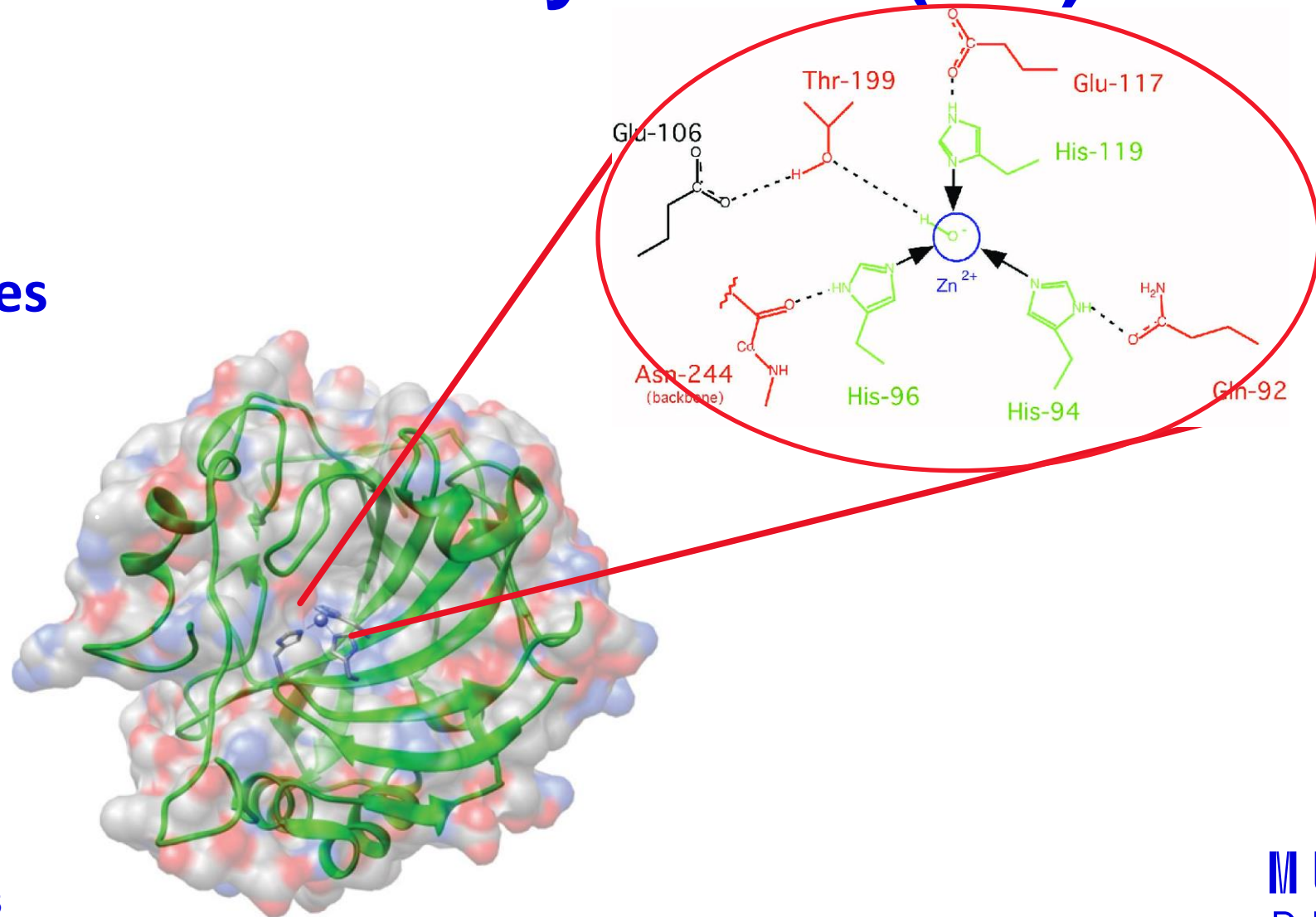


Carbonic anhydrases: One target for multiple diseases

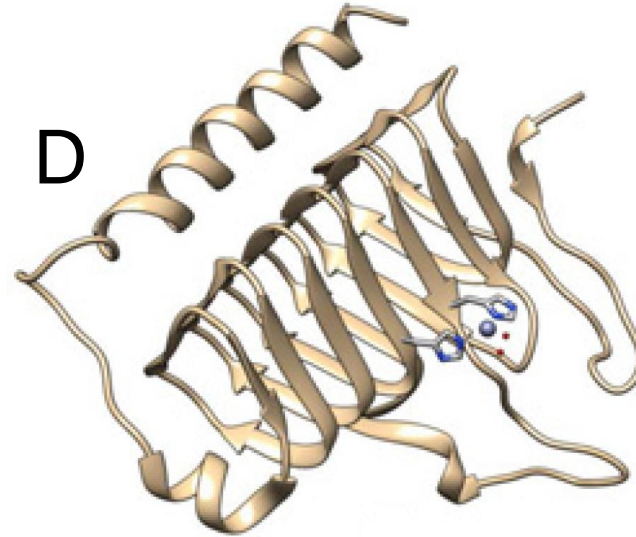
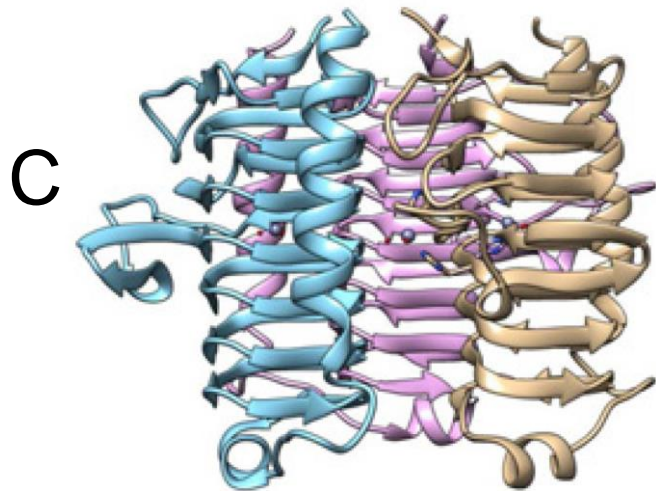
RNDr. Eva Havránková, PhD.

What are carbonic anhydrases (CA)

- CA, EC 4.2.1.1
- Metalloenzymes



Structure



CA families

α -CAs	vertebrates, protozoa, algae, cytoplasm of green plants, Gram ⁻ bacteria
β -CAs	Gram ⁻ bacteria, Gram ⁺ bacteria, algae, chloroplasts of mono- and dicotyledons, fungi, <i>Archaea</i>
γ -CAs	<i>Archaea</i> , 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

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

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

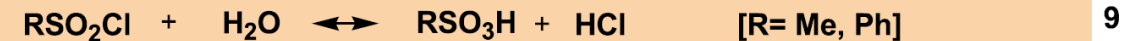
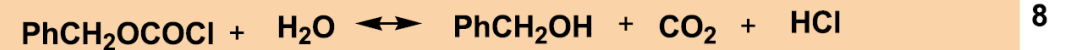
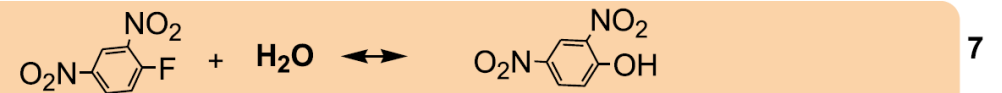
Kidney, brain, lung, gut, and reproductive tract

CA XIV

Kidney, brain, liver, and eye

hCAs are involved in...

- Respiration
- Transportation of CO₂ and bicarbonate
- pH and CO₂ homeostasis
- Electrolyte secretion in various tissues
- Biosynthetic reactions
- Gluconeogenesis
- Adipogenesis
- Ureagenesis
- Bone resorption
- Calcification



