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Librarii constitutionale dinamice aplicate la descoperirea de inhibitori ai anhidrazei carbonice

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

5 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

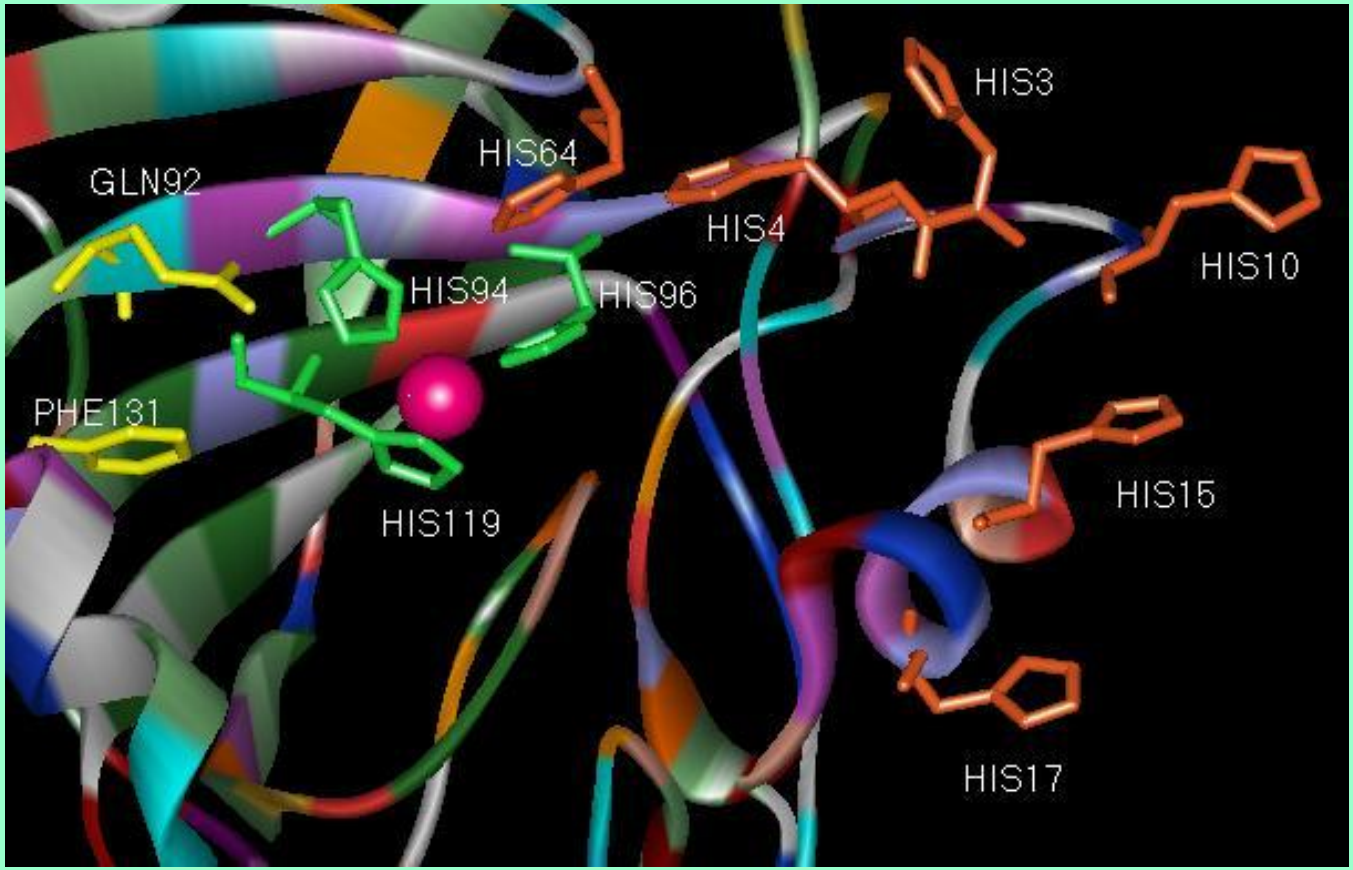
These gene families are evolutionary unrelated.

(Supuran, CT. *Bioorg Med Chem Lett* **2010**, *20*, 3467-3474.)

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**
hCA XIII	**low**	**high**	**cytosol**
hCA XIV	**low**	**high**	**transmembrane**
mCA XV	**high**	**high**	**plasma membrane**

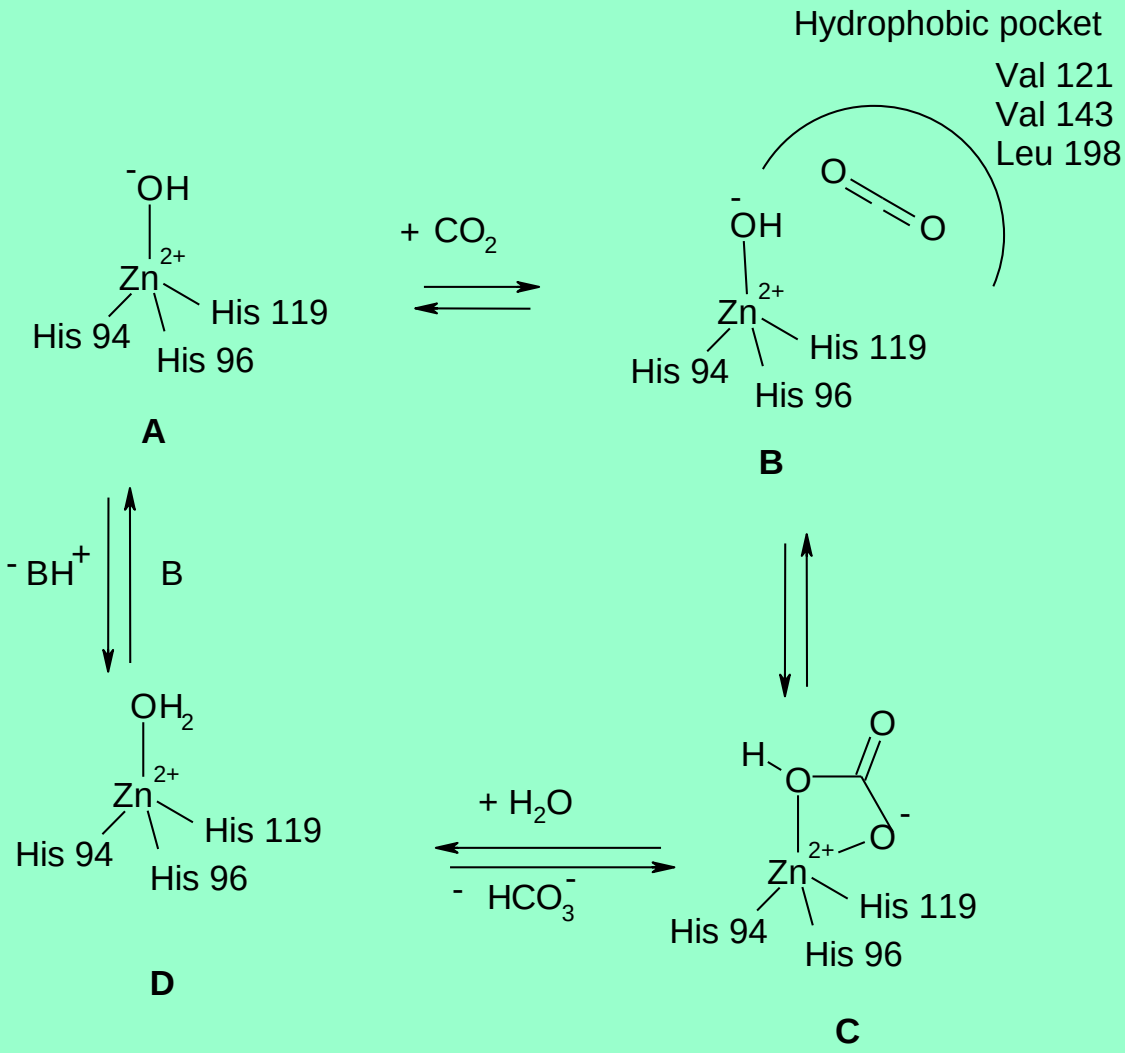
h = human, m = murine isoforms



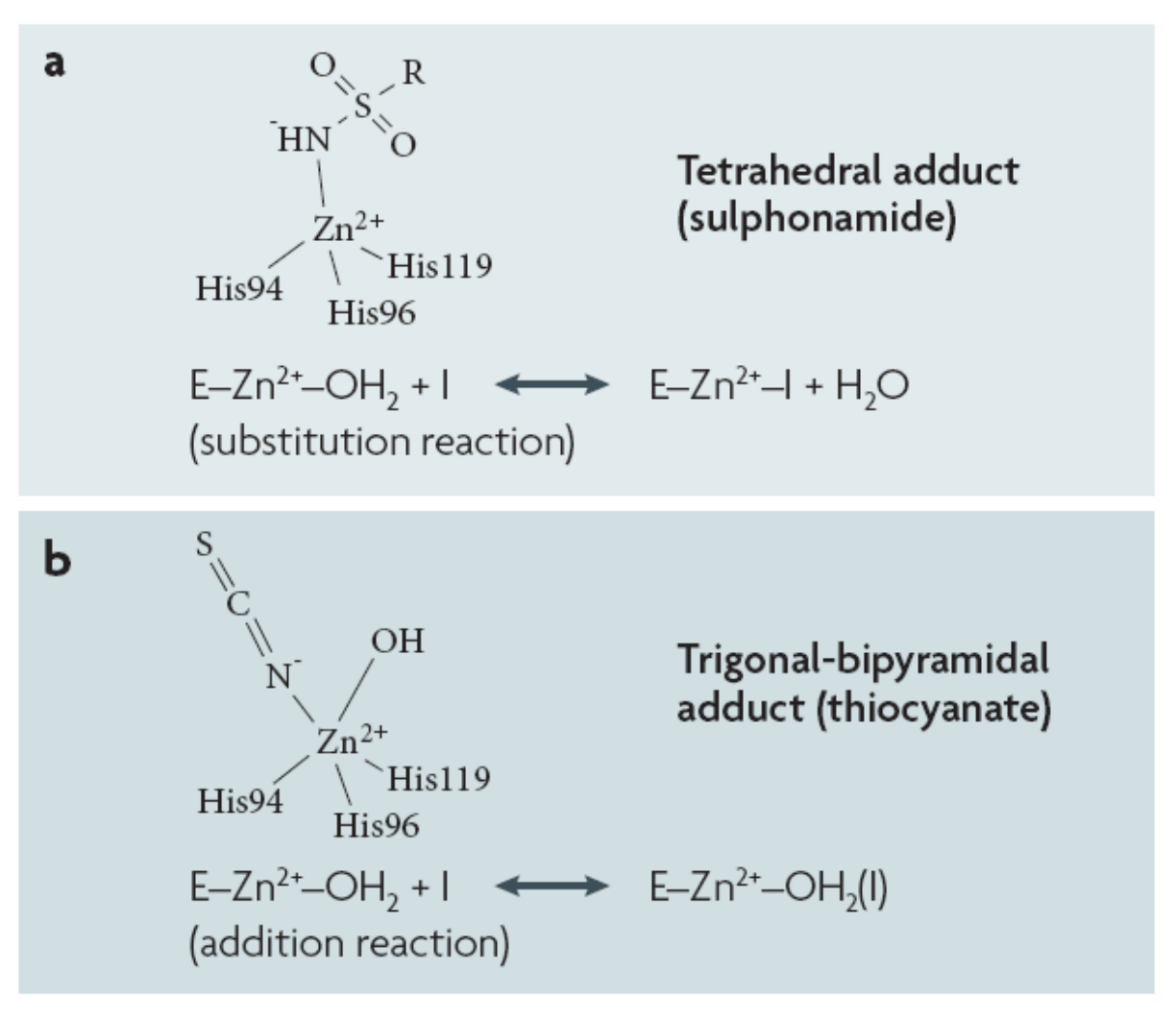
hCA II active site, with the Zn(II) ion (pink sphere), its three histidine ligands (His 94, His 96 and His 119, in green), the proton shuttle residue His 64 as well as the histidine cluster extending from the rim of the active site to the surface of the protein, comprising residue 3, 4, 10, 15 and 17,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

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

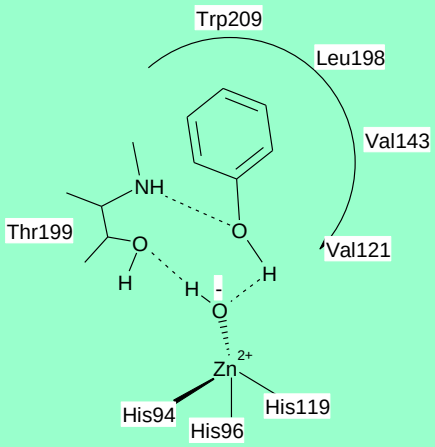
Catalytic mechanism of α-CAs



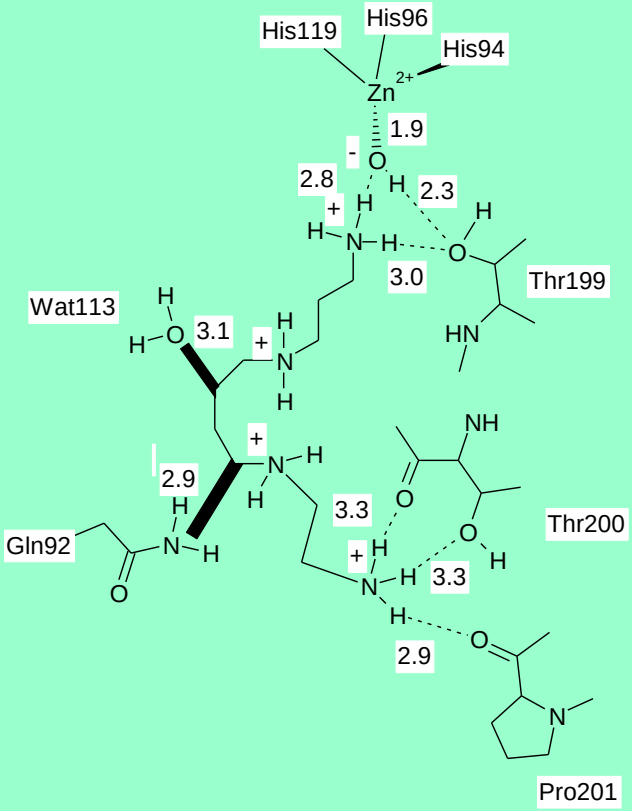
CA inhibition mechanism (by sulfonamides (a) and anions (b), cf. Supuran, CT. *Nature Rev Drug Discov* **2008**, *7*, 168-181; Supuran, CT. *Bioorg Med Chem Lett* **2010**, *20*, 3467-3474.

