

Matching (20 pts). Place the letter of the best answer in the blank on the left (use each answer only once)

1. Mechanism-based inactivator __G__

2. Reversible enzyme inhibition __J__

3. K_M __F__

4. Affinity labeling agent __H__

5. Enzyme __S__

6. K_i __C__

7. IC_{50} __L__

8. Effective molarity __P__

9. Endogenous substrate __O__

10. Nucleophilic catalysis __A__

- A. Reaction with the catalyst generates an intermediate that must be more reactive than the starting material
- B. Better nucleophiles will always be superior in this role
- C. Dissociation constant for enzyme-inhibitor complex
- D. Association constant for enzyme+inhibitor
- E. Higher value indicates better binding of substrate to enzyme
- F. Lower value indicates better binding of substrate to enzyme
- G. Converted to a reactive electrophile by the target enzyme
- H. Contains an inherently reactive electrophile
- J. Enzyme activity returns when excess drug is removed from solution
- K. Binds inversely to active site pocket
- L. Concentration required to cut enzyme activity in half
- M. Equal to K_i at high substrate concentration
- N. Inactivation coefficient at a concentration of 50 μ M.
- O. Can serve as a lead compound for inhibitor design
- P. A measure of how proximity accelerates a given chemical reaction
- Q. Interacts with the biological target primarily by electrostatic forces
- R. Molar concentration required to effectively shift the reaction equilibrium in the forward direction
- S. A protein catalyst

11. (10 pts) Imagine that the active site pocket of an enzyme is lined with leucine, valine and phenylalanine side chains.

Leu, Val, Phe are all "hydrocarbon" side chains

(a) Do you expect the "effective dielectric constant" on the interior of this binding pocket to be... (circle the best answer)

These side chains make the interior of the pocket like organic solvent, what is the dielectric constant of an organic solvent? More or less than water?

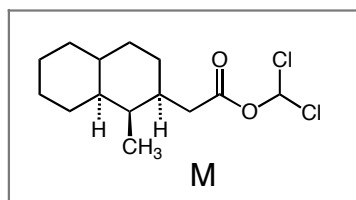
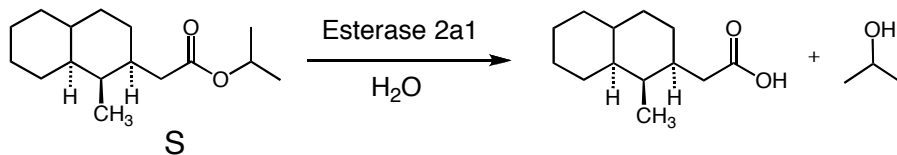
greater than 78 less than 78 about 78 condensed expanded preorganized

(b) This active site would favor binding by which type of inhibitor?

Which of the properties listed indicates that a small molecule has affinity for (solubility in) organic solvent?

positive LogP negative LogP LogP = 0 flexible macroscopic rigid

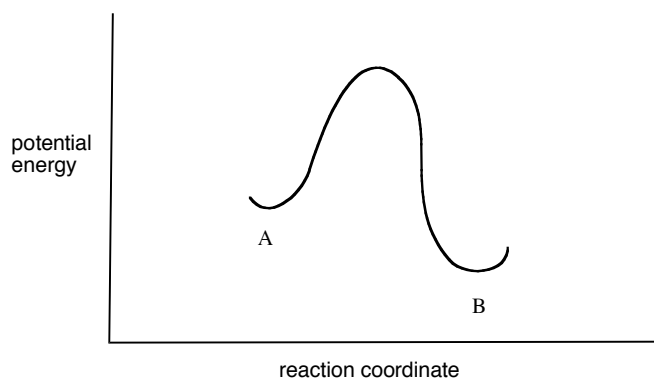
12. (10 pts) The enzyme "esterase 2a1" catalyzes the reaction shown below. Compound **M** causes time-dependent inactivation of esterase 2a1. That is, the enzyme activity decreases over a period of about 1-2 hours. The inactivation of esterase 2a1 by compound **M** is *slowed* by the presence of a substrate (**S**) in the reaction mixture. Explain what is happening here (in words) and provide a chemical mechanism (drawing structures)



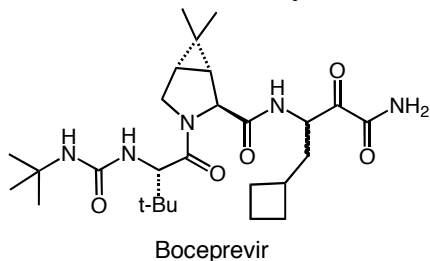
Time dependent inactivation indicates covalent modification of the enzyme. This inactivator is very similar to the zinc-dependent protease inactivator that we discussed in class. Also similar to the one shown in the paper posted on the website. Also similar to one problem on the homework. It begins as a normal ester hydrolysis, but then “blows up”.

