20.201 Take-home exam, October 2013

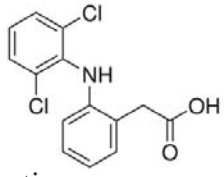
Question 2

When thinking about toxicity, we can think about drug metabolizing enzymes that a xenobiotic encounters during both phase I and phase II of metabolism, which generally consists of hydrolysis, reduction, oxidation and conjugation reactions. If the drug encounters an enzyme that can convert xenobiotics to reactive electrophilic metabolites, these metabolites can therefore react with nucleophiles in the cell such as DNA or proteins and covalently bind, leading to toxicity. The enzyme most often associated with the process of generating reactive electrophiles is cytochrome p450. My approach therefore for this problem will be to analyze how many sites in each drug can be recognized by a metabolic enzyme and subsequently converted to an electrophile.

A) Structures and approach are attached on a separate PDF file. I outline the possible reactive site in each molecule and which I think will form the most reactive intermediates. Hypo1 can form the following potent metabolites nitrogen cation electrophile, a sulfinic acid, an ortho-quinone and an epoxide group and a ketone intermediate. Hypo 2 can form the following potent metabolites: a para-quinone, an α,β-unsaturated γ-dicarbonyl compound, an epoxide, and a ketone intermediate. It seems as though Hypo2 could be less toxic than Hypo1. Because you are losing the toxicity from the heterocyclic tertiary amine oxidation and the sulfur oxidation, but you still have major quinone toxicity. I would say that the most important electrophile for each structure is phenol rings which can easily be converted to quinones. Quinones are powerful electrophilic Michael Acceptors that can react with cellular nucleophiles and form protein adducts. For example, neurotoxicity associated with Parkinson's disease can be explained by para-quinone formed from the oxidation of dopamine, (Labenski et al 2009).

B) Diclofenac is an NSAID (or nonsteroidal anti-inflammatory drug) which is used to reduce inflammation.

This is its chemical structure:



Dicflofenac can get extensively metabolized into well characterized metabolites including 4'-hydroxydiclofenac, 5-hydroxydiclofenac (5-OHdic), 3′-hydroxydiclofenac, 4′,5-dihydroxydiclofenac, and 3′-hydroxy-4′-methoxydiclofenac. 4' and 5'-hydroxydiclofenac are known to form highly reactive quinone imines.

Without cytochrome p450, usually diclofenac is less toxic in vitro because there is no formation of the reactive metabolites. In this case, however, diclofenac has some toxicity associated with itself. Diclofenac in vitro has been shown to impair ATP synthesis by the mitochondria, (Bort et al 1999). Impairing ATP synthesis would essentially cause cell death. While diclofenac's metabolites can also impair ATP synthesis to some extent, usually the metabolites are present with some concentration of the parent molecule as well. Here, we just have a large concentration of diclofenac that can all react with the mitochondria and essentially is not titrated away by a CYP.

On the other hand, diclofenac could be metabolized by another enzyme in the cell. UDPGTs, for example, have been shown to react with diclofenac to produce a reactive electrophile that can form protein adducts, (from Prof. Tannenbaum's Drug Metabolism II Lecture). Perhaps, we do not normally observe as much of this reaction because diclofenac binds with a higher affinity to CYPs than UGTs, but in the absence of CYP we can observe this affect, assuming CHO cells express UGTs.

C) Cholestasis is a condition where bile cannot flow from the liver to the duodenum. In testing for Drug Induced Liver Injury, according to the FDA's standard of Hy's Law, a drug that shows hepatocellular injury will show greater than 3-fold elevation of ALT or AST above the normal and greater then 2x the upper limit of normal for total bilirubin levels. However, since cholestasis is an obstructive condition, it can also cause increases in serum bilirubin levels but it has not been characterized to cause high elevation of ALT or AST. Therefore, by just testing for ALT and AST, cholestasis will be overlooked. However, testing for ALP levels is more specific to cholestasis over general DILI. Since in cholestasis, the bile ducts can become blocked, ALP levels will rise in the serum because it is expressed in the edges of the cells that join together to form the bile ducts. In general, hepatocellular injury induced necrosis can vastly increase the serum levels of ALT and AST. However, cholestasis is functionally a blockage disorder, caused by inhibition of a transporter such as BSEP. ALT and AST will not be majorly transported into the serum during cholestasis.

