Glucuronidation and Sulfonation

Bill Atkins, H172Q; winky@uw.edu

Glucuronidation

I. UDPGA Metabolism

II. Reactions of UDPGA/glucuronidation

III. UGTs

IV. Reactions of Glucuronides

Sulfonation

I. PAPS Metabolism

II. Sulfonation Reactions

III. PAPS-dependent SULTs

IV. Reactions of Sulfate Conjugates

Suggested Reading

Chemistry and Biodiversity, vol 5: 2171-336 (2008)

Glucuronidation

Guillemette, C. Pharmacogenomics of human UDP glucuronosyltransferase enzymes. Pharmacogenomics J. 2003;3(3):136-58.

Rowland, A., Minors, JO., Mackenzie, PI. (2013) The UDP-glucuronosyl transferases: Their role in drug metabolisma nd detoxication. Int. J. Biochem. Cell Biol.45: 1121-1132.

Wells PG, Mackenzie PI, Chowdhury JR, Guillemette C, Gregory PA, Ishii Y, Hansen AJ, Kessler FK, Kim PM, Chowdhury NR, Ritter JK. Glucuronidation and the UDP-glucuronosyltransferases in health and disease. Drug Metab Dispos. 2004, 32(3):281-90.

Shipkova, M., et. al. Acyl glucuronide drug metabolites; toxicological and analytical implications. Therapeutic Drug Monitor. 2003, 25: 1-16.

Wu B, et al. First pass metabolism via UDP-glucuronosyl transferases:a barrier to oral bioavailability of phenolics, J. Pharm Sci.100: 3655. 2011.

Argikar, UA. Unusual Glucuronides. Drug Metab Disp. 40: 1239. 2012.

Kaivosaari et al. N-Glucuronidation of drugs and other xenobiotics . . . Xenobitoica 41: 652-669, 2011.

Sulfonation

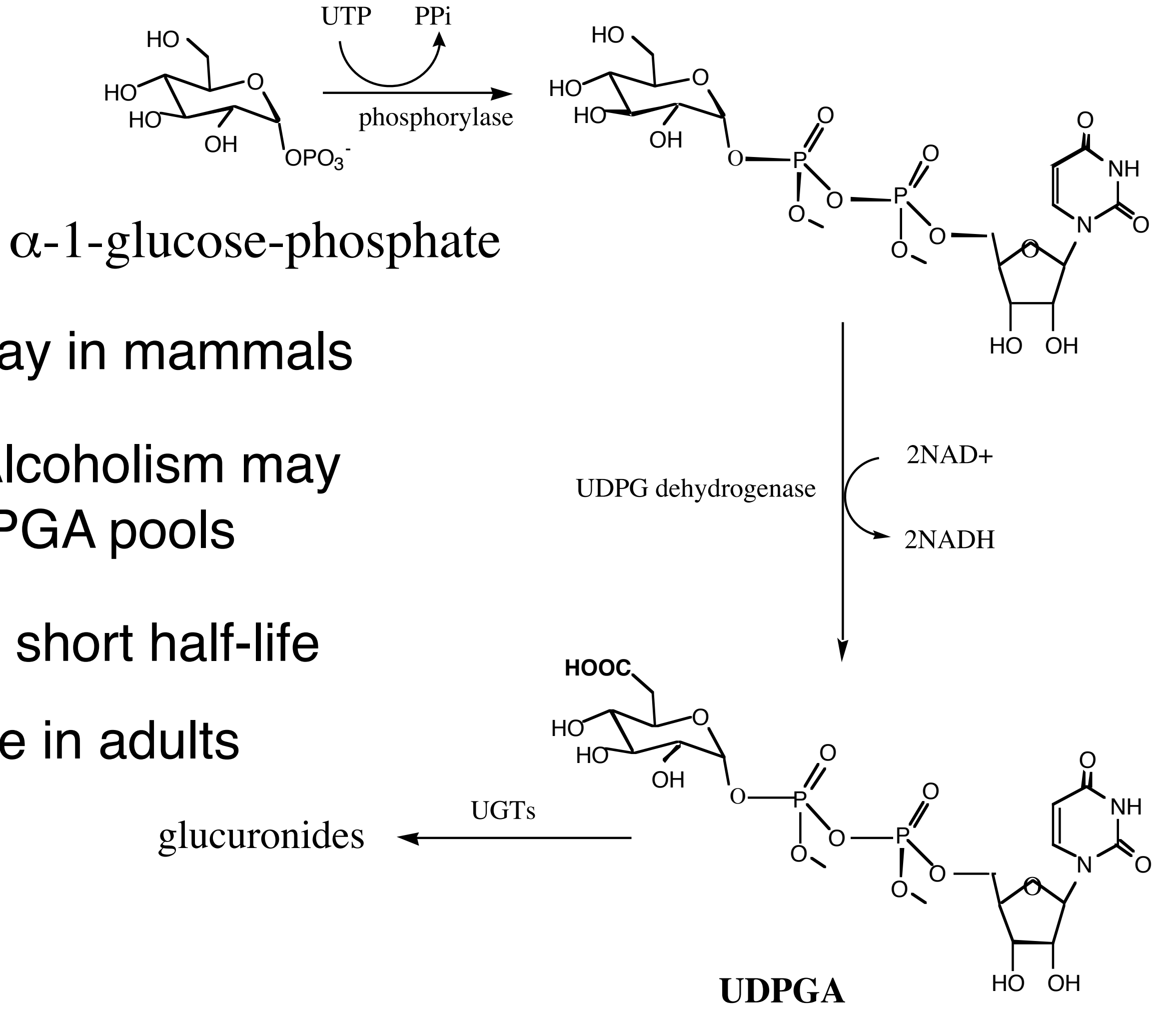
E. Chapman et. al. Sulfotransferases: structure, mechanisms, biological activity, inhibition and synthetic utility. Angewandte Chemie, 2004, 43: 3526-3548.

Glatt H., et. al. Human cytosolic sulphotransferases: genetics, characteristics, toxicological aspects. Mutation Res. 2001: 27-40.

Gamage et al. Human Sulfotransferases and Their Role in Chemical Metabolism. 2006, Toxicol. Sci. 90:5-22.

Nowell and Falany. Pharmacogenetics of human cytosolic sulfotransferases. Oncogene 25:1673 (2006)

I. UDPGA Metabolism: biosynthesis



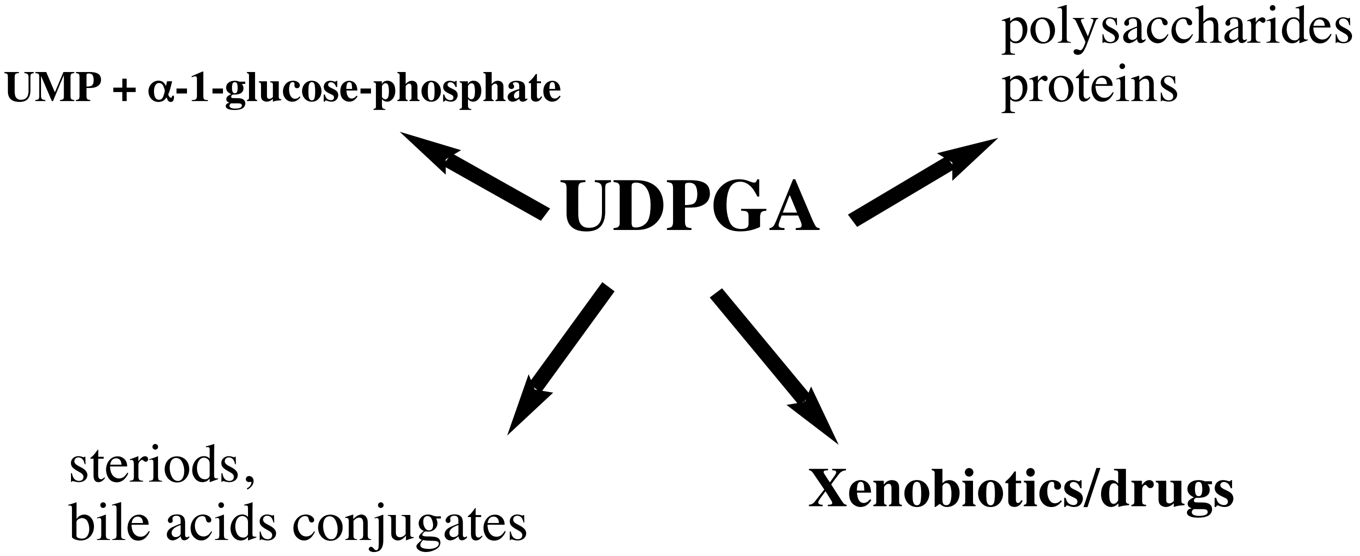
α-1-glucose-phosphate

UDPGA

- Major pathway in mammals
- Starvation/Alcoholism may decrease UDPGA pools
- UDPGA has short half-life

~5 g/day made in adults

I. UDPGA Metabolism: Fate of UDPGA



UMP + α-1-glucose-phosphate

polysaccharides
proteins

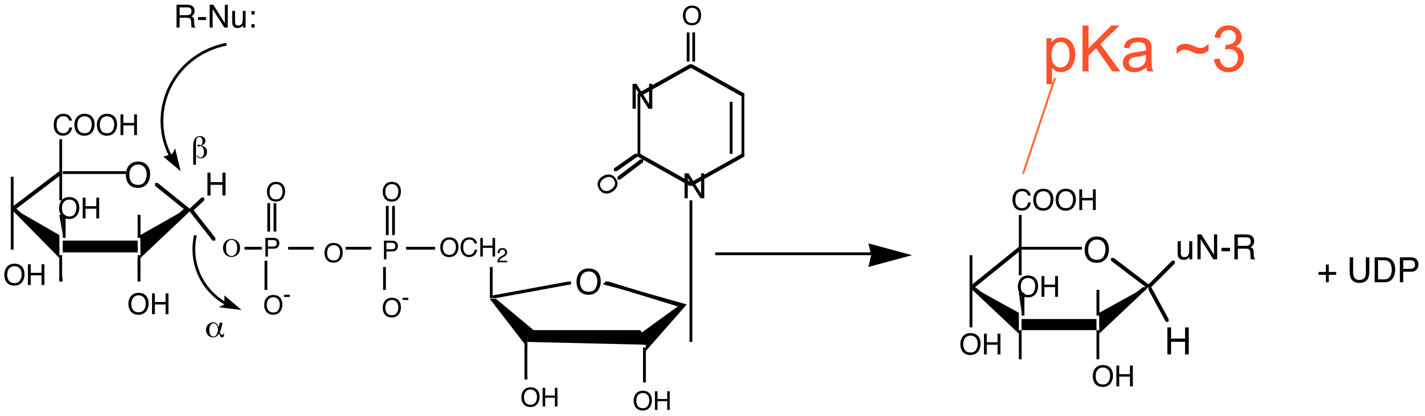
UDPGA

steriods,
bile acids conjugates

Xenobiotics/drugs

Many ‘endogenous’ glucuronide acceptors: e.g. Crigler-Najjar disease, Gilbert’s disease result from insufficient conjugation of bilirubin (UGTA1); impairs biliary excretion, hyperbilirubinemia.

II. Reactions/glucuronidation



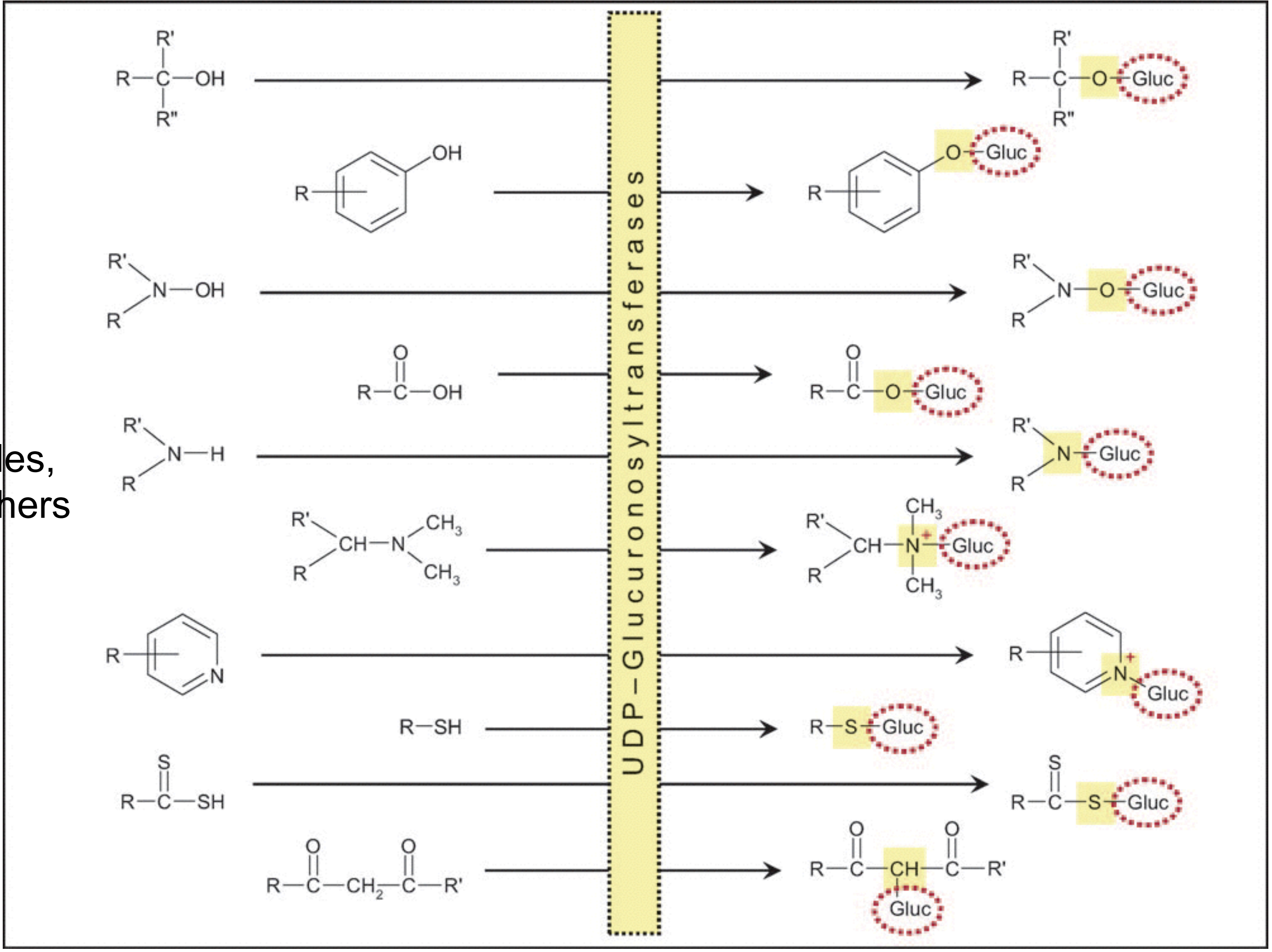
pKa ~3

UDPGA

β-D-glucuronide

Chemical strategy of glucuronidation is to create a good electrophile, by providing a good leaving group, on a hydrophilic co-factor. Nucleophilic drugs react.

II. Glucuronidation: Nucleophilic Acceptors



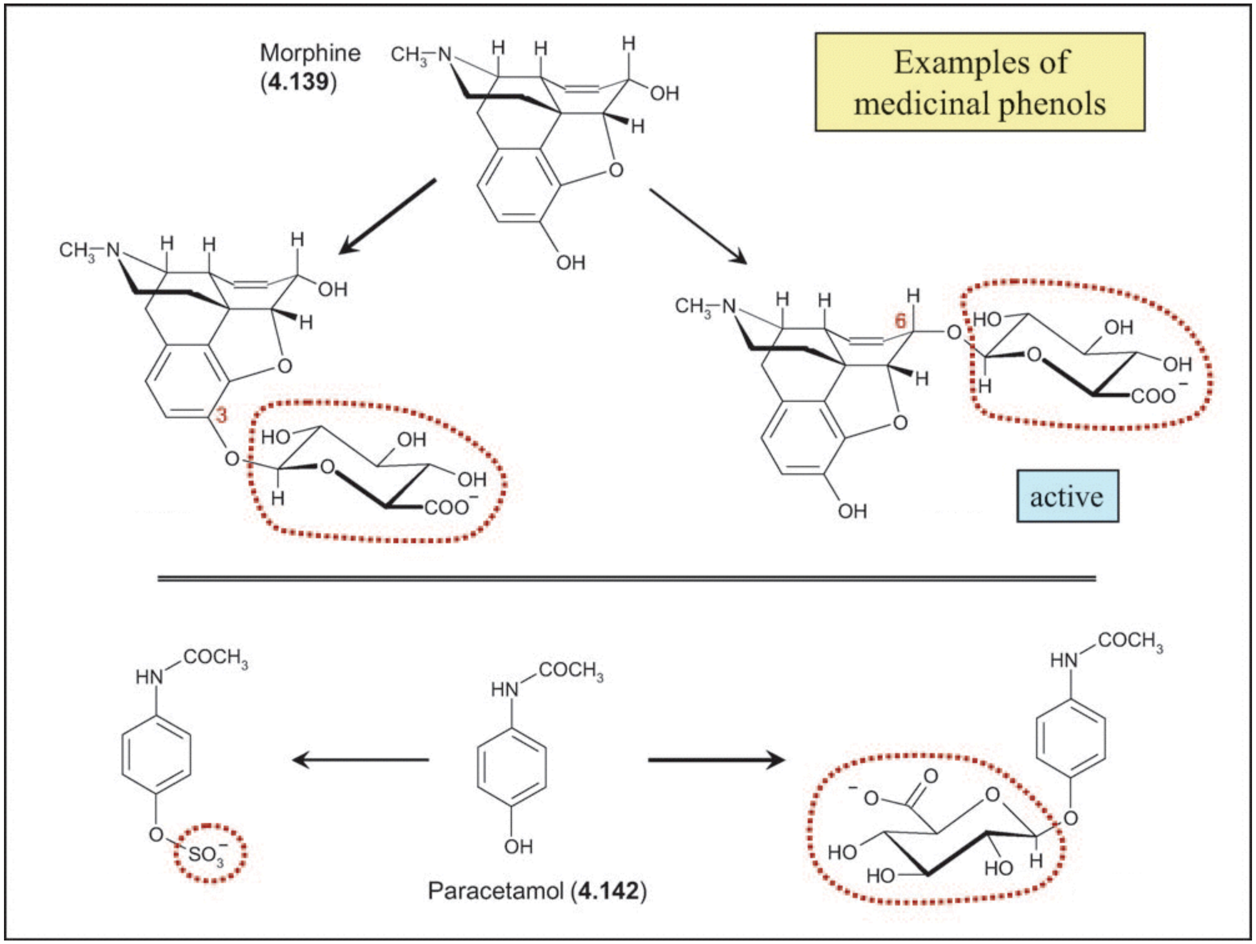
Includes sulfonamides, amides, others

Perhaps the most versatile conjugation reaction.

Examples of nearly every type of nucleophile-glucuronide

II. Glucuronidation of Phenols: Example Morphine, Paracetamol

Both by UGT2B7

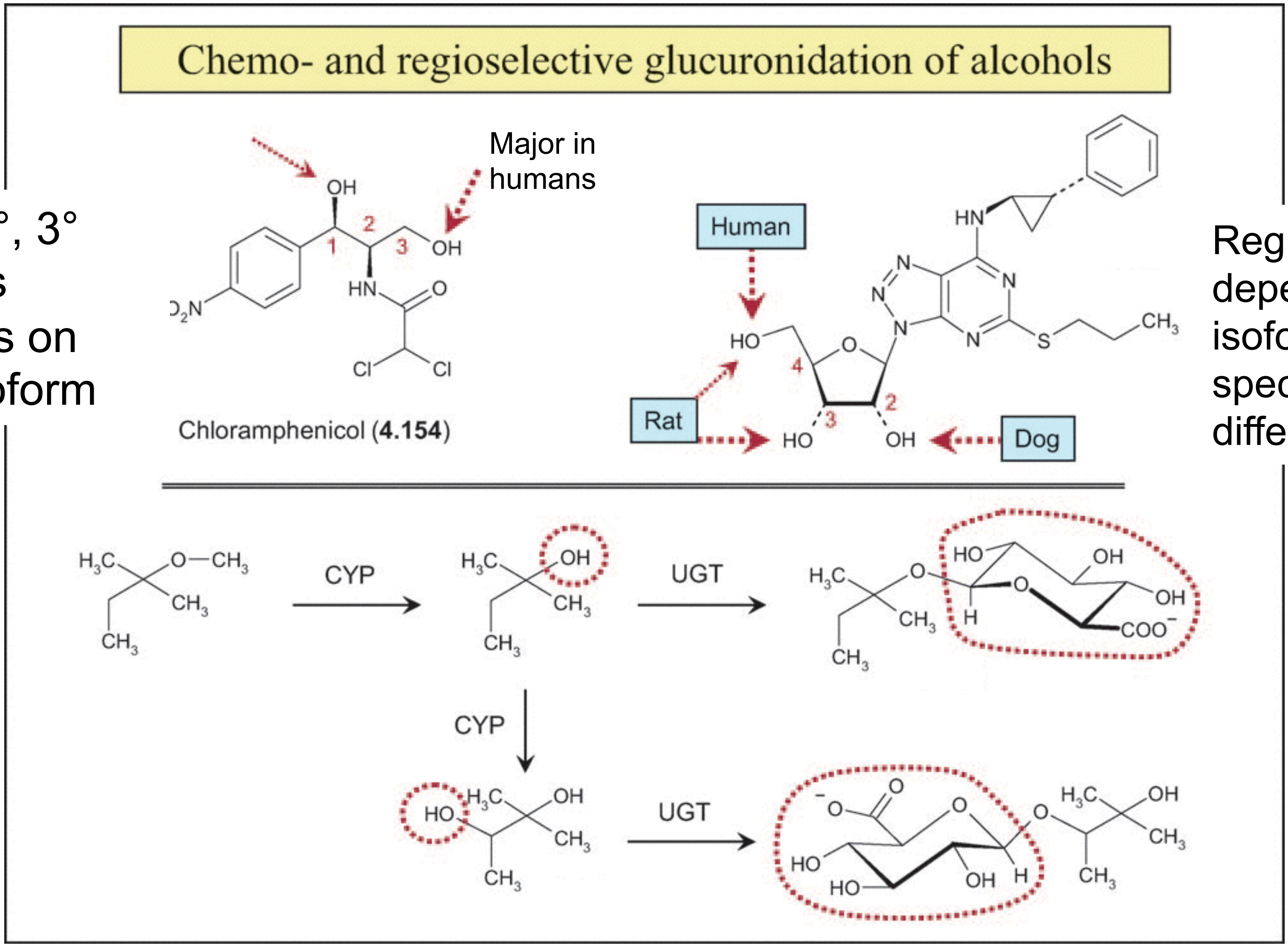


Several UGT1A's

Competition between sulfonation-glucuronidation is common for phenols

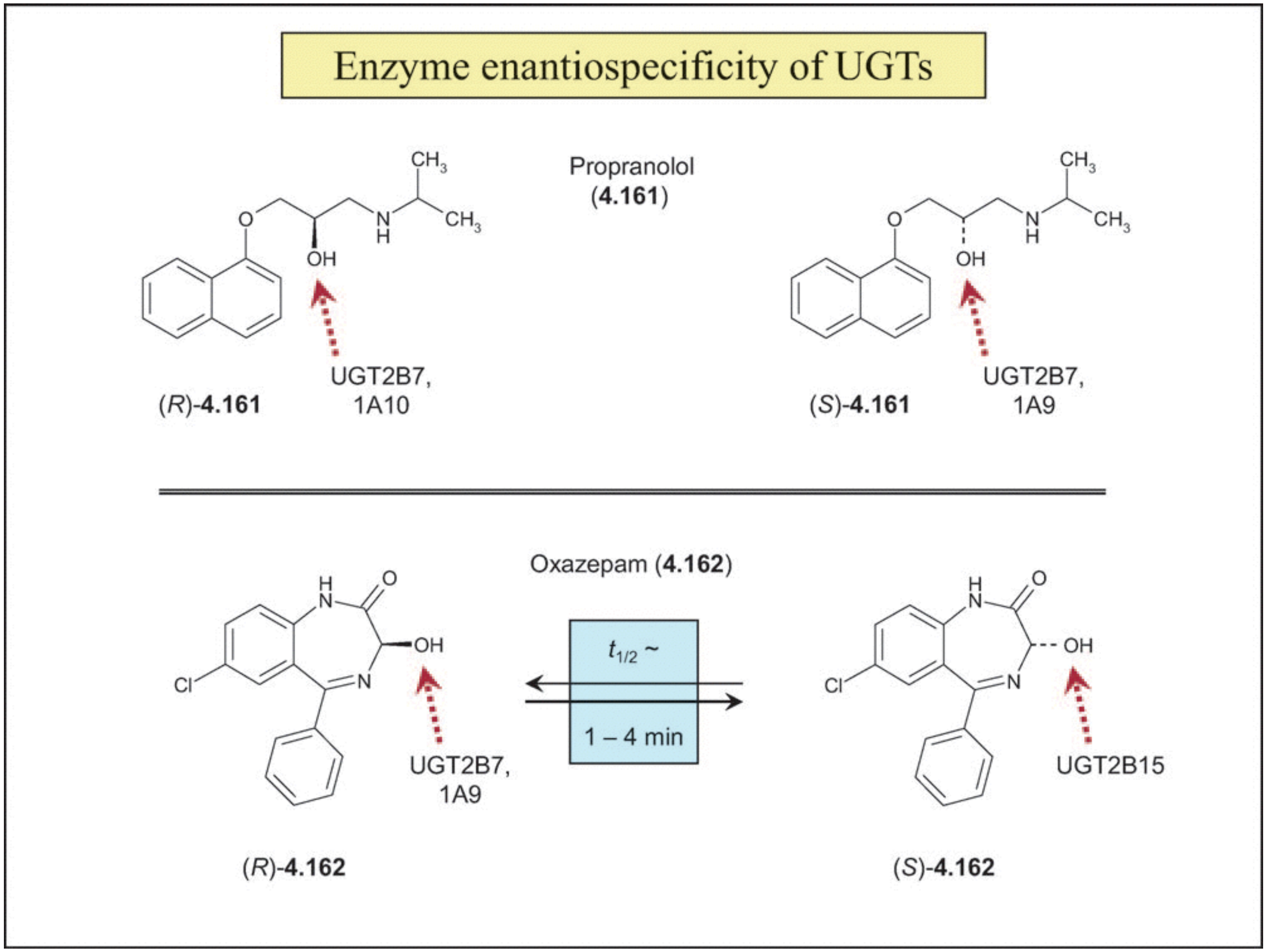
II. Glucuronidation of Alcohols: Selectivity

