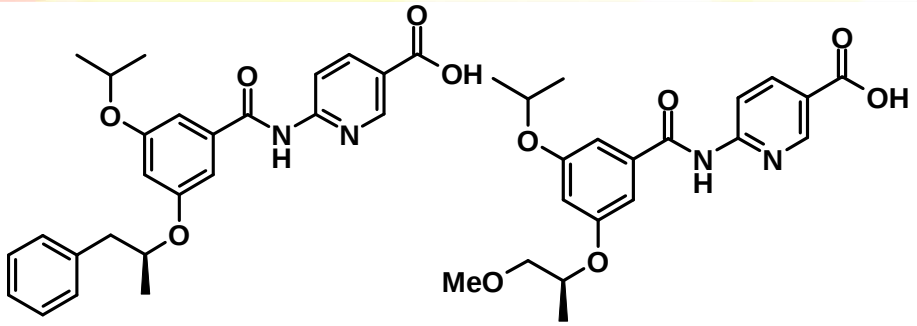
You can predict in vivo activity!

	A	B
IC50	0.02	0.07
PPB	99.7%	98%
Oral Cmax	2.0uM	4.5uM
Free Cmax	0.3% of 2.0 = 0.006uM	2% of 4.5 = 0.09uM
Multiple of IC50	0.006/0.02 =0.3	0.09/0.07 =1.3
Predicted in vivo activity	<15%	>15%

Balancing Potency and % Free: Real example

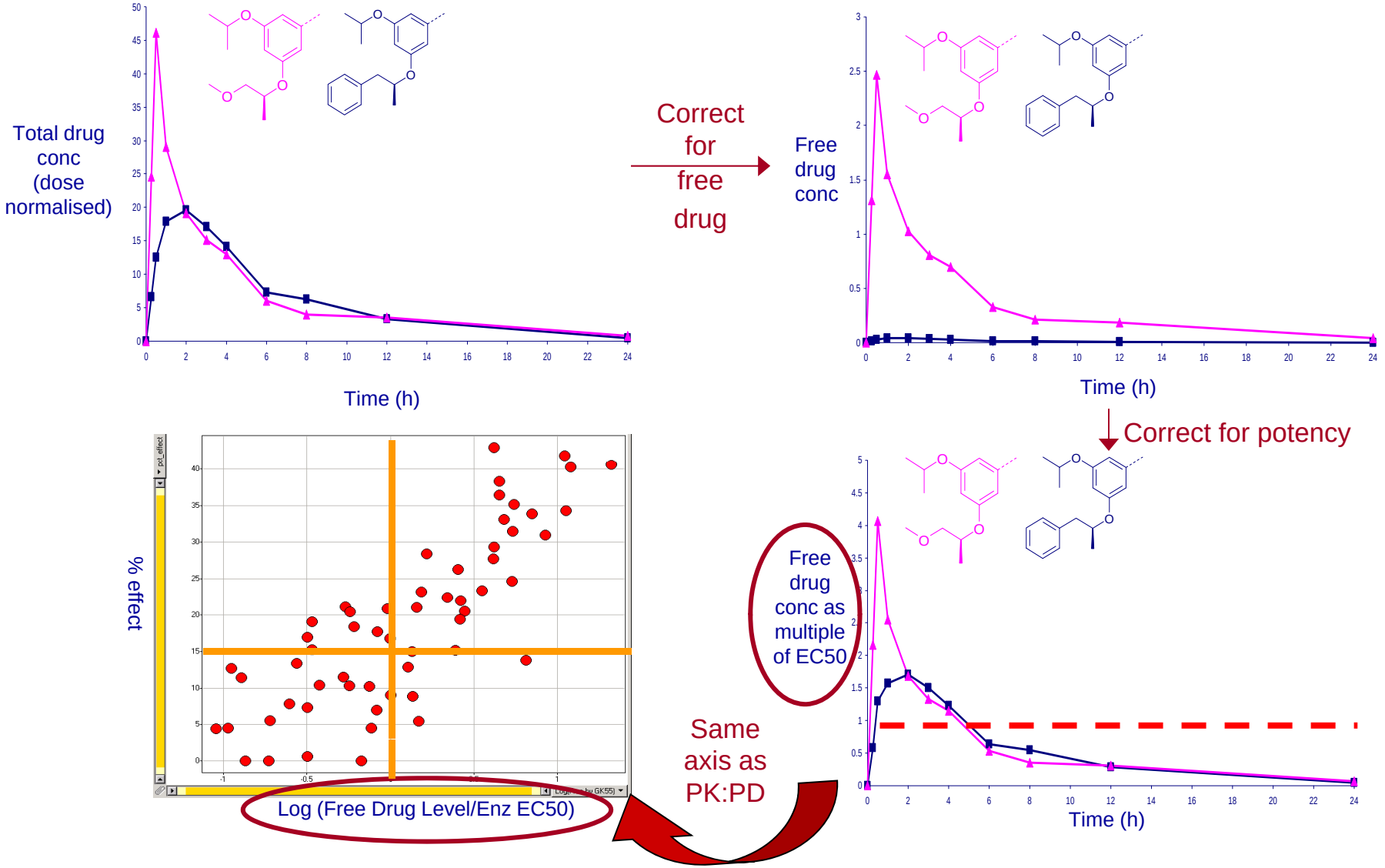


	GKA 31	GKA 30
Enzyme EC_{50} (μM)	0.02	0.61
% free (Rat)	0.23	5.34
Solubility (μM)	8	3140
Cl (ml/min/kg)	3.3	2.3
Unbound Clearance	20	45
F (%)	100	85

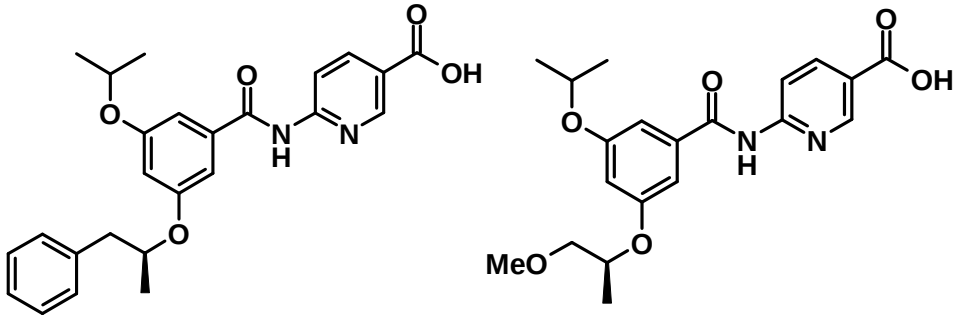
Biorg. Med. Chem. Lett., **2006**, 16, 2705

How to rank compounds?

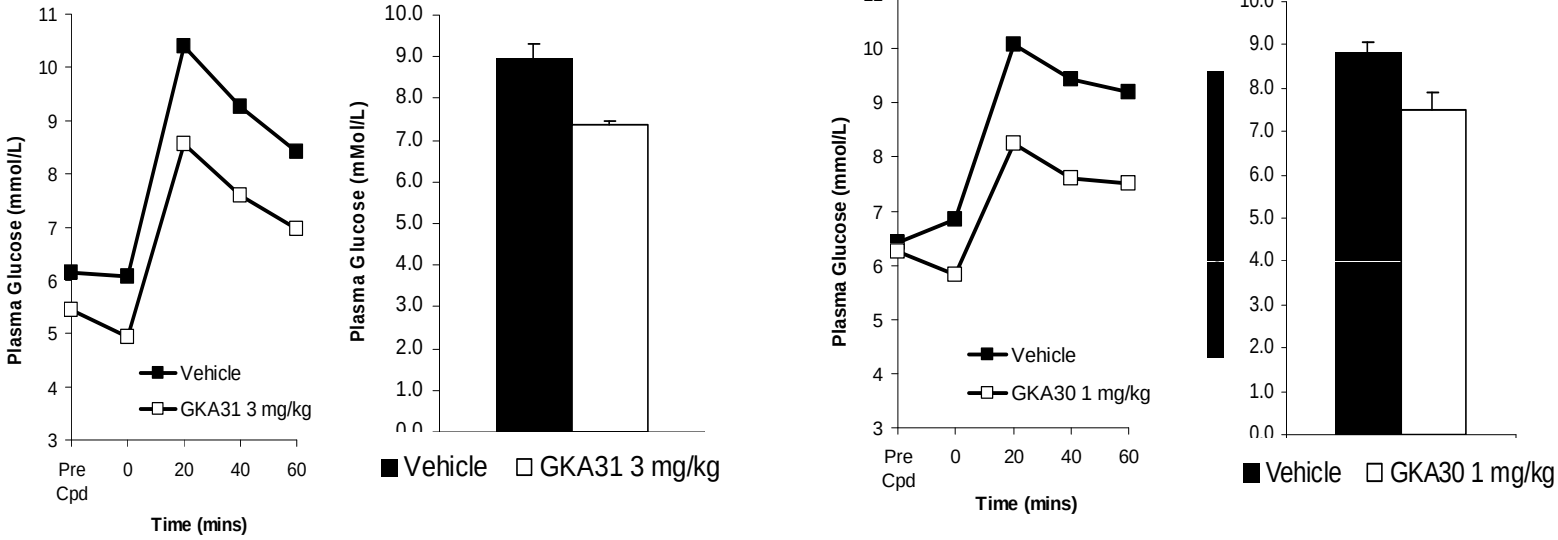
Best cpds will have best coverage above PKPD free drug multiple



In vivo efficacy data



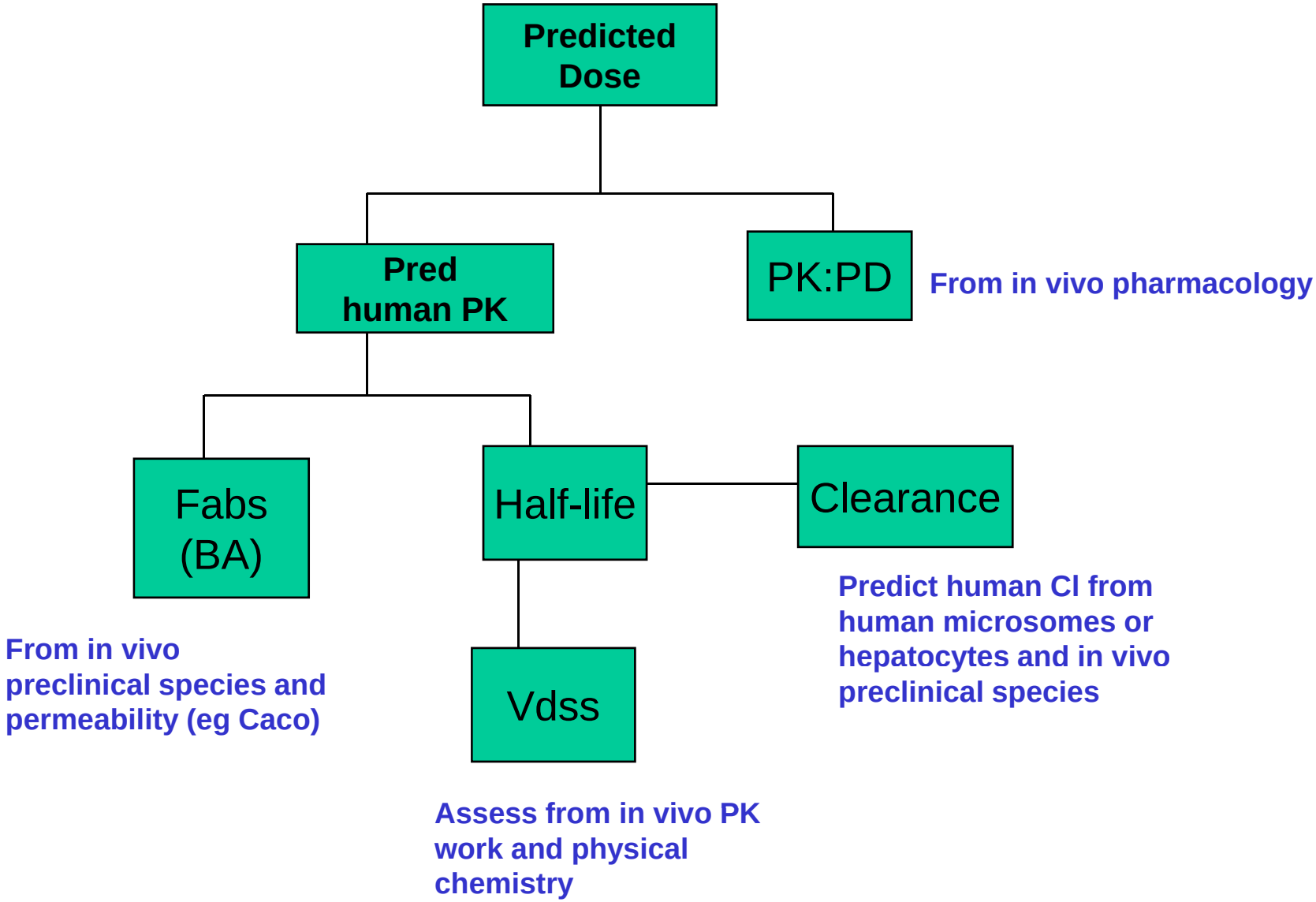
	GKA 31	GKA 30
Enzyme EC_{50} (μM)	0.02	0.61
% free (Rat)	0.23	5.34
Solubility (μM)	8	3140
In vivo activity	3mg/kg	1mg/kg



