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# Enzymes FAD-dépendantes impliquées dans l'oxydation métabolique des xénobiotiques

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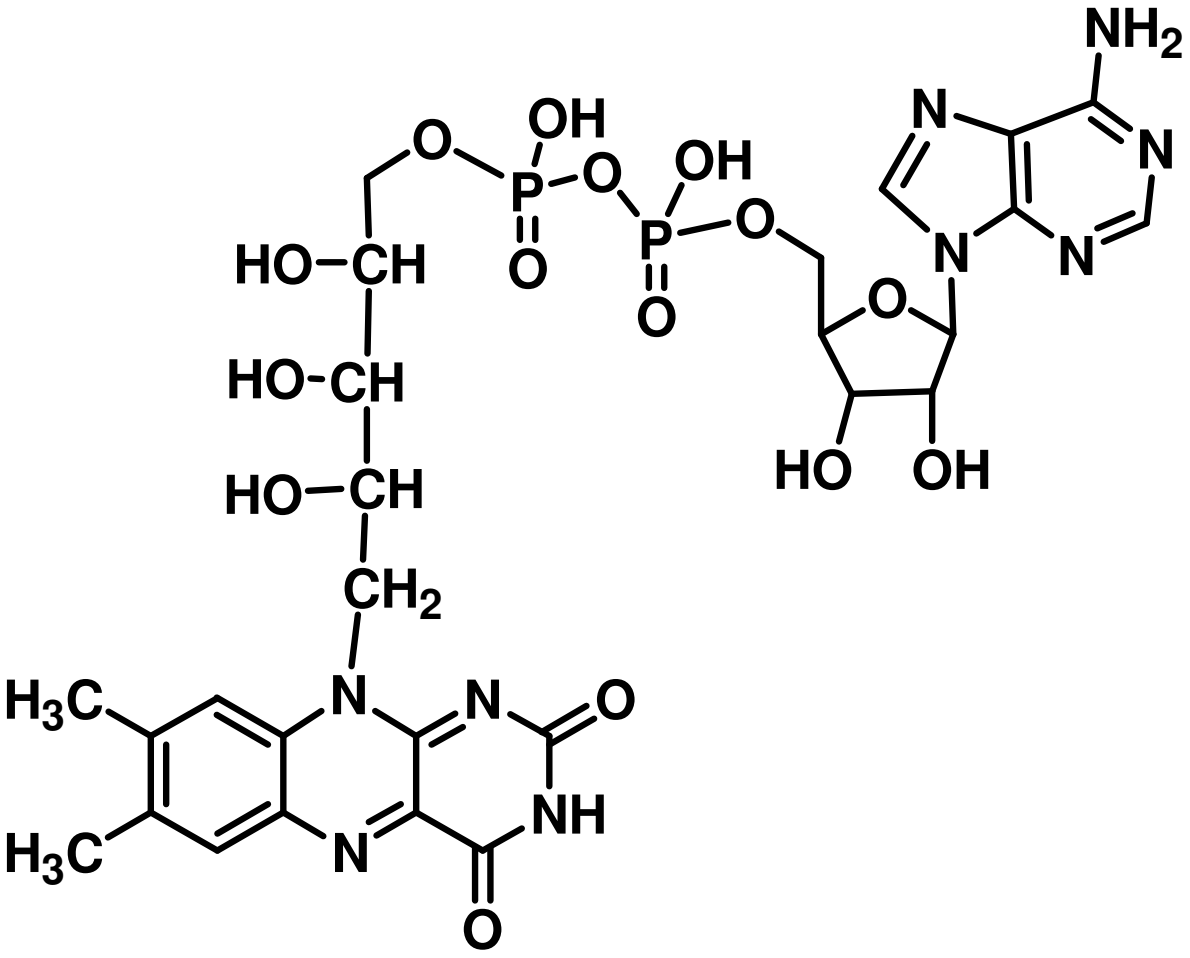
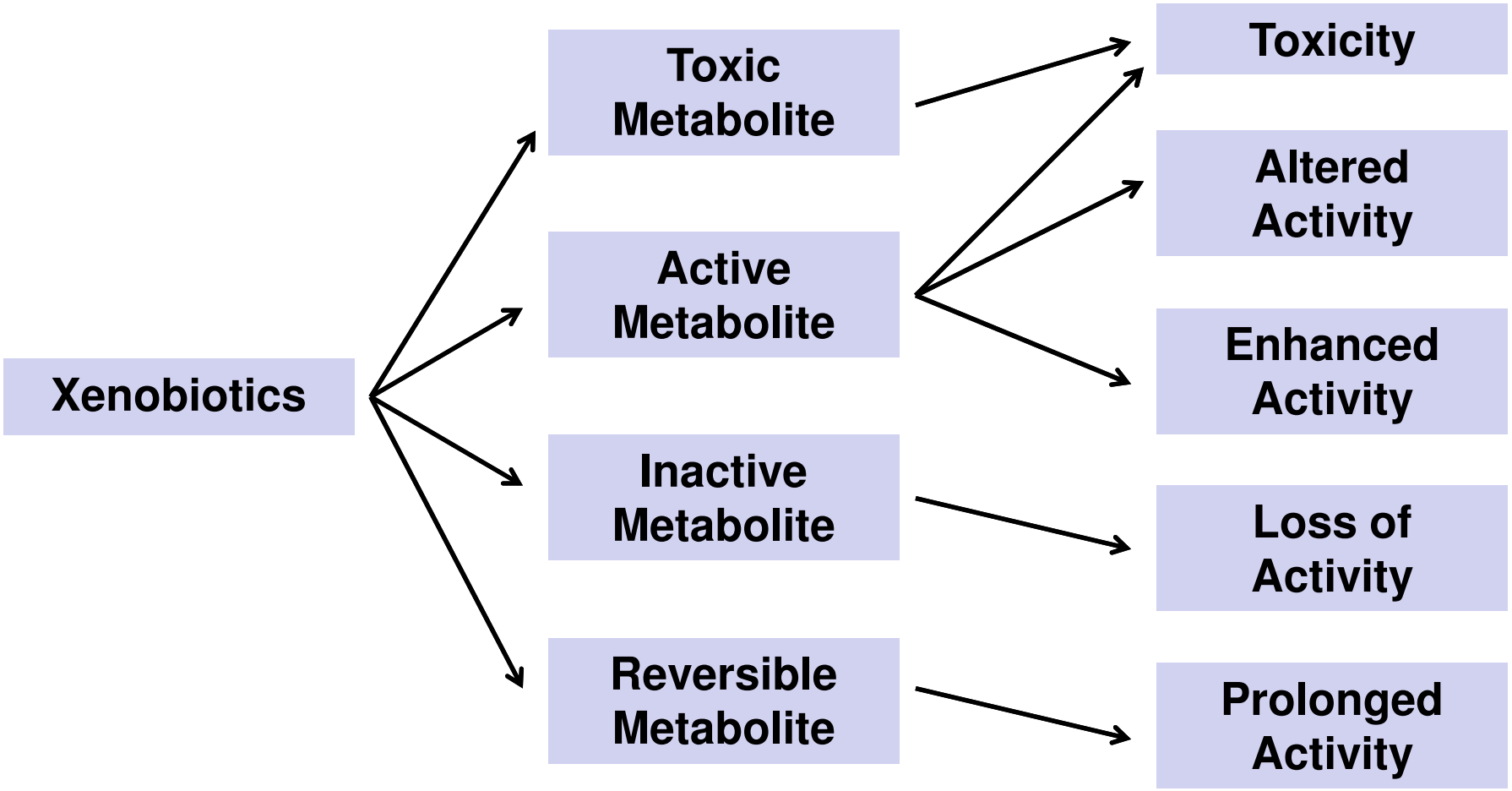
# Structure of Flavine-Adenine Dinucleotide (FAD)



