Medicinal Chemistry Name:_____
CHEM 515
Mid-term Exam
March 6, 2025

There are 12 lucky questions on the exam. You should complete any 10 of the 12 questions.
Each question is worth 10 points. You are encouraged to answer the questions where you feel you have the greatest understanding or competence. You may use blank paper provided to write your answers. Please label the additional paper with your name and the question number. You can staple the answers to this exam at the end. If you answer additional questions beyond the 10 requested, then bonus points *may* be awarded.

Read each question carefully before answering. Be certain you understand everything the question is requesting. Do the easy questions first. If questions appear confusing or exceedingly complex, then you may need to rethink the question. Keep in mind the intended examination topics.

In medicinal chemistry, hand-drawn pictures convey specific information. Be sure the drawing you have made conveys the essential information required to answer the question. Make certain that three-dimensional pictures display the correct atom arrangements. Don't forget to include formal charges when appropriate.

1. Lead compound development

Pick three of the six most common methods for finding new drug leads to begin a therapy program and briefly (one to three sentences or phrases) describe what these three involve. (10 pts.)

Six most common methods for lead drug discovery:
(1) Random screen or high throughput screen (HTS)
(2) Directed screen
(3) Structure-based drug design (SBDD)
(4) Fragment screen or Fragment based lead generation (FBLG):
(5) DNA-encoded library screening (DEL):
(6) Known compounds

(1) Random screen or high throughput screen (HTS): screening of small-molecules through collections of random compounds that can be large compound databases (10^5–10^6) for a large pharmaceutical organization but may also be smaller sets.

(2) Directed screen: screening of smaller sets of compounds that are selected based on prior knowledge of the target or chemical class. Also known as focused, targeted, or biased screening.

(3) Structure-based drug design (SBDD): in silico screening of compound collections, including the use of the target protein 3D structure, in this context used to generate novel chemical equity in a hit-finding campaign.

(4) Fragment screen or Fragment based lead generation (FBLG): typically libraries with a few thousand compounds of low molecular weights (<200 Da), screened at high concentrations.

(5) DNA-encoded library screening (DEL): screening of very large collections (typically >108) of small molecule compounds, using a technology that involves the conjugation of chemical compounds to DNA fragments.

(6) Known compounds: project started based on a prior disclosure of an active compound, endogenous ligand or previous program.

D. G. Brown et al., Where Do Recent Small Molecule Clinical Development Candidates Come From?, J. Med. Chem. **2018**, 61, 9442–9468.

2. Molecular interactions

The following figure and K_i values for factor X_a inhibitors were described in the article entitled, "A Medicinal Chemist's Guide to Molecular Interactions" (*J. Med. Chem*. **2010**, *53*, 5061-5084).

a) What is the name of the intermolecular interaction shown in the picture for the most potent of the inhibitors in the list? (3 pts.)

A cation-pi interaction.

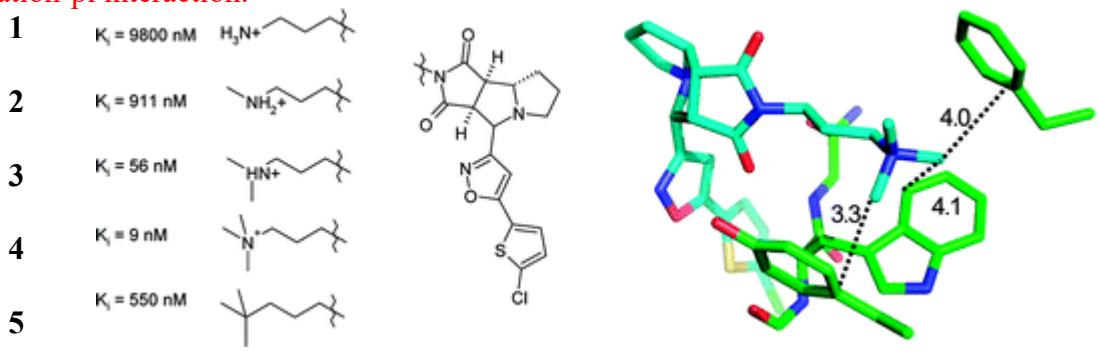


Figure 19. Example of cation−π interactions: a factor Xa inhibitor scaffold with systematically varied S4 pocket side chains (PDB code 2bok, distances in Å).(209)

b) The trend demonstrated in the first four inhibitors (**1-4**) highlights the impact of desolvation on drug potency. Explain. Your answer should include a description of drug desolvation. (4 pts.)

