Rational Drug Design lecture 7

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Enzyme inhibitors

- **Covalent - react with amino acid residues of the enzyme. Covalent inhibitors may be irreversible or reversible.**
- **Non-covalent - interact with the enzyme.**

- Covalent inhibitors may react with:
- nucleophiles **of** the active site (involved in the enzymatic reaction) - mechanism-based inhibitors;
- nucleophiles **near** active site - Targeted covalent inhibitors (TCI).

Covalent inhibitors

Advantages

- strong interaction with the enzyme, often irreversible;
- combination of non-covalent (specificity) and covalent (high energy) interactions;
- increased biochemical efficiency:
- Non-equilibrium constraints restrict competition with natural substrates of ligands;
- Slow removal from the body;
- Lower doses required;
- If the target is deactivated rapidly, drug concentration is rapidly reduced and drug toxicity and drug interactions are limited.
- Potentially longer action time depending on the synthesis of new enzyme (less dosage).
- The most effective strategy for goals that require complete deactivation.

Covalent inhibitors

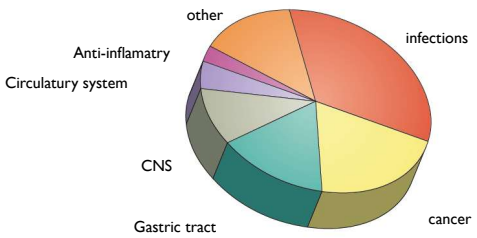
- **Disadvantages**
  - Greater probability of the lack of selectivity;
  - Potential immunogenicity of protein adduct with ligand leading to allergic response;
  - idiosyncratic reaction (hypersensitivity);
  - Possible problems with reactive metabolic products;
  - Not suitable for molecular purposes requiring short-acting or partial inhibition;

Covalent inhibitors

- **Idiosyncrasia** (gr. Ιδιοσυγκρασία, idiosynkrasia, idios "his own" and syn-krasis "mixture") - state of increased organism's perception of a particular chemical compound.
- Drug response, associated with individual sensitivity, is due to abnormalities in the biochemical metabolism of the drug in the body: abnormal metabolism, accumulation or blocking of metabolism of other substances.
- This is not an allergic reaction (no antigen-antibody interaction).

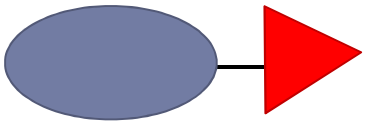
Covalent inhibitors

- Due to the difficult to predict side effects, covalent inhibitors are reluctantly used as leading substances in pharmaceutical projects.
- Most of the covalent inhibitors used were commercially available prior to the discovery of their covalent mechanism of action.
- Approx. 30% of the enzyme inhibitors used act by covalent modification of molecular target.



Covalent inhibitors

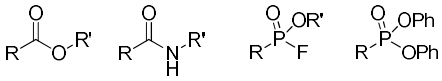
- The simplest way to design:
  - Fragment analogous to the substrate,
  - group reacting with Ser or Cys residues in active site or its surroundings.



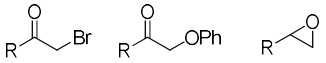
warhead

Covalent inhibitors

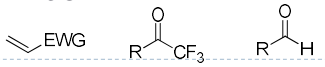
- Nucleophiles in enzyme:
  - Cys, -SH
  - Ser, -OH
- Electrophiles in inhibitor:
  - Acylation reaction



  - Alkylation reaction



  - Addition



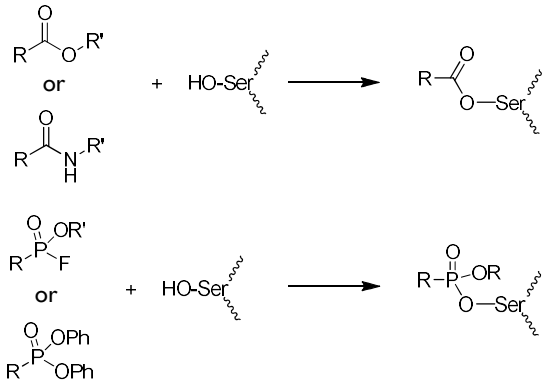
Covalent inhibitors

- **Nucleophile** - a group with an excess of electrons, which in reaction can be an electron donor**:**
  - -SH
  - -OH
- **Electrophile** - an electron deficient group that reacts with electron acceptor:

- The electrophile can not be too reactive (can not react with water).

