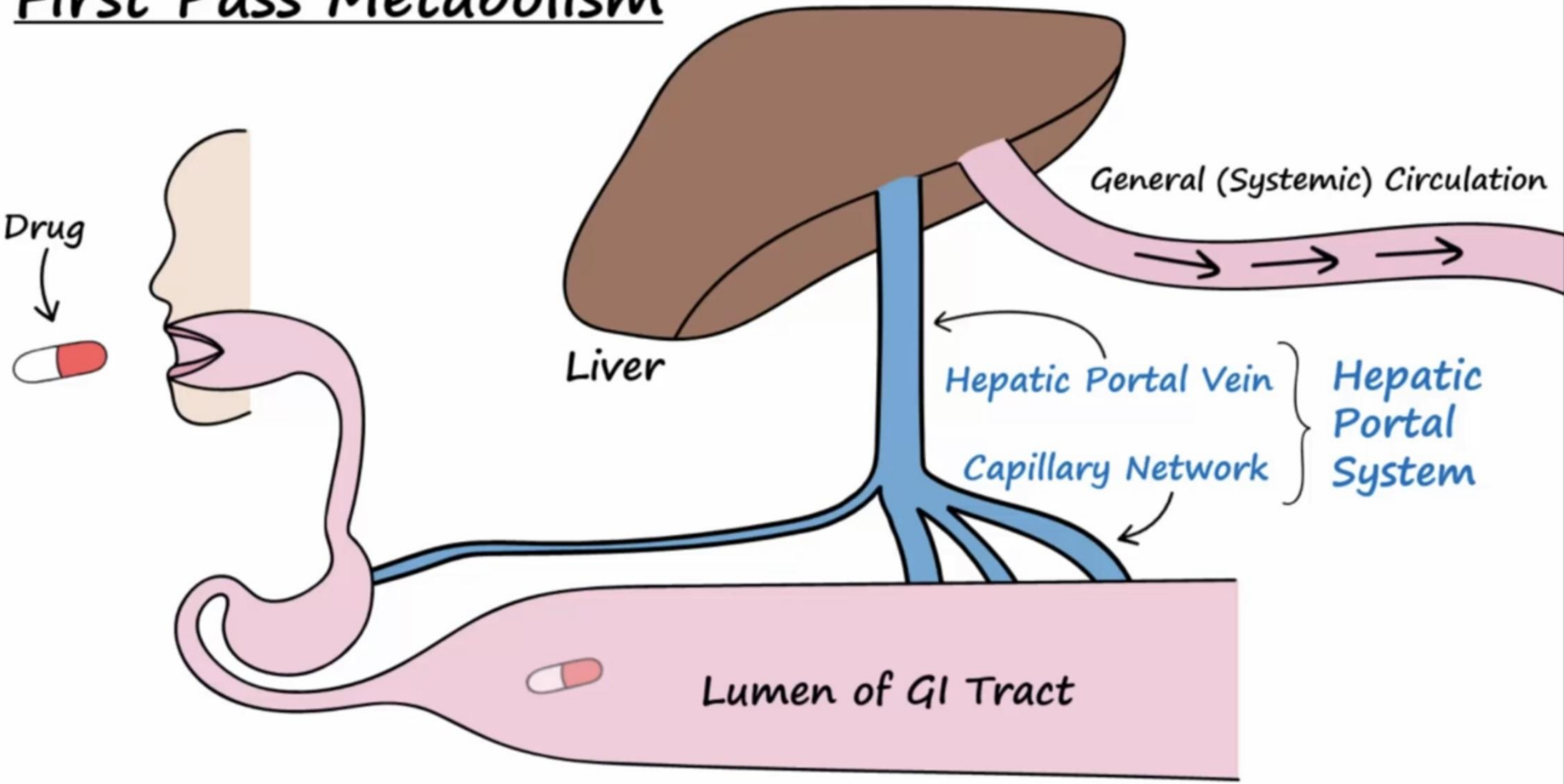
First Pass Metabolism



ROLE OF METABOLISM

- The role of metabolism step is to degrade **or modify the foreign structure, such that it can be more easily excreted**. As a result, most drugs undergo some form of metabolic reaction, resulting in structures known as **metabolites**.

Metabolites may:

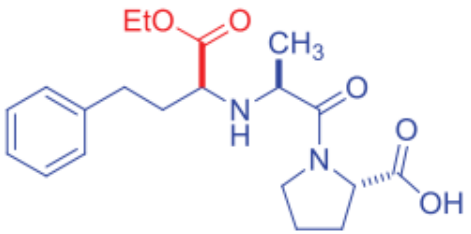
1- Lose the activity of the original drug (**DETOXIFICATION**).

2- Retain a certain level of activity.

3- Be more active than the parent drug (**BIOACTIVATION: PRODRUGS**)

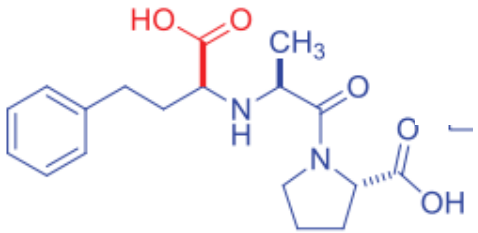
- Aside from water and most hydrophilic dugs, all other molecules/drugs are metabolized. This is actually essential because lipophilic drugs would circulate in the body for a long time, causing untoward side effects if not eliminated in due course. In most cases, metabolism converts lipophilic compounds to hydrophilic metabolites, which are then eliminated/excreted from the body.
- Metabolism is chemical alteration of the drugs in the body. The primary site for drug metabolism is the liver, which is of the uttermost importance with regard to a drug's biotransformations. Other sites of metabolism are the kidney, intestine, lungs, and plasma.

Examples

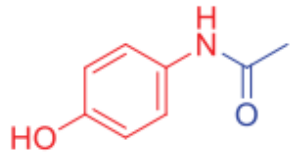


enalapril (Vasotec, **106**)

pro-drug

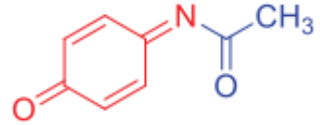


enalaprilat (**105**)

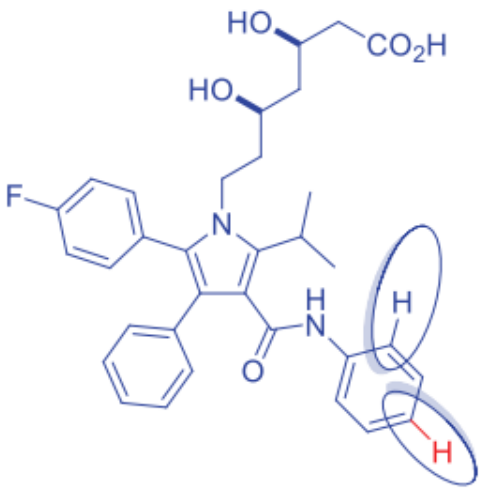


acetaminophen (Tylenol, **109**)
OK

CYP3A4
CYP2E1

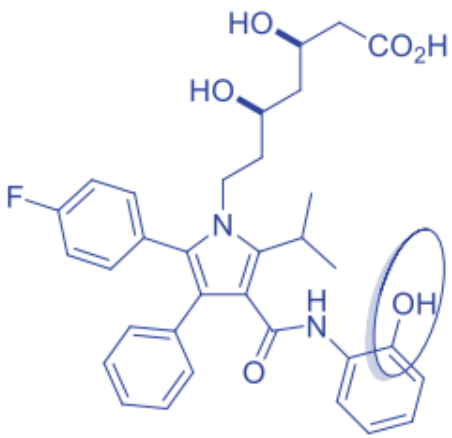


NAPBQI (**110**)
Bad!

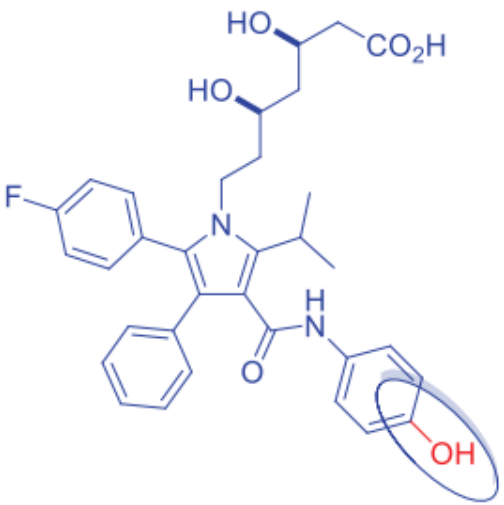


atorvastatin (Lipitor, **2**)

CYP450 3A4

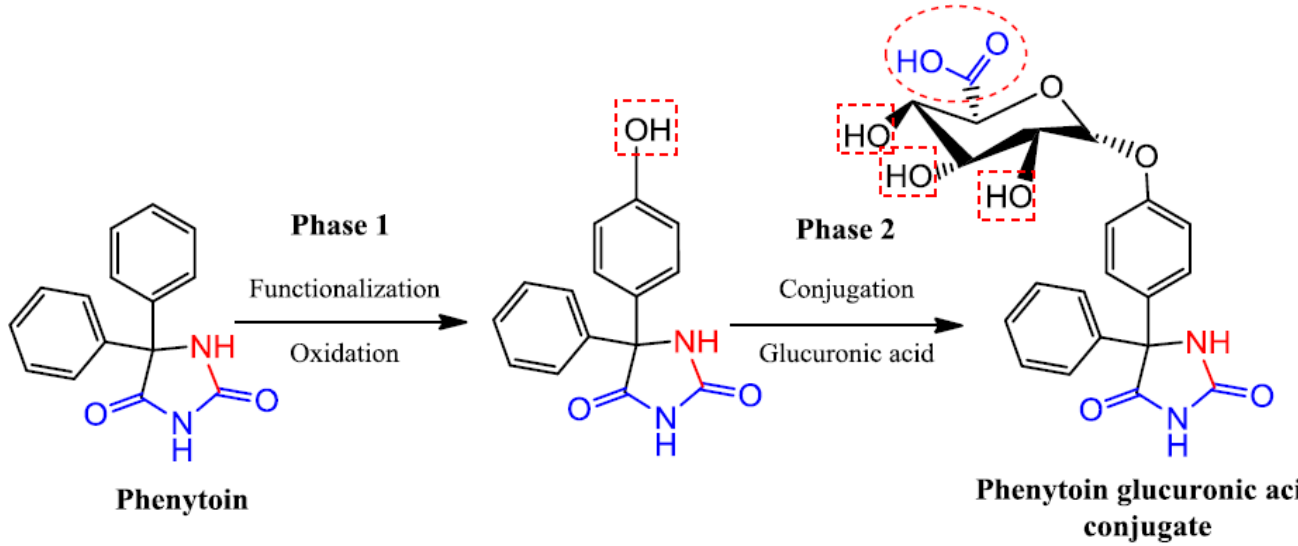


2-hydroxy-atorvastatin (**103**)



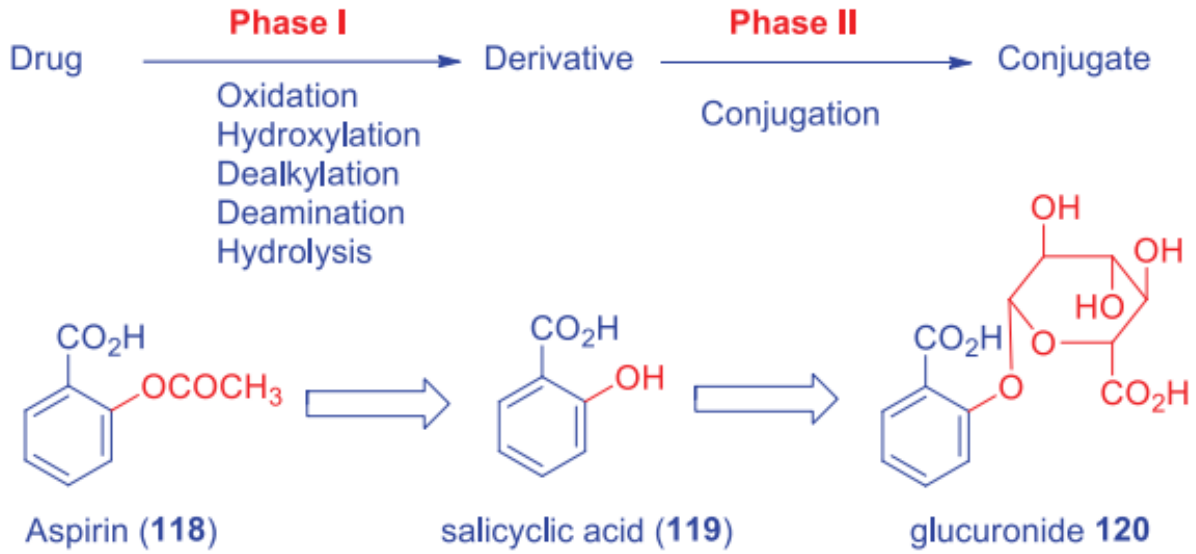
4-hydroxy-atorvastatin (**104**)

- Hydrophobic drugs like Phenytoin are poorly eliminated by the kidneys because they can be reabsorbed after filtration due to balanced hydrophobic/hydrophilic properties (optimal log P ≈ 2).
- These molecules usually have few hydrogen bond donors/acceptors and lack ionic groups, allowing them to cross membranes easily and be reabsorbed in renal tubules.
- Phenytoin has a long residence time (~8 hours), so the body must chemically modify it in the liver to facilitate elimination.
- So far the resultant metabolite...
- Became very hydrophilic and do not cross the blood brain barrier or any other tissues; protection was assured.
- Once it's renally filtered, it will not be re-absorbed as it's ionized.
- It became good candidate for biliary secretion as its M.wt has increased.



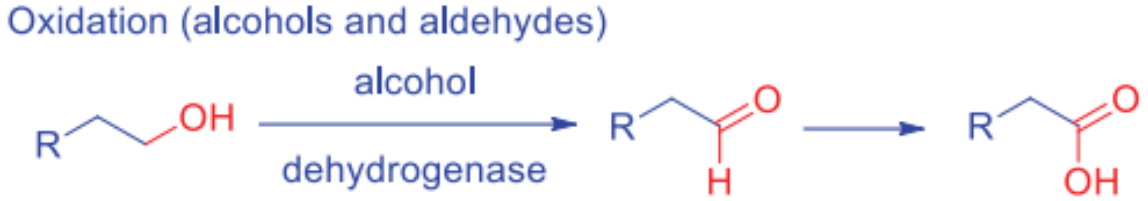
Phases of Metabolism

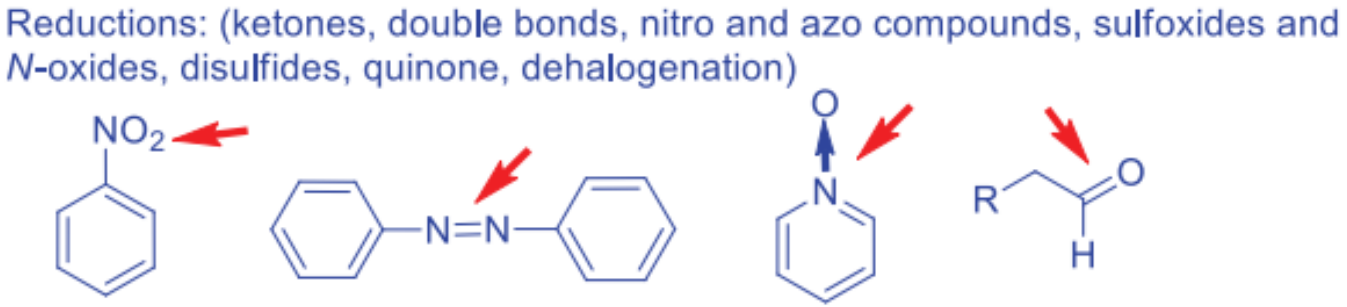
- Drug metabolism may be divided to two phases: Phase I metabolism and Phase II metabolism.
- *Phase I metabolism* refers to **functional group transformations** of the original drug, which involves oxidation, reduction or hydrolysis; at this stage we attach a handle on the compound which is used in phase 2 to attach a hydrophilic moiety
- *Phase II metabolism*, also known as conjugation, is the process of appending **a very polar and highly hydrophilic molecule** (glucuronic acid) to appropriately functionalized parent compound or Phase I metabolite.

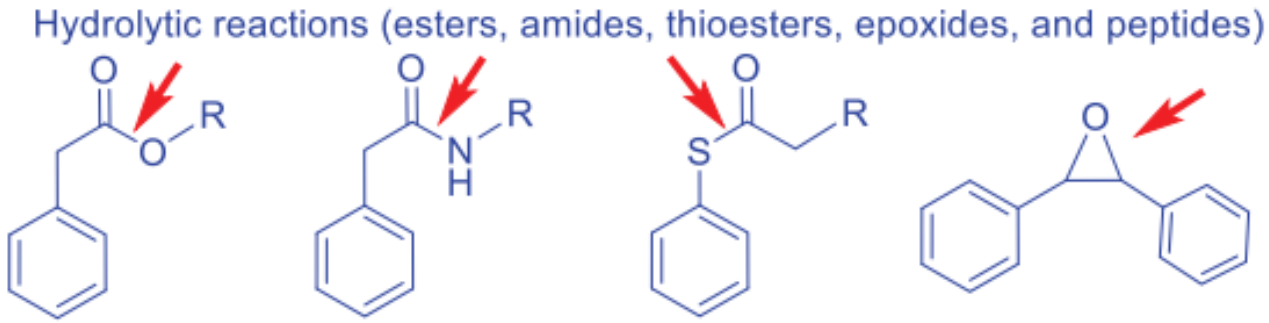


Phase I metabolism

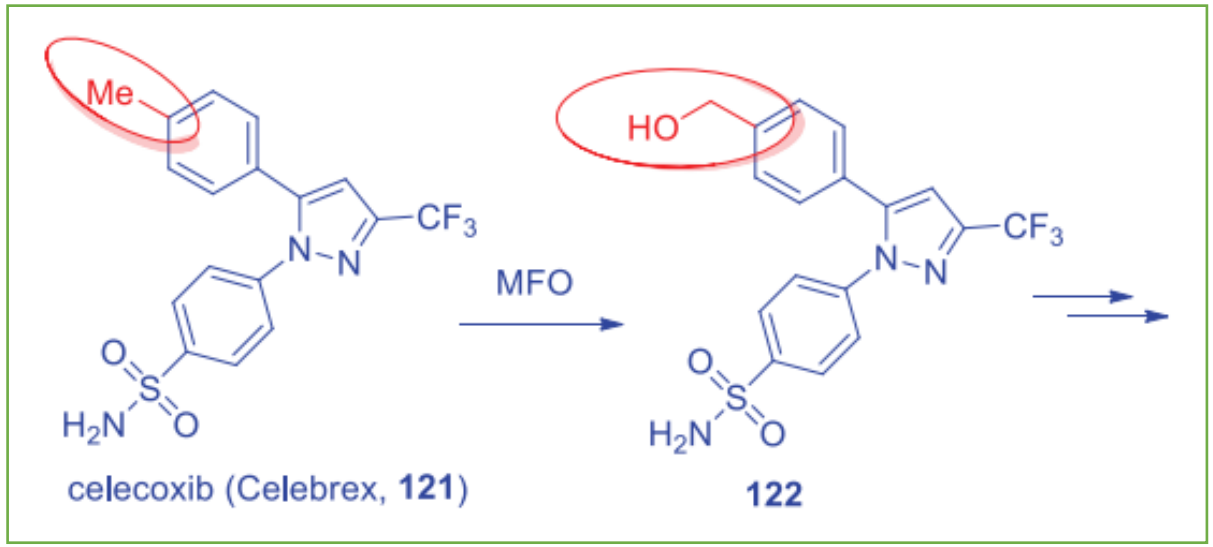
- The types of reactions for Phase I metabolism are oxidation, reduction, hydrolysis, cyclization, and de- cyclization.



