Ectosteric Inhibitor Concept and the Selective Inhibition of Cathepsin K Collagenase Activity

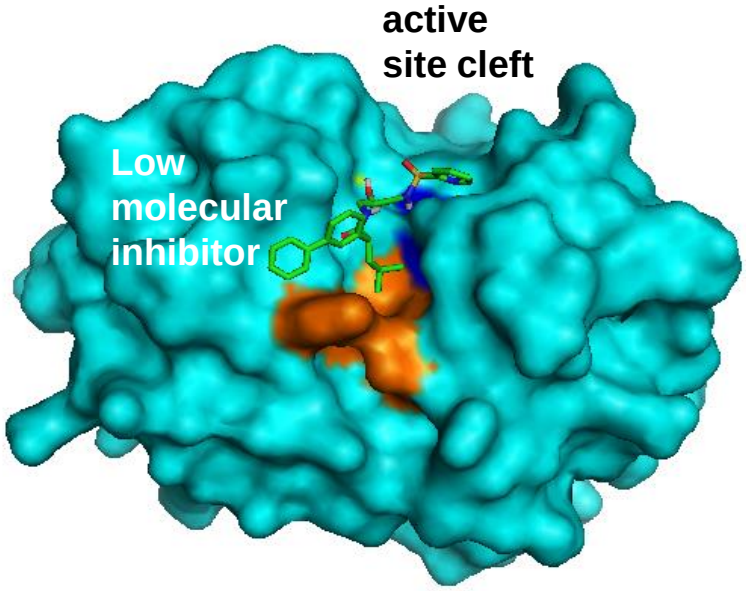
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What are Cathepsins?

- cysteine proteases of the papain-family
- lysosomal protein turnover (specific or non-specific)
- extracellular matrix degradation



Cathepsin L
Cathepsin O
Cathepsin S
Cathepsin K
Cathepsin V
Cathepsin F
Cathepsin W

endopeptidases

Cathepsin B
Cathepsin X
Cathepsin H
Cathepsin C

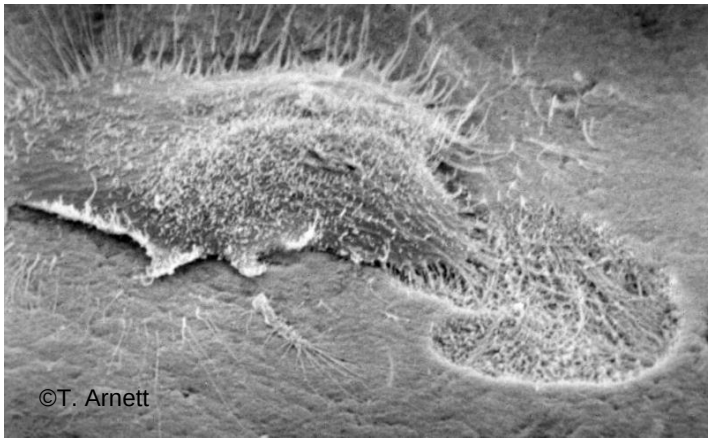
exopeptidases

tissue-specific

ubiquitous

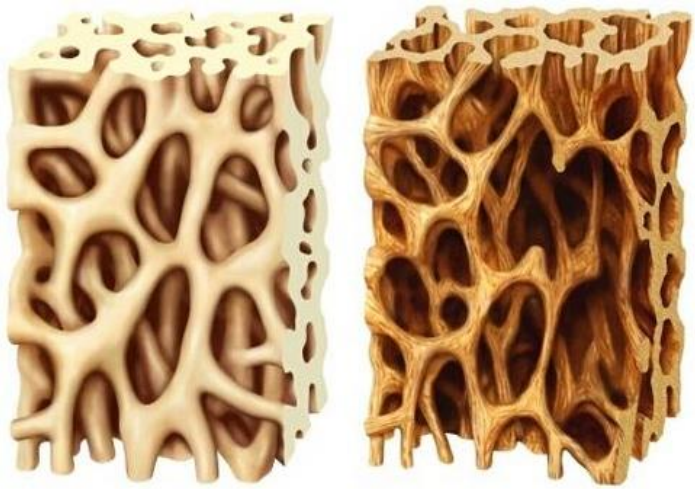
Cathepsin K (catK) is a potent collagenase and elastase

Osteoporosis and Cathepsin K



Excessive degradation

normal osteoprotic

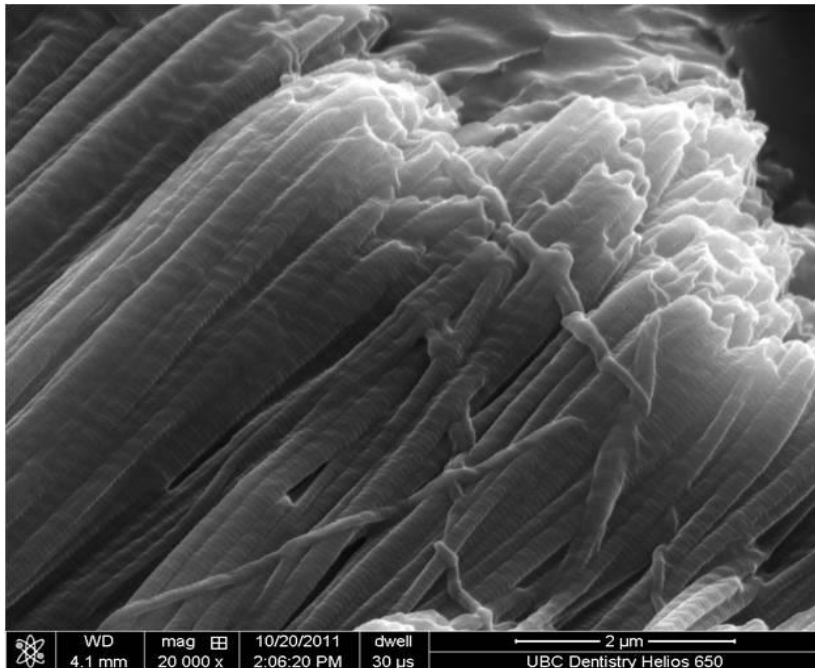


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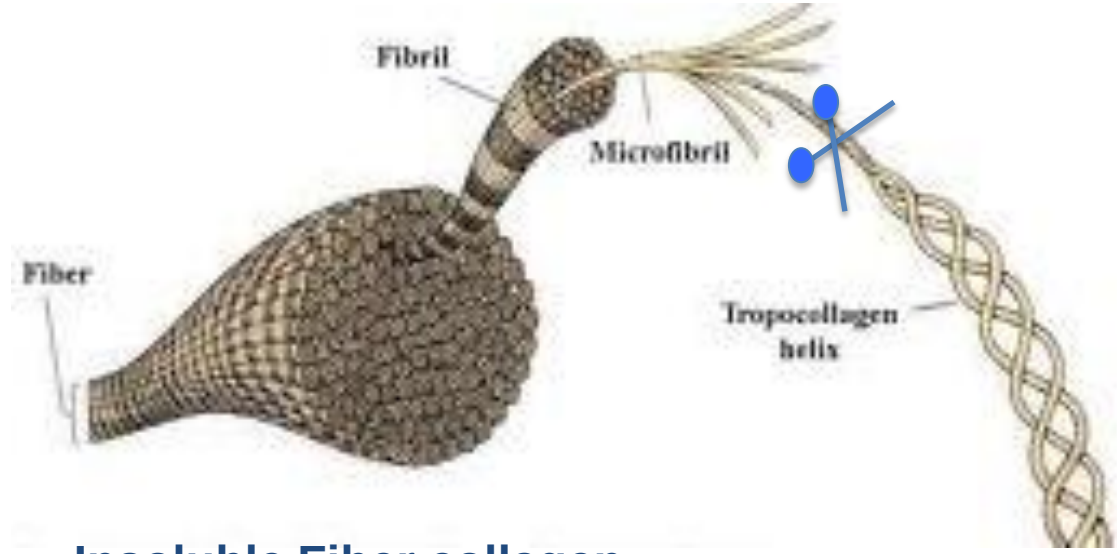
Cathepsin K:

- predominantly expressed in osteoclasts
- represents >95% of total cysteine protease activity
- major collagenolytic activity
- excessive collagen degradation: osteoporosis

How does Cathepsin K degrade fibrillar collagen?



