MATHEMATICS

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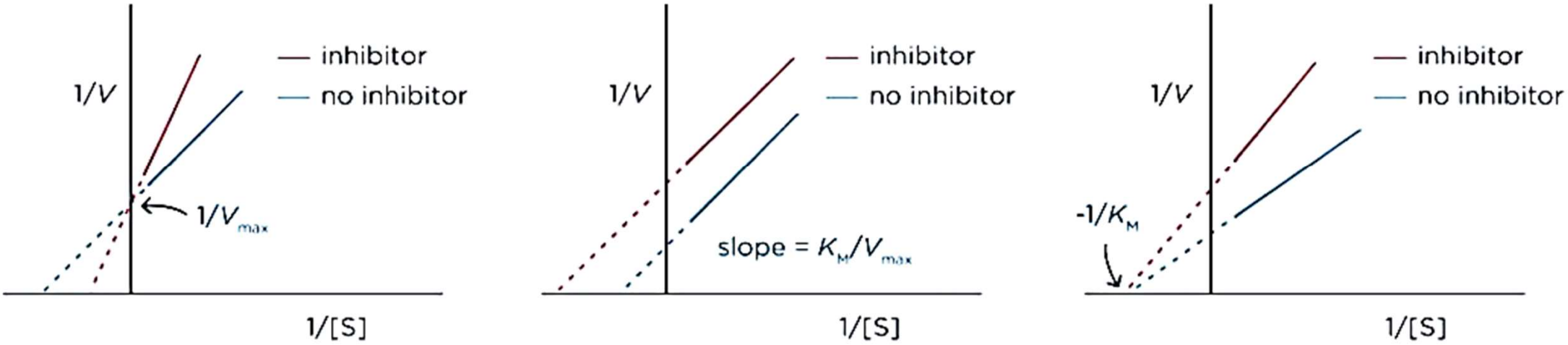
ENZYME INHIBITION



Competitive inhibition

K_M increased

V_{max} unaffected

Uncompetitive inhibition

K_M reduced

V_{max} reduced

Noncompetitive inhibition

K_M unaffected

V_{max} reduced

source: Biochemistry, epathshala, A Gateway to All Post Graduate Courses, MHRD

ENZYME INHIBITION

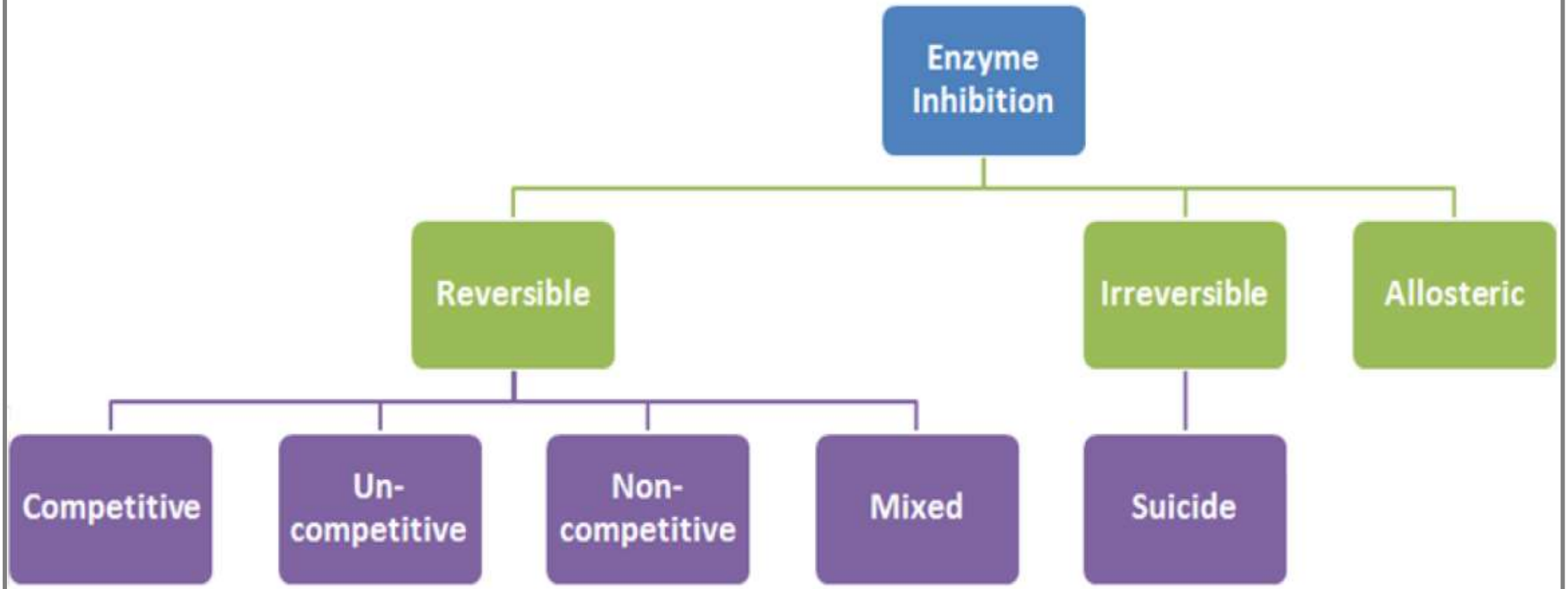
- **Introduction – Enzyme Inhibition**

An enzyme catalyzed reaction can be hindered or reduced by a number of substances. Some others like **urea** are known as ***denaturants***, being ***non-specific*** in their mode of action.

But if any compound act in a fairly ***specific way*** in inhibiting the catalysis of a particular enzyme they are called **inhibitors**.

ENZYME INHIBITION

- **Enzyme Inhibition - *Types***



ENZYME INHIBITION

- **Enzyme Inhibition - *Types***

The loss in activity can either be of two types -

(1) Reversible - where the activity ***can be restored*** by the *removal* of the inhibiting compound. *It's temporary*.

(2) Irreversible - where the loss of activity ***cannot be recovered within the stipulated time*** of interest. *It is permanent*. …

ENZYME INHIBITION

- **Enzyme Inhibition - *Types***

… Irreversible inhibition behave as ***time dependent loss of enzyme concentration*** with **lowered V_{max}** or ***incomplete in activation*** with ***time dependent change*** in both **K_m** and **V_{max}**.

…

ENZYME INHIBITION

- **Enzyme Inhibition - *Types***

… **Heavy metal ions** like **mercury**, **lead** etc. *cause irreversible inhibition*, which bind strongly to the ***amino acid backbone*** termed as "**suicide inhibition**".

ENZYME INHIBITION

- **Studying Enzyme Inhibition Mechanisms**

There are several **reasons** behind the need for ***studying enzyme inhibition mechanisms***. They are –

- Exploring potential mechanisms in multi-substrate reactions.
- Studying the relative binding affinity of competitive inhibitors to the enzyme active …

ENZYME INHIBITION

- **Studying Enzyme Inhibition Mechanisms**

There are several **reasons** behind the need for ***studying enzyme inhibition mechanisms***. They are –

… site, in the absence of 3-D structure information.

- For **understanding** various **control mechanisms** - how the balance of protease …

ENZYME INHIBITION

▪ Studying Enzyme Inhibition Mechanisms

There are several **reasons** behind the need for ***studying enzyme inhibition mechanisms***. They are –

… enzymes and their inhibitors in tissues achieve **homeostasis**.

- For various **commercial applications** like ***pesticide, insecticide, weed-killers, pharmaceutical compounds*** like ***drugs*** etc.