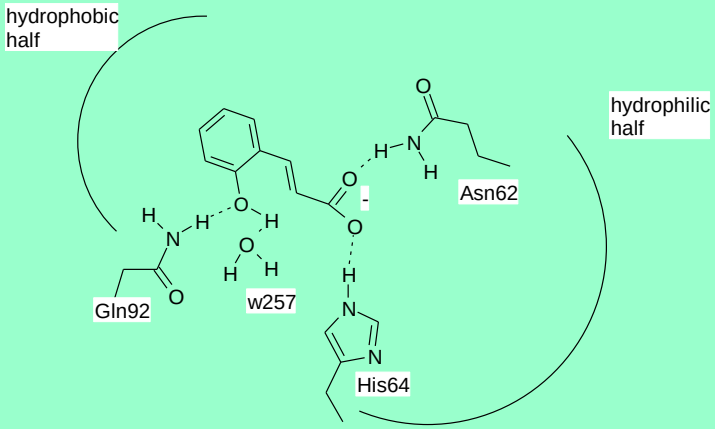


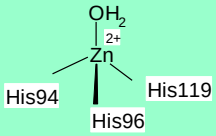
A



B







C

Inhibition mechanisms with:
A: Phenols
B: Polyamines (spermine)
C: Coumarins

Sulfonamide CAIs in clinical use :

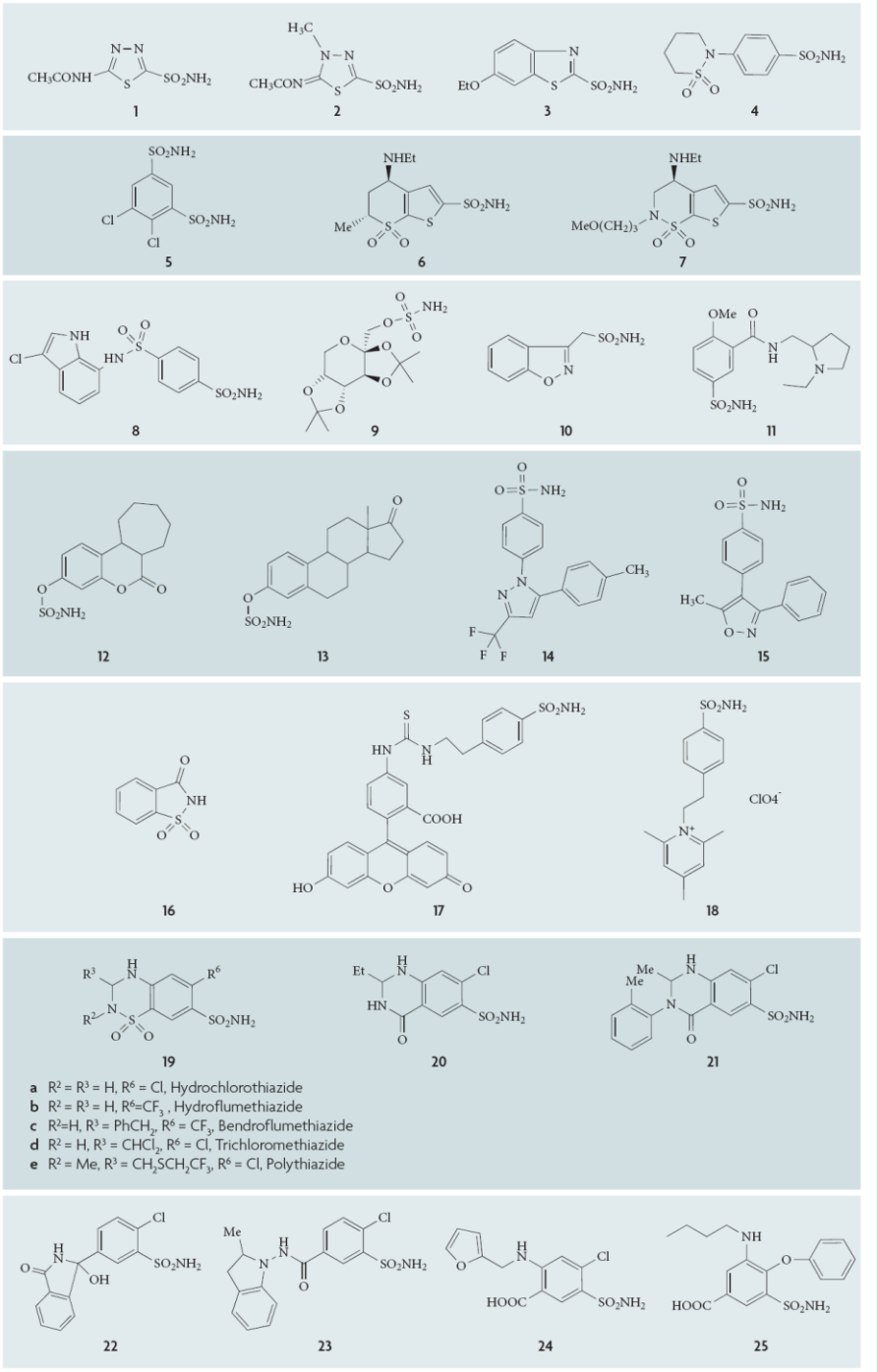
Classical use (since 1954):

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. Anti-infectives

Different isozymes/enzyme classes are targeted by such drugs



Sulfonamides/sulfamates used clinically (more than 30 compounds)

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

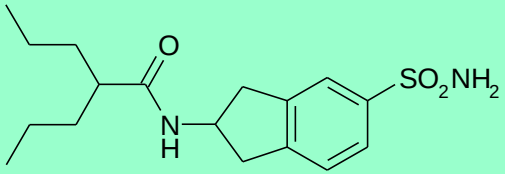
Table 1 | **Inhibition data with selected sulphonamides/sulphamates/sulphamides 1–25 against isozymes I–XIV***

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

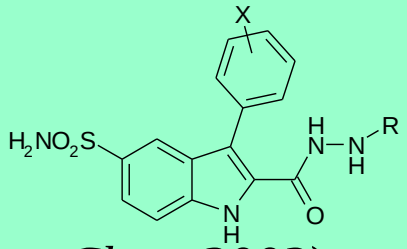
Drug design of CAIs



I. Clasical inhibitors (sulfonamides and their bioisosteres)

1. The ring approach

Indanesulfonamides (Masereel et al);
Indolesulfonamides (Guzel et al)



2. The tail approach

a) Click-tailing (Poulsen et al., based on Sharpless et al, *Angew Chem* 2002)

b) Sugar approach (Winum et al., *Med. Res. Rev*. 2009)

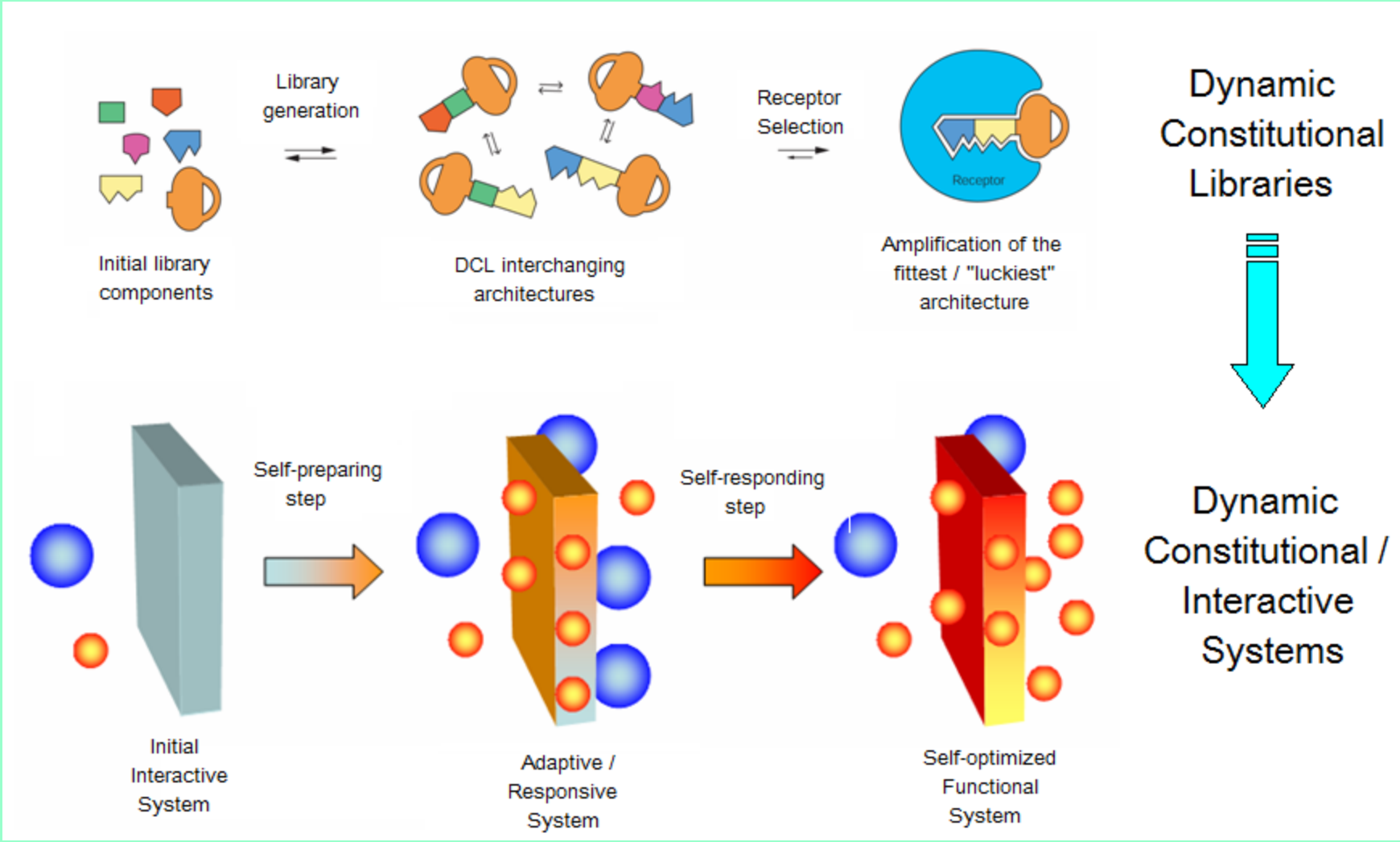
c) Two-prong (based on Scozzafava et al., *J Med Chem*. 2002)

3. Dynamic constitutional libraries (DCL) – Barboiu et al 2009

II. Non-classical CAIs, new chemotypes

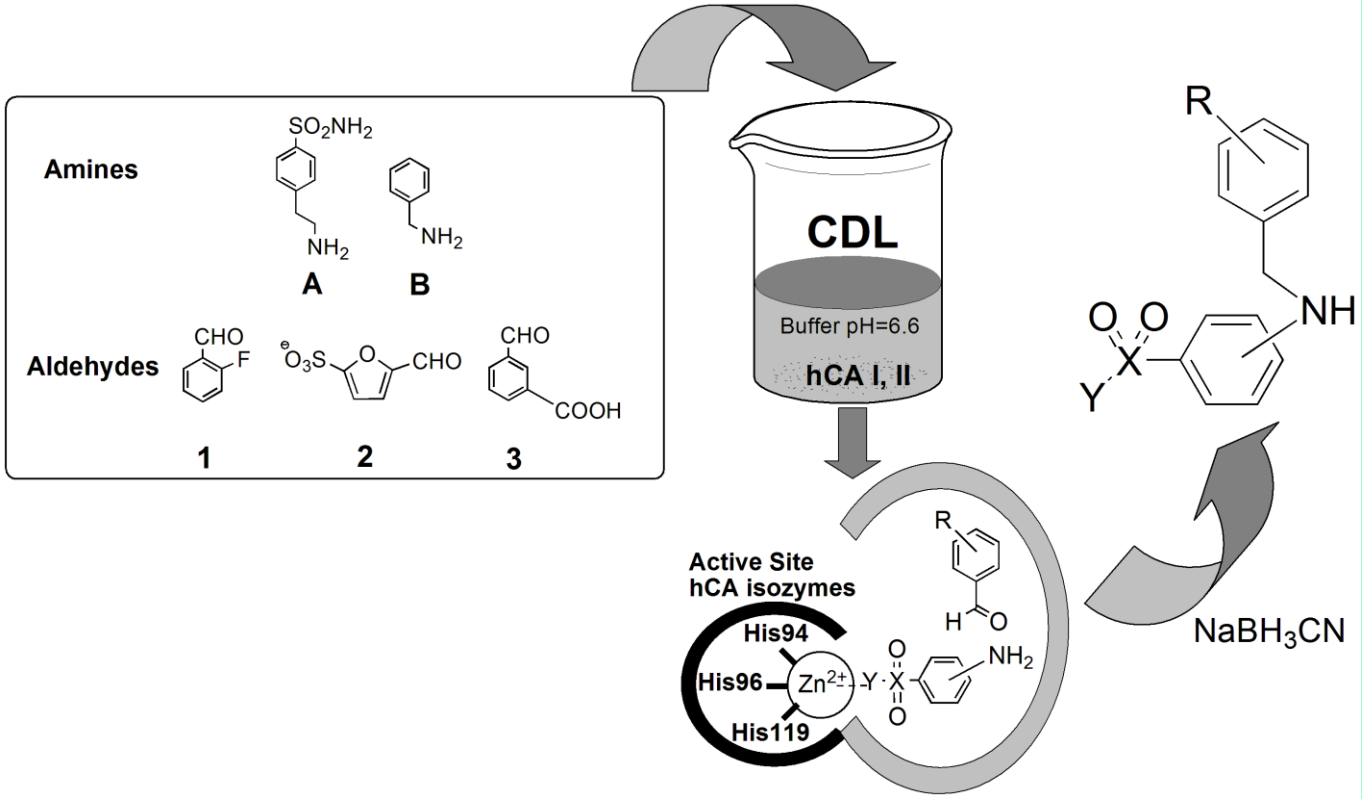
a) coumarins/thiocoumarins (Maresca et al., *JACS* 2009) - lacosamide
b) phenols (based on Christianson et al, *JACS* 1994) and polyamines
c) fullerenes (*Bioorg Med Chem* 2010)
d) imatinib, nilotinib, etc (based on Parkkila et al., *BMCL* 2009)
e) boronic acids (Winum et al., *BMC* 2009)

DCL – general principle (Barboiu, Chem Comm 2010)



