IASOC 2016,
September 25-29, 2016

Carbonic Anhydrase Inhibitors: Synthesis and Mechanism of Action

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$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$$

CAs are highly effective catalysts:

hCA IX: $k_{cat}/K_M = 1.5 \times 10^8\ M^{-1} \times s^{-1}$
(Hilvo, De Simone et al., *JBC* **2008**, 283, 27799)
SazCA: $k_{cat}/K_M = 3.5 \times 10^8\ M^{-1} \times s^{-1}$
(extremophilic bacterium *Sulfurihydrogenibium azorense*
Vullo et al., *BMC* **2013,** *21*, 4521)
hSOD: $k_{cat}/K_M = 7.0 \times 10^9\ M^{-1} \times s^{-1}$

7 Carbonic anhydrase (CA) gene families

α-CAs (*Bacteria*, algae, cytoplasm of green plants, protosoa (e.g. *Plasmodium*), animals – including vertebrates)

β-CAs (*Bacteria*, algae, chloroplasts of mon-/dicotyledons)

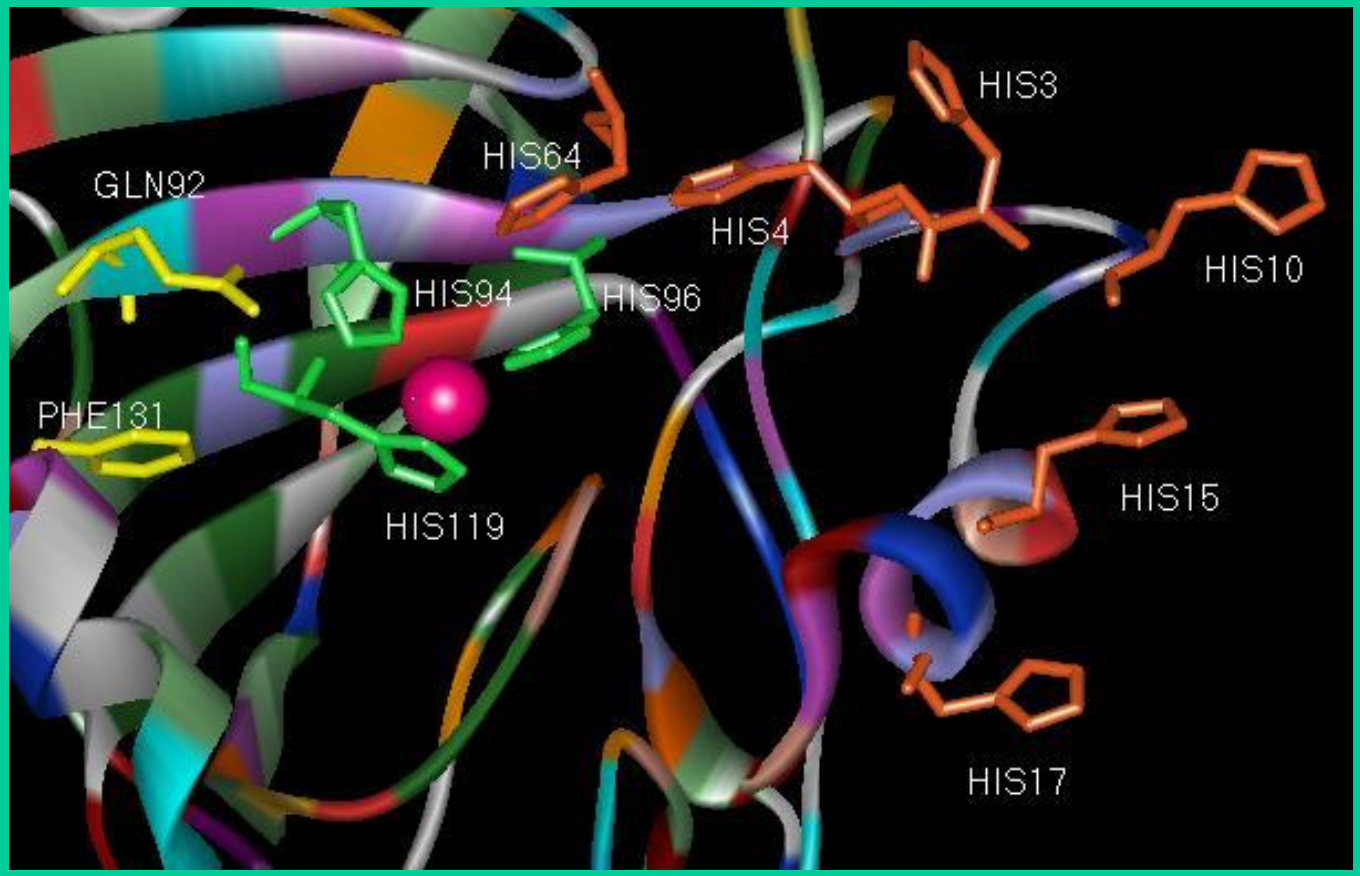
γ-CAs (*Archaea*, *Bacteria*)

δ- CAs – marine diatoms and algae (e.g., *Thalassiosira weissflogii* TWCA1 and related organisms)

ζ- CAs –Cd or Zn enzymes from marine diatoms

η-CAs – in *Plasmodium* spp.

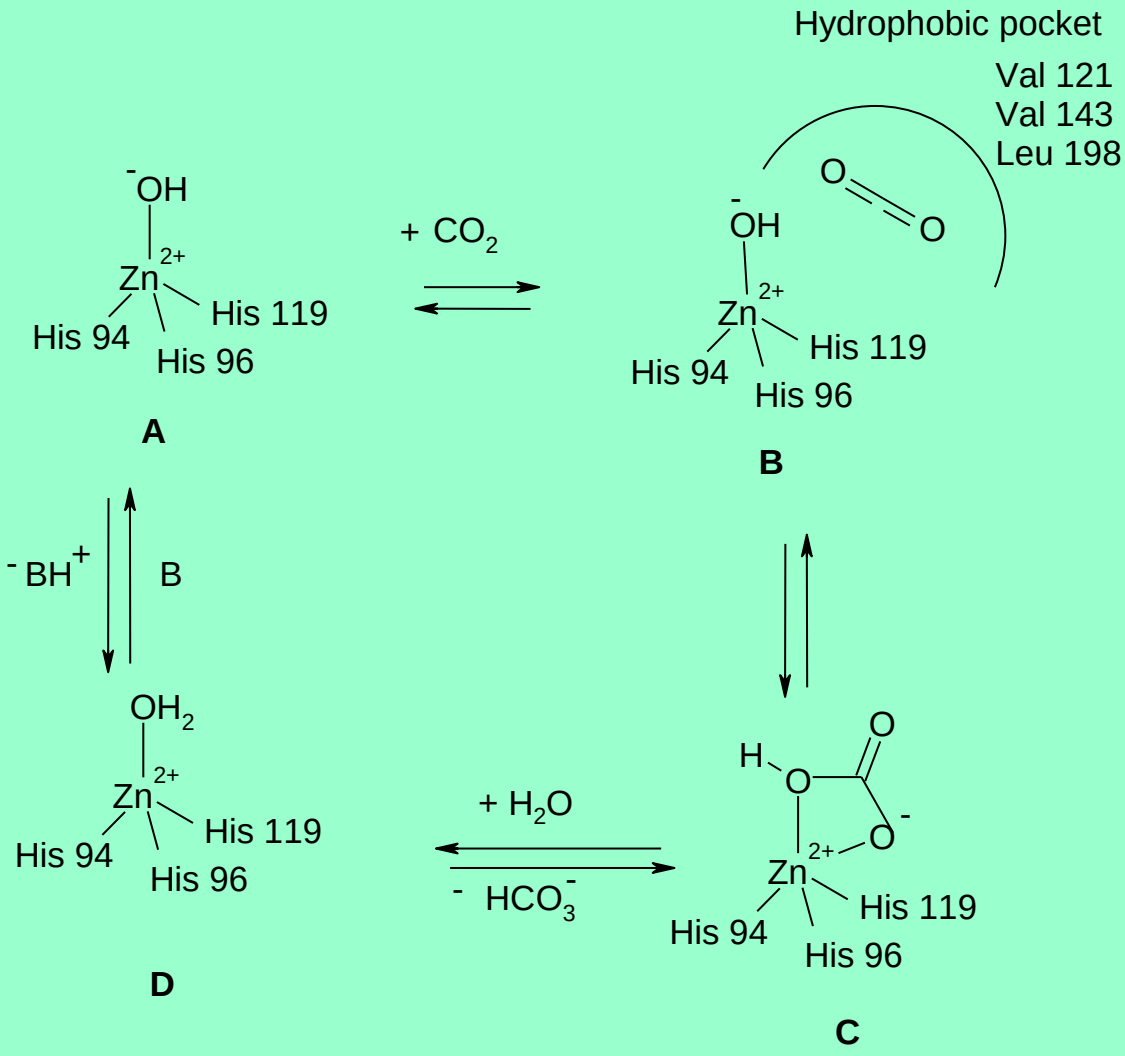
Θ-CAs – thylakoid of diatoms (*PNAS* **2016**, 113,9828-33)



hCA II active site, with the Zn(II) ion (pink sphere), its three histidine ligands (His 94, His 96 and His 119, in green), the proton shuttle residue His 64 as well as the histidine cluster extending from the rim of the active site to the surface of the protein, comprising residue 3, 4, 10, 15 and 17, in orange)

Supuran, CT. *Nature Rev Drug Discov* **2008**, *7*, 168-181

Catalytic mechanism of α-CAs



$O=C=O + H_2O \leftrightarrow HCO_3^- + H^+$	(*1*)
$O=C=S + H_2O \leftrightarrow H_2S + CO_2$ $S=C=S + 2H_2O \leftrightarrow 2\,H_2S + CO_2$	(*2*) (*3*)
$HN=C=NH + H_2O \leftrightarrow H_2NCONH_2$	(*4*)
$RCHO + H_2O \leftrightarrow RCH(OH)_2$	(*5*)
$RCOOAr + H_2O \leftrightarrow RCOOH + ArOH$	(*6*)
$RSO_3Ar + H_2O \leftrightarrow RSO_3H + ArOH$	(*7*)
$ArOPO_3H_2 + H_2O \leftrightarrow ArOH + H_3PO_4$	(*8*)
$R_2NCSSR' + H_2O \leftrightarrow R_2NH + R'SH + COS$	(9)
$PhCH_2OCOCl + H_2O \leftrightarrow PhCH_2OH + CO_2 + HCl$	*(10)*
$RSO_2Cl + H_2O \leftrightarrow RSO_3H + HCl$	(*11*)

Reactions catalyzed by the CAs (Supuran CT, *Biochem J*. **2016**, *473*,2023-32)

DOI: 10.1021/ml500470b
ACS Med. Chem. Lett. 2015, 6, 292−295

ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

α-Carbonic Anhydrases Possess Thioesterase Activity

Muhammet Tanc,[†,‡] Fabrizio Carta,[‡] Andrea Scozzafava,[‡] and Claudiu T. Supuran*[,†,‡]

[†]Laboratorio di Chimica Bioinorganica, Università degli Studi di Firenze, Polo Scientifico, Room 188, Via della Lastruccia 3, 50019 Sesto Fiorentino, Florence, Italy

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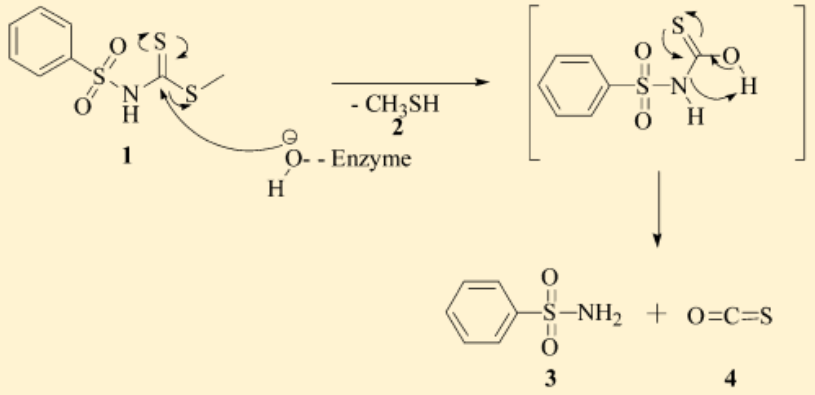
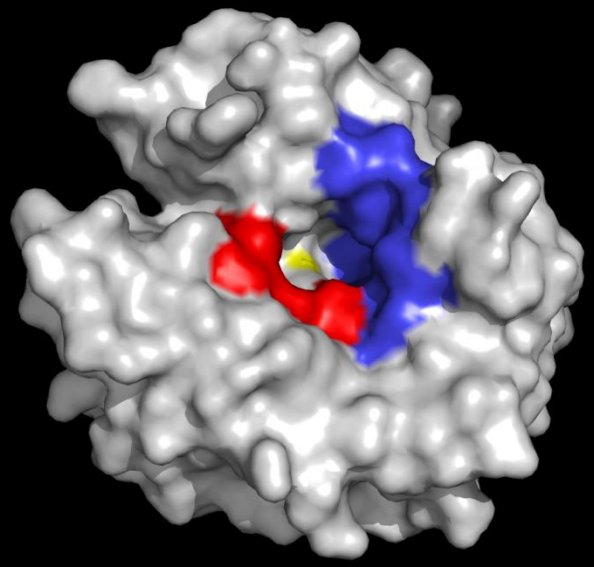
Supporting Information

ABSTRACT: The α-carbonic anhydrases (CAs, EC 4.2.1.1) show catalytic versatility acting as esterases with carboxylic, sulfonic, and phosphate esters. Here we prove by kinetic, spectroscopic, and MS studies that they also possess thioesterase activity with a dithiocarbamate ester as a substrate ($PhSO_2NHCSSMe$). Its CA-mediated hydrolysis leads to benzenesulfonamide, methyl mercaptan, and COS. The CA thioesterase activity may be useful for designing prodrug enzyme inhibitors, whereas some CA isoforms may use this activity for modulating physiologic/pathologic processes, which are possibly amenable to drug discovery of agents with multiple mechanisms of action.

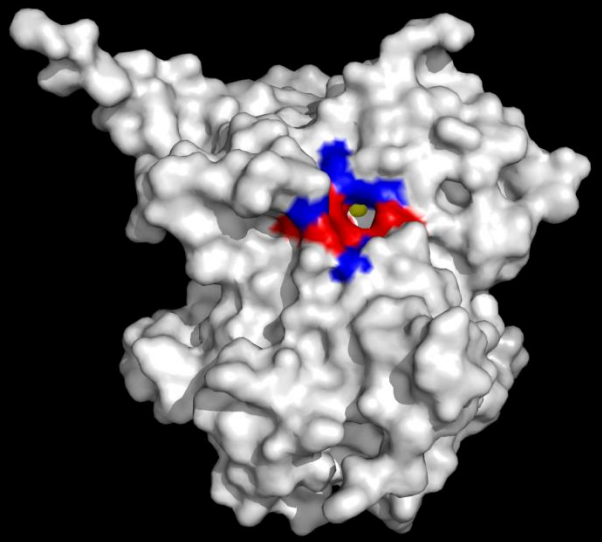


KEYWORDS: *Carbonic anhydrase, thioesterase, inhibitors, prodrugs*

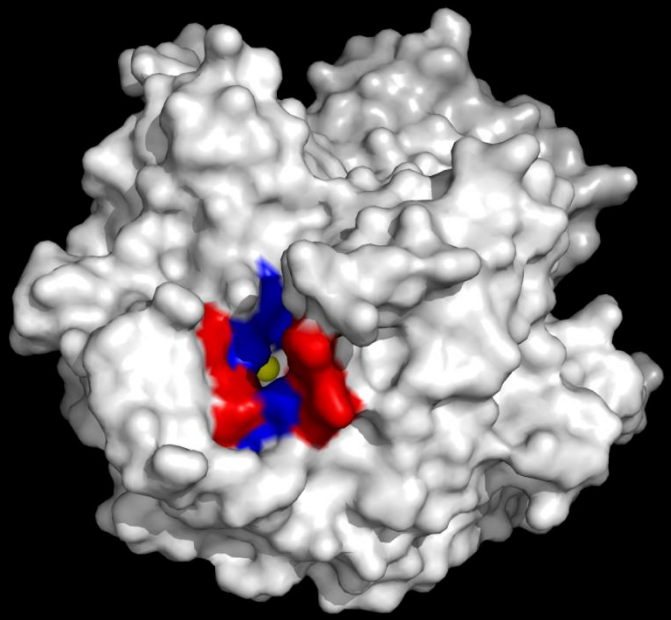


α-CA
(hCA II)

β-CA
(Can2)

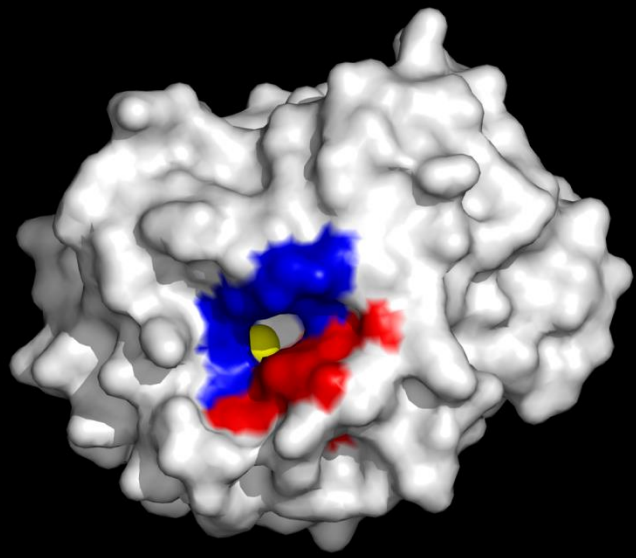


Blue= Hydrophobic
Red = Hydrophilic
Yellow = metal



γ-CA
(Cam)

ζ-CA
(R3-CdCA)



α-CAs in higher vertebrates including *Homo sapiens*

Isozyme	Catalytic activity (CO_2 hydration)	Affinity for sulfonamides	Sub-cellular localization
CA I	**medium**	**medium**	**cytosol**
CA II	**high**	**very high**	**cytosol**
CA III	**very low**	**very low**	**cytosol**
CA IV	**high**	**high**	**plasma membrane**
CA VA	**moderate**	**high**	**mitochondria**
CA VB	**high**	**high**	**mitochondria**
CA VI	**medium**	**high**	**secreted (saliva/milk)**
CA VII	**high**	**very high**	**cytosol**
CA VIII	**acatalytic**	**-**	**cytosol**
CA IX	**high**	**high**	**transmembrane**
CA X	**acatalytic**	**-**	**cytosol**
CA XI	**acatalytic**	**-**	**cytosol**
CA XII	**medium**	**very high**	**transmembrane**
CA XIII	**medium**	**high**	**cytosol**
CA XIV	**low**	**high**	**transmembrane**
mCA XV	**high**	**high**	**plasma membrane**

m = mouse isoform; all other are human CAs

CHEMICAL REVIEWS

Review

pubs.acs.org/CR

Multiple Binding Modes of Inhibitors to Carbonic Anhydrases: How to Design Specific Drugs Targeting 15 Different Isoforms?

Vincenzo Alterio,[†] Anna Di Fiore,[†] Katia D'Ambrosio,[†] Claudiu T. Supuran,[*,‡] and Giuseppina De Simone[*,†]

[†]Istituto di Biostrutture e Bioimmagini-CNR, via Mezzocannone 16, 80134 Nap
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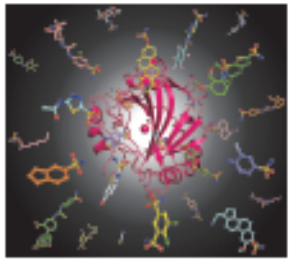

Corresp
Notes
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Acknowled
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References

1. INTROD

Carbonic an
enzymes, p
encoded by

Alterio et al.
Chem. Rev. **2012**, 112, 4421-4468.

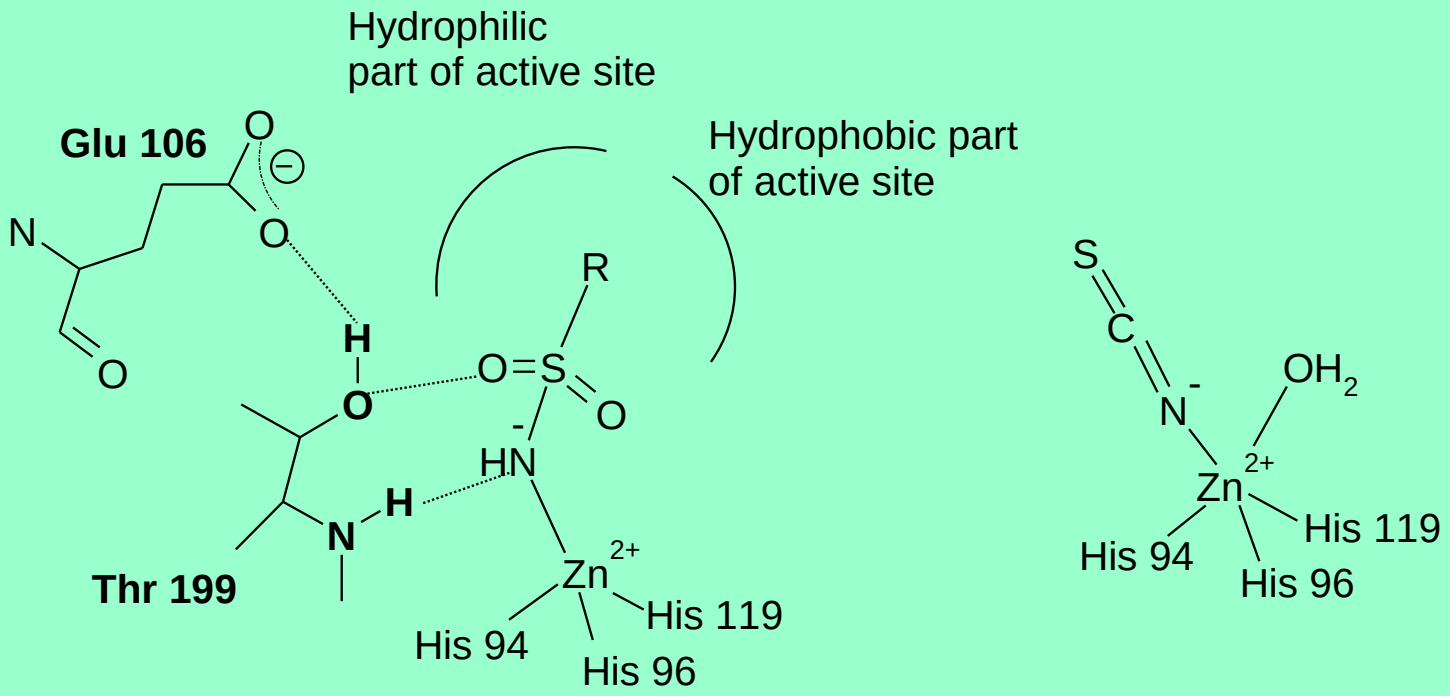
CA inhibition mechanism by sulfonamide and anionic inhibitors

$E\text{-}Zn^{2+}\text{-}OH_2 + I \Leftrightarrow E\text{-}Zn^{2+}\text{-}I + H_2O$ **(substitution)**

Tetrahedral adduct

$E\text{-}Zn^{2+}\text{-}OH_2 + I \Leftrightarrow E\text{-}Zn^{2+}\text{-}OH_2(I)$ **(addition)**

Trigonal-bipyramidal adduct



Journal of Enzyme Inhibition and Medicinal Chemistry

www.tandfonline.com/ienz
ISSN: 1475-6366 (print), 1475-6374 (electronic)

J Enzyme Inhib Med Chem, 2016; 31(3): 345–360
 DOI: 10.3109/14756366.2015.1122001

Taylor & Francis
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REVIEW ARTICLE

How many carbonic anhydrase inhibition mechanisms exist?

