STUDY MATERIAL

VIVEKANANDA COLLEGE THAKURPUKUR

NAAC ACCREDITED GRADE—'A'

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Subject: ENZYMOLOGY
Topic: Enzyme Inhibition

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Enzyme Inhibitor

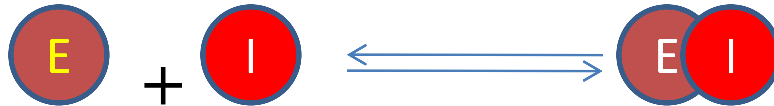
Enzyme Inhibitor:

- ❖ An Enzyme inhibitor is a compound that decreases or tends to decrease the rate of an enzyme catalyzed reaction by influencing the binding of S and /or its turnover number.

Reversible Inhibition

- weak non-covelent Inhibitor binds to Enzyme reversibly through weak non-covelent interactions

- An Equilibrium is established between the free inhibitor & EI Complex and is defined by an equilibrium constant (Ki)



- The activity of Enzyme Is fully restored on removing the Inhibitor by dialysis.

- Reversible Inhibitors depending on concentration of E, S and I, show a definite degree of inhibition which is reached fairly rapidly and remains constant when initial velocity studies are carried out. constant when initial velocity studies are carried out.

Irreversible Inhibition

- Inhibitor binds at or near the active site of the enzyme irreversibly, usually by covalent bonds, so it can't dissociate from the enzyme.

- No equilibrium exits.

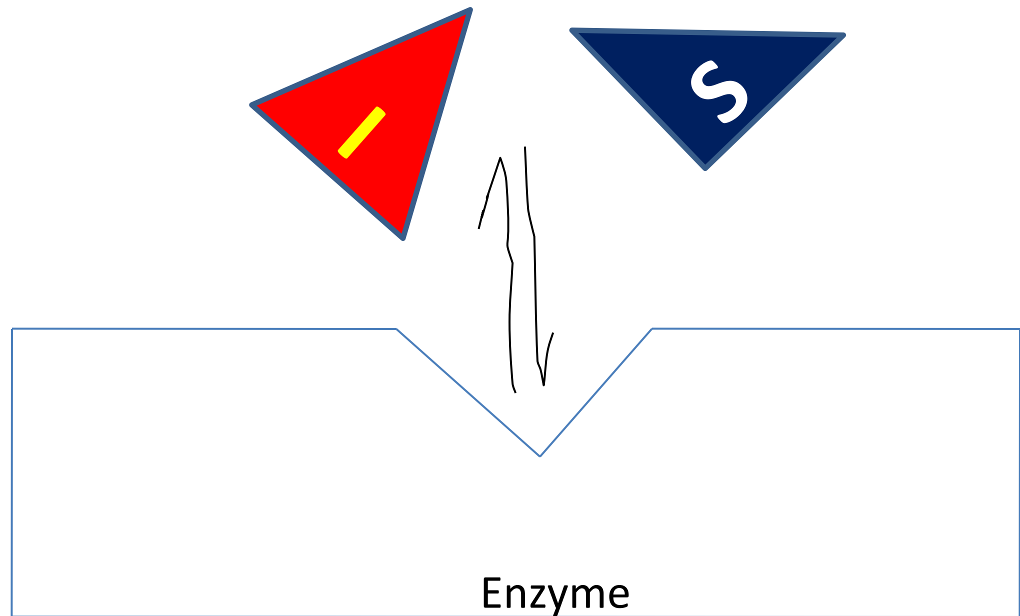
- Enzyme activity is not regained on dialysis.

- Effectiveness of I is expressed not by equilibrium constant but by a velocity constant, which determines the fraction of the enzyme inhibited in a given period of time by a certain concentration of the I

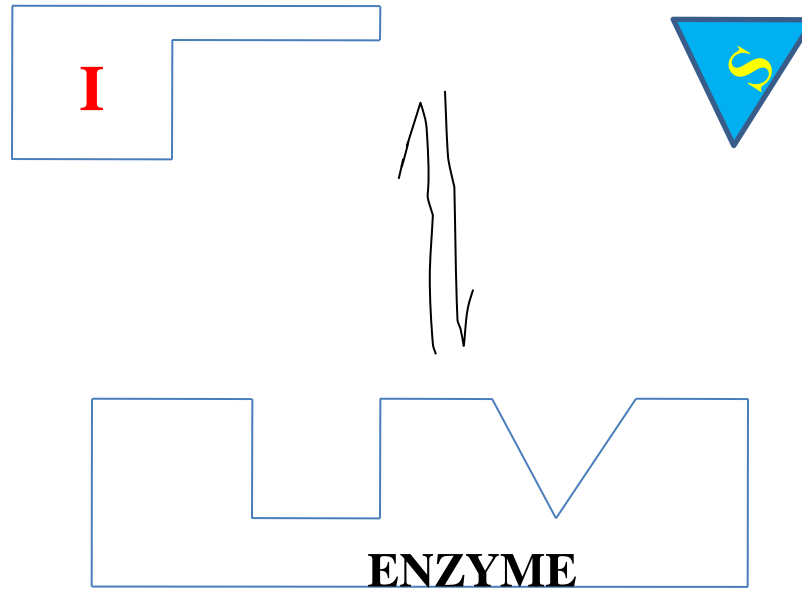
- A competitive I combines with the free enzyme to form an EI complex in a manner that prevents S binding.
- Binding of S & I is mutually exclusive.
- Inhibition can be reversed by increasing the concentration of S at a constant [I].
- Degree of inhibition will depend on the concentrations of S & I and on the relative affinities of the enzyme for S & I

