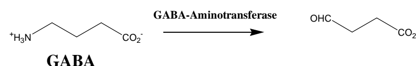
Enzymes as Drug Targets

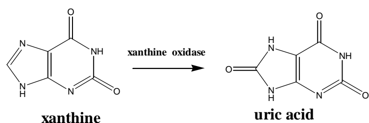
Rationale I

- Chemical deficiencies can be corrected by inhibiting an enzyme that uses the chemical as a substrate
  - Seizures can be caused by insufficient γ-aminobutyric acid (GABA)
  - GABA is degraded by GABA-aminotransferase
  - GABA-aminotransferase inhibitors are anticonvulsant



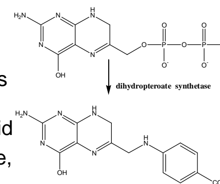
Rationale II

- Chemical excess can be corrected by inhibiting an enzyme that produces the molecule
  - Gout results from excess uric acid
  - Xanthine oxidase converts xanthine to uric acid
  - Inhibitors of xanthine oxidase reduce levels of uric acid



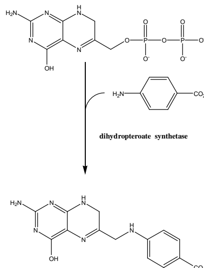
Rationale III

- Inhibition of biochemical pathways unique to a pathogen (bacteria, virus or parasite) can reduce growth or kill the pathogen
  - Bacteria, but not humans, synthesize folic acid
  - Dihydropteroate synthetase is a required enzyme in the bacterial synthesis of folic acid
  - Sulfa drugs (eg. sulfanilamide, 1935) inhibit dihydropteroate synthetase and are bacteriostatic



An Organic Interlude

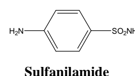
- Propose a mechanism for the reaction catalyzed by dihydropteroate synthetase
- What role does the diphosphate group play?
- Provide a structural reason for that role

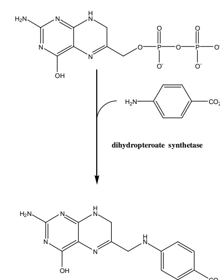


You may work together

Sulfanilamide

- Speculate how sulfanilamide can inhibit dihydropteroate synthetase





Types of Enzyme Inhibition

- Reversible: generally non-covalent interactions
  - Competetive
  - Noncompetetive
  - Uncompetetive

  These categories are based on experimental observation of their kinetic differences
- Irreversible: generally covalent interactions
  - Affinity labeling agents
  - Mechanism-based inactivators

General Enzyme Mechanism

- E = Enzyme
- S = Substrate
- ES = Enzyme-substrate complex (Michaelis complex)
- P = Product

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

General Enzyme Kinetics

- Enzyme reactions are generally studied at low [E] and high [S]
- This results in a steady-state [ES]
- Measurements generally include rate of product formation, v, at various [S]
- The resulting plot can be described by the following equation (Michaelis-Menten equation):

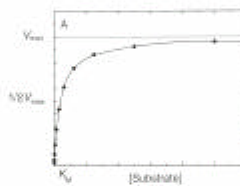
$$v = \frac{v_{\max}[S]}{[S] + K_m}$$

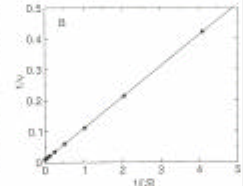
- Given a plot of v versus [S], where will K_m be found?

Enzyme Kinetics Plots

$$v = \frac{v_{\max}[S]}{[S] + K_m}$$

$$\frac{1}{v} = \frac{1}{v_{\max}} + \frac{K_m}{v_{\max}[S]}$$

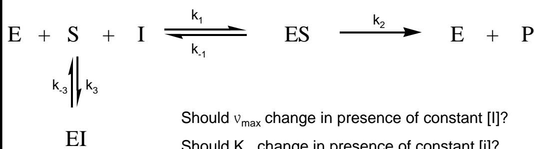




double reciprocal plot
Lineweaver-Burke plot

Competetive Inhibition

- Inhibitor competes with substrate for a single binding site – both cannot bind simultaneously



