Metabolic Transformations of Xenobiotics
(Introduction of Biotransformation reactions)

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Bioanalytical Course
University of Connecticut
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Chemistry Building T309
11:00-12:15

OUTLINE

- Definition of xenobiotics
- Major Categories of Xenobiotics
- Biotransformation vs Metabolism
- Fate of Drugs
- Why is Drug Biotranformation necessary?
- General Outline of Biotransformation Reactions
- Summary

Xenobiotics

Xenobiotics: Foreign chemicals (substances not native to the body)

Major Categories of Xenobiotics

- Drugs
- Food constituents
- Food additives
- Chemicals of abuse (ethanol, coffee, tobacco..etc)
- Agrochemicals (fertilizers, insecticides, herbicides…etc)
- Industrial chemicals (solvents, dyes, monomers, ploymers..etc)
- Pollutants

Biotransformation vs Metabolism

• The terms biotransformation and metabolism are often used synonymously; particularly when applied to drugs.

• The term metabolism is often used to describe the total fate of a xenibiotic which includes absorption, distribution, biotransformation and excretion.

• Metabolism is commonly used to mean biotransformation from the standpoint that the products of xenobiotic biotransformation are called metabolites

Fate of Drugs

The knowledge of the metabolic fate of a drug is highly important, since the metabolism can generate toxic species, activate the drug or lead to the loss of pharmacological activity.

Pharmacokinetic phase

What the body does to the drug

Absorption
Distribution
Metabolism (Biotransformation) ★
Excretion

Elimination

Pharmacodynamic phase

What the drug does to the body

- Interaction between the drug and its site(s) of action (receptors, enzymes membranes, nucleic acids..etc)

- Also describes the site and the mechanism the drug acts on the body

Pharmacokinetic phase

(I) Absorption

- Drugs administered orally are absorbed from the GI tract
- Absorption takes place mostly in the small intestine
- To reach systemic circulation (bloodstream), the drug must pass through first the intestinal wall and then the liver (via portal vein).
- The intestinal wall and liver chemically alter (metabolize or biotransform) many drugs.
- In contrast, drugs injected IV reach the general circulation without passing through the intestinal wall and liver and thus they have quicker and more consistent response.

(II) Distribution

• After the drug is absorbed into the bloodstream, it rapidly circulates through the body.

• Most drugs don’t spread out evenly through the body. Some drugs remain within the watery tissues of the blood and tissues while others concentrate in specific tissues such as liver and kidneys.

• Some drugs bind tightly to blood protein and leave the bloodstream very slowly while others leave bloodstream quickly into other tissues.

• Distribution of a given drug may also vary among different persons.

(III) Elimination

All drugs are either Metabolized (Biotransformed) or Excreted intact

Metabolism (Biotransformation)

• Metabolism (Biotransformation) is the process by which a drug is chemically altered by the body. The liver is the principal, but not the only, site of drug metabolism.

• The products of metabolism are called metabolites. Metabolites may be inactive or they may have similar or different degrees of therapeutic activity or toxicity than the original drug.

• The liver has enzymes that facilitate chemical reactions such as oxidation, reduction, and hydrolysis of drugs. It has other enzymes that attach substances to the drug, producing reactions called conjugations. The conjugates (drug molecules with the attached substances) are excreted in the urine.

Excretion

• Excretion refers to the processes by which the body eliminates a drug.

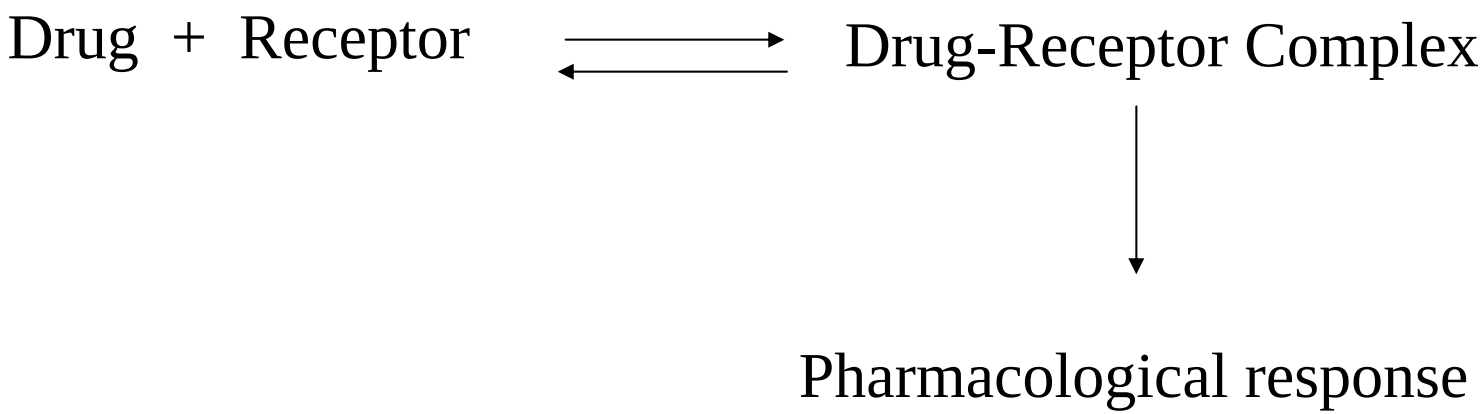
• The kidneys are the major organs of excretion. They are particularly effective in eliminating water-soluble drugs and their metabolites. The kidneys filter drugs from the bloodstream and excrete them into the urine.

• The liver excretes some drugs through bile. These drugs enter the GI tract and end up in the feces if they are not reabsorbed into the bloodstream or decomposed.

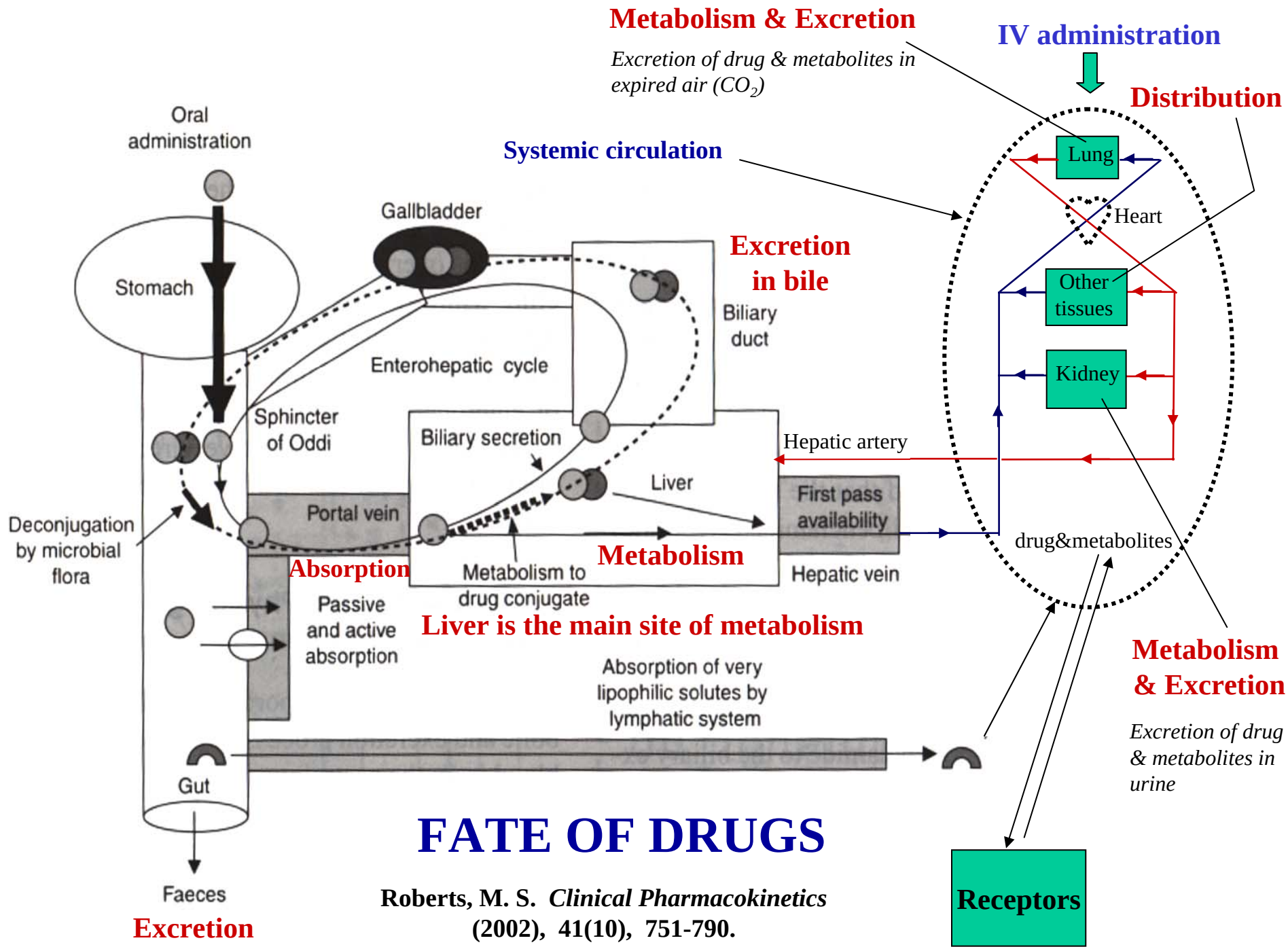
• Small amounts of drugs are also excreted in saliva, sweat, breast milk, and even exhaled air.

Pharmacodynamic phase

Following introduction into the body, a drug must pass through many Barriers, survive sites of attachment and storage and avoid significant metabolic destruction before it reaches the site of action, usually a receptor. At the receptor, the following equilibrium usually holds



The ideal drug will show favorable binding characteristics to the receptor, such that the equilibrium lies to the right. At the same time the drug will be expected to dissociate from the receptor and re-enter the systemic circulation to be excreted.



FATE OF DRUGS

Roberts, M. S. *Clinical Pharmacokinetics* (2002), 41(10), 751-790.

Why is Drug Biotranformation necessary?

• During the process of biotransformation, the molecular structure of a drug is changed from one that is absorbed (lipophilic, or capable of crossing the lipid core of membranes) to one that can be readily eliminated from the body (incapable of crossing the lipid core of membranes, or hydrophilic).

• If lipophilic drugs are not metabolized, they will remain in the body for longer than intended, and their cumulative biological effects will eventually cause harm. Thus, the formation of water-soluble metabolites not only enhances drug elimination but also leads to compounds that are generally pharmacologically inactive and relatively nontoxic.

Biotransformation Reactions

Phase I (Functionalization)

Oxidative Reactions

Hydrolytic Reactions

Reductive Reactions

Phase II (Conjugation)

Glucuronidation

Sulfation

Acetylation

Methylation

Amino Acid Conjugation

Glutathione Conjugation

Oxidative Reactions

- *Oxidation is the most common and an important route of metabolism of xenobiotics.*
- *It is a process of electron abstraction followed by incorporation of oxygen into the molecule. The source most often is molecular oxygen. Sometimes the oxygen is acquired from water.*
- *Most organic compounds undergo a 1 electron or 2 electron redox reactions. Some undergo a 4e^- oxidation.*
- *The most common electron acceptor is molecular oxygen. This can undergo a 2 e^- reduction to generate H_2O_2 or 4 e^- reduction to generate water.*

Common Oxidative Reactions

Hydroxylation of aromatic carbons

Hydroxylation of aliphatic carbons

Dehydrogenation: involves a hydride abstraction.

Oxidations Involving Carbon-Heteroatom Systems

Main Enzymes Involved in the Oxidative Reactions

Aldehyde oxidase (AO)

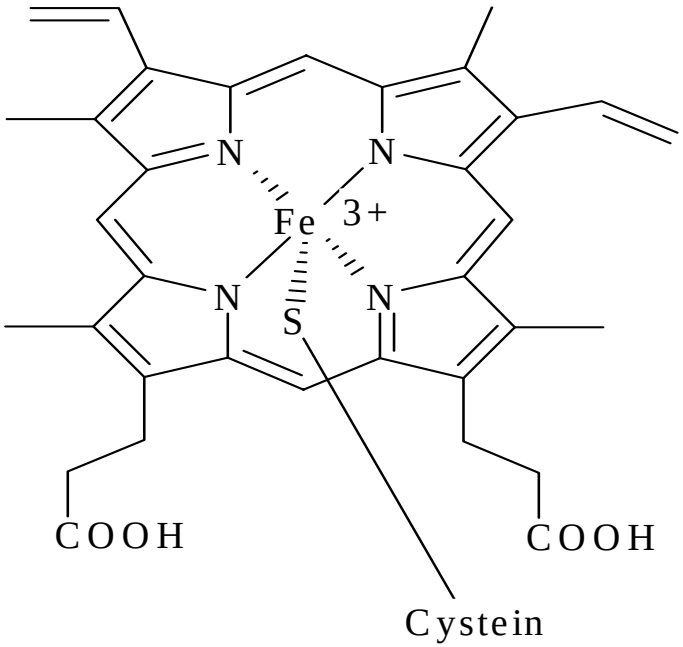
Xanthine oxidase (XO)

Monoamine oxidases (MAOs)

Flavin-containing monooxygenases (FMOs)

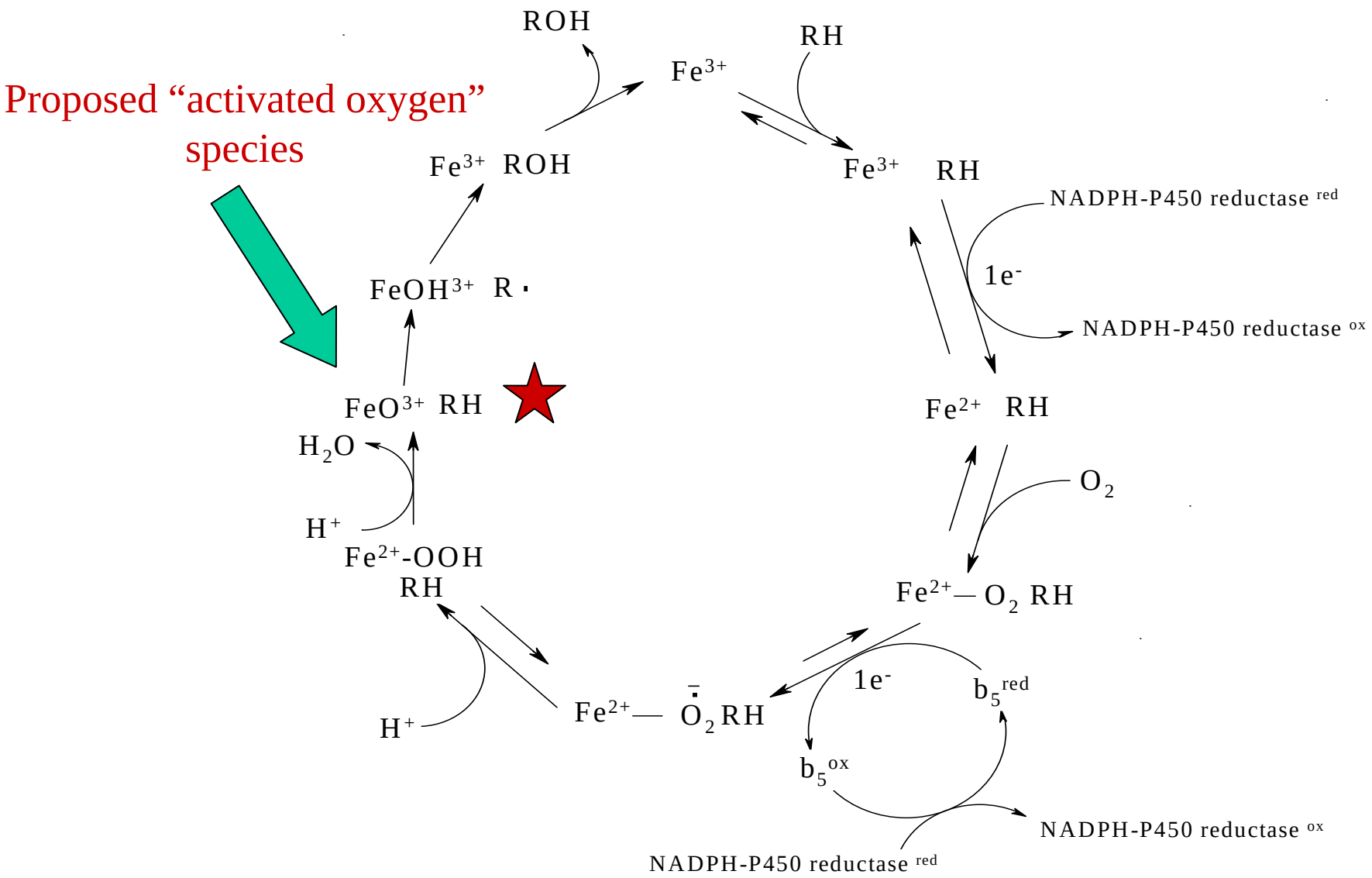
Cytochrome P450s (P450s)