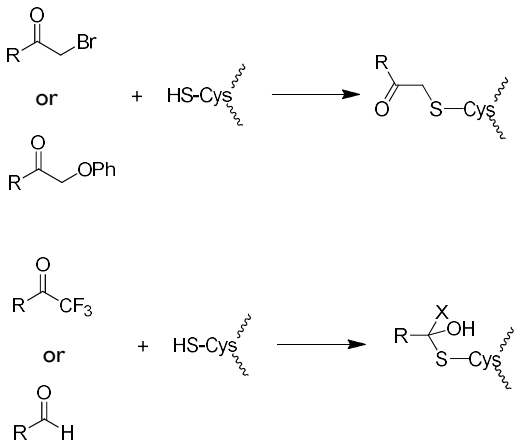
Covalent inhibitors

- Acylation or phosphonylation of serine



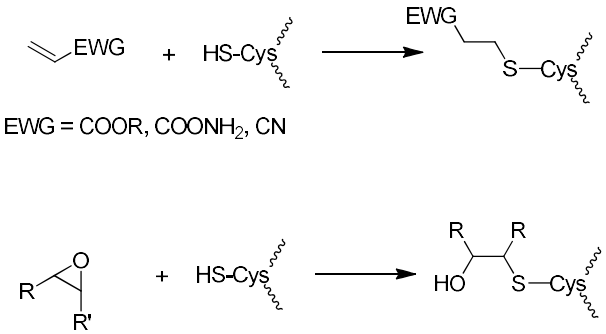
Covalent inhibitors

- Alkylation or addition to cysteine



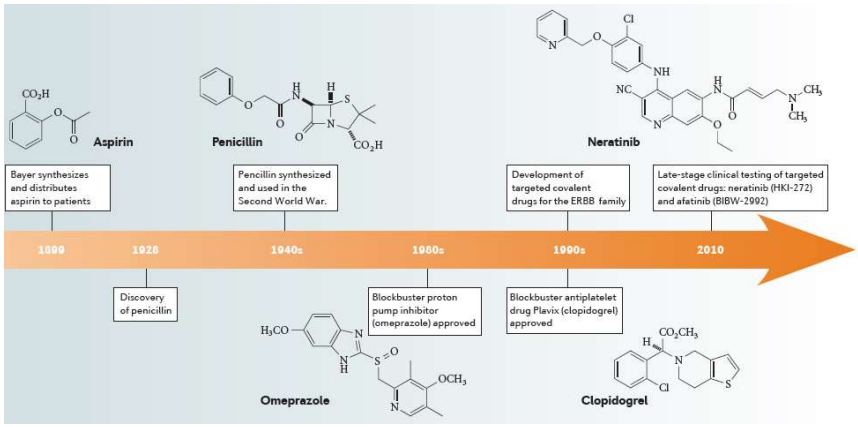
Covalent inhibitors

- Alkylation or addition to cysteine

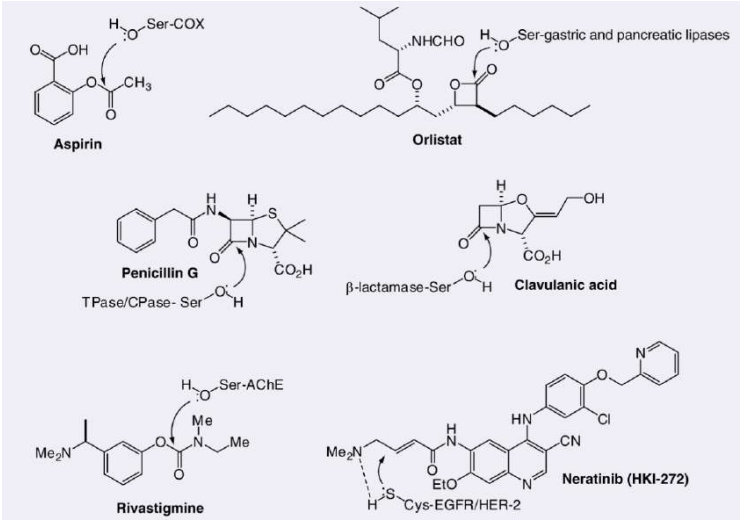


Covalent inhibitors

- Covalent inhibitors are used to treat many diseases.

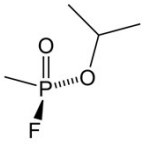


Examples

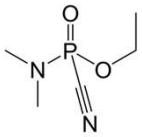


Acetylcholinesterase inhibitors

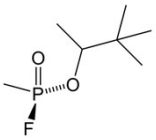
- Sarin was discovered in Germany in 1938 (IG Farben) in research aimed at finding pesticides.
- Since 1939 Germany produced sarin and its analogues as chemical weapons (never used by them).
- The name sarin comes from the names of the discoverers: **S**chrader, **A**mbros, **R**üdiger i Van der L**in**de.



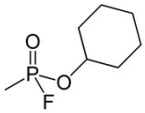
sarin



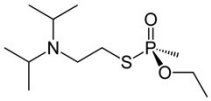
tabun