Sources:

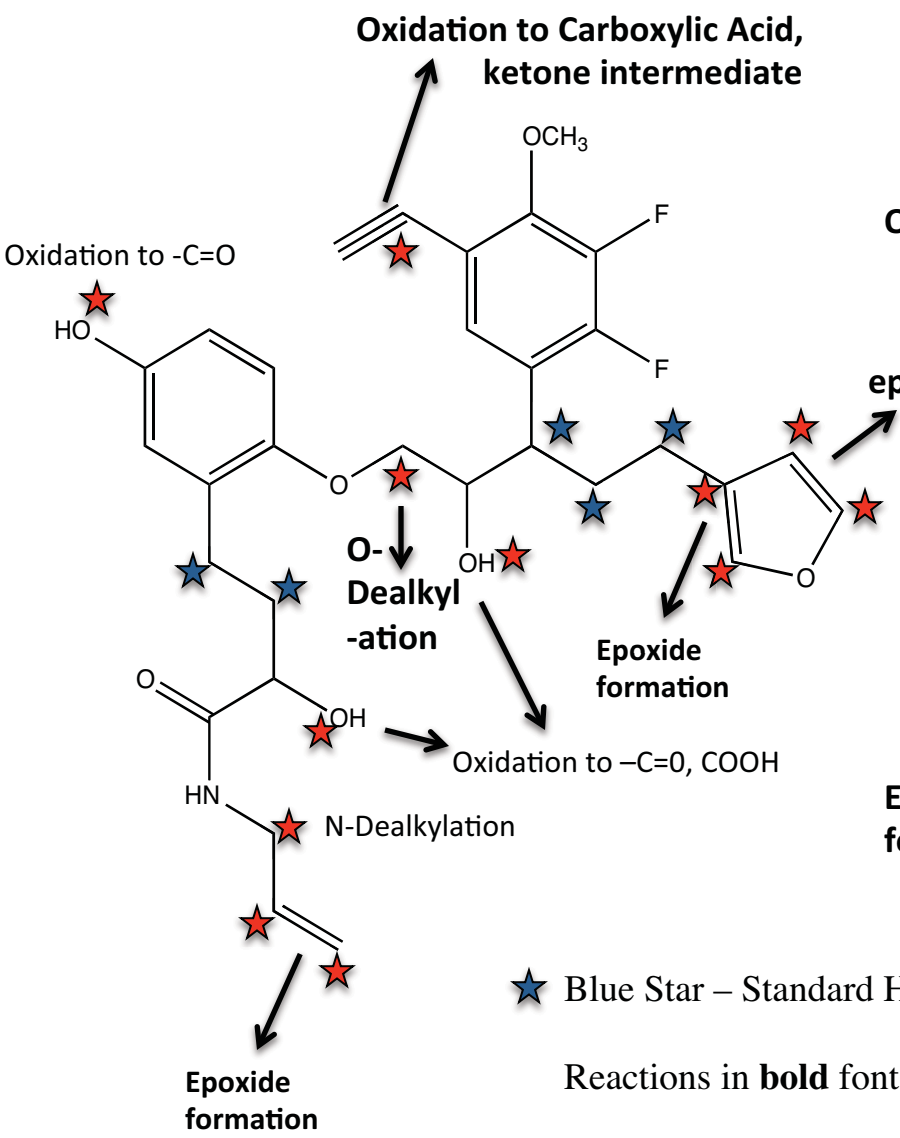
Bort, R. et al. "Diclofenac Toxicity to Hepatocytes: A Role for Drug Metabolism in Cell Toxicity." *Pharmacology*. (1999): 288(1)

Regan, SL. Et al. "Acyl glucuronides: the good, the bad and the ugly." *Biopharmaceutics and Drug Disposition*. (2010): 31(7)

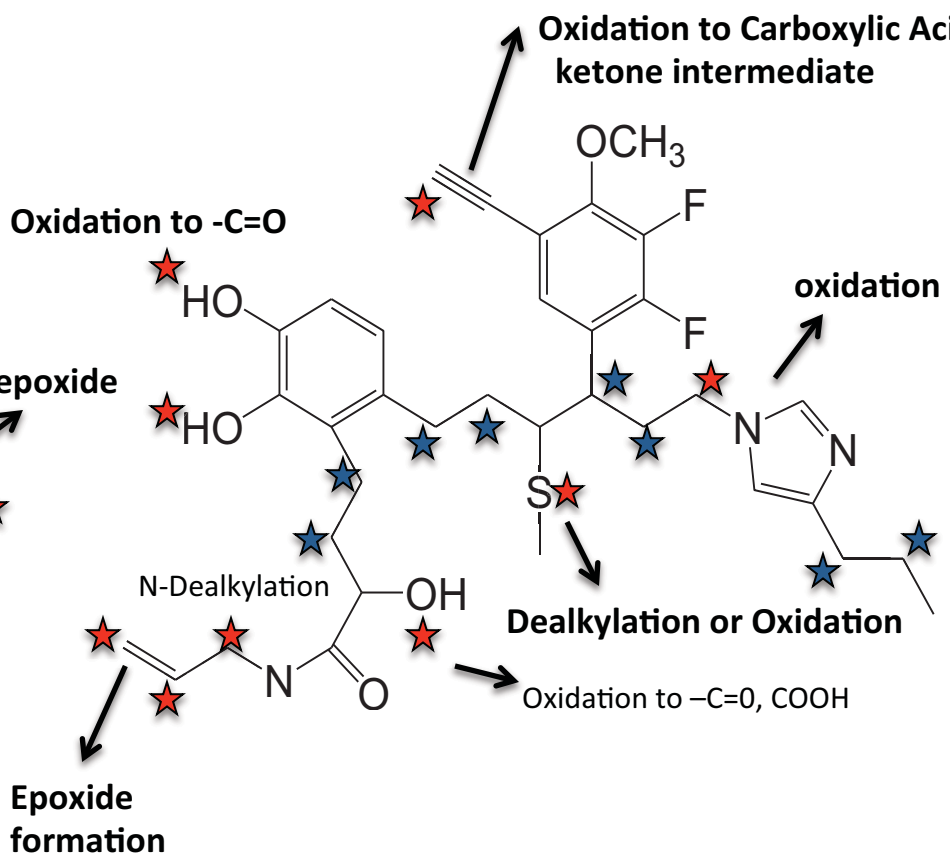
http://labtestsonline.org/understanding/analytes/alp/tab/test

