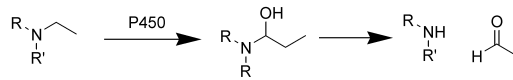


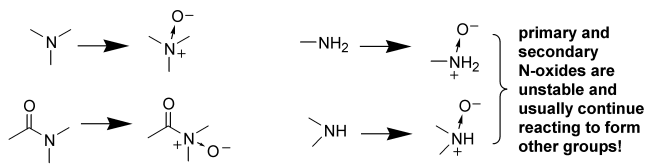
Drug Metabolism: Phase I

N-oxygenation

Earlier that we showed the **oxidative dealkylation** of alkyl amines:



Here we discuss the metabolic reaction where the nitrogen atom is oxidized directly. This is called **N-oxygenation** and the compounds produced are **N-oxides**:



Two enzyme systems are utilized for N-oxidation reactions:

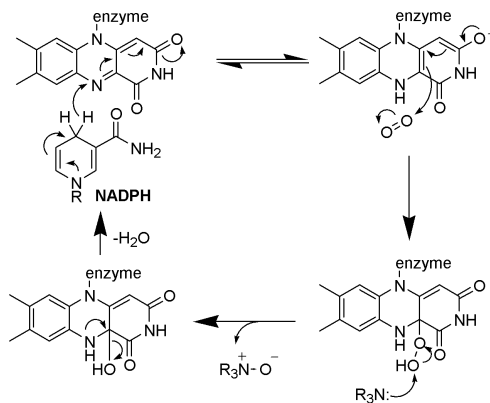
- **Flavin-containing monooxygenase (FMO):** amines (pKa 8-11).
- **Cytochrome P450:** amides and other nonbasic nitrogen species

Drug Metabolism: Phase I

N-oxygenation

Although less prevalent than P450, **Flavin-Containing Monooxygenase (FMO)** is important for the oxygenation of xenobiotics (especially N and S).

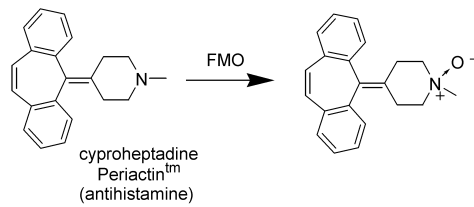
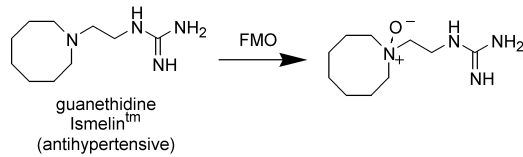
- One oxygen atom is incorporated from O_2 into the substrate.
- A **flavin hydroperoxide** is the putative **intermediate** in this process, which gets attacked by the substrate nucleophile.



Drug Metabolism: Phase I

N-oxygenation

Tertiary amines are generally oxidized to the corresponding **N-oxides**, which tend to be stable species.

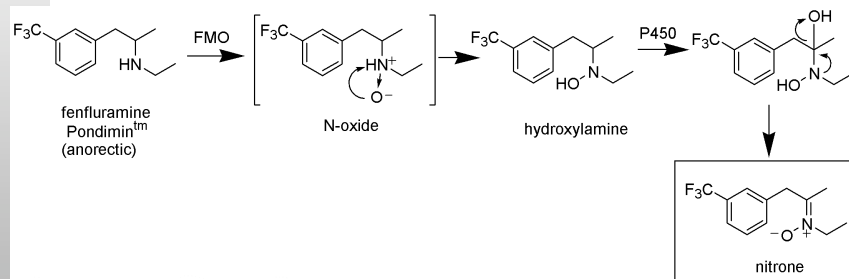


Drug Metabolism: Phase I

N-oxygenation

Secondary amines are commonly oxygenated to **hydroxylamines** but these are usually further oxidized to **nitrones**.

