Drug Metabolism

Hugo Kubinyi

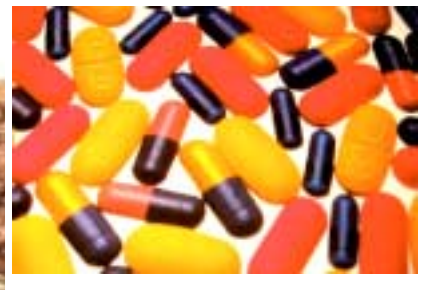
Germany

E-Mail kubinyi@t-online.de
HomePage www.kubinyi.de

Hugo Kubinyi, www.kubinyi.de

Pharmacodynamics

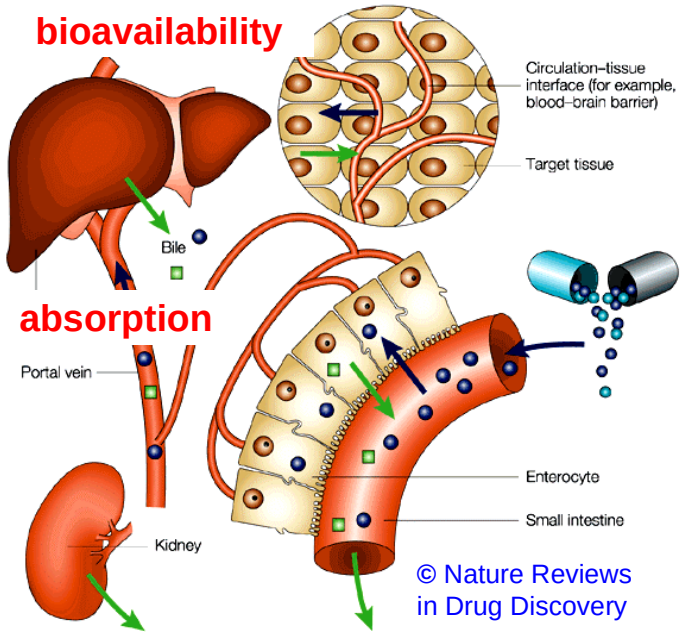
the action of the drug on the body



Metabolism

the action of the body on the drug

bioavailability



© Nature Reviews in Drug Discovery

Sites of Drug Metabolism:

(intestinal wall), liver, (organs)

Sites of Drug Elimination:

kidneys (polar compounds), bile, feces (lipophilic analogs), lung

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Drug Metabolism

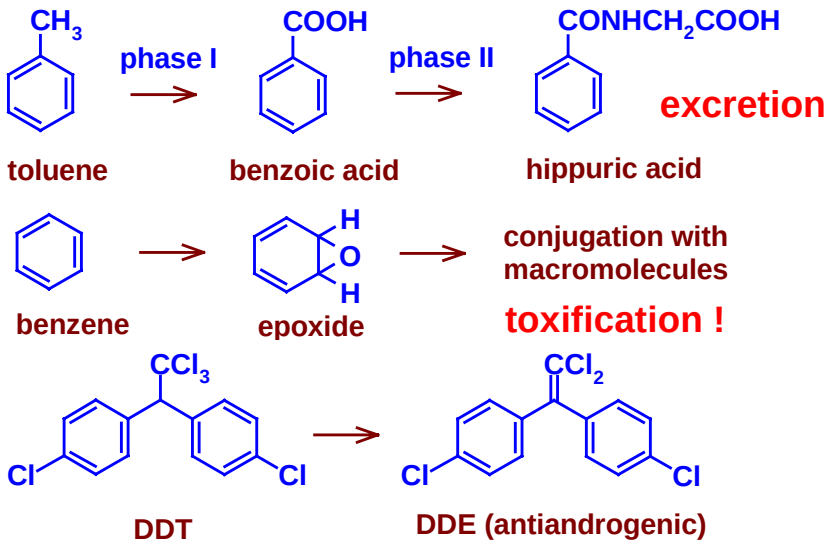
For the elimination of xenobiotics, mainly in the liver.

- **oxidations, reductions and hydrolyses (phase I reactions),** and
- **conjugations with small molecules (phase II reactions).**
- drug elimination by transporters is sometimes defined as **phase III reaction.**

The most important phase I enzymes are the group of **cytochrome P450 enzymes (or CYPs).**

First pass effect: extensive metabolism and/or biliar elimination of drugs of (either) lipophilic character, MW >500-600, or specific affinity to transporters, in their **first liver passage.**

Metabolic Pathways of Xenobiotics



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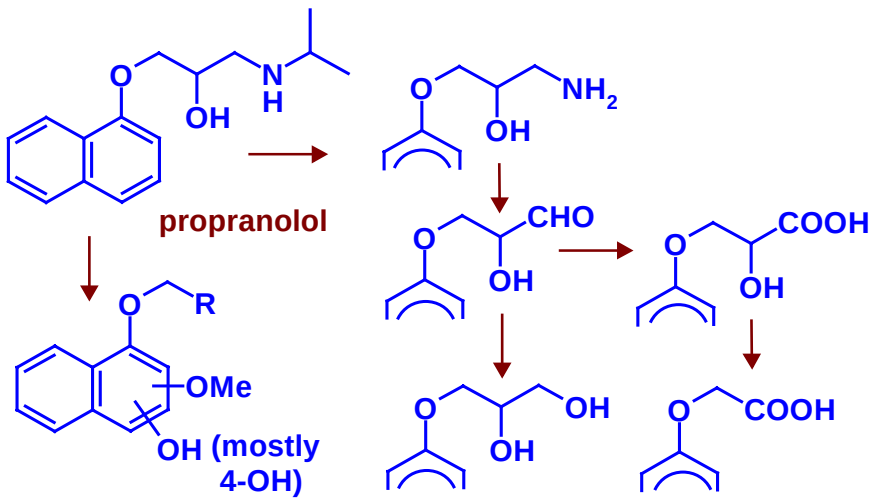
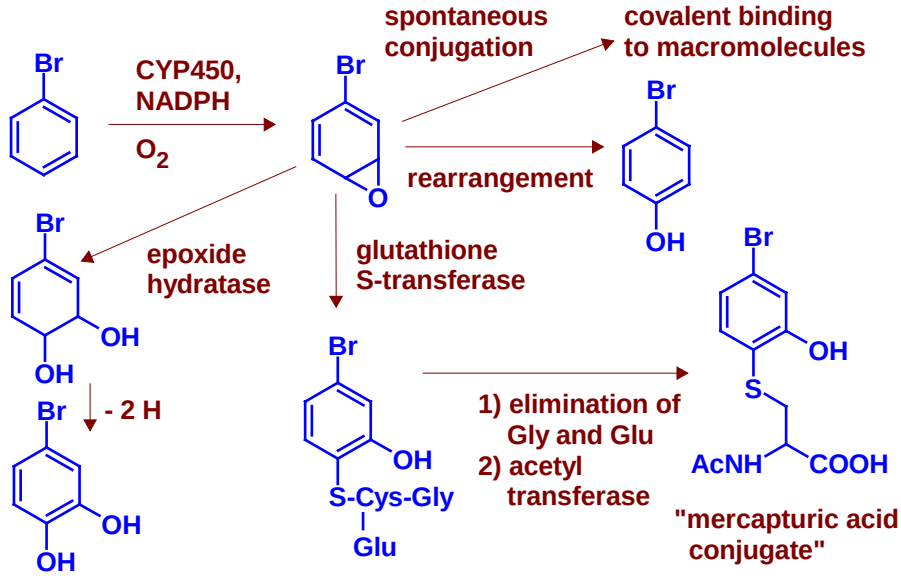
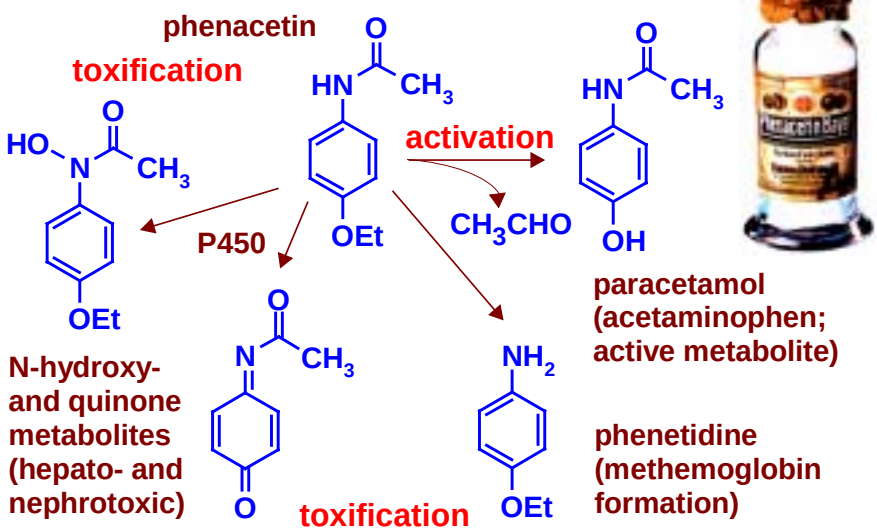
Metabolism of Propranolol



