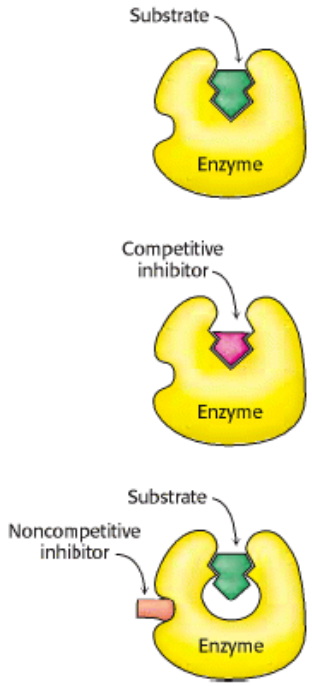
Enzyme Inhibition

1. Enzymes are inhibited by specific molecules/ions
2. Allosteric enzymes regulate enzyme actions by this mechanism.
3. Drugs, toxic agents, transition-state analogues.
4. Two types: Reversible and irreversible.
5. An **irreversible inhibitor** dissociates very slowly from its target enzyme because it has become **tightly bound** to the enzyme, either covalently or noncovalently. Some irreversible inhibitors are important drugs. Penicillin acts by covalently modifying the enzyme transpeptidase, thereby preventing the synthesis of bacterial cell walls and thus killing the bacteria. Aspirin acts by covalently modifying the enzyme cyclooxygenase, reducing the synthesis of inflammatory signals.
6. **Reversible inhibition**, is characterized by a rapid dissociation of the enzyme inhibitor complex.
7. In **competitive inhibition**, an enzyme can bind substrate (forming an ES complex) or inhibitor (EI) but not both (ESI). The competitive inhibitor resembles the substrate and binds to the **active site of the enzyme**. The substrate is thereby prevented from binding to the same active site. *A competitive inhibitor diminishes the rate of catalysis by reducing the proportion of enzyme molecules bound to a substrate*. At any given inhibitor concentration, competitive inhibition can be relieved by increasing the substrate concentration. Under these conditions, the substrate "outcompetes" the inhibitor for the active site. Eg. Methotrexate is a structural analog of tetrahydrofolate, a coenzyme for the enzyme dihydrofolate reductase, which plays a role in the biosynthesis of purines and pyrimidines. It binds to dihydrofolate reductase 1000-fold more tightly than the natural substrate and inhibits nucleotide base synthesis. It is used to treat cancer.
8. In **noncompetitive inhibition**, which also is reversible, the inhibitor and substrate can bind simultaneously to an enzyme molecule **at different binding sites**. A noncompetitive inhibitor acts by decreasing the **turnover number** rather than by diminishing the proportion of enzyme molecules that are bound to substrate. This cannot be overcome by increasing the substrate concentration.
9. **Mixed inhibition:** when a single inhibitor both hinders the binding of substrate and decreases the turnover number of the enzyme.

The **turnover number of an enzyme** (k_{cat} or catalytic rate constant) is the maximal **number** of molecules of substrate converted to product per active site per unit time of several different substrates to different products.



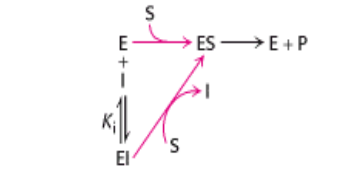
(1) In competitive inhibition, the inhibitor competes with the substrate for the active site. The dissociation constant for the inhibitor is given by $K_i = [E][I]/[EI]$

Because increasing the amount of substrate can overcome the inhibition, V max can be attained in the presence of a competitive inhibitor. The hallmark of competitive inhibition is that it can be overcome by sufficiently:

high concentration of substrate. However, the apparent value of K_M is altered; the effect of a competitive inhibitor is to increase the apparent value of K_M. This new value of K_M, called K_M^{app} = $K_M^{app} = K_M(1 + [I]/K_i)$ where [I] is the concentration of inhibitor and K_i is the dissociation constant for the enzyme-inhibitor complex. As the value of [I] increases, the value of K app M increases. In the presence of a competitive inhibitor, an enzyme will have the same Vmax as in the absence of an inhibitor.

Irreversible inhibitors can be divided into three categories: group-specific reagents, substrate analogs, and suicide inhibitors.

(1) **<u>Group-specific reagents</u>** react with specific R groups of amino acids. Two examples of group-specific reagents are diisopropylphosphofluoridate and iodoacetamide. DIPF modifies only 1 of the 28 serine residues in the proteolytic enzyme chymotrypsin, implying that this serine residue is especially reactive. It is indeed the case that this serine residue is at the active site. DIPF also revealed a reactive serine residue in acetylcholinesterase, an enzyme important in the transmission of nerve impulses. Thus, DIPF and similar compounds that bind and inactivate acetylcholinesterase are potent nerve gases.



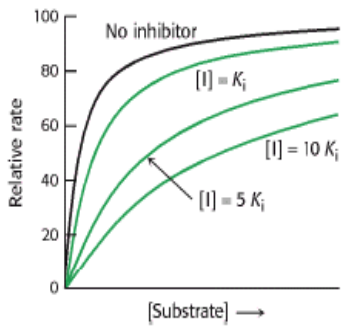


Figure 8.17. Kinetics of a Competitive Inhibitor. As the concentration of a competitive inhibitor increases, higher concentrations of substrate are required to attain a particular reaction velocity. The reaction pathway suggests how sufficiently high concentrations of substrate can completely relieve competitive inhibition.

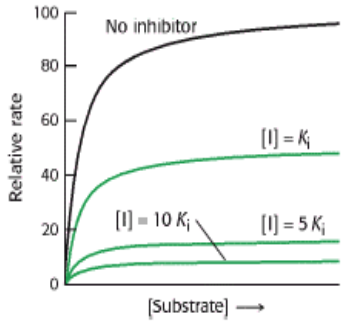


Figure 8.18. Kinetics of a Noncompetitive Inhibitor. The reaction pathway shows that the inhibitor binds both to free enzyme and to enzyme complex. Consequently, V_{max} cannot be attained, even at high substrate concentrations.

Affinity labels are the molecules that are structurally similar to the substrate for the enzyme that covalently modify active site residues. They are thus more specific for the enzyme active site than are group-specific reagents.

Eg. Tosyl-l-phenylalanine chloromethyl ketone (TPCK) is a substrate analog for chymotrypsin. TPCK binds at the active site; and then reacts irreversibly with a histidine residue at that site, inhibiting the enzyme.

Suicide inhibitors, or mechanism-based inhibitors are modified substrates that provide the most specific means to modify an enzyme active site. The **inhibitor binds to the enzyme** as a substrate and is initially processed by the normal catalytic mechanism. The mechanism of catalysis then generates a **chemically reactive intermediate** that inactivates the enzyme through covalent modification. The fact that the enzyme participates in its own irreversible inhibition strongly suggests that the covalently modified group on the enzyme is catalytically vital.

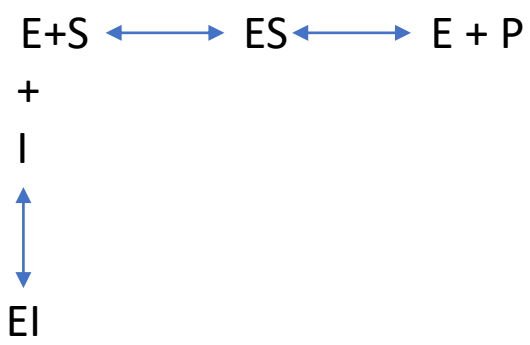
Eg. N,Ndimethylpropargylamine. A flavin prosthetic group of monoamine oxidase (MAO) oxidizes the N,Ndimethylpropargylamine, which in turn inactivates the enzyme by covalently modifying the flavin prosthetic group by alkylating N-5.