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

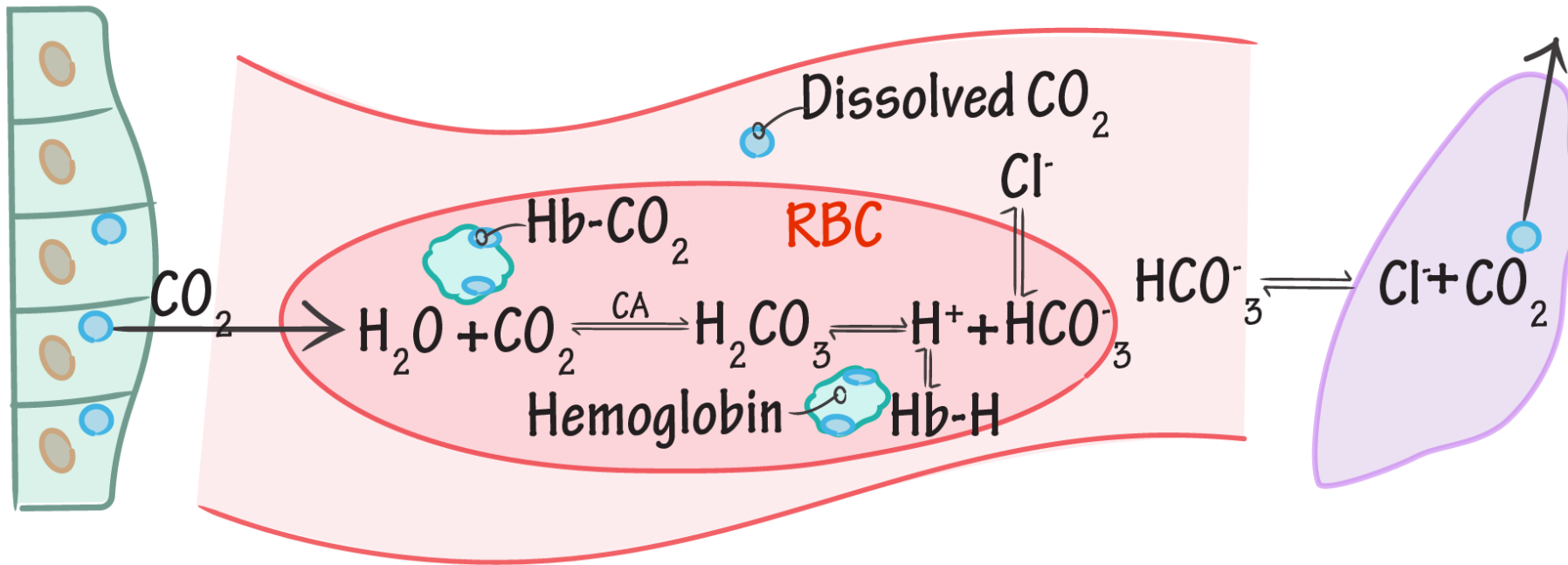
CA in the breathing cycle

Tissues

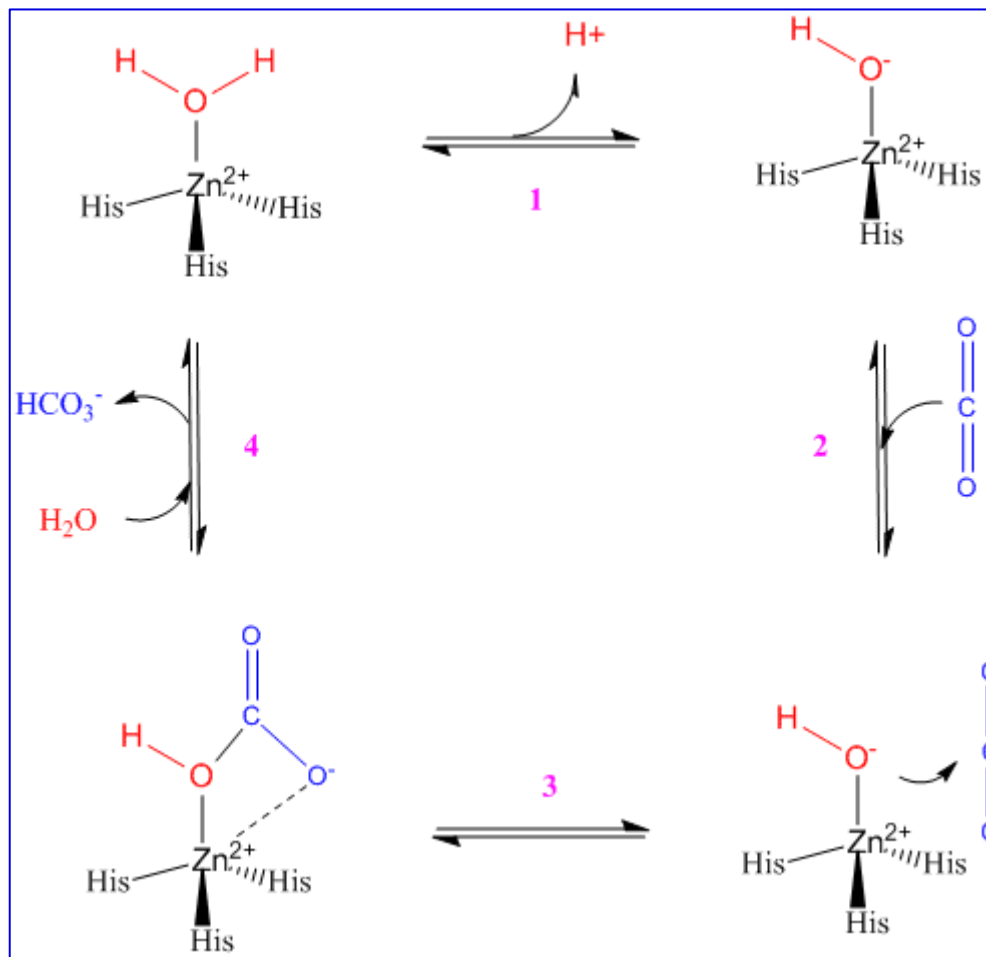
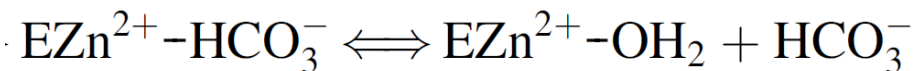
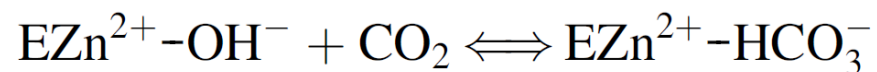
Blood

Lungs

CA II
CA I



CA Mechanism of Action



How CA can be used in therapy?

