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Carbonic anhydrases: One target for multiple diseases

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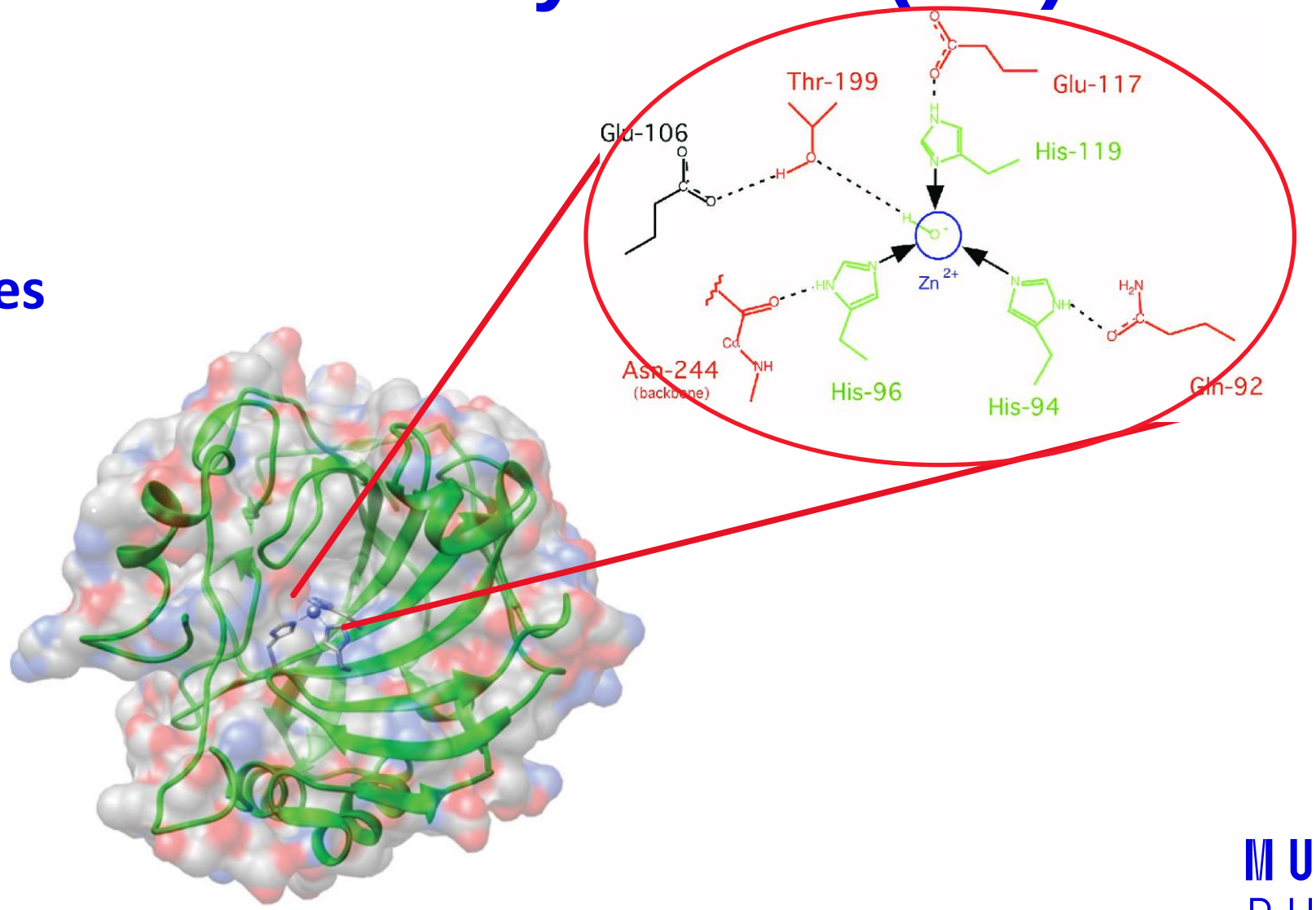
What are carbonic anhydrases (CA)

- CA, EC 4.2.1.1
- **Metaloenzymes**



 Med Res Rev. 2020;40:2485–2565

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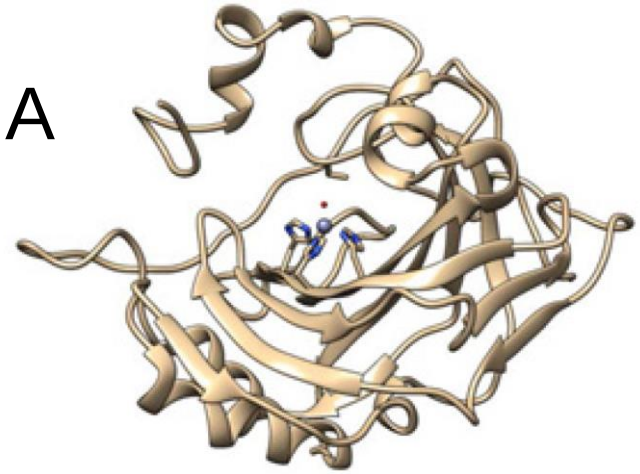
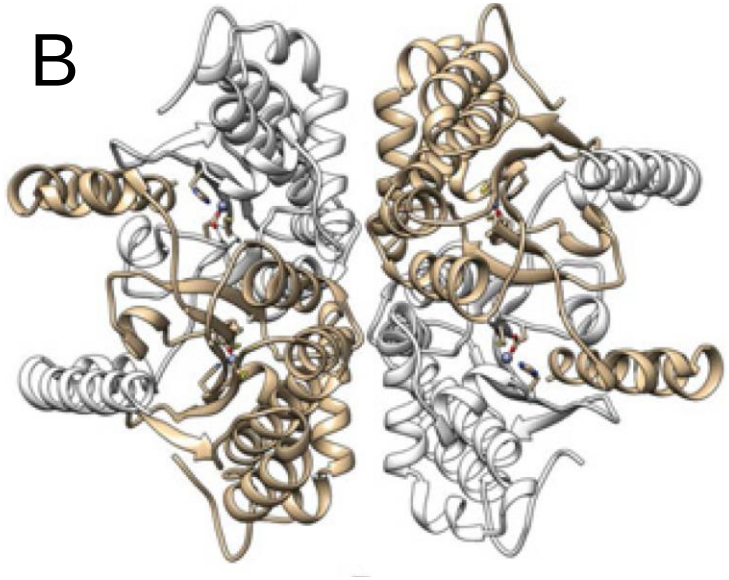
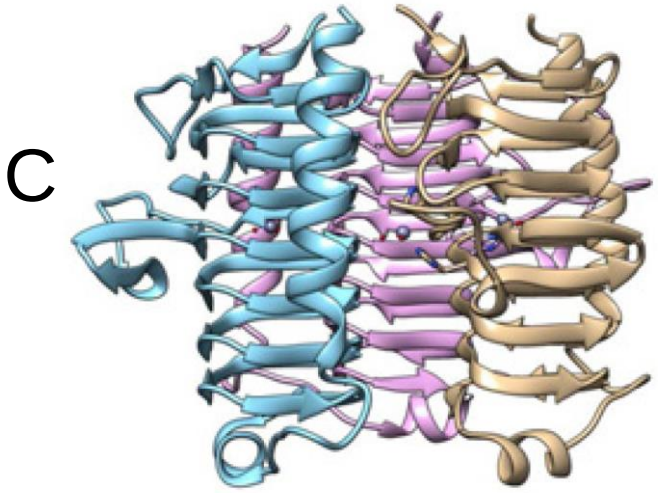
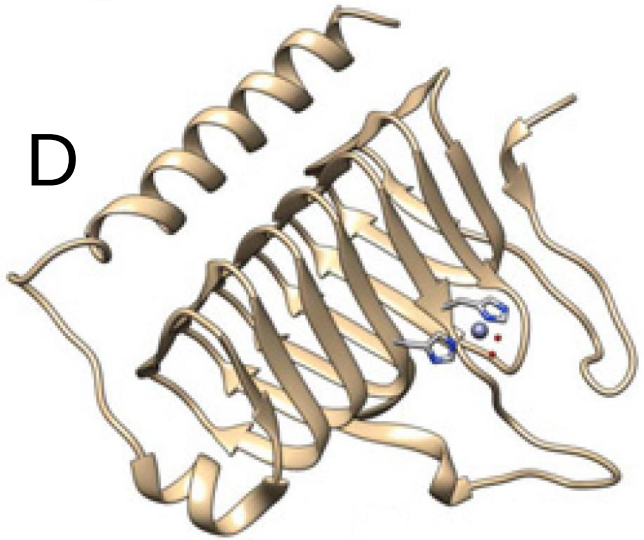
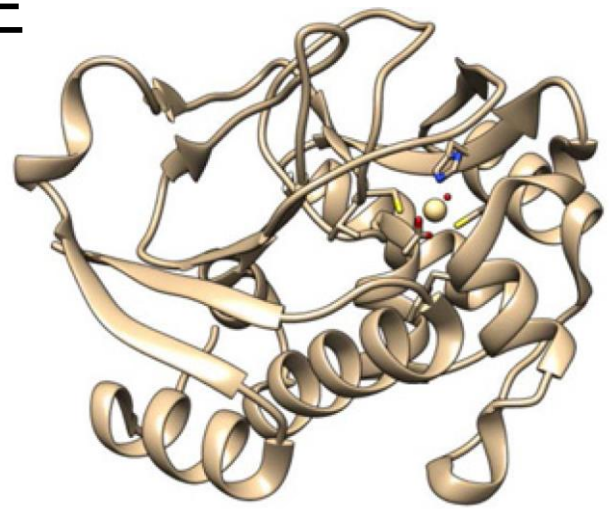
Structure











Biochem. J. (2016) **473**, 2023–2032

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CA families

α-CAs	vertebrates, protozoa, algae, cytoplasm of green plants, $Gram^-$ bacteria
β-CAs	$Gram^-$ bacteria, $Gram^+$ bacteria, algae, chloroplasts of mono- and di-cotyledons, fungi, *Archaea*
γ-CAs	*Archaea*, cyanobacteria and most types of bacteria
δ-CAs	marine diatoms
ζ-CAs	marine diatoms
η-CAs	protozoa
θ-Cas	marine diatoms
ι-CAs	marine diatoms

