

Answer Key

Homework 1, 4170

1. Hansch proposed that a balance of lipid (organic) and water solubility is required for "good" drugs. Too much lipid solubility and drugs will either be completely insoluble or will localize in the lipid bilayer of membranes. Too little lipid solubility and the drug will not be capable of passing *through* the lipid bilayer to get inside the cell. These arguments can be summarized by saying that "a balance of water and lipid solubility is important to achieve good pharmacokinetics".

2. Why are there different possible answers to these log P calculations? This is best answered with examples. Consider the following calculations for π_{COOH} . $\log P_{\text{ICH}_2\text{COOH}} - \log P_{\text{CH}_3\text{I}} = -0.82$ whereas, $\log P_{\text{NCCH}_2\text{COOH}} - \log P_{\text{CH}_3\text{CN}} = 0.01$ Clearly very different estimates are obtained. If one wants an accurate π_x value, it probably must be obtained by averaging the values calculated for that substituent from many different molecules. If you use a molecule where the functional group in question can be involved in intramolecular (internal) interactions with another functional group the calculation may give faulty estimates (e.g. intramolecular H-bonding of group could lead to an underestimate of the water-loving properties of a functional group).

A. $\pi_{\text{benzene}} + 4(\pi_{\text{CH}_2}) + \pi_{\text{CH}_3} = 3.63$

B. $\log P_{\text{Me}_3\text{N}} - \pi_{\text{CH}_3} + \log P_{\text{CH}_3\text{CH}_2\text{Br}} = 1.51$

C. $\log P_{\text{"core"}} + \pi_{\text{CH=CH}} + \pi_{\text{PhCH}_2} = \text{answer}$
 $\pi_{\text{CH=CH}} = 1/3(\log P_{\text{benzene}}) = 0.71$
 $\pi_{\text{PhCH}_2} = \log P_{\text{PhCH}_2\text{NH}_2} - \pi_{\text{NH}_2} = 2.16$
 $\pi_{\text{NH}_2} = \log P_{\text{CH}_3\text{NH}_2} - \pi_{\text{CH}_3}$

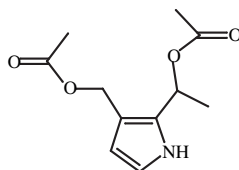
D. $\log P_{\text{EtOEt}} - 2(\pi_{\text{CH}_3}) - 2(\pi_{\text{CH}_2}) = -1.23$

E. $\log P_{\text{HC=CCOOH}} - \pi_{\text{COOH}} = \text{answer}$ depends strongly on value you calculate for π_{COOH} (calculate several π_{COOH} values and see how your answer changes)

F. $\log P_{\text{CH}_3\text{CO}_2\text{CH}_3} + \pi_{\text{CH}_3} + \log P_{\text{CH}_3\text{CN}} - 0.2(\text{branching}) = 0.14$

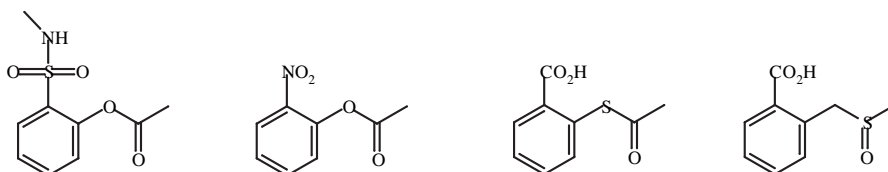
G. we worked this one in class

3. The SMALLEST "substructure" that is present in all of the active analogs looks like this:



4. Don't know where number four went...

5. Many are possible... here are a few

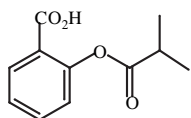


6. Some bioisosteric changes are structurally conservative (the molecule will retain the same size and shape) but substantially alter the chemical properties of the molecule. For example, your textbook (correctly) lists -NH- and -CH₂- as a bioisosteric pair. They are structurally similar (size, bond angles); however, at physiological pH the -NH- group will likely exist predominantly in the protonated form (-NH₂⁺- this is addressed further in problem 20). The charge on the nitrogen will obviously change its pharmacodynamic and pharmacokinetic properties relative to the -CH₂- group. Similarly, a guanidinium group (as seen in arginine) may be isosteric for an amino group, in that both are positively charged at pH 7. However, these two groups are substantially different in their size and shape. In the end, there is no such thing as a "perfect bioisosteric substitution".

