

Lecture 13: Inhibitors, Measuring K_i , Rational Drug Design

Goals:

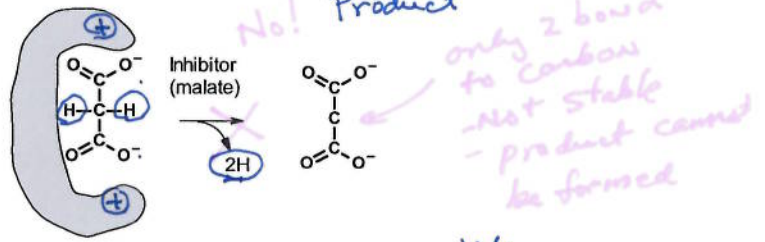
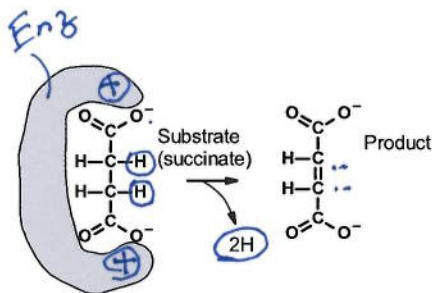
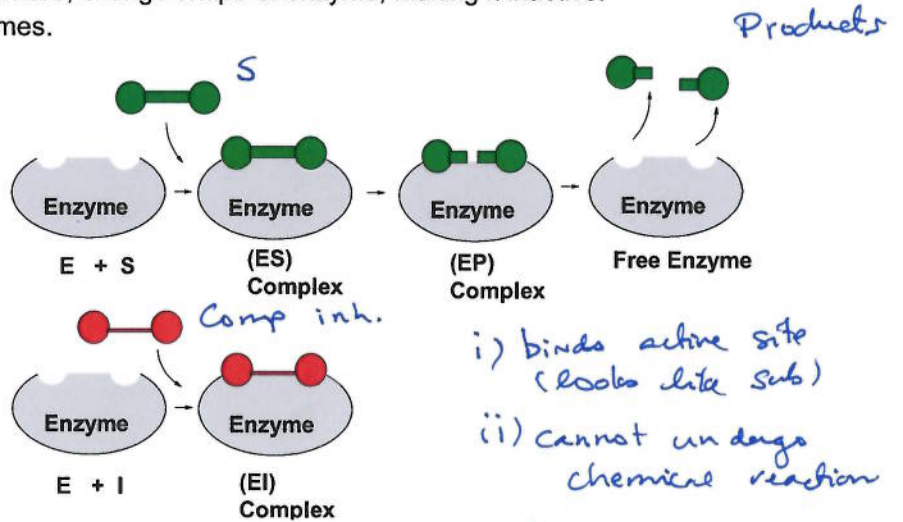
- Distinguish between competitive, allosteric, covalent inhibitors
- Determine K_i from enzyme kinetic data
- Understand principles of rational drug design
- Understand effect of inhibitors on enzyme reaction rates

Enzyme Inhibitors:

1. Competitive Inhibitor: Bind at the active site, block substrate
2. Allosteric inhibitors: Bind elsewhere, change shape of enzyme, making it inactive.
3. Covalent modification of enzymes.

1. Competitive inhibitors:

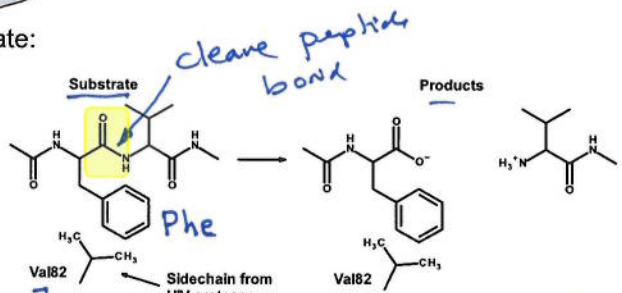
- These are compounds that are similar in structure to the substrate.
- Bind at the active site.
- Cannot undergo a reaction (otherwise they would be substrates).
- Many drugs are competitive inhibitors.