Med Res Rev. 2020;40:2485–2565; Biochem. J. (2016) **473**, 2023–2032

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CA presence in the cells

Cytosol
- CA I
- CA II
- CA III
- CA VII
- CA VIII
- CA X
- CA XI
- CA XIII

Membrane-bound
- CA IV

Trans-membrane
- CA IX
- CA XII
- CA XIV

Mitochondria
- CA VA
- CA VB

Saliva and milk
- CA VI

Med Res Rev. 2020;40:2485–2565

CA presence in organs/tissue

CA I Erythrocytes, gastrointestinal tract (GIT), and eye	**CA II** Erythrocytes, eye, GIT, bone osteoclasts, kidney, lung, testis, and brain	**CA III** Skeletal muscle and adipocytes	**CA IV** Kidney, lung, pancreas, brain capillaries, colon, heart muscle, and eye	**CA V** Liver, Heart and skeletal muscle, pancreas, kidney, spinal cord, and GIT
CA VI Salivary and mammary gland	**CA VII** Central nervous system (CNS)	**CA VIII** Central nervous system (CNS)	**CA IX** Tumors and gastrointestinal mucosa	**CA X** Central nervous system (CNS)
CA XI Central nervous system (CNS)	**CA XII** Kidney, intestine, reproductive epithelia, eye, tumors, and CNS	**CA XIII** Kidney, brain, lung, gut, and reproductive tract	**CA XIV** Kidney, brain, liver, and eye	

Med Res Rev. 2020;40:2485–2565

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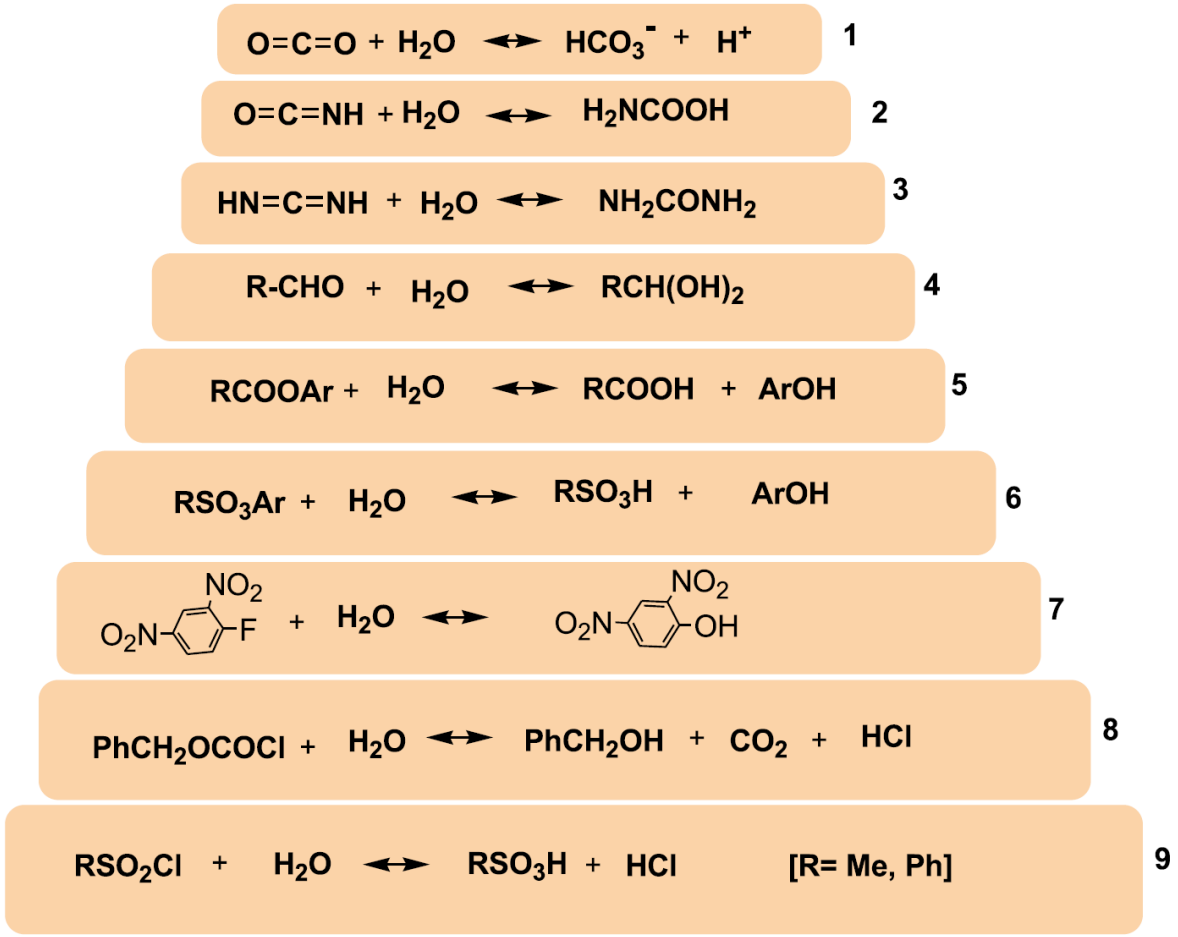
hCAs are involved in…

- Respiration
- Transportation of CO_2 and bicarbonate
- pH and CO_2 homeostasis
- Electrolyte secretion in various tissues
- Biosynthetic reactions
- Gluconeogenesis
- Adipogenesis
- Ureagenesis
- Bone resorption
- Calcification



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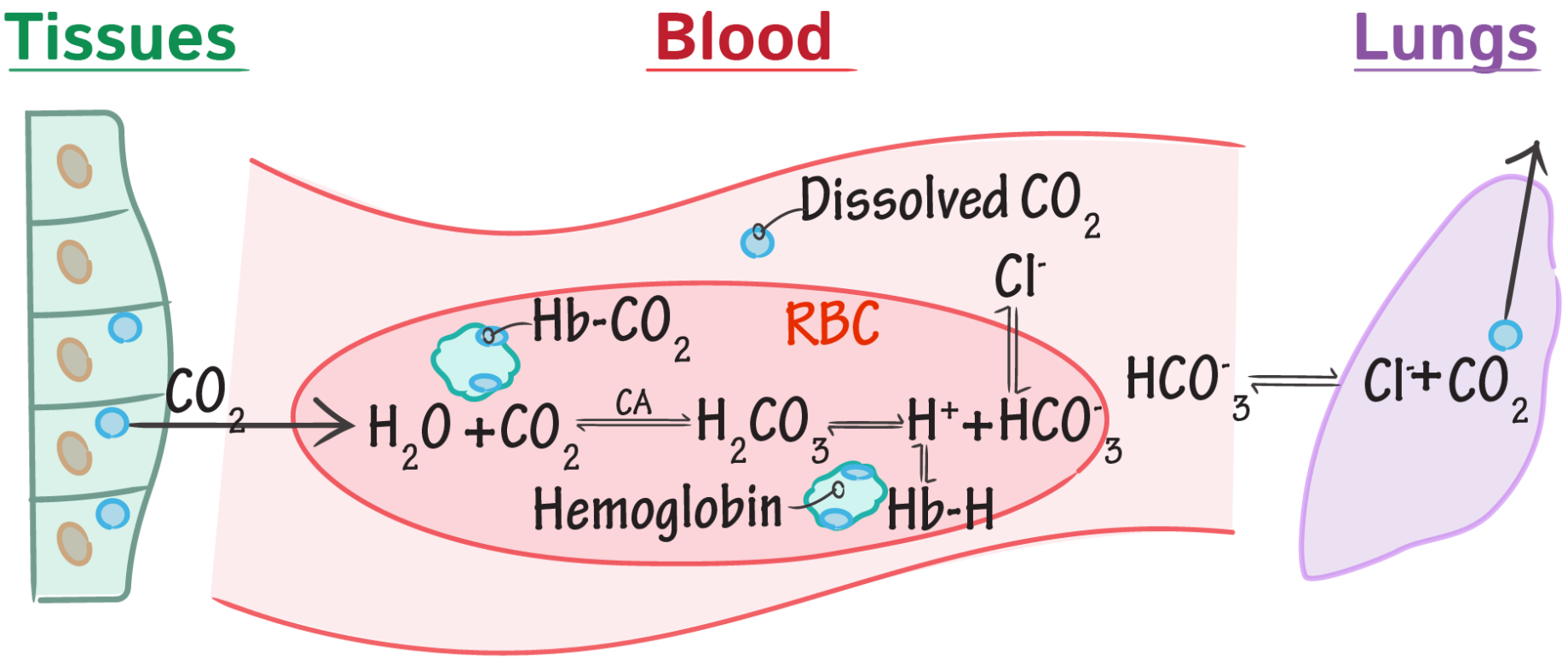
In which form is most of the carbon dioxide transported through the bloodstream?

- dissolved in plasma
- Bonded to haemaglobine in form of carbaminohaemaglobine
- Converted into hydrogencarbonate ions and then transported in plasma

CA in the breathing cycle

CA II
CA I



https://drawittoknowit.com/course/physiology/glossary/physiological-process/carbon-dioxide-transport

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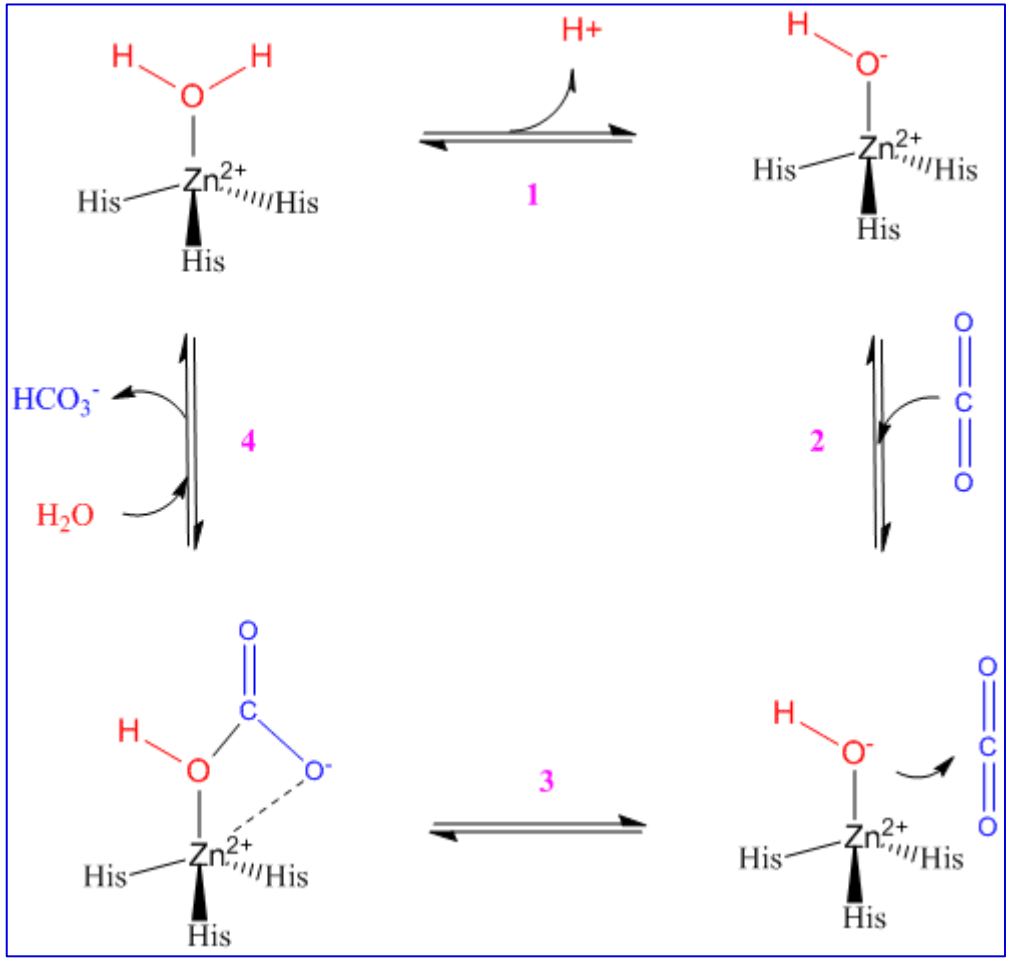
CA Mechanism of Action

$$EZn^{2+}\text{-}OH^- + CO_2 \Longleftrightarrow EZn^{2+}\text{-}HCO_3^-$$

$$EZn^{2+}\text{-}HCO_3^- \Longleftrightarrow EZn^{2+}\text{-}OH_2 + HCO_3^-$$

$$EZn^{2+}\text{-}OH_2 \Longleftrightarrow EZn^{2+}\text{-}HO^- + H^+$$



 J. Enzyme Inhib. Med. Chem.2016;31: 345-360

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How CA can be used in therapy?