SEM of mouse tail collagen fiber
(diameter ~40µm)

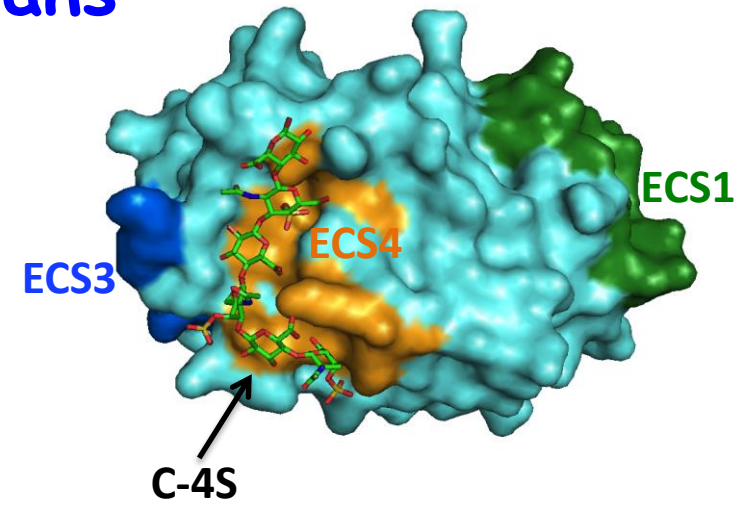


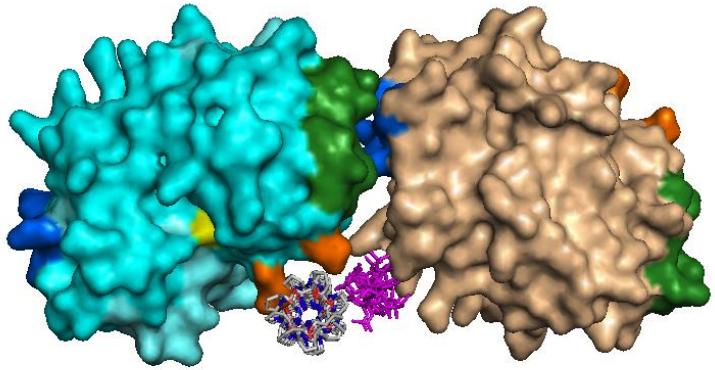
Insoluble Fiber collagen
(degraded by CatK dimer)

Soluble Collagen
(degraded by CatK tetramer)

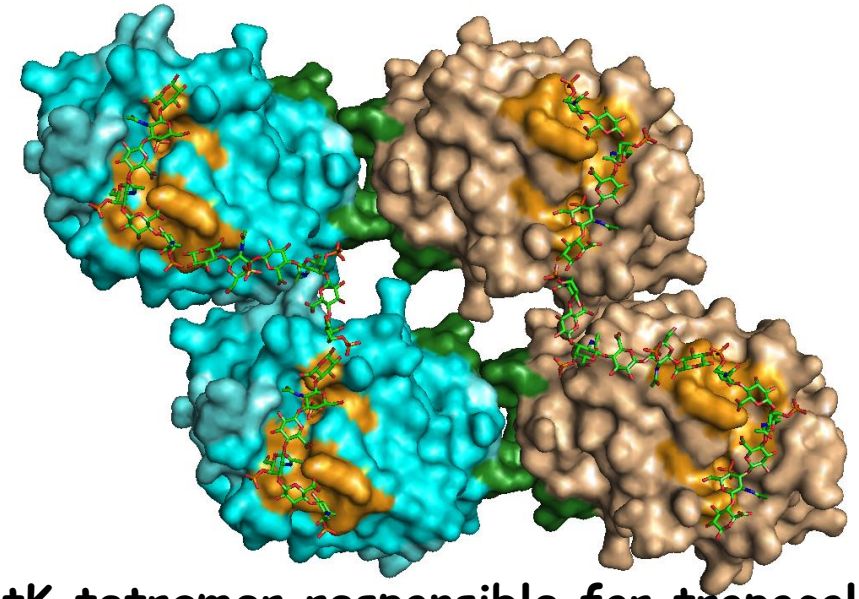
Collagenase Activity of CatK Requires Oligomerization in the Presence of Glycosaminoglycans

Specific protein-protein interaction sites (ECS1, ECS3) and ligand binding sites (ECS4 for GAGs) allow oligomerization





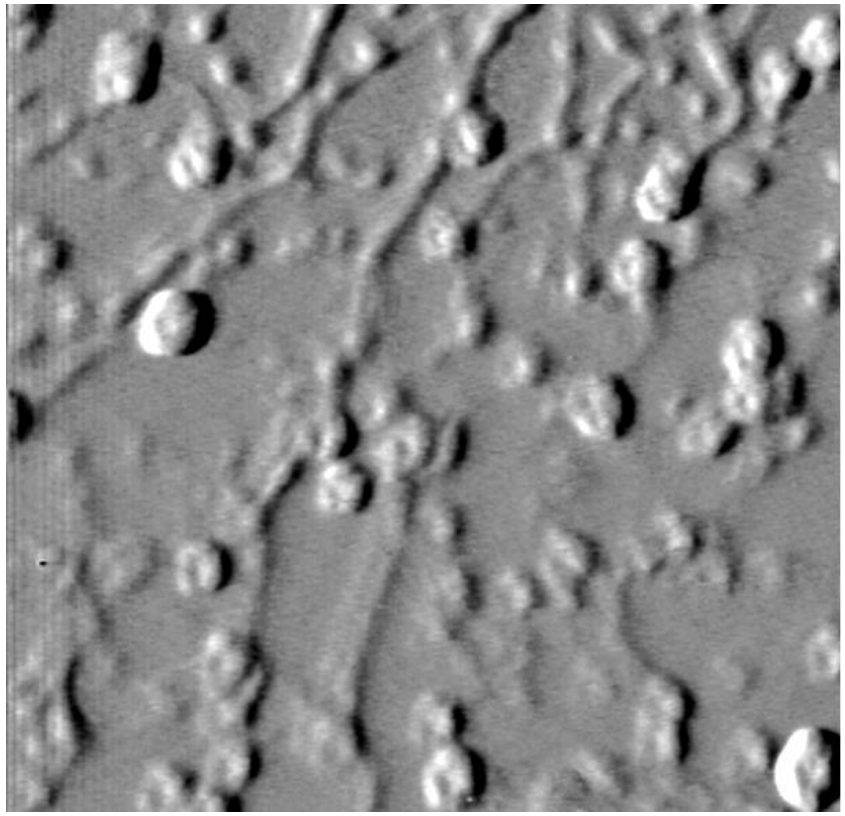
CatK dimer responsible for collagen fiber degradation (ESC1, ESC3)



CatK tetramer responsible for tropocollagen degradation (ESC1, ESC4)