Binding of S & I in different Situations

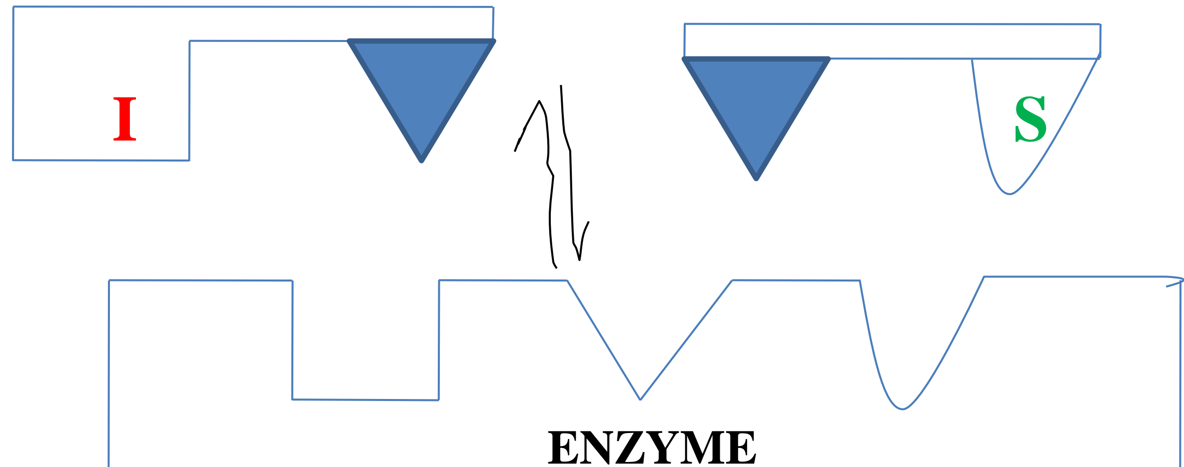
1. Classical Competitive Inhibition (S & I compete for the same binding site)



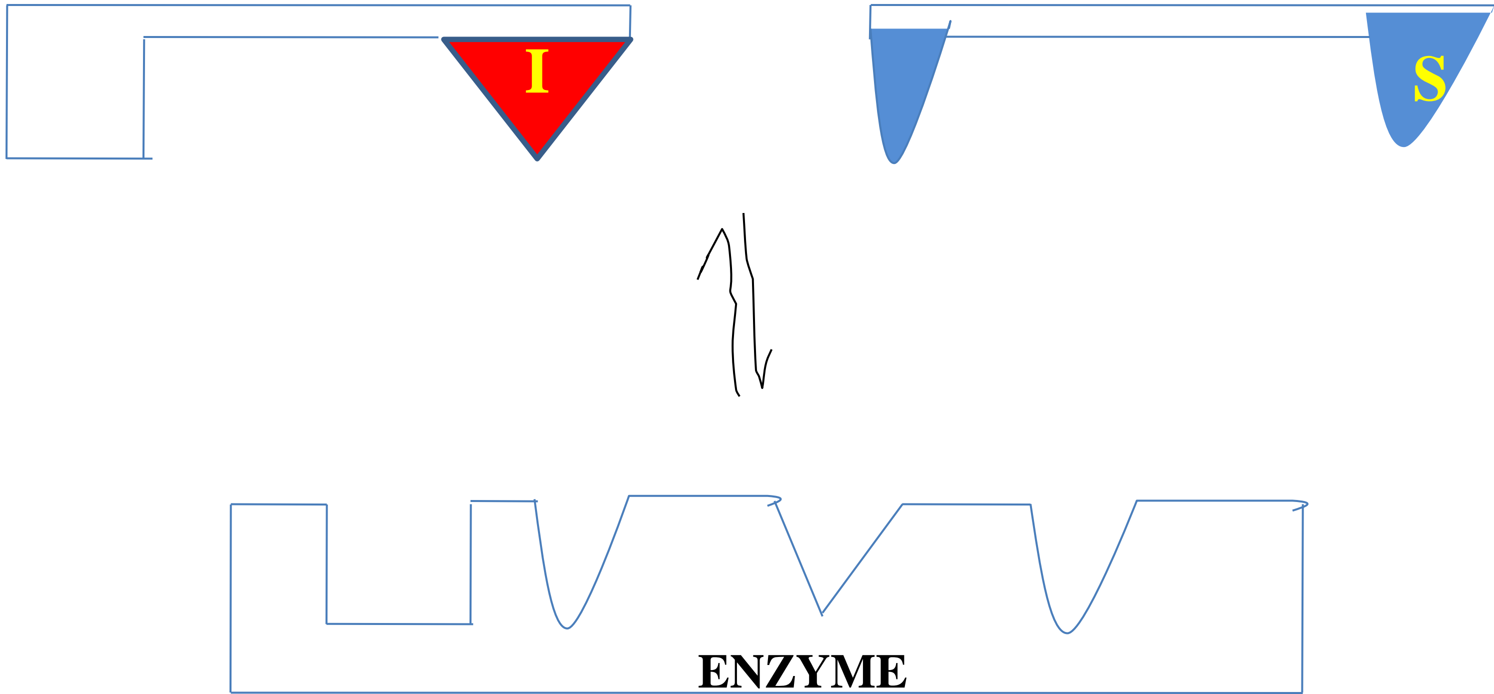
2. S & I are mutually exclusive because of steric hindrance.



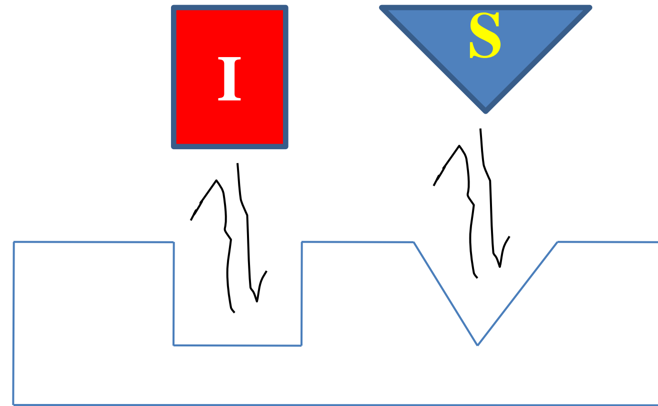
3. S & I have a common binding group on the enzyme.