$$E + S + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

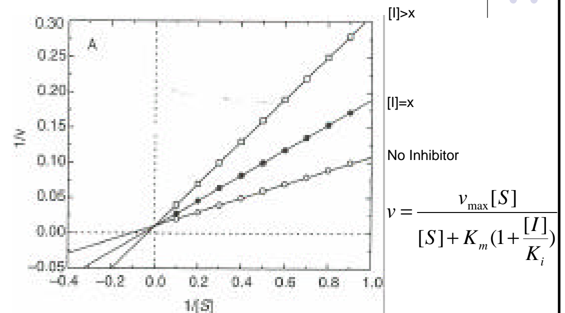
k_{-3} k_3 (E + I ⇌ EI)

EI

Should v_{max} change in presence of constant [I]?

Should K_m change in presence of constant [i]?

Consequence of Competetive Inhibition



[I]>x

[I]=x

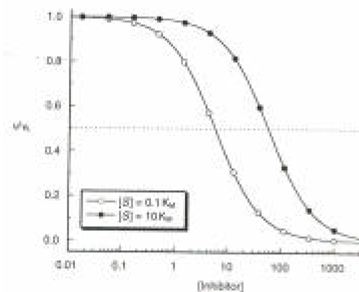
No Inhibitor

$$v = \frac{v_{\max}[S]}{[S] + K_m(1 + \frac{[I]}{K_i})}$$

Practical Assay of Enzyme Inhibition

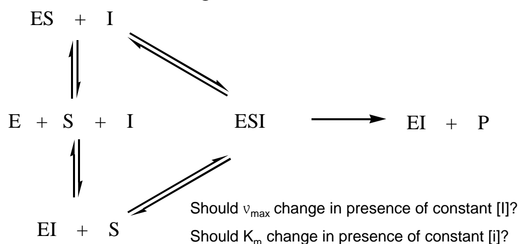
- Assess fractional activity (v/v_0) as a function of [I] at constant [S] and [E]
- Plot dose-response curve (v/v_0 versus –log[I])
- Determine concentration of [I] giving 50% inhibition ($v/v_0 = 0.5$), IC_{50}
- For competetive inhibitors: $IC_{50} = K_i\left(1+\frac{[S]}{K_M}\right)$

Variability in IC_{50}



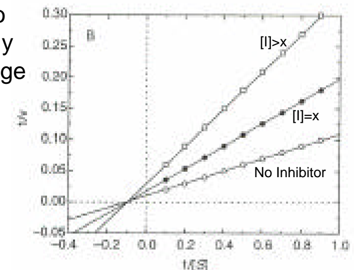
Noncompetetive Inhibition

- Inhibitor binds to a site distinct from the substrate binding site



Non-competetive Inhibitor Kinetics

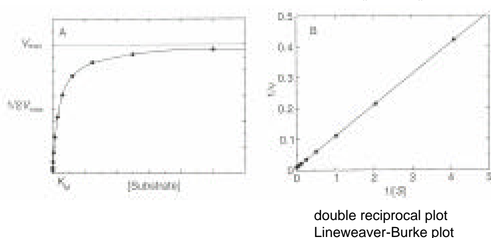
- If inhibitor binds equally well to E and ES, only V_{max} will change



Enzyme Kinetics Plots

$$v = \frac{v_{\max}[S]}{[S]+K_m}$$

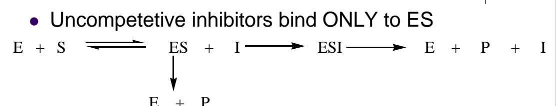
$$\frac{1}{v} = \frac{1}{v_{\max}} + \frac{K_m}{v_{\max}[S]}$$



double reciprocal plot
Lineweaver-Burk plot

Uncompetetive Inhibition

- Uncompetetive inhibitors bind ONLY to ES

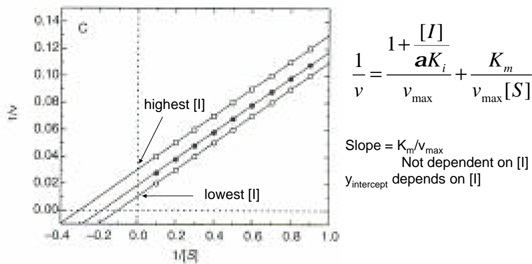


- Uncompetetive inhibition velocity equation:

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

- Given this velocity equation, how will the double-reciprocal plot (1/v vs. 1/[S]) change when the inhibitor concentration is doubled?