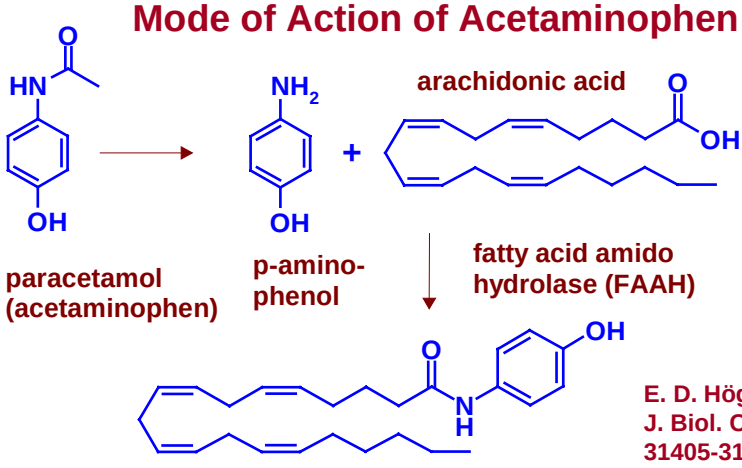
Metabolism of Bromobenzene



Metabolic Activation and Toxification



Mode of Action of Acetaminophen



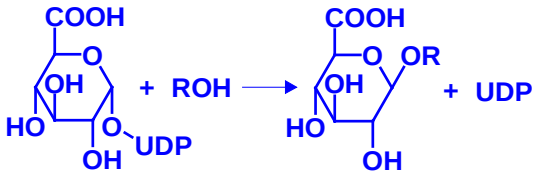
paracetamol (acetaminophen)

p-amino-phenol

E. D. Högestätt et al., J. Biol. Chem. 280, 31405-31412 (2005)

N-arachidonoyl phenolamine, a potent TRPV1 (transient receptor potential vanilloid 1, vanilloid receptor) agonist, pEC_{50} = 7.80 (about 16 nM), binds also to the cannabinoid CB_1 receptor and inhibits cellular anandamide uptake.

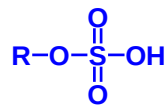
Phase II Metabolic Processes



glucuronidation

acetaminophen, morphine, diazepam, trichloroethanol

RXH + 3'-phospho adenosine-5'-phospho-sulfate (PAPS)



+ PAP

sulfatation (X = O, NH)

phenol, steroids, acetaminophen, methyldopa

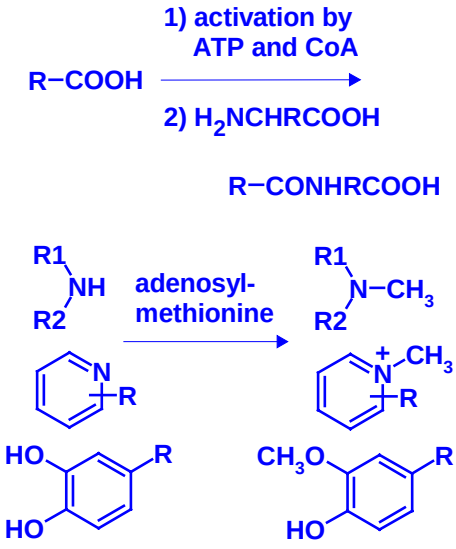
acetylation

sulfonamides, isoniazide, dapsone, clonazepam

epoxide → mercapturic acid

mercapturic acids (via glutathione addition)

Phase II Metabolic Processes



conjugation with glycine (R = H)

benzoic acid,
isonicotinic acid

conjugation with glutamine ($R = -(CH_2)_2-CONH_2$)

indolylacetic acid,
phenylacetic acid

O-, N- and S-methylation

methadone, nicotinamide,
norepinephrine

catecholamines
(catechol-O-methyl
transferase)

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Phase I Metabolic Processes

Hydrolysis

- of esters and amides by esterases and amidases
- of epoxides by epoxide hydrolases
- of acetals by glycosidases
- of glucuronides by glucuronidases

Decarboxylation of e.g. amino acids

Reduction

- of carbonyl compounds by alcohol dehydrogenases or aldo-keto reductases
- of azo compounds (via hydrazo compounds to amines) by NADPH-cycochrome P450 reductase and others
- of nitro compounds

Reductive dehalogenation of aliphatic compounds

Phase I Metabolic Processes

Oxidation Reactions

of alcohols and aldehydes: $RCH_2OH \rightarrow RCHO \rightarrow RCOOH$

of aliphatic chains: $R\text{-}CH_2CH_3 \rightarrow R\text{-}CH(OH)CH_3$ (e.g. in barbiturates)

of aromatic amines: $R\text{-}NH_2 \rightarrow R\text{-}NHOH \rightarrow R\text{-}N{=}O$

of tertiary amines: R1-N(R2)-R3 → R1-N(→O)(R2)-R3

of sulfides: R1-S-R2 → R1-SO-R2 → $R1\text{-}SO_2\text{-}R2$

of alkenes to epoxides

of aromatic compounds to phenols (para-hydroxylation)

Oxidative O- and N-dealkylation: $R1\text{-}X\text{-}CH_2\text{-}R2 \rightarrow$ R1-X-H + R2-CH=O (X = O, NH)

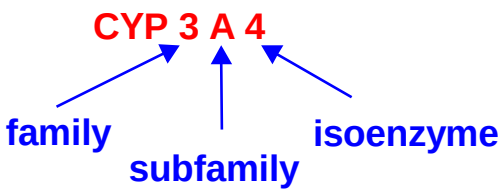
Oxidative deamination (MAO): $R\text{-}CH_2\text{-}NH_2 \rightarrow R\text{-}CH{=}O$

Oxidative desulfuration: R1-C(=S)-R2 → R1-C(=O)-R2

Phase I Metabolic Processes: Oxidases

Cytochrome P450 isoenzymes

are (microsomal) haem monooxygenases with MW = 35-45 kD; there are 51 CYP families (homology >40%), with up to 10 subfamilies (homology >55%).