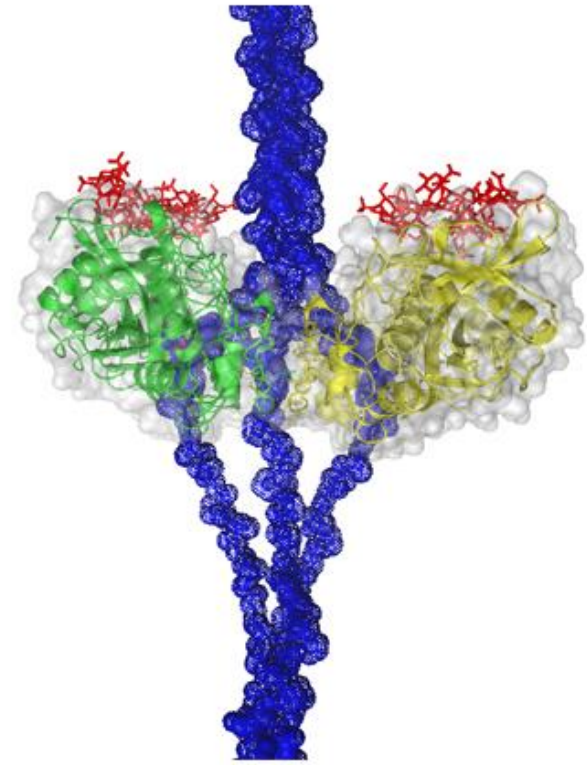
Type I Collagen Association with Tetrameric CatK

"donut"like tetrameric CatK/GAG complexes associate with collagen molecules



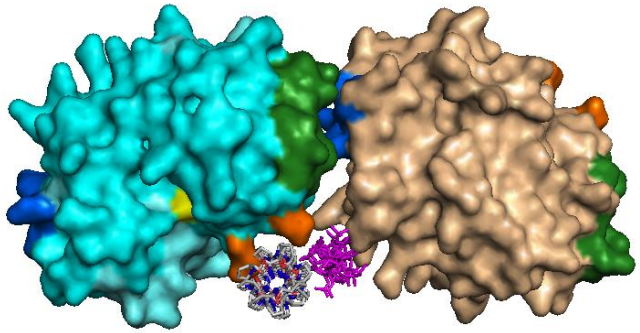
AFM

Model of collagen unfolding by tetramer

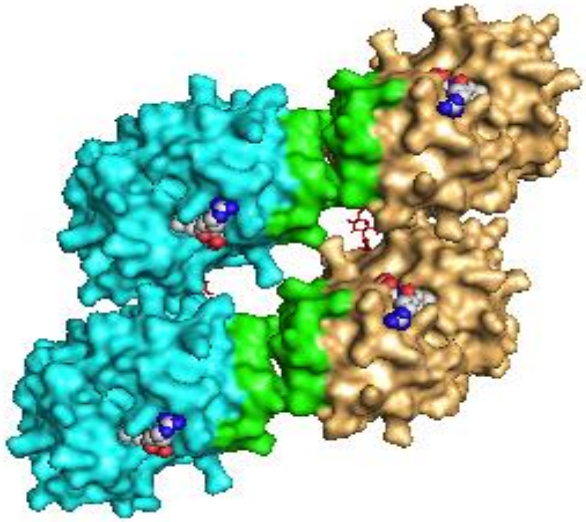


Tetramer likely acts as a helicase to unfold triple helical collagen

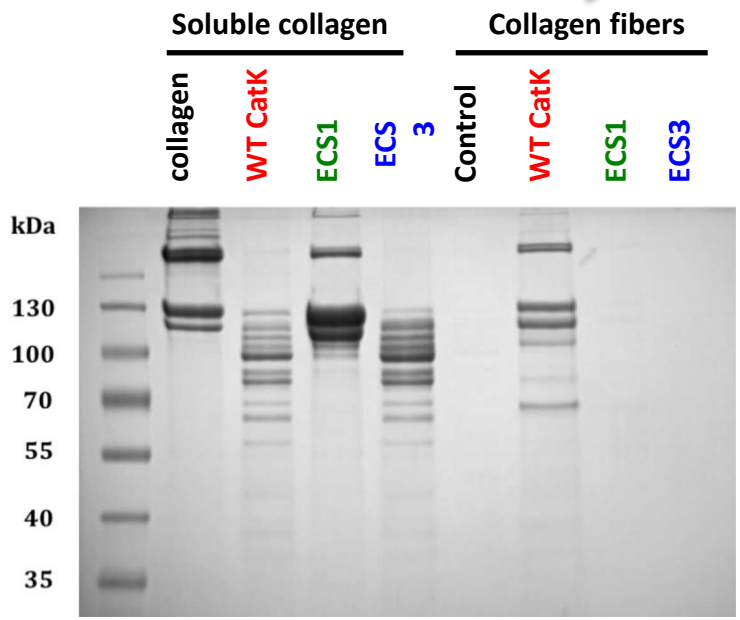
Inhibition of Collagenase Activity in CatK/CatL Chimerases

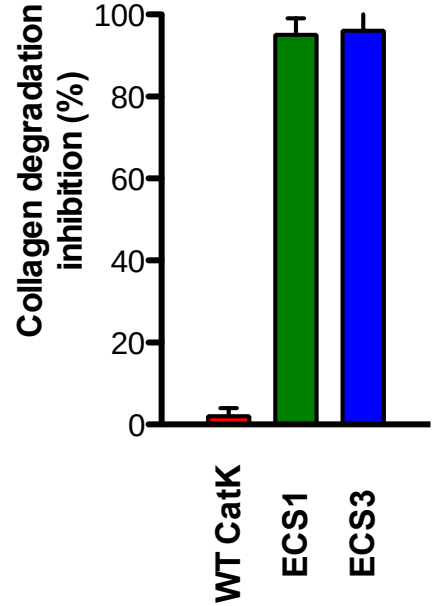


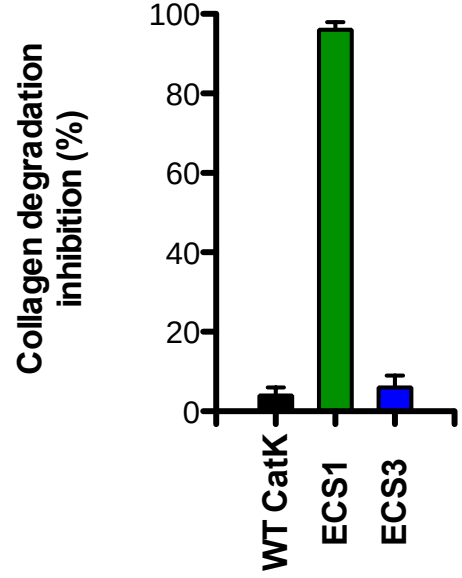
CatK dimer responsible for collagen fiber degradation (ESC1, ESC3)



CatK tetramer is only able to degrade soluble collagen



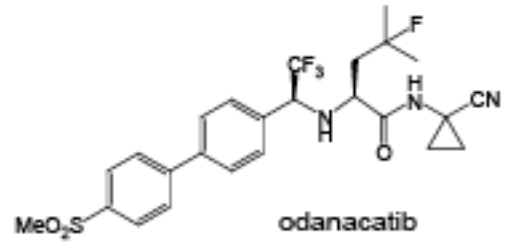




Synthetic Active site-directed Inhibitors of Cathepsin K as Anti-Osteoporotic Drugs

At least four compounds advanced into clinical trials:

- **Odanacatib**
- **Balicatib**
- **Relacatib**
- **ONO3445**



odanacatib

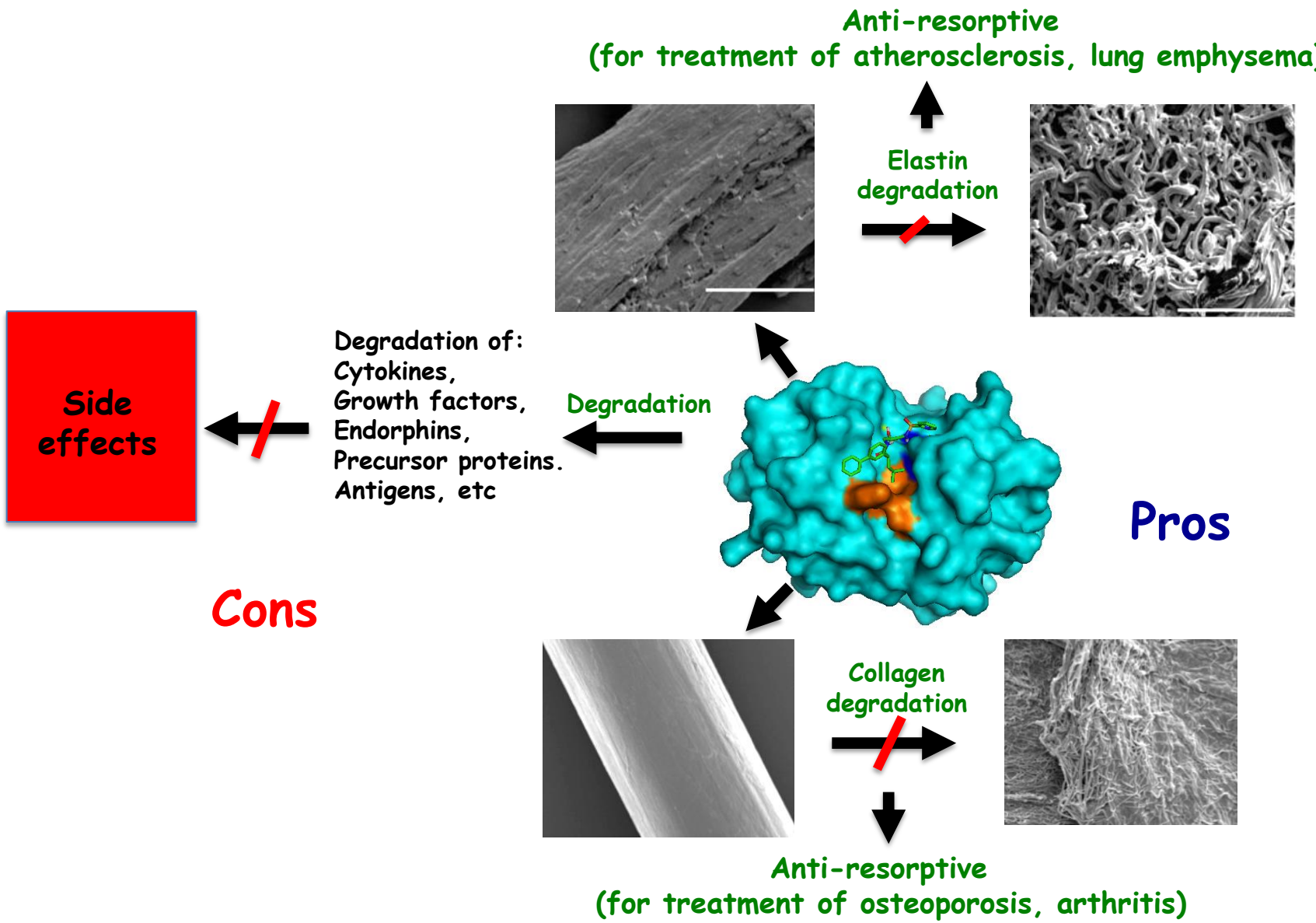
"catib" cathepsin inhibitor

Odanacatib completed successfully phase III testing

All inhibitors were effective in reducing surrogate bone resorption marker in women

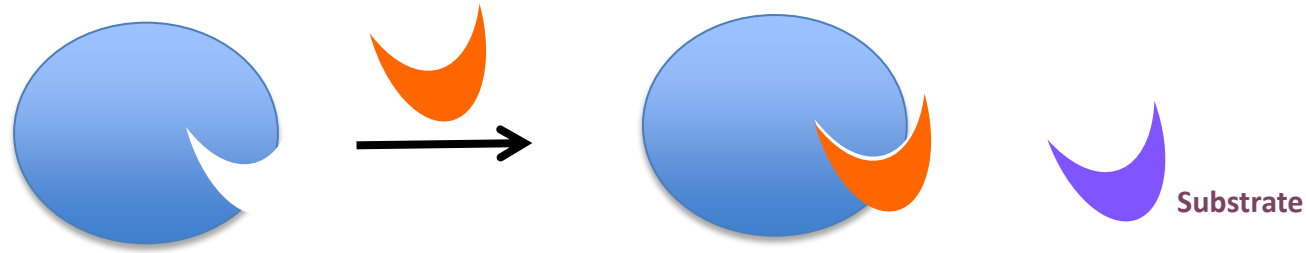
However,
trials with Balicicatib and Relacatib were discontinued due to side effects.

Pros and Cons of Active Site-Directed Cathepsin K Inhibitors



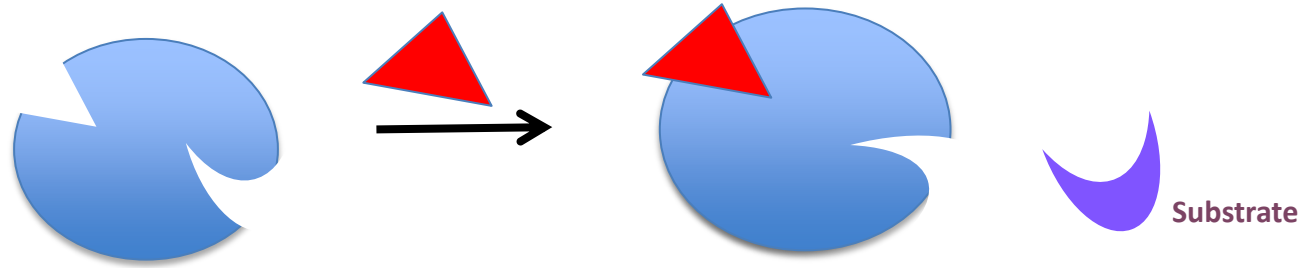
Protease inhibitors and the ectosteric concept

