RATIONAL IRREVERSIBLE ENZYME INHIBITION

Sarmd Dheyaa

Inhibitors

- Inhibitors are chemical substance that reduce the rate of enzymic reactions
- The are usually specific and they work at low concentrations
- They block the enzyme but they do not usually destroy it
- Many drugs and poisons are inhibitors of enzymes in the nervous system

TYPE OF ENZYME INHIBITOR

- **Irreversible inhibitors:** Combine with the specific groups of the enzyme in the active site, irreversibly,
- Irreversible inhibitors bind covalently to the enzyme and permanently inhibit it.

Examples: nerve gases and pesticides, containing organophosphorus, combine with serine residues in the enzyme acetylcholine esterase

The effect of enzyme inhibition

- **Reversible inhibitors:** These can be washed out of the solution of enzyme by dialysis.
- Competative , uncompetative , non competative .

Basis of Irreversibility

- • **Irreversible**
- • Irreversible inhibitors binds to enzyme via covalent bonds and prevent enzyme from further performing of catalytic acts.
- • It is necessary to understand, that irreversible inhibitors act on some selected group of enzymes, with no effect on other enzymes and proteins.
- Can not be reverse inhibition by increase substrate level
- Recovery possible only by synthesis of new enzyme

TYPE OF IRREVERSIBLE INHIBITOR

- Group specific inhibitor
- Affinity label inhibitor
- Suicide inhibitor

Irreversible enzyme inhibition

1. Group-specific inhibitors

- react with specific amino acid side chains like **(DIPF),** For example, only 1 of the 28 serine residues in chymotrypsin is modified by DIPF. This means that this specific residue is especially reactive; moreover, it is implied that this specific residue lies in the active site of the enzyme **chymotrypsin**. DIPF has also provided data that suggests, through its binding with active serine residues, that there is indeed a reactive serine residue contained within the active site of the enzyme Acetylcholinesterase. The inactivating functionality of DIPF and similarly-shaped molecules in acetylcholinesterase is representative of a group of compounds, known as nerve agents.