Low specificity, different sites, different CYPs at same drug, at same site.

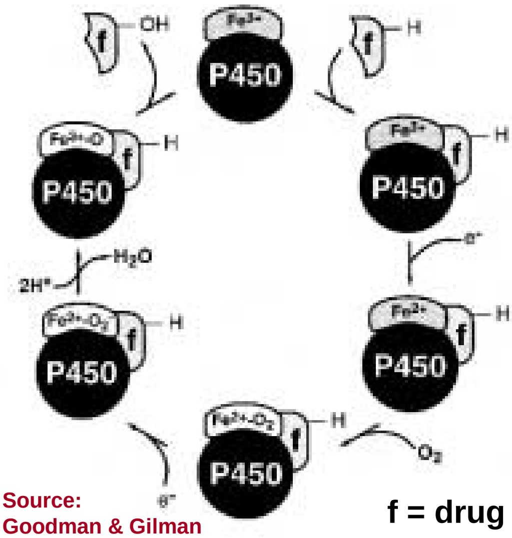
Flavin monooxygenase isoenzymes

Alcohol dehydrogenase

Aldehyde oxidase

Monoamine oxidase (MAO)

Mechanism of Cytochrome P450 Oxidation



Source:
Goodman & Gilman

CYP 450s are no real cytochromes, they are (microsomal) mono-oxygenases (only one oxygen atom of O_2 is introduced into the sub-strate). The Fe^{3+}-haem complex is reduced by an electron that is pro-vided by an electron transfer chain, in which an NADPH-flavoprotein (oxidation to NADP) is involved. Binding of O_2 and uptake of an electron produces a metabolite; CYP 450 is regenerated.

Cytochrome P450 Enzyme Families

> 1000 genes discovered so far; complete?

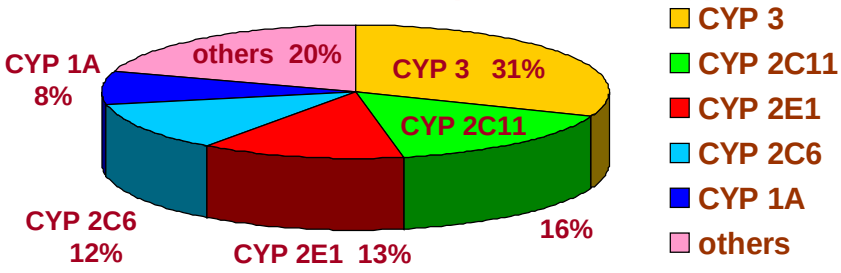
Human CYP families (about 50 isoforms in 17 families)

CYP 1-5, 7, 8, 11, 17, 19, 21, 24, 26, 27, 39, 46 and 51
- hCYPs that mainly degrade xenobiotics (drugs):
  CYP 1, 2A...E, 3
- hCYPs in steroid metabolism:
  CYP 2G1, 7, 8B1, 11, 17, 19 (aromatase), 21, 27A1, 46, 51
- hCYPs in fatty acid metabolism:
  CYP 2J2, 4, 5, 8A1
- other hCYPs:
  CYP 2R1 (?), 2S1 (?), 24 (vitamine D), 26 (retinoic acid), 27B1 (vitamine D), 39 (?)

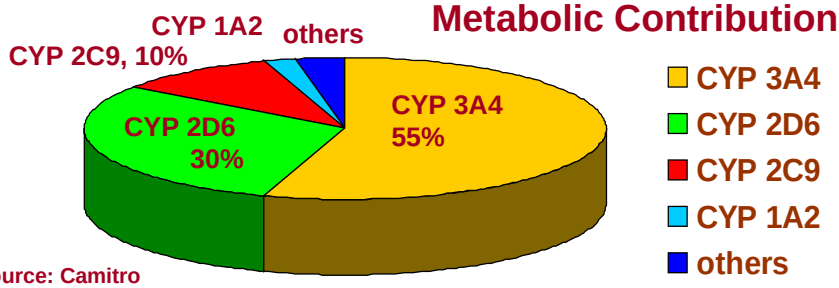
The dual, ambivalent character of CYPs: oxidative degradation of natural products for better elimination; toxification of chemicals and drugs to chemically highly reactive compounds.

→ there were only about 8 human generations since 1800

hCYP450 Concentrations (high variability)

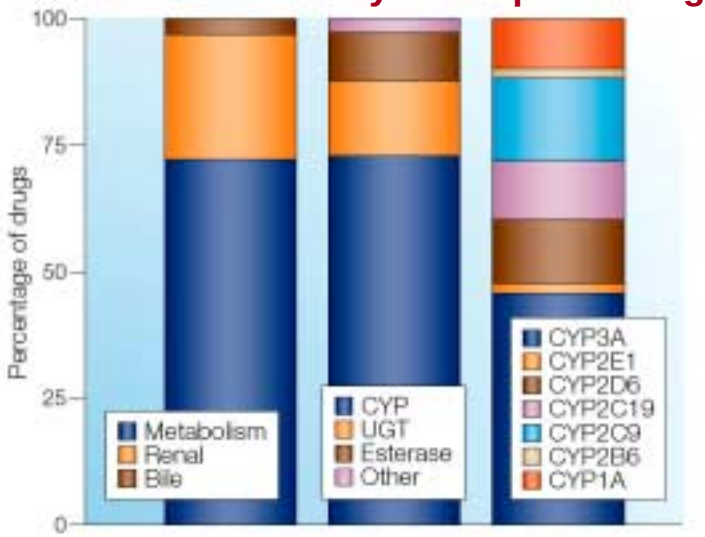


Metabolic Contributions



Source: Camitro

Elimination Pathways of Top 200 Drugs of 2002



UGT = uridine diphosphate glucuronyl transferase

L. C. Wienkers and T. G. Heath, Nature Rev. Drug Discov. 4, 825-833 (2005)

Specificity of Cytochromes (incomplete list)

CYP 1A2: amitryptiline, caffeine, imipramine, paracetamol, theophylline, verapamil
CYP 2A6: nicotine
CYP 2B6: cyclophosphamid
CYP 2C9: diclofenac, naproxen, piroxicam, tolbutamide, warfarin
CYP 2C19: diazepam, omeprazole, S-mephenytoin, phenytoin, propranolol
CYP 2D6: amitryptiline, captopril, chlorpromazine, codeine, debrisoquine, dextromethorphan, flecainide, fluoxetine, imipramine, metoprolol, mianserin, paroxetine, perhexilene, propafenone, thioridazine, venlafaxine.
CYP 2E1: dapsone, ethanol, halothane, paracetamol
CYP 3A4: alprazolam, amiodarone, amitryptiline, carbamazepine, ciclosporin, cisapride, clarithromycin, dexamethasone, erythromycin, ethinyl estradiol, ketoconazole, midazolam, nifedipine, paclitaxel, paracetamol, terfenadine, verapamil, warfarin