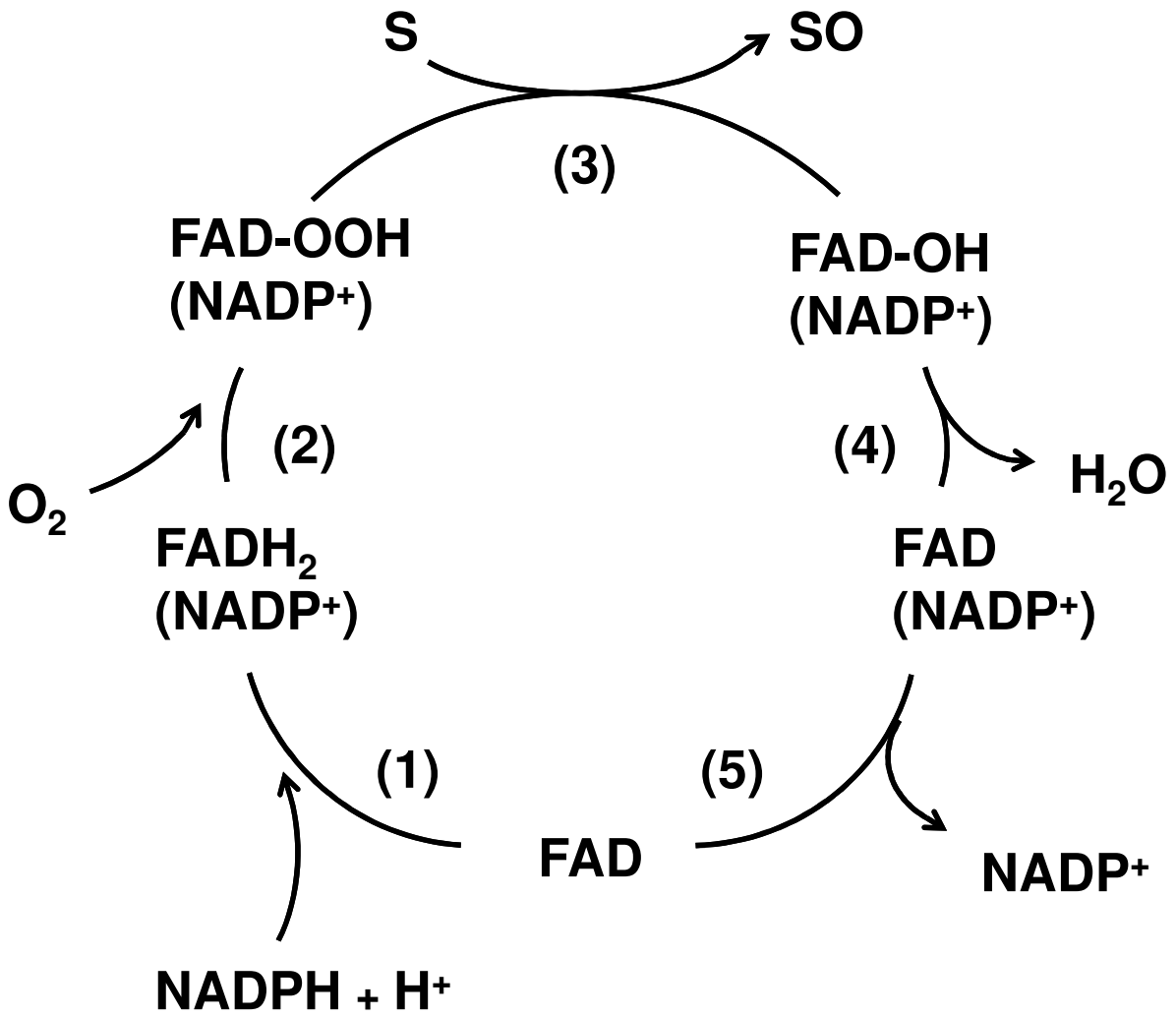
# Subcellular localization of enzymes involved in the metabolic oxidation of xenobiotics

| Enzymes | Subcellular localization |
|---|---|
| CYP | Endoplasmic reticulum (microsomes) |
| FMOs | Endoplasmic reticulum (microsomes) |
| AO et XO | Cytosol |
| MAOs | Mitochondria |
| PAOs | Cytosol, peroxisomes |
| CuAOs | Serum/Plasma and plasma membranes |

# Characteristics of metabolites produced in the metabolism of xenobiotics



# Catalytic cycle of flavin-containing monooxygenase



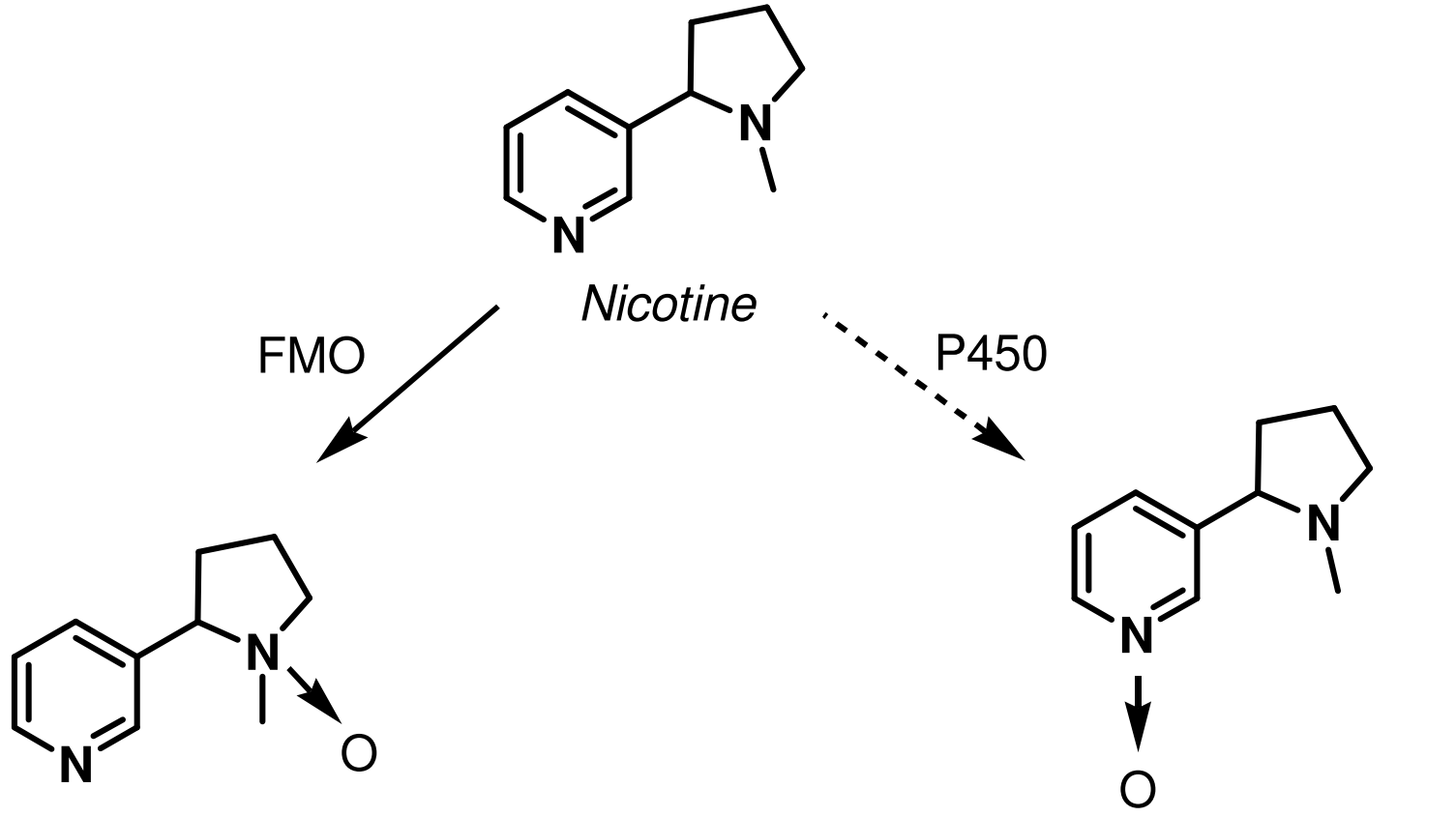
# Differences and similarities of the human flavin-containing (FMOs) and cytochrome P450 (CYPs) monooxygenases

- Both FMOs and CYPs catalyze the NADPH-dependent N-or S-oxygenation of heteroatom-containing compounds
- While FMOs require nucleophiles as substrates, CYPs can oxidize non-nucleophilic substrates
- Both FMOs and CYPs generally convert lipophilic compounds to more hydrophilic materials
- FMOs are much more sensitive to temperature then CYPs (e.g.FMOs are quite unstable at 50℃, while under similar conditions about 85% of the functional activity of CYPs is retained)
- CYPs are often inducible, whereas very few reports of FMOs induction have appeared
- In contrast to CYPs, FMOs are rarely inhibited

# Amines oxidized by FMOs

| SUBSTRATE | FMOs |
|---|---|
| PRIMARY AMINES | - |
| SECONDARY AMINES | + |
| TERTIARY ALIPHATIC AMINES | + |
| TERTIARY AROMATIC AMINES | - |