Uncompetitive inhibition, also known as **anti-competitive inhibition**, takes place when an enzyme inhibitor binds only to the complex formed between the enzyme and the substrate (the E-S complex) at a site other than active site. Uncompetitive inhibition typically occurs in reactions with two or more substrates or products.

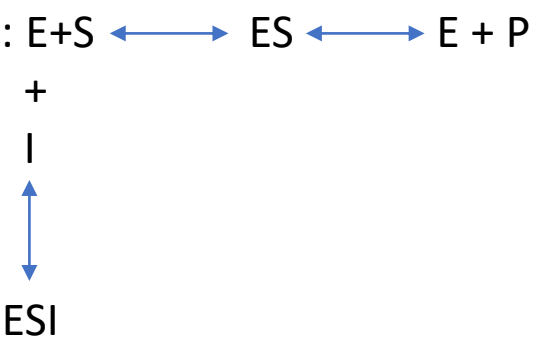
While uncompetitive inhibition requires that an enzyme-substrate complex must be formed, non-competitive inhibition can occur with or without the substrate present.

Uncompetitive inhibition is distinguished from competitive inhibition by two observations: first uncompetitive inhibition cannot be reversed by increasing [S] and second, as shown, the Lineweaver–Burk plot yields parallel rather than intersecting lines. This behavior is found in the inhibition of acetylcholinesterase by tertiary amines (R_3N). Such compounds bind to the enzyme in its various forms, but the acyl-intermediate-amine complex cannot break down into enzyme plus product

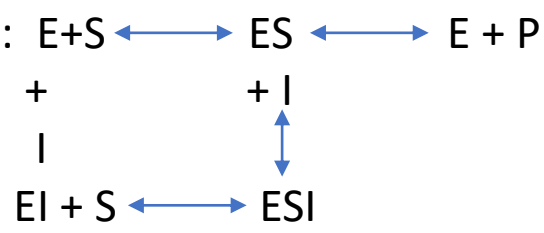
(1) Competitive inhibition:



(2) Uncompetitive inhibition:



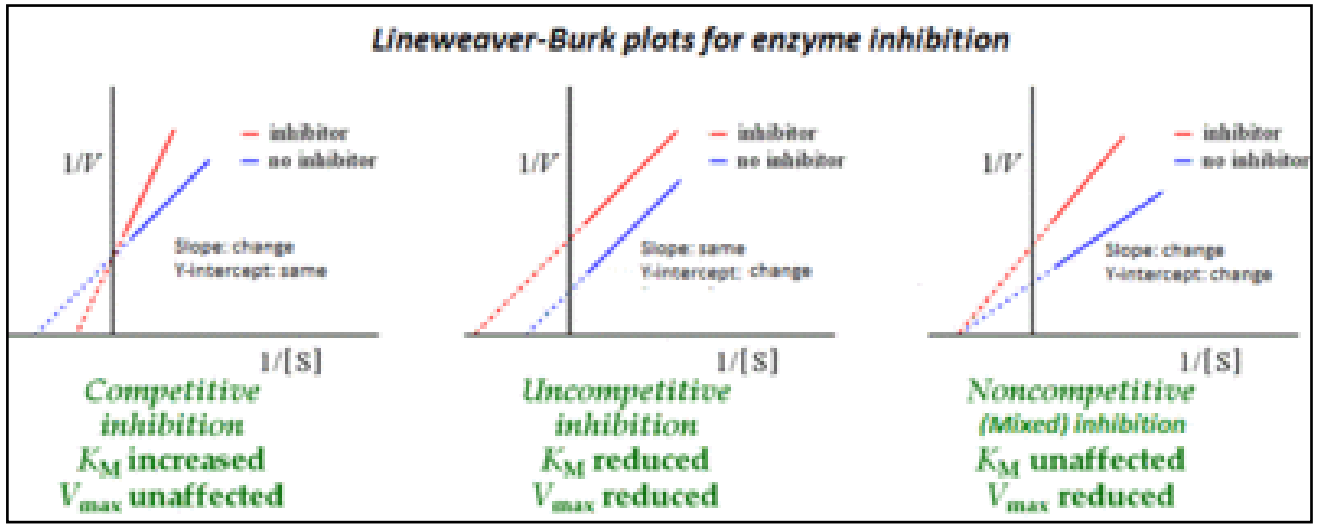
(3) Mixed Inhibition:



Michaelis-Menten Equation		
Competitive Inhibition	$V_0 = \frac{Vmax[S]}{\alpha Km + [S]}$	$\alpha = 1 + [I]/K_I$; $K_I = \frac{[E][I]}{[EI]}$
Uncompetitive inhibition	$V_0 = \frac{Vmax[S]}{Km + \alpha'[S]}$	$\alpha' = 1 + [I]/K'_I$; $K'_I = \frac{[ES][I]}{[ESI]}$
Mixed inhibition	$V_0 = \frac{Vmax[S]}{\alpha Km + \alpha'[S]}$	
Non-competitive inhibition	$V_0 = \frac{Vmax[S]}{\alpha Km + \alpha'[S]}$	Here, $\alpha = \alpha'$

Km is the concentration of substrate which permits the **enzyme** to achieve half Vmax. An **enzyme** with a high **Km** has a low affinity for its substrate, and requires a greater concentration of substrate to achieve Vmax."

Vmax = maximum velocity obtained with increase in substrate concentration and it does not increase any further by increasing the concentration of the substrate.



	Competitive	Uncompetitive	Non-competitive
Vmax	Unaffected	Decrease	Decreases
Km	Increases	Decrease	Unaffected
Turn over no	Unaffected	Affected	Unaffected

Lineweaver-Burk Equation	
Competitive Inhibition	$1/V = \frac{\alpha Km \ 1}{Vmax \ [S]} + 1/Vmax$
Uncompetitive inhibition	$1/V = \frac{Km \ 1}{Vmax \ [S]} + \alpha'/Vmax$
Non-competitive inhibition	$1/V = \frac{\alpha Km \ 1}{Vmax \ [S]} + 1/Vmax$

1. Why Ki is important?
At low concentrations, lowest Ki values will cause the greatest degree of inhibition.

2. Important for drugs.

Enzyme poisons: eg. Iodoacetamide, heavy metal, oxidizing agents

(1) Effect amino acid residues in the enzymes .
(2) Not readily reversed
(3) No structural resemblance with the substrates
(4) Site of chemical attack may or may not be in the active site. If it is on the active site, then substrate/product offers protective effect.

For competitive inhibition

(1) Y-intercept = 1/Vmax
At very high [S], I/S = 0
Vi= same for both inhibition and non-inhibition.
Km varies with [I] and increases and becomes a larger number.
Uninhibition: -1/Km
Comp inh: -1/K'm
-1/K'm < -1/Km
Thus, Km increases in presence of comp inh.

(2) Since Km = substrate concentration at which free [E] = bound [E] of [ES].
X-intercept = 1/ αKm

For non-competitive inhibition

(1) Since [I] and [S] bind at two different regions therefore, [EI] & [ESI] both are formed.
(2) [ESI] breaks down slowly to give [P]
(3) [S] has equal affinity for both [E] and [S].

