Metabolism and drug design

2018

AD**M**E - **Metabolism**

Organisms posesses a large set of enzymes able to modificate xenobiotics

Original issue is to protect internal environment from toxic agents

Compounds are generally made more hyrophilic to enhance renal or hepatobiliar excretion

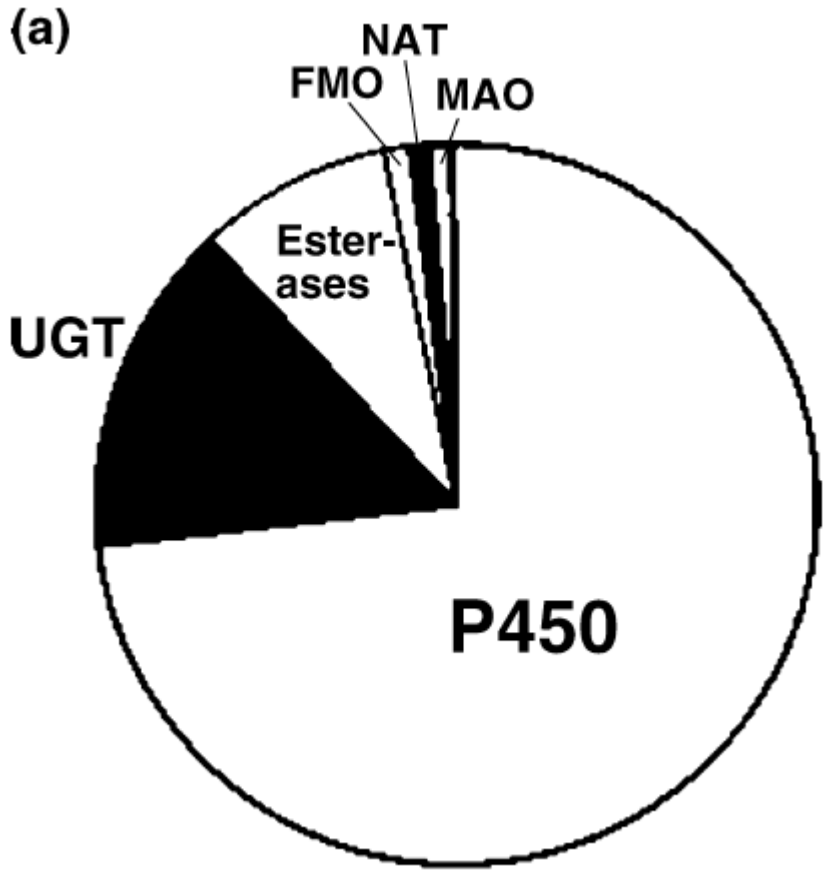
ADME - **Metabolism**

Knowledge of metabolic pathways is crucial

By metabolization, drug can be
- deactivated (most metabolites)
- made more potent (prodrugs)
- converted to reactive form (toxicity)

Enzyme set ready for metabolisation

Fraction of drugs metabolized by various systems

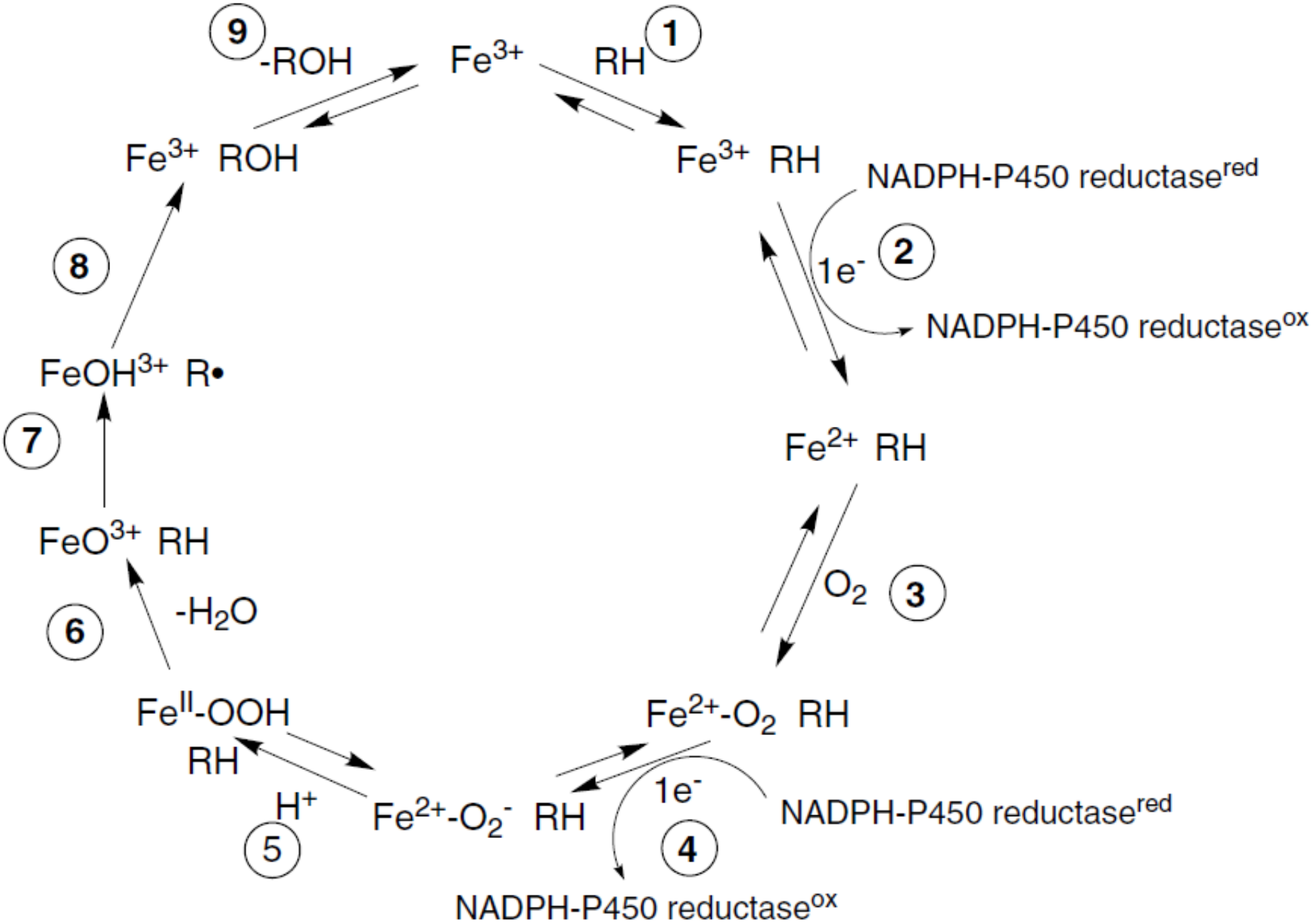


Oxidation enzymes
Cytochrome P450 (CYP, P450)

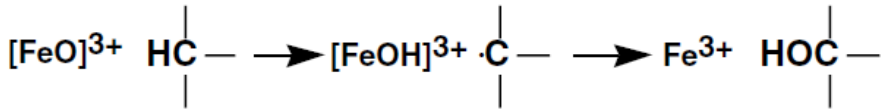
$$NADPH + O_2 + RH \rightarrow ROH + NADP^+ + H_2O$$

often occures rearrangements in structure after primary oxidation

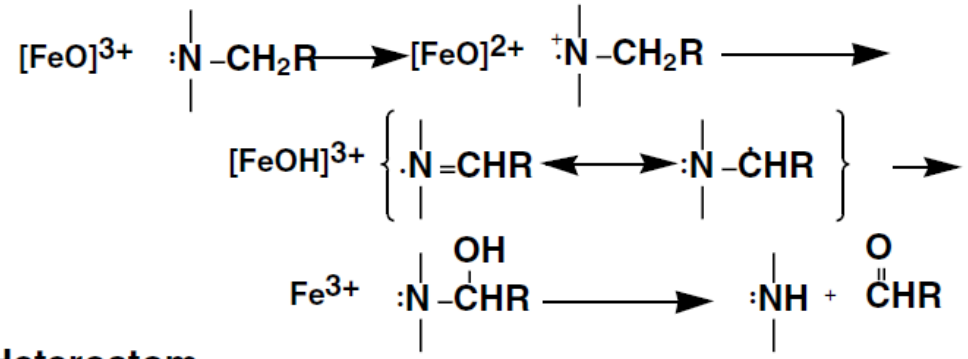
FeO^{3+} involved in mechanism


9
-ROH
Fe3+
RH
1
Fe3+ ROH
Fe3+ RH
NADPH-P450 reductasered
1e-
2
NADPH-P450 reductaseox
8
FeOH3+ R•
7
Fe2+ RH
FeO3+ RH
O2
3
6
-H2O
FeII-OOH
RH
Fe2+-O2 RH
H+
Fe2+-O2- RH
1e-
NADPH-P450 reductasered
5
4
NADPH-P450 reductaseox

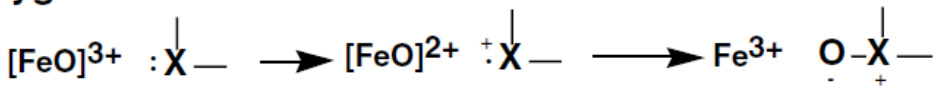
Carbon hydroxylation:



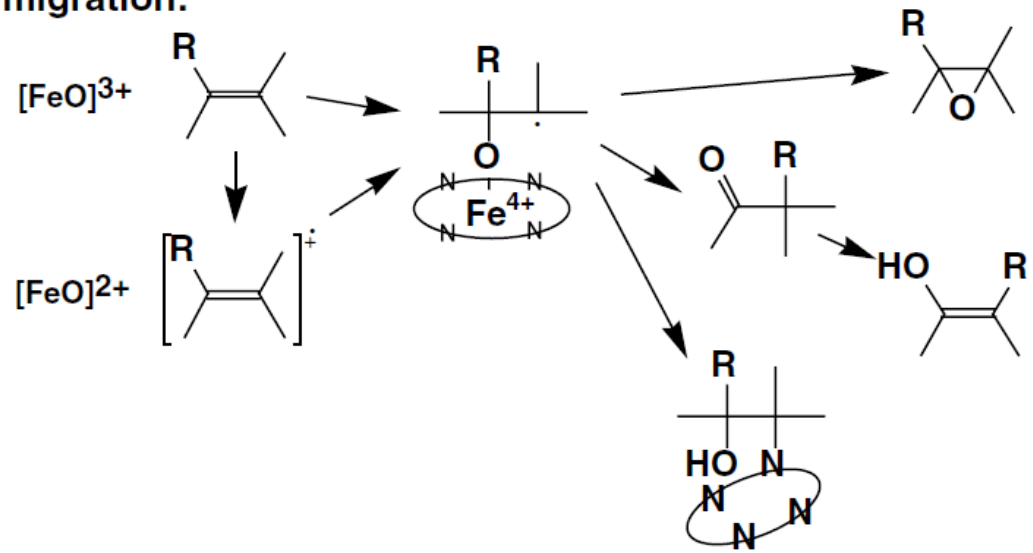
Heteroatom release:



Heteroatom oxygenation:



Epoxidation and Group migration:



Oxidation enzymes

Cytochrome P450 (CYP, P450)

human genome has 57 P450 genes

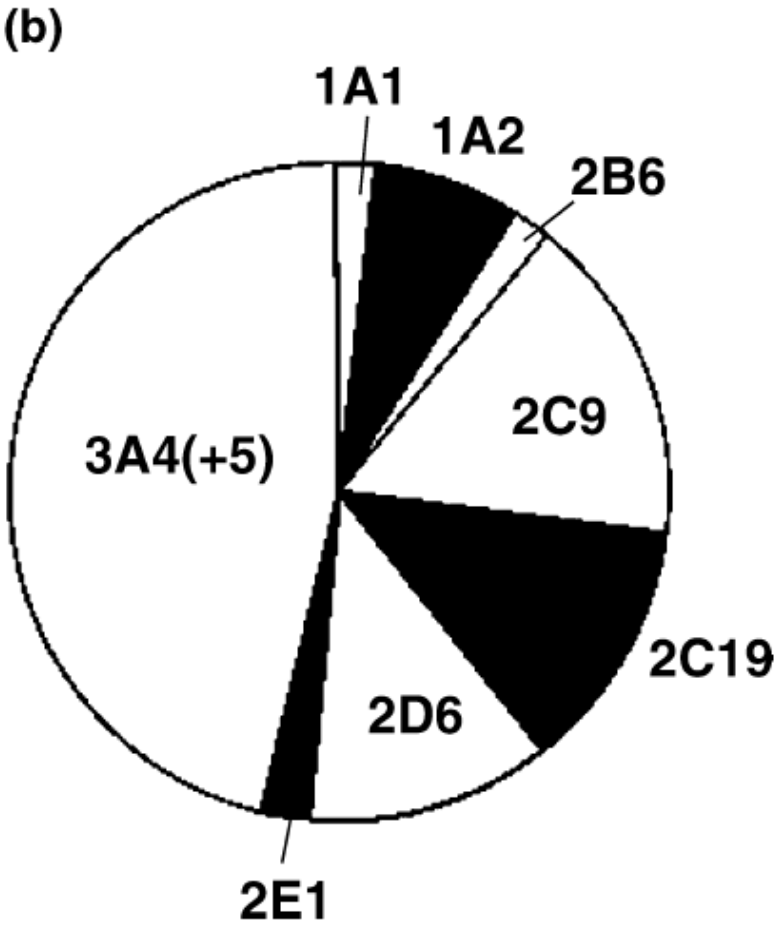
TABLE 2.1 Classification of human P450s based on major substrate class (Guengerich, 2005; Guengerich et al., 2006).

