

INIBIDORES DA ENZIMA CONVERSORA DE ANGIOTENSINA (ECA)

REDSHAW, S. *Angiotensin-converting enzyme (ACE) inhibitor and the design of cilazapril*. In: GANELLIN, C. R & ROBERTS, S. M. (eds) "Medicinal chemistry: the role of organic chemistry in drug research", Academic Press, 2nd. ed., p. 163-185, **1993**.

SILVERMAN, R. B. "The organic chemistry of drug design and drug action", Academic Press, p. 162-174, **1992**.

HARROLD, M. *Agents affecting the renin-angiotensin pathway and calcium blockers*. In: LEMKE, T.L. & WILLIAMS, D.A. (Eds.) "Foye's principles of medicinal chemistry", Lippincott Williams & Wilkins, 5th ed., p. 815-840 (Cap.25), **2013**.

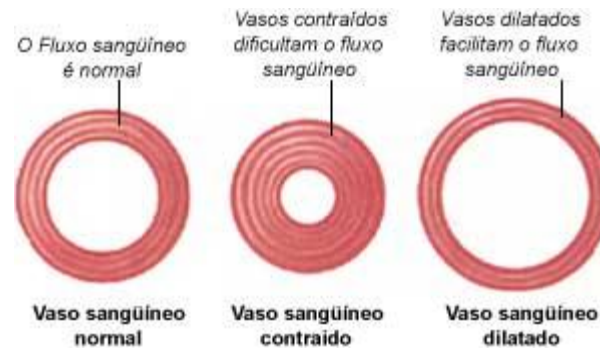
HARROLD, M. *Angiotensin converting enzyme inhibitors, antagonists and calcium blockers*. In: LEMKE, T.L. & WILLIAMS, D.A. (Eds.) In: "Foye's principles of medicinal chemistry", Lippincott Williams & Wilkins, 6th ed., p. 738-768, **2008**.

NATESH, R., SCHUWAGER, S.L.U., EVANS, H.R., STURROCK, E.D., ACHARYA, K.R. Structural details on the binding of antihypertensive drugs captopril and enalaprilat to human testicular angiotensin I-converting enzyme. *Biochemistry* **2004**, 43, 8718-8724.

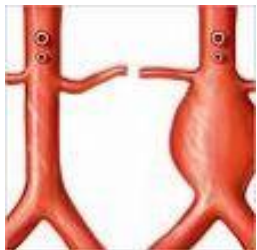
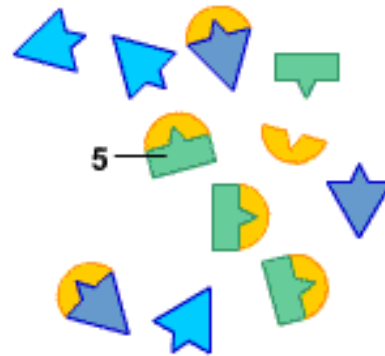
NATESH, R., SCHUWAGER, S.L.U., STURROCK, E.D., ACHARYA, K.R. Crystal structure of the human angiotensin-converting enzyme-lisinopril complex. *Nature* **2003**, 421, 551-554.

ACHARYA, K.R.; STURROCK, E.D.; RIORDAN, J.F.; EHLERS, M. R.W. ACE revisited: a new target for structure based drug design. *Nature Reviews Drug Discovery* **2003**, 2, 891-902.

Em situações estressantes, as artérias que suprem funções não essenciais se contraem, causando aumento da pressão arterial.

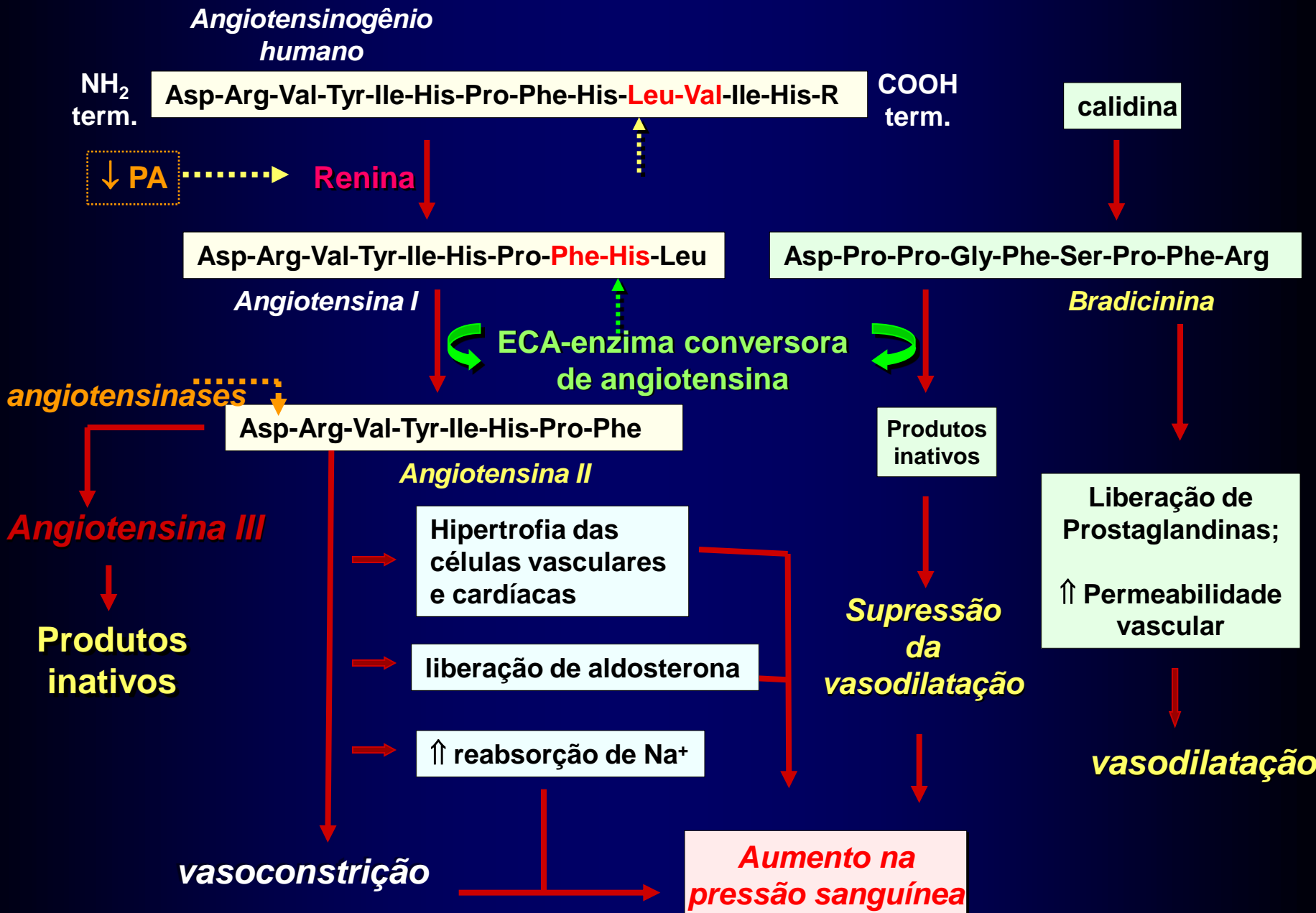


A enzima conversora de angiotensina (ECA) leva à formação de angiotensina II, que provoca a constrição dos vasos sanguíneos



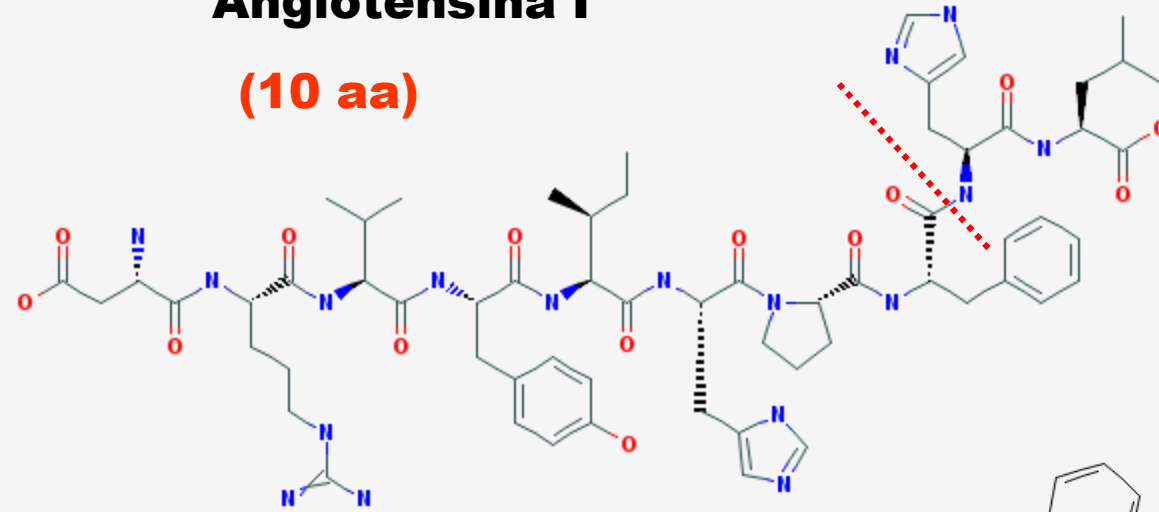
Os inibidores da ECA também previnem a expansão e ruptura da aorta em modelos com animais. Esses fármacos reduzem o risco de ruptura em pacientes com aneurismas da aorta abdominal



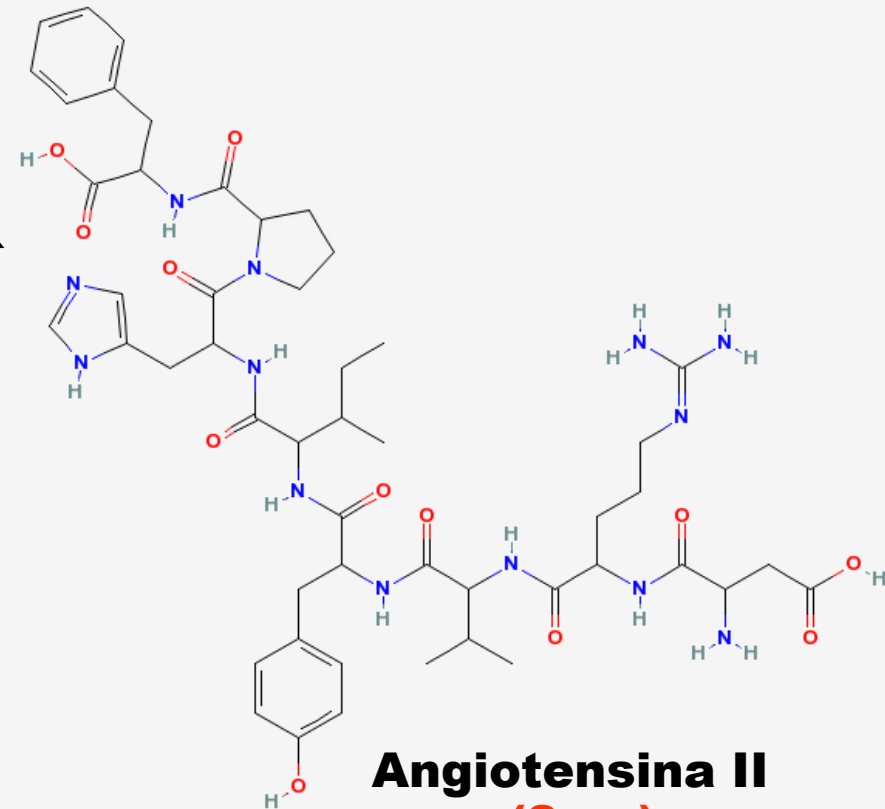


Angiotensina I

(10 aa)



ECA

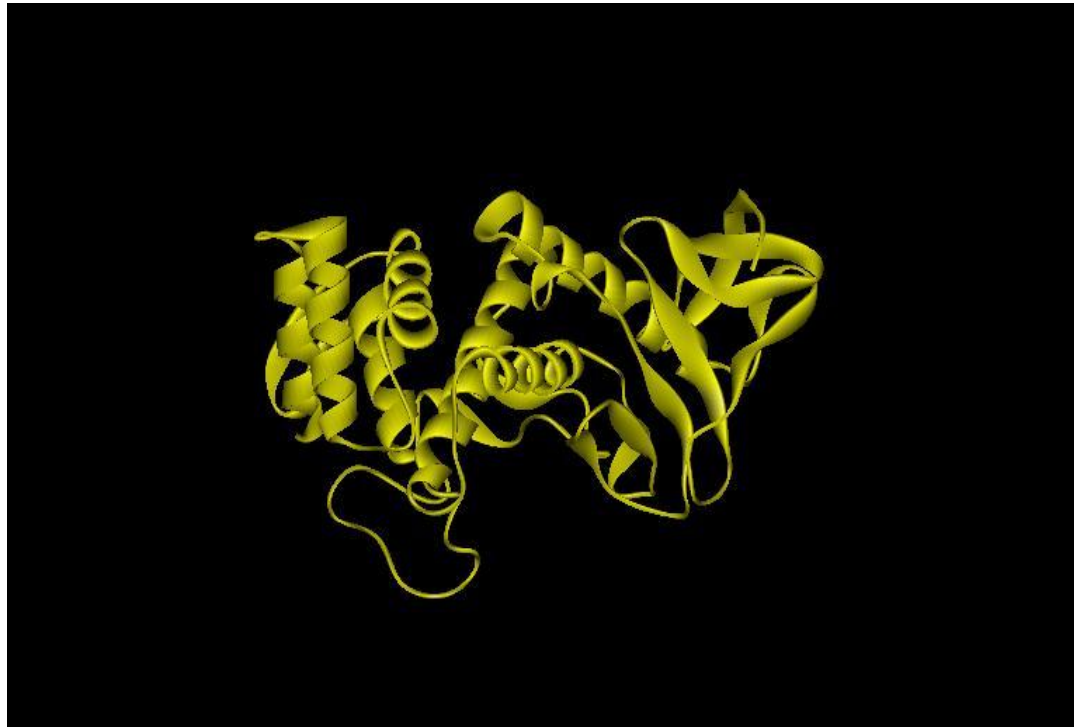


Angiotensina II

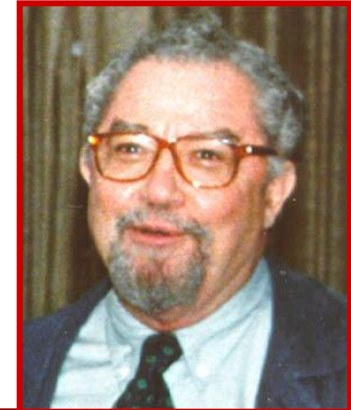
(8 aa)

