Drug Metabolism 2

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20.201

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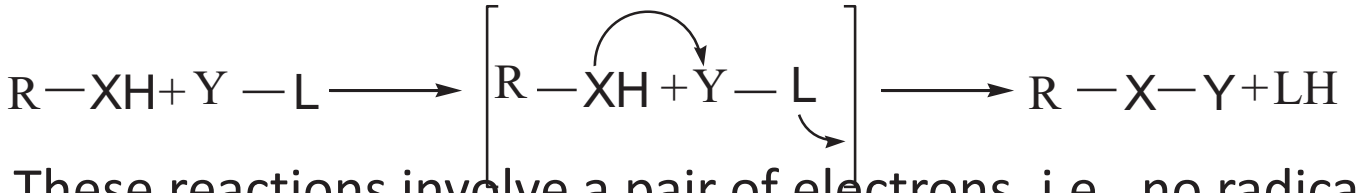
Conjugation Reactions

- “Phase II” biotransformations attach a hydrophilic moiety onto drug/metabolite. The resulting product is too polar to re-cross membranes back into tissues and is readily excreted.
- These type of reactions most often abolish biological activity

Chemistry

- Unlike P450, Phase II reactions are concerted reactions that involve an electrophile (Y), a nucleophile (X) and a leaving group (L)



- These reactions involve a pair of electrons, i.e., no radical intermediate

- Electron donating groups enhance nucleophilicity of substrate-favors reaction whereas, electron withdrawing groups decrease nucleophilicity-hinders reaction

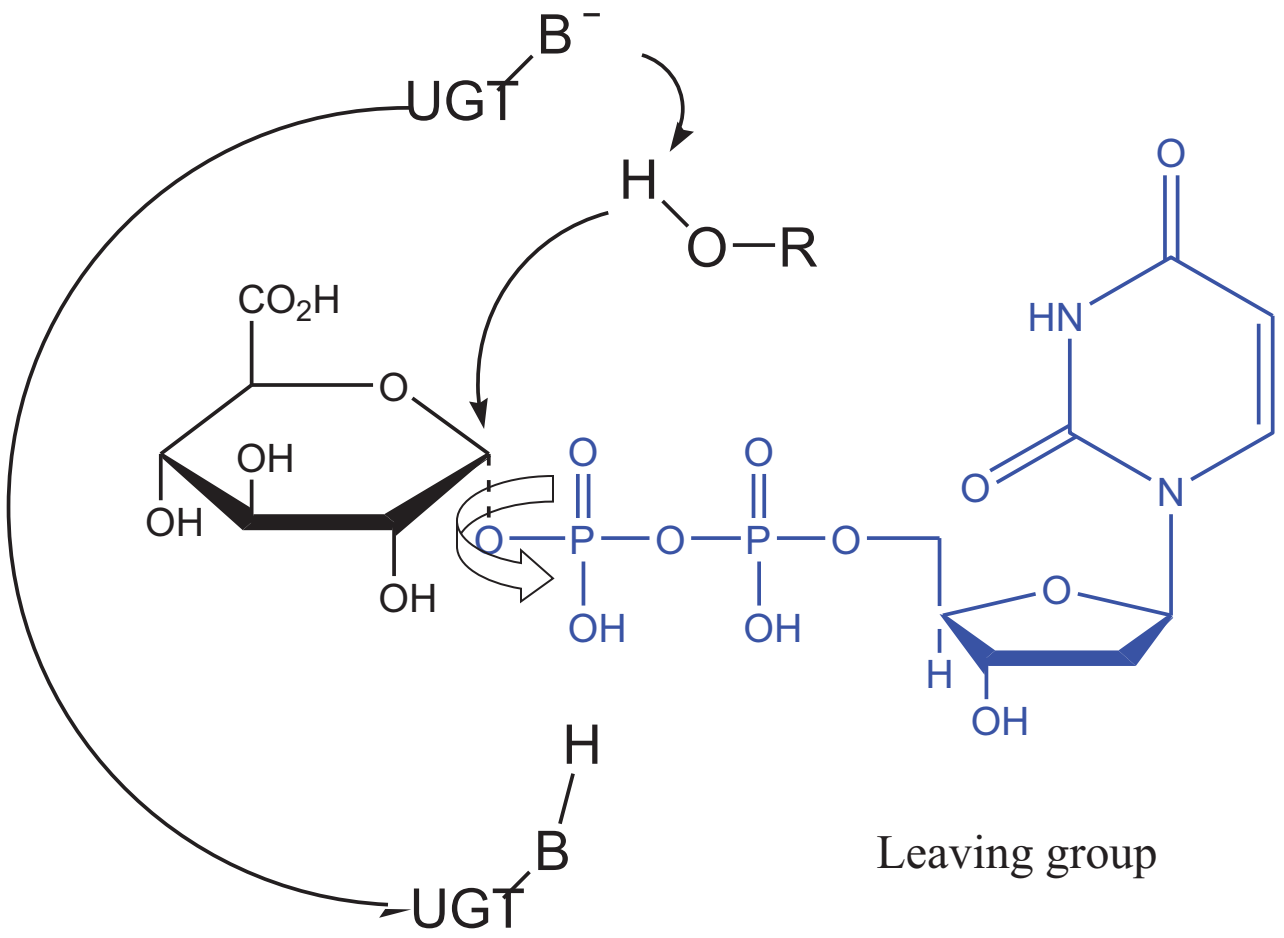
Types of Conjugation Reactions

- Glucuronidations (UGTs)
- Sulfations (STs)
- Glutathione transferases (GSH, GST)
- Methylations
- Acetylations
- Amino acid conjugations

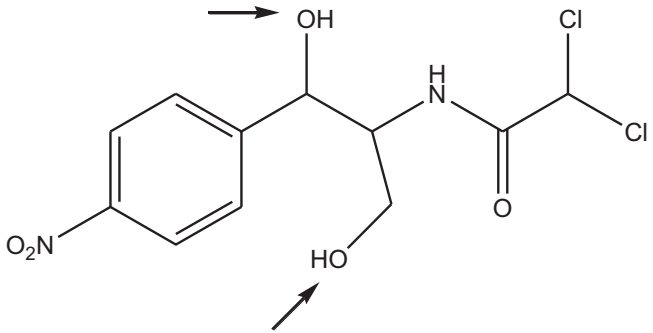
Uridine Diphosphoglucuronic Acid Transferases (UGT)

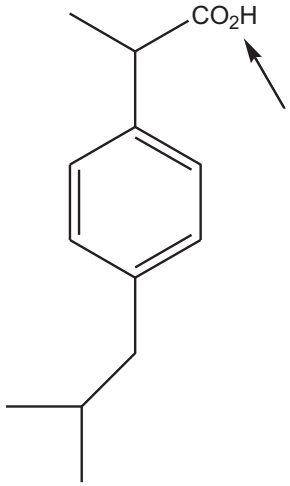
- UGTs are a family of enzymes, with two groups (UGT1A and UGT2B)
- Found in same tissues as P450, microsomal
- Uses uridine-5'-diphospho-α-D-glucuronic acid (UDPGA) as a cofactor
- One of the most common conjugative reactions
- Catalyze the glucuronidation of electron-rich nucleophilic heteroatoms/sites
  - Alcohols (ROH)
  - Phenols (phenolic hydroxy groups, Ar-OH)
  - Carboxylic acids (R-COOH)
  - Aromatic Amines (Ar-NH_2 or Ar-NHR)
  - Tertiary amines (R_3-N)
  - Sulfhydryl groups (R-SH)
  - Carbons w/ sufficient nucleophilicity

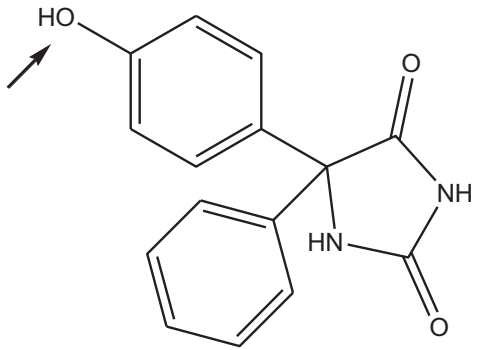
Glucuronidation Reaction

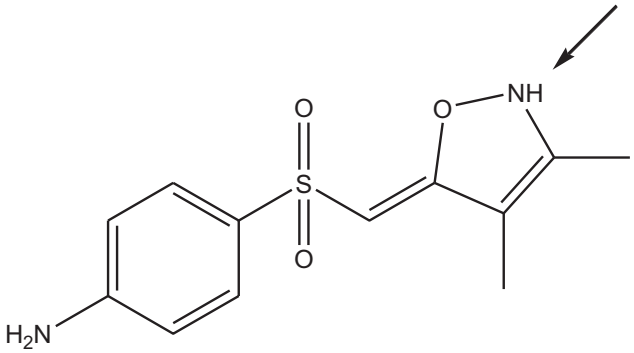


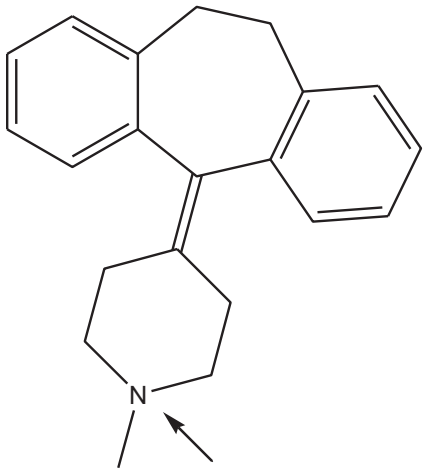
Xenobiotics that are Glucuronidated

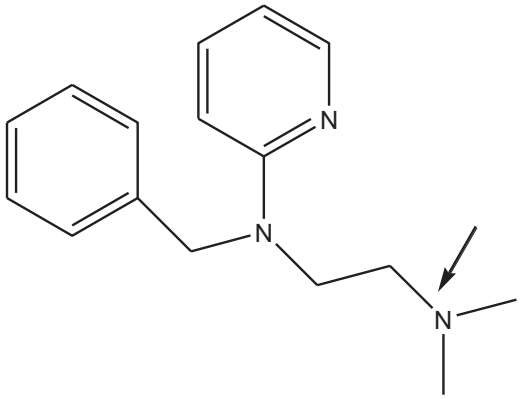








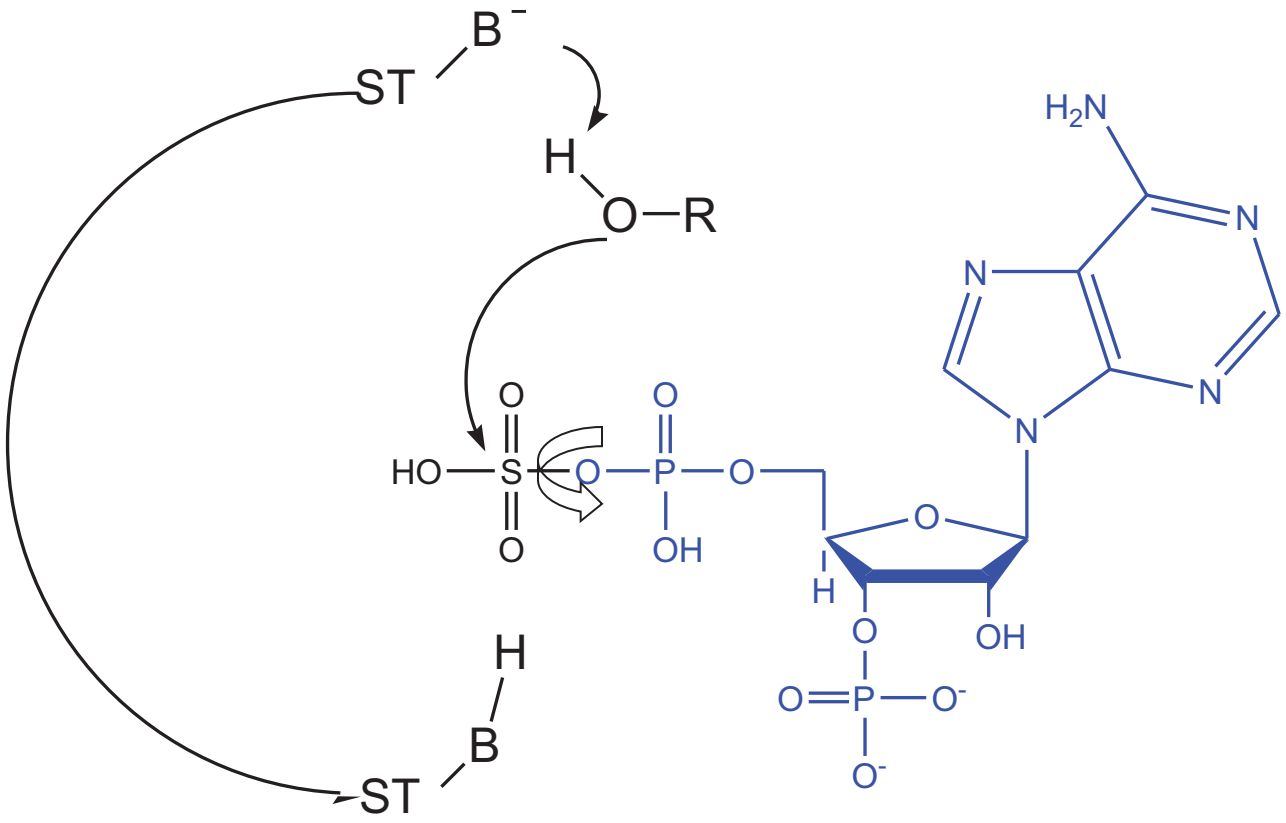




Sulfotransferases (ST)

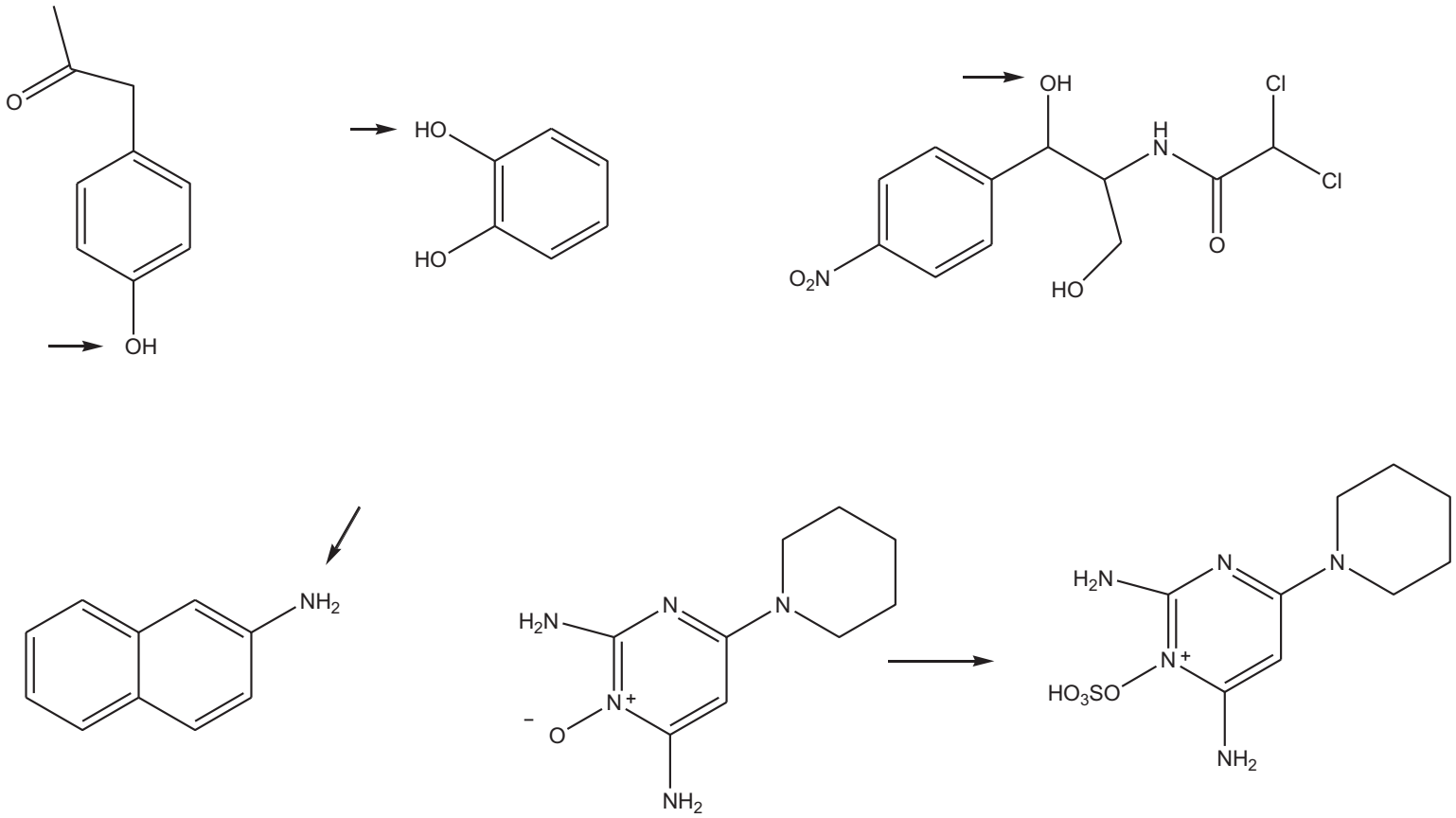
- STs are a family of enzymes with five members (SULT1-5, SULT1 and 2 are most important in the metabolism of drugs
- Found in liver, kidney, intestine, in cytosol
- Catalyze the sulfation of
  - Alcohols (R-OH)
  - Phenols (main group of substrates, Ar-OH)
  - Arylamines (Ar-NH_2)
  - N-hydroxy compounds (R-NH-OH)
- Cofactor (3'-phosphoadenosine-5'-phosphosulfate, PAPS) is in limited supply, drug can overwhelm the system
- Low activity in some individuals thought to be the cause of wine induced headache

