impacted.

These results definitively demonstrated the role of mitochondrial CA isoforms in fatty acid production and intermediate metabolism. The idea of targeting mitochondrial CAs for anti-obesity therapy only started to gain traction in the early 2000s, when Solvay Pharmaceuticals and a number of academic groups launched drug discovery programs aimed at creating CAIs with anti-obesity activity, despite this solid mechanistic foundation. However, the field has encountered a great deal of resistance and criticism from the scientific community, just like other therapeutic approaches related to CA (such as CAIs as anti-infective or anticancer medicines). The adoption of this novel therapy

approach has reportedly been slowed by critics who have tried to impede the spread of these findings including biased peer review and publishing challenges.

Nonetheless, the growing body of research keeps emphasizing the possible therapeutic advantages of CA inhibition in the treatment of obesity. It's also important to remember that the cytosolic isoform CA III was previously thought to play a role in obesity and lipogenesis. Due in large part to its poor catalytic efficiency in converting CO_2 to carbonate and its poor affinity for sulfonamide or sulfamate inhibitors, later research definitively showed that CA III does not play an important function in lipogenesis, despite early findings suggesting its involvement. Because of these factors, CA III is no longer thought to be a promising target in the hunt for treatments that combat obesity.



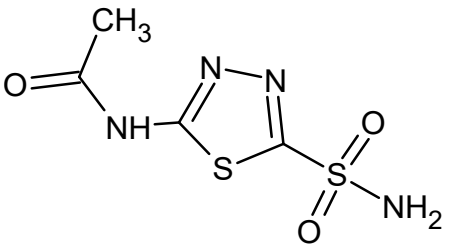
Drug Design of CA Inhibitors as Antiobesity Agents

Although the pharmacological design of carbonic anhydrase inhibitors (CAIs) is well known for illnesses like infections, glaucoma, cancer, epilepsy, and neuropathic pain, antiobesity applications are still little studied. Repurposing medications from other therapeutic areas, evaluating natural or synthetic compounds using virtual or experimental testing methods, and de novo design based on known leads or comprehensive enzyme–inhibitor structural data, mostly from X-ray crystallography, are the three primary approaches that have emerged over the past 20 years.

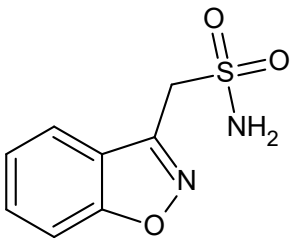
- **Drug Repurposing**

The most researched of carbonic anhydrase inhibitors (CAIs) are sulfonamides and related isosteres, including sulfamates and sulfamides. Many of these compounds have been used in clinical settings for decades as medications for antiglaucoma,

antiepileptic medications, and even anti-infective drugs. Numerous additional compounds are presently being studied for new uses in clinical studies. The antiepileptic drugs zonisamide (ZNS) and sugar sulfamate topiramate (TPM) are classic examples, as is acetazolamide (AAZ), the first non-mercurial diuretic introduced in 1954. It's interesting to note that despite being created initially as antiepileptic medications, ZNS and TPM have both been demonstrated to cause notable weight reduction in anecdotal stories, controlled clinical trials, and animal models.



Acetazolamide (AAZ)



Zonisamide (ZNS)

One significant but intricate example of medication repositioning is the use of AAZ, ZNS, and TPM for obesity. In addition to helping obese individuals lose weight, these CAIs also lower blood glucose levels when taken either by itself or in conjunction with medications like metformin, bupropion, or phentermine. Inhibition of human the enzyme carbonic anhydrase (hCA) isoforms associated with fatty acid production and de novo lipogenesis is primarily responsible for their antiobesity actions (Table 1). With inhibition constants ranging from 10 to 63 nM, all three medications efficiently suppress hCA II, hCA VA, and hCA VB; ZNS's action against hCA VB is in the micromolar range.