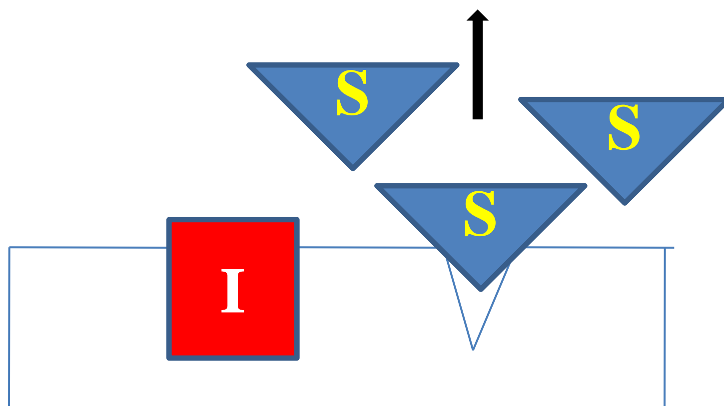


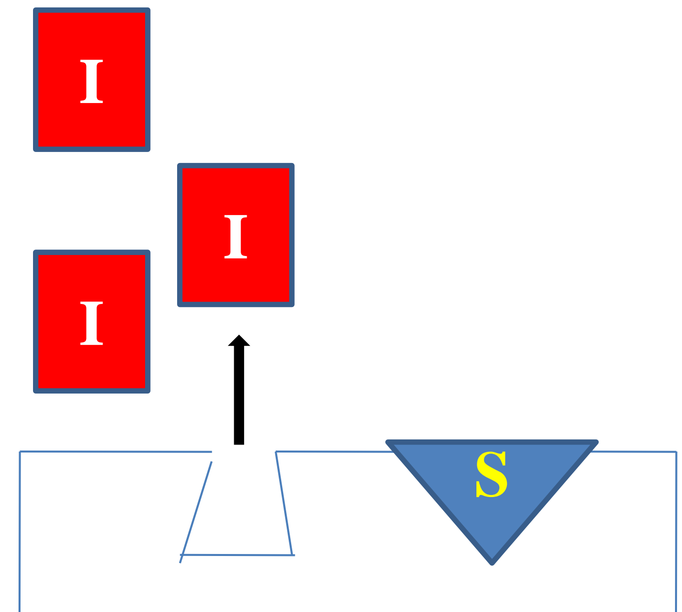
4. The binding sites for S & I are distinct but overlapping



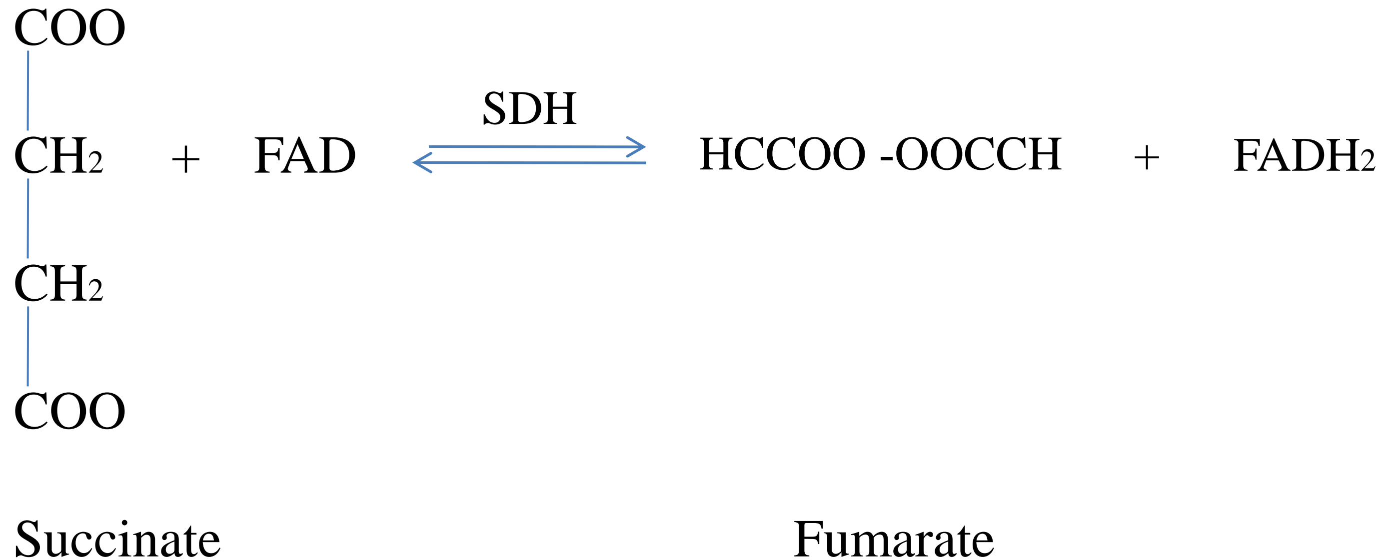
5. Binding of I to a distinct inhibitor site causes a conformational change in the enzyme that distorts conformational change in the enzyme that distorts or masks the S binding site or vice versa.







Examples for Competitive Inhibition



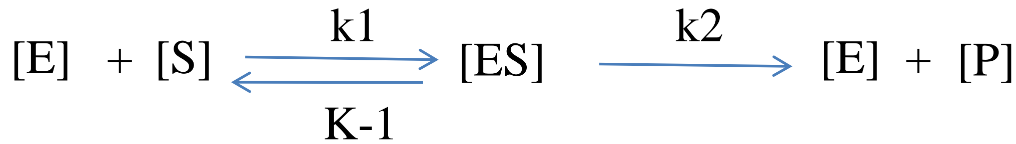
Succinate Fumarate

Malonate is a competitive inhibitor of SDH.

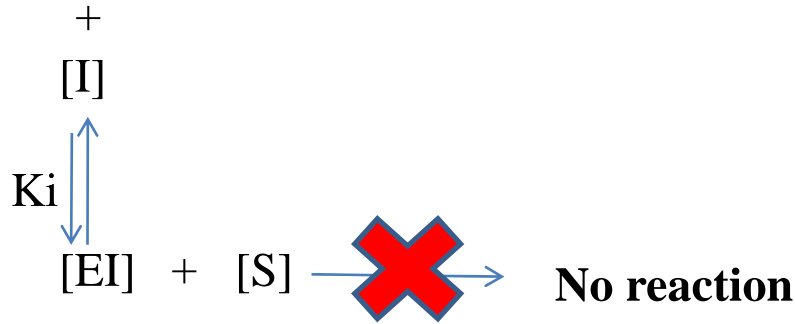
ii) Cometitive inhibition accounts for the antibacterial action of sulfanilamide which is a structural analog of PABA.

Sulfanilamide inhibits the bacterial enzyme dihydropteroate synthetase which catalyzes the incorporation of PABA into 7,8-dihydropteroic acid.