ENZYME INHIBITION

- **Reversible Inhibition**

In this type of inhibition, the ***hindrance is temporary*** and thus **noncovalent interactions** like **hydrogen bonds**, **ionic bonds** or **hydrophobic bonds** form between inhibitors and the enzyme. Even though these are ***weak bonds***, multiple such bonds cause strong and specific binding. …

ENZYME INHIBITION

- **Reversible Inhibition**

… Despite absence of any chemical reactions, the inhibitors can easily remove or exchange by dilution or dialysis. After removing the inhibitor, **enzyme** can be fully **restored** in reversible inhibition. ***Equilibrium*** is established between ***free inhibitor*** and ***enzyme-inhibitor*** **[EI]** ***complex*** (Figure 2).

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- **Reversible Inhibition**

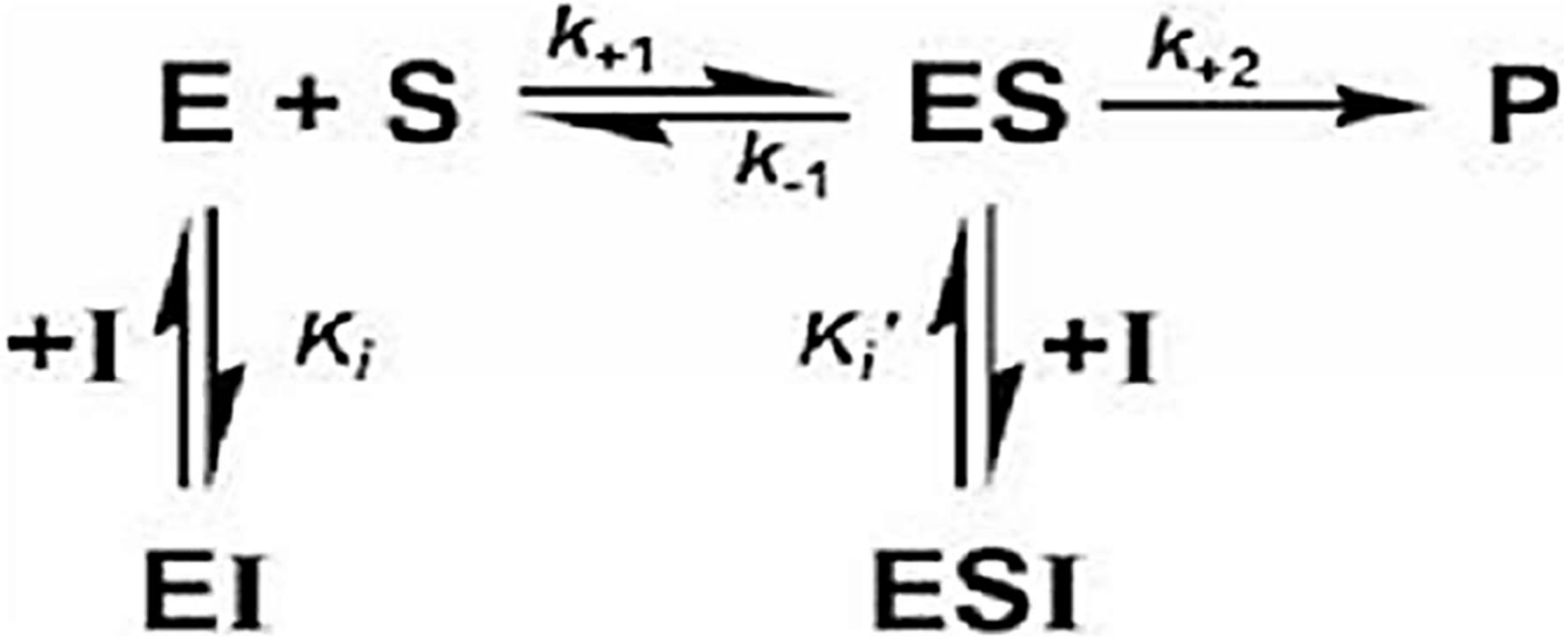

Figure 2. Mechanism of reversible inhibition

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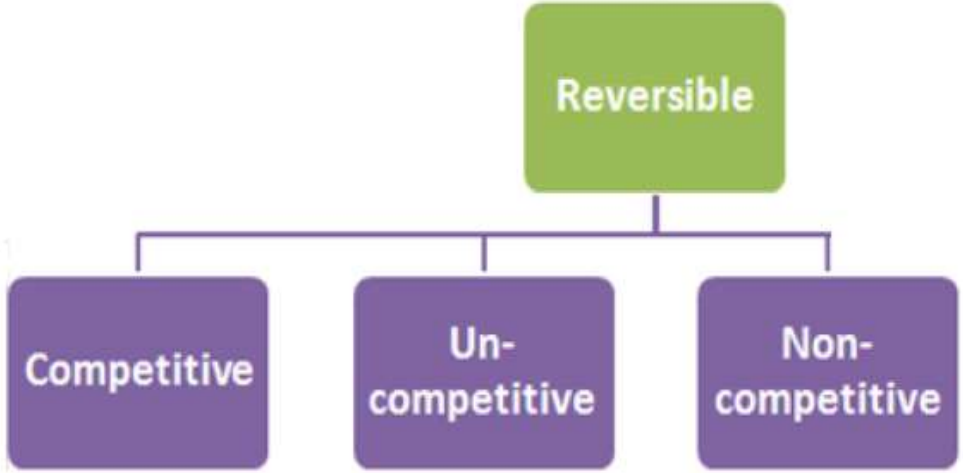
- **Reversible Inhibition - Types**

Reversible inhibitions are of different types. The classification is **based** according to the effect of varying the concentration of the enzyme's substrate on the inhibitor.



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- **Competitive Inhibition**

In this type of **reversible inhibition**, both the **substrate** and its **inhibitor** ***cannot bind*** to the enzyme ***at the same time*** to the **allosteric / active site**. This normally occurs ***due to*** the **structural similarity** of **substrate** and the **inhibitor**, which results with ***affinity*** for the active site. …

ENZYME INHIBITION

- **Competitive Inhibition**

… The inhibition ***can be recovered by*** the presence of **high concentration of substrate**, outcoming the competing inhibitor. $\mathbf{V_{max}}$ of the reaction is **unchanged**, while $\mathbf{k_d}$, the **dissociation constant** is apparently **increased**. Competitive inhibitors can also be used to find the enzyme active site.

ENZYME INHIBITION

- **Competitive Inhibition**

Example-1: N-(Phosphonoacetyl)-L-asparate also known as **PALA** is a **competitive inhibitor** for *Aspartate Transcarbamoylase*.

Example-2: **Malonate** is a **competitive inhibitor** of enzyme *succinate dehydrogenase*, and *competes with succinate*.

