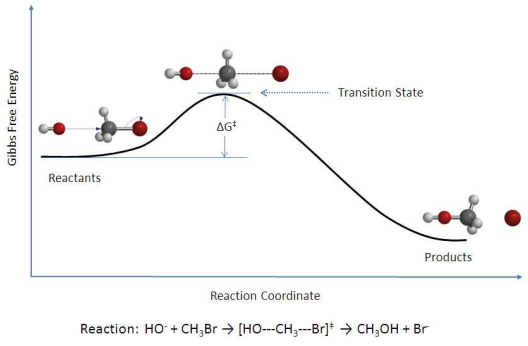
Rational Drug Design lecture 8

Łukasz Berlicki

Theory of transition state

- **Transition state** - the state with the highest potential energy on the reaction path.
- **Activation energy -** energy difference between substrates and transition state. It affects the reaction speed (k) - the Arrenius equation.

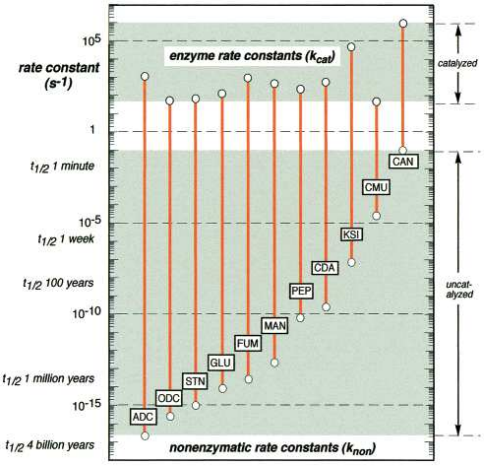


$$k = Ae^{-E_a/RT}$$

Enzymes

- The increase in reaction rate (k_{cat} / k_{non}) obtained by enzymes is in the range of:

10^7 - 10^{19}.



ADC = arginine decarboxylase;
ODC = orotidine 5ɡ-phosphate decarboxylase;
STN = staphylococcal nuclease;
GLU = sweet potato *alpha*-amylase;
FUM = fumarase;
MAN = mandelate racemase;
PEP = carboxypeptidase B;
CDA = E. coli cytidine deaminase;
KSI = ketosteroid isomerase;
CMU = chorismate mutase;
CAN =carbonic anhydrase.

Enzymatic catalysis

- **Linus Pauling** suggested that the efficiency of enzymes results from the transition state.

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Molecular Architecture and Biological Reactions

LINUS PAULING
Chairman, Division of Chemistry and Chemical Engineering,
California Institute of Technology, Pasadena, Calif.

Answers to many basic problems of biology—nature of growth, mechanism of duplication of viruses and genes, action of enzymes, mechanism of physiological activity of drugs, hormones, and vitamins, structure and action of nerve and brain tissue—may lie in knowledge of molecular structure and intermolecular reactions

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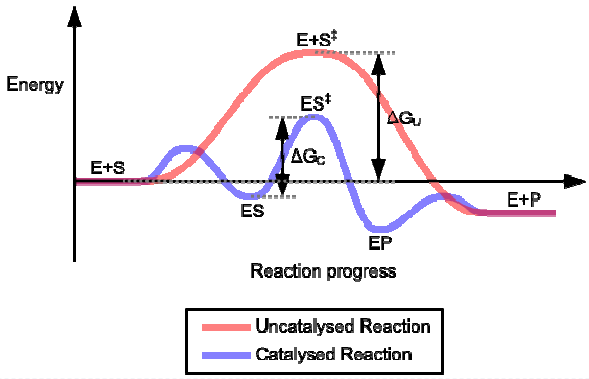
rom the standpoint of molecular
cture and the quantum mechanical
y of chemical reaction, the only rea-
ble picture of the catalytic activity of
mes is that which involves an active
n of the surface of the enzyme which
ely complementary in structure not
e substrate molecule itself, in its
al configuration, but rather to the
rate molecule in a strained configura-
corresponding to the "activated
ex" for the reaction catalyzed by
nzyme: the substrate molecule is
cted to the enzyme, and caused by
orces of attraction to assume the
ed state which favors the chemical
on—that is, the activation energy of
action is decreased by the enzyme to
an extent as to cause the reaction to

Enzymatic catalysis

- The reduction of activation energy results from its efficient binding by the enzyme.
- Substrates and products are associated with significantly lower energy.



Transition state analogues

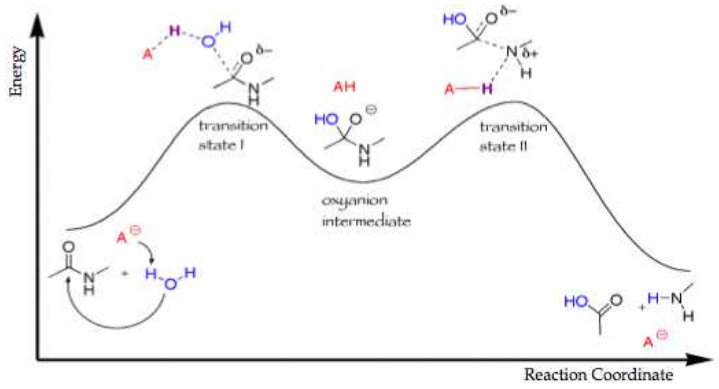
- **Transition state analogues** – compounds exhibiting structural and electronic features similar to the transion state.
- transition state analogues are associated with much higher energy compared to substrates / products and their analogues.

Enzymes bind
"transition state",
NO substrates or products

Transition state analogues
are good inhibitors.