Derivation of velocity equation



$$[E] + [S] \underset{K_{-1}}{\overset{k_1}{\rightleftharpoons}} [ES] \xrightarrow{k_2} [E] + [P]$$



$$+ \; [I] \underset{}{\overset{K_i}{\updownarrow}} [EI] + [S] \rightarrow$$ **No reaction**

$$K_i = \frac{[E]\,[I]}{[EI]}$$

$$\text{Or, } [EI] = \frac{[E]\,[I]}{K_i}$$

In the steady state assumption In the steady state assumption $\frac{[E]\,[S]}{[ES]} = \frac{k_{-1} + k_2}{k_1} = \mathbf{Km}$

$$[ES] = \frac{[E]\,[S]}{\mathbf{Km}}$$

$V = K_2\,[ES]$, $V_{max} = K_2\,[E]_T$

Now, $[E]_T = [E] + [ES] + [EI]$

$V_{max} = K_2\,([E] + [ES] + [EI])$

$$V_{max} = k_2 ([E] + [ES] + [EI])$$

$$\frac{v}{V_{max}} = \frac{k_2 [ES]}{k_2 ([E] + [ES] + [EI])} = \frac{[ES]}{[E] + [ES] + [EI]}$$

Putting the value of [ES] and [EI}

$$\frac{v}{V_{max}} = \frac{\frac{[E] [S]}{Km}}{[E] + \frac{[E] [S]}{Km} + \frac{[E] [I]}{K_i}}$$

$$\frac{v}{V_{max}} = \frac{\frac{[S]}{Km}}{1 + \frac{[S]}{Km} + \frac{[I]}{K_i}}$$

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Multiplying by k_m both in the numerator and the denominator

$$\frac{v}{V_{max}} = \frac{[S]}{K_m + [S] + \frac{[I]}{Ki} K_m}$$

$$\frac{v}{V_{max}} = \frac{[S]}{K_m \left(1+\frac{[I]}{Ki}\right) + [S]}$$

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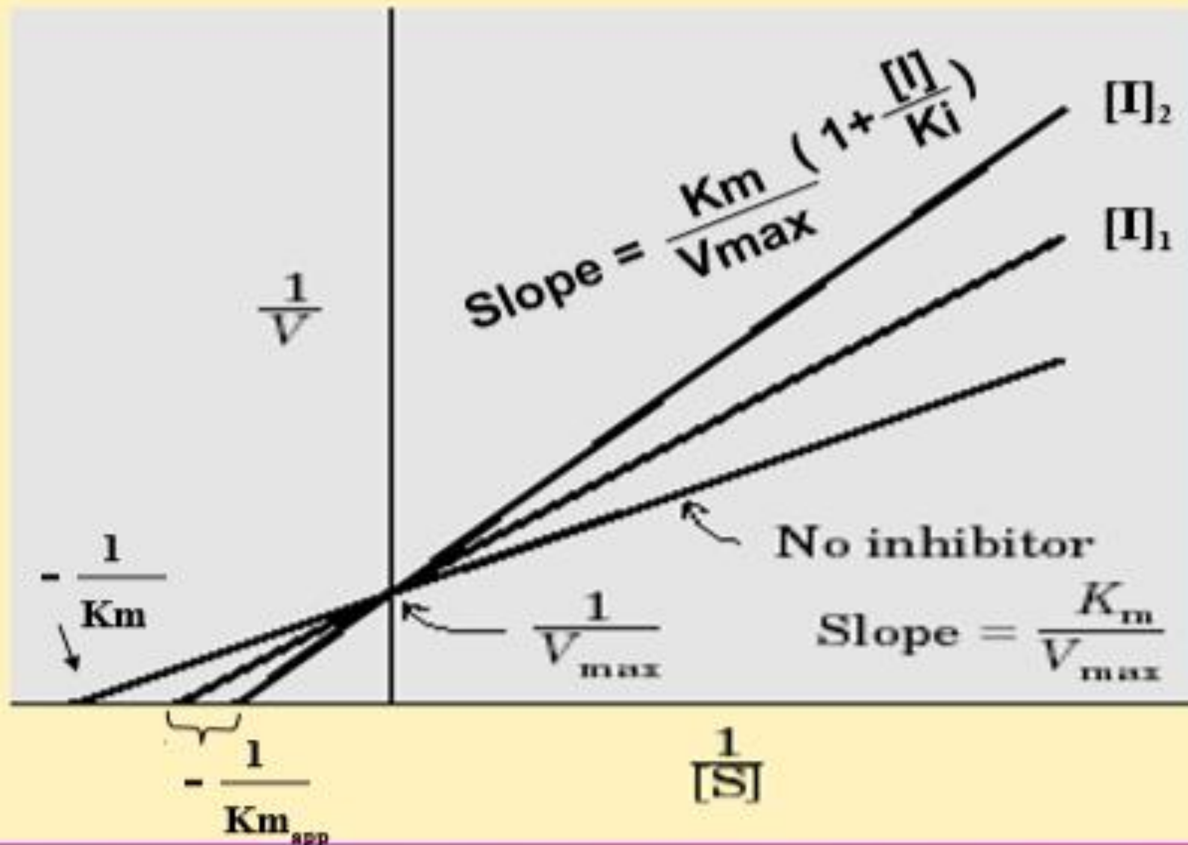
Lineweaver Burk plot

$$\frac{1}{v} = \frac{Km}{Vmax}\left(1+\frac{[I]}{Ki}\right)\frac{1}{[S]} + \frac{1}{Vmax}$$



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Non-competitive Inhibition

- An inhibitor that binds to an enzyme to form a dead end complex, whether or not the active site is occupied by a substrate is termed as a substrate is termed as a Non-Competitive Inhibitor.
- Can bind either to E or ES complex
- Since I doesn't bear structural resemblance to the S, it must bind to the enzyme at a site distinct from the S binding site
- The presence of I does not affect S bonding but does interfere with the catalytic functioning of the enzyme
- The binding of I often deforms the E so that it doesn't form ES complex at a normal rate and once formed, ES complex doesn't decompose at to yield products
- A Non-Competitive I doesn't affect the Km because the binding of I does not block S binding or vice-versa
- I effectively lowers the concentration of active enzyme and hence decreases the apparent V hence decreases the apparent Vmax
- since there is no competition between S & I, the inhibition is not reversed by increasing the [S]