# N-Oxidation of nicotine

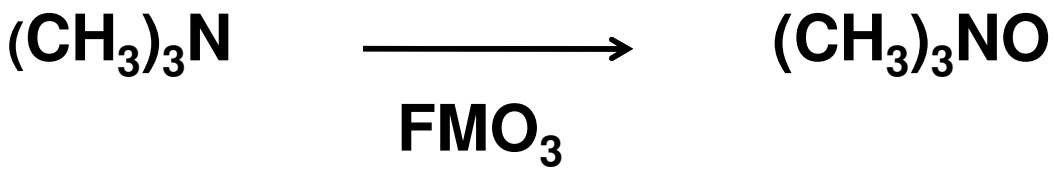


# Summary of tissue mRNA levels of human flavin-containing mononoxygenases in adult humans

| Enzyme | Tissue |
|---|---|
| FMO1 | kidney >> lung, small intestine >> liver >> brain |
| FMO2 | lung >> kidney > liver, small intestine >> brain |
| FMO3 | liver >> lung > kidney >> small intestine >> brain |
| FMO4 | liver > kidney > lung > small intestine >> brain |
| FMO5 | liver >> small intestine, lung, kidney >> brain |

FMO : Flavin-containing monooxygenase

# Xenobiotic amine metabolism : detoxication and deodorization process for trimethylamine



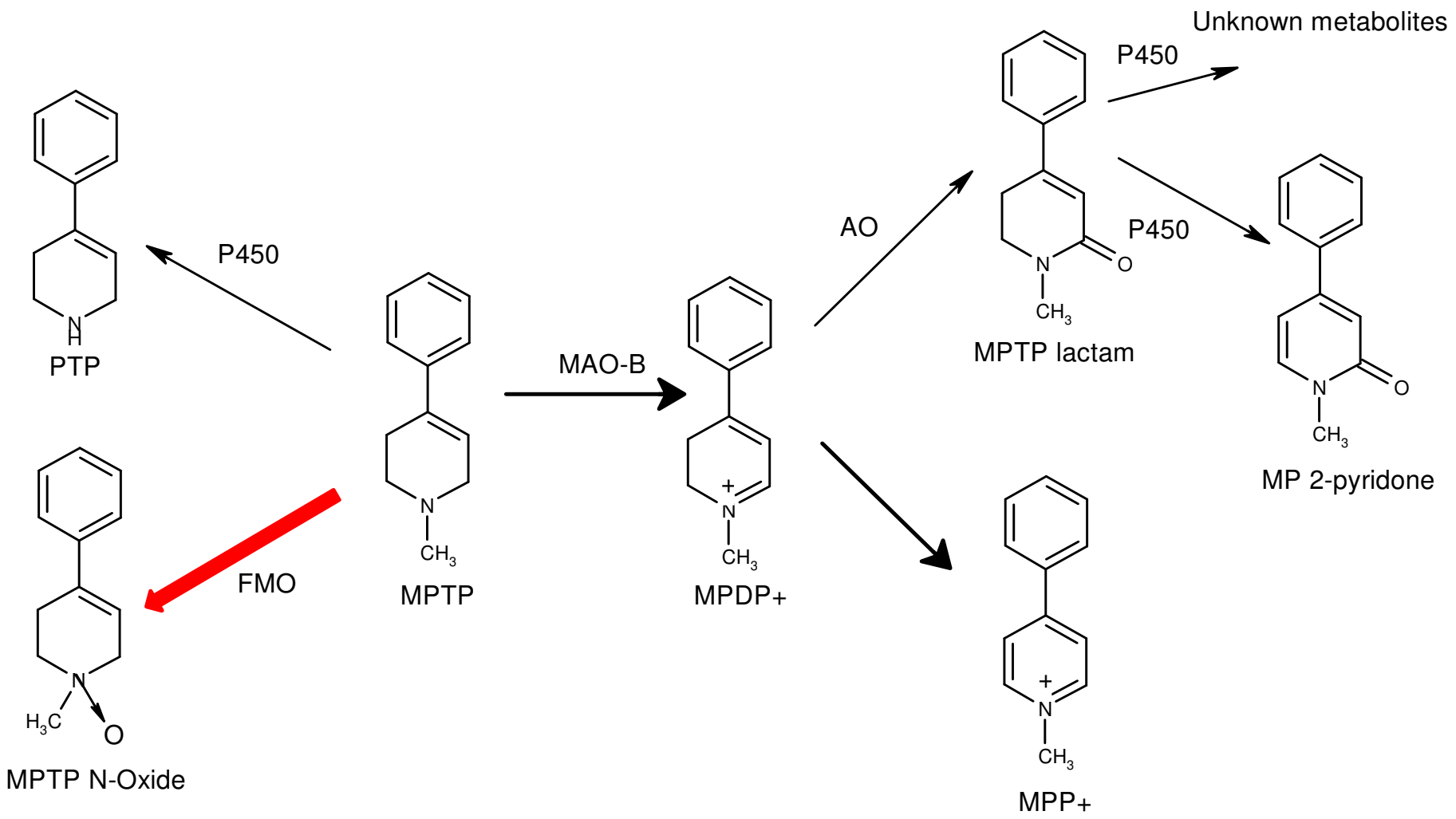
## METABOLISM OF TRIMETHYLAMINE (TMA) TO TMA N-oxyde

| Subjects | Urinary TMA N-oxyde/TMA ratio |
|---|---|
| Normal | 97/3 |
| Severely FMO3-deficient | 10/90 |

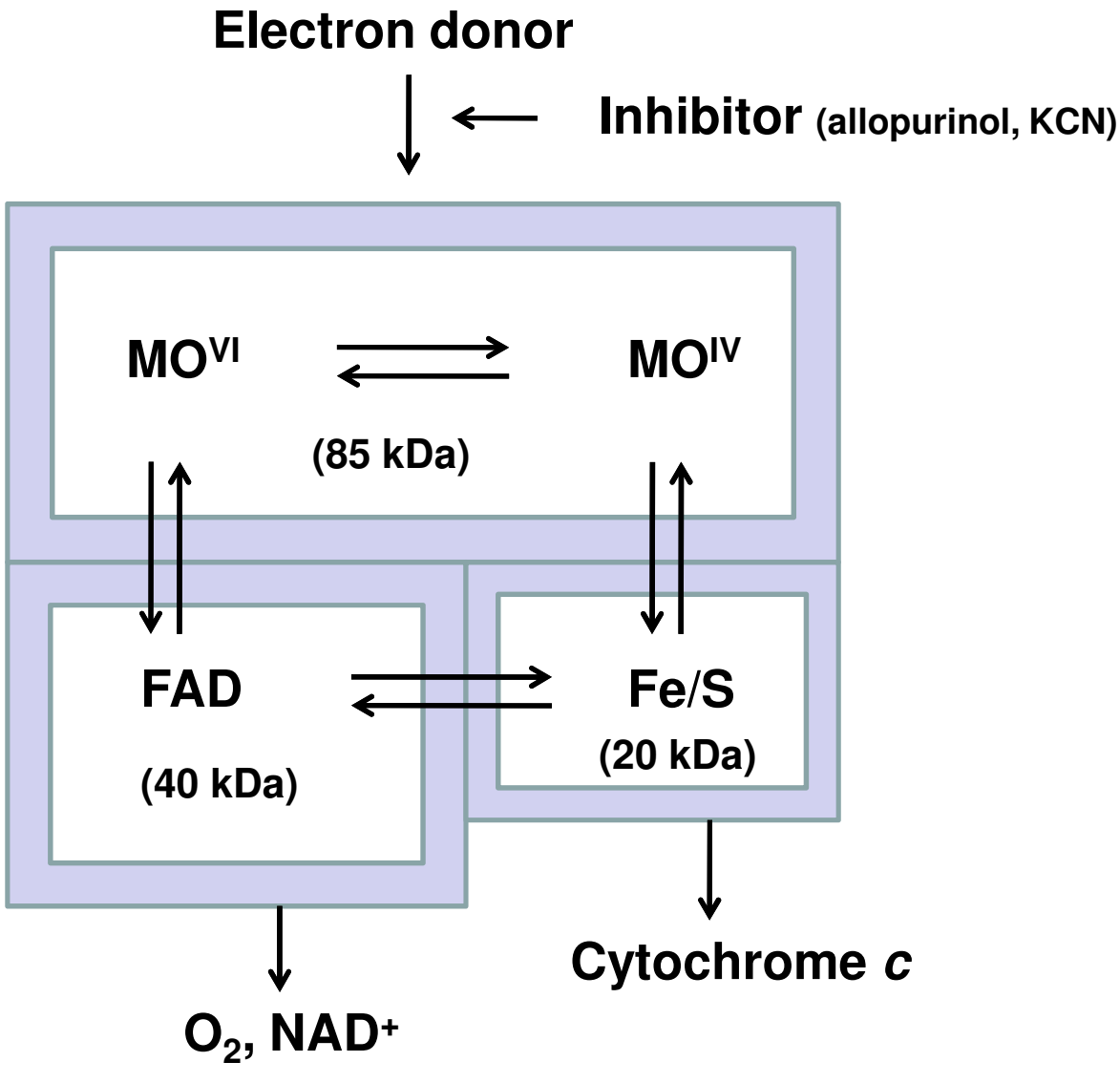
# Xenobiotics metabolized by FMOs

| Xenobiotic | Metabolite |
|---|---|
| Itopride | N-oxide |
| Nicotine | N-oxide |
| Trimethylamine | N-oxide |
| Cimetidine | S-oxide |
| Sulindac | S-oxide, sulphone |
| Ranitidine | N-oxide, S-oxide |
| Olopatadine | N-oxide |
| Clozapine | N-oxide |
| Tamoxifen | N-oxide |
| MPTP *(N-methylphenyl-4-tetrahydro-1,2,3,6-pyridine)* | N-oxide |

