Carbonic Anhydrase Inhibitors: Newer Chemical Scaffolds and SAR

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Abstract

Carbonic anhydrases (CAs) are ubiquitous zinc metalloenzymes that interconvert carbon dioxide and bicarbonate and thereby regulate pH, ion transport, secretion and metabolism. Fifteen α-CA isoforms occur in humans, and several are validated drug targets in glaucoma, epilepsy, altitude sickness, oedema, obesity and cancer. Classical primary sulfonamide inhibitors such as acetazolamide are potent but poorly isoform-selective, which causes systemic side effects. Over the last two decades, medicinal chemistry has produced a broad set of newer scaffolds: tail-approach sulfonamides (ureido, triazole, glycosyl and multi-tailed), coumarins and sulfocoumarins, dithiocarbamates, benzoxaboroles, carboxylic acids, phenols, polyamines, selenium compounds, metal complexes, biologics and small-molecule drug conjugates. This report reviews the biology of CAs, the catalytic and inhibition mechanisms, the major classes of inhibitors, the synthetic routes used to make them, their structure–activity relationships (SAR), the role of CA IX/XII in cancer, clinical status and future directions. [1,2]

Keywords: Carbonic anhydrase inhibitors, newer chemical scaffolds, structure–activity relationship (SAR), sulfonamides, heterocyclic compounds, coumarins, indoles, quinolines, zinc-binding groups, isoform selectivity, molecular docking, drug design.

Introduction and Background

1.1 Historical perspective

- 1933: Meldrum and Roughton discovered CA in erythrocytes, the first zinc enzyme to be described. [3]
- 1940: Mann and Keilin showed that sulfanilamide inhibits CA, the first demonstration that a sulfonamide drug acts on an enzyme target. [4]
- 1950s: Observations that sulfonamides cause alkaline diuresis led to acetazolamide (introduced in 1953), the first non-mercurial oral diuretic and the prototype CA inhibitor (CAI). [1]
- 1990s: Structure-based design produced the topical antiglaucoma agents dorzolamide (1995) and brinzolamide (1998). Cloning of the tumour-associated isoforms CA IX and XII opened the oncology field. [1,5
- 2000s–present: The “tail approach”, discovery of non-sulfonamide zinc binders and new mechanisms (2009 onwards), and clinical development of SLC-0111. [6,7]

1.2 What carbonic anhydrases do

Carbonic anhydrases (CAs, EC 4.2.1.1) catalyse the reversible hydration of carbon dioxide: CO2 + H2O ⇌ HCO3 − + H . +The uncatalysed reaction is slow, while hCA II turns over at about 106 s−1, close to the diffusion limit. [8] Eight evolutionarily unrelated families are known (α, β, γ, δ, ζ, η, θ, ι). Humans have only

α-CAs. β-CAs occur in bacteria, fungi, algae, plants and some invertebrates, and γ-CAs in archaea and some bacteria, which makes them targets for anti-infective drugs. [9]

Physiological roles include respiration and CO2 transport, acid–base balance, aqueous humour and cerebrospinal fluid secretion, renal bicarbonate reabsorption, gastric acid secretion, bone resorption, and supply of bicarbonate for biosynthetic reactions (lipogenesis, gluconeogenesis, ureagenesis).

1.3 Human isoforms

The table below summarises the 15 human α-CA isoforms, their tissue location, relative catalytic activity and disease relevance. [1,10]

1.4 Active site architecture (hCA II numbering)

- Zn2+ sits at the bottom of a conical cavity about 15 Å deep, coordinated by His94, His96, His119 and a water/hydroxide molecule (tetrahedral geometry).
- The Thr199–Glu106 hydrogen-bond network (the "gatekeeper") orients substrate and inhibitors.
- His64 shuttles protons from the zinc-bound water to the buffer, which is the rate-limiting step.
- The cavity has a hydrophobic half (Val121, Phe131, Val135, Leu198, Pro202, Leu204) and a hydrophilic half (Tyr7, Asn62, His64, Asn67, Gln92).
- Residues at the rim (positions 91, 92, 130, 131, 135) differ most between isoforms. They are the basis of selectivity design. [2,11]

1.5 Catalytic mechanism (zinc–hydroxide mechanism)

- Step 1, nucleophilic attack: the zinc-bound hydroxide attacks CO2, which is held in a hydrophobic pocket, forming zinc-bound bicarbonate.
- Step 2, release: bicarbonate is displaced by a water molecule.

