CYP450 Oxidations

Oxidation at Carbons in Heteroatom Systems

(N-dealkylation, oxidative deamination and N-oxidation)

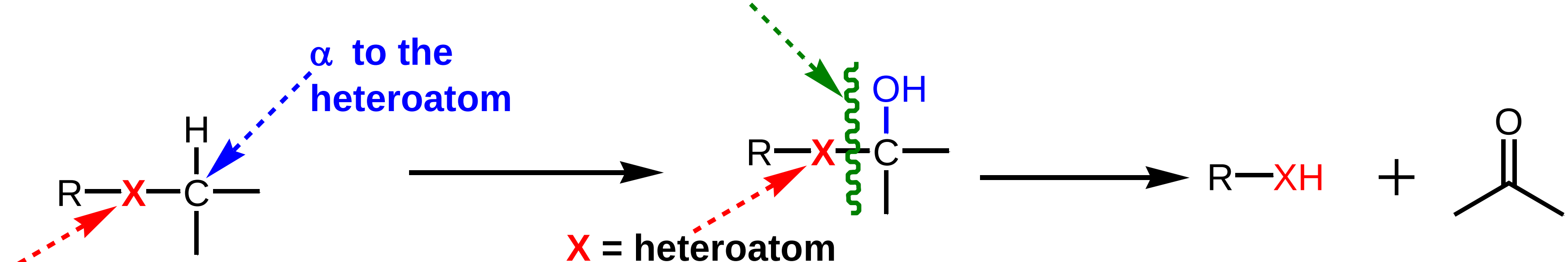
Oxidation at Carbons in Heteroatom Systems

- **Heteroatom system contains hetero-atom instead of carbon (i.e. N, O, S).**
- **Nitrogen and oxygen functionalities are common in the majority of drugs and foreign compounds, where sulfur functionalities occur only occasionally.**

- **Metabolic oxidation of these systems involves principally two basic types of biotransformation processes:**

1) **Oxidation at the carbon atom attached to the heteroatom ((N, O, S) ➔ (The intermediates resulted is unstable and decomposes with the cleavage of the carbon-heteroatom bond) ➔ dealkylation (N- O- and S-dealkylation)**

2) **Oxidation of the heteroatom (N and S only)➔ N- S- oxidation)**
(e.g. N-oxidation , N-oxide formation, sulfoxide and sulfone formation)



carbon-heteroatom bond break

α to the heteroatom

X = heteroatom
(O, N, S)

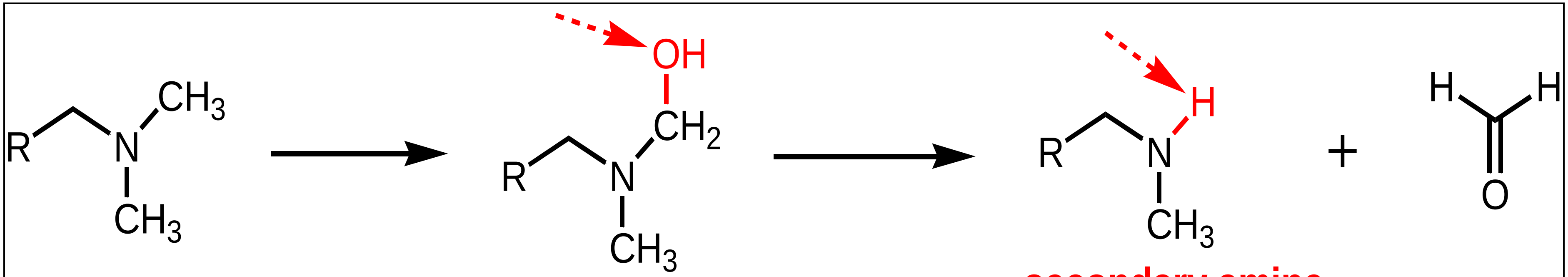
X = heteroatom
(O, N, S)

hydroxylated intermediate
(unstable ==> break down)

Oxidation Involving Carbon- Nitrogen Systems

Aliphatic (tertiary , secondary and primary) and Alicyclic Amines

- **The oxidative removal of alkyl groups (particularly methyl) from tertiary amines referred to as oxidative N-dealkylation.**



tert. amine

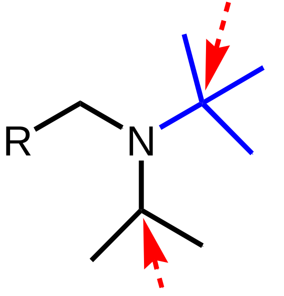
carbinolamine [intermediate]

secondary amine

- **In general small alkyl groups (like methyl, ethyl and isopropyl) are rapidly removed, while N-dealkylation of the tertiary butyl group is not possible by the carbinolamine pathway since the α-carbon oxidation can not occur**

- **Removal of the first alkyl group from a tert. amine occurs more rapidly than removal of the second one**

can not be hydroxylated

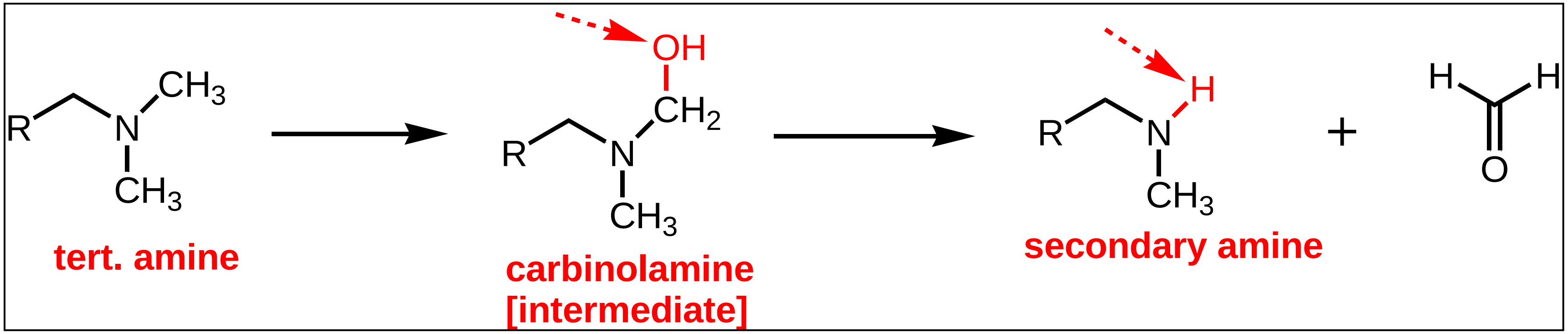


can be hydroxylatedr

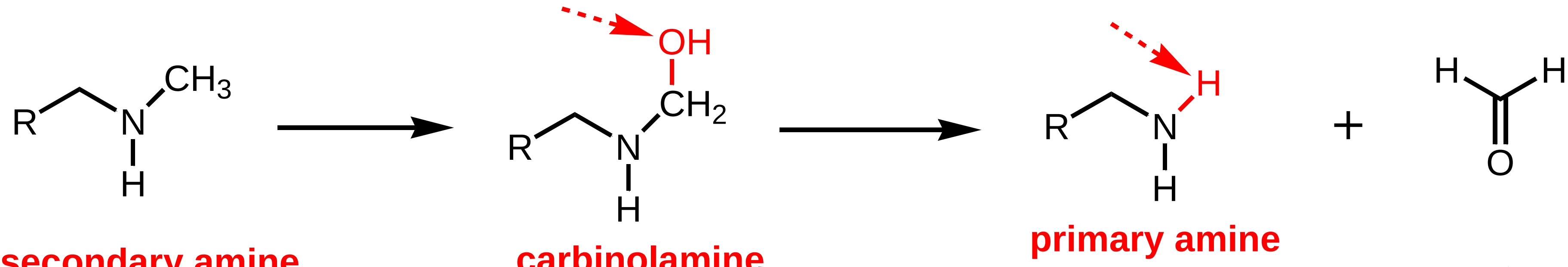
Oxidation Involving Carbon- Nitrogen Systems

Aliphatic (tertiary , secondary and primary)

(N-dealkylation)



- ➢Oxidative N- dealkylation of a tertiary amine produces secondary amine
- ➢Oxidative N- dealkylation of a secondary amine produces primary amine
- ➢Removal of the first alkyl group from a tert. amine occurs more rapidly than removal of the second one

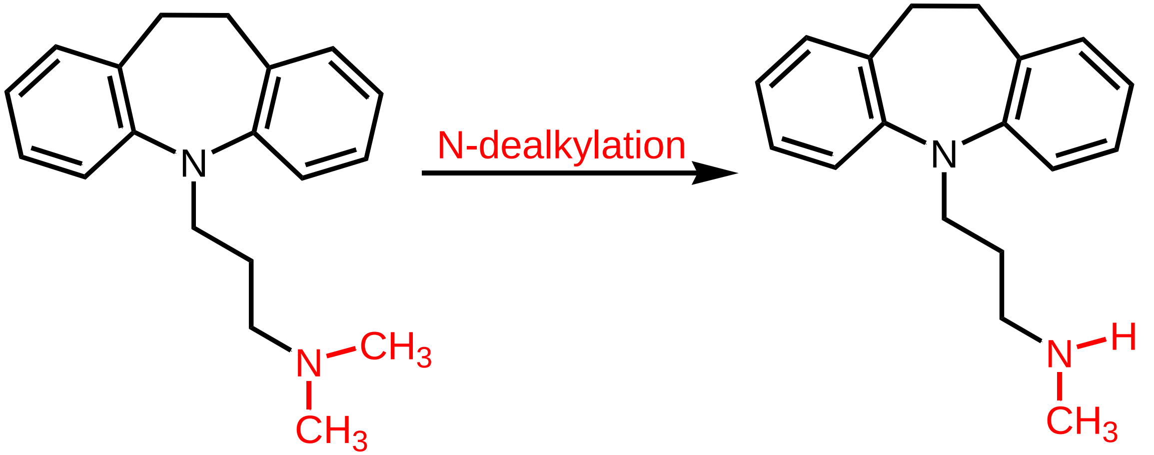


Oxidation Involving Carbon- Nitrogen Systems

Aliphatic (tertiary , secondary and primary)

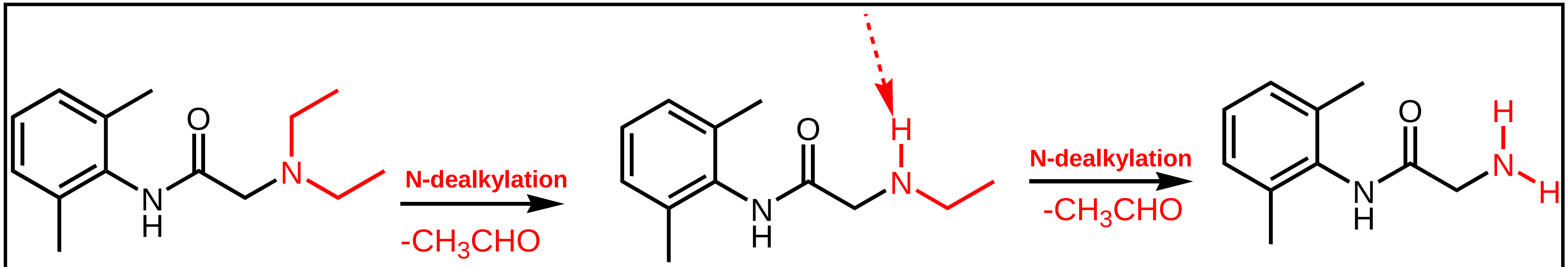
<u>(N-dealkylation)</u>

(Examples)



Imipramine
(tricyclic antidepressant)

desimipramine
(active metabolite)



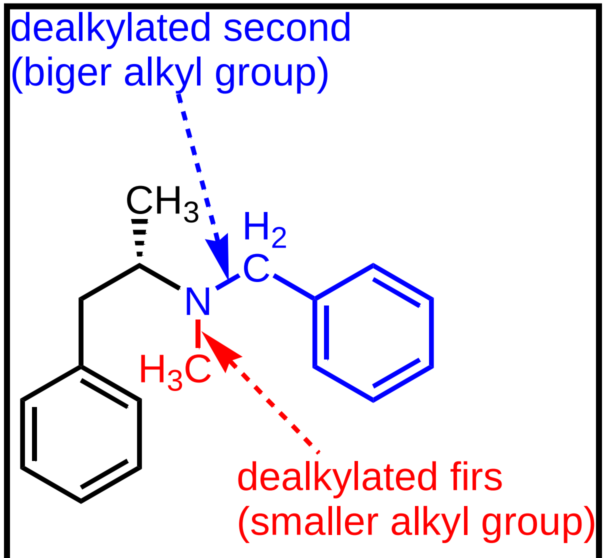
lidocaine
(local anasthesia)

Oxidation Involving Carbon- Nitrogen Systems

Aliphatic (tertiary , secondary and primary)
(N-dealkylation)
(Examples)

- **If there is two different groups (e.g. methyl and benzyl), the smaller alkyl group (methyl in this case) is preferentially and more rapidly removed.**

e.g. benzphetamine: the methyl group is removed much more rapidly than the benzyl.