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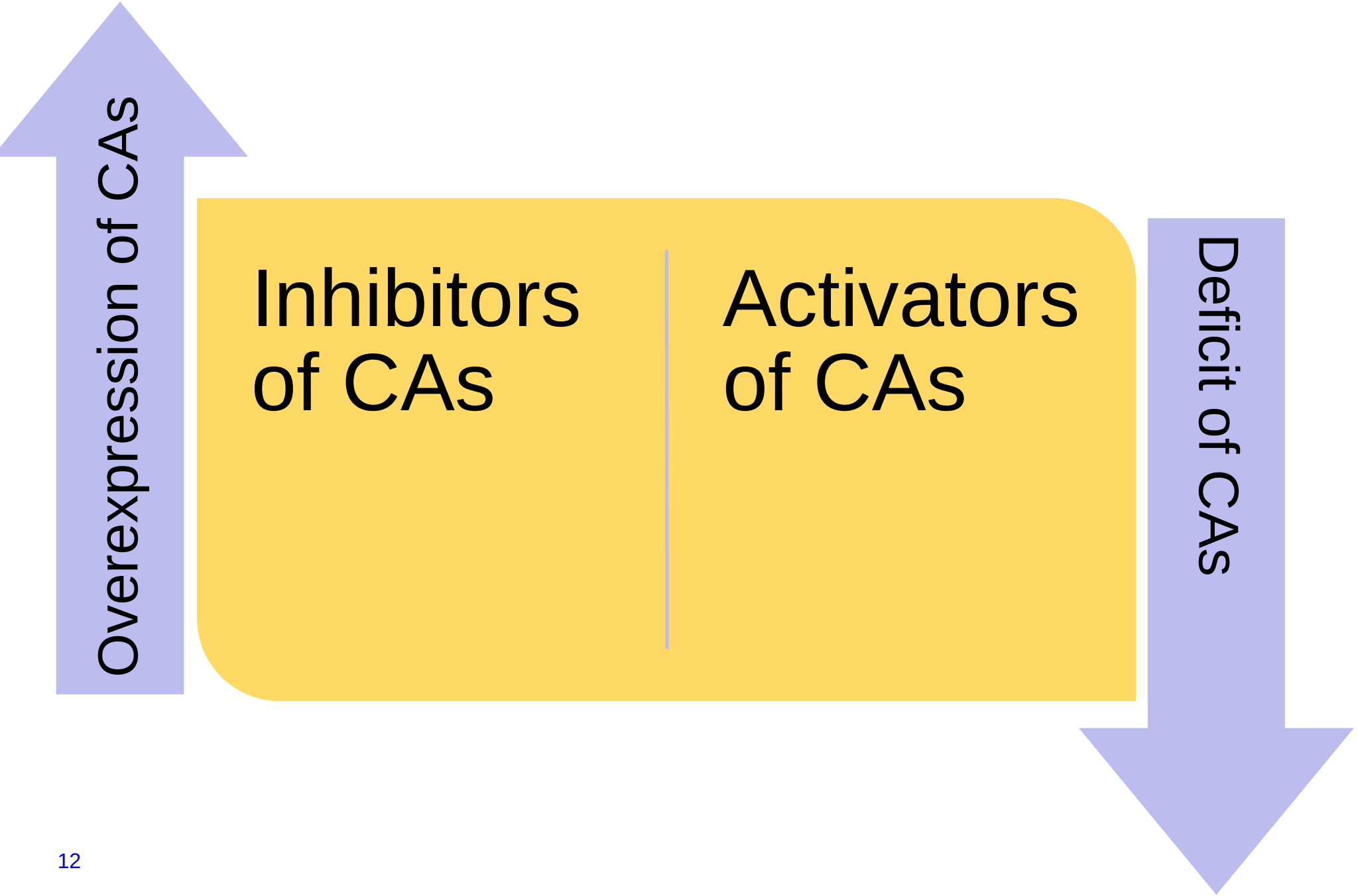
Overexpression of CAs
Inhibitors of CAs
Activators of CAs
Deficit of CAs

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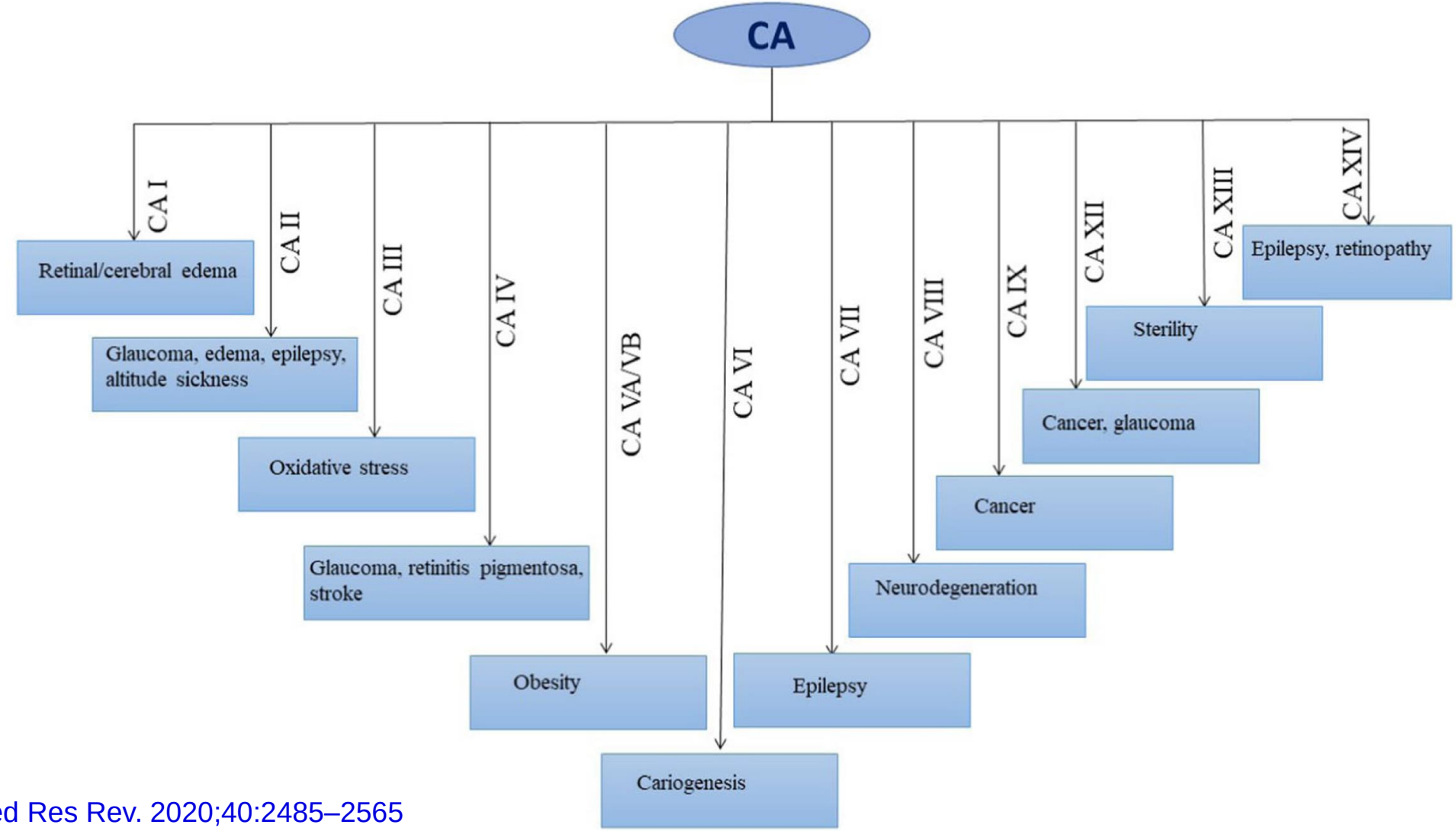
Overexpression/deficit of CAs



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How many mechanism of inhibition of carbonic anhydrases do we know?