Possible Reactive Sites

Hypoamericillin 2



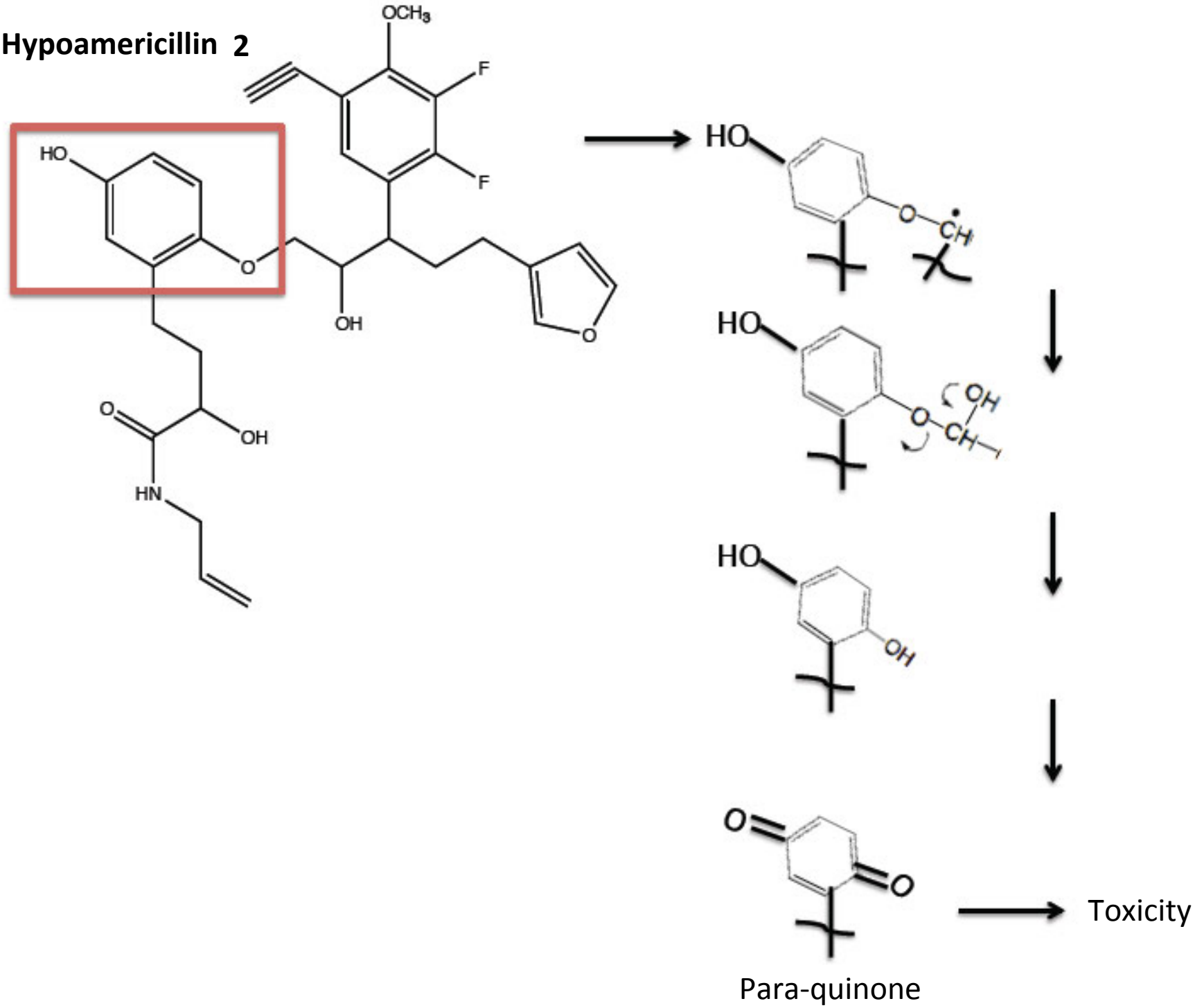
Hypoamericillin 1



★ Blue Star – Standard