DRUG Metabolism Holds its Destiny in its own Hands

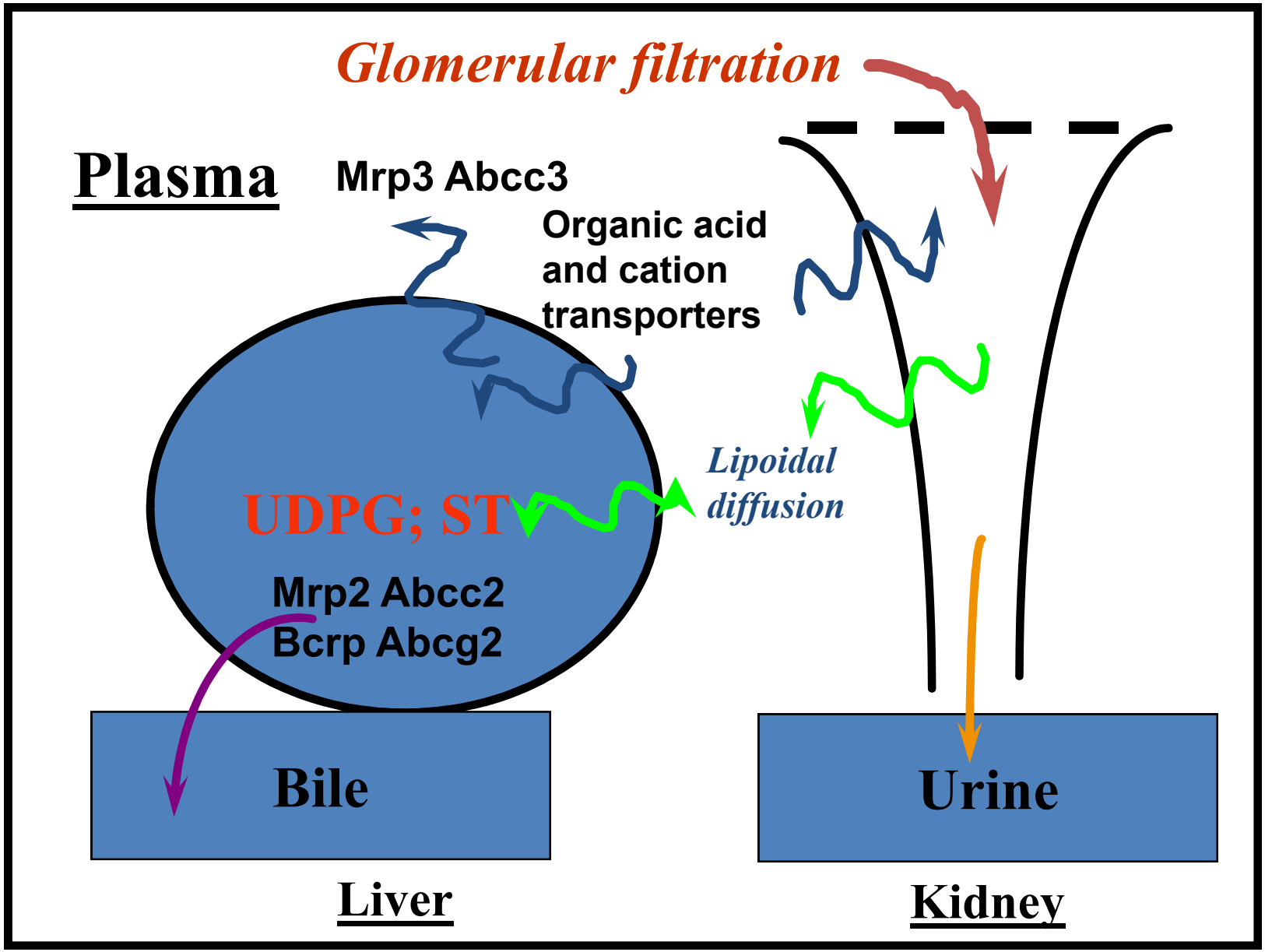
Dennis A. Smith, 2010

In future drug metabolism will have evolved into a set of separate sections and disciplines capable of being outsourced and multiplexed into partner lines thus providing the science with a robust future.

Wrong !

How permeable is the molecule ?
I don’t know, I do the PK / PD, you better ask the screening group in China…

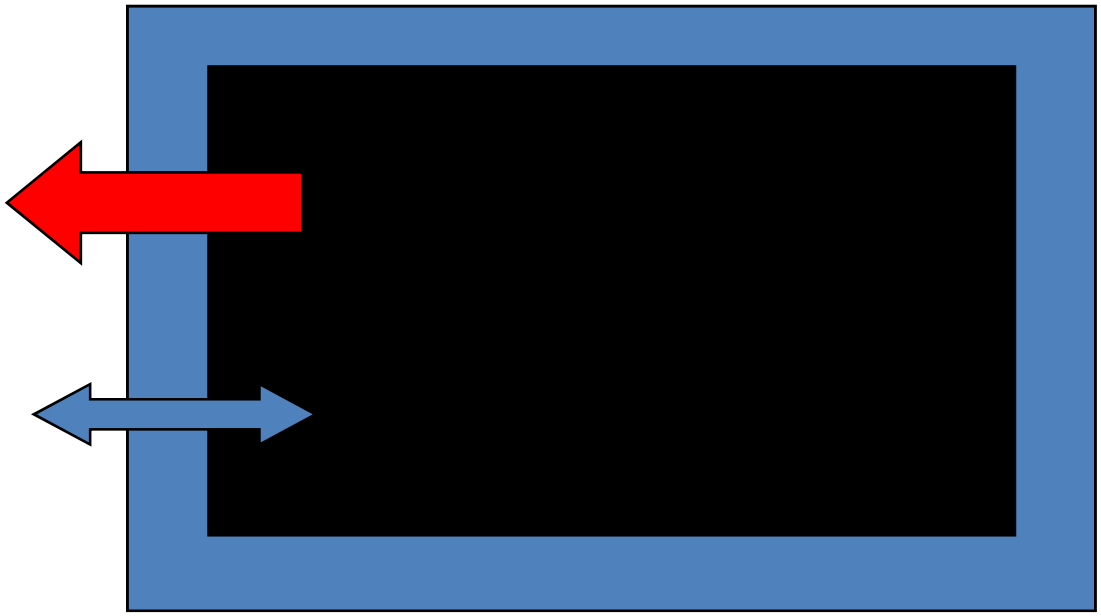
Is permeability central to small molecule drug metabolism ?


Glomerular filtration
Plasma
Mrp3 Abcc3
Organic acid
and cation
transporters
Lipoidal
diffusion
UDPG; ST
Mrp2 Abcc2
Bcrp Abcg2
Bile
Urine
Liver
Kidney

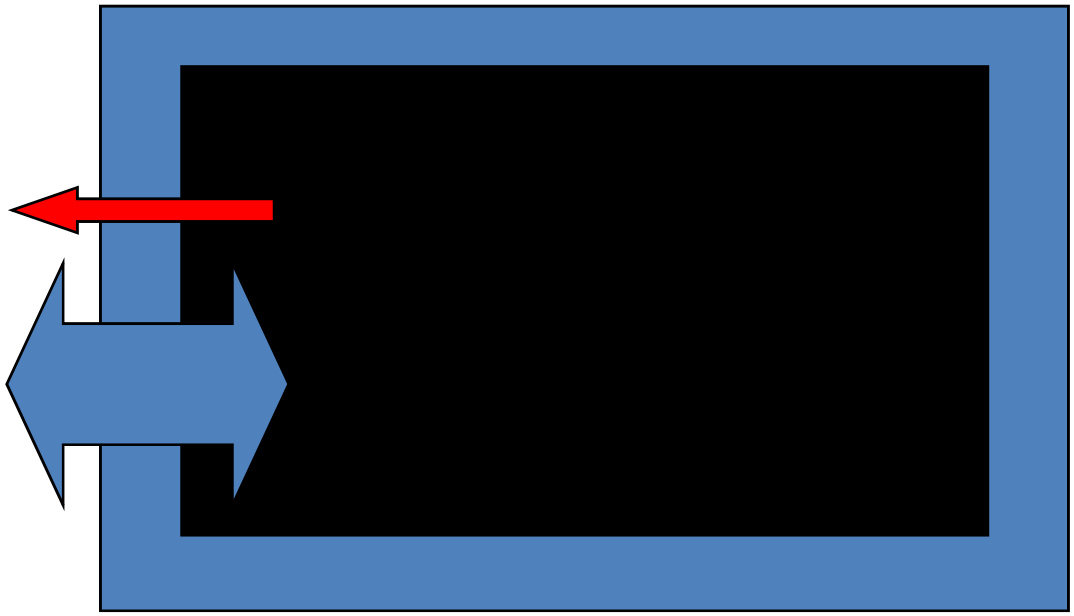
Permeability: pivotal to ADME fate

Permeability	Low	Medium	High
PSA/LogP	High	Medium	Low
Absorption	Low (*aliskeran*) unless MWt less than 250 daltons and absorbed by paracellular route *(atenolol)*	Variable. Influenced by permeability and transporters *(nelfinavir)*	High via transcellular route *(propranolol)*
Bioavailability	As for absorption	As for absorption and metabolism	Variable. Influenced by metabolism
Clearance	Renal or Biliary (possible transporter involvement)	Transporters and metabolism	Metabolism