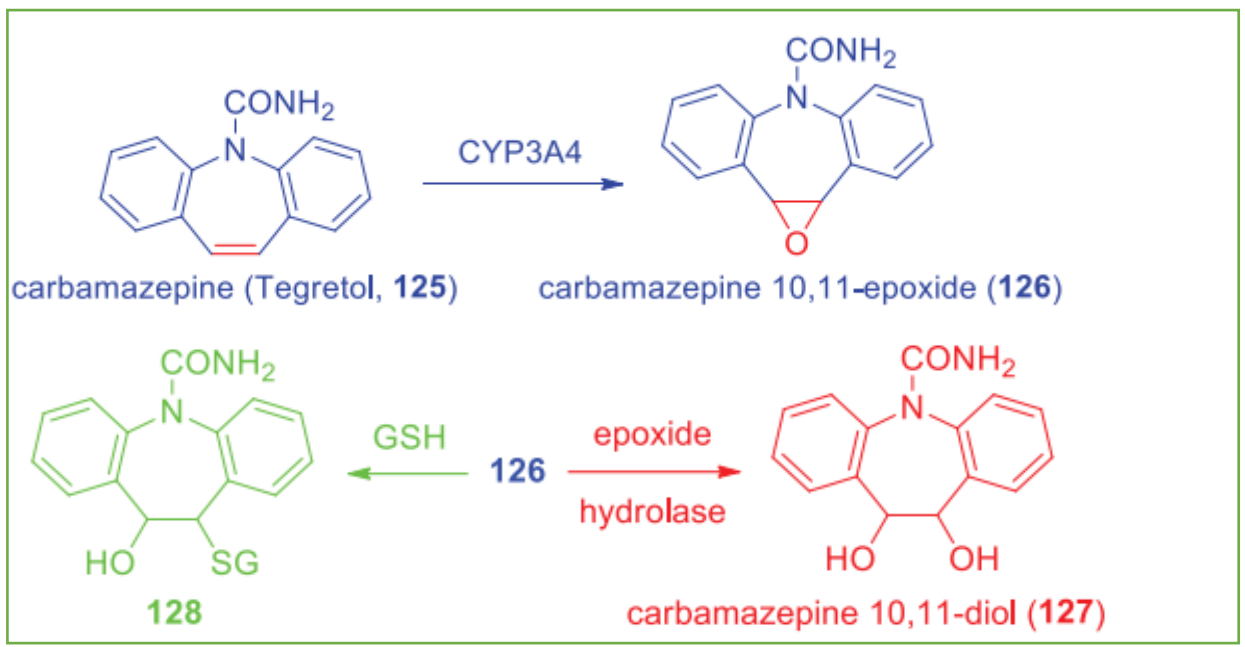




EXAMPLES

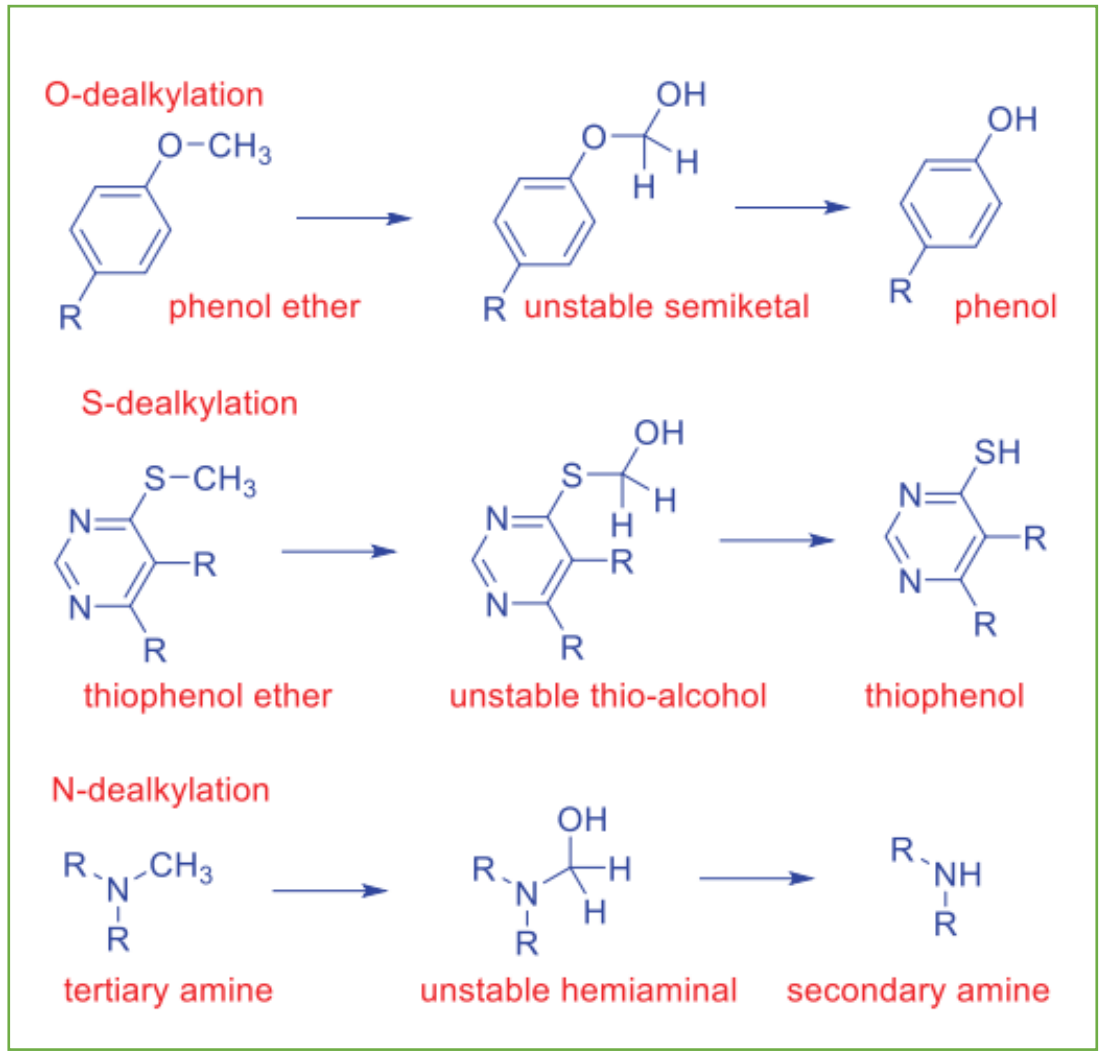


celecoxib (Celebrex, **121**) **122**



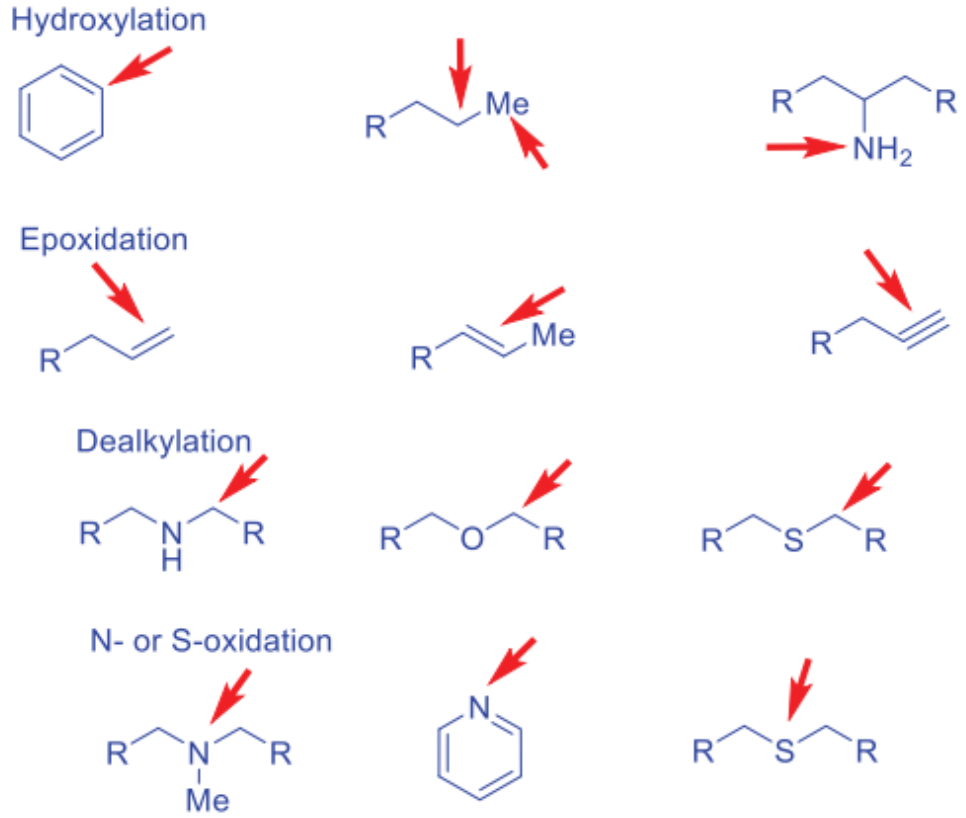
carbamazepine (Tegretol, **125**) carbamazepine 10,11-epoxide (**126**)

128 carbamazepine 10,11-diol (**127**)



OXIDATION

- **Oxidation** is the most important drug-metabolizing reaction.
- Phase I metabolism is largely an oxidative process.
- Various oxidative metabolisms are hydroxylation; oxygenation at carbon, nitrogen, or sulfur atoms; N-dealkylation or O-dealkylation, oxidative deamination, etc.
- **Hydroxylation is a prevalent oxidation process for Phase I metabolism.**



The oxidation process involves a group of enzymes known by three names:

Microsomal Oxidases

Liver-specific enzymes; "microsomes" "microsomes" are active liver particles. particles.

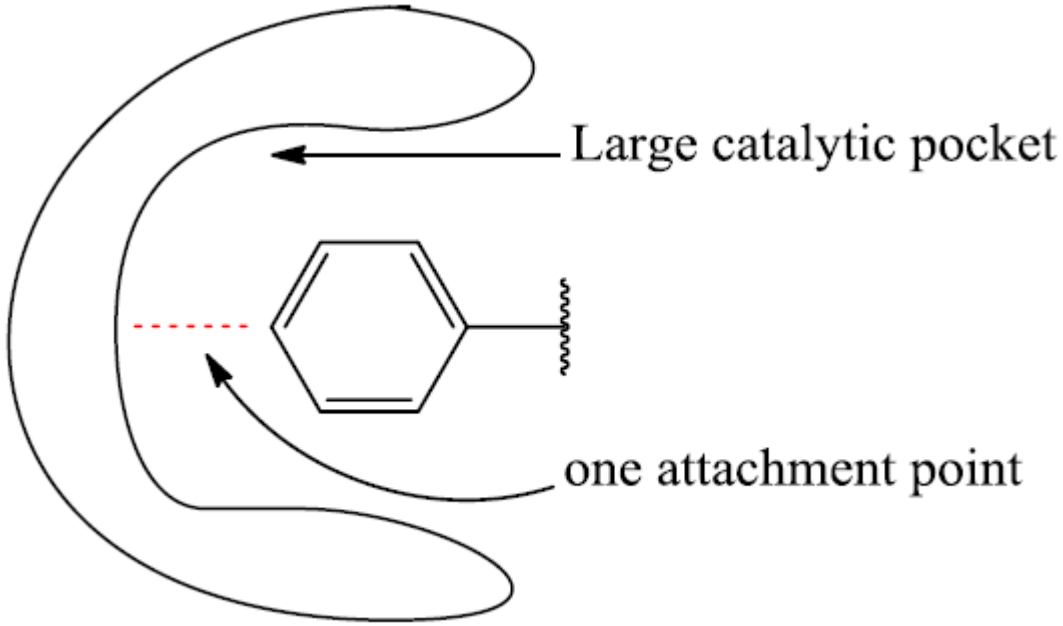
Mixed Function Oxidases

Oxidize many substrates; not substrate-substrate-specific. Low affinity, slow slow turnover (~hours). Drug must remain >2 hrs for metabolites to appear. appear.

Cytochrome P450 Isozymes

Multiple enzyme forms differing in amino acid sequence but catalyzing the catalyzing the same oxidation. Grouped Grouped into families by structural similarity.

- **Mixed-function oxidases**
- They can oxidize various substrates in the same way; they're not 'substrate-specific'. Why?
- 1) They have large catalytic pockets that allow anything to go inside and any
- chemical structure can fit in; no steric clashes.
- 2) Binding occurs only by one or two attachment points instead of three, only make
- Pi-Pi stacking for example
- This low number of attractive interactions leads to low affinity, low specificity, no selectivity, and less turnover therefore they're slow rate enzymes.

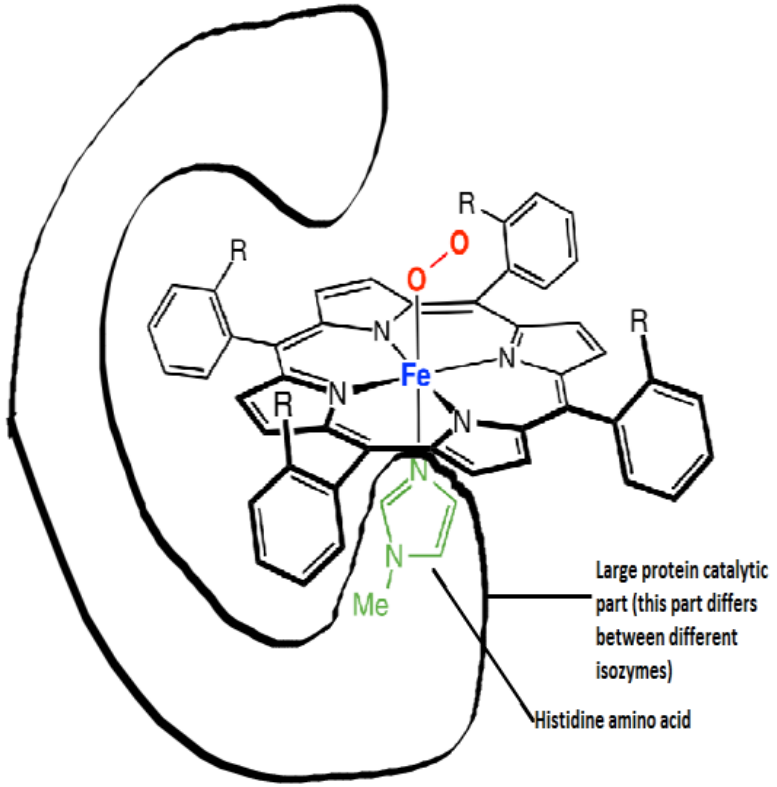


- Other metabolic enzymes involved in Oxidative reactions :
- **flavin-containing Monooxygenases**
- **Monoamine Oxidases**
- Alcohol Dehydrogenases
- Aldehyde Dehydrogenases
- Xanthine Oxidase

Cytochrome P450 (CYP450) Enzymes

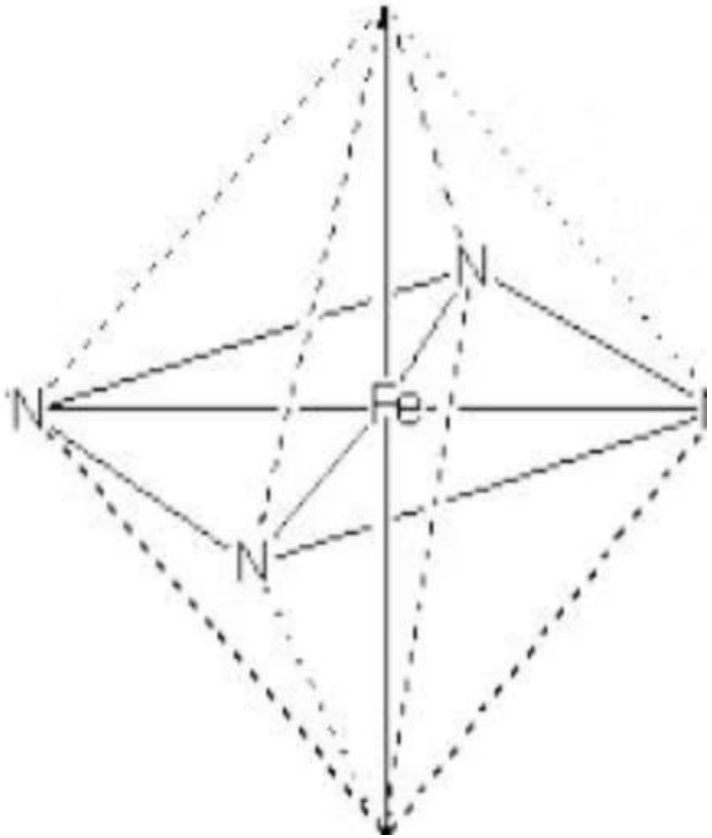
- Isozymes are multiple forms of enzymes that have different chemical structures (they differ in the amino acid sequence), but catalyze the same reaction (perform similar oxidation reaction).
- They are so named because they are bound to membranes within a cell (cyto) and contain a heme pigment (chrome and P) that absorbs light at a wavelength of 450 nm when exposed to carbon monoxide.
- Isozymes are classified into families and subfamilies based on their structural similarity. Chief among them are **CYP450 3A4 and 2D6**.
- CYP 3A4 carries **out bio transformations** of the largest number (~50%) of drugs.
- In addition to the liver, these isoforms are expressed in the intestine and the kidney too.
- **Different isozymes specialize in metabolizing different substrates**, for example:
- Some are specialized in **steroid oxidation**.
- Others metabolize **aromatic ring–containing compounds**.