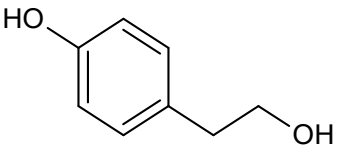
While the structure of the crystal of hCA VA is yet unknown, X-ray crystallographic studies on hCA II have shown different binding mechanisms for TPM and ZNS to interact (Figure 3). TPM stabilizes the enzyme–inhibiting complex (KI = 10 nM) by primarily binding to the hydrophilic part of the active site and generating many hydrogen bonds with receptors including Thr199, Thr200, the amino acids Asn62, Asn67, and Gln92. ZNS, on the other hand, is more oriented toward the hydrophobic half. Its sulfur-containing zinc-binding group (ZBG) forms a hydrogen bond with Thr199, while the rest of the molecule mostly interacts with hydrophobic residues such as Val121, Phe131, and Leu198 through van der Waals interactions.

Notwithstanding these variations, both substances have inhibitory potencies against hCA II that are comparable to those of AAZ, which binds extensively within the enzyme's active region via its SO_2NH^- moiety.

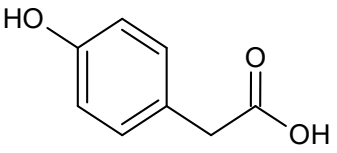
Despite having well-characterized interactions with hCA II, TPM, and ZNS, as well as with hCA I, these compounds have not been used as lead structures for the development of more effective antiobesity CAIs, most likely because derivatization has proven difficult. For example, the inhibitory activity was reduced when TPM's sulfamate ZBG was changed to a sulfonamide. However, computational research has shed light on how they bind to hCA VA, which helps to explain why the potencies of the cytosolic and mitochondrial isoforms differ.

- **Screening of Natural Products/Synthetic Libraries**

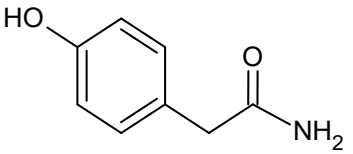
Davis and colleagues published the first screening study to use a phenolic library created from natural products to find inhibitors of human the mitochondrial carbonaceous anhydrases, which include hCA VA and hCA VB. The hydroxyl group of phenol interacts with the zinc-anchored water molecule in the enzyme's active site, making it a weak carbon dioxide anhydrase inhibitor with a different binding mechanism than traditional sulphonamides. Using phenol as the lead scaffold, a broad range of natural product-based phenols with simple to structurally complex structures were tested for inhibitory efficacy against hCA I and hCA II as off-target isoforms and hCA VA and hCA VB as the main targets.



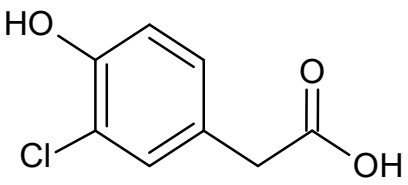
1



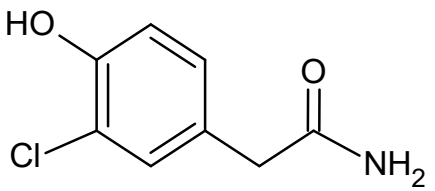
2



3



4



5

With inhibition coefficients (K_i) in the nanomolar domain of roughly 90–105 nM, a number of compounds demonstrated significantly increased activity regarding the mitochondrial isoforms hCA VA and VB, whereas many of the tested phenolic derivatives only showed micromolar detrimental potency against hCA I and II.

Gidaro and colleagues assessed the inhibitory effect of a natural product library that included polyphenols, flavones, and specific glycosylated derivatives against many isoforms of carbonic anhydrase, including the mitochondrial isoform hCA VA. Low micromolar range inhibitory actions against hCA VA were demonstrated by a number of the investigated drugs; nevertheless, limited selectivity was indicated by the molecules' activity toward other isoforms, including hCA I, IX, and XII.

With K_i values of 0.30 μM and 0.15 μM, respectively, apigenin (14) and eriocitrin (16) were shown to be the most effective inhibitors of mitochondrial carbonic anhydrase among the compounds that were examined.