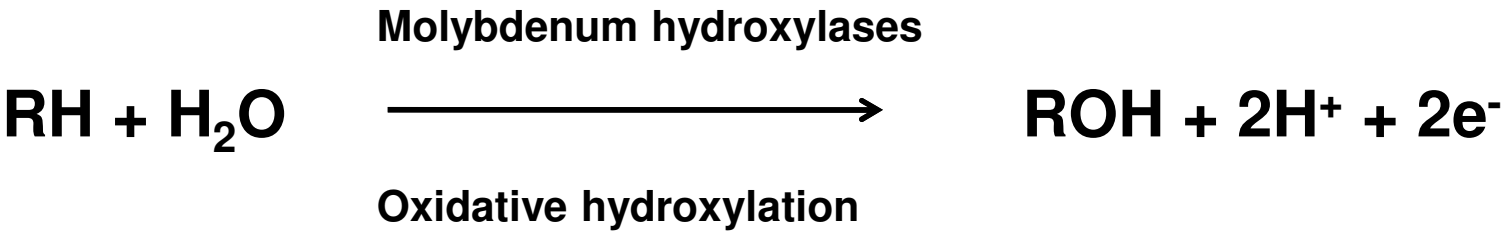
# Proposed metabolic pathways of MPTP in perfused rat liver



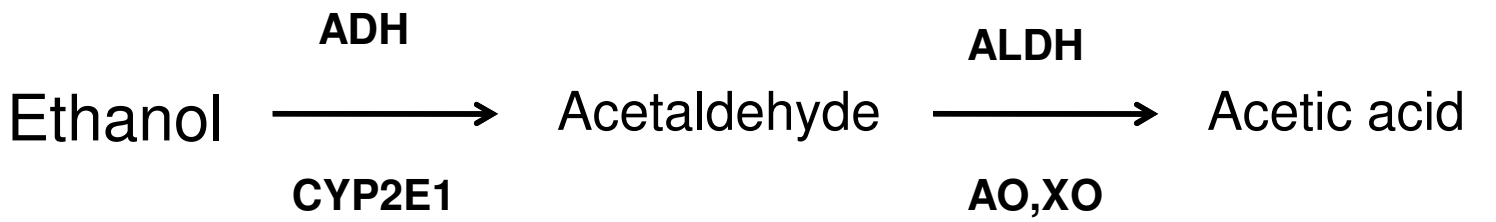
# Intramolecular electron transport system of molybdenum hydroxylases, aldehyde oxidase and xanthine oxidoreductase



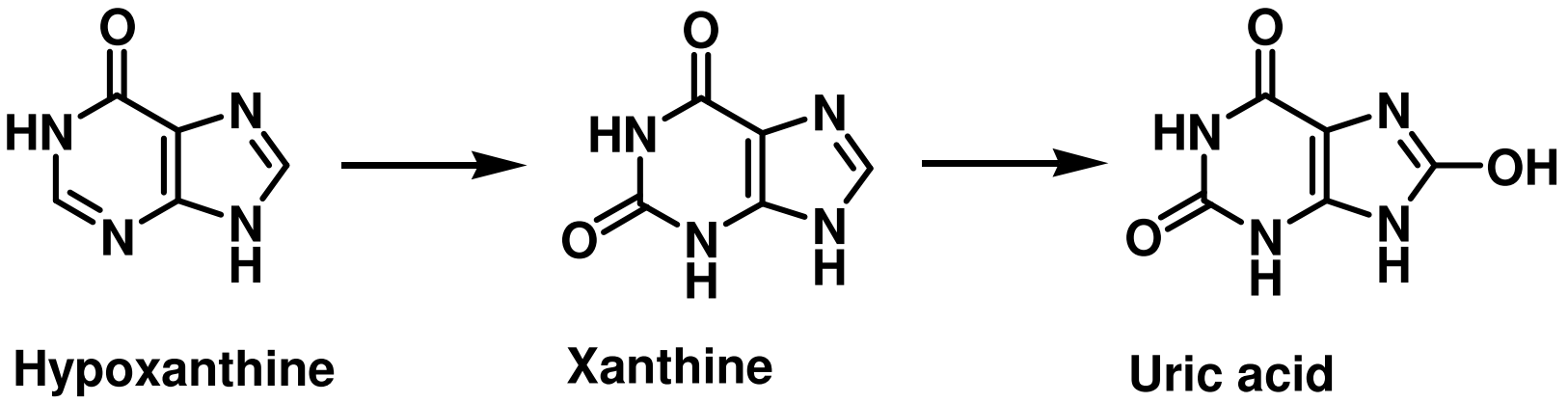
# Reaction catalyzed by Molybdenum hydroxylases



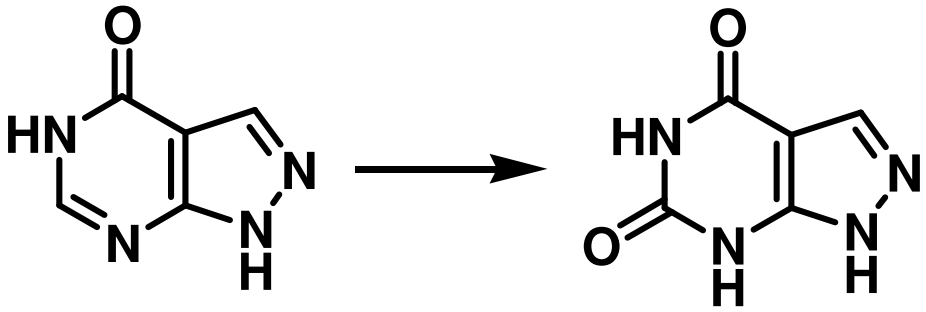
# Enzymes involved in ethanol metabolism