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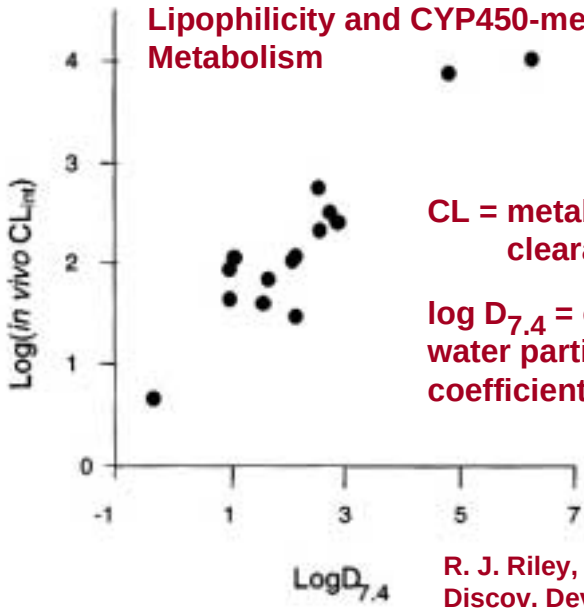
Lipophilicity and CYP450-mediated Drug Metabolism

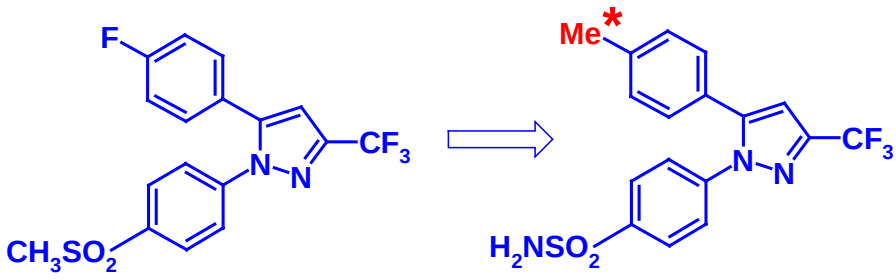


CL = metabolic clearance

log $D_{7.4}$ = octanol/ water partition coefficient at pH = 7.4

R. J. Riley, Curr. Opin. Drug Discov. Dev. 4, 45-54 (2001)

Oxidative Metabolism and Drug Design

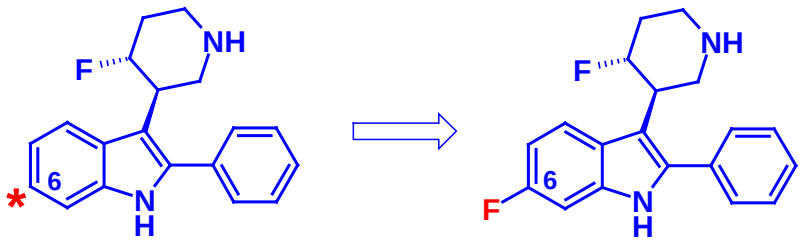


early COX2 inhibitor
$t_{1/2}$ (rat) up to 220 h

celecoxib
$t_{1/2}$ (rat) = 3.5 h

T. D. Penning et al., J. Med. Chem. 40, 1347-1365 (1997);
D. A. Smith, H. van de Waterbeemd and D. K. Walker, Pharmacokinetics and Metabolism in Drug Design, Wiley-VCH, 2001, p. 83

Oxidative Metabolism and Drug Design



K_i h5-HT_{2A} = 0.43 nM

rat:
bioavailability = 18%
$t_{1/2}$ = 1.4 h

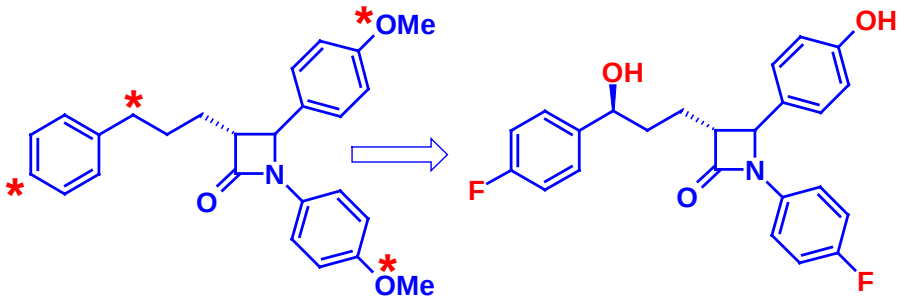
major metabolite: 6-OH

K_i h5-HT_{2A} = 0.06 nM

rat:
bioavailability = 80%
$t_{1/2}$ = 12 h

M. Rowley et al., J. Med. Chem. 44, 1603-1614 (2001)

Oxidative Metabolism and Drug Design



SCH 48461
ED_{50} (hamster) = 2.2 mg/kg

Ezetimib (SCH 58235, oral cholesterol absorption inhibitor)
ED_{50} (hamster) = 0.04 mg/kg

M. van Heek et al., J. Pharmacol. Exp. Ther. 283, 157-163 (1997); D. A. Smith, H. van de Waterbeemd and D. K. Walker, Pharmacokinetics and Metabolism in Drug Design, Wiley-VCH, 2001, p. 85

Metabolite Databases and Predictive Models

Accelrys (www.accelrys.com/chem_db/biotrans.html)
electronic version of D. Hawkins, Biotransformations (7 volumes), Royal Society of Chemistry, London.

Camitro Corporation (www.camitro.com; now ArQule)
models for bioavailability, BBB penetration, metabolic degradation by CYP 1A2, 2C9, 2D6, 3A4

CompuDrug (www.compudrug.com)
software MetabolExpert + metabolite database

Lhasa (www.chem.leeds.ac.uk/LUK/meteor/meteor.htm)
software METEOR + literature database

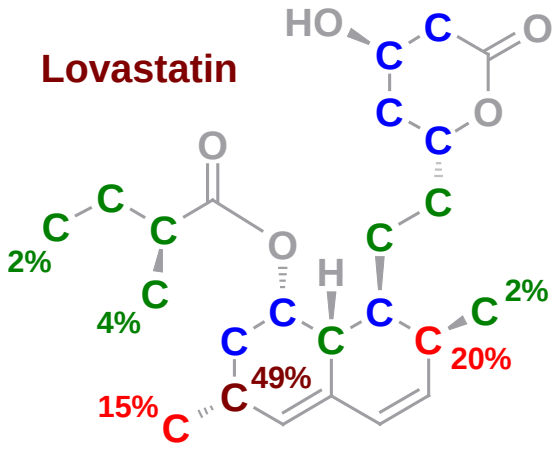
MDL Metabolite Database (www.mdl.com/products/)
metabolism information system

MULTICASE (www.multicase.com/start.htm)
rule-based expert system + metabolite database

Camitro Prediction of CYP450 Regioselectivity

(D. Janssen, Drug Discov. Dev., January 2002)

