BIOL 485 homework due at the start of class on <u>Wednesday, August 7</u>
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Read "Triazolopyrimidine-based dihydroorotate dehydrogenase inhibitors with potent and selective activity against the malaria parasite *Plasmodium falciparum*" (PubMed ID 18522386) and answer the questions below.

1. What are the purine and pyrimidine salvage pathways? What is their function in the parasite?
These pathways essentially "recycle" purines and pyrimidines, as opposed to synthesizing them "from scratch" (i.e., from non-purine and non-pyrimidine precursors). The pathways provide purine and pyrimidine bases to be used in the synthesis of nucleic acids (DNA and RNA). Note that Plasmodium does not have a pyrimidine salvage pathway and thus is dependent on de novo (from-scratch) synthesis of pyrimidines.

2. The article mentions "Lipinsky's Rule of Five" as a general guideline in the drug discovery process. Wikipedia "Lipinsky's Rule of Five" and assess whether any of these compounds violate these "rules" and also, where in the drug discovery process this information is most important.
The compounds listed in Table 1 are sufficiently small (molecular weight < 500) and hydrophobic (few hydrogen bond donors or acceptors) to be in compliance with Lipinski rules. The presence of the multiple nitrogen atoms limits their lipophilicity enough to keep their logP's under 5. Researchers disagree as to the importance of initial hits from a screen being Lipinski-compliant, but the rules are especially useful during lead optimization.

3. What is the difference between compound 6 seen in *High-throughput Screening for Potent and Selective Inhibitors of Plasmodium falciparum Dihydroorotate Dehydrogenase* and compound 7 in this paper? Why did they decide to continue the study with compound 7?
Aside from the large structural difference between compound 6 of Baldwin et al. 2005 and compound 7 of Phillips et al. 2008, the compounds were very different in that compound 6 had essentially no effect on growth of P. falciparum cells, whereas compound 7 strongly inhibited growth (EC_{50} = 79 nM). The potency against P. falciparum cells suggested that further work was warranted.

4. Refer to last week's reading of "In vitro and in vivo antimalarial activity of peptidomimetic protein farnesyltransferase inhibitors with improved membrane permeability" by D. Carrico et al. Do you think the compounds in Table 1 of this week's reading had any issues with membrane permeability? Why or why not?
The compounds are small, uncharged, and lipophilic (see #2 above), so membrane permeability should be OK. This idea is supported by the fact that many of the compounds do kill the parasite (and thus apparently are able to pass through cell membranes).

5. Why do you think several of the compounds in Table 1 have an EC_{50} that is lower than their IC_{50}? Conversely, what could have lead to others' EC_{50} be higher than their IC_{50}?

If an EC_{50} is lower than an IC_{50}, the usual suspicion is that the compound is killing cells by a mechanism unrelated to the particular target being studied. This is because, as a general rule, a particular protein needs to be inhibited by >50% for cell growth to be inhibited by 50%. Another way of stating this is that most individual proteins have excess functional capacity beyond the minimum needed to support the growth of cells at their usual rate.