Grand Challenges
African Drug Discovery Accelerator

Drug Discovery and Development Course

Introduction to ADME Profiling (Metabolism and Excretion)

Franco Lombardo
February 13, 2025

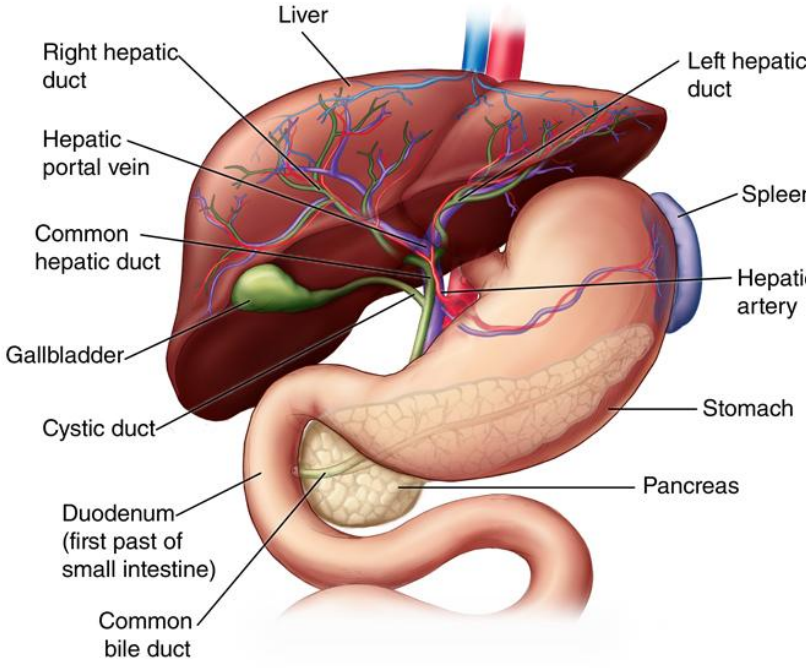
Supported by

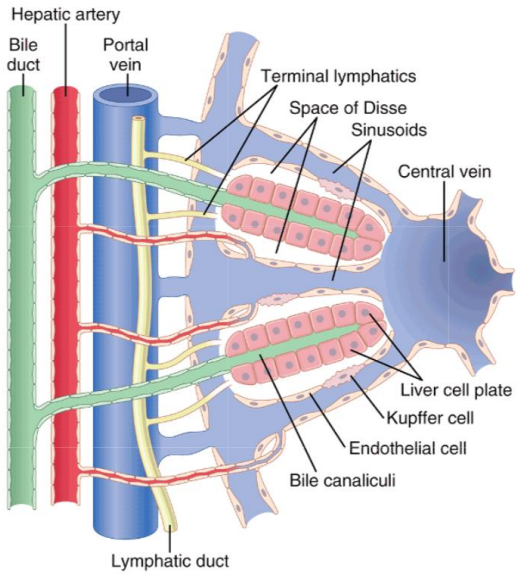
Gates Foundation LifeArc H3D FOUNDATION

Key objectives

1. Oxidative and conjugative enzymes (yes, phase I and II).
2. What is clearance?
3. How do we estimate it in vitro?
4. ECCS (no worries we will learn its meaning!)
5. Are transporters involved?
6. What is DDI?
7. Some renal physiology and excretion points to remember.

Metabolic and excretory guardian of the body





Species	Hepatic portal blood flow (mL/min/kg)
Mouse	90
Rat	55 (70)
Dog	30.9
Monkey	43.6
Human	20.7

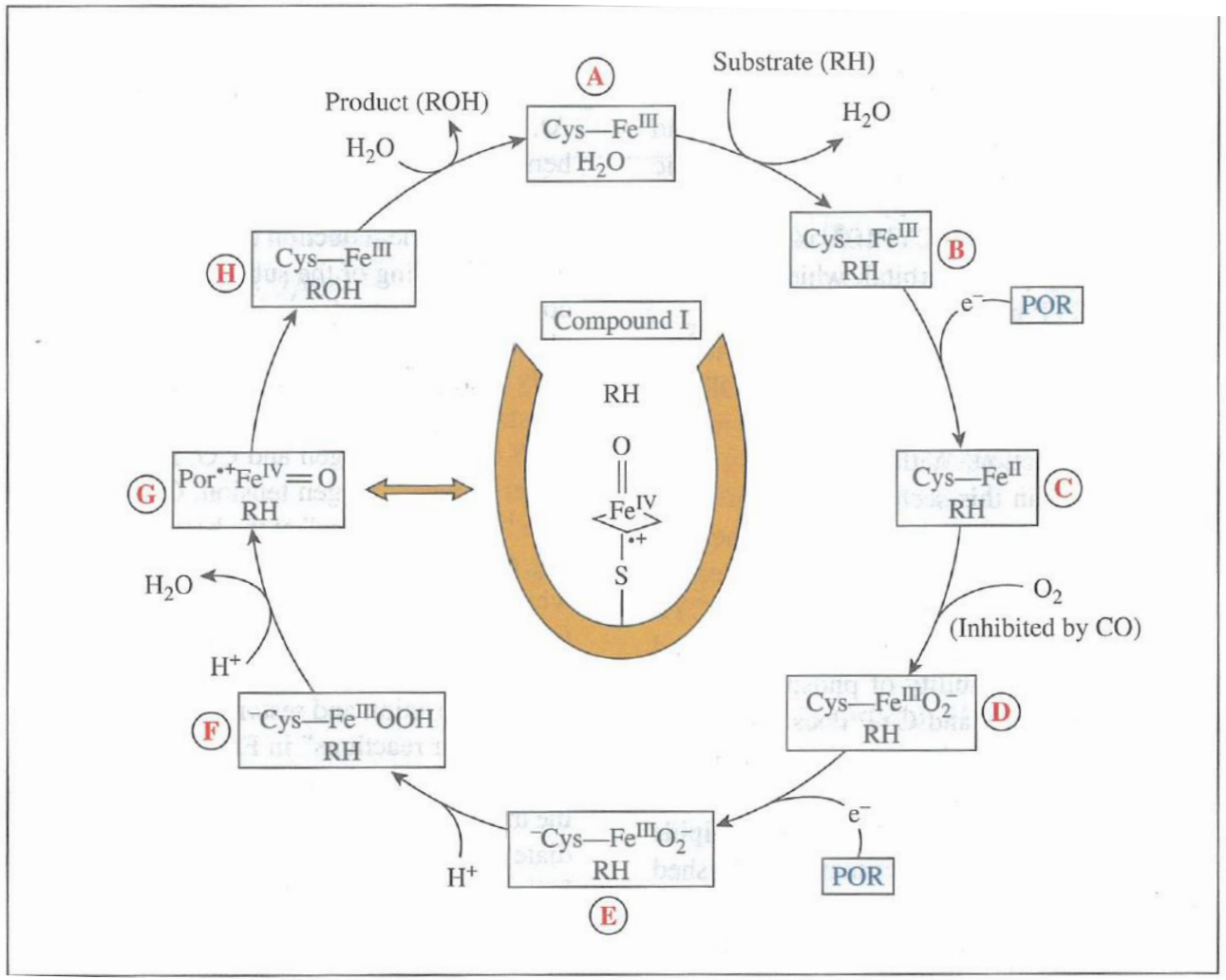
Data from Davies B, Morris T.
Physiological parameters in laboratory animals and humans. *Pharm Res* **1993**, *10*, 1093-1095

Some authors use 70 mL/min/kg as HBF in rat considering the 55 mL/min/kg too low. The values for other species are generally reported as slightly different but they are generally within a fraction of a mL, although larger values (77 mL/min/kg in dog and 152 mL/min/kg in mouse) can be found in literature.

Handbook of Drug Metabolism, 3rd Ed., P.G. Pearson and L. C. Wienkers Eds, CRC Press, Boca Raton, FL 2019

Guyton and Hall Textbook of medical physiology, Unit V, 14th Ed. J. E. Hall and M. E. Hall, Elsevier Philadelphia, 2021

The catalytic cycle of CYP450



The basic reaction catalyzed by CYP enzymes is a monooxygenation

Substrate (**RH**) +O2 +NADPH + H^+ ➡ Product (**ROH**) + H2O + $NADP^+$

Of all the xenobiotic-biotransforming enzymes the CYP enzyme system (57 human enzymes) ranks first in terms of catalytic versatility and the sheer number of xenobiotics it detoxifies or activates to reactive intermediates.

The highest levels of CYP enzymes are found in liver endoplasmic reticulum (microsomes) but CYP enzymes are present in virtually all tissues and some in mitochondria.

Some classification, examples and.. book!