The binding relationship between eriocitrin and murine mitochondrial mitochondrial mitochondrial VA (mCA VA), for which a trimmed X-ray crystal structure is known, was examined using computational docking techniques in the same paper. Subsequent X-ray crystallographic studies showed that catecholic compounds preferentially interact with the zinc-bound the water molecule in order and a deeply

located water molecule within the active site of hCA II, casting doubt on the authors' proposal that the catechol moiety of eriocitrin directly coordinates with the active-site zinc ion. Furthermore, blueberry-derived polyphenolic compounds and their glycosidic forms were tested for mitochondrial CA inhibition and shown inhibitory efficacy in the micromolar concentration range.

In a different study, Costa and associates assessed the inhibitory effect of natural products made from oil constituents of different botanical resources (compounds 24–33) against the enzyme mitochondrial isoform hCA VA, the carbonated anhydrase isoforms hCA I, and hCA II. Tropolone (24) was shown to be a unique chemotype with carbonic anhydrase inhibitory action within this series. According to computational research, tropolone interacts with the active site of the enzyme by generating a bidentate coordination between the catalytic zinc ion and its CO–CHOH functional group.

A straightforward tropolone–CA complex was analyzed using X-ray crystallography to confirm this suggested binding mechanism. With inhibition constants (K_i) less than 5 μM, 2-hydroxyisobutyric acid twenty-five and three Z-nonenoic acidic substances (29) were shown to be the most effective inhibitors of hCA VA among the substances examined. Interestingly, these substances also showed some selectivity for the subcellular isoform human CA VA over the off-target variant hCA II, which is widely expressed.

Alcaro and colleagues have used virtual screening (VS) techniques to find possible anti-obesity compounds by inhibiting the mitochondrial carbonic anhydrase isoform hCA VA. Using this method, 12 potential hits were chosen from a compound library of 93,522 molecules after they underwent in silico screening.

- **De Novo Drug Design**

Using zinc-binding groups like sulphonamides and associated bioisosteres, several studies have concentrated on the de novo generation of inhibitors that target the mitochondrial hydrocarbon anhydrase versions hCA VA and hCA VB. The so-called "tail approach," which entails functionalizing the carbonic anhydrase inhibitors' core scaffold with extra substituents intended to reach beyond the outside regions of the enzyme active site, is frequently used in these attempts. This approach provides chances to improve isoform selectivity by facilitating interactions with the catalytic cavity entry and its surrounding rim, regions that exhibit notable amino acid sequence variability among various CA isoforms.

One of the first studies in this field[68] assessed the inhibitory activity of 46 aromatic and heterocyclic sulphonamides against murine CA VA, while including human CA I, II, and IV as off-target isoforms. Many of these compounds were structurally simple analogues of the benzenesulfonamide and 1,3,4-thiadiazole-5-sulphonamide. Many substances showed strong inhibitory effects in the microscopic variety, with KI values usually ranging from 6 to 95 nM.

The most potent compounds had a zinc-binding headgroup of benzenesulfonamide coupled with either acylamido or amino tail substituents that were affixed to a sulphanilamide substrate scaffold and further embellished with alkyl or synthetic residues. The discovered lead structures served as a useful basis for the later

creation of subsequent-generation mitochondrial CA inhibitors, despite the fact that this chemical set was not evaluated directly against human CA VA or VB. A wide range of aromatic and heterocyclic sulphonamides with phenacetyl, pyridylacetyl, and thienylacetyl tails were later reported by Guzel and colleagues[69] in this context (compounds 40–44, Table 3). Several of these compounds are among the most powerful and isoform V-selective sulphonamide inhibitors that have been reported to date.

Conclusion & Future Directions

Investigations into the basic mechanism causing these effects were spurred by clinical observations showing that some antiepileptic medications, including the drugs topiramate and zonisamide, together with the contraceptive acetazolamide, encourage weight loss and decrease levels of glucose in obese patients. Their capacity to block certain carbonic anhydrase (CA) isoforms, such as the enzymes found in mitochondria hCA VA and hCA VB, which are known to be involved in fatty acid biosynthesis and de novo lipogenesis (DNL), has been primarily responsible for this phenomena. Importantly, despite sporadic misunderstandings in the literature, these substances are not linked to mitochondrial toxicity. The metabolic advantages of topiramate and zonisamide have sparked interest in their potential repurposing for the treatment of obesity, despite their complex polypharmacological profiles.

