

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.

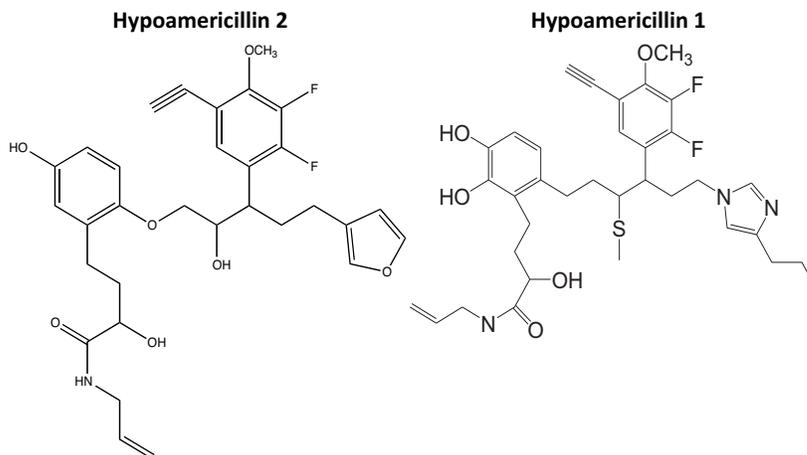
Question 1 - 10 points total

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.

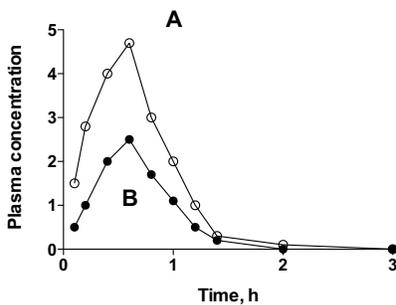
Question 3 – 30 points total

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.

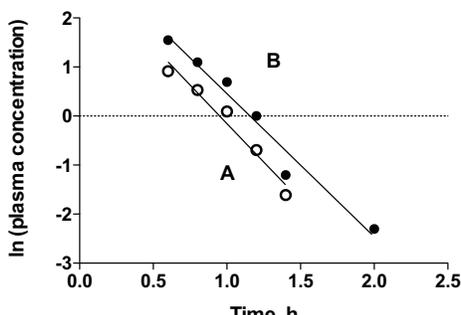


The non-linear increase in plasma concentration from injection to $t=0.6$ hr reflects absorption from the peritoneal compartment into the systemic blood circulation – crossing the epithelial lining of the peritoneal cavity into the intestinal tissues or directly into the blood vessels that form the portal circulation. Once the drugs have reached peak concentration in the plasma at $t=0.6$ hr, there is a non-linear decrease in plasma concentration for both drugs until the drugs are undetectable at 2-3 hr.

The data have subtle non-linear behavior that many people assumed represented zero-order kinetics. I accepted this conclusion as long as you were able to argue the mechanistic basis for either first- or zero-order kinetics.

A.2) Explain the different pharmacokinetic behaviors of A and B. As noted above, the plasma concentration profiles for both drugs are showing non-linear absorption from the peritoneal cavity and then non-linear elimination from the plasma. Drug B clearly has a lower AUC than drug A. Since blood flow from the peritoneal cavity (blood vessels associated with the intestinal tract!) mainly enters the portal circulation, it is possible that the lower AUC for B results from B being metabolized to a greater extent than A in the liver. However, only one person caught the fact that the drugs are excreted entirely unchanged in the urine! I gave credit to any good explanation for the different AUCs. It is possible that B is “trapped” in the peritoneal cavity by protein binding to a greater extent than A. Other reasonable explanations would also be accepted (e.g., B is phagocytized by peritoneal macrophages – highly unlikely but biologically reasonable). It is also possible that the rate of plasma elimination of B is much higher than that of A, to cause lower plasma levels at every measurable time point – unlikely but possible.