ECA

- glicoproteína ligada à membrana das células endoteliais vasculares
- estrutura determinada em 1988 (metaloproteínase)



Busca de inibidores da ECA



1. Descoberta do protótipo

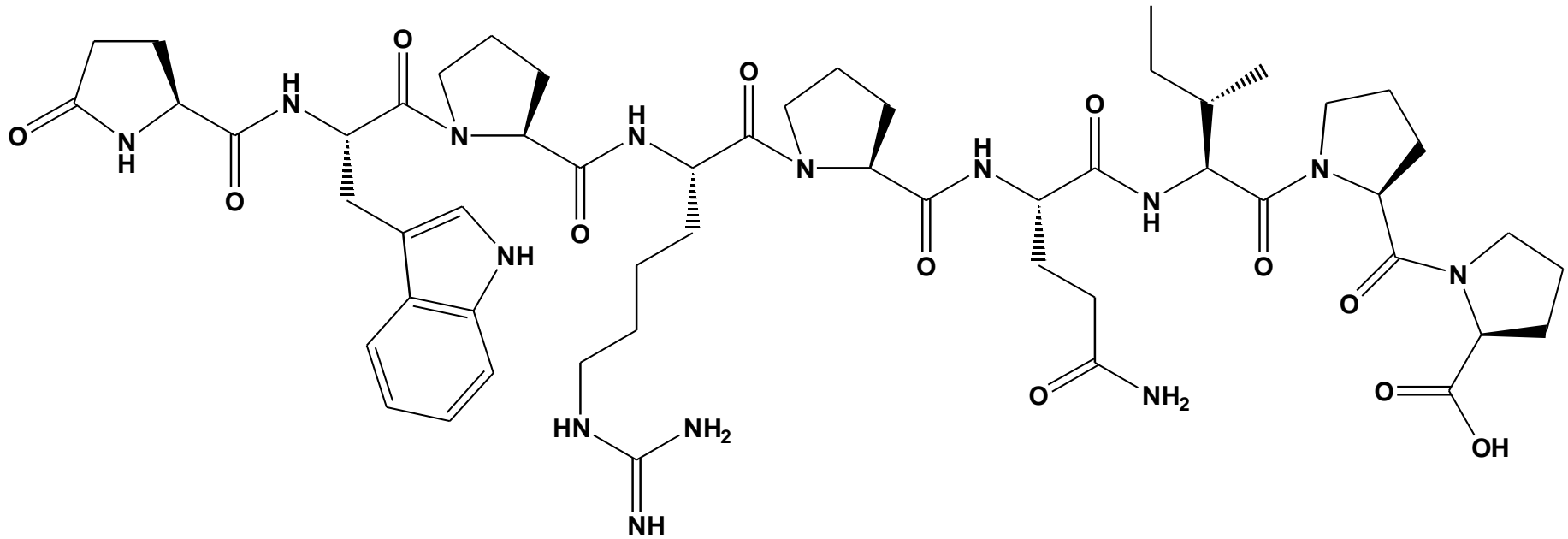
1965 - prof. Dr. Sérgio H. Ferreira - relatou que uma mistura de peptídeos (5-13 aa) da cobra *Bothrops jararaca* potencializava a ação da bradicinina pela inibição de alguma bradicininase



1968 - *Bakhle* e colaboradores – mostraram que estes peptídeos também inibiam a conversão de angiotensina I em angiotensina II

1979 - *Ondetti* e colaboradores - isolamento de 9 peptídeos desta mistura – **nonapeptídeo (TEPROTÍDEO)** ⇒ apresentou a maior potência *in vivo* e foi efetivo na diminuição da pressão sanguínea (inativo por via oral)

TEPROTÍDEO (SQ 20.881)



O TEPROTÍDEO e outros peptídeos foram usados para estudar as propriedades enzimáticas da ECA