Claudiu T. Supuran

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Abstract

Six genetic families of the enzyme carbonic anhydrase (CA, EC 4.2.1.1) were described to date. Inhibition of CAs has pharmacologic applications in the field of antiglaucoma, anticonvulsant, anticancer, and anti-infective agents. New classes of CA inhibitors (CAIs) were described in the last decade with enzyme inhibition mechanisms differing considerably from the classical inhibitors of the sulfonamide or anion type. Five different CA inhibition mechanisms are known: (i) the zinc binders coordinate to the catalytically crucial Zn(II) ion from the enzyme active site, with the metal in tetrahedral or trigonal bipyramidal geometries. Sulfonamides and their isosters, most anions, dithiocarbamates and their isosters, carboxylates, and hydroxamates bind in this way; (ii) inhibitors that anchor to the zinc-coordinated water molecule/hydroxide ion

Keywords

Anchoring to zinc-coordinated water, carbonic anhydrase, inhibition mechanism, inhibitors, occlusion of the active site entrance, out of the active site binding, zinc binder

History

Received 11 November 2015

Biochem. J. (2016) 478, 2023–2032 doi:10.1042/BCJ20160115

REVIEW ARTICLE

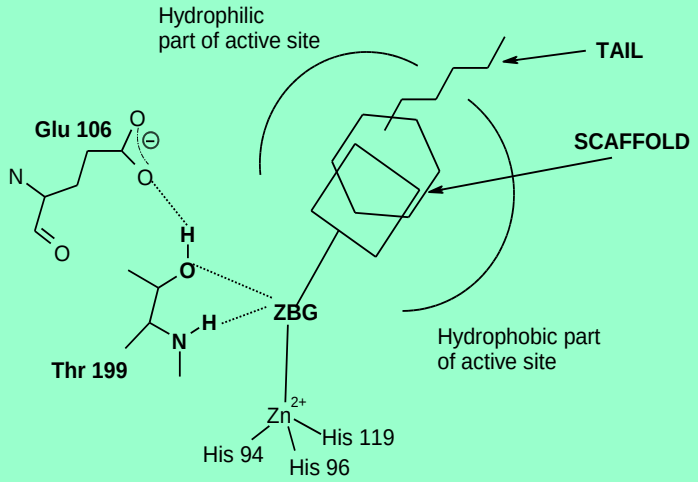
Structure and function of carbonic anhydrases

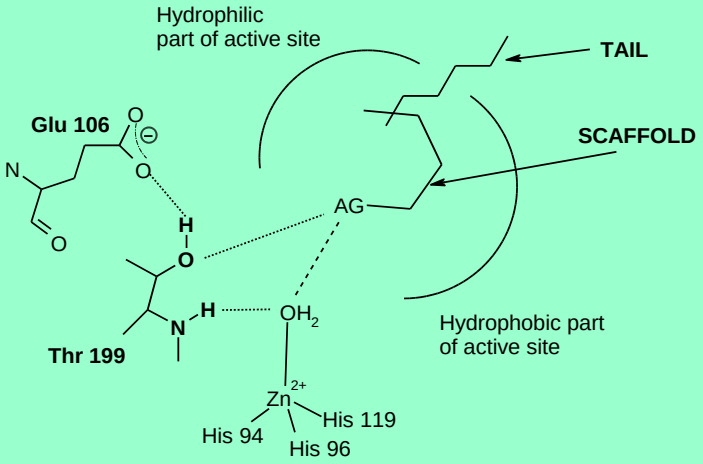
Claudiu T. Supuran*[1]

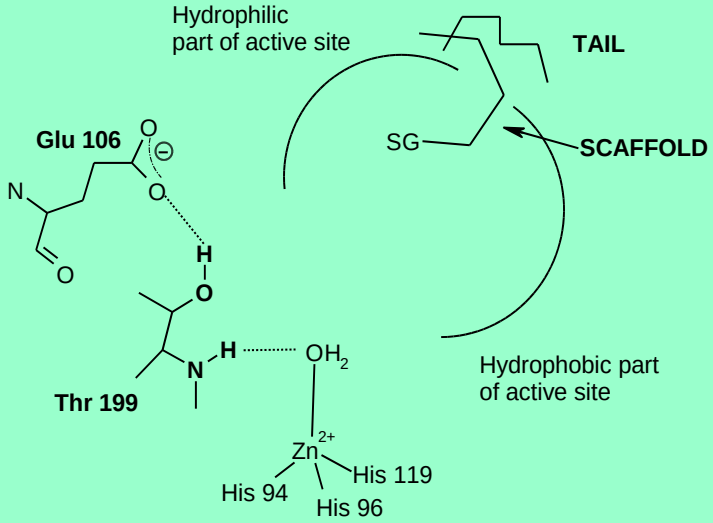
*Neurofarba Department and Laboratorio di Chimica Bioinorganica, Sezione di Chimica Farmaceutica e Nutraceutica, Università degli Studi di Firenze, Via U. Schiff 6, 50019 Sesto Fiorentino (Florence), Italy

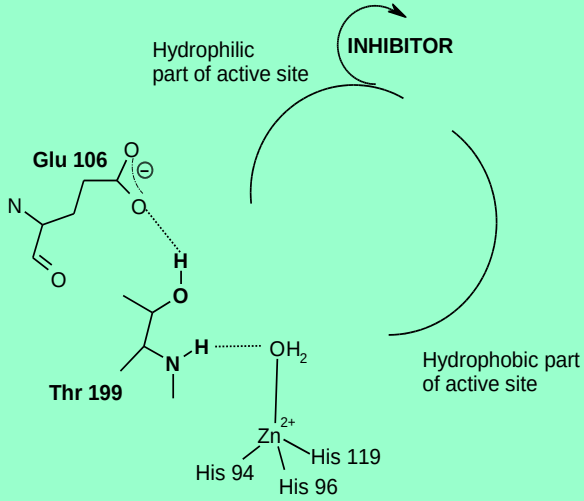
Figure 5 CA inhibition mechanisms

(**A**) Zinc binding, (**B**) Anchoring to the metal ion coordinated water, (**C**) Occlusion of the active site entrance, (**D**) Out of the active site binding [61].

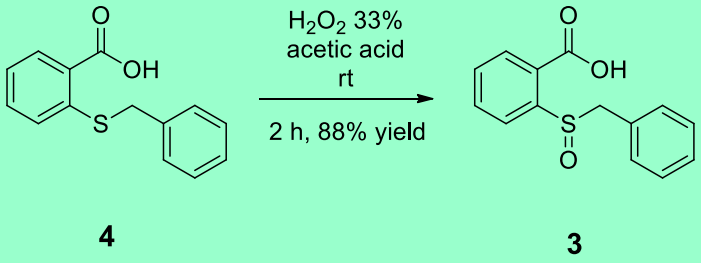








New CA inhibition mechanism



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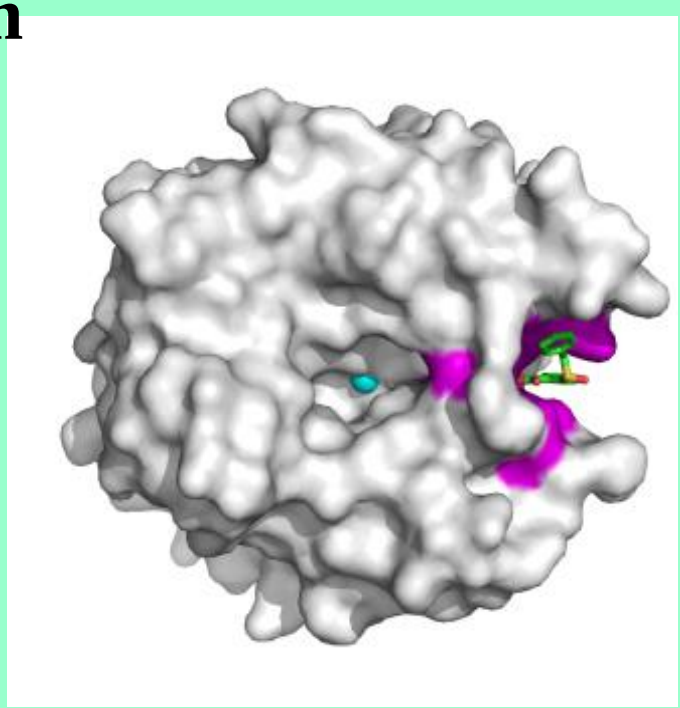
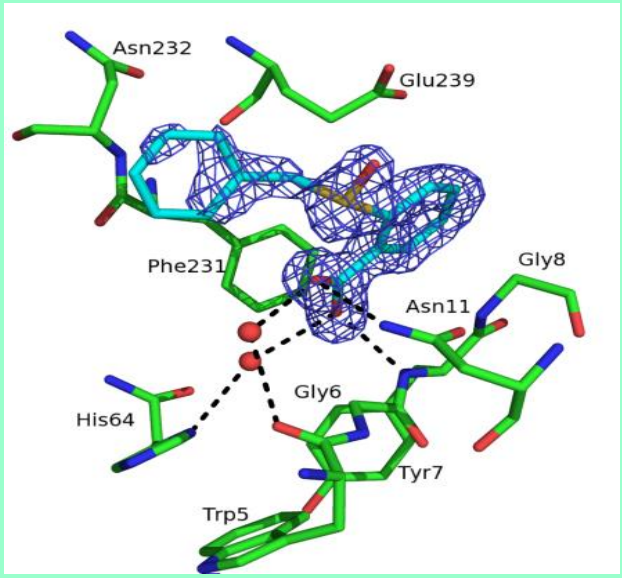
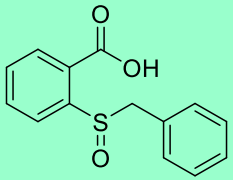


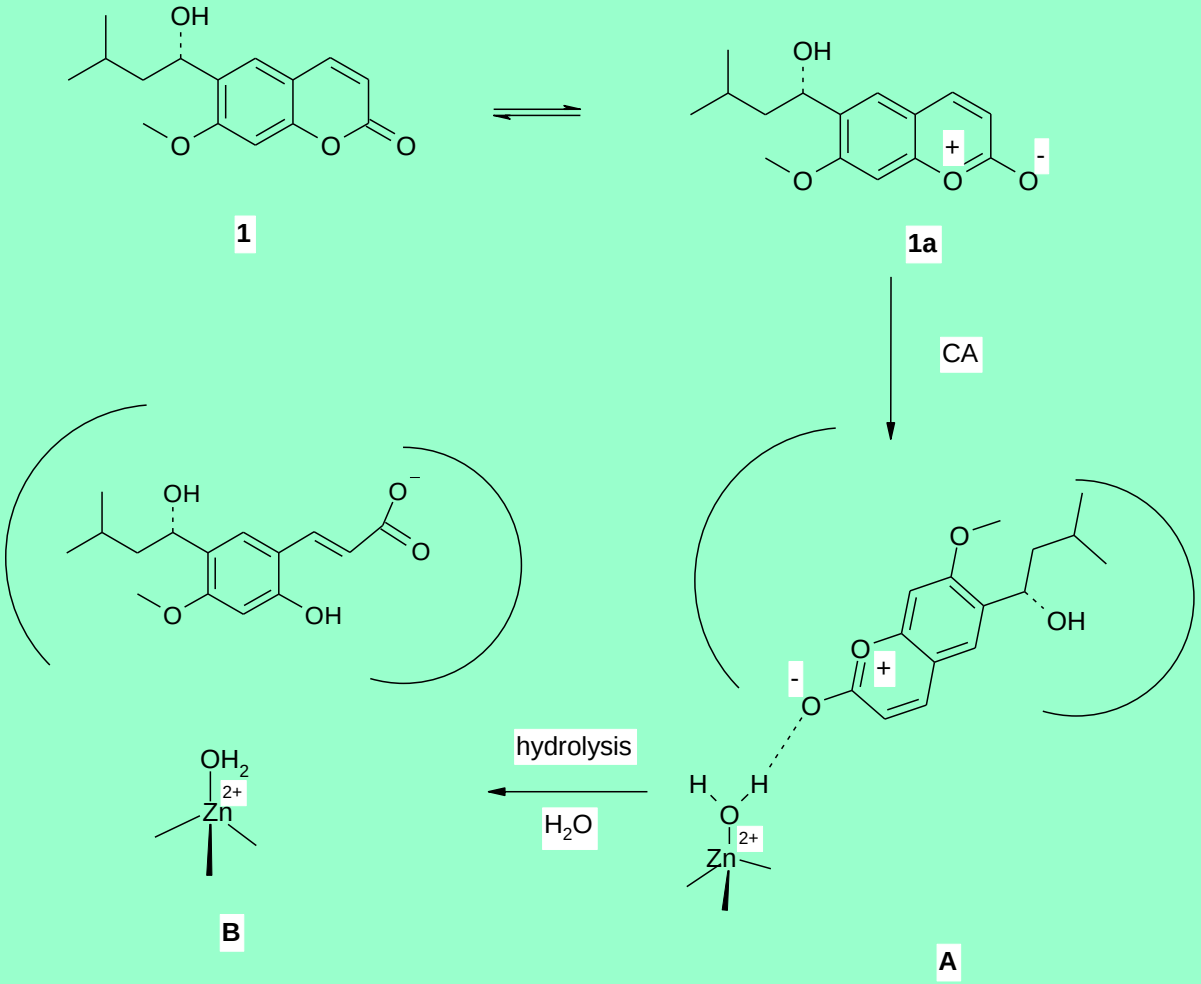


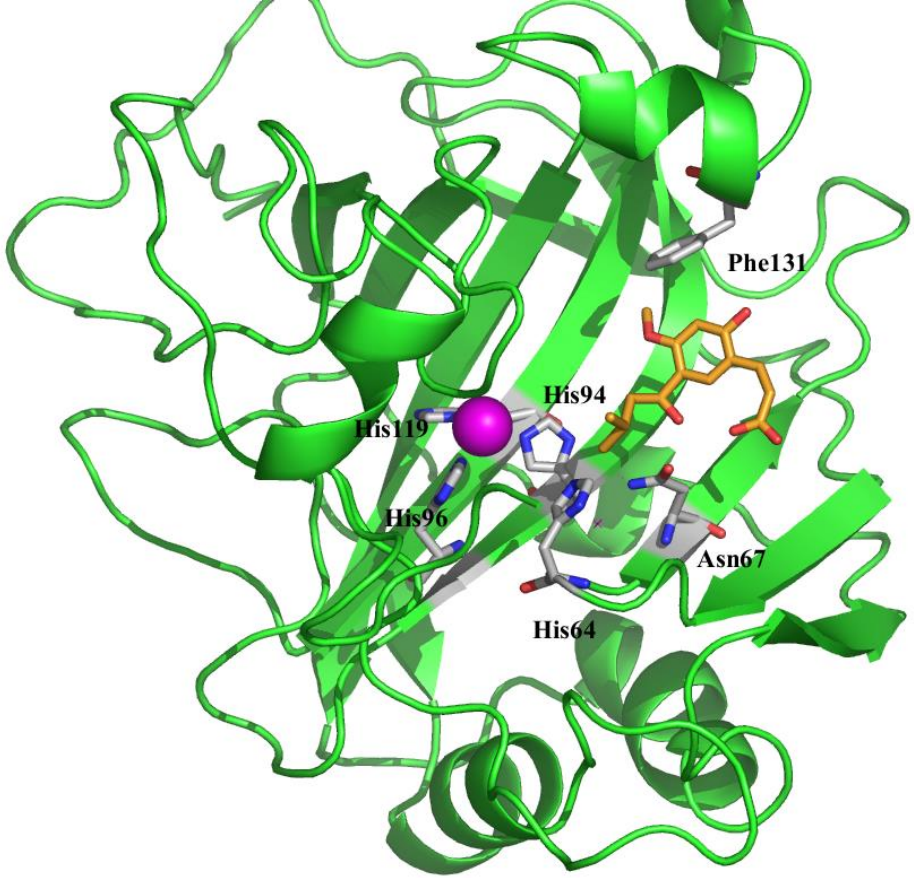


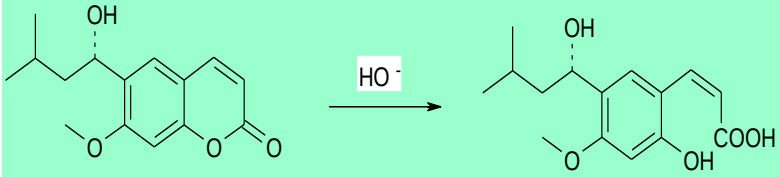
OUT OF THE ACTIVE SITE BINDING

D'Ambrosio, Carradori, et al., *Chem Commun* **2015**, *51*, 302-305

CA inhibition mechanism by Coumarins (Maresca et al, JACS 2009) (Inhibition Mechanism no. 3: occlusion of active site entrance)

