

20.201 Take-Home Exam

Distributed: October 16, 2013, at 1:30 p.m.

Due: October 21, 2013, at 10:00 a.m.

Directions: This take-home exam is to be completed without help from any other individual except: Drs. Dedon, Tannenbaum, Hoffmaster, and Murcko, and ~~other students~~. This includes help from other students (whether or not they are taking 20.201), professors, people via the Internet, and so on. You are permitted to use your books, class notes, library resources, internet resources, and other inanimate materials.

Please prepare your answers to all questions as a single PDF document - labeled with your name. Please prepare your answers using word processing software and insert either digitized hand-drawn graphics or computer-generated graphics. **Please submit your answers by email to all of the following email addresses by 10:00 a.m. on October 21, 2013**" Late exams, without prior approval, will not be accepted.

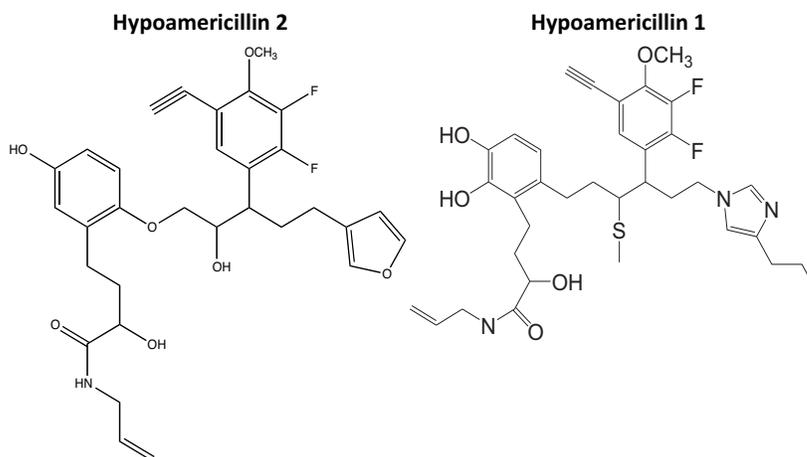
Question 1 - 10 points total

We discussed how many widely used drugs such as prozac and thiorazine interact with multiple proteins in the body. Briefly describe the pros and cons of such multi-target drugs, including, where possible, specific examples to highlight your points. *Please limit your answer to one page.*

Question 2 - 30 points total

Do your reading, review your homework and lectures. For questions B and C, use reference material and don't be afraid to think outside the box. You may use the images in the Powerpoint file (20.201 '13 Question 2 Structures.pptx) to draw structures for your answers. Use PPT drawing tools to decorate the images. Then select the image and its associated decorations, copy, and paste in your Word document answer sheet as a PDF or JPEG image. Alternatively, submit the PPT file with your answer document.

- A. (10 points)** Hypo 1 was found to be toxic, so the chemists came up with Hypo 2. You have been asked to predict whether 2 will be more or less toxic than 1, or about the same. Ignore the changes in bond angles, it's my mistake. Back up your answer with chemistry. Including what you think is the most important electrophile for each structure.



- B. (10 points)** In preclinical toxicity testing of Diclofenac in Chinese Hamster Ovary Cells containing the entire complement of human cytochrome p450 metabolic activity, the drug was only mildly toxic. When all of the p450 enzymes were killed (inactivated) by exposing the cells briefly to carbon monoxide (CO), the drug was even more toxic. What was going on?
- C. (10 points)** In testing for DILI *in vivo*, it is common to monitor blood for an aminotransferase (e.g., ALT, AST, *etc.*), but this fails when the drug causes cholestasis, and testing for alkaline phosphatase is the preferred assay. Please explain.

Question 3 – 30 points total

Soon after being graduated from MIT, you were hired as Director of ADME at ACME Pharmaceuticals. One of the teams under your supervision is responsible for characterizing the pharmacokinetic properties of a series of new antibiotic candidates. The team has already determined that both lead compounds A and B are 20% bound to plasma proteins and both are excreted unchanged in the urine.