Sulfation Reaction



Leaving group

Xenobiotics that are Sulfated



Minoxidil
Produces hair growth

Active metabolite responsible for its effects

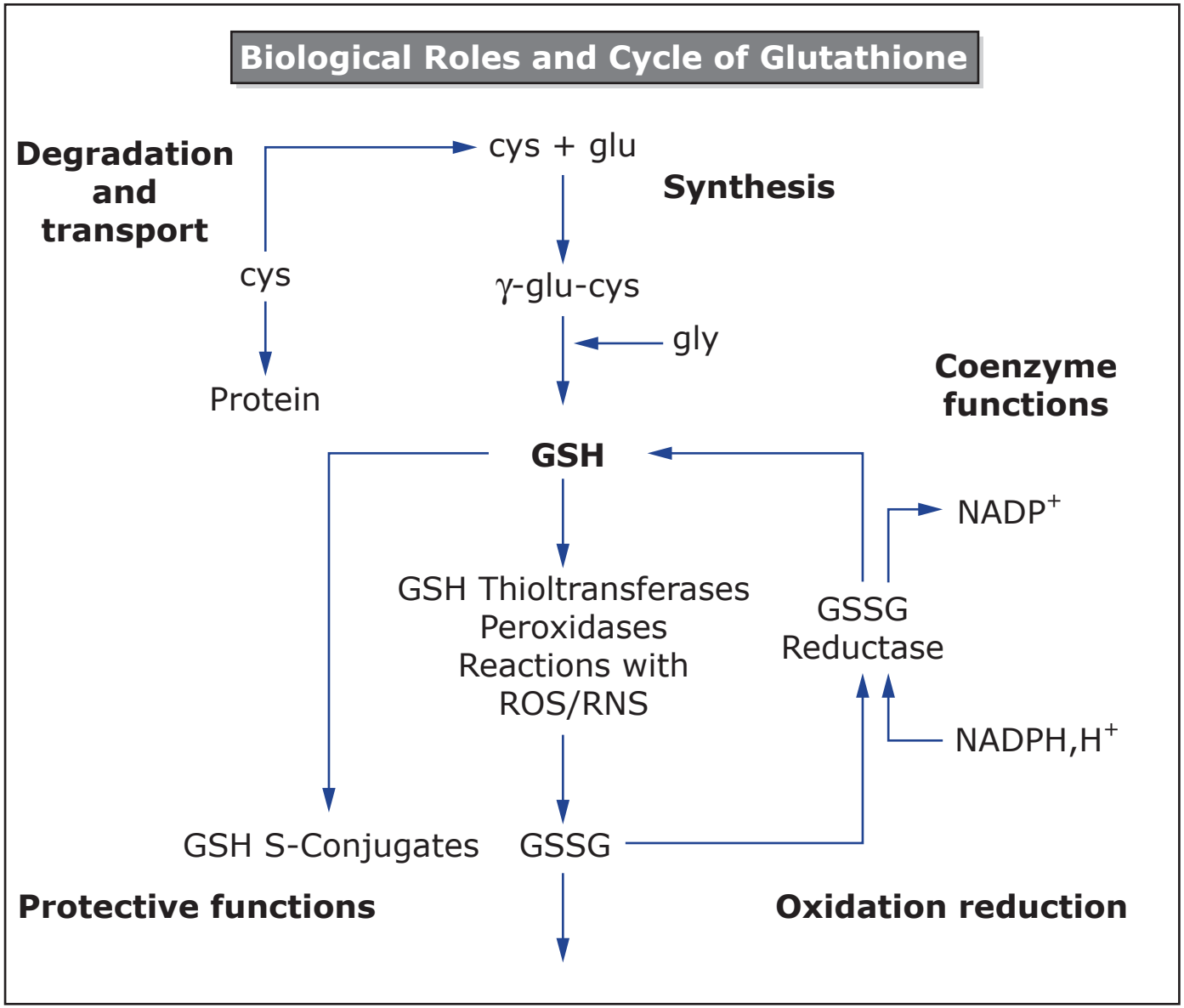
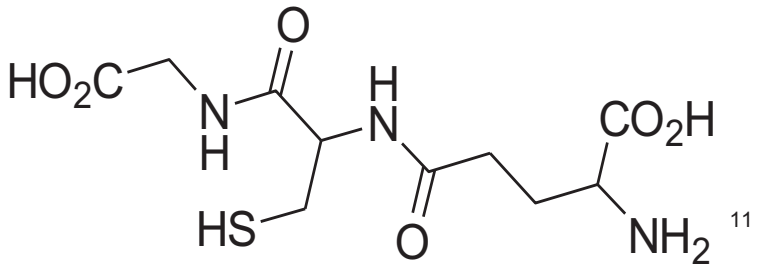


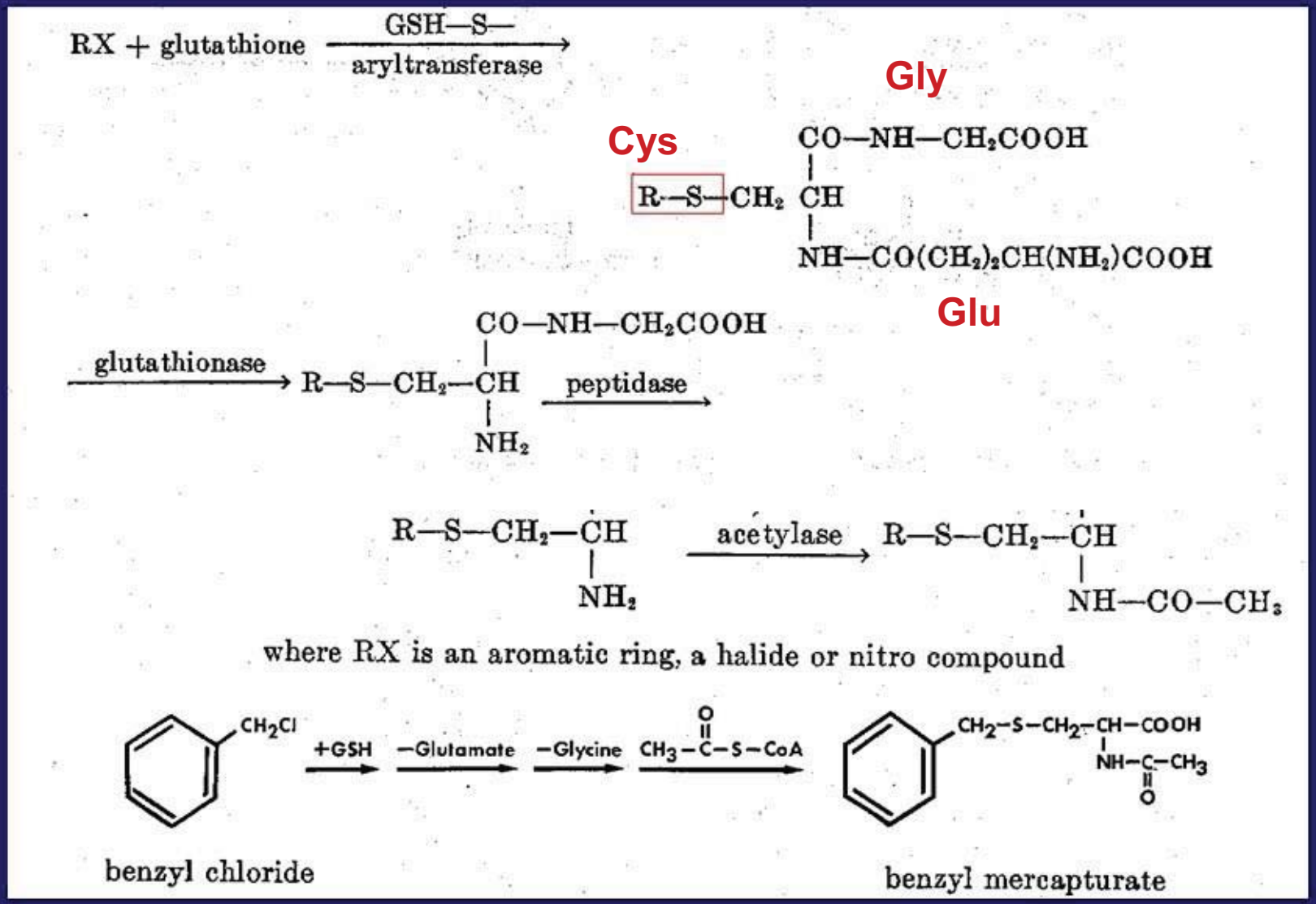
Image by MIT OpenCourseWare.



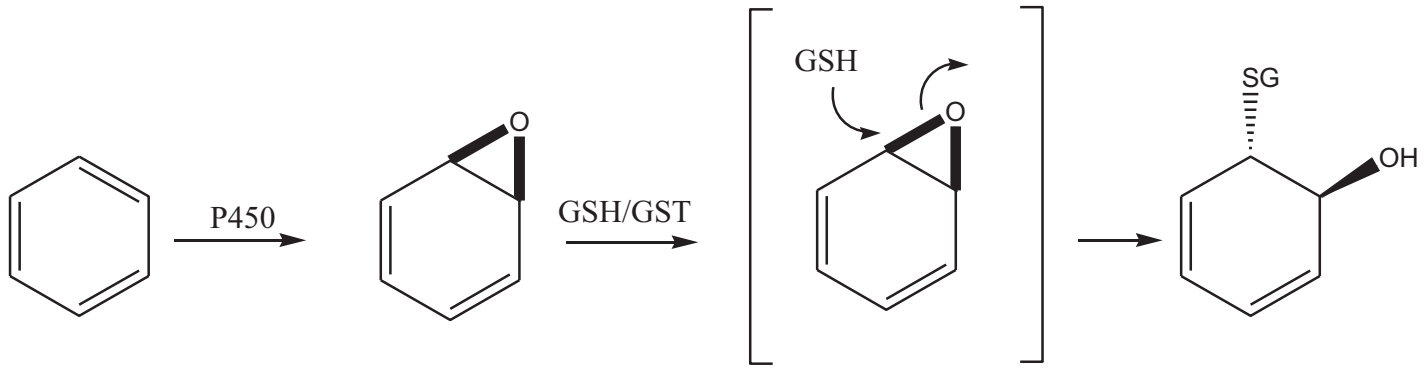
Glutathione Transferases (GST)

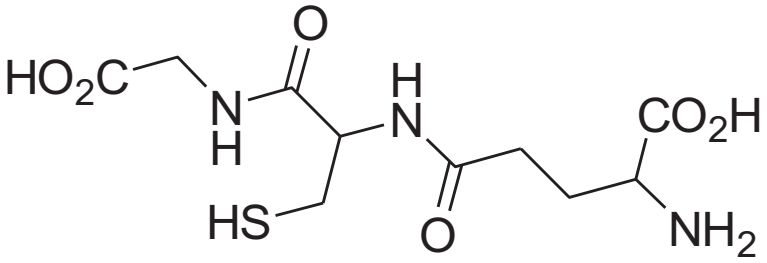
- **Family of enzymes (2 microsomal and 7 cytosolic)**
- **Referred to as an "electrophile killing" enzyme**
- **Uses reduced glutathione (GSH) as a cosubstrate ; Cysteine sulfhydryl group is the nucleophilic (attacking) moiety**
- **Glutathione can exist in a reduced form (GSH) or can be oxidized to a dimer (GSSG) via a disulfide bond**
- **GST catalyze the reaction of GSH with electrophiles**
  - **GSH is reactive on its own; the enzyme holds the substrate in place for an increased reaction rate**

Glutathione Conjugation:

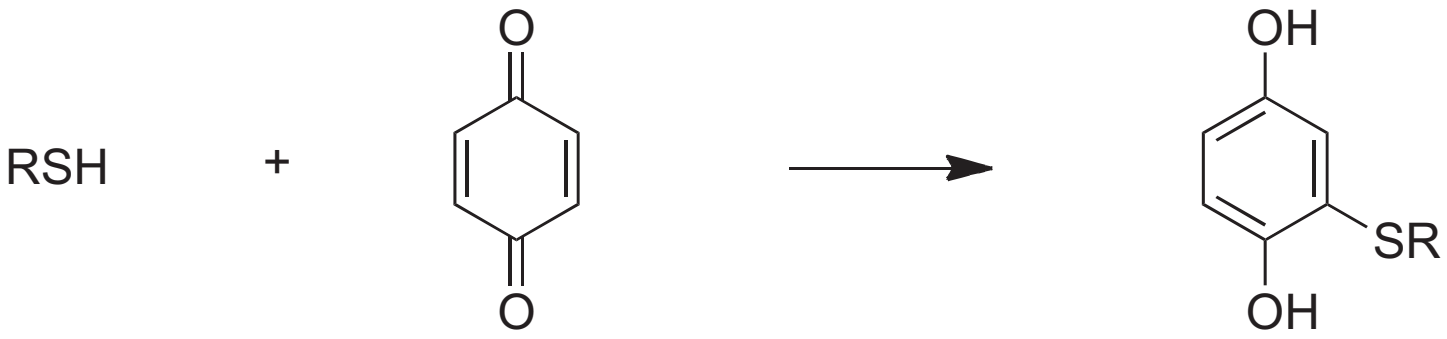


Glutathione Transferase Reaction





Michael Reaction



Important Points

- **Most often, metabolic activation is a result of Phase I (oxidation or reduction) metabolism**
- **Phase II metabolism can also play a role in metabolic activation**
- **Metabolic activation could involve one enzyme or could be a result of multiple enzymes**

Reactive Intermediates

How stable?

How far can they diffuse?

Organ to Organ transport

(heart to lungs=4.3s)

$HO^{\cdot}$ ~ 10(-9) s 1 bond length

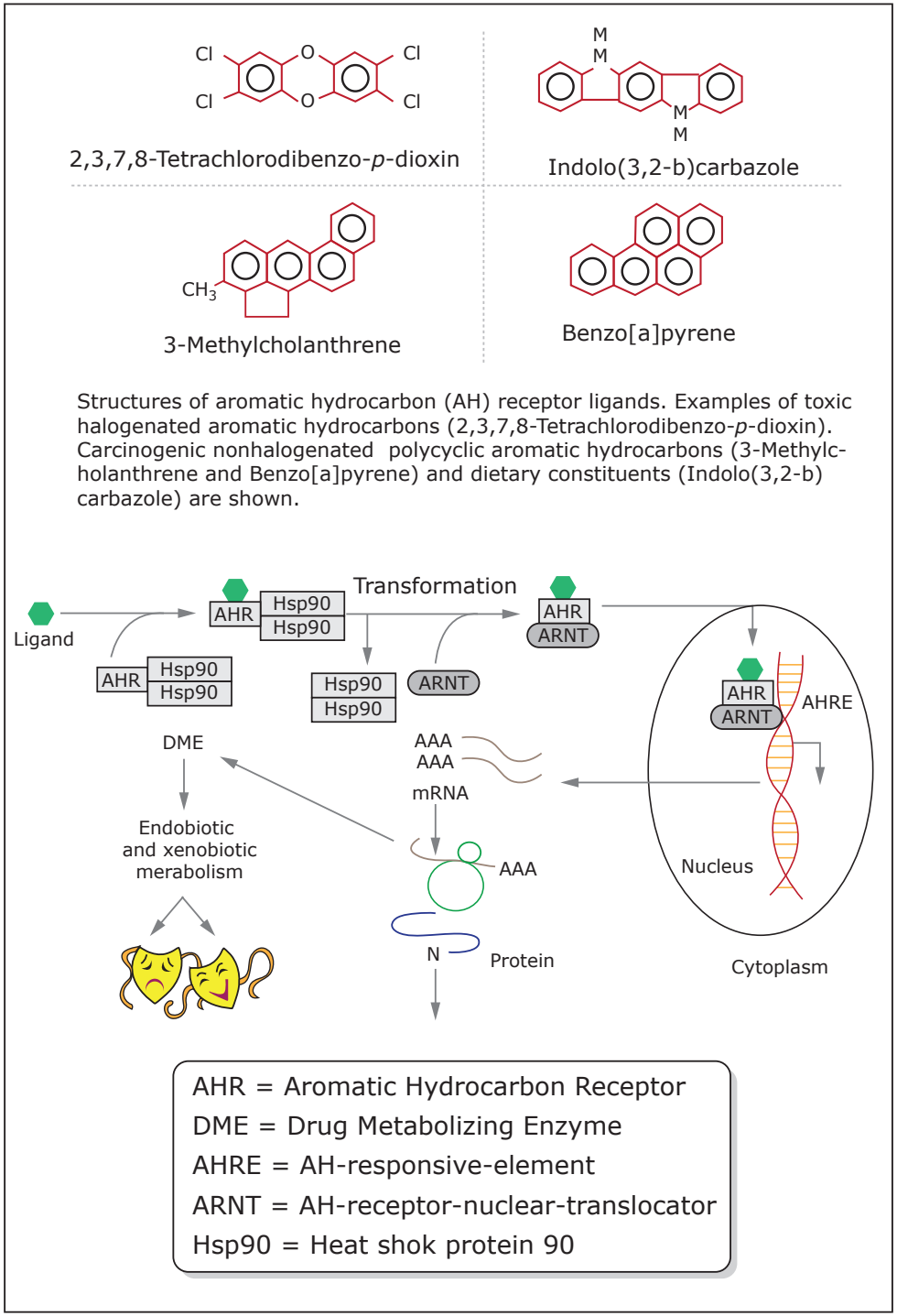
ArNH+ 10(-6 to -4) s within cell

$CH_3N{=}NOH$ ~1s cell to cell

ArNHOH mins organ to organ

P450 Regulation

P450	Receptor	Constitutive	Inducer	Organ
1	AhR	steroids	PAH	liver, lung
2	CAR	androstane steroids	PB	liver
3	PXR	pregnane steroids	Dexamethasone Rifampin –IL-6	liver, intestine
4	PPARα Peroxisome-Proliferator-Activated	fatty acids	fibrates	liver, kidney, heart



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Nuclear receptor	Uptake transporter	CYPs	UGTs	Export transporter
AhR	n.d.	CYP1A1 CYP1A2	UGT1A1 UGT1A6 (rUGT1A7)	n.d.*
CAR	OATP2 (mOatp2)	CYP2B6 (rCYP2Bl) (mCyp2b10)	UGT1A1 (rUGT2B1)	MRP2 (mMrp2)
PXR	OATP2 (mOatp2)	CYP3A4 (rCYP3A23) (mCyp3a11)	UGT1A1 (mUgt1a6) (mUgt1a9)	MRP2 MRP3

Inducible rodent enzymes are listed in parenthesis.
*n.d., not detected.

Selected human and rodent CYPs, UGTs and glucuronide transporters induced by the Ah receptor. CAR and PXR.