Examples for Non- Competitive Inhibition

1. Enzymes requiring divalent metal ions (e.g. Mg^{2+} & Ca^{2+} etc) for their etc) for their activity are inhibited non-competitively by chelating agents like EDTA which removes metal ions from the enzyme

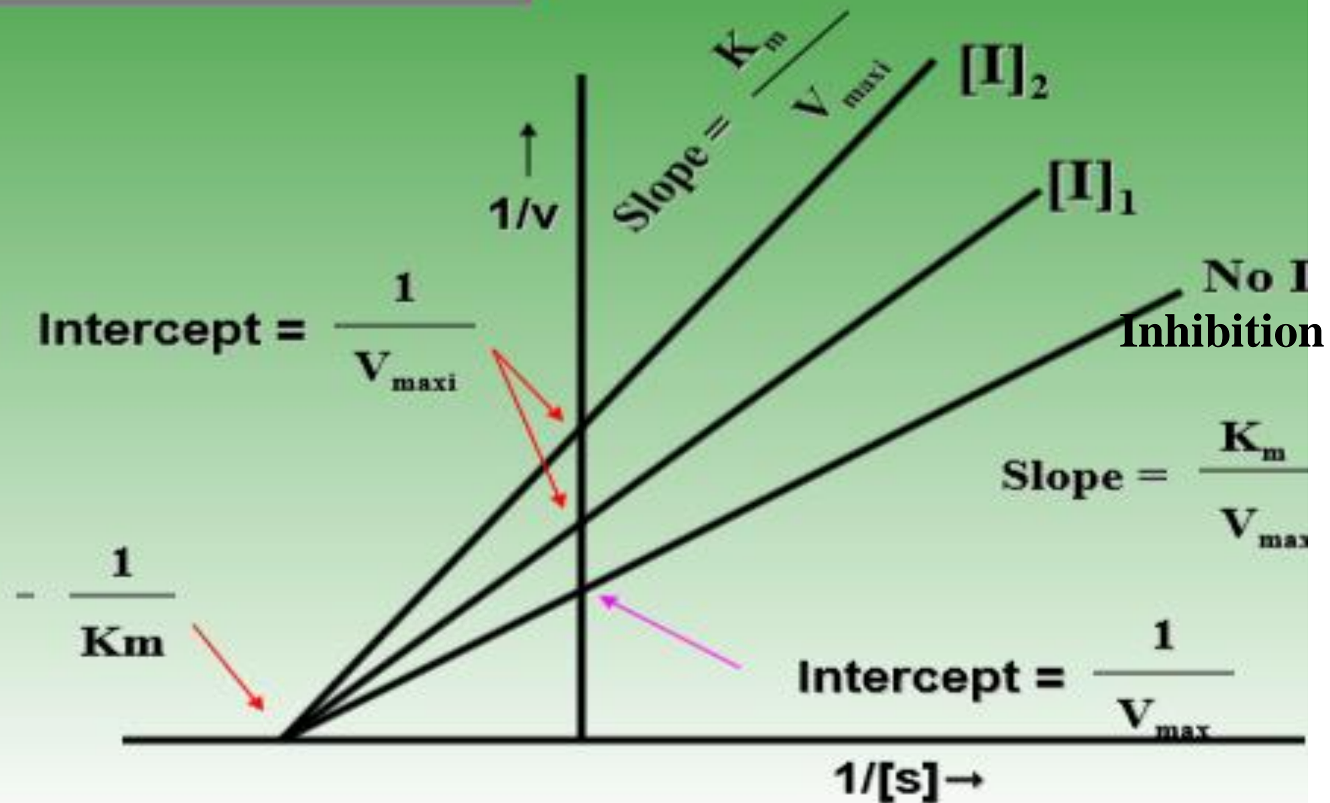
2. Enzymes with -SH groups that participate in the maintenance of the three dimensional conformation of the molecule are non-competitively inhibited by heavy metal ions.

❑ Lineweaver – Burk Plot

$$\frac{1}{v} = \frac{Km}{Vmaxi}\frac{1}{[S]} + \frac{1}{Vmaxi}$$

Both slope & Intercept Increased By the factor

$$\left(1+\frac{[I]}{Ki}\right)$$



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Uncompetitive Inhibition

- I doesn't bind to the free E rather it binds to the ES complex

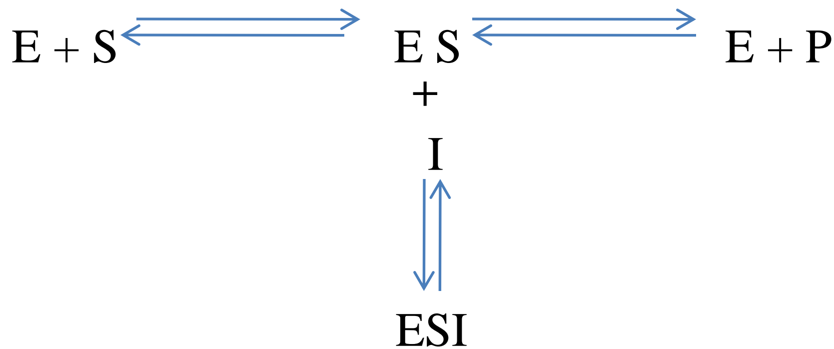
- The binding of an Uncompetitive I is presumed to cause structural distortion of the active site making the enzyme catalytically inactive

- The binding of S could cause a conformational change in the E thereby revealing an I binding site

- Inhibition can’t be reversed by increasing the [S] since I doesn't compete with S for the same binding site

UC Inhibition is rare in single-substrate reactions.

for e.g. Inhibition of intestinal alkaline phosphatase by L- phenylalanine. It is common in multisubstrate reactions phenylalanine.



- The equilibria show that at any [I] an infinitely high [S] will not drive all the enzyme to ES form; some non productive ESI complex will always be present. Consequently an Uncompetitive I will decrease the Vmax.