- Note in the figure the large protein catalytic part; this is the part that differs in different oxidases (different amino acid sequence).
- However, they all **share the non-protein part (heme cofactor)**
- As you can also see the **porphyrin ring** in the catalytic ring; made up of 4 pyrrole rings in a highly conjugated system.
- There's an Iron in the middle of the ring that forms coordinate bonds with Nitrogen atoms [Coordinate bond: a bond between an electron donor (Nitrogen in this case) that gives the electrons to the empty d-orbitals of a metal (Iron in this case)].
- The Iron in the porphyrin ring forms the **heme;**
- The Iron is in the ferric state (Fe^{+3}).
A similar molecule that contains a heme group is hemoglobin but the Iron in hemoglobin is a ferrous iron (Fe^{2+}).



6 Coordinate Bonds (Bipyramidal)

- Bonds 1–4: Iron bonds with 4 nitrogen atoms in the pyrrole rings pyrrole rings of the porphyrin.
- Bond 5: Nitrogen of histidine's imidazole group — anchors the porphyrin in the catalytic pocket.
- Bond 6: Oxygen molecule (O_2) — the oxygen donated in the oxidation reaction. Under slightly acidic liver conditions, it exists as —O—OH, donating —OH to metabolized compounds → R—OH.



$$\text{Drug}-\text{H} + \text{O}_2 + \text{NADPH} + \text{H}^+ \xrightarrow{\text{Cytochrome P450 enzymes}} \text{Drug}-\text{OH} + \text{NADP} + \text{H}_2\text{O}$$

The oxidation systems

There are 5 groups that are oxidized with CYP450 system: ***Carbon*** *systems,* ***Nitrogen*** *systems,* ***Sulphur*** *systems,* ***Oxygen*** *systems and* ***Halogen*** *systems.*

I. Carbon systems,

7 C-systems get oxidized by CYP450 which are:

1. aromatic ring,
2. benzylic carbon,
3. allylic carbon,
4. double bond,
5. alicyclic,
6. aliphatic
7. and carbons α to carbonyls oxidations.

Oxidative Reactions

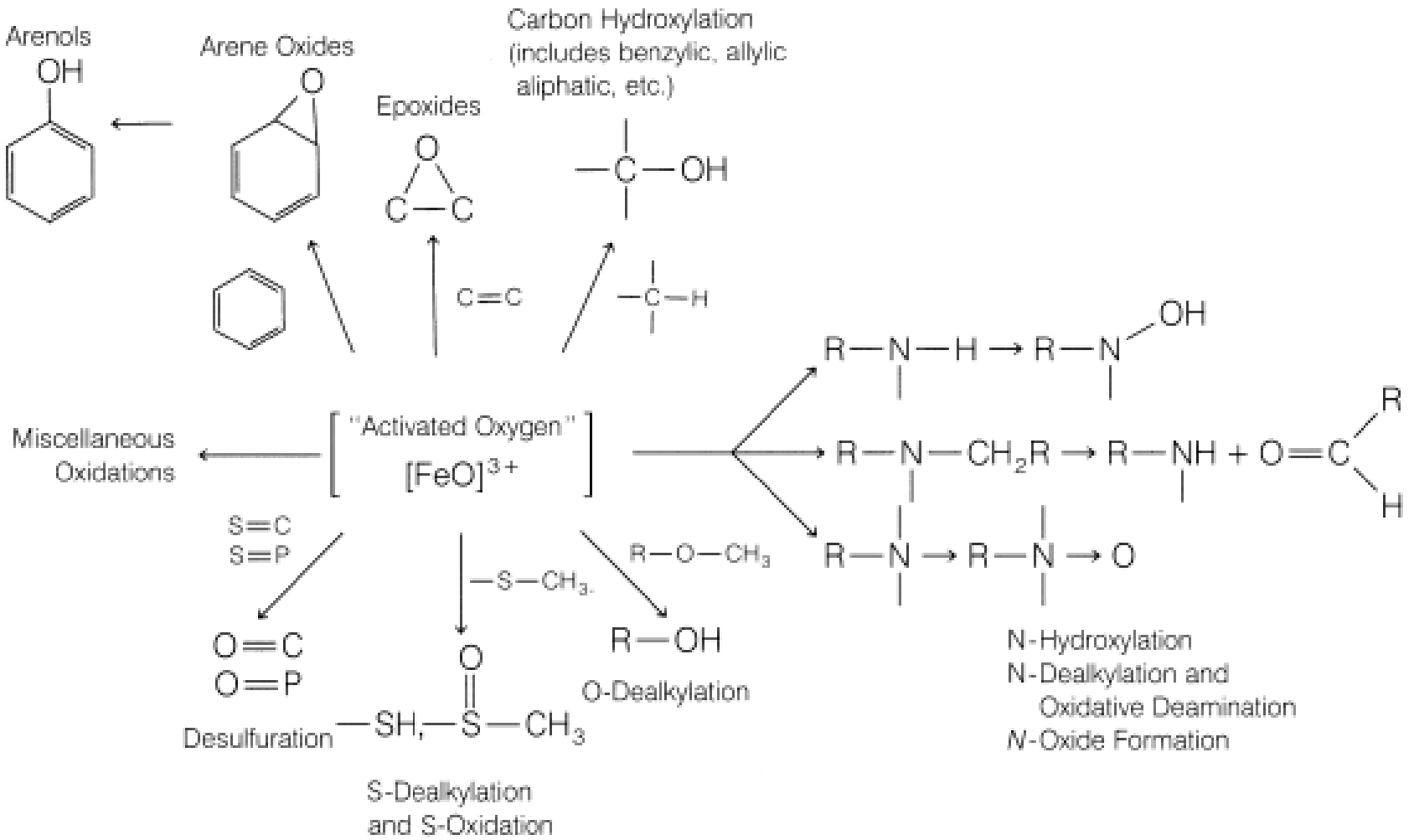
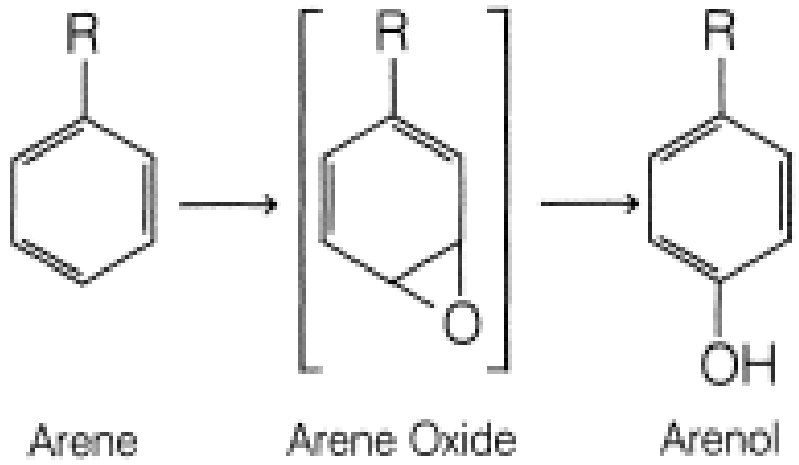


Figure 3.3 ● **Schematic summary of cytochrome P450-catalyzed oxidation reactions. (Adapted from Ullrich, V.: Top. Curr. Chem. 83:68, 1979.)**

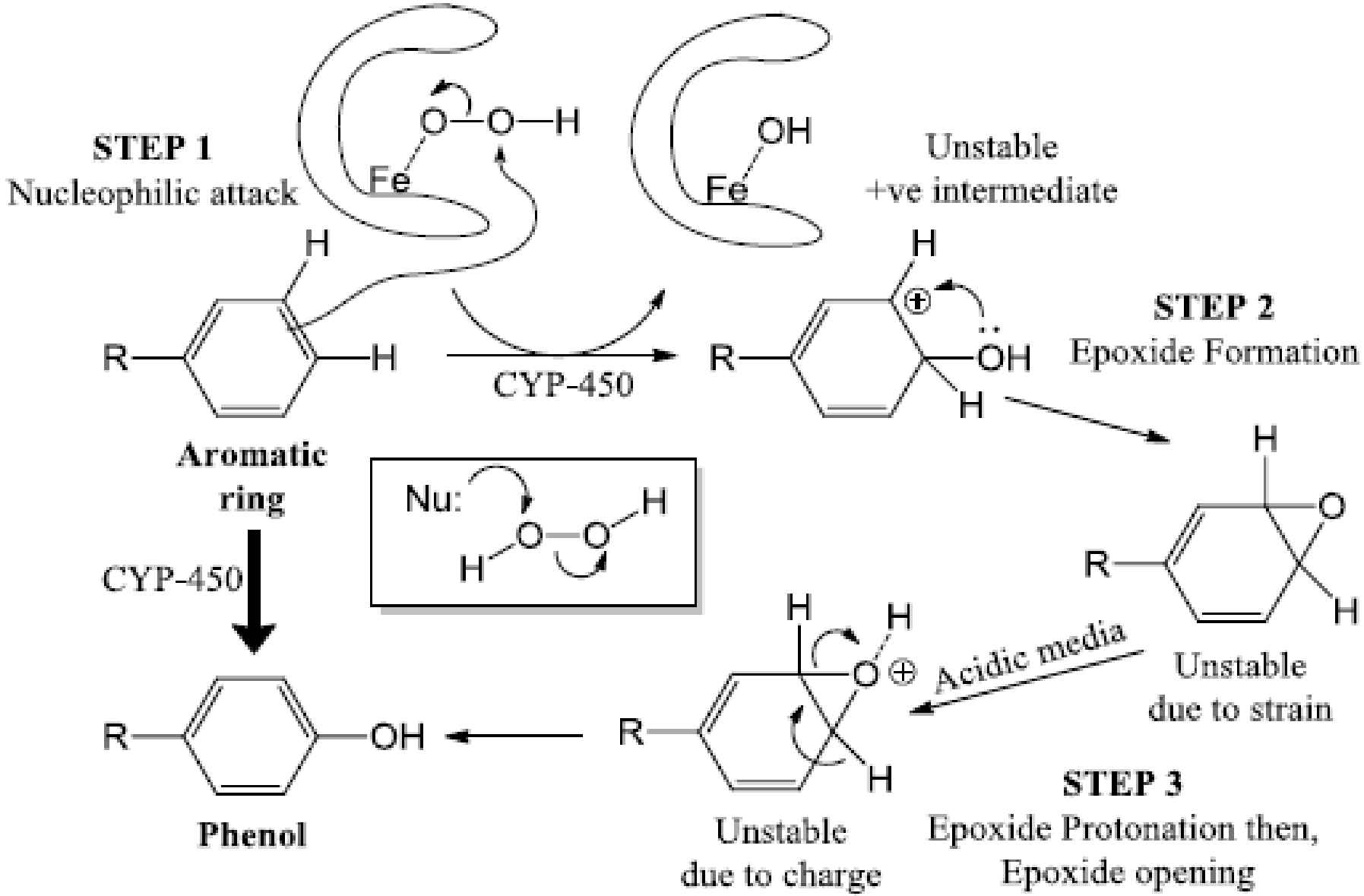
1.Aromatic oxidation



The term aromatic hydroxylation refers to the mixed functional oxidation of aromatic compounds (arenes) to their corresponding phenolic metabolites (arenols). It is believed that almost all aromatic hydroxyl reactions proceed initially through an epoxide intermediate called "arene oxide". which quickly and spontaneously rearranges to the arenol product in most cases.

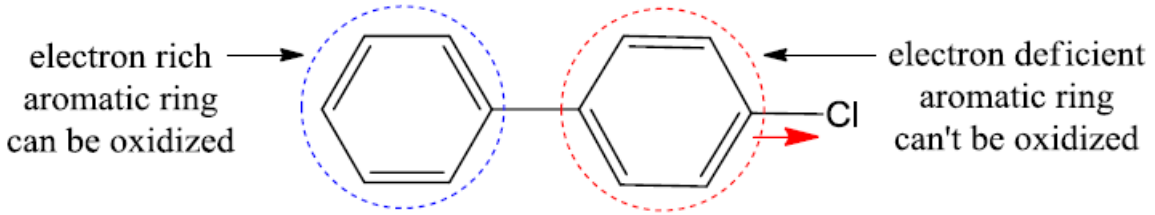
Most foreign compounds containing aromatic moieties are susceptible to aromatic oxidation. In humans, aromatic hydroxylation is a major route of metabolism for many drugs containing phenyl groups.

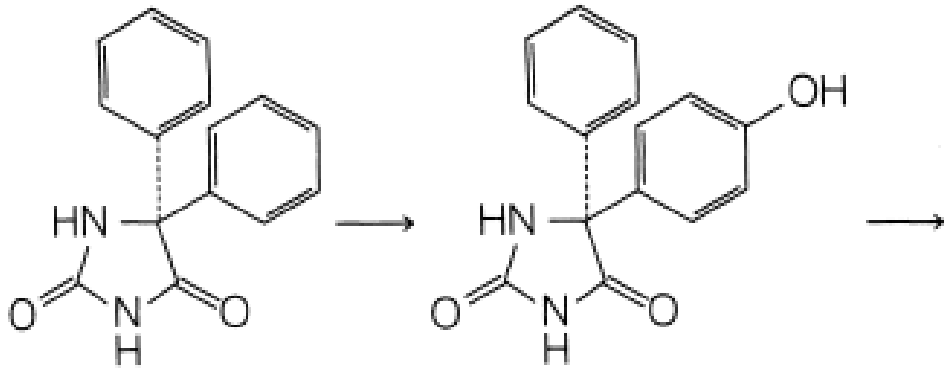
Important therapeutic agents such as propranolol, phenobarbital, phenytoin- , atorvastatin, 17α- ethinylestradiol and S- warfarin undergo extensive aromatic oxidation. In most of the drugs just mentioned, hydroxylation occurs at the para position.



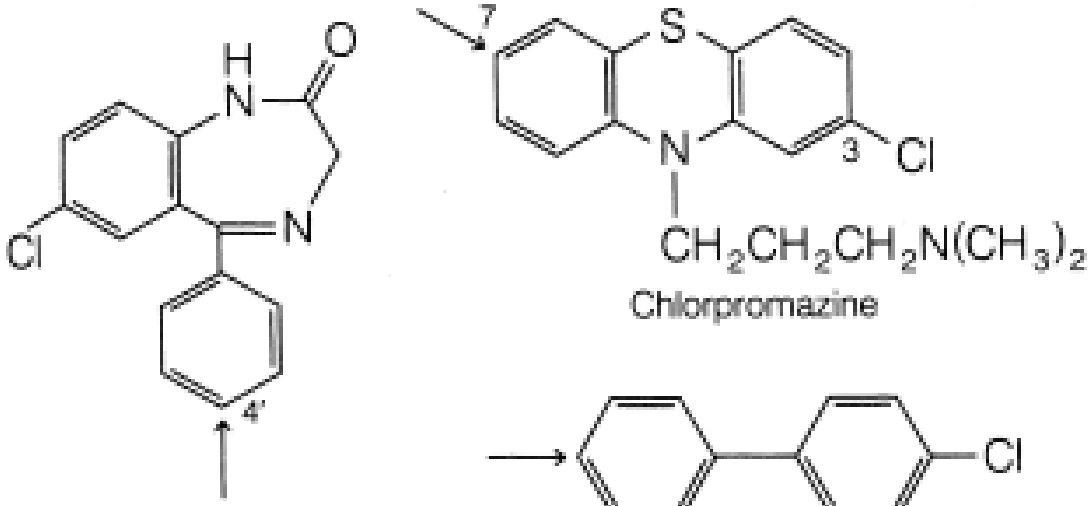
- CYP450 activates oxygen (unstable O–O system)
- Aromatic ring attacks oxygenPositive intermediate forms
- **Arene oxide (epoxide)** intermediate forms Epoxide opens in acidic conditions
- Aromaticity restored → **phenol formed**

In compounds with **two aromatic rings**, hydroxylation occurs preferentially in the **more electron-rich ring**. For example, aromatic hydroxylation of diazepam (Valium) occurs primarily in the more activated ring to yield 4'-hydroxydiazepam. A similar situation is seen in the 7-hydroxylation of the antipsychotic agent chlorpromazine (Thorazine) and in the para-hydroxylation of p-chlorobiphenyl to p-chloro-p'-hydroxybiphenyl.

