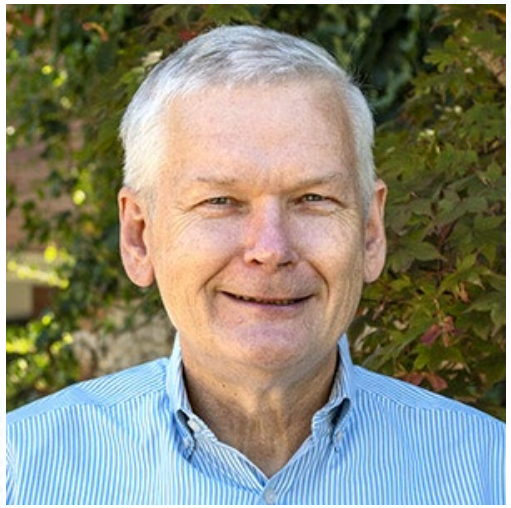
Role of UDP-Glucuronosyltransferases in Drug Metabolism and Drug-Drug Interactions



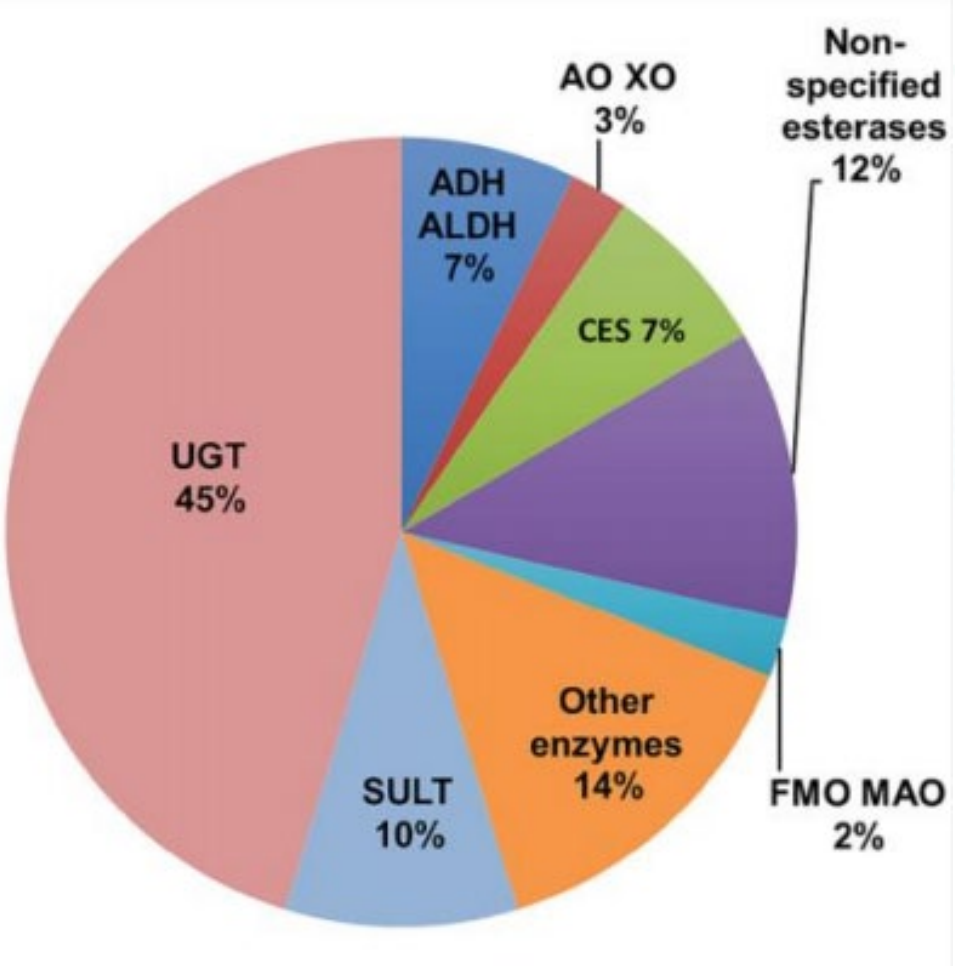
Maciej Czerwinski, Ph.D.
Director of Scientific Consulting

Outline

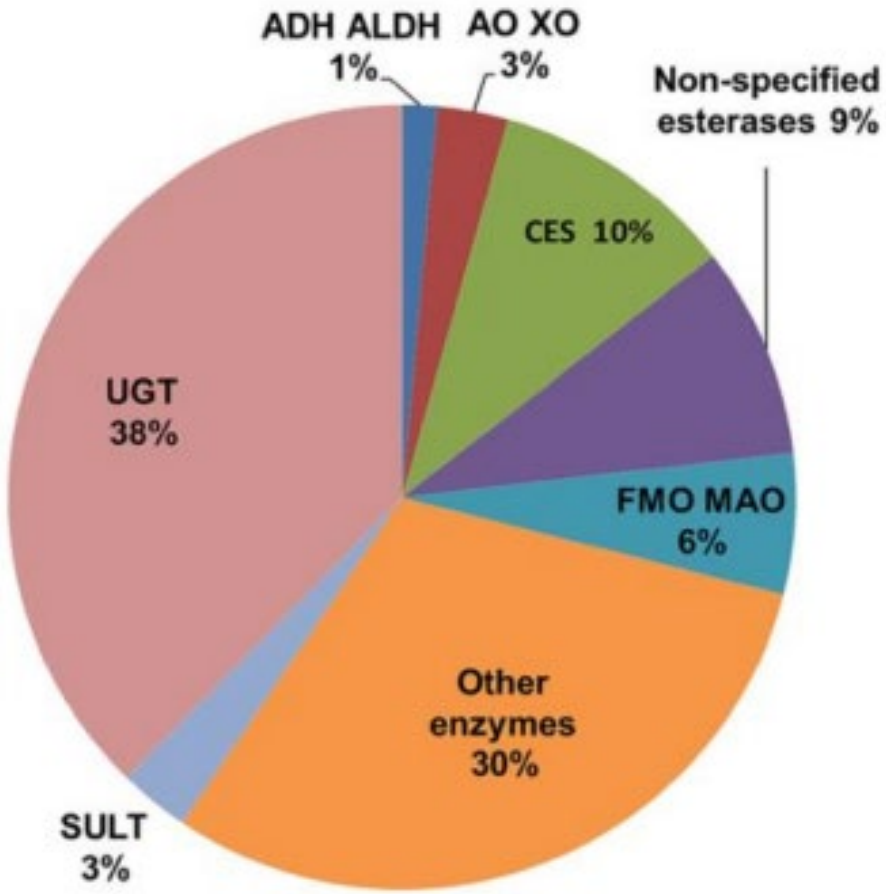
- Introduction to UDP-glucuronosyltransferases
- Examples of reaction catalyzed by UGT
- Cases - Gemfibrozil, Irinotecan
- Highlights of important properties of the UGT enzymes and their products

Glucuronides as a major metabolites of drugs

Most prescribed drugs

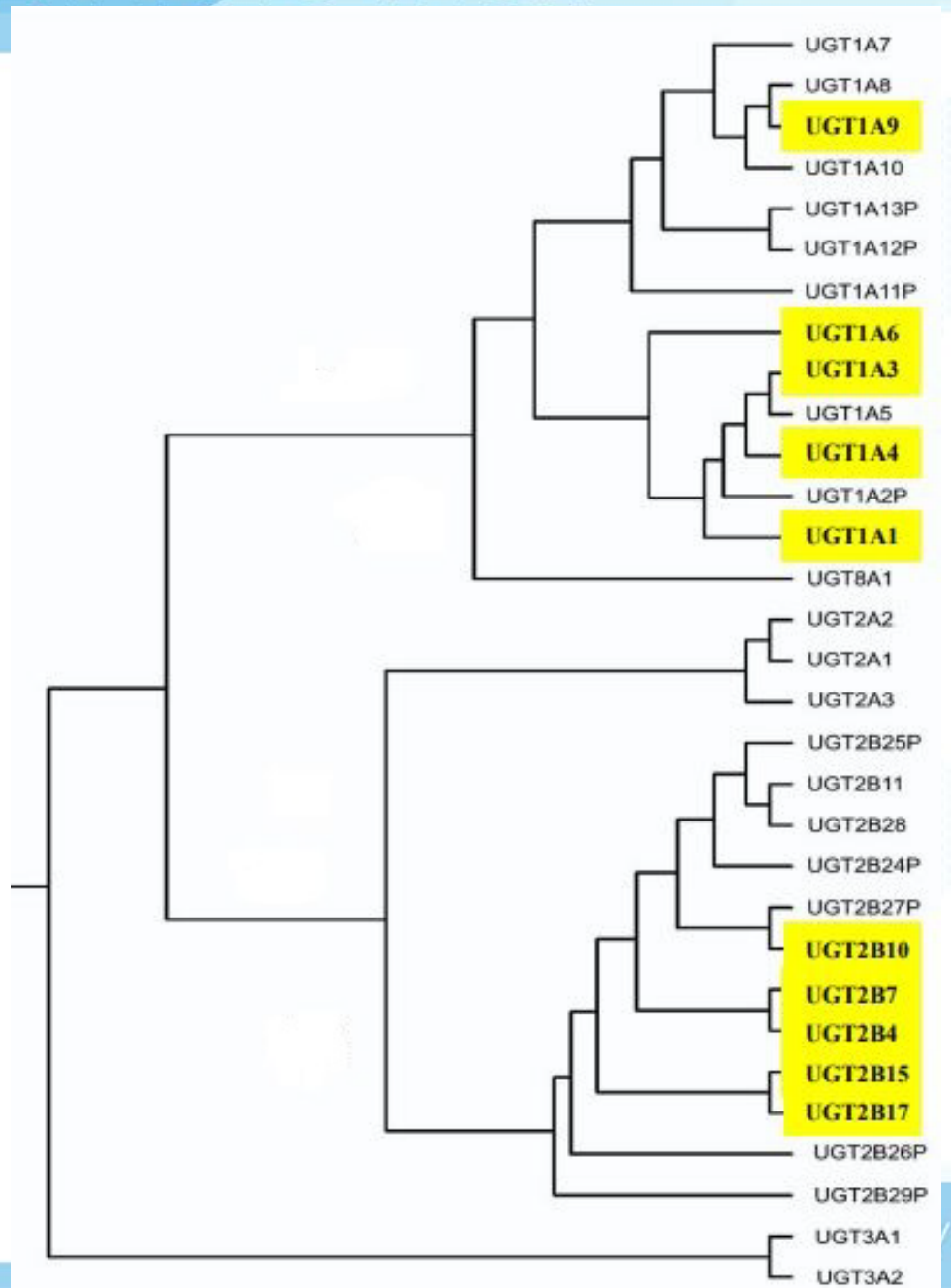


FDA approved drugs 2005 - 2016



Saravanakumar A et al. Clin Pharmacokin. 2019; 58:1281-94

UGTs superfamily



1A, 2A and 2B family enzymes mainly utilize UDPGA as a cofactor (UDP-glucuronosyltransferases);