Cytochrome P450

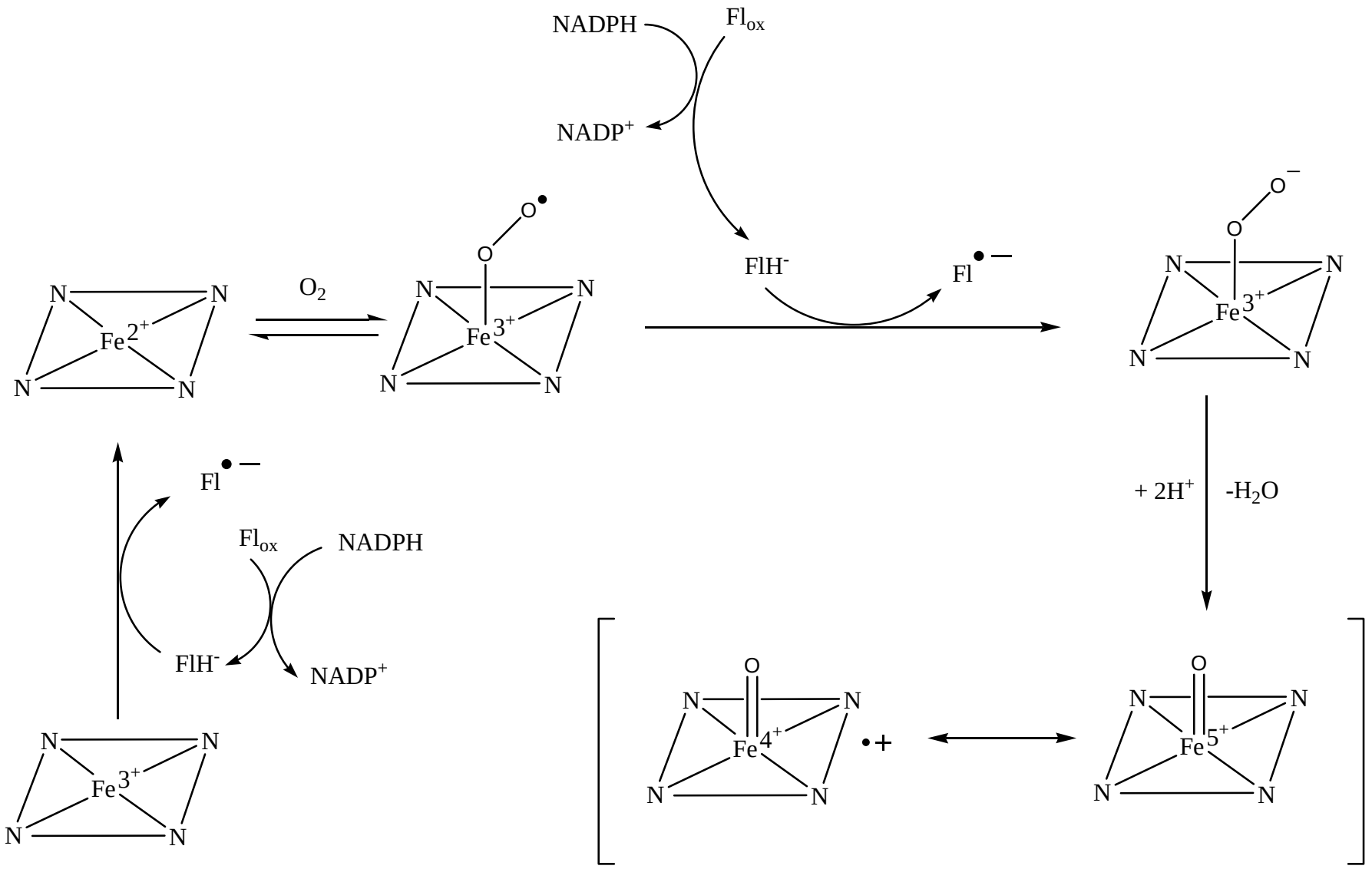


Heme (protoporphyrin IX)

All P450 enzymes are heme-containing proteins. The heme is Ferric (Fe^{3+})-containing porphyrin cofactor with cystein as the fifth ligand, leaving the sixth coordination site to bind and activate molecular oxygen

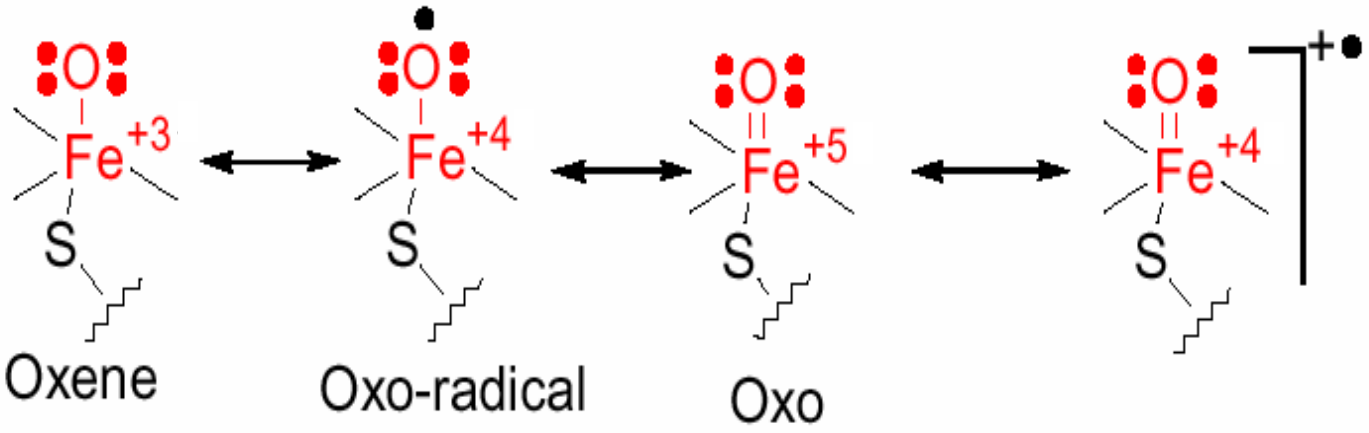


Catalytic Cycle of Cytochrome P450



Mechanism for the formation of the high-energy iron-oxo species in heme-dependent oxygenases. *The organic Chemistry of drug design and Drug Action-Richard Silverman*

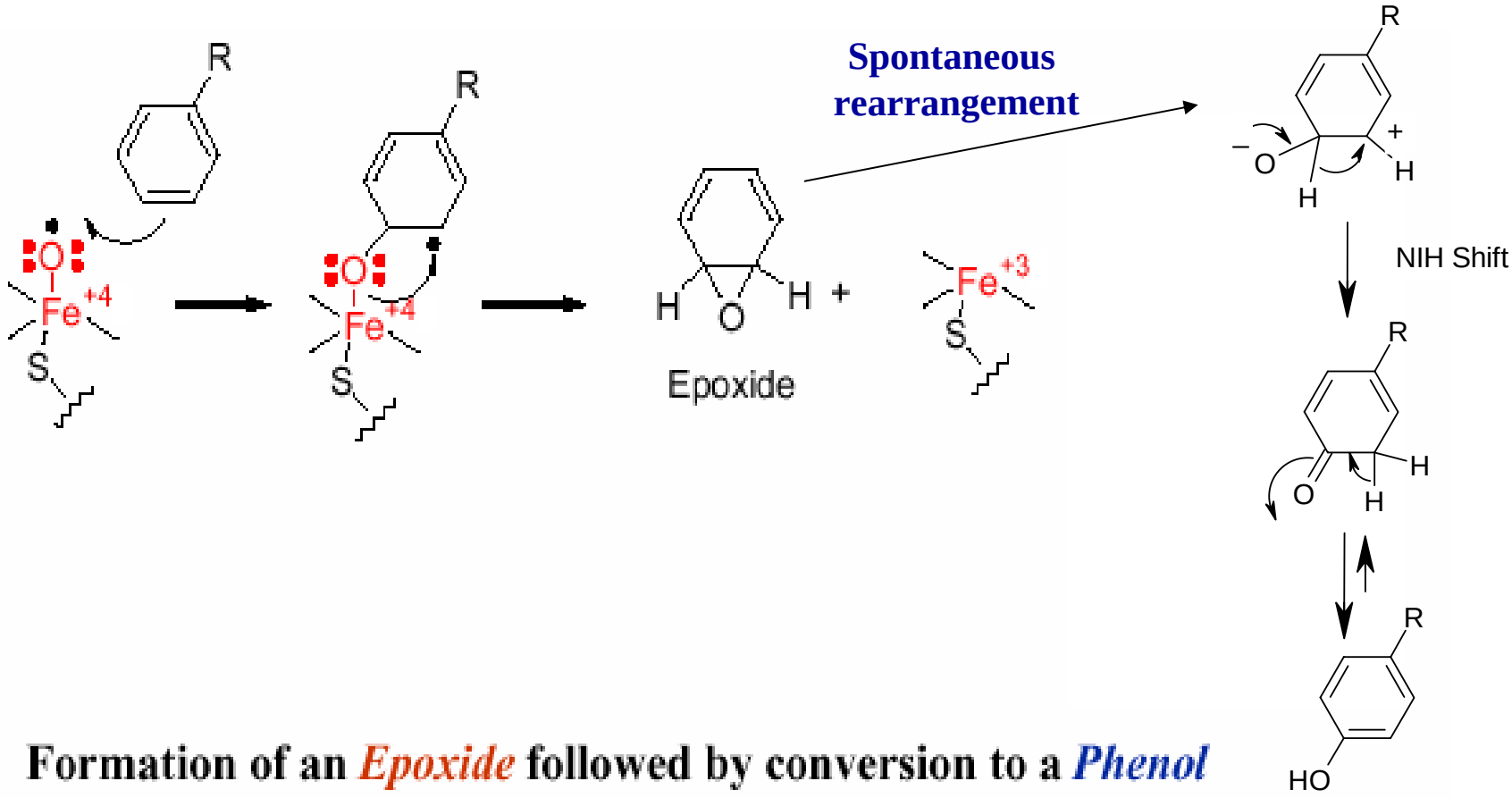
Oxidation of compounds by P450 involves activation of oxygen to an oxene



Iron(V) is such an exceedingly high oxidation state

Iron(IV) with a radical cation in the porphyrin ring (more likely)

Hydroxylation of Aromatic Carbons

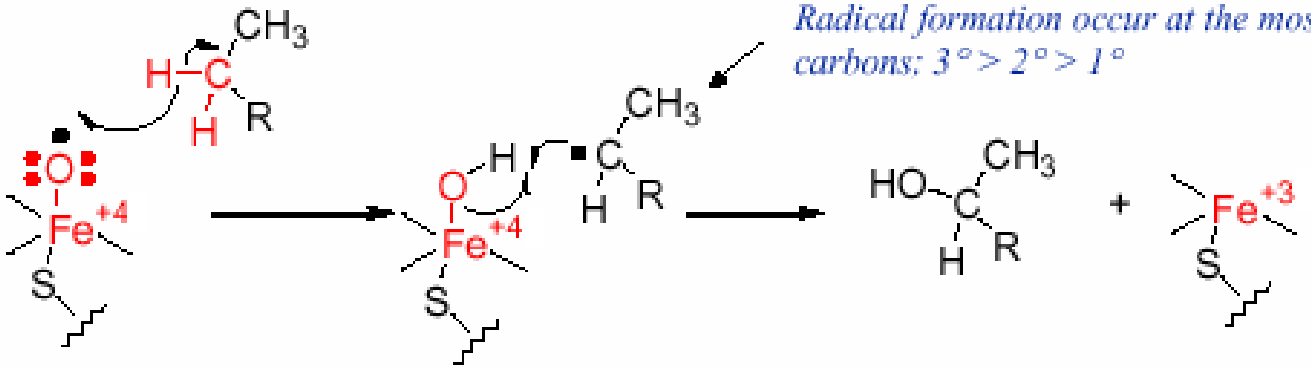


Formation of an *Epoxide* followed by conversion to a *Phenol*

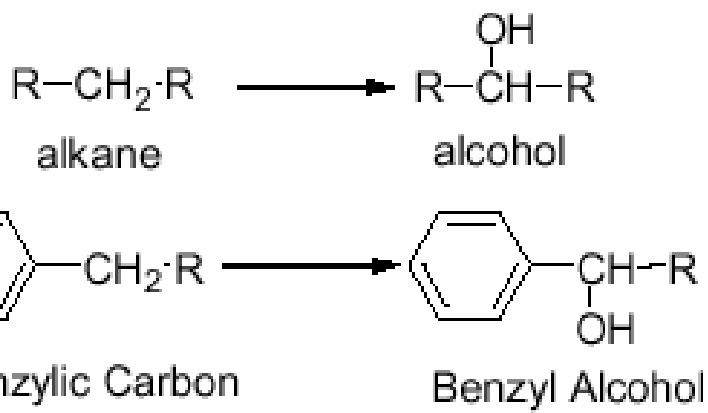
Hydroxylation of Aliphatic Carbons

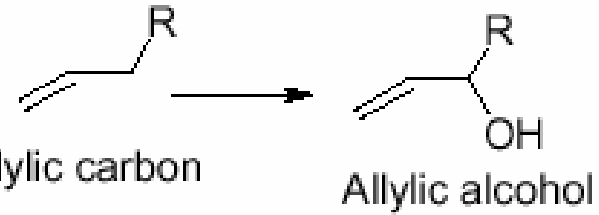
(I) Alkanes

Alkane Oxidation or Oxidation of sp^3 Carbons



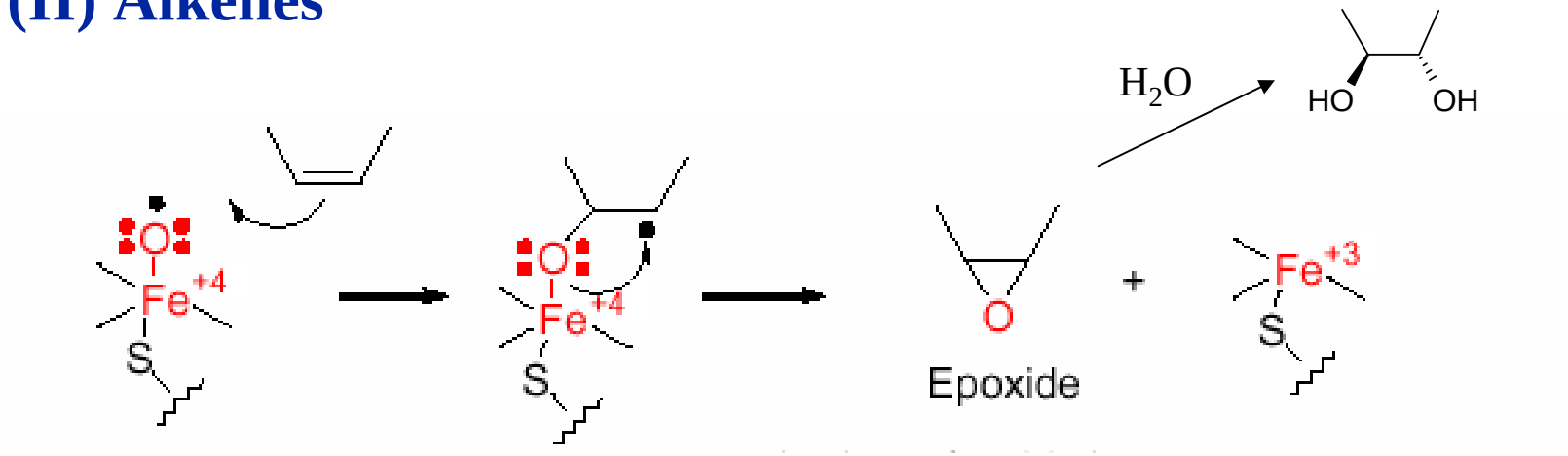
Some common oxidation reactions of sp^3 carbons