Isoform	Location	Activity	Disease relevance
I	Cytosol (erythrocytes, GI tract)	Moderate	Retinal and cerebral oedema (emerging)
II	Cytosol (almost ubiquitous)	Very high	Glaucoma, oedema, epilepsy; main off-target
III	Cytosol (skeletal muscle, adipose)	Low	Oxidative stress; poorly studied
IV	Membrane-anchored (kidney, lung, eye)	High	Glaucoma, retinitis pigmentosa
V	Mitochondria (liver; widely)	Moderate	Taste and dental caries
VI	Secreted (saliva, milk)	Moderate	Taste and dental caries
VII	Cytosol (brain)	High	Epilepsy, neuropathic pain
IX	Transmembrane(hypoxic tumours)	High	Hypoxic solid tumours, metastasis
XII	Transmembrane (kidney, tumours)	Moderate	Cancer, glaucoma
XIII	Cytosol (kidney, gut, reproductive tract)	Moderate	Not well defined
XIV	Transmembrane (brain, retina)	Moderate	Epilepsy (possible)
XI	Brain (acatalytic CA-RPs)	None	Cerebellar function; no catalytic role

- Step 3, regeneration: His64 transfers a proton from the zinc-bound water to bulk buffer, restoring Zn–OH− for the next cycle. This is the proton-transfer, rate-limiting step. [8]

Sulfonamides and other zinc-binding inhibitors displace the nucleophilic Zn–OH−/H O and so stop the cycle.

1.6 Therapeutic relevance

Inhibition of different isoforms is useful in a wide range of conditions, as shown below. [1,5]

Disease area	Target isoforms	Rationale
Glaucoma	II, IV, XII	Lower aqueous humour secretion and intraocular pressure
Epilepsy, neuropathic pain	VII, II, XIV	Modulate neuronal pH and GABA-mediated excitability
Altitude sickness, sleep apnoea	II, IV	Metabolic acidosis stimulates ventilation
Idiopathic intracranial hypertension	II	Reduce CSF production
Obesity, metabolic disease	VA, VB	Reduce de novo lipogenesis
Hypoxic solid tumours	IX, XII	Counter tumour acidosis, invasion and metastasis
Infections (TB, H. pylori, fungi)	Bacterial and fungal β/γ-CAs	Impair pathogen growth and virulence

2. Classical Inhibitors and Their Limitations

Primary sulfonamides (R–SO2NH2) and their bioisosteres , sulfamates (R–OSO2NH2) and sulfamides (R–NHSO2NH2), form the established class. Several non-CA-targeted drugs, including topiramate (sulfamate), celecoxib, hydrochlorothiazide and furosemide, also inhibit CAs, which partly explains their side-effect profiles. [7,12]

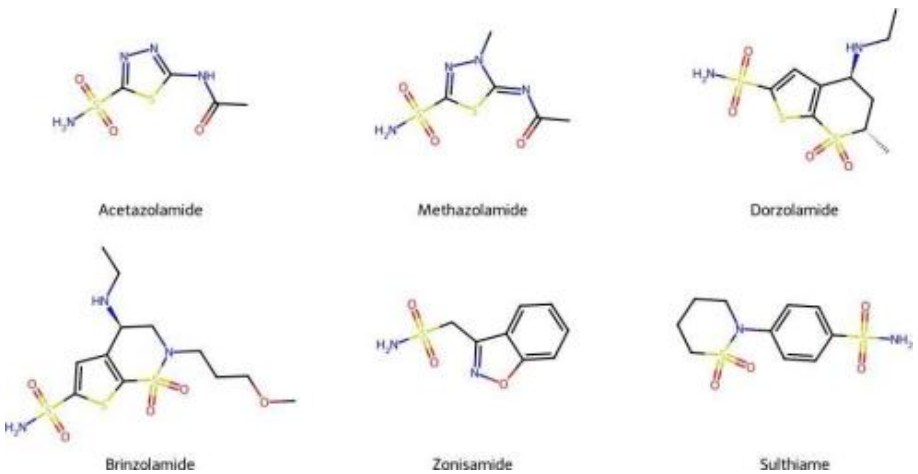

Figure 1. Structures of clinically used sulfonamide CA inhibitors.