Hepatic enzymes involved in drug metabolism: 1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7, 2B10, 2B15, 2B17;

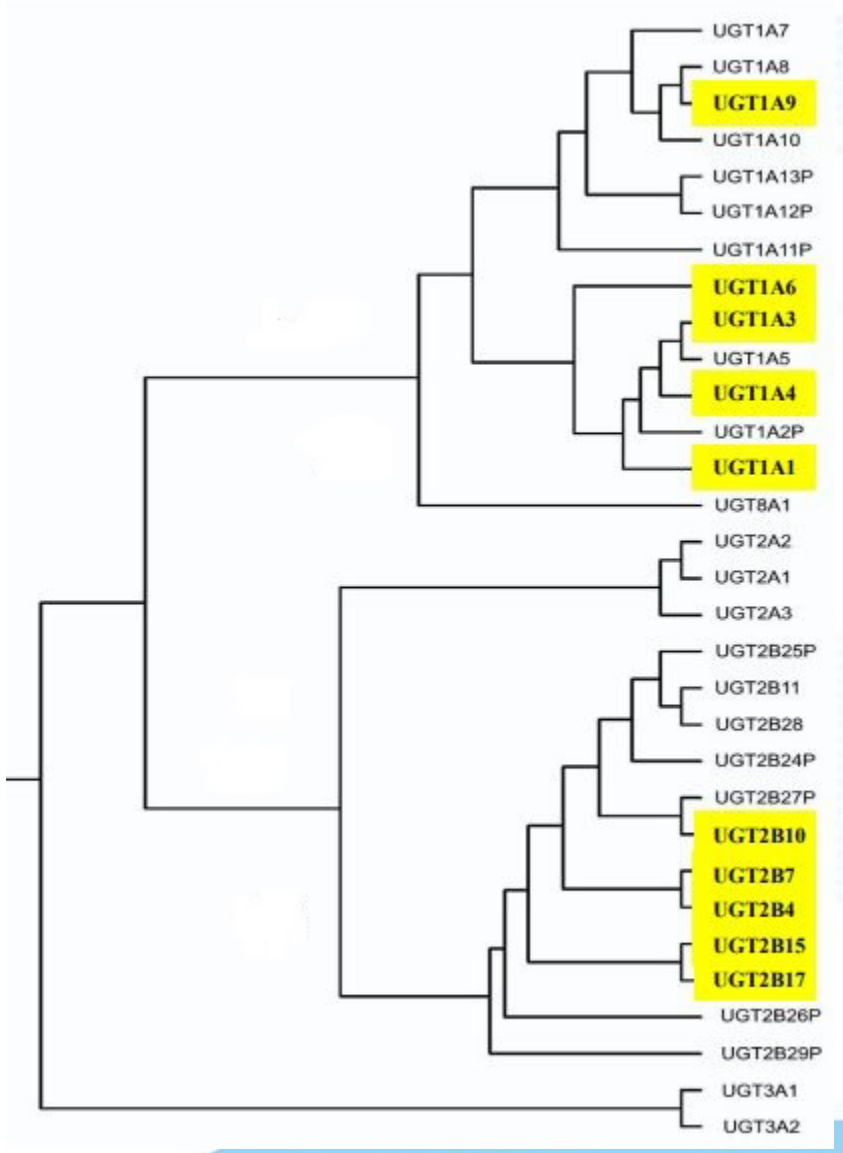
Intestinal enzymes: 1A1, 1A3, 1A6, 2B7, 2B17;

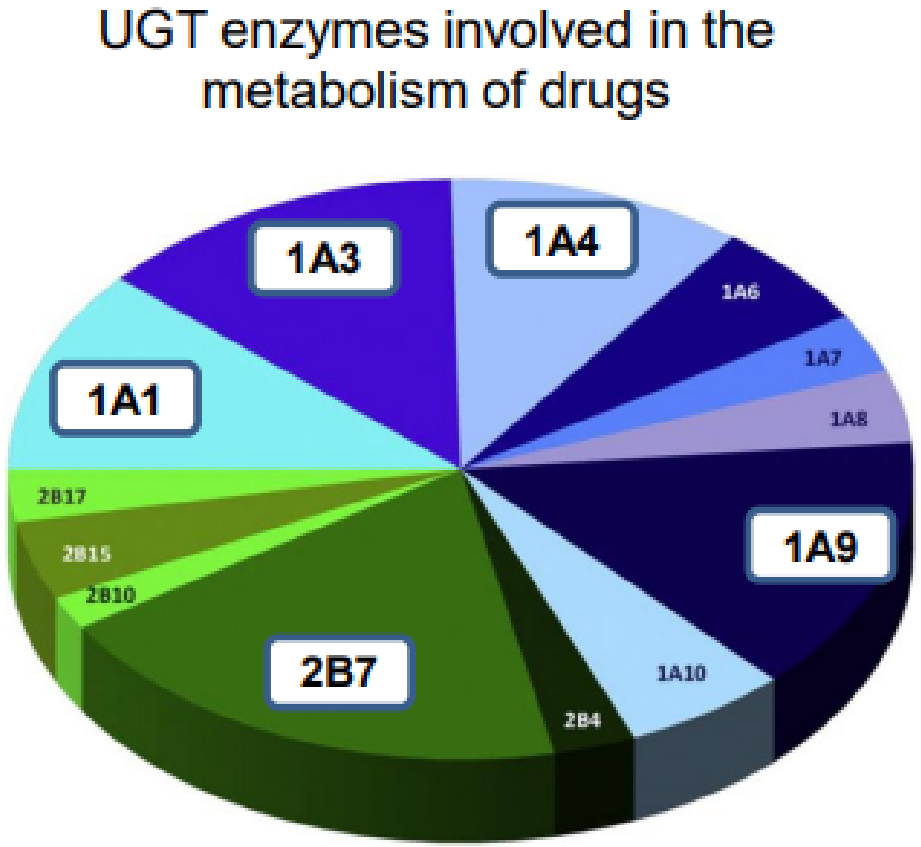
UGTs 1A7, 1A8 and 1A10 are also expressed in the gastro-intestinal tract, but their contribution to drug metabolism is largely unknown;

Renal enzymes: 1A9>2B7>>1A6

In vitro assessment of the DDI liability of glucuronidated drugs… John O. Miners, University Adelaide

UGTs superfamily





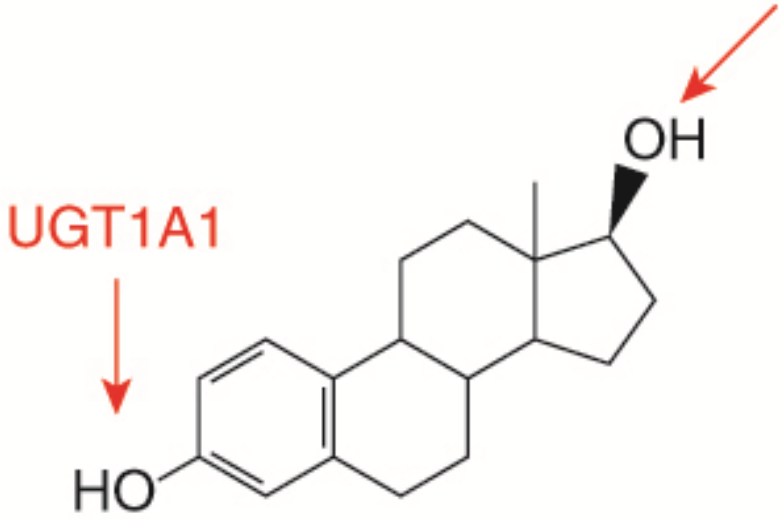
Stingl et al, Pharmacol Ther, 2014, 141;92-116

UGT-Mediated Metabolism

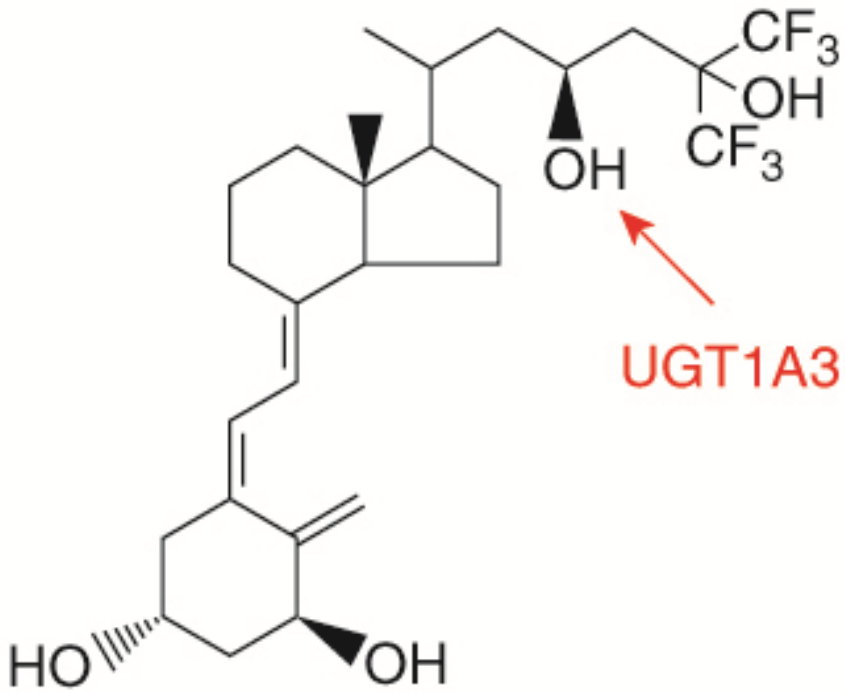
- UGTs are mainly located in the endoplasmic reticulum of liver, kidney, GI tract, lung, prostate, mammary gland, skin, brain, spleen, nasal mucosa
- Endobiotics metabolized by UGT include bilirubin, steroid hormones and thyroid hormones
- Dependence on uridine-5'-diphospho-α-D-glucuronic acid formed by UDP-glucose dehydrogenase. Alternative cofactors include UDP-glucose, UDP-xylose, UDP-galactose.
- Generally, site of glucuronidation is an electron rich (nucleophilic) O, N, or S heteroatom.