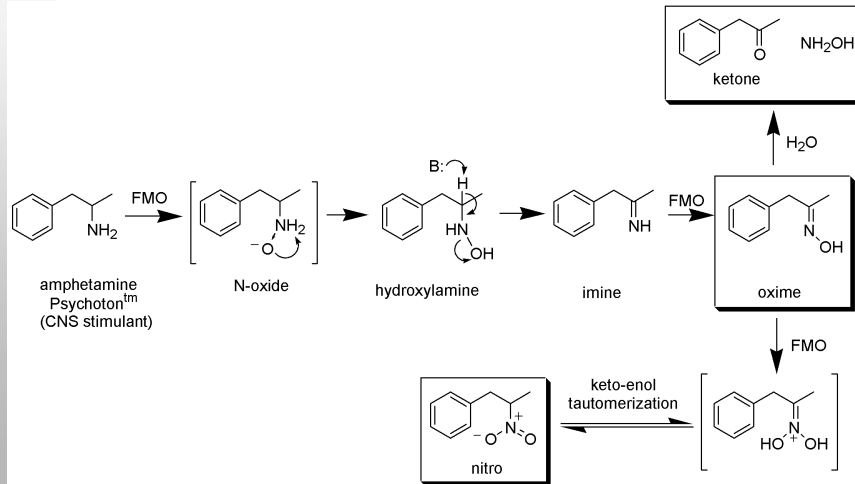
An example of this is shown below for the *anorectic drug* (appetite-suppressant) **fenfluramine** (one half of fen-phen).



Drug Metabolism: Phase I

N-oxygenation

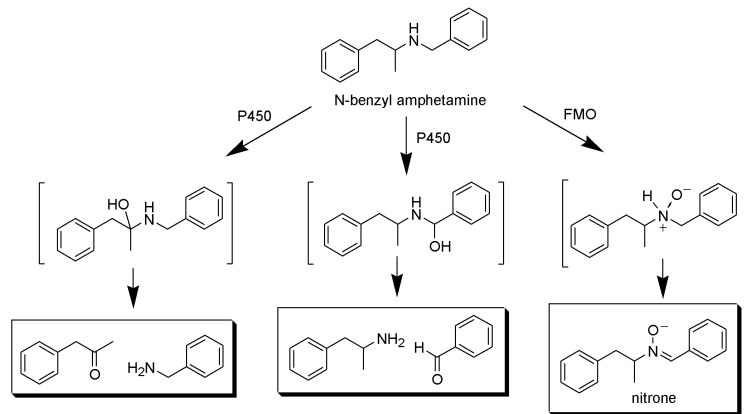
Primary Amines are oxidized to **N-oxides** that quickly rearrange to **hydroxylamines** and then to **oximes**. These can be converted to **ketones** or to **nitro** species.



Drug Metabolism: Phase I

N-oxygenation

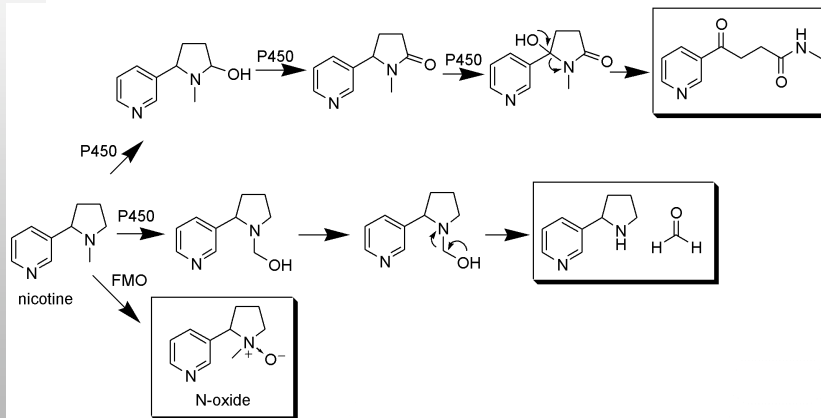
- **Drugs (xenobiotics) rarely undergo a single metabolic pathway.**
- Several metabolic pathways may compete to detoxify the compound, ultimately allowing its excretion.
- As examples, consider **N-benzylamphetamine** and **nicotine** (next slide).



Drug Metabolism: Phase I

N-oxygenation

The metabolism of **nicotine** can proceed through these three pathways (among others)



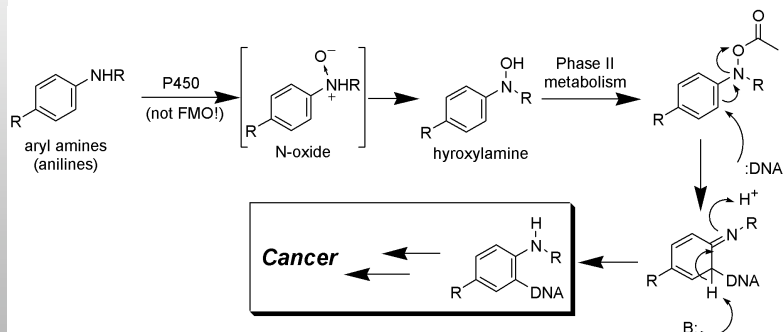
Drug Metabolism: Phase I

N-oxygenation

Aryl amines (anilines) are oxidized to **N-oxides** which activate the aryl ring for attack by cellular nucleophiles (**DNA, etc.**).