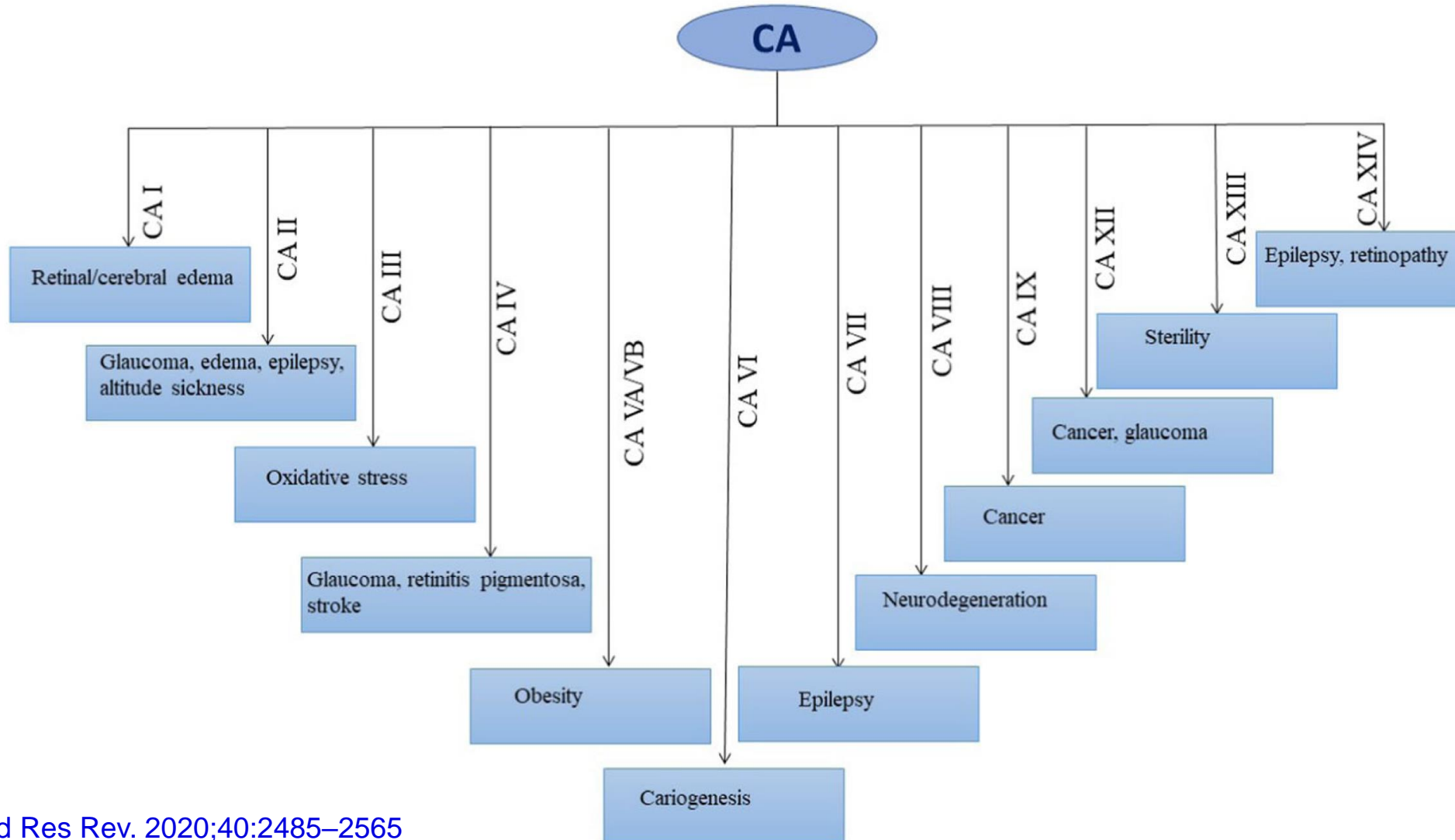
Overexpression of CAs

Inhibitors
of CAs

Activators
of CAs

Deficit of CAs

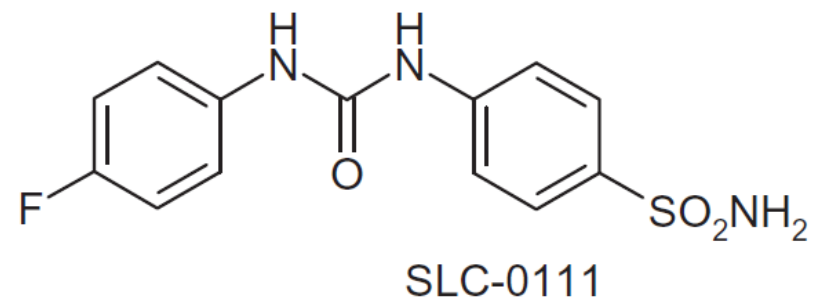
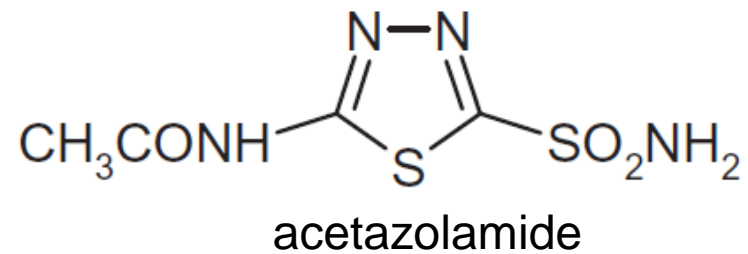
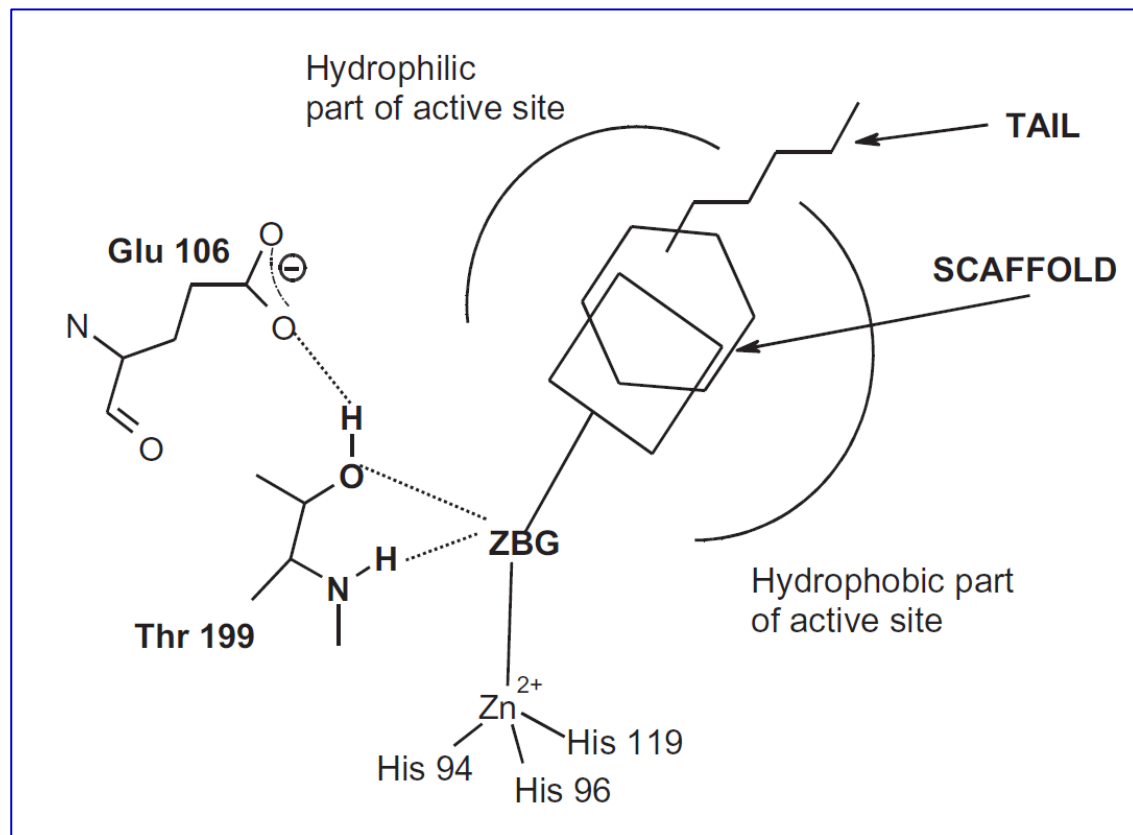
Overexpression/deficit of CAs



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

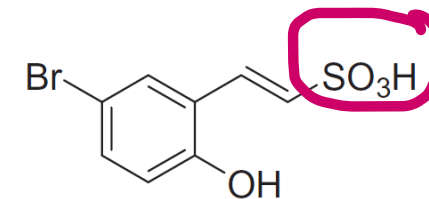
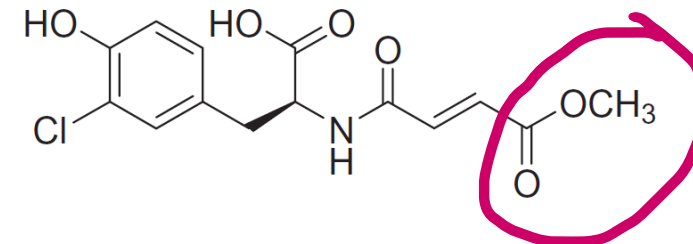
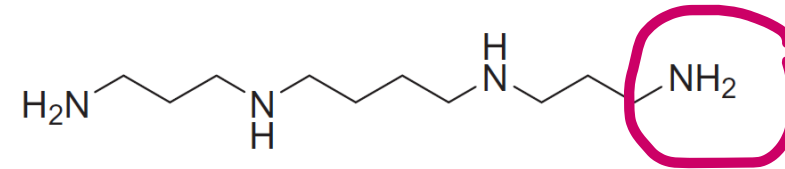
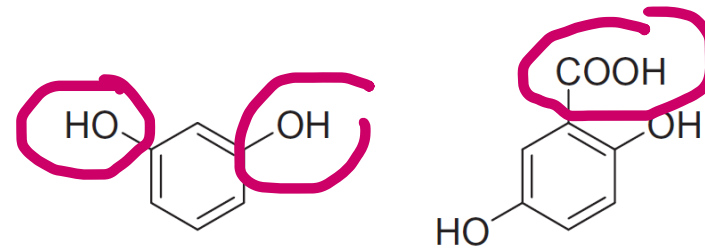
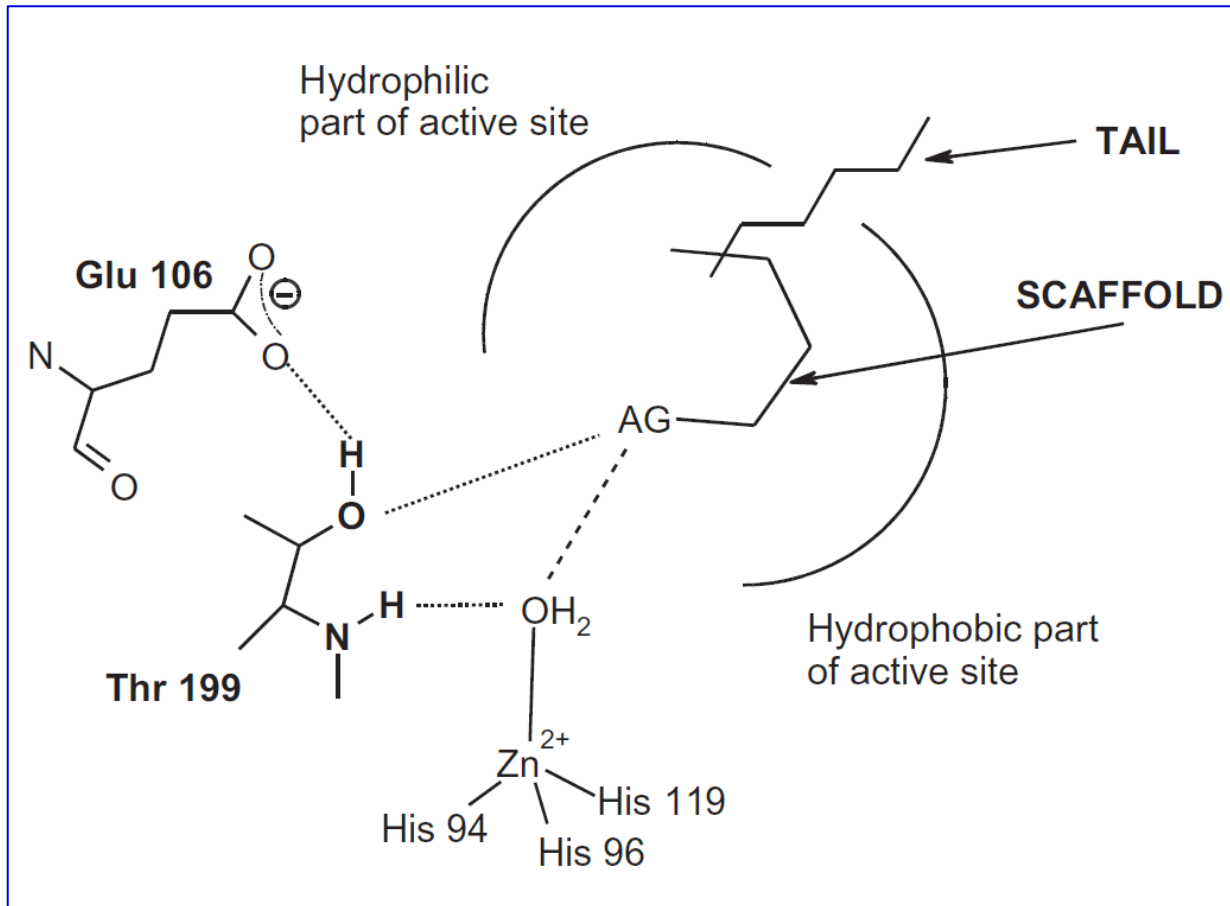
CA Inhibitors Mechanism of Action