Transition state vs intermediate

- The mechanism of the amide bond hydrolysis reaction usually involves two transition states.

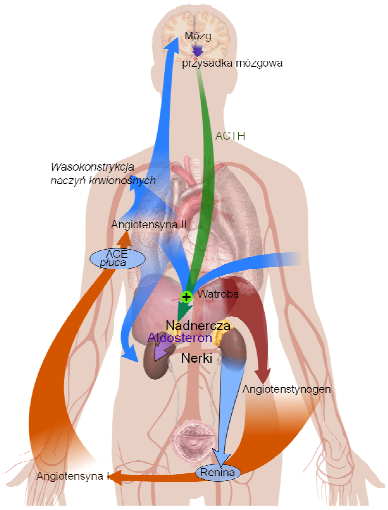


Transition state analogues

- **Advantages:**
  - high binding energy - high inhibitory activity;
  - high specificity;
  - simple design with a known transition state structure.
- **Disadvantages:**
  - the enzymatic reaction mechanism is not always known;
  - it is often very difficult to determine the structure of the transition state;
  - Transition state analogs do not always have the characteristics necessary for the drug (pharmacokinetics, farmocodynamics, etc.).

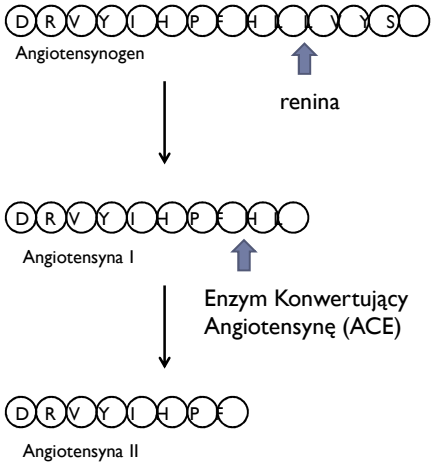
Regulation of blood pressure

- Blood pressure is precisely regulated by the interaction of many organs and the action of several hormones.
- **Angiotensin II** increases blood pressure and causes aldosterone to be released.
- **Bradykinin** reduces blood pressure.



The renin-angiotensin-aldosterone system

- Renin is secreted in the blood vessels in the kidneys and hydrolyses angiotensinogen.
- Angiotensin I is converted to angiotensin II by ACE.
- Angiotensin II is a hormone with a strong effect on increasing blood pressure.
- Renin and ACE are molecular targets for hypertension drugs.



Bothrops jararaca

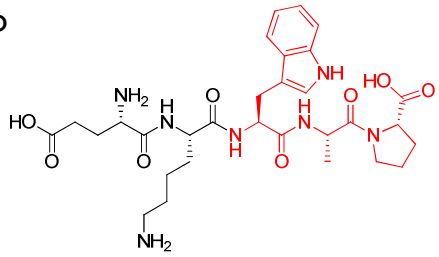
- Film

Bothrops jararaca

- *Jararaca* is one of the most poisonous snakes in the world.
- He lives in South America.
- A significant portion of the treated bites in South America is associated with this snake.
- Its venom causes a very rapid drop in blood pressure.

Peptides from jararaca venom

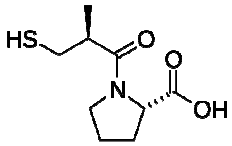
- In jararaca venom, there is a mixture of peptides that inhibit the angiotensin converting enzyme (ACE) activity.
- The active motif of these peptides is the Trp-Ala-Pro sequence.



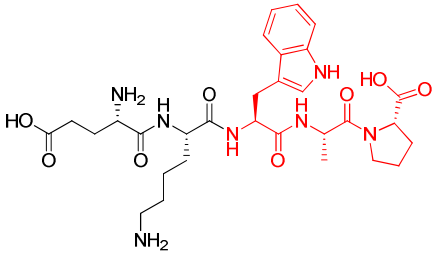
BPP5

Inhibitors of ACE

- Peptides are not good candidates for drugs because they can not be administered orally.
- The first ACE inhibitor approved as a drug was captopril.

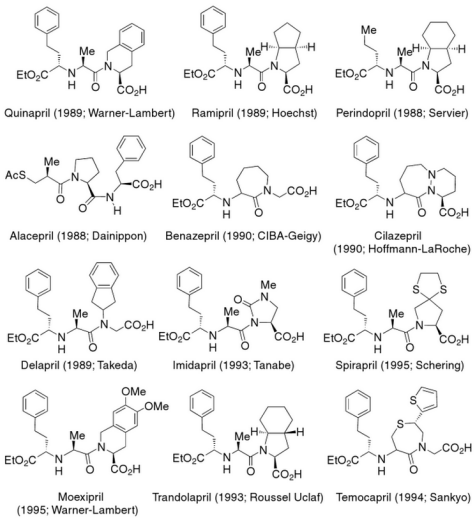


captopril



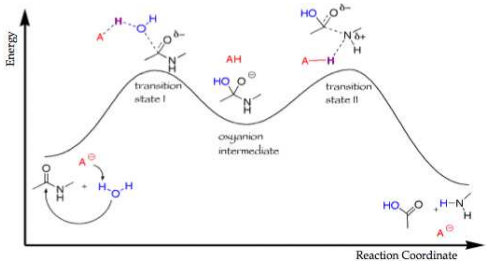
Inhibitors of ACE

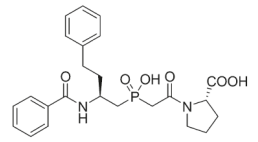
- A large number of ACE inhibitors have been developed, of which over a dozen were subject to clinical trials.
- Most have two characteristic structural elements: proline analog and homophenylalanine.



