

Protease Inhibitors

February 11, 2025

I. Introduction

*importance of proteases—> _____

-used in natural metabolic processes to _____

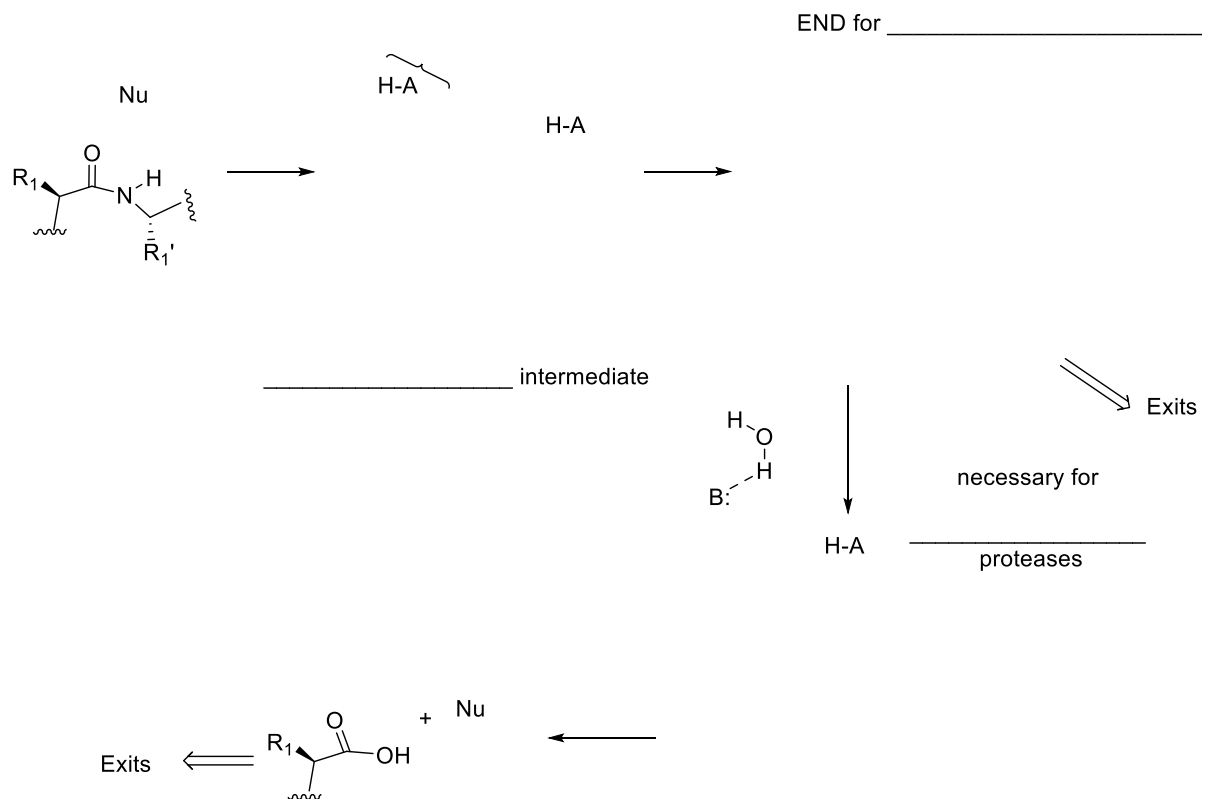
**Example:

-used by viruses to _____ into active enzymes or proteins

*Very important _____

A. Protease enzyme mechanisms

Generic protease/amidase process



KEY Transition State Theory: Enzyme _____ and facilitate catalytic process

-therefore enzyme binds TS with _____

- _____ is close in energy and structure to _____
 (_____ postulate)

* For serine and cysteine protease, Nu= _____ and _____, respectively

* For aspartic and metalloproteases, Nu= _____

* 4 specific mechanisms:

Serine, e.g. _____

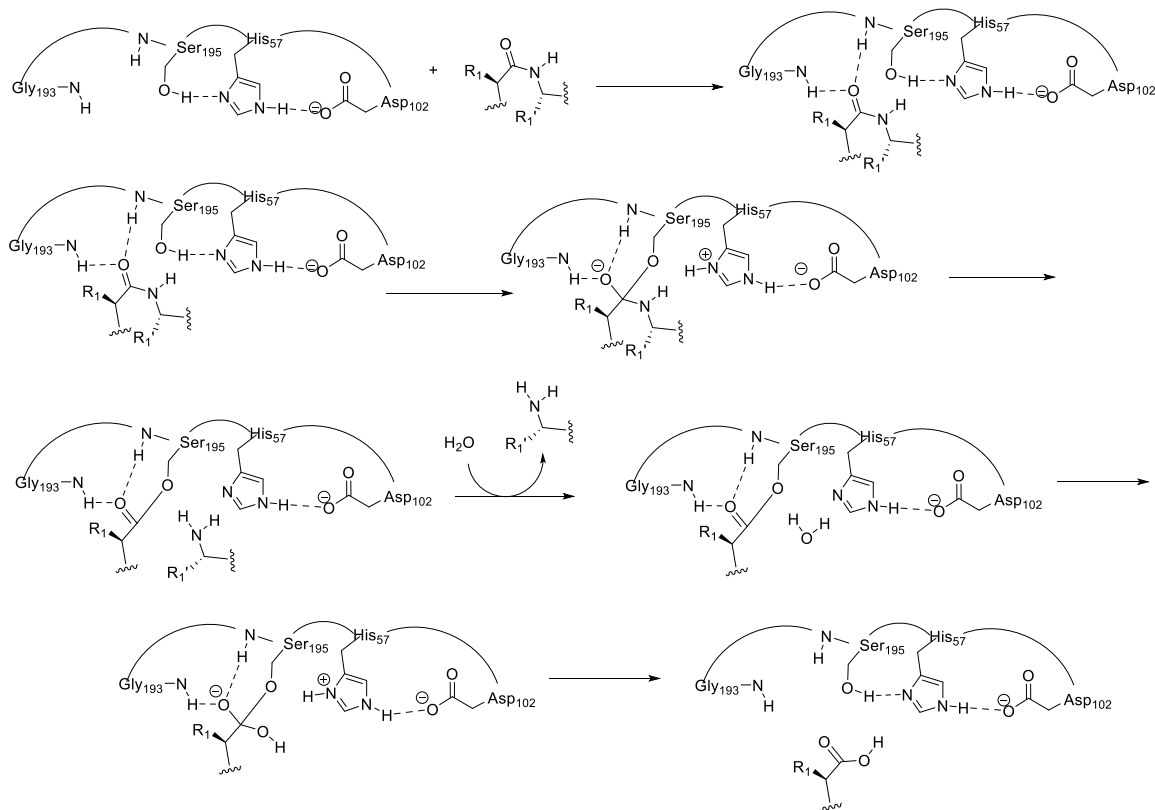
Cysteine, e.g. _____

Aspartic, e.g. _____

metallo-proteases, e.g. _____

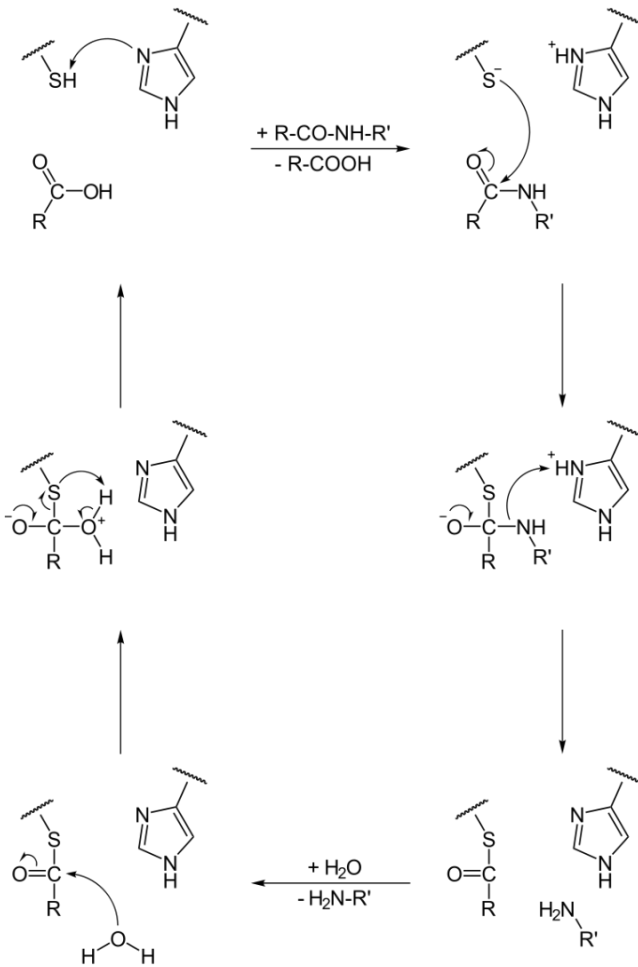
1) _____ protease mechanism:

* archetype: Chymotrypsin



2) protease mechanism:

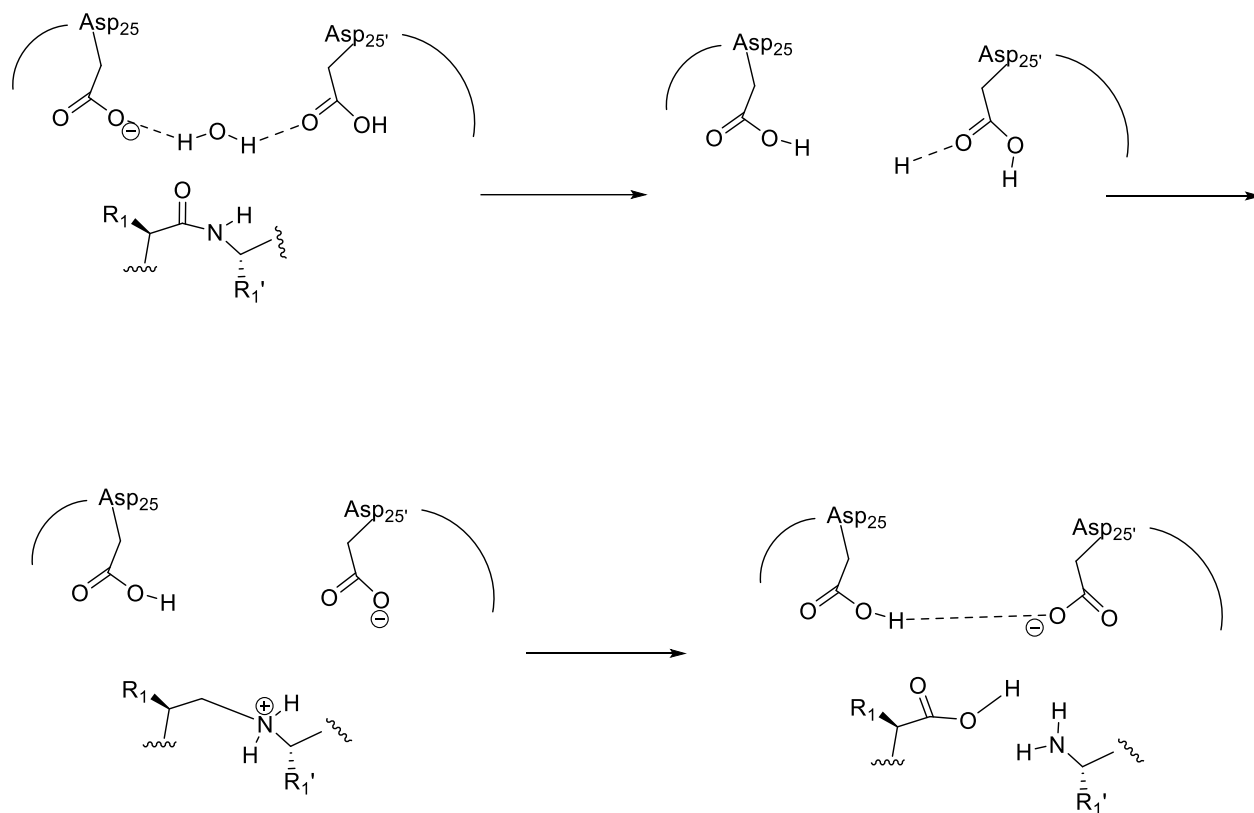
*Thiols/Cys is one of the _____