The Zinc Binders



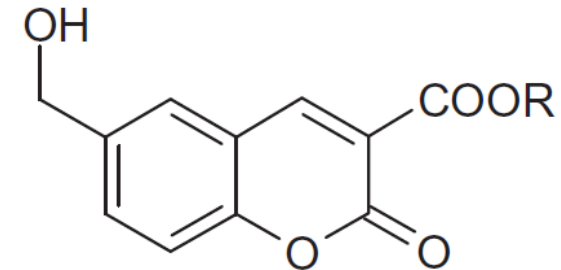
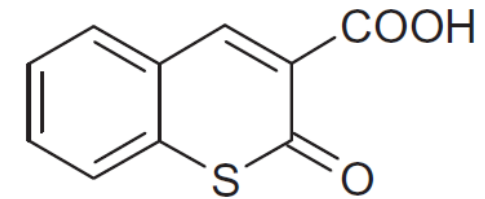
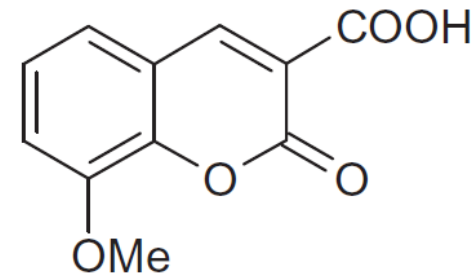
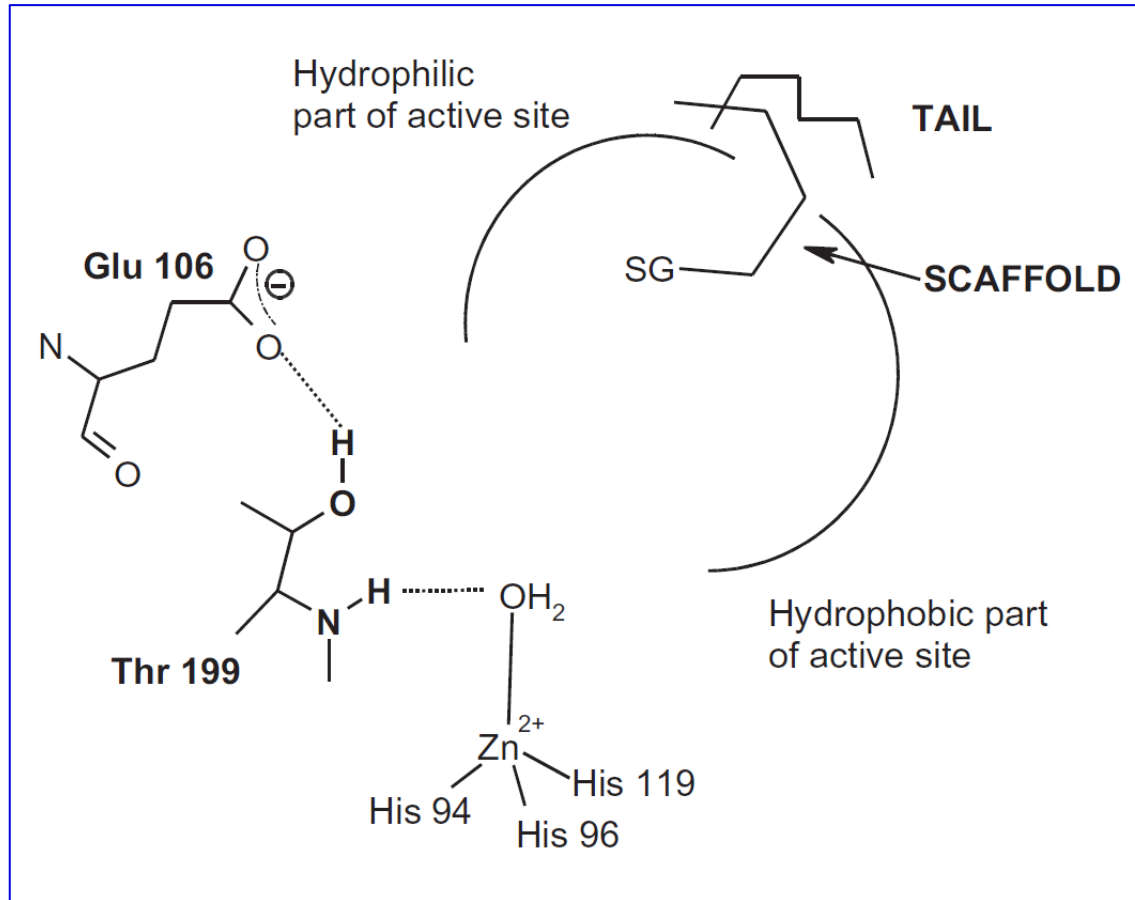
CA Inhibitors Mechanism of Action