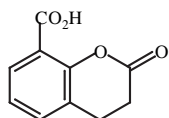
7. See book for definitions (page 26). Pharmacokinetics and pharmacodynamics are equally important. A clinically used drug must be excellent in both categories. Many drugs that appear promising in early assays that emphasize pharmacodynamics fail in clinical trials due to unforeseen pharmacokinetic problems. This occurs at great expense to the pharmaceutical industry.

8. The Log P has been increased by addition of a methylene group (-CH₂-). This may adversely affect pharmacokinetics. Alternatively, a key drug-target interaction in the binding pocket may have been lost by "walking" the acetyl group out by a distance of two carbons (~3 Å).

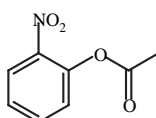
Possible Lead Modifications....



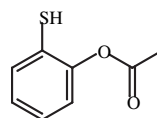
Chain-Branching
(not a great example,
but it works)



Ring-Chain
Analog



Isosteric
Substitution



Func. Group
Exchange

NOTE: No chiral centers to be modified

9. Use the "USEFUL equation" from the handout that I gave you to calculate the concentration required to achieve 90% bound target:

$$1/(K_{eq}D_{free} + 1) = T_{free}/T_{total} = 0.1 \text{ (10\% of target in remains unbound or free)}$$

Plug and chug to solve for D (drug concentration required to reach 90% bound target). I get $9 \times 10^{-6} \text{ M}$ for the required drug concentration. To calculate dose, use: \underline{M} = moles/L. $9 \times 10^{-6} \underline{M} = \text{mol}/50\text{L} = 4.5 \times 10^{-4} \text{ moles}$. Use the average molecular weight for a drug that I gave you in class - MW = 250 g/mole. Dose required: 0.113 gm or 113 mg. Look in your medicine cabinet... what is a "typical" dose for a drug? Of course, it depends on the drug. Just before writing this answer key I took 10 mg of allegra-D and 600 mg of ibuprofen (allergies).

at $K_B = 9 \times 10^9 \text{ M}^{-1}$ the required drug dose is 0.1 mg. Just to give you a feel... that is a mass equal to about one or two grains of salt.

10. (a) 1×10^{-9} M (1 nanomolar) (b) $K_D = 1 \times 10^{-9}$ M Ah Ha! The K_D = the concentration at which half of the biological target is bound (under conditions of excess drug).

11. (a) It is likely that **P** will bind to the target more poorly than **M**. Why? See part (b)...

(b) **P** will bind more poorly than **M** (have a smaller K_B). This is because **P** is less rigid and must "pay an entropic penalty" (suffer an entropically unfavorable loss of conformational freedom) upon binding to the biological macromolecule. ΔS of binding for **P** will be more unfavorable than ΔS of binding for **M**. The enthalpy of binding (ΔH can be envisioned to result primarily from formation of weak bonds between the drug and target) may be largely the same for the two compounds.

(c) Use the equation that relates equilibrium to free energy:

$\Delta G = -RT \ln K_{eq}$ to calculate that $K_{eq} = 2.5 \times 10^4$ (use RT values from the handout that I gave you). Then use $K_{eq} = \frac{[conf1]}{[conf2]}$ to calculate that there is 0.004% of conf1 present at equilibrium.

Notice that the "active" conformation of **P** (with the functional groups in the correct position for interaction with the binding pocket) is present in very low amounts!

(d) Hydrophobic forces are probably more important here. Because hydrogen bonding is a "transfer" process. The free drug is hydrogen bonded to water and this hydrogen bond must be broken (at a cost of about 2-3 kcal/mol) before the hydrogen bond inside the binding pocket can be formed (at a "payoff" of about 2-3 kcal/mol). Net energy payoff for the formation of a hydrogen bond to a biological target may be about zero.

(e) At low (physiological) temperatures the hydrophobic effect is largely driven by ENTROPIC effects (ΔS) as discussed in class (and in the text). Basically, dissolving an organic molecule in water results in formation of a highly ordered shell of water around the molecule. Association of two hydrophobic partners leads to an entropically favorable "release" of water molecules into the bulk solution.