Hydroxylation by CYP450

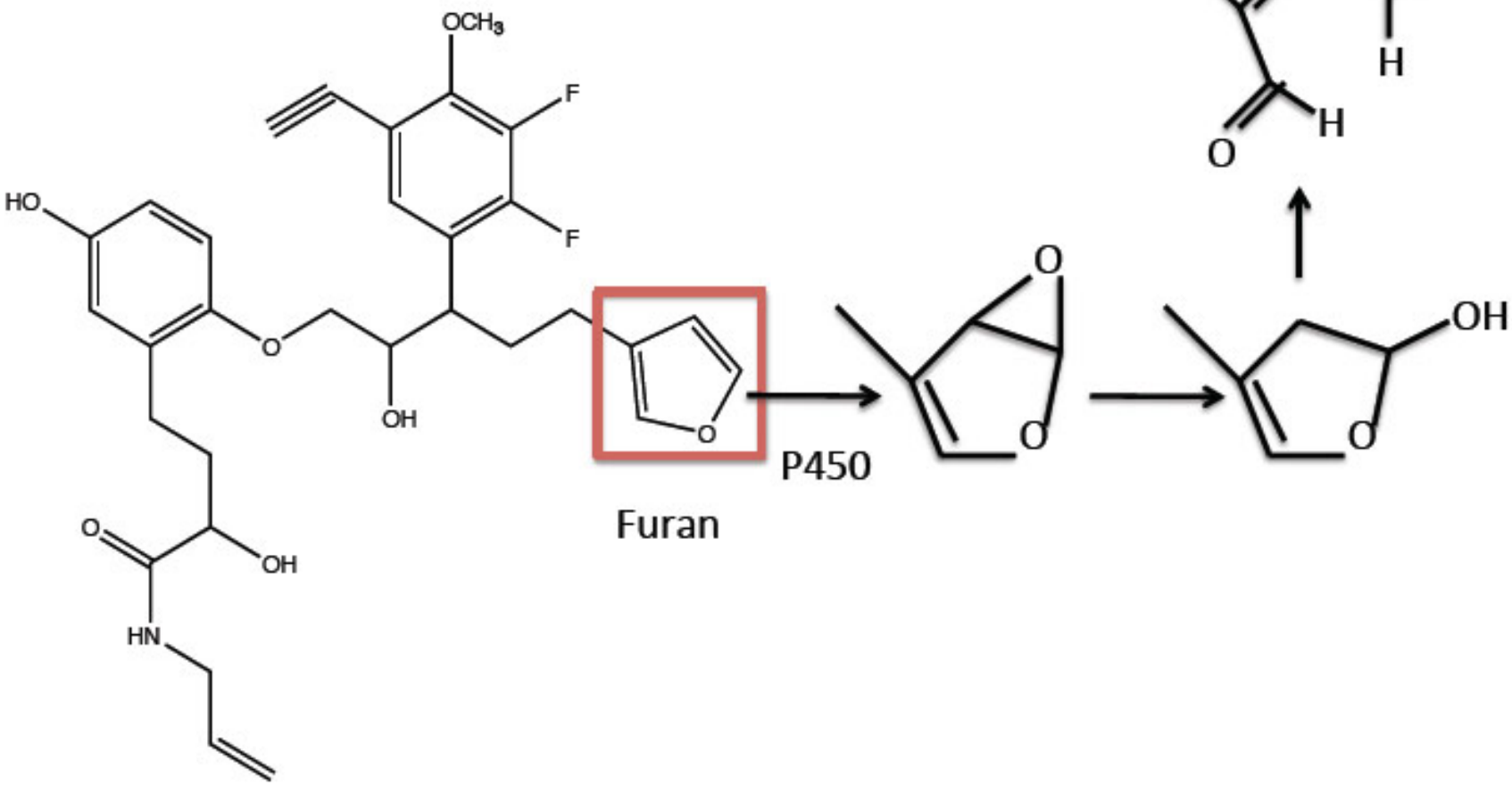
Reactions in **bold** font lead to possible toxic intermediates