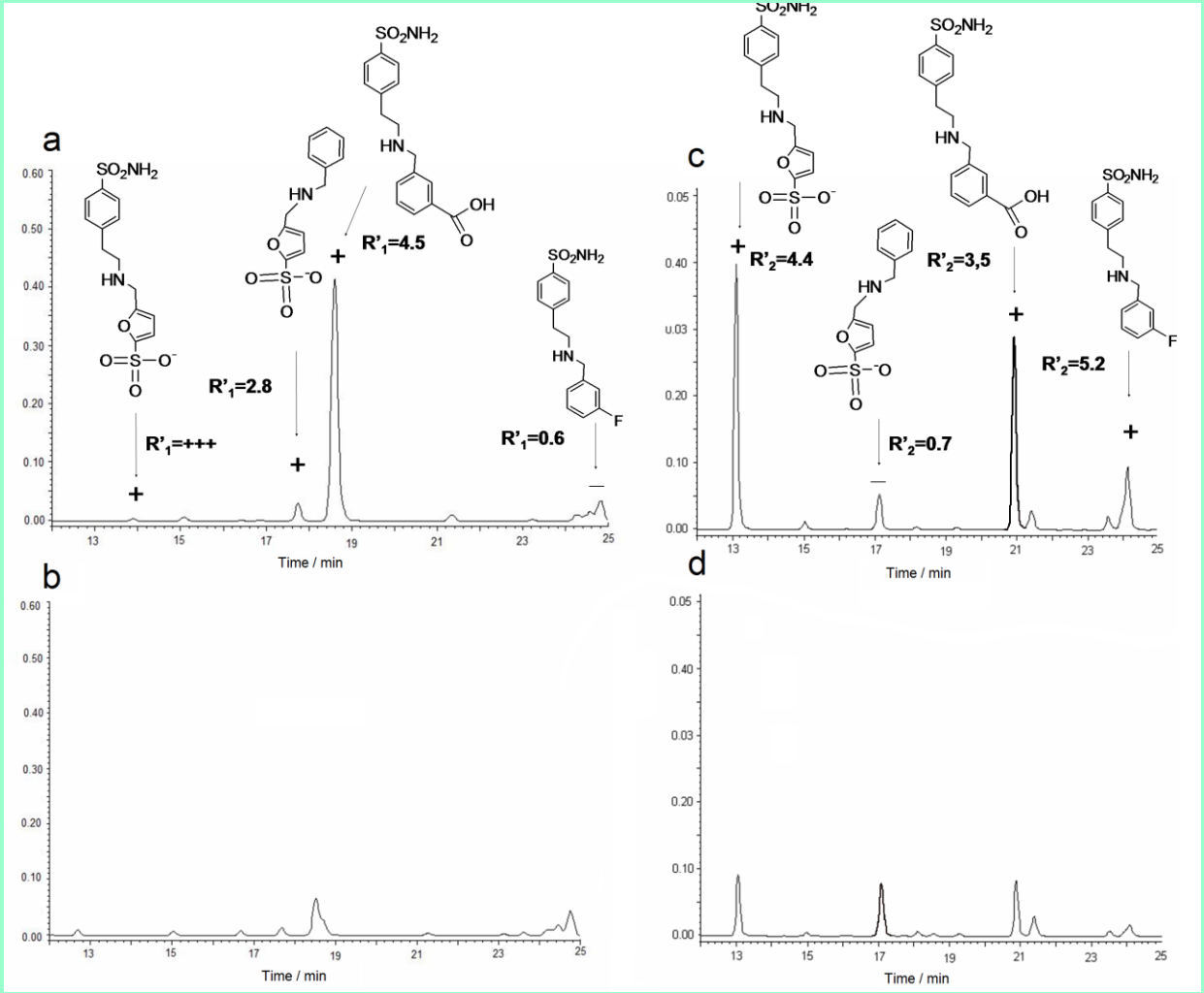
Nasr et al., J Med Chem 2009

Schiff's base chemistry (based on Lehn et al, PNAS 1997)



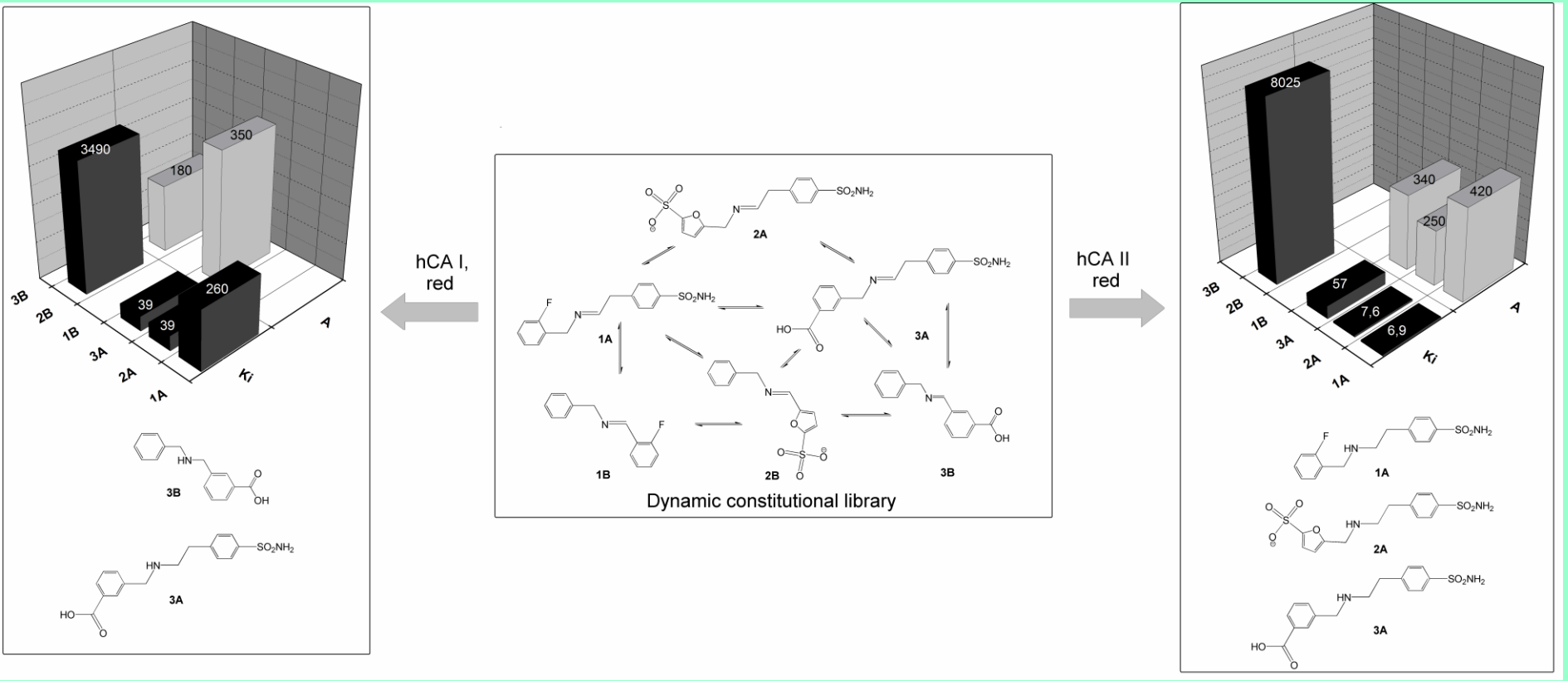
Constitutional dynamic chemistry applied to human carbonic anhydrase hCAI and hCA II isozymes and elaboration of constitutional dynamic library (CDL)

Nasr et al., J Med Chem 2009



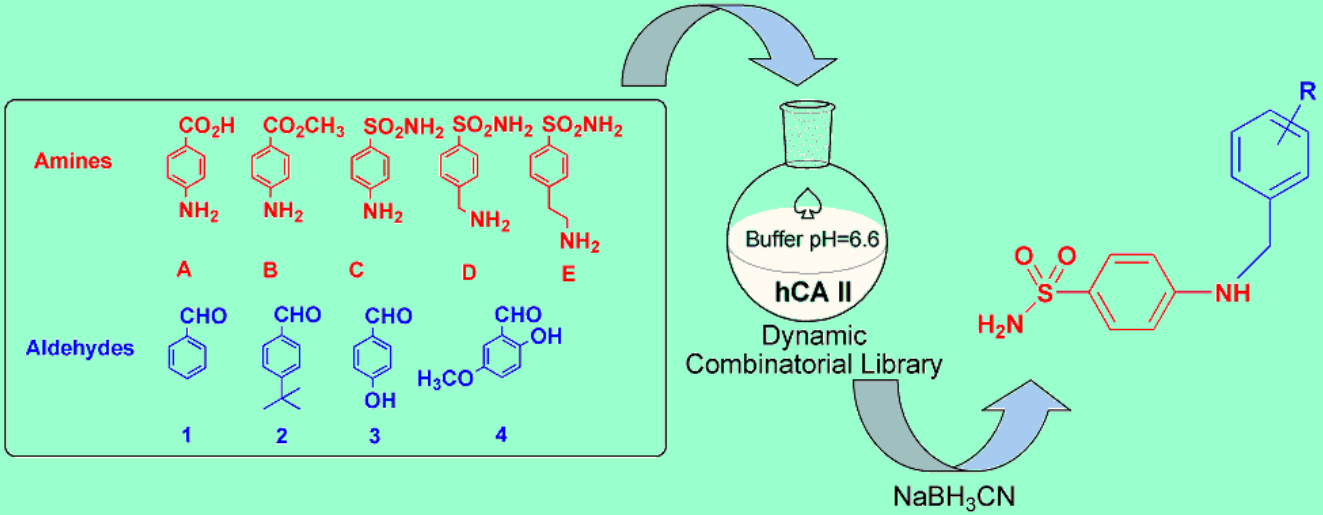
HPLC analysis of generation and screening of the CDL library: a) in the presence and b) in the absence of human hCA I isozyme and c) in the presence and d) in the absence of human hCA II isozyme

Nasr et al., J Med Chem 2010



Inhibition constants Ki and the amplification of the constitutional dynamic library (CDL) against catalytically active human hCA I and hCA II cytosolic isosymes.

Nasr et al., Bioorg Med Chem Lett 2009

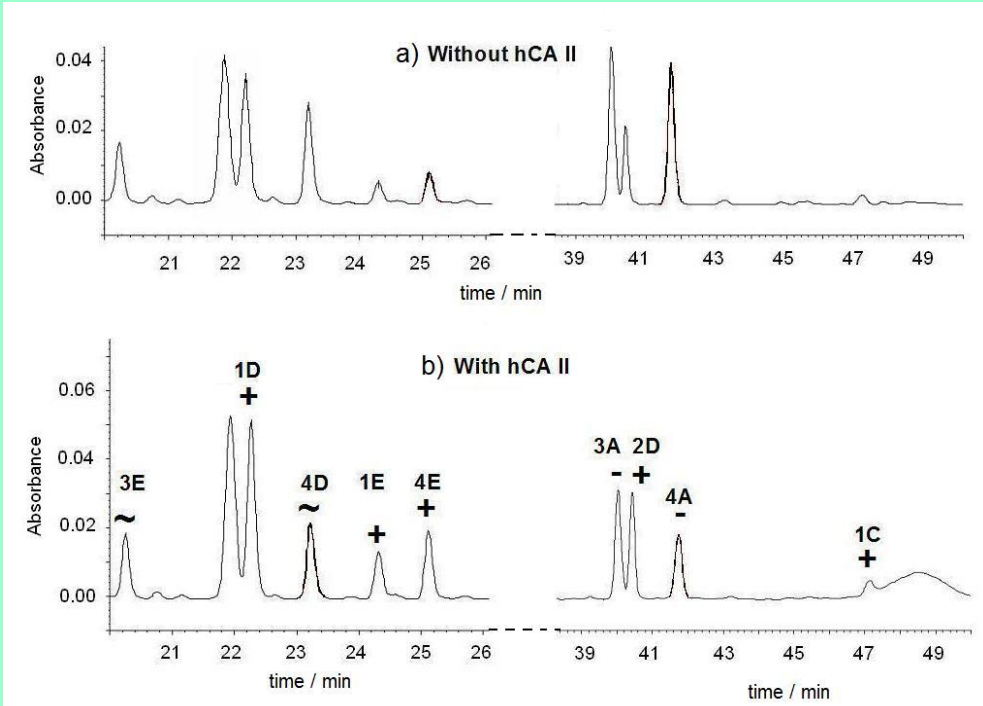


HPLC analysis of generation and screening of the DCL generated

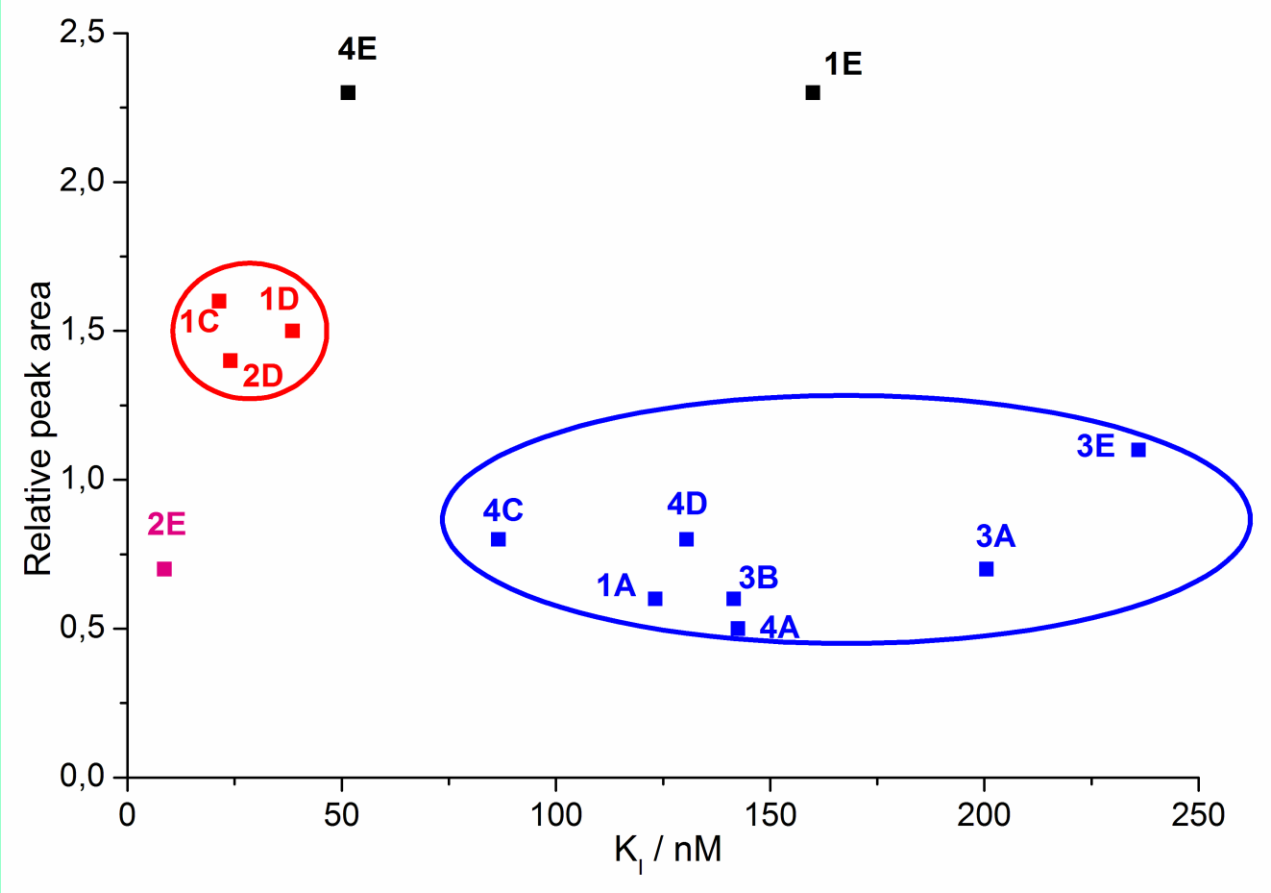
a) in the absence and b) in the presence