Transport v. passive diffusion
Low permeability: large impact of transporter



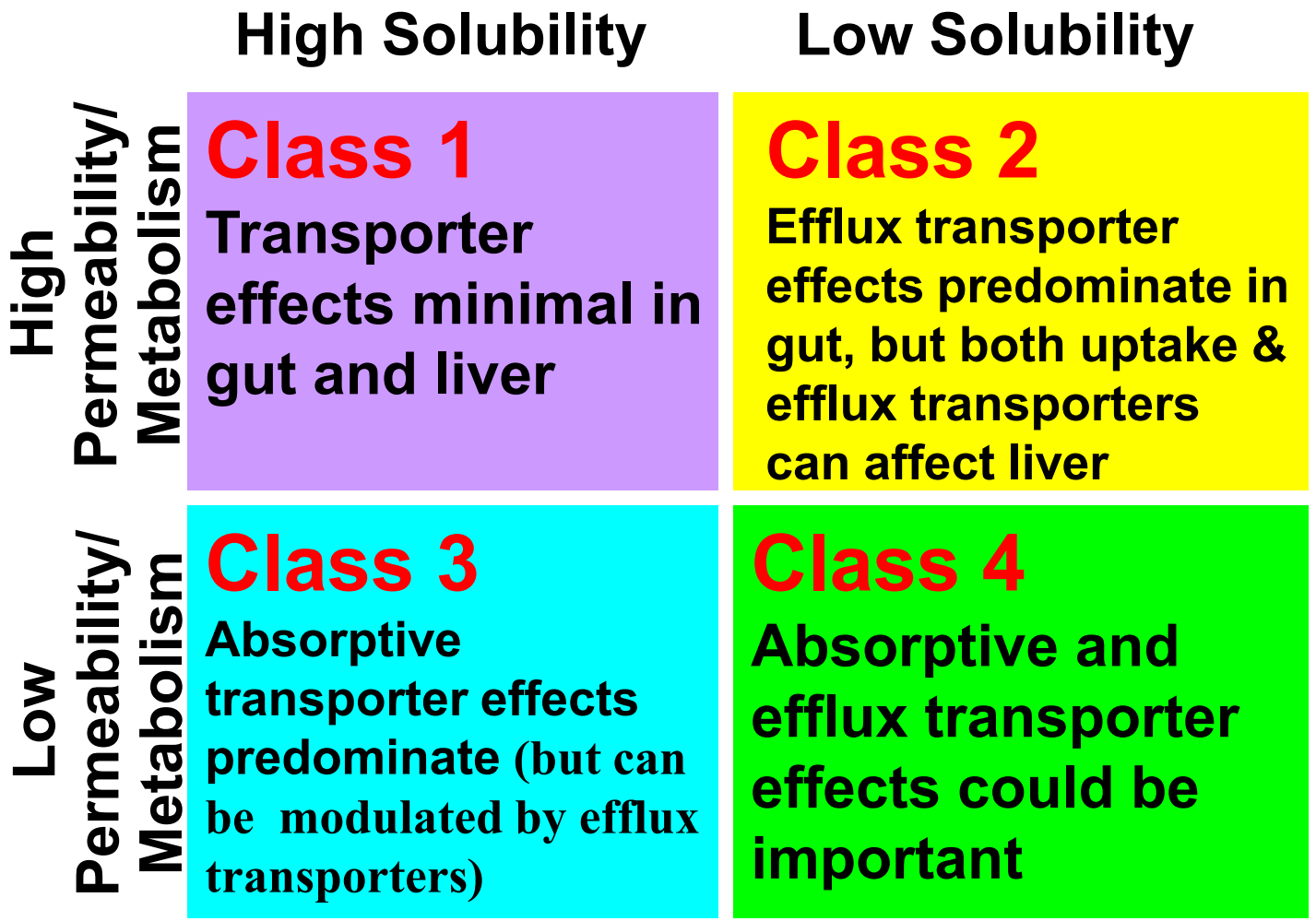
Transport v. passive diffusion
High permeability: small impact of transporter



P-gp influenced flux rates-how do we measure permeability: deconvolution or convoluted guess?

	Log P	PSA	A –B $Nm.s^{-1}$	`B-A $Nm.s^{-1}$
Propanolol	3.0	42	450	700
Saquinavir	4.4	167	2	395
Ritonavir	5.3	202	16	852
Nelfinavir	7.0	127	35	786

BCS and Oral Dosing Transporter Effects



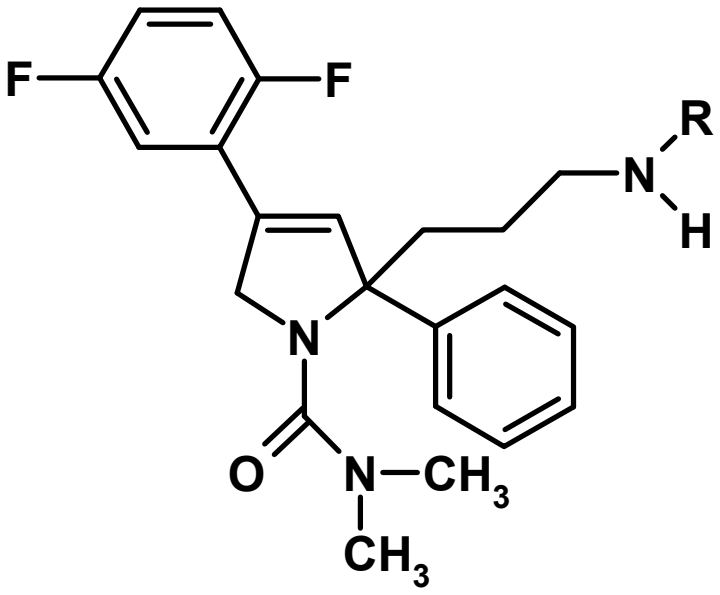
Slide provided by Les Benet

SAR- Phenomena or target based

- **Attempts to change the influence of transporters, particularly Pgp and brain or tumour entry are now being published.**

2,4-diaryl-2,5-dihydropyrrole kinesin spindle protein inhibitors,

Data from Cox et al., Biorg. Mwed. Chem. Lett. 17 (2007) 2697-2702



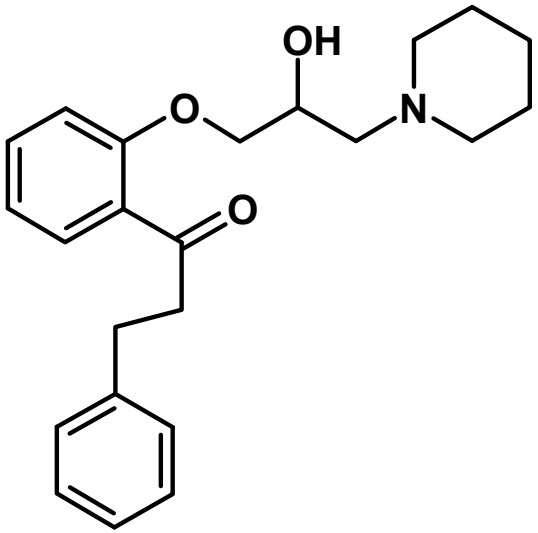
R	MDR ratio	pKa	Log P
H	1200	10.3	1.2
CH_2CH_3	135	10.7	1.6
CH_2CH_2F	32	8.8	2.6
CH_2CHF_2	2	7.0	3.4
CH_2CF_3	1	5.2	>3.2

SAR- Phenomena or target based

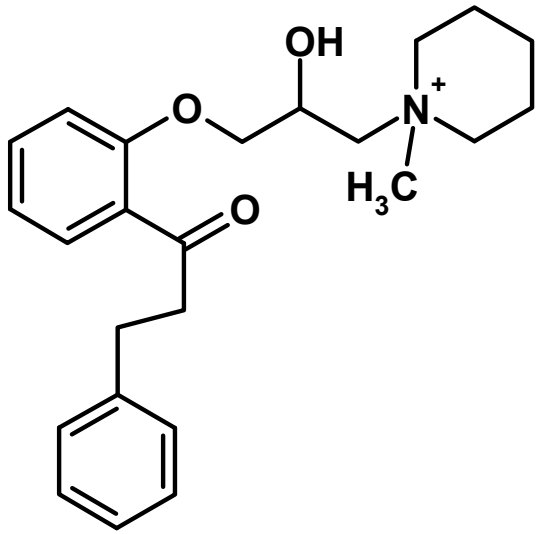
- Attempts to change the influence of transporters, particularly Pgp and brain or tumour entry are now being published.
- **In almost all cases it is impossible to separate increased intrinsic permeability from decreased transporter affinity or rate.**
- Quoted from the publication

1. Penetration to the target was increased by modulation of the basicity of the side chain by b-fluorination.
2. With these improvements (there are some reductions in potency) in access to the target it is not possible to separate if this is due to decreased Pgp activity or on intrinsic permeability.

