Inhibition of Enzyme Activity

Types of Inhibition:

Competitive

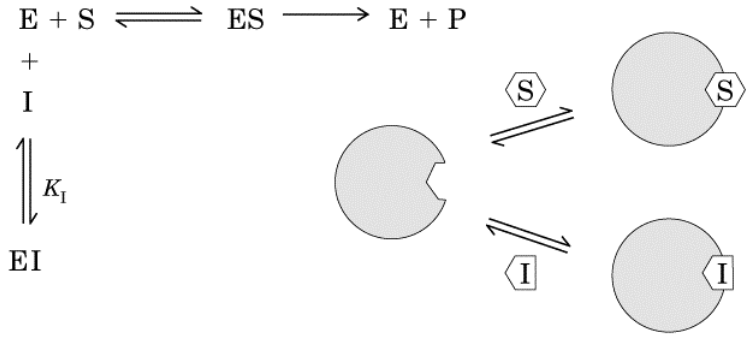
Noncompetitive

Uncompetitive

Product Inhibition

Suicide Inhibition

Competitive Inhibition



(a) Competitive inhibition

Fig 8-15

Competitive Inhibition

COMPETITIVE

- **$-I_c$ structrually resembles S, but is not an S**
- **$-I_c$ binds to free E at active site where S binds**
- **$-I_c$ competes with S for free E**
- **High S overcomes inhibition because all E is bound in ES complex; since rate [ES] and [ES] is max, rate is max; no EI_c is present**

Equilibria Scheme

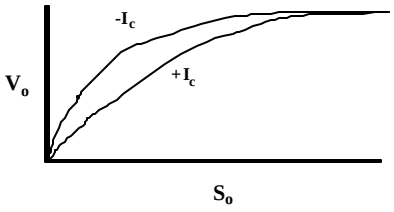
$$E + S \underset{K_m}{\rightleftharpoons} ES \longrightarrow P + E$$

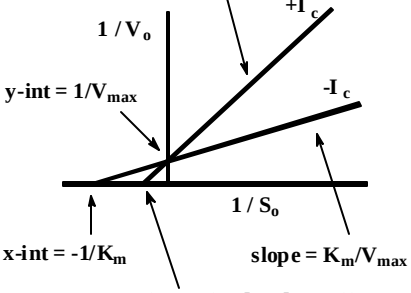
$$+ \quad I_C$$

$$K_c \updownarrow$$

$$EI_c$$

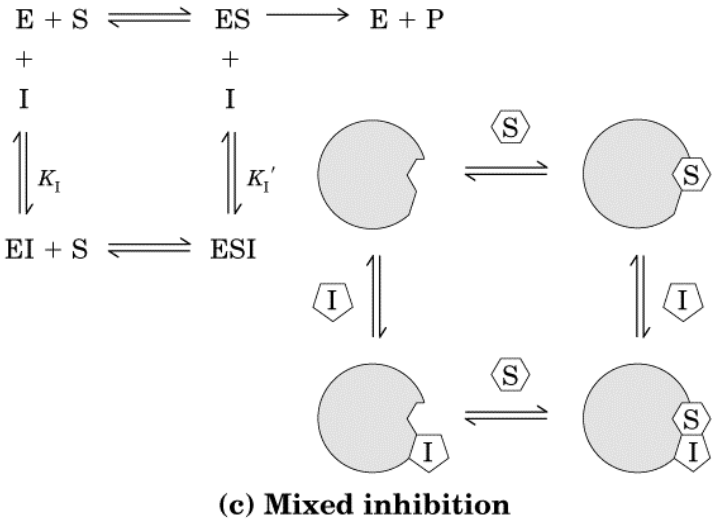
slope = $K_m \cdot (1 + [I_c] / K_c) / V_{max}$





yint = $-1 / (K_m \cdot (1 + [I_c] / K_c))$

Noncompetitive Inhibition



(c) Mixed inhibition

Fig 8-15

Noncompetitive Inhibition

NONCOMPETITIVE

- $-I_{nc}$ is not structurally similar to S; is not an S
- $-I_{nc}$ binds to free E or ES at a site where S does not bind
- $-I_{nc}$ does NOT compete with S for free E
- High S cannot overcome inhibition because I_{nc} binds to ES complex, inactivating it