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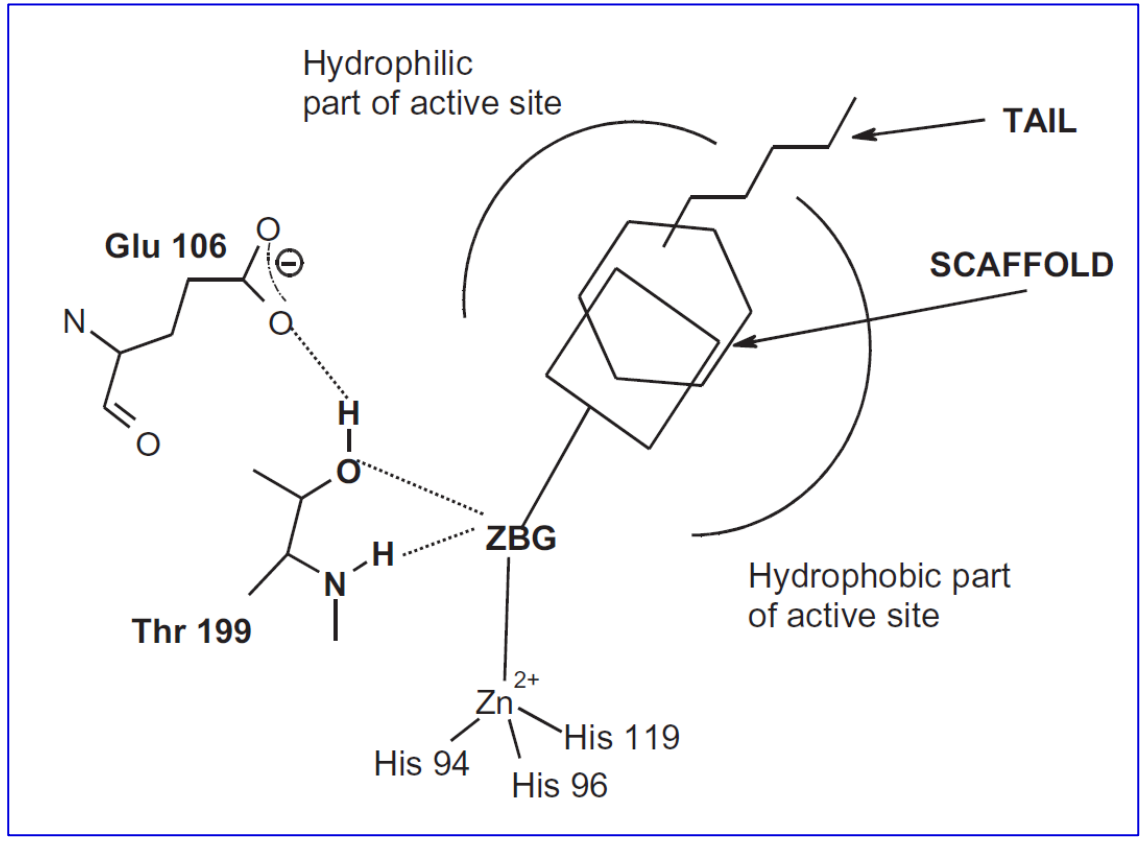
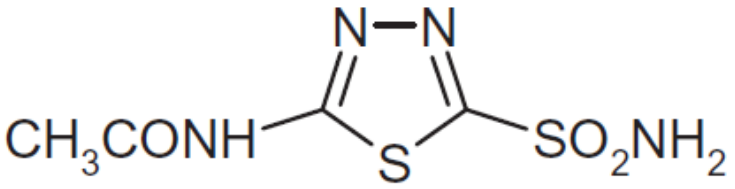
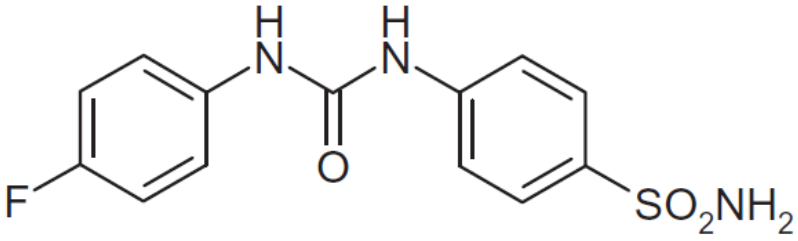
CA Inhibitors Mechanism of Action

The Zinc Binders





acetazolamide



SLC-0111

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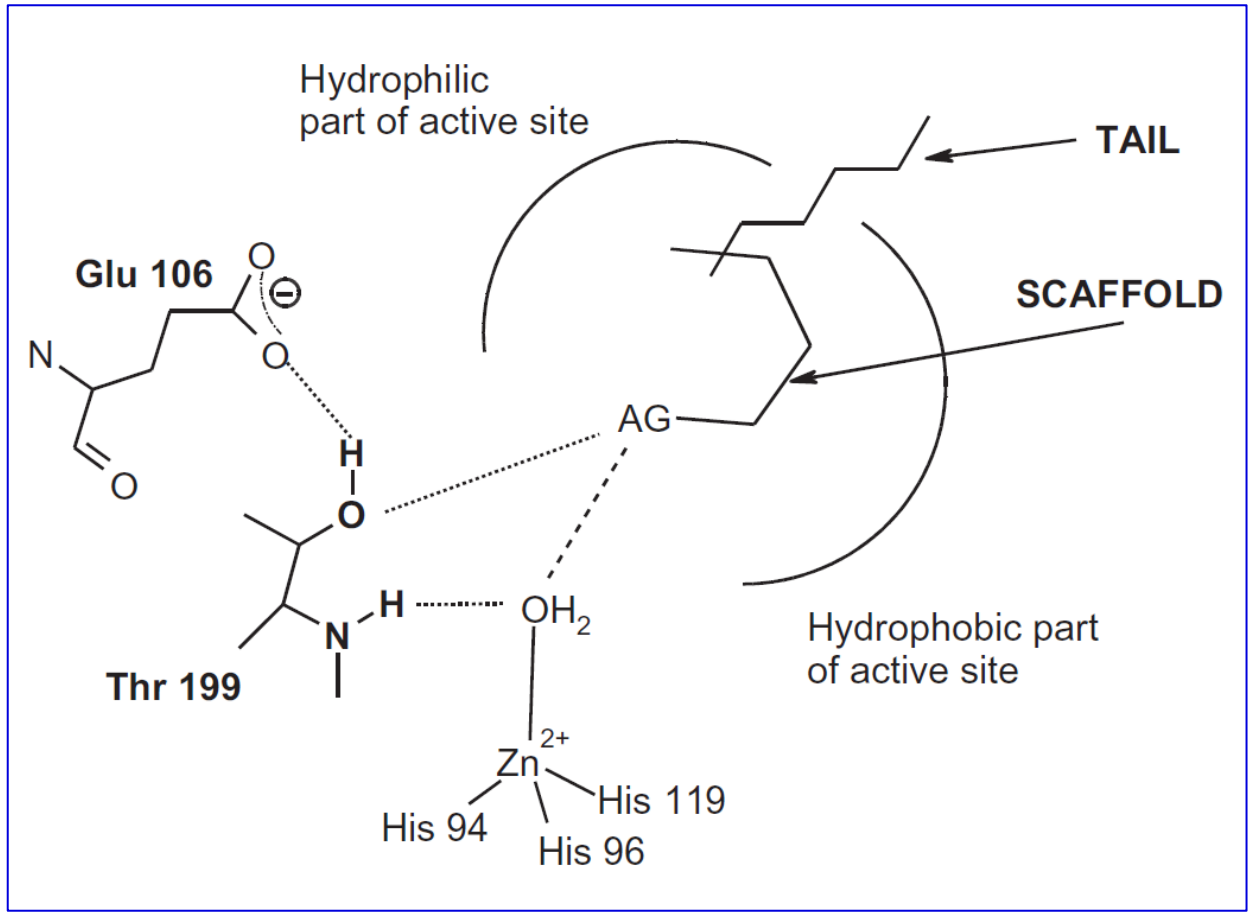
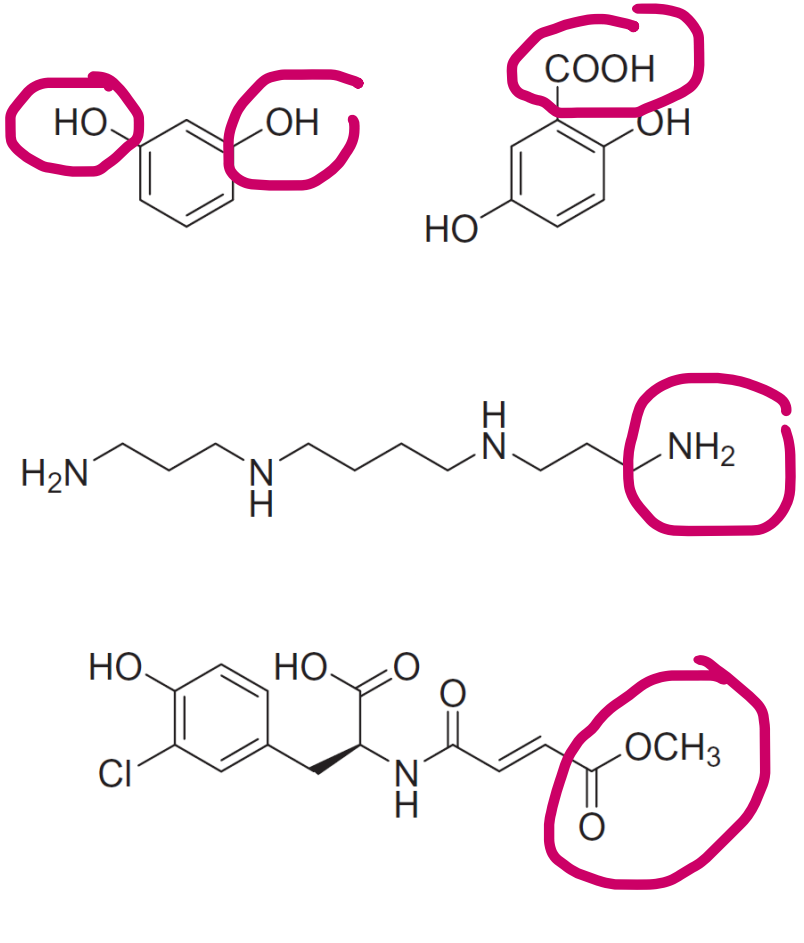
CA Inhibitors Mechanism of Action