Examples of irreversible inhibition

1. Penicillin (group of antibiotics, derived originally from common moulds known as Penicillium moulds; discovered by Alexandar Flemming)	Irreversible inhibitor of glycopeptide transpeptidase (glycoprotein peptidase) Binds to serine residue in the active site Glycopeptide transpeptidase: crosslink the peptidoglycan chains during bacterial wall synthesis

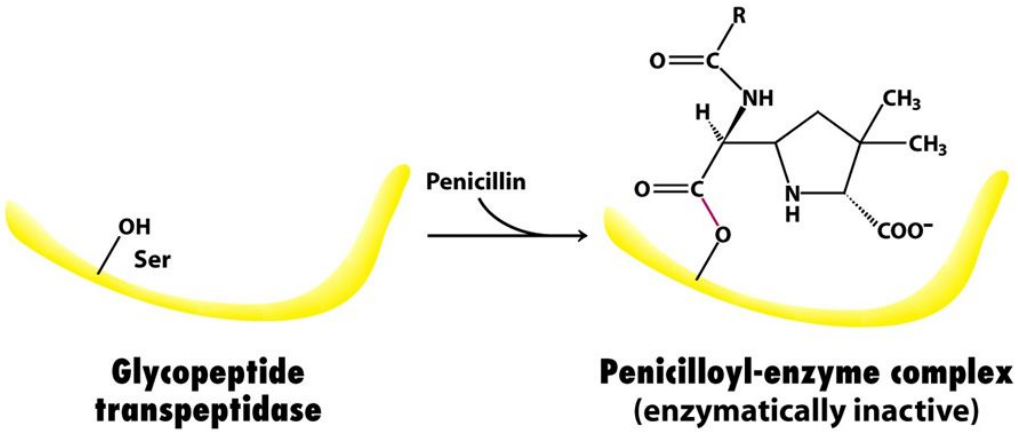
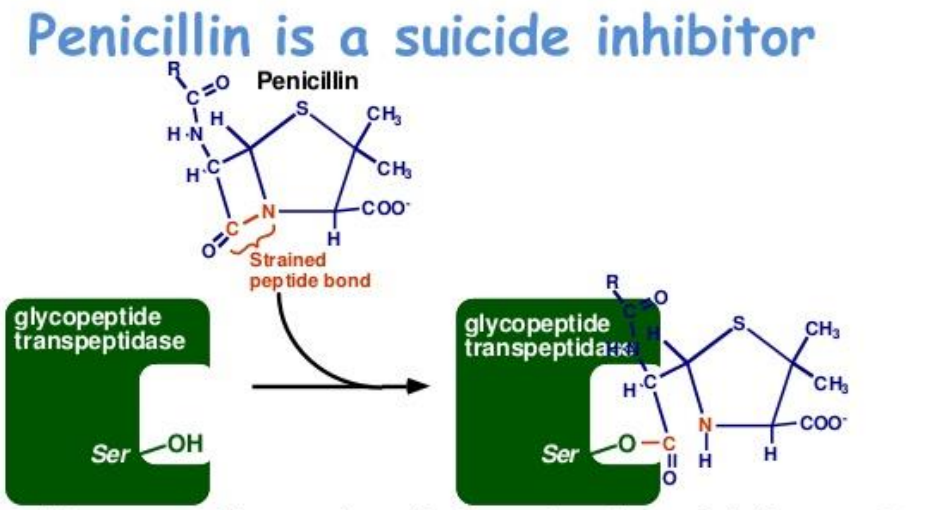


Figure 8-35

-On binding to the transpeptidase, the serine residue at the active site attacks the carbonyl carbon atom of the lactom ring of the penicillin to from the penicilloyl-serine derivative.
-Penicillin acts as a suicide inhibitor.



Glycopeptide transpeptidase catalyzes the formation of cross-links between D-amino acids in the cell walls of bacteria. This enzyme also catalyzes the reverse reaction, the hydrolysis of peptide bonds. During the course of hydrolyzing the strained peptide bond in penicillin, the enzyme activates the inhibitor (penicillin), which then covalently modifies an active site serine in the enzyme. In effect, the enzyme "commits suicide" by hydrolyzing the strained peptide bond in penicillin.

Transpeptidation mechanism

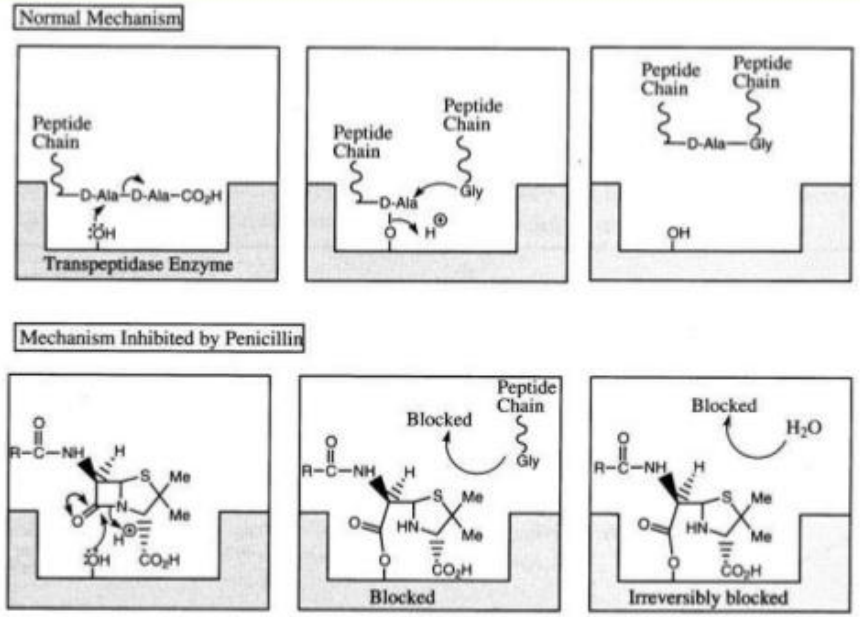
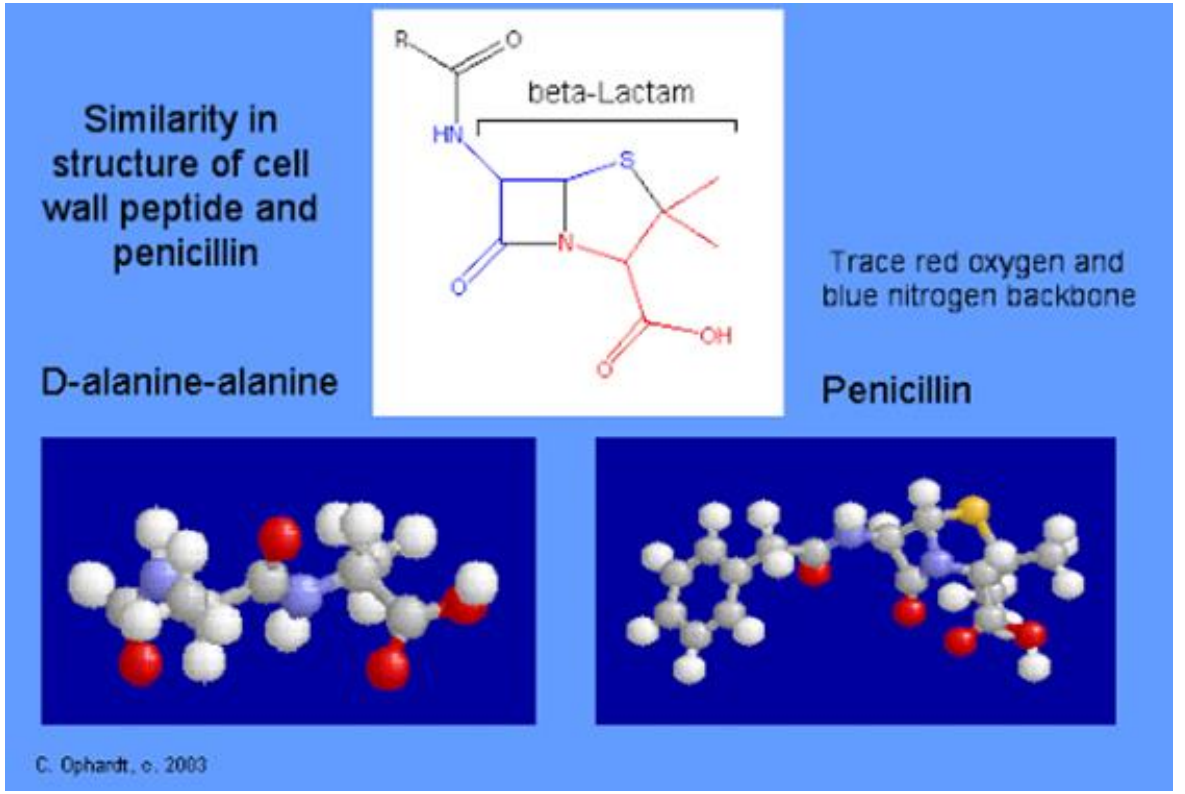
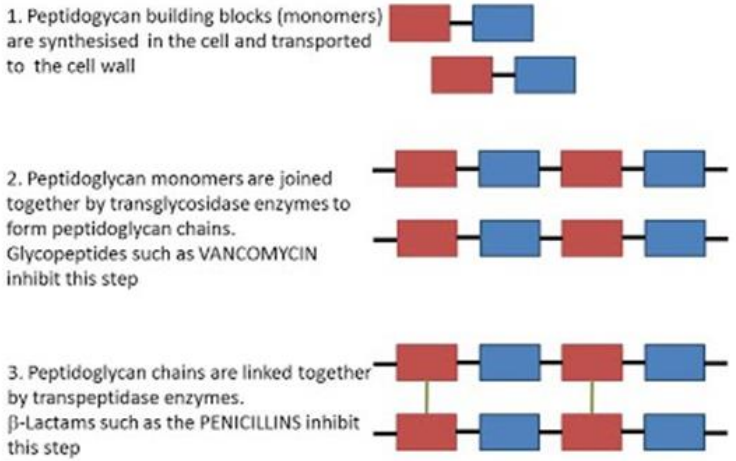
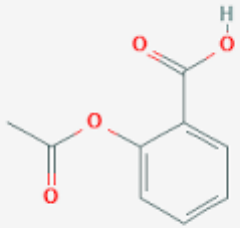