Table 6-14

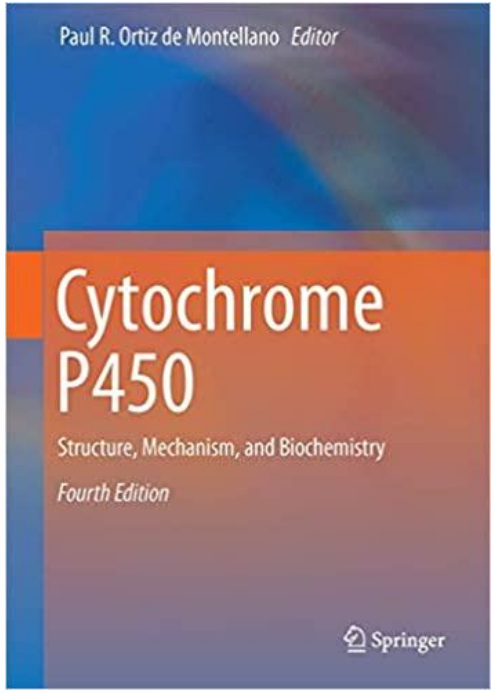
Classification of the 55 Functional Human CYP Enzymes

XENOBIOTICS		FATTY ACIDS/ EICOSANOIDS	STEROIDOGENIC	BILE ACIDS	VITAMIN D	RETINOIC ACID	UNKNOWN
CYP1A1	CYP2F1	CYP2U1	CYP11A1	CYP7A1	CYP2R1	CYP26A1	CYP4A22
CYP1A2	CYP2J2*,†	CYP4A11	CYP11B1	CYP7B1	CYP24A1	CYP26B1	CYP4X1
CYP1B1	CYP2S1	CYP4F2‡,§	CYP11B2	CYP8B1	CYP26C1**		CYP20A1
CYP2A6	CYP2W1	CYP4F3§	CYP17A1	CYP27A1††	CYP27B1		CYP27C1
CYP2A13	CYP3A4†,§,†	CYP4F8	CYP19A1	CYP39A1			
CYP2B6	CYP3A5	CYP4F11	CYP21A2	CYP46A1			
CYP2C8*	CYP3A7	CYP4F12§		CYP51A1‡‡			
CYP2C9	CYP3A43	CYP4F22					
CYP2C18		CYP4V2					
CYP2C19		CYP4Z1					
CYP2D6		CYP5A1§§					
CYP2E1		CYP8A1***					

Aromatase

Lanosterol 14α-demethylase

*Also involved in fatty acid and eicosanoid metabolism.
†Also involved in vitamin D metabolism.
‡Also involved in vitamin E and vitamin K metabolism.
§Also involved in xenobiotic metabolism.
Also involved in retinoic acid metabolism.
††Also involved in bile acid synthesis.
‡‡Also involved in cholesterol biosynthesis.
**Thromboxane A synthase (TBXAS1).
***Prostaglandin I_2 (prostacyclin) synthase (PTGIS).
Note: CYP2A7 and CYP4B1 are full length genes that probably encode inactive enzymes due to lack of heme incorporation.



Cytochrome P450: Structure, Mechanism, and Biochemistry P. Ortiz de Montellano Ed. 4th ed. 2015 Springer

13 chapters in 2 volumes.

Authoritative book on this very important enzyme family.

AOX

Soluble Mo enzyme localized in cytosol and some species (**most notably dog**) are devoid of it. Both aspects can be diagnostic.

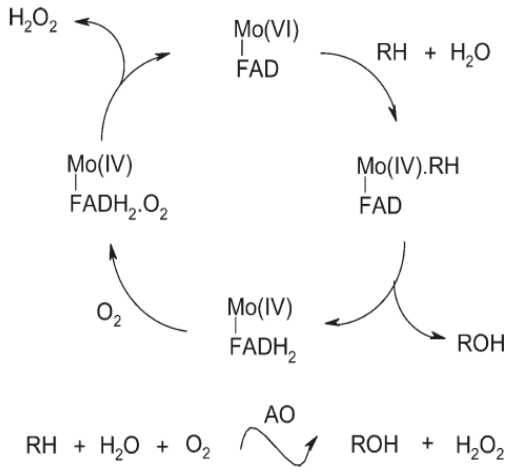


Figure 4. Proposed mechanism of AO-catalyzed oxidation of a substrate RH and the overall transformation catalyzed by AO.

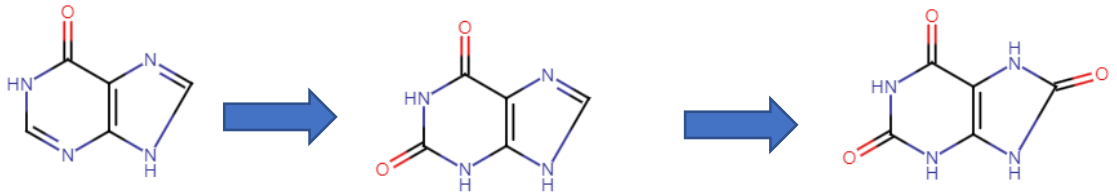
Obach R. S. POTENT INHIBITION OF HUMAN LIVER ALDEHYDE OXIDASE BY RALOXIFENE
Drug Metab Dispos **2004**, *32*, 89-97.
(K_i 0.87 to 51 nM depending on substrate and mechanism)

Pryde, Dalvie, Hu, Jones, Obach and Tran. Aldehyde Oxidase An Enzyme of Emerging Importance in Drug Discovery
J. Med. Chem. **2010**, *32*, 8441-8460.

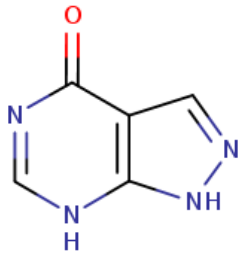
Tang, DaSilva, Lapham, Obach R. S. Evaluation of Icotinib as a Potent and Selective Inhibitor of Aldehyde Oxidase for Reaction Phenotyping in Human Hepatocytes.
Drug Metab Dispos **2024**, *52*, 565-573.
(time-dependent inhibitor, selective)

XO

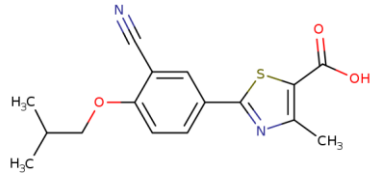
Soluble Mo enzyme localized in cytosol. It catalyzes the oxidation of hypoxanthine to xanthine and of xanthine to uric acid, in the breakdown of purine nucleotide. High concentration of uric acid leads to gout. Inhibited by **allopurinol**, **oxypurinol**, **febuxostat** and **topiroxostat**.



Hypoxanthine → Xanthine → Uric acid



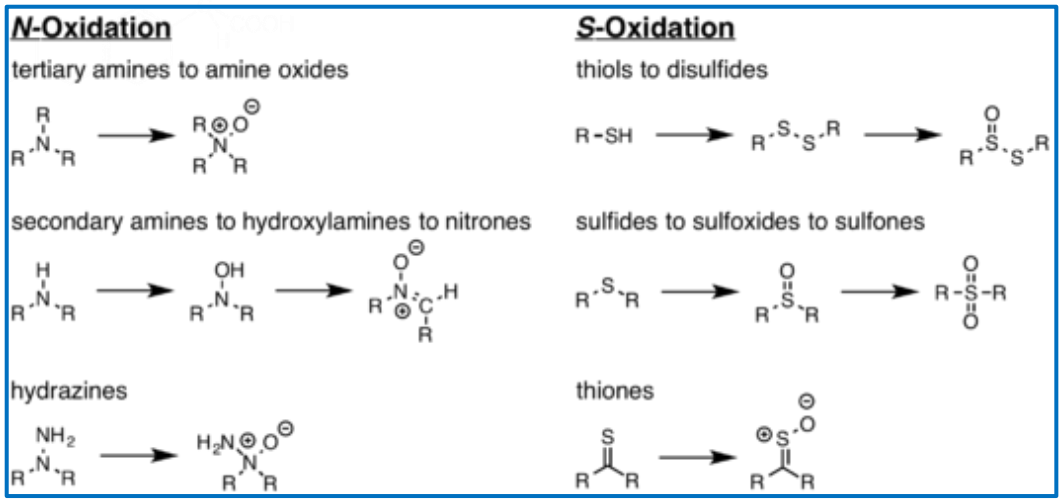
Allopurinol

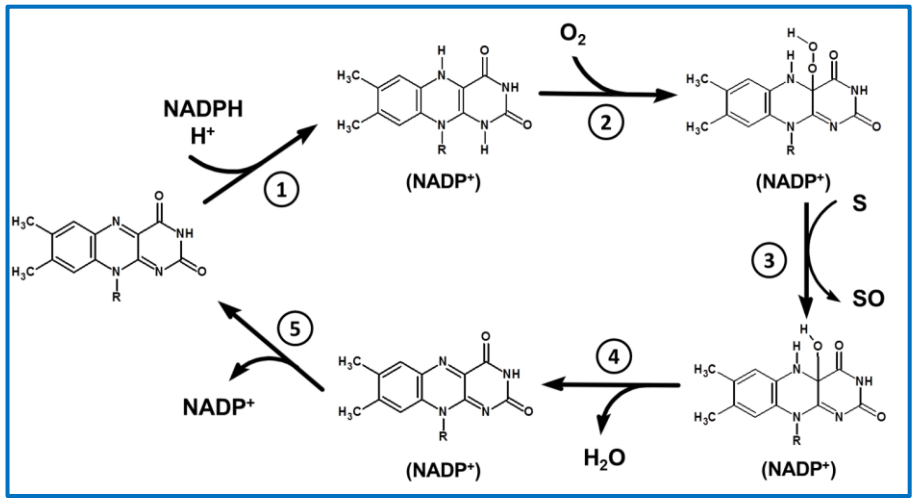


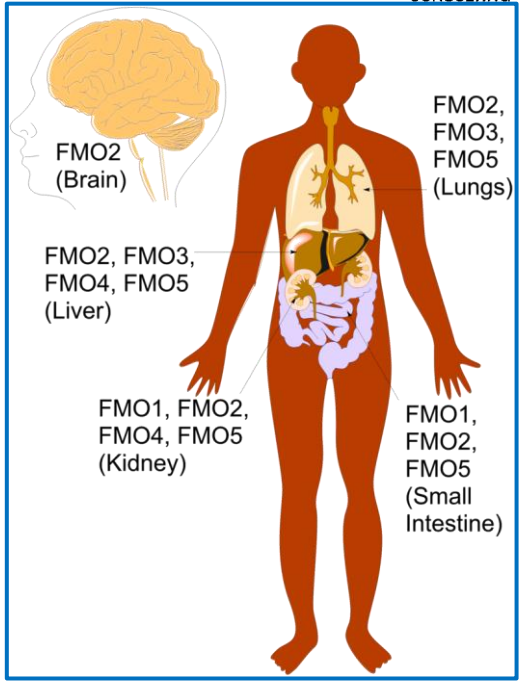
Febuxostat

XO inhibitors

FMOs







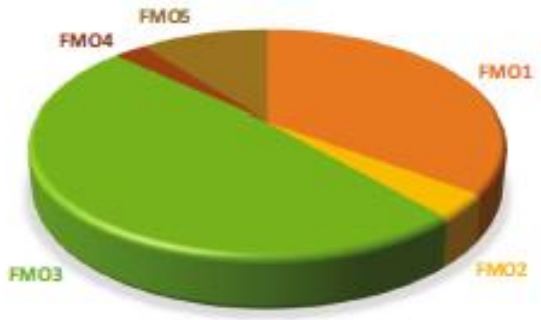


Fig. 1 Human FMO enzymes participating in the metabolism of drugs (data calculated for major and minor enzymes from Table 3; a total of 114 drugs used in calculations)

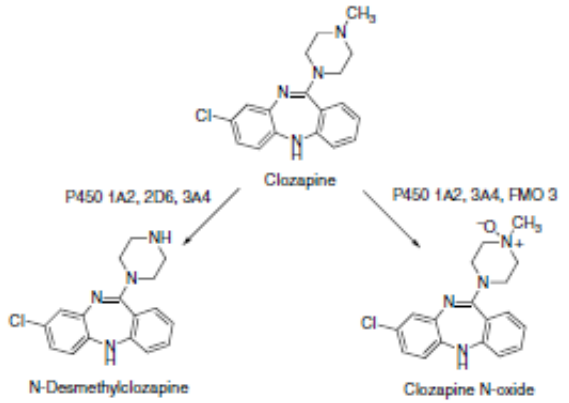


Fig. 7 Clozapine metabolism by human FMO and P450 enzymes (Fang et al. 1998; Tugnait et al. 1999, 1997)

Rendic, Crouch and Guengerich. *Arch. Toxicol.* ***2022****, 96,* 2145-2246.

