

Name: Paul Newman

Date: Dec. 14, 2005

**Topics in Organic Chemistry: Modern Medicinal Chemistry
(CHEM 515)
Final Examination**

Profs. Malachowski and White

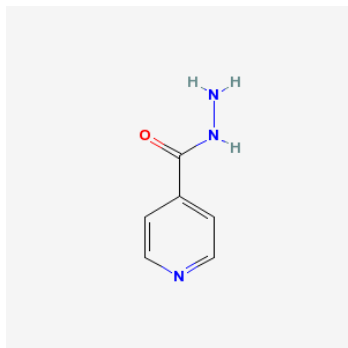
Due: December 14, 2005, 5 PM

Honor Code: You may take this examination while consulting your course lecture notes and the information posted on the course Blackboard site. You are not to consult any other electronic or written material during the exam. You have 3 consecutive hours to complete the exam. You may word process your answers. You may use molecular models to assist you in answering the exam questions. You should not discuss the exam with anyone until all students have handed in their exam. There are a total of twelve questions on 12 pages.

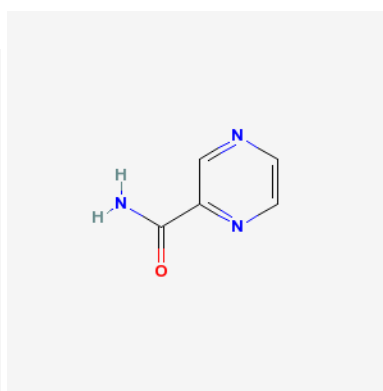
No answers to questions 1-9.

10. Three drugs commonly prescribed for the treatment of tuberculosis reportedly target cell wall biosynthesis. Name these three drugs, draw their structures and describe why cell wall biosynthesis is a common target for tuberculosis drugs.

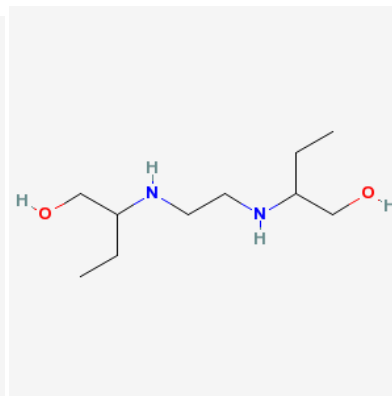
Isoniazid, pyrazinamide and ethambutol are all believed to inhibit cell wall biosynthesis. Their structures are shown below:



Isoniazid



Pyrazinamide



Ethambutol

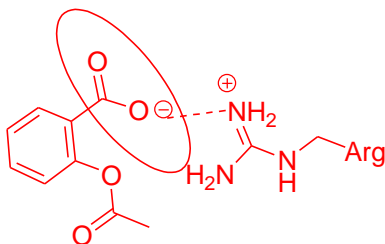
The cell wall of *Mycobacterium tuberculosis* is a central defense line for the bacteria, shielding it from the hazards of the external environment. Therefore, disrupting cell wall synthesis severely hampers the growth of mature tuberculosis bacteria.

11. Explain why Concepcion Gonzalez-Bello and co-workers used C-2/C-3 sp^2 hybridized cyclohexene analogs in their recent attempts to make inhibitors of the *Mycobacterium tuberculosis* type II dehydroquinase (*J. Med. Chem.* **2005**, *48*, 4871).

Enzyme catalysis theory holds that enzymes bind and stabilize the transition state and/or intermediate in chemical reactions, thereby lowering the energy of the transition state/intermediate and facilitating the reaction. Consequently, the enzyme structure has evolved to bind the transition state/intermediate more tightly than the substrate. As a result, stable structures that closely mimic the transition state/intermediate should bind strongly to the enzyme.

Since the proposed mechanism of type II dehydroquinase involves a planar enol intermediate in a cyclohexene ring, the intermediate has two sp^2 hybridized carbons. The analogs with two sp^2 hybridized carbons did apparently mimic this planar portion of the cyclohexane ring since one had an inhibition constant (K_i) of 54 nM.

12. a) Draw the structure of aspirin as it would exist at physiological pH and circle the portion that interacts with the arginine group present at the active site of COX-1 and COX-2.



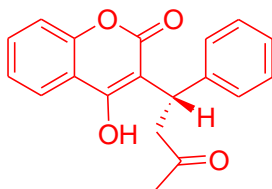
b) What type of intermolecular force is this interaction and approximately how many kcal/mole binding energy would this represent?