Some Examples of Bioactivation of Drugs

Drug Metabolism

Problem 2 Answers

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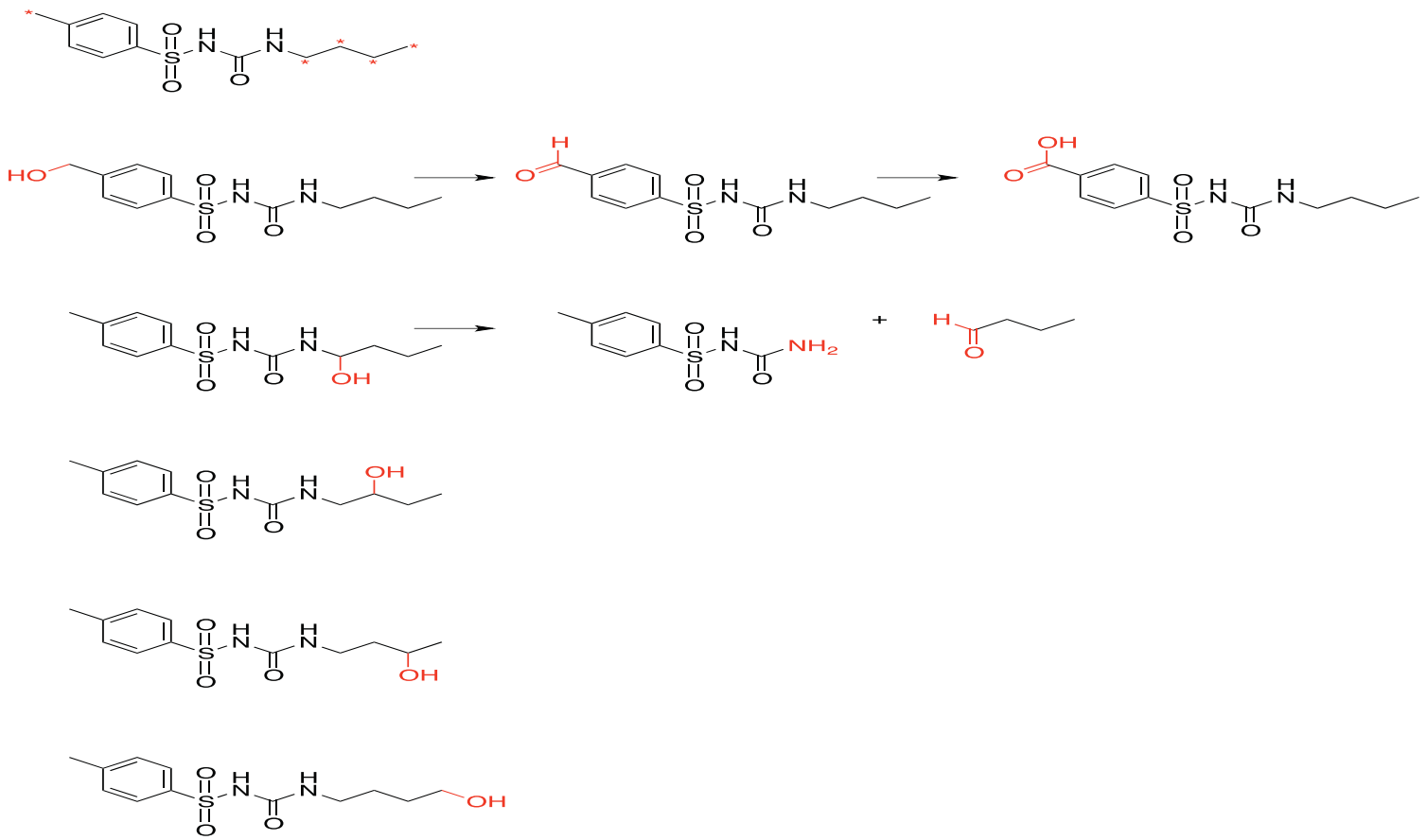
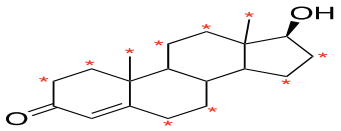
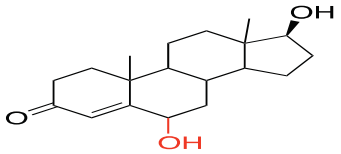
September 16, 2013

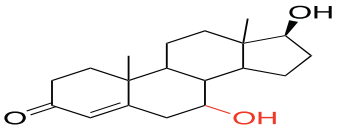
Tolbutamide

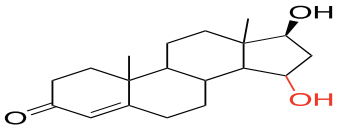


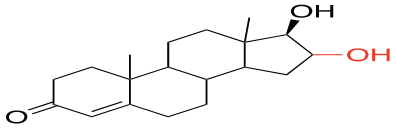
Testosterone

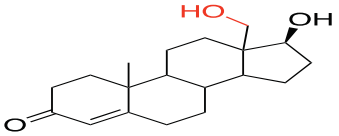


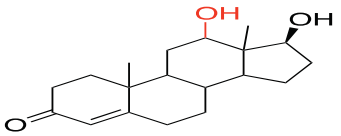


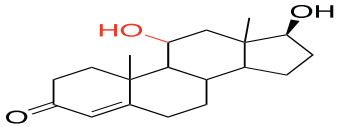


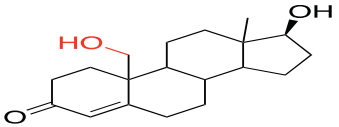


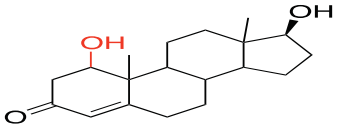


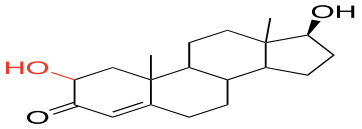




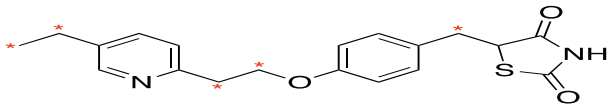


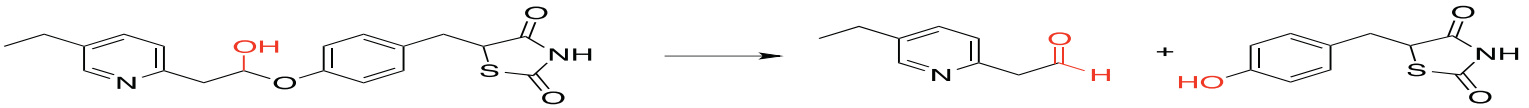


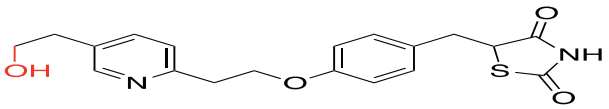


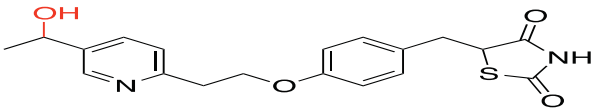


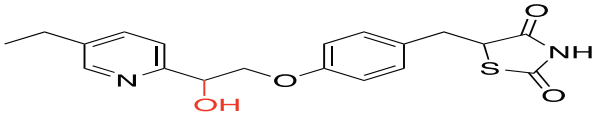
Pioglitazone

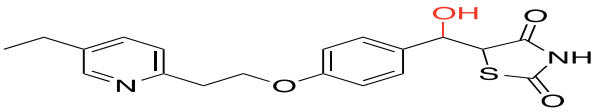


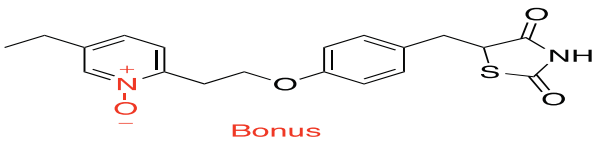


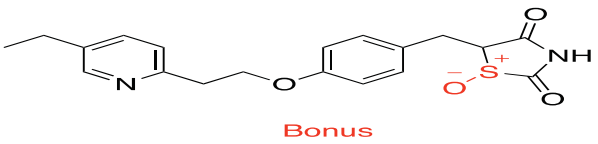




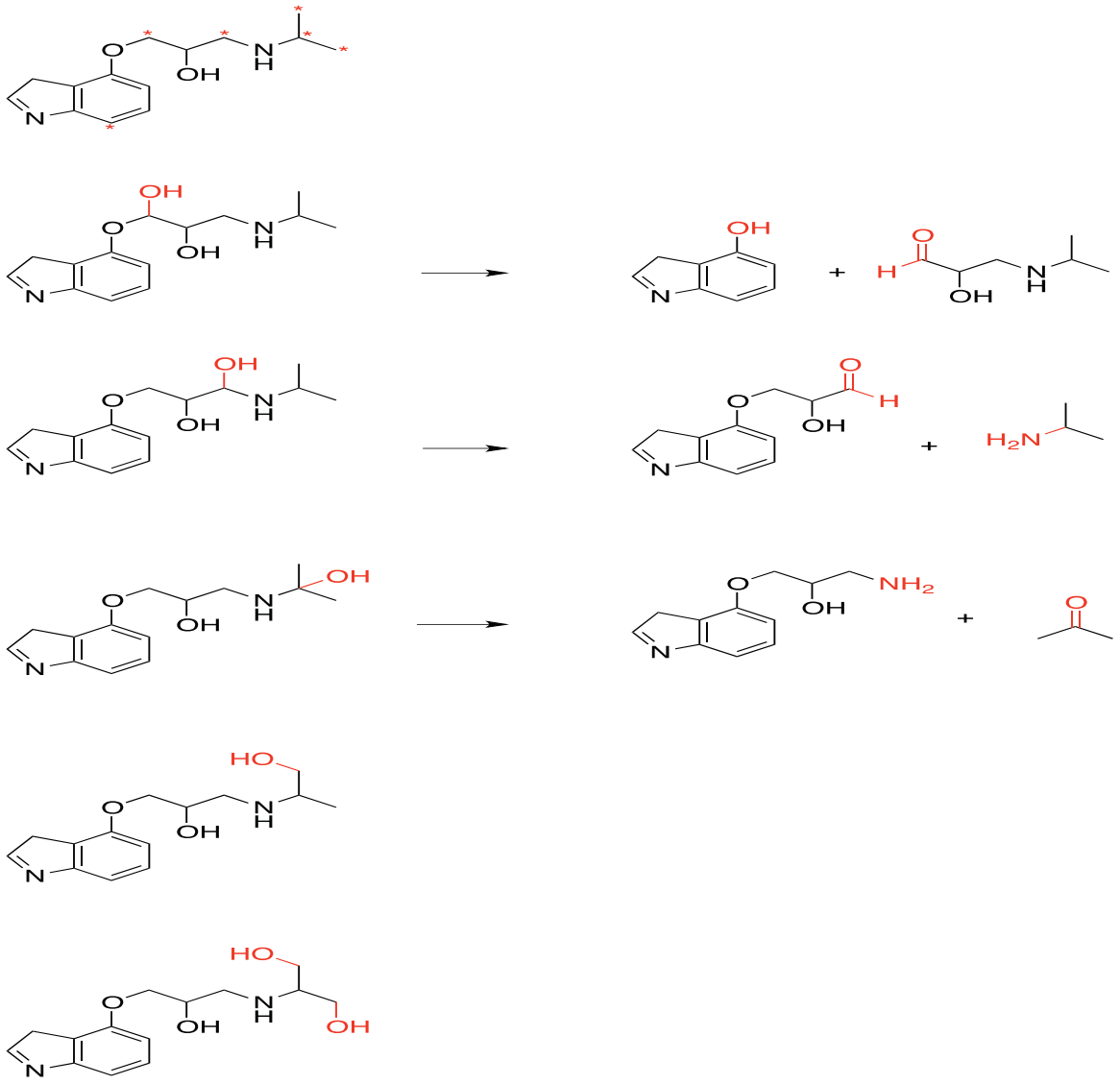


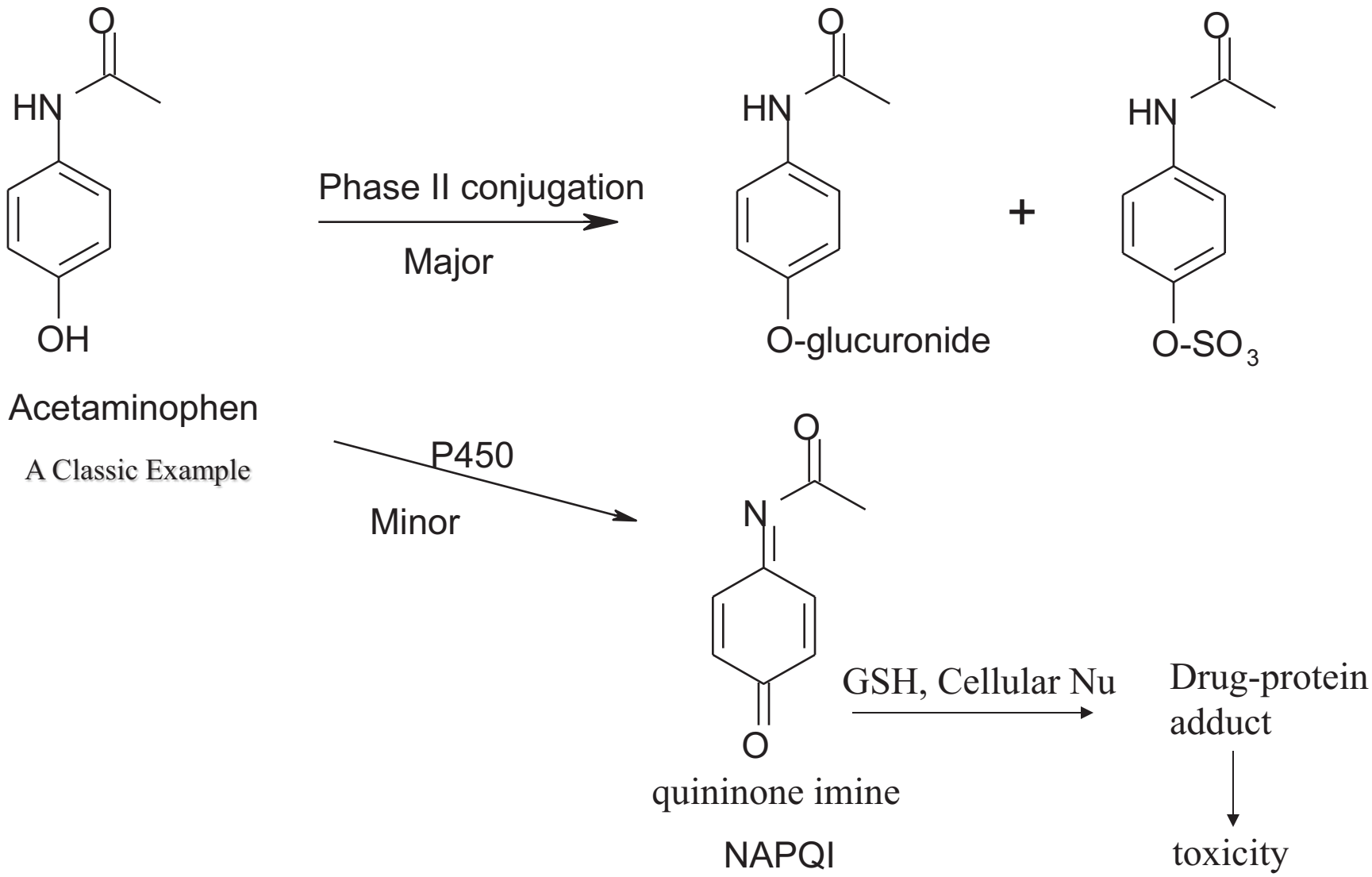


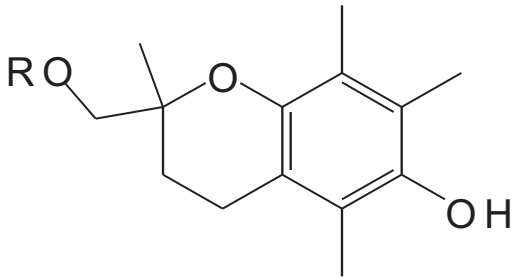




Pindolol

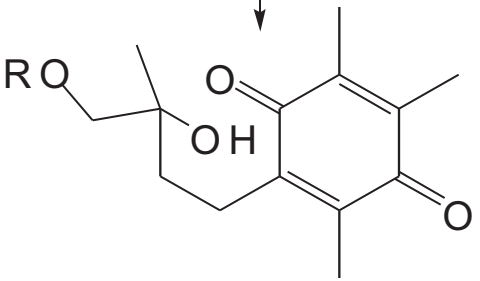



O
HN
OH
Acetaminophen
A Classic Example
Phase II conjugation
Major
O
HN
O-glucuronide
+
O
HN
O-SO3
P450
Minor
O
N
O
quininone imine
NAPQI
GSH, Cellular Nu
Drug-protein adduct
toxicity



Troglitazone

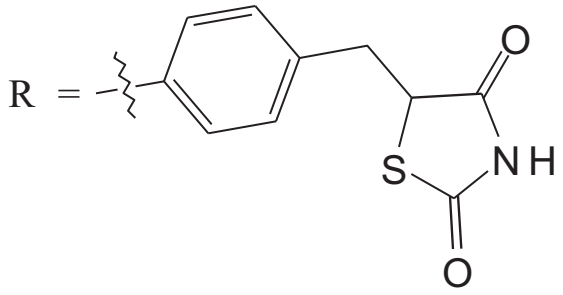
P450

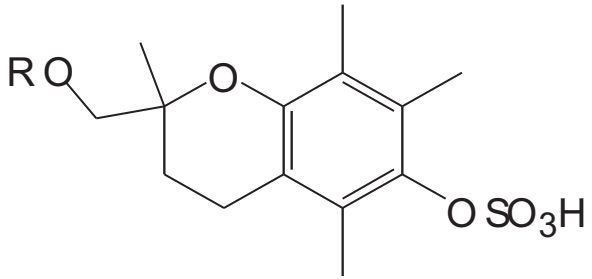


Quinone

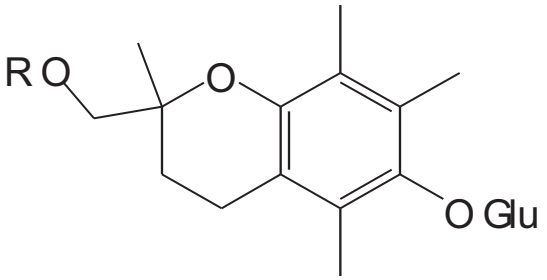
C VB

Toxicity?