CAIs anchoring to the zinc-coordinated water/hydroxide ion





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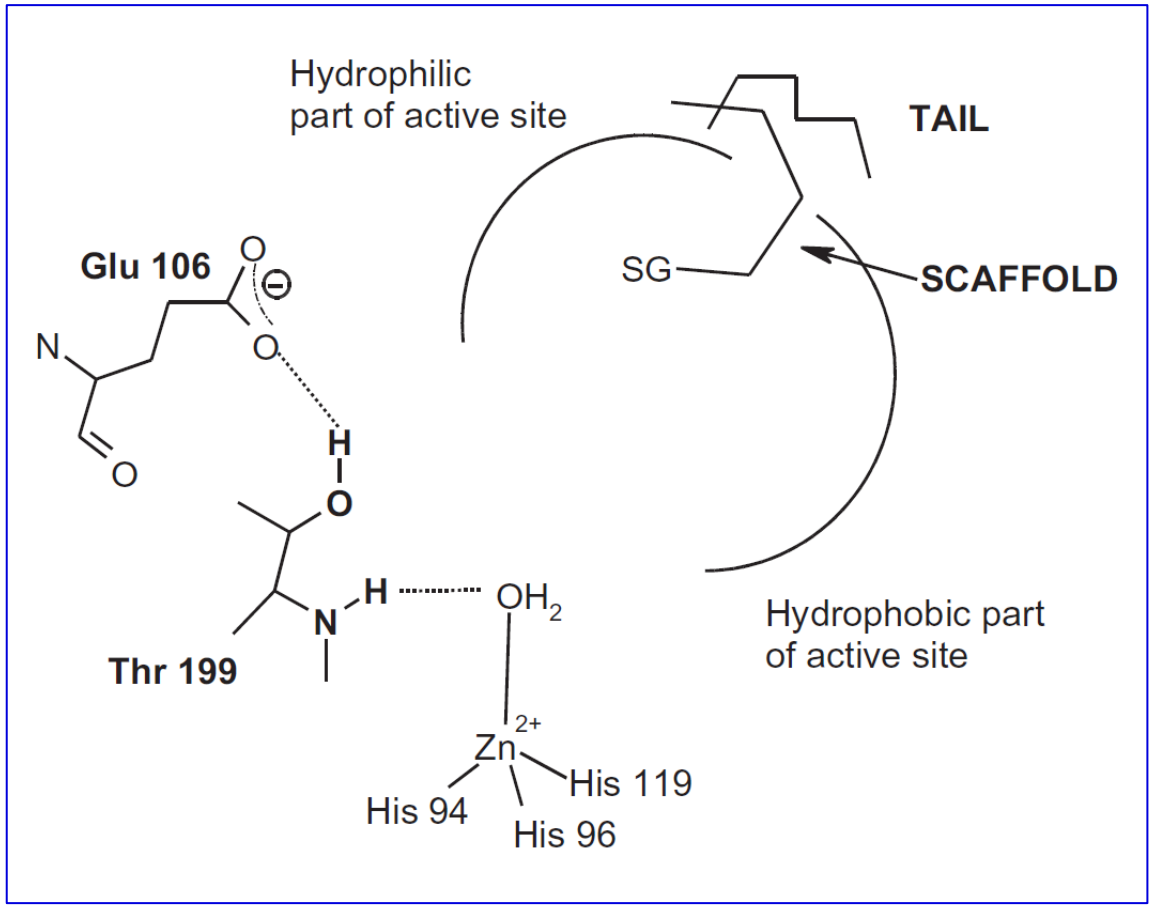
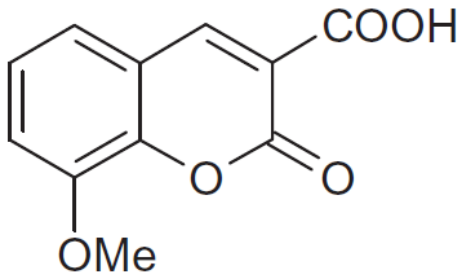
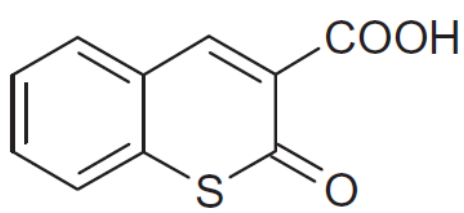
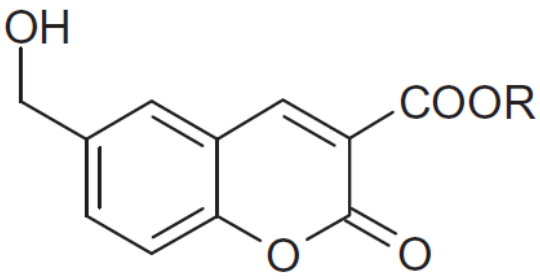
CA Inhibitors Mechanism of Action

CA inhibition by occlusion of the active site entrance









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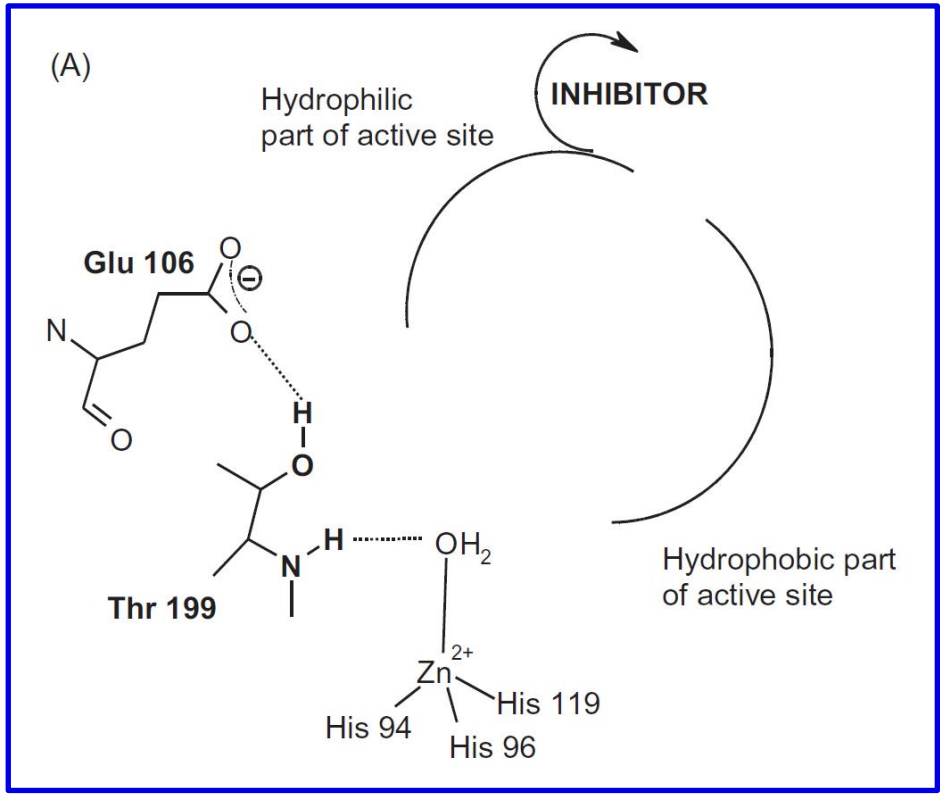
CA Inhibitors Mechanism of Action

Out of the active site binding as a CA inhibition mechanism



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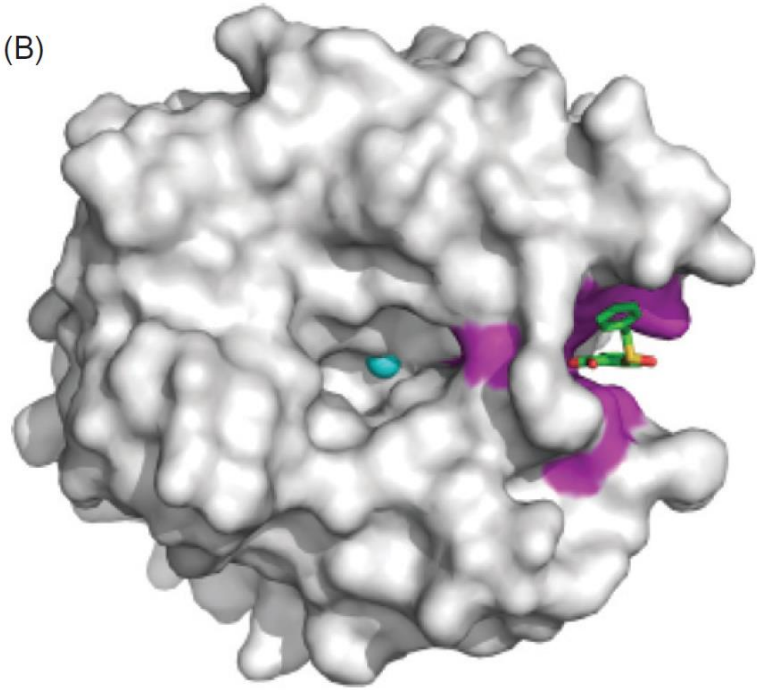

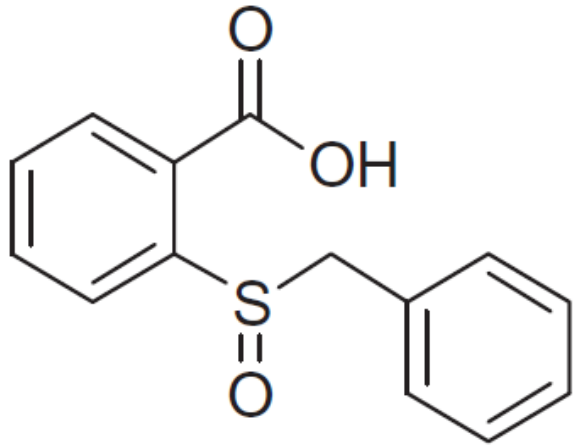

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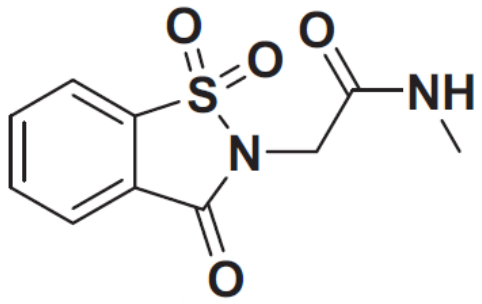
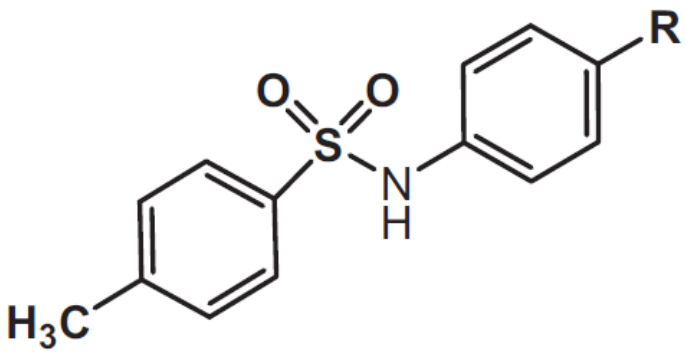
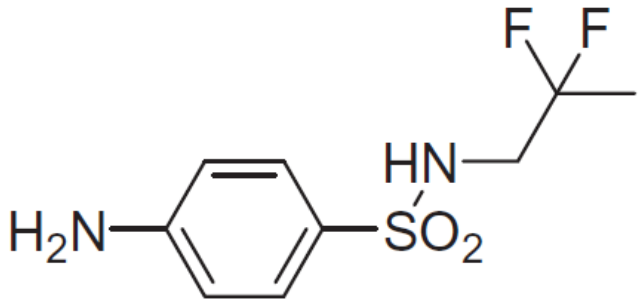
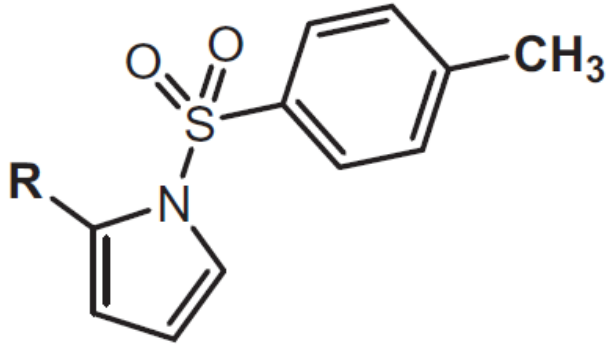
CA Mechanism of Action