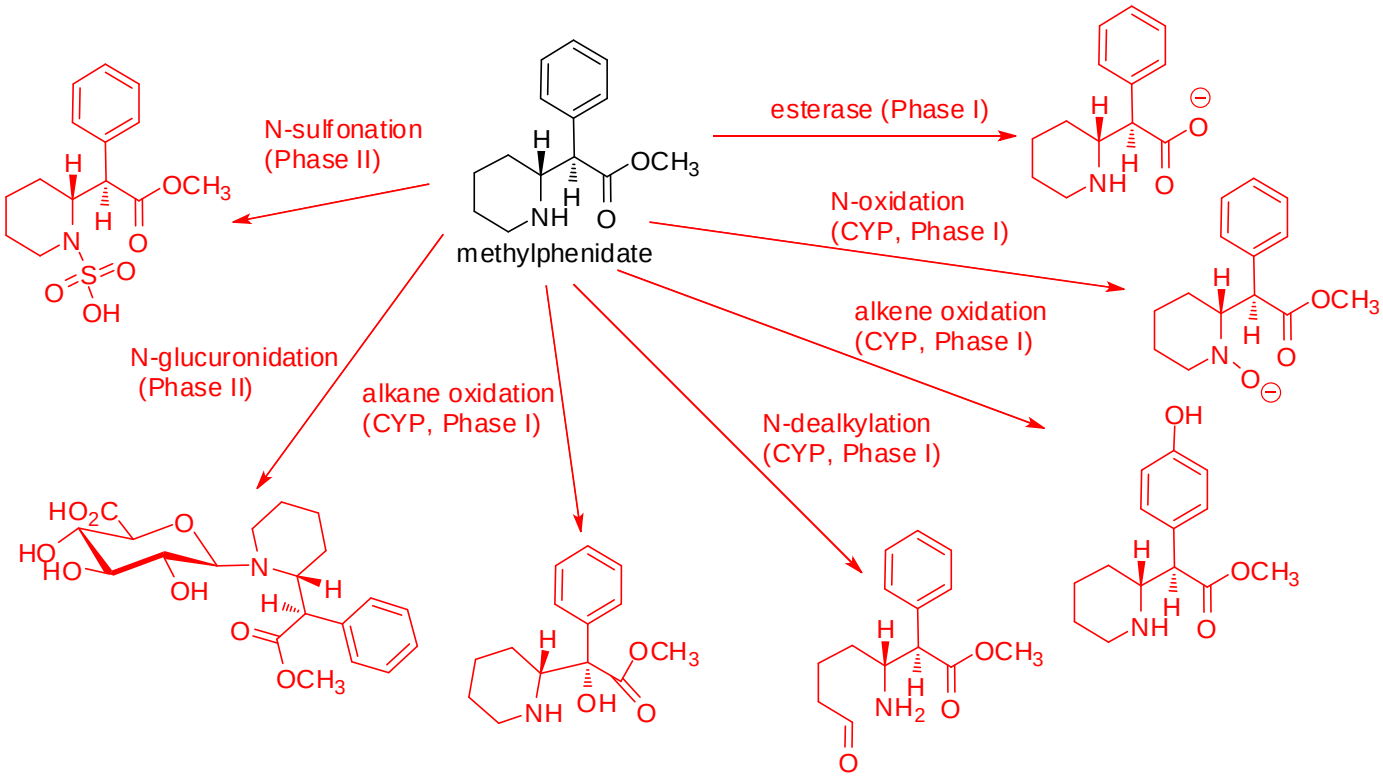
The elimination of hydrogen bond donors, i.e. N-H's, made the inhibitor more potent. This shows that without the need to desolvate the inhibitor with hydrogen bond donors, the inhibitor is more potent. Desolvation involves the removal of water molecules from around a drug as it enters the lipophilic environment of a protein. The presence of hydrogen bonding groups, such as N-H, increase the energy necessary to remove the water because it involves breaking the favorable intermolecular hydrogen bonds. This effect makes the drug binding process less favorable.

c) What is learned from the fifth inhibitor (**5**) with a K_i= 550 nM? (3 pts.)

The fifth inhibitor illustrates that the increase in potency isn't just from hydrophobic or van der Waals interactions and is actually the result of the cation charge interacting with the hydrophobic aromatic rings.

3. Metabolism of Ritalin

Methylphenidate (Ritalin) is the most commonly prescribed treatment for attention deficit hyperactivity disorder (ADHD). Based on our discussions of drug metabolism, propose three potential metabolic products of methylphenidate. You must have at least one example of a Phase I metabolic product and at least one example of a Phase II metabolic product. You may show a Phase II step that occurs after one of your Phase I metabolic reactions. Each metabolic reaction should be titled with the type of reaction and the Phase (I or II). Reactions catalyzed by cytochrome P-450 should be noted with a 'CYP' designation. (10 pts.)