MAO A and B

Localization: mitochondria. Based on FAD co-factor

The MAOs are mitochondrial, membrane-bound enzymes, and are located in many tissues, of which the most significant may be the brain. The enzymes are present also in the liver, where they catalyze the oxidative deamination of some xenobiotics.

MAO A is present in the brain, small intestine, heart, placenta, liver, portal system, and peripheral adrenergic neurons, and it is selective for the metabolism of norepinephrine and serotonin. MAO B is found in blood platelets, cerebral glial cells, and hepatic cells and is relatively selective for the metabolism of benzylamine and phenylethylamine. Physiological substrates are amines that are oxidized to aldehydes, which may be reduced by aldehyde reductase to alcohols.

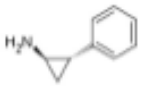
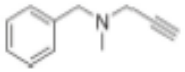
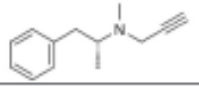
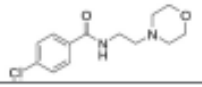
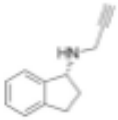
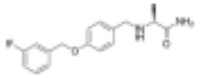
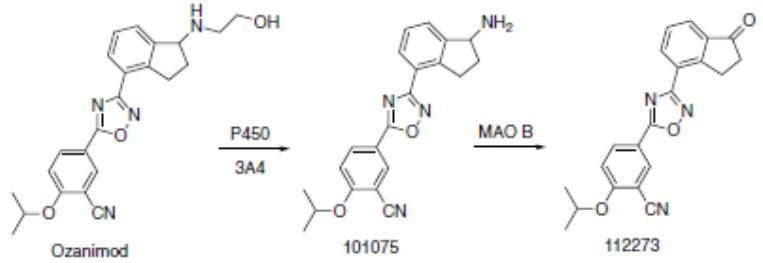
Tranylcypromine	Irreversible MAO-A + MAO-B	Used as antidepressant with dietary control	
Pargyline	Irreversible MAO-A and MAO-B	Antidepressant and antihypertensive Currently not in clinical use	
Selegiline	Irreversible MAO-B selective (R- enantiomer) Selectivity is dose dependent *in vivo*	Metabolism to amphetamines	
Clorgyline	Irreversible highly MAO-A selective	Antidepressant effect demonstrated in humans but not in clinical use	
Moclobemide	Reversible highly MAO-A selective	Moderately effective antidepressant drug	
Rasagiline	Irreversible MAO-B selective (R+ enantiomer) Selectivity is dose dependent *in vivo*	Neuroprotective *in vitro*, anti-Parkinson drug, metabolism to 1-aminoindan	
Safinamide	Reversible highly MAO-B selective	Anti-Parkinson drug, glutamate receptor antagonistic and Na+ channel blocking properties	

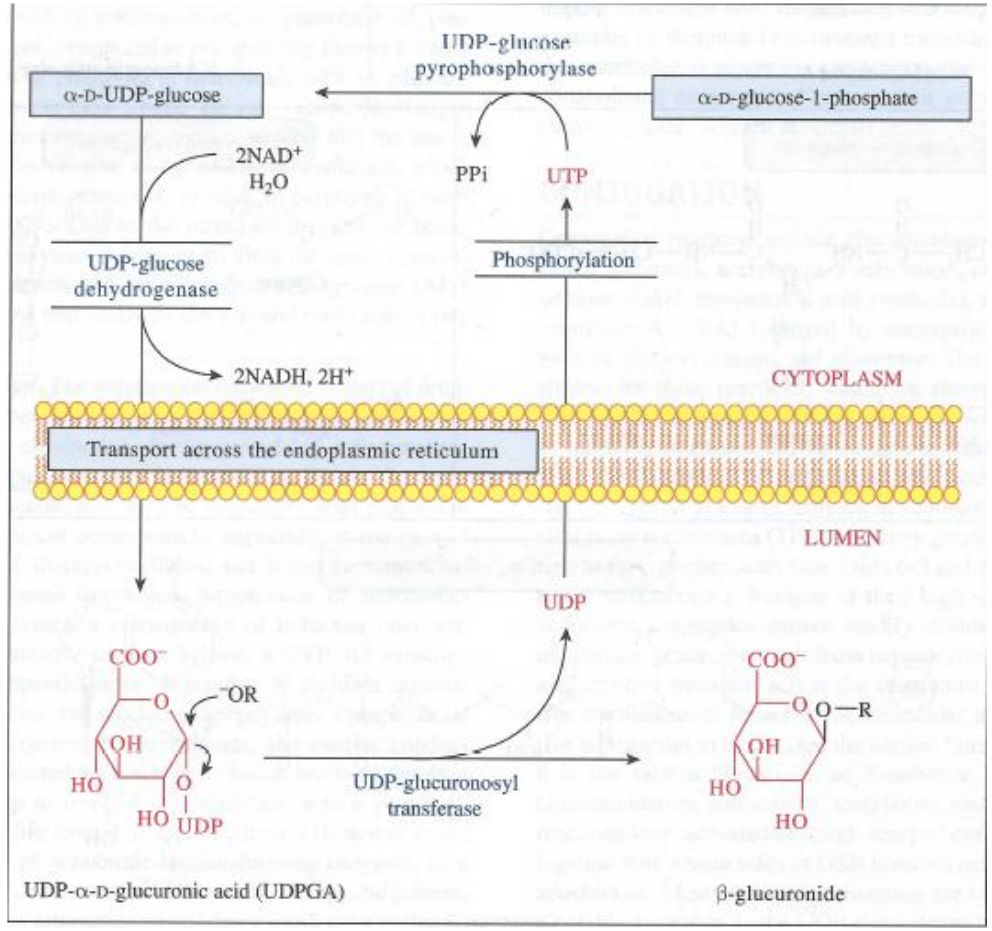
Fig. 22 Ozanimod metabolism by P450 and MAO enzymes



Rendic, Crouch and Guengerich. Arch. Toxicol. 2022, 96, 2145-2246

Finberg et al. Front. Pharmacol. October 2017, 7 article 340

UGTs
(Uridine 5'-diphospho-glucuronosyltransferases)



Conjugative enzymes located in the endoplasmic reticulum largely in the hepatocytes but also small intestine and colon and other (minor amounts) tissues. They catalyze the conjugation of D-glucuronide acid to detoxify endo- and xenobiotics. Highly variable contents of same enzyme in different tissues

Isoforms generally sought-after and characterized

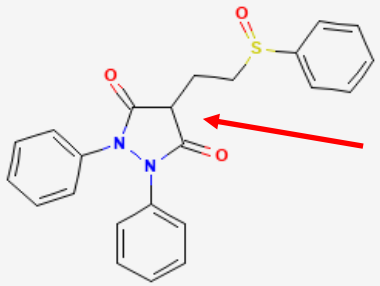
1A1 1A4 1A6 1A9 1A10 2B4 2B7

But many others present.

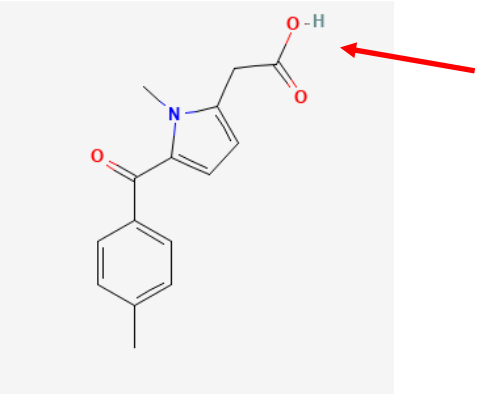
UGT1A3, UGT1A5, UGT1A7, UGT1A8
UGT2A1, UGT2A2, UGT2A3
UGT2B10, UGT2B11, UGT2B15, UGT2B17, UGT2B28

General reference: Biotransformation of Xenobiotics, A. Parkinson, B. W. Ogilvie, D. B. Buckley, F. Kazmi, O. Parkinson. Ch. 6 in Casarett and Doull's *Toxicology, The Basic Science of Poisons*, Ninth Edition, C. Klaassen Ed. 2018, McGraw-Hill Education.

UGTs at work! C.O.N.S.



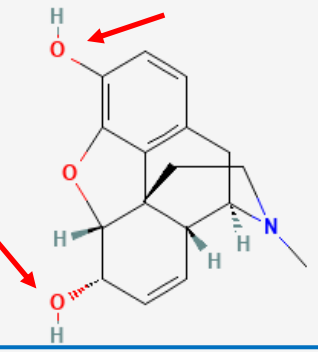
Example of C-glucuronide:
phenylbutazone and sulfinpyrazone
(UGT1A9)
Kerdpin et al.,
Drug Metab Disp **2006**, *34*, 1950-1953.