Uncompetetive Inhibition



$$\frac{1}{v} = \frac{1+\frac{[I]}{aK_i}}{v_{max}} + \frac{K_m}{v_{max}[S]}$$

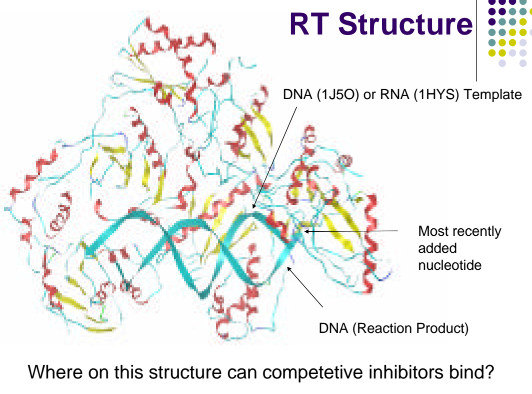
Slope = K_m/v_{max}
Not dependent on [I]

$y_{intercept}$ depends on [I]

Enzyme Inhibitors as Drugs

- Example 1: Human Immunodeficiency Virus (HIV) Reverse Transcriptase (RT)
  - RT catalyzes the production of viral DNA from the viral RNA template
  - Both competetive and noncompetetive inhibitors are known
  - Both competetive and noncompetetive inhibitors are used clinically

RT Structure



DNA (1J5O) or RNA (1HYS) Template

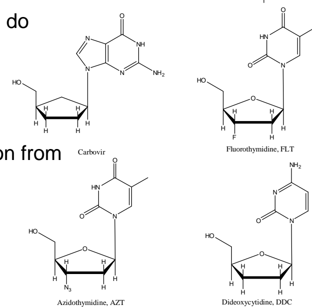
Most recently added nucleotide

DNA (Reaction Product)

Where on this structure can competetive inhibitors bind?

Competetive RT Inhibitors

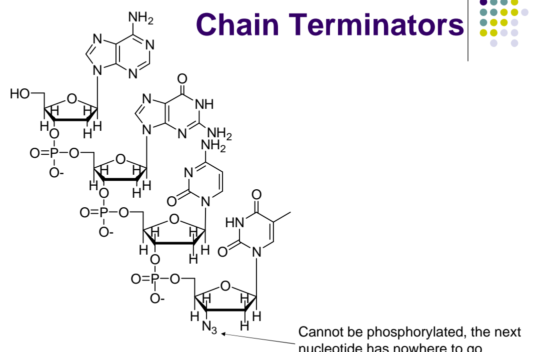
- What biomolecules do these structures resemble?
- Can you infer a mechanism of action from these structures?



Competetive RT Inhibitor Mechanism

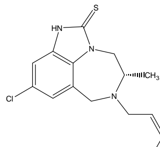
- Download and view 1N5Y from www.rcsb.org
- The residue abbreviated ATM in chain 6 is AZT – view this residue and its immediate vicinity (Note, MOE doesn't include all the bonds involving the phosphates in this structure for some reason)
  - Take note of nearby amino acid UIDs for later reference
- Does this help you to propose a mechanism for the inhibition?

Chain Terminators

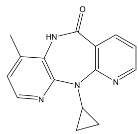


Cannot be phosphorylated, the next nucleotide has nowhere to go

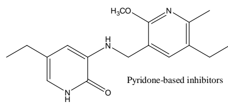
Noncompetetive RT Inhibitors



Tetrahydro-imidazobenzodiazepinone (TIBO) derivatives



Dipyridodiazepinones (Nevirapine)



Pyridone-based inhibitors

Comment on these structures compared with the competitive inhibitors.