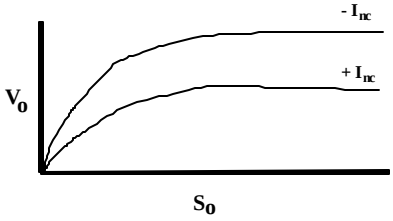
Equilibria Scheme

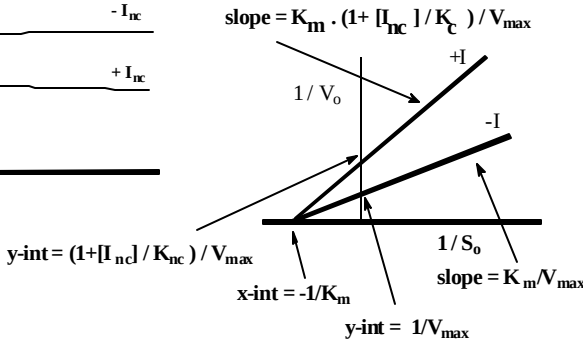
$$E + S \underset{K_m}{\rightleftharpoons} ES \longrightarrow P + E$$

$$+ \quad I_{nc} \qquad + \quad I_{nc}$$

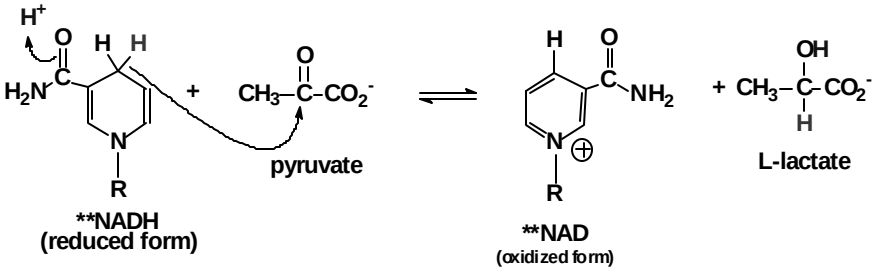
$$K_{nc} \updownarrow \qquad K'_{nc} \updownarrow$$

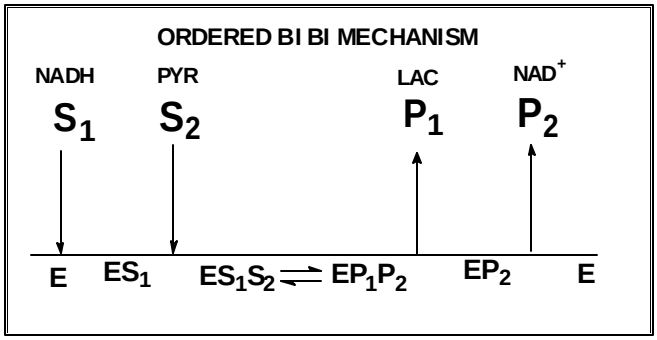
$$EI_{nc} + S \rightleftharpoons ESI_{nc} \quad \text{(inactive)}$$





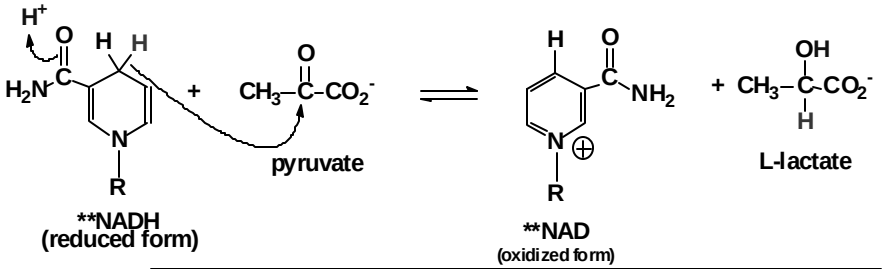
Examples: Competitive and Noncompetitive Inhibition





Examples: Competitive and Noncompetitive Inhibition

LACTATE DEHYDROGENASE



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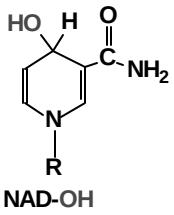
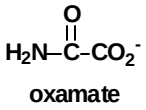
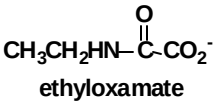




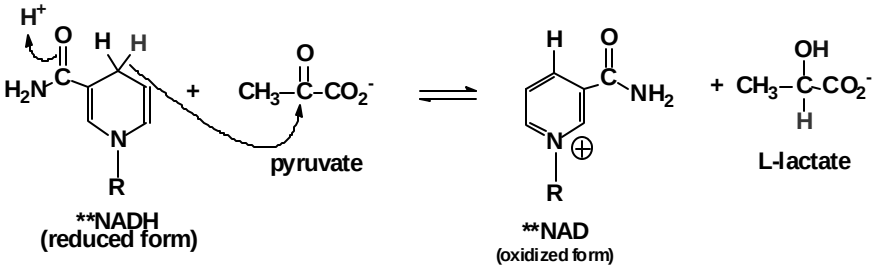
oxamate



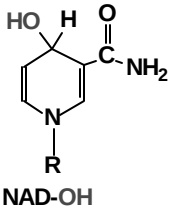
ethyloxamate

For multisubstrate rxns, the type of inhibition depends upon the substrate that is varied in the inhibition experiment!