Tolmetin

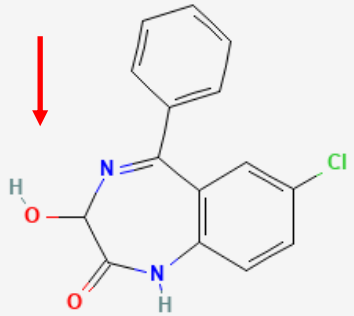
Example of O-glucuronides: acylglucuronides (esters).
Intramolecular migration of acyl group across sugar OH groups.
Potentially toxic and reactive toward proteins.

More complex species (di-glucuronides) and sertraline carbamoyl glucuronides with involvement of HCO_3^- have also been reported



Morphine, UGT2B7

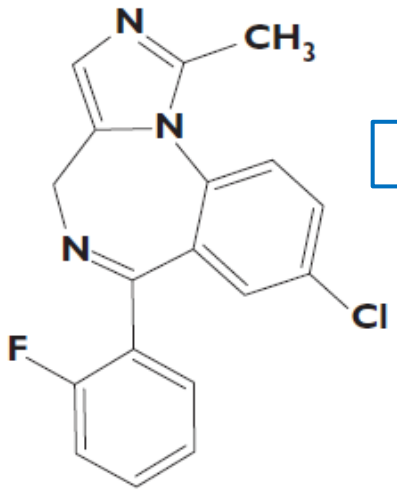
Oxazepam UGT2B15



Examples of O-glucuronides: acetals

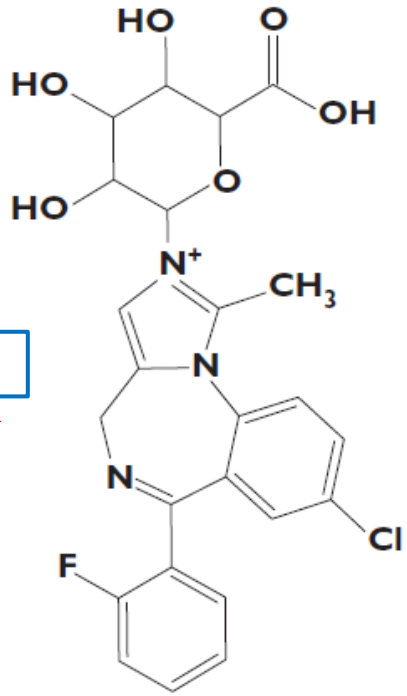
General reference: Biotransformation of Xenobiotics, A. Parkinson, B. W. Ogilvie, D. B. Buckley, F. Kazmi, O. Parkinson. Ch. 6 in Casarett and Doull's *Toxicology, The Basic Science of Poisons*, Ninth Edition, C. Klaassen Ed. 2018, McGraw-Hill Education.

UGTs at work! C.O.N.S.



Midazolam

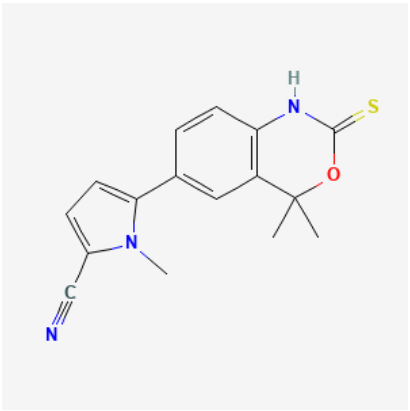
UGT1A4



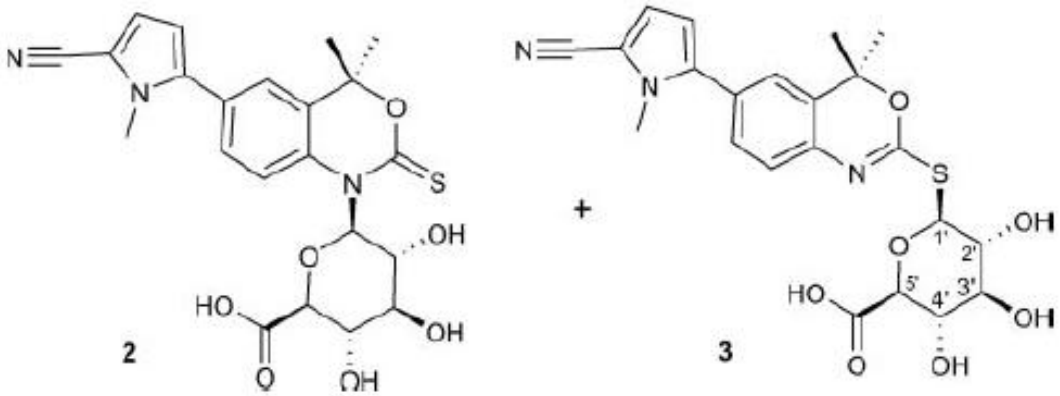
Midazolam-N-glucuronide

Hyland et al., *Br J Clin Pharmacol* **2009**, *67*, 445–454

UGT1A9 and 2B7



Tanaproget



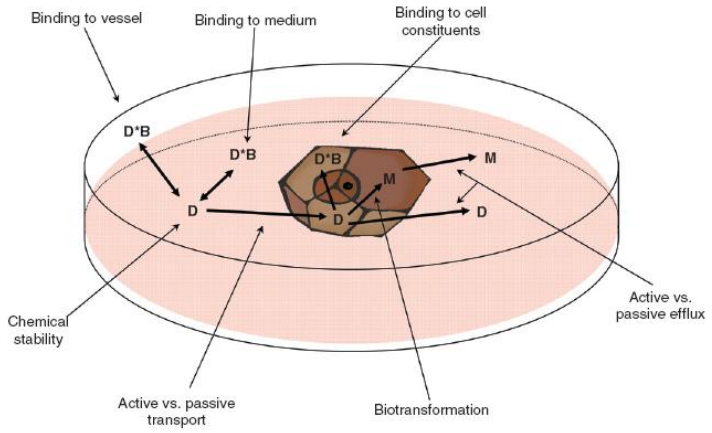
2 + 3

Keating et al., *Drug Metab Disp* **2006**, *34*, 1283-1287

Other examples of N-glucuronides:
Camonsertib (*primarily* UGT1A4) Papp et al., *Drug Metab Disp* ***2024***, *52, 368-376*
Cediranib (N+ glucuronide, UGT1A4), Lenz et al., *J. Pharm. Biomed. Anal.* **2010**, *53*, 526-536.

General reference: Biotransformation of Xenobiotics, A. Parkinson, B. W. Ogilvie, D. B. Buckley, F. Kazmi, O. Parkinson. Ch. 6 in Casarett and Doull's *Toxicology, The Basic Science of Poisons*, Ninth Edition, C. Klaassen Ed. 2018, McGraw-Hill Education.

In vitro clearance prediction



Prediction of In Vivo Hepatic CL (CL_h) from In Vitro Data

Cl_{int} in µL/min/mg protein **or** µL/min/mg 10^6 cells

Calculation:

1) Scale *in vitro* $t_{1/2}$ to *in vivo* intrinsic clearance

$$CLint,H\ (mL/min/kg) = 0.693 * \frac{1}{T1/2(min)} * \frac{g\ liver}{kg\ body} * \frac{mL\ inc}{million\ cells} * \frac{120\ million\ cells}{g\ liver}$$

$\frac{45\ mg\ mic\ prot}{g\ liver}$

2) Use Well Stirred Model

$$CL_H\ (mL/min/kg) = \frac{Q * fu * CL_{int,H}}{Q + fu * CL_{int,H}}$$

Where Q is the liver blood flow, fu is the unbound fraction and $CL_{int,H}$ is the scaled hepatic CL_{int}

	Mouse	Rat	Dog	Monkey	Human
Body weight (kg)	0.02	0.25	10	5	70
Liver weight (g/kg)	87.5	40	32	30	25.7
Liver Blood Flow (ml/min/kg)	90	55.2	30.9	43.6	20.7
(L/hr/kg)	5.4	3.3	1.9	2.6	1.2

Extraction Ratio = CL_H/Q

Clearance Classification	ER
High CL	> 0.75
Medium CL	0.25-0.75
Low CL	<0.25

Obach, R., et al.,(1997). *J Pharmacol Exp Ther.* 283:46-58
Davies and and Morris (1993). Pharm Res. 10: 1093-1095

Parallel tube model

$$CL_{blood} = Q \cdot \left(1 - e^{\left(\frac{-CL_{int}}{Q}\right)}\right) \quad (4)$$

$$CL_{blood} = Q \cdot \left(1 - e^{\left(\frac{-f_{u(blood)} \cdot CL_{int}}{Q}\right)}\right) \quad (5)$$

$$CL_{blood} = Q \cdot \left(1 - e^{\left(\frac{-f_{u(blood)} \cdot CL_{int}}{Q \cdot f_{u(mic)}}\right)}\right) \quad (6)$$

Obach R. S. ,
Drug Metab. Dispos. **1999**, *27*, 1350-1359

In vitro clearance prediction

$$CL_{blood} = \frac{Q \cdot f_{u(blood)} \cdot \frac{CL'_{int}}{f_{u(mic)}}}{Q + f_{u(blood)} \cdot \frac{CL'_{int}}{f_{u(mic)}}}$$

Rb = C_{bl}/C_{pl} (blood-to-plasma ratio)

Generally, between 0.5 and 2 but it could be very high in some cases. It cannot be lower than ≈ 0.5

More often than not we measure concentrations in plasma, then $Cl_p = Cl_b * C_b/C_p$

that is, if $C_b/C_p > 1$ clearance in plasma will be higher than clearance in blood.

Also, $f_{u,b} = f_{u,p}/ C_b/C_p$

Well-stirred model (**WS** most frequently used)

Obach R. S. et al. *J. Pharmacol. Exp. Ther.* **1997**, *283,* 46-58

Stringer R. A. et al., *Drug Metab. Dispos.* **2009**, *37,* 1025-1034.

Hepatocytes or microsomes: what if they differ with microsomes predicting > clearance?

Obach R. S. , *Drug Metab. Dispos.* **1999**, *27,* 1350-1359

Well, **microsomes!**
According to
Keefer, Chang, Carlo et al.
Eur. J. Pharm. Sci. **2020**, *155*,105541

*Better extrapolation with either **both** binding factors or **none**!*

Jones, Leung et al. *Drug Metab. Dispos.* **2022**, *50,* 1053-1063.