Fig. 14.63 Cross-linking mechanism by transpeptidase enzyme.

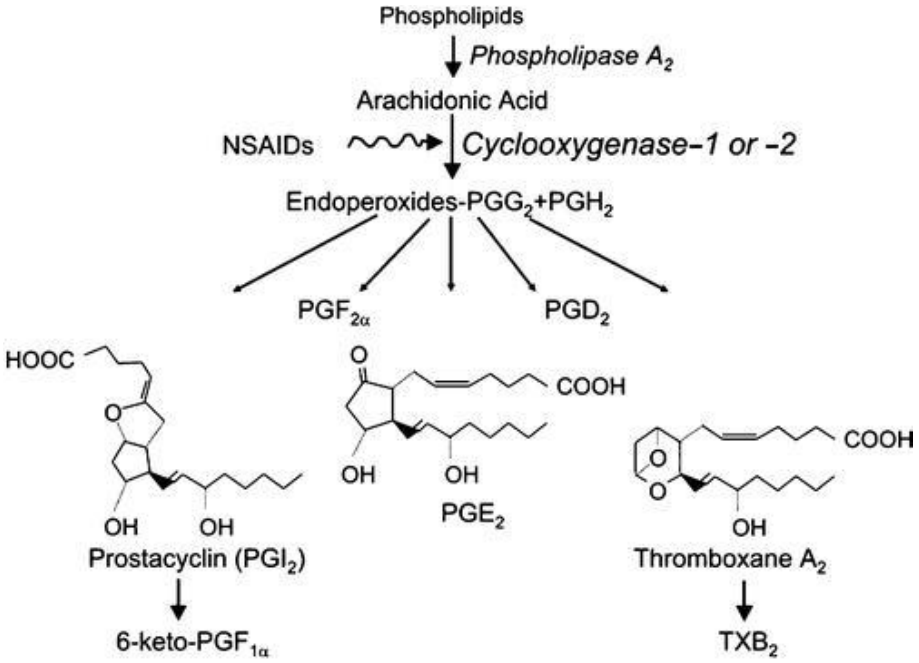




2. Aspirin	Cyclooxegenase: reduces prostaglandin synthesis



Structure of aspirin



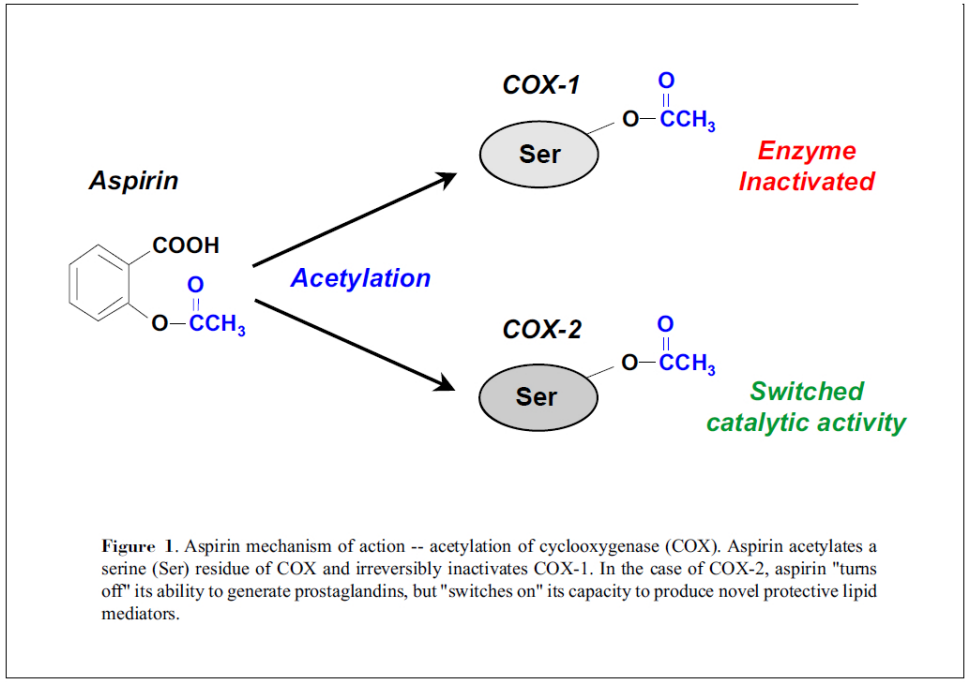
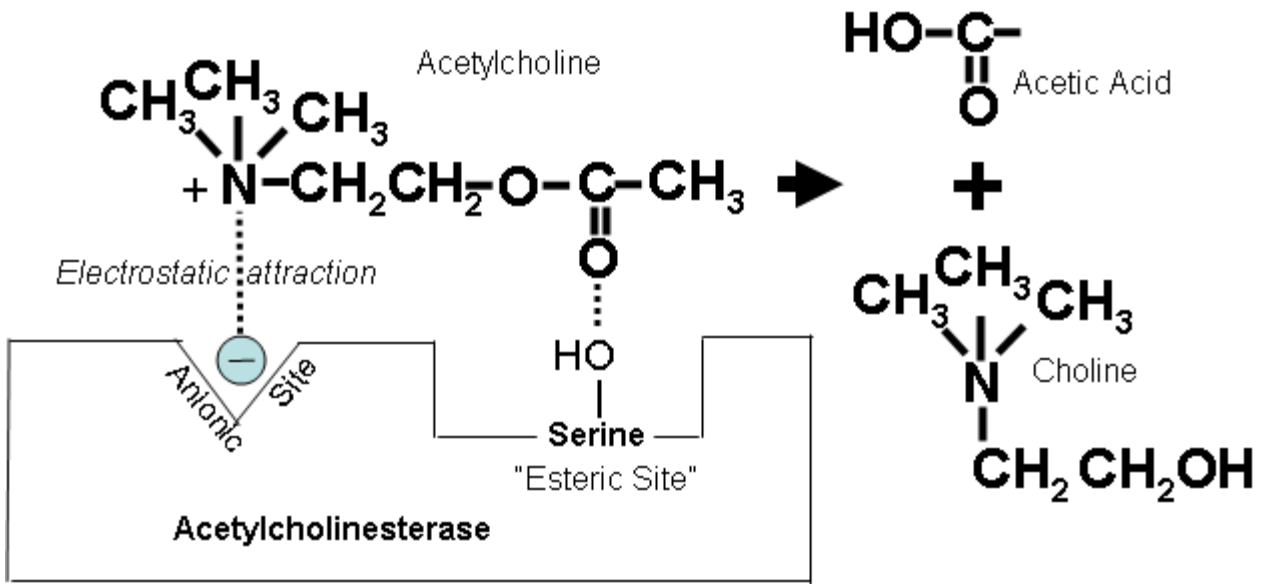
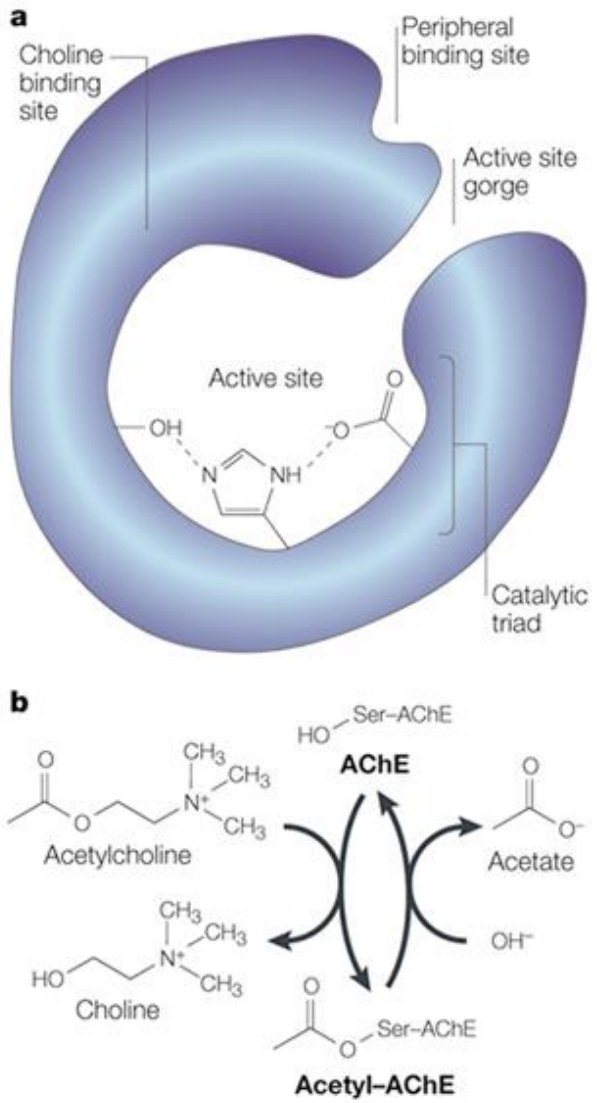


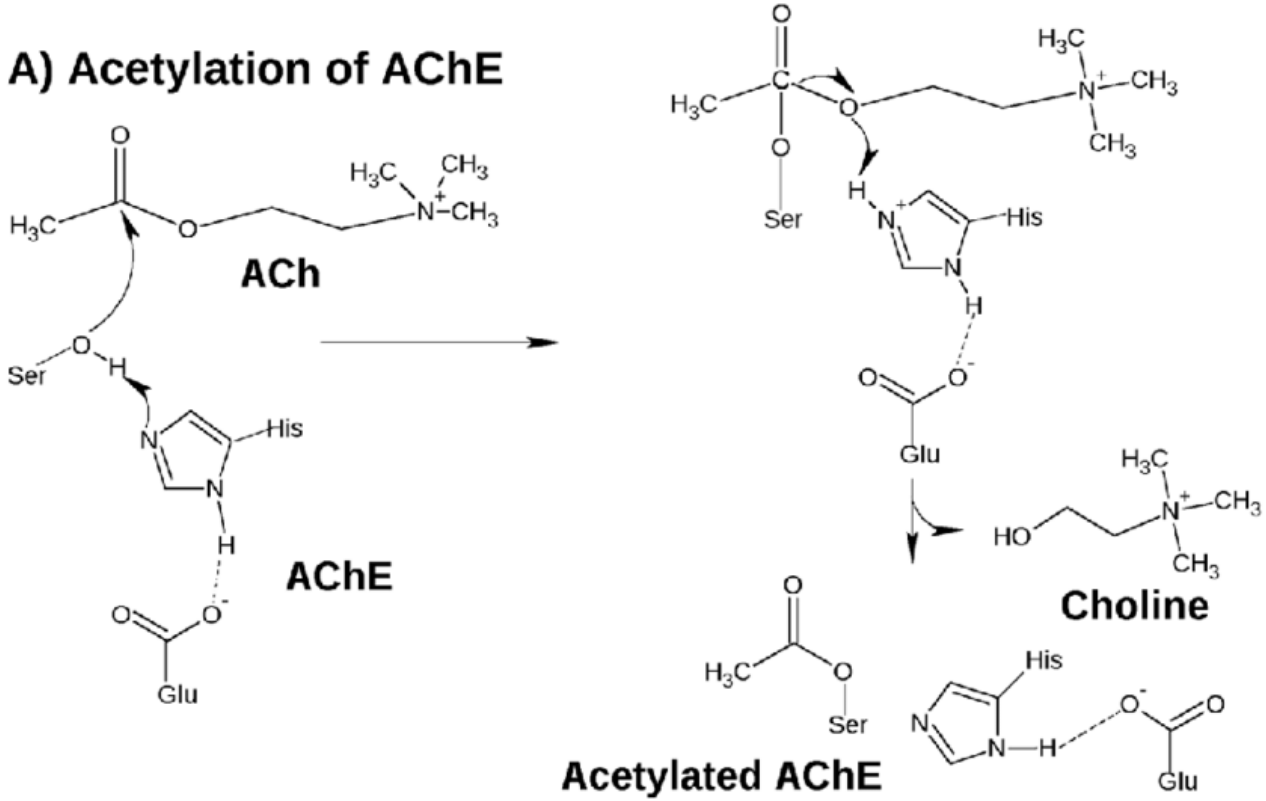
Figure 1. Aspirin mechanism of action -- acetylation of cyclooxygenase (COX). Aspirin acetylates a serine (Ser) residue of COX and irreversibly inactivates COX-1. In the case of COX-2, aspirin "turns off" its ability to generate prostaglandins, but "switches on" its capacity to produce novel protective lipid mediators.