Lovastatin



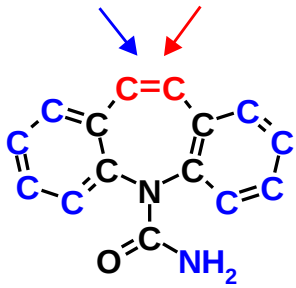
brown = labile

red = moderately labile

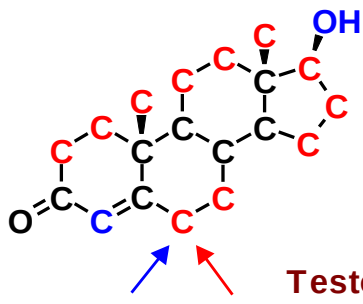
green = modera-tely stable

blue = stable

Semiquantitative Model for CYP 3A4-Metabolism



Carbamazepine



Testosterone

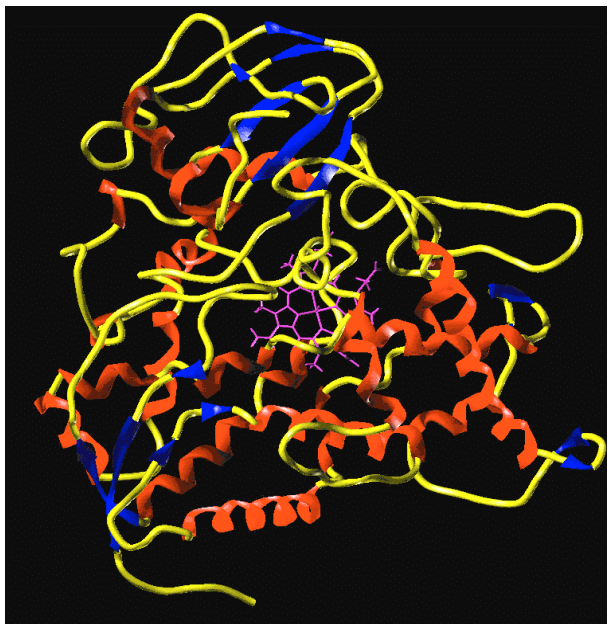
AM1 hydrogen abstraction energies + accessible surface. Red = accessible, low energy; blue = high energy; red arrows = predicted sites, blue arrows = experimental sites of CYP 3A4 metabolism.

S. B. Singh et al., J. Med. Chem. 46, 1330-1336 (2003)

CYP 3D Structures

Rabbit CYP 2C5 (1dt6)

CYP450cam camphor complex (1akd)



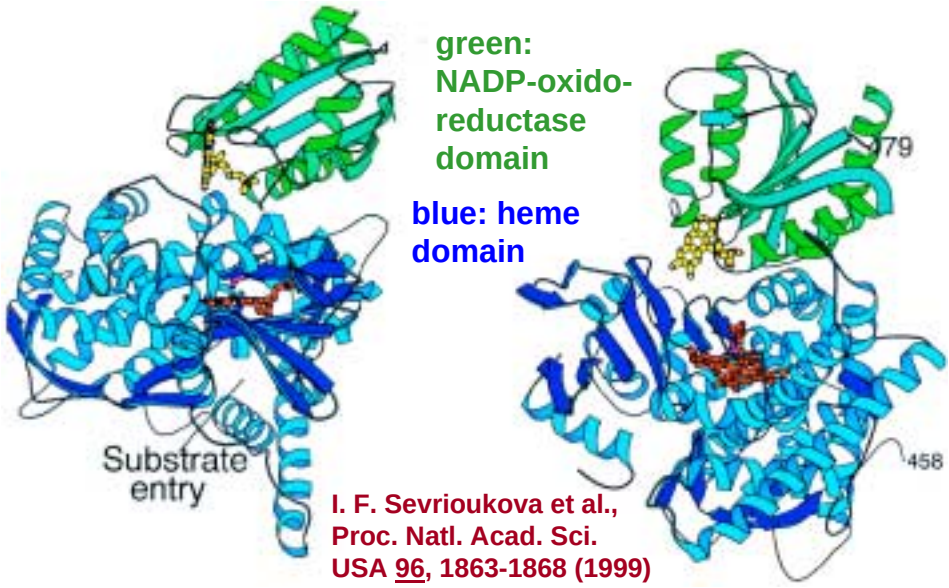
3D Model of human CYP 1A2

N.V. Belkina,
V. S. Skvortsov,
A. S. Ivanov

www.imbh.msk.su

3D structures of the most important hCYPs are already elucidated by protein crystallography (T. Blundell, Astex)

Bacterial CYP450BM-3 Heme and Reductase Domains

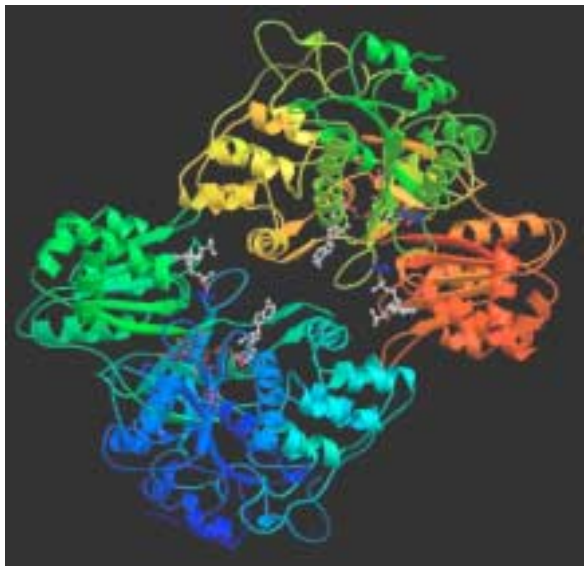


green: NADP-oxido-reductase domain

blue: heme domain

I. F. Sevrioukova et al., Proc. Natl. Acad. Sci. USA 96, 1863-1868 (1999)

NADPH-Cytochrome P450 Reductase (rat; 1amo)

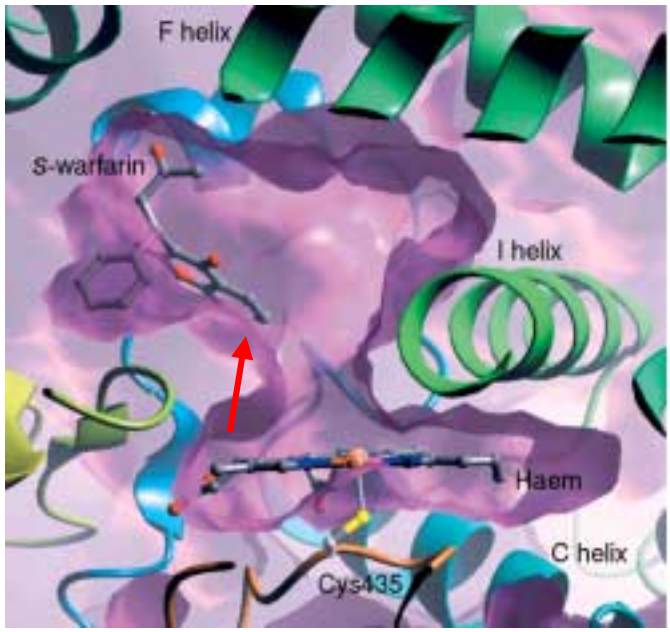


Cofactors:

flavine adenine dinucleotide (FAD)

flavine mononucleo-tide (FMN)

nicotine adenine dinucleotide (NADP)