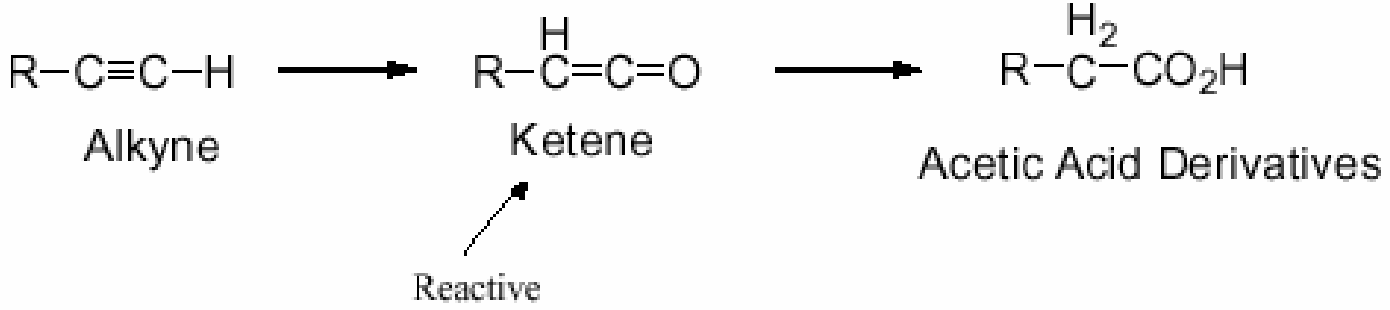


Hydroxylation of Aliphatic Carbons (cont'd)

(II) Alkenes



(III) Alkynes

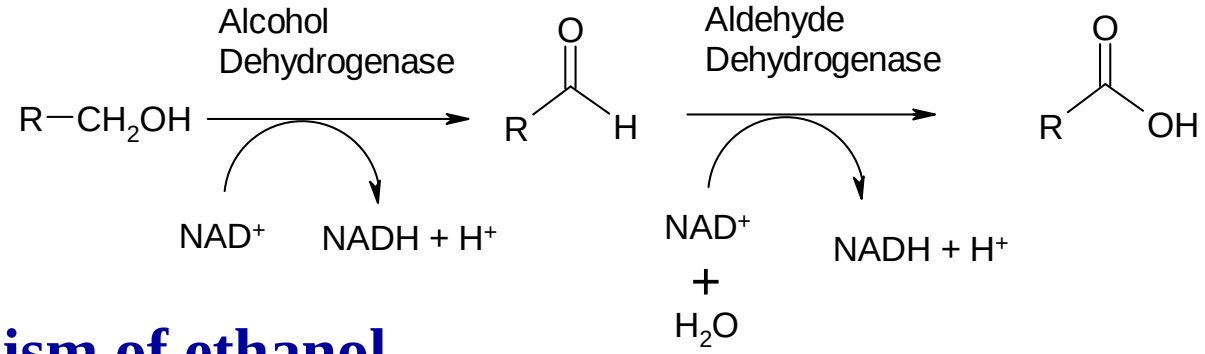


Most terminal alkyne containing compounds are inactivators of P450

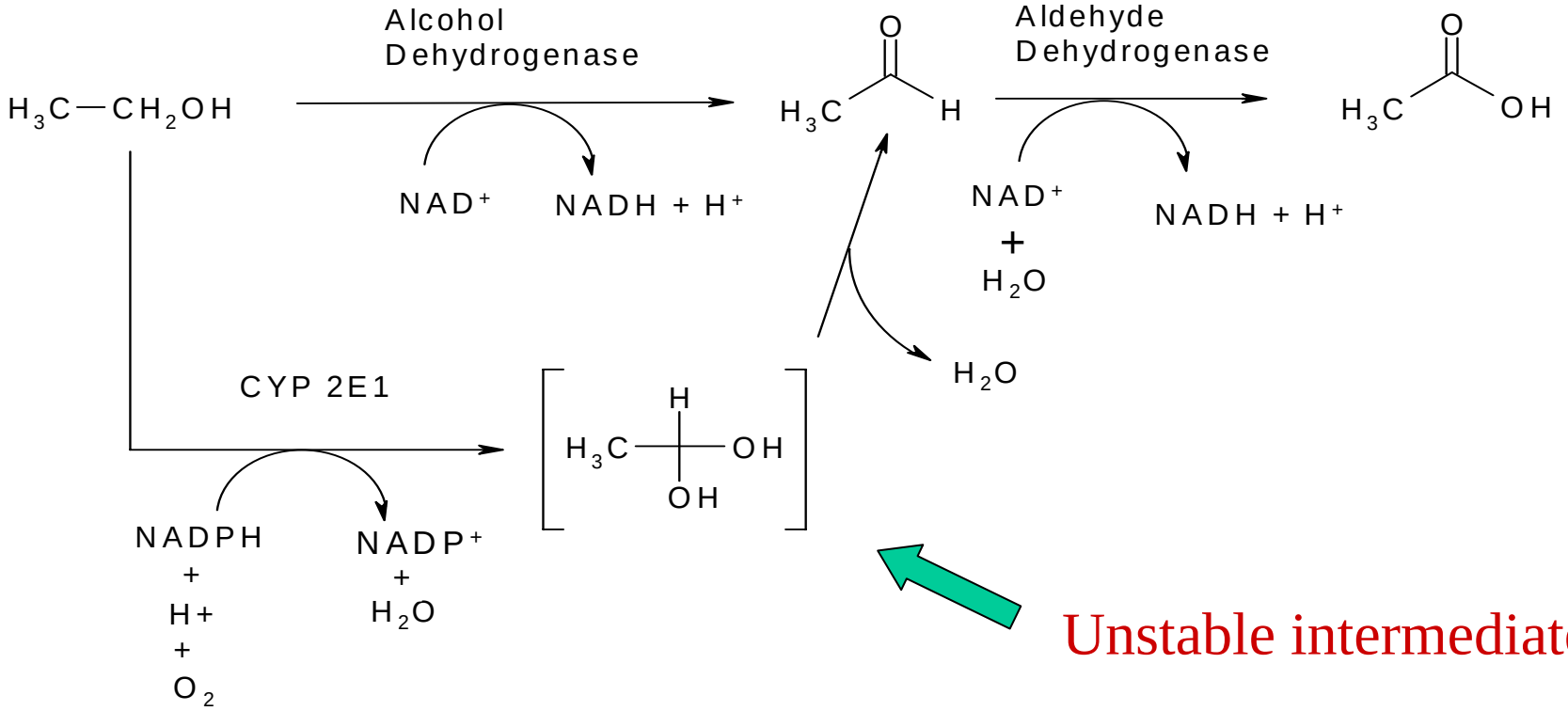
Inactivate P450 by heme alkylation

Oxidation (Dehydrogenation) of Alcohols and Aldehydes

Alcohols are oxidized to aldehydes which are further oxidized to carboxylic acids



Metabolism of ethanol

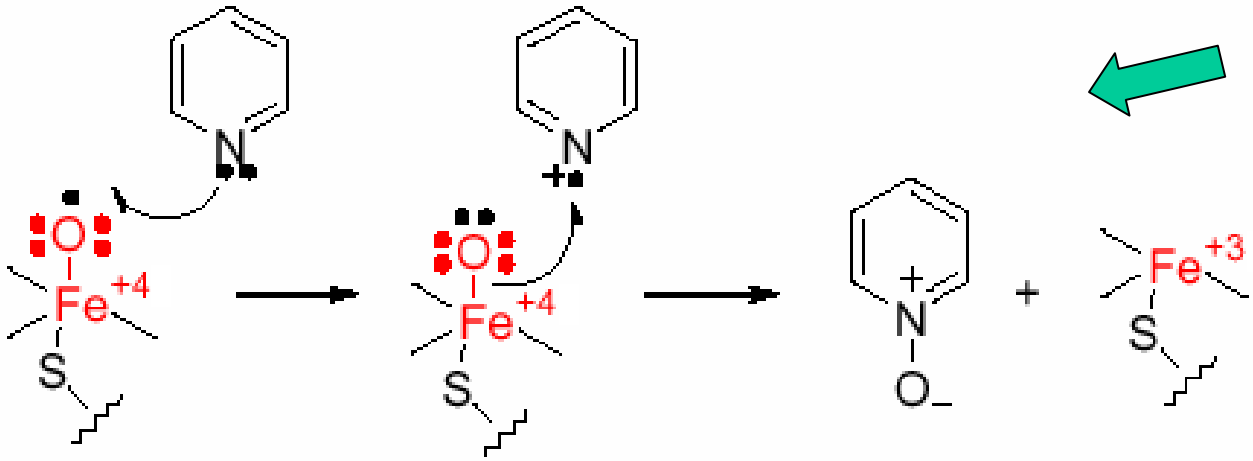


Oxidations Involving Carbon-Heteroatom Systems

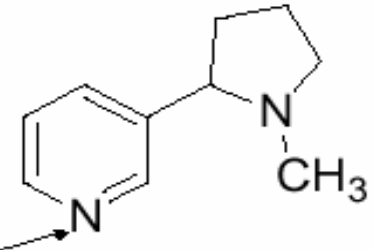
(I) The heteroatom oxidation (N- and S-oxide formation)

Nitrogen Oxidations By P450

Less nucleophilic nitrogens undergo N-oxidations by P450



Mechanism



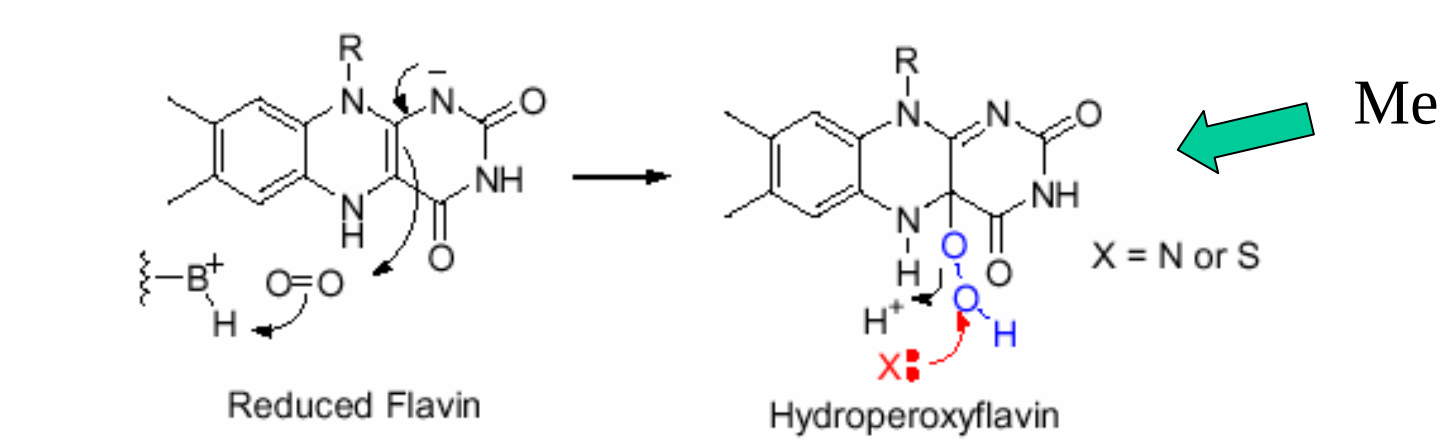
P450 catalyzes N-oxidation of pyridine nitrogen

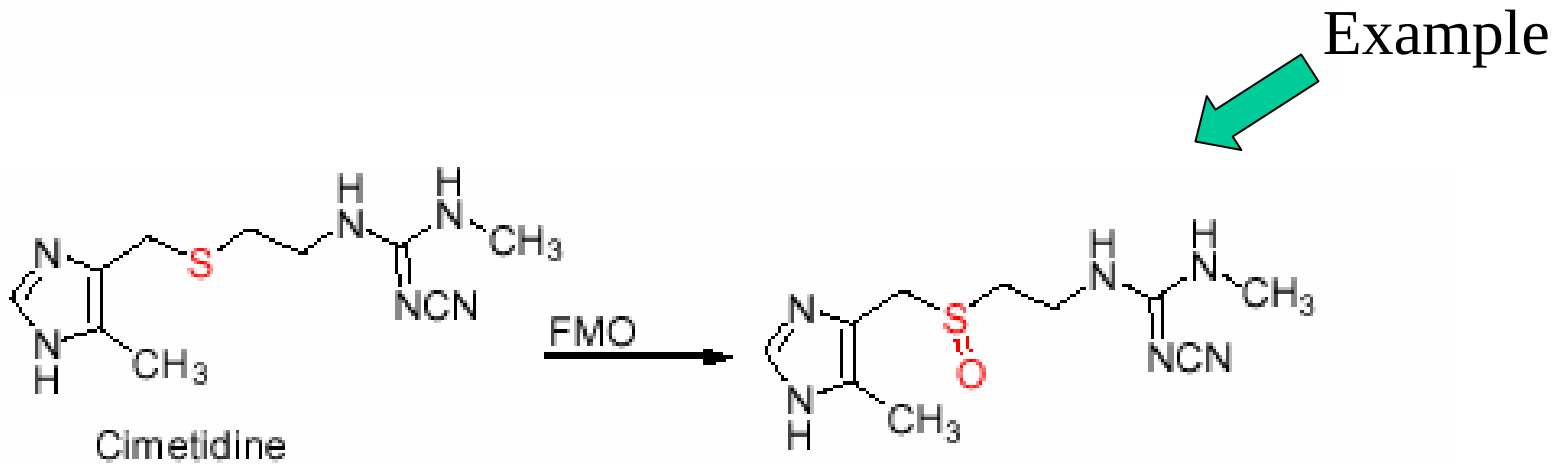
Nicotine

Example

(I) The heteroatom oxidation (N- and S-oxide formation), cont'd

Nitrogen and sulfur Oxidations By FMO

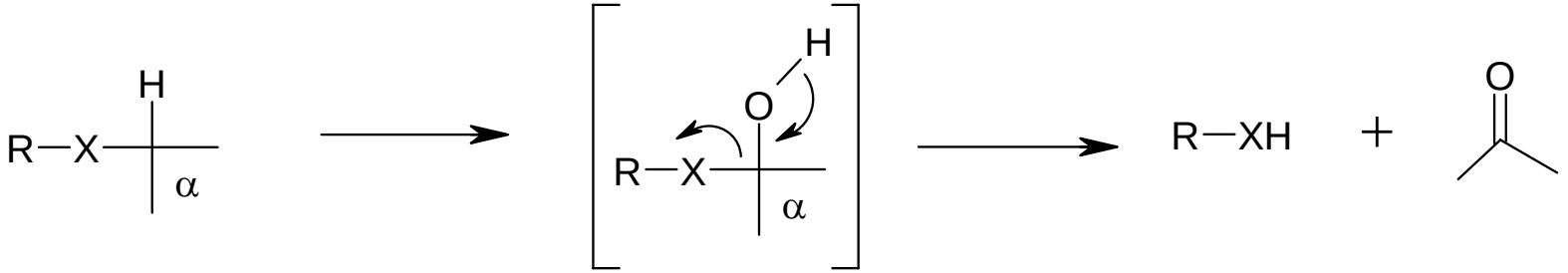




Important Note: ***Many if not all reactions by FMO are also catalyzed by CYP450***