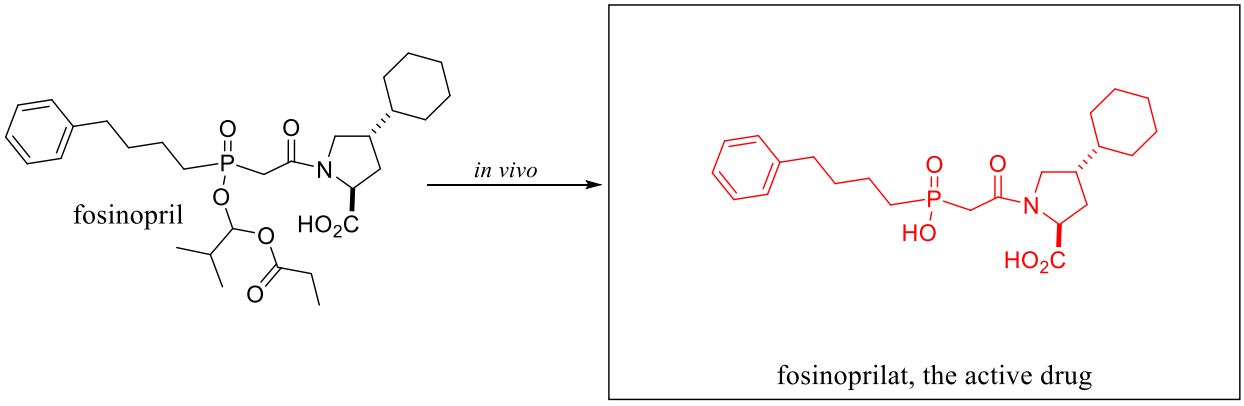
[Metabolomics of Methylphenidate and Ethylphenidate: Implications in Pharmacological and Toxicological Effects | European Journal of Drug Metabolism and Pharmacokinetics]

4. Angiotensin-converting enzyme (ACE) inhibitors

In class we talked about ACE inhibitors such as captopril and enalapril, which are used for the treatment of hypertension. Another example of an ACE drug is fosinopril (structure shown below). *In vivo* fosinopril is converted into fosinoprilat, the active drug and an inhibitor of ACE by the action of an esterase, which releases both propanoic acid and 2-methylpropanal.

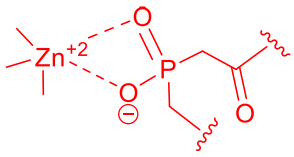
[Structural basis for the inhibition of human angiotensin-1 converting enzyme by fosinoprilat - Cozier - 2022 - The FEBS Journal - Wiley Online Library]

a) Provide the structure of fosinoprilat in the box below based on the description of the in vivo transformation of fosinopril. (3 pts.)



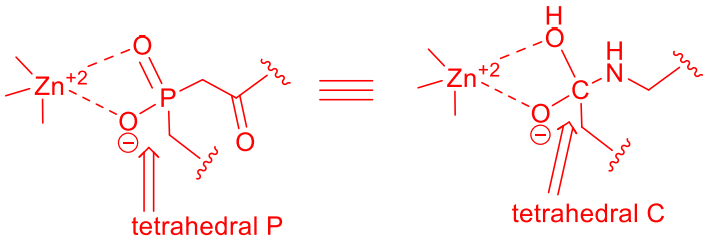
b) What is the general medicinal chemistry name applied to fosinopril given that it is transformed in vivo into the active drug? (1 pt.)
Fosinopril is a pro-drug of fosinoprilat.

c) Fosinopril binds in the active site of the metalloprotease ACE in a manner similar to captopril and enalaprilat, the active form of enalapril. Draw a picture of this interaction. You only need include the critical potions of fosinopril and the ACE. (3 pts.)



d) Unlike captopril and enalapril, fosinopril's inhibition of the ACE enzyme can also be considered a "transition state analog" inhibition event. How is fosinopril acting as a transition state analog and why is this a useful mode of inhibition of enzymes? Pictures might assist in your explanation. (3 pts.)

Fosinopril has a tetrahedral phosphorus atom that can mimic the tetrahedral intermediate of the ACE metalloprotease enzyme as shown below.



Enzymes generate their catalytic benefits by lowering the energy of transition states and tetrahedral intermediates in a reaction path thereby providing easier access to the products. This is primarily accomplished by creating an environment around a particular substrate that serves to stabilize the high energy transition state or intermediate. Since the enzyme has evolved to bind and have strong affinity for this transition state/intermediate, the enzyme will also likely have strong affinity for a stable mimic of this transition state/intermediate. A transition state/intermediate mimic can therefore be a quite potent inhibitor of the enzyme.

5. HIV protease inhibitors

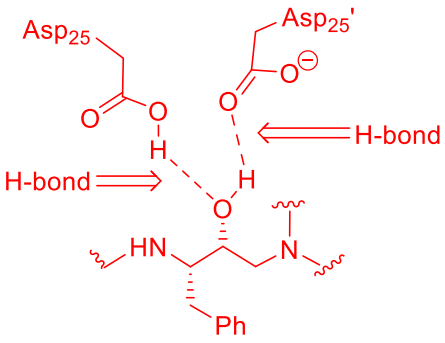
Saquinavir is an HIV protease inhibitor developed by Roche pharmaceutical company with the structure shown below. It has many of the same properties as the two other HIV protease inhibitors, ritonavir and indinavir, that were discussed in class.