soman



cyclosarin



VX

Acetylcholinesterase inhibitors

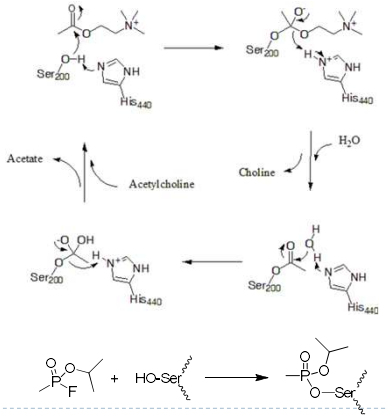
- Sarin was used in 1988 against Kurds in Iraq (5,000 victims).
- In 1995, sarin was used in the assassination of the metro in Tokyo by the sect of the Supreme Truth.

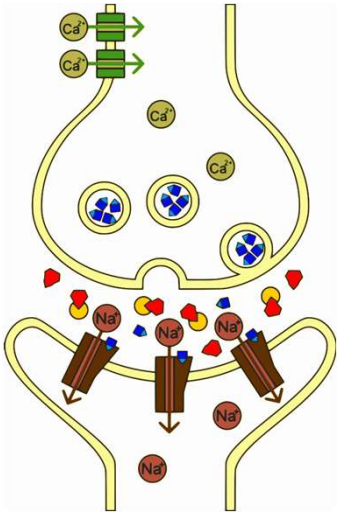
Acetylcholinesterase inhibitors

- The high toxicity of paralytic-convulsive fighting gases: sarin, soman, cyclosarin, tabun and VX results from their irreversible inhibition of acetylcholinesterase.
- LD_{50} = 172 µg/kg

Acetylcholinesterase inhibitors

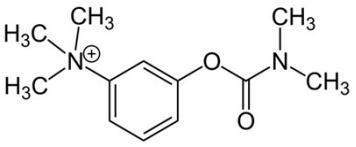
- Inhibitors react with the rest of the serine at the active site of the enzyme.



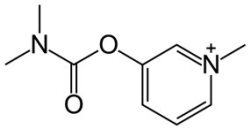


Acetylcholinesterase inhibitors

- **Myasthenia gravis** - An autoimmune disease characterized by rapid pain in the skeletal muscles. It is associated with blockade of acetylcholine receptors.
- Medicines are **reversible covalent** acetylcholinesterase inhibitors.