Scalars for f_{up} and Cl_{int} in a WS model with good results

Scaling factors to account for variation and lability of enzymes and transporter, donor phenotypic differences, binding. Hallifax and Houston proposed scaling factors on the basis of segregated Cl_{int} values. *Drug Metab. Dispos.* **2019**, *47*, 320-327 (and references therein). Concerns over errors and scaling methods but through a retrospective analysis with data for 98 compounds from various sources. Wood, Houston and Hallifax et al. *Drug Metab. Dispos.* **2022**, *50,* 1053-1063.

Some of the scaling systems used

- Hepatocytes
  - Closest to an *in vivo* system (contain cell membrane and majority of the enzymes/transporters and cofactors involved in drug metabolism)
  - Could be expensive as a primary screening assay
  - Generally used in suspension. Limited viability (4 h). Relay methods proposed to extend time.
- Liver Microsomes
  - Most commonly used *in vitro* system for because of its accessibility and ease of handling
  - Contain Phase I oxidative enzymes (cytochrome P450 enzymes)
  - May underpredict clearance if the compounds are metabolized by cytosolic enzymes
- Recombinant enzymes
  - *Available for many CYPs and other enzymes.*
  - *Intersystem extrapolation and relative activity factors are substrate dependent especially for 3A4 and 2C9.*
  - *Non-specific binding differences between recombinant enzymes and HLM/HHEP.*
  - *Lack of cooperativity between CYPs which is important for turnover of some substrates.*
- Liver/Intestinal S9
  - Contain both cytosolic and microsomal enzymes but have lower enzyme activity
  - Intestinal S9 used for gut metabolism assessment
  - Not commonly used for IVIVE (mostly for qualitative-mechanistic studies)
- Whole Blood/Plasma
  - Contain esterases, amidases, glutathione transferases (GSTs), proteases, etc.
  - Mostly used for pro-drug metabolic assessment (or if hydrolysis is a major metabolic pathway)

COMMENTS ON RECOMBINANT ENZYMES

Li Di *Expert Opin Drug Discov*, 2017, 12, 1105-1115.

RELAY METHOD FOR LOW CLEARANCE COMPOUNDS

Li Di et al. *Drug Metab Dispos* **2012**, *40*, 1860-1865.

Li Di et al. *Drug Metab Dispos* **2013**, *41*, 2018-2023.

Perspective on the topic:

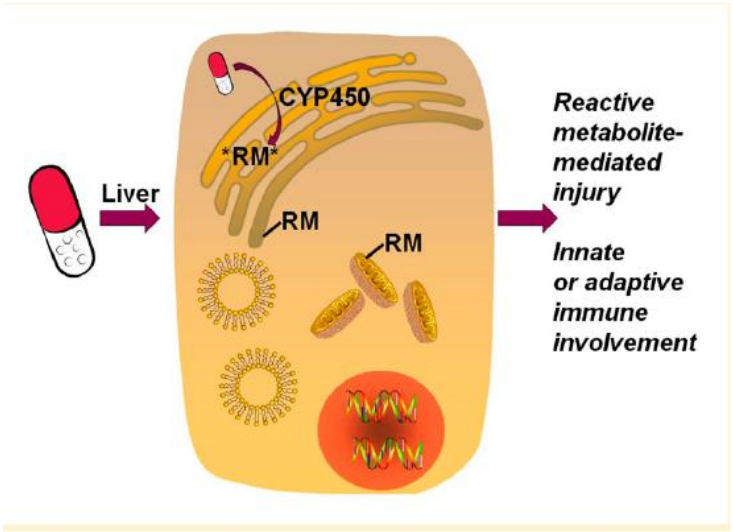
Li Di *Expert Opin Drug Discov*, 2023, 18, 12091-219.

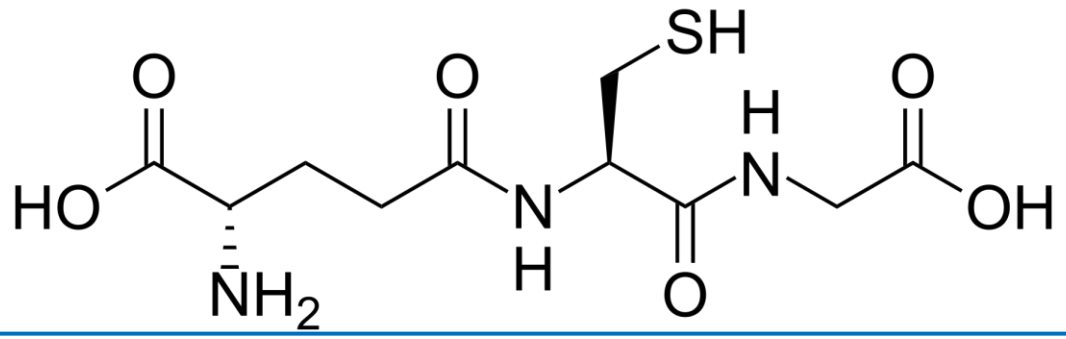
TWO-TIER PHENOTYPING APPLICATION (qualitative-then-quantitative)

Doran, Burchett et al. *Drug Metab Dispos* **2022**, *50*, 1259-1271.

Doran, Dantonio et al. *Drug Metab Dispos* **2022**, *50*, 1272-1286.

Glutathione…there for a purpose

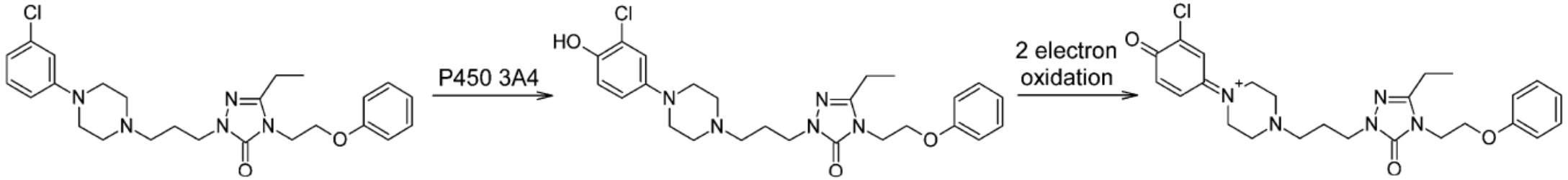




Could be trapped by glutathione (GSH, transferred by GSTs) present at mM levels in cells. Elimination of ROS (peroxides and free radicals, GSH to GSSG) and conjugation of reactive metabolites.

In vitro other RSH (soft) nucleophiles e.g., for Michael acceptors or CN- for (hard) electrophiles, e.g., iminium ions can be used.

Nefazodone



Kalgutkar A et al. A comprehensive listing of bioactivation pathways of organic functional groups. *Curr. Drug Metab.* **2005**, *6*, 161−225./Dalvie D et al. Practical approaches to resolving reactive metabolite liabilities in early discovery. Drug Metab Rev, 2014, Early Online: 1–15/Thompson R A et al. Reactive Metabolites: Current and Emerging Risk and Hazard Assessments *Chem. Res. Toxicol.* **2016**, *29*, 505−533

Extended Clearance Classification System (ECCS)