b) of human hCA II isosyme,

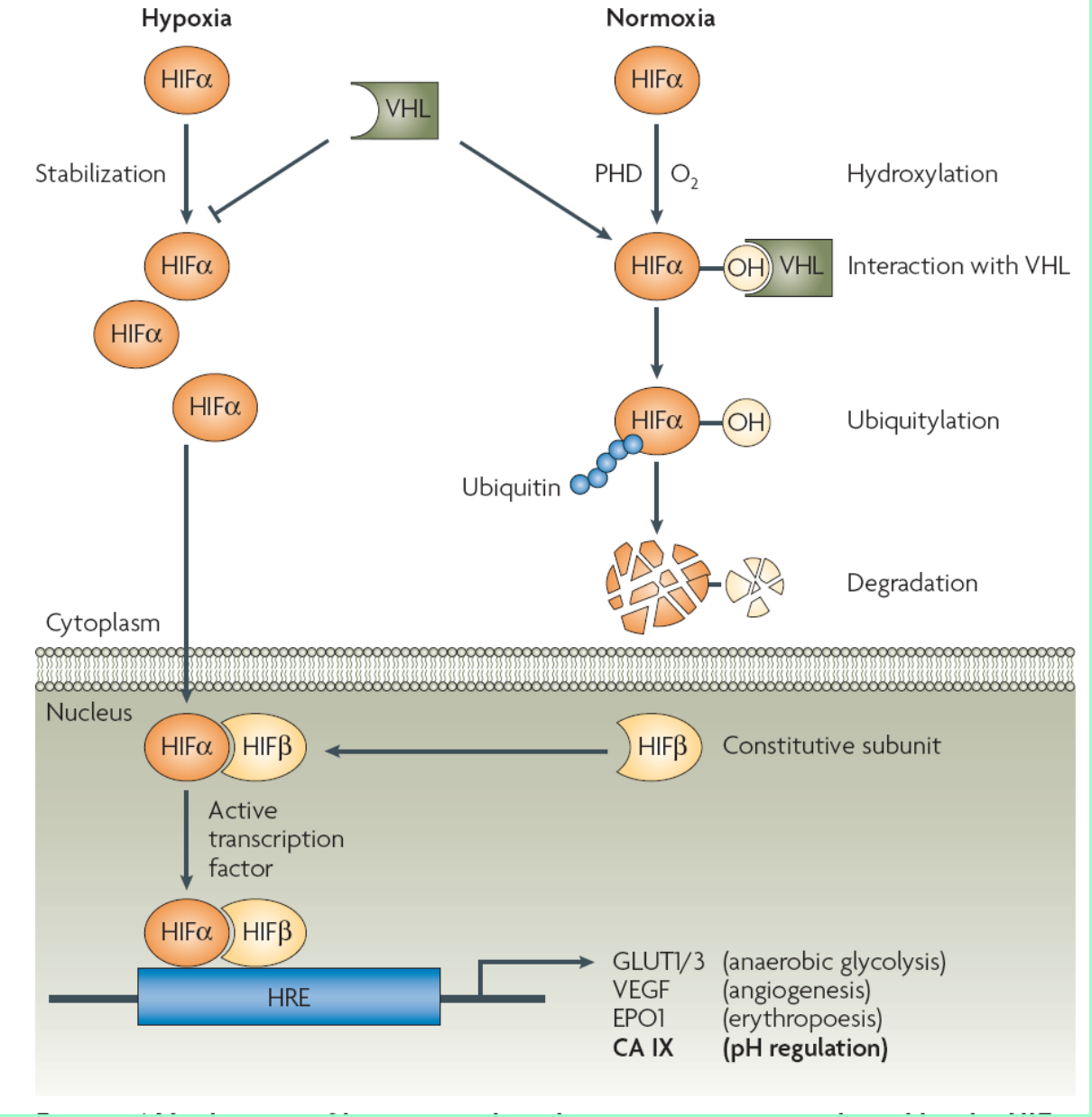
at equilibrium



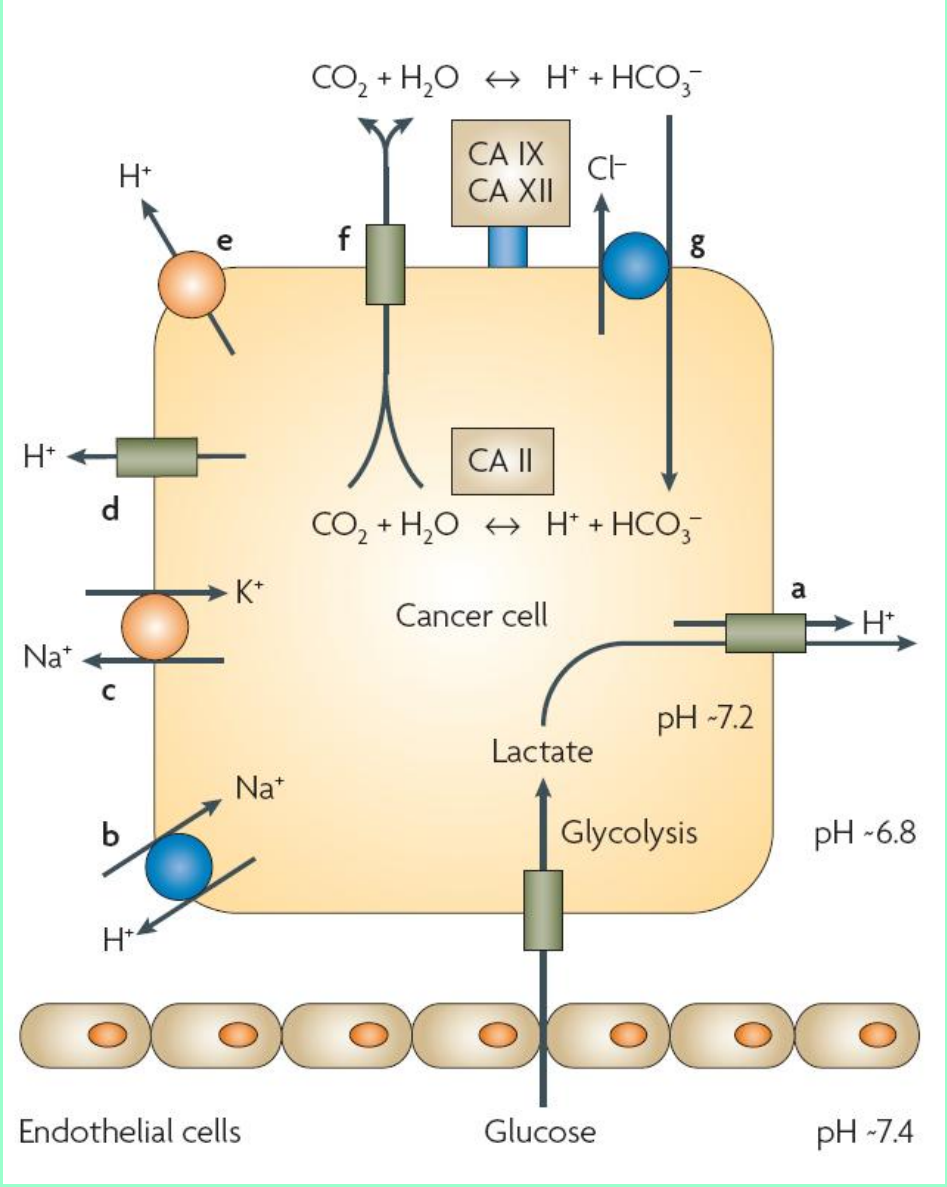
Nasr et al., BMCL 2010



Relative peak area expressing the amplification of the DCL function of inhibitory power (Ki) against hCA II

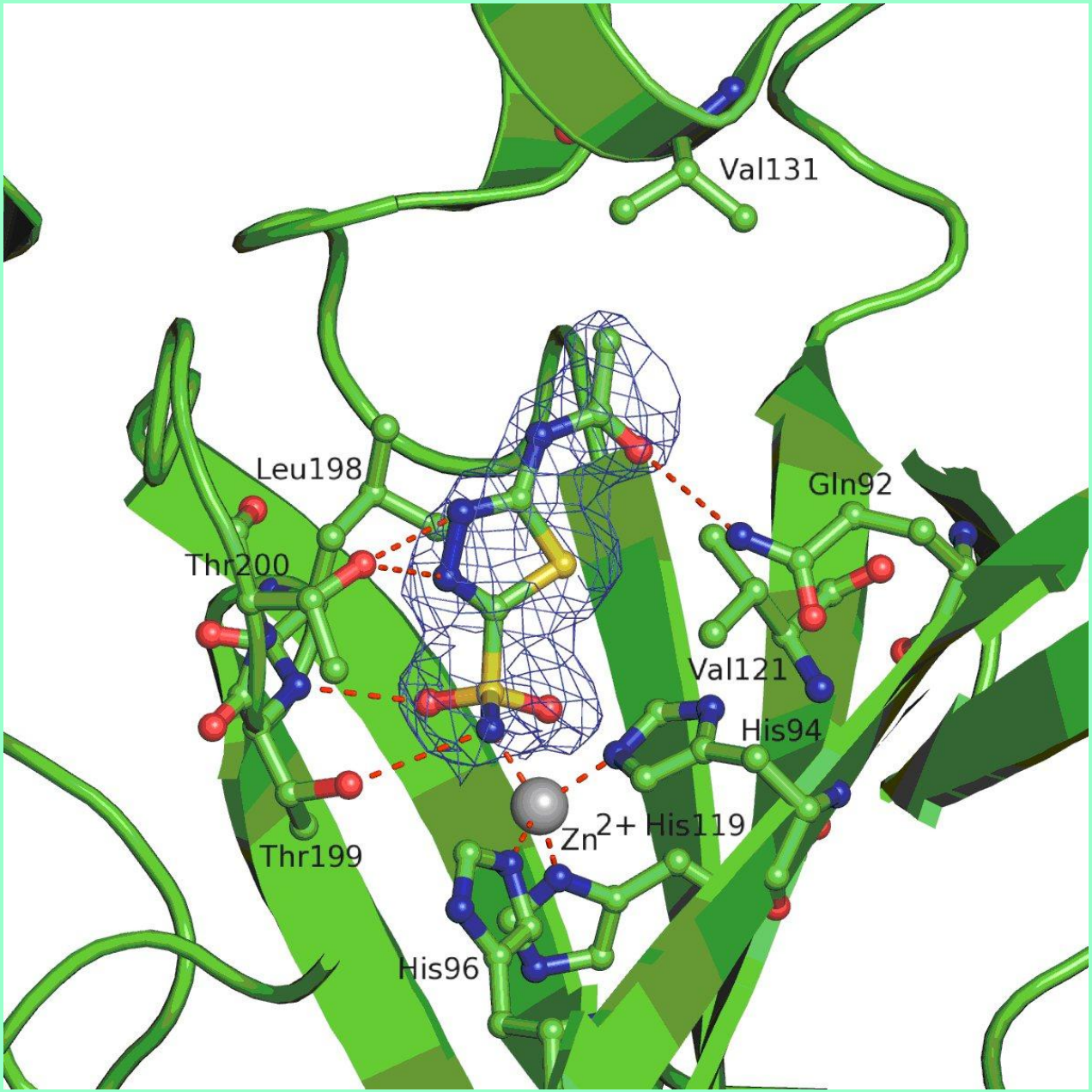


Mechanism of hypoxia-induced gene expression mediated by HIF transcription factor (Supuran, CT. *Nature Rev Drug Discov* **2008**, *7*, 168-181**).**