Oxidations Involving Carbon-Heteroatom Systems (cont'd)

(II) carbon-heteroatom bond cleavage (N-, O- and S- dealkylations)

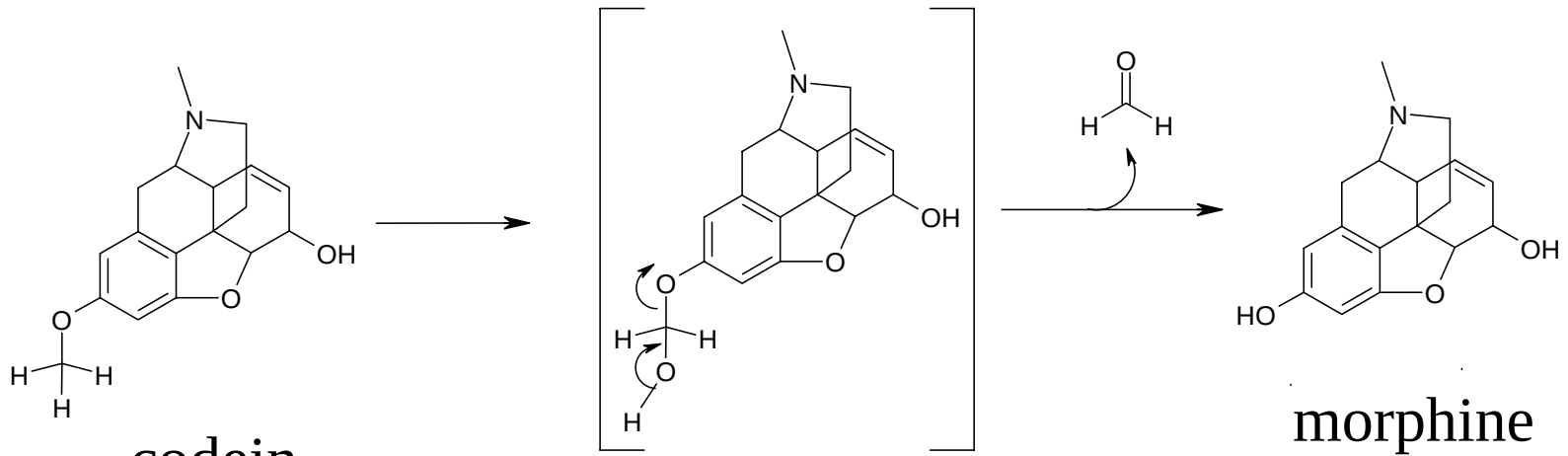


Where X = N, O, S

Usually unstable

N- and O- Dealkylations are quite common whereas S-dealkylation reactions are rare

Example: O-demethylation of Codein



codein

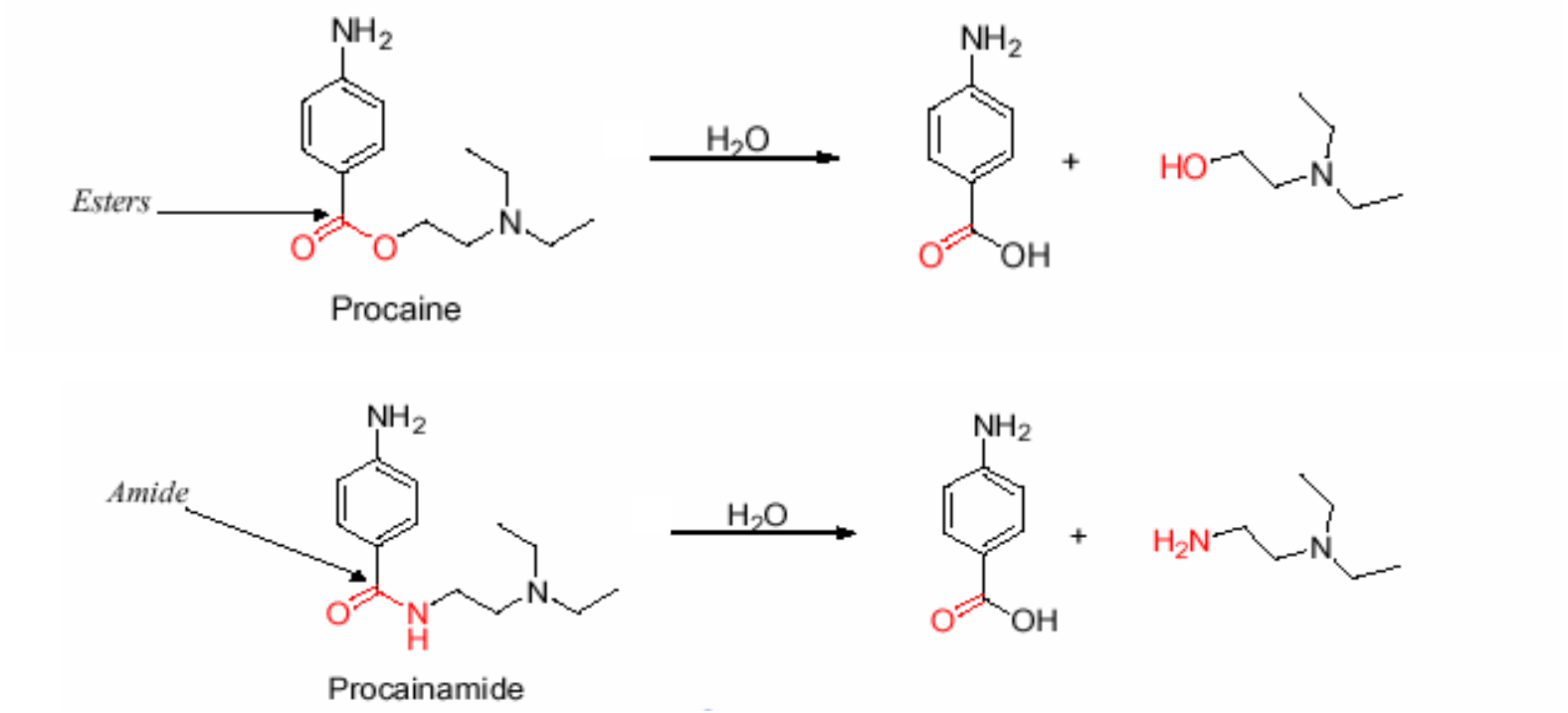
morphine

Hydrolytic Reactions

Primary enzymes are carboxylesterases, peptidase and epoxide hydrolase. Other enzymes are cholinesterases and paraoxonases.

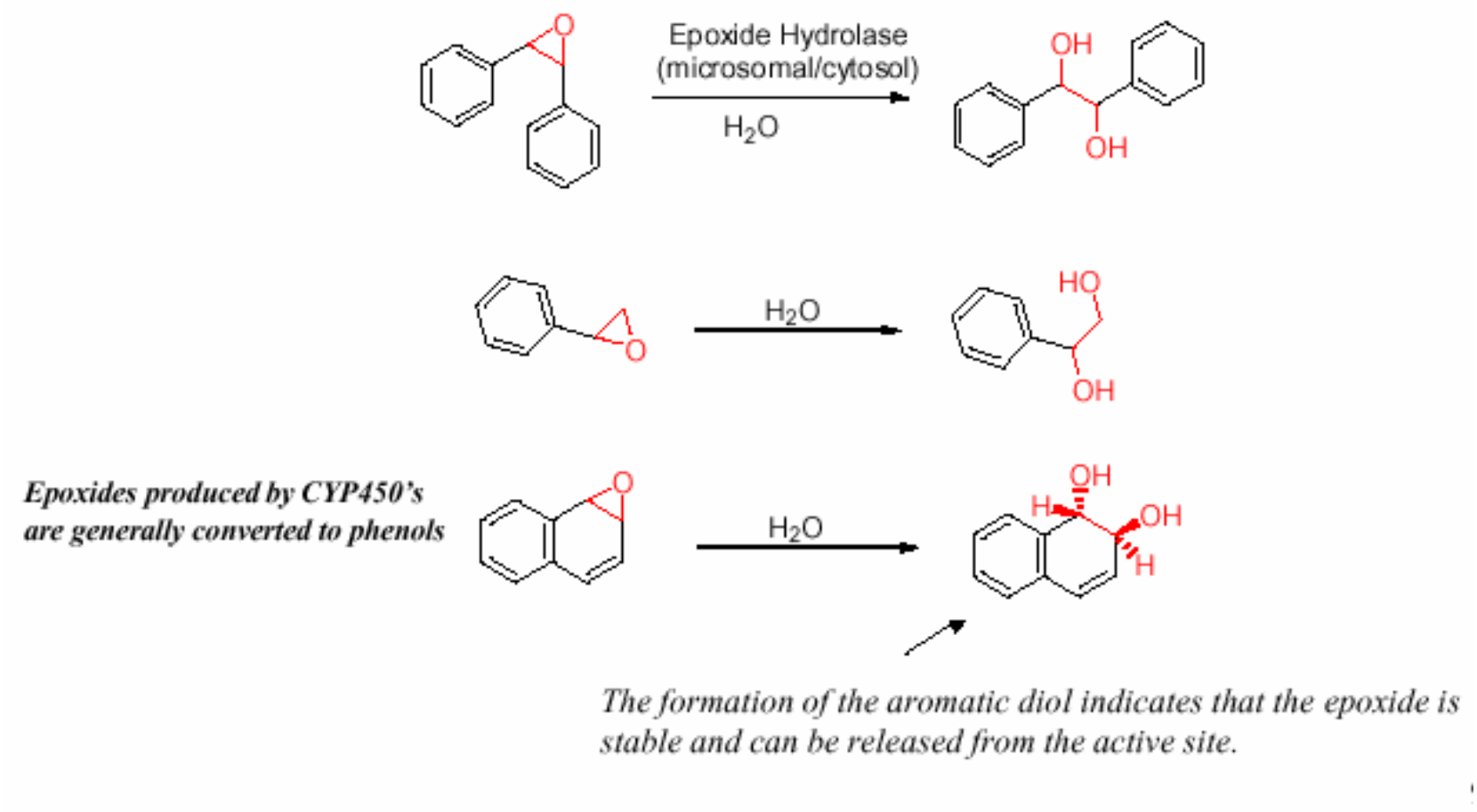
(I) Hydrolysis of esters and amides

- Hydrolysis involves cleavage of ester or amide bonds resulting in carboxylic acids. Generally, hydrolysis of an amide is slower than esters.
- Water is primarily responsible for the cleavage.



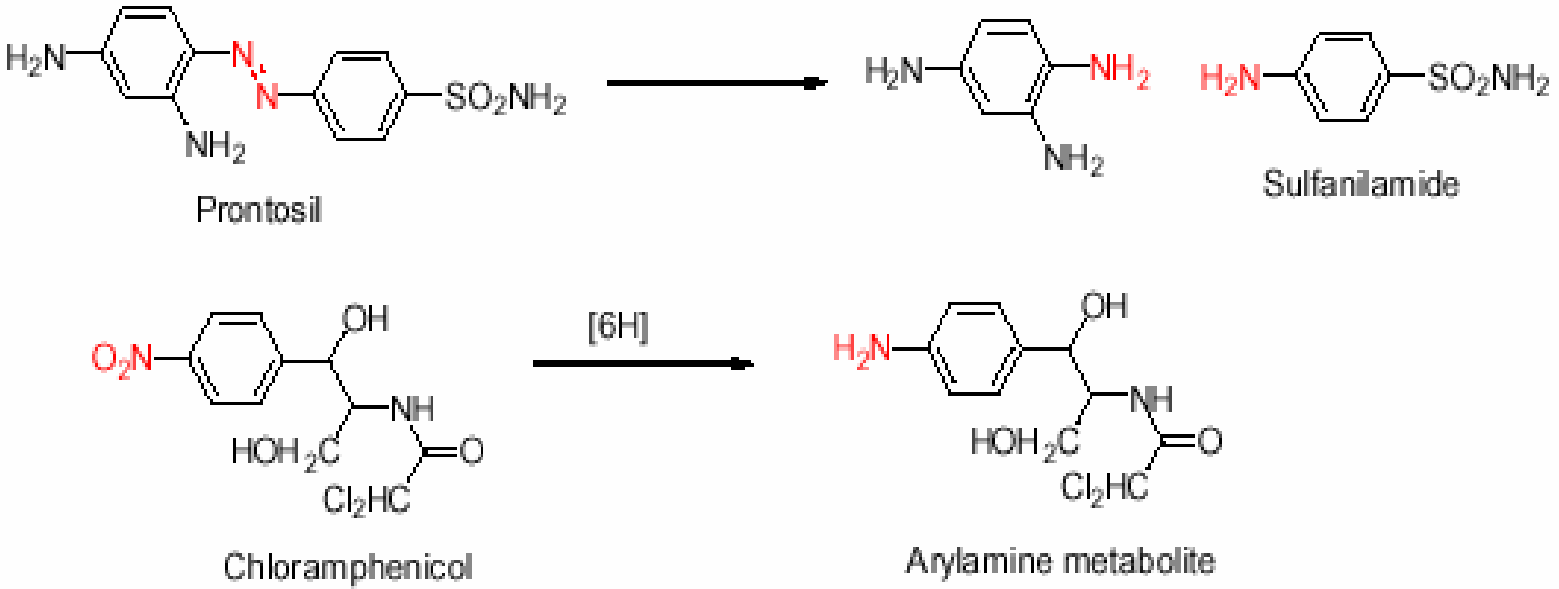
(II) Hydration of Epoxides

Hydrolytic cleavage of epoxides involves addition of water to alkene epoxides and arene oxide to form the corresponding diols