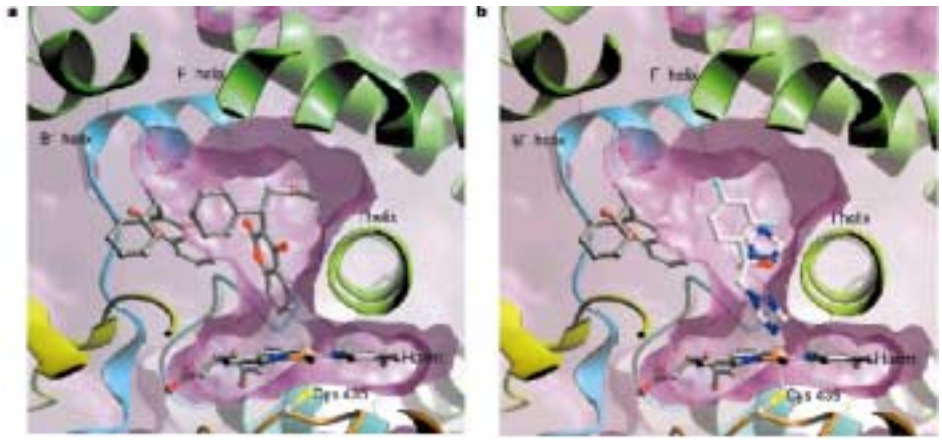


Binding Mode of (S)-Warfarin to hCYP 2C9

the red arrow indicates the site of oxidation (7- but also 6-position)

P. A. Williams et al., Nature 424, 464-468 (2003)

Binding of (S)-Warfarin to hCYP 2C9, Complexes with

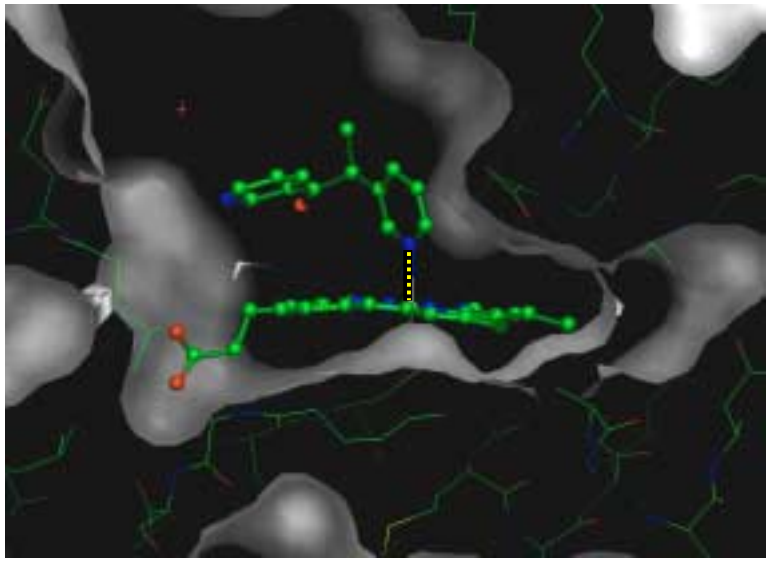


a) a second warfarin molecule

b) the CYP inhibitor fluconazole (modelled from CYP51 complex)

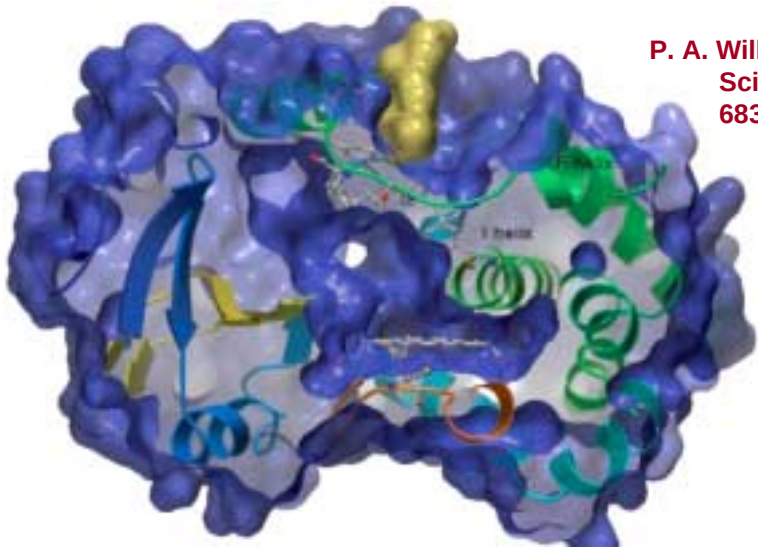
P. A. Williams et al., Nature 424, 464-468 (2003)

Binding Mode of Metyrapone to hCYP 3A4



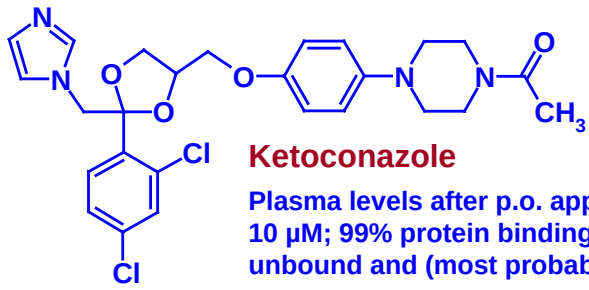
1w0g

Binding Mode of Progesterone to hCYP 3A4



P. A. Williams et al.,
Science 305,
683-686 (2004)

Ketoconazole and Other Azoles Inhibit CYP 3A4



Ketoconazole

Plasma levels after p.o. application = about 10 µM; 99% protein binding → about 100 nM unbound and (most probably) intracellular concentration. K_i **CYP 3A4 = 15 nM**

Side effect of ketoconazole: enhanced nephrotoxicity of cyclosporin (a CYP 3A4 substrate)

Therapeutic application of ketoconazole (or diltiazem, another CYP 3A4 inhibitor): combination with cyclosporin reduces the dose of this (expensive) drug!

Cytochrome P450 Inhibitors (incomplete list)

CYP 1A2: cimetidine, fluvoxamine, grapefruit juice, quinolone antibiotics (ciprofloxacin, enoxacin, norfloxacin)

CYP 2C9: amiodarone, chloramphenicol, cimetidine, fluconazole, fluoxetine, isoniazid, omeprazole, sertraline, sulfaphenazole, sulfinpyrazone

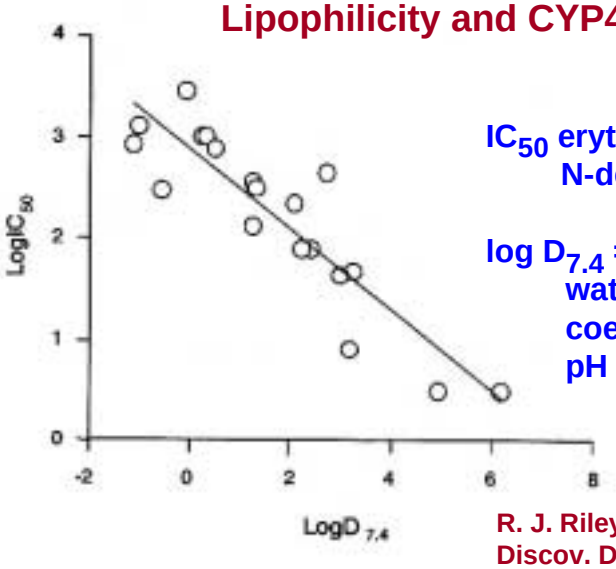
CYP 2C19: fluoxetine, fluvastatin, fluvoxamine, isoniazid, omeprazole, sertraline, ticlopidine, tranylcypromine

CYP 2D6: amiodarone, cimetidine, fenfluramine, haloperidol, mibefradil, quinidine, propafenone, ritonavir, all SSRIs (fluoxetine and paroxetine being most potent), thioridazine, yohimbine

CYP 2E1: cimetidine, disulfiram (ethanol intoxication!)