O-glucuronides formed by UGT1A

O-Glucuronides (acetals)



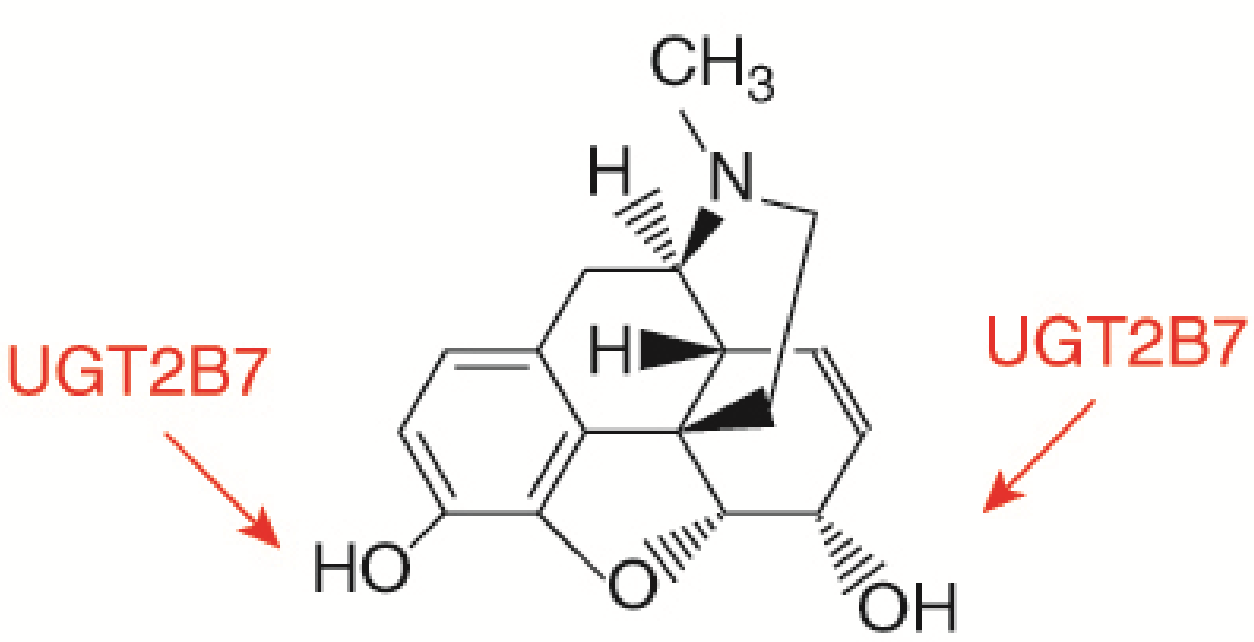
17β-Estradiol



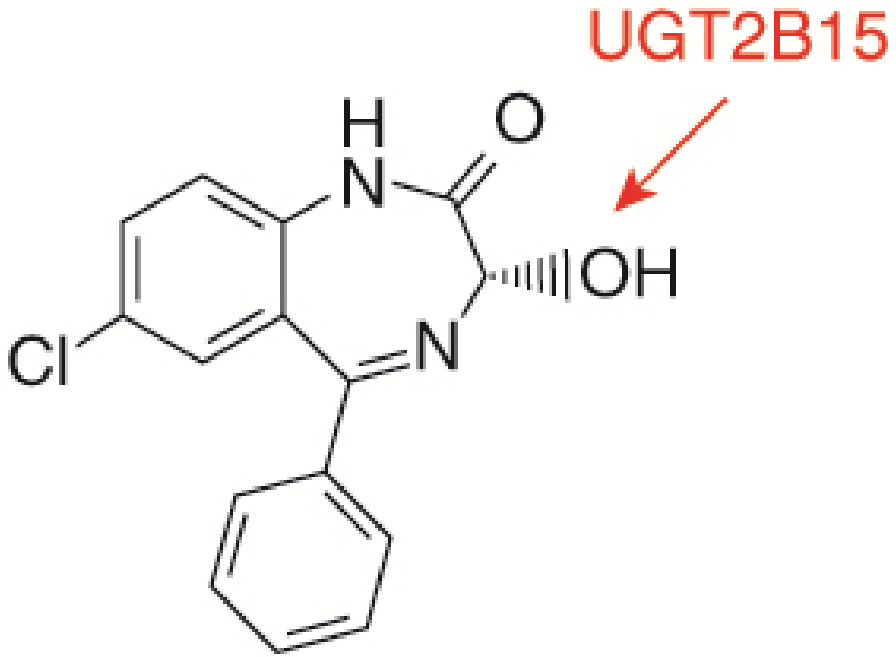
26,26,26,27,27,27,-hexafluoro-
1α,23,25-trihydroxyvitamin D_3

Parkinson et al.; Casarett & Doull's Toxicology: The Basic Science of Poisons, 9th edition; 2018

O-glucuronides formed by UGT2B enzymes



Morphine

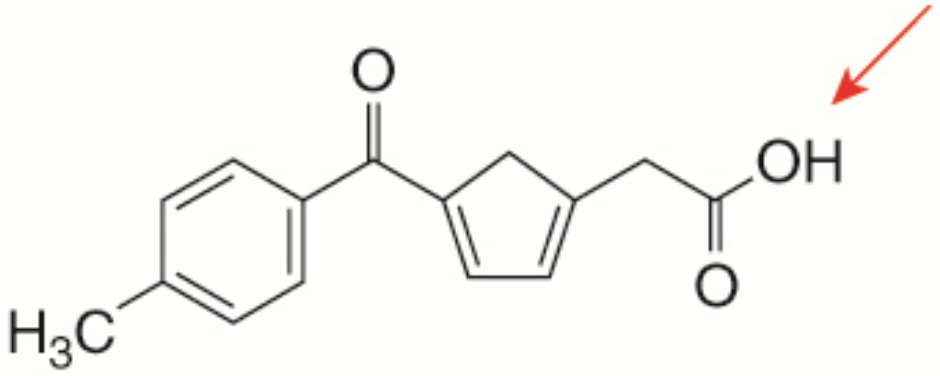


S-Oxazepam

Parkinson et al.; Casarett & Doull's Toxicology: The Basic Science of Poisons, 9th edition; 2018

Acyl-glucuronide

Acyl-glucuronides
O-Glucuronides (esters)

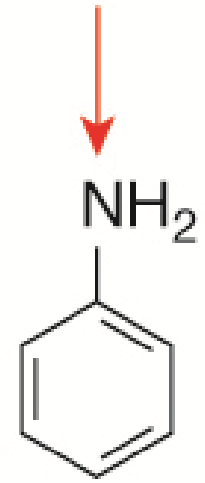


Tolmetin

Parkinson et al.; Casarett & Doull's Toxicology: The Basic Science of Poisons, 9th edition; 2018

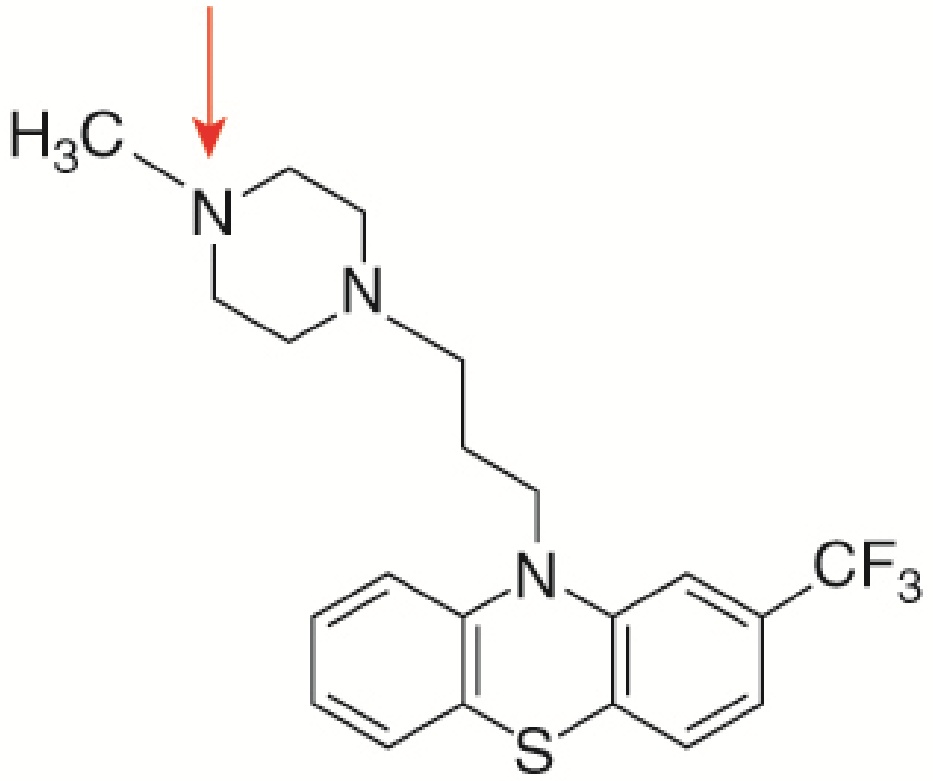
N-glucuronides

N-Glucuronides



Aniline

UGT1A4

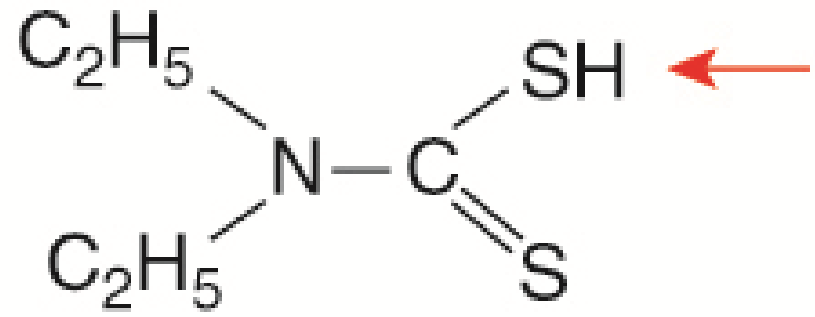


Trifluoperazine

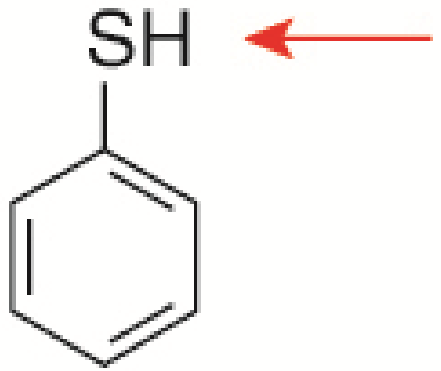
Parkinson et al.; Casarett & Doull's Toxicology: The Basic Science of Poisons, 9th edition; 2018

