Enzyme Inhibition and Drug Action

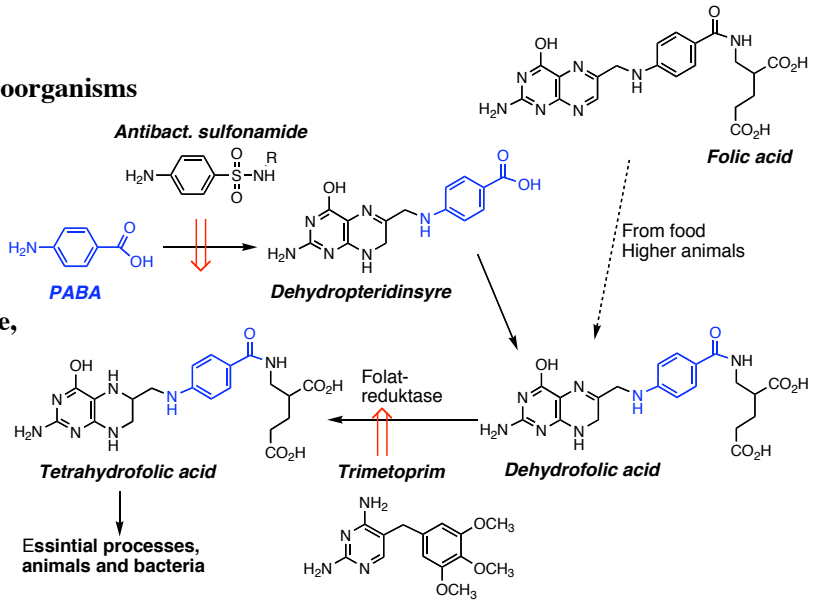
- Malfunction of enzyme
- Introduction of enzyme by microorganism

} Disease

Inhibition of enzyme - Interesting but difficult drug strategy

Inhib. of enzymes from microorganisms

- Enzyme only in microorg.
- Different structure of enzyme, human and microorg



Enzyme inhibition

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [E\text{-}S] \overset{k_3}{\rightleftharpoons} [E\text{-}P] \rightleftharpoons E + P$$

E: Enzyme
S: Substrate
P: Product

Two last steps ≈irreversible, E-S to E-P rate limiting

Reaction velocity, $V=k_3\,[E\text{-}S]$ Rate of form. ES: $k_1[E][S]$
Rate of decomp. ES: $(k_1 + k_3)[E][S]$

Assume steady state ([E-S] doesn't change)

$$k_1[E][S] = (k_1 + k_3)[E][S]$$

$$\Downarrow$$

$$[E\text{-}S] = \frac{[E]\,[S]}{(k_2 + k_3)\,/\,k_1}$$ Michaelis const.: $K_M = (k_2 + k_3) / k_1$

$$\Downarrow$$

$$[E\text{-}S] = \frac{[E]\,[S]}{K_M}$$ $[E] = [E_{tot}] - [E\text{-}S]$

$$\Downarrow$$

$$[E\text{-}S] = \frac{([E_{tot}] - [E\text{-}S]\,[S]}{K_M} \Longrightarrow [E\text{-}S] = \frac{[E_{tot}]\,[S]}{[S] + K_M}$$

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [E\text{-}S] \overset{k_3}{\rightleftharpoons} [E\text{-}P] \rightleftharpoons E + P$$

E: Enzyme
S: Substrate
P: Product

$$[E\text{-}S] = \frac{[E_{tot}]\,[S]}{[S] + K_M} \qquad V = k_3\,[E\text{-}S]$$

$$\Downarrow$$

$$V = \frac{k_3[E_{tot}]\,[S]}{[S] + K_M}$$

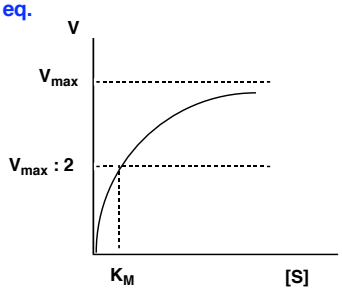
V_{max}: All enzyme sites occupied by S

$[S] \gg K_M$, $\frac{[S]}{[S] + K_M} \approx 1$ $\quad V_{max} = k_3\,[E_{tot}]$

$$\Downarrow$$

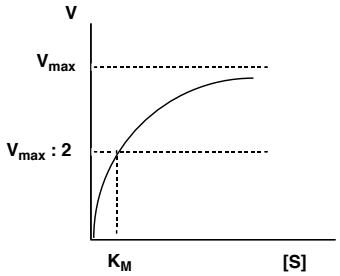
$$V = \frac{V_{max}\,[S]}{[S] + K_M}$$

Michaelis Menten eq.