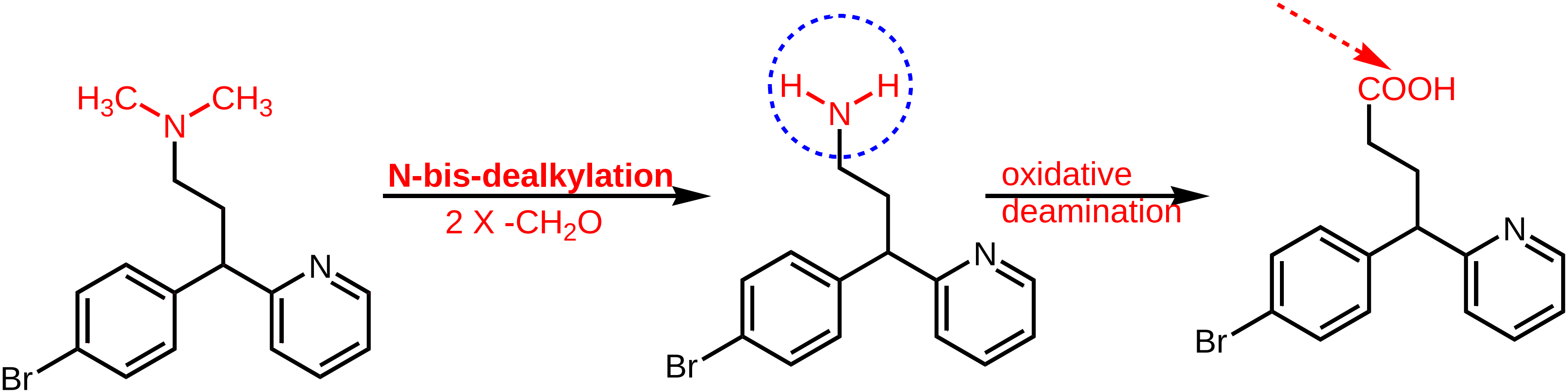
Benzphentamine
(anti-obesity drug)

Oxidation Involving Carbon- Nitrogen Systems

(N-dealkylation)

- **In many cases bis-deakylation of a tertiary amine leads to the corresponding primary aliphatic amine metabolite, which is susceptible to further oxidation**

- **e.g. Brompheniramine (sedative antihistamine): The N-bis-dealkylation of it produces the primary amine metabolite, which then undergoes oxidative de-amination to produce the carboxylic acid metabolite**



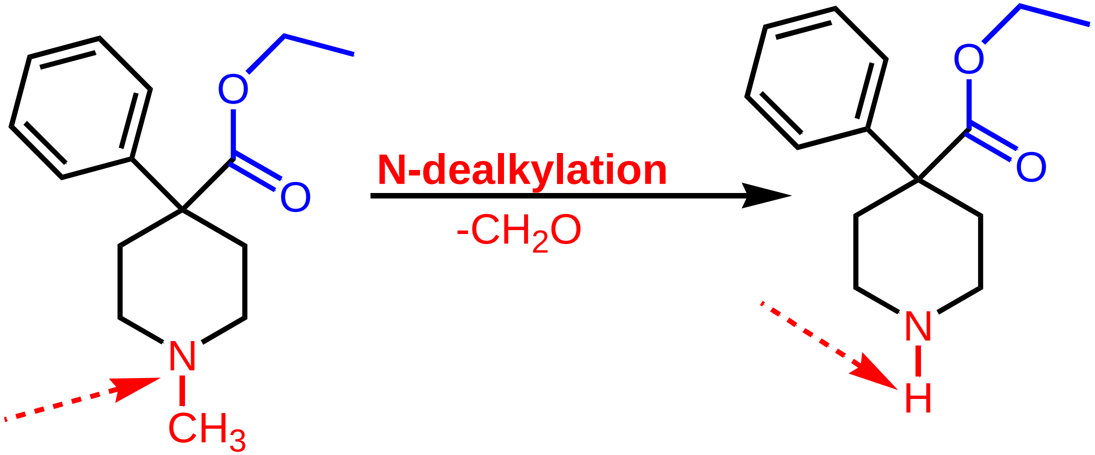
Brompheniramine
(sedative anti-histamine)

Oxidation Involving Carbon- Nitrogen Systems

Alicyclic Amines
(N-dealkylation)
(Pethidine)

- ➢Like their aliphatic counterparts, alicyclic tertiary amines are susceptible to N-dealkylation reactions

- ➢e.g. Pethidine (meperidine in US) (narcotic analgesic agent) is metabolized by the following pathway



Pethidine
(narcotic analgesic)

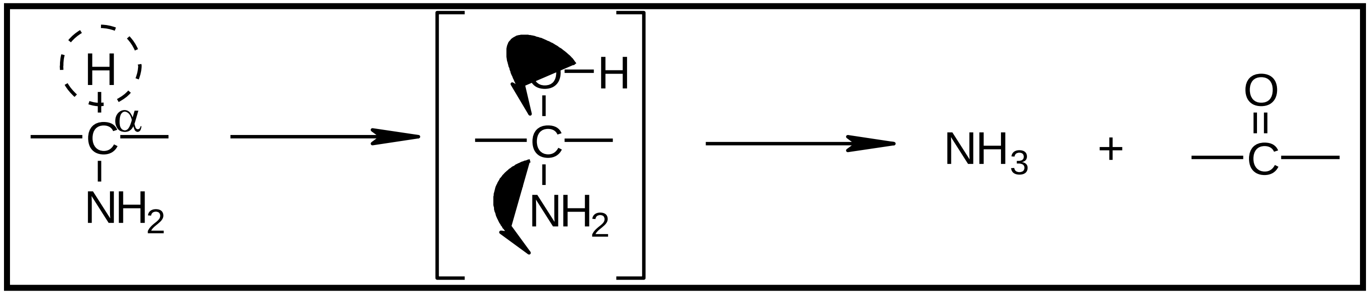
Oxidation Involving Carbon- Nitrogen Systems

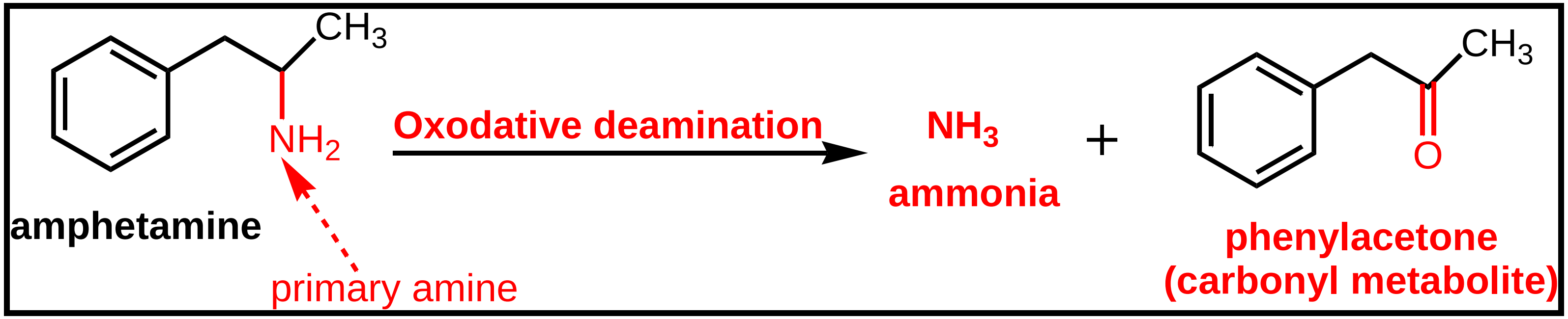
Oxidative Deamination

- **The primary amine metabolites formed from oxidative N-dealkylation are susceptible to oxidative deamination.**

- **Similar to N-dealkylation, oxidative deamination involves an initial α-carbon oxidation reaction to form the carbinolamine intermediate which then undergoes to C - N cleavage to produce carbonyl metabolite and ammonia.**

- **If α-carbon oxidation cannot occur, then oxidative deamination is not possible (cf. norketamine)**

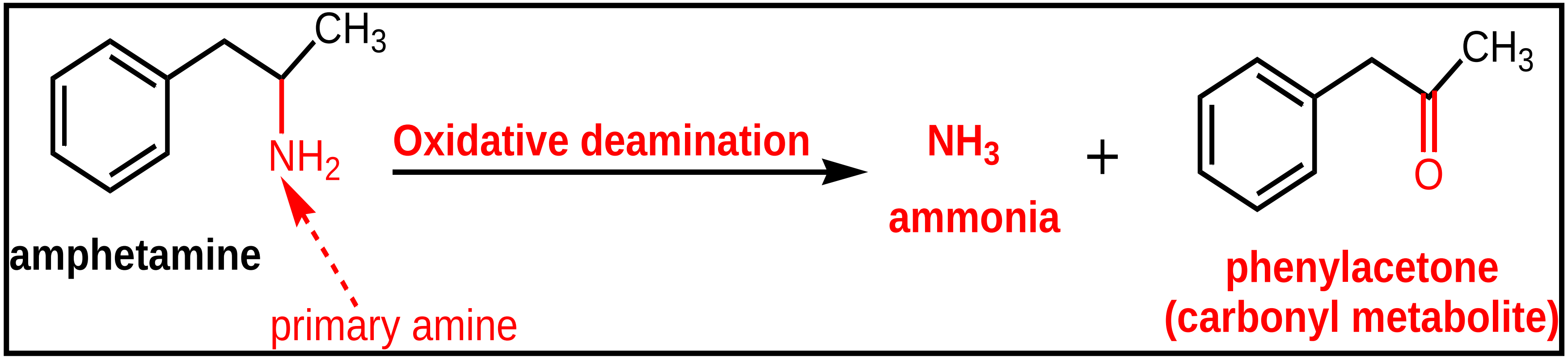




Oxidation Involving Carbon- Nitrogen Systems

Oxidative Deamination

- **If α-carbon oxidation cannot occur, then oxidative deamination is not possible (cf. norketamine)**



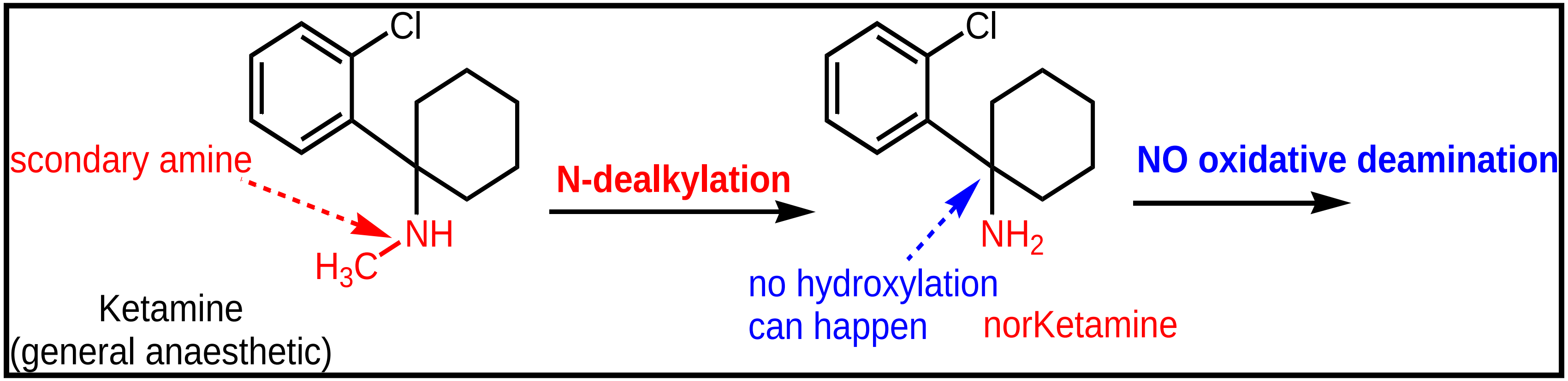
Oxidative deamination

ammonia

amphetamine

primary amine

phenylacetone (carbonyl metabolite)