S-glucuronides

S-Glucuronides



Diethyldithiocarbamate

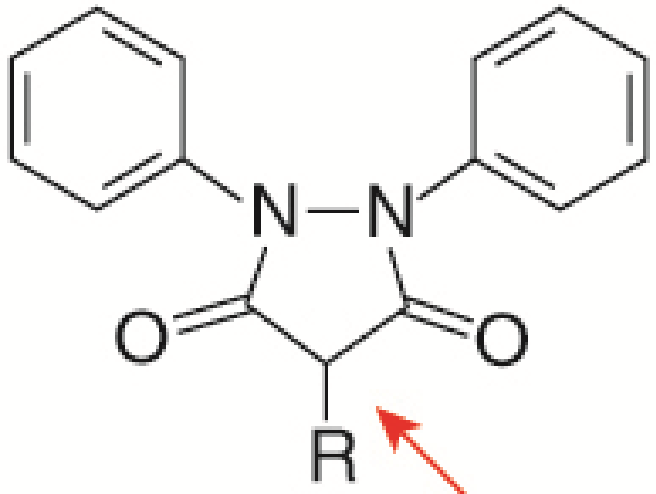


Thiophenol

Parkinson et al.; Casarett & Doull's Toxicology: The Basic Science of Poisons, 9th edition; 2018

C-glucuronides

C-Glucuronides

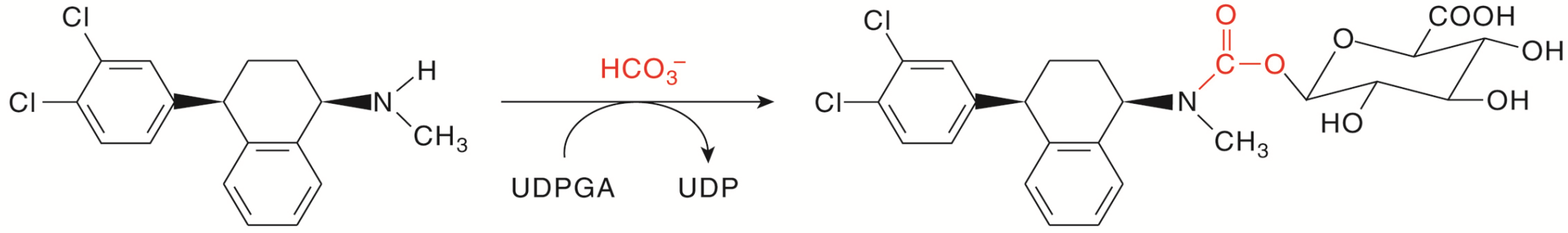


Phenylbutazone $R = C_4H_9$

Sulfinpyrazone $R = (CH_2)_2SOC_6H_5$

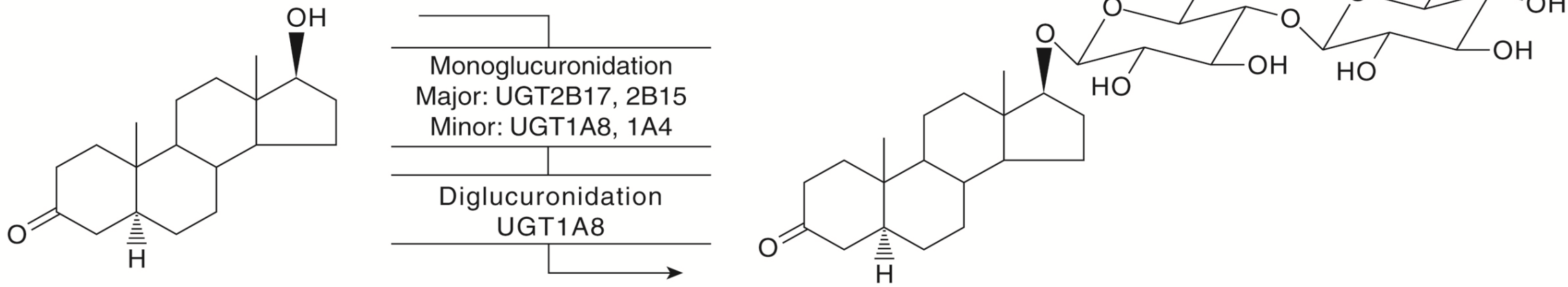
Parkinson et al.; Casarett & Doull's Toxicology: The Basic Science of Poisons, 9th edition; 2018

Less common glucuronide conjugates



Sertraline

Sertraline *N*-carbamoyl glucuronide



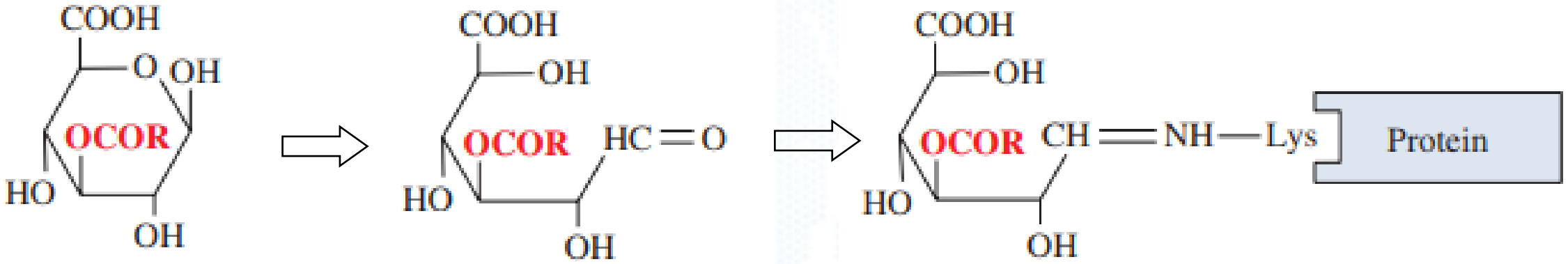
5α-Dihydrotestosterone

5α-Dihydrotestosterone diglucuronide

Parkinson et al.; Casarett & Doull's Toxicology: The Basic Science of Poisons, 9th edition; 2018

Acyl glucuronides can be reactive metabolites

- Due to the phenomenon of acyl migration among carbons of glucuronic acid, acyl glucuronides are subject to ring opening and subsequent covalent binding to cellular proteins

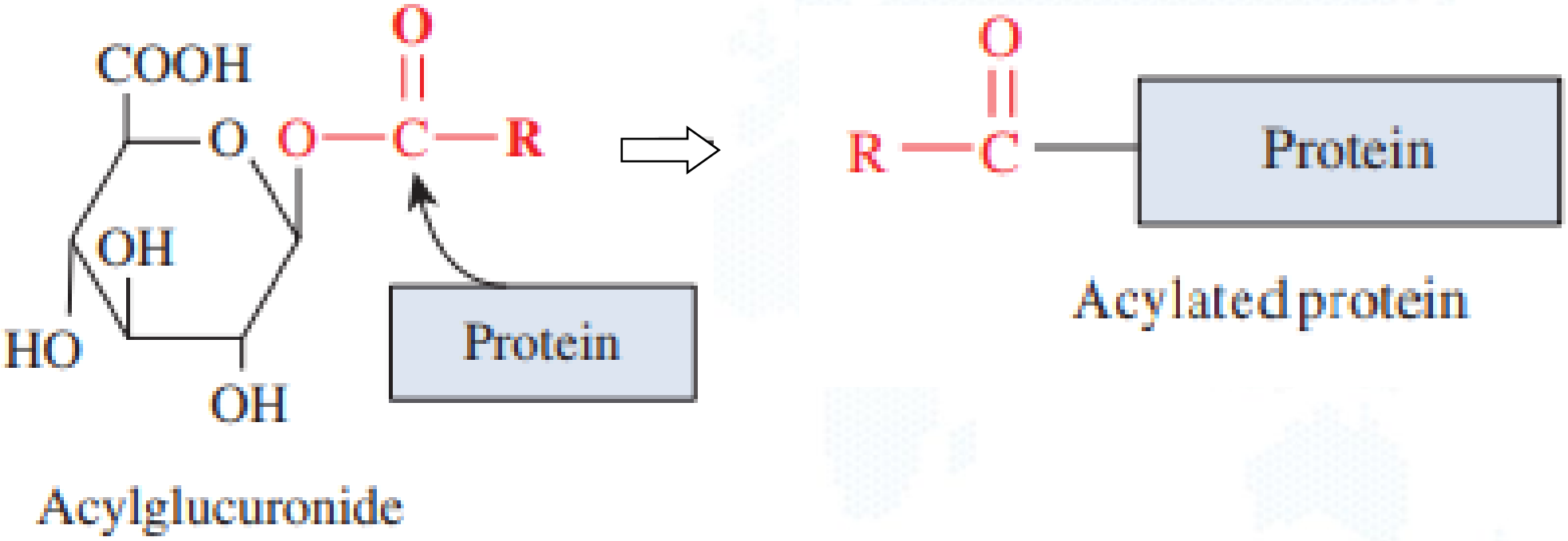


The 2-*O*-β-, 3-*O*-β-, and 4-*O*-β-isomers also form and undergo ring opening and protein binding

Glycated protein containing a rearranged acyl glucuronide

Parkinson et al.; Casarett & Doull's Toxicology: The Basic Science of Poisons, 9th edition; 2018

Acyl glucuronides can be reactive metabolites (2)



Parkinson et al.; Casarett & Doull's Toxicology: The Basic Science of Poisons, 9th edition; 2018
TA Baillie Acyl Glucuronides – Causative Factors in Idiosyncratic Drug Toxicity? ISSX 24th North American Meeting

Approach to acyl glucuronide safety evaluation

The Guidance (Safety Testing of Drug Metabolites, Guidance for Industry, US FDA, 2020) recognized that reactive metabolites can be difficult to detect and measure because of their short half-lives. The Guidance suggests that in some cases, however, they can form stable products (e.g., glutathione conjugates) that can be measured.

"However, if the conjugate forms a potentially toxic compound such as acyl glucuronide, additional safety assessment may be needed."

The additional studies may include characterization of acyl-glucuronide stability and reactivity.

Approach to acyl glucuronide safety evaluation

Hazard mitigation with question-based approach:

* Do metabolites formed indicate activation by CoA or oxidative pathways?
* Is AG formation a major or a minor pathway?
* Are AGs detectable in circulation in human and tox species?
* Does AG show acyl migration in vitro and in vivo?
* What is the in vitro reactivity of AG?