Irreversible enzyme inhibition

1. Group-specific inhibitors

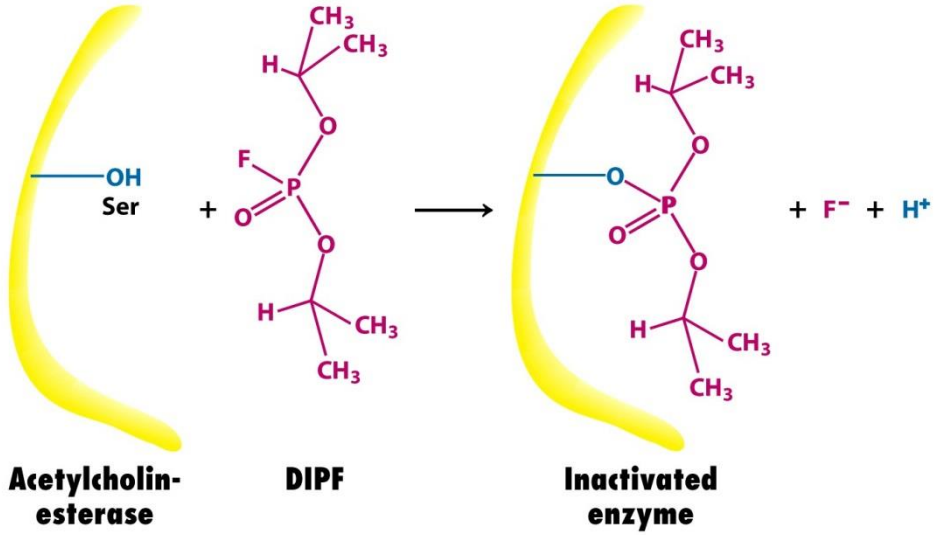

Figure 8-23
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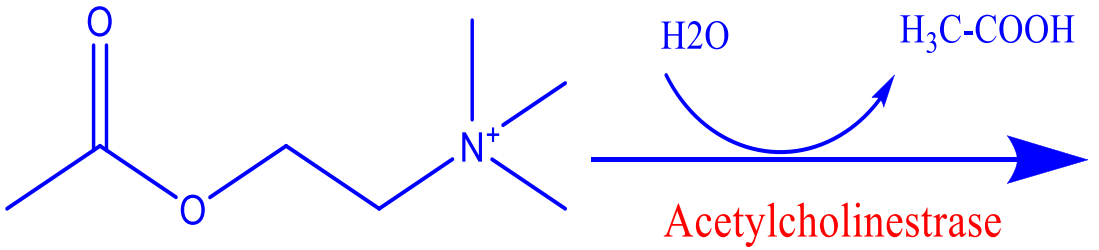
- React with amino acid side chains;
- Lead to inhibition by interfering with the catalysis (e.g. by reacting with side-chains important for the catalysis);
- E.g. diisopropyl fluorophosphate (DIPF);
  - Nerve gas
  - Inhibits acetylcholine esterase (and many other proteases with Ser at the active site)