3. Diisopropyl phosphofluoridate	Inhibits ser at active sites of the enzymes (acetylcholinesterase)
4. Sarin (nerve gas)	Same
5. Physostigmine	Same
6. Parathion	Same (insect acetylcholinesterase)

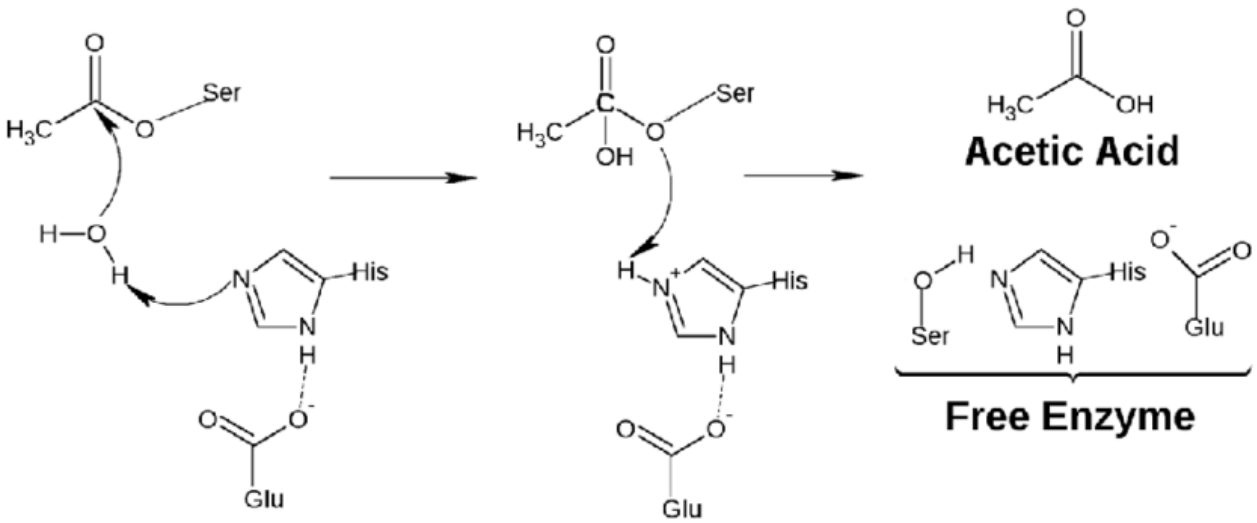


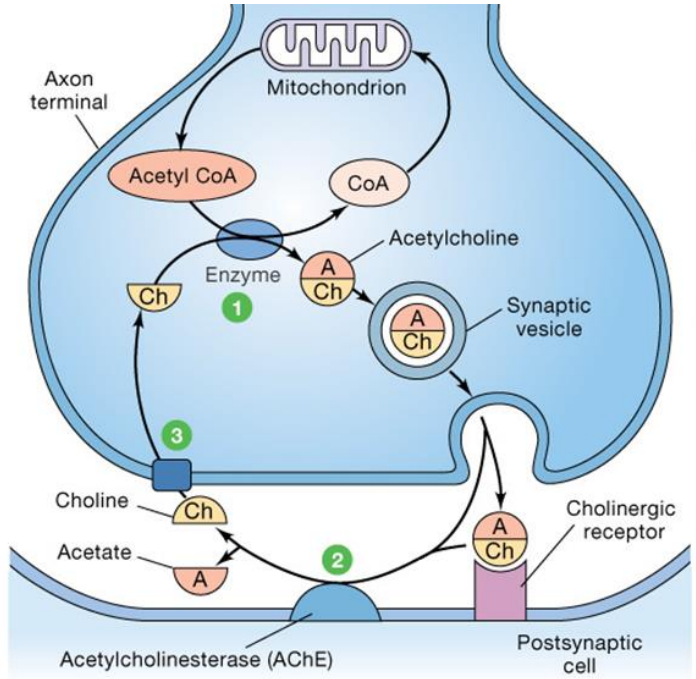


A) Acetylation of AChE



B) Deacetylation of AChE

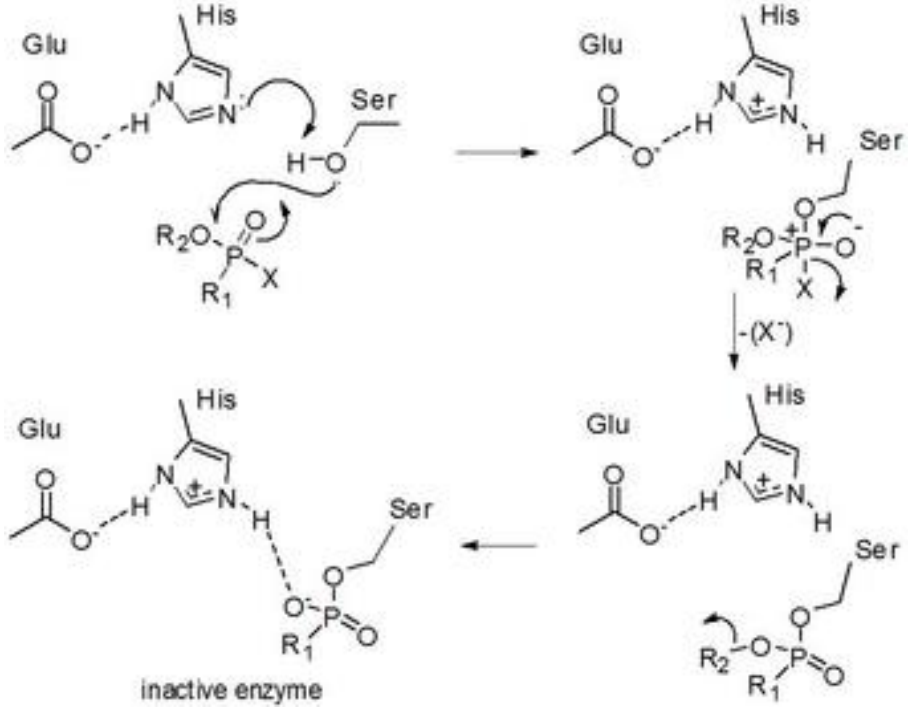




1. **Acetylcholine** (ACh) is made from choline and acetyl CoA.
2. In the synaptic cleft ACh is rapidly broken down by the enzyme **acetylcholinesterase.**
3. Choline is transported back into the axon terminal and is used to make more ACh.