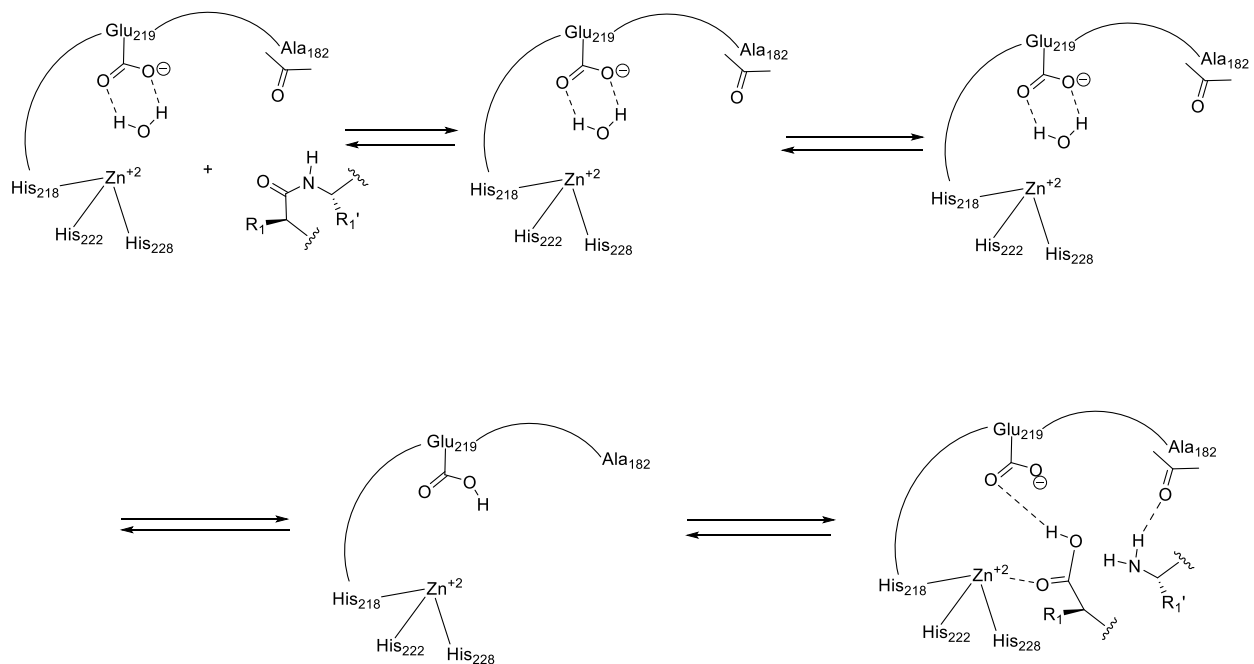
https://en.wikipedia.org/wiki/Cysteine_protease

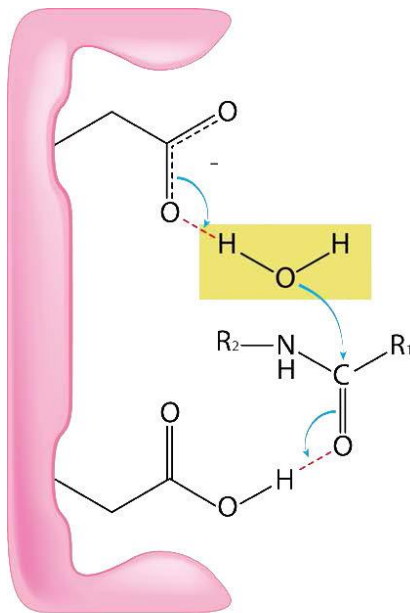
3) Aspartic protease mechanism:

Aspartic Protease Enzyme Mechanism

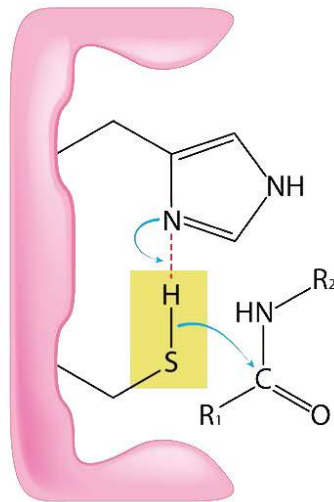


4) Metalloprotease mechanism:

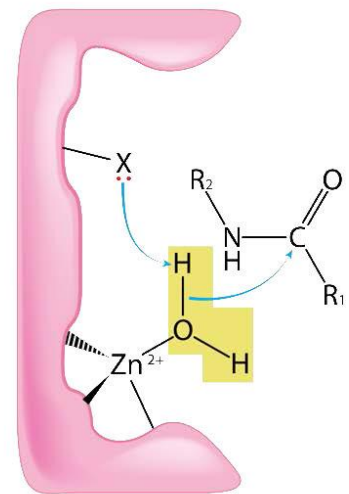




Aspartyl Proteases



Cysteine Proteases



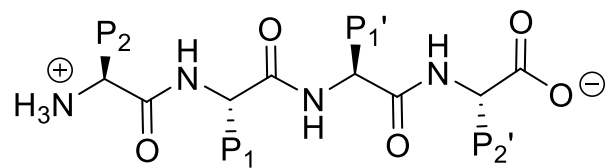
Metalloproteases

from bio.libretexts.org

B. Substrate recognition

*Structure of _____

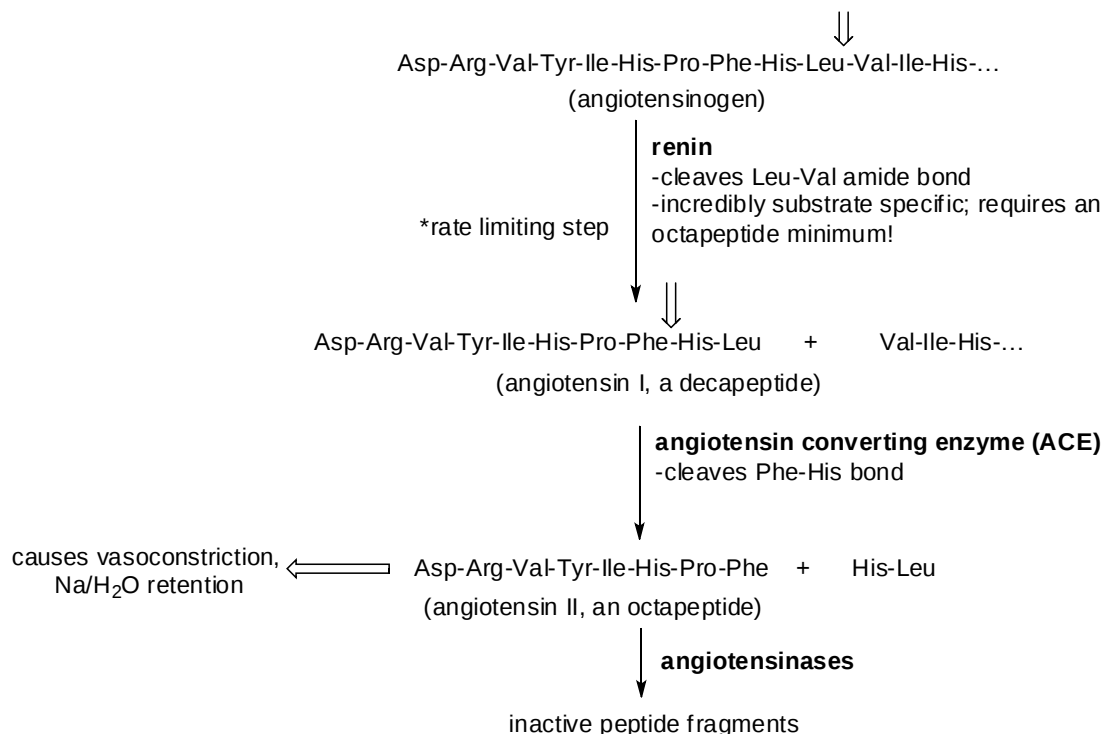
-nomenclature (Schechter-Berger)