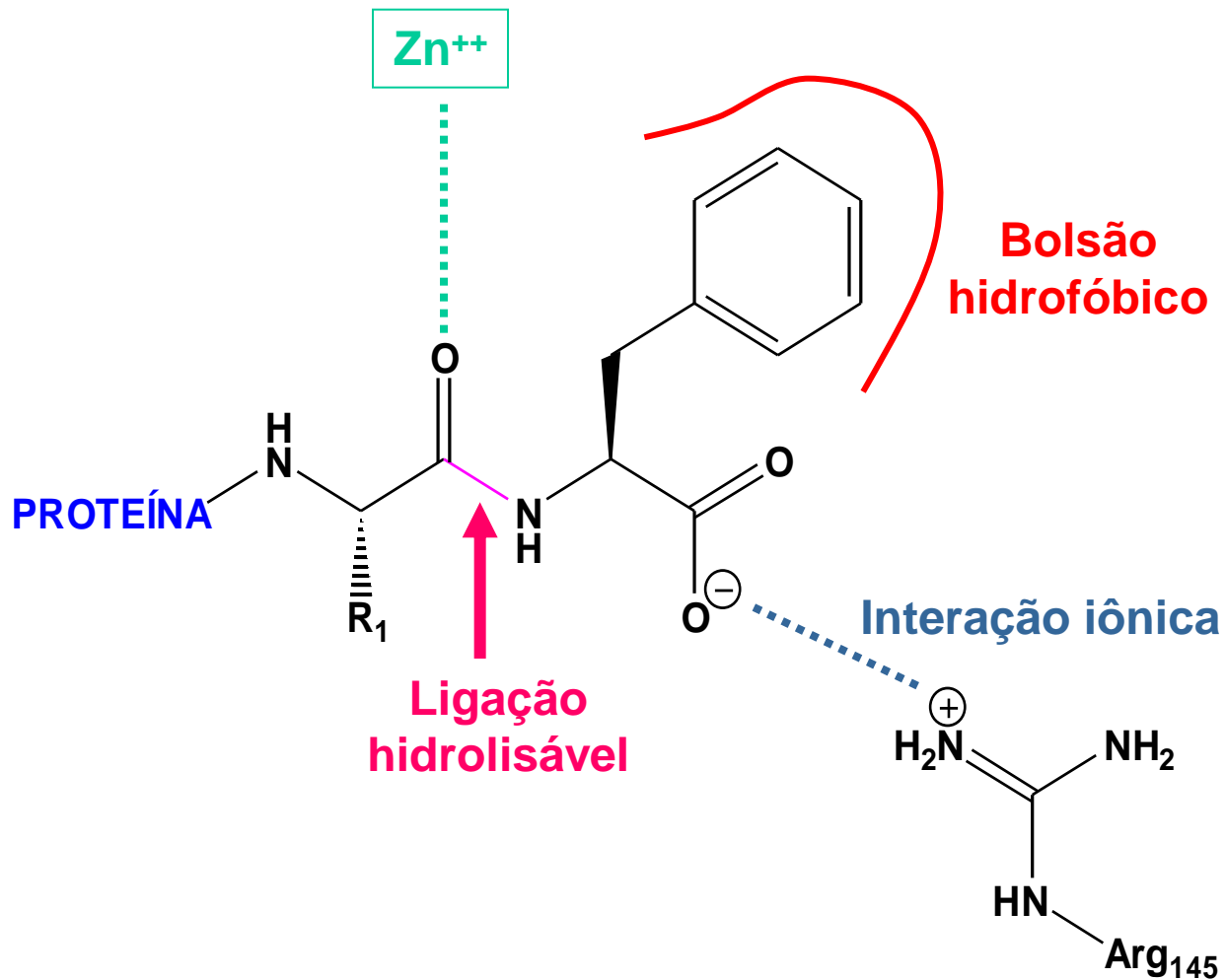


propriedades semelhantes de outras carboxipeptidases

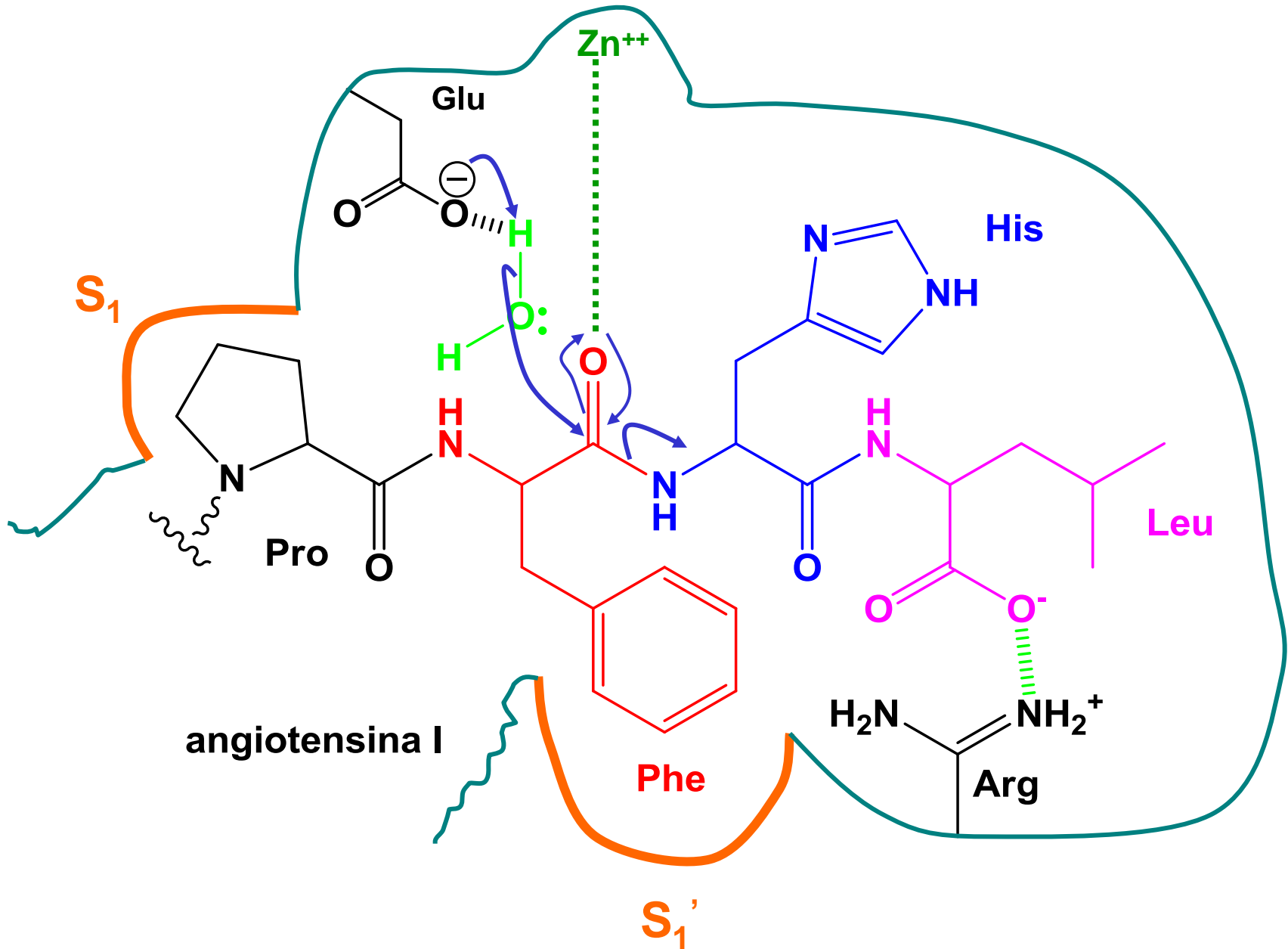


MODELO HIPOTÉTICO DO SÍTIO ATIVO DA ECA

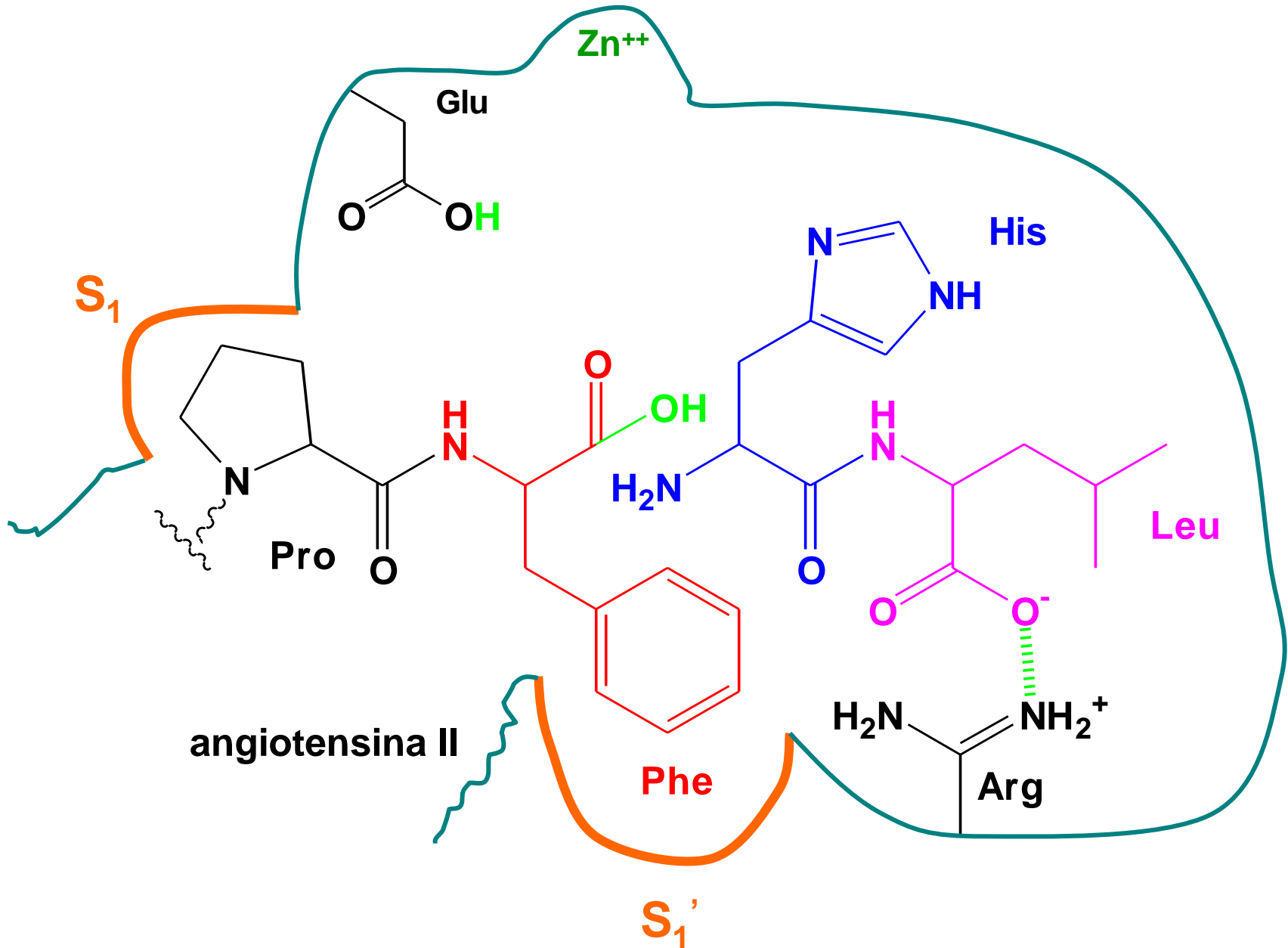
Modelo de ligação da carboxipeptidase



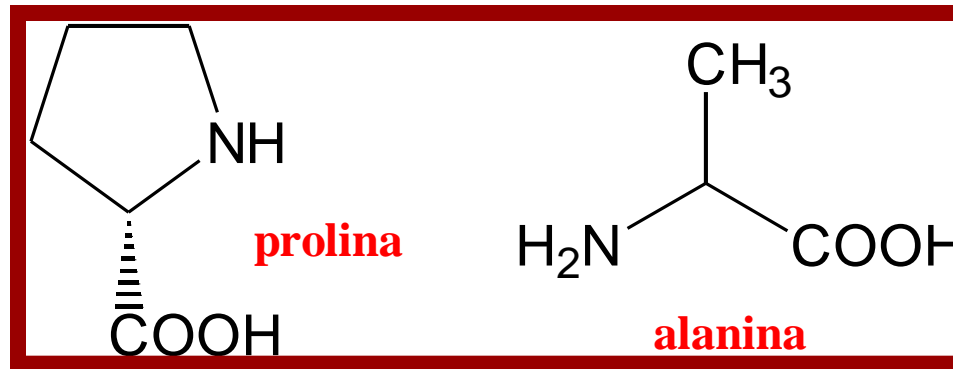
Reação catalisada pela ECA



Produtos da reação catalisada pela ECA



Requerimento estrutural mínimo para ligação e quebra pela ECA: ser tripeptídeo com COO⁻ livre ⇒ vários inibidores pequenos foram preparados

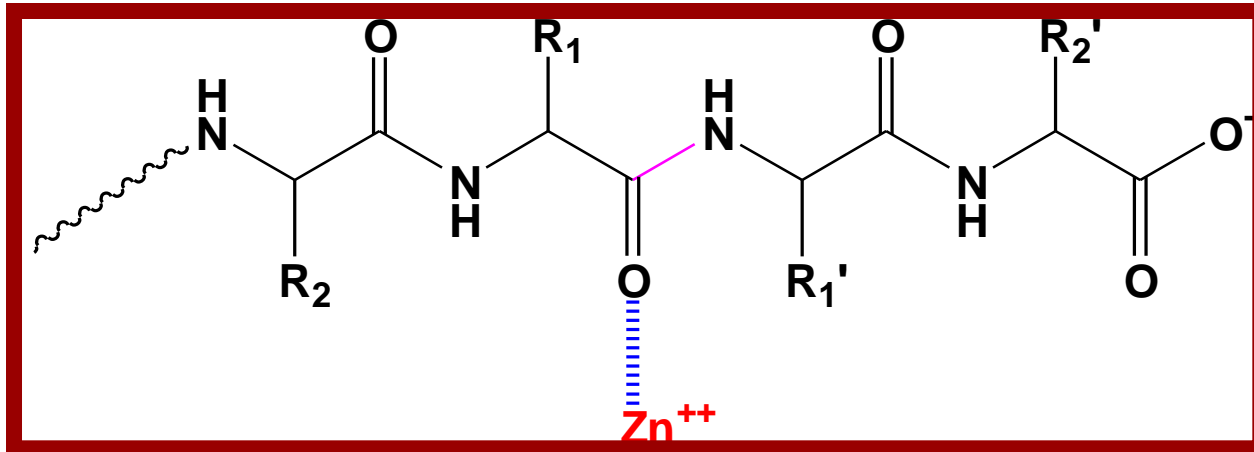


Melhor C terminal: prolina

Penúltima posição: alanina

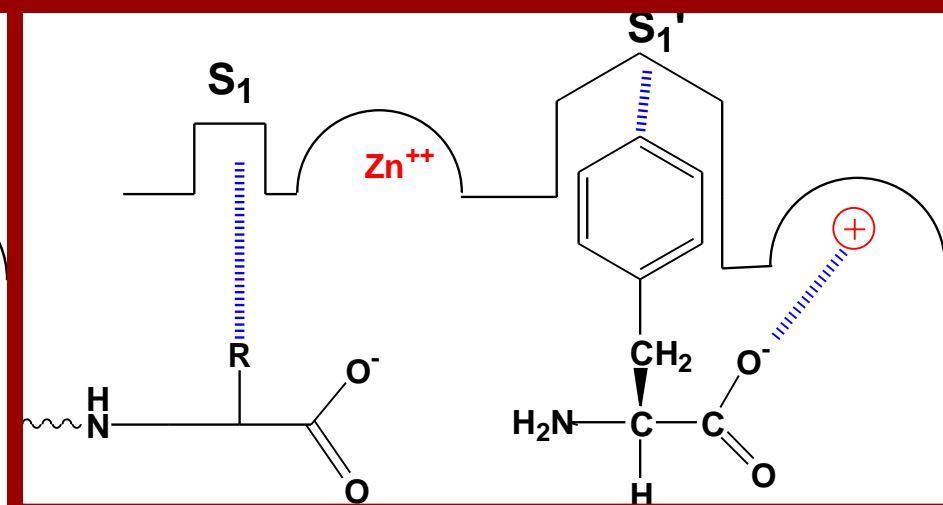
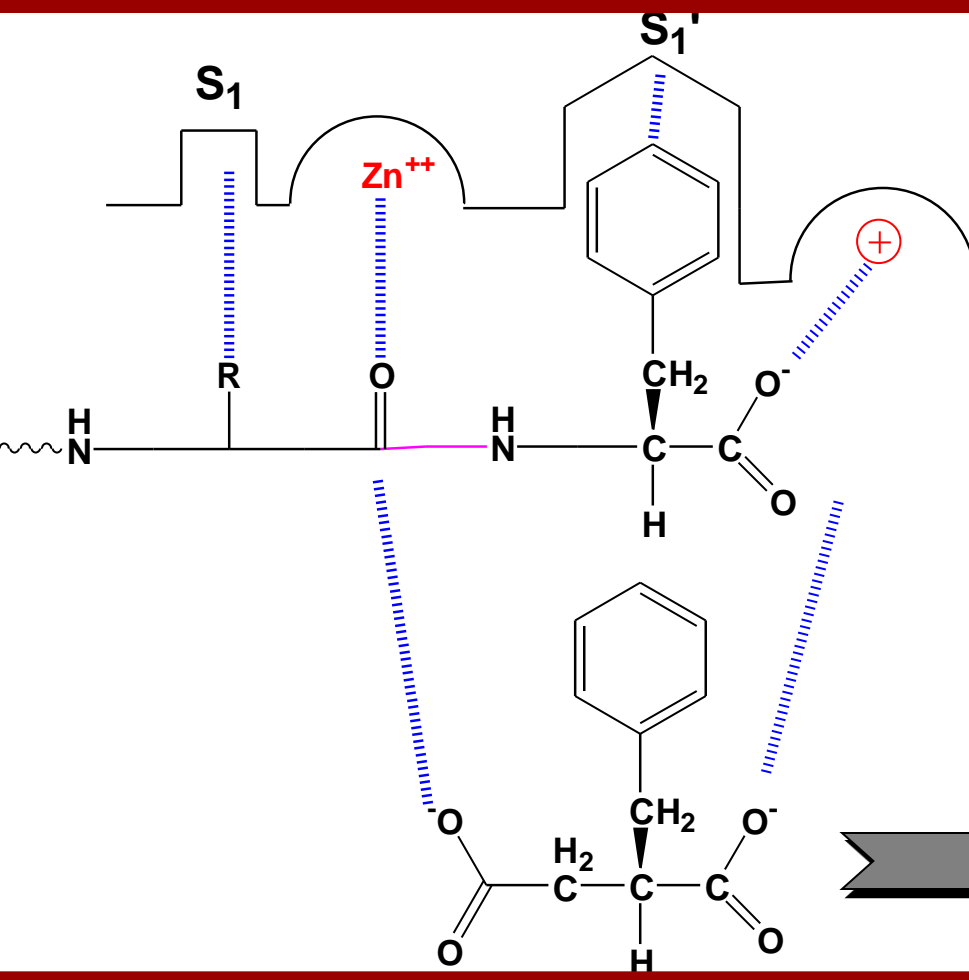
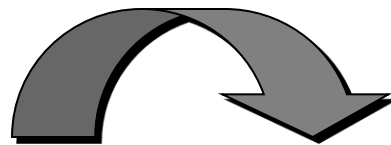
Antepenúltima posição: aminoácido aromático

Presença do **Zinco** é essencial para atividade da ECA



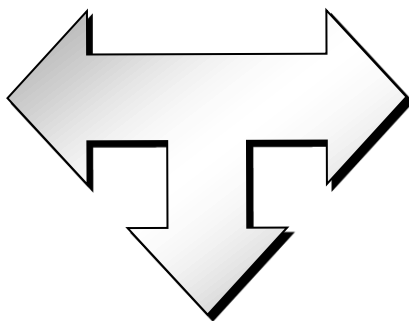
Comparação com carboxipeptidase A

hidrólise



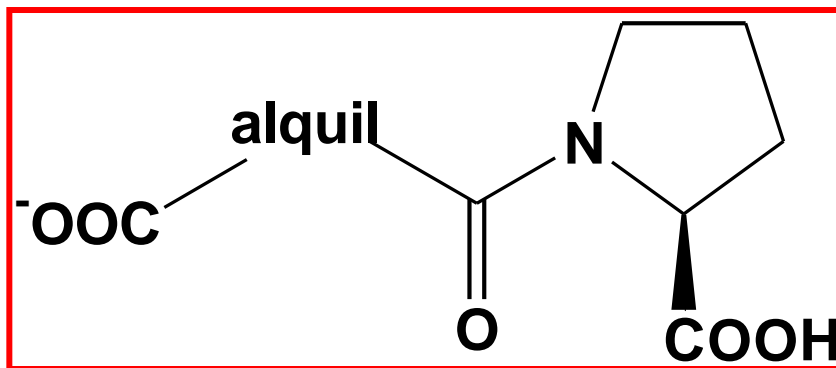
Ácido (*R*)-2-benzilsuccínico
(*inibidor*)

Ácido-(*R*)-2-
benzilsuccínico
(modelo)



Eficiência de uma
prolina no C terminal na
inibição da ECA

Testes de várias carboxialquilprolinas como inibidores da ECA

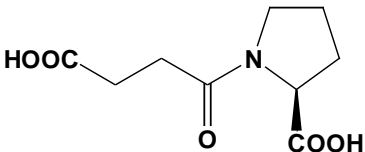
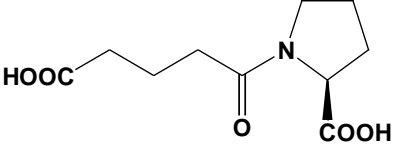
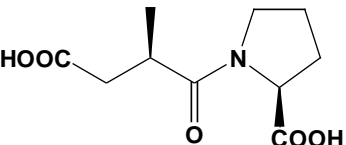
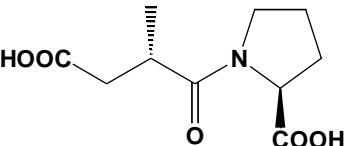
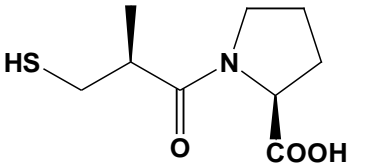