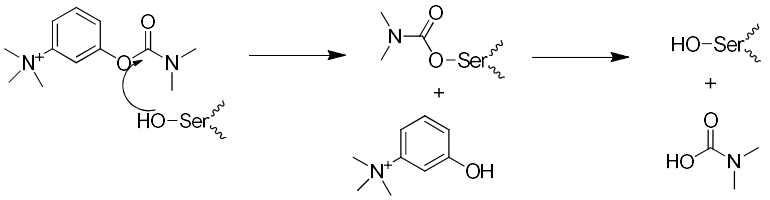


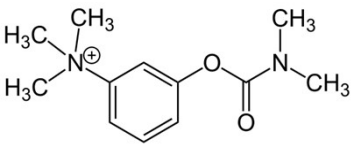
Neostygmin



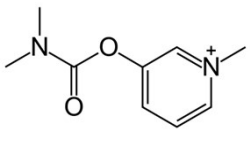
Pirydostygmin

Acetylcholinesterase inhibitors





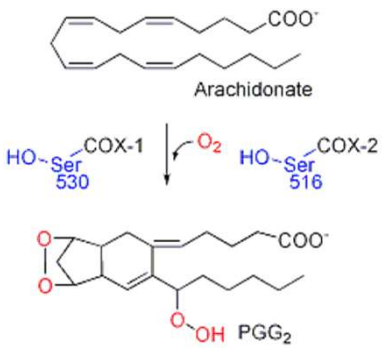
Neostygmine



Pirydostygmine

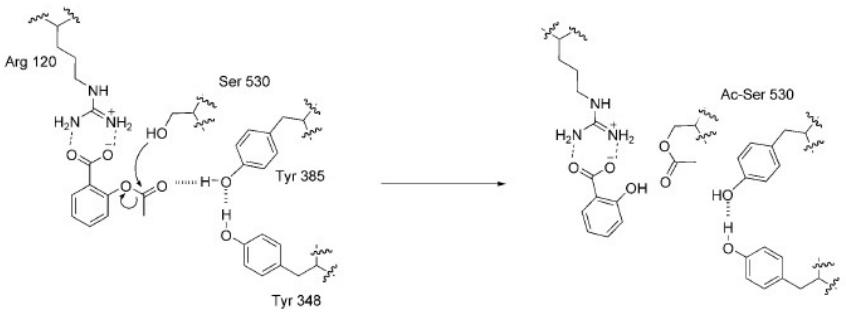
Aspirin

- Aspirin is an **irreversible covalent** cyclooxygenase inhibitor of COX-1 and COX-2.
- Cyclooxygenases catalyze the synthesis of prostaglandin from arachidonic acid.



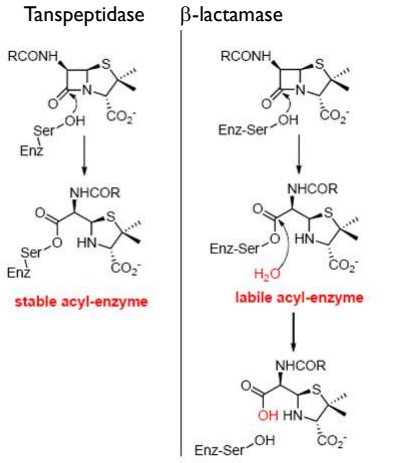
Aspirin

- Aspirin acetylates the serine residue at the active site of the enzyme.