The carboxylate ion and guanidyl group have an electrostatic interaction which should have a binding energy of approximately 5 kcal/mole.

c) There is a fundamental difference in the way aspirin inhibits COX (prostaglandin synthase) and the way all other NSAID's that we discussed act. What is this difference?

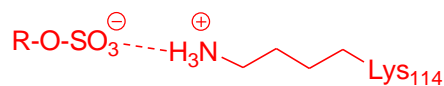
Aspirin causes an acetylation of a serine residue at the active site. This represents a reaction with the COX enzyme. The result is the formation of a new covalent bond between the enzyme and a portion of aspirin. None of the other NSAID's react with COX. All other NSAID's that we discussed are termed reversible inhibitors, whereas aspirin is a form of irreversible inhibitor.

13. a) Draw the more active S isomer of warfarin.

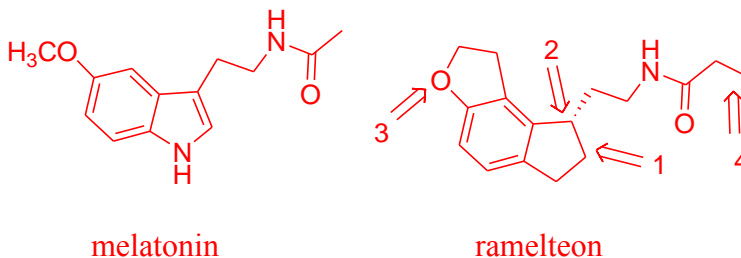


b) What type of interactions are involved in the binding of oligosaccharide 12 to thrombin and anti-thrombin? Draw an example of the groups on each of the molecules that are involved in this binding.

The interactions between oligosaccharide 12 and thrombin/anti-thrombin are electrostatic in nature. One example is the interaction between a sulfonate group on oligosaccharide 12 and a lysine side chain of antithrombin.

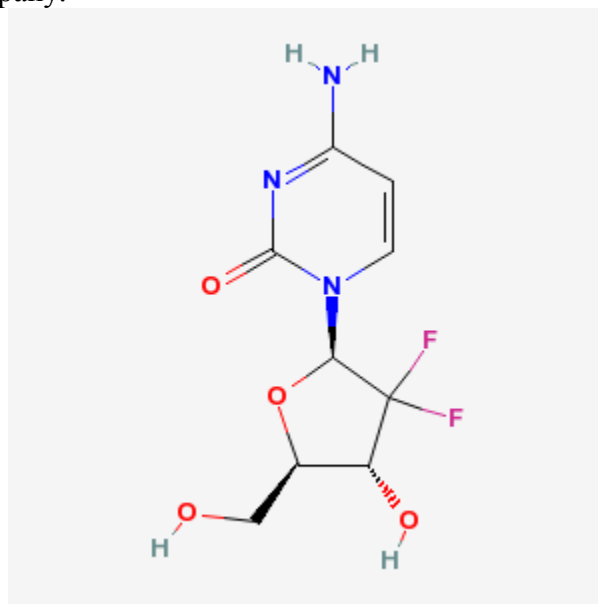


14. Draw the structure of melatonin, the natural substrate for MT₁/MT₂ and ramelteon, the potent MT₁/MT₂ agonist. Identify three modifications to the ramelteon structure relative to melatonin and describe the effect these changes have on the receptor site binding or bioactivity of ramelteon.



1. Elimination of pyrrole to allow for control of the side chain conformation. This might also reduce rapid melatonin metabolism.
2. Stereochemistry is introduced at the side chain connection to the rings to find the optimal interaction between the amido group and hydrogen bonding residues in the MT₁/MT₂ receptor site (possibly Tyr175 and the Ser182).
3. The methoxy group is restricted to create the optimum orientation for the oxygen lone pair to form a hydrogen bond, possibly with His195 of MT₁/MT₂.
4. The propanoyl group extends the acetyl group of melatonin making favorable hydrophobic interactions in the MT₁/MT₂ receptor site (possibly with Pro265 and Val261).

15. Gemcitabine is an anti-cancer and anti-viral drug developed by Eli Lilly Pharmaceutical Company.



gemcitabine (GEM)

In November 2005, the American Association for Cancer Research (AACR) held a conference in Philadelphia and one of the meeting presentations involved gemcitabine. The abstract for that presentation is pasted below. Read this abstract and answer the questions that follow.