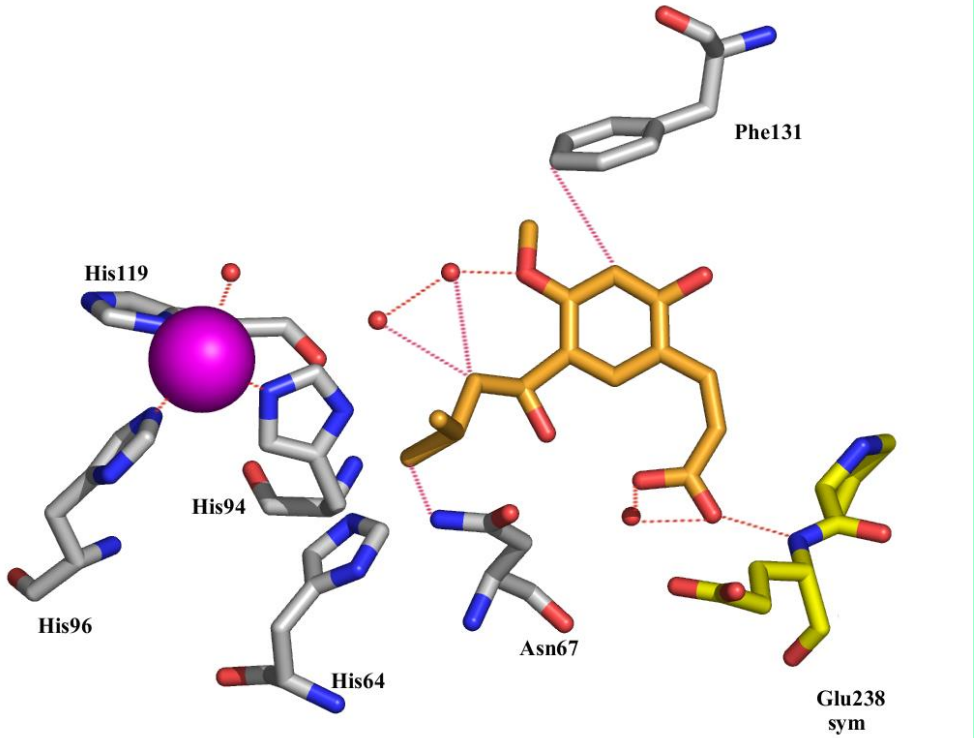


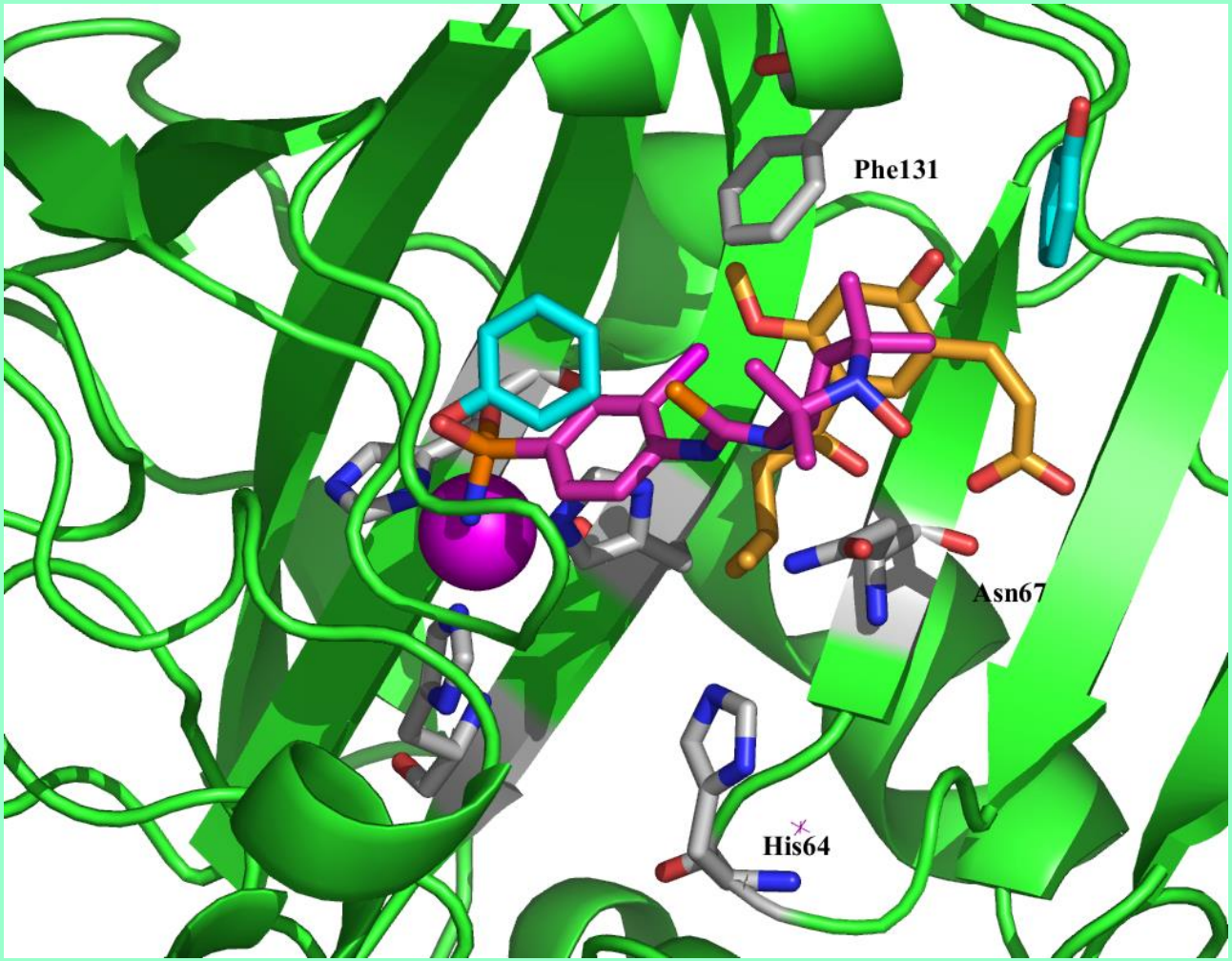




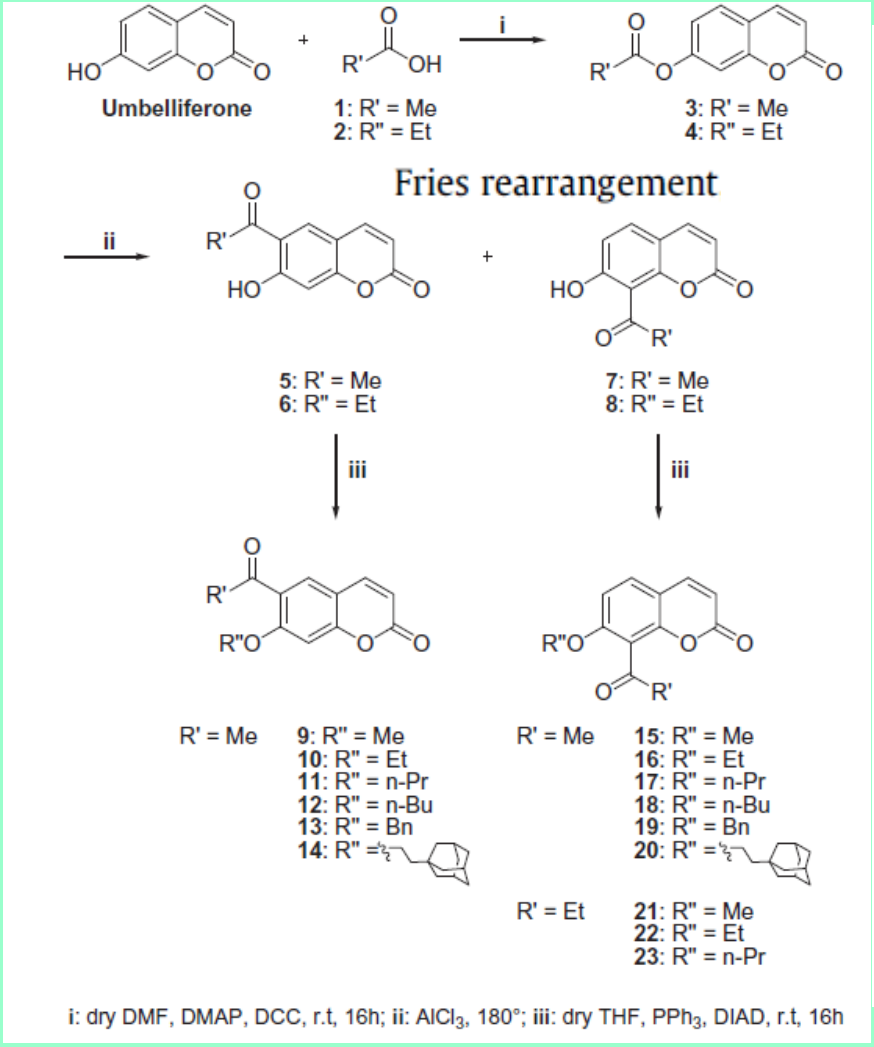
Binding of the *CIS*-2-hydroxy-cinnamic acid (in gold) hydrolysis product of the coumarin NP within hCA II active site

Maresca et al, JACS 2009





Superposition of hCA II – 5 (coumarin hydrolysis product) – gold – with hCA II – phenol adduct (sky) and hCA II – sulfonamide adduct (possessing a TEMPO tail) (magenta).



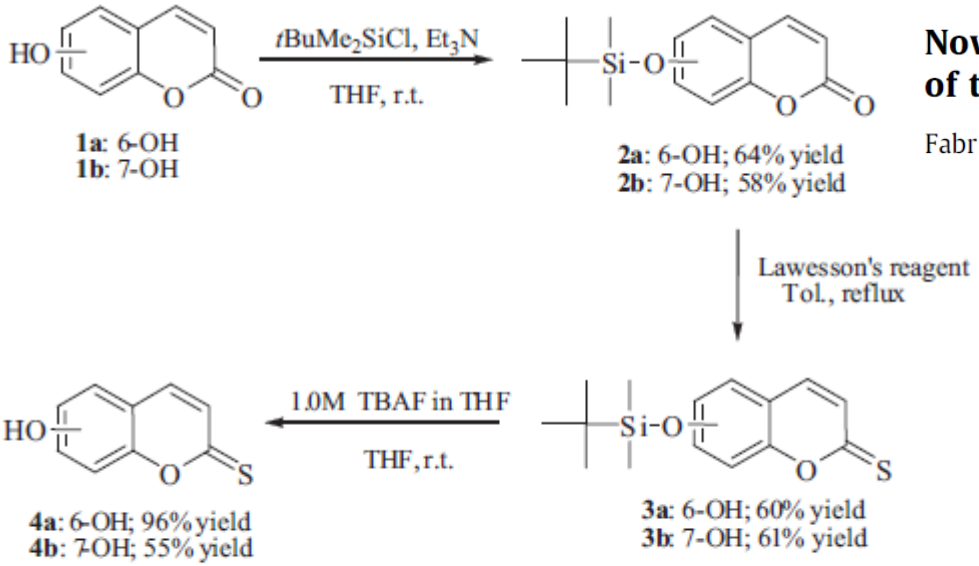
hCA I, II, IX and XII inhibition data with coumarins **5–23** and **A–C** (as standard inhibitors), by a stopped-flow, CO_2 hydration assay method (6 h incubation time between enzyme and coumarin)[17]

Compound	K_I^*			
	hCA I[a] (μM)	hCA II[a] (μM)	hCA IX[b] (nM)	hCA XII[b] (nM)
A	0.078	0.059	54.5	48.6
B	3.1	9.2	>100	>100
C	58.4	>100	482	754
5	>100	>100	8030	>100,000
6	>100	>100	8015	>100,000
7	>100	>100	73.0	61.9
8	>100	>100	58.2	61.7
9	>100	>100	7800	6540
10	>100	>100	7400	>100,000
11	>100	>100	7580	>100,000
12	>100	>100	>100,000	>100,000
13	>100	>100	>100,000	>100,000
14	>100	>100	>100,000	77,700
15	>100	>100	78.3	60.9
16	>100	>100	70.8	1.0
17	>100	>100	56.7	0.98
18	>100	>100	61.2	8.8
19	>100	>100	72.3	22.4
20	>100	>100	63.9	31.5
21	>100	>100	37.8	26.3
22	>100	>100	46.7	33.2
23	>100	>100	50.2	38.4

7,8-Disubstituted- but not 6,7-disubstituted coumarins selectively inhibit the transmembrane, tumor-associated carbonic anhydrase isoforms IX and XII over the cytosolic ones I and II in the low nanomolar/subnanomolar range

Alfonso Maresca, Andrea Scozzafava, Claudiu T. Supuran *

Bioorganic & Medicinal Chemistry Letters 20 (2010) 7255–7258



Scheme 1. Preparation of silylated coumarins/2-thioxo-coumarins incorporating 6- and 7-hydroxy moieties.

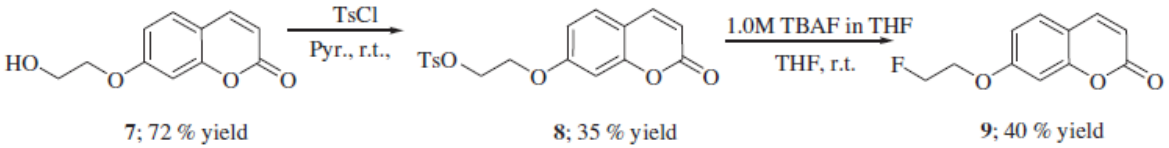
Novel coumarins and 2-thioxo-coumarins as inhibitors of the tumor-associated carbonic anhydrases IX and XII

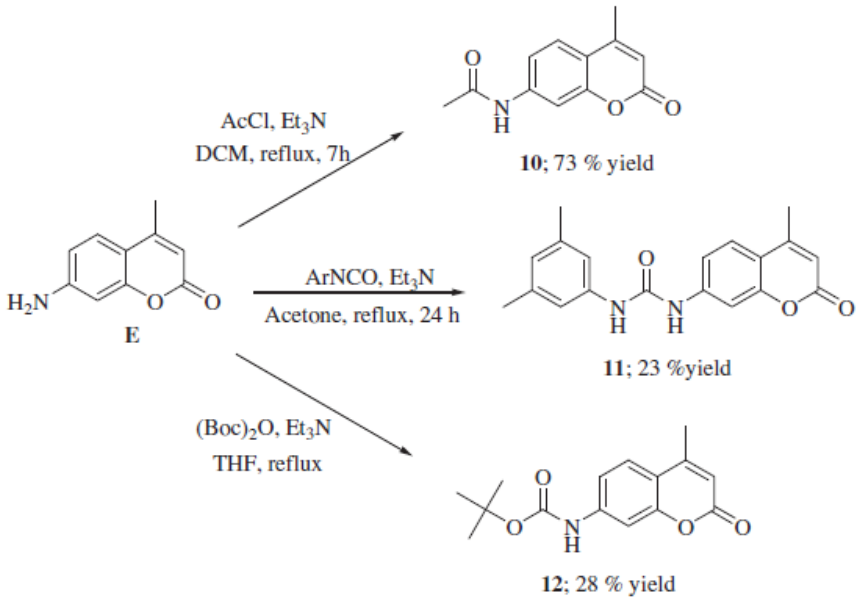
Fabrizio Carta, Alfonso Maresca, Andrea Scozzafava, Claudiu T. Supuran *

Bioorganic & Medicinal Chemistry 20 (2012) 2266–2273

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

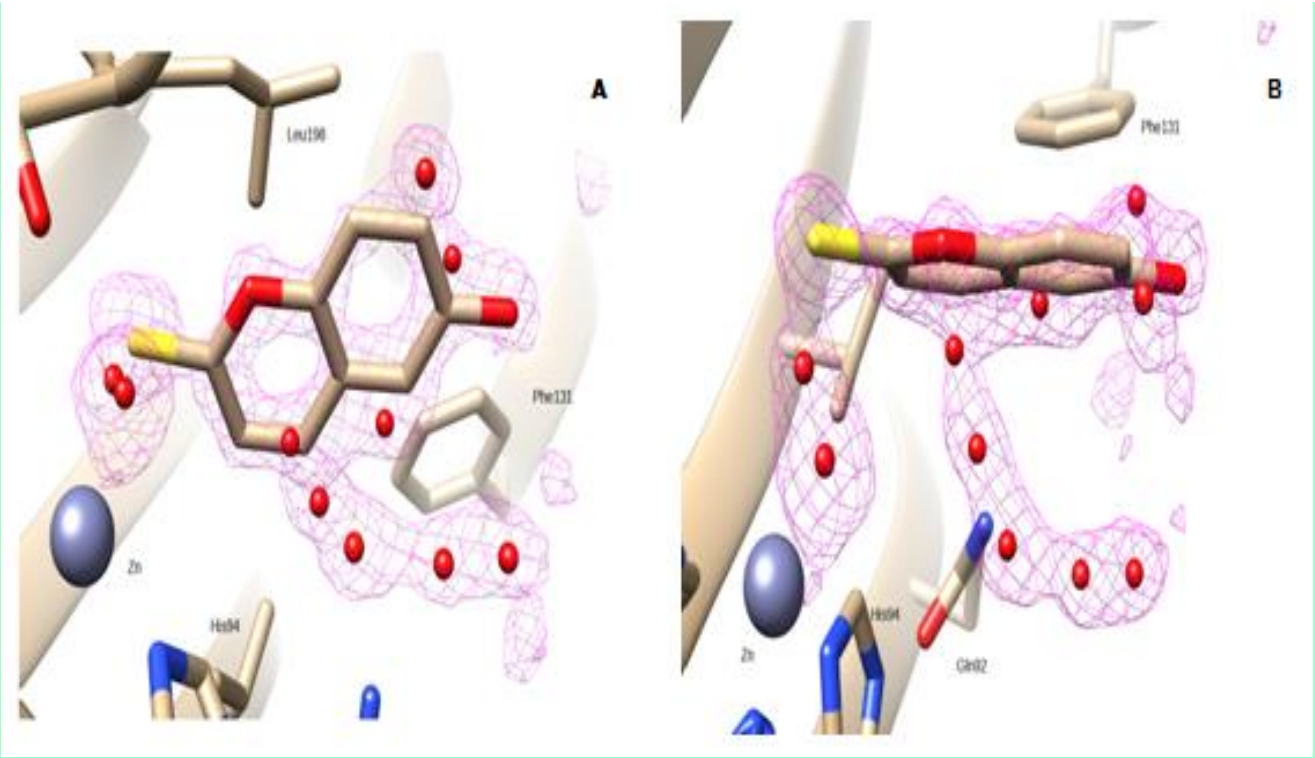




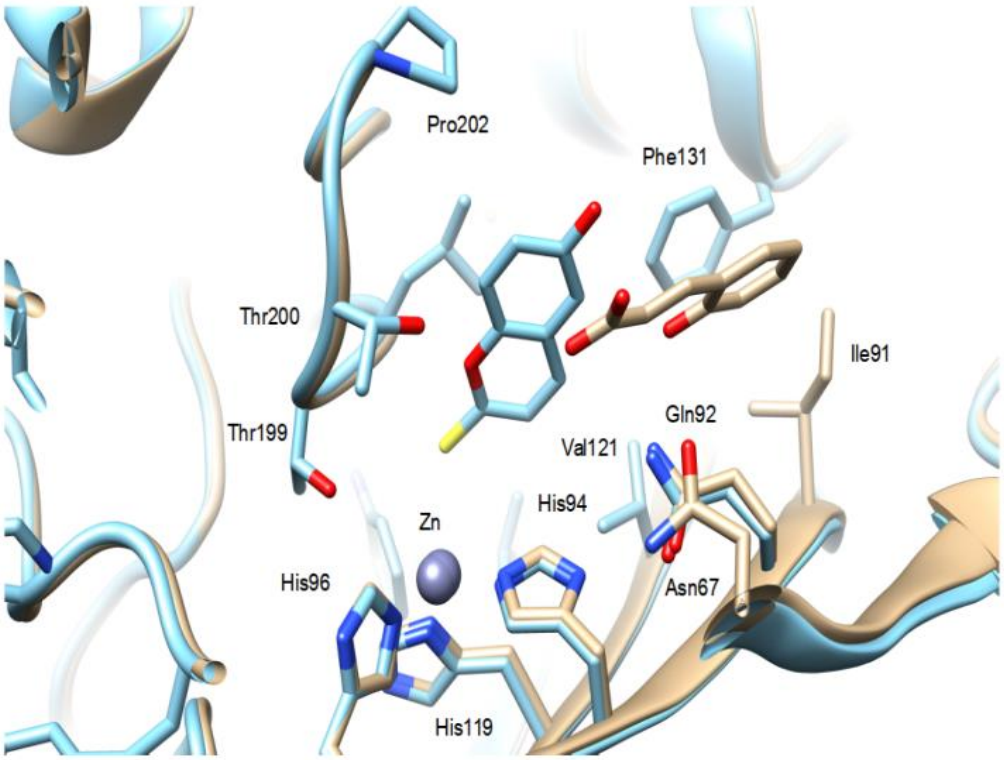
These coumarins/thiocoumarins were effective CA IX/XII inhib. No inhibition of CA I and II

Thioxocoumarins Show an Alternative Carbonic Anhydrase Inhibition Mechanism Compared to Coumarins

Marta Ferraroni,[†] Fabrizio Carta,[†] Andrea Scozzafava,[†] and Claudiu T. Supuran*[,†,‡]

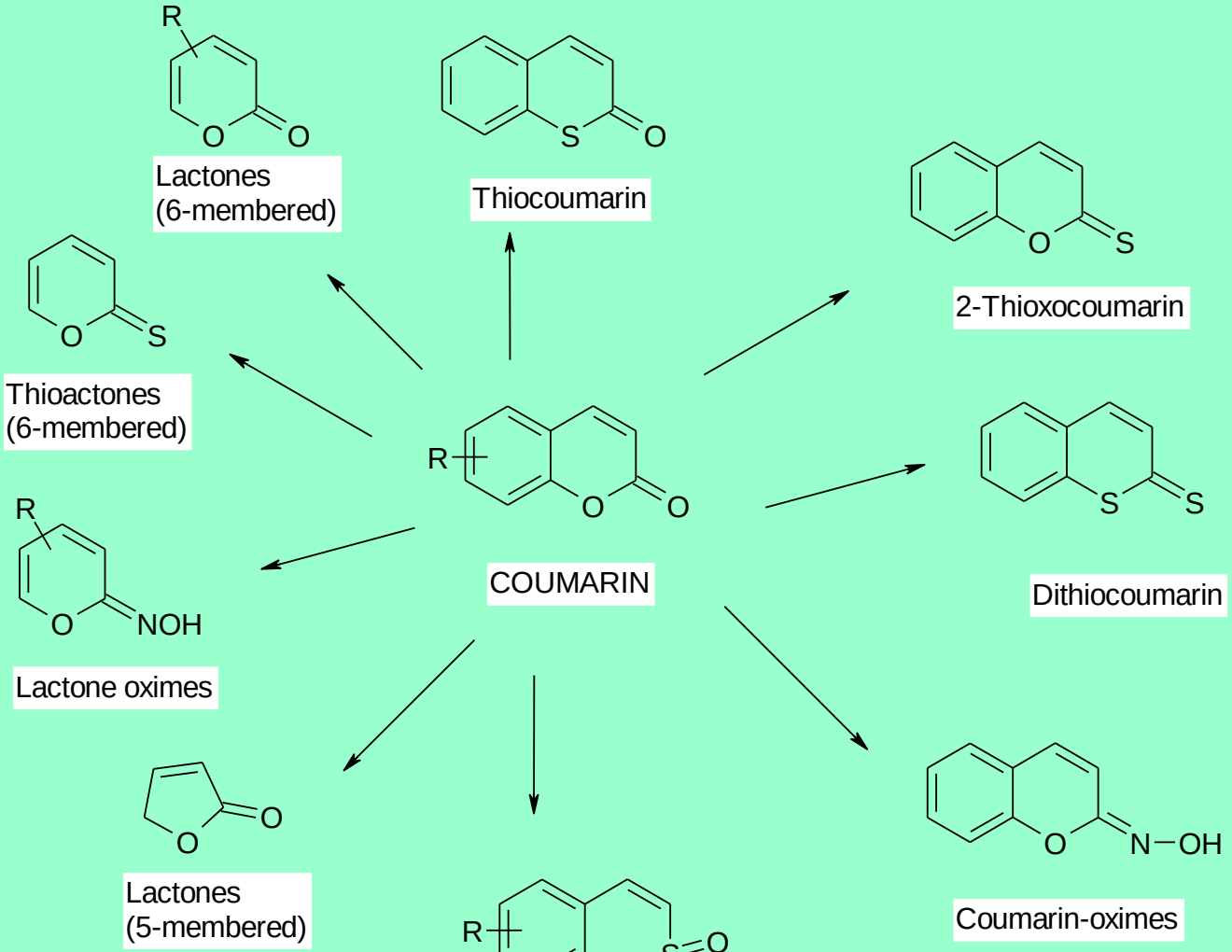


Fo-Fc omit map of **6-hydroxyl-2-thioxocoumarin 8a** and water molecules within the hCA II active site in the hCA II – **8a** adduct; **B**: Tilted view of the electron density of 8a and water molecules within the hCA II active site. Ferraroni et al. *J Med Chem* **2016**, *59*, 462-73.



Superposition of the hCA II - **8a** adduct (sky blue, 4WL4) with the hCA II - hydrolyzed coumarin adduct (5BNL) (silver). The zinc ion, its three His ligands and amino acid residues involved in the binding of inhibitors are shown. Ferraroni et al. *J Med Chem* **2016**, *59*, 462-73.