III. Two classic drug examples:

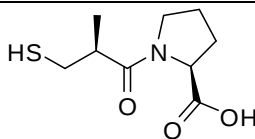
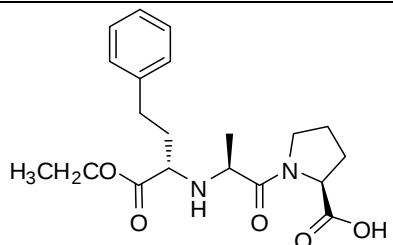
A. Metabolic diseases: _____

*two metabolic enzymes in the renin-angiotensin system targeted: ACE (angiotensin converting enzyme) and renin



Key role in hypertension: Angiotensin II then _____, increases the secretion of ADH and aldosterone, and stimulates the hypothalamus to activate the thirst reflex, each leading to an _____.

ACE= _____ $\rightarrow$ a metalloprotease

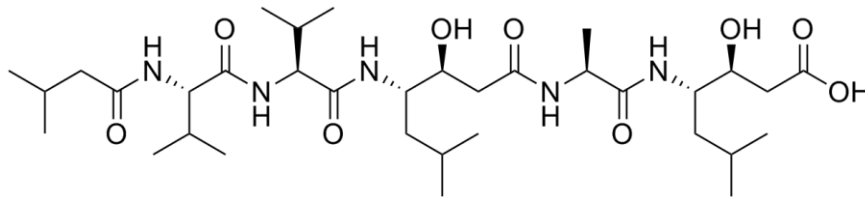
 captopril (Capoten) $\text{IC}_{50} = \text{_____}$ ($\text{K}_i = \text{_____}$) * _____ Brazilian pit viper venom peptide sequence: <u>Glu-Trp-Pro-Arg</u>-Pro-<u>Gln-Ile</u>-Pro-Pro	 ethyl ester called _____, $\text{IC}_{50} = 1.2 \text{ uM}$; but acid has $\text{IC}_{50} = 0.0012$ ($\text{K}_i = 0.0002$) *60-70% absorption in humans (due _____ NOT _____!); *cleaved by _____
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Renin $\rightarrow$ an _____ also a focus of considerable research

B. HIV protease → _____

*an _____ protease

a) inspiration from a natural product:



_____ : isopentatanoyl-Val-Val-Sta-Ala-Sta

-two copies of an unnatural amino acid: _____

(NOT to be confused with _____ !!)

-discovered by Umezawa in bacteria cultures (Actinomyces)

-found by Umezawa to be an excellent (_____ !) inhibitor of _____, a stomach aspartic protease

original report: Umezawa H, Aoyagi T, Morishima H, Matsuzaki M, Hamada M (1970). "Pepstatin, a new pepsin inhibitor produced by Actinomycetes". *J. Antibiot.* **23** (5): 259–62. PMID 4912600;

related article: Marciniszyn J, Hartsuck JA, Tang J (1976). "Mode of inhibition of acid proteases by pepstatin". *J. Biol. Chem.* **251** (22): 7088–94.

-pepstatin also found to inhibit a large array of _____ !

b) subsequent development by other pharmaceutical companies:

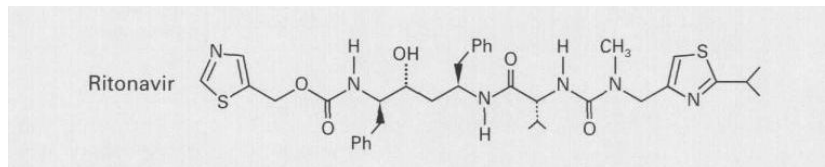
* _____ **inhibitors** (really intermediate inhibitors!)