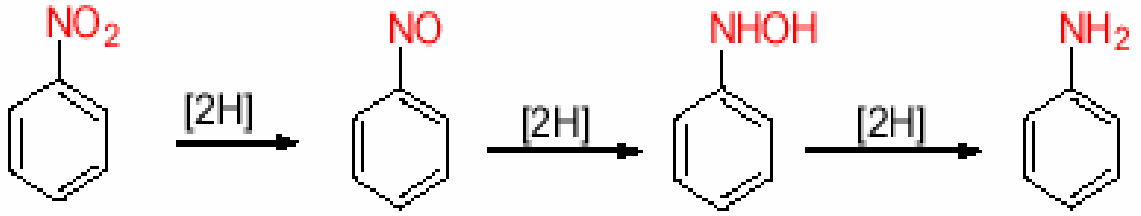


Reductive Reactions

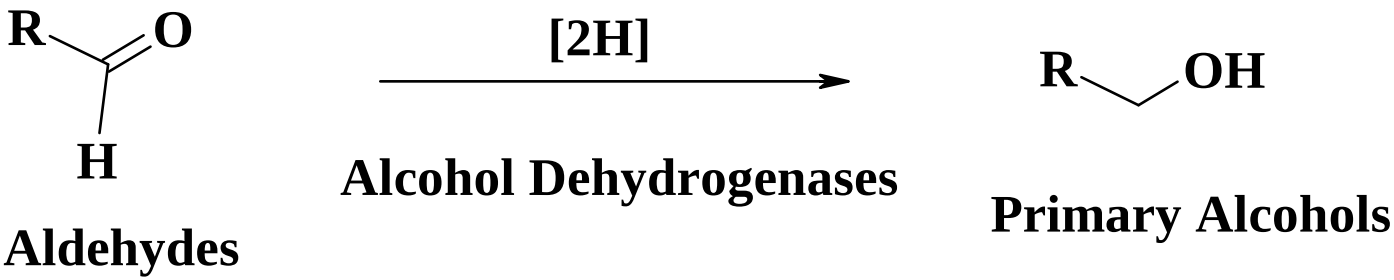
(I) Reduction of Azo and Nitro groups

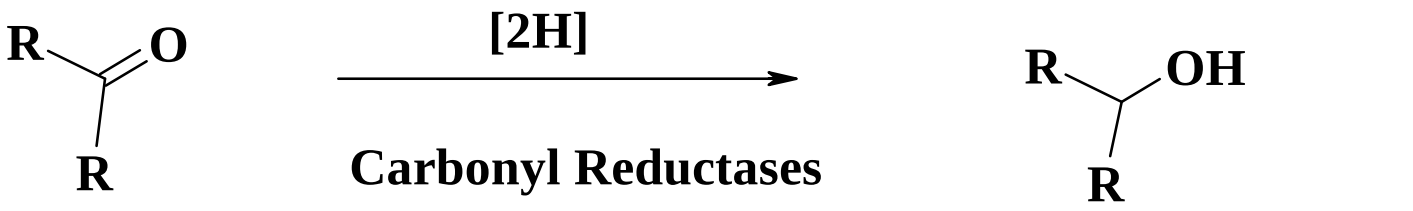


Sequential Reduction of Nitro group by Intestinal Flora



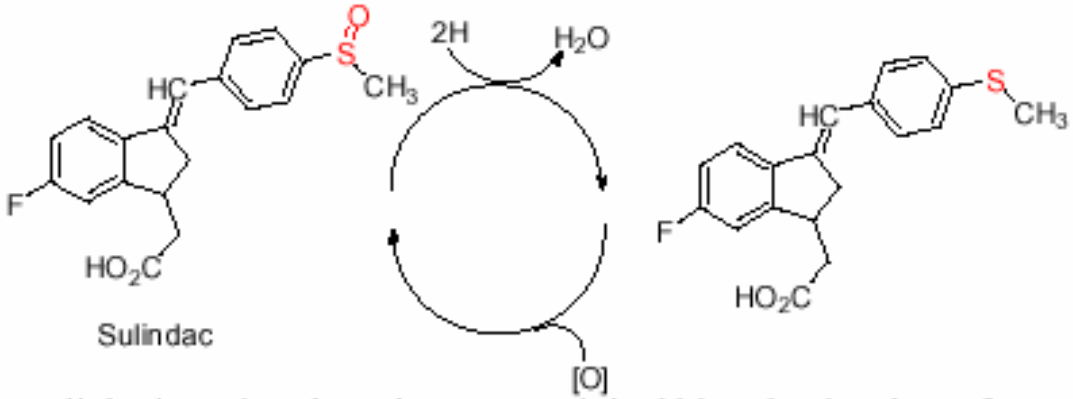
(II) Reduction of Carbonyl Groups



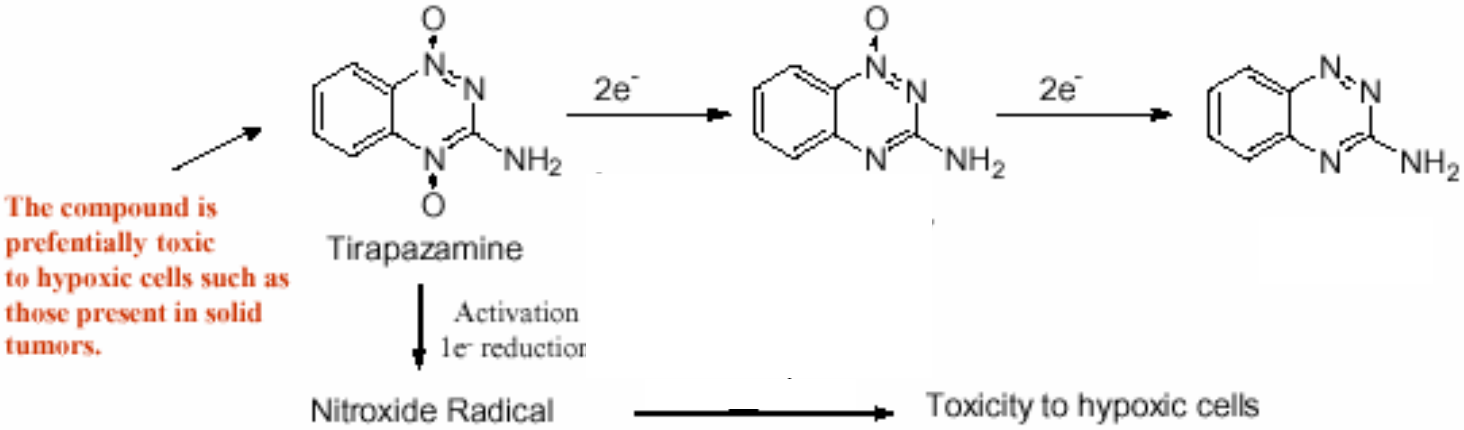


(III) Reduction of Sulfoxides and N-oxides

Sulfoxides are reduced to sulfides but can be reoxidized to sulfoxides.

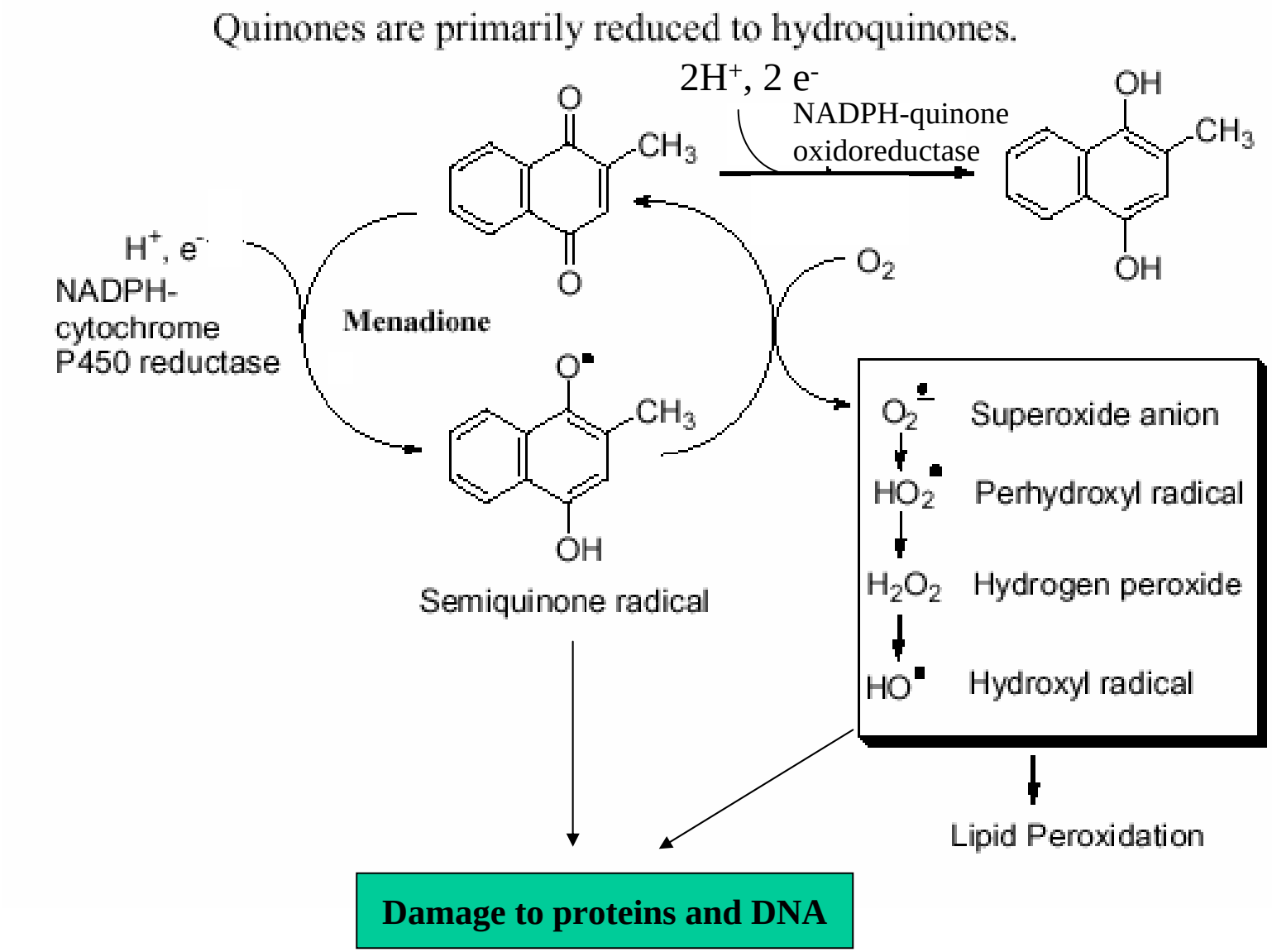


N-oxides of aromatic or aliphatic amines have been as exploited bioreductive drugs for treatment of cancers and infectious diseases

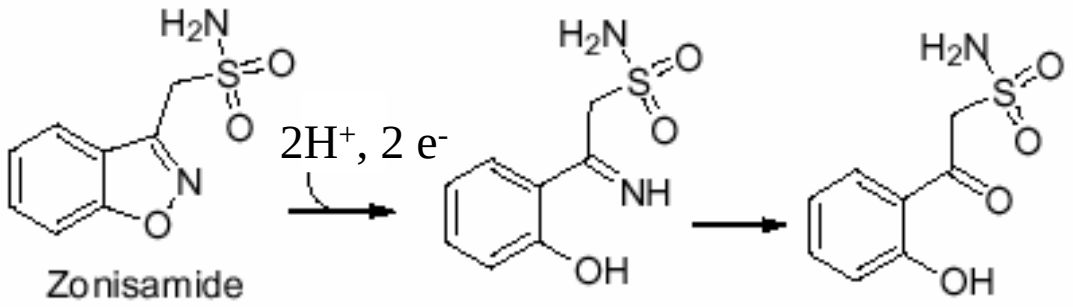


The compound is prefentially toxic to hypoxic cells such as those present in solid tumors.

(IV) Reduction of Quinones

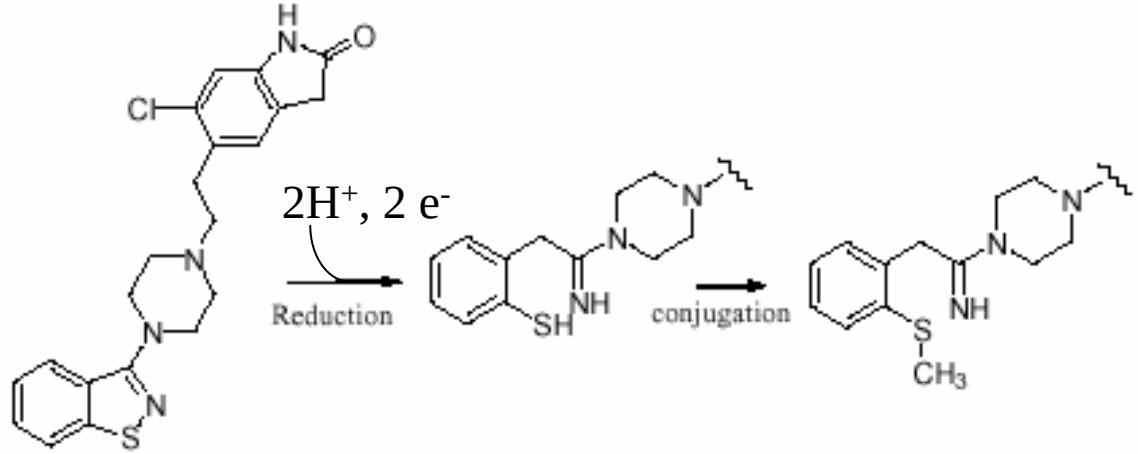


(V) Reductive Cleavage of Heteroaromatic Compounds



Zonisamide

Isoxazoles and isothiazoles are reduced to ring opened metabolites



Ziprasidone

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(VI) Others: Disulfide reduction and reductive dehalogenation

Phase II (Conjugation)

- **Sugar Conjugation (Glucuronidation + others)**
- **Sulfation**
- **Methylation**
- **Acetylation**
- **Amino Acid Conjugation**
- **Glutathione Conjugation**

Conjugation Reactions

Sugar Conjugation

Conj. with Glucuronic acid
(Glucuronidation)

Most important & common sugar conj. in most species

O-Glucuronides (***R-OH, Ar-OH, R-COOH***)

N-Glucuronides (***Ar-NH_2, Ar-NH-R, -$CONH_2$, SO_2NH_2, R3 –N***)

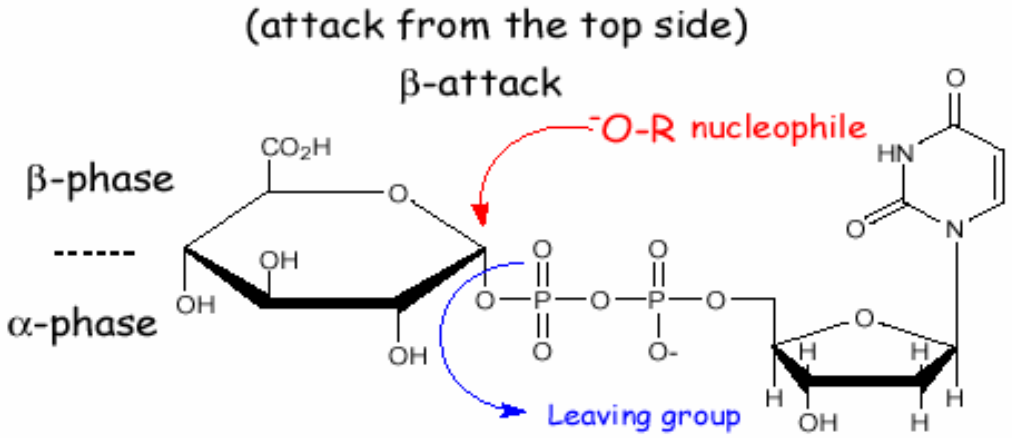
S-Glucuronides (***-SH***)

C-Glucuronides (**C-H, direct attachment to carbon**)