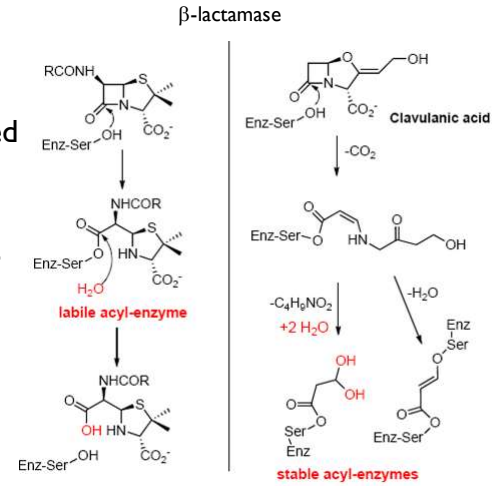
β-lactames

- β-lactam antibiotics are irreversible D-Ala-D-Ala-transpeptidase inhibitors.
- They form a stable adduct to the Ser residue at the active site of the transpeptidase.
- Penicillins are effectively hydrolysed by β-lactamase



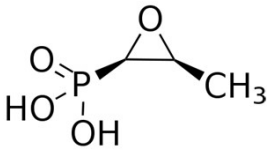
Clavulanic acid

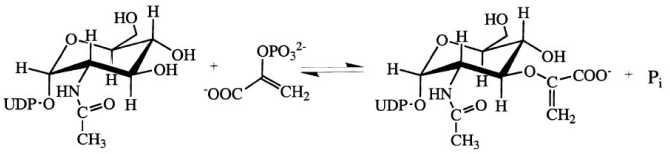
- Clavulanic acid is an irreversible inhibitor of β-lactamase.
- Clavulanic acid was isolated from the *Streptomuces clavuligerus* bacteria.
- It is used together with β-lactam antibiotics.



Fosfomycin

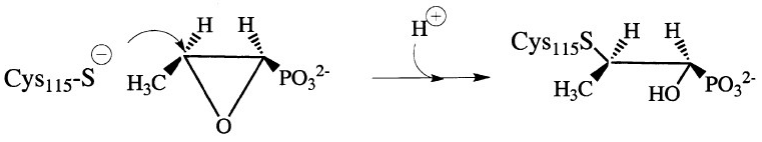
- **Fosfomycin** was isolated from *Streptomyces* bacteria.
- It is a **covalent** MurA inhibitor - UDP-N-acetylglucosamine enolpyruvyl transferase.
- Enzyme is essential for the biosynthesis of peptidoglycan - the bacterial cell wall component.



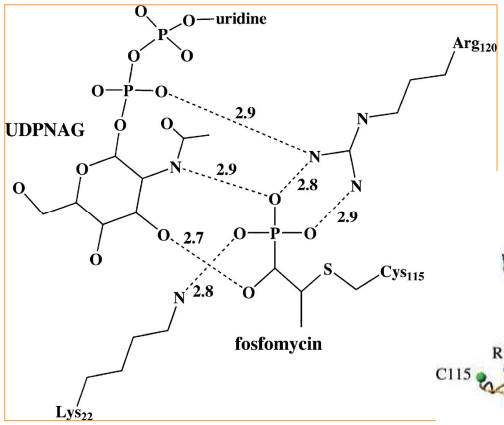


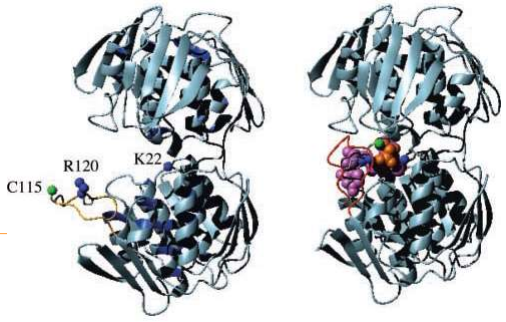
Fosfomycin

- Fosfomycin is an effective antibiotic used to treat urinary tract infections.
- Typically, 1 mega-dose (2-3 g of medicine) is taken.
- Fosfomycin reacts with Cys115 enzyme to form covalent adduct.