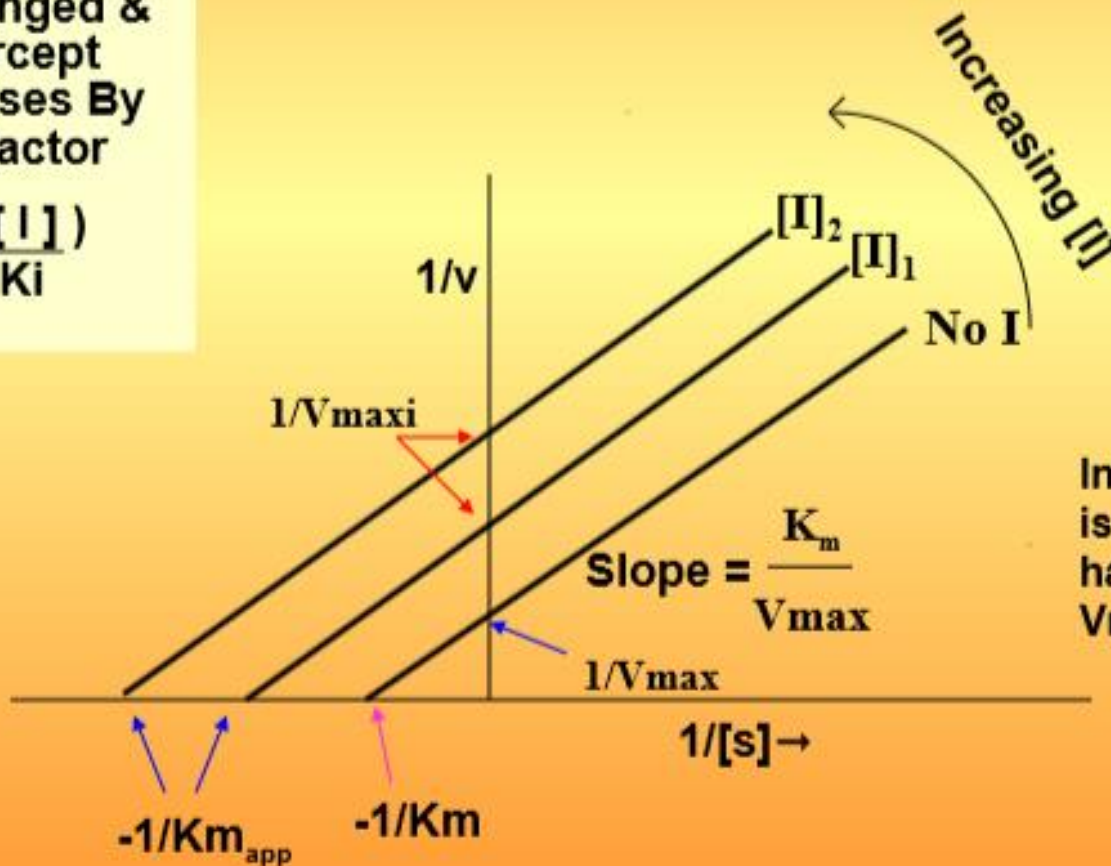
- An Uncompetitive I will also decrease the Km(app) because the reaction ES + I ⟶ ESI removes some ES causing the reaction E + S ⟶ ES to proceed to the right.

Lineweaver Burk plot

$$\frac{1}{v} = \frac{Km}{Vmax}\frac{1}{[S]} + \frac{1}{Vmax}\left(1+\frac{[I]}{Ki}\right)$$

Slope remains Unchanged & Intercept Increases By the factor

$$\left(1+\frac{[I]}{Ki}\right)$$



Incase of UC Inhibition Ki is that conc^n of I which halves the value of both Vmax and Km

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Irreversible Inhibition

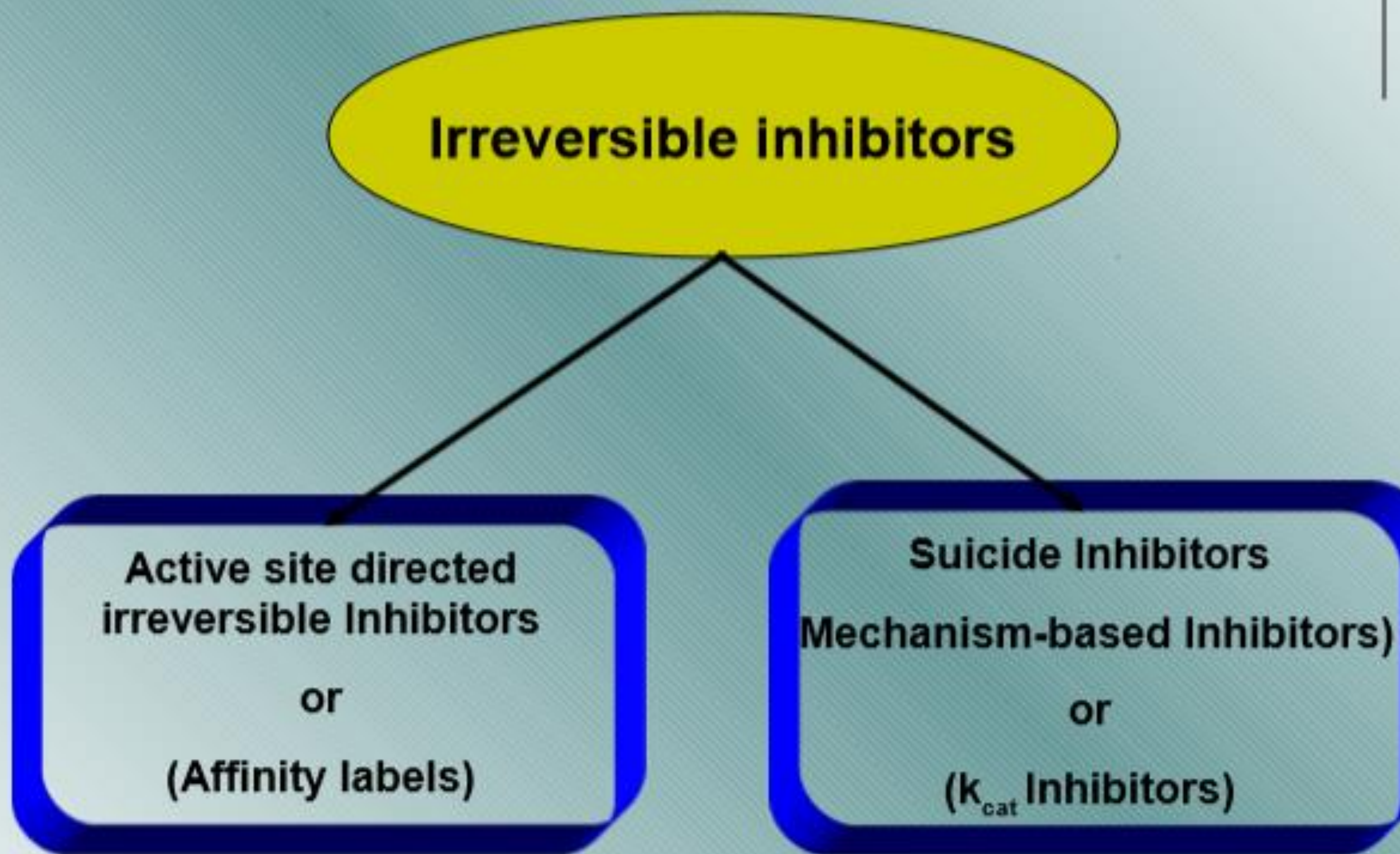
- An irreversible Inhibitor binds at or near the active site of the enzyme irreversibly, usually by covalent bonds, so that it can’t subsequently dissociate from the enzyme