$$V = \frac{V_{max}\,[S]}{[S] + K_M}$$

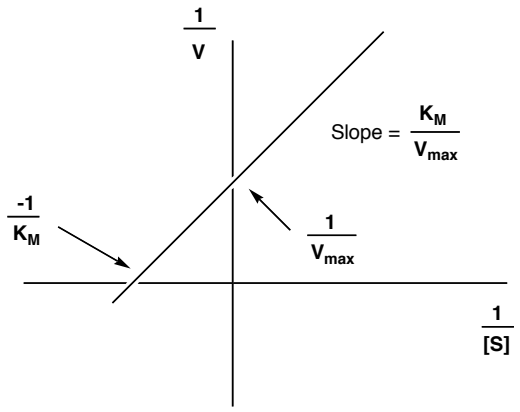
Michaelis Menten eq.



$$\Downarrow$$

$$\frac{1}{V} = \frac{K_M}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

Lineweaver-Burk eq.

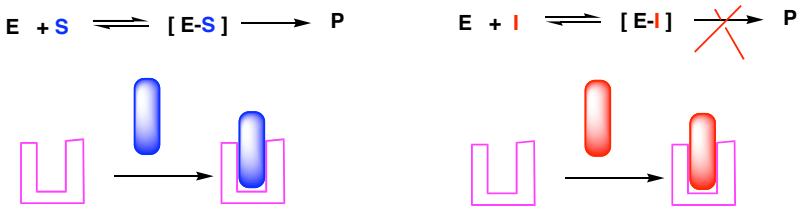


Measure rate at different [S]:
Determine K_M and V_{max}

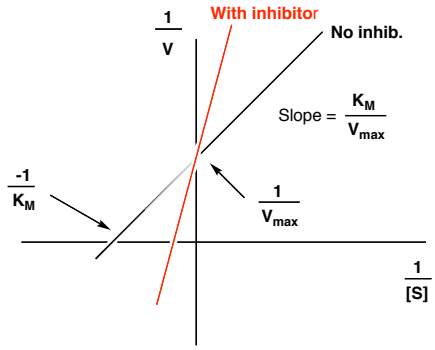
Reversible and irreversible enzyme inhibitors

Reversible inhibition
- **Competitive**
- **Non-competitive**

If covalently bond to enzyme, bond relatively easily be broken i.e. hydrol. of ester