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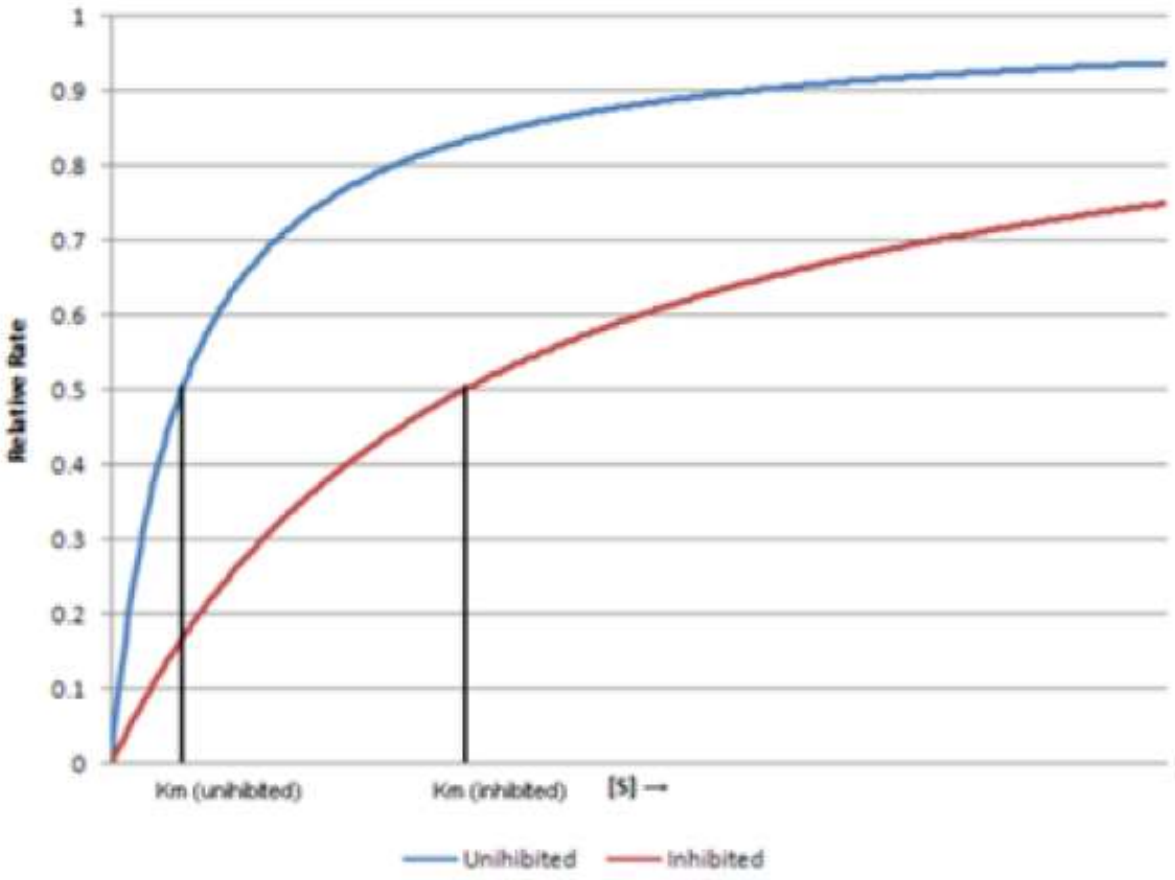

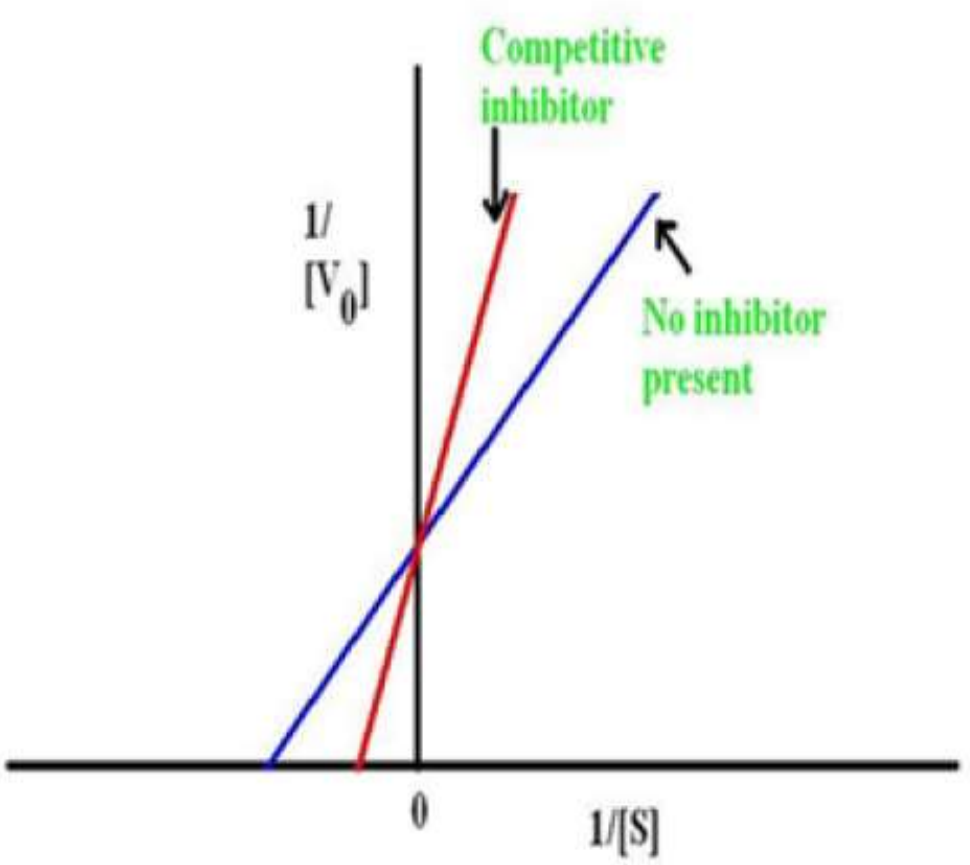

Figure 3. Kinetics of competitive inhibition

ENZYME INHIBITION

Table 1. Clinical use of competitive inhibition

Drugs	Target Enzyme	Therapeutic use
STATINS, Atorvastatin Simvastatin	HMG CoA reductase	Involved in the reduction of plasma cholesterol level- Anti-hyperlipidemic agents
Allopurinol	Xanthine oxidase	Used in case of prevention of gout attacks
Methotrexate	Dihydrofolate reductase	Used as canecr treatment drug
Captopril, Enalapril	Angiotensin converting enzyme	Treatment of high blood pressure
Discoumarol	Vit K-epoxide reductase	Used as an anti-coagulant

ENZYME INHIBITION

- **Uncompetitive Inhibition**

It's an **anti-competitive inhibition**; where the inhibitor binds **only** to the substrate-enzyme complex. According to its kinetics, $\mathbf{V_{max}}$ and $\mathbf{K_m}$ **decrease**. This type of inhibition works best in case of ***high concentration*** of the ***substrate***. The substrate and the uncompetitive inhibitor **does not resemble** each other.

Example - *Lithium and phosphoinositide cycle.*

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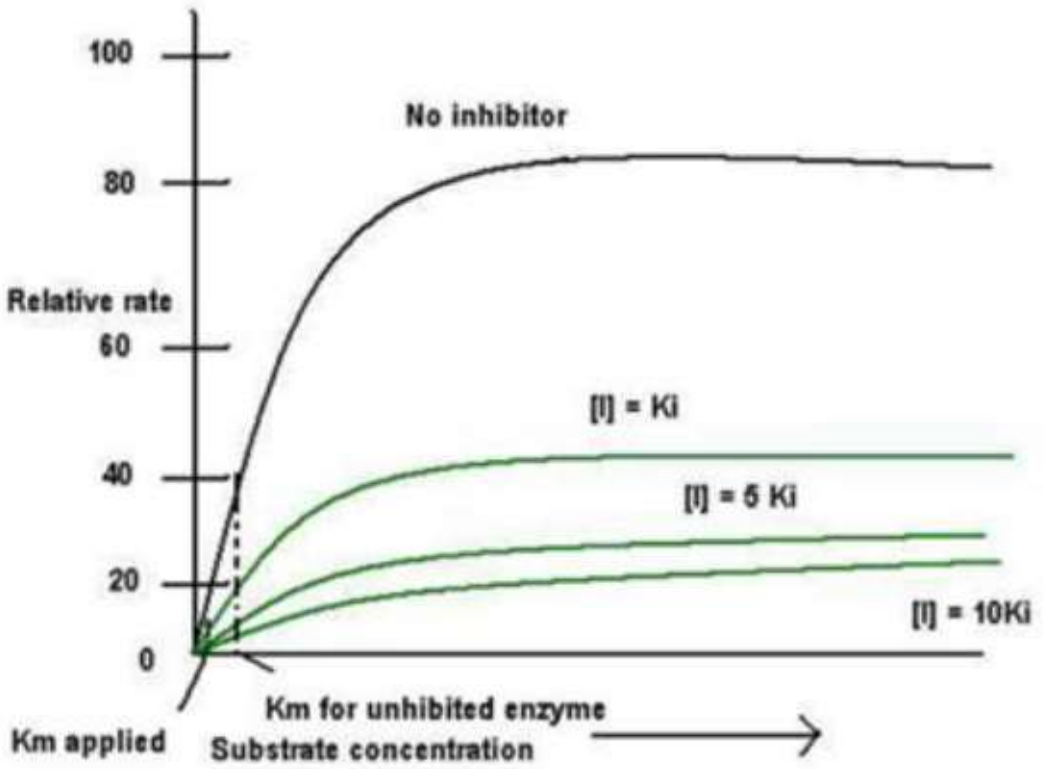