LACTATE DEHYDROGENASE



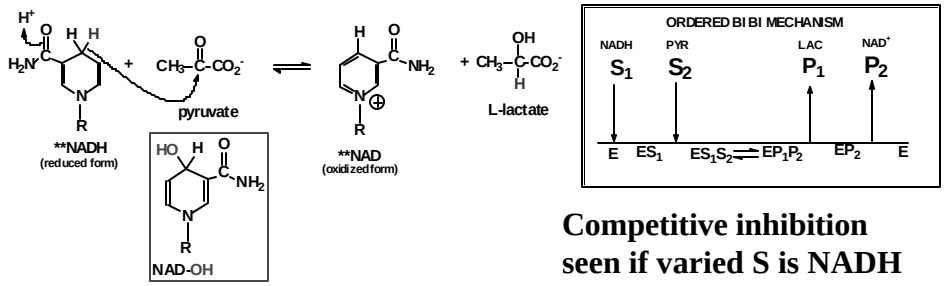
INHIBITORS:



What substrate does this inhibitor resemble?

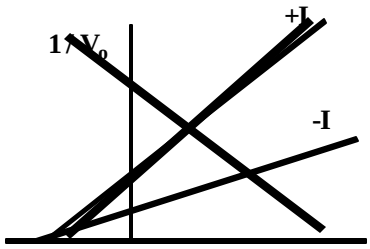
NADH

Which plot describes the inhibition of lactate dehydrogenase by NAD-OH when NADH is the varied substrate?

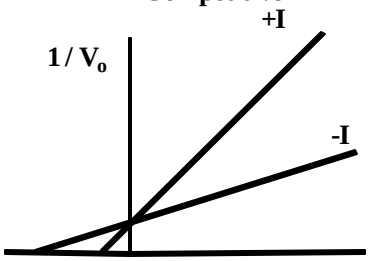


Competitive inhibition seen if varied S is NADH

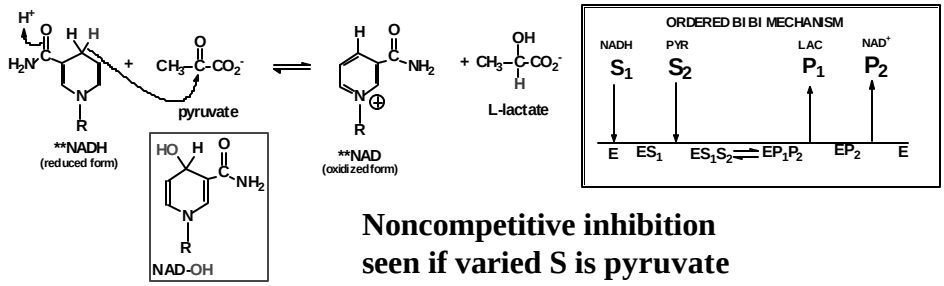
Noncompetitive



Competitive

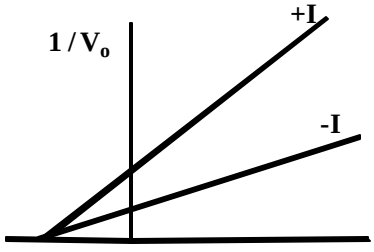


Which plot describes the inhibition of lactate dehydrogenase by NAD-OH when pyruvate is the varied substrate?