Sterols	Xenobiotics	Fatty acids	Eicosanoids	Vitamins	Unknown
1B1	1A1	2J2	4F2	2R1	2A7
7A1	1A2	4A11	4F3	24A1	2S1
7B1	2A6	4B1	4F8	26A1	2U1
8B1	2A13	4F12	5A1	26B1	2W1
11A1	2B6		8A1	26C1	3A43
11B1	2C8			27B1	4A22
11B2	2C9				4F11
17A1	2C18				4F22
19A1	2C19				4V2
21A2	2D6				4X1
27A1	2E1				4Z1
39A1	2F1				20A1
46A1	3A4				27C1
51A1	3A5				
	3A7				

Oxidation enzymes

Cytochrome P450 (CYP, P450)

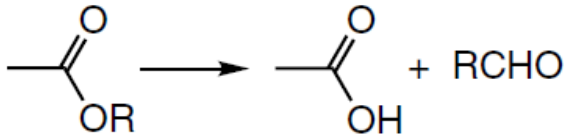
only few are of high importance

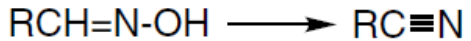


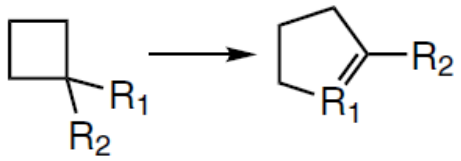
Oxidation enzymes

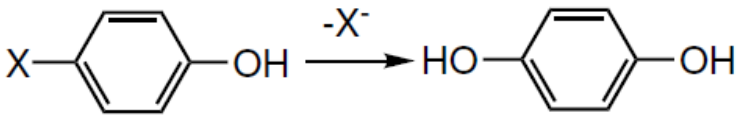
Cytochrome P450 (CYP, P450)

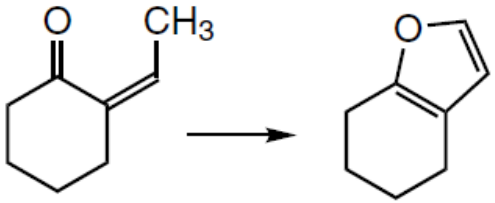
some uncommon reactions

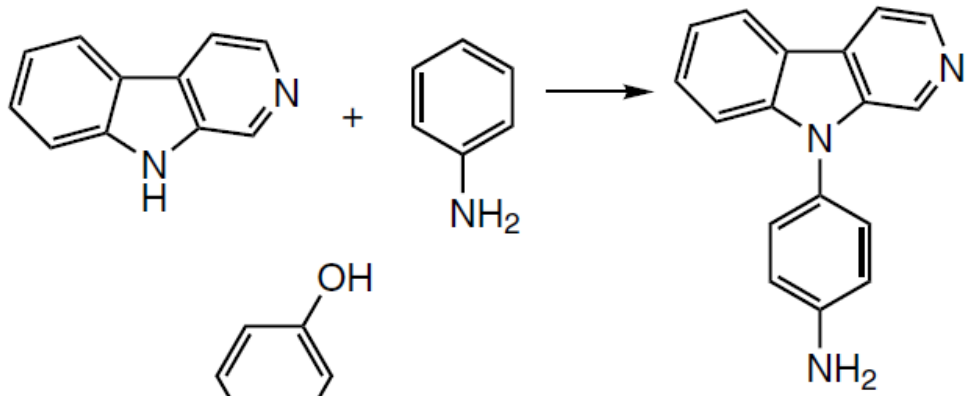


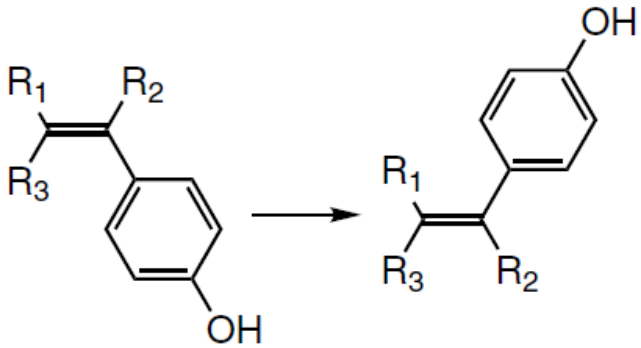












Oxidation enzymes
Cytochrome P450 (CYP, P450)

Reaction	Product	Typical example
Aromatic hydroxylation	Phenyl to phenol	Phenytoin
Aliphatic hydroxylation	Methyl to carbinol	Ibuprofen
N-dealkylation	Tertiary to secondary amine	Lidocaine
O-dealkylation	Ether to alcohol	Naproxen
S-dealkylation	Thioether to thiol	6-methylthiopurine
N-oxidation	Pyridine to pyridine *N*-oxide	Voriconazole
S-oxidation	Sulphoxide to sulphone	Omeprazole
Alcohol oxidation	Alcohol to carboxylic acid	Losartan

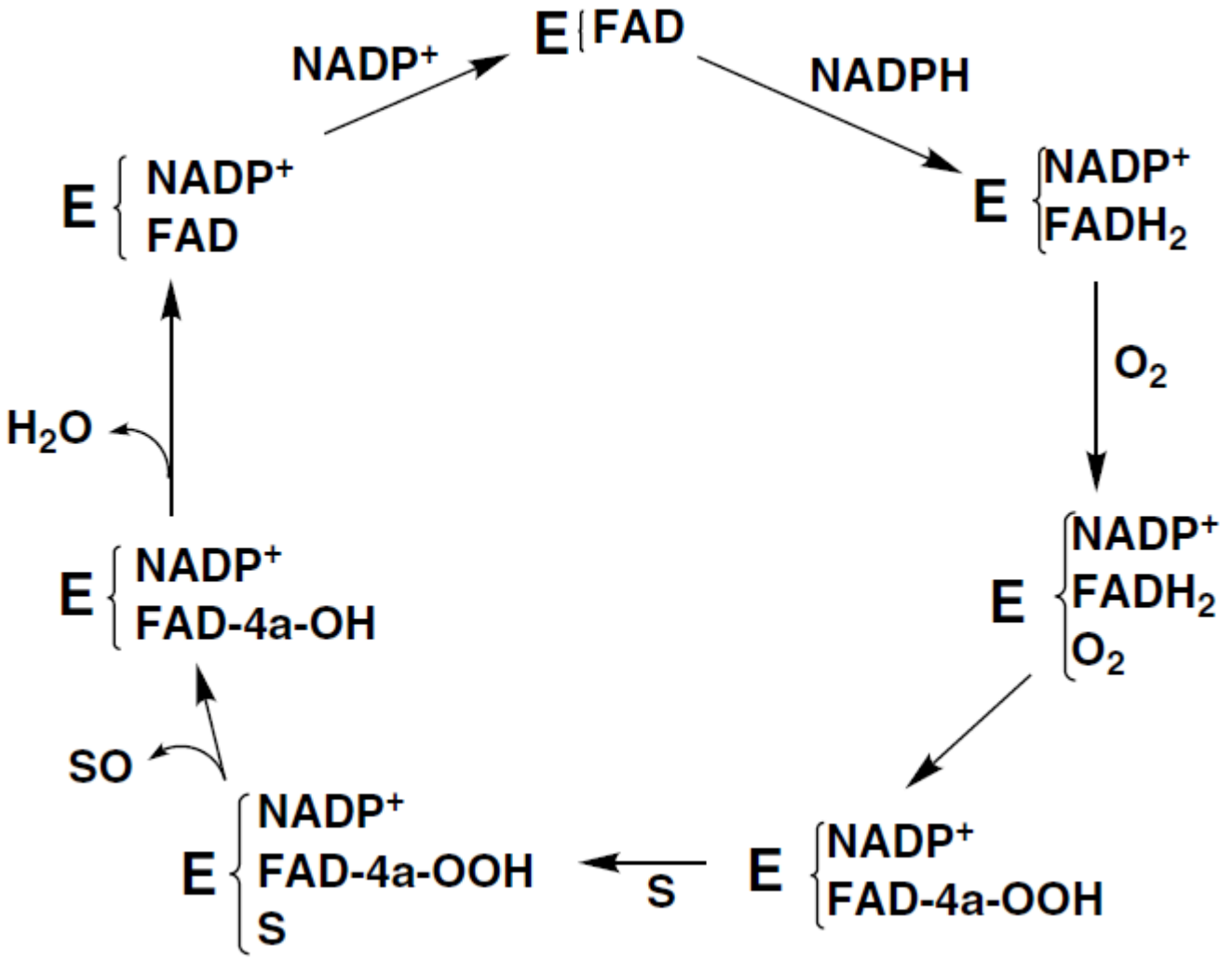
Oxidation enzymes
Flavin-containing Monooxygenase (FMO)

7 forms of FMO, located in endoplasmatic reticulum

distincly from CYP P450, only soft nucleophiles are substrates (N, S, P in phosphines)

$$\mathrm{NADPH} + \mathrm{O_2} + \mathrm{RX} \rightarrow \mathrm{RXO} + \mathrm{NADP^+} + \mathrm{H_2O}$$
$$(\mathrm{X} = \mathrm{N}, \mathrm{X}, \mathrm{P})$$

Oxidation enzymes
Flavin-containing Monooxygenase (FMO)



Oxidation enzymes
Monoamine Oxidase (MAO)

two forms – MAO A and MAO B

present in mitochondrial membrane of hepatocytes and neurons

flavoprotein oxidase protein as FMO, but with different mechanism releasing ammonia and hydrogen peroxide

$$RCH_2NH_2 + O_2 \rightarrow RCHO + H_2O_2 + NH_3$$

Oxidation enzymes
Aldehyde Oxidase
Xanthine Dehydrogenase

contains molbdenum and iron in active center
present in cytosol, mainly in hepatocytes

$$RH + H_2O \rightarrow ROH + 2e^-$$

electron can be transferred to an oxidized pyridine nucleoside or to oxygen forming H_2O_2

substrates are: various aldehydes
heterocycles containing N
(purines are substrate for both enzymes)

Oxidation enzymes
Peroxidases

similar mechanism with CYP system involving FeO generating of radical

$$RX + RX^{\bullet} \rightarrow \ldots$$

radical further undergoes propagation, dimer formation or dealkylation

Oxidation enzymes
Alcohol Dehydrogenases (ADH)

concentrated in liver, specifity for primary and some secondary alcohols
reaction is generally reversible

$$RCH_2OH + NAD^+ \leftrightarrow RCH{=}O + NADH + H^+$$

at least 7 different genes for ADHs

Oxidation enzymes
Aldehyde Dehydrogenases (ALDH)

concentrated in liver, mainly in mitochondria

$$RCHO + NAD^+ \rightarrow RCO_2H + NADH + H^+$$

at least 19 genes for ALDHs are known

Reduction enzymes
CYP P450, ADH

both can catalyze reductions of aldehydes and imines

$$RCHO + NAD(P)H + H^+ \rightarrow RCH_2OH + NADP^+$$

these reverse reactions occures in environments with low O_2 tension (e.g. venous section of liver)

Reduction enzymes
CYP P450, ADH

$$RNO_2 \rightarrow R{-}N{=}O$$

$$R{-}N{=}O \rightarrow RNHOH$$

$$RNHOH \rightarrow RNH_2$$

$$R_3N^+O^- \rightarrow R_3N$$

$$R{-}N{=}N{-}R' \rightarrow R{-}NH_2 + H_2N{-}R'$$

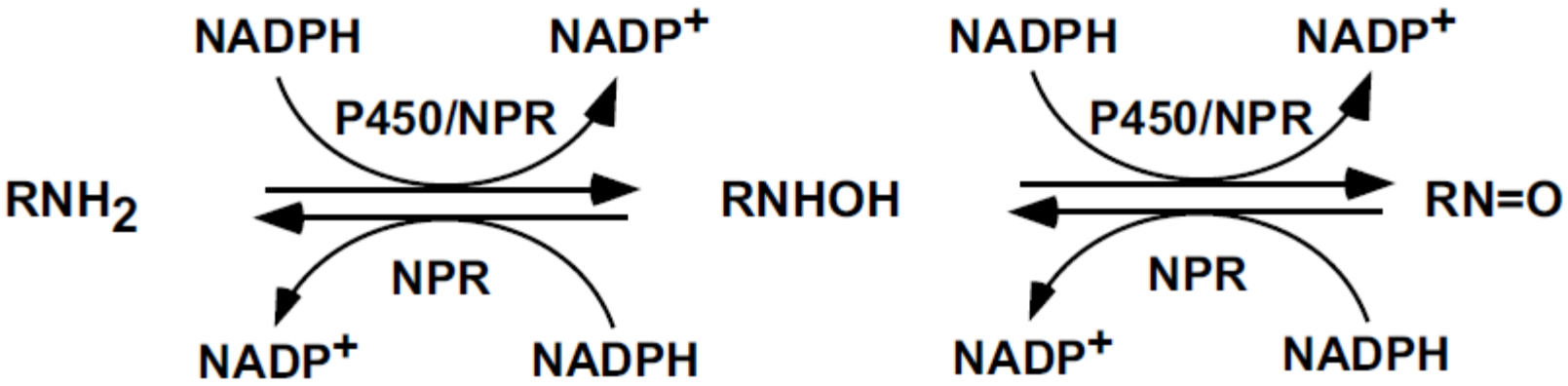
$$CCl_4 \rightarrow CCl_3 \cdot + Cl^-$$

Benzo[*a*]pyrene 4,5-oxide → benzo[*a*]pyrene

Halothane → 2-Cl-l,l,l-F_3 ethane + 2-C1-1,l-F_2 ethylene

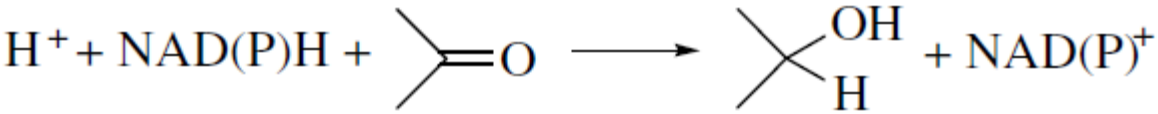
Reduction enzymes
NADPH-P450 Reductase

main function is to restore P450 system, but has enzymatic activity to some P450 substrates as well