Hypoamericillin 2

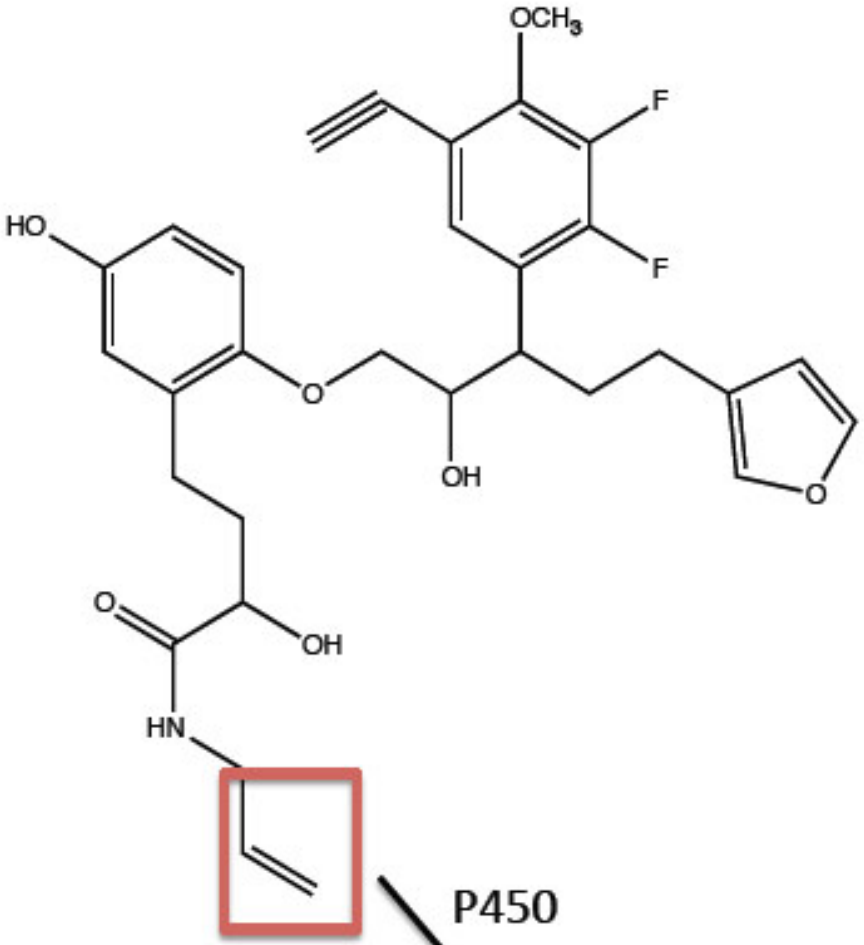


Para-quinone

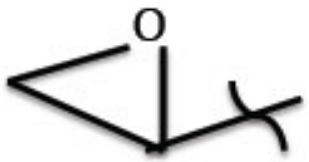
Hypoamericillin 2



Hypoamericillin 2



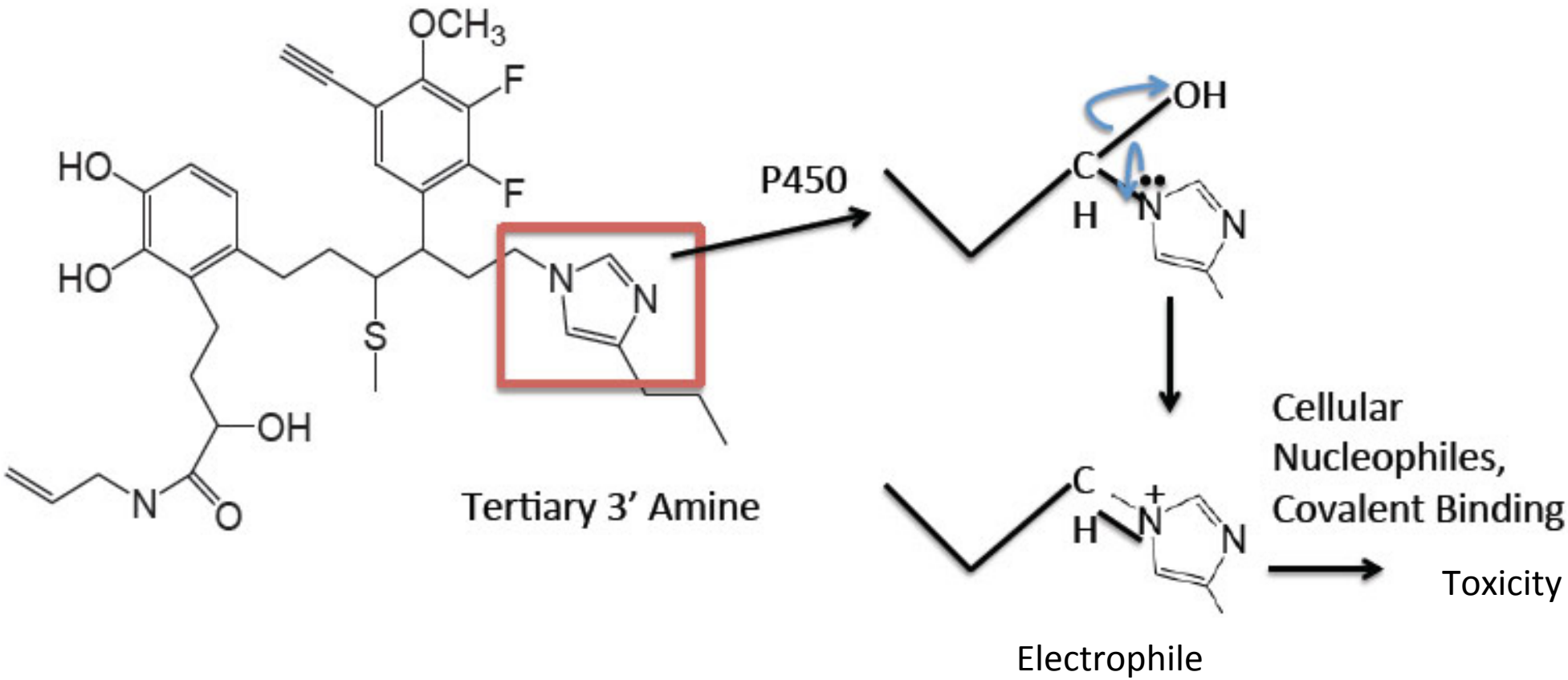
P450



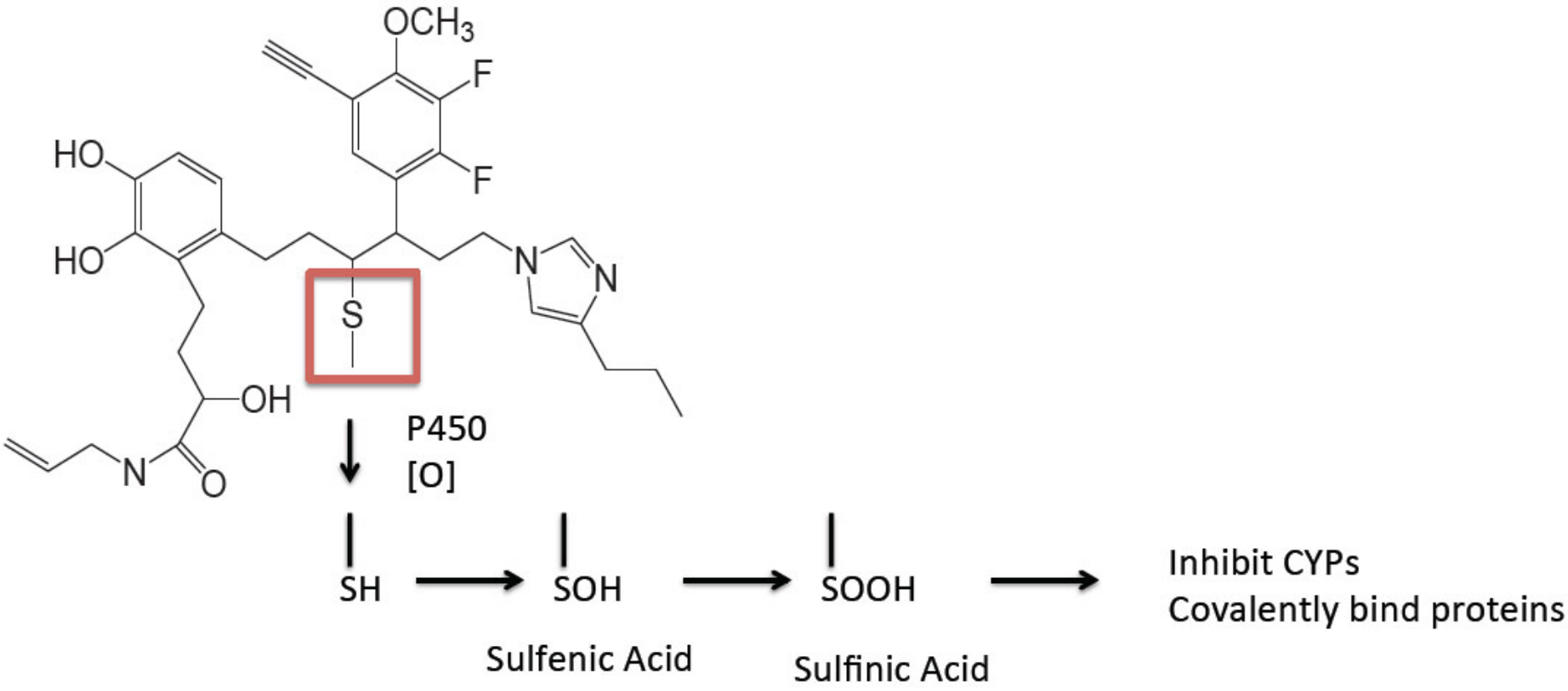