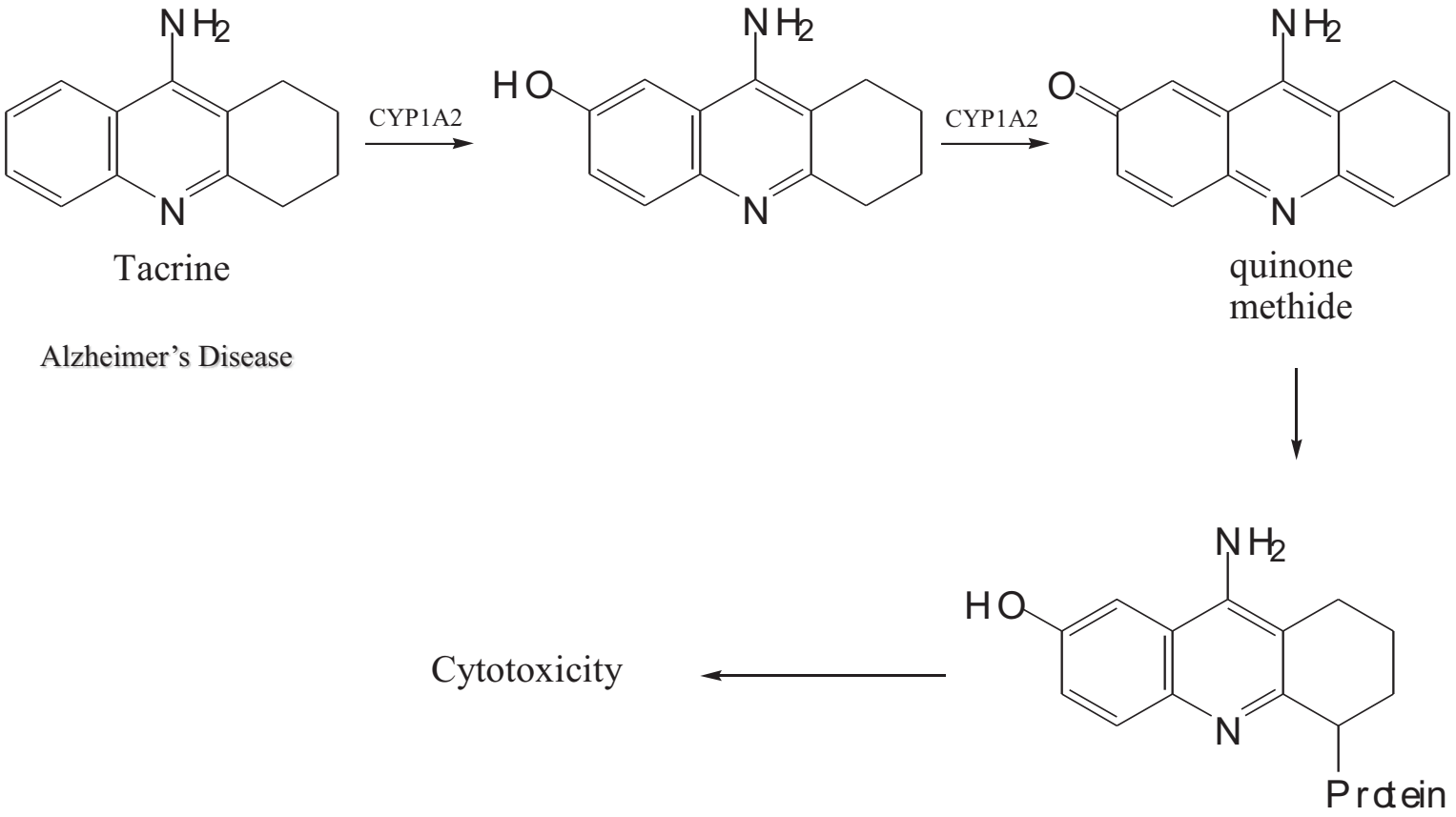




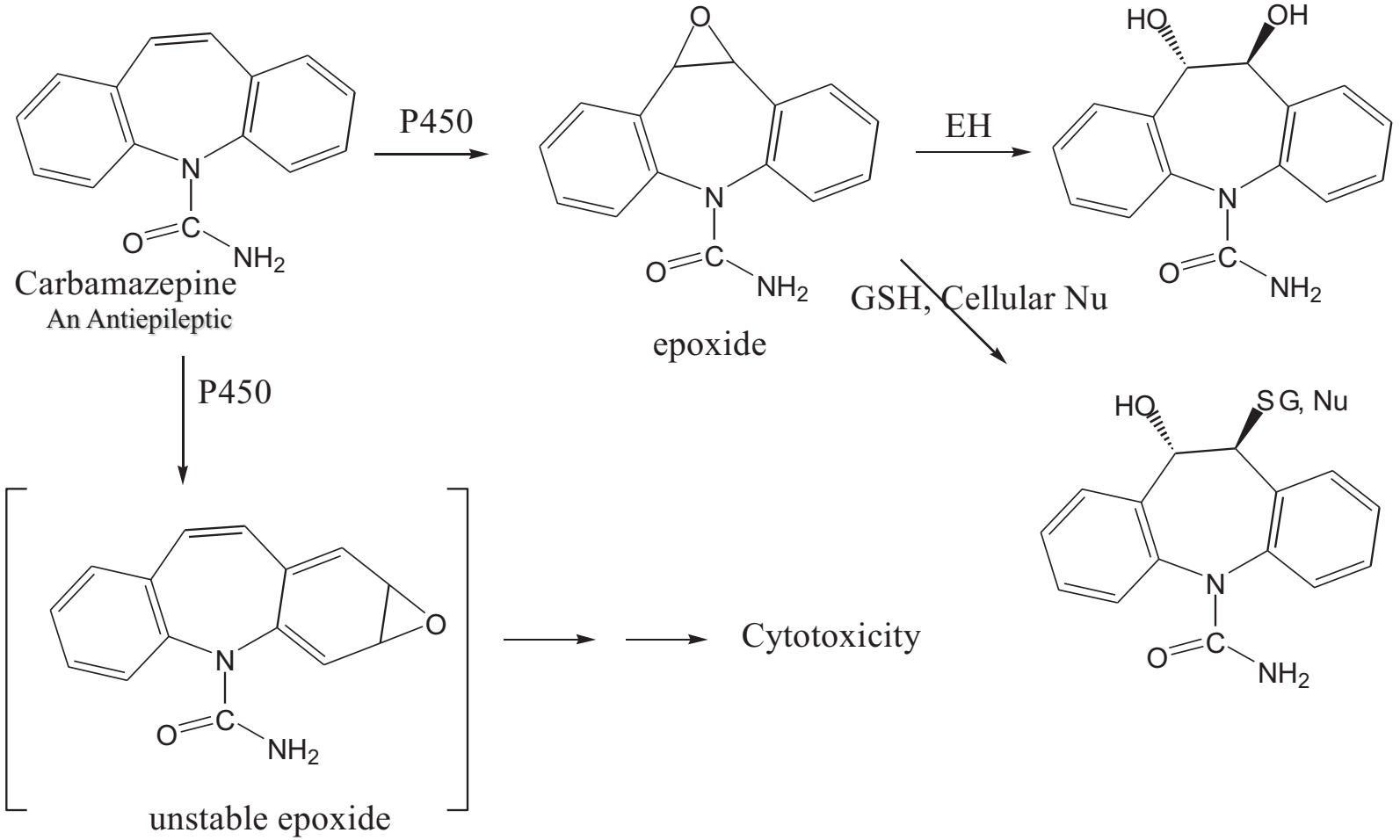
Sulfate

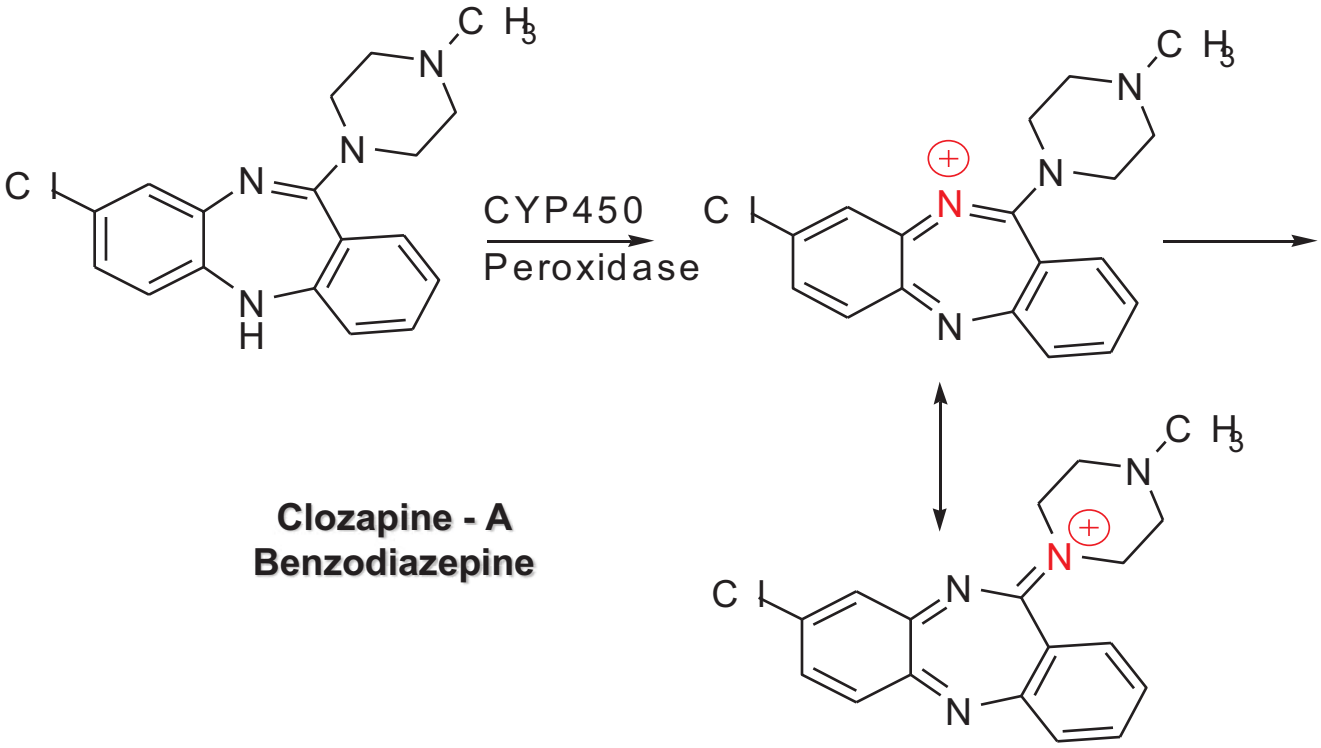


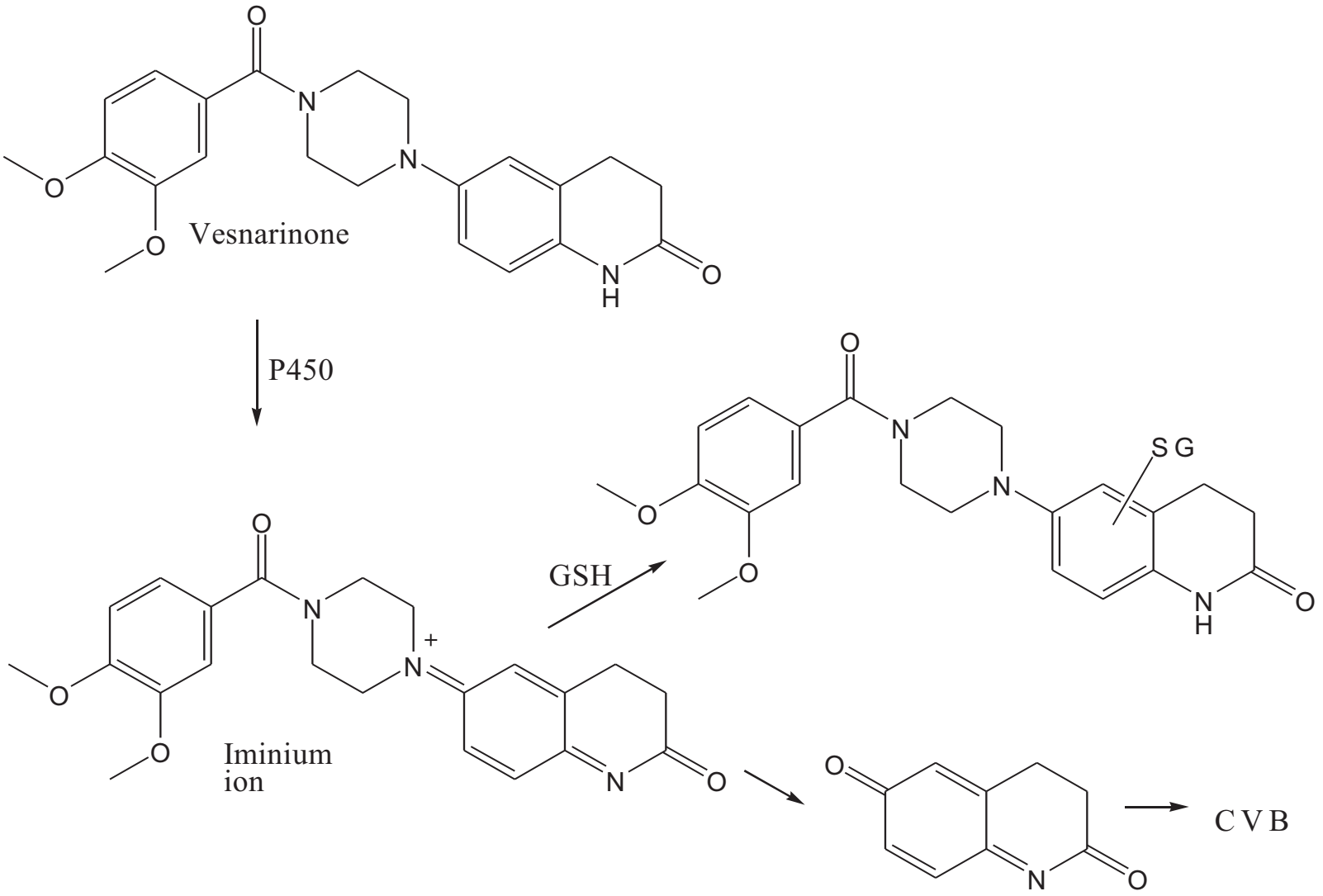
Glucuronide



Alzheimer's Disease


P450
EH
GSH, Cellular Nu
Carbamazepine
An Antiepileptic
epoxide
P450
unstable epoxide
Cytotoxicity
HO
OH
SG, Nu

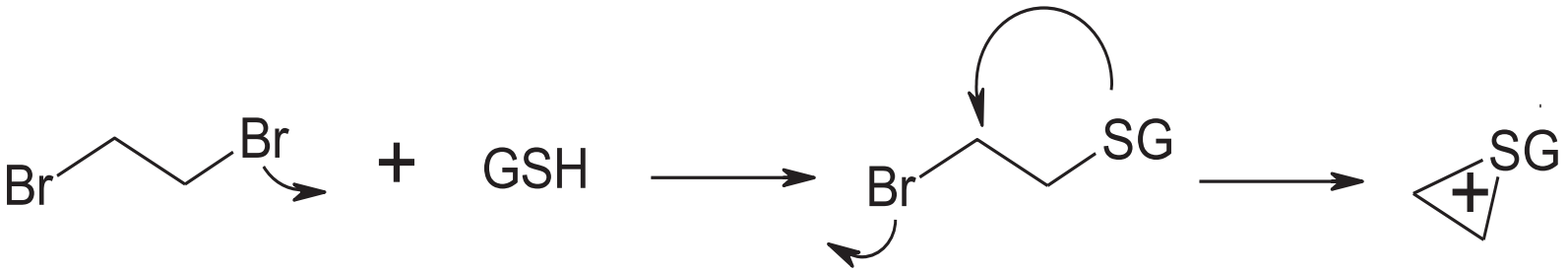

CYP450
Peroxidase
Covalent binding to proteins
Clozapine - A Benzodiazepine
Reactive Intermediate


Vesnarinone
P450
GSH
Iminium ion
SG
CVB

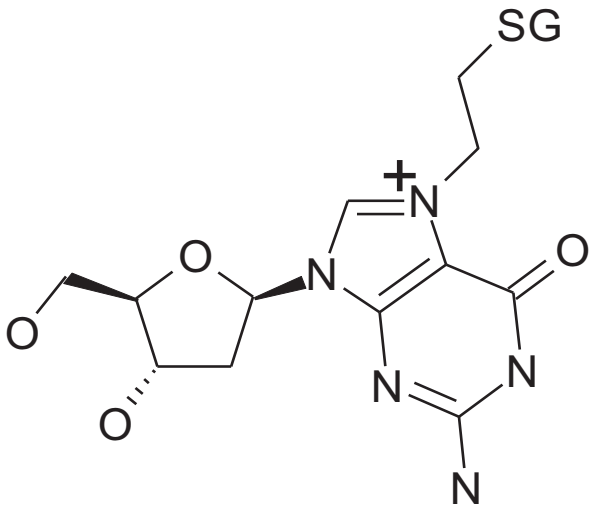
Bioactivation via Conjugative Enzymes

- GSH conjugate
- Acyl glucuronide
- Sulfation

GSH-Conjugate Mediated Toxicity



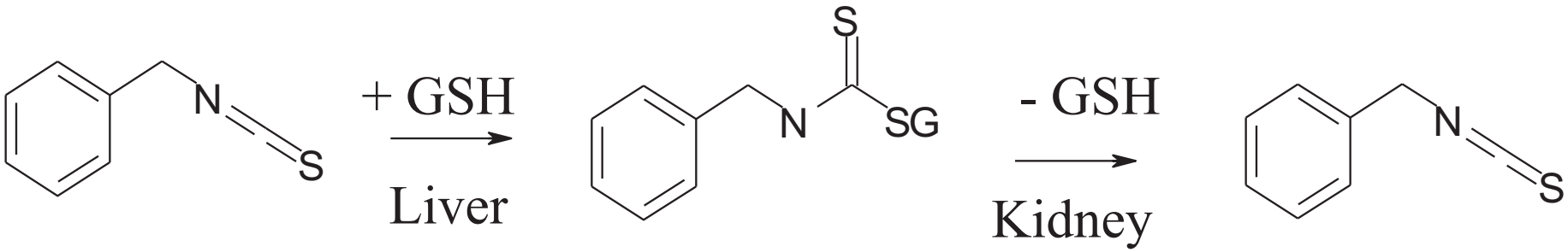
Mutagenicity



DNA Adduct

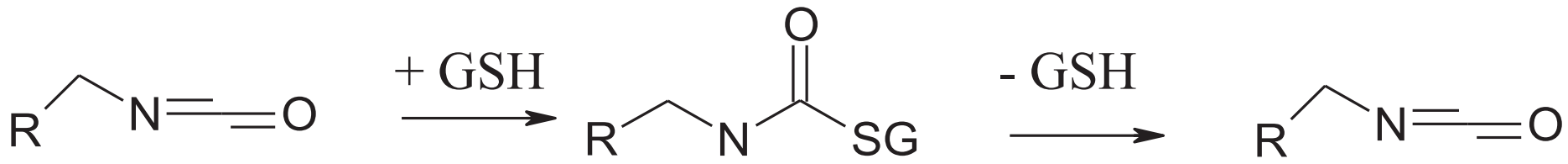
Toxicity of Reversible GSH-Conjugates

A reactive molecule may initially be detoxified via conjugation with GSH, if the GSH conjugates are unstable they may revert back to the reactive moiety at a different site/organ