CAIs anchoring to the zinc-coordinated water/hydroxide ion



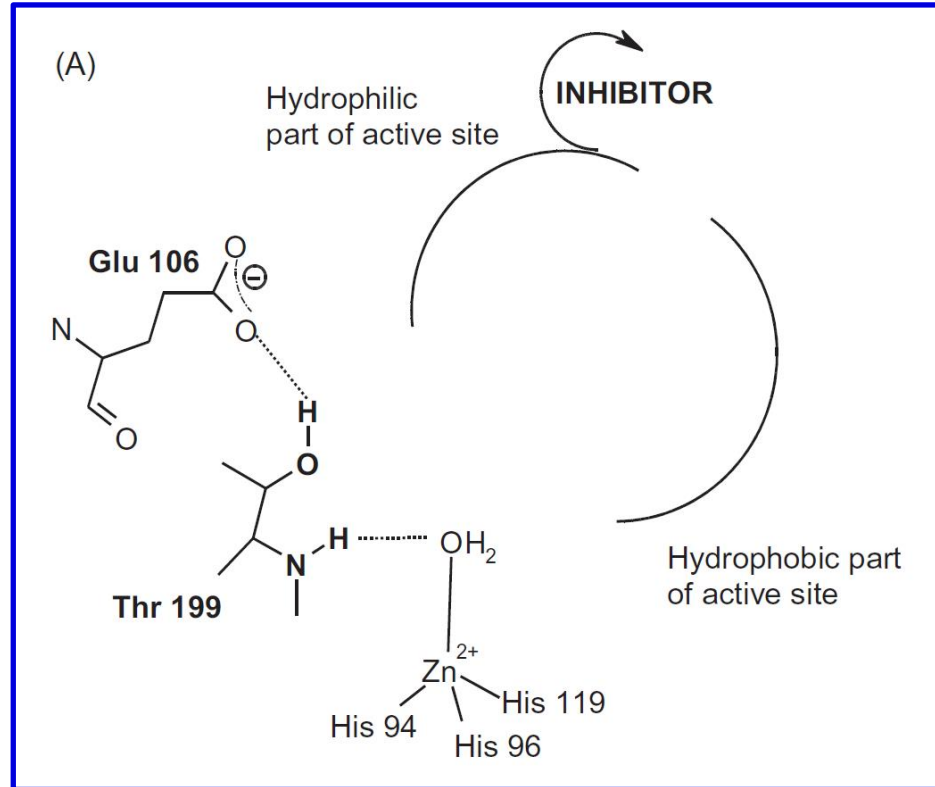
CA Inhibitors Mechanism of Action

CA inhibition by occlusion of the active site entrance

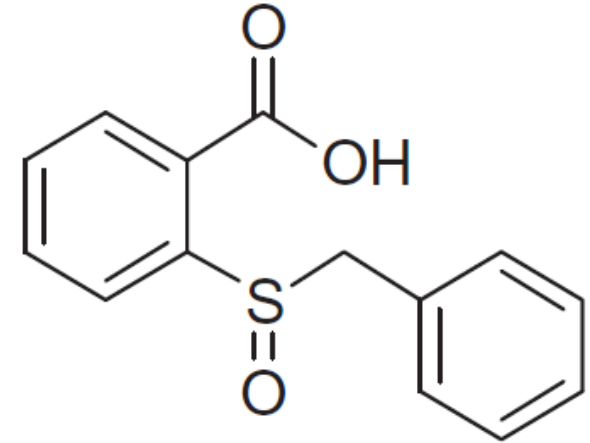
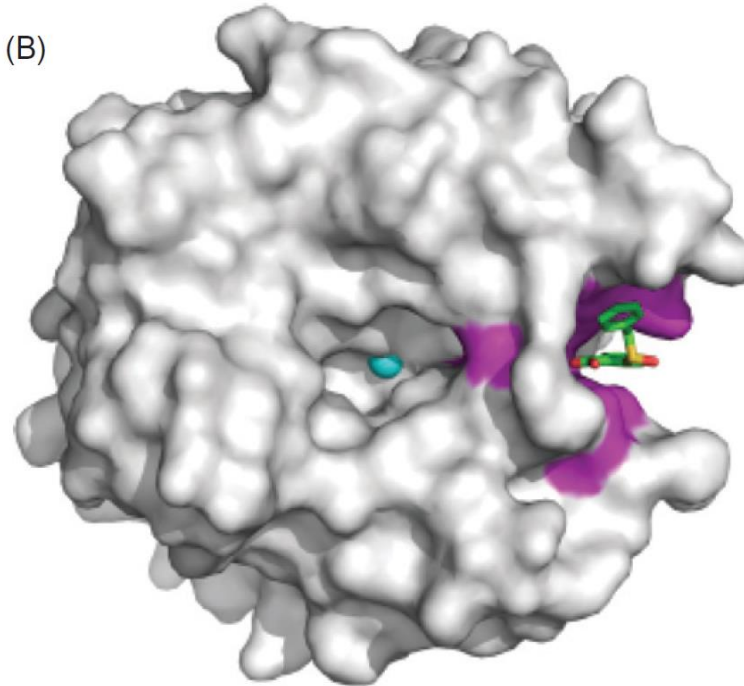


CA Inhibitors Mechanism of Action

Out of the active site binding as a CA inhibition mechanism

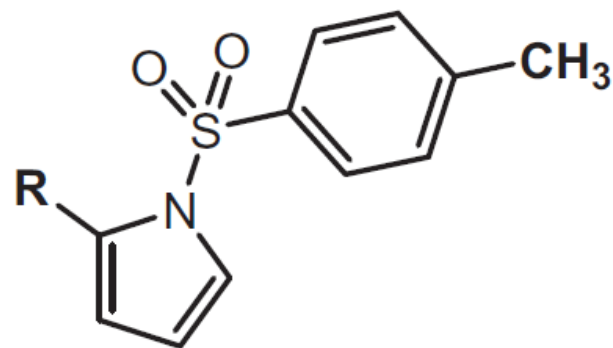
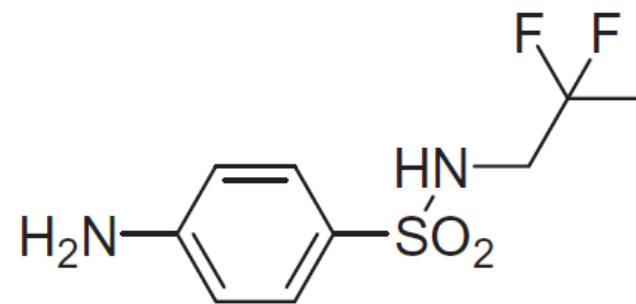
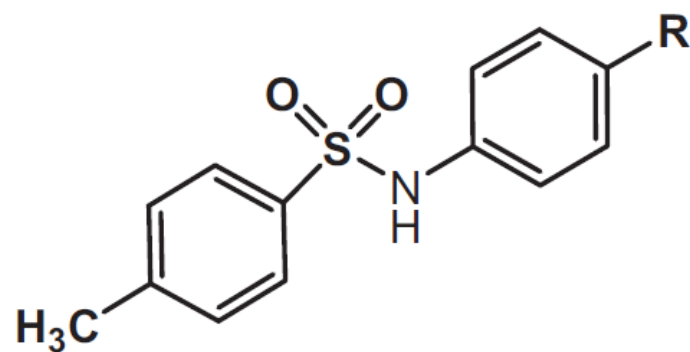
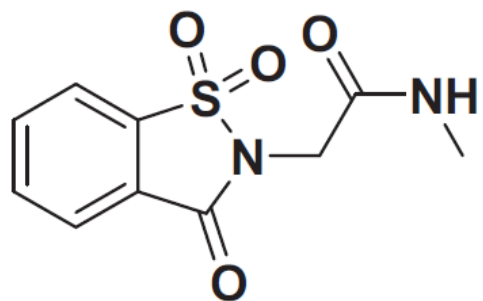


(B)

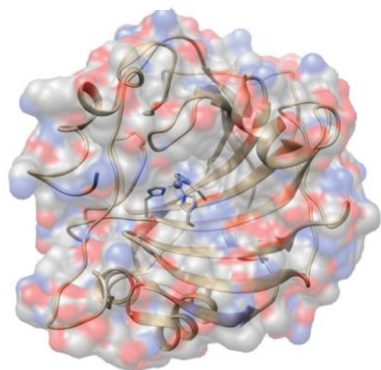


CA Mechanism of Action

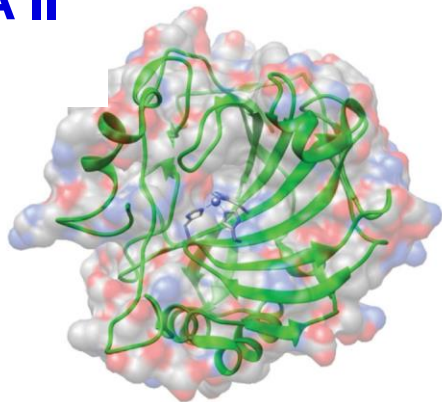
Compounds acting as CAIs with an unknown mechanism of action