1° vs. 2°, 3° alcohols depends on UGT isoform

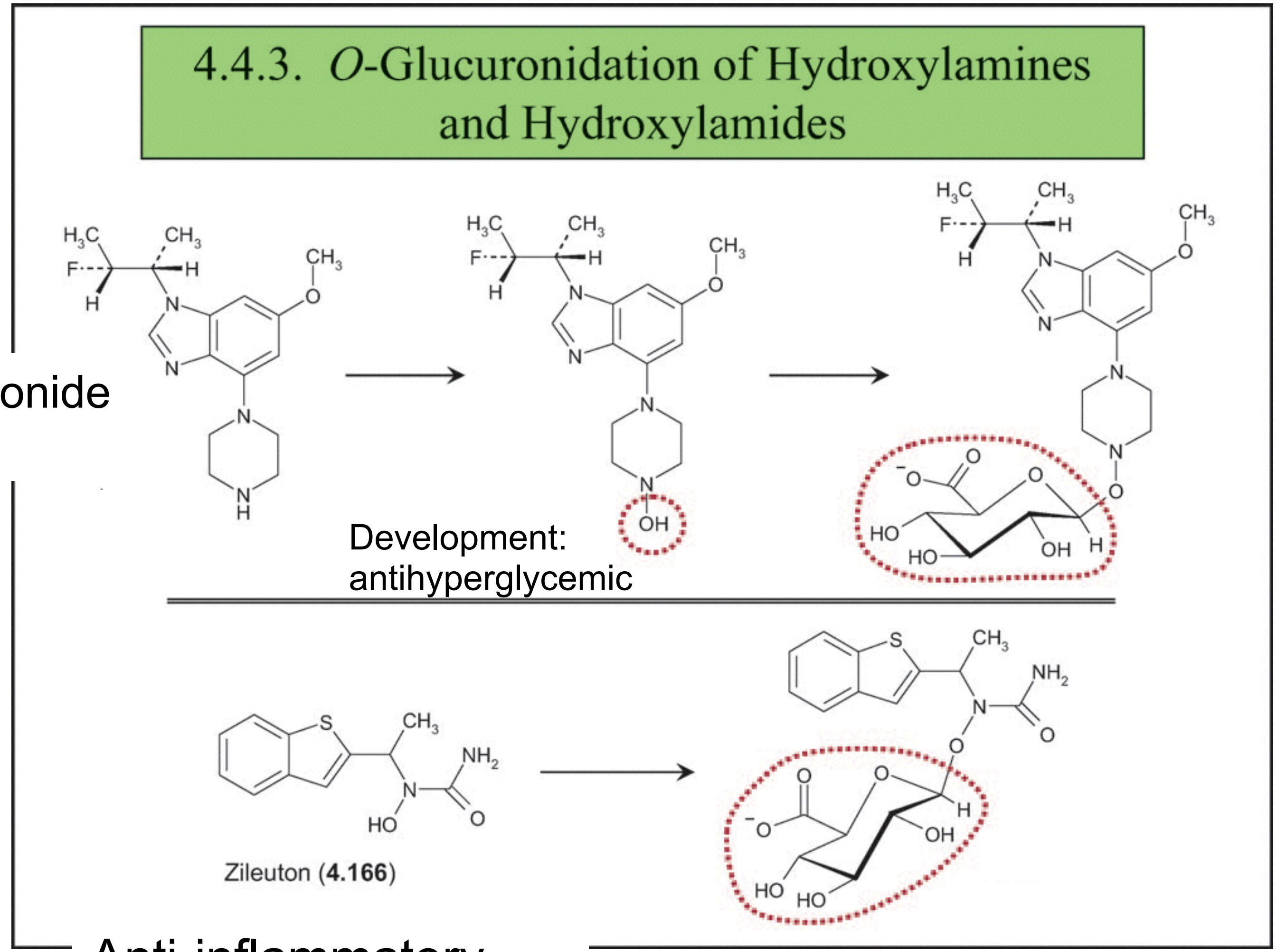


Regioselectivity depends on isoform - species differences.

II. Glucuronidation of Alcohols: Enantioselectivity



II. Glucuronidation of Hydroxylamines


4.4.3. *O*-Glucuronidation of Hydroxylamines and Hydroxylamides

No N-glucuronide detected

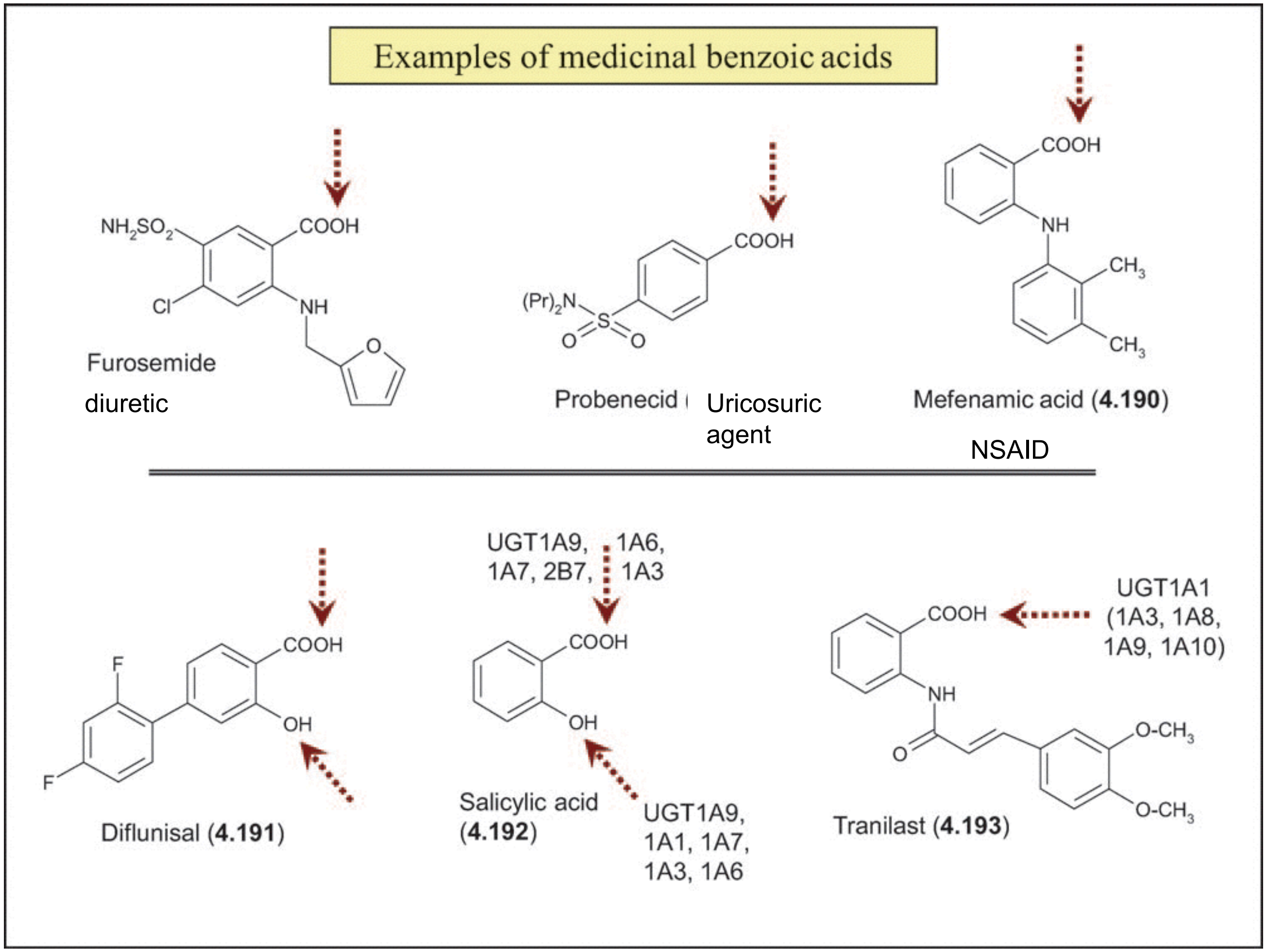
Development: antihyperglycemic

Zileuton (**4.166**)

Anti-inflammatory

Asthma

II. Glucuronidation of Carboxylic Acids: Aryl Acids



Examples of medicinal benzoic acids

Furosemide
diuretic

Probenecid Uricosuric agent

Mefenamic acid (**4.190**)
NSAID

Diflunisal (**4.191**)

UGT1A9, 1A7, 2B7, 1A6, 1A3

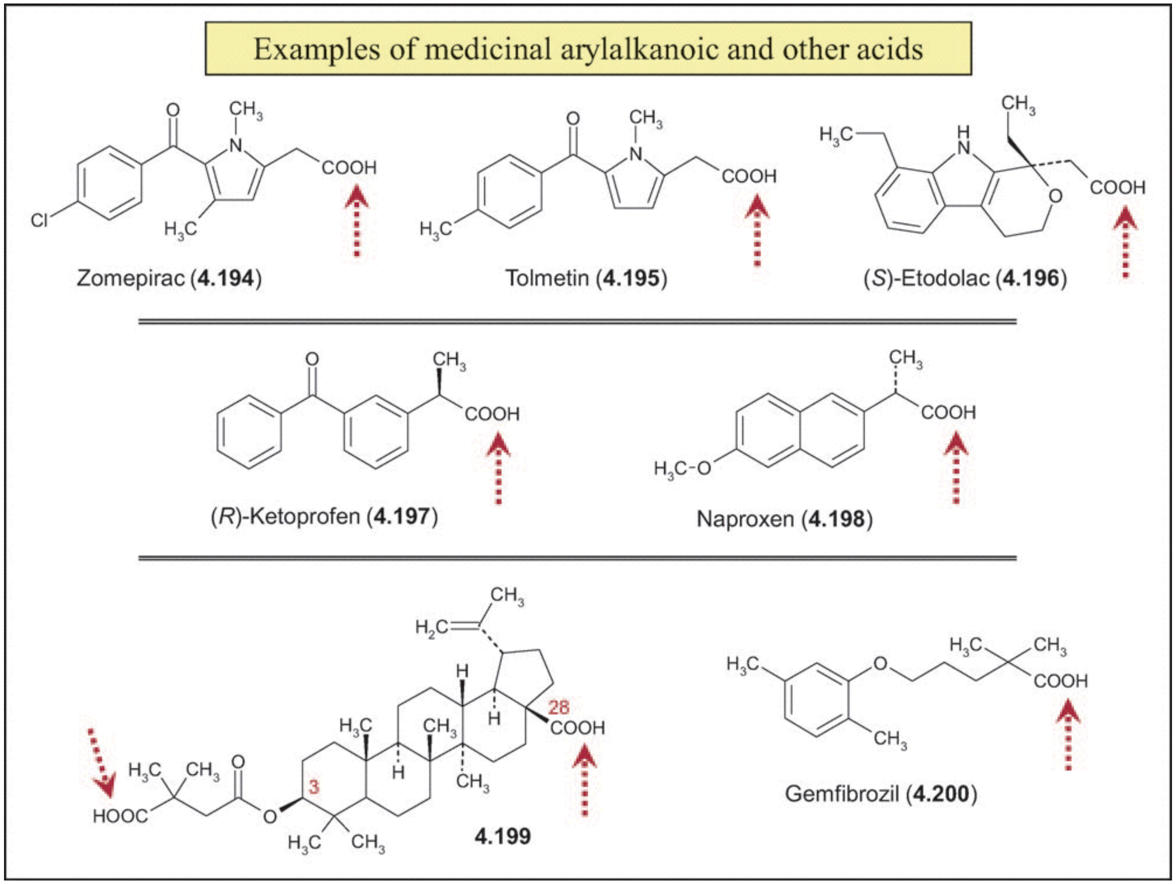
Salicylic acid (**4.192**)

UGT1A9, 1A1, 1A7, 1A3, 1A6

UGT1A1 (1A3, 1A8, 1A9, 1A10)

Tranilast (**4.193**)

II. Glucuronidation of Carboxylic Acids



Examples of medicinal arylalkanoic and other acids

Zomepirac (**4.194**)

Tolmetin (**4.195**)

(*S*)-Etodolac (**4.196**)

(*R*)-Ketoprofen (**4.197**)

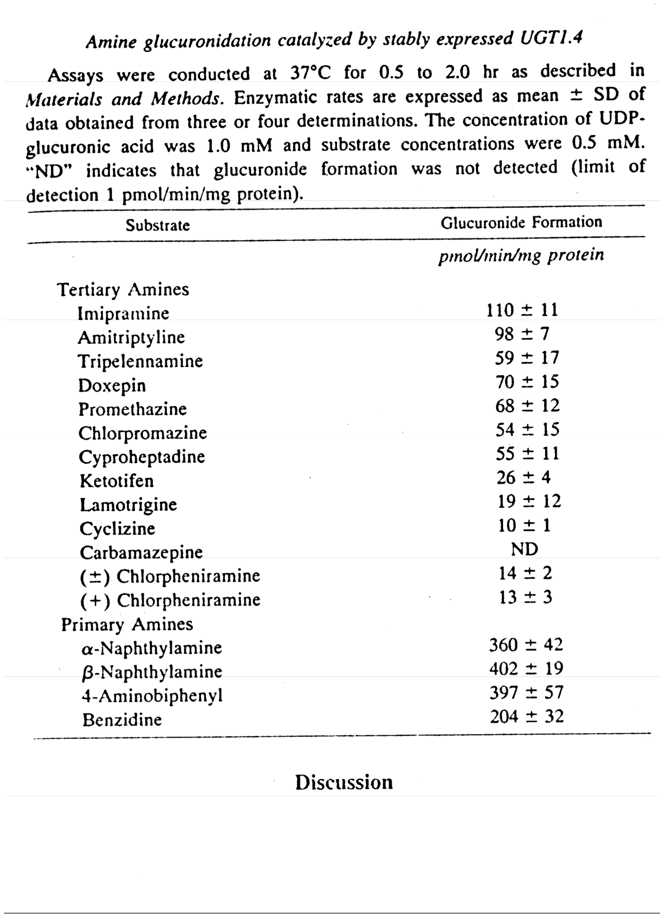
Naproxen (**4.198**)

4.199

Gemfibrozil (**4.200**)

II. Glucuronidation: Amines

Amine glucuronidation, including formation of quaternary N-glucuronides, has received lots of attention because so many drugs contain imidazoles, tetrazoles, etc, and because aryl amine glucuronides may contribute to colon and bladder cancer (see below).



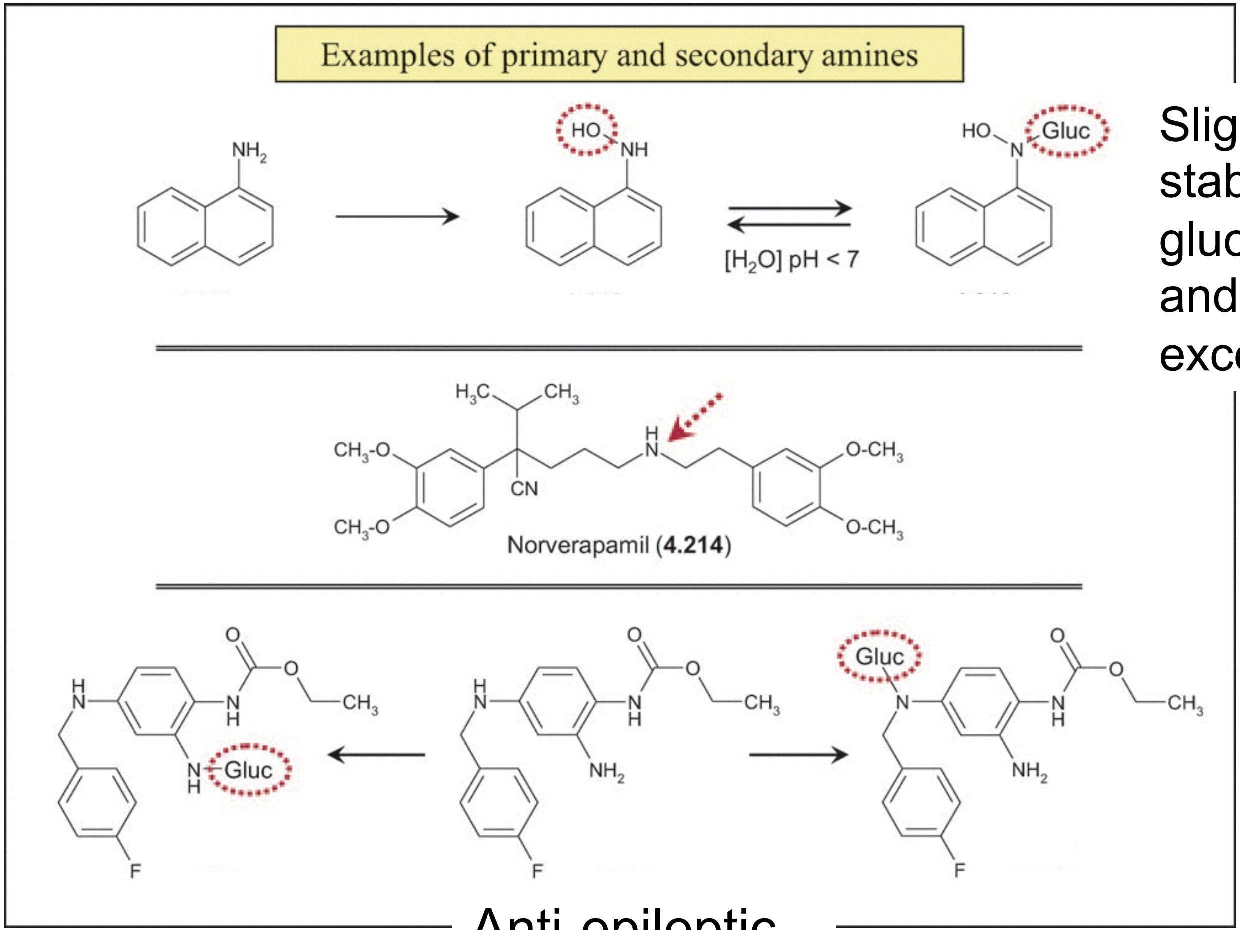
Amine glucuronidation catalyzed by stably expressed UGT1.4

Assays were conducted at 37°C for 0.5 to 2.0 hr as described in *Materials and Methods*. Enzymatic rates are expressed as mean ± SD of data obtained from three or four determinations. The concentration of UDP-glucuronic acid was 1.0 mM and substrate concentrations were 0.5 mM. "ND" indicates that glucuronide formation was not detected (limit of detection 1 pmol/min/mg protein).

Substrate	Glucuronide Formation
	pmol/min/mg protein
Tertiary Amines	
Imipramine	110 ± 11
Amitriptyline	98 ± 7
Tripelennamine	59 ± 17
Doxepin	70 ± 15
Promethazine	68 ± 12
Chlorpromazine	54 ± 15
Cyproheptadine	55 ± 11
Ketotifen	26 ± 4
Lamotrigine	19 ± 12
Cyclizine	10 ± 1
Carbamazepine	ND
(±) Chlorpheniramine	14 ± 2
(+) Chlorpheniramine	13 ± 3
Primary Amines	
α-Naphthylamine	360 ± 42
β-Naphthylamine	402 ± 19
4-Aminobiphenyl	397 ± 57
Benzidine	204 ± 32

Discussion

II. Glucuronidation of Aliphatic Amines, Aryl Amines, Hydroxyl Amines



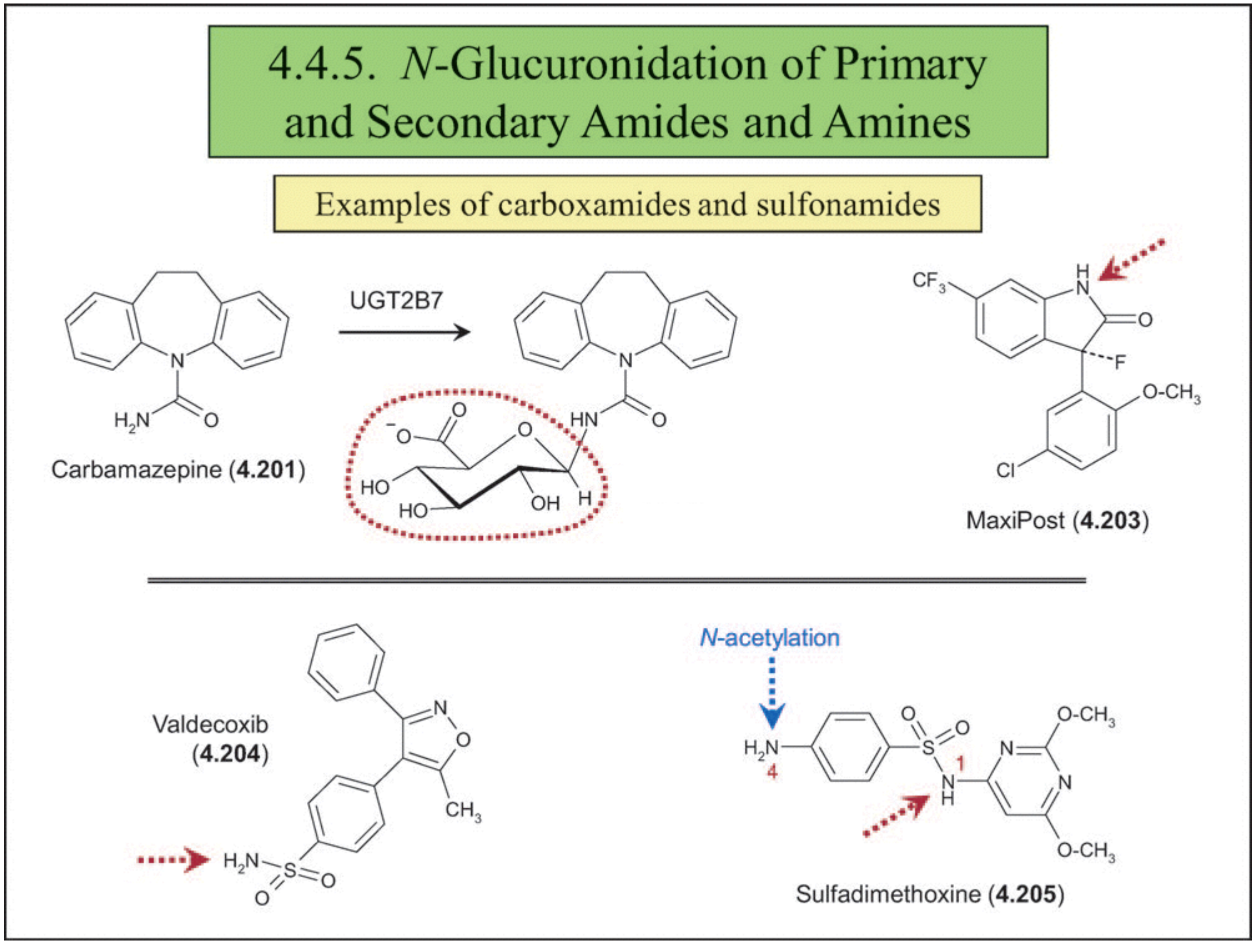
Norverapamil (**4.214**)