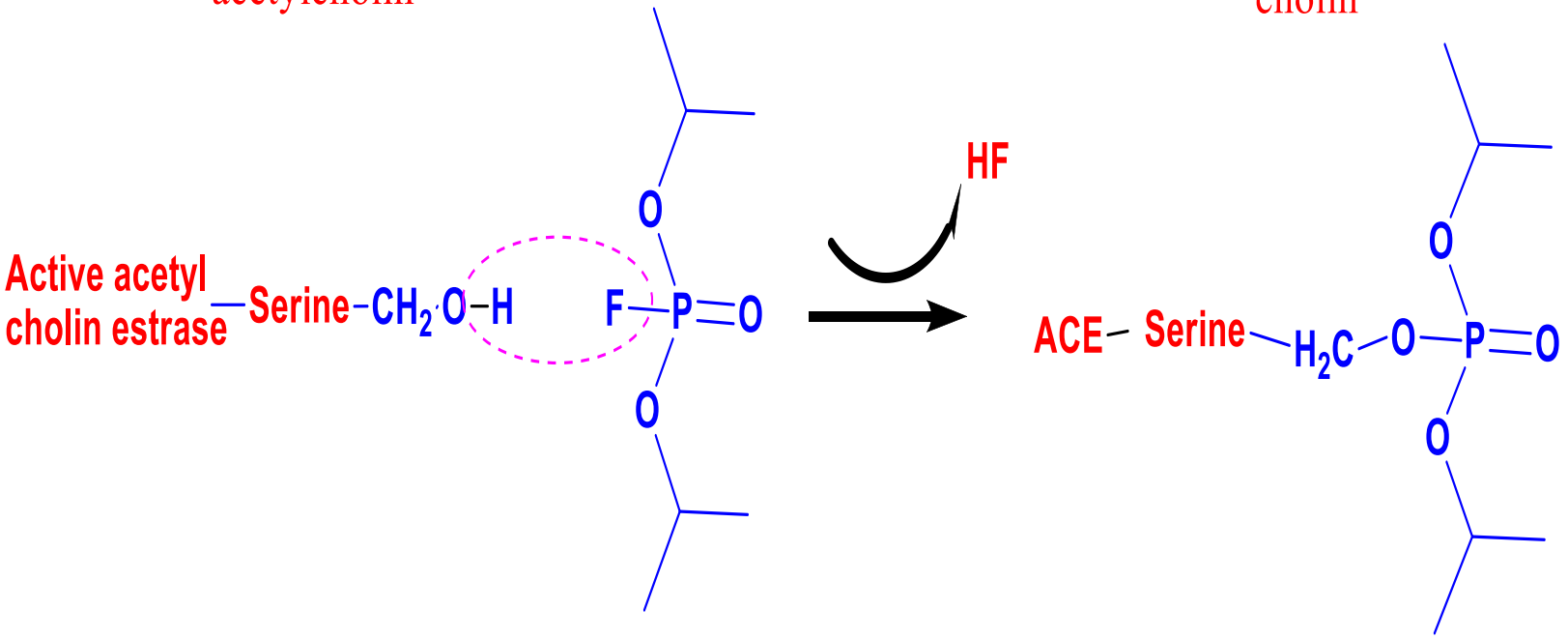
Irreversible enzyme inhibition

1. Group-specific inhibitors



acetylcholin

cholin



Inactive enzyme

Irreversible enzyme inhibition

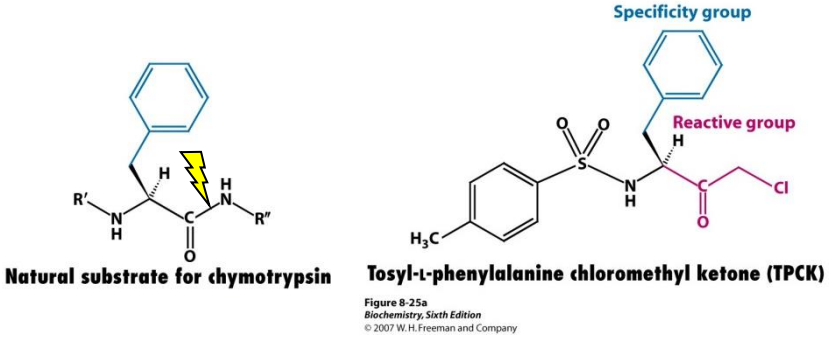
2. Affinity labels

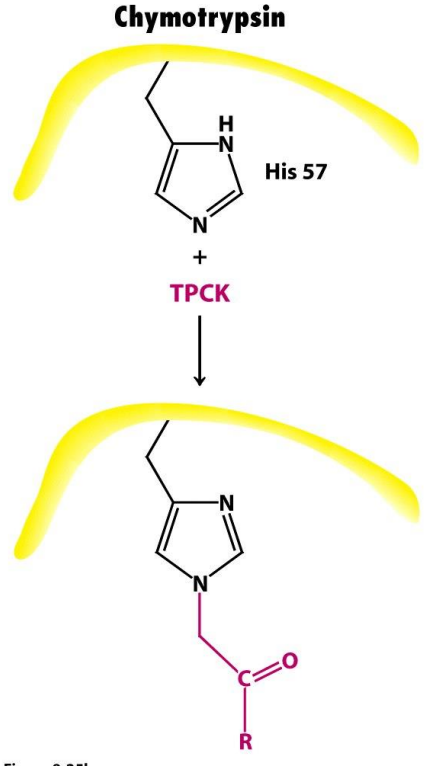
- (Reactive substrate analogs) are structurally similar to the substrate that can covalently bind to the active site and are therefore more specific than group specific reagents. An example is **Tosyl-L-phenylalanine chloromethyl ketone (TPCK)** which is an analog for chymotrypsin which binds to the active site and reacts irreversibly with the histidine residue to inhibit the enzyme.

Irreversible enzyme inhibition

2. Affinity labels

- Inhibitor is ***structurally similar*** to Substrate
- Reacts with active site residues;
- I reacts with E to form a covalent bond that ***cannot*** be hydrolysed;





Irreversible enzyme inhibition

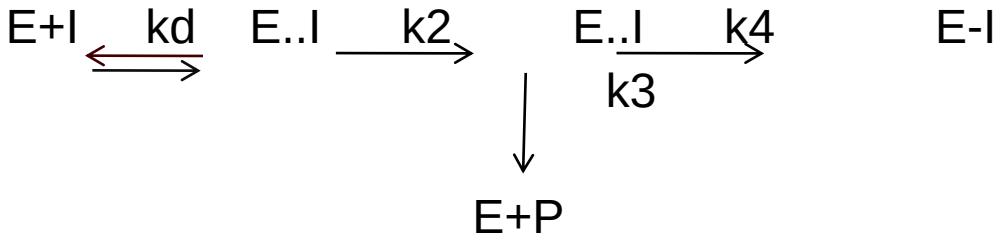
3. Suicide inhibitors

- A suicide inhibitor is a relatively inert molecule that is transformed by an enzyme at its active site into a reactive compound that irreversibly inactivates the enzyme (mechanism - based inhibitor)
- Relatively unreactive until they bind to the active site of the enzyme