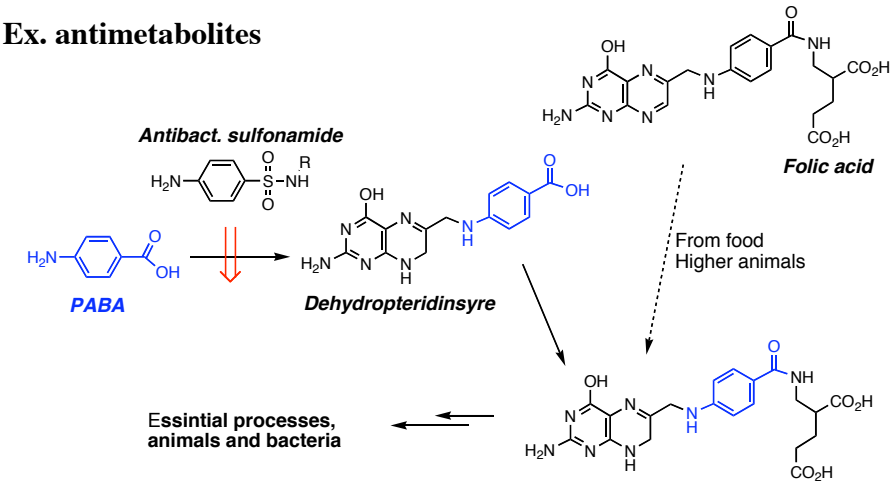


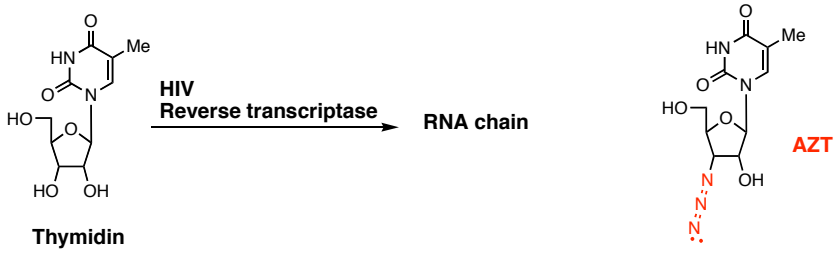
Binding to the same site
Inhib. can be reversed by increasing [S]
V_{max} unchanged
K_M increase



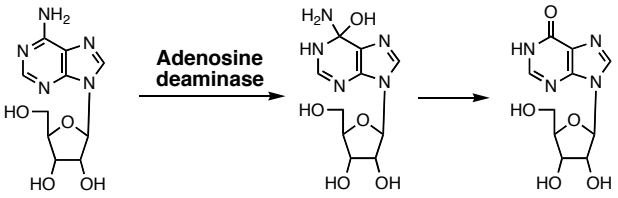
Structural resemblance S and I
Designed I drugs - Antimetabolites

Ex. antimetabolites



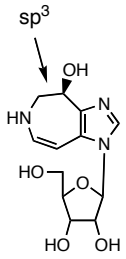


Ex. transition state analogs



Adenine → Inosine

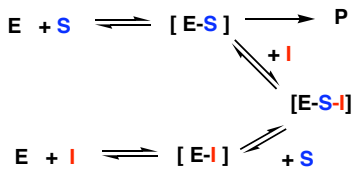
Also metab. of anticancer / antiviral adenine deriv.

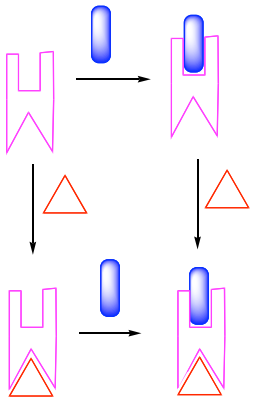


Coformycin

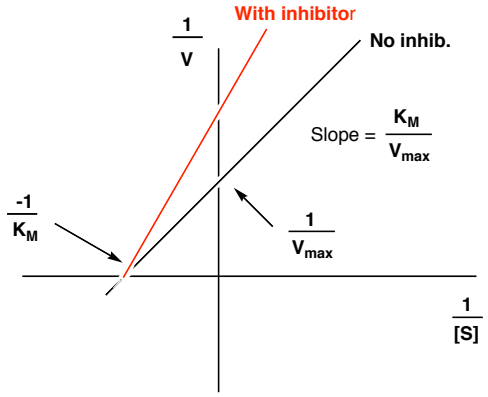
Reversible inhibition

- **Competitive**
- **Non-competitive**





Binding to different sites
Inhib. can be reversed by increasing [S]
V_{max} decrease
K_M unchanged



Inhib. and substrate very different structures
Difficult to design inhib.

Irreversible enzyme inhibitors

E + I ⟶ [E-I]

Often covalent bonds between E and I
Enzyme is permanently modified and inactivated

- **Affinity labels and active site directed irreversible inhibitors**
- **Mechanism based irreversible enzyme inactivators**

Structural recemblance with substrate

Electrophilic - alkylate nucleophilic subst in enzyme active site

Low selectivity - generally highly toxic

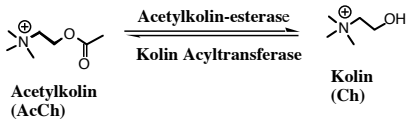
From last chapter

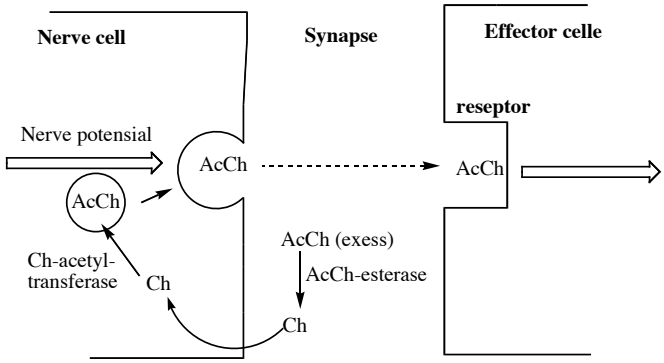
Binding of ligand to receptor

- **Covalent bond**
- **Ionic bond**
- **Hydrogen bond**
- **Hydrophobic interaction**