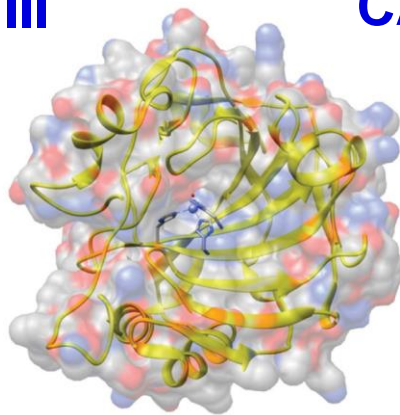
CA I



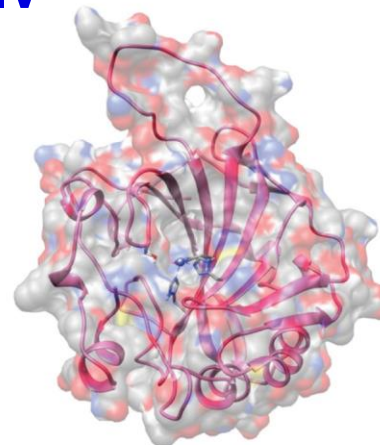
CA II



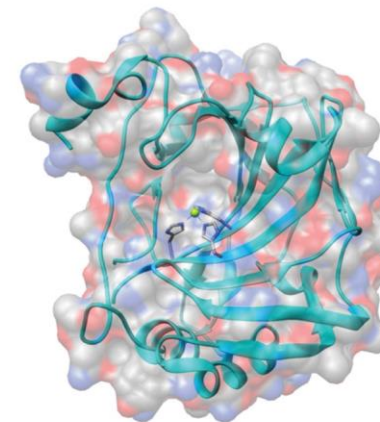
CA III



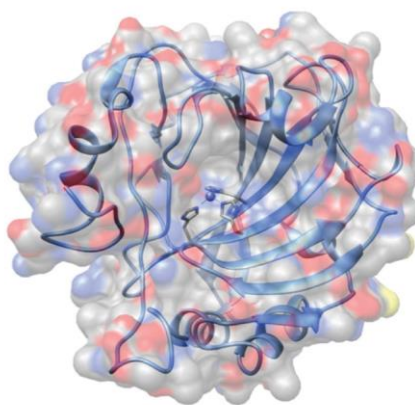
CA IV



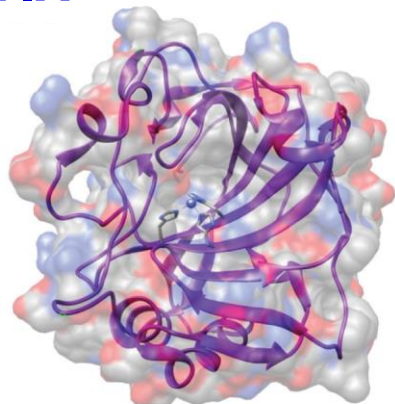
CA VI



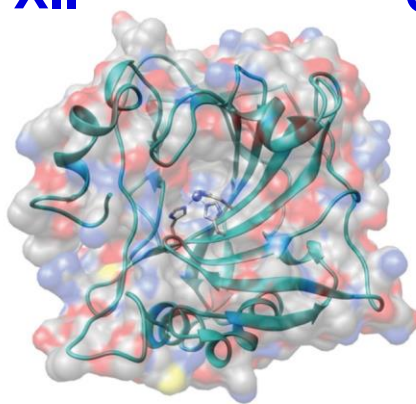
CA VII



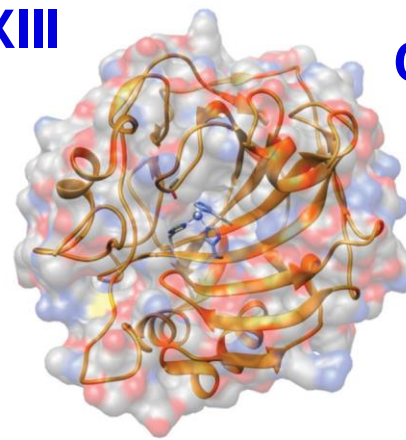
CA IX



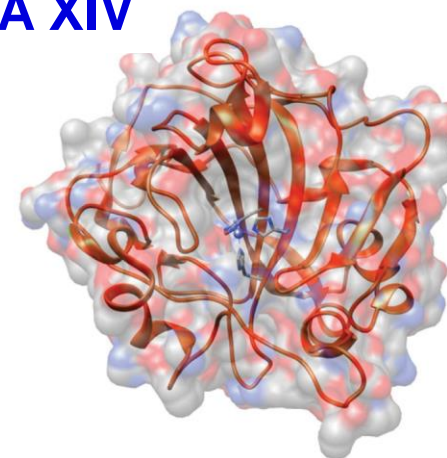
CA XII



CA XIII



CA XIV



How to discover new selective inhibitors ?

**How to
discover new
selective
inhibitors ?**

Knowledge of the
structure

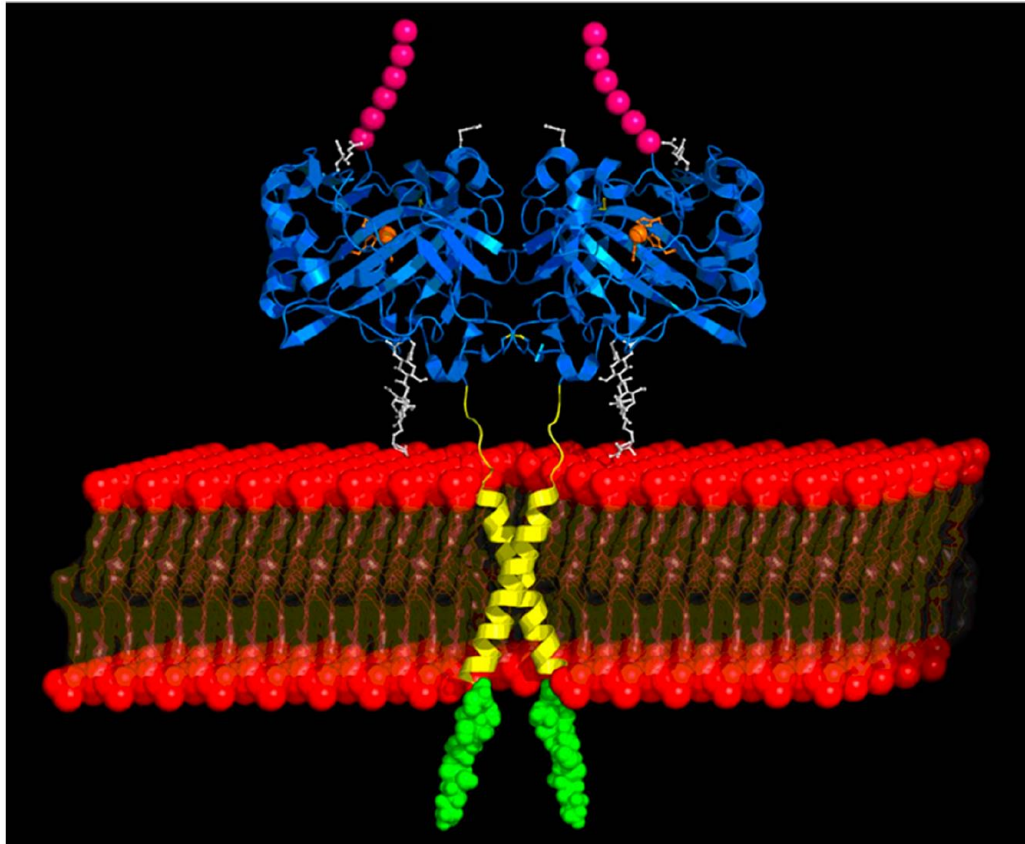
Screening of
anions

Screening of
drugs

Combination of
approaches

Steal other
people's work

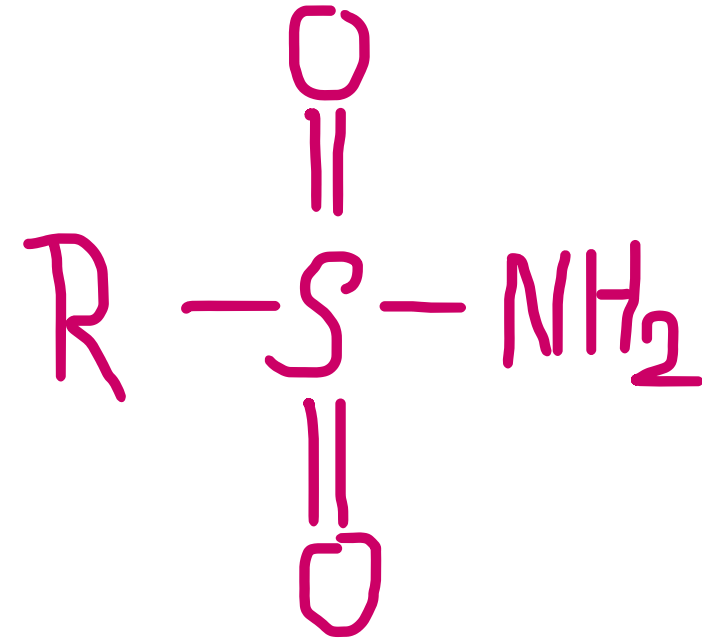
Knowledge of the structure



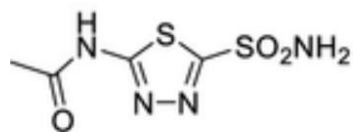
- X-ray crystal structure of CA IX
- NMR
- other

Screening of anions

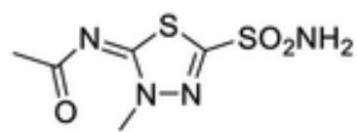
	K _i [mM]					
	hCA I ^a	hCA II ^a	hCA III ^a	hCA VII ^f	hCA IX ^g	hCA XII ^h
F ⁻	> 300	> 300	78.5	1.24	48	0.56
Cl ⁻	6	200	0.98	1.84	33	73
Br ⁻	4	63	0.96	1.06	16	82
I ⁻	0.3	26	0.90	0.25	7	215
CN ⁻	0.0005	0.02	0.06	9.2	0.004	0.001
CNO ⁻	0.0007	0.03	0.57	15.2	0.043	0.73
SCN ⁻	0.1	1.6	0.09	0.17	0.13	0.80
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 ₂ NSO ₃ ⁻	0.021	0.39	31.1	0.0095	0.09	0.70
H ₂ NSO ₂ NH ⁻	0.31	1.13	1.09	0.0068	0.096	0.83



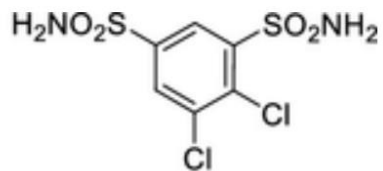
Screening of known drugs



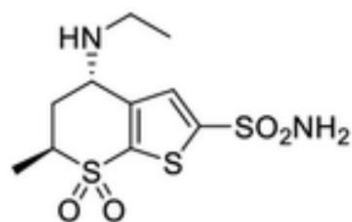
AAZ



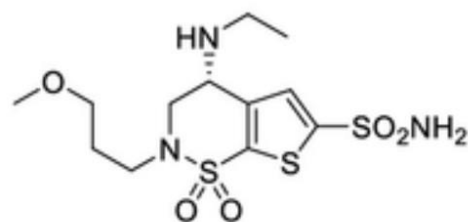
MTZ



DCP



DZA

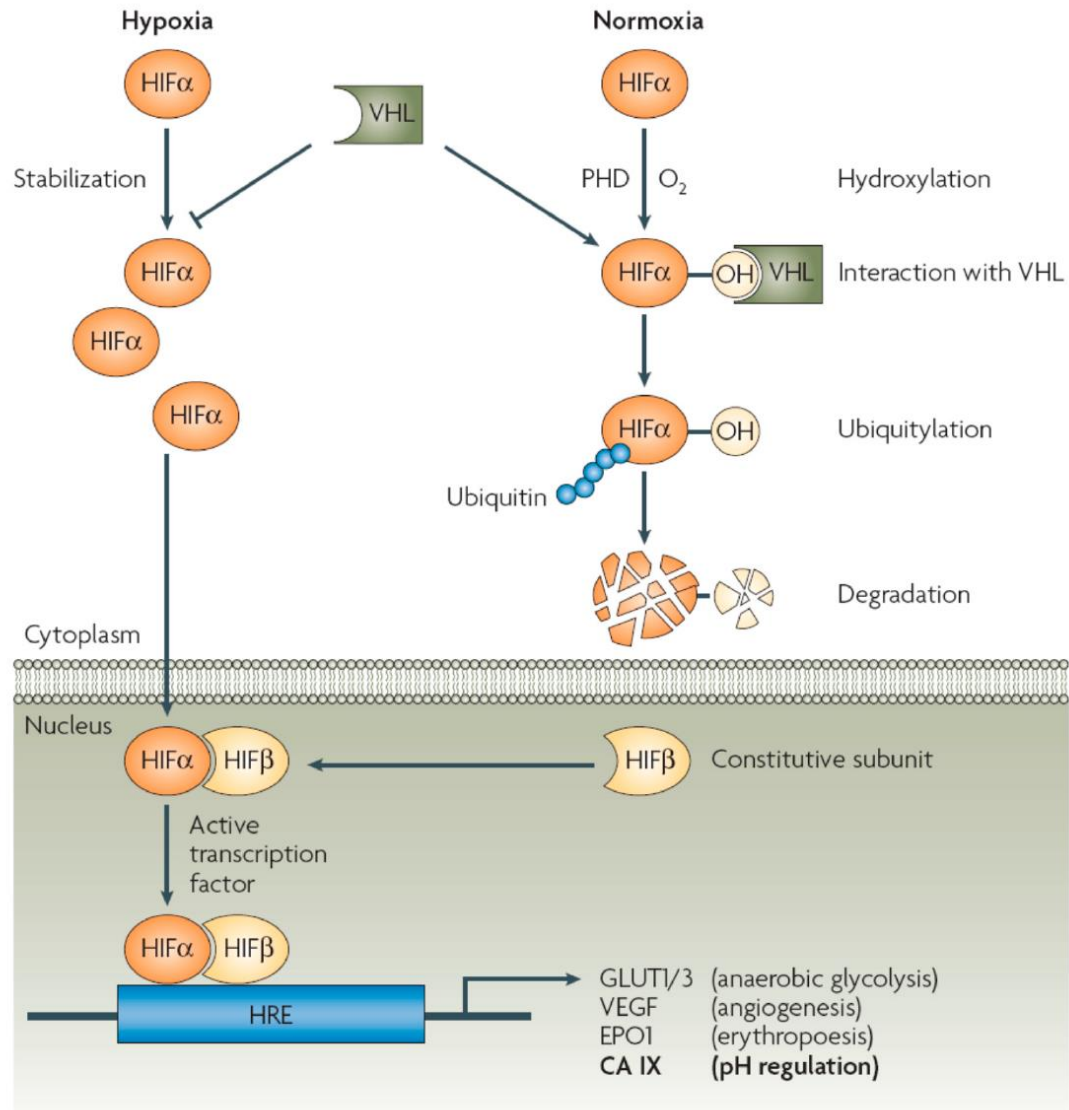


BRZ

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

How to treat cancer – Inhibition of hCA IX ?

Which isoenzyme is involved in the cancer?



Hypoxia → HIF-1 → aggressive tumor

hCA IX – How it helps the tumor

Catalytic
hydration of
CO₂

Maintaining
pHe and pHi

Up-regulation of
metabolism

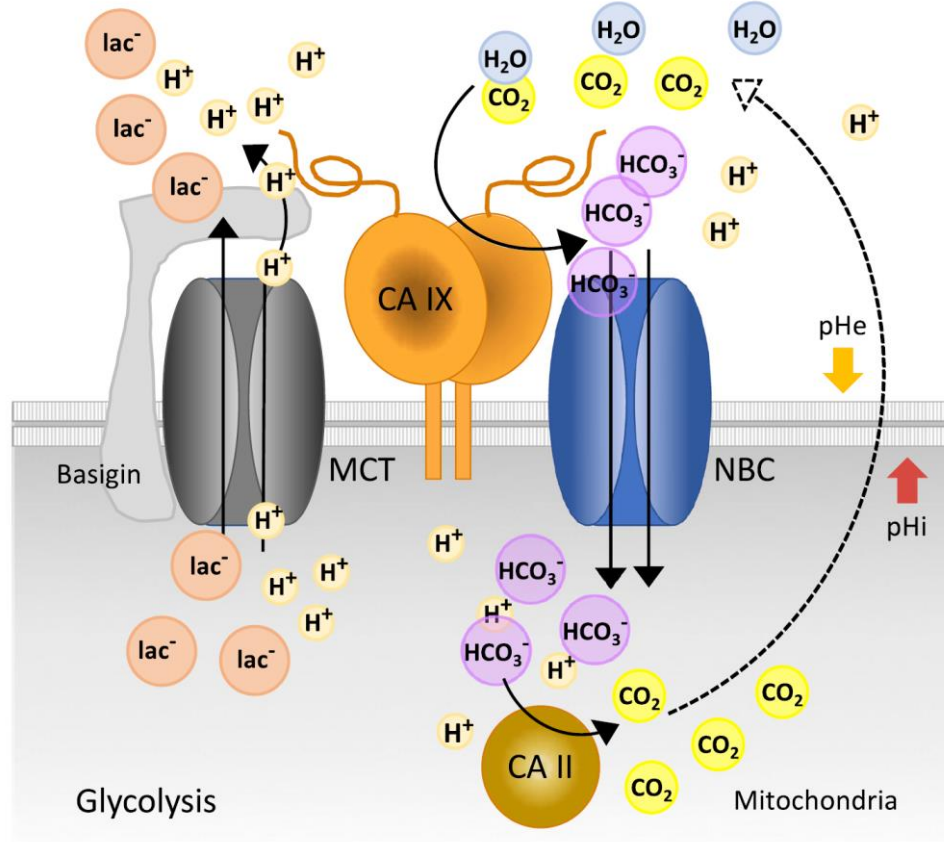
Increased
adhesion ability

Migration
Invasion
Metastasis

Interaction with
signal cascades

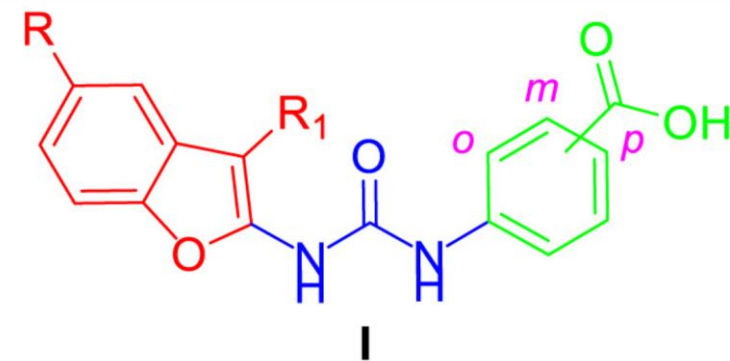
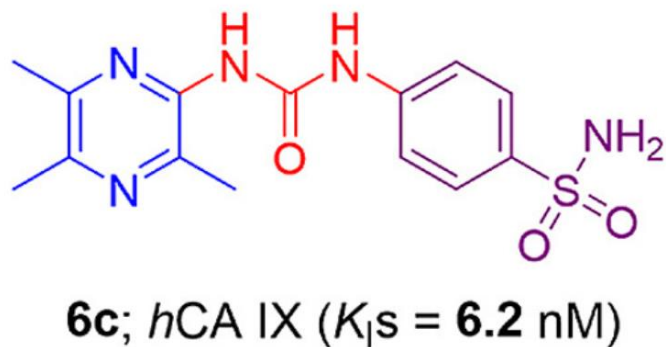
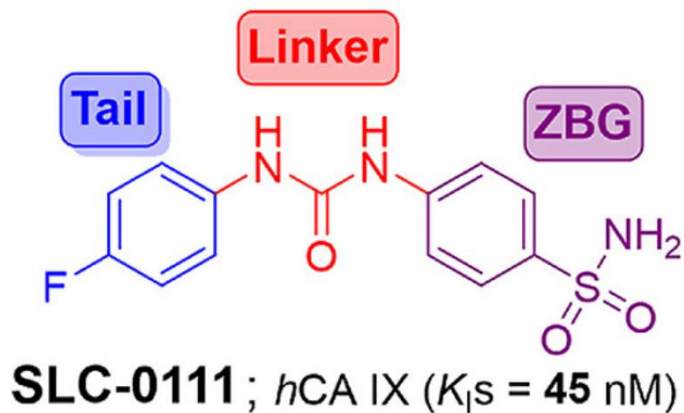
Support of
angiogenesis

What the hCA IX inhibitors do?

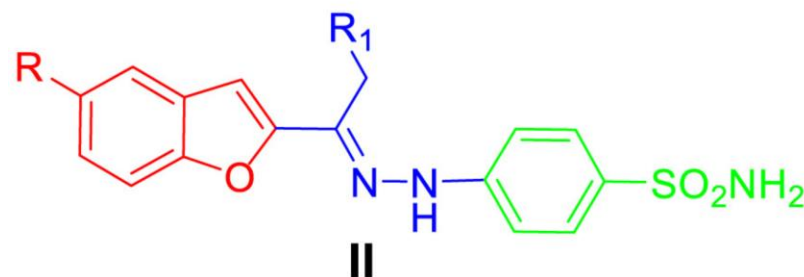


- No cytotoxicity (cytostatic effect)
- Inhibit all of the previously mentioned processes
- Prevents the formation and spread of metastasis
- Multiply increases sensitivity to existing treatments

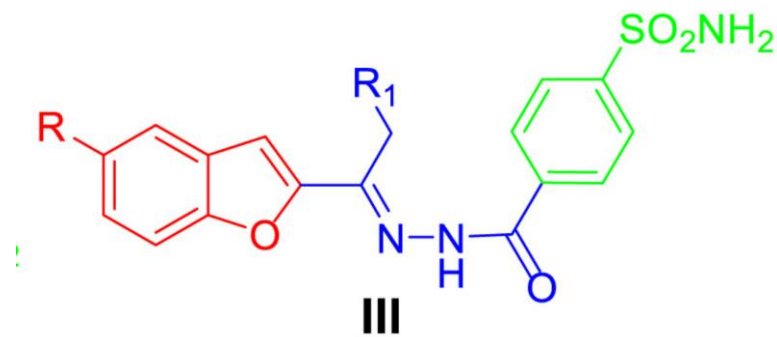
Inhibitors of CA IX



hCA IX: K_{iS} range 0.56 - 5.1 μ M



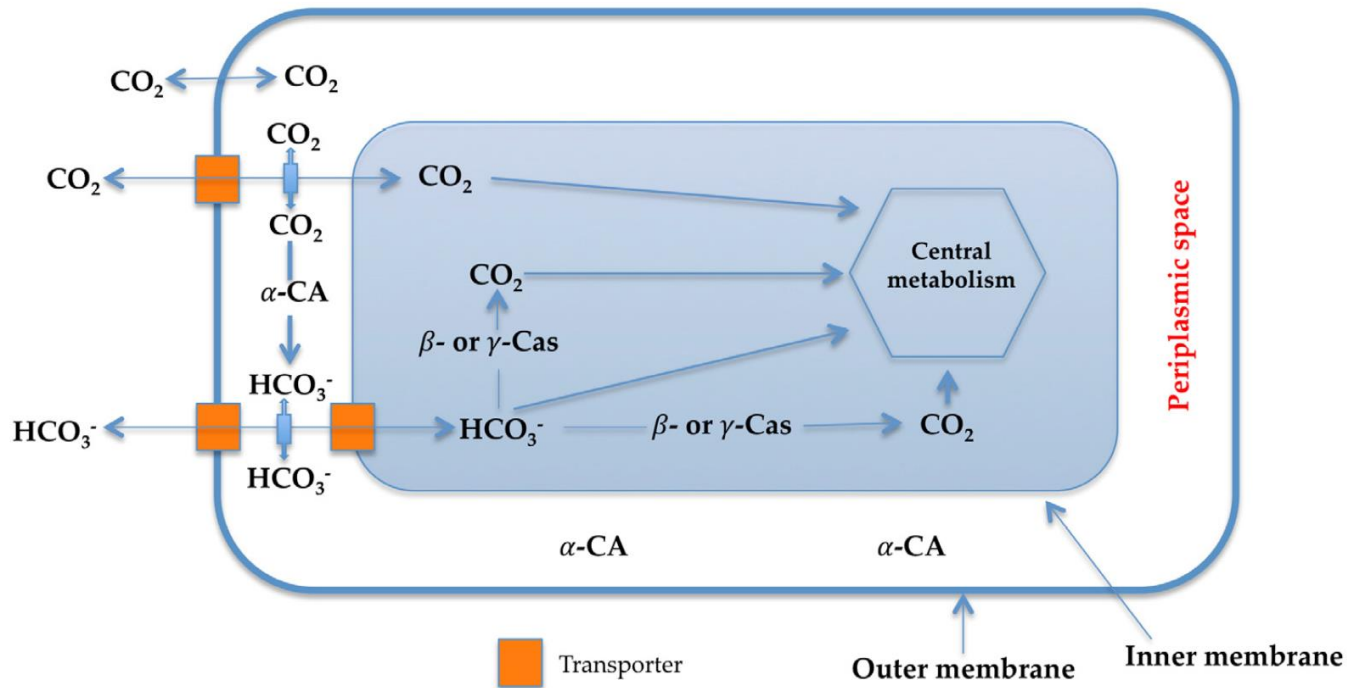
hCA IX: K_{iS} range 10.0 - 76.6 nM



hCA IX: K_{iS} range 27.7 - 97.5 nM

What about bacterial CAs ?

Bacterial CA

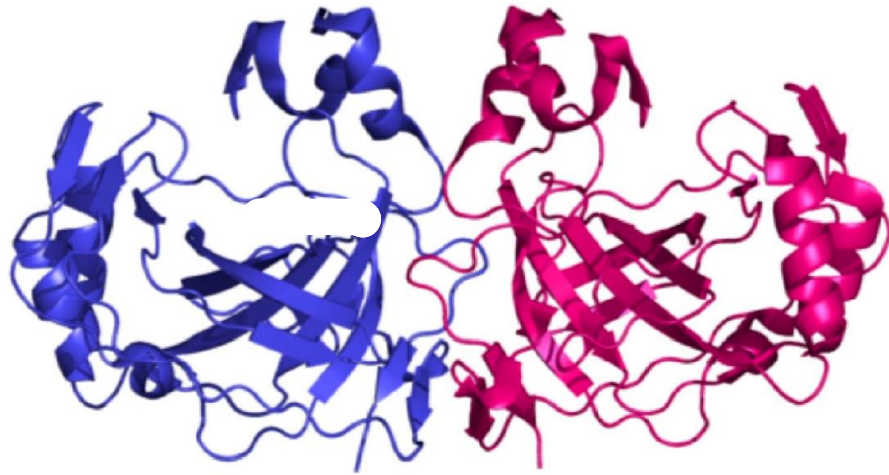


Inhibition of CA:

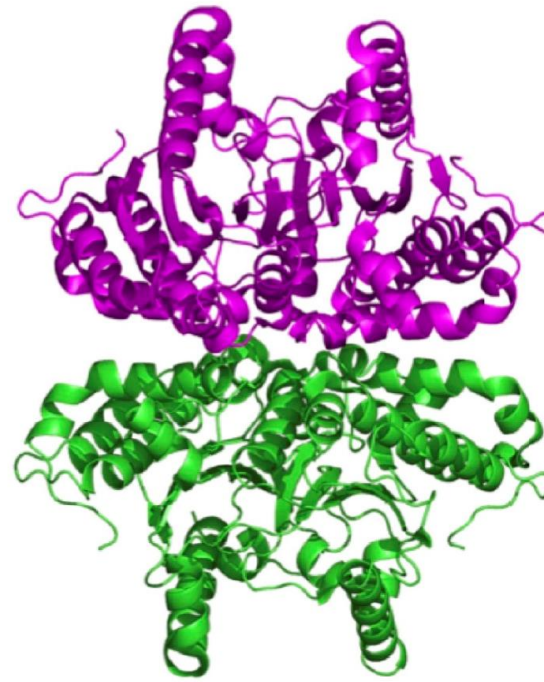
- disruption of essential metabolic pathways
- growth retardation
- growth defects
- vulnerable to host defense mechanism

Bacterial CA

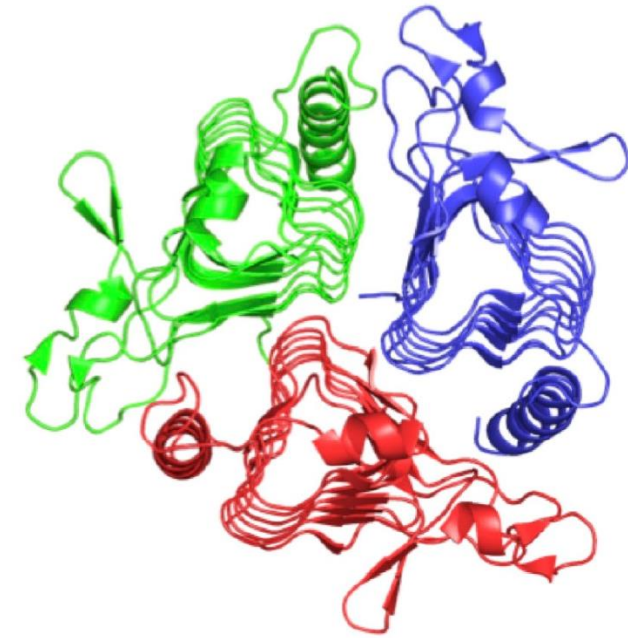
α -CA



β -CA



γ -CA



Does it have potential?

- Wide range of diseases
- Effective treatment
- Great potential
- But.....

M U N I
P H A R M

Thank you for your attention.