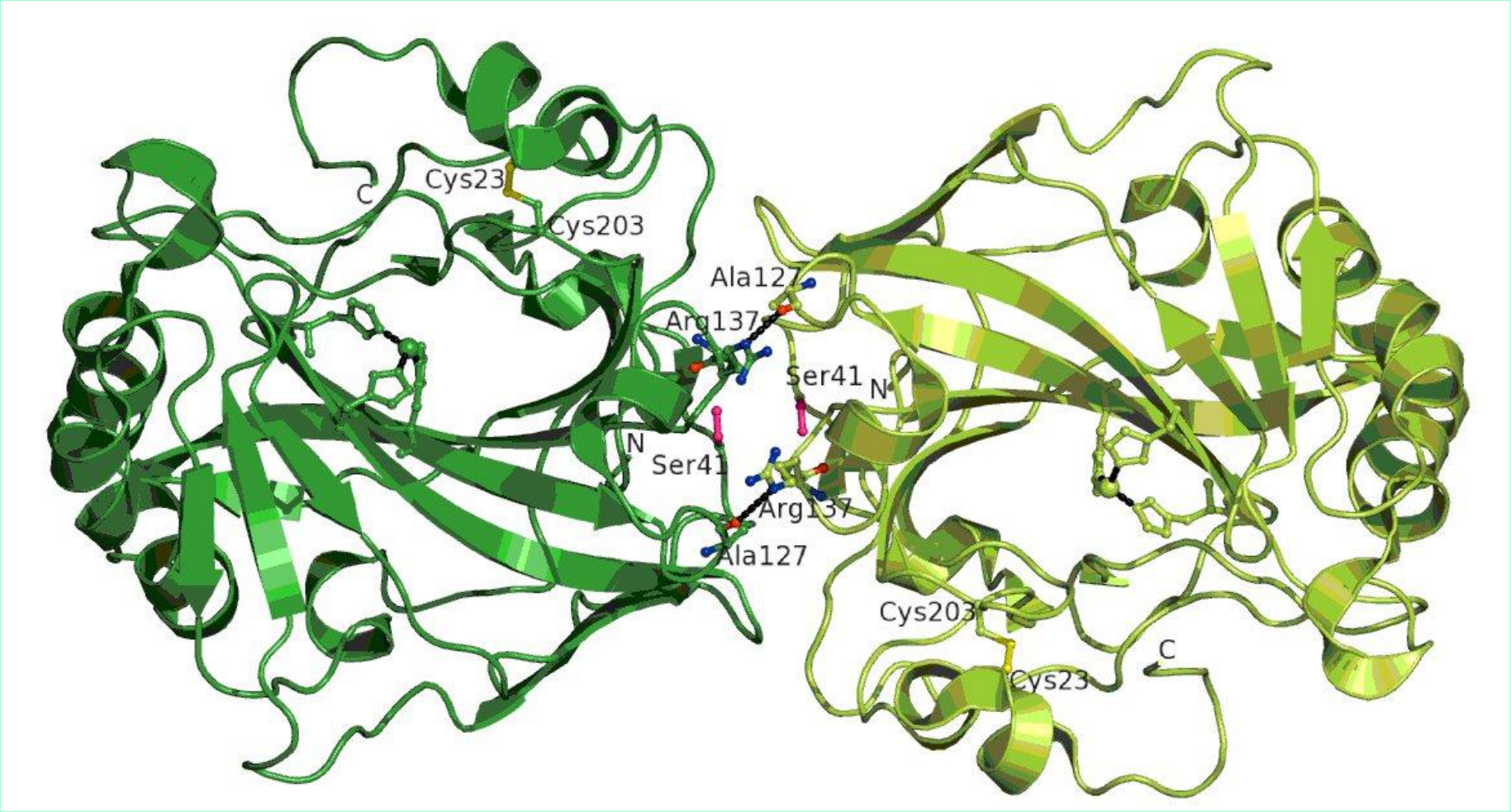


pH regulation in a tumor cell. Normal cell: pHi = 7.2, pHe = 7.4
Supuran, CT. *Nature Rev Drug Discov* **2008**, *7*, 168-181

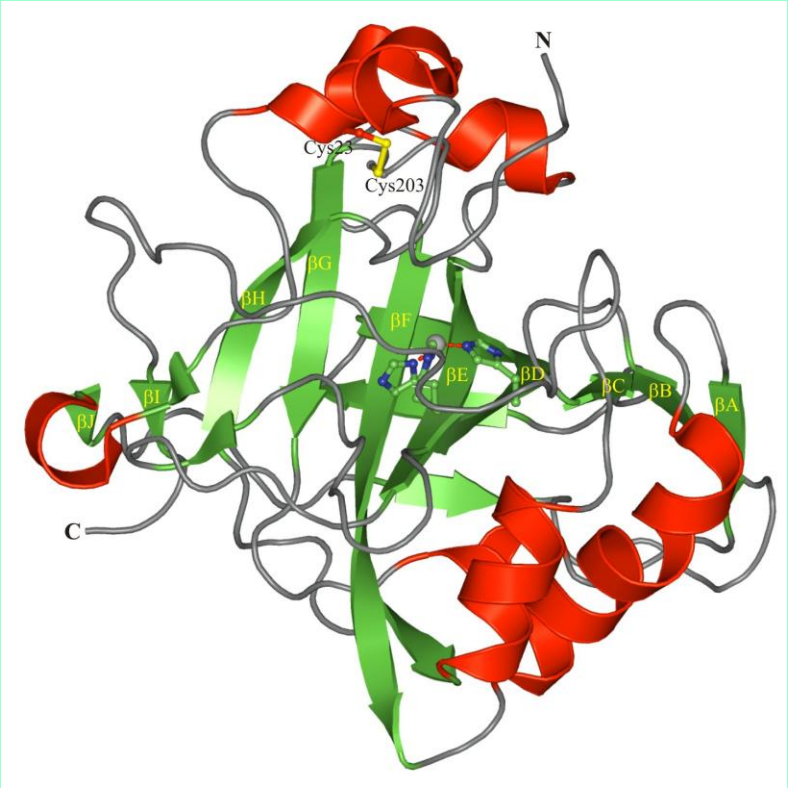
The hCA IX – acetazolamide adduct (Alterio et al., PNAS 2009)

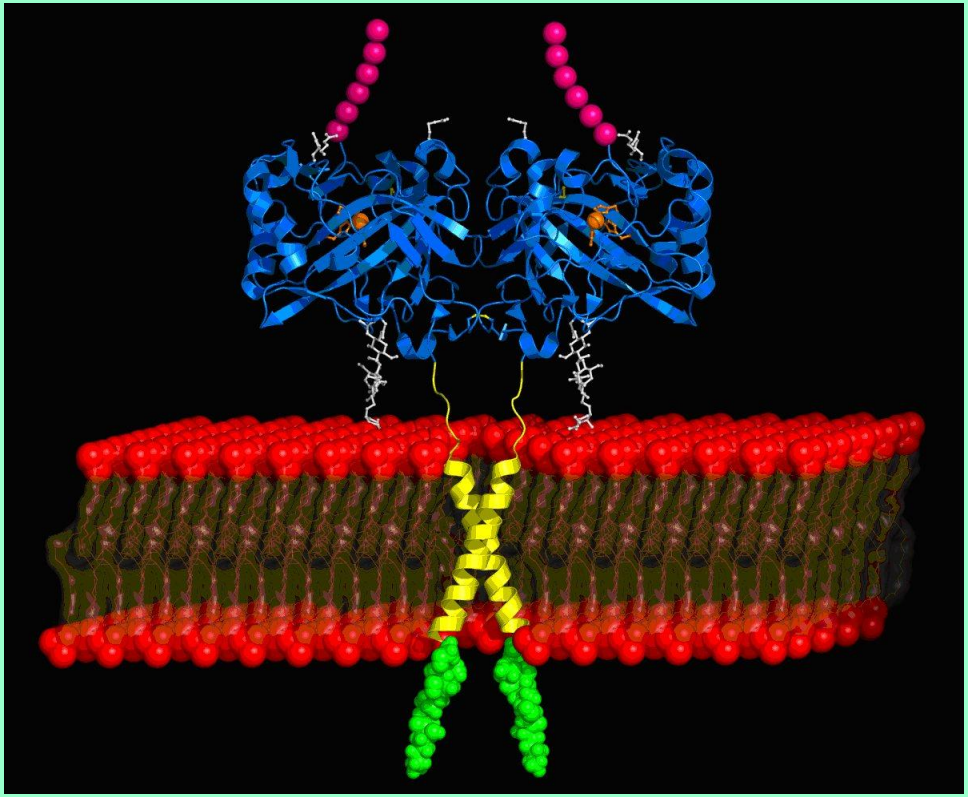


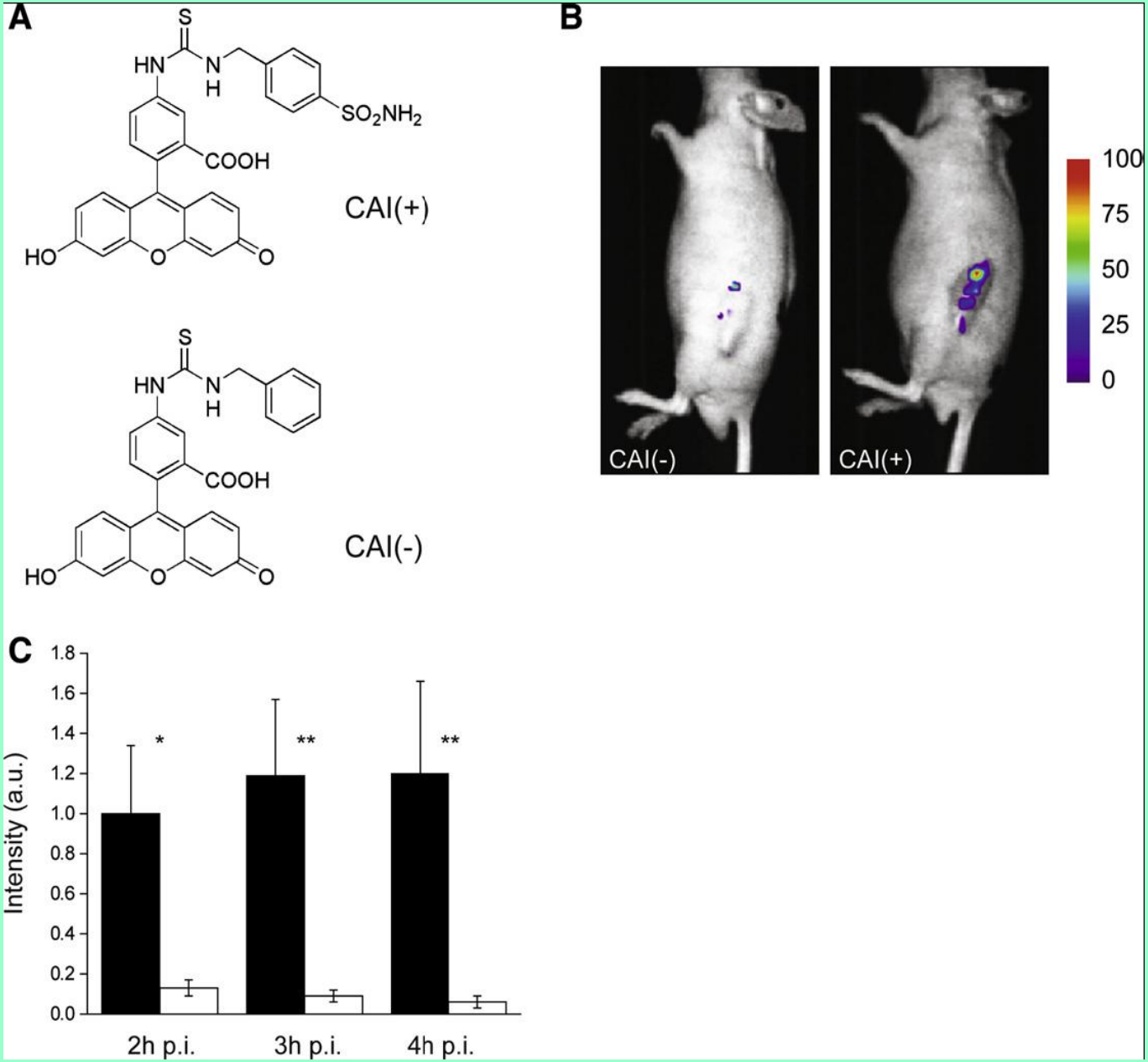
hCA IX is a homodimeric protein (Alterio et al., PNAS 2009)



Crystal structure of hCA IX (Alterio et al., *PNAS* **2009, *106*,** 16233-16238)