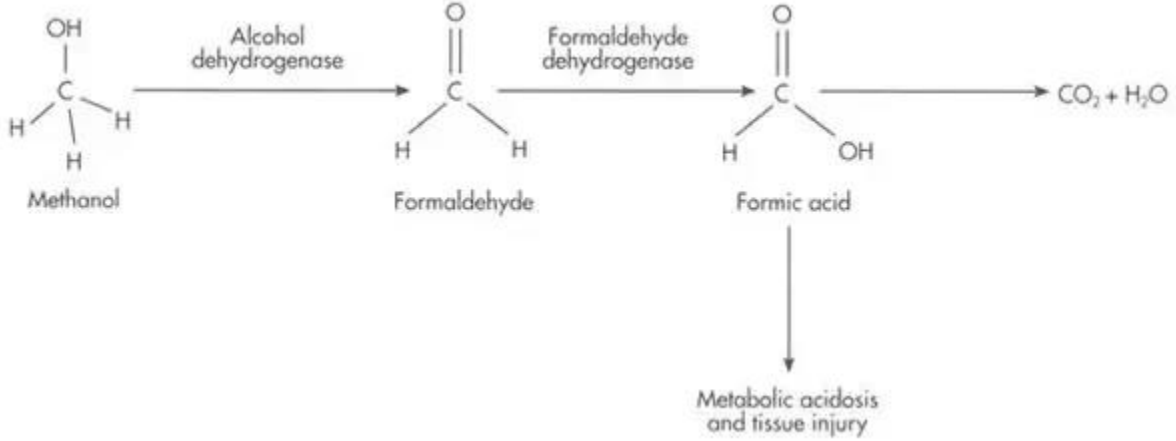
Mechanism of action

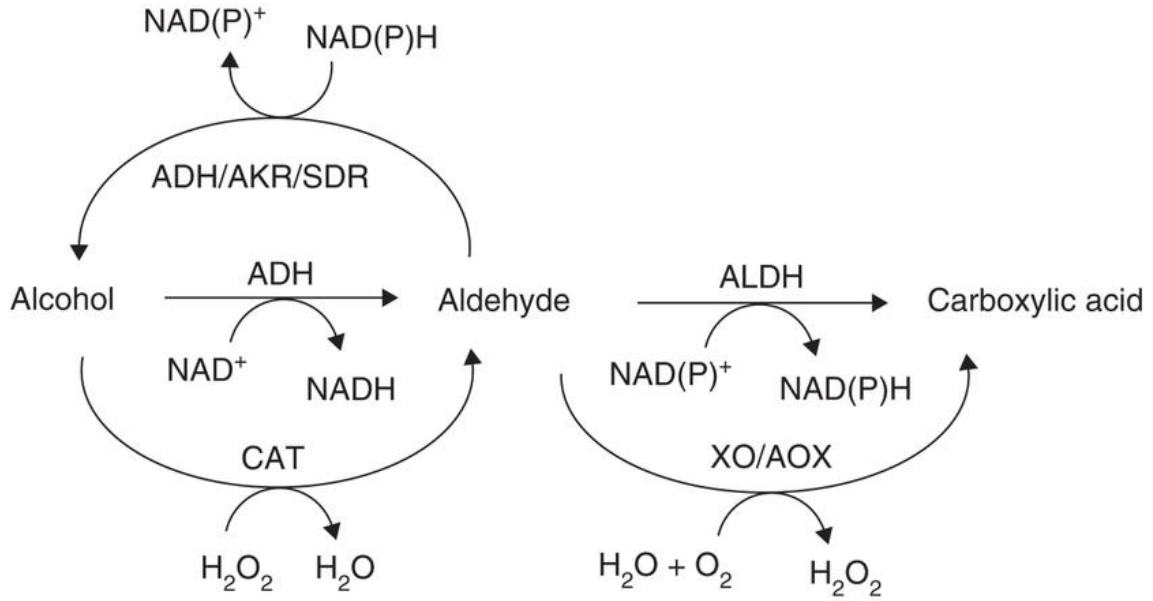
- Acetylated enzyme reacts with water very rapidly and the esteretic site is freed in fraction of milli sec.
  - Carbamylated enzyme reacts slowly (reversible inhibitors)
  - Phosphorylated enzyme reacts extremely slowly or not at all.
- OPPs attaches only to the esteretic site whereas drugs like Tacrine & Endrophonium attaches to the anionic site.