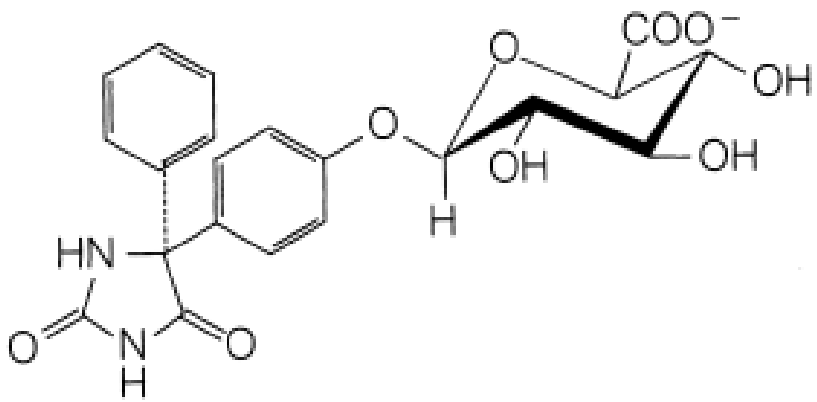




Phenytoin *p*-Hydroxyphenytoin

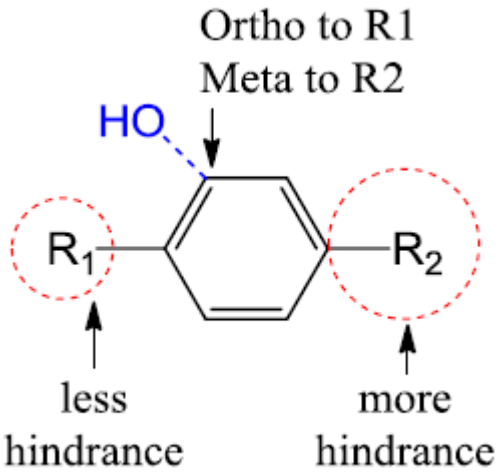


Diazepam Chlorpromazine *p*-Chlorobiphenyl



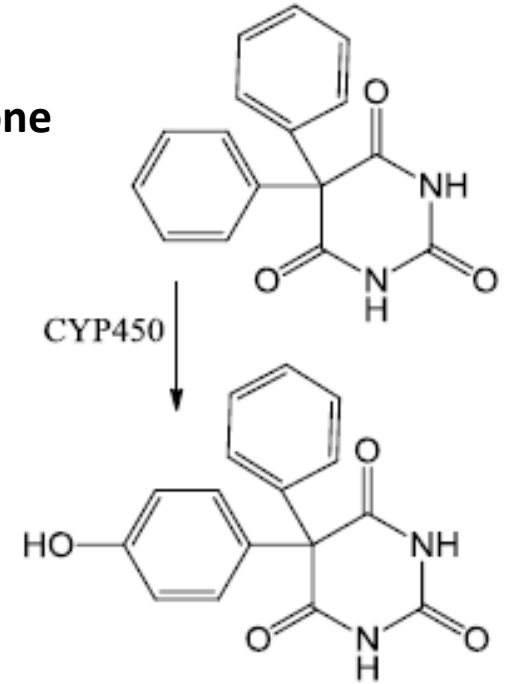
O-Glucuronide Conjugate

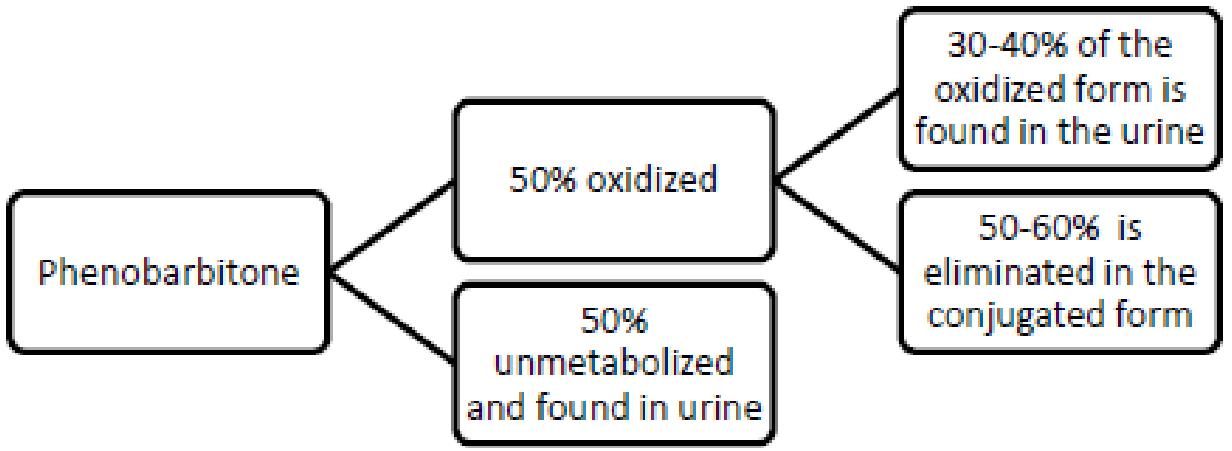
•Aromatic oxidation by Cytochrome P450 is sterically controlled.
•The enzyme prefers the least crowded position on the aromatic ring.
•Usually, oxidation occurs at the para position because it is least sterically hindered.
•If the para position is already occupied, oxidation occurs at the next least hindered site (meta or ortho).
•The –OH group forms at the position farthest from bulky substituents like R_1 or R_2.
, usually at the para position, before conjugation occurs.



- Oxidation always occur at para position, if there is a substitution on the **para** then it will happen on the **meta** position , if both positions are being occupied then no oxidation will occur.
- Aromatic ring oxidation is moderate in speed usually not more than 50%, other kind of oxidations are faster, so their products will be extremely metabolized.

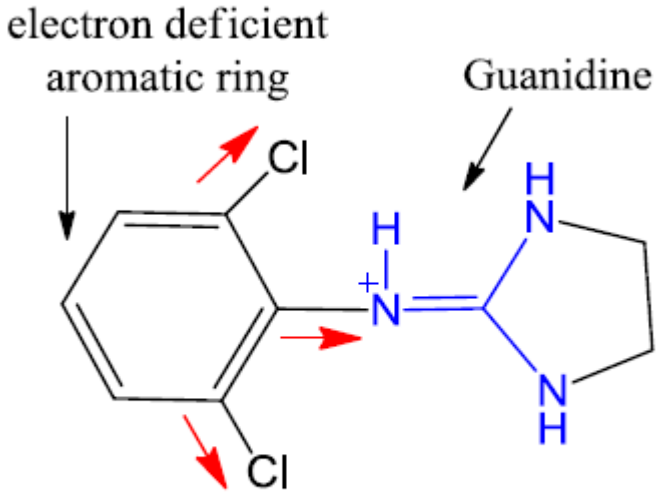
Phenobarbitone





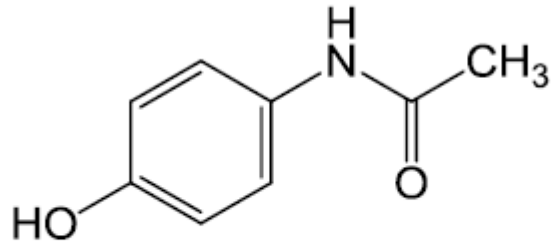
Clonidine is an antihypertensive drug with an electron-deficient aromatic ring due to the presence of a positively charged guanidine group and two chlorine atoms, both of which exert electron-withdrawing effects. This makes the aromatic ring resistant to aromatic oxidation metabolism.

However, the guanidine group increases the drug's water solubility and gives it a positive charge at physiological pH, preventing renal tubular reabsorption after filtration. As a result, the drug is rapidly excreted by the kidneys before significant metabolism occurs, illustrating the principle that drugs readily eliminated by the renal system tend to undergo less metabolic transformation.



Clonidine

Another example: **Paracetamol**
Paracetamol compound is already hydroxylated; there is no need for further oxidation.
Therefore, it's eliminated in the urine as it is or as its conjugated form (the most important conjugate is glucuronic acid).



Paracetamol

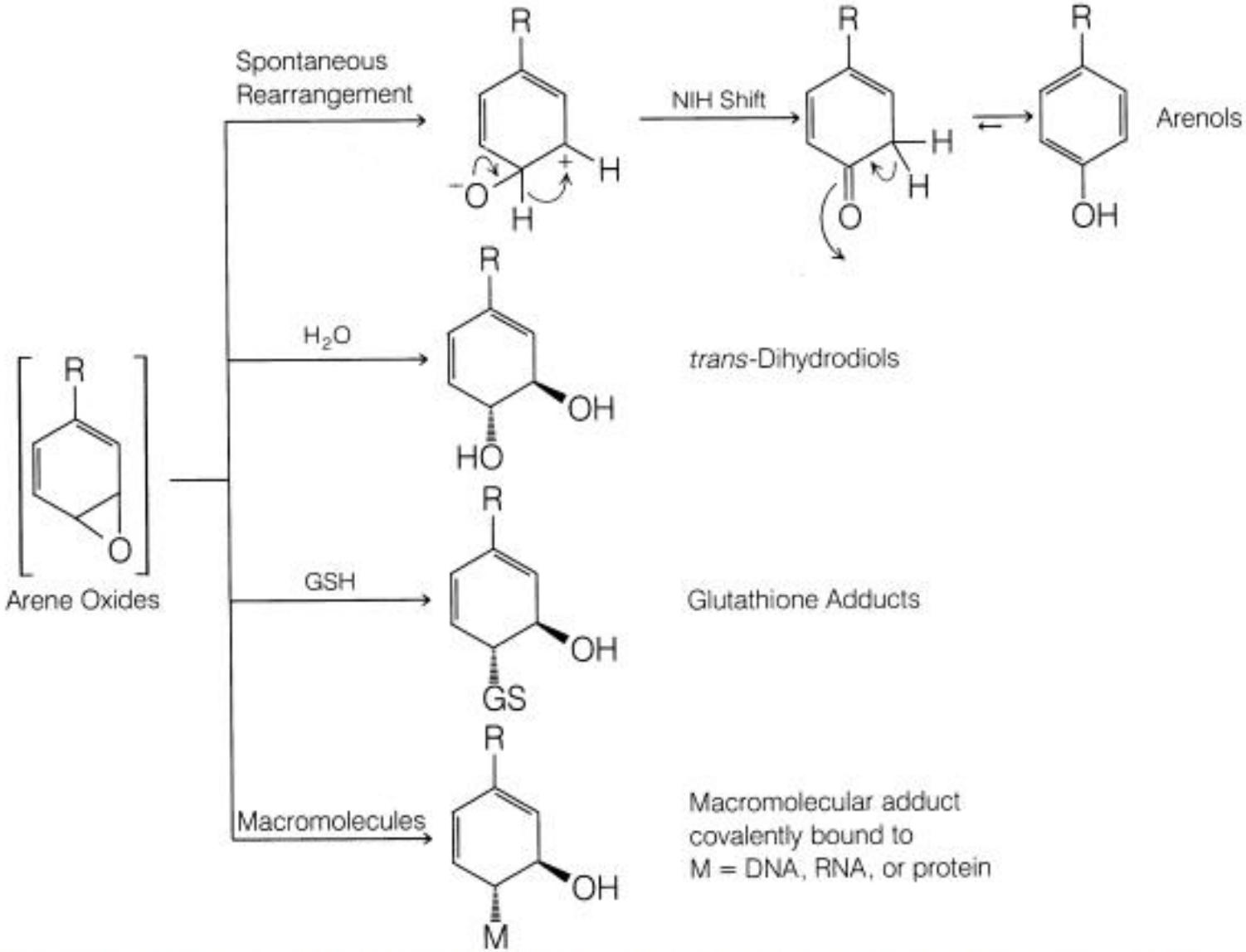
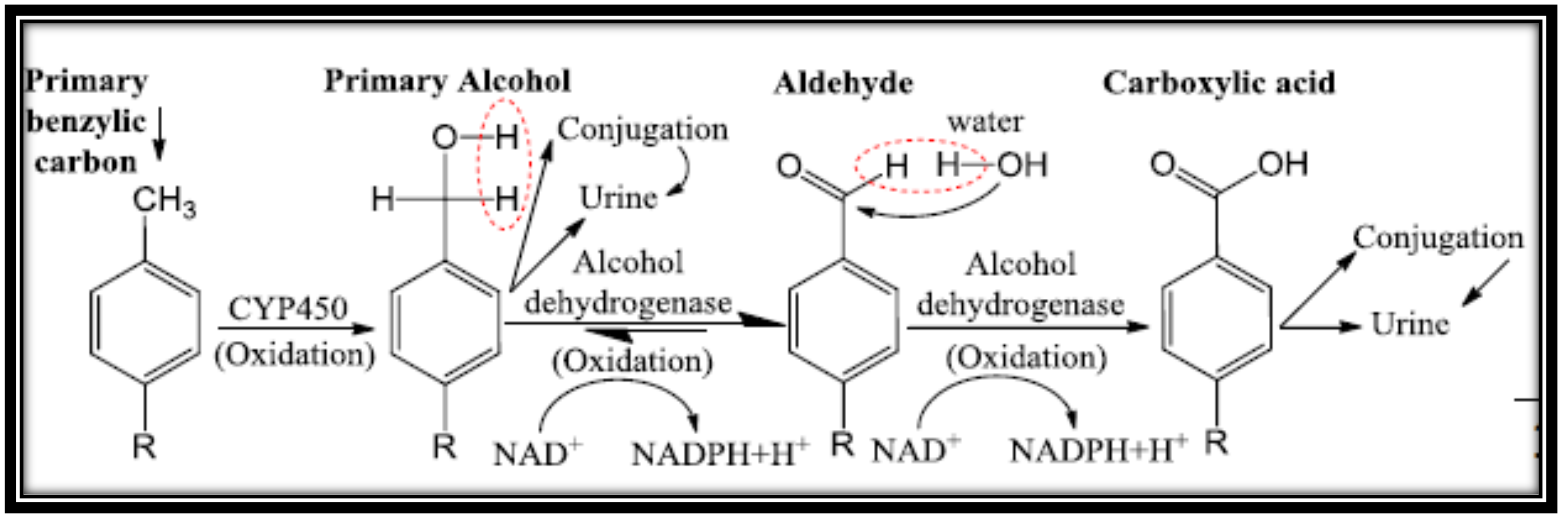


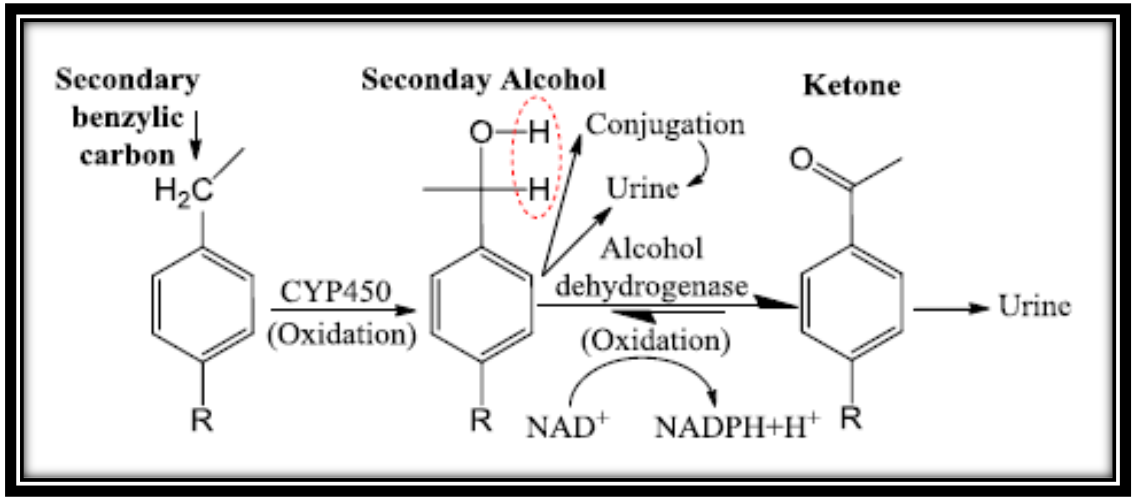
Figure 3.5 • Possible reaction pathways for arene oxides. (Data are from Daly, J. W., et al.: Experientia 28:1129, 1972; Jerina, D. M., and Daly, J. W.: Science 185:573, 1974; and Kaminsky, L. S.: In Anders, M. W. [ed.]. Bioactivation of Foreign Compounds. New York, Academic Press, 1985, p. 157.)

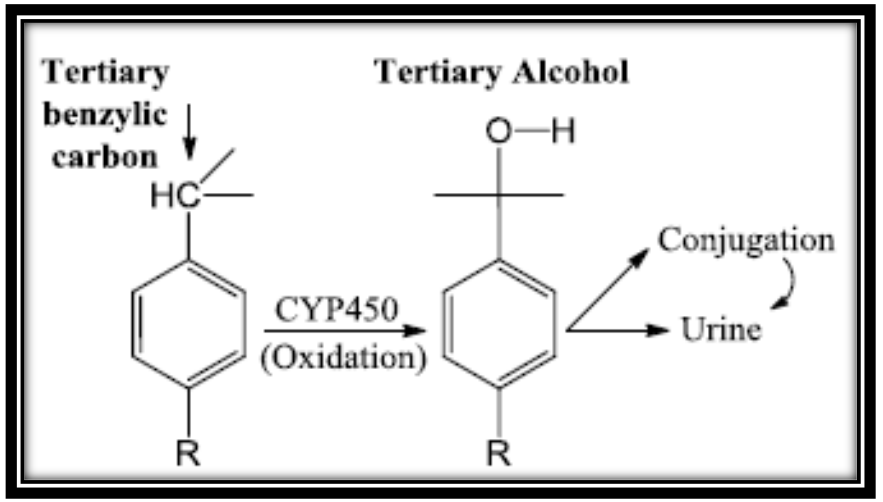
2. Benzylic oxidation



❖ Properties of the reaction

- The reaction is very fast with a 100% yield; in around 3 hours the drug is fully metabolized.
- The benzylic carbon must have hydrogen bonded to it in order for it to be replaced by –OH.





4° carbon, no oxidation because there is no H to replace (remember that H must exist to replace it with –OH).