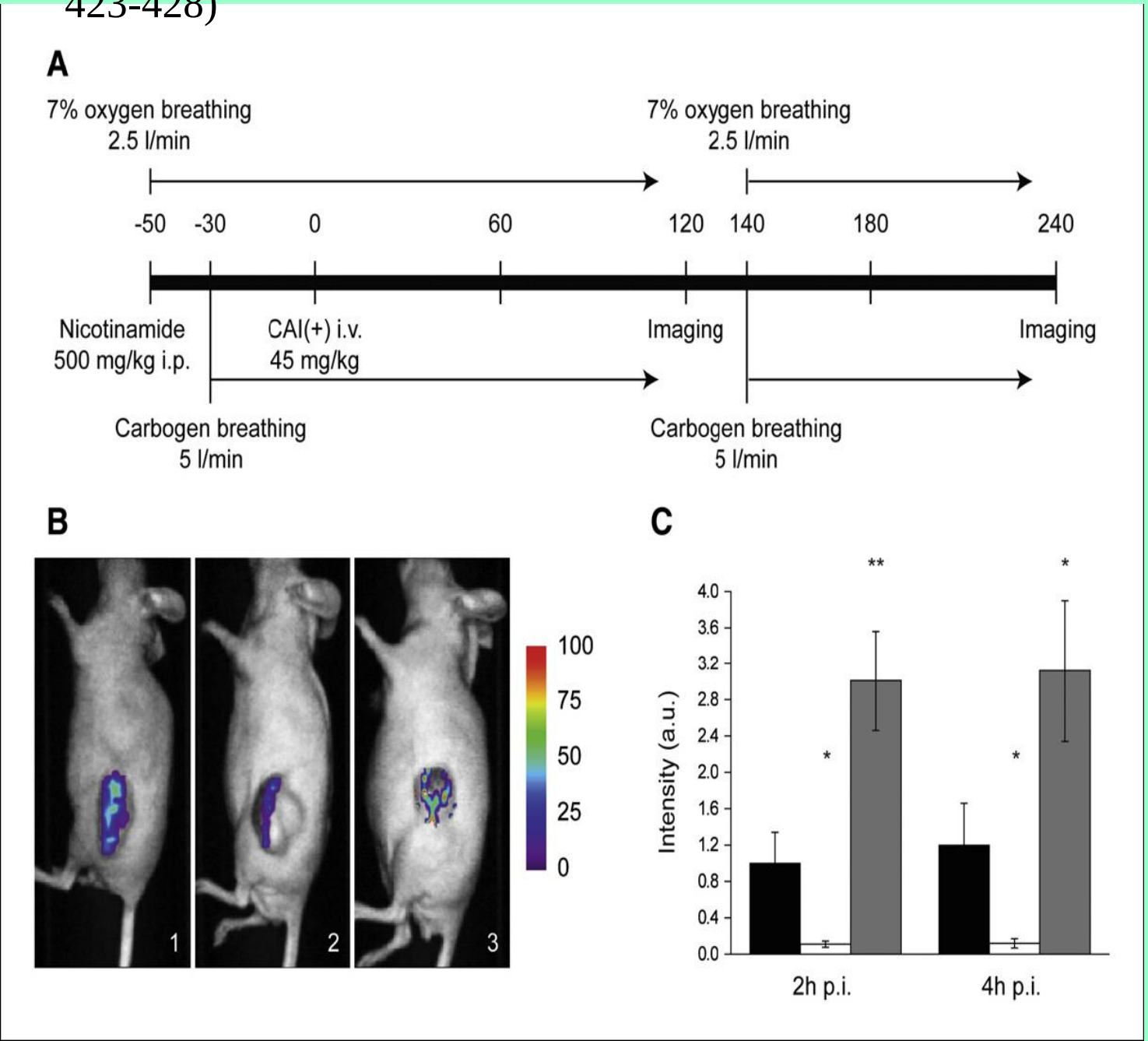


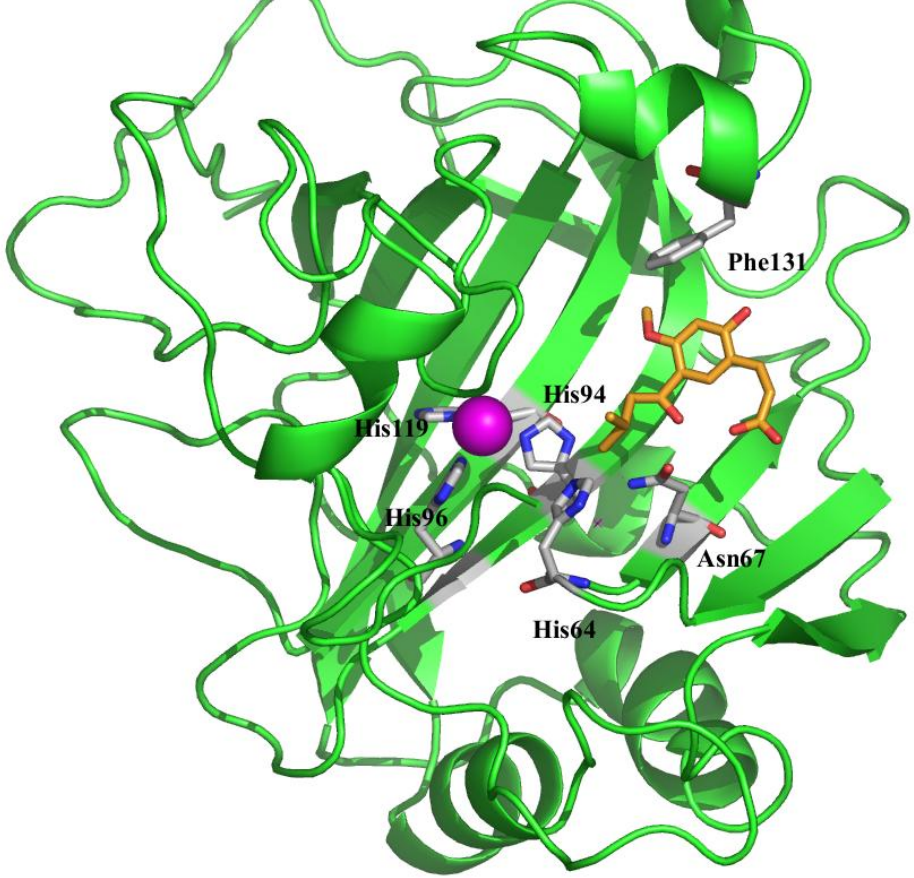


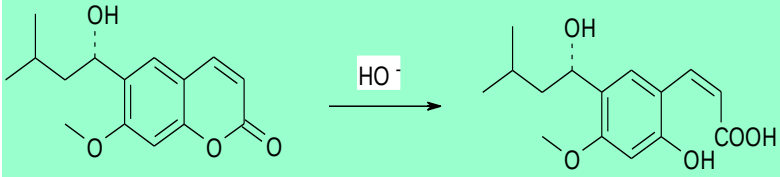
HT-29 bearing mice injected with fluore-scent compounds

Dubois et al., *Radiother. Oncol*. **2009**, *92*, 423-428

Treatment schedule/experimental design (Dubois et al., *Radiother. Oncol*. **2009**, *92*, 423-428)

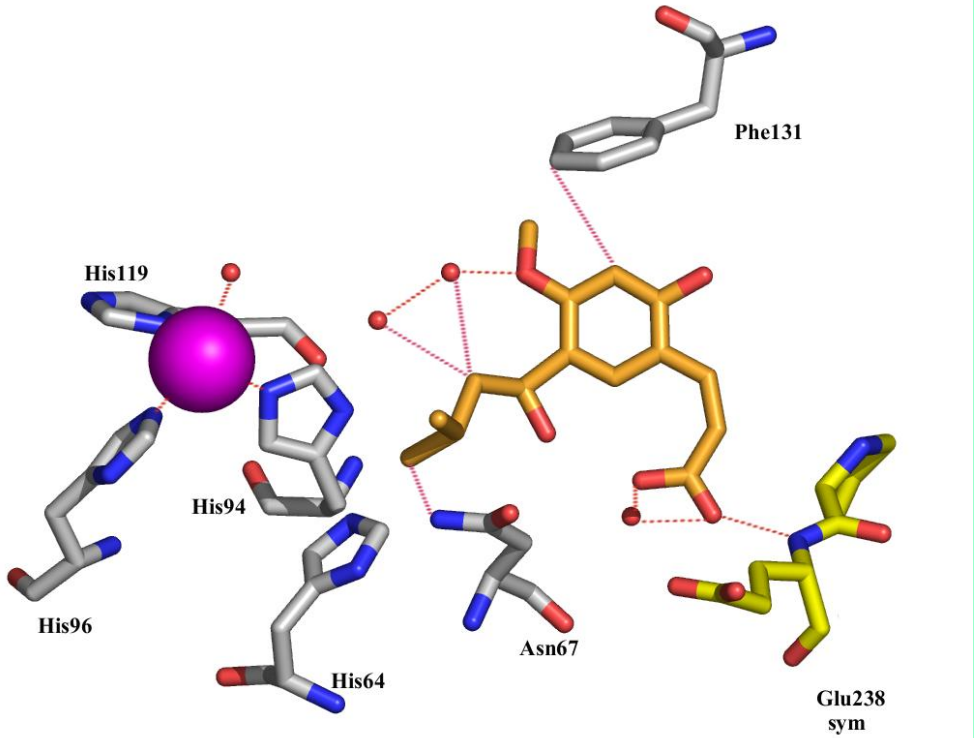


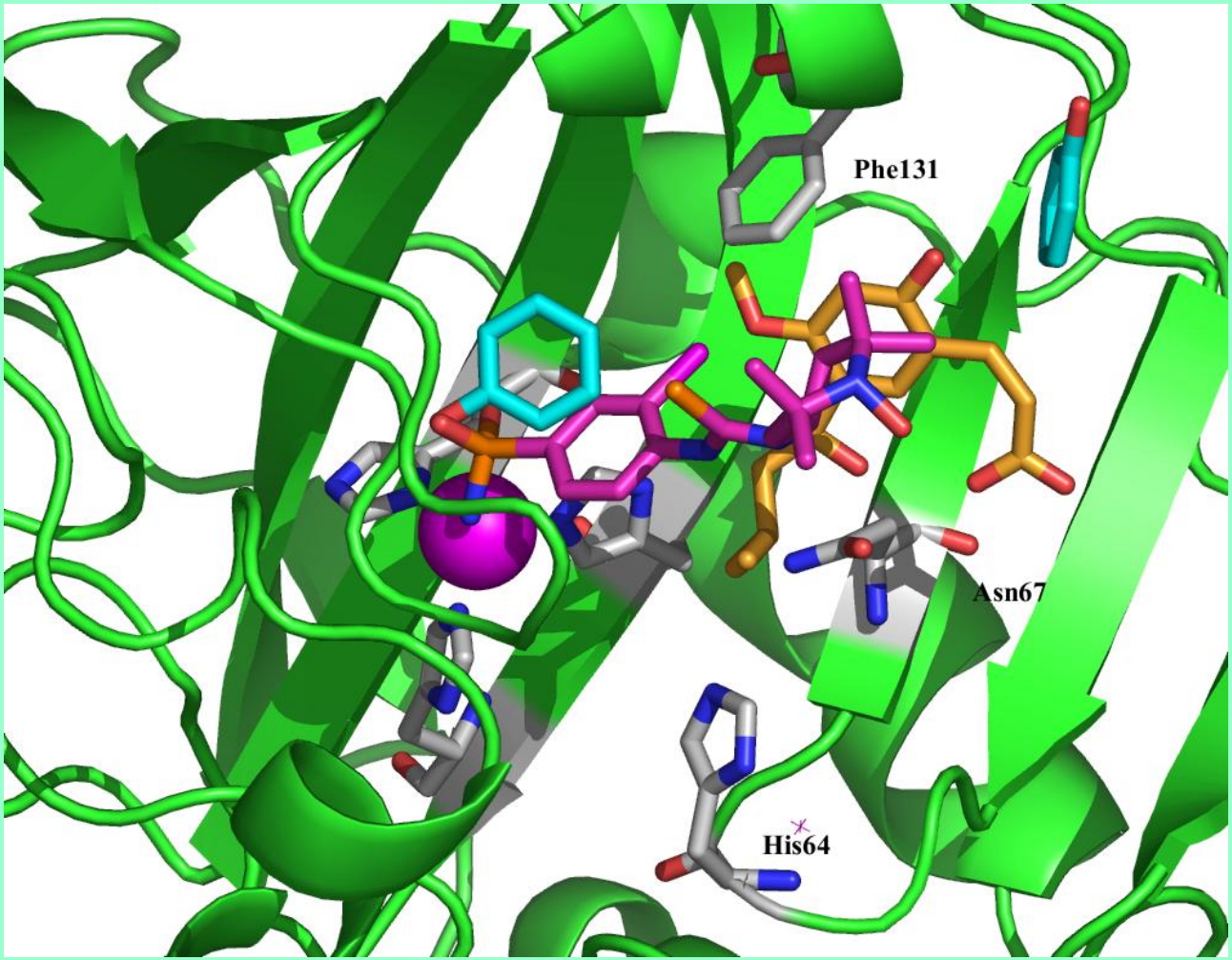




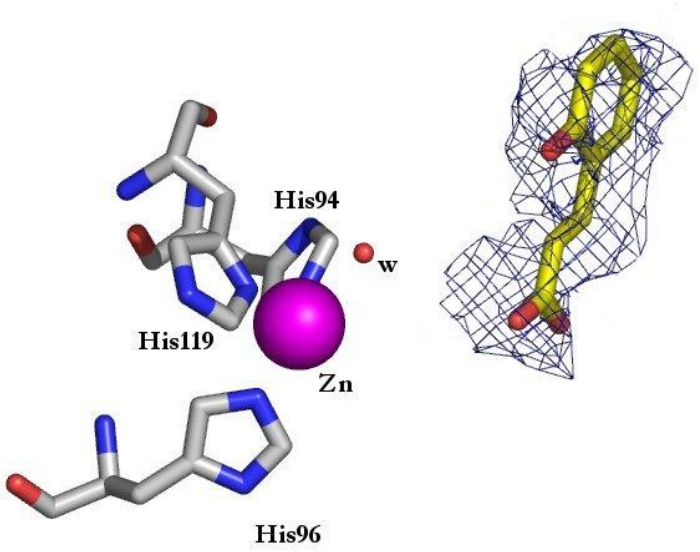
Binding of the *CIS*-2-hydroxy-cinnamic acid (in gold) hydrolysis product of the coumarin NP within hCA II active site

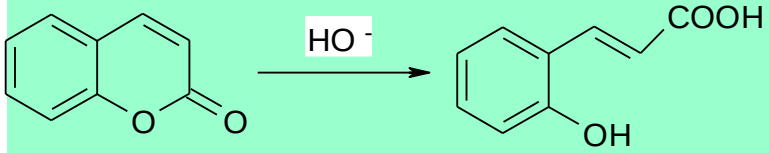
Maresca et al., JACS 2009

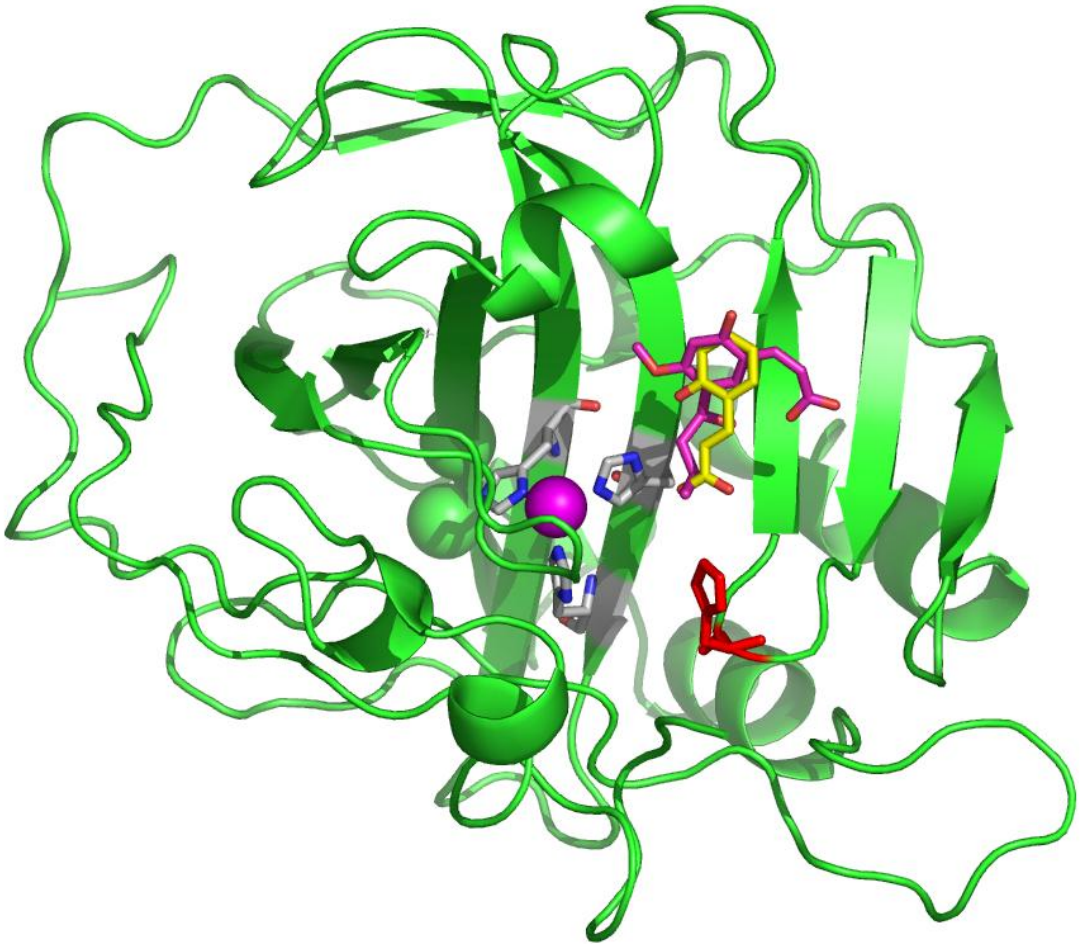




Superposition of hCA II – 5 (coumarin hydrolysis product) – gold – with hCA II – phenol adduct (sky) and hCA II – sulfonamide adduct (possessing a TEMPO tail) (magenta).