# Oxidation of hypoxanthine, xanthine, and allopurinol by xanthine oxidase




Hypoxanthine Xanthine Uric acid

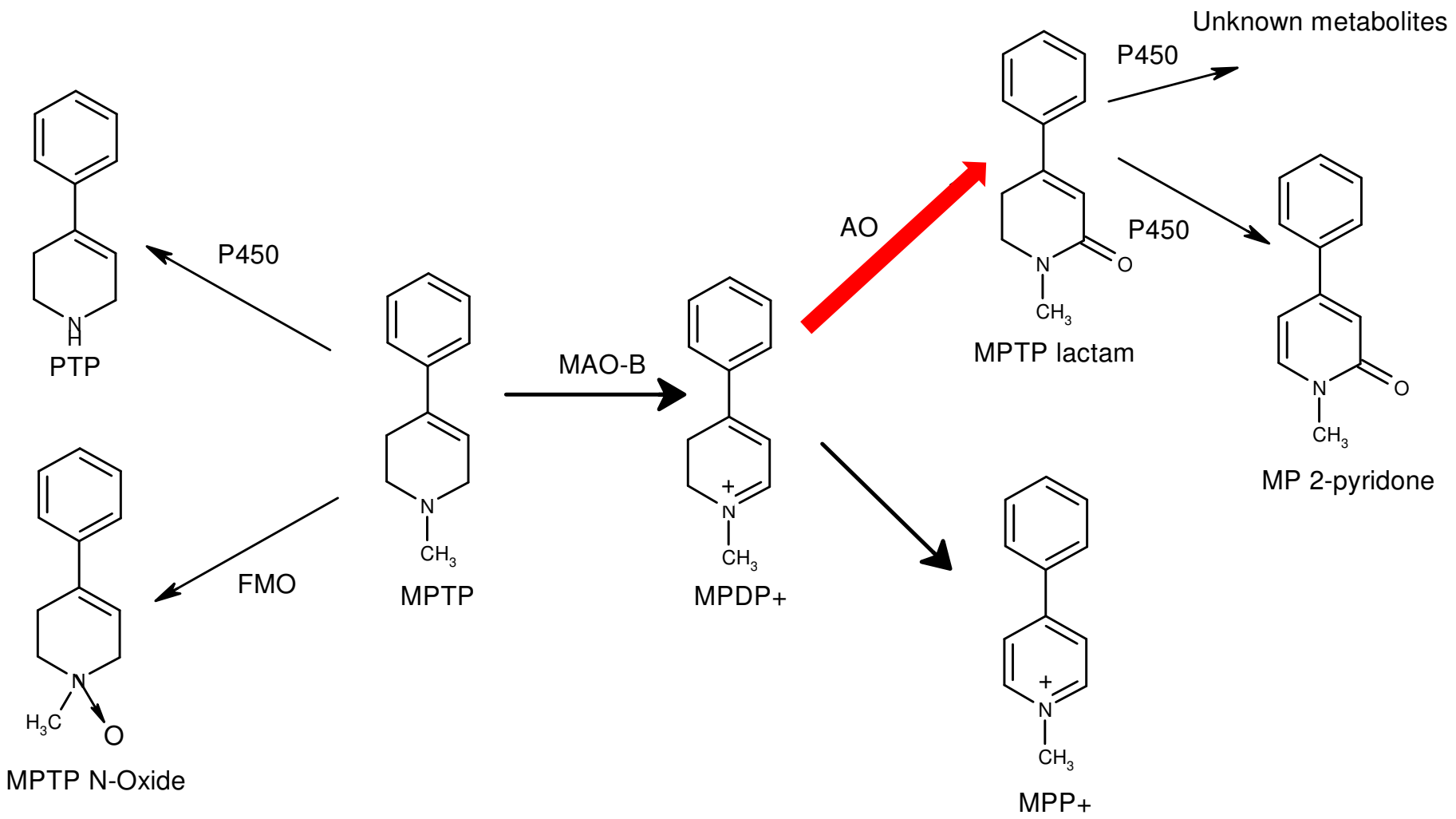



Allopurinol Alloxanthine

# Inhibitors of molybdenum hydroxylases

| COMPOUND | Ki (µM) | IC (µM) |
|---|---|---|
| MYRICETIN | <1 | <1 |
| QUERCETYN | <1 | <1 |
| ALLOPURINOL (XO inhibitor) | 0.34 | |
| MENADIONE (AO inhibitor) | | 0.20 |

# Proposed metabolic pathways of MPTP in perfused rat liver

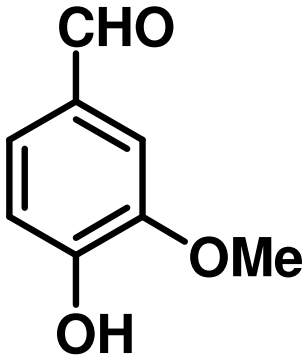


# Tissue distribution of XO and AO in humans

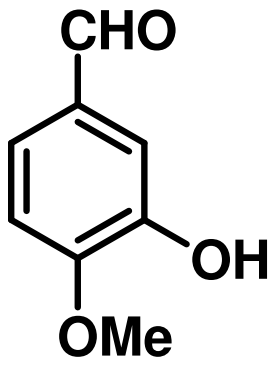
| Tissue | XO | AO |
|---|---|---|
| Liver | + (K, E) | + (E) |
| Kidney | + (K) | + (E) |
| Heart | NA (I) +(K) | |
| Adrenals | + (K) | |
| Spleen | +(K) | |
| Skeletal muscle | + (I,K) | |
| Small intestine | ++ (I, K, E) | (much lower than XO) |
| Lung | NA (I, K) | + (E) |
| Brain | NA (I) +(K) | ++ (E) |
| Mammary Gland | ++ (I) | NA (E) |

XO and AO detected with kinetic measurement (K), immunocytochemistry (I) or mRNA expression(E).
NA=no activity found