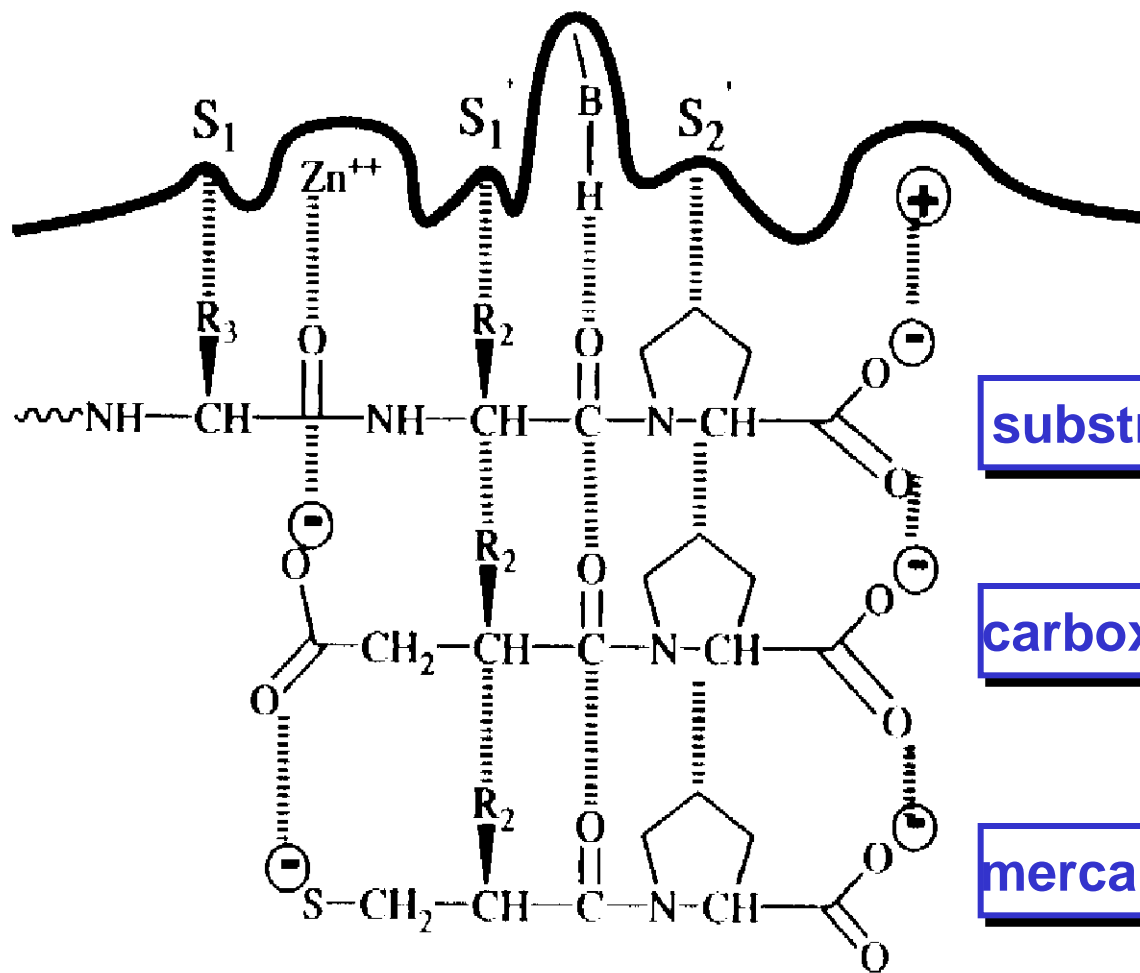
Atividade oral

Atividades *in vitro* de inibidores da ECA

Estratégia:
aumentar a potência
a partir da estrutura
da
(S)-succinilprolina (2)

captopril

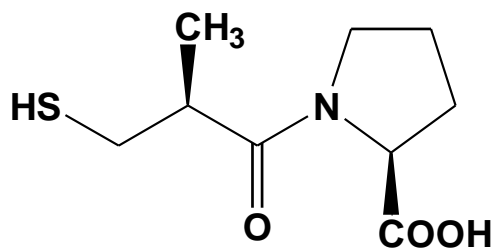
	Estrutura	IC ₅₀ (μM)
1	<Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro (teprotídeo)	0,56
2		630
3		70
4		52
5		1470
6		0,023



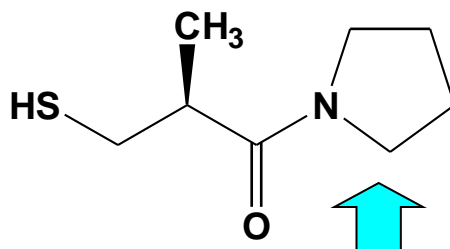
substrato

carboxialquilprolina

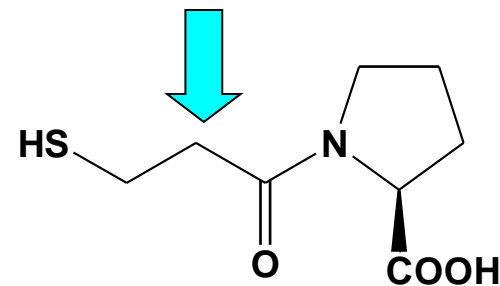
mercaptoalquilprolina



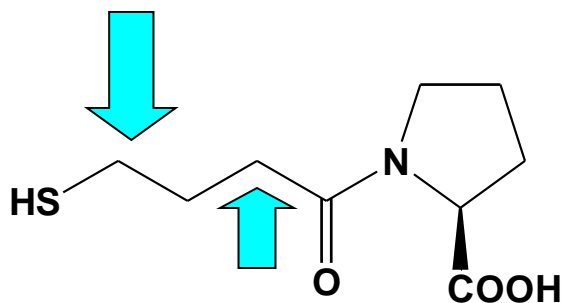
captopril $K_i = 1$



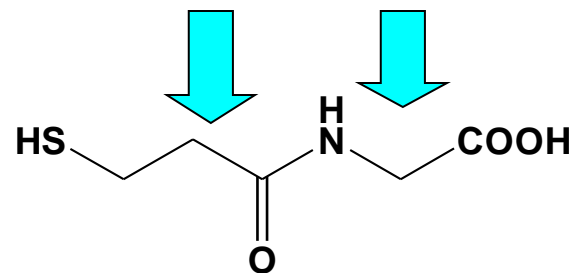
$K_i = 12.500$



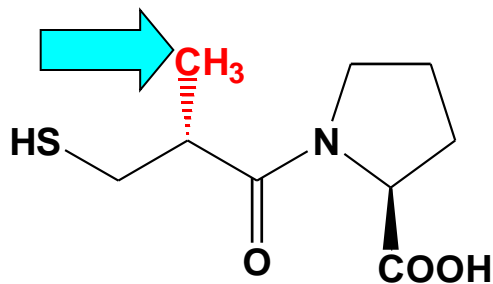
$K_i = 10$



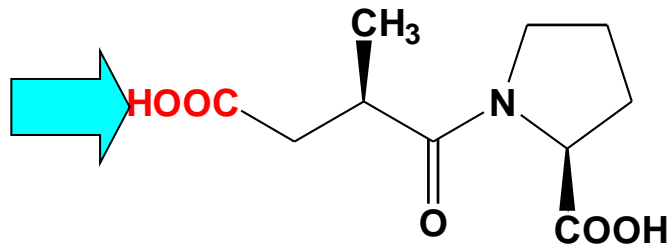
$K_i = 12.000$



$K_i = 120$

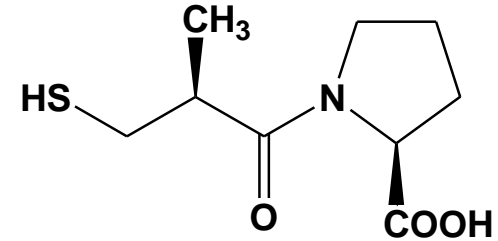


$K_i = 120$



$K_i = 1.100$

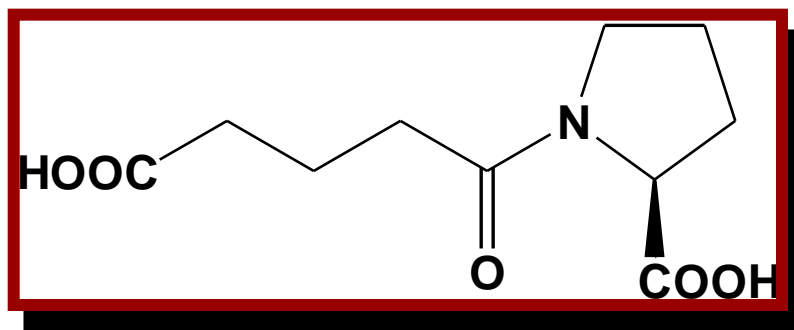
CAPTOPRIL (Squibb)



- **Primeiro inibidor da ECA a ser comercializado**
- **Efeito contra hipertensão e ICC**
- **Normaliza 50% dos casos de hipertensão como monoterapia**
- **Efeitos adversos: erupções cutâneas, diminuição do paladar**
- **Hipertensão é assintomática: difícil adesão ao tratamento devido aos efeitos adversos**

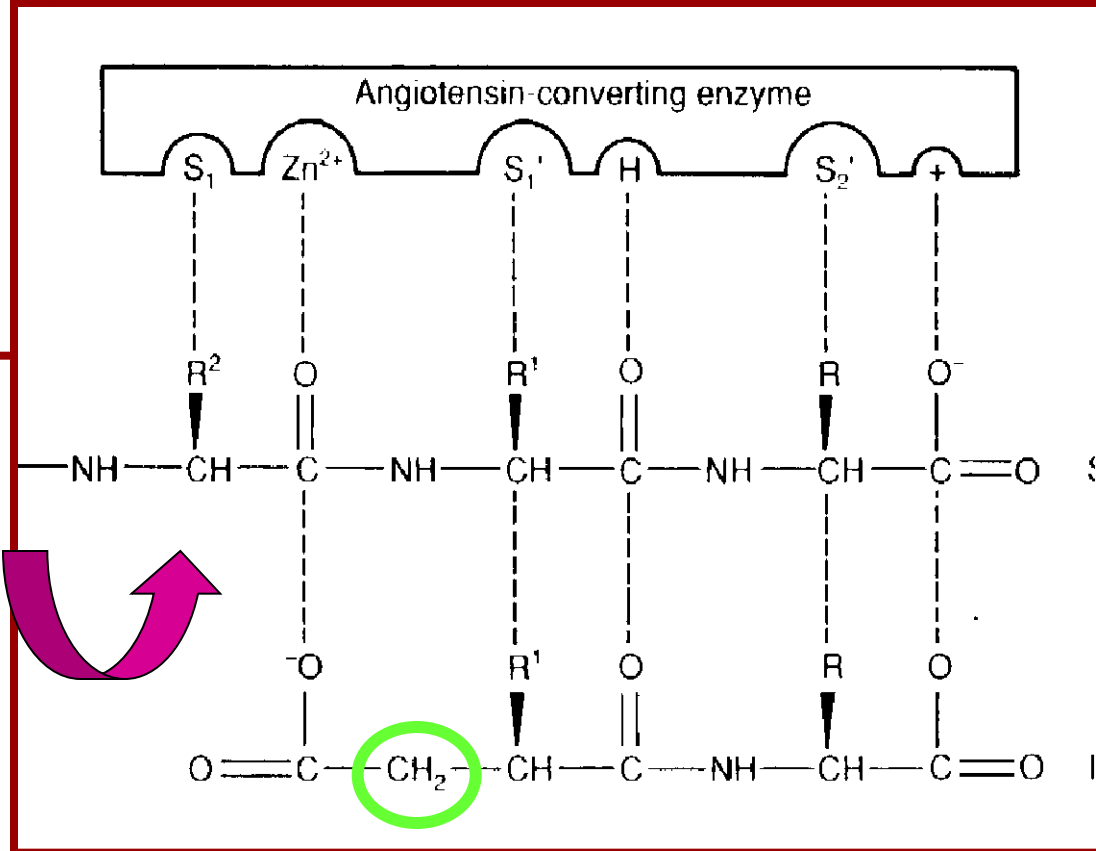
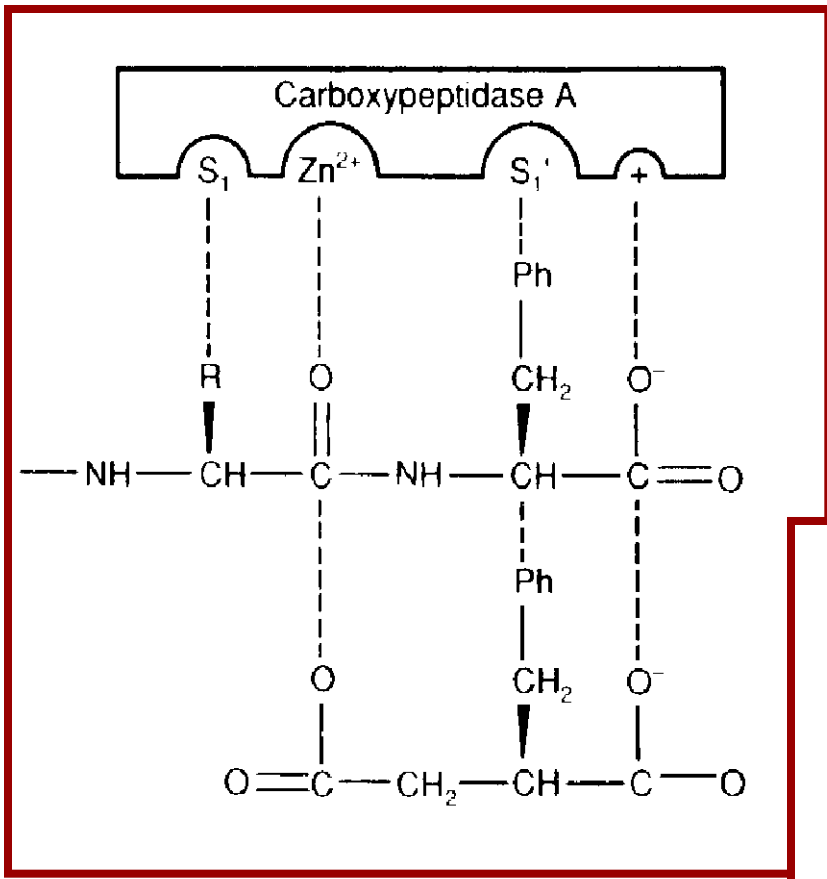
MERCK

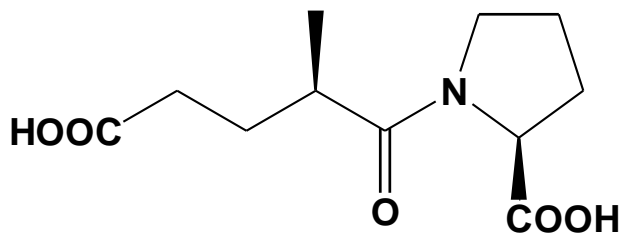
- Efeitos adversos do captopril relacionados ao grupo tiol (-SH)
- Compostos com grupo -SH sofrem oxidação *in vivo* a dissulfetos
- Estratégia: aumentar a potência da glutarilprolina