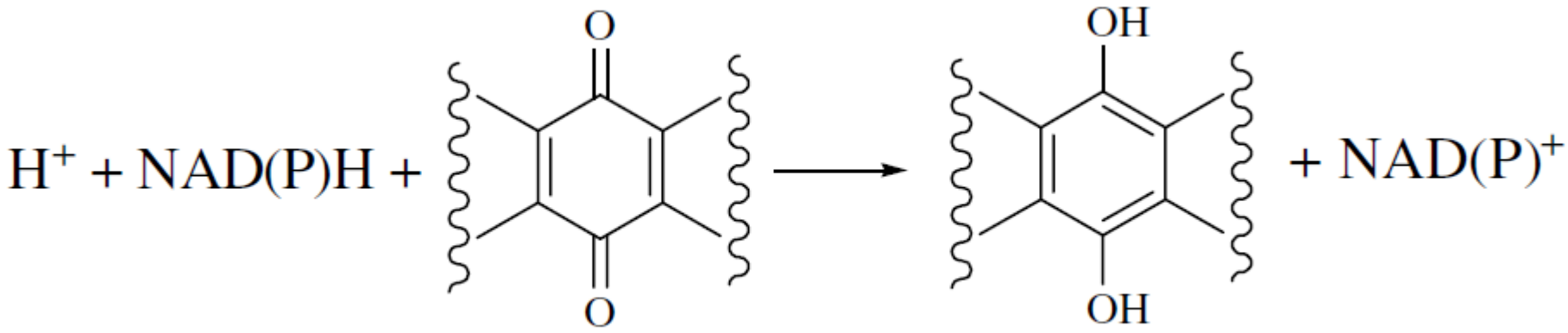
Reduction enzymes
Aldo-Keto Reductase (AKR)

substrates are mainly sugar aldehydes, steroids, prostaglandines



Reduction enzymes
Quinone Reductase (NQO)

present in cytosol, substrates are quinones (both ortho- and para-), iminoquinones, nitro- and azo-compounds



Reduction enzymes
Glutathione Peroxidase (GPX)

reduces hydroperoxides including H_2O_2
six different enzymes, most of them contains selenocystein in the active site

$$2GSH + ROOH \rightarrow GSSG + ROH$$

restored by GSH reductase

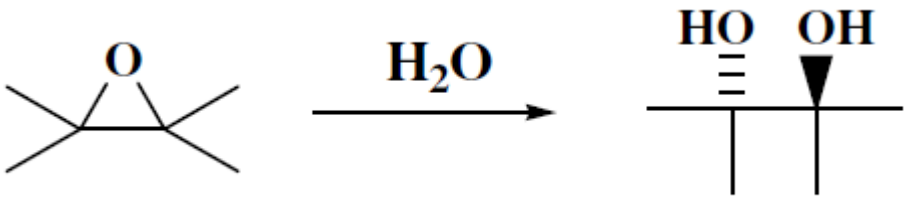
$$NADPH + H^+ + GSSG \rightarrow NADP^+ + 2GSH$$

overall reaction can be written as

$$NADPH + H^+ + ROOH \rightarrow NADP^+ + ROH$$

Hydrolysis enzymes
Epoxide Hydrolase (GPX)

located in microsomes and endoplasmic reticulum
catalyses simple addition of water to epoxide:



Hydrolysis enzymes
Esterases and Amidases

heterogenous group of enzymes with similar basic mechanism of action
present in all environments in the organism

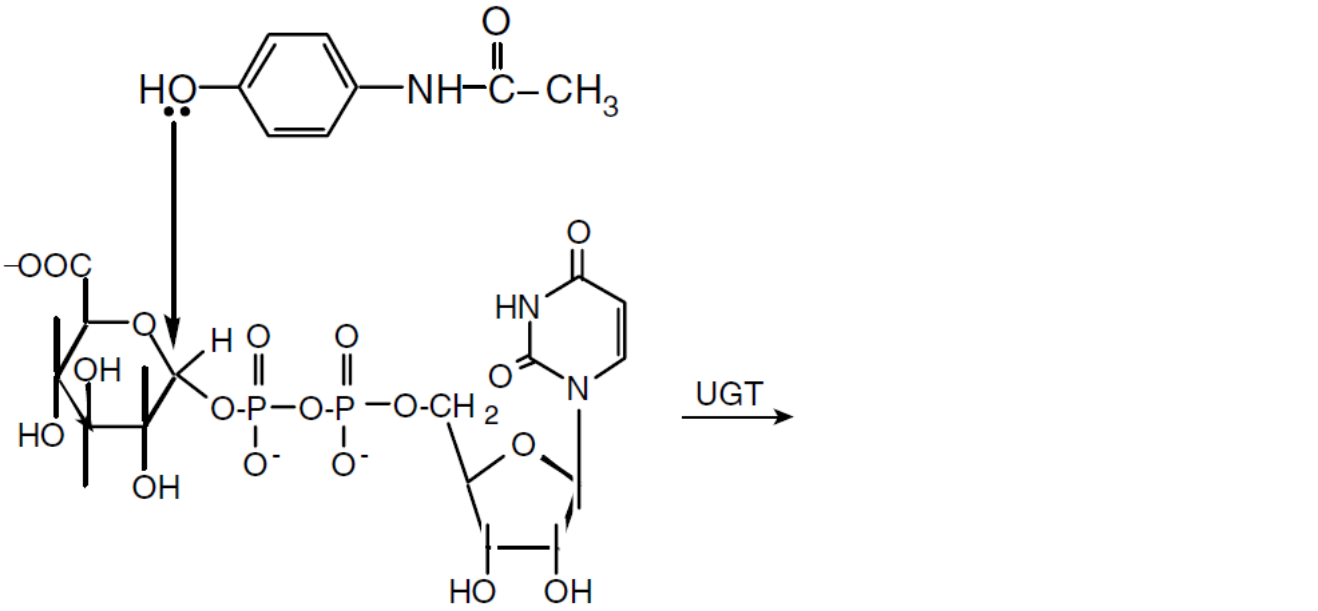
most important:
lipases
acetylcholin esterase (ACHE)
butylcholin esterase

Conjugation enzymes
UDP-Glucuronosyl Transferases (UGT)

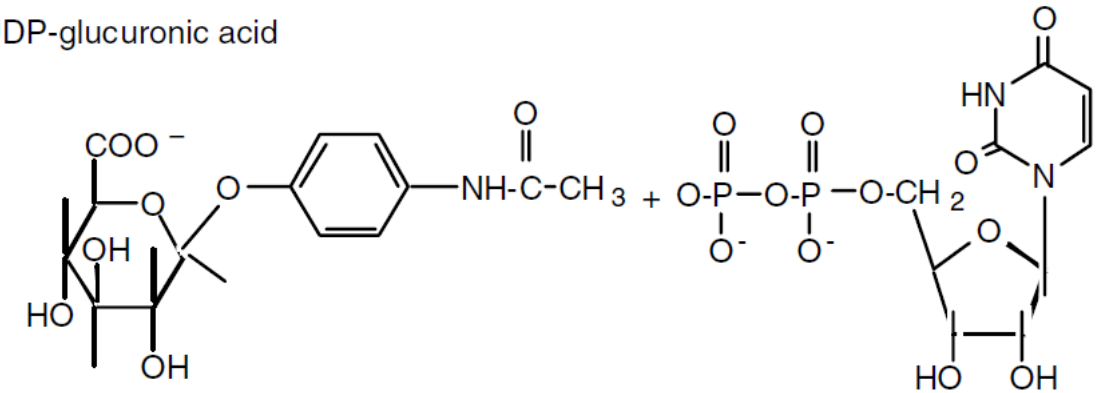
conjugation of glucuronic acid to various functional groups:
alcohols, phenols, amines, heterocyclic nitrogens, amides thiols, acids

conjugates are:
- more polar
- ionized at physiological pH
- have increased Mr
- are actively excreted by carriers in liver and kidney

Conjugation enzymes
UDP-Glucuronosyl Transferases (UGT)



UDP-glucuronic acid



Acetaminophen glucuronide

UDP

Conjugation enzymes
UDP-Glucuronosyl Transferases (UGT)

endogenous substrates:
bilirubin, steroids, lipids, leucotrienes, thyroid hormones, vitamines A, D

three major gene families:

- UGT1—various forms catalyze conjugation of planar phenols, bulky phenols, amines, tertiary amines, and bilirubin. (Nine active human forms now cloned are expressed, i.e., 1A1, 1A3–1A10).
- UGT2A—olfactory (nasal) UGTs.
- UGT2B—xenobiotics, steroids and bile acids ($\geq$4 human active enzymes, i.e., 2B4, 2B7, 2B10, 2B15, 2B17).

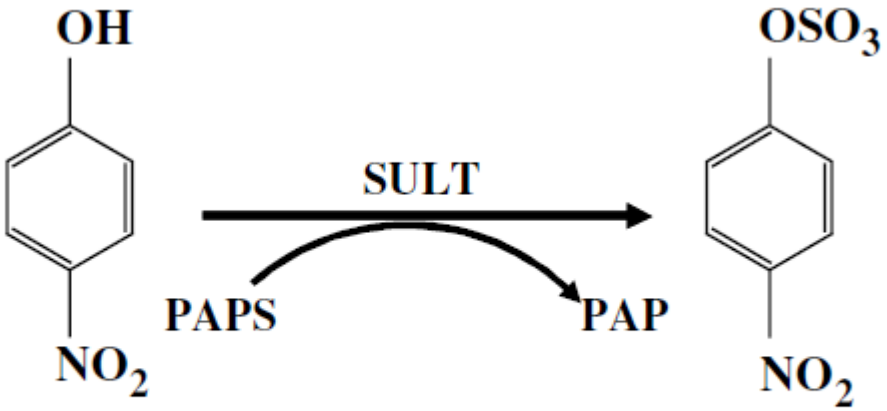
Conjugation enzymes
UDP-Glucuronosyl Transferases (UGT)

Function	Typical example
Aliphatic hydroxyl	Tiaramide
Phenol	Morphine
Aromatic carboxyl	Furosemide
Aromatic tetrozole	Losartan
Aliphatic carboxyl	Benoxaprofen
Immidazole	Tioconazole
Aromatic amine	Dapsone
Tertiary amine	Chlorpromazine
Triazine	Lamotrigine

Conjugation enzymes
Cytosolic Sulfotransferases (SULT)

works with cosubstrate PAPS (3-phosphoadenosin-5-phosphosulfate)

both O-sulfates and N-sulfates can be formed

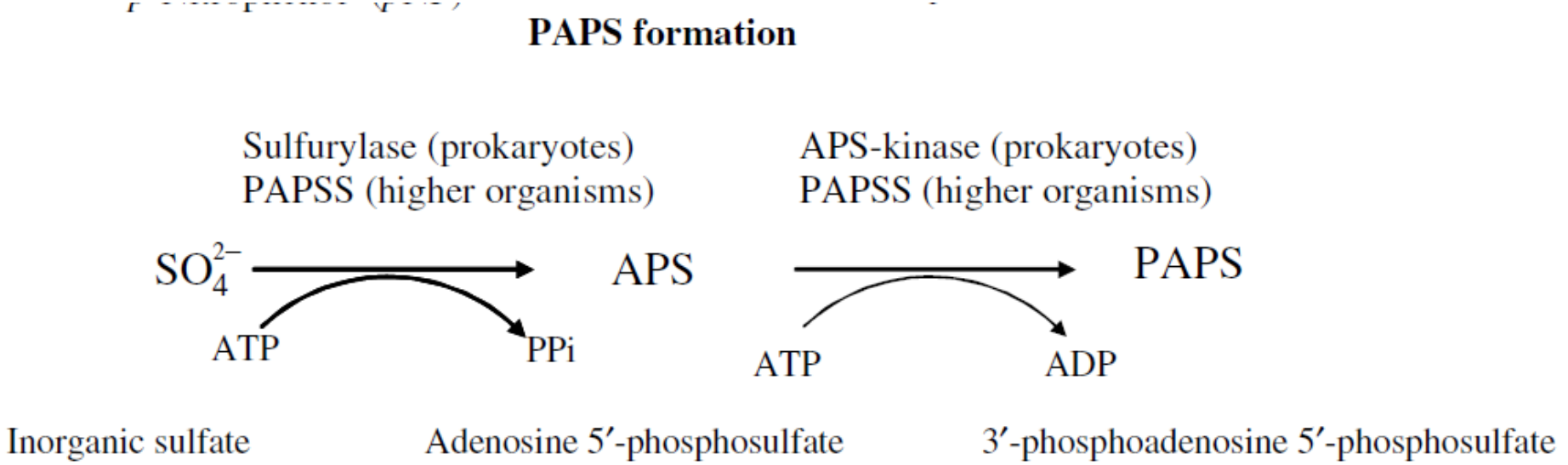


p-Nitrophenol (*p*NP)

*p*NP-sulfate

Conjugation enzymes
Cytosolic Sulfotransferases (SULT)

works with cosubstrate PAPS (3-phosphoadenosin-5-phosphosulfate)



Conjugation enzymes
Cytosolic Sulfotransferases (SULT)

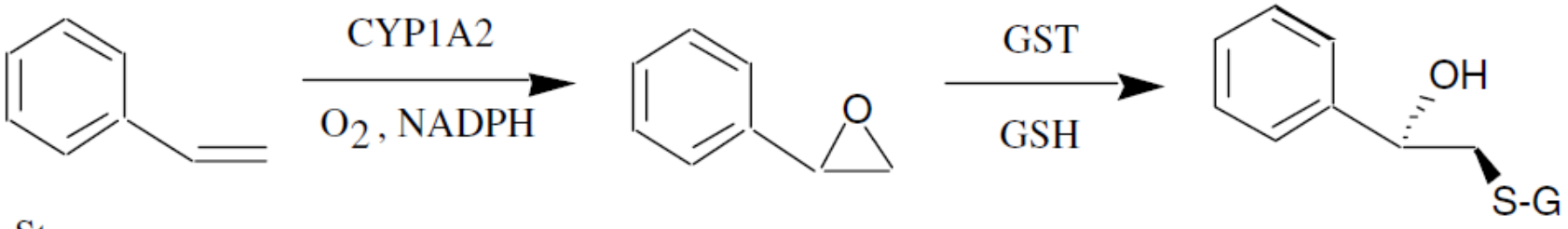
Human SULT cDNA	Substrates
SULT1A1	Simple phenols 17β-estradiol
	Iodothyronines Acetaminophen
	Minoxidil
	17α-ethinylestradiol Isoflavones
	Hydroxy-tamoxifen
SULT1A2	Catecholestrogens
	Simple phenols
SULT1A3	Dopamine (catecholamines)
	Tyramine
	Serotonin
	Salbutamol
	Isoprenaline
	Dobutamine
	Hydroxylated tibolone
	4-Hydroxypropranalol
SULT1B1	Simple phenols
	Catechols
	Iodothyronines
	0-desmethylnaproxen