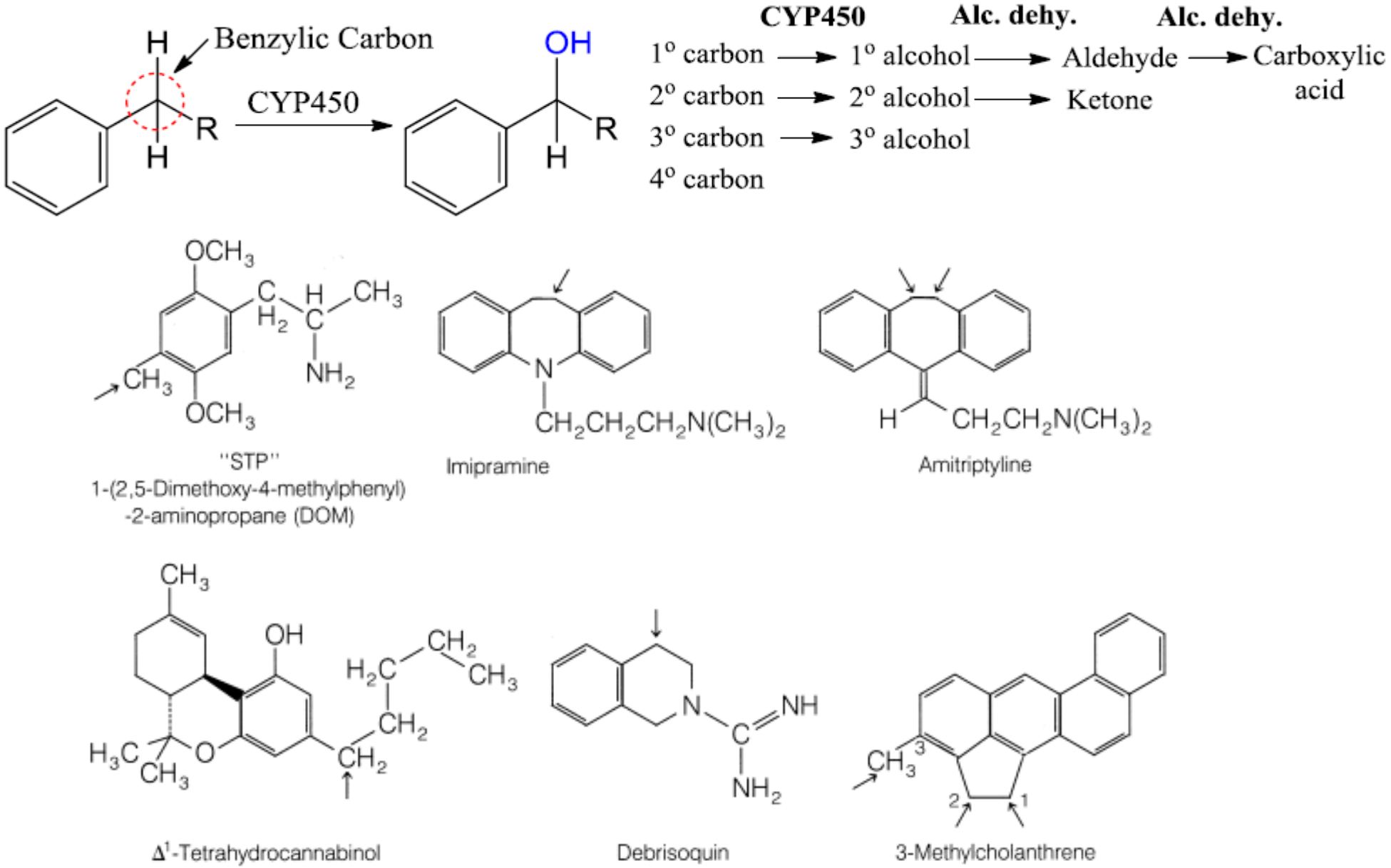
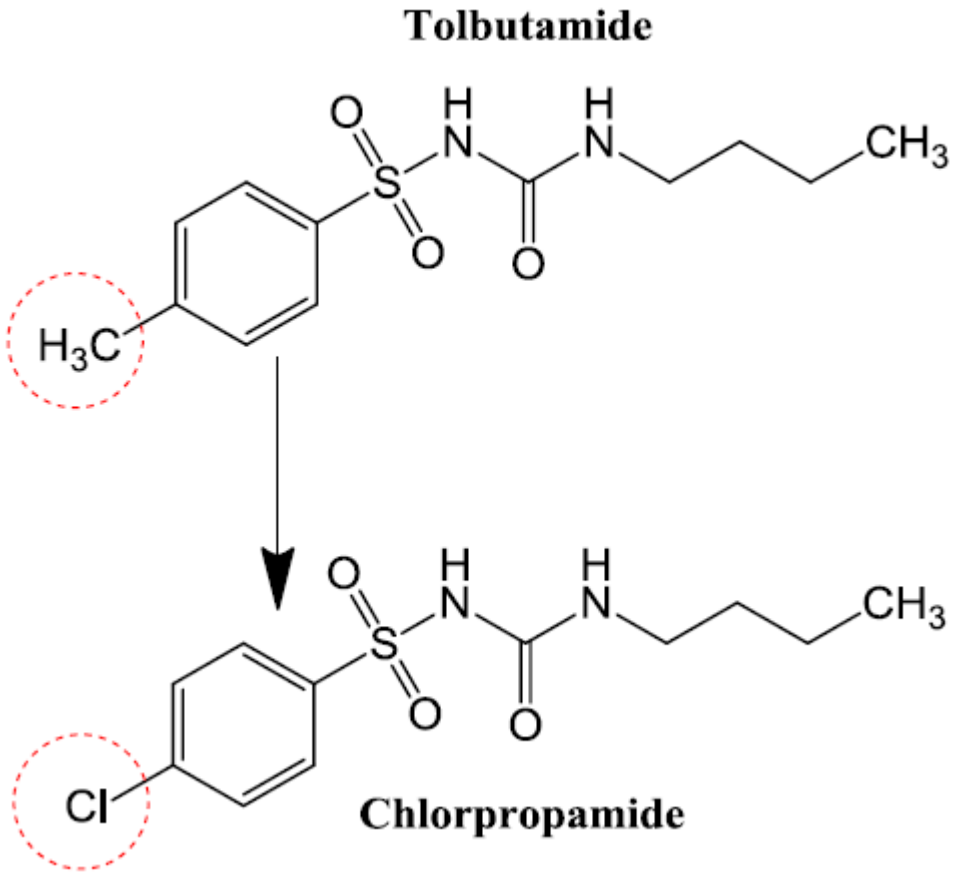


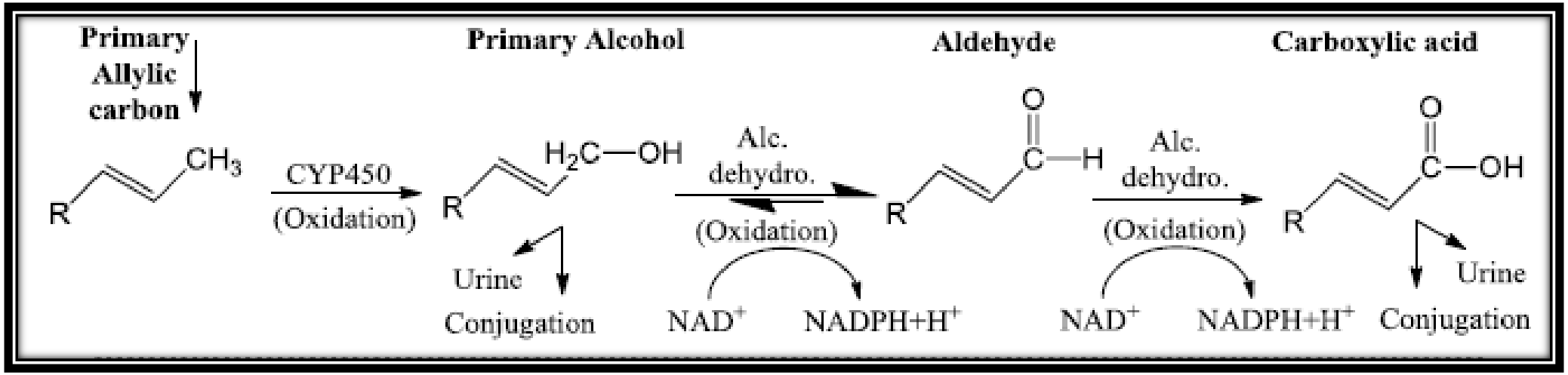
Figure 3.8 • Examples of drugs and xenobiotics undergoing benzylic hydroxylation. *Arrow* indicates site of hydroxylation.

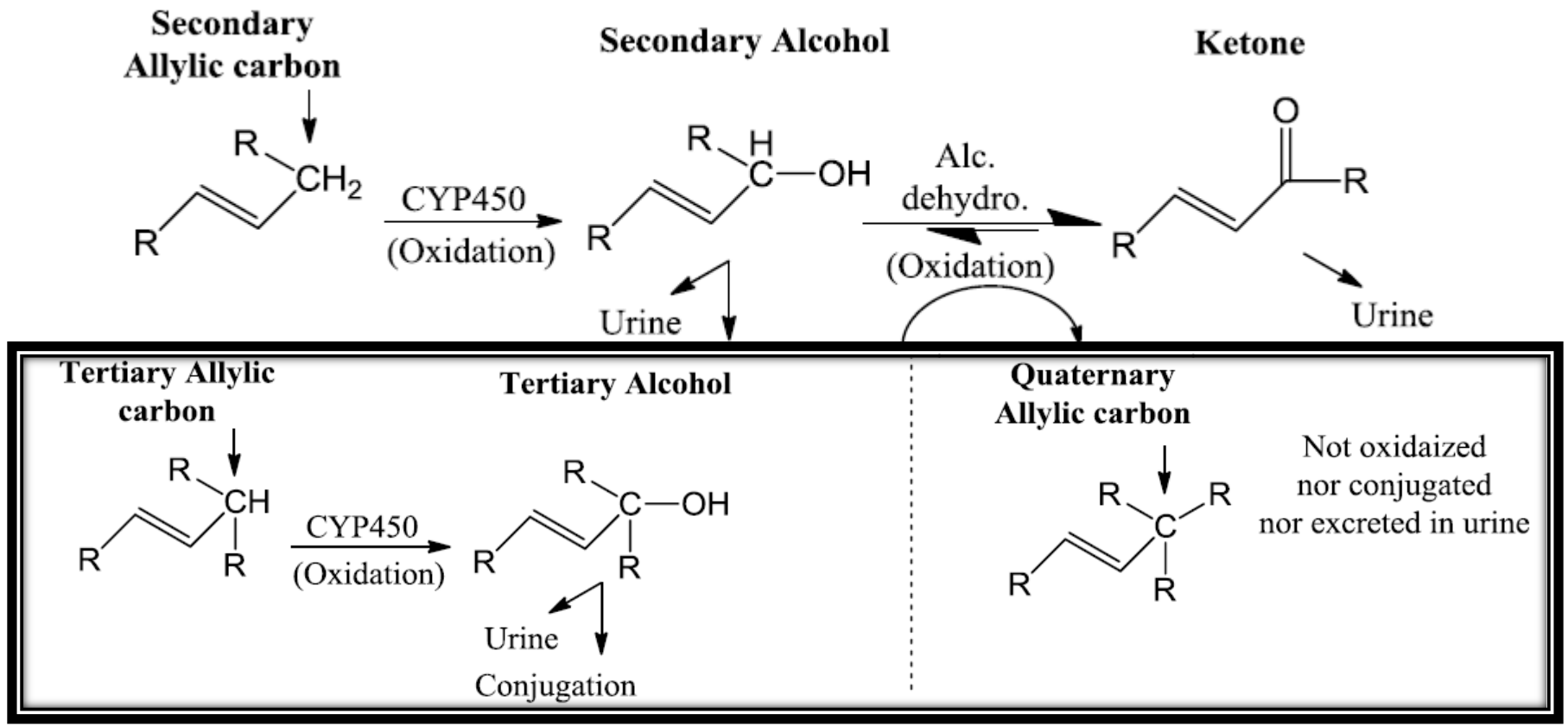
The benzylic carbon (carbon next to the aromatic ring) is the site that gets oxidized.
Oxidation converts the methyl group → alcohol → carboxylic acid.
In this drug, metabolism results in:~70% carboxylic acid and its conjugates~30% alcohol and its conjugates in urine
The drug was previously used to treat Diabetes Mellitus, but it was rapidly oxidized, so patients had to take it every 3 hours, which was inconvenient.
To solve this, the methyl group was replaced with chlorine (isosteric replacement).Chlorine is electron-withdrawing, which resists oxidation and slows metabolism.
The modified drug Chlorpropamide lasts longer in the body and is taken twice daily (morning and evening).

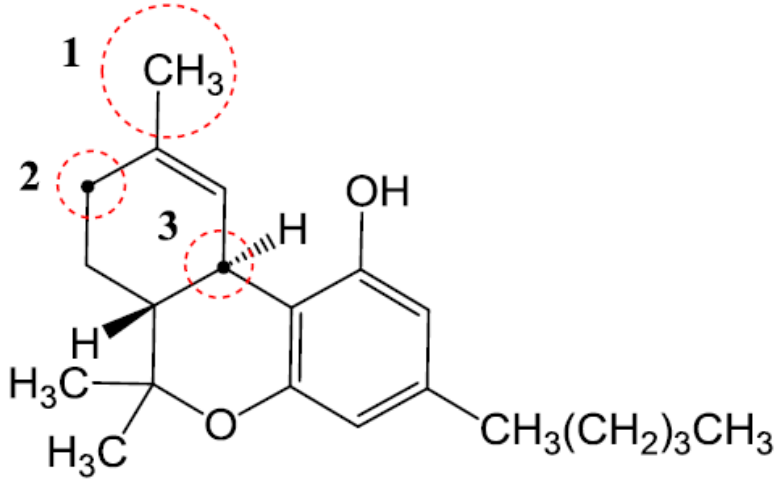


3. Allylic oxidation

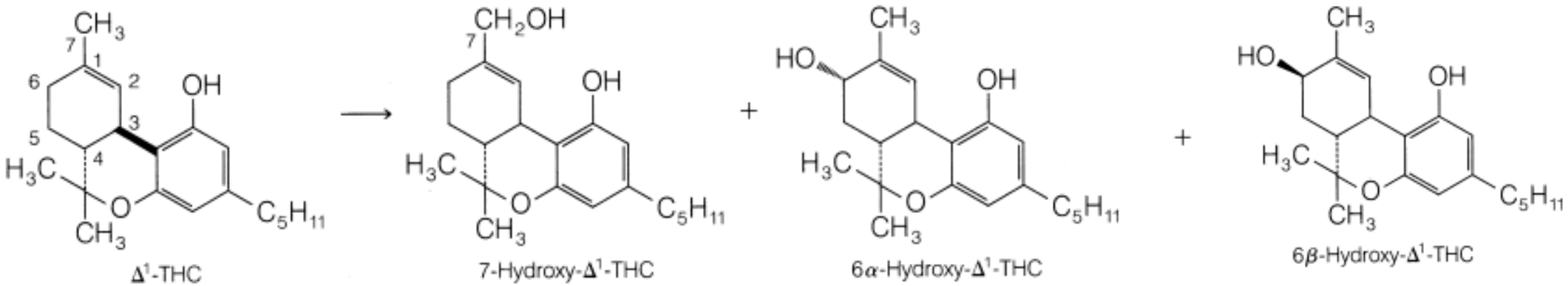
Allylic carbon is a carbon atom bonded to another carbon atom, which in turn is bonded doubly to another carbon atom
Considered as fast oxidation reaction.




Secondary
Allylic carbon
Secondary Alcohol
Ketone
R
CH2
CYP450
(Oxidation)
H
C
OH
Alc.
dehydro.
(Oxidation)
O
Urine
Urine
Tertiary Allylic
carbon
Tertiary Alcohol
Quaternary
Allylic carbon
CH
CYP450
(Oxidation)
Urine
Conjugation
Not oxidaized
nor conjugated
nor excreted in urine



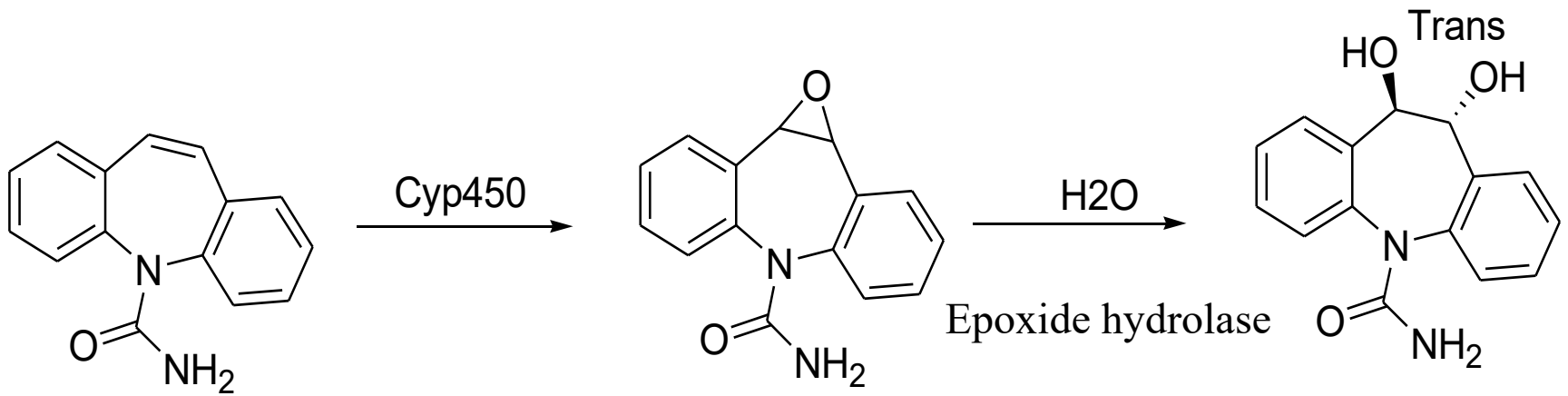
Tetrahydrocannabinol



CYP450 favors oxidation of the allylic carbon number 1 rather than positions 2 and 3 due to steric hindrance; oxidation happens only once.

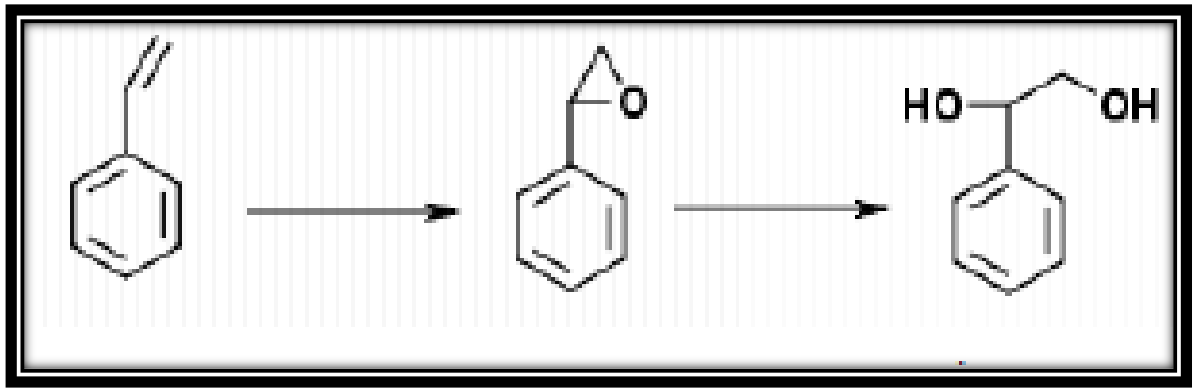
4.Double bond oxidation

- Alkene epoxidation:
- In order for a double bond to be oxidized it **has to be conjugated to at least one aromatic ring**
- - Rate is similar to aromatic ring oxidation but slightly higher; still moderate rate
- - Yield 50-60%.