Irreversible enzyme inhibition

3. Suicide inhibitors

- TActive-site directed reagent (unreactive) binds to the enzyme active site → transformed to a reactive form. Once activated, a covalent bond between the inhibitor and the enzyme forms
- his approach minimizes side reactions (non specific covalent bond formation) which may occur with an affinity reagent

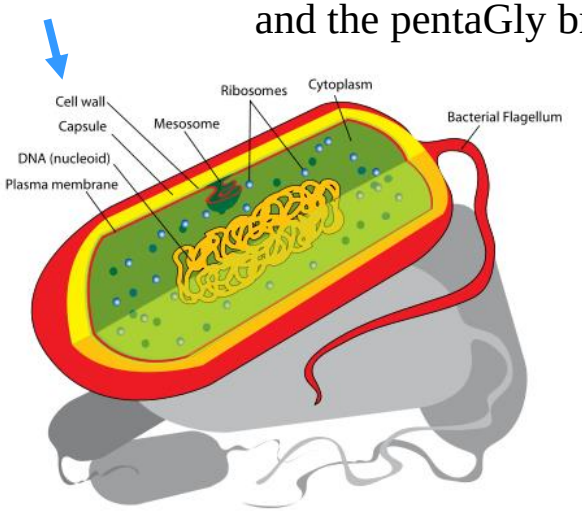


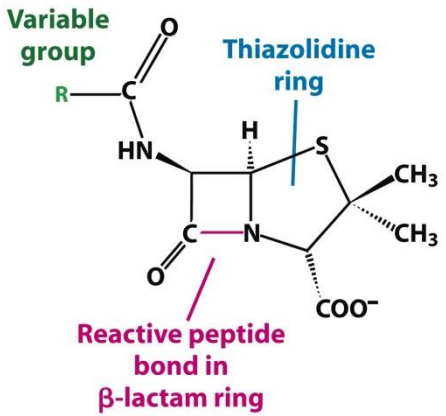
- 1) inhibitor binds to active site
  2) converted to reactive compound via enzyme's catalytic capabilities
  3) reactive form covalently reacts with the enzyme
- Inactivation (covalent bond formation, k4) must occur prior to dissociation (k3) otherwise the now reactive inhibitor is released into the environment

Irreversible enzyme inhibition

3. Suicide inhibitors - penicillin

- Interfere with the synthesis of the bacterial cell wall
  - Makes bacteria much less resistant to stress;
- Cell wall:
  - Peptidoglycan
  - Penicillin blocks the formation of the link between the tetrapeptide and the pentaGly bridge;





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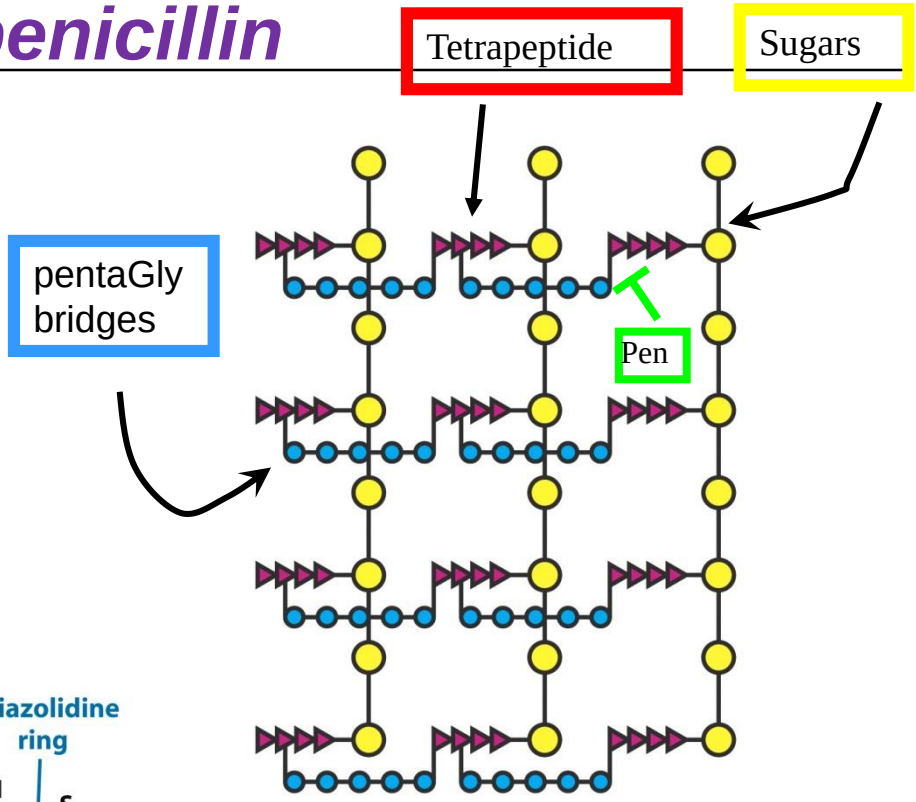