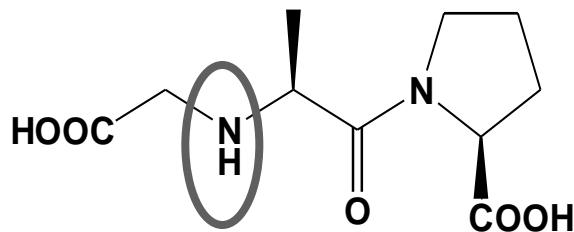
$$IC_{50} = 70 \mu M$$

Elaborar uma molécula com maior número de interações com a ECA

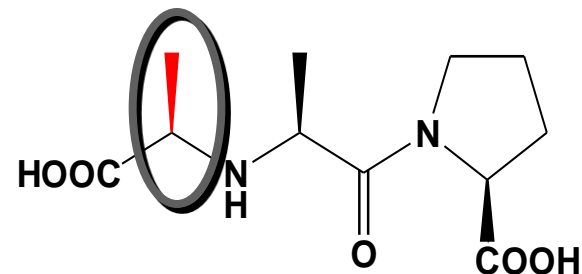




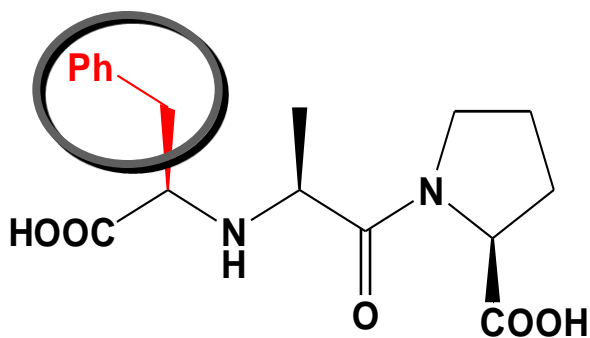
$IC_{50} = 4,9 \mu M$



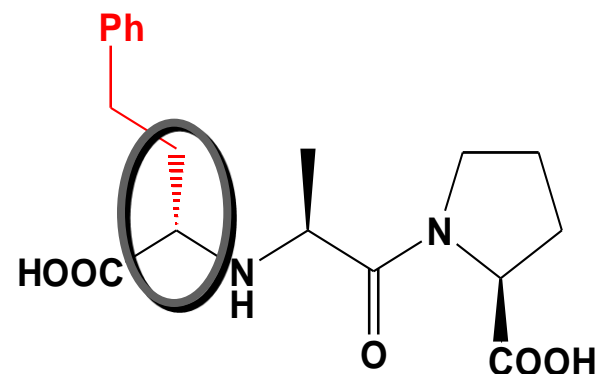
$IC_{50} = 2,4 \mu M$



$IC_{50} = 0,09 \mu M$

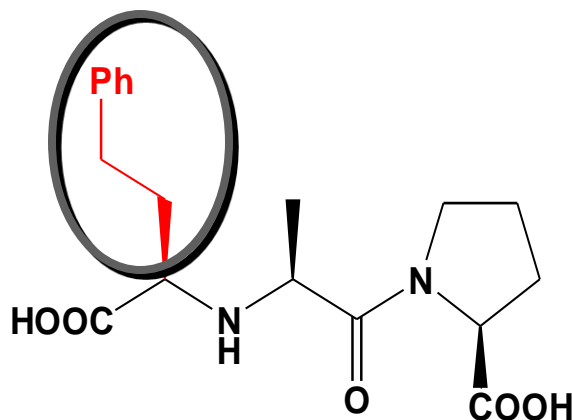


$IC_{50} = 0,04 \mu M$



$IC_{50} = 0,08 \mu M$

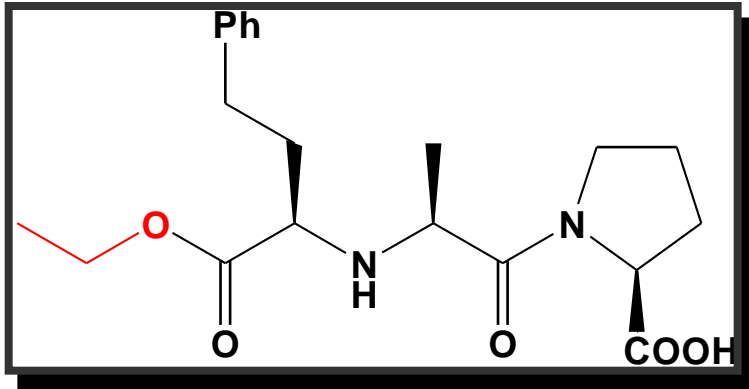
Fracamente absorvido
administração i.v.



$IC_{50} = 0,001 \mu M$



ENALAPRILATO



ENALAPRIL

PRÓ-FÁRMACO

excelente atividade oral

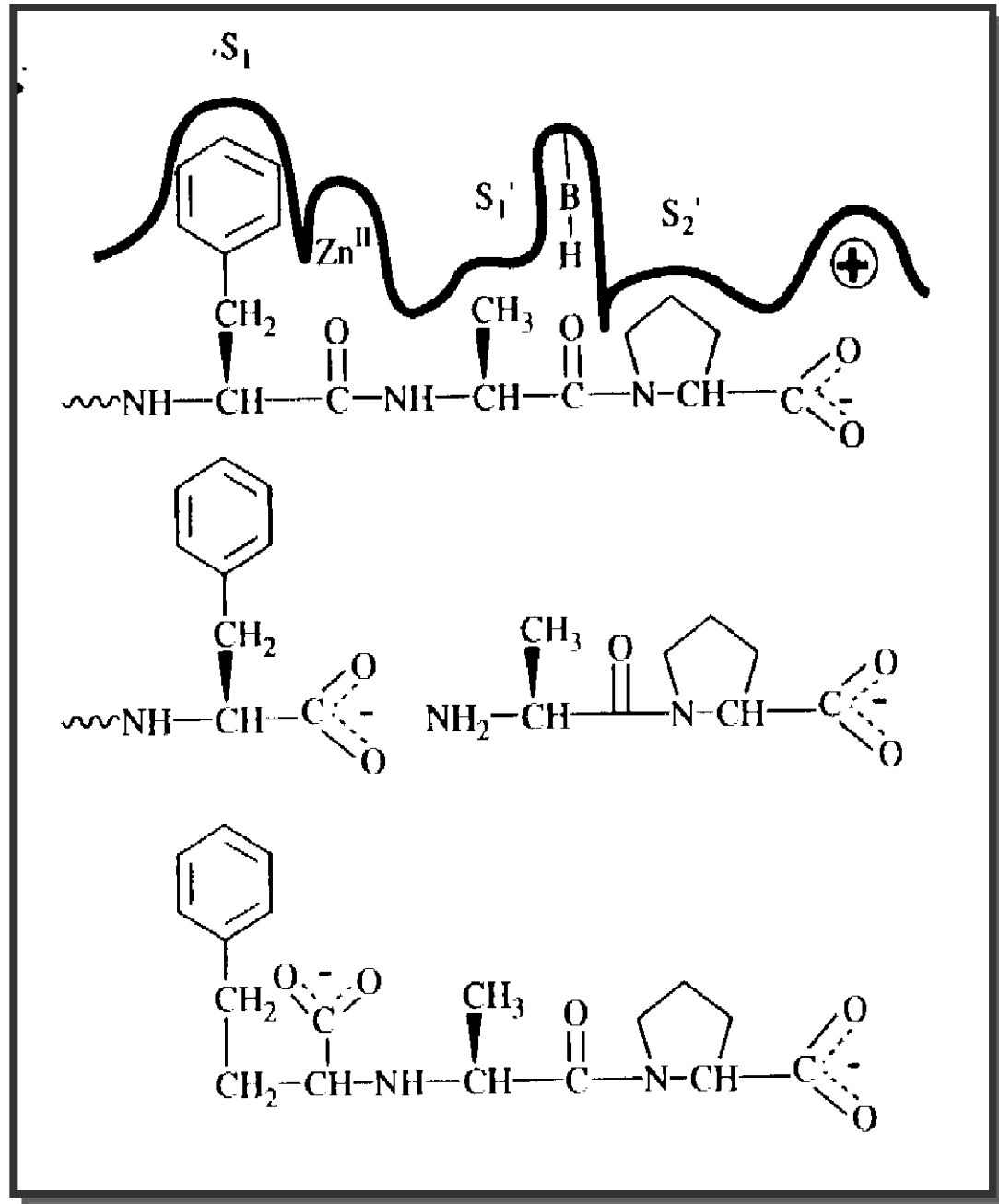
atravessa mais facilmente
as membranas

enalapril

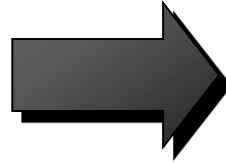
dose única diária (20-40mg)

captopril

doses 25-50 mg (2-3 X / dia)

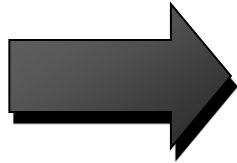


Modelo 2D do sítio ativo



**captopril (Squibb)
e enalapril (Merck)**

Roche

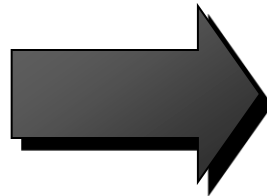


**Possível melhorar a potência dos inibidores da
ECA se a conformação bioativa pudesse ser
usada numa molécula mais rígida**

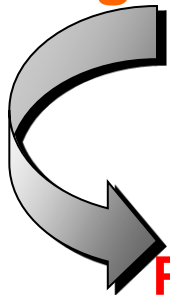
COOH

amida

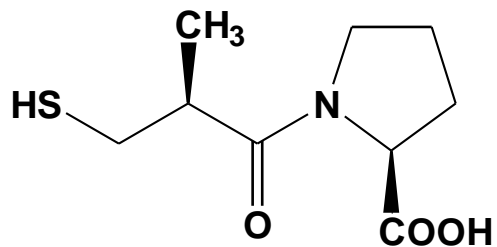
Ligante do Zn⁺⁺



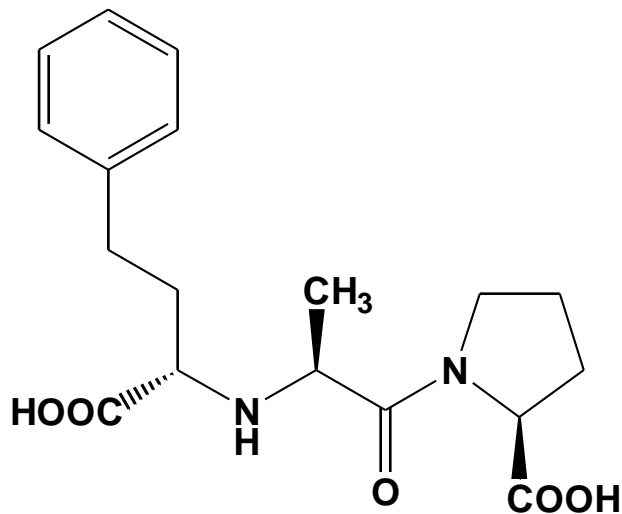
Grupos chave na atividade



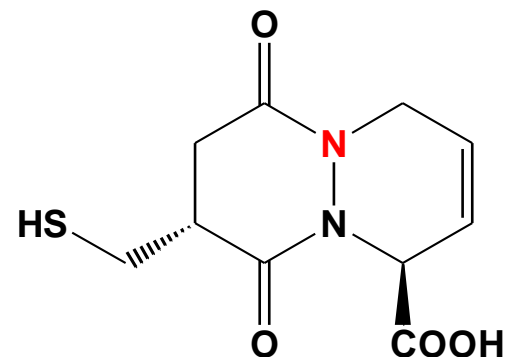
**Planejamento de modelos rígidos, onde a orientação espacial
destes grupos pudesse ser variada sistematicamente**