=mimic the structure of the _____
since that is the structure that enzymes have evolved to bind most tightly

**many subsequent drug development efforts sought to _____

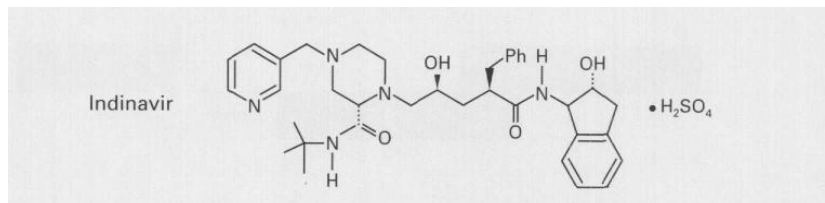
(limit _____), increase _____ (add _____) and
_____ molecular weight.

*note common core of "statine-like" transition state/intermediate mimic



- _____ (Norvir), Abbott, 1996 (7th anti-retroviral drug approved)

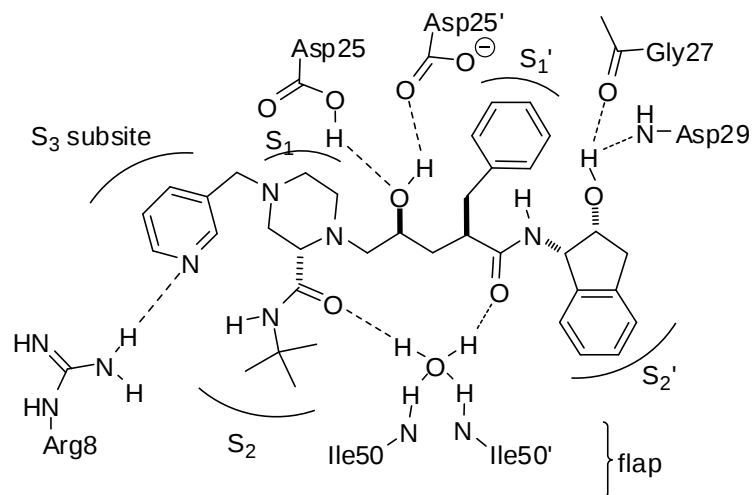
-note _____ in hydroxyethylamine



- _____ (Crixivan), Merck, 1996

-note _____ in hydroxyethylamine piece

-PDB crystal structure: 2R5P

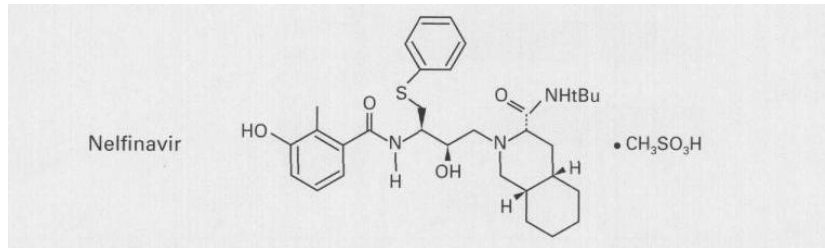


Crixivan's Interactions in HIV-2 proteases active site

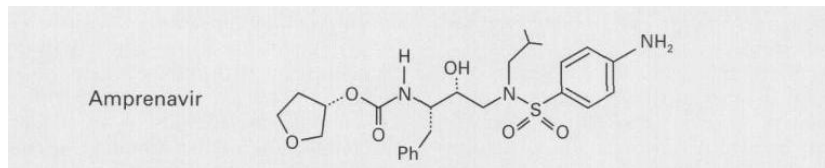
Z. Chen et al. (Merck) *JBC* **1994**, 269, 26344-26348.

For more biochemical details see: <http://www.pnas.org/content/91/9/4096.abstract>

"L-735,524: an orally bioavailable human immunodeficiency virus type 1 protease inhibitor" J P Vacca, B D Dorsey, et al. *PNAS* **1994**, vol. 91, 4096-4100



-nelfinavir (Viracept), Agouron, 1997



-aprenavir (Agenerase), Vertex/Glaxo, 1999

IV. Targeting coronaviruses

3CL-like protease (3-chymotrypsin-like protease)

*a _____ protease

-thiols are the _____