The COUMARINS and their derivatives



10
different
new classes of CAIs

Chemical diversity generated using coumarins as lead (PRODRUG CAIs)

Journal of Medicinal Chemistry

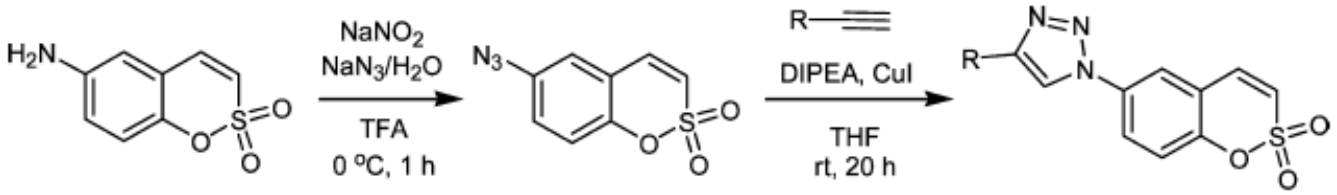
dx.doi.org/10.1021/jm301625s | J. Med. Chem. 2013, 56, 293−300

Article

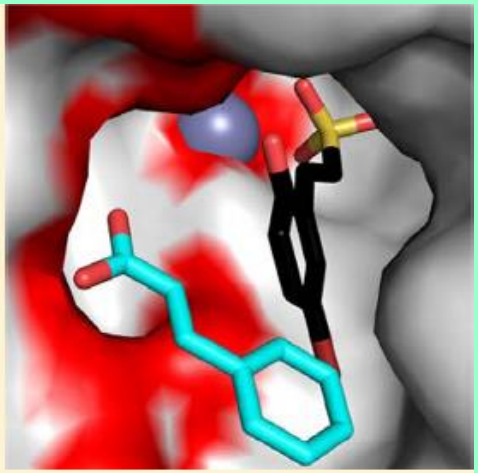
pubs.acs.org/jmc

Sulfocoumarins (1,2-Benzoxathiine-2,2-dioxides): A Class of Potent and Isoform-Selective Inhibitors of Tumor-Associated Carbonic Anhydrases

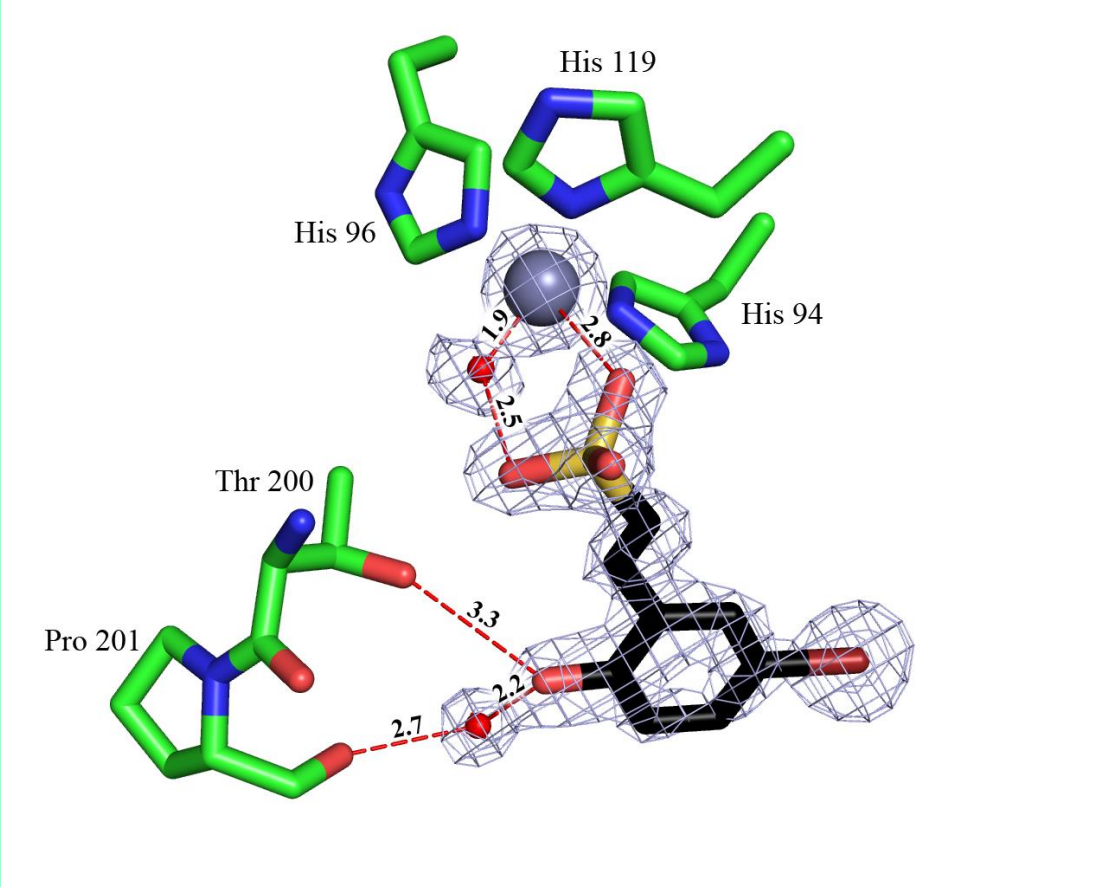
Kaspars Tars,[†] Daniela Vullo,[‡] Andris Kazaks,[†] Janis Leitans,[†] Alons Lends,[§] Aiga Grandane,[§] Raivis Zalubovskis,*[,§] Andrea Scozzafava,[‡] and Claudiu T. Supuran*[,‡,∥]



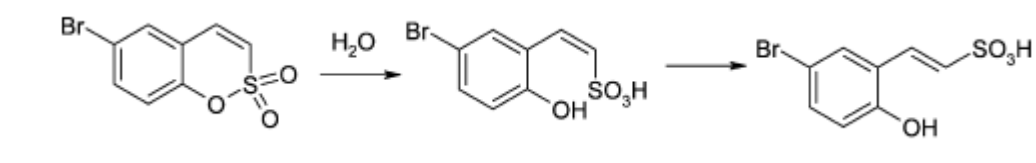
ABSTRACT: Coumarins were recently shown to constitute a novel class of mechanism-based carbonic anhydrase (CA, EC 4.2.1.1) inhibitors. We demonstrate that sulfocoumarins (1,2-benzoxathiine 2,2-dioxides) possess a similar mechanism of action, acting as effective CA inhibitors. The sulfocoumarins were hydrolyzed by the esterase CA activity to 2-hydroxyphenyl-vinylsulfonic acids, which thereafter bind to the enzyme in a region rarely occupied by other classes of inhibitors. The X-ray structure of one of these compounds in adduct with a modified CA II enzyme possessing two amino acid residues from the CA IX active site, allowed us to decipher the inhibition mechanism. The sulfonic acid was observed anchored to the zinc-coordinated water molecule, making favorable interactions with Thr200 and Pro201. Some other sulfocoumarins incorporating substituted-1,2,3-triazole moieties were prepared by using click chemistry and showed low nanomolar inhibitory action against the tumor-associated isoforms CA IX and XII, being less effective against the cytosolic CA I and II.



Sulfocoumarins as CAIs (J Med Chem 2013)



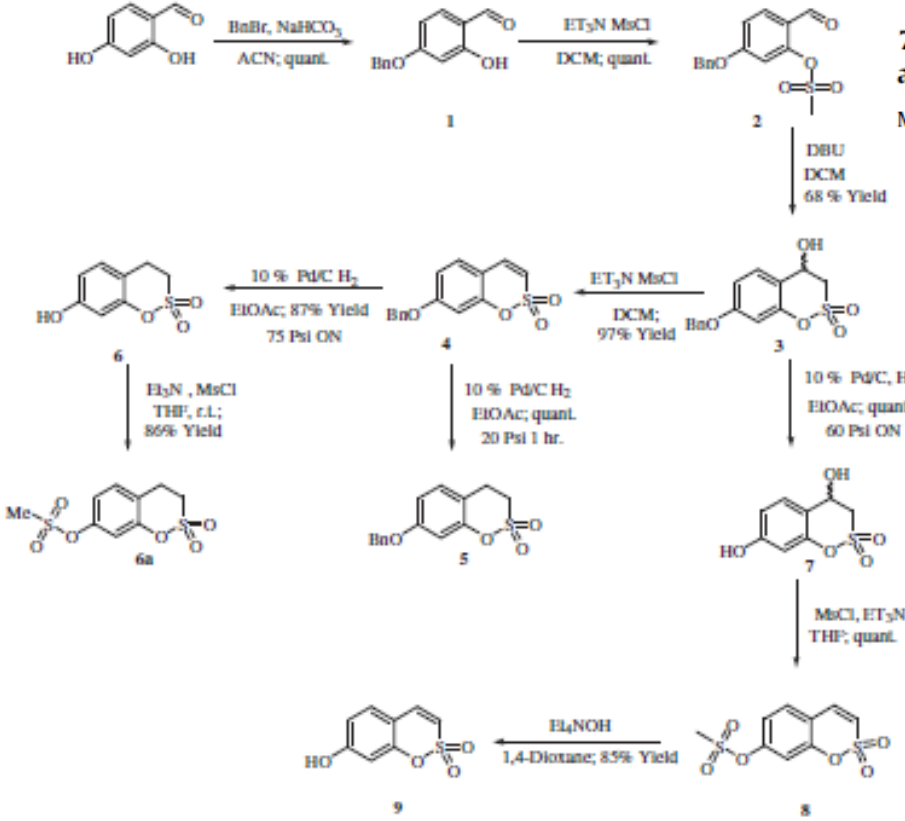
Anchoring to the zinc-coord water



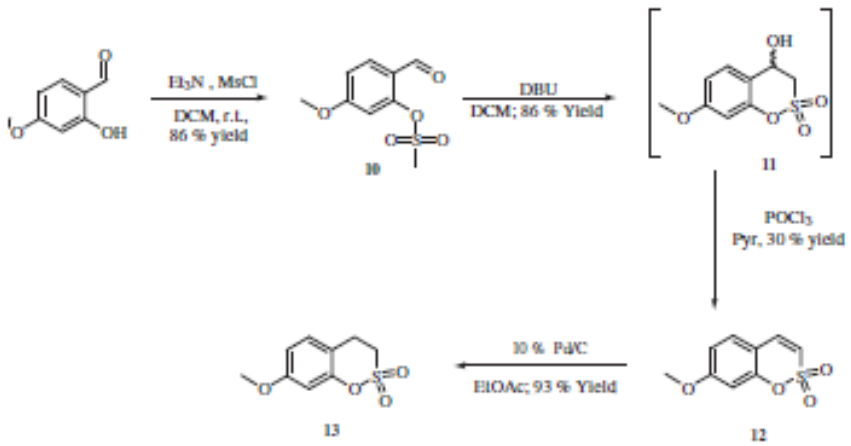
6-substituted sulfocoumarins are CA IX/XII – selective inhibitors

7-Substituted-sulfocoumarins are isoform-selective, potent carbonic anhydrase II inhibitors

Muhammet Tanc [a,b], Fabrizio Carta [b], Murat Bozdag [b], Andrea Scozzafava [b], Claudiu T. Supuran [a,b,*]



Scheme 1. Synthesis of sulfocoumarins **3**–**9**.



Scheme 2. Synthesis of sulfocoumarins **12** and **13**.

Table 1
hCA I, II, VA, IX and XII inhibition data with sulfocoumarins and their derivatives of types **3**–**19** synthesized in this work, by a stopped-flow CO_2 hydrase assay.[16] The sulfonamide inhibitor acetazolamide (**AAZ**, 5-acetamido-1,3,4-thiadiazole-2-sulfonamide) was used as standard

Compound	K_I^* (nM)				
	hCA I	hCA II	hCA VA	hCA IX	hCA XII
3	>10000	2.3	591	>10000	>10000
4	>10000	2.5	835	>10000	>10000
5	>10000	2.0	2150	>10000	>10000
6	>10000	7.6	327	>10000	>10000
6a	>10000	1.8	517	>10000	>10000
7	>10000	1.6	5100	>10000	>10000
8	>10000	2.1	5090	>10000	>10000
9	>10000	8.4	788	>10000	>10000
12	>10000	1.5	9960	>10000	>10000
13	>10000	2.4	91	>10000	>10000
14	>10000	1.5	1675	>10000	>10000
15	>10000	2.2	206	>10000	>10000
16	>10000	3.4	141	>10000	>10000
17	>10000	2.2	716	>10000	>10000
18	>10000	2.6	3910	>10000	>10000
19	>10000	1.9	7060	>10000	>10000
AAZ	250	12	64	25	5.7

Primary sulfonamide CAIs in clinical use since 1954:

Classical use:

1. Diuretics
2. Antiglaucoma systemic drugs

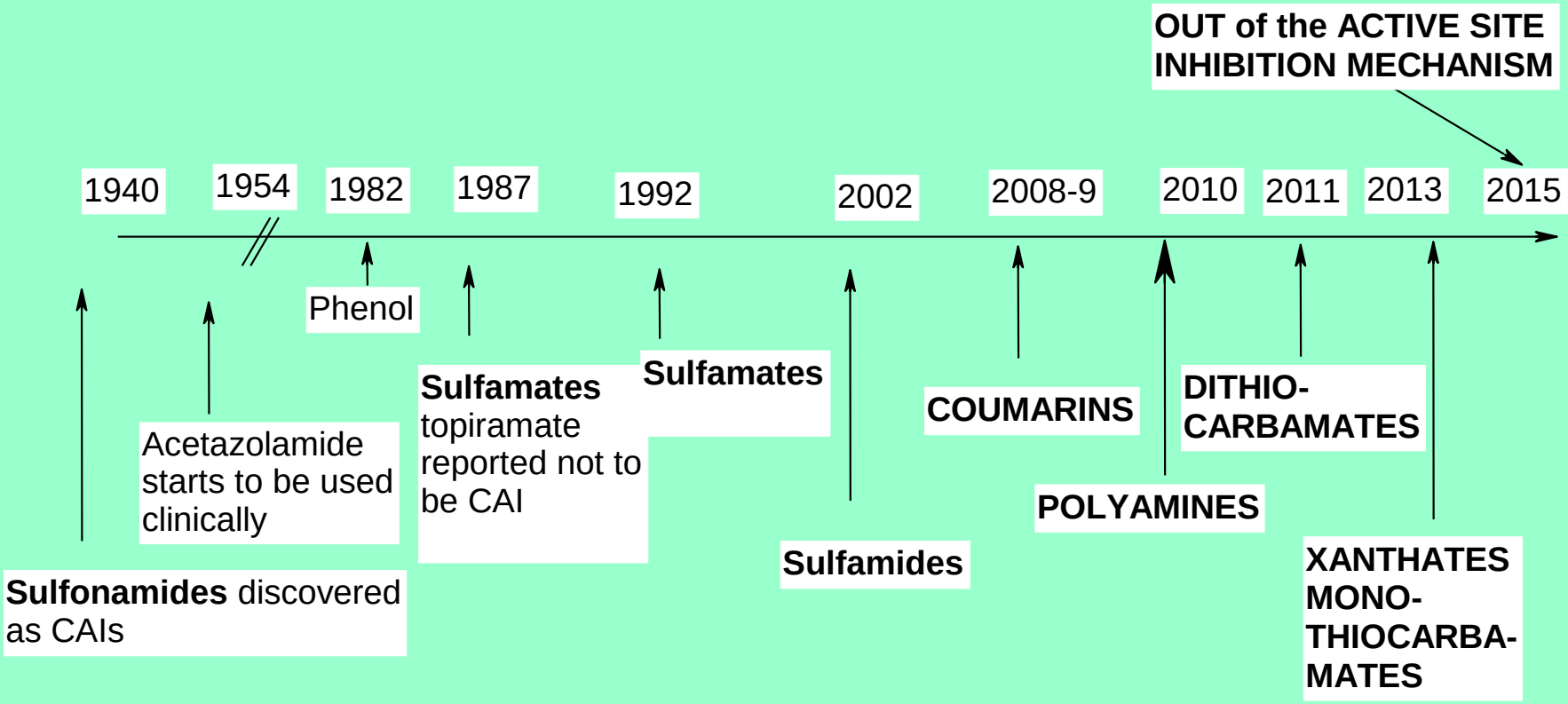
"Modern" use/applications:

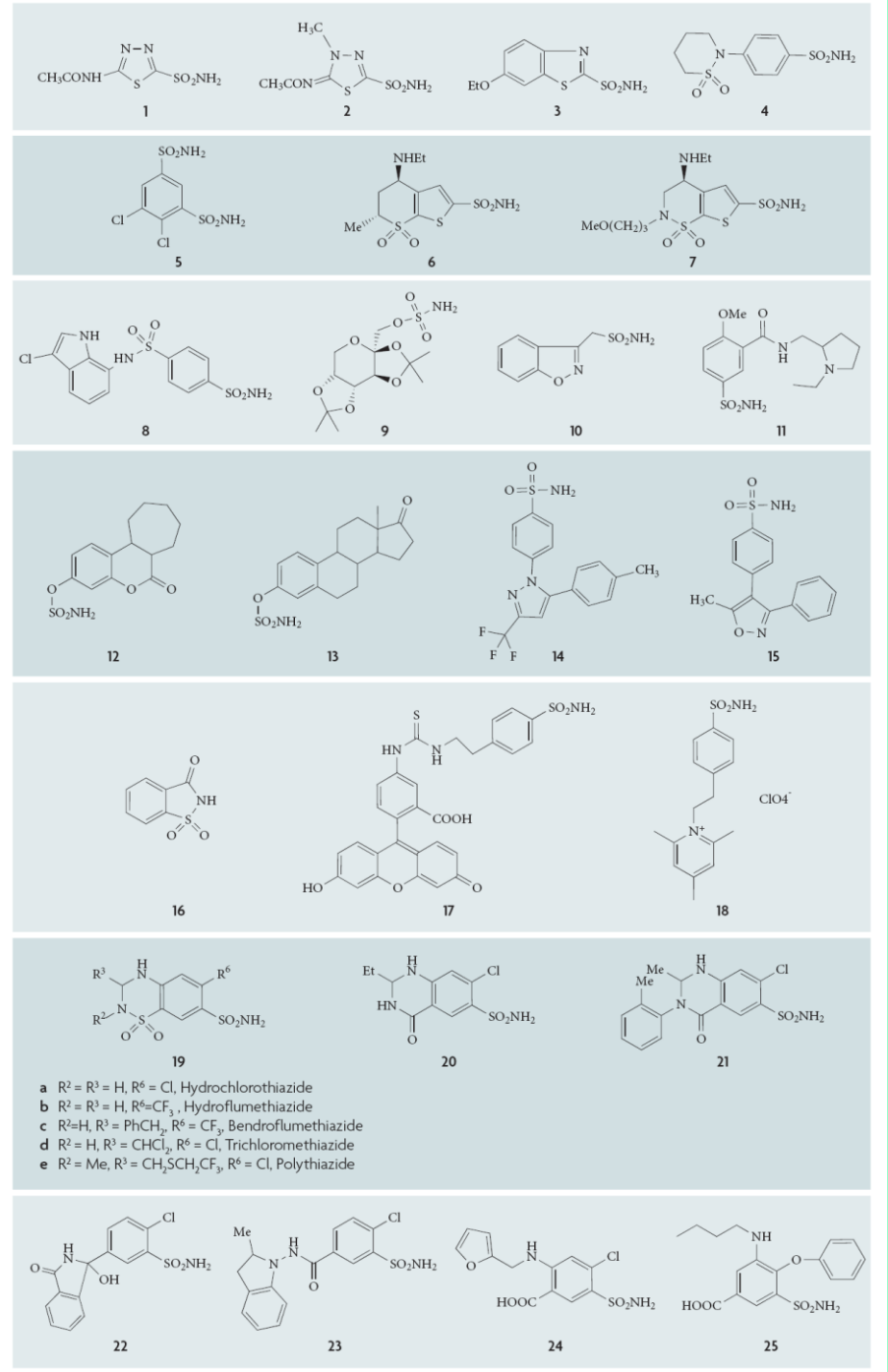
1. Topical antiglaucoma drugs
2. Anticonvulsants/antiepileptics
3. Antiobesity agents
4. Antitumor therapies/diagnostic tools
5. Agents for the treatment of neuropathic pain

Different isozymes are targeted by such drugs

Inhibitors design – novel classes of CAIs

Hystoric overview on CAI drug discovery





Sulfonamides/sulfamates used clinically (more than 30 compounds)

Supuran, CT. *Nature Rev Drug Discov* **2008**, *7*, 168-181

Table 1 | **Inhibition data with selected sulphonamides/sulphamates/sulphamides 1–25 against isozymes I–XIV***

K_I (nm)	Isozyme (h = human, m = mouse)											
	hCA I‡	hCA II‡	hCA III‡	hCA IV‡	hCA VA‡	hCA VB‡	hCA VI‡	hCA VII‡	hCA IX§	hCA XII§	mCA XIII‡	hCA XIV‡
1	250	12	2×10^5	74	63	54	11	2.5	25	5.7	17	41
2	50	14	7×10^5	6,200	65	62	10	2.1	27	3.4	19	43
3	25	8	1×10^6	93	25	19	43	0.8	34	22	50	2.5
4	374	9	6.3×10^5	95	81	91	134	6	43	56	1,450	1,540
5	1,200	38	6.8×10^5	15,000	630	21	79	26	50	50	23	345
6	50,000	9	7.7×10^5	8,500	42	33	10	3.5	52	3.5	18	27
7	45,000	3	1.1×10^5	3,950	50	30	0.9	2.8	37	3.0	10	24
8	31	15	10,400	65	79	23	47	122	24	3.4	11	106
9	250	10	7.8×10^5	4,900	63	30	45	0.9	58‖	3.8	47	1,460
10	56	35	2.2×10^6	8,590	20	6,033	89	117	5.1	11,000	430	5,250
11	12,000	40	10,600	6.5×10^5	174	18	0.8	3,630	46	3.9	295	110
12	3,450	21	7.0×10^5	24	765	720	653	23	34	12	1,050	755
13	37	10	6.5×10^5	NT	NT	NT	NT	NT	30	7.5	NT	NT
14	50,000	21	7.4×10^4	880	794	93	94	2,170	16	18	98	689
15	54,000	43	7.8×10^4	1,340	912	88	572	3,900	27	13	425	107
16	18,540	5,950	1.0×10^6	7,920	10,060	7,210	935	10	103	633	12,100	773
17	1,300	45	1.3×10^6	650	134	76	145	18	24	5	76	33
18	4,000	21	3.1×10^5	60	88	70	65	15	14	7	21	13
19a	328	290	7.9×10^5	427	4,225	603	3,655	5,010	367	355	3,885	4,105
20	35,000	1,260	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
21	54,000	2,000	6.1×10^5	216	750	312	1,714	2.1	320	5.4	15	5,432
22	348	138	1.1×10^4	196	917	9	1,347	2.8	23	4.5	15	4,130
23	51,900	2,520	2.3×10^5	213	890	274	1,606	0.23	36	10	13	4,950
24	62	65	3.2×10^6	564	499	322	245	513	420	261	550	52
25	4,930	6,980	3.4×10^6	303	700	NT	NT	NT	25.8	21.2	2,570	250

*The isoforms CA VIII, X and XI are devoid of catalytic activity and probably do not bind sulphonamides as they do not contain Zn^{2+} ions. ‡Full-length enzyme. §Catalytic domain. ‖The data against the full-length enzyme is of 1,590 nM. NT, not tested, data not available.