scondary amine

N-dealkylation

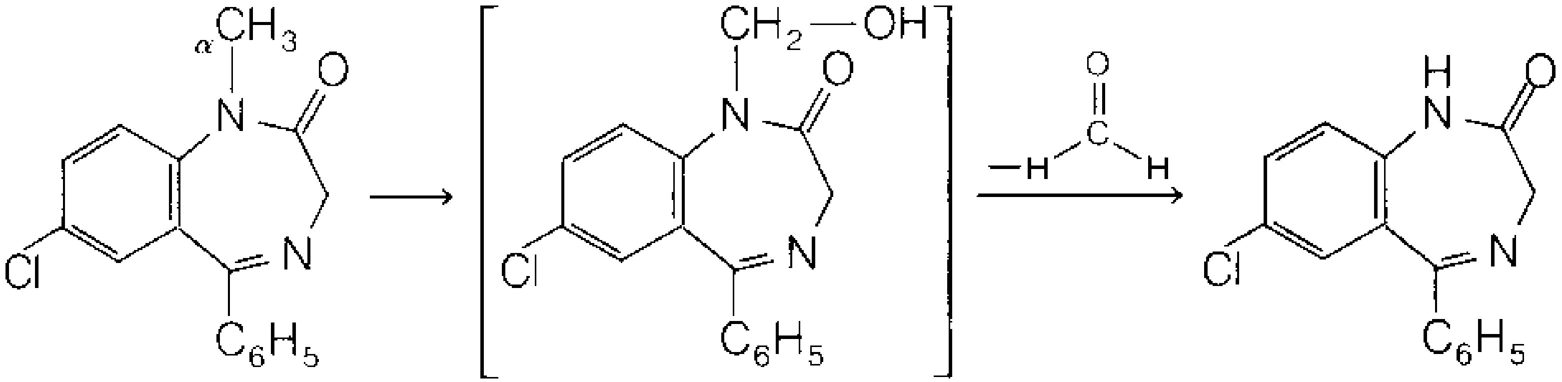
NO oxidative deamination

no hydroxylation can happen

norKetamine

Ketamine
(general anaesthetic)

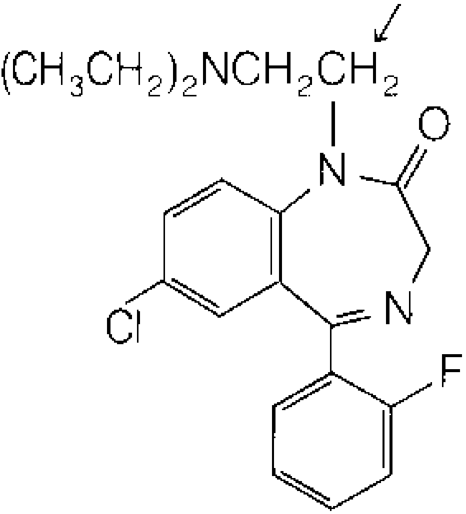
Metabolic Oxidation of amides



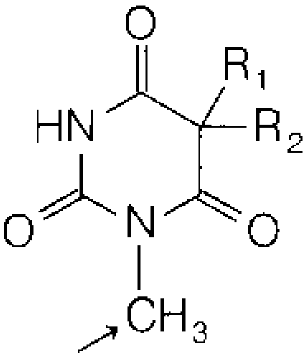
Diazepam

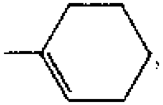
Carbinolamide

Desmethyldiazepam

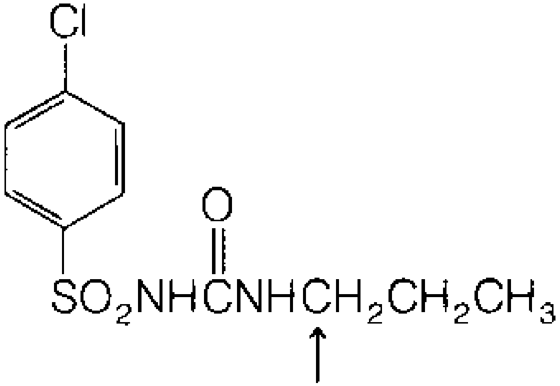


Flurazepam



Hexobarbital $R_1 =$ , $R_2 = CH_3$

Mephobarbital $R_1 = C_6H_5$, $R_2 = CH_2CH_3$



Chlorpropamide

Oxidation involving carbon- oxygen systems

O-dealkylation

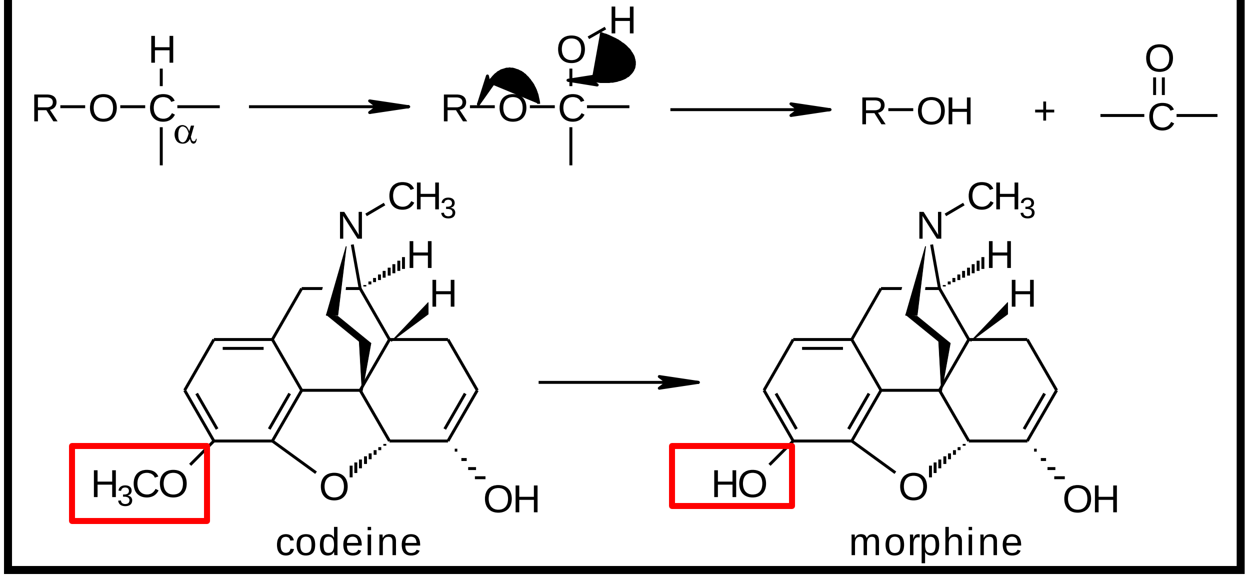
- Oxidative O-dealkylation of carbon-oxygen systems (principally ethers) is catalyzed by microsomal mixed function oxidases.

- It involves an initial α– carbon oxidation to form either a hemiacetal or hemiketal, which undergoes spontaneous carbon - oxygen bond cleavage to

➔yield the dealkylated oxygen species (phenol or alcohol) and a carbon moiety (aldehyde or ketone)

➔ Small alkyl group attached to oxygen are rapidly O-dealkylated

➔ e.g. morphine is the metabolic product resulting from codeine.



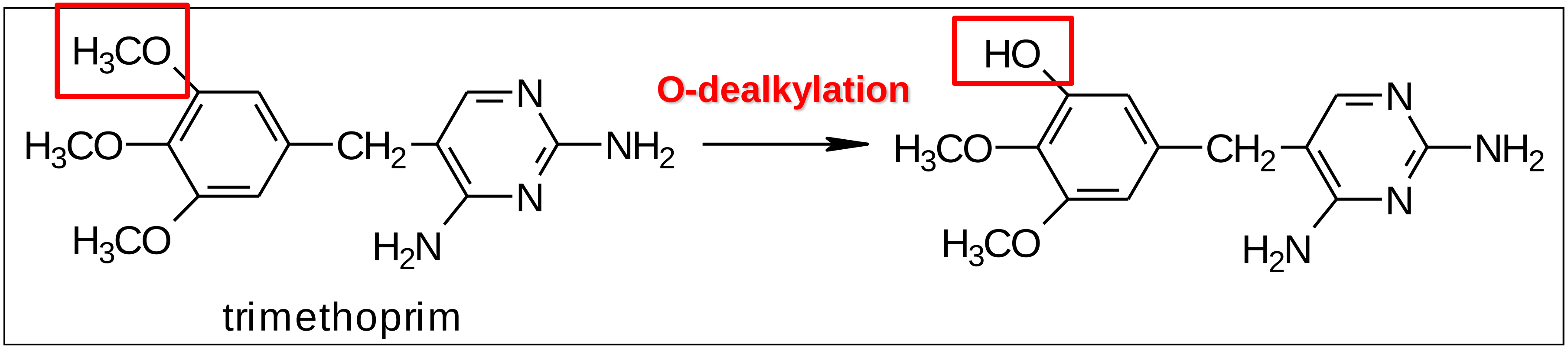
Oxidation involving carbon- oxygen systems

O-dealkylation

- **In many drugs which have several non-equivalent methoxy groups , one particular methoxy group often appears to be selectively or prefrentially O- demethylated**

- **e.g. trimethoprim undergoes O-demethylated to give predominatly the corresponding 3-O-demethylated metabolite.**

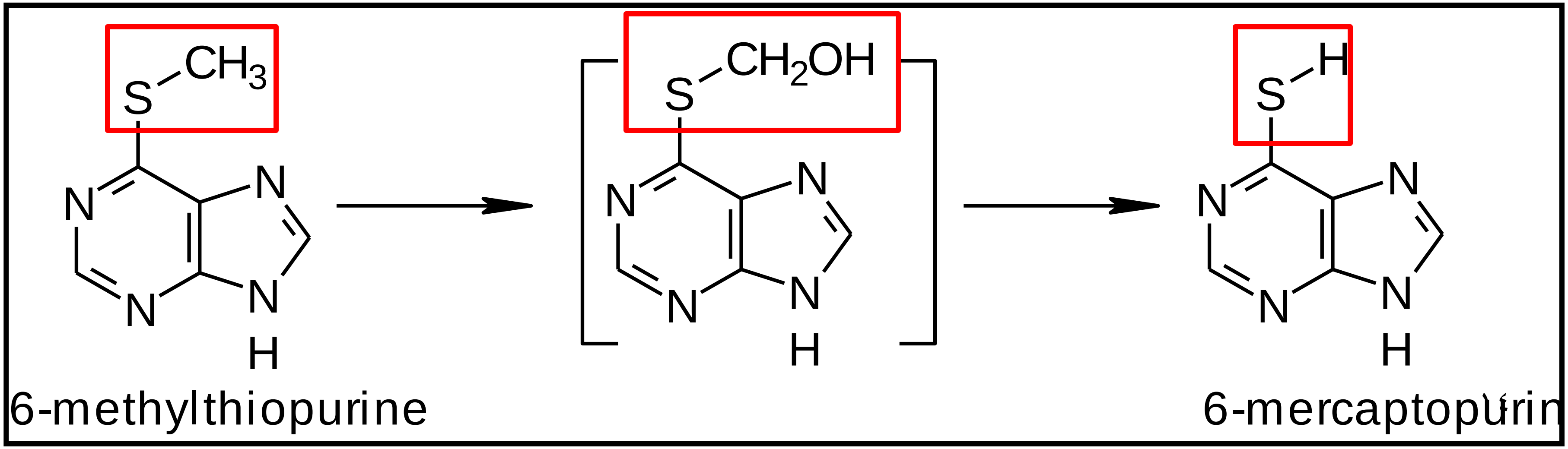
- **4-O-demethylated metabolite also occurs but to a minor extent.**



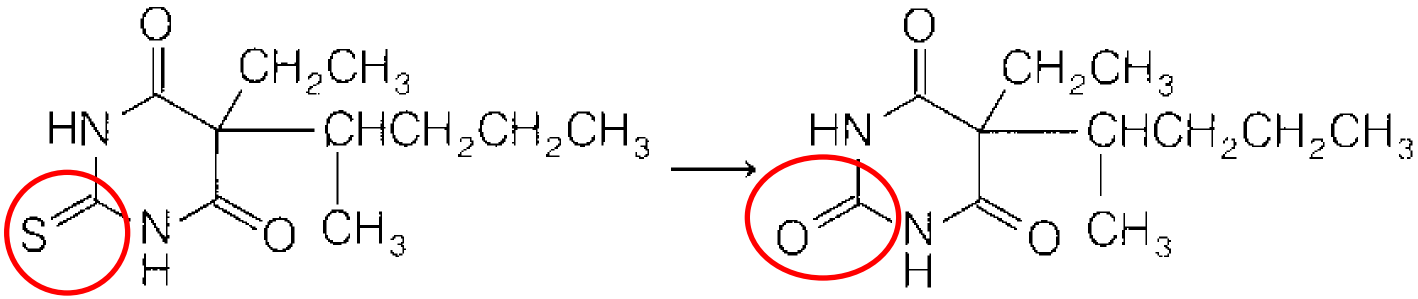
Oxidation involving carbon- sulfur systems

S-dealkylation

- ➢Carbon-sulfur functional groups are susceptible to metabolic ➔ S-dealkylation, desulfuration and S-oxidation reactions .
- ➢The first two invole oxidative carbon-sulfur bond cleavage.
- ➢S-dealkylation is analogues to O and N- dealkylation (α- carbon oxidation)
- ➢e.g. 6-mercaptopurine is the S-demethylated product of 6-(methylthio)-purine.