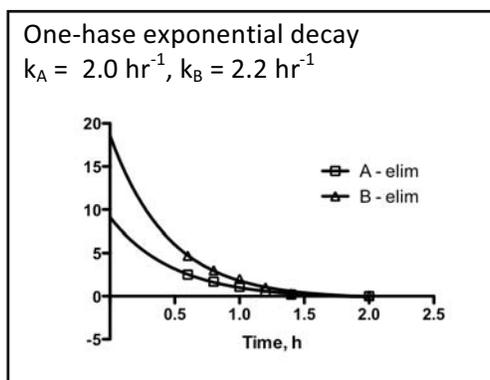
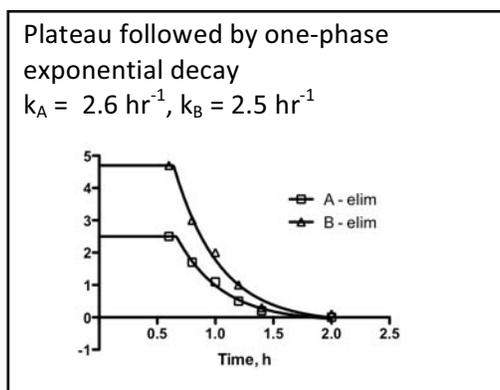
A.3) Estimate the plasma elimination rate constants and plasma half-lives for A and B. What must you assume in these calculations?



You could estimate the plasma elimination rate constant by plotting the natural logarithm of the plasma concentration versus time using plasma concentration data past the peak concentration at $t=0.6$ hr. If the drug elimination is first order, which we usually assume it is, then the plot should be linear with a slope equal to the negative value of the elimination rate constant. Indeed, the plots show good linearity, so our

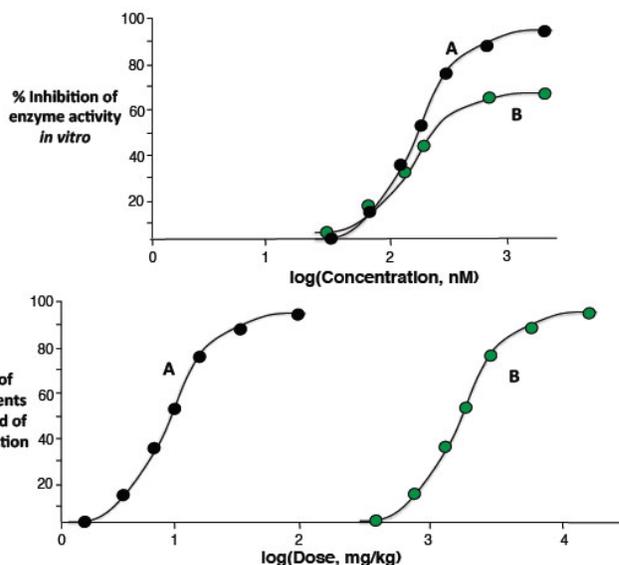
assumption of first order behavior is justified. The rate constants are as follows: **A**, $3.1 (\pm 0.4) \text{ hr}^{-1}$; **B**, $2.9 (\pm 0.3) \text{ hr}^{-1}$. The values are not significantly different. The half-lives ($0.693/k$) are: **A**, 0.22 hr; **B**, 0.24 hr.

You could also analyze the data using non-linear regression approaches. Be careful with these algorithms since they make assumptions such as extrapolating to “t=0” if the peak concentration at t=0.6 is not used as “t=0”. I will accept rate constants and half-lives determined by non-linear regression methods here, even though the results are slightly different than the linear regression with ln-transformed data, since the data are artificial and I am more concerned about the argument of first-order kinetics. See examples below of non-linear fits and results:



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. **Both drugs cause similar levels of enzyme inhibition at low concentrations, but, at higher concentrations, drug B does not completely inhibit the enzyme as does drug A. The plateaus in enzyme inhibition apparent for both drugs probably relates to saturation of the drug binding site, whether this is at the active site of the enzyme or at some other site on the protein. The lower “efficacy” of drug B at inhibiting the enzyme could be due to binding at a different site than drug A or the same site as A, with complete saturation by B producing a protein conformational change that still allows activity of the enzyme on its substrate. B would be a partial antagonist and A would be a complete antagonist.**



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.