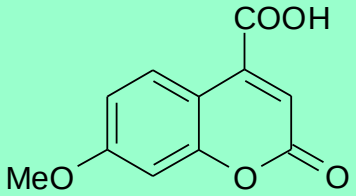




hCA II – unsubstituted “coumarin” adduct (*TRANS*-2-hydroxy-cinnamic acid

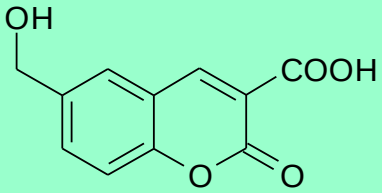
Maresca et al., J Med Chem 2010

Isoform selective CAIs based on the coumarins (Maresca et al., J Med Chem 2010)



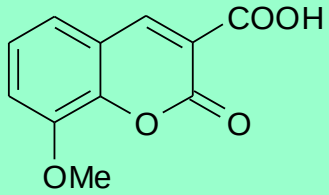
11

Ki = 99 nM (CA II)
Ki-s > 4 uM all other CAs



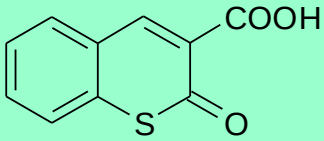
15

Ki = 48 nM (CA XIII)
Ki-s > 5 uM all other CAs



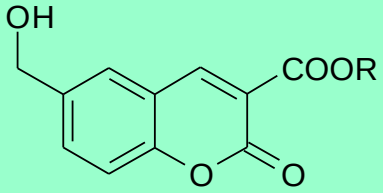
16

Ki = 1 uM (CA XIV)
Ki-s > 6 uM (all other CAs)



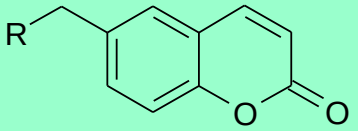
17

Ki = 47 nM (hCA IX)
kI = 42 nM (CA XIII)
Ki-s > 4 uM (other CAs)



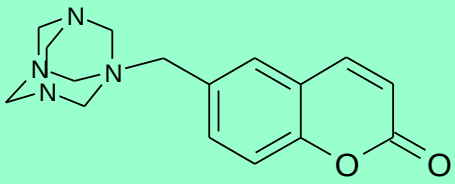
19: R = Et

Ki = 48 nM (CA IV)
Ki = 45 nM (CA VII)
Ki = 47 nM (CA IX)
Ki = 46 nM (CA XV)
Kis > 3 uM other CAs



22: R = NH_2

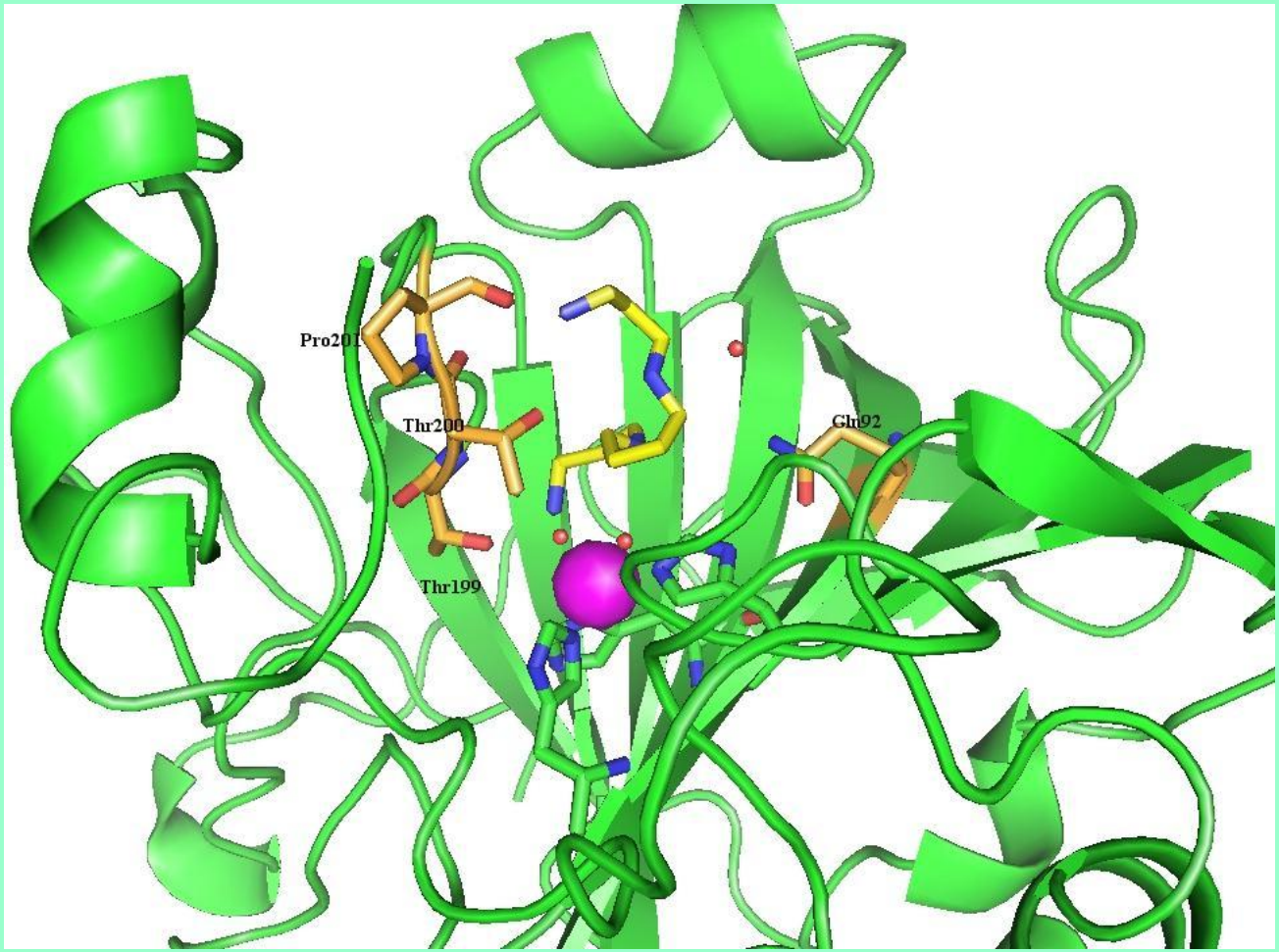
Ki = 40 nM (CA XIII)
Kis > 4 uM all other CAs

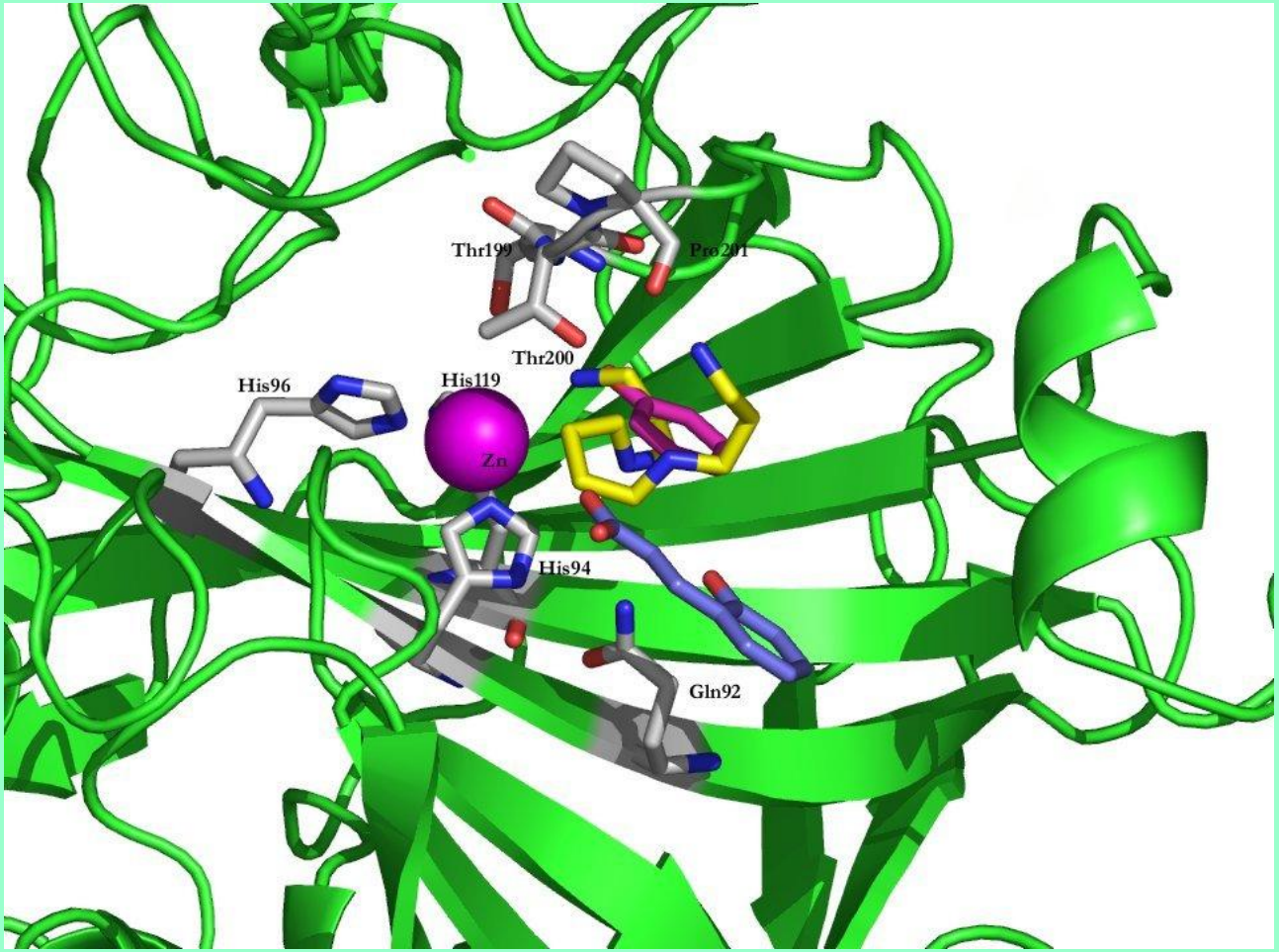


23

Ki = 48 nM (CA IX)
Ki-s > 3 uM all other CAs

hCA II – spermine adduct (Carta et al, J Med Chem 2010)



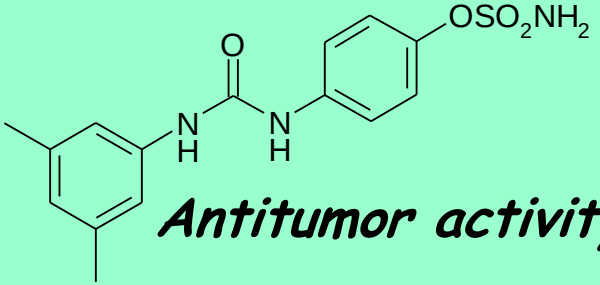


Spermine Ki-s (uM)

hCA I: 230
hCA II : 84
hCA III: 167
hCA IV: 10 nM
hCA VA: 0.84
hCA VB: 0.83
hCA VI: 0.99
hCA VII: 0.71
hCA IX: 13.3
hCA XII: 27.6
hCA XIII: 22.5
hCA XIV: 0.86
mCA XV: 74

Superimposition of the spermine (yellow), phenol (magenta) and *trans*-2-hydroxycinnamic acid (violet) adducts with hCA II. The Zn(II) ion is the violet sphere.

In vivo antitumor activity of CAIs



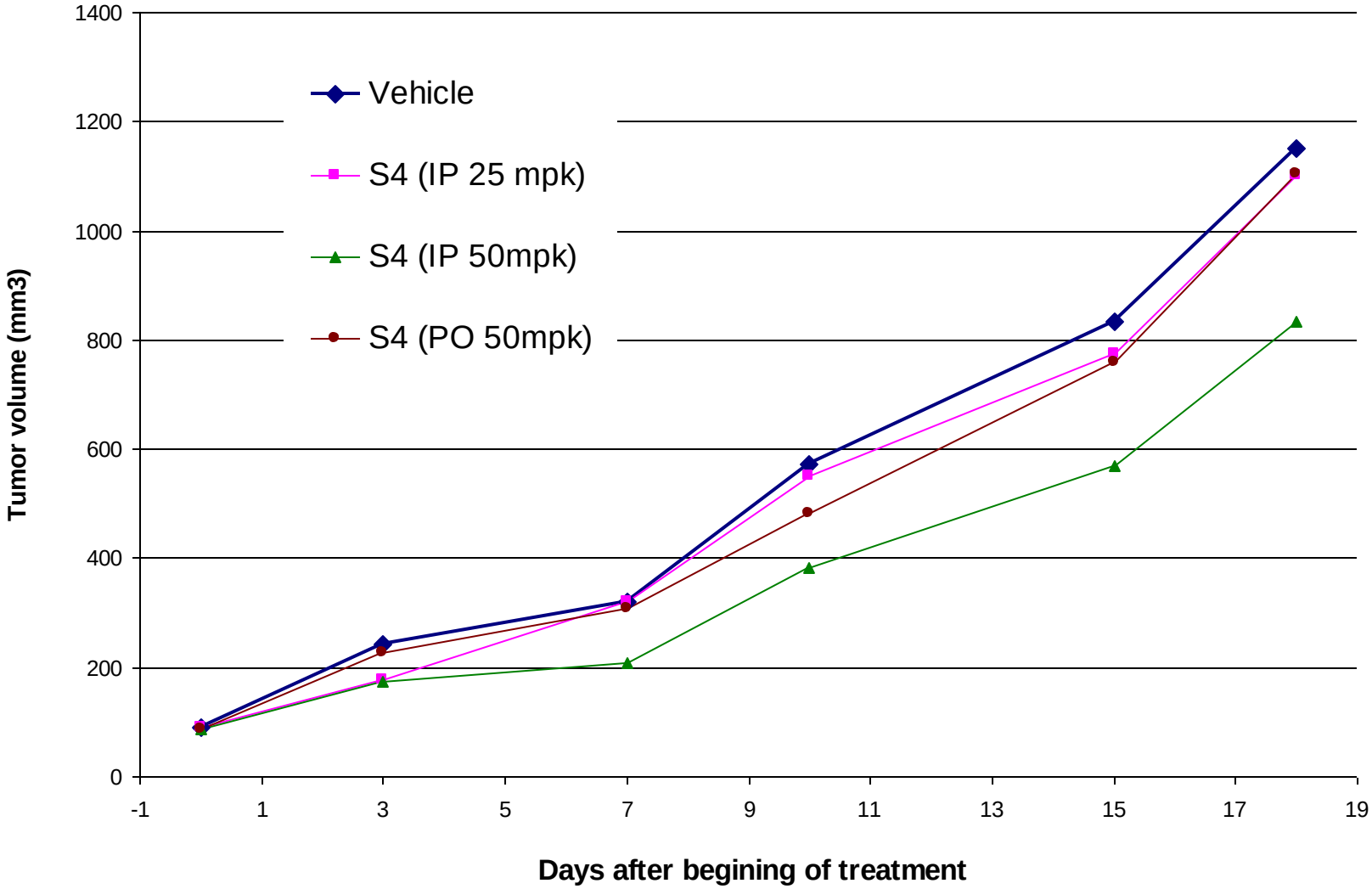
S4, is a strong CAI

Ki = 2 nM (CA IX)
Ki = 7 nM (CA XII)