Anti-epileptic

Slightly Greater stability than O-gluc’s, chemical and enzymatic, except at low pH

II. Glucuronidation of Carboxamides and Sulfonamides

Greater stability than O-gluc’s, chemical and enzymatic, except at low pH



4.4.5. *N*-Glucuronidation of Primary and Secondary Amides and Amines

Examples of carboxamides and sulfonamides

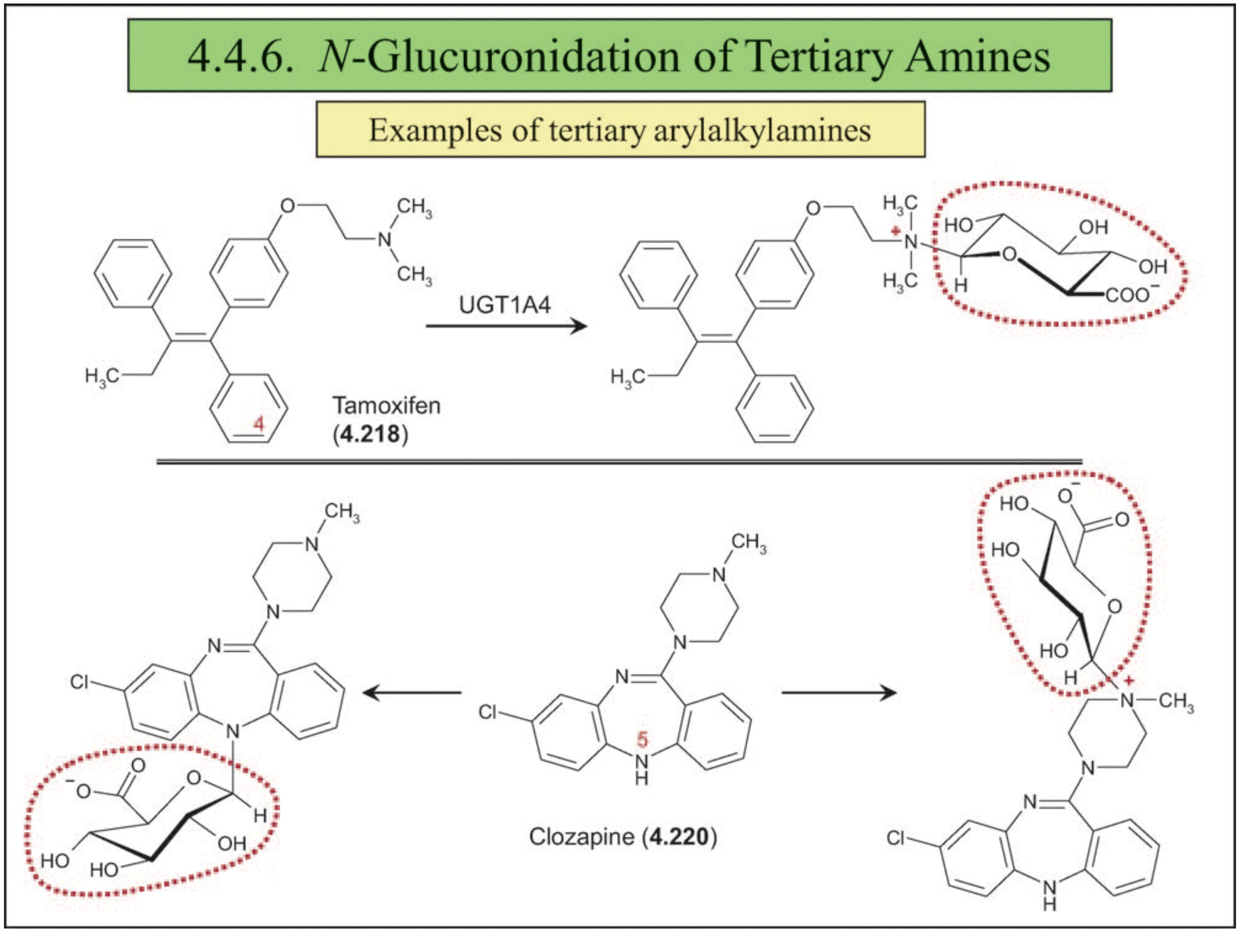
Carbamazepine (**4.201**)

MaxiPost (**4.203**)

Valdecoxib (**4.204**)

Sulfadimethoxine (**4.205**)

Glucuronidation of Tertiary Amines



4.4.6. *N*-Glucuronidation of Tertiary Amines

Examples of tertiary arylalkylamines

Tamoxifen (**4.218**)

Clozapine (**4.220**)

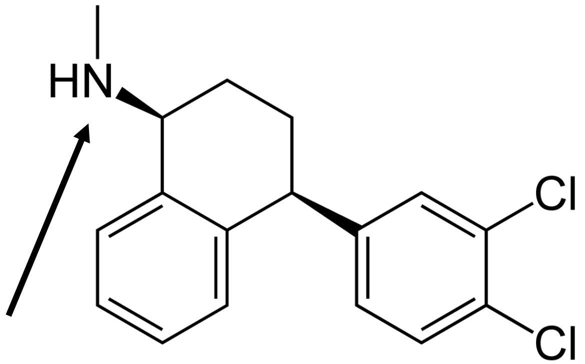
Stable toward glucuronidase, but hydrolyzed in acid

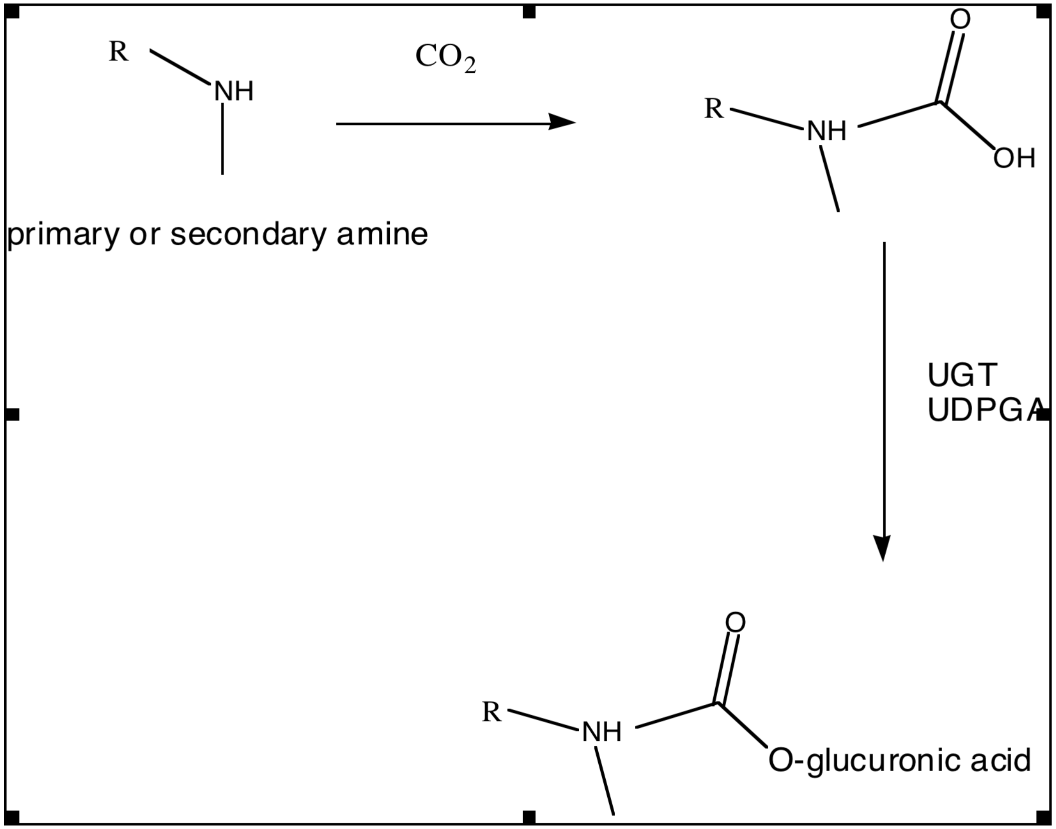
Usually, 4° N-glucuronides are less susceptible to hydrolysis by glucuronidase but not always!.

Stable to acid, but hydrolyzed by glucuronidases

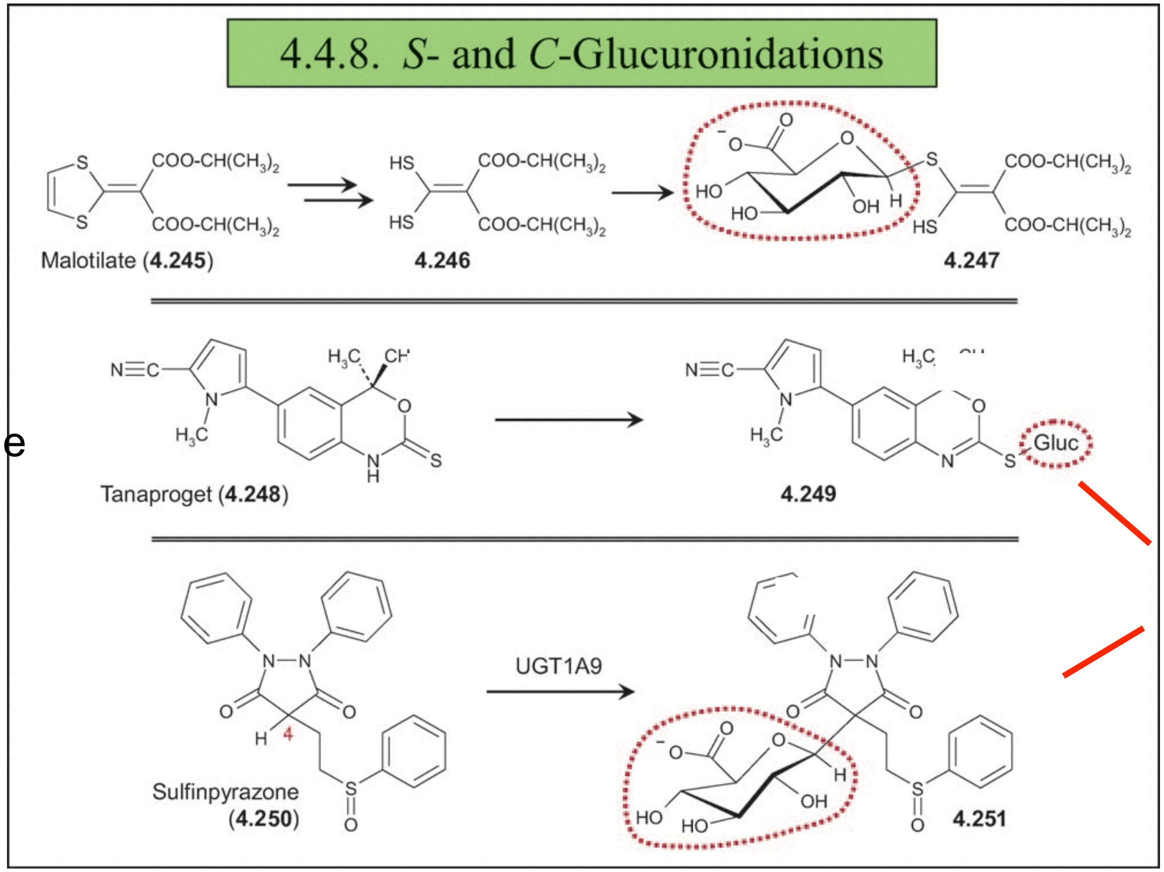
II. “Indirect” Glucuronidation of Amines: Carbamates

Sertraline (Zoloft) is N-carbamylated, then O-gluronidated





II. C- and S-Glucuronidation



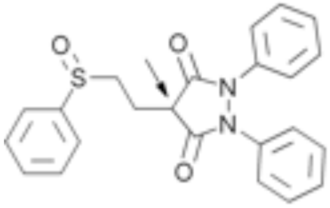
Progesterone antagonist

Major metabolite in humans

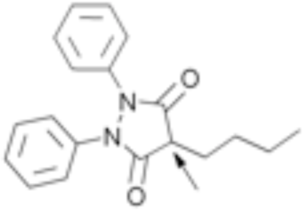
Rare compared to O- or N-glucuronidation

S-glucuronidation is rare because thiol containing drugs are rare
C-glucuronides are rare because not many nucleophilic carbons

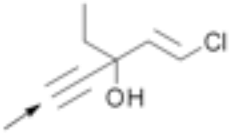
II. C- and S-Glucuronidation



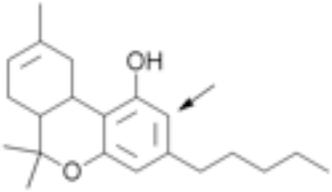
sulfinpyrazone



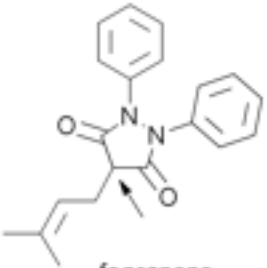
phenylbutazone



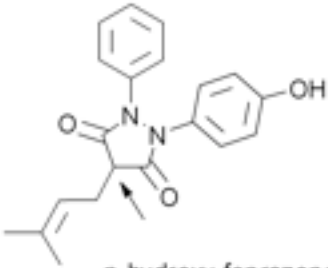
ethchlorvynol



Δ^6-tetrahydrocannabinol



feprazone

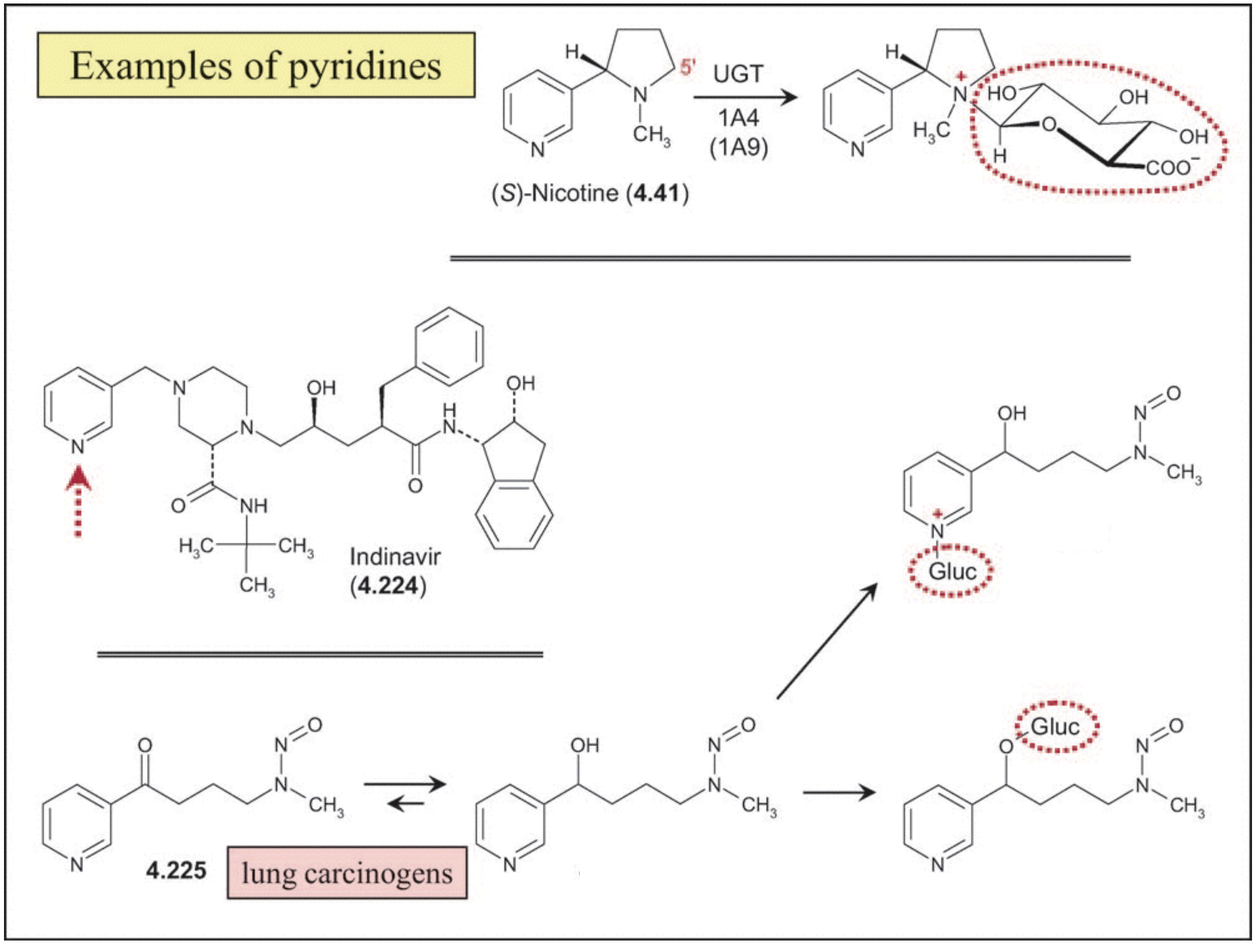


p-hydroxy feprazone

C-glucuronides can be more prevalent than phenolic glucuronides in the same molecule.

II. Glucuronidation of Pyridines

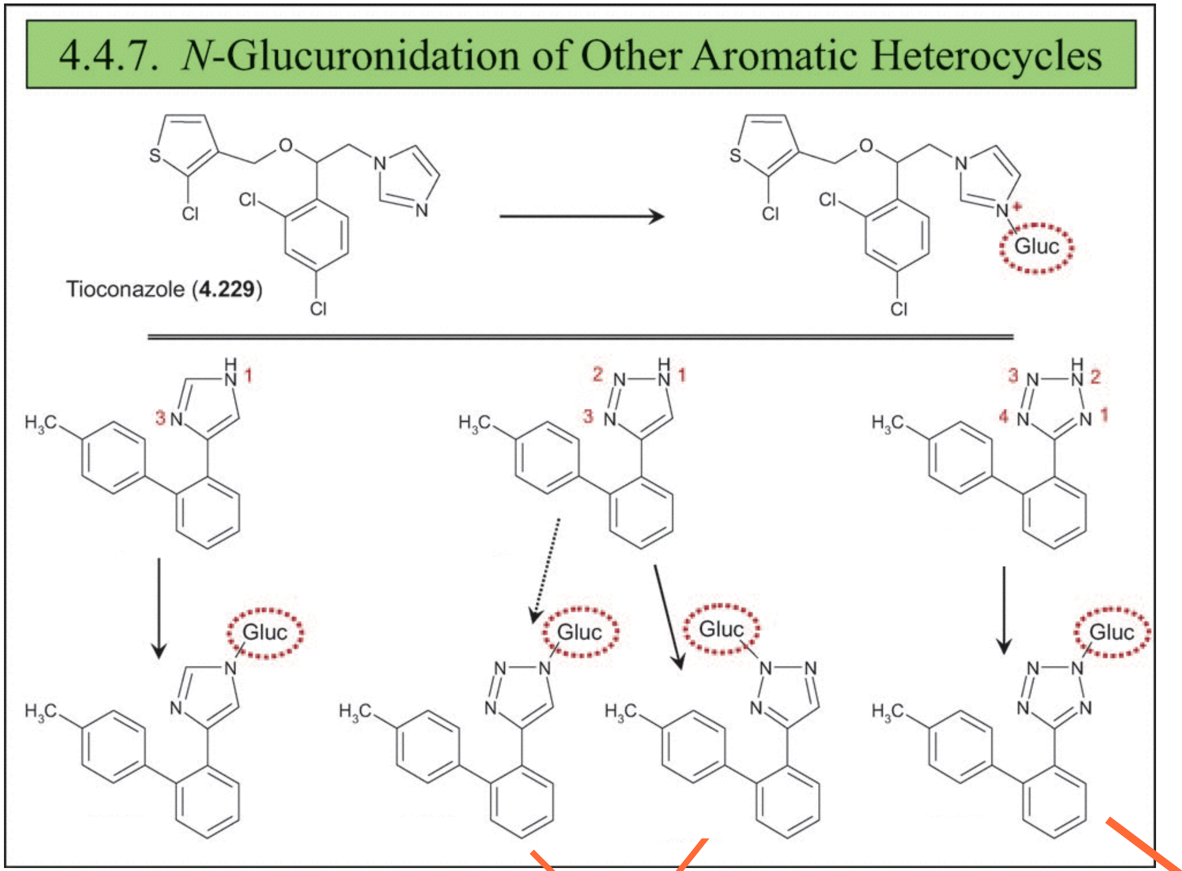
‘heterocyclic amines’



Pyridine may not be preferred site.

But, many examples of pyridine glucuronidation.

II. N-Glucuronidation of Heterocyclic Amines


4.4.7. *N*-Glucuronidation of Other Aromatic Heterocycles

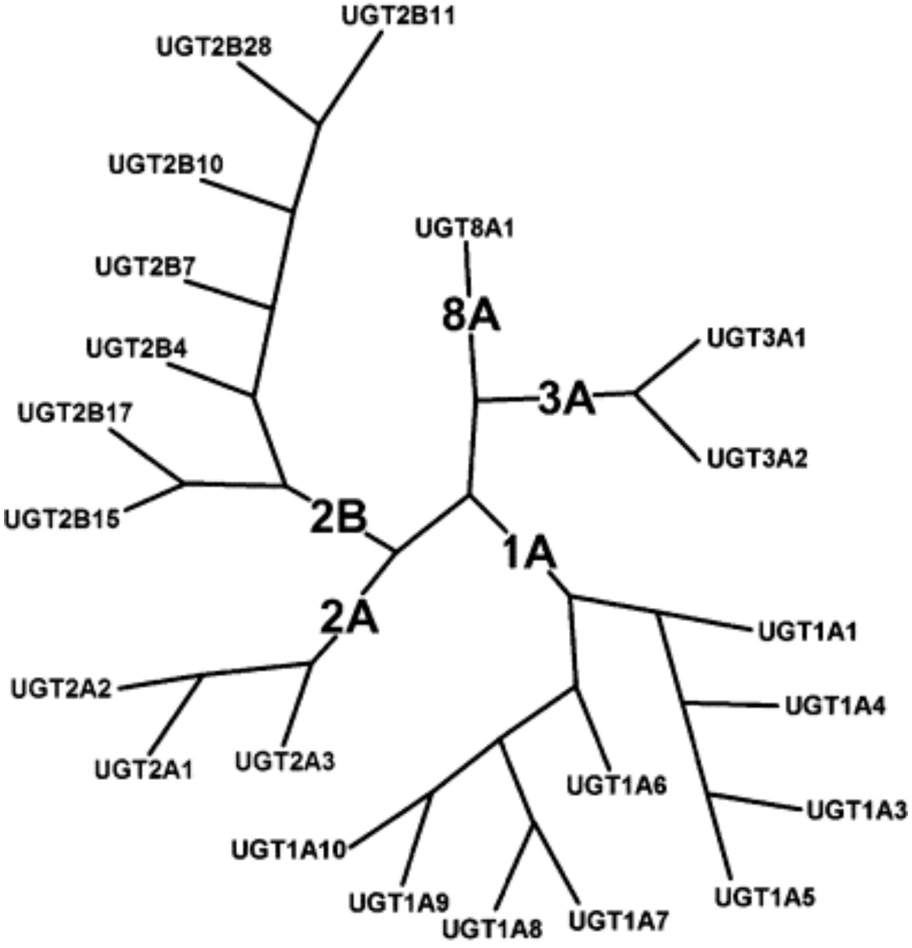
Tioconazole (**4.229**)

Besides the pyridine derivatives, a marked number of aromatic diaza- and polyazaheterocyclic compounds are known to undergo N-glucuronidation.

Model imidazole, triazole, tetrazoles yield tertiary N-glucuronides. Steric hindrance a major determinant of N-selectivity.