Access to Pgp is from the cytosol not the membrane (propafenone analogues)

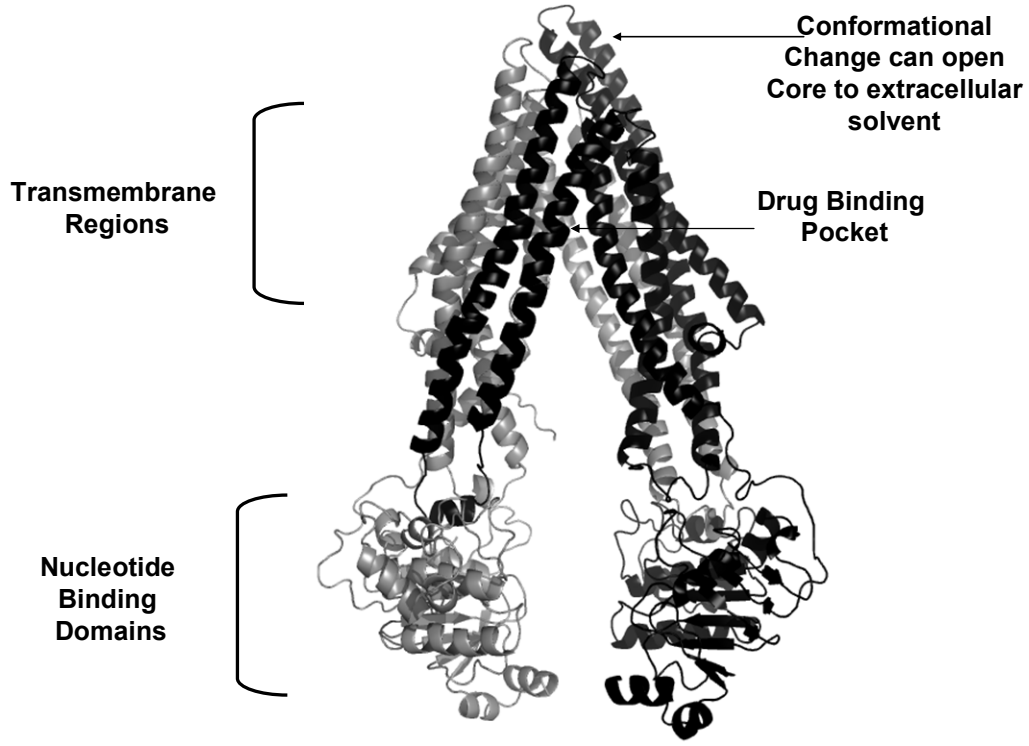


CCRF-CEM cells
Membrane association
Rapid steady state across membrane



CCRF-CEM cells
No membrane association
No transfer across membrane
Inside out CCRF-ADR5000 cells
Accumulation in presence of ATP
No accumulation in absence of ATP

Schmid *et al*. Biochem Pharmacol. 58, 1447-1456, 1999



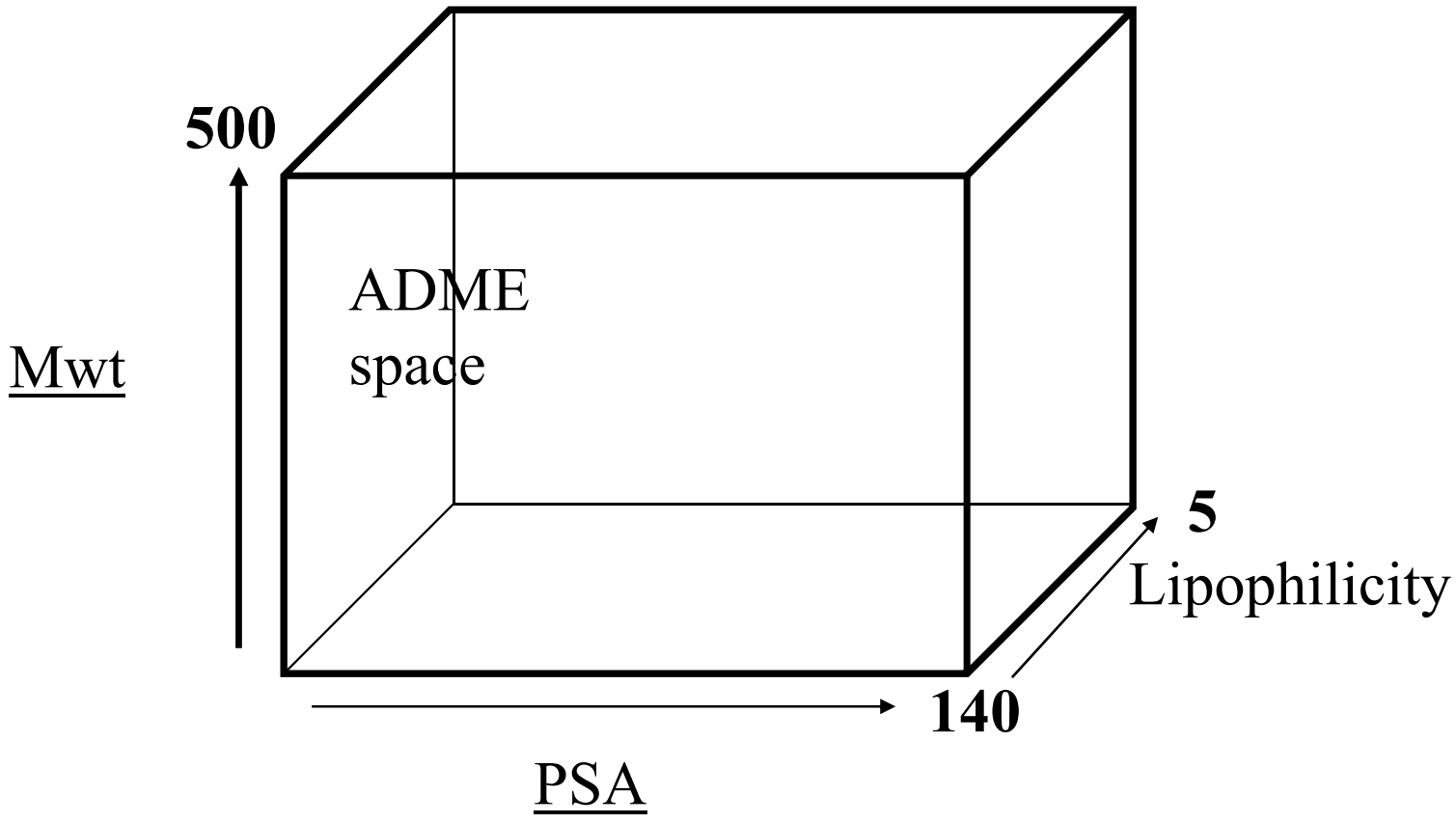
Substrate binding site open to cytosol with lipophilic residues exposed

Lipophilic regions of substrate bind to protein

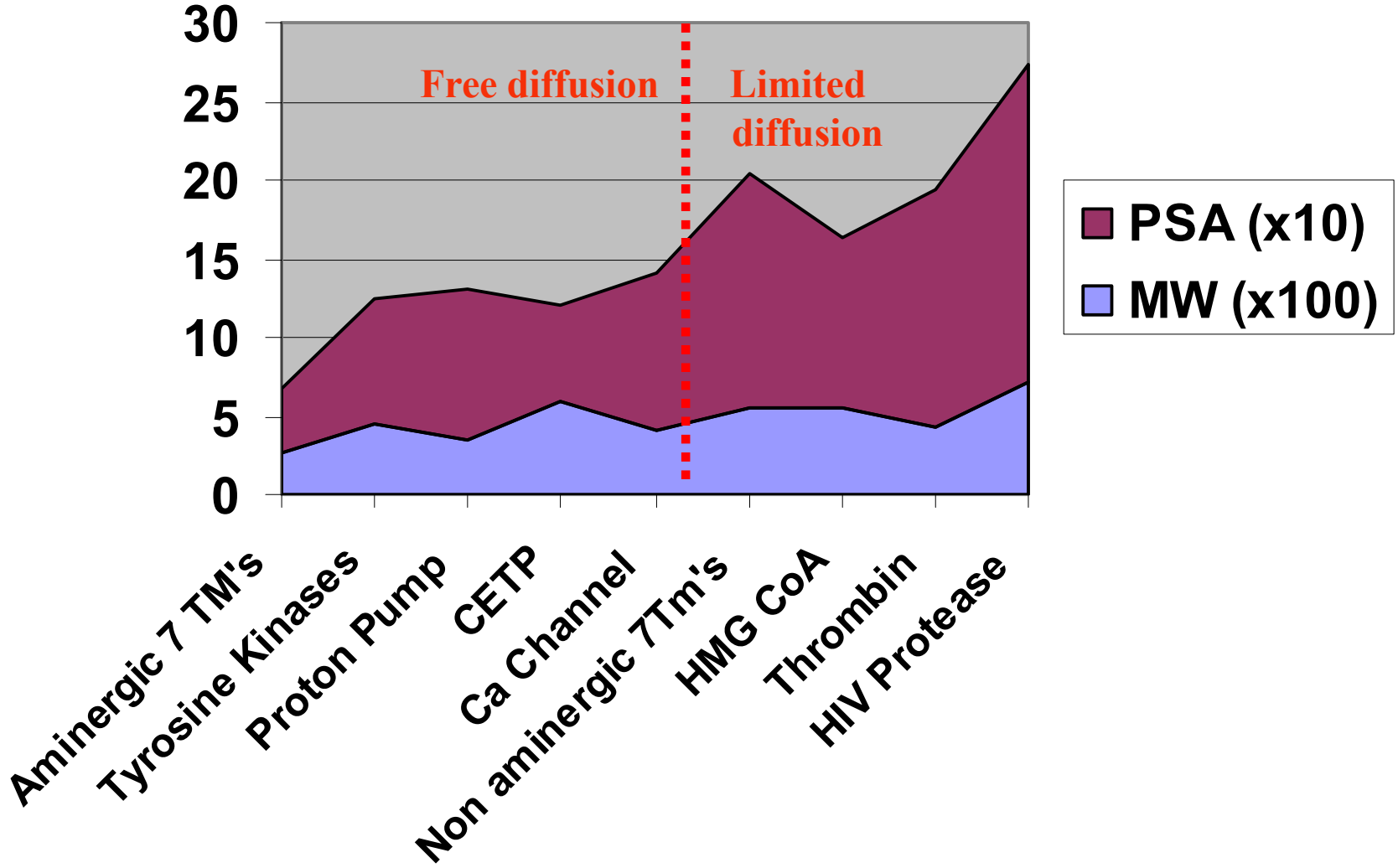
ATP consumption triggers protein conformational change due to hydrophobic collapse