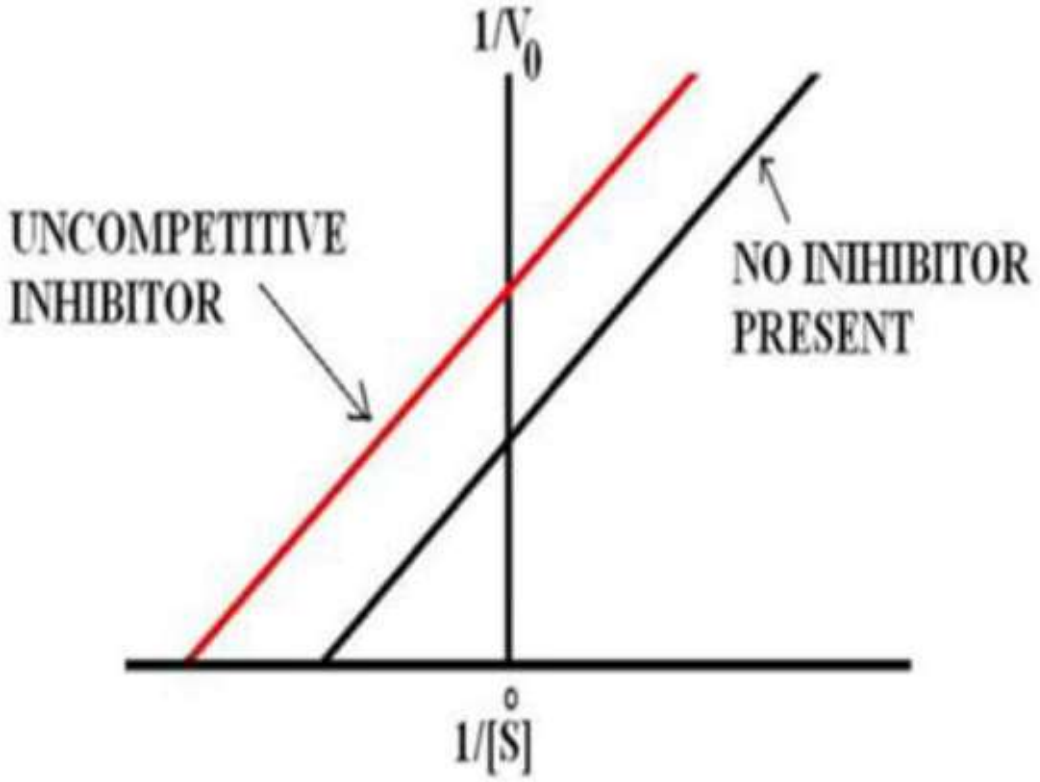


Figure 4. Kinetics of un-competitive inhibition

ENZYME INHIBITION

- **Non-Competitive Inhibition**

A non-competitive inhibitor is one which reacts with enzyme-substrate or [ES] complex. It does **not affect the binding of the substrate**, but **slows down the reaction rate for formation of the enzyme-product [EP] complex**. …

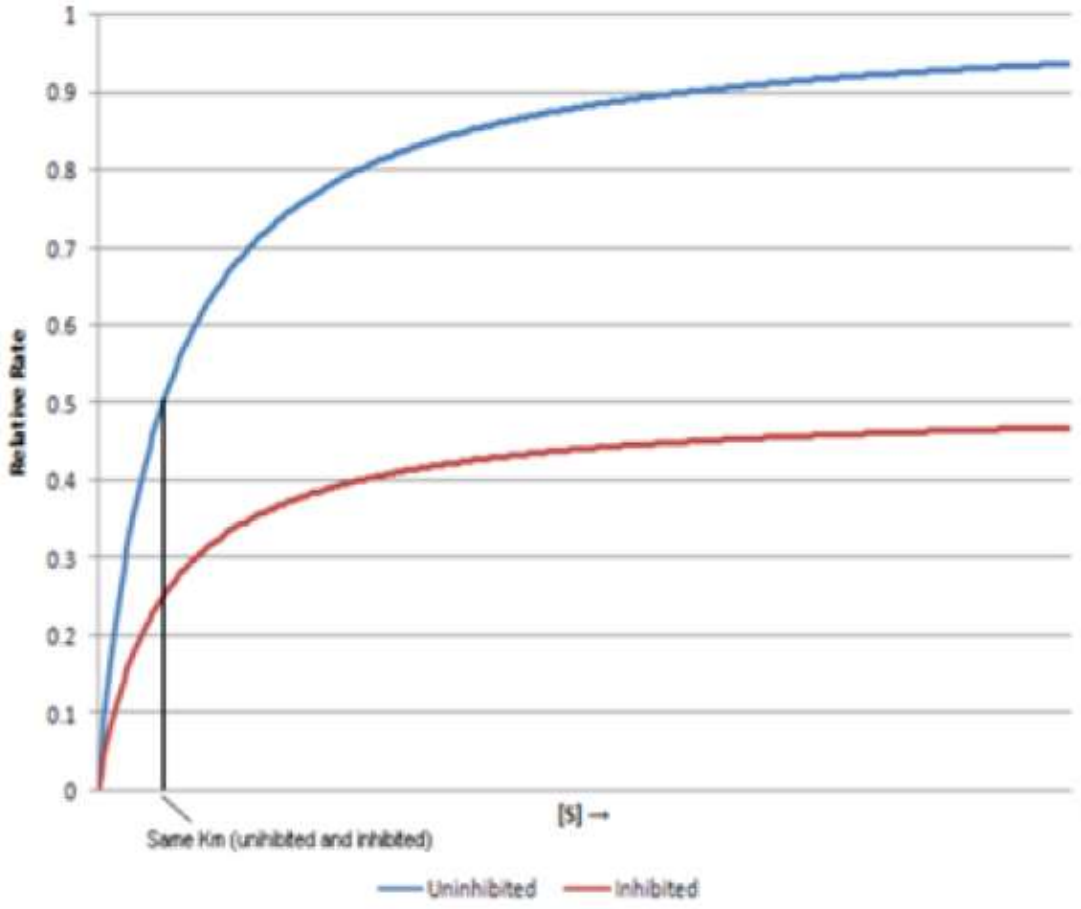
ENZYME INHIBITION

- **Non-Competitive Inhibition**

… The only factor on which the extent of hindrance or inhibition **depends** is the **inhibitor concentration**. There will be a *decrease* in $\mathbf{V_{max}}$ but $\mathbf{K_m}$ will remain the *same*.

Example: **Alanine** non competitively inhibits the enzyme **pyruvate kinase**.