Compounds acting as CAIs with an unknown mechanism of action









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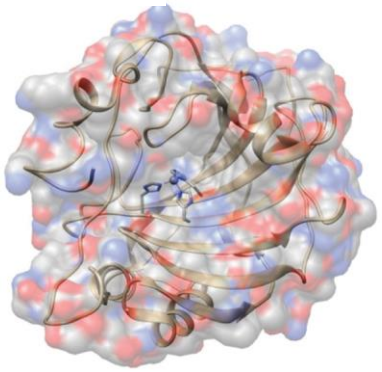
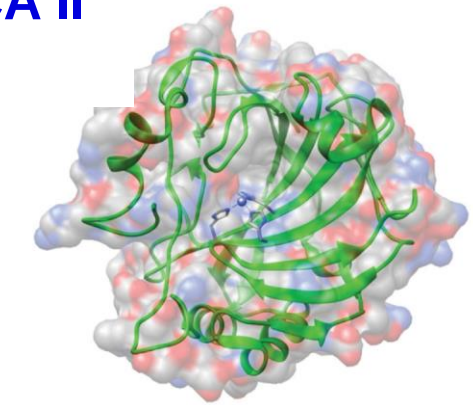
CA I



CA II



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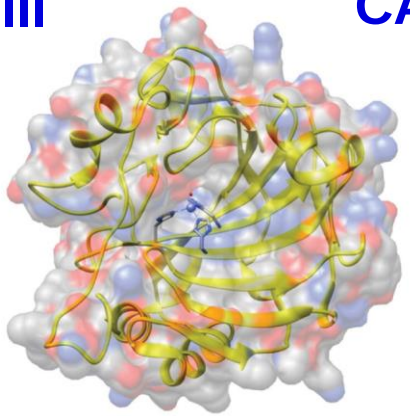
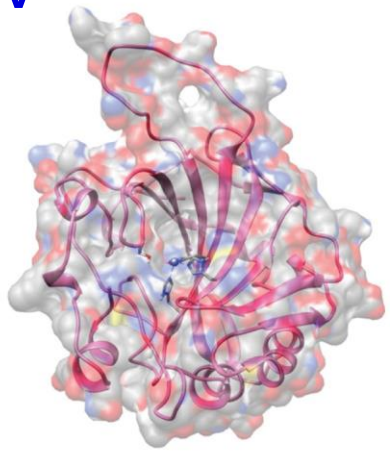
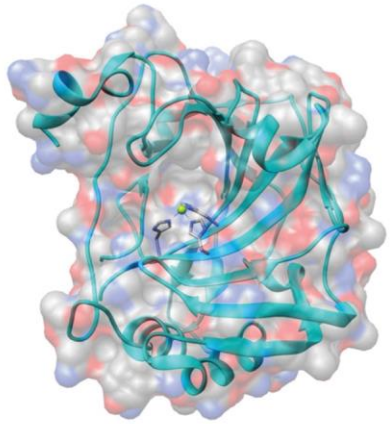


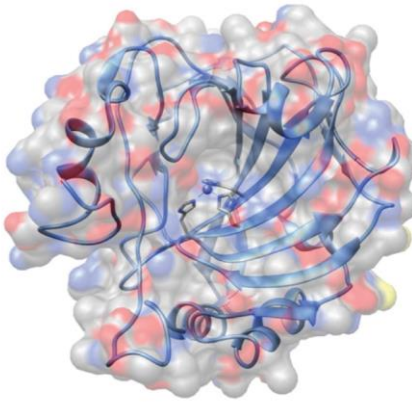
CA IV



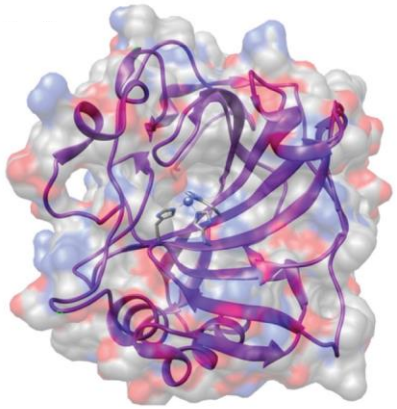
CA VI



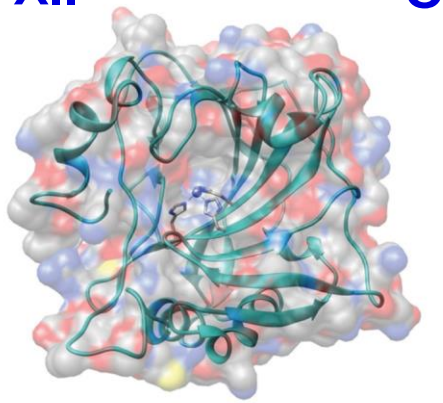
CA VII



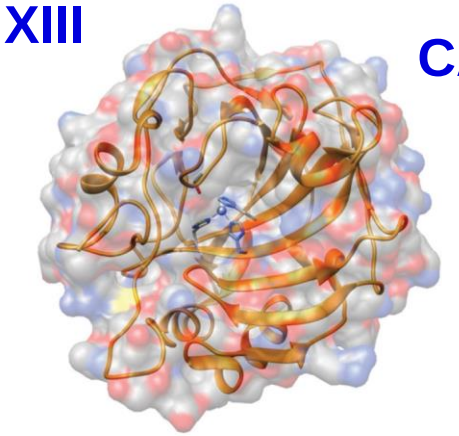
CA IX



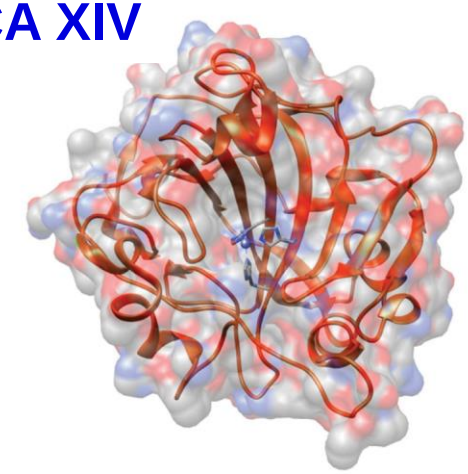
CA XII



CA XIII



CA XIV



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How to discover new selective inhibitors ?

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How to discover new selective inhibitors ?

- Knowledge of the structre
- Screening of anions
- Screening of drugs
- Combination of approaches
- Steal other people's work

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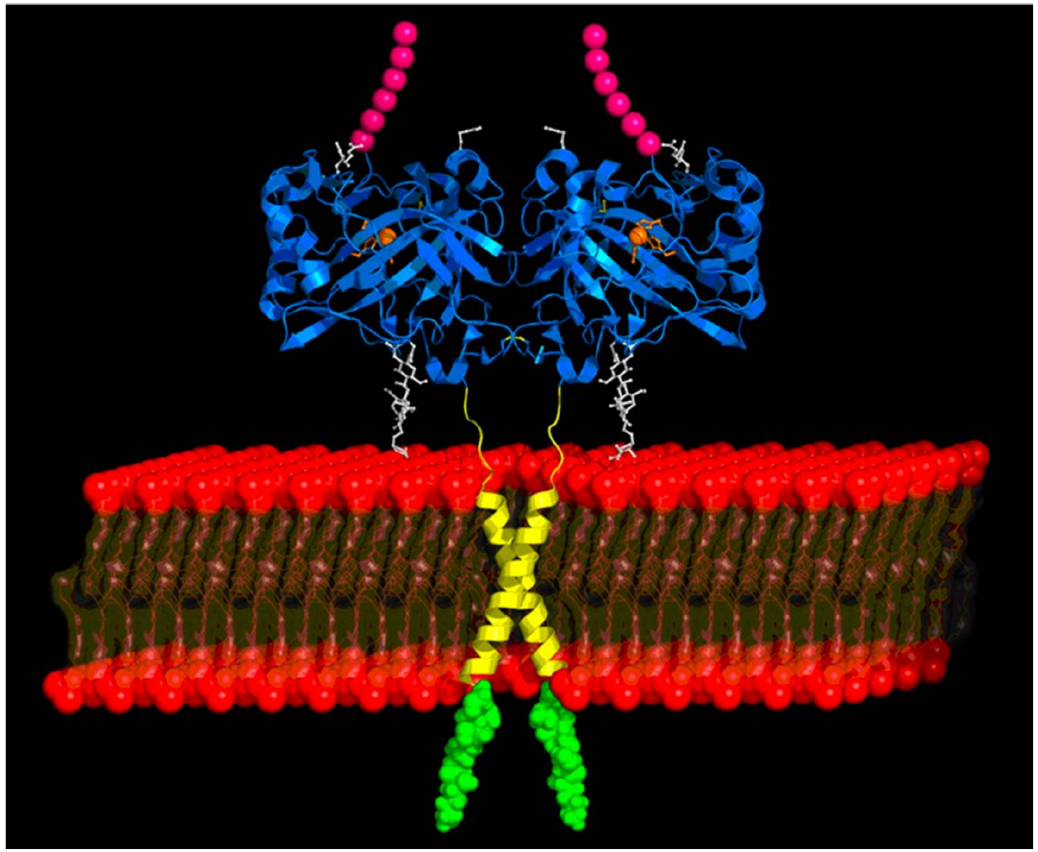
Knowledge of the structure



- X-ray crystal structure of CA IX
- NMR
- other

 FEBS Lett. 2004;577:439–445; Metabolites. 2017;7:48

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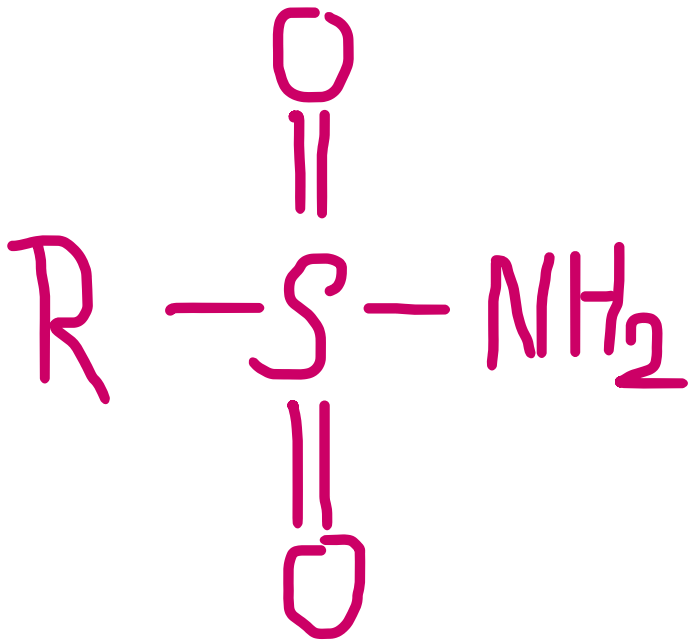
Screening of anions