Epoxide
electrophile

Possible toxicity

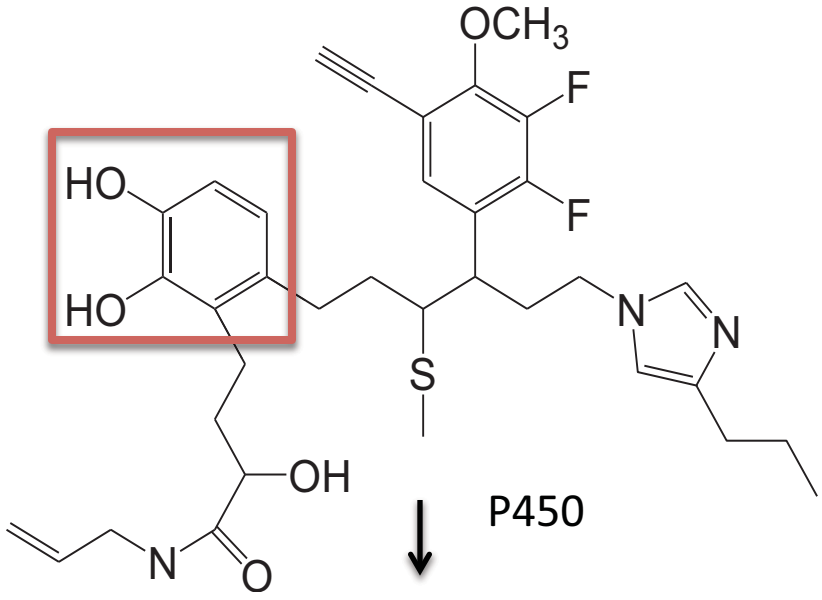
Hypoamericillin 1



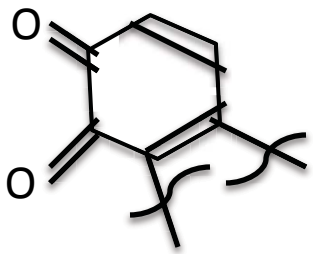
Hypoamericillin 1



Hypoamericillin 1



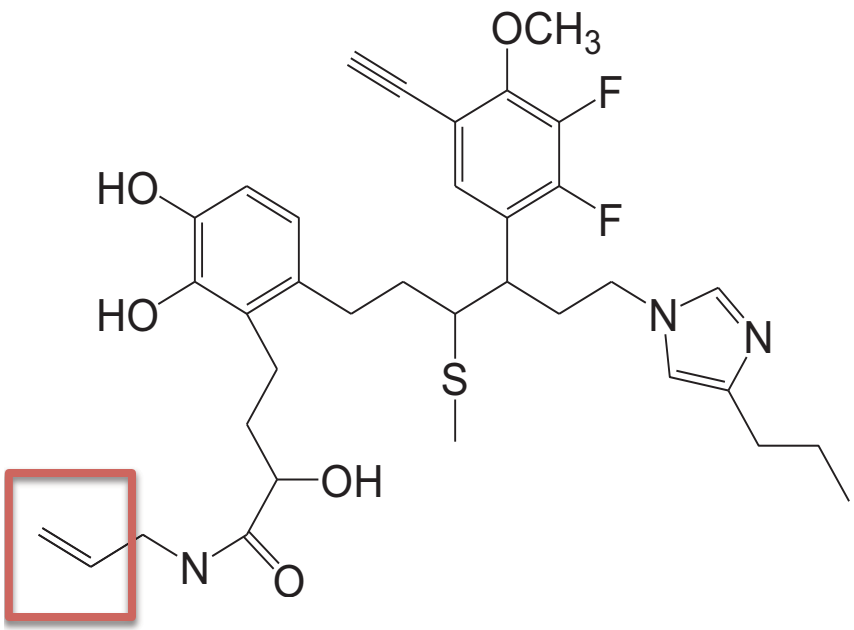