Drug	Principal use	Notes
Acetazolamide	Glaucoma, epilepsy, altitude sickness, oedema	Prototype; oral; pKa about 7.2; poor selectivity
Methazolamide	Glaucoma	More lipophilic; better tissue penetration
Ethoxzolamide	Historic diuretic / glaucoma	Lipophilic; research tool
Dichlorphenamide	Primary periodic paralysis	Oral
Dorzolamide	Glaucoma (topical)	Thienothiopyran sulfonamide; water-soluble; 1995
Brinzolamide	Glaucoma (topical)	Thienothiazine sulfonamide; suspension; 1998
Topiramate, zonisamide, sulthiame	Epilepsy (CA inhibition is partial)	Sulfamate / sulfonamide anticonvulsants

2.1Binding mode of sulfonamides

The deprotonated sulfonamide nitrogen (SO_2 NH−) displaces the zinc-bound water/hydroxide and coordinates Zn^{2+} in a tetrahedral[2] geometry. The NH group forms a hydrogen bond with the Thr199 hydroxyl, and one sulfonyl oxygen hydrogen-bonds with the Thr199 backbone NH. The aromatic or heteroaromatic ring lies in the hydrophobic half of the cavity. [2]

2.2Limitations of classical drugs

- Poor isoform selectivity. Most inhibit the ubiquitous off-target CA II at low-nM concentrations (acetazolamide KI roughly 250 nM for hCA I, 12 nM for II, 25 nM for IX and 5.7 nM for XII). [1,13]
- Systemic side effects: paresthesia, taste disturbance, fatigue, metabolic acidosis, hypokalaemia, renal stones, and rarely blood dyscrasias.
- Sulfonamide hypersensitivity in some patients (cross-reactivity between antibiotic and non-antibiotic sulfonamides is low, but it still limits use).
- Poor tissue and membrane targeting and poor oral tolerance, which led to topical ocular agents.

These problems drive the search for new scaffolds and zinc-binding groups (ZBGs) that give isoform selectivity.

3.Mechanistic Classification of Inhibitors

Supuran's classification recognises four inhibition mechanisms, shown in the table below.[6] The classification matters for design, because a compound that does not touch zinc is not limited by the very conserved zinc environment and can discriminate between isoforms more easily.

Mechanism	Description	Examples
1. Zinc binders	Replace the Zn-bound water/hydroxide; tetrahedral or trigonal-bipyramidal Zn	Sulfonamides, sulfamates, sulfamides, DTCs, xanthates, hydroxamates, benzoxaboroles, carboxylates (some)
2. Anchored to zinc-bound water	H-bond to the Zn-bound water/hydroxide without displacing it	Phenols, polyamines, some carboxylic acids, thioxocoumarin hydrolysis products
3. Entrance occluders	Bind at the mouth of the active site, blocking access	Coumarins, sulfocoumarins (after hydrolysis), some lactones
4. Out-of-active-site	Bind outside the cavity (hydrophobic pocket)	Certain carboxylic acids and other fragments

4.Newer Chemical Scaffolds

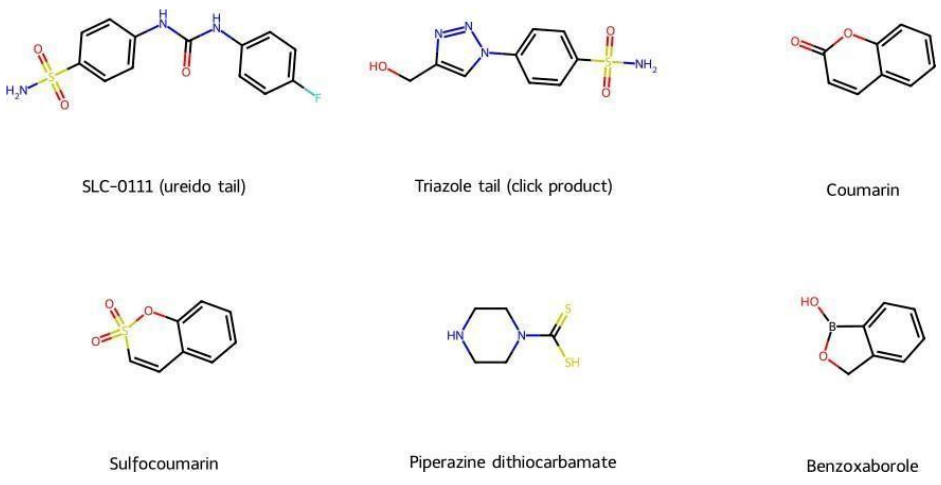

Figure 2. Representative newer CA inhibitor scaffolds (SLC-0111, triazole tail, coumarin, sulfocoumarin, dithiocarbamate, benzoxaborole).