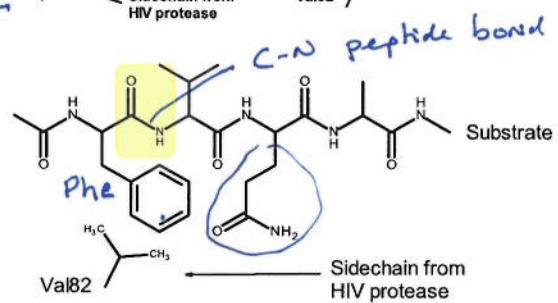
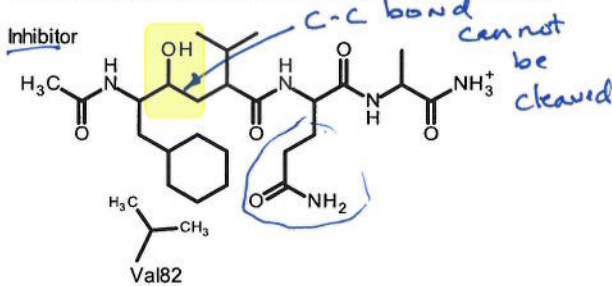
Example: Malate inhibits enzymes that use succinate:

Review- HIV protease:

- Binds to non-polar amino acids (Phe) and cleaves the peptide bond after them.
- Specificity defined by non-polar "specificity pocket" in enzyme active site (Pro, Val, Leu).

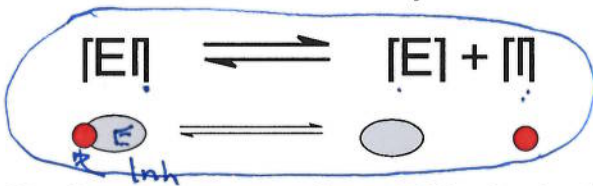


Competitive Inhibitors of HIV Protease:



Identify one difference between the inhibitor (left) and the substrate (right). In what ways are they similar?

Quantitative measure of Competitive Inhibitor Binding:



$$K_I = \frac{[E][I]}{[EI]}$$

when $[I]$, since $[E] = [EI]$

The dissociation constant for an inhibitor leaving the enzyme is defined as:

$$K_I = \frac{[E][I]}{[EI]}$$

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$\frac{1}{2}$ of Enz will have Inh bound when $[I] = K_I$

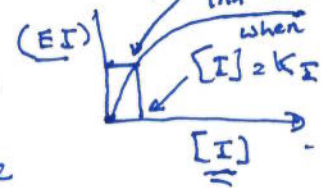
The K_I is the amount of inhibitor required to $\frac{1}{2}$ saturate the enzyme (in the absence of substrate).

When $[E] = [EI]$, $K_I = [I]$

If the $[I]$ is less than K_I less than 50% of the enzymes will have inhibitor bound.

If the $[I]$ is greater than K_I more than 50% of the enzymes will have inhibitor bound.

If the $[I]$ is $10 \times K_I$ 90% of the enzymes will have inhibitor bound.

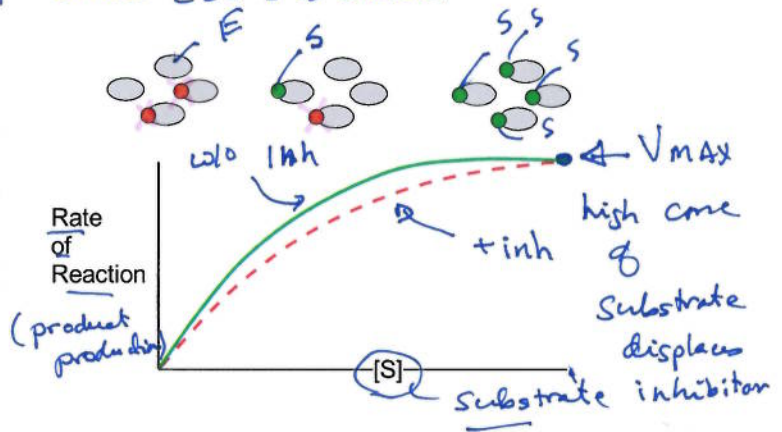


If the inhibitor binds tightly, K_I will be: *small*, since $[EI]$ is large

If the inhibitor binds weakly, K_I will be: *large*, since $[EI]$ is small.