CYP 3A4: amiodarone, cannabinoids, cimetidine, clotrimazole, diltiazem, erythromycin, norfluoxetin (metabolite of fluoxetin), fluvoxamine, grapefruit juice, ketoconazole, metronidazole, miconazole, paroxetine, HIV protease inhibitors (ritonavir being most potent), and many others.

Lipophilicity and CYP450 Inhibition

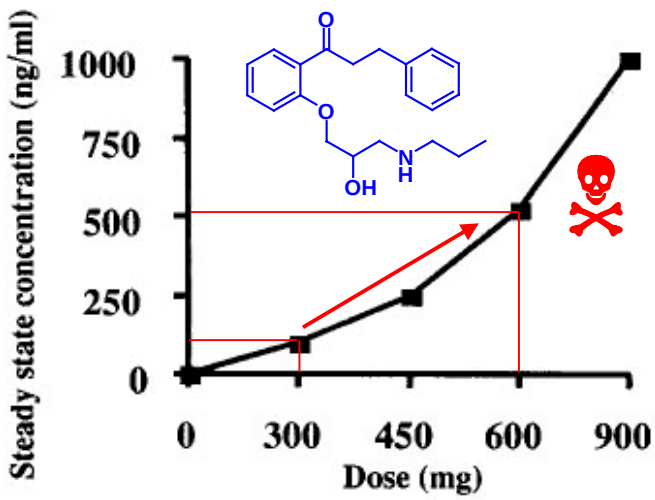


IC_{50} erythromycin N-demethylation

log $D_{7.4}$ = octanol/water partition coefficient at pH = 7.4

R. J. Riley, Curr. Opin. Drug Discov. Dev. 4, 45-54 (2001)

„Saturation“ of Propafenone Metabolism

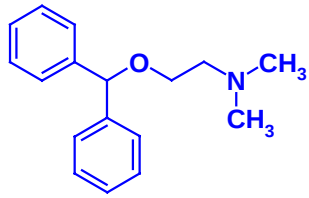


A dose increase by a factor 2 increases plasma levels by a factor 5-6 (propafenone is a CYP 2D6 inhibitor)

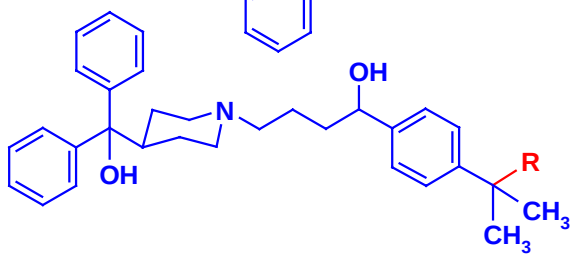
D. A. Smith, H. van de Waterbeemd and D. K. Walker, Pharmacokinetics and Metabolism in Drug Design, Wiley-VCH, Weinheim, 2001, p. 79.

Oxidative Metabolism and Drug Design

diphenhydramine
lipophilic H_1 antagonist
(sedative side effect)

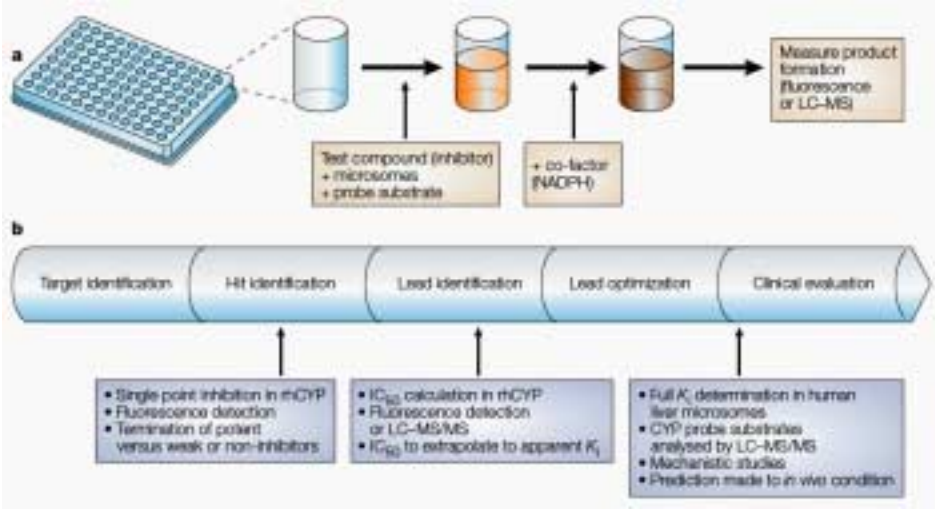


terfenadine (Seldane®), R = CH_3: polar H_1 antagonist (originally designed as an antipsychotic agent; no sedative side effect but cardiotoxic, especially in combination with CYP 3A4 inhibitors)



fexofenadine (Allegra®), R = COOH: active terfenadine metabolite (no sedative side effect, no cardiotoxicity)

In vitro Tests for CYP Inhibition in Drug Development



L. C. Wienkers and T. G. Heath, Nature Rev. Drug Discov. 4, 825-833 (2005)

A Clinical Case Study

(H. Schneemann, L.Y. Young and M. A. Koda-Kimple, Angewandte Arzneimitteltherapie, Springer-Verlag, Berlin 2001, p. 110)

B. D., a 32-year old man, suffers since 18 years from chronic pain. In addition, he developed stomach ulcers and a grand mal epilepsy. In the past he received opioid as well as non-narcotic analgesics. Recently he was treated with 10 mg methadone, every 6 hours, with good success. However, after some time withdrawal symptoms and insufficient pain control were observed. In the mean-time, a neurologist had prescribed phenytoin.

Phenytoin increases methadone metabolism by CYP 450 enzyme induction. The methadone dose has to be increased to 20 mg per 6 hours, under clinical control.

Hugo Kubinyi, www.kubinyi.de

Cytochrome P450 Induction by Xenobiotics

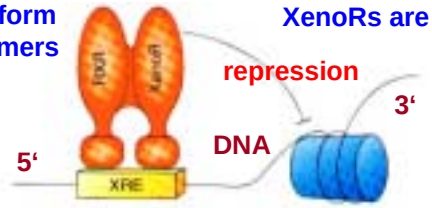
CYP 450 induction is a special case of drug-drug interaction. In addition, cigarette smoking and dietary-derived substances can induce CYP 450s and thus, increase metabolic degradation of certain drugs

(coffee drinkers, who decide to stop smoking, experience headache and agitation, due to an increase of plasma caffeine concentrations).