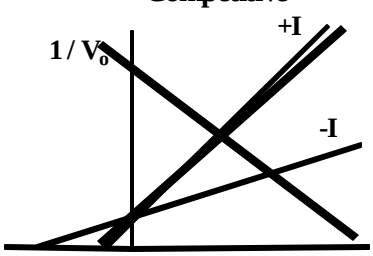


Noncompetitive inhibition seen if varied S is pyruvate

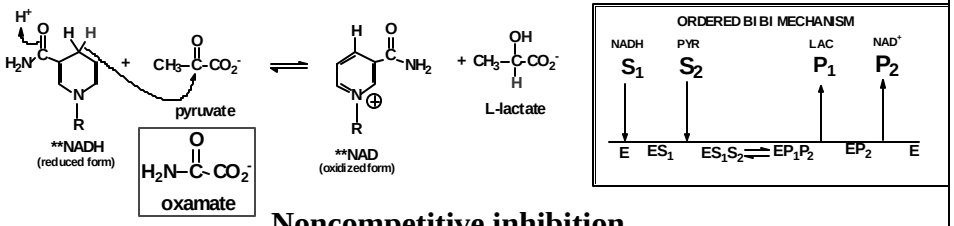
Noncompetitive



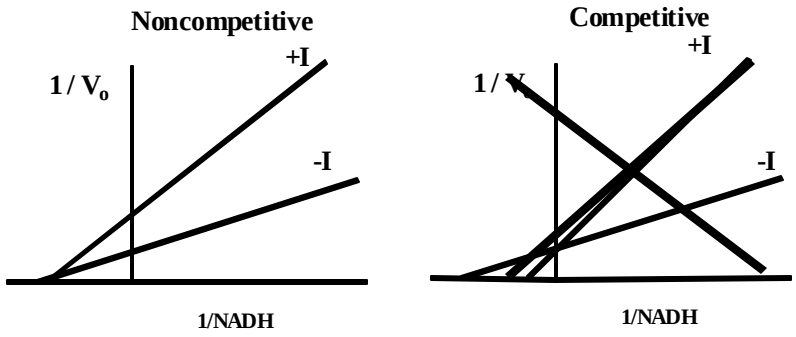
Competitive



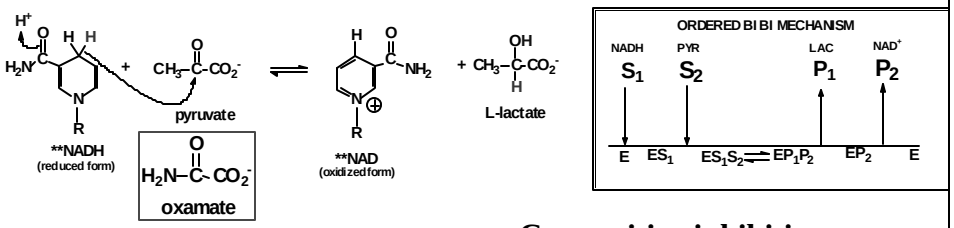
Which plot describes the inhibition of lactate dehydrogenase by oxamate when NADH is the varied substrate?



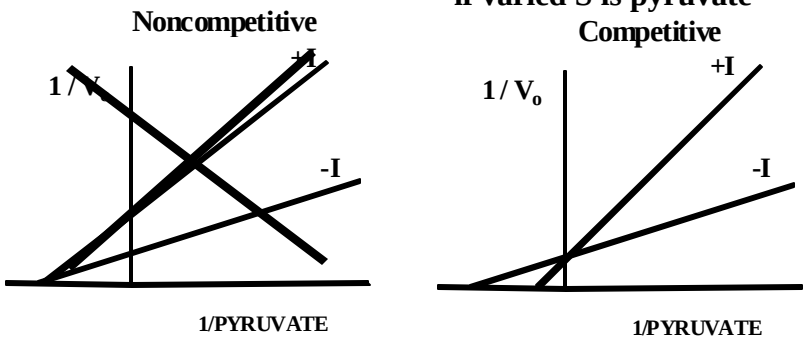
Noncompetitive inhibition seen if varied S is NADH



Which plot describes the inhibition of lactate dehydrogenase by oxamate when pyruvate is the varied substrate?

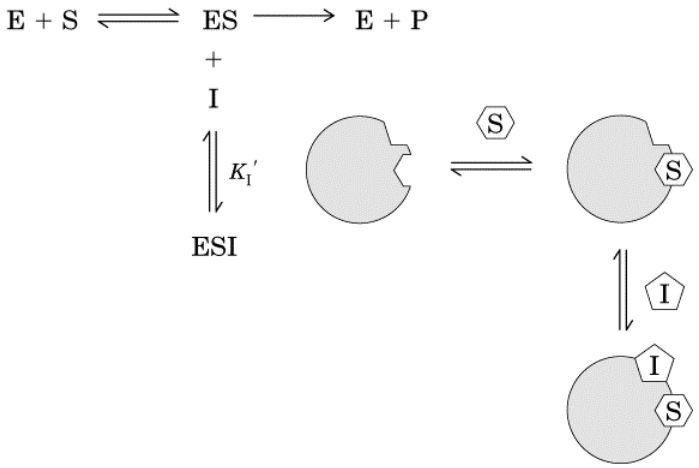


Competitive inhibition seen if varied S is pyruvate



Uncompetitive Inhibition

This type of inhibition requires that one or more substrates bind to E before the inhibitor can bind