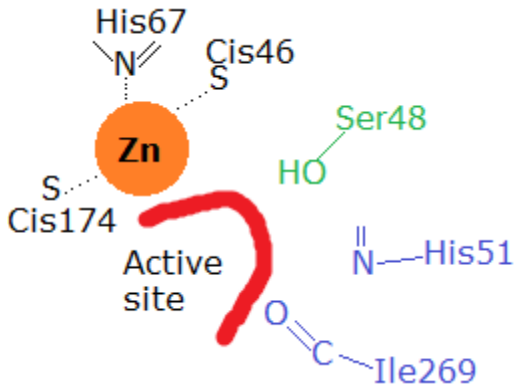


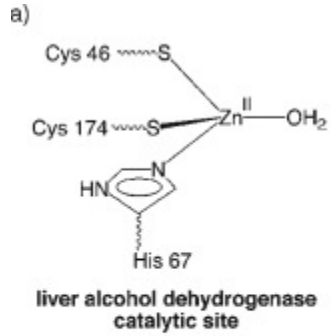
1

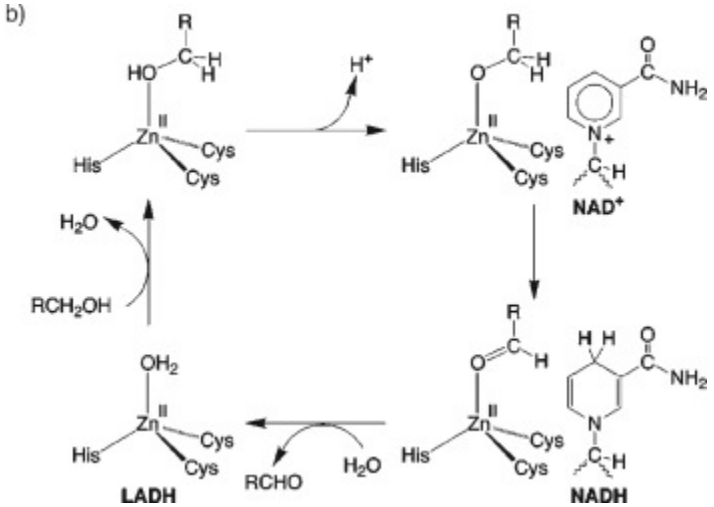
Examples of competitive inhibition

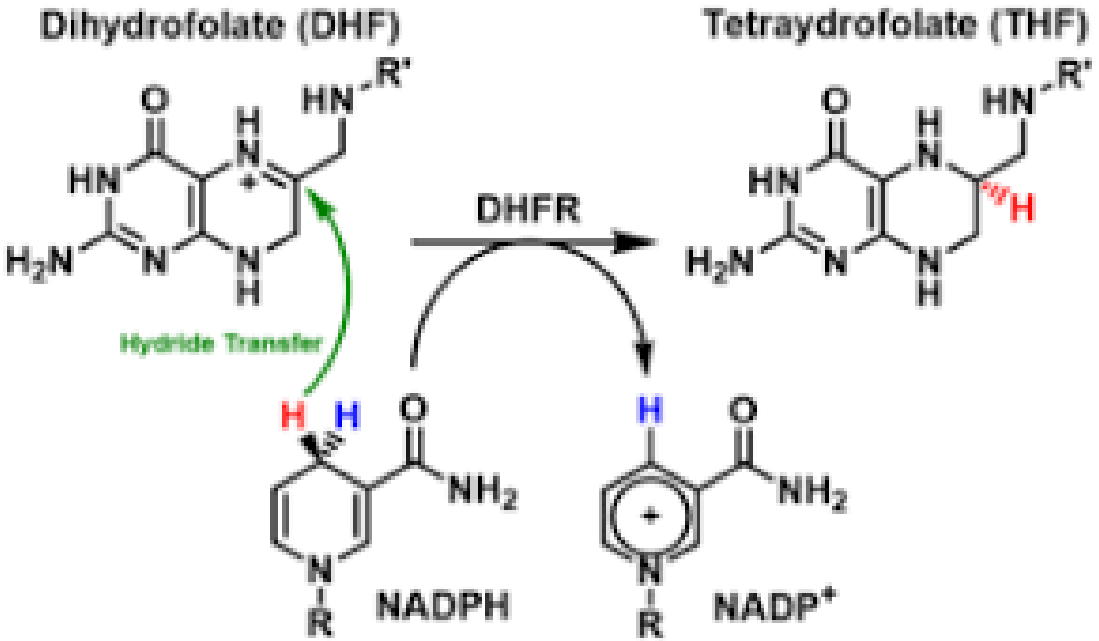


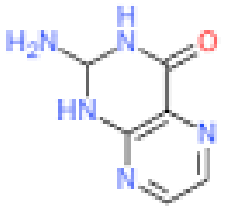


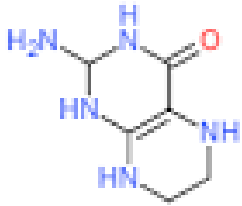






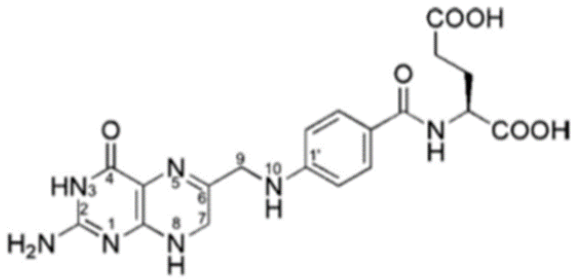




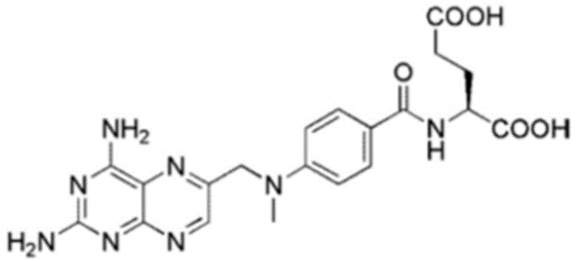


Folic Acid Folate

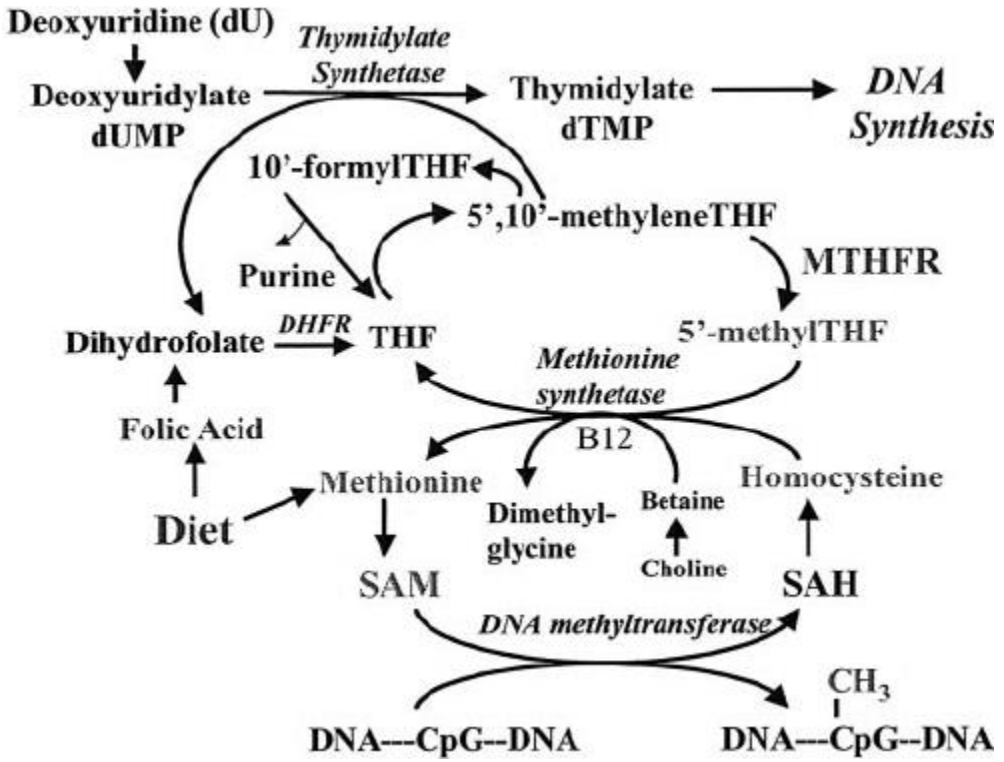
2

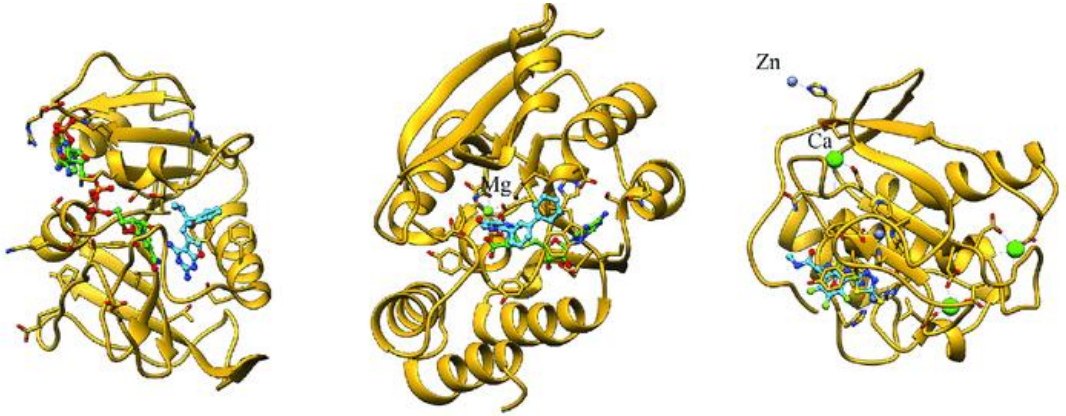


Dihydrofolate (DHF)



Methotrexate (MTX)

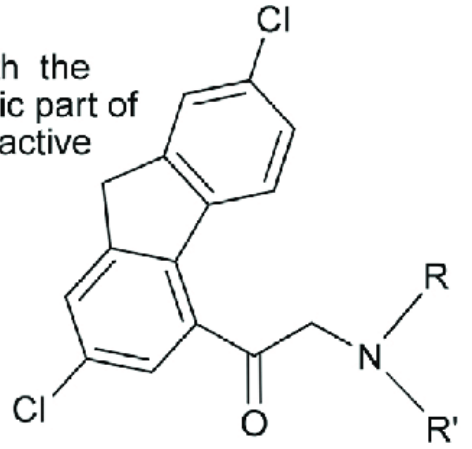




Dihydrofolate Reductase (no-metal) | **Catechol-O-methyltransferase (one-metal)** | **Stromelysin-1 (many-metal)**

A central eight-stranded beta-pleated sheet makes up the main feature of the polypeptide backbone folding of DHFR.[9] Seven of these strands are parallel and the eighth runs antiparallel. Four alpha helices connect successive beta strands. Residues 9 – 24 are termed "Met20" or "loop 1" and, along with other loops, are part of the major subdomain that surround the active site. The active site is situated in the N-terminal half of the sequence, which includes a conserved Pro-Trp dipeptide; the tryptophan has been shown to be involved in the binding of substrate by the enzyme

interact with the hydrophobic part of the DHFR active site



interact with the hydrophobic part of the DHFR active site

interact with the hydrophilic part of the DHFR active site