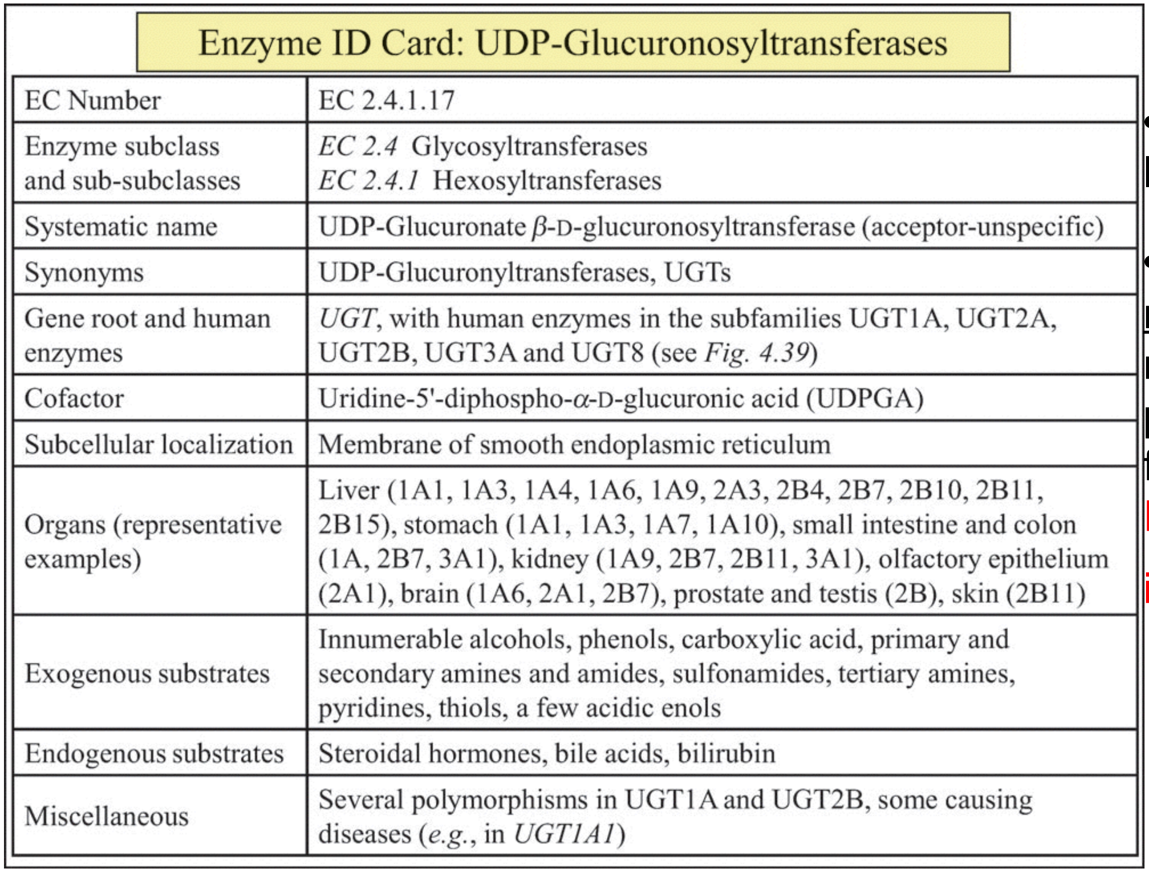
Triazole: 2 ‘distinct’ products, tetrazole 1product because two N’s are equivalent.

III. UDP-glucuronosyl Transferases UGTs: Isoforms and Phylogeny



- 22 human isoforms identified.
- 4 gene families; 1, 2, 3 and 8; 2 has subs A,B
- family 3 only recently discovered/ characterized – NOT much participation in drug metabolism; endogenous substrate?
- Family 1 isoforms share a common C-terminus, but are differentiated by N-terminal sequences.
- Family 2 isoforms have differences throughout the sequence.

III. UGT’s - Overview of Distribution, Substrate Selectivity



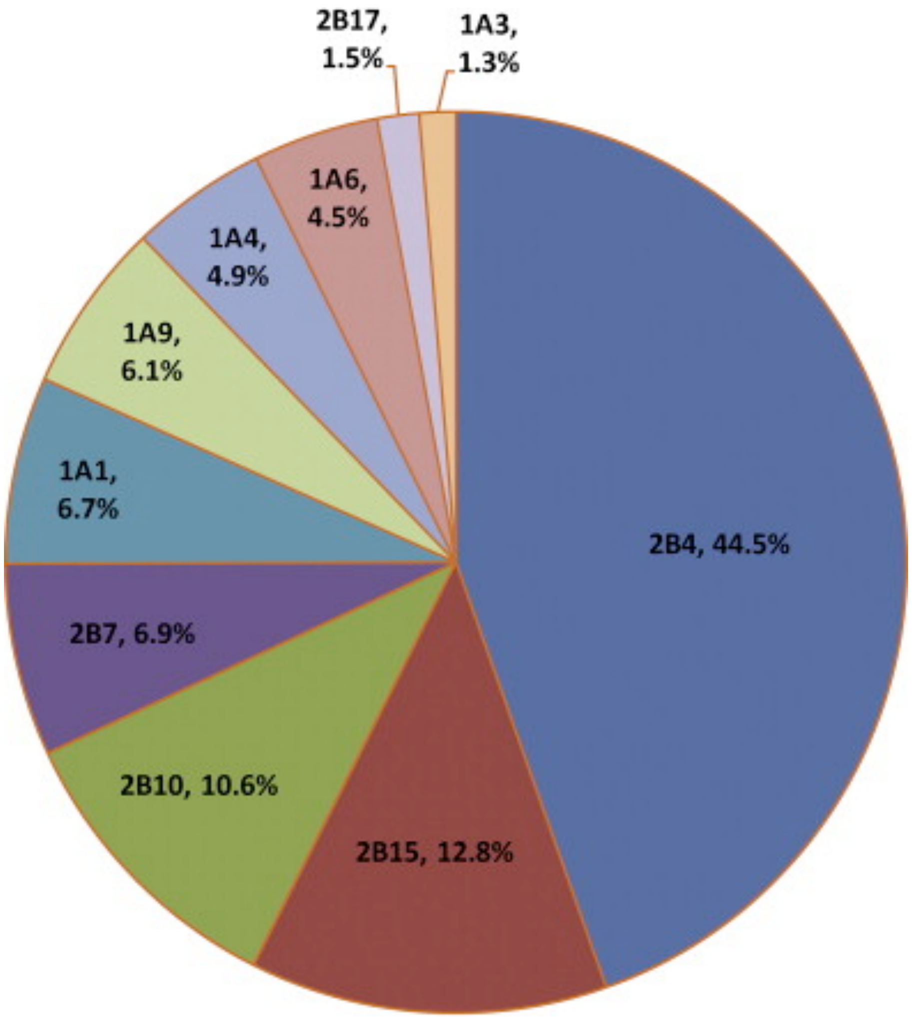
Enzyme ID Card: UDP-Glucuronosyltransferases

EC Number	EC 2.4.1.17
Enzyme subclass and sub-subclasses	*EC 2.4* Glycosyltransferases *EC 2.4.1* Hexosyltransferases
Systematic name	UDP-Glucuronate β-D-glucuronosyltransferase (acceptor-unspecific)
Synonyms	UDP-Glucuronyltransferases, UGTs
Gene root and human enzymes	*UGT*, with human enzymes in the subfamilies UGT1A, UGT2A, UGT2B, UGT3A and UGT8 (see *Fig. 4.39*)
Cofactor	Uridine-5'-diphospho-α-D-glucuronic acid (UDPGA)
Subcellular localization	Membrane of smooth endoplasmic reticulum
Organs (representative examples)	Liver (1A1, 1A3, 1A4, 1A6, 1A9, 2A3, 2B4, 2B7, 2B10, 2B11, 2B15), stomach (1A1, 1A3, 1A7, 1A10), small intestine and colon (1A, 2B7, 3A1), kidney (1A9, 2B7, 2B11, 3A1), olfactory epithelium (2A1), brain (1A6, 2A1, 2B7), prostate and testis (2B), skin (2B11)
Exogenous substrates	Innumerable alcohols, phenols, carboxylic acid, primary and secondary amines and amides, sulfonamides, tertiary amines, pyridines, thiols, a few acidic enols
Endogenous substrates	Steroidal hormones, bile acids, bilirubin
Miscellaneous	Several polymorphisms in UGT1A and UGT2B, some causing diseases (*e.g.*, in *UGT1A1*)

- Prominent in hepatic, renal, gut, lung, olfactory tissue.

- Cellular location: ER and nuclear membrane, not in mitochondria, lysosomes or plasma membranes. No soluble forms reported in mammals (yet). Found on the luminal side of ER, in contrast to CYPs.

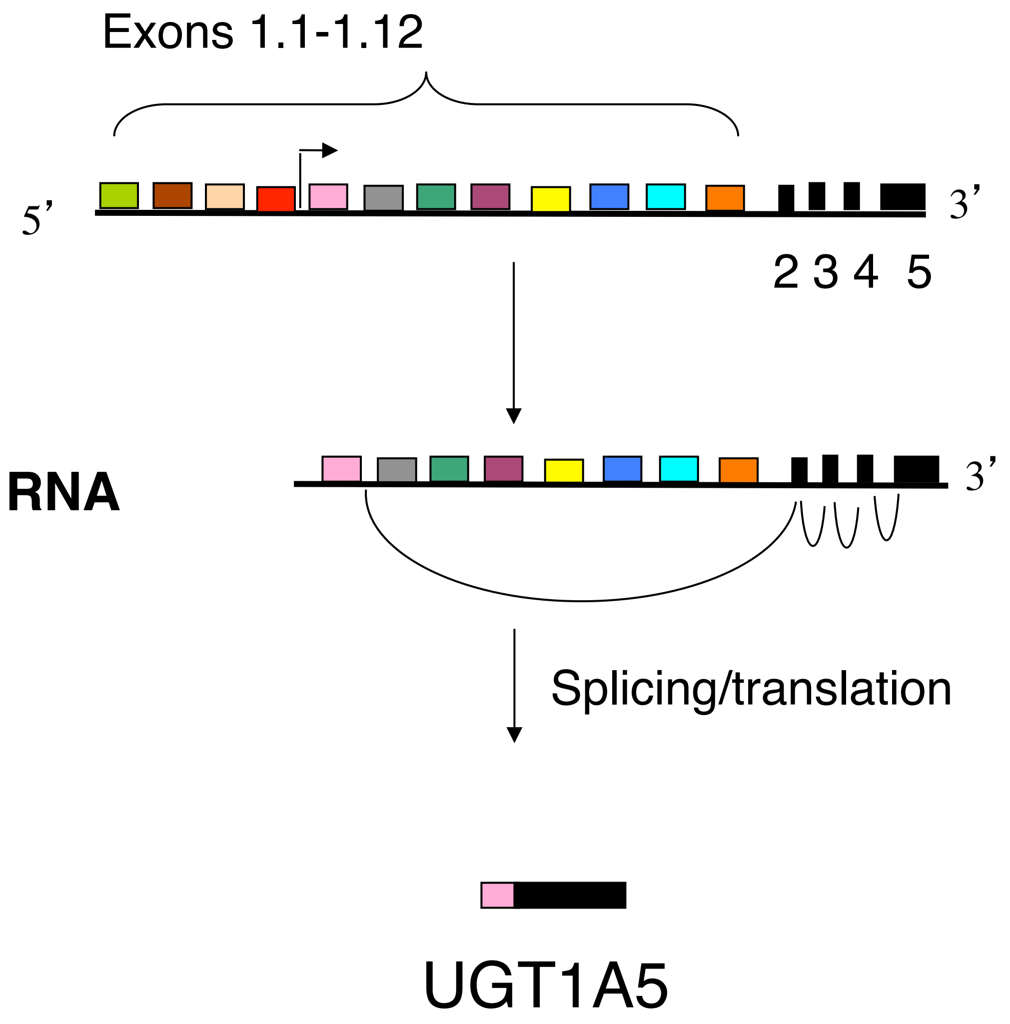
Relative Hepatic Expression in Humans



UGT2B4 is the major isoform in the liver, by a wide margin. But not so important in drug metabolism: Metabolizes deoxycholic acids and catechol estrogens.

UGTs also expressed in the gut: isoform-dependent expression along the stomach, small intestine, colon.

III. UDP-glucuronosyl Transferases UGTs:Genetic Structure



The UGT1A locus yields different isoforms via differential splicing of a single variable N-terminal exon, with 4 common exons (exons 2-5). Thus, UGT1A’s have an identical C-terminus, approximately 245 amino acids.

The UGT2 families have distinct genes for each isoform.

III. UGTs: Isoform Substrate Selectivity

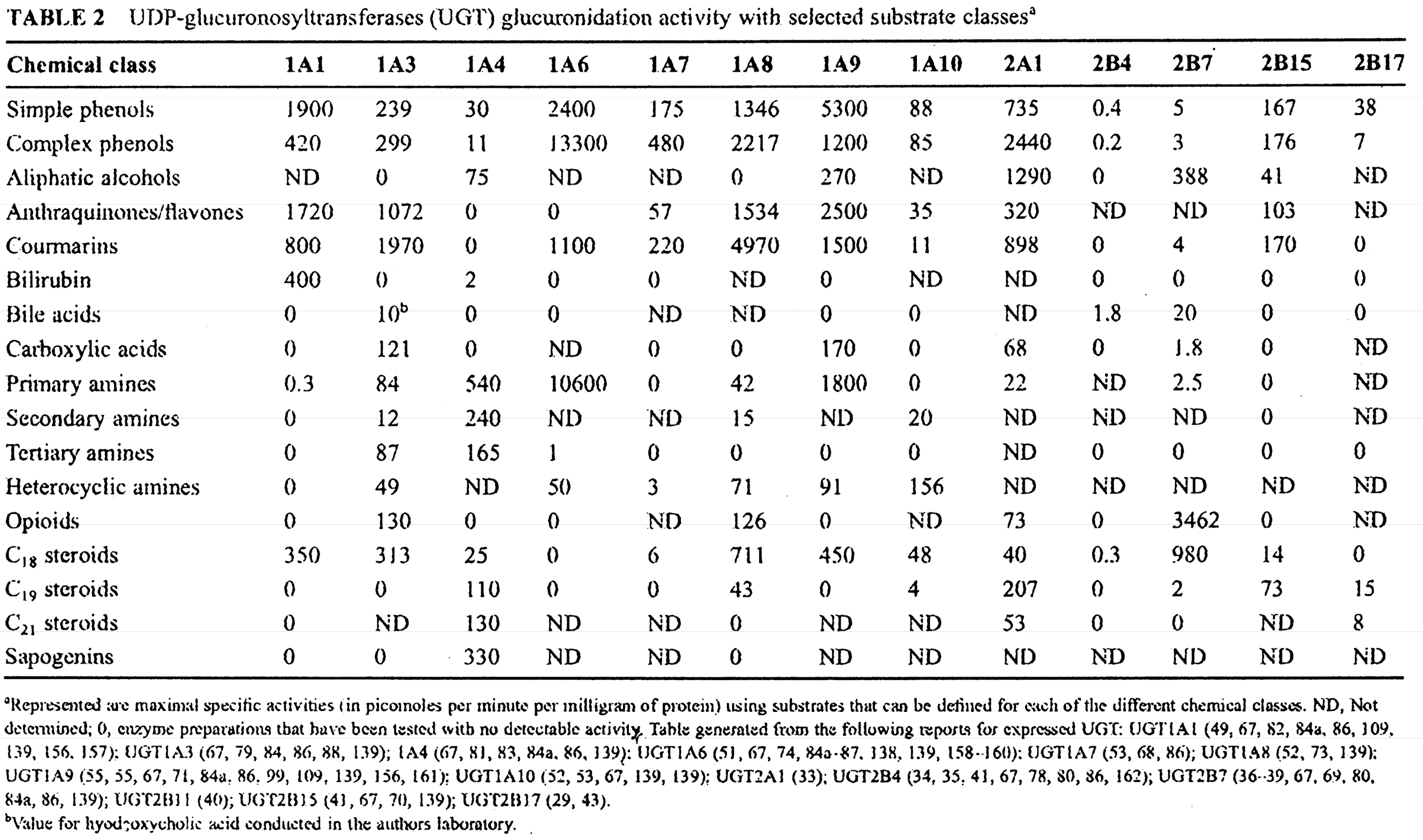


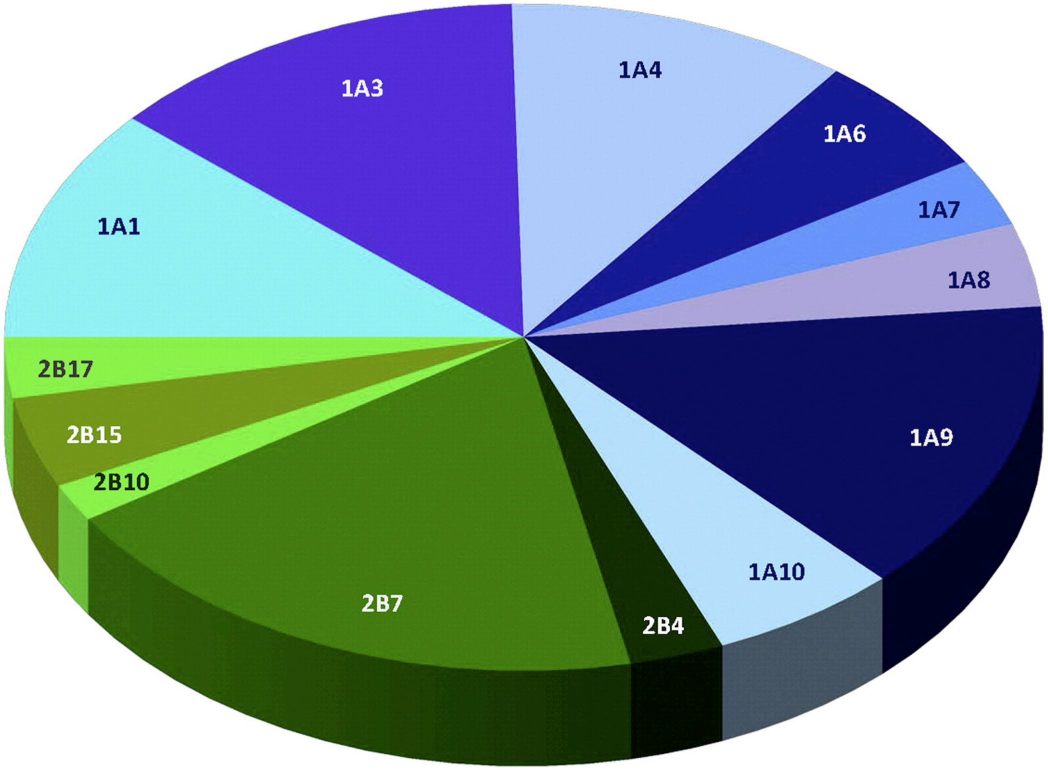
TABLE 2 UDP-glucuronosyltransferases (UGT) glucuronidation activity with selected substrate classes[a]

Chemical class	**1A1**	**1A3**	**1A4**	**1A6**	**1A7**	**1A8**	**1A9**	**1A10**	**2A1**	**2B4**	**2B7**	**2B15**	**2B17**
Simple phenols	1900	239	30	2400	175	1346	5300	88	735	0.4	5	167	38
Complex phenols	420	299	11	13300	480	2217	1200	85	2440	0.2	3	176	7
Aliphatic alcohols	ND	0	75	ND	ND	0	270	ND	1290	0	388	41	ND
Anthraquinones/flavones	1720	1072	0	0	57	1534	2500	35	320	ND	ND	103	ND
Courmarins	800	1970	0	1100	220	4970	1500	11	898	0	4	170	0
Bilirubin	400	0	2	0	0	ND	0	ND	ND	0	0	0	0
Bile acids	0	10[b]	0	0	ND	ND	0	0	ND	1.8	20	0	0
Carboxylic acids	0	121	0	ND	0	0	170	0	68	0	1.8	0	ND
Primary amines	0.3	84	540	10600	0	42	1800	0	22	ND	2.5	0	ND
Secondary amines	0	12	240	ND	ND	15	ND	20	ND	ND	ND	0	ND
Tertiary amines	0	87	165	1	0	0	0	0	ND	0	0	0	0
Heterocyclic amines	0	49	ND	50	3	71	91	156	ND	ND	ND	ND	ND
Opioids	0	130	0	0	ND	126	0	ND	73	0	3462	0	ND
C_{18} steroids	350	313	25	0	6	711	450	48	40	0.3	980	14	0
C_{19} steroids	0	0	110	0	0	43	0	4	207	0	2	73	15
C_{21} steroids	0	ND	130	ND	ND	0	ND	ND	53	0	0	ND	8
Sapogenins	0	0	330	ND	ND	0	ND	ND	ND	ND	ND	ND	ND

[a] Represented are maximal specific activities (in picomoles per minute per milligram of protein) using substrates that can be defined for each of the different chemical classes. ND, Not determined; 0, enzyme preparations that have been tested with no detectable activity. Table generated from the following reports for expressed UGT: UGT1A1 (49, 67, 82, 84a, 86, 109, 139, 156, 157); UGT1A3 (67, 79, 84, 86, 88, 139); 1A4 (67, 81, 83, 84a, 86, 139); UGT1A6 (51, 67, 74, 84a-87, 138, 139, 158–160); UGT1A7 (53, 68, 86); UGT1A8 (52, 73, 139); UGT1A9 (55, 55, 67, 71, 84a, 86, 99, 109, 139, 156, 161); UGT1A10 (52, 53, 67, 139, 139); UGT2A1 (33); UGT2B4 (34, 35, 41, 67, 78, 80, 86, 162); UGT2B7 (36–39, 67, 69, 80, 84a, 86, 139); UGT2B11 (40); UGT2B15 (41, 67, 70, 139); UGT2B17 (29, 43).

[b] Value for hyodeoxycholic acid conducted in the authors laboratory.

III. UGT Isoform Contributions to Drug Metabolism



Relevance of UDP-glucuronosyltransferase polymorphisms for drug dosing: A quantitative systematic review. Stingl JC, Bartels H, Viviani R, Lehmann ML, Brockmöller J. Pharmacol Ther. 2014, 141(1):92-116.

The UDP-glucuronosyltransferases: their role in drug metabolism and detoxification. Rowland A, Miners JO, Mackenzie PI.
Int J Biochem Cell Biol. 2013, 45(6):1121-32.

III. UGT Substrate/Inhibitor Isoform Specificity

UGT Isoform	Substrate	Inhibitor	IC_{50} (μM)
1A1	SN-38, metabolite of irinotecan	Atazanavir	1.9
1A3	Zolasartan	Buprenorphine	40-50
1A4	Imipramine, Trifluorperazine	Hecogenin	1.5
1A6	Serotonin	Troglitazone	20
1A7	Octylgallate	phenylbutazone	3.9
1A8	Dihydroxy TST	emodin	15.6
1A9	propofolol	Niflumic Acid	0.0275
1A10	dopamine	tacrolimus	0.034
2B7	AZT	fluconazole	146
2B15	Oxazepam	ibuprofen	120

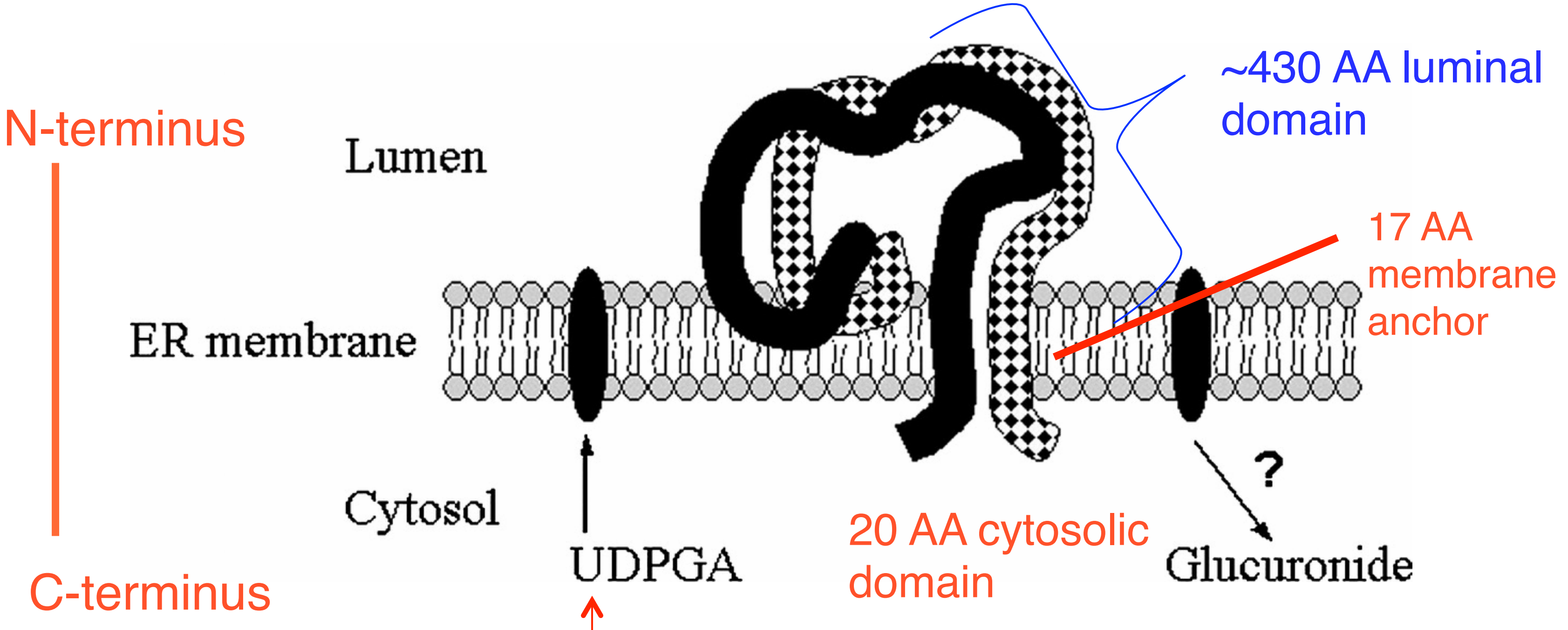
III. UGTs: Structure and Function

in microsomal preps UGT's exhibit "latency", i.e. low activity which can be increased by small molecule "activators" such as lubrol. This "latency" of activity is due to:

Compartmentation hypothesis claims that the active site is near the lumen of the ER and highly charged UDPGA requires specific transport across the membrane. This transport doesn't occur in microsomes, but addition of membrane disrupters allows leakage. A UDP-N-acetyl-galactosamine-stimulated transport protein recently has been characterized which transports UDPGA into ER. Several ER transport proteins may be important for UGT function. Probably NOT MRP's which are found in the plasma membrane and do transport glucuronide conjugates.