ENZYME INHIBITION



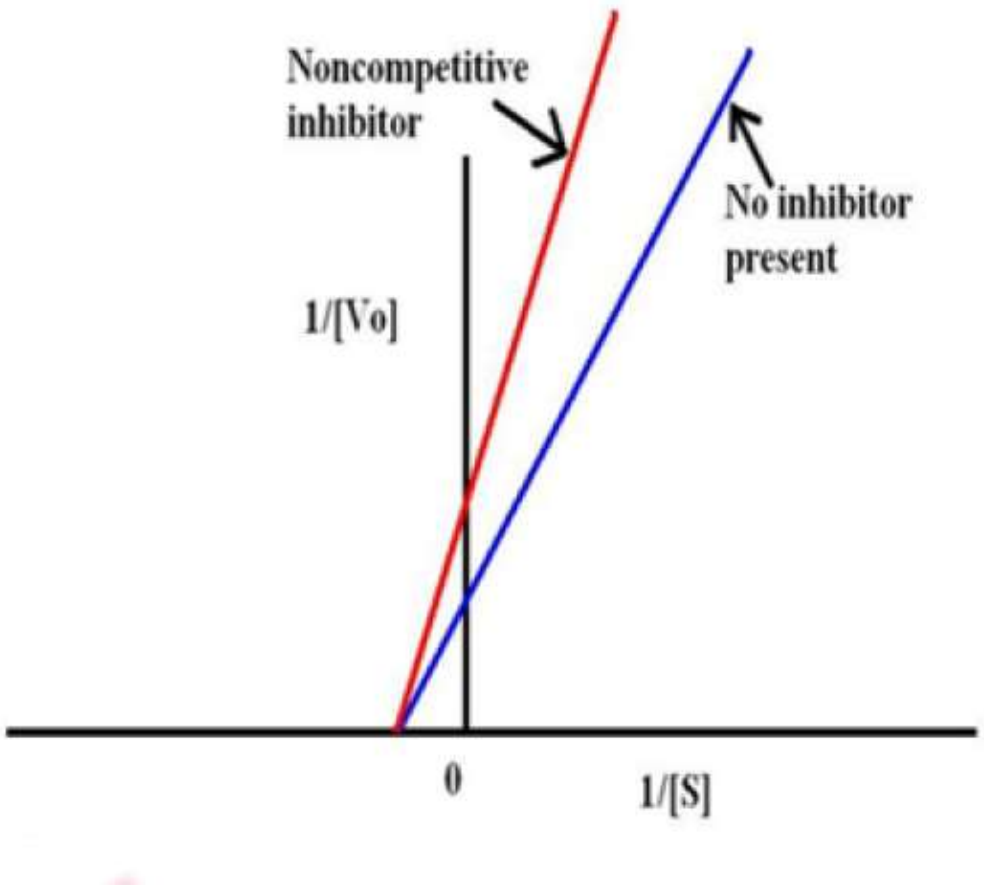


Figure 5. Kinetics of non-competitive inhibition

ENZYME INHIBITION

Table 2. Examples of common non-competitive inhibitors

Inhibitor	Enzyme inhibited
Heavy metals – Ag^{2+}, Hg^{2+}, Pb^{2+}	Heavy metals bind with cysteinyl SH group of enzyme
Pepstatin	Pepsin
Soybean trypsin inhibitor	Trypsin
Ethanol/narcotic drugs	Acid phosphatase

ENZYME INHIBITION

- **Mixed Inhibition**

In this type of inhibitor, **inhibitor** is capable of **binding to both *free enzyme*** as well as ***enzyme-substrate complex***. In this case, $\mathbf{V_{max}}$ and $\mathbf{K_{max}}$ varies. **Mixed inhibitor binds to the *allosteric site***. This type of inhibition **cannot overcome by increasing substrate concentration S**, but can be reduced. …

ENZYME INHIBITION

- **Mixed Inhibition**

… The inhibitor binding to the ***allosteric site*** *changes the* structural confirmation to reduce the *affinity* of the substrate.

Example: Mixed inhibition is observed on case of *oxidoreductase activity* of ***Xanthine oxidase*** **by Pd^{2+} ion**.

ENZYME INHIBITION

$$\frac{1}{v} = \frac{ks}{v\,max}\left(1 + \frac{[I]}{ki}\right)\frac{1}{[S]} + \frac{1}{v\,max}\left(1 + \frac{[I]}{\alpha ki}\right)$$