Carbamazipine

Styrene (polystyrene monomer)



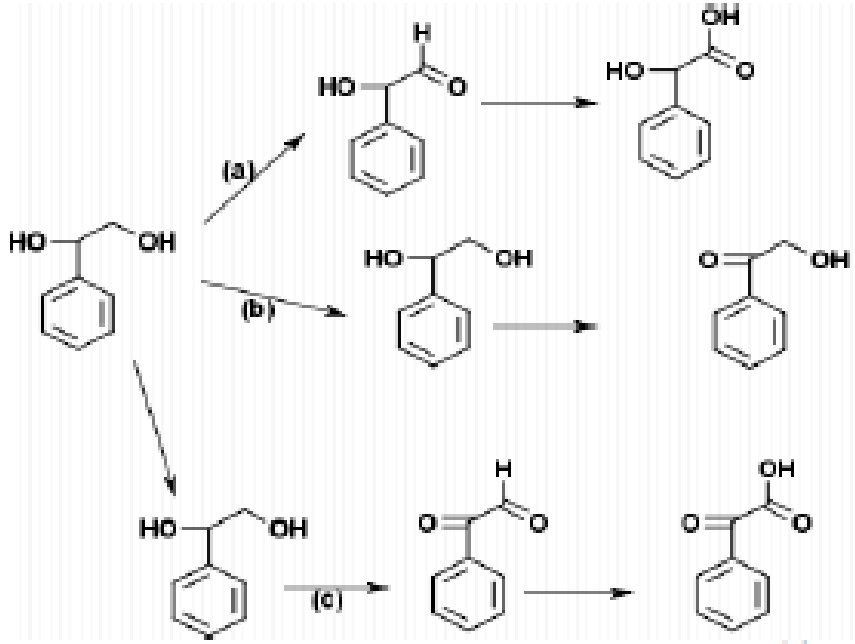
What is the fate of this styrene glycol?

(1) Further oxidation by **<u>alcohol dehydrogenase</u>** on one of the OH or both. It could give:

a. An aldehyde which gets further oxidized into a carboxylic acid. An alcohol carboxylic acid is found in the urine.

b. A ketone. An alcohol ketone is eliminated in the urine.

c. A ketone and a carboxylic acid. (eliminated in the urine).

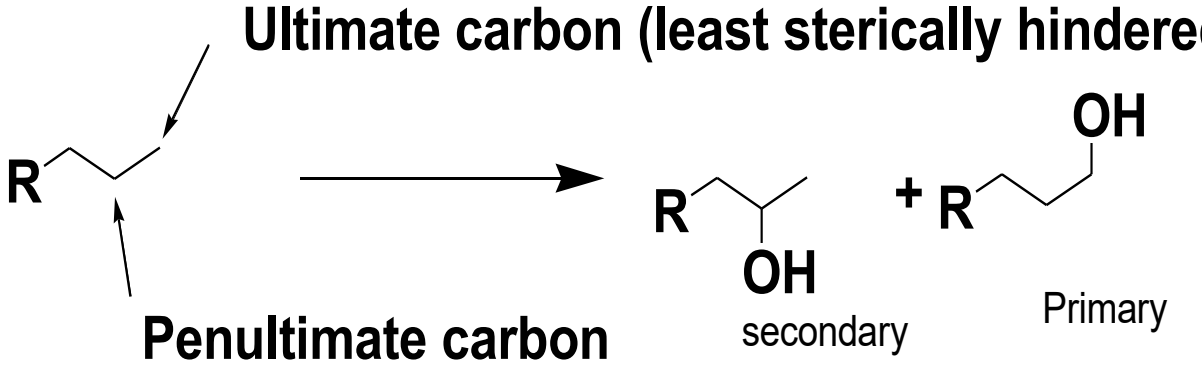


(2) Gets conjugated on one of the hydroxyls with glucuronic acid and eliminated.

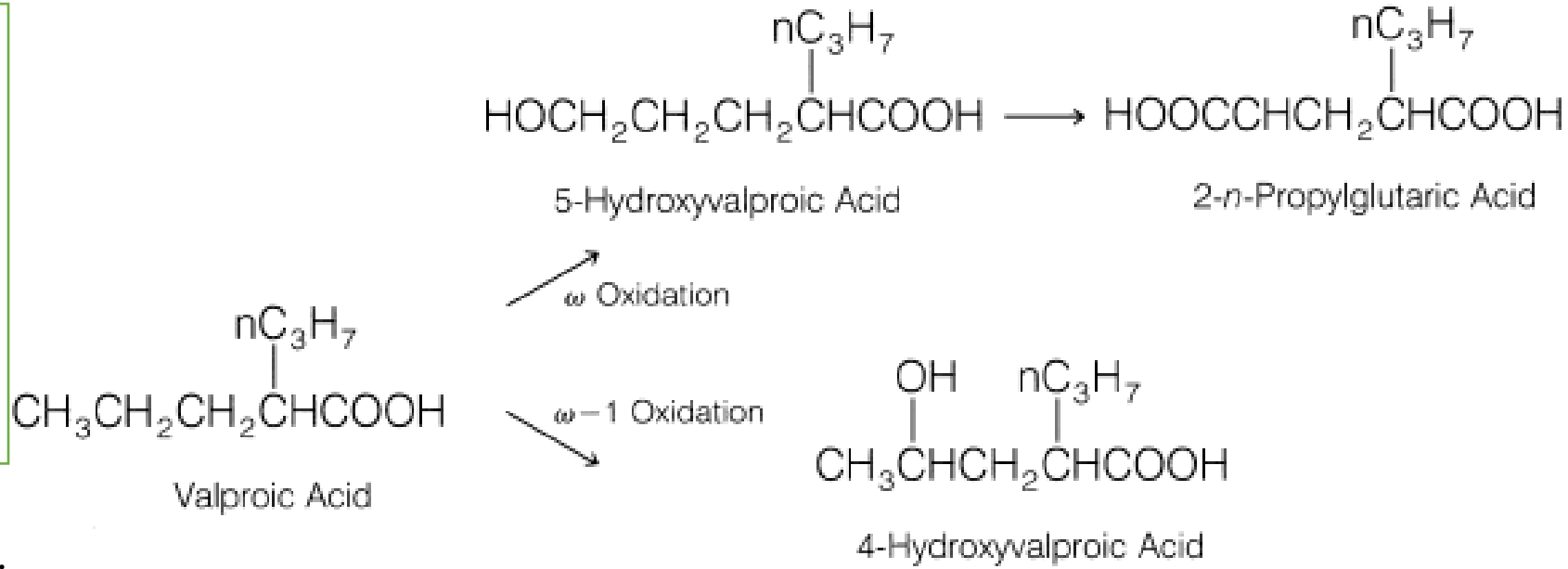
(3) Eliminated in the urine as the oxidized form (glycol).

5.Oxidation at Aliphatic and Alicyclic Carbon Atoms

- Alkyl or aliphatic carbon centres are subject to mixed-function oxidation. Metabolic oxidation at the terminal methyl group often is referred to as ω -oxidation, and oxidation of the penultimate carbon atom (i.e., next-to-the-last carbon) is called ω -1 oxidation.
- The initial alcohol metabolites formed from these enzymatic ω and ω -1 oxidations are susceptible to further oxidation to yield aldehyde, ketones, or carboxylic acids. Alternatively, the alcohol metabolites may undergo glucuronide conjugation.

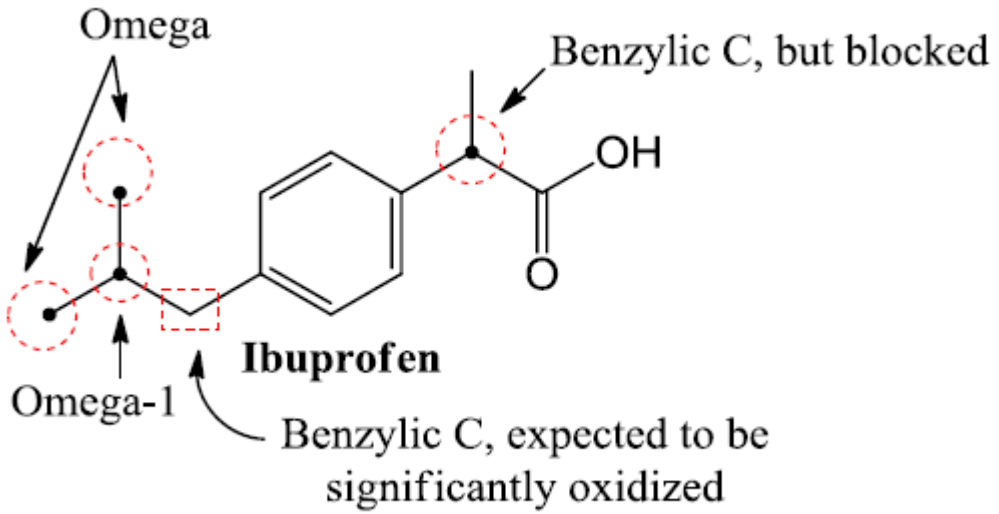


Valproic acid (Anti-seizure; Depakin®)
This drug has a carboxylic acid group so it's readily filtered and actively secreted to be eliminated in the urine unchanged.
It also gets oxidized, around 5%, in a ω and ω-1 oxidation. But mostly is eliminated unchanged.



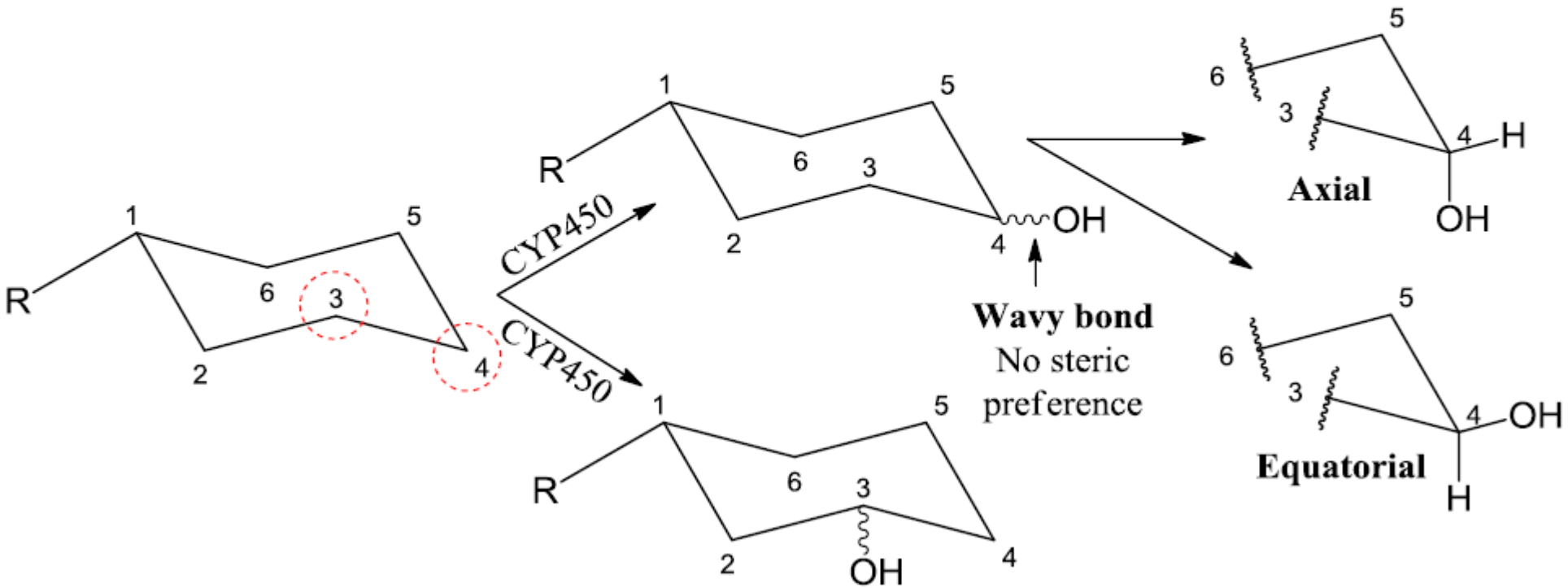
Ibuprofen

- Gets oxidized by ω or ω-1 oxidation; but notice if ω-1 oxidation happens a tertiary alcohol forms which cannot be further oxidized by alcohol dehydrogenase.
- Due to the carboxylic acid group it gets eliminated unchanged in the urine.
- Notice the benzylic carbon, α to aromatic ring; this oxidation reaction is more likely to happen than ω and ω-1 oxidations.
- **Weak candidate for aliphatic chain oxidation**



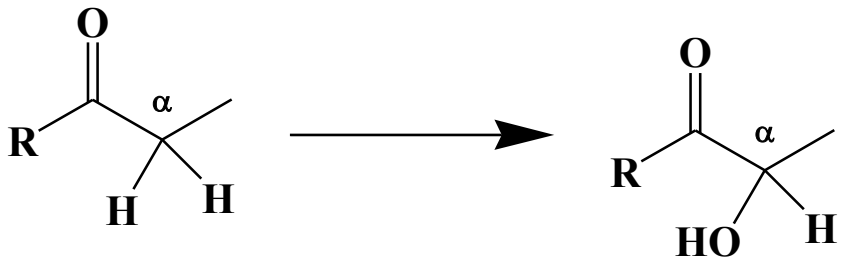
(6) Alicyclic Oxidation

This reaction involves a cyclic system.

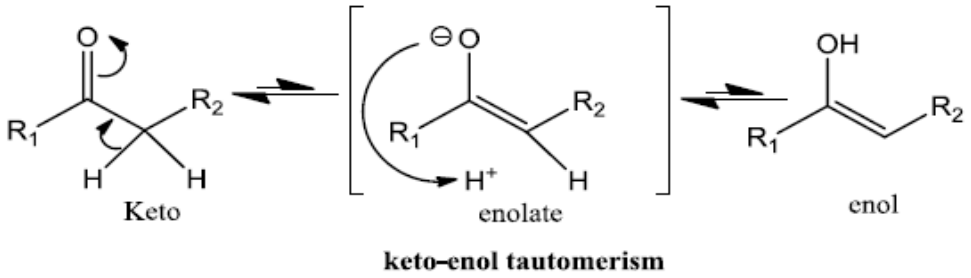


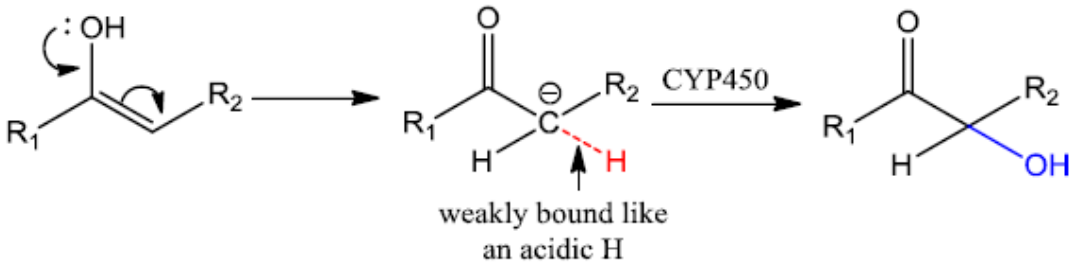
7.Oxidation to α carbon to carbonyl

- **Aldehyde, Ketone, Ester , Enole , Carboxylic acid, Thioester**
- Carbonyl is electron withdrawing group→ α carbon is electron rich
- Very fast reaction: 90-100% yield
- Fate of the oxidized drug:
- Eliminated in its oxidized form in the urine
- Good candidate for conjugation to glucuronic acid

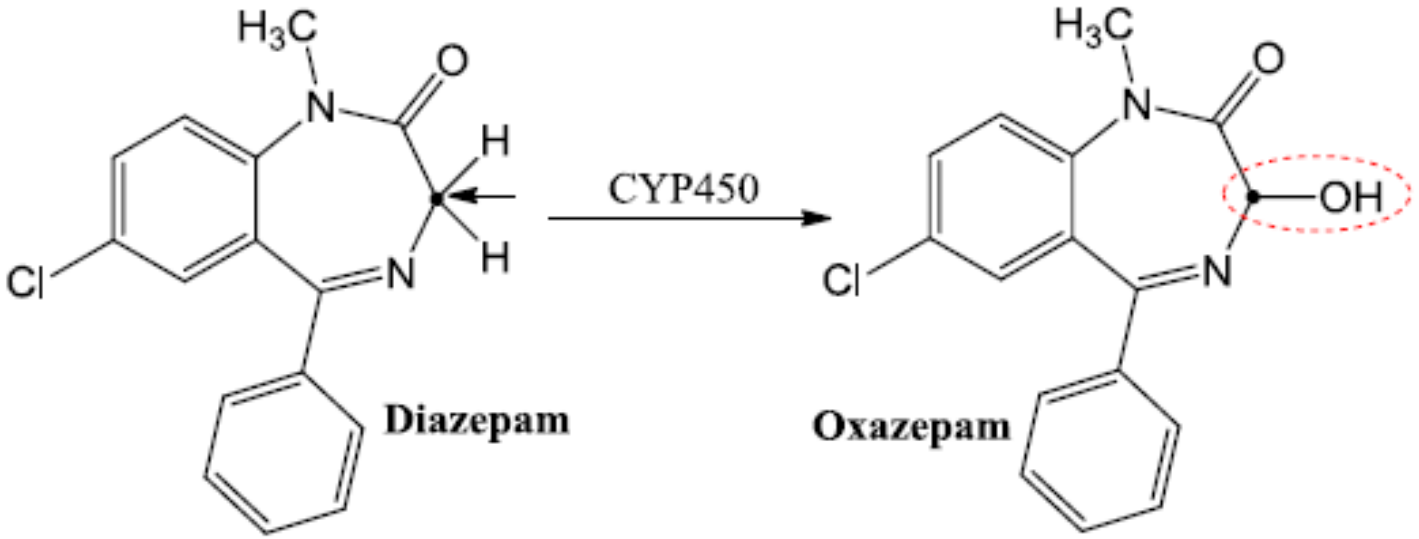


- Further oxidation by alcohol dehydrogenase: If a primary alcohol it's oxygenated to a carboxylic acid and gives a carboxylic acid ketone group in the urine.
- If it's a secondary alcohol it's oxidized to a ketone, giving a diketone in the urine.





Example

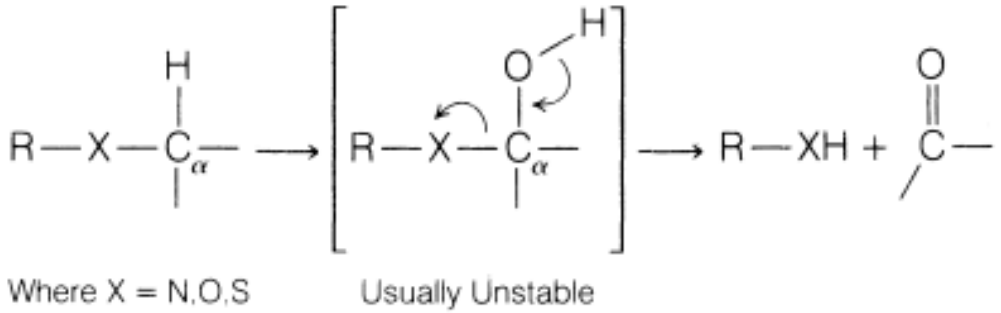


Aromatic ring oxidation is very minor because it's slower. The oxidation of the alpha carbon is much faster and more likely to happen.