In fact, the FDA approved topiramate and phentermine together in 2012 for the treatment of obesity, and zonisamide is presently being studied in clinical studies either by itself or in conjunction with other medications like metformin or bupropion. However, their broad pharmacological activities and low isoform selectivity frequently result in unwanted side effects, which prevent their widespread usage as specific anti-obesity medicines.

The development of strong and isoform-selective inhibitors of the mitochondrial carbonic anhydrases hCA VA and hCA VB has thus been the focus of significant drug discovery efforts during the last 20 years.

In addition to medication repurposing strategies, scientists have used virtual screening (VS) and experimental screening of large libraries of synthetic as well as natural compounds to find novel inhibitory scaffolds. These studies have produced a number of intriguing and occasionally surprising chemotypes, such as flavones, polyphenols that are and their glycosidic derivatives, as well as tropolones, a unique family of CA inhibitors. Additionally, the CA-inhibitory potential of some therapeutically licensed medications has been identified, including rufinamide, lenvatinib, and fludarabine. Concurrent with these endeavors, conventional structure-based design approaches have predominantly focused on the well-known sulphonamide, sulfamate, and sulfamide pharmacophores, which continue to be the most extensively investigated zinc-binding groups for CA inhibition.

Despite these developments, only a small number of controlled investigations have been published, and while a number of compounds show outstanding in vitro potency alongside selectivity, thorough pharmacological analyses proving their capacity to inhibit new-onset lipogenesis and encourage weight loss in experimental unhealthy weight models are still lacking.

References

1. Batchuluun B, Pinkosky SL, Steinberg GR. Lipogenesis inhibi- tors: therapeutic opportunities and challenges. Nat Rev Drug Discov 2022; 21:283–305.
2. Burki T. European Commission classifies obesity as a chronic disease. Lancet Diabetes Endocrinol 2021; 9:418.
3. de Luis D, Primo D, Izaola O, Aller R. A pilot study of gene expression analysis in peripheral blood mononuclear cells in response to a hypocaloric mediterranean diet. Dis Markers 2022; 2022:3706753.
4. Redmond IP, Shukla AP, Aronne LJ. Use of weight loss medications in patients after bariatric surgery. Curr Obes Rep 2021; 10:81–9.
5. Czepiel KS, Perez NP, Campoverde Reyes KJ, et al. Pharmacotherapy for the treatment of overweight and obes- ity in children, adolescents, and young adults in a large health system in the US. Front Endocrinol (Lausanne) 2020; 11:290.
6. Supuran CT. Emerging role of carbonic anhydrase inhibitors. Clin Sci 2021; 135:1233–49.
7. De Simone G, Supuran CT. Antiobesity carbonic anhydrase inhibitors. Curr Top Med Chem 2007; 7:879–84.
8. De Simone G, Di Fiore A, Supuran CT. Are carbonic anhy- drase inhibitors suitable for obtaining antiobesity drugs? Curr Pharm Des 2008; 14:655–60.
9. Supuran CT, Di Fiore A, De Simone G. Carbonic anhydrase inhibitors as emerging drugs for the treatment of obesity. Expert Opin Emerg Drugs 2008; 13:383–92.
10. Supuran CT. Carbonic anhydrase inhibitors as emerging drugs for the treatment of obesity. Expert Opin Emerg Drugs 2012; 17:11–5.
11. Scozzafava A, Supuran CT, Carta F. Antiobesity carbonic anhydrase inhibitors: a literature and patent review. Expert Opin Ther Pat 2013; 23:725–35.
12. Supuran CT. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. Nat Rev Drug Discov 2008; 7:168–81.
13. Supuran CT. Advances in structure-based drug discovery of carbonic anhydrase inhibitors. Expert Opin Drug Discov 2017; 12:61–88.
14. Aspatwar A, Tolvanen MEE, Barker H, et al. Carbonic anhydrases in metazoan model organisms: molecules, mechanisms, and physiology. Physiol Rev 2022; 102:1327–83.
15. Supuran CT. Carbonic anhydrases and metabolism. Metabolites 2018; 8:25.

☯☯☯