(b) Uncompetitive inhibition

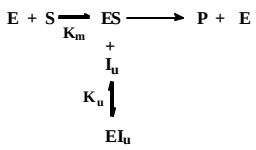
Uncompetitive Inhibition

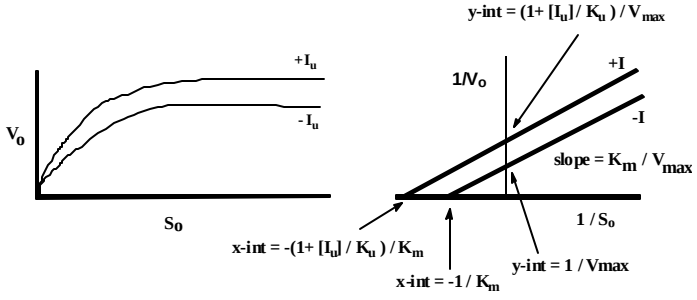
This type of inhibition requires that one or more substrates bind to E before the inhibitor can bind

UNCOMPETITIVE

- $-I_u$ is not structurally similar to S; is not an S
- $-I_u$ binds to ES only; S opens up a site for I_u
- $-I_u$ binding site may be in active site but binding of I_u requires prior binding of S
- High S cannot overcome inhibition because presence of S is required to provide a site for binding of I_u

Equilibria Scheme

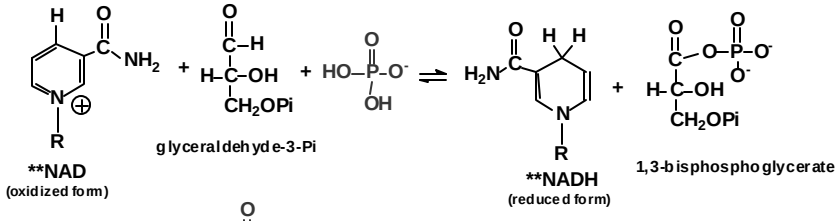




Example: Uncompetitive Inhibition

This type of inhibition requires that one or more substrates bind to E before the inhibitor can bind

GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE



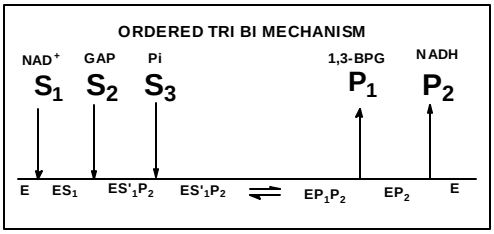
**NAD (oxidized form)

glyceraldehyde-3-Pi

**NADH (reduced form)

1,3-bisphosphoglycerate

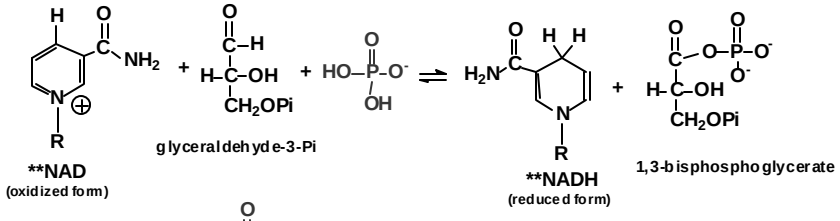
INHIBITOR: 



Predict the type of inhibition by $H_2AsO_4^-$ when each of the substrates is varied in inhibition experiments

This type of inhibition requires that one or more substrates bind to E before the inhibitor can bind

GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE



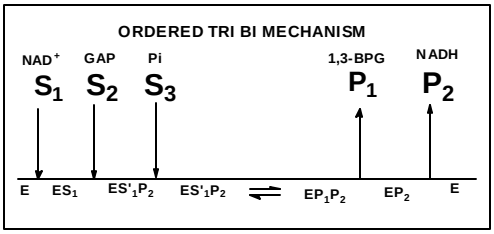
**NAD (oxidized form)

glyceraldehyde-3-Pi

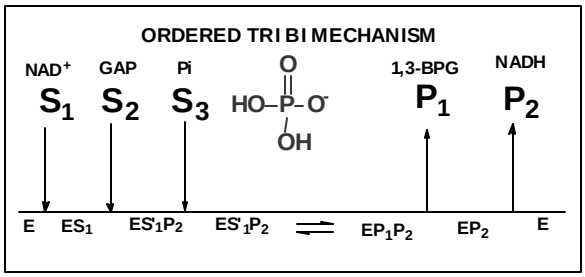
**NADH (reduced form)