Covalent bond
strong; 50-150 kcal/mol,
Normally irreversible bonding

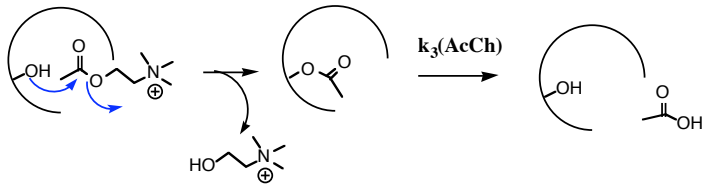
ex. Acetylcholine esterase (enzyme) inhibitors



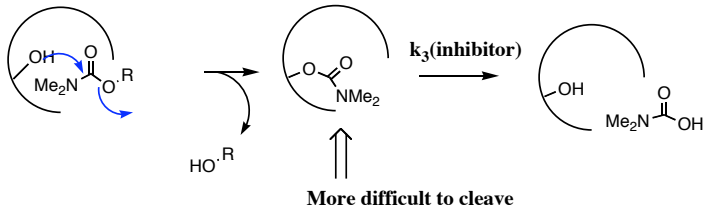


Reversible inhibitors

AcCh



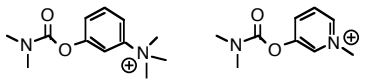
Inhibitor



More difficult to cleave

Reversible inhibitor (drugs): k_3 (inhib) < k_3(AcCh)

Neostigmin ***Pyridostigmin***



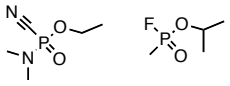
Myastenia gravis (weak muscles, reduced sensitivity to Acetylcholine)

Irreversible Inhibitors

Not drugs, nerve gasses, insecticides etc.

Gen structur mustard gasses

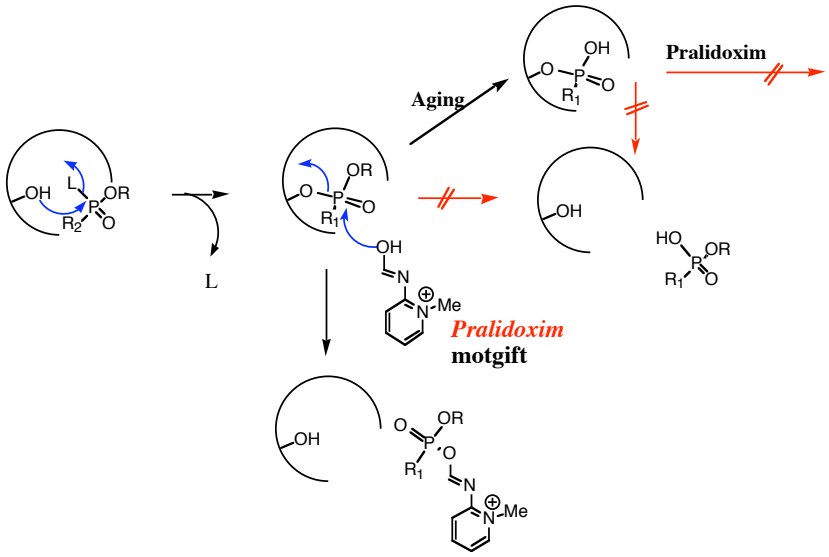
L: Leaving group
R1: alkoksy
R2: alkyl, alkoksy, amino



Tabun *Sarin*

Malation ***Malaoxon***

not tox. **Act as mustard gasses**



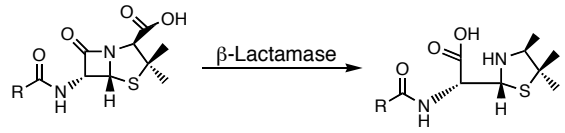
Pralidoxim
motgift

•**Affinity labels and active site directed irreversible inhibitors**

•**Mechanism based irreversible enzyme inactivators**

Suicide substrate - kcat inhibitors - Trojan horse inhib. - latent alkylating agent
≈ Pro-drug, must be activated by the enzyme

Penicillins are cleaved by β-lactamase



Clavulanic acid irreversibly inhibits β-lactamase