Human SULT cDNA	Substrates
SULT1C2	*N*-hydroxy-2-acetylaminfluoren
SULT1C4	*N*-hydroxy-2-acetylaminfluoren
SULT1E1	Estrone
	17β-Estradiol
	17α-Ethinylestradiol
	Equilenin
	Diethylstilbestrol
	Thyroxine
	0-desmethylnaproxen
	3-OH-benozo[*a*]pyrene
	Phytoestrogens
SULT2A1	DHEA
	Pregnolone
	Cholesterol
	Cortisol
	Testosterone
	Bile salts
	PAHs (benzylic alcohols)
	Hydroxy-tamoxifen
SULT2B1_v1	DHEA
	Pregnenolone
	3β-Hydroxy steroids
SULT2B1_v2	DHEA
	Pregnenolone
	3β-Hydroxy steroids

Conjugation enzymes
Glutathione-S-Transferases (GTS)

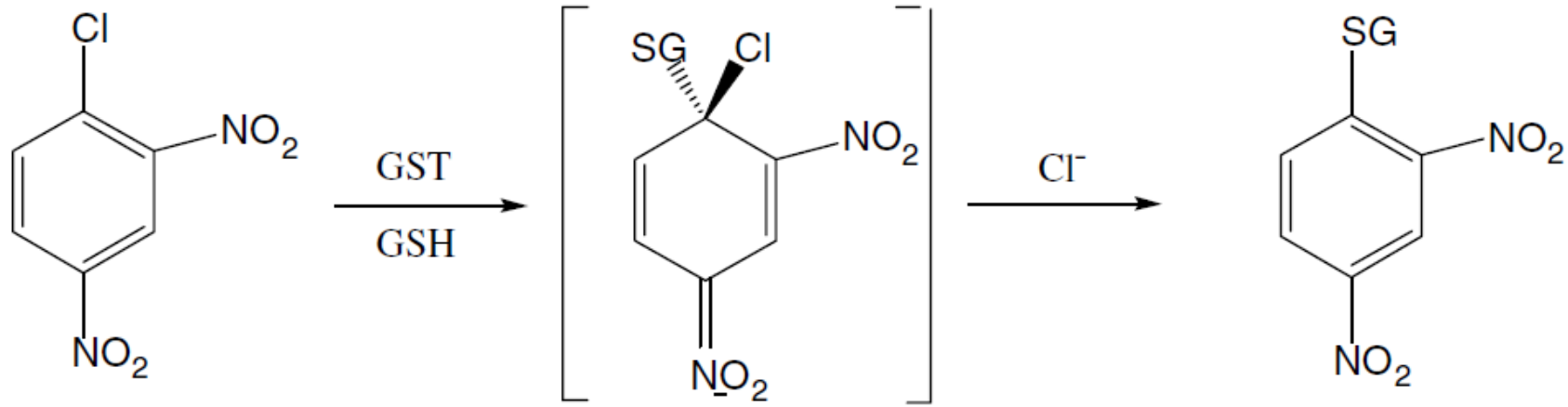
- glutathione is endogenous nucleiophile
- attacs electrophilic molecules
- glutathione conjugates are excreted by transporters into bile
- six major classes if GTS

Conjugation enzymes
Glutathione-S-Transferases (GTS)



Styrene

Styrene oxide

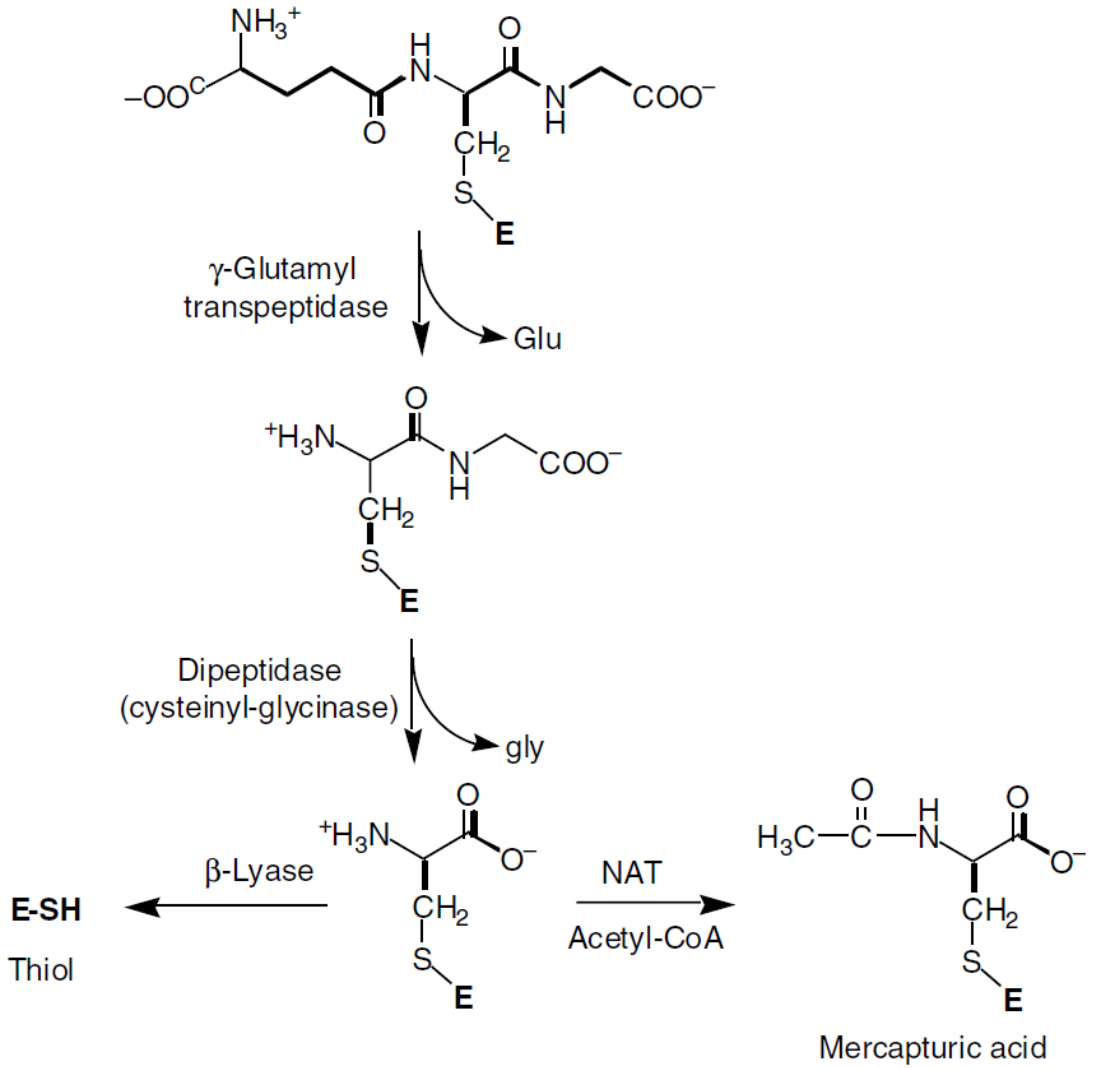


1-Chloro-2,4-dinitrobenzene (CDNB)

Intermediate σ-complex

Conjugation enzymes
Glutathione-S-Transferases (GTS)

conjugates may
be further converted
to N-acetylcystein,
mercaptouric
or thiol derivatives
and excreted
by urine



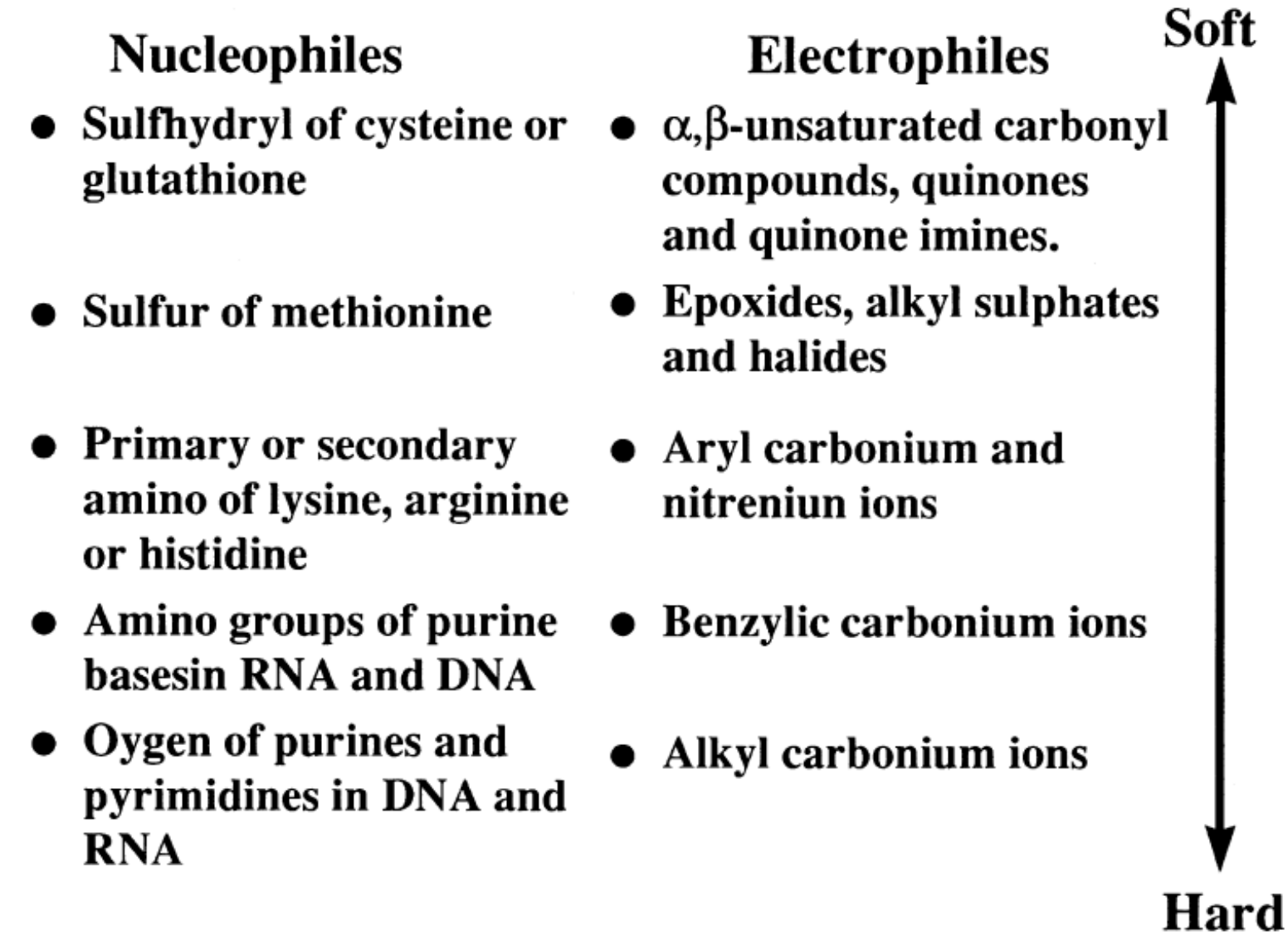
Metabolism-induced toxicity

Part of molecule is altered by oxidation, reduction or conjugation to form a reactive electrophile

Electrophiles reacts with internal nucleophiles (proteins, nucleic acids, small peptides)

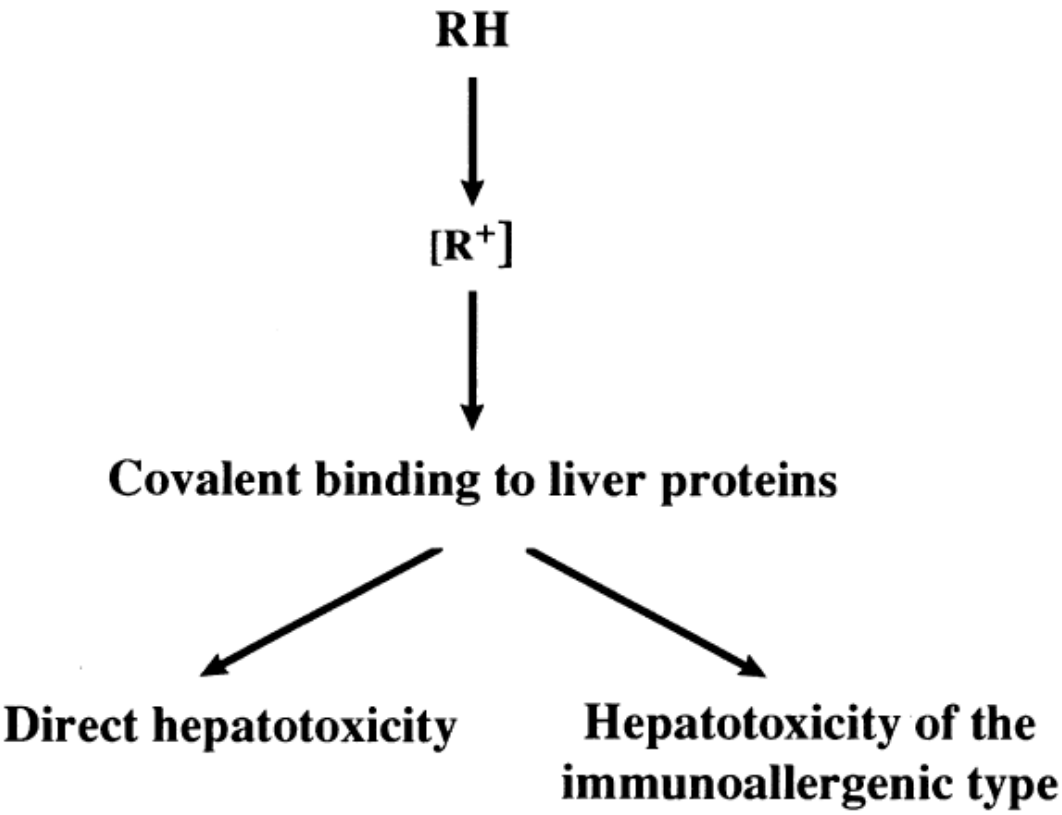
Alkylation of such structures may cause organ toxicity and cancerogenity.