Supuran, CT. *Nature Rev Drug Discov* **2008**, *7*, 168-181

Neri, Supuran
Nature Rev. Drug Discov. **2011**, 10, 767

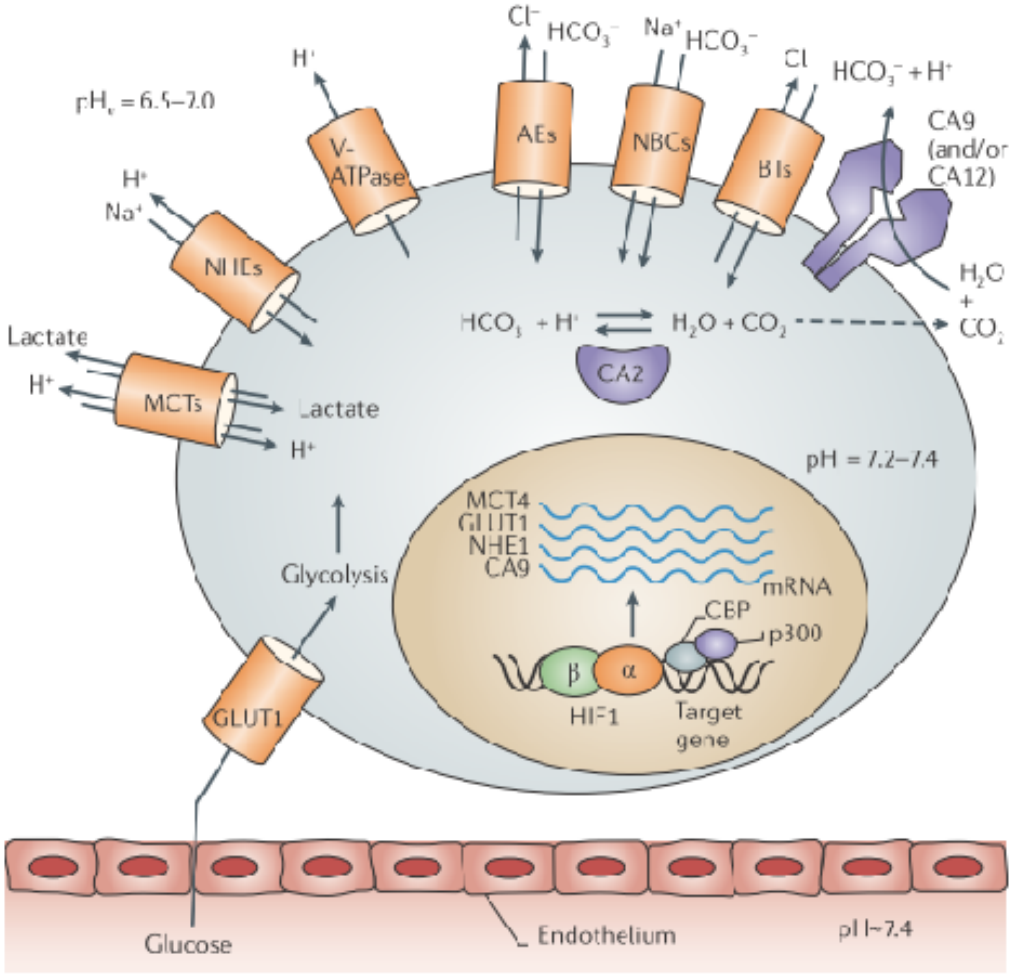


Figure 1 | **Proteins involved in pH regulation within a tumour cell.** The figure shows various proteins that are involved in regulating pH within tumours, including: monocarboxylate transporters (MCTs), which transport lactic acid and other monocarboxylates formed by the glycolytic degradation of glucose; Na^+/H^+ exchangers (NHEs); the plasma membrane proton pump vacuolar ATPase (V-ATPase); anion exchangers (AEs); Na^+/HCO_3^- co-transporters (NBCs); and carbonic anhydrases (CA2, CA9 and/or CA12). The glucose transporter GLUT1 (which is upregulated in most tumours) transports glucose into tumour cells. The intracellular tumour pH (pH_i) is slightly alkaline (pH 7.2–7.4), whereas the extracellular pH (pH_e) is slightly acidic (pH 6.5–7.0). BT, HCO_3^- transporter; CBP, cyclic AMP-responsive element-binding (CREB) protein; HIF1, hypoxia-inducible transcription factor 1; p300, histone acetyltransferase p300.

REVIEWS

nature REVIEWS

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Interfering with pH regulation in tumours as a therapeutic strategy

Daric Neri and Claudiu T. Supuran‡*

Abstract | The high metabolic rate of tumours often leads to acidosis and hypoxia in poorly perfused regions. Tumour cells have thus evolved the ability to function in a more acidic environment than normal cells. Key pH regulators in tumour cells include: isoforms 2, 9 and 12 of carbonic anhydrase, isoforms of anion exchangers, Na^+/HCO_3^- co-transporters, Na^+/H^+ exchangers, monocarboxylate transporters and the vacuolar ATPase. Both small molecules and antibodies targeting these pH regulators are currently at various stages of clinical development. These antitumour mechanisms are not exploited by the classical cancer drugs and therefore represent a new anticancer drug discovery strategy.

Warburg effect
The ability of cancer cells to predominantly produce energy by a high rate of glycolysis followed by lactic acid fermentation in the cytosol, rather than by glycolysis followed by oxidation of pyruvate in the mitochondria, as most normal cells do.

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doi: 10.1038/nrd3554
Published online
16 September 2011

The growth of solid tumours is characterized not only by the uncontrolled proliferation of cancer cells but also by changes in the tumour environment that support the growth of the neoplastic mass and the metastatic spread of cancer cells to distant sites[1]. The formation of new blood vessels from pre-existing ones (angiogenesis) provides oxygen and nutrients to the tumour, which are essential for tumour growth[2]. A complex dynamic interplay exists between the expanding neoplastic mass and the tumour environment, which is affected by the metabolic requirements of cancer cells and by their products, such as secreted proteins (for example, extracellular matrix components and proteases) and metabolites.

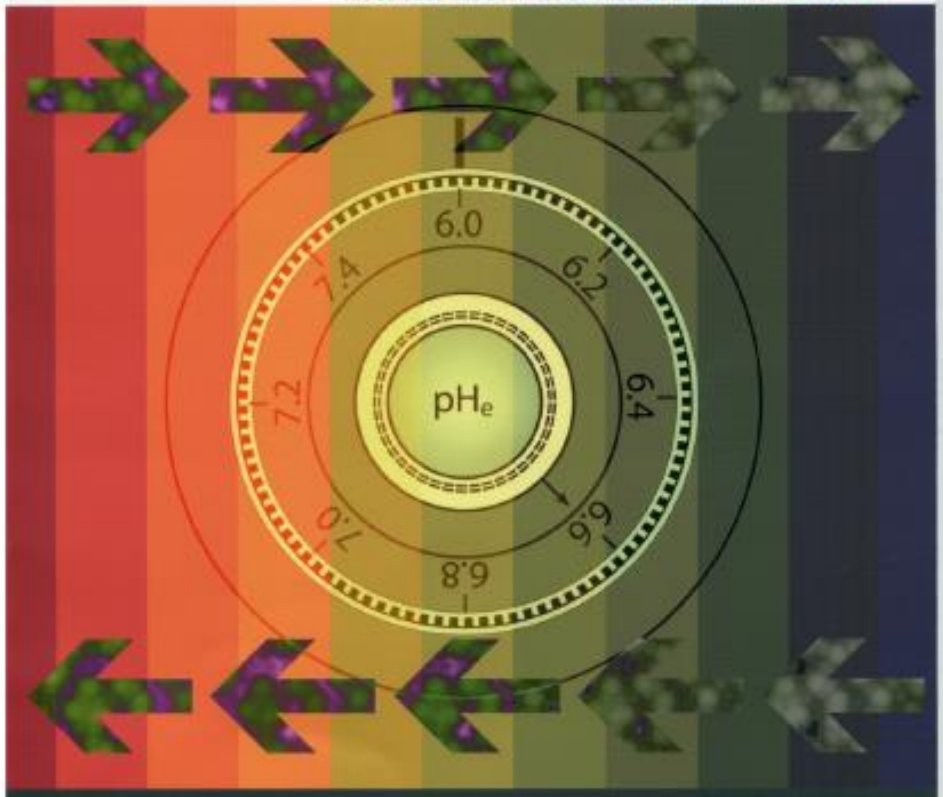
Upregulated glucose metabolism is a hallmark of invasive cancers[3]. In normal cells glucose is converted to glucose-6-phosphate and subsequently to pyruvate, which is then oxidized in the mitochondria to carbon dioxide and water; this releases 38 moles of ATP per mole of glucose[3]. However, inadequate oxygen delivery to some regions of tumours leads to hypoxia, which restricts oxidative phosphorylation. As a consequence, hypoxic tumour cells shift their metabolism towards glycolysis so that the pyruvate generated in the first step of the process is reduced to lactate, generating only 2 moles of ATP per mole of glucose. This is less energy-efficient process but it does not depend on oxygen. Furthermore, glycolysis often persists even after reoxygenation because the obtained metabolic intermediates (that is, lactate and pyruvate) can be used for the biosynthesis of amino acids, nucleotides and lipids, thus providing a selective advantage to proliferating tumour cells. This explains Warburg's observation of high glucose consumption and high lactate production in tumour tissues[3–5] (known as the Warburg effect).

Oncogenic metabolism also generates an excess of protons and carbon dioxide, which are kept in equilibrium with carbonic acid by the enzyme carbonic anhydrase[6–7]. Thus, increased glucose metabolism in tumour cells leads to enhanced acidification of the extracellular milieu, which is frequently accompanied by various levels of hypoxia. This phenotype confers a substantial Darwinian growth advantage to tumour cells over normal cells, which undergo apoptosis in response to such an acidic extracellular environment[1].

Tumour cells have evolved various mechanisms to cope with the acidic and hypoxic stress mentioned above. They eliminate acidic catabolites by ion transporters and pumps to preserve a slightly alkaline intracellular pH (pH_i), which is optimal for cell proliferation and tumour survival[8–10]. Acid export leads to a reduction in the extracellular pH (pH_e) to values as low as 6.0 (the usual pH_e in tumours is in the range of 6.5–7.0)[8], which is a salient feature of the tumour microenvironment[1,8]. As well as triggering the overexpression of many proteins involved in glucose metabolism — such as the glucose transporter GLUT1 (also known as SLC2A1) and pH-regulating proteins such as carbonic anhydrase 9 (CA9)[3,4,10] — hypoxia also constitutes a detrimental feature for radiotherapy, as oxygen is needed to oxidize the radiation-induced DNA free radicals that subsequently lead to tumour cell death[4].

pH homeostasis in any cell type is a complicated process that involves many proteins and buffer systems[4]. In tumour cells, these processes are even more complex owing to the internal compartment being slightly more alkaline (pH 7.4 or more) and the external compartment being more acidic than in normal cells[4–8]. A variation

DRUG DISCOVERY
THE SCIENCE AND BUSINESS OF DRUG DISCOVERY AND DEVELOPMENT

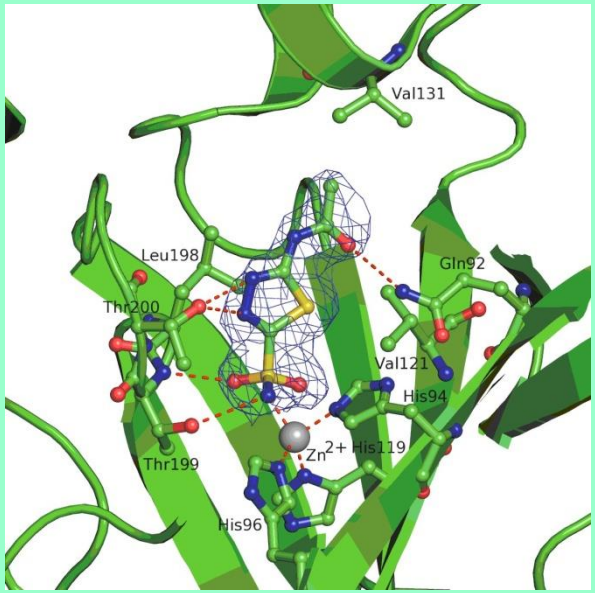


ANTICANCER TARGETS
Interfering with tumour pH regulation as a therapeutic strategy

Compound quality
The influence of the 'organizational factor' in drug design

The X-Ray structure of hCA IX reported by our

PNAS | September 22, 2009 | vol. 106 | no. 38 | 16233–16238

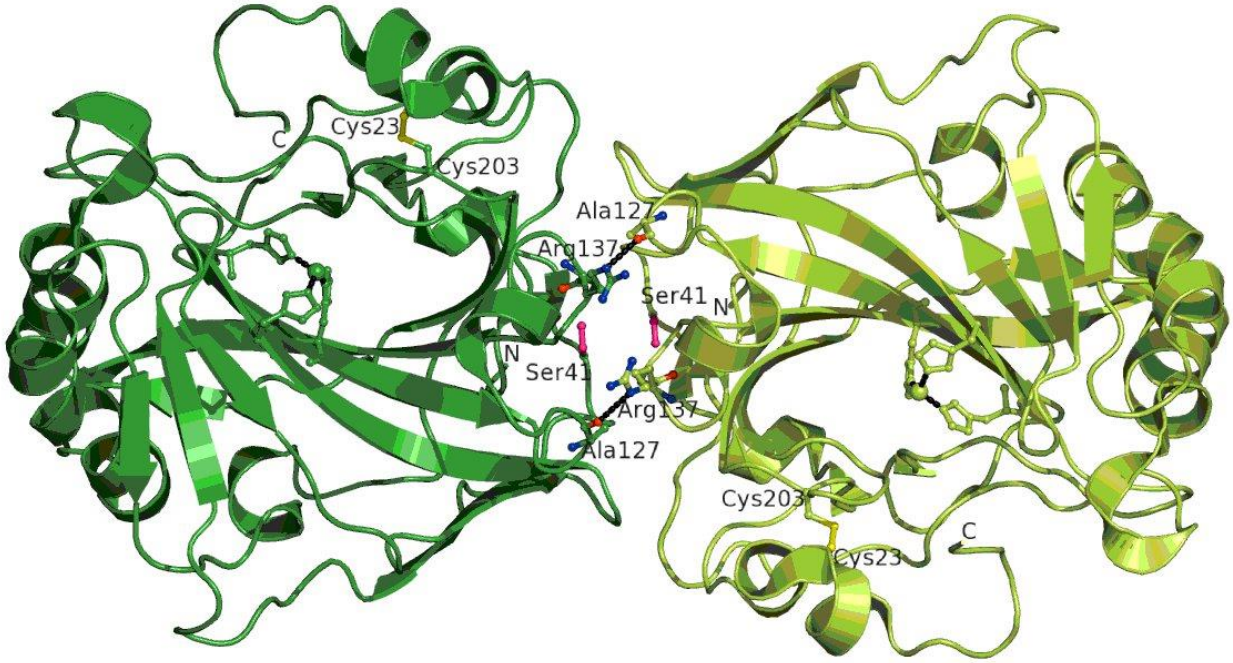


Crystal structure of the catalytic domain of the tumor-associated human carbonic anhydrase IX

Vincenzo Alterio[a,1], Mika Hilvo[b,c,1], Anna Di Fiore[a], Claudiu T. Supuran[d,2], Pelwen Pan[b], Seppo Parkkila[b], Andrea Scaloni[e], Jaromir Pastorek[f], Silvia Pastorekova[f], Carlo Pedone[a], Andrea Scozzafava[d], Simona Maria Monti[a,2], and Giuseppina De Simone[a,2]

hCA IX dimer

hCA IX-acetazolamide adduct



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Therapeutics, Targets, and Chemical Biology

Cancer Research

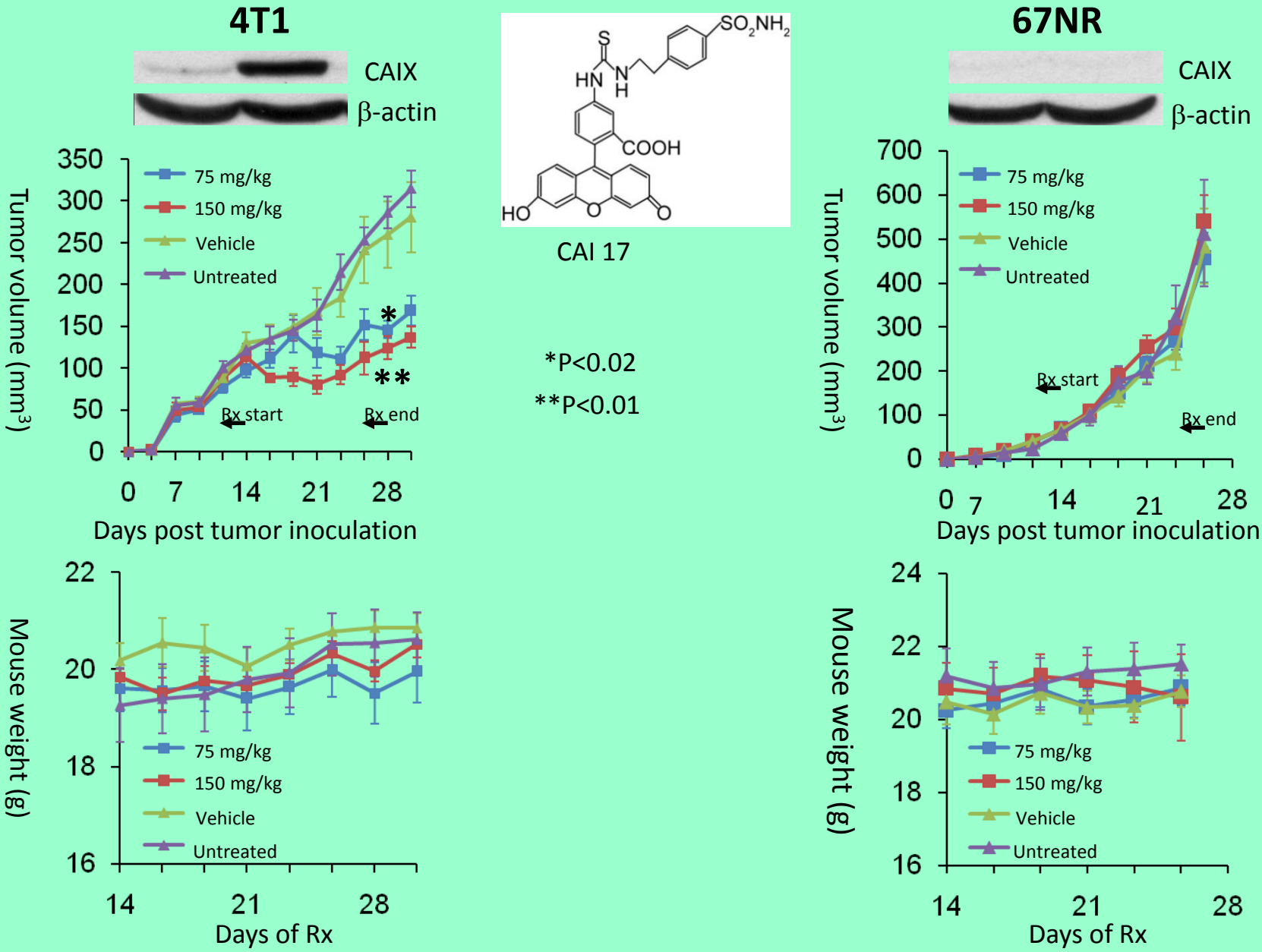
Targeting Tumor Hypoxia: Suppression of Breast Tumor Growth and Metastasis by Novel Carbonic Anhydrase IX Inhibitors

Yuanmei Lou[1], Paul C. McDonald[1], Arusha Oloumi[1], Stephen Chia[2], Christina Ostlund[1], Ardalan Ahmadi[1], Alastair Kyle[1], Ulrich auf dem Keller[5], Samuel Leung[7], David Huntsman[3,7], Blaise Clarke[7,8], Brent W. Sutherland[4], Dawn Waterhouse[4], Marcel Bally[4], Calvin Roskelley[6], Christopher M. Overall[5], Andrew Minchinton[1], Fabio Pacchiano[9], Fabrizio Carta[9], Andrea Scozzafava[9], Nadia Touisni[10], Jean-Yves Winum[10], Claudiu T. Supuran[9], and Shoukat Dedhar[1,5]