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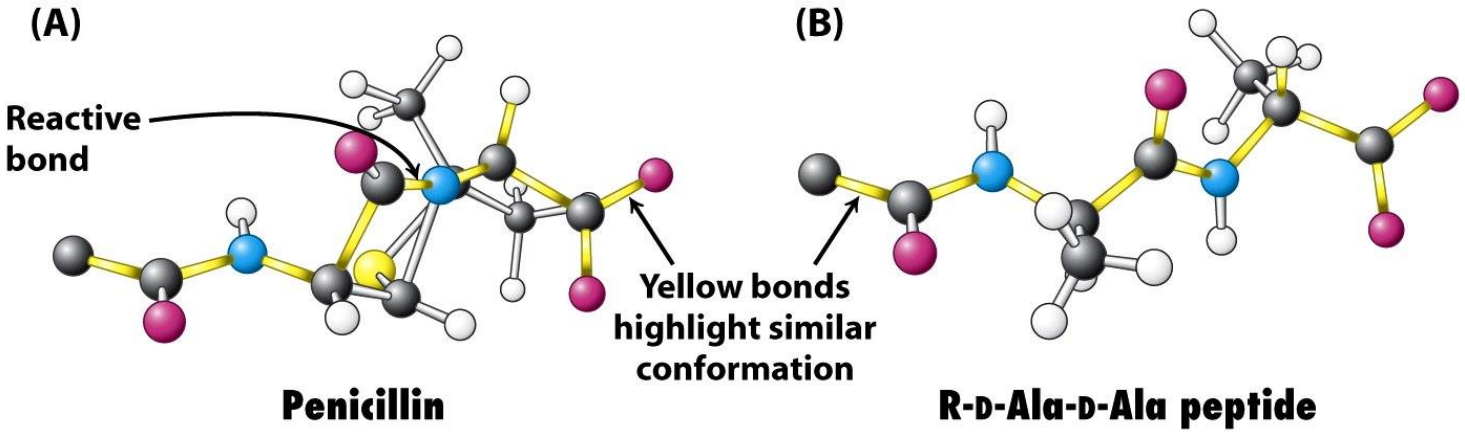
Structure of the bacterial cell wall