4.1 Tail-approach sulfonamides

The "tail approach", introduced by Supuran and co-workers [7], attaches a variable tail to a benzenesulfonamide or heterocyclic sulfonamide head. The head binds zinc, while the tail reaches the isoform-variable rim and gives selectivity. Subtypes include:

- **Ureido-benzenesulfonamides, notably SLC-0111 (U-104),**

4-(3-(4-fluorophenyl)ureido)benzenesulfonamide. It is selective for CA IX/XII (low-nM KI) over CA I/II

(µM), reduces primary tumour growth and metastasis in preclinical models, completed Phase I, and has been evaluated in a Phase Ib/II study with gemcitabine in advanced pancreatic cancer. It is the leading CA IX clinical candidate. [13,14,15]

•**1,2,3-Triazole tails** assembled by copper-catalysed azide–alkyne cycloaddition (CuAAC, "click" chemistry), which allows rapid parallel library synthesis.

•**Glycoconjugates** (glucose, galactose, mannose or sugar-like tails). The sugars make the molecule polar and membrane-impermeant, so it selectively targets extracellular CA IX/XII and avoids intracellular CA I/II.

•**Dual-tail and three-tail designs** that add extra rim contacts and increase selectivity.

•**Hybrids** with pyrazolines, thiazolidinones, isatins, quinazolinones, chalcones, benzothiazoles and imidazoles, which often add anticancer or anti-inflammatory activity.

•**Positively charged tails** (pyridinium, quaternary ammonium) restrict the compounds to the extracellular space, which suits membrane-bound CA IV, IX and XII.

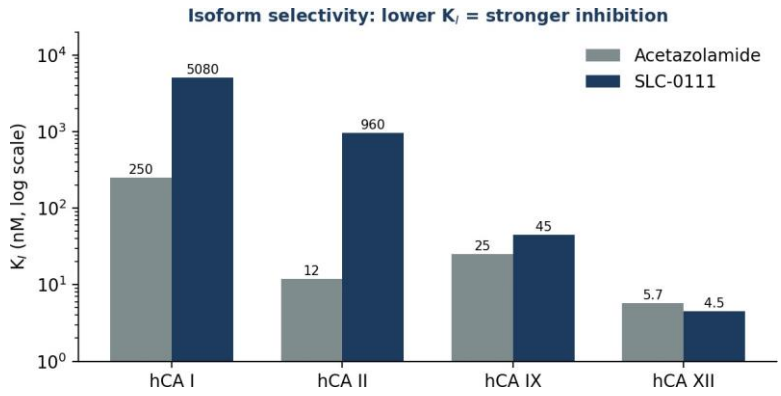

Figure 3. Inhibition constants of acetazolamide and SLC-0111 against four hCA isoforms

4.2Coumarins, thiocoumarins and sulfocoumarins

•**Coumarins** were found in 2009 as "suicide" or prodrug-like inhibitors. The CA esterase activity hydrolyses the lactone to a cis-2-hydroxycinnamic acid. That acid binds at the active-site entrance and does not touch zinc. [16]

•They are very selective: CA IX/XII are inhibited at nM to sub-µM levels, while CA I/II are barely affected (KI > 10 µM).

•**Sulfocoumarins** (1,2-benzoxathiine 2,2-dioxides) and homosulfocoumarins hydrolyse to sulfonic acids and show similar or improved selectivity. [17]

•Isocoumarins, psoralens, thiocoumarins and related lactones are also CA-active.

•**Natural products** such as coumarins from plants are a rich source for lead discovery.