Hydrophilic residues now prominent In binding cavity open to exterior aqueous environment of cell

Log P= Mwt-PSA



Properties of typical antagonists

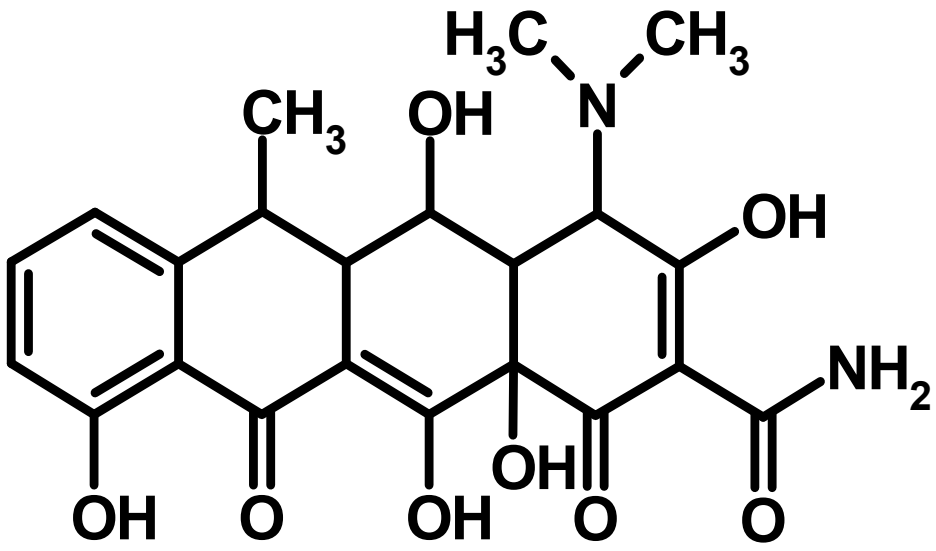


Is this drug going to be an oral drug ?
What we miss with TPSA calculations

Log D	0.5
Log P	4.4
pKa	10.8
PSA	182
MW	444
H bond	17
Freely rotatable bonds	7

Not an Oral Drug

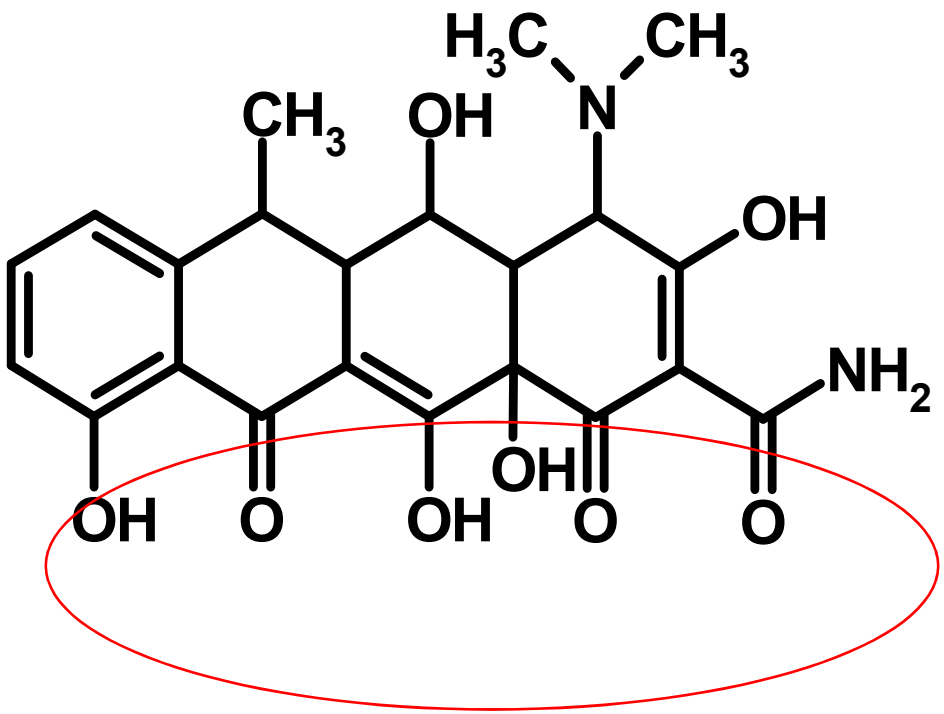
Doxycycline



Log D	0.5
Log P	4.4
pKa	10.8
PSA	182
MW	444
H bond	17
Freely rotatable bonds	7

98% bioavailability

Doxycycline

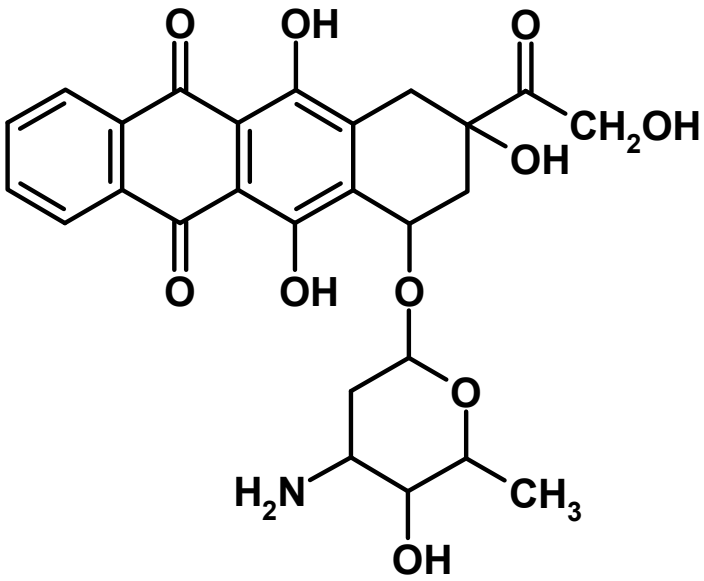


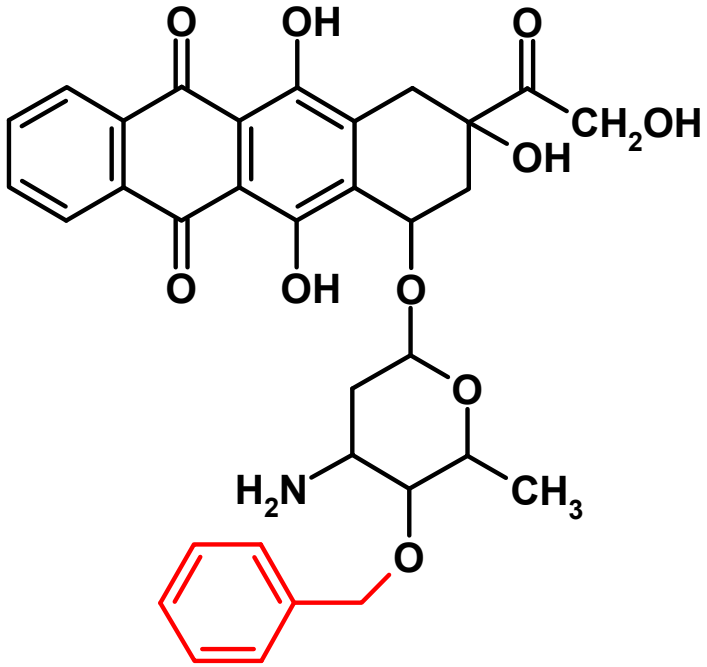
Log D	**0.5**
Log P	**4.4**
pKa	**10.8**
PSA	**182**
MW	**444**
H bond	**17**
Freely rotatable bonds	**7**

Cyclosporine A

- Mwt 1200
- **Log P oct 2.9 Log P hep 1.4**
- CaCo flux 2.3
- Baskbone N-H groups involved in intramolecular H bonds in aprotic solvent
- In aqueous solution all N-H groups point towards solvent
- Low energy cost of N-H desolvation