Metabolism-induced toxicity



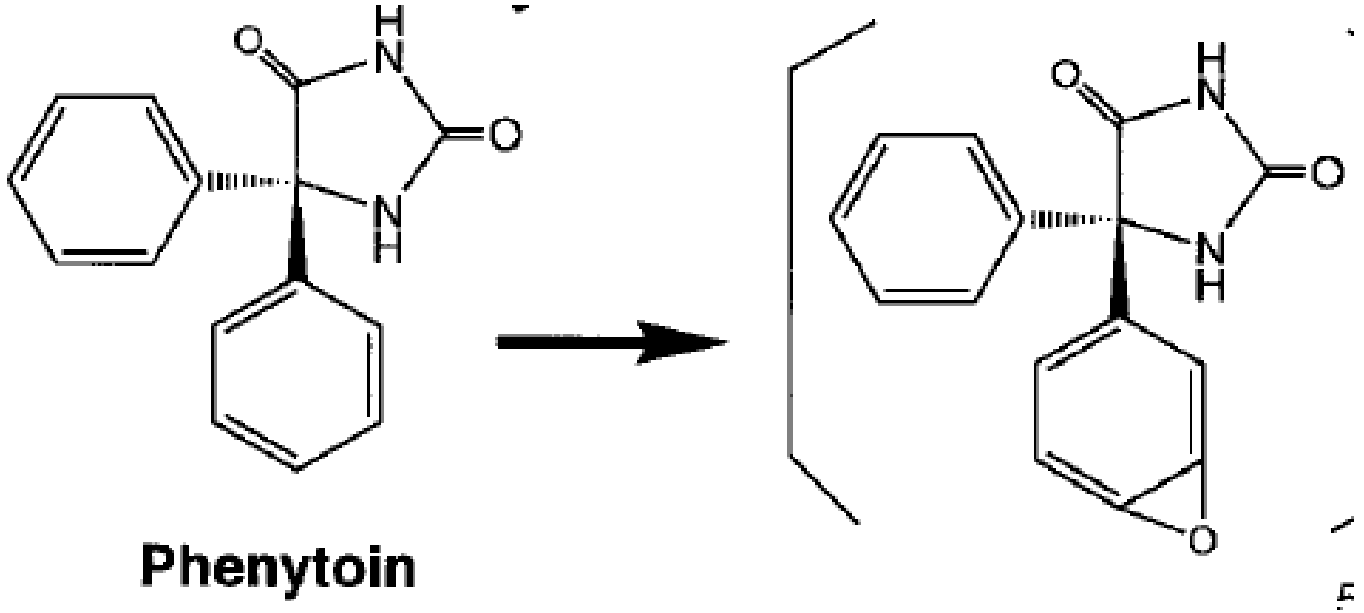
Metabolism-induced toxicity

two kinds of toxicity – altering protein functions
- triggering immunity reaction



Toxicophores
Epoxides

phenytoin is metabolized by P450 to epoxide metabolite causing hepatic necrosis and aplastic anaemia



Toxicophores

Epoxides

carbamazepine epoxide causes teratogenicity and skin rash

oxcarbamazepine is much less toxic

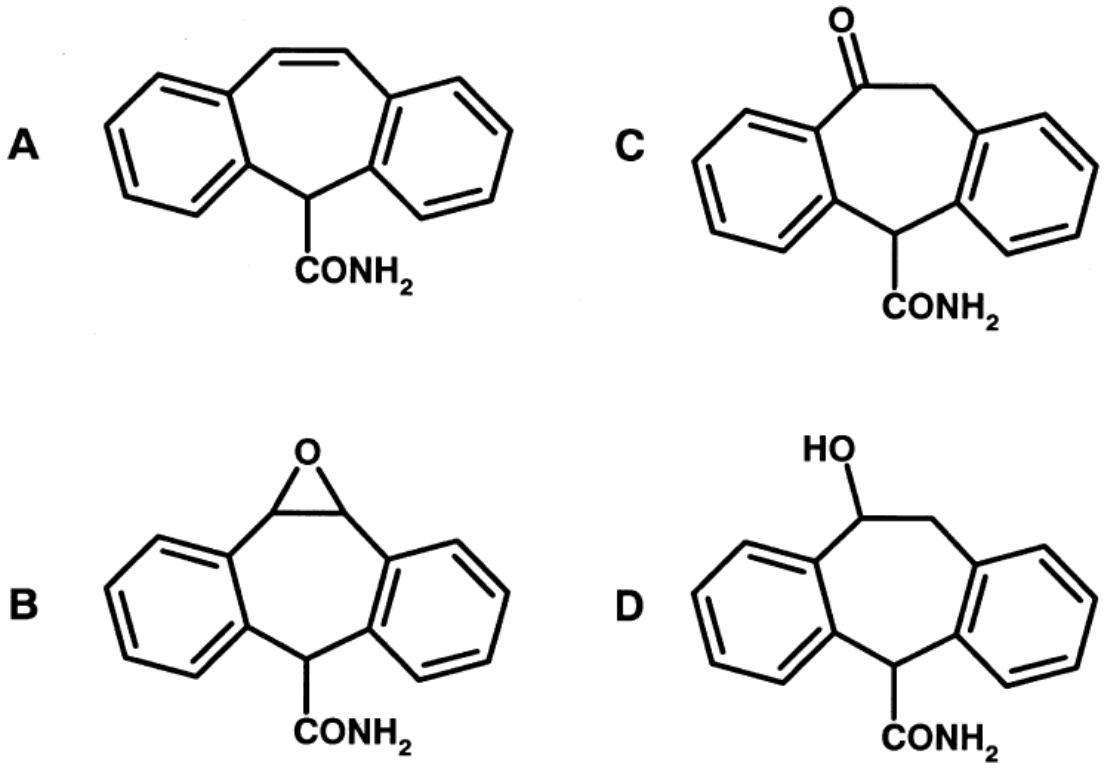
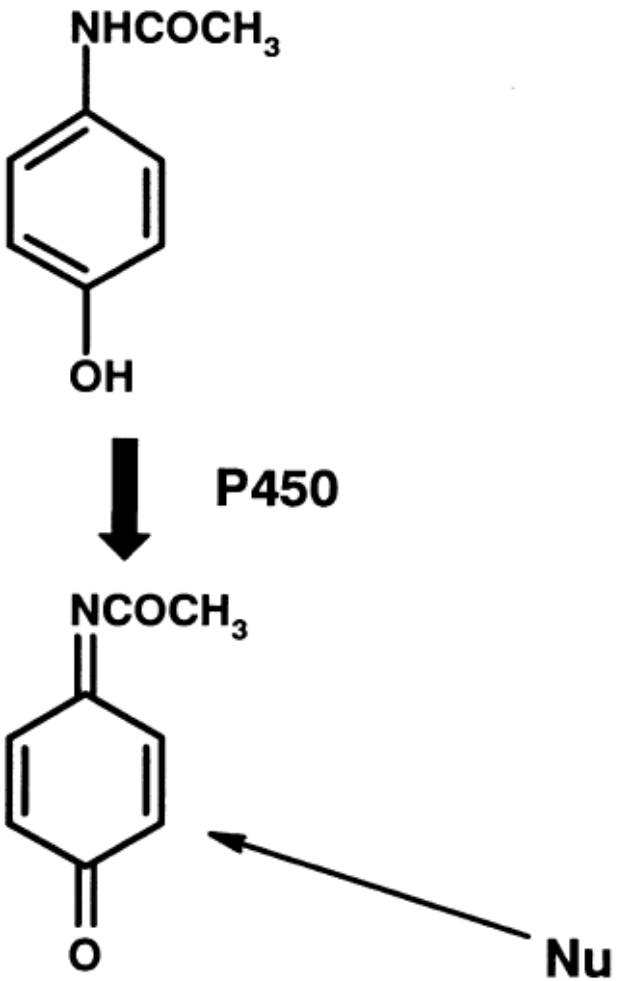


Fig. 8.7 Structures of carbamezepine (A), its 10-11-epoxide metabolite (B), and oxcarbazepine (C) and its hydroxyl metabolite (D).

Toxicophores

Quinone Imines

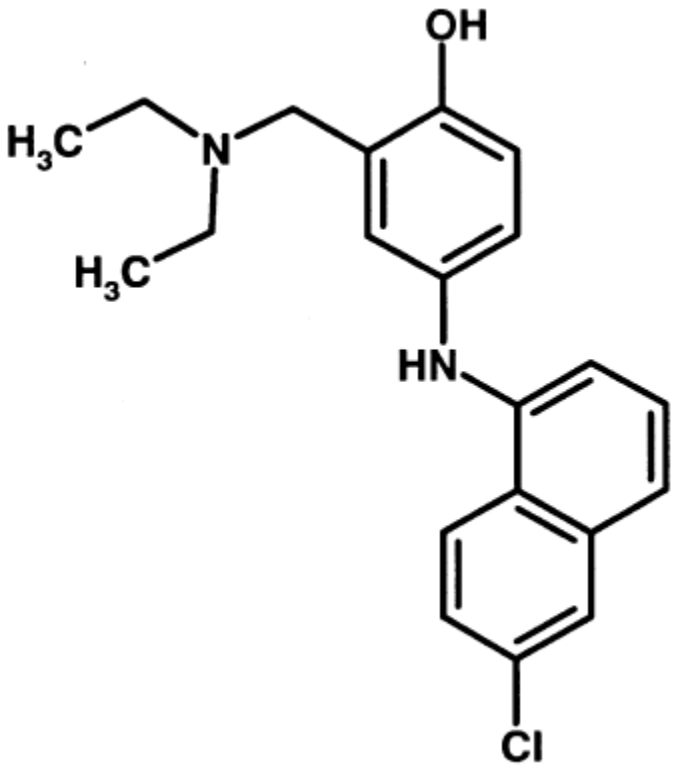
phenacetin is oxidated by P450 and causes cellular hepatotoxicity