- The I destroys as essential functional group on the enzyme that participates in normal S binding or catalytic action. As a result the enzyme is rendered permanently inactive.

- Compounds which irreversibly denature the enzyme protein or cause non-specific inactivation of the active site are not usually regarded as irreversible inhibitors.

- Organophosphorus compounds (such as DFP) irreversibly react with the –OH group of essential serine residue of some enzymes

- DFP (Diisopropylphosphofluoridate) is a nerve poison since it inactivates acetylcholinesterase that plays an important role in the transmission of nerve impulses.

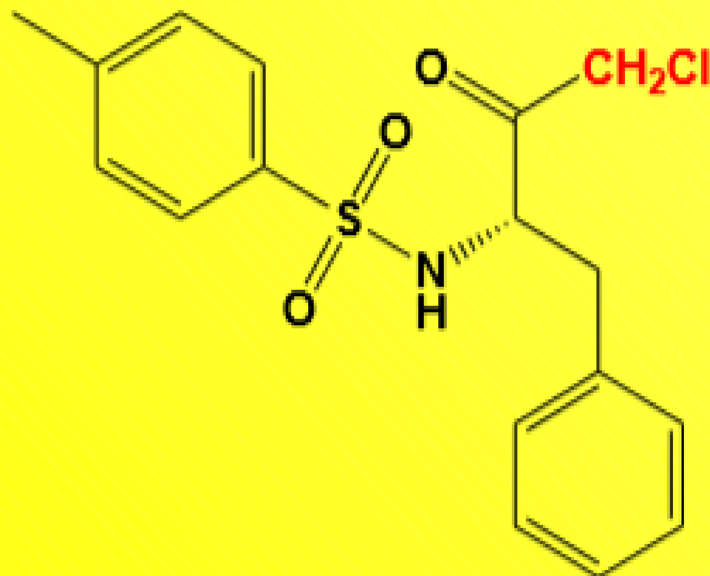

Types of Irreversible Inhibitors
Irreversible inhibitors
Active site directed irreversible Inhibitors
or
(Affinity labels)
Suicide Inhibitors
Mechanism-based Inhibitors)
or
(k_{cat} Inhibitors)

Affinity labels

An affinity label is a chemically reactive compound that is designed to resemble the substrate of an enzyme so that it binds at the active site and forms a stable covalent bond with a susceptible group of the nearby residue in the enzyme protein.

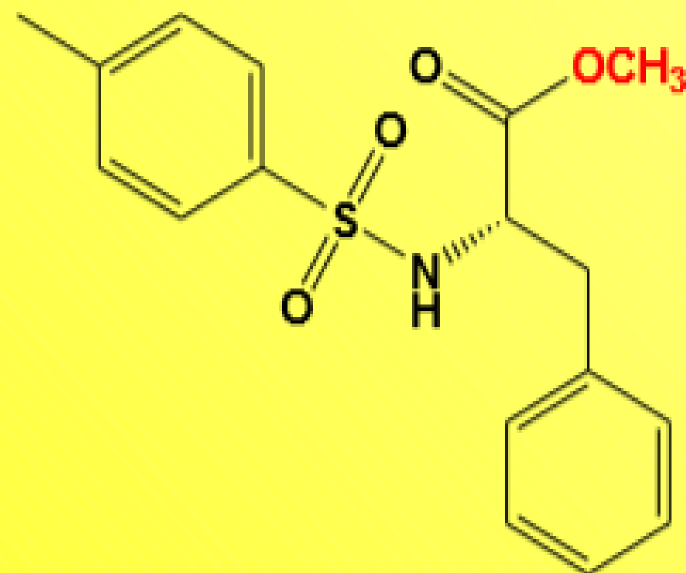
Affinity labels are very useful for identifying catalytically important residues

TPCK acts as an affinity label for Chymotrypsin; even at very low concⁿ TPCK quantitatively inactivates chymotrypsin; TPCK is identical in structure to a substrate of this enzyme i.e. tosyl-L-phenylalanyl methyl ester, except that the carboxylic ester is replaced by the chloromethyl group.