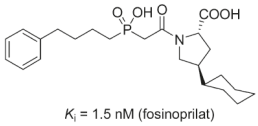
Inhibitors of ACE

- Fosinoprilat is an analog of the intermediate product of the enzymatic reaction.
- Fosinopril is a prodrug. The phosphino function blocking group is hydrolyzed *in vivo*.

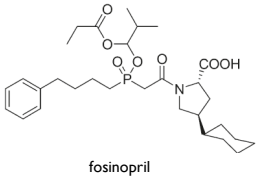




IC_{50} = 12 nM



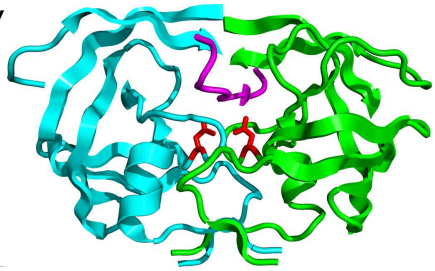
K_i = 1.5 nM (fosinoprilat)



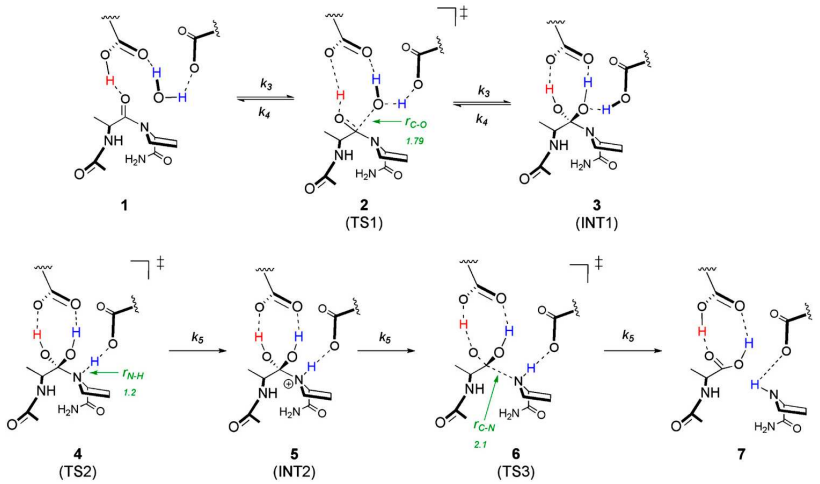
fosinopril

HIV protease

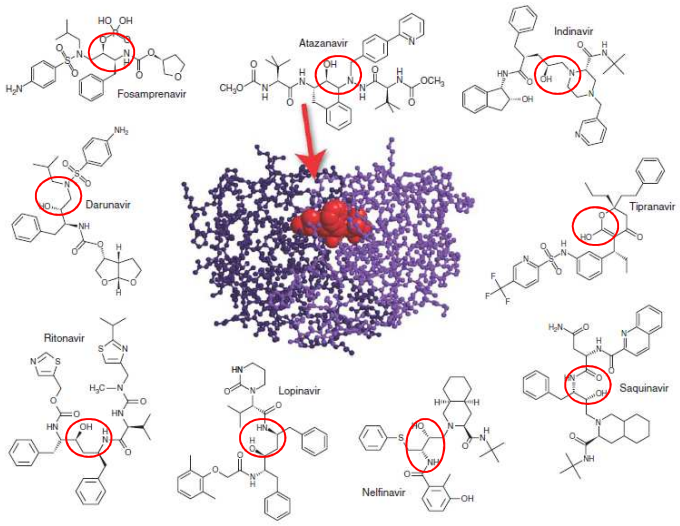
- Aspartyl protease
- It cuts the polypeptide chain to obtain the proteins necessary to create a mature virus.
- Dimer, two chains of 99 amino acid residues
- C2 symmetry
- Two catalytic triads Asp25-Thr26-Gly27
- It cuts through most effectively
  peptide bonding
  Phe-Pro or Tyr-Pro



HIV protease

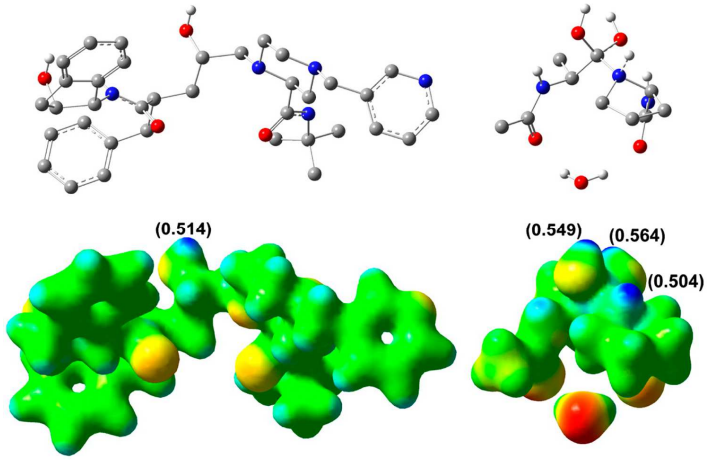


HIV protease inhibitors



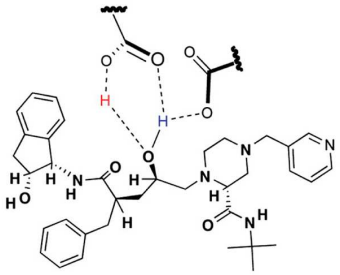
HIV protease inhibitors

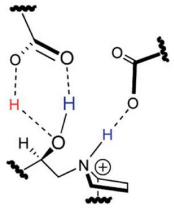
- Conformation and electrostatic potential of HIV protease inhibitor and TS3 transition state.