Active site-directed inhibitor

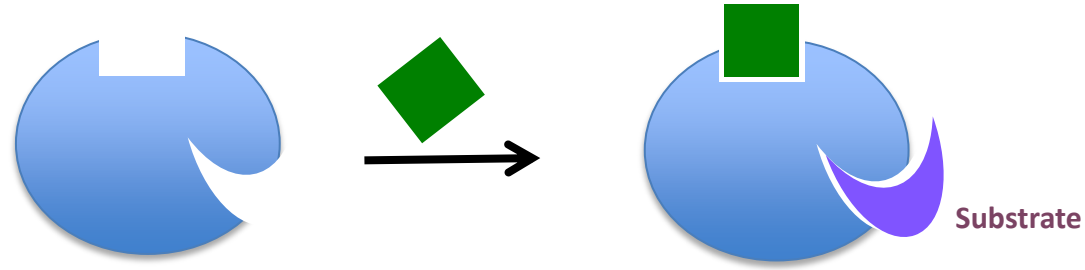


Exosite Inhibitors

Allosteric inhibitor

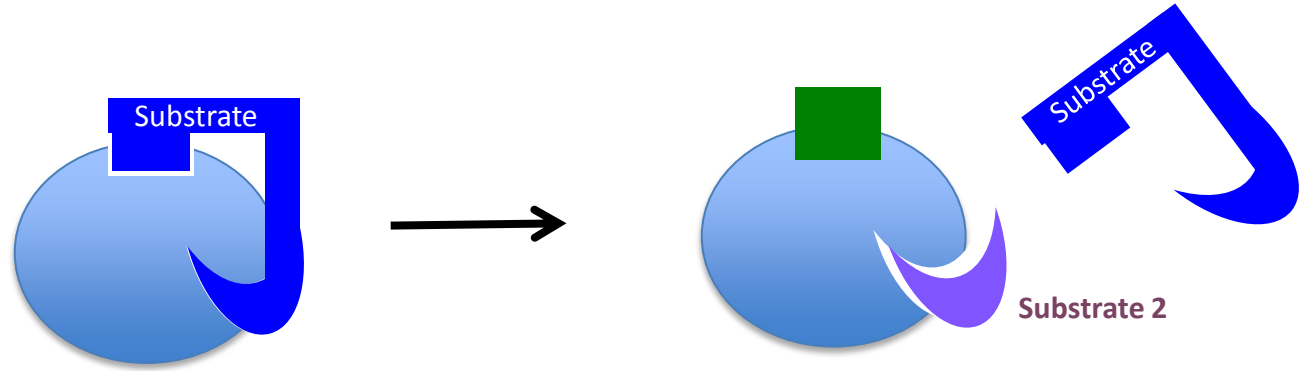


Ectosteric inhibitor

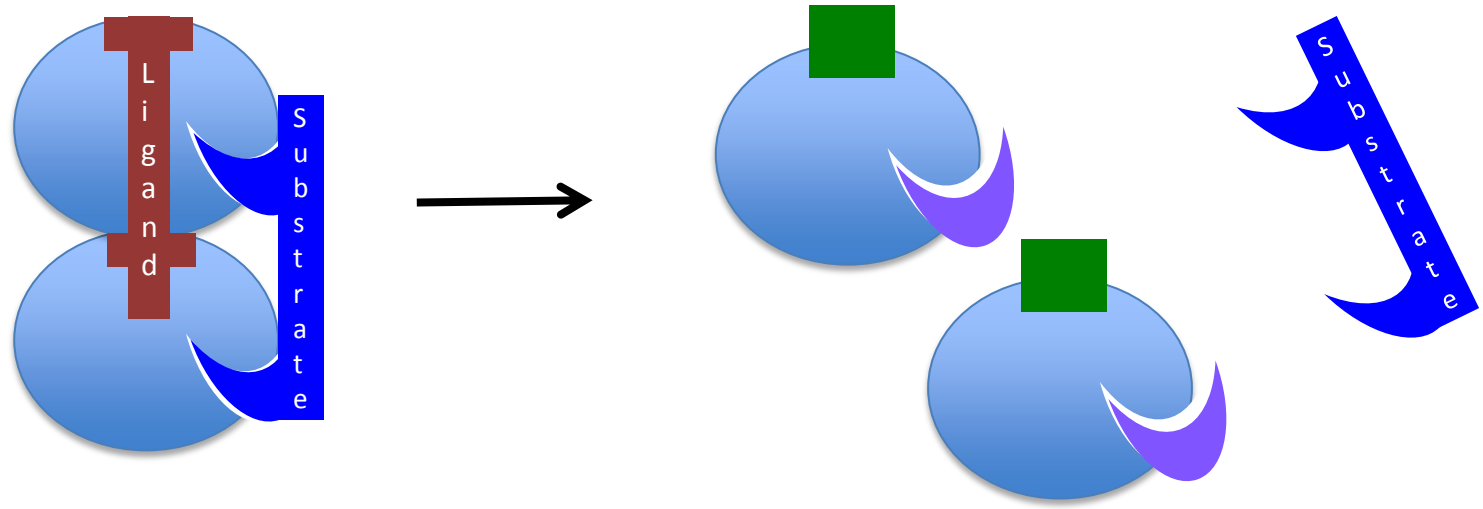


Ectosteric Inhibitors

Substrate binding site

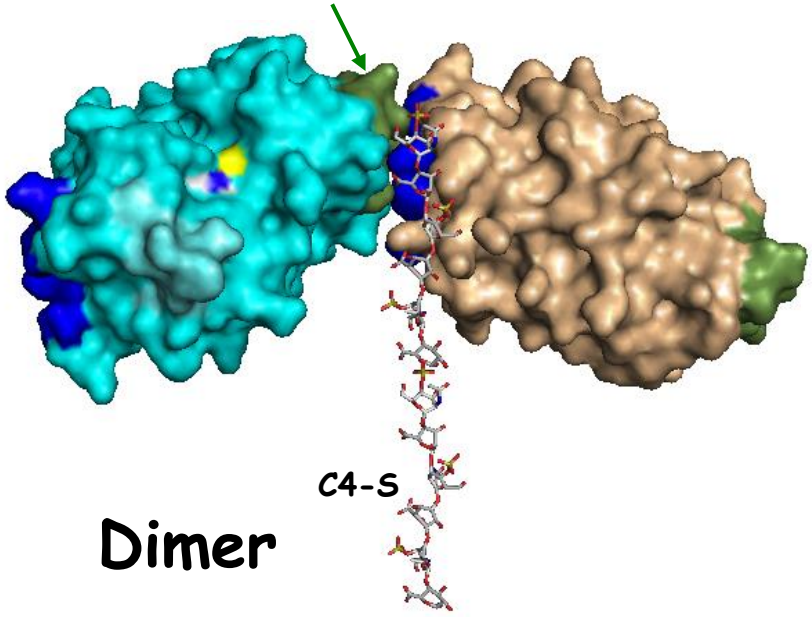


Oligomerization Ligand binding



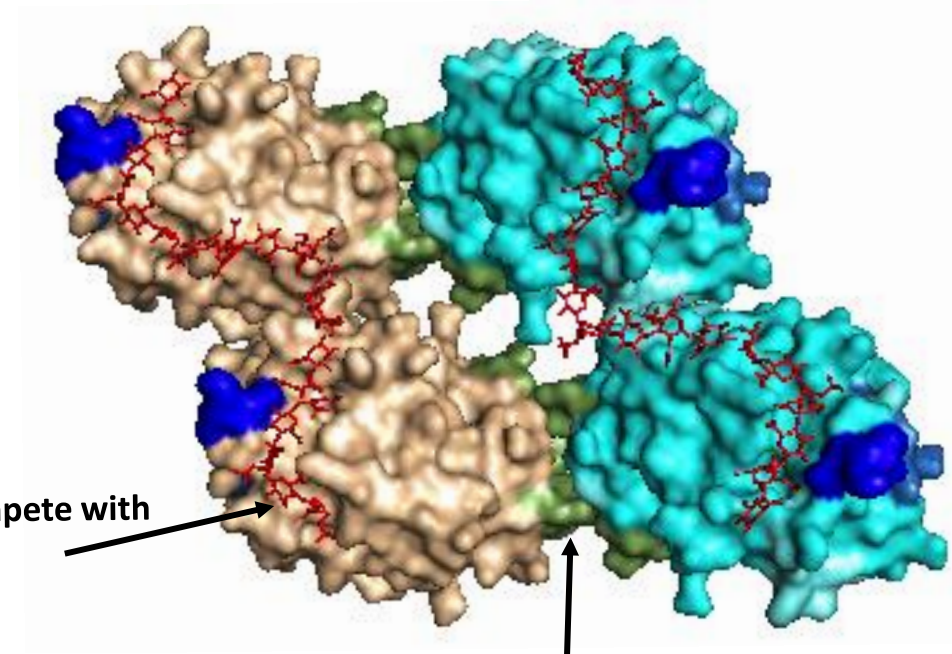
Strategy: To identify complex formation inhibitors which specifically block the collagenase and/or elastase activity of cathepsin K