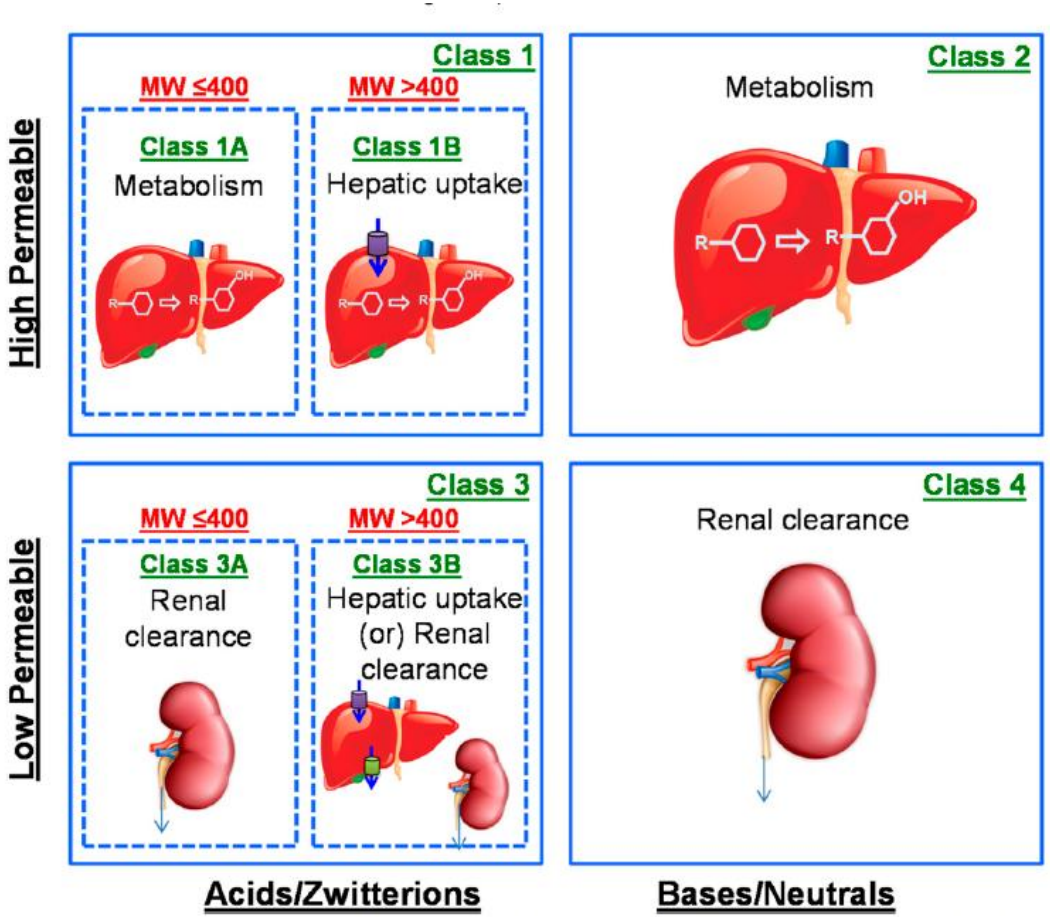
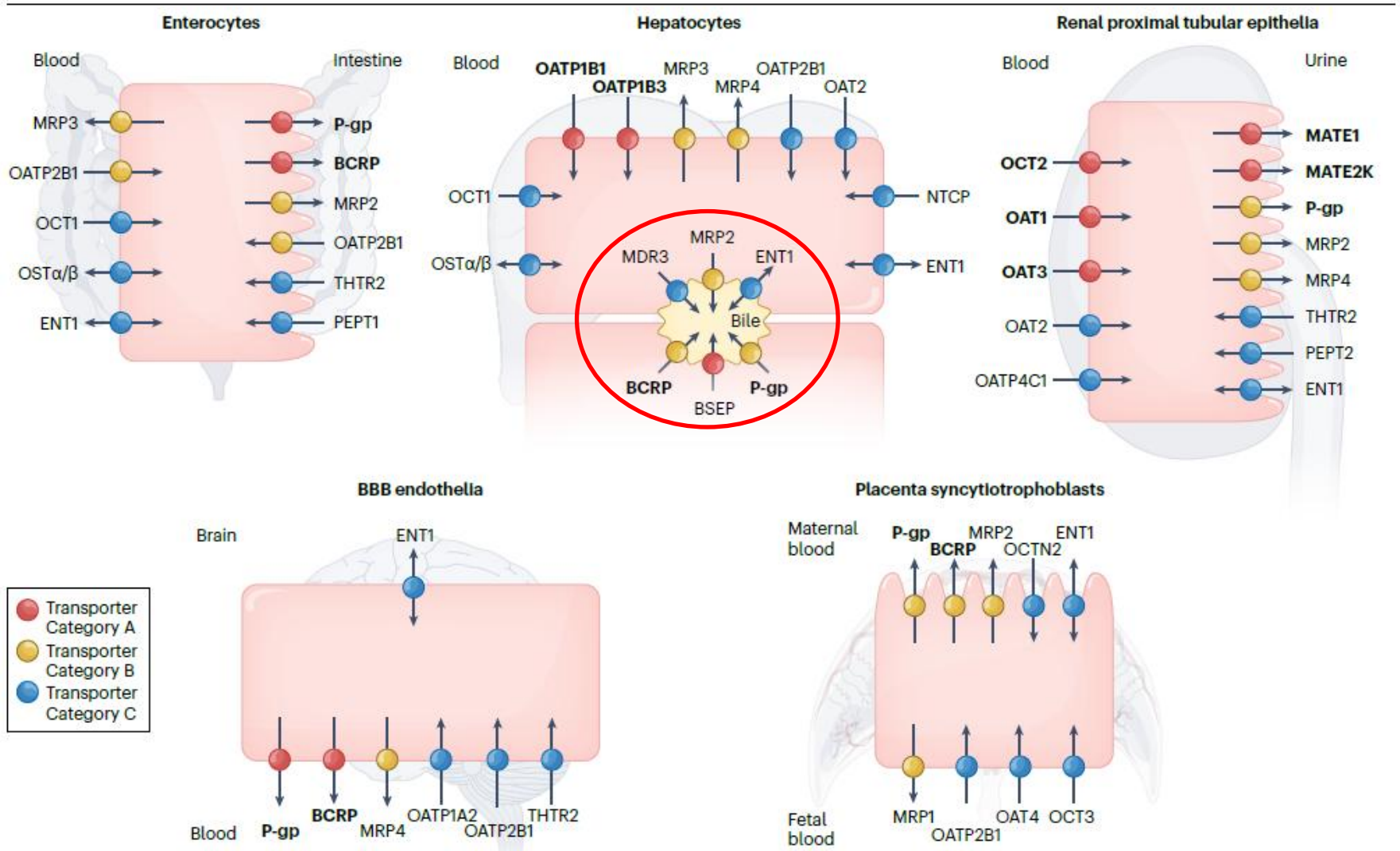


Fig. 1. ECCS for predicting the clearance mechanism (rate-determining process) (Varma et al., 2015b). Hepatic uptake mediated by OATPs is likely the rate-determining step in the clearance of class 1B and 3B compounds. Renal transporters, OAT1 and OAT3, contribute to the active secretion of class 3A, 3B, and 4 compounds, while OCT2 and/or MATE proteins are involved in renal secretion of class 4 compounds.

Application of ECCS framework in mapping the role of clinically relevant transporters. "Assignment" of (likely) transporters to each class.

Varma et al., *Pharm. Res.* **2015**, *32*, 3785-3802.; El-Kattan et al, *ibid.* **2016**, *33*, 3021-3030.;
El-Kattan and Varma, *Drug. Metab. Dispos.* **2018**, *46*, 729-739.

TRANSPORTERS



Galetin A. et al.
Nat. Rev. Drug Disc. **2024**, *23*, 255-280

Hillgren, K. et al.
Clin. Pharm. Ther. **2013**, *94*, 52-63

Zamek-Gliszczynski M. et al.
Clin. Pharm. Ther. **2018**, *104*, 890-899

The Transporter Book 4th edition, April 2021, edited by Solvo.
Very nice compendium of techniques, references, regulatory requirements and tools

INHIBITION AND INDUCTION

- Drug-drug interactions (DDI) occur when 2 or more drugs interact in such a way that the effectiveness or toxicity of one or more of the drugs is altered.
- DDI can lead to severe side effects and have resulted in early termination of development, refusal of approval, and withdrawal of drugs from the market.
- CYP-mediated DDI is one of the most common mechanisms of clinically important DDI.
  - CYP inhibition – reduced enzyme activity (through reversible or time-dependent inhibition)
  - CYP Induction – increased enzyme activity (through nuclear receptors): generally studied in hepatocytes focusing on **1A2**, **2B6** and **3A4.**

The compound that causes the change (induction or inhibition) is generally called the "perpetrator" or "precipitant" while the compound whose metabolism is changed is called "victim" or "object"

Also, there are natural products, like bergamottin and naringenin, that can inhibit metabolizing enzymes while hyperforin (*H. perforatum*) can induce both CYP3A4 and P-gp

DDI of human in vitro and clinical database was maintained by the University of Washington:
https://www.druginteractionsolutions.org/
Now acquired by CERTARA

https://www.fda.gov/regulatory-information/search-fda-guidance-documents

Common types of CYP inhibition

- Direct inhibition (Co-incubation Assay)
  - ➢ Reversible inhibition
    - Occurs when a drug inhibits CYP enzymes without requiring biotransformation.
- Time-Dependent Inhibition (Pre-incubation Assay)
  - ➢ Metabolism-based inhibition
    - Compounds are first metabolized by CYP enzymes to generate metabolites which then cause ***reversible inhibition of CYP enzymes***.
  - ➢ Mechanism-based inhibition (Dilution Assay)
    - Compounds are first metabolized by CYP enzymes to generate reactive metabolites which ***inactivate CYP enzymes via covalent binding***.

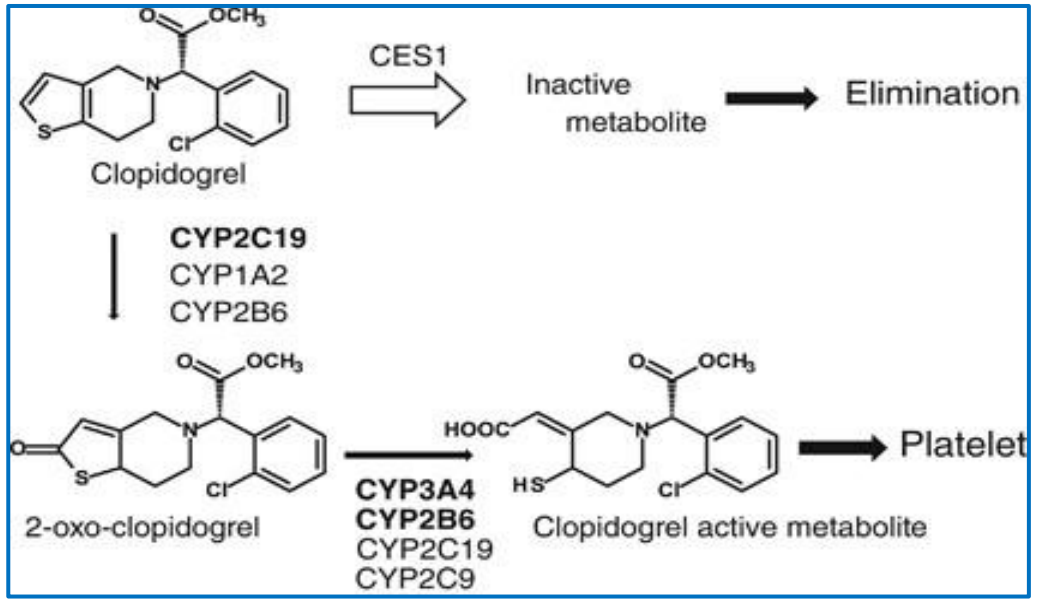
FDA Guidance for Industry – ICH M12 August 2024 ICH–Multidisciplinary

Genetic Polymorphism PM, IM, EM and UM

There is genetic polymorphism across ethnic groups. Several isoforms, such as, **CYP 1A1, 2C9, 2C19**, **2D6, 3A5** are expressed in widely varying amount in different ethnic groups and the results could be toxicity (PM) or lack of efficacy (EM and UM). Also, **UGT1A1** and **NAT** are among polymorphic enzymes.

PM : poor metabolizers
IM : Intermediate metabolizers
EM: Extensive metabolizers
UM: Ultra-rapid metabolizers
Not limited to CYPs: also observed with conjugative enzymes and transporters

5-10% of Caucasians
3.8% of African Americans
0.9% of Asians
1% of Arabs
Are poor 2D6 metabolizers



Patients who are poor metabolizers of the drug, **do not effectively convert Plavix** (**clopidogrel**, a platelet $P2Y_{12}$ inhibitor) **to its active form**. In these patients, Plavix has less effect on platelets, and therefore less ability to prevent heart attack, stroke, and cardiovascular death.