Therefore **anilines** are often **carcinogens** (and usually bad functional groups in drugs)

Note: the oxidation of aryl amines is done by **P450**, not **FMO**!

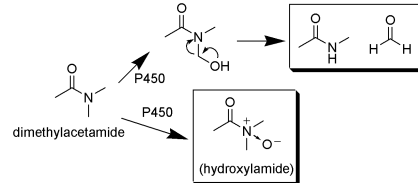


Drug Metabolism: Phase I

N-oxygenation

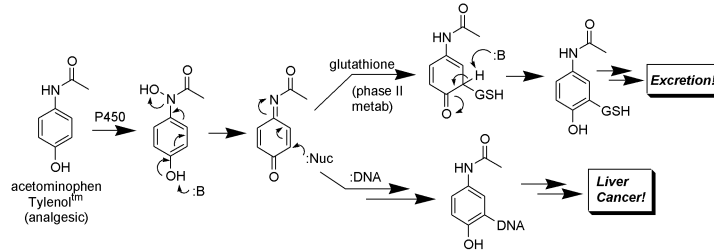
Alkyl amides are metabolized by **oxidative dealkylation** or **N-oxidation**.

Both reactions are catalyzed by **P450** (not **FMO**).



Aromatic amides are also subject to **oxidative dealkylation** and **N-oxidation**.

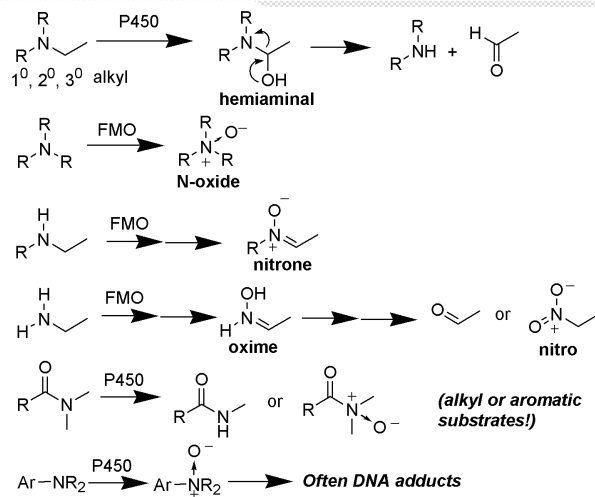
In the latter case an **electrophilic** species can be formed and result in **toxicity!**



Drug Metabolism: Phase I

N-oxygenation

Summary of reactions of amines and amides:



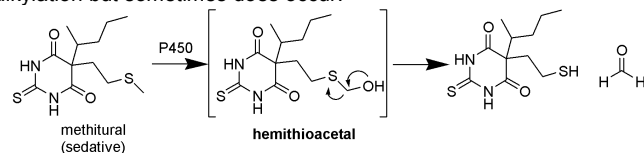
Drug Metabolism: Phase I

S-Oxygenation

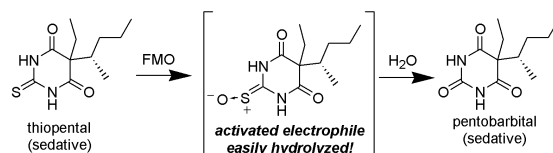
There are 3 principle ways in which sulfur-containing drugs are biotransformed.

- Oxidative S-dealkylation (P450)
- Desulfuration (FMO)
- S-oxidation (FMO)

Oxidative S-dealkylation is not as prevalent as the analogous oxidative N- or O-dealkylation but sometimes does occur:



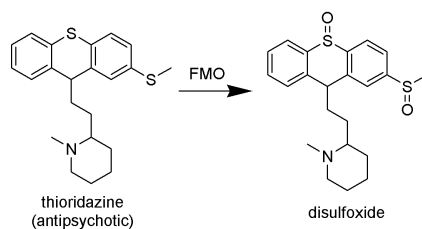
Desulfuration is often done to metabolize **thioketones** to **ketones**:



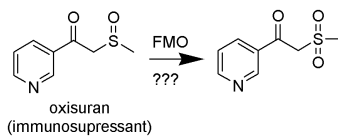
Drug Metabolism: Phase I

S-Oxygenation

- **S-Oxidation of sulfides to sulfoxides** is one of the most common metabolic transformations of sulfur-containing drugs.