In vitro, recent studies have demonstrated the utility of membrane 'pore-formers', such as alamethicin.

III. UGTs: Structure Function



N-terminus

C-terminus

~430 AA luminal domain

17 AA membrane anchor

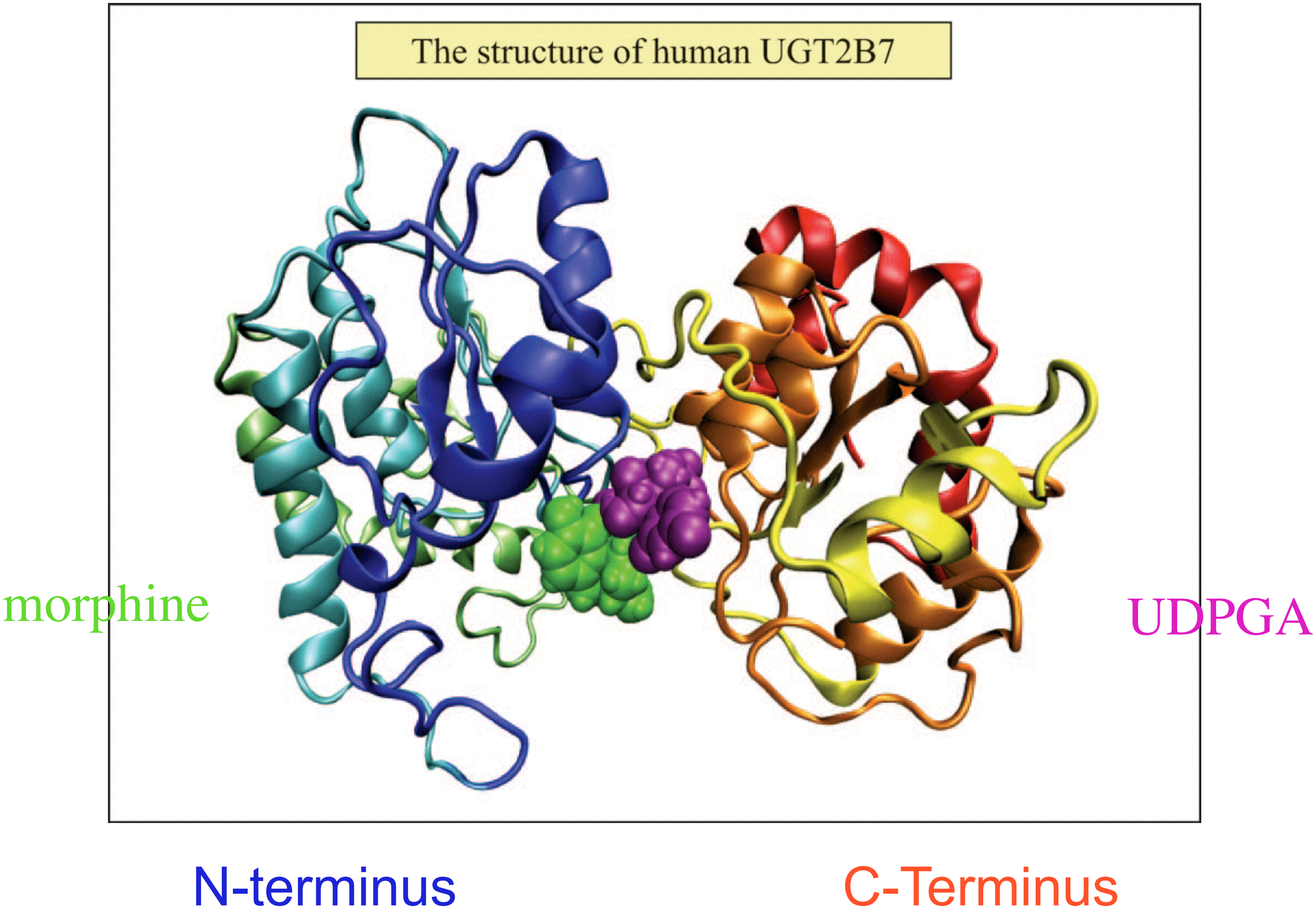
20 AA cytosolic domain

Recently, there has been some evidence for a monomer-dimer equilibrium, with the dimer being the functional UGT. Co-expression of mutants containing single amino acid substitutions or chimeras with decreased activity, individually, leads to ‘complementation’ and restoration of activity. Based on analysis of chimeric UGTs, the ‘dimerization’ domain is proposed to be N-terminal regions. It appears that heterodimers are functional.

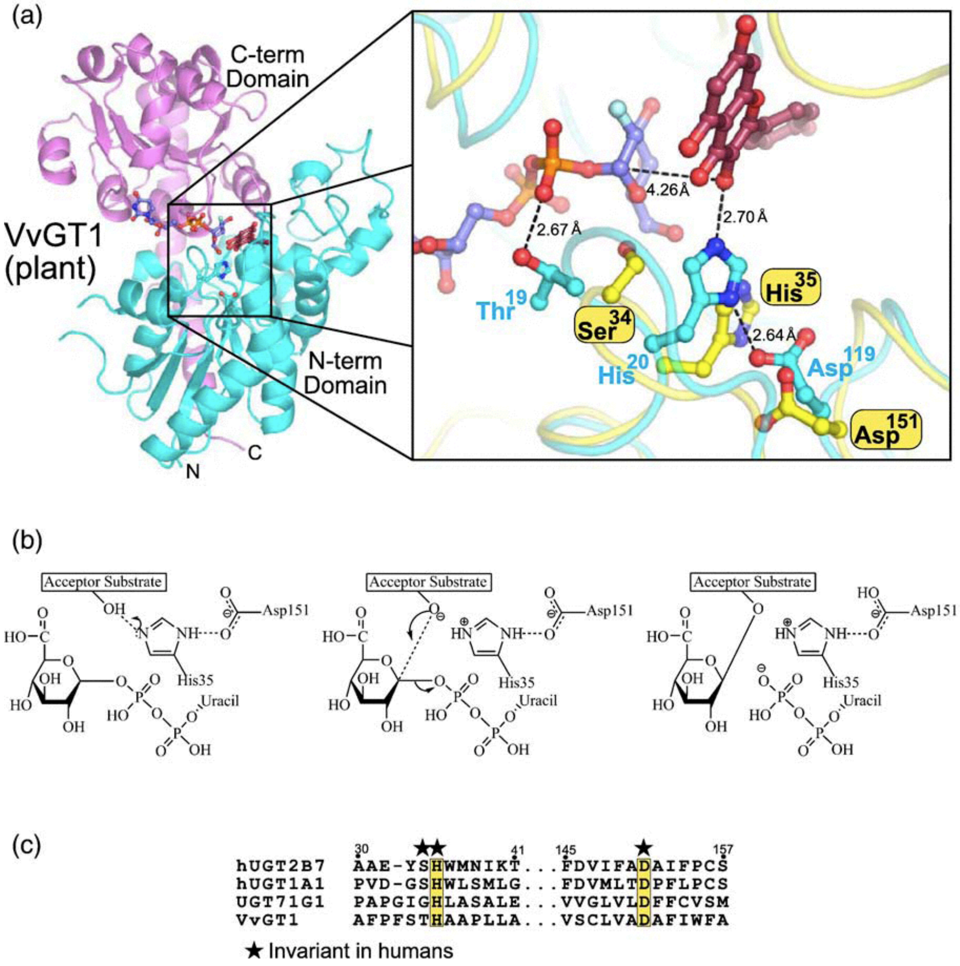
Will revisit this transport later

III. UGT Structure:Model Structure of UGT2B7 Based on Crystal Structure of C-terminal Domain Combined with Homology Model of N-terminus based on Glycosyl transferase

Miley et al., J. Mol. Biol. (2007) 369: 498



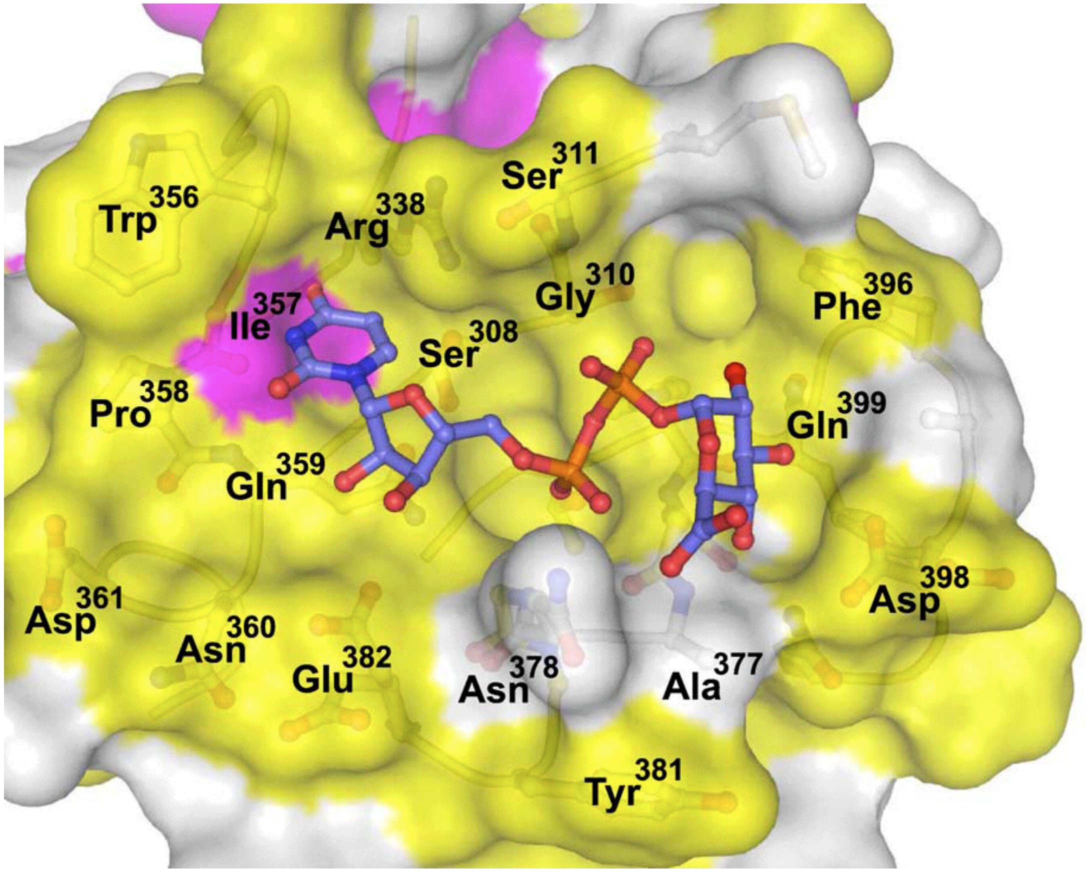
III. UGTs: Structure and Function, Catalytic Mechanism



The crystal structure of a C-terminal fragment of UGT2B7 and work using mutagenesis and chemical modification studies with pure enzymes - a general acid-base mechanism is postulated involving His-35 to deprotonate the attacking nucleophile.

His-35 is invariant in human UGTs.

III. UGTs: Structure/Function



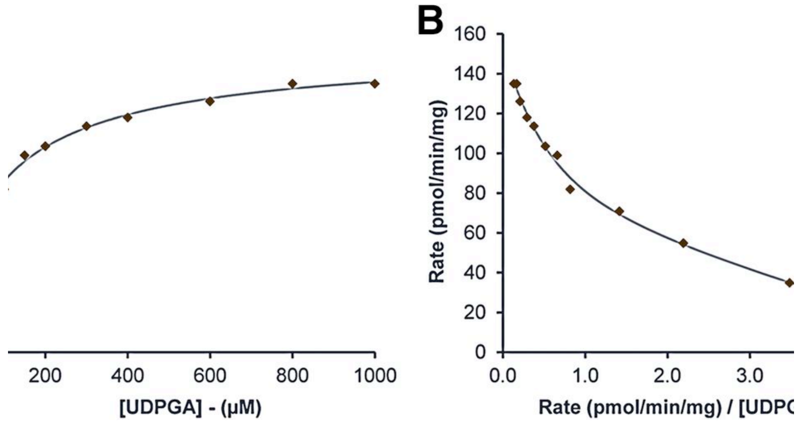
Glucuronic acid binding site, based on docking to apo enzyme 2B7structure, indicates the site is nearly invariant among all human UGTs.

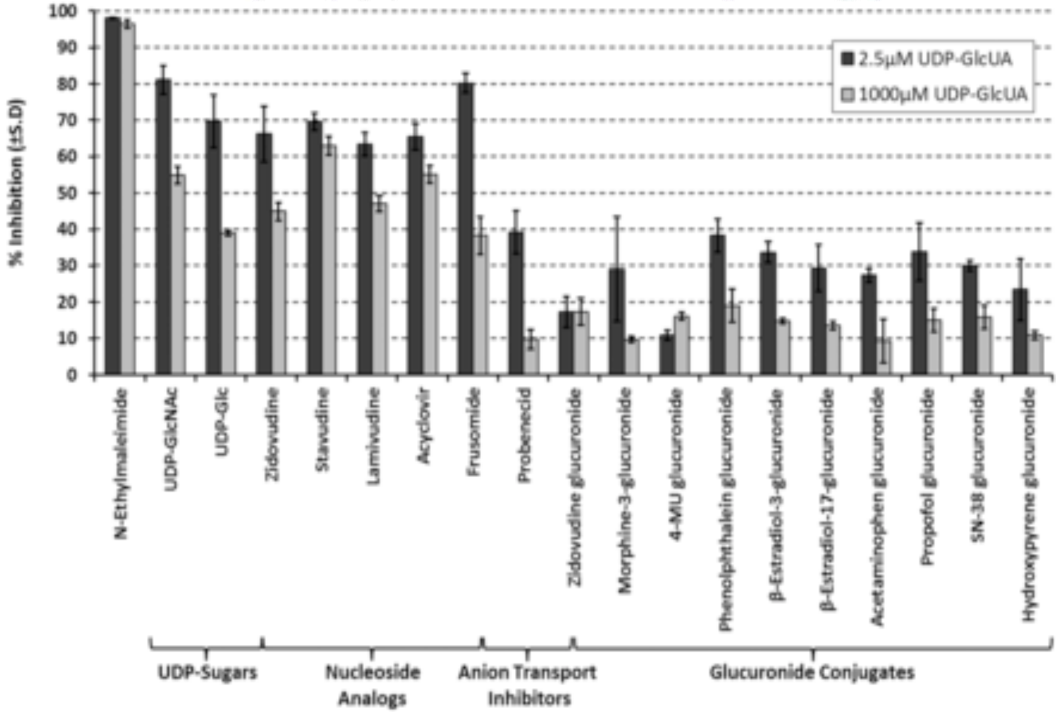
Yellow: completely conserved.

White: “high conservation”

Pink: low conservation, but note only backbone interactions.

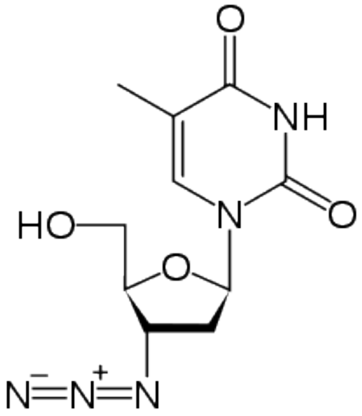
III. Revisit UDPGA Transport: How Does UDPGA Get into ER Lumen?





A UDPGA transporter in ER has been partially characterized in human microsomes. It is multicomponent, with different K_M and similar V_{max}

It is inhibited by glucuronide conjugates and nucleoside drugs. This could limit UDPGA pools in the ER lumen and limit glucuronidation. Note Zidovudine, lamivudine etc are inhibitors – reverse transcriptase inhibitors/HIV drugs.
Rowland et al., DMD: 43:147-153, 2015.



III. UGTs: Polymorphisms

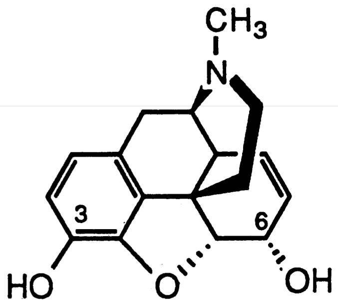
- UGT1A1 is primarily responsible for the glucuronidation of bilirubin in vivo, and phenotypic differences occurring as a consequence of altered expression and activity of this enzyme are readily discernible. Three forms of inheritable unconjugated hyperbilirubinaemia exist in man; Crigler–Najjar syndromes type I and II, and Gilbert syndrome The former are rare genetic traits characterized by absent or very low UGT1A1 activity, and arise from mutant coding region alleles and promoter polymorphisms. Gilbert's syndrome is a chronic, mild hyperbilirubinaemia inherited as an autosomal recessive trait.

- UGT1A6 Two missense mutations in exon 1 of UGT1A6 result in Thr181Ala and Arg184Ser substitutions. UGT1A6 glucuronidates many xenobiotic phenols, and rates of in vitro metabolism recombinant UGT1A6*2 were lower than the wild-type enzyme. It is unknown whether these differences translate to altered metabolism in vivo.

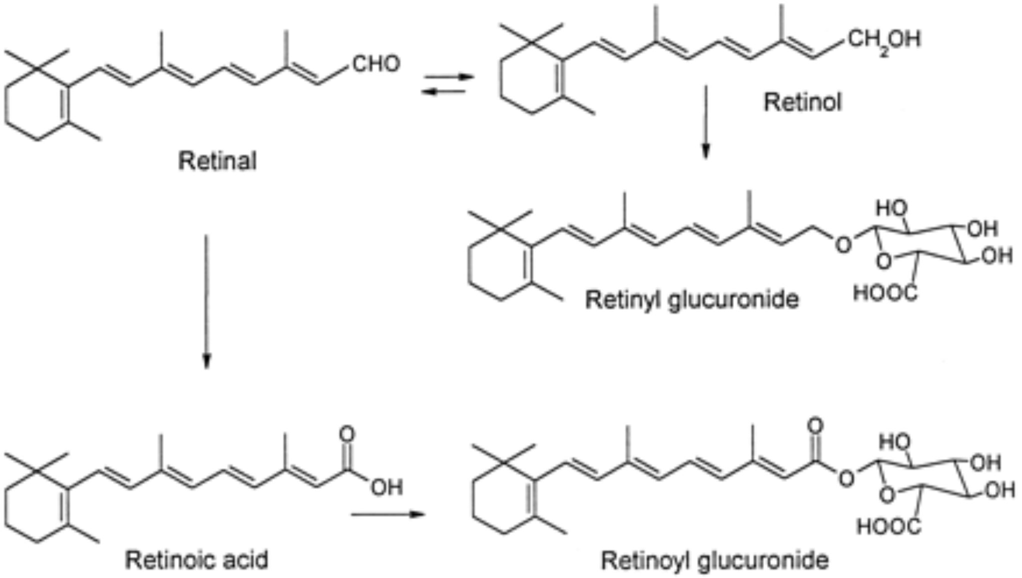
III. UGTs: Polymorphisms

- UGT1A7 Three missense mutations in exon 1 of UGT1A7 result in the existence of four alleles; UGT1A7*1 (Asn129, Arg131, Trp208), UGT1A7*2 (Lys129,131, Trp208), UGT1A7*3 (Lys129,131, Arg208), and UGT1A7*4 (Asn129, Arg131,208). UGT1A7 is expressed in human lung, but not liver, and hence individuals homozygous for the low activity UGT1A7*3 allele (~15% of the population) may be at increased risk to polycyclic aromatic hydrocarbon exposure in the lungs.
- UGT2B7 forms glucuronides from a wide range of xenobiotics and hydroxy-steroids. A C to T transversion at nucleotide 802 of the UGT2B7 coding region gives rise to enzymes with either His (UGT2B7*1) or Tyr (UGT2B7*2) at residue 268. Although studies with the expressed variants have suggested that UGT2B7*1 may be more active towards some substrates (e.g. zidovudine), differences were not apparent in rates of metabolism of a number of UGT2B7 substrates by microsomes from genotyped livers.

IV. Reactions of Glucuronides



Morphine



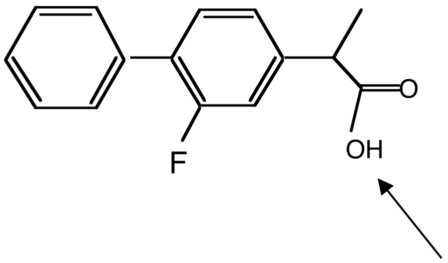
A. Pharmacological Effects:

Glucuronide conjugates may themselves be directly pharmacologically active. e.g. morphine 3-OH and 6-OH glucuronides are formed and the 6-OH may be as or more active than morphine, at least with some classes of opiate receptors.

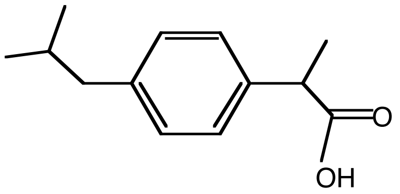
Other eg.'s include cardiac glycosides, retinoids.

IV. Reactions of Glucuronides

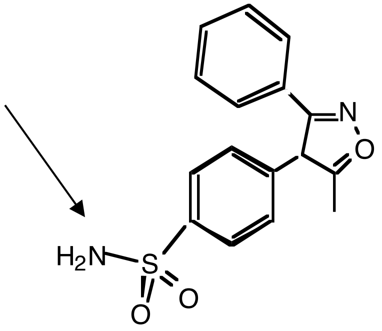
NSAIDS



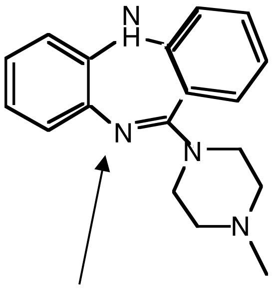
Flurbiprofen



Ibuprofen



Valdecoxib



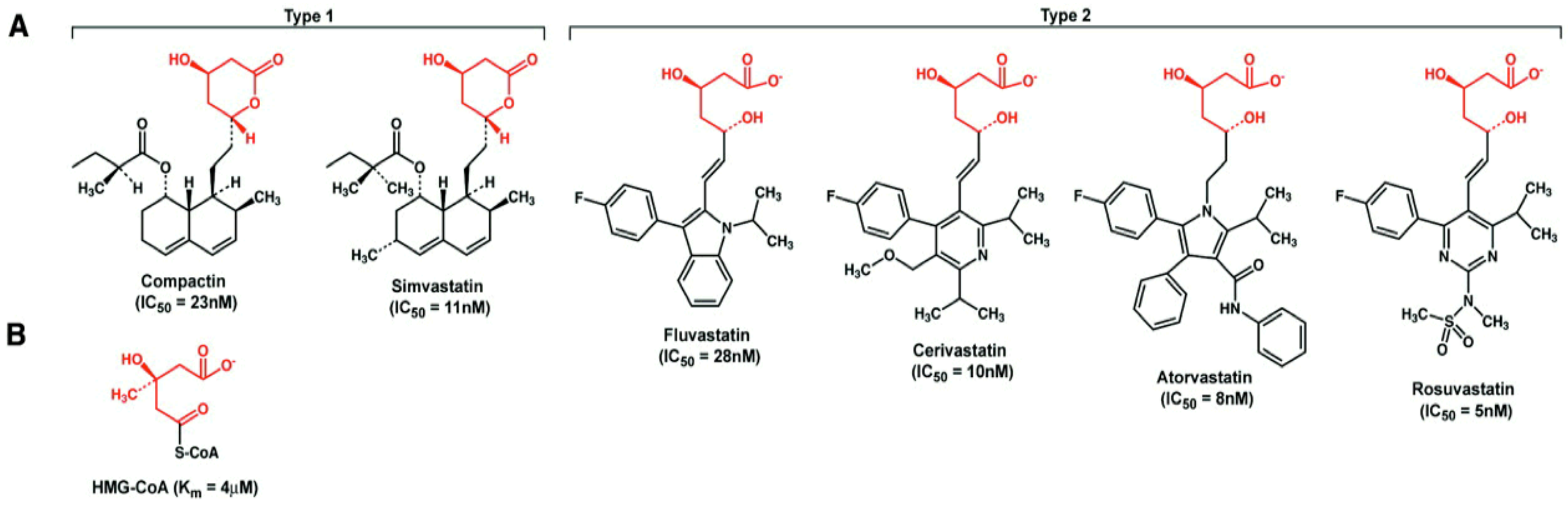
Clozapine

B. Regeneration of aglycone. Non-enzymatic hydrolysis or β-Glucuronidases may lead to "futile cycle", due to regeneration of aglycone. e.g.'s include NSAIDS, zomepirac, gemfibrozil, clofibrate, statins, and N-glucuronides others.