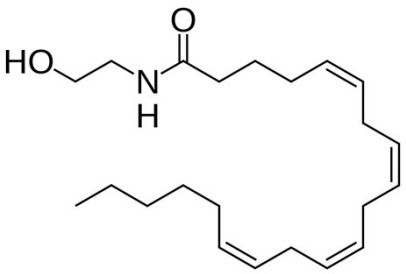
Fosfomycin





fatty acid amides hydrolase

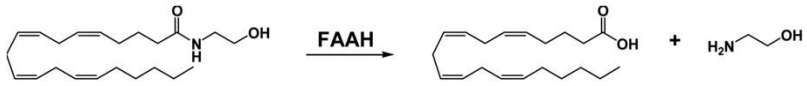
- The main substrate for fatty acid hydrolase (FAAH) is anandamide.
- Anandamide is endocannabinoid.
- Anandamide activates cannabinoid receptors CB1 and CB2.
- Anandamide has similar activity to THC.



Anadamide
(Sanskrit, *ananda* – happiness)

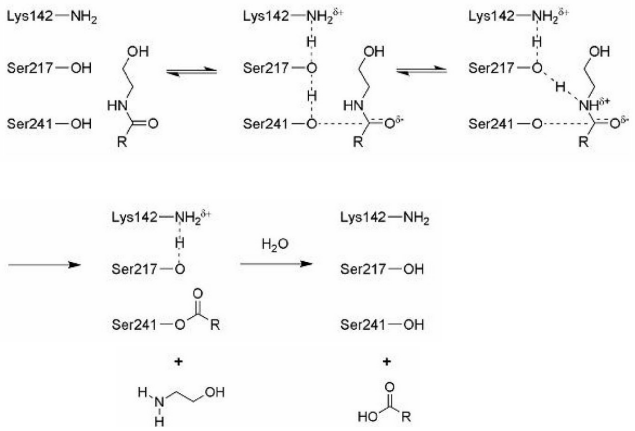
FAAH

- FAAH - is the molecular target of painkillers.
- Failure of FAAH results in increased anadamide concentrations and hence analgesic effect.
- FAAH inhibition does not have side effects observed with direct activation of CB1 and CB2 receptors (recognition and motor dysfunction).



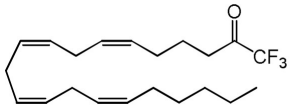
FAAH

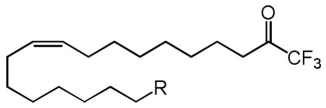
- Mechanism of hydrolysis

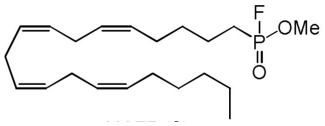


FAAH inhibitors

- Inhibitors that are substrate analogues

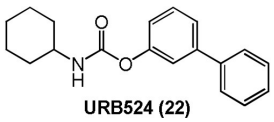






FAAH inhibitors

- Carbamate-based inhibitors acylate enzyme.



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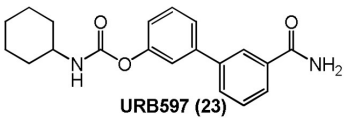

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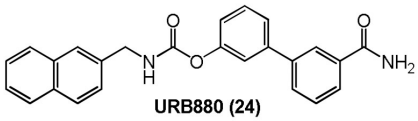

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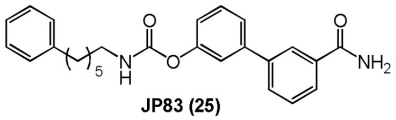

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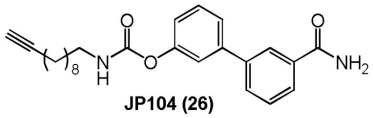
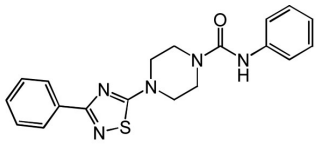


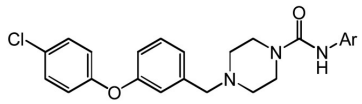
JP104 (26)

FAAH inhibitors

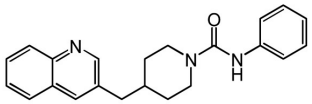
- Urea-based inhibitors acylate enzyme.