Biorg. Med. Chem. Lett., **2006**, 16, 2705

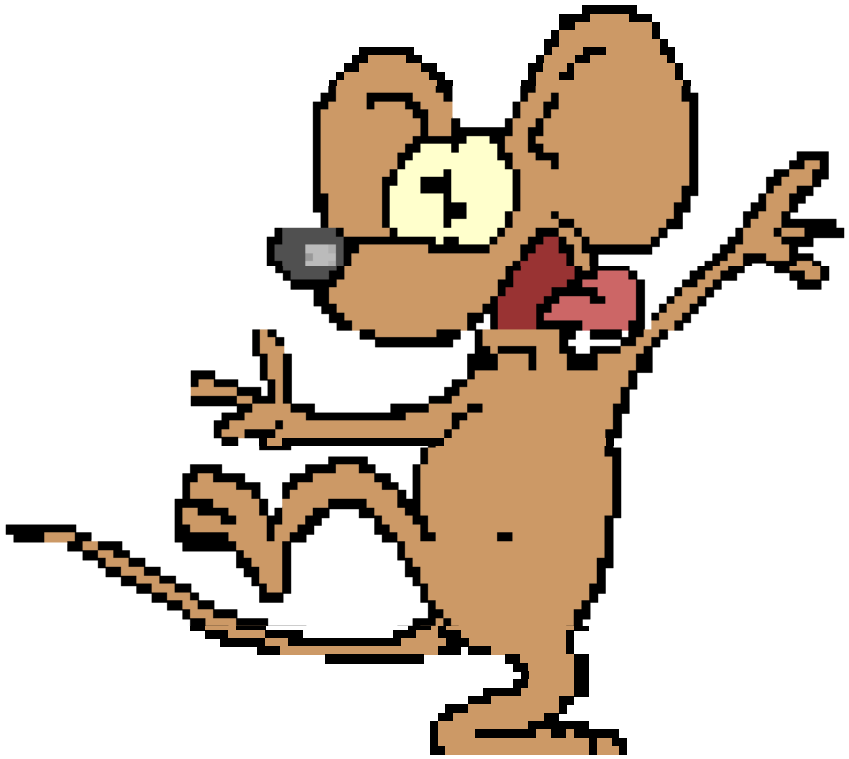
And if you can predict in vivo activity, perhaps you can predict the human dose too!

Prediction of Human Dose - Factors



Toxicity

How do you know you have a problem?



Safety Assessment (Benefit vs Risk)

- Likely side effects have to be identified and minimised
- For drugs, there has to be a *benefit* to the patient
  - ie any side-effects suffered have to be out-weighed by the beneficial effects of the drug
  - This will depend on the seriousness of the disease!
- For healthy volunteers in PhI trials, there is **no net benefit**, so the compound has to be extremely safe, or given at low doses!

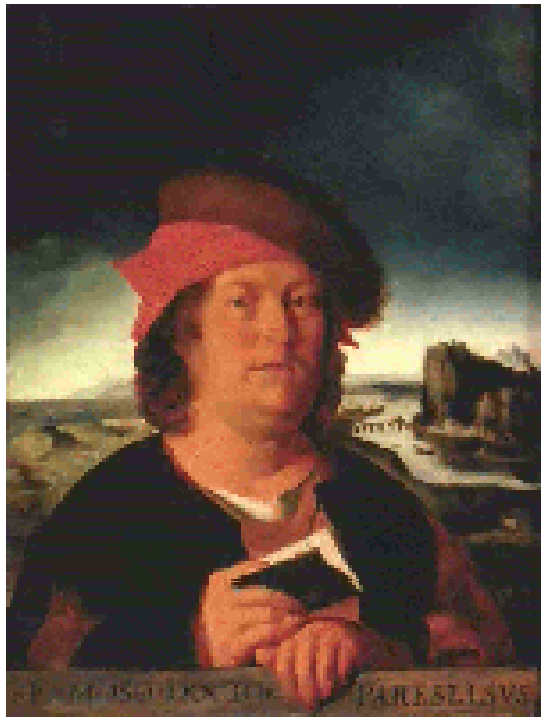
The Role of Toxicology

- **Identify Hazards**
  - Need to identify potential target organs
  - Need to know of consequences of overdosing

- **Assess Risk to Man**
  - Key is to understand the worst scenario in human - not what happens at efficacious dose
  - Need (a regulatory requirement!) to dose as high as possible
    - 1g/kg(/day) or MTD or max. solubility or max. total plasma levels are reached
    - This can be several hundred fold higher than the efficacious dose
    - But, to put in context, need to know margin of safety
  - Need to look at reversibility of any toxicities
  - Is the toxicity premonitorable?

- **To assess risk you must understand:**
  1. Hazard
  2. Margins
  3. Relevance to man

The Concept of "*Margin of Safety*"



Philippus Aureolus Theophrastus
Bombastus von Hohenheim

Paracelsus

(1493 - 1541)

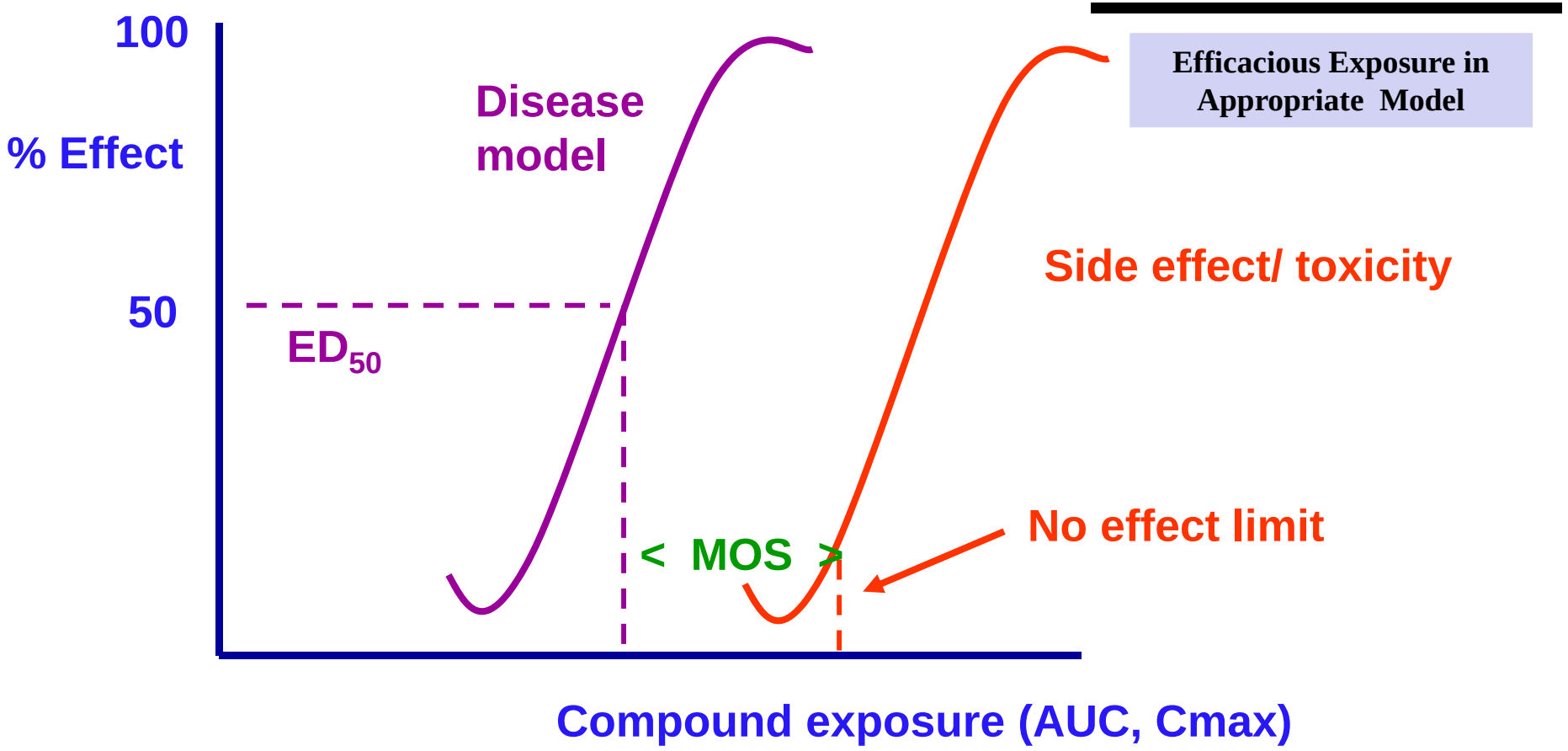
"All substances are [toxic];

There is none which is not [toxic].

It is the dose that differentiates a poison from a remedy."

Margin of Safety

Highest Exposure Associated with No Toxicity (NOEL) or "Manageable" Toxicity (NOAEL)

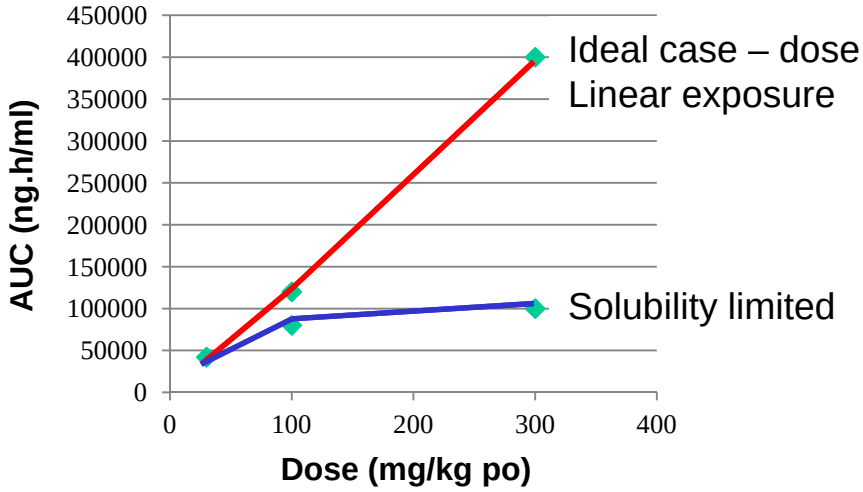


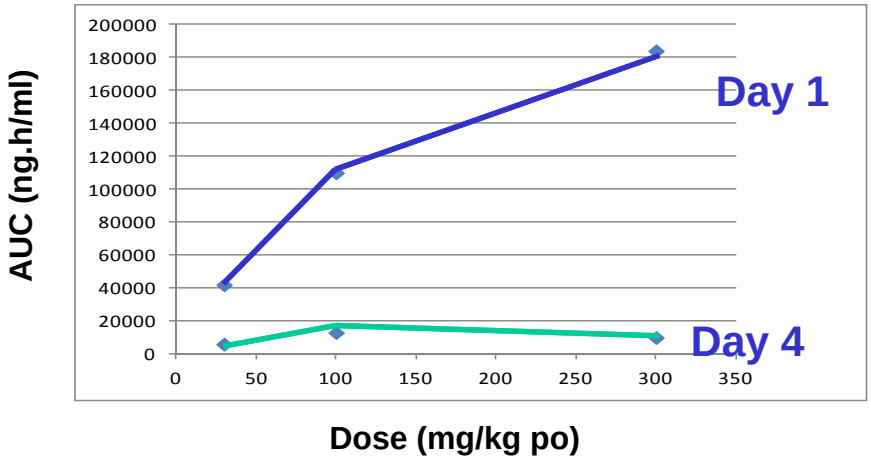
- Based on exposure, not dose!

Margin of Safety – an aside

Toxicology exposures need to exceed efficaceous exposures

- Ideally want high (>100x) multiples over efficacy exposures
- More often: absorption is limiting and exposure plateaus
- Metabolic processes may be saturated or induced

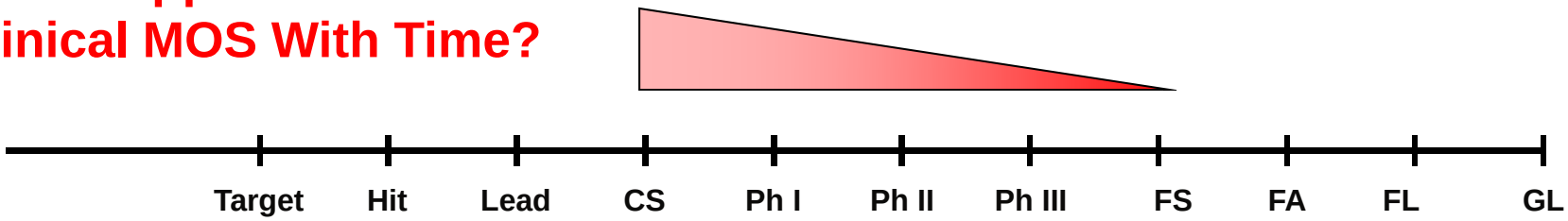




Compound is metabolised by CYP1A2 but induces this enzyme in liver over 3 days

A Narrow Margin of Safety in Non-Clinical Species Does Not Kill Compounds

What Happens to Non-Clinical MOS With Time?



What Does Kill Compounds?