FDA Safety Communication
(accessed August 31, 2022).

If an investigational drug is a substrate *in vitro* for a particular CYP, *in vivo* interaction studies with a strong inhibitor and inducer for that CYP are recommended to determine the extent of changes in the investigational drug's pharmacokinetics."

Why Conduct CYP Reaction Phenotyping ?

- Useful for explaining or predicting pharmacokinetic variability, which may occur when a drug is metabolized by a ***polymorphically*** expressed P450 enzyme. **Whether polymorphic or not**, we generally study **CYP1A2**, **CYP2B6**, **CYP2C8**, **CYP2C19**, **CYP2C9** and **CYP2D6** ,**CYP3A4**.
- Useful for explaining or predicting DDI, which may occur with concomitantly administered drugs

$$DDI = \frac{1}{\left(\frac{f_m}{1+\left(\frac{[I]}{K_i}\right)}\right) + (1 - f_m)}$$

TWO-TIER PHENOTYPING APPLICATION
(qualitative-then-quantitative)

Doran, Burchett et al. *Drug Metab Dispos* **2022**, *50*, 1259-1271.
Doran, Dantonio et al. *Drug Metab Dispos* **2022**, *50*, 1272-1286.

"Interactions between an investigational new drug and other drugs should be defined during drug development, as part of an adequate assessment of the drug's safety and effectiveness.

The objective of drug-drug interaction (DDI) studies is to determine whether potential interactions between the investigational drug and other drugs exist and, if so, whether the potential for such interactions indicates the need for dosage adjustments, additional therapeutic monitoring, a contraindication to concomitant use, or other measures to mitigate risk"

The contribution of a specific metabolizing enzyme to an investigational drug's clearance is considered significant if the enzyme is responsible for > 25% of the drug's elimination based on the in vitro phenotyping studies and human PK data.

FDA Guidance for Industry – ICH M12 August 2024 ICH–Multidisciplinary

Some renal physiology and excretion

$$Cl_R = fu * GFR + ClTS - TR$$

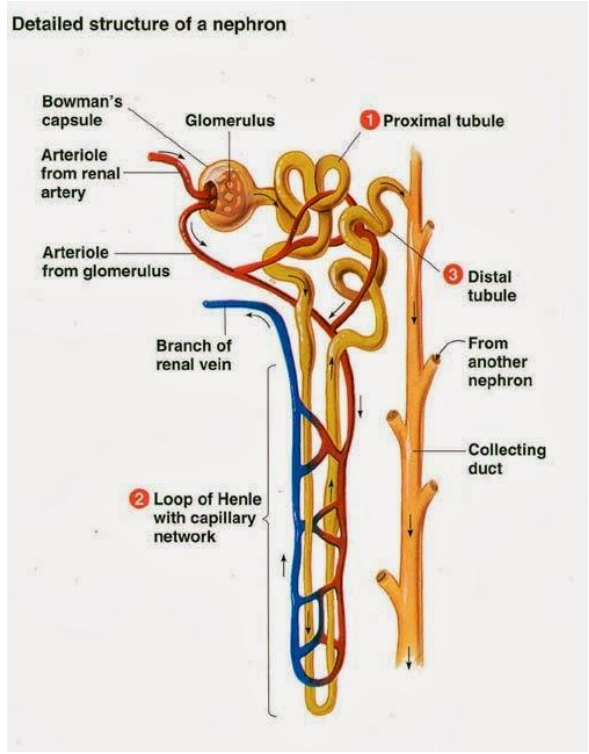
Where Cl_{TS} is clearance by tubular secretion and *TR is the extent of tubular reabsorption.*

As the urine progresses through the proximal tubule **water is reabsorbed**. Thus, a gradient is formed which is directed from the apical to the basolateral side since the concentration in urine is higher than the concentration of the compound in blood. Reabsorption is governed by **three main factors**.

Lipophilicity. Lipophilic compounds have a higher chance to pass through lipoidal membranes and it is part of the reason why metabolism transforms lipophilic compounds into more hydrophilic compounds, which are therefore excreted more easily.

Charge. Generally charged species have more difficulty passing through lipoidal membranes although there are equilibria that depend on urine pH, which may also vary (within some drugs and within limits).

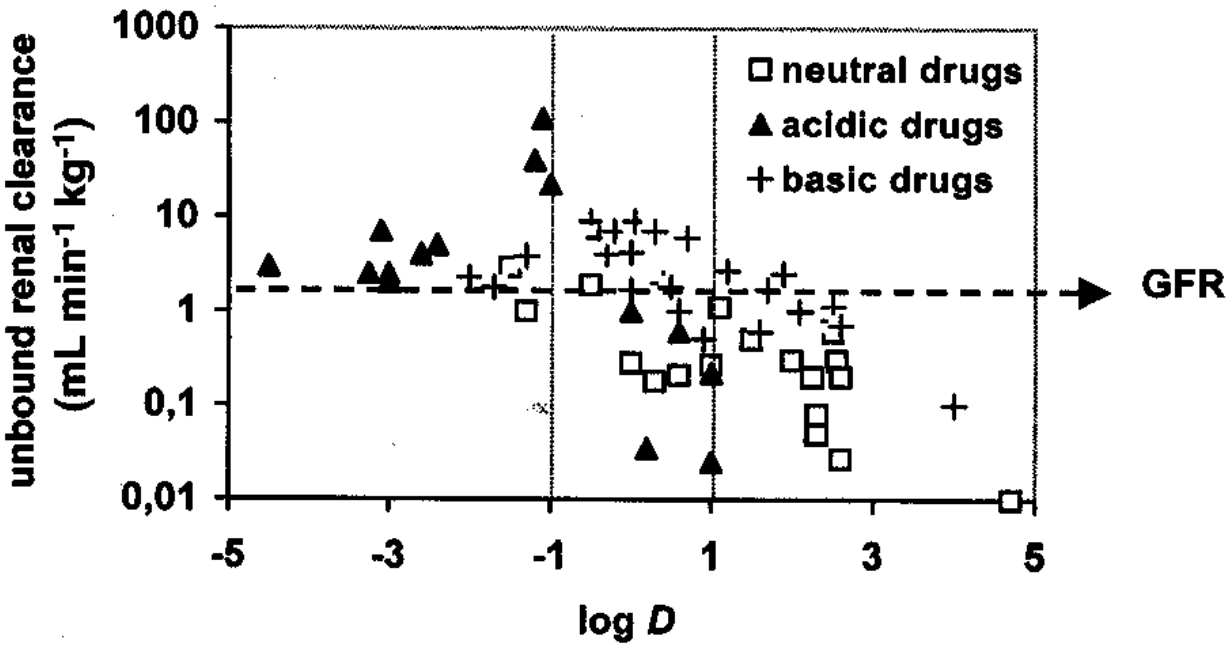
Urine/filtrate flow. The faster the flow the lower the opportunity for reabsorption. Urine flow can be increased by consumption of large amounts of water and/or diuretics such as caffeine.



GFR glomerular filtration rate 120 mL/min in a young male

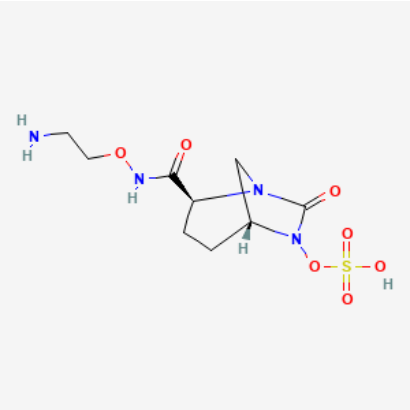
Basic Pharmacokinetics and Pharmacodynamics, 2nd ed. S. Rosenbaum Ed., Wiley, NY 2017.
Guyton and Hall Textbook of medical physiology, Unit V, 14th Ed. J. E. Hall and M. E. Hall, Elsevier Philadelphia, 2021

Renal excretion

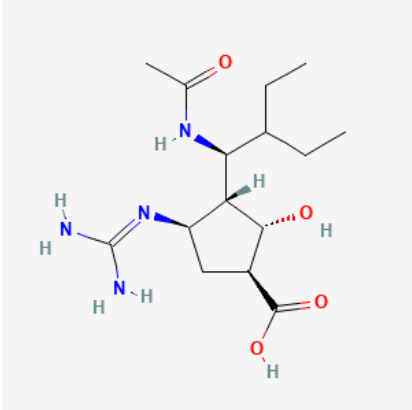


- As lipophilicity increases renal clearance decreases

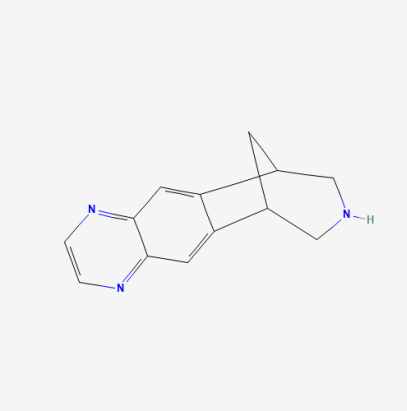
- Acidic compounds most sensitive to increase in LogD (LogD = -1)
- Neutral compounds moderately sensitive to LogD (LogD = 0)
- Basic compounds least sensitive to LogD (logD = +1)



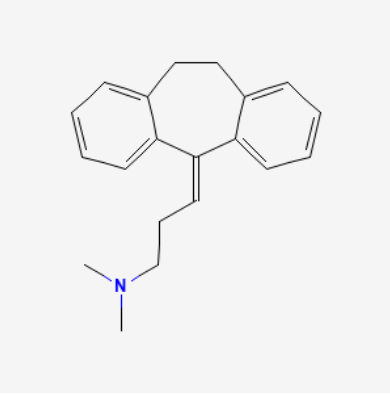
Nacubactam
88-95% excreted
MoKa v.5 $logD_{7.4}$ = - 5.4