Abstract

Carbonic anhydrase IX (CAIX) is a hypoxia and HIF-1–inducible protein that regulates intra- and extracellular pH under hypoxic conditions and promotes tumor cell survival and invasion in hypoxic microenvironments. Interrogation of 3,630 human breast cancers provided definitive evidence of CAIX as an independent poor prognostic biomarker for distant metastases and survival. shRNA-mediated depletion of CAIX expression in 4T1 mouse metastatic breast cancer cells capable of inducing CAIX in hypoxia resulted in regression of orthotopic mammary tumors and inhibition of spontaneous lung metastasis formation. Stable depletion of CAIX in MDA-MB-231 human breast cancer xenografts also resulted in attenuation of primary tumor growth. CAIX depletion in the 4T1 cells led to caspase-independent cell death and reversal of extracellular acidosis under hypoxic conditions *in vitro*. Treatment of mice harboring CAIX-positive 4T1 mammary tumors with novel CAIX-specific small molecule inhibitors that mimicked the effects of CAIX depletion *in vitro* resulted in significant inhibition of tumor growth and metastasis formation in both spontaneous and experimental models of metastasis, without inhibitory effects on CAIX-negative tumors. Similar inhibitory effects on primary tumor growth were observed in mice harboring orthotopic tumors comprised of lung metatstatic MDA-MB-231 LM2-4^{Luc+} cells. Our findings show that CAIX is vital for growth and metastasis of hypoxic breast tumors and is a specific, targetable

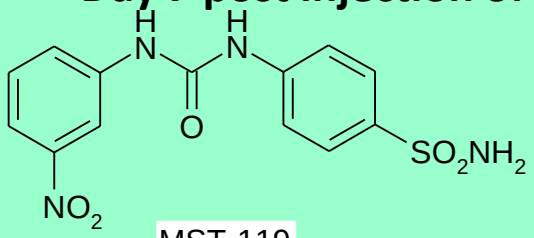
Specific Inhibition of CAIX-positive 4T1 Tumor growth by a CAIX small molecule Inhibitor (Lou et al, *Cancer Res*. **2011**, *71*, 3364-76)



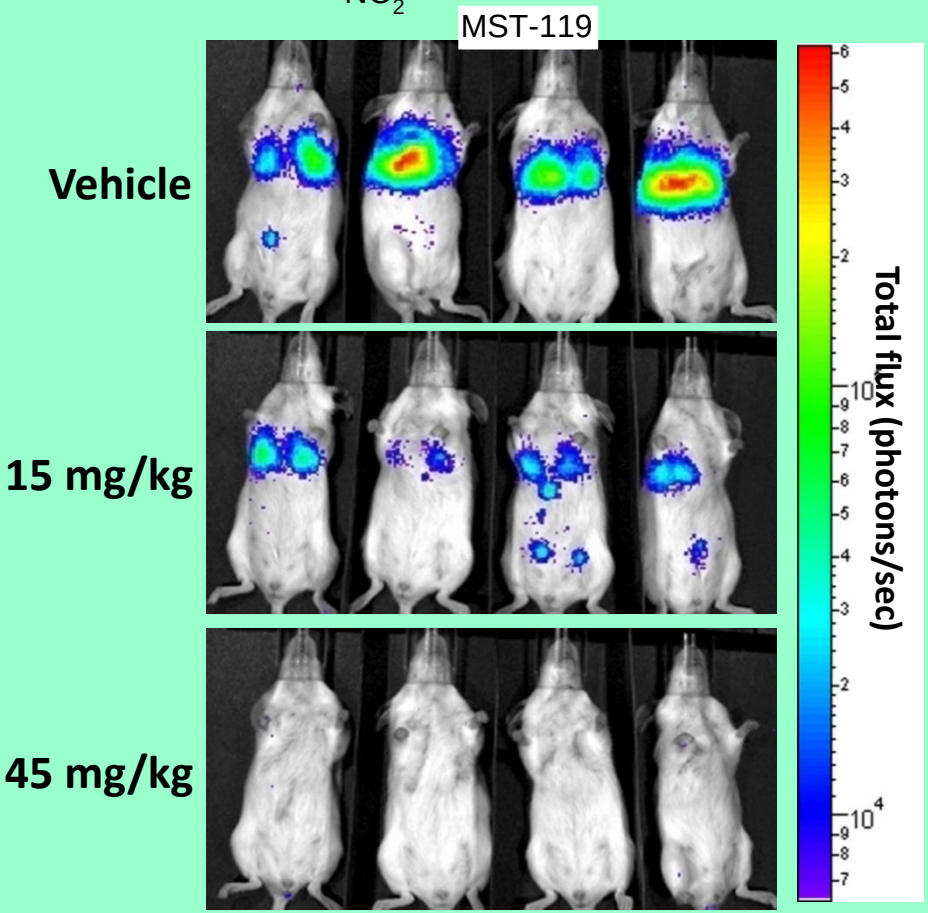
MST-119 inhibits the formation of metastases by 4T1 mammary tumor cells

Day 7 post injection of tumor cells ($5x10^5$ cells/animal)

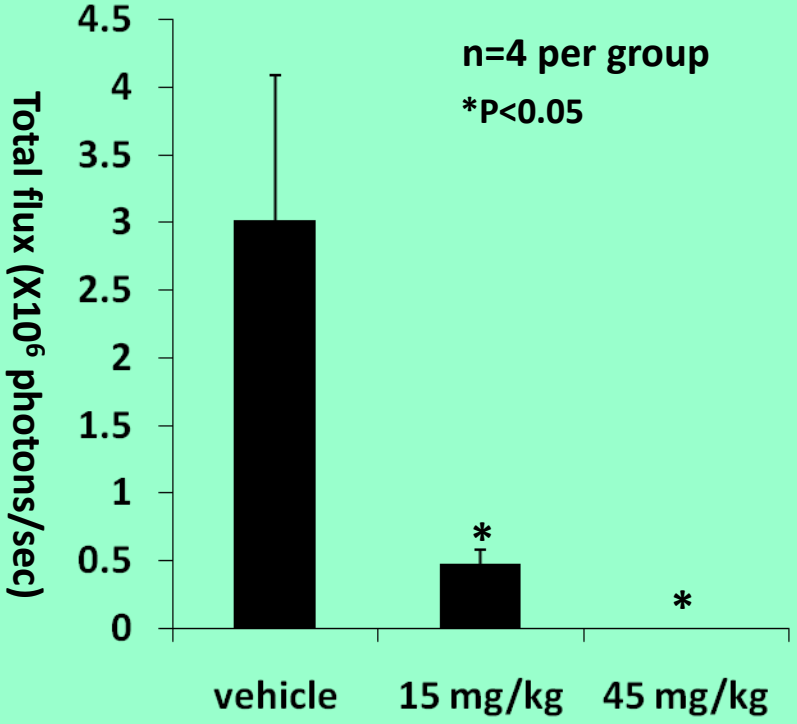
MST-119



Pacchiano et al., J. Med. Chem. 2011, 54, 1896-902.

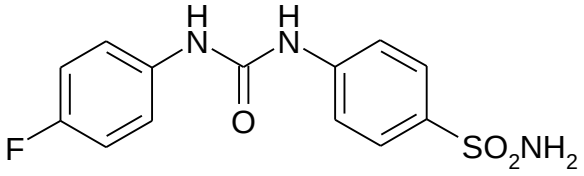


3 doses (eod x 3 i.p.)



A Phase I, multi-center, open-label study to investigate the safety, tolerability and pharmacokinetics of SLC-0111 in subjects with advanced solid tumours was successfully completed (2016)

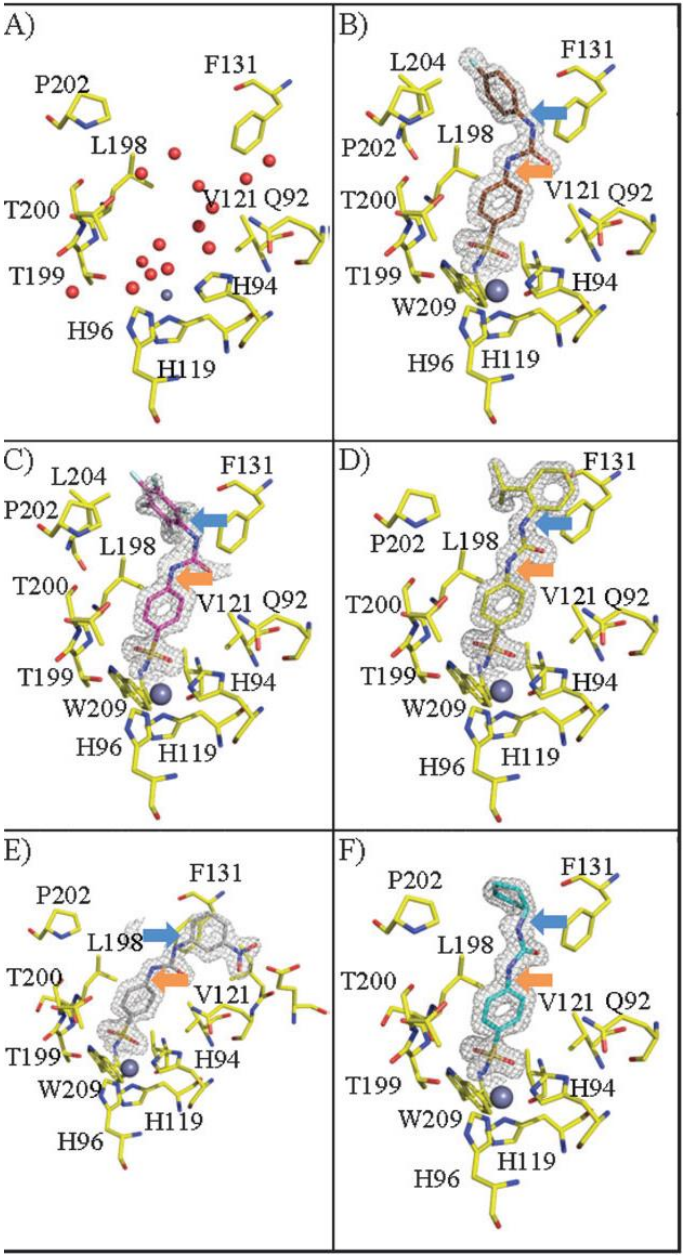
ClinicalTrials.gov Identifier:
NCT02215850
Sponsor Protocol No. SLC0111-14-C01
Ozmosis Study No. OZM-055



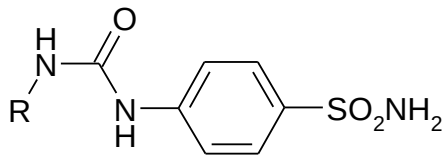
SLC-0111

Sponsor: Wellichem Corporation, Vancouver, Canada

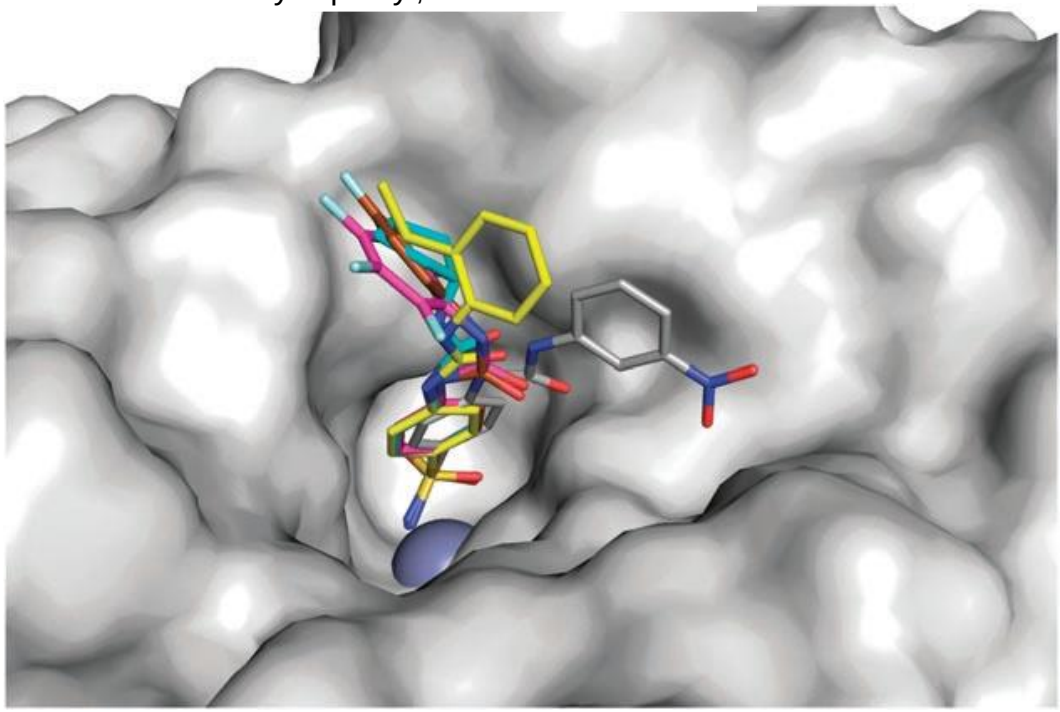
SLC-0111 is presently in Phase Ib clinical trials



Pacchiano et al., *Chem. Commun.* **2010**



1: R = 4-F-C_6H_4 (SLC-0111); Ki = 960 nM
2: R = C_6F_5; Ki = 50 nM
3: R= 2(i-Pr-)-C_6H_4; Ki = 3.3nM
4: R = 3-O_2N-C_6H_4 (U-119); Ki = 15 nM
5: R = cyclopentyl; Ki = 226 nM



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X-ray crystallography-promoted drug design of carbonic anhydrase inhibitors†

Jekaterina Ivanova,‡[a] Janis Leitans,‡[b] Muhammet Tanc,‡[cd] Andris Kazaks,[b] Raivis Zalubovskis,*[a] Claudiu T. Supuran*[cd] and Kaspars Tars[be]

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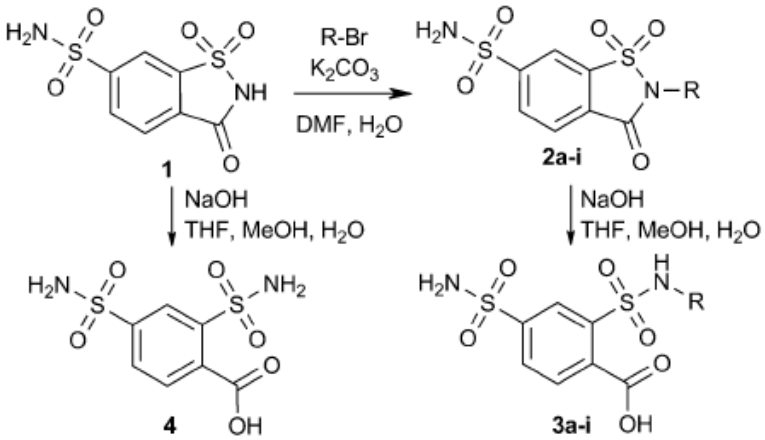


Scheme 1 Synthesis of 1-*N*-substituted 6-sulfamoylsaccharins **2** and their hydrolysis products **3** and **4**.

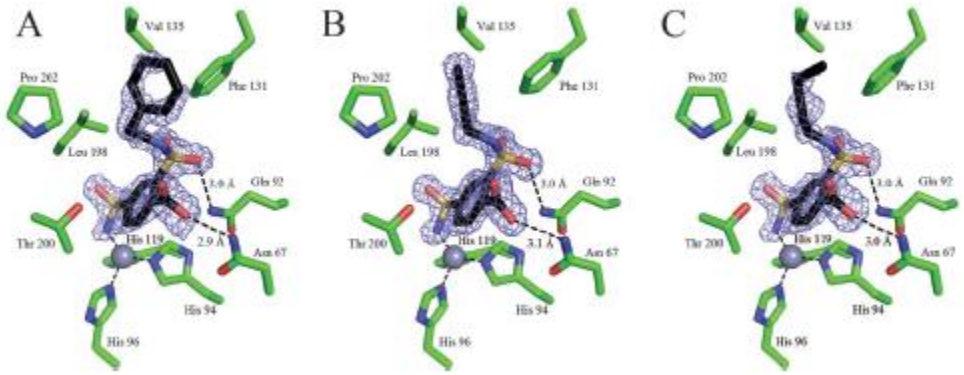
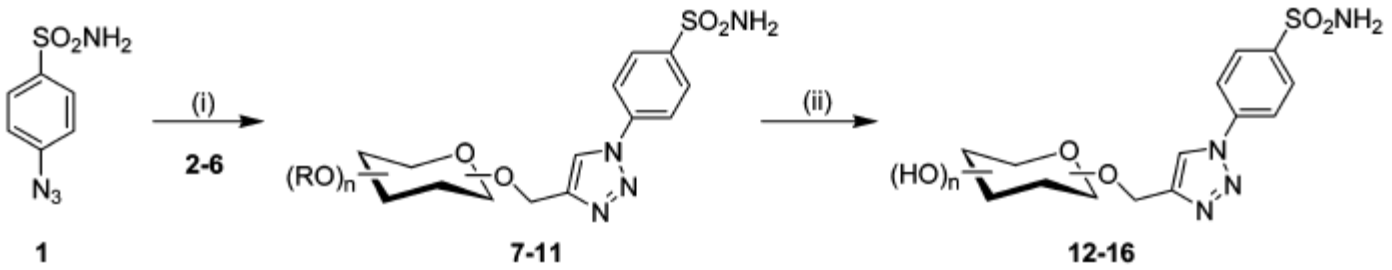


Fig. 2 Comparison of binding modes of compounds **2i**, **2e** and **2d** within the hCA II active site. Compound **2i**/**3i** is shown in panel A, **2e**/**3e** is shown in panel B and **2d**/**3d** is shown in panel C. The zinc ion is the gray sphere and its

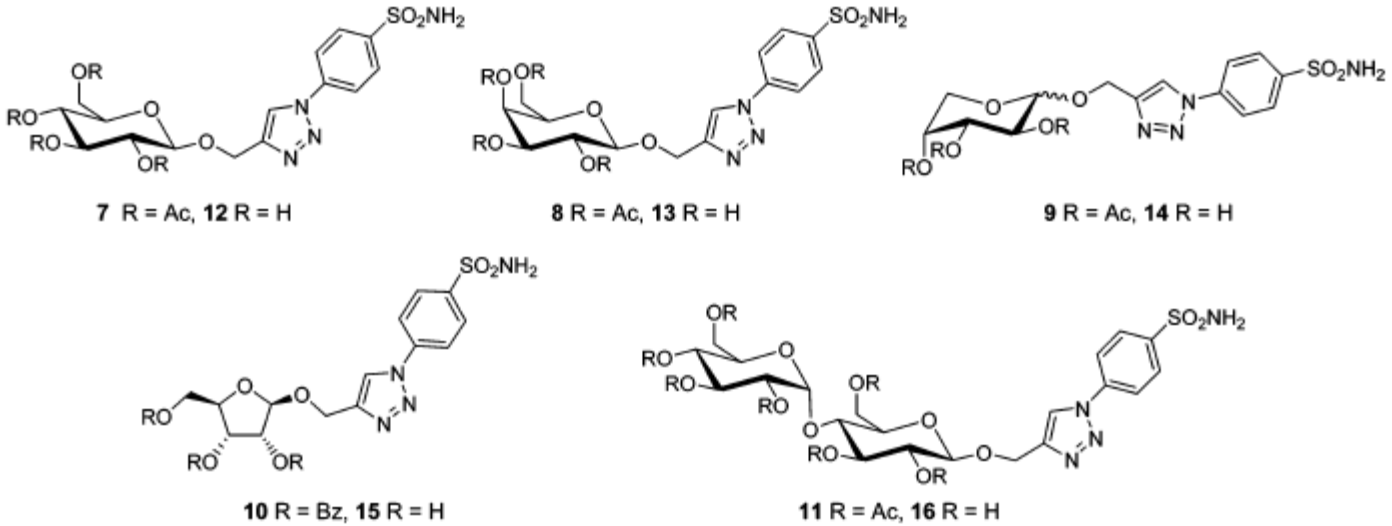
Carbonic Anhydrase Inhibitors: Inhibition of Isozymes I, II, and IX with Triazole-Linked *O*-Glycosides of Benzene Sulfonamides

Brendan L. Wilkinson,[†] Laurent F. Bornaghi,[†] Todd A. Houston,[‡] Alessio Innocenti,[§] Daniela Vullo,[§] Claudiu T. Supuran,*[,§] and Sally-Ann Poulsen*[,†]

Scheme 1[a]



where



[a] Reagents and conditions: (i) azide **1** (0.2−0.5 M), *O*-propynyl glycoside **2**−**6** (1 equiv), $CuSO_4 \cdot 5H_2O$ (0.1−0.2 equiv), sodium ascorbate (0.2−0.4 equiv), 1:1 *t*-BuOH/H_2O, 40 °C, 30 min to 1 h, 77−92%; (R = Ac, Bz) (ii) $NaOCH_3$, CH_3OH, room temperature, 15 min to 2 h, quantitative.