12. This one is straight off the handout that I gave you regarding the relationship of equilibrium to free energy.

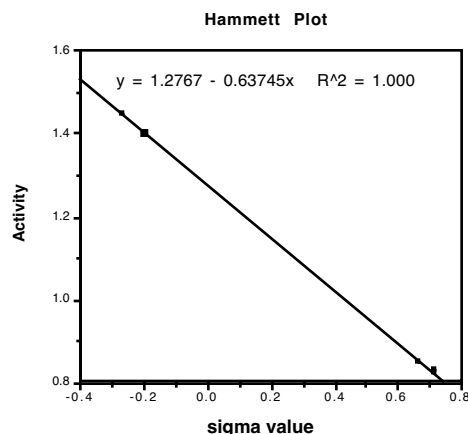
(a) Larger K_B (also known as K_{eq}) means better binding of drug to target.

(b) Use the equation that allows you to calculate the change in the free energy of binding ($\Delta\Delta G$). Use $K_B/K_A = 2.5$ to calculate that $\Delta\Delta G = -0.54$ kcal/mol. Drug B enjoys an additional -0.54 kcal/mol favorable binding interactions with the target.

(c) This is basically a repeat of calculations like those in problem 9 where we calculated $\frac{[target_{free}]}{[target_{bound}]}$. In order to work this problem, we have to assume a drug concentration. On an exam, I'd give you a concentration to use. But, for this problem, let's use a drug concentration of 1×10^6 M.

A = target is 10% in the drug-bound form (target is 90% in the free form) and, for B = target is 20% in the drug-bound form (target is 80% free). Amount of target bound doubled even though we only "added" 0.5 kcal/mol of binding energy!

13. If you are unsure what a Hammett plot is, look back at your notes, at the textbook, or at the handout that I posted on the course website. The handout on the website gives necessary lists of substituent constants (σ). I would give you necessary σ values to solve problems on the exam. (a) First, you can see that the plot gives a nice linear fit. This indicates that a linear free energy relationship exists. That is, the effects that electron donating and withdrawing substituents have on drug action can be related back to the effects that these substituents have on the ionization of benzoic acid. The rho value (ρ) = -0.64



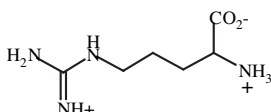
(b) Evidently, groups with electron-donating properties (groups with negative sigma values) favor activity. One would expect that installation of -OH and -NH₂ substituents on the pharmacophore might yield very active analogs.

(c) Compound **Y** clearly does not lie on the line. For this compound the σ -value is apparently not a good predictor of activity. Why? Perhaps because the Log P is drastically altered by this charged substituent.

14. Three amino acids and their potential weak bonding interactions....

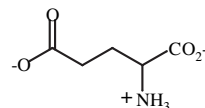


Valine,
Hydrophobic
Interactions



Arginine,
Hydrogen Bonding
Ionic Interactions

(Also, possible hydrophobic interactions with the alkyl chains)



Glutamic Acid,
Hydrogen Bonding,
Ionic Interactions

15. Drug lead compounds can come from historical medicines, accidental or deliberate exposure to poisons, and through observation of side effects of clinically used drugs or developmental drugs. Most modern drug leads are discovered through a more systematic approach screening large numbers of compounds in a cell-based or isolated biomolecule-based bioassay.

16. Three types of bioassay:

1. Animal assay. Expensive. Ethical considerations lead companies to limit the use of animals. Probably predictive of both pharmacokinetics and pharmacodynamics in mammals. Interesting side effects and toxicity may be observed. No information regarding the molecular origin of the biological effects can be gained (unless using something sophisticated like knockout mice)

2. Cell culture. Cheaper and faster than animal studies. Does not model all aspects of pharmacokinetics, though it may provide information on some aspects (solubility, ability to cross cell membranes, cellular drug metabolism). Appropriate choice of control experiments can model toxicity (e.g. one might test whether experimental antibacterial agents also kill normal human cells).

3. Isolated biological macromolecule. Can be very cheap and fast. Chemical, biochemical, and biophysical studies may reveal detailed information regarding the interaction of the drug with the target. Such studies may lead to better drugs. These assays do not claim to reveal information regarding pharmacokinetics. Obviously, one must have a reasonable understanding of the disease process before one can determine which biological targets to use in drug screens.

17. I listed the rule of fives in class. In addition you can find them listed in the conclusions of the paper posted on the course website that discusses the role of hydrophobic effects in drug binding.

Penicillin G: MW = 334.4; logP = 1.67; total H-bond donors = 2; total N,O atoms = 6

So, yes, penicillin G is a "good" drug as judged by the rule of 5's.