Noncompetetitive Inhibitors

- In general, noncompetetive inhibitors are more structurally diverse than competetive inhibitors – Why do you think this is?
  - Competetitive inhibitors are often developed by analogy to the reaction substrate, products, or expected transition state
  - Competetitive inhibitors bind in the same location as the substrate (as well as other competetive inhibitors)
  - Noncompetetive inhibitors bind to allosteric sites, and may each have distinct binding sites

Noncompetetive RT Inhibitor Binding Site

- Download and view structure 1IKW (TIBO) and/or 1HNV (Efavirenz) – coordinate with your neighbors to make sure both are viewed by someone in your vicinity
- How far is the binding site of the inhibitor in your crystal structure from the amino acids you found near AZT in the 1N5Y structure?
- Do TIBO and Efavirenz bind in the same site?

Drug Resistance in HIV

- Resistance to the nucleoside analogs in treated patients develops over the course of about six months
- Resistance to non-nucleoside analogs in treated patients develops over the course of about six weeks
- Discuss with your neighbors possible reasons for the time difference

Drug Synergism

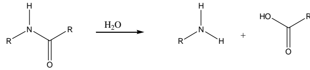
- HIV is now commonly treated with drugs that act synergistically (effect of combination is greater than sum of individual drug effects)
- Synergism can arise:
  - Through inhibition of a drug-destroying enzyme
  - Sequential blocking
    - HIV treatments often include inhibitors of reverse transcriptase and protease
  - Inhibition of enzymes in multiple pathways
  - Use of multiple drugs for the same target
    - HIV treatments often include both nucleoside and non-nucleoside inhibitors

Transition State Isosteres

- Enzymes often lower activation energy by stabilizing transition states to a greater degree than either substrates or products
- This indicates that compounds which won't undergo the reaction catalyzed by the enzyme, but resemble the transition state, should bind at the active site and inhibit the enzyme competetively

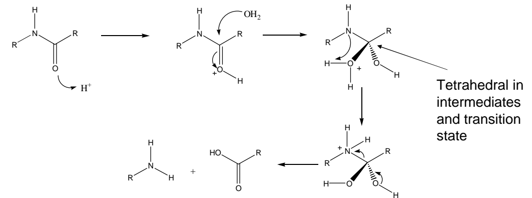
Transition State Isostere Example

- Isostere = same size/shape
- HIV protease
  - catalyzes hydrolysis of peptide bonds in viral polyprotein to form functional viral proteins



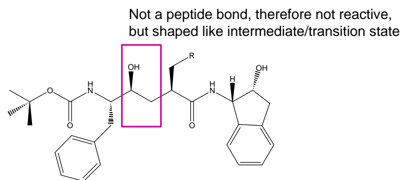
  - What mechanistic steps occur in acid-catalyzed peptide bond hydrolysis?

Peptide Hydrolysis Mechanism



HIV Protease Inhibitor

Not a peptide bond, therefore not reactive, but shaped like intermediate/transition state



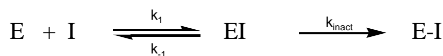
Where is the transition state isostere?

Irreversible Inhibitors

- Affinity labeling agents
  - Contain a reactive group
  - First form noncovalent EI complexes, then undergo reaction with enzyme to form a covalent bond (E-I)
  - Example: acylating agents such as penecillins
- Mechanism-based inactivators
  - Initially unreactive
  - Enzyme acts on them to generate a reactive compound, which inactivates the enzyme

Affinity Labeling Agents

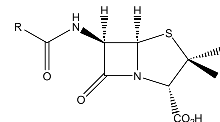
- Effective inactivation requires $k_{inact} >> k_{-1}$
- This means that I must have affinity (favorable interactions) with E



$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EI \xrightarrow{k_{inact}} E\text{-}I$$

Penecillins – Acylating Agents

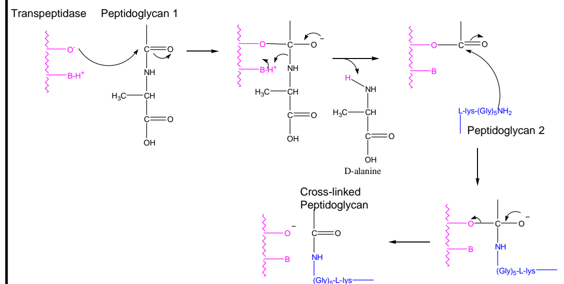
- Penecillins are a family of structures produced by molds in the *Penecillium* family
- Their activity was discovered in 1928 by Alexander Fleming
- They were not widely used until the late 1940's
- Their structures were eludicated in 1943 by Robinson and Folkers
- Two are still used today
  - R=$PhOCH_2$ is penecillin V
  - R-$PhCH_2$ is penecillin G