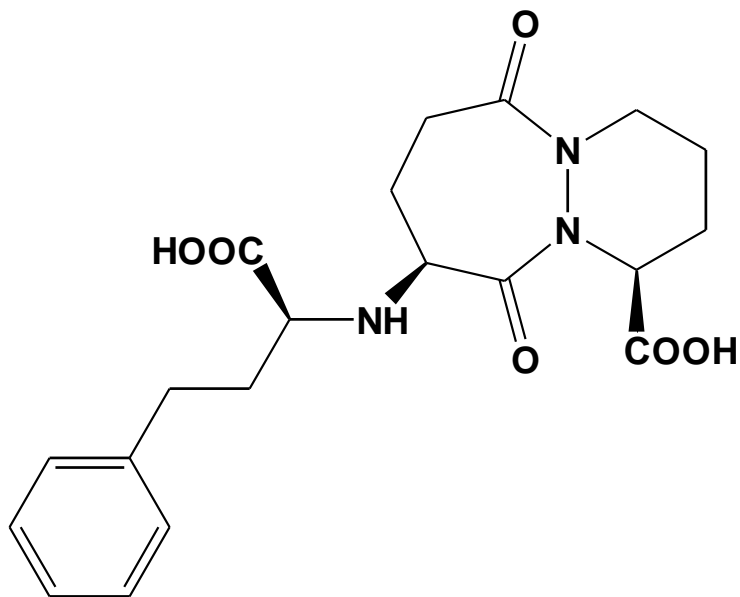
captopril
IC₅₀ = 23,0 nM



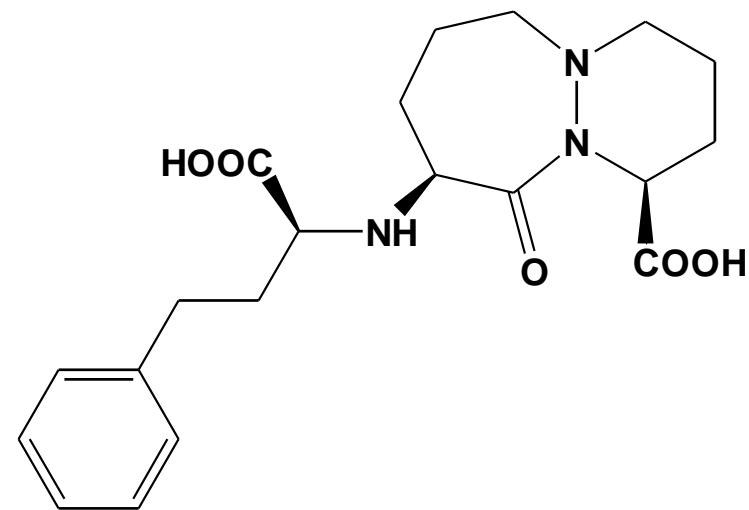
enalaprilato
IC₅₀ = 1,0 nM



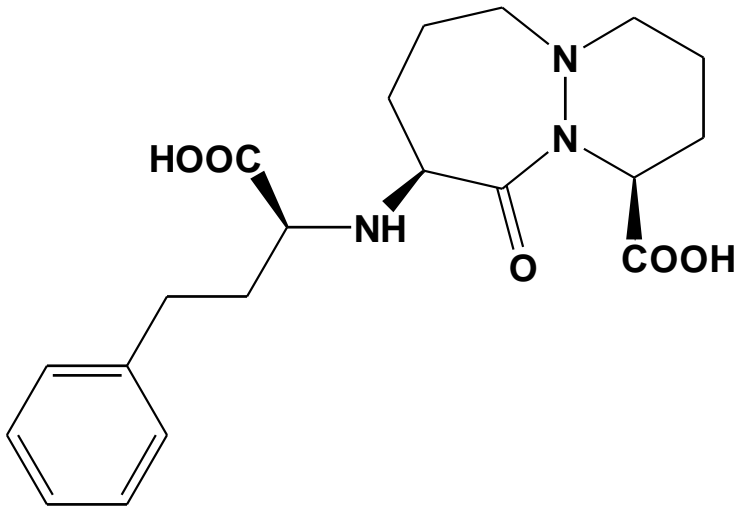
IC₅₀ = 40,0 nM



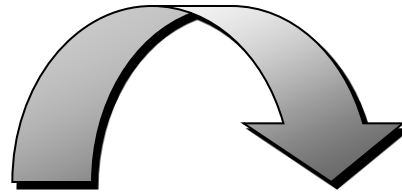
IC₅₀ = 4,0 nM



cilazaprilato
IC₅₀ = 1,6 nM

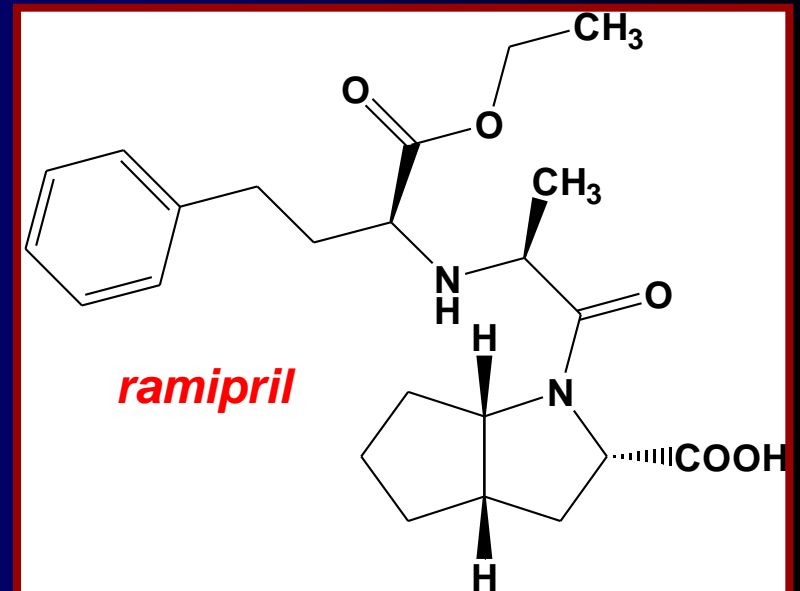
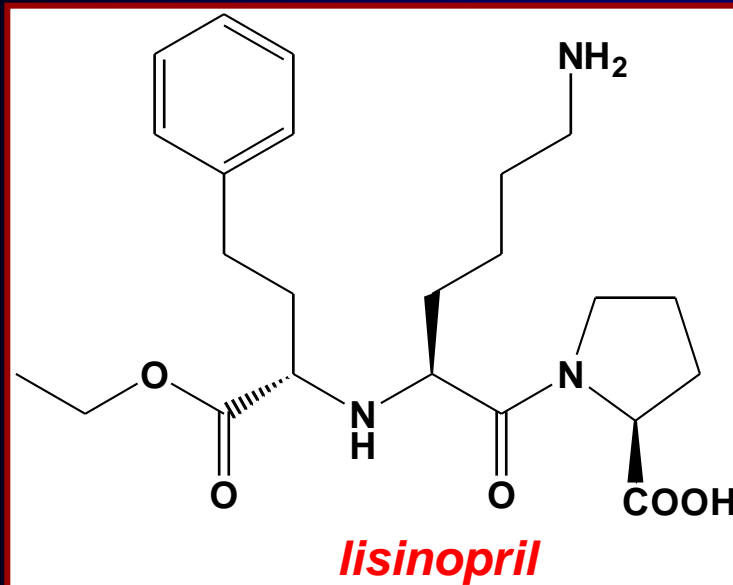
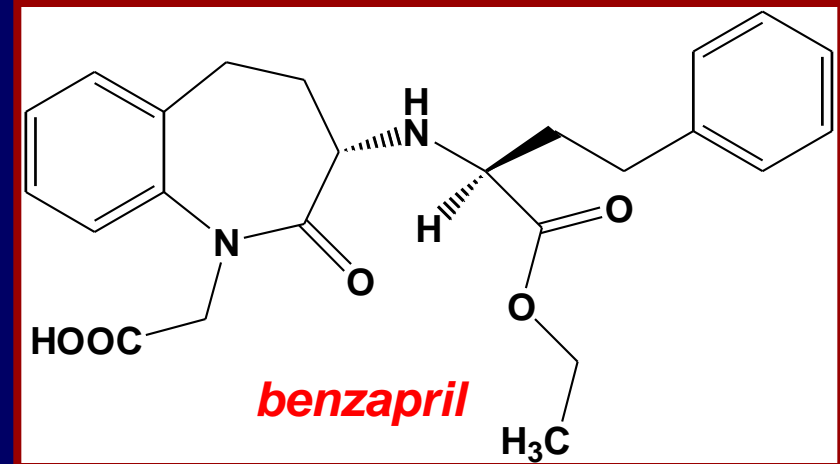
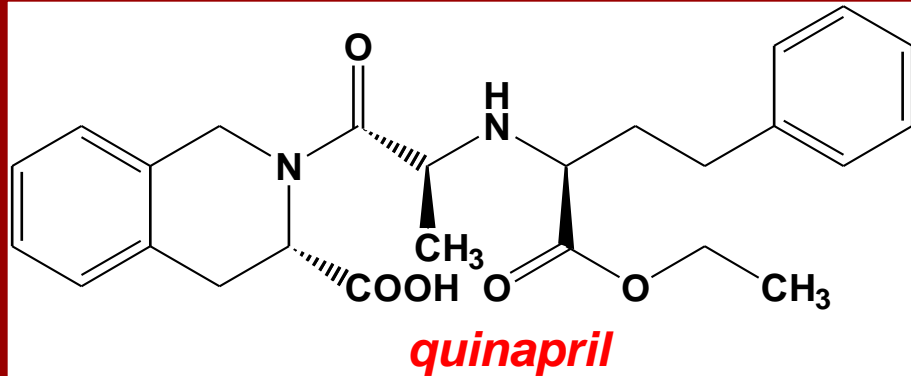


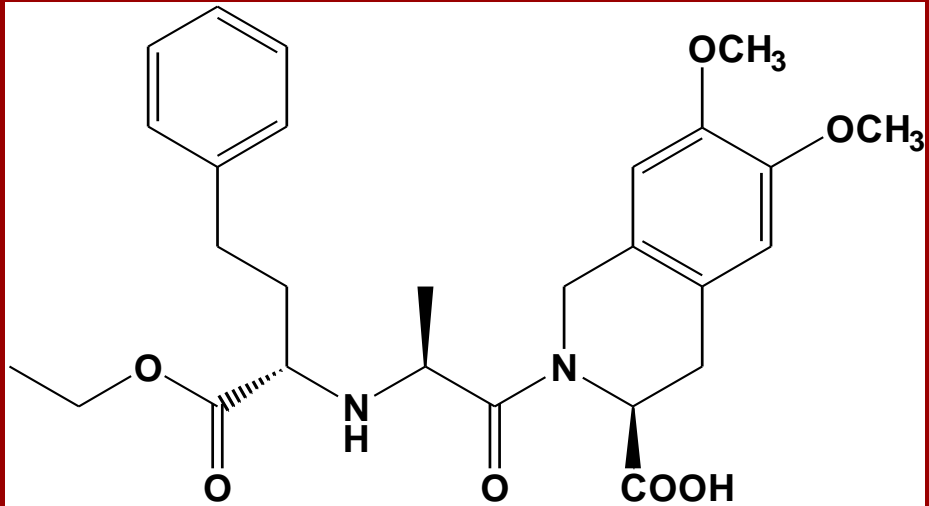
cilazaprilato



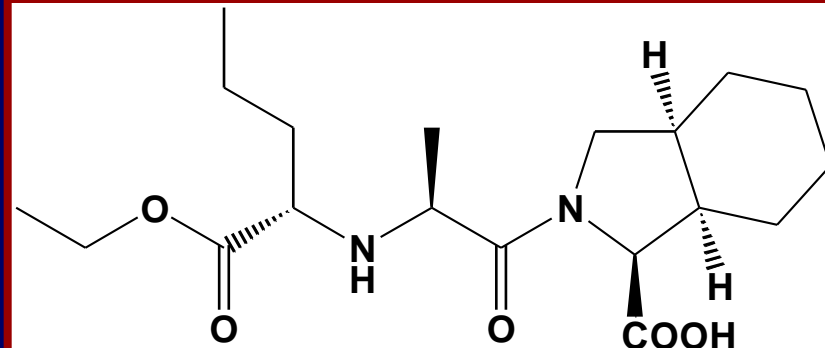
Maior especificidade pela ECA
Menor afinidade por outras neuropeptidases
Diminui incidência de tosse seca

Outros dicarboxilatos inibidores da ECA (S,S,S)