Binding at ectosteric site 1 site to block dimerization



Dimer

Tetramer

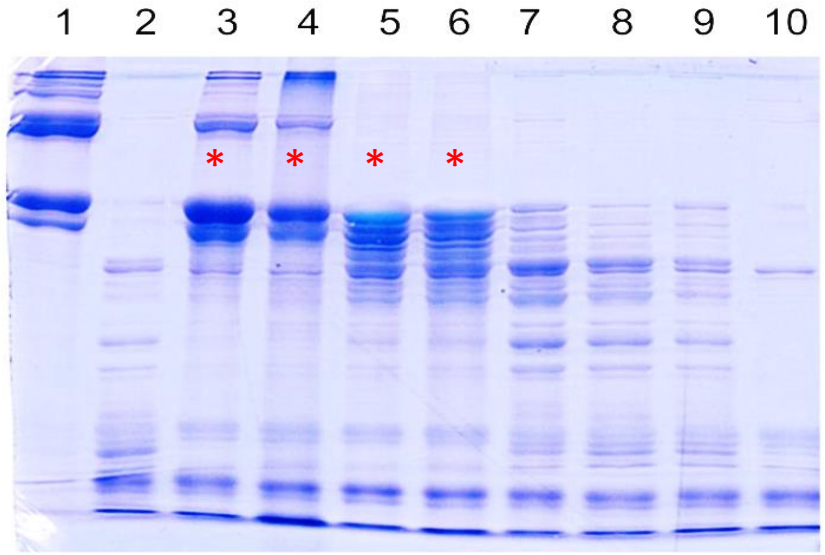


Binding site to compete with GAG binding

Binding at ectosteric site 1 site to block tetramerization

Screening for ectosteric CatK inhibitors in anti-osteoporotic Chinese herbal medicine

Screening of Herbal Extracts for Anti-Collagenase Inhibitors (I)



	CK	Inhibitor (methanol extracts) 100μg/mL
1	-	-
2	+	-
3	+	Dan Shen (Radix & Rhizoma Salviae Miltiorrhizae).
4	+	Dan Shen (Radix & Rhizoma Salviae Miltiorrhizae)
5	+	Di Huang (Radix Rehmamniae)
6	+	Di Huang (Radix Rehmamniae)
7	+	Bai Zhu (Rhizoma Atractylodis Macrocephalae)
8	+	Bai Zhu (Rhizoma Atractylodis Macrocephalae)
9	+	Huang Qi (Radix Astragali)
10	+	Huang Qi (Radix Astragali)

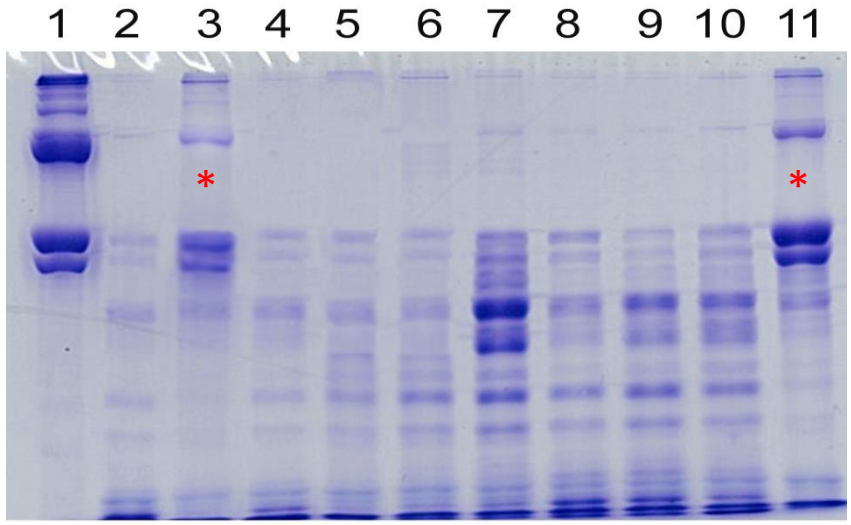


Dan Shen



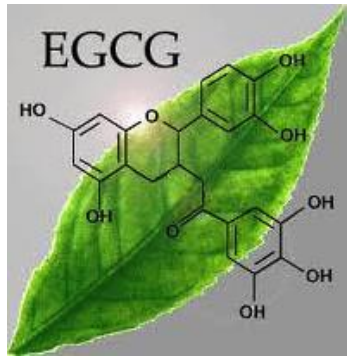
Di Huang

Screening of Herbal Extracts for Anti-Collagenase Inhibitors (II)





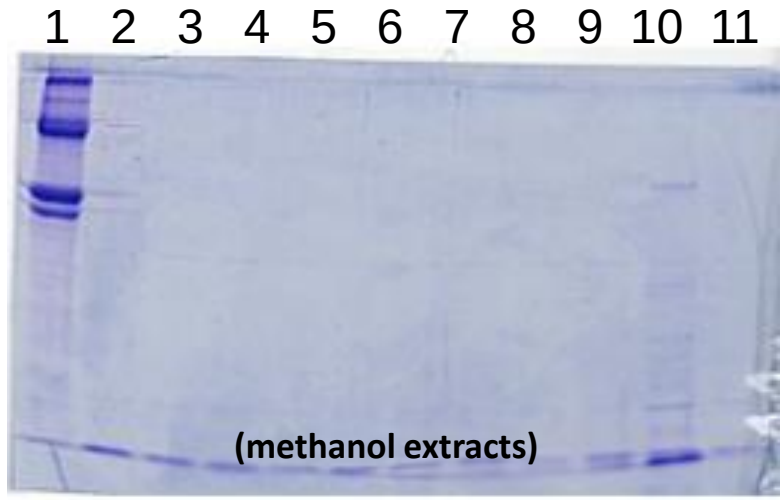
Gu Sui Bu

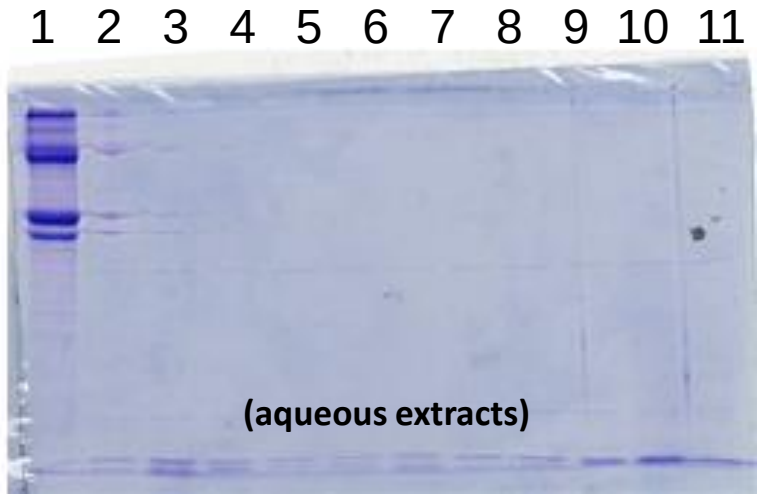


Green tea

	CatK	Inhibitor (aqueous extracts) 100μg/mL
1	-	-
2	+	-
3	+	Gu Sui Bu (Rhizoma Drynariae)
4	+	Huai Niu Xi (Radix Achyranthis Bidentatae)
5	+	Bu Gu Zi (Fructus Cnidii)
6	+	Chuan Niu Xi (Radix Achyranthis)
7	+	San Qi (Panax notoginseng)
8	+	Tu Si Zi (Cuscuta chinensis seed)
9	+	Xi Yang Shen (American Ginseng)
10	+	Ren Shen (Radix Ginseng)
11	+	EGCG

Anti-Collagenolytic Herbs do not Inhibit the Gelatinase Activity of Cathepsin K





No.	Name
1	Gelatin
2	Gelatin+CatK
3	Dan Shen (Radix & Rhizoma Salviae Miltiorrhizae)
4	Di Huang (Radix Rehmamniae)
5	Du Zhong (Eucommia ulmoides)
6	Juan Bai (Herba Selaginellae Involvens)
7	Xu Duan (Dipsacus asper)
8	Nv Zheng Zi (Fructus Ligustri Lucidi)
9	Gu Sui Bu (Rhizoma Drynariae)
10	San Qi (Panax notoginseng)
11	EGCG