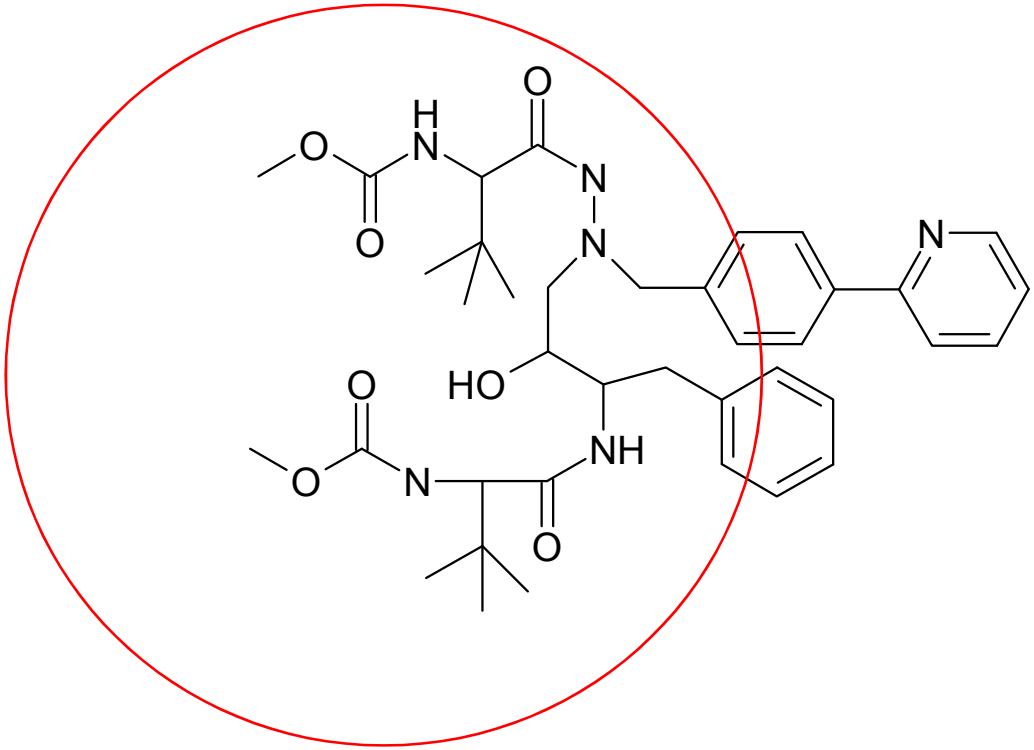
Doxurubicin (PSA 206 A2, cLog P 3.1) analogue with low Pgp flux





Brooks et al. Invest New Drugs, 25, 115-122, 2007

Atazanavir-H bonding networks in modern drugs



How do we put permeability into its rightful central role ?

Is the metabolism of drugs PK / PD?

The hunt for oxidised october

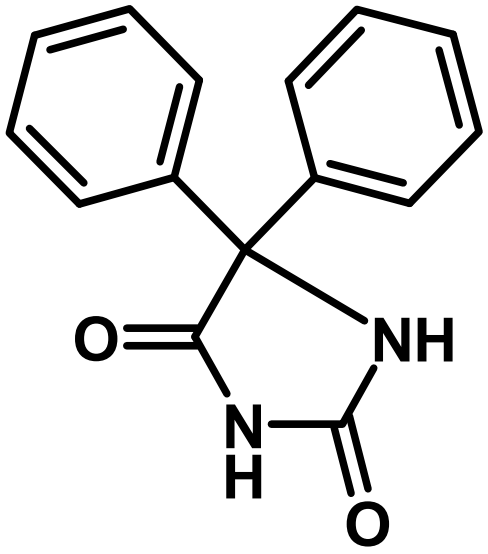
- Rule 1 All unexpected pharmacodynamic events of any molecule or any project are due to a previously undetected or uncharacteried metabolite.
- Rule 2 Drug metabolism will set off gleefully to do as its name suggests and return empty handed

The hunt for oxidised october

- Meanwhile we will convey plasma concentration data as
- C max ng / ml
- AUC ng.h/ml

What information does this impart instantaneously to scientists ?

Phenytoin

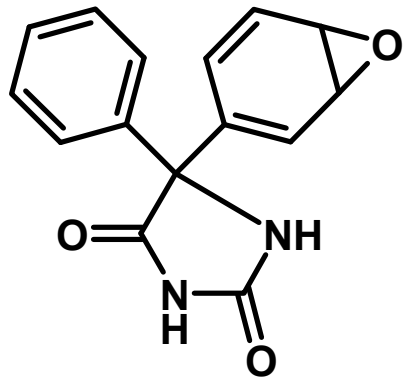


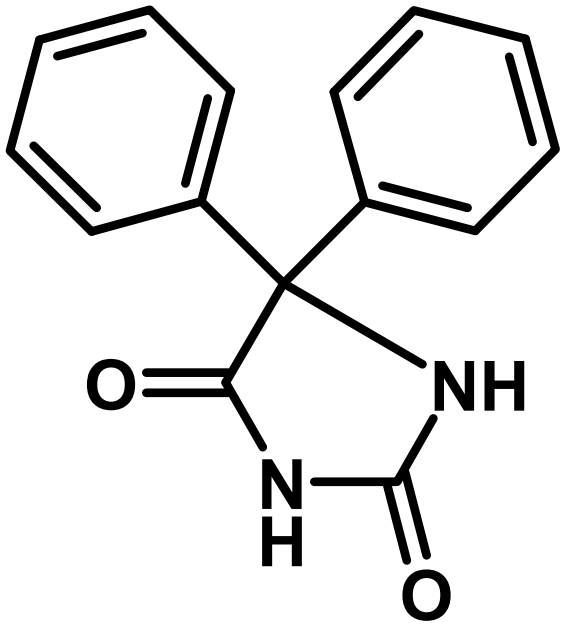
- Phenytoin used as a anticonvulsant
- Therapeutic action due to sodium channel blockade
- Phenytoin is a teratogen

Rodent teratology has consistent findings:

Decreased foetal weights
Cleft lip
Distal digital effects
Cardiovascular abnormalities

Phenytoin
Must be metabolites





Phenytoin activity due to Na^+ channel block. Activity against binding site 2 of the sodium channel receptor IC_{50} is 47 μM

Phenytoin is also an I_{Kr} channel blocker (HERG ED_{50} around 50 μM)

Danielsson et al., ***Current Pharm. Des. 7,*** 787, 2001
Salvati et al., ***JPET., 288***, 1151, 1999
Kallen et al.. ***Reprod. Toxicol., 20***, 209, 2005

I_{Kr} present in fetal but not adult rat hearts

I_{Kr} blockers at concentrations not affecting the adult cause bradycardia, arrhythmia and cardiac arrest in the fetus leading to:

- Hypoxia (embryonic death and growth retardation)
- Reoxygenation and reactive oxygen species generation (orofacial clefts and distal digital reduction)
- Alterations in embryonic blood flow (cardiovascular defects)

Unbound drug concentrations of phenytoin in pregnant rats and resultant effects Data converted to Cmax and C_{av} values.
Decrease in in vitro foetal heart rate first observed at 12μM

Route	Dose level mg/kg	C_{max} μM	$C_{av(0\text{-}24\ h)}$ μM	Effects
Oral	150	7	5	No effects
IP	100	18	12	Small decrease in foetal weights
IP	150	33	29	Embryonic death, decreased foetal weight, teratogenicity

Instantaneous PK/PD

- Insist on molar units throughout drug discovery, development and drug research
- Supplement AUC values with Cav

D.A. Smith et al., The use of Cav rather than AUC in safety assessment. Reg Tox and Pharmacol., 57, 70-73, 2010

Metabolites-why are we interested, has anyone crisply articulated it

- **“Circulating metabolites are of interest primarily because they can directly and probably reversibly interact with macromolecules, particularly proteins and cause a change in conformation and function of the protein to elicit a biological effect (beneficial or hazardous).**
- **These effects can be similar and additional to the parent molecule or may in some rare cases be different (usually as a result of elevated concentrations). Identifying and analysing these metabolites in the same matrix as the parent allows concentrations to be measured and thereby assessment of PK / PD.”**

Circulating (stable) metabolites-whats important

- **Circulating concentrations**
- **Structure (relationship to parent and known structure activity relationships)**
- **Physicochemistry (In particular lipophilicity, polar surface area and charge)**

Smith, D.A. and Obach R.S. (2005) Seeing through the MIST. Commentary on Metabolites in safety testing. Drug Metab. Dispos. 33, 1409-141

Smith, D.A. and Obach R.S. (2006) Metabolites and Safety: What Are the Concerns, and How Should We Address Them? Chem. Res. Toxicol. 19, 1570-1579

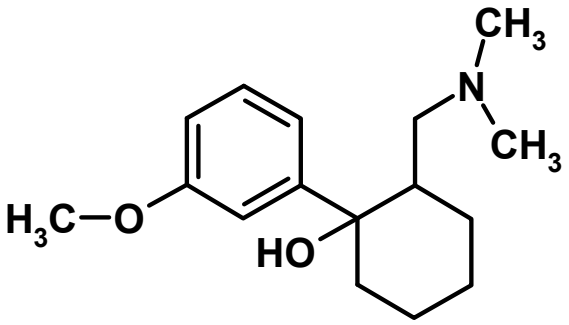
Smith D.A., Obach, R.S., Williams, D.P. and Park, B.K. (2009) Clearing the MIST (Metabolites in Safety Testing) of time: the impact of duration of administration on drug metabolite toxicity. Accepted for publication Chem Biol. 179, 60-67

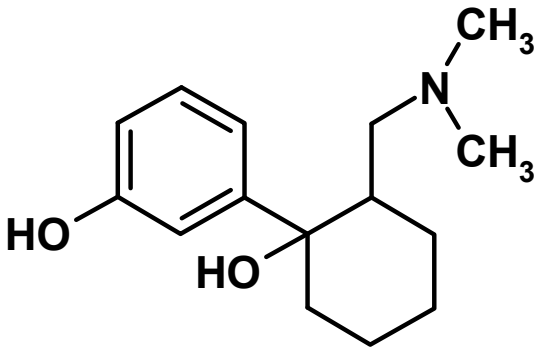
Smith D.A. and Obach R.S.(2009) Metabolites in Safety Testing (MIST): Considerations of Mechanisms of Toxicity with Dose, Abundance, and Duration of Treatment. Chem Res. Toxicol. 22, 267-279

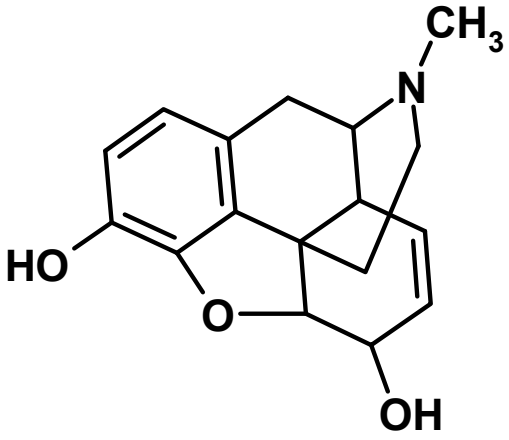
The facts (mine) are

- Most metabolites are inactive
- SAR accounts for the few times metabolites are more potent
- Metabolites with similar structures to the parent may have similar receptor binding properties against known targets (selectivity); this can reasonably be extended to the whole proteome.
- Inactive metabolites including those with different structure to the parent; many secondary metabolites, N-dealkylation of central nitrogens, loss of a key functional group (e.g deamination of a GPCR ligand) will probably be devoid of pharmacological or toxicological effects; unless they are present at reasonably high concentrations (above 1μM unbound).