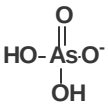
1,3-bisphosphoglycerate

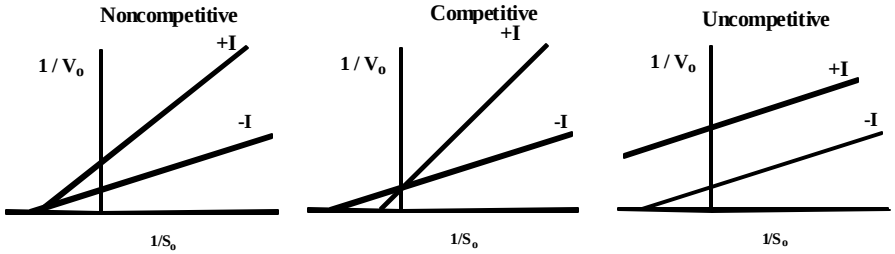
INHIBITOR: 



Predict the type of inhibition by $H_2AsO_4^-$ when each of the substrates is varied in inhibition experiments







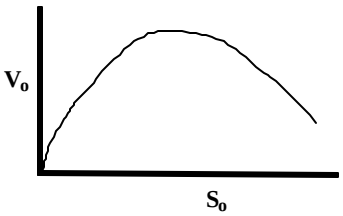
If S_o = Pi

If S_o = NADH or GAP

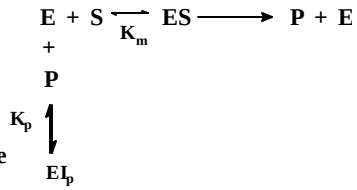
Product Inhibition

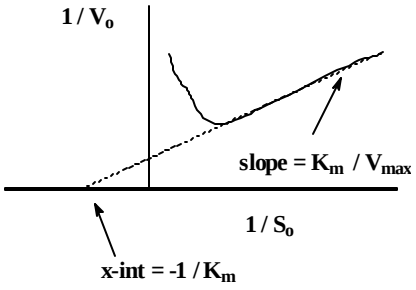
PRODUCT INHIBITION

- Ip is structurally similar to S
- I_p binds to free E at active site where S binds
- I_p competes with S for free E
- At low S, resembles competitive inhibition
- However, at high S, the inhibition is not overcome because higher levels of P are generated which inhibit the enzyme



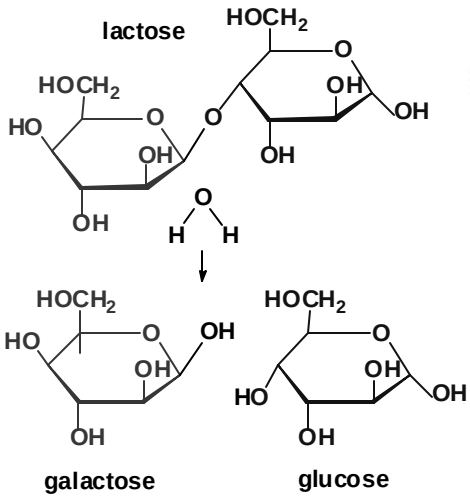
Equilibria Scheme

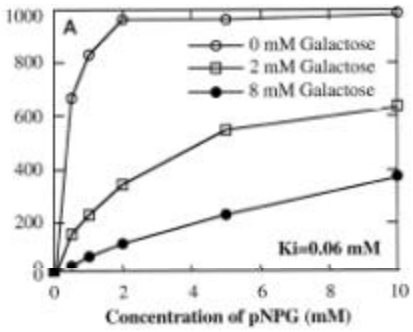




Example: Product Inhibition

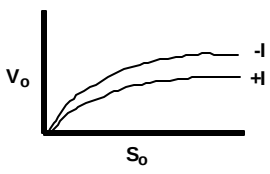
b-galactosidase (lactase)

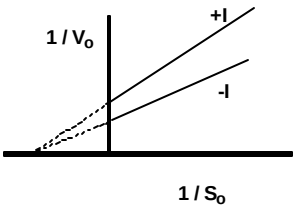




Suicide Inhibition

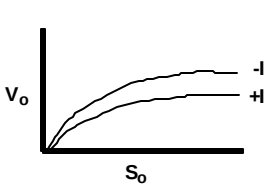
This type of enzyme inhibition results in the stoichiometric covalent modification of a side chain on an amino acid in the active site of an enzyme. The inhibitor chemically resembles a (one of the) substrate(s) and binds in the active site in the same way as the substrate(s) binds. The inhibitor, however, has a functional group, ususally a leaving group, that is replaced by a nucleophile in the enzyme active site. This covalent enzyme-inhibitor complex forms irreversibly, thereby irreversibly inactivating the enzyme. Therefore this type of inhibition is called "suicide inhibition" or affinity labeling and the inhibitor is called a "suicide inhibitor". This reaction with the suicide inhibitor removes active enzyme from the system; this removal is measured as inhibition. Since active enzyme is lost, the inhibition is not relieved at high substrate levels. The rate, at high substrate in the presence of the inhibitor,is still proportional to the amount of the enzyme-substrate complex. However, the maximum amount of that complex is limited by the remaining amount of active enzyme, not by the total enzyme added to the system.