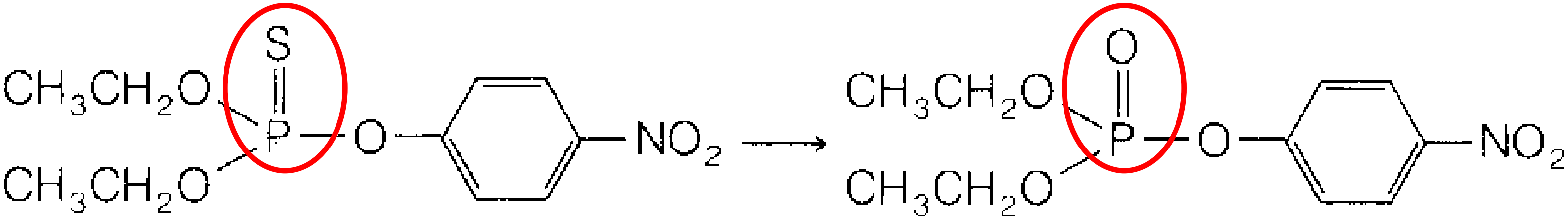


Desulfuration



Thiopental

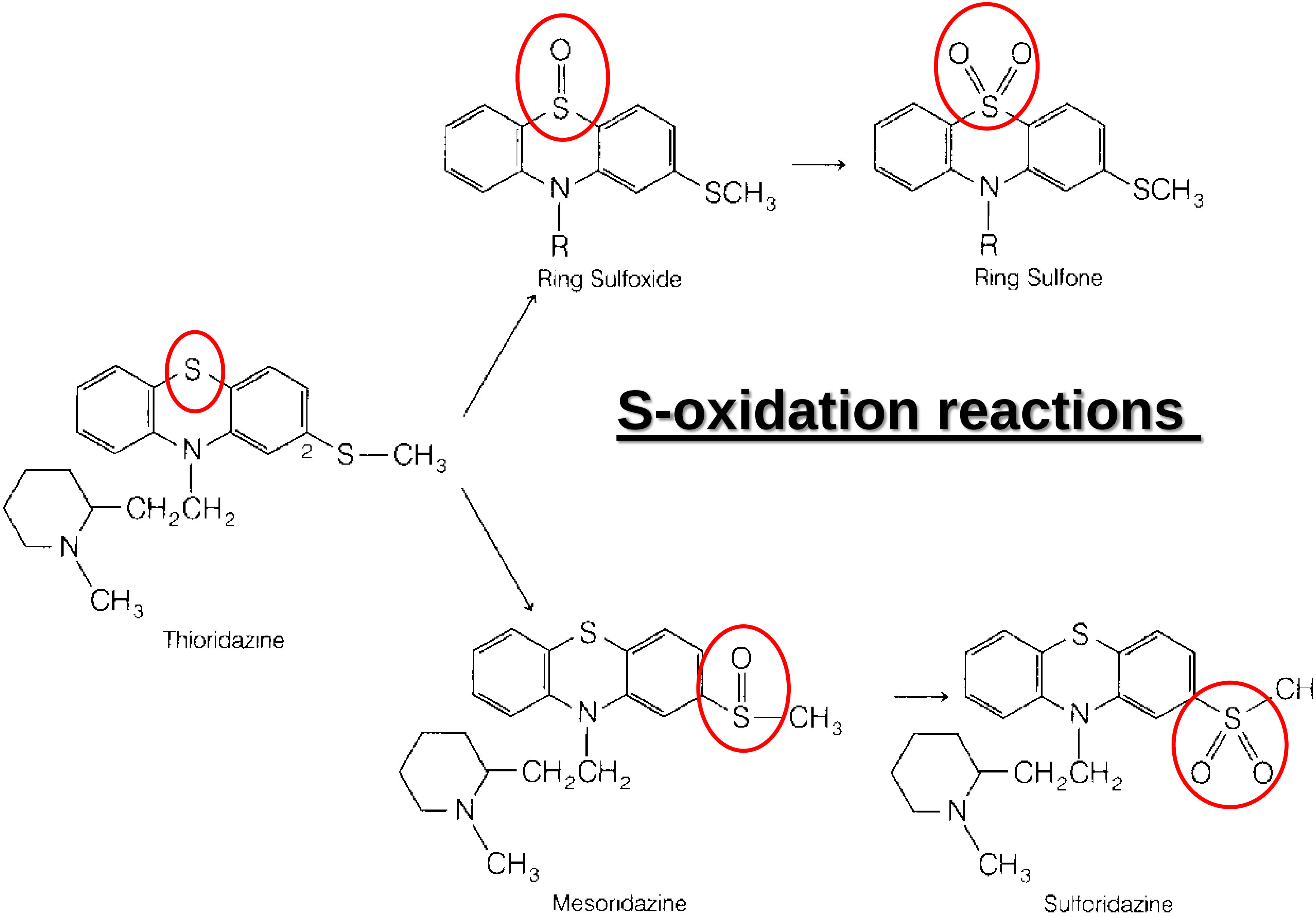
Pentobarbital



Parathion

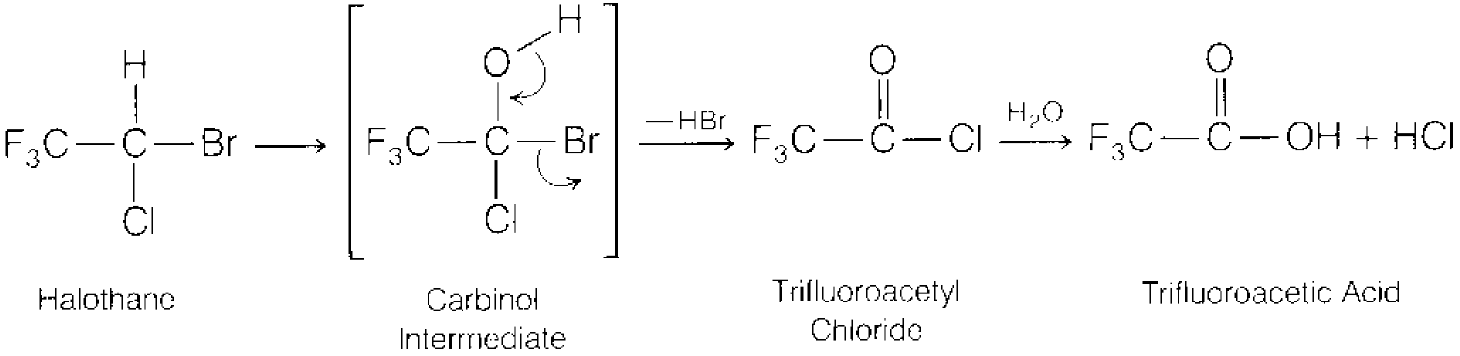
Paraoxon

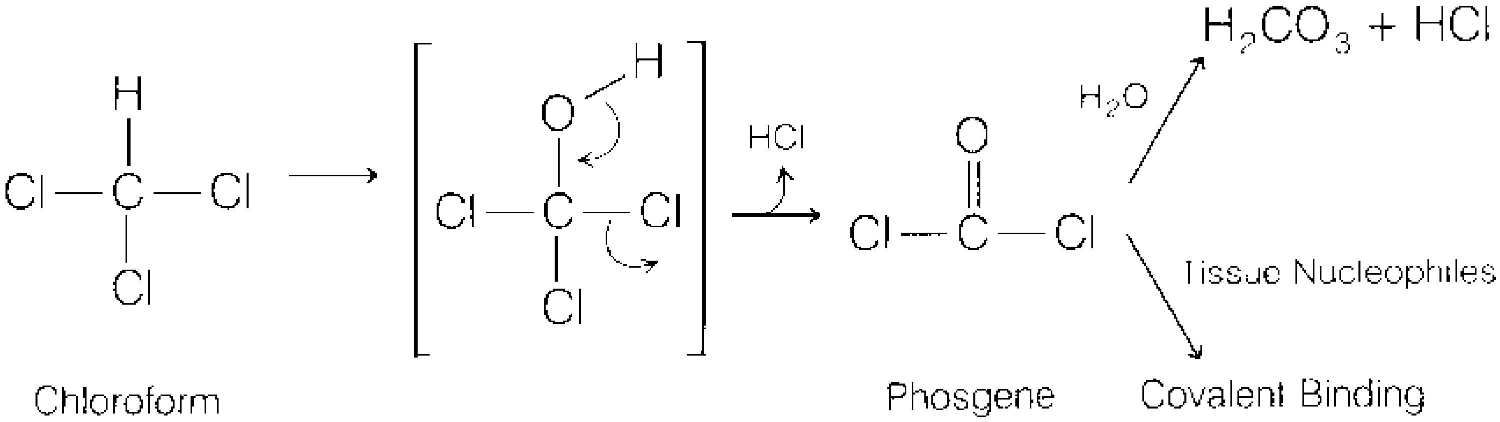
S-oxidation reactions



Dehalogenation

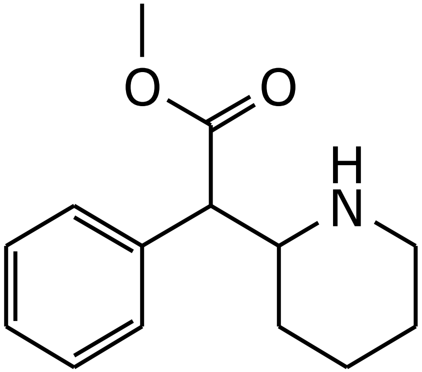
Example: carbon tetrachloride, others include. halothane, methoxyflurane





Exercise

Methylphenidate (Ritalin®) is a central nervous system stimulant. All of the following metabolic reactions for this drug are logical <u>EXCEPT</u>:

a) *para*-aromatic oxidation *b)* *N*-oxidation c) benzylic oxidation d) ester hydrolysis e) Ketone reduction	

Oxidation of alcohols and aldehydes

- **Many oxidative process (e.g. benzylic , allylic , alicyclic or aliphatic oxidation) generate alcohols (carbinols) as intermediate products if not conjugated ➔ these alcohols are further oxidized to aldehyde (if primary) or ketones (if secondary).**

- **Aldehyde metabolites resulting from oxidation of primary alcohol or from oxidative deamination of primary aliphatic amines often undergo facile oxidation to generate polar carboxylic acid derivatives.**

$$RCH_2OH \rightleftharpoons RCHO \longrightarrow RCOOH$$

- **Although secondary alcohols are susceptible to oxidation, this reaction is not often significant, since the reverse reaction occurs quite readily, in addition the secondary alcohol group being polar and functionalized is more likely to be conjugated than the ketone moiety.**

Reductive reactions

- In addition to the oxidative system, liver microsomes also contain enzyme system that that catalyze the reduction of:

- Carbonyl compounds to alcohol derivatives (hydroxyl moieties)

- Nitro and azo compounds to primary amine derivatives

- The hydroxyl and amino moieties of the metabolites are much more susceptible to conjugation than the functional groups of the parent compounds

- Thus reductive processes facilitate drug elimination.