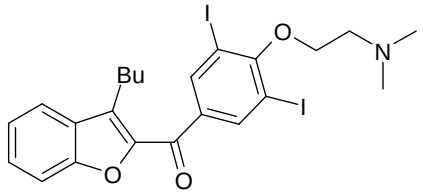
1. Lack of Monitorability
2. Lack of Reversibility
3. Uncertainty Regarding the Translation to Man
4. Idiosyncratic Drug Reactions (unpredictable, dose independent)

Common Toxicities

- Cardiovascular
  - Blockade of hERG potassium channel
  - Prolong QT interval – arrhythmias, death
  - Early alert: Binding assays and ion channel electrophysiology
- Hepatotoxicity
  - Irreversible CYP450 inhibition
  - Reactive metabolites
  - Early alert: In vitro studies in hepatocytes/ liver slices
- Reactive metabolites
  - Toxicity derived from pathway/ intermediates
  - In vitro reactive metabolite screens
  - In vivo studies to detect glutathione adducts (bile, urine)

Common Toxicities

- Genetic toxicity/ Mutagenicity
  - Mini-Ames, in vitro micronucleus tests
  - GreenScreen – human cell based gene reporter assay
  - Run + or – S9 liver fraction to assess metabolites
- Phospholipidosis/ phospholipid accumulation in cells
  - Cationic amphiphilic drugs
    - Eg: amiodarone - lung and liver toxicity
    - Lipophilic ring + hydrophilic chain bearing cationic group
  - In vitro cellular assays and chromatographic methods
  - High Vd can be a warning



- CNS side effects
  - BBB penetration
  - Off target pharmacology
  - Early alert: broad CNS receptor and enzyme screening

Toxicity – what can chemists do?

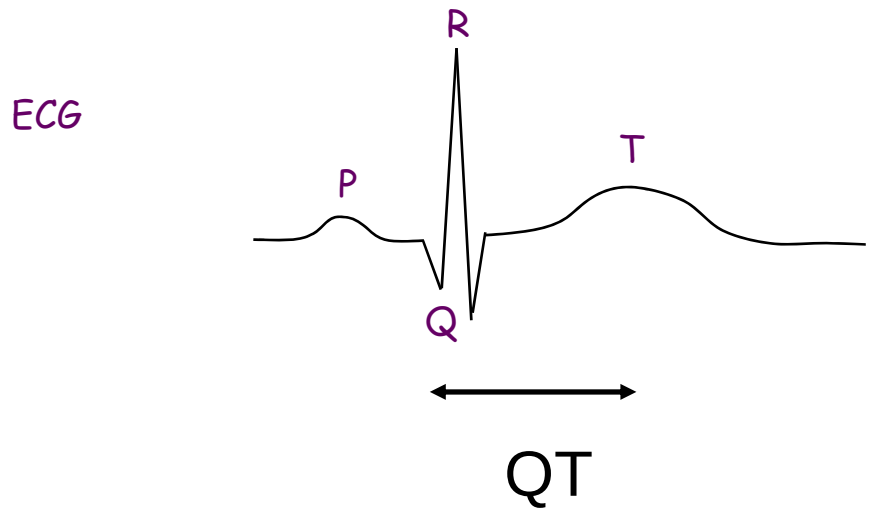
- Ideally, want efficacious compounds with no side effects
- More often…
- Observe side effects in one or more species
- Mechanism related
  - Exaggerated pharmacology (hypoglycaemia when taking glucose lowering agents or positional hypotension when taking blood-pressure lowering agents)
  - Undesirable consequence of biology (cytotoxics in cancer therapy)
- Secondary Pharmacology
  - Lack of selectivity against another target
- Compound-related
  - Parent or metabolite

Not a lot chemists can do!

Maybe something chemists can do!

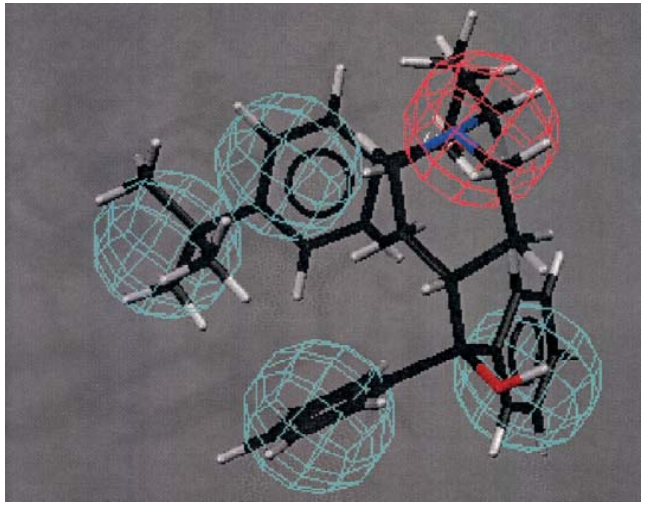
hERG - Background

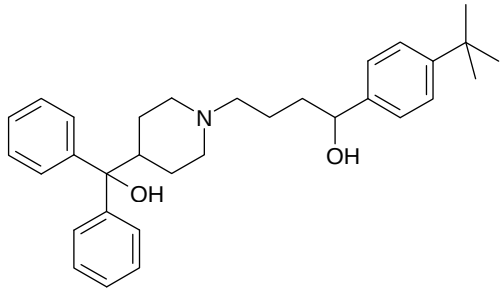
- Human *Ether-a-Go-Go*-Related Gene
- Potassium ion channel expressed in heart
- Associated with QT interval prolongation
- Can cause arrythmia and sudden death!
- Terfenadine, cisapride and astemizole withdrawn due to Herg blockade



hERG – What can chemists do?

- Most potent hERG inhibitors seem to be strongly basic + highly lipophilic molecules – reduce logP and attenuate basicity (pKa)
- Avoid hERG pharmacophores
- Ability to form π-stacking and hydrophobic interactions with aromatic residues on hERG is important – these can be disrupted
- J. Med Chem (2006) 49(17) 5029-5046 for review of assays and strategies for reducing hERG activity.



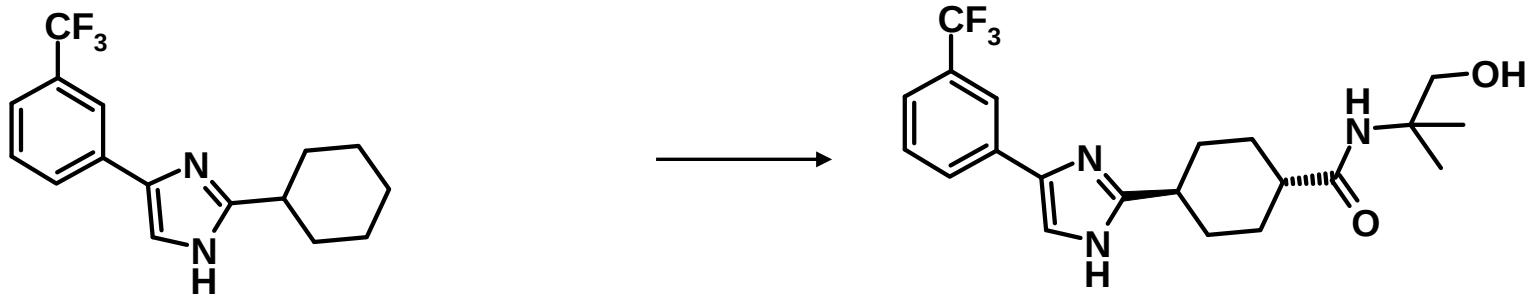


Terfenadine fitted to a QSAR derived Herg Pharmacophore
Hydrophobic regions in cyan
Positive ionizable regions in red

Reducing Activity at hERG

Neurogen: Neuropeptide Y-Y5 antagonists

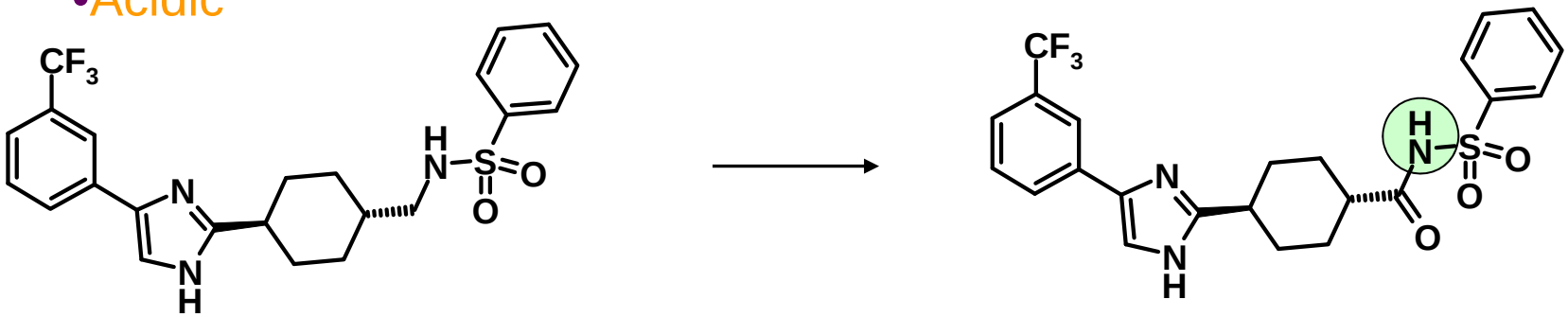
- Lower lipophilicity-adding hydrophilic groups



hERG 60% @ 3µM
logP = 3.34

hERG 6% @ 3µM
logP = 2.3

- Acidic



hERG 87% @ 300nM

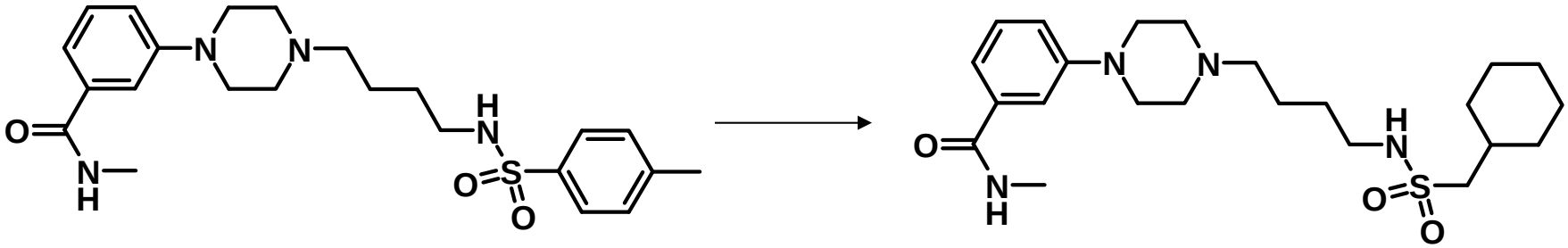
hERG 7% @ 3µM

J. Med. Chem **2004,** 47, 2318-2325

Reducing Activity at hERG

Predix Pharm: 5HT1A agonists-anxiety

- Removing aromatic interactions



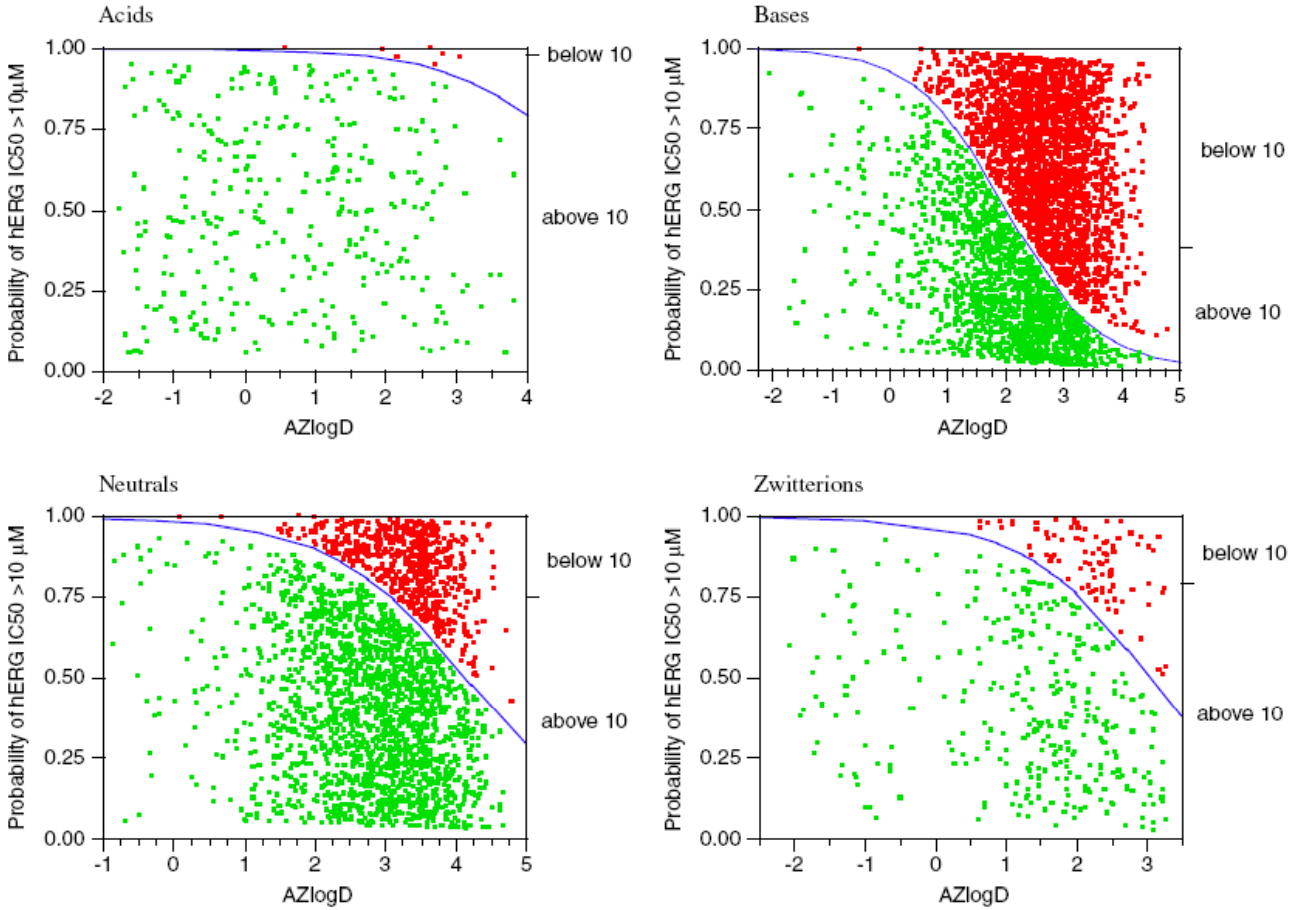
hERG IC_{50}= 300nM
ACDpKa = 6.8
ACDLogP = 0.66
ACDLogD = 0.6

hERG IC_{50}= 3800nM
Removing interaction to Ph656
ACDpKa = 6.8
ACDLogP = 0.87
ACDLogD = 0.8

Insilico based methods as primary tool
-Model 3D hERG channel

J.Med. Chem. 2006, 49, 3116-3135

LogP component to Herg liability



Logistic regressions showing how the probability of a compound achieving a hERG IC_{50} of >10 µM changes with AZlog *D* for each ionisation class. Those compounds with IC_{50} values above 10 µM are shown in green; those below 10 µM are in red.