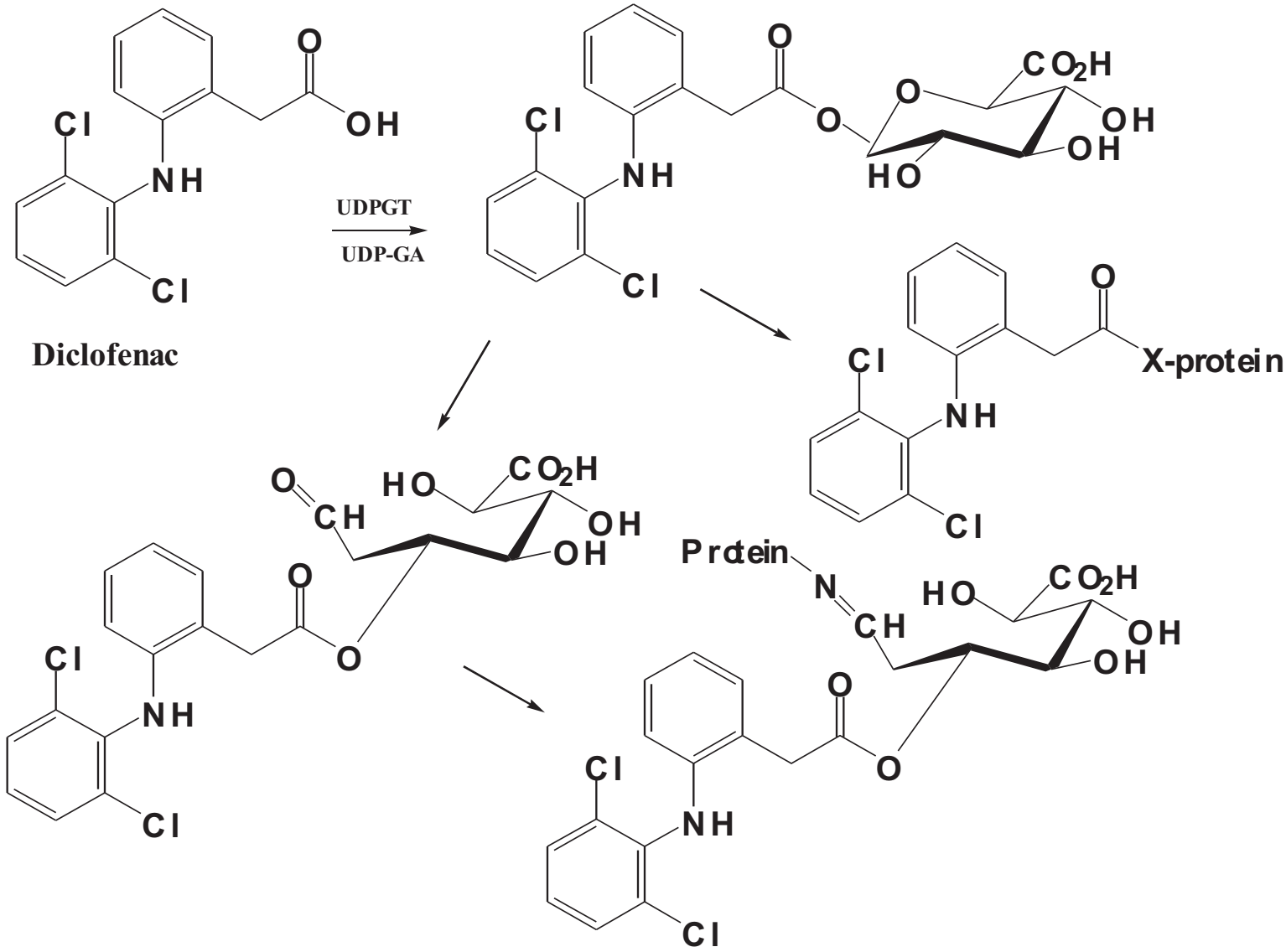
Benzyl isothiocyanate

Nephrotoxicity



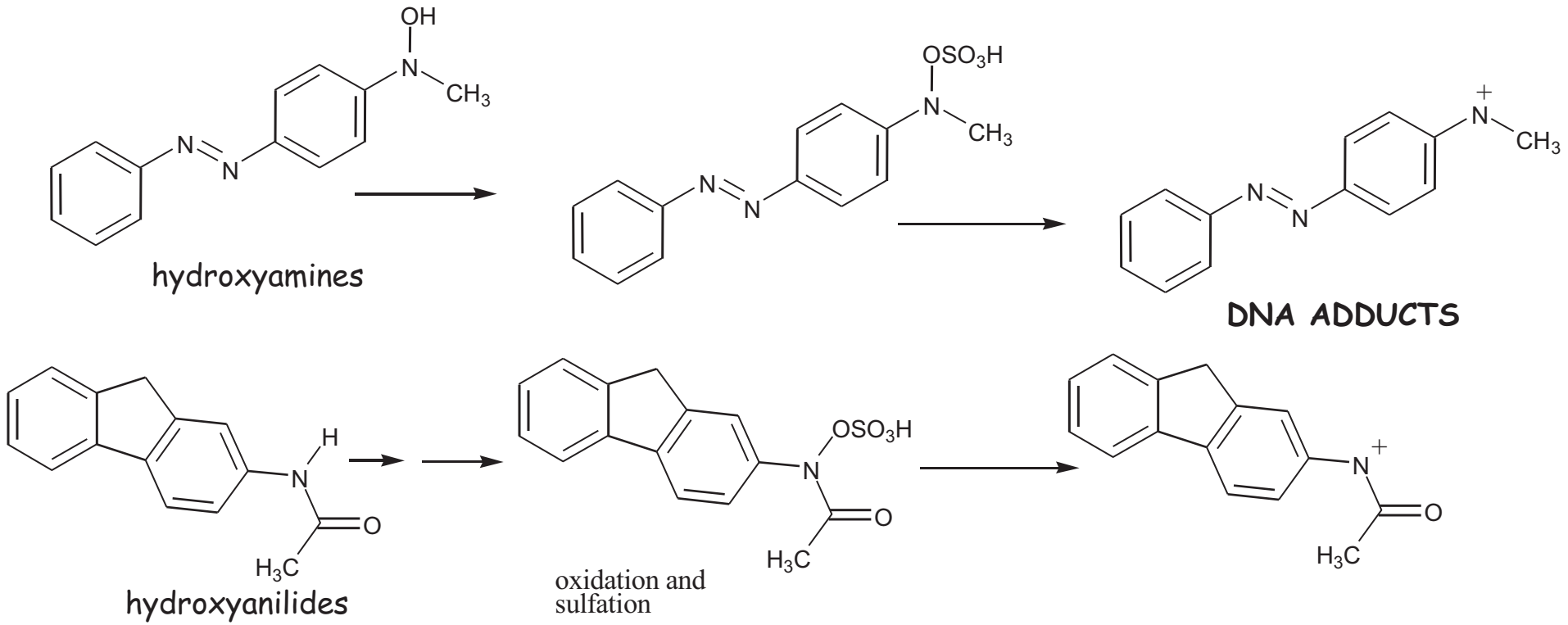
Isocyanate

Acyl Glucuronide Migration



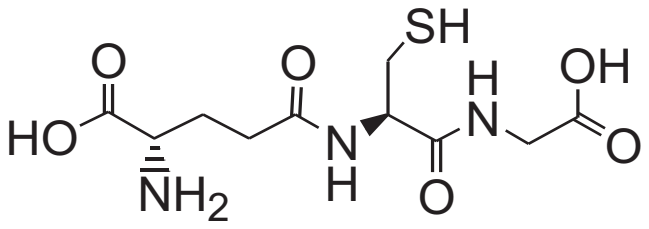
Bioactivation by Sulfation

Sulfate conjugates play an important role in the metabolic activation of *N*-hydroxylamines and *N*-hydroxylamides to reactive intermediates



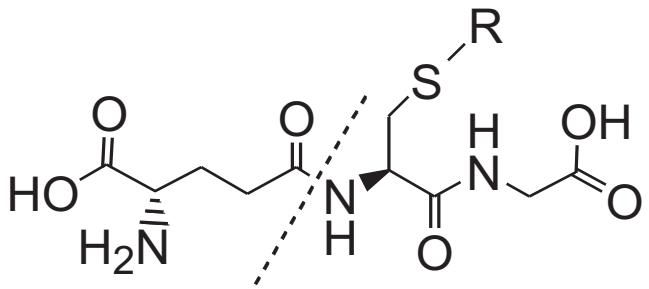
Generic Method for Identifying Compounds that form Reactive Intermediates

Drug +



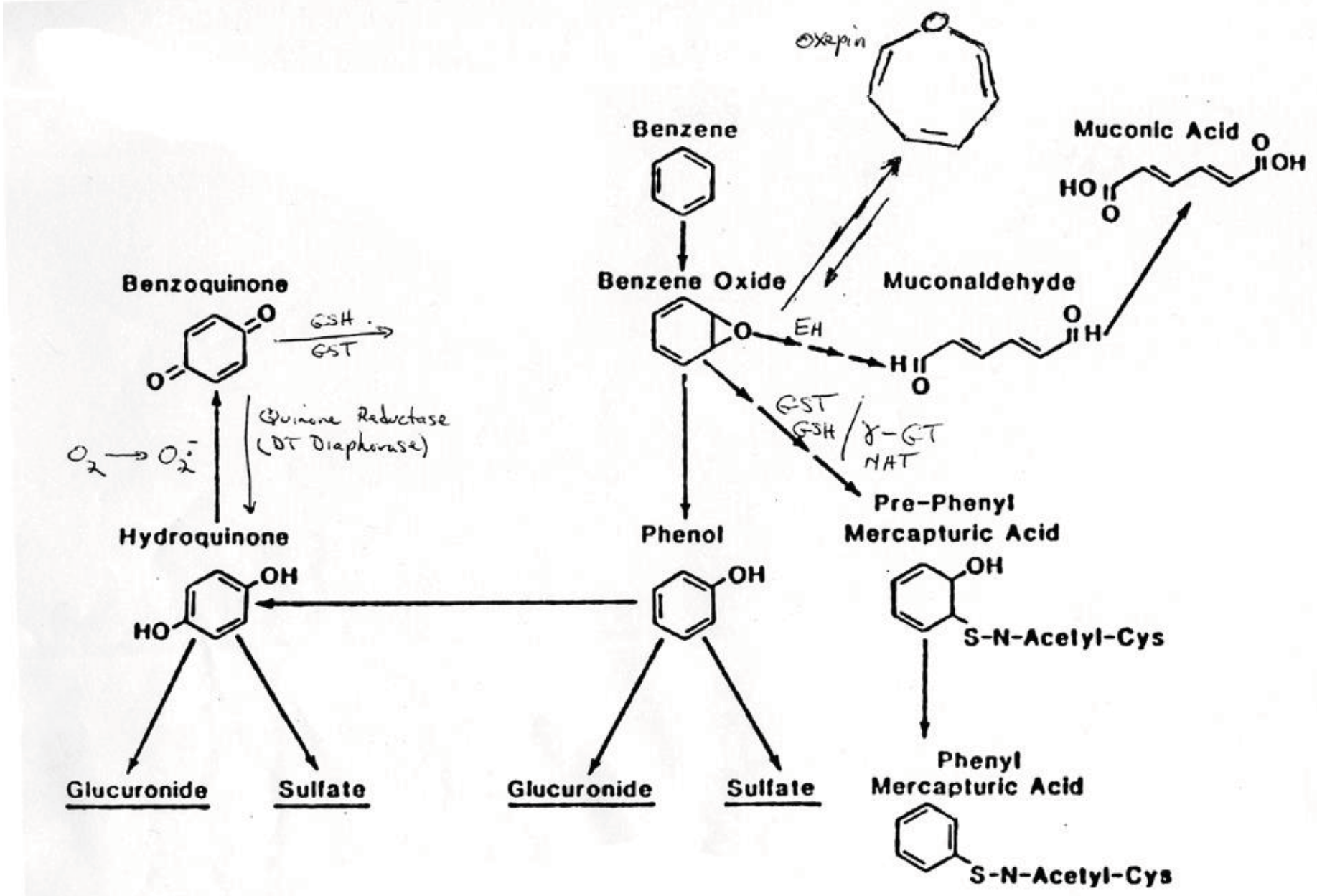
Drug of interest is incubated with human liver microsomes in the presence of GSH

Detect with mass spectrometry (m/z 129)

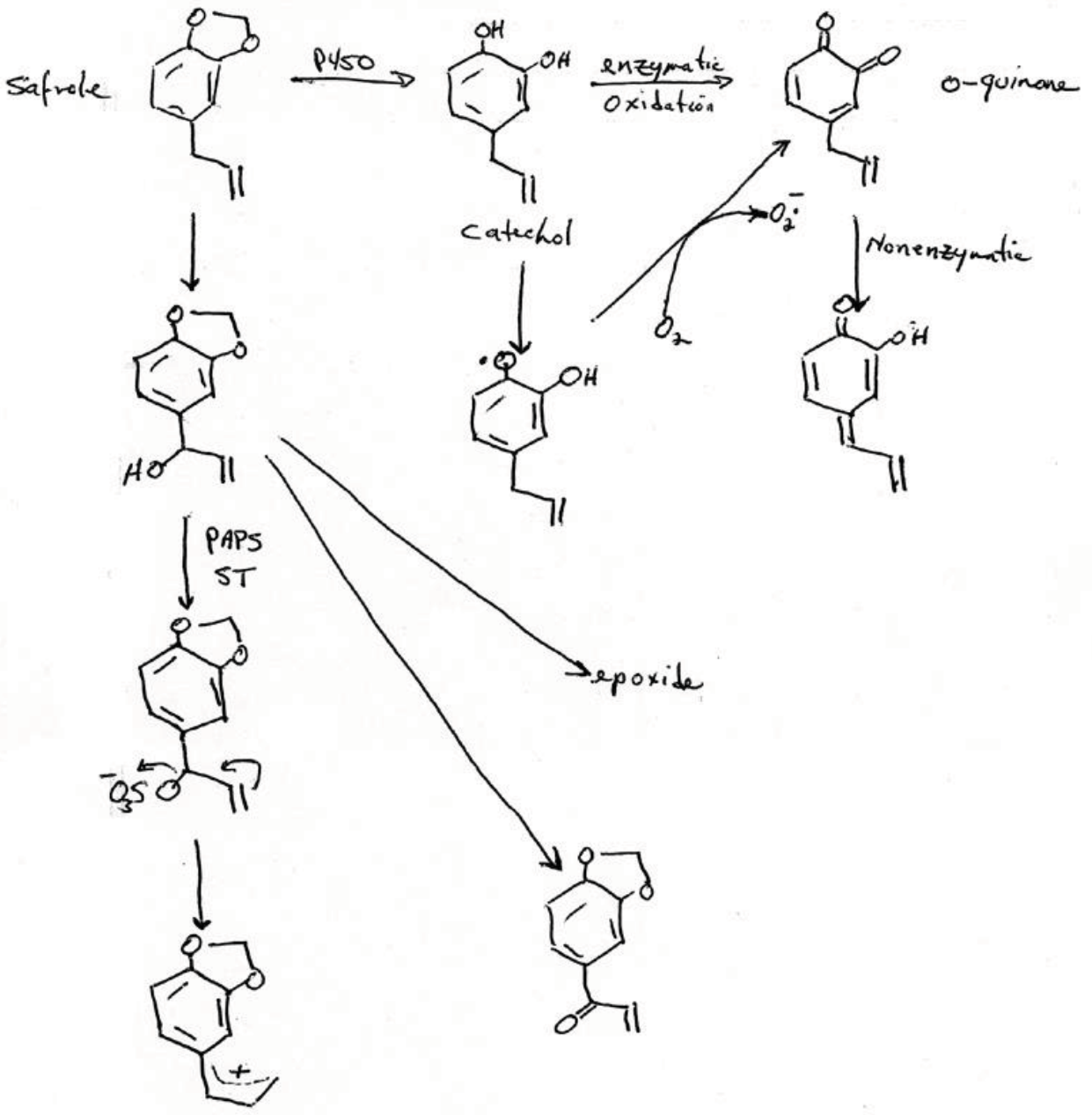


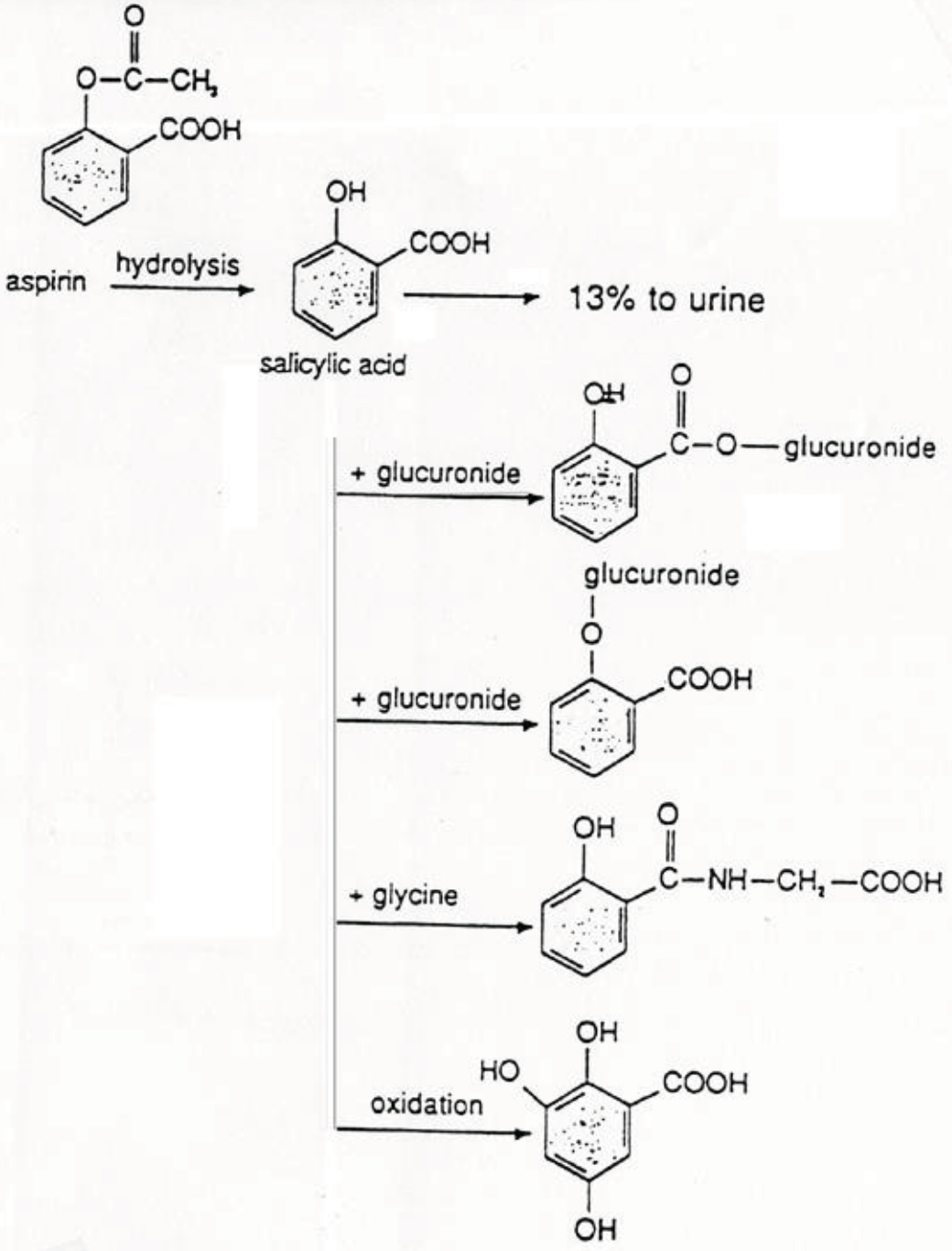
Reactive Metabolite (R)