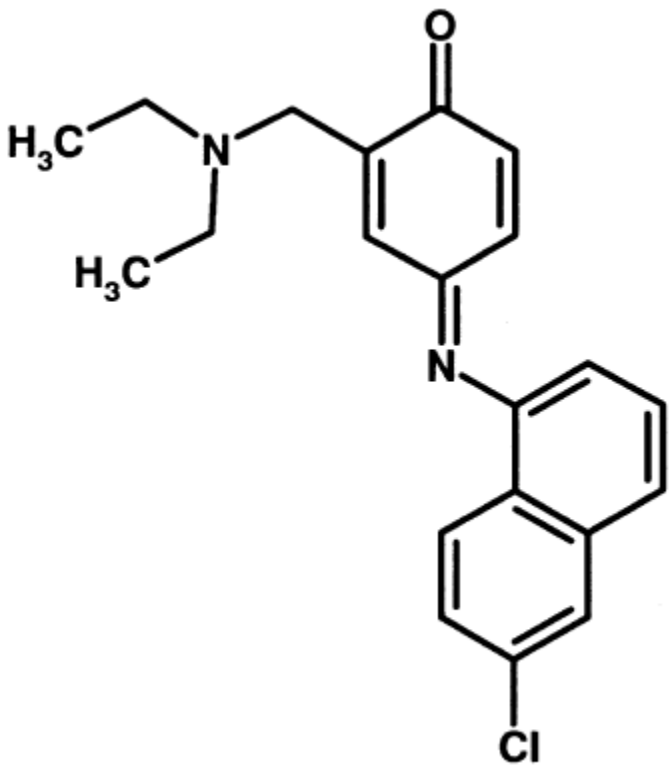


Toxicophores

Quinone Imines

Antimalaric agent amodiaquine causes hepatotoxicity

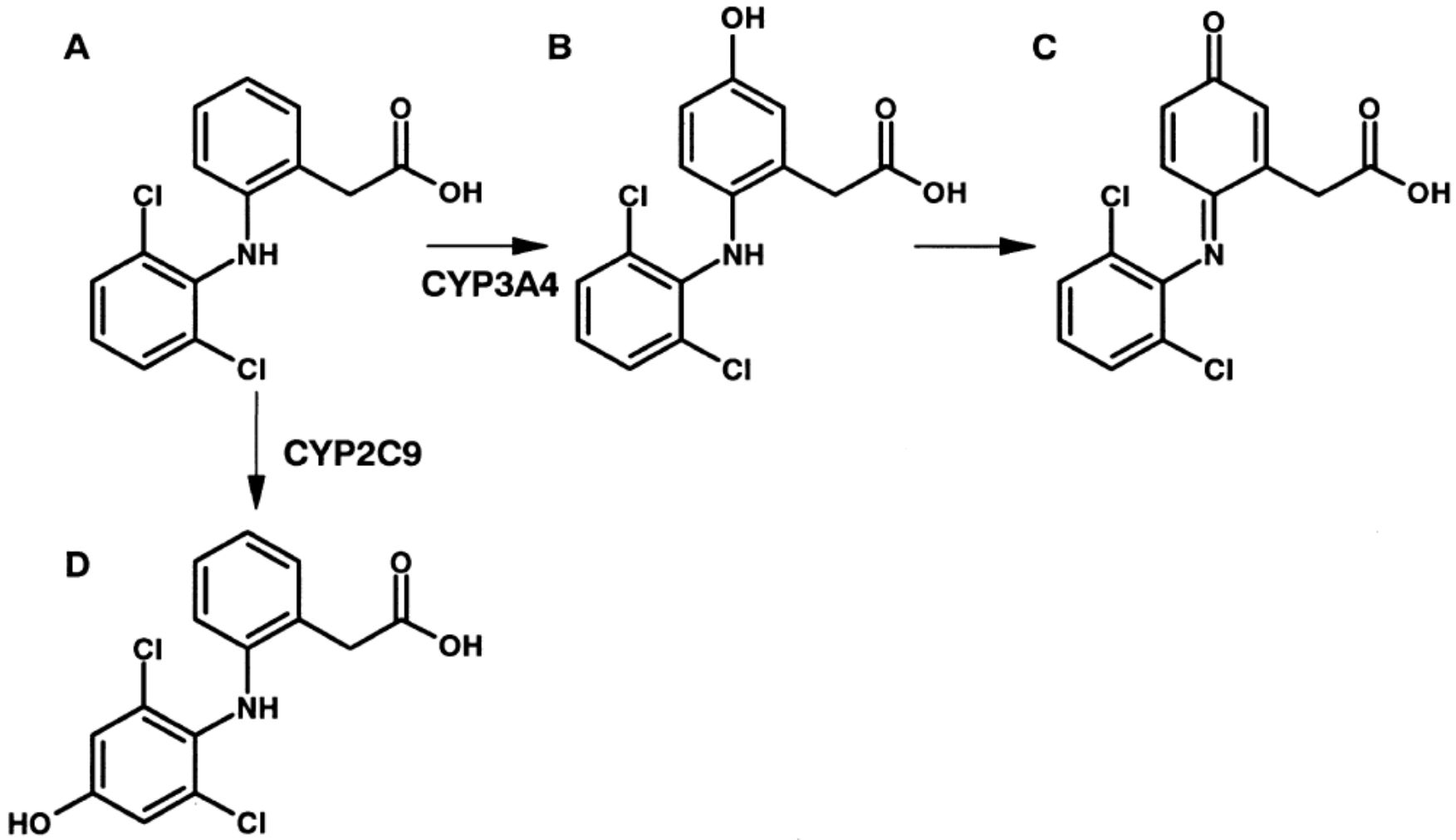




Toxicophores

Quinone Imines

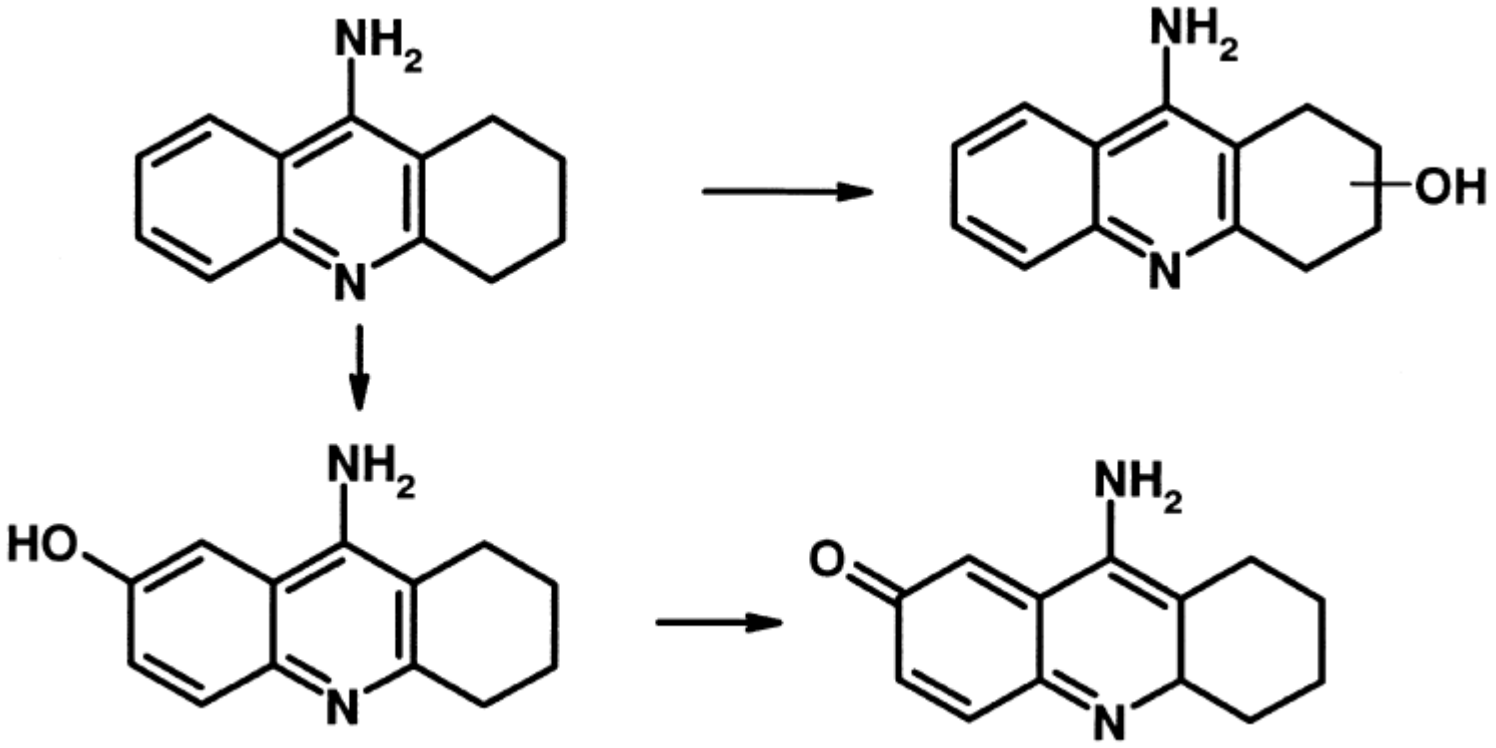
One pathway for diclofenac causes hepatotoxicity



Toxicophores

Quinone Imines

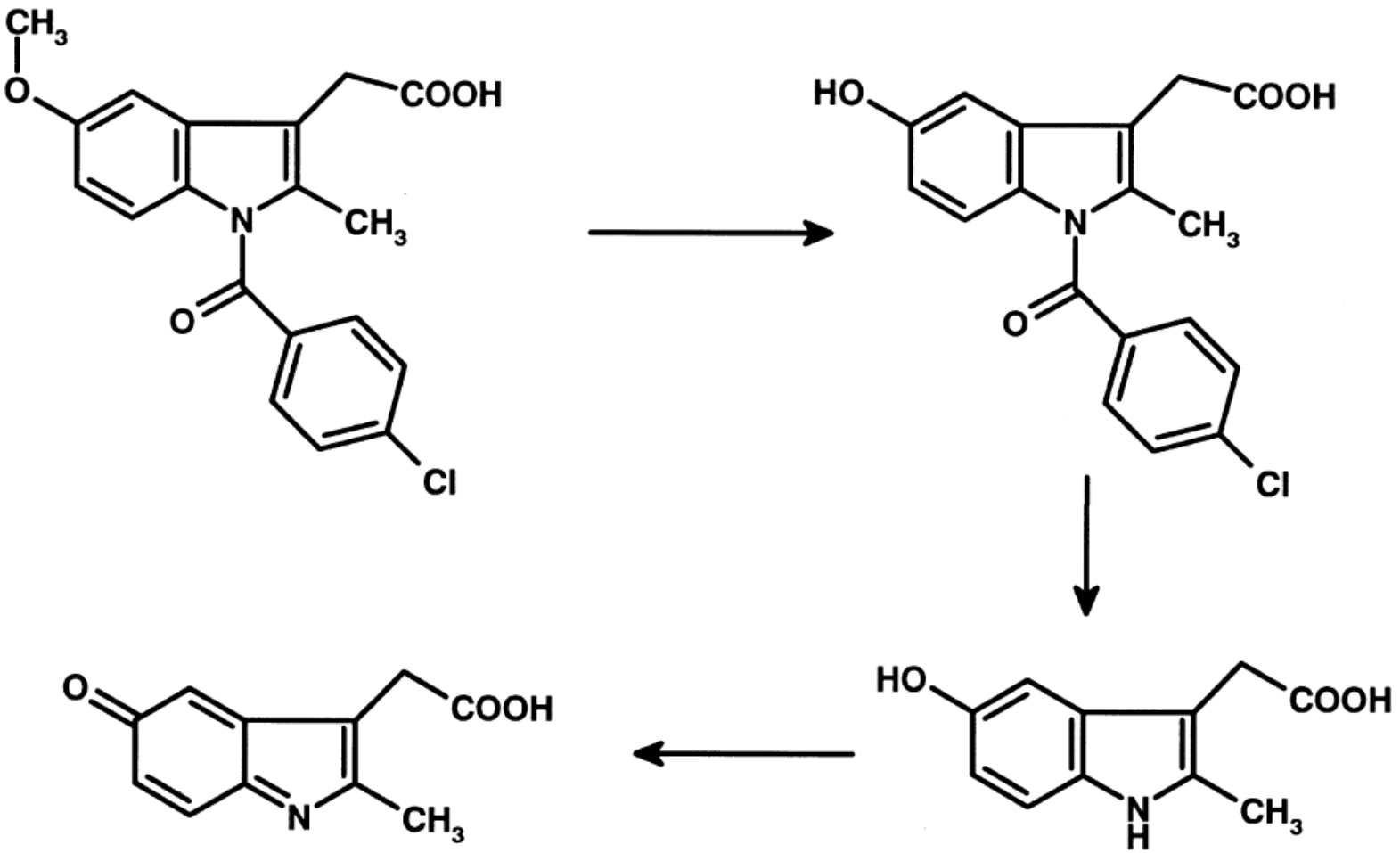
ACHE inhibitor tacrine causes hepatotoxicity