Reduction of nitro and azo compounds

- **The reduction of aromatic nitro and azo compounds leads to primary amine metabolites**

$$Ar-NO_2 \xrightarrow{\text{nitro reduction}} Ar-NH_2$$

nitro

primary amine

azo group

$$Ar-N=N-Ar \xrightarrow{\text{azo reduction}} Ar'-NH_2 + Ar-NH_2$$

primary amine

primary amine

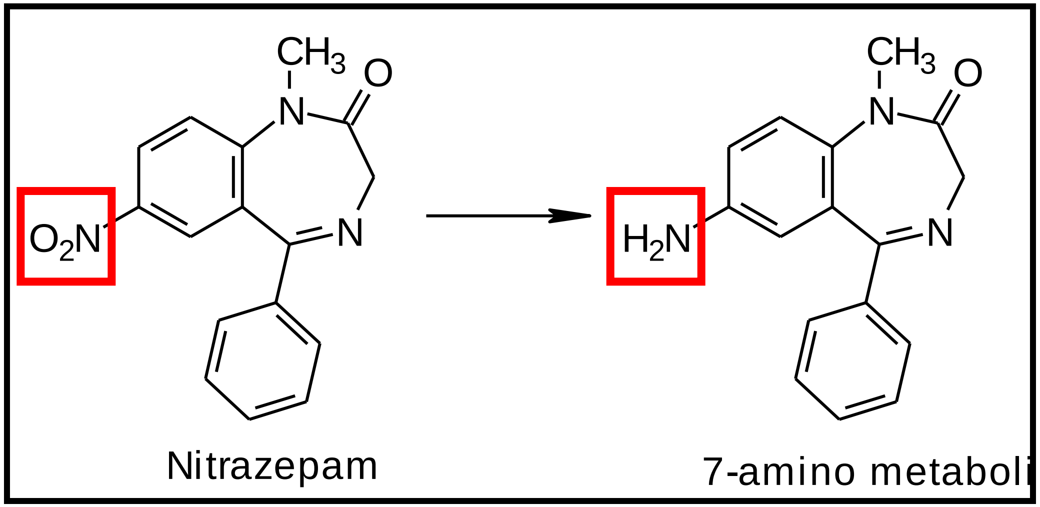
- **Azo reduction is believed to proceed via a hydrazo intermediate (NH–NH).**

$$Ar-N=N-Ar` \longrightarrow Ar-NH-NH-Ar` \longrightarrow Ar`-NH_2 + Ar-NH_2$$

Reduction of nitro and azo compounds

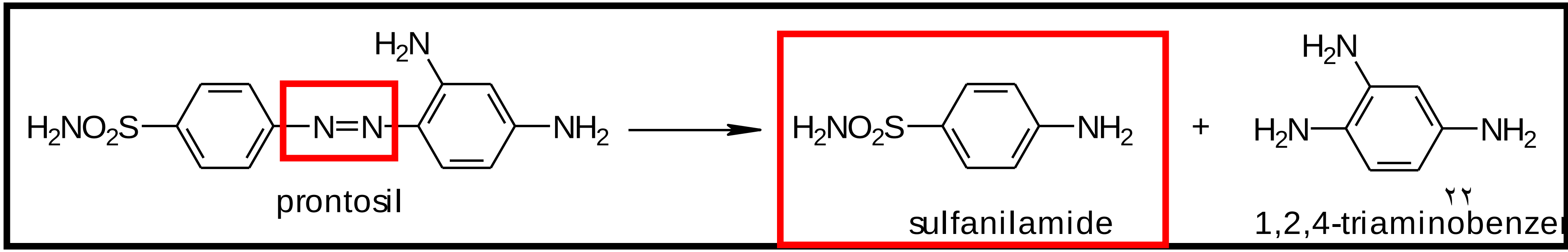
➢**Nitro reduction:**

e.g. nitrazepam is metabolized to its amino metabolite.



➢**Azo reduction:**

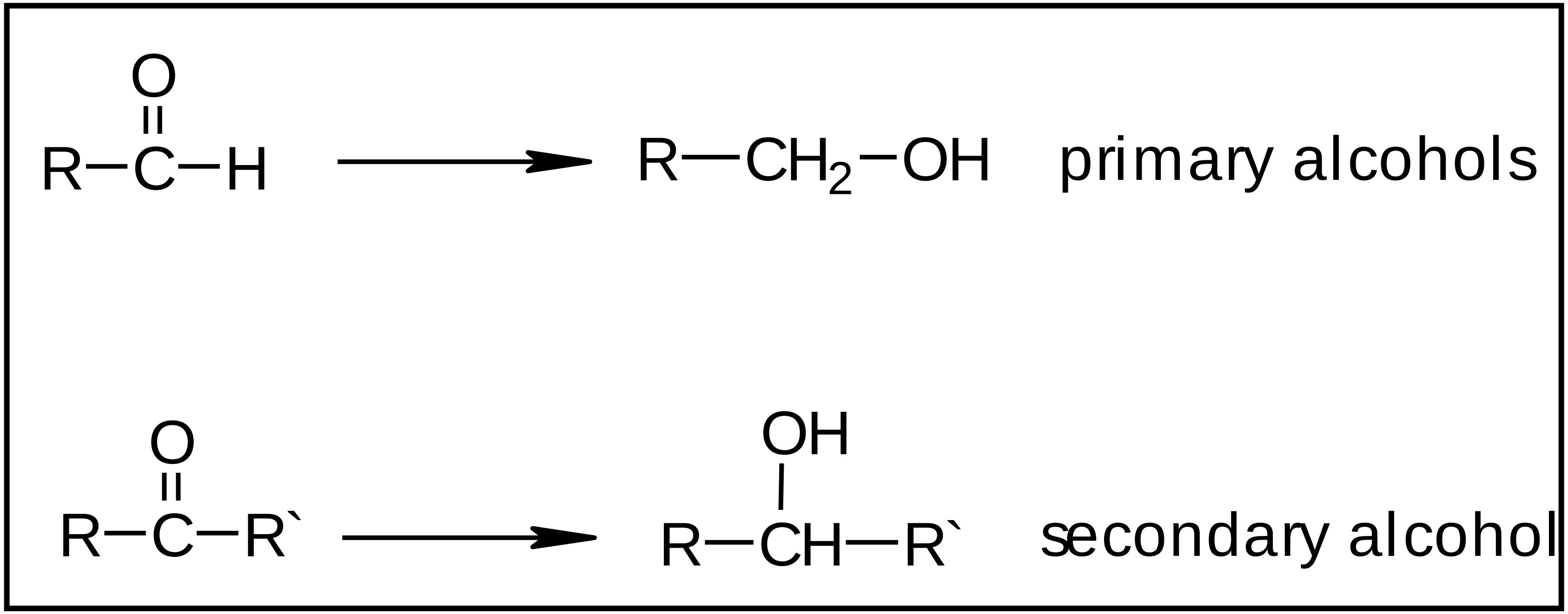
e.g. Prontosil (pro-drug) to its active sulfanilamide metabolite.



Reduction of aldehyde and ketone carbonyls

- **Aldehydes** are reduced to primary alcohols

- **Ketones** on the other hand are generally resistant to oxidation and are primarily reduced to secondary alcohols, which undergo further conjugation (glucouronidation)



$$R-\overset{\displaystyle O}{\overset{\|}{C}}-H \longrightarrow R-CH_2-OH$$

primary alcohols

$$R-\overset{\displaystyle O}{\overset{\|}{C}}-R` \longrightarrow R-\overset{\displaystyle OH}{\overset{|}{C}}H-R`$$

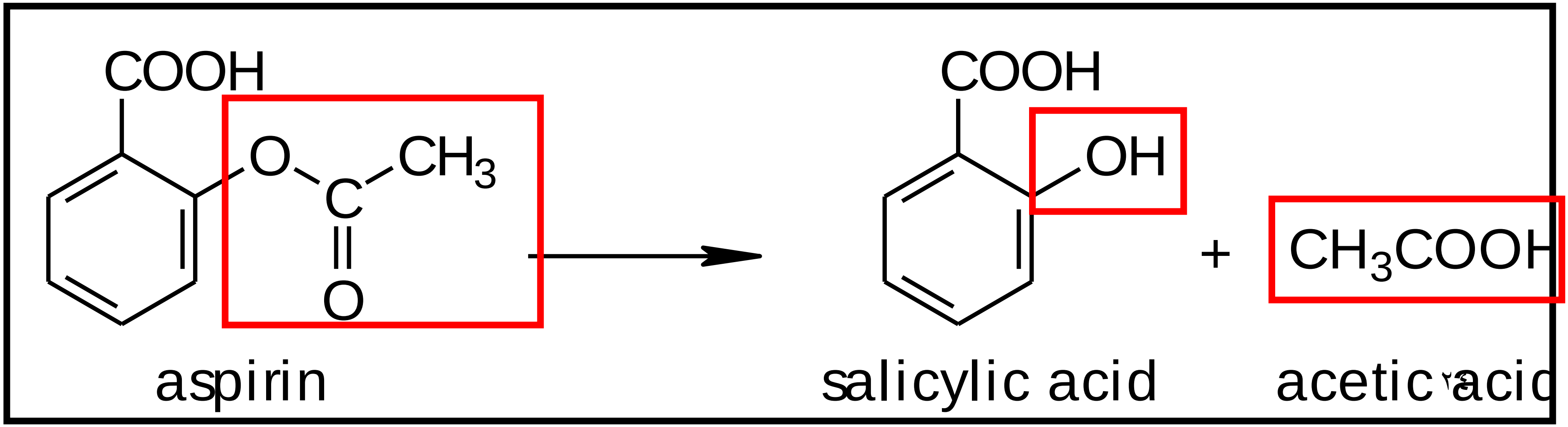
secondary alcohol

Hydrolysis of esters and amides

- The hydrolysis of ester and amide linkage in many drugs is catalyzed by hydrolytic enzymes present in various tissues, and in plasma.

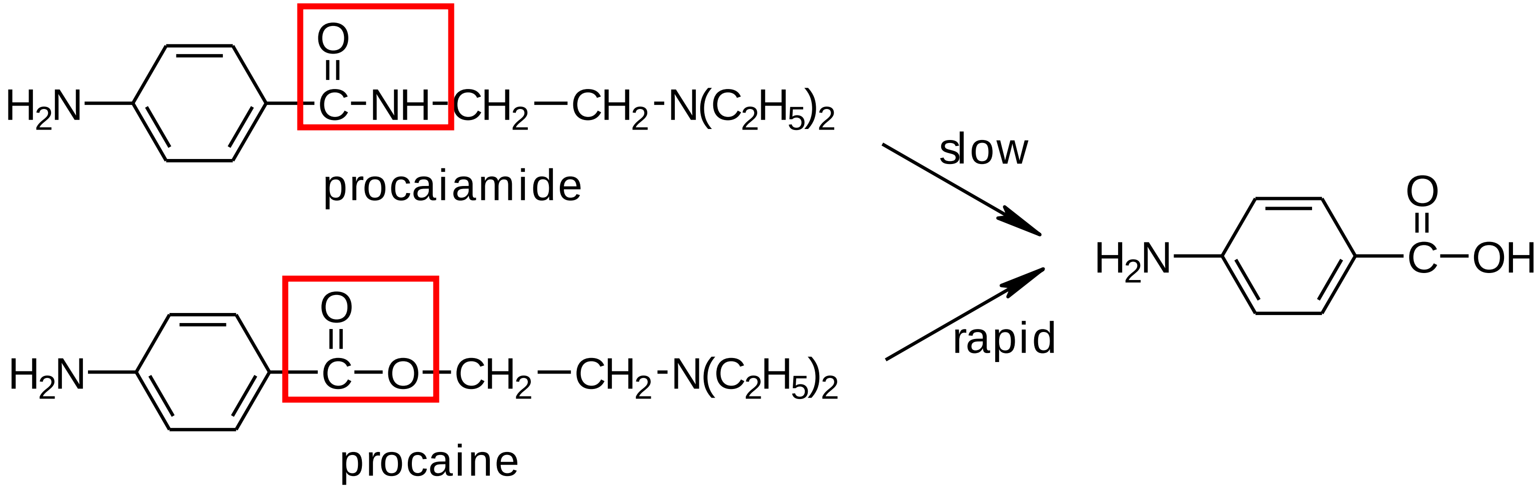
- The metabolic products formed, namely carboxylic acids ,alcohols, phenols and amines generally are polar and more susceptible to conjugation and excretion than the parent ester or amide drugs.

- A classical example of ester hydrolysis is the metabolic conversion of aspirin to salicylic acid.