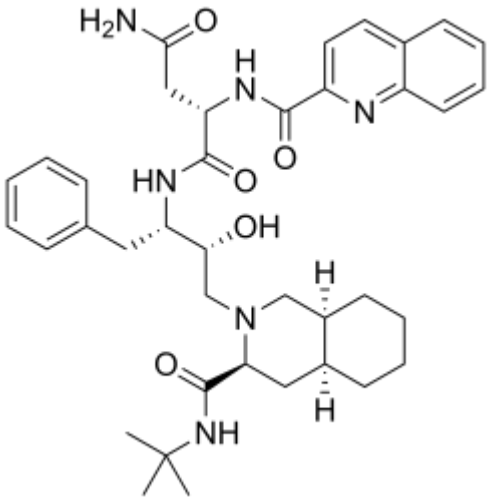
a) Sketch the key interaction between saquinavir and the amino acids involved in catalysis in the HIV protease active site. (5 pts.) You should abbreviate your structures but be sure to:

(i) Show the side chains of the key enzyme amino acids involved in catalysis.

(ii) Show the portion of saquinavir interacting with these catalytic amino acids.

(iii) Identify key intermolecular forces involved in the interaction between the amino acids and saquinavir.





saquinavir

PROTEINS: Structure, Function, and Bioinformatics | Protein Science Journal | Wiley Online Journal

b) On the structure of saquinavir drawn above, identify the approximate location of the components of saquinavir in the subsites S_1, S_1', S_2, S_2', and S_3. (5 pts.)

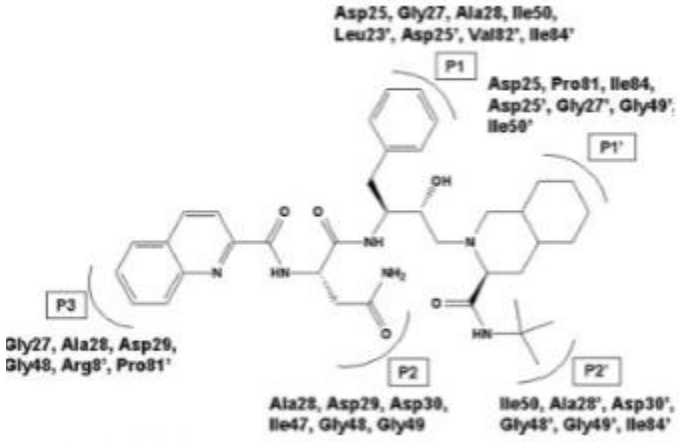
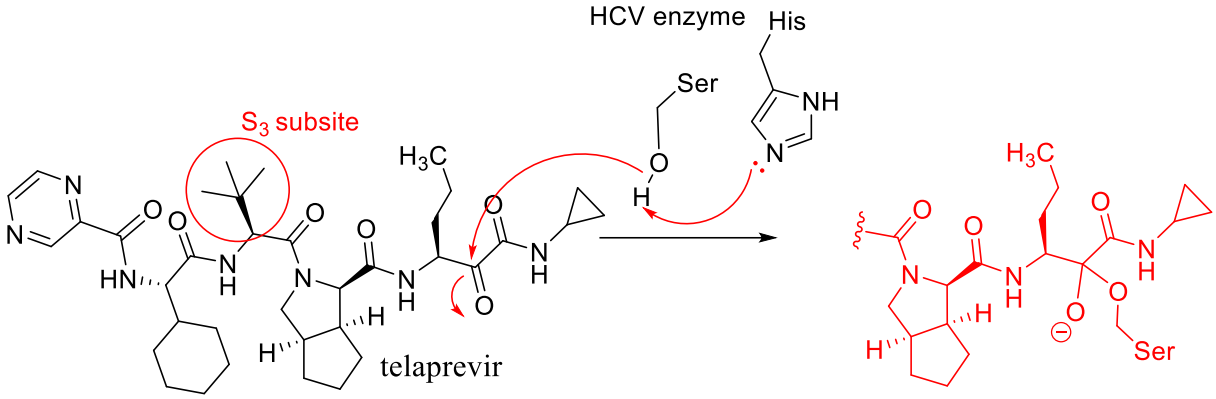


Fig. 2. SQV structure and PR residues forming van der Waals contacts.

6. Hepatitis C virus (HCV) protease inhibitors

Telaprevir was an HCV protease drug developed by Vertex pharmaceutical company. It has a mechanism of action similar to boceprevir, which was discussed in problem set 5.

a) On the structure below, draw the curved electron flow arrows and the product that results from the interaction of telaprevir with the HCV serine protease, called NS3/4A. You are encouraged to abbreviate your product structure but be sure to include the key components of the reaction outcome. (6 pts.)



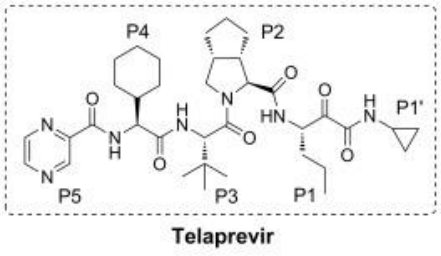
Acc. Chem. Res. **2008**, *41*, 50-59.

Substrate-Envelope-Guided Design of Drugs with a High Barrier to the Evolution of Resistance | SpringerLink

b) Based on the reaction that you showed between telaprevir and the HCV serine protease, what general class of inhibitors does telaprevir represent? (2 pts.)

Because a covalent bond is formed, telaprevir represents an irreversible or covalent inhibitor.

c) On the structure above, circle the substituent or group that would likely occupy the S_3 subsite of the HCV protease. (2 pts.)