Tanshinone Inhibitors Screened for CatK Collagenase Inhibition



Salvia miltiorrhiza

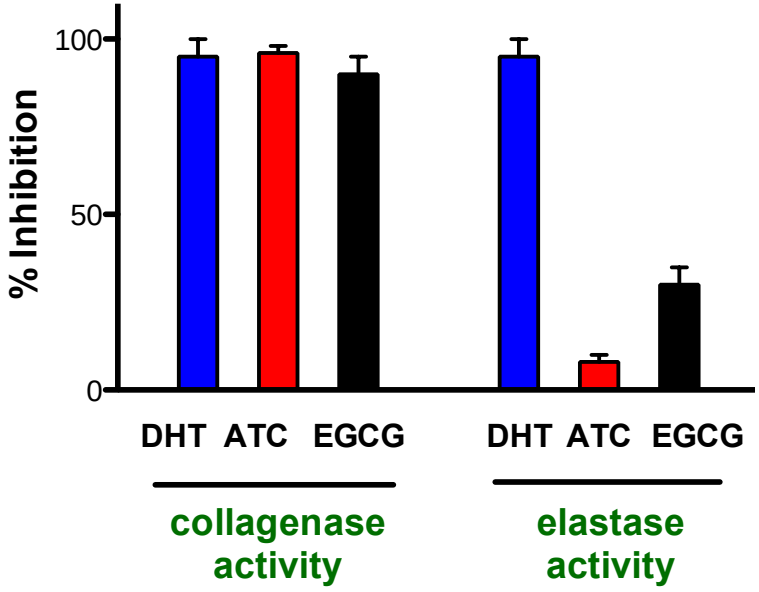
Inhibitor	Name	Structure	Z-FR-MCA Inhibition (%)	Collagen Degradation IC50 (μM)
T01	Miltirone		7.14	29.5
T02	15,16-Dihydrotanshindiol		21.07	9.4
T03	Danshenol A		1.77	20.5
T04	Epi-cryptoacetalide		16.31	64.7
T05	1,2,5,6-Tetrahydrotanshinone I		29.23	4.5
T06	Tanshinone IIA Sulfonic Sodium		16.35	2.1
T07	Methylenedihydrotanshinquinone		38.42	13.2
T08	Salvianan		21.35	7.3
T09	Trijuganone C		9.87	9.4
T10	Epidanshenspiroketal lactone		1.13	>50
T11	Dihydrotanshinone		30.25	15.5
T12	Dihydrotanshinone I		18.30	7.1
T13	Cryptotanshinone		2.57	25.6
T14	Tanshinone I		29.2	13.3
T15	Tanshinlactone		11.30	>100

Inhibitor	Name	Structure	Z-FR-MCA Inhibition (%)	Collagen Degradation IC50 (μM)
T16	Tanshinone IIA		7.67	>100
T17	Isotanshinone II		30.50	4.4
T18	Danshenxinkun D		16.22	>50
T19	Przewaquinone A		41.63	13.2
T20	Neocryptotanshinone II		49.35	5.7
T21	Salviolone		33.25	15.7
T22	Sageone		7.26	17.6
T23	Methylenetanshinquinone		47.61	4.3
T24	Neocryptotanshinone		6.44	>50
T25	1,2-Didehydrocrypto-tanshinone		66.42	>50
T26	15,Epi,Danshenol-A		49.21	>50
T27	Tanshindiol A		6.20	16.3
T28	6,7-Dehydroferruginol		0	>100
T29	Salvinolone		19.76	>100
T30	Sugiol		17.38	>100
T31	Hydroxymethylenetanshinquinone		1.60	>100

http://www.itmonline.org/arts/salvia.ht

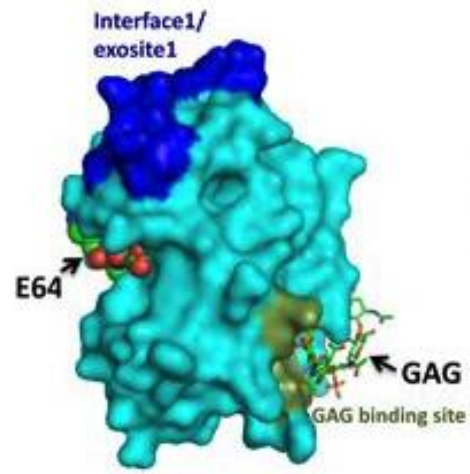
Collagenase and elastase inhibition of cathepsin K by exosite inhibitors

Docking of DHT and EGCG on putative binding sites of cathepsin K

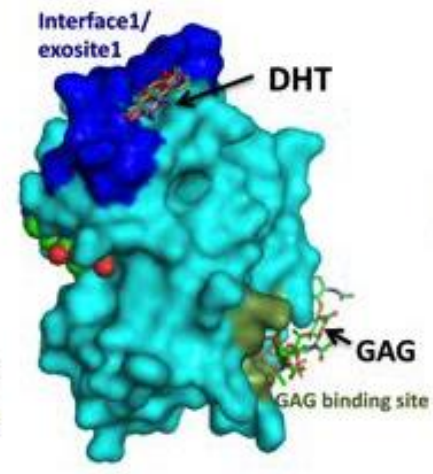


Collagenase and elastase inhibition

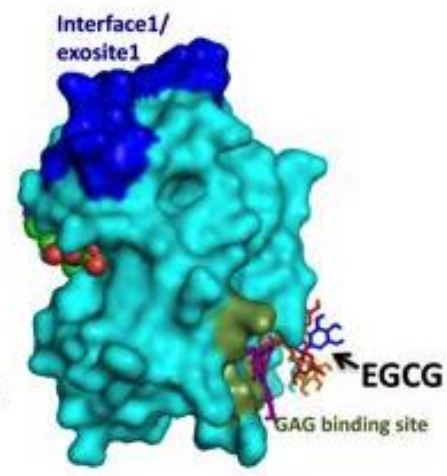
Collagenase but weak to no elastase inhibition

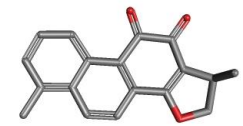


CatK/GAG

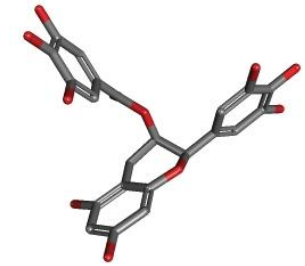


CatK/DHT



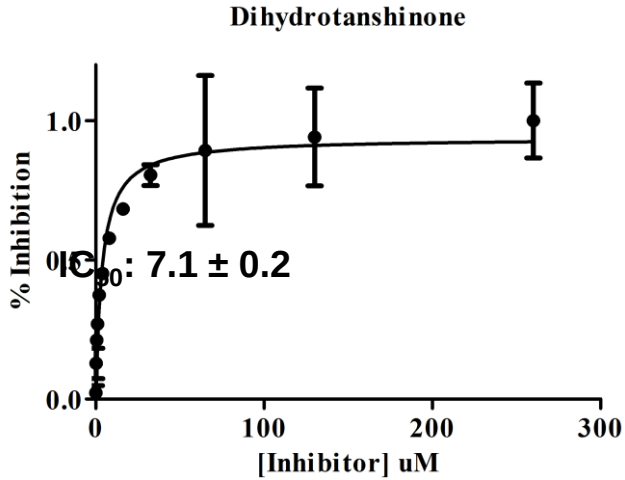


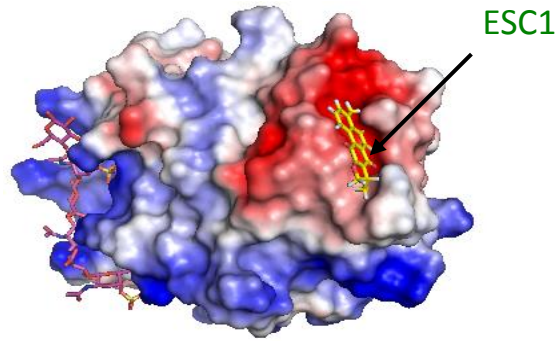
Dihydrotanshinone (DHT)