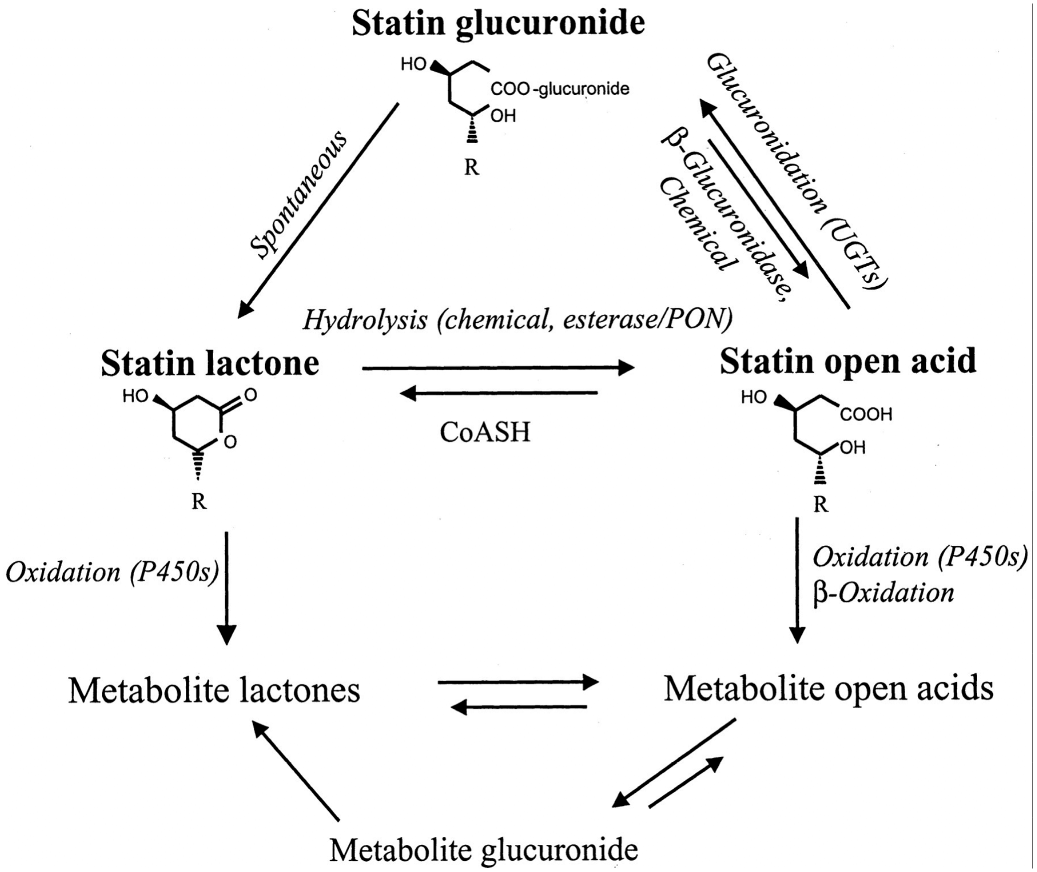
Analytical difficulties result from non-enzymatic hydrolysis. N-Glucs more stable than O- or S-Glucs,except at low pH.

It has been claimed that some tumors demonstrate elevated levels of β-glucuronidase, so glucuronide conjugates of antitumor compounds may provide a targeted delivery system.

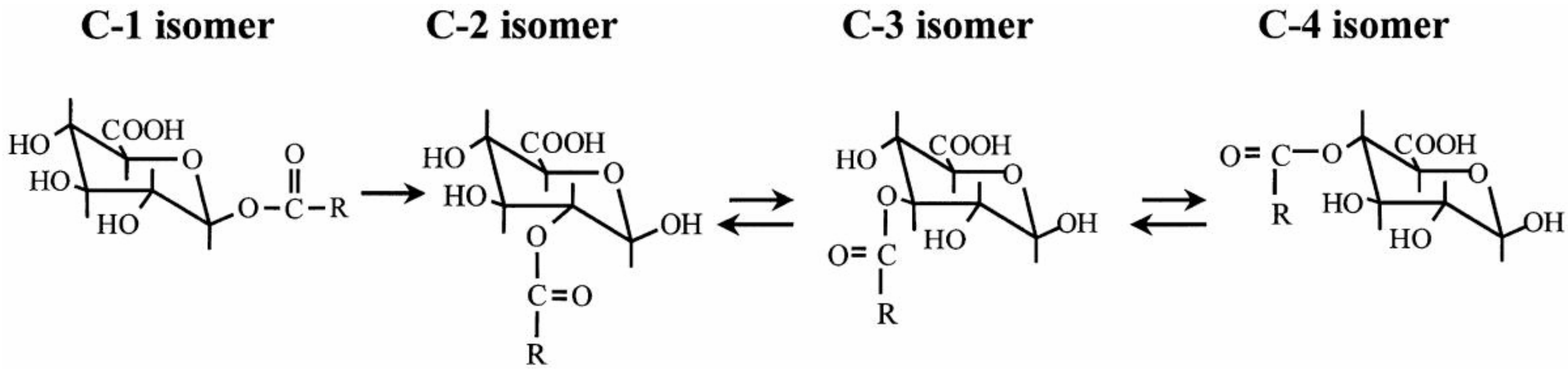
IV. Reactions of Glucuronides: Statins as an example of futile cycling



IV. Reactions of Glucuronides: Statins as an example of futile cycling



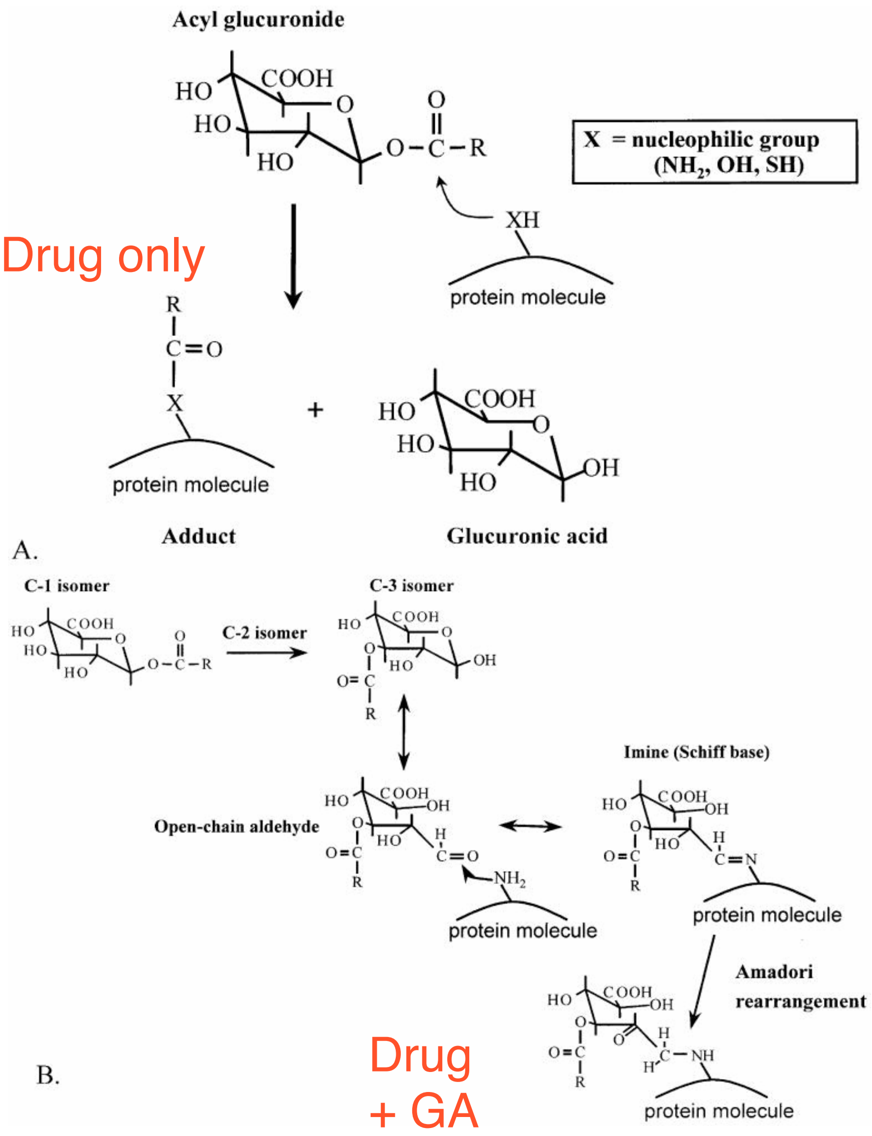
IV. Reactions of Glucuronides:



C. Intramolecular Rearrangement of Acyl glucuronides.
Glucuronides formed from carboxylic acids exhibit ‘acyl migration.’

- base catalyzed
- complicates analysis
- regioisomers other than 1-O-acyl are resistant to β-glucuronidases

IV. Reactions of Glucuronides



Drug only

Drug + GA

D. Acylation of protein nucleophiles, mainly at cys but also N-, O- nucleophiles. GSH a good 'acceptor.'

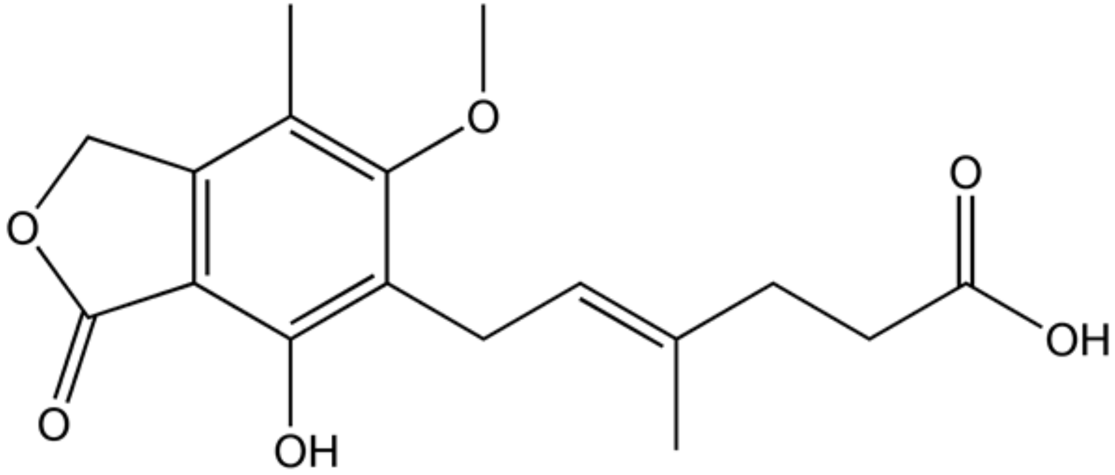
E. Acyl migration, followed by ring-opening and reaction with amines. e.g.'s Clofibric acid, benoxaprofen, form imine linkage with albumin and form stable α-keto-amine.

IV. Reactions of Glucuronides

Examples of toxic acylglucuronides:

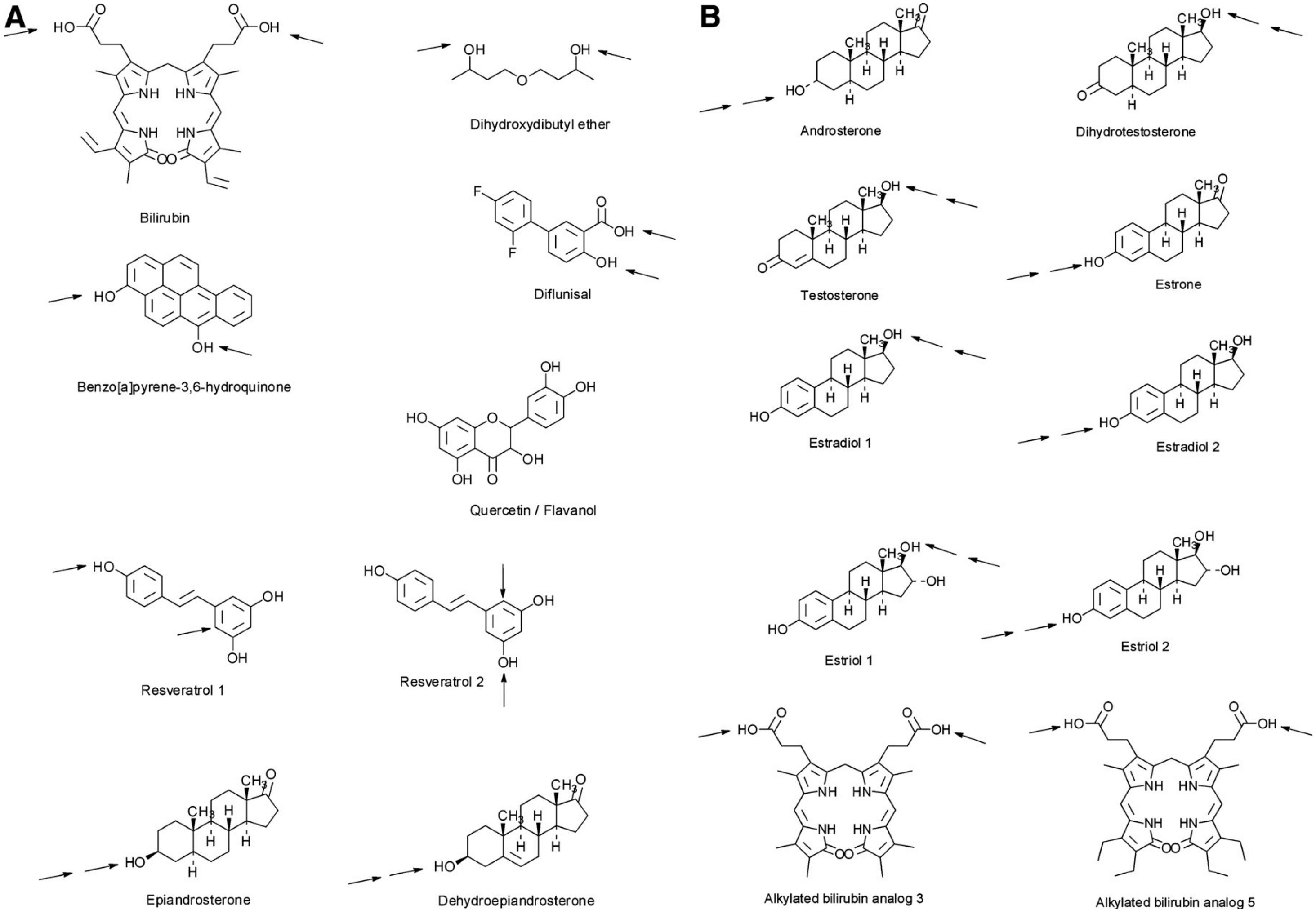
NSAIDs

Mycophenolic acid - immunosuppressant, protein adducts identified in rat models include ATP synthetase, protein disulfide isomerase, and selenium binding protein - whether these adducts are causally related to to toxicity is not known, and if so the mechanism is not established. In humans, only albumin adducts in plasma, so far.



IV. Reactions of Glucuronides

F. Formation of bis-glucuronides or diglucuronides



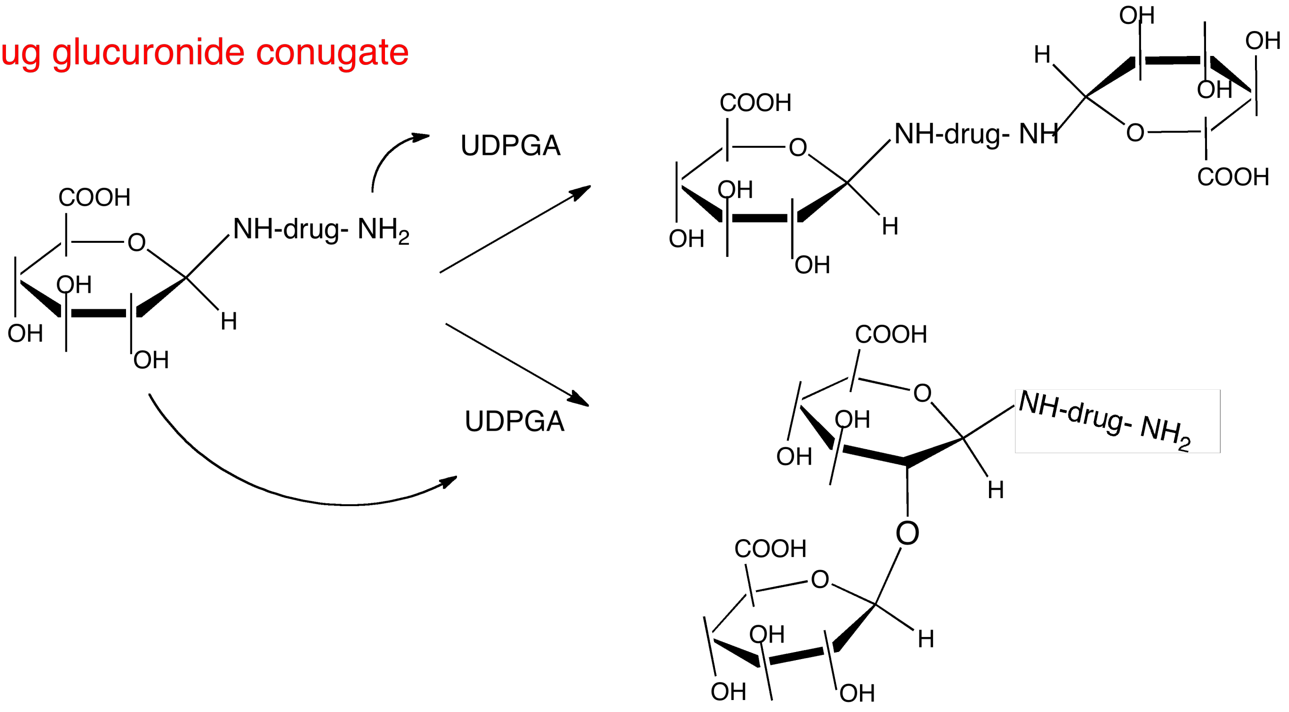
Head-to-tail arrows: ‘diglucuronides,’ via 2-OH group of first

Separate arrows: ‘bis-glucuronides.’ Or ‘discreet’ vs. ‘linked’

IV. Reactions – Glucuronides reacting with UDPGA

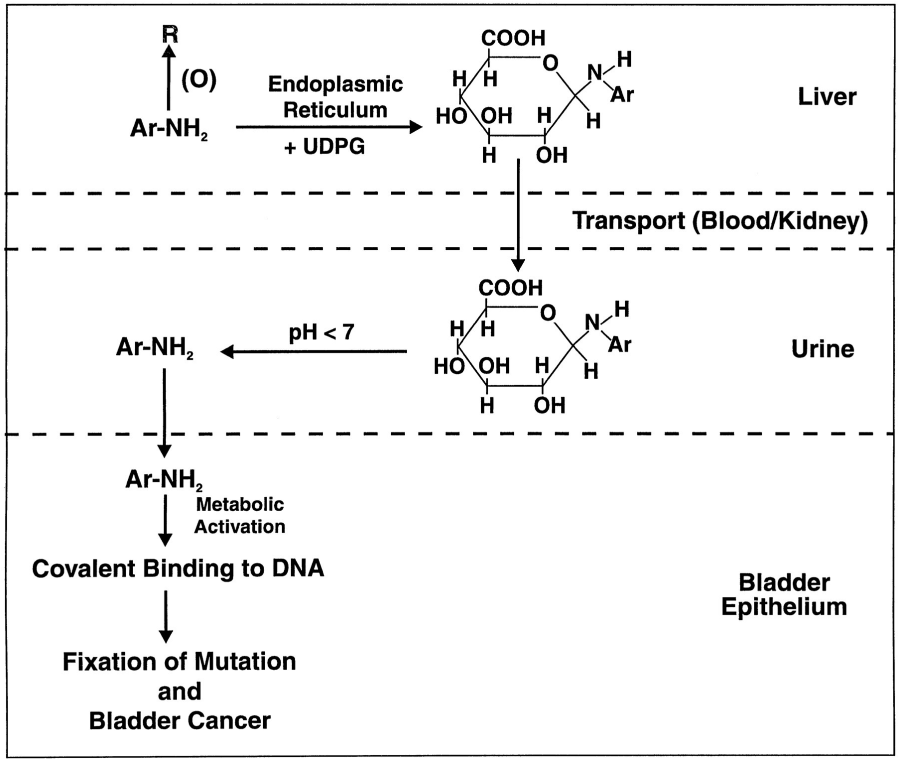
discreet diglucuronide, or 'bis'

drug glucuronide conugate



linked diglucuronide, or just "di'

IV. Reactions of Glucuronides: role of N-glucuronides of aryl amines in bladder cancer



N-glucuronidation has received much attention due to potential roles in toxicity /detoxification of aryl amines. N-glucuronides may act as vehicles for transport of the conjugates to bladder and kidney. N-glucuronides of aryl amines are acid labile, and regeneration of parent aryl amine in the urine occurs. Oxidative activation of the aryl amine generates the toxic N-hydroxy metabolite in the bladder.

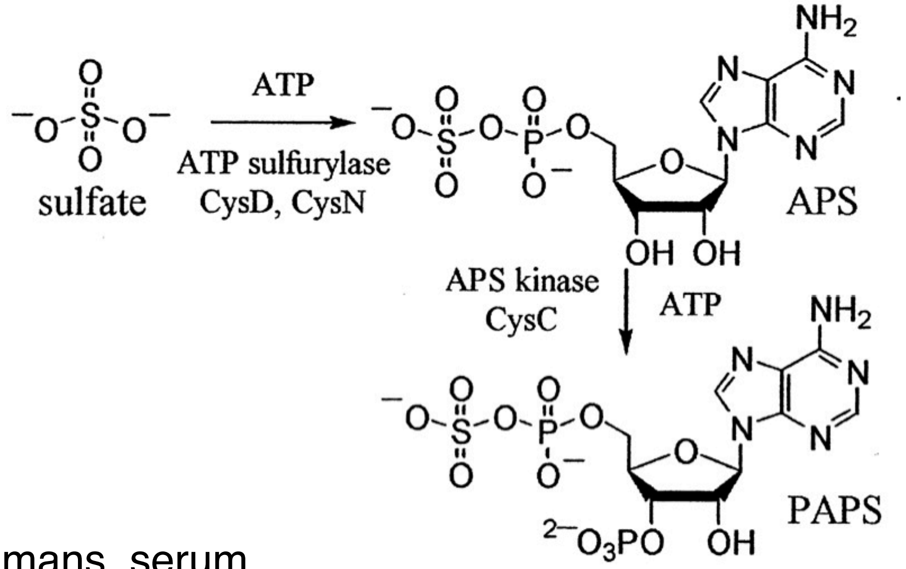
Sulfonation and Sulfotransferases

- PAPS Metabolism
- Sulfonation Reactions
- PAPS-dependent SULTs
- Reactions of Sulfate Conjugates

Overview:

- Sulfonation less extensive than glucuronidation
- similar preference for functional groups - a subset of the acceptor types that glucuronidation has e.g. phenols, alcohols, arylamines.
- Many endogenous substrates,steroids, bile acids and phenols, neurotransmitters, proteins, carbohydrates, etc.
- Sulfonation is a low capacity, high affinity system: Glucuronidation is high capacity, low affinity.