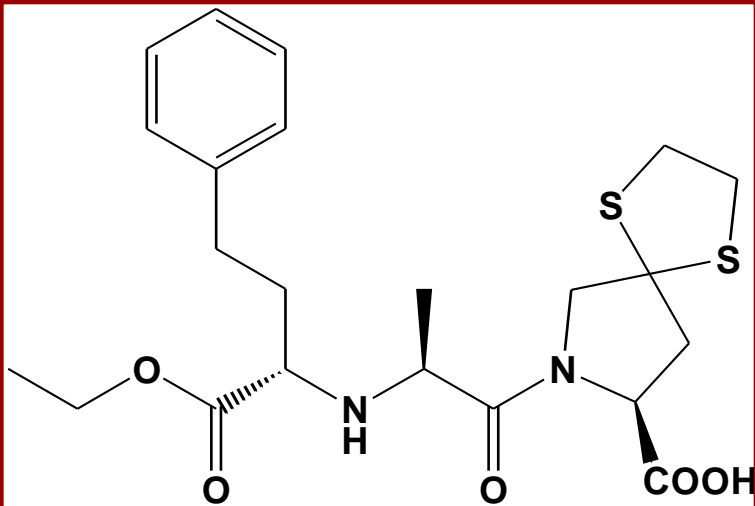




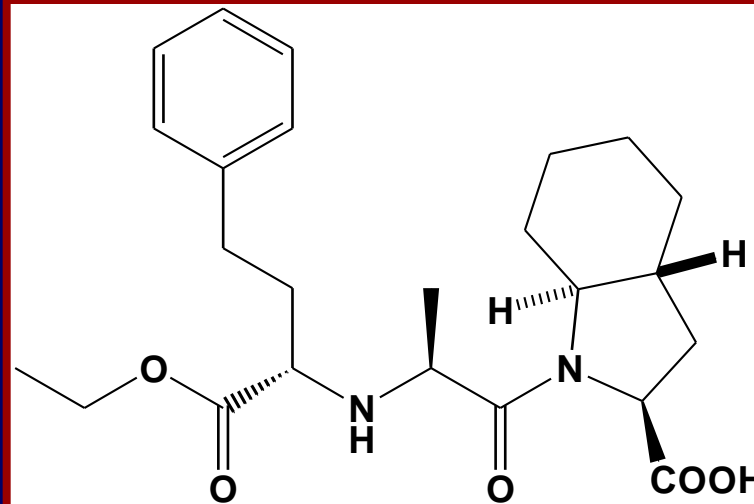
Moexipril



Perindopril



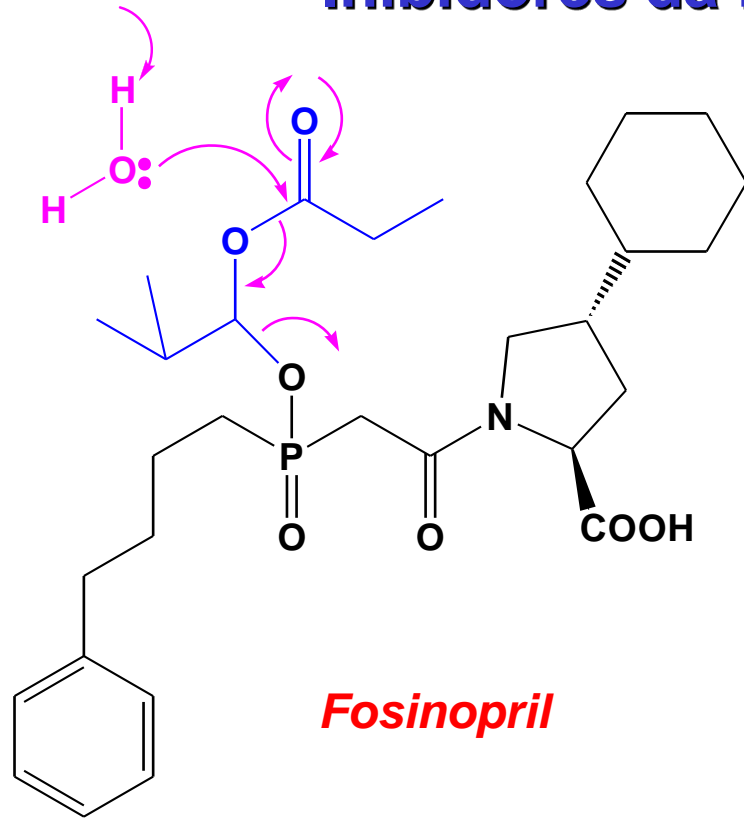
Espirapril



Trandolapril

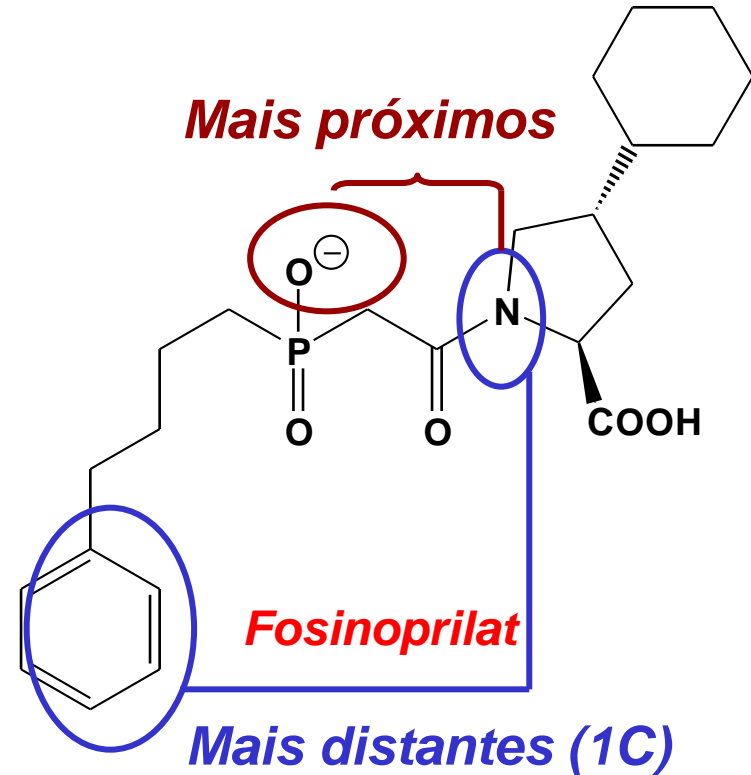
esterase

Inibidores da ECA contendo fosfonato



Fosinopril

esterase



Mais próximos

Fosinoprilat

Mais distantes (1C)

Mais potente que captopril e menos potente que enalaprilato

ECA

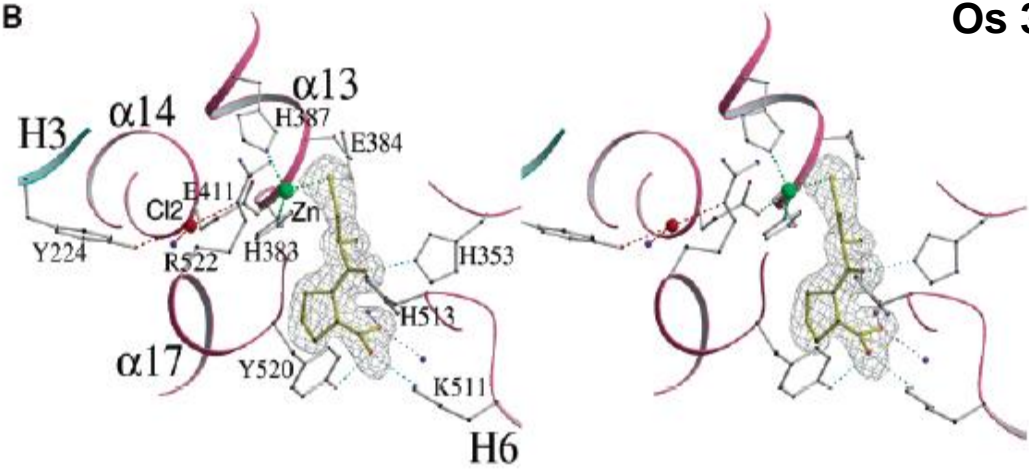
Domínio C – envolvido na regulação da pressão sanguínea – a inibição deste domínio é suficiente para tratamento de certas doenças cardio-renais

Domínio N – controle hematopoiético da diferenciação e proliferação de células tronco

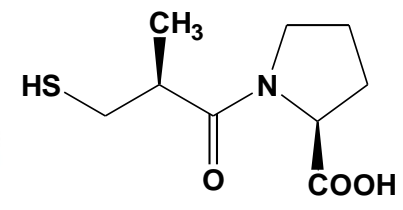
A inibição de apenas um dos domínios inibe a hidrólise da angiotensina

A inibição dos 2 domínios é necessária para abolir a conversão da bradicinina em produtos inativos

B



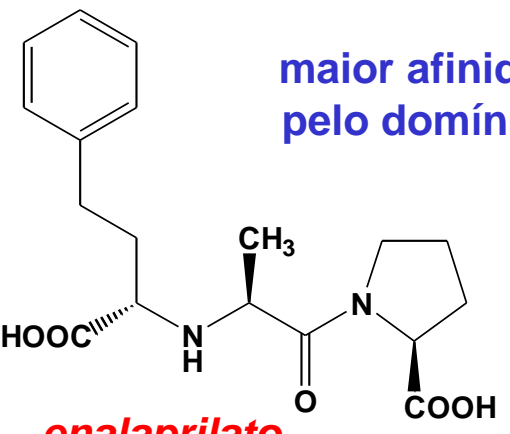
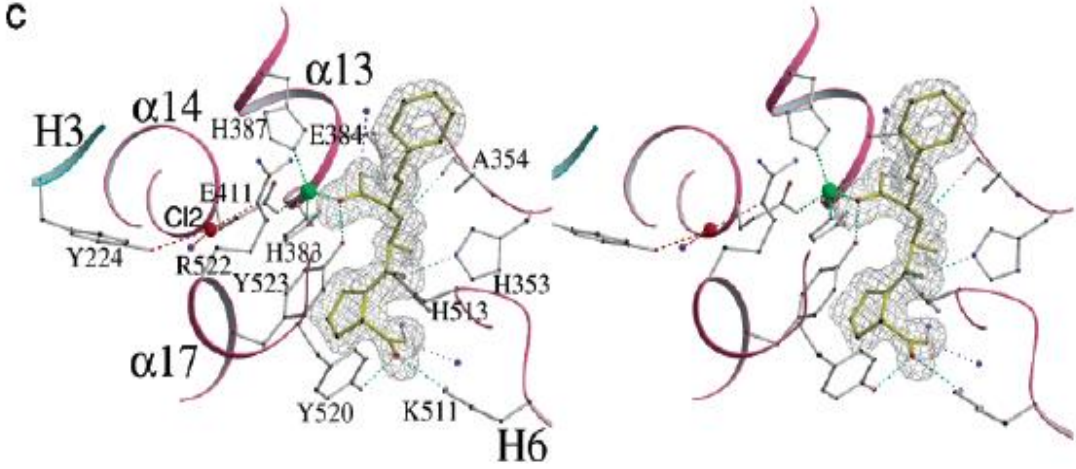
Os 3 são inibidores altamente potentes dos domínios N e C



maior afinidade pelo domínio N

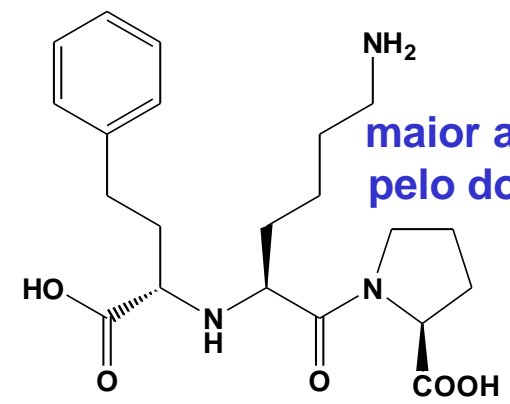
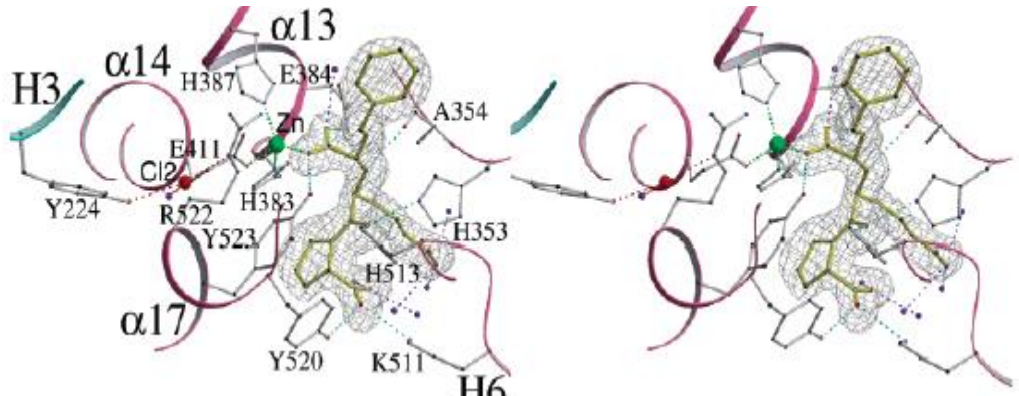
captopril