Peramivir
90% excreted
MoKa v.5 $logD_{7.4}$ = - 2.2



Varenicline
92% excreted
MoKa v.5 $logD_{7.4}$ = - 0.5



Amitriptyline
5% excreted
MoKa v.5 $logD_{7.4}$ = 3

Physicochemical property space of hepatobiliary transport

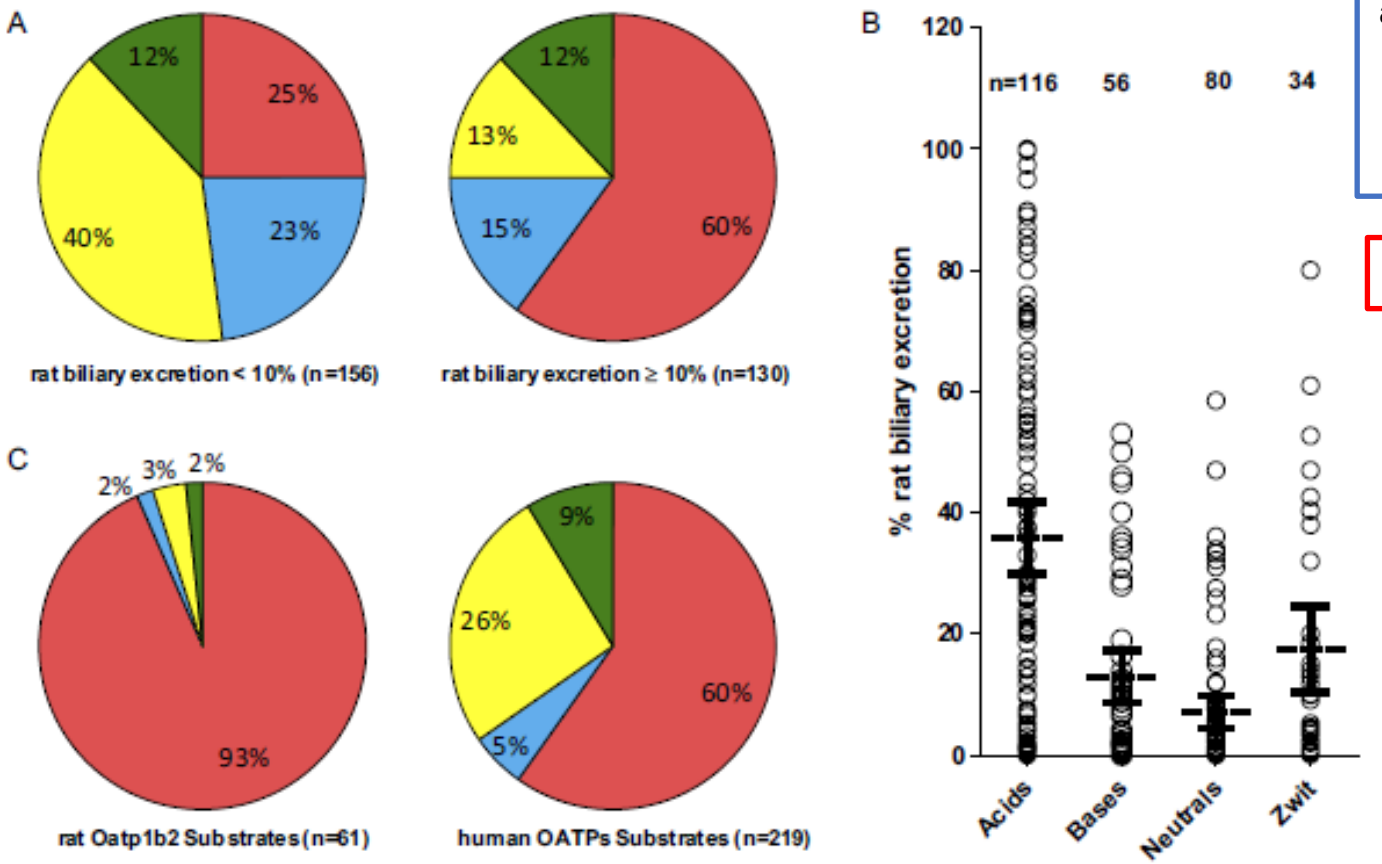


FIG. 1. A, ionization state distribution of compounds showing BE of <10% and ≥10% in BDC rat model. B, ionization state versus rat BE for 286 compounds. Mean ± 95% CI is depicted for each bin. C, ionization state distribution of rat Oatp1b2 and human OATP transporters (hOATP1B1, hOATP1B3, hOATP2B1) substrates. Red, anions; blue, cations; yellow, neutrals; green, zwitterions.

Physicochemical Property Space of Hepatobiliary Transport and Computational Models for Predicting Rat Biliary Excretion

Manthena V. S. Varma, George Chang, Yurong Lai, Bo Feng, Ayman F. El-Kattan, John Litchfield, and Theunis C. Goosen

Drug Metab Dispos **2012**, *40*, 1527-1537

Only 27% of compounds with BE > 36 % are non-acids

Examples

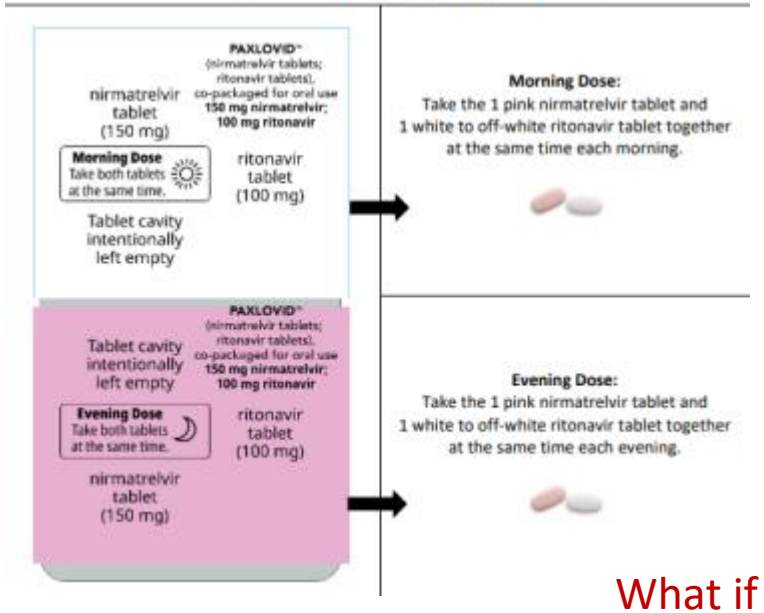
Clazosentan
Irbesartan
Linerixibat
Susalimod
Telmisartan
Tezosentan
Valsartan
(all acidic compounds)

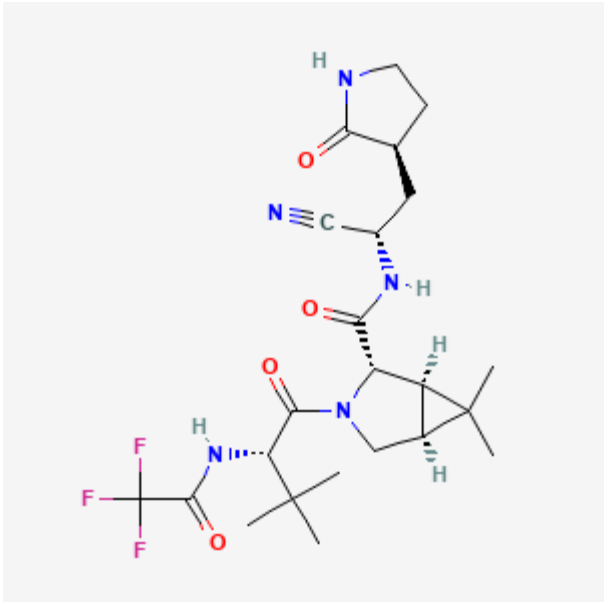
Let us look at a real life case…of a “benign” DDI

Figure B

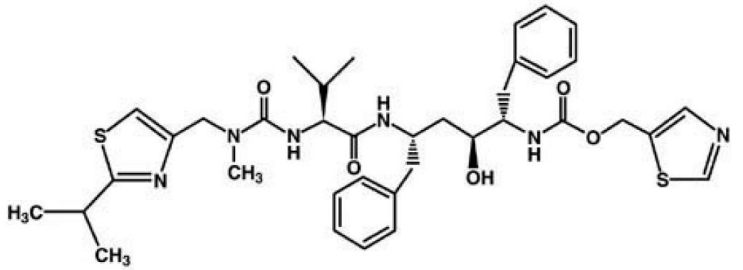
If you have a PAXLOVID 150 mg; 100 mg **Dose Pack**: each dose contains 2 tablets.

How to take PAXLOVID 150 mg; 100 mg Dose Pack





CYP3A4 Substrate



CYP3A4 Inhibitor

What if we took an inducer (e.g. rifampicin) or an inhibitor (e.g. voriconazole) of CYP3A4?

Let us check what we learned (DDI)

If we gave a daily dose of midazolam (f_m for CYP3A4 ~ 0.93, MDZ) to a human on other medications and after a few days MDZ plasma level was significantly lower than day 1 what might that be due to?

If a drug is a substrate (with very high f_m) for aldehyde oxidase would its plasma level be higher or lower in dog than, say, in rat? If the f_m would instead be low what might be the outcome?

Intestinal uptake transporters: if inhibited, may be because of food components, what might be the result in terms of bioavailability?

If a drug is largely metabolized by CYP2D6 (e.g. dextromethorphan) and the subject is a PM what might be the action to be taken on dose? And what if the subject is an EM?

Points to take home

1. CYP450 are the main metabolic “guardians” of the body, but not the sole oxidative transformation enzymes.

2. FMOs, MAO, AOX and XO also concur to the “defense”.

3. Among conjugative enzymes UGTs are the most important but others, such as SULTs, NATs are also involved.

4. There is redundancy among metabolic enzymes: many isoforms can do the same transformations. But **not every ethnic group** has the same enzymatic capabilities. Important for dose adjustment.

5. ECCS: a useful construct, based on physicochemical properties and permability.

6. Transporters have an important role too, whether uptake or efflux.

7. Beware of DDI (inhibition/induction) whether through metabolic enzymes or transporters…study it!

8. Renal excretion important for intact compounds and metabolites alike. Generally more polar compounds are largely excreted unchanged.