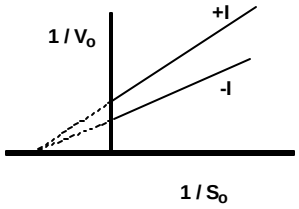


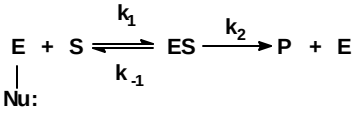


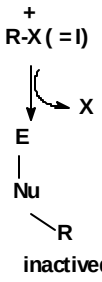
Michaelis-Menten and Lineweaver-Burk plots "look" noncompetitive

Suicide Inhibition





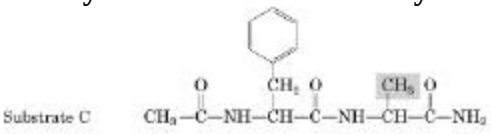


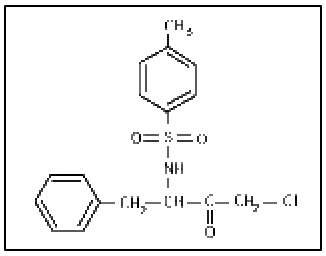


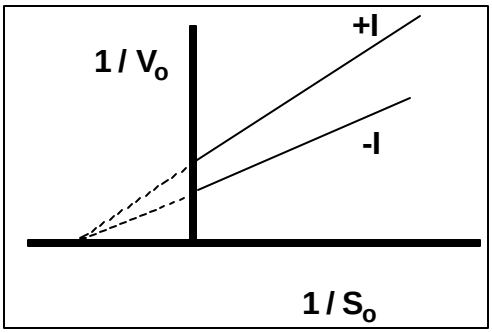
The suicide inhibitor removes E so that the [ES] is lower, V_{max} is lower, and inhibition cannot be overcome at high S_o

Example: Suicide Inhibition with Chymotrypsin

One synthetic substrate for chymotrypsin





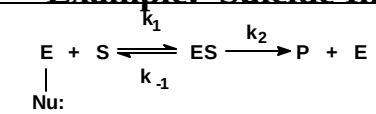


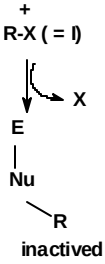
Chymotrypsin Inhibitor

tosyl phenylalanyl chloromethylketone

tpck

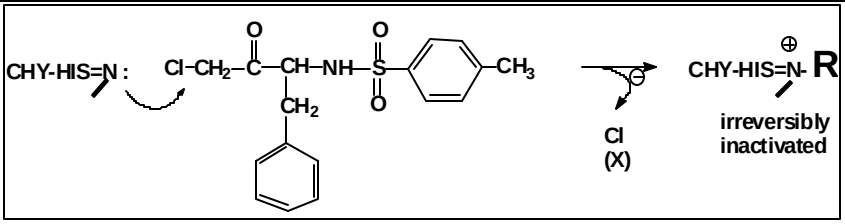
Example: Suicide Inhibition with Chymotrypsin





inactived enzyme

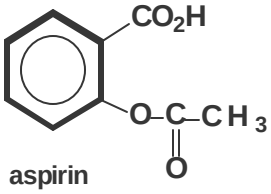
- at all S_o, V_o % [ES]
- I chemically resembles S (I added first, S second to start reaction)
- I reacts 1:1 with enzyme usually
- I lowers [E], therefore lowering [ES] and V_o
- I lowers maximum amount of [ES] because it removes E
- V_o can never reach V_{max}
- resembles noncompetitive inhibition because inhibition cannot be relieved at high S



Suicide Inhibitors: Aspirin

Drug

Aspirin

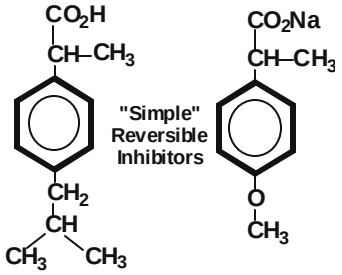


aspirin

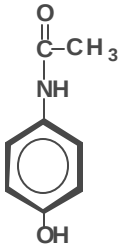
Target Enzymes

(Box 21-1)