C



maior afinidade pelo domínio C

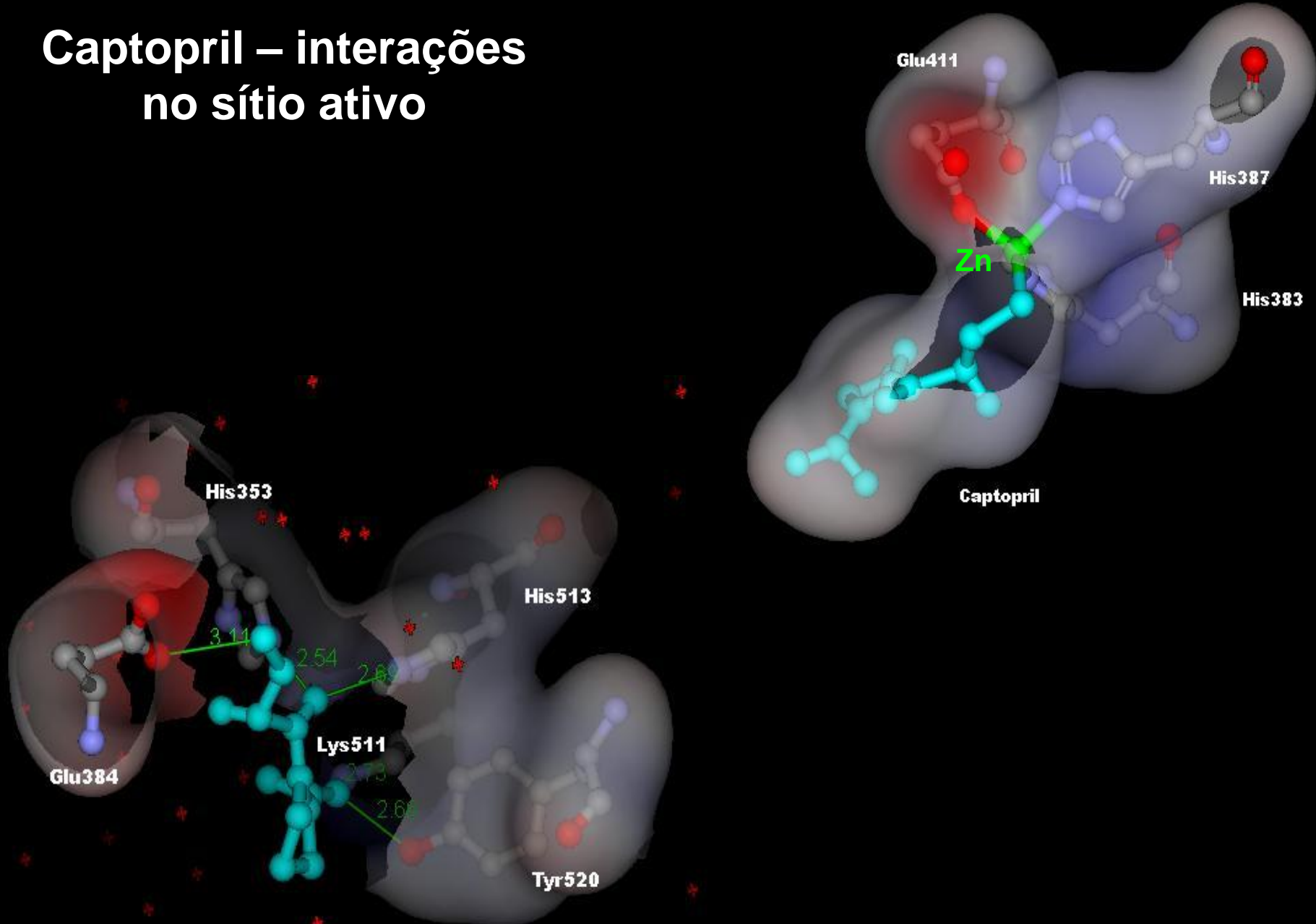
enalaprilato



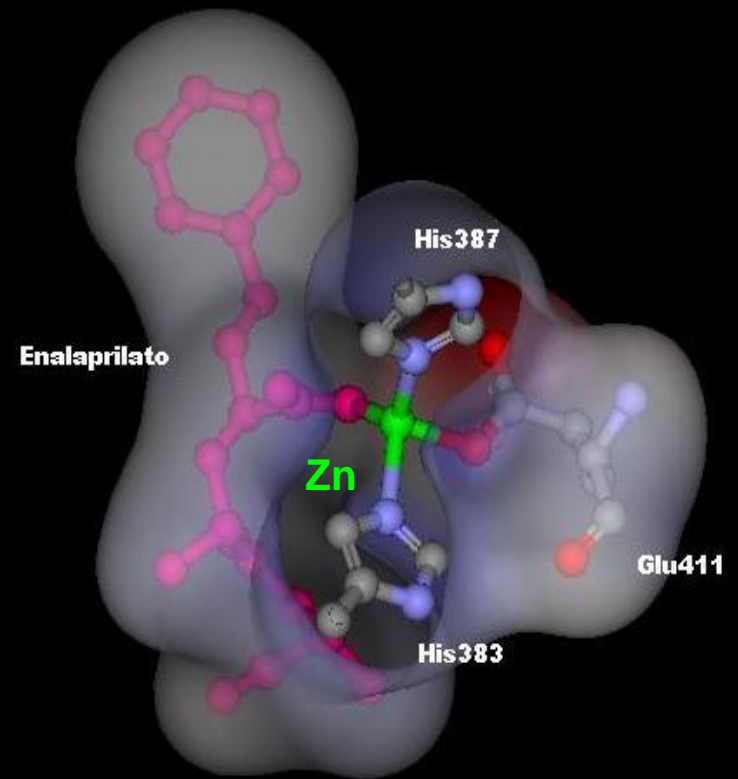
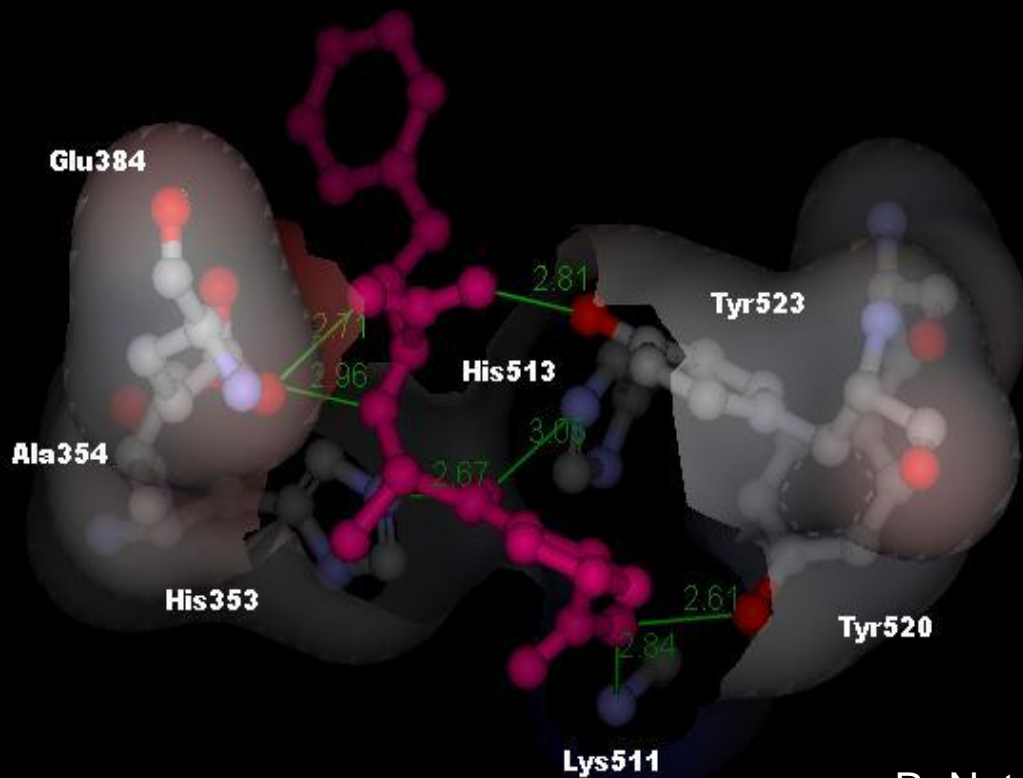
maior afinidade pelo domínio C

lisinopril

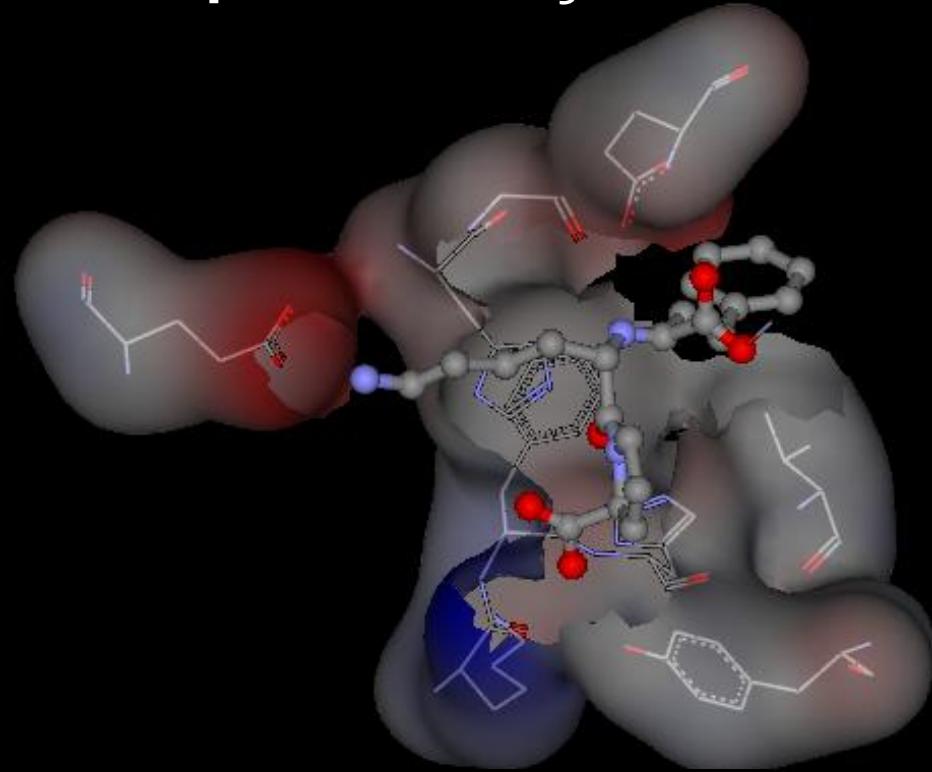
Captopril – interações no sítio ativo



Enalaprilato – interações no sítio ativo

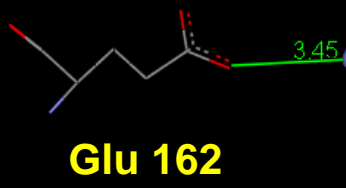
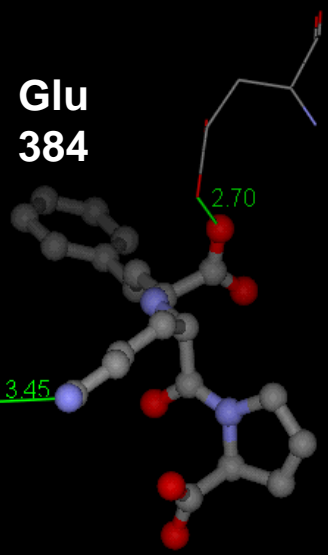


Lisinopril – interações no sítio ativo



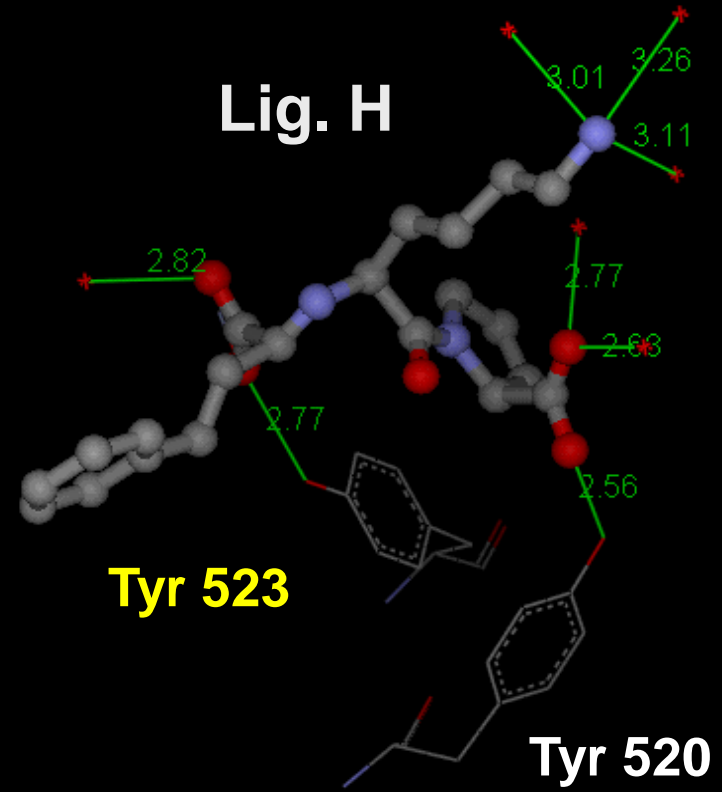
Interações eletrostáticas

Glu 384



Glu 162

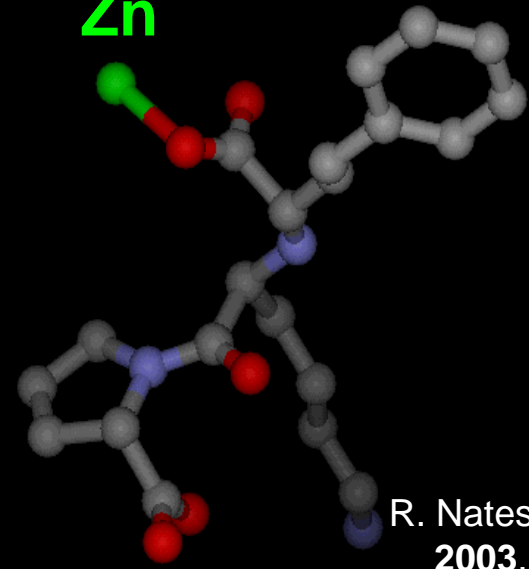
Lig. H



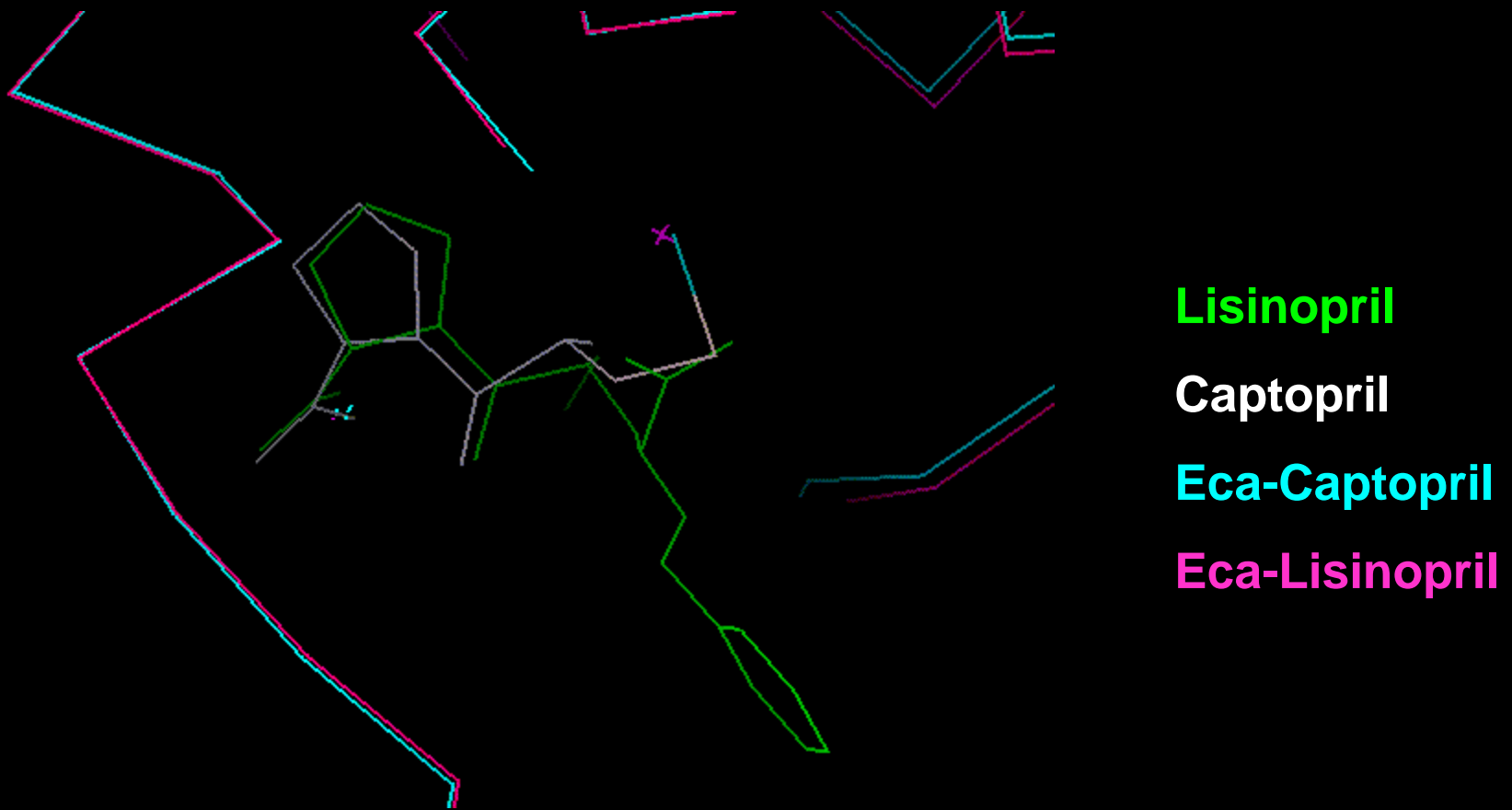
Tyr 523

Tyr 520

Zn



Sobreposição dos complexos enzima-inibidores



Sobreposição dos complexos enzima-inibidores

ECA-Enalaprilato

ECA-Captopril