4.3 Dithiocarbamates, monothiocarbamates, xanthates and trithiocarbonates

- Sulfur-based zinc binders show that CAs can be inhibited without a sulfonamide, which avoids sulfonamide hypersensitivity.
- Dithiocarbamates (DTCs), formed from secondary or primary amines and CS2, give nM inhibition of CA I, II, VII, IX and XII. Piperazine, piperidine, amino-acid and polyamine DTCs are common. [18]
- Crystal structures show monodentate and bidentate zinc coordination.
- Some DTC drugs in clinical use for other purposes (e.g., disulfiram metabolites) also inhibit CAs.

4.4 Benzoxaboroles and other boron-based inhibitors

Benzoxaboroles bind zinc as a tetrahedral boronate after deprotonation of the boron hydroxyl. They are sulfonamide-free, small, drug-like and sometimes show improved isoform selectivity. The scaffold is already present in approved antifungal drugs, which supports its safety profile. [19]

4.5 Hydroxamates, carboxylic acids and phenols

- Hydroxamates bind zinc in a bidentate fashion.
- Carboxylic acids (benzoic acids, cinnamic acids, NSAID-related acids) coordinate zinc directly or anchor to the zinc-bound water.
- Phenols anchor to the zinc-bound water and give µM, often isoform-selective inhibition (some series favour CA VII, IX or XII). Natural polyphenols and related compounds fall in this class. [6]

4.6 Polyamines

Spermine and spermidine anchor to the zinc-bound water through their ammonium groups. They are weaker inhibitors (µM) but are mechanistically instructive and inspire polyamine-conjugate design.[6]

4.7 Selenium derivatives

Selenols, selenoureas, selenides and selenium-containing sulfonamides show strong inhibition. They have been explored for combined CA-inhibitory and chemopreventive or antioxidant effects.

4.8 Bioisosteres and other zinc-binding-group variants

Sulfamates (e.g., topiramate), sulfamides, N-hydroxysulfonamides, hydrazides, ureas and certain heterocycles that coordinate zinc have been developed as alternatives to the primary sulfonamide. [7,11]

4.9 Metal complexes and organometallics

Ru(II), Au(I) and Pt complexes and ferrocene derivatives bearing sulfonamides combine CA inhibition with cytotoxicity.

4.10 Fluorinated benzenesulfonamides (Matulis series)

Tetra- and trifluoro-substituted benzenesulfonamides with 2,4-disubstitution show high intrinsic affinity (often sub-nM Kd) and good CA IX selectivity, as measured by fluorescent thermal shift assay (FTSA). The fluorine atoms lower the sulfonamide pKa and strengthen zinc binding.

4.11 Biologics, peptides and conjugates

- Anti-CA IX antibodies (girentuximab, G250) are used for imaging (PET with radiolabelled girentuximab in clear-cell renal carcinoma) and for radioimmunotherapy. [20]
- Bicyclic peptides targeting CA IX.
- Small-molecule drug conjugates (SMDCs) use acetazolamide- or sulfonamide-based CA IX ligands to deliver cytotoxins (e.g., monomethyl auristatin E) or radionuclides, partly discovered through DNA-encoded libraries.
- Fluorescent and radiolabelled probes for hypoxia imaging.

4.12 Dual-target and multi-target hybrids

Hybrids pair a CA-binding head with a second pharmacophore, giving CA + COX-2, kinase (sunitinib/pazopanib-type), HDAC, cholinesterase (Alzheimer's disease), PI3K inhibition, or hypoxia-activated prodrugs (e.g., nitroimidazoles). The aim is to overcome resistance and to hit parallel pathways.

4.13 Anti-infective-oriented CA inhibitors

- Mycobacterium tuberculosis encodes three β-CAs (Rv1284, Rv3273, Rv3588c). Their inhibition affects growth and persistence.
- Helicobacter pylori uses α- and β-CAs to survive in gastric acid. Acetazolamide and ethoxzolamide inhibit its growth.
- Sulfonamides and DTCs also inhibit CAs from Neisseria gonorrhoeae, vancomycin-resistant enterococci, Candida and Cryptococcus, and protozoan CAs (Plasmodium, Leishmania, Trypanosoma).
- The key challenge is selectivity over human CAs, and drug penetration into the pathogen. [9]

4.14 CA activators (for completeness)

Amines and amino acids (histamine, L-/D-histidine, L-adrenaline) activate CAs by acting as proton shuttles. They are studied for memory and cognition, but they are not inhibitors and are outside the scope of this report.