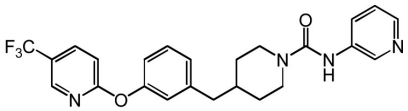
JNJ-1661010/Takeda-25 (**32**)



33, Ar = phenyl
34, Ar = pyridazin-3-yl
35, Ar = benzoisoxazol-3-yl



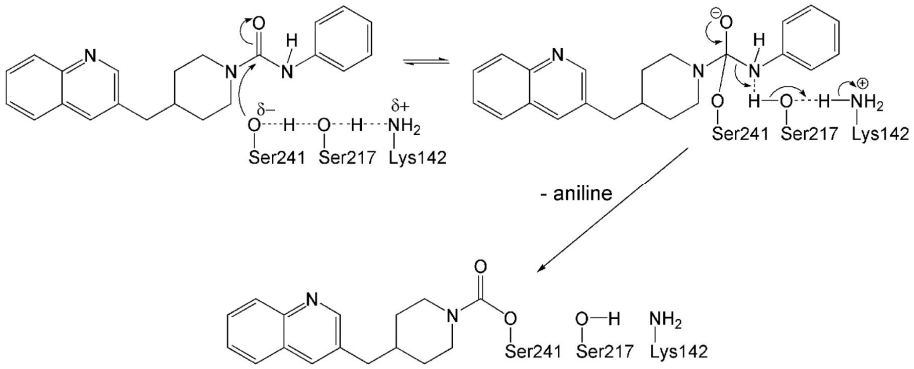
PF-750 (**36**)



PF-3845 (**37**)

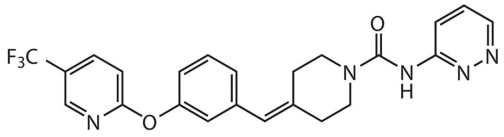
FAAH inhibitors

- Mechanism of enzyme acylation



FAAH inhibitors

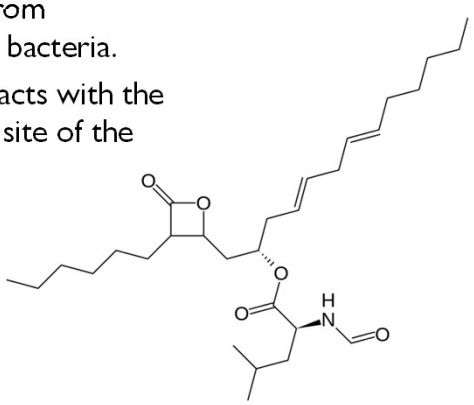
- Compound PF-04457845 is in Phase II clinical trials as a painkiller



PF-04457845

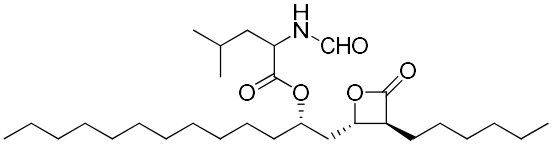
Lipostatin

- Lipostatin is an irreversible inhibitor of pancreatic lipase.
- Lipostatin was isolated from Streptomyces toxytricini bacteria.
- The β-lactone system reacts with the Ser residue at the active site of the enzyme.



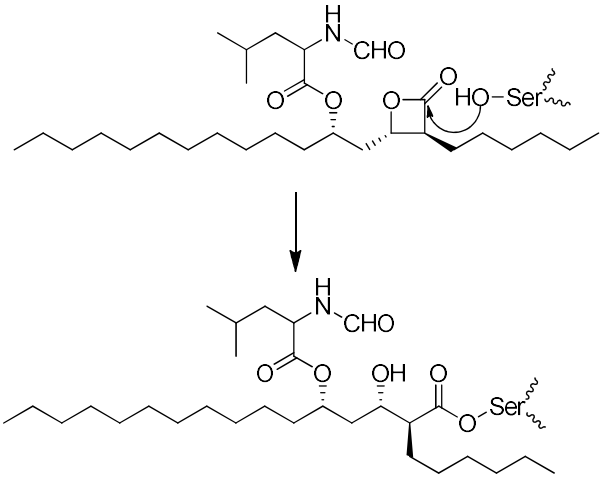
Lipostatin

- Saturated lipostatin analogue - **orlistat** is a commercially available drug for obesity.
- Inhibition of lipase enzyme activity reduces the absorption of fats.
- With no additional treatments (exercise and diet) the effect of the drug is moderate: about 2-3kg per year.