Irreversible enzyme inhibition

3. Suicide inhibitors - penicillin



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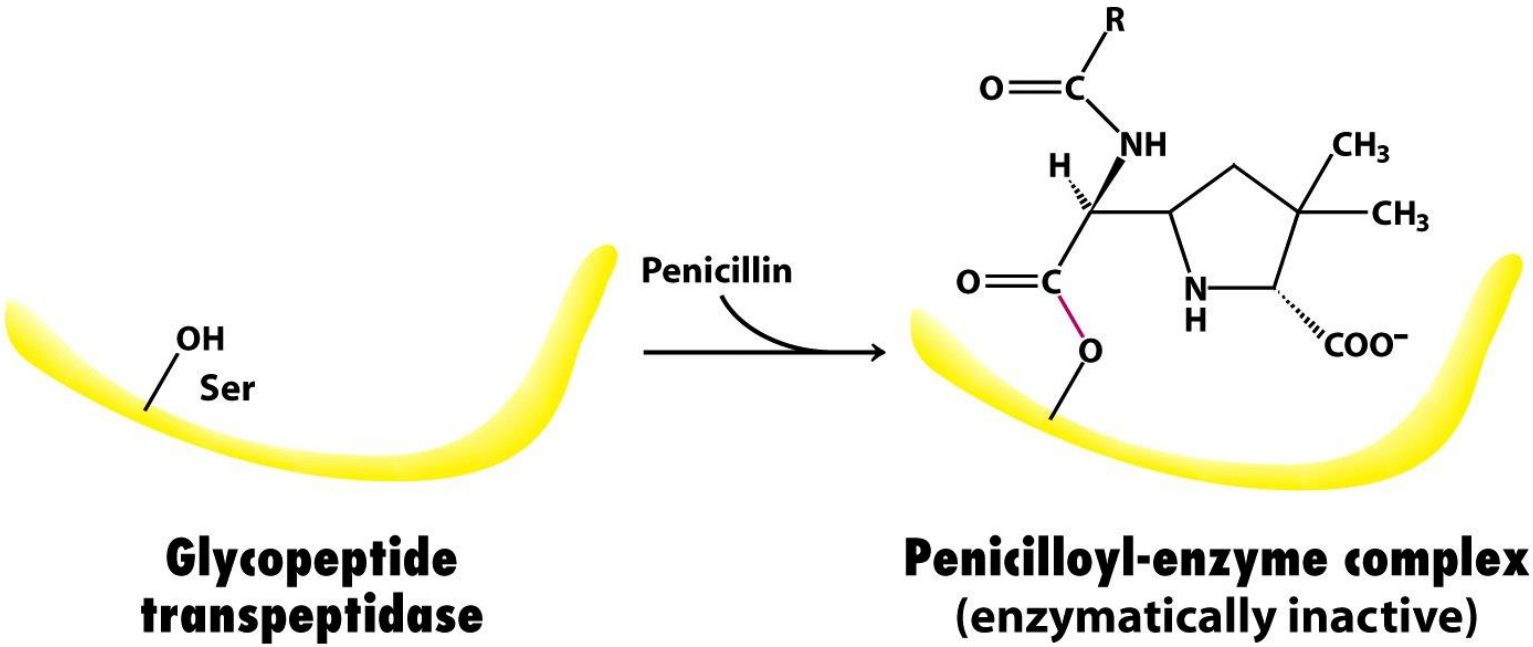
Irreversible enzyme inhibition

3. Suicide inhibitors - penicillin



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Allopurinol as suicide enzyme inhibitor