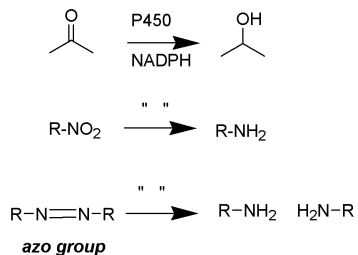
- Further oxidation of **sulfoxides to sulfones** occurs with some drugs:



Drug Metabolism: Phase I

Reductions

- **Oxidative** processes constitute the majority of the metabolic pathways for drugs and xenobiotics.
- However, some **reductive** reactions have also been identified and may be of great pharmacological importance when they generate active or toxic metabolites.
- Typically the **P450** enzymes are used in the presence of **reductive cofactors** (often **NADPH**).
- We will only address these three reductive transformations:

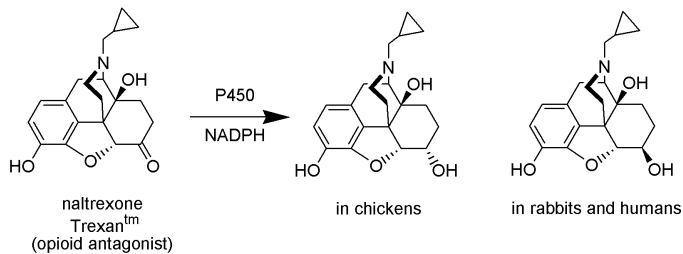


Drug Metabolism: Phase I

Reductions

Reductions at Carbon Atoms

- The reduction of **saturated ketones** leads to **alcohols**.
- Often a **single stereoisomer** is formed in the reduction: **stereospecific!**
- Note the **species-dependent stereospecificity** that results!

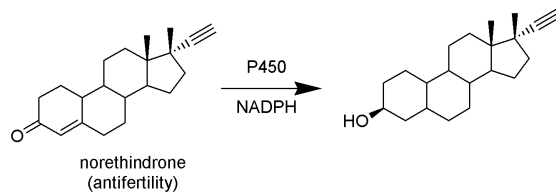
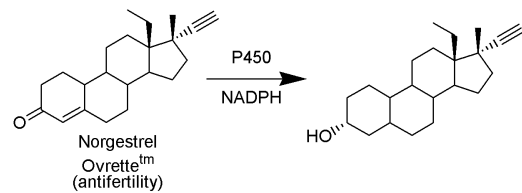


Drug Metabolism: Phase I

Reductions

Reductions at Carbon Atoms

- The reduction of α,β -unsaturated ketones leads to **alcohols**.
- The **C=C** and **C=O** bonds are both reduced.
- Note the effect of a **single extra carbon** on the **stereospecificity** of this rxn!

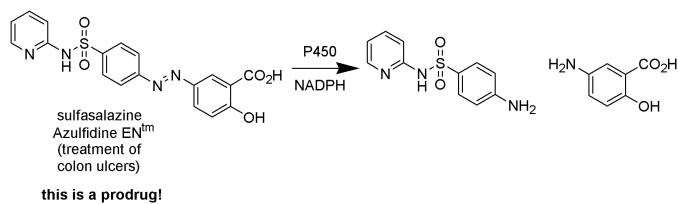
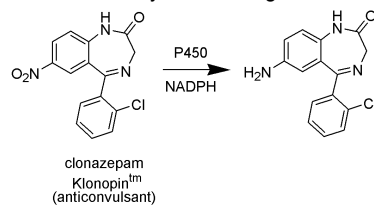


Drug Metabolism: Phase I

Reductions

Reductions of Nitro and Azo groups:

- The reduction of these groups lead to **amines**.
- In both cases, the reactions are catalyzed by **P450** and the reductive cofactor **NADPH**. (other metabolic enzymes in the gut are also important)

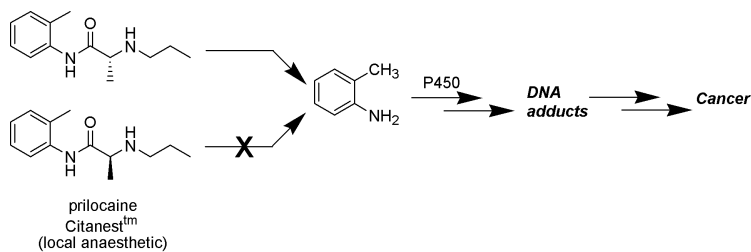


Drug Metabolism: Phase I

Hydrolytic Reactions

The hydrolysis of a drug may be stereospecific.