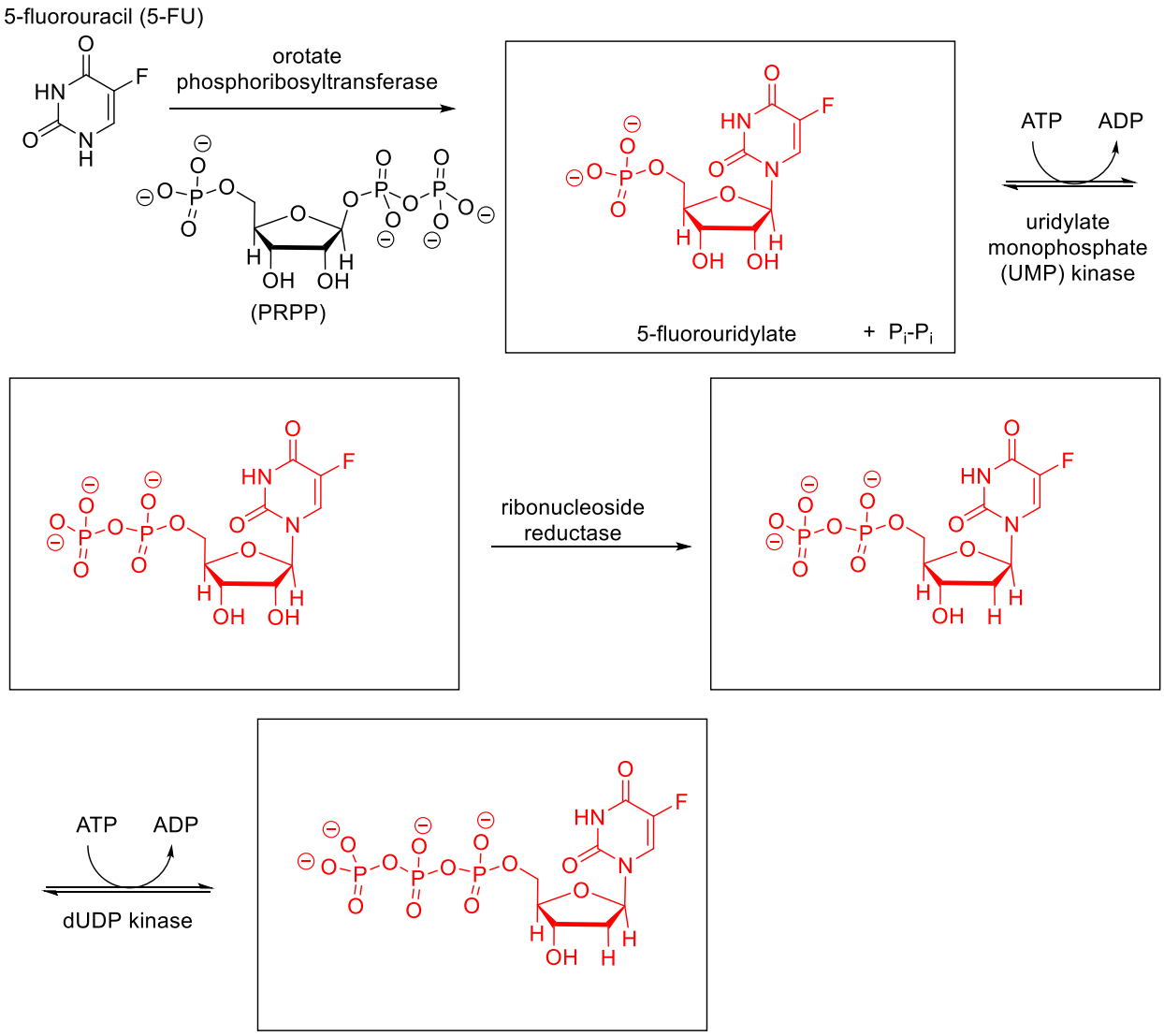


Telaprevir

7. Nucleic acid drugs

Most nucleic acid drugs that interact with DNA or RNA polymerase require the activation process shown below for the well-known anti-cancer drug, 5-fluorouracil.

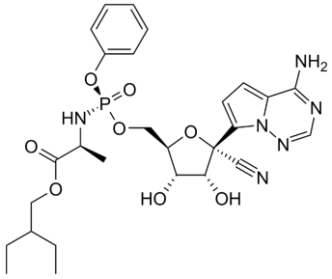
a) Provide the products for the four reactions shown. (8 pts.)



[Intracellular Pharmacokinetics of Pyrimidine Analogues used in Oncology and the Correlation with Drug Action | Clinical Pharmacokinetics]

b) Which step in this process is often the slowest or rate-limiting and the reason for the Pro-tide technology used with the COVID-19 anti-viral remdesivir? (2 pts.)