	K_I [mM]					
	hCA I[a]	hCA II[a]	hCA III[a]	hCA VII[f]	hCA IX[g]	hCA XII[h]
F^-	>300	>300	78.5	1.24	48	0.56
Cl^-	6	200	0.98	1.84	33	73
Br^-	4	63	0.96	1.06	16	82
I^-	0.3	26	0.90	0.25	7	215
CN^-	0.0005	0.02	0.06	9.2	0.004	0.001
CNO^-	0.0007	0.03	0.57	15.2	0.043	0.73
SCN^-	0.1	1.6	0.09	0.17	0.13	0.80
N_3^-	0.0012	1.5	0.087	1.41	0.005	0.08
HCO_3^-	12	85	0.74	0.16	13	0.75
CO_3^{2-}	15	73	0.01	0.27	29	0.64
HSO_3^-	18	89	1.06	7.3	75	0.84
NO_3^-	7	35	117	0.19	46	79
NO_2^-	8.4	63	53	1.78	42	94
HS^-	0.0006	0.04	0.08	1.24	0.007	4.8
SO_4^{2-}	63	>200	1.00	1.38	>200	0.77
$H_2NSO_3^-$	0.021	0.39	31.1	0.0095	0.09	0.70
$H_2NSO_2NH^-$	0.31	1.13	1.09	0.0068	0.096	0.83



 J. Inorg. Biochem. 2012; 111:117-129

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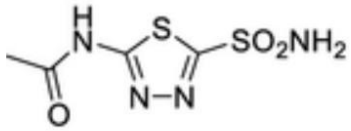
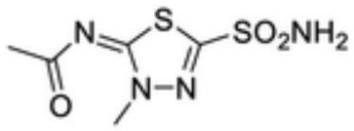
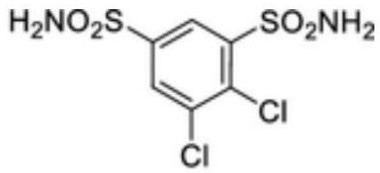
Screening of known drugs



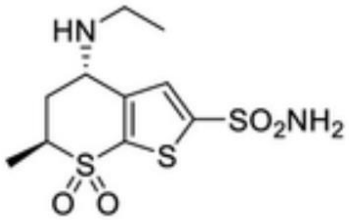
AAZ



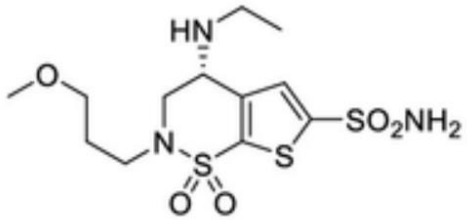
MTZ



DCP



DZA



BRZ

Compound	K_I* (nM)		
	hCA I	hCA II	hCA IX
AAZ	250	12.1	25.8
BRZ	NT	3	37
DCP	1200	38	50
DZA	50 000	9	52
EZA	25	8	34
IND	31	15	24
MZA	50	14	27

J. Med. Chem. 2020; (63)13: 7422−7444

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How to treat cancer –

Inhibition of hCA IX ?

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Which isoenzyme is involved in the cancer?

 J. Med. Chem. 2020; (63)13: 7422−7444

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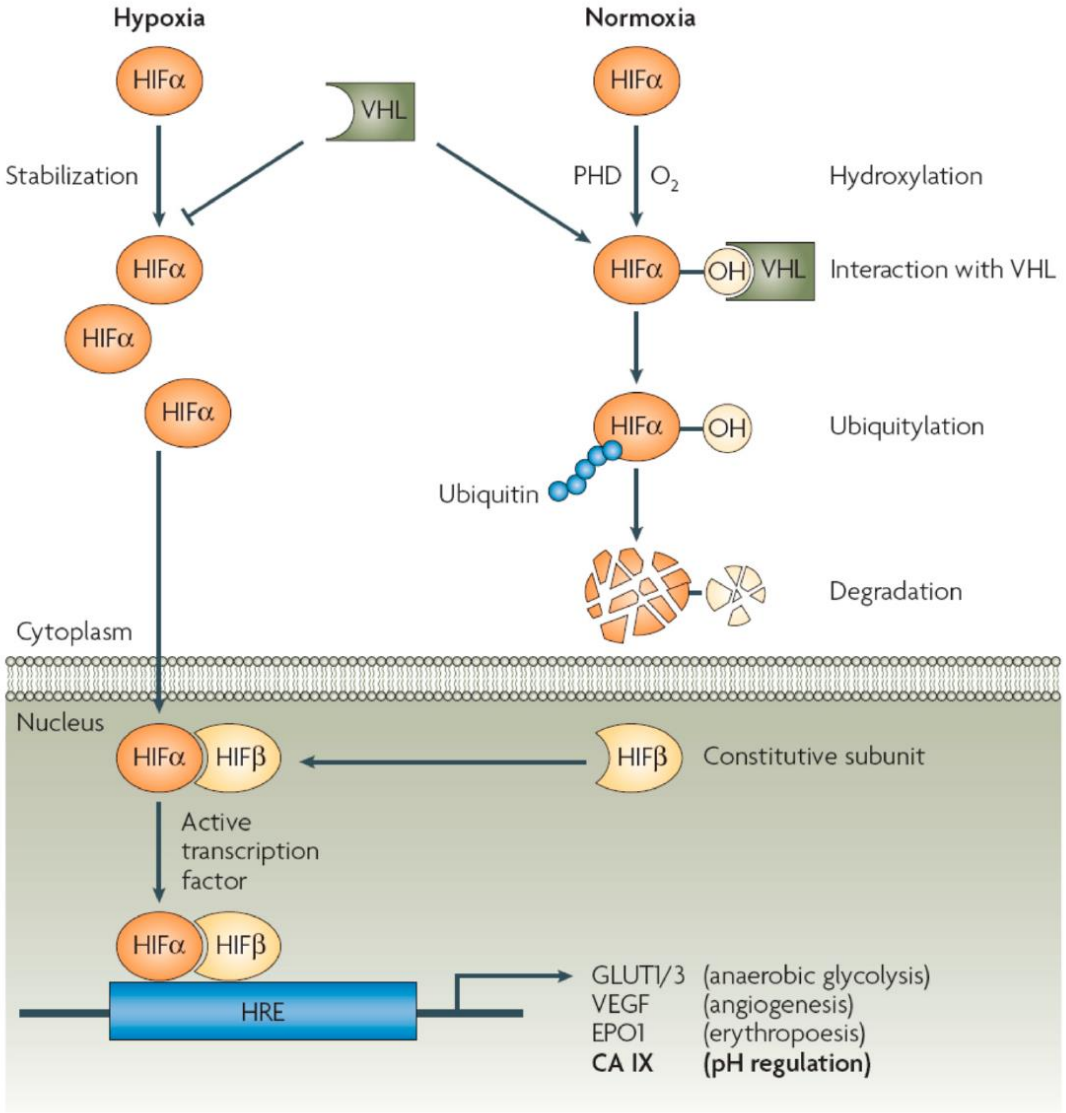

Hypoxia → HIF-1 → aggressive tumor

Metabolites. 2017;7:48; Cancer Metastasis Rev. 2019;38:65–77; Natl. Cancer Inst. 2001;93;266–276.; Cancer Cell. 2008;13:472–482; Nat. Rev. Drug Discov. 2008;7:168–181; Nat. Rev. Drug Discov. 2011;10:767–777.

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hCA IX – How it helps the tumor

Catalytic hydration of CO_2

Maintaining pHe and pHi

Up-regulation of metabolism

Increased adhesion ability

Migration
Invasion
Metastasis

Interaction with signal cascades

Support of angiogenesis

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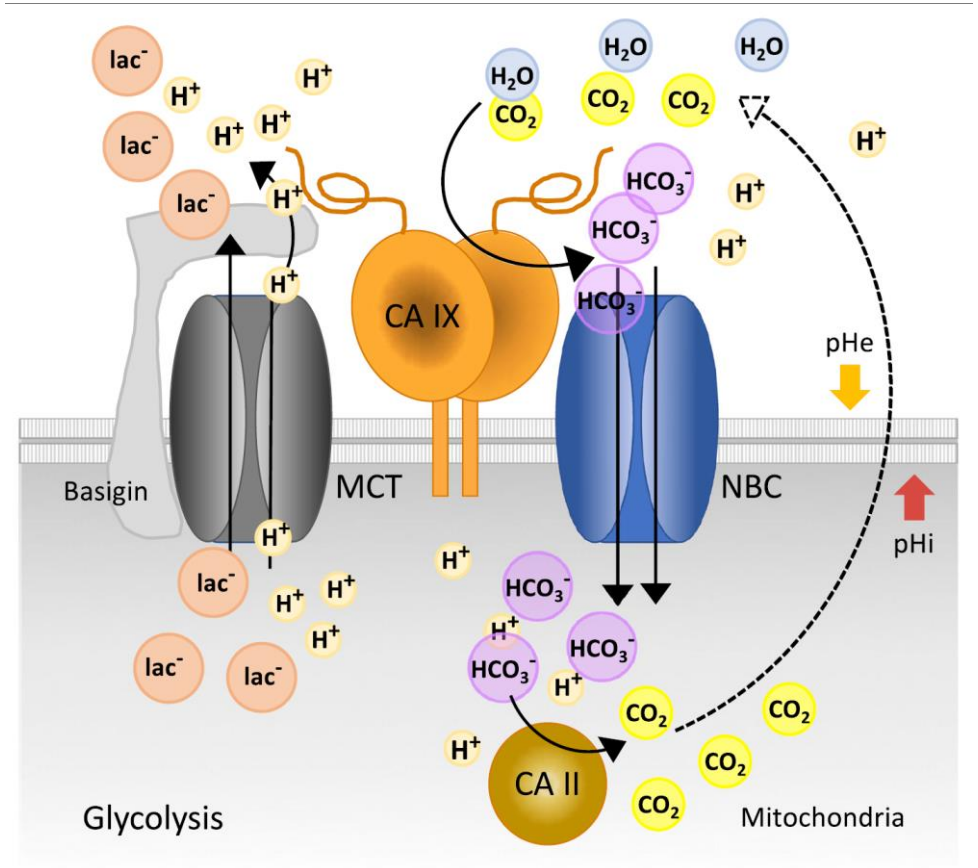
What the hCA IX inhibitors do?



