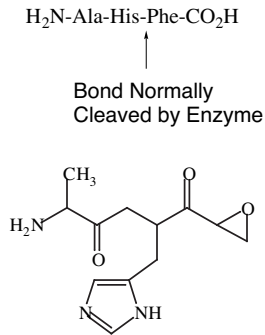


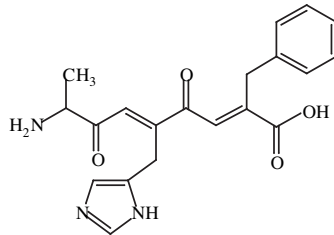
CHEM 4170
Homework 3 - ANSWERS

1. Consider the following (imaginary) example. An endogenous (naturally occurring) peptide called intelligensin is shown below. Intelligensin is responsible for learning and storage of memories in mammals. Some animals, such as Golden Retrievers, seem to express high levels of an enzyme that degrades intelligensin. This enzyme is called intelligensinase, it is a protease enzyme that degrades the peptide as shown below. Design an affinity-labeling agent for intelligensinase.



The left side of the molecule provides "affinity" the right side (the epoxide) reacts with some active site nucleophile to "label" the enzyme covalently.

2. Design a peptidomimetic reversible inhibitor for the protease described above (intelligensinase).

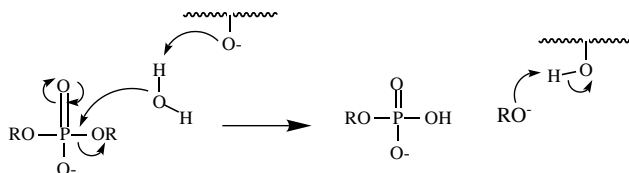


Peptidomimetic retains key features of peptide (usually side chains), but does not contain amide bonds that can be hydrolyzed by the protease enzyme.

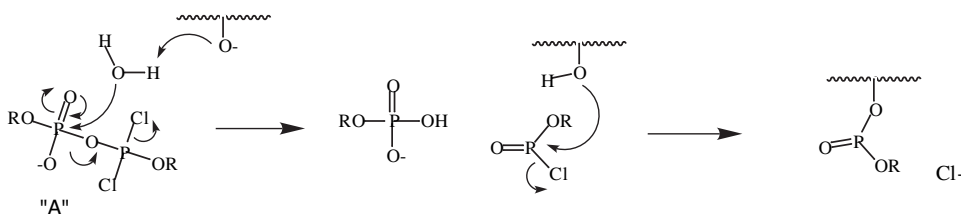
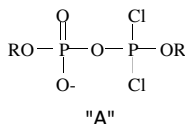
3. What is the expected biological effect of the inhibitor described above? Will it make my dog smarter or (even) less intelligent?

It will make my dog more smarter. A much needed drug in veterinary medicine.

4. Enzymes called phosphodiesterases catalyze the hydrolysis of phosphodiesters as shown below.



Offer a mechanism that explains why "A" is a mechanism-based inactivator of phosphodiesterases. Show exact how the enzyme is covalently modified. (Hint One: enzyme inactivators are usually electrophiles... what is the electrophile generated here? Hint Two: remember the reactivity of acid chlorides that we saw in lecture: RCOCl .)

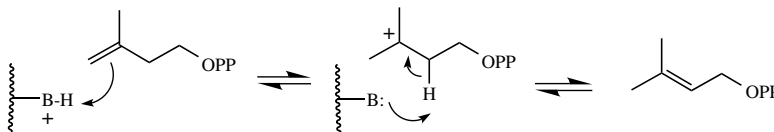


As we saw in the lecture regarding mech based inact, acid chlorides are reactive electrophiles that can "label" active site amino acid side chains. This is just a phosphorus analog of an acid chloride... does the same type of chemistry

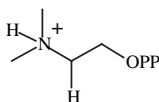
5. When taking a reversible enzyme inhibitor, why is it necessary to take frequent doses?

Because the drug is in equilibrium with the "free" (unbound) form. The unbound form can be excreted and/or metabolized and excreted. So drug concentration drops over time. To maintain the drug at concentrations required for enzyme inhibition (enzyme binding) it is necessary to take another dose.

6. Design a transition state analog inhibitor for isopentenyl phosphate isomerase, whose mechanism is shown below.



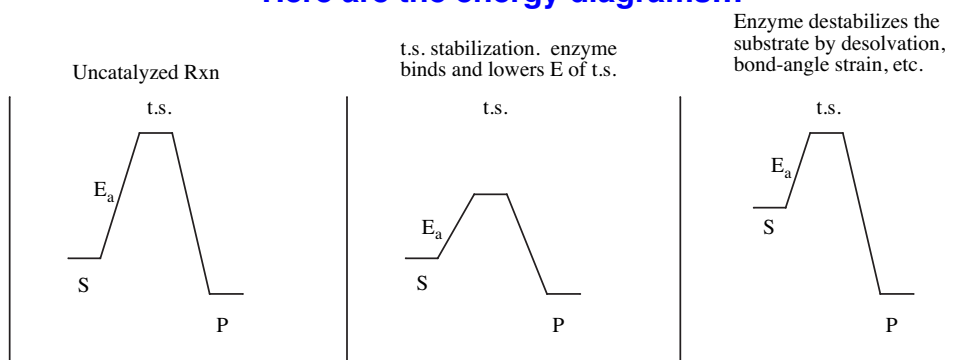
We cannot synthesize the true transition state/intermediate of this (or any) enzymatic reaction and use IT as an inhibitor because transition states and (to a lesser extent) intermediates are unstable, fleeting species. We need to design a STABLE compound that retains key structural elements expected to be present in the transition state of the reaction.