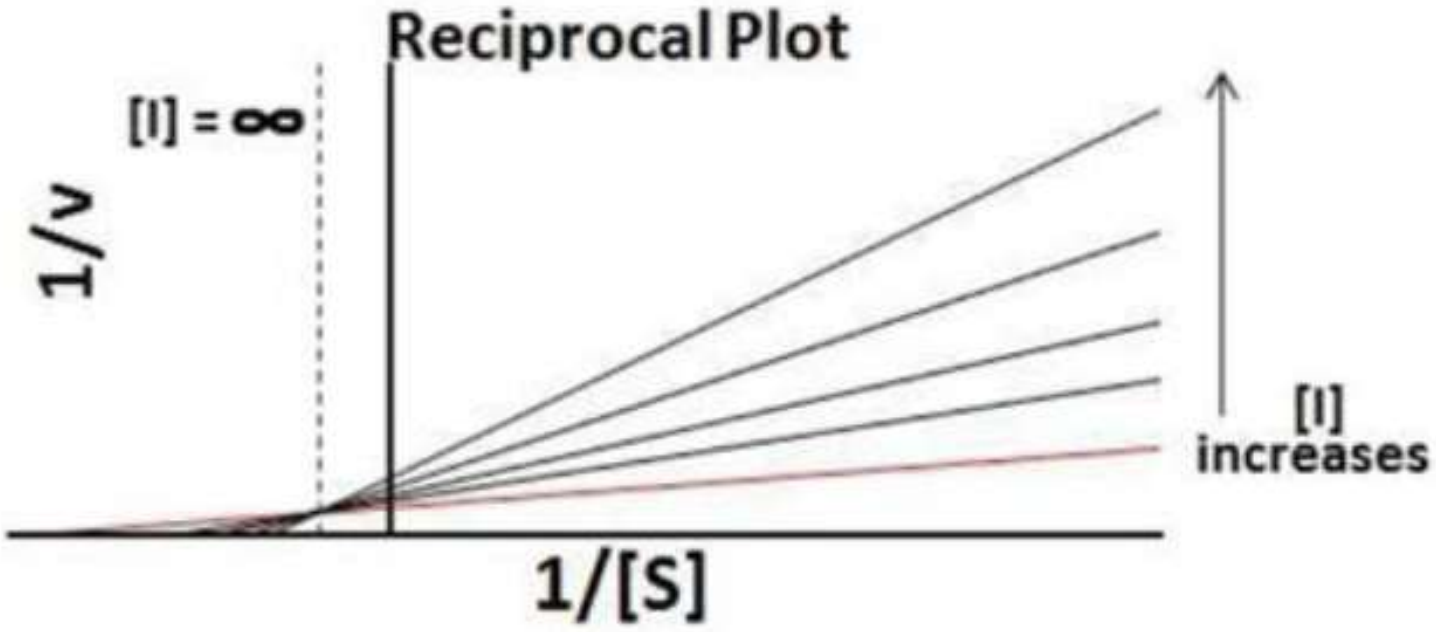


Figure 6. Kinetics of Mixed Inhibition

ENZYME INHIBITION

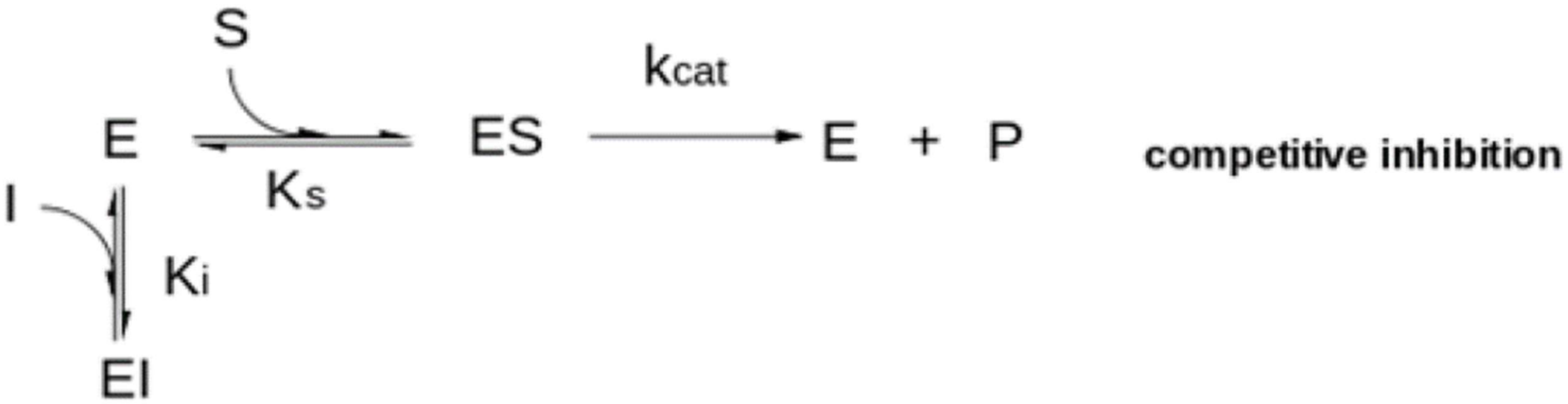
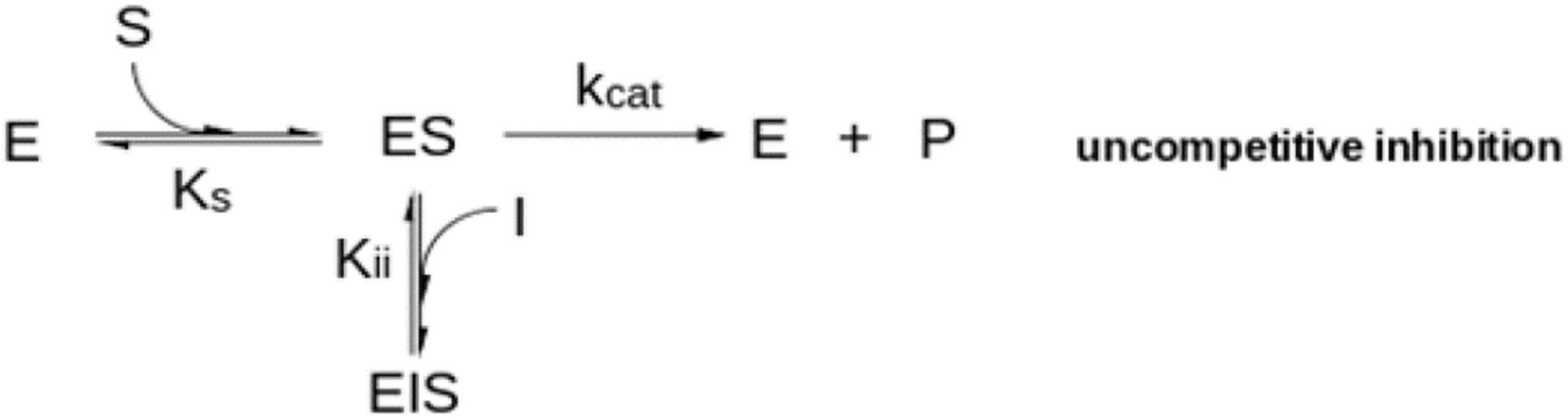


Figure 7. Different types of reversible inhibitions

ENZYME INHIBITION



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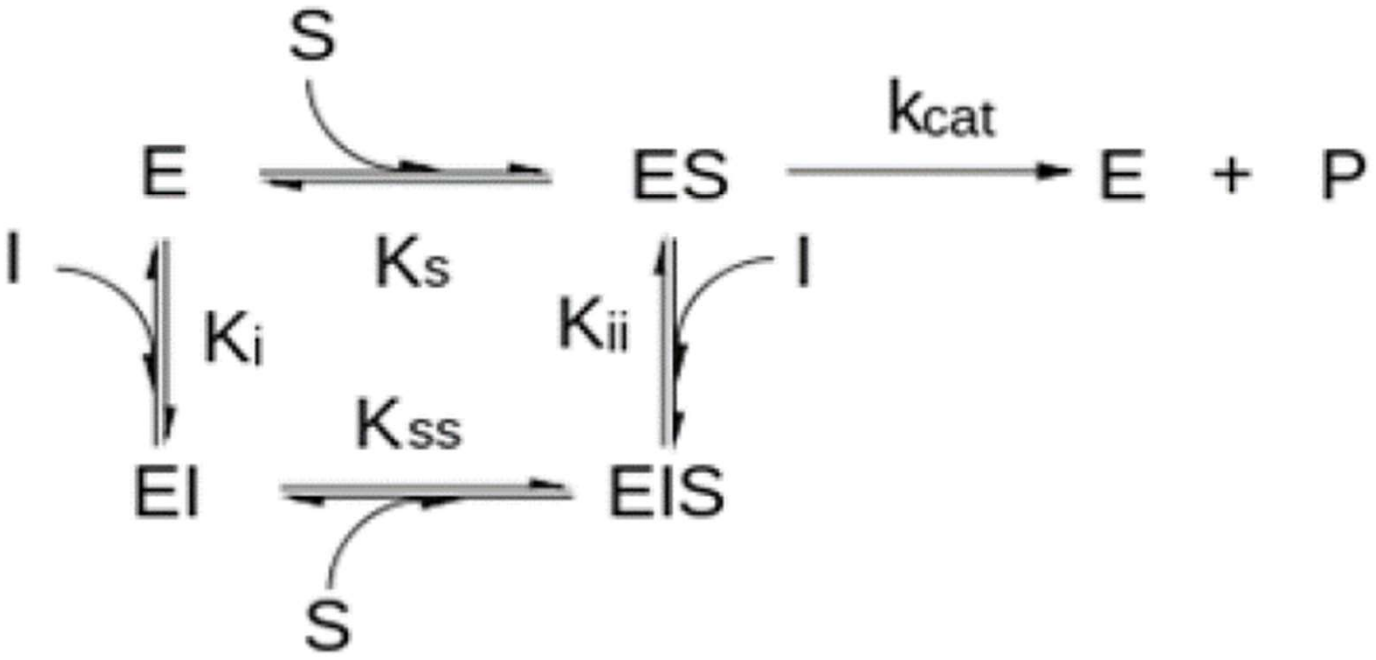
ENZYME INHIBITION