New Perspectives on Drug-Induced Liver Injury Risk Assessment of Acyl Glucuronides. *Chemical research in toxicology* (2020) 33:7 1551-1560

In Vitro Stability of Acyl Glucuronides

The stability of acyl glucuronides is assessed in 100 mM phosphate buffer, pH 7.4, at 37° C

↓

The half-life of the parent acyl glucuronide is determined

↓

If the half-life is less than 0.5 hour, the acyl glucuronide is likely to cause liver toxicity (or possibly other adverse effects)

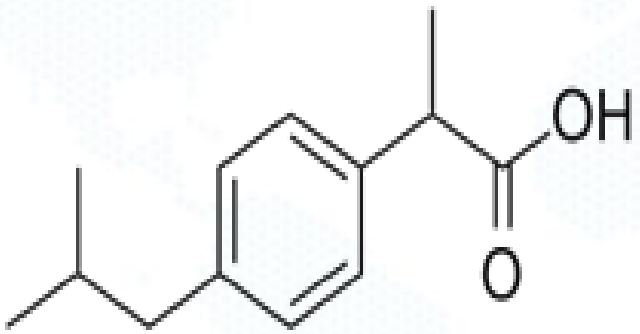
Acidic drug	Half-life of acyl glucuronide (h)
Tolmetin	0.26
Isoxepac	0.29
Probenecid	0.40
Zenarestat	0.42
Zomepirac	0.45
Diclofenac	0.51
Diflunisal	0.67
(R)-Naproxen	0.92
Salicylic acid	1.3
Indomethacin	1.4
(S)-Naproxen	1.8
Ibuprofen	3.3
Bilirubin	4.4
Furosemide	5.3
Flufenamic acid	7.0
Clofibric acid	7.3
Mefenamic acid	16.5
Telmisartan	26
Gemfibrozil	44
Valproic acid	79

Ebner *et al., Drug Metab Dispos* 27: 1143-1149, 1999
Boelsterli *et al., Current Drug Metabolism* 3: 439-50, 2002

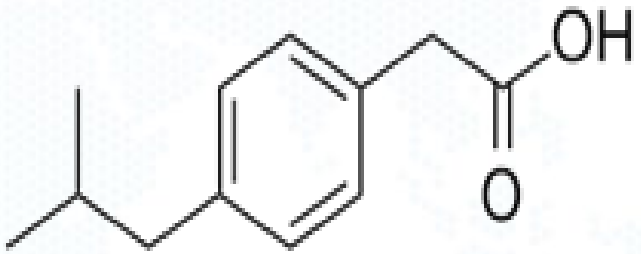
Structural alerts

Structural alerts for the bioactivation to reactive metabolites that cause toxicity and/or CYP inhibition are acetic and propionic acid.

Two nonsteroidal anti-inflammatory drugs with very similar structures are shown below (ibufenac and ibuprofen).



Ibuprofen



Ibufenac

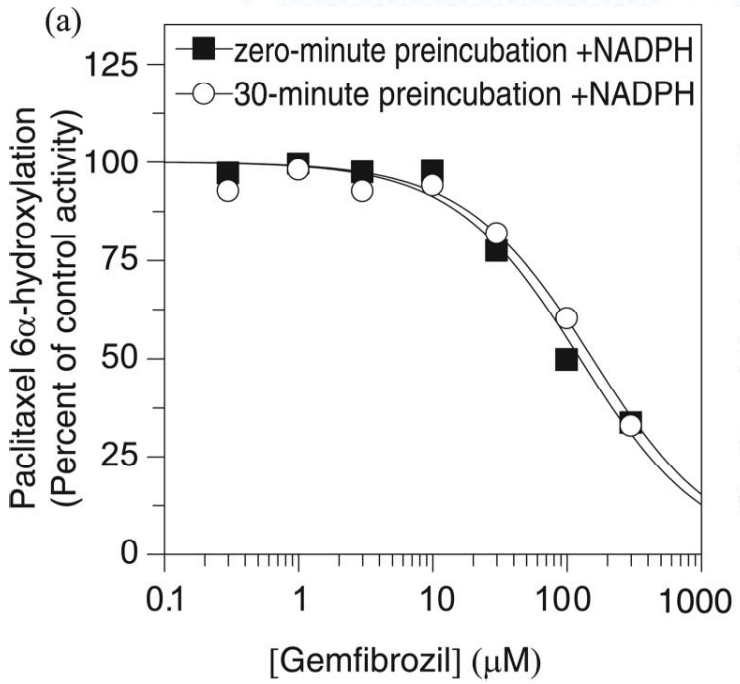
Both drugs produced an acyl glucuronide, but the ibufenac glucuronide was much more reactive due to the small change in chemical structure.

CYP2C8 inhibition by acyl-glucuronides

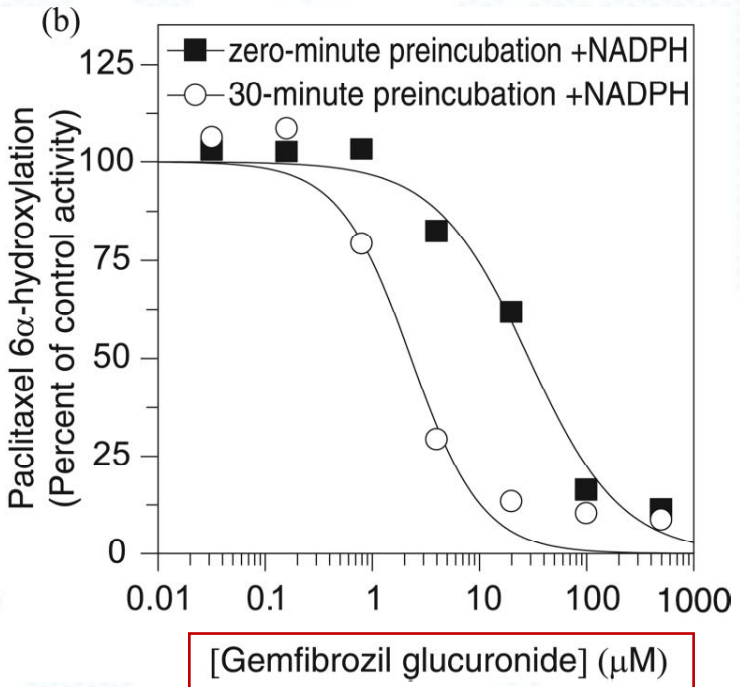
Acyl glucuronides			Major CYP
Gemfibrozil UGT2B7	Metabolism dependent (irreversible)	Acid containing drug	CYP3A4
Clopidogrel UGT2B7, 2B17, 2B4	Metabolism dependent (irreversible)	Ester (CES1)	CYP1A2, 2C9, 2C19, CYP2B6, 3A4/5
Deleobuvir	Metabolism dependent (irreversible)	Acid containing drug	(reductive metabolism by gut microflora)
Simvastatin, Steviol, Mefenamic acid, Diclofenac, rac-Ketoprofen, Indomethacin, Atorvastatin, Ibuprofen, Naproxen, dihydro-Ketoprofen	Reversible	Acid containing drug	

Expert Opinion on Drug Metabolism & Toxicology, Vol. 13, pp 83-95, 2017 - Issue 1
Drug Metabolism and Disposition February 2018, 46 (2) 141-150
Drug Metabolism and Disposition December 2011, 39 (12) 2421-2430

Gemfibrozil acyl glucuronide is MDI of CYP2C8



0 min IC_{50}	30 min IC_{50}
120 μM	150 μM



0 min IC_{50}	30 min IC_{50}
24 μM	1.8 μM

Fatal interactions of Gemfibrozil occurred with cerivastatin, which is a CYP2C8 substrate. Cerivastatin, but not Gemfibrozil, was withdrawn in 2001.

Ogilvie et al., *DMD* **34**:191, 2006

UGTs in FDA Guidance on in vitro interaction studies

Is the investigational drug a substrate of metabolizing enzymes?

Phase II enzymes including UDP glucuronosyl transferases and sulfotransferases are to be considered.

General consideration for evaluation of drug candidates UGT victim and perpetrator potential

Are the UGTs main metabolic pathway?

Are one or more UGTs involved? Are they polymorphically expressed?

What is likelihood of co-administration with other UGT inhibitors?

Are glucuronide conjugates pharmacologically active?

Are glucuronide conjugates chemically reactive?

Evaluation of DDIs Mediated by UGTs: Regulatory Perspectives Xinning Yang, Ph.D. ISSX Short Course, July 12, 2021

UGT reaction phenotyping

Stepwise approach

- Initial qualitative screen in the recombinant enzymes (UGT 1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7, 2B10, 2B15 and 2B17);
- Confirmation of enzyme involvement with specific chemical inhibitors, estimation of f_m in vitro;
- Correlation method using a panel of individual HLMs;
- For polymorphically expressed UGTs, variants with low or no activity can be examined.

UGT reaction phenotyping

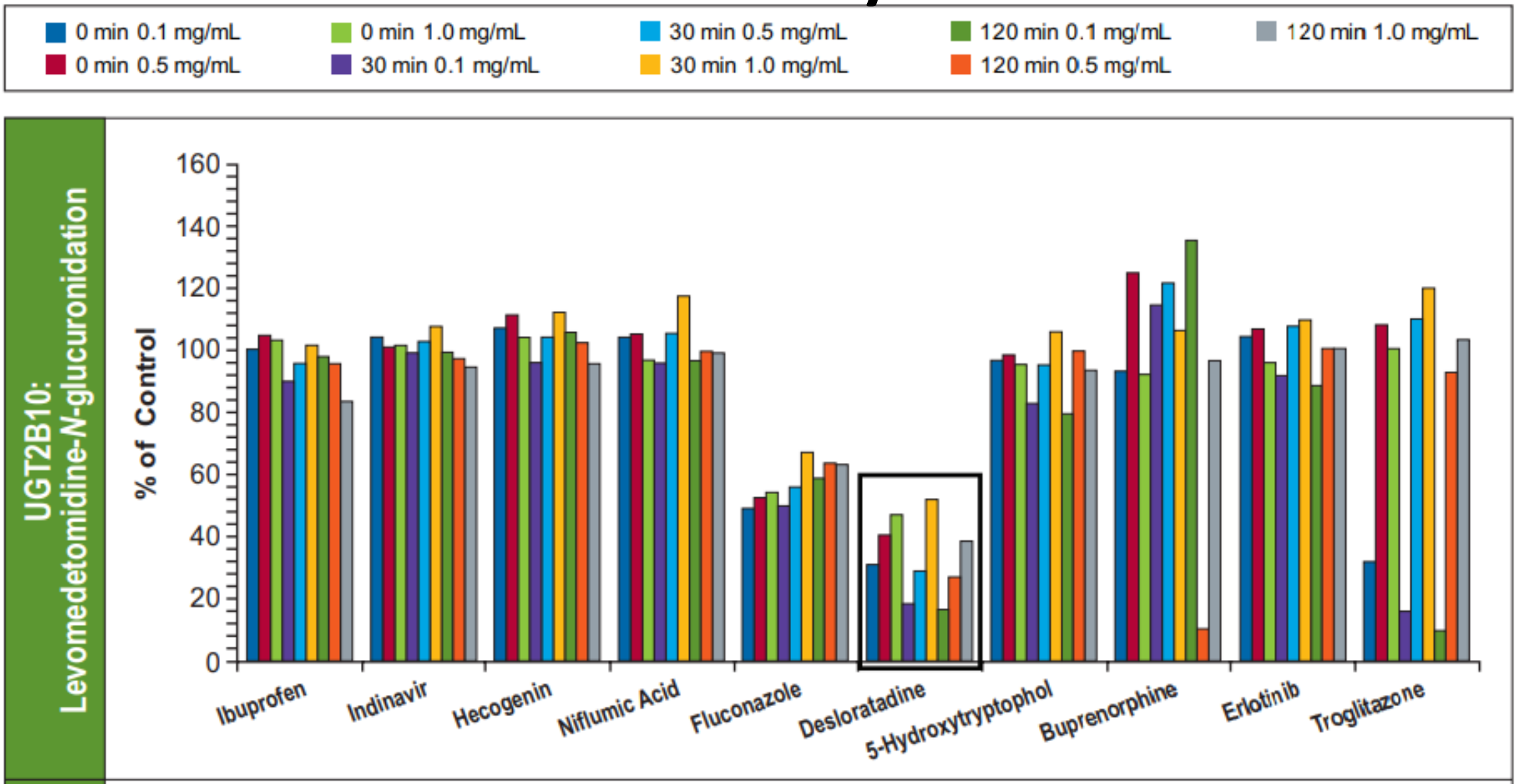
"Evaluation of Chemical Inhibitors for UDP-glucuronosyltransferase (UGT) Reaction Phenotyping Assays in Human Liver Microsomes"