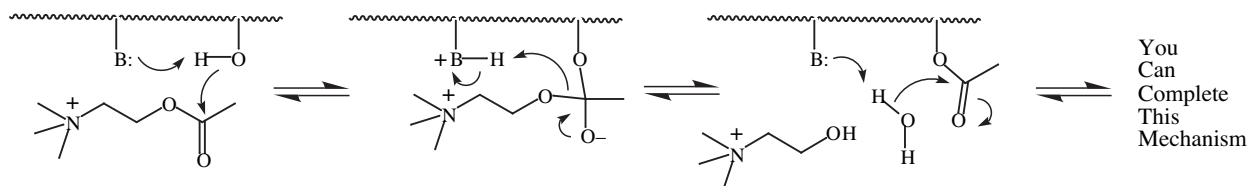
How about this one above? This N-containing compound is good because it has a positive charge (at pH 7). It doesn't have the sp^2 -hybridized, planar geometry found in the transition state (which is "bad"), but still has a K_i of 14 pM (picomolar! 14×10^{-12} M). Reardon and Abeles *Biochemistry* 1986, 25, 5609.

7. Use a reaction coordinate diagram (plot of ΔG vs rxn coordinate) to graphically depict two modes of enzymatic catalysis: transition state stabilization and substrate destabilization by strain and distortion.

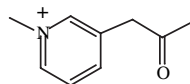
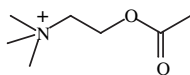
Here are the energy diagrams...



8. Acetylcholinesterase is the enzyme responsible for deactivating (destroying) the neurotransmitter acetylcholine. The mechanism of this enzyme is shown below.

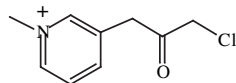
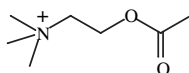


(a) using principles developed in this course, design a reversible inhibitor of this enzyme



Reversible enzyme inhibitors typically resemble the normal substrate of the enzyme. The structural analogies to the normal substrate are clear in the inhibitor that I've designed, I think

(b) design an affinity labelling agent for this enzyme (not the one I showed in class)



The affinity labelling agent that I've designed retains ability to bind noncovalently to the enzyme active site and contains a mildly reactive electrophile that can react with an active site nucleophile when held in close proximity to a nucleophilic center at the active site.

9. The K_i of drug **A** for its target enzyme is 6 nM. The K_i of drug **B** for its target enzyme is 10 μ M.

(a) based upon the data that you are given, which drug would you predict to be more potent?

Smaller K_i means better binding to the enzyme active site. A is better.

(b) Let's assume that these drugs diffuse completely and randomly through all the fluid space of the human body. Let's also assume that there are 50 liters of fluid in a human (as we have calculated before). How much drug **A** will you need to administer to achieve 50% inhibition of the target enzyme? How much drug **B**? ASSUME THAT AN "AVERAGE" DRUG HAS A MW = 250 gm/mol.

At LOW substrate concentrations ($[S] \ll K_M$) the "rule of thumb" says K_i is the concentration required to cause 50% inhibition of the target enzyme. To convince ourselves that this is true, look at the Equation 22 on page 570 of the enzyme kinetics handout (the second one) on the course website. See the equation for v :

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

And note that when $[S] \ll K_M$:

$$K_M \left(1 + \frac{[I]}{K_i}\right) + [S] \sim K_M \left(1 + \frac{[I]}{K_i}\right)$$

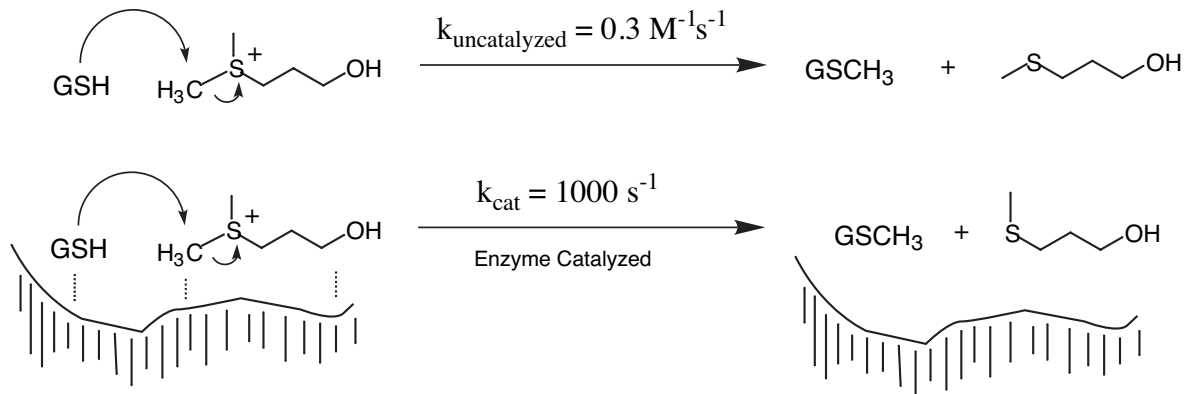
And when $[I] = K_i$, $[I]/K_i = 1$ so:

$$v = \frac{V_{\max} [S]}{2K_M}$$

v = in the presence of inhibitor (at $[I] = K_i$) is half of what it would be without the inhibitor.

So, we need to achieve a 6 nM concentration in the 50 L of human body fluid. $6 \times 10^{-9} \text{ M} = x \text{ mol}/50 \text{ L}$ $x = 3 \times 10^{-7} \text{ mol}$ if MW=250 this is 0.075 mg of drug is required. That is probably less than the weight of a typical grain of salt.

10. One way that enzymes catalyze chemical reactions is by lowering the entropy of activation. In lecture, and in your textbook, this has been called "catalysis by proximity". A high "local concentration" of the reaction partners is created by holding the two molecules together in close proximity. This effect is quantitatively assessed using a term called "effective molarity". Consider the reactions of glutathione (GSH) with the sulfonium ion shown below and calculate the effective molarity of glutathione in the enzyme-catalyzed reaction.



$$k_{\text{enzymatic}}/k_{\text{uncatalyzed}} = 1000 \text{ s}^{-1} / 0.3 \text{ M}^{-1}\text{s}^{-1} = \text{Effective Molarity is } 3333 \text{ M}$$

In other words, in order to achieve the same reaction rate that as that which occurs on at the enzyme active site would require over 3000 M concentration of GSH. This is a theoretical concept. This concentration of GSH cannot actually be achieved in aqueous solution.

21. 1. For the system:



The reciprocal of an expanded form of the Michaelis-Menten equation is often written as follows:

$$\frac{1}{v} = \frac{k_{-1} + k_3 + k_1[S]}{k_1 k_3 [E_T][S]}$$

where: S is the substrate, E_T is the total enzyme, and v is defined as:

$$v = \frac{dP}{dt} = -\frac{dS}{dt}$$

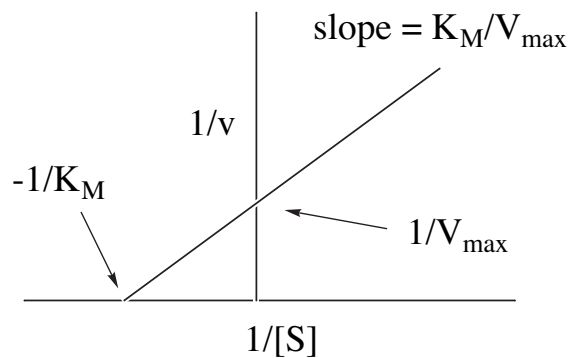
a. Define the terms K_m and V_{max} using the parameters that are shown above.

$$V_{max} = k_3[E_T] \quad K_M = \frac{k_{-1} + k_3}{k_1}$$

b. Rewrite the Michaelis-Menten equation using the terms K_m and V_{max} .

$$1/v = \frac{K_M + [S]}{V_{max} [S]}$$

c. Draw a plot and annotate it so that it shows how you can obtain the values of K_m and V_{max} from a series of measurements of the initial reaction velocities v_o at a set of initial $[S_o]$, in other words $v_o [S_o]$ pairs.



22. Look at the handout regarding the kinetics of enzyme inhibition (posted on the website). Under the following conditions, calculate the enzyme velocity (v_0) in terms of V_{\max} .

$$\text{enzyme } K_M = 1 \times 10^{-3} \text{ M}$$

$$[S] = 1 \times 10^{-3} \text{ M (1 mM)}$$

$$\text{drug } K_i = 1 \times 10^{-6} \text{ M}$$

$$[\text{Drug}] = 1 \times 10^{-6} \text{ M (1 } \mu\text{M)}$$

Use Eqn 22 from page 570 of the handout on enzyme kinetics posted on the course website (the second one related to enzyme kinetics):

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

Using this equation you can calculate that, under these conditions, in the *absence* of inhibitor ($[I]=0$), v would equal $V_{\max}/2$. In the presence of inhibitor $v = V_{\max}/3$. Thus, when $[S]=K_M$ addition of inhibitor at a concentration equal to K_i leaves the enzyme working at 66% of its original rate. One thing to learn here is that the effect of an enzyme inhibitor depends, in part, upon the substrate concentration. This is because the inhibitor and the substrate are competing for binding to the active site. Whether drug or substrate "wins" this competition is dependent upon drug concentration, substrate concentration, K_M and K_i , just like we saw in Question 7 (for receptor binding).