A drug-glutathione cojugate will arise if drug is metabolized to a reactive intermediate

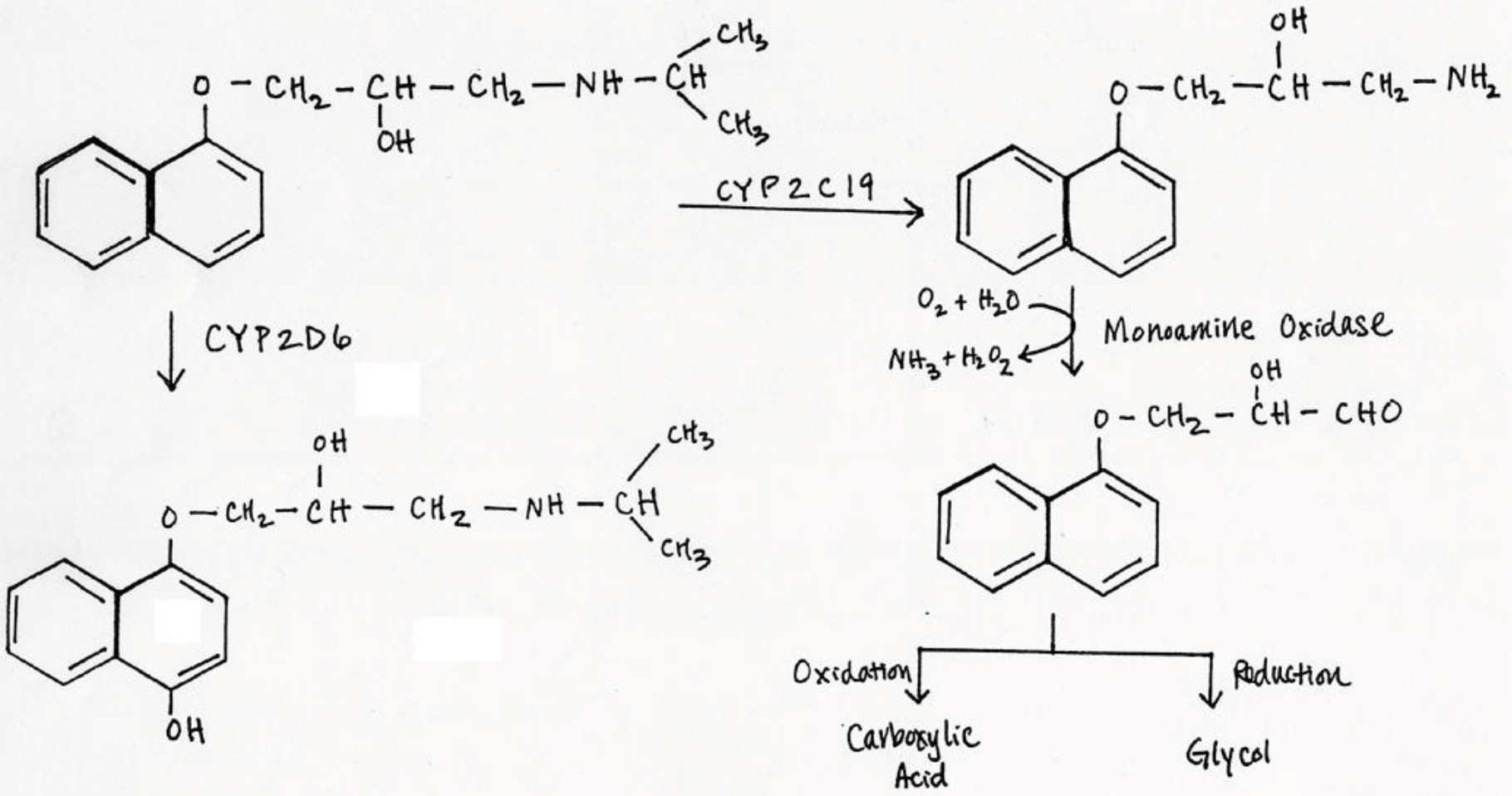

Oxepin
Benzene
Muconic Acid
Benzoquinone
Benzene Oxide
Muconaldehyde
GSH
GST
EH
Quinone Reductase
(DT Diaphorase)
GST
GSH
γ-GT
NAT
Pre-Phenyl
Mercapturic Acid
Hydroquinone
Phenol
S-N-Acetyl-Cys
Glucuronide
Sulfate
Glucuronide
Sulfate
Phenyl
Mercapturic Acid
S-N-Acetyl-Cys

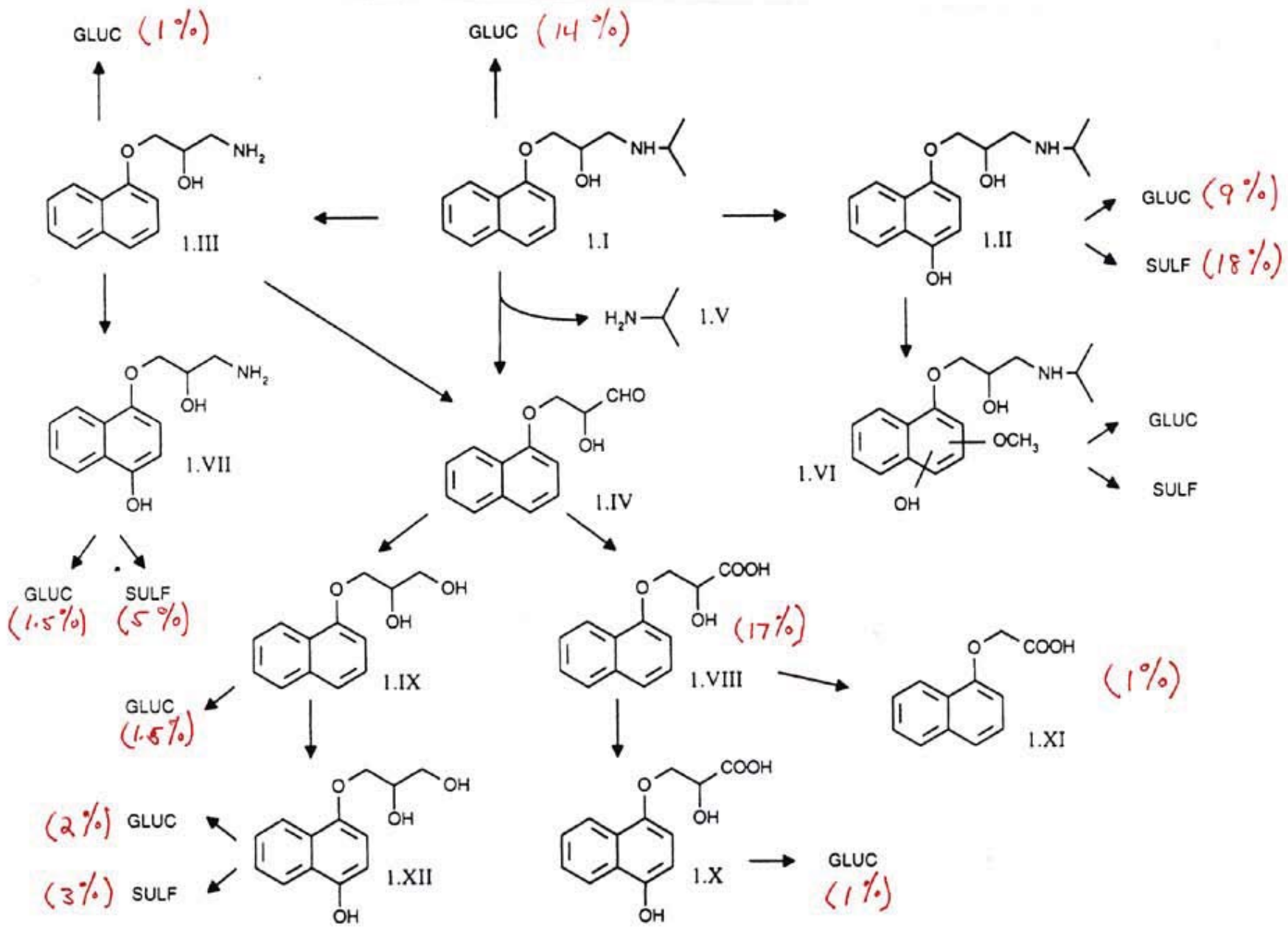
Quinones and Quinone Methides



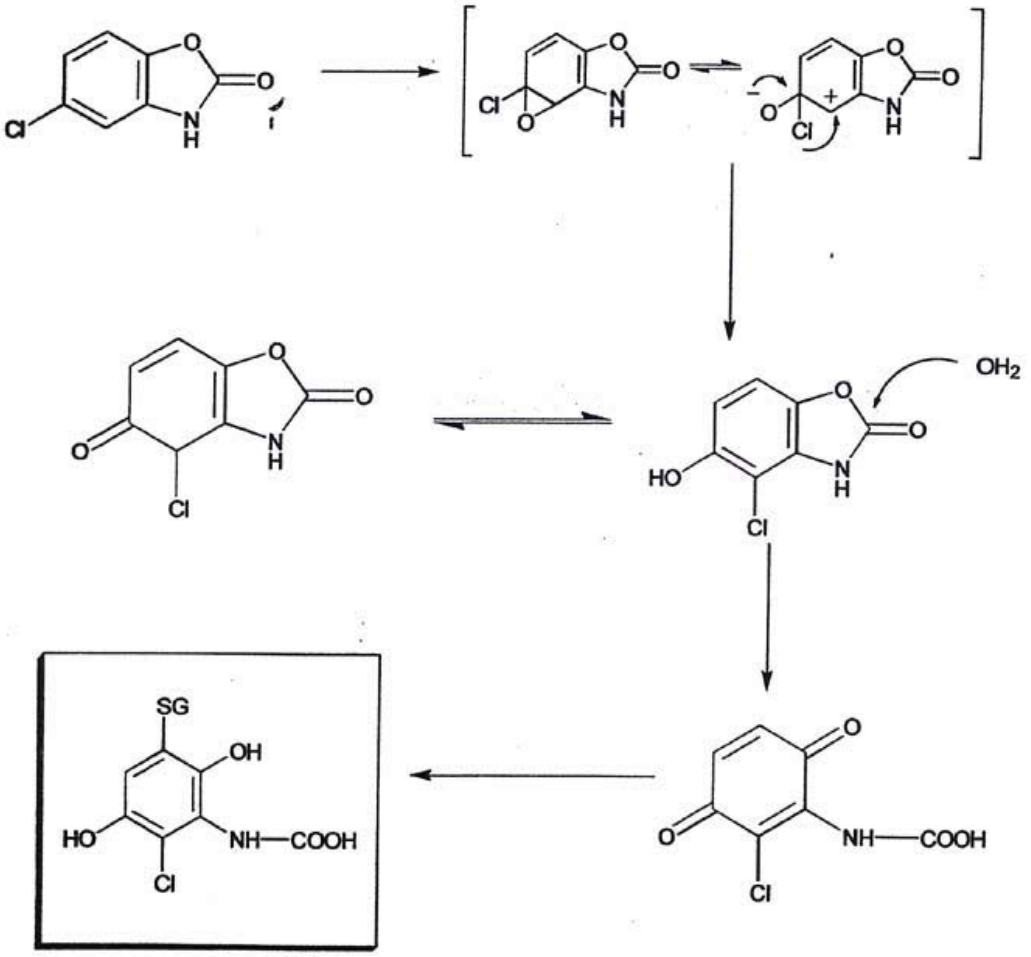

aspirin
hydrolysis
salicylic acid
13% to urine
+ glucuronide
glucuronide
+ glucuronide
+ glycine
oxidation

Propanolol

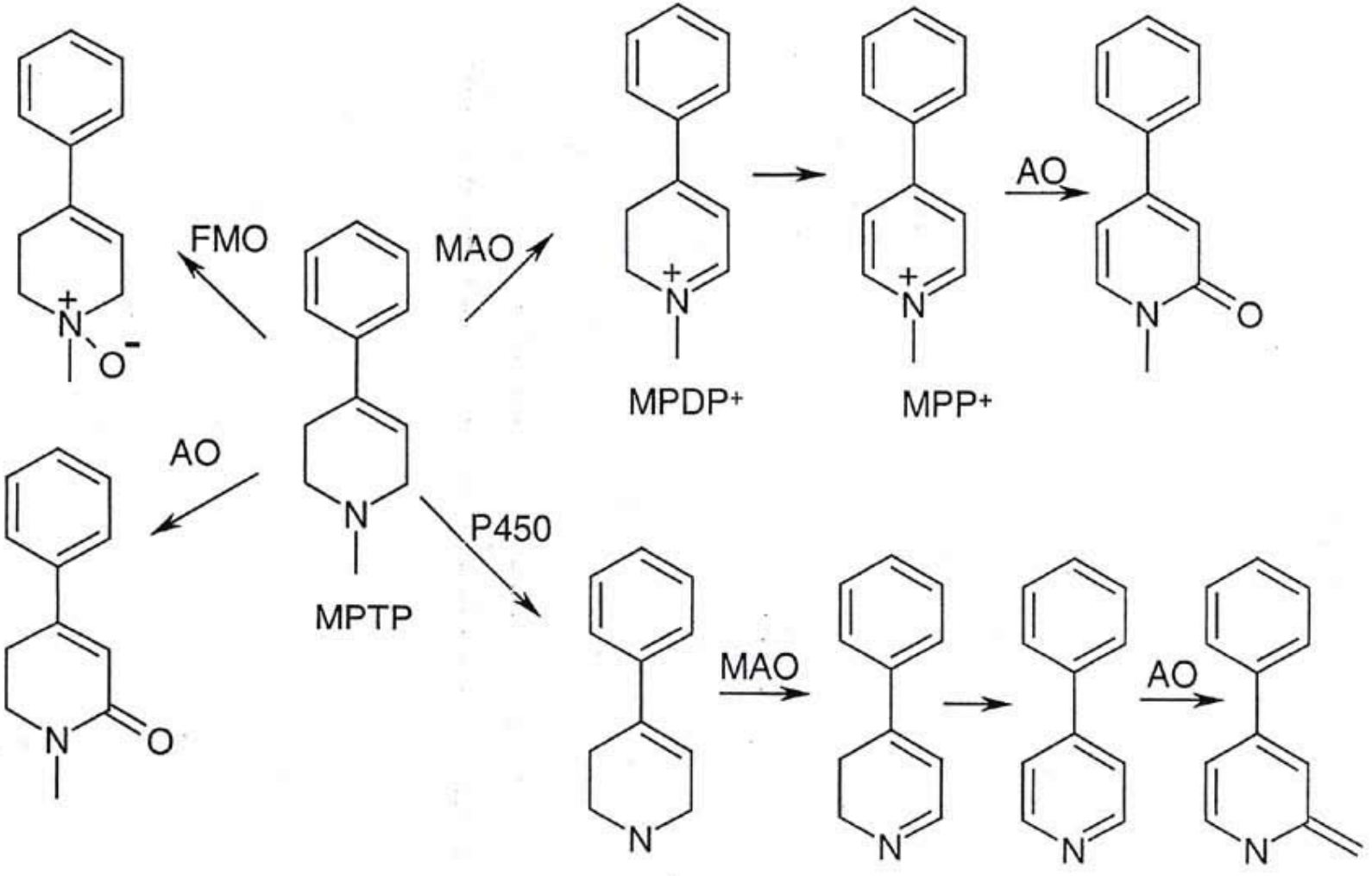

CYP2C19
CYP2D6
$O_2 + H_2O$
$NH_3 + H_2O_2$
Monoamine Oxidase
Oxidation
Reduction
Carboxylic Acid
Glycol