# Differential reactivity of isomeric hydroxyl-benzaldehydes towards aldehyde oxidases

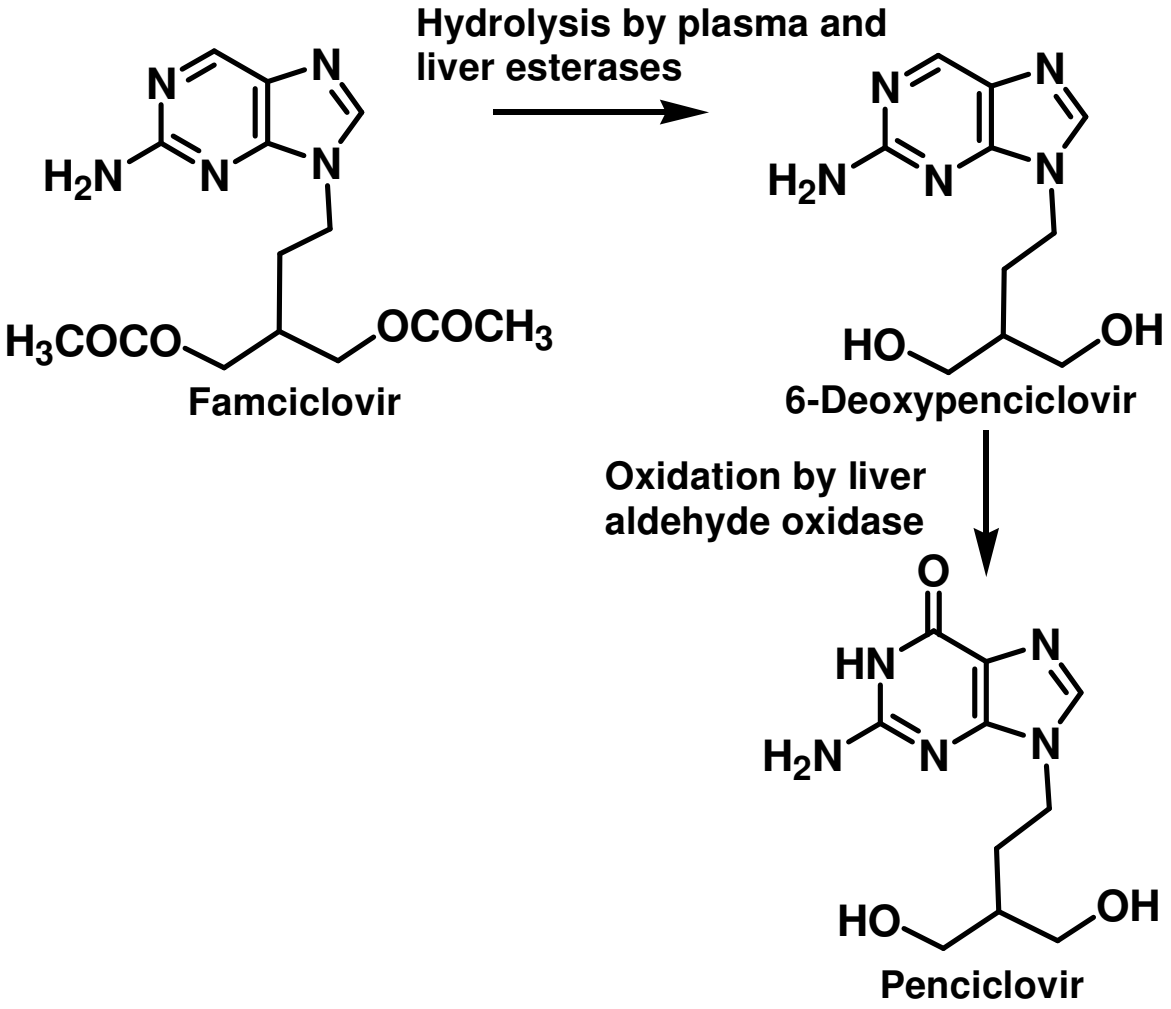



Vanillin




Isovanillin

# Major metabolic route for the conversion of famciclovir to the active antiviral agent, penciclovir



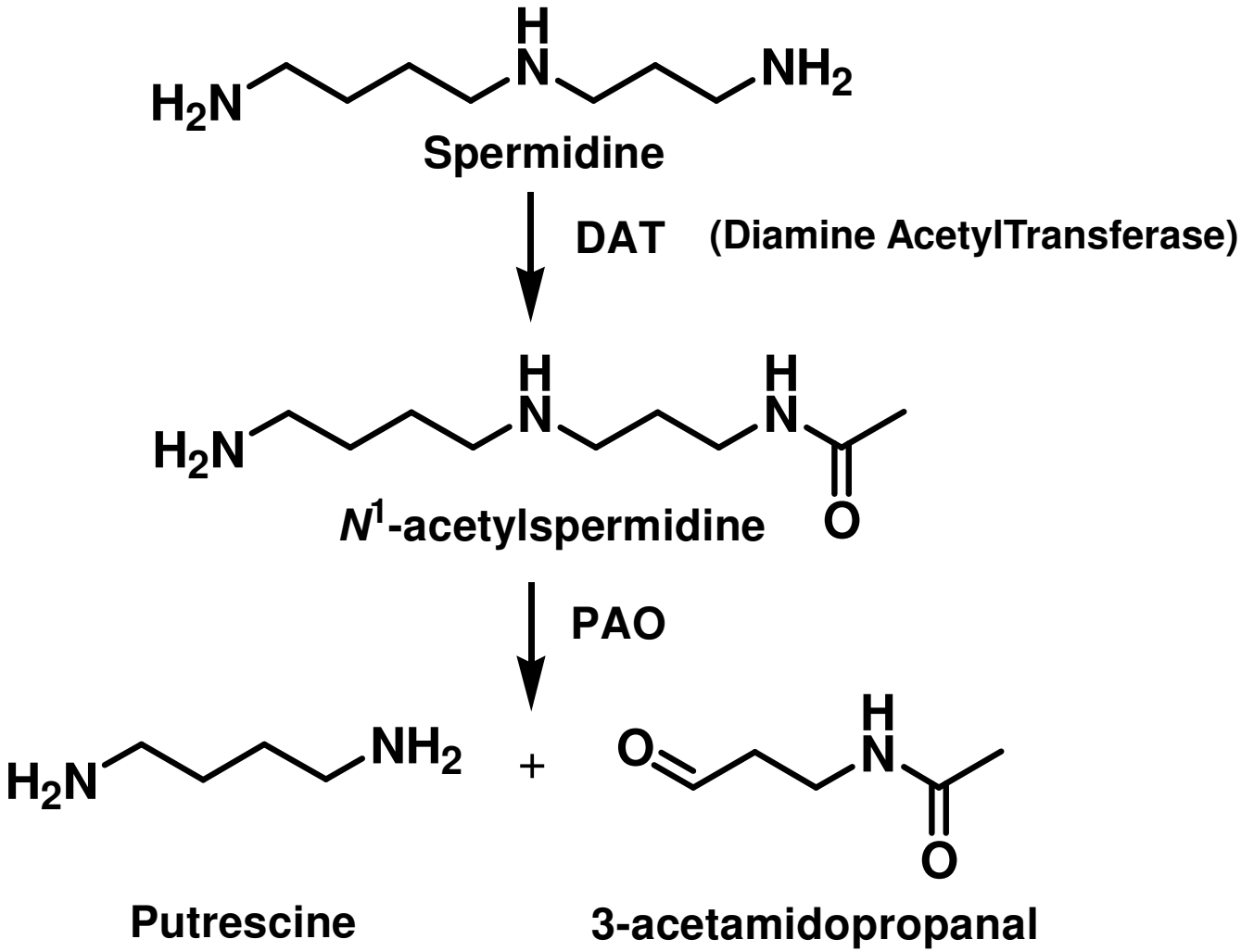
# FAD-dependent Amine Oxidases (AOs)

| FAD-dependent AOs | Tissue and subcellular distribution |
|---|---|
| Monoamine oxidases:<br>MAO-A, MAO-B | All tissues except red cells<br>(outer mitochondrial membrane, some activity reported in microsomes) |
| Polyamine oxidases | All tissues (cytosol, peroxisomes) |

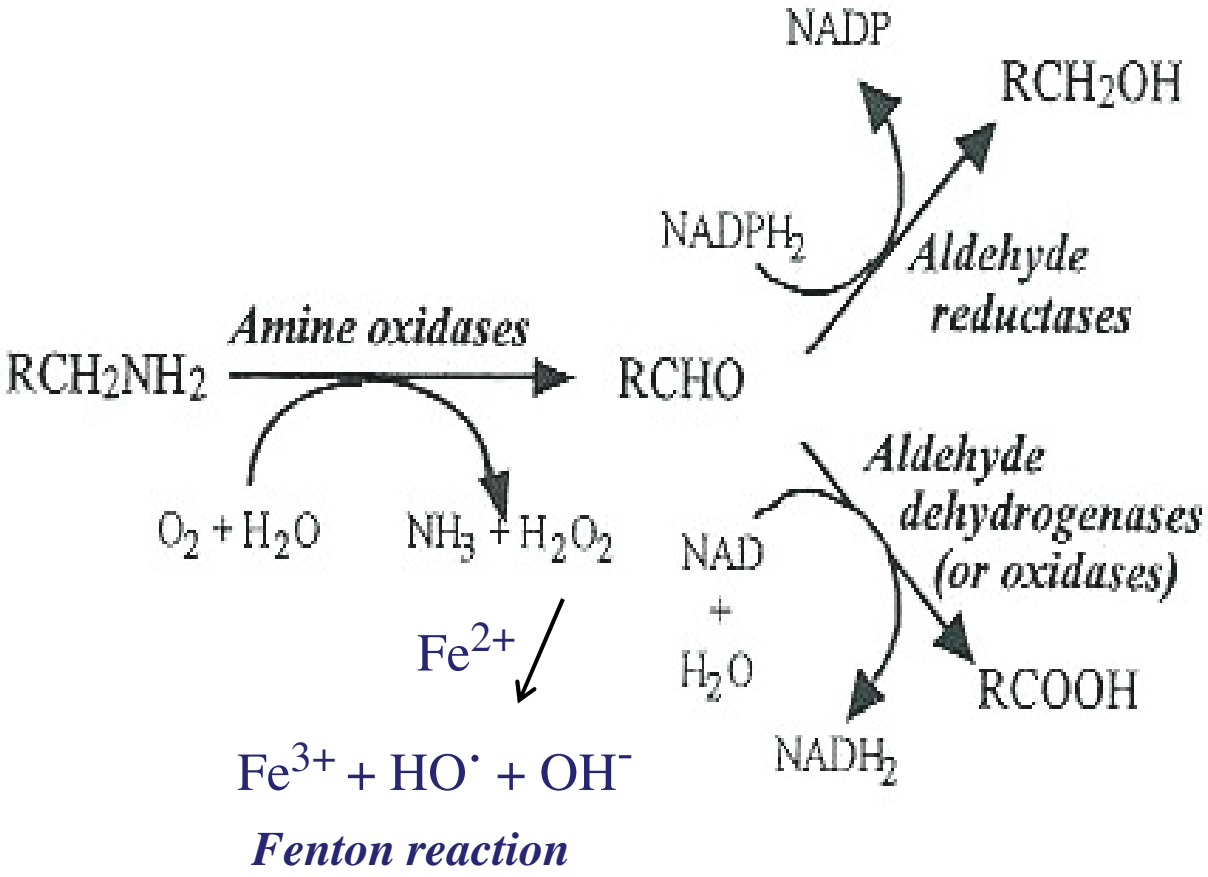
# Amines oxidatively deaminated by the different FAD-dependent AOs

| Substrate | Enzyme | | |
|---|---|---|---|
| | MAO-A | MAO-B | PAO |
| **Primary amines** | × | × | - |
| **Diamines** *(primary amino group)* | - | - | - |
| **Polyamines** *(primary amino group)* | - | - | - |
| **Secondary amines** | × | × | - |
| **Tertiary amines** | × | × | - |
| **Polyamines** *(secondary amino group)* | - | - | × |