CYP 1A2: broccoli (?), cigarette smoking, insulin, omeprazole, phenobarbitone, aromatic hydrocarbons (e.g. char-broiled meat)
CYP 2C9: rifampicin, secobarbital
CYP 2C19: carbamazepine, norethindrone, prednisone, rifampicin
CYP 2D6: dexamethason, rifampicin (?)
CYP 2E1: ethanol, isoniazid
CYP 3A4: carbamazepine, efavirenz, ethosuximide, glucocorticoids, phenobarbitone, rifampicin, St. John's wort, sulfadimi-dine, nevirapine, sulfinpyrazone, troglitazone

CYP450 Induction is Nuclear Receptor-Mediated

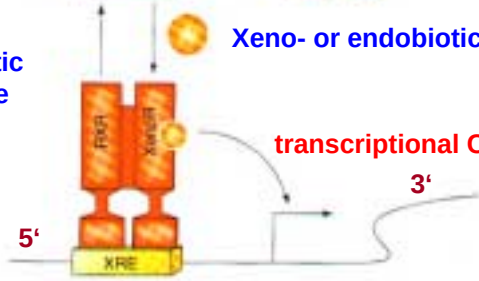
XenoRs form heterodimers with the retinoid receptor (RXR)



XenoRs are the pregnane X receptor (PXR), the constitutive androgen receptor (CAR) and the peroxisome proliferator-activated receptor (PPAR).

XRE = xenobiotic response element of DNA

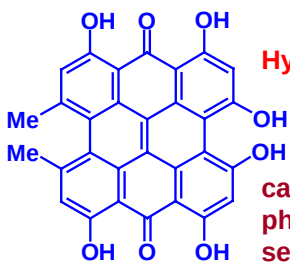
Xeno- or endobiotics, e.g. drugs



W. Xie and R. M. Evans, Drug Discov. today 7, 509-515 (2002)

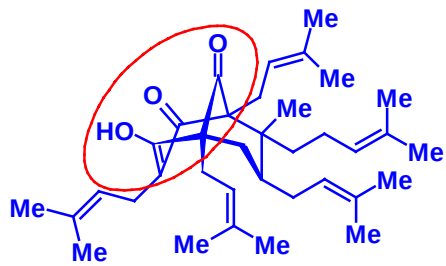
St. John‘s Wort (Johanniskraut, *Hypericum performatum*),

originally a herb for healing wounds (“doctrine of signatures“), is commonly used as „mild antidepressant“



Hypericin

causes photosensitivity



Hyperforin, a six-fold prenylated phloroglucin, is the highest affinity ligand of hPXR, K_d = 27 nM.

Humanized Mouse Model for the PXR Receptor

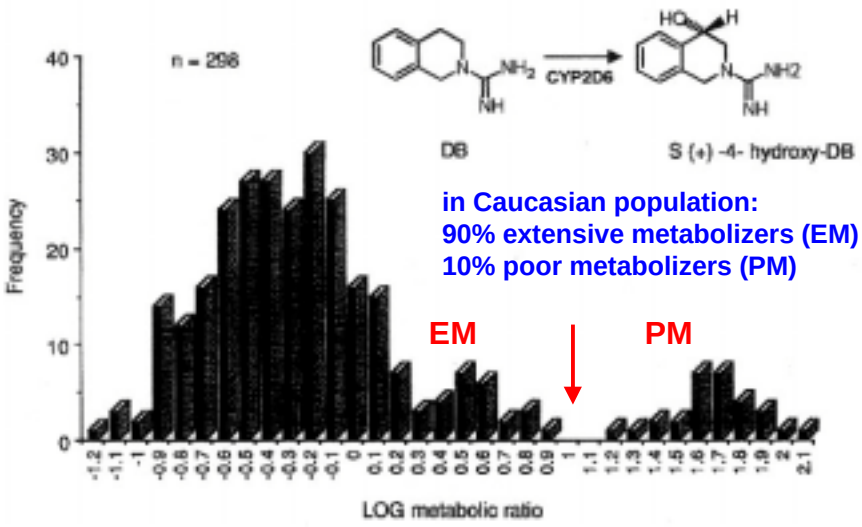
(W. Xie and R. M. Evans, Drug Discov. today 7, 509-515 (2002))

wild type
mPXR+
transgenic
mPXR+, hPXR+
transgenic k.o.
mPXR-, hPXR+

mPXR and hPXR show **species-specific ligand profiles. A humanized mouse model (mPXR-, hPXR+)** displays a human drug-response profile, with drug-induced over-expression of CYP 3A isozymes. This xeno-sensor allows the investigation of **drug-drug interactions in humans.**

Genetic Variability of Drug Metabolism

(CYP 2D6 = „debrisoquine-4-hydroxylase“)



Genetic Variability in CYP Expression (incomplete)

CYP 1A2: significant inter-individual variability; e.g. high, intermediate, and low caffeine metabolizers

CYP 2B6: lack of enzyme in 3-4% caucasians

CYP 2C9: deficiency (1-3% in Caucasians) leads to an inability to metabolize (S)-warfarin (doses have to be reduced from 5-10 mg to 0.5 mg/day); codeine, tramadol and losartan will not be activated.

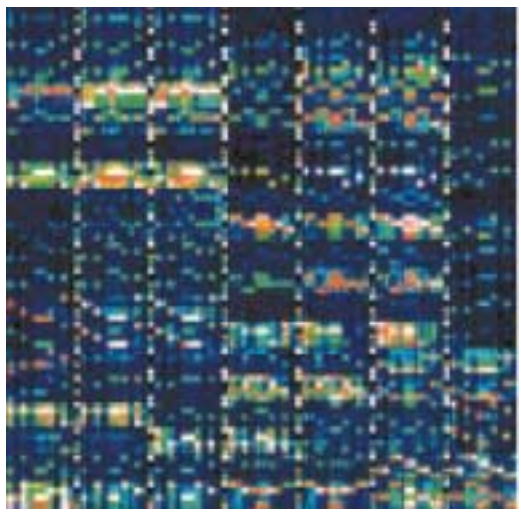
CYP 2C19: individuals with defective enzyme (3-6% Caucasians, 15-20% Asians) experience greater healing rates of their peptic ulcers, after treatment with omeprazole.

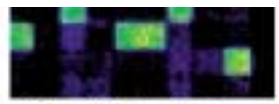
CYP 2D6: most extensively studied example of genetic polymorphism („sparteine-debrisoquine polymorphism"); poor metabolizers in about 10% of Caucasian population, less then 1% in Japanese population. High expression in many Ethiopians and Saudi Arabians (multiple gene copies).

CYP 3A4: only certain mutations (associated with MDR1 gene polymorphism)

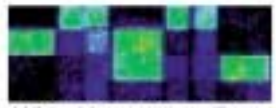
Genotyping with Cytochrome P450 Arrays

Affymetrix GeneChip® CYP 450 Assay for genotyping 2D6 and 2C19 gene variants (18 known mutations)

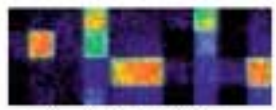




WM Wildtype Target Hybridization Image



WM Heterozygote Target Hybridization Image



WM Mutant Target Hybridization Image

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Gentest Human P450 Metabolism Database (www.gentest.com/human_p450_database/index.html)

Aspet Division for Drug Metabolism / Cytochrome P450 (www.aspet.org/public/divisions/drugmetab/cytochrome_p450.htm)

Cytochrome P450 Allele Nomenclature Committee (www.imm.ki.se/CYPalleles)

CYP 450 Drug Interactions (http://medicine.iupui.edu/flockhart)

CYP 450 Gene Databases (http://drnelson.utmem.edu/Databases.html)

Brookhaven Protein Database (www.rscb.org/pdb or www.biochem.ucl.ac.uk/bsm/pdbsum)