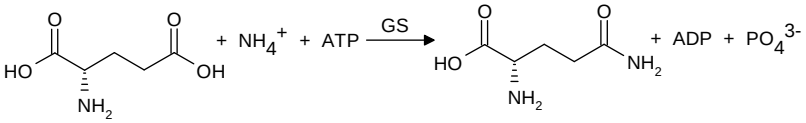
HIV protease inhibitors

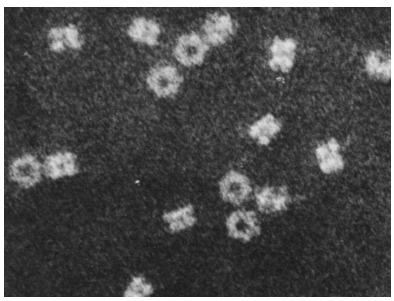
- A thorough analysis of the reaction pathway showed that the mode of binding HIV inhibitors is different than the transition state.





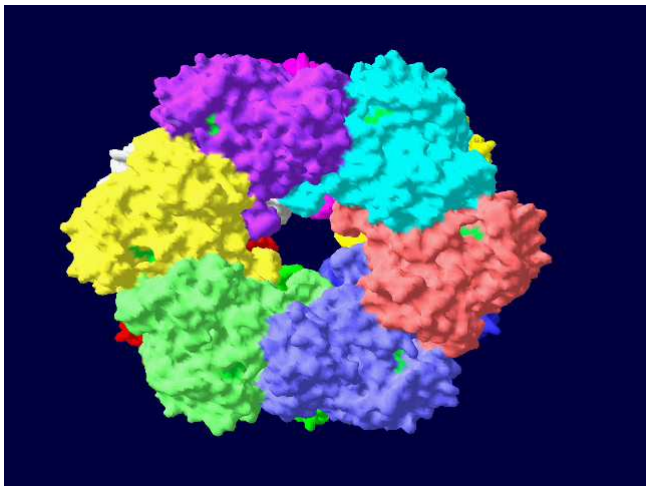
Glutamine synthetase



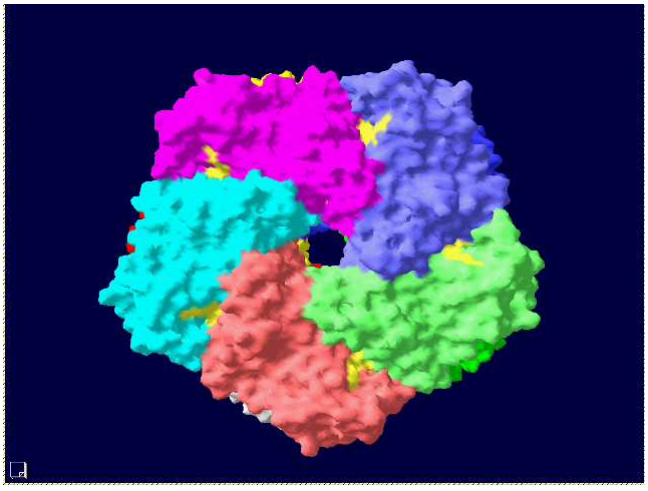


Glutamine synthetase (GS, EC 6.3.1.2) is a key enzyme of nitrogen metabolism.

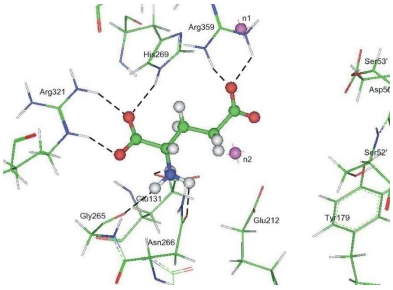
The structure of the enzyme

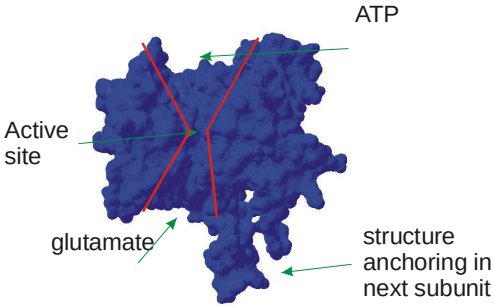


The structure of the eucariotic enzyme



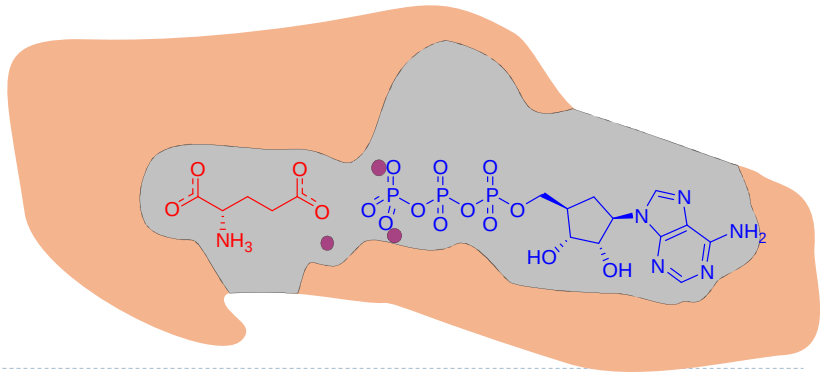
Active site





Reaction mechanism

- The mechanism of reaction of glutamate synthetase is multistep.