13. (10 pts) Label the transition state and activation energy on the diagram. Draw an energy diagram showing for this same reaction *catalyzed by an enzyme*. (OVERLAY your drawing on the one provided below).

T.S. is at the peak between A and B. The “distance” A→T.S. is E_a . The enzyme catalyzed reaction has the same A and B energies but *lower* T.S. energy.



14. (10 pts) What is the role of the α -ketoamide group in boceprevir? A complete answer requires chemical structure/enzyme mechanism diagrams.

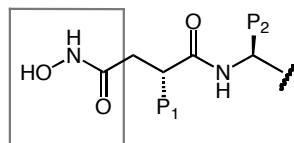


See the paper posted on the website describing the boceprevir story. This shows the chemistry of inactivation.

15. SHORT ANSWER (5 pts) Does the mechanism by which boceprevir blocks the NS3 protease of HCV fit neatly into any of the enzyme inhibitor/inactivator categories that we discussed in class? Briefly discuss why it does or does not.

It does not fit into any of the categories that we discussed. Covalent, yet reversible enzyme inhibition. For the mechanism see the boceprevir story.

16. (5 pts) Matrix metalloproteinases (MMPi) are Zn(II)-dependent protease enzymes that are involved in tumor metastasis. Thus, inhibitors of MMPi's are desired. Many of the successful lead compounds in this area include hydroxamic acid groups attached to peptidic scaffolds. Propose a likely role for the hydroxamic acid group (in the box).



We talked about zinc-dependent proteases in class. We defined the meaning of P_1 and P_2 sites in proteases. Look at the ACE inhibitors like captopril and enalaprilat. What is the role of the functional groups on the "left side" of the P_1 site in these inhibitors? The hydroxamic acid group fulfills the same role. Why is the hydroxamic acid group good in this role?

17. (10 pts) The SARS virus encodes a cysteine protease that is necessary for viral replication, the 3CL protease (*Chem. Biol.* **2006**, *13*, 261-268). Let's imagine that the natural reaction of this enzyme involves cleavage of a ...Phe-Ala... linkage in a key viral protein. Design an affinity-labeling agent for this enzyme and show the inactivation reaction.

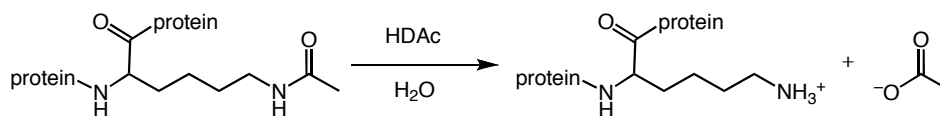
We discussed the structure of affinity-labeling functional groups in class. One of these groups can be employed for "labeling". For affinity, chemical structures attached to the labeling agent should resemble the natural substrate. See the homework set for an example. (If you don't know the structure of Phe or Ala you could use the abbreviation "Phe" or "Ala" dangling off of an amide-like scaffold).

Name _____

Exam 2

4 pages... all answers *must* fit in space provided!

18. Histone deacetylase (HDAC) is a Zn(II)-dependent mammalian enzyme that removes acetyl groups from lysine residues found in the histone proteins that are associated with DNA in the cell nucleus. The reaction is shown below. Inhibitors of HDAC are in clinical trials as potential anticancer agents.



(a) (10 pts) Propose a detailed mechanism for how this enzyme carries out its reaction. Label the types of catalysis that you envision.

HDAC is a zinc-dependent enzyme that cleaves an amide bond. The mechanism is just like that discussed for ACE in class.

(b) (10 pts) Design a potential reversible inhibitor of this enzyme. Briefly explain your strategy.

Same general strategies used for the design of ACE inhibitors should work. Design small molecules (MW ~ 200) that contain functional groups found on the normal substrate but LACK the amide bond. Transition state analogs can also be designed using the strategies employed for the design of ACE inhibitors.