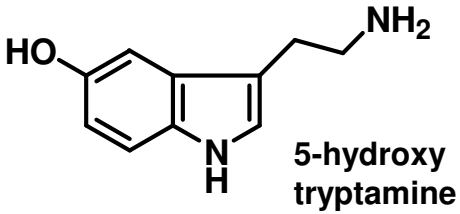
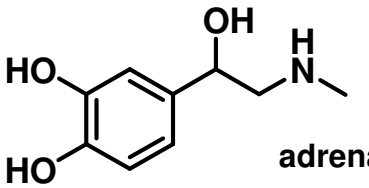
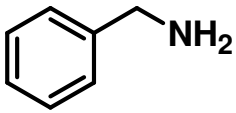
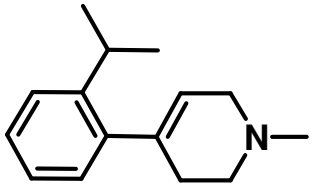
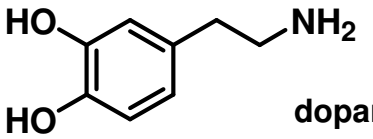
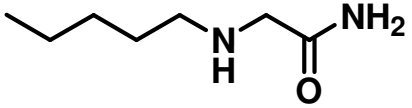
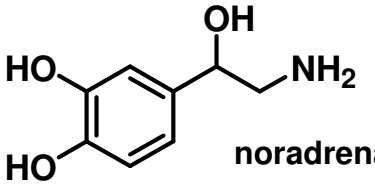
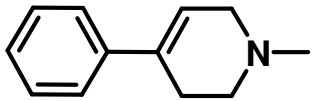
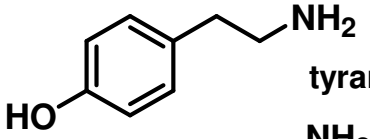
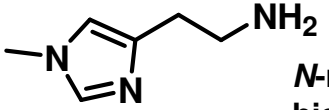
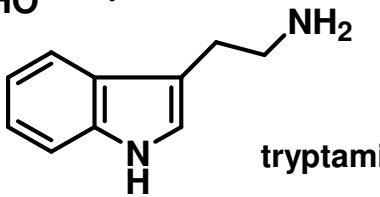
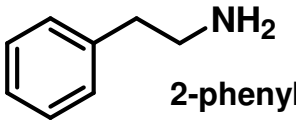
# Outline of the main reactions in the metabolism of spermidine



# General outline scheme of amine metabolism through AOs



# Some common MAO substrates arranged according to their preference for MAO-A or MAO-B

| MAO-A | Common | MAO-B |
|---|---|---|
|  5-hydroxy tryptamine |  adrenaline |  benzylamine |
|  2'-isopropyl-MPTP |  dopamine |  milacemide |
| |  noradrenaline |  MPTP |
| |  tyramine |  *N*-methyl histamine |
| |  tryptamine |  2-phenylethylamine |

Common : good substrates for both isoenzymes

# MAO-A and MAO-B specific activities in human peripheral tissues

| Tissue | Specific activity (pmol/min/mg protein) | |
|---|---|---|
| | MAO-A *(5-HT 100 μM)* | MAO-B *(benzylamine 100 μM)* |
| Duodenum | 1158 ± 301 | 209 ± 75 |
| Liver | 1073 ± 112 | 322 ± 55 |
| Kidney | 1003 ± 133 | 261 ± 50 |
| Adrenal | 596 ± 140 | 104 ± 26 |
| Heart | 583 ± 229 | 222 ± 71 |
| Lung | 409 ± 165 | 23 ± 11 |
| Thyroid | 372 ± 89 | 14 ± 2 |
| Aorta | 99 ± 42 | 47 ± 20 |
| Pancreas | 3 ± 1 | 5 ± 3 |

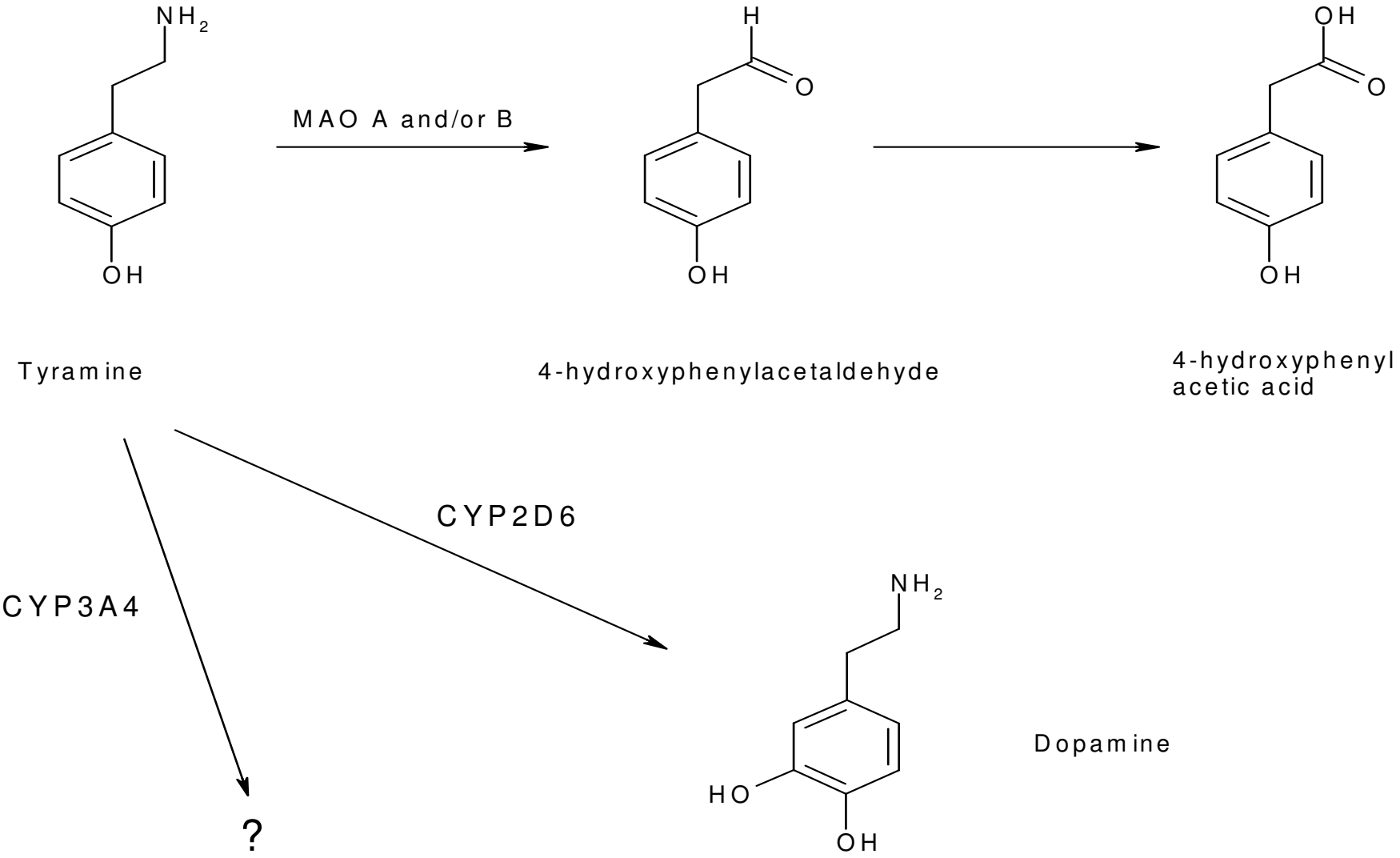
mean ± SD, number of samples = 2-7

# PAO activity in various human organs

| Organ | PAO activity (nmol/g wet weight/ 30 min) |
|---|---|
| Liver | 398 ± 83 |
| Testis | 339 ± 80 |
| Kidney | 263 ± 34 |
| Spleen | 140 ± 48 |
| Small intestine | 41.4 ± 11.7 |
| Heart | 37.6 ± 4.9 |
| Brain | 34.5 ± 5.1 |
| Lung | 31.7 ± 6.2 |
| Pancreas | 15.9 ± 6.9 |