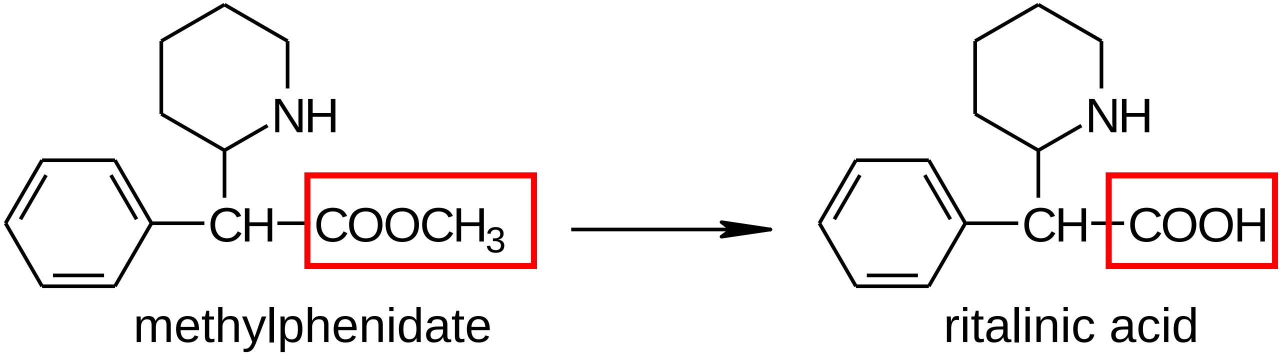


Hydrolysis of esters and amides

- ➢Amides are slowly hydrolyzed in comparison to esters e.g. procainamide and procaine.

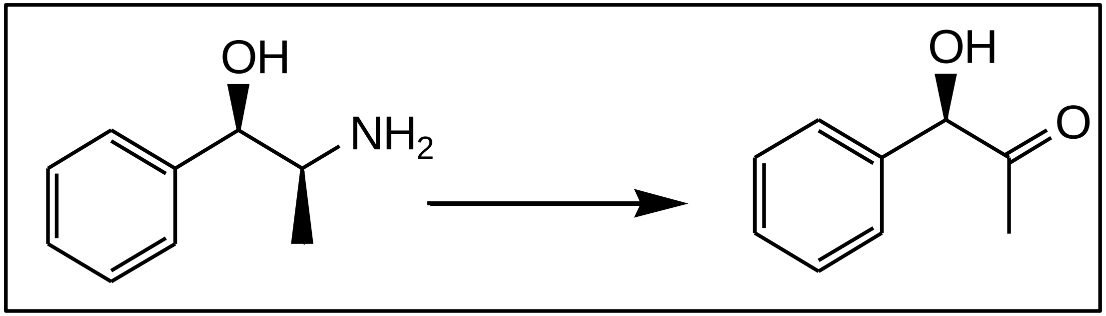


- ➢Methylphenidate is biotransformed to ritalinic acid.



Exercise I

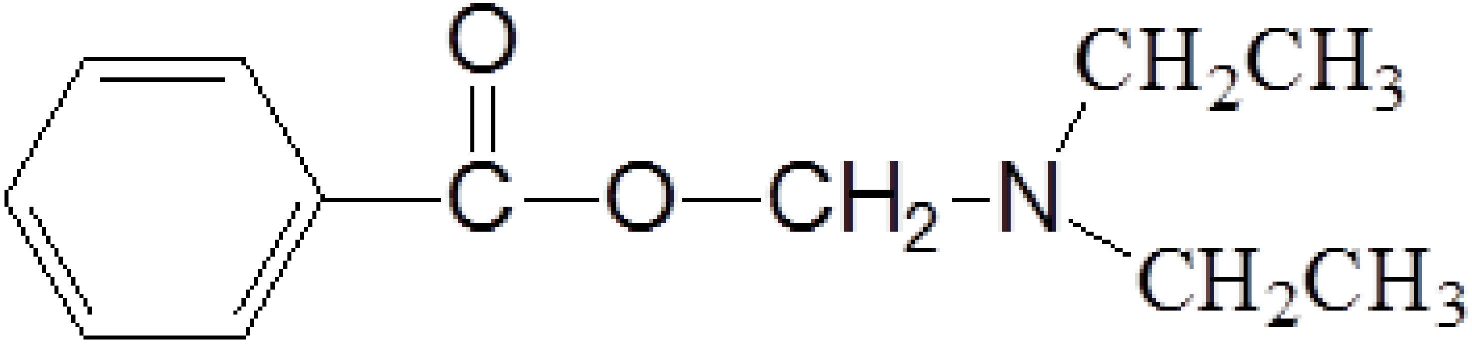
- **The best term used to define the following metabolic reaction is:**



a) Aromatic hydroxylation	a) O-dealkylation
a) Oxidative deamination	a) α-hydroxylation
a) Hydrolysis	

Exercise III

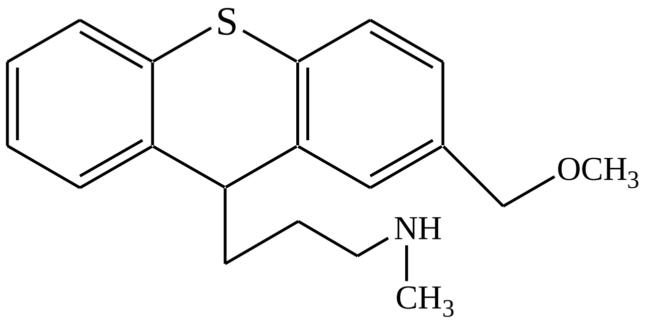
- Give five possible metabolites for this compound, indicating in each the enzyme involved and weather it is a major or minor metabolic pathway:



Exercise IV

Observe the following structure and operate the requested metabolic biotransformation:

a) Major metabolic pathway at the sulfide group.
b) Major metabolic pathway at the amine group
c) Major metabolic pathway at the ether group.
d) Minor metabolic pathway at the amine group.
e) One phase II metabolism.



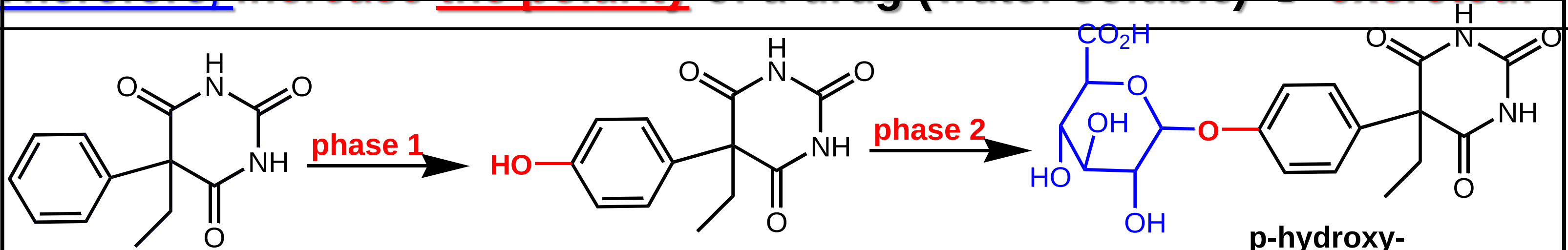
Phase II

- **Phase II (conjugation reactions):** ➔ condensation of two independent molecules

- **Reaction Types Including:** glucuronidation, sulfate formation, acetylation,, GSH conjugation, methylation, glycine and glutamate conjugation

- **In which:** a functional group (hydroxyl, carboxylic acid, amine) is masked by the addition of a new group (glucuronic acid, sulfate, acetyl)
  - ✓ Acts on parent drug or
  - ✓ Acts on phase 1 metabolite?
  - ✓ Links to endogenous, polar, ionizable compound
  - ✓ Purpose: enhance excretion?

- **Therefore, increase the polarity** of a drug (water soluble) ➔ excreted!



phenobarbital
(water insoluble)

p-hydroxy-
phenobarbital
(water insoluble)

p-hydroxy-
phenobarbital
glucuronide
(water soluble)

Phase II reactions (Conjugation)

- Phase I or functionalization reactions do not always produce hydrophilic or pharmacologically inactive metabolites,

- However various phase II or conjugation reactions are capable of converting these metabolites to more polar and water soluble products.

- The conjugated products are relatively water soluble and readily excretable.

- In addition , they generally are biologically inactive and non-toxic

- Phase II include conjugation with:
  - glucouronic acid,
  - sulfate ,
  - amino acids (glycine and glutamine)
  - glutathione

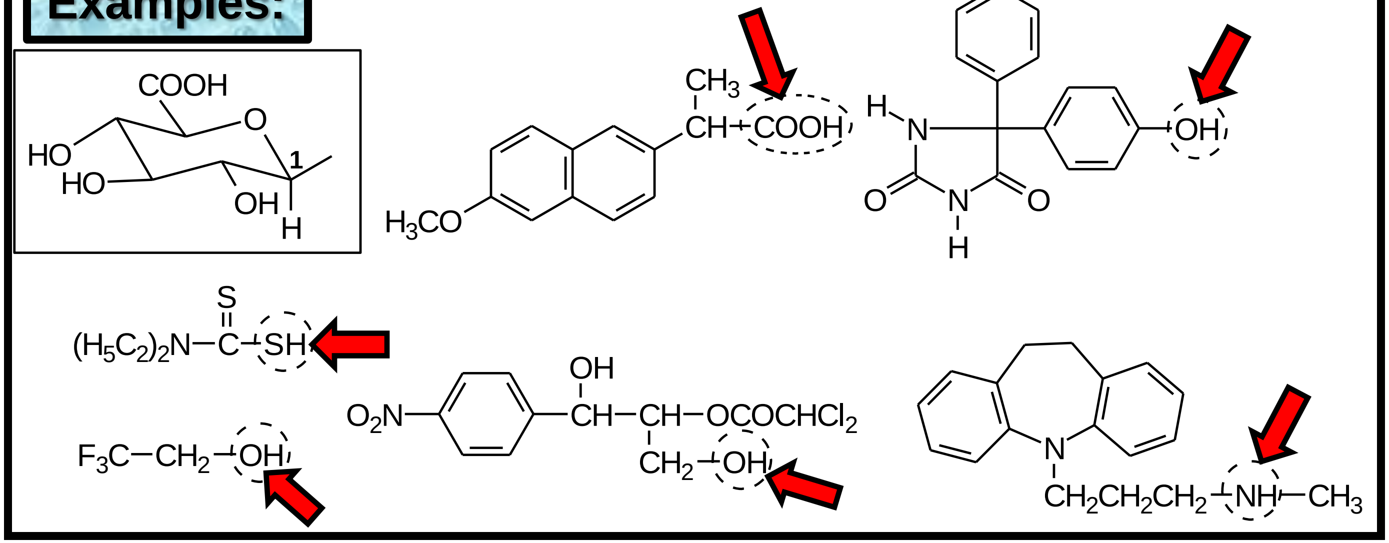
- Other phase II reaction such as acetylation and methylation don’t increase solubility in water but serve mainly to terminate pharmacological activity.

(Glucouronic acid conjugation)

- **Glucouronidation is the most common conjugative pathway in drug metabolism because**
  - A readily available supply of D-glucuronic acid
  - A large number of functional groups that can combine enzymatically with glucuronic acid.
  - The glucuronyl moiety when attached to xenobiotic substances , greatly increases the water solubility.

- **Metabolic products are classified as oxygen , nitrogen , sulfur or carbon glucuronide according to the heteroatom attached to C-1 atom of the glucuronyl moiety**

Examples:



(Sulfate conjugation)

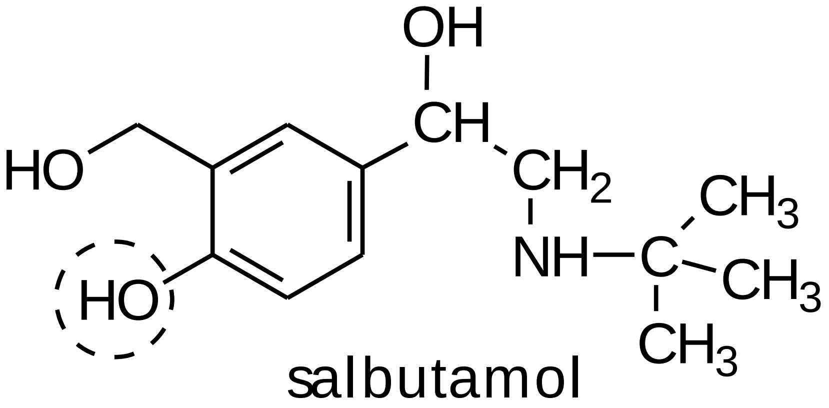
- **It occurs primarily with phenols and occasionally with alcohol, aromatic amines and N-hydroxy compounds.**

In contrast to glucuronic acid, the amount of the available sulfate is limited and sulfate is provided through coenzyme called (PAPS, 3’-phosphoadenosine-5’-phosphosulfate) with the enzyme sulfo transferase.