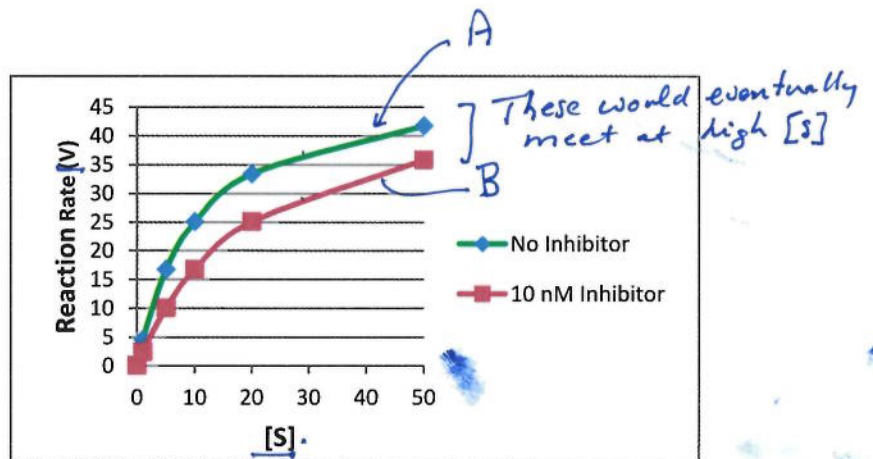
Effect of Competitive Inhibitors on Enzyme Reaction Rates:

- Competitive inhibitors only inhibit at low substrate.
- When the substrate is high the inhibitor cannot bind since both inhibitor and substrate bind to the same place. The high concentration of substrate displaces the inhibitor.
- The effect of the inhibitor on the reaction rate can be used to determine K_I .



Obtaining K_I :

- Measure the reaction rate as function of substrate, with no inhibitor.
- Measure the reaction rate as function of substrate with a fixed amount of inhibitor, $[I]$.
- Plot $1/v$ versus $1/[S]$ for both data (**double reciprocal plot**).
- Obtain the ratio of the slopes = α .



$$K_I = \frac{[I]}{(\alpha - 1)}$$

[S]	rate (I=0) A	Rate (I=10 nM) B
0	0	0
1	4.5	2.4
5	16.7	10.0
10	25.0	16.7
20	33.3	25.0
50	41.7	35.7