mean ± SE, number of samples = 4-7,
substrate N1-monoacetylspermine 0.2 mM

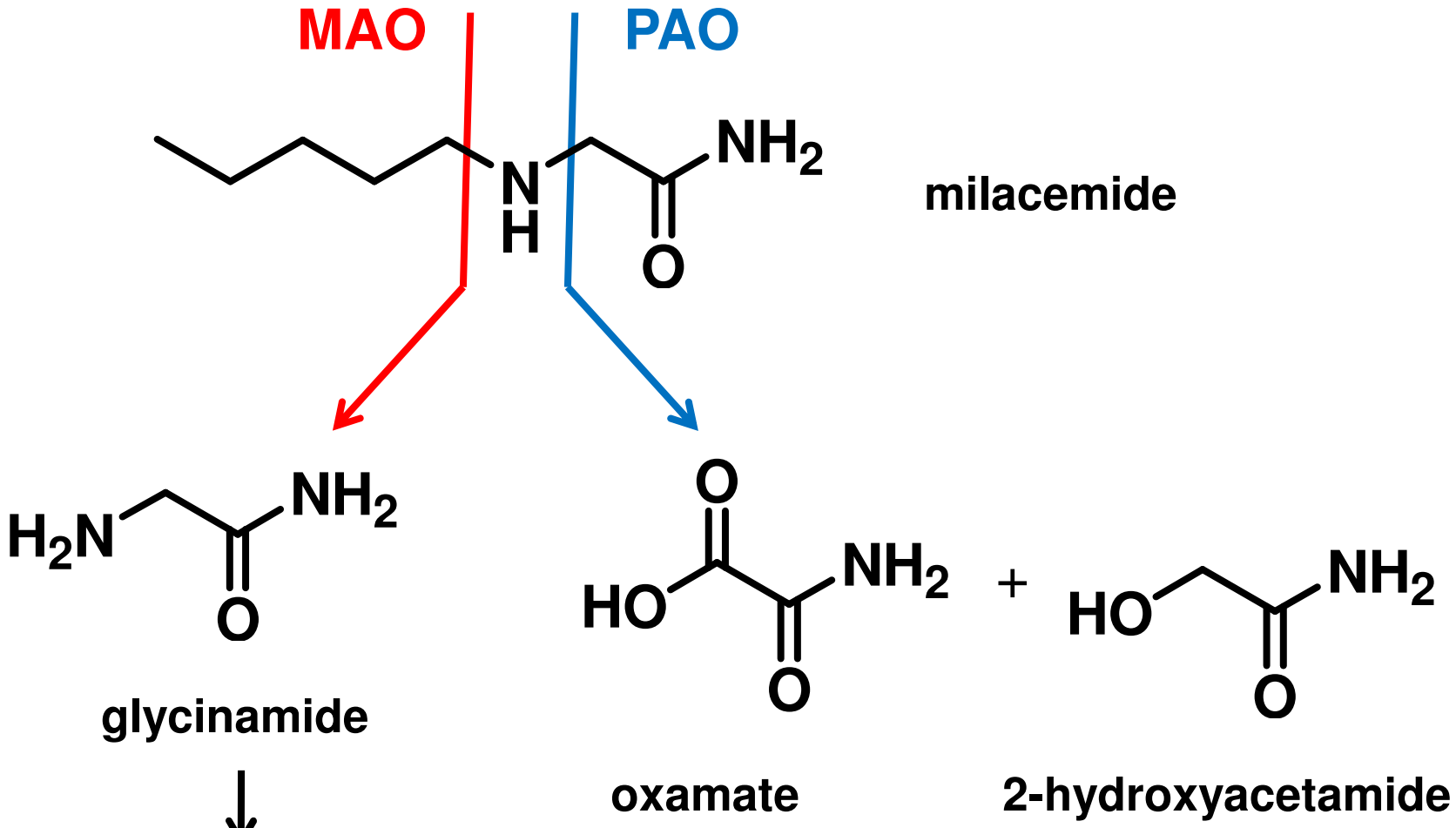
# Metabolism of oral tyramine by MAOs and CYPs



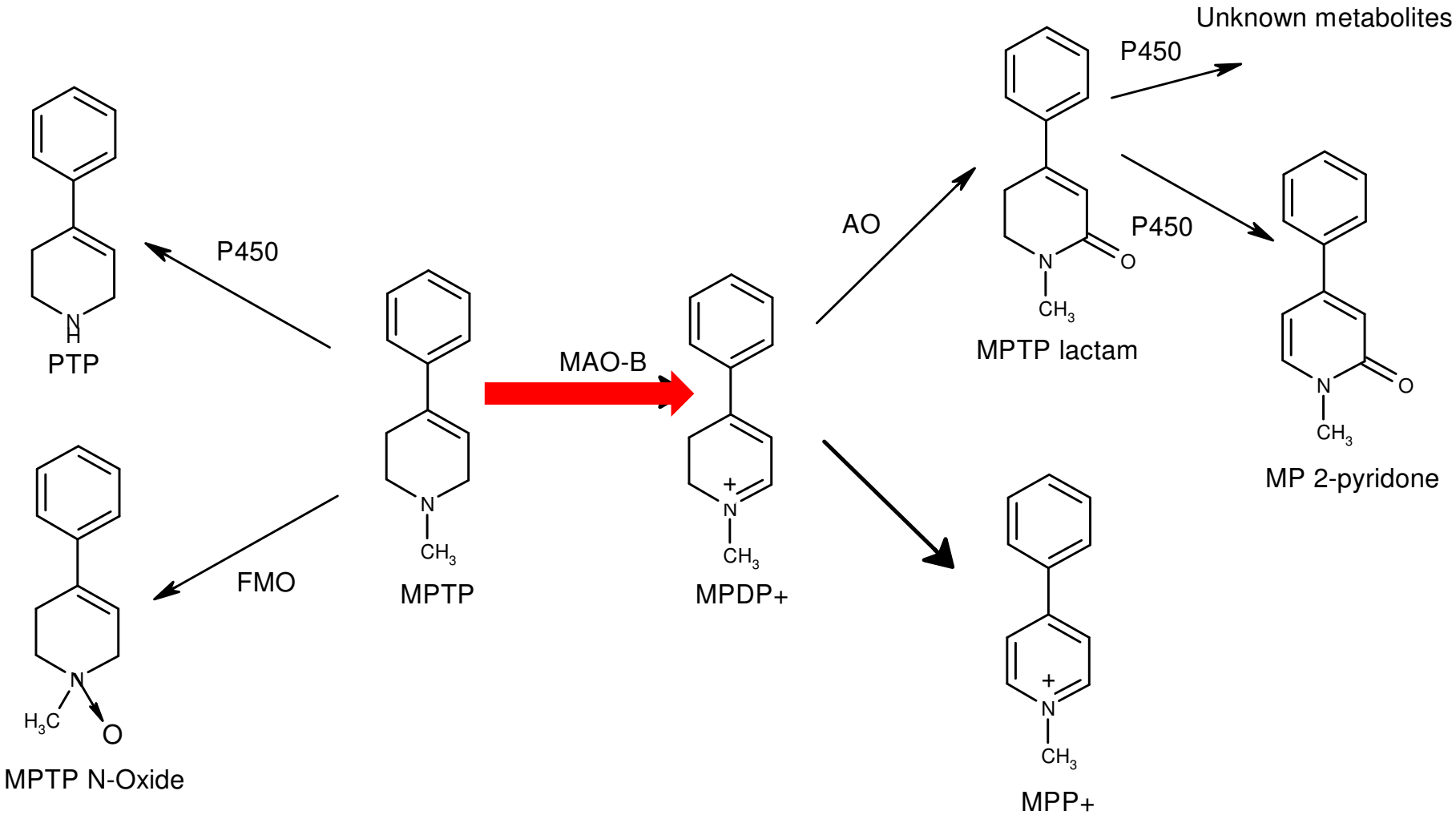
# Xenobiotics metabolized by MAO-A and/or MAO-B

| Class | Xenobiotic | Isozyme |
|---|---|---|
| Toxins | MPTP | MAO-B > MAO-A |
| β-Adrenoceptor agonists and antagonists | Phenylephrine<br>Propranolol | MAO-A<br>MAO-A |
| Phenylethylamine derivatives | Ibopamine<br>Docarpamine | MAO-A and MAO-B<br>MAO-A and MAO-B |
| MAO inhibitors and structurally related compounds | Phenelzine<br>Procarbazine<br>Almoxatone<br>Lazabemide | <br><br>MAO-A > MAO-B<br>MAO-B |
| Milacemide and related compounds | | MAO-B |
| Serotonin 5-$HT_1$-receptor agonists | Triptans | MAO-A |
| Selective serotonin re-uptake inhibitors | Sertraline<br>Citalopram | MAO-A and MAO-B<br>MAO-A and MAO-B |
| Miscellaneous drugs | Primaquine<br>Flurazepam | |

# Metabolism of milacemide



# Proposed metabolic pathways of MPTP in perfused rat liver



# Conclusion

**Factors affecting the relative importance of cytochrome P-450 enzymes, flavin containing monooxygenases, molybdenum hydroxylases and amine oxidases in the in vivo metabolism of xenobiotics in humans**

1. **The route of administration of the drug/xenobiotic**
2. **The physico-chemical/molecular properties of the xenobiotic expected a priori to account for the steps of penetration (absorption, tissue distribution, cellular and subcellular localization), binding and catalysis towards a given enzyme**
3. **The relative amount of isoenzyme of each family present in the tissues and in the cells/organelles where the xenobiotic preferentially distributes**
4. **The location of the active site of the enzyme in the extracellular or intracellular domain**
5. **The number of reactions which a given isoenzyme/enzyme family can carry out**