TPCK

(Affinity label)



tosyl-L-phenylalanine methyl ester

(Substrate)

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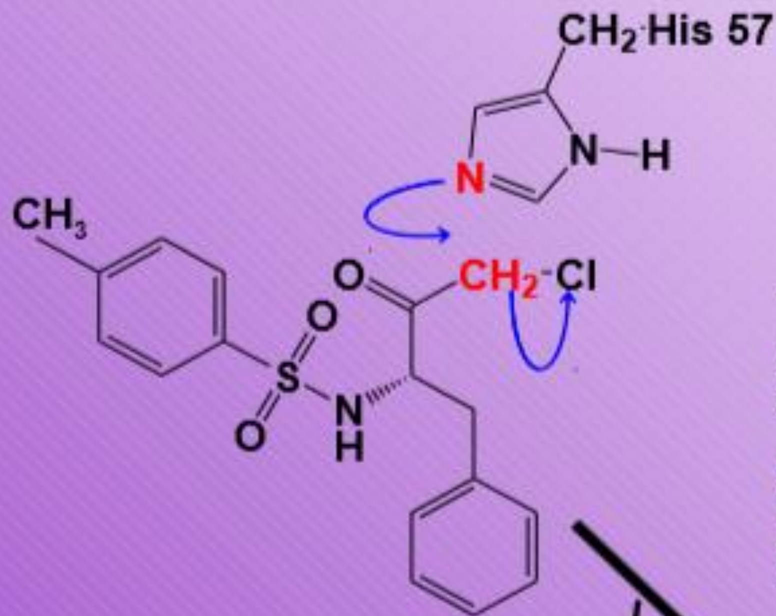
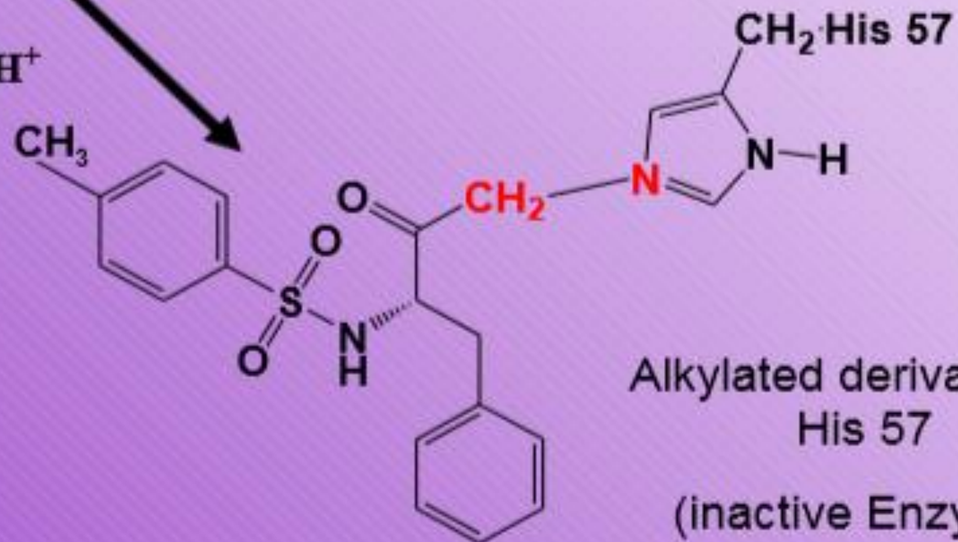


TPCK is attacked in a nucleophilic reaction by the N atom of the imidazole side chain of His57. the binding of TPCK to the Enz Brings the reactive –Cl group in close proximity to the His57 residue and facilitates the formation of a covelent bond between the I & imidazole side chain

phenylpropionate

Excess conc^n of this prevent the inactivation by TPCK



Alkylated derivative of His 57

(inactive Enzyme)

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Suicide Inhibitors

A suicide inhibitor is a relatively inert molecule that is transformed by enzyme at its active site into a reactive compound that irreversibly inactivates the enzyme

They are substrate analogs designed so that via normal catalytic action of the enzyme, a very reactive group is generated.

The latter forms a covalent bond with a nearby functional group within the active site of the enzyme causing irreversible inhibition.

Such inhibitors are called suicide inhibitors because the enzyme appears to commit suicide.

e.g. FdUMP is a suicide inhibitor of thymidylate synthase.

During thymidylate synthesis, N^5,N^{10}- methyleneTHF is converted to 7,8-dihydrofolate; methyleneTHF is regenerated in two steps

FdUMP

dUMP

dTMP

thymidylate synthase

7,8-Dihydrofolate

N^5,N^{10}-Methylene H_4 folate

$NADPH + H^+$

Glycine

serine hydroxymethyl-transferase

dihydrofolate reductase

Methotrexate
Aminopterin
Trimethoprim

PLP

H_4 folate

$NADP^+$

Serine

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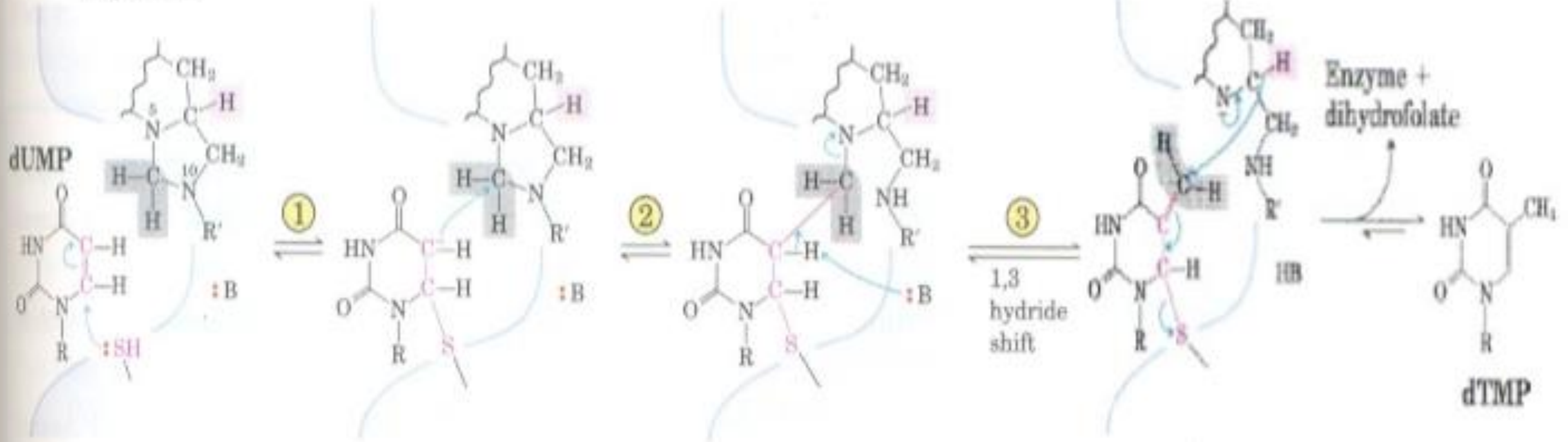
Conversion of dUMP to dTMP and its inhibition by FdUMP



FdUMP

Dead-end
covalent
complex

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