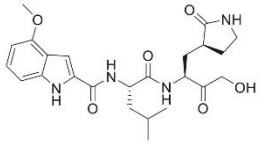
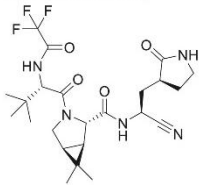
The first step, which puts the first monophosphate on the ribose ring, is often the slowest step. Pro-tide technology allows for the release of the monophosphate drug derivative once the drug has entered the body.



remdesivir

8. Paxlovid drug development

The table below shows important drug pharmacodynamic (PD) and pharmacokinetic (PK) parameters of the COVID-19 Pfizer drug called nirmatrelvir (PF-07321332) and the lead compound 1 (PF-00835231). Answer the questions below based on the table's data and our class discussions. Your answers should identify the data that you are using to draw your conclusions (i.e. the table column). (10 pts.)

Number/Structure	SARS-CoV2 M^{pro} K_i (nM)*	VeroE6-enACE2 CPE EC_{50} (nM)†	MDCK-LE P_{app} (x 10^{-6} cm/sec)‡	HLM CL_{int} (µl/min/mg)§	Rat CL_p (mL/min/kg)¶	Oral F (%)¶#	F_a x F_g (%)¶††
1 (PF-00835231) 	0.271 (0.155 – 0.471, n=6)	231 (158 – 338, n=8)	< 0.207 ± 0.048 (n=6)	7.47 ± 0.88	27.0 ± 3.1	1.4 ± 0.8	3.3
6 (PF-07321332) 	3.11 (1.47 – 6.59, n=6)	74.5 (66.5 – 83.4, n=20)	1.71 ± 0.28 (n=4)	24.5 ± 0.2	27.2 (22.5, 31.9)	50 (30, 71), 34 ±19**	95, 65**

D. Owen, C. M. Allerton, ***J. Lee*** et al., An oral SARS-CoV-2 Mpro inhibitor clinical candidate for the treatment of COVID-19, Science **2021**, 374, 1586-1593.

a) Is nirmatrelvir more potent than the lead compound in isolated enzyme assays or cell-based assays? Approximately how different are the values, i.e. five-times, ten-times, one-hundred-times?

Nirmatrelvir is not more potent in the isolated enzyme assay (SARS-CoV2 M^{pro} Ki, column 1) than compound **1**, but is more potent in the cell-based assay, VeroE6-enACE2 CPE EC_{50}. Nirmatrelvir is approximately 10-times less potent in the isolated enzyme assay and roughly three-times more potent in the cell-based assay.

b) Which compound has better bioavailability and by approximately how much?

Nirmatrelvir has much better bioavailability than compound **1** by two measures, Oral F (column 6) and F_a x F_g (column 7). By both analyses, nirmatrelvir has 20-30 times greater bioavailability.

c) Which compound is better able to penetrate cells and by approximately how much?

Nirmatrelvir has approximately eight-times greater cell permeability than compound **1** based on the P_{app} in MDCK-LE cells (third column). The permeability is a measure of the ability of the compound to enter cells.

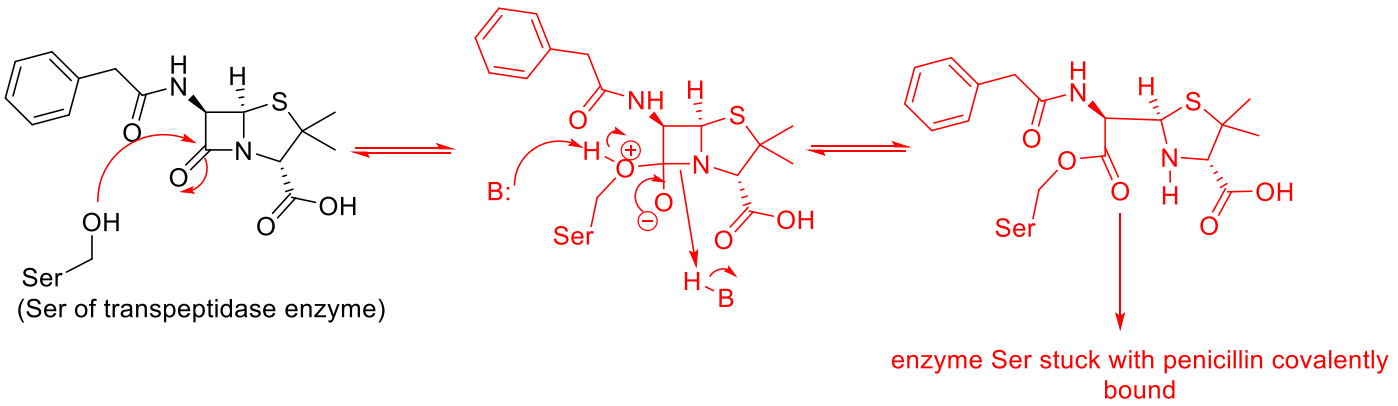
d) Which compound is degraded most rapidly and removed from the system? Estimate the difference.

Nirmatrelvir is cleared more quickly (roughly three-times faster) than compound **1** in human liver microsomes (HLM CL_{int}, column 4), but is cleared at about the same rate in rats (Rat CL_p, column 5).