Tramadol and o-desmethyl metabolite

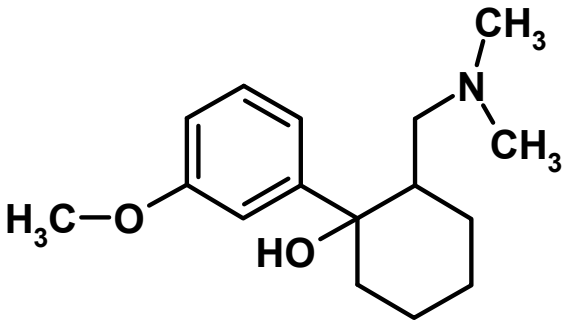


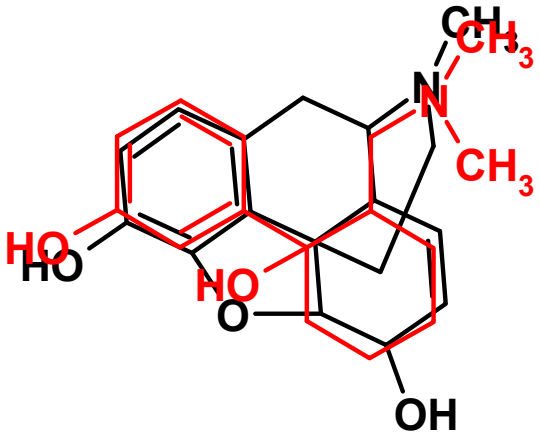




Morphine
μ-receptor partial agonist

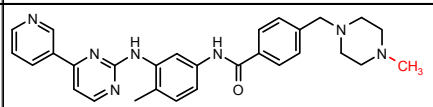
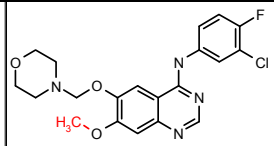
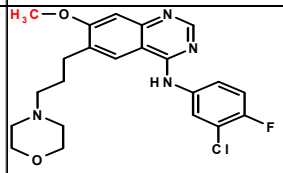
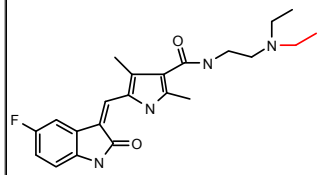
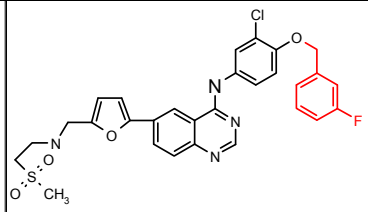
Tramadol and o-desmethyl metabolite





Morphine

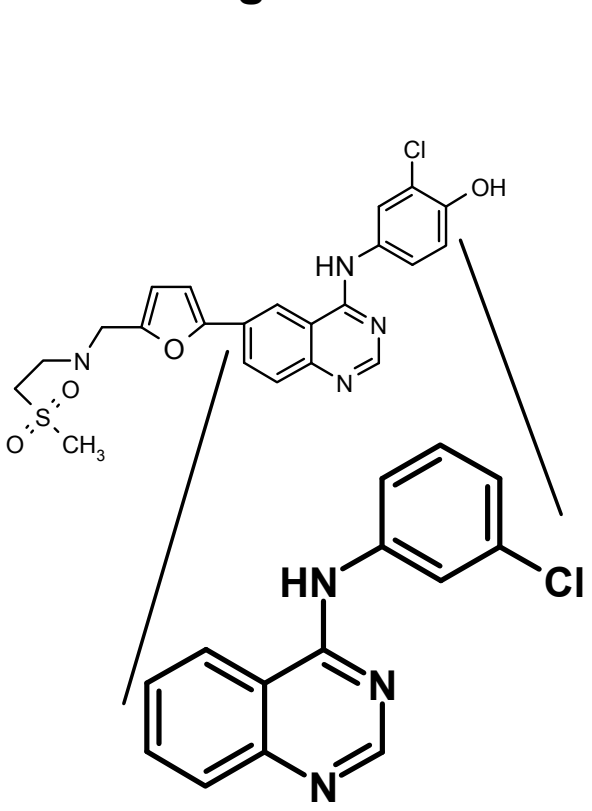
The circulating metabolite facts for kinase inhibitors

Drug		Active metabolite	Potency and selectivity of metabolite
Imatinib		CGP74588	Same potency and selectivity as parent, but lower concentrations *in vivo*
Erlotinib		M523595	Same potency and selectivity in isolated enzyme assays, but much lower activity in cell based assays
Gefitinib		Desmethyl-gefitinib	Same potency and selectivity in isolated enzyme assays, but much lower activity in cell based assays
Sunitinib		SU12662	Same potency for PDGFR-α and –β, VEGF2 and KIT. Accumulates 7-10 fold compared to parent (3-4 fold) and has higher free fraction indicative of role in anti-cancer effects of drug
Lapatinib		GW690006	Similar potency against EGFr but low activity against C-erbB-2. Low circulating concentrations

The SAR case for change in selectivity

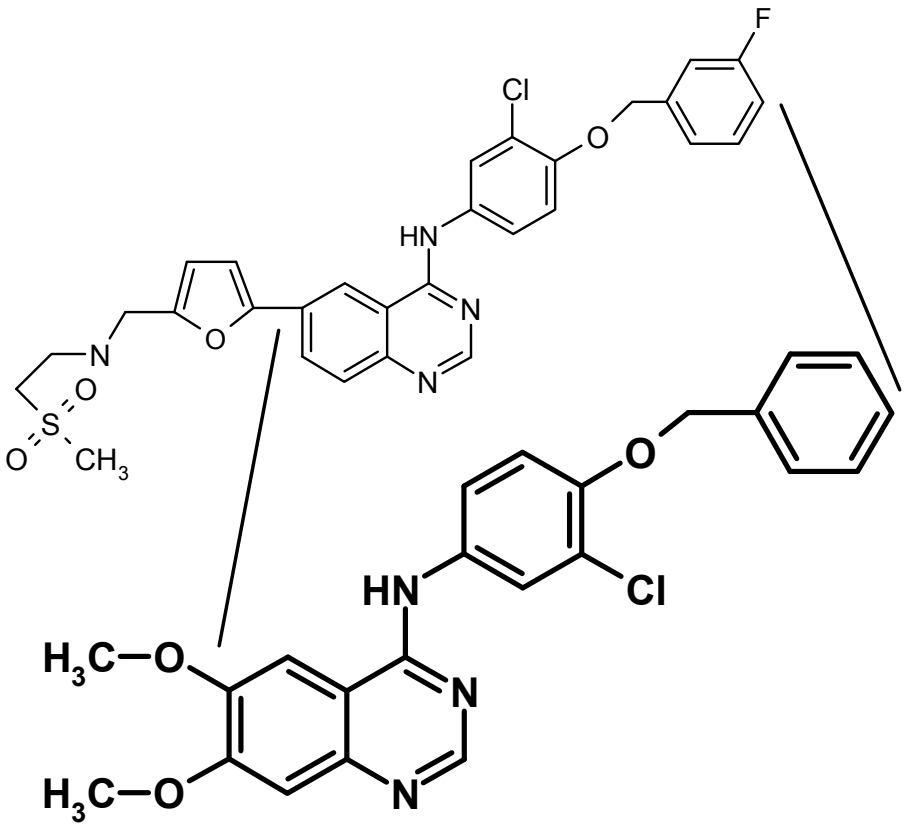
PSA 127 A2
cLog P 2.8

PSA 115 A2
cLog P 5.1



CAQ

EGFr inhibition



4557 W

EGFr / C-erbB-2 inhibition

Circulating (stable) metabolites-whats important

- Circulating concentrations
- Structure (relationship to parent and known structure activity relationships)
- Physicochemistry (In particular lipophilicity, polar surface area and charge)

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Observed odds for *in vitro* promiscuity and toxicity

(Defined as multilple receptor interactions at 10 μM for 108 compounds and *in vivo* toxicity defined as effects above 1μM free drug)

in vitro Promicuity*	TPSA>75 A^2	TPSA<75 A^2
Clog P < 3	0.2	0.8
Clog P > 3	0.4	6.2
in vivo Toxicity		
Clog P < 3	0.4	0.5
Clog P > 3	0.8	2.6