11 inhibitors and 9 UGTs were evaluated using specific substrates,

Selective UGT inhibitors were identified -

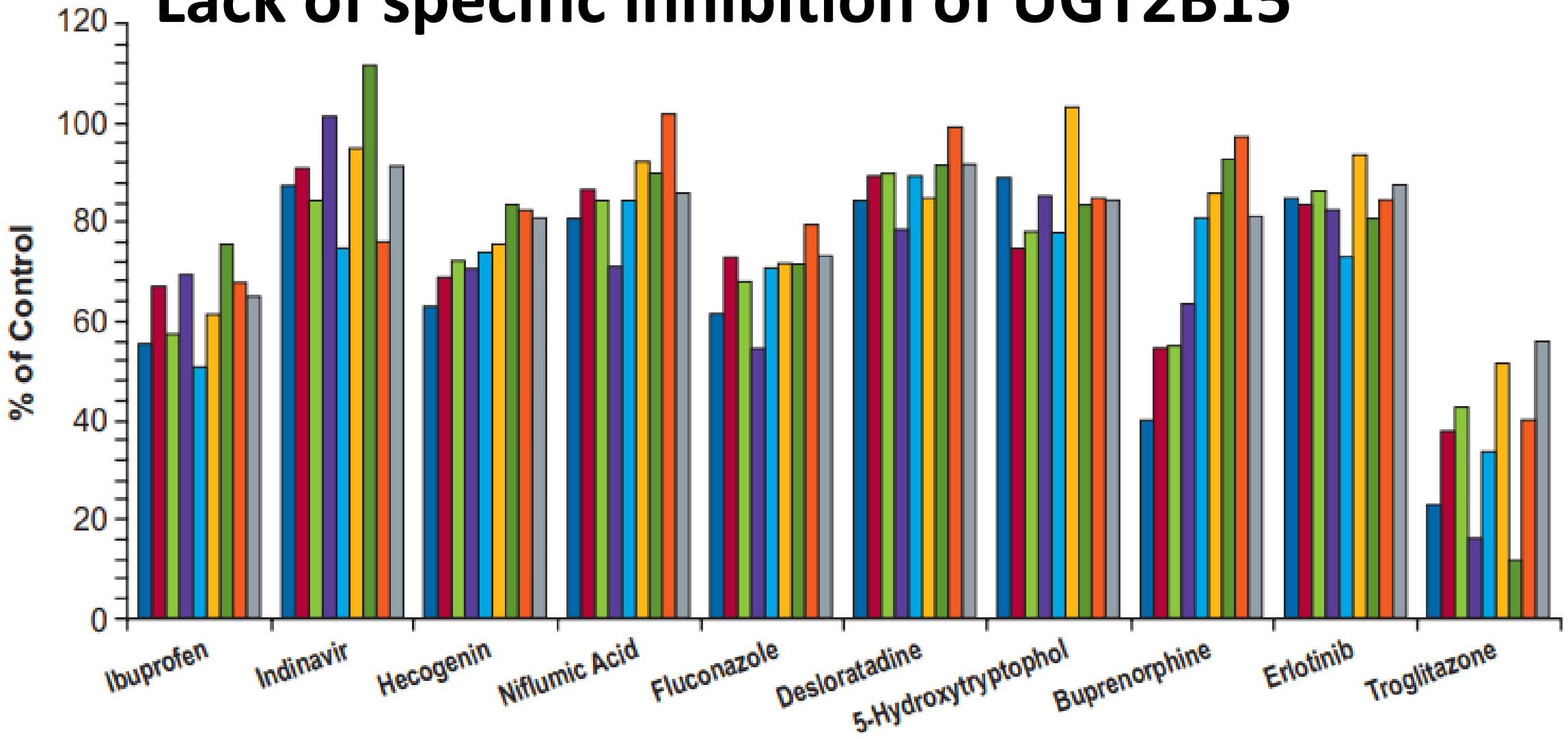
Erlotinib	UGT1A1
Hecogenin	UGT1A4
Nifumic acid	UGT1A9
Desloratadine	UGT2B10

Inhibition of UGT2B10 by Desloratadine



UGT2B15:
S-oxazepam-17-O-glucuronidation

Lack of specific inhibition of UGT2B15



Reaction phenotyping: UGT enzyme selective inhibitors

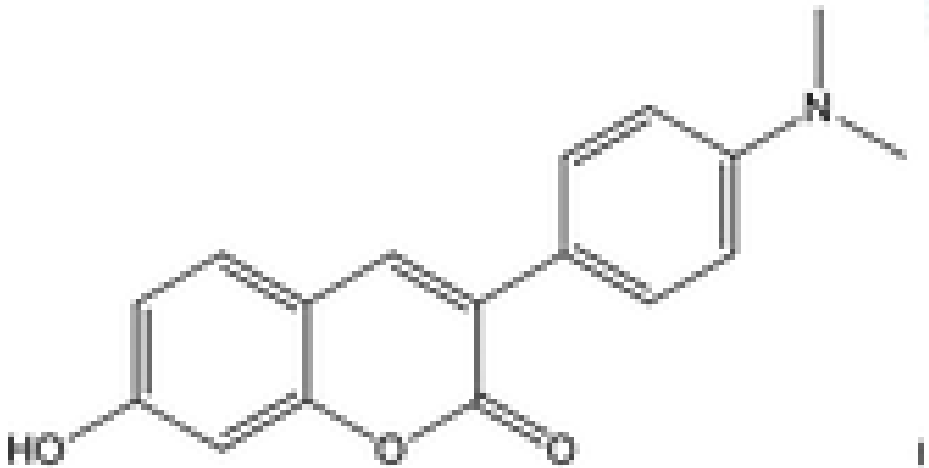
ENZYME	SELECTIVE INHIBITOR(S)	OPTIMAL CONCENTRATION (μM)	OTHER ENZYMES INHIBITED	POTENTIALLY SELECTIVE INHIBITORS
1A1	Nilotinib Regorafenib	2 – 5 (total) 0.25 (unbound)	1A9	Atazanavir, erlotinib, sorafenib
1A4	Hecogenin	10 (total)	1A3	
1A9	Magnolol Niflumic acid	1 (total) 3.5 (total)	1A1	Digoxin, ginsenoside Rc, tranilast
2B7	Fluconazole	2.5 mM (total)	2B4, 2B10	16α- and 16β-Phenyllongifolol
2B10	Desloratidine	10 (total)	2B4, 2B17	Nicotine

Pharmacol. Ther., 218: 107689 (2021)

Reaction phenotyping: UGT enzyme selective substrates

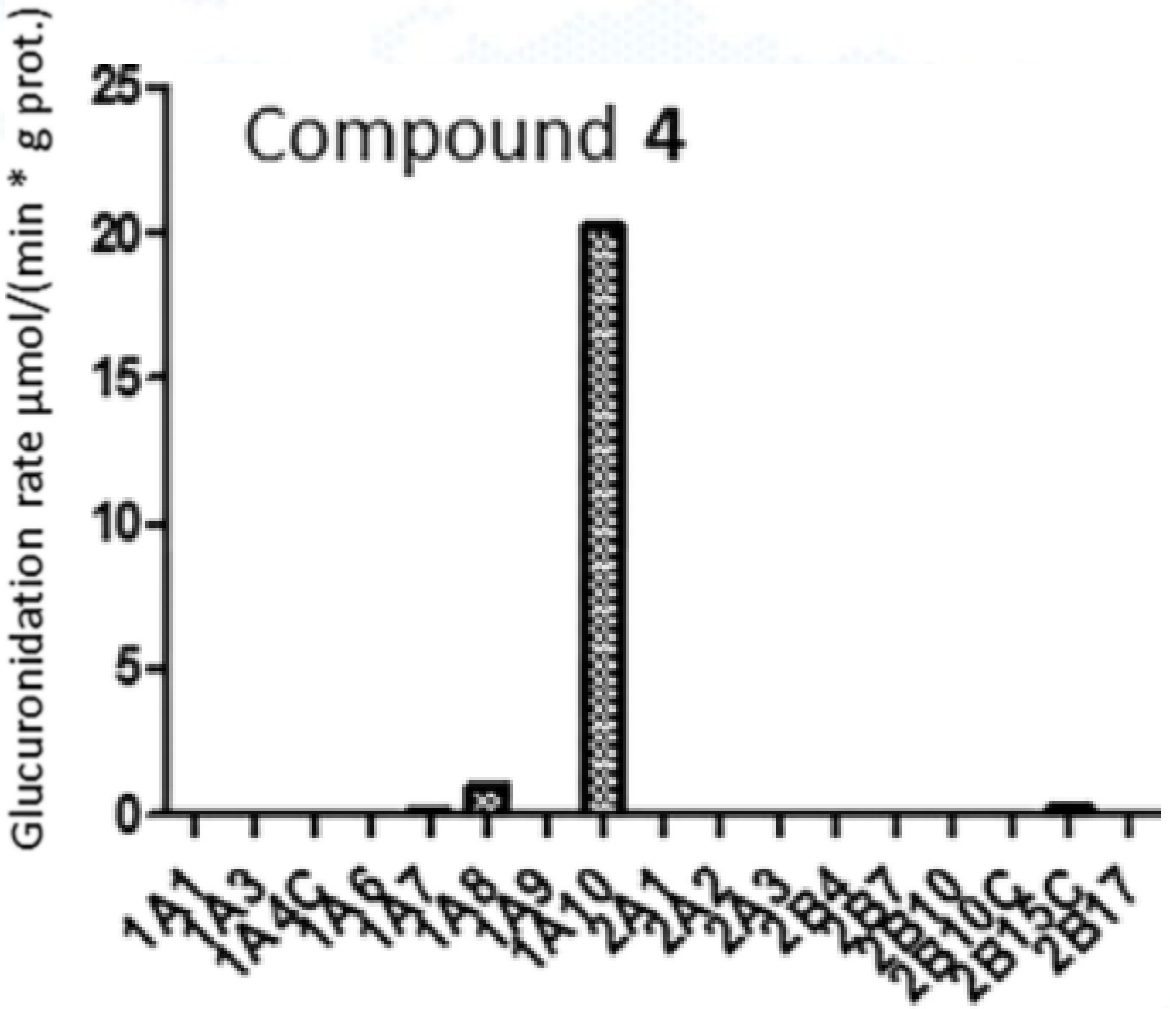
	Selective substrate	Other substrates
UGT1A1	β-estradiol	PF-0640577, 17α-ethinylestradiol, NHPN, SN-38
UGT1A3	chenodeoxycholic acid	telmisartan, fasiglifam, fimasartan, hyodeoxycholic acid, lithocholic acid, montelukast, , ursdeoxycholic acid
UGT1A4	trifluoroperazine	desacetylcinobufagin
UGT1A6	naphthol	5-hydroxytryptophol, serotonin
UGT1A9	propofol	mycophenolic acid, psoralidine
UGT2B7	morphine	zidovudine, chloramphenicol, 6α-hydroxyprogesterone
UGT2B10	levomedetomidine	cotinine, RO-5263397, amitriptyline, chlorcyclizine, cyclizine, levomedetomidine, mirtazapine
UGT2B15	oxazepam	S-lorazepam
UGT2B17	testosterone	MK-7246