5. Synthetic Approaches

Most CA inhibitors are made in one to three steps from commercially available sulfonamides, phenols or amines, which is a major reason why the field has produced thousands of analogues. Representative routes are summarised below. [7,11]

Class	General synthetic route	Key points
Ureido-benzenesulfonami des	(SLC-0111) Sulfanilamide + aryl isocyanate in acetonitrile or THF	One step, high yield; many isocyanates available for tail variation
Triazole tails	4-Azidobenzenesulfonamide + terminal alkyne, Cu(I) catalyst (CuAAC)	Regioselective 1,4-triazole; click chemistry; library friendly
Amide / thiourea tails	Sulfanilamide + acid chloride, or + isothiocyanate	Simple, modular
Glycoconjugates	Sulfonamide amine or azide + protected sugar, then deprotection	Sugar hydroxGlycoconjugatyls must be deprotected at the end
Coumarins	Pechmann condensation of phenols with β-keto esters; Knoevenagel; Perki	Substituents at 6/7 introduced from the phenol
Sulfocoumarin	Phenol + sulfonylating reagents then ring closing, or from salicylaldehyde derivatives	Ring-closing step is key
Dithiocarbamates	Amine + CS2 + base (KOH, Et3N) in solvent, 0 °C to room temperature	Salts are often used directly; stability can be an issue
Benzoxaboroles	Borylation of 2-bromobenzyl alcohol, ring closure	Specialised organoboron chemistry

6. Case Studies: Rational Design of Marketed and Clinical Compounds

6.1 From acetazolamide to dorzolamide and brinzolamide

Oral acetazolamide lowers intraocular pressure, but it has systemic side effects and cannot be given topically because it is too hydrophilic to cross the cornea. Structure-based design used the thiophene-2-sulfonamide scaffold, which has a more acidic sulfonamide, and added a secondary amine and a fused sulfone ring. These changes balanced water solubility (to allow formulation as eye drops) and lipophilicity (to allow corneal penetration), and they added contacts in the hydrophobic cavity. The result was dorzolamide (hCA II KI in the low-nM range), which could be applied locally with minimal systemic exposure. Brinzolamide followed with a similar strategy. [1,7]

6.2 SLC-0111 and CA IX

SLC-0111 came from the ureido-benzenesulfonamide series. The 4-fluorophenyl-urea tail fits the broader hydrophobic region of CA IX and XII, and it clashes with Phe131 in CA II. In preclinical models, it reduced primary tumour growth and metastasis and sensitised tumours to chemotherapy and radiotherapy. It remains the most advanced selective CA IX/XII inhibitor. [13,14,15]

6.3 Coumarins: a mechanistic discovery

Coumarins were identified when screening natural products. Crystallography of hCA II with a hydrolysed coumarin showed the 2-hydroxycinnamic acid bound at the cavity entrance. Isoforms IX and XII, with a wider mouth, tolerate bulkier hydrolysis products, which explains the selectivity and gives a way to tune compounds by ring substituents. [16,17]

7. Summary Table of Scaffolds

Scaffold	ZBG / binding mode	Typical selectivity trend
Ureido/tail benzenesulfonamides (SLC-0111)	SO NH− → Zn 2	IX/XII > II > I
Heterocyclic sulfonamides (acetazolamide type)	Zn	Broad, II-potent
Sulfamates / sulfamides	Zn	Broad
Coumarins	Entrance occlusion (hydrolysed product)	Strong IX/XII selectivity
Sulfocoumarins	Hydrolysed sulfonic acid, entrance	Strong IX/XII

DTCs / xanthates	Zn (mono/bidentate)	Variable, often nM on VII, IX, XII
Benzoxaboroles	Tetrahedral boronate → Zn	Variable
Carboxylic acids	Zn or anchored to water	Variable
Phenols	Anchored to zinc-bound water	I/II weak, IX/XII better
Polyamines	Anchored to water	µM, moderate
Hydroxamates	Bidentate Zn	Moderate
Selenium-based	Zn / other	Often potent, broad