Design and synthesis of thiourea compounds that inhibit transmembrane anchored carbonic anhydrases

Janina Moeker [a], Kanae Teruya [a], Sabine Rossit [a], Brendan L. Wilkinson [a], Marie Lopez [a], Laurent F. Bornaghi [a], Alessio Innocenti [b], Claudiu T. Supuran [b,*], Sally-Ann Poulsen [a,*]

J. Moeker et al. / Bioorg. Med. Chem. 20 (2012) 2392–2404

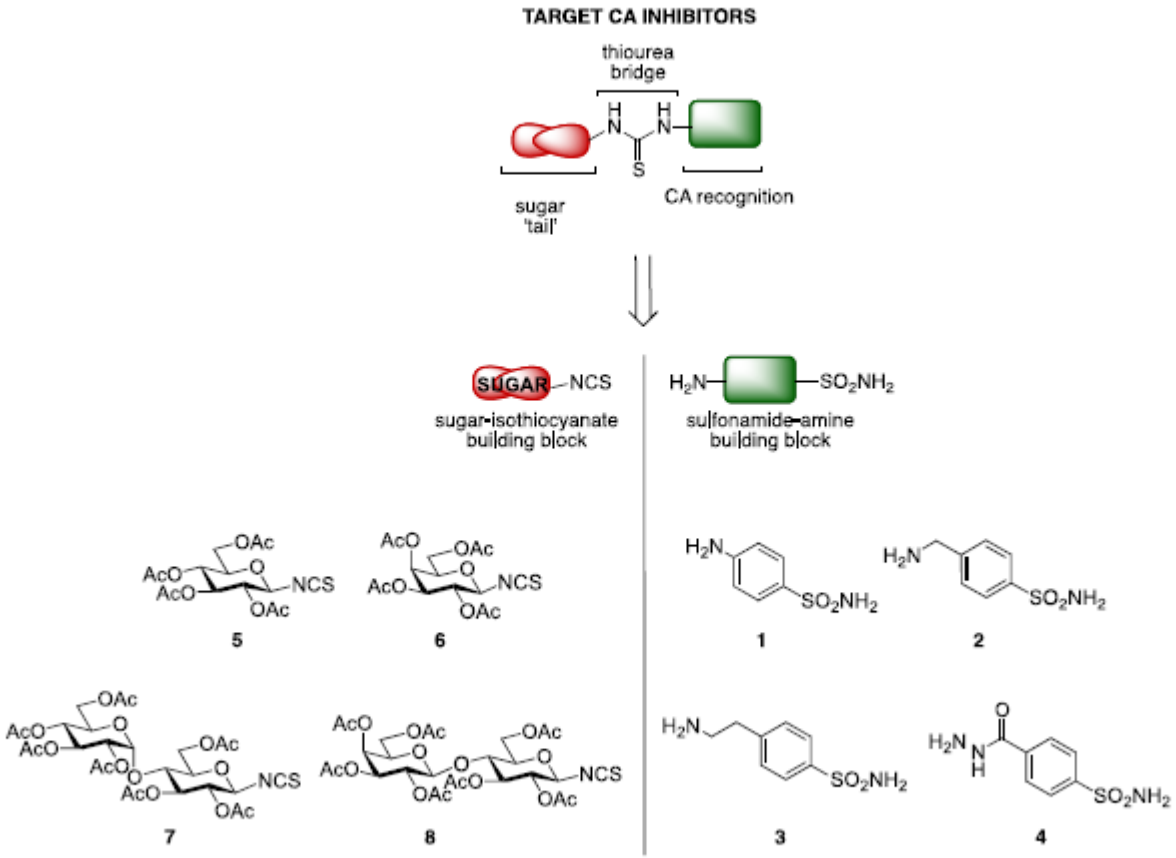


Chart 1. Amino (**1–4**) and isothiocyanate (**5–8**) building blocks used for the synthesis of target thiourea-bridged glycoconjugate CA inhibitors.

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Carbonic Anhydrase Inhibitors with Dual-Tail Moieties To Match the Hydrophobic and Hydrophilic Halves of the Carbonic Anhydrase Active Site

Rajendra P. Tanpure,[†] Bin Ren,[‡] Thomas S. Peat,[‡] Laurent F. Bornaghi,[†] Daniela Vullo,[§] Claudiu T. Supuran,*[,§] and Sally-Ann Poulsen*[,†]

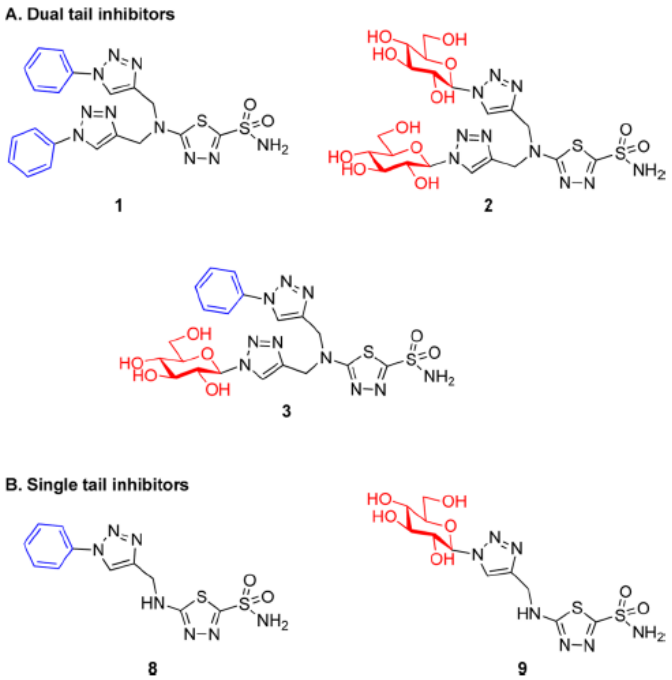
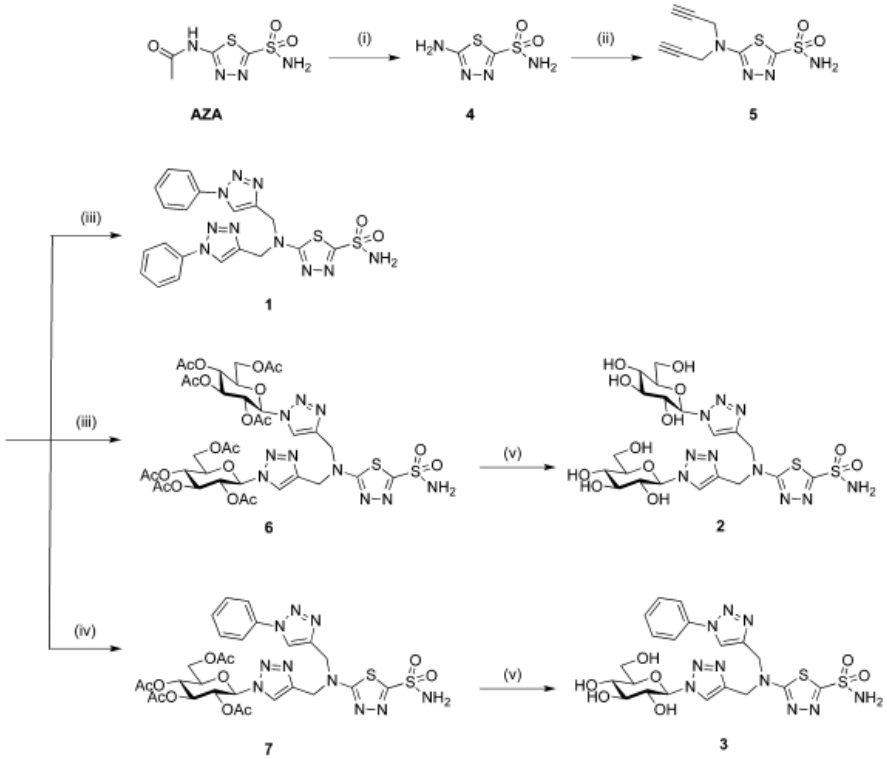


Figure 1. (A) Target CA inhibitors with dual-tail moieties derived from acetazolamide (**AZA**). The tail combinations include (i) two hydrophobic moieties (blue) **1**, (ii) two hydrophilic moieties (red) **2**, and (iii) one hydrophobic (blue) and one hydrophilic (red) moiety **3**. (B) Corresponding single-tail CA inhibitors **8** and **9**.

Scheme 1. Synthetic Route for the Synthesis of Target CA Inhibitors 1–3 with Dual-Tail Moieties[a]



DOI: 10.1021/jm501798g
J. Med. Chem. 2015, 58, 1494−1501

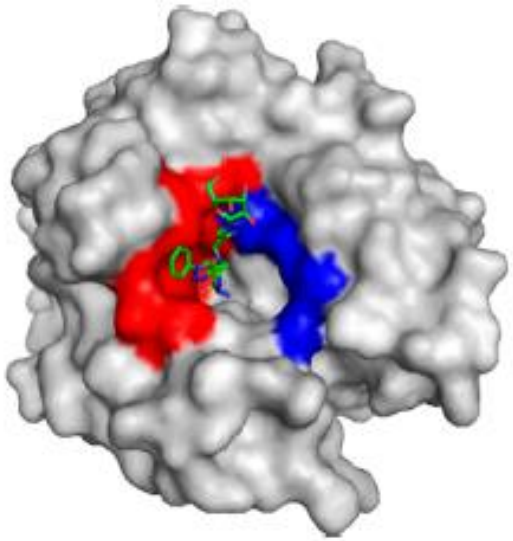


Figure 3. Surface representation of hCA II with the binding of the heterogeneous dual-tailed compound **3**. Hydrophobic half, red; hydrophilic half, blue. The picture was prepared with PyMOL.[23]

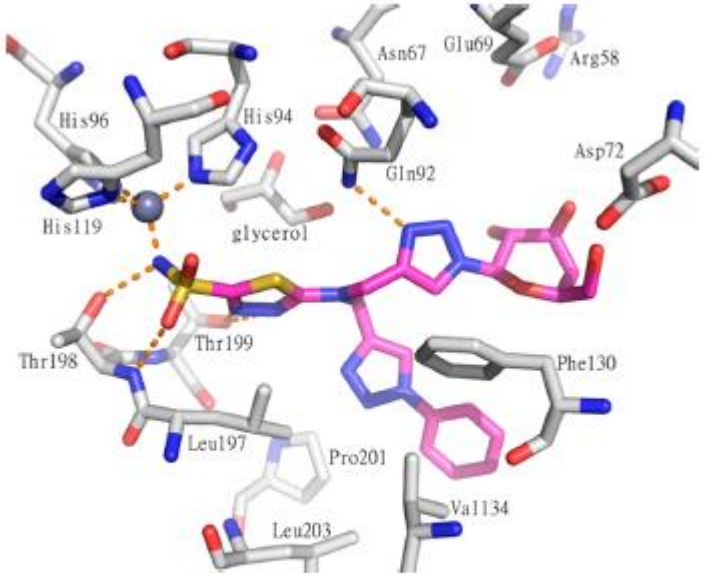


Figure 2. Interactions and environment at the binding site in the structure of hCA II/**3** complex. Hydrogen bonds are shown as broken lines, and the Zinc ion, as a sphere. The picture was produced with PyMOL.[23]

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An Unusual Natural Product Primary Sulfonamide: Synthesis, Carbonic Anhydrase Inhibition, and Protein X-ray Structures of Psammaplin C

Prashant Mujumdar,[†] Kanae Teruya,[†] Kathryn F. Tonissen,[†] Daniela Vullo,[‡] Claudiu T. Supuran,[‡] Thomas S. Peat,[§] and Sally-Ann Poulsen*[,†]

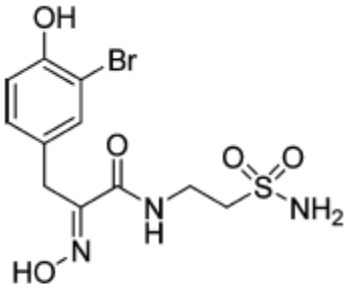
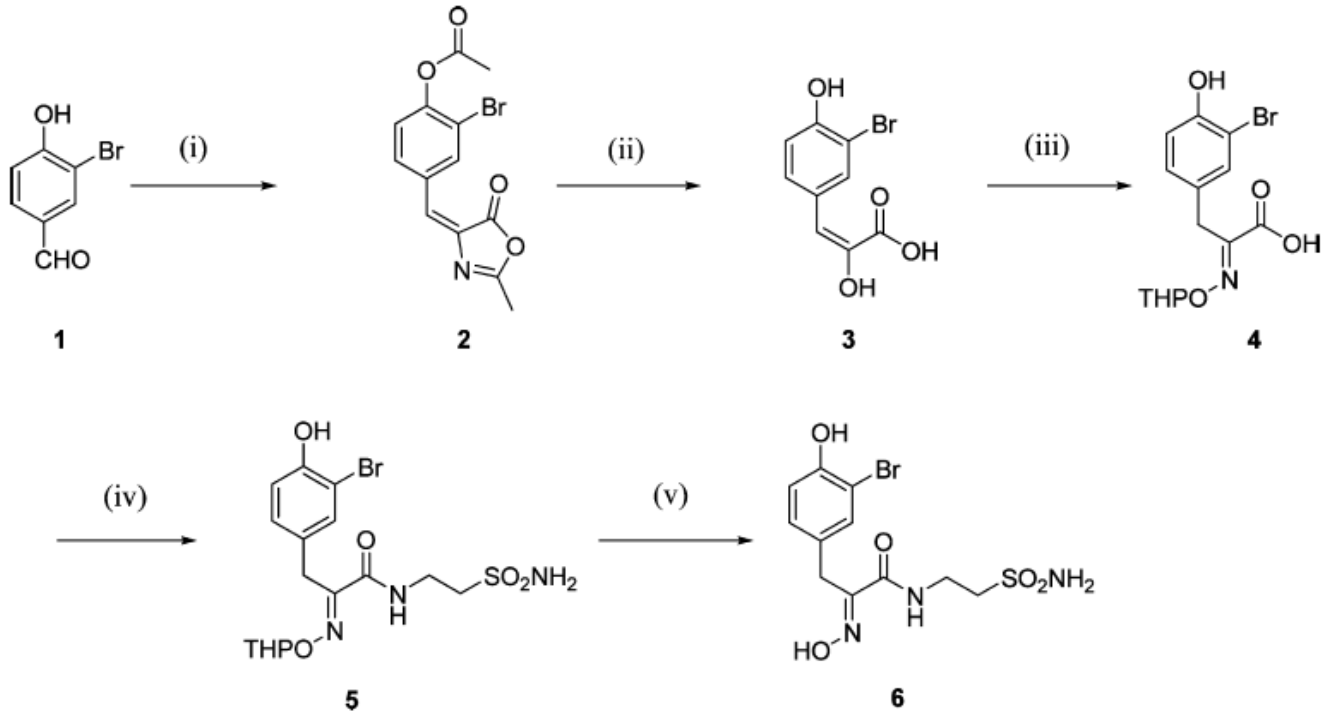


Figure 1. Psammaplin C, an unusual natural product comprising a primary sulfonamide group.

DOI: 10.1021/acs.jmedchem.6b00443
J. Med. Chem. 2016, 59, 5462−5470

Scheme 1. Synthesis of NP 6[a]



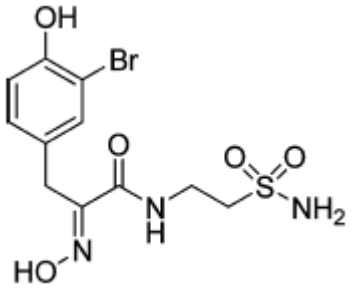
1 2 3 4 5 6

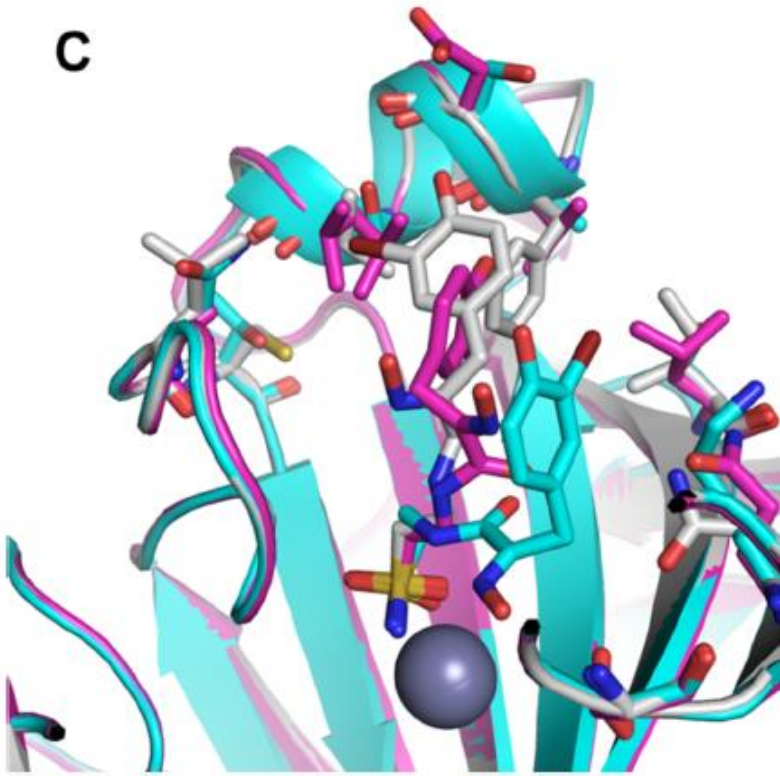
[a]Reagents and conditions: (i) *N*-acetyl-glycine, NaOAc, Ac_2O, 120 °C, 3 h, 85%; (ii) aq HCl (10%), reflux, 15 h, 65%; (iii) H_2NOTHP, dry pyridine, rt, 15 h, 54%; (iv) (a) EDC·HCl, HOSu, dry 1,4-dioxane, 2−3 h, rt; (b) *β*-aminoethanesulfonamide hydrochloride **11**, dry NEt_3, dry MeOH, rt, 12 h, 46% (over 2 steps); (v) 4.0 M HCl in 1,4-dioxane, 0 °C, 6−8 h, 80%.

Table 1. Human CA Inhibition Profile for NP 6 and the Reference CA Inhibitor AZA

		K_i (nM)[a]	
entry	hCA isoform	6	AZA
1	hCA I	48.1	250
2	hCA II	88.0	12
3	hCA IV	75.3	74.2
4	hCA VA	154	63.1
5	hCA VI	9680	11.0
6	hCA VII	1.7	2.5
7	hCA IX	12.3	25.0
8	hCA XII	0.79	5.7
9	hCA XIII	10630	16.4
10	hCA XIV	379	41.3

[a]Errors ±10% of the reported values (from three separate assays).





Angewandte International Edition Chemie

Cancer Therapy

DOI: 10.1002/anie.201310709

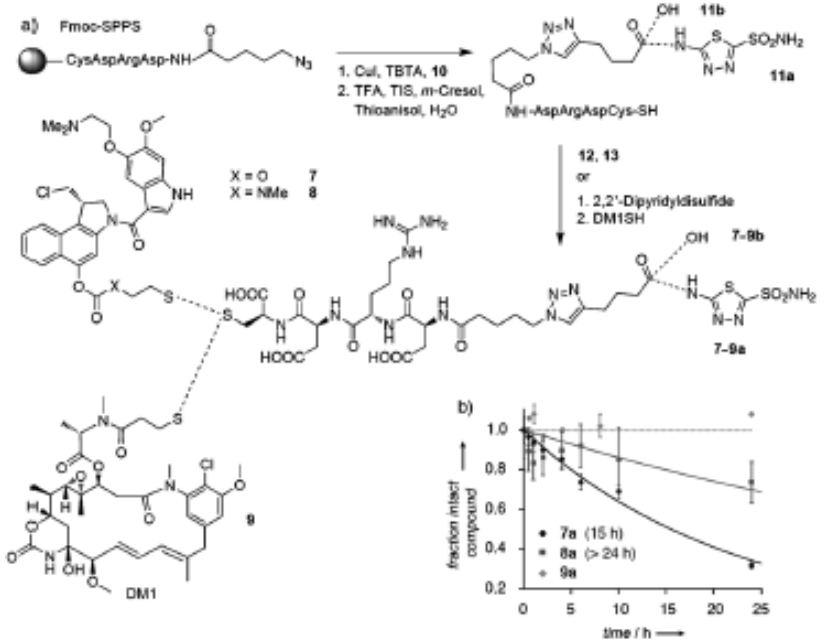
A Small-Molecule Drug Conjugate for the Treatment of Carbonic Anhydrase IX Expressing Tumors**

*Nikolaus Krall, Francesca Pretto, Willy Decurtins, Gonçalo J. L. Bernardes, Claudiu T. Supuran, and Dario Neri**

maytansinoid DM1 as the cytotoxic payload

The sulfonamide – DM1 conjugate

exhibited a potent antitumor effect in SKRC52 renal cell carcinoma in vivo. It was furthermore superior to sunitinib and sorafenib, both small-molecule standard-of-care drugs for the treatment of kidney cancer.



Scheme 2. Structure, synthesis, and stability of drug conjugates. a) A Cu^{I}-catalyzed azide–alkyne cycloaddition was used to couple the targeting ligand **10** to the charged linker, which was prepared by fluorenylmethoxycarbonyl (Fmoc) solid-phase peptide synthesis (SPPS). Therapeutic payloads were installed by disulfide exchange. b) Hydrolytic stability of drug conjugates in PBS pH 7.4 at 37 °C as determined by mass spectrometry (**7a** and **8a**) and high-performance liquid chromatography (**9a**). The carbamate derivative of duocarmycin conjugate **8a** ($t_{1/2}$ > 24 h) was found to be more stable than the carbonate ($t_{1/2}$ = 15 h). DM1 conjugate **9a** was found to be hydrolytically stable. TBTA = tris(benzyltriazolylmethyl)amine, TFA = trifluoroacetic acid, TIS = triisopropylsilane.