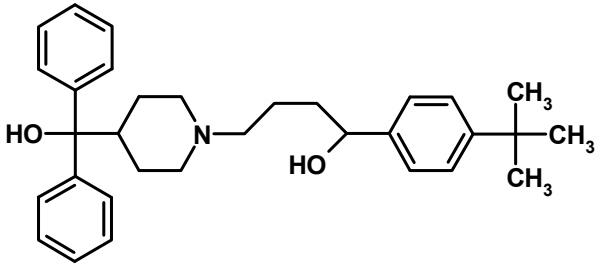
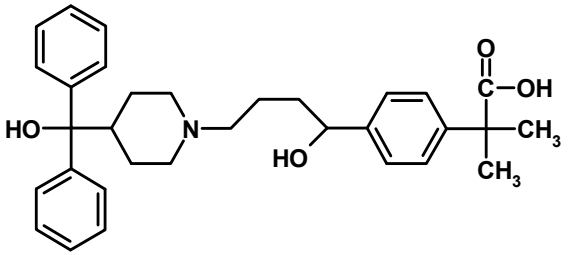
Price DA, Blagg J, Jones L. et al. Physicochemical drug properties associated with in vivo toxicological outcomes: a review. Exp. Opin. Drug Met. Toxicol. 5 (8), 921-931 (2009)

Hughes J.D., Blagg J., Price DA et al. Physicochemical drug properties associated with in vivo toxicological outcomes. Biorg. Med. Chem. Letts. 18 (17) 4872- 4875 (2008)

Azzaoui K, Hamon J, Faller B, Whitebread S. et al. Modeling promiscuity based on in vitro safety pharmacology profiling data. ChemMedChem 2 (6) 874-880 (2007)

Comparison of terfendine and its carboxylic acid metabolite fexofenadine.

IKr blockade is estimated to be 100μM for the metabolite.

		PSA A^2	Log P	Log $D_{7.4}$	Activities <100 nM	Activities <1μM
Terfenadine		**44**	**6.5**	**4.2**	**H1 (5nM) IKr (50nM)**	**Ca++ channel Na+ channel (site 2) DA transporter 5HT2A 5HT2B**
Fexofendine		**81**	**4.8**	**2.3**	**H1 (12nM)**	

Physicochemical changes associated with metabolism

Metabolic Step	Increase in TPSA	Reduction in cLog P	Ionisation, log D
Aliphatic hydroxylation	**20.23 A^2**	**-1.99**	
Aromatic hydroxylation	**20.23 A^2**	**-0.67**	
Dealkylation of tertiary amine	**8.8 A^2**	**-0.6 for a methyl group: increases with fragmental value of leaving function**	**Increase in basicity of approximately +1pKa. Decrese in Log $D_{7.4}$ of 1 unit**
Dealkylation of secondary amine	**14 A^2**	**-0.6 for a methyl group: increases withfragmental value of leaving function**	
Oxidation of hydroxyl to carboxylic acid	**17 A^2**	**Little change in cLog P**	**Introduction of acidic charge and pKa 3-5. Reduction in log $D_{7.4}$ of 3-5 units. Formation of a zwitterions for basic parent molecules.**

Manner C N, Payling D W, Smith, D A, Distribution coefficient, a convenient term for the relation of predictable physico-chemical properties to metabolic processes, Xenobiotica. 18 (3), 331-350, 1988

- Do we carefully analyse our metabolism data in terms of concentration, structure against target SAR, and physicochemistry?

Excreted Metabolites

- Excreted metabolites are of interest primarily, in human, because they allow the proportion of the parent converted to a particular metabolite to be determined and thereby support the *in vitro* enzymological evaluations for population variations and drug-drug interactions.
- In addition they allow the detection of the downstream products of reactive metabolites and, moreover, allow an estimation of the amount (mass) formed. Recommendation is the total of these products in human needs to be >10mg to be considered for further study.

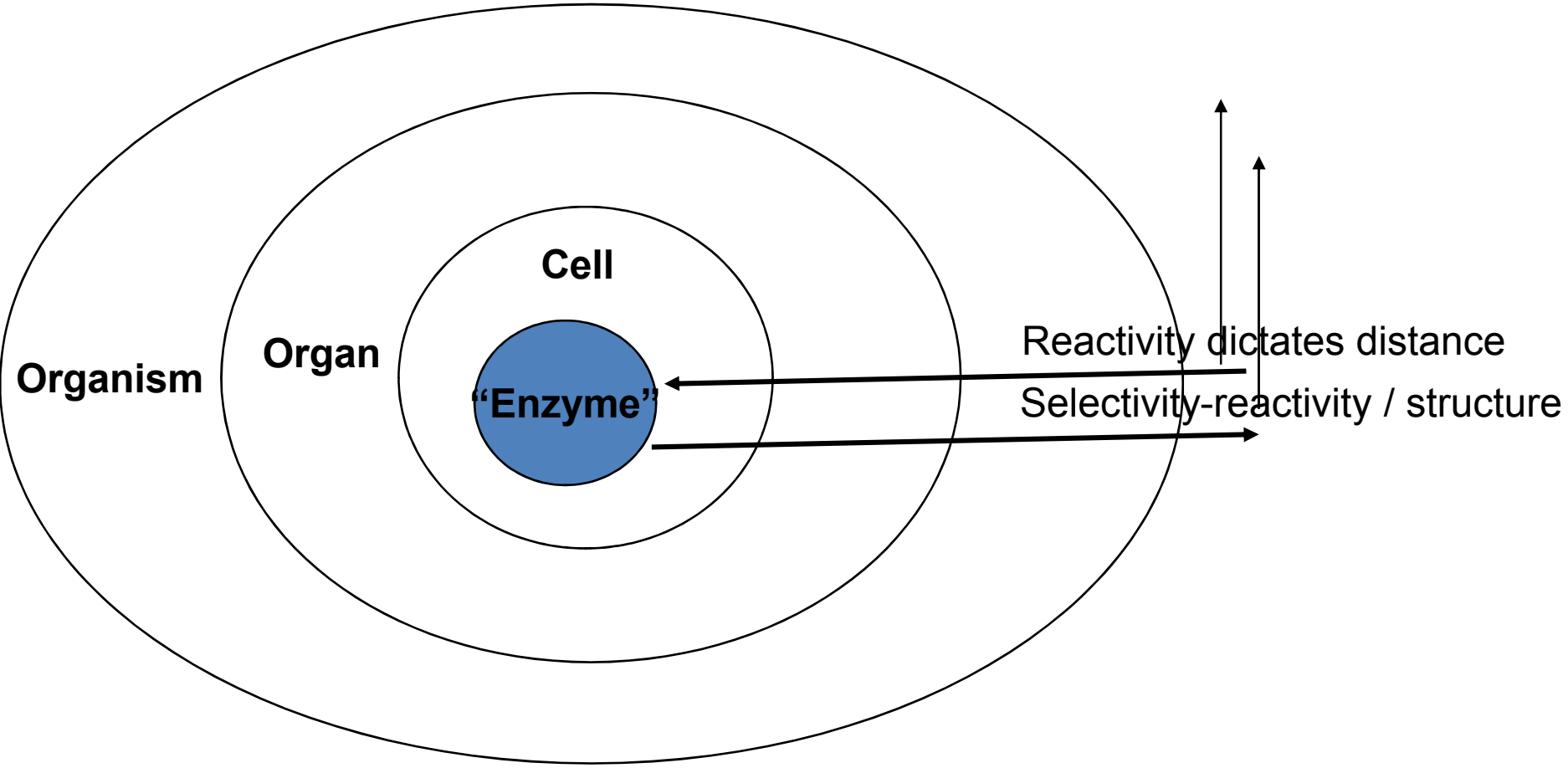
Observations

- That despite an earlier belief, to the contrary, all toxicity caused by reactive metabolites shows a **dose response relationship**. The earlier confusion was prompted by the relative rarity of immunoallergenic events and the difficulty in obtaining any useful dose relationship over very sparse data and a limited dose range
- **Structural alerts**. These are chemical groups which have historically been associated with reactive metabolites and leading to toxicity. Incorporation of such grouping into a molecule increases the risk of the formation of reactive metabolites

Reasons for withdrawal

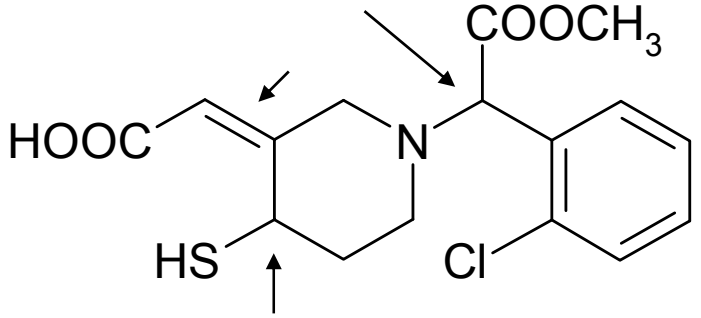
Primary Pharmacology		**Secondary Pharmacology**		**Idiosyncratic Toxicity-reactive metabolites**	
Generic name	Daily dose mg	Generic name	Daily dose mg	Generic name	Daily dose mg
Alosetron	1	Astemizole	10	**Benoxaprofen**	**600**
Cerivastatin	0.3	Cisapride	40	**Bromfenac**	**100**
Encainide	150	Dexfenfluramine	15	**Nomifensine**	**125**
Flosequinan	100	Fenfluramine	15	**Remoxipride**	**300**
Rofecoxib	25	Grepafloxacin	400	**Suprofen**	**800**
		Mibefradil	100	**Temafloxacin**	**600**
		Rapacuronium	100	**Ticrynafen**	**400**
		Terfenadine	120	**Tolcapone**	**300**
				Troglitazone	**400**
				Trovafloxacin	**200**
				Zomepirac	**400**

Are reactive metabolites selective. Is it the nature of the reactive species or the overall shape of the molecule



Pharmacological targets and selectivity

- Clopidogrel reactive metabolite is an irreversible inhibitor of platelet purinergic P2Y12 receptor formed in the liver (CYP3A4 and CYP2C19). Only one isomer of the eight isomers exhibits in vitro antia-ggregating activity



Pereillo, J.M. et.al. Structure and stereochemistry of the active metabolite of clopidogrel, Drug Met. Disp. 30, 11, 1288-1295, 2002

Can we categorise reactive metabolites systematically-have I MIST it?