- Both enantiomers of **prilocaine** have local anesthetic action.
- Only the **(R)-isomer** of is **hydrolyzed to toluidine**, which causes toxic side-effects.
- The **(S)-isomer** is not hydrolyzed and does not cause this side effect.

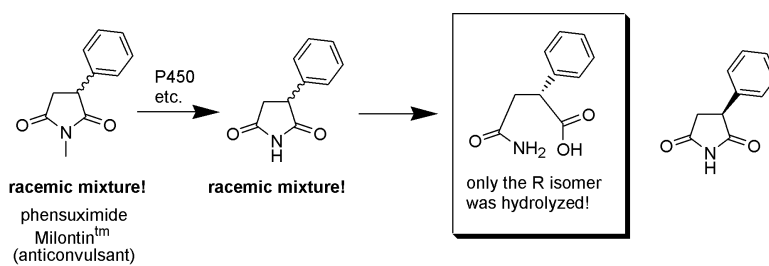


Drug Metabolism: Phase I

Hydrolytic Reactions

The hydrolysis of a drug may be stereoselective.

- **Phensuximide** is metabolically N-demethylated followed by stereoselective hydrolysis.

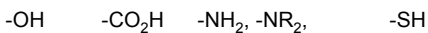


Drug Metabolism

Phase II Transformations: Conjugation Reactions

Phase II reactions generally involve the attachment (or **conjugation**) of small polar endogenous molecules to drugs or **Phase I** metabolites. The result is further deactivation of the drug and the production of **water-soluble metabolites** that are readily excreted in the urine or bile.

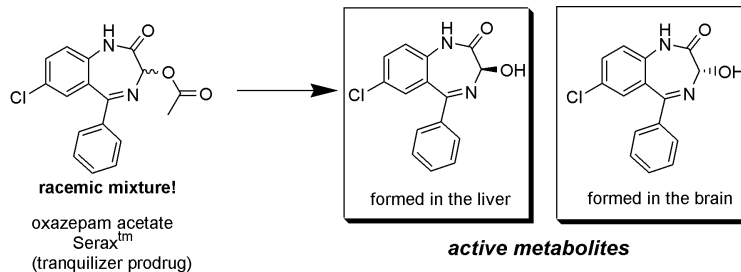
- Common conjugates include **glucuronic acid**, **sulfate**, and **amino acids**.
- Conjugation reactions with **glutathione** function to quench highly electrophilic drugs or metabolites before they covalently modify, and consequently damage, biological macromolecules (proteins, RNA, DNA).
- Some Phase II reactions (**methylation** and **acetylation**) do not necessarily produce more polar metabolites but rather function to terminate biological activity.
- Conjugation reactions will generally take place with available nucleophiles on drugs or xenobiotics such as **alcohols**, **carboxylic acids**, **amines** (including heterocyclic amines), and **thiols**. If these functional groups are not present in a drug, they may be *introduced or revealed* by **Phase I** reactions.



Drug Metabolism: Phase I

Hydrolytic Reactions

Hydrolytic stereoselectivity may be **organ dependent** as different enzymes may be present.

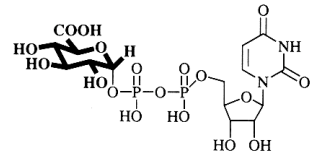
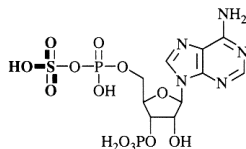


Drug Metabolism

Phase II Transformations

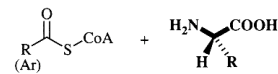
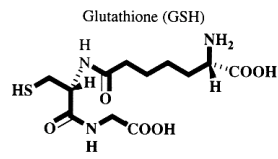
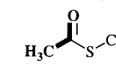
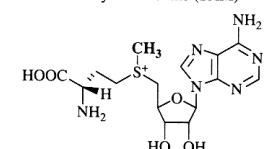
For many of these reactions, the **conjugating group** is an endogenous molecule that is first **activated** in a **coenzyme form** prior to transfer to the drug. The enzymes that catalyze these reactions are known as **transferases**.