No inhibitor present

Inhibitor concentration = 10 nM

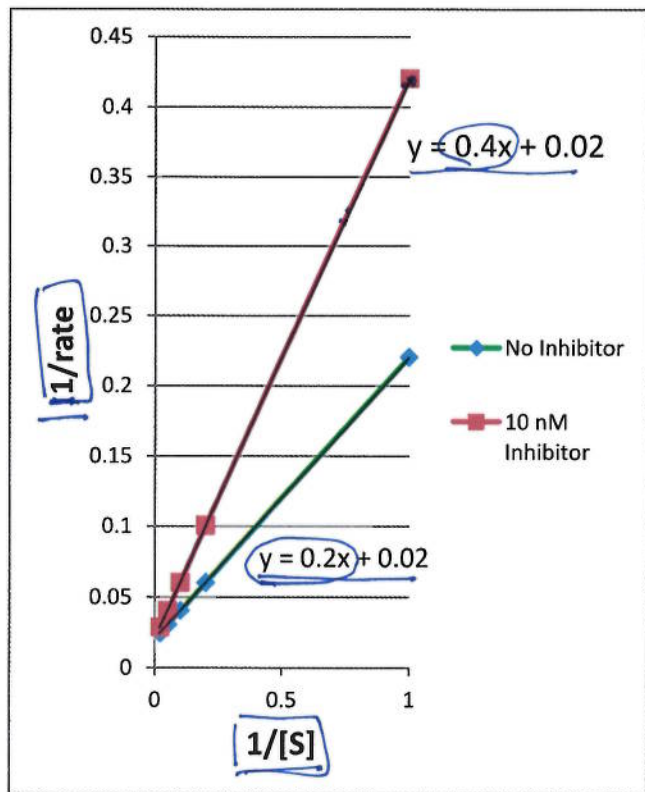
Slope w/o inhibitor = 0.2

Slope with inhibitor = 0.4

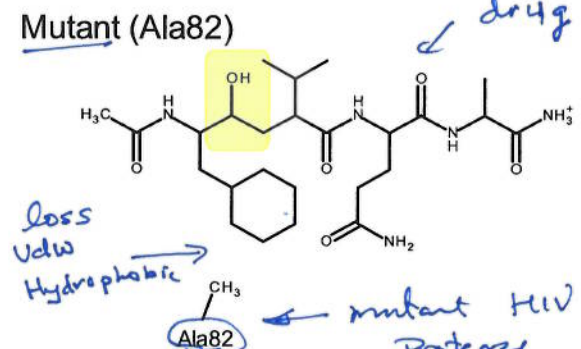
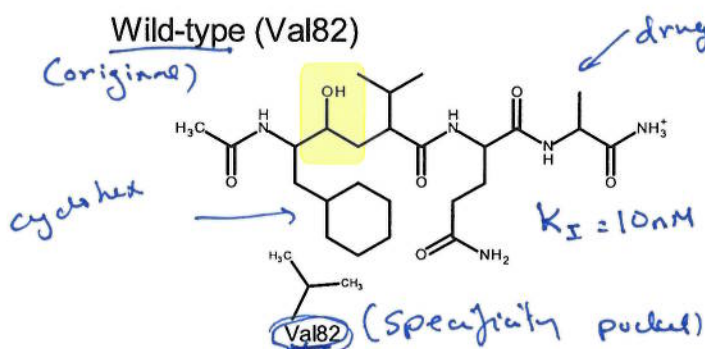
$$\alpha = \frac{0.4}{0.2} = 2$$

$$K_I = \frac{[I]}{(\alpha - 1)} = \frac{10 \text{ nM}}{2 - 1}$$

$K_I = 10 \text{ nM}$



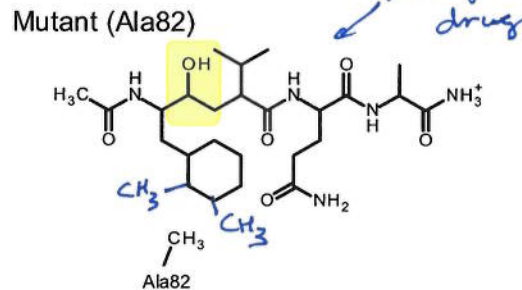
Rational Drug Design – Redesign of a drug in response to mutations in HIV protease.



1. Why will the mutation of Val82 to Ala82 reduce the effectiveness of the HIV protease inhibitor?

higher K_I

2. What could be done to the drug to increase the effectiveness of the inhibitor?



3. How would you experimentally test whether the new drug would be effective at inhibition of the mutant protease?

- measure K_I , should be small.

$$K_I \approx 10 \text{ nM}$$

(Same as original cyclohexane drug & wild type protein)

2. Allosteric (non-competitive) Inhibitors:

These do not bind at the active site, but elsewhere. The change the shape of the enzyme (allo=different, steric=shape). By changing the shape they can decrease the rate of reaction.

- Many of the newer drugs are allosteric inhibitors.