Valium: MW 284.7; logP = 3.86; total H-bond donors = 0; total N,O atoms = 3

Again, valium is a "good" drug as judged by rule of 5's

Reasons why good drugs follow the rule of fives: The rule of fives is essentially a semi-quantitative restatement of Hansch's feeling that a balance of hydrophobicity and water solubility is necessary for a drug to have "good" properties. In the article discussing hydrophobic effects in drug-target binding, the authors suggest that good K_B values are the result of hydrophobic effects. Thus bigger logP values will, in general, provide better K_B s. In their view, appropriate numbers of heteroatoms must be installed simply to provide sufficient water solubility. Remember, the rule of 5's says nothing about whether the drug has an appropriate structure to bind to the appropriate biological target. The rule of 5's is simply a general set of physical requirements that need to be met in order for a compound to have potential as a successful drug.

18. Most drugs are reasonably complex organic molecules. Early humans did not possess the ability to synthesize complex organic molecules. In early times, plants were the only readily available source of complex organic molecules. Therefore it is logical that plants and plant extracts were the first historical medications.

19. Drug companies often seek to screen large numbers of compounds in their bioassays. This increases the odds of getting a "hit". Combinatorial chemistry can be used to prepare large libraries of compounds. An even better approach is to construct libraries that contain substructures thought to be appropriate for interaction with the given biological target. This is called a "directed library"... made especially for the target of interest.

20. The pK_a for the $-NH_3^+$ side chain of lysine is 10.8. Here is how to do a very quick calculation: Remember that when $pH=pK_a$ there are equal amounts of the protonated and unprotonated forms. This means that at pH 10.8 there will be equal amounts of protonated and unprotonated amine. As the pH goes down (reflecting a greater concentration of protons) there will be large amounts of the protonated form. So at pH 8.0 there will be considerably more of the protonated form of lysine compared to the unprotonated ($-NH_2$) form.

Calculation: Look at the acid-base calculation handout posted on the course website. There you'll see the following relationship:

$$K_{eq}/[H^+] = [RNH_2]/[RNH_3^+]$$

Because you know that $pK_a = -\log K_a$ and $pH = -\log [H^+]$

The values for K_{eq} and $[H^+]$ are known:

$$1.6 \times 10^{-11} / 1 \times 10^{-8} = [RNH_2]/[RNH_3^+] = 0.0016 \text{ (not much } RNH_2, \text{ as expected)}$$

To calculate the percent RNH_2 we'll say that $RNH_2 + RNH_3^+ = 100\%$

thus, $RNH_3^+ = 100 - RNH_2$. So $[RNH_2]/[100 - RNH_2] = K_{eq}/[H^+] = 100$ solve this to find that:

At pH 8, lysine's amino side chain is 99.99% in the protonated form.

21. Regarding the paper by Rebek and coworkers (posted on the course website). In the more polar, hydrogen-bonding solvent water, the hydrogen bonding interactions shown in the diagram on the first page of the paper would probably NOT contribute significantly to the free energy of binding. As discussed earlier in problem, hydrogen bonding of a small molecule to a binding pocket in water is an energetically neutral "transfer" process. This is not to say that hydrogen bonds are unimportant, these hydrogen-bonding interactions may impart *specificity*. That is, a "guest" molecule (drug) that *can* form hydrogen bonds in the binding pocket of a "host" molecule (biological macromolecule) will bind better than a similar guest that *cannot* form these hydrogen bonds.

In water, the noncovalent binding interactions between the aromatic rings in these molecules is likely to be much larger than in chloroform. The interaction will be driven by the hydrophobic effect and so-called pi-stacking interactions. Another one of the papers posted on the course website discusses how hydrophobic interactions probably are the primary driving force for the association of drugs to their biological targets. These ideas were also discussed above in problem 11. Based upon this idea, it is likely that the molecules shown on the first page of the Rebek paper would have higher affinity for each other (a larger K_a) in water.

Note: There is a practical problem here. It might be difficult to actually prove that the K_a of these molecules is larger in water - because they probably don't dissolve very well in water.

22. The ideas regarding how weak bonding interactions change as a function of solvent are highly relevant to the interaction of drugs with binding pockets of biomolecules. In some ways, the transfer of drugs from water into the binding pocket of a biomolecule is something like the transfer of a hydrophobic organic molecule from water into organic solvent (like an extraction in organic lab). In this hydrophobic, nonpolar, organic solvent-like environment hydrogen bonds and ionic bonds are stronger than they would be if they occurred in an aqueous environment.