Table 7.7 Mammalian Phase II Conjugating Agents^a

Conjugate	Coenzyme Form	Groups Conjugated	Transferase Enzyme
Glucuronide	<p style="text-align: center;">Uridine-5'-diphospho-α-D-glucuronic acid (UDPGA)</p> 	-OH, -COOH, -NH ₂ , -NR ₂ , -SH, C-H	UDP-Glucuronosyl-transferase
Sulfate	<p style="text-align: center;">3'-Phosphoadenosine-5'-phosphosulfate (PAPS)</p> 	-OH, -NH ₂	Sulfotransferase

Drug Metabolism

Phase II Transformations

Glycine and glutamine	<p style="text-align: center;">Activated acyl or aroyl coenzyme A cosubstrate</p> 	-COOH	Glycine <i>N</i> -acetyltransferase Glutamine <i>N</i> -acetyltransferase
Glutathione	<p style="text-align: center;">Glutathione (GSH)</p> 	Ar-X, arene oxide, epoxide, carbocation or related	Glutathione <i>S</i> -transferase
Acetyl	<p style="text-align: center;">Acetyl coenzyme A</p> 	-OH, -NH ₂	Acetyl-transferase
Methyl	<p style="text-align: center;">S-Adenosyl methionine (SAM)</p> 	-OH, -NH ₂ , -SH, heterocyclic N	Methyl-transferase

^a The bold-faced parts are transferred to the drug or metabolite.

Drug Metabolism

Phase II Transformations

Methylation is a relatively minor component to drug or xenobiotic metabolism but is rather important in the biosynthesis of endogenous compounds such as **epinephrine** and **melatonin**.

Usually the cofactor **SAM (S-adenosyl methionine)** serves as a methyl donor.

Methylation (like **acetylation**) differs from most conjugation reactions in that it produces **products with lower hydrophilicity**.

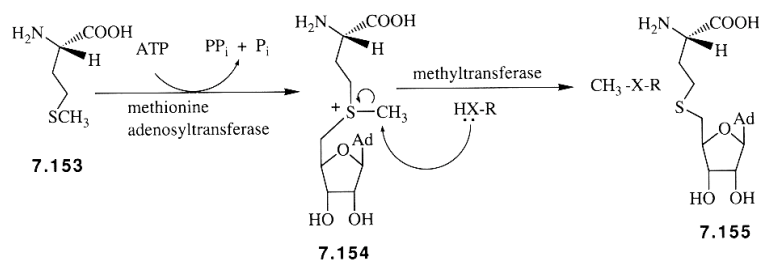
The exception is methylation of **tertiary** or **pyridine-type** nitrogens resulting in a charged, **quaternary ammonium** salts:



Drug Metabolism

Phase II Transformations

- **Methylation** is a 2-step process whereby the cofactor **S-adenosylmethionine (SAM)** which transfers a methyl group is first biosynthesized from **methionine**.
- Once available, **SAM** is utilized by a **methyltransferase** to transfer an activated methyl group to an acceptor molecule (nucleophile = **alcohol, amine, thiol**).
- A variety of transferases are used depending on the nature of the acceptor.

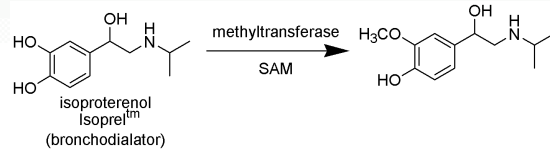


Scheme 7.48. Methylation of xenobiotics.

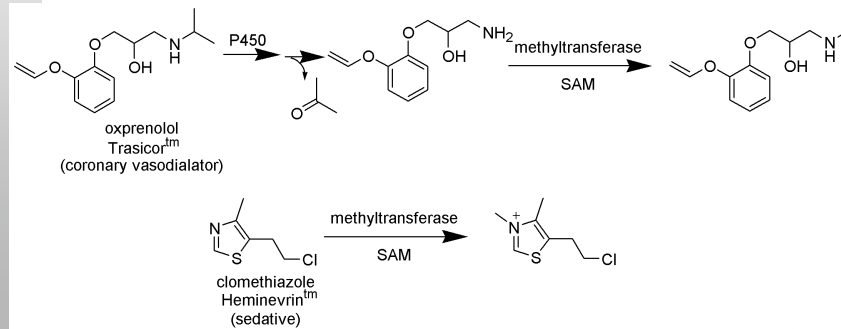
Drug Metabolism

Phase II Transformations

- O-Methylation** occurs mostly with catechols and at the *meta* position.



- N-Methylation** is less common but does occur. Heterocyclic nitrogen atoms are also candidates for methylation.



Drug Metabolism

Phase II Transformations

- S-Methylation** is common for both **aromatic** and **aliphatic sulfhydryl** groups. Once they are formed, they may be further oxidized to **sulfoxides** or **sulfones** (Phase I transformations).