- **Sulfate conjugation generally leads to water soluble and inactive metabolites**

- **However the O-sulfate conjugates of some N-hydroxy compounds give rise to chemically reactive intermediates that are toxic**

- **Phenolic moieties are often susceptible to sulfate formation e.g. salbutamol.**

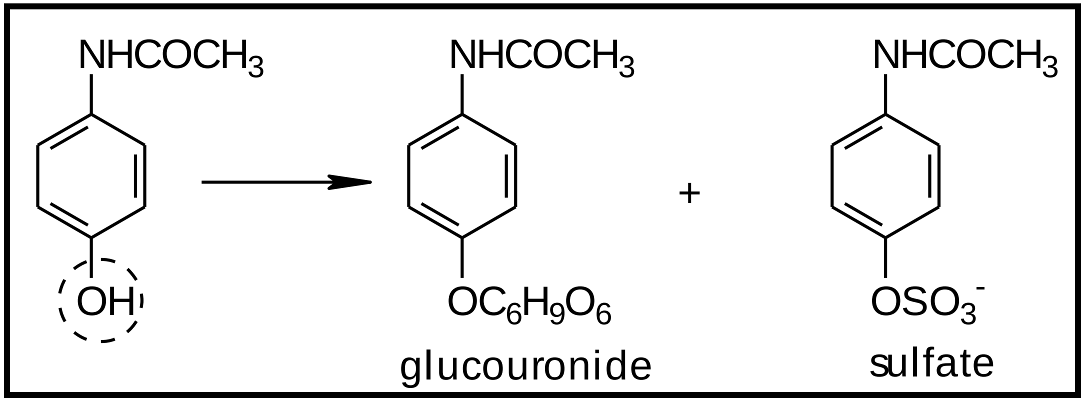


salbutamol

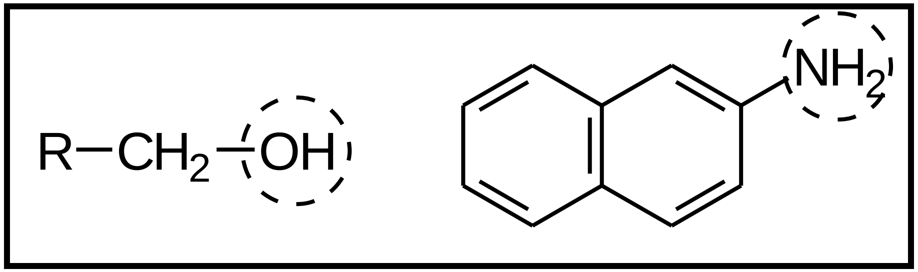
(Sulfate conjugation)

- In adults the major urinary metabolite of acetaminophen is the O-glucuronide conjugate, with O-sulfate conjugate being formed in small amounts.

- Interestingly in infants, O-sulfate is the main metabolite, this is because the neonates and children have a decreased glucuronating capacity owing to undeveloped glucuronyl transferase or low level of glucuronyl transferase.



- Other functionalities such as alcohols e.g. aliphatic alcohols and aromatic amines are also capable of forming sulfate conjugates.

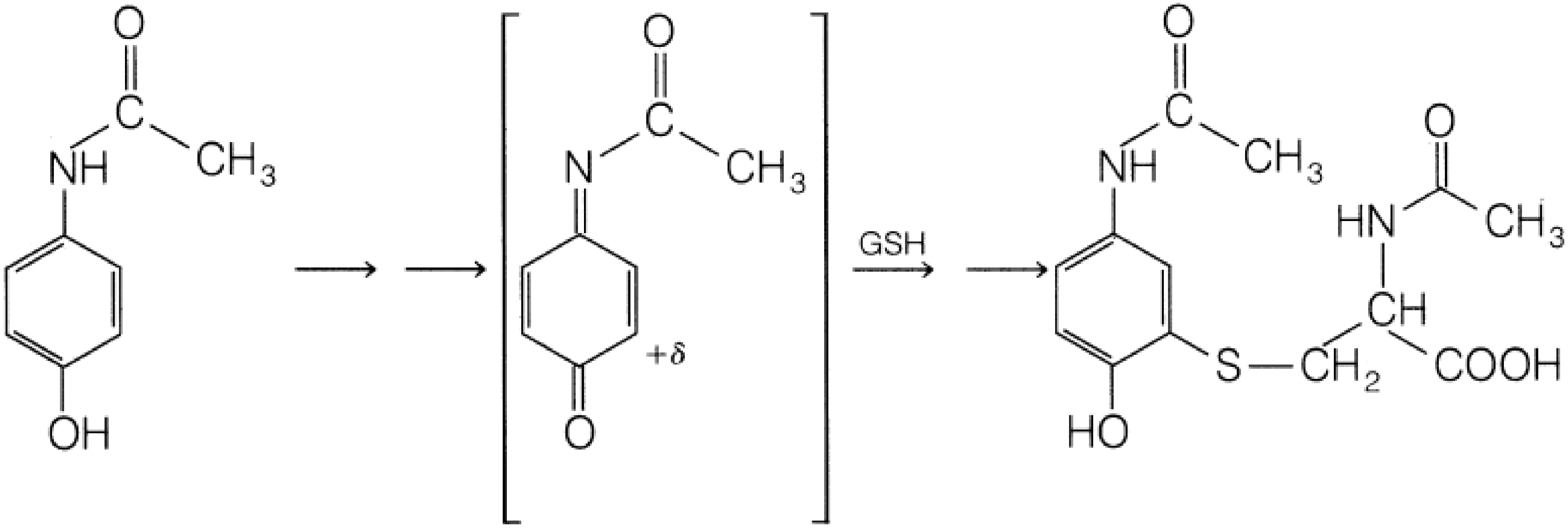


(GSH conjugation)

- GSH conjugation is an important pathway for detoxifying chemically reactive electrophilic compounds. It is now generally accepted that reactive electrophilic species manifest their toxicity (e.g. tissue necrosis. carcinogenicity, mutagenicity, teratogenicity) by combining covalently with nucleophilic groups present in vital cellular proteins and nucleic acids.
- Many serious drug toxicities may be explained also in terms of covalent interaction of metabolically generated electrophilic intermediates with cellular nucleophiles.

- GSH protects vital cellular constituents against chemically reactive species by virtue of its nucleophilic sulfhydryl (SH) group. The SH group reacts with electron-deficient compounds to form S-substituted GSH adducts chemically reactive species by virtue of its nucleophilic sulfhydryl (SH) group.

Detoxification of the toxic metabolite of Acetaminophen (N-Acetylimidoquinone) through the intervention of GSH



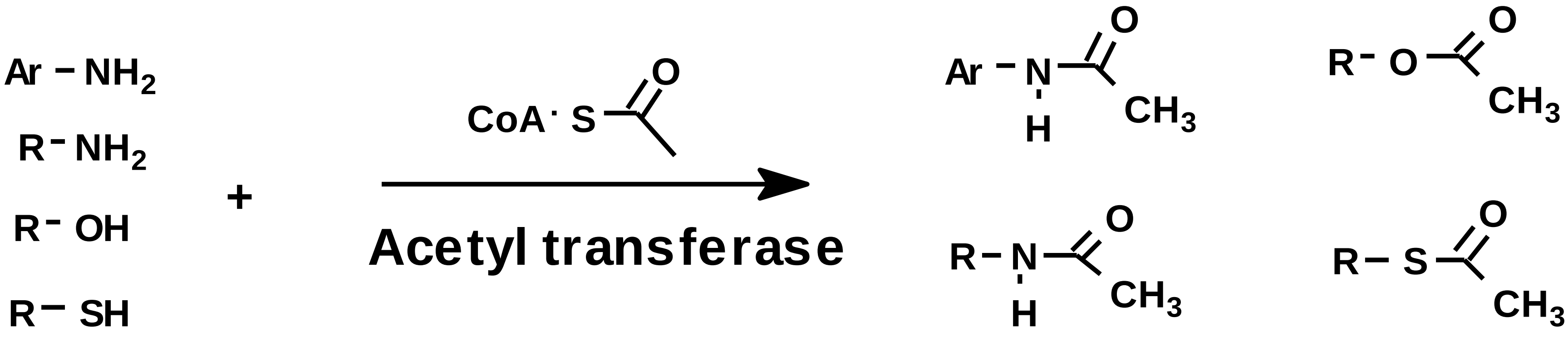
Acetaminophen

N-Acetylimidoquinone

Mercapturic Acid Derivative

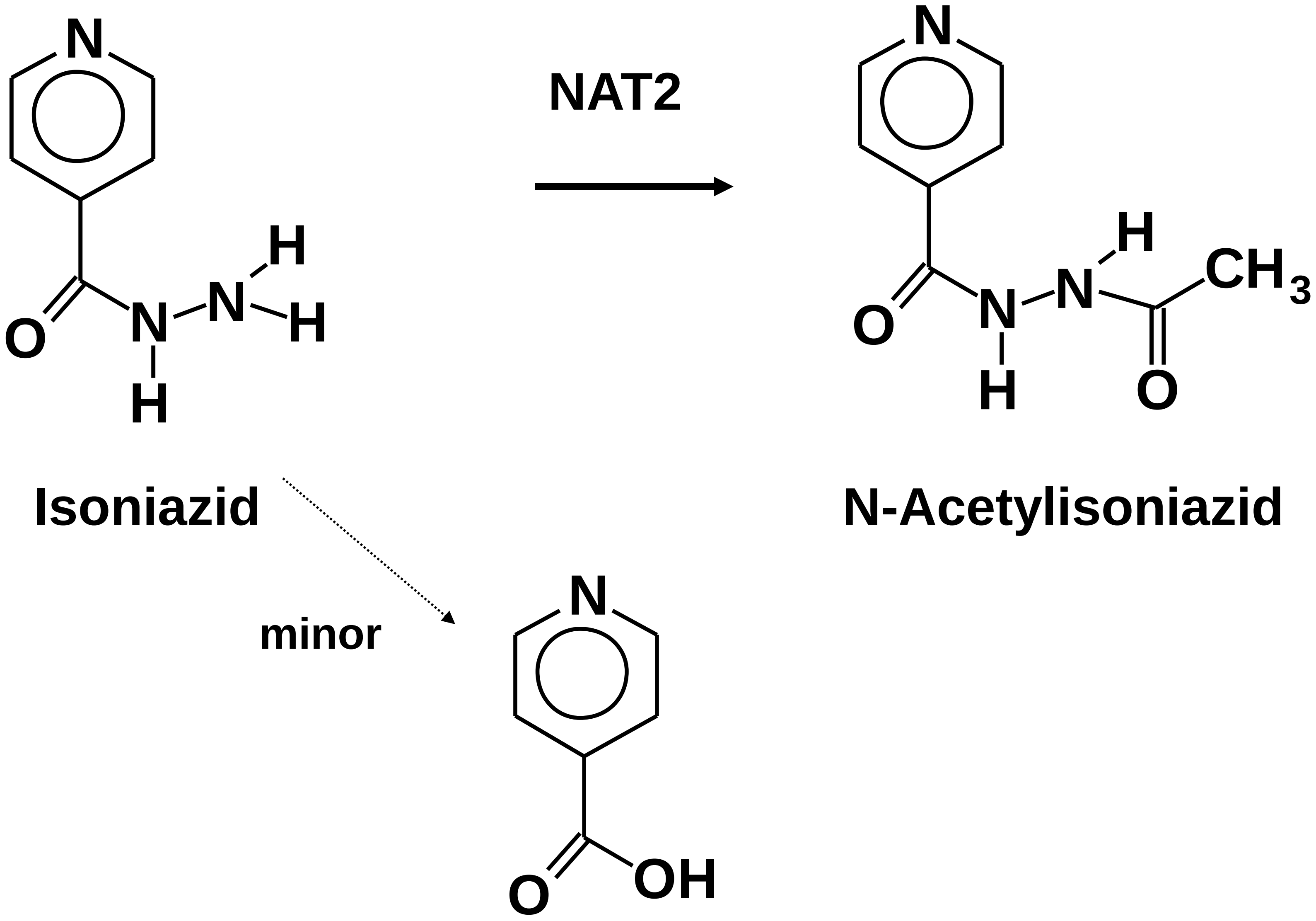
(Acetylation)

Acetylation constitutes an important metabolic route for drugs containing primary amino groups. This encompasses primary aromatic amines (ArNH2), Sulfonamides (H2NC6H4SO2NHR), hydrazines (—NHNH2), hydrazides (—CONHNH2), and primary aliphatic amines. The amide derivatives formed from acetylation of these amino functionalities are generally inactive and nontoxic.