- No cytotoxicity (cytostatic effect)
- Inhibit all of the previously mentioned processes
- Prevents the formation and spread of metastasis
- Multiply increases sensitivity to existing treatments

Cancer Metastasis Rev. 2019;38:65–77

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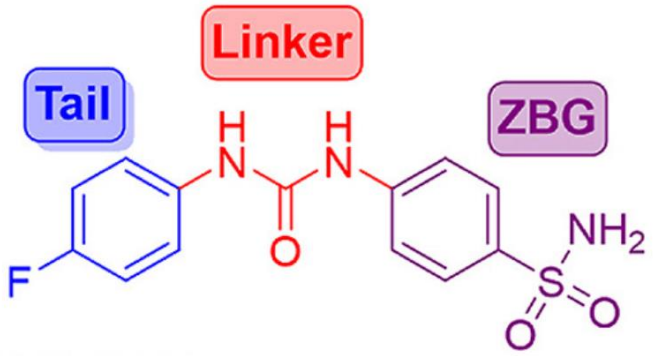
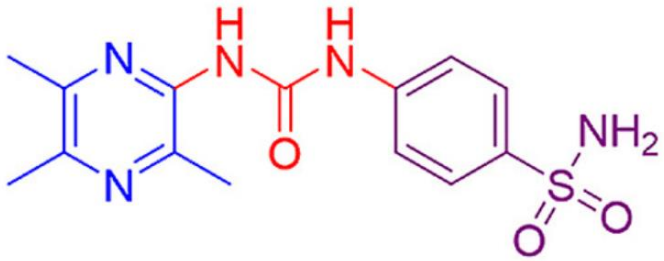
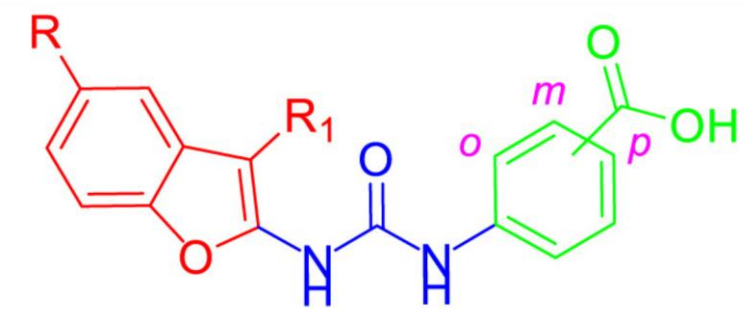
Inhibitors of CA IX



SLC-0111 ; *h*CA IX (K_Is = **45** nM)

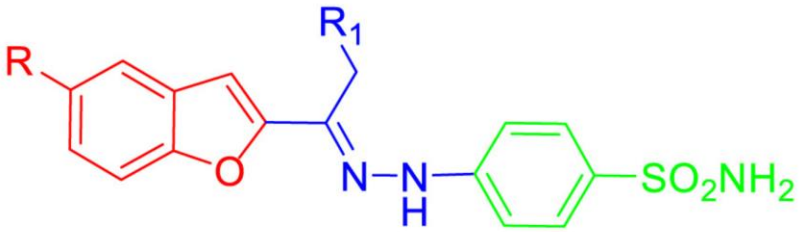


6c; *h*CA IX (K_Is = **6.2** nM)



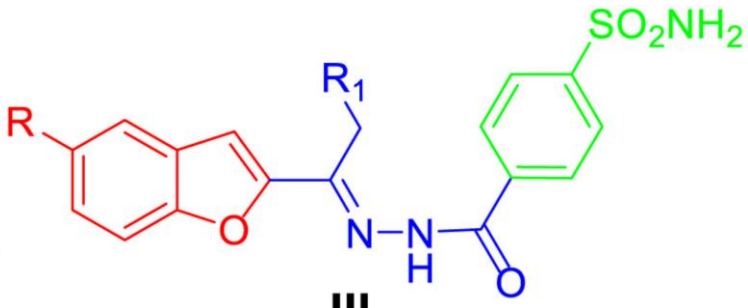
I

*h*CA IX: K_Is range 0.56 - 5.1 μM



II

*h*CA IX: K_Is range 10.0 - 76.6 nM



III

*h*CA IX: K_Is range 27.7 - 97.5 nM

Eur. J. Med. Chem. 2022;228:114008; Eur. J. Med. Chem. 2021;216:113283

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What about bacterial CAs ?

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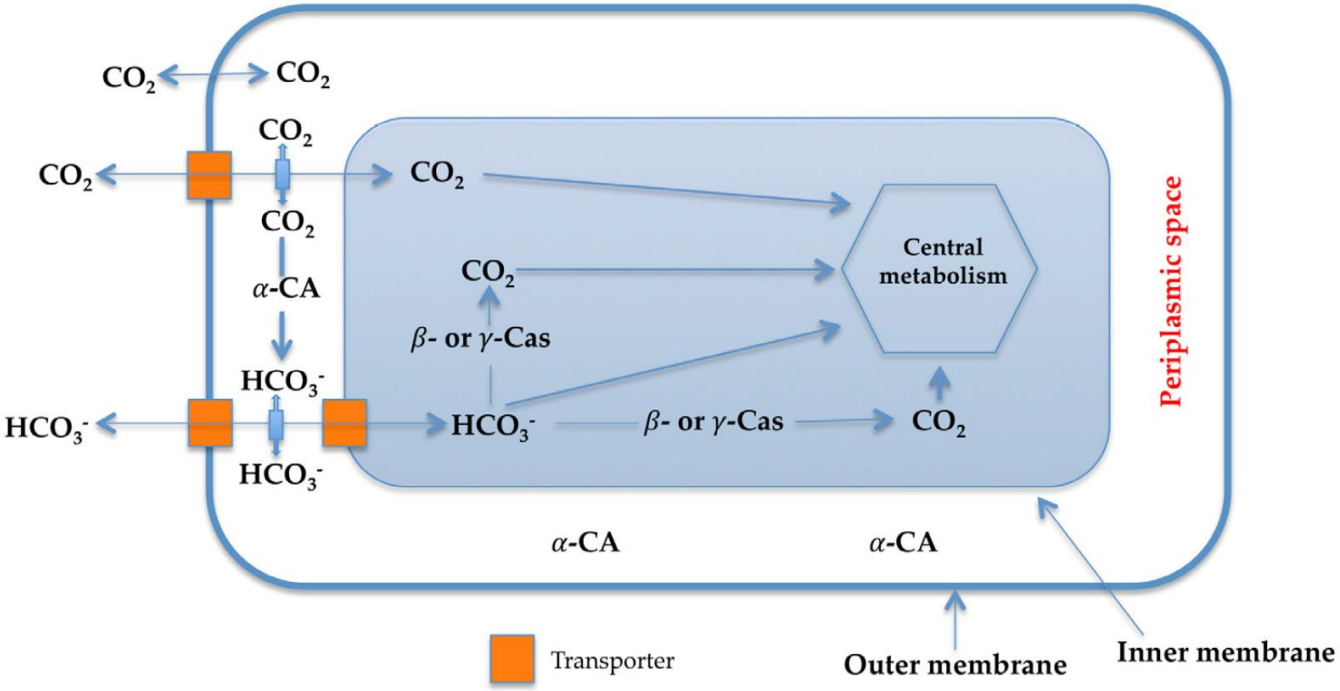
Bacterial CA



Inhibition of CA:

- disruption of essentials metabolic pathways
- growth retardation
- growth defects
- volnurable to host defense mechanism

 Expert Opin. Ther. Pat. 2018; 28:745-754

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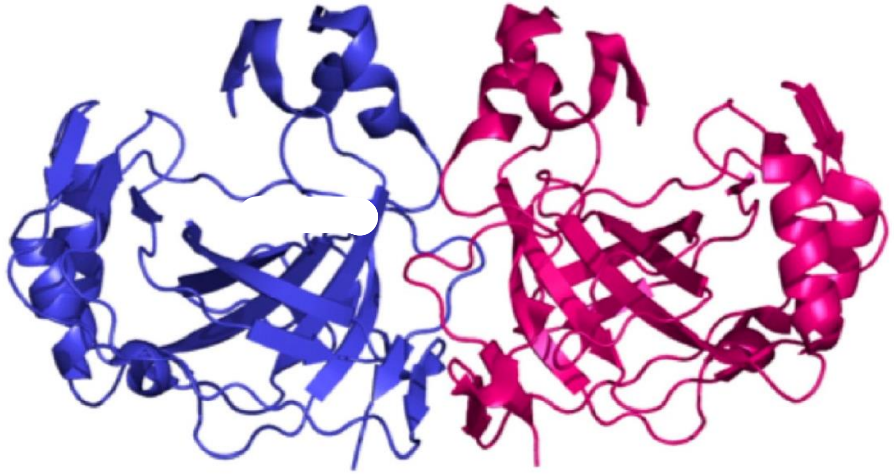
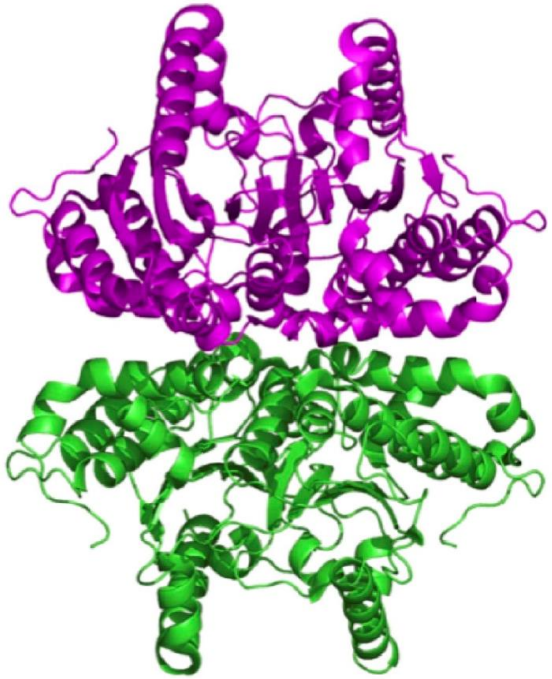
Bacterial CA

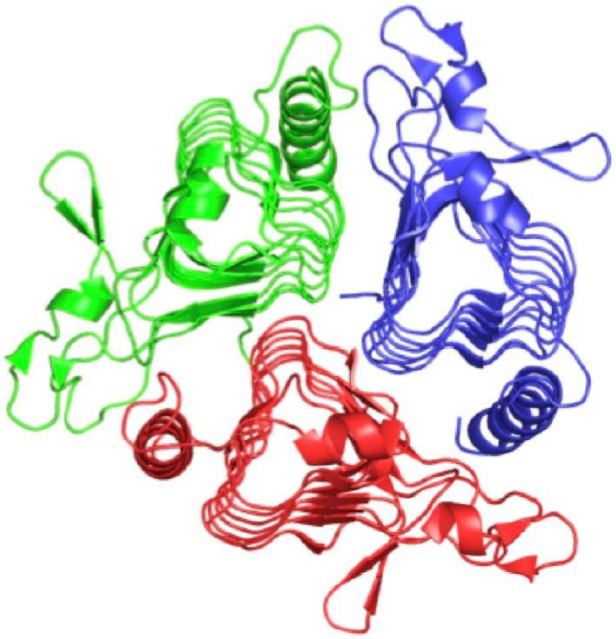
α-CA



β-CA



γ-CA



 Expert Opin. Ther. Pat. 2018; 28:745-754

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Does it have potential?

- Wide range of disseases
- Effective treatment
- Great potential
- But……..

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Thank you for your attention.