Bioorganic & Medicinal Chemistry Letters 17 (2007) 1759–1764

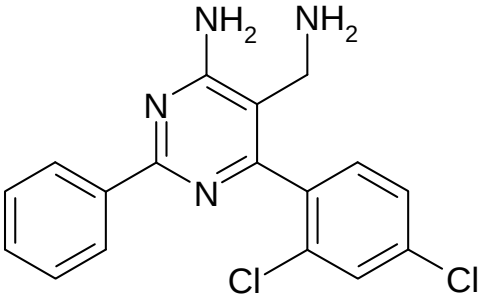
Target upper limits of logD and clogP to ensure >70% of compounds achieve a hERG IC50 of greater than 10 µM

	Acids	Bases	Neutrals	Zwitterions
logD	>4	1.4	3.3	2.3
clogP	>9	1.9	4.0	4.4

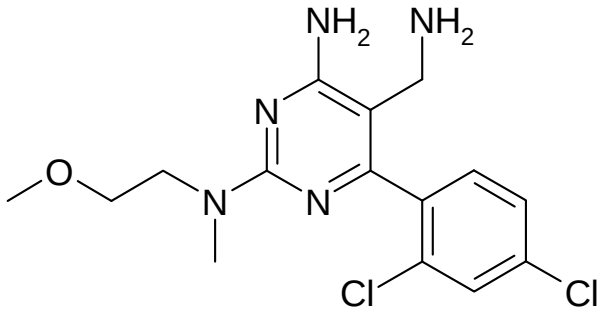
Phospholipidosis – What can chemists do?

- Reduce amphiphilic nature of compound (can be predicted or measured)
  - Reduce lipophilicity and basicity
- Increase steric hindrance around the amine
- Reduce or replace multiple Cl or CF_3 groups on an Ar ring

Roche DPP-IV inhibitors. Bio Med Chem Lett (2004) 14(13) 3575-3578



DPP-IV IC_{50} = 10 nM
$logD_{7.4}$ = 3.0, pKa = 7.8
Phospholipodosis in fibroblasts



DPP-IV IC_{50} = 9 nM
$logD_{7.4}$ = 1.6
No Phospholipodosis

Reviews

Drug-Induced Phospholipidosis: Are There Functional Consequences? Exp Biol Med, 226(9), 825-830, 2001.
In Silico Assay for Assessing Phospholipidosis Potential of Small Druglike Molecules J. Med. Chem. 2012, 55, 126−139

And sometimes it seems that there’s not a lot that chemists can do….

But look more closely!

Liver toxicity – Example from GSK

Background

GSK had series of compounds which suffered liver toxicity

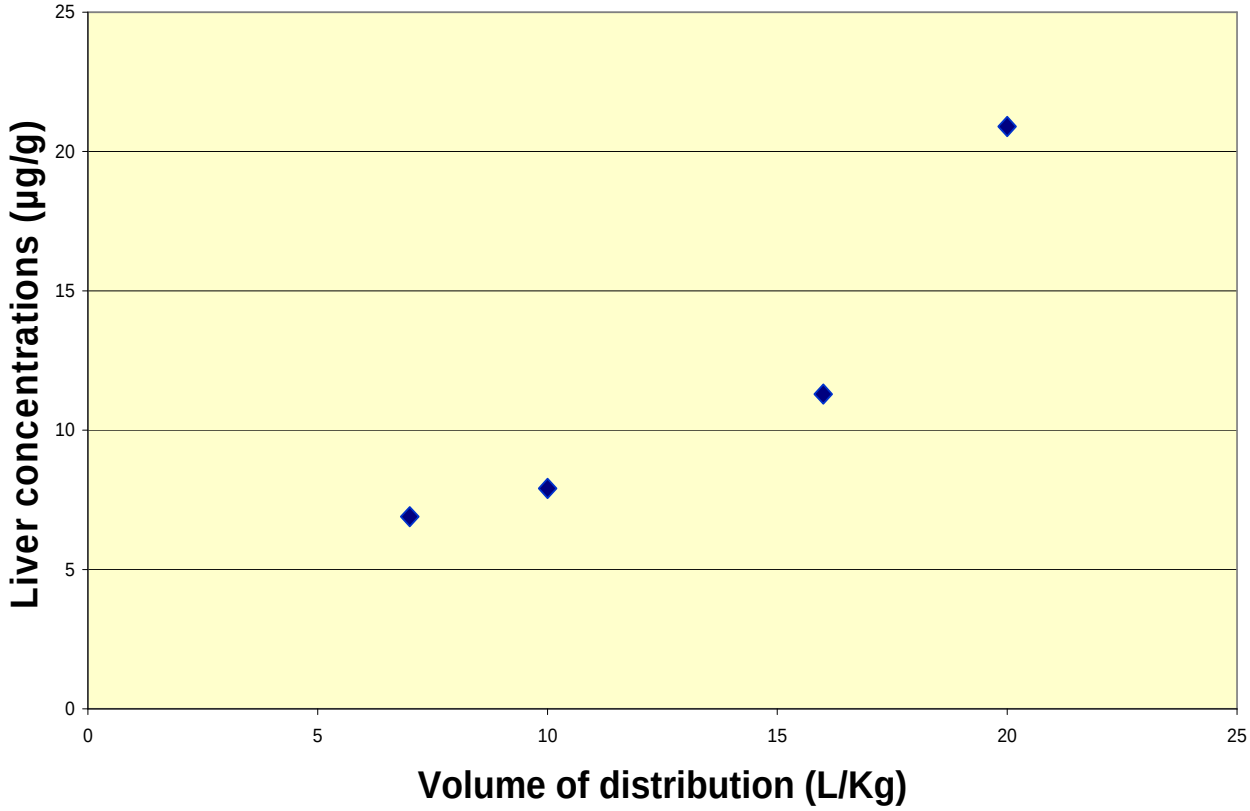
Compounds were lipophilic bases, and were intended to act centrally (penetrate blood-brain barrier)

Drug levels in plasma and liver were determined at end of 7d tox study ….

	Liver/plasma concentration ratios		
	30 mg/kg	**100 mg/kg**	**300 mg/kg**
GW *AAAAAA*	**70**	**499**	**383**
GW *BBBBBB*	**173**	**565**	**1140**
GW *CCCCCC*	**1100**	**7800**	**5200**
GW *DDDDDD*	**51**	**103**	**110**

⇒ *liver accumulation is compound specific and is not related to plasma exposure (AUC)*

Correlation of volume of distribution and liver concentrations after a single low dose (<10mg/kg)



⇒ *Relationship between Vd and liver disposition could be useful to design compounds with lower liver accumulation and hopefully toxicity*

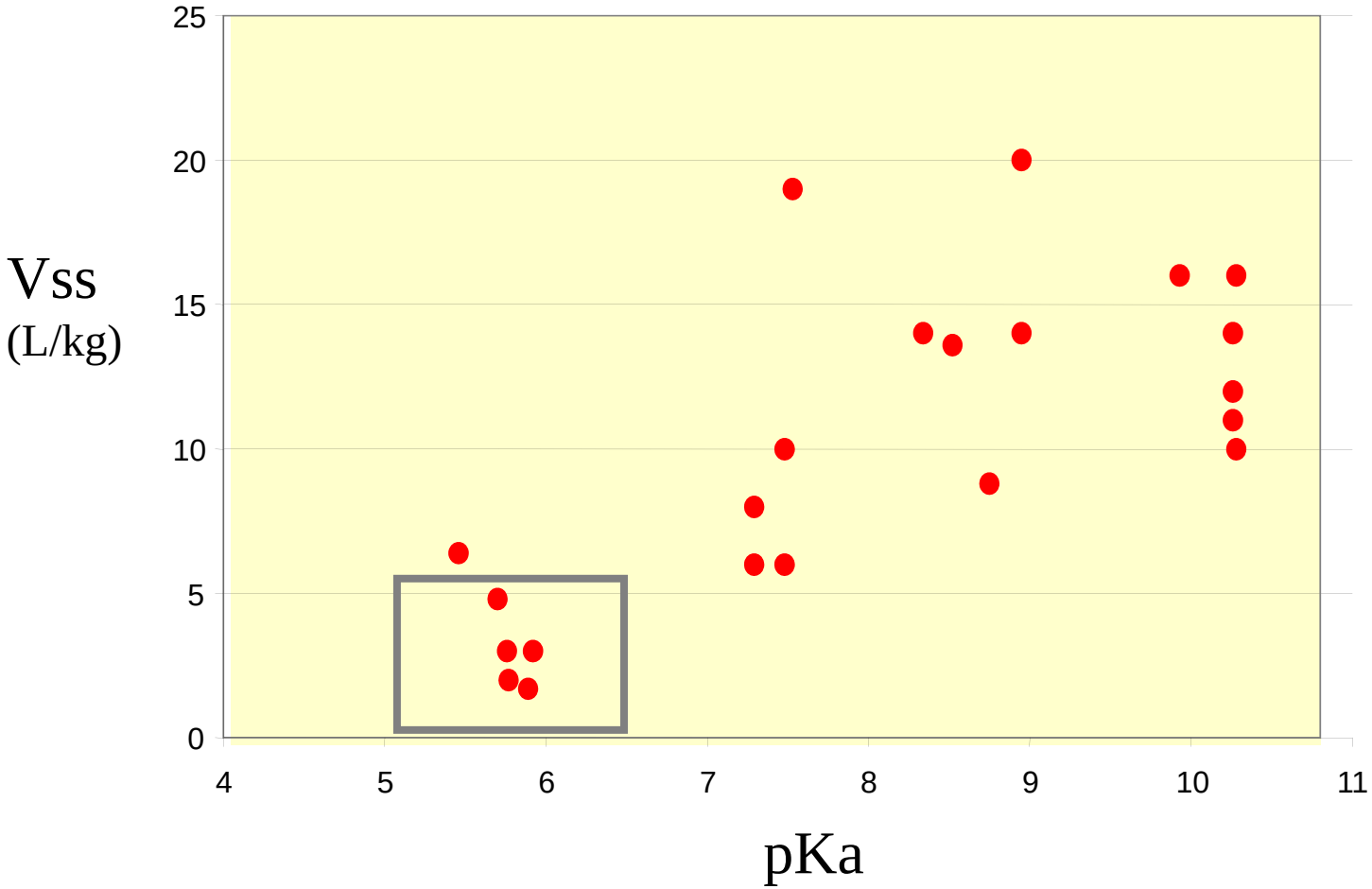
Volume of Distribution

- Factors affecting volume are:
  - Lipophilicity
    - increase logD, increase Vdss
  - Plasma protein binding
    - increase PPB, decrease Vdss
  - pKa
    - generally bases > neutrals > acids

- (strong lipophilic bases tend to have high Vd because of their interaction with cell membranes and lysosomal trapping (Low pH environment)

Basicity and volume of distribution
- piperidine based antagonists

- 24 compounds with known Vss



Success!

- Lower pKa compounds identified and tested
- Low liver/plasma ratios (1-5) in acute low dose studies
- Best compounds gave improved brain penetration and no hepatotoxicity in tox studies at any dose.

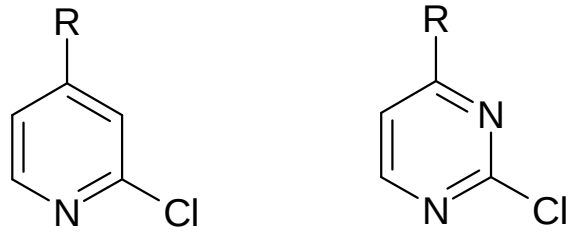
- Compound selected for phase 1 clinical studies

Reactive molecules and metabolites

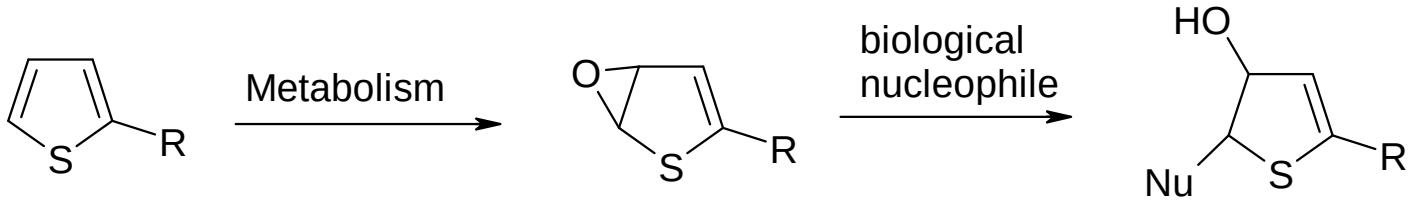
- The body is full of mild nucleophiles (proteins, peptides, glutathione etc)
- Reaction between small molecules and proteins or peptides can give rise to foreign adducts
- These adducts can cause immunological responses or further organ toxicities
- This kind of toxicology is often spotted late – very expensive!