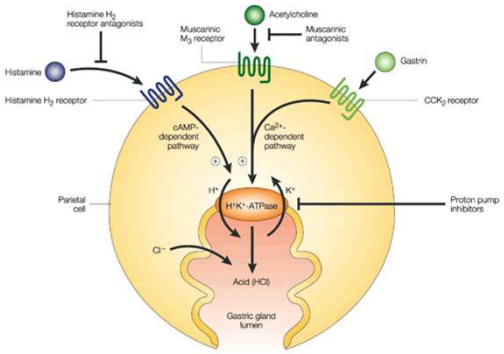
orlistat

Lipostatin - orlistat



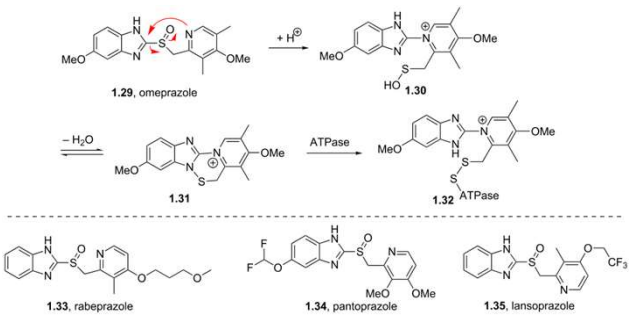
Proton pump inhibitors

- In gastric and duodenal ulcers and gastroesophageal reflux disease, it is desirable to reduce the acidic effect of gastric juice.
- Inhibition of H + / K + ATPase (proton pump) in cells of the stomach causes reduction of secretion hydrogen ions.



Proton pump inhibitors

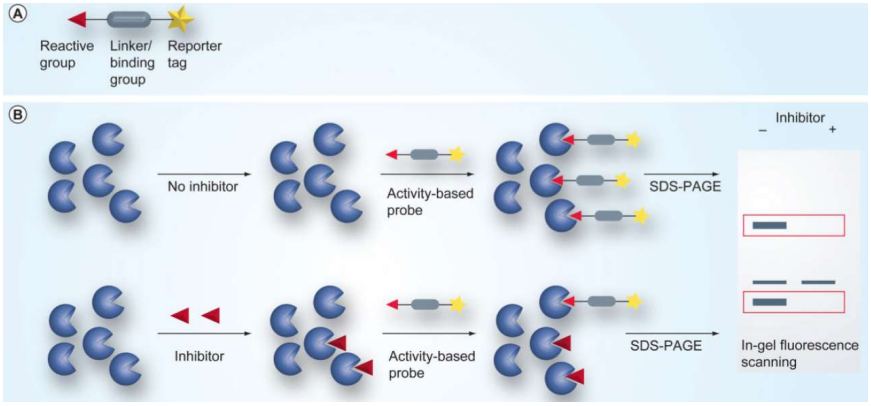
- Omeprazole and its analogues are covalent ATPase inhibitors.



Checking selectivity

- Because of the reactivity of covalent inhibitors, it is necessary to check their selectivity for all enzymes in a given class.
- Tests can be performed using substances that detect activity-based probes.

Checking selectivity



Summary

- Irreversible enzyme inhibitors are an important class of **widely used drugs**.
- Irreversible inhibitors have many advantages over non-equilibrium behavior with respect to the enzyme.
- They can cause difficult to predict **side effects**.