8. Structure–Activity Relationships (SAR)

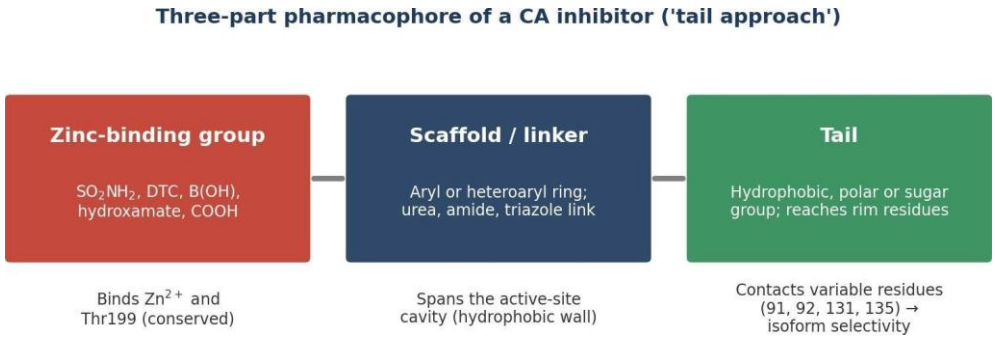

Figure 4. General three-part pharmacophore model used for CA inhibitor design.

8.1 Sulfonamide pharmacophore

- A primary, unsubstituted SO2NH2 is essential; N-substitution usually abolishes or sharply reduces activity.
- Electron-withdrawing groups that lower the sulfonamide pKa (raising the SO2NH fraction) generally increase affinity. Benzenesulfonamide has pKa about 10, while thiadiazole sulfonamides such as acetazolamide are near 7, and polyfluorinated benzenesulfonamides are lower still. [7]
- Five-membered heteroaryl sulfonamides (thiophene, thiadiazole, thiazole, benzothiazole) are generally more potent than benzenesulfonamides.
- The aromatic scaffold matters. Para-substituted benzenesulfonamides are most common; meta and ortho isomers change tail trajectory and often selectivity.
- Too much lipophilicity increases non-specific binding, while too little reduces cell and tissue penetration.

8.2 The tail approach

- Tails reach the rim and interact with isoform-variable residues. Position 131 is Phe in hCA II but smaller in IX (Val) and XII (Ala), giving room for bulkier tails and hence IX/XII over II selectivity. [2,11]
- Positions 91, 92, 130 and 135 are also selectivity determinants.
- Linker type sets tail orientation. Ureas, amides, thioureas, triazoles and hydrazones add H-bonds and rigidity.
- Linker length: too short gives no rim contact; too long increases flexibility, entropic penalty and loss of selectivity.
- Hydrophobic tails contact the hydrophobic wall (Phe131, Val135, Leu198). Polar/charged tails (sugars, carboxylates, ammonium) improve solubility and prevent membrane permeation, targeting extracellular CA IV, IX and XII.
- Halogenated aryl tails (e.g., 4-fluorophenyl in SLC-0111) often improve CA IX/XII binding through lipophilic and halogen contacts.
- Positional isomerism (ortho, meta, para) of the tail on the benzene ring changes isoform preference.

8.3 Coumarin and sulfocoumarin SAR

- Substituents at positions 6 and 7 (and 3/4 in some series) tune the fit of the hydrolysis product at the pocket entrance.
- Bulky or lipophilic groups at 6/7 enhance CA IX/XII selectivity because their entrance accommodates them better than the narrower CA I/II.
- Blocking lactone hydrolysis reduces activity, confirming the prodrug mechanism. [16]
- Sulfocoumarins: substituents at 6 and 7 similarly shape selectivity, and many reach nM KI for IX/XII. [17]

8.4 DTCs and other non-sulfonamide ZBGs

- The amine substituent determines potency. Cyclic amines (piperidine, piperazine, morpholine) and amino-acid-derived DTCs often give the most potent inhibition. [18]
- Extra H-bond donors/acceptors can interact with Thr199 or Gln92 and improve KI.
- Replacing the primary sulfonamide with a weaker ZBG usually costs affinity, but it can improve selectivity and safety.

8.5 Phenols and carboxylic acids

- Typically weaker (µM). Ortho-substitution, acidity and the ability to H-bond with Thr199/Thr200 matter.
- Hydroxyl positioning and lipophilic substituents tune selectivity (e.g., for CA VII/IX/XII).