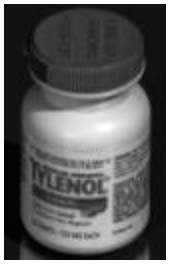
Cyclooxygenases 1 and 2

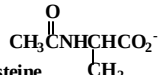


ibuprofen naproxen



tylenol



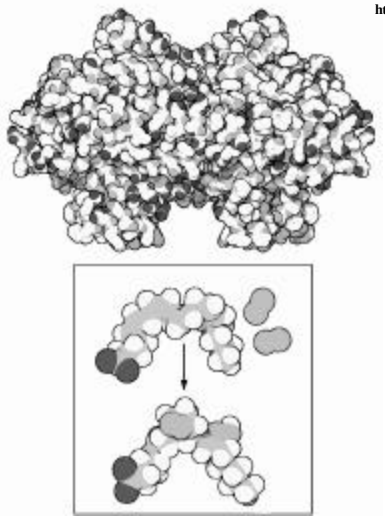


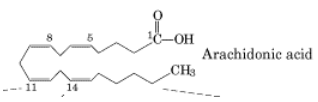
N-acetylcysteine
antidote for
tylenol poisoning

http://cti.itc.virginia.edu/~cmg/Demo/pdb/cycox/cycox_2.html

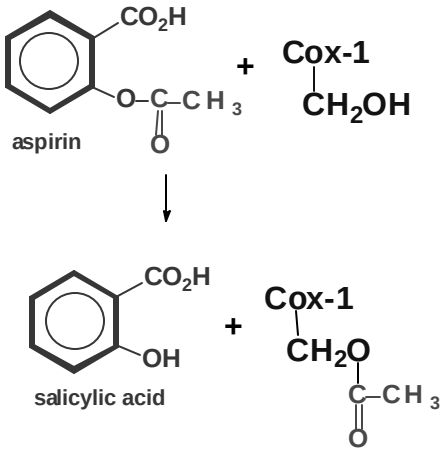
Cyclooxygenase and Modification by Aspirin

http://www.chem.uwec.edu/Webpapers_F98/bruce/Pages/aspcommands.html



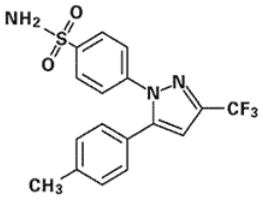


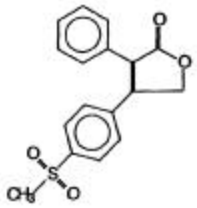
http://www.scripps.edu/pub/goodsell/pdb/pdb17/pdb17_1.html

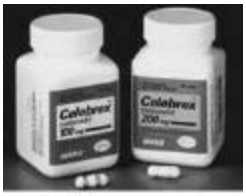


Suicide Inhibitors: New NSAIDs

Drug	Target Enzyme
Celebrex	Cyclooxygenase 2
Vioxx	







Celebrex & Voixx

Tylenol and Vioxx, two other medications commonly used for arthritis, were similarly tested … Both groups showed no competitive interaction with aspirin.

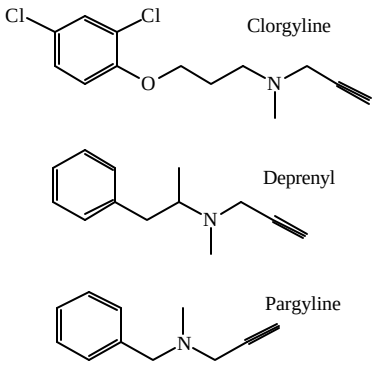
http://www.pslgroup.com/dg/1e8fa2.htm

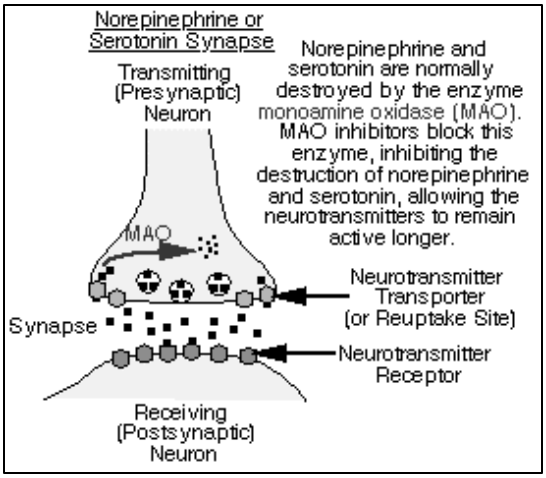
Two phases of inhibition:

1. **Rapid competitive inhibition**
2. **Slower irreversible inhibition (covalent modification)**

Suicide Inhibitors: Inhibitors of monoamine oxidase (MAO) for treatment of depression

www.chem.vt.edu/chem-dept/office/jwolfe/DRUGCHEM1.ppt





http://www.csusm.edu/DandB/AD.html