C273 Pharmacodynamics of Gemcitabine in Glioblastoma Multiforma. Godefridus J. Peters, Jennifer Sigmond, Sandra De Lange, Adrie C. Laan, Richard J. Honeywell, Tjeerd J. Postma, Clemens M. Dirven, Johannes C. Baaijen, Cornelius J. Van Groeningen, Giuseppe Giaccone. VU University Medical Center, Amsterdam, The Netherlands.

Glioblastoma Multiforma (GBM) has a poor prognosis and is poorly sensitive to cytotoxic drugs. Gemcitabine (GEM) is a very potent radiosensitizer. However, in order to apply GEM in combination with radiation in the treatment of GBM, the drug needs to be taken up by the brain into the tumor and metabolised to its active nucleotides. The aim of our study was to investigate whether GEM would pass the blood-tumor barrier and would be taken up at sufficiently high concentrations in the tumor to enable radiosensitization. In addition, we investigated whether critical enzymes in GEM metabolism would be expressed in GBM: deoxycytidine kinase (dCK), responsible for GEM activation, and deoxycytidine deaminase (CDA), converting GEM to difluorodeoxyuridine (dFdU), which is supposed to be an inactive metabolite. GEM was administered just before surgery or during anesthesia to 10 patients with recurrent GBM, at two doses of 500 and 1000 mg/m², each group consisting of 5 patients. Tumor samples were obtained between 2-4 hr after administration. GEM levels in plasma at the time of the biopsy varied from 0.9-9.2 μM, dFdU from 25-72 μM and that of the active metabolite, gemcitabine triphosphate, dFdCTP in white blood cells from 2-108 pmol/10⁶ cells. GEM levels could be measured in tumor samples of 5 patients by LC-MS-MS and

varied between 2.5-47 pmol/g tissue; no difference was observed between the 500 and 1000 mg/m² group. In 7 patients dFdU was measured by HPLC and varied from 29-60 nmol/g tissue in the 500 and from 49-72 in the 1000 mg/m² group (t-test, not significant). From the other patients insufficient material was available to evaluate GEM and dFdU. The relative gene expression of dCK compared to β -actin as measured with real-time light cycler PCR varied from 0.44-2.56. The dCK activities were in a relatively small range of 1.1-2.32, while that of CDA were between 1.5-5.5 nmol/hr/mg protein. Since the CDA levels are very low compared to e.g. liver (100-fold lower) and dCK close to that of intermediately sensitive xenografts, GEM is likely to be phosphorylated under these conditions, although we were not able to measure the active nucleotide, since this is rapidly degraded during tissue preparation. These data demonstrate for the first time that GEM passes the blood-tumor barrier in GBM-patients. In tumor samples both the levels of GEM and even of dFdU are high enough to enable radiosensitization and warrant clinical studies using GEM in combination with radiation.

©2005 American Association for Cancer Research.

Abstracts published as a Supplement to *Clinical Cancer Research* (Volume 11, Issue 23, December 1, 2005).

Page 1 Pharmacodynamics of Gemcitabine in Glioblastoma Multiforma of 1

file://D:\rawabs\3193.html 11/22/2005

a) Based on the structure of gemcitabine, suggest a mechanism of action, i.e. what does it mimic and what action of cancer cells or viral cells might it inhibit?

Gemcitabine mimics cytidine or cytosine, one of the DNA bases (replace the two fluorine atoms with hydrogens and you have cytidine). It inhibits DNA synthesis in cancer or a virus.

b) Gemcitabine is another example of a pro-drug. Based on the abstract above, what must happen to GEM to make it an active drug?

GEM must be converted into a triphosphate by deoxycytidine kinase (dCK). As the triphosphate it can act as a substrate (or mimic of a substrate) for DNA synthetase.

c) According to PubChem, GEM has a log P value of -1.318. Why might the researchers at the AACR conference think it necessary to study the viability of GEM in the treatment of glioblastomas, a common form of brain cancer?

For anti-cancer drugs to act on brain cancer, they must be able to penetrate the blood-brain barrier to get to the tumor. With a log P value of -1.318, GEM is very hydrophilic and therefore unlikely to passively diffuse through the lipophilic blood-brain barrier.

d) The fluorine atoms on GEM are very important for its activity. List one property of fluorine that might explain the basis of GEM's bioactivity.

1. Fluorine atoms are very electronegative, so the strong inductive effect generated by the two fluorines might be involved in GEM's bioactivity.
2. Fluorine is a hydrogen bond acceptor. The geminal fluorine atoms might be involved in hydrogen bonding.

Since GEM simply replaces the hydrogen atoms of cytidine with the fluorines, it is very likely that one of these properties is responsible for GEM's bioactivity.