P450



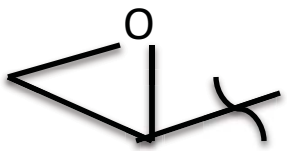
Ortho-Quinone

Oxidative Stress
DNA Damage

Hypoamericillin 1



P450

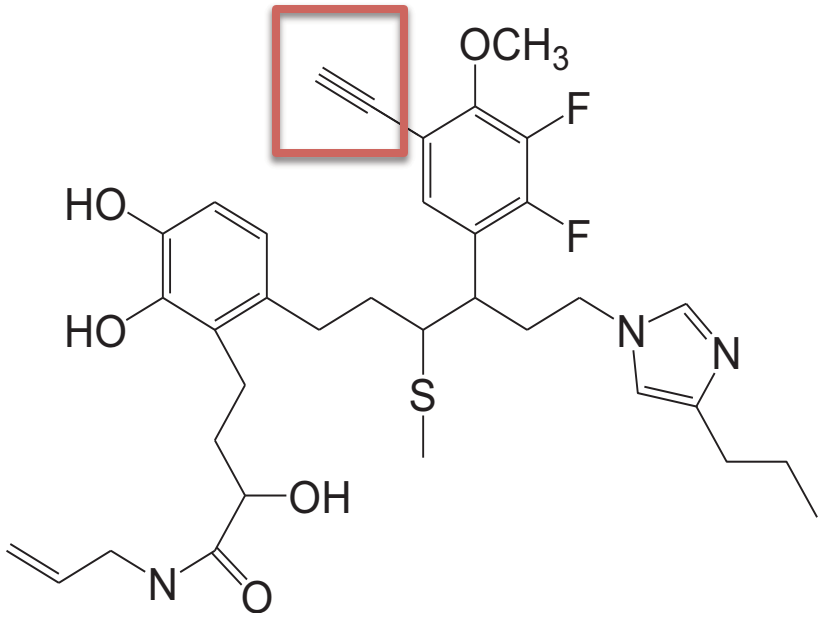


Possible toxicity

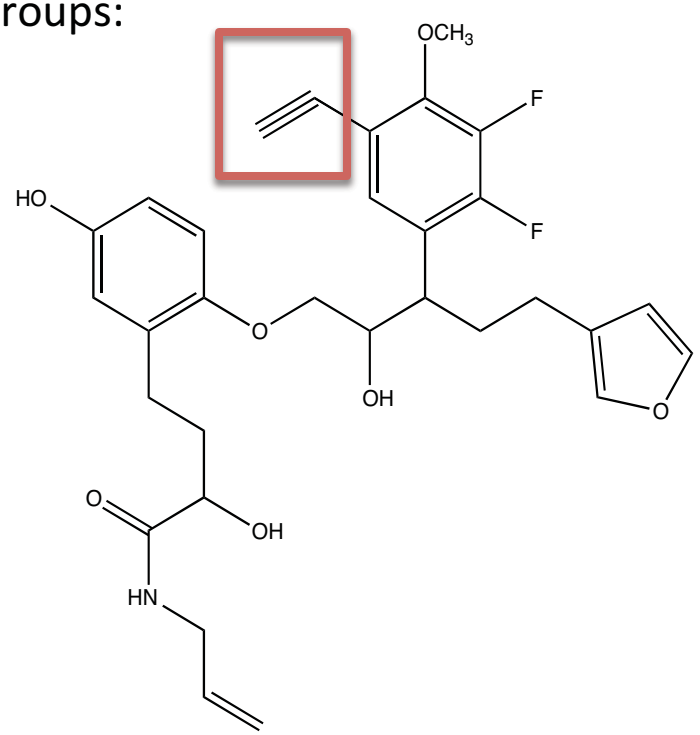