Journal of Medicinal Chemistry

Article

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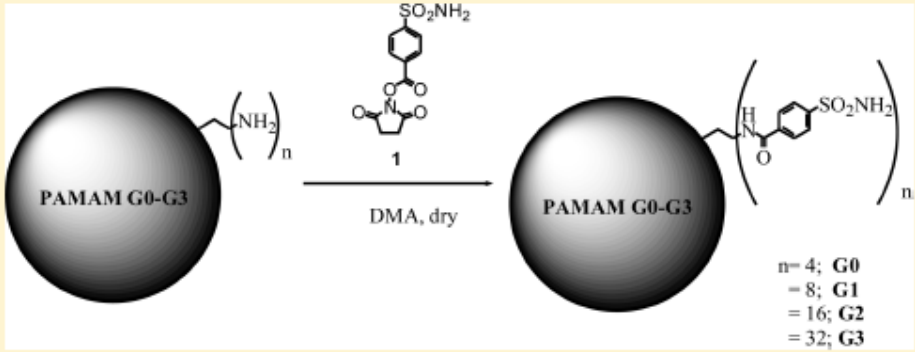
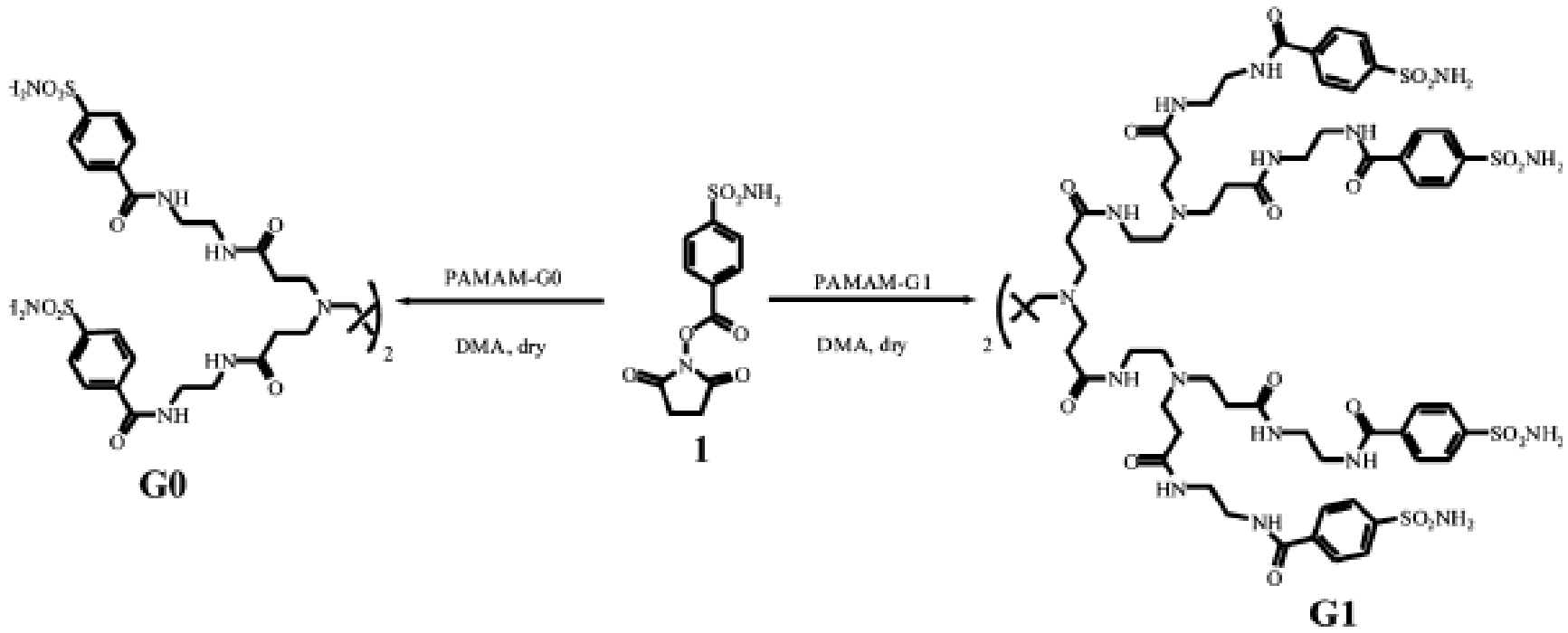
Poly(amidoamine) Dendrimers with Carbonic Anhydrase Inhibitory Activity and Antiglaucoma Action

Fabrizio Carta,[†] Sameh M. Osman,[‡] Daniela Vullo,[†] Antonella Gullotto,[†] Jean-Yves Winum,[§] Zeid AlOthman,[‡] Emanuela Masini,[∥] and Claudiu T. Supuran*[,‡,⊥]

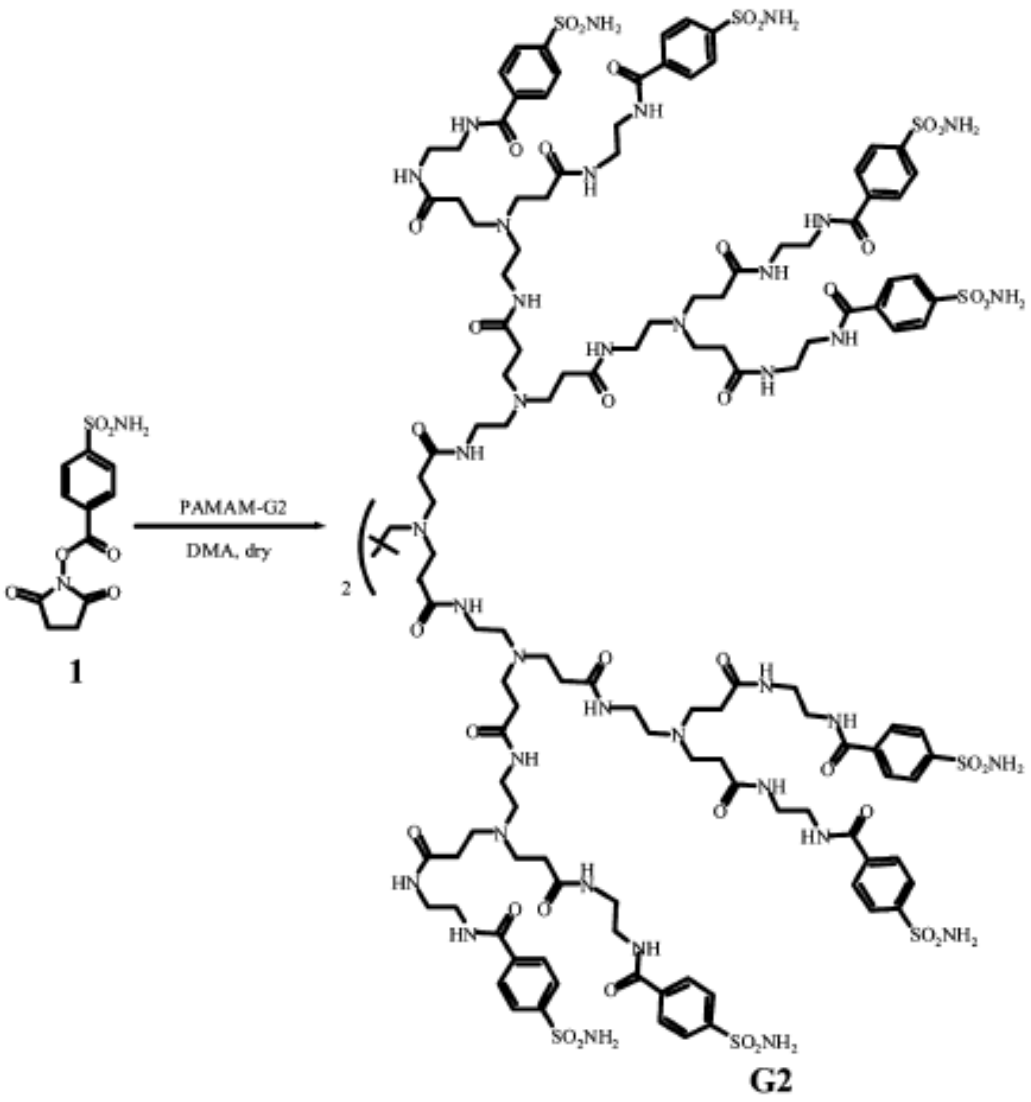
ABSTRACT: Four generations of poly(amidoamine) (PAMAM) dendrimers decorated with benzenesulfonamide moieties were prepared by derivatizing the amino groups of the dendrimer with 4-carboxy-benzenesulfonamide functionalities. Compounds incorporating 4, 8, 16, and 32 sulfonamide moieties were thus obtained, which showed an increasing carbonic anhydrase (CA, EC 4.2.1.1) inhibitory action with the increase of the number of sulfamoyl groups in the dendrimer. Best inhibitory activity (in the low nanomolar−subnanomolar range) was observed for isoforms CA II and XII, involved among others in glaucoma. In an animal model of this disease, the chronic administration of such dendrimers for 5 days led to a much more efficient drop of intraocular pressure compared to the standard drug dorzolamide.

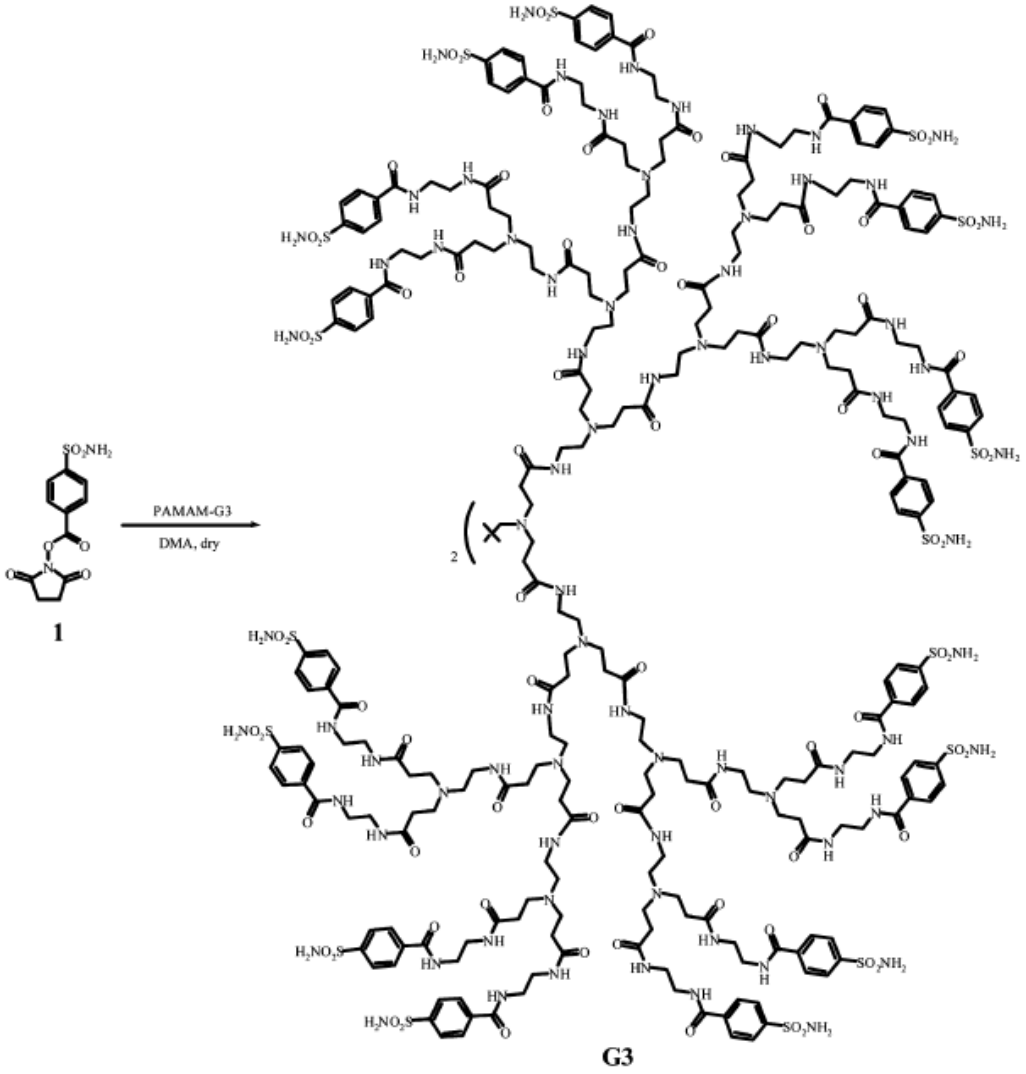


Poly(amidoamine) Dendrimers with CAI Activity and Antiglaucoma Action



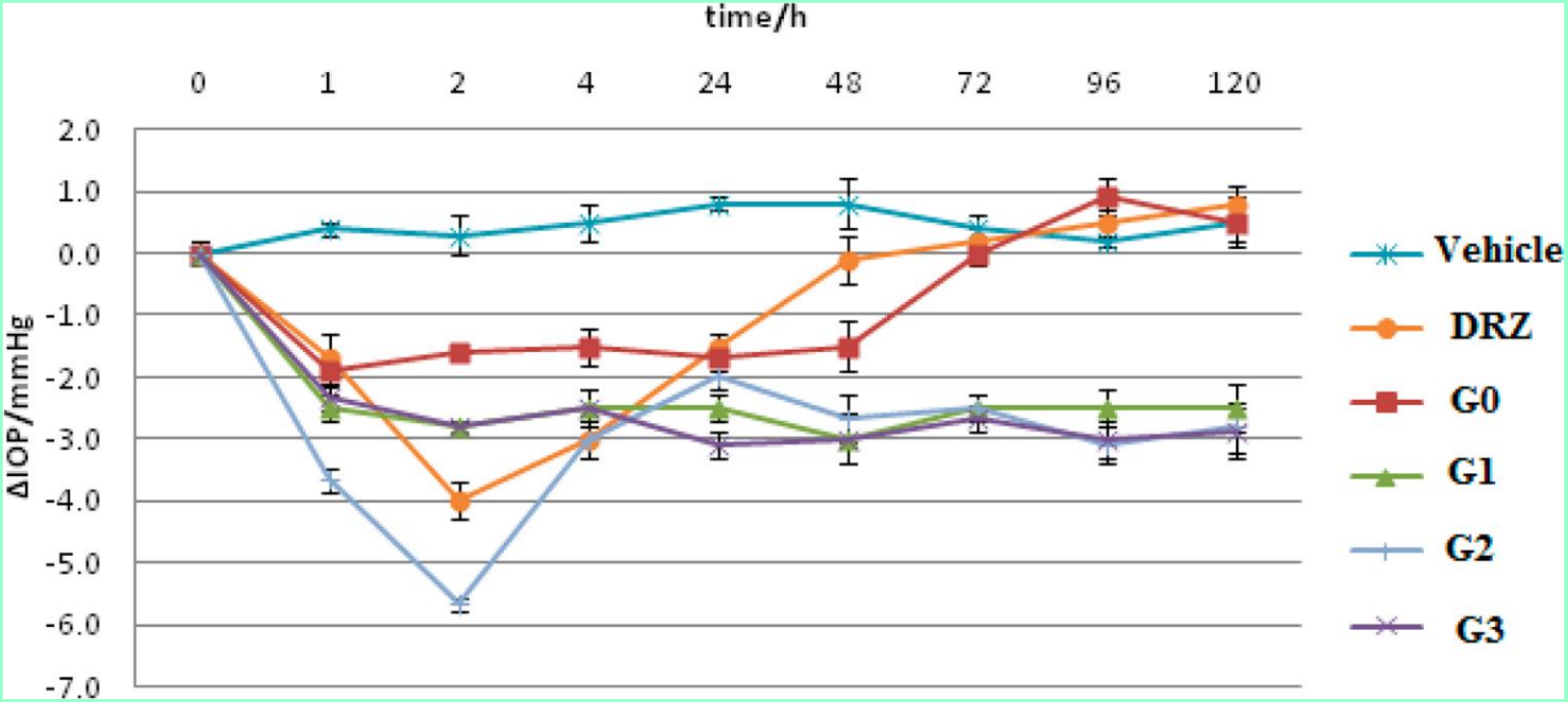
Carta, Osman, AlOthman, et al. J. Med. Chem, 2015, 58, 4039-45.


PAMAM-G2
DMA, dry
1
G2


PAMAM-G3
DMA, dry
1
G3

compd	K_i (nM)[a]			
	hCA I	hCA II	hCA IX	hCA XII
1	7800	640	475	380
G0	24.1	10.4	34.7	9.3
rp	323	61	13	40
rp/n	80.9	15.3	3.4	10.2
G1	12.0	3.1	20.5	1.1
rp	650	206	23	345
rp/n	81.2	25.8	2.8	43.1
G2	10.8	0.93	8.6	0.94
rp	722	688	55	404
rp/n	45.13	43	3.45	25; 26
G3	10.5	0.07	5.1	0.06
rp	742	9142	93	6333
rp/n	23.21	285.7	2.9	197.9
AAZ	250	12	25	5.7
DRZ	50000	9	52	3.5

Carta et al. J. Med. Chem, 2015,



Carta et al. J. Med. Chem, 2015,.

Carta et al. J. Med. Chem, 2015,.

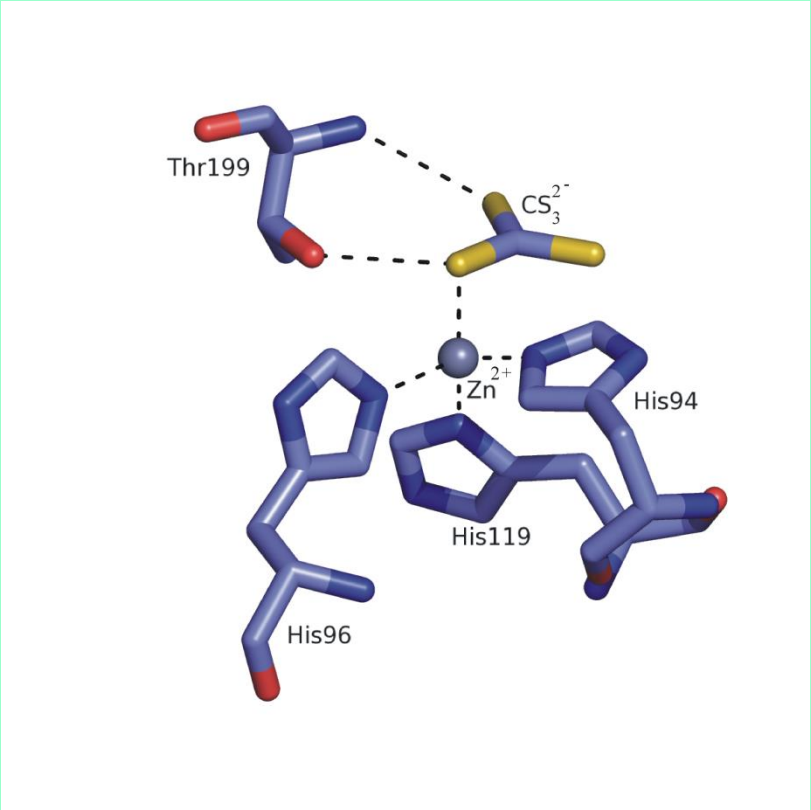
Dendrimers incorporating benzenesulfonamide moieties strongly inhibit carbonic anhydrase isoforms I-XIV.

Carta F, Osman SM, Vullo D, AlOthman Z, Supuran CT. Org Biomol Chem. 2015 Jun 21;13(23):6453-7.

Poly(amidoamine) dendrimers show carbonic anhydrase inhibitory activity against α-, β-, γ- and η-class enzymes.

Carta F, Osman SM, Vullo D, AlOthman Z, Del Prete S, Capasso C, Supuran CT. Bioorg Med Chem. 2015 Nov 1;23(21):6794-8.

Novel Chemotypes: Dithiocarbamates/XANTHATES, new classes of CAIs



Trithiocarbonate $(CS_3)^{2-}$ was shown recently to be a CAI (Innocenti et al. BMCL19 (2009) 1855-1857

TTC is thus a NEW ZBG (zinc-binding group)

$RNH_2 + CS_2$ = DTCs
$ROH + CS_2$ = XANTHATES

TTC is however a weak or very weak CAI, Ki-s in the micro-millimolar range (depending on the isoform)

CAN we design more effective CAIs considering TTC as lead ? YES

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COMMUNICATION

Dithiocarbamates: a new class of carbonic anhydrase inhibitors. Crystallographic and kinetic investigations†

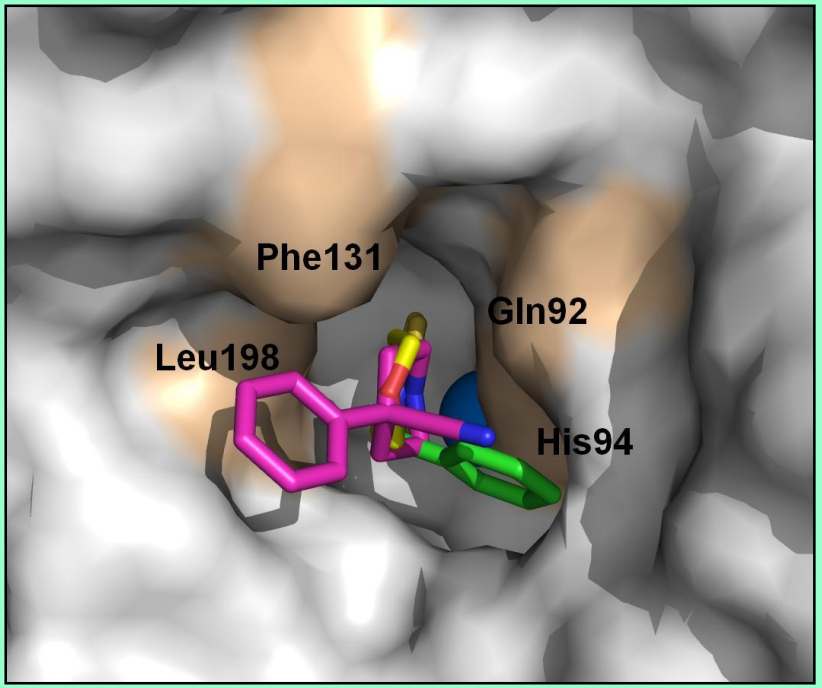
Fabrizio Carta,[a] Mayank Aggarwal,[b] Alfonso Maresca,[a] Andrea Scozzafava,[a] Robert McKenna*[b] and Claudiu T. Supuran*[a]

$RNH_2 + CS_2 = DTCs$

$ROH + CS_2 = XANTHATES$

Table 1 CA inhibition data with DTCs **1–9** ($R1R2NCS_2^-Na^+$) and acetazolamide **10** (as standard inhibitor) against the cytosolic isoforms hCA I and II and transmembrane, tumor-associated one hCA IX

DTC, (R^1,R^2 groups)	K_I (hCA I)/ nM	K_I (hCA II)/ nM	K_I (hCA IX)/ nM
1 (Me, Me)	699	6910	714
2 (Et, Et)	790	3100	1413
3 (nPr, nPr)	1838	55.5	53.8
4 (nBu, nBu)	43.1	50.9	50.3
5 (iBu, iBu)	0.97	0.95	4.5
6 (Et, nBu)	157	27.8	25.9
7 (Me, Ph)	39.6	21.5	28.2
8 (Me, $PhCH_2$)	69.9	25.4	53.0
9 ($NC(Ph)C(CH_2CH_2)_2$	48.4	40.8	757
10 (acetazolamide)	250	12	25



Superposition of the three hCA II – DTCs (**7-9**) X-ray structures showing a very variable orientation/conformation of the bound inhibitors within the active site

CONCLUSIONS

CA IX is a validated antitumor/antimetastatic target

Sulfonamide, sulfamate, sulfamide and coumarins actings as CA IX inhibitors show significant effects in vitro and in vivo (primary tumors/metastases)

Treatment with CA IX inhibitors reduces the number of cancer stem cells

A sulfonamide CA IX inhibitor entered Phase I clinical trials in 2014

Imaging of CA IX-positive tumors feasible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