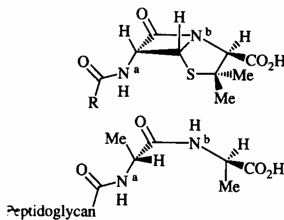
Penecillin Activity/Mechanism

- Penecillins are antibiotic compounds
- Penecillins disrupt bacterial cell wall cross-linking, for which there is no analagous process in humans
- Penecillins inactivate the enzyme transpeptidase

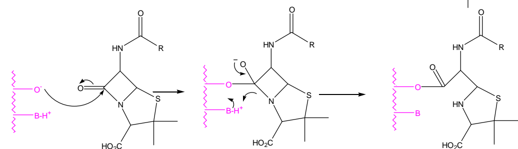
Transpeptidase Mechanism



Penecillin: Substrate Analog



Transpeptidase Inactivation



This reaction stops at the acylated enzyme
Propose an explanation

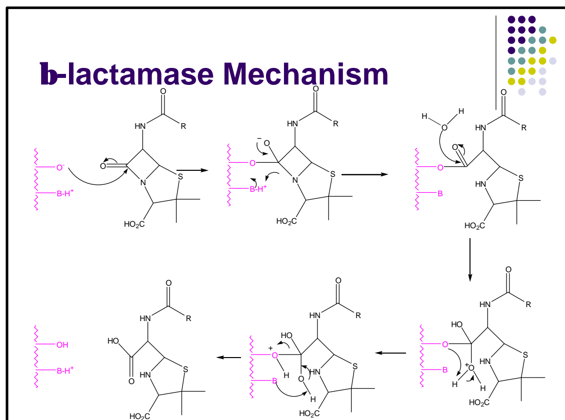
Mechanisms of Drug Resistance

- Altered drug uptake
- Overproduction of target enzyme
- Altered target enzyme
- Production of drug-destroying enzyme
- Overproduction of target enzyme substrate
- Deletion of prodrug-activating enzyme
- Target enzyme product formation by alterate path

Penecillin Resistant Bacteria

- Some bacteria have developed penecillin resistance by expressing an enzyme called β-lactamase
- β-lactamase opens the 4-membered lactam ring of penecillins

β-lactamase Mechanism



Mechanism-Based Enzyme Inactivators

- Initially unreactive
- Enzyme converts to a reactive compound

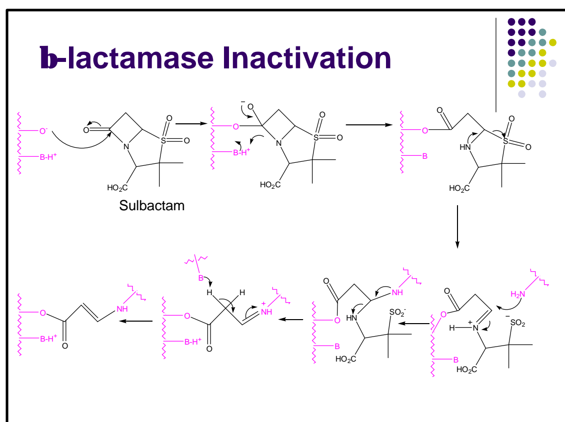
$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EI \xrightarrow{k_2} EI' \xrightarrow{k_{inact}} E\text{-}I'$$

$$EI' \xrightarrow{k_3} E + P$$

Benefits: I unreactive, other proteins safer (more selective)

Disadvantages: P may cause problems if k_{inact} is not >> k_3

β-lactamase Inactivation



Reading

- Chapter 5 – The Organic Chemistry …
  - All chapter 5 problems
- Chapter 12 – Textbook …