What can chemists do?

- Avoid electrophilic compounds
  - eg electron deficient aromatic rings with leaving groups

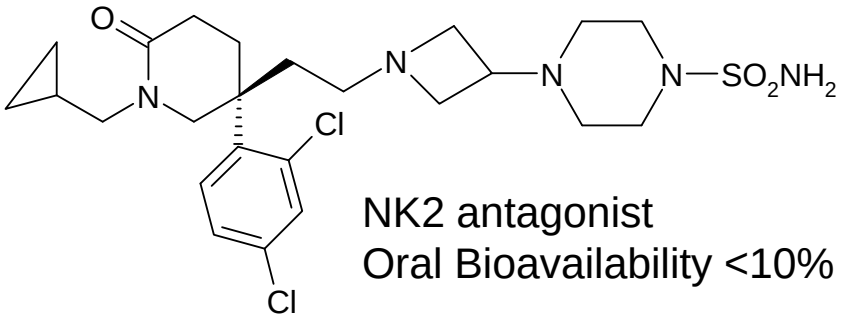


- And motifs/ groups which could give reactive metabolites
  - Eg thiophenes, furans



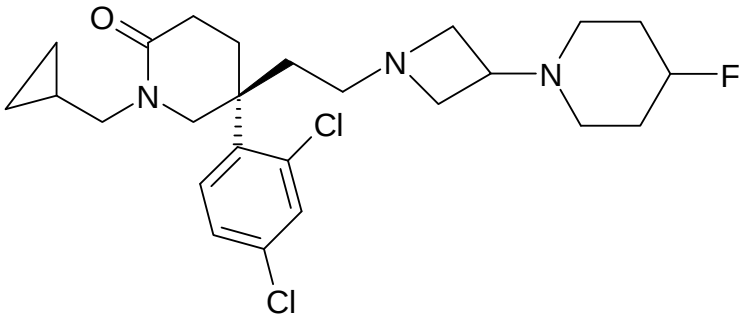
Reviews - A Comprehensive Listing of Bioactivation Pathways of Organic Functional Groups A.S.Kalgutkar et al , Current Drug Metabolism, 2005, 6, 161-225.
- Biotransformation Reactions of Five-Membered Aromatic Heterocyclic Rings, Chem. Res. Toxicol., 2002, 15, 269-299

Reactive metabolite example from Pfizer



NK2 antagonist
Oral Bioavailability <10%

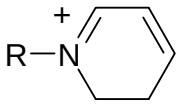
Absorption increased
by raising logP



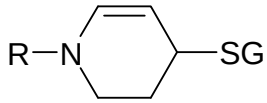
Compound stopped due to testicular toxicity

Oxidation

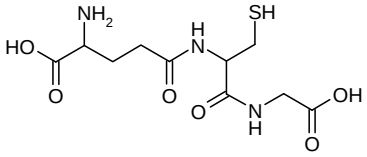
-F



GSH

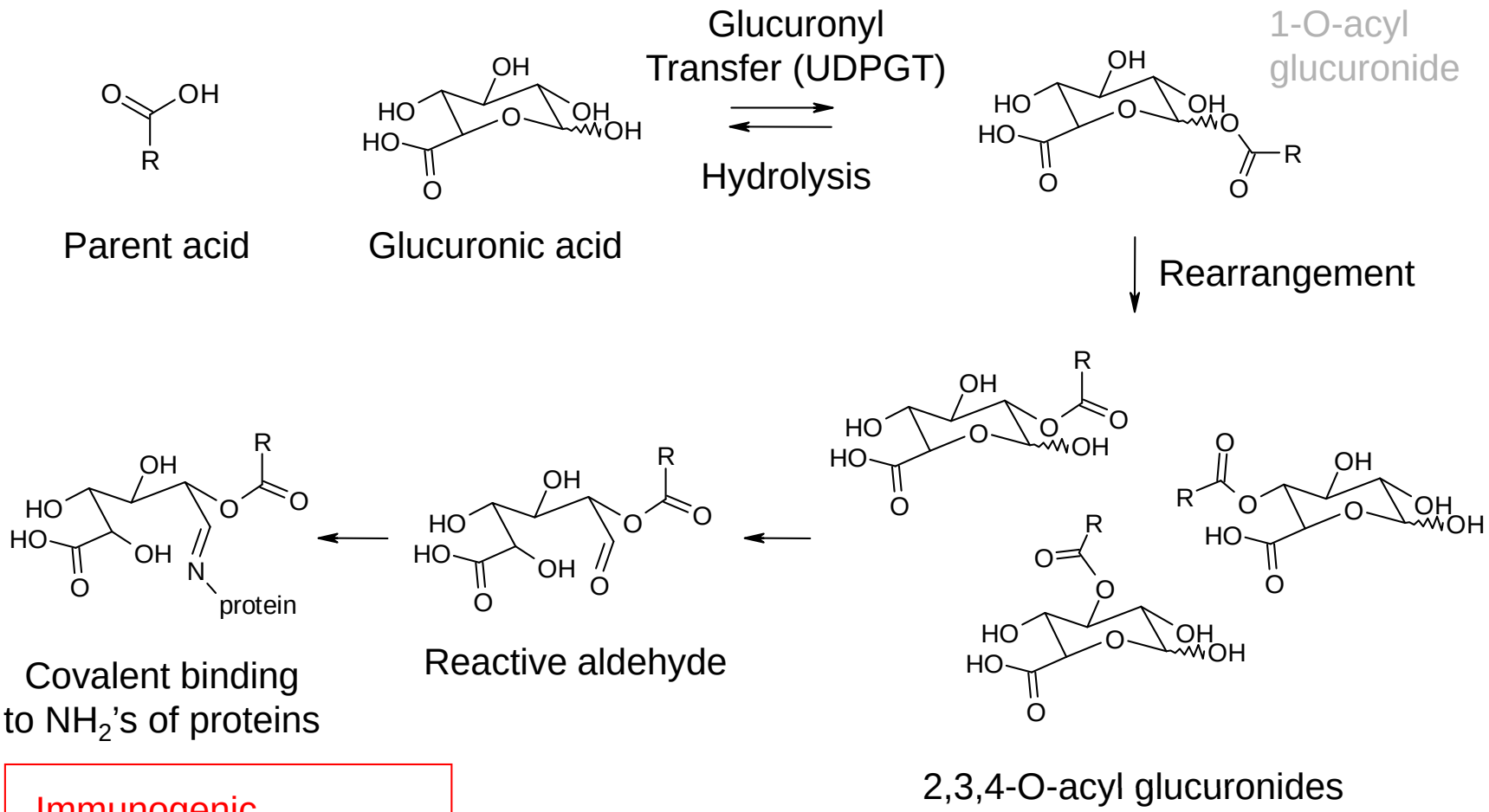


Glutathione
GSH =



Acyl glucuronides

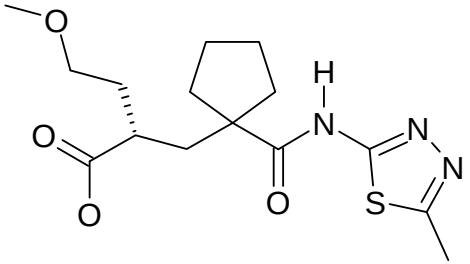
Acyl Migration and Covalent Binding



Immunogenic
Implicated in GI toxicity

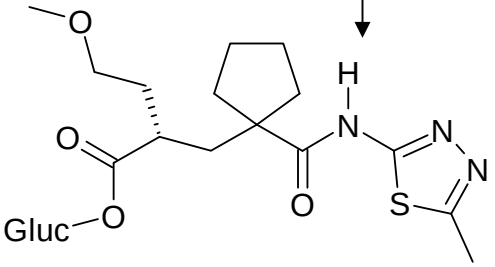
For a review Current Opinion in Drug Discovery & Development 2007 **10**(1):58-66

Reactive metabolite example from Pfizer

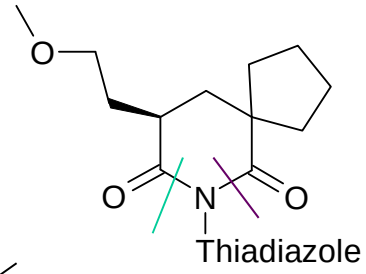


UK 414,495
Potent NEP inhibitor
Prototype candidate
stopped due to GI tox

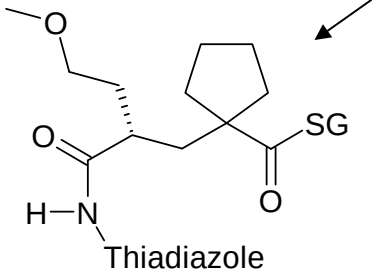
pKa 9.6



$t_{1/2}$ = 0.17h

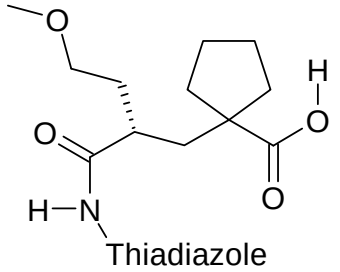


GSH



Toxic glutathione conjugate

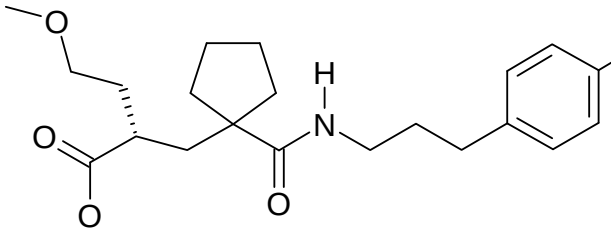
$t_{1/2}$ = 8.7h



Major circulating Metabolite in dog

Rapid formation of reactive diimide *in vivo* via acyl glucuronide
Driven by pKa of amide?

SOLUTION – identify equipotent analogue with amide pKa >10
UK 447,841 – stable acyl glucuronide and cyclic imide
- successful candidate in P1.



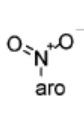
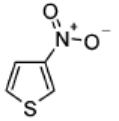
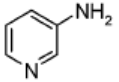
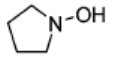
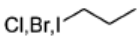
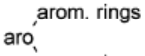
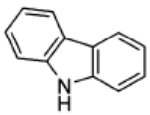
Calc pKa = 16

Thanks to David Pryde

Toxicophores for Mutagenicity

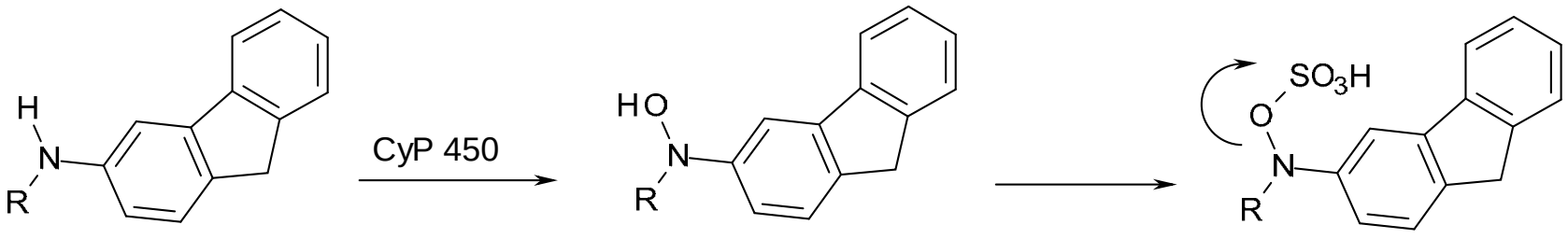
Structural alerts for DNA Reactivity

- DNA adducts
- Base deletions, insertions and mutations
- Distortion of DNA structure
- Intercalation eg of polyclic aromatics
- Parent or metabolites

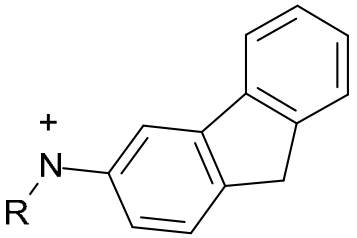
Toxicophore name	Substructure representation	Example compound
aromatic nitro		
aromatic amine		
three-membered heterocycle		
nitroso		
unsubstituted heteroatom-bonded heteroatom		
azo-type		
aliphatic halide		
polycyclic aromatic system		

J. Med. Chem. **2005,** *48,* 312-320

Toxicity of anilines and derivatives

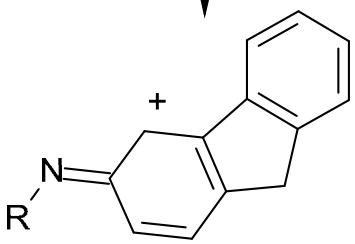


The more electron rich the aniline, the greater the risk!