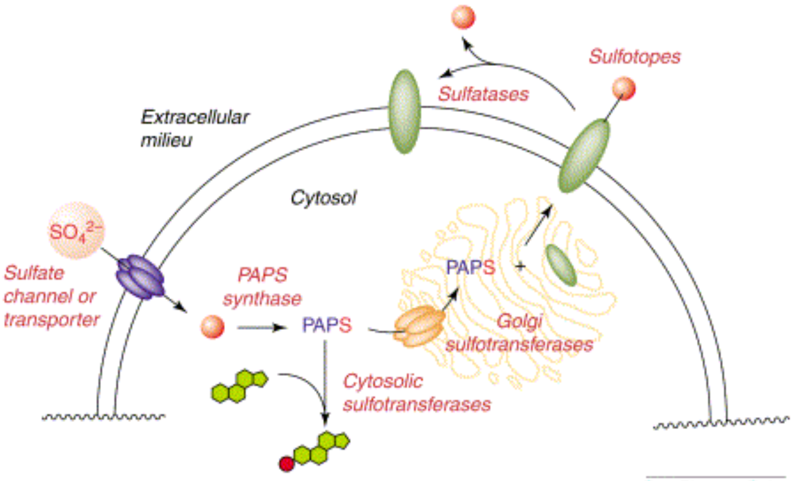
I. PAPS Metabolism: Biosynthesis



in humans, serum
$[SO_4]^{-2}$ is ~0.3 mM

3’-phospho-adenosine-5’-phosphosulfate

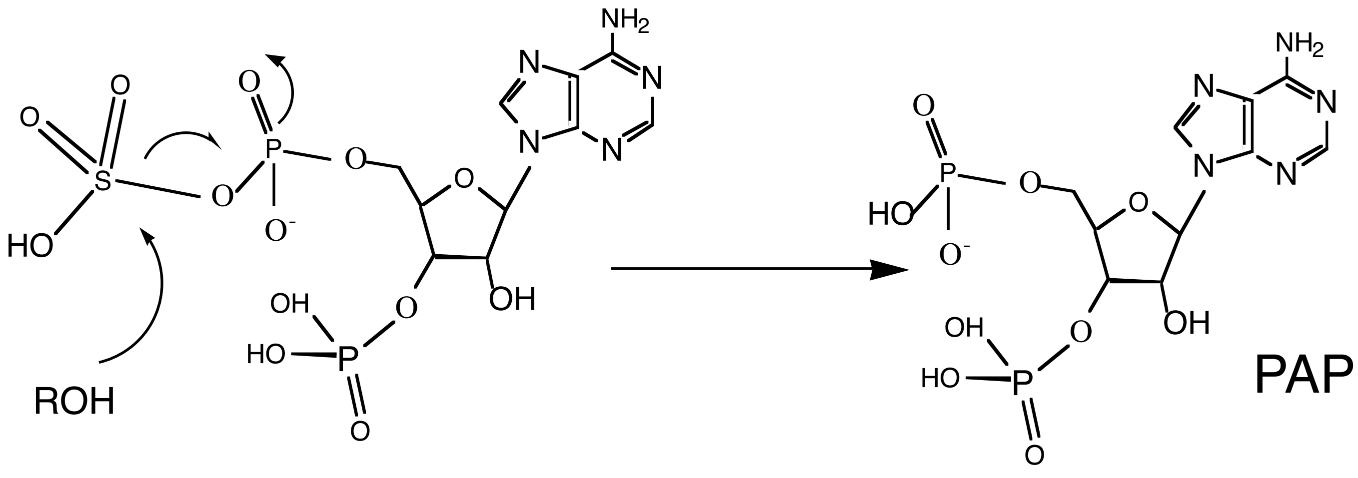
I. PAPS Metabolism



Drug Discovery Today

- Specific transporters take up sulfate.
- PAPS can be used in the cytosol or transported into Golgi.
- Cytosolic SULTs metabolize small molecules.
- Golgi SULTs metabolize glycoproteins, glycolipids.

II. Sulfonation Reaction: Chemical Strategy



PAPS

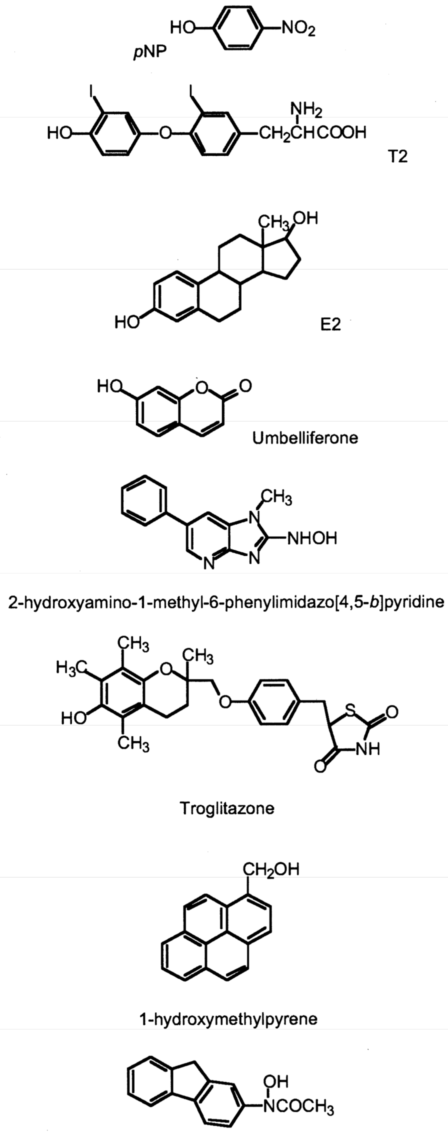
PAP

+ R-O-SO$_3$H

Chemical strategy is to provide an electrophilic site on a water soluble co-factor by providing a good leaving group.

II. Sulfonation Reactions:Typical Substrates

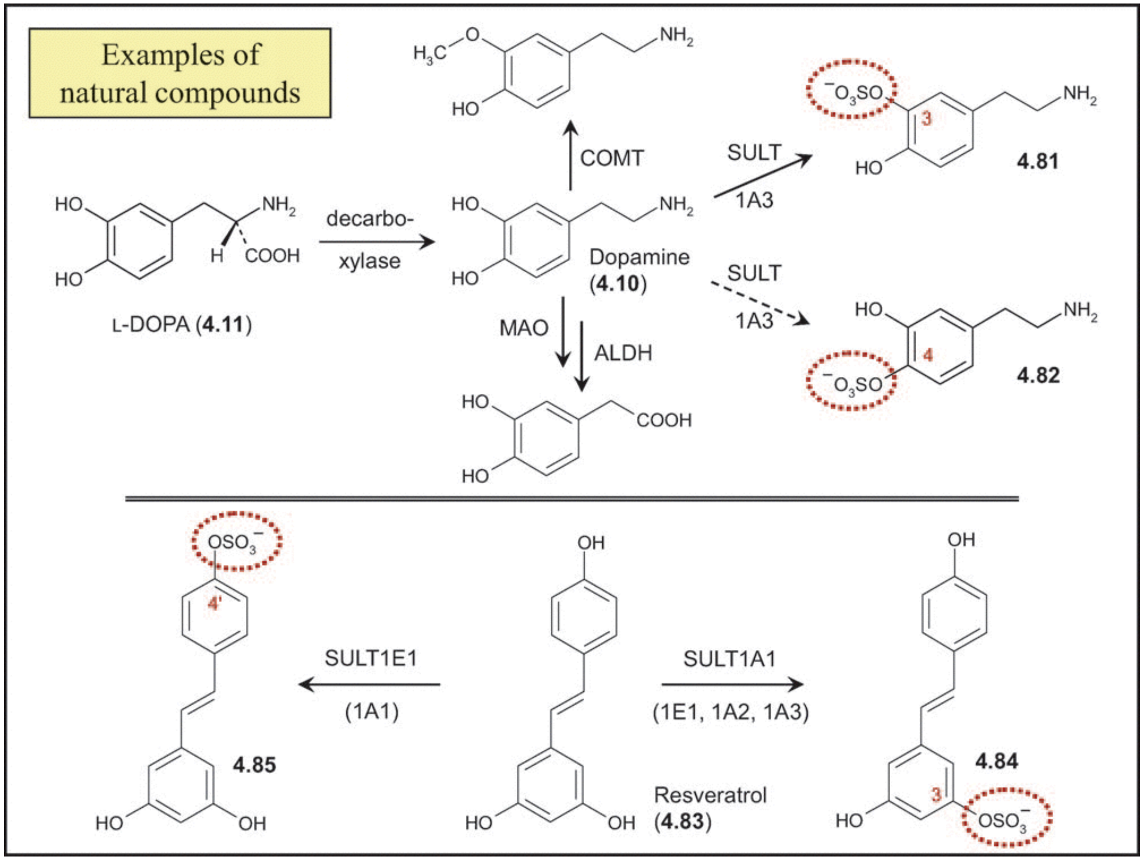
Fig. 1



- Others include minoxidil, apomorphine, tamoxifen:

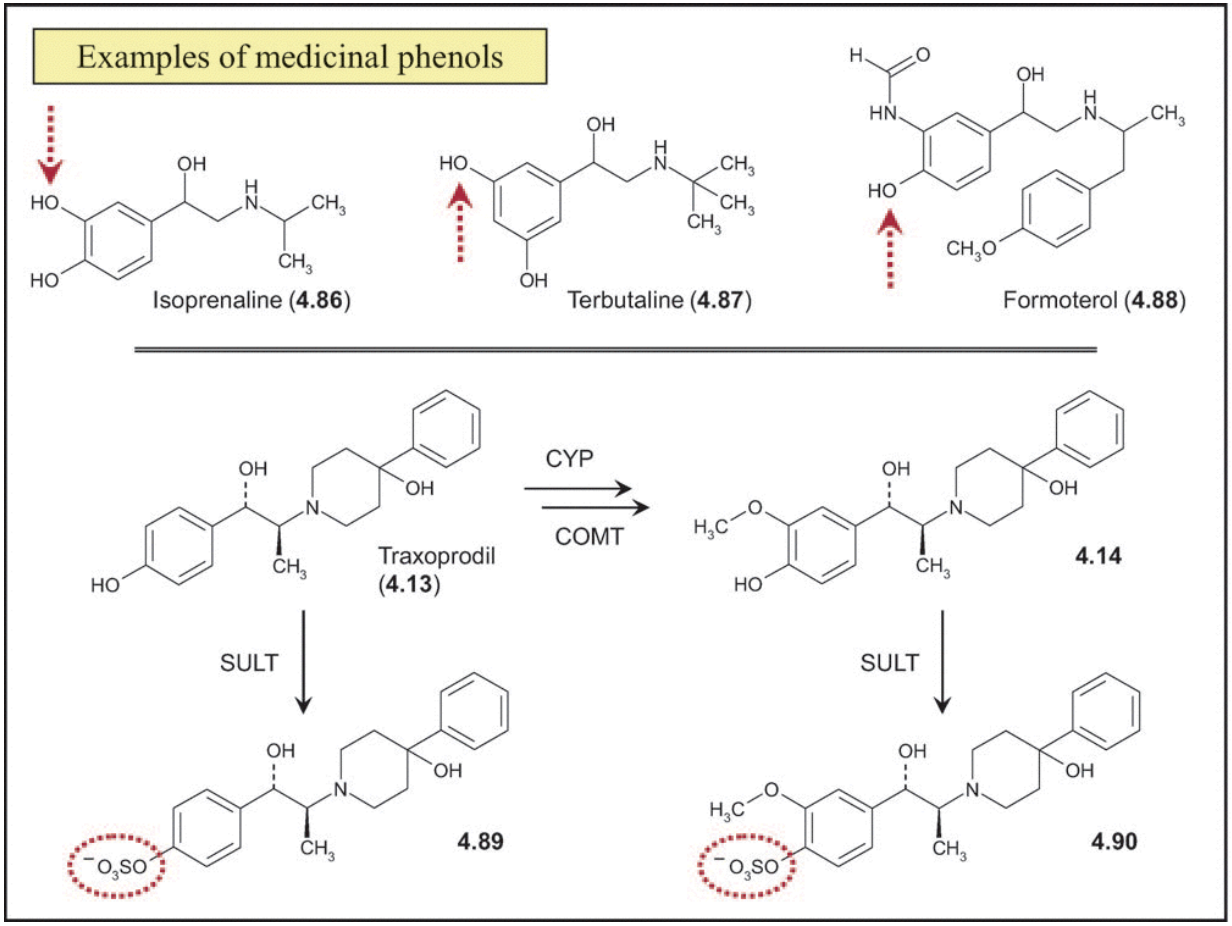
No thiols, carbanions, carboxylates

II. Sulfonation Reactions
O-Sulfonation

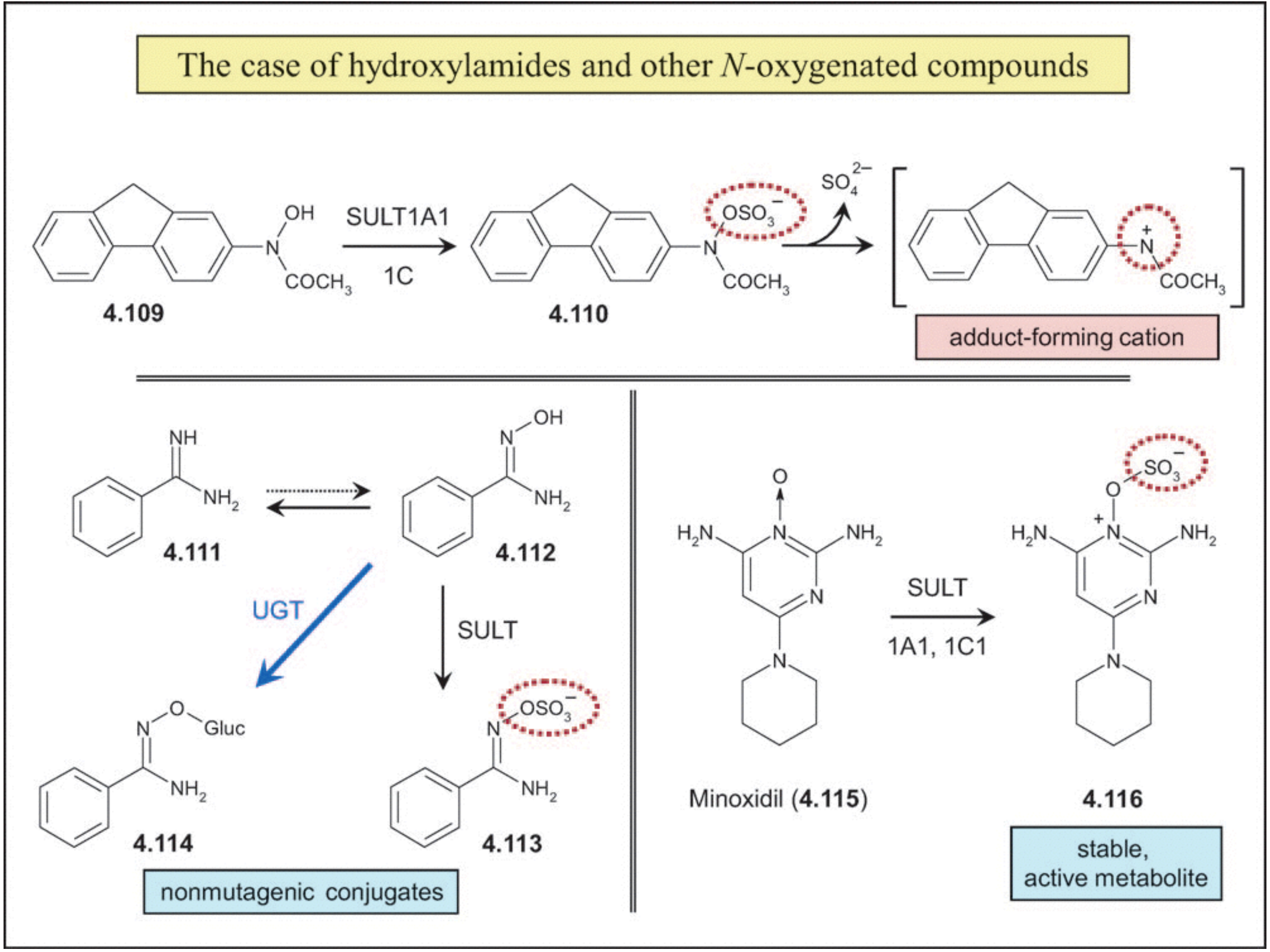


- ‘endogenous’ O-sulfates formed from catecholic neurotransmitters.
- Natural compounds in diet are O-sulfonated - resveratrol

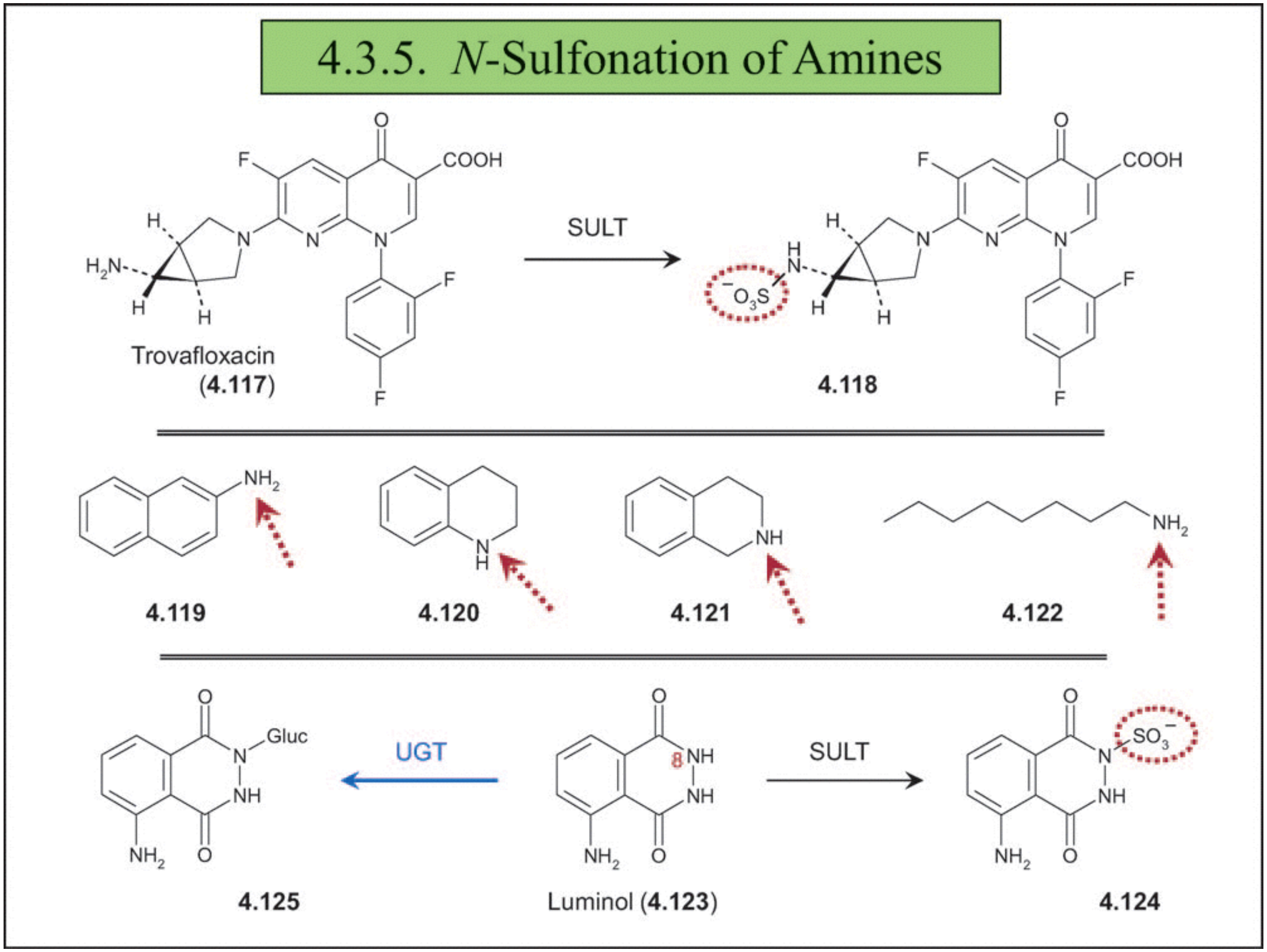
II. Sulfonation Reactions: O-Sulfonation of Drugs