Potential UGT1A10-selective substrate



Compound 4

4-(dimethylamino) phenyl



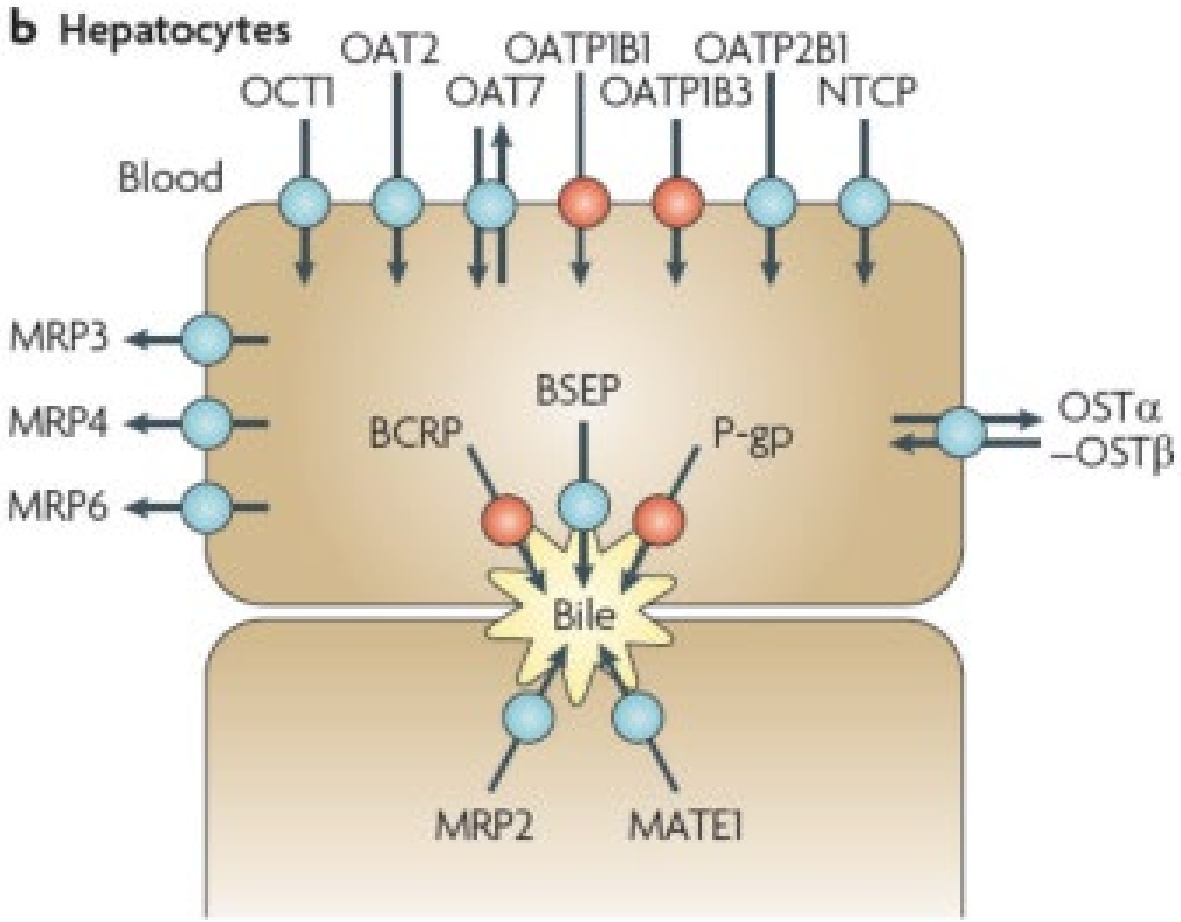
Mol. Pharmaceutics 2018, 15, 3, 923-933

UGT induction

Nuclear receptor	Response element(s)	Receptor activators	Regulated UGTs
AhR	XRE	PAHs, TCDD, β-NF, omeprazole, lansoprazole	1A1, 1A6
CAR	DR-3, DR-4, ER-6	Phenobarbital, phenytoin, carbamazepine, CITCO	1A1
PXR	DR-3, DR-4, ER-6, ER-8	Bile acids, carbamazepine, dexamethasone, hyperforin (SJW), omeprazole, PCBs, phenobarbital, simvastatin, troglitazone	1A1, 1A3, 1A4, 1A6
PPARα	DR-1	Fibrates, WY-14643, perfluorodecanoic acid	1A9, (2B4 in rodents)
Nrf2	ARE	β-NF, oltipraz, acetaminophen	UGTs
FXR	IR-1	Bile acids, GW4064	2B4
HNF-1α			1A6, 1A8 (GI), 1A9, 1A10 (GI)

Parkinson et al.; Casarett & Doull's Toxicology: The Basic Science of Poisons, 9th edition; 2018

Transporters in disposition of glucuronides



Under prediction of UGT contribution to hepatic clearance

Variations in UGT reaction conditions

- Reaction buffers - Tris-HCl, phosphate;
- Co-factor concentration, saturating concentration is recommended;
- Addition of saccharolactone, an inhibitor of β-glucuronidases;
- Addition of $MgCl_2$ to sequester UDP formed as the glucuronidation reaction co-product that is a competitive inhibitor for binding of UDPGA;
- Instability of acyl glucuronides;
- Addition of protein to bind long-chain fatty acids that inhibit UGTs.
- Use of membrane disruptive agents such as alamethicin or detergent CHAPS.

Effects of Alamethicin

UGT Inhibition Studies in the Presence or Absence of Alamethicin: Evaluation of UGT1A1 and UGT2B7 Inhibition in HLM and Recombinant Enzymes

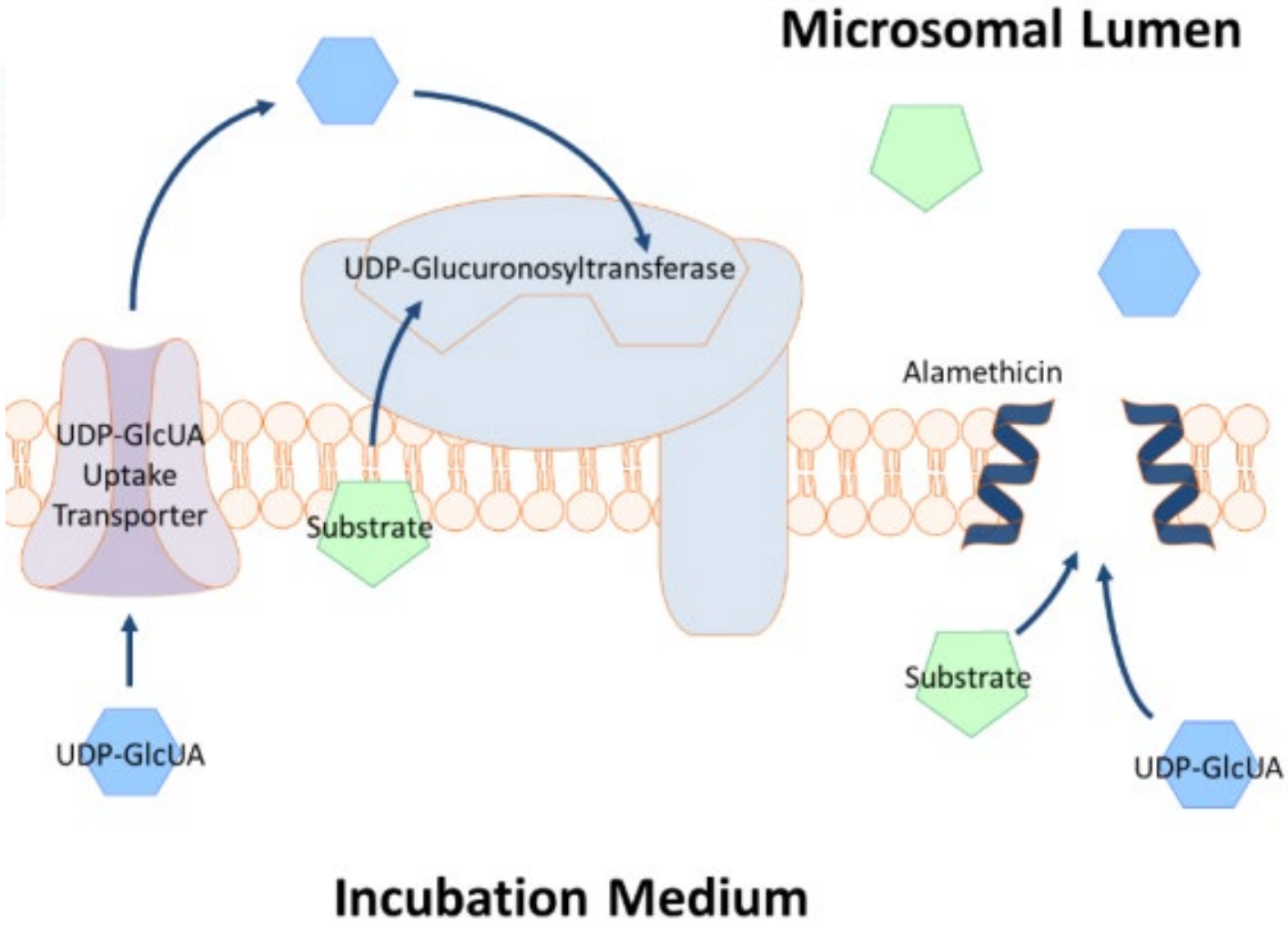
		HLM		rUGT	
	Substrate	**K_m – Ala (μM)**	**K_m + Ala (μM)**	**K_m – Ala (μM)**	**K_m + Ala (μM)**
UGT1A1	Estradiol	19.1 ± 6.5	12.1 ± 0.8	11.8 ± 1.1	11.5 ± 0.5
UGT2B7	Morphine	401 ± 25	384 ± 56	339 ± 37	403 ± 21