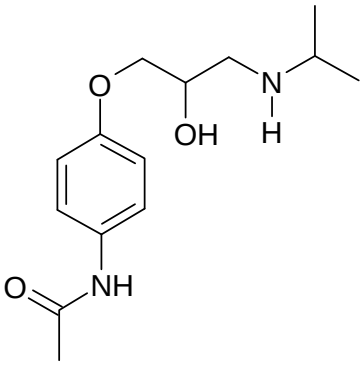


Interaction with proteins or DNA

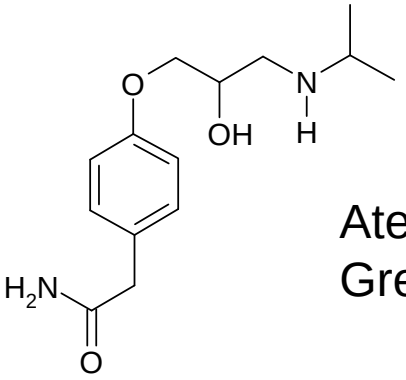
Organ Toxicity
Genetic Toxicity



Look for alternatives

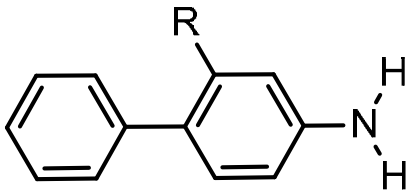


Practolol
Ocular toxicity



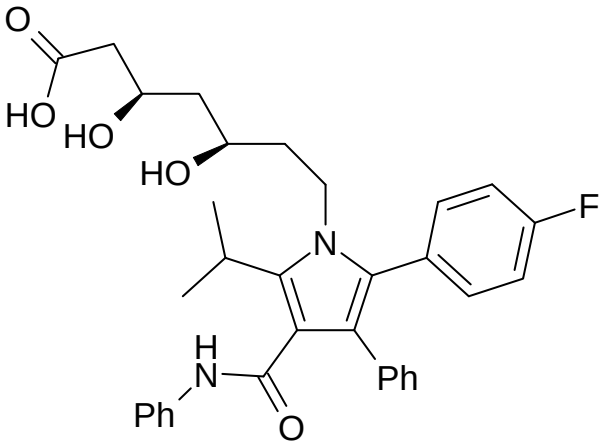
Atenolol
Greater Safety

OR….



Reduce liability to metabolism
R = H : Ames +ve (+ S9)
R = Cl : Ames –ve (+S9)
- electronic/ conformational effects

J. Med. Chem (2012), 55(8), 3923-3933.

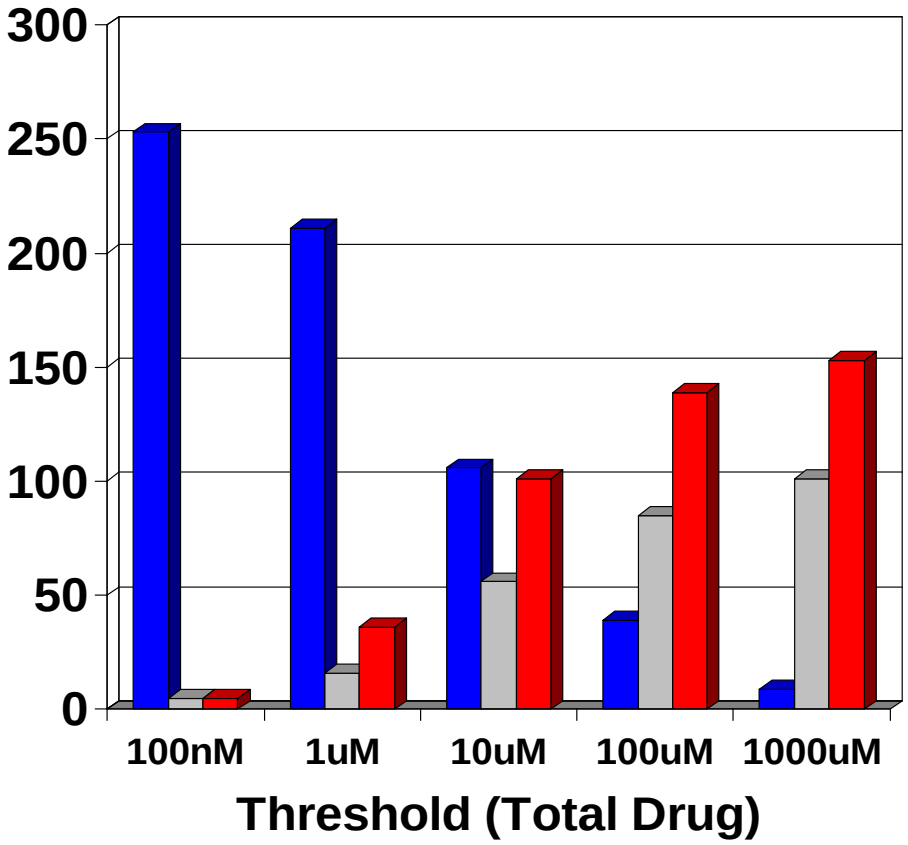


Atorvastatin
Anilide NH is hindered

Tony Wood (Pfizer)

In vivo Toxicity

- Results of an analysis of 349 studies on 315 compounds covering 90 targets at 985 doses with >10,000 organ evaluations in 4 species
- PK known for all cases - strong correlation between AUC and Cmax
- Compound set has similar diversity to Pfizer file



- exposure thresholds were chosen to obtain a balance of toxicity/non-toxicity.
- set to 10uM for the total-drug threshold.
- approx 40% of evaluations above threshold & 40% below.

Clean
Uncertain
Toxic

- similar analysis for free drug levels gives a threshold of 1 uM.

Bioorganic & Medicinal Chemistry Letters 18 (2008) 4872–4875

Pfizer in vivo Toxicology Findings: PSA/cLogP

Total Drug	TPSA>75	TPSA<75
ClogP<3	1.35 (61)	2.47 (59)
ClogP>3	1.18 (37)	13.5 (87)

10-fold higher risk toxic outcome

Free Drug	TPSA>75	TPSA<75
ClogP<3	1.06 (33)	1.00 (24)
ClogP>3	2.43 (24)	28.5 (59)

27-fold higher risk toxic outcome

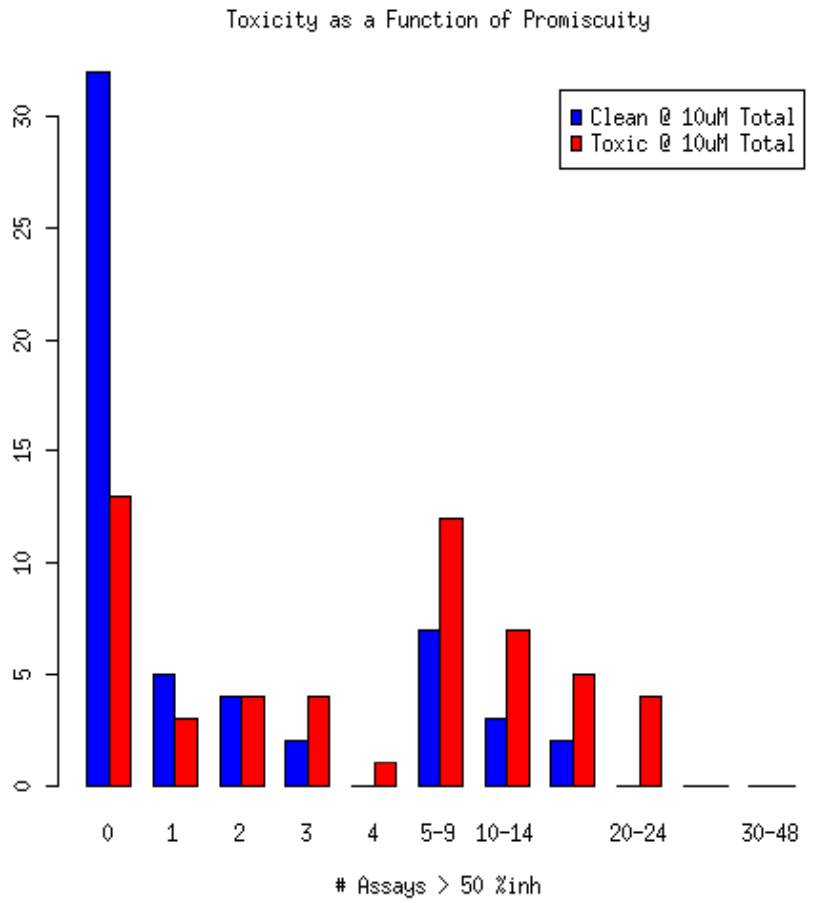
Significantly higher risk of toxicity findings when cLogP>3 AND TPSA<75Å^2

- Numbers in parentheses indicate number of outcomes in database
- Holds for both free-drug or total-drug thresholds

Hughes *et al*. (2008) *Bio Med Chem Letts 18*, 4872

Thanks to Tony Wood (Pfizer)

Toxicity and Promiscuity



ratio of promiscuous to non-promiscuous compounds

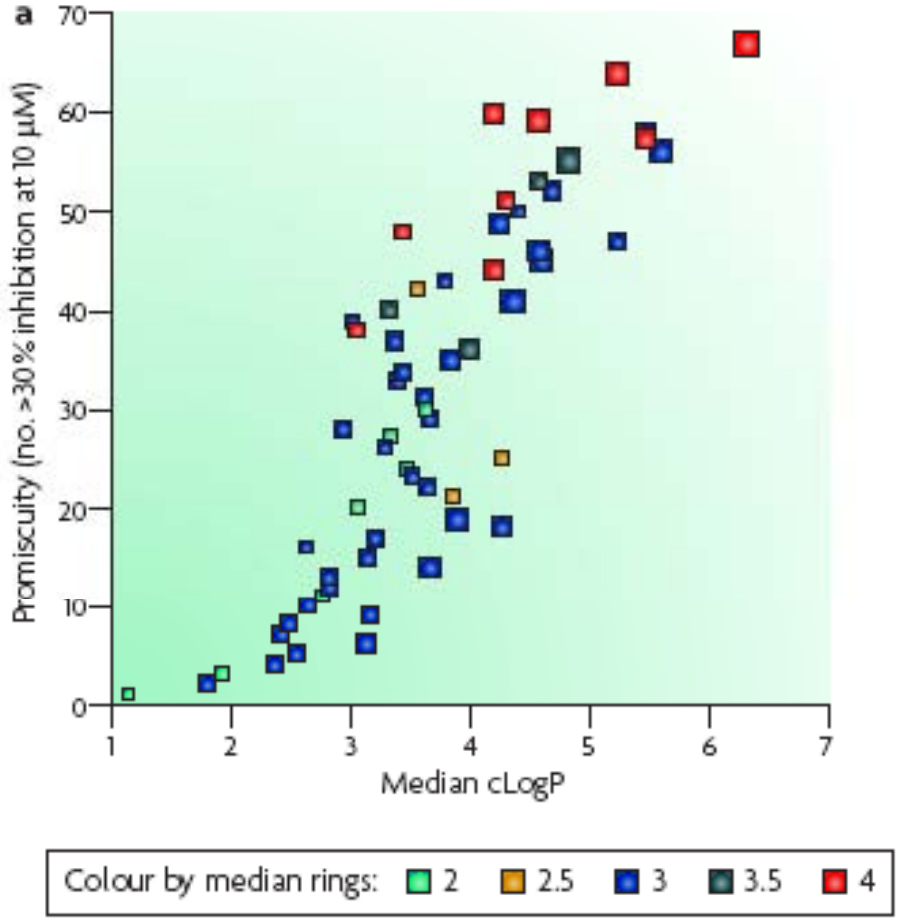
	TPSA>75	TPSA<75
ClogP<3	0.25 (25)	0.80 (18)
ClogP>3	0.44 (13)	6.25 (29)

- promiscuity defined as >50% activity in >2 Bioprint assay out of a set of 48 (selected for data coverage only)

Lipophilicity and Promiscuity

cLogP *vs*. Promiscuity 2133 Cpds in 200 CEREP assays

- Promiscuity = # Compounds with >30% inhibition at [10 µM]
- Greater propensity for off-target binding for compounds with cLogP≤3