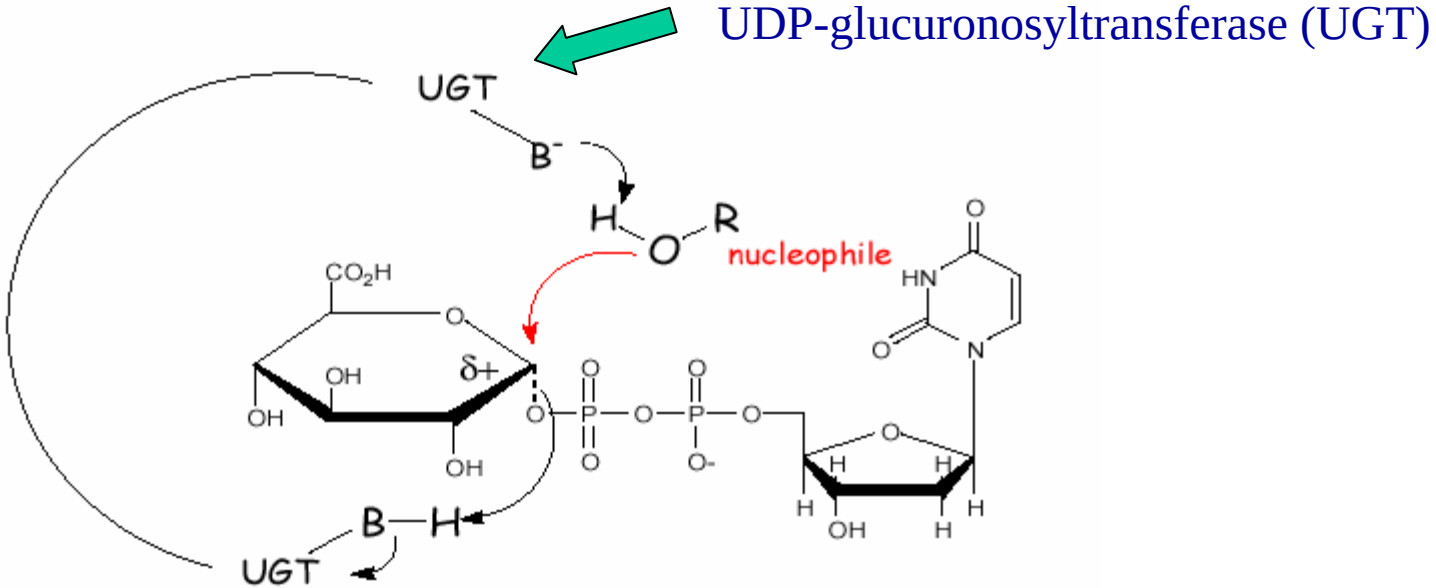
Conj. with glucose, ribose and xylose

Less common conjugation

Conjugation with Glucuronic acid (Glucuronidation)



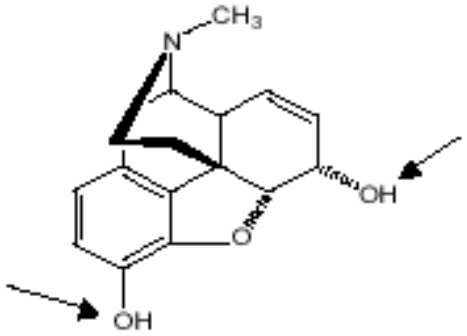
Cofactor(coenzyme): Uridine diphospahte-glucuronic acid (UDPGA)



O-Glucuronides

Morphine

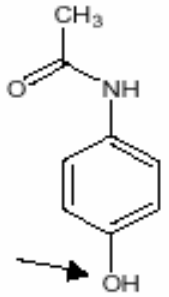
Has the potential to undergo glucuronidation at 2 sites



PHENOLS:

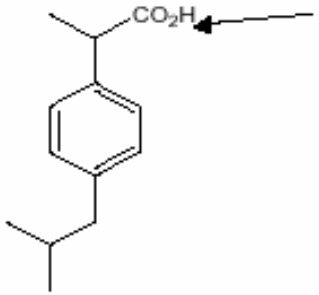
Acetaminophen

Several other examples of phenols are known.



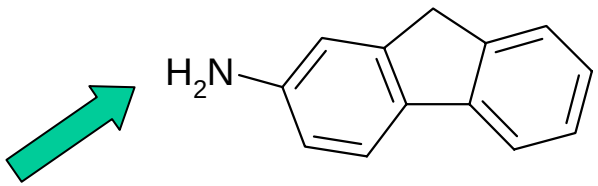
Carboxylic Acids: Ibuprofen

Several carboxylic acids undergo glucuronidation



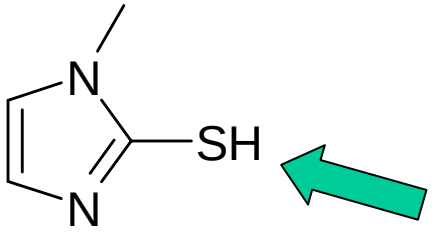
N-Glucuronide

2-Aminofluorene



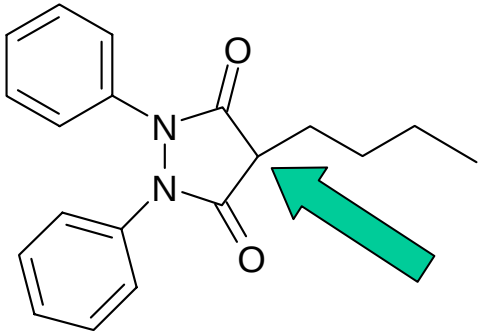
S- Glucuronide

Methimazole



C- Glucuronide

Phenylbutazone

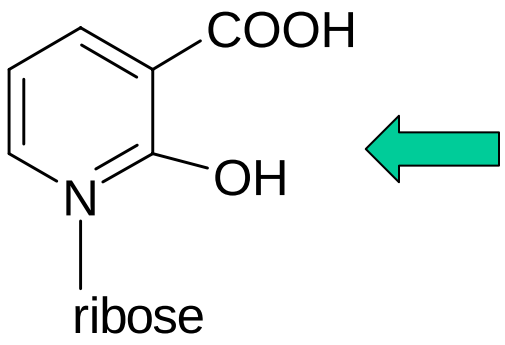


Conjugation with other sugars

• In most species conjugation with glucuronic acid is by far the most important sugar conjugation.

• In insects conjugation with glucose is more prevalent and UDP-glucose is used instead of UDPGA and glucosides are formed (O-, N- and S-glucosides). Such rxns are also of importance in plants and have been found in mammals to a limited extent.

• In certain circumstances UDP-xylose or UDP-ribose can be formed giving the corresponding xyloside or riboside.



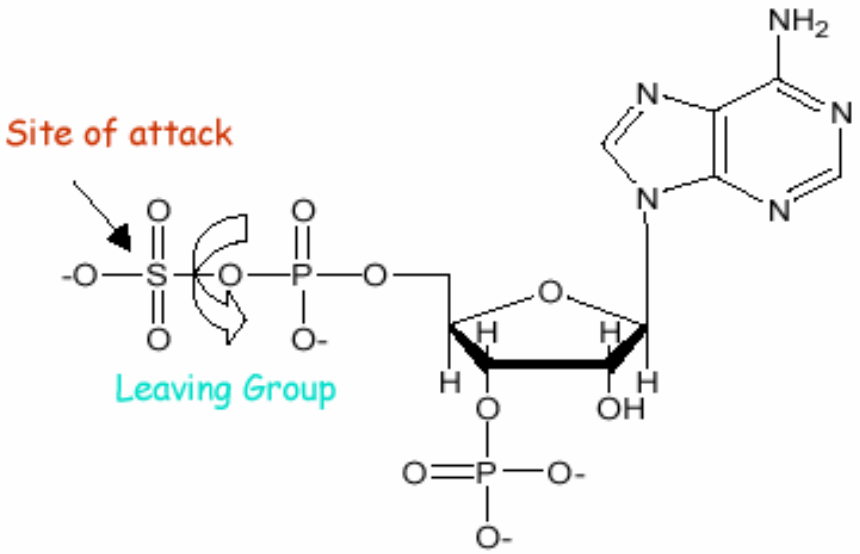
N-ribosylation of 2-hydroxynicotinic acid

Sulfation

• Sulfation is a major conjugation pathway for phenols but can also occur for alcohols, arylamines, N-hydroxy compounds and, to a lesser extent, thiols.

• As with sugar conjugation an active donor is required: 3'-phosphoadenosine-5'-phosphosulfate (PAPS)

• Sulfation occurs by interaction of the drug with PAPS in the presence of the cytosolic enzyme sulfotransferase



Cofactor (coenzyme): 3'-phosphoadenosine-5'-phosphosulfate (PAPS)

• Sulfate conjugation occurs less frequently than does glucuronidation presumably because of:

➢PAPS cellular concentration is considerably lower (75 mM) than UDPGA (350 mM). Hence the capacity of sulfation is low.

➢Fewer number of functional groups that undergo sulfate conjugation

• Functional groups that can be sulfated are

Phenols: **Ar-OH**

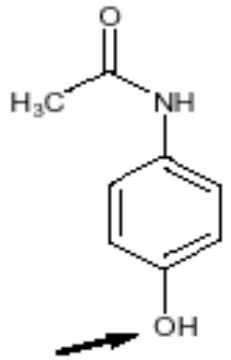
Alcohols: **R-OH**

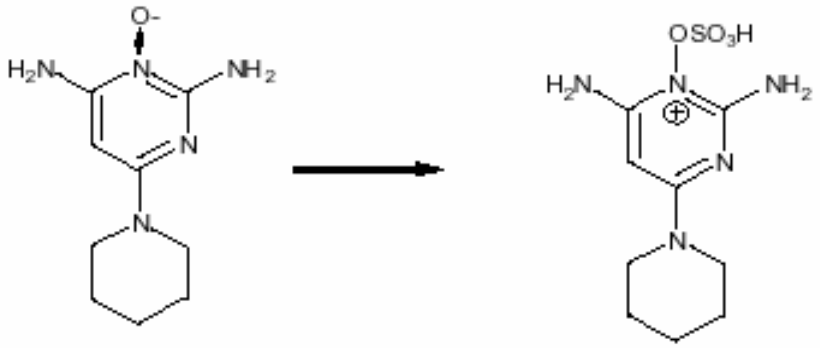
Arylamines: **Ar-NH_2**

N-hydroxy compounds: **R-NH-OH**

Examples of Sulfation

Acetaminophen





Minoxidil
produces hair growth

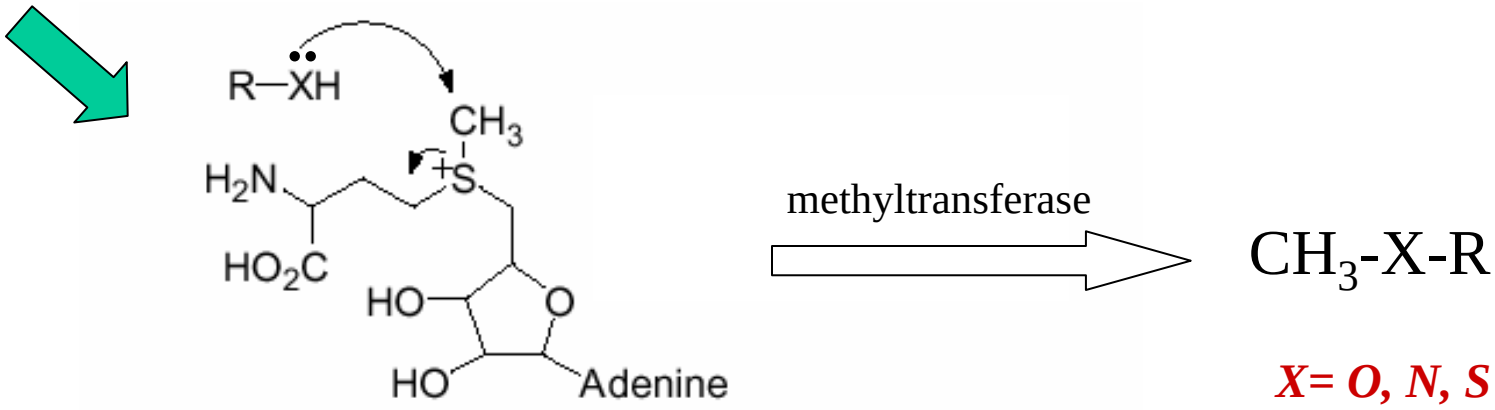
Active form responsible for its effects

Biochem. Pharmacol. 45, 271-279 (1993)

Methylation

- Methylation is a relatively minor conjugative pathway in drug metabolism
- Methylation rxns are mainly involved with endogenous compound such as melatonin, histamine, serotinin, dopamine…etc)
- Methylation differs from almost other conjugation rxns (excluding acetylation) in that it reduces the polarity and hydrophilicity of the substrates.
- The purpose of methylation is to deactivate the biological activity

Mechanism



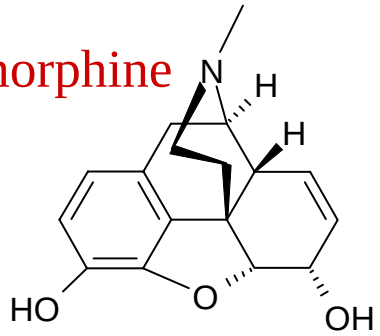
Coenzyme: S-adenosylmethionine (SAM)

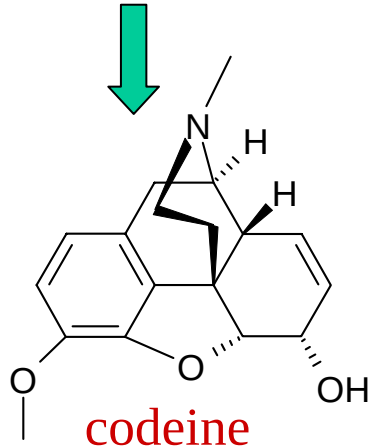
Functional groups that can be methylated

- Phenols and catachols → ***O-Methyl***
- Aliphatic and aromatic amines, N-heterocylics → ***N-Methyl***
- Sulfhydryl-containing compounds → ***S-Methyl***