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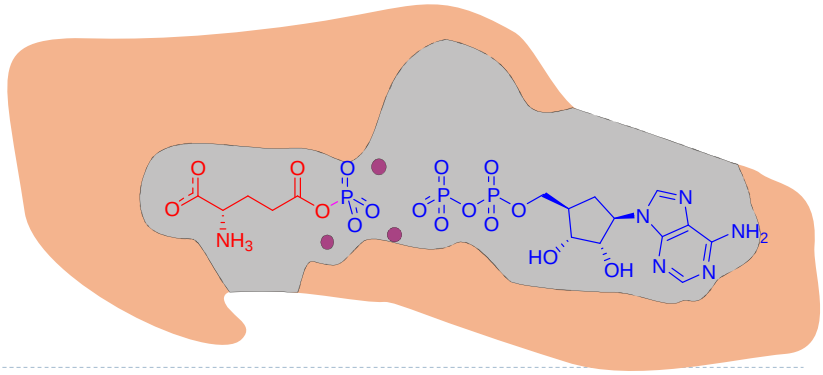

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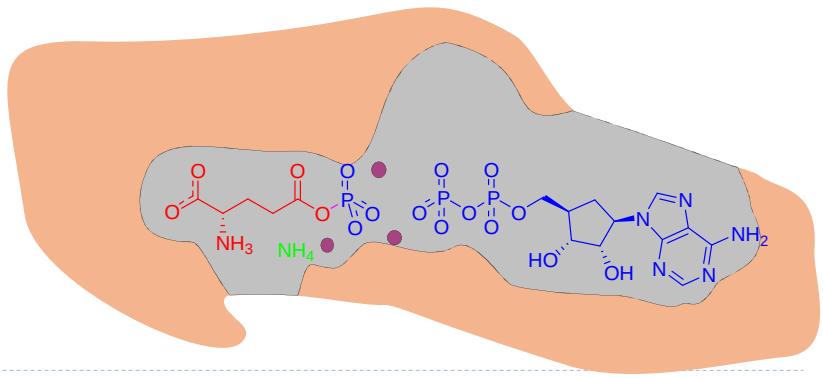

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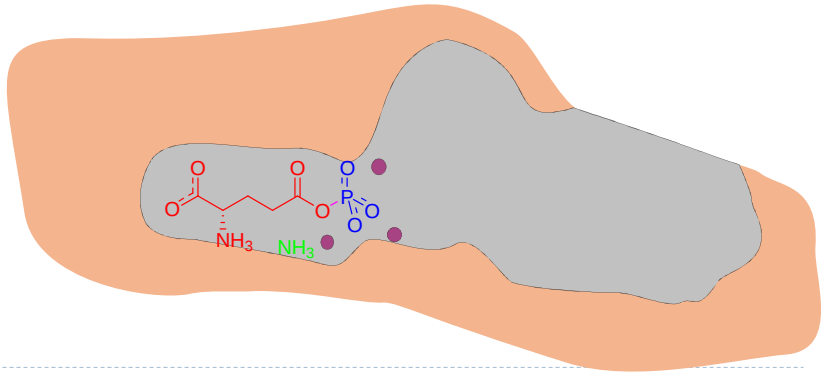

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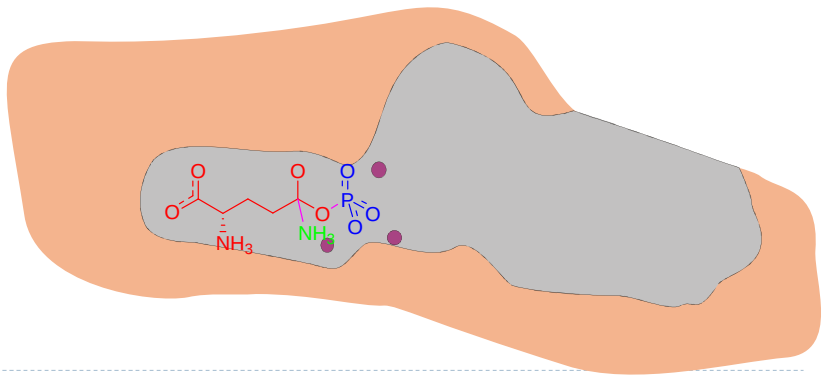

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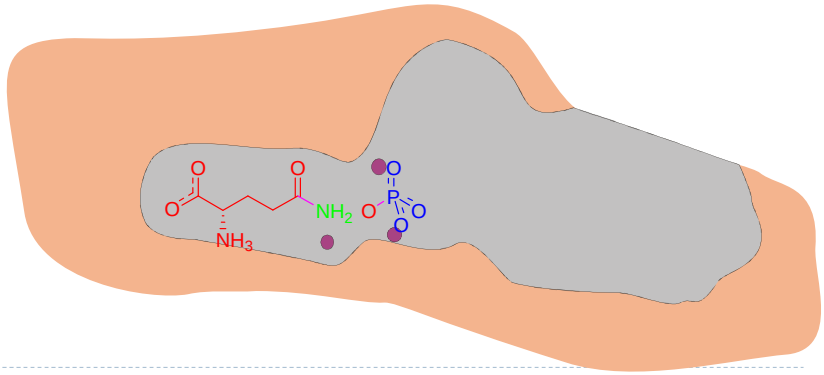