Both drugs cause complete (100%) cures of the infection at high doses (i.e., both are equally efficacious), but A does so at lower doses than B (i.e., A is more potent than B). The difference in potency could be due to poorer bioavailability of B from the intraperitoneal site of administration, perhaps by entrapment in the peritoneal cavity by covalent binding to proteins or, more likely, by metabolism in the liver when the drugs enter the portal circulation and then reach the liver before reaching the systemic circulation (blood flow from the peritoneum enters the portal circulation!). Perhaps drug A is absorbed into the lymphatic circulation that is prominent in the peritoneum and thus bypasses the portal circulation altogether – I would not have expected you to come up with this unlikely mechanism; just thinking “outside the box”. It is also possible that B does not enter the infected tissue or the inside of the bacterium as well as A. Lots of possible reasons here. Another from one of you (brilliant!): Another difference is that the IC_{50} concentration is the same for both drugs in vitro, but different in vivo. A possible explanation is a shift in binding affinity. In an in vivo environment, there are many differences compared to in vitro (temperature, pH, etc.), all of which can affect the affinity, or K_d value. Therefore, in this situation, Drug A has a higher affinity and lower K_d for the enzyme compared to Drug B.

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? As discussed in B.2, A is more potent than B and both drugs are equally efficacious in curing the infection. The enzyme inhibition data show that both drugs are roughly equally potent, though B does not completely inhibit enzyme activity (i.e., less efficacious at inhibiting enzyme activity). So the lower potency of B in the infection cure dose-response relationship is unlikely to be due to a lower affinity for the target enzyme. The fact that both drugs achieve 100% cure at the highest doses suggests that the incomplete enzyme inhibition by B is not an important issue. It appears that even partial enzyme inhibition by B is enough to kill the bacteria or to halt their growth so that the immune system can clean up the infection. Perhaps B has other targets that augment its antibacterial activity. The most likely explanation for the lower potency of B in curing infections is bioavailability.

B.4) Provide a mechanistic explanation for the relationship between the dose-response data here and the plasma pharmacokinetic data presented above. B has both a lower AUC than A and it is less potent at curing infection than A. The most likely explanation for these differences is bioavailability – lower levels of B reach the systemic circulation than A. The fact that both drugs achieve 100% cure at the highest doses suggests that the incomplete enzyme inhibition by B is not an important issue. It appears that even partial enzyme inhibition by B is enough to kill the bacteria or to halt their growth so that the immune system can clean up the infection.

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.

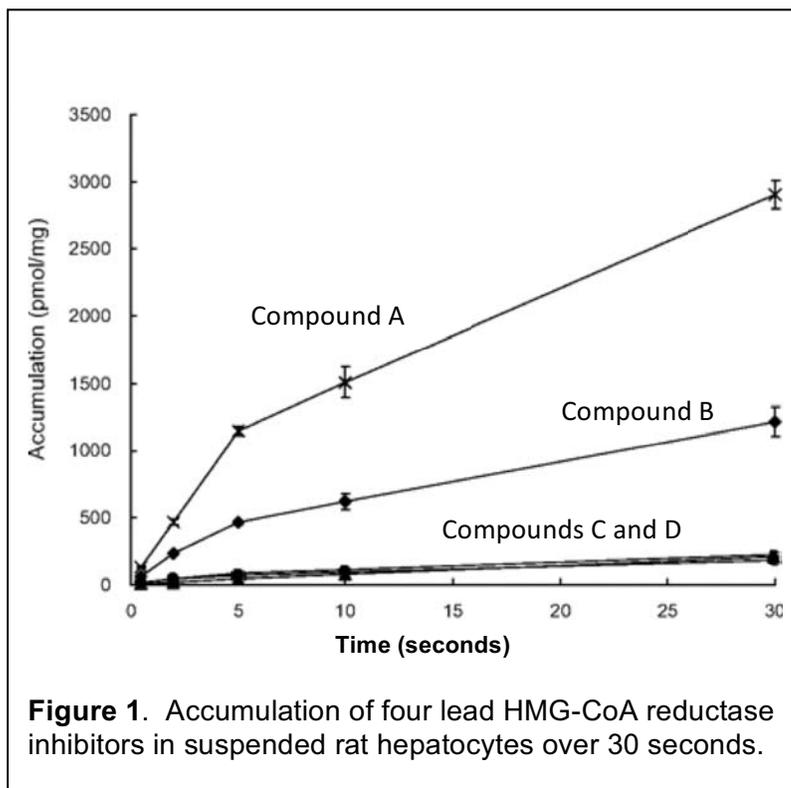


Figure 1. Accumulation of four lead HMG-CoA reductase inhibitors in suspended rat hepatocytes over 30 seconds.

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:

- A.** 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?

- B.** 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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