- Reactivity-stability
- Structural desciptors of molecule
- Physicochemistry
- Amount formed

- Drug Metabolism…..leading personalised medicine from the back of the field?

Back to clopidogrel-Personalised medicine?

- Separating fact from fiction…once you rely on others then
  - Estabishing facts=1/number of papers^2

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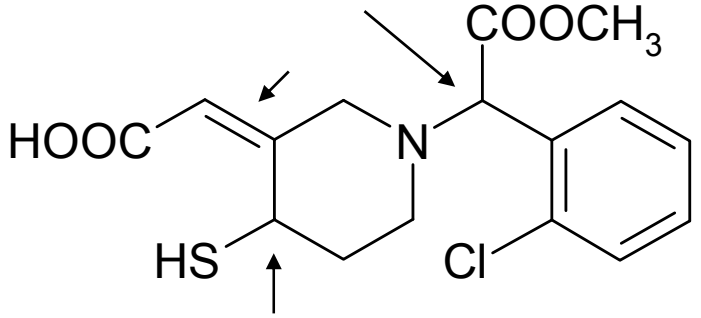

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More questions than answers

- 2-oxo clopidogrel formed mainly be CYP3A4
- Formation of active thiol by hydrolysis or further oxidation ?
- Further oxidation by multiple CYPs or is CYP2C19 selective for the active isomer of thye metabolite?
- Is the lack of response in CYP2C19*2 due to metabolism or a link to polymorphism in the P2Y12 receptor?
- **All the above have had positive and negative views in the plethora of papers**

Black Box Warning of Clopidogrel

- **WARNING: DIMINISHED EFFECTIVENESS IN POOR METABOLIZERS**
- **Effectiveness of Plavix depends on activation to an active metabolite by the cytochrome P450 (CYP) system, principally CYP2C19.**
- **Poor metabolizers treated with Plavix at recommended doses exhibit higher cardiovascular event rates following acute coronary syndrome (ACS) or percutaneous coronary intervention (PCI) than patients with normal CYP2C19 function.**
- **Tests are available to identify a patient's CYP2C19 genotype and can be used as an aid in determining therapeutic strategy.**
- **Consider alternative treatment or treatment strategies in patients identified as CYP2C19 poor metabolizers.**

Too late now (maybe), but easy to do earlier in China (14% 2C19 PMs)

- **CYP2C19 poor metabolizer status is associated with diminished antiplatelet response to clopidogrel.**
- **Although a higher dose regimen (600 mg loading dose followed by 150 mg once daily) in poor metabolizers increases antiplatelet response an appropriate dose regimen for this patient population has not been established in clinical outcome trials**

P2Y12 receptor gene variation is major factor in direct antagonist variation

Bourman et al. Thrombosis and Haemostasis 103, 379-386, 2010

Would drug metabolism lead this from the front now ?

Because once the bandwagon gets rolling it starts to go only downhill

Is this CYP2C19 inhibition or something else ?

- **72 healthy subjects were administered Plavix (300 mg loading dose followed by 75 mg per day) alone and with omeprazole (80 mg at the same time as Plavix) for 5 days. The exposure to the active metabolite of clopidogrel was decreased by 46% (Day 1) and 42% (Day 5) when Plavix and omeprazole were administered together. Mean inhibition of platelet aggregation was diminished by 47% (24 hours) and 30% (Day 5).**

- **72 healthy subjects were given the same doses of Plavix and omeprazole but the drugs were administered 12 hours apart; the results were similar, indicating that administering Plavix and omeprazole at different times does not prevent their interaction.**

Is this CYP2C19 inhibition?

- **Suggestions of accumulative mechanism based inhibition by esomeprazole (s-enantiomer of omeprazole) on its own clearance. No effect of R-enantiomer**

  McColl, Kennerley. Digest. Liver Dis., 34, 461-467, 1992

- **Esomeprazole showed less inhibitory potency compared with omeprazole and its R-enantiomer as reversible inhibitors.**

  Xue-Qing et al. Drug Met Disposit., 32, 821-827, 2004

- **Omeprazole is a time-dependent inhibitor of CYP2C19 in human hepatocytes**

  Paris et al. Drug Met Rev 40, 89, Abstract, 2008

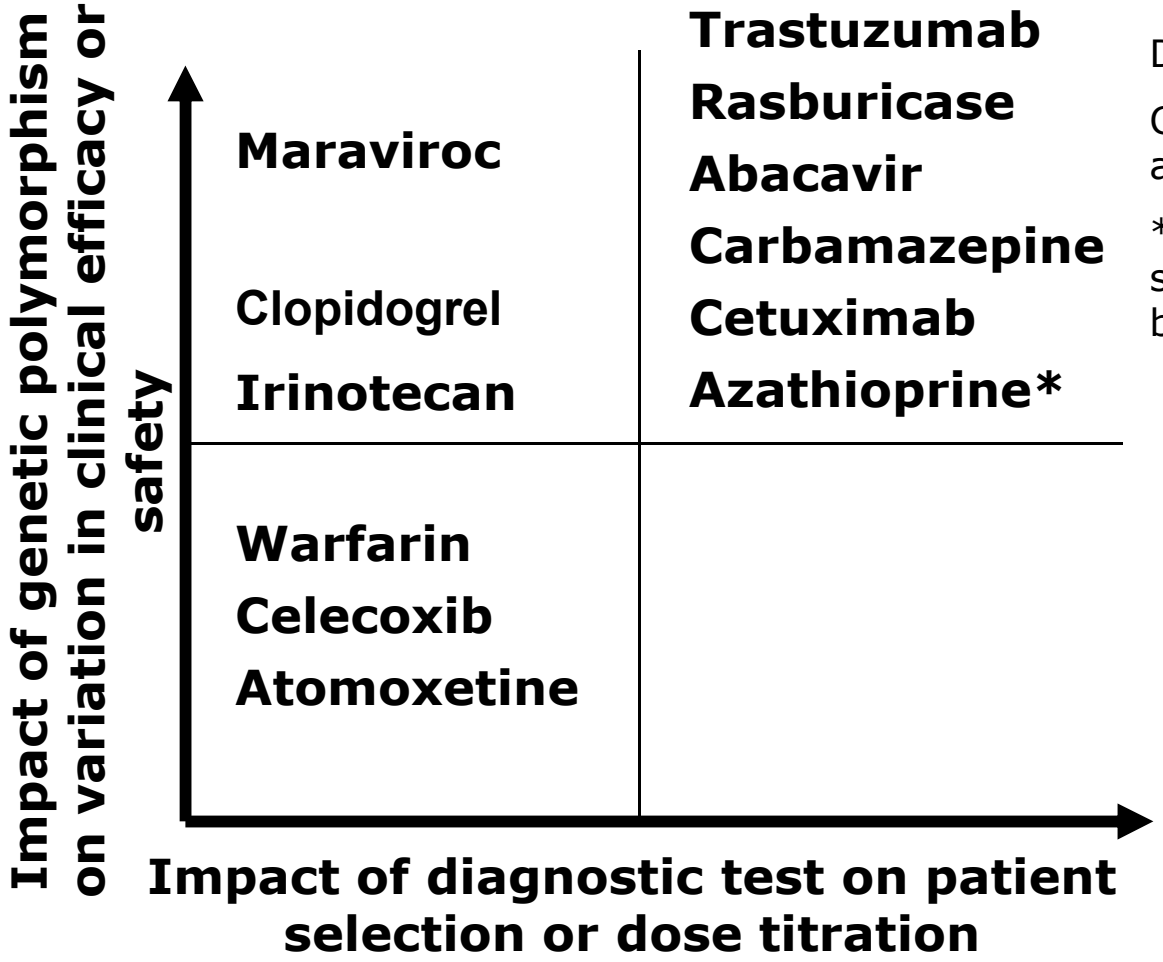
- **Omeprazole classified as a moderate reversible inhibitor of CYP2C19**

  Isoherranen et al. Chem. Res. Toxicol., 22, 294-298, 2009

- **Multi-factorial interaction proposed including the PPI and clopidogrel inhibition of CYP2C19**

  Zhang et al. Drug Met Letts.,3, 287-289, 2009

Classification of drugs with PGx in product label



Diagnostic is a guide:

Clinical signs still regarded as most important:

*TPMT testing cannot substitute for complete blood count monitoring

Conclusions

- Drug Metabolism must be integrated and not seen as separate functions
- Only this way will it lead (and survive)
- Future directions must include a closer relationship with clinical outcomes in terms of safety and efficacy
- Probably can be the biggest influence on personalised medicine if we start early enough in the drug discovery / development cycle