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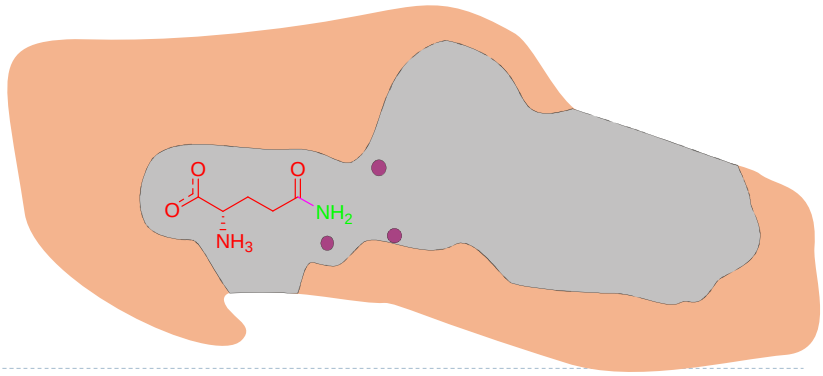
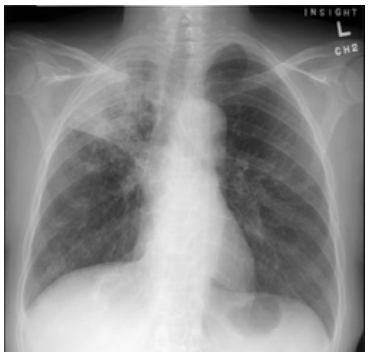


the use of GS inhibitors

- potential anti-tuberculosis drugs,



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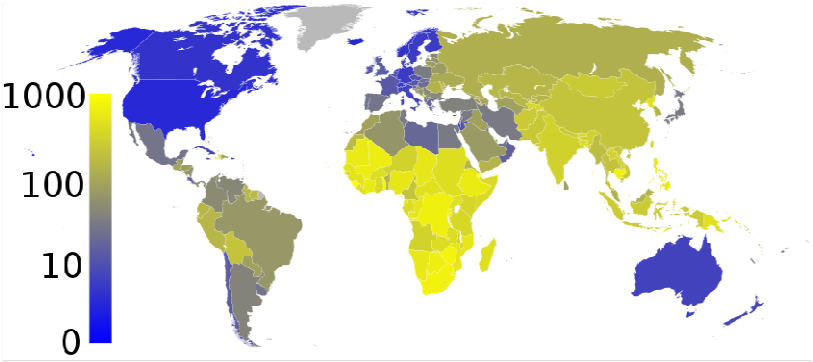
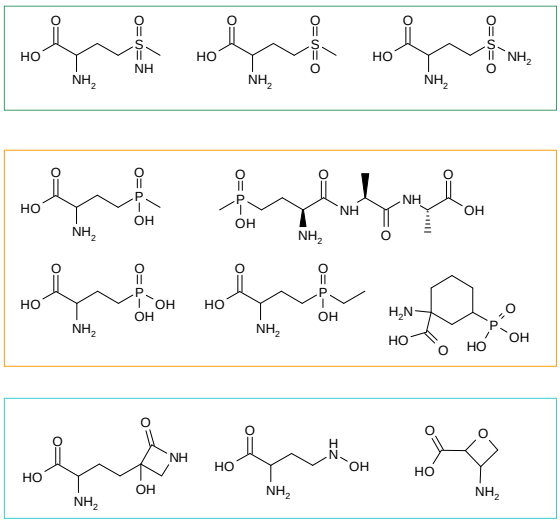
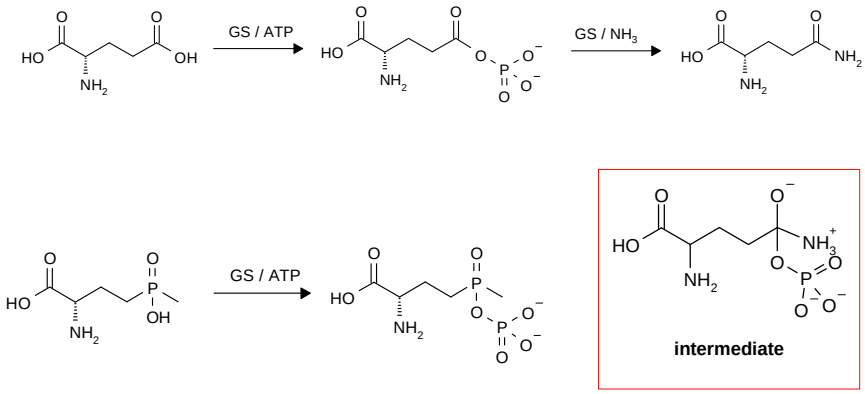


Known inhibitors

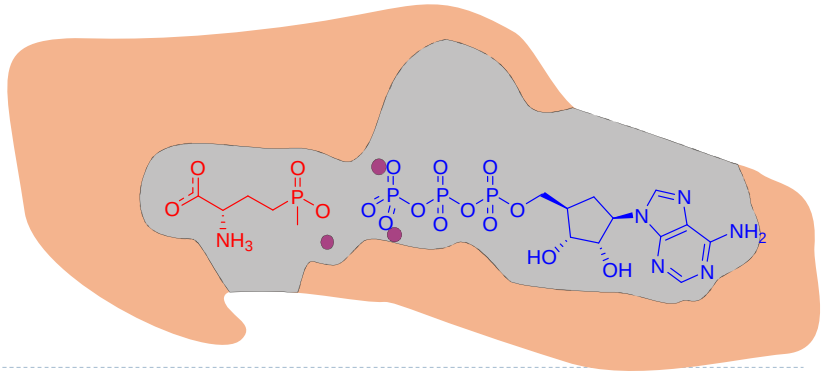


Inhibition mechanism



Inhibition mechanism

- The mechanism of inhibition of glutamine synthetase by phosphinotricin is based on phosphorylation of the inhibitor in the active site.