9. Penicillin mechanism of action

Penicillin is another antibacterial that we discussed. It reacts with the bacterial transpeptidase enzyme, a serine protease.

a) Show the curved arrow electron flow mechanism of penicillin's interaction with bacteria in the space below. The necessary starting components, penicillin and the important side chain of the bacterial transpeptidase enzyme, are given. Be sure to include any important intermediates. You may use generic acids and bases to accomplish the reaction. (7 pts.)



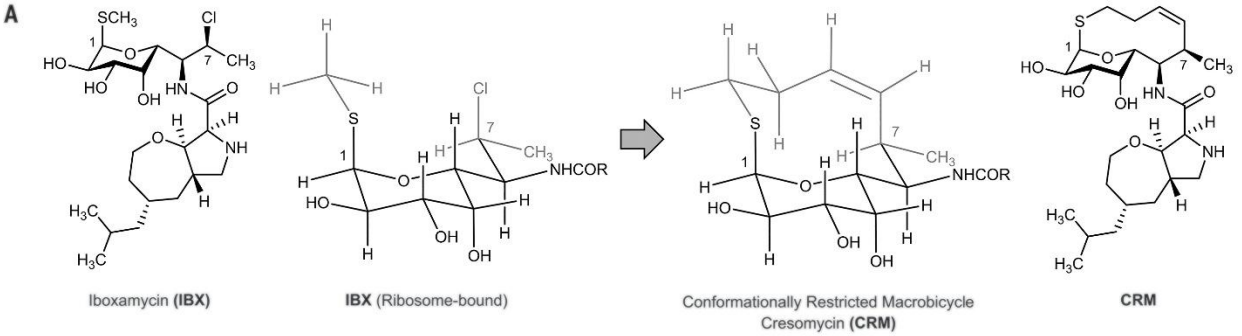
The mechanism of action of penicillin on JSTOR

b) How does this reaction with the bacterial transpeptidase enzyme kill the bacteria? (3 pts.)

Since the transpeptidase is a critical enzyme in the formation of the bacterial cell wall, the irreversible inhibition of this enzyme leads to an aborted cell wall formation. Without a complete cell wall, the bacteria spills its guts into solution and never forms a viable cell.

10, Iboxamycin antibacterial drug

A 2024 report in *Science* described a second generation of an iboxamycin drug, which further evolved the antibacterial drug structure by the addition of an alkyl/alkenyl bridge that restricted the conformation of the iboxamycin molecule. This new antibacterial, called cresomycin (CRM), was found to further enhance the potency of the antibacterial drug, IBX.
(An antibiotic preorganized for ribosomal binding overcomes antimicrobial resistance | Science)



a) Cresomycin, like IBX and clindamycin, interacts with bacteria by inhibiting the same critical operation of the bacteria. Where did IBX and clindamycin bind and how did that kill the bacteria? (3 pts.)

IBX and clindamycin bind in the ribosome of the bacteria. As such, they prevent bacterial protein synthesis.

b) What benefits would the conformational restriction shown for CRM provide, in terms of enthalpy and entropy, in binding to the ribosome in the same location as IBX? (7 pts.)

If the conformation is pre-organized in the active conformation of the drug, then there is a lower entropy penalty, i.e. less negative ΔS, for the drug to enter the target and bind. There would be no need for additional structural organization since the drug is already in the proper conformation.

With regards to enthalpy, there would be benefits if the hydrocarbon piece installed had additional intermolecular forces or interactions, i.e. van der Waals interactions, with a portion of the binding site location. Greater occupation of empty space can also benefit the binding energy enthalpy.

11. Antibacterial drug properties

An article published in the Journal of Medicinal Chemistry offered an analysis of antibacterials and their unique properties in an effort to facilitate the successful development of new antibacterials. The results are reported in the table below. Review the data in the table and answer the following questions based on our discussions of similar drug properties in other molecules.
(Physicochemical Properties of Antibacterial Compounds: Implications for Drug Discovery)

Table 1. Comparison of Average (Mean) Compound Property Values of Three Drug Data Sets Representing General (CMC, Excluding Antibiotics) and Antibacterial Drugs

parameter	CMC data set	antibacterials (only Gram-positive activity)	antibacterials (Gram-negative activity)
MW	338	813	414
clogp	2.7	2.1	−0.1
$clogD_{7.4}$	1.6	−0.2	−2.8
PSA ($Å^2$)	70	243	165
rel PSA (%)	22	30	42
H-donor	1.6	7.1	5.1
H-acceptor	4.9	16.3	9.4

CMC= comprehensive medicinal chemistry, which is all drugs that are not antibacterials

a) Compare the general properties of drugs with those of the antibacterials, both gram positive and gram negative. Which properties are different and what do these properties represent, i.e. your answer should include a description of what these properties mean. It would be appropriate for you to define acronyms that are used in the list of parameters. (5 pts.)