non-competitive inhibition

Figure 7. Different types of reversible inhibitions

ENZYME INHIBITION

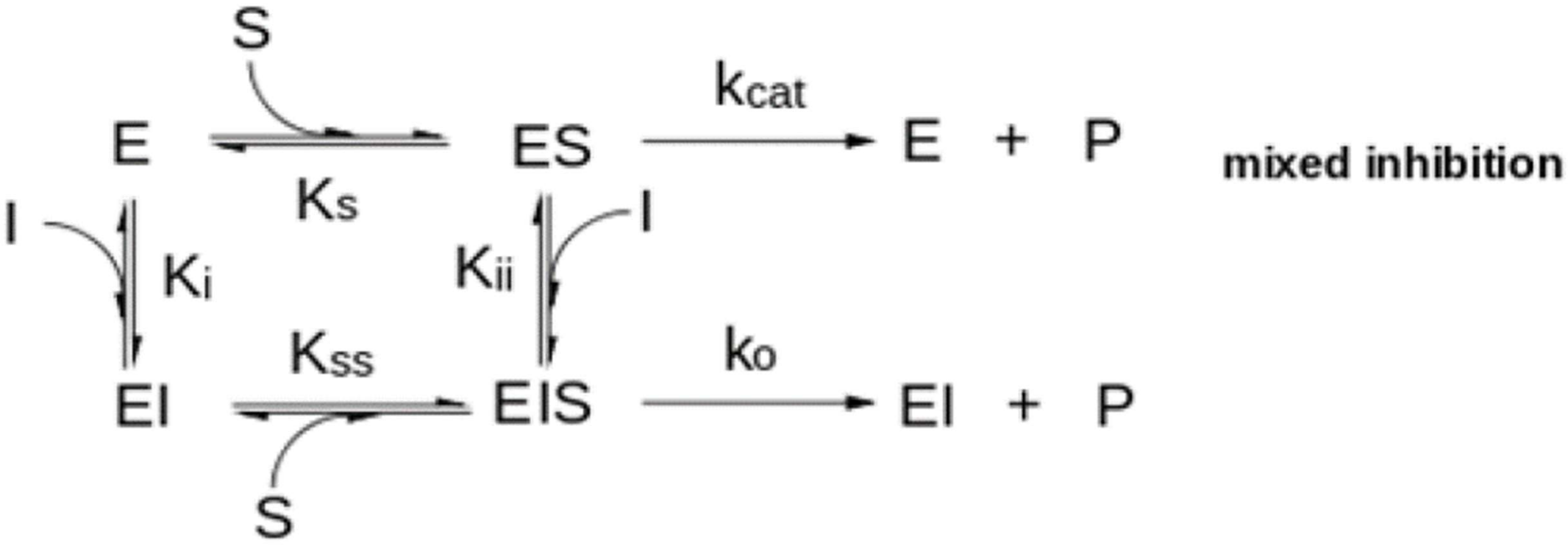


Figure 7. Different types of reversible inhibitions

ENZYME INHIBITION

- **Irreversible Inhibition**

In this type of inhibition, the **hindrance** is of **permanent** nature by ***modifying enzyme covalently***. These types of inhibitors often contain **electrophilic functional groups** like **fluorophosphates**, **aldehydes**, **haloalkanes**, **alkenes**, **nitrogen mustards**, **phenyl sulfonates**, **Michael acceptors etc.**, which react with amino acid side chains having ***nucleophilic residues***.

ENZYME INHIBITION

- **Irreversible Inhibition**

These inhibitors are **very specific** in the mechanism of inactivation for a particular class of enzyme. They do irreversible ***inhibition by specially altering the active site***. They display **inhibition which is time-dependent**. …

ENZYME INHIBITION

- **Irreversible Inhibition**

… Their **potency** cannot be characterized by **IC_{50}** value. These inhibitors ***increase*** **K_m** and ***decrease*** **V_{max}**.

Example: Di isopropyl flurophosphate (**DFP**) is an irreversible **protease inhibitor**.

ENZYME INHIBITION

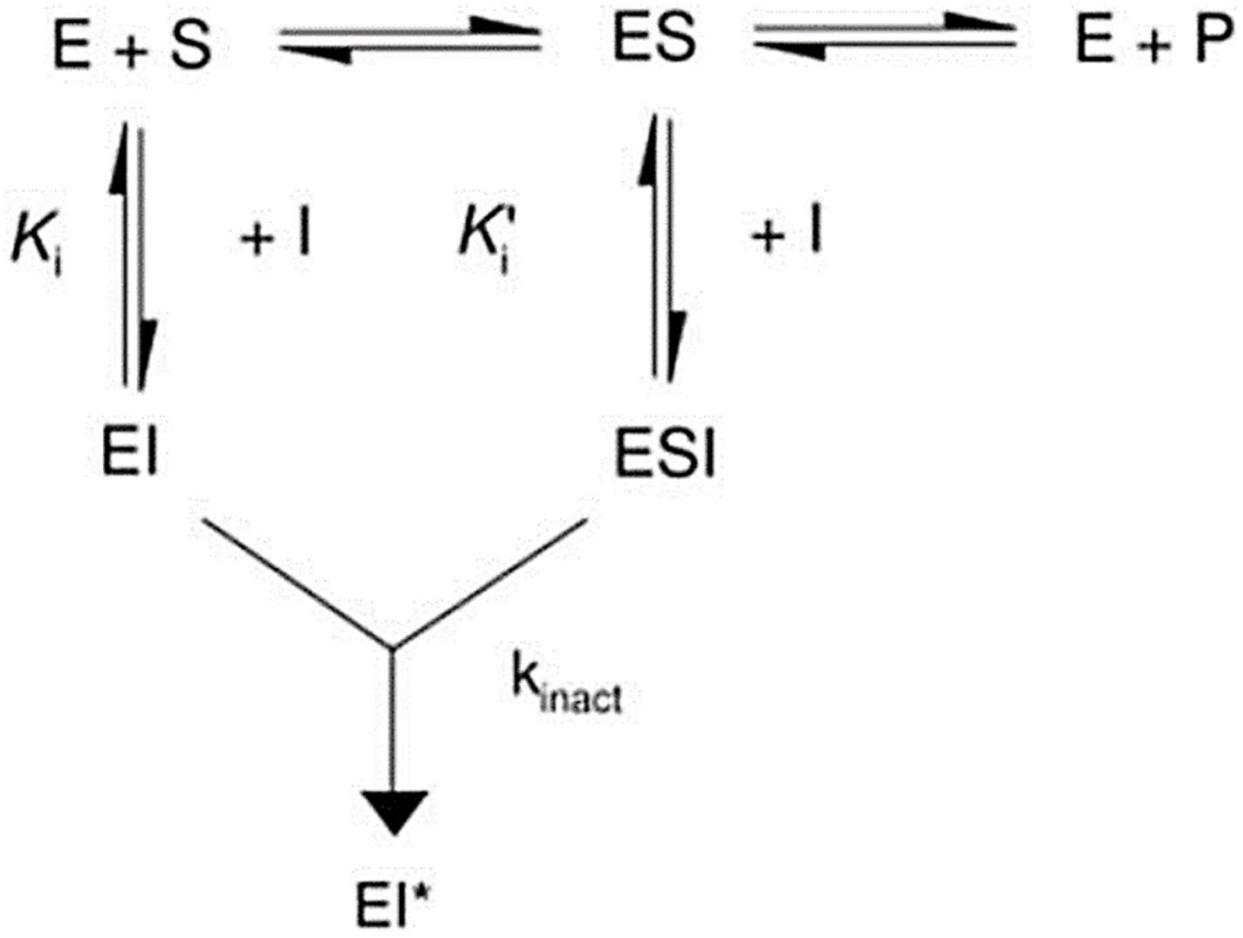


Figure 8. Kinetics of irreversible inhibition

ENZYME INHIBITION

Table 3. Therapeutic uses of irreversible inhibitors.