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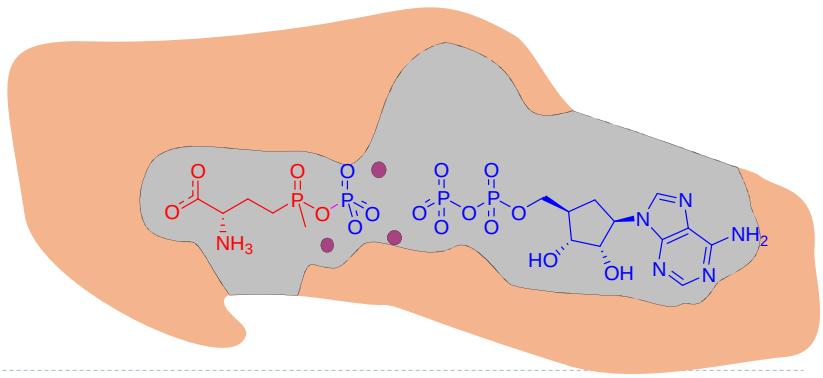

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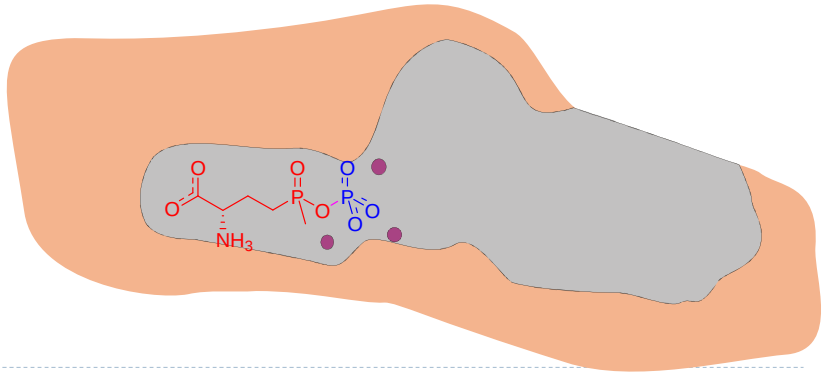
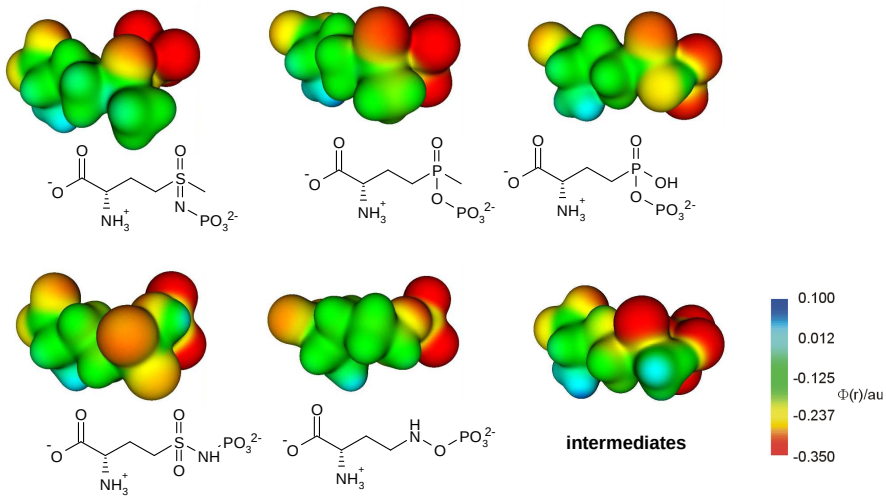


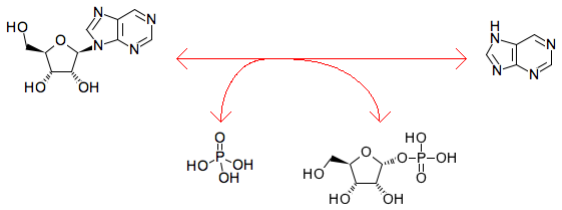
Structures of inhibitors



Electrostatic potentian on inhibitors' surface.

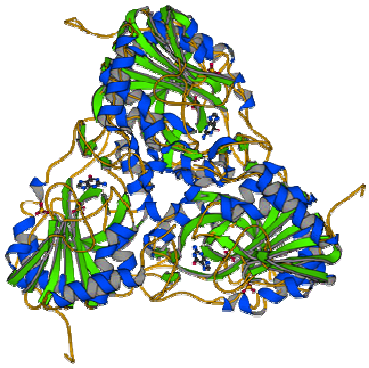
Purine nucleoside phosphorylase

- Purine nucleoside phosphorylase (PNP) catalyzes the breakdown of nucleosides to the corresponding bases and phosphoribose.
- The damage to this enzyme results in lack of immunity due to apoptosis of T lymphocytes.
- PNP is a molecular target for drugs for diseases whose development is associated with T lymphocytes.