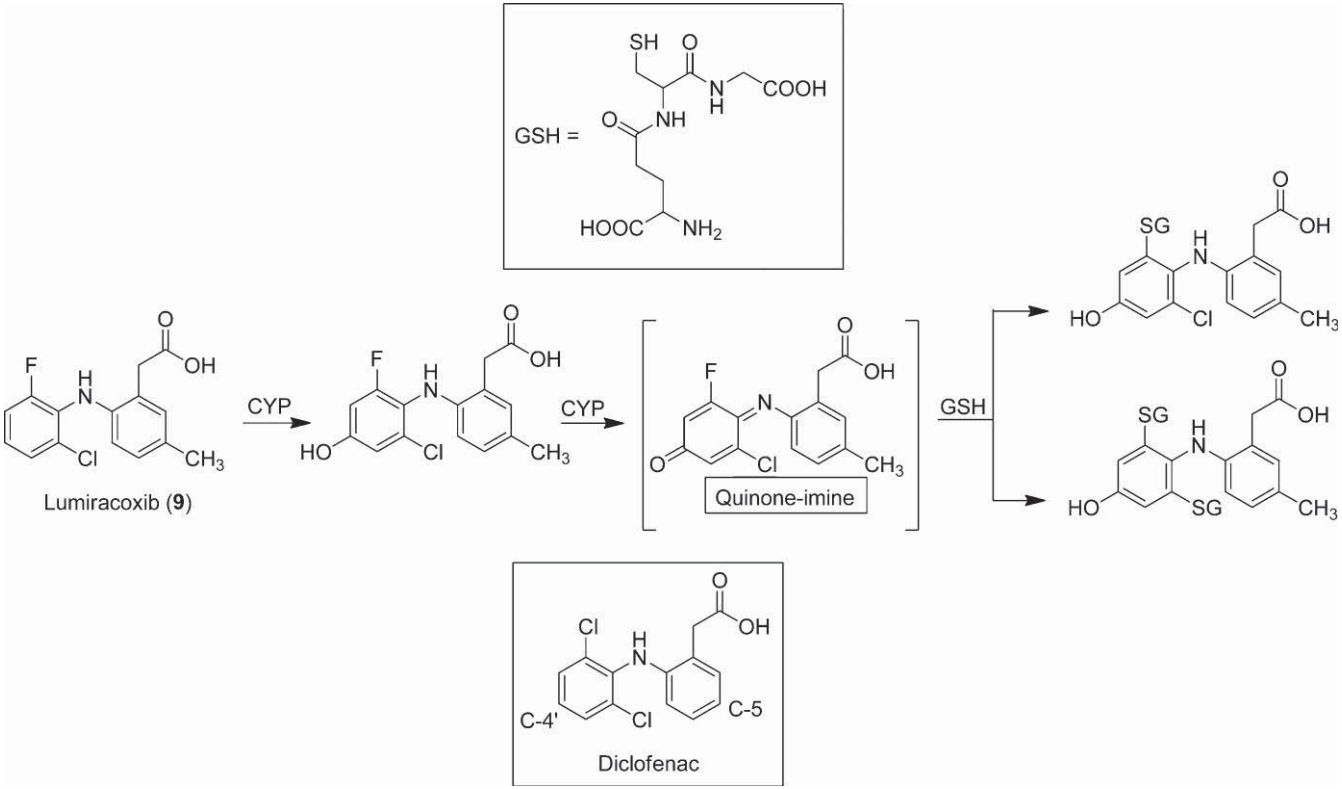

GLUC (1%)
GLUC (14%)
1.III
1.I
1.II
GLUC (9%)
SULF (18%)
1.V
1.VII
1.IV
1.VI
GLUC
SULF
GLUC (1.5%)
SULF (5%)
1.IX
1.VIII
(17%)
GLUC (1.5%)
1.XI
(1%)
(2%) GLUC
(3%) SULF
1.XII
1.X
GLUC (1%)

Chlorzoxazone



Multiple pathways for biotransformation of drugs - the case of MPTP





Source: Stepan, Antonia F., Daniel P. Walker, et al. "Structural Alert / Reactive Metabolite Concept as Applied in Medicinal Chemistry to Mitigate the Risk of Idiosyncratic Drug Toxicity: A Perspective Based on the Critical Examination of Trends in the Top 200 Drugs Marketed in the United States." *Chemical Research in Toxicology* 24, no. 9 (2011): 1345-410.

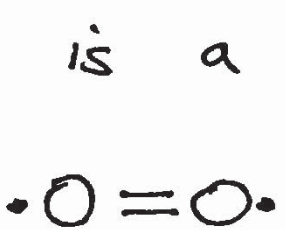
Published in: Antonia F. Stepan; Daniel P. Walker; Jonathan Bauman; David A. Price; Thomas A. Baillie; Amit S. Kalgutkar; Michael D. Aleo; *Chem. Res. Toxicol.* **2011,** 24, 1345-1410.

Oxidative Stress and Antioxidation

20.201

November 12, 2008

Oxygen is a diradical



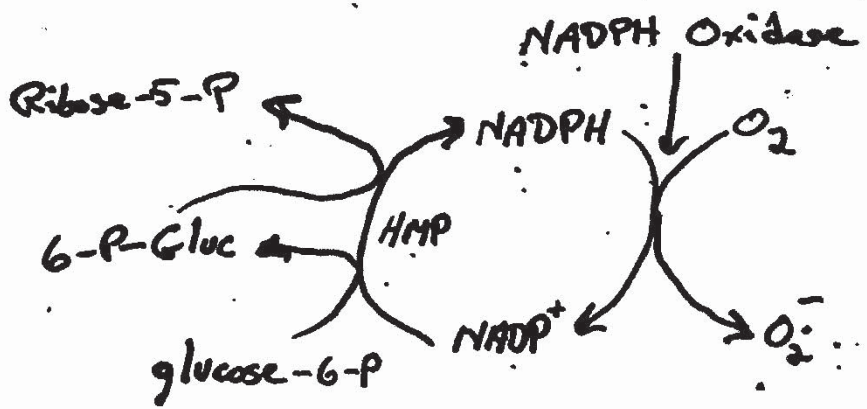
Conversion of O_2 to H_2O_2 1 e^- at a time

$$\cdot O_2 \xrightarrow{e^-} O_2^{\cdot -} \xrightarrow{e^-} H_2O_2 \xrightarrow{2e^-} H_2O$$

Sources of $O_2^{\cdot-}$

Mitochondria – inefficiency in the electron transport chain

Phagocytic cells – e.g. Macropheges



in phagosomes and cell membranes

Cytochome P450 – uncoupling cycling in absence of substrate

Oxygen Radicals

$O_2^{\cdot -} + H^+ \rightleftharpoons HO_2\cdot \qquad pK_a = 4.6$

$2O_2^{\cdot -} + 2H^+ \xrightarrow[\text{Dismutase}]{\text{Superoxide}} H_2O_2 + O_2 \quad (k_2 = 10^9\ M^{-1}s^{-1})$

$2\,H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$

$H_2O_2 + 2RH \xrightarrow[\text{peroxidase}]{\text{glutathione}} R\text{-}R + 2H_2O$

(Se-Cys)

$H_2O_2 + O_2^{\cdot -} \longrightarrow HO\cdot + OH^- + O_2$ Haber–Weiss Reaction

$O_2^{\cdot -} + Fe^{+3} \longrightarrow Fe^{+2} + O_2$

$Fe^{+2} + H_2O_2 \longrightarrow HO\cdot + OH^- + Fe^{+3}$

$O_2^{\cdot -} + H_2O_2 \longrightarrow O_2 + OH^- + HO\cdot$

} Fenton Reaction

$Cu^+ = 5000\ Fe^{+2}$

$Fe^{+2} + H_2O_2 + H^+ \longrightarrow Fe^{+3}\text{–}\dot{O}H + H_2O$ Ferryl

$Fe^{+3}\text{–}\dot{O}H \longrightarrow Fe^{+3} + HO\cdot$

$Fe^{+2} + H_2O_2 + H^+ \longrightarrow Fe^{+3} + HO\cdot + H_2O$

HO· has $t_{1/2}$ so short that it can diffuse only about a few Angstroms

Lipid Oxidation

initiation: $h\nu, M^{n}$ ⟶ free radicals $R\cdot$

propagation:

$R\cdot + O_2 \longrightarrow ROO\cdot$

$ROO\cdot + RH \longrightarrow ROOH + R\cdot$

termination:

$R\cdot + R\cdot \longrightarrow$

$R\cdot + ROO\cdot \longrightarrow$

$ROO\cdot + ROO\cdot \longrightarrow$

products: $ROOH \longrightarrow RO\cdot + HO\cdot$

↓

aldehydes, ketones, etc

react with proteins, nucleic acids

e.g. linoleate $CH_3(CH_2)_4CH=CH-CH_2-CH=CH-(CH_2)_7COOR$

↓

$-CH=CH-\dot{C}H-CH=CH-$

$-CH=CH-CH=CH-\dot{C}H-$

etc

↓

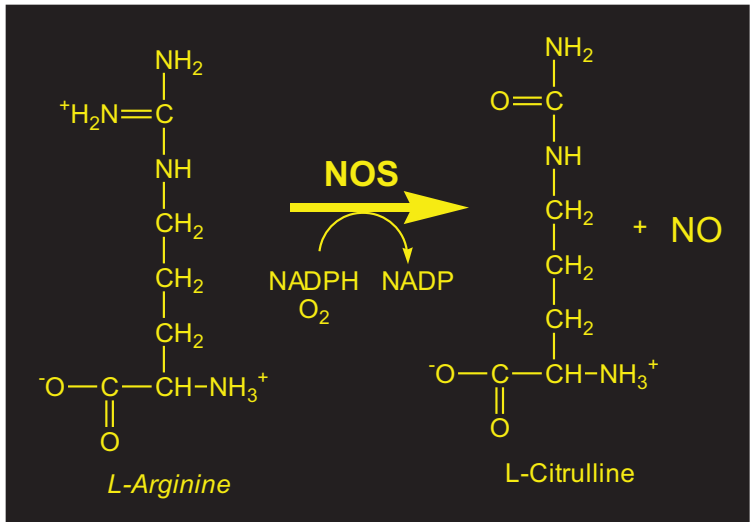
$-CH=CH-CH=CH-CH(OOH)-$ ⟵ $-CH=CH-CH=CH-\dot{C}H-$

$-CH=CH-CH=CH-\dot{C}H-$ (with $O\cdot$)

↓

$-CH=CH-CH=CH\cdot \xrightarrow{O_2}$ ⟶ 3- monenal

Nitric Oxide $NO^{\bullet}$



- Ubiquitous radical produced by
  - ~ *Neurons*: neurotransmitter
  - ~ *Macrophages*: immune response
  - ~ *Vascular endothelial cells*: blood pressure control
- Generated by reaction of nitric oxide synthases (NOS) with *L*-arginine, NADPH and O_2. Reaction inhibited by *N*-methylarginine
- Physiological role of NO is balanced by toxic effects
  - ~ Reacts with O_2 to form N_2O_3: *nucleobase deamination and nitrosamine formation*
  - ~ Reacts with superoxide to form $ONOO^-$: *nucleobase oxidation, protein oxidation and nitration*

Infection, Injury, and Immunity

- Infection and injury, including toxicity cause an inflammatory response
- Inflammation involves the immune system
- The Immune system consists of both innate and adaptive responses
- Innate immunity involves cells that produce reactive chemicals and cytokines
- Adaptive immunity includes antibodies that are formed from neoantigens

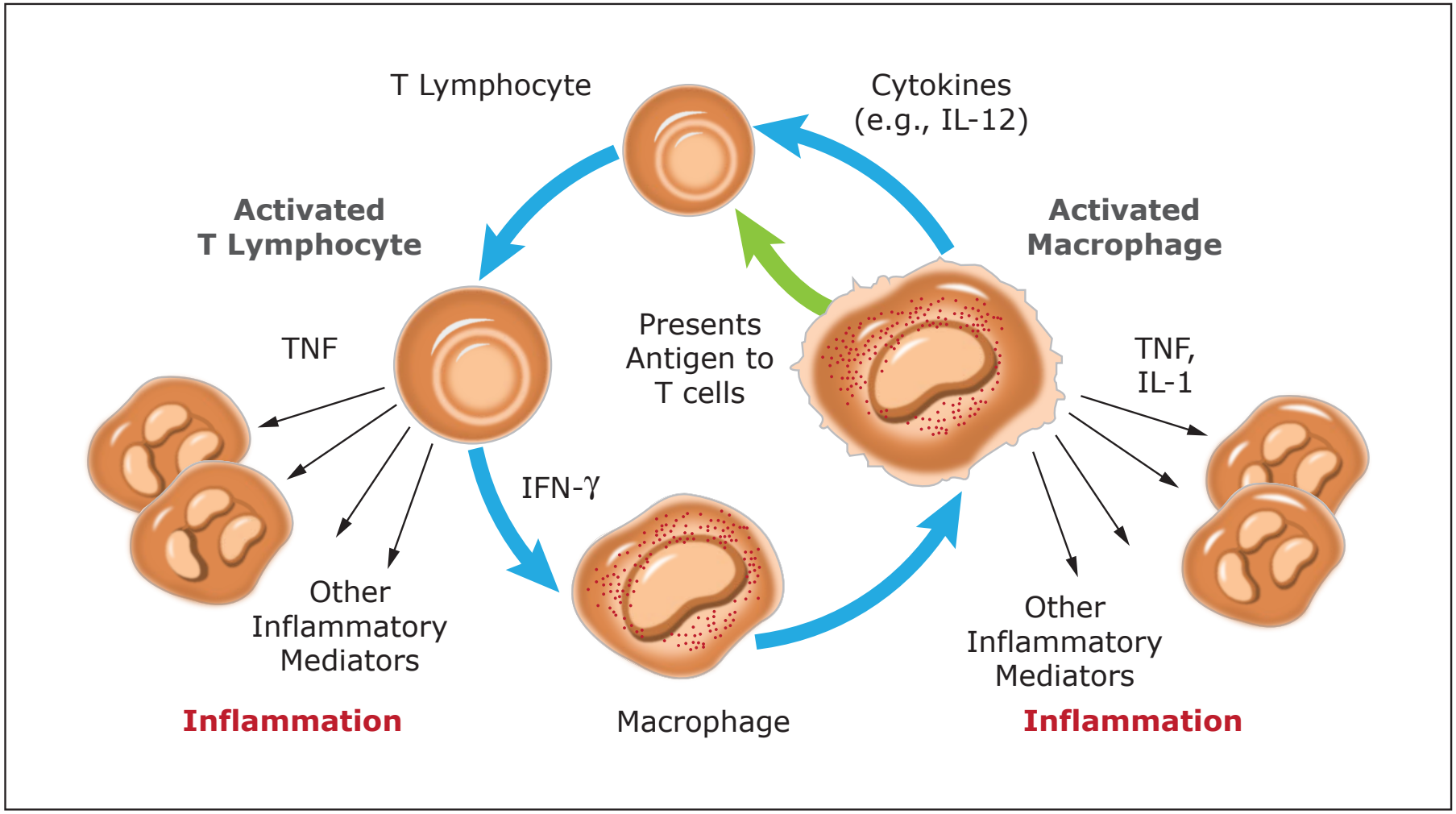


Image by MIT OpenCourseWare.