Epoxide
electrophile

Hypoamericillin 1

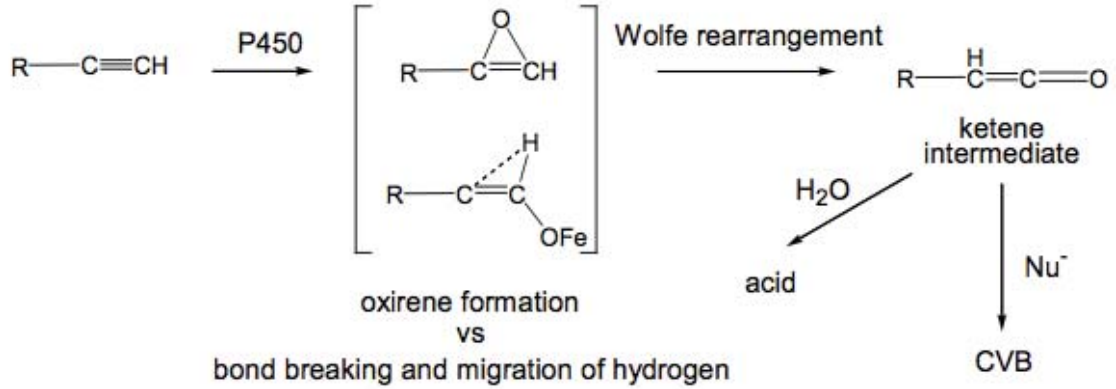
Terminal Acetylene Groups:



Hypoamericillin 2



From Professor Tannenbaum's Lecture Notes:



20.201 Mechanisms of Drug Actions

Fall 2013