8.6 Isoform-selectivity design principles

Target	Design strategy
CA IX / XII (tumour)	Bulky, polar, membrane- impermeant tails; coumarins and sulfocoumarins; ureido linkers
CA II / IV / XII (glaucoma)	Balanced lipophilicity and water solubility for corneal penetration (as with dorzolamide, brinzolamide)
CA VII (CNS)	Blood–brain barrier penetration: smaller, more lipophilic scaffolds
CA VA / VB (obesity)	Mitochondria-selective designs (still scarce)
Bacterial CAs	Exploit structural differences between β/γ and α classes

9. CA IX and CA XII in Cancer

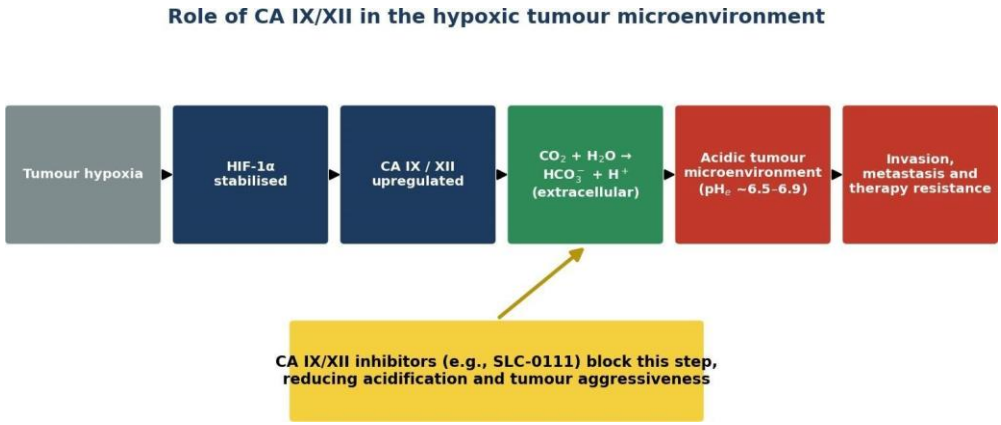

Figure 5. Pathway linking tumour hypoxia to CA IX/XII-driven acidosis and aggressive behaviour.

- Regulation: CA IX is strongly induced by hypoxia-inducible factor 1α (HIF-1α), and expression is low in most normal tissues (mainly gastrointestinal epithelium). [21]
- Structure: CA IX has an extracellular catalytic domain, a proteoglycan-like domain, a transmembrane helix and a short intracellular tail. It acts as a dimer.
- Function: CA IX and XII to HCO_3^- and H+ outside the cell. Bicarbonateconvert CO_2 is + imported to keep intracellular pH alkaline, while H acidifies the surroundings, which promotes invasion, matrix degradation and immune evasion. [5,20]
- Clinical correlation: high CA IX expression is linked to poor prognosis and treatment resistance in many carcinomas (breast, lung, cervix, kidney, brain). [21]

- Therapeutic strategies: small-molecule inhibitors (SLC-0111), antibodies, SMDCs and radioligands, and CA IX as an imaging biomarker of hypoxia. [15,20]

10. **Future Directions**

- Isoform-selective, non-sulfonamide ZBGs with better safety. [10,20]
- CA IX/XII-targeted theranostics: SMDCs, radioligands and imaging agents.
- Multi-target hybrid drugs.
- Combination with chemo-, radio- or immunotherapy, since CA IX inhibition counters tumour acidosis.
- CA VII and CA VA/VB inhibitors for pain, epilepsy and obesity.
- Anti-infective CA inhibitors, including against drug-resistant bacteria.
- Computational and AI-driven design, fragment-based discovery and DNA-encoded libraries.
- Nanocarrier and prodrug delivery of CA inhibitors.

11. Conclusion

Newer CA inhibitor scaffolds go well beyond classical primary sulfonamides. Tail-approach sulfonamides (ureido, triazole, glyco- and dual-tail), coumarins and sulfocoumarins, dithiocarbamates, benzoxaboroles, selenium compounds, phenols, carboxylic acids and metal complexes all exploit distinct binding modes. The central SAR principles are to retain an acidity-tuned zinc-binding head and to use tails or entrance-occluding fragments that exploit variable rim residues (especially position 131) for isoform selectivity. The remaining work lies in clinical translation, selectivity over CA II, and extension into anti-infective and CNS indications.

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Note: KI values and clinical-trial status are approximate and change over time. Verify specific values, trial status and full citation details against the primary literature before final submission.

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