Allopurinol is xanthine oxidase inhibitor

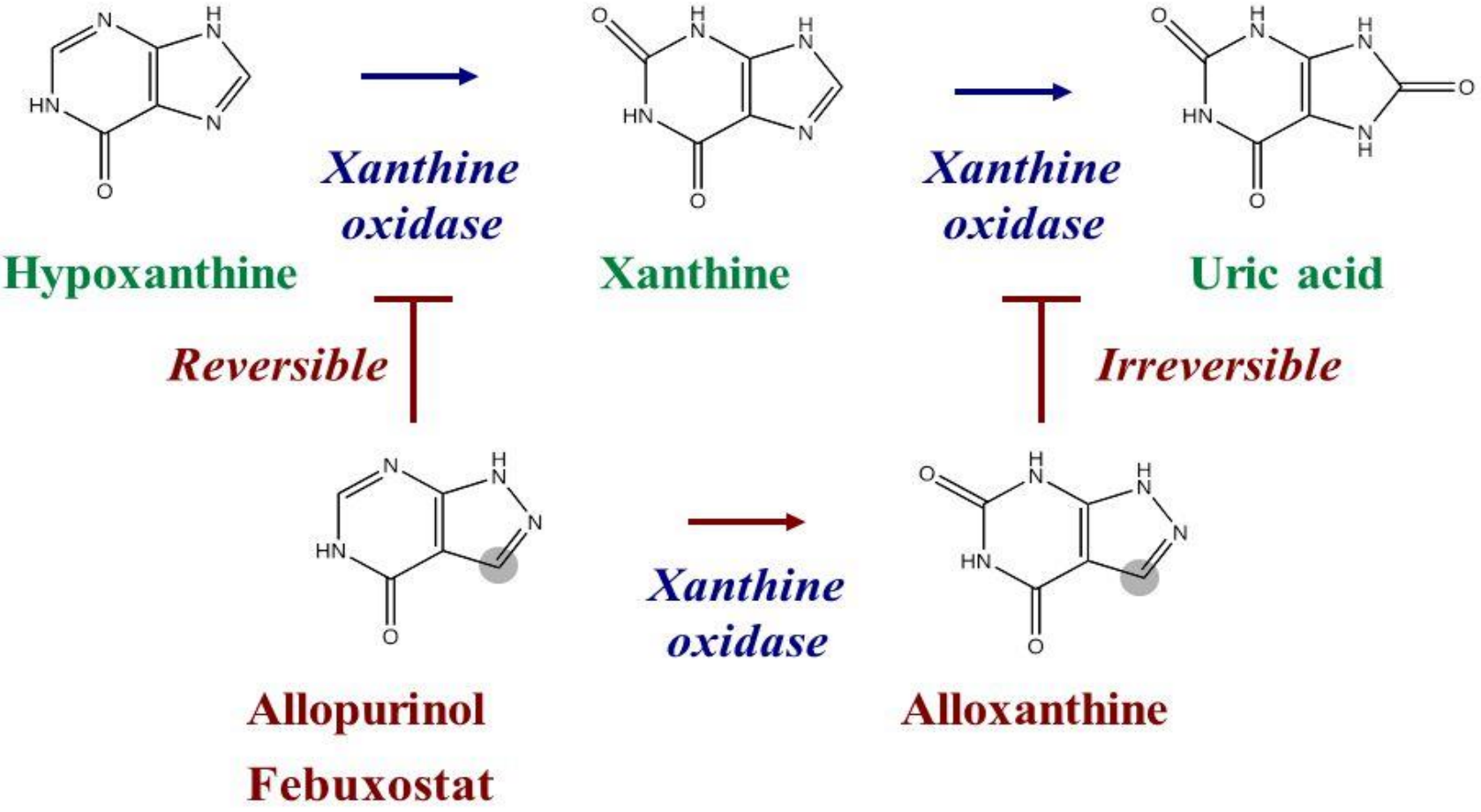
Allopurinol is similar to hypoxanthine in structure

Allopurinol is a substrate for xanthine oxidase, but the product binds so tightly to enzyme

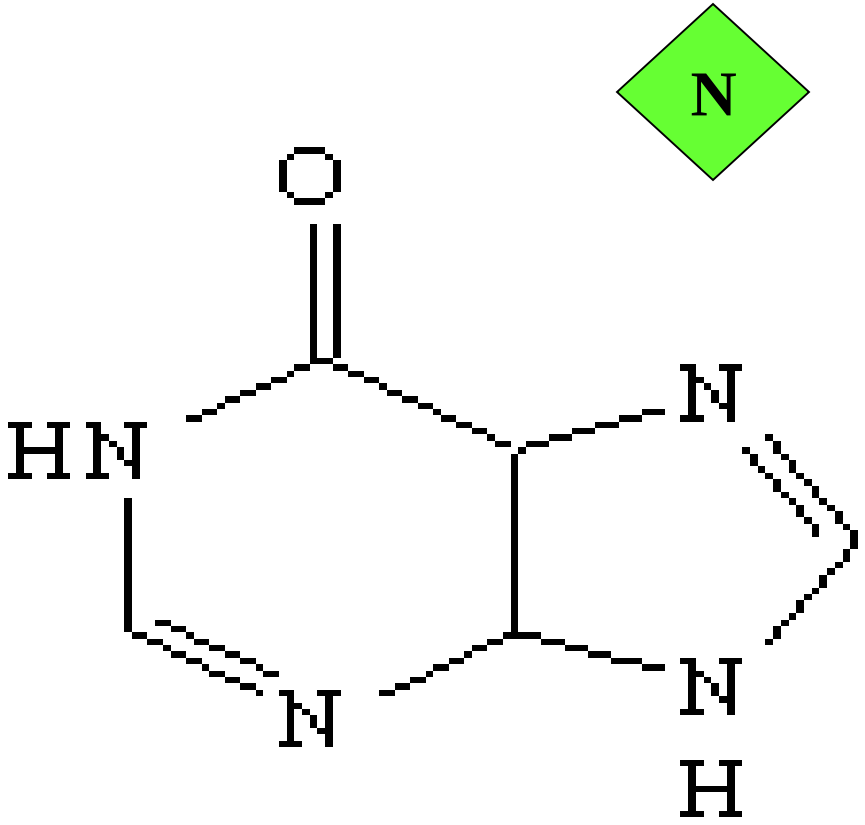
Xanthine oxidase enzyme is now unable to act on normal substrate.