7. Oxidation Involving Carbon-Heteroatom Systems

- Metabolic oxidation of carbon-nitrogen, carbon-oxygen, and carbon-sulfur systems involve two basic types of biotransformation processes:

1. Hydroxylation of the carbon atom directly attached to the heteroatom (N, O, S). The resulting intermediate is often unstable and decomposes with the cleavage of the heteroatom-carbon bond:

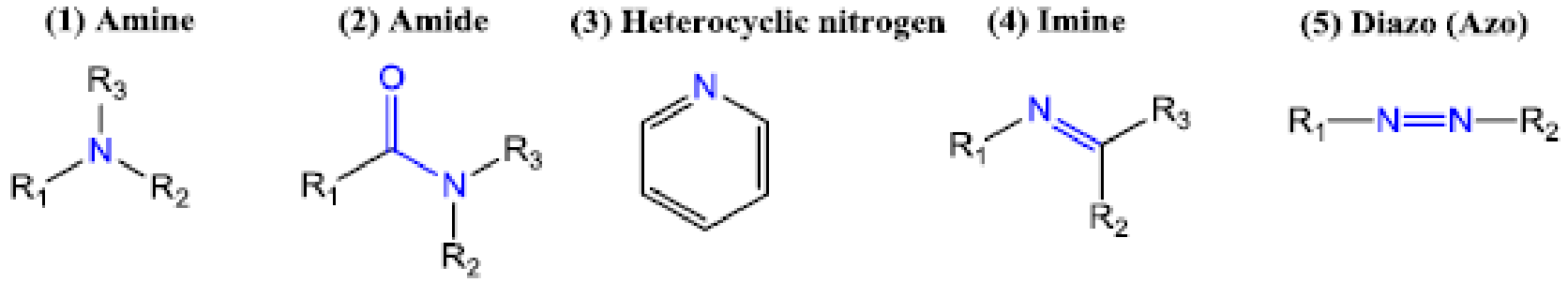


nitrogen is more electrophilic than carbon, the α-carbon is likely to get oxidized and is easier to be oxidized than the nitrogen

Oxidative N-, O-, and S-dealkylation as well as oxidative deamination reactions fall under this mechanistic pathway.

2. Hydroxylation or oxidation of the heteroatom (N, S only, e.g., N-hydroxylation, N-oxide formation, sulfoxide, and sulfone formation).

N- systems



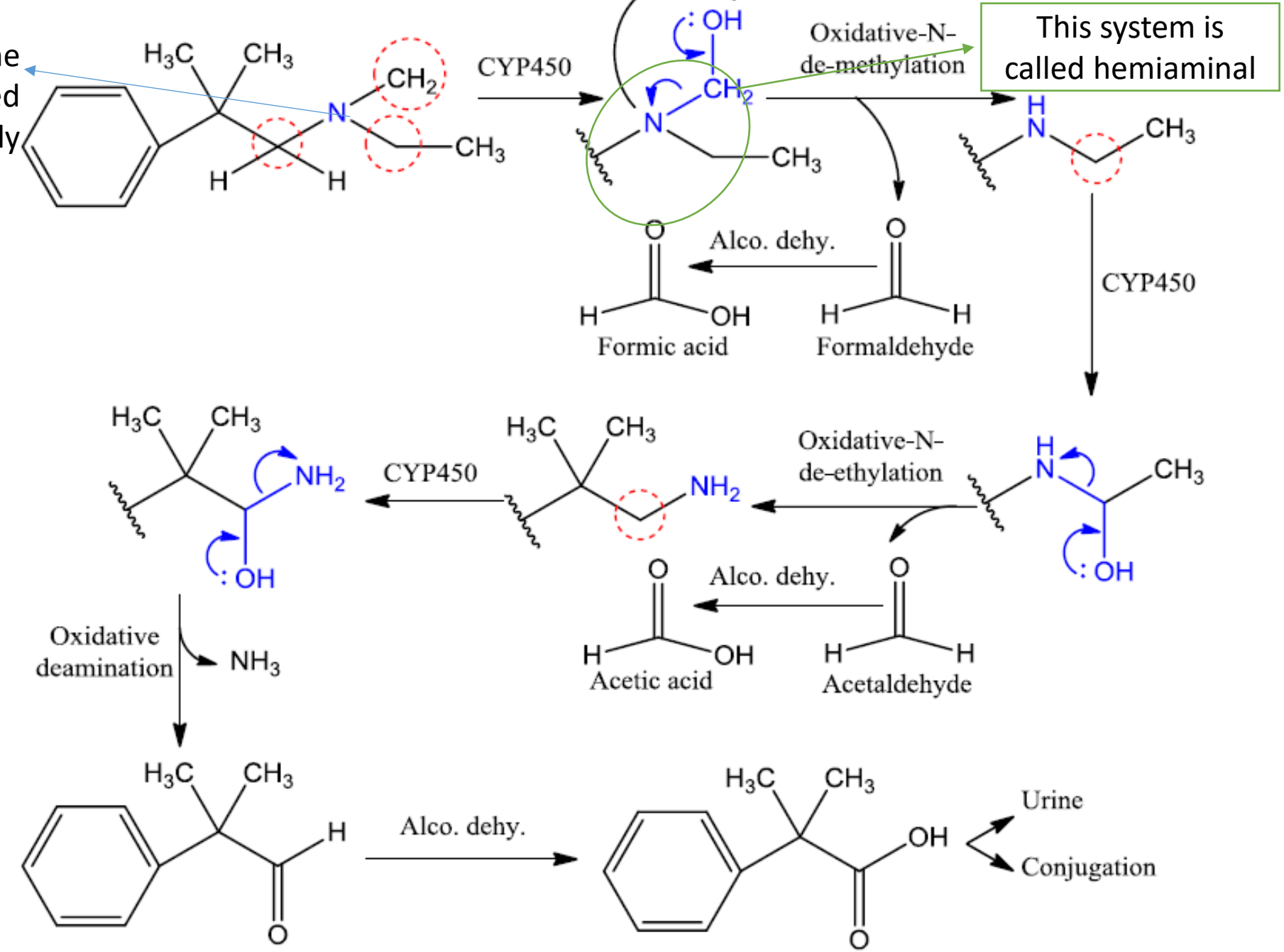
Amines are ***ionizable*** groups with a pKa= 9.5. The plasma's pHa= 7.5 so amines are ionized in physiological conditions.

How does that affect its renal elimination?

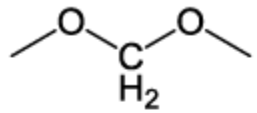
Since it's ionizable that means it's water soluble and it's more likely to get eliminated in the urine **mainly** unchanged than reabsorbed. around **80%** of the amines get eliminated unchanged in the urine

- and around **10%** get conjugated. This means that amines aren't good candidates for metabolism by **CYP450**.

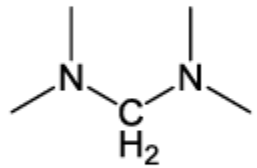
methyl will be the group to be oxidized because it's sterically the smallest



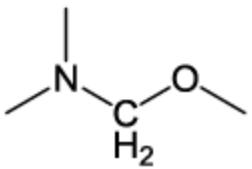
Acetal



Aminal
"Mannich base"



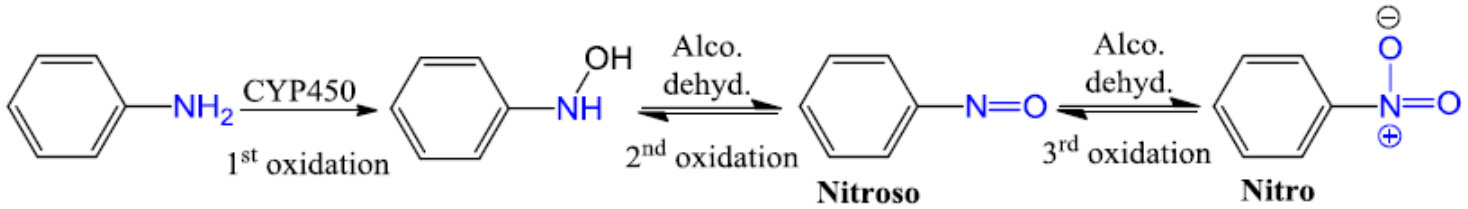
Hemiaminal

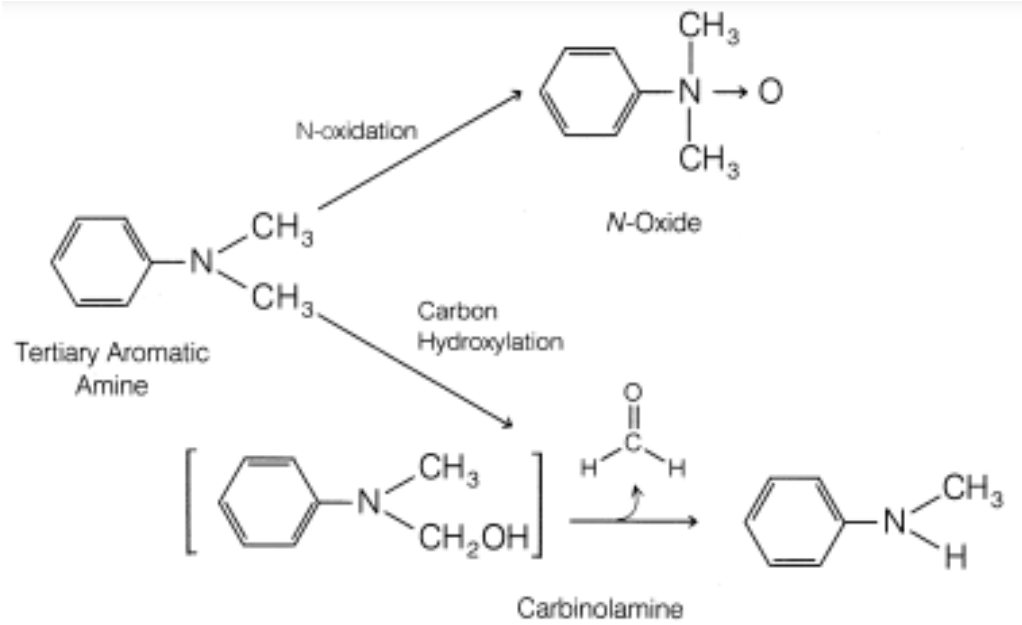


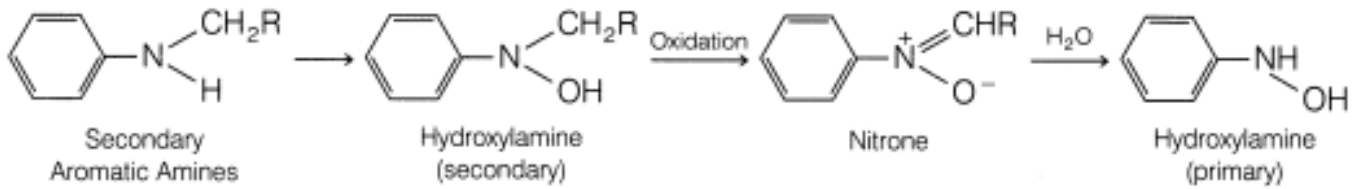
N-systems: What do we find in the urine?

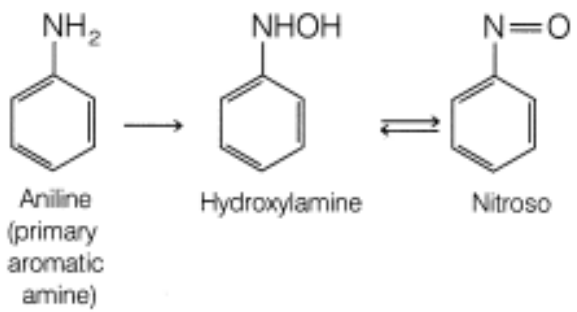
- Ionized drug unchanged (mostly)
- - Conjugated drug (to a lesser extent)
- To a much lesser extent:
- Ionized secondary and primary amines and their conjugates
- Ammonia
- Carboxylic acids (formic acid, acetic acid and the final carboxylic acid product of the last reaction)
- **NO** aldehyde (unstable)
- **NO** hemi-aminal (unstable)

N-oxidation

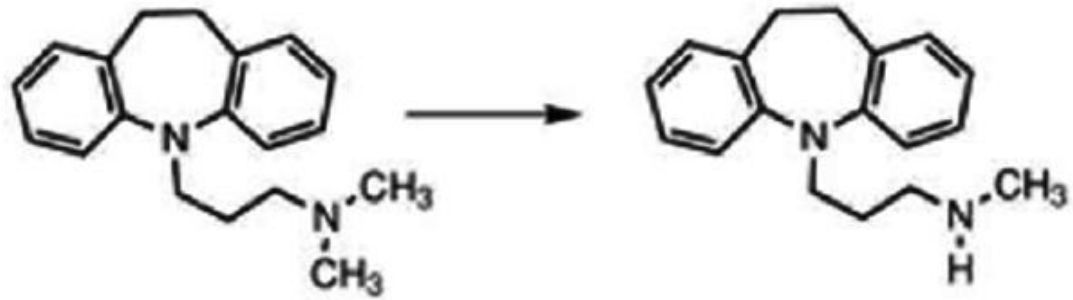

NH2
CYP450
1st oxidation
OH
NH
Alco. dehyd.
2nd oxidation
N=O
Nitroso
Alco. dehyd.
3rd oxidation
O
N=O
Nitro


CH3
N → O
CH3
N-oxidation
N-Oxide
CH3
N
CH3
Tertiary Aromatic Amine
Carbon Hydroxylation
O
C
H
H
CH3
N
CH2OH
CH3
N
H
Carbinolamine


CH2R
N
H
Secondary Aromatic Amines
CH2R
N
OH
Hydroxylamine (secondary)
Oxidation
CHR
N+
O−
Nitrone
H2O
NH
OH
Hydroxylamine (primary)


NH2
Aniline (primary aromatic amine)
NHOH
Hydroxylamine
N=O
Nitroso

Example



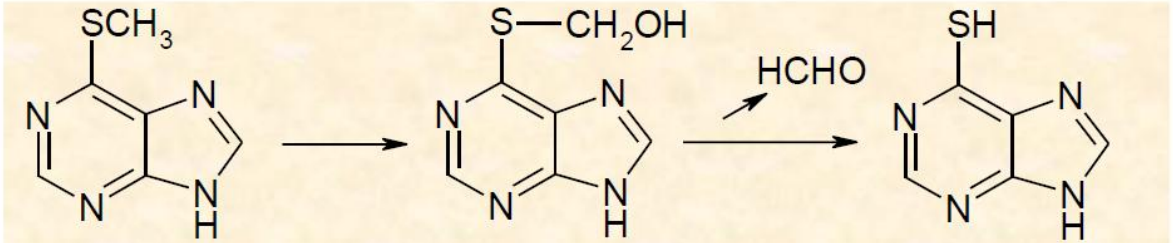
Imipramine Desimipramine

Nicotine Cotinine

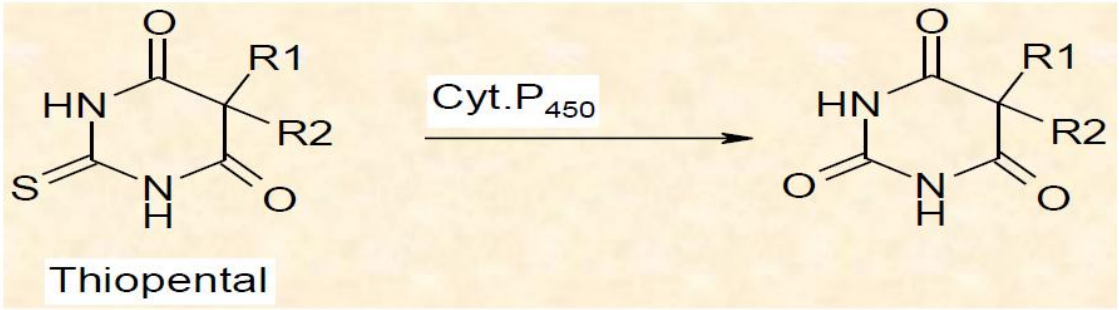
Nornicotine Norcotinine

S-Oxidation

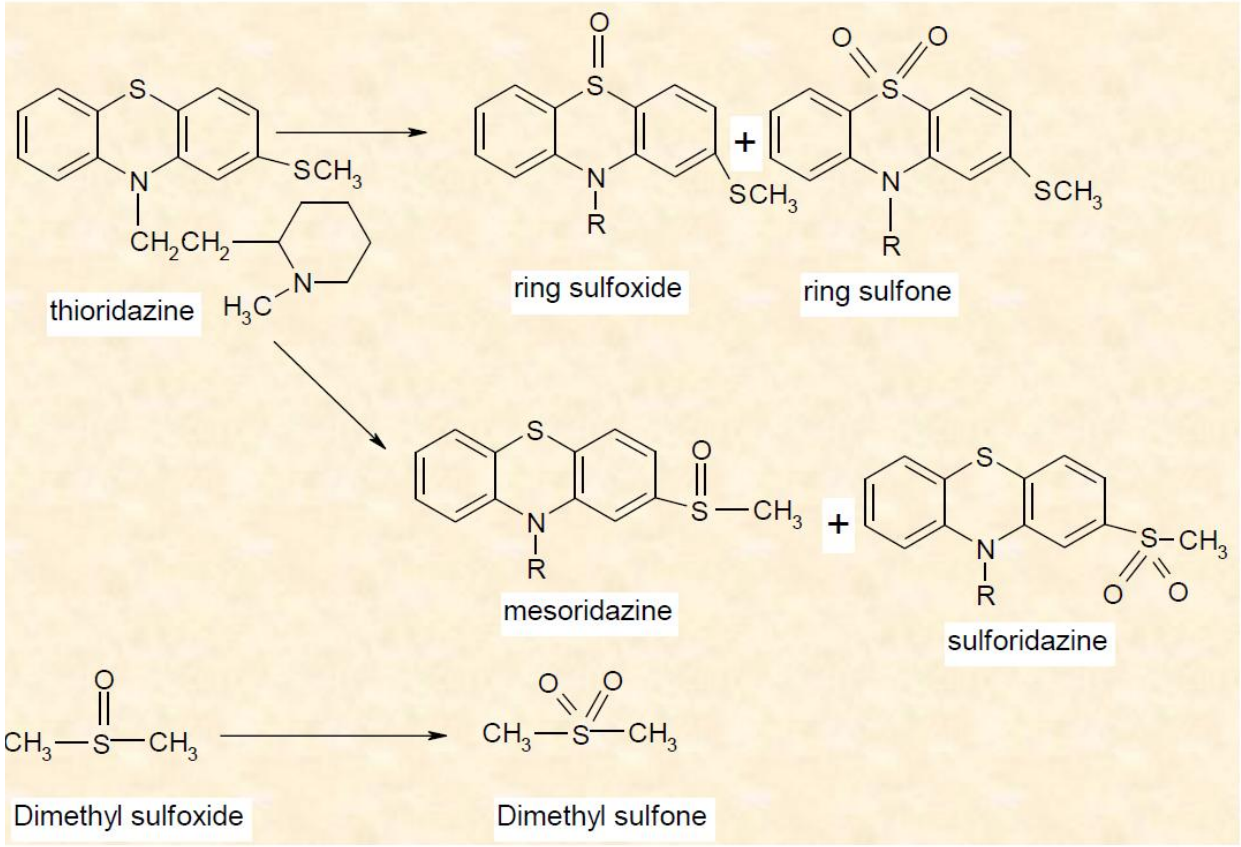
1) S-dealkylation:



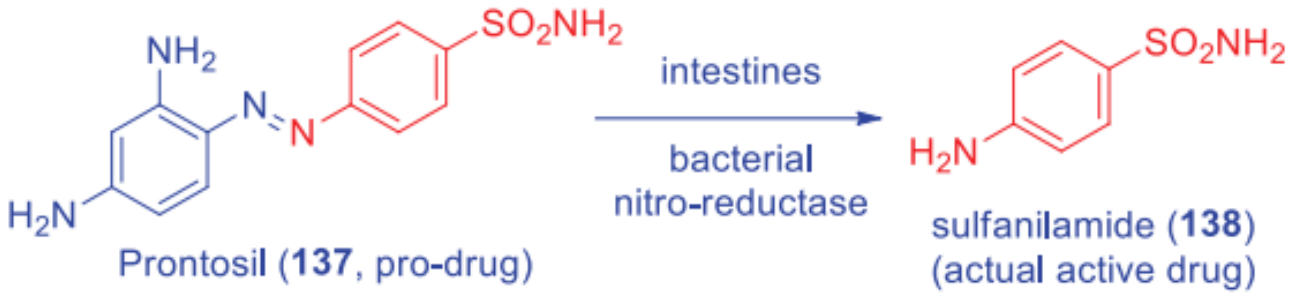
2) Desulphuration:



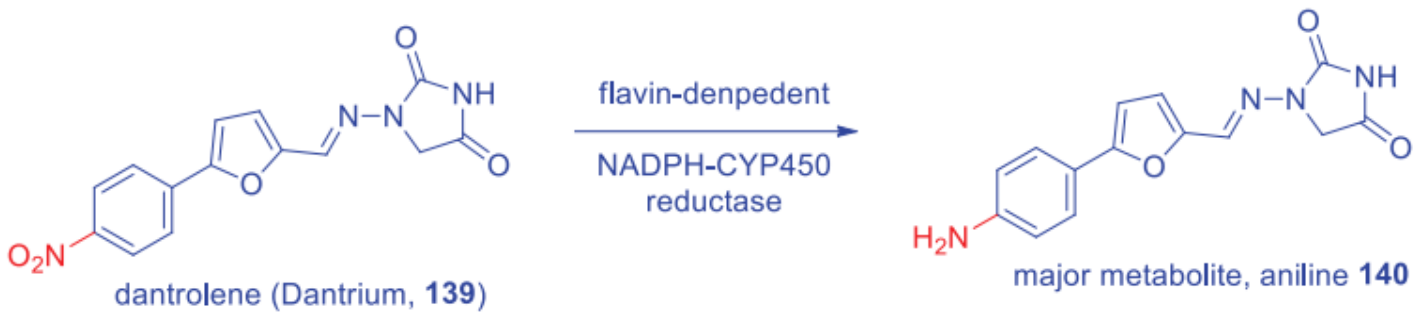
3) S-oxidation:

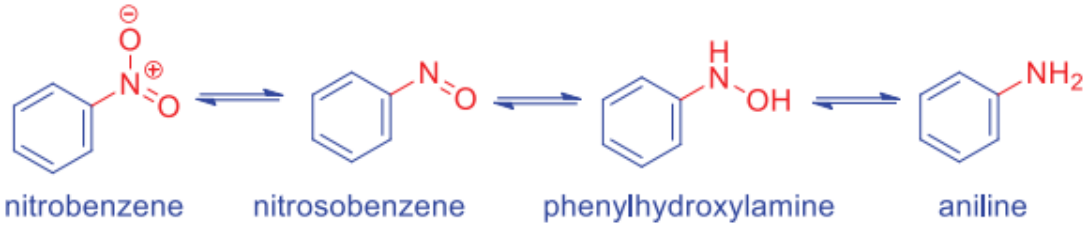


REDUCTION



- Reduction is the reverse of oxidation and involves CYP450 enzymes working in the opposite direction.





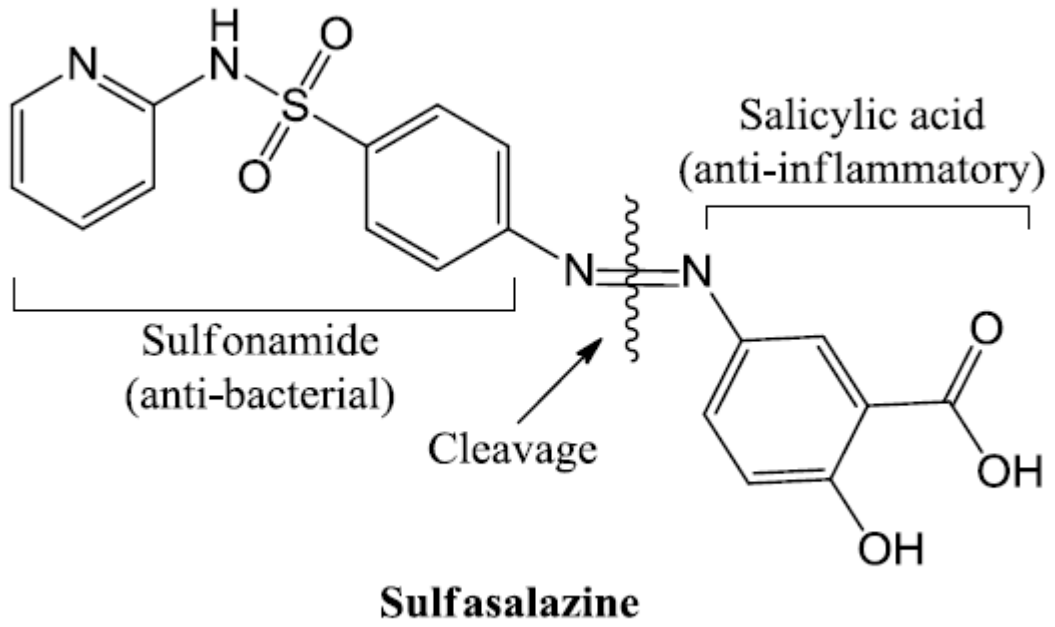
Example on reduction reactions

- An enzyme called " **floral diazo reductase** ", is involved in the metabolism of **Diazo compounds** , actually it's the major one.
- floral diazo reductase enzymes are present in the large intestine

Sulfasalazine

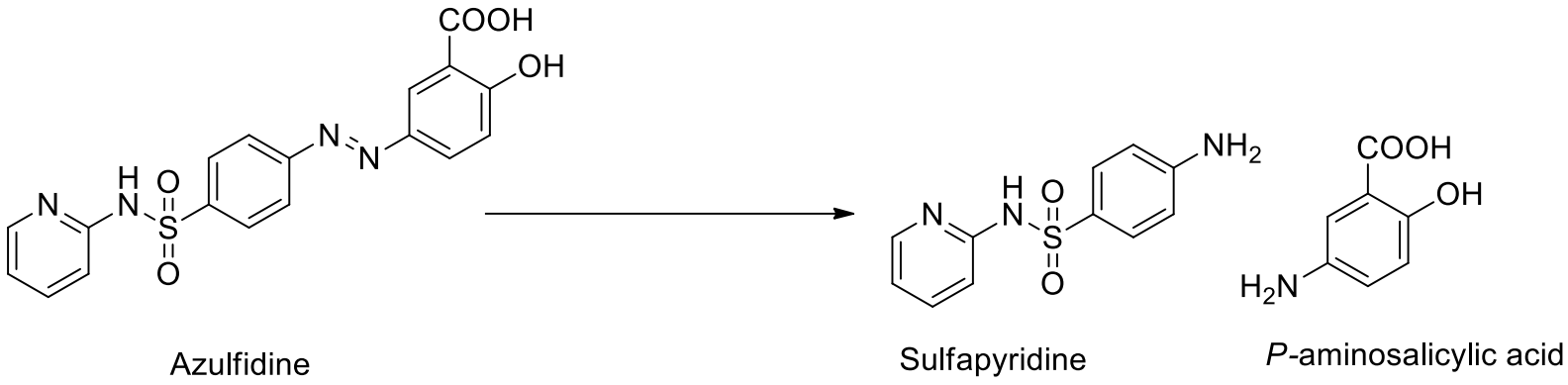
- The presence of the floral Diazo reductase enable us to build prodrug to treat a disease called Crohn's disease (autoimmune disease leads to colon ulcers , these ulcers make the colon prone to infectious agents like bacteria) , our drug which is actually a prodrug is **Sulfasalazine**
- Normal flora will reduce sulfasalazine (prodrug) by "floral Diazo reductase ", the reduction will cleave our drug in the line position. (At the Diazo group, where the reductase enzyme acts)

Sulfasalazine is not absorbable, so it acts locally
at the large intestine .and it is a prodrug that has to be activated in vivo,



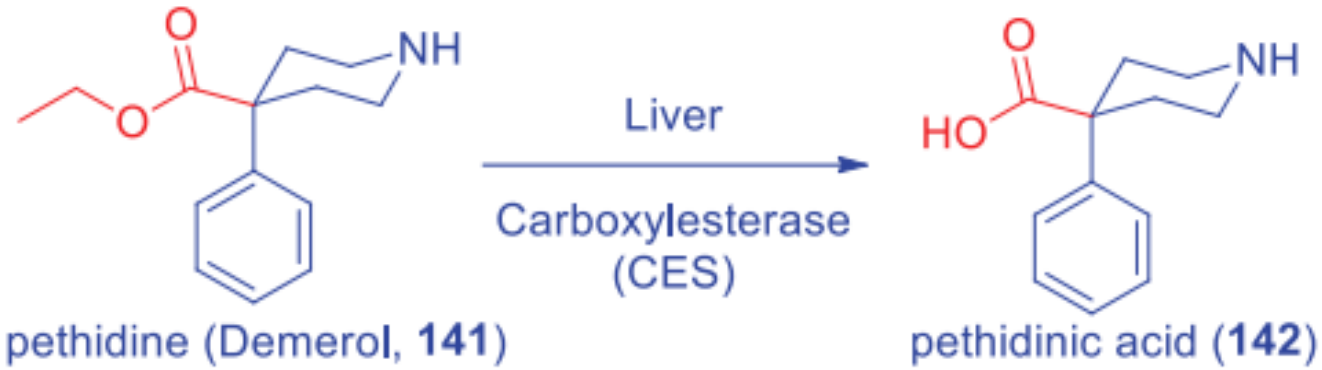
Azo and Nitro Reduction

- A number of azo compounds, such as Prontosil and sulfasalazine, are converted to aromatic primary amines by **azoreductase**



HYDROLYSIS

- Hydrolysis means adding water. For an ester-containing drug, hydrolysis is cleavage of the ester by taking up a molecule of water employing esterase. Similarly, amides and polypeptides are hydrolyzed by **amidases and peptidases,** respectively. Hydrolysis occurs in the liver, intestines, plasma, and other tissues.



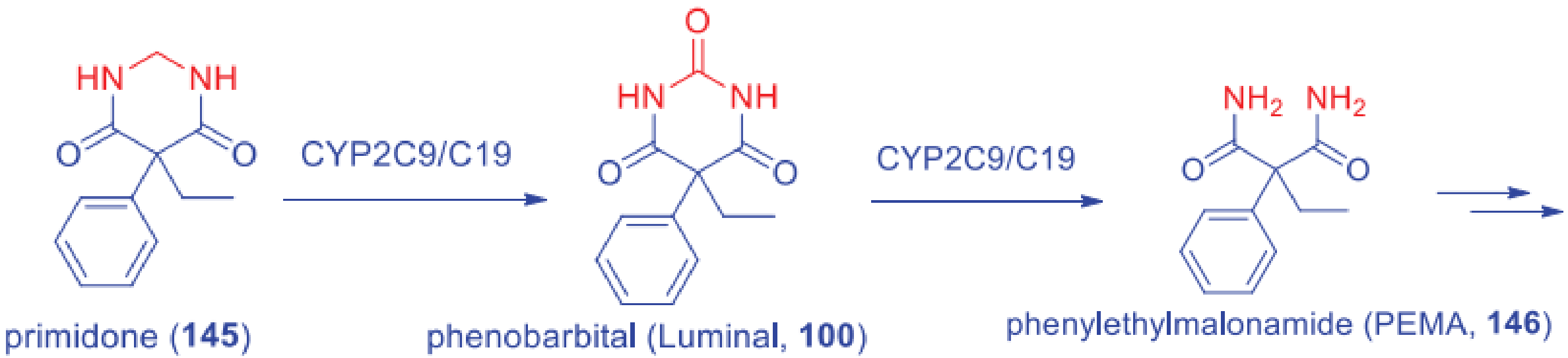
pethidine (Demerol, **141**) pethidinic acid (**142**)

CYCLIZATION

- Metabolic cyclization is formation of a **ring structure** from a **straight-chain compound**

DECYCLIZATION

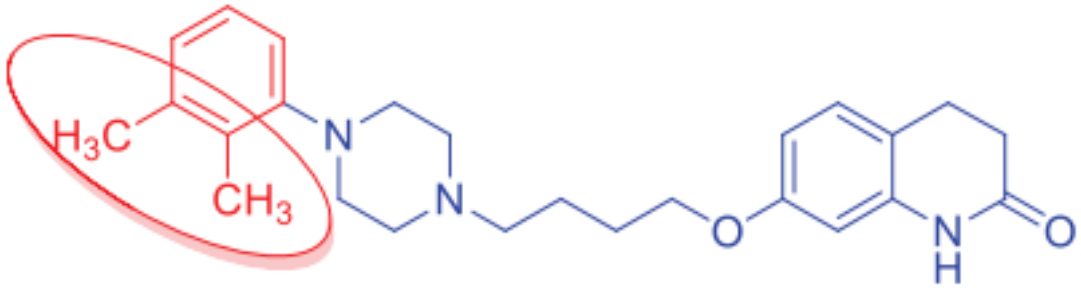
- Metabolic decyclization is **ring-opening** of a cyclic molecule such as phenytoin and barbiturates.



primidone (**145**) phenobarbital (Luminal, **100**) phenylethylmalonamide (PEMA, **146**)

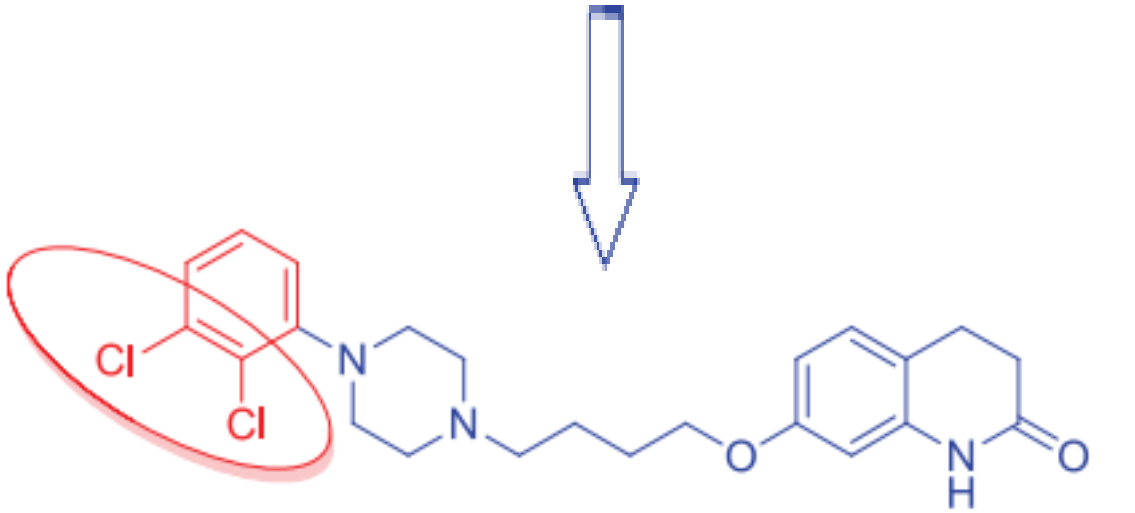
- Phase I metabolism is often problematic if the compound undergoes extensive metabolism to afford inactive metabolites, or worth still, reactive metabolites. There are many approaches to address Phase I metabolism issues:

- (i) Reducing the lipophilicity of the drug;
- (ii) Blocking a site of hydroxylation by replacing the hydrogen(s) with fluorine(s);
- (iii) Blocking a site of metabolism through cyclization;
- (iv) Eliminating or replacing a functional group with an isostere less susceptible to metabolism; or
- (v) Changing the chirality near or at the site of metabolism. This makes sense because **the CYP enzymes are chiral**, therefore metabolize different chirality differently. If the (*R*)-stereochemical center is metabolized, chances are the corresponding (*S*)-stereochemical center may be resistant to the metabolism.



OPC-4392 (**151**), ED_{50} = 41 μmol/kg, p.o.

The two methyl groups readily underwent hydroxylation and the diols were further oxidized to the corresponding inactive carboxylic acids.



aripiprazole (Abilify, **152**), ED_{50} = 0.6 μmol/kg, p.o.
Otsuka/BMS, 2002

Switching the two methyl groups to two chlorine atoms led to a molecule that is more resistant to the metabolism. The resulting compound OPC-14597 (aripiprazole, Abilify, **152**) is more efficacious with an ED_{50} of 0.6 mmol/kg, p.o.
It was approved by the FDA in 2002 as an effective and unique antipsychotic.