A. (15 points) Rats were given intraperitoneal injections of 500 mg of either A or B and plasma concentrations (C_p) were quantified at various times, as shown in the adjacent table.

Time, hr	C_p , $\mu\text{g/mL}$	
	A	B
0.1	1.5	0.5
0.2	2.8	1.0
0.4	4.0	2.0
0.6	4.7	2.5
0.8	3.0	1.7
1.0	2.0	1.1
1.2	1.0	0.5
1.4	0.3	0.1
2.0	0.1	0
3.0	0	0

A.1) Create a graph of the two data sets and explain the basis for the shape of the curves.

A.2) Explain the different pharmacokinetic behaviors of A and B.

A.3) Estimate the plasma elimination rate constants and plasma half-lives for A and B. What must you assume in these calculations?

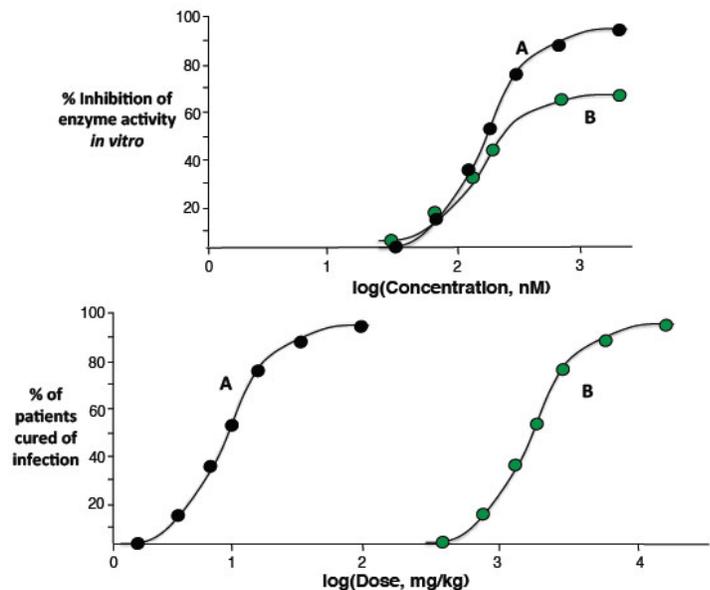
B. (15 points) You then compared the dose-response behavior for compounds A and B in infected rats, as shown in the figure below. The upper panel shows the concentration-dependence of inhibition of the activity of the drug target: a transamidase responsible for cell wall cross-linking in the bacteria. The enzyme inhibition data were obtained with purified enzyme *in vitro*. The lower panel shows the *in vivo* dose-response behavior for intraperitoneal injections of the drugs, with complete cure of the infection (i.e., no culturable bacteria) as the response end-point.

B.1) Provide mechanistic explanations for the differences and similarities observed in concentration-dependent enzyme inhibition by A and B in the top panel.

B.2) Discuss two mechanisms (other than the mechanism provided in part B.4 below) that could account for the observed differences in the bottom dose-response curves for curing the infection.

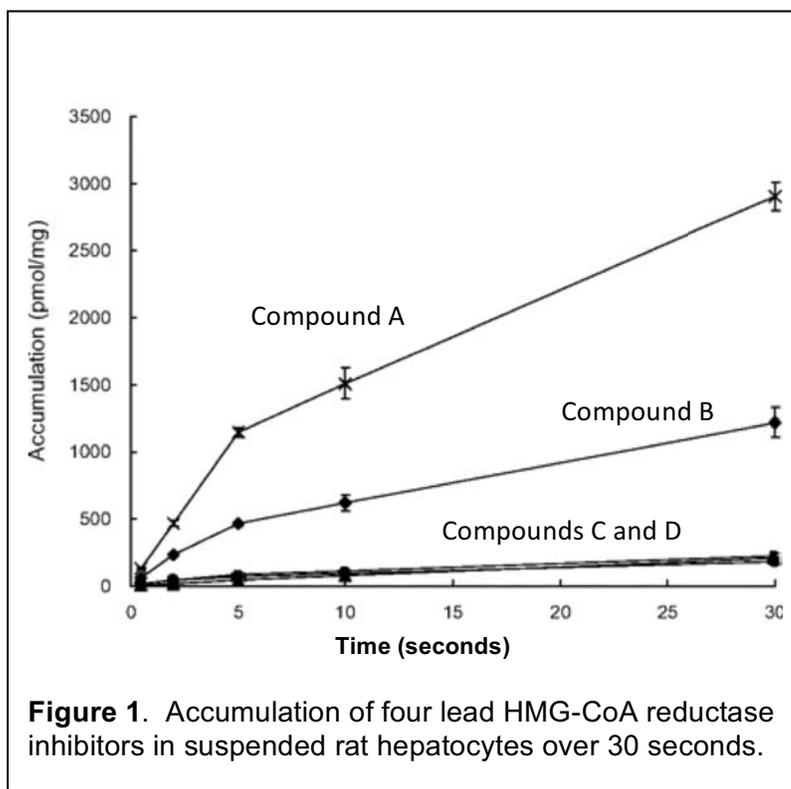
B.3) In terms of curing the infection, which drug is more potent and which is more efficacious? How do you explain the differences between the enzyme inhibition data and the infection cure dose-response data?