Toxicophores

Quinone Imines

Indomethacin caused agranulocytosis

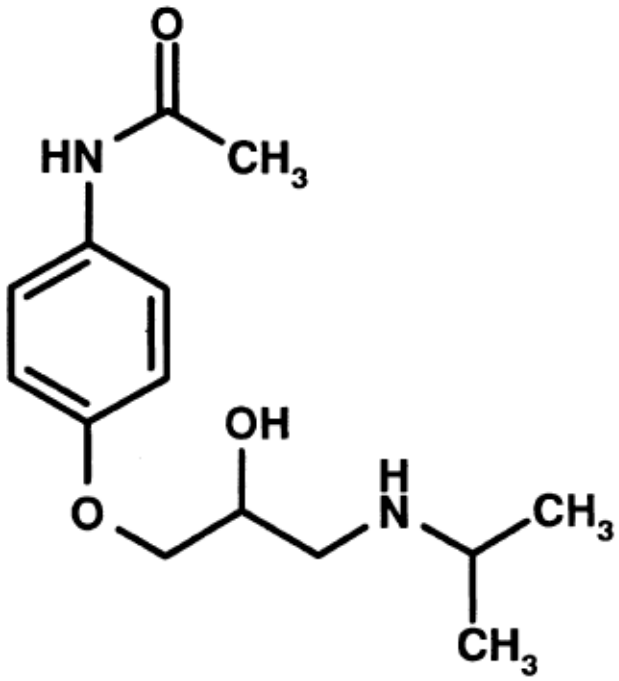


Toxicophores

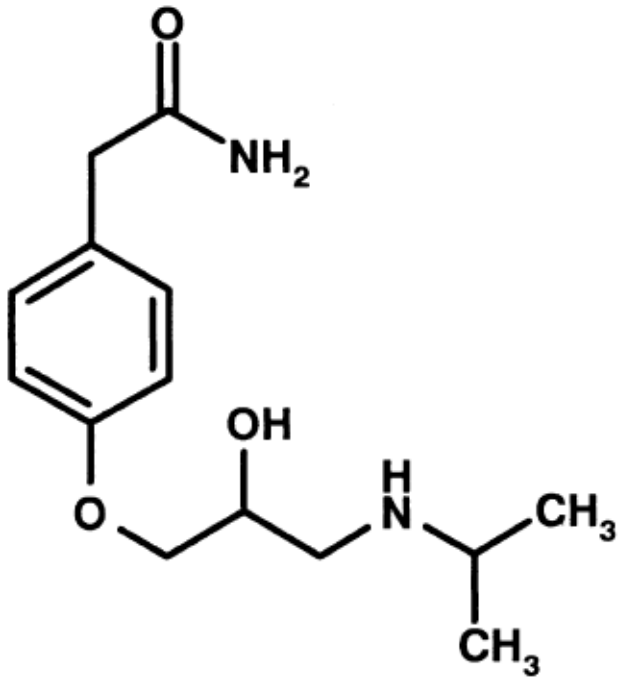
Quinone Imines

Practolol had to be withdrawn from the market due to skin and eye lesions

Atenolol have not toxic acetanilide moiety



practolol

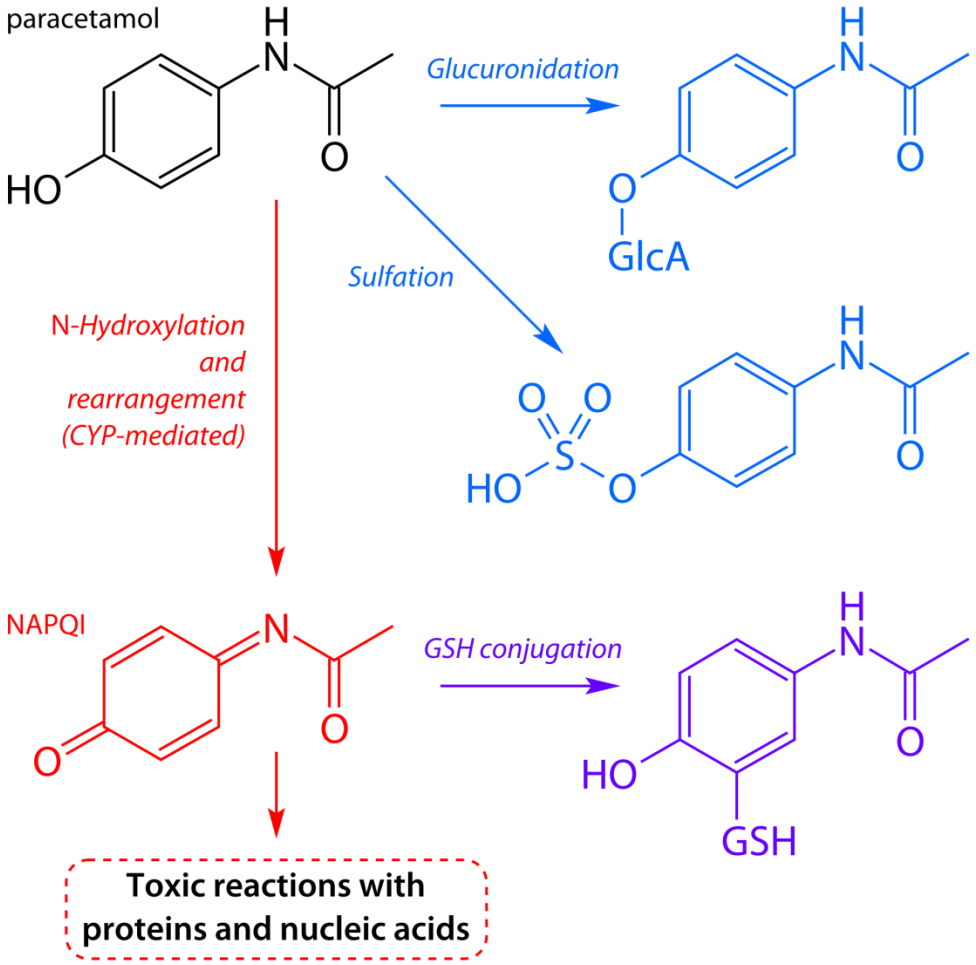


atenolol

Toxicophores

Quinone Imines

Paracetamol has the same toxicologic issue

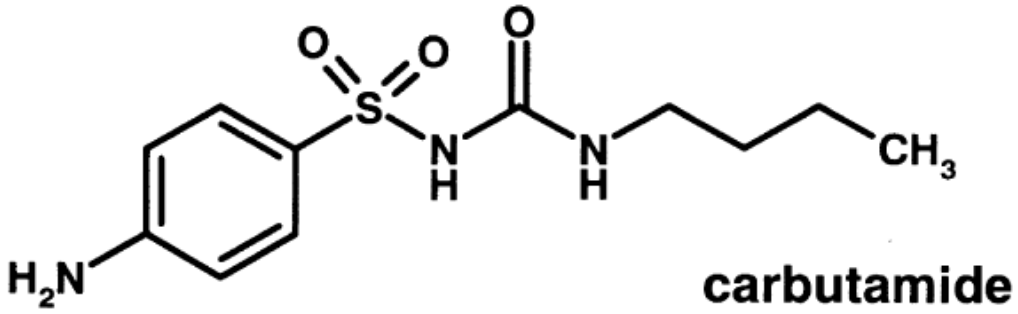


Toxicophores

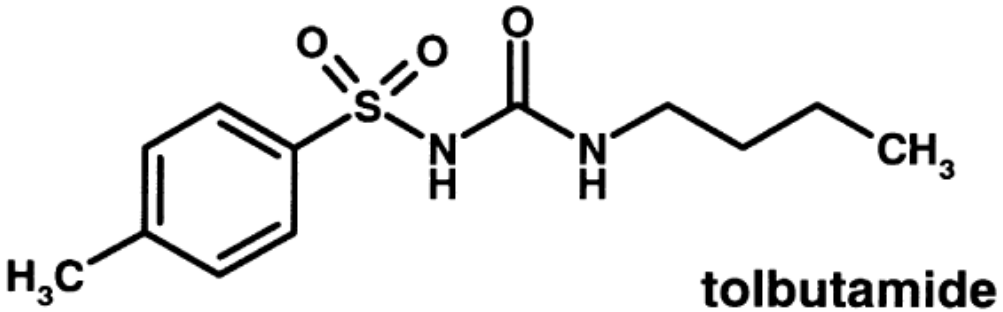
Quinone Imines

Carbutamid caused bone marrow toxicity and was withdrawn

Tolbutamid have not this problem



carbutamide



tolbutamide

Toxicophores

Quinone Imines

Lidocain as antiarrhytmic has short biological half-time. Longer acting analogues procainamide and tocainide are toxic, flecainid do not so.

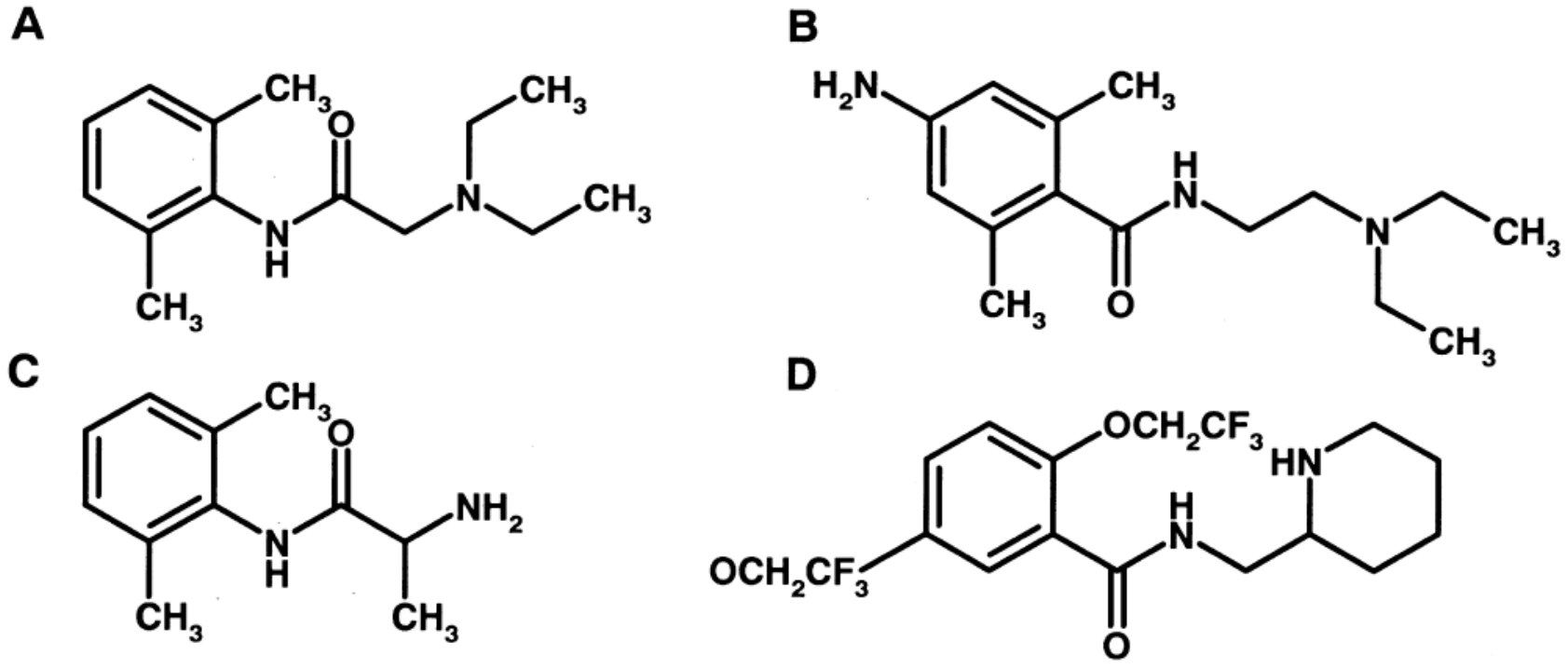
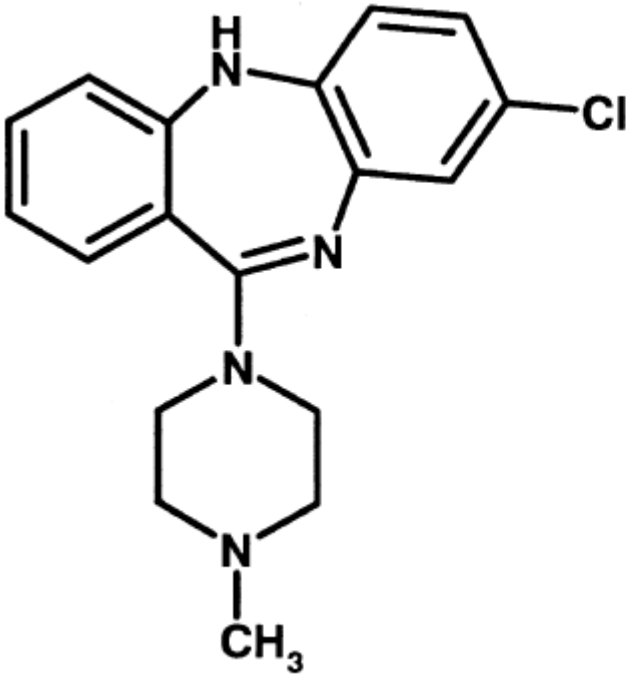
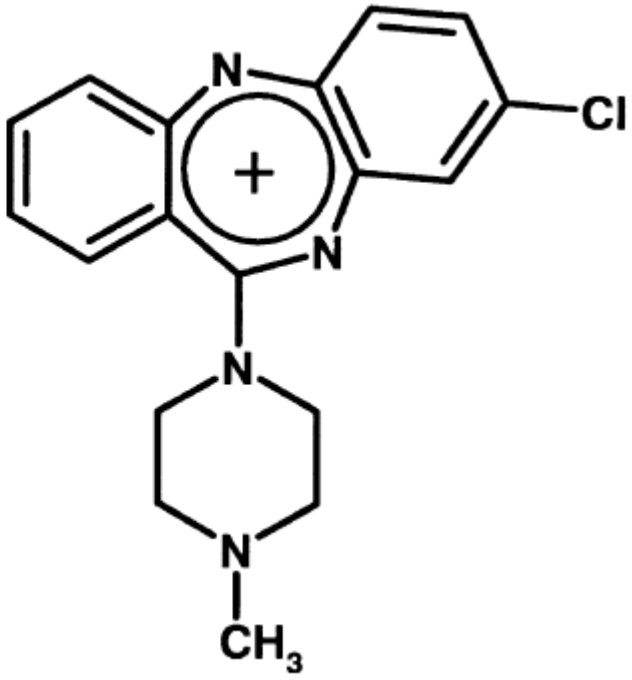


Fig. 8.16 Structures of Na^+ channel blocker antiarrythmics: lidocaine (A), procaineamide (B), tocainide (C) and flecainide (D).

Toxicophores
Nitrenium Ions

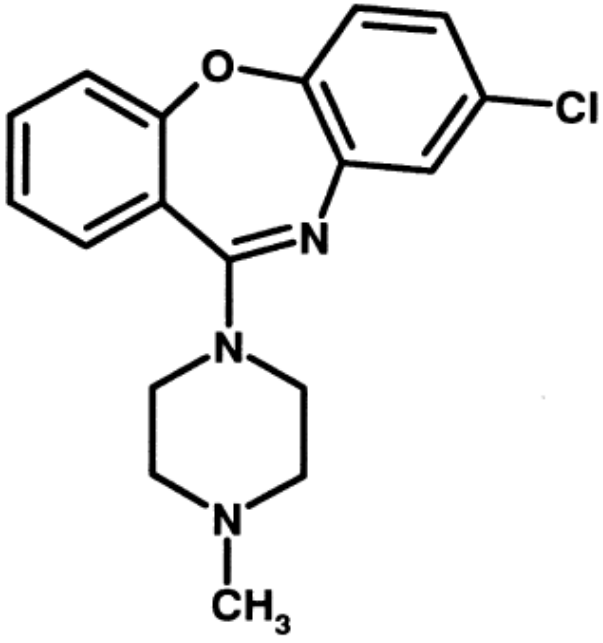
Clozapine forms reactive nitrenium ions causing agranulocytosis