O-Methylation

morphine

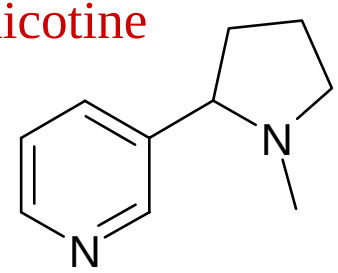


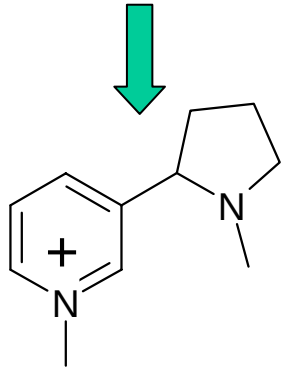


codeine

N-Methylation

nicotine

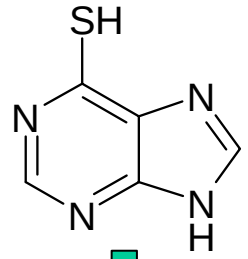


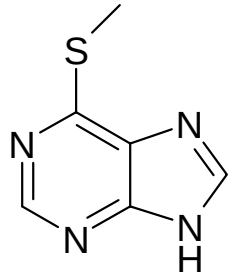


N-methylnicotinium ion

S-Methylation

6-mercaptopurine





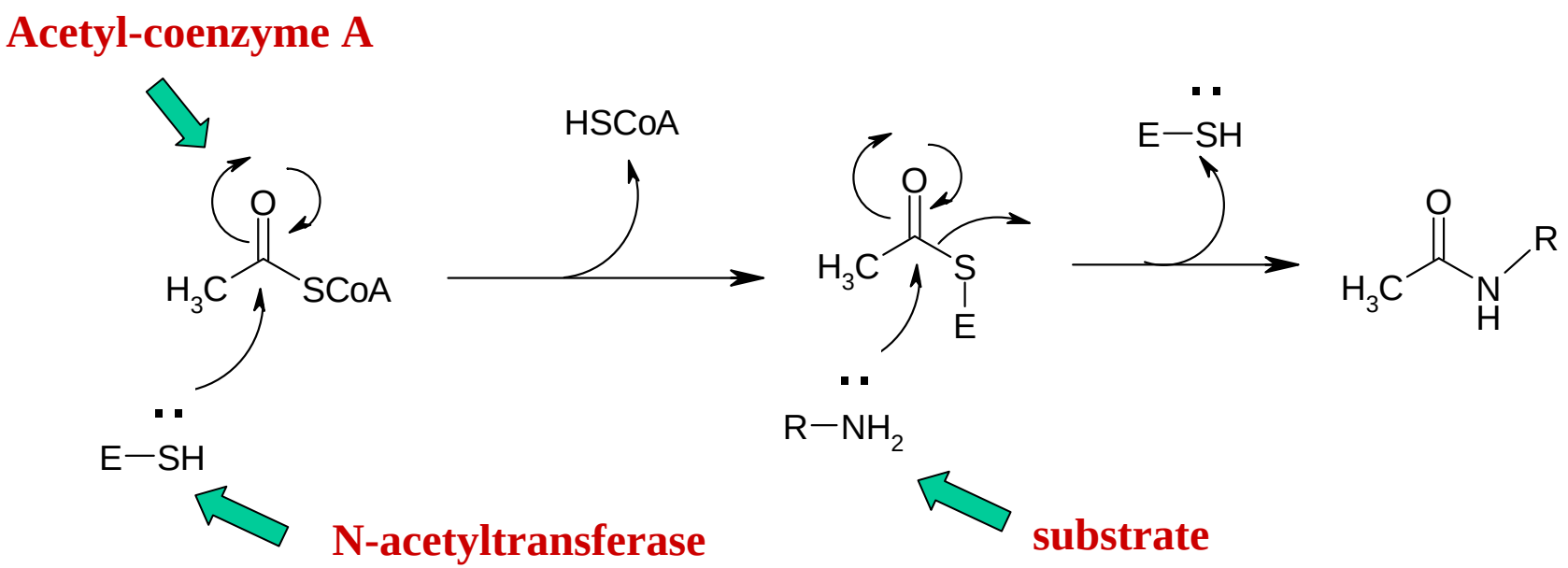
6-methylmercaptopurine

Acetylation

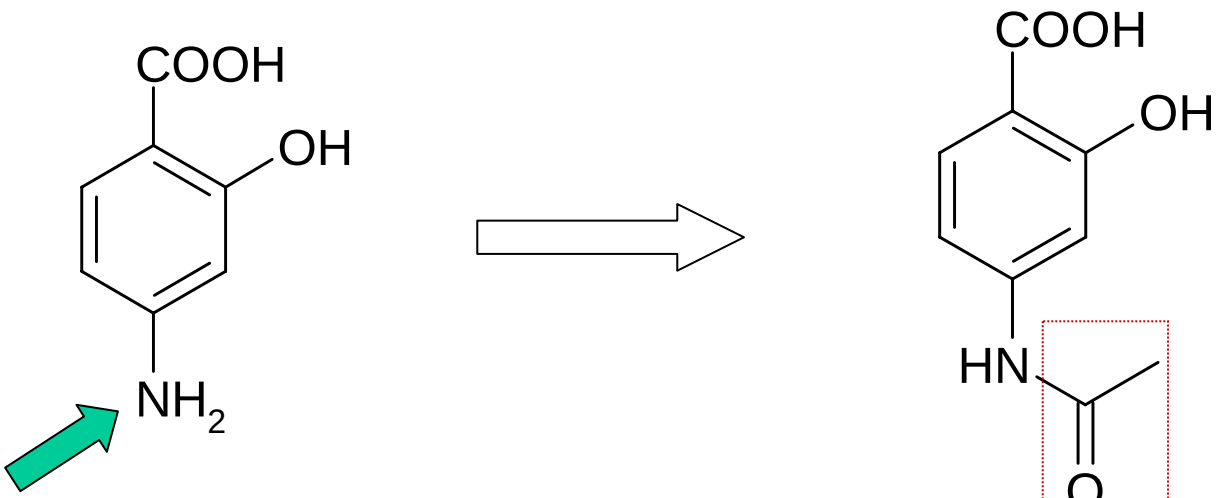
• Acetylation is an important rout of metabolism for xenobiotics containing an aromatic amine (R-NH_2) or a hydrazine group (R-NH-NH_2), which are converted to aromatic amides (R-NH-$COCH_3$) and hydrazides (R-NH-NH-$COCH_3$), respectively.

• Primary aliphatic amines are rarely substrates for N-acetylation, a notable exception being cystein conjugates which are converted to mercapturic acids by N-acetylation

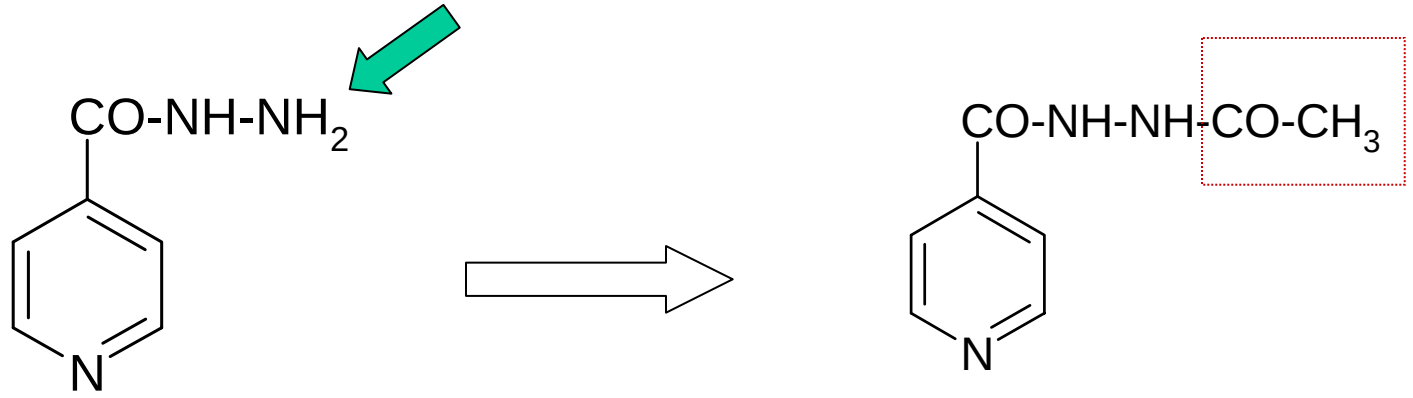
• Like methylation, N-acetylation masks an amine with a nonionizable group, so that many N-acetylated metabolites are less water soluble than the parent compound.



Examples of N-acetylation



aminosalicylic acid



isoniazid

Conjugation with Amino Acids

• Carboxylic acids particularly aromatic acids and arylacetic acids are conjugated with polar endogenous amino acids

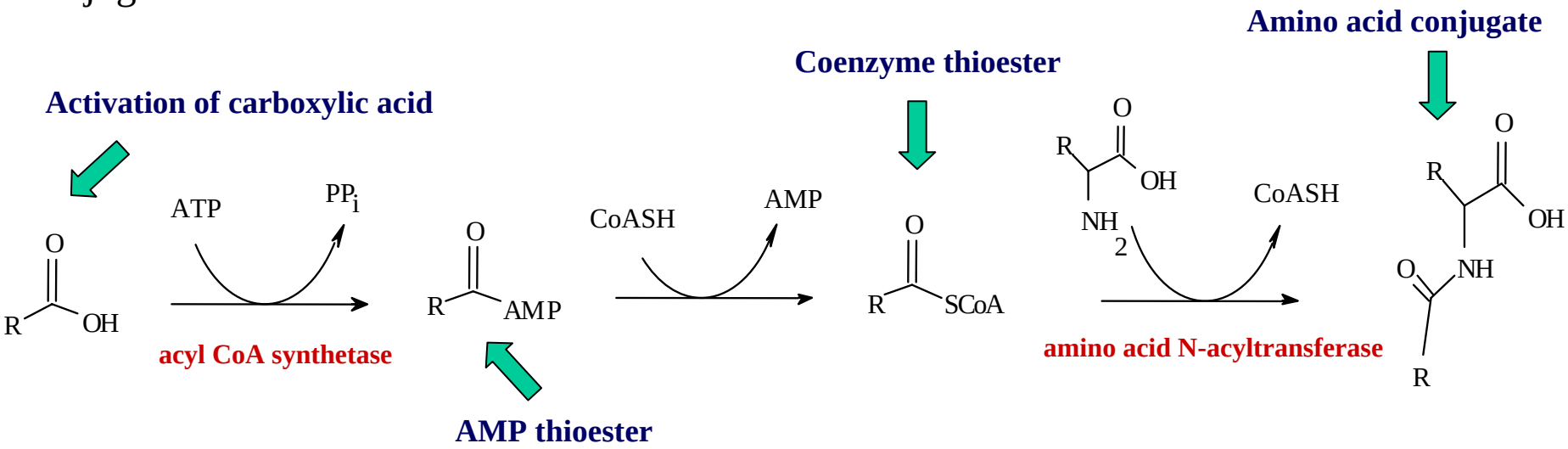
• The quantity of amino acid conjugation is minute because of the limited availability of amino acids in the body and competition with glucuronodation for carboxylic acid substrates

• Amino acids conjugation of carboxylic acids leads to amide bond formation

• Glycine conjugates are the most common amino acid conjugates in animals

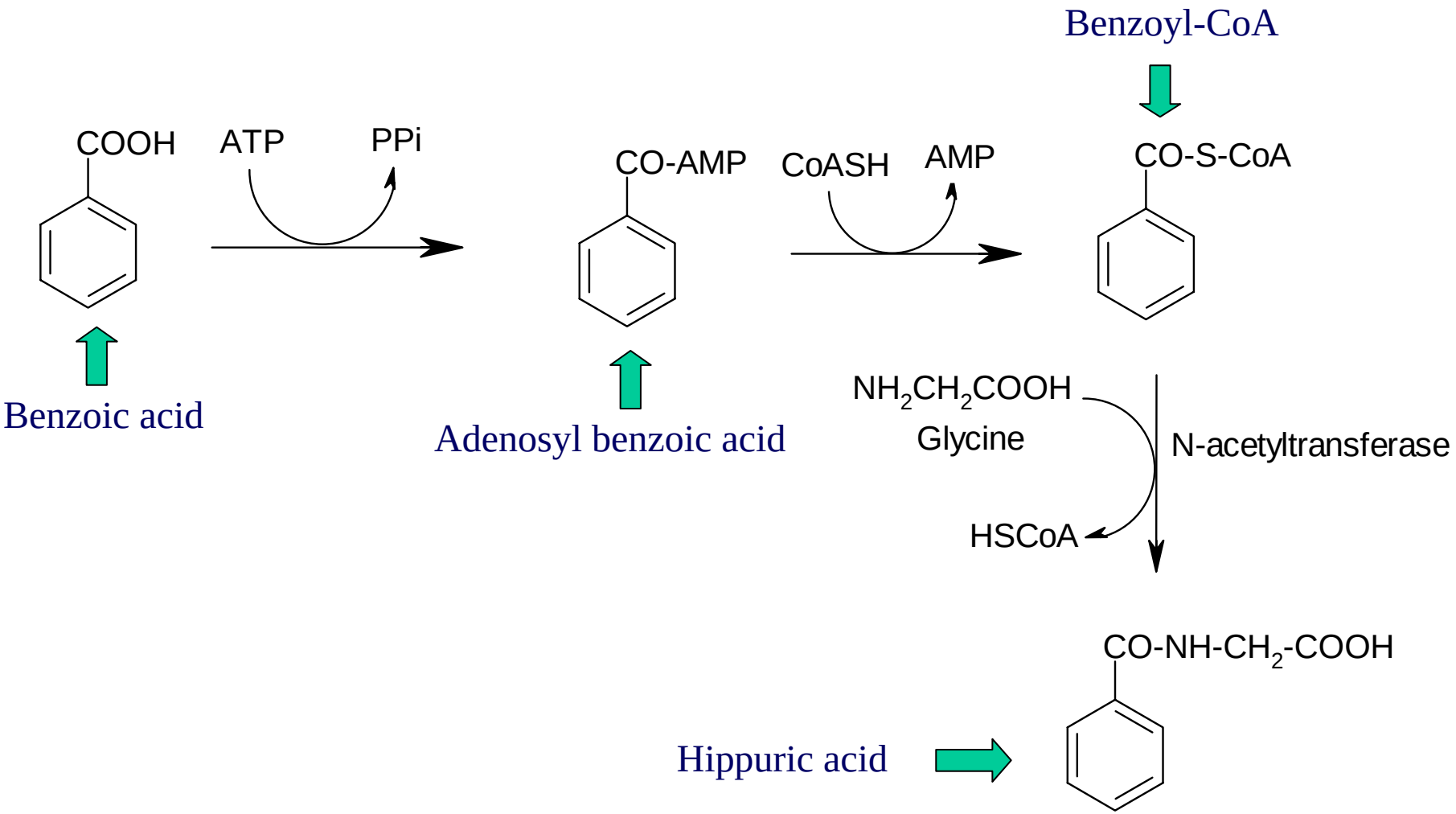
• Conjugation with L-glutamine is most common in humans and other primates, it does not occur to any significant extent in non-primates.

• Taurine, arginine, asparagine, histidine, lysine, glutamate, aspartate, alanine and serine conjugates also have been found in mammals.



Example of Amino Acid Conjugation

glycine conjugation of benzoic Acid



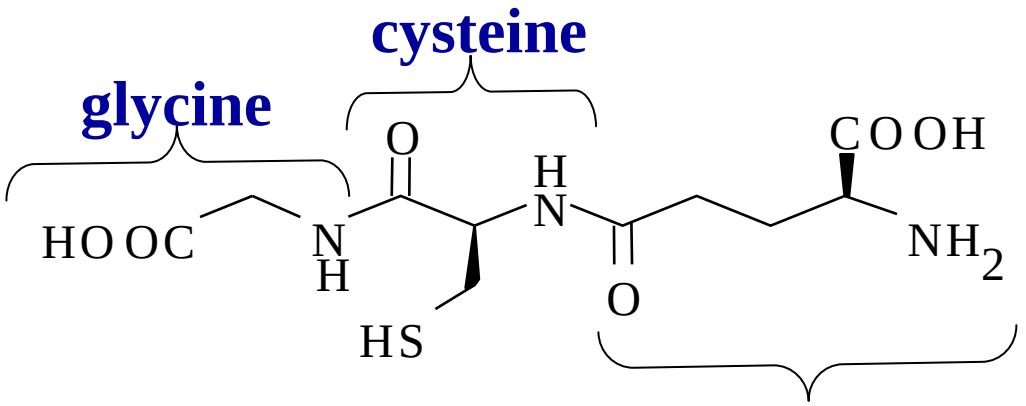
Glutathione Conjugation

• The tripeptide glutathione (**GSH**) is found in virtually all mammalian tissues and it contains a potent nucleophilic thiol group

• Glutathione function appears to be as a scavenger of harmful electrophilic compounds ingested or produced by metabolism

• Xenobiotics that are conjugated with glutathione are either highly electrophilic as such or are first metabolized to an electrophilic product prior to conjugation

• Drug toxicity can result from the reaction of cellular nucleophiles with electrophilic metabolites if glutathione does not first intercept these reactive compounds



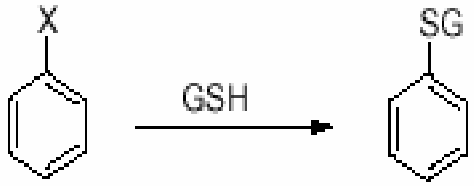
Glutathione (GSH)

Categories of Substrates Undergoing GSH Conjugation

At Saturated Carbon atoms

X = leaving gp
Cl, Br, I, sulfate etc..