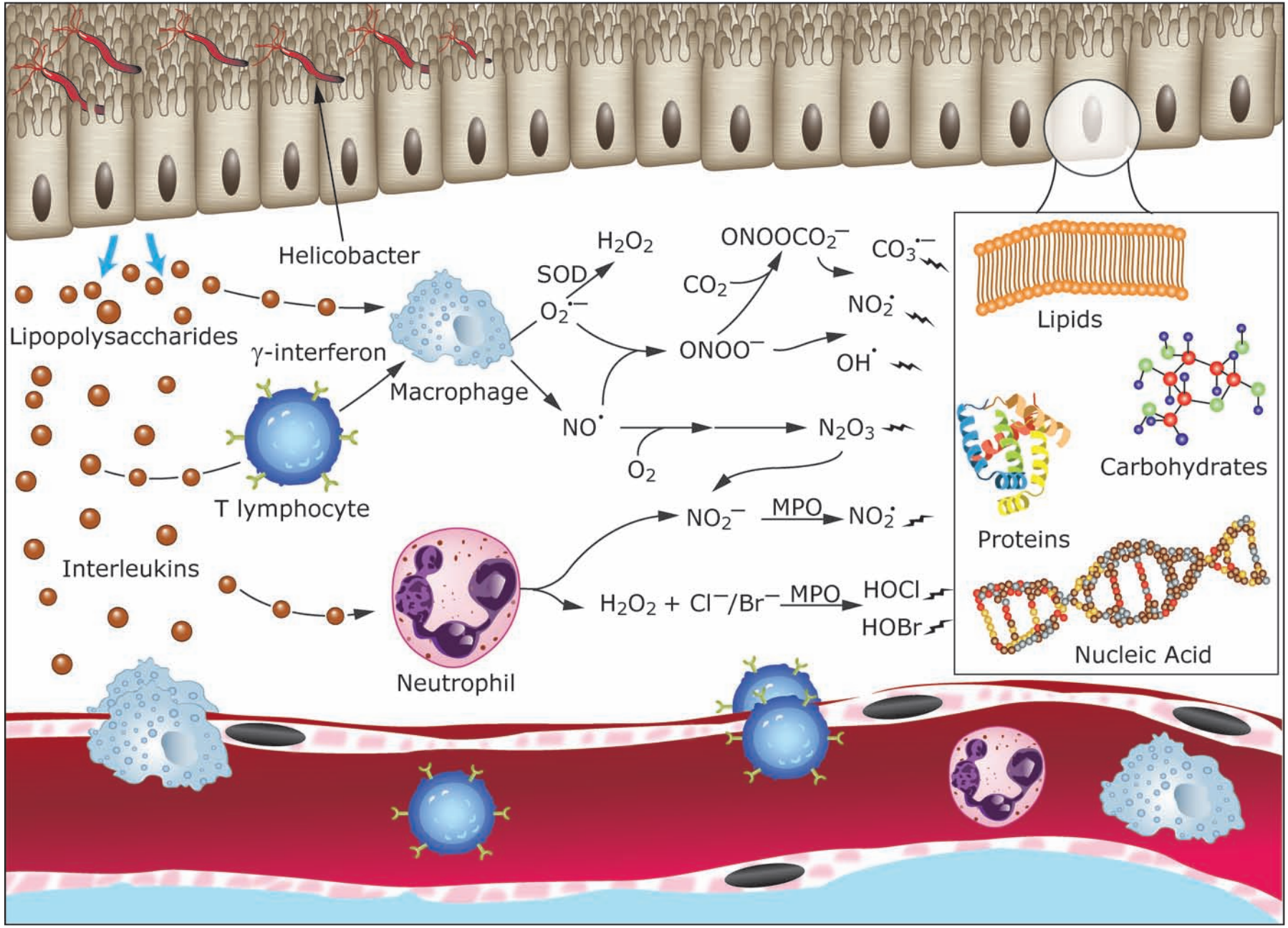
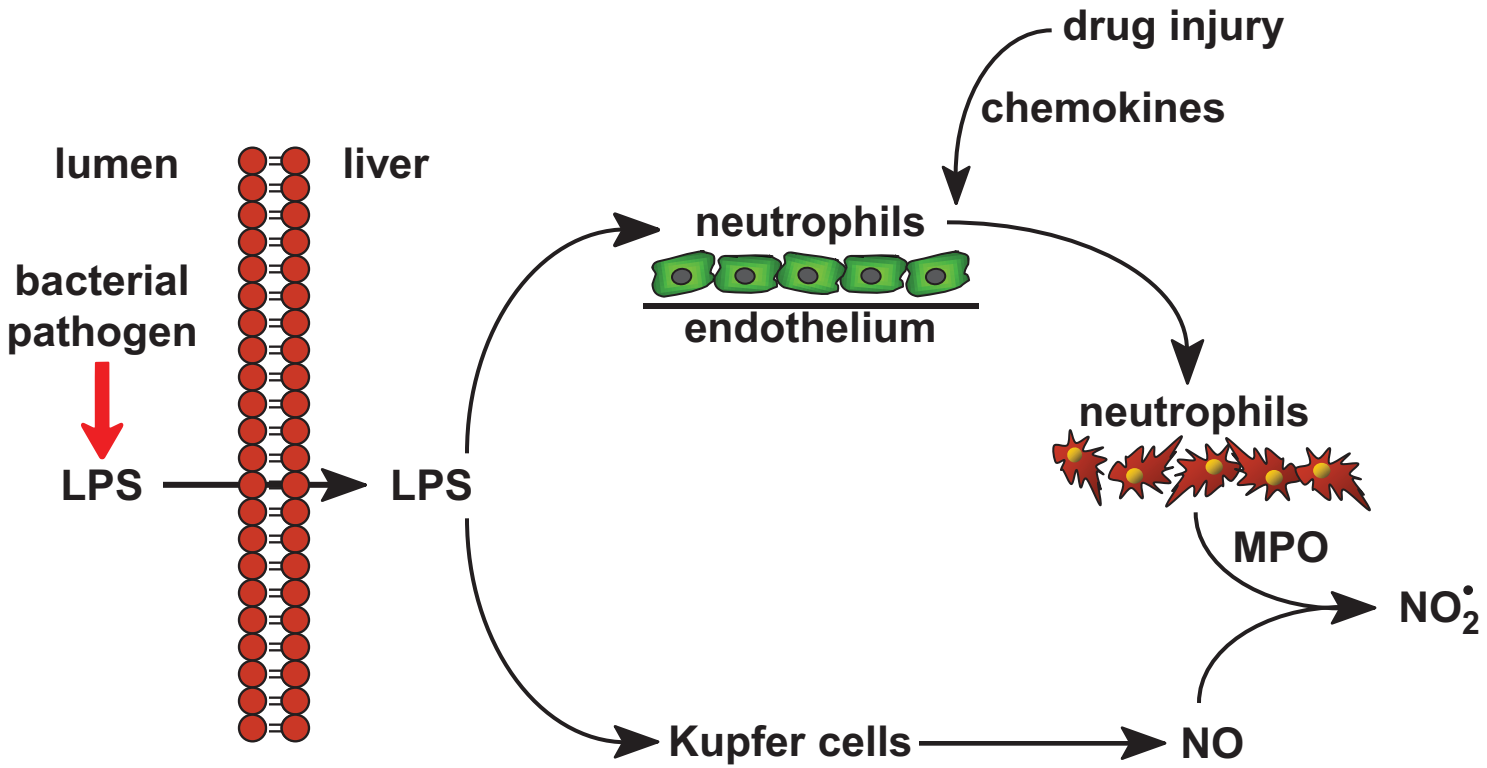
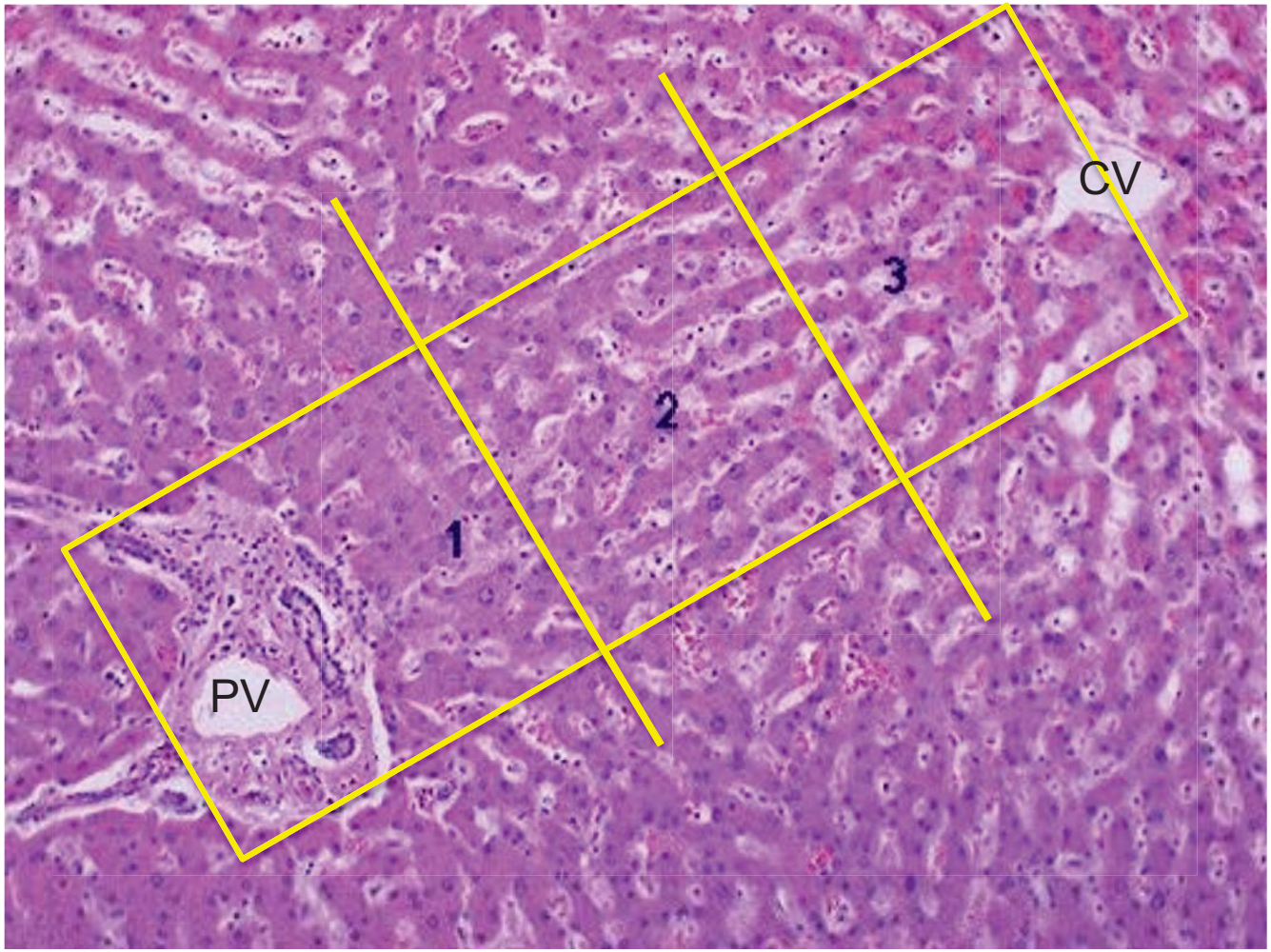


Image by MIT OpenCourseWare.

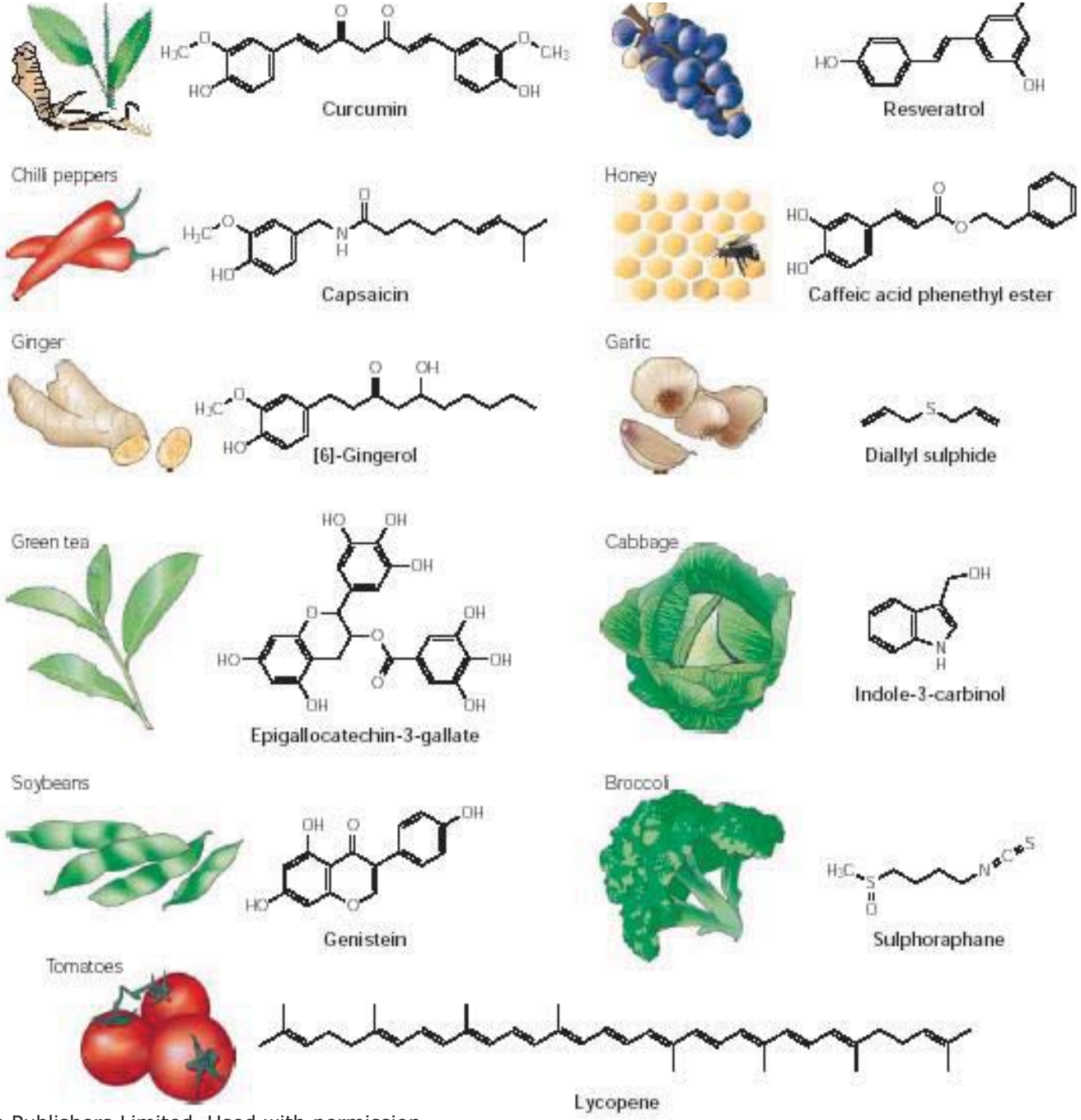

drug injury
chemokines
lumen
liver
neutrophils
bacterial
pathogen
endothelium
neutrophils
LPS
LPS
MPO
$NO_2^{\bullet}$
Kupfer cells
NO

Overview of liver zonal analysis

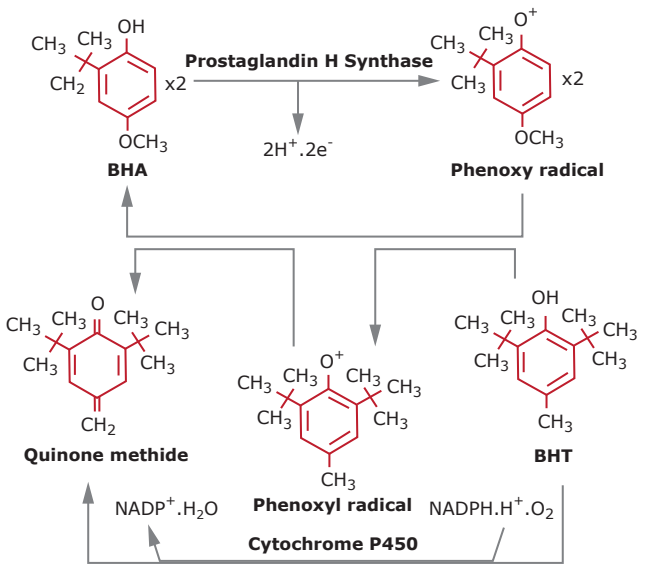


Ascorbic acid

Tocopherols

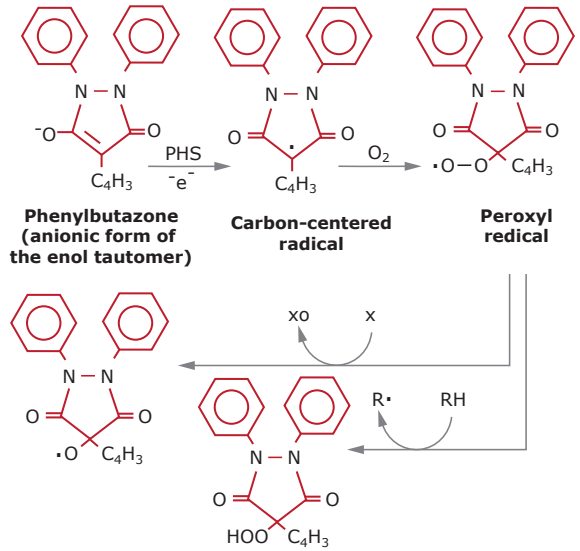


Source: Surh, Young-Joon. "Cancer Chemoprevention with Dietary Phytochemicals." *Nature Reviews Cancer* 3, no. 10 (2003): 768-80.



Metabolic interaction between the phenolic antioxidants, butylated hydroxytoluene (BHT) and butylated hydroxya-nisole (BHA)

Note that activation of BHT to a toxic quinone methide can be catalyzed by cytochrome P450 or. In the presence of BHA, by prostaglandin H synthase.



Oxidation of phenylbutazone by prostaglandin H synthase (PHS) to a carbon-centered radical and peroxyl radical.

Note that the peroxyl radical can oxalic xenobiotics (X) in a peroxidative manner.

Image by MIT OpenCourseWare.

Transcriptional regulation mediated by the Antioxidant Response Element

Background

Rat *GST-A2* and *NAD(P)H:quinone oxidoreductase* (*NQO1*) genes cloned and characterized.

Transcriptional regulation is mediated by two distinct enhancers:

1. **The xenobiotic response element (XRE): confers inducibility to planar aromatic hydrocarbons mediated by Ah receptor.**

2. **The antioxidant response element (ARE): confers inducibility to prooxidants, electrophiles, phenolic antioxidants…**

Glutathione Transferases

- **Three Mammalian Glutathione Transferase Families:**
  - **Cytosolic**
  - **Mitochondrial**
  - **Microsomal**

- **Catalyze the conjugation of glutathione to non polar compounds containing an electrophilic carbon, nitrogen or sulphur atom.**

- **Cytosolic glutathione transferase are encoded by seven gene families:**
  - **In addition to their catalytic activity bind hydrophobic non-substrate ligands**

Glutathione Transferases

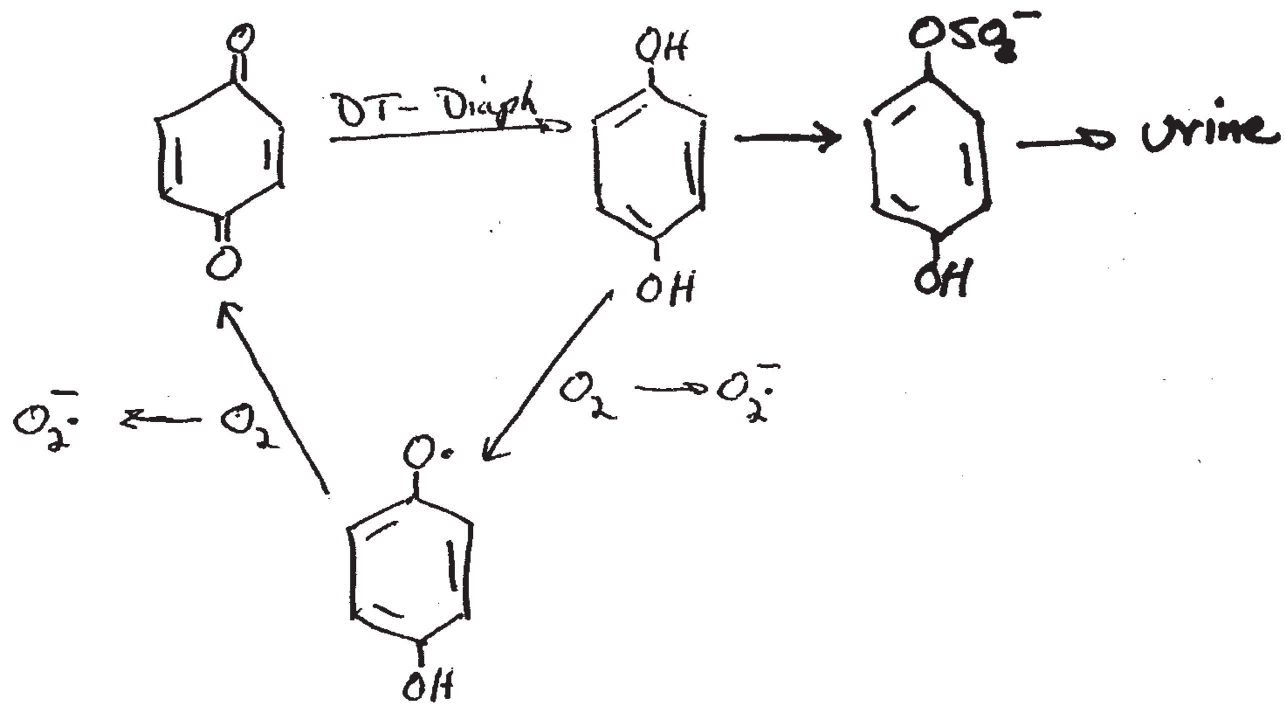
- **Some cytosolic glutathione transferases are elevated in livers of animals exposed to various xenobiotics**

- **Level of expression of glutathione transferases is critical in determining sensitivity of cells to a broad spectrum of toxic compounds**
  - **Carcinogens**
  - **cytotoxic chemotherapeutic drugs**

- **Human glutathione transferases M-1 and T-1 are deleted in 50% and 16% of population, respectively.**
  - **Increased susceptibility to bladder, colon, skin and possibly lung cancer**

NAD(P)H – Quinone Oxidoreductase

- **Cytosolic enzymes (NQO1 and NQO2) that catalyze two electron reduction and detoxication of highly reactive quinones.**
- **NAD(P)H – quinone oxidoreductase maintains the endogenous lipid soluble anti oxidants, alpha tocopherol – hydroquinone and ubiquinol in their reduced and active forms.**
- **Enzymes are elevated in livers of animals exposed to various xenobiotics**
- **NQO1 knockouts have increased toxicity to benzene and benzo(a)pyrene induced skin carcinogenesis**


DT- Diaph
OH
OH
OSO3-
OH
Urine
O2
O2-
O·
OH

20.201 Mechanisms of Drug Actions

Fall 2013