B.4) Provide a mechanistic explanation for the relationship between the dose-response data here and the plasma pharmacokinetic data presented above.



Question 4 - 30 points total

Your lab is developing a new drug for the treatment of high cholesterol. The approach is to inhibit a key enzyme involved in the cholesterol biosynthesis pathway. This enzyme, HMG-CoA reductase, is localized to the liver and serves as the rate controlling enzyme of the mevalonate pathway, the metabolic pathway that produces cholesterol and other isoprenoids. As the new head of the lab (and an expert in drug transport), your main strategy is to utilize the liver's capacity for active uptake of xenobiotics to target the liver. You have received the following data from some recent studies with your best compounds. **Figure 1** shows the uptake of four compounds into suspended rat hepatocytes. Each of the compounds was incubated at 37 °C for up to 30 seconds.



After discussion amongst the project team, you decide that these compounds warrant further investigation. Follow up *in vivo* studies in rats showed significant differences in bioavailability following oral administration, acute hyperbilirubinemia with compound A, and a transient increase in serum bile acids with compound D (**Table 1**).

Despite these observations, the pharmacologist on the project team was keen on determining the *in vivo* efficacy of these four compounds given their excellent *in vitro* potency (**Table 1**). Results from those *in vivo* studies in rats showed that when administered at 5 mg/kg orally, the compounds rank ordered as follows: A (most efficacious) > D >> B >> C (least efficacious).

Table 1: *In vivo* data for compounds A, B, C, and D following oral administration of 30 mg/kg in rats.

Compound	In Vitro Potency (nM)	Oral Bioavailability (%F)	In Vivo Observations
A	35	3.1	Elevated conjugated bilirubin
B	41	28	No adverse events
C	0.2	2.2	No adverse events
D	121	97	Increased serum bile acids

The project team is confused on how to interpret all of these data and is seeking your expert guidance on which compound to select to move forward. Based upon the data you have in front of you (and any relevant literature/publications), answer the following questions to help guide the team:

- Interpret the data in **Figure 1** – how do you think each of the four compounds is getting into the hepatocyte? For compound A, how might you prove your hypothesis with a follow up study?
- Explain why you think the bioavailability observed for compounds A and C is so low and why compound D is so high.

- C.** Compound A is acidic and is charged at physiologic pH. One of your fellow transporter experts suspects active biliary elimination as its mechanism of clearance. What two active transport mechanisms (uptake and excretion) would likely be responsible for the excretion of compound A from the plasma into the bile? (Be specific here.)
- D.** Why do you think that compounds A and D are causing hyperbilirubinemia and cholestasis, respectively? Propose a mechanism for each and suggest how one might test this experimentally to prove your hypothesis.
- E.** The team has a budget to put only one of your four lead compounds into clinical trials. What compound will you put into patients and why? For each of the other experimental compounds, state at least one key reason why you would not put that compound into the clinic over your choice.

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20.201 Mechanisms of Drug Actions
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