At Carbon atoms of Aromatic or Heteroaromatic Rings

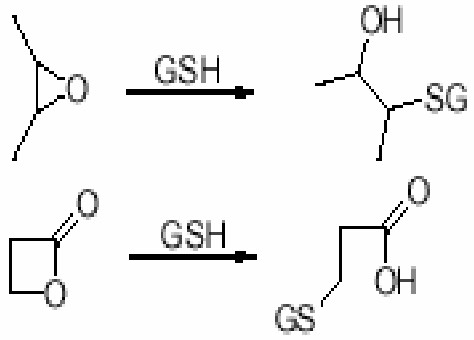


X = NO_2, Cl, Br etc...

α, β - Unsaturated Systems (Michael Acceptors)

Z = COR, CO_2R, CN, NO_2

At Carbons of Strained Rings

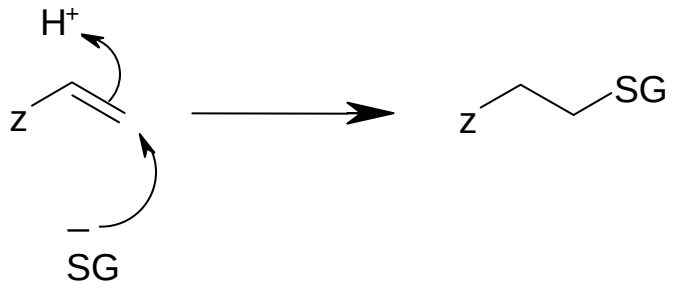


Other Activated Double Bonds

Isocyanates, Thioisocyanates

R–N=C=O R–N=C=S

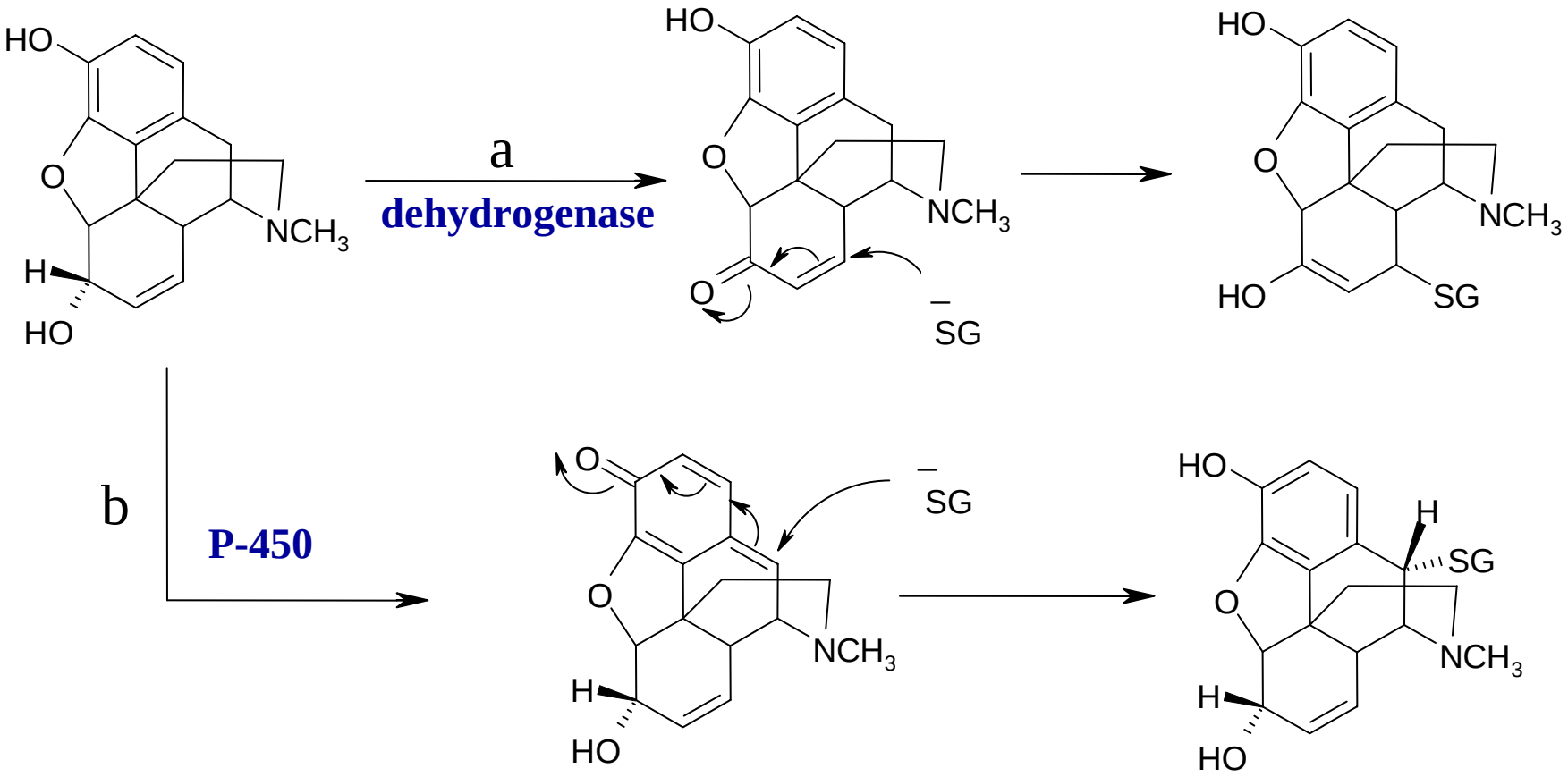
An Example of glutathione conjugation: Michael addition



Z = RCO, RCO_2, CN, NO_2

morphine

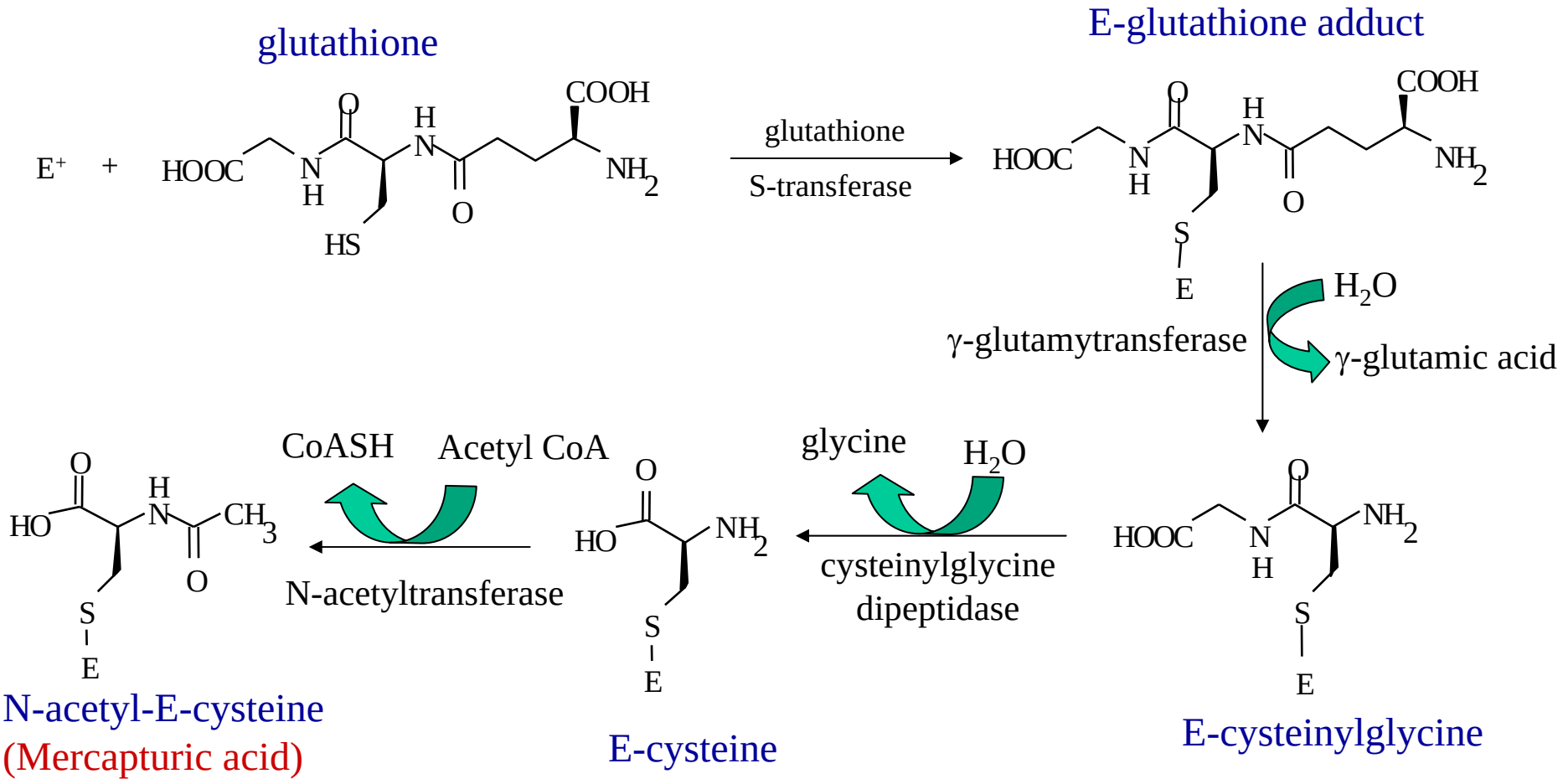
morphinone



Metabolism of GSH conjugates to mercapturic acid conjugates (Phase III metabolism)

• GSH conjugates are rarely excreted in urine due to their high MW, when they are eliminated, it is in the bile

• Most typically, however, they are not excreted and instead they are metabolized further and are excreted ultimately as mercapturic acid conjugates

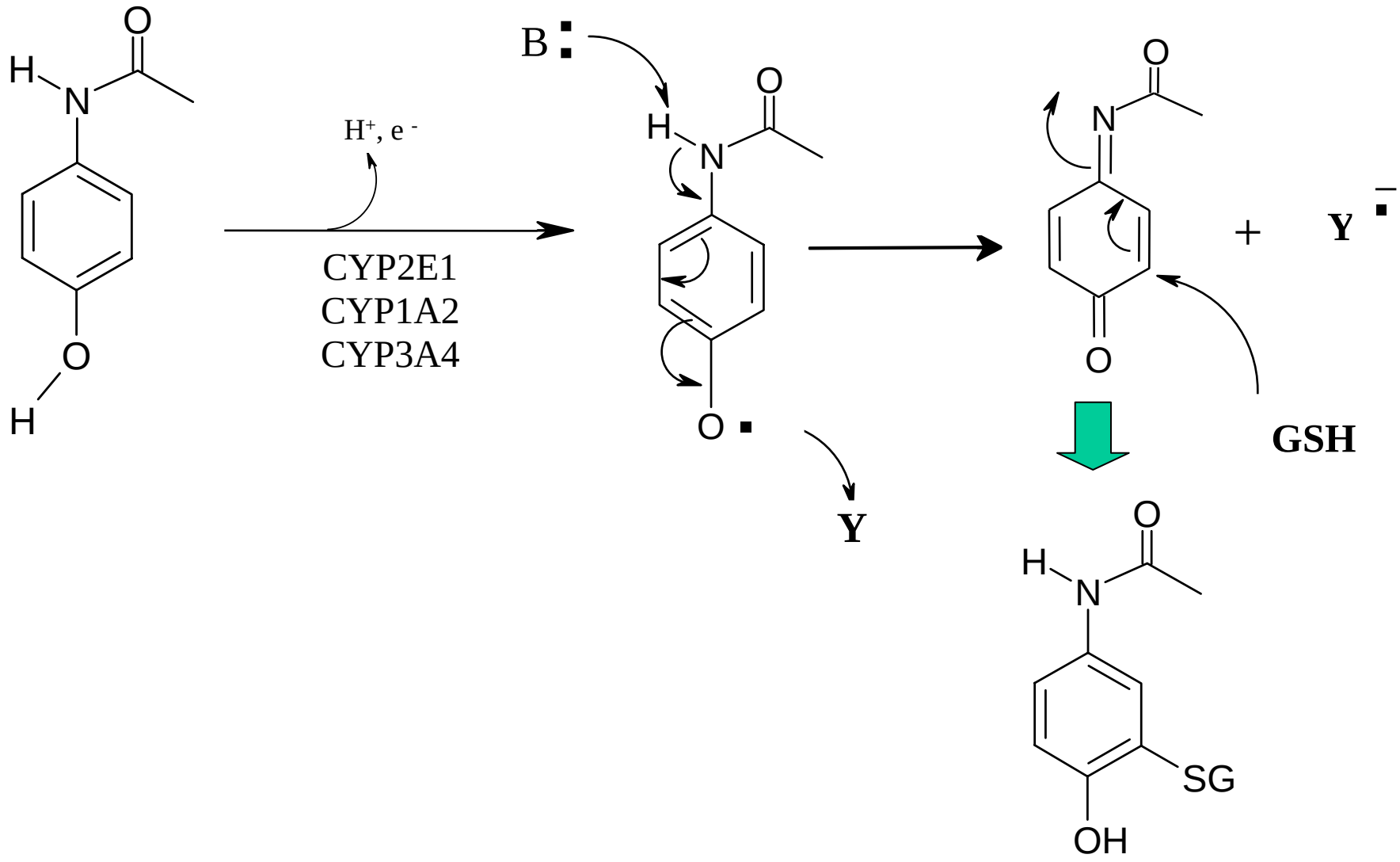


Bioactivation of Actaminophen

Hepatotoxic metabolite

Acetaminophen

N-acetylbenzoquinoneimine



Summary

• Biotransformation is the process by which a drug is chemically altered by the body.

• The liver is the principal, but not the only, site of drug biotransformation. The liver has enzymes that facilitate chemical reactions such as oxidation, reduction, and hydrolysis of drugs (phase I). It has other enzymes that attach substances to the drug, producing reactions called conjugations (phase II).

•The products of biotransformation (metabolism) are called metabolites. Metabolites may be inactive or they may have similar or different degrees of therapeutic activity or toxicity than the original drug.

• During the process of biotransformation, the molecular structure of a drug is changed from one that is absorbed (lipophilic) to one that can be readily eliminated from the body (hydrophilic or water soluble).

•If lipophilic drugs are not metabolized, they will remain in the body for longer than intended, and their cumulative biological effects will eventually cause harm. Thus, the formation of water-soluble metabolites not only enhances drug elimination but also leads to compounds that are generally pharmacologically inactive and relatively nontoxic.

SUGGESTED REFERENCES

- *Biochemistry of reactions by Bernard Testa*

- *Biotransformation of Xenobiotics - Andrew Parkinson -in Casarett and Doull's Toxicology, 5th edition.*

- *The organic Chemistry of drug design and Drug Action- Richard Silverman*

- *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical chemistry- Edited by J. N. Delgado and W. A. Remers.*