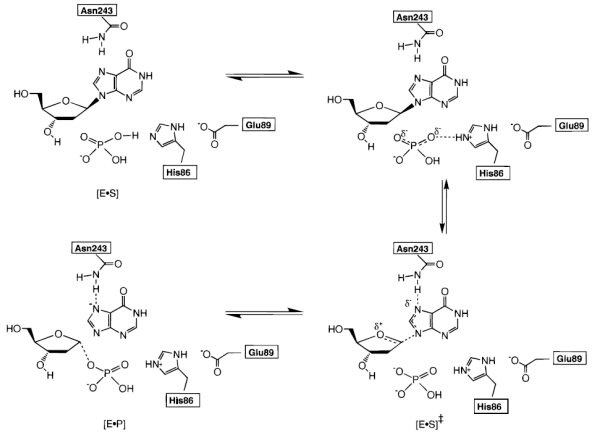
Purine nucleoside phosphorylase

- **PNP inhibitors** are potential drugs for:
  - acute myeloid leukemia (T-cellular),
  - cutaneous (T-cell) lymphoma
  - psoriasis,
  - rheumatoid arthritis,
  - systemic lupus erythematosus,
  - Crohn's disease,
  - type I diabetes,
  - gout.



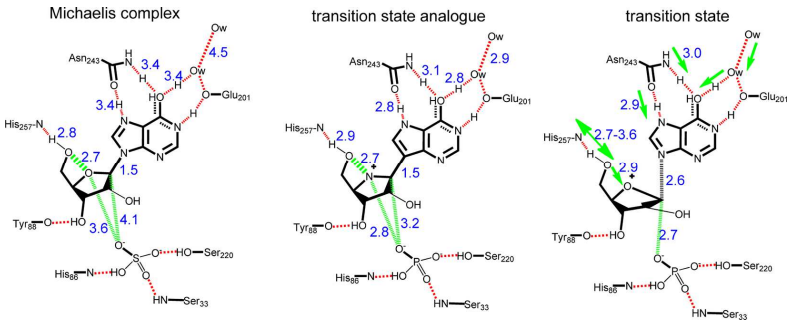
Purine nucleoside phosphorylase

- Mechanism of enzymatic reaction of PNP



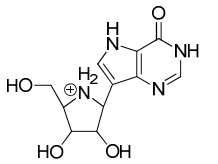
Analogues of transition states

- Highly active analog of transition state constructed on the basis of transtion state analysis.



Analogues of transition states

- The PNP inhibitor (Immunilin) is undergoing clinical trials as a leukemia drug.
- Since the inhibitory activity was higher for the bovine enzyme, an analysis of the transtion state for the human enzyme (very structurally similar) was also carried out.

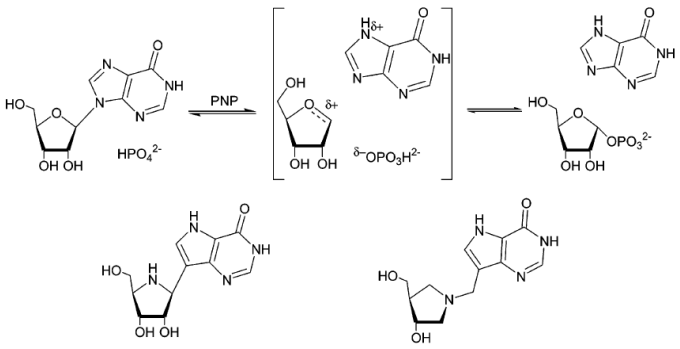


K_i = 56 pM (enzym ludzki)
K_i = 23 pM (enzym wolowy)

Immunilin

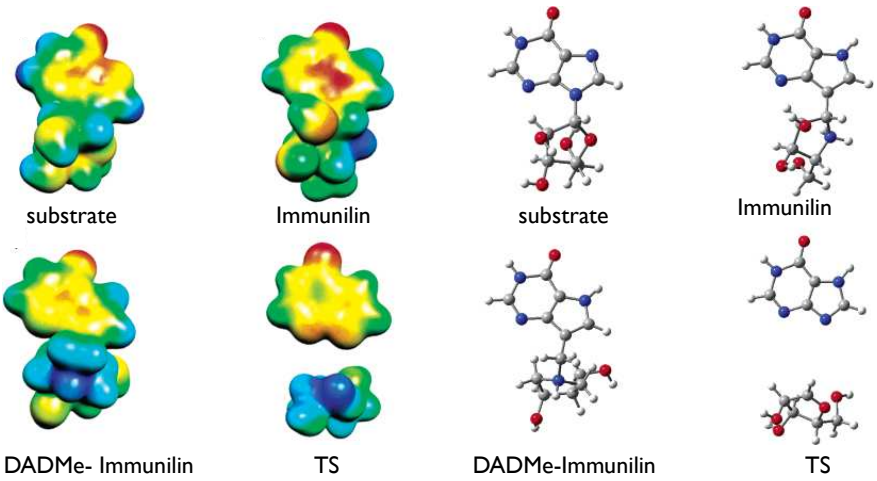
Mechsim of reaction of human PNP

- It has been shown that the transtion state for the human enzyme is different from that previously tested for the beef enzyme.



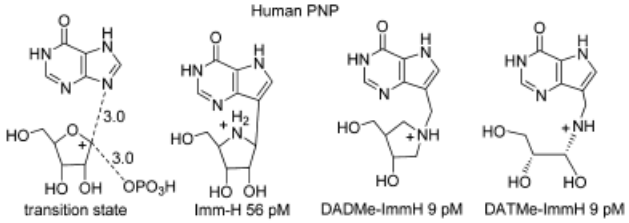
Immunilin DADMe-Immunilin

Substrate, transition state and analogues



Purine nucleoside phosphorylase

- DADMe-Immucillin is an analogue of the transition state of the reaction catalyzed by the human enzyme. It exhibits extremely high inhibitory activity (9 pM).
- DADMe-Immucillin is the subject of clinical trials as a gout remedy.



Summary

- Transtion state analogs are very effective enzyme inhibitors.
- It is not always easy to find real structures of the transition state.