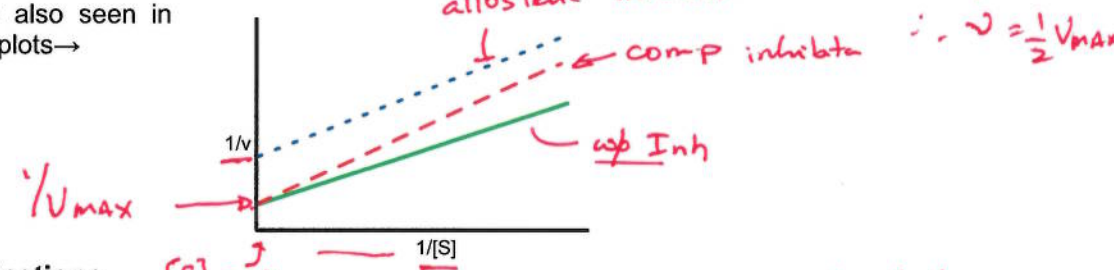
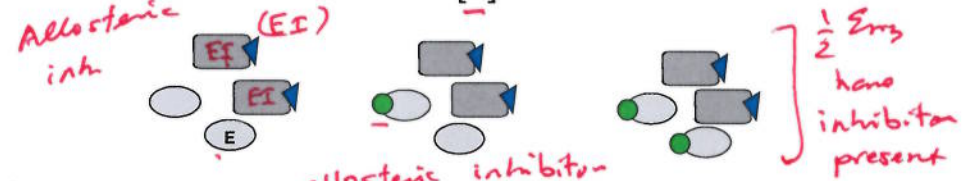
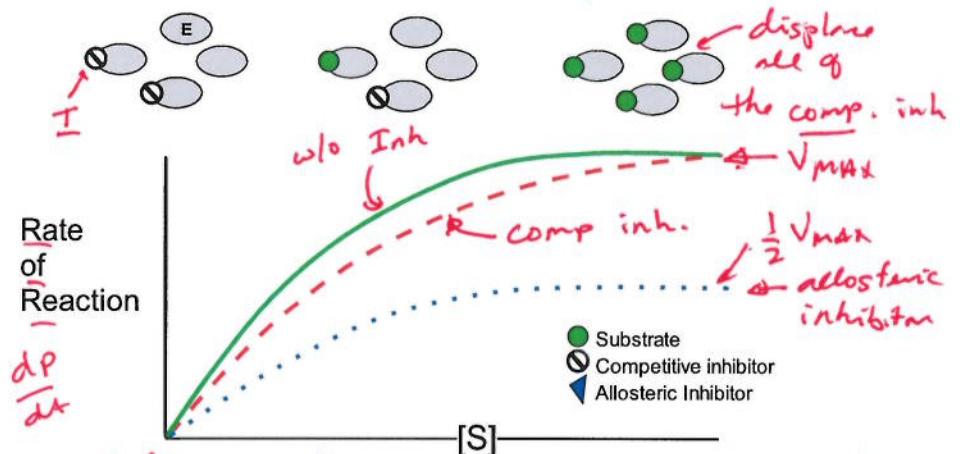
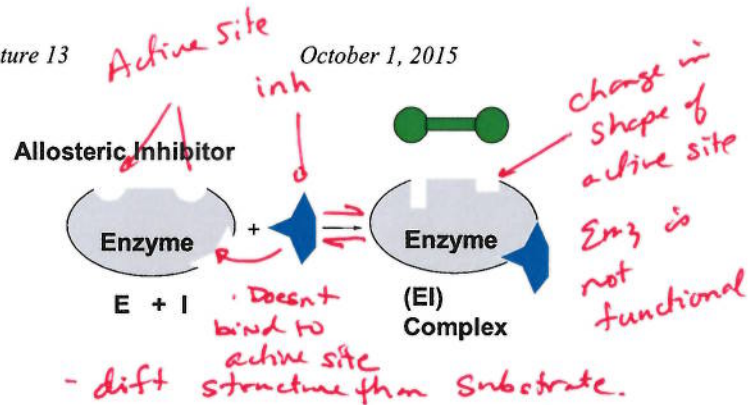
Effect of Allosteric Inhibitors on Enzyme Kinetics:

Allosteric inhibitors will reduce the rate at all substrate concentrations. The bound inhibitor is not displaced by the substrate because it binds at a different location.

This is different than competitive inhibitors which are displaced by high substrate concentrations. Thus allosteric inhibitors are less sensitive to substrate concentration.

The different effect on kinetics can be used to distinguish one type of inhibitor from another.

The difference is also seen in double reciprocal plots →



3. Covalent modifications.

- A chemical group is transferred from the drug to the enzyme.
- The modified enzyme is inhibited.

Example: Aspirin

- Inhibits enzymes (cyclooxygenases, COX) involved in inflammation & pain.
- A serine residue in the active site (required for activity) is acetylated

Expectations:

- Compare and contrast different types of inhibitors (comp, allosteric, covalent)
- Determine K_i from enzyme kinetic data (competitive inhibitors)
- Interpret K_i in terms of molecular interactions (lower K_i , more favorable interactions).
- Redesign of drugs to restore effectiveness of drug by favorable drug-enzyme interactions.