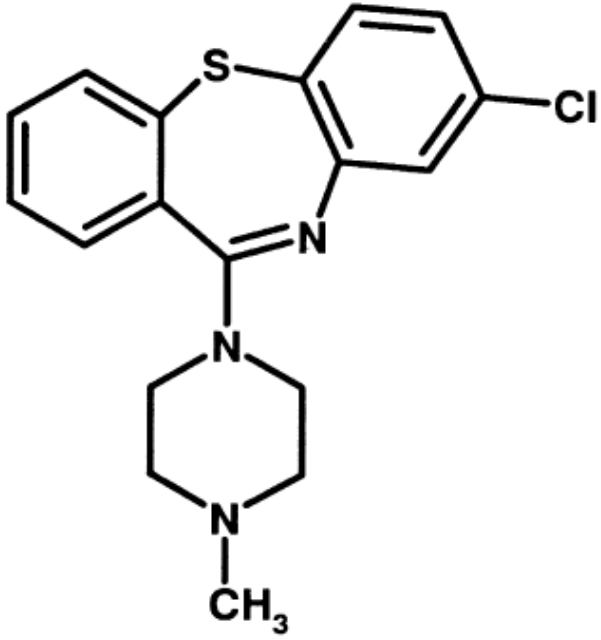


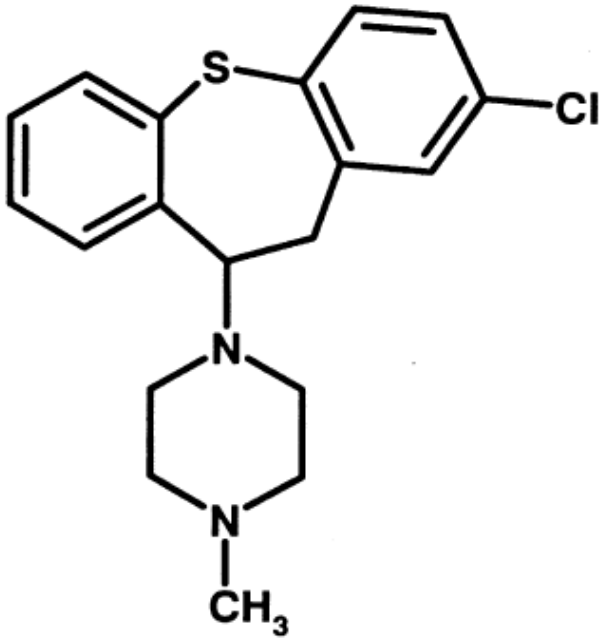


Toxicophores
Nitrenium Ions

Modification of circle brought non-toxic analogues





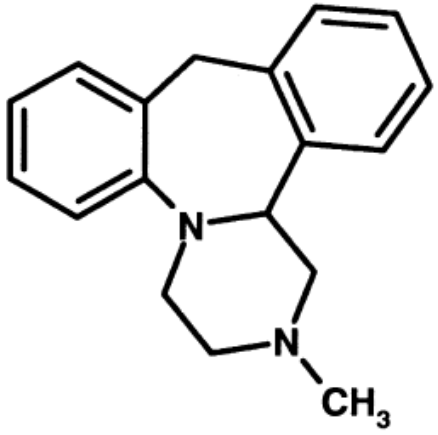


Toxicophores

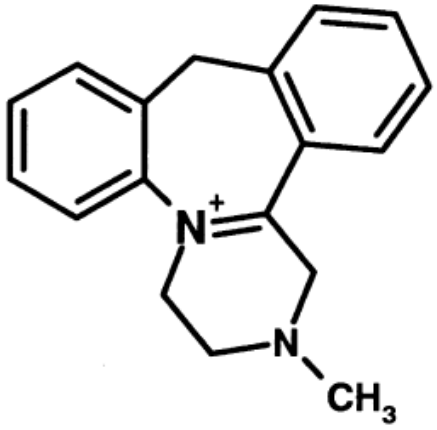
Iminium Ions

Antidepressant mianserin causes agranulocytosis

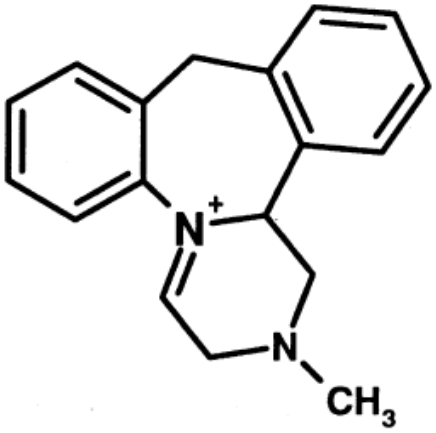
A



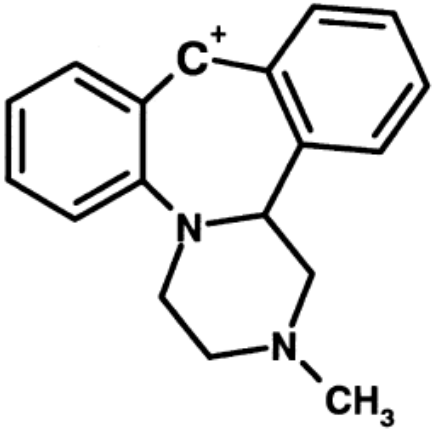
B



C



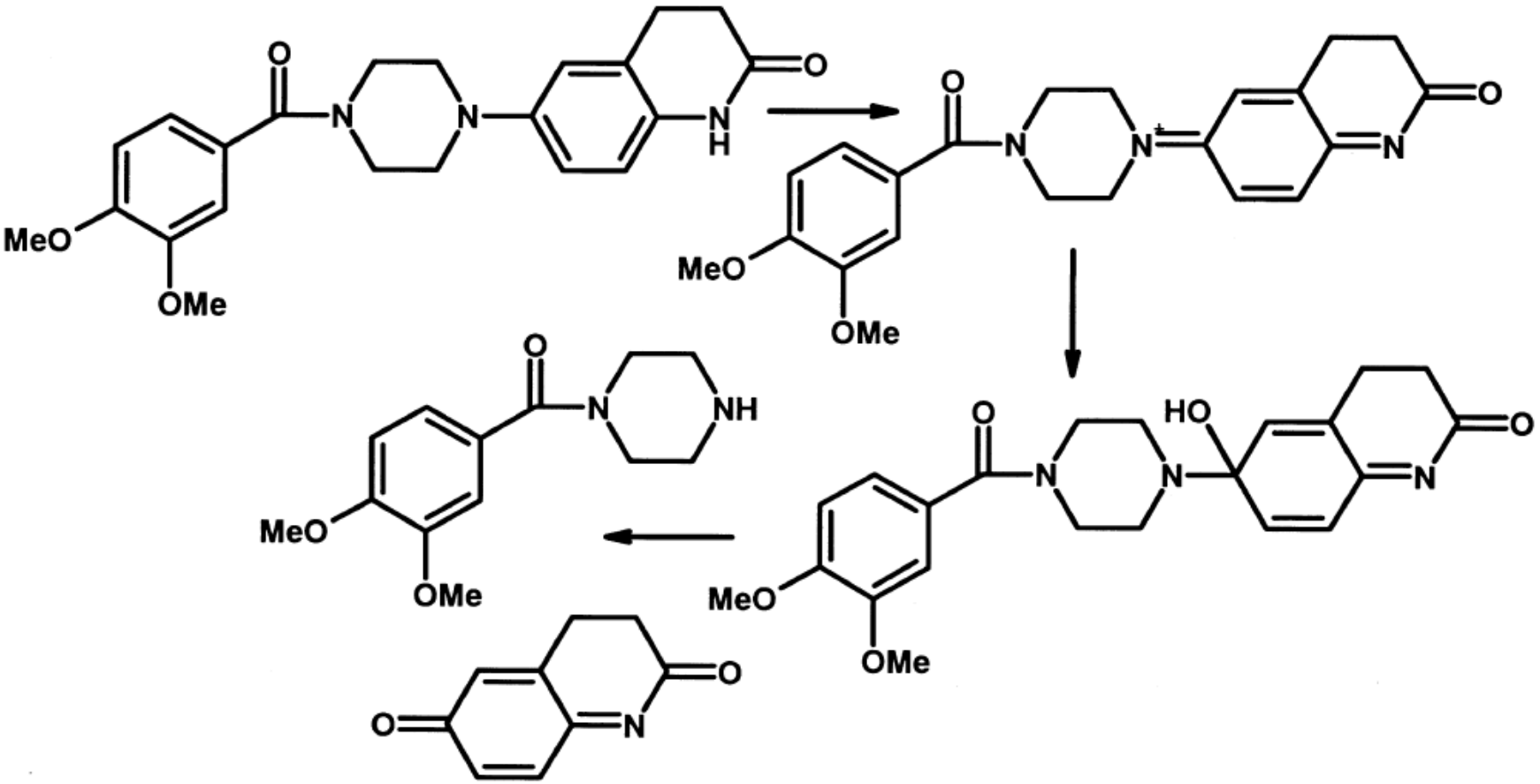
D



Toxicophores

Iminium Ions

Cardiotonic vesnarinon causes agranulocytosis both due to iminium and quinone imine formation

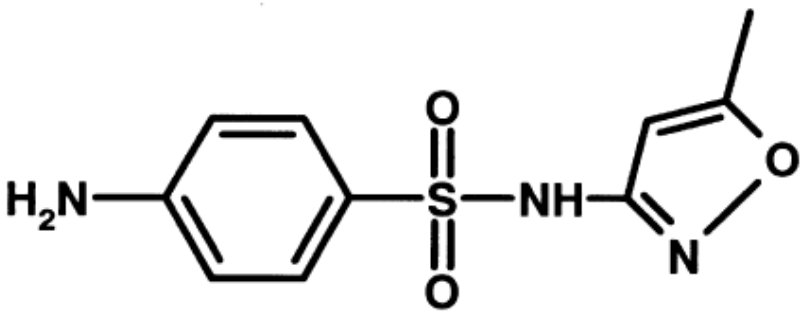


Toxicophores

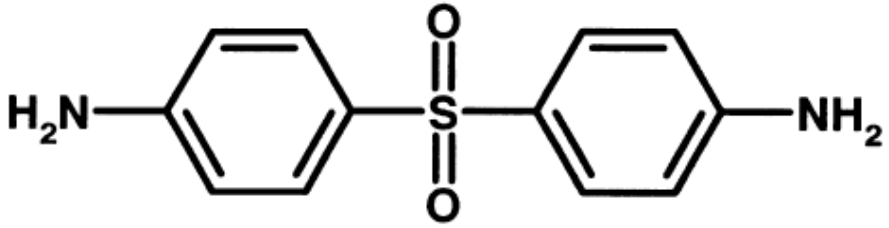
Hydroxylamines

hydroxylamines are further metabolized to reactive nitroso derivatives

Sulphonamides and dapson are metabolized that way - causes agranulocytosis and skin hypersensitivity



Sulphamethoxazole



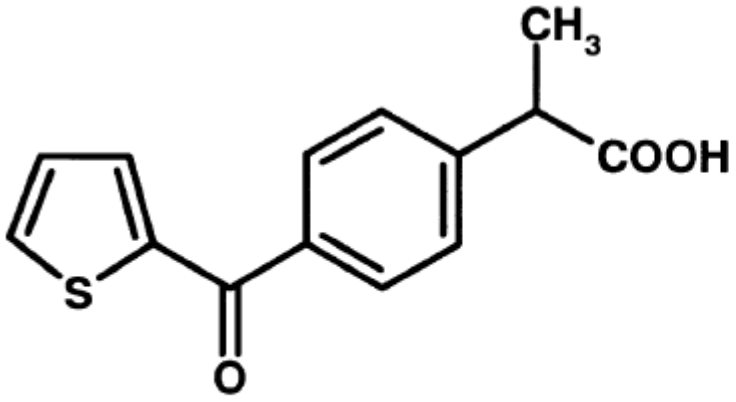
Dapsone

Toxicophores

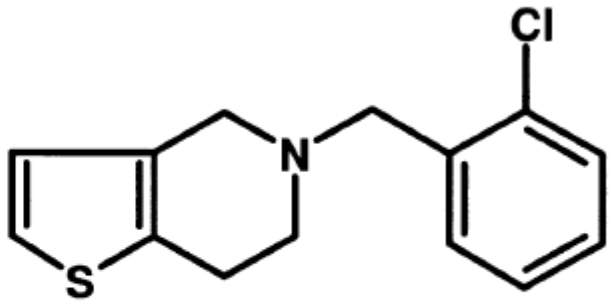
Thiophene ring

thiophene S-oxides and S-chlorides causes covalent protein bonding

agranulocytosis by ticlopidine (platelet inhibitor)
nephrotoxicity of suprofen (NSA, withdrawn)



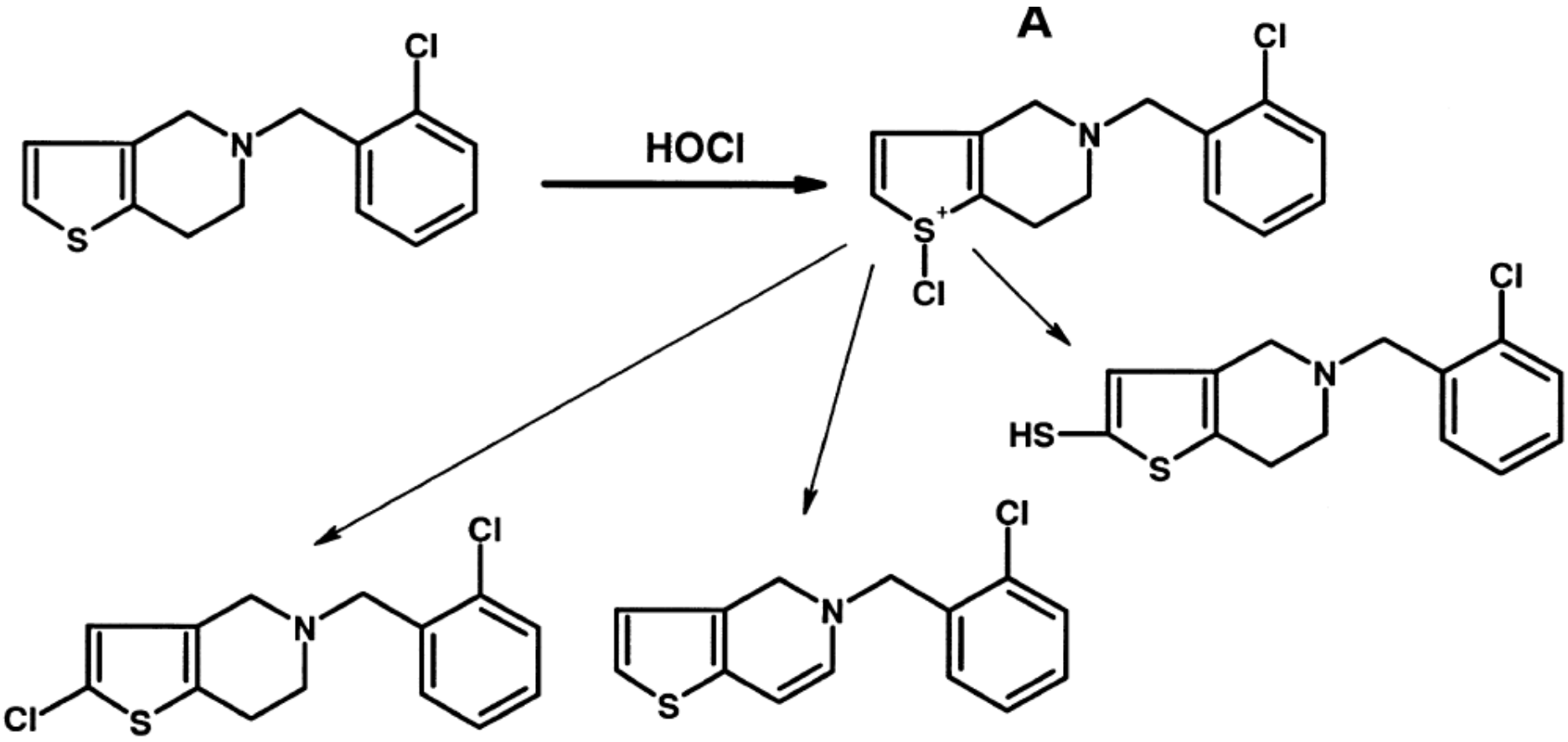
Suprofen



Ticlopidine

Toxicophores
Thiophene ring

ticlopidine metabolism in white blood cells



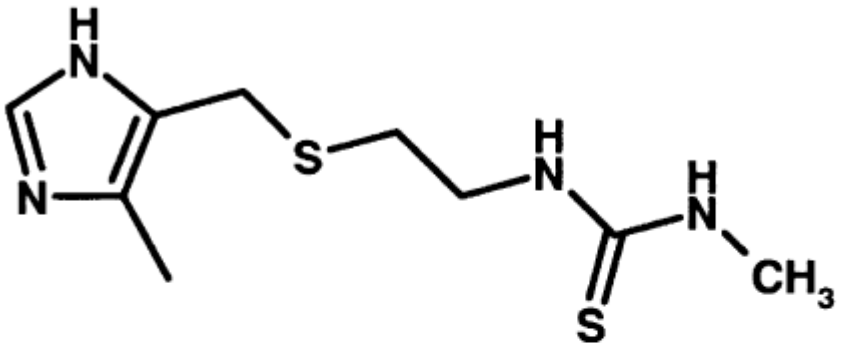
Toxicophores

Thioureas

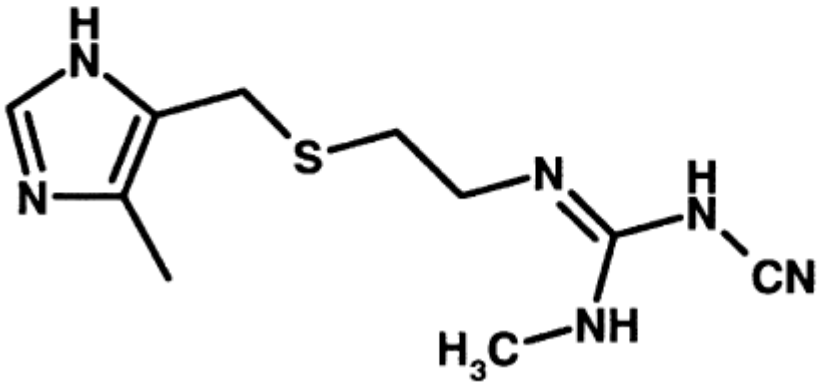
thioureas are metabolized to sulfon acids

Metiomid was withdrawn due to blood dyscrasias

Cimetidine not shows this problem



Metiamide



Cimetidine