Uric acid production is diminished and xanthine and hypoxanthine levels in the blood rise.

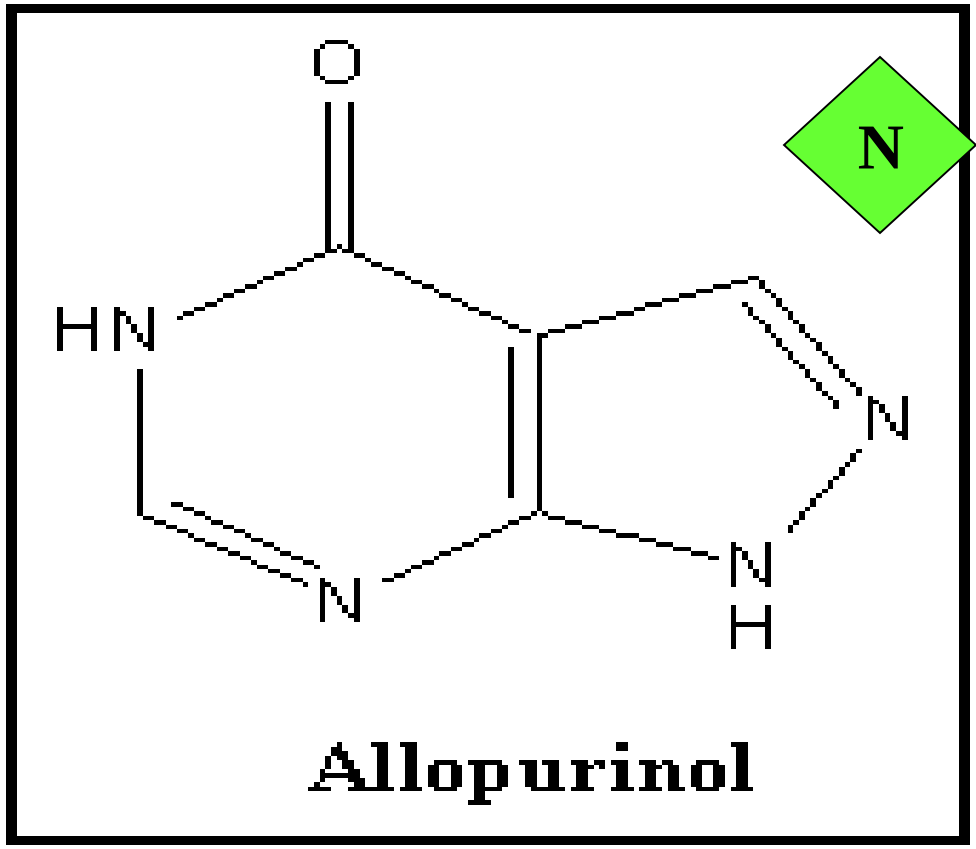
Allopurinol Inhibits Uric Acid Production



Toxicity: ***Acute gout, rash, hematologic reactions, drug interactions***



Hypoxanthine



Allopurinol

conclusion

- Inhibitor binds at or near the active site of the enzyme irreversibly, usually by covalent bonds, so it can't dissociate from the enzyme.
- Irreversible inhibitors combine with the functional groups of the amino acids in the active site, irreversibly.
- Irreversible inhibitors occupy or destroy the active sites of the enzyme permanently and decrease the reaction rate.
- Enzyme activity is not regained on dialysis

Thanks for your attention