Effects of Alamethicin

Inhibition of UGT1A1 (estradiol-3-O-glucuronidation) in HLM and rUGT1A1 in the presence or absence of alamethicin

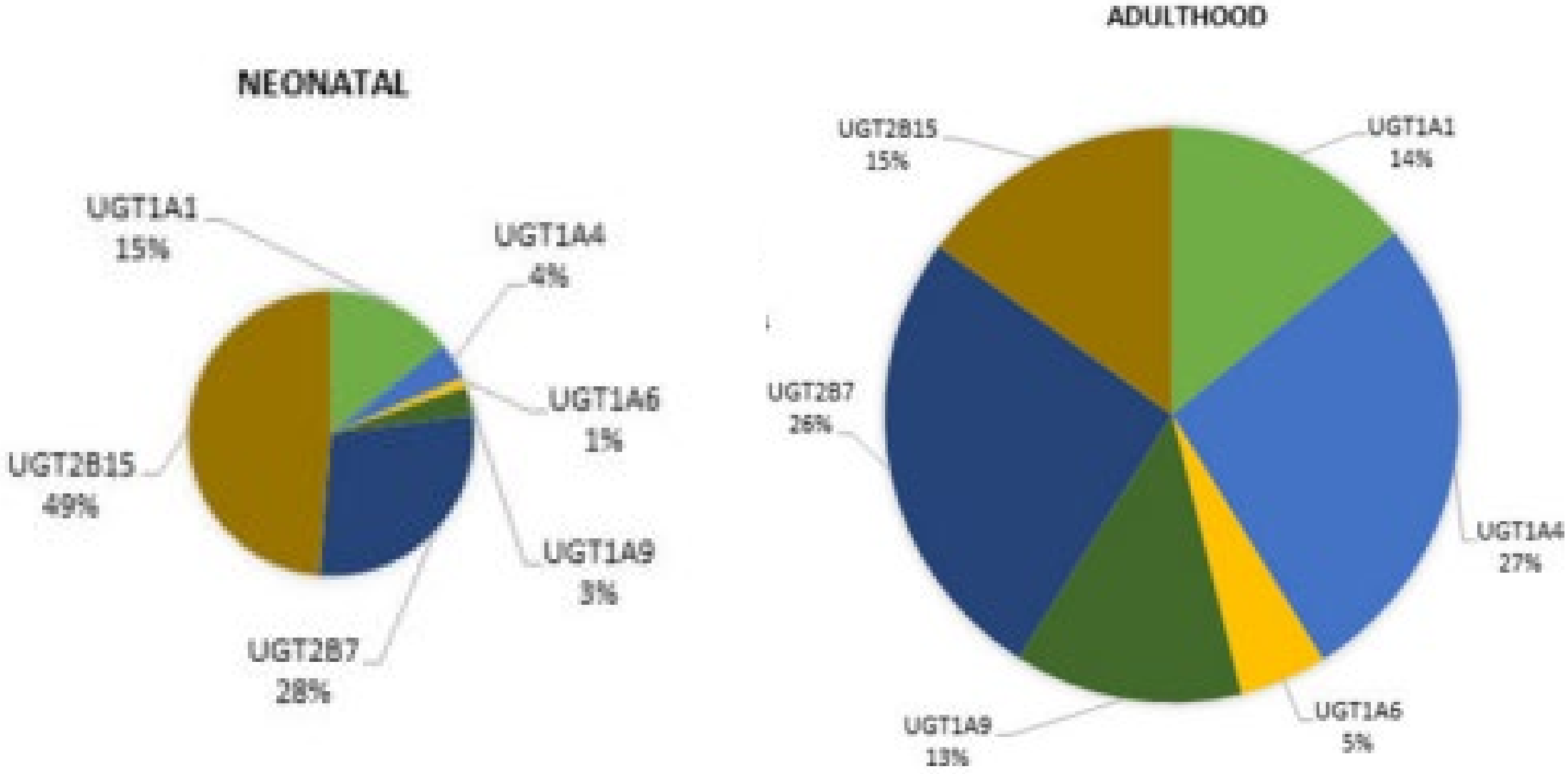
	HLM		rUGT1A1	
Inhibitors	IC_{50} – Ala (μM)	IC_{50} + Ala (μM)	IC_{50} – Ala (μM)	IC_{50} + Ala (μM)
Bilirubin	2.75 ± 0.14	2.70 ± 0.47	2.78 ± 0.23	2.49 ± 0.20
Cyclosporin	43.7 ± 15.4	44.3 ± 11.9	46.4 ± 14.4	55.5 ± 14.7
Ethynylestradiol	45.2 ± 2.9	46.2 ± 3.8	47.9 ± 2.4	46.9 ± 3.0
Erlotinib	3.14 ± 1.58	3.22 ± 0.79	1.40 ± 0.20	1.63 ± 0.22
Itraconazole	10.0 ± 4.0	7.05 ± 2.75	4.46 ± 1.75	3.13 ± 1.14

Effects of Alamethicin



In vitro assessment of the DDI liability of glucuronidated drugs… John O. Miners, University Adelaide

Human UGT ontogeny



Clin Pharmacol Ther. 2019 January ; 105(1): 131–141

Human UGT ontogeny

	Neonatal, %	Infant, %		Age_{50}
1A4	1.8	16	3.6	0.5
2B7	13	41	2.8	2.18
2B15	38.6	60	Not estimated	
1A1	12.2	43	7.5	
1A9	3.0	2.4	8.2	2.18
1A6	2.9	15	10.3	
1A3	Limited age related changes			
2B4	Limited age related changes			
2B10	Limited age related changes			
2B17				17.4

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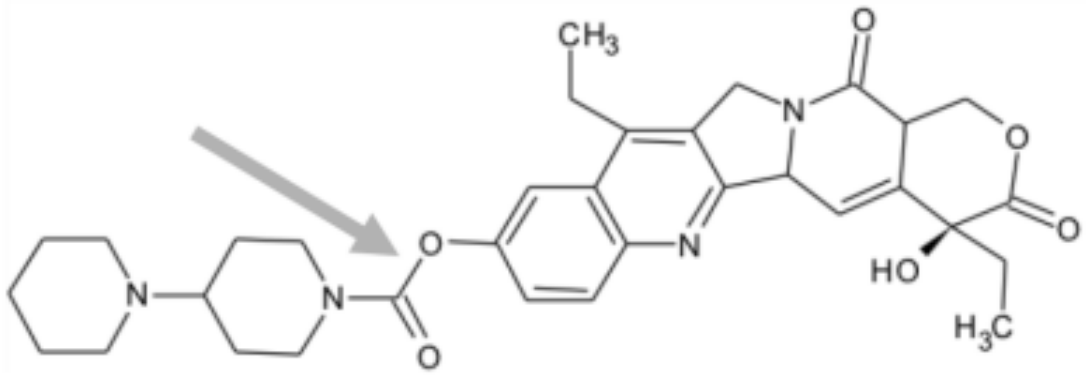
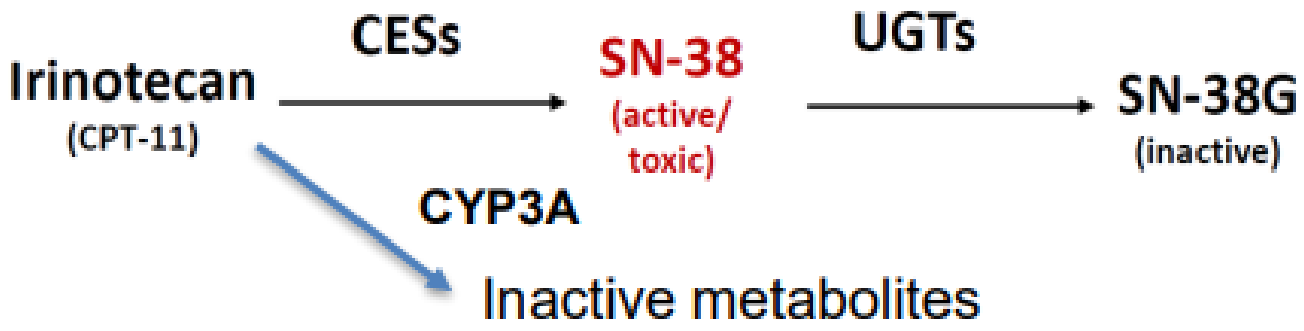
Irinotecan





Hepatic UGT1A1 and UGT1A9 inactivate Irinotecan.
The UGT1A1*28/*28 patients are at higher risk for side effects.

The intestinal bacteria β-glucuronidases de-conjugate SN-38G to SN-38 resulting in entero-hepatic re-circulation of toxic moiety and gastro intestinal side effects of the drug.

Sacituzumab govitecan (Trodelvy), an antibody-drug conjugate of SN-38, is approved for two forms of metastatic cancer with a warning.

UGT genotyped human liver microsomes

- **UGT1A1**
  - High Activity (*1/*1)
  - Moderate Activity (*1/*28)
  - No Activity (*28/*28)
- **UGT1A9**
  - High Activity (*1/*1)
  - Moderate Activity (*1/*3)
  - No Activity (*3/*3)

Dogs are good glucuronidators, but cats are better acetylators than dogs.

Acknowledgement

- I would like to acknowledge all scientists whose data was referenced on the slides used in the presentation.
- I am also happy to acknowledge past and present XenoTech scientist who contributed to this field.

Thank you!

Questions or Comments?