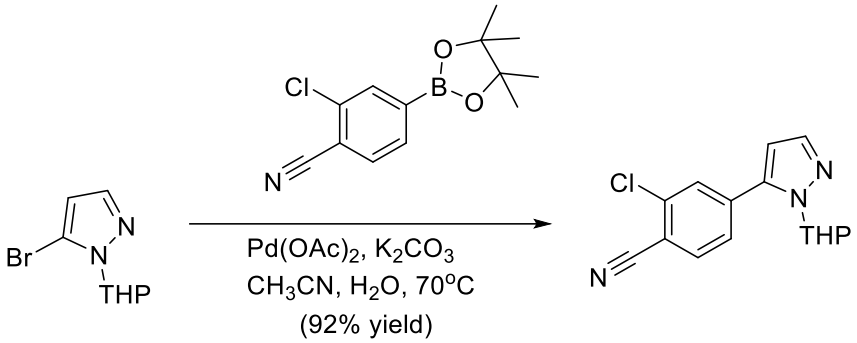
-much higher molecular weight (mw)
-generally more polar (clogp and clogD; partition coefficients), with a greater polar surface area (PSA); more H-bond donors (H-donor) and H-bond acceptors (H-acceptor).

b) Are there any properties of the antibacterials that break Lipinski's rule of five? Are there any properties that adhere to Lipinski's rule of five? (5 pts.)

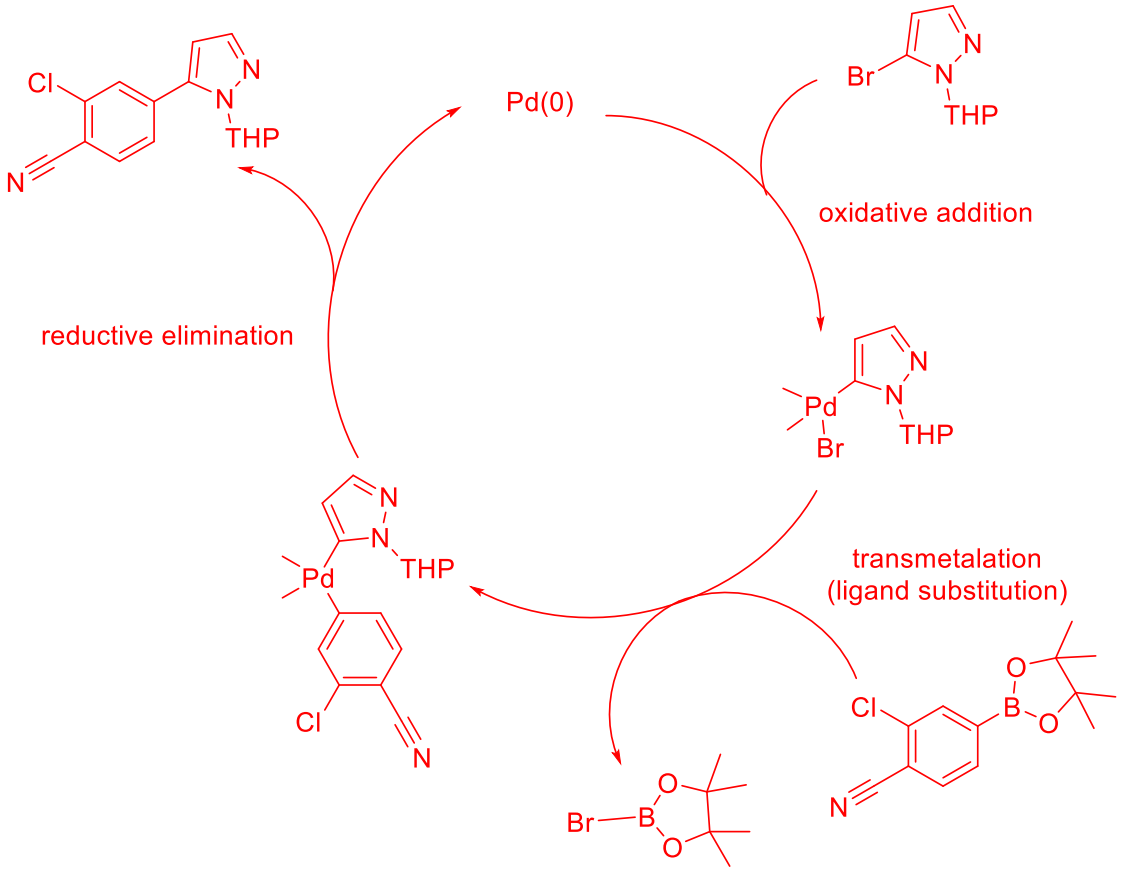
-H-donor too high (5 max)
-H-acceptor too high for gram positive group (10 max)
-molecular weight exceeds the 500 maximum suggested by Lipinski's rule
-the clogp is below 5, which is suggested by Lipinski's rule of five

12. Suzuki-Miyaura reaction mechanism

Provide a mechanism for the following cross-coupling reaction. Be sure to include intermediate structures and the mechanistic name of the steps in the catalytic cycle. You are encouraged to abbreviate the intermediate structures but be sure the key components of the molecules are shown. (10 pts.)



Possible catalytic cycle



Mechanism of Palladium-Catalyzed Suzuki–Miyaura Reactions: Multiple and Antagonistic Roles of Anionic "Bases" and Their Countercations - Amatore - 2013 - Chemistry – A European Journal - Wiley Online Library