Leeson and Springthorp (2007) *Nature Rev./Drug Disc. 6*, 881

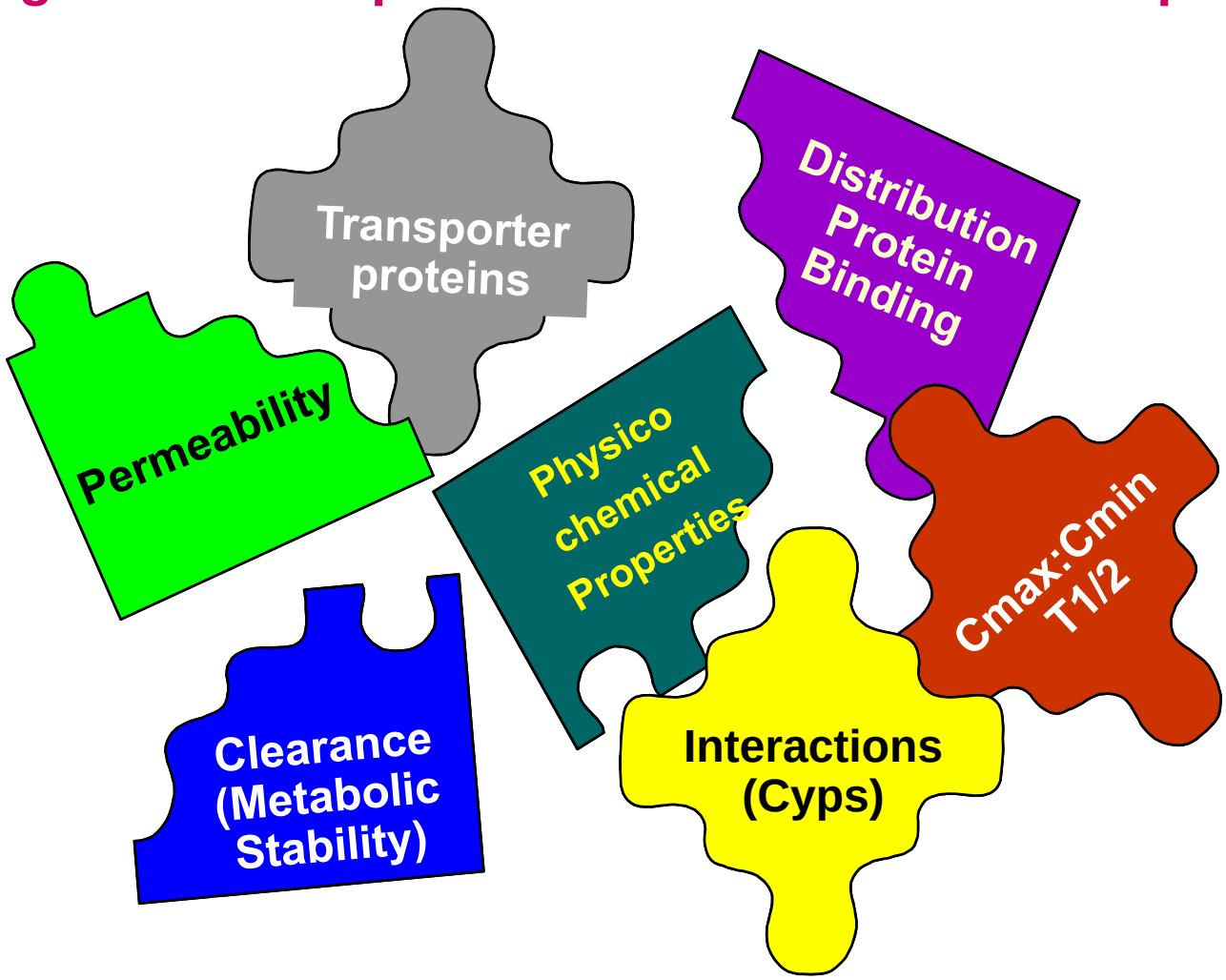
Summary – chemistry and toxicology

- Avoid hERG pharmacophores
  - Modulate pKa and lipophilicity
- Avoid amphiphilic species
- Avoid electrophilic (reactive) compounds
- Consider potential reactive metabolites
- Avoid electron-rich or unhindered anilines
  - Or avoid anilines completely!
- Combining low PSA and high LogP may increase the risk of toxicity

Closing Remarks

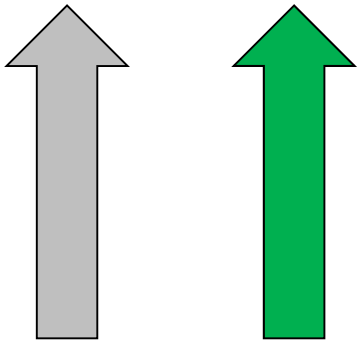
DMPK & Candidate Drugs

Candidate Drugs need good predicted human PK & minimal drug-drug interaction potential to have a chance of progress



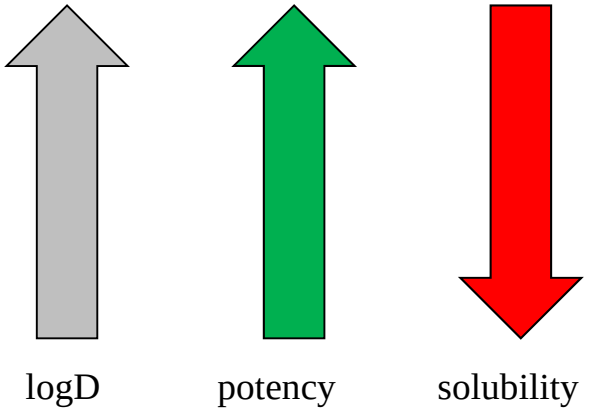
Drug Design Criteria for Medicinal Chemists to be worried about

Lipophilicity - Potency



- Lipophilicity needs to be optimised
- In general, increasing lipophilicity increases potency (increased binding to ‘fatty protein’ target)

Lipophilicity - Solubility

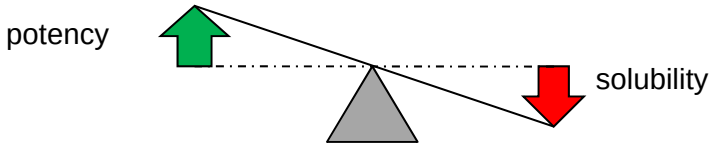


Tactics to improve solubility
- Reduce lipophilicity
- Disrupt crystal packing

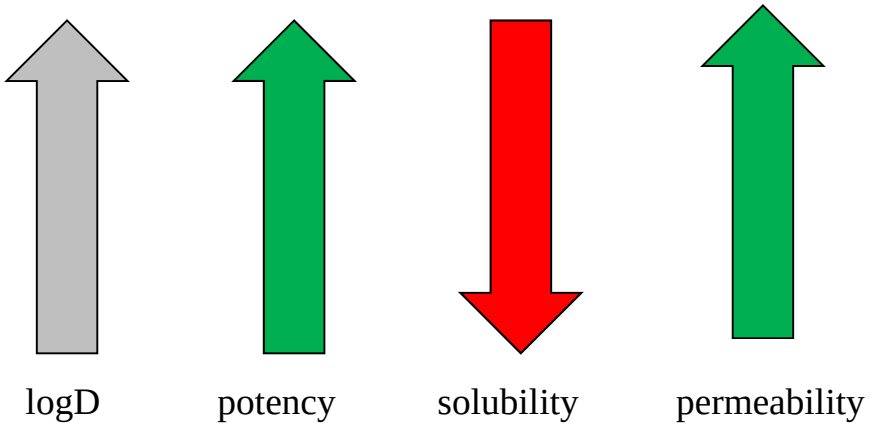
- Lipophilicity needs to be optimised

- Two properties are heading in opposing directions!
- Increasing logD could increase your potency but lower solubility!

- Need to strike a balance…….



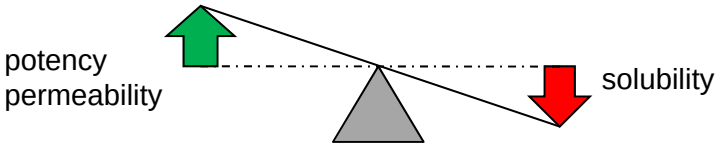
Lipophilicity - Permeability



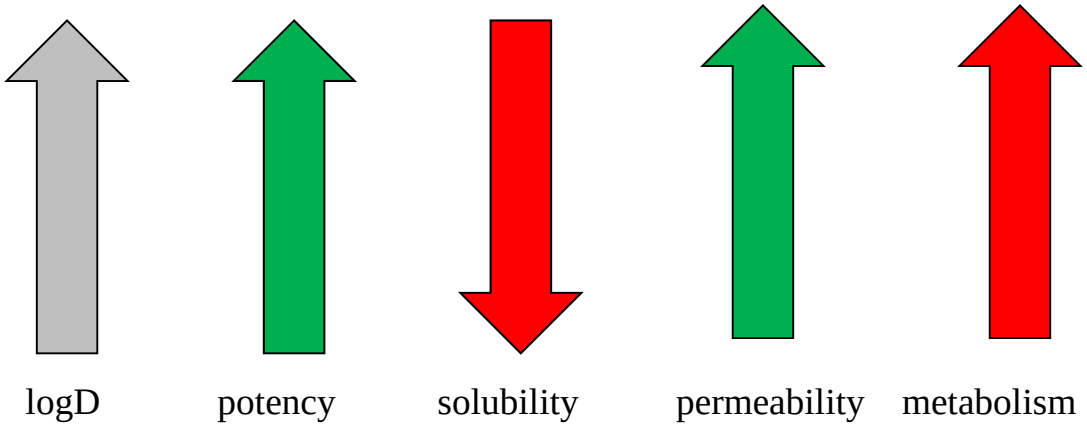
Tactics to improve permeability
- Increase lipophilicity
- Remove H-bond donors (NH,OH)
- Keep size small

- Lipophilicity needs to be optimised

- Increasing lipophilicity generally increases permeability (higher partition into membranes)



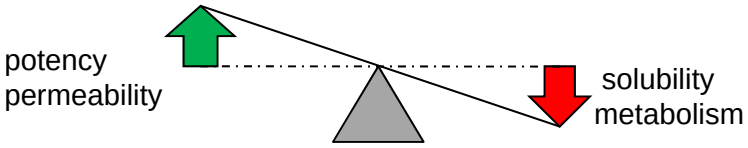
Lipophilicity - Metabolism



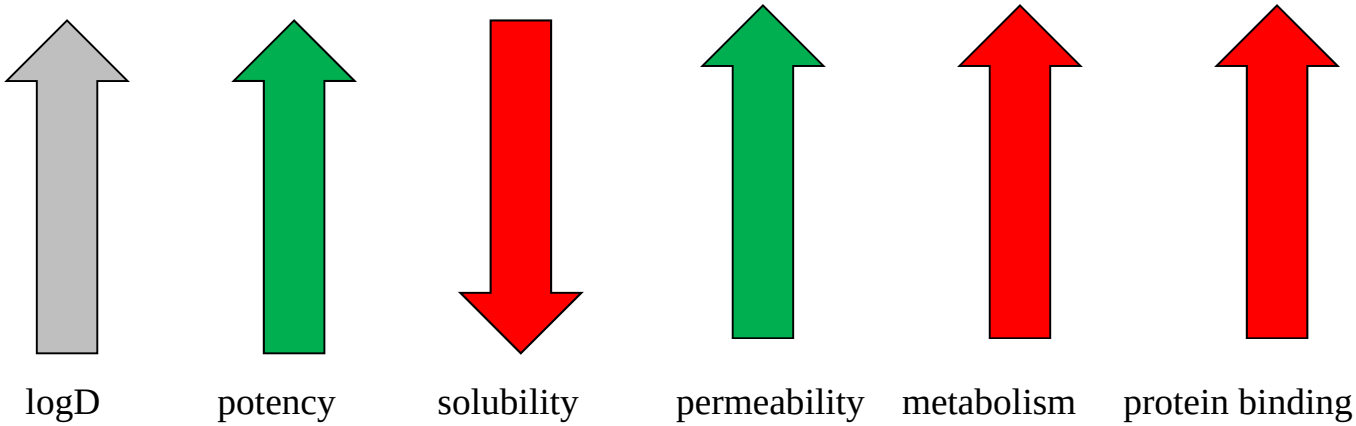
Tactics to reduce metabolism
- Decrease lipophilicity – add polar atoms, charged groups
- Add metabolic blocking groups (eg F for H)

- Lipophilicity needs to be optimised

- Increasing lipophilicity usually increases metabolism (more points of metabolism)



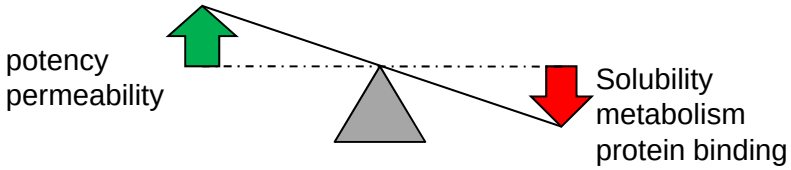
Lipophilicity – Plasma Protein Binding



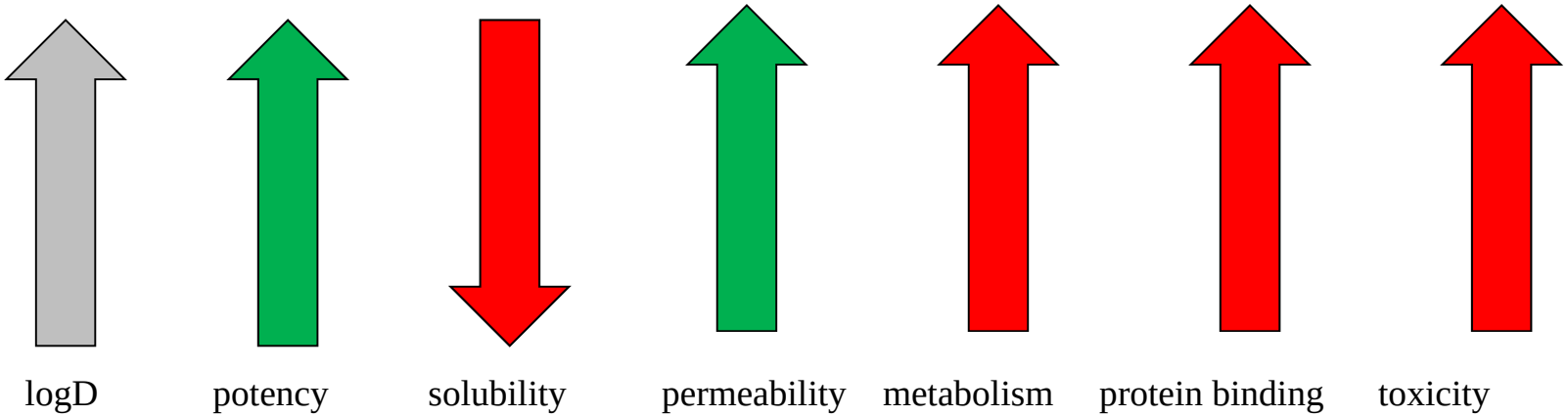
- Lipophilicity needs to be optimised

- In general, increasing lipophilicity increases plasma protein binding (increased binding to ‘fatty protein’)