Epigallocatechin gallate (EGCG)

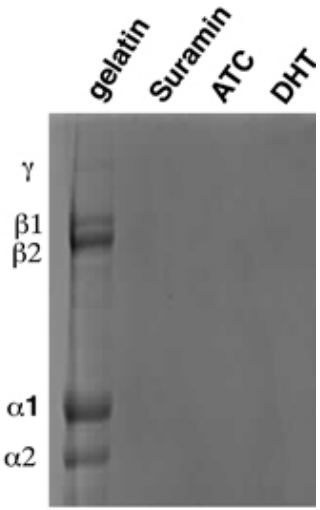
Inhibition of CatK collagen degradation by dihydrotanshinone I



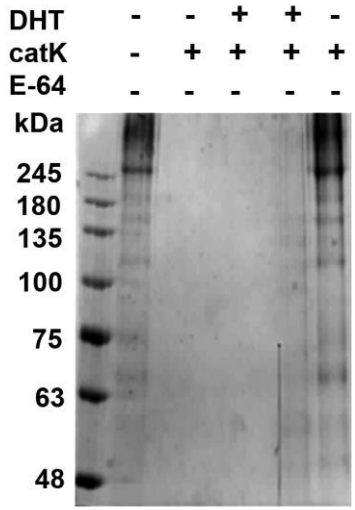


Putative binding of DHT in ESC1

Ectosteric site inhibitors (at 50μM) do not block the degradation of non-ECM substrates

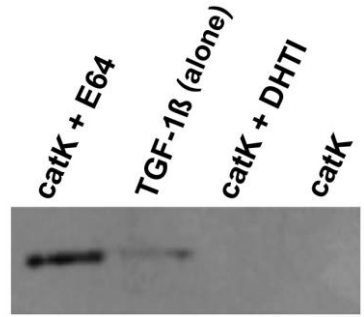


No effect on gelatin degradation by catK



DHTI does not inhibit the degradation of thyroglobulin by catK

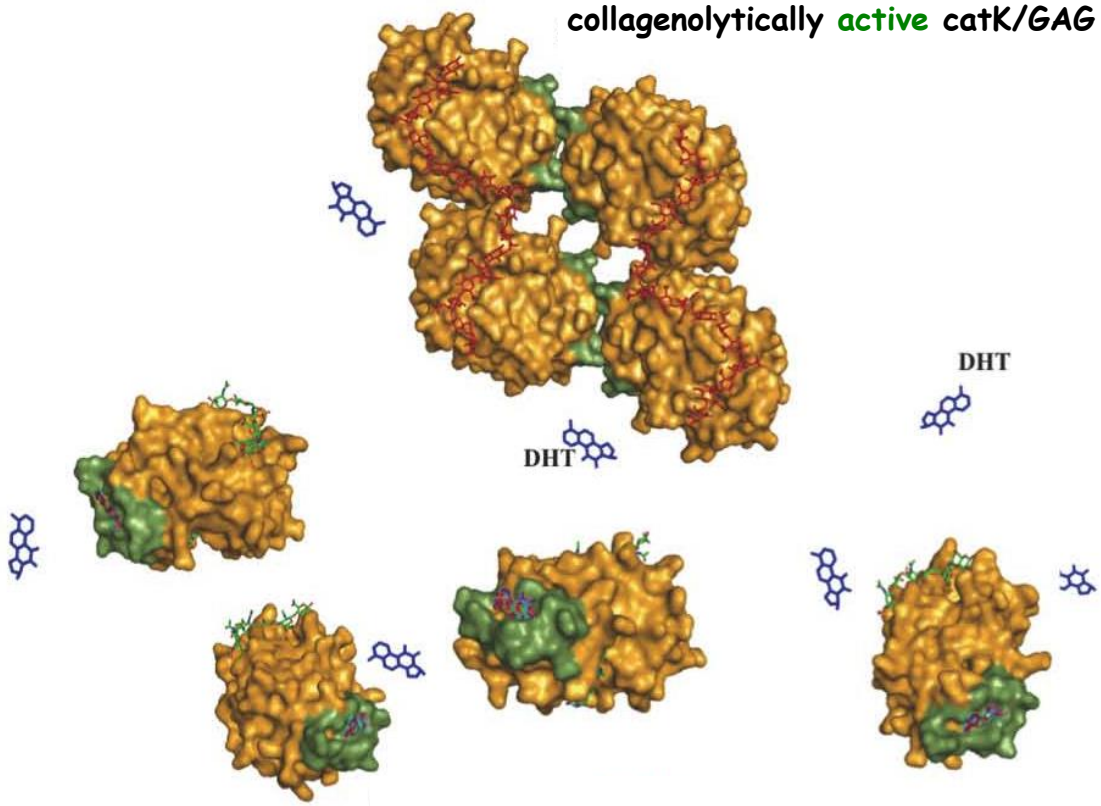
(implicated in cognitive malfunction in ctsk$^{-/-}$)

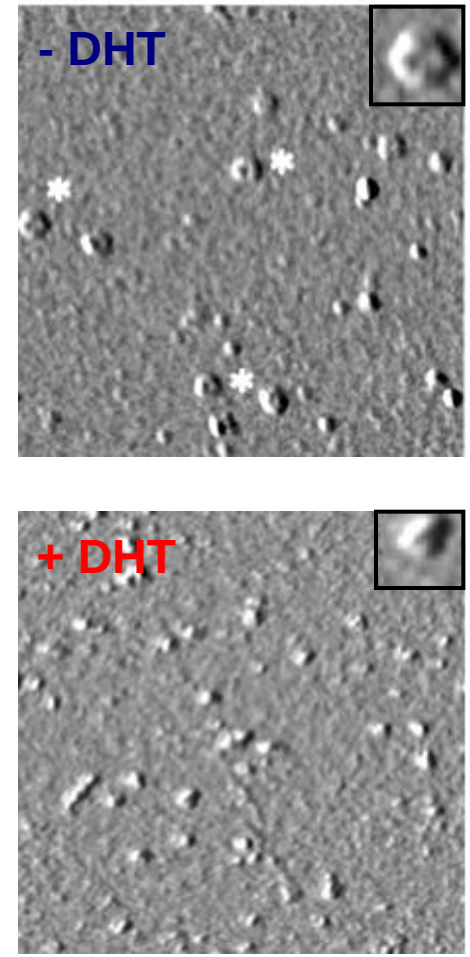


DHTI does not inhibit the degradation of TGF-1ß

(implicated in airway abnormalities in ctsk$^{-/-}$)

Scheme of inhibition of cathepsin K oligomer by dihydrotanshinone





Atomic force microscopy images of catK/C4-S complexes in the absence and presence of DHT. DHT prevents the formation of ring-like catK complexes (*).

Acknowledgments

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Nham Nguyen

CDRD/UBC:
Tom Pfeifer



University of Southern Denmark
Jean-Marie Delaisse
Kent Soe

Financial Support: CIHR, NSERC, CRC

Inhibition of cathepsin K-mediated collagen fiber degradation by ectosteric site inhibitors

Cathepsin K	-	+	+	+
Ectosteric site inhibitor	-	-	EGCG	DHTI