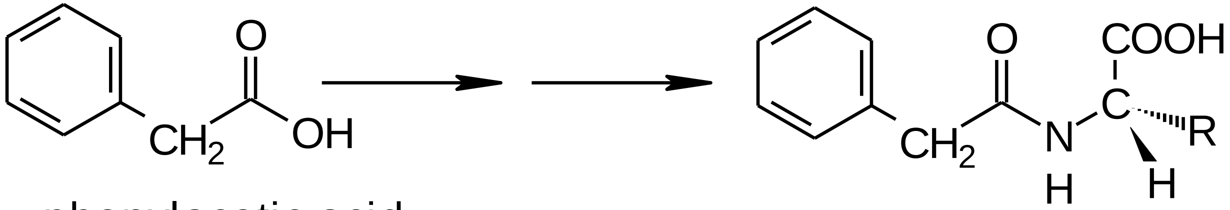
Examples: Procainamide, isoniazid, sulfanilimide, histamine

NAT enzyme is found in many tissues, including liver


NAT2
Isoniazid
N-Acetylisoniazid
minor

(Glycine Conjugations)

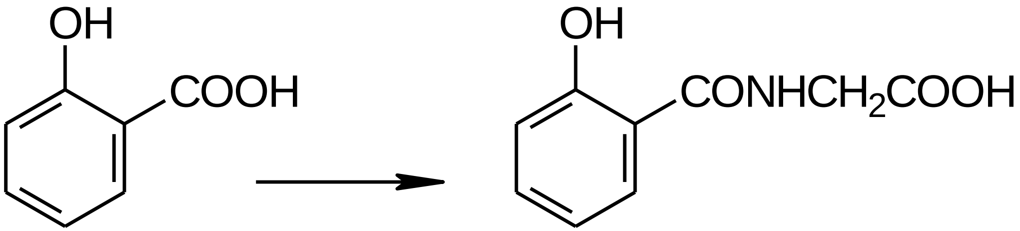
- They conjugate aromatic and aryl-alkyl (aralkyl) acids, the quantity of amino acid conjugates formed from xenobiotics is quite small because of the limited availability of amino acids in the body and competition with glucuronidation for carboxylic acid and substrates.



phenylacetic acid

R = H , glycine congugate
R = $CH_2CH_2CONH_2$, glutamine congug

- **Aromatic acids and aralkyl acids are the major substrates undergoing glycine conjugation**



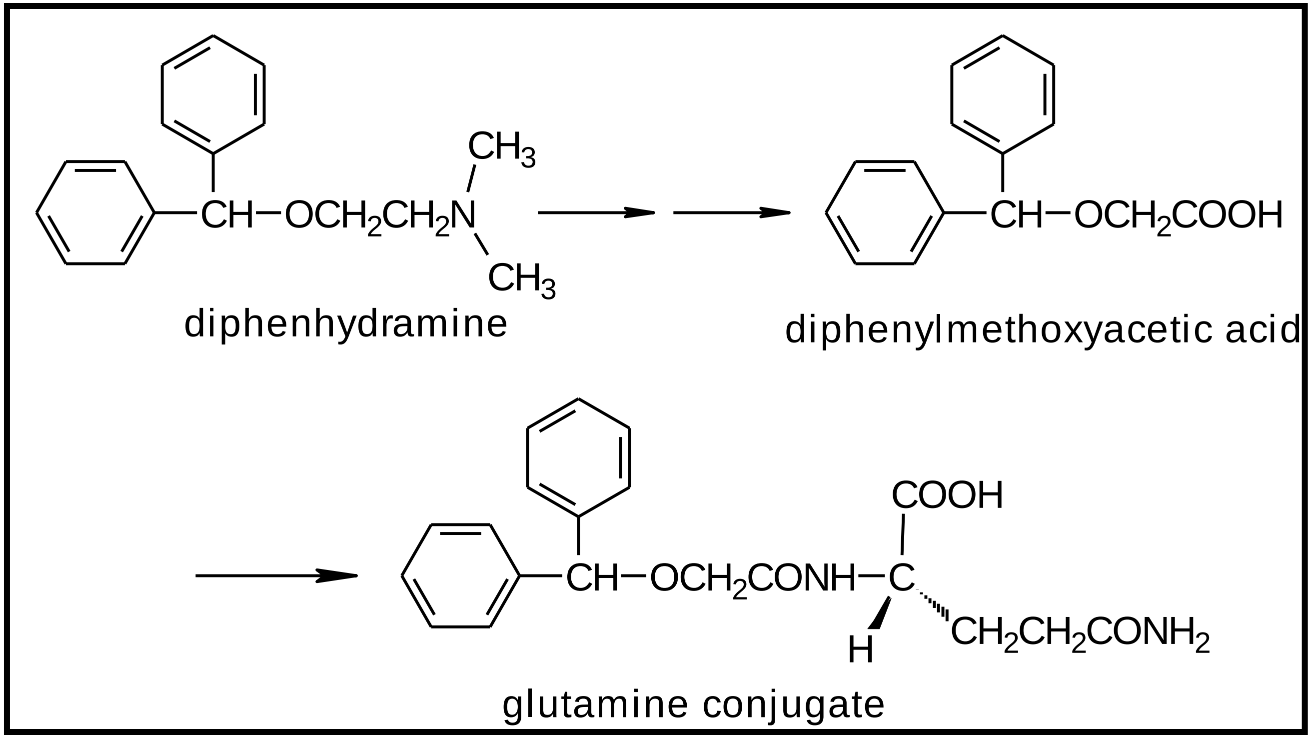
salicylic acid

salicyluric acid

(Glutamine conjugates)

- **Glutamine conjugates occur mainly with arylacetic acids including endogenous phenylacetic and indol-acetic acids.**

- **A few of glutamine conjugates of drug metabolites have been reported e.g. diphenylmethoxyacetic acid a metabolite of the antihistamine diphenhydramine.**



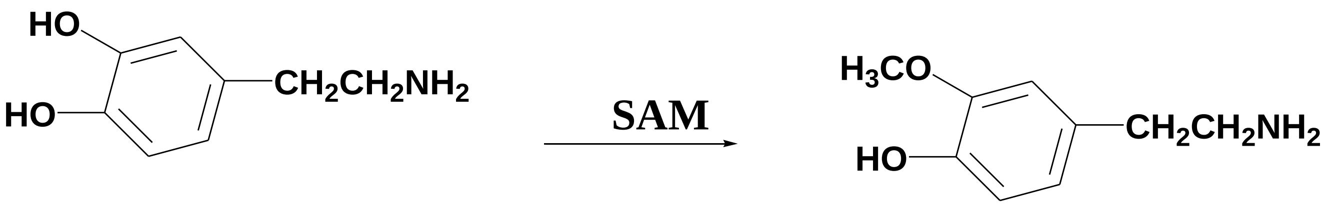
Methylation Reactions

Substrates: Phenol, thiol, NH_2, some heterocycles are metabolized by this route.

Catalyzed by specific methyltransferases

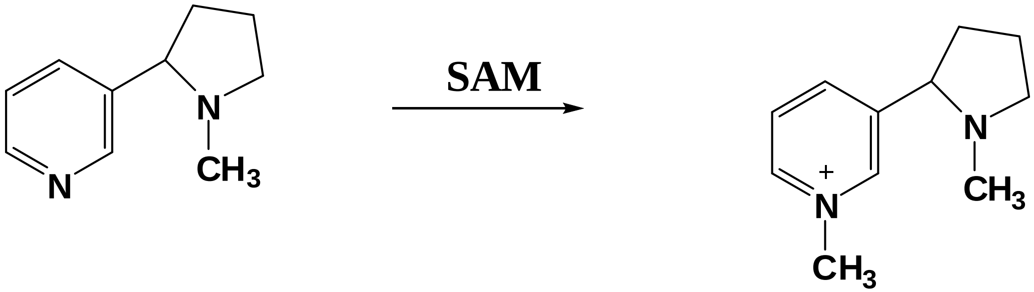
The formed methylated metabolites, except with amines are less water soluble and pharmacologically inactive

The co-enzyme is: S-adenosylmethionine



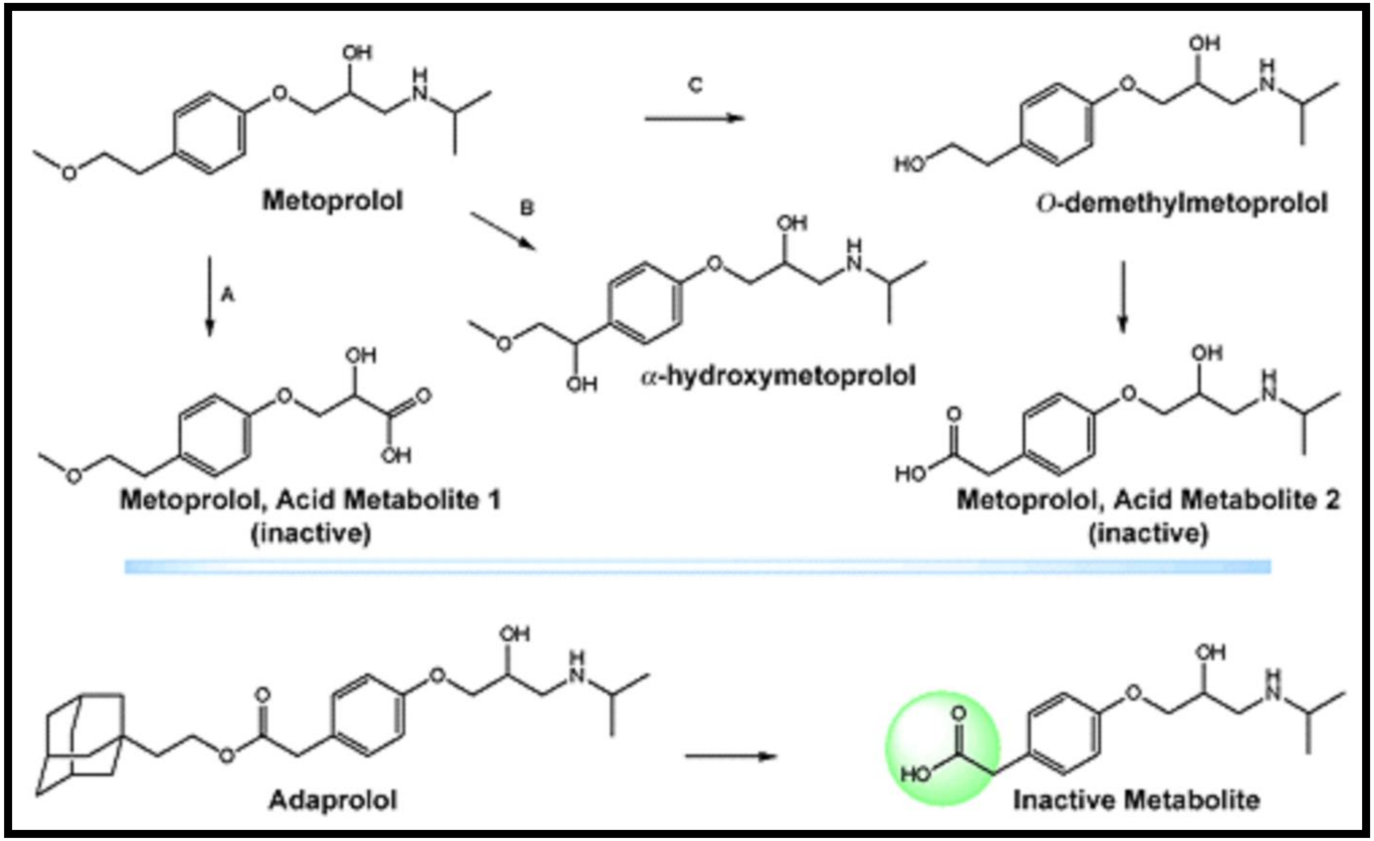
(Dopamine)
a neurotransmitter

3-Methoxy
(Selective)



Quaternary ammonium salts
(water solube)

N.B: Catechol –O-Methyltransferase COMT is the enzyme responsible for methylation of the *m*-OH group in catechol-type biogenic amines


Metoprolol
C
O-demethylmetoprolol
B
A
α-hydroxymetoprolol
Metoprolol, Acid Metabolite 1
(inactive)
Metoprolol, Acid Metabolite 2
(inactive)
Adaprolol
Inactive Metabolite

Thank you
Please smile