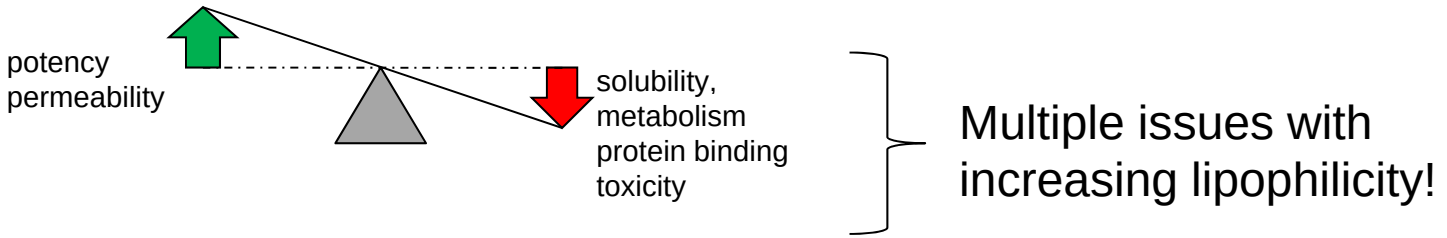
Tactics to reduce plasma protein binding
- Decrease lipophilicity
- Avoid acidic functionality



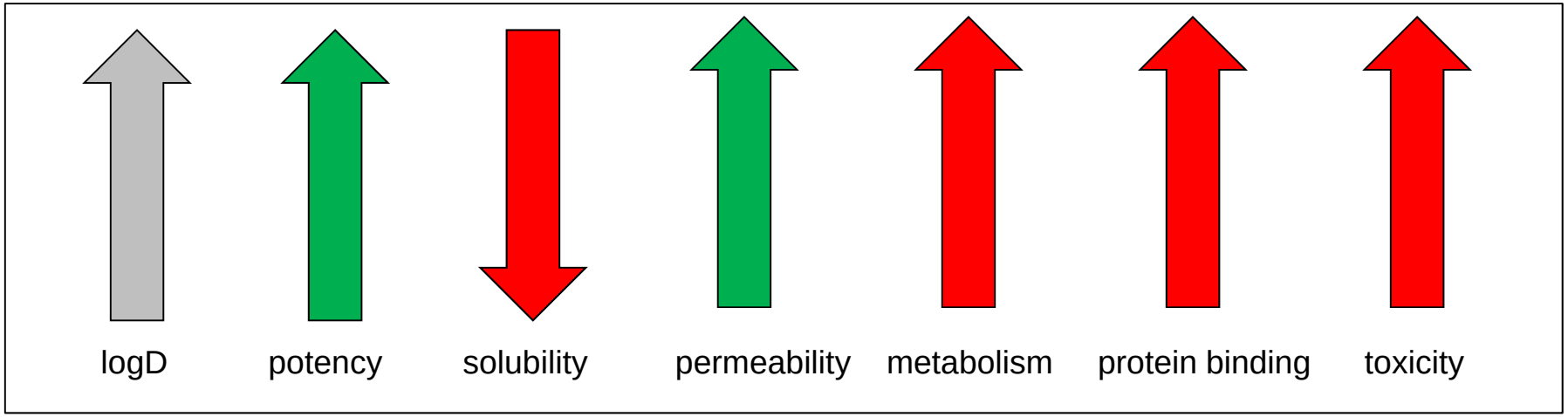
Lipophilicity - Toxicity



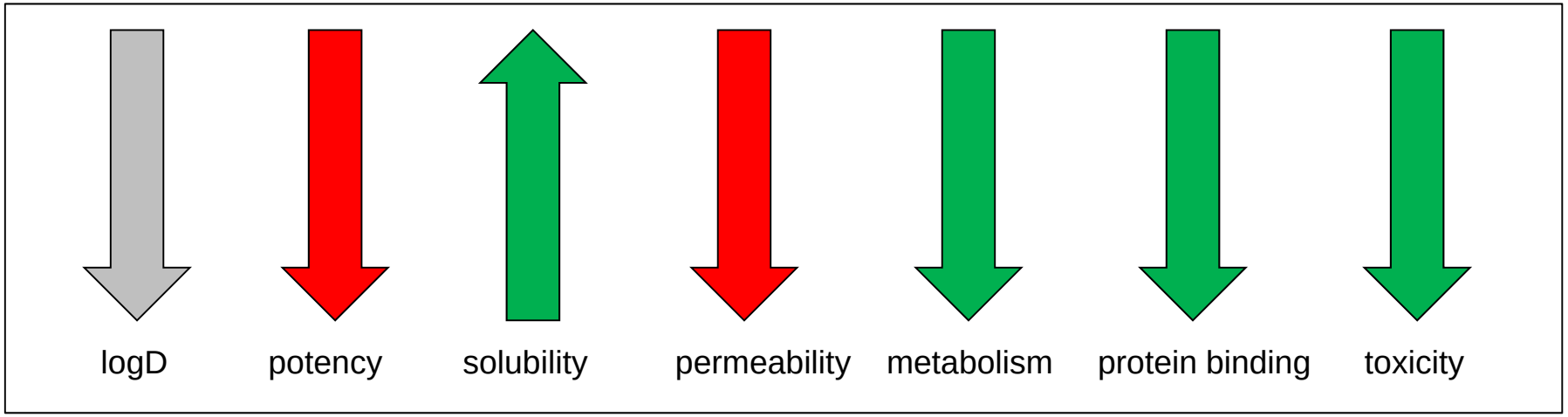
- Lipophilicity needs to be optimised
- In general, increasing lipophilicity increases chances of toxicity (increased binding to other targets)



Lipophilicity - Toxicity



- Lipophilicity needs to be optimised
- The reverse is also true…..



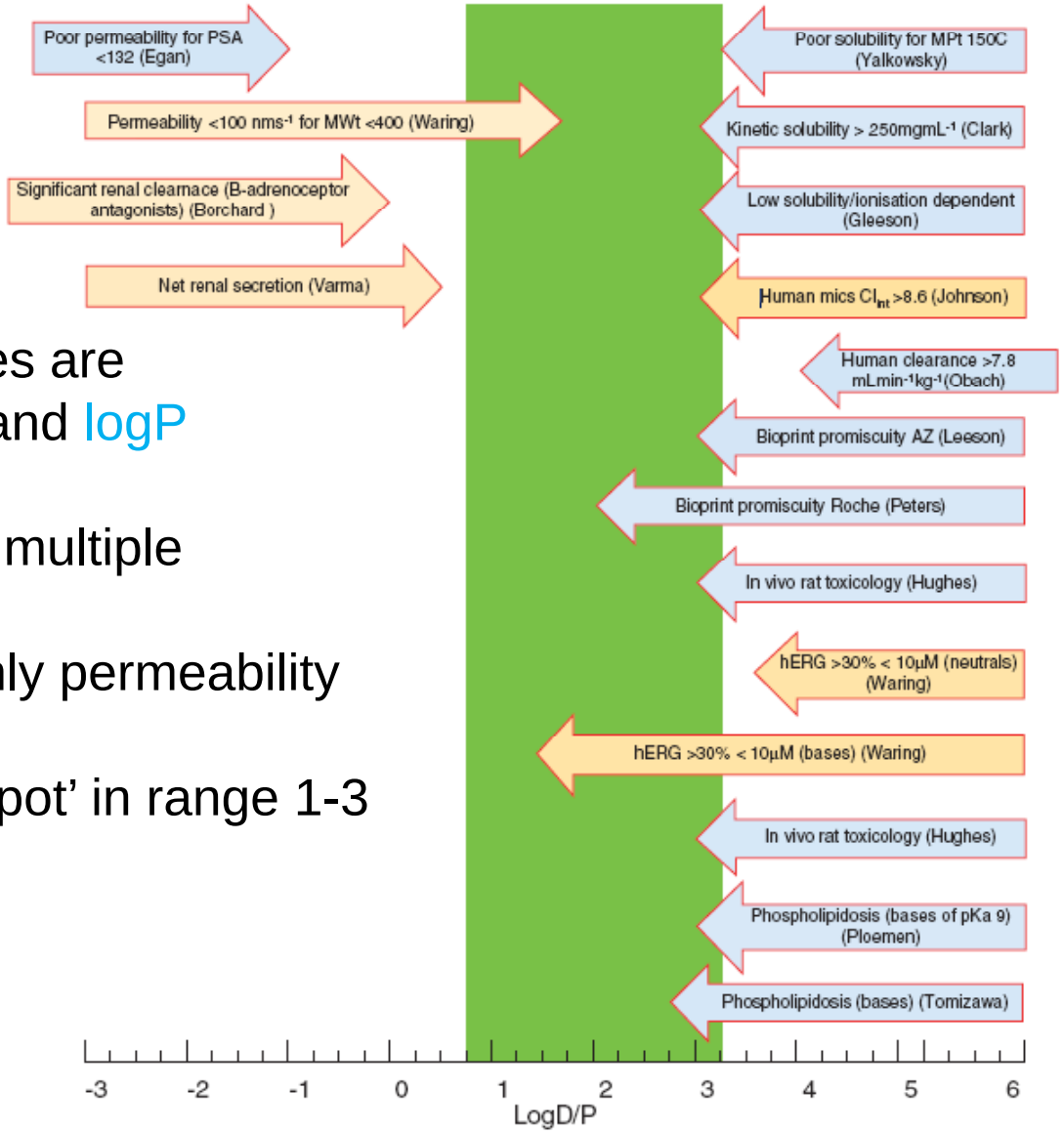
- lowering logD, you ‘only’ need to worry about potency and permeability

‘Optimum’ Lipophilicity

- Plot showing where properties are compromised based on logD and logP

- Problems with high logD are multiple (and increasing)
- Problems with low logD mainly permeability

- Analysis shows that ‘sweet spot’ in range 1-3

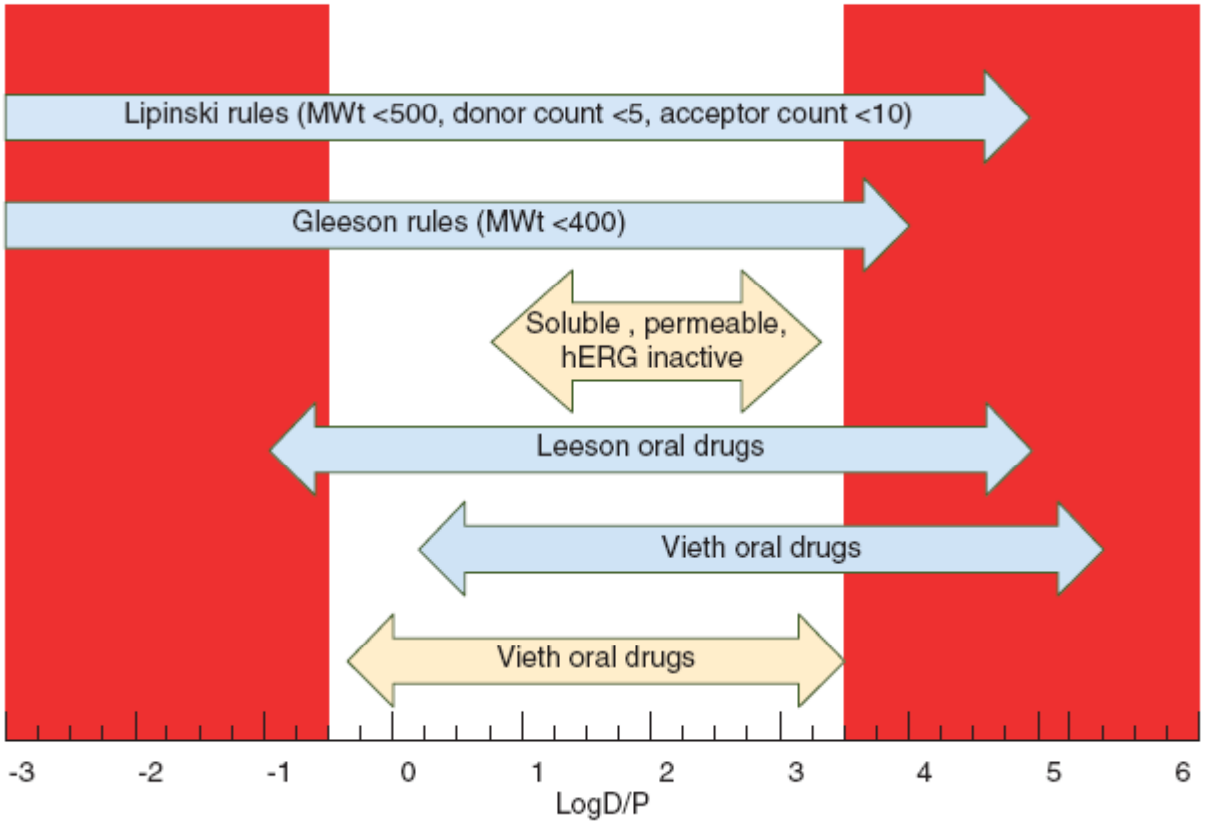


[Waring, M. J. *Expert Opinion on Drug Discovery*, 2010, *5*, 235]

‘Paradise’ between a rock and a hard place?

• All recent cpds entering clinical studies from AZ CVGI group within defined logD range

‘Optimum’ Lipophilicity



& this lipophilicity window is where high-quality drugs are found …..

[Waring, M. J. *Expert Opinion on Drug Discovery*, 2010, *5*, 235]

More often than not..

- Solubility is too low
- Hepatic Clearance is too high
- Duration is too short
- Selectivity is a problem
- Toxicology is a problem

Reduce lipophilicity!