Inhibitors	Enzyme inhibited	Therapeutic uses
Disulfiram	Aldehyde dehydrogenase	Treats alcoholism
Cyanide ions	Cytochrome oxidase	Inhibition of the respiratory chain
Fluoride ions	Enolase	Inhibition of Glycolysis
Melathion	Acetyl choline esterase	Used as an organophosphorus insecticide
Di-isopropyl fluorophosphate	Serine proteases, Acetyl choline esterase	Used as a nerve gas
British Anti Lewisite (BAL)	Reaction with the thiol (-SH) group of the enzyme	Used as an antidote in case of poisoning due to heavy metal

ENZYME INHIBITION

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ENZYME INHIBITION

- **Suicide inhibition**

This is another type of ***irreversible inhibition***. In this case, the target **enzyme converts** the **inhibitor compound into a reactive form** in its **active site**. They are also known as mechanism based inhibitors or transition state analogs.

ENZYME INHIBITION

- **Suicide inhibition**

Example: **DFMO** [*α-difluromethyl ornithine*], an analogue of ***ornithine*** inhibits ***ornithine decarboxylase***.

Example: ***Allopurinol*** is a suicide inhibitor of ***xanthine oxidase***

Example: Aspirin inhibits cyclooxygenase.

ENZYME INHIBITION

Table 4. Therapeutic uses of suicide inhibitors

Drugs	Product	Target Enzyme	Therapeutic use
Allopurinol	Alloxanthin	Xanthine oxidase	Treatment of gout
5-Fluorouracil	Fluoro-deoxy uridylate	Thymidylate synthase	Cancer treatment
Aspirin	Acetylates the serine residue present in the cyclooxygenase active site	Cyclooxygenase	Used as a non-steroidal anti-inflammatory drug
Difluro methyl ornithine (DFMO)	Forms an irreversible covalent complex with the co-enzyme	Ornithine decarboxylase	Used for treating Sleeping sickness (trypanosomiasis)

ENZYME INHIBITION

Table 5. Differences between reversible & irreversible inhibitions

Reversible	Irreversible
Binds via non-covalent interactions	Binds via covalent interactions
Do not perform any chemical changes	Inhibitor binds to the substrate and prevent catalytic activity of enzymes
As there is no bonding between the inhibitor and substrate, reversible inhibition can be reversed,	Irreversibility due to strong covalent bonding

ENZYME INHIBITION

- **Allosteric Inhibition**

Allosteric inhibition is a type of ***enzyme regulation***, in which allosteric inhibitor binds to a site **other than the active site** of the enzyme. This *additional site* to which effector binds is called **allosteric site**. **When** these effectors bind to the **protein**, results with **conformational change** and …

ENZYME INHIBITION

- **Allosteric Inhibition**

… cause ***enhancement in activity*** is known as ***allosteric activators***. **When** they ***decrease the activity*** of the protein, they are known as **allosteric inhibitors**. Allosteric enzymes are **K** or **V types**.

ENZYME INHIBITION

- **Models of Allosteric Regulation**

The allosteric effects or **mechanism** is well described by the **concerted MWC model**, which was put forth by *Monod, Wyman and Changeux*. Another model called the **sequential model**, proposed by *Koshland, Nemethy and Filmer*, also possibly explains the allosteric regulation. …

ENZYME INHIBITION

- **Models of Allosteric Regulation**

… Both these models postulate that **enzyme subunits** exist in one two conformations - **tensed (T) or relaxed(R) states**.

ENZYME INHIBITION

▪ Concerted model

This model is known as **symmetry** or **MWC model**. According to this model, **enzyme subunits** exist in same conformation, they **are connected** and **a slight conformational change** in any one of the subunits is conferred to all other subunits of the enzyme. When any **ligand** or **substrate** is absent, the equilibrium favors towards either of the conformational states. Among the **tensed** and **relaxed states**, the **'R' state has higher affinity than 'T' state**. The most successful application of this model is this **regulation of hemoglobin**.

ENZYME INHIBITION

▪ Sequential model

In contrary to Concerted model, **Sequential model** states that **enzymes subunits** are **not connected**, such that any **change** in the **enzyme conformation leads to induction** of a similar change in the others. When a subunit randomly collides with substrate, an **induced fit** converts a subunit from the **'T' state to 'R' state**.

ENZYME INHIBITION

▪ Morpheein model

The third model is a **dissociative concerted model** known as **Morpheein model**. This is **physiologically significant homo-oligomeric tetramer structure**. Transition in the morpheein model are assisted by dissociation of oligomer, conformational change in dissociated state and reassembly of oligomers.

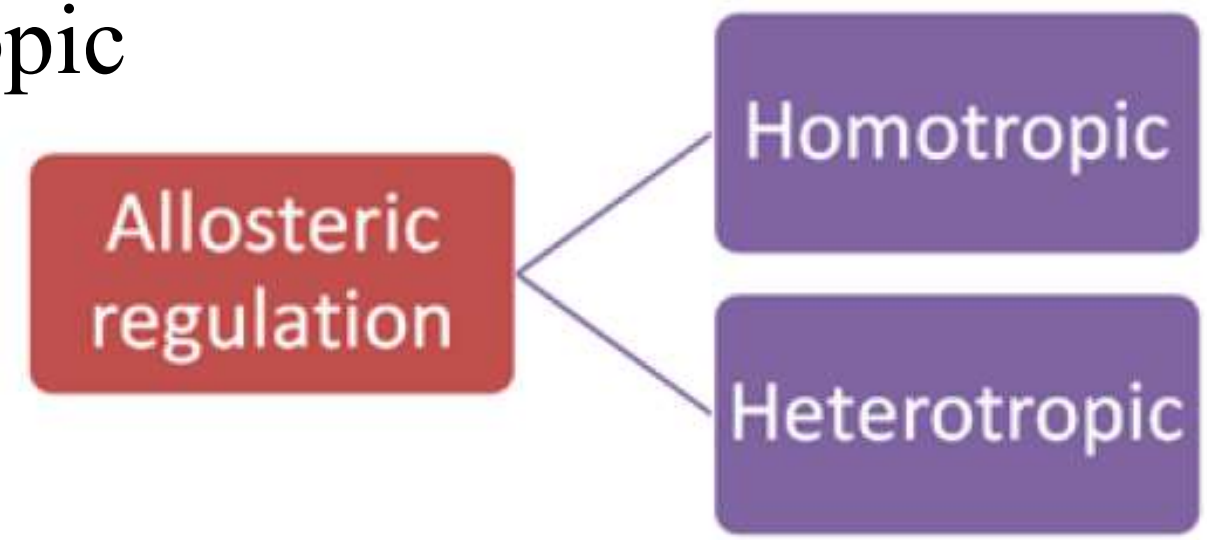
So far, one of the best characterized morpheein is the enzyme **porphobilinogen synthase**.

ENZYME INHIBITION

▪ Types of Allosteric Regulation

There are mainly **two types** of allosteric regulation.

- Homotropic
- Heterotropic



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▪ Types of Allosteric Regulation

▪ **Homotropic regulation**: It's a **positive modulation** - The modulator acts not only as a **substrate** but also as a **regulatory molecule** of the target enzyme.

Example: **O_2** and **CO** are homotropic allosteric modulator of **haemoglobin**.

▪ **Heterotropic regulation**: It can either be a **positive or negative modulation**. Here the modulator is a **regulatory molecule** but **not** an **enzymes substrate**.

Example: **CO_2** is a heterotropic modulator of **haemoglobin**.

ENZYME INHIBITION

- **Importance of enzyme inhibition**
- Understanding regulation of enzyme activity in living cells.
- Elucidation of the cellular metabolic pathways by accumulation of intermediates.
- Helps in identification of catalytic or functional groups present at the enzyme active site.

ENZYME INHIBITION

- **Importance of enzyme inhibition**
- Helps in providing information on enzyme's substrate specificity.
- Helps in studying the mechanism of catalytic activity.
- Competitive or suicide inhibitors also find therapeutic applications.

Thank you!

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