II. Sulfonation: O-Sulfonation at N-hydroxyl Amines, N-hydroxy Amides

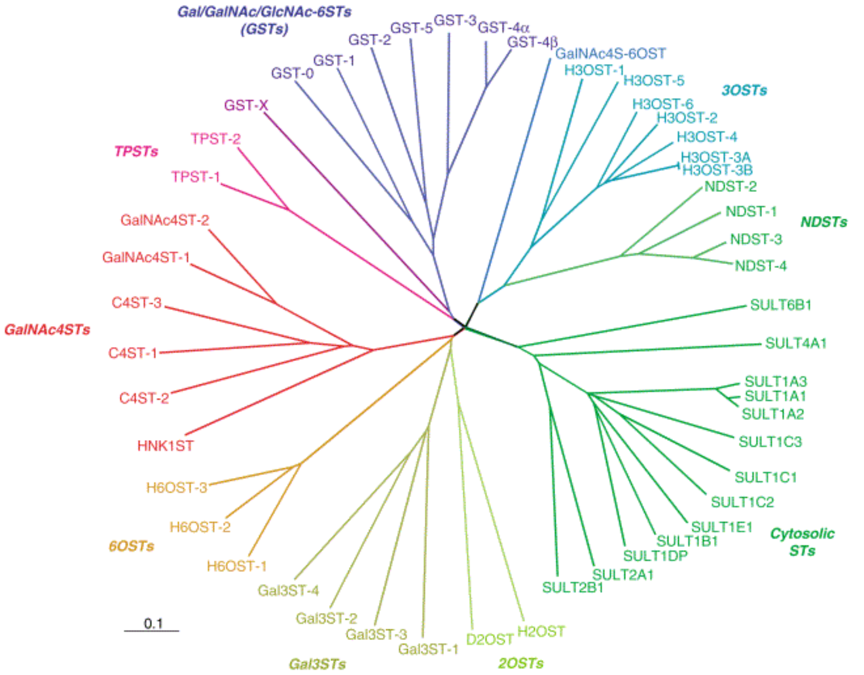


II. Sulfonation Reactions: N-Sulfonation

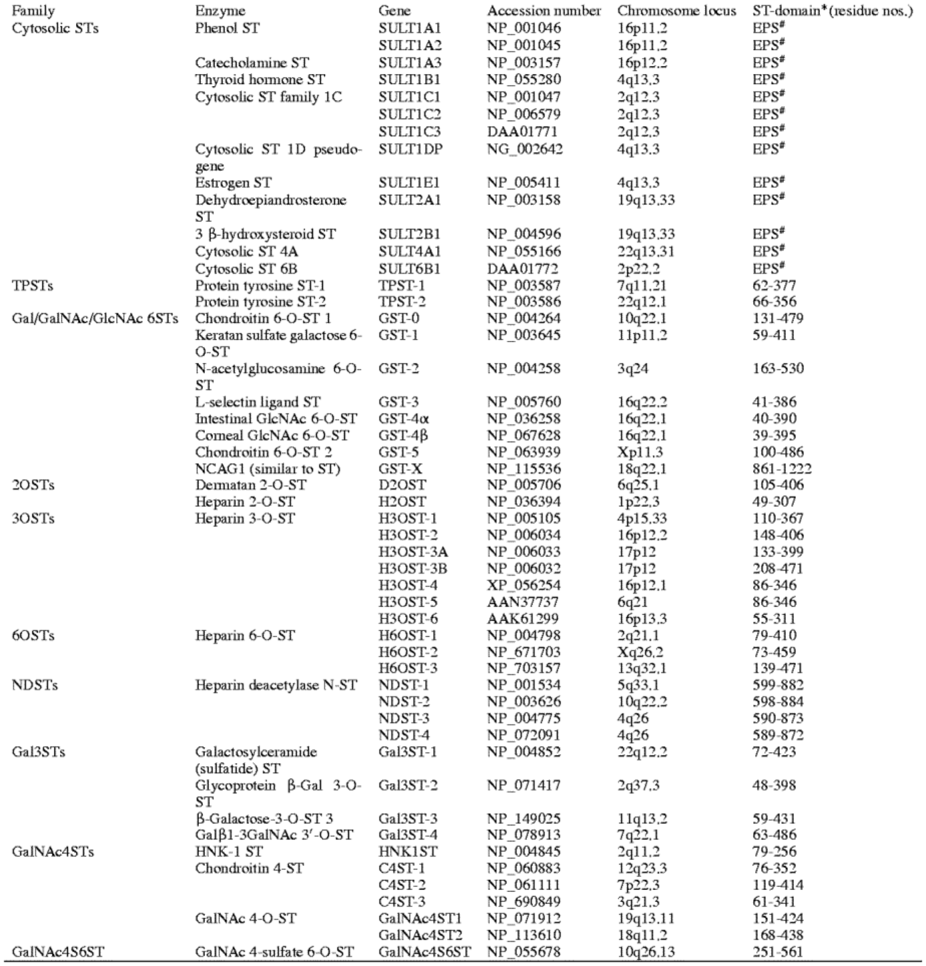


4.3.5. *N*-Sulfonation of Amines

III. Sulfotransferases: SULT’ s



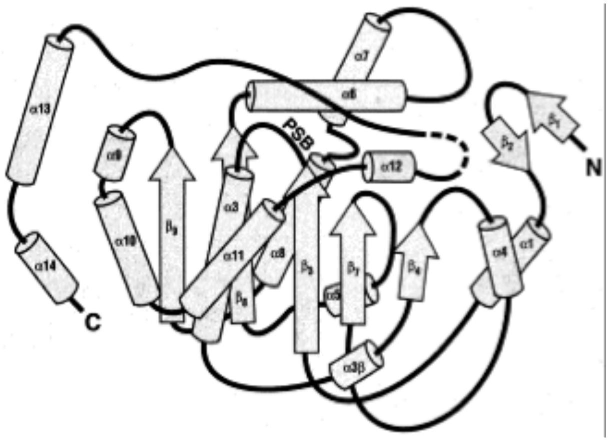
Drug Discovery Today



Family	Enzyme	Gene	Accession number	Chromosome locus	ST-domain* (residue nos.)
Cytosolic STs	Phenol ST	SULT1A1	NP_001046	16p11.2	EPS[#]
		SULT1A2	NP_001045	16p11.2	EPS[#]
	Catecholamine ST	SULT1A3	NP_003157	16p12.2	EPS[#]
	Thyroid hormone ST	SULT1B1	NP_055280	4q13.3	EPS[#]
	Cytosolic ST family 1C	SULT1C1	NP_001047	2q12.3	EPS[#]
		SULT1C2	NP_006579	2q12.3	EPS[#]
		SULT1C3	DAA01771	2q12.3	EPS[#]
	Cytosolic ST 1D pseudogene	SULT1DP	NG_002642	4q13.3	EPS[#]
	Estrogen ST	SULT1E1	NP_005411	4q13.3	EPS[#]
	Dehydroepiandrosterone ST	SULT2A1	NP_003158	19q13.33	EPS[#]
	3 β-hydroxysteroid ST	SULT2B1	NP_004596	19q13.33	EPS[#]
	Cytosolic ST 4A	SULT4A1	NP_055166	22q13.31	EPS[#]
	Cytosolic ST 6B	SULT6B1	DAA01772	2p22.2	EPS[#]
TPSTs	Protein tyrosine ST-1	TPST-1	NP_003587	7q11.21	62-377
	Protein tyrosine ST-2	TPST-2	NP_003586	22q12.1	66-356
Gal/GalNAc/GlcNAc 6STs	Chondroitin 6-O-ST 1	GST-0	NP_004264	10q22.1	131-479
	Keratan sulfate galactose 6-O-ST	GST-1	NP_003645	11p11.2	59-411
	N-acetylglucosamine 6-O-ST	GST-2	NP_004258	3q24	163-530
	L-selectin ligand ST	GST-3	NP_005760	16q22.2	41-386
	Intestinal GlcNAc 6-O-ST	GST-4α	NP_036258	16q22.1	40-390
	Corneal GlcNAc 6-O-ST	GST-4β	NP_067628	16q22.1	39-395
	Chondroitin 6-O-ST 2	GST-5	NP_063939	Xp11.3	100-486
	NCAG1 (similar to ST)	GST-X	NP_115536	18q22.1	861-1222
2OSTs	Dermatan 2-O-ST	D2OST	NP_005706	6q25.1	105-406
	Heparin 2-O-ST	H2OST	NP_036394	1p22.3	49-307
3OSTs	Heparin 3-O-ST	H3OST-1	NP_005105	4p15.33	110-367
		H3OST-2	NP_006034	16p12.2	148-406
		H3OST-3A	NP_006033	17p12	133-399
		H3OST-3B	NP_006032	17p12	208-471
		H3OST-4	XP_056254	16p12.1	86-346
		H3OST-5	AAN37737	6q21	86-346
		H3OST-6	AAK61299	16p13.3	55-311
6OSTs	Heparin 6-O-ST	H6OST-1	NP_004798	2q21.1	79-410
		H6OST-2	NP_671703	Xq26.2	73-459
		H6OST-3	NP_703157	13q32.1	139-471
NDSTs	Heparin deacetylase N-ST	NDST-1	NP_001534	5q33.1	599-882
		NDST-2	NP_003626	10q22.2	598-884
		NDST-3	NP_004775	4q26	590-873
		NDST-4	NP_072091	4q26	589-872
Gal3STs	Galactosylceramide (sulfatide) ST	Gal3ST-1	NP_004852	22q12.2	72-423
	Glycoprotein β-Gal 3-O-ST	Gal3ST-2	NP_071417	2q37.3	48-398
	β-Galactose-3-O-ST 3	Gal3ST-3	NP_149025	11q13.2	59-431
	Galβ1-3GalNAc 3′-O-ST	Gal3ST-4	NP_078913	7q22.1	63-486
GalNAc4STs	HNK-1 ST	HNK1ST	NP_004845	2q11.2	79-256
	Chondroitin 4-ST	C4ST-1	NP_060883	12q23.3	76-352
		C4ST-2	NP_061111	7p22.3	119-414
		C4ST-3	NP_690849	3q21.3	61-341
	GalNAc 4-O-ST	GalNAc4ST1	NP_071912	19q13.11	151-424
		GalNAc4ST2	NP_113610	18q11.2	168-438
GalNAc4S6ST	GalNAc 4-sulfate 6-O-ST	GalNAc4S6ST	NP_055678	10q26.13	251-561

1. cytosolic- drugs, steroids, neurotransmitters
2. Golgi - glycolipids, proteins, glycoproteins

III. SULTs: Structure and Function



membrane bound forms with MW ~ 90-95 kD. Sulfates heparins, tyrosine's in proteins, proteoglycans, glycolipids. No known activity with drugs.

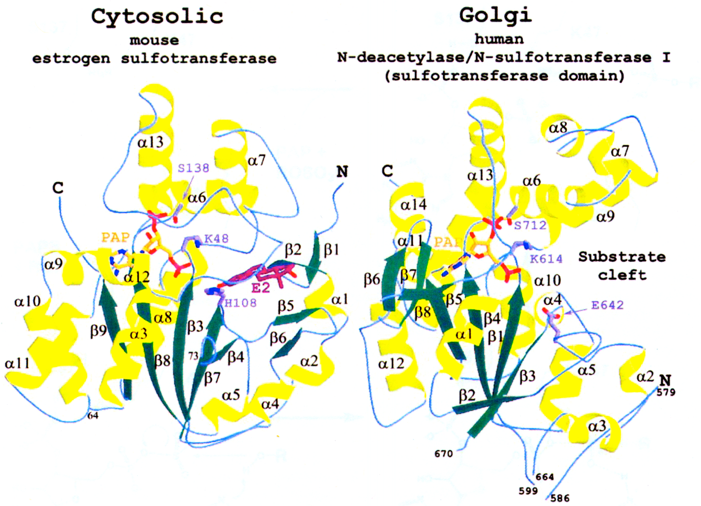
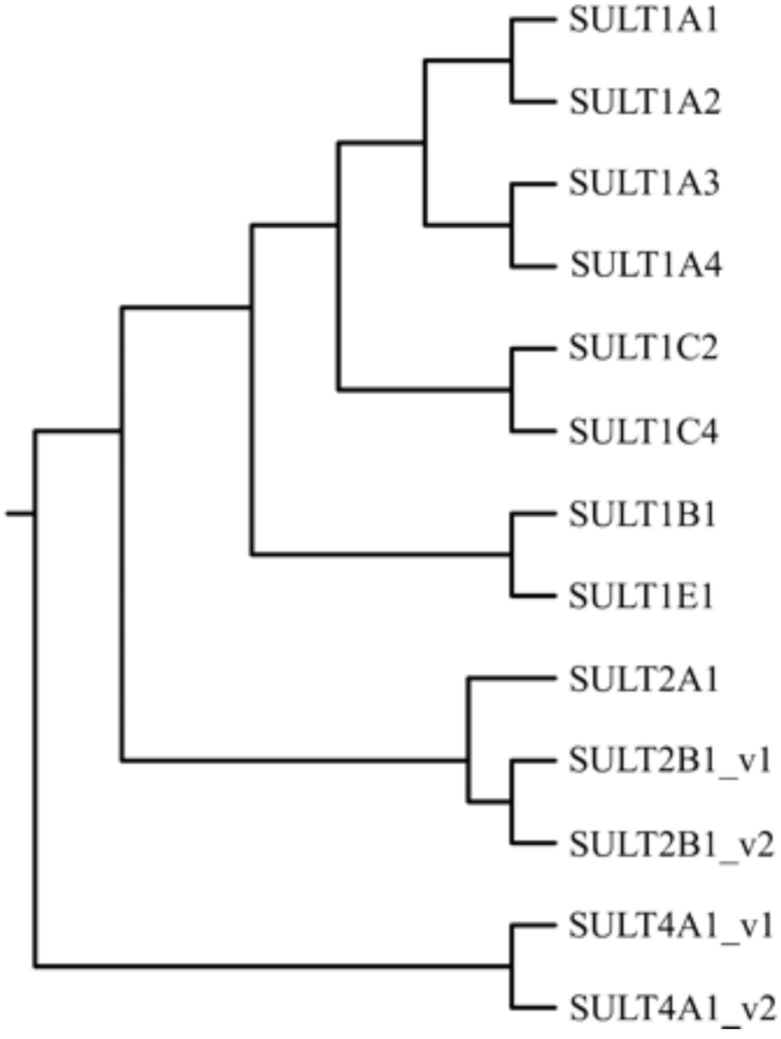


FIG. 2. Ribbon representation of X-ray crystal structures of ST enzymes. The structure of mEST is the ternary complex with PAP and E2, while the NST-1 structure contains only the PAP. Region between residues 586 and 599 is disordered in the NST-1 structure. This figure is created using Molscript (44) and Raster3D (45).

cytosolic forms with MW ~ 30 kD. sulfonation of drugs, steroids, bile acids, catechols. In humans, 13 isoforms known in 4 families

BUT: structurally related ! Share a common fold around a conserved active site.

III. SULTs: Phylogeny and Nomenclature



Isoforms important in drug metabolism have variable but overlapping tissue distribution:

SULT1A1 is highly expressed in hepatic tissue, with lower levels in extrahepatic sites.

SULT1A2 is found in the liver, but no extraheptic sites detected yet.

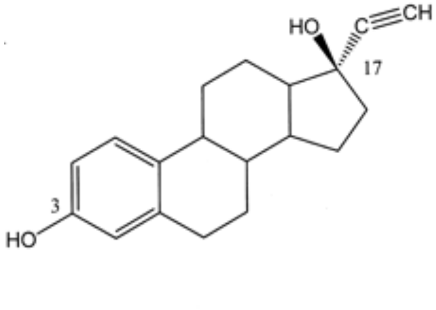
SULT1A3 is highly expressed in gut, with lower expression in other sites and negligible expression in hepatic tissue.

SULT1B1 is expressed in liver and colon.

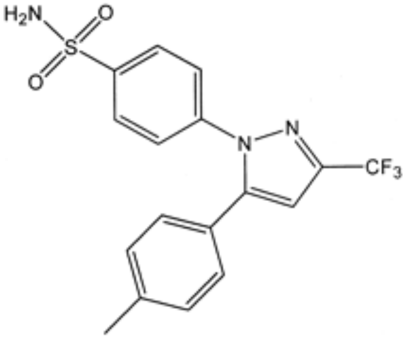
III. SULT’s: Substrate Selectivity

isoform	Alias	substrates	Homology with 1A1
ULT1A1	P-PST, HAST-1, H-PST, TS-PST-1	phenols, (hydroxysteroids)	
ULT1A2	HAST-4, TS-PST-2	phenols, (hydroxysteroids)	95%
ULT1A3	M-PST, HAST-3, TL-PST, AST-3	catecholamines	93%
ULT1B1	SULT1B2, hydroxylamine-ST	Thyroid hormones	54%
ULT1C1	ST1C2	Aryl hydroxylamines	37%
ULT1C2	ST1C3	Aryl hydroxylamines	41%
ULT1E1	EST	estrogens	51%
ULT2A1	DHEA-ST, DST, HSST-1, STa	hydroxysteroids	37%

III. SULT's: Kinetic Mechanism, Heterotropic Effects

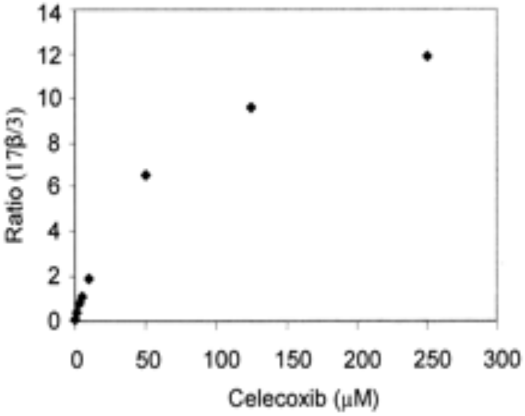


17α-Ethynylestradiol

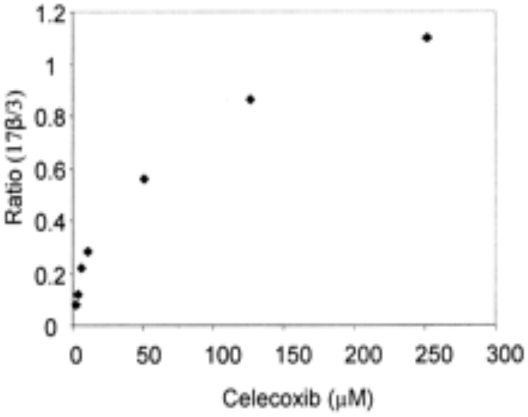


Celecoxib

(a) Expressed Human SULT2A1



(b) Human Liver Cytosol

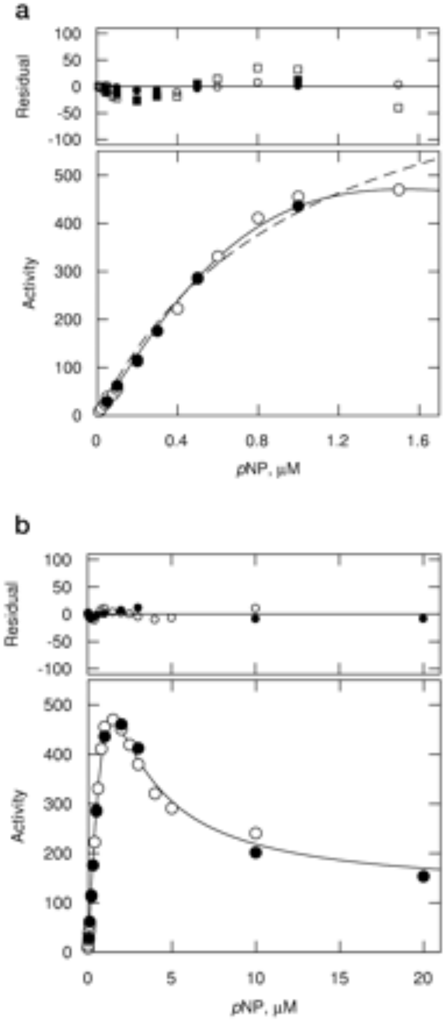


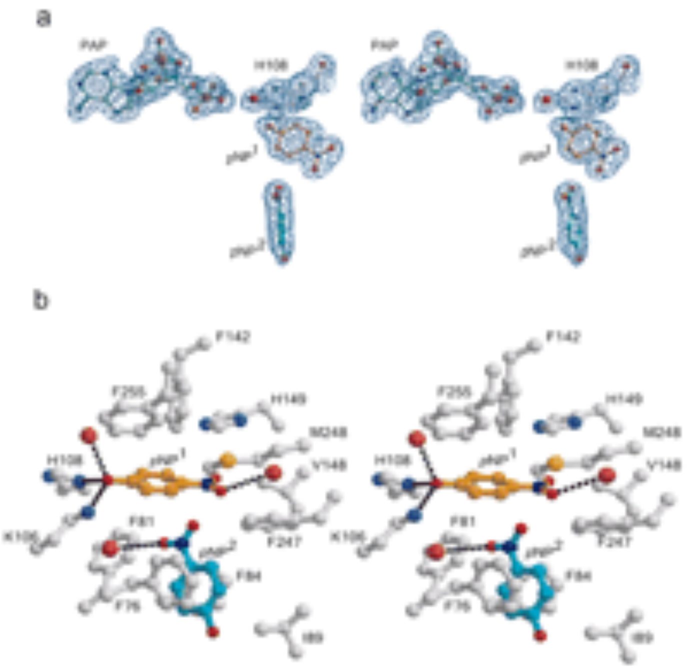
SULTs exhibit heterotropic effects - multiple binding on a single SULT.

e.g. celebrex alters regioselective sulfonation of ethynylestradiol

Cui, et. al. (2004) DMD 32:1260-1264.

III. SULT’s: Kinetic Mechanism: Homotropic Effects





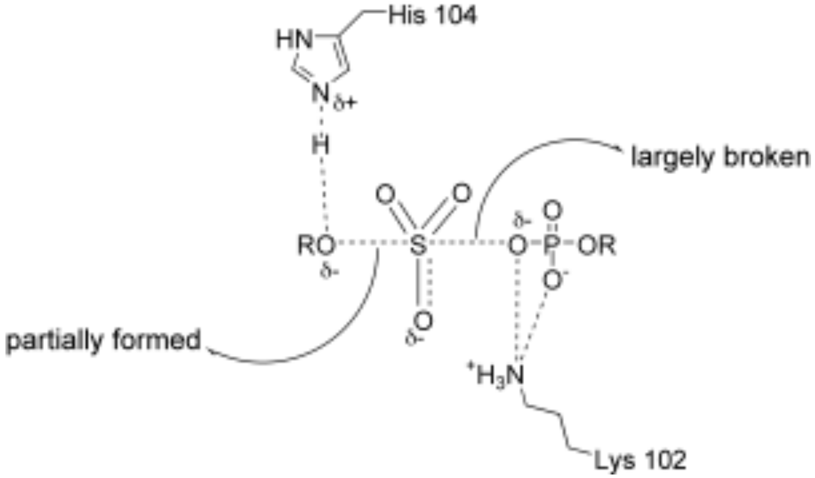
SULT’s exhibit homotropic effects - multiple binding to a single SULT.

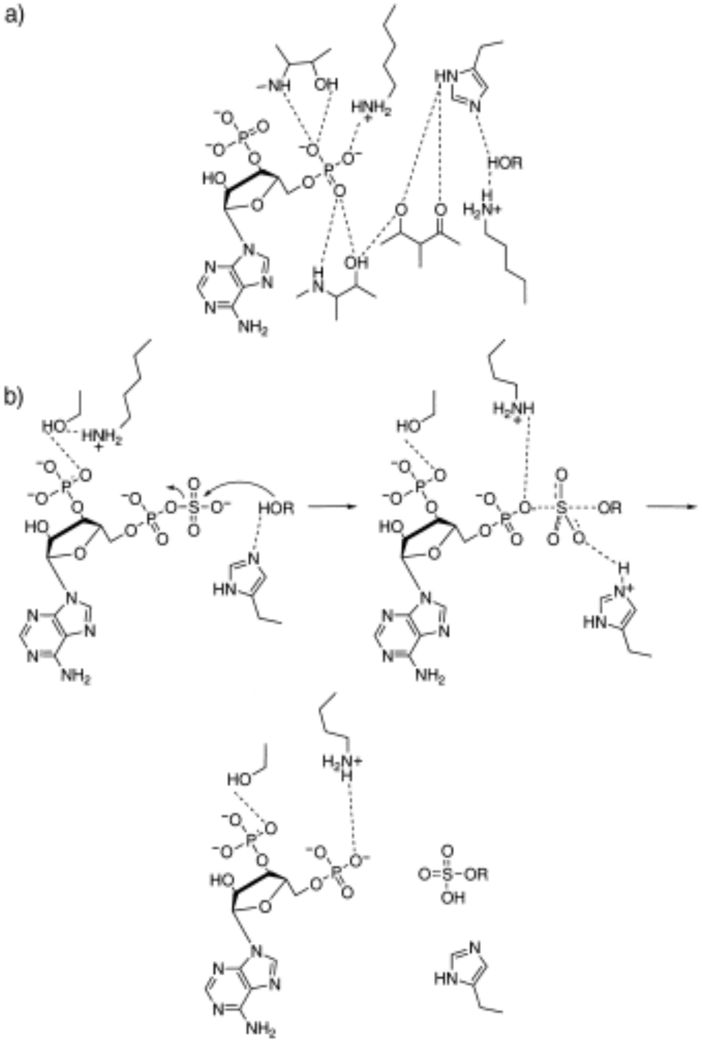
e.g. p-nitrophenol/SULT1A1

Gamage et. al. (2003) JBC 278: 7655-7662.

III. SULTs: Chemical Mechanism

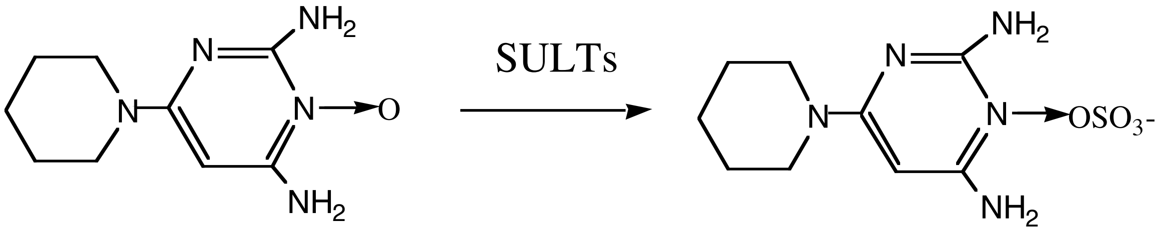
Conserved lysine moves to stabilize transition state, which results from in line attack - transition state is trigonal bipyramid





IV. Reactions of Sulfated Drugs

A. Pharmacological/therapeutic activity.



minoxodil

Pro-drug

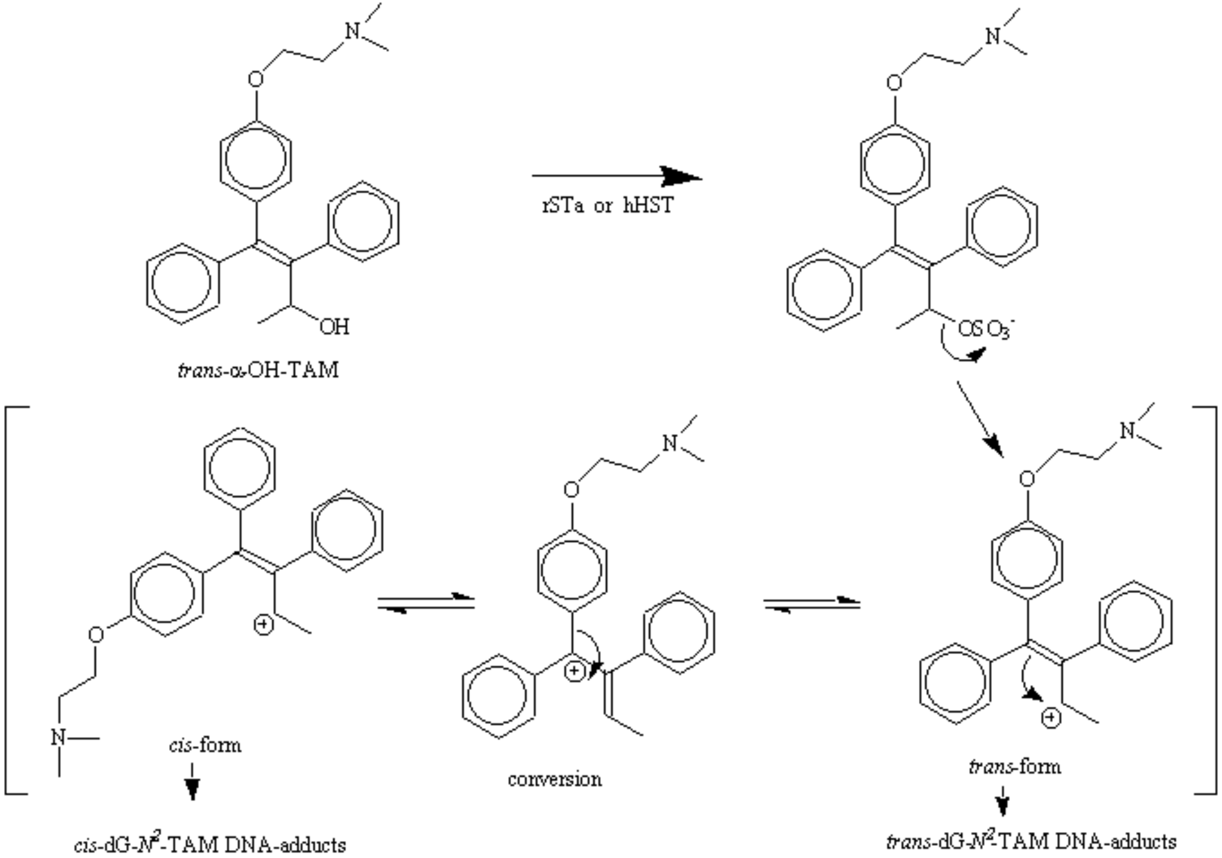
active
antihypertensive and hair
restorer

IV. Reactions of Sulfated Drugs

B.
 ‘bioactivation’
to toxins.

Sulfates of
Allylic, benzylic
alcohols -
decompose to
electrophilic
carbocations

Tamoxifen: anti-estrogen



IV. Reactions of Sulfated Drugs

2-aminofluorene is ‘activated’ to N-hydroxy or N-hydroxy, N-acetyl metabolites. These are sulfated and breakdown to electrophilic nitrenium ions.