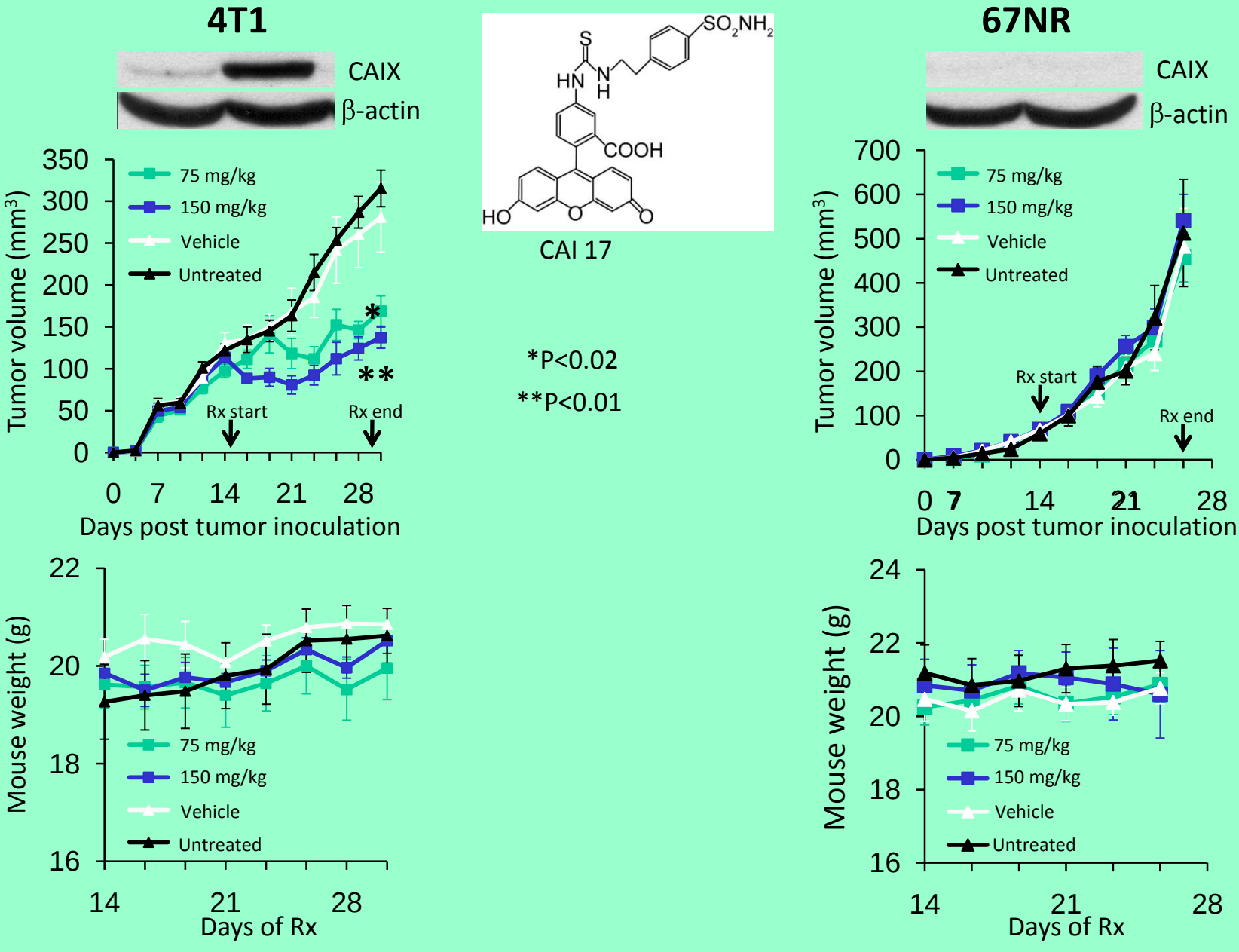
Antitumor activity of S4 in the HCT116 xenograft model

Nude female CD1 mice, 7 week old (Charles River France) were injected subcutaneously with 4 millions of HCT116 colon carcinoma cells in physiologic suspension (200 ul). 14 days after injection of cells; animals began treatment (5 ml/kg). The treatment continued for 18 days.
Animals were divided into **four** separate groups (10 animals per group):

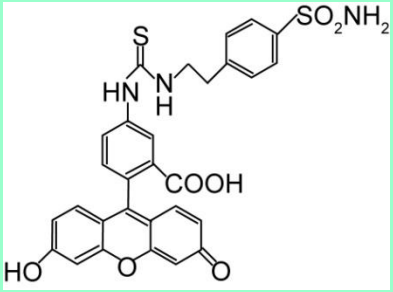
Compound	*Dose & Regime*	*Via*	*duration*
Vehicle (10%DMSO/45%PEG400/45%Water)	QD 5 days a week	I.P.	3 weeks
S4	25 mpk QD 5 days a week	I.P.	3 weeks
S4	50 mpk QD 5 days a week	I.P.	3 weeks
S4	50 mpk QD 5 days a week	**PO**	3 weeks


Vehicle
S4 (IP 25 mpk)
S4 (IP 50mpk)
S4 (PO 50mpk)
Tumor volume (mm3)
0
200
400
600
800
1000
1200
1400
-1
1
3
5
7
9
11
13
15
17
19
Days after begining of treatment

Specific Inhibition of CAIX-positive 4T1 Tumor growth by a CAIX small molecule Inhibitor

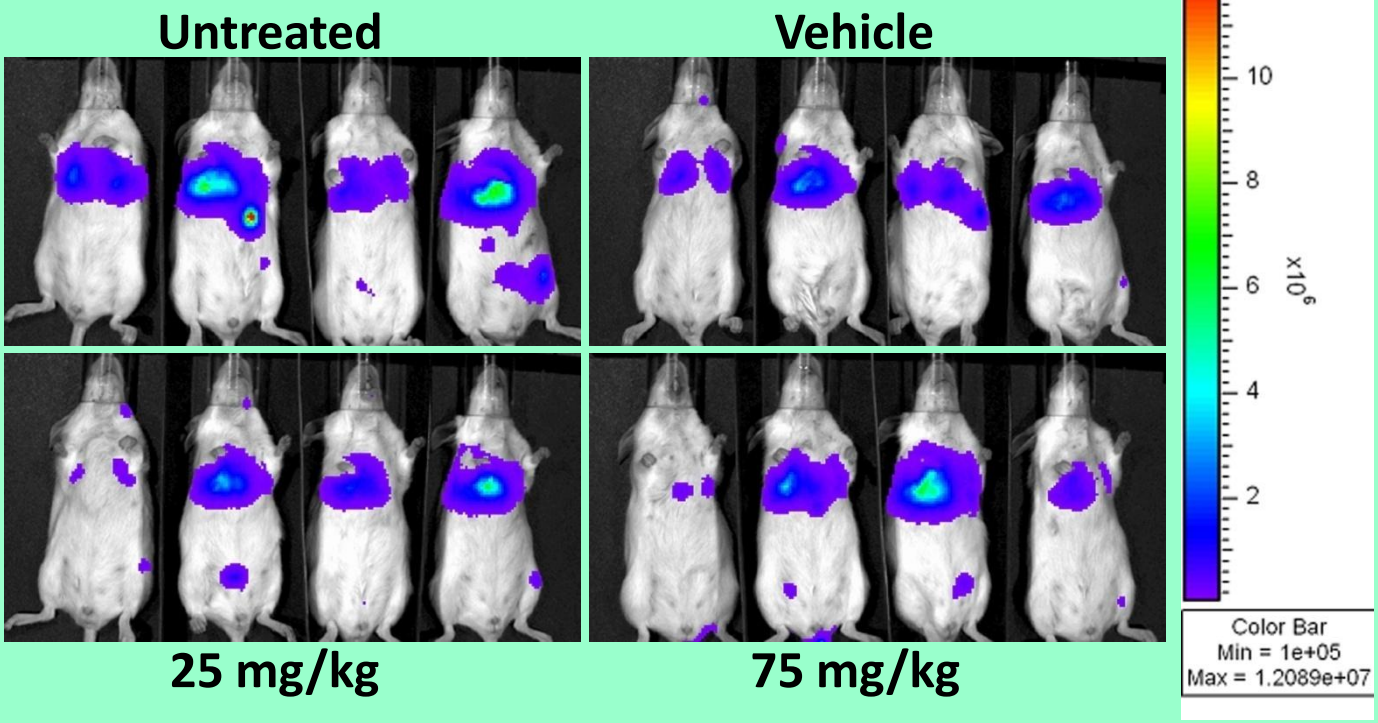


CAI17 inhibits the formation of lung metastases by 4T1 mammary tumor cells



12 days post-iv injection of 4T1 cells

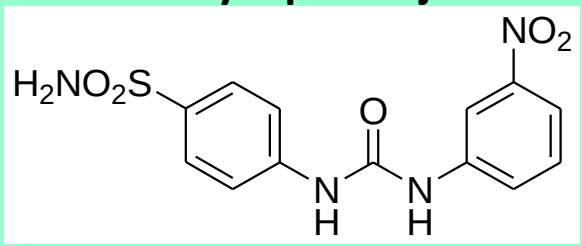
5 doses ip



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

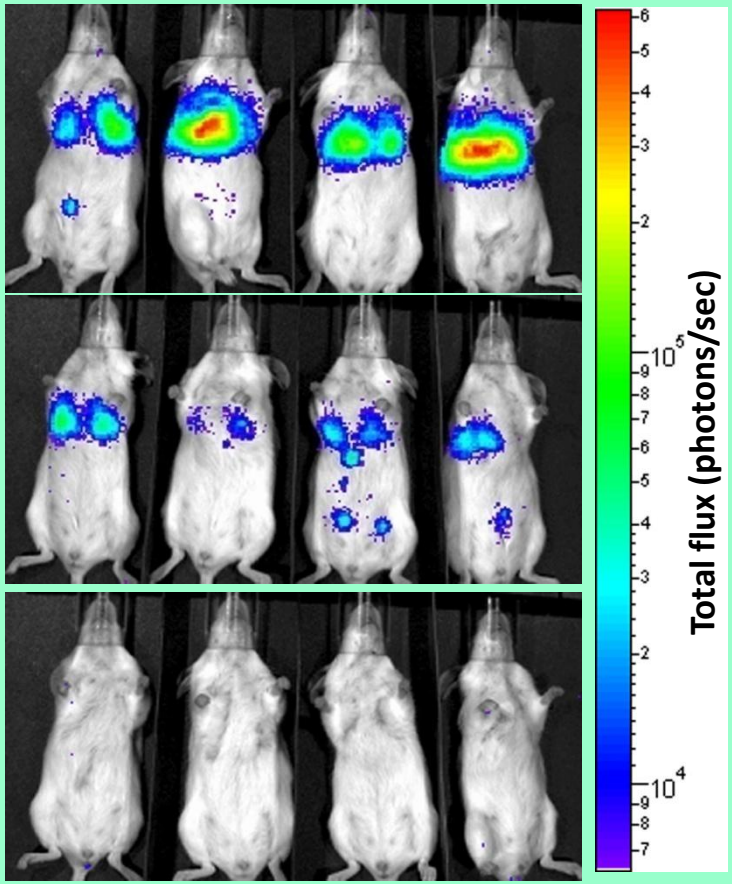


Supuran et al., WO Patent 2010

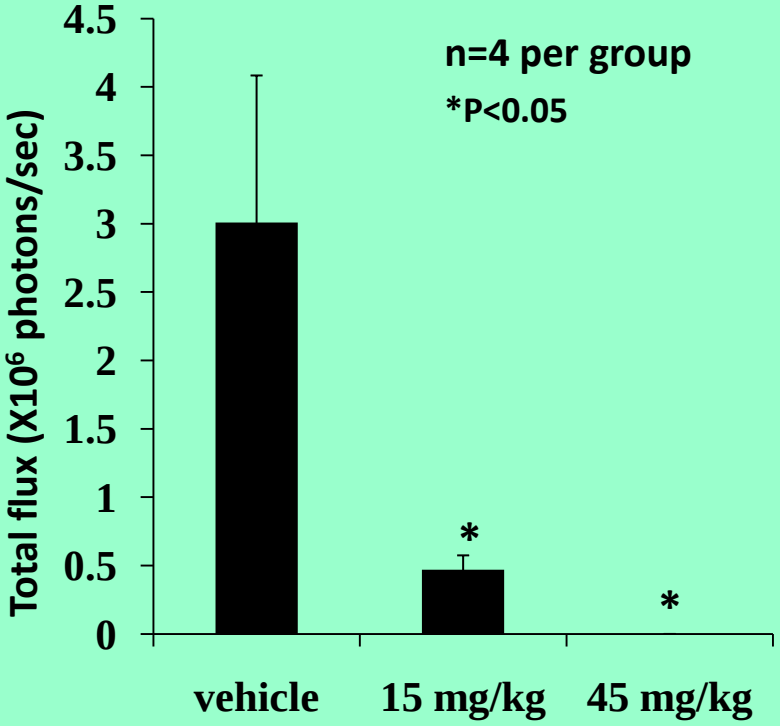
Vehicle

15 mg/kg

45 mg/kg



3 doses (eod x 3 i.p.)



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- E. Merck (Darmstadt, DE) – antitumor sulfamates

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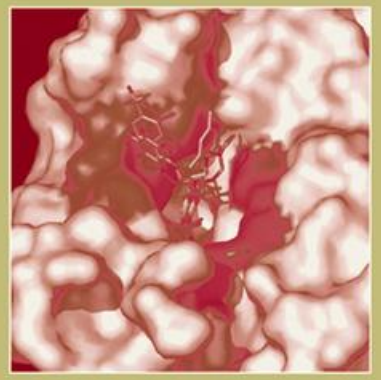
-Novartis (Basel, CH) – novel chemotypes

Wiley Series in Drug Discovery and Development
Binghe Wang, Series Editor

Drug Design